An ESR Study of the Effect of Adsorbed Species on the Radiolysis of Cellulose

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Senior Comprehensive Paper

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Abstract: The presence of absorbed free radical initiators, such as benzophenone, benzoyl peroxide and bromine may enhance the number of free radicals formed by the radiation of cellulose. These radicals may then become available to initiate the break-up of the cellulose structure. Experimental methods for studying the degradation of cellulose were developed with a particular emphasis on the effect of absorbed species. The first approach focused on determining the amount of glucose present after exposing cotton balls saturated with hydrogen peroxide to gamma radiation dose of 49kGy. The amount of glucose that was present would show if hydrogen peroxide aided in the degradation of cellulose. This method, which used Benedict's reagent to test for the presence of glucose, failed to show results consistently and the ESR approach was then taken. This is studied by comparing samples of pure cellulose to samples of cellulose with each of the three different initiators. One group of these samples is then subjected to gamma radiation while the other is used as a control. The samples were then analyzed using ESR (Electron Spin Resonance) to determine the free radical production. The spectra of the samples that contained a mixture of benzophenone and cellulose showed unique peaks with the sample of 0.2g benzophenone having peaks at 3347G and 3330G with another smaller one at 3320G while the sample with 0.5 g of benzophenone showing peaks at the same point in the magnetic field but with a stronger intensity. Although there was not sufficient time to obtain all the results, the data that was gathered shows promising results about the concentration of free radicals present in the sample.

Introduction: The degradation of cellulose is important to study because it is at the forefront of biofuel research. Cellulose is one of the most widespread natural polymers and is a key component of plant's cell walls, but it is not easily freed from the cell wall's complex and rigid structure[1]. When cellulose is exposed to radiation it acts as a pre-treatment to break-up the cellulose into fragments prior to enzymatic or hydrolytic treatment. The process of decomposing cellulose to glucose is as follows in Fig.1.

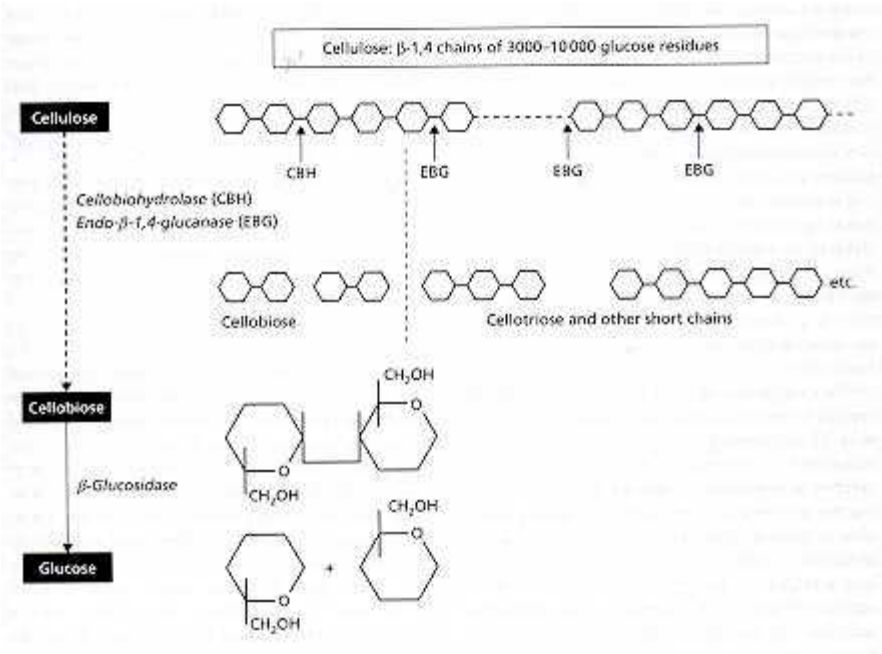


Figure.1 Breakdown of Cellulose [2]

This glucose can then be used to create ethanol which in turn is a viable source of biofuel. The adoption of using radiation to treat cellulose would replace ecologically hazardous steps for the pre-treatment of cellulose, such as pre-treatments with concentrated ammonia of H_2SO_4 by treatment with ionizing radiation leading to reduction of the number of technological stages and a decrease in energy consumption[1]. Using ionizing radiation results in a scission of the

polymeric chain of cellulose, which is made up linked D-glucose units[3]. These glucose units are the source of the glucose that is produced from the degradation of cellulose. By degrading cellulose with radiation, the process of creating glucose and, subsequently, ethanol could be made much more efficient and affordable. However, degrading cellulose is much more difficult than decomposing starch. The second method used in my research focused on using ionization radiation to facilitate the break-up of cellulose which could allow for different chemicals to incorporate themselves into the cellulose structure.

Adsorbing free radical initiators onto cellulose and exposing them to radiation may enhance the number of free radicals that are formed upon irradiation and thus enhance the degradation of cellulose. By enhancing the degradation of cellulose, perhaps more glucose units could be freed from the cellulose structure and therefore produce a higher yield. The different species used as intiators in the present study have been benzoyl peroxide, bromine water and benzophenone and the technique used to analyze the free radical formation has been ESR. The intiator of benzoyl peroxide works in such a way that two radicals are produced as seen in the reaction scheme below.

The free radical initiator of bromine water works by removing hydrogen from the cellulose and thus creating a radical. The benzophenone initiator works by once again abstracting a hydrogen from cellulose and creating a free radical.

The structure of cellulose is very rigid and strong, which is why radiation must be used to facilitate its break-down[4]. The monomer units of cellulose consist of pyranose rings in the chair conformation with axial hydrogen atoms[4]. This structure is characterized by high rigidity and restricts the conformational transitions as seen in Figure 2.

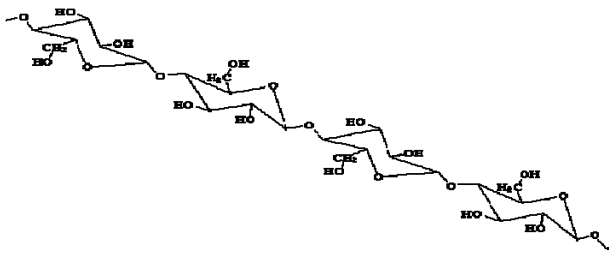


Figure 2. Cellulose structure[2]

In most valence-saturated compounds, such as this, the carbon atom has four sp^3-hybrid orbitals[5]. When one C-H bond is cleaved, sp^2-hybridization occurs and the unpaired electron occupies a $2p_z$ orbital[5]. If the carbons in Figure 2 are numbered 1-5, 1 being the C atom on the right and then moving to the left, it is seen that the radical centers at C-1 and C-4 cannot be obtained without the cleavage of the polymeric chain of cellulose. Radical structures could also be found on C-2 and C-3 but it is less likely because the C-H bonds at C-1 and C-4 are the weakest and therefore most susceptible to hydrogen abstraction[5]. In a study of the influence of gamma irradiation on the structure of cellulose, an NMR study showed that irradiation caused breakage of inter- and intra-molecular H-bonds and chemical bonds with the formation of a macro-radical[4]. Another study dealing with irradiated cellulose shows the formation of radicals having an unpaired electron in the 1 and 4 positions in the pyranose ring. These radicals were accompanied by cleavage of the glycoside bond as well[1]. The following shows a mechanism for the breakdown of cellulose when using gamma radiation:

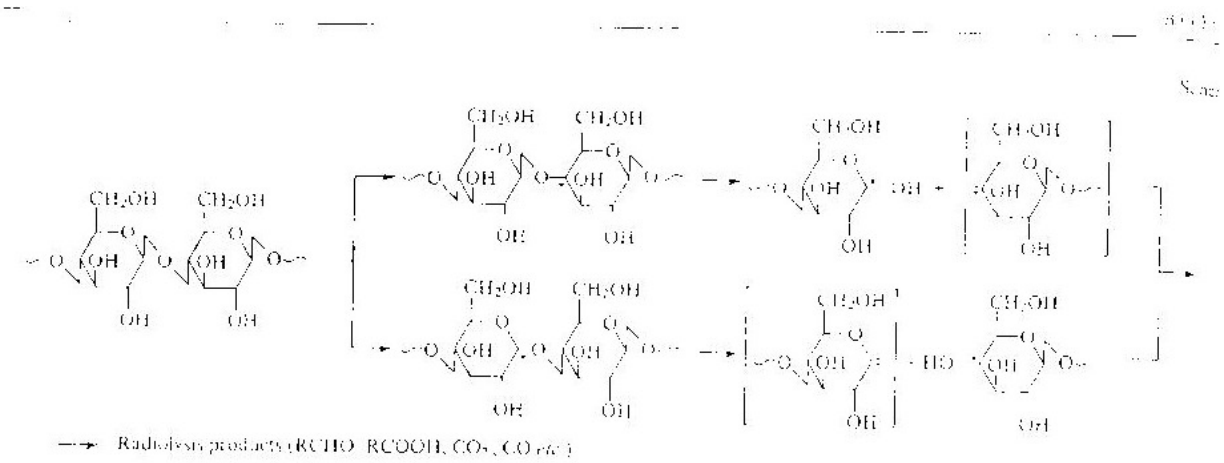
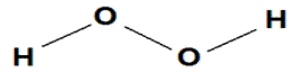


Figure 3. Degradation of cellulose by gamma radiation[5]

Throughout my research on the degradation of cellulose I have taken different approaches into analyzing this degradation. The two techniques that were used to monitor the degradation of cellulose were 1) by measuring glucose yields using Benedict's reagent and 2) using ESR (Electron Spin Resonance) to determine the free radical production. The first method used to analyze the degradation of cellulose was done by using cotton balls that were soaked in different concentrations of hydrogen peroxide and exposed to gamma radiation. This method used Benedict's reagent to determine the amount of glucose present after the samples were exposed to gamma radiation. The Benedict's Reagent test is used to analyze the content of reducing sugars in solutions, which in this case would be glucose[6]. The reaction for the Benedict's reagent is as follows:

$$\mathbf{CHO + 2\ Cu^{2+} + H_2O \rightarrow COOH + 2\ Cu^{+} + 2\ H^{+}}$$

In this experiment we are analyzing whether more glucose is present in the cellulose samples when they are irradiated in the presence of hydrogen peroxide than when H_2O_2 is absent.



HYDROGEN PEROXIDE

Figure 4. Hydrogen peroxide structure[9]

If glucose is present the solution will turn anywhere from a green (representing the Cu^{2+} in the reagent) to a brick red color (representing the Cu^{+} product depending on the amount of glucose present, the green being the lesser while the brick red being the greater[6]. The analysis of the samples was done by decomposing the excess H_2O_2 with catalase and then allowing the glucose product to react with Benedict's solution (Cu^{2+}) and measuring the spectral changes resulting from the conversion of Cu^{2+} to Cu^{+} using a GenTech UV/Vis Spectrophotometer. Catalase is a common enzyme that catalyzes the breakdown of hydrogen peroxide into water and oxygen[7]. The decomposition of hydrogen peroxide with catalase is as follows:

$$2\ H_2O_2 \rightarrow 2\ H_2O + O_2$$

Catalase was inserted into the samples before running them in the spectrophotometer in an effort to try and remove the hydrogen peroxide from the sample.

The method of ESR (Electron Spin Resonance) proves to be a viable method for analyzing the production of free-radicals in cellulose because it allows for chemical species that have one or more unpaired electrons to be studied (such as free radicals)[8]. ESR is very similar to NMR, except that unlike NMR which studies the excitation of atomic nuclei spins, ESR studies

the excitation of electron spins[8]. In particles with an even number of electrons the total spin number is equal to 0, according to Pauli's principle which says that the numbers of electrons with spin quantum numbers of + ½ and – ½ are equal[8]. In particles with an odd number of electrons, the total spin magnetic moment is not equal to 0 and the particles display magnetic properties. According to the theory of magnetism, diamagnetic materials have paired electrons while paramagnetic materials have unpaired electrons[8]. Paramagnetic substances display magnetic properties whereas diamagnetic substances have no permanent magnetic moment. Paramagnetic substances exhibit ESR signals because of this[8]. In the ESR technique, a uniform magnetic field is produced allowing the individual magnetic fields of the paramagnetic structure to orient themselves at a certain angle[8].

Since the substances that are studied using ESR are paramagnetic, the lone electron will be in transition between two magnetic levels, m_s=+ ½ and m_s=- ½. The field is split because in the presence of an external magnetic field, the electron spin may be aligned parallel or anti-parallel to the field. The unpaired electron can move between the two energy levels by either absorbing or emitting electromagnetic radiation of energy[8]. This transition is accompanied by an absorption or emission of energy, which is determined by the direction of the transition[8].

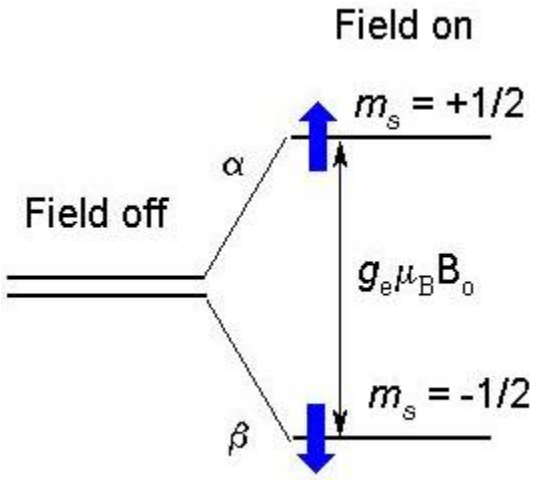


Figure 5. ESR magnetic field[9]

In order to study the feasibility of increasing efficiency of the degradation of cellulose, the free radical initiator benzophenone was added to the cellulose as a liquid. When benzophenone is irradiated, a free radical is created and this free radical seeks to abstract a hydrogen atom.

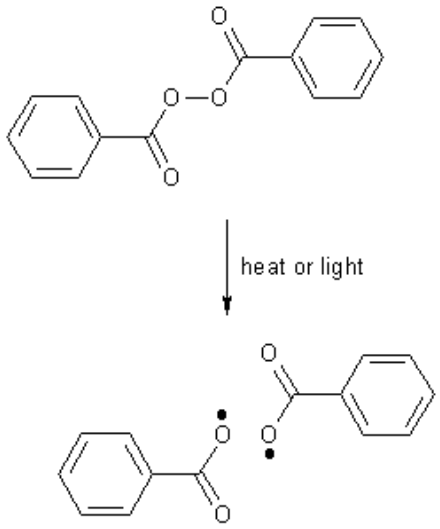


Figure 6. Benzophenone structure[10]

Experimental Design: The first method used to analyze the degradation of cellulose involved soaking cotton balls with differing concentrations of 30% hydrogen peroxide, which will provide a source of OH radicals upon irradiation. Hydrogen peroxide has a very high oxidizing capacity and is used in this experiment to help facilitate the breakdown of cellulose. OH radicals are very powerful oxidizing agents and they produce radicals capable of initiating chain reactions. Cotton balls are used because they are made up primarily of cellulose fibers, which is the species that is under study. As mentioned before, when cellulose is exposed to radiation one of the byproducts of its degradation is glucose. If more glucose is observed to form than hydrogen peroxide can be said to increase the radiation-induced degradation of cellulose.

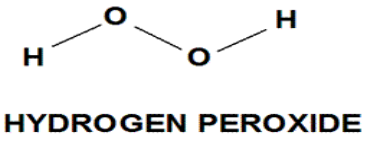


Figure 7. Hydrogen peroxide structure[10]

The experiment was carried out by making dilutions of a stock solution of SigmaAldrich 30% hydrogen peroxide. Dilutions were made with DI water in the following concentrations: 1 H_2O_2: 2 H_2O, 1 H_2O_2: 4 H_2O, and 1 H_2O_2: 24 H_2O. These samples were each 10mL total, with the respective amount of water and hydrogen peroxide in each. A cotton ball was also placed in each solution and allowed to saturate with the solution. Once this saturation took place, the sample was then exposed to gamma radiation at a dose of 49.01 kGy. After the samples were irradiated, 2mL of Benedict's reagent was added to each sample. The Benedict's solution was

made in the lab by the following process: 10 g of sodium carbonate and 17.3 g of sodium citrate dihydrate were dissolved in a final volume of 85 mL of water. While stirring the solution, 1.73 g of copper sulfate pentahydrate in 10 mL of water was added. The solution was then brought to a total of 100 mL by adding water[3].

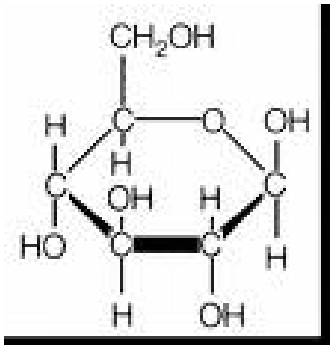


Figure 8. Glucose structure[10]

Once the Benedict's reagent was added, the solution was placed in a hot water bath for 5-20 minutes to allow for the Benedict's reagent to react. The samples were then run in the GenTech UV Spectrophotometer. A calibration of the instrument was done first by adding different amounts of a 5% glucose solution and diluting to a total volume of 4.5 mL and then adding 2mL of Benedict's reagent to each. After the calibration plot was completed the samples were run using a GenTech Spectrophotometer to analyze the absorbance of each from the reaction with the Benedict's reagent. The data obtained from the first trials showed a consistent error that was believed to be caused by the presence of the hydrogen peroxide. In order to try and remove the residual H_2O_2 interferences the samples were boiled first for 5 minutes and then for 20. Both of these approaches did not obtain better results. The next method used was to use the enzyme catalase which was previously explained.

For the second method in order to test for free radical formation, samples of cellulose were tested with free radical initiators present. The samples prepared throughout the analysis were prepared using pure cellulose powder obtained from the Sigma Aldrich Company. The control sample for the experiment contained pure cellulose. For each sample that contained an initiator along with the cellulose, 1g of cellulose was placed in the sample and then the initiator was added in solution. In order to dissolve the initiators, 10mL of 95% ethanol was used for each 0.1g of substance used. Once the initiators were mixed with the cellulose they were allowed to mix and then settle for one day, after which the liquid was decanted off and the sample was allowed to dry under a hood. Once the sample dried, part of it was used as an unirradiated control and the other as the experimental substrate. The experimental group was then subjected to gamma irradiation. The cobalt-60 radiation source at University of Maryland was used and the samples were exposed to a radiation dose of 100 kGy. The samples that were exposed to radiation were pure cellulose, cellulose with benzoyl peroxide, cellulose with benzophenone and cellulose with bromine water. Once irradiated, both the control and experimental groups were analyzed using ESR. In order to analyze the samples using ESR, it was necessary to transfer the samples to small tubes that could fit into the ESR slot. The ESR showed the nature and extent of free radical formation in each sample and allowed for comparisons to be made.

Results: The results for the first experimental method are shown in Figs.6-11

Figure 8. Glucose calibration data

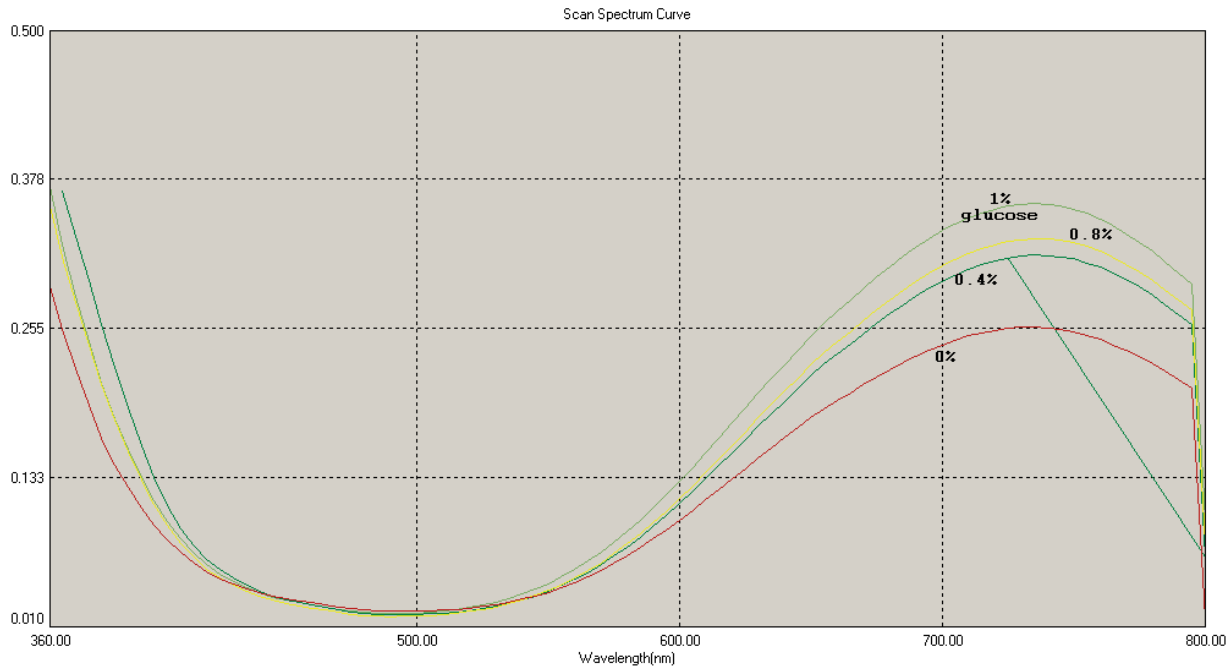
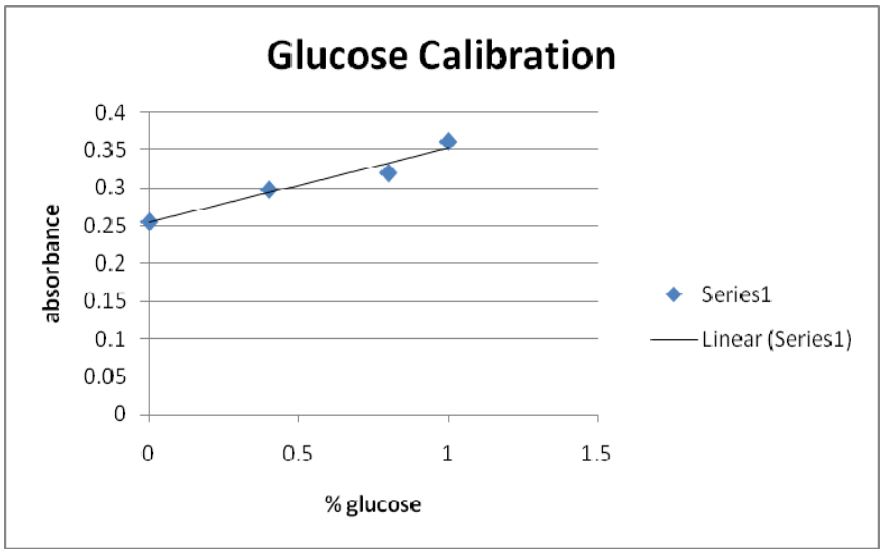


Figure 9 shows the glucose calibration done for the experiment. The values used for the calibration plot were found by finding the differences between the absorbance of each solution at the wavelengths of 725 nm and 500 nm. These two wavelengths served as the maximum and minimum respectively of the plots.

Figure 9. Calibration plot



The calibration plot provided shows increased absorbance as the solution is more concentrated.

The absorbance of the sample increases as the % of glucose in the sample increases.

Figure 10. Samples after 5 minute boil

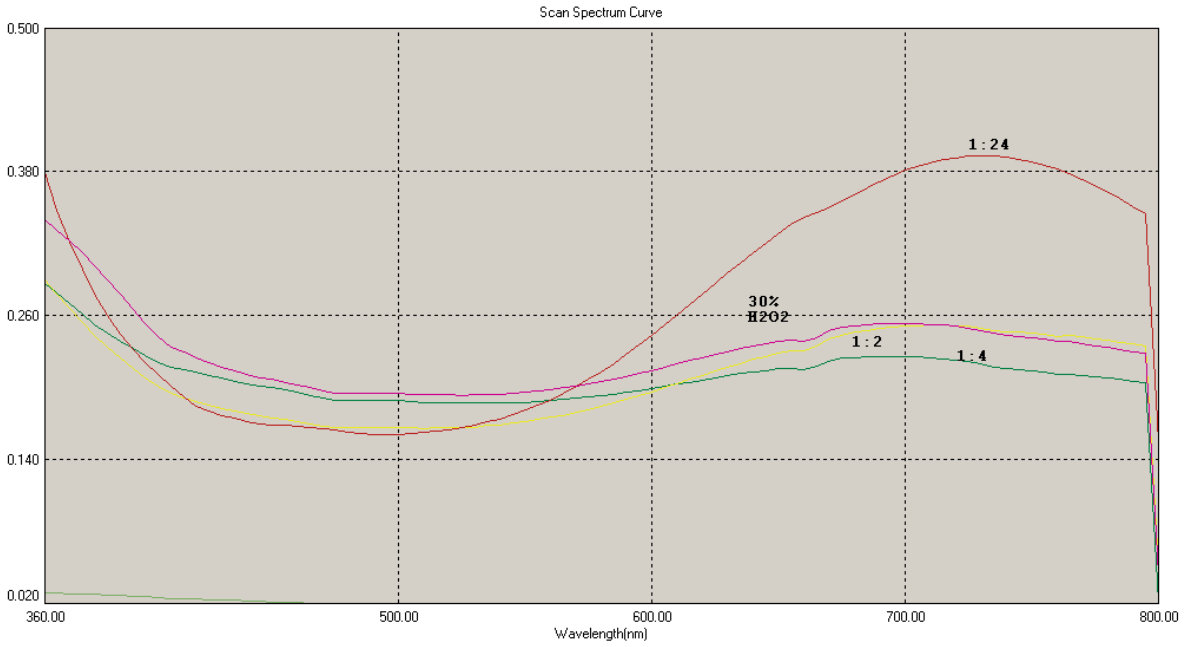
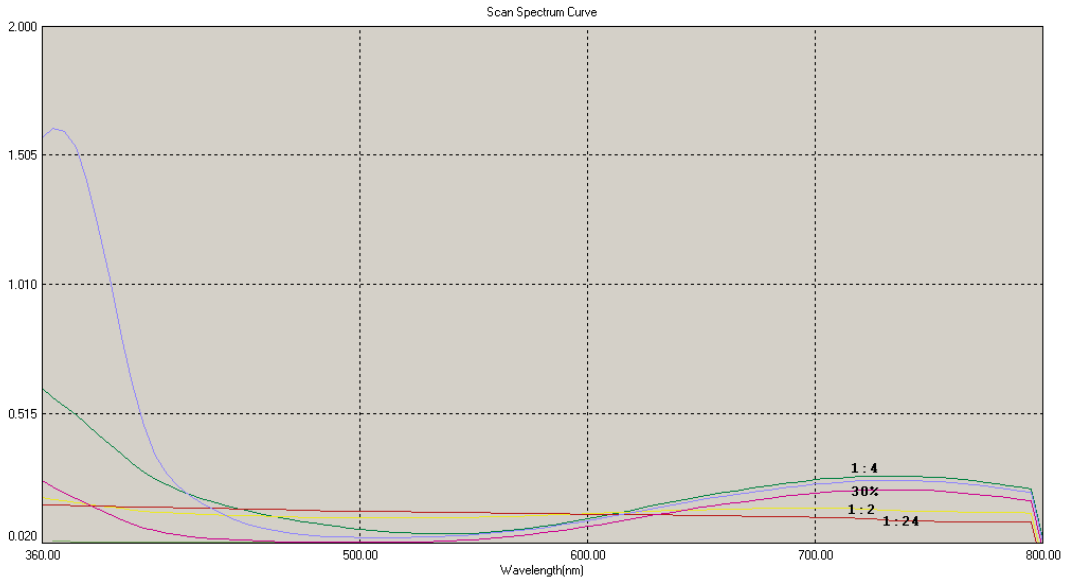


Figure 10 shows the solutions after they were boiled for 5 minutes to allow for the solution to react with the Benedict's reagent. The data does not correlate with the hypothesis that as the concentration of H_2O_2 increase so does the amount of glucose present. The highest absorbance in this plot corresponds to the 1:24 dilution. The inconsistency in the absorbance readings showed that this technique did not work and perhaps more time was needed for the sample to boil and react completely with Benedict 's reagent.

Figure 11. Samples after 20 minute boiling



This figure shows the data that was obtained after boiling the solutions for 20 minutes to allow the Benedict's reagent to react longer. The data did not show any correlation consistently as seen in Fig. 11. The 1:4 dilution which once again does not correlate with the hypothesis.

Figure 12. Samples with catalase enzyme

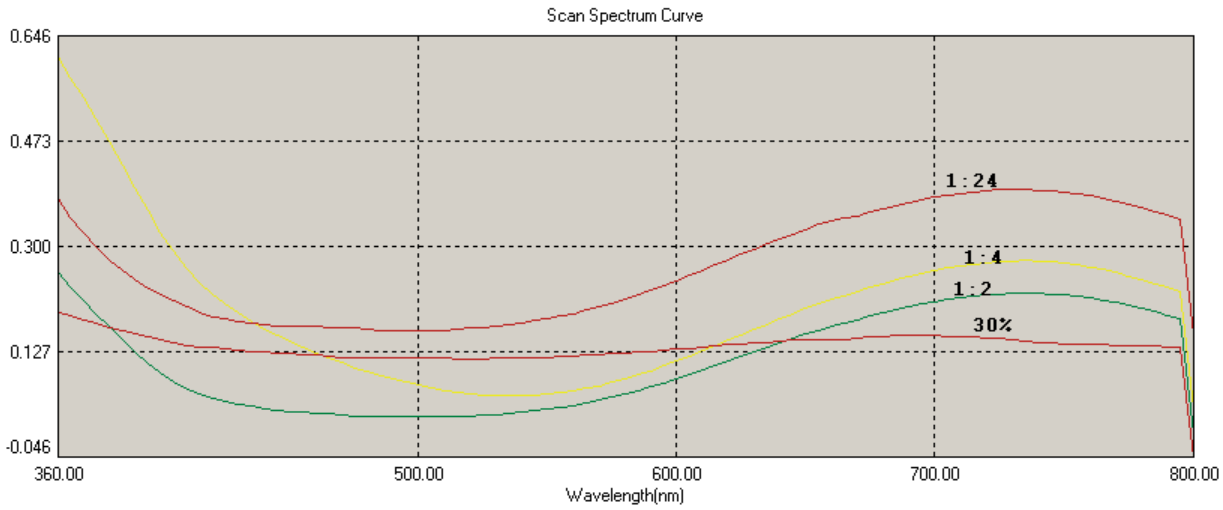


Figure 12 shows the data for the samples that had catalase added . This data agrees with the hypothesis in that as the H_2O_2 is more diluted, the absorbance decreases. This shows that as the concentration of H_2O_2 increases so does the amount of glucose.

The following results show the data for the ESR approach, the second method.

Figure 13. Pure irradiated benzophenone

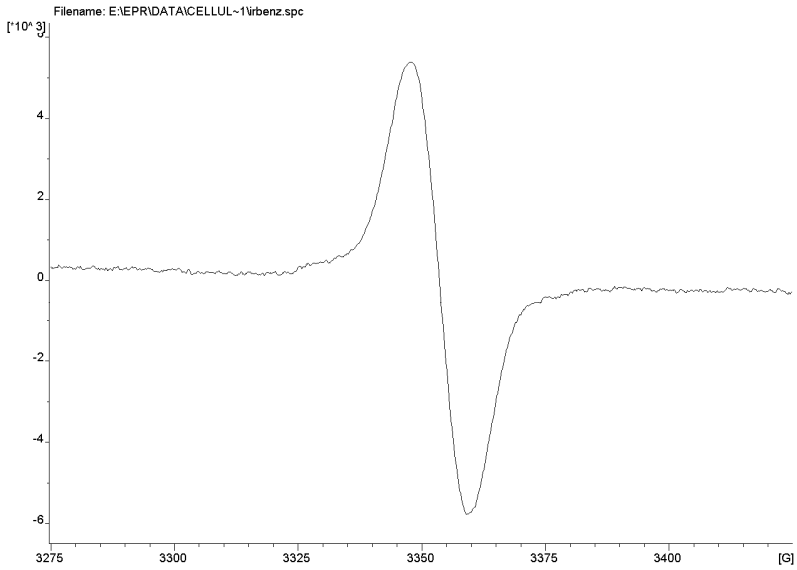


Figure 13 shows the ESR spectra of pure irradiated benzophenone with a peak at 3350 gauss.

Figure 14. Unirradiated benzophenone with cellulose

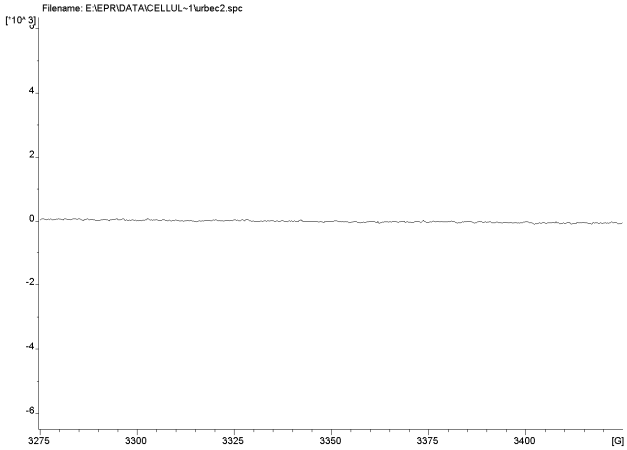


Figure 15. Pure irradiated cellulose

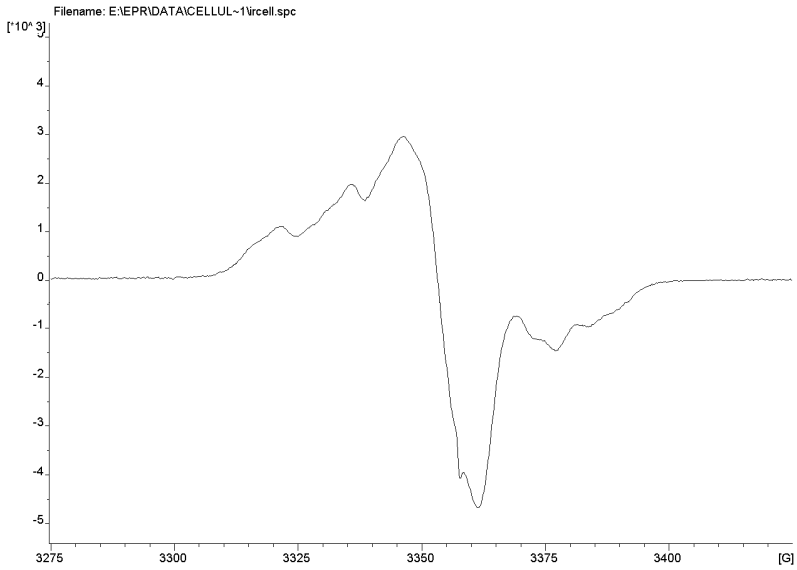


Figure 15 shows the ESR spectra of pure irradiated cellulose with a maximum peak around 3345G and some splitting peaks at 3320G and 3335G.

Figure 16. Irradiated cellulose with 0.2 g of benzophenone

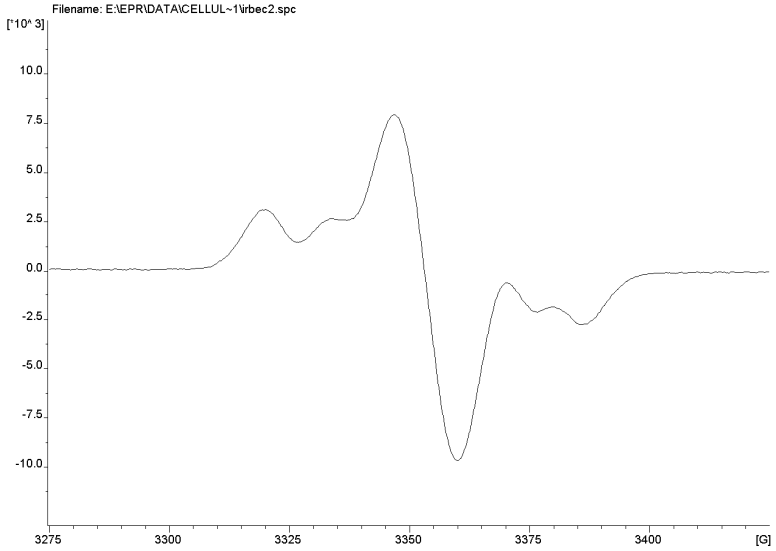


Figure 16 shows a peak at 3345 with a larger magnitude than that of cellulose and benzophenone as well as a peak at 3320G.

Figure 17. Irradiated cellulose with 0.2 g of benzophenone after 2 months

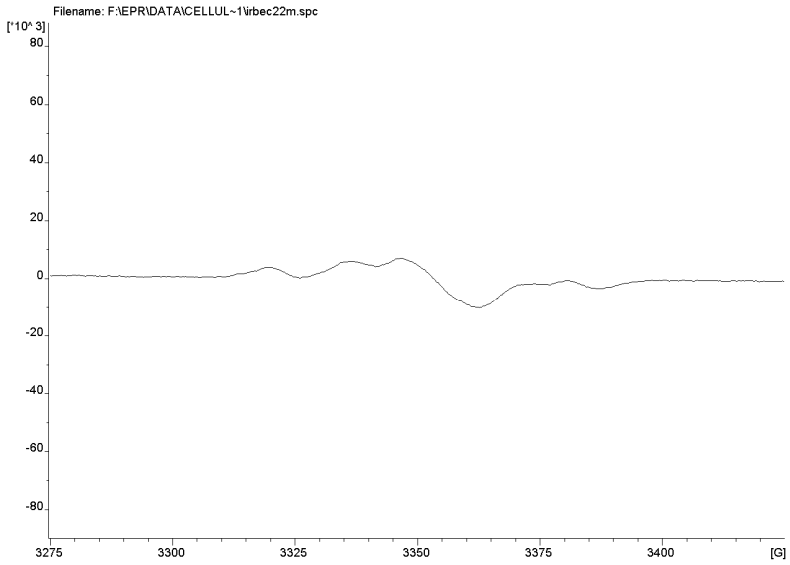
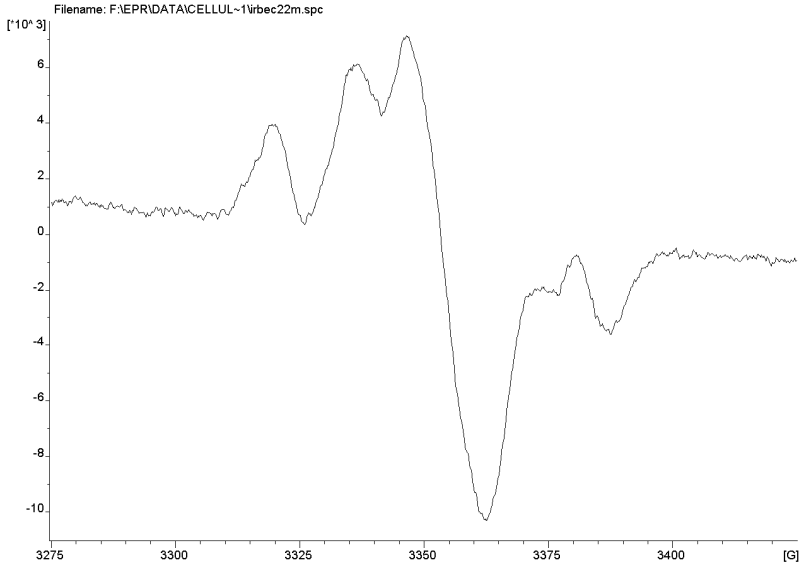


Fig 18. Irradiated cellulose with 0.2 g of benzophenone after 2 months (full scale)



Figs. 17 & 18 show a changed spectra from 2 months previous. Fig. 16 displays the spectra in the same scale as the other spectra in the paper. Fig. 17 shows two large peaks at 3347G and 3330G with another smaller one at 3320G.

Fig. 19 Irradiated cellulose with 0.5 g of benzophenone

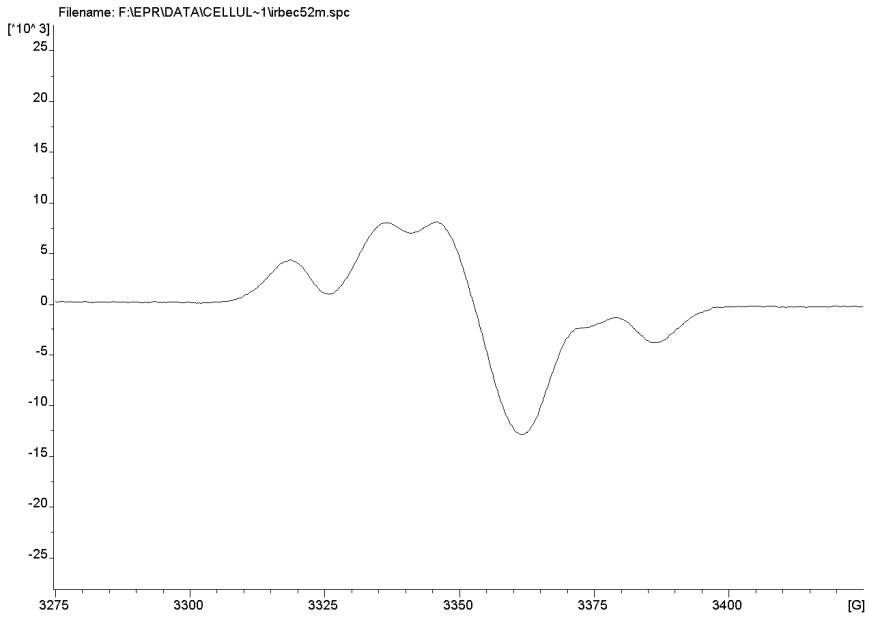


Fig.19 shows a split peak at 3340G with smaller peak at 3320G.

Fig. 20 Irradiated cellulose with 0.5 g of benzophenone after 2 months (full scale)

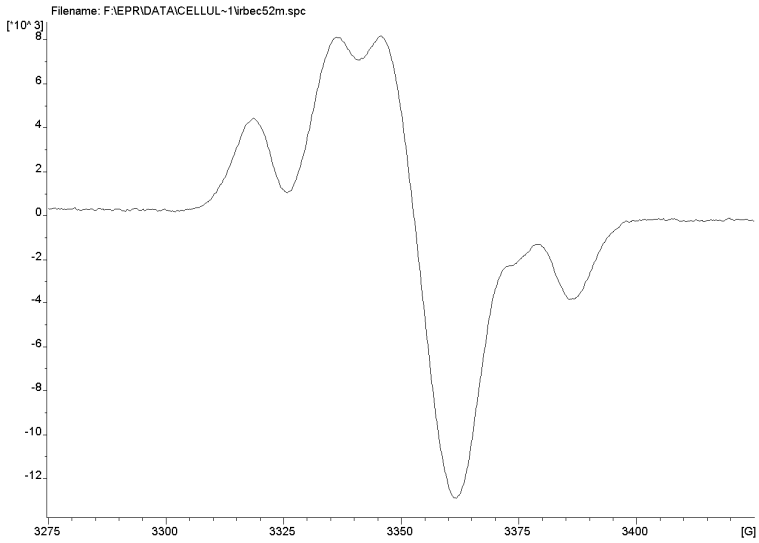


Fig.20 shows a peak at 3345G and another at 3320G.

Figs. 19 & 20 show a changed spectra from 2 months previously. Fig. 18 displays the spectra in the same scale as the other spectra in the paper.

Discussion: The two experimental methods that were used during the research both provided good results, but both of them had problems. During the first experimental method of using H_2O_2 to degrade cellulose and test for the glucose present, the actual presence of the H_2O_2 in the sample posed a problem when using the spectrophotometer. Figs. 9&10 both show data that does not agree with the hypothesis in that as the H_2O_2 concentration increases the absorbance increases. The initial thought that more time was needed for the Benedict's to react in a boiling water bath did not show any improvement in the results. Since the hydrogen peroxide posed a problem to the analysis of the solutions, the enzyme catalase was used to remove the H_2O_2 from the sample. Fig.12 shows that when the catalase was added it seemed to decompose the H_2O_2 being that as the samples that more diluted, showed a decreased absorbance. This shows that the stronger the concentration of the H_2O_2 present, the larger the absorbance is. This can be

justified because the amount of glucose present in the sample can be determined by how dark the color change is when using Benedict's reagent. Although the sample with catalase present seemed to decompose the hydrogen peroxide, there was an unknown peak that appeared at 500 nm. This peak did not appear in the previous samples that were run which prevented a linear plot from being obtained since the glucose calibration plot was constructed by finding the differences in the absorbance at wavelengths of 725 nm and 500 nm. This method showed some promising results with the use of catalase, but the amount of glucose present could not be accurately obtained because of the peak at 500 nm.

The approach of allowing free radical initiators to absorb into cellulose structure through radiation did not focus on studying the formation of glucose, but rather on the generation of free radicals upon irradiation. The ESR technique showed free radicals that were present in the structure. The samples that were not exposed to radiation showed no results on the ESR spectra because no free radicals were present. The samples that were exposed to radiation each showed unique spectra caused by the free radicals present in that structure. The free radical intiator of benzophenone gave the most distinctive differences in ESR spectra out of the free radical initiators used. Benzophenone was hence studied the most out of the free radical initiators because of lack of time. The spectra of the pure irradiated benzophenone and cellulose each had distinctive features and when benzophenone was added, elements of each ones spectra can be seen in the spectra of the mixture. This mixture also showed unique elements of its own that were not present in either the benzophenone or cellulose ESR. This supports the idea that perhaps the benzophenone incorporated itself into the structure of cellulose. When looking at the spectra after two months from when the original samples were analyzed, the spectra of the 0.2g and 0.5g of benzophenone showed interesting changes in the shape of the spectra. This shows

that the structure has changed and that the free radical may have incorporated itself into the cellulose structure. This cannot be said with complete certainty however because of the other possibilities of what may have occurred. Being that the sample was tested again after 2 months, it is possible that the free radicals may have been abstracted by atmospheric oxygen and thus removed. Another possibility is that the radical may have just reacted with air and lost its stability. If more time were available, this could have tested by removing the sample from air and keeping it in an environment, such as nitrogen or any inert gas, so that air could not abstract the free radical. Samples of the pure irradiated cellulose and pure irradiated benzophenone were supposed to be obtained, but due to a mechanical failure in the ESR, these results could not be presented in the paper at this time.

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