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## Kinetic Characterization of B-Carotene in n-hexane at Various Oxygen Concentrations

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**Kinetic Characterization  
of B – Carotene in *n*-hexane  
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Honors Thesis

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Department: Chemistry

Advisor: Mark Masthay, Ph.D.

May 2015

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## Abstract

We have characterized the photo – degradation of  $\beta$ C in oxygenated and deoxygenated *n*-hexane solvent and in the solid phase using broadband (BB;  $\lambda \geq 200$  nm) and 313 nm filtered output of a 100 W Hg Arc lamp. Oxygen concentrations of 0, 10, 20, and 100% were used in the solution phase studies.  $\beta$ C(*s*) did not photo – degrade upon exposure to BB light under air atmospheres; the mass and appearance of the samples, TLC chromatograms, and the absorption spectra were all identical pre- and post-irradiation. In the solution phase, the order of the photo – degradation with respect to  $\beta$ C concentration was of the order  $\frac{1}{2} \leq n \leq \sim 1$  in the presence of O<sub>2</sub> and 0<sup>th</sup> order in the absence of O<sub>2</sub>. Our results indicate that  $\beta$ C(*s*) photo – degradation proceeds via a different mechanism than  $\beta$ C(*s*) thermal degradation,[1, 2] as the photo – degradation and thermal degradation reactions of  $\beta$ C(*s*) yield different products. These results (i) indicate unequivocally that solvent plays a critical role in the photo – degradation process, and (ii) strongly suggest that solvent radicals resulting from UV irradiation are responsible for the photo – degradation, which is especially important in the absence of O<sub>2</sub>. [3-5]

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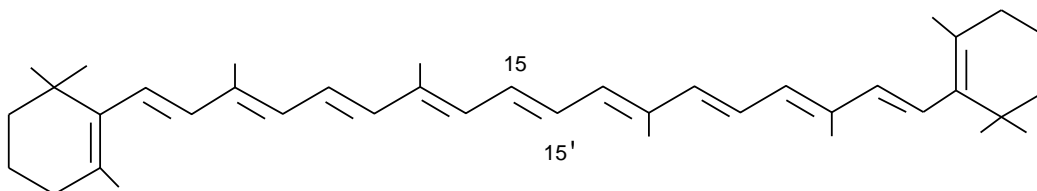
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## Introduction:

$\beta$ -carotene ( $\beta$ C), an orange polyisoprenoid molecule with 11 conjugated double bonds, is an important antioxidant that quenches reactive oxygen species (ROS) free radicals both in plants[6-10] and in the human body.[11-18]

**Figure 1: Structure of  $\beta$ -carotene ( $C_{40}H_{56}$ )**



Because it is non-polar,  $\beta$ C localizes within the  $O_2$ -rich, hydrophobic interior of cellular phospholipid bilayers. When exposed to ultraviolet (UV) light, solutions of  $\beta$ C in *n*-hexane— a hydrocarbon solvent that mimics the hydrophobic interior of cell membranes—turn colorless in both the presence and absence of  $O_2$ . [19] These  $\beta$ C photoproducts have been implicated as causative agents of UV-induced immune suppression [11, 12, 20, 21] and carcinogenesis. [22]. Surprisingly—and in distinct contrast to  $\beta$ C:*n*-hexane solutions— solid  $\beta$ C is photochemically inert. [23]

The primary focus of this research was mechanistic: To characterize the rate law

$$-\frac{d[\beta C]}{dt} = k [\beta C]^n [O_2]^m [hex]^p I_\lambda^q$$

for the photo – degradation of  $\beta$ C in *n*-hexane at various oxygen concentrations. Though our studies are not clinical in design, they may provide new insights into the biological effects of carotenoids in the presence of  $O_2$  and their photoproducts on immune suppression [11, 12, 20, 21] and carcinogenesis. [22]

**Experimental:**1. Solvent Purification

a. Following a procedure by Turro and co-workers,[4] we employed an acid wash to purify the *n*-hexane. Stock *n*-hexane and concentrated sulfuric acid were added in a 1:2 ratio, respectively, to a 125 mL Erlenmeyer flask with a ground glass joint. The mixture was stirred with a large stir bar for at least 12 hours (prepared the night before for use the following day). The solvent-acid mixture separated into two layers: a cloudy looking sulfuric acid waste layer on the bottom and a transparent, purified *n*-hexane layer on top. The purified *n*-hexane was carefully removed with a Pasteur pipette immediately prior to each experiment

2. Modified/improved technique for purifying  $\beta$ C

a. A glass column was packed in increments of ~1-5 inches of a gel benzene (Silica Gel 60) slurry to a height of ~ 7 inches, which was kept submerged under a small “head” of benzene (Sigma-Aldrich, HPLC Grade).

b. The concentrated  $\beta$ C: benzene solution was prepared using ~ 0.148 mg  $\beta$ C/ mL benzene (Sigma-Aldrich, >93% [UV]).

c. The concentrated solution was added to the column a few drops at a time using a Pasteur pipet, with brief waiting periods between aliquots to allow the  $\beta$ C to adhere to the column.

d. Once all of the  $\beta$ C had been added, the column was filled to the top with benzene, the flash attachment was placed on the top of the column, and nitrogen gas was used to push  $\beta$ C through the column at a rate of 1-2 drops per second.

- e. The yellowish leading band was collected in a waste beaker before switching beakers to collect the purified  $\beta$ C (assumed to be present on in the dark orange middle band).
- f. After  $\sim 30$  minutes of collection the beaker was stirred with a large stir bar while gently blowing nitrogen gas over it to evaporate benzene at room temperature. The final dried product was stored in a vial with a septum screw cap under  $N_{2(g)}$  or  $Ar_{(g)}$  in the freezer.

### 3. Degassing Technique

- a. Different in vitro oxygen concentrations were achieved by bubbling samples with compressed gas (Argon, 10% Oxygen, and 100% Oxygen) for 25 minutes using a hypodermic syringe long enough to reach the bottom of the cuvette to ensure thorough solubilization of the gas.

### 4. Solution Studies with BB and 313 nm Filter

- a. A 1cm pathlength quartz sample cuvette filled with  $\sim 3$  mL of purified, air-saturated *n*-hexane was used as blank before bubbling it with 99.999% Ar, 10% O<sub>2</sub>/90% Ar, air, and 100% O<sub>2</sub> atmospheres from pressurized gas cylinders to obtain ostensible O<sub>2</sub> concentrations of  $2.95 \times 10^{-7}$  M,  $1.475 \times 10^{-3}$  M,  $3.09 \times 10^{-3}$  M (for air saturated solutions), and  $1.475 \times 10^{-2}$  M. The Henry's Law constant,  $k_{Henry}^{(O_2 \text{ in } n\text{-hexane})} = 1.475 \times 10^{-2}$  M, was used to specify O<sub>2</sub> concentrations.
- b. An  $\sim 10^{-5}$  M  $\beta$ C:*n*-hexane solution ( $A_{450} \sim 3.0$ ) was transferred to a septum-sealed quartz cuvette and a syringe was used to extract a 0.2 to 0.3 mL aliquot and inject it into the sample cuvette ( $A_{450} \sim 1.0$ ). The bubbling procedure used on the control cuvette was repeated to ensure accurate O<sub>2</sub> concentrations.

c. Samples were irradiated with UV light from the 100W Oriel Hg Arc lamp either in combination with a 313 nm line filter or in the absence of a filter (BB).

d. Absorption spectra were obtained using an Agilent Model 8453 Photodiode Array UV-Visible Spectrophotometer at ambient temperatures of ~21-22° C.

#### 5. Photo-Induced Dark Reactions

a. All experiments were performed with purified *n*-hexane and small irradiation periods (15 or 30 seconds) as compared to earlier experiments (0, 1, 6, 15, 30, 45 min). Air-saturated photo-degradation experiments using stock *n*-hexane usually took at least 30 minutes; comparable photo-degradation experiments with purified *n*-hexane occurred in less than 10 minutes.

b. Purified air-saturated *n*-hexane was used for the blank. Both the control and experimental cuvettes were filled to ~ 1/3 full height with purified air-saturated *n*-hexane and injected with about 0.2 mL of concentrated  $\beta$ C solution. The control was not irradiated with broadband light and stored in the dark. The experimental cuvette was irradiated with broadband light for 30 seconds before being placed in the dark on a magnetic stir plate for 10 minutes. Spectra of each were obtained and the irradiation and dark periods were repeated for a second time and both cuvettes were kept in the dark for 20 minutes.

#### 6. Intermittent vs. Long Irradiation Periods

a. A purified sample of air saturated *n*-hexane and  $\beta$ C was continuously irradiated with broadband light for 6 minutes before obtaining a spectrum.



b. A sample prepared in the same manner was irradiated for intermittent time intervals of 30 – 60 seconds for a total of 6 minutes irradiation with broadband light. Spectra were obtained after each intermittent irradiation period.

#### 7. Septum Cap Tests

a. Purified *n*-hexane was sparged (i.e. bubbled for 25 minutes with highly purified 99.999 %Ar<sub>(g)</sub>) to eliminate dissolved O<sub>2</sub> before obtaining taking a blank. A first spectrum was obtained before leaving the cuvette overnight stirring in the hood (16 hours).

b. The following morning a second spectrum was obtained using the original baseline from the previous day; the UV-Visible spectrometer was left running over night to maintain the baseline.

c. Another sample of purified *n*-hexane was sparged using the same needles as before to ensure septum punctures of the same diameter. After the blank was obtained, the septum cap remained on the cuvette for the first spectrum. The septum cap was then removed and the open cuvette was stirred for one hour, with spectra being obtained every 15 minutes.

d. The absorbance of the spectra obtained in part (b) and part (c) in the 190-220 nm range, which originates from a solvent-O<sub>2</sub> charge transfer band,[3-5] was compared.

#### 8. Solid State Studies

a. 7.1 – 9.2 mg samples of solid, purified βC in septum sealed quartz cuvettes and in a beaker open to the air were irradiated with the collimated broadband output of a 100 W Hg arc lamp. The front and back polished faces of the cuvettes were rotated by 180

degrees frequently to ensure equal exposure to the light at both faces. Attempts were made to note qualitative changes at the times the cuvette was rotated; no changes were observed.

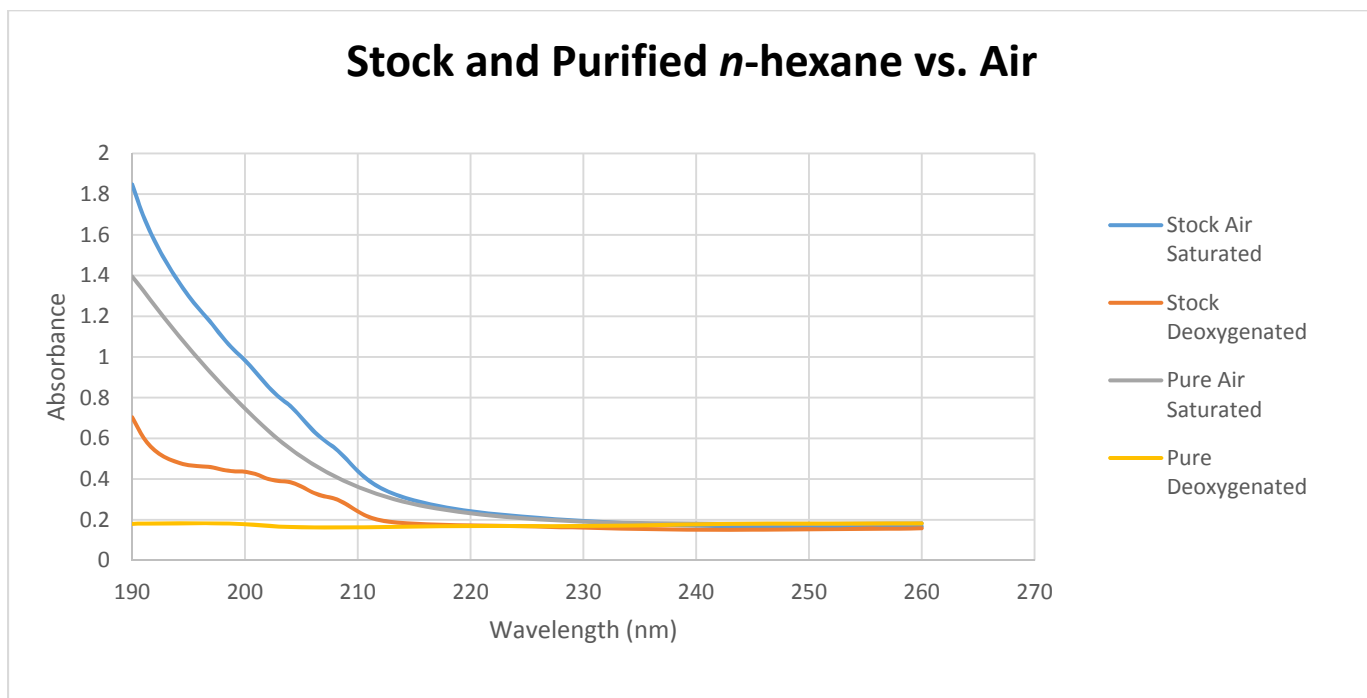
b. To test for photoproducts a sample of the irradiated solid  $\beta\text{C}$  was dissolved in benzene and its thin layer chromatogram with that of dissolved non-irradiated solid  $\beta\text{C}$  as a reference spot was obtained. Benzene was used as the mobile phase.

## **Results and Discussion:**

### 1. Purification of the Solvent

Although the stock *n*-hexane is reported to be 99.0% pure as received, impurities in the solvent must be removed so that a valid mechanism for  $\beta\text{C}$  photo – degradation can be specified. The impurities are likely short chain olefins and may react with oxygen or  $\beta\text{C}$ .

Spectra of pure and stock *n*-hexane under air saturated and sparged conditions were obtained. In Figure 2, it is apparent that the absorbance in the 190 – 220 nm range became significantly less intense when using purified *n*-hexane. The most dramatic difference in absorbance in this range is seen with the deoxygenated purified *n*-hexane, indicating that impurities contribute to the absorbance in this region. We attribute the residual absorbance in purified *n*-hexane to a solvent – to – oxygen charge transfer transition.

**Figure 2: Comparison of stock and purified *n*-hexane**

After several purifications, spectra of the accumulated, yellow sulfuric acid waste were obtained to observe the band in the 190 - 220 nm range. The waste had an intense peak around 300 nm with vibronic structure, suggesting the presence of an alkene. A smaller, unstructured band was also observed at ~450 nm; this band is also suggestive of the presence of a conjugated molecule, and was responsible for the yellow color of the solution.

Experiments performed using purified *n*-hexane were all faster than experiments performed using stock *n*-hexane, suggesting that the impurities partially “quench” the photo – degradation of  $\beta\text{C}$  perhaps by acting as free radical chain – breaking antioxidants.

## 2. Importance of solution preparation

Initially solutions were prepared by adding *n*-hexane to a blanking cuvette then adding a small amount of solid  $\beta$ C directly to that cuvette and stirring. The problem with this procedure is that we were unsure of the mass of  $\beta$ C being added and thought that the solid was dissolving instantly, as that is how it appeared visually. It actually takes much longer for  $\beta$ C to fully dissolve, leading to increasing absorbance with time.

We believed that we were observing induction periods because the absorbance values at 450 nm would increase before falling. In fact this observation was due to the  $\beta$ C continuing to dissolve, leading to increasing concentrations and absorbance measurements.

This issue was resolved by preparing a concentrated  $\beta$ C solution in a 10 mL or 5 mL volumetric flask, transferring a portion of this solution to a septum – capped cuvette and using a syringe to extract a small aliquot (usually 0.2 to 0.3 mL), and then injecting this aliquot into the blanking cuvette. This method also eliminated any zeroing inaccuracies caused by using different cuvettes for the blank and the sample.

The majority of oxygen in our samples was eliminated upon sparging with purified argon due to Henry's Law

$$P_{O_2} = k_H^{O_2-hexane} [O_2],$$

in which  $P_{O_2}$  is the partial pressure of a  $O_2$  above the solvent and is  $k_H^{O_2-hexane}$  is the

Henry's Law constant for  $O_2$  in *n*-hexane. Sparging with argon increases  $P_{Ar}$  above the solvent and decreases  $P_{O_2}$  to essentially zero, causing  $O_2$  to leave the sample solution.

### 3. Solution Studies with BB and 313 nm Filter

The following results were obtained:

(1) Reaction orders  $n$  for  $\beta\text{C}$  in  $\beta\text{C}:n$ -hexane solutions irradiated with BB light were as follows: sparged,  $n=0$ ; air-saturated,  $n=1/2$ ; and  $\text{O}_2$ -saturated,  $n=1/2$

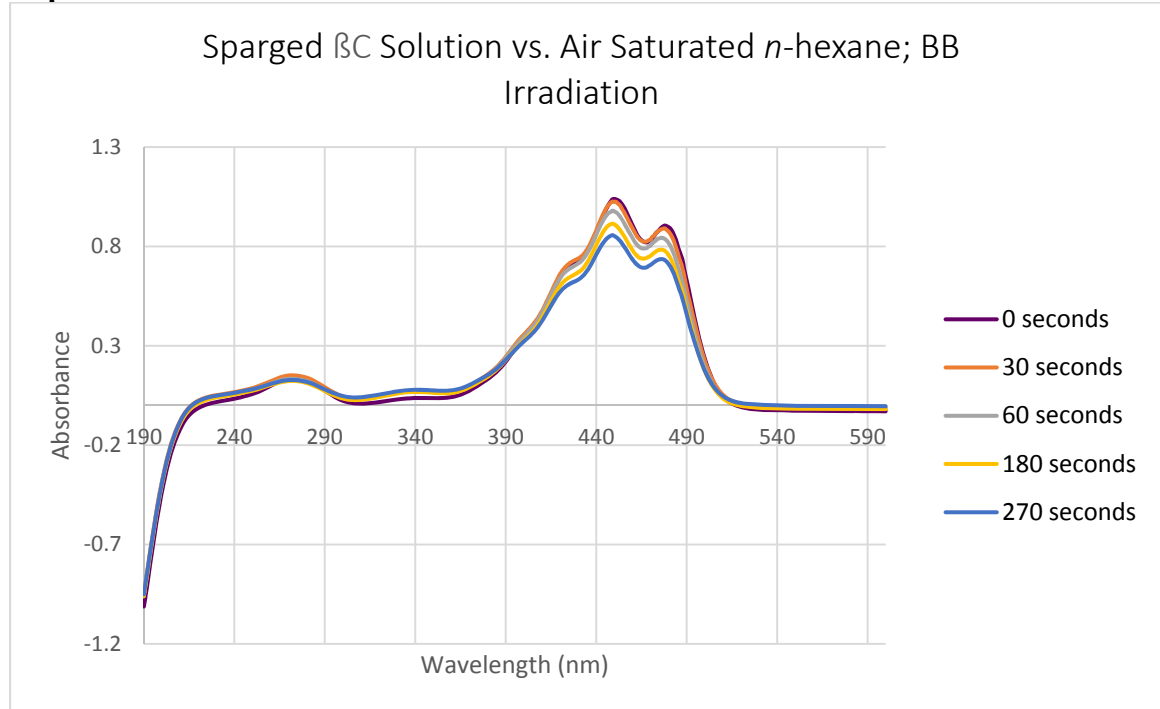
(2) Reaction orders  $n$  for  $\beta\text{C}$  in  $\beta\text{C}:n$ -hexane solutions irradiated with the 313nm Hg line filter were as follows: sparged,  $n=0$ ; air-saturated,  $n=1/2$ ; and  $\text{O}_2$ -saturated,  $1/2 \leq n \leq 1$ .

(3) The order  $n$  of photo – degradation rate with respect to  $[\beta\text{C}]$  increases with increasing  $[\text{O}_2]$ .

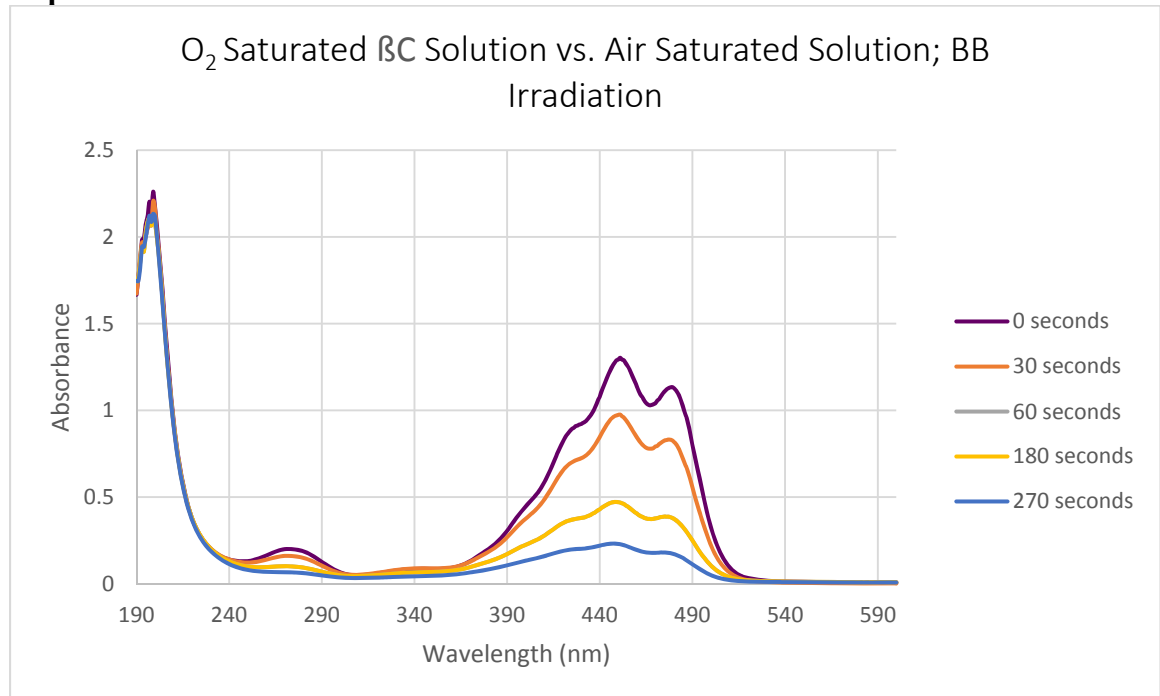
(4) Rates of photo – degradation of  $\beta\text{C}$  in  $n$ -hexane solvent are significantly faster with BB ( $\lambda \geq 200\text{nm}$ ) than 313nm irradiation;

(5) Sparged  $\beta\text{C}:n$ -hexane solutions degraded at a rate which is zero<sup>th</sup> order in  $[\beta\text{C}]$ , suggesting the involvement of alkyl radicals which result from cleavage of C-C bonds in  $n$ -hexane upon irradiation with short-wavelength( $\lambda < 200\text{nm}$ ) UV light.

**Figure 3: Typical Spectrum Obtained for a Sparged Solution Photo-degradation Experiment**



**Figure 4: Typical Spectrum Obtained for O<sub>2</sub> Saturated Photo-degradation Experiment**



Figures 3 and 4 are representative of typical spectra obtained for sparged and O<sub>2</sub> saturated concentrations upon BB irradiation. By comparing Figures 3 and 4, it is obvious that βC: *n*-hexane solutions that are O<sub>2</sub> saturated degrade more quickly than sparged solution. After 270 seconds of irradiation, O<sub>2</sub> - saturated solutions appeared almost colorless, corresponding to a low A<sub>450</sub> as seen in Figure 4 (Note: Figure 3 is not completely representative of a typical experiment; usually spectra were obtained until the absorbance had decreased past two half – lives in order for our kinetic studies to be reflective of the actual photo – degradation reaction).

One way to determine an order of a reaction is to see which kinetic plot gives the best linear fit for a particular O<sub>2</sub> – concentration. In this spirit, data from BB irradiation experiments are plotted in Figures 5a,b, and c to model 0, ½, and 1<sup>st</sup> order kinetics, respectively.

**Figure 5a: Fitting to 0<sup>th</sup> Order; A<sub>450</sub> vs. Time (Rate  $\propto$  [βC]<sup>0</sup>)**

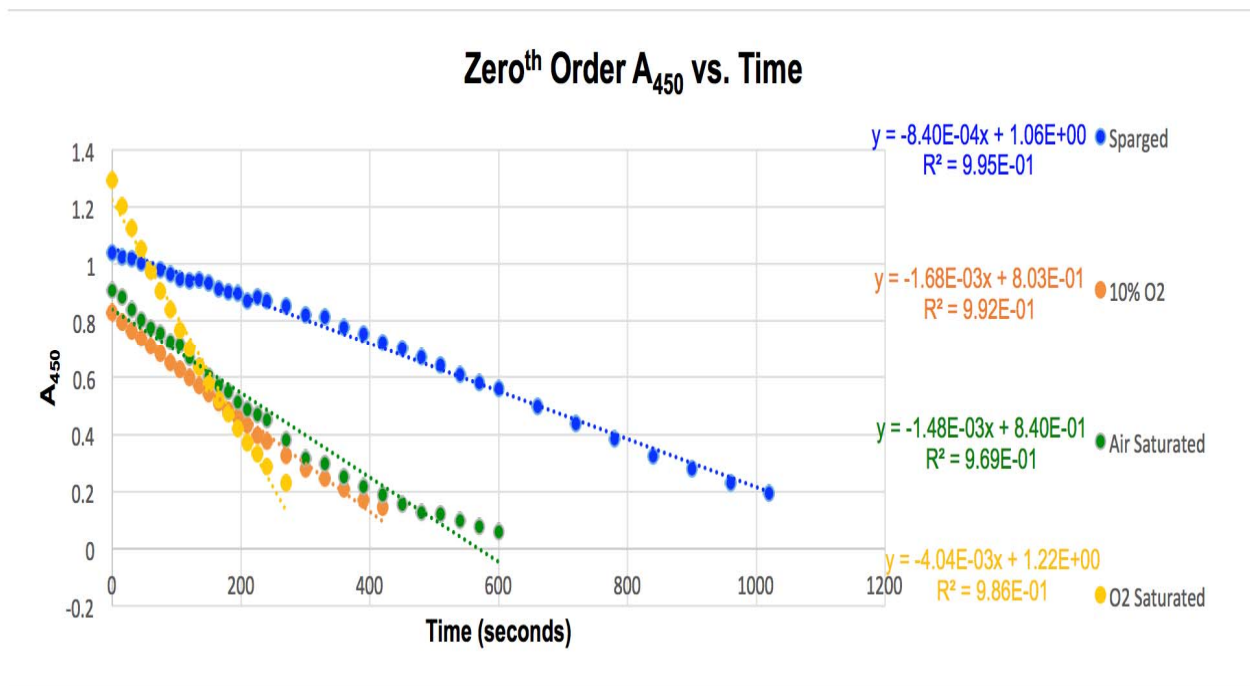
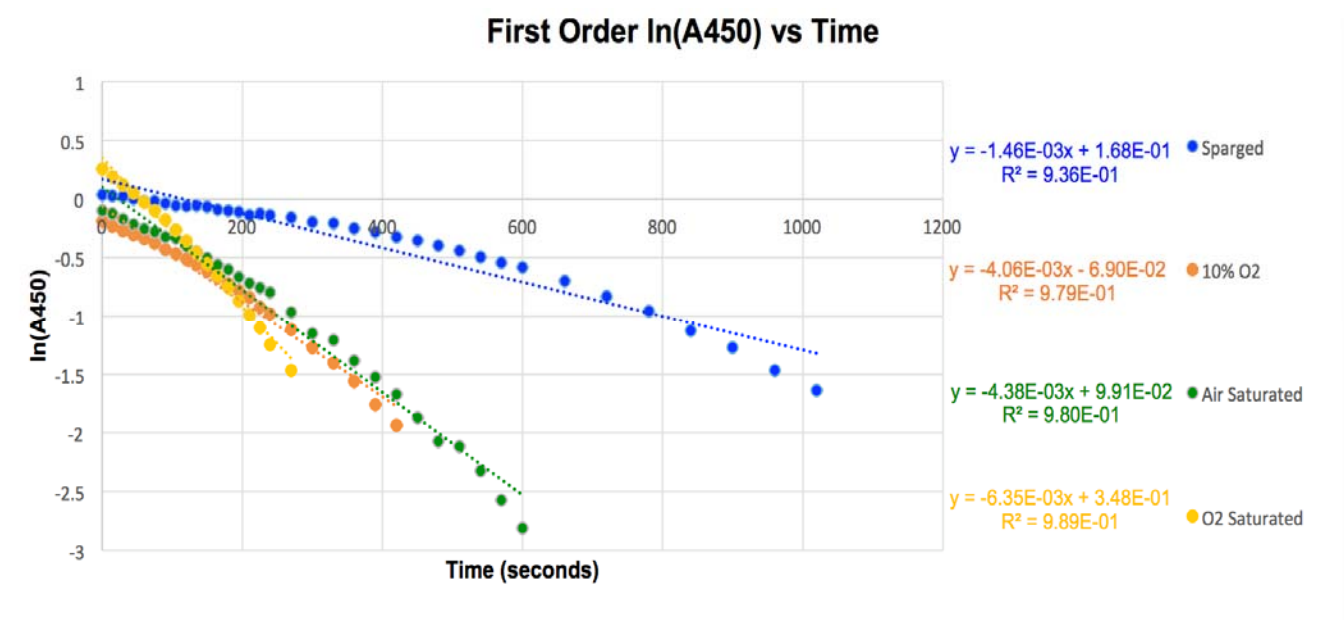


Figure 5b: Fitting to  $\frac{1}{2}$  Order;  $A_{450}^{1/2}$  vs. Time (Rate  $\alpha [\beta C]^{1/2}$ )



Figure 5c: Fitting to 1<sup>st</sup> order;  $\ln(A_{450})$  vs. Time (Rate  $\alpha [\beta C]^1$ )



On each Figure, each experimental oxygen concentration is included. The sparged solution (blue circles) most closely fits the linear trend line in Figure 5a, the 0<sup>th</sup> order



kinetic plot. By comparing Figures 5a, b, and c, it can be seen visually that as the reaction order increases, the linear fit becomes poorer.

Correlation coefficients were used to see how precisely the data fits the linear trend line. From these plots the reaction order with respect to  $\beta\text{C}$  is 0 for sparged,  $\frac{1}{2}$  for 10% and 20%  $\text{O}_2$  concentrations, and  $\frac{1}{2} - 1$  for  $\text{O}_2$  saturated solutions.

As illustrated in Figures 5a, b, and c, the order,  $n$ , of the photo – degradation rate with respect to  $[\beta\text{C}]$  increases with increasing  $[\text{O}_2]$ . The same  $[\text{O}_2]$  trend was observed with the 313 nm line filter as seen in Figures 6a,b and c below.

**Figure 6a: Fitting to Zero Order,  $A_{450}$  vs. Time (Rate  $\propto [\beta\text{C}]^0$ )**

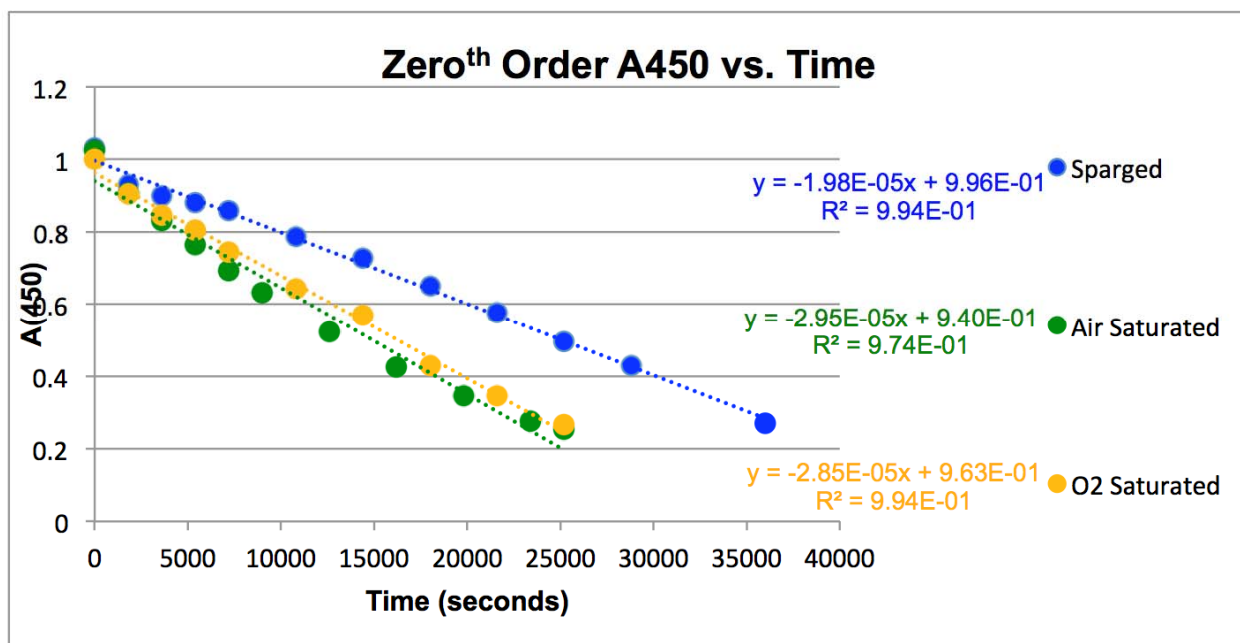


Figure 6b: Fitting to  $\frac{1}{2}$  Order;  $A_{450}^{1/2}$  vs. Time (Rate  $\alpha$   $[BC]^{1/2}$ )

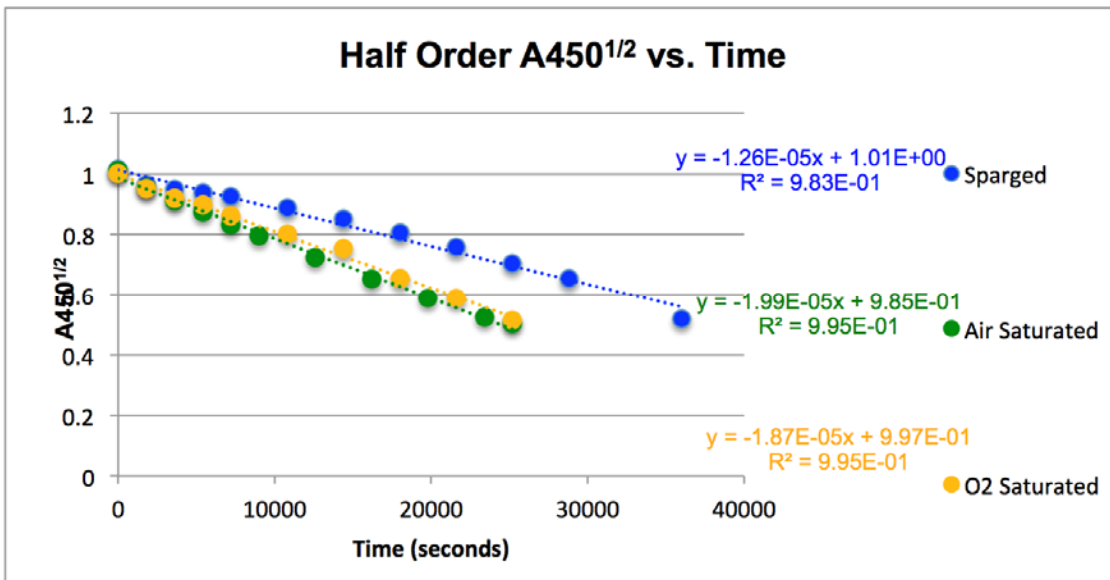
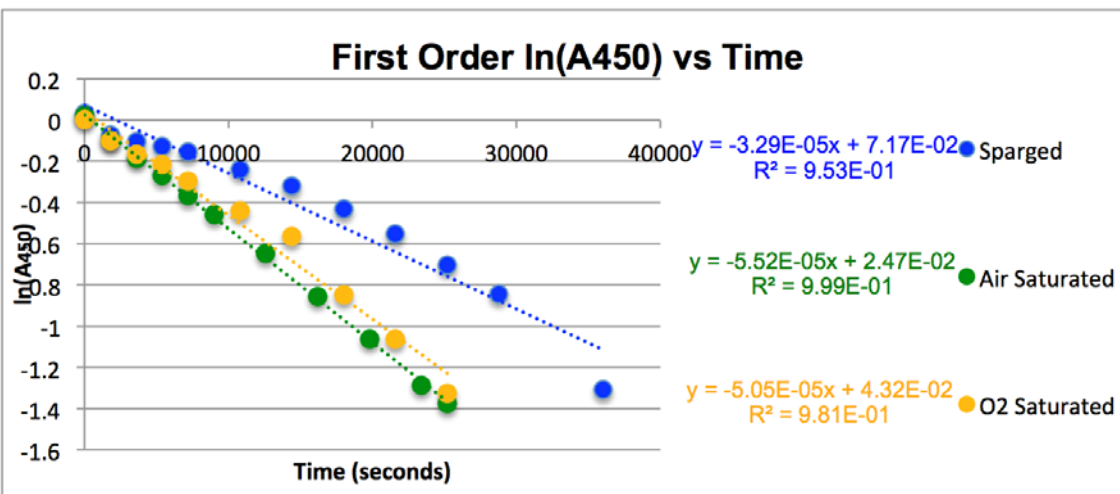


Figure 6c: Fitting to 1<sup>st</sup> order;  $\ln(A_{450})$  vs. Time (Rate  $\alpha$   $[BC]^1$ )



The order of the reaction was also confirmed by examining the half – life trends. For any 0 order reaction, the half – life decreases as the concentration of the reactant decreases. For any 1<sup>st</sup> order reaction, the time to reach each half-life is constant.

**Table 1: Examining half – life trends in 313 nm line filter data**

	Sparged		Air Saturated		Oxygen Saturated	
	Time (sec)	A(450)	Time (sec)	A(450)	Time (sec)	A(450)
$T_0$	0	1.031	0	1.024	0	1.005
1 <sup>st</sup> $T_{1/2}$	25200	0.495	12600	0.523	14400	0.567
2 <sup>nd</sup> $T_{1/2}$	10800	0.270	12600	0.253	10800	0.266

The data Table 1 indicate that the second half-life (10,800 sec) is very roughly 50% shorter than the first half-life (25,300 sec) in sparged ( $[O_2] \sim 0$ ) solution, providing strong support for  $n = 0$  order kinetics with respect to  $[\beta C]$ . For air – saturated and  $O_2$  saturated concentrations the first and second half-lives are approximately equal, indicating an order  $n \sim 1$  in  $[\beta C]$ ; this was particularly apparent with the air-saturated solutions.

As previously noted with Figures 5a, b, and c,  $n$  increases with increasing  $[O_2]$ . All reactions were faster with BB irradiation vs. the use of the 313 nm line filter. Both of these trends are observed in the data in Table 2 below; the bold numbers represent the orders with the best linear fit.

**Table 2: Ratio of the Rate Constants BB: 313 nm**

Kinetic Order	Sparged	Air Saturated	Oxygen Saturated
0	<b>42.42</b>	50.06	141.83
1/2	43.18	<b>61.32</b>	<b>132.75</b>
1	44.43	<b>86.81</b>	<b>115.06</b>

The final aspect of the solution phase studies was the examination of why  $\beta\text{C}$  still photo – degrades in the absence of  $\text{O}_2$ . We propose that alkyl radicals play an important role in this process. *n*-hexane absorbs at low wavelengths ( $\lambda < 200\text{nm}$ ) and it is this source of high energy that is capable of generating alkyl radicals from *n*-hexane. Our experimental results agree with this notion because when BB light ( $\lambda \geq 200\text{nm}$ ) was used,  $\beta\text{C}$  in a sparged solution degraded past 2 half – lives in approximately 16 minutes. However when the 313 nm line filter was used, it took approximately 10 hours for  $\beta\text{C}$  to degrade past 2 half – lives.

Once alkyl radicals are generated, it is likely that most will recombine back to *n*-hexane since they cannot diffuse far in solution due to their inherent instability and short lifetimes. However, the small fraction of alkyl radicals that are in close proximity to a  $\beta\text{C}$  molecule may react with  $\beta\text{C}$  via (i) addition across the double bonds in the  $\beta\text{C}$  conjugation pathway or (ii) cleavage of the conjugation pathway, and the absorbance will decrease as a result.

#### 4. Photo-induced Dark Reactions

Since the cuvette was being transferred back and forth from the lamp to the spectrometer between irradiation intervals, it was possible that photo-induced dark

reactions were occurring and artificially increasing the observed photo – degradation rate constant and rate. To see if this was occurring we prepared a control and experimental sample and monitored their absorbance.

$A_{450}$  remained approximately constant for the non – irradiated control throughout, decreasing from 0.69374 to 0.68719. The absorbance for the experimental decreased after 30 seconds of broadband irradiation from 0.60575 to 0.45474. This absorbance value did not decrease significantly during its 10 minute incubation in the dark – 0.45474 to 0.43552. After another 30 second irradiation period the absorbance decreased from 0.43552 to 0.33372, and again this absorbance value did not decrease significantly after another 10 minute incubation in the dark – 0.33372 to 0.32769.

Significant decreases in absorption were only observed during irradiation. An incubation period in the dark between irradiation did not affect the absorption significantly. Hence photo – induced dark reactions are not occurring or are negligible.

##### 5. Intermittent vs Long Irradiation Periods

BB irradiation intervals were typically 0,1,6,15,30, and 45 minutes in earlier experiments with stock *n*-hexane; spectra were obtained at the end of each irradiation period. The impact of short, intermittent irradiation intervals were characterized by taking spectra after 15 second irradiation intervals for a total irradiation exposure of 3 minutes using purified *n*-hexane. These experiments were performed in an attempt to observe a possible sigmoidal dependence of reaction rate on  $[\beta\text{C}]$  that may have been missed in experiments with longer irradiation periods. We did not expect the reaction to occur faster.

Since there is no evidence of photo-induced dark reactions, we tested to see if more frequent irradiations verses one continuous period of equal time affected absorbance.

Prior to irradiation the absorbance at 450 nm was 1.23270. After 6 minutes of irradiation the absorbance had dropped by 71.57 % to 0.35047. These results were compared to those from the purified air saturated *n*-hexane with  $\beta$ C experiment performed a few days prior. After 20 irradiation periods, a total of 6 minutes irradiation time, the percent difference in the absorbance was 72.37%, indicating that both intermittent and long irradiation intervals yield essentially the same total photo – degradation percentages for identical total irradiation intervals. Additionally sigmoidal behavior was not observed when the data was plotted in *Excel*, thus precluding autocatalysis during the photo – degradation.

#### 6. 10% O<sub>2</sub> / 90% Ar Gas Mixture

The 10% O<sub>2</sub> / 90% Ar mixed gas cylinder was custom made by Weiler, a local compressed gas distributor.

A photo – degradation experiment with broadband light was performed following the standard procedure. However, a drastic decrease in volume ( $\sim \frac{1}{4}$  of the total cuvette volume) and temperature was observed after bubbling the purified *n*-hexane cuvette for 10 minutes and bubbling was stopped to ensure enough solution would remain to obtain a spectrum. Since vaporization is an endothermic process both the volume and temperature decreased during the bubbling. The cuvette remained on the stir plate until it returned to room temperature before spectra were obtained to reduce effects from temperature – induced changes in solvent density.

The degradation was monitored for a total irradiation time of five minutes during which more than two half-lives elapsed. This rapid photo – degradation rate was surprising because it was expected that the total irradiation time would be between that observed for the air – saturated (10 minutes for 2 half – lives) and sparged solutions (17 minutes for 2 half – lives).

Since the bubbling period was shortened during this first experiment, the experiment was repeated. In the second experiment new *n*-hexane was bubbled with a longer hypodermic needle for a full 25 minutes and  $\beta\text{C}$  in the  $\beta\text{C}:\textit{n}$ -hexane solution for 15 minutes; the total irradiation time required to reach two half-lives was 7 minutes. This data resulted in a first order rate constant slightly lower than that for air saturated conditions, corresponding to the expected direct dependence of photo – degradation rate on  $\text{O}_2$  concentration.

#### 7. Septum Cap Tests

Since it is important for the relative concentration of oxygen in our samples to remain constant, we characterized the permeability of a septum cap to oxygen that had been pierced twice during the sparging technique.

We found that the total increase of  $A_{190}$ , originating from the solvent – to – oxygen charge transfer band, after 30 minutes of air exposure is comparable to the increase in absorbance after 16 hours incubation with the septum cap on. Hence, the septum cap slows the reentry of  $\text{O}_2$  into the solvent from air by a factor of  $\sim 32$ .

#### 8. Solid State Studies

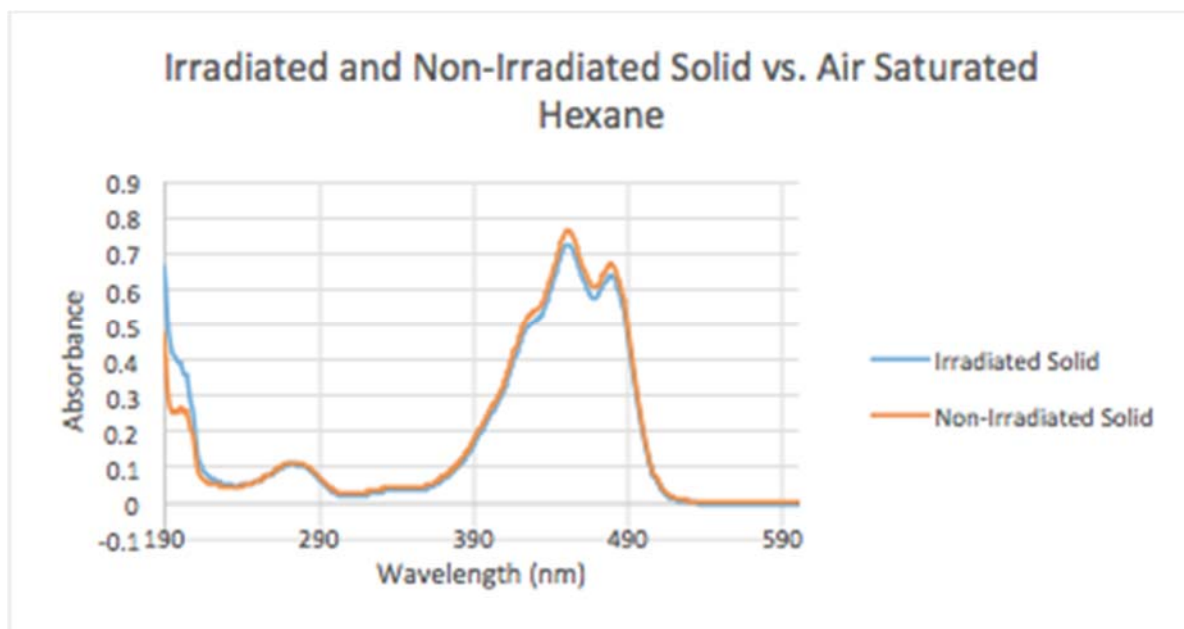
When irradiated in the open beaker, the initial loose  $\beta\text{C}_{(s)}$  powder developed a hard outer shell; when disturbed with a spatula this outer shell fragmented. The  $\beta\text{C}$

underneath the shell was powder-like but it was of a firmer consistency than the original loose powder, possibly indicating that the  $\beta\text{C}$ , being fully exposed to the atmosphere, had taken on components from the atmosphere and/or had undergone a reaction. However, no change in mass pre – and post – irradiation was observed, therefore if  $\beta\text{C}_{(s)}$  had taken on anything from the atmosphere it must have released components of equal mass. It is also possible that the  $\beta\text{C}$ , upon direct UV light exposure, heated up then cooled and hardened post – irradiation. No other physical changes were observed in all trials.

Minimal changes in the absorption spectra of the  $\beta\text{C}$ : *n*-hexane solutions post – irradiation compared to pre – irradiated  $\beta\text{C}$  :*n*-hexane solutions were observed as illustrated in Figure 6. The spectrum for these samples were essentially identical across the full spectrum except at  $\lambda \sim 200$  nm; at these very short wavelengths the absorbance of the irradiated sample was greater than that for the non – irradiated sample as shown in Figure 6. In previous experiments of our own, it was confirmed that this band  $\sim 190 - 220$  nm is characteristic of  $\beta\text{C}$ / *n*-hexane absorption spectra and is largely due to oxygen present in the system. It is possible therefore that the greater absorbance at  $\lambda \sim 200$ nm for the irradiated sample may be attributed to a greater amount of oxygen entering the sample open to the atmosphere during the irradiation of the solid.



**Figure 6: Comparing Irradiated and Non – irradiated Solid Absorption Spectrum**



TLC Studies of  $\beta\text{C}_{(s)}$  Pre – and Post – Irradiation:

No changes were observed in the TLC plates. The fluorescent TLC plates were spotted with concentrated samples of the irradiated and non-irradiated solids dissolved in benzene and then developed using a benzene mobile phase. A hand-held UV lamp was used to study the plates. Both pre – and post – irradiation spots travelled the same distance; and only these two spots were observed under visible, short-band and long-band UV light.

Our absorption spectra and TLC data indicate that solid  $\beta\text{C}$  is significantly more stable upon irradiation than  $\beta\text{C}$  dissolved in *n*-hexane. The gravimetric data indicate that non-volatile photoproducts are not generated during BB irradiation of  $\beta\text{C}_{(s)}$ .

## Future Studies

Continued studies will be performed both for solution and solid phase  $\beta$ C.

For the solution phase studies, 2,3- dimethylbutane (DMB, a liquid at ambient temperature) and 2,2,3,3-tetramethylbutane (TMB, a solid at ambient temperature) have already been purchased and will be used instead of pure *n*-hexane. Solutions of  $\beta$ C in neat DMB solvent and a saturated solution of TMB in *n*-hexane will be irradiated with the BB output of our Hg lamp. These branched alkanes will produce alkyl radicals more easily than a straight – chain alkane, and rather than primary radicals ( $\bullet R_{1o}$ ), they will generate secondary ( $\bullet R_{2o}$ ), and tertiary ( $\bullet R_{3o}$ ). Increasing the substitution increases radical stability and lifetime so that  $\bullet R_{2o}$  and  $\bullet R_{3o}$  will diffuse further in solution before recombining, thus making reaction with  $\beta$ C more likely. Using these solvents, especially with sparged solutions, should allow for better characterization of the role of alkyl radicals in the photo – degradation of  $\beta$ C. In addition to a change in solvent, a change in the light source would improve the radical focused studies. The Hg lamp's broadband profile extends down into short wavelength UV region but the light is not as intense at low wavelengths. The use of a D<sub>2</sub> or Xe lamp would allow for greater intensity at low wavelengths, which is the range important for radical production.

For the solid phase studies, changes in the experimental apparatus will allow for more uniform and complete irradiation of the solid. To avoid the issue of the light only irradiating the surface layer of a solid, a thin film will be prepared by creating a concentrated  $\beta$ C:benzene solution, evenly spreading it on the intended surface of a polished cuvette face, and gently blowing N<sub>2(g)</sub> over it to evaporate the solvent, leaving a thin, dried film of  $\beta$ C residue. Such an arrangement will facilitate obtaining changes in

the absorption spectrum of the thin filament itself as the irradiation proceeds, as well as characterization of volatile products using gas chromatography of the post – irradiation atmospheres in the septum – capped cuvettes. Also an IR filter will be incorporated in the experimental apparatus to eliminate wavelengths that cause the reaction vessel to heat up over the course of hours of UV irradiation exposure. The use of a quartz cuvette filled with copper sulfate solution placed in front of the reaction vessel would filter out IR wavelengths without absorbing in the UV range. Elimination of IR exposure will help demonstrate if the hardened shell observed in our studies was due to a physical heating and re – cooling process or a chemical process.

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