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Degradation of Melanosomes in Human Hair

by

Camie Parsons

A thesis

submitted in partial fulfillment

of the requirements for the degree of

Masters of Science in the Department of Chemistry

Idaho State University

May 2020

To the Graduate Faculty:

The members of the committee appointed to examine the thesis of Camie Parsons find it satisfactory and recommend that it be accepted.

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Dr. Rene Rodriguez, Major Advisor

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Acknowledgements

I would like to thank Drs. Rene Rodriguez, Karl DeJesus and Caryn Evilia for their patience and willingness to mentor me through the process of this work, even though it is outside their personal areas of expertise and interest.

To my children Jon, Karl, Luke and Emma, I would like to thank them for being sounding boards, unpaid researchers and stalwart supports. I promise I will not talk about melanin or the CMC at the dinner table again!

I would also like to thank my dear friends Christopher Fasel, Mark Waddoups and Dr. Jordan Wade and his beautiful wife Bethany Wade for encouragement in the very dark hours of doubt that this research would ever see the light of day. You have meant more to me than you can possibly know.

To Wyatt Parsons, thank you for the encouragement and financial support. This is a long way from K-mart.

To Jeremy Wade, thank you for building the arrays, and for introducing me to people who could help get this out of my head and into the lab.

Most of all to my sister, Holly Harrison, the strongest woman I know.

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List of Abbreviations

C13	Carbon 13
CO_3	Carbonate
DAA	Dehydroascorbic Acid
DOPA	Dihydroxyphenylalanine
IR	Infrared
H_2O_2	Hydrogen peroxide
M	Molar
nm	Nanometer
NMR	Nuclear Magnetic Resonance
PTCA	Pyrrole-tricarboxylic Acid
ROS	Reactive Oxygen Species
SO_4	Sulfate
UV	Ultraviolet

Degradation of Melanosomes in Human Hair

Thesis Abstract--Idaho State University (2020)

The hypothesis of this work is that if a reactive oxygen species is present in a melanosome in hair that is still attached and then excited by the highest quantum yield wavelengths of light ultraviolet then the melanosome will degrade and create a localized bleach. The theory will be tested in solution and then in hair samples. Melanin degradation in this manner would be far less damaging to the hair structure and skin than current forms of hair lightening. This testing shows that the basis of this theory is correct and this method can degrade melanin in hair.

Key words: melanin, hair, bleach, ultraviolet, degradation, reactive oxygen species, hydrogen peroxide

1. Introduction and Literature Review - Overview of Melanin

Melanin is a biologically ubiquitous molecule. It is found throughout the animal kingdom and organisms use it for many different reasons. Before delving deeper, an understanding of the term “melanin” is in order. Melanin is the common name of any animal pigmentation, in scales, fur, feathers or skin. It also refers to the individual monomer units of dihydroxyindole derivatives and benzothiazine derivatives. These monomer units will oligomerize and form filaments that makeup part of the larger pigment organelle, the melanosome. In this work, I will describe the general monomer units as melanin, the specific units I will refer to by their individual names and the complete organelle as a melanosome.

In humans, there are three main types of melanin; pheomelanin, eumelanin and neuromelanin. Neuromelanin is found solely in the brain and is thought to be a byproduct of the cells efforts to rid itself of toxic levels of quinone derivatives and has been linked to neural degradation leading to Parkinson’s disease1. Eumelanin is the pigment that is the major contributor to brown or black coloring. It is made up mainly of dihydroxyindole (DHI) and dihydroxyindole carboxylic acid (DHICA). The final type of melanin is pheomelanin, the pigment responsible for light brown and red coloring. The individual units are benzothiazine derivatives that have only recently been fully characterized. The basic units are shown below in Figure 1.

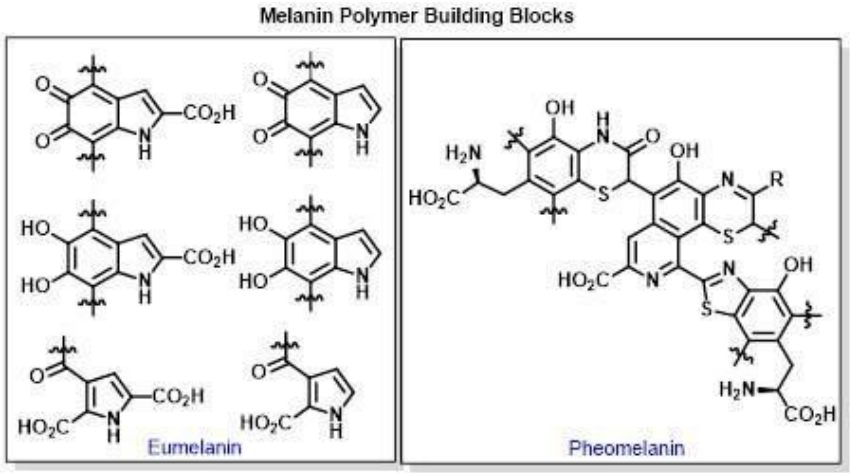

Figure 1. Eumelanin and pheomelanin polymer units[2].

All melanin is formed from the precursor tyrosine that reacts with the enzyme tyrosinase. The product from the tyrosinase is dopa-quinone which will then be formed into leucodopachrome by the intramolecular addition of the amino group, if cysteine is not present. This dopa species can then either be formed into the DHI or into the DHICA. When the DHICA is formed then this dopachrome reacts with the protein tyrosinase-related protein-1. If there are cysteine amino acids present the dopaquinone will react with it to give the intermediate cysteinyl dopa. This species is then oxidized by dopaquinone to give the benzothiazine intermediates that react further to become pheomelanin. This process is shown in Figure 2 below.

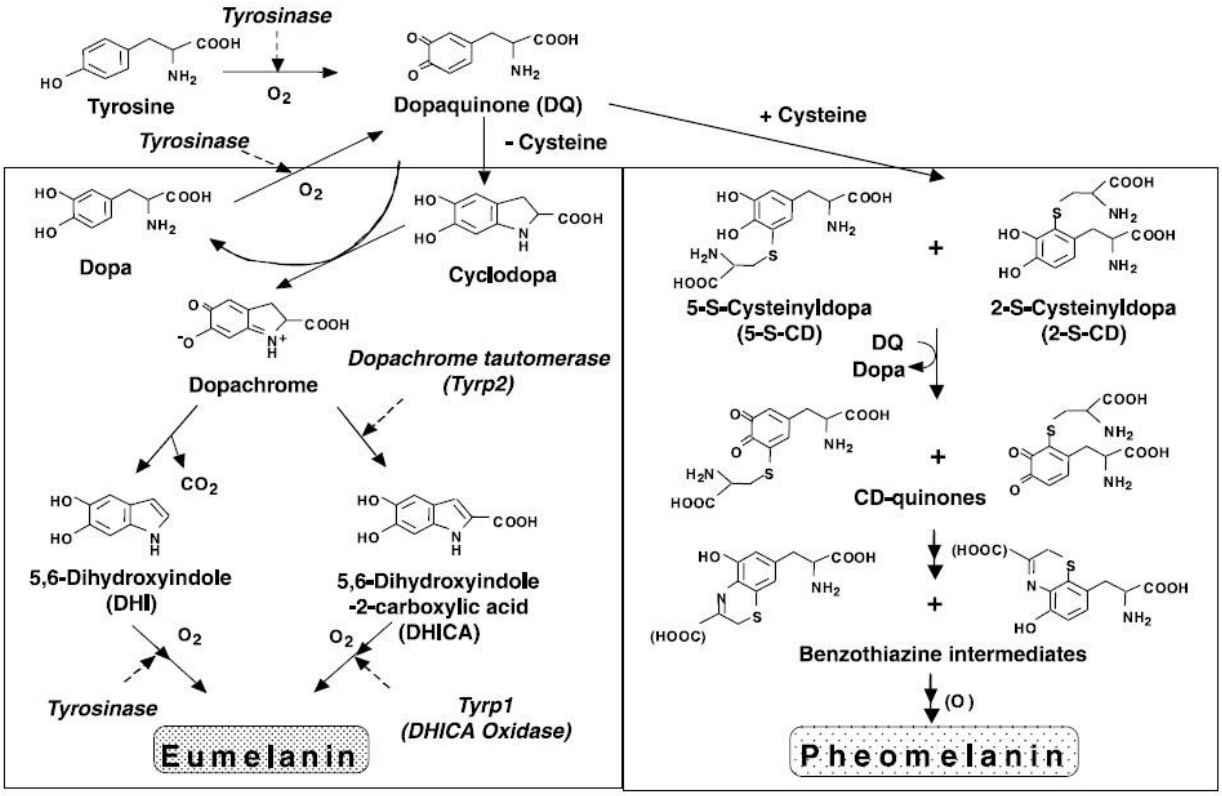

Figure 2. The pathway for melanogenesis[3].

After melanin is formed the monomers oligomerize into sheets. It is believed that the sheets are made up of 4-8 monomer units. These sheets then pi stack to form filaments. The distance between the polymer sheets is about 3.5 angstroms4. These filaments are then incorporated into the larger organelle, the melanosome. It is thought that the eumelanin monomer units oligomerize at specific spots, namely the 4 and 7 carbons on the dihydroxyindole as well as the dihydroxyindole carboxylic acid that add to the aromaticity of the larger structure. This means that there is a high degree of conjugation not only in the oligomer but in the larger macromolecular structure as well. The structure of melanin, unlike other natural chromophores, will absorb across the entire light spectrum with a telltale increase as the

wavelength decreases from the visible toward the UV range. This phenomenon is shown in Figure 3, below. Pheomelanin and eumelanin have been shown to have different absorptive abilities but they both follow the same trend. Other natural chromophores such as chlorophyll and beta carotene will have distinctive peaks where they absorb light at the greatest percentage. The absorption spectra for other natural chromophores is shown in Figure 4, as an example of the ease of identification and characterization. Melanin's broad range absorption combined with an unusually low emission spectra has made it difficult to get an exact picture of the macromolecular structure mainly because most spectroscopy is based on differences in light absorption and emission.

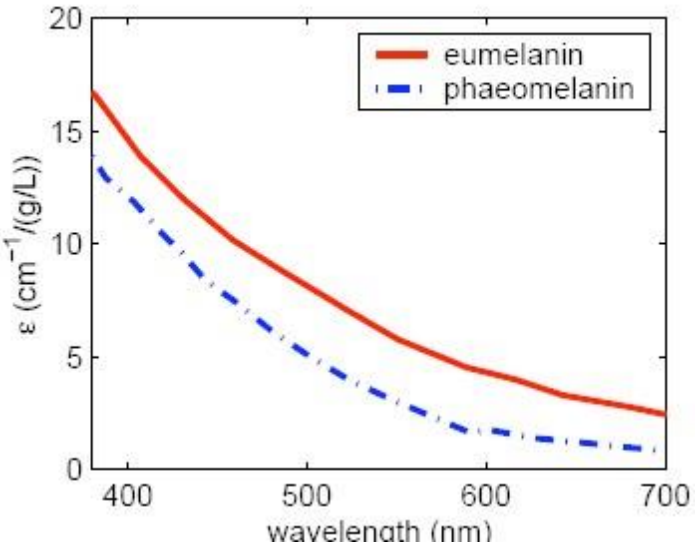

Figure 3. Absorption spectra for eumelanin and pheomelanin molecules[4].

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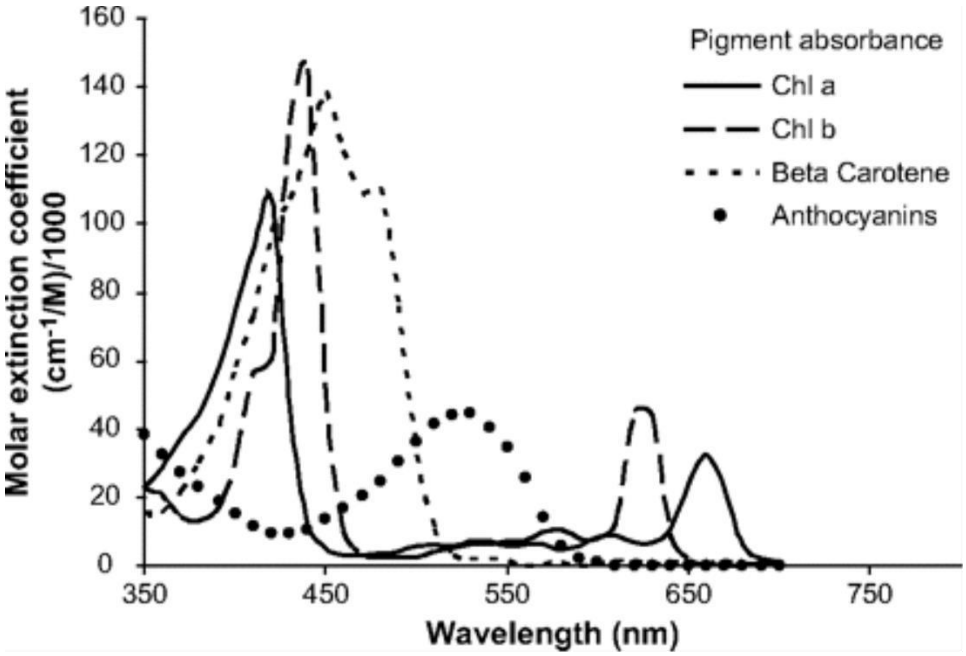


Figure 4. Absorption spectra of natural chromophores. Chl a is chlorophyll a, chl b is chlorophyll b[5]

2. Melanin in the Animal Kingdom

The uses for melanin in the animal kingdom are based on the fact that melanin has some unique properties. It is an excellent absorbing agent of emitted energy, less than 0.1% of absorbed energy will emit off as light, the majority is transformed into heat1. There are types of fungus that use melanin's ability to absorb energy to survive in truly inhospitable environments including the reactor at Chernobyl, nuclear reactor cooling ponds, extremely high altitudes, the Arctic and Antarctic and the International Space Station. These fungi use the absorbed energy to carry out biological processes[6].

There is a canyon in Israel dubbed "Evolution Canyon" that has two vastly different slopes; one slope faces Africa towards the south and receives 200-800% more solar radiation than the slope that face Europe towards the north. There is are species of fungus that inhabit the canyon and have evolved to deal with the different environments. Aspergillius niger was found to have on average three times more melanin on the south facing slope than its near relatives on the north facing slope. This species from the African slope, along with others from the families Alternaria, Humicola, Oidiodendron and Staphiotrichum were found to grow faster when exposed to 4000 Gy of radiation[6]. To put that into perspective, a dose of 30-80 Gy is toxic to humans and will result in death in 2 days to 2 weeks.

Melanin is used by pathogenic fungi including A. fumigatus and C. neoformans in their conidia cell walls. The conidia is the spore of each fungus and the melanin is thought to protect the

developing spore and add strength to the cell wall. The fungi will extract the needed phenolic precursors from the host and use those to synthesize melanin that will protect the spore against UV light, radicals and reactive oxygen and antifungal agents that could cause cell lysis[7].

The wood tiger moth, p. plantaginis uses melanin to enhance thermoregulation. In species that live in more northern climates, the amount of melanin is greater and it is thought to be used to absorb more energy from available light sources to keep the moth warm. The downside of this is that the moths are more visible to predators[8]. Bird feathers that have melanin in them have been proven to be stronger against abrasions and less susceptible to parasites and bacterial degradation[9]. This is due to melanin filling in the spaces between keratin fibers as well as a symbiotic relationship with a bacteria that is only found on feathers that contain melanin.

Melanin will also act as a pseudo dismutase for turning harmful O2 radicals into less harmful species. In human eyes there is a layer of melanin underneath the photoreceptors that are believed to protect the macula from photo oxidative and radical damage. These ocular melanosomes have been shown to be a factor in age related macular degeneration, the more melanosomes in the macula, the less age-related macular degeneration. Albinism in humans is characterized by a lack of melanin. Those who have this genetic mutation have little to no coloration in skin, hair and eyes. This is because of a lack of the enzyme tyrosinase which will convert dopamine to DOPA, the precursor of melanin. Because of this lack of melanin, people afflicted with albinism are more prone to vision problems[10].

Melanin has been shown to be an excellent sunscreen when the largest amount of the chromophore is eumelanin but found to be a factor in an increased risk of skin cancer when the pheomelanin moiety is the largest percentage present. People of African descent have skin cancer rates of 1-2% vs Caucasian people who have a 35-45% chance of developing the same cancers. It is interesting to note that Caucasian people have much higher percentages of pheomelanin than African descendants. Pheomelanin will break down into thiazole-2,4,5-tricarboxylic acid when exposed to the combination of UV and visible light much faster than eumelanin, which will degrade into pyrole-2,3,5tricarboxylic acid[11]. Eumelanin deposits in the skin have proven to be a protection against light degradation of keratinocytes while pheomelanin will break down into species that will react quickly and damage the structure of the melanocyte or keratinocyte. When these proteins are repeatedly damaged it can cause melanoma or the deadlier basal cell and squamous cell carcinomas.

Melanin has been shown to absorb emitted energy very efficiently, yet has very low emission rates. The question is then raised, what does melanin do with the energy it absorbs. The complex pi structure of the polymerized melanin will accept energy and move to a higher energy state. While in the excited state, melanin will pass energy along the pi system which makes the entire system more reactive. Melanin in this excited state is more reactive than the insoluble polymer molecule in the ground state. There have been inroads into using melanin as a renewable source for solar cells and energy storage[12]. In this excited state, melanin in solution has been proven since the 1960s to act as a pseudo superoxide dismutase. Melanin is not a true

enzyme because the reaction ends up breaking the melanin down instead of returning to the original structure as is the case with a true enzyme.

3. Melanin in Human Hair

The melanosome that occurs in human hair has been the main focus of this study. The melanosome is formed in melanocytes in the bulb of hair. The melanocytes are intermingled with the keratinocytes so that as the melanosomes are formed they are deposited into the forming keratin matrix. The hair has two or three distinct layers; the cuticle, cortex and medulla. The cuticle is made up of 2-10 sheets of lipids bonded on both sides of a single protein layer. The cortex is made of micro fibrils of keratin protein called cortical cells that wind in helices. Surrounding the cortical cells is a layer of lipids called the cell membrane complex (CMC). The final layer is not present in all hair types, which is the differentiation between two or three layers. The medulla has not been well studied so most things known about the medulla are conjecture. There are no melanosomes found in the cuticle, there are melanosomes scattered throughout the cortex and the highest concentration is in the medulla. It has been theorized that the medulla has a higher concentration of melanosomes than the cortex because of its structure, but there is no evidence to support that claim. Hair structure is approximately 95% keratin, 3.8% CMC and 0.7% melanosomes[2]. The structure of keratin is that of an alpha helix made up of primarily of sulfur containing amino acids. The exact composition is shown in Table 1 below.

Amino Acid	Percent in Human Hair
Alanine	4.5
Arginine	6.3
Aspartic acid	5.3
Asparagine	0.1
Citrulline	0.3
Cysteic acid	32.9
Glutamic acid	12.5
Glycine	5.8
Histidine	0.8
Isoleucine	2.6
Leucine	5.9
Lysine	2.4
Methionine	0.5
Phenylalanine	1.7
Proline	8.2
Serine	11.9
Threonine	7.4
Tyrosine	2.1
Valine	5.4

Table 1. Percentages of amino acids in human hair keratin from Bradbury.

Melanosomes in hair do not have strictly one type of melanin in them, the many shades of natural hair color are derived from mixing the amount of the macromolecules into the melanosome structure. This mixture, combined with the natural metals that the melanin scavenges from blood during melanogenesis, gives hair its color. Although the makeup of melanosomes is almost as individual as the person, there are a few things that are common in all melanosomes. Pheomelanin melanosomes are spherical while eumelanin melanosomes are ovoid as shown in these atomic force microscopy images[17] in Figure 5.

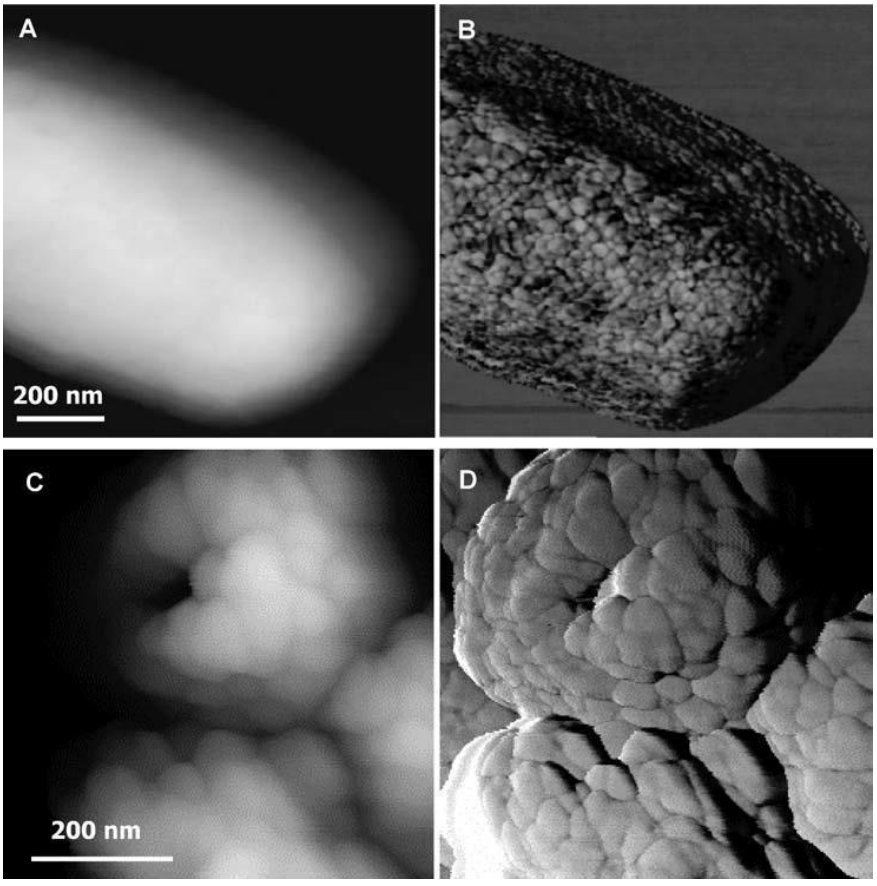


Figure 5. Atomic force microscopy images of red hair melanosomes, A and B, and black hair melanosomes, C and D. A and C are to show height, B and D are to show detail[13].

All melanosomes are distributed throughout the hair shaft as shown in Figures 6 and 7 which are hair samples from the same person, given at the same time. These are microscopic images of bleached hair as compared to regular brown hair. Note that bleached hair still has intact melanosomes in it, there are simply fewer in number than in the darker sample. The bleached hair sample, Figure 7, shows the spread of melanin when melanosomes burst. When melanosomes react with bleach, the membrane surrounding the organelle will burst releasing the melanin and protein into the hair shaft. The yellowish haze seen throughout the bleached sample is evidence of this. The image of bleached hair shows that the medulla, the center of the hair, still appears to have the greatest concentration of intact melanosomes in the sample.

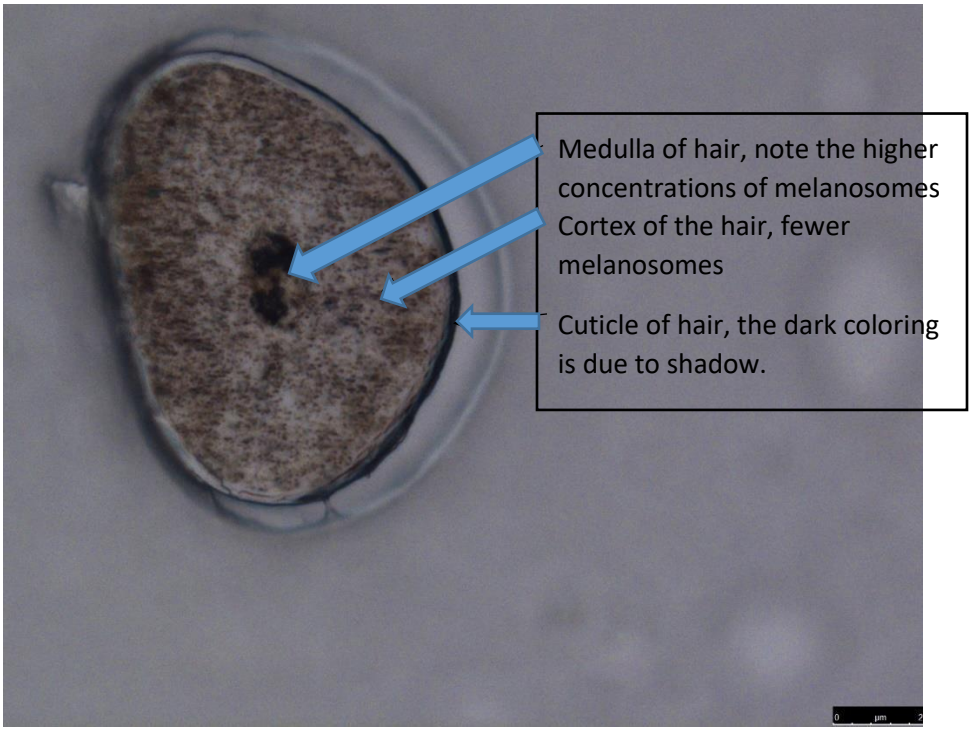


Figure 6. Dark brown hair sample taken from female age 25, no previous coloring, 40X magnification cut to 5 µm width.

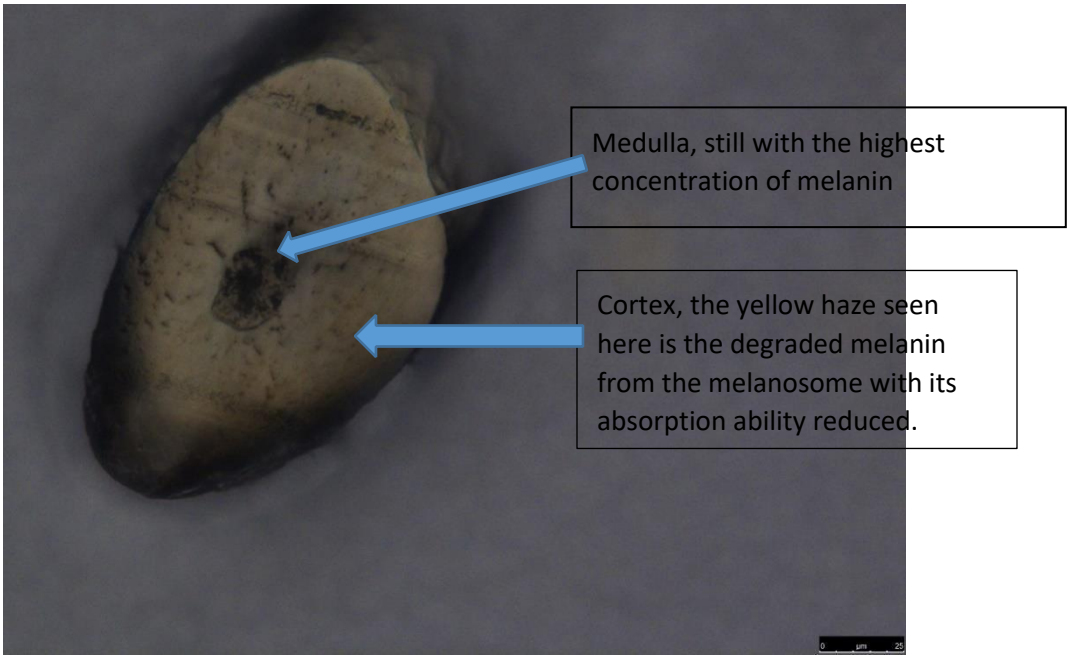


Figure 7. Hair sample from same subject, hydrogen peroxide and potassium persulfate bleach applied to destroy melanin, 40X magnification 5 µm width.

Melanosomes in hair are organelles, they are made up of proteins intermingled with the melanin molecules. In Table 2 the differences are shown between the amino acid content of red hair melanosomes as compared to black hair melanosomes[14].

Amino Acid	Percent nmol (mean±SEM)		
	Black hair	Red hair	Red hair/ black hair
Lysiene	4.41±0.10	4.61±0.30	1.05
Histidine	1.25±0.02	1.11±0.12	0.89
Arginine	7.38±0.10	6.81±0.25	0.92
Aspartic acid	10.08±0.13	9.70±0.04	0.96
Threonine	6.40±0.11	5.96±0.19	0.93
Serine	7.58±0.14	8.03±0.43	1.06
Glutamic Acid	18.05±0.42	21.52±0.67	1.19
Proline	1.13±0.05	1.31±0.17	1.16
Glycine	4.73±0.40	4.35±0.23	0.92
Alanine	7.89±0.10	7.79±0.36	0.99
Valine	8.08±0.14	7.74±0.38	0.93
Methionine	1.27±0.07	1.21±0.27	0.95
Isoleucine	5.01±0.10	4.61±0.11	0.92
Leucine	10.83±0.25	11.9±0.36	1.11
Tyrosine	2.74±0.16	2.82±0.37	1.03
Phenylalanine	3.10±0.09	2.95±0.04	0.95

Table 2. Percentages of amino acids in eumelanin, black hair, melanosomes versus pheomelanin, red hair, melanosomes.

The amount of protein in the human hair as well as the melanosome is a hindrance to traditional bleaching methods because of the sheer amount of possible reactants for the bleach. The table does show that there is not a large statistical difference between red hair and black hair melanosome structure. There is a difference in the volume and number of melanosomes in the different hair colors. Blonde hair has fewer and smaller melanosomes then brown hair does and black hair has the highest concentration and largest macrostructures of all.

4. Degradation of Melanin

Melanin has been tested and shown to produce hydrogen peroxide and hydroxyl radicals by acting as a pseudo dismutase. These radical species can scavenge oxygen in order to produce the superoxide or they will auto oxidize, they will be destroyed in the process of oxidation. There have been different theories of how the hydrogen peroxide is produced but there is evidence to show that it goes through the superoxide anion. The rate of production of the O2 radical is 104-105 M-1 s-1. When superoxide dismutase was added to melanin solution in a phosphate buffer and irradiated with light, the amount of hydrogen peroxide increased[15].

In 2018 a study was published theorizing the exact intermediate in the high energy system of excited melanin. When melanin was exposed to visible light while in water, electron paramagnetic resonance (EPR) showed that a semiquinone radical is formed as well as 2 equivalents of H3O+ [16]. Other studies have shown that oxygen consumption lead to an increase in oxidation of both melanin moieties, both in speed of reaction and number of oxidized particles[17]. Oxygen has come from air, O2, or from reactive oxygen species (ROS). It is worth noting that in the presence of UV-B light keratinocytes will produce ROS, the same keratinocytes that make up the major portion of hair[18].

The amount of oxygen consumed is based on the wavelength used to excite the species. It should be noted that the pigment used in this experiment used synthetic and natural melanin and the natural melanin showed a higher rate of oxygen consumption than the synthetic kind[17].

The rate of oxygen consumption as compared to wavelength would indicate that the rate of the reaction is dependent on the reaction of a single photon with a monomer melanin molecule. Therefore, the higher the energy of the photon, the faster the reaction would proceed. The data in Tables 3 and 4 from Sarna and Sealy shows the quantum yield for oxygen consumption parallels the absorption spectrum in Figure 4. Quantum yield is a term in physical chemistry that is defined as the number of times a specific event, usually a chemical reaction, takes place as compared to the number of photons absorbed. A very common example of this is chlorophyll absorbing light and using the photon energy to make glucose from carbon dioxide. It is generally a ratio of the amount of the completed reaction to the number of photons absorbed at a certain intensity, $\Phi = \frac{mol\ CO2\ converted}{einsteins\ photons}$. Einsteins of light are the measurement of photons, equivalent to moles of a substance. It is common to see Φ as less than 1, except in cases where the light acts as the beginning of a chain reaction. When a species is being degraded, such as the degradation of melanin, it is expected that the number will be less than 1 because it will take the energy of many photons to achieve the desired reaction.

When melanin is oxidized, the overall pi structure is damaged and leads to the destruction of the macrostructure. This loss of the macrostructure reduces its ability to absorb light and is then considered to be “bleached”. The main conclusion drawn from the tables shown below is that as the wavelength decreases, the amount of oxygen used is increased in the reaction. The logical next step is that as more oxygen is consumed, more melanin is degraded. This was the inspiration behind the use of UV light in the reactions that were conducted in this research.

Table 3 shows DOPA melanin, a synthetic variety; AB melanin, a different synthetic melanin and finally melanosomes. Table 4 is a continuation of this wherein Sarna and Sealy used pheomelanins and eumelanins from varying sources under similar conditions to see what differences existed between the chromophores. To quote the conclusion, “The main conclusion from the present work is that natural pheomelanins and eumelanins are of equal efficiency in promoting oxygen consumption and are thus equally subject to net photo oxidation.” If the type of melanin is not a factor in the consumption of oxygen, then melanosomes in hair should use an equal amount and theoretically degrade at a consistent rate.

λ (nm)	DOPA melanin (103 ϕ)	AB-Melanosomes melanin (103 ϕ)	(103 ϕ)
230		4.9	11.5
235	2.7		
240	1.9	10.3	9.3
250	1.2	3.9	5.1
260	1.1	3.3	5.6
270	1.1	2.5	5
280	0.93	2.4	3
290	0.85	1.5	
300	0.66	1.7	
320	0.53	1	1.8
340	0.44	1.3	
360	0.3.	0.84	0.81
380	0.25		
400	0.16	0.4	0.43
420	0.15	0.31	0.31
440	0.09		0.4
460		0.19	
480	0.06	0.16	0.22
500			
540	0.04	0.1	0.19
560			0.22
600	0.05		0.21

Table 3. Quantum yield of melanin of oxygen consumption at varying wavelengths[3].

	Cysteineldopa Melanin	Red Feather Pheomelanin	Black Feather Eumelanin	Red Hair Pheomelanin	Black Hair Eumelanin
λ (nm)	l03 ϕ)	(103 ϕ)	(103 ϕ)	(103 ϕ)	(103 ϕ)
225	9.2	6.5			
230	8.4	4.8		7.3	
235	7.1				
240	6.3	3.7	7	6.7	6.6
250	4	2.6		4.9	
260	2.4	1.9	3.1	4.1	3.9
270	2.1			2.9	
280	1.7	1.2	2.8	2.5	3
290	1.3	0.95			
300	102	0.73		2.2	
320	0.88	0.59	1.6	1.5	1.2
340	0.81	0.48		1.4	
360	0.71	0.37	1.2	1.3	0.53
380	0.47			0.91	
400	0.71	0.29		0.53	0.59
420	0.51			0.3	
440		0.2			
460	0.51				
480	0.31	0.15		0.21	
540		0.11		0.16	
600				0.18	

Table 4. Quantum yield of oxygen consumption using a wider range of melanin varieties[19].

The proposed reactions for oxygen consumption with photo excited melanin are shown below. As can be seen from the proposed reaction of excited melanin in Figure 8, photon excited melanin in the presence of water will create a melanin radical as well as a hydronium ion.

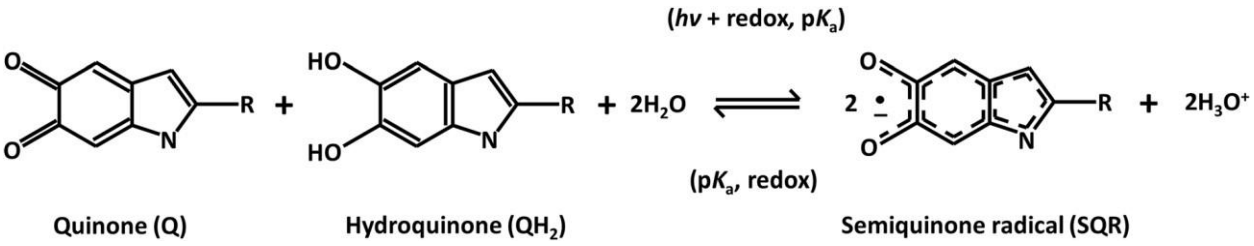


Figure 8. Melanin reacting with water and light to form the radical and hydronium ions[16].

As an example of the reaction and kinetics of an ROS and melanin, Korytowski et al proposed the following reaction of reactive oxygen with melanin. At a rate of 109 M^{-1} s^{-1} this should qualify as a rapid reaction. The proposed reaction is shown below in Figure 9.

$$O_2^- + O_2^- + 2H^+ \xrightarrow{kd} H_2O_2 + O_2$$

$$O_2^- + mel - red \rightarrow K_{red}2H \rightarrow H_2O_2 + melanin\ radical$$

$$O_2^- + mel - ox \rightarrow k_{ox} \rightarrow O_2 + melanin\ radical$$

In the presence of SOD, O_2^- dismutates predominately via the following eq.

$$O_2^- + O_2^- + 2H \rightarrow k_{SOD} \rightarrow O_2^- + H_2O_2$$

(The rate constants are $k_d = 10^7\ M^{-1}s^{-1}$; $k_{sod} = 10^9\ M^{-1}s^{-1}$)(Fridovich, 1977, Korytowski *et al.* in press.)

Figure 9. Proposed reaction of negatively charged oxygen with melanin[15].

There are two components to light, wavelength and intensity. The wavelength is the distance between the successive peaks of the wave, in light this is usually measured in nm. Intensity of light can be measured in irradiance units, power received per surface area. A common unit in light is watts/meter2. Sunlight hitting a beach at sea level when the sun's rays are perpendicular to the ground will deliver about 1000 W/m2. One watt is equal to a joule per second so an equivalent amount of irradiance would be 1000 J/s m2. Of that light approximately 52-55% is infrared, 42-43% is visible light and 3-5% is ultraviolet light. Of the ultraviolet light that makes it through Earth's atmosphere, 95% is UVA and 5% is UVB and UVC. A breakdown of the wavelengths of light is shown below in Figure 10.

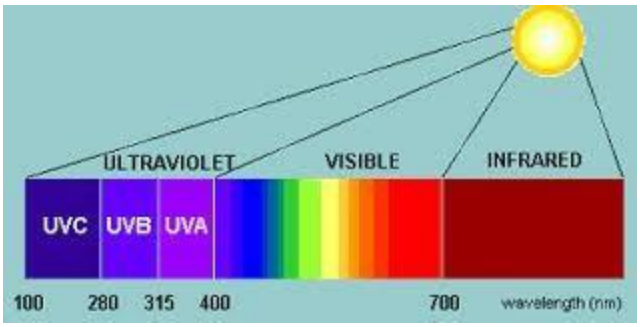


Figure 10. The wavelengths, in nm, of sunlight. Image from californialightwork.com

The amount of energy that is used to excite melanin naturally is mainly made up of visible light if you were using sunlight as a base.

In human skin the absorption of light triggers a response in the melanocyte to produce more melanosomes, hence tanning. In hair, the absorption of light will break down the melanin and in turn the melanosomes and lighten hair, this process is commonly referred to as sun bleaching or sun lightening. Hair is essentially non-living, hair cannot repair itself or change itself once it leaves the follicle so light on the hair does nothing to the physiology of the body. We most often associate hair lightening with excessive time in the sun. The sun bleached, tanned image is one that is highly sought after even though it is associated with prematurely aged skin and higher rates of skin cancer. Anecdotally, it seems that sun bleaching occurs more quickly when

time is spent in the ocean or a swimming pool. Based on the role that ions and water play in the destruction of melanin, this would make sense.

Multiple teams that have researched how light alone reacts with melanin in hair and the data shows that visible light is thought to degrade melanin more than UV light[4,5]. This seemed contrary to the optical properties of melanin but closer examination of the methods reveals the reason behind this apparent disconnect. The researchers used light sources that mimicked sunlight, but as stated previously sunlight is overwhelmingly visible light. In sunlight the small percentage of UV light means there were far fewer photons at the UV energies than at the visible light energies.

Takahashi et al. were successful at destroying melanosomes in hair using light but the energies for UV as compared to visible were 60 W/m^2 and 608 W/m^2, respectively[6]. Even then when they destroyed the melanin the hair was not significantly lighter until the strands were washed. An interesting side note is that the samples were lighter after being washed, theoretically because once destroyed melanin becomes free pyrrole tricarboxylic acid. PTCA, which has multiple sites to hydrogen bond and is polar enough that it should be water soluble. When UV light was used to degrade eumelanin and pheomelanin, the authors noted that when UVB was used at 20% of the intensity of UVB, the degradation occurred at 1/3 of the rate of UVA. This would indicate that UVB should degrade melanin faster than UVA when used at similar intensities[11]. This is supported by the quantum yields found by Sarna et al.

5. Current Methods of Bleaching

The current method of bleaching is to use 6, 9 or 12% hydrogen peroxide combined with persulfate, usually a mixture of ammonium and potassium persulfate. The persulfate is in a mixture that is proprietary to each company but the basics are the same. In a paper published in 2011[20], Imai studied the effect of current bleaching procedures focusing mainly on identifying damage to the superstructure of hair through transmission electron microscopy and energy dispersive X-ray spectrometry equipped with transmission electron microscopy.

The tests were performed using high lift bleach, the kind that utilizes persulfates and a gentler, less effective mixture that uses simply ammonia and hydrogen peroxide to degrade melanin. The study used melanin free hair, referred to as white hair, and black hair. Both sets of samples had no previous chemical damage done. As previous works have noted, the process of applying strong oxidizing agents to hair not only effects the melanosomes in hair but will also attack keratin proteins, most specifically the disulfide bonds of cystine linkages. It also degrades the cell membrane complex which is the protective layer.

The work shows that bleaching hair will decrease tensile strength, increase friction on hair strands and decrease the amount of intact cystine and as a result increase cysteic acid. One interesting thing noted in this work was that white hair showed less damage than black hair. The author theorized this was because of the relatively high concentration of metals in the melanosomes due to metal scavenging. When this higher concentration of metals would react

with the hydrogen peroxide and persulfate, the metal would catalyze the reaction and cause further damage.

The final statement of this work is very important and the reason I undertook this work-

“Further study will lead us to the edge of the development of a new bleaching agent, which reacts only with melanin granules and causes the minimum of damage to outside the melanin granules.”

The danger that people face to lighten their hair goes beyond just damage to the hair. The combination of chemicals frequently does nerve damage to stylists who use the product, severe scalp and skin burns, damage to mucus membranes and lungs. This is a solution that is desperately needed.

6. Scope of work

The purpose of this research was to use light and biologically safe chemicals to degrade melanin only, without damaging the rest of the hair structure. To degrade melanin there either needs to be an extended period of light exposure or a combination of hydrogen peroxide and a reactive oxygen species. As has been shown, melanin when exposed to light will create hydrogen peroxide in the melanosome as long as water is present. The hypothesis is that if an ROS is present when the melanin is excited by the highest quantum yield wavelengths then the melanosome will degrade and create a localized bleach. This would be far less damaging to the hair structure and skin than current forms of hair lightening.

This research will include testing multiple biologically gentle ROS, sodium carbonate, ascorbic acid, glycine and potassium bisulfate. The pKa of the bisulfate ion solution is 1.99, the pKa of the conjugate acid of carbonate is 6.4 and ascorbic acid has an initial pKa value of 4.10, the secondary is 11.6. These species were chosen because they all have reactive oxygen sites and have a history of safe use in haircare products[21].

In the paper by Marsh et al. they use hydrogen peroxide, ammonium carbonate and glycine to destroy melanin. It is proposed that hydrogen peroxide reacting with carbonate will form a new oxidant species, peroxymonocarbonate ion. In a paper by Clarke et al. the carbonate radical was found to react in the presence of ammonia and oxygen to carbon dioxide and NH_2O^{-7}. When this radical was reacted with synthetic eumelanin the second order rate of reaction was found to be

3 x 105 dm^3/mol s and the second order rate of reaction with synthetic pheomelanin was 2 x 108 dm^3/mol s. When reacting hydrogen peroxide with sulfate, the peroxymonosulfate ion is formed, but this reaction has not been found by the author. Ascorbic acid reacting with hydrogen peroxide is thought to create threonic acid, but it is possible that reacting ascorbic acid with melanin in the excited state could be enough to cause further depolymerizing of the melanin structure, however this is purely theoretical, no supporting work has been found. The other species used were potassium citrate, potassium hydroxide, citric acid, and glycerin.

The aim of this work is to first test the differing strength ROS with hydrogen peroxide and melanin in solution, then test the same melanin solution with varying strengths of hydrogen peroxide and stable amounts of ROS. The amount of hydrogen peroxide strengths are to first mimic the amount that would be produced by irradiating with light and then increasing in strength to mimic the concentrations used in the haircare industry.

Spectroscopic methods will be used to gather data on kinetics and products, UV-Vis to study kinetics and IR and NMR to identify products. From there, tests will be repeated using differing intensities and sources of visible and UV lights, specifically UVB light at 290 nm with an intensity of 1.0 W/m^2 and full spectrum visible light with an intensity of 2 W/m^2. This will be compared to the kinetics and products of the hydrogen peroxide reactions. Next the solutions of ROS species will be tested on donated hair samples to compare to the solutions, having made predictions about the reactions from the solution tests. Through experimentation it was

concluded that neither the ROS alone or the hydrogen peroxide alone, whether externally or created in situ, alone will be an effective method of degradation. The aim is to test if together they will be enough to create a relatively safe, rapid method of destroying melanin superstructure and thereby bleaching hair.

7. Methods and Results

The method for proving or disproving the hypotheses was through a series of tests: proof of concept, molarity variations, hydrogen peroxide variations, kinetics, light and dark reactions and finally identification of products. The reactants in each set of tests depended on determining which reactants from previous tests were successful. For this work, the tests will be listed first as an overall group, first describing the methods and then the results will be reported. Using the successful reactants and concentrations from the previous tests, the next step was to test the melanin and successful ROS using UV only lights, visible only lights and a combination of both to test efficacy and time. Then it was tested if the ROS could be absorbed be absorbed into the hair and finally to test if the ROS inside the hair would react with hydrogen peroxide and in a separate test if the ROS/hair would react with the lights. The products from hydrogen peroxide and light catalyzed reactions were filtered and dried. These were analyzed IR. The supernatant from the UV was analyzed using carbon-13 NMR to look for evidence of formation of the peroxy ions formed.

It should be noted that the light used was left on a hair sample over the course of a weekend with no visible change and ROS was placed in solution with melanin with no visible change after 30 minutes, the same test was done with hydrogen peroxide and melanin only with no visible reaction.

7.1 Hydrogen Peroxide Catalyzed Reactions

The first set of tests was to determine which of the ROS would be the most effective catalyst in the reaction with hydrogen peroxide present. These solutions were tested by performing the series of reactions in Chart 1.

ROS	pKa	Hydrogen Peroxide Strength
Sodium Carbonate	6.4	12%
Ascorbic Acid	4.10/11.6	12%
Potassium Bisulfate	1.99	12%
Dehydroascorbic Acid	3.9	12%
Citric Acid	3.1, 4.7, 6.4	12%
Potassium Citrate	8.5	12%
Potassium Hydroxide	10.98	12%
Glycerol	14.15	12%

Chart 1. List of Possible ROS for hydrogen peroxide catalyzed reactions.

Test 1. Proof of Concept Method

The first set of tests were a proof of concept- whether using a simple ROS and hydrogen peroxide is sufficiently oxidizing enough to delaminate melanin into a lighter colored species. This test was run using hydrogen peroxide, 12%, and 1 molar concentrations of each ROS. The following section lists describes the procedure and the reactants for the first set of tests. For the test to be deemed successful, the solution of 1 mL each of 0.7% melanin, 1M ROS and 12% hydrogen peroxide when combined in a test tube should become significantly lighter in color. The only element that changed was the ROS, all other variables such as temperature, ambient light and concentrations remained the same.

Test 2. 1M concentrations of ROS, Varying H_2O_2 Percentages Method-

The next set of tests moved the variable from molarities of ROS to percentages of hydrogen peroxide. There is a limited amount of hydrogen peroxide that can be formed from degrading melanin so if that is a limiting factor it will inhibit completion of the result. The conditions were the same as with other tests, 1mL of each of the three solutions; melanin in solution, hydrogen peroxide and ROS. The tests were first to determine if the reactions would be successful in producing a significantly lighter solution and secondly to determine, in a broad sense, kinetics. The tests were timed, and timing started as soon as all three reactants are in solution together. The reaction were determined to be complete when the solution is equivalent to a standard that was prepared using commercially available bleach, 12% hydrogen peroxide and 0.7% melanin in DMF. The ROS, molarities and percentages are listed in Chart 2 below.

Molarity	Hydrogen Peroxide Strength
1 M	12%
1 M	6%
1 M	3%
1 M	1.5%

Chart 2. Solutions strengths needed testing varying hydrogen peroxide in hydrogen peroxide catalyzed reactions.

Test 3. Molarity variations, 1M, 0.5M. 0.1M and 0.05M ROS Method- This test was done with varying concentrations of the viable species of ROS to determine if the reaction is dependent on ROS concentration, again using visual confirmation with 1 mL of each solution at room temperature and ambient light. The solutions are listed in Chart 3 below.

Molarity	Hydrogen Peroxide Strength
1 M	12%
0.5 M	12%
0.1 M	12%
0.05 M	12%

Chart 3. Solutions needed for varying molarities of ROS in hydrogen peroxide catalyzed reactions.

Test 4. Kinetics Method-

These series of tests were performed to determine the dependence of the rate of the reaction on the ROS concentration, the hydrogen peroxide concentration, and/or the melanin concentration assuming the rate law for the melanin degradation is:

$$\text{rate} = k\,[\text{ROS}]^x\,[\text{H2O2}]^y\,[\text{melanin}]^z$$

Given this, the tests looked for concentrations that the reaction is dependent upon.

The only light in this set of reactions was from the instrument. The chart below shows the concentrations used in the cuvette, naturally the final concentration of each reagent was 1/3 of the initial because it was diluted in combining each, but for convenience the original concentrations will be listed.

Figure 11, shown below, is the chart of the reaction parameters as well as a spectrum of the absorbance of the production Figure 11. The product of the reaction was filtered and the absorbance was tested, this is Figure 12.

% H2O2	W/W Melanin to DMF	Molarity of CO3	Test
6	2.1 x 10 ^-3	1 M	1
6	1.05 x 10^-3	1 M	2
6	5.25 x 10^-4	1 M	3
12	1.05 x 10^-3	1M	4
3	1.05 x 10^-3	1M	5
6	1.05 x 10^-3	0.5 M	6
6	1.05 x 10^-3	0.25M	7

Figure 11. Solutions needed for kinetics tests.

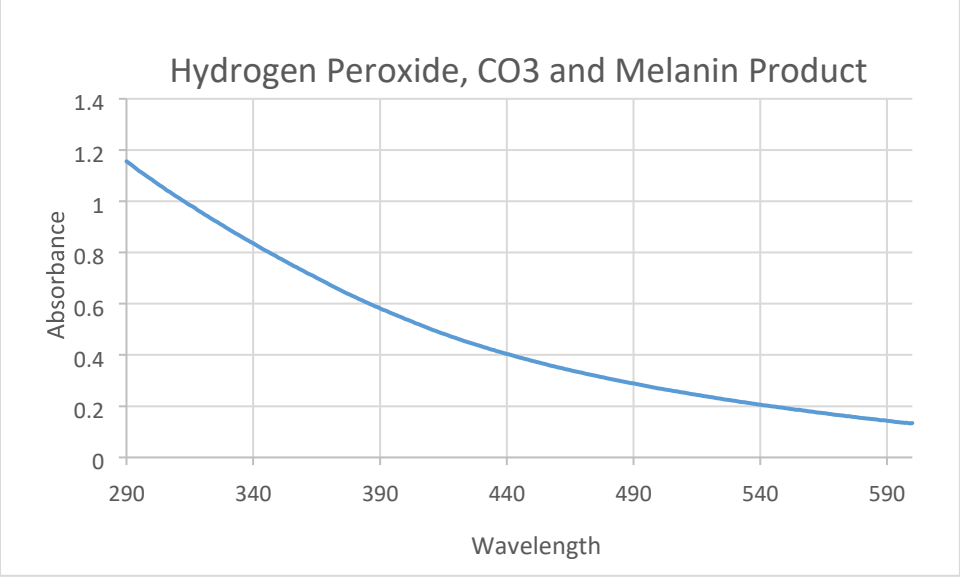


Figure 12. UV-Vis spectrum of the product of the first set of reactions.

Test 1. Proof of Concept Result

Sodium carbonate and potassium bisulfate were the most reactive species as is shown in Table 5. The reaction time was determined through calculating the difference between the time when it started and the time when the solution stopped reacting. The reactions would initiate by the formation of bubbles, solution beginning to lighten and large, brown precipitate formation. As it proceeded the solution would continue to bubble and the precipitate would dissolve. When the reaction finished the solution would be a light, golden brown with no visible precipitate. This was done at ambient light and temperature, so any help with the reaction would be the same for every test. Previously, control tests with ROS and melanin only were run as well as another test of only hydrogen peroxide and melanin. Neither of these tests showed any reaction so they were dismissed.

Species	rxn begins	rxn complete	rxn begins	rxn complete	rxn begins	rxn complete
Na2CO3	20	135	22	135	24	135
KHSO4	45	200	42	210	45	200
Dehydroascorbic acid	NR	NR	NR	NR	NR	NR
citric acid	NR	NR	NR	NR	NR	NR
potassium citrate	NR	NR	NR	NR	NR	NR
potassium hydroxide	NR	NR	NR	NR	NR	NR
glycerin	NR	NR	NR	NR	NR	NR

Table 5. Reaction times for different ROS, each 1M, 12% hydrogen peroxide and melanin solution; time in seconds.

This data is consistent with the information found by Marsh et al that found using a peroxycarbonate ion is sufficiently reactive to cause the destruction of melanosomes in hair[22].

The dehydroascorbic (DAA) acid showed no reaction, no lightening or bubble formation. The potassium citrate, citric acid, glycerin and potassium hydroxide showed were the same, they no reaction.

Test 2. Molarity Variations Results-

The ROS that were shown to be effective were tested in solutions of melanin and 12% hydrogen peroxide. The times were again recorded of when the reaction began and when it ended. The visual confirmation of the reaction and of the different concentrations of ROS and their efficacy is shown in Figures 13 and 14 below. The first image shows the beginning of the reaction, all test tubes were filled at approximately the same time, the second shows the reaction at 250 seconds. This is shown as visual proof of the reaction to enlighten the reader. As is shown in Table 5, the time for reaction completion using sulfate was significantly slower than carbonate. This led to focusing mainly on the peroxymonocarbonate ion as the ROS species rather than peroxymonosulfate or any of the other ions created by reacting the ROS with hydrogen peroxide.

The results of the reactions are shown below, Figure 15 shows a chart of the reaction times, Table 6 shows the reaction times of the sulfate reaction in seconds and Table 7 shows the averages and standard deviations of the carbonate reactions.

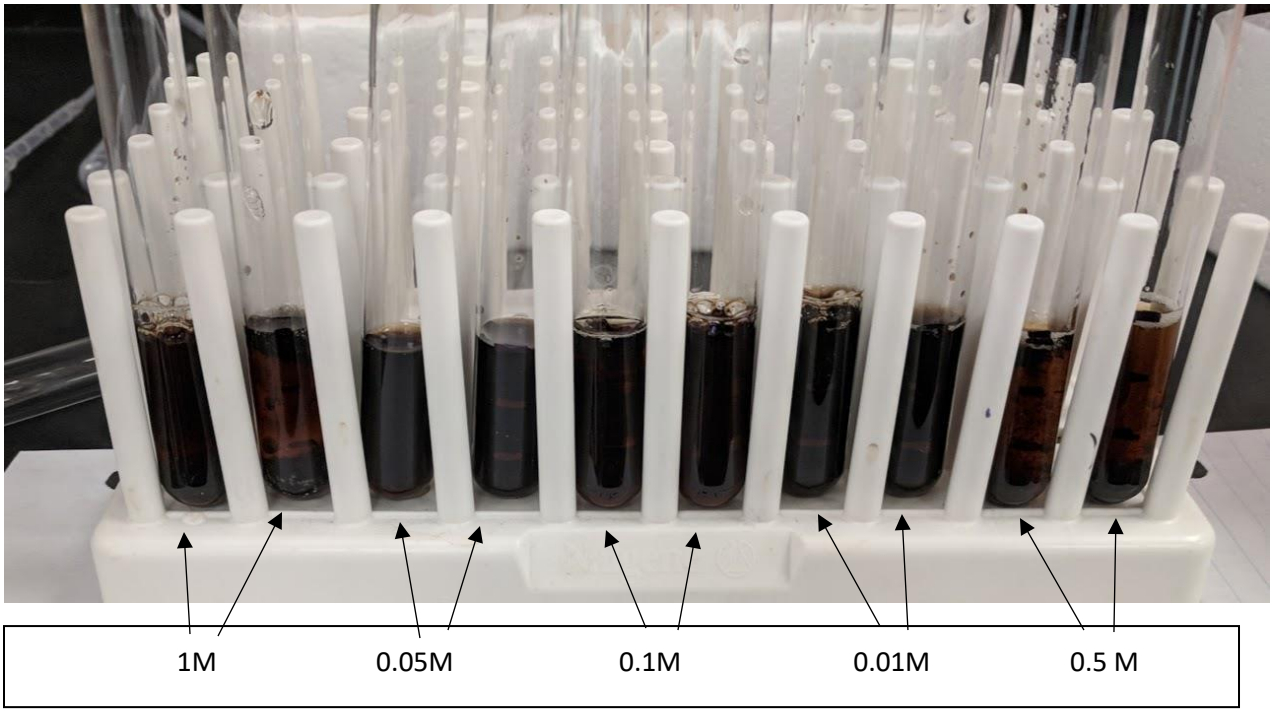


Figure 13. The solutions with 1 mL each of sodium carbonate, melanin in solution and 12% hydrogen peroxide as the reaction begins.

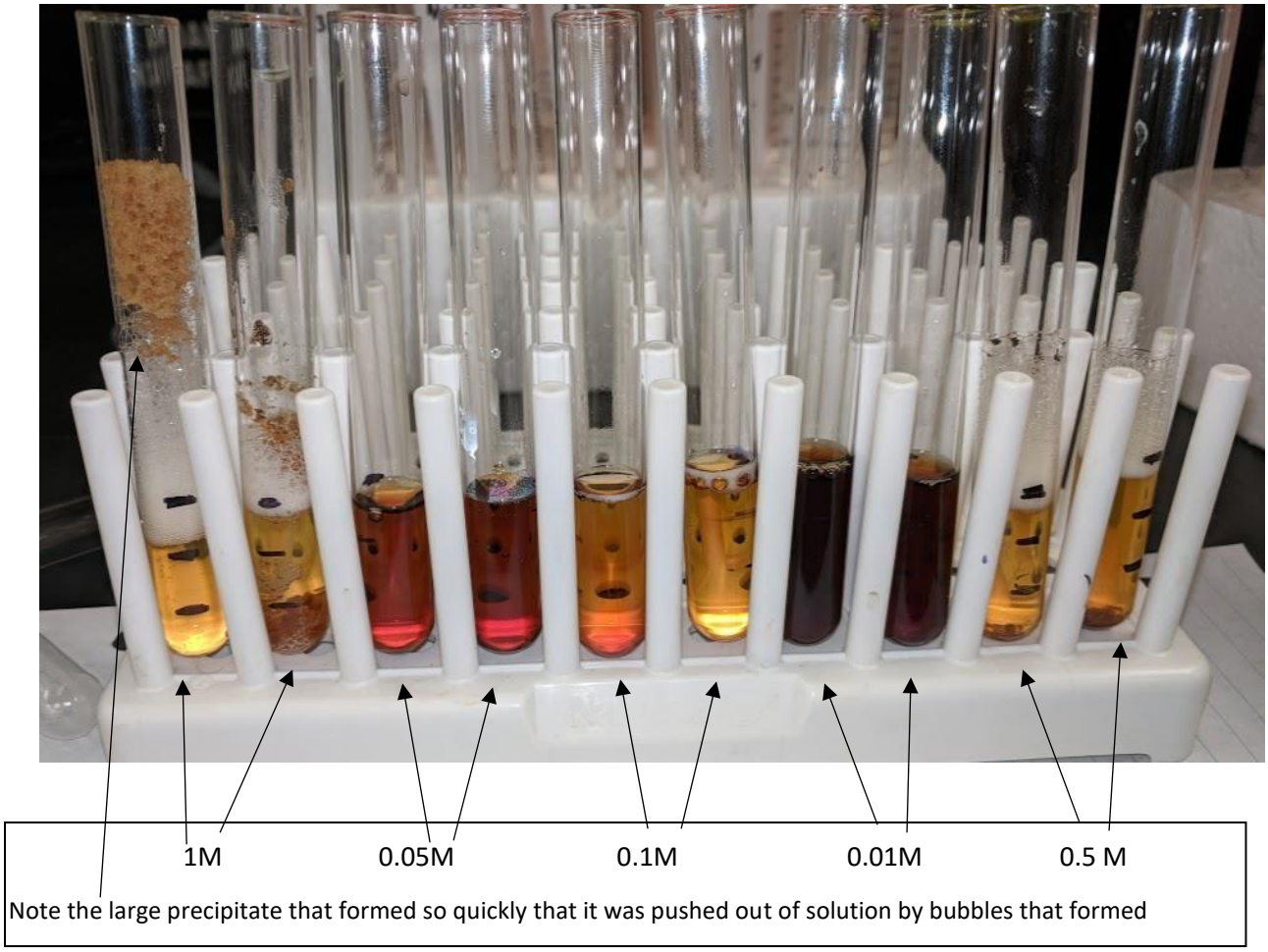


Figure 14. The same tests as above at 250 seconds.

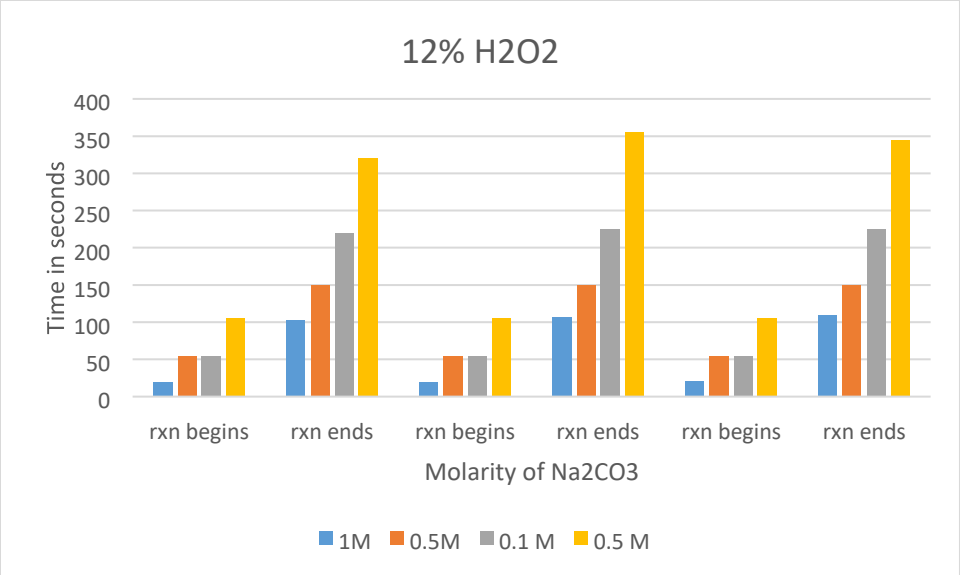


Figure 15. The reaction times for 12% hydrogen peroxide and differing molarities of sodium carbonate.

sulfate solution	rxn start, sec	rxn end, sec
1M	120	300
0.5 M	200	375
0.1M	320	420
0.05M	NR	NR

Table 6. Time of reaction for 1M KH2SO4 using 1 mL each of sulfate, 12% hydrogen peroxide and melanin solution, time is in seconds.

Na2CO3	H2O2 %	averages	std dev
1M	12	86.67	2.52
0.5M	12	96.00	0.00
0.1 M	12	168.33	2.89
0.5 M	12	235.00	18.03

Table 7. Average reaction times and standard deviation for 12% hydrogen peroxide, melanin and varying Na2CO3.

Test 3. Percent Hydrogen Peroxide Variation Results-

When the percentage of hydrogen peroxide was varied there was no clear advantage to using a higher concentration of the hydrogen peroxide. The data is shown in Figure 16 below. The data follows the previous pattern, showing when the reaction begins and when the reaction is complete. Table 8 is the average and standard deviation of the reaction times as well.

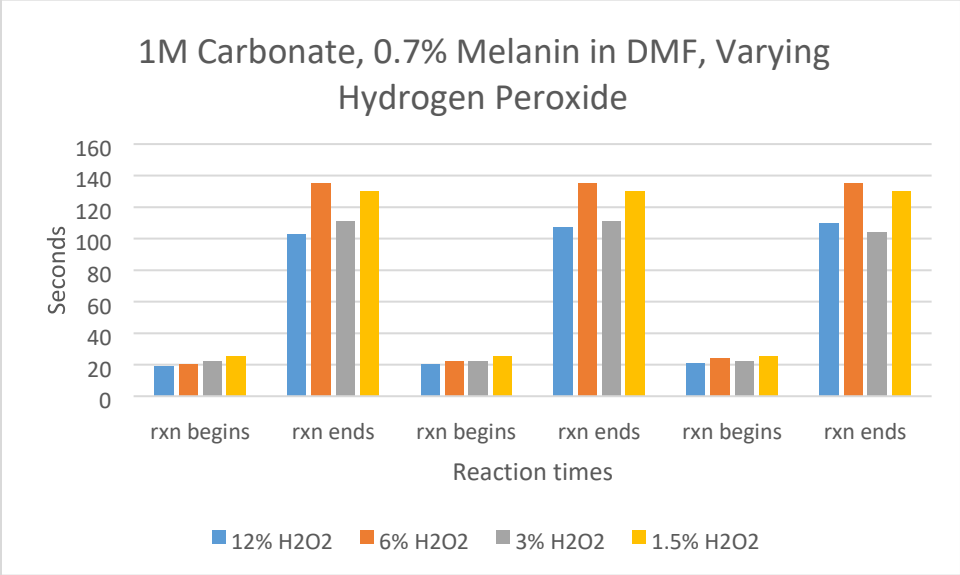


Figure 16. 0.7% Reaction times of melanin in DMF with 1 M Na_2CO_3 and 1.5, 3, 6 and 12 % H_2O_2

Na_2CO_3	H_2O_2 %	Avg. Reaction Completion Time (s)	std dev
1M	12	86.67	2.52
1 M	6	113.00	2.00
1M	3	86.67	4.04
1M	1.5	105.00	0.00

Table 8. Average times for completion of reaction, including standard deviations for 1M Na_2CO_3, melanin solution and varying hydrogen peroxide tests.

Test 4. Kinetics Results

The absorbance of the reaction mixture, at a particular wavelength, was recorded with the different initial molarities of ROS, percentages of H_2O_2 and percentages of melanin to monitor the rate of degradation. The instrument used was a Thermo Scientific Genesys 10S UV-Vis, each scan was set for 5 seconds and the slit width was 1.5 nm. This was done using a quartz cuvette to ensure that UV and visible light could penetrate and wouldn't be absorbed, as is the case with glass, or that the solvent wouldn't degrade the cuvette, as is the case with plastic. The

tests were performed at 550 nm, with a consistent total scan time of 2 minutes with data collected every 5 seconds. The initial concentrations are listed in Table 9.

% H_2O_2	W/W Melanin to DMF	Molarity of CO_3	Test
6	2.1 x 10 ^-3	1 M	1
6	1.05 x 10^-3	1 M	2
6	5.25 x 10^-4	1 M	3
12	1.05 x 10^-3	1M	4
3	1.05 x 10^-3	1M	5
6	1.05 x 10^-3	0.5 M	6
6	1.05 x 10^-3	0.25M	7

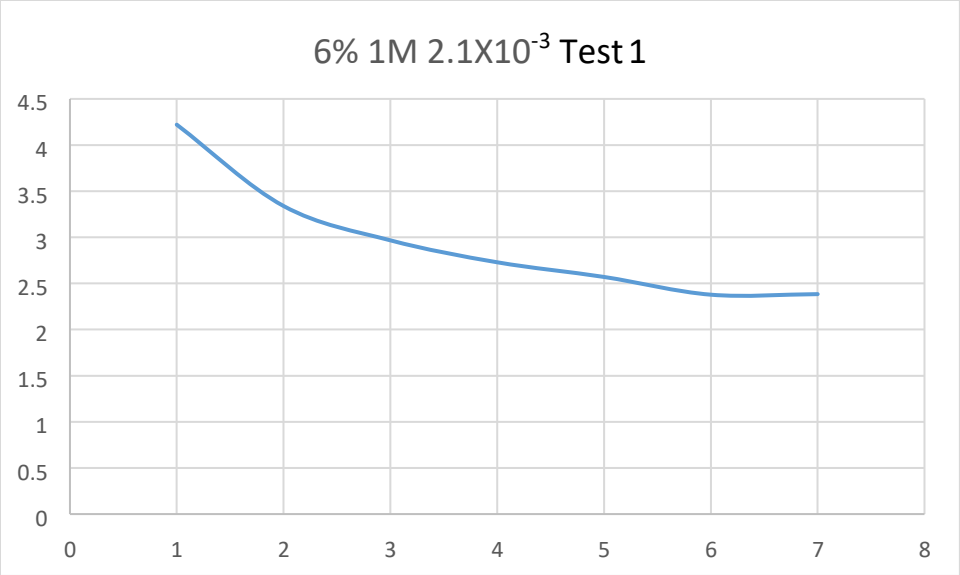
Table 9. Solutions used for kinetics tests.

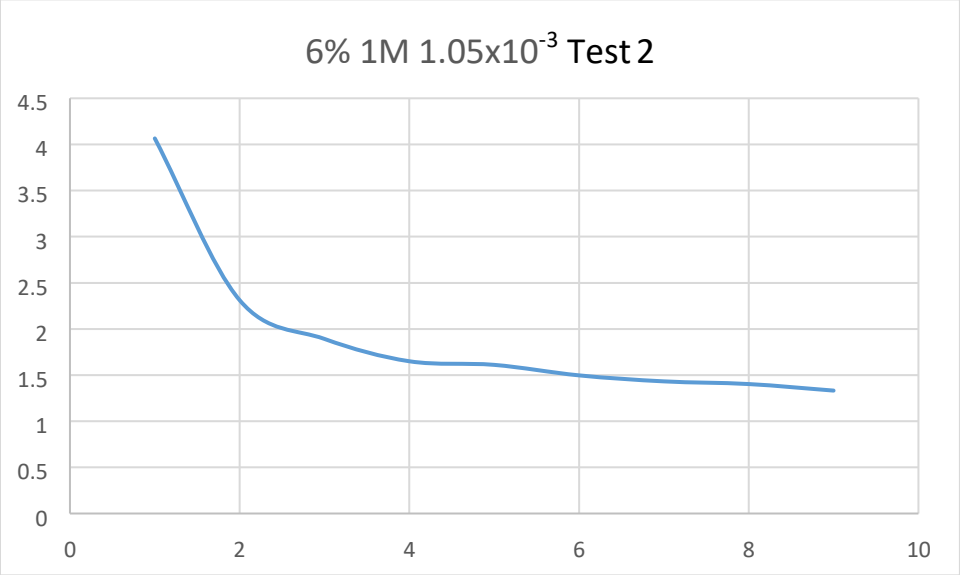
These conditions were chosen in an attempt to use the initial rate method to determine the rate law for the reaction assuming that it was of the form:

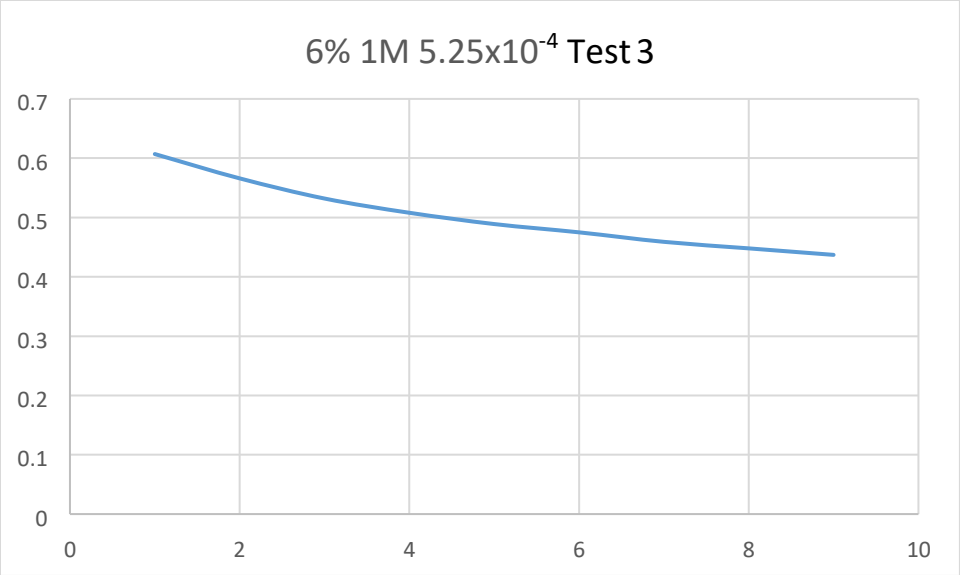
$$\text{rate} = k\,[\text{ROS}]^x\,[H_2O_2]^y\,[\text{melanin}]^z$$

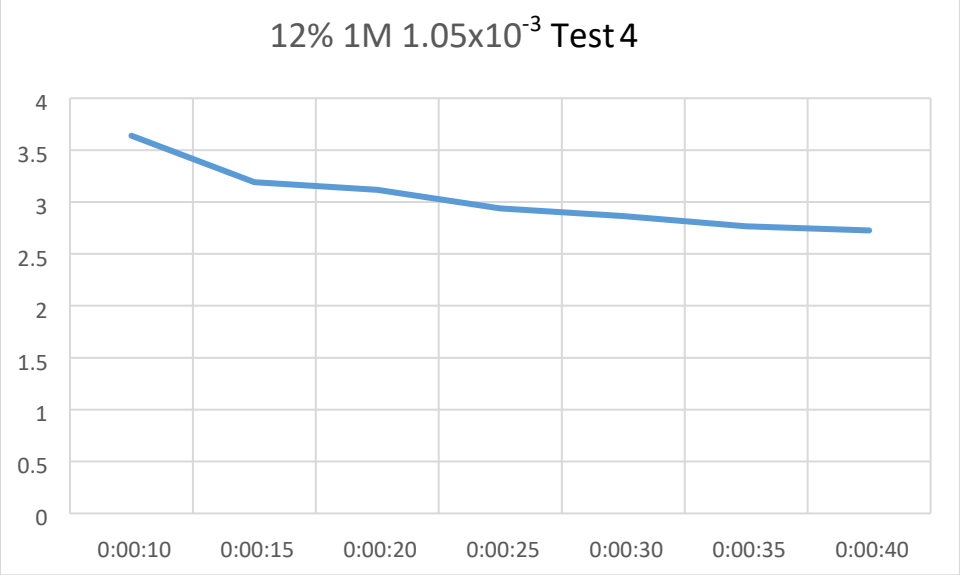
Note that in experiments 1, 2, and 3 the concentrations of the carbonate and hydrogen peroxide were held constant and only the melanin concentration was varied to allow for the determination of the value of z. In tests 2, 4, and 5, the concentations of the melanin and the carbonate were held constant, and only the hydrogen peroxide concentration was varied to allow determination of the value of y. Lastly, in tests 2, 6, and 7, the concentrations of melanin and hydrogen peroxide were held constant and only the carbonate concentration was varied to allow determination of the value of x.

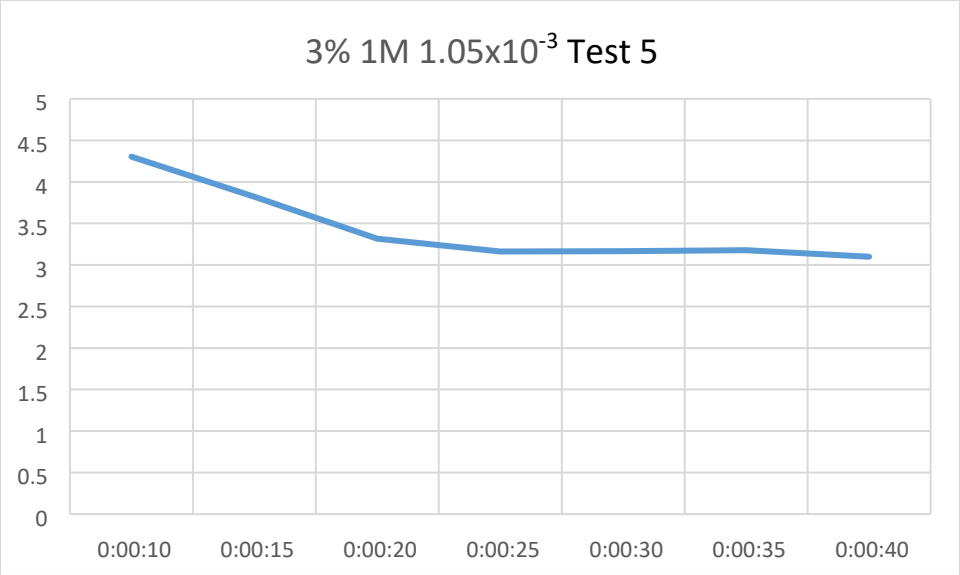
The initial rates were determined from the scans, in Figure 17, and these showed that melanin will absorb consistently, even as it degrades. Below are the scans and the calculations to determine absorbance.

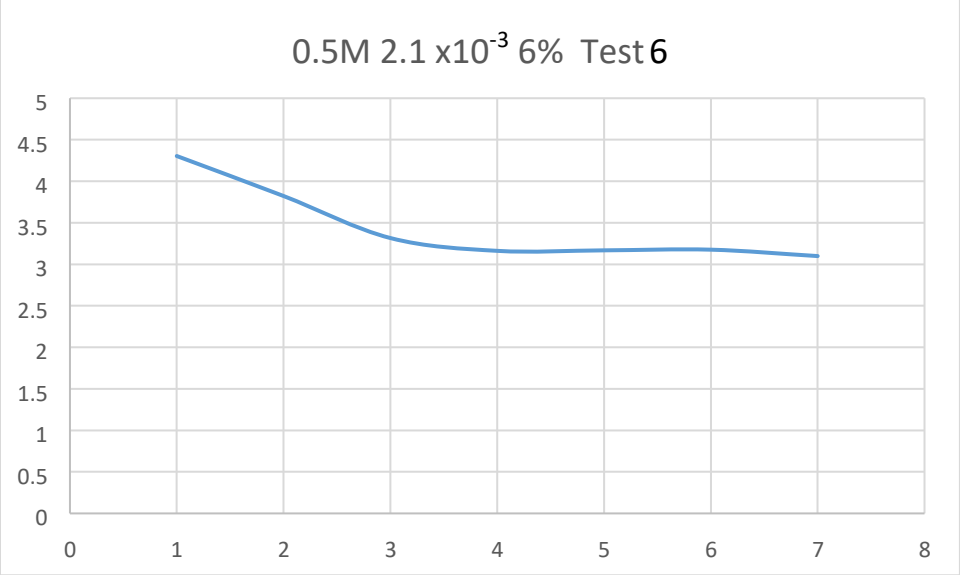

6% 1M 2.1X10-3 Test 1
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
0
1
2
3
4
5
6
7
8


6% 1M 1.05x10-3 Test 2
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
0
2
4
6
8
10


6% 1M 5.25x10-4 Test 3
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
2
4
6
8
10


12% 1M 1.05x10-3 Test 4
4
3.5
3
2.5
2
1.5
1
0.5
0
0:00:10
0:00:15
0:00:20
0:00:25
0:00:30
0:00:35
0:00:40


3% 1M 1.05x10-3 Test 5
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
0:00:10
0:00:15
0:00:20
0:00:25
0:00:30
0:00:35
0:00:40


0.5M 2.1 x10-3 6% Test 6
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
0
1
2
3
4
5
6
7
8

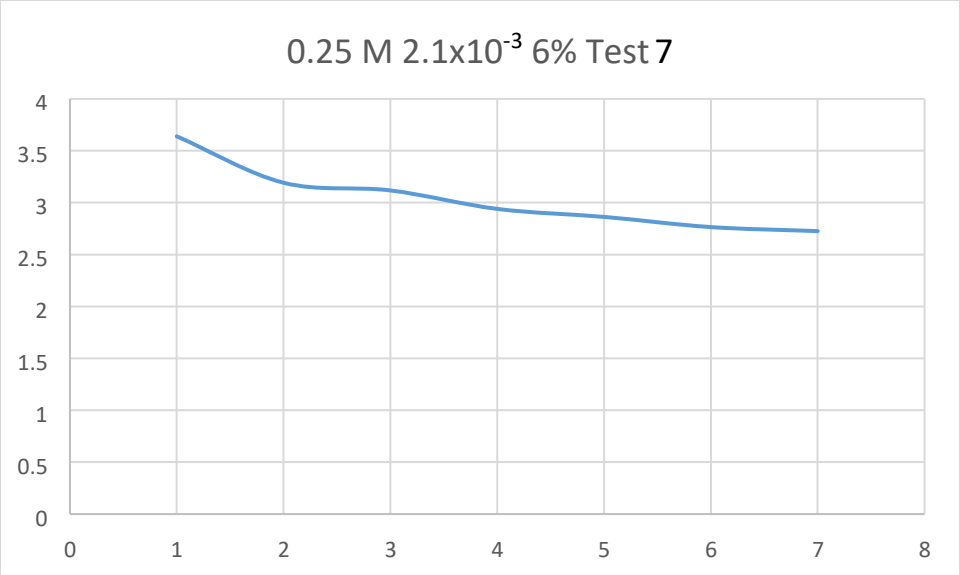


Figure 17. Scans of kinetic tests Tests 1-7

As the calculations in Table 10 show, the data is inconsistent so no real conclusions can be drawn. The equation used to solve for the coefficient of the rate of reaction is as follows. Using Beer's Law, $A=\varepsilon lc$, A is absorption, ε is the molar absorptivity or extinction coefficient, l is the path length of the sample and c is the concentration. Using this equation you can compare the concentration of a known substance, like the melanin at the beginning and the absorbances to find the concentration of melanin at the end of the reaction. Comparing those we can find the coefficients of the reaction of this equation.

Solutions	Delta Abs	Test	1 and 2	2 and 4	2 and 6
6% 1M $2.1x10^{-3}$	1.837	1	-0.573133833	1.181452313	1.181452313
6% 1M $1.05x10^{-3}$	2.733	2			
6% 1M $5.25x10^{-4}$	0.17	3	2 and 3	2 and 5	2 and 7
6% .5M $1.05x10^{-3}$	1.205	4	4.006878808	0.790899347	-1.581798695
6% .25M $1.05x10^{-3}$	0.913	5			
3% 1M $1.05x10^{-3}$	1.205	6	1 and 3	4 and 5	6 and 7
12% 1M $1.05x10^{-3}$	0.913	7	1.716872487	0.400346381	-0.200173191

Table 10. The calculations to determine the coefficient for the rate of reaction equation.

As is evident from this table, since the ratios of the equations used to determine x, y and z do not have consistent values, no real conclusions can be formed regarding the values of x, y, and z in the rate equation. One possible explanation for this can be seen in the absorbance spectrum, see Figure 18 below, of the new, water soluble product. It is similar to the absorbance of unreacted melanin, so it is unlikely that true kinetics could be understood using this method.

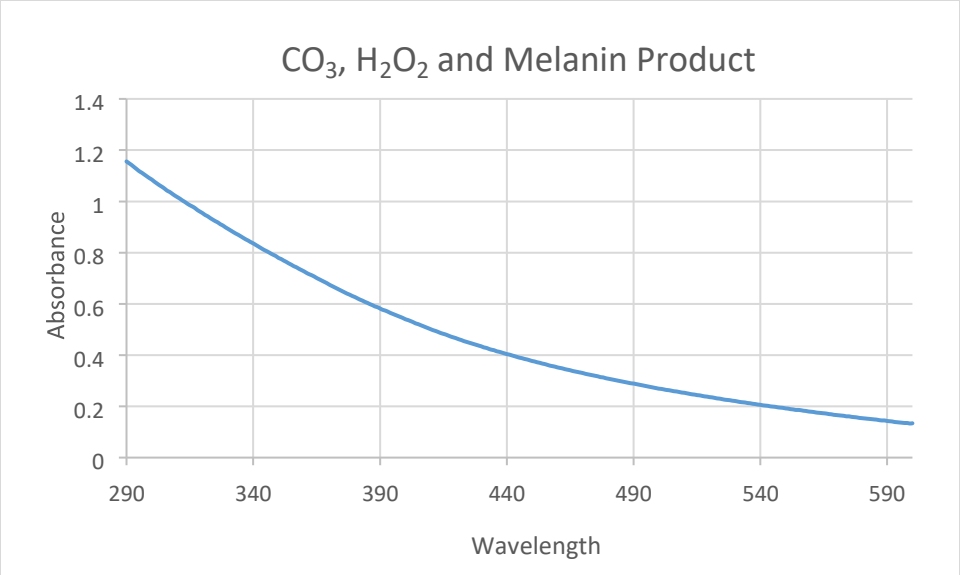


Figure 18. Absorbance spectrum of water soluble product of carbonate, melanin and hydrogen peroxide.

7.2 Absorption of ROS in Hair

Test 5. Absorption of Carbonate into Hair Method-

To see if this is a species that would be absorbed into hair via the ion channels of the cell membrane complex, samples were weighed on a Mettler Toledo balance before and after soaking in 1M Na_2CO_3. The samples were weighed at room humidity, soaked in the solution for 45 minutes, taken out and left to air dry. As soon as the samples were removed from ROS solution, they were rinsed with deionized water to ensure that any weight change came from absorption of the ROS into the interior of the hair and not just collection on the exterior of the shaft. When the samples dried, they were weighed again. The test was performed at room

temperature because heat will affect the absorption rates of ions into the interior of the hair. The samples were then left to dry overnight to assure that any water in the sample was due to ambient humidity and was approximately equivalent to that of beginning weights. The samples came from volunteers, and have had no previous chemical services. There were three samples at hair level 5 and three at hair level 7. The hair level scale is standard in the beauty industry, level 1 is dark black and level 10 is white blonde.

Test 5. Hair and Carbonate Reaction with Hydrogen Peroxide Method-

The hair samples that absorbed reactive species were divided and one set was placed in a room temperature hydrogen peroxide solution and a the other set was placed in a hydrogen peroxide solution that was heated 90° C. Both solutions were of 12% and 1.5% strength to see if the strength of hydrogen peroxide made a difference in the rate of reaction.

Test 5. Absorption of Carbonate in Hair Results

The data is shown in Figure 18 and shows that the hair will absorb the carbonate species through the channels in the cell membrane. This figure also shows a control of hair that was soaked in deionized water and left out to dry overnight.

	Test		Control	
Sample	Initial Mass CO3 sample, g	Initial Mass CO3 sample, g	Initial Mass DI Sample, g	Final Mass DI Sample, g
Blonde 1	0.468	0.621	0.574	0.570
Blonde 2	0.268	0.388	0.345	0.346
Blonde 3	0.435	0.528	0.644	0.645
Black 1	0.378	0.501	0.834	0.832
Black 2	0.426	0.622	0.512	0.512
Black 3	0.371	0.498	0.872	0.870

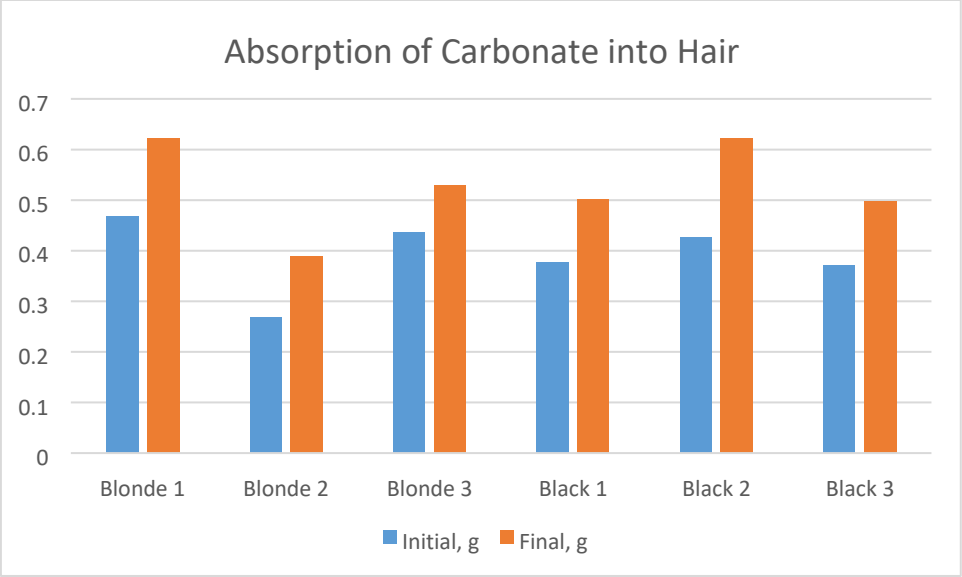


Figure 19. Absorption of sodium carbonate and control in untreated hair

Test 6. Hair and Carbonate Reaction with Hydrogen Peroxide Results-

At 10 minutes using room temperature hydrogen peroxide there was no change in the color, most likely due to the inability of hydrogen peroxide to reach the carbonate species. When the test was repeated with the addition of heat at approximately 3:00 minutes the samples in 12% hydrogen peroxide began reacting by producing bubbles and lightening in color. The entire process was finished at 5:30 minutes and achieved the average color of normal bleaching at 5:30 minutes, images are below. The test with 1.5% hydrogen peroxide produced similar results, no reaction until 3:10 and then the process was complete at 5:18. The method of determining the extent of bleaching is to use the hair color scale mentioned above. This was done visually with calibrated eyes (tongue in cheek). The images below, Figures 19 and 20, are the visual proof of the extent of lightening.

Figure 20. Results of sodium carbonate and hydrogen peroxide on level 5 brown hair.

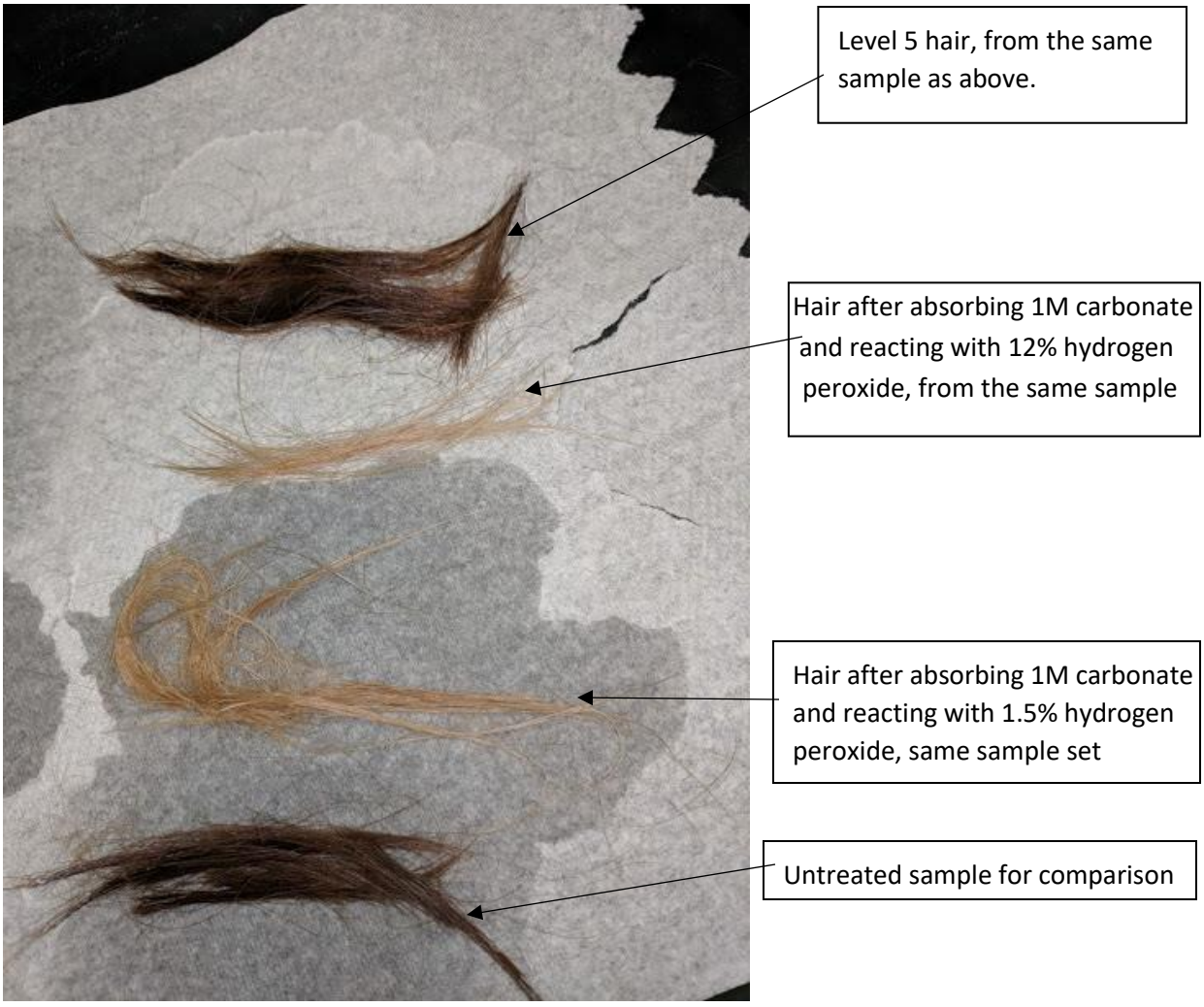


Figure 21. Results of sodium carbonate and hydrogen peroxide on level 5 hair, before and after.

7.3 UV and Visible Light as Catalyst

Test 7. Using Light as a Catalyst Method-

Previously all tests have an external source of hydrogen peroxide as opposed to having it come from the degraded melanin that Sarna, Sealy and others have proposed, see degradation of melanin, pg. 18 of this work. The tests from this point forward all used light in varying wavelengths to excite aqueous melanin to induce degradation and create hydrogen peroxide by reacting with the ROS. If these tests prove valid then this method of bleaching will be significantly less damaging to the hair, the scalp and the stylist using it.

The ROS species tested were using UV as the catalyst to produce hydrogen peroxide from melanin. Na_2CO_3, $KHSO_4$ and dehydroascorbic acid (DAA) were used as the ROSs. Initial tests showed that 1M was too concentrated and gave reaction times were too quick to read accurately so the concentration were reduced to 0.5M. The light source is from an array of 290 nm, LEDs at an intensity of 1W/cm^2 and a visible light array across the spectrum at an intensity of 3 W/cm^2. The samples were placed in a quartz cuvette that will allow all wavelengths of light to pass through. 1 mL each of the melanin in solution and ROS were added to the cuvette. The light arrays were affixed to strips of copper in an effort to transfer heat away from the reaction and keep the arrays in a configuration that will allow light to reach the entire height of the cuvette. The cuvette and light array were enclosed in a reflective chamber so that the only light in the reaction was coming from the array and any light that passed through the cuvette would then be reflected back to the experiment.

The rationale behind using both UV and visible is that melanin absorbs across the entire spectrum of light. Tests were performed using UV light only, UV and visible light and in a dark environment, limiting all ambient light. The purpose of the dark experiment was as a control, to determine if the reaction would go to completion without light as the catalyst. With the dark experiment all conditions were identical except light and the created heat. Theoretically the heat would not be a deciding factor, but future testing could prove or disprove this.

Test 7. Using Light as a Catalyst Result-

The reactions proceeded in a similar fashion to the hydrogen peroxide catalyzed reaction. The solution would bubble, precipitate would form and if left alone the precipitate would dissolve and the solution would be a light golden color. If the reaction was filtered while precipitate was still present it could be analyzed. There was heat produced from the lights, but the researcher couldn't find a way to have lights not produce any ambient heat. It was thought that any created heat would also be useful in completing the reaction. Using an IR thermometer, the heat found to be produced was never allowed to be higher than 38°C, on advice of the engineer who fabricated the light arrays. This temperature is far below the regular temperature for tools used on hair, which can range from 93°C to 176°C.

When this method was tested on hair, difficulty getting the reaction to go to completion was encountered because the samples would dry out and this would inhibit the reaction from

completing. When hair was washed it would lighten significantly, again confirming the conclusion that the new species created was water soluble even in hair. Figure 21 shows the test of carbonate saturated hair exposed to UV light for 3:00 after washing. Tables 11 and 12 show the results, products and times from the light catalyzed reactions.

Figure 22. Results of sodium Carbonate and UV on level 7 hair, before and after. The top is the control, untreated sample and the bottom is the test sample.

ROS	Visible light	UV Light	UV and Vis
Sodium carbonate	Precip, water soluble	Precip, water soluble	Precip, water soluble
Potassium Bisulfate	Precip, water soluble	Precip, water soluble	Precip, water soluble
Dehydroascorbic Acid (DAA)	precip, not water soluble	precip, not water soluble	N/A

Table 11. Results and products of light catalyzed reactions.

	Source	ROS	exp 1 in min	exp 2 in min	exp 3 in min
Melanin	UV	Na2CO3	1:50	2:00	1:50
Melanin	UV/Vis	Na2CO3	1:50	1:50	1:52
Melanin	Dark	Na2CO3	10:00	NR	NR
Melanin	UV	KHSO4	2:30	2:35	2:40
Melanin	UV/Vis	KHSO4	2:50	3:00	2:40
Melanin	Dark	KHSO4	12:50	NR	NR
Melanin	UV	DAA	2:10	2:40	2:35
Melanin	UV/Vis	DAA	2:40	3:00	2:30
Melanin	Dark	DAA	NR	NR	NR

Table 12. Reaction times of different species ROS, 0.5M, reacting with melanin, 0.7%, under different light conditions.

7.4 Analysis of Solid Intermediate Formed During Melanin Degradation

The product that was formed, if filtered quickly before completely degraded, would create a solid that was water soluble. This solid could give clues as to how the reaction proceeded and why the new product was water soluble. The analysis includes a comparison of starting material versus the product.

IR of product

Using a Thermo Scientific Nicolet IR iS5 with the iD7 ATR spectrometer, the starting melanin (SM), the solids formed from reacting melanin with carbonate under UV light (M/CO3) and the solids formed from reacting melanin with sulfate under UV light (M/SO4) were analyzed. The spectra are shown in Appendix 1 and are labeled IR 1-4, IR 7-8 in the appendix of this work.

The infrared of SM showed distinctive peaks at 1350 cm^{-1}, 1550 cm^{-1}, 2000 cm^{-1}, 2350 cm^{-1} and a broad, tongue peak from 3050-3450 cm^{-1}. The peak at 1350 cm^{-1} is likely due to the C-N bond of the indole, the 1550 cm^{-1} peak is due to the carbon-carbon double bonds of the aromatic rings. The 2000 cm^{-1} and 2350 cm^{-1} peaks are overtones, peaks that result from the electrons jumping from the base level to the second level, bypassing the first excitation level. The wide tongue peak is in the O-H region, which is due to the dihydroxy groups.

The M/SO4 spectrum is relatively featureless, only showing a significant peak at about 1100 cm^{-1}. Not many conclusions can be drawn from this, it is possible that it could be a C-N bond or a C-

O bond. Given the stability of the indole to oxidation, it is most likely the intact indole, but without more peaks this is only a surmise.

The M/CO3 had a single spike peak 1450 cm^{-1} and a small side peak at 1400 cm^{-1}. The spectrum also showed peaks at 1650 and 1750 cm^{-1}. There was a small peak at 2980 but was missing the wide tongue peak upfield of the O-H region. The downfield peaks are likely C-N stretch at 1650 cm^{-1} and a C=C peak at 1450 cm^{-1}. The 1750 cm^{-1} peak is in all likelihood due to a C=O stretch. The spectrum indicates that the product still has double bonds, the intact indole and a carbonyl group where the phenol groups were. This change is enough to delaminate the melanin and the product to lighten. However, some of these peaks are unlike typical peaks, so this is a broad interpretation. The basis for this interpretation is the proposed mechanism and similar product found by other researchers[12].

NMR of Supernatant

The solution of degraded melanin and carbonate, if hydrogen peroxide was formed, should contain the new ion, peroxy monocarbonate. The reaction hinges on the creation of this ion, if the ion is present then it confirms that melanin will produce hydrogen peroxide in a sufficient quantity to complete the reaction. Carbon-13 would be an ideal test to run to look for this species.

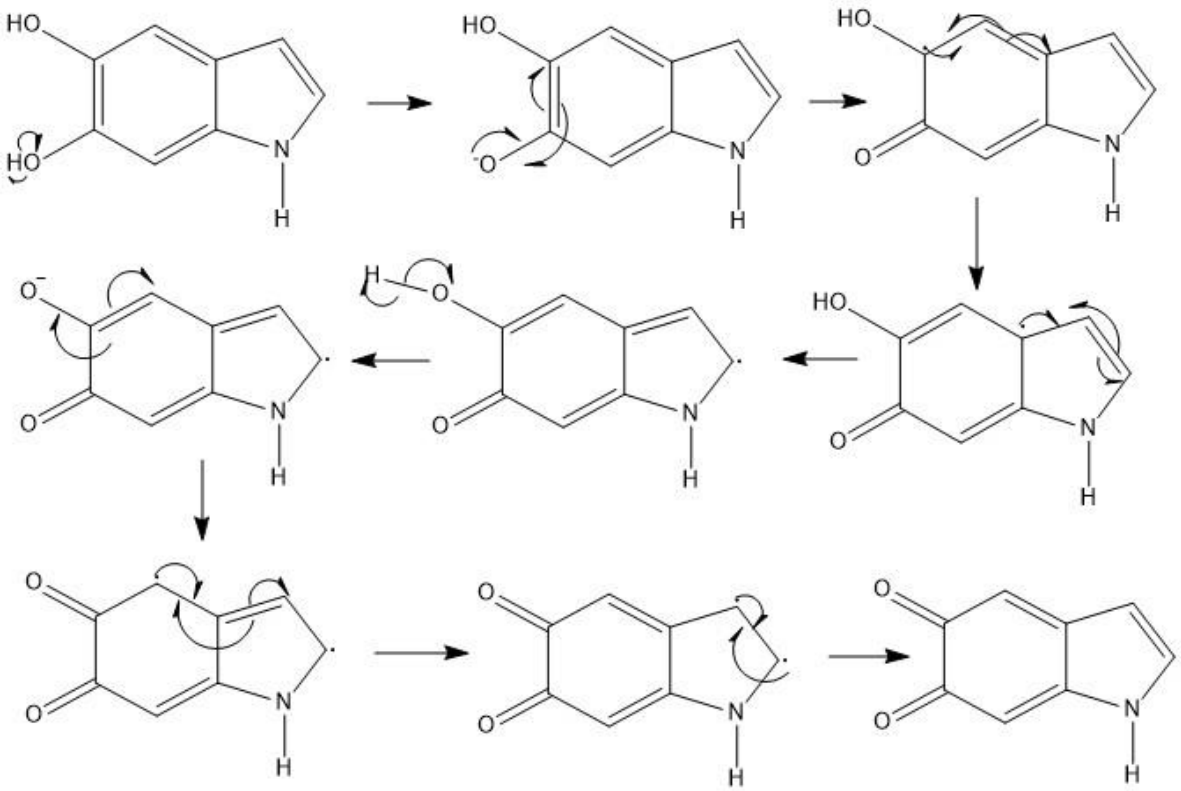
The supernatant from these reactions was run through C13 NMR using a 300 Hz Jeol instrument for the purpose of identifying the peroxymonocarbonate species. This spectrum is shown in

appendix 1 and is labeled as NMR 1. As was shown in the Figures 23, 24 and 25, the peroxy ion is sufficient to break down the melanin to an appropriate level that would be commercially desired. The purpose of the NMR testing was just to prove that the melanin under UV light conditions would degrade enough to produce hydrogen peroxide in a sufficient quantity to react with the carbonate and produce the peroxy ion.

When carbonate is processed in C13 NMR, the expected single peak is at 160[23]. In the spectrum of the supernatant there was the expected carbonate peak at 160.8. The spectrum also showed a similar sized peak at 170.3. A spectra simulator showed that the peroxymonocarbonate should be at 166 but the simulations do not allow for the two partial double bonds that will further de-shield the carbon which would move it downfield, so the peak at 170.3 could be interpreted to be the product of carbonate and hydrogen peroxide produced from the degraded melanin.

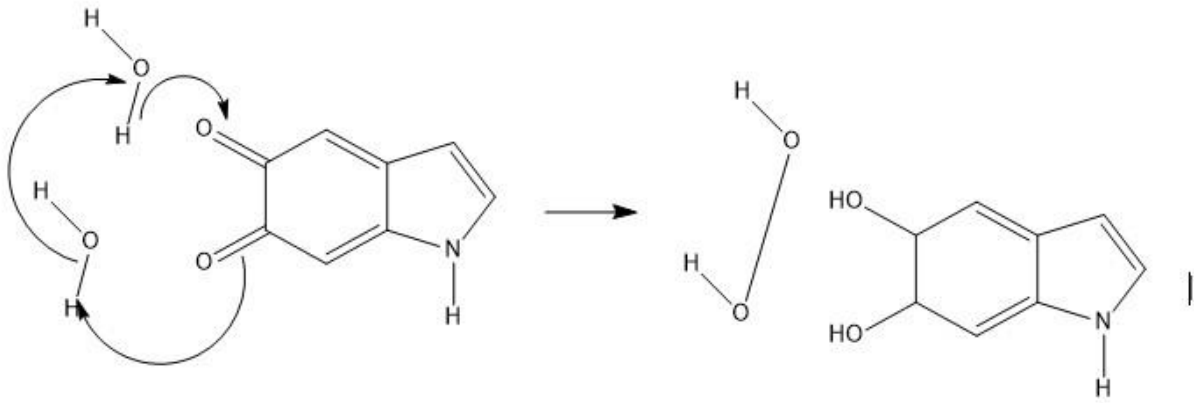
8. Proposed Mechanism

The proposed mechanisms for these set of reactions is as follows. First, we have the excitation and degradation of the melanin monomer units. It has been shown that excited melanin will transition from the hydroquinone to the semiquinone and from the semiquinone to the quinone. The hydroquinone does not deprotonate, it will dehydrogenate meaning the hydrogen will take its electron with it. The second hydroxy group will also dehydrogenate, and this molecule is more reactive than the incredibly stable melanin starting material as shown in Mechanism 1 below.

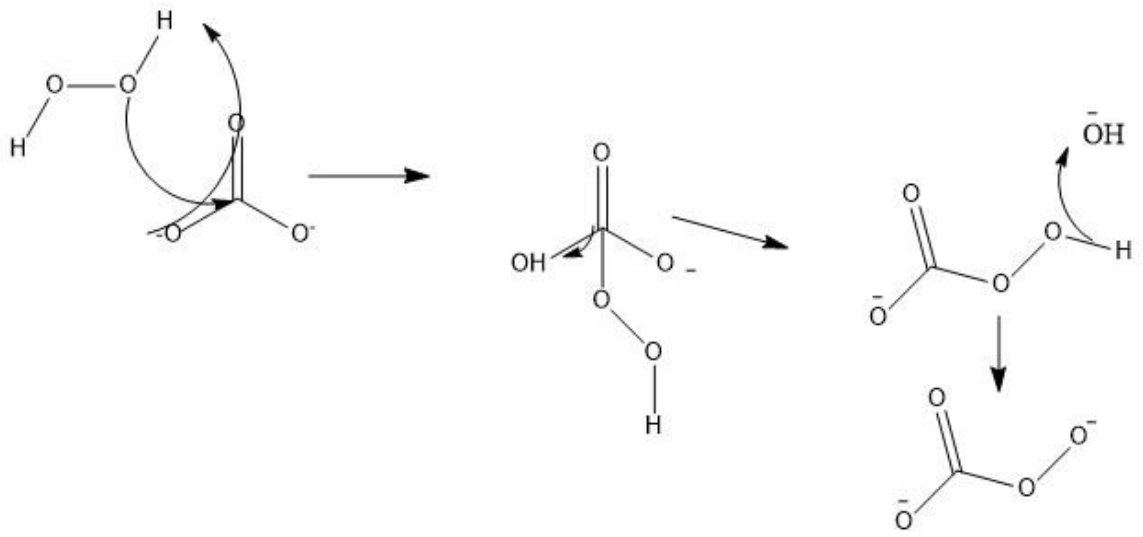


Mechanism 1. Excitation and degradation of starting material.

The second step of the proposed mechanism uses the excited product using 2 equivalents of water. The excited product from mechanism 1 would react in an electron pushing reaction that would result in 1 equivalent of hydrogen peroxide and the melanin starting material forming a molecule that is no longer aromatic. In order for bleaching to occur, the aromaticity has to be disrupted only enough to delaminate the filaments. Theoretically, once the hydrogen peroxide forms it will combine with the carbonate to make the peroxycarbonate species. The formation of hydrogen peroxide is shown in mechanism 2 and mechanism 3 shows the reaction of carbonate and hydrogen peroxide to form the peroxy species.

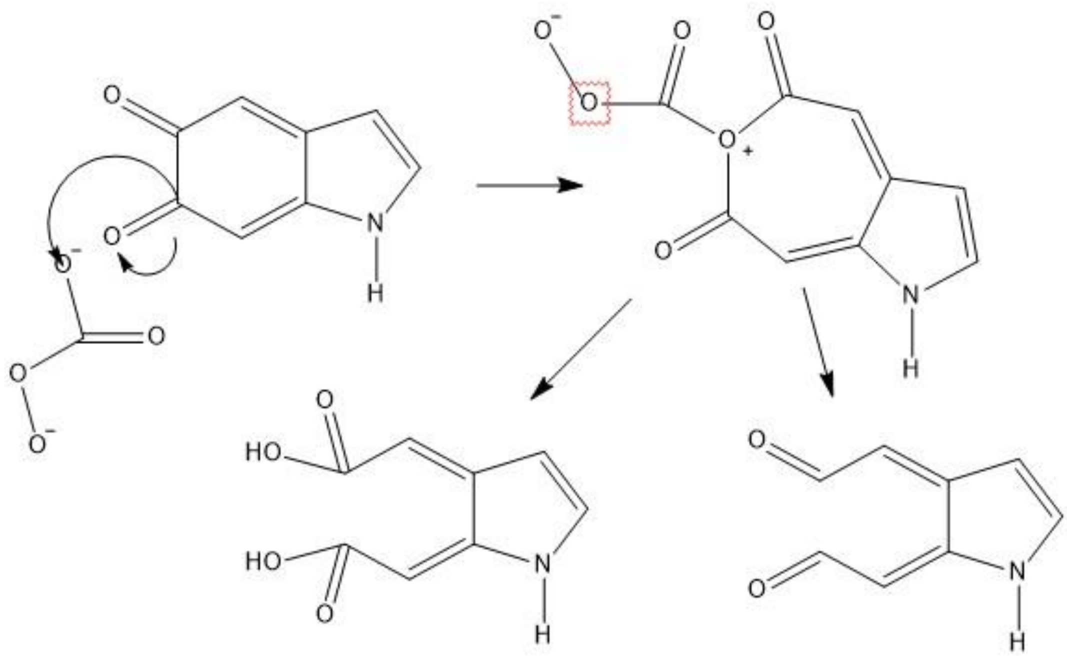


Mechanism 2. Creation of Hydrogen Peroxide.



Mechanism 3. Formation of Peroxymonocarbonate.

The final proposed step is the degradation of excited melanin by the peroxycarbonate species. It is proposed that this will go through a 7 membered ring and it will then form a dialdehyde or dicarboxylic acid, assuming the species is still in water. This species was verified by electron spin resonance by Wakamatsu[12], so it is logical to assume that this species could be one form of the final degradation. Again, for bleaching to occur, just enough of the aromaticity to be removed for delamination of the filaments. This proposed final step in shown in mechanism 4.



Mechanism 4. Degradation of Melanin through Peroxycarbonate.

9. Conclusion and Future Work

The hypothesis that light and biologically safe reactive oxygen species could destroy melanin and lighten hair was proven correct. The two most effective combinations were first, UVB light and sodium carbonate and second, UVB light and potassium sulfate. The damage done to the hair from the process was significantly less than current methods for multiple reasons. First, hydrogen peroxide was produced inside the melanosome only so the reactive materials were not reacting with all structures in the hair. Second, the time period for the reaction to go to completion was under 10 minutes compared to 20-25 minutes for conventional methods. Finally, the pH range of the hair was not drastically altered as conventional methods. The combination of light and ROS created a localized bleaching that was diminished if not completely disabled as soon as the light source was removed. The product formed from the carbonate reaction seems to consist of an indole/carbon ring with one or more aldehydes projecting from it based on the IR spectrum. The bisulfate product is largely without feature based on the IR, but both products are water soluble.

Future work on this could include a more in depth understanding of how the ROS reacts with intact melanosomes. If the mode of penetration could be more clearly understood, then perhaps it could be improved upon. The role of water in the reaction could also be more defined. Water is a key player in the reaction, and it was difficult keeping the hair wet enough during the process for the reaction to be complete.

10. Materials

Melanin samples were obtained from Biomedics, lot number Q5281, dissolved in DMF at a concentration of 0.7% w/w which was meant to simulate the average amount of melanin in Asian hair. Sodium carbonate, dehydroascorbic acid, potassium bisulfate, citrate, citric acid, potassium hydroxide, glycerin, and DMF were obtained from either Fisher Scientific or the stockroom at Idaho State University. Hydrogen peroxide was obtained from a supply store and tested for efficacy.

Hair samples were obtained from friends or colleagues who had previous arrangements to cut their hair. Before any testing it was cleaned using a mild detergent and air dried.

Cuvettes of visible and UV reactions were quartz to prevent inhibition of absorption of light by the chemicals.

Appendix 1

Figures

13C NMR of supernatant of melanin and carbonate catalyzed by UV light

13C NMR of supernatant with peaks picked

13C NMR enlarged spectrum of carbonate and peroxymonocarbonate peaks

IR of sepia melanin, the starting material

IR of melanin and carbonate product when catalyzed by UV light

IR of starting material and melanin/carbonate product on the same chart

IR of starting material and melanin/carbonate product subtracted

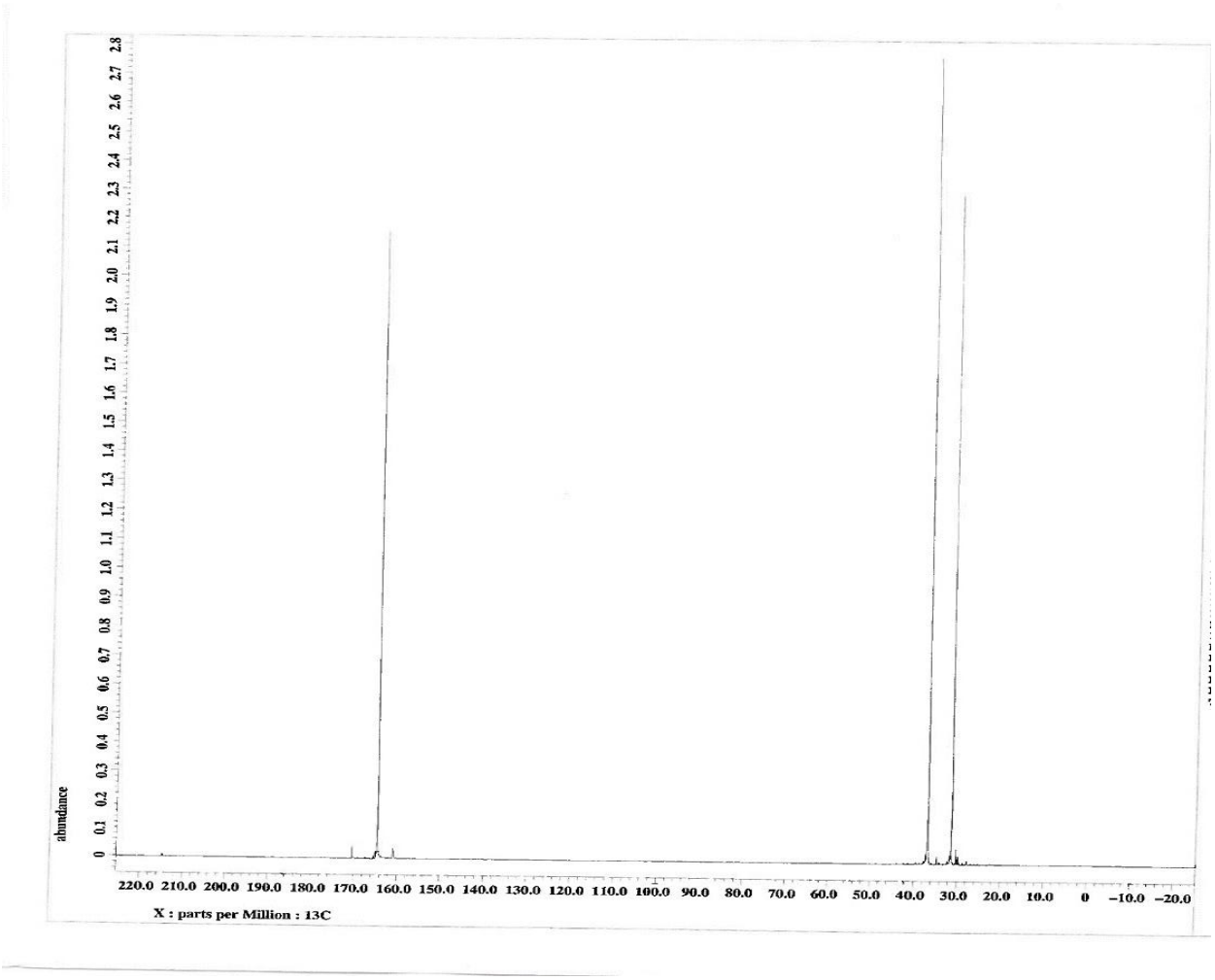
IR of carbonate subtracted from product

NIST standard of sodium carbonate

NIST standard of potassium bisulfate

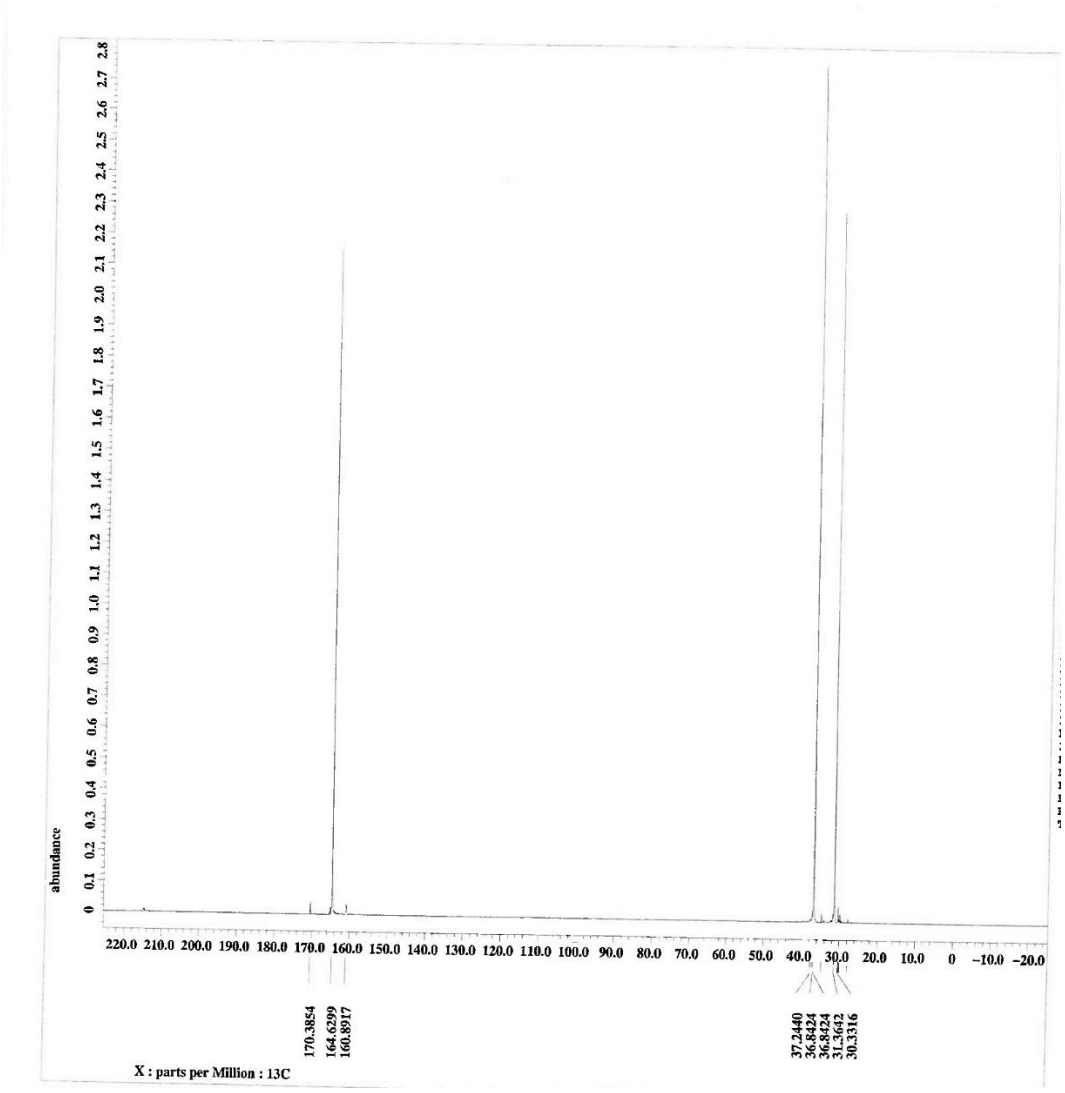
IR of bisulfate product

IR of bisulfate product and starting material

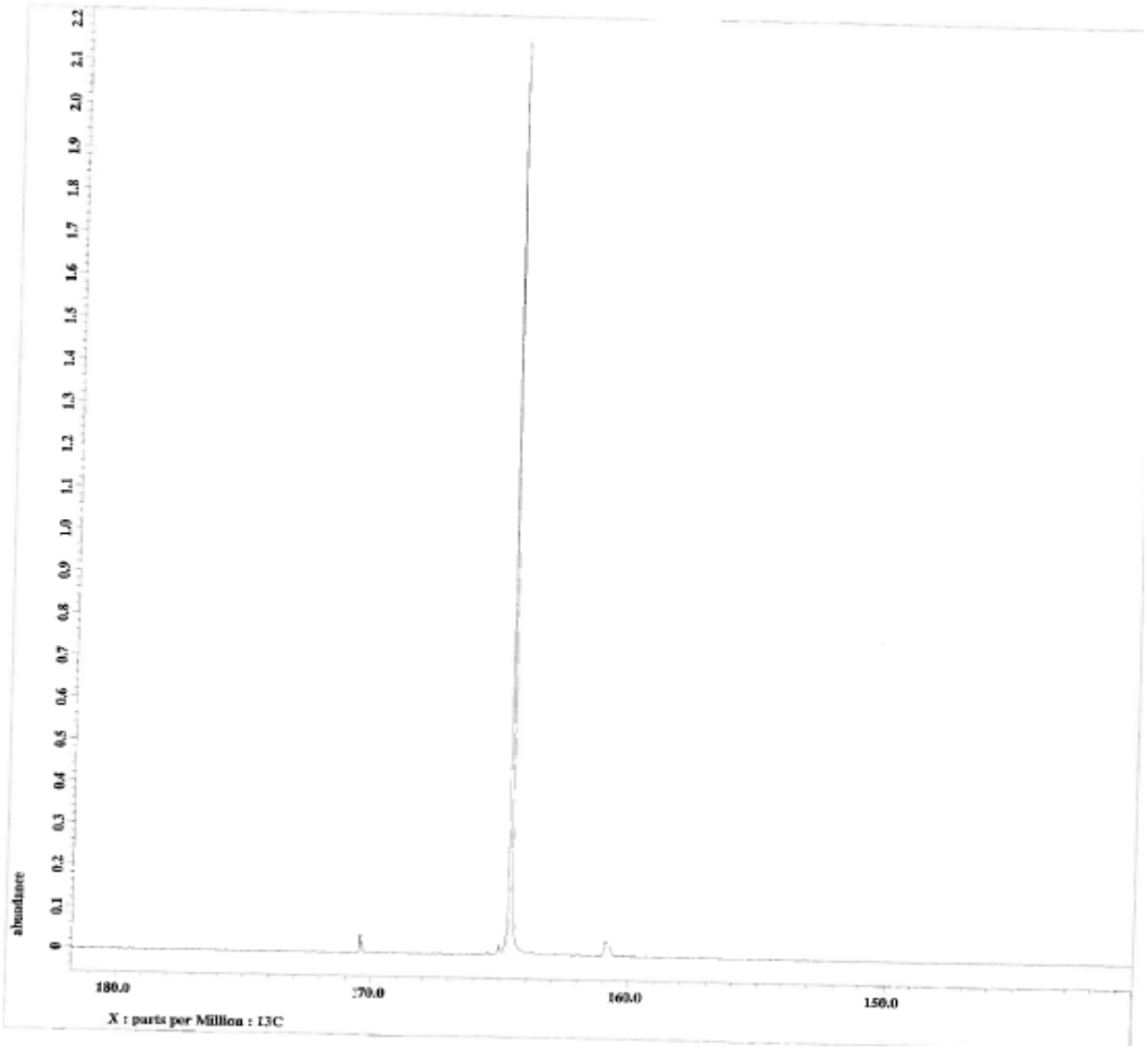


This is the C-13 NMR spectrum of the supernatant of the UV catalyzed reaction of melanin and carbonate. The melanin is dissolved in DMF and that is evidenced by the large peaks at ~36, 31

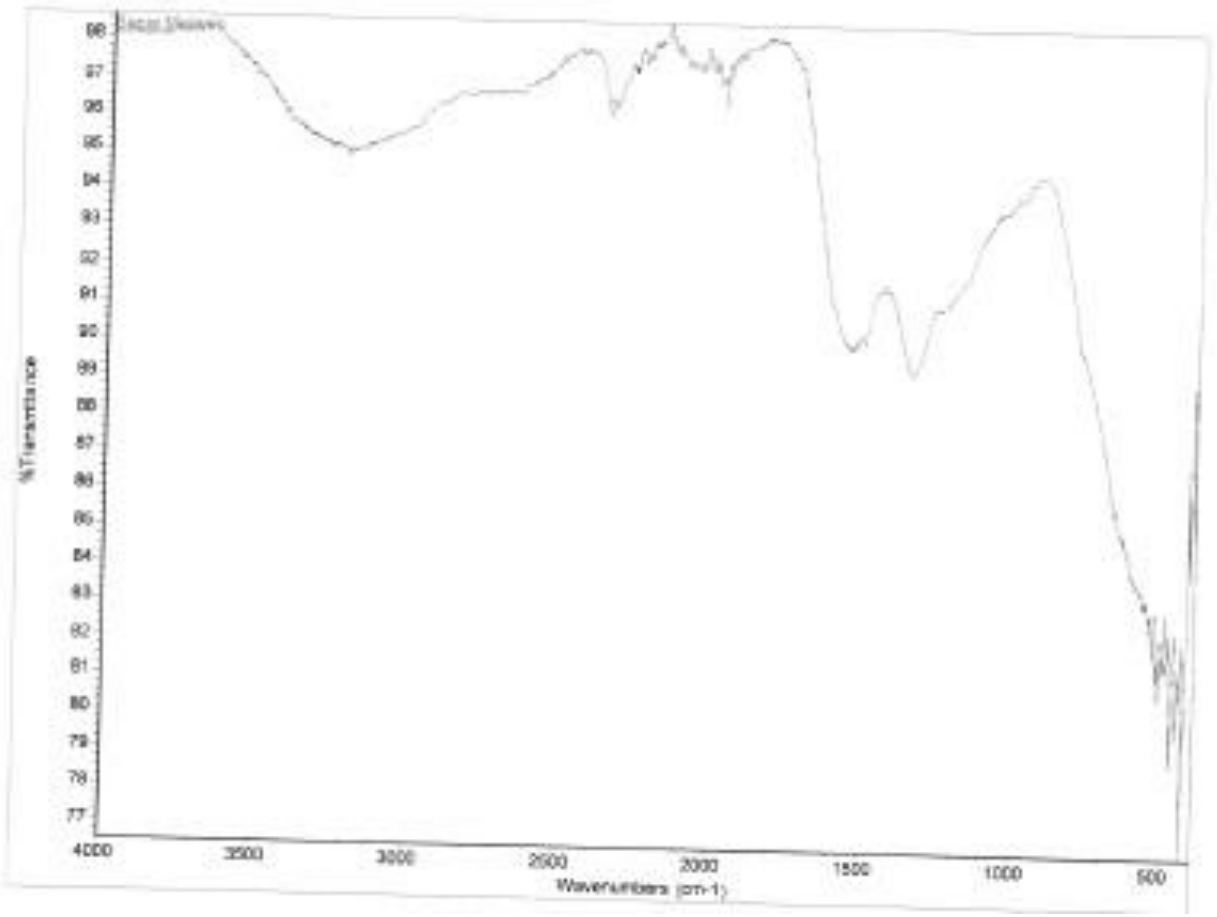
and 164ppm. The two smaller peaks at 160 and 170 ppm are thought to be the carbonate and peroxymonocarbonate peaks.



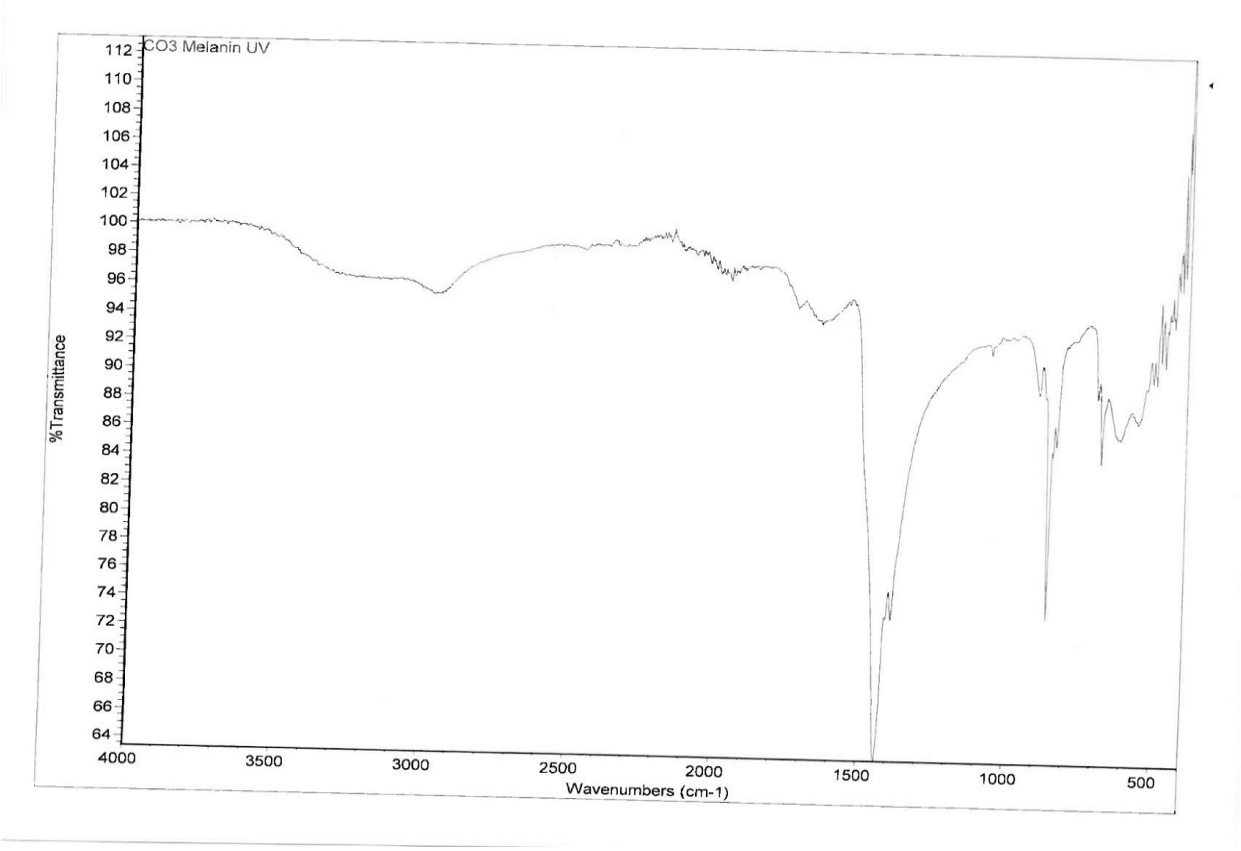
This is the same spectrum above with peaks picked.



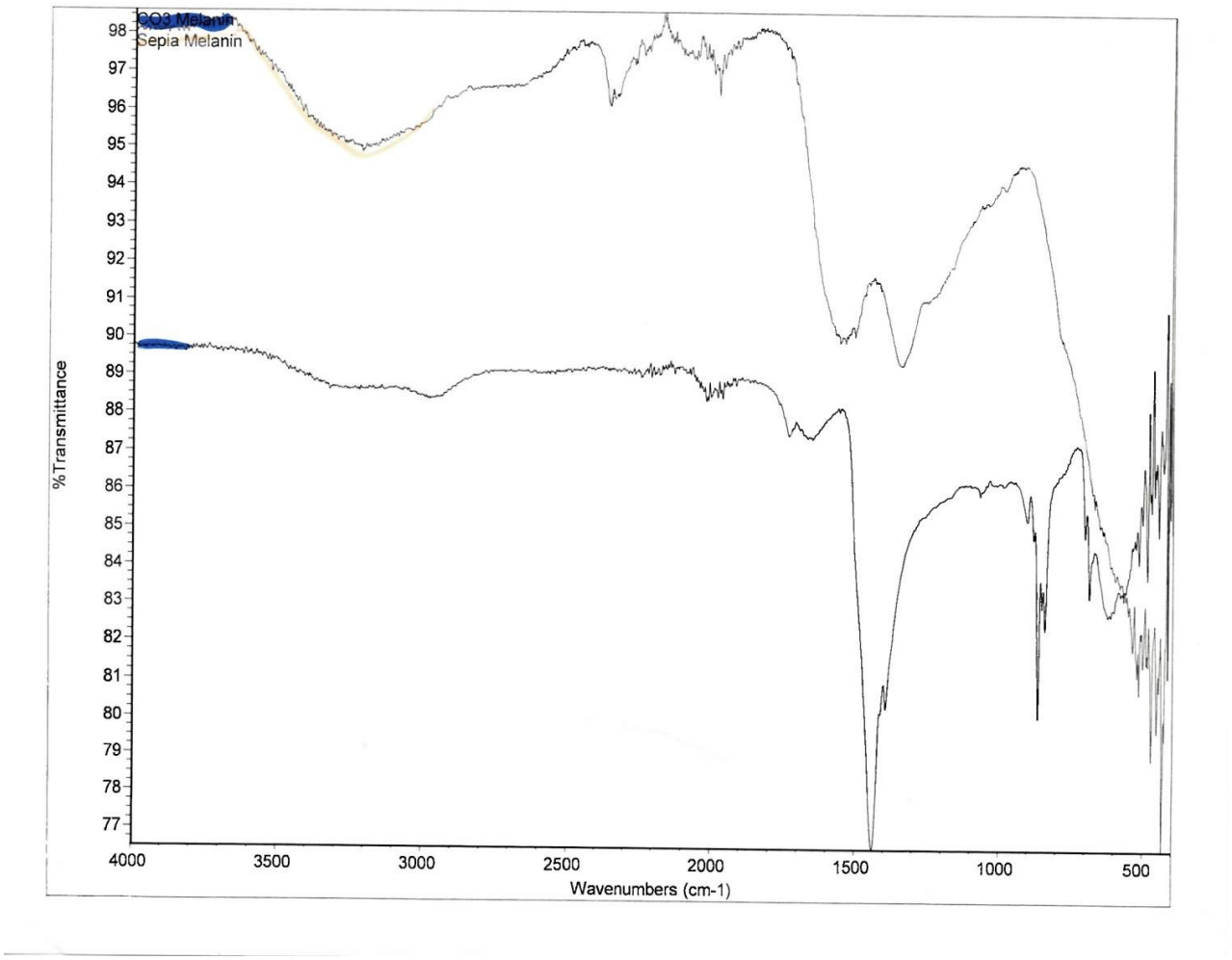
This NMR image shows the same peaks from the previous NMR that has been expanded to show detail of the relevant peaks.



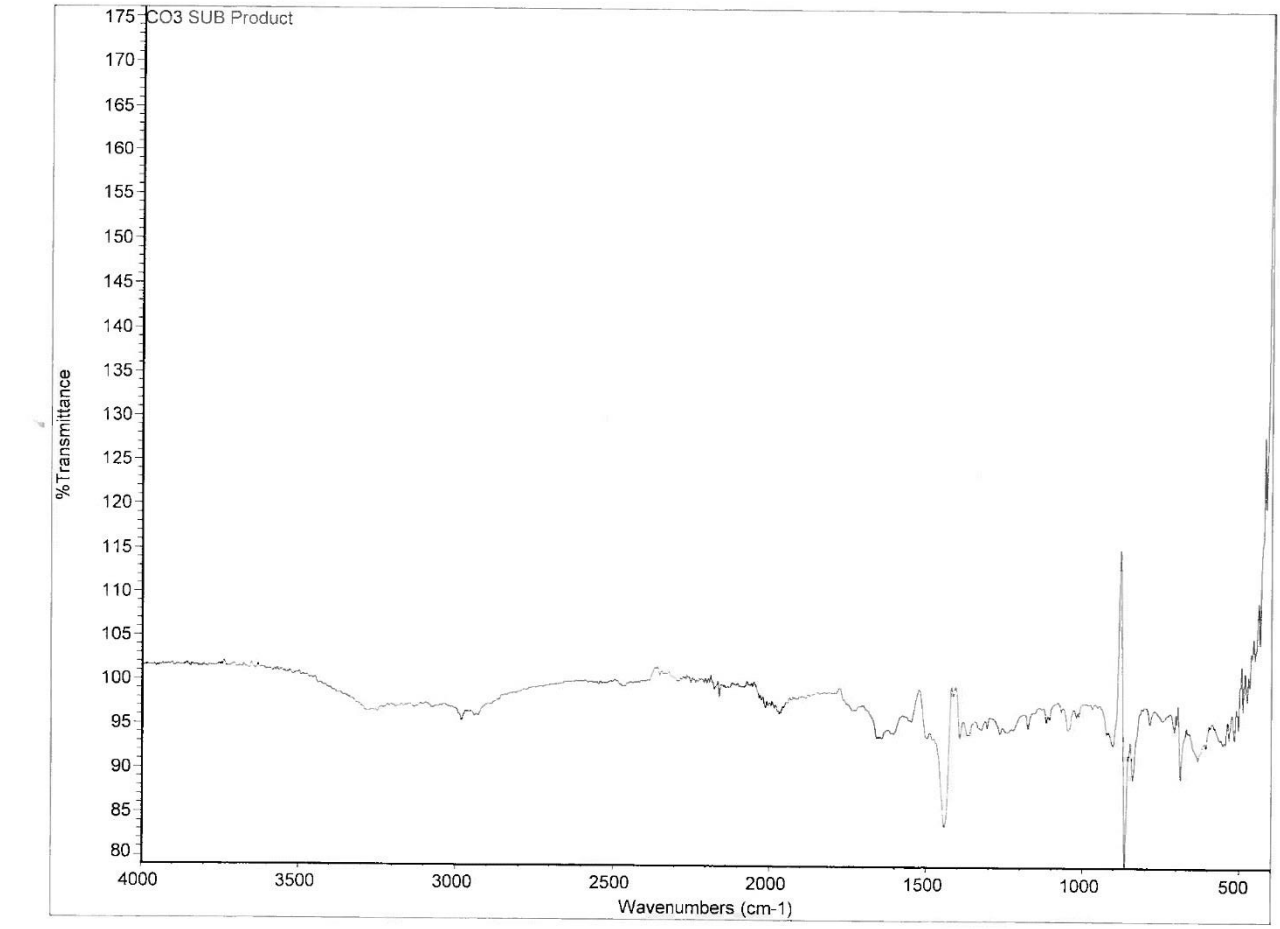
IR spectrum of the starting material, sepia melanin. Note the wide “tongue” peak from 3000-3800 that is the result of the dual hydroxy groups as well as the indole peak at about 1450. The aromatic carbon carbon double bond peak is thought to be above 1500, at about 1580.



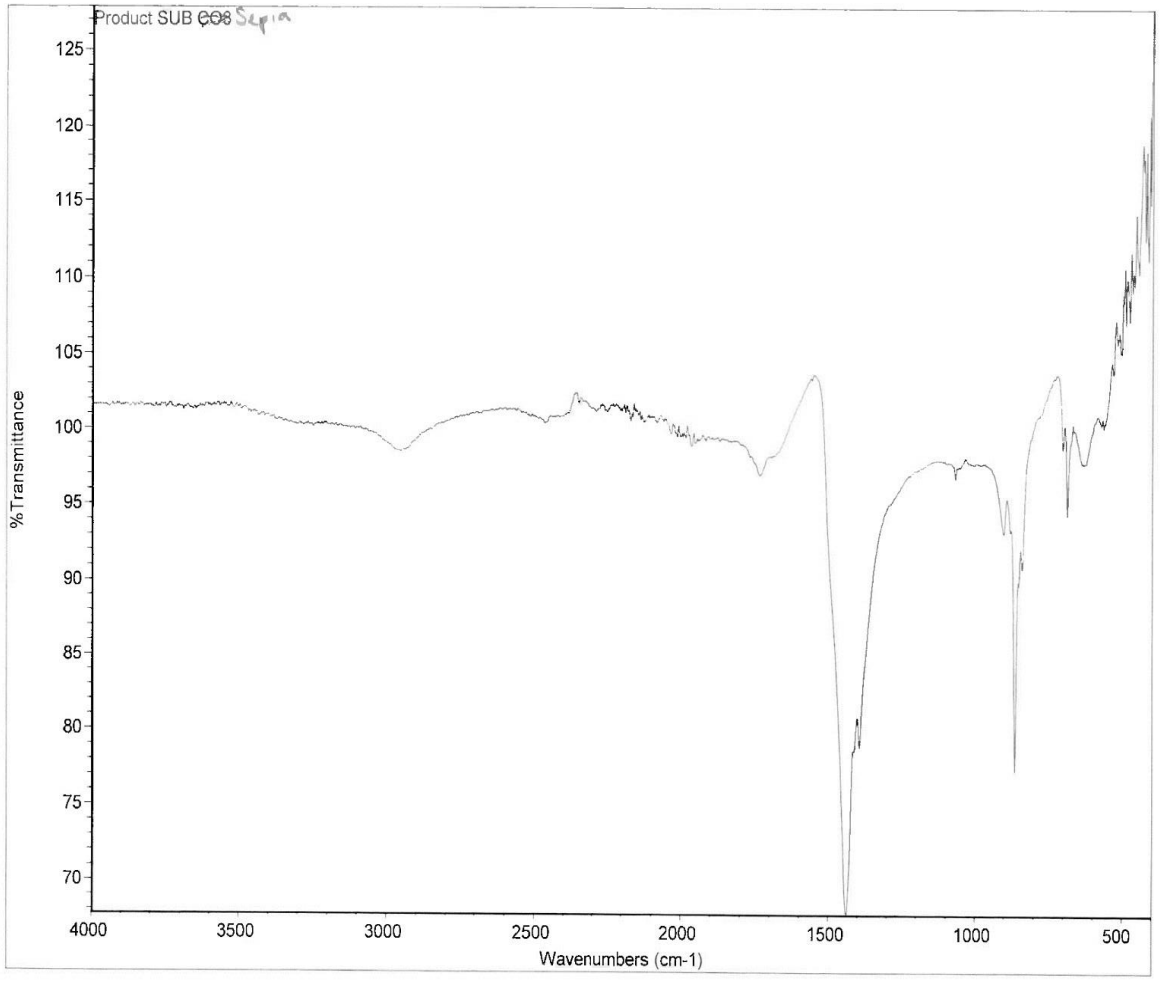
IR spectrum of the melanin after being catalyzed with UV light. The indole peak is more pronounced, and the hydroxy tongue peak has been reduced drastically as well as the aromatic peak. There is not much data on this and although these are not typical peaks for this and the following spectra, this is what best fits the structures, proposed mechanisms and other published results.



This spectra is a comparison of the starting material and the light catalyzed carbonate product.

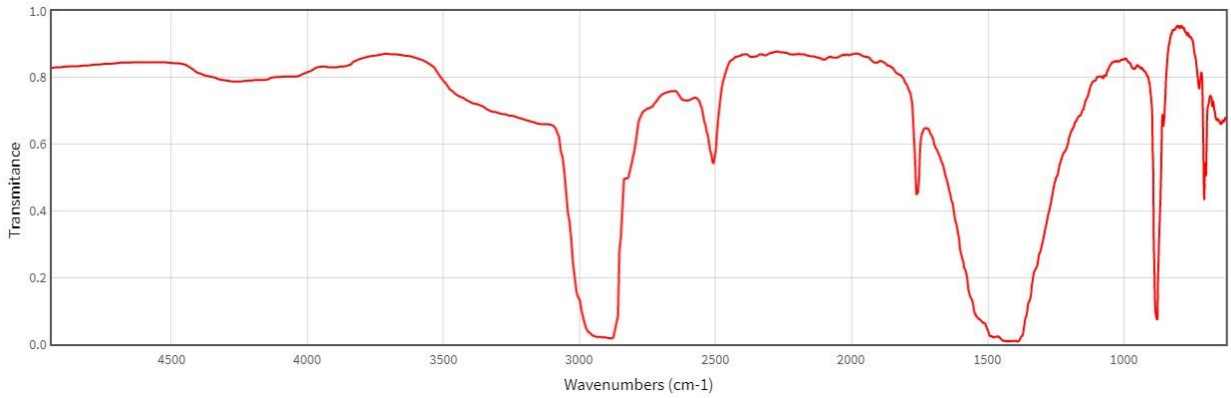


This spectra is a subtraction of the starting material from the product. Again, the indole peak is very strong with the carbon carbon double bond peak being reduced significantly.

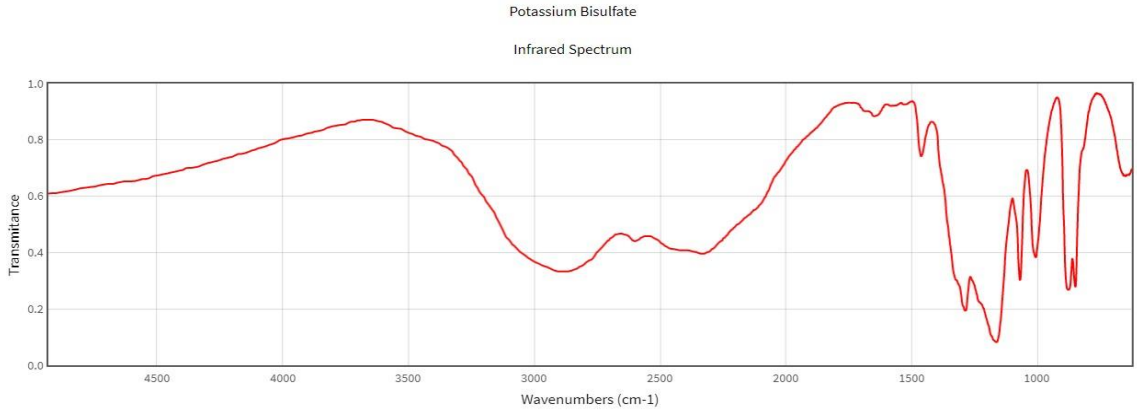


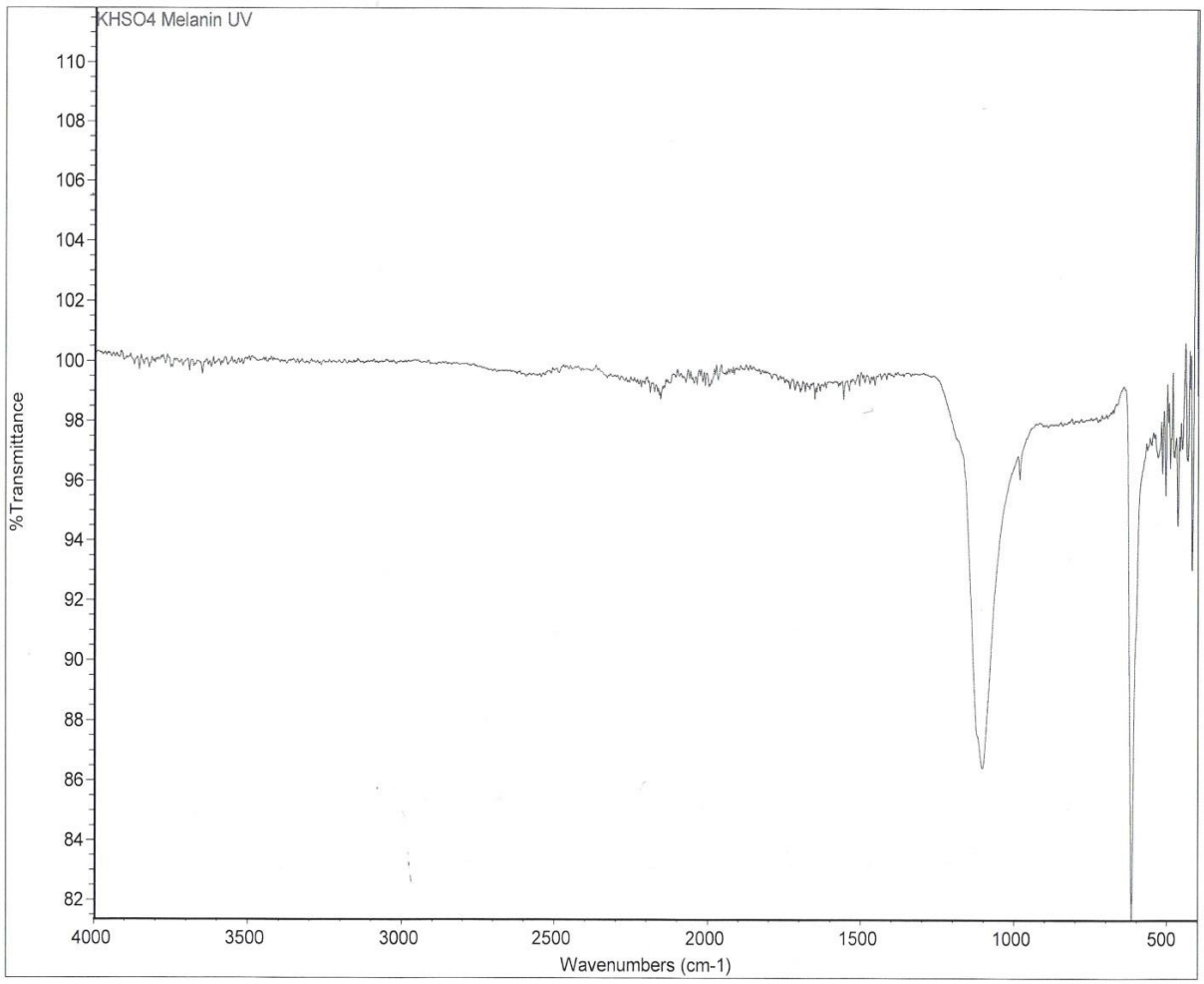
This is the light catalyzed carbonate product subtracted from the starting material. The most notable feature here is the large reduction in what was thought to be the hydroxyl tongue and the emergence of the N-H peak.

IR From NIST of sodium carbonate

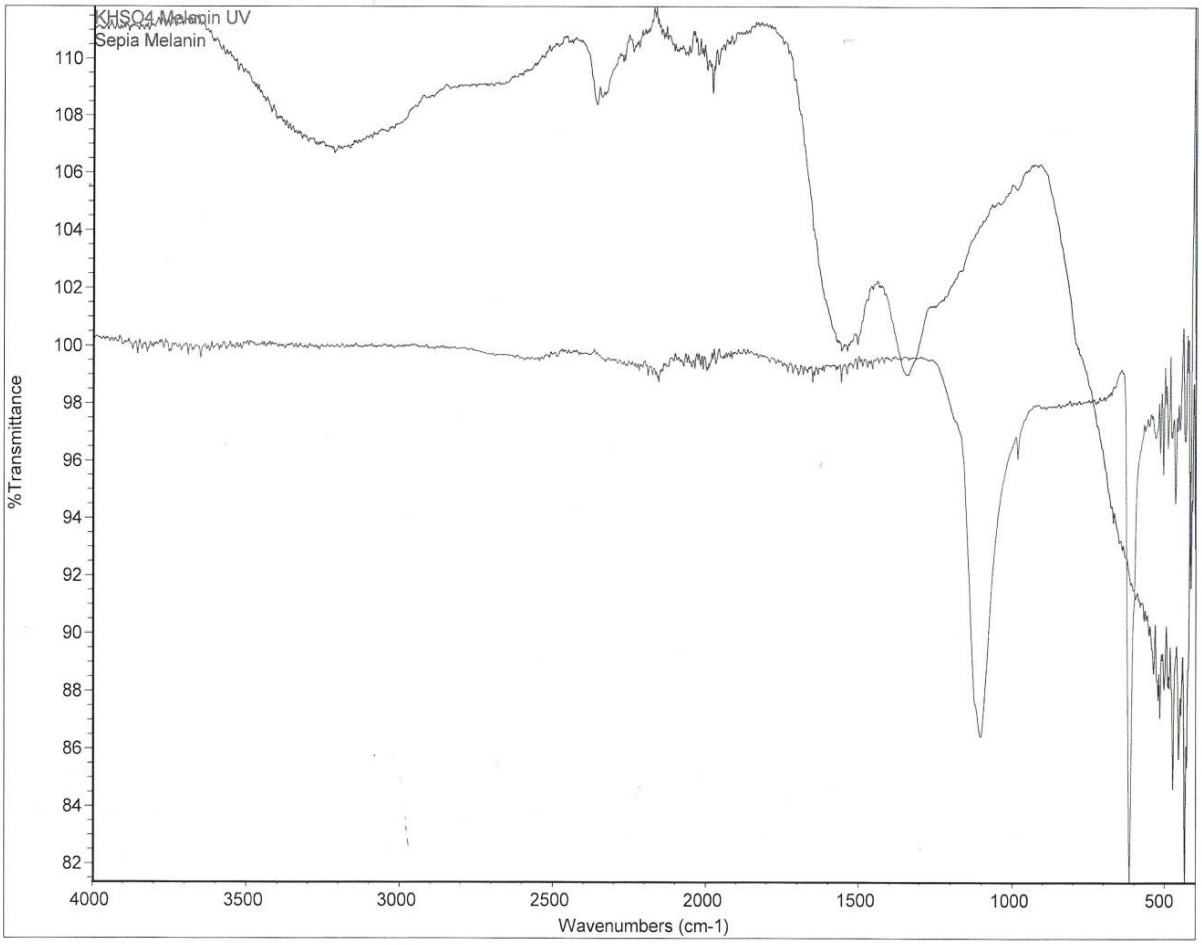


IR from NIST of potassium bisulfate





This is the IR spectra of the UV product of potassium bisulfate in the reaction. Note the only large peak is at about 1200 in the fingerprint region. This would indicate almost complete destruction of the starting material.



This spectra is a comparision of the starting material, melanin, and final product of the UV catalyzed KHSO4 reaction. This is the clearest spectra althought the most likely peaks are from carbon dioxide in the sample.

Appendix 2

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