Investigation of the mode of action of succinic acid and amino acids during hair bleaching treatment

Dissertation

zur Erlangung des

Doktorgrades der Naturwissenschaften (Dr. rer. nat.)

der

Naturwissenschaftlichen Fakultät I – Biowissenschaften –

der Martin-Luther-Universität

Halle-Wittenberg,

vorgelegt

von Frau Jing Hodes

geb. am 24.September 1986 in Ping Ding Shan, China

Gutachter:

1 Prof. Dr. Dr. h.c. Reinhard H. H. Neubert

2 Prof. Dr. Thomas Gassenmeier

3 Prof. Dr. Volkmar Vill

Tag der Verteidigung: Halle (Saale), 19.08.2019

Contents

Abbreviations

AFM	Atomic force microscopy
CMC	Cell membrane complex
DHI	5,6-Dihydroxyindole
DEPMPO	5-(Diethylphosphono)-5-methyl-1-pyrroline N-Oxide
DIPPMPO	5-Diisopropoxy-phosphoryl-5-methyl-1-pyrroline-N-oxide
DMPO	5,5-Dimethyl-1-pyrroline N-oxide
DSC	Differential scanning calorimetry
EDTA	Ethylendiaminetetaacetic acid
EDDS	Ethylenediamine-*N*, *N'*-disuccinic acid
EPR	Electron paramagnetic resonance
ESR	Electron spin resonance spectroscopy
FT-NIR	Fourier transform near infrared spectrometer
GLDA	N,N-bis(carboxymethyl)glutamic acid
IF	Intermediate filaments
IDS	Tetrasodium-iminodisuccinate
IR	Infrared spectroscopy
KAP	Keratin associated protein
18-MEA	18-Methyleicosanoic acid
NMR	Nuclear magnetic resonance spectroscopy
ROS	Reactive oxygen species
SEM	Scanning electron microscopy

TEM	Transmission electron microscopy
UV	Ultraviolet
XPS	X-ray photoelectron spectroscopy

Table of tables

Table of figures

Chapter 1 Introduction

1.1 Human hair fibre

All human hair is ethnic, which is categorized into 3 major distinct groups according to ethnic origin: African, Asian, and Caucasian [1]. Categorizing diverse hair types makes it easier to recognize characteristics specific to each hair type, such as curliness, color, and cross-sectional parameters. All hair, however, regardless of its ethnic origin, exhibits common characteristics of morphology, chemical composition and molecular structure. [2] Therefore, in chapter 1 of this thesis, it provided a brief review of the salient features of hair morphological structure, chemical compositions, and the fundamental interactions that contribute to the properties of hair fibre and its response to different chemical, mechanical, or environmental treatments.

1.1.1 Morphological components of human hair fibre

Human hair fibre can be divided into three general components [3]: an outer cuticle cell layer, an inner cortex, and a central medulla (in some cases). All are composed of dead cells, which are mainly filled with keratin protein [2]. Its hierarchical structure is displayed in Fig. 1.1 [4].

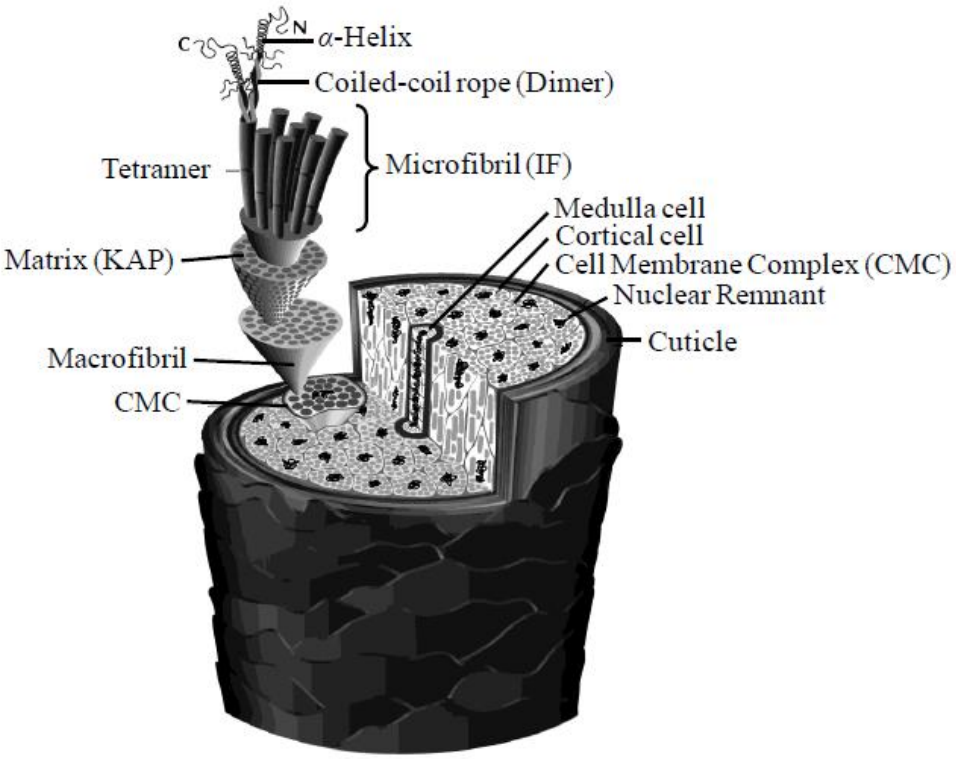


Figure 1. 1 Schematic of hierarchical structure of human hair fibre [4].

The cuticle is composed of proteins of a high cross-link density and consists of the epicuticle, the A-Layer, the exocuticle, the endocuticle and the cuticular cell membrane complex (CMC), as shown in Fig. 1.2, which protects the inner tissues of human hair fibre. The cuticle layer is generally 5-10 scales thick. Each cuticle cell is 0.3-0.5 µm thick and the visible length is between 5 and 10 µm. [5, 6] The A-layer is highly cross linked by disulfide bonds of cystine (of high cystine content ~30 %) which is responsible for its considerable mechanical toughness and chemical resilience [6]. Regarding the inner layers, the exocuticle is also of high cystine content (~15 %) and the endocuticle is low in cystine (~3 %) containing much of the non-keratinous cellular debris and a high content of basic and acidic proteins [2, 6]. The cuticle CMC is a lamellar structure, which consists of the inner β-layer, the δ–layer and the outer β-layer. The central core δ–layer is bound on both sides by two lipid-endowed β-layers [6, 7]. The out β-layer of CMC

separates the cuticle cells from each other. The layers of overlapping cells constitute a diffusion barrier that need to be considered during the processes for chemical modification of human hair. In conclusion, low crosslinked regions of cuticle, the intercellular material and endocuticle combined with its hydrophilic character can function as preferred diffusion pathways because of their high ability to swell in aqueous environment [8]. Under non-aqueous conditions, molecules diffuse through intercellular pathways which can be explained by the high mobility of lipid and protein molecules of the CMC at room temperature [5]. Additionally, it is well known that a monolayer of covalently bonding lipids, mainly 18-methyleicosanoic acid (18-MEA), a branched-chain fatty acid, is grafted onto the outermost surface of each cuticle [9]. As indicated in Fig. 1.2, the 18-MEA is covalently bound to the amorphous proteins of cuticle surface via thioester or ester linkages with cysteine residues [9]. The presence of such a lipid film plays important roles in such as hair's smoothness to the touch and its surface hydrophobic [10]. Epicuticle, containing different amino acid components, is a thin protein layer existing between the lipid layer and A-layer [11].

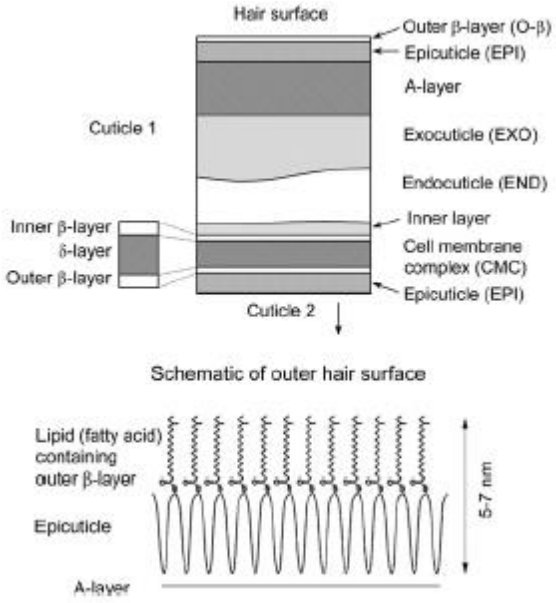


Figure 1. 2 Schematic of cross section of a human hair cuticle [2, 6, 12, 13].

The morphologically dominant component of human hair fibre is cortex, which is made up of closely packed spindle-shaped macrofibrils and whose axis orient parallel to the axis of human hair fibre as it is shown in Fig. 1.1 [4]. The macrofibrils have two main structures, the intermediate filaments (IF), previously called microfibrils and the matrix, known as keratin associated protein (KAP), which are distinguished by their structures and amino acid compositions [14]. The microfibril is a crystalline fibrous protein that is mainly composed of α-helical proteins with low cystine content (~6 %) and whose axis is parallel to the fibre axis and embedded in an amorphous matrix with rich cystine content (~21 %) [15]. The matrix comprises the largest structural subunit of the cortex of human hair fibre. The helical structure is stabilized by the hydrogen bonds inside the helix chain (Fig. 1.3a), causing the chain to twist and exhibit a helical shape. Two isolated α-helix chains form a coiled-coil (the dimer) by disulfide cross links (S-S), then dimers aggregate end-to-end and stagger side-by-side via disulfide bonds (S-S) to form a protofilament, two protofilaments laterally associate into a protofibril, four protofibrils combine into a circular or helical IF (Fig. 1.3b). [16]. Then, the IFs pack into a supercoiled

conformation, and link with the matrix proteins. Therefore, hair keratins can be considered as a polymer/polymer composite of crystalline filaments embedded in an amorphous matrix. This is known as the two-phase model and it has been often used to investigate and explain the mechanical and thermal properties of hair fibre.

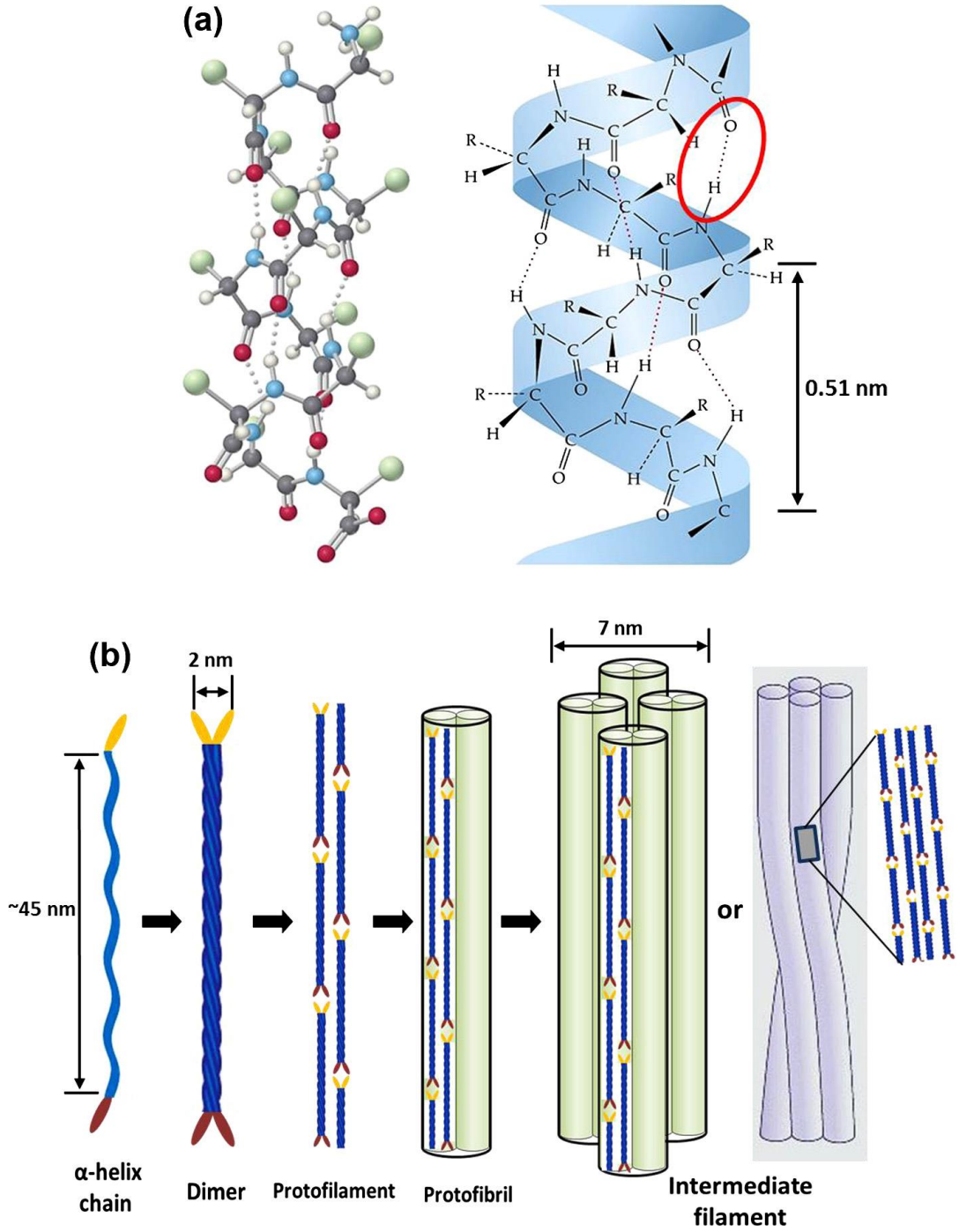


Figure 1. 3 Intermediate filament structure of α-keratin: (a) α-helix with hydrogen bonds (red ellipse) insides the polypeptide chain; (b) schematic displaying the intermediate filament formation [16].

Meanwhile, D. Istrate el at. [17] hypothesized a three-phase model, as shown in Fig. 1.4, to describe the behavior of hard alpha-keratins in human hair fibre, in which the interface phase, made of non-helical terminal domains of keratin, lies between IF and KAP. The non-helical terminal domains of keratin, which project into the IF, link with the

KAP through disulfide bonds (S-S). The terminal domains contain besides cystine, glycine, threonine, valine, alanine and serine, acidic sites as glutamic and aspartic acid [17]. The scaffolding structure at the IFs surface, made by the side-chain interactions that anchored microfibrils to matrix, controls and enhances the thermal properties of keratin filaments [17].

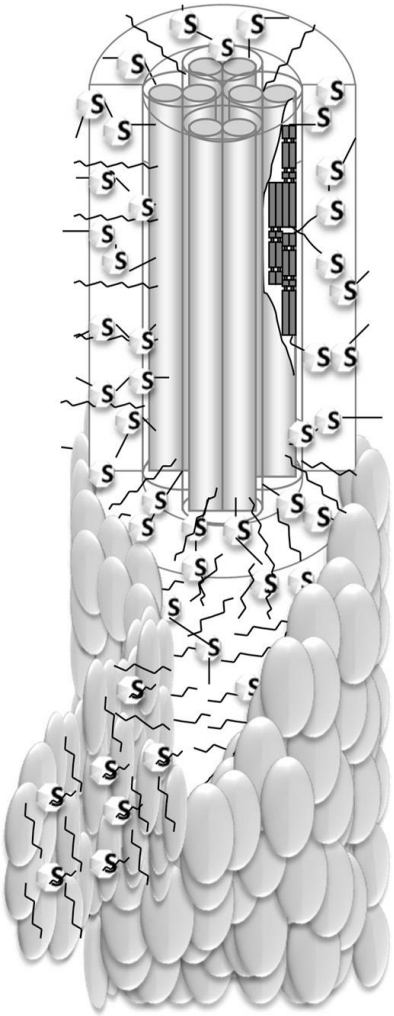


Figure 1. 4 Schematic of hypothesized network structure of the IF-KAP structural unit of human hair fibre with the “interface phase” [17].

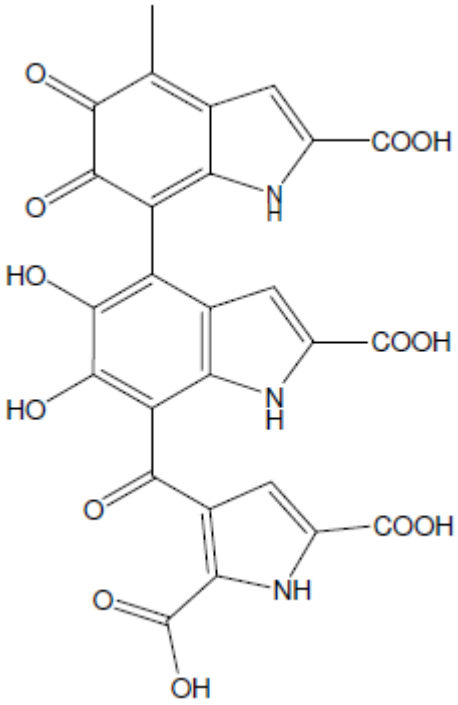
The cell membrane complex (CMC) consists of cell membranes and adhesive material that binds the cuticle and cortical cells together. The CMC is primarily non-keratinous protein and is low in cystine content (~2 %). [7]. Together with the endocuticle, the CMC

forms the non-keratinous regions, which is nowadays regarded as the primary pathway for entry or diffusion of cosmetic products into hair fibre [18].

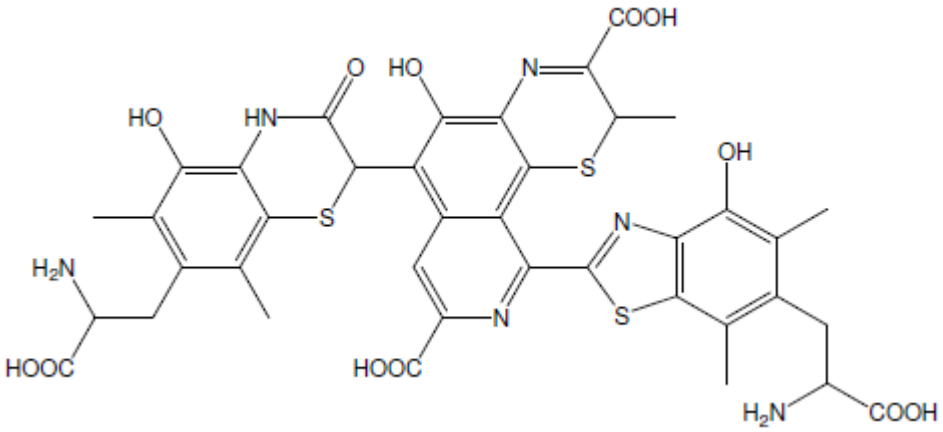
The medulla of human hair, if present, consists of only a small percentage of the mass of whole hair fibre, and is believed to contribute negligibly to the mechanical properties of human hair fibre [6]. Medulla is a thin cylindrical layer in the center of hair fibre containing high amount of lipid and low amount of cystine [19]. Medullary cells are loosely packed, and during formation, they leave a series of vacuoles along the fiber axis, which are believed to be caused by a defect in the synthesis of the microfibril-matrix complex in the cortex, most likely with less being produced. This effect creates cavities or air spaces in hair. The function of medulla in human hair fibre is, however, not yet completely elucidated. S. Nagase et al. [20, 21] suggested that the medulla seems to play a role in gray hair. The medulla may also be involved in the splitting of hairs since in addition to the CMC it also provides a pathway or an area of weakness for the propagation of cracks along the axis of the fibre.

The color of human hair varies from nuances of blond and red to brown, black, grey and unpigmented. Melanin pigments are responsible for the color in human hair, which are mainly found in the cortex of human hair fibre (in the keratin macrofibrils and in the microfibrillar matrix) and present in granular form [2]. The melanin granules (melanosomes) [22] contain small amounts of protein and varying proportions of two types of highly heterogeneous polymeric pigment, eumelanin and pheomelanin (Fig. 1.5). Eumelanin is responsible for black and brown hair color and is insoluble in solvents and chemically intractable to all but powerful oxidizing agents such as hydrogen peroxide. Eumelanin (Fig. 1.5a) is believed to be a polymer derived from oxidative copolymerization of 5,6-dihydroxyindole (DHI) and 5,6-dihydroxyindole-2-carboxylic

acid (DHICA), which are aggregated into nanoparticles and further encapsulated into melanosomes [23]. These DHI derivatives are synthesized in the body from tyrosine in a process known as melanogenesis [23, 24]. Pheomelanin is responsible for yellow and brownish-red hair color and contains significant amounts of sulfur and is soluble in strong alkali. [22] Pheomelanin (Fig. 1.5b) is composed of tyrosine and cysteine-derived units constructed into benzothiazine monomers that produce the polymer [25, 26]. Melanin granules, which can capture free radicals and adsorb ultraviolet (UV) and visible (Vis) radiation, are expected to play a role in natural photoprotection.[27] The absence of melanin granules in unpigmented hair is the reason why unpigmented hair is normally more prone to display the effects of photodegradation during light exposure than dark hair. Additionally, photo yellowing has also been observed in grey hair as tryptophan is degraded to 3-hydroxykynurenine that has a yellow color [27].



(a)



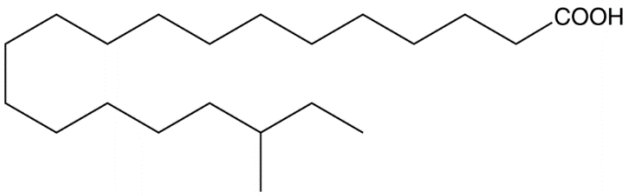
(b)

Figure 1. 5 Chemical structure of (a) eumelanin and (b) pheomelanin [25].

1.1.2 Chemical composition of human hair fibre

Human hair, as a type of keratin fibre, depending on its moisture content, consists of approximately 65 % to 95 % proteins which are condensed polymers of 24 different amino acids [24]. These amino acids are classified in five groups: acidic amino acids, basic amino acids, amino acids with hydroxyl groups, sulfur-containing amino acids, and amino acids with no reactive groups in the side chains [13]. The other components are lipids, water, sugars, melanin pigments and trace metals which represent only a minor fraction [2]. Table 1.1 displays a summary of chemical composites of human hair fibre [24]. In addition, trace metals detected in human hair fibre may play an important role in the composition of pigments. The most frequently found trace metals are Ca, Mg, Cd, Cr, Cu, Hg, Zn, Pb, Fe, As and Si. Most of them are incorporated in hair from extraneous source but are probably integrated in the fibre structure as a salt linkages or co-ordination complexes with side-chains of melanin pigment or proteins. [28]

Table 1.1 Summary of chemical composites present in human hair [24].

Chemical components in human hair	Content or chemical structure
Keratin (protein)	65-95 %
Amino acids	NH^{3+} — CH — R | CO^{2-} (R: functional group)
Cystine	NH^{3+}-CH-CH_2-S-S-CH_2-CH-NH^{3+} | | CO^{2-} CO^{2-}
Lipids	Structural and free
18-Methyleicosanoic acid (18-MEA)	
Water	Up to 30 %
Pigment and trace elements	Melanin, Fe, Cu etc.

Human hair protein, primary composed of α-helix keratin, like all proteins, contains both cationic and anionic groups and is therefore amphoteric [8]. The macromolecular structure of keratin derives its stability from a variety of intra-chain and inter-chain interactions holding the individual peptide chains together to form a super-helical structure (Fig. 1.3b) [16]. The individual peptide chains in hair fibre are held together by various types of covalent bonds and non-covalent interactions, as shown in Fig. 1.6 [28]. A high cystine content corresponds to rich disulfide cross-links. These disulfide bonds (S-S) contribute to physical and mechanical properties as well as structural stability of human hair, if it is not exposed to reducing, oxidizing and hydrolytic agents or to weathering [29, 30]. Western blot and amino-acid analysis revealed the presence of γ-glutamyl-ε-lysine isopeptide linkages in human hair fibre that could constitute a second covalent network [31]. The formation of isopeptide protein-protein cross-links are catalyzed by the calcium-dependent enzymes such as the family of transglutaminases,

which are key enzymes involved in the construction of this structure [31, 32]. This second covalent bonds provide an additional physical and mechanical stability for hair fibre. The non-covalent bonds consist of three main groups: hydrogen bonds, ionic bonds (or salt bridges), and the hydrophobic effect. Although relatively weak and easier broken by water, the hydrogen bonds are the most numerous in human hair. The hydrogen bonds can be realized between –CO and –NH groups between one peptide chain and another (intermolecular) and the amino and carboxyl groups within a peptide chain (intramolecular). The ionic bonds are formed between cationic and anionic side chain groups giving rise to coulombic interactions that are relatively stable in aqueous environments but are readily broken by acids and alkalis. The hydrophobic effect results from the approach of two non-polar side groups.[28]

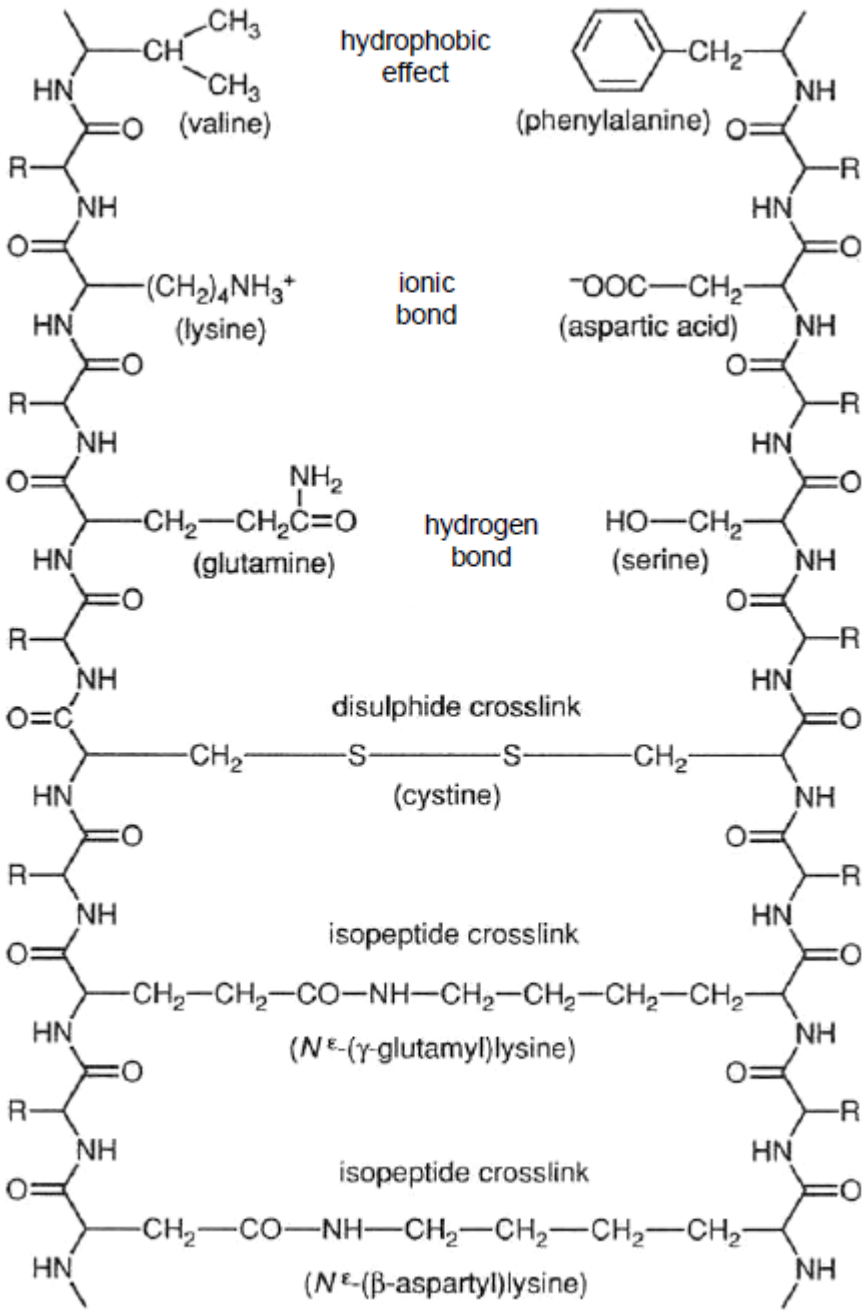


Figure 1. 6 Schematic representation of covalent and non-covalent bonds between segments of two hypothetical peptide chains [28].

1.1.3 Metal contents in human hair fibre

A number of studies has reported that there is a range of levels of metal ions detected in human hair fibre such as calcium, magnesium, iron, copper, zinc and etc [13]. However,

certain transition metals such as iron and copper can catalyse the formation of free radicals in oxidative reactions involved in hair oxidative coloring and bleaching processes. This fact has been of high interest in cosmetic science. The transition metals in human hair come both from an endogenous and exogenous source, for example the environment, in which hair is exposed to and in particular to the water used during the shampoo and rinsing-off processes; and the final levels of copper in hair can vary from 20 ug g^{-1} to > 1000 ug g^{-1} [33]. The endogenous metals are incorporated in the human hair fibre at the hair follicle and are thought to act as a pathway for the body to excrete unwanted metals. It was also reported that copper from swimming pool can turn blond hair green at even low concentration. [34]

A previous study performed by Procter and Gamble where human hair was collected from 450 women across nine countries (colorant users and non-users) clearly showed that there are significant levels of copper present in human hair and that these levels increase from root to tip as hair increases and coordination sites for copper increases [27]. In addition, it should be not ignored that hair dye products can also serve as an external source of metal ions which are mainly introduced as mordanting agents in the coloring system [35]. Metal complex/oxides, such as titanium dioxide and iron oxide, are also often used as colorants in the process [36]. It has been reported that the coloring process can alter the levels of many transition metals and cause an increase in the concentration of iron, copper, magnesium, calcium and etc. in the hair samples from female consumers [35, 37]. Although the calcium does not directly take part in the bleaching chemistry via Fenton or Fenton-like reaction, it can affect the shine and combing properties of hair by forming insoluble calcium salts and soaps on the surface of hair fibre [34]. For instance, human hair is rich in sulphur containing components;

therefore, it is believed that metal ions are bonded through metal-sulphur interaction, such as salt linages or coordination complexes with the side chains of the proteins or pigments. Hydroxyl groups of serine and nitrogen groups may also provide metal binding sites. A previous EPR study also proposed nitrogen and oxygen binding sites for copper in hair fibre [25]. The carboxylate anions of dicarboxylic acids (aspartic acid and glutamic acid) can also chelate metal ions. This fact was proved by the observation that hair absorbs more alkaline earth metals at neutral pH due to deprotonation of carboxylate [25]. The regions of high carboxylic acid content of hair fibre like the endocuticle, the CMC of the cortex and the medulla are likely to have a high affinity for divalent and trivalent metals [13].

It has been reported that oxidative damaged hair like dyed or bleached hair which are washed multiple times in tap water, accumulates high concentrations of metal ions in the sulfonate rich regions of the hair such as A-layer, exocuticle, and the matrix of the cortex [13]. As such oxidative hair treatments oxidize cystine and cysteine, major components of hair keratin, to cysteic acid and these sulfonate groups complex with hard water metal ions such as calcium and magnesium and transition metal ions such as copper and iron from the wash water. The metal uptake is proportional to the level of oxidative damage in the hair and the pH of the wash water, and the level of calcium ions in hair from a regular colorer can be up to 10 000 ug g^{-1} [34]. Previous reviews suggested that Ca^{2+} has a higher affinity for carboxylic acid and sulfonate groups, while Cu^{2+} has a preference for binding with primary amine groups -NH_2 and Cu^{+} has a higher affinity for thiol groups -SH [38].

As described above, metals like Fe^{2+} and Cu^{2+} which bind to polar groups in hair fibre can participate in oxidation-reduction reactions such as in Fenton or Fenton-like reactions

respectively by generating active oxygen compounds. The increased uptake of copper ions can also contribute to further fibre damage during subsequent coloring or bleaching due to its ability to take part in metal-induced radical chemistry [34].

Melanin granules contain highest concentration of metal elements, particular transition metals such as iron and copper and have the property of being able to coordinate metal elements [39]. Besides that, Y. Liu et al. [40] demonstrated that there are significant amounts of Cu and Zn bound to both black-hair and red-hair melanin granules, however, the Fe content is four times higher in red-hair melanin granules. Moreover, there was a study performed with *sepia* melanin. When purified *sepia* melanin without metal elements is added to an alkaline hydrogen peroxide solution, the reaction is very mild. Whilst, when *sepia* melanin coordinated with metal element is added to the alkaline hydrogen peroxide solution, the sepia is quickly bleached. Based on these findings, it was supposed [39] that the melanin granules react more easily with hydrogen peroxide than the other hair proteins, because the decomposition speed of hydrogen peroxide solution with brown hair is faster than with white hair. Therefore, it was considered that the metal elements in the melanin granules act as a decomposition catalyst for hydrogen peroxide and this provides an evidence to support the suppositions. Once the melanin granules have been completely solubilized, the metals coordinating to the melanin granules would flow out during the bleaching process and become a catalyst for hydrogen peroxide in other areas than the melanin granules; radical chemistry involved metal elements such as copper or iron appears to enable the bleaching to proceed to a much greater extent, leading to promote an increasing hair damage [39].

1.2 Oxidative hair treatment–hair bleaching and oxidative hair coloring

Cosmetic oxidative hair coloring and bleaching depend on the degradation melanin pigments to change the hair color, usually by treating hair with hydrogen peroxide under alkaline conditions at pH=9-10. Principally, there are at least three steps that need to take place for the degradation of melanin granules within the hair to occur: 1) Diffusion of oxidants/base into the hair to access the melanosomes. 2) Rupture of the melanosomal membrane and the solubilisation of the released melanin nanoparticles and 3) Action of the oxidant/oxidants in the melanin pigments. [23] Hydrogen peroxide is the principal oxidizing agent used in bleaching compositions, and salts of persulfate are often added as "accelerators" to achieve more lightening effect [41]. Therefore, from the cosmetically application-technical viewpoint, hair lightening is usually considered as the cosmetic procedure using alkaline hydrogen peroxide; whilst hair bleaching is considered as the cosmetic procedure additionally using salts of persulfate as "booster", besides alkaline hydrogen peroxide. The conventional cosmetic hair bleaching product generally consists of two different parts, the hair alkaline lightener base containing salts of persulfate and the lotion developer containing the hydroxide peroxide. The mixture applied to the hair will be prepared just prior to use by mixing approximately 50 g of the lightener base with 100 g of the lotion developer. However, from the scientific viewpoint, hair bleaching can also generally be considered as the procedure only using alkaline hydrogen peroxide.

Permanent hair coloring, also called oxidative hair coloring, accounts for the major share of hair color market. It is very popular among the customers due to its excellent grey coverage, wide range of color shades available and better wash-off and light fastness

properties offering a long-lasting and intense color result. Oxidative hair coloring involves a diffusion-controlled process where active ingredients penetrate the hair fibre first and then react to form a new chromophore inside hair fibre. During the oxidative hair coloring process, there are two key oxidative chemical processes taking place that contribute to the final coloring effect: the first reaction is the oxidation of the natural melanin to lighten the underlying color of hair; the second reaction is the oxidative activation of primary dye precursors (Fig. 1.7a and 1.7b) to enable a chemical reaction with couplers (Fig. 1.7c and 1.7d) for color formation [25].

A range of dye precursors is available which are used to develop various color shades. They are aromatic molecules and generally classified as precursors and couplers in hair color industry. Primary precursors are aromatic diamines or amino phenols with amino ($-NH_2$) or hydroxy (-OH) group in the *ortho* or *para* positions of the aromatic ring (Fig. 1.7a and 1.7b). These groups and their positions on benzene ring are important in determining the reactivity and rate of reaction for these molecules. The nature of these groups may also influence the development of color shade. [25, 42] The couplers are also aromatic diamines or amino phenols with a similar structure though with substitution at the meta position (Fig. 1.7c and 1.7d). They don't develop significant color themselves, however, when mixed with primary precursors under alkaline hydrogen peroxide, they form intense color shades upon a chemical reaction [42].

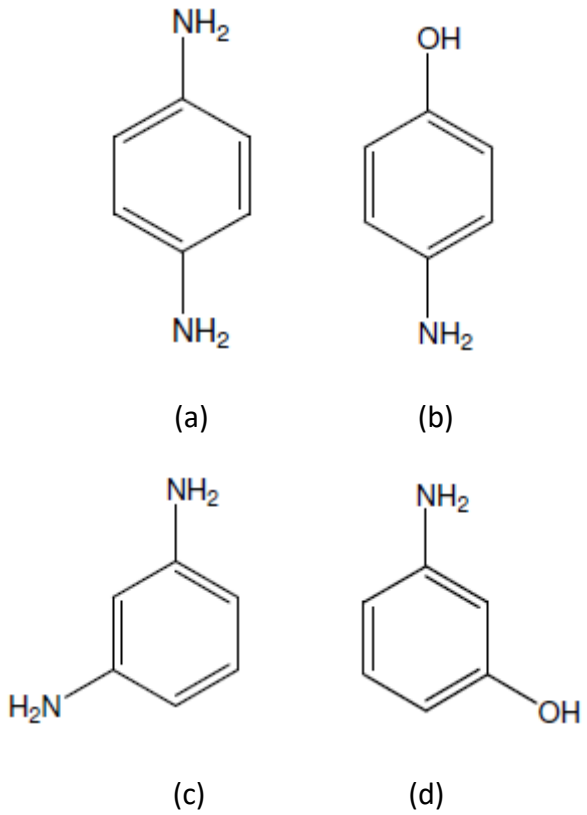


Figure 1. 7 Chemical structure of (a) primary dye precursor *p*-phenylenediamine (PPD); (b) primary dye precursor 4-aminophenol; (c) coupler dye precursor *m*-phenylenediamine; (d) coupler dye precursor *m*-aminophenol (MAP) [25, 42].

When hydrogen peroxide is combined with a para-dye, the highly active electrophilic intermediates "imin" structure such as diiminium or quinoniminium ions develops. Oxidation dye couplers are electron-rich aromatic species, which can further condense with the active intermediates creating a new dye. These reactions are usually carried out at alkaline pH, generally from 8 to 10. Commercial oxidative hair coloring application consist of two components, a color cream and an oxidising developer lotion. Oxidative color cream contains dye precursors formulated at high alkaline pH of 8-10. Ammonia is considered as the most effective alkaline agent. Developer lotion contains an oxidising agent such as hydrogen peroxide at acidic pH. These two components are mixed together just before the application. High amount of alkaline agent in the oxidative coloring cream ensures alkaline pH=8-10 condition of the final mixture which activated

hydrogen peroxide to oxidize melanin granules and dye precursors. Additionally, the alkaline agent within the mixture also helps to soften the hair, opening up the cuticle layer and allowing dyestuffs to enter the cortex.

Melanin pigments are also bleached by alkaline hydrogen peroxide in oxidative dyeing, as a result of which the hair color becomes brighter than the underlying hair color. The development of colored chromophores staying inside hair fibre and decolorization of melanin pigments during oxidative dyeing determine the final hair color after oxidative dyeing.[43]

A high alkaline pH is necessary for the deprotonation of H_2O_2 (pK_a=11.65) and the formation of the hydroperoxide anion ^-OOH, which is believed to be an important oxidant in oxidative hair coloring/bleaching processes [23]. The hydroperoxide anion ^-OOH can carry out nucleophilic attacks on melanin granules to lighten hair color but can also cause undesired hair damage such as poor hair feel and look, reduced hair strength, increased incidents of split ends and breakage [44]. It has been, however, reported [44, 45] that hydrogen peroxide at extreme high alkaline pH can also generate a variety of reactive oxygen species (ROS), which would be an additional source of hair damage.

$$H_2O_2 + OH^- \leftrightarrow HOO^- + H_2O \quad pK_a = 11.65 \qquad (1)$$

$$H_2O_2 + HOO^- \rightarrow \cdot OH + O_2^{\cdot -} + H_2O \qquad (2)$$

$$HOO^- + \cdot OH \leftrightarrow O_2^{\cdot -} + H_2O \qquad (3)$$

$$\cdot OH + H_2O_2 \rightarrow HO_2^{\cdot} + H_2O \qquad (4)$$

Figure 1. 8 Decomposition of hydrogen peroxide under alkaline condition [44].

Ammonia is the most widely used alkalizing agent in oxidative hair coloring/bleaching process and it is also known that the ammonia/hydrogen peroxide combination is an

effective melanin-bleaching agent. Previous literature suggested that the key function of ammonia in hair bleaching is to rupture the melanosome membrane leading to the release of melanin nanoparticles; whereas other alkalizing agents such as sodium hydroxide or sodium carbonate at identical pH conditions (e.g. pH=10) do not induce morphological changes to melanosomes like ammonia does [26]. It is presumably due to the small, non-polar and uncharged nature of the ammonia molecule, which allows it to rapidly diffuse through the melanosome membrane. Moreover, it is reported that once melanin has been solubilized by ammonia, the identity of the alkalizing agent used in hair bleaching plays no further mechanistic role in bleaching beyond deprotonation of hydrogen peroxide to give the hydroperoxide anion [23]. It is well known that ammonia can be oxidized to amino radicals $\cdot NH_2$ by hydroxyl radicals $\cdot OH$. The reaction between amino radicals $\cdot NH_2$ and oxygen O_2 resulting in amino-peroxyl radicals $NH_2OO\cdot$ is more efficient than the reaction between amino radicals $\cdot NH_2$ and amino acids [46]. Based on these results, it was proposed that during the bleaching of dark hair, amino radicals $\cdot NH_2$ cause only a minor damage of hair protein, and they are mainly responsible for initiating bleaching of melanin granules to make them more susceptible to oxidative attacks by alkaline hydrogen peroxide [46]. The formation of diverse radical species during bleaching process was reported to further lead to hair structure damage as measured by protein loss [33].

Human hair consists primarily of keratin protein, which contains a large percentage of oxidative groups. In addition to bleaching melanin granules and oxidising precursors with couplers, these oxidative treatments with alkaline hydrogen peroxide can induce various chemical modifications of hair protein which lead to change of the physicochemical and morphological properties of human hair. For example, hair

contains thioester bonds at the surface and between cuticle cells. When hair is exposed to chemical processes such as oxidative coloring or bleaching, changes occur firstly in the surface layers and in the removal of the 18-MEA. The reaction with 18-MEA results in the formation of acidic sulfur compounds, such as cysteic acid residues. Hair fibre properties, especially the hair surface, change from a hydrophobic to more hydrophilic characteristics. Bleach also oxidizes cystine residues of the hair matrix in the cortex and other hair regions rich in cystine such as the A-Layer and the exocuticle inside cuticle cells. These reactions result in the formation of cysteic acid residues and in the breakdown of the CMC, the cuticle and cortex components and ultimately dissolving proteins in these regions [13]. As the disulphide bonds contributes to the strength and stability of hair, so its cleavage leads to hair breakage and split up.

1.3 Methods for damage assessment of human hair fibre

Hair is a complex assortment of many different proteins and has multiple structural layers that can be modified by a given treatment in multiple ways. Therefore, diverse damage products and locations are expected in human hair after the chemical treatments such as oxidative hair coloring and bleaching. The damage assessment of human hair can be achieved by a series of methods.

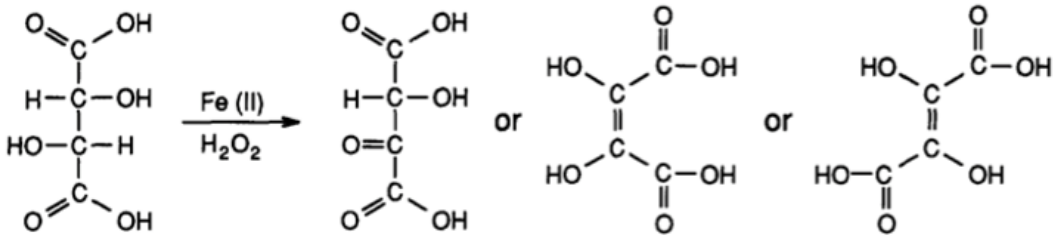
Scientific test methods such as tensile strength test, hair breakage test, fatigue test, wet and dry combing test have been established as standard methods to evaluate the degree of hair damage due to chemical treatments in order to compare the performance of different formals and products. Chemical methods have been established to analyze the amino acid composition of human hair. Cysteic acid residues, the oxidation product of cystine or disulfide bonds, is as an indicator of hair damage resulted from bleaching- and oxidative coloring processes. The changes in electrophoretic patterns of cosmetically treated hair contribute also to damage evaluation [28]. Spectroscopic methods, like Raman [14, 15] and infrared spectroscopy (IR) [47], X-ray photoelectron spectroscopy (XPS) [9] are widely employed for quantifying structural and chemical changes at the molecular level. The morphological changes at the microstructural level can be investigated by approaches of scanning electron microscopy (SEM) [18, 39, 48], transmission electron microscopy (TEM) [18, 39, 49] and atomic force microscopy (AFM) [6]. Taking in account all those observations, these methods can be employed together in order to better understand the mechanism of hair damage, assess the location and the grad of hair damage from different aspects.

1.4 Fenton chemistry

The oxidation of organic substrates by iron(II) and hydrogen peroxide is called the "Fenton chemistry", which was first published by H.J.H Fenton more than 100 years ago [50]. He observed the oxidation of tartaric acid by H_2O_2 in the presence of ferrous iron ions and proposed the reaction mechanism, as shown in Fig. 1.9. He described that *"When tartaric acid in aqueous solution interacts with certain oxidizing agent in the presence of a trace of ferrous salt, a solution is obtained which gives a beautiful violet color on the addition of a caustic alkali" [25].* Alternatively, the name of "Fenton reaction" or " Fenton reagent" is also often used. The system Fe(II)-H_2O_2 is an efficient oxidation agent for various organic substrates. It is well known that Fenton reagent is effective in treating various industrial wastewater components including aromatic amines, a wide variety of dyes, pesticides, surfactants, explosives as well as many other substances. As a result, the Fenton reagent has been applied to treat a variety of wastes such as those associated with the textile industry, chemical manufacturing, refinery and fuel terminals, engine and metal cleaning etc. The Fenton reagent can also effectively be used for the destruction of toxic wastes and non-biodegradable effluents to render them more suitable for secondary biological treatment. [50]

Fenton mentioned two important conditions in his findings for the reaction: firstly, the presence of an oxidizing agent e.g. hydrogen peroxide or chlorine water; and secondly, a heavy metal such as iron (II) in its reduced form, but in low concentration. Almost 20 years later he identified the structure of the products. The violet color that Fenton observed is due to Fe complex of dihydroxy maleic acid. Later, Haber investigated the iron catalysed decomposition of hydrogen peroxide and proposed the reaction

mechanism through free radical pathway [25]. However, there is still a debate about the reaction mechanism whether through radical intermediate or not for around 80 years.



L-(+)-Tartaric acid 2-hydroxy-3-oxobutanedioic acid 2,3-dihydroxymaleic acid 2,3 dihydroxyfumaric acid

Figure 1. 9 Oxidation of tartaric acid in a Fenton reaction [25].

1.4.1 Mechanism of Fenton or Fenton-like reaction

The chemistry of Fenton reaction revolves around the transition metal such as copper or iron in its lower oxidation state such as Fe^{2+}, which is oxidized to a higher oxidation state Fe^{3+} using an oxidizing agent such as hydrogen peroxide, and then is reduced back to the original lower oxidation state Fe^{2+}. The important outcome of the reaction is the formation of new oxidizing intermediate species, which is much more reactive and powerful than the original oxidizing agent. [25, 51]

The earliest Fenton reaction mechanism was proposed that the decomposition of hydrogen peroxide in the presence of Fe^{2+} is a chain reaction which generates hydroxyl radical as intermediate species. The hydroxyl radical is the new oxidant formed in Fenton reaction and is much more reactive and powerful than hydrogen peroxide [51]. It is a highly reactive and very short-lived species which may undergo a reaction with hydrogen peroxide further yielding superoxide. The Fe^{2+} is oxidized to Fe^{3+} which in turn is reduced back to the original state Fe^{2+} by superoxide to enter in a new reaction cycle, as shown in Fig. 1.10. The Fe^{3+} catalytically decomposes hydrogen peroxide following a similar

mechanism converting Fe^{3+} to Fe^{2+} generating superoxide. The Fe^{2+} and superoxide then enter the cycle for the decomposition of hydrogen peroxide.

$$Fe^{2+} + HOOH \rightarrow Fe^{3+} + {}^{-}OH + {}^{\cdot}OH$$

$$^{\cdot}OH + HOOH \rightarrow H_2O + {}^{\cdot}OOH$$

$$^{\cdot}OOH \leftrightarrow O_2^{\cdot-} + H^+$$

$$O_2^{\cdot-} + H_2O_2 \rightarrow {}^{\cdot}OH + {}^{-}OH + O_2$$

$$O_2^{\cdot-} + Fe^{3+} \rightarrow Fe^{2+} + O_2$$

$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + {}^{\cdot}OOH + H^+$$

Figure 1. 10 Iron (II) catalyzed decomposition in a Fenton reaction.

According to this theory mentioned above the chemistry related to the use of Fenton reagent is the chemistry of this radical. Therefore, taking into consideration that the Fenton reaction can also involve several other transition metal ions (M^{n+}) like Cu^{2+}, the processes connected with the reactions similar to Fenton reaction may be characterized as follows [50]:

$$(M^{n+}) + H_2O_2 \rightarrow (M^{n+1}) + OH^- + {}^{\cdot}OH$$

Due to the high reactivity and short half-life of hydroxyl radical, it is extremely difficult to detect and quantify hydroxyl radical by any direct method. Almost at the same time as Haber proposed the Fenton reaction mechanism through free radical pathway, the hypothesis of reaction mechanism though non-radical pathway was also reported [52]. An iron-oxo intermediate or a complex in high oxidation state was proposed (Fig. 1.11).

$$Fe^{2+} + H_2O_2 \rightarrow [Fe(IV)=O^{2+}] + H_2O$$

$$FeO^{2+} + H_2O_2 \rightarrow Fe^{2+} + H_2O + O_2$$

Figure 1. 11 Formation of iron-oxo intermediate in a Fenton reaction [25, 53].

Recently, there are a few of studies which have demonstrated that the Fenton reaction may proceed through various pathways [54, 55]. These various pathways in a given reaction are dependent on many factors such as: nature of the metal, nature of the ligand and solvent, the concentrations of the reactants, the nature and the concentration of any substrate, the ratio of metal to hydrogen peroxide and pH of the reaction mixture [51, 56]. All these factors play an important role in understanding the mechanism of Fenton reaction. The hydroxyl radical could be formed under the specific conditions, whereas ferryl ion might be the dominant intermediate under different conditions. What is interesting to us is that regardless of whether hydroxyl radical or iron-oxo complex is formed in Fenton reaction, the intermediate species is a high reactive and powerful oxidant which plays an important role in biology, medicine, ecology, organic chemistry and biochemistry [53]. The reactive $HO^{\cdot}$, H_2O_2 and other ROS oxidants are connected to aging and severe human diseases such as cancer, cardiovascular disorders, and Alzheimer's, and related neurodegenerative diseases. It is well known that the exposition to certain noxious risk factors, such as some xenobiotics, infection agents, pollutants, UV light, cigarette smoke, and radiation, may lead to the production of ROS. ROS, and also non-radicals such as peroxynitrite annion ($ONOO^-$), peroxynitrous acid (ONOOH), nitosoperoxycarbonate anion ($ONOOCOO^-$), nitronium cation ($^+NO_2$), and dinitrogen trioxide (N_2O_3) are continuously generated in small quantities on normal cellular processes. Endogenously produced ROS are essential to

life, being involved in many different biological functions. However, when overproduced, or when the levels of antioxidants become severely depleted, these reactive species become highly harmful, causing oxidative stress through the oxidation of biomolecules. The very important fact is that Fenton chemistry plays a crucial role in both physiological and pathological process in living organisms. The Fenton and Fenton-like reactions are probably the earliest chemical means of ROS generation by Nature [50, 53].

1.4.2 The role of copper ions in hair bleaching

Transition metal ions in human hair fibre [57], such as iron or copper ions, are known to bind to the protein polymers and are concentrated in the outer three or four cuticle layers in hair [58]. Copper (II) catalyses decomposition of hydrogen peroxide by following a mechanism like Fenton-like reaction (Fig. 1.12) [25]. It reacts with hydrogen peroxide to give Cu^{+} and superoxide. The reduced Cu^{+} decomposes hydrogen peroxide to generate hydroxyl radical and lead to oxidation of Cu^{+} back to Cu^{2+} which completes the cycle. The superoxide also reduces Cu^{2+} to Cu^{+} to continue the metal recycling.

$$Cu^{2+} + H_2O_2 \rightarrow Cu^{+} + HOO\cdot + H^{+}$$

$$Cu^{+} + H_2O_2 \rightarrow Cu^{2+} + HO^{-} + HO\cdot$$

$$Cu^{2+} + HOO\cdot \rightarrow Cu^{+} + H^{+} + O_2$$

Figure 1. 12 Copper (II) catalyzed decomposition of hydrogen peroxide in a Fenton-like reaction [44].

These transition metals like iron or copper can catalytically decompose hydrogen peroxide under alkaline condition via the Fenton chemistry or Fenton-like reaction respectively during oxidative hair coloring or bleaching treatment, which results in the formation of reactive oxygen species (ROS) such as hydroxyl radicals $HO\cdot$ and

hydroperoxyl radicals $HOO^{\cdot}$ or superoxide $O_2^{\cdot -}$ radicals even in the presence of low levels of iron or copper ions [44]. Hydroxyl radical $^{\cdot}OH$ is highly reactive towards organic matrix such as hair proteins, leading to significant hair damage; however, hydroperoxyl/superoxide radicals $^{\cdot}OOH/O_2^{\cdot -}$ were reported to display a lower reactivity towards hair proteins [44]. These copper metal-induced radical species can further cause protein degradation leading to loss of hair fibre strength and consequent hair breakage [33, 58].

The harmful effects of sun radiation on untreated human hair are considered to be a photosensitized oxidation of the structural proteins via the formation of activated reactive oxygen species (ROS) driven by the UVA radiation (315-400 nm) of the solar spectrum. Incorporation of transition metal ions can further increase the photosensitivity of the system and thereby significantly increase the photodegradation. [35]. The role of copper in further increasing the photodegradation and thereby significantly increasing the level of hair damage from UV exposure has also been confirmed in the previous studies [27, 35]. Therefore, especially in oxidative hair coloring and bleaching process, it is necessary to attempt to eliminate the transition heavy metals like copper and iron as much as possible to prevent accumulation of metals in hair. Besides hair damage resulted from redox metal-induced radical formation, the high amount of transition metals accumulated in hair can also cause the burn injury of scalp during the application of hair bleaching product. This was a customer complain from United States in 2014. The bleaching-treated hair fibres were collected and analysed. There were 8100 ppm calcium and 770 ppm copper detected in the hair fibres. This level of copper ions and calcium ions in the hair was much higher than the level in untreated hair which was shown in Table 3.2. Additionally, regarding the storage stability of hair

developer lotion containing hydrogen peroxide, stabilizers (e.g. chelating agents) and separate containers are often used to reduce the rate of decomposition of the peroxide induced by transition metals and to provide satisfactory shelf life [41].

1.4.3 Introducing chelating agents in bleaching process

In order to reduce transition metal-induced hydroxyl radical ·OH formation during bleaching process, the ligands are often used with the aim to chelate metal to stabilise hydrogen peroxide. This State-of-the-art technology has been listed in the previous most important literatures [25, 27, 33, 58] and patent references (in the chapter 8). The main criteria for choosing a particular chelating agent in hair bleaching system are based on the regulatory concerns, commercial availability, biodegradability, binding strength with the metal and stability of the metal-chelating agent complex under the reaction conditions. Besides transition metals like copper and iron, human hair fibre contains different other metals e.g. calcium ,magnesium, sodium and potassium. These alkaline earth metal ions compete with transition metal ions for the added chelating agent which may have a great effect on the catalytic activity of the transition metal ions like copper or iron in the decomposition of hydrogen peroxide. These differences may reflect differences in stability constants. The stability constant (K) [59], expressed as log K, is used to describe the strength of the complex formed between the metal ion and the chelating agent. The higher the log K values, the more tightly the metal ion will be bound to the chelating agent and the more likely the complex will be formed. Ethylenediaminetetraacetic acid (EDTA) was chosen for this work because it represents one of the most important groups of chelating agents containing amino carboxylate group. However, EDTA will preferably bind to calcium than to copper if both metal ions

present [33]. In contrast, IDS, EDDS, N,N-bis(carboxymethyl)glutamic acid (GLDA) have a higher binding constant for copper ions over calcium ions (Table 1.2).

Table 1.2 Comparison of the stability constants of M(II)-L=1:1 complexes with EDTA, IDS, EDDS, GLDA (- data not available) [60].

M(II)	EDTA	IDS	EDDS	GLDA
Ca(II)	10.7	5.2	4.6	5.2
Mg(II)	8.8	6.1	6.0	6.1
Cu(II)	18.8	13.1	18.4	13.1
Fe(II)	14.3	8.2	-	8.7

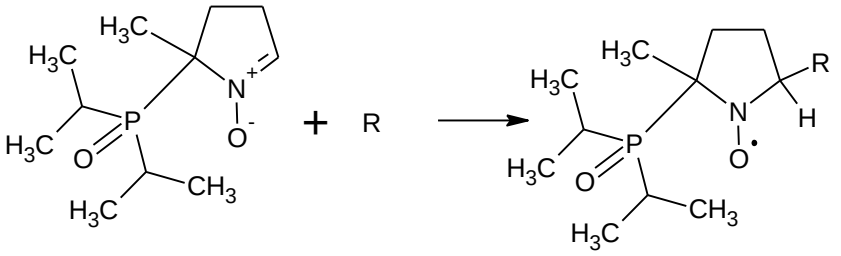
Nowadays, there are, however, many discussions concerning its poor biodegradability and the direct or indirect potential effects of the presence of the complexions in the environment [61]. The ligand-metal complexes may significantly increase the bioavailability of extremely dangerous heavy metals, for example, Cu (II)-EDTA and Cd (II)-EDTA complexes which are more toxic than their respective free metals [61]. There is a few of alternative products on market which are claimed to be as effective as EDTA but most of them have also their restrictions. For example, organophosphonates were found to be not readily biodegradable. Others are readily biodegradable, such as citrated and gluconates, but do not have a sufficiently strong chelating power compared to EDTA [61]. A series of new diethanolamine derivatives such as complexing agents have been designed. Ethylenediamine-*N*, *N*'-disuccinic acid (EDDS) and tetrasodium-iminodisuccinate (IDS) have also been proposed. According to recent investigations, especially EDDS is viable replacement ligand in pulp and paper industry, in cosmetics, etc. and IDS is also comparable to EDTA [33, 61].

1.5 Detection and quantification of radical species formation in bleaching process

Free radicals play an important role in medicine, biology and organic chemistry. However, the detection and quantification of free radical species formed in oxidizing process have an extreme challenge due to the extreme high reactivity of radical species and very short life-time involved (e.g. 10^{-9} s for ·OH) [62]. Various spectrophotometric methods haven been developed and employed during the last thirty years in order to identify and quantify such species, but overall, there are still serious methodological limitations such as lack of specificity and low sensitivity. The spin-trapping technique has been widely used for detection of various radical species. The technique is based on the reaction in which the generated radical species reacts with specific nitrone or nitroso containing spin-trapping reagents to yield more persistent nitroxide spin-adducts. These adducts can be readily detected by electron paramagnetic resonance (EPR) or electron spin resonance (ESR) spectroscopy or any other analytical method. [44, 62] A variety of spin-trapping reagents such as 5,5-Dimethyl-1-pyrroline N-oxide (DMPO), 5-(Diethylphosphono)-5-methyl-1-pyrroline N-Oxide (DEPMPO), 5-Diisopropoxy-phosphoryl-5-methyl-1-pyrroline-N-oxide (DIPPMPO) was developed in the last years [63]. More recently, it was demonstrated [45] that phosphorus-containing spin trapping reagents give rise to radical adducts that have longer half-lives compared to other spin traps. Unfortunately, these radical adducts degrade with time and therefore, cannot be reliably detected by EPR. Additionally, it is always difficult to perform radical detection in complex systems in which very different radical species of varying lifetimes are to be detected and quantitatively analyzed over a range of times. EPR spectroscopy cannot be applied easily or reliably in these cases [44]. A well-studied spin trap system is the

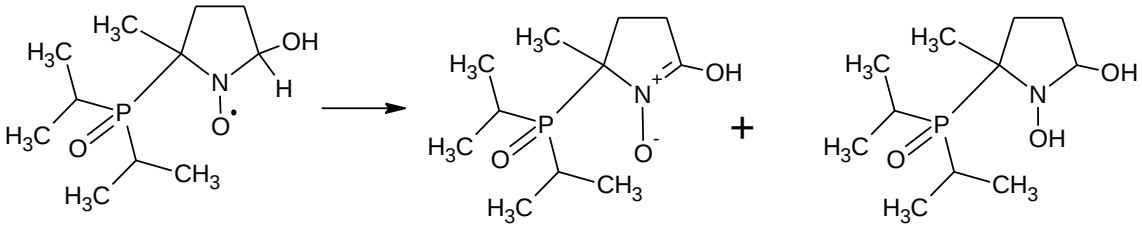
DIPPMPO)/^{31}P nuclear magnetic resonance spectroscopy (NMR) system; this system is well-known for the analysis of oxygen-centered radicals [64]. The use of phosphorus-containing spin traps allows for the detection of diamagnetic products by ^{31}P NMR without the complexity of multiple signal overlap spectra usually encountered when common nuclei, such as proton or carbon, are examined [44]. Overall, however, a possible draw-back of this technique could be the reduced sensitivity of NMR compared to that of EPR. This is partly overcome by the acquisition of more NMR signals with time [65]. Due to the presence of the phosphorous atom in the DIPPMPO spin trapping reagent, the different spin trap adducts show different chemical shifts of the ^{31}P atom, which depends on the nature of the adduct forming radicals. Therefore, ^{31}P NMR spectroscopy can be conveniently employed for both identification and quantification, if a suitable internal or external standard is present in the system. Free radicals such as hydroxyl radicals $HO^{\cdot}$, hydroperoxyl radicals $HOO^{\cdot}$, superoxide radicals $O_2^{\cdot -}$ and amino radicals $^{\cdot}NH_2$ can react with DIPPMPO to form stable adducts with a longer half-life, respectively. The basic reactions between DIPPMPO and the various oxygen-based radicals, and the evolving species are shown in Scheme 1, 2, 3 [63]. Depending on the nature of the adducts formed, different radicals can be distinguished by showing different chemical shifts of the ^{31}P atom in NMR spectra. As proposed by V. Khramstov et al., [63] when the concentration of the radical adducts is high, disproportionation and rearrangement reactions occur (Scheme 2), affording a new nitrone and the original spin trap DIPPMPO. Therefore, the amount of new nitrone 5 (Scheme 2) observed in the ^{31}P NMR spectra represents only half of the total spin trapped hydroxyl radicals. The other half of the hydroxyl radical adducts which are originally formed are transformed back into the DIPPMPO via the loss of water of the product 4 in Scheme 2. In conclusion, in

order to accurately quantify the real value of trapped hydroxyl radicals, the NMR integral of the corresponding radical adduct peak in the ^{31}P NMR spectrum should be doubled.

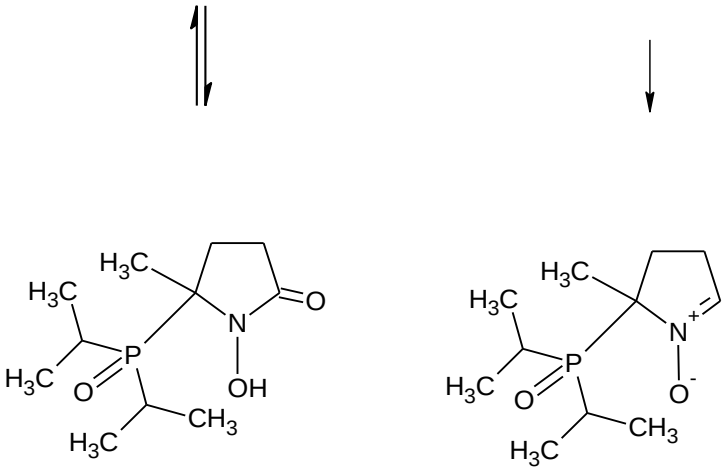


DIPPMPO (1) DIPPMPO radical adduct

Scheme 1. Spin trapping reaction between DIPPMPO and different radical species.
R: different radical species: such as hydroxyl, hydroperoxyl or amino radicals

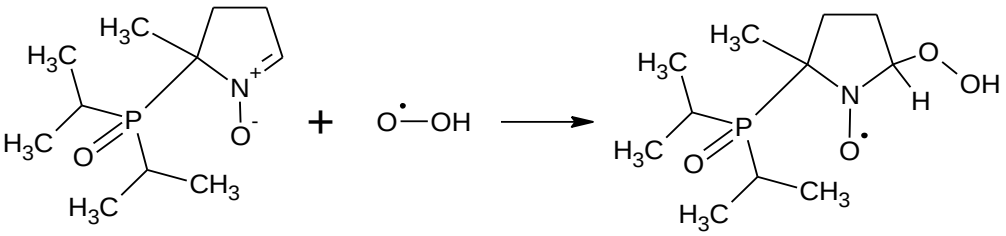


DIPPMPO/·OH radical adduct (2) (3) (4)



(5) DIPPMPO (1)

Scheme 2. Spin trapping reaction between DIPPMPO and hydroxyl radicals.



DIPPMPO (1) DIPPMPO/·OOH radical adduct (6)

Scheme 3. Spin trapping reaction between DIPPMPO and hydroperoxyl radicals.

1.6 Repair of damaged hair fibres versus damage protection

There is a variety of approaches developed to repair the damaged hair resulted from oxidative coloring or bleaching processes, such as leave-on and rinse-off products. Hair shampoos or conditioners formulated with cationic polymers or cationic surfactants are used oft after oxidative hair coloring or bleaching processes to give the hair a soft, smooth feel which results in easier hair combing. Since bleached hair is more negative charged at and near the hair surface because of the formation of cysteic acid residues after the oxidative cleavage of both thioester bond in the fatty acid 18-Methyleicosanoic acid and disulfide bonds of cystine in hair keratin. Cationic ingredients will be through electrochemical bonding attracted to the negative charged damaged hair more readily than anionic ingredients fixing a mono-molecular film which results in reducing static electricity and the frizz effect on hair surface. [66]

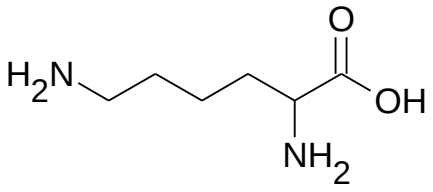
On the other hand, there is the interference of the pH value of hair shampoos or conditioners needed to be considered. Hair is extremely sensitive to the pH variation of the products applied on its surface. The isoelectric point is defined as the moment of charge neutrality in a determined pH. The isoelectric point of human hair is around a pH=3.67. In bleached hair, however, the isoelectric point reached at an even more acid pH because of the formation of cysteic acid residues. The pH of the scalp, however, is around 5.5, which is more alkaline than the isoelectric point of hair fibre. It is of notice that a pH higher than 5.5 may cause irritation of the scalp. [67] Any hair products applied on hair that have pH higher than 3.67 can cause an increase in the negativity of the electric network of hair, that is, an increase of static electricity and the repulsion between tresses.

Therefore, cationic ingredients muss be added to the formula in order to be attracted by the negatively charge net. If a hair shampoo or conditioner formula is above pH 3.67 and has no cationic ingredients added, the electrostatic forces will considerably increase the tangling and the attrition forces, increasing the damage to the surface of hair fibre. In conclusion, besides the inclusion of antistatic agents like cationic ingredients in the shampoo and conditioner formulas, the formula and ingredients must create a final pH no higher than 5.5 in order to avoid the scalp damage and avoid a significant increase in static electricity and consequently, in the negatively of hair fibre that causes frizz. [67]

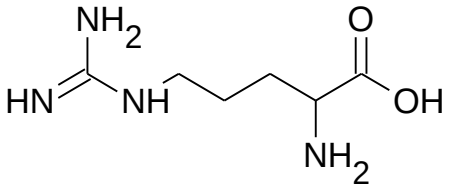
Thus, the most important interactions for hair shampoos or conditioners are those that occur at or near the fibre surface or near the first new cuticle layers. Of course, if the hair surface is damaged to the extent that the cortex is exposed then conditioners interact with exposed cortex too [6].

With bright blond hair colors becoming more fashionable in recent years, prevention of hair damage due to bleaching treatments has become a strong consumer need. A hair care product “Olaplex” was launched as a first line of hair repair and “bonding rebuilding” treatments, which can be mixed in with bleaching products to minimize damage. The patent [68] proposed an amount of details into the mechanism of how it is supposed to work in hair straightening and perming in which reducing agents are commonly used. Reduction of the disulfide cross-linker (-S-S-) produces thiol groups (K-SH). The two ends of bisaminopropyl diglycol dimaleate, the main active ingredient used in “Olaplex”, which is well known as Michael acceptors, may react with reduced thiol (K-SH) group each in a Michael reaction to form covalent bonds. And so, a new disulfide link may be formed between the two sulfurs. If the mode-of-the-action of “Olaplex” works in the same way in bleaching process, there is still a doubt. Because in hair bleaching process,

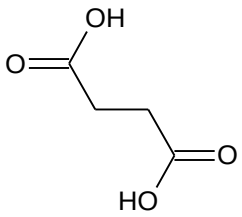
the oxidation of disulfide cross-linkers with alkaline hydrogen peroxide or even with the addition of persulfate to cysteic acid residue may be so fast that the intermediate thiol group SH group may not exist in this process. Recently, another patent and a literature were published concerning a hair bleaching system containing succinic acid in combination with lysine and arginine (Fig. 1.13). It was reported that the addition of this mixture can better protect hair fibres from damage during bleaching treatment in comparison to the conventional bleaching product [69, 70]. Results of tensile strength evaluations as well as multiple grooming tests and differential scanning calorimetry (DSC) and finally test salon evaluations proved this protective effect. The mechanism of this protective effect is, however, still uncertain and not yet fully understood. As a possible working hypothesis, it was proposed by T. Förster el at. [69] that the organic di-acids like succinic acid which was used as one of the active ingredients could be absorbed by the hair cortex and rebuild salt bridges and/or bridges with hydrogen bonds interactions in hair fibre, which resulted in enforcement of the hair bonds. T. C. Schlenkermann [71] employed multidiscipline spectroscopic methods to attempt to provide evidence for the formation of new ionic bonds between di-carboxylic acids and keratin chains in hair. However, no any interaction of di-carboxylic acids in hair was detected to support this hypothesis. Additionally, the effect of another key ingredients lysine and arginine has not been reported in any previous study.



(a): Lysine



(b): Arginine



(c): Succinic acid

Figure 1. 13 Chemical structures of the active ingredients used in the patented hair bleaching system [70].

Chapter 2 Objectives of this study

The commercially available ammonia-based alkaline bleaching system is widely used in human hair oxidative colorants or bleaching products. However, over time the consumers can experience undesired hair damage such as poor hair feel and look, reduced hair strength, increased incidents of split ends and breakage. Scientific test methods like tensile strength evaluations as well as multiple grooming tests and differential scanning calorimetry (DSC) allow a quantitative assessment of human hair damage. A hair bleaching product formulated with succinic acid in combination with lysine and arginine demonstrated that it protects hair fibre from damage during bleaching in comparison to the conventional bleaching products [69, 70]. However, the mechanism of this protective effect was not yet fully understood. Therefore, the understanding of the mode of action was a first step in further designing a more efficient technology to protect hair fibre. As a hypothesis of the mechanism of this protective effect, there are two options: A) Interaction of the components with hair fibre and direct stabilization of the damaged sites by molecular interaction/reaction during bleaching [69, 71] or B) Modification of the reaction mechanism towards milder conditions.

Therefore, the objectives of this dissertation were:

1. In order to gain deep insight into the protective effect, a multidisciplinary approach is needed in order to characterize systematically the hair damage during cosmetic bleaching. The holistic appearance at the macroscopic level such as changes in color, and tensile strength is to be examined. At the microstructural level, the morphological changes in cuticle layer and even in internal structure should be evaluated using

scanning electron microscopy and transmission electron microscopy, respectively. At the molecular level, the amount of oxidized cystine (and cysteine) product, cysteic acid residue is to be quantified using Fourier transform near-infrared spectroscopy (FT-NIR).

2. To gather evidence for hypothesis A (binding of molecules) or hypothesis B (modification of the reaction mechanism).
3. Development a model to investigate the mechanism of the protective effect due to the addition of the mixture (succinic acid, lysine and arginine) during bleaching treatment. The favourite model (milder bleaching conditions) provides high motivation to have a deeper look into radical chemistry. To achieve this goal, a spin trap NMR technology based on the agent 5-Diisopropoxy-phosphoryl-5-methyl-1-pyrroline-N-oxide (DIPPMPO) is to be explored and employed in this study to identify and semi-quantify free radical species formed in bleaching systems. The role of arginine, lysine and succinic acid in the decomposition of alkaline hydrogen peroxide will be investigated. At last, the mechanism of the protective effect during hair bleaching will be elucidated in this study.
4. As it is more practical relevant to test the radical formation directly in the presence of human hair itself. The spin trap technique will be applied in the current research, with suitable modifications, to study the activity of radical species involved in bleaching process in the presence of human hair under cosmetically relevant conditions.

Chapter 3 Results and discussion

3.1 Characterization and assessment of hair damage resulted from bleaching treatments

3.1.1 Tensile strength measurement

It has been demonstrated by Robbins and Crawford [72] that the cortex is primarily responsible for the tensile properties of human hair and that the cuticle has little involvement. It was considered that the tensile properties could be primarily regarded as an indicator of cortex damage. Moreover, the tensile properties are highly dependent on the condition of hair fibre and climatic conditions during tensile testing [73].

In this study, a stress-strain test was performed. Most often, the purpose of performing this type of tests is to detect and quantitatively evaluate hair cortex damage induced by chemical and environmental treatment. The moisture content of human hair, however, has a large effect on the tensile properties. A major side reaction in the bleaching of hair involves the oxidation of cystine cross-links to cysteic acid residues. The breakage of disulfide cross-links in the cortex of hair has a major effect on the wet tensile properties of hair; furthermore, the wet properties decreased almost linearly with the cystine content. In contrast, the dry tensile properties were virtually unaffected by disulfide cross-links rupture. [74] In summary, hair bleaching caused significantly decrease in the wet tensile properties, but the changes caused by hair bleaching in the dry tensile properties were very small and close to the limits of detection [41].

The elastic modulus for stretching human hair, Young's Modulus is defined as the ratio of stress over strain in the Hookean region. Hooke’s law of elasticity states that the longitudinal change of a material body (the strain) is linearly related to the force causing the deformation (the stress). For wet hair this region lies between a strain of approximately 0 and 2 %. The Young’s Modulus represents the elastic region of hair fibre

and is a measure for the strength of a fibre (the higher the Young's Modulus the stronger the fibre) [69]. It was confirmed that no substantial structural change is introduced during the elastic region since the deformation is completely reversible and initial properties could be reset [6]. Its evaluation therefore does not need fibres to be extended out to their break point. The other advantage to evaluate Young's Modulus is that measurements at each condition such as bleach treatment can be performed on the exact same set of 50 replicate hair fibres.

In this study, a set of 50 different hair samples was examined to validate the results. The E-modulus (=Young's Modulus) before (on untreated hair) and after the application of bleaching products (on bleached hair) was calculated, respectively. The difference in Young's Modulus of the same hair fibre between after and before bleaching application was calculated. The results of Young's Modulus changes after bleaching treatments were shown in Fig. 3.1. Bleaching treatments led to a modification of hair fibre with a significant decrease in the values of Young's Modulus in comparison to untreated hair. In other words, this damage induced by bleaching treatments resulted in a loss of mechanical strength and stability of hair fibre. Twofold powder-bleach treatment decreased Young's Modulus even more than three times in comparison to onefold lightener-bleach treatment. The elasticity of hair fibre reduced continuously as hair bleaching progressed. However, the hair fibres treated with the bleaching mixture plus the combination of succinic acid (1 %), lysine (0.2 %) and arginine (0.2 %) showed the tendency that the loss of Young's Modulus was less, suggesting its ability to restore the tensile properties of hair fibre during the bleaching process. The effect was, however, not statistically significant.

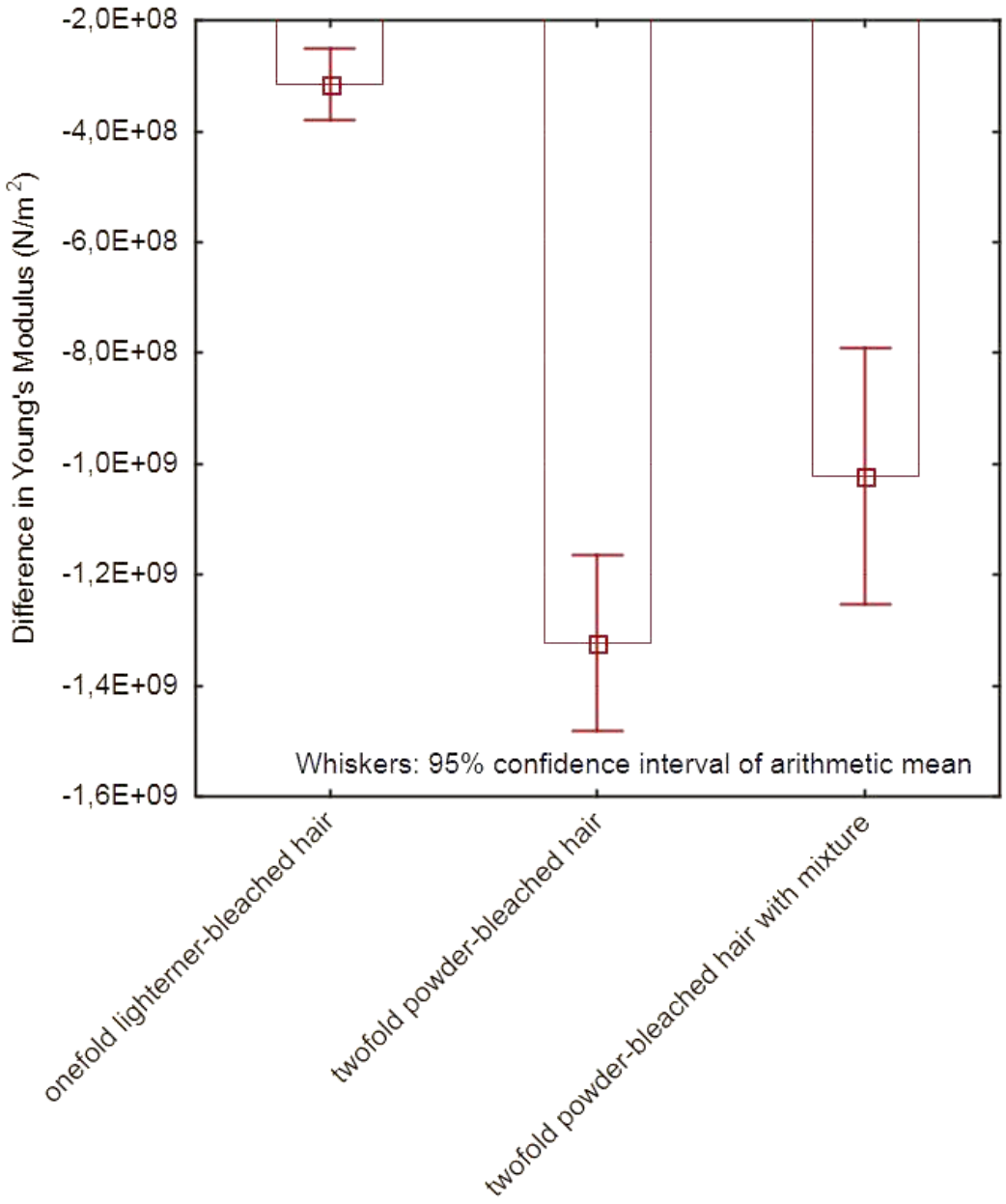


Figure 3. 1 Wet state Young's modulus results as a function of bleaching treatments (N=50).

3.1.2 Fourier transform near infrared spectrometer (FT-NIR) measurement

After evidence for a hair fibre stabilizing effect during bleaching treatments was obtained by the analysis of elasticity, the hair tresses were further analyzed by a Fourier transform near infrared spectrometer (FT-NIR). The purpose of this measurement was to characterize the chemical changes at molecular level after bleaching treatments and

identify potent hair protective effect by addition of the mixture of succinic acid (1 wt.%), lysine (0.2 wt.%) and arginine (0.2 wt.%) during bleaching treatment, because this is the most rapid and valuable screening tool so far in hair research and development for cosmetic industries to quantitatively assess hair damage. The band intensity of the S=O at 1040 cm^{-1} observed in the FT-NIR spectra has been reported to relate directly to the cysteic acid residue content of hair because of oxidative cleavage of both thioester bond in the fatty acids 18-Methyleicosanoic acid (18-MEA) and disulfide bonds in the cystine amino acid residues of hair keratin [75]. The content of cysteic acid residue could be as an indicator of hair damage after bleaching treatment.

In Fig. 3.2, the FT-NIR spectra of untreated hair, onefold lightener-bleached hair, twofold powder-bleached hair and twofold powder-bleached hair with the combination of succinic acid and basic amino acids were displayed. As expected, the results indicated that the level of hair damage depended on the strength of oxidants which were used in bleaching treatment and the number of the applications. The amount of cysteic acid residue increased as hair bleaching progressed. The hair tresses treated with the bleaching mixture plus succinic acid and both amino acids showed significantly lower amount of cysteic acid present in hair in comparison to the hair tresses treated with bleaching mixture alone (Fig. 3.3) ($p < 0.05$; t-test), which suggested that the addition of the mixture significantly reduced oxidative hair damage.

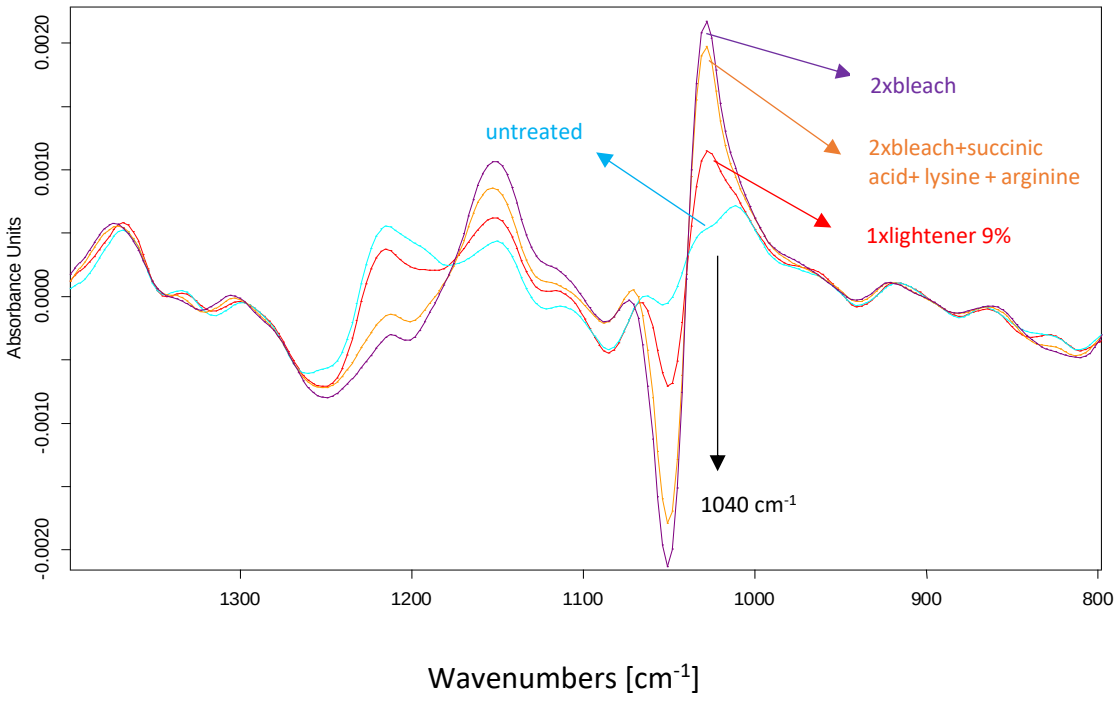


Figure 3. 2 FT-NIR spectra of untreated hair and bleached hair and hair sample, which is twofold powder bleached with the combination of succinic acid, lysine and arginine.

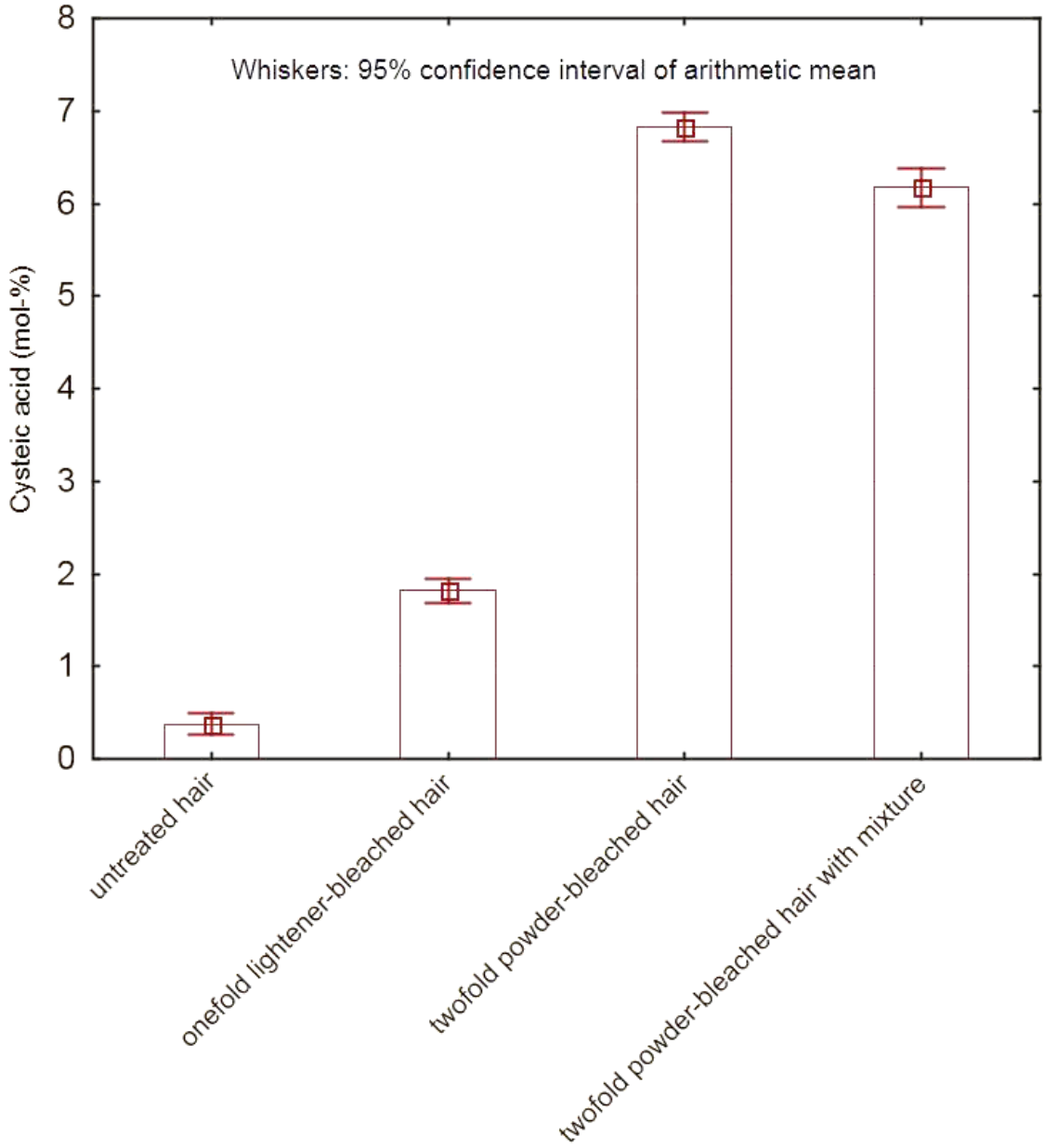


Figure 3. 3 The amount of cysteic acid measured after bleaching treatment (N=18).

3.1.3 LAB measurements

Fig. 3.4 showed the lightening data (dL) as a function of bleaching treatment. The lightening of the hair after these treatments was measured and the result showed that the lightening effect was dependent on the strength of oxidants used in bleaching treatment. The powder bleaching treatment provided more lightening effect than a bleaching treatment with a weak lightener. Per naked eye observations, the color of hair

tresses subjected to onefold lightener treatment changed from dark brown to medium brown, and from dark brown to light blond with the powder-bleach treatment.

Although the customers aspire to obtain the health hair look, they would not like to lose the lightening performance. These data in Fig. 3.4 showed that the bleaching mixture plus succinic acid (1 wt.%) and both amino acids (lysine 0.2 wt.% and arginine 0.2 wt.%) achieved the similar lightening to conventional bleaching system but led to less hair damage which was already proved by tensile strength measurement and quantification of cysteic acid amount in this study.

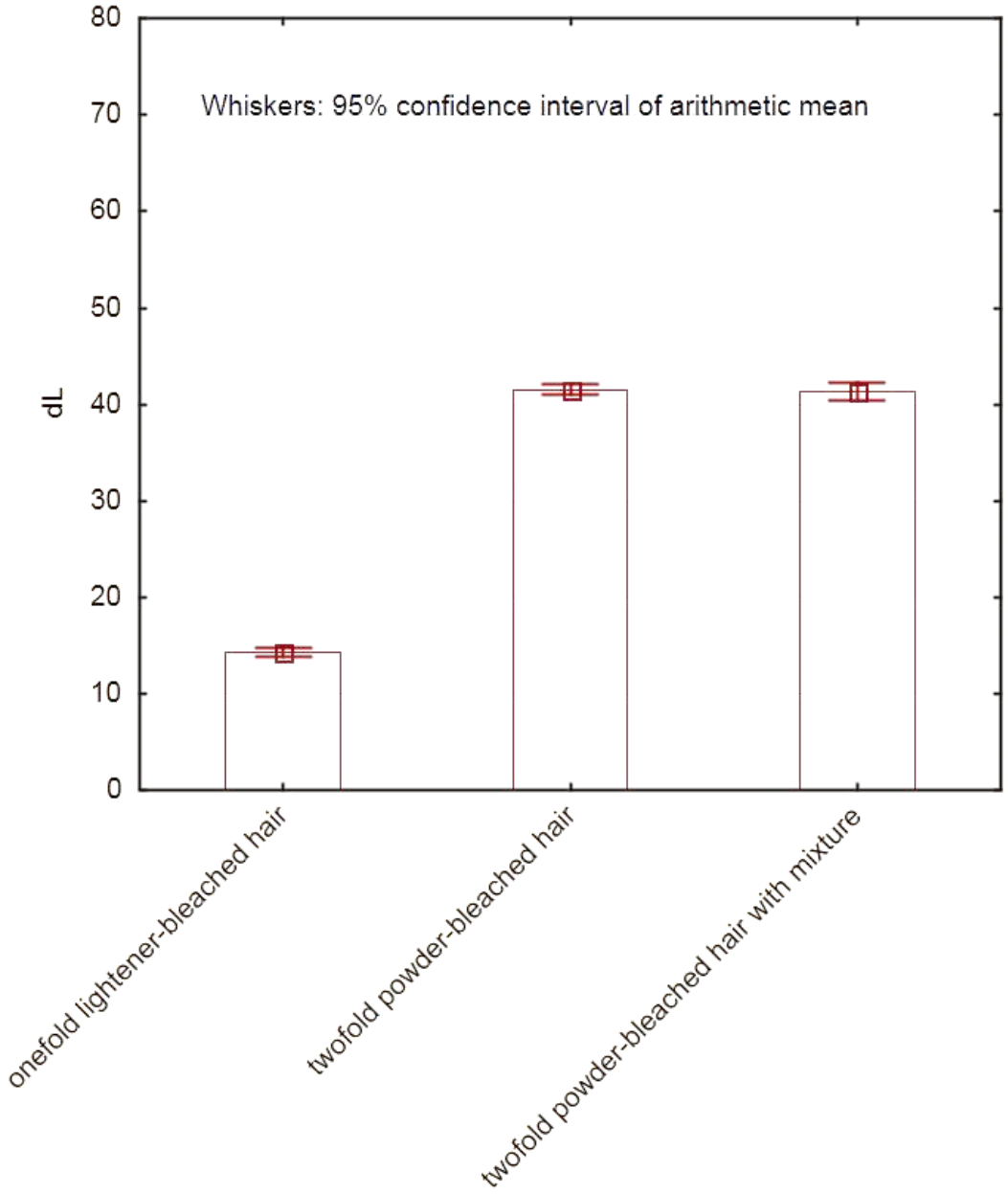


Figure 3. 4 Lightening effect as a function of bleaching treatments (N=12).

3.1.4 Morphological observation of the ultrastructure of hair fibre–cuticular surface and inner structures

Current methods of analysis of hair damage resulted from bleaching treatment consist mainly of macroscopic testing such as combing, tensile strength measurements. However, these lack visualized characterization and tell little about the actual hair morphology. Most detailed information about the structure of human hair fibre can be

obtained from scanning electron microscope (SEM) and transmission electron microscope (TEM) observations [18, 39, 49]. Both SEM and TEM techniques use an electron beam to give a “photographic” image of the sample and provide the visual details about the changes of the cuticular surface and inner structures of human hair resulted from bleaching treatment, respectively. However, both techniques cannot provide quantitative data about the hair morphology, which means that it is not possible to quantitatively evaluate hair damage due to bleaching treatment.

3.1.4.1 Morphological observation of the cuticular surface of hair fibre using SEM

The morphology of the cuticular surface, which is the most external layer of hair fibre and thus the most exposed to chemical damages, was firstly analyzed using SEM. Fig. 3.5 showed representative SEM images observed on the surface of different hair samples: untreated hair (Fig. 3.5a), hair after twofold powder-bleach treatment alone (Fig. 3.5b), hair after twofold powder-bleach treatment combined with succinic acid, lysine and arginine (Fig. 3.5c). These images were obtained in the central area of the hair tress.

Fig. 3.5a showed that the cuticular surface in untreated hair exhibited entire cuticles, in a good general condition, although it was possible to notice some border areas from where broken pieces were removed such as little cracks, as indicated by the arrows and ring in Fig. 3.5a. It was estimated that these features could be intrinsic to the sample morphology and result from the hair’s previous history and not from the bleaching treatments applied in this work. In samples of dark brown hair after twofold-bleaching treatment with bleach powder alone (Fig. 3.5b), SEM revealed more clear damages such as irregular overlay, lift of the cuticle cells, the deep fractures, loss of cuticle edges and

holes in cuticle cell surface ,as indicated by the arrows and rings in Fig. 3.5b. In samples of dark brown hair after twofold-bleaching treatment combined with the above-mentioned mixture, some broken border areas and uneven and full of cavities were still found, as indicated by arrows in Fig. 3.5c. However, a relative smoother cuticular surface was observed compared to the SEM images in Fig. 3.5b. This result provided visual evidence that the addition of the mixture of succinic acid, lysine and arginine during the bleaching treatment was likely to protect hair surface from damage which resulted in a relative smoother cuticular surface compared to the bleaching mixture alone.

Taken together, irregular overlay, lift and even loss of the cuticle cells, the large cracks and holes in cuticle cell surface were observed in both hair bleach-treated samples (Fig. 3.5b and Fig. 3.5c), whereas the cuticle cells in untreated hair (Fig. 3.5a) appeared to be closed and in good general conditions. It is tempting to speculate that that the degradation of the inner layers of the cuticle due to bleaching treatment could be responsible for the morphological changes of the cuticular surface, such as the partial detachment and lifting of the cuticle cells etc., which in turn, could be responsible for macroscopically noticeable optical effects, such as shine loss in hair tresses, due to light scattering by irregular cuticle cells and loss of smooth feel. Previous publication by T. Förster et al. [69] proved the following aesthetic properties of hair in test salon: feel of wet hair, combing ease of wet hair, feel of dry hair, combing ease of dry hair, level of lift. The bleaching product containing the mixture of active ingredients performed better in all other categories. However, the better performance could be only obtained for combing ease of wet hair. In summary, these microscopic observations indicated that the bleaching treatment had an important effect of weakening the cuticle cells, thus making them more fragile and susceptible to break. However, these morphological

changes could be to a certain degree inhibited by the addition of succinic acid, lysine and arginine during bleaching treatment, as indicated in Fig. 3.5c that the hair sample showed a relative smooth surface. This improvement of cuticular surface was, however, not able to be quantified using SEM.

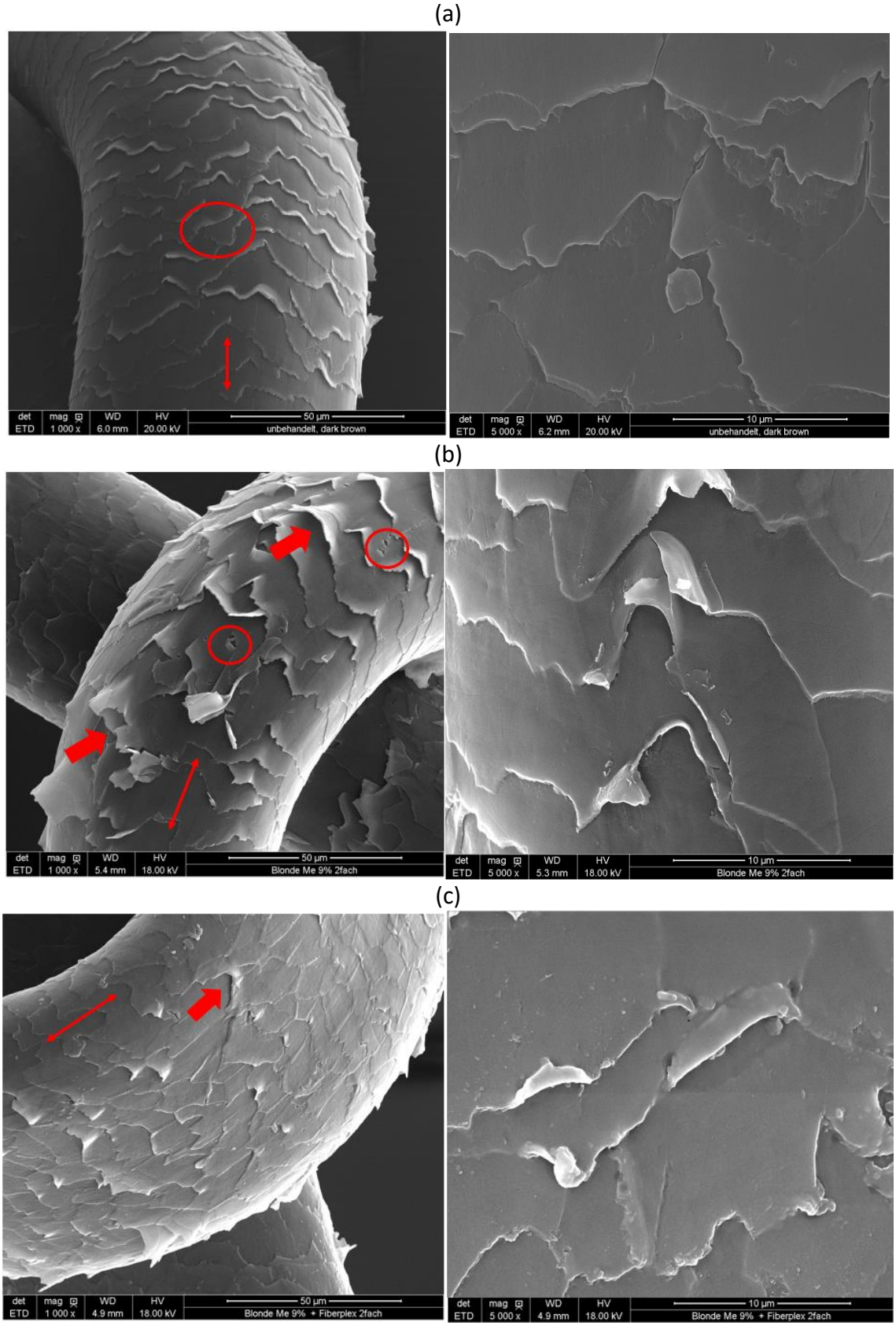


Figure 3. 5 Scanning electron microscope (SEM) micrographs of the surface of (a) untreated hair; (b) twofold-powder bleached hair; (c) twofold-bleached hair with treatment of mixture (succinic acid, lysine and arginine).

3.1.4.2 Morphological observation of the inner structures of hair fibre–cuticle and cortex using TEM

TEM images provided information on morphological changes of human hair fibres (cross-sections). Around 1000 transmission electron microscopic findings of normal and of various kinds of damaged hairs were analyzed. When the ultrathin cross sections of the untreated hair were stained with uranyl acetate and lead citrate, as shown in Fig. 3.6, it was observed that the exocuticle was a uniform structure with low electron density (brighter areas), while the endocuticle was a structure with substantially high electron density (darker areas). It was not possible to clearly distinguish between the A-layer and the exocuticle in Fig. 3.6a. The cortex region was shown in Fig. 3.6b in which the cortical cell membrane complex (CMC) showed a slightly lower electron density than the structures around them. The macrofibrils were embedded in the intermacrofibrillar matrix and the electron density of the intermacrofibrillar matrix was higher (darker areas) than the macrofibrils (brighter areas) (Fig. 3.6c). Melanin granules were the structures with the highest electron density observed in the cortex (Fig. 3.6b and Fig. 3.6c). It was described before that melanin granules have the property of being able to coordinate metal elements, and actually metal elements like transition metal copper or iron have been detected in the melanin granules of human hair by EDS-TEM [76]. Moreover, T. Imai [39] used TEM without fixation and electronic staining and proved that the melanin granules were still the structures with the highest electron density inside hair fibre. Additionally, it is well known that the main chemical composition of hair is keratin, and its elemental component with the largest atomic number is sulfur. Based on these facts and findings, it was considered that there were metal-like elements with larger atomic number present in melanin granules than sulfur [39]. The extremely

high electron density in melanin granules observed could be extra due to staining with uranyl acetate and lead citrate besides the natural presence of metal elements with larger atomic number.

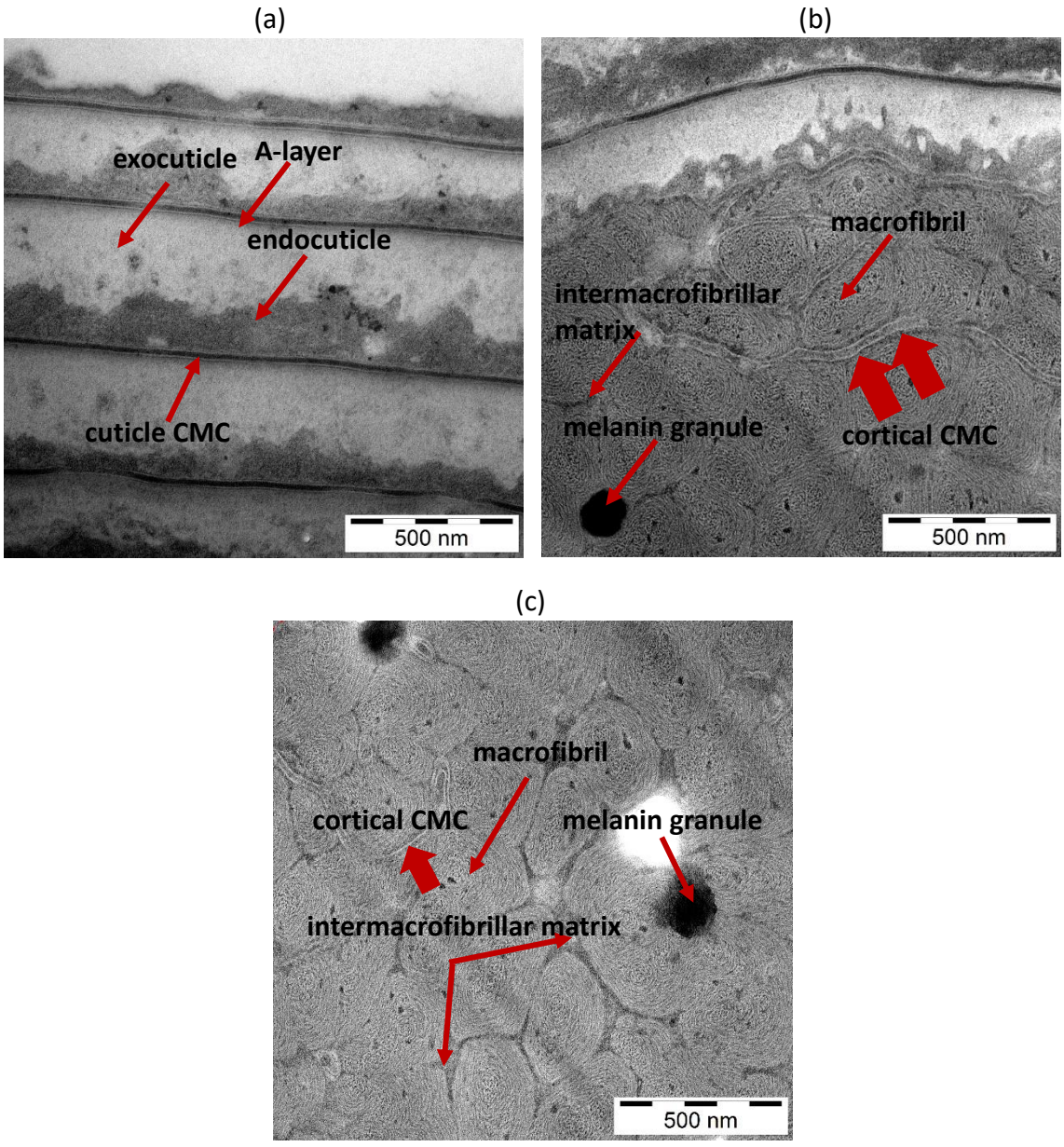


Figure 3. 6 TEM micrographs of untreated hair stained with uranyl acetate and lead citrate procedure. (a) TEM micrograph showed clearly intact cuticle structure containing the normal complement and distribution of exocuticle and endocuticle, (b) the membrane-like structure located at the interface between cuticle and cortex. Cortex CMC (arrows) and intermediate filaments (IFs) arrangement were also clearly discernable. (b and c) The macrofibrils are main components of the cortex and are separated from each other by an intermacrofibrillar matrix. The melanin granules have extreme high electron density and are in the intermacrofibrillar matrix. It was speculated that these bright spot in untreated hair could be intrinsic to the sample morphology and result from the hair's previous history.

The TEM observations of the hair treated with onefold lightener-bleach, using a weaker bleaching agent (Fig. 3.7) and twofold powder-bleach, using a stronger bleaching agent (Fig. 3.8) were shown. In contrast to the untreated hair, bright spots of variable size ("hole-like") were observed in the sub-structures of cuticles and cortex of both bleach- and lightener-treated hair as a consequence of oxidative treatment and damage. Bright spots represent the regions in which few electrons were absorbed and therefore were obviously brighter than surrounding regions. Generally, these bright spots observed in TEM images may result from less dense material or from a change in the local hydrophilicity, since the stain (uranyl acetate) used for sample preparation acts preferably on hydrophilic areas [48].

The ultrastructure of the cuticular CMC was obviously altered by bleaching (Fig. 3.7a and 3.8a). It appeared diffuse and the trilaminar structure was not clearly differentiated in comparison to unbleached cuticle CMC (Fig. 3.6a), suggesting the degradation of the cuticle CMC by bleaching. The high sulfur A-layer was clearly distinguished from the rest of the exocuticle after twofold powder bleaching treatment, as a very electron-dense layer (Fig. 3.8a). The increased electron density of the cuticle A-layer after bleaching presumably resulted from the oxidation of the cystine and consequent an increase of the amount of cysteic acid residues. Because uranyl acetate/lead citrate stain preferably on hydrophilic areas [48], the increase of permeability of the staining agents occurs in the oxidized cuticle A-layer with an increased cysteic acid groups.

As the melanin granules exist in the cortex, bleach compositions would have a considerable impact on the proteins inside hair fibre on their way to react with melanin granules. As expected, these bright spots or hole-like structures were especially observed in the endocuticles of the cuticle cells (Fig. 3.7a and 3.8a), the

intermacrofibrillar matrix (Fig. 3.7b, 3.8a and 3.8b) and along the cortical CMC of the hair modified by bleach (Fig. 3.8c), suggesting that the regions have less electron density and a possible loss of integrity due to the degradation of the hair proteins induced by bleaching. The cuticle and cortical CMC, together with the endocuticle, are believed to be the principle pathway for cosmetic products to penetrate into hair fibres [18]; so, some oxidation of the CMC components would be expected. This was supported in this study by the observation of the increase of hole-like structures in the endocuticle and the cortical CMC using TEM. Since cysteine, a very oxidation-sensitive amino acid residue, is heavily affected by bleaching, cysteine-rich hair components are also expected to be most affected [49]. This became evident in this study, as the hole-like structures were clearly observed in the endocuticle and the intermacrofibrillar matrix (containing high amount of cysteine), very likely resulting from the oxidation and/or subsequent loss of some matrix proteins. Surprisingly, after bleaching treatments variable sized hole-like structures (bright spots) were often observed in regions with a high amount of non-keratinous or amorphous structures like in endocuticle, intermacrofibrillar matrix and along the cortex CMC. Therefore, it was demonstrated that the regions of hair fibre containing much of non-keratinous amorphous structure could have a weaker resistant against chemical treatments and be more susceptible to the damage caused by bleaching treatments. Moreover, it was reported that the endocuticle together with the CMC layers and intermacrofibrillar matrix is considered as the primary pathway for entry or penetration of cosmetic products into hair fibre. Based on this fact, it is tempting to speculate that the active ingredients like oxidants would be more accumulated in these above-mentioned areas than in other regions; thus, these regions would be more susceptible to the damage induced by the oxidants.

Since hair bleaching procedures are designed to oxidize the melanin pigments to lighten the underlying color of hair, as expected, the most obvious effect of bleaching on the hair ultrastructure was degradation of the melanin granules. Some hole-like structures (bright spots) were clearly seen in the melanin granules of both bleach-modified hair (Fig. 3.7c and 3.8b), which revealed the severe degradation of melanin granules by the oxidants. Melanin granules were severely degraded after even onefold lightener treatment (Fig. 3.7b and 3.7c) and even completely degraded after twofold bleach treatment (Fig. 3.8b). Since transition metals like copper or iron naturally exist in melanin granules of hair, they are likely to flow out during the bleaching treatment and become a catalyst for the oxidants in other areas than the melanin granules to promote an increasing hair damage and the consequent augmentation of permeability and reactivity of the hair [39].

In conclusion, bleaching agents have a considerable impact on the inner ultrastructural components of hair. After bleaching treatments, variably-sized hole-like structures (bright spots) which could be due to the degradation of the components of inner hair fibre were often observed in endocuticle, intermacrofibrillar matrix and along the cortex CMC in which the regions have non-keratinous or amorphous structures. However, it was difficult to quantitatively evaluate hair damage according to these above-mentioned morphological changes of the cuticle and cortex. Simple or descriptive observation of either the cuticle or the cortex cannot be enough to evaluate thoroughly. Because morphological studies have been described using varying terms and subjective descriptions by different researcher.

Additionally, these variably-sized hole-like structures, as expected, were also clearly seen in melanin granules of both bleach-modified hair samples. The size of hole-like

structure observed in bleach modified hair seems to be dependent on the strength of the bleaching agents applied. This propose was supported by the findings that melanin granules were even completely replaced by holes that formed inside the cortex of the powder-bleached hair. Besides this, it has been proved in the previous studies that the melanin granules react more easily and faster with hydrogen peroxide than hair proteins, as there is a level of transition metal ions like copper or iron naturally existing in melanin granules and these metal elements act as a catalyst for the decomposition of hydrogen peroxide. Moreover, these metal ions naturally coordinating to melanin granules can flow out during the bleaching treatment and become a catalyst for hydrogen peroxide in other areas than the melanin granules, which results in the formation of free radicals [39, 44]. Free radicals can attack and break the bonds which are responsible for the hair integrity, to proceed to a much greater extent, causing protein degradation leading to loss of hair fibre strength and consequent hair breakage [33, 58]. Therefore, the obtained results from TEM observations could enable to assess hair damage by evaluating the morphological changes of the melanin granules. In other words, it is reasonable to consider that the level of degradation of melanin granules by the oxidants indicates the level of hair damage resulted from bleaching treatment, although it is not possible to quantify the amount of damage.

Finally, the morphological changes inside hair fibre resulted from the bleaching treatment may be responsible for the macroscopically measurable changes such as loss of the stability and strength of hair fibre, as discussed earlier in tensile strength measurements.

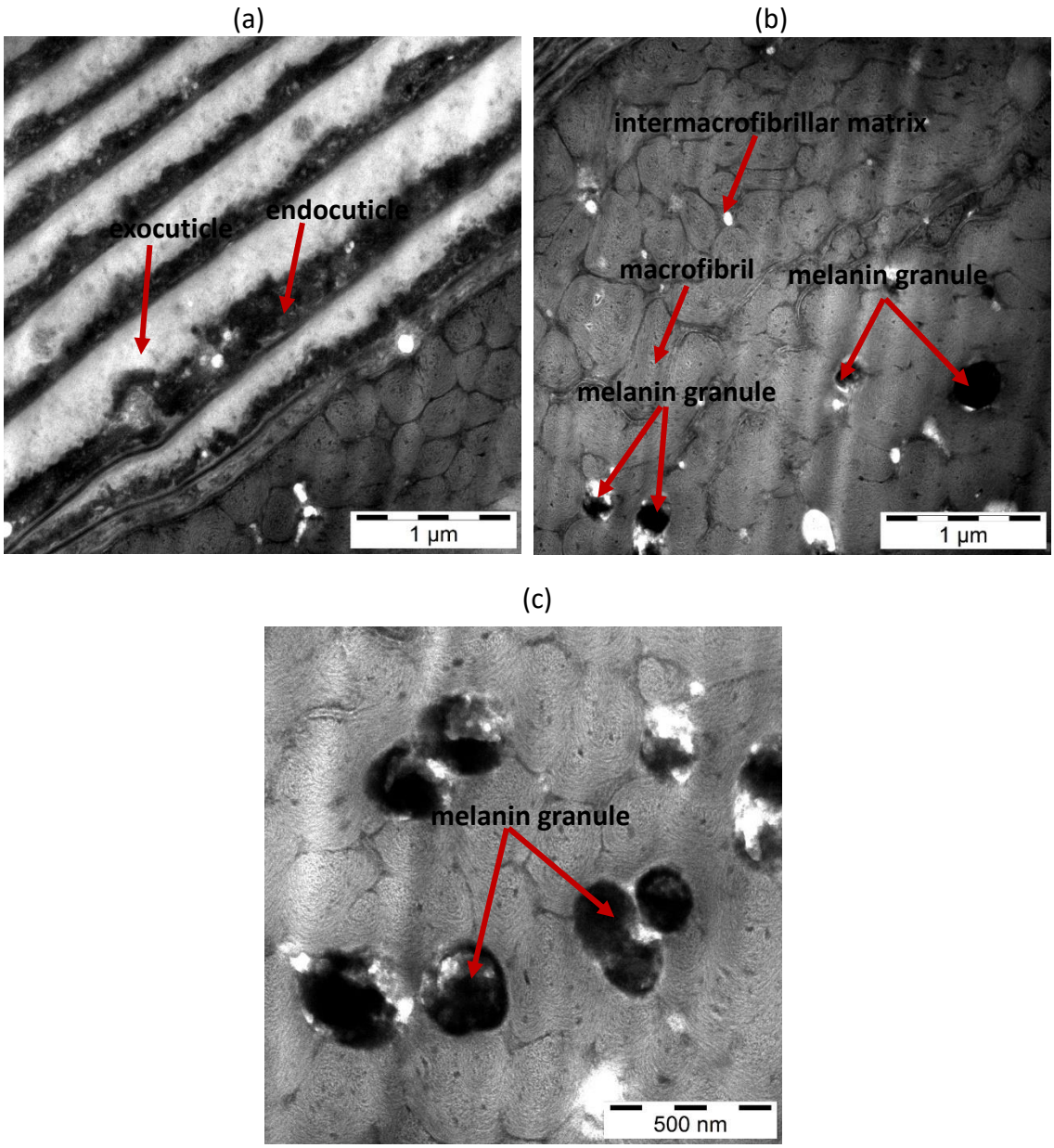


Figure 3. 7 TEM observation of hair samples onefold treated with lightener (a-c), which were stained by uranyl acetate and lead citrate.

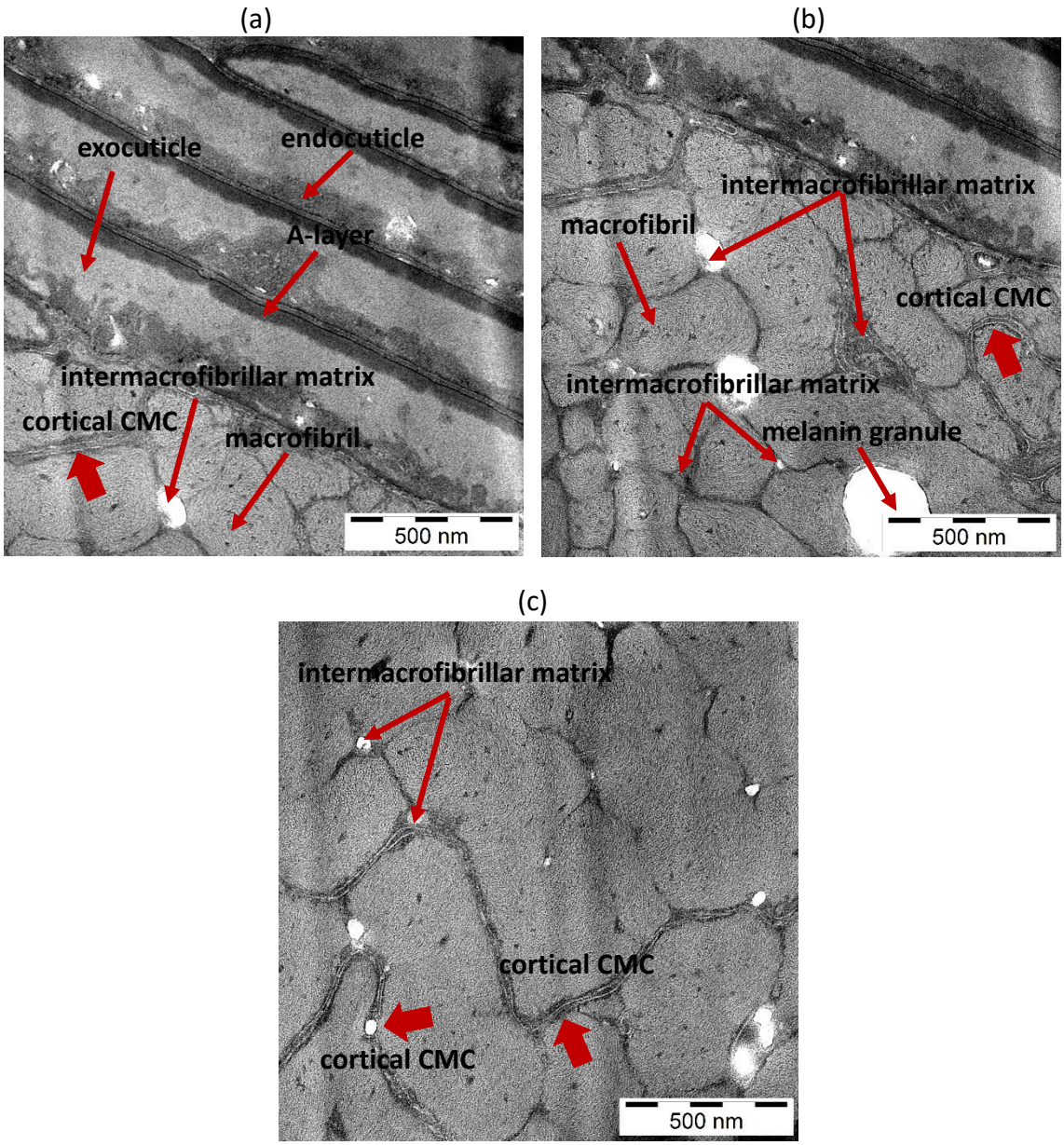


Figure 3. 8 TEM observations of hair samples twofold treated with powder-bleach (a-c), which were stained by uranyl acetate and lead citrate.

To further examine the protective effect of the addition of the mixture of succinic acid, lysine and arginine during bleaching treatment visually, the images of internal hair were analyzed using TEM. The changes of the ultrastructure were similar to those observed in the powder-bleach-treated hair, as shown in Fig. 3.8. The hole-like structures were also clearly seen in endocuticle, intermacrofibrillar matrix, along the cortex CMC and melanin granules. However, it was surprising to see that there was still significant

remaining of melanin granules observed in TEM images (Fig. 3.9b and 3.9c), which provided direct evidence that the addition of succinic acid, lysine and arginine may prevent the complete degradation of melanin granules. These residual melanin granules revealed the involvement of these substances in bleaching mechanism that does not achieve the complete destruction of melanin components. It has been reported that there is a range of transition metals like copper or iron present in human hair and they can catalytically decompose hydrogen peroxide via Fenton chemistry, which results in the formation of free radicals [39, 44]. Free radicals can attack and break the bonds which are responsible for the hair integrity, to proceed to a much greater extent, causing protein degradation leading to loss of hair fibre strength and consequent hair breakage [33, 58]. As the experimental results shown above, the hair damage was reduced by adding the combination of succinic acid and basic amino acids during the bleaching treatment. As a working hypothesis, it was proposed these substances like succinic acid, lysine and arginine may act as chelating agents via the carboxylic group -COO- and amino groups NH_2 to deactivate transition metals like copper metals existing in hair fibres during the bleaching treatment. In this way, the amount of highly reactive free radicals induced by the presence of copper or iron would be reduced to better protect hair bonds and reduce damage. This hypothesis was investigated and proved using a bleaching model system in our previous published research [77].

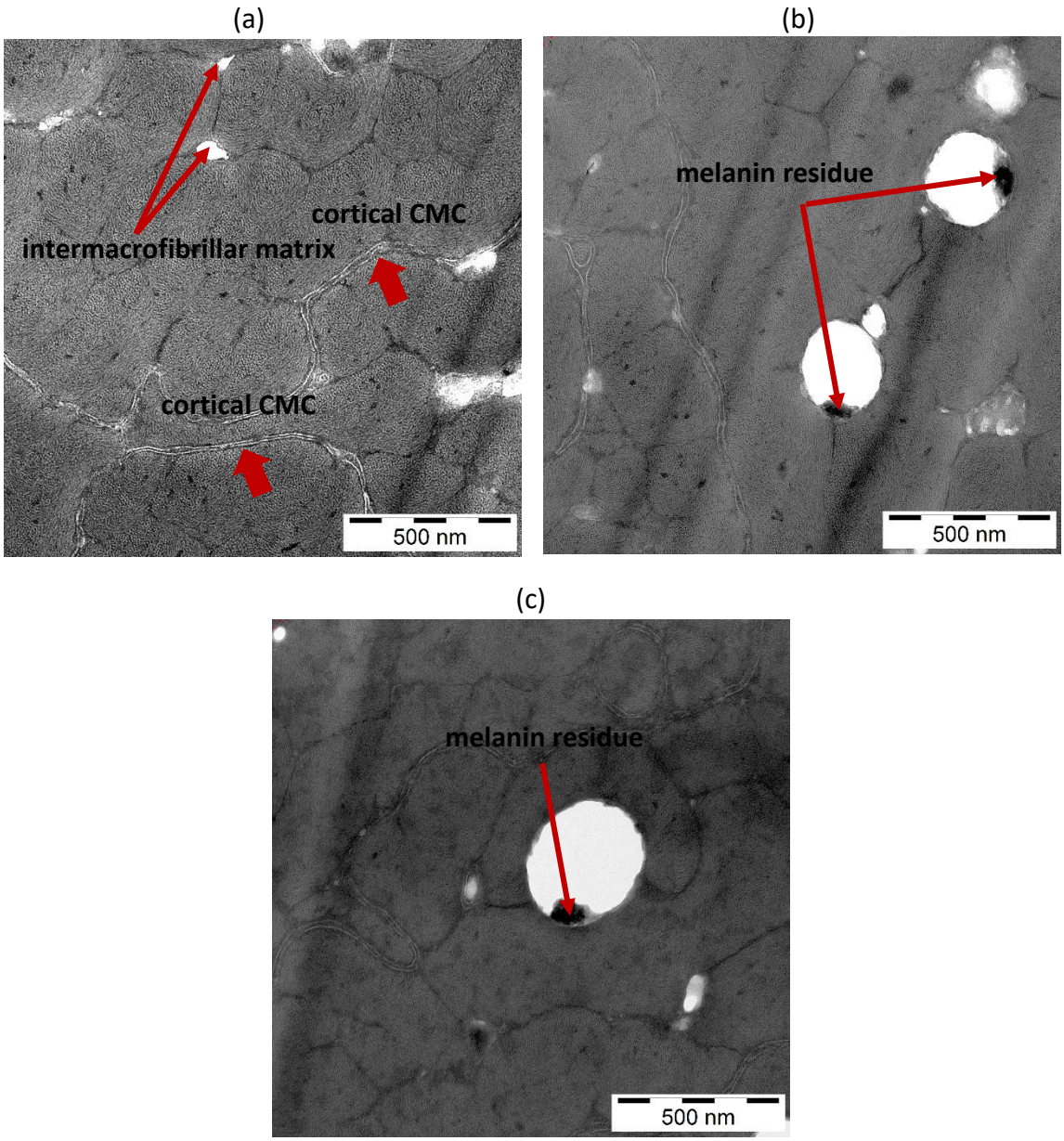


Figure 3. 9 TEM observation of twofold powder-bleach-treated hair combined with the mixture of succinic acid, lysine and arginine.

3.1.5 Conclusion

In this part of the current study, a variety of methods was combined to characterize the extent of hair damage resulted from bleaching treatment. Conventional human hair bleaching treatment resulted in changes of morphological structures in the cuticular surface and the cortex region. The results of SEM observations showed that irregular

overlay, lift and even loss of the cuticle cells, the large cracks and holes in cuticle cell surface were observed in the bleach modified hair. TEM images showed that variably-sized bright spots (hole-like structures) were predominant forms of oxidative damage observed in the inner cuticle and cortex of the bleach modified hair. A decrease in Young's Modulus of hair fibre in wet condition after bleaching treatment was confirmed using tensile strength measurement. An increase in the amount of cysteic acid residues resulted from oxidation of disulfide bonds present in hair were found after bleaching treatment by using FT-NIR spectroscopy. The cleavage of disulfide bonds, which give a high degree of physical and chemical stability to the hair fibre, caused a loss of structural integrity. The overall changes at the molecular level and at the microstructural level after bleaching were likely to contribute to the poor mechanical properties which was proved by the tensile strength measurements in this study. In conclusion, it was demonstrated that the morphological changes at the microstructural level and the chemical changes at the molecular level inside hair fibre due to bleaching is very likely to be responsible for the macroscopically measurable changes in the loss of strength.

Next, the study was conducted with bleaching agents in which part of succinic acid, lysine and arginine were formulated. The results showed that a hair bleaching product with the addition of the above-mentioned mixture restored mechanical properties loss of bleach modified hair and reduced amount of cysteic acid generated by bleaching treatments. The observation of the cuticular surface with SEM showed that the addition of the mixture provided a relative smoother hair surface after bleaching treatment compared to the conventional bleaching mixture alone. Moreover, the TEM observations indicated that the melanin granules were not completely degraded by the addition of this mixture. However, this color difference due to the undegraded melanin

residues was not detected at the macroscopic level, which was supported by the data of LAB measurement. The residual melanin granules could hint at the involvement of these substances such as succinic acid, lysine and arginine in bleaching reaction leading to a milder bleaching condition which causes less hair damage.

Taken together, the results obtained from the multidiscipline approach provided evidence that the addition of the combination of succinic acid, lysine and arginine during bleaching treatment offers fibre protective properties compared to the conventional bleaching mixture alone. The lightening performance was, however, not impaired, despite TEM showed residual melanin. This different obtained from TEM observations was not able to be distinguished by human eyes at marcoscopical level.

A possible mechanism of the effect of this mixture during hair bleaching previous proposed by T. Förster [69] and T. C. Schlenkermann [71] was that the organic di-acids like maleic acid, succinic acid could be absorbed by the hair cortex and rebuild salt bridges and/or bridges with hydrogen bonds interaction inside hair fibre, which resulted in enforcement of the hair bonds, thus helping to strengthen hair fibre. Before proposing our favourite mechanism, we attempted to further verify this mechanism concerning Interaction of the components with hair fibre in this work. Untreated hair tresses, the hair tresses treated with bleaching formula containing succinic acid, and bleached hair tresses were extracted using a solution mixture containing methanol water and then the eluates were analysed using ion chromatography, respectively. However, no extra succinic acid was detected in or on hair tresses. Additionally, in order to further trace succinic acid in or on hair, succinic acid was replaced by 99 % isotopic enriched succinic acid. Because a $^{13}C_4$-enriched succinic acid would provide stronger signals on NMR spectroscopy [78]. Another advantage is that ^{13}C NMR allowed the measurements to

perform directly on hair tresses. However, no any succinic acid was detected in or on hair using ^{13}C NMR to support this hypothesis. Moreover, the effect of another key ingredients lysine and arginine has not been investigated in the previous studies and should not be ignored.

Till now it can be concluded that the addition of the mixture of active ingredients containing succinic acid, lysine and arginine led to a physically stabilized hair fibre. Secondly, the oxidation of disulfide cross-linkers was reduced but not completely inhibited. Thirdly, there was no enrichment of di-carboxylic acid like succinic acid in or on hair fibre after bleaching treatment. However, there was still significant remaining of melanin granules observed after bleaching treatment with the combination of the mixture. In consideration of all experimental findings above, the favourite hypothesis was proposed. That could be, the mixture containing succinic acid, lysine and arginine may interfere with bleaching reaction and lead to a milder bleaching condition. These substances like succinic acid, lysine and arginine may act as chelating agents via the carboxylic group -COO- and amino groups NH_2 to deactivate transition metals like copper metals existing in hair fibre [79], especially in melanin granules during the bleaching treatment. In this way, the amount of highly reactive free radicals induced by iron or copper via Fenton or Fenton-like reaction during bleaching process would be reduced and hence hair damage would be reduced correspondingly. A deeper investigation of mechanism of this protective effect was further discussed in the next part of this research, as only with this knowledge might enable us to find other more efficient substances suitable for a more efficient hair bleaching product with less side effects.

3.2 Detection and semi-quantification of radical species in different bleaching solution systems

In order to understand how these substances like succinic acid, lysine and arginine modify the bleaching reaction, a better understanding of the reaction mechanism of bleaching is needed. Alkaline hydrogen peroxide is the main oxidant used in human hair bleaching products. It has been demonstrated that hydrogen peroxide generates hydrogen peroxide anion (HOO^-) at pH=10 and above. Metal ions in human hair fibres, such as copper ions, can catalytically decompose alkaline hydrogen peroxide via the Fenton chemistry during bleaching, which results in the formation of reactive oxygen species. Especially, hydroxyl radical is a highly reactive and non-selective oxidant which can react with almost any organic compounds with rate constants approaching 10^8-10^{10} $M^{-1}S^{-1}$ [80]. Free radical-mediated oxidation of proteins and lipids is well known [81, 82]. These copper ions-induced radical species can further cause protein degradation which leads to hair damage. Therefore, the well-known Fenton-like reaction was chosen in this study to build a model. ^{31}P NMR-spectroscopy-based spin trap technique was employed to identify and quantify radical species formed during bleaching processes based on ammonium hydroxide/hydrogen peroxide alkaline model systems at pH=10. Here, the bleaching system with the addition of salts of persulfate was not investigated and discussed in this work, because of the intense reaction which was difficult to control and quantify using ^{31}P NMR-spectroscopy. The aim was to obtain detailed knowledge of the specific radical species generated in different bleaching solutions, their kinetics and selectivity. This knowledge is the essential scientific base for the further development of more efficient and safe products for hair bleaching and laundry detergents. Next, I employed this approach to investigate the formation of radical species in the presence

of succinic acid, lysine and arginine in bleaching system to verify the proposed working mechanism.

3.2.1 Identification and semi-quantification of radical species in H_2O_2/NH_4OH+NH_4Cl model solution at pH=10

Firstly, a blank sample was analysed as control. It only contained the key oxidant hydrogen peroxide in an ammonia buffered solution. The ammonia buffered solution was employed in order to ensure the pH maintenance of all bleaching systems in this work. As the decomposition of hydrogen peroxide is dependent on the pH condition of bleaching system [83], the buffered solution was used to avoid the influence of various pH on the formation of radical species. The identified fragmentation patterns together with specific chemical shifts are presented in Table 3.1. The representative spectrum of the blank sample (Fig. 3.10) showed peaks that correspond to the radical reaction products of $NH_2OO^{\cdot}$/DIPPMPO, $HOO^{\cdot}$/DIPPMPO or $O_2^{\cdot-}$/DIPPMPO, which have been identified before in previous studies [44]. A strong signal at 27.4 ppm was clearly detectable, which was, however, not detected at all in the bleaching system with NaOH (0.1 mmol/l) instead of ammonium salt as alkalizing agent. This signal was supposedly related to the formation of DIPPMPO adducts with nitrogen containing radical species $\cdot NH_2$. It is well known that hydroxyl radicals react with ammonia to generate amino radical $\cdot NH_2$ [46] However, in previous literature, the chemical shift of the DIPPMPO/$\cdot NH_2$-adduct was detected at 26.6 ppm [44]. This difference in chemical shift could be due to different alkaline buffer solutions used in these studies and a different composition of the model system.

Moreover, the peak at 30.6 ppm found in the spectra, was assigned to the DIPPMPO/$\cdot OONH_2$ radical adduct based on literature data. This radical species is known

to be formed upon the reaction of the amino radical $\cdot NH_2$ with oxygen O_2. The DIPPMPO/$\cdot$OH radical adduct with a chemical shift of 25.3 ppm was not detected at all in the blank sample. This indicated that the bleaching agent was associated with HOO$\cdot$ only, if hydrogen peroxide was in contact with air under strong alkaline conditions at pH=10.

Table 3.1 ^{31}P NMR signals for DIPPMPO reaction adducts.

Species	Chemical shift (ppm)
DIPPMPO	22.2
DIPPMPO/$\cdot$OH-adduct	25.3
DIPPMPO/$\cdot$OOH-adduct	16.9, 17.1
DIPPMPO/$\cdot NH_2$-adduct*	27.4
DIPPMPO/$\cdot OONH_2$-adduct	30.7

*The chemical shift of DIPPMPO/$\cdot NH_2$ adduct of 26.6 ppm was documented in previous literature [44].

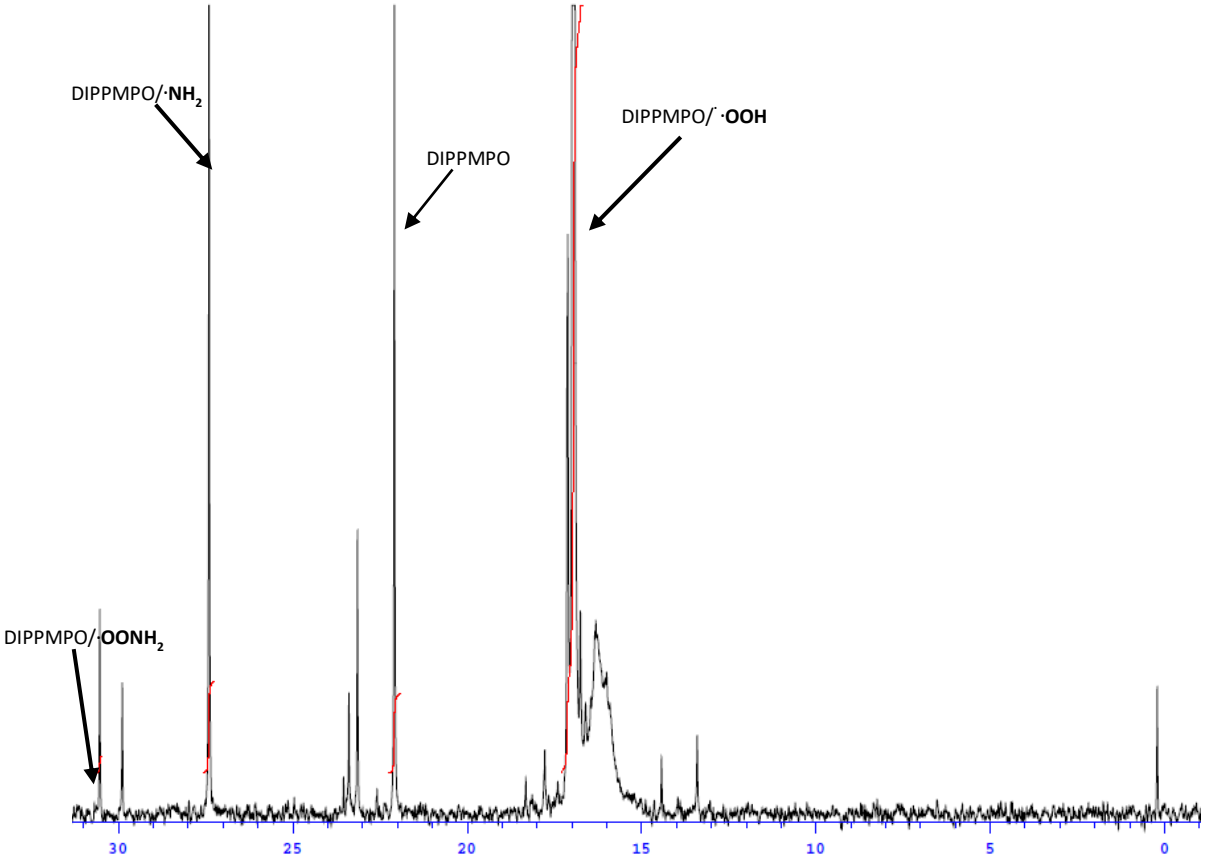


Figure 3. 10 Representative ^{31}P NMR spectrum of the radical adducts formed in a H_2O_2/NH_4OH+NH_4Cl system at pH=10 with DIPPMPO spin trap.

If not reported differently, the spin trapping experiments were performed at least three times in order to examine the quantitative reliability of the approach. Based on assignment of the ^{31}P NMR signals, kinetic analysis of the generation of the DIPPMPO-radical adducts and their relative concentrations were evaluated at 26 °C over a time span of 45 min. This corresponds to a typical application time of hair bleaching processes. The kinetic analysis of detected radical spin adducts was shown in Fig. 3.11. The formation of radical species occurred during the first minutes of the reaction. A steady state situation of radical adducts was quickly reached, except for the superoxide radical adduct, which decreased over time. The DIPPMPO/·OOH radical adduct was trapped in an amount of around 31 mmol/l after ca.7 min of measurement, and decreased to about 19 mmol/l after ca. 45 min. Nevertheless, the superoxide radical was the most abundant

radical species detected in this bleaching system. Congruent with this, the following equations elucidate the high amount of superoxide radicals generated under high alkaline conditions at pH=10 [23, 45]:

$$H_2O_2 + OH^- \leftrightarrow HOO^- + H_2O \quad pK_a = 11.65 \qquad (1)$$

$$H_2O_2 + HOO^- \rightarrow \cdot OH + O_2^{\cdot -} + H_2O \qquad (2)$$

$$HOO^- + \cdot OH \leftrightarrow O_2^{\cdot -} + H_2O \qquad (3)$$

$$\cdot OH + H_2O_2 \rightarrow HO_2^{\cdot} + H_2O \qquad (4)$$

In the absence of transition metal ions such as copper ions, the decomposition of hydrogen peroxide depends on pH. In bleaching processes at pH=10 and above, hydrogen peroxide (pK_a=11.65) is present in the form of the hydroperoxide anion HOO^- (Eq. (1)). The hydroxyl radicals formed (Eq. (2)) can subsequently react with hydroperoxyl anions to give superoxide radicals (Eq. (3)).

The hydroxyl radical species, however, were not detected at all in the blank sample, so this leads to the assumption that most of the hydroxyl radicals readily reacted with hydroperoxyl anions or hydrogen peroxide to form superoxide radicals and hydroperoxyl radicals, respectively (Eq. (3), Eq.(4)), while the remaining low amount of hydroxyl radicals was readily trapped by ammonia to generate amino radicals. As expected, DIPPMPO/$\cdot NH_2$ and DIPPMPO/ $\cdot OONH_2$ radical adducts were present in a low amount in the blank sample.

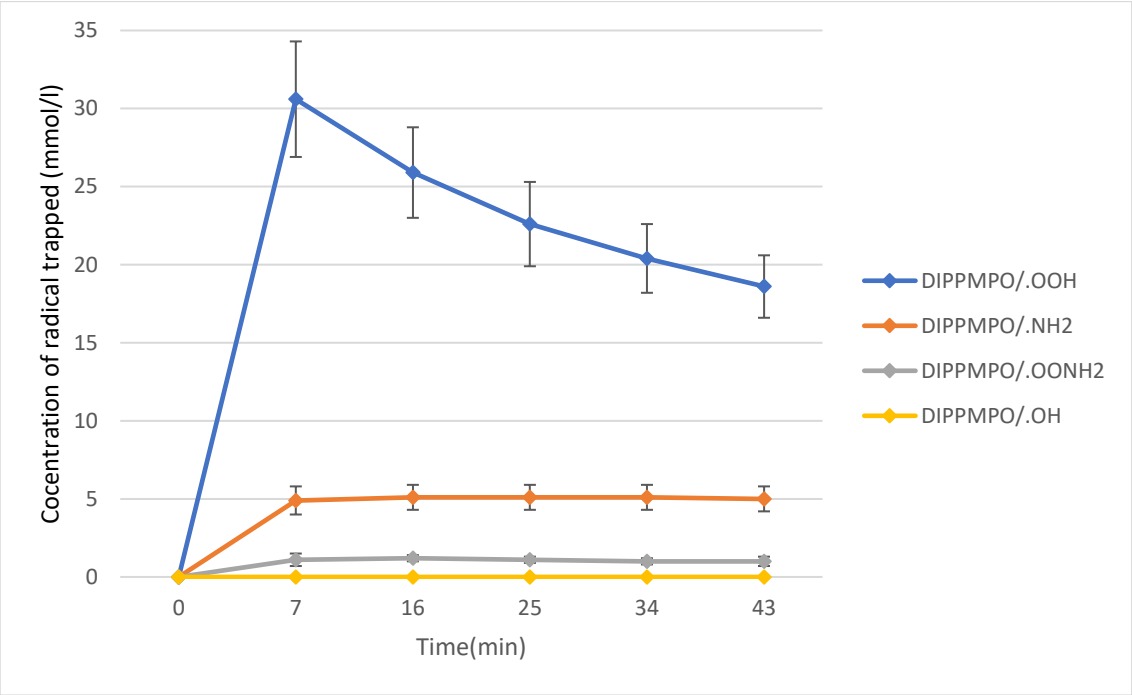


Figure 3. 11 DIPPMPO radical adducts (concentration of radical trapped in mmol/l) trapped at 26 °C in a H2O2/NH4OH+NH4Cl system. Concentration of radical adducts trapped as a function of reaction time (N=3).

3.2.2 Identification and semi-quantification of radical species in a H_2O_2/NH_4OH+NH_4Cl/Ca^{2+} model solution at pH=10

It is well known that Ca^{2+} ions are not able to participate the decomposition reaction with hydrogen peroxide. In agreement with this, the results showed (Fig. 3.12) that there was no hydroxyl radical detected at all and the reaction kinetics of decomposition of hydrogen peroxide at pH =10 was independent on the addition of Ca^{2+} (8 mmol/l, 320 ppm) and corresponded to the blank system without metal ions. The superoxide radical adducts were the most frequent species present in this system, second-most frequent species was the amino radical adduct. Overall, the most abundant species profiles were similar to the blank system which did not contain any metal ions, as well as the general kinetic trends for the build-up of the equilibrium concentrations.

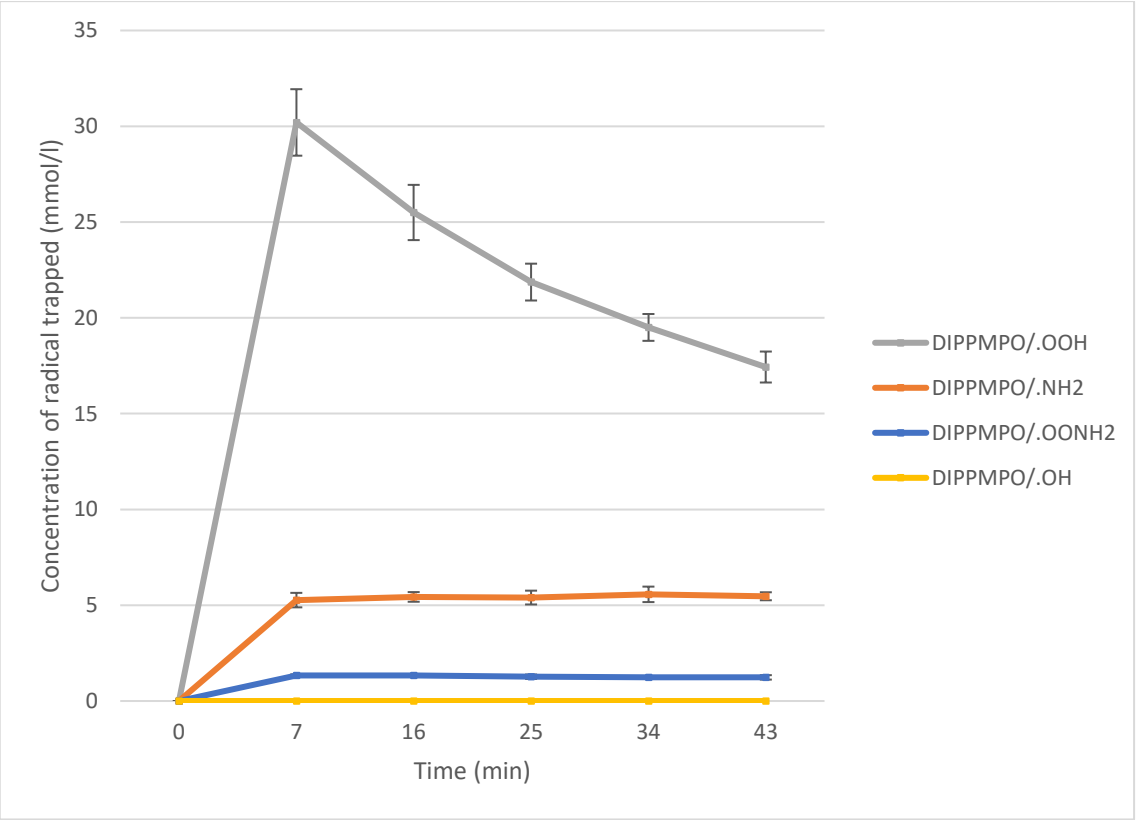


Figure 3. 12 DIPPMPO radical adducts (concentration of radical trapped in mmol/l) trapped at 26 °C in a $H_2O_2/NH_4OH+NH_4Cl/Ca^{2+}$ system. Concentration of radical adducts trapped as a function of reaction time (N=3).

3.2.3 Identification and semi-quantification of radical species in a $H_2O_2/NH_4OH+NH_4Cl/Cu^{2+}$ model solution at pH=10

The addition of a low amount of transition metal ions, Cu^{2+} (0.3 mmol/l, 19.2 ppm), to bleaching solutions described before, had an effect on the formation and stability of radical species in the time-course of the reaction (Fig. 3.14). The signal from DIPPMPO/·OH adducts at 25.3 ppm was clearly observed (Fig. 3.13). It was not detectable in the absence of Cu^{2+} (Fig. 3.10). The identification and quantification of DIPPMPO/hydroxyl radical adducts ·OH as well as DIPPMPO/nitrogen radical species NH_2· appeared to be more accurate and reproducible than the corresponding determination of superoxide radical species ·OOH or $O_2^{\cdot-}$, as the superoxide radical adducts appeared in form of two distinct, but relatively broad peaks as shown in Fig. 3.10 and 3.13.

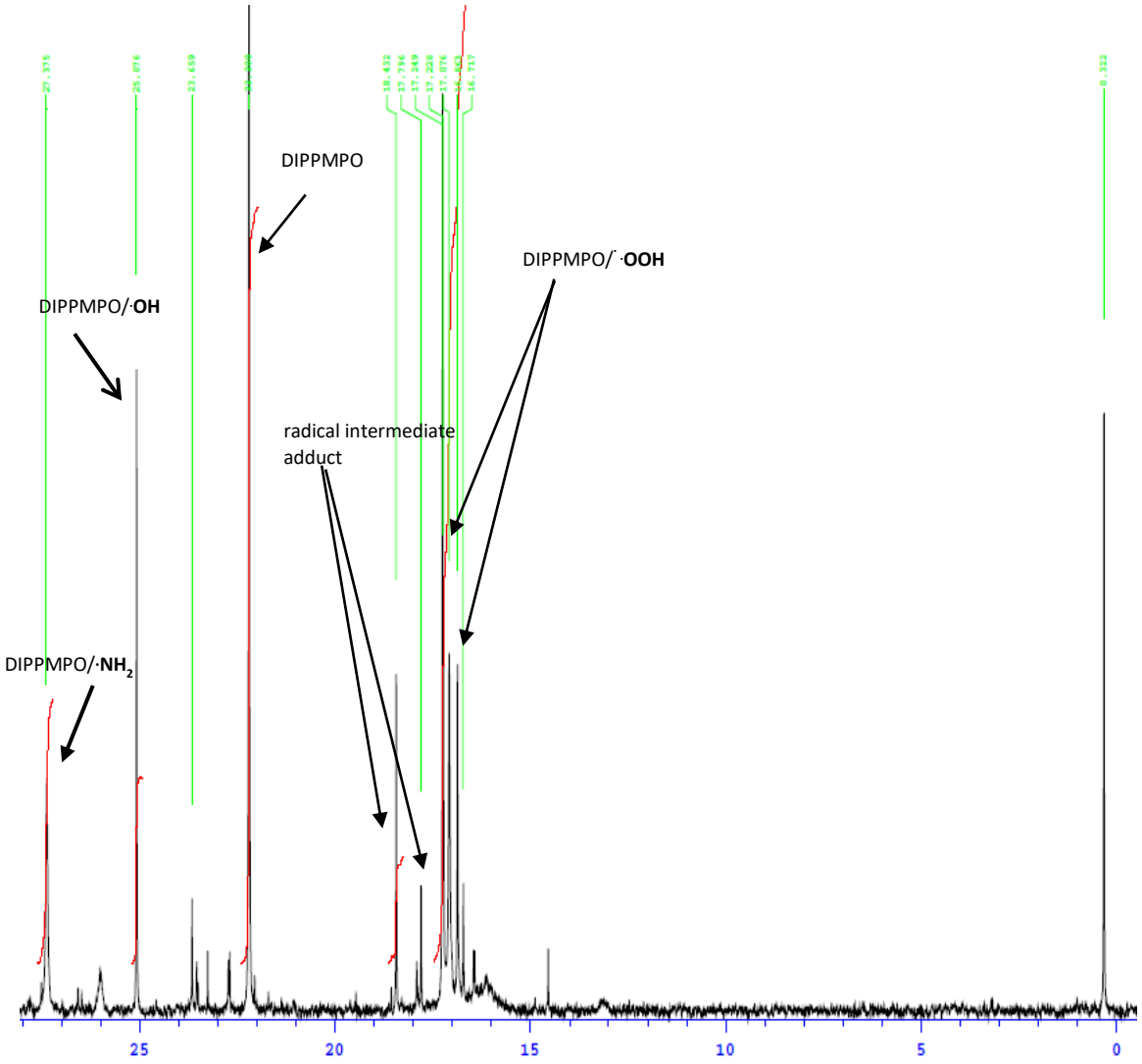


Figure 3. 13 Representative ^{31}P NMR spectrum of the radical adducts formed in a $H_2O_2/NH_4OH+NH_4Cl/Cu^{2+}$ system at pH10 with DIPPMPO spin trap.

Regarding reaction kinetics, the result in Fig. 3.14 clearly showed an increased amount of hydroxyl radicals ·OH formed during the first minutes. The data showed strong evidence that hydroxyl radical ·OH formation was induced by Cu^{2+} in a Fenton-like reaction (Eq. (5-7)). Hydroxyl radical ·OH , due to its extremely high reactivity towards hair proteins, is believed to be one of the main radical species responsible for oxidation of hair proteins in alkaline peroxide [44]. As proposed by V. Khramstov et al. [63] when the concentration of the radical adducts is high, disproportionation and rearrangement reactions occur (Scheme 2), affording a new nitrone and the original spin trap DIPPMPO.

Therefore, the amount of new nitrone 5 (Scheme 2) observed in the ^{31}P NMR spectra represents only half of the total spin trapped hydroxyl radicals. The other half of the hydroxyl radical adducts which are originally formed are transformed back into the DIPPMPO via the loss of water of the product 4 in Scheme 2. In conclusion, in order to accurately quantify the real value of trapped hydroxyl radicals, the NMR integral of the corresponding radical adduct peak in the ^{31}P NMR spectrum should be doubled.

The DIPPMPO/·OOH adduct was still the dominant species present in this bleaching system in the presence of Cu^2. This system with Cu^{2+} also had a clear effect on the amount of superoxide radicals. The amount of detected superoxide radical adducts reached only ca. 20 mmol/l after ca. 7 min of measurement, and then its amount appeared to be constant, whereas in the bleaching solution in the absence of Cu^{2+}, the superoxide radical adducts were firstly trapped in the amount of around 31 mmol/l after ca. 7 min of measurement (Fig. 3.11). From these results, it was concluded that the addition of Cu^{2+} to the bleaching solution led to a decrease of superoxide radical formation (Fig. 3.11 and Fig. 3.14). This result can easily be explained by the following Fenton-like reactions [25]:

$$Cu^{2+} + H_2O_2 \rightarrow Cu^{+} + HOO\cdot + H^{+} \qquad (5)$$

$$Cu^{+} + H_2O_2 \rightarrow Cu^{2+} + HO^{-} + HO\cdot \qquad (6)$$

$$Cu^{2+} + HOO\cdot \rightarrow Cu^{+} + H^{+} + O_2 \qquad (7)$$

Transition metal ions such as copper ions in their low oxidation state can participate in and catalyse hydrogen peroxide decomposition. Cu^{2+} reacts with hydrogen peroxide to give Cu^{+} and the superoxide radical (Eq. (5)). The reduced Cu^{+} further decomposes hydrogen peroxide to generate hydroxyl radicals and leads to the oxidation of Cu^{+} back to Cu^{2+} (Eq. (6)). The superoxide radicals reduce Cu^{2+} to Cu^{+} to continue the cycle (Eq.

(7)). Our data are in agreement with the notion that superoxide radicals continuously joined the cycle reaction to reduce Cu^{2+} back to Cu^{+}, therefore the amount of trapped superoxide radicals in bleaching systems with Cu^{2+} was lower than that in the system without Cu^{2+}. Fig. 3.14 showed that the DIPPMPO/·NH_2 adducts are still present in an amount that is comparable to the experiment in which Cu^{2+} is absent (Fig. 3.11). The amount of DIPPMPO/·OH increased up to 10 mmol/l between ca. 25 min and 45 min in the course of measurement. Overall, the most abundant species were DIPPMPO/·OOH, DIPPMPO/·NH_2, and DIPPMPO/·OH in bleaching systems in the presence of Cu^{2+}. The general kinetic trends for the build-up of the equilibrium concentrations of all detected radical adducts (Fig. 3.14) showed a different behaviour from the system without copper ions. A steady state situation of all detected radical adducts was quickly reached the same as before in the H_2O_2/NH_4OH+NH_4Cl system. However, the H_2O_2/NH_4OH+NH_4Cl system showed that after 7 min, the amount of DIPPMPO/·OOH radical adduct decreased at a faster rate (Fig. 3.11), whereas its amount in the bleaching system with Cu^{2+} was found to be relatively constant (Fig. 3.14).

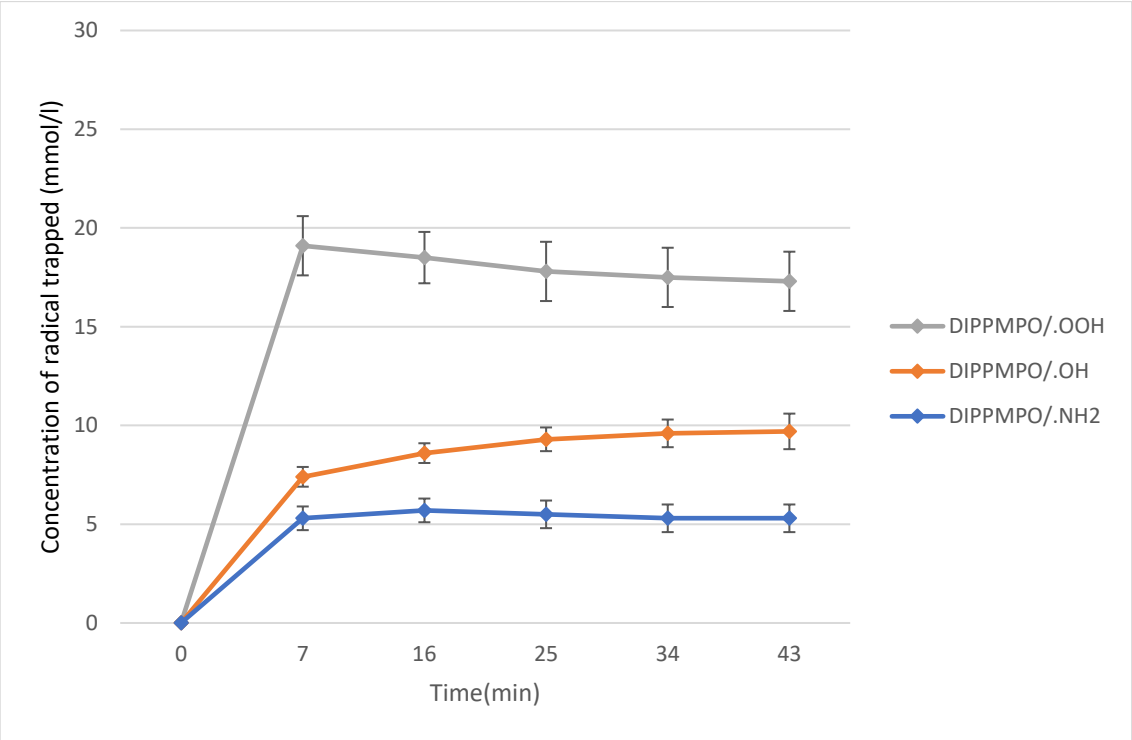
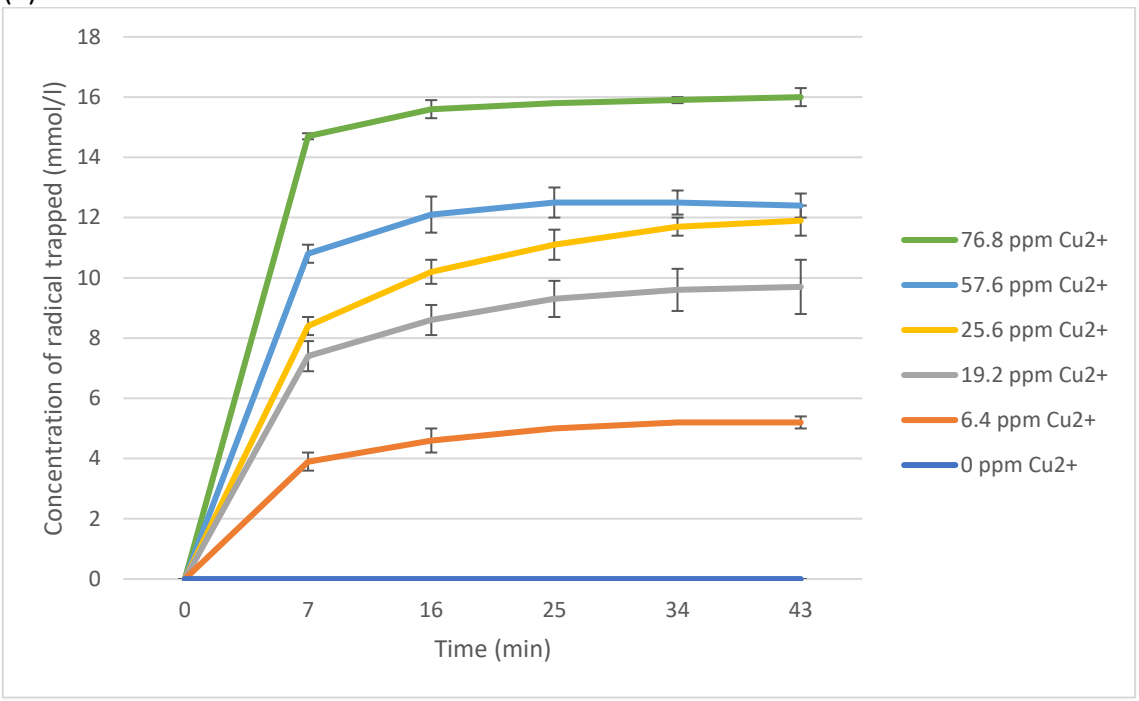


Figure 3. 14 DIPPMPO radical adducts (concentration of radical trapped in mmol/l) trapped at 26 °C in a $H_2O_2/NH_4OH+NH_4Cl/Cu^{2+}$ system. Concentration of radicals trapped as a function of reaction time(N=3).

Fig. 3.15a showed the hydroxyl radical formation for the solution system without and with added copper at concentration between 6.4 ppm and 76.8 ppm. According to the previous research data, the level of Cu^{2+} in human hair can vary from 50 to 80 ppm. Therefore, experiments with copper levels under typical use conditions were performed. The results clearly showed an increase amount of hydroxyl radicals formed when copper ions were added. The data provides strong evidence again that hydroxyl radical formation was induced by copper ions in a Fenton-like reaction. Fig. 3.15b showed the superoxide radicals formation for the solution system without and with added copper at concentration between 6.4 ppm and 76.8 ppm. The results clearly showed a decrease amount of superoxide radicals formed when copper ions were added. The finding is consistent with the notion that superoxide radicals continuously joined the cycle

reaction to reduce Cu^{2+} back to Cu^{+}, therefore the amount of trapped superoxide radicals in bleaching systems decreased when the amount of Cu^{2+} increased.

(a)



(b)

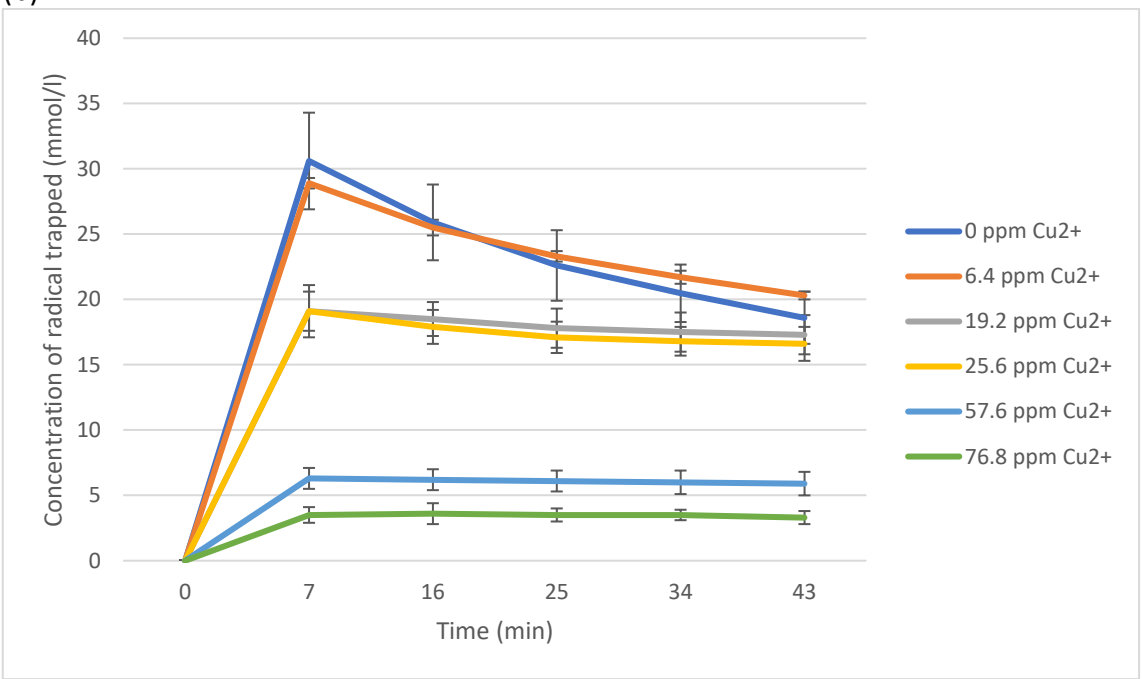


Figure 3. 15 Concentration of radical species trapped as a function of added copper in aqueous model system. DIPPMPO (100 mmol/l) was added to trap the radicals at pH 10. ^{31}P NMR was used to collect the quantitative data after the mixture was thoroughly stirred continuously under air for 10 min. (a) DIPPMPO/·OH spin adducts formation; (b) DIPPMPO/·OOH spin adducts formation (N=3).

3.2.4 Identification and semi-quantification of radical species in a $H_2O_2/NH_4OH+NH_4Cl/Cu^{2+}$ /EDTA model solution at pH=10

As reported in the previous experiment above, an increase of hydroxyl radical species was seen if transition metal ions such as copper ions Cu^{2+} were present in the bleaching system. It has been reported that the addition of chelating agents to alkaline peroxide systems can prevent transition metal-induced hydroxyl radical formation [84].

Upon addition of the chelating agent EDTA (8 mmol/l) to the system with Cu^{2+} (0.3 mmol/l, 19.2 ppm), there was no hydroxyl radical detected at all (Fig. 3.16) and the reaction kinetics corresponded to the one of the blank system without Cu^{2+} and EDTA. The superoxide radical adducts were the most frequent species present in this system, second-most frequent species was the amino radical adduct. Overall, the most abundant species profiles were similar to the blank system which did not contain copper ions and chelating agent, as well as the general kinetic trends for the build-up of the equilibrium concentrations. This result showed clearly that EDTA had the ability to effectively chelate Cu^{2+} and thus prevent Cu^{2+} from joining the redox-cycle reaction if Cu^{2+} was present in the bleaching system alone.

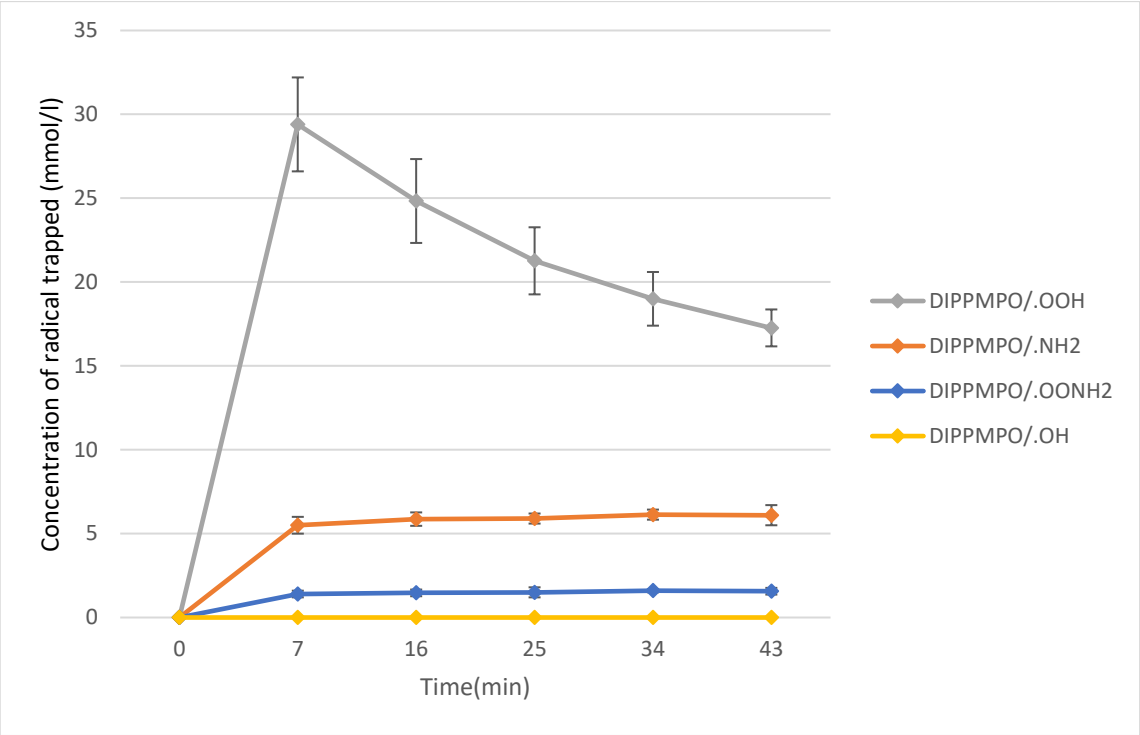
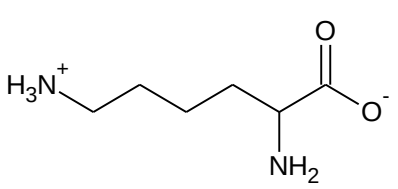


Figure 3. 16 DIPPMPO radical adducts (concentration of radical trapped in mmol/l) trapped at 26 °C in a H_2O_2/NH_4OH+NH_4Cl/Cu^{2+}/EDTA system. Concentration of radicals trapped as a function of reaction time (N=3).

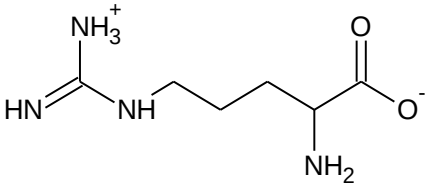
3.2.5 Decomposition of hydrogen peroxide in the presence of Cu^{2+} at pH=10 and various chelating agents

Recently, a hair bleaching product formulated with succinic acid in combination with lysine and arginine was introduced to the market. It was proved that this formulation led to a relevant hair strengthening benefit perceived by consumers as better grooming properties and protection against hair breakage [69]. However, the mechanism of this protective effect is not yet fully elucidated and understood. The solution system in the present study can be used as a good model to predict the mechanism if and how these substances modulate the bleaching reaction and profile radical formation. To do this, the bleaching system in the presence of Cu^{2+} (0.3 mmol/l, 19.2 ppm) and a mixture of sodium succinate (24.0 mmol/l), lysine (8.0 mmol/l) and arginine (8.0 mmol/l) was studied next. The mixture was comprised of lysine, arginine and succinic acid, which

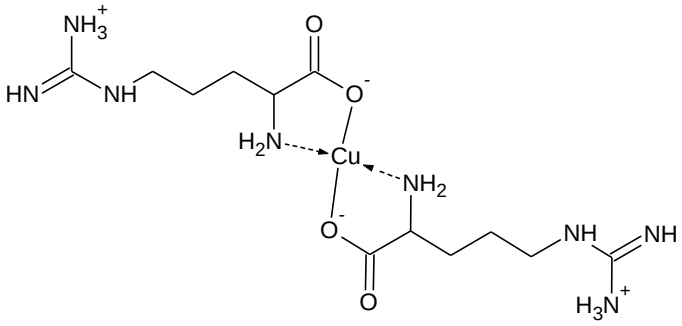
contain carboxylic acid groups -COOH and amino groups -NH_2. It is well known that lysine and arginine are basic amino acids [85]. Lysine contains an α-amino group (pK_a=8.90), an α-carboxylic acid group (pK_a=2.20), and a side chain with ε-amino group (pK_a=10.28). As shown in Fig. 3.17a, at pH=10, the predominant form of lysine has a negative carboxylate (-COO^-) and neutral α-amino group (-NH_2). The ε-amino group is protonated to become the positive ε-ammonium group (-NH_3^+), as long as the pH of the system (pH=10) is still below its respective pK_a value (pK_a=10.28). Like lysine, arginine is also a basic amino acid, which contains an α-carboxylic acid group (pK_a=2.0), an α-amino group (pK_a=9.0), and a side chain ending in a guanidine group (pK_a=12.1). At pH=10, the α-carboxylic acid is deprotonated (-COO^-), the α-amino group stays neutral (-NH_2), and the guanidine group is protonated to give the guanidinium group (-C-$(NH_2)_2^+$) (Fig. 3.17b). The carboxylic group of succinic acid is negatively charged at pH=10. It is proposed here that Cu^{2+} could be chelated via the carboxylic groups -COO^- and amino groups -NH_2 to form stable complexes (Fig. 3.17c) [79].



(a): Lysine



(b): Arginine



(c): Amino acid-copper(II) complex, e.g. arginine

Figure 3. 17 Charge of the amino acids under alkaline condition of pH=10.

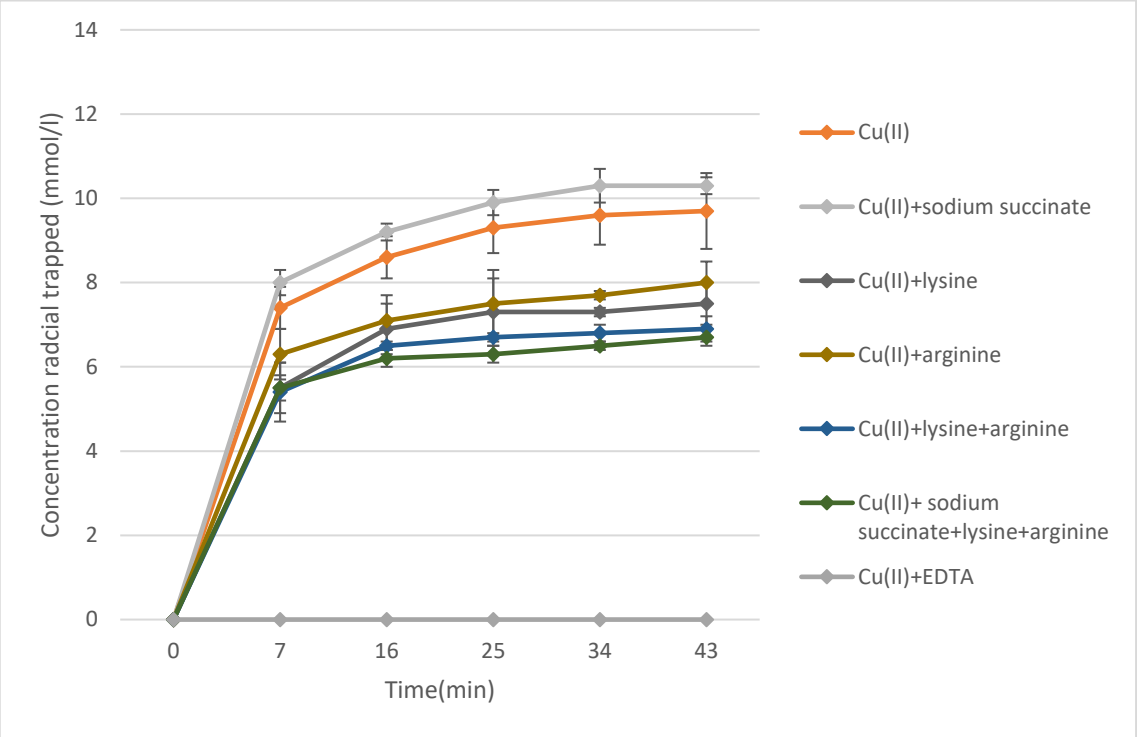
The results in Fig. 3.18a show that the presence of the mixture containing sodium succinate, lysine and arginine led to a reduction of the formation and concentration of hydroxyl radicals compared to the presence of copper ions alone; in contrast, EDTA is able to completely suppress hydroxyl radical formation. Sodium succinate alone, being a dicarboxylic acid, showed only a very weak chelating effect on copper ions and had no effect on reducing the formation of hydroxyl radicals in the system (Fig. 3.18a) even though the use concentration of it was much higher than other chelating agents. An explanation would be that copper may form complex with succinic acid similar to Fenton reagent and this complex may decompose hydrogen peroxide resulting in production of reactive oxygen species [86, 87]. It was also reported [87] that succinic acid /copper (II) / hydrogen peroxide system efficiently decolorize synthetic dyes. The system, similar to Fenton reagent, produces hydroxyl radicals. This fact was in agreement with the result

of model system copper(II) / succinate /hydrogen peroxide shown in Fig. 3.18a. The role of succinic acid lies probably in complexation of coper ions and consequent binding of produced radicals [87]. However, the combination of basic amino acids and sodium succinate showed the best chelating effect on copper ions in comparison to the single substances or even the combination of both basic amino acids only. This result based on model system was consistent with the results reported in chapter 3.1 that the addition of succinic acid, lysine and arginine to bleaching formula reduced hair damage. However, besides the propose that carboxylic groups in succinic acid could assist with chelating copper ions in model system, there could be another speculation about the role of succinic acid in hair bleaching. As mentioned before, in order to achieve more lightening effect, salts of persulfate are usually added as “booster” in hair bleaching products. In chapter 3.1, the bleaching products with the addition of salts of persulfate were used to assess hair damage. A. Ocampo [88] reported that the organic compound like succinic acid deactivated salt of persulfate under high alkaline condition. Thus, it is tempting to speculate that the addition of succinic acid may deactivate persulfate to some extent, and thus lead to less hair damage.

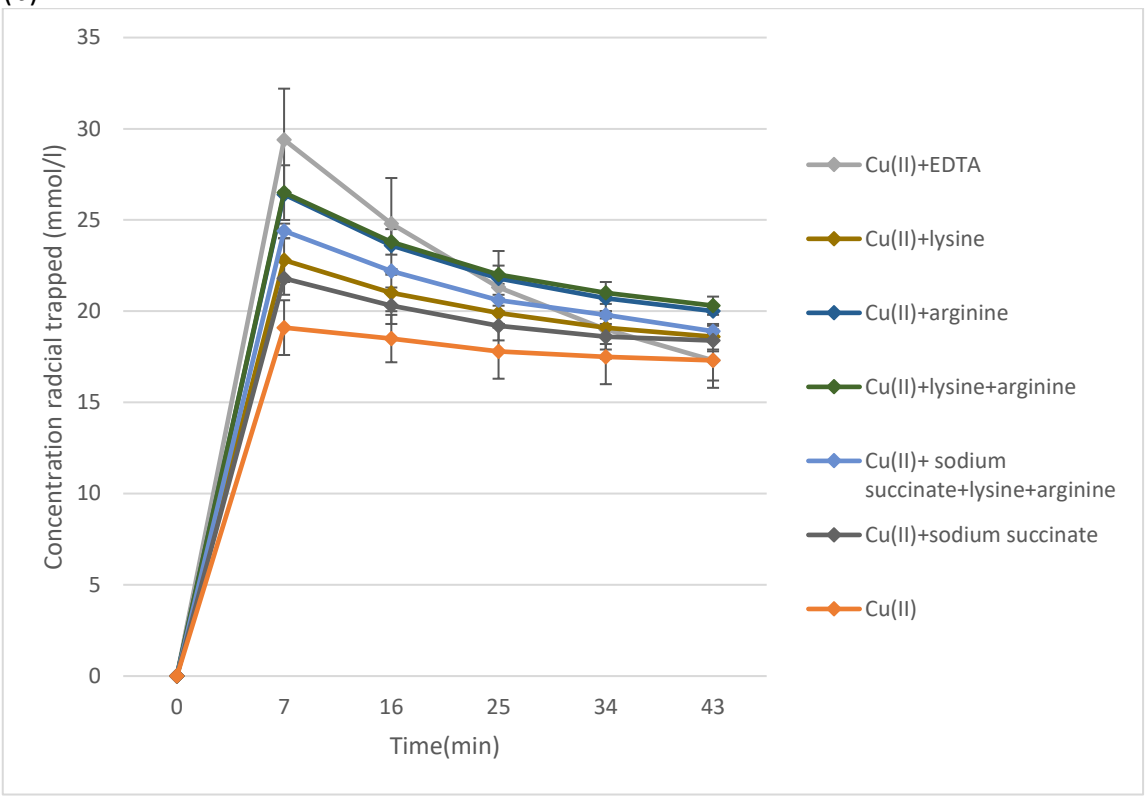
The superoxide-radical ·OOH adduct was the dominant species in all three systems, and it was assumed that their amount could be directly correlated with the different concentrations of non-chelated copper ions (active copper) in the systems (Fig. 3.18b). A steady state situation of superoxide-radical adduct was quickly reached after 7 minutes of measurement, and then its concentration decreased over reaction time. Overall, a higher concentration of superoxide-radicals was detected in the system containing lower levels of active copper ions. Quantitatively, the amount of NH_2· - radical did not differ strongly at different levels of active copper ions present in the systems.

The concentration level of NH_2· - radicals after the first 7 minutes was essentially stable over the observed time-span (Fig. 3.18c).

(a)



(b)



(c)

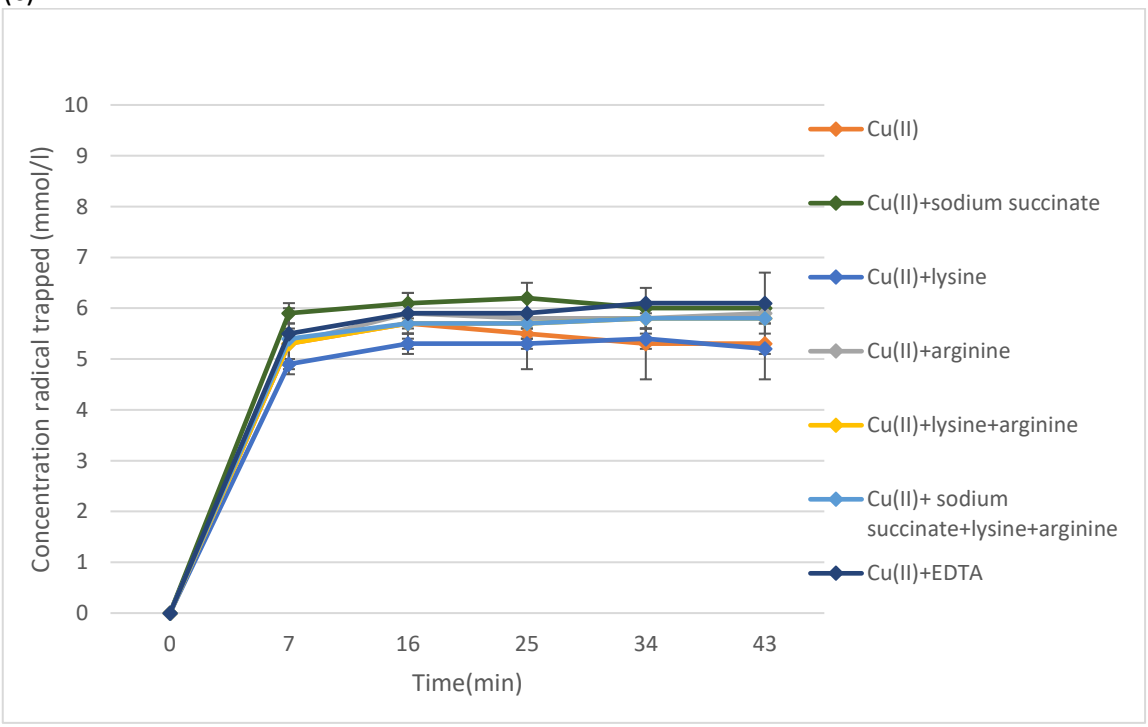


Figure 3. 18 Radical species (concentration of radical trapped in mmol/l) trapped in Cu^{2+} systems with different chelating agents under alkaline conditions as a function of reaction time. (a) DIPPMPO/·OH spin adducts formation; (b) DIPPMPO/·OOH spin adducts formation; (c) DIPPMPO/·NH_2 spin adducts formation (N=3).

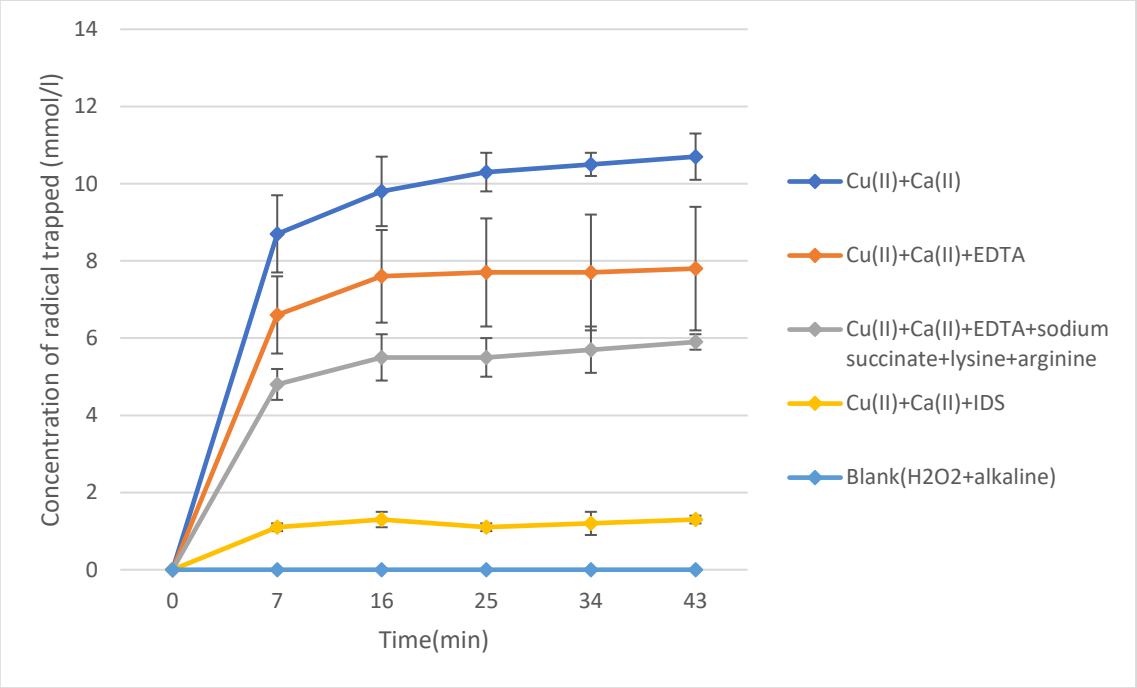
3.2.6 Decomposition of hydrogen peroxide in the binary Cu^{2+}-Ca^{2+} system at pH=10 and various chelating agents

The previous experiments discussed showed that transition metal ions such as copper ions Cu^{2+} catalysed the decomposition of hydrogen peroxide under alkaline conditions at pH=10 and induced hydroxyl radical formation. These experiments were performed using a simple model system containing a single metal ion. However, a realistic system for hair bleaching is much more complex. Metal content in hair samples was measured in this work by inductively coupled plasma optical emission spectroscopy Agilent ICP-OES 720. The metal content in hair is well shown in Table 3.2. The range of about 1200-1400 ppm of calcium and 110 ppm of magnesium ions were detected in untreated European-Caucasian hair samples. Besides transition metals such as copper or iron, human hair fibres contain calcium, magnesium, etc. Calcium is the most abundant metal present in hair fibres which could lie on the calcium-dependent enzymes involved in the construction of isopeptide protein-protein cross-links in human hair [32]. Therefore, in human hair fibres a competition between different ion types for the chelating agent could occur. As model system for such a reaction, a set of experiments containing a Cu^{2+}-Ca^{2+} mixture was performed. Again, a chelating agent-free solution under alkaline conditions was tested followed by addition of EDTA, a mixture of lysine, arginine and sodium succinate or IDS. A binary ion system, 0.3 mmol/l (19.2 ppm) of copper ions Cu^{2+} and 8 mmol/l (320 ppm) of calcium ions Ca^{2+} (as calcium nitrate), was added. The chelating agents were added to ammonia buffer solution (pH=10) at 8 mmol/l concentration level. The concentrations of the DIPPMPO-adducts from ·OH radical, $\cdot OOH/O_2^{\cdot -}$ radical and $\cdot NH_2$ in different systems are shown in Fig. 3.19a, 3.19b and 3.19c.

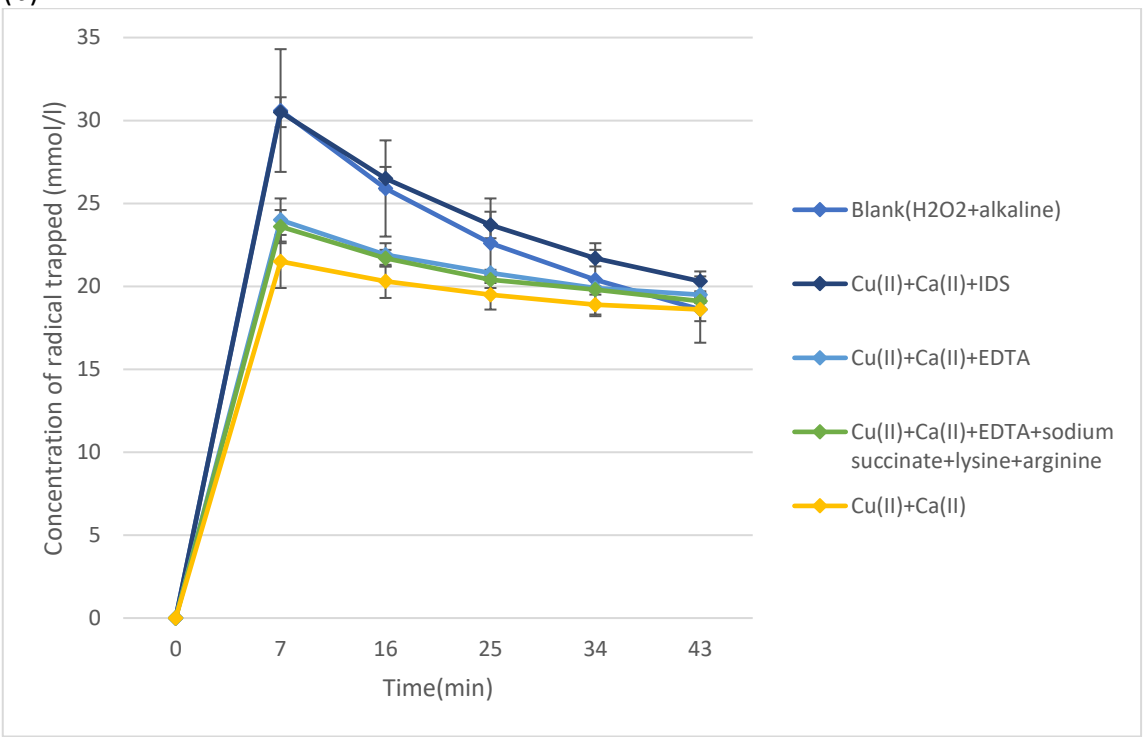
Table 3.2 Summary of the range and values of the metals detected in untreated hair fibre (N=3).

Hair sample	Ca(mg/kg)	Cu (mg/kg)	Fe (mg/kg)	Mg (mg/kg)	Zn (mg/kg)
Untreated hair					
No.1	1212±88	66±9	37±2	109±2	245±4
No.2	1354±107	82±8	70±15	112±2	268±2
No.3	1225±47	75±7	51±4	107±3	260±16

(a)



(b)



(c)

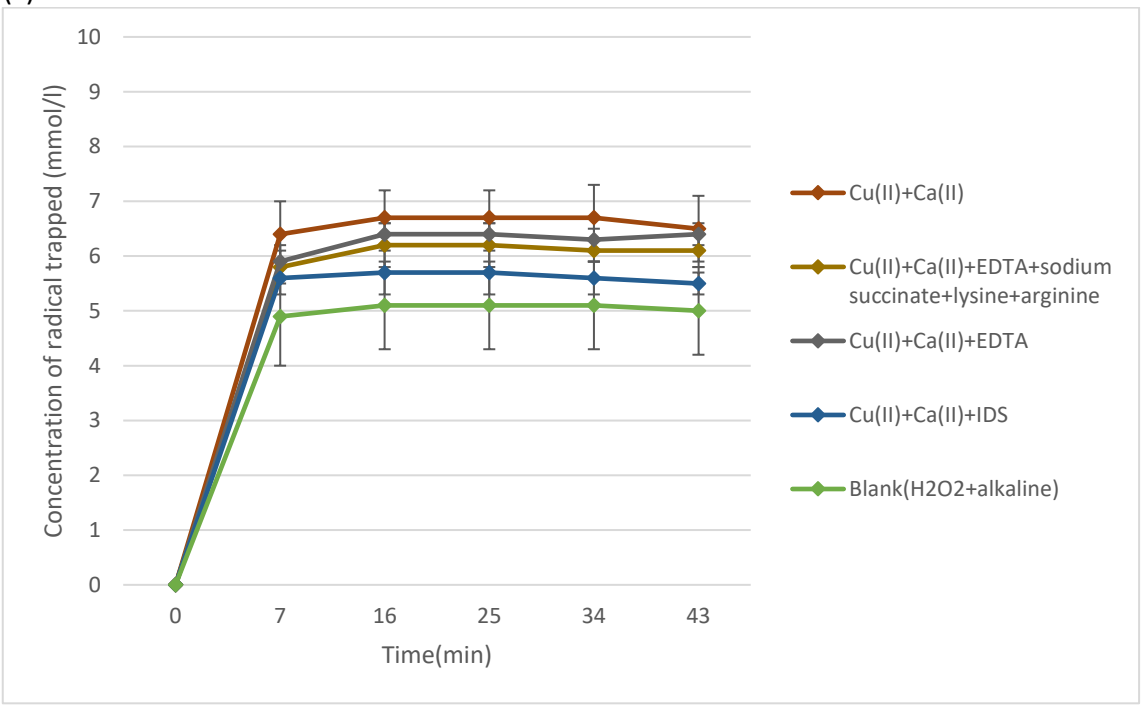


Figure 3. 19 Radical species (concentration of radical trapped in mmol/l) trapped in Cu^{2+}- Ca^{2+} binary systems with different chelating agents under alkaline conditions as a function of reaction time. (a) DIPPMPO/·OH spin adducts formation; (b) DIPPMPO/·OOH spin adducts formation; (c) DIPPMPO/·NH_2 spin adducts formation (N=3).

The hydroxyl radical formation in the binary systems was shown in Fig. 3.19a. The chelating agent-free system, as expected, produced the highest level of hydroxyl radicals due to the presence of Cu^{2+} in an active form. The addition of IDS chelating agent was found to significantly reduce hydroxyl radical formation even in the presence of large amount of calcium ions, whereas a high level of hydroxyl radical formation was still detected, if EDTA was present in this binary Cu^{2+}- Ca^{2+} ion system. However, the result in Fig. 3.16 showed that in the presence of copper ions alone, EDTA was able to suppress hydroxyl radical formation. These data indicated that in binary Cu^{2+}-Ca^{2+} ion systems, IDS was selective in binding active copper ions over calcium ions and thus more efficient in preventing the hydroxyl radical formation in the presence of calcium in comparison to EDTA. These differences may reflect differences in stability constants (Table 3.3). The stability constant (K) [59] expressed as log K, is used to describe the strength of the complex formed between the metal ion and the chelating agent. The higher the log K values, the more tightly the metal ion will be bound to the chelating agent and the more likely the complex will be formed. IDS has a higher binding constant for copper ions over calcium ions (Table 3.3). Moreover, it was proved in previous studies [33] that the EDTA will preferably bind to calcium than to copper and leave copper ions in an active form if both ions are present in the system. The obtained result in Fig. 3.19a is accordant with this fact. The binary Cu^{2+}-Ca^{2+} ion system containing the mixture of lysine, arginine and sodium succinate was studied next. The result in Fig. 3.19a shows that the presence of this mixture does have a further reducing effect on the concentration of hydroxyl radical formation in the copper-calcium binary system compared to the system with EDTA alone.

Table 3.3 Conditional stability constants log K at 25 °C and pH=10 [84].

	Cu(II)	Ca(II)
EDTA	16.0	10.2
IDS	11.0	3.5

The DIPPMPO/·OOH species remained the major species in the binary system (Fig. 3.19b). The amount of ·OOH - radical formation in these binary systems was also found to differ at different levels of Cu^{2+} content in an active form, which is in accordance with the results proved in the previous systems with copper ions alone. It has been proved in the previous experiments (Fig. 3.14) that the addition of Cu^{2+} to the bleaching solution led to a decrease of the superoxide radical adduct. In Fig. 3.19b, the metal-free system and the system with IDS, both with the lowest level of active copper ions, showed the highest amount of ·OOH-radical trapped, whereas the chelating agent-free binary Cu^{2+}-Ca^{2+} system, with the highest level of active copper ions, showed the lowest amount of ·OOH-radical trapped.

A less clear trend was observed for the concentration of radical spin adduct generating from $\cdot NH_2$ -radicals in the binary system (Fig. 3.19c): the concentration level of amino radical $\cdot NH_2$ can be correlated with the different levels of copper content present in the systems. Overall, higher concentrations of $\cdot NH_2$ - radicals were detected in the binary Cu^{2+} -Ca^{2+} system without chelating agents, which contained the most amount of non-chelated copper ions, whereas in the blank sample without metal ions, the concentration of $\cdot NH_2$ - radicals was lowest. However, the concentration of $\cdot NH_2$-radicals did not vary upon different active copper content. In other words, the DIPPMPO-NH_2·-adduct was present in amounts comparable to each other, regardless of the metal ions present in the different bleaching systems.

3.2.7 Detection and quantification of radical species in the presence of human hair during bleaching process

The model solution system can act as a good model to predict the radical species formation in different bleaching systems and the ability of different chelating agents to suppress this chemistry. However, it is more practical relevant to investigate the radical formation directly in the presence of human hair itself. In order to achieve this, hair tresses containing different levels of copper were used for this study (Table 3.4). The untreated hair tress which contained ca. 80 ppm copper was employed. To dose the hair with a various level of copper, untreated hair tresses were soaked in a standard aqueous solution of copper(II) sulphate (200 ppm) to produce hair samples with ca. 360 ppm copper ions.

Fig. 3.20 showed the reaction kinetic with the hair added to the solution as the only source of copper. Results showed that hair containing different levels of copper decomposed alkaline hydrogen peroxide and generated free radicals. An increase in the level of copper in hair led to an increase of the hydroxyl radical adduct formation, as shown in Fig. 3.21a, which provided strong evidence for the source of these radicals being via Fenton metal-induced radical chemistry. As the Fenton reaction is catalytic, the low levels of copper can produce a high flux of radicals and hydrogen peroxide decomposition.

The superoxide-radical ·OOH adduct was the dominant species in the system present with human hair, and its amount did not differ strongly at different levels of copper ions present in the hair. A steady state situation of both superoxide-radical adduct (Fig. 3.21b) and amino-radical adduct (Fig. 3.21c) was quickly reached after 7 minutes of measurement, and then its concentration was essentially stable over the observed time-

span and did not differ strongly at different levels of active copper ions present in the hair.

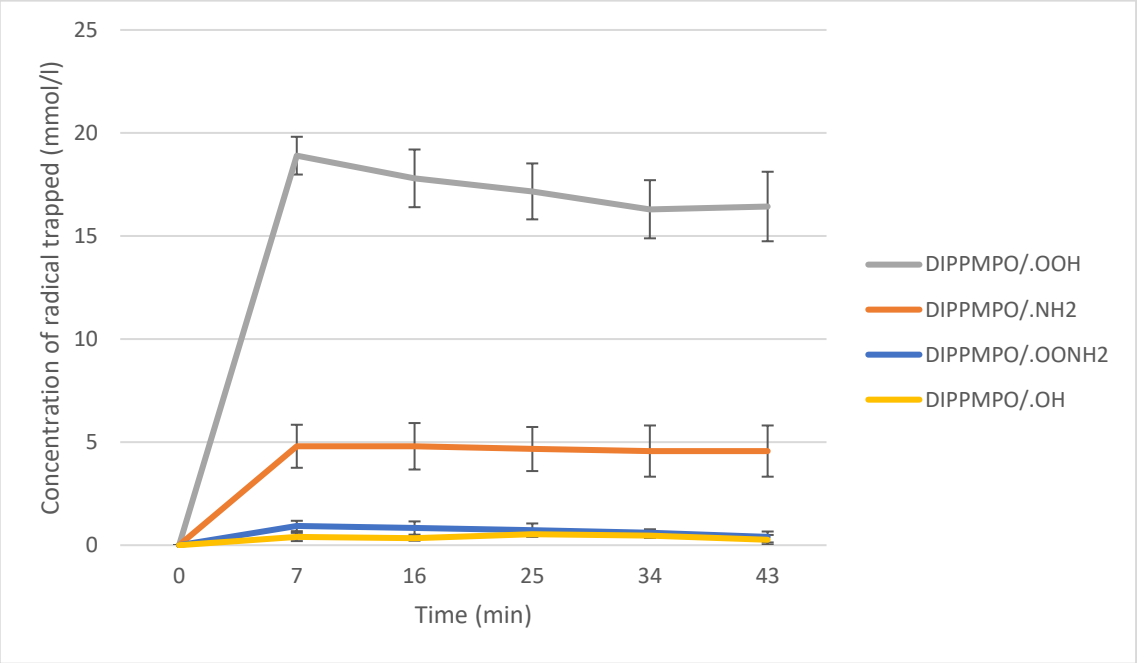
Comparison of these results with the previous data on copper solution systems, showed that the concentration of hydroxyl radical adduct was much less detected in the hair experiments (Fig. 3.21a) as compared to the model solutions (Fig. 3.15a); whereas the concentration of superoxide radical adduct was much higher measured in the hair experiments (Fig. 3.21b) in comparison to the model solutions (Fig. 3.15b). However, both systems had a similar level of copper ions (ca. 80 ppm copper present in hair). This inconsistency can be explained by the low concentration of DIPPMPO probe reached inside hair fibre and hence lower efficiency of radical detection. Another possibility is that copper ions adsorbed inside the hair fibre may have limited accessibility and may not reach into the bulk bleaching solution to join in the decomposition of hydrogen peroxide. Moreover, the highly active ·OH radicals could react faster with hair protein than with DIPPMPO, and thus DIPPMPO/·OH could not be thoroughly detected. Recent studies reported that calcium is mainly present in the outer layer of cuticles while copper is abundant in the cortex of hair fibre, especially in melanin granules. The location of metal ions may influence their diffusion behaviour into the reaction solution. As a result, the actual amount of accessible copper ions might be less than the total copper present in or on the hair fibre. In any case, the results suggested that the DIPPMPO can be successfully used to detect radical species formation in the hair fibre.

Table 3.4 Summary of the range and values of the metals detected in copper-treated hair fibre (N=3).

Hair sample	Ca (mg/kg)	Cu (mg/kg)	Fe (mg/kg)	Mg (mg/kg)	Zn (mg/kg)
Untreated hair*	1354±107	82±8	70±15	112±2	268±2
Copper-treated hair	1231±95	359±20	63±13	104±3	246±6

*The No. 2 hair tress documented in Table 3-2 was chosen as the untreated hair sample for this study. The data of metal amount in this hair tress were already documented in Table 3-2.

(a)



(b)

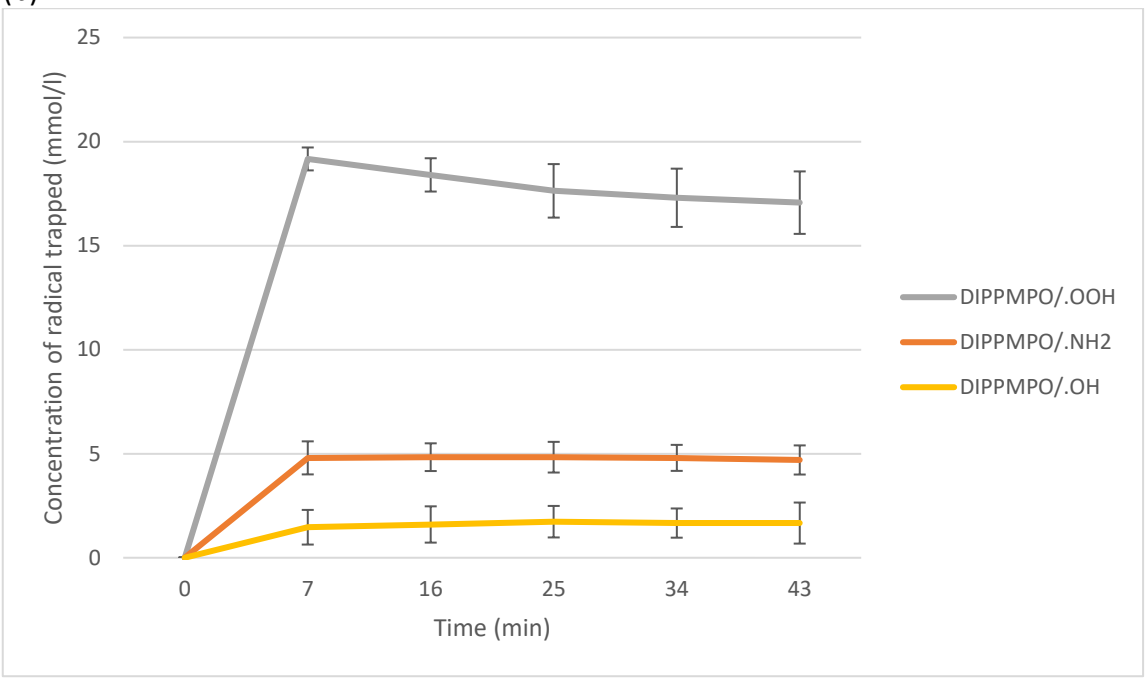
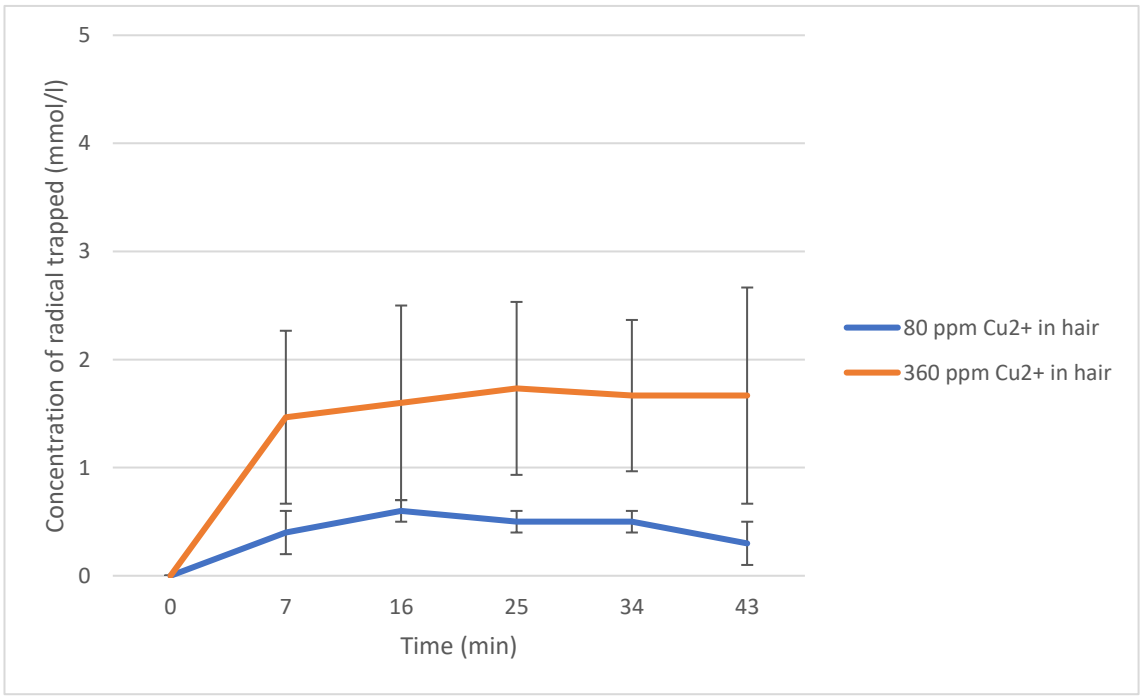
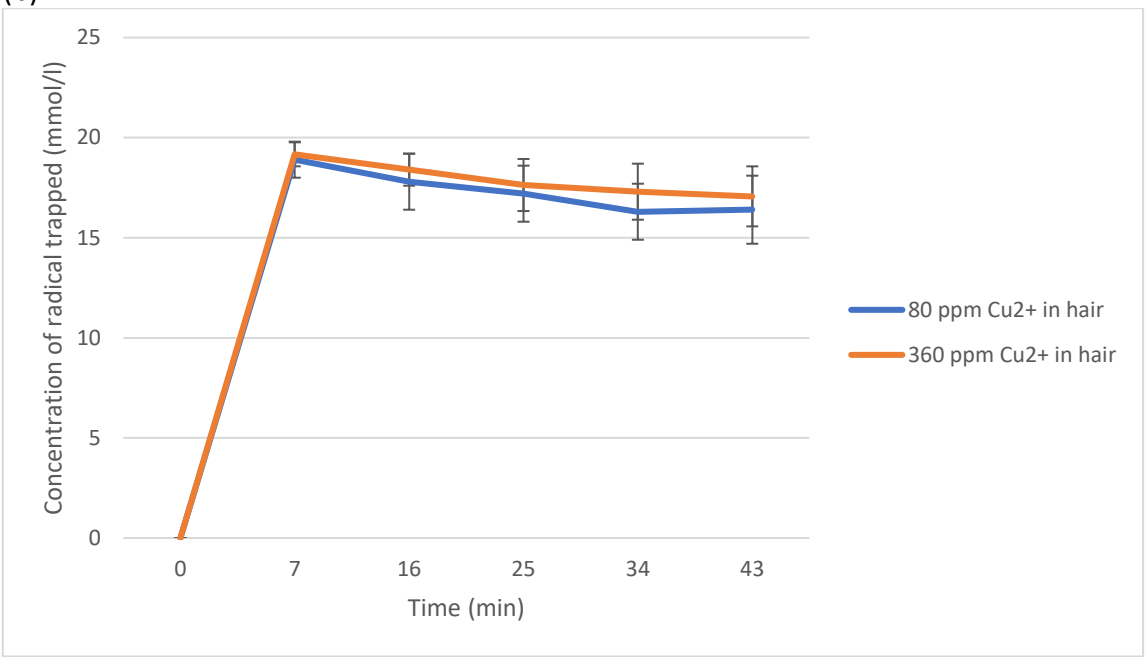


Figure 3. 20 DIPPMPO radical adducts (concentration of radical trapped in mmol/l) trapped at 26 °C in the presence of copper-treated human hair (N=3). Concentration of radical adducts trapped as a function of reaction time. (a) a level of copper in untreated hair (ca. 80 ppm, Table 3.4); (b) a level of copper in copper-treated hair (ca. 360 ppm, Table 3.4).

(a)



(b)



(c)

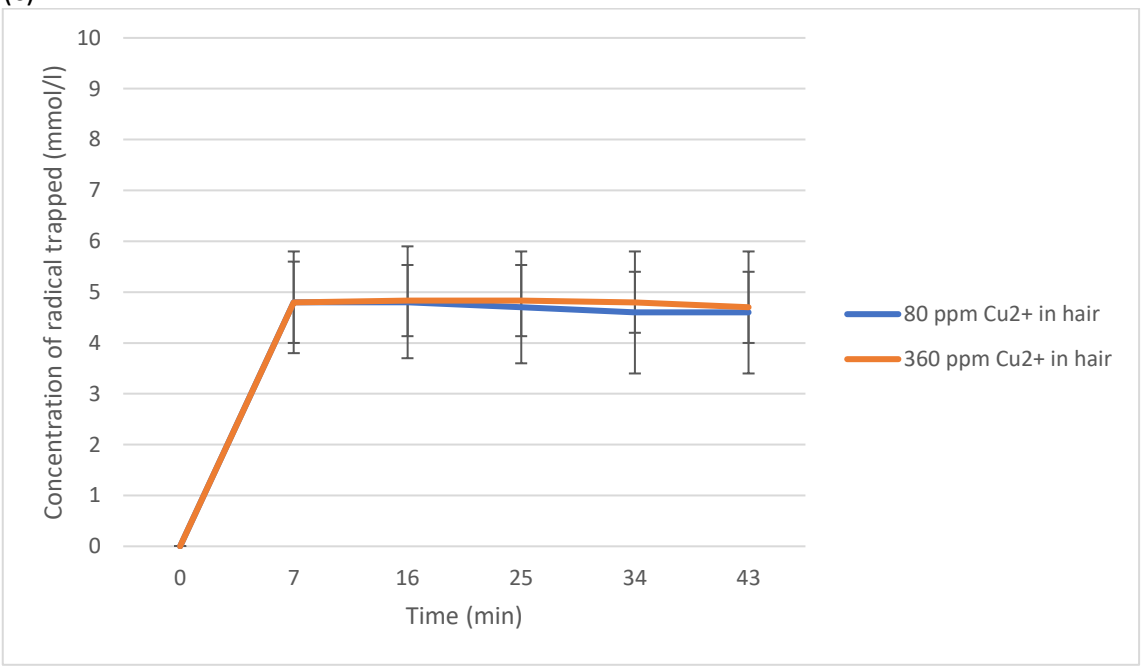


Figure 3. 21 Concentration of DIPPMPO radical adducts trapped in systems with the different copper level present in human hair under alkaline conditions as a function of reaction time (N=3). (a) DIPPMPO/·OH spin adducts formation; (b) DIPPMPO/·OOH spin adducts formation; (c) DIPPMPO/·NH_2 spin adducts formation.

3.2.8 Conclusion

The DIPPMPO/^{31}P NMR spin trap technique was explored and successfully employed to identify the formation of radical species during bleaching processes based on ammonium hydroxide/hydrogen peroxide alkaline model systems at pH=10. It is the first time to use spin trap technique to systematically characterize the role of copper ions and the role of binary copper-calcium ions in a bleaching system under high alkaline conditions (pH=10). Such types of systems are frequently used in many practical applications, e.g. cosmetics or laundry care. This study allowed for the semi-quantification of relative abundance of radical species as a function of time.

The main radical species involved in the process were the superoxide radicals ·OOH and the amino radicals $\cdot NH_2$ in the absence of transition metals such as copper ions. The superoxide-radical ·OOH adduct was the dominant species. Addition of copper ions to the system significantly induced hydroxyl radical ·OH formation. In addition to the effect of copper ions on hydroxyl radical formation, the presence of copper ions had a reducing effect on the overall concentration of superoxide radicals in the bleaching solutions, which was demonstrated for the first time. The concentration of $NH_2\cdot$ radicals did not very clearly vary with different levels of active copper ions present in this ammonium hydroxide/hydrogen peroxide based bleaching system.

Addition of chelating agents induced a change of the radical species' profile. A (partly) suppression of hydroxyl radicals ·OH formation was observed. EDTA had the ability to effectively chelate Cu^{2+} and thus prevent Cu^{2+} from joining the redox-cycle reaction if Cu^{2+} was present in the bleaching system alone. EDTA is able to completely suppress hydroxyl radical formation.

Besides transition metals such as copper or iron, human hair fibres contain calcium, magnesium, etc. Calcium is the most abundant metal ion present in hair fibres. Therefore, in human hair fibres a competition between different ion types for the chelating agent could occur. As model system for such a reaction, a set of experiments containing a Cu^{2+}-Ca^{2+} mixture was performed. The chelating agent-free system produced the highest level of hydroxyl radicals due to the presence of Cu^{2+} in an active form. It was also proved in this study that Ca^{2+} did not join the Fenton-like reaction to influence the formation of hydroxyl radical. The addition of IDS chelating agent was found to significantly reduce hydroxyl radical formation even in the presence of large amount of calcium ions, whereas a high level of hydroxyl radical formation was still detected, if EDTA was present in this binary Cu^{2+}-Ca^{2+} ion system. The results indicated that in the binary Cu^{2+}-Ca^{2+} ion system, IDS was selective in binding active copper ions over calcium ions and thus more efficient in preventing the hydroxyl radical formation in the presence of calcium in comparison to EDTA. And EDTA preferably binds to calcium than to copper and leave copper ions in an active form if both ions are present in the system. This finding may reflect differences in stability constants of EDTA and IDS.

As described in the second part of this study, the introduction of the mixture of succinic acid, lysine and arginine during the bleaching treatment has allowed this system to deliver this lightening without the previously observed negatives of hair damage such as tensile strength loss, high amount of cysteic acid present in hair etc. The solution system can be used as a good model to predict the mechanism how these substances modulate the bleaching reaction and profile radical formation. First, the studies were employed using the bleaching system in the presence of copper ions alone. Sodium succinate alone, being a dicarboxylic acid, had only a very weak chelating effect on copper ions and had

no effect on reducing formation of hydroxyl radicals in the system. This result was supported by the fact that copper could form complex with succinic acid and this complex could decompose hydrogen peroxide resulting in production of reactive oxygen species [86, 87]. The combination of basic amino acids and sodium succinate had the best chelating effect on copper ions in comparison to the single substances or the combination of both basic amino acids only. Second, the binary Cu^{2+}-Ca^{2+} ion system containing the mixture of lysine, arginine and sodium succinate was also studied. The presence of the mixture showed a further reducing effect on the concentration of hydroxyl radical's formation in the copper-calcium binary system compared to the system with EDTA alone. The results provided evidence to support the hypothesis discussed before in the second part of the study. The substances like succinic acid, lysine and arginine act as chelating agents via the carboxylic group -COO- and amino groups NH_2 to deactivate transition metals like copper ions existing in hair fibre. In this way, the high active free radicals induced by transition metals such as copper or iron during bleaching process was reduced. This result based on model system was consistent with the results reported in chapter 3.1 that the addition of succinic acid, lysine and arginine to bleaching formula reduced hair damage. However, besides the propose that carboxylic groups in succinic acid assist with chelating copper ions in model system, there could be another speculation about the role of succinic acid in hair bleaching. As mentioned before, in order to achieve more lightening effect, salts of persulfate are usually added as "booster" in hair bleaching products. In chapter 3.1, the bleaching products with the addition of salts of persulfate were used to assess hair damage. A. Ocampo [88] reported that the organic compound like succinic acid deactivated salt of persulfate under high alkaline condition. Thus, it could be speculated that the addition

of succinic acid may deactivate persulfate to some extent, and thus lead to less hair damage.

All the obtained results in the third part of this study indicated that each bleaching solution has its characteristic performance and damage profile; whereas the reactivity can be controlled by the addition of copper ions and the usage of chelating agents. An ideal chelating agent for bleaching system would be one that has a very strong copper-chelating ability and a relatively weak calcium or magnesium-chelating ability.

Additionally, the DIPPMPO/^{31}P NMR spin trap technique had allowed to further study free radical chemistry of bleaching systems in the presence of human hair, which greatly improved the understanding of this commercially important process. Copper ions present in the hair were shown to trigger free radical chemistry and the concentration of hydroxyl radical formation increased as the amount of copper ions in hair increased. This current study provided evidence for some likely mechanisms involved in alkaline peroxide bleaching systems, especially in pulp bleaching, textile bleaching and laundry detergents.

Chapter 4 Experimental part

4.1 Materials

The spin trapping reagent DIPPMPO was purchased from Enzo Life Sciences, Inc. (Farmingdale, NY, USA) and stored at -25 °C in a deep freeze refrigerator. Trimethyl phosphate and deuterium oxide were from Sigma-Aldrich Chemicals, Inc. (St. Louis, MO, USA). 25 % aqueous ammonia was purchased from Merck Chemicals GmbH (Darmstadt, Germany). Ammonium chloride was purchased from BASF SE (Ludwigshafen, Germany). 50 % aqueous hydrogen peroxide and copper (II) sulphate pentahydrate were purchased from Sigma-Aldrich Chemicals, Inc. (St. Louis, MO, USA). Calcium nitrate tetrahydrate was purchased from VWR International BVBA (Leuven, Belgium). Ethylenediamine tetra-acetic acid, tetra-sodium salt (EDTA) was from BASF SE (Ludwigshafen, Germany). Sodium iminodisuccinate (IDS) was from LANXESS AG (Köln, Germany) under the trade name Baypure® CX-100. Sodium succinate, lysine and arginine were purchased from Sigma-Aldrich Chemicals, Inc. (St. Louis, MO, USA).

Ammonia buffer solution (pH=10) was prepared following the instruction: 5.4 g of ammonium chloride was dissolved in 20 ml of distilled water, and 35 ml of 10 M ammonia hydroxide were added and diluted with distilled water to 100 ml.

All chemicals purchased from suppliers are in appropriate analysis grades and were used as received without further purification.

All bleaching solutions were made up with demineralised water.

Hair samples: European Natural Hair dark blond 7/0 tresses were purchased from KERLING International Haarfabrik GmbH (Backnang, Germany).

All pH measurements were performed using a 766-laboratory pH meter from Knick Elektronische Messgeräte GmbH and Co.KG (Berlin, Germany), which were calibrated using buffer solutions from Fisher (pH 4.0, 7.0 and 10.0) on the day of use.

1,2-Propylenoxide and Epoxy embedding medium Fluka 45345 were from Merck KGaA (Darmstadt, Germany). Uranyl acetate was from SERVA Feinbiochemica (Heidelberg, Germany) and lead (II) citrate trihydrate was purchased by Alfa Aesar by Thermo Fisher Scientific (Karlsruhe, Germany). Succinic acid was purchased from ESIM Chemical GmbH (Linz, Austria), lysine was from Altlantic Chemicals Trading GmbH (Hamburg, Germany) and arginine was purchased from Lipotec USA, Inc. (Lewisville, TX, USA).

Commercially available hair bleaching products were used as follows: lightener (BlondMe Premium Developer, 9 %, Schwarzkopf and Henkel Beauty Care, Düsseldorf, Germany) and bleaching powder (BlondMe Premium Lift 9+, Schwarzkopf and Henkel Beauty Care, Düsseldorf, Germany).

4.1.1 Hair samples

European Natural Hair dark blond 7/0 was purchased from KERLING International Haarfabrik GmbH (Backnang, Germany).

Hair clamps: plastic taps, code 900.0320 (Dia-Stron Ltd, Andover, UK)/hair clamped with liquid epoxy resin.

4.1.2 Bleaching treatments

Hair treatments: The damaged hair was created by treating European natural hair with commercial bleaching products (BlondMe Premium 9+ bleaching powder and BlondMe 9 % H_2O_2 bleaching lotion). The amount of bleaching agent used was four times the weight of hair tress. The active ingredients of bleaching treatment are shown in Table

4.1. The hair was soaked in the bleaching mixture for 45 minutes at 32 °C. Then, all these hair samples were rinsed with tap water for 3minutes. Afterwards the hair samples were blow-dried for 60 minutes. This procedure was repeated once. The treated hair fibres were stored for at least 48 hours before the measurements.

Table 4.1 Hair samples and their chemical treatment.

Hair sample	Bleaching agents (active ingredients)	Treatment conditions
Untreated hair	-	-
Onefold lightener-treated hair	9 % hydrogen peroxide, ammonia	32 °C, 45 min
Twofold powder-bleach-treated hair	Potassium, ammonium persulfate, hydrogen peroxide	32 °C, 45 min
Twofold powder-bleach-treated hair with succinic acid, lysine and arginine	Potassium, ammonium persulfate, hydrogen peroxide, succinic acid, lysine, arginine	32 °C, 45 min

4.2 Analytical methods

4.2.1 Tensile strength measurement

The tensile measurements were performed using stress-strain-system MTT 686 with control unit UV 1000 (Dia-Stron Ltd, Andover, UK) and a Fibre Dimensional Analysis Unit (Laser Scan Micrometer LSM 6000, Mitutoyo Corporation, Kanagawa, Japan). 50 single fibres were tested for each sample at a stretching rate of 10 mm/min, as initial condition. At the beginning of the test the mean cross-sectional area of each single hair was determined at a temperature of 22 °C and a relative humidity of 50 %. Data thus obtained were used for the stress calculation before and after product application.

The tensile measurements were performed in wet conditions, considered to reflect best the changes at the level of intermediate filament [73]. All the hair fibres were soaked in water for 1 hour before they were stretched with a constant speed rate of 10 mm/min within the elastic phase (0-1.5 % elongation). Afterwards the E-modulus (=Young's Modulus) before the application of products was calculated with UvWin Software 1.32.1000 (Dia-Stron Ltd, Andover, UK).

The hair fibres were soaked in water for at least 1 hour. Afterwards they were stretched with a constant speed rate of 10 mm/min within the elastic phase (0-1.5 % elongation). The E-modulus (=Young's modulus) after the application of the products was calculated.

4.2.2 LAB measurement

Three hair tresses of each sample were treated for LAB measurement. The color of the hair tresses was measured with a spectrophotometer Spectraflash 450 (Datacolor, X-Rite, Regensdorf, Switzerland). Lab values were calculated under D65 illuminant. The

lightening value (dL) was calculated as the difference in L value between the final color and the starting color on the untreated hair.

4.2.3 Scanning electron microscopy (SEM)

Three hair tresses were randomly chosen from the samples and a knot was carefully hand made in the central region of each hair tress (around 5 cm from the root end). Then the hair tresses were fixed to the sample holder stub by the length containing the knot, and the cut with a stylet above and below the knot to have a 2 cm final length. Individual hair fibre (3 tresses/sample) were coated with gold by a sputter coating machine. The hair was inserted into the FEI Nova NanoSEM 450 (ThermoFischer Scientific, Hillsboro, OR, USA; 20 kV) for viewing and photography. 24 Images were obtained on different areas of hair tresses.

4.2.4 Transmission electron microscopy (TEM)

Each hair sample was placed in 1:1 propylene oxide and Epoxy embedding medium Fluka 45345 purchased from Merck KGaA (Darmstadt, Germany) mixture overnight at 60 °C. Hair cross section approximately 70-100 nm was cut with a diamond knife. The ultrathin sections were put on a formvar covered copper grid, and electronic stained with 2 % aqueous uranyl acetate and 0.2 % lead citrate. Grids were placed on drops of 2 % aqueous uranyl acetate (in darkness for one hour) and washed on three drops of ultrapure water for about 1 minute each, followed by a short rinse under ultrapure water. The grids were then stained on drops of 0.2 % lead citrate for 15 min. Finally, grids were washed for 1 minute each with ultrapure water and dried (as above). The specimens were viewed with a transmission electron microscope at a lower magnification (Philips Tecnai 10, Thermo Fischer SCIENTIFIC Inc., Eindhoven, Netherland,

Acceleration voltage 80 kV). At least 150 images were taken for untreated hair and 150 images for each bleach-treated hair.

4.2.5 Fourier transform near infrared spectra (FT-NIR)

Analysis of hair damage using FT-NIR spectroscopy was performed and studied per Chandra M. Pande and Brian Yang [47]. The spectra were taken using a MPA FT-NIR spectrometer (Bruker Optik GmbH, Ettlingen, Germany). Spectra of interest for this study were taken from 500 cm^{-1} – 4000 cm^{-1}. Calibration was performed with untreated hair and bleach-treated hair using onefold lightener-treated hair, twofold powder-bleach-treated hair, twofold powder-bleach-treated hair with succinic acid and amino acids, respectively.

4.2.6 Detection and quantification of radical species in different bleaching solution systems

4.2.6.1 Determination of metal elements in hair

Metal content in hair samples was measured by inductively coupled plasma optical emission spectroscopy Agilent ICP-OES 720 (Santa Clara, CA, USA). Samples of 0.4 g of hair were immersed overnight with high purity concentrated nitric acid HNO_3/HCl. Three hair tresses were chosen, and each hair tress was analysed in triplicate (N=3).

To dose the hair with a various level of copper, untreated hair tresses were soaked in a standard aqueous solution of copper(II) sulphate (200 ppm) for 1 min and dried in the air. The procedure was repeated three times to produce hair samples with ~ 360 ppm copper ions.

4.2.6.2 ^{31}P Nuclear magnetic resonance (NMR) spectra

^{31}P NMR spectra were measured with an Agilent 400 MHz spectrometer (Santa Clara, CA, USA) and recorded via the software VnmJ4.2 NMR. The spectra were recorded using an inverse gated decoupling sequence, acquiring 64 scans at a pulse relaxation delay D1 of 5 s and pulse angle of 45°. Chemical shifts were relative to the shift of spin trap reagent DIPPMPO (δ = 22.2 ppm). Areas under the peaks at a chemical shift specific for detected radical adducts were recorded. Quantification was carried out against an external standard trimethyl phosphate (100 mmol/l) using the tools provided by the spectrometer software ("qEstimate"). The stated data were average of three replicate experiments (N=3). The NMR parameters were chosen in such a way, that the obtained spectra showed a decent signal-to-noise-ratio after a measurement time of 7 minutes with a delay of 120 s. The determined concentrations for each trapped species were the average values during each measurement, which were recorded after 7 min, 16 min, 25 min, 34 min, 43 min, respectively. These conditions do not guarantee a complete relaxation of all detected spins, so that some of the determined values might underrepresent the actual concentration of the respective species. However, since all measurements were carried out using the same parameters, it is possible to compare them to each other, and a semi-quantitative evaluation can be carried out.

4.2.6.3 Trapping free radicals' experiment

Experiments using bleaching model solutions were performed in NMR tubes at an ambient temperature of 26 °C in the presence of spin trap reagent DIPPMPO at a concentration of 100 mmol/l. Ammonia buffer solution (pH=10) was used in all

experiments. The different reaction systems were designed and prepared to study different bleaching solutions.

4.2.6.4 Trapping radical formation in solution system in the absence of metal ions

DIPPMPO (100 mmol/l) was thoroughly mixed with hydrogen peroxide (300 mmol/l) in an ammonia buffer solution (pH=10, 120 µl)). Prior to measurement, the reaction mixture was filled up to 0.6 ml with deuterium oxide (D_2O).

4.2.6.5 Trapping radical formation in solution systems in the presence of copper ions or binary copper-calcium ions

DIPPMPO (100 mmol/l) was mixed with copper ions (0.3 mmol/l) or binary copper-calcium ions (0.3 mmol/l, 8.0 mmol/l respectively) in an ammonia buffer solution (pH=10, 120µl). Hydrogen peroxide (300 mmol/l) was added to the solution and mixed thoroughly. Chelating agents were added to the bleaching solution at 8.0 mmol/l concentration level. Prior to measurement, the reaction mixture was filled up to 0.6 ml with D_2O.

Bleaching systems tested in this set of experiments were: 1) ammonium hydroxide/ammonium chloride-hydrogen peroxide; 2) ammonium hydroxide/ammonium chloride-hydrogen peroxide in the presence of Cu^{2+}; 3) ammonium hydroxide/ammonium chloride-hydrogen peroxide in the presence of Cu^{2+} and ethylenediaminetetraacetic acid (EDTA); 4) ammonium hydroxide/ammonium chloride-hydrogen peroxide in the presence of binary Cu^{2+} - Ca^{2+} ions 5) ammonium hydroxide/ammonium chloride-hydrogen peroxide in the presence of binary Cu^{2+} - Ca^{2+} ions and EDTA; 6) ammonium hydroxide/ammonium chloride-hydrogen peroxide in the presence of binary Cu^{2+} - Ca^{2+} ions and EDTA and a mixture of amino acids and

dicarboxylic acid; 7) ammonium hydroxide/ammonium chloride-hydrogen peroxide in the presence of binary Cu^{2+} - Ca^{2+} ions and tetrasodium iminodisuccinate (IDS).

4.2.6.6 Trapping radical formation in the presence of human hair

DIPPMPO (100 mmol/l) was mixed with 1.3 mg copper-treated human hair (containing copper ca. 80 ppm, 360 ppm respectively) in an ammonia buffer solution (pH=10, 120 µL). Hydrogen peroxide (300 mmol/l) was added to the solution and mixed thoroughly for 30 min. Prior to measurement, the reaction mixture was filled up to 0.6 ml with D_2O.

Chapter 5 Summary

The commercially available ammonia-based alkaline bleaching system is widely used in human hair oxidative colorants or bleaching products. From the cosmetically application-technical viewpoint, hair bleaching is usually considered as the cosmetic procedure using salts of persulfate as “booster”, besides alkaline hydrogen peroxide. However, from the scientific viewpoint, hair bleaching can be generally known as the process only using alkaline hydrogen peroxide. However, over time the consumers can experience undesired hair damage such as poor hair feels and look, reduced hair strength, increased incidents of split ends and breakage.

In this work, first of all, a multidisciplinary approach was employed to characterize and assess hair damage resulted from bleaching treatment. Conventional human hair bleaching treatment resulted in changes of morphological structure outside and inside hair fibre. According to the observations on hair surface using SEM, lift and even loss of the cuticle cells, the large cracks and holes in cuticle cell surface were observed in the bleach- modified hair. TEM images showed that variable sized bright spots (hole-like-structures) were predominant forms of damage observed in the inner cuticle and cortex of the bleach modified hair. Melanin granules were even completely replaced by holes, if a stronger bleaching agent applied. A decrease in Young’s Modulus of hair fibre in wet condition was confirmed using tensile strength measurement. At the molecular level, an increase in the amount of cysteic acid residues resulted from oxidation of disulfide bonds present in hair were found after bleaching treatment. Collectively these results showed that the conventional bleaching treatments have detrimental effects on the mechanical properties and the integrity of human hair fibre. The combination of

different scientific techniques and experiments performed under different conditions led to a very comprehensive indication and systematically analyze the effect of bleaching on hair.

Recently, a hair bleaching product formulated with succinic acid in combination with lysine and arginine was applied for patent [70], which demonstrated to be able to better protect hair fibre during bleaching treatment in comparison to the conventional bleaching product. However, the mechanism of this protective effect was still not fully understood.

As a next step, this protective effect was proved in the second part of this study. The results showed that the addition of succinic acid, lysine and arginine during bleaching treatment is able to prevent the loss of mechanical strength of the hair and to reduce the amount of cysteic acid. The observation of the hair surface with SEM demonstrated that the typical morphological changes like irregular overlay, lift and even loss of the cuticle cells, the large cracks and holes in cuticle cell surface resulted from bleaching treatments could be inhibited by the addition of the above-mentioned mixture to the bleaching powder. As a result, the hair samples showed a much smoother surface. Taken together, the results obtained from the multidiscipline approach provided evidence that the addition of the combination of succinic acid, lysine and arginine during bleaching treatment offers fibre protective properties compared to the conventional bleaching mixture alone. The lightening performance was, however, not impaired. Furthermore, TEM images of the inner hair fibre revealed that there was still significant remaining of melanin granules observed. In other words, melanin granules were not completely degraded.

Considering the mechanism of this protective effect, there was a hypothesis proposed previously by T. Förster [69] and T. C. Schlenkermann [71] that the organic di-acids like maleic acid, succinic acid could be absorbed by the hair cortex and rebuild salt bridges and/or bridges with hydrogen bonds interaction inside hair fibre, which resulted in enforcement of the hair bonds, thus helping to strengthen hair fibre. T. C. Schlenkermann [71] employed multidiscipline spectroscopic methods to attempt to provide evidence for the formation of new ionic bonds between di-carboxylic acids and keratin chains in hair. However, no any interaction of di-carboxylic acids in hair was detected to support this hypothesis. In order to further verify this mechanism in this work, the eluates of hair tresses treated with the bleaching formula containing succinic acid were analysed using ion chromatography. Additionally, ^{13}C NMR was employed in this study to directly track the presence of ^{13}C-enriched succinic acid in or on hair. However, no enrichment of succinic acid was detected in or on hair tresses. Therefore, there was no direct evidence to support the hypothesis of binding molecular. Moreover, the effect of another key ingredients lysine and arginine has not been reported in previous studies and should not be ignored.

In consideration of all experimental findings above ,it can be concluded that the addition of the mixture of active ingredients containing succinic acid, lysine and arginine led to a physically stabilized hair fibre. Secondly, the oxidation of disulfide cross-linkers was reduced but not completely inhibited. Thirdly, there was no enrichment of di-carboxylic acid like succinic acid in or on hair fibre after bleaching treatment. However, there was still significant remaining of melanin granules observed after bleaching treatment with the combination of the mixture. These findings indicated that the addition of the mixture of succinic acid, lysine and arginine could modify the bleaching reaction. This

modification of the bleaching reaction could enable the product to deliver excellent lightening with less hair damage compared to the conventional bleaching product. This was also the main mechanism proposed in this work.

In order to understand how these substances like succinic acid, lysine and arginine modify the bleaching reaction, a better understanding of the reaction mechanism of bleaching is needed. Alkaline hydrogen peroxide is the main oxidant used in human hair bleaching products. Metal ions in human hair fibres, such as copper ions, can catalytically decompose alkaline hydrogen peroxide via the Fenton chemistry during bleaching, which results in the formation of reactive oxygen species. These copper ions-induced radical species can further cause protein degradation which leads to hair damage. Therefore, the well-known Fenton-like reaction was chosen in this study to build a model. ^{31}P NMR-spectroscopy-based spin trap technique was explored and successfully employed to identify the formation of radical species during bleaching processes based on ammonium hydroxide/hydrogen peroxide alkaline model systems at pH=10. Here, the bleaching system with the addition of salts of persulfate was not able to be investigated and discussed in this work, because of the intense reaction which was difficult to control and quantify using ^{31}P NMR-spectroscopy. It is the first time to use spin trap technique to systematically characterize the role of copper ions and the role of binary copper-calcium ions in a bleaching system under high alkaline condition. This study also achieved to semi-quantify radical species. The results demonstrated that the main radical species involved in bleaching systems, were hydroperoxyl/superoxide radicals $HO_2\cdot$ / $O_2^{\cdot-}$, amino radicals $\cdot NH_2$ in the absence of transition metals such as copper ions. The detected amino radicals $\cdot NH_2$ provided evidence based on radical chemistry that the identity of the alkalizing agent is important for mechanistic considerations. The

presence of copper ions significantly induced hydroxyl radical and amino radical formation, whereas the presence of copper ions had a reducing effect on the overall concentration of superoxide radicals. These findings were demonstrated for the first time. Moreover, hydroxyl radical formation increased with an increase in concentration of copper ions which suggests that higher amount of copper ions in bleaching may lead to higher amounts of hydroxyl radical formation and hence more oxidative hair damage. Addition of chelating agents induced a change of the radical species' profile. EDTA was effective in chelating copper ions and able to completely suppress the hydroxyl radical formation., if the copper ions were present alone in bleaching system.

Besides copper ions, human hair fibres also contain calcium, magnesium ions, etc. Calcium is the most abundant metal ion present in hair fibres. Therefore, a competition between different ion types for the chelating agent could occur in hair. As model system for such a reaction, a set of experiments containing a binary copper-calcium mixture was performed. The chelating agent-free system produced the highest level of hydroxyl radicals due to the presence of copper ions in an active form. Additionally, the impact of calcium ions was investigated, and the result revealed that calcium ions did not induce the formation of hydroxyl radical. The addition of IDS significantly reduced hydroxyl radical formation even in the presence of large amount of calcium ions, whereas a high level of hydroxyl radical formation was still detected, if EDTA was present in this binary copper-calcium ion system. The results indicated that in the binary copper-calcium ion system, IDS was selective in binding active copper ions over calcium ions and thus more efficient in preventing the hydroxyl radical formation in comparison to EDTA. Moreover, thanks to its biodegradable property, IDS has a better sustainable perspective than EDTA.

Therefore, IDS can be considered as a better alternative chelating agent for human hair bleaching product in the future.

As a next step, the role of these substance (succinic acid, lysine and arginine) in decomposition of alkaline hydrogen peroxide was studied. Sodium succinate had only a very weak chelating effect on copper ions and had no effect on the formation of hydroxyl radicals. This result was supported by the fact that copper could form complex with succinic acid that could decompose hydrogen peroxide resulting in production of reactive oxygen species [86, 87]. The combination of basic amino acids (lysine and arginine) and sodium succinate had the best chelating effect on copper ions in comparison to the single substances or the combination of both basic amino acids only. The presence of the mixture showed a further reducing effect on the concentration of hydroxyl radical's formation in the copper-calcium binary system compared to the system with EDTA alone. The results provided evidence to support the propose discussed before in the second part of the study. The substances like succinic acid, lysine and arginine may act as chelating agents via the carboxylic group -COO- and amino groups NH_2 to deactivate transition metals like copper ions existing in hair fibre. Thanks to the addition of this mixture, the highly active hydroxyl radicals which lead to more oxidative hair damage were reduced. This result based on model system was consistent with the results reported in chapter 3.1 that the addition of succinic acid, lysine and arginine to bleaching formula reduced hair damage. However, besides the propose that carboxylic groups in succinic acid assist with chelating copper ions in model system, there could be another speculation about the role of succinic acid in hair bleaching. As mentioned before, in order to achieve more lightening effect, salts of persulfate are usually added as "booster" in hair bleaching products. In chapter 3.1, the bleaching

products with the addition of salts of persulfate were used to assess hair damage. A. Ocampo [88] reported that succinic acid deactivated salt of persulfate under high alkaline condition. Thus, it could be speculated that the addition of succinic acid may deactivate persulfate to some extent, and thus lead to less hair damage.

Additionally, in order to validate the results obtained with the model systems, hair fibres treated with copper were used as source of metal ions to decompose alkaline hydrogen peroxide. The DIPPMPO/^{31}P NMR spin trap technique had allowed to further study free radical chemistry of bleaching systems in the presence of human hair, which greatly improved the understanding of this commercially important process. Copper ions present in the hair were shown to trigger free radical chemistry and the concentration of hydroxyl radical formation increased as the amount of copper ions in hair increased.

In conclusion, the results in this study indicated that each bleaching solution has its characteristic performance and damage profile; whereas the reactivity can be controlled by the addition of copper ions and the usage of chelating agents. An ideal chelating agent for bleaching system would be one that has a very strong copper-chelating ability and a relatively weak calcium or magnesium-chelating ability.

Chapter 6 Zusammenfassung

Das kommerziell verfügbare Ammoniak-basierte alkalische System zum Bleichen findet bei der menschlichen, oxidativen Haarfärbung oder bei Bleichprodukten weitverbreitet Anwendung. Aus kosmetisch anwendungstechnischen Standpunt wird Haarbleichen üblicherweise als kosmetisches Verfahren unter Anwendung von Persulfatsalzen als „Booster“ außer der Anwendung der alkalischen Wasserstoffperoxide. Jedoch haben die Konsumenten mit der Zeit unerwünschte Haarschädigungen wie schlechter werdendes Aussehen und Gefühl des Haares, reduzierte Haarstärke, erhöhte Schädigung durch Haarspleissen und Haarbruch festgestellt.

In dieser Arbeit wurde zunächst ein interdisziplinärer Ansatz verfolgt, um Haarschädigungen, welche durch die Anwendung von Bleichmitteln hervorgerufen wurden, zu charakterisieren und zu bewerten. Konventionelles Bleichen menschlichen Haares führt zu Veränderung der morphologischen Struktur außerhalb und innerhalb der Haarfasern, einer niedrigeren Rissfestigkeit unter feuchten Bedingungen und einem erhöhten Wert an Cysteinsäure, welcher durch die Oxidation von Disulfidbindungen in den Haarfasern hervorgerufen wird. Nach der Betrachtung der Haaroberfläche mittels SEM wurden geöffnete oder verloren gegangene Curticularschichten und große Risse und Löcher in der Haaroberfläche des gebleichten Haares beobachtet. Aufnahmen von gebleichtem Haar mittels TEM haben gezeigt, dass unterschiedlich große weiße Punkte (lochartige Struktur) überwiegend Formen von Haarschädigung der inneren Curticularschicht und des Cortex darstellen. Melanin Granulat wurde sogar komplett durch Löcher ersetzt, wenn ein starkes Bleichmittel angewendet wurde. Eine Reduzierung von Young’s Modulus in der Haarfaser unter feuchten Bedingungen konnte

unter Anwendung von Zugdehnungsmessungen gemessen werden. Auf molekularer Ebene wurde ein erhöhter Gehalt an Cysteinsäure, hervorgerufen durch Oxidation von Disulfidbindungen im Haar, nach dem Bleichen mittels FT-NIR-Spektroskopie nachgewiesen. All diese Untersuchungen zeigen, dass das konventionelle Bleichen schädliche Effekte auf die mechanischen Eigenschaften der Haarfasern und deren Unversehrtheit hat. Die Kombination von verschiedenen wissenschaftlichen Techniken und die unter verschiedenen Bedingungen durchgeführten Experimente führten zu einem sehr umfassenden Hinweis und systematischer Analyse der Wirkung des Bleichens auf das Haar.

Vor Kurzem wurde ein Haar-Blondierungsprodukt welches Bernsteinsäure in Kombination mit Lysin und Arginin beinhaltet, zum Patent angemeldet [70]. Dieses Produkt wirbt damit, die Haarfasern während des Bleichens besser als herkömmliche Blondierungsprodukte zu schützen. Jedoch wurde der Wirkmechanismus noch nicht vollständig aufgeklärt und verstanden. Als nächster Schritt wurde im zweiten Teil dieser Arbeit der beworbene schützende Effekt nachgewiesen. Die Ergebnisse zeigen, dass ein Blondierungsprodukt für Haare, kombiniert mit Bernsteinsäure, Lysin und Arginin in der Lage ist, den Verlust von mechanischen Eigenschaften des Haares zu verhindern und den Gehalt von Cysteinsäure, welches beim Prozess des Bleichens entsteht, zu reduzieren. Die Untersuchung der Haaroberfläche mittels SEM hat gezeigt, dass die typischen morphologischen Änderungen wie geöffnete oder verloren gegangene Curticularschichten und große Risse und Löcher in der Haaroberfläche mittels Zugabe von Bernsteinsäure, Lysin und Arginin abgemildert werden können. Die untersuchten Haarproben weisen im Ergebnis eine deutlich glattere Oberfläche auf. Die erzielten Ergebnisse belegen, dass die Zugabe von Bernsteinsäure, Lysin und Arginin zum

Blondierungsprodukt die Haarfasern effektiver schützen als konventionelle Blondierungsprodukte. Die aufhellende Wirkung des Blondierungsproduktes wurde durch die Zugabe der drei genannten Substanzen nicht negativ beeinträchtigt. Die Untersuchung der inneren Struktur der Haarfasern mittels TEM haben gezeigt, dass Melanin-Pigmente im Haar, welches mit einer Blondierungsformel inklusive der oben genannten Zusätze behandelt wurde, nicht komplett zerstört wurden. Das verbleibende Melanin könnte auf eine direkte Beeinflussung durch die hinzugefügten Substanzen auf die Bleich-Reaktion hinweisen.

Es gab jedoch noch einen weiteren möglichen Mechanismus, der von T. Förster [69]und T. C. Schlenkermann [71] vorgeschlagen wurde, dass die Dicarbonsäure wie Maleinsäure und Bernsteinsäure vom Haar Cortex absorbiert werden könnten und Salzbrücken und /oder Brücken mit Wechselwirkung mit Wasserstoffbrücken innerhalb der Haarfaser wieder aufgebaut werden könnten. Dies führte zum Aufbau der Haarbindungen, wodurch die Haarfaser gestärkt wurde. T. C. Schlenkermann [71] setzte multidisziplinäre spektroskopische Methoden ein, um die neuen ionischen Bindungen zwischen Dicarbonsäuren und Haar nachzuweisen. Es wurde jedoch keine Wechselwirkung von Dicarbonsäuren im Haar nachgewiesen, um diese Hypothese zu stützen. Um diesen Mechanismus in dieser Arbeit weiter zu verifizieren, wurden die Eluaten von Haarsträhne in dieser Arbeit, die vorher mit Bleichmittel mit der Zugabe von Bernsteinsäure wurden, mittels Ion Chromatographie analysiert. Zusätzlich wurde ^{13}C-NMR in dieser Arbeit verwendet, um die Anwesenheit von ^{13}C-angereicherter Bernsteinsäure in oder an Haaren zu verfolgen. Es wurde jedoch keine Bernsteinsäure nachgewiesen, um diese Hypothese zu stützen. Darüber hinaus wurde in keiner früheren

Studie über die Wirkung anderer Hauptbestandteile Lysin und Arginin berichtet und sollte die Wirkung nicht ignoriert werden.

Um zu verstehen wie die Substanzen (Bernsteinsäure, Lysin und Arginin) die Bleich-Reaktion verändern muss ein besseres Verständnis des Reaktionsmechanismus beim Bleichen im Allgemeinen hergestellt werden. Es ist bekannt, dass alkalisches Wasserstoffperoxid das Haupt-Oxidationsmittel ist, welches in Blondierungsprodukten genutzt wird. Metallionen in menschlichen Haarfasern, wie z.B. Kupferionen, können alkalisches Wasserstoffperoxid mittels Fenton Reaktion beim Bleichen katalytisch zersetzen. Dies führt zu einer Entstehung von reaktiven Sauerstoffspezies. Diese durch Kupferionen erzeugten Radikale können die Zersetzung von Proteinen verursachen, was zur Haarschädigung führen kann. Daher wurde die bekannte Fenton-artige Reaktion ausgewählt, um im Rahmen dieser Arbeit ein Modell zu entwickeln. Eine ^{31}P NMR-Spektroskopie basierte „Spin trap“ Technik wurde erforscht und erfolgreich angewendet, um die Bildung von radikalen Spezies während des Blondierungsprozesses basierend auf alkalischem Ammoniumhydroxid/ Wasserstoffperoxid System unter alkalischen Bedingungen (pH=10) zu identifizieren. Das Bleichsystem mit Zusatz von Persulfatsalzen konnte in dieser Arbeit wegen der intensiven Reaktion, die mit ^{31}P-NMR-Spektroskopie schwer zu kontrollieren und zu quantifizieren war, nicht untersucht und diskutiert werden. Es ist das erste Mal, dass die „Spin trap“ Technik benutzt wurde, um die Rolle von Kupferionen und die Rolle von binären Kupfer-Kalzium Ionen im alkalischen Bleich-System systematisch zu charakterisieren. Im Rahmen dieser Arbeit wurde ebenfalls eine Methode zur halb-quantitative Bewertung radikaler Spezies entwickelt. In Abwesenheit von Übergangsmetallen wie Kupferionen, die im Bleichsystem involviert sind, sind Hydroperoxyl/Superoxide Radikale $HO_2{\cdot}/O_2{\cdot}^-$, Aminoradikale $\cdot NH_2$ die radikalen

Hauptspezies. Die entdeckten Aminoradikale $\cdot NH_2$ liefern einen Beleg basierend auf der Radikalchemie dafür, dass die Anwesenheit des eingesetzten Alkalisierungsmittels wichtig für mechanistische Bedingung ist. Die Zugabe von Kupferionen führt zu einer signifikanten Bildung von Hydroxylradikalen und Aminoradikalen, hingegen führt die Zugabe vom Kupferionen zu einer deutlichen Reduktion von Superoxidradikalen. Diese Entdeckungen wurden zum ersten Mal aufgezeigt. Außerdem kann festgehalten werden, dass die Konzentration von Hydroxylradikalen steigt, wenn die Konzentration von Kupferionen steigt. Dies führt zu der These, dass ein höherer Gehalt von Kupferionen im Bleichsystem zu einem höheren Gehalt von Hydroxylradikalen führt und somit zu einer größeren oxidativen Haarschädigung. Die Zugabe von Komplexbildner führt zu einem veränderten Profil von radikalen Spezies. EDTA hat sich als effektiver Komplexbildner für Kupferionen erwiesen und ist darüber hinaus dazu in der Lage, die Bildung von Hydroxylradikalen vollständig zu unterbinden unter der Voraussetzung, dass nur Kupferionen und keine weiteren metallischen Ionen im Bleichsystem präsent sind.

Neben Kupferionen enthalten menschliche Haarfasern auch beispielsweise Kalzium- und Magnesiumionen. Kalzium ist das Metallion, welches am häufigsten in Haarfasern vorkommt. Dadurch kann eine Konkurrenz zwischen verschiedenen Kationen im Haar für Komplexbildner entstehen. Als Modellsysteme für solch eine Konkurrenz wurden Experimente wie z.B. Einsatz einer binären Kupfer- und Kalziummischung durchgeführt. Ein Komplexbildner freies System bewirkt die höchste Konzentration von Hydroxylradikalen auf Grund der Präsenz von Kupferionen in einer aktiven Form. Zusätzlich wurde der Einfluss von Kalziumionen untersucht mit dem Ergebnis, dass Kalzium Ionen nicht zu einer Bildung von Hydroxylradikalen beitragen. Die Zugabe von IDS reduziert die Bildung von Hydroxylradikalen signifikant, sogar bei dem Präsenz von

großen Mengen von Kalzium Ionen. Hingegen kann bei der Zugabe von EDTA in ein binäres Kupfer- und Kalziumionen System ein hohes Level von Hydroxylradikalen festgestellt werden. Die Resultate weisen darauf hin, dass in einem binären Kupfer-Kalziumionen-System IDS die Bindung von aktiven Kupfer Ionen gegenüber Kalzium Ionen bevorzugt und deswegen in der Lage ist, effizienter als EDTA ist die Bildung von Hydroxylradikalen zu verhindern. Darüber hinaus ist IDS biologisch abbaubar und erfüllt damit den Anspruch auf Nachhaltigkeit deutlich besser als EDTA. Daher kann IDS eindeutig als der bessere Komplexbildner für oxidative Haarfärbe- und Blondierungsprodukte angesehen werden.

Als nächstes wurde die Rolle der Substanzen (Bernsteinsäure, Lysin und Arginin) bei der Zersetzung von alkalischem Wasserstoffperoxid untersucht. Natriumsuccinat hat nur einen sehr schwachen Komplexeffekt auf Kupferionen und keinen Effekt auf die Bildung von Hydroxylradikalen. Die Kombination von basischen Aminosäuren (Lysin und Arginin) und Natriumsuccinaten hatte den besten Komplexierungseffekt auf Kupferionen im Vergleich zu Einzelsubstanzen oder der Kombination von beiden Aminosäuren. Dieses Ergebnis wurde dadurch unterstützt, dass Kupfer mit Bernsteinsäure einen Komplex bilden kann, und der Komplex der Wasserstoffperoxid zersetzen kann, wodurch reaktive Sauerstoffspezies gebildet werden [86, 87]. Der Präsenz dieses Mix zeigt eine weitere Reduzierung der Konzentration von Hydroxylradikalen im binären Kupfer-Kalzium-System im Vergleich zum System mit EDTA. Die Resultate unterstützen die zuvor diskutierte Hypothese aus dem zweiten Teil der Arbeit. Die Substanzen wie Bernsteinsäure, Lysin und Arginin agieren als Komplexbildner mittels der Carboxylgruppen -COO- und Aminogruppen NH_2 für Übergangsmetalle wie Kupferionen in Haarfasern und sind in der Lage, diese zu deaktivieren. Auf Grund der Zugabe der

Mischung wurden die hochaktiven Hydroxylradikale, welche zu erhöhter oxidativer Haarschädigung führen, reduziert. Dieses Ergebnis des Modelsystems stimmte mit den in Kapitle 3.1 berichteten Ergebnissen überein. Die Zugabe von Bernsteinsäure, Lysin und Arginine zur Bleichrezeptur reduzierte die Haarschädigung. Außer der Hypothese, dass Carboxylgruppen in Bernsteinsäure als Komplexbildner von Kupferionen im Modellsystem unterstützen, könnte es jedoch zu weiteren Spekulationen über die Rolle von Bernsteinsäure bei der Haarbleiche kommen. Wie bereits erwähnt, werden in den Haarbleichmitteln üblicherweise Persulfatsalzen als "Booster" zugegeben, um einen stärkeren Aufhellungseffekt zu erzielen. In Kapitel 3.1 wurden die Bleichmittel unter Zusatz von Persulfatsalzen zur Beurteilung der Haarschädigung verwendet. A. Ocampo [88] berichtete, dass Bernsteinsäure die Persulfatsalzen unter stark alkalischen Bedingungen deaktivieren kann. Man könnte deswegen spekulieren, dass der Zusatz von Bernsteinsäure Persulfate in gewissem Maße deaktivieren und somit zu weniger Haarschäden führen könnte.

Um die erzielten Resultate aus dem Modellsystem zu validieren, wurden mit Kupferionen behandelte Haarfasern als Quelle von Metallionen genutzt, um alkalisches Wasserstoffperoxid zu zersetzen. Die DIPPMPO/^{31}P NMR Spin-Trap-Technik erlaubt uns, die Chemie von freien Radikalen in Bleichsystemen unter Zugabe von menschlichem Haar tiefergehend zu erforschen, was zu einem verbesserten Verständnis dieses kommerziell wichtigen Prozesses führt. Es wurde gezeigt, dass im Haar befindliche Kupferionen zu einer vermehrten Bildung freier Radikale führen und dass sich die Konzentration von Hydroxylradikalen erhöht, wenn sich der Gehalt von Kupferionen im Haar erhöht.

Die Resultate dieser Arbeit weisen darauf hin, dass jede Bleichlösung ihre eigene charakteristische Performance und ihr eigenes Schädigungsprofil aufweist. Die Reaktivität jeder einzelnen Bleichlösung kann durch die Zugabe von Kupferionen und den Einsatz von Komplexbildnern gesteuert werden. Ein idealer Komplexbildner für ein Bleichsystem würde einer sein, der eine sehr starke kupferbindende Eigenschaft aufweist und eine relativ schwache kalzium- oder magnesiumbindende Eigenschaft aufweist.

Chapter 7 Outlook

DIPPMPO/^{31}P NMR spin trap technique opens a room for further research in the field of free radical chemistry in the future. The followings would be interesting for future investigation:

1. The activity of deposits of copper ions in hair in decomposition of alkaline hydrogen peroxide and the formation of free radicals had been discussed in the present study. The effect of various chelating agents on the decomposition of alkaline hydrogen peroxide can be further investigated in the presence of human hair, which could enhance our understanding of this commercially important process.
2. In an oxidative hair coloring system, where aromatic dye precursors and couplers are also present, copper ions may influence color development. This potential involvement of copper ions deposited in hair in the oxidative coloring can be interesting to explore in the future research.
3. There is a range of factors which can cause and enhance hair damage. Environmental factors that impact human hair include exposure to UV and visible light, besides the presence of copper ions in human hair. Exposure of human hair to UV is well known to enhance oxidation degradation, mainly through the generation of reactive oxygen species [89]. The spin trap technique explored in the present study may enable to further investigate the free radical chemistry under UV exposure.

Chapter 8 Appendices

Appendix A: Results of hair damage evaluation

Table A-1 Cysteic acid amounts (mol %) on hair sample

Untreated hair	Onefold lightener-treated hair	Twofold powder-bleached hair	Twofold powder-bleached hair with succinic acid and both amino acids
0.3	1.6	6.4	6.9
0.3	1.9	6.8	6.6
0.9	1.7	7.1	5.8
0.4	2.1	7.3	5.8
0.5	1.6	6.6	6.0
0.1	1.9	7.0	5.9
0.5	2.1	6.8	5.8
0.3	1.2	6.5	6.1
0.3	1.8	6.3	6.6
0.1	1.9	7.0	5.5
0.6	1.9	7.3	5.9
0.2	1.6	6.5	6.7
0.5	1.9	6.6	5.9
0.4	2.1	7.2	6.3
0.2	1.5	6.7	6.6
0.1	1.9	7.0	6.1
0.2	2.3	6.9	6.8
0.1	1.7	6.9	5.9

Table A-2 Lightening values of bleach-modified hair

Onefold lightener-treated hair	Twofold powder-bleached hair	Twofold powder-bleached hair with succinic acid and both amino acids
15.11	42.04	42.20
14.03	41.88	42.01
15.05	42.94	44.18
14.47	40.93	41.54
15.17	42.46	42.32
12.45	41.61	40.72
17.37	41.42	39.41
13.94	40.23	38.83
14.02	42.04	40.70
14.43	41.05	39.93
14.62	40.02	41.25
13.83	41.49	42.17

Table A-3 Young's Modulus of hair fibre

Onefold lightener-treated hair		Twofold powder-bleached hair		Twofold powder-bleached hair with succinic acid and both amino acids	
EMOD(V) N/m^2 (Pa)	EMOD(N) N/m^2 (Pa)	EMOD(V) N/m^2 (Pa)	EMOD(N) N/m^2 (Pa)	EMOD(V) N/m^2 (Pa)	EMOD(N) N/m^2 (Pa)
2.23E+09	1.70E+09	2.51E+09	6.08E+08	2.42E+09	1.31E+09
2.18E+09	1.96E+09	2.42E+09	1.15E+09	2.81E+09	1.03E+09
2.51E+09	2.33E+09	2.42E+09	1.01E+09	2.52E+09	1.27E+09
2.36E+09	2.13E+09	2.45E+09	8.22E+08	2.39E+09	2.09E+09
2.51E+09	2.08E+09	2.43E+09	3.62E+08	2.65E+09	1.73E+09
2.49E+09	2.24E+09	2.37E+09	1.45E+09	2.66E+09	5.25E+08
2.45E+09	2.30E+09	2.14E+09	8.81E+08	2.43E+09	1.88E+09
2.36E+09	2.17E+09	2.48E+09	1.46E+09	2.46E+09	2.12E+09
2.45E+09	2.15E+09	2.26E+09	1.23E+09	2.57E+09	5.38E+09
2.62E+09	2.32E+09	2.36E+09	1.25E+09	2.80E+09	1.29E+09
2.24E+09	1.89E+09	2.50E+09	1.43E+09	2.43E+09	1.62E+09
2.22E+09	1.99E+09	2.16E+09	4.00E+08	2.56E+09	9.31E+08
2.18E+09	1.93E+09	2.08E+09	2.73E+08	2.35E+09	1.77E+09
2.32E+09	2.03E+09	2.35E+09	1.22E+09	2.49E+09	1.27E+09
2.48E+09	2.13E+09	2.22E+09	1.18E+09	2.61E+09	1.71E+09
2.27E+09	1.97E+09	2.51E+09	1.27E+09	2.61E+09	1.36E+09
3.52E+09	2.04E+09	2.39E+09	1.31E+09	2.52E+09	2.38E+09
1.86E+09	2.19E+09	2.15E+09	9.89E+08	2.65E+09	1.78E+09
2.29E+09	1.98E+09	2.14E+09	1.13E+09	2.59E+09	1.01E+09
2.22E+09	2.06E+09	2.33E+09	3.99E+08	2.36E+09	1.14E+09
2.67E+09	2.17E+09	2.40E+09	1.27E+09	2.39E+09	1.56E+09
		2.33E+09	1.24E+09	2.48E+09	8.97E+08

2.49E+09	2.26E+09	5.31E+09	1.41E+09	2.51E+09	1.84E+09
2.41E+09	2.21E+09	2.44E+09	1.40E+09	2.53E+09	8.19E+08
2.48E+09	2.16E+09	1.86E+09	1.06E+09	2.17E+09	2.09E+09
2.63E+09	2.34E+09	2.29E+09	1.26E+09	2.47E+09	8.14E+08
2.31E+09	2.10E+09	2.24E+09	1.20E+09	2.42E+09	1.36E+09
2.51E+09	2.26E+09	2.48E+09	1.54E+09	2.50E+09	1.93E+09
2.27E+09	1.90E+09	2.45E+09	1.28E+09	2.16E+09	1.17E+09
2.49E+09	1.95E+09	2.44E+09	1.35E+09	2.35E+09	3.27E+08
2.59E+09	2.12E+09	2.35E+09	3.46E+08	2.79E+09	1.74E+09
2.57E+09	2.13E+09	2.30E+09	1.42E+09	2.38E+09	1.88E+09
2.43E+09	2.16E+09	2.38E+09	1.51E+09	2.57E+09	1.03E+09
2.40E+09	2.08E+09	2.20E+09	1.00E+09	2.57E+09	2.42E+09
2.81E+09	2.46E+09	2.48E+09	9.08E+08	2.56E+09	1.31E+09
2.61E+09	2.34E+09	2.41E+09	1.27E+09	3.03E+09	2.16E+09
2.61E+09	2.26E+09	2.58E+09	1.47E+09	2.11E+09	6.14E+08
2.47E+09	2.18E+09	2.24E+09	1.25E+09	2.74E+09	4.53E+08
2.49E+09	2.01E+09	2.48E+09	1.32E+09	2.64E+09	1.50E+09
2.55E+09	2.28E+09	2.25E+09	1.41E+09	2.43E+09	1.93E+09
2.62E+09	2.28E+09	2.39E+09	1.35E+09	2.07E+09	2.19E+09
2.41E+09	2.09E+09	2.41E+09	1.46E+09	2.52E+09	6.56E+08
2.44E+09	2.13E+09	3.55E+09	7.45E+08	2.22E+09	4.65E+08
2.59E+09	2.30E+09	2.32E+09	2.40E+08	2.21E+09	4.25E+08
2.61E+09	2.28E+09	2.42E+09	1.54E+09	2.55E+09	1.40E+09
2.09E+09	1.99E+09	2.07E+09	1.24E+08	2.47E+09	1.33E+09
2.43E+09	2.12E+09	2.37E+09	1.32E+09	2.04E+09	8.18E+08
2.44E+09	2.23E+09	1.78E+09	8.58E+08		
		2.49E+09	1.23E+09		

Appendix B: Results of microscopic observations

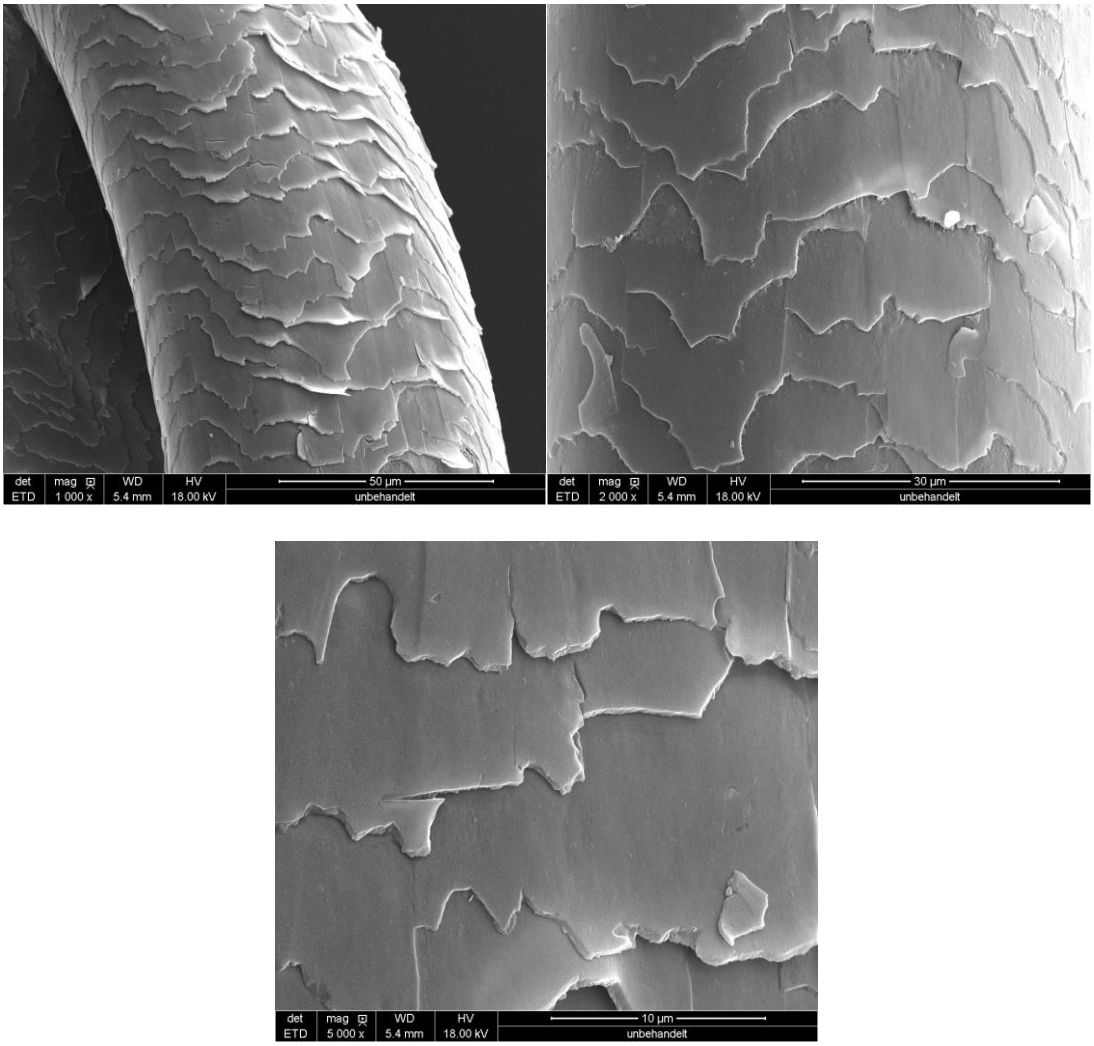


Figure B-1 SEM observations of hair samples (European hair light blond 10/0).

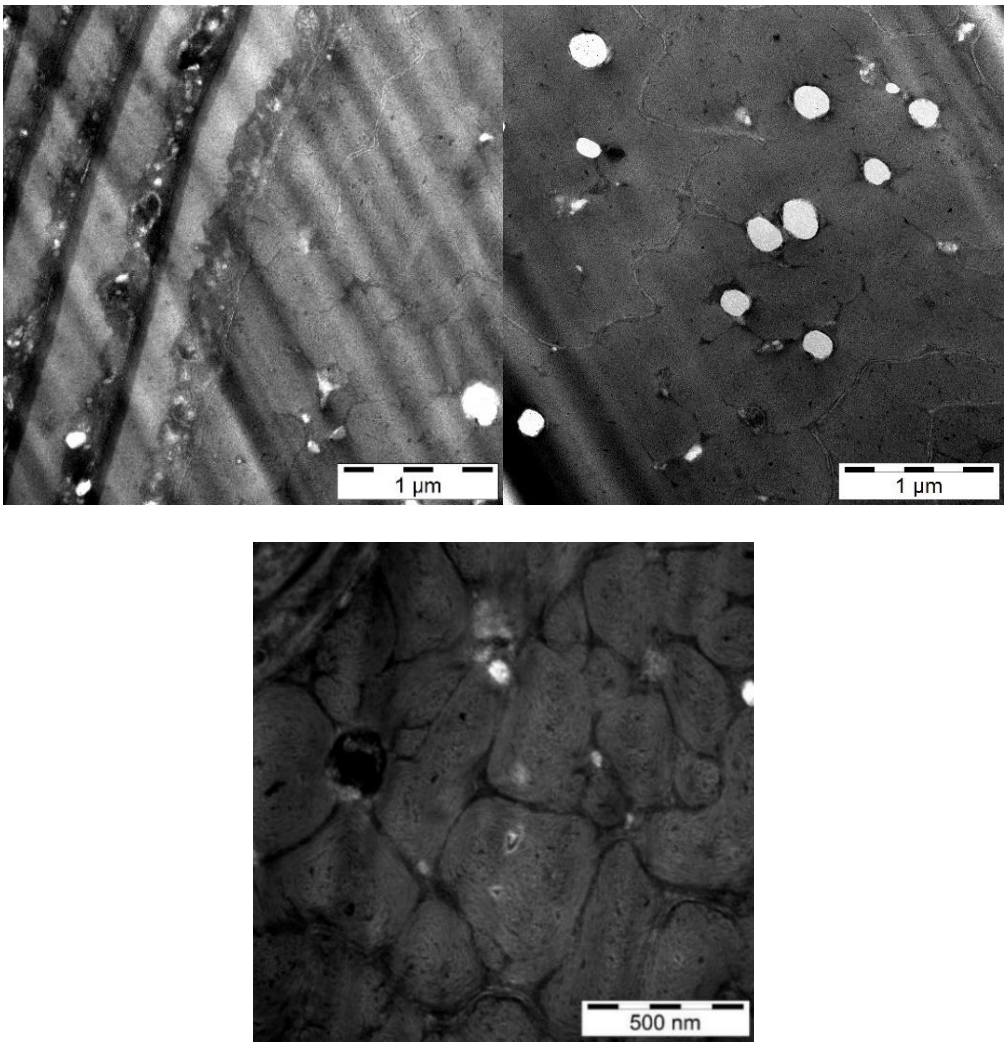


Figure B-2 TEM observations of hair samples onefold treated with powder-bleach, which were stained by uranyl acetate and lead citrate.

Appendix C: Results of eluates of hair tresses analysis using ion chromatography

Table C-1 Amount of succinic acid in hair eluates.

Sample	Amount of succinic acid in hair extract
Untreated hair	50 mg/kg (50 ppm)
Twofold bleached hair	30 mg/kg (30 ppm)
Twofold bleached hair with succinic acid in formula	50 mg/kg (50 ppm)

Appendix D: Results of ^{13}C-NMR on hair

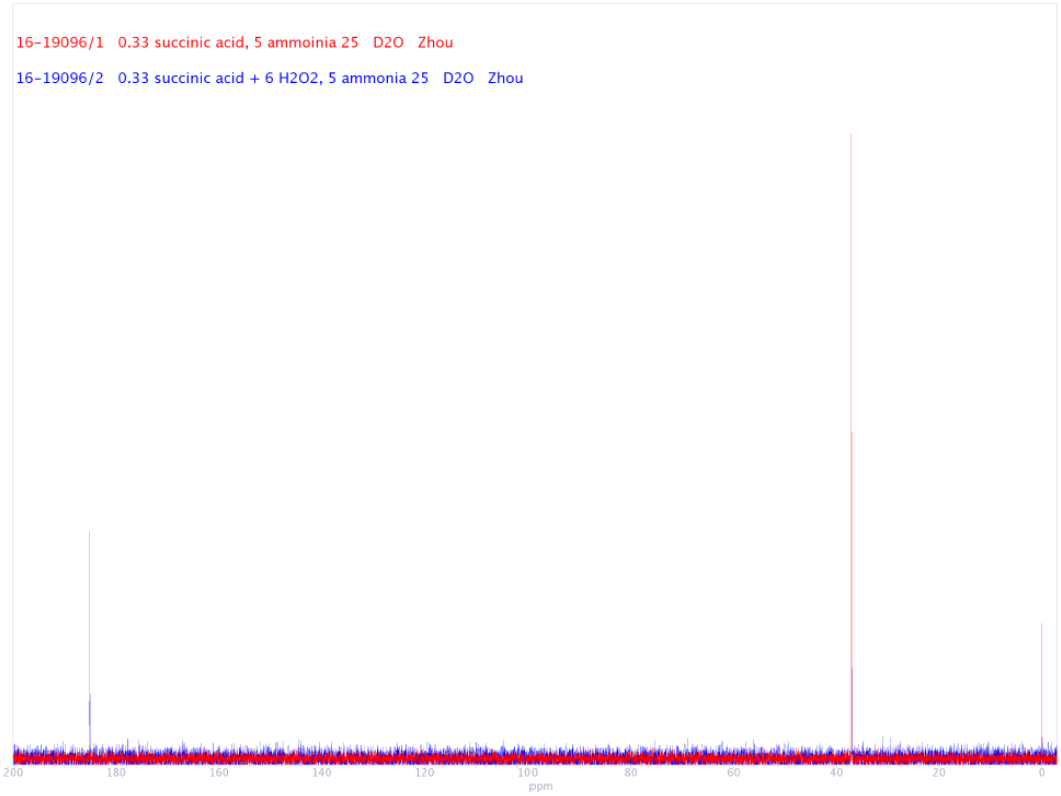


Figure D-1 NMR spectrum of hair tresses treated with bleaching formula in combination with C-enriched succinic acid.

Appendix E: Results of DIPPMPO/^{31}P NMR measurements

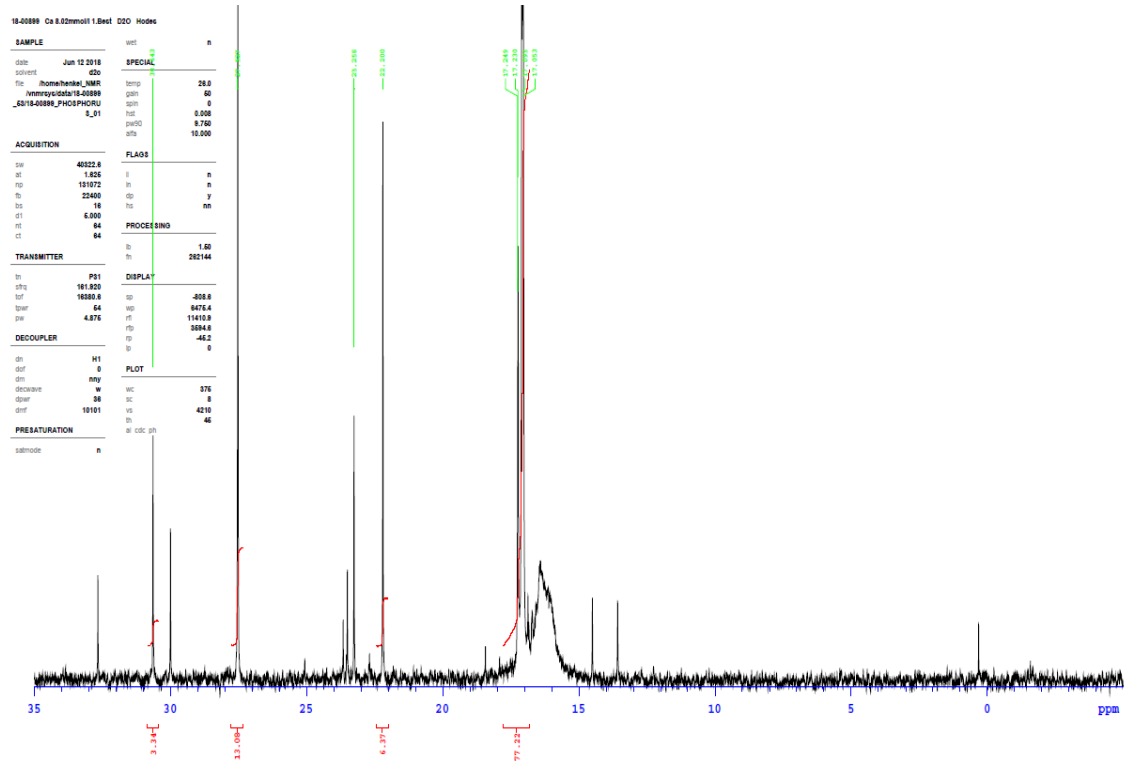


Figure E-1 Representative ^{31}P NMR spectrum of the radical adducts formed in a $H_2O_2/NH_4OH+NH_4Cl/Ca^{2+}$ system at pH=10 with DIPPMPO spin trap.

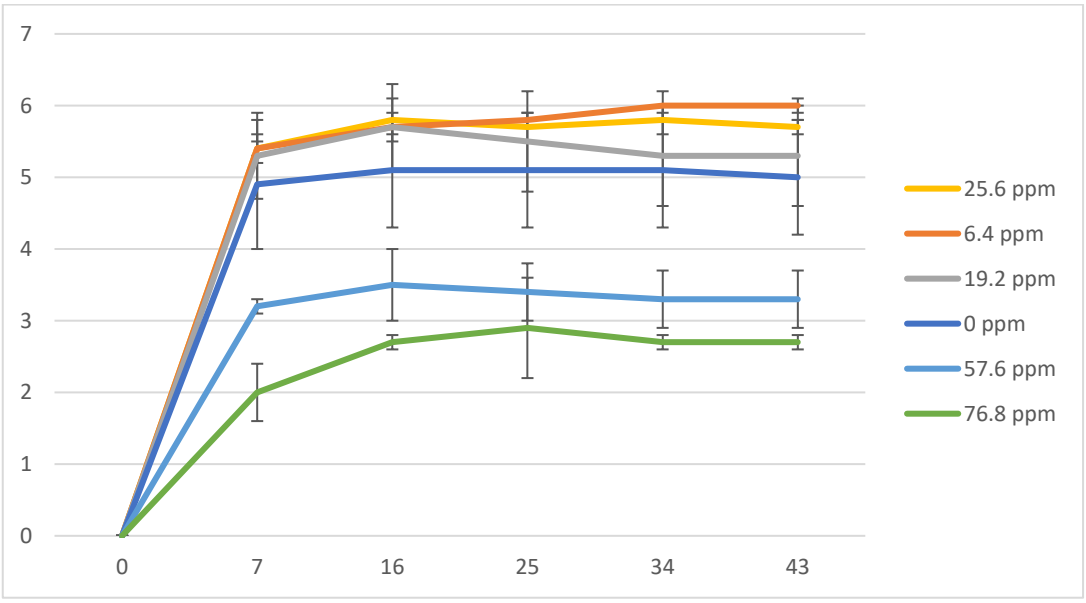


Figure E-2 Concentration of DIPPMPO/·NH_2 spin adducts trapped as a function of added copper in aqueous model system. DIPPMPO (100 mmol/l) was added to trap the radicals at pH 10. ^{31}P NMR was used to collect the quantitative data after the mixture was thoroughly stirred continuously under air for 10 min (N=3).

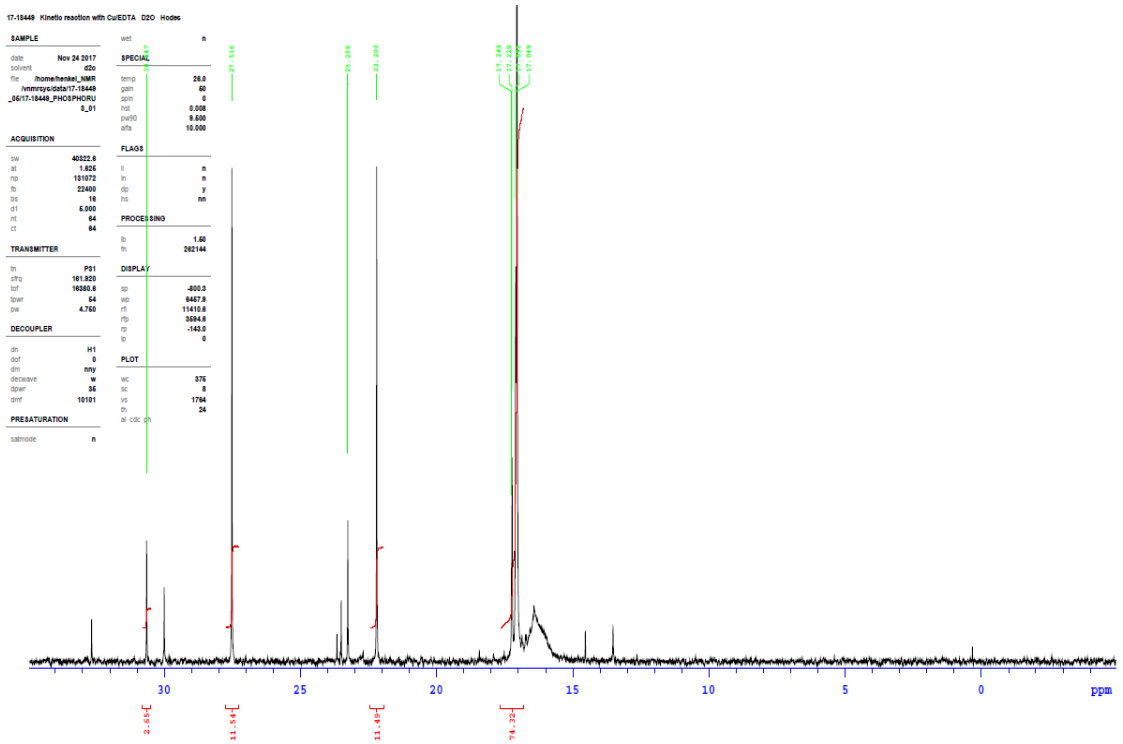


Figure E-3 Representative ^{31}P NMR spectrum of the radical adducts formed in a $H_2O_2/NH_4OH+NH_4Cl/Cu^{2+}$/EDTA system at pH=10 with DIPPMPO spin trap.

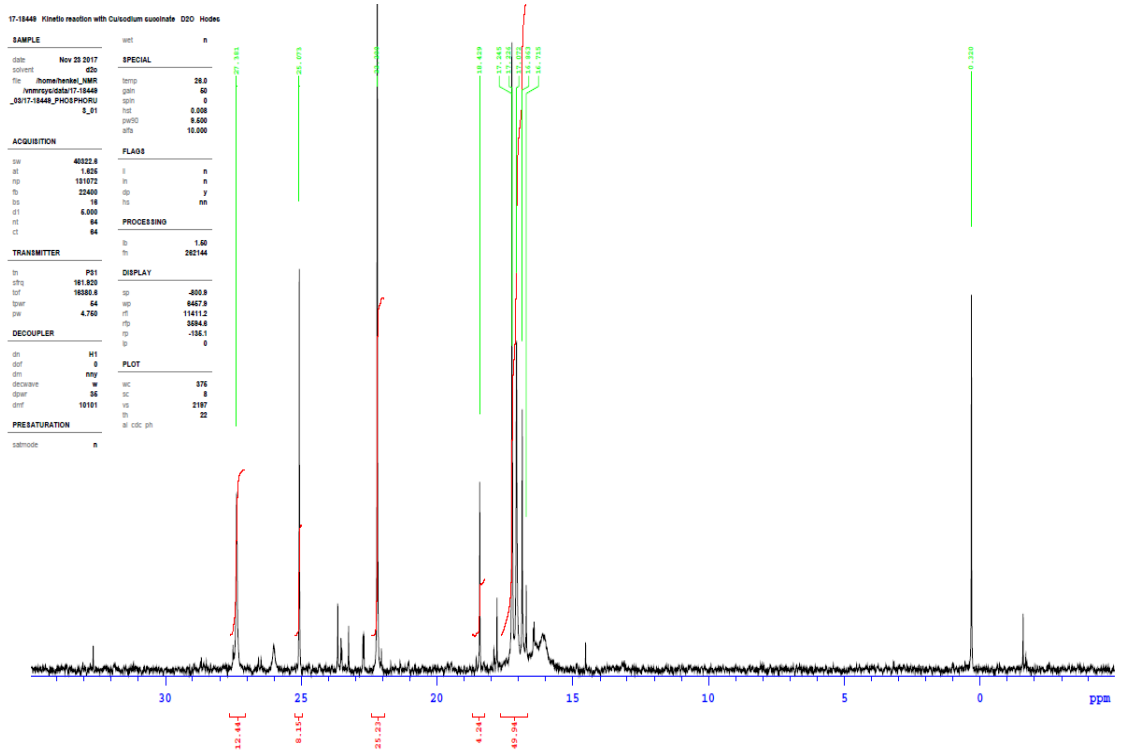


Figure E-4 Representative ^{31}P NMR spectrum of the radical adducts formed in a $H_2O_2/NH_4OH+NH_4Cl/Cu^{2+}$/sodium succinate system at pH=10 with DIPPMPO spin trap.

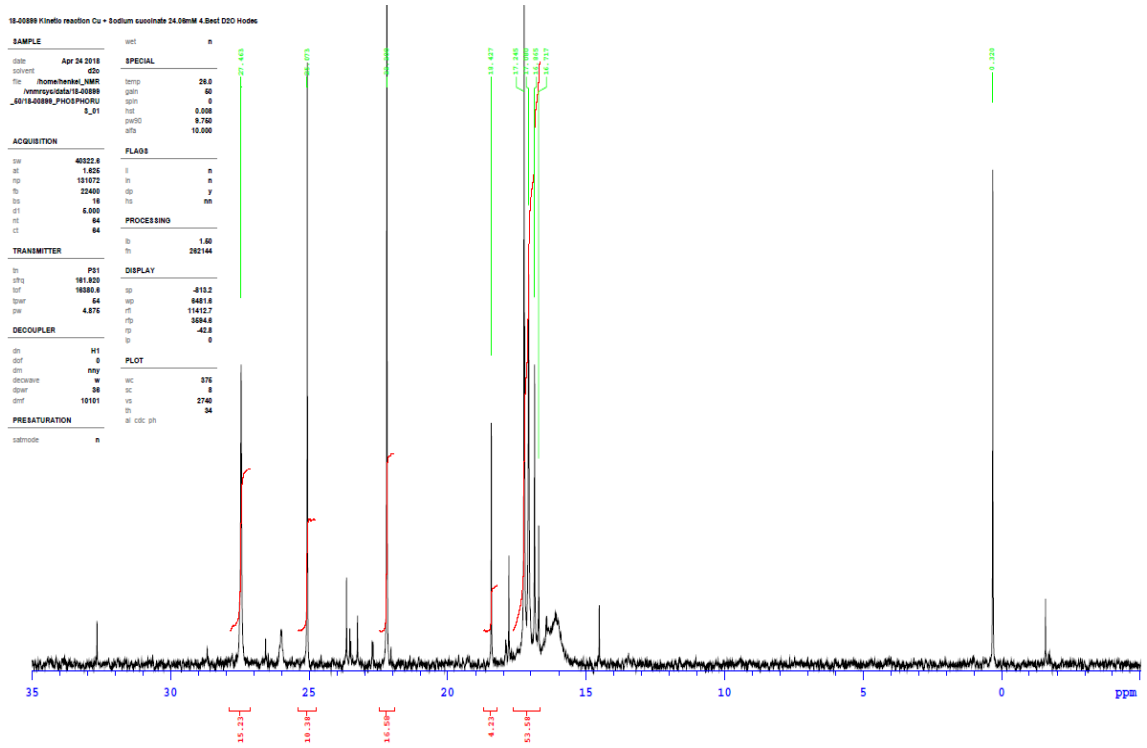


Figure E-5 Representative ^{31}P NMR spectrum of the radical adducts formed in a $H_2O_2/NH_4OH+NH_4Cl/Cu^{2+}$/sodium succinate (24.08 mmol/l) system at pH=10 with DIPPMPO spin trap.

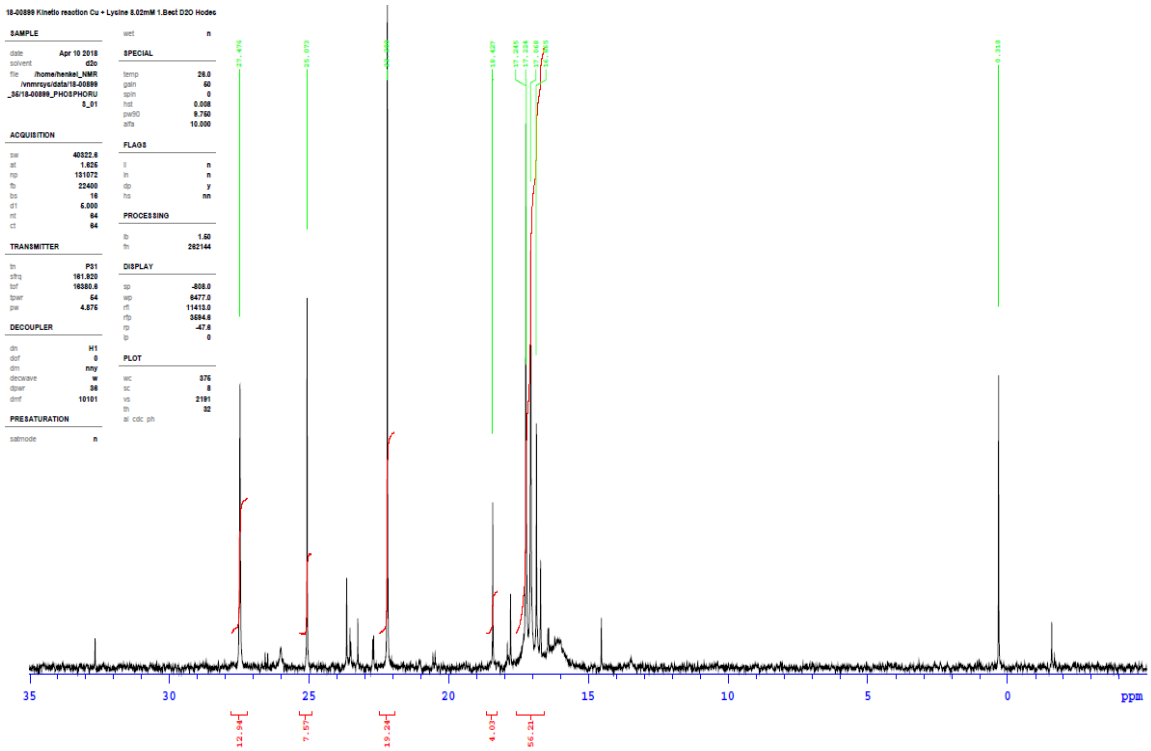


Figure E-6 Representative ^{31}P NMR spectrum of the radical adducts formed in a $H_2O_2/NH_4OH+NH_4Cl/Cu^{2+}$/lysine system at pH=10 with DIPPMPO spin trap.

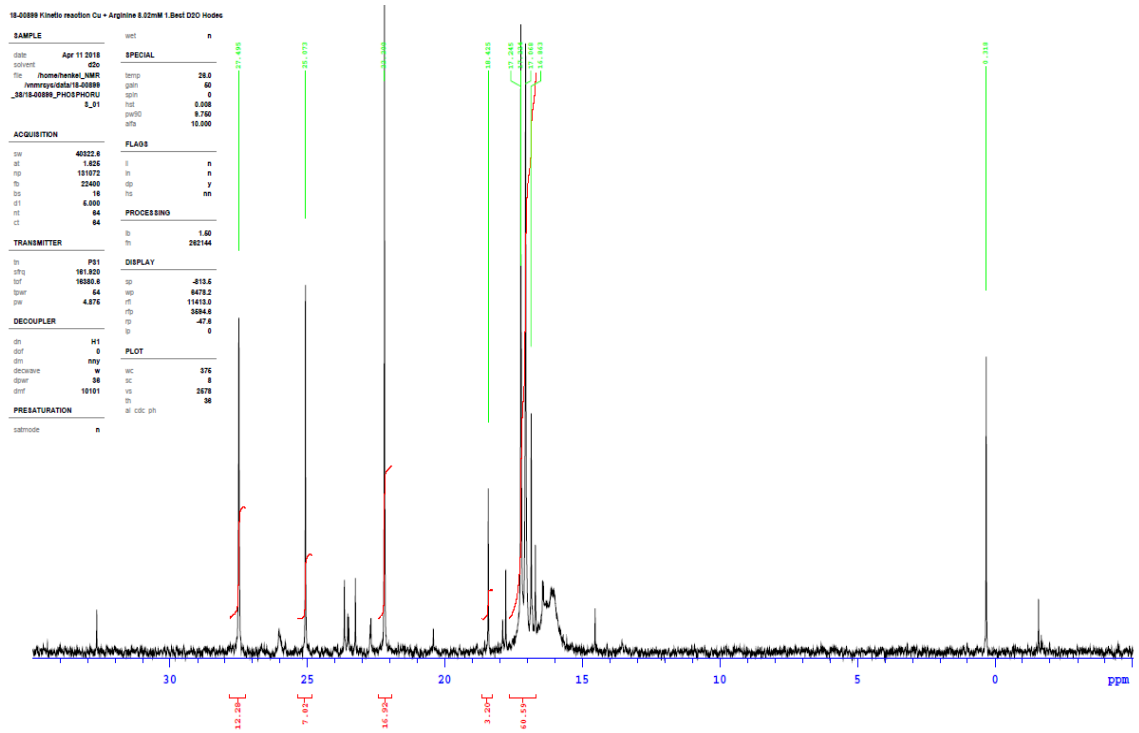


Figure E-7 Representative ^{31}P NMR spectrum of the radical adducts formed in a $H_2O_2/NH_4OH+NH_4Cl/Cu^{2+}$/arginine system at pH=10 with DIPPMPO spin trap.

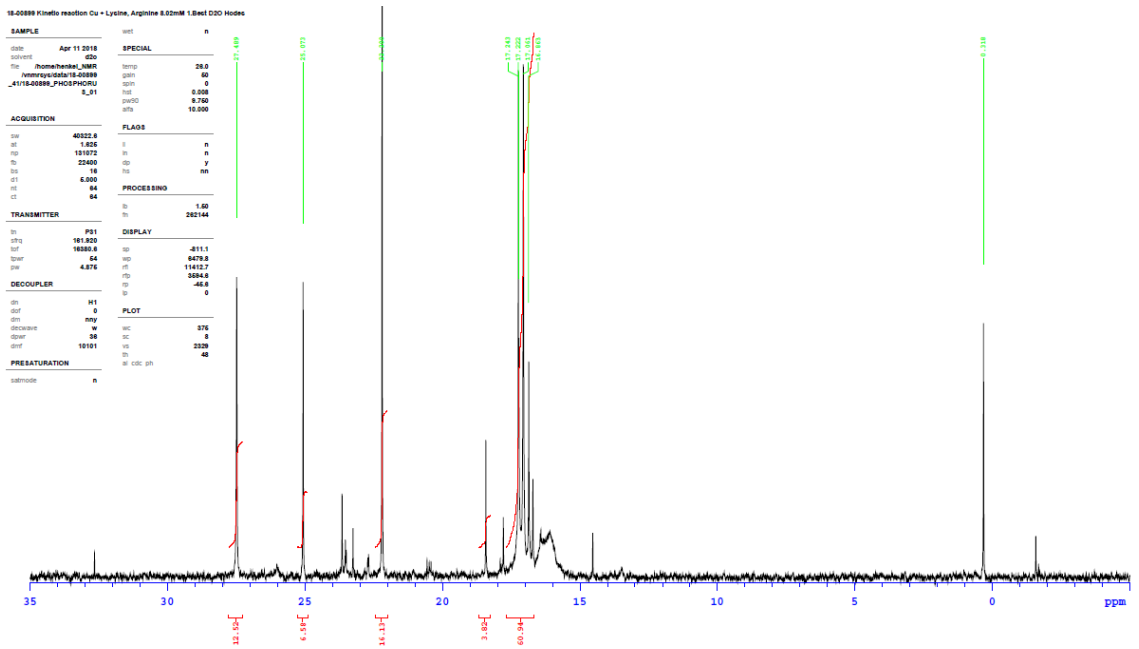


Figure E-8 Representative ^{31}P NMR spectrum of the radical adducts formed in a $H_2O_2/NH_4OH+NH_4Cl/Cu^{2+}$/arginine + lysine system at pH=10 with DIPPMPO spin trap.

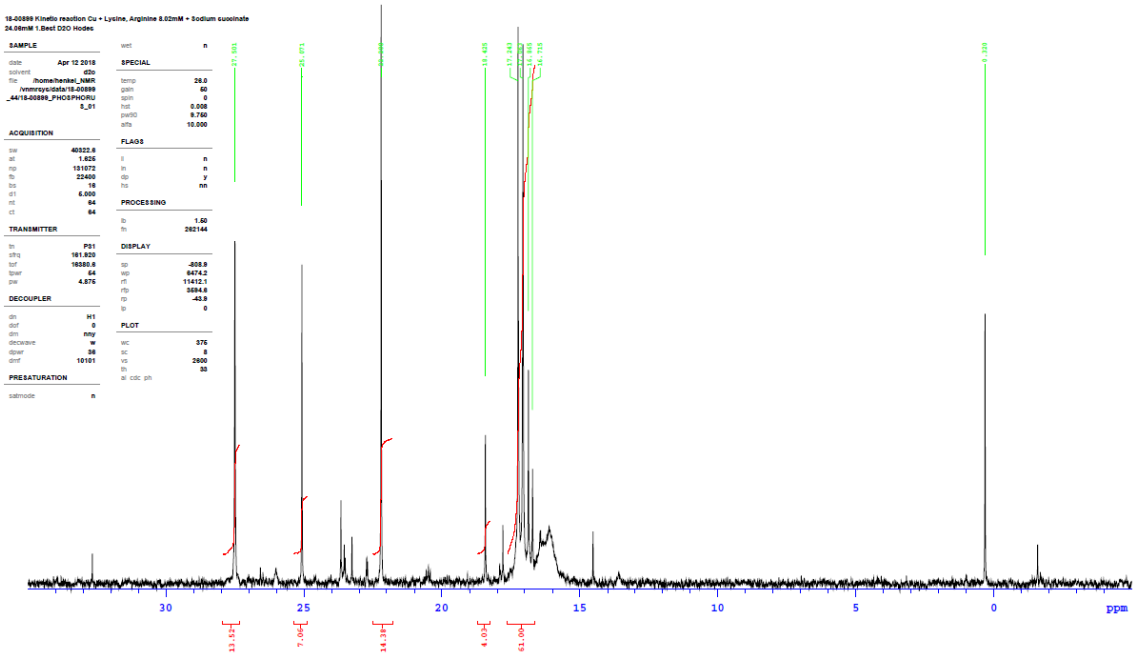


Figure E-9 Representative ^{31}P NMR spectrum of the radical adducts formed in a $H_2O_2/NH_4OH+NH_4Cl/Cu^{2+}$/arginine + lysine + sodium succinate system at pH=10 with DIPPMPO spin trap.

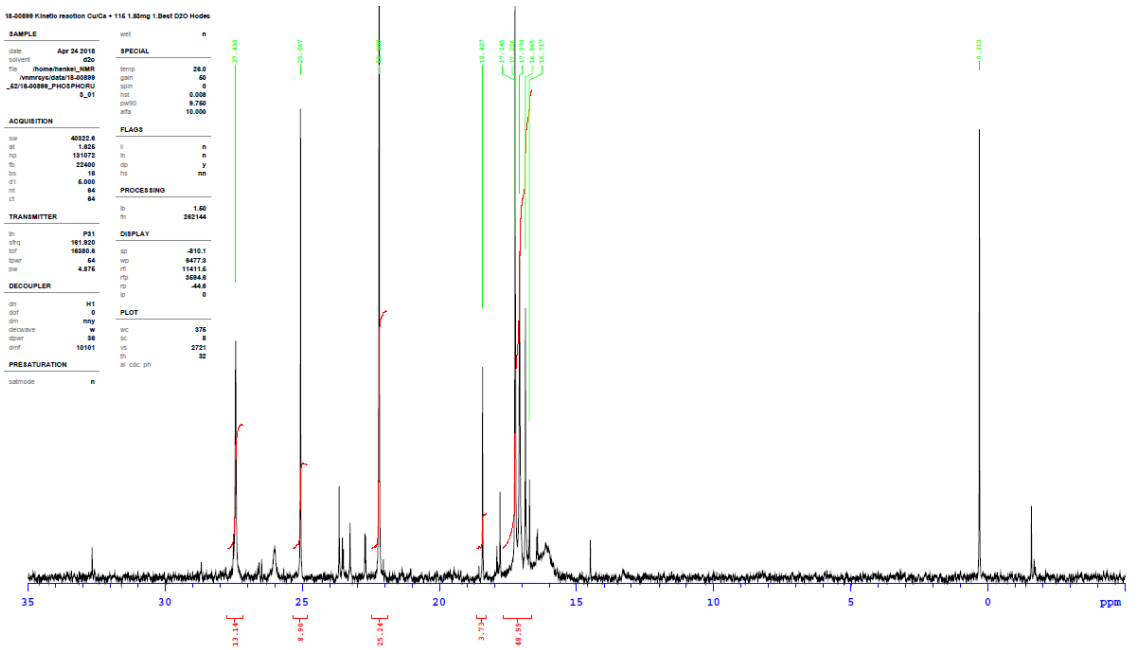


Figure E-10 Representative ^{31}P NMR spectrum of the radical adducts formed in a $H_2O_2/NH_4OH+NH_4Cl/Cu^{2+}$ + Ca^{2+} at pH=10 with DIPPMPO spin trap.

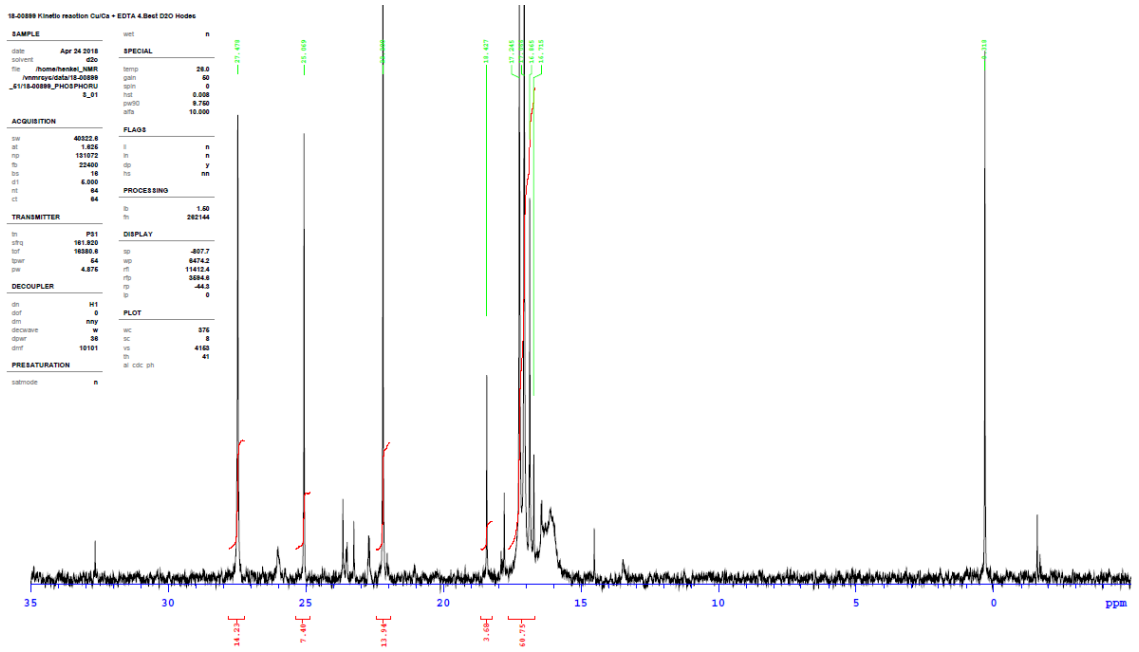


Figure E-11 Representative ^{31}P NMR spectrum of the radical adducts formed in a $H_2O_2/NH_4OH+NH_4Cl/Cu^{2+}$ + Ca^{2+}/EDTA at pH=10 with DIPPMPO spin trap.

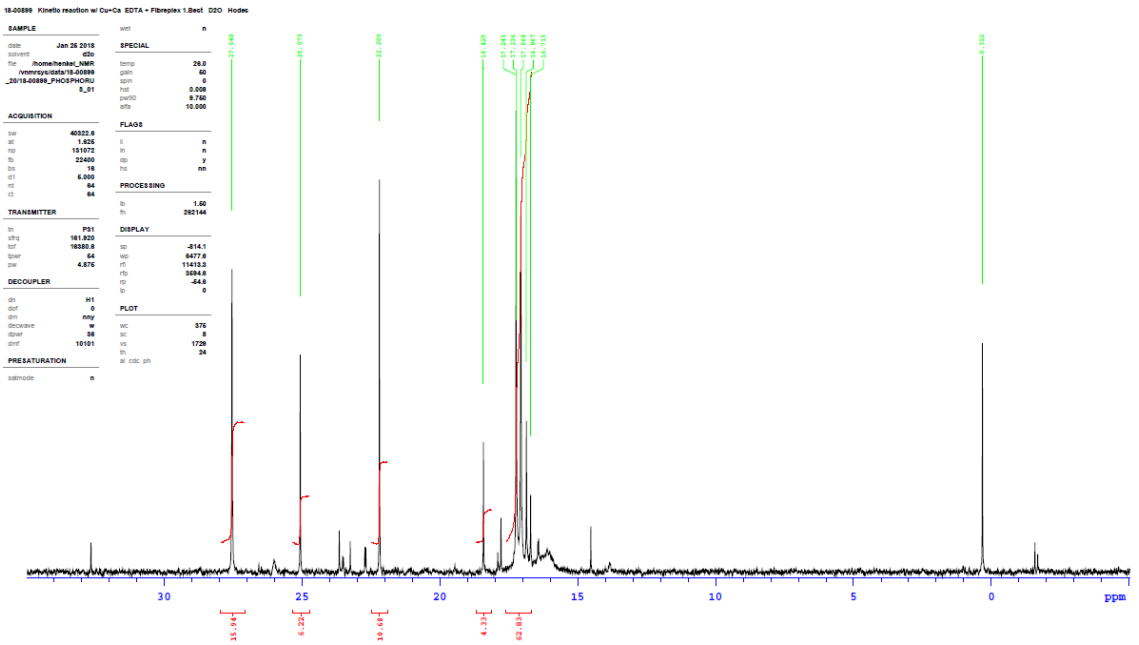


Figure E-12 Representative ^{31}P NMR spectrum of the radical adducts formed in a $H_2O_2/NH_4OH+NH_4Cl/Cu^{2+}$ + Ca^{2+}/EDTA/sodium succinate + lysine + arginine at pH=10 with DIPPMPO spin trap.

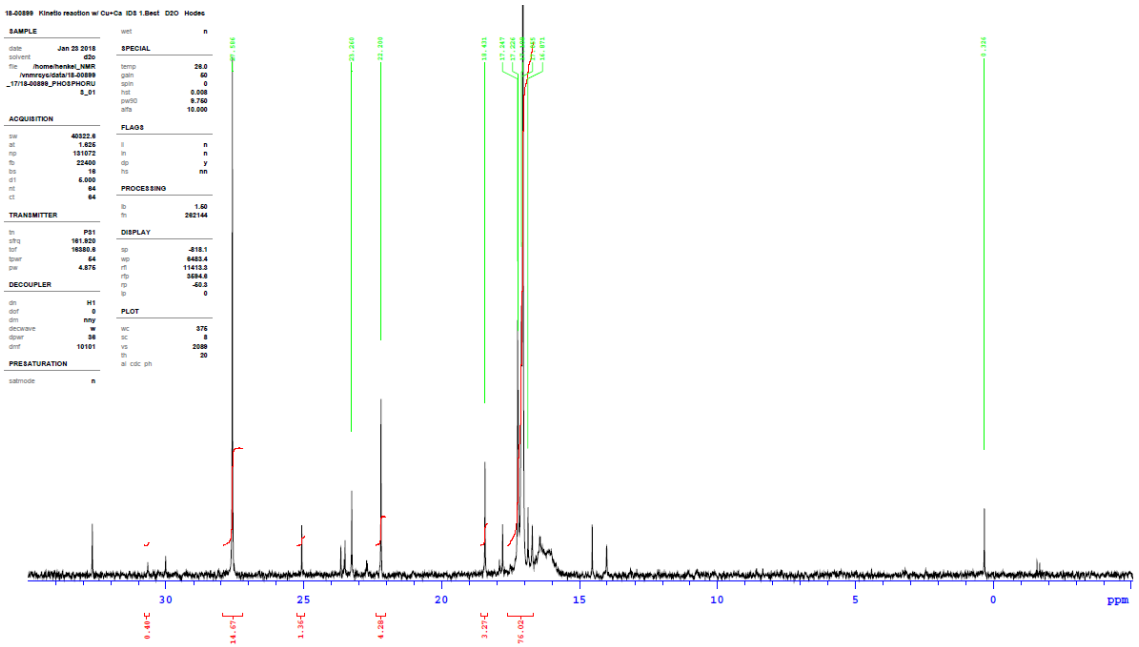


Figure E-13 Representative ^{31}P NMR spectrum of the radical adducts formed in a $H_2O_2/NH_4OH+NH_4Cl/Cu^{2+}$ + Ca^{2+}/EDTA/IDS at pH=10 with DIPPMPO spin trap.

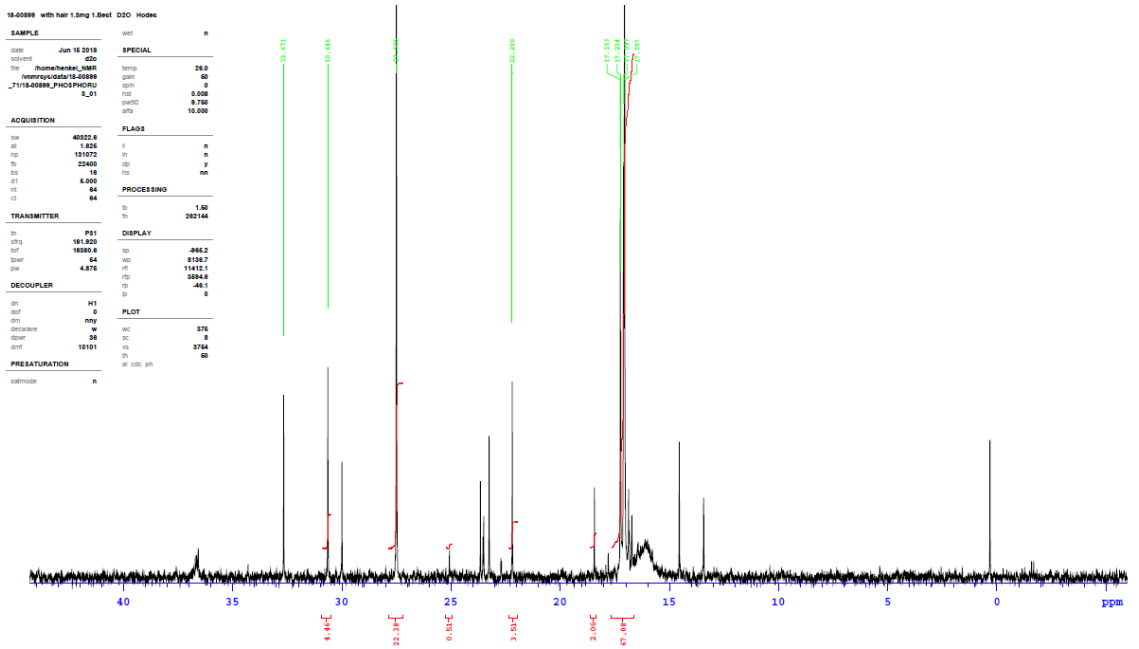


Figure E-14 Representative ^{31}P NMR spectrum of the radical adducts formed in a H_2O_2/NH_4OH+NH_4Cl system at pH=10 with DIPPMPO spin trap in the presence of human hair containing ca. 80 ppm copper.

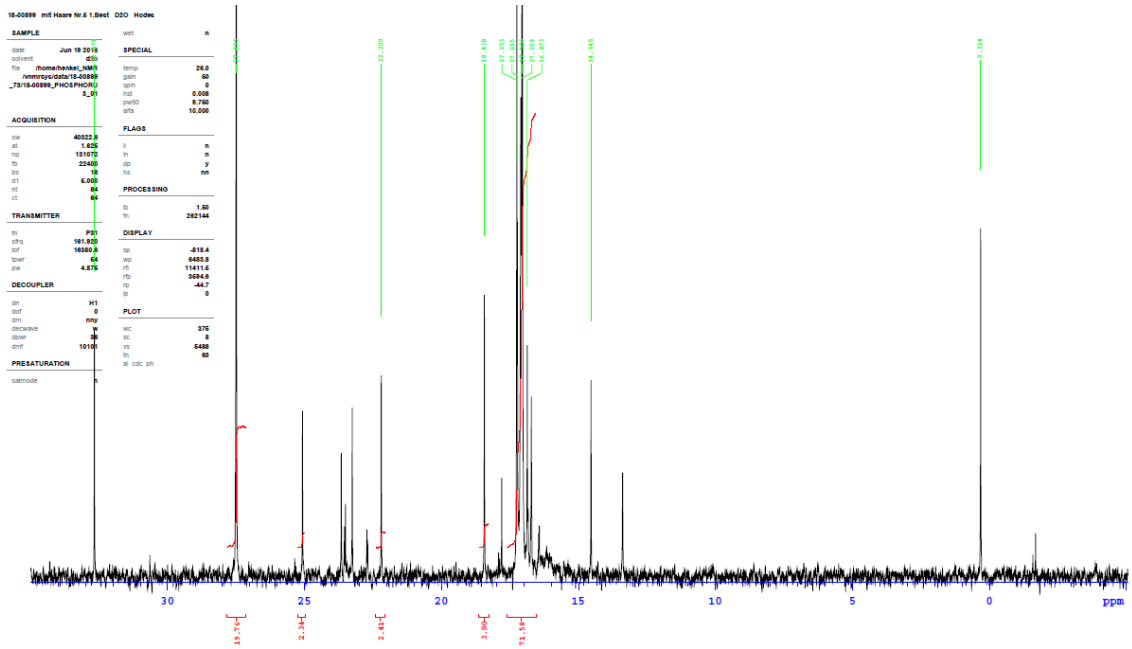


Figure E-15 Representative ^{31}P NMR spectrum of the radical adducts formed in a H_2O_2/NH_4OH+NH_4Cl system at pH=10 with DIPPMPO spin trap in the presence of human hair containing ca. 360 ppm copper.

Appendix F: Patent reference

1. Höffkes, H., Knübel, G., Brockmann, C., and Fuhr, D., Bleaching and coloring agents well tolerated by the skin, DE102006016817 A1, Henkel AG and Co. KGaA, 2006
2. Legrand, F. and Millequant, Jean M., Verwendung von Polycarbonsäuren und deren Salzen als Komplexbildner in oxidierenden Zusammensetzungen zum Färben, Entfärben und zur dauerhaften Verformung von Keratinfasern, EP 1475074 A1, L'Oreal GmbH, 2003
3. Gentschev, P., Doering, S., Breyer, J. and Machin, A., Use of support-fixed bleaching catalyst complex compounds as catalysts for peroxide compounds, WO200354128 A1, Henkel AG and Co. KGaA, 2001
4. Schmiedel, P., Buzzacchi, M., Kaiser, H., Von Rybinski, W., and Orlich, B., Percarboxylic acid-based polyelectrolyte capsule system having a long shelf life, WO2004110612 A1, Henkel AG and Co. KGaA, 2003
5. Glogowski, Mark W., Hartman, Frederick A., Heinzman, Stephen W., and Perkins, Christopher M., N, N'-(1-oxo-1,2-ethanediyl)-bis (aspartic acid), salts and use in detergent compositions, US4983315 A, Procter and Gamble GmbH, 1989
6. Müller, R., and Desenne, P., Composition for treating keratinous materials comprising a polycarboxylic acid and a protecting or conditioning agent. EP1462087 A2, L'Oreal GmbH, 2003
7. Maignan, J., Use of alpha-phenylcinnamic acid derivatives as melanogenesis inhibitor for lightening human skin or hair and for removing brownish pigmented spots and senescence spots from human skin, FR2852840 A1, L'Oreal GmbH, 2003
8. Pressly, Eric D., and Hawker, Craig J., Methods for fixing hair and skin , CA2916985 A1 , Liqwd, Inc., Santa Barbara, CA (US), 2013
9. Lalleman, B., and Lagrange, A., Method for extracting metal ions from keratinous fibres by repeated application of an acid composition, WO2017109186 A1, L'Oreal GmbH, 2015
10. Wood, J., and Aechtner, A., Hair colouring composition comprising gluconic acid and/or its salts, EP2658509 B1, KAO Germany GmbH, 2013

11. Wood, J., and Aechtner, A., Bleaching composition comprising a polyhydroxy carboxylic acid, US9233058 B2, Kao Germany GmbH, 2012
12. Smith, Liam R., and Long, Stewart P., Hair colouring compositions, WO03/015734 A2, The boots company PLC, 2003
13. De Boer, R., Mol, R., and Van Ommen, J., Low-hygroscopic particulate composition comprising one or more aminopolycarboxylate chelating compounds, EP2392638 B1, Dalli-Werke GmbH and Co. KG, 2017
14. Marsh, Jennifer M., Iwata, T., and Lim, Phan S., Hair care conditioning composition comprising histidine , US20140335036 A1 ,Procter and Gamble GmbH, 2013
15. Boswell, Henry D., Marsh, Jennifer M., Olshavsky, Michael A., and Park, John S., Oxidative treatment of hair with reduced hair damage, Procter and Gamble GmbH, 2013
16. Desenne, P., and Millequant, Jean M., Hair dying composition comprising at least one polycarboxylic acid or a salt thereof, ready-to-use composition comprising it, process and apparatus, EP1462093 A2, L'Oreal GmbH, 200

List of publications

Research articles

Hodes, Jing; Sielaff, Patric; Metz, Hendrik; Kessler-Becker, Daniela; Gassenmeier, Thomas; Neubert, Reinhard H. H.: *The role of chelating agents and amino acids in preventing free radical formation in bleaching systems*, Free Radical Biology and Medicine 129(2018) 194-201

Hodes, Jing; Eschen, Burkhard; Kessler-Becker, Daniela; Gassenmeier, Thomas; Neubert, Reinhard H. H.: *Oxidative damage of human hair fibre - Protection effect of succinic acid and amino acids, in International Journal of Cosmetic Science, under revise*

Invention disclosure

1. Hodes, J. and D. Kessler-Becker, *Verbesserte Blondiermittel*, 2017, Henkel AG and Co. KGaA: amtliches Aktenzeichen:102017222516.5
2. Hodes, J., D. Kessler-Becker, and B. Banowski, *Haarschonende Blondierung mit GLDA*, 2018, Henkel AG and Co. KGaA: amtliches Aktenzeichen:10201823507.0
3. Hodes, J., D. Kessler-Becker, and B. Banowski, *Haarschonende Blondierung mit Glucoheptonate*, 2018, Henkel AG and Co. KGaA: amtliches Aktenzeichen:102018123454.6
4. Hodes, J. and D. Kessler-Becker, *Na-Gluconat in Blondiermitteln*, 2018, Henkel AG and Co: KGaA: amtliches Aktenzeichen:102018123526.7
5. Hodes, J., et al., *Amine-based reaction products as functional additives*, 2018 Henkel AG and Co. KGaA: amtliches Aktenzeichen:1812032.9
6. Hodes, J., et al., *Improved the quality and durability of color in human colored hair*, 2018, Henkel AG and Co. KGaA: amtliches Aktenzeichen: 102018221959.1

7. Hodes, J and D. Kessler-Becker, Avoid hair color shift, 2018, Henkel AG and Co. KGaA: amtliches Aktenzeichen: 102018222222.3

Acknowledgements

First, I would like to express my deepest gratitude and appreciation to my supervisor Prof. Dr. Dr. h.c. Reinhard H. H. Neubert for your continuous support and scientific guidance. I appreciate your willingness to accept to supervise my PhD project. Especially your continuous readiness to discuss the results and the permanent input of ideas were impressive and very helpful for finishing this thesis.

I am deeply grateful to my supervisor, Prof. Thomas Gassenmeier, for your guidance and constructive feedback for my PhD project. Due to your immense experience in the world of hair cosmetic science, it was possible for me to benefit from that in every sense. I will always be grateful to you for your scientific as well as personal advice, support, encouragement and positive influence in my life, in general.

Additionally, my thanks are addressed to Dr. Daniela Kessler-Becker, who was supervising this project directly. Without all your high technical knowledge and immense experience in the world of hair cosmetic science, this project would be not finished yet. Especially I am also deeply grateful for your continuous readiness and constructive feedback.

Furthermore, I would like also to thank Dr. Hendrik Metz for advising me on some key scientific aspects of my work and his thoroughly readiness for my publications.

Many thanks to Mr. Burkhard Eschen and Ms. Yvonne Fellmer, without all their practical background about electron microscopy (SEM and TEM), this project would not be finished yet. Thanks a lot for all their support during this work.

I would like to offer my special thanks to Patric Sielaff for his excellent technical assistance and the intensive collaboration regarding the ^{31}P NMR studies, which complemented the given results fruitfully.

I also acknowledge Meike Voss-Klimke, Gerhard Maas for measuring the cysteic acid residue amount and the metal amount in hair, respectively.

I would like to acknowledge Dr. Anja Reichert and Dr. Oliver Seiler for their helpful comments on my publication.

I greatly acknowledge the financial support provided by Henkel AG and Co KGaA, Germany.

Finally, I am very grateful to my families and friends for their emotional and moral supports and understanding. Special thanks to my husband for his stunning support and help and encouragement.

Curriculum Vitae

Personal Details:

Name:	Jing Hodes
Maiden name:	Zhou
Date of birth:	24th of September 1986
Location of birth:	Ping Ding Shan, Henan, China
Nationality:	German
Marital status:	Married

Education:

May 2016 – present	PhD student under the supervision of Prof. Dr. Dr, h.c. R. Neubert, at Department of Pharmaceutical Technology and Bio pharmacy, Institute of Pharmacy, Martin Luther University Halle-Wittenberg, Germany
Feb 2010 – Mar 2012	M.Sc. in Applied Chemistry, Reutlingen University, Germany
Sep 2005 – Jun 2009	Bachelor in Textile Chemistry, Dyeing and Finish Engineering, in College of Chemistry, Chemical Engineering and Biotechnology, Donghua University, Shanghai, China

Work Experience:

May 2016 – present	Doctoral candidate at Department Beauty Care Research and Development, Advanced research and product development for Hair Coloration and bleaching, Henkel AG and Co KGaA, Düsseldorf, Germany
Nov 2014 – May 2016	Junior Manager at Department Beauty Care Research and Development, Product development for hair coloration and bleaching, Henkel AG and Co KGaA, Hamburg, Germany
Feb 2013 - Nov 2014	Junior Manager at Department Beauty Care Research and Development, Product development for Antiperspirants and Deodorants, Henkel AG and Co KGaA, Düsseldorf, Germany
Aug 2011 – Aug 2012	Intern at Department Process engineering for functional materials, Fraunhofer Institute for Manufacturing Engineering and Automation IPA, Stuttgart, Germany

Eidesstattliche Erklärung

Hiermit erkläre ich entsprechend der Promotionsordnung der Naturwissenschaftlichen Fakultät I (Biowissenschaften) der Martin-Luther-Universität Halle-Wittenberg, dass ich die Ergebnisse der vorliegenden Dissertationsarbeit:

Investigation of the mode of action of succinic acid and amino acids during hair bleaching treatment

am Institut für Pharmazie selbständig und ohne fremde Hilfe erarbeitet und verfasst habe und dass ich alle Informationen in dieser Arbeit und alle beschriebenen Materialien ordnungsgemäß zitiert habe. Ich habe mich zu keinem früheren Zeitpunkt um den Doktortitel an einer anderen Einrichtung beworben. Weiterhin habe ich die vorliegende Arbeit bisher keiner anderen Prüfungsbehörde vorgelegt.

Halle (Saale), im August 2019 Jing Hodes

Reference

1. Franbourg, A., et al., *Current research on ethnic hair.* Journal of the American Academy of Dermatology, 2003. **48**(6): p. 115-119.
2. Wolfram, L.J., *Human hair: A unique physicochemical composite.* Journal of the American Academy of Dermatology, 2003. **48**(6): p. 106-114.
3. Yang, F., Y. Zhang, and M.C. Rheinstädter, *The structure of people's hair.* Journal of Life and Environmental Sciences, 2014. **2**: p. 619.
4. Suzuta, K., *Studies on the disulfide cross-linked structure and mechanical properties of keratin fibers*, PhD thesis, in *Shinshu University*2015, Shinshu University: Japan.
5. Wortmann, F.J., G. Wortmann, and H. Zahn, *Pathways for dye diffusion in wool Fibers.* Textile Research Journal, 1997. **67**(10): p. 720-724.
6. Bhushan, B., *Nanoscale characterization of human hair and hair conditioners.* Progress in Materials Science, 2008. **53**(4): p. 585-710.
7. Robbins, C.R., *The cell membrane complex: Three related but different cellular cohesion components of mammalian hair fibers.* Journal of Cosmetic Science, 2009. **60**(4): p. 437-465.
8. Reis, G.D.M.F., *Hair cosmetics: An overview.* International Journal of Trichology, 2015. **7**(1): p. 2-15.
9. Okamoto, M., et al., *Investigation of the damage on the outermost hair surface using ToF-SIMS and XPS.* Surface and Interface Analysis, 2012. **44**(6): p. 736-739.
10. Breakspear, S., J.R. Smith, and G. Luengo, *Effect of the covalently linked fatty acid 18-MEA on the nanotribology of hair's outermost surface.* Journal of Structural Biology, 2005. **149**(3): p. 235-242.
11. Lshikawa, K., M. Okamoto, and S. Aoyagi, *Structural analysis of the outermost hair surface using TOF-SIMS with gas cluster ion beam sputtering.* Biointerphases, 2016. **11**(2): p. 02A315.
12. Smith, J.R. and J.A. Swift, *Lamellar subcomponents of the cuticular cell membrane complex of mammalian keratin fibres show friction and hardness contrast by AFM.* Journal of Microscopy, 2002. **206**(3): p. 182-193.
13. Robbins, C.R., *Chemical composition of different hair types*, in *Chemical and Physical Behavior of Human Hair*, C.R. Robbins, Editor. 2012, Springer Berlin Heidelberg: Berlin, Heidelberg. p. 105-176.
14. Kuzuhara, A., *Analysis of internal structure changes in black human hair keratin fibers resulting from bleaching treatments using Raman spectroscopy.* Journal of Molecular Structure, 2013. **1047**: p. 186-193.
15. Kuzuhara, A., *Analysis of structural change in keratin fibers resulting from chemical treatments using Raman spectroscopy.* Biopolymers, 2005. **77**(6): p. 335-344.
16. Wang, B., et al., *Keratin: Structure, mechanical properties, occurrence in biological organisms, and efforts at bioinspiration.* Progress in Materials Science, 2016. **76**: p. 229-318.
17. Istrate, D., et al., *The effect of pH on the thermal stability of fibrous hard alpha-keratins.* Polymer Degradation and Stability, 2013. **98**(2): p. 542-549.
18. Kim, Y.D., et al., *Development of a classification system for extrinsic hair damage: Standard grading of electron microscopic findings of damaged Hairs.* The American Journal of Dermatopathology, 2010. **32**(5): p. 432-438.

19. Velasco, M.V.R., et al., *Hair fiber characteristics and methods to evaluate hair physical and mechanical properties.* Brazilian Journal of Pharmaceutical Sciences, 2009. **45**: p. 153-162.
20. Robbins, C.R., *Morphological, macromolecular structure and hair growth*, in *Chemical and Physical Behavior of Human Hair*, C.R. Robbins, Editor. 2012, Springer Berlin Heidelberg: Berlin, Heidelberg. p. 89-94.
21. Nagase, S., et al., *Influence of internal structures of hair fiber on hair appearance. I. Light scattering from the porous structure of the medulla of human hair.* Journal of Cosmetic Science, 2002. **53**(2): p. 89-100.
22. Brenner, M. and V.J. Hearing, *The protective role of melanin against UV damage in human skin.* Photochemistry and Photobiology, 2008. **84**(3): p. 539-549.
23. Smith, R.A.W., et al., *Mechanistic insights into the bleaching of melanin by alkaline hydrogen peroxide.* Free Radiccal Biology and Medicine, 2017. **108**: p. 110-117.
24. Chen, N. and B. Bhushan, *Morphological, nanomechanical and cellular structural characterization of human hair and conditioner distribution using torsional resonance mode with an atomic force microscope.* Journal of Microscopy, 2005. **220**(2): p. 96-112.
25. Naqvi, K.R., *Role of transition metal ions in oxidative hair colouring*, PhD thesis, in *The University of York > Chemistry (York)*2014, University of York: United States.
26. Prem, P., et al., *New insights into the physicochemical effects of ammonia/peroxide bleaching of hair and Sepia melanins.* Journal of Cosmetic Science, 2003. **54**(4): p. 395-409.
27. Marsh, J.M., et al., *Role of copper in photochemical damage to hair.* International Journal of Cosmetic Science, 2014. **36**(1): p. 32-38.
28. Mitu, A.M., *Damage assessment of human hair by electrophoretical analysis of hair proteins*, PhD thesis, in *Fakultät für Mathematik, Informatik und Naturwissenschaften*2004, RWTH Aachen University: Germany.
29. Kim, M.M., *Effect of procyandin oligomers on oxidative hair damage.* Skin Research and Technology, 2011. **17**(1): p. 108-118.
30. Fernandez, E., et al., *Efficacy of antioxidants in human hair.* Journal of Photochemistry and Photobiology B: Biology, 2012. **117**: p. 146-156.
31. Thibaut, S., et al., *Transglutaminase-3 Enzyme: A putative actor in human hair shaft scaffolding?* Journal of Investigative Dermatology, 2009. **129**(2): p. 449-459.
32. Eckert, R.L., et al., *Transglutaminase function in epidermis.* Journal of Investigative Dermatology, 2005. **124**(3): p. 481-492.
33. Naqvi, K.R., et al., *The role of chelants in controlling Cu(II)-induced radical chemistry in oxidative hair colouring products.* International Journal of Cosmetic Science, 2013. **35**(1): p. 41-49.
34. Smart, K.E., et al., *Copper and calcium uptake in colored hair.* Journal of Cosmetic Science, 2009. **60**(3): p. 337-345.
35. Tang, Y., et al., *Trace metal ions in hair from frequent hair dyers in China and the associated effects on photo-oxidative damage.* Journal of Photochemistry and Photobiology B: Biology, 2016. **156**: p. 35-40.
36. Samanta, A., et al., *Nanomechanical responses of human hair.* Journal of the Mechanical Behavior of Biomedical Materials, 2016. **56**: p. 229-248.

37. Wei, Z.L., Y.K. Rui, and L. Shen, *Effects of hair dyeing on the heavy metals content in hair.* Spectroscopy and Spectral Analysis, 2008. **28**(9): p. 2187-2188.
38. Kempson, I.M., W.M. Skinner, and K.P. Kirkbride, *The occurrence and incorporation of copper and zinc in hair and their potential role as bioindicators: A review.* Journal of Toxicology and Environmental Health B: Critical Review, 2007. **10**(8): p. 611-622.
39. Imai, T., *The influence of hair bleach on the ultrastructure of human hair with special reference to hair damage.* Okajimas Folia Anatomica Japonica, 2011. **88**(1): p. 1-9.
40. Liu, Y., et al., *Comparison of structural and chemical properties of black and red human hair melanosomes.* Photochemistry and Photobiology, 2005. **81**(1): p. 135-144.
41. Robbins, C.R., *Bleaching and oxidation of human Hair*, in *Chemical and Physical Behavior of Human Hair*, C.R. Robbins, Editor. 2012, Springer Berlin Heidelberg: Berlin, Heidelberg. p. 263-328.
42. Franca, S.A.d., et al., *Types of hair dye and their mechanisms of action.* Cosmetics, 2015. **2**: p. 110-126.
43. Robbins, C.R., *Dyeing human hair*, in *Chemical and Physical Behavior of Human Hair*, C.R. Robbins, Editor. 2012, Springer-Verlag: Berlin. p. 444-488.
44. Crestini, C., et al., *Identification and quantification of radical species by ^{31}P NMR-based spin trapping — A case study: NH_4OH/H_2O_2-based hair bleaching.* Microchemical Journal, 2015. **121**: p. 112-121.
45. Argyropoulos, D.S., et al., *Quantitative ^{31}P NMR detection of oxygen-centered and carbon-centered radical species.* Bioorganic and Medicinal Chemistry, 2006. **14**(12): p. 4017-4028.
46. Clarke, K., et al., *Direct observation of NH_2 reactions with oxygen, amino acids, and melanins.* Journal of Physical Chemistry A, 2008. **112**(6): p. 1234-1237.
47. M. Pande, C., B. Yang, and B.M. Squibb, *Near-infrared spectroscopy: Applications in hair research.* Journal of Cosmetic Science, 2000. **51**(3): p. 183-192.
48. Richena, M. and C.A. Rezende, *Morphological degradation of human hair cuticle due to simulated sunlight irradiation and washing.* Journal of Photochemistry and Photobiology B: Biology, 2016. **161**: p. 430-440.
49. Grosvenor, A.J., et al., *The physical and chemical disruption of human hair after bleaching – Studies by transmission electron microscopy and redox proteomics.* International Journal of Cosmetic Science, 2018. **40**(6): p. 536-548.
50. Barbusinski, K., *Fenton reaction-controversy concerning the chemistry.* Ecological Chemistry and Engineering S: Society, 2009. **16**(3): p. 347-358.
51. Wardman, P. and L.P. Candeias, *Fenton chemistry: An introduction.* Journal of Radiation Research, 1996. **145**(5): p. 523-531.
52. Bray, W.C. and M.H. Gorin, *Ferryl Ion, a compound of tetravalent Iron.* Journal of the American Chemical Society, 1932. **54**(5): p. 2124-2125.
53. Prousek, J., *Fenton chemistry in biology and medicine.* Pure and Applied Chemistry, 2007. **79**(12): p. 2325-2338.
54. Ensing, B., F. Buda, and E.J. Baerends, *Fenton-like chemistry in water: Oxidation catalysis by Fe(III) and H_2O_2.* The Journal of Physical Chemistry A, 2003. **107**(30): p. 5722-5731.

55. Freinbichler, W., et al., *Mechanistic aspects of the Fenton reaction under conditions approximated to the extracellular fluid.* Journal of Inorganic Biochemistry, 2009. **103**(1): p. 28-34.
56. Rachmilovich-Calis, S., et al., *New mechanistic aspects of the Fenton reaction.* Chemistry, 2009. **15**(33): p. 8303-8309.
57. Chittleborough, G., *A chemist's view of the analysis of human hair for trace elements.* Science of the Total Environment, 1980. **14**(1): p. 53-75.
58. Marsh, J.M., et al., *Preserving fibre health: Reducing oxidative stress throughout the life of the hair fibre.* International Journal of Cosmetic Science, 2015. **37**(2): p. 16-24.
59. Albert, A. and E.P. Serjeant, *Chelation and the stability constants of metal complexes*, in *The Determination of Ionization Constants: A Laboratory Manual*, A. Albert and E.P. Serjeant, Editors. 1984, Springer Netherlands: Dordrecht. p. 176-191.
60. Kołodyńska, D., *Chelating Agents of a New Generation as an Alternative to Conventional Chelators for Heavy Metal Ions Removal from Different Waste Waters*. Expanding Issues in Desalination, ed. R.Y. Ning. 2011, Poland: InTech.
61. Kołodyńska, D., *Iminodisuccinic acid as a new complexing agent for removal of heavy metal ions from industrial effluents.* Chemical Engineering Journal, 2009. **152**(1): p. 277-288.
62. Bačić, G., et al., *Spin-trapping of oxygen free radicals in chemical and biological systems: New traps, radicals and possibilities.* Spectrochimica Acta A: Molecular and Biomolecular Spectroscopy, 2008. **69**(5): p. 1354-1366.
63. Khramtsov, V.V., L.J. Berliner, and T.L. Clanton, *NMR spin trapping: Detection of free radical reactions using a phosphorus-containing nitrone spin trap.* Magnetic Resonance in Medicine, 1999. **42**(2): p. 228-234.
64. Khramtsov, V.V. and T.L. Clanton, *NMR spin trapping: Insight into the hidden life of free radical adducts.* Applied Magnetic Resonance, 2011. **41**(2): p. 305-323.
65. Zoia, L., et al., *Understanding the radical mechanism of lipoxygenases using ^{31}P NMR spin trapping.* Bioorganic and Medicinal Chemistry, 2011. **19**(9): p. 3022-3028.
66. Hossel, P., et al., *Conditioning polymers in today's shampoo formulations - Efficacy, mechanism and test methods.* International Journal of Cosmetic Science, 2000. **22**(1): p. 1-10.
67. Gavazzoni Dias, M.F.R., et al., *The Shampoo pH can affect the hair: Myth or reality?* International Journal of Trichology, 2014. **6**(3): p. 95-99.
68. Pressly, E.D. and C.J. Hawker, *Methods for fixing hair and skin*, 2015, Liqwd, Inc., Santa Barbara, CA(US): United States, US9144537 B1.
69. Förster, T., T. Hippe, and G. Knübel, *Is repair of lightened hair feasible? Myths and facts on different hair bonding treatments.* SOFW Journal, 2018. **144**: p. 2-9.
70. Anderheggen, B. and B. Müller, *Bleaching agent and method for gentle oxidative hair lightening*, 2017, Henkel AG and Co. KGaA: United States, US20170340549 A1.
71. Schlenkermann, T.C., *Investigation of damages of the keratinous hair structure by oxidative bleach treatments and development of methods for its minimization*, Master thesis, in *Kosmetikwissenschaft*2016, University of Hamburg: Germany.

72. Robbins, C.R. and R.J. Crawford, *Cuticle damage and the tensile properties of human hair.* Journal of the Society of Cosmetic Chemists, 1991. **42**(1): p. 59-67.
73. Yu, Y., et al., *Structure and mechanical behavior of human hair.* Materials Science and Engineering C: Materials for Biological Applications, 2017. **73**: p. 152-163.
74. Robbins, C.R., *The physical properties of hair fibers*, in *Chemical and Physical Behavior of Human Hair*, C.R. Robbins, Editor. 2012, Springer Berlin Heidelberg: Berlin, Heidelberg. p. 555-557.
75. Strassburger, J. and M.M. Breuer, *Quantitative fourier transform infrared spectroscopy of oxidized hair.* Journal of the Society of Cosmetic Chemists, 1985. **36**(1): p. 61-74.
76. Eibl, O., et al., *Quantitative chemical analysis of ocular melanosomes in the TEM.* Micron, 2006. **37**(3): p. 262-276.
77. Hodes, J., et al., *The role of chelating agents and amino acids in preventing free radical formation in bleaching systems.* Free Radical Biology and Medicine, 2018. **129**: p. 194-201.
78. Marsh, J., et al., *A new oxidant for hair coloring.* International Journal of Cosmetic Science, 2010. **32**(2): p. 158-158.
79. Hakimi, M. and T.S. Aliabadi, *Coordination chemistry of copper α-amino acid complexes.* World Applied Programming, 2012. **2**(10): p. 431-443.
80. Munter, R., *Advanced oxidation processes-current status and prospects.* Proceedings of the Estonian Academy of Science. Chemistry, 2001. **50**(2): p. 59-80.
81. Xu, G. and M.R. Chance, *Hydroxyl radical-mediated modification of proteins as probes for structural proteomics.* Chemical Reviews, 2007. **107**(8): p. 3514-3543.
82. Yin, H., L. Xu, and N.A. Porter, *Free radical lipid peroxidation: Mechanisms and analysis.* Chemical Reviews, 2011. **111**(10): p. 5944-5972.
83. Sharma, H. and D.S. Sharma, *Detection of hydroxyl and perhydroxyl radical generation from bleaching agents with nuclear magnetic resonance spectroscopy.* Journal of Clinical Pediatric Dentistry, 2017. **41**(2): p. 126-134.
84. Fowles, E.H., et al., *The effects of chelating agents on radical generation in alkaline peroxide systems, and the relevance to substrate damage.* Free Radical Research, 2007. **41**(5): p. 515-522.
85. Bolger, M.B., *Computational techniques in macromolecular structural analysis*, in *Introduction to Biophysical Methods for Protein and Nucleic Acid Research*, J.A. Glasel and M.P. Deutscher, Editors. 1995, Academic Press: San Diego. p. 433-490.
86. Sursyakova, V.V., G.V. Burmakina, and A.I. Rubaylo, *Composition and stability constants of copper(II) complexes with succinic acid determined by capillary electrophoresis.* Journal of Coordination Chemistry, 2017. **70**(3): p. 431-440.
87. Shah, V., et al., *Decolorization of dyes with copper(II)/organic acid/hydrogen peroxide systems.* Applied Catalysis B: Environmental, 2003. **46**(2): p. 287-292.
88. Ocampo, A.M., *Persulfate activation by organic compound*, PhD thesis, in *Department of Civil and Environmental Engineering*2009, Washington State University: United States.
89. Grosvenor, A.J., et al., *Oxidative modification in human Hair: The effect of the levels of Cu (II) ions, UV exposure and hair pigmentation.* Photochemistry and Photobiology, 2016. **92**(1): p. 144-149.