## ABSTRACT

ZHANG, WENWEN. The Study of the Synthesis of a Water Soluble Antimicrobial Polymer. (Under the direction of Stephen Michielsen.)

In this study we have tried to synthesize a water soluble antimicrobial polymer in order to produce an effective, reusable and skin friendly antimicrobial fabric while also lowering the production cost. The soluble polymer is a copolymer of acrylic acid, vinyl benzyl chloride, and 4-stryrene sulfonic acid. The antimicrobial agent, Rose Bengal, was expected to attach to the copolymer through a reaction with benzyl chloride, but it was found that this did not occur. Rather, a large amount of free dye was found at the completion of the reactions. To eliminate the free dye, the reaction conditions were adjusted. After testing several methods for quantitatively determining the amount of free dye in our samples, it was found that dialysis provided the best quantitation. The amount of free dye was measured at several points during the polymerization to understand the cause of residual free dye. We believe it is due to competition between –COOH groups on Rose Bengal and on acrylic acid. By modifying the polymerization conditions and the benzyl Rose Bengal monomer synthesis, water soluble and antimicrobial polymer was successfully polymerized with very little free dye remaining. This was accomplished by attaching Rose Bengal to vinyl benzyl chloride. Next this monomer was copolymerized with acrylic acid to enhance its water solubility. While the polymerization was occurring, the reaction mixture was added to an aqueous solution of 4-styrene sulfonic acid and the polymerization continued.

# The Study of the Synthesis of a Water Soluble Antimicrobial Polymer

by
Wenwen Zhang

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APPROVED BY:

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| _____ | _____ |
| Dr. Stephen Michielsen<br>Committee Chair | Dr. David Hinks |
| _____ | _____ |
| Dr. Melissa Pasquinelli | Dr. Jan Genzer |

## DEDICATION

This thesis is dedicated to my parents and my advisor Dr. Stephen Michielsen

## BIOGRAPHY

The author was born in Weifang, Shandong province, China in January 22th, 1989. She received the Shanghai Excellent Graduate award and received her Bachelor of Engineering in Textile Engineering (Textile Testing and Commerce) from Donghua University, China. In January, 2011, she joined the College of Textiles at North Carolina State University to pursue a Master of Science degree in Textile Engineering. She will continue onto her PhD degree in Fiber and Polymer Science in College of Textile, NC State.

## ACKNOWLEDGMENTS

I appreciate Dr. Michielsen's help and encouragement throughout my research and life in the US. Without him, I would not be a master student in NC State. He guided me at every moment that I needed suggestions. He encouraged me to think and work by myself in order to get prepared for my PhD study. He offered me and my friends very nice help in my life when I needed his advice. He also taught me science knowledge and American English. I feel so lucky to be one of his students.

I also acknowledge the members of my committee Dr. David Hinks, Dr. Melissa A. Pasquinelli, and Dr. Jan Genzer for their interest. I would like to thank Dr. Beck, Elton R. Lawrence and Brooke L. Kaznowski for their help during the study of vinyl benzyl Rose Bengal chemical structure. I would like to thank Jeff Kraus and Vicki Stocksdale for their help through my research. I would like to thank Jialong Shen and Joo Ram Kim for their help in conducting singlet oxygen production experiments and processing FTIR results, respectively. I would also like to thank College of Textiles' staff, who helped me during my research. I wish to express my appreciation to Textile Engineering, Chemistry and Science department and LaamScience for funding this research.

I thank my parents, brother and sister-in-law for their support throughout my life and during my study in the United States from overseas. I thank my friend Qian Jiang,Yuqiang Fu, Gabrielle Mineo and Elizabeth Phelps for their endless support throughout my study.

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## 1. INTRODUCTION

With the increasing concern over health and disease control, antimicrobial fabrics are playing a more and more important role in both protecting people who are working with life-threatening diseases and creating a healthy environment for people. Recently, the medical market, furniture market, tourist market, and numerous family housewives are in need of a highly effective, good durability, low cost antimicrobial fabric.(*1*)

The current commercial antimicrobial fabrics have been widely used in house cleaning, surgical room maintenance, and public health system. Most of them are antibiotics applied to nonwoven fabrics, which have poor durability, high potential to damage skin, and are not always effective because the scope of some antibiotics is very limited. S. Michielsen et al. proposed grafting the antimicrobial agent onto a copolymer, which was attached to a nylon surface to produce the desired antimicrobial fabric.(2) The plan of his proposal was to attach antibiotics on a soluble copolymer first, and then graft the copolymer on nylon and other fabrics. Rose Bengal has been proved to be an effective antimicrobial dye.(*3*) Unlike other types of common antibiotics, it can release singlet oxygen while being exposed to light. The amount of Rose Bengal does not decrease with increasing time. More importantly, once Rose Bengal attached copolymer is grafted onto a fabric surface, it won't be washed off. It is not only good for cost, but also good for sustainability.

Singlet oxygen is short lived and can only exist for 1~2 μs in water(*4*). It should be safe for human skin because the mean thickness of epidermis layer of human skin is around 11 μm at the shoulder(*5*). Singlet oxygen can kill almost all microorganisms for it can oxidize structural proteins in them(*6*). Also, it won't motivate drug resistance of the microorganisms.

Therefore, making water soluble Rose Bengal attached copolymer became the final target of this study.

In 2011, S. Michielsen's student Halil Ibrahim Akyildiz produced a water soluble copolymer, poly (acrylic acid-co-styrene -co-styrene sulfonic acid) (*7*). Based on this work, the current study was supposed to attach Rose Bengal onto a similar copolymer using vinyl benzyl chloride in place of styrene. After the polymer was formed, the Rose Bengal containing copolymer was to be attached to nylon, and then the leaching properties and antimicrobial performance of the final fabric were to be tested. Unfortunately, at the beginning of this study, it was unexpectedly found that there was a large amount of free dye in the copolymer solution. Therefore the target of the study was changed to reduce the free Rose Bengal in the copolymer water solution. Three different approaches were attempted to reduce the free dye and increase the solubility of the polymer. Esterification and addition polymerization was involved in every experiment, which makes the reaction difficult to control. We found that a 2-step reaction could lead to the effective, soluble, copolymer with little or no free dye.

The objectives of this study are listed as follows.

(1) Develop a free dye evaluation procedure.

(2) Copolymerize PBRB (polymer-bonded Rose Bengal) copolymer without free dye.

(3) Improve water solubility of PBRB copolymer.

(4) Understand the behavior of Rose Bengal at different pH values.

(5) Compare singlet oxygen production efficiency of PBRB with Rose Bengal.

(6) Understand chemical structure of VBRB synthesized in this study.

In the following sections, we will describe the synthesis and characterization of PBRB.

# 2. LITERATURE REVIEW

## 2.1 Antimicrobial Fabric

### 2.1.1 Introduction

An antimicrobial is a material that can kill or inhibit the growth of microorganisms such as viruses, bacteria, fungi, or protozoans. People from ancient Egypt and China used juices from certain plants as drugs to kill microorganisms from the beginning of their cultures. The term "antimicrobial" originated with Pasteur and Joubert (*8*), who found one type of microbe can prevent the growth of another type. Antimicrobial materials can be heavy metals, poisons, or highly developed antimicrobial agents. However, some microbes almost always survive from some antimicrobials, especially the heavy metal and poisons type. To avoid developing antimicrobial resistance, more and more studies are focusing on the development of new antimicrobials. The use of antimicrobials has experienced rapid growth in many fields such as medical applications, agricultural industry and even the food industry. The combination of textiles and antimicrobial agents has inspired many applications in many fields, especially in medical applications.

### 2.1.2 Development

The first generation of textiles with antimicrobial agents was made by immersion of fabric in a solution containing an antimicrobial agent. This type of product does not retain its activity for long periods as we desire. The second generation of antimicrobial textiles was the treatment of the fiber with a coating(*9*). After applying a thin coat on fibers, we can weave them to get a fabric. The final antimicrobial fabric can last a little bit longer but still does not satisfy our needs. The third generation is based on spinning technology. In melt spinning, a

master batch containing antimicrobials was added to the melt solution and then spun into fibers.(*10*) Antimicrobials inside the fiber can diffuse in final product during the application period. These antimicrobial fibers can last longer than the previous two generations. However, it is hard to control the concentration of the drugs on the fiber surface in application and to make it last as long as the fabric. And what's more, the safety issue could come up when we use them as undergarments. Therefore, a new approach is urgently needed to solve this problem.

## 2.2 Singlet oxygen

### 2.2.1 Basic information

The first electronic excited state of molecular $O_2$, singlet oxygen is much more active than ground state, triplet oxygen because it has higher energy. This enables singlet oxygen to readily oxidize microorganisms. Singlet oxygen can be produced by photosensitization process, which is a light-activated process requiring a light-absorbing substance i.e. photosensitizer. (*11*) In Dr. Leonard I. Grossweiner's opinion, in addition to its reaction with microbes and tumor cells, singlet oxygen is believed to mediate atmospheric airglow, lipid peroxidation, atmospheric pollution, photo hemolysis, and to react with many organic compounds such as olefins, hetero-aromatic, and fatty acids.

### 2.2.2 Biological Application

With the increasing attention on environment and public health issues, singlet oxygen is more and more popular in water purification and tumor treatment based on its reaction with microbes and tumor cells. A. J. Acher and I. Rosenthal described one way to purify organic wastewater by singlet oxygen produced by photosensitization. In their experiment, Rose

Bengal (RB) and methylene blue (MB) were used as photosensitizers. They found MB was more effective for destroying fecal coliform population under UV light. They also determined the effect of MB concentration, the amount of energy supplied, removal of dye-sensitizer and dye removal on oxidation performance.(*3*) They provided a good method to purify water especially in arid areas with the participation of sunlight, air and photosensitizer. However, for this method, a matrix in water for MB or RB to attach stably is necessary so that they can be reused as photosensitizer for purifying water.

Another important application is photodynamic therapy (PDT), which has been known for more than a hundred years. Usually, a photosensitizer can be injected into tumors. Then with radiation from a laser or other light, the photosensitizer produces singlet oxygen. However, a precise control of singlet oxygen concentration is a must for tumor treatment in order to get a complete cure with no side effects on nearby organs in the human body. Philip B. Keating and his colleagues developed a singlet oxygen concentration sensor for photodynamic cancer therapy. An IR photomultiplier tube was used as the sensor. They built a time gated system to detect the concentration and lifetime of the singlet oxygen. The lifetime of singlet oxygen in chloroform/HPD1 solution was 207 μs and in acetone/HPD1 solution was 40.8 μs according to his paper. (*12*) The advantage of this approach for measuring singlet oxygen is that they excluded interference from dye fluorescence by making the time gate delay until the dye fluorescence had decayed to a very low level. Later on, Toshiski compared the singlet oxygen releasing efficiency of ATX-S10 and photofrin in solution by a similar method and found ATX-S10 was more effective. Photofrin was used in clinics at that time, so ATX-S10 was a new drug.  The efficiency is important for oncologists.(*13*) At that time, several

researchers had been focused on the mechanism of singlet oxygen in killing microorganisms and tumor cells. Dr. Ledias found that singlet oxygen generated from Rose Bengal in Myeloid Leukemia Cells can oxidize human catalase. Human Myeloid Leukemia (U937) cells have high catalase activity and high resistant to oxidative stresses. (Even hydrogen peroxide could not modify the electrophoretic mobility of catalase at a high concentrations in cells that were already damaged.)(*14*) In his experiments, U937 cells were treated under different stress conditions and catalase activity and their electrophoretic mobility were monitored. He showed that photosensitization reactions using Rose Bengal modified catalase to a more acidic conformer of catalase.(*14*) He also suggested catalase modification can be used as a detection agent of intracellular singlet oxygen. However, detection of catalase is equally hard. In bio-chemical engineering, hydrogen peroxide is used as a detection agent and looking for any bubbles to judge if there is any catalase in the biological cells. It is an easy method to perform qualitative analysis for catalase, but it is difficult to measure the concentration of catalase quantitatively, even with regard to the concentration of singlet oxygen.

Lars-Oliver Klotz, A Klaus-Dietrich Kroncke B and Helmut Sies found two mechanisms of signaling pathways by singlet oxygen, which are the generation of positive regulators as well as the inactivation of negative regulators.(*15*) Shulaz found singlet oxygen induces predominantly oxidative guanine modifications by comparing three oxidants' effect on various repair endonucleases of DNA, which are [4-(tert-butyldioxycarbonyl) benzyl] triethylammonium chloride (BCBT), a photochemical source of tert-butoxyl radicals, disodium salt of 1,4-ethyl-2,3-benzodioxin-1,4-dipropanoic acid (NDPO2), a chemical

source of singlet oxygen, and riboflavin, a type-I photosensitizer.(*16*) There are also many studies based on microorganisms and tumor cells. Tatsuzawa, Maruyama, and Misawa found singlet oxygen can cause a significant loss of *E. coli* due to inactivation of membrane respiratory chain enzymes. In this study, singlet oxygen was generated by chemical and enzymatic systems at pH 4.5. So they suggested that phagocytic leukocytes produce singlet oxygen as a major bactericidal oxidant in the phagosome.(*17*) From all of these studies based on biological effects in human cells and bacterial cells, there is strong evidence of singlet oxygen's antimicrobial property.

### 2.2.3 The production of singlet oxygen

Some photosensitizers can produce singlet oxygen under light in the presence of oxygen. They can be classified as natural photosensitizers and anthropogenic sensitizers. Natural photosensitizers include chlorophyll, non-iron prophyrins, and plant pigments. Anthropogenic sensitizers include dyes, pharmaceuticals, and cosmetics. (*18*)
Photosensitization is a reaction to light that is mediated by photosensitizers. Photosensitizers are not usually consumed during photosensitization procedures. They return to their original state once the photosensitization reaction is complete. However, photosensitizers can be photobleached by light to change to another from which does not absorb light.(*19*)
Rose Bengal and other types of dyes are used in medical treatments as drugs that can release singlet oxygen under visible light, which can kill microbes and viruses effectively. What's more, Rose Bengal can be reused many times, which means a much lower cost and much better convenience for industrial production and further application. Rose Bengal is a good

photosensitizer, which has the highest production rate or quantum yield for producing singlet oxygen compared with most other dyes.

### 2.2.4 The test for singlet oxygen

Quantitative measurement of singlet oxygen concentrations is complicated because of the properties of singlet oxygen. Basically, there are two ways to test for singlet oxygen, direct and indirect detection. There are also two indirect approaches. The first one uses a special colored chemical which reacts with singlet oxygen directly. The amount of reaction with singlet oxygen is measured by the absorption of the solution. From the reaction conditions, the amount of singlet oxygen produced can be measured. This method has been used since the 1960s. In 1979, Botsivali and Evans found anthracene-9,10-bisethanesulphonic acid to be a good trapping agent of singlet oxygen in water solution.(*20*) In1980, Lindig, Rodagers and Schapp found a new water soluble singlet oxygen monitor, disodium salt of 9, 10-anthracenedipropionic acid (ADPA). Their work is not only a trapping agent invention, but also an important way to determinate the lifetime of singlet oxygen in $D_2O$ (*21*).

In 2002, Naoki Umezawa and Tetsuo Nagano synthesized new chemical traps named DPAX to react with singlet oxygen to yield the corresponding endo peroxides, DPAX-EP. They obtained the concentration of singlet oxygen by the fluorescence intensity. However, in this report, the authors said the trap agent has very weak fluorescence and the DOAX-EPs are strongly fluorescent. So it is not so precise when the concentration of the singlet oxygen in solution is quite low. (*22*)

In1996, Veronique Nardello et al, synthesized sodium 1, 3-cyclohexadiene-1,4-diethanoate, a new colorless and water soluble trap of singlet oxygen. The advantage of this chemical is that

it is colorless under both visible and UV light. So it is better than the method we just mentioned explained by Naoki Umezawa and Tetsuo Nagano.(*23*)

The second indirect approach is to react singlet oxygen in solution with a trapping agent first and then measure the concentration change of the dissolved oxygen in water solution with exposure time. This system should be closed to prevent exchange of oxygen with the atmosphere. Haag et al performed a systemic study of singlet oxygen trapping and testing. His study made great contribution, which is still meaningful for us to test the production of singlet oxygen. In1984, he found furfuryl alcohol (FFA) is a good trapping agent since it reacts only with singlet oxygen and remains as a soluble product. In their study, Haag et al. used FFA to trap singlet oxygen and then measured the concentration of dissolved oxygen in solution. (*24*) Then they studied the concentration of singlet oxygen in various type of water with the assistance of FFA.(*25*) They also measured the effect of the wavelength of light on singlet oxygen production in surface waters. (*26*) Charles Tanielian and Christan Wolff performed similar work to determine the possible parameters that affect singlet oxygen production by oxygen and heavy-atom enhancement of triplet yields.(*27*)

Nowadays, a singlet oxygen probe, trans-1-(2'-methoxyvinyl) pyrene, is commercially available. (*28*) It does not react with other oxygen active species. However this chemical is just commercially available with a very high price around $ 145 for 1 mg.

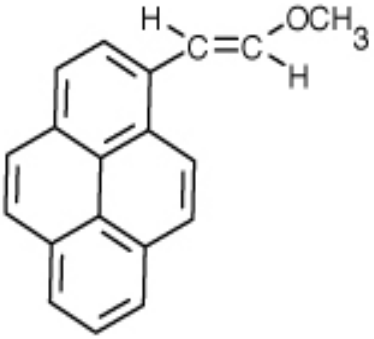



Figure 2.1. Chemical structures of Trans-1-(2'-methoxyvinyl) pyrene (*28*)

## 2.3 Rose Bengal

### 2.3.1 Properties

Rose Bengal, is a synthetic dye, 2,4,5,7-tetraiodo-3', 4', 5', 6'-tetrachlorofluorescein. (*29*) Rose Bengal is a typical photosensitizer to produce singlet oxygen, which can be used to kill microbes. Rose Bengal has many derivatives. The most common one is the sodium salt form, which is highly water soluble. Its structure is shown in Figure 2.2.

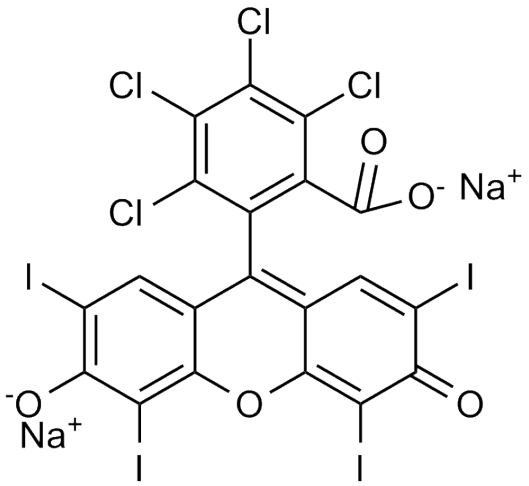



Figure 2.2. Chemical structure of Rose Bengal sodium salt.

Rose Bengal is sensitive to many environment factors such as pH, temperature, and concentration of the solution. In Tsuboi et al.'s paper, a higher activity of the Rose Bengal occurs when temperature is decreased.(*30*) When the concentration of Rose Bengal is increased, aggregation can occur. For example, Rose Bengal starts to aggregate when the concentration exceeds 0.148 mM at pH=7. (*31*)

### 2.3.2 Test method- Beer-Lambert law

Rose Bengal solution is a clear pink liquid. The Beer-Lambert law can be used to determine the concentration of Rose Bengal in solution. Beer-Lambert law states that the molar concentration of a colored liquid can be determined from the absorption of the solution for low concentrations:

$$A = \varepsilon L c$$

where A is the absorbance, c is the concentration of Rose Bengal in solution, L is the length of the path as shown in Figure 2.3, and ε is the molar absorption coefficient. The wavelength of maximum absorbance for Rose Bengal is near 555 nm at pH 7.(*29*) So the concentration of the unknown solution can be obtained by measuring the absorbance provided that a calibration curve for known concentrations has been performed.

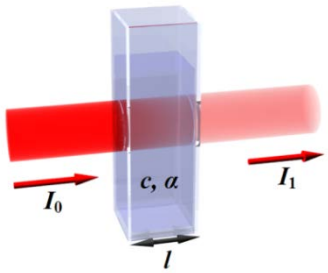



Figure 2.3. Absorption of light according to Beer-Lambert law(*32*)

## 2.4 Polymerization

### 2.4.1 Introduction

Generally, long chain polymer molecules are synthetized by two types of polymerization, step growth and chain growth. Step growth is based on chemical reactions such as esterification or amidation. For example, the polymerization of nylon can be achieved by mixing equal parts of a diamine and a dicarboxylic acid. (*33*) Chain growth is an alternative way to get long chain polymers. Chain growth is based on the opening of unsaturated bonds on monomers by initiators. Basically, chain growth has three steps, which are chain initiation, chain propagation, and chain termination. Compared with step growth, chain growth more easily generates high molecular weight polymers. Many functional polymers can be polymerized by chain copolymerization.

### 2.4.2 Redox Chain Copolymerization

Chain copolymerization refers to a single polymer with two or more kinds of monomers polymerized simultaneously. (*33*) The copolymerization product can vary unlimitedly by variations in the nature and relative amounts of the monomers in the polymerization procedure. However, chain growth requires initiation by initiators to generate reactive species, which can be free radicals, cations, or anions. As the polymer adds a monomer by opening the π-bond, it forms a new radical, cation, or anion center. However, these initiators cannot be used randomly because all three types of initiation cannot be used on all monomers. Generally, radical initiators can be applied to most monomers. Ionic initiators and cationic initiators are strictly selected for specific monomers. Propagation is the next step after initiation. The rate of propagation is determined by monomers and initiators involved in the

polymerization system. Compared with other types of initiators, redox initiators can be used over a wide range of temperatures. At the same time, initiators can be organic or inorganic, which makes it more flexible when dealing with different monomer systems. More importantly, it can be thermally or photolytic initiated, which makes the polymerization procedure much easier. After getting the molecular chain we want, termination ends the polymerization. Disproportionation and coupling are the two models of termination. Basically, the final molecular weight from coupling termination is twice that from disproportionation. In a word, we can get the desired polymers by choosing monomers and polymerization conditions.

## 2.5 Surface Modification

### 2.5.1 Introduction

The fiber surface properties are very important to fiber performance in traditional textiles and novel textiles. Surface modification is a technology that aims at improving the properties of fiber surfaces. By modifying the surface of fiber, many novel fibers are created, such as~~,~~ antimicrobial fibers(*34*), hydrophobic fibers(*35*), and fiber sensors(*36*). Traditionally, there are three different types of modification based on fibers including coating technology, plasma treatment, and surface grafting. Coating can improve the fiber adhesion, wettability, corrosion resistance and wear resistance but coated fiber surface modification is usually not sufficiently durable. Plasma treatment is capable of changing the adhesion of fiber surface but it is famous for its high cost. Chemical grafting on surfaces is more and more popular with the increasing demand of high performance fiber including fireproofing fiber (*37*), antifouling membrane (*38*) and antimicrobial fiber.(*39*) For example, through chemical

grafting, inactive polypropylene fiber can be made to be very active against bacteria when antibacterial groups are attached on polypropylene fibers. (*38*)What's more, hydrophilic cellulose can be modified to be super hydrophobic by modifying the surface with low-energy compounds together with building a good surface micro-and nanometer structure.(*40*) Another advantage of chemical grafting of fiber surface is its stability and reasonable cost. Plasma treatment plus surface chemical grafting and plasma plus surface polymerization are described as a new generation of surface modification. In this thesis, different methods of chemical surface grafting on different surfaces with different applications will be compared and analyzed.

Currently, there are four types of surface chemical treatments including chemicals applied on fiber surfaces directly, chemical treatment plus polymerization on fiber surfaces, polymerization on fiber surfaces directly and plasma treatment plus polymerization on fiber surfaces. Surface properties of fibers are not only important to clothing application, but also crucial to industrial application such as composite, filtration and medical applications. All these applications will be covered in specific approaches for improving fiber surface properties.

### 2.5.2 Direct chemical treatment on fiber surface

The first method is to apply chemicals directly on the fiber surface. Covalent bonds are formed between the active groups on the fiber surface and the active group on the chemicals. Recent studies on direct chemical treatment involve natural fibers like cotton, hemp and synthetic fibers like PAN, nylon, polypropylene, and even carbon fiber as the most basic fiber used in high performance composite materials.(*41*)(*42*)(*38*)(*43*)

Using direct chemical treatment on fiber surfaces to improve the absorption properties have seen great improvements in recently years. In Zhao's study, polyacrylonitrile (PAN) fiber was pretreated with NaOH solution and $SOCl_2$ solution for 13 minutes, and then reacted with soy bean milk solution to make the soy bean protein solution attached on the surface of PAN fiber. In this case, the CN group on PAN surface can react with soy milk protein. (*42*) After grafting, several types of polar groups, such as amine groups (−NH2, −NH−), −OH, −COOH, and so on, have been introduced onto the chemical fiber, which contributed to the increase of the moisture absorption. We can see the data provided on Table 1 shows a 3x increase in the moisture absorption. Using this technology, synthetic fibers can become biocompatible. Sometimes these fibers can be used in place of natural fibers.

Table 2.1. Mechanical and moisture absorption properties of PAN fiber before and after grafting (*42*)

| *Grafting efficiency (%)* | *Breaking Strength (cN/dtex)* | *Breaking Elongation (%)* | *Moisture absorption (%)* |
|---|---|---|---|
| 0 | 2.74 | 29.32 | 2.11 |
| 1.78 | 2.66 | 29.22 | 3.76 |
| 2.93 | 2.65 | 29.05 | 4.78 |
| 3.67 | 2.72 | 28.74 | 5.11 |
| 4.27 | 2.69 | 28.91 | 5.36 |
| 6.52 | 2.71 | 28.96 | 6.08 |

The author claims that this procedure is easy to industrialize. However, environmental factors need to be taken into consideration in bulk production because of the use of chemical solvents. Another concern about protein attached PAN is that proteins are good food source for some bacteria and may provide a good environment for bacterial growth.

At the same time, the surface of natural fibers also has received great attention for its defects when applied to specific applications. For example, wool fiber has good water adsorption and good insolation properties when used as clothing. However, the wash durability is not acceptable. There were numerous studies on improving wool's wash durability. The most recent described in Dong Chen's paper is to treat wool fiber with aminopropyltrimethoxysilane (APTMS) which serves as an anchoring layer and then grafting silica nanoparticles by a hydrolysis reaction between the alkylsilane functionalities on the silanized wool fiber surface and tertraethoxysilane, please see Figure 3.4. (*44*)

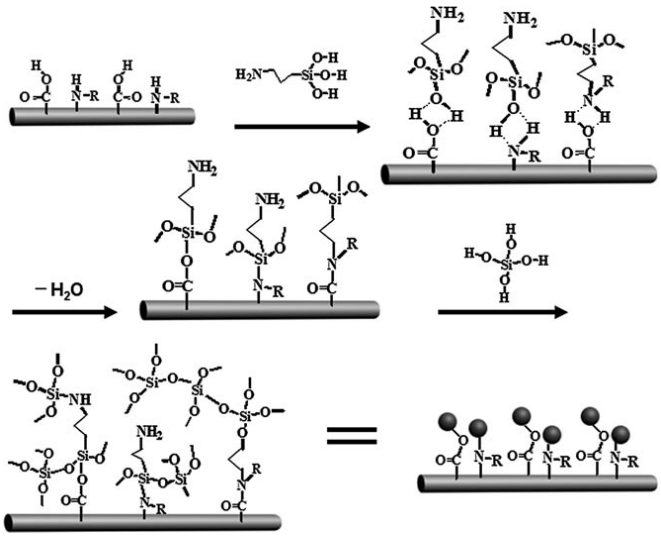



Figure 2.4. Possible formation mechanism of a durable ultrathin silica layer on a wool fiber. (*44*)

After treatment, the wool fabric showed excellent water adsorption and quick drying properties when tested in accordance with the Chinese National Standard GB/T 21655.1-2008 and 21655.2-2009. This will improve clothing thermophysiological comfort by taking perspiration away from human skin. More importantly, the hydrophilic properties can be maintained after washing 20 times in a washing machine. This study suggests a very good area for further development of wool. However, the grafting of nano particle needs 30 minutes in solution. It is not a practical way to use for clothing production, which is supposed to be quick and low cost.

Surface modification of engineering fibers used in composite materials including carbon fiber and hemp fiber also has been studied. Hemp has been widely used in composites because of its high strength. However, its use is limited because moisture can invade composite materials if hemp fiber is used as reinforcing material. Ali Rachini changed hemp fiber's surface hydrophilic property by silane coupling agent (Y-methacryloxypropyltrimethoxysilane and Y-aminopropymtriethoxysilane) directly. Hemp fibers used in this study were cut into 5 cm length pieces and then treated with hydrolysed silane for 2h at 120°C.(*41*) After sufficient curing, the condensation reaction between hydrolyzed silane and hydroxyl groups of hemp were evaluated by ATR-FTIR and $^{29}$Si-NMR analysis.

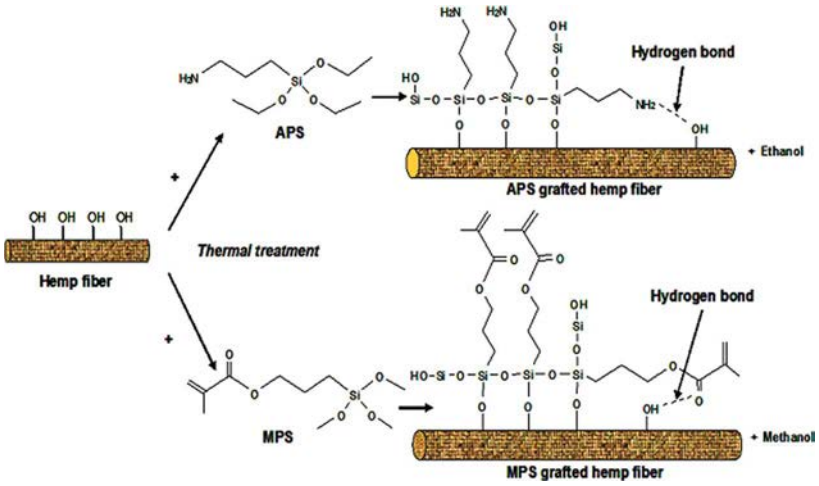



Figure 2.5. Simplified illustration of silane molecules grafting (APS and MPS) on hemp fiber surfaces (*41*)

Figure 2.5 illustrates silane molecules grafting onto hemp fiber. From the illustration, the hydrophobic groups are supposed be increased after grafting. However, from Figure 2.6 we can see that the strong absorbance at wavenumber 1060 $cm^{-1}$ was increased after silane grafting, which means the number of C-OH groups increased after treatment. To some degree, it should have decreased the hydrophobic property of hemp fiber. So for this study, the measurement of contact angle of hemp fiber after treatment is more persuasive. Besides, though this work can improve the adhesion between hemp fiber and the matrix, only changing the surface should not change the moisture adsorption very much.

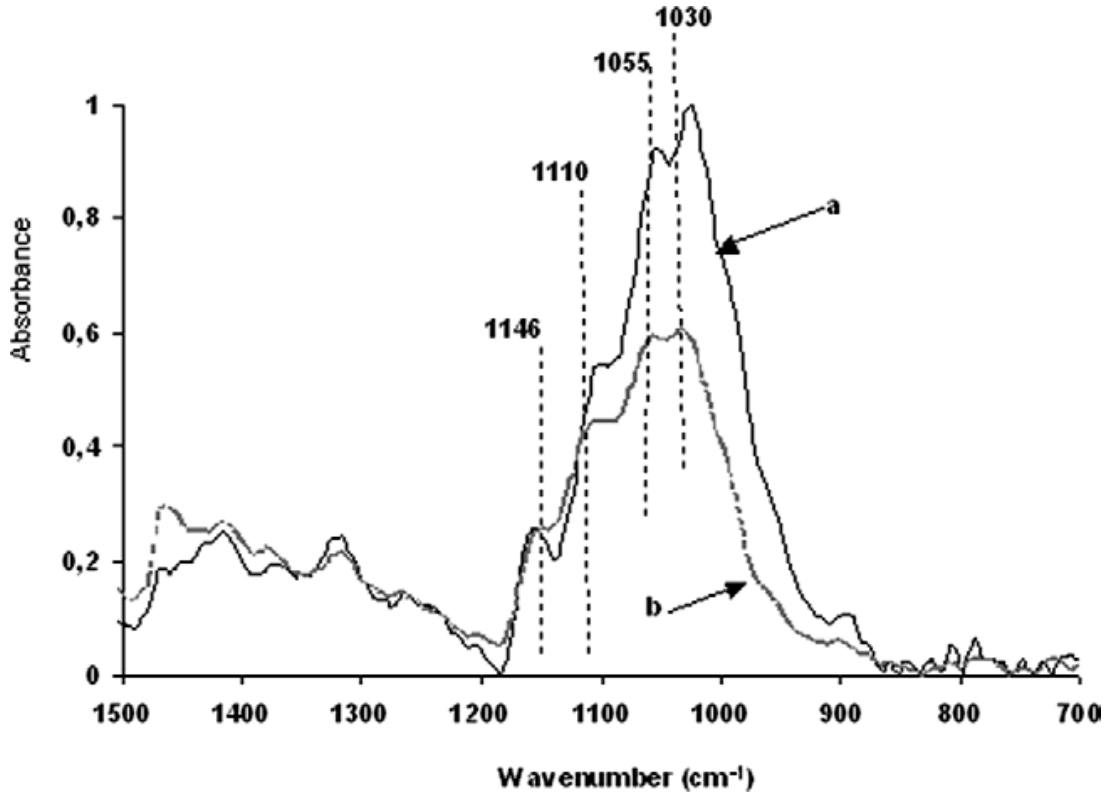



Figure 2.6. ATR-FTIR (1500–700 $cm^{-1}$) spectra of (a) ethanol/water extracted hemp fibers and (b) APS (0.2 mol/L1) treated hemp fibers. (*41*)

Another type of fiber that is often used in composite materials is carbon fiber. Sometimes, a secondary grafting is used on reinforcing fibers to improve physical and electrical properties. In this work, a carboxyl functionalized surface is created by the reaction between naturally existing surface hydroxyl groups on the fiber surface and isopropylidenemalonate.(*43*) X-ray photoelectron spectroscopy showed the relative surface coverage by carboxylic acid groups increased from an initial 5.2% up to 9.2%, and the strength was not affected by surface grafting. It offered a great chance for carbon fiber to be functionalized with further grafting. The figure below shows the mechanism of reaction proposed in this work.

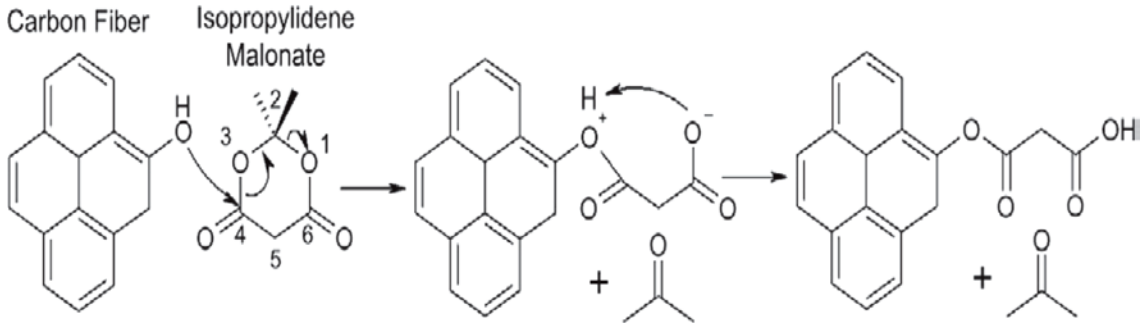



Figure 2.7. Ring opening reaction of isopropylidenemalonate (Meldrum's Acid) with pendent hydroxyl group (*43*). Numbers near the ring indicate the ring position. Carbon atoms 4 and 6 are subject to nucleophilic attack, which causes the ring to open leaving a terminal malonic ester.

From the study above, direct chemical treatment is very easy so it is good for bulk production. At the same time, the potential limitation for fiber used in this approach is the surface activity. All fibers that can react with chemicals must have certain number of reactive group such as OH, -CN, and –COOH.

### 2.5.3 Direct Polymerization on fiber surface

In addition to treating the fiber with chemicals directly, polymerization on fiber surfaces has also been studied. Compared with direct chemical treatment, polymerized polymers on fiber surfaces offer more chances for fibers to have a unique property by covering the surface of fiber through covalent bonds. Nystrom and his coworkers created a superhydrophobic and self-cleaning cellulose via atom transfer radical polymerization (ATRP) plus post-modification.[35] A washed filter paper was immobilized in a solution containing 2-bromoisobuturyl bromide, triethylamine (TEA) and a catalytic amount of 4-(dimethylamino)pyridine(DMAP) in THF for 12 hours. The hydroxyl groups on the cellulose

surface were converted to ATRP initiators. Then, glycidyl methacrylate (GMA) was grafted on the initiator-modified paper at 30 °C for 40-120 minutes. After washing and drying, the sample was hydrolyzed for 60 minutes at 50 °C. Then pentadecafluoro-octanolyl chloride and bis (3-aminopropyl)-terminated PDMS were attached to hydrolyzed PGMA-grafted filter paper. Next the author tried a graft-on-graft, which is a post modification after the first graft, see scheme 2. The author found that the contact angle (CA) of the graft-on-graft sample had a higher CA than the other materials, as shown in Figure 2.10.

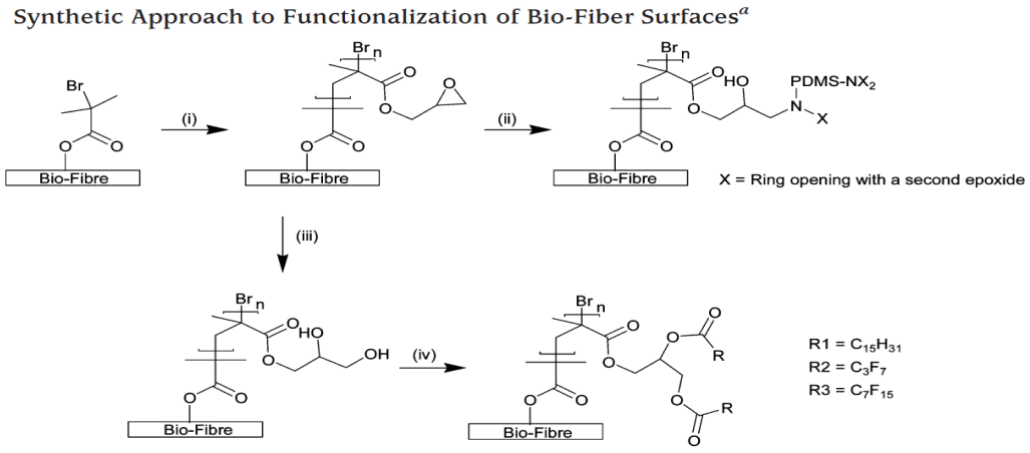



Figure 2.8. Synthetic approach to functionalization of bio-fiber surfaces.(*45*)

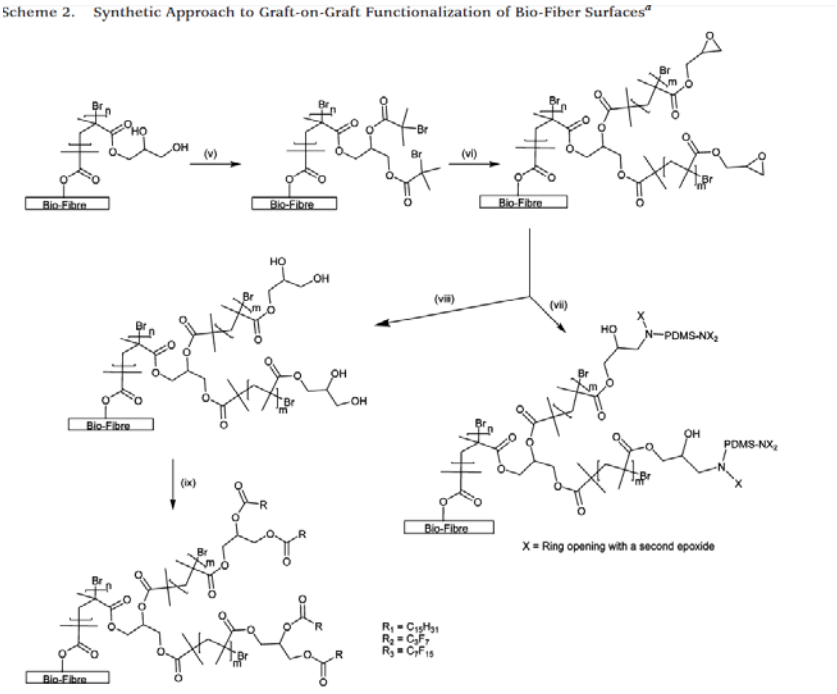



Figure 2.9. Synthetic graft on graft approach to functionalization of bio-fiber surfaces.(*45*)

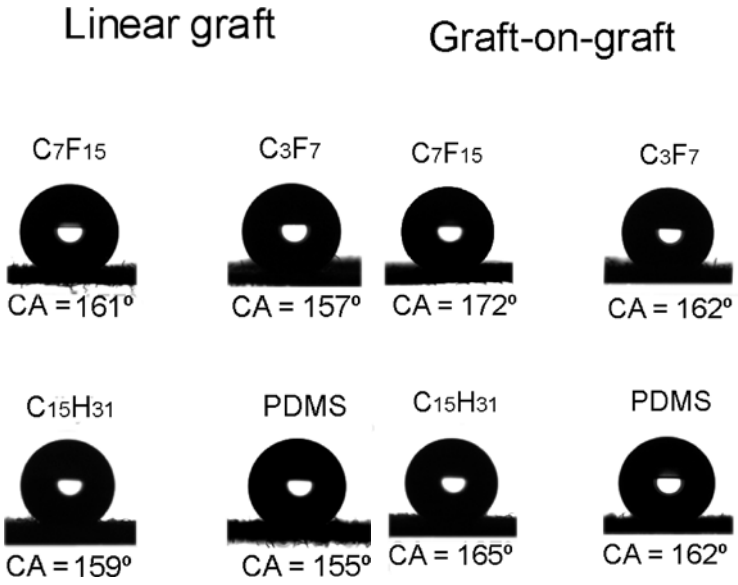



Figure 2.10. CA images for PGMA linear graft and graft-on-graft modified with different low-energy compounds.(*45*)

From Figure 2.10, we can see $C_7F_{15}$-modified graft-on-graft architecture can achieve a 172° CA. It was also verified that it has a high self-cleaning ability. This project has the following advantages: 1) it is based on cellulose, which is an abundant, inexpensive, biodegradable, and sustainable fiber. 2) ATRP can control the length of the grafted polymer chains and has a dormant end, which can be reactivated for further modification. However, we can still see the reaction time is long, which needs further improvement for industrial production. Hao et al also performed polymerization on the surface of hemp fiber in order to improve their fixation percentage.(*46*) The reaction they used on hemp fiber surface is shown below.

$$nCELL-OH \rightarrow CELL-O\square + nCH_2=CH-CN \rightarrow CELL-O-\left[CH_2-CH(CN)-\right]_n$$

After free radical polymerization, the effect of monomer density, the effect of CAN (cerous ammonium nitrate) density, the effect of grafting temperature and the reaction time were studied. After polymerization the dyeing property of the hemp fiber improved as shown in Figure 2.11.

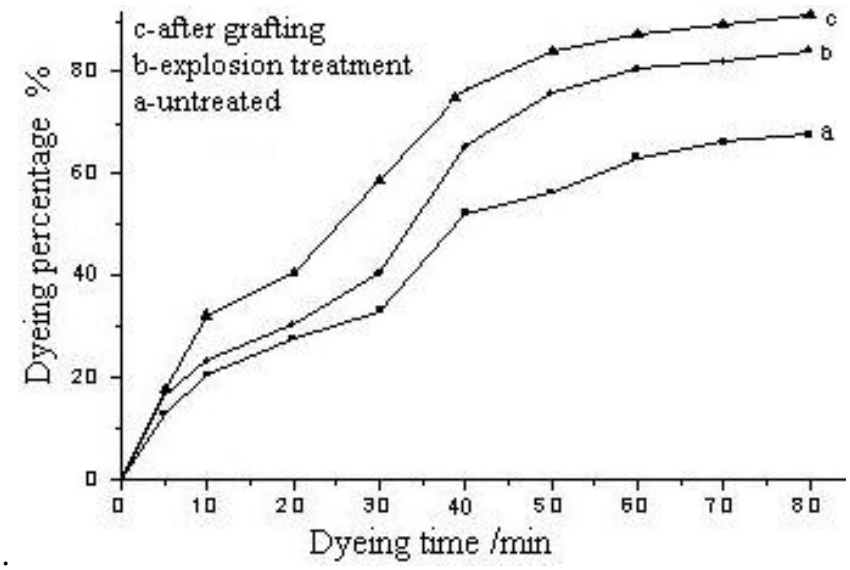



Figure 2.11. Dyeing percentage before and after grafting of hemp fiber. (*46*)

Compared with ATRP modification, this method can save considerable time. However, we see there is only a small change of dyeing with this approach, with only a 10% increase. Dyeing can be improved by other, easier methods rather than polymerization, so cost will be an issue when being applied to industry. However, in the membrane industry, many studies are focusing on direct polymerization on the fiber surface.(*47*) Hydrophilic poly(poly(ethylene glycol) methyl ether methacrylate) (P(PEGMA)) brushes were grown on chloromethylated polyethersulfone (CMPES) hollow fiber membrane surface by surface-initiated atom transfer radical polymerization (SI-ATRP) in order to increase the hydrophilic property of the membrane, which is supposed to be used in filtration and purification. The CMPES hollow fiber membrane was prepared by phase inversion process. The benzyl chloride groups on the CMPES membrane surface could be active macro initiators for

surface grafting. The grafting of P(PEGMA) chains was verified by attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy.

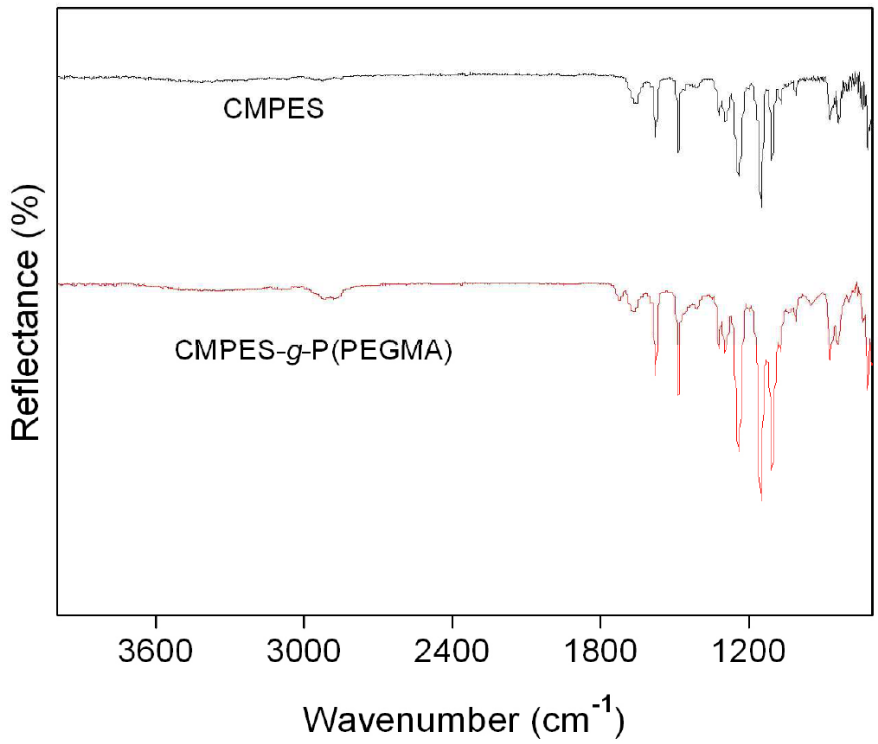



Figure 2.12. ATR-FTIR spectroscopy of CMPES and CMPES-g-P. (*47*)

They also used field emission scanning electron microscopy (FESEM) to characterize the surface morphology of the CMPES membrane and modified membrane. The change of morphology of the membrane is shown in Figure 2.13.

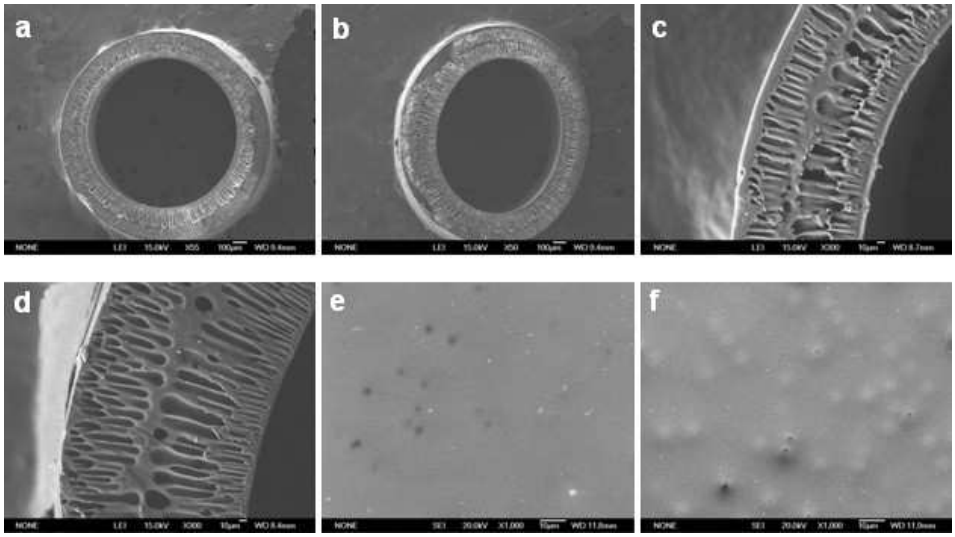



Figure 2.13. FESEM images of CMPES membrane cross-section (a, c), membrane surface (e) and CMPES-g-P (PEGMA) membrane cross-section (b, d), and membrane surface (f). (*47*)

The grafting of P(PEGMA) was found to have higher pure water flux, improved water uptake ratio and better anti-protein absorption for the CMPES modified membrane. The rejection properties of polysulfone hollow fiber ultrafiltration membranes were also improved by UV-irradiation graft polymerization of trimethylallylammonium chloride (TMAAC) and diallyldimethylammonium chloride (DMDAAC) (*48*) This paper compared the polymer grafted membrane's filtration performance with blank membrane from the aspect of streaming potential, flux and rejection of Lys and positively charged ions as shown below, Figure 2.14-2.16.

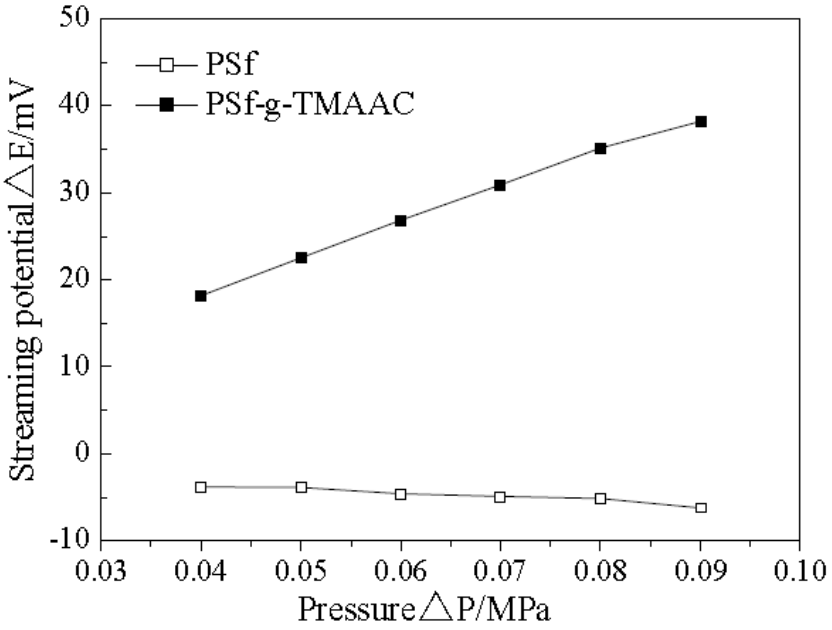



Figure 2.14. Effects of operating pressure on streaming potential of membranes.(*48*)

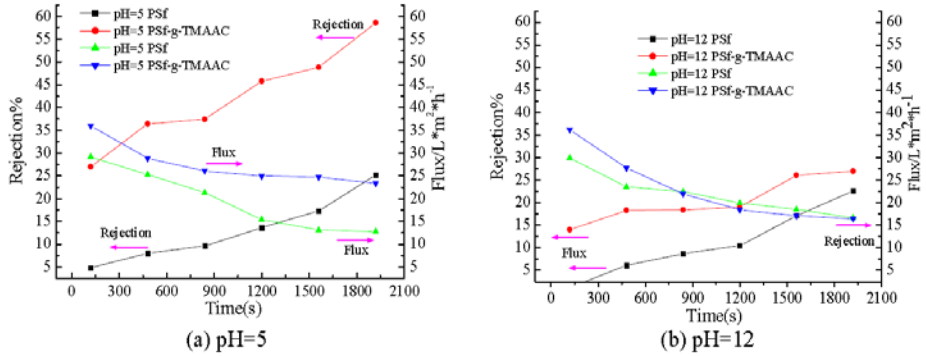
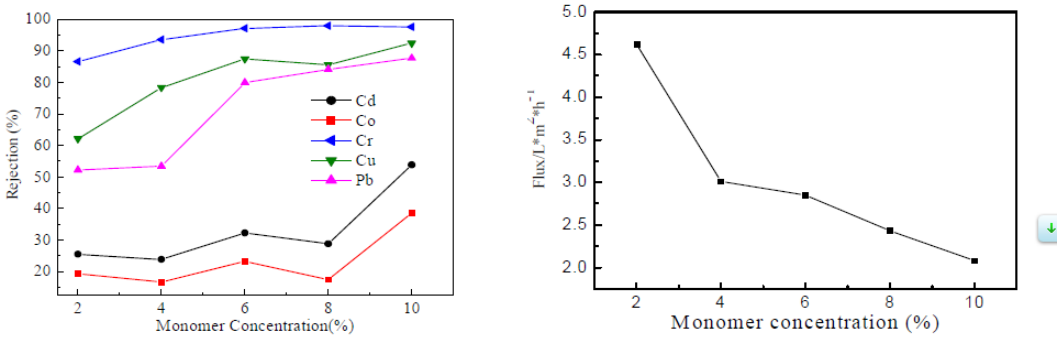



Figure 2.15. Flux and rejection of Lys for PSf and PSf-g-TMAAC membrane. (*48*)




(a) Rejection (b) Flux

Figure 2.16. Rejection and flux of mixed metal ions by PSf-g-DMDAAC membrane at 0.2MPa.(*48*)

Appling monomers to the surface of fibers and then initiating chain growth polymerization on the surface also offered some unique properties just because of the properties of the polymer. For example, when acrylamide monomers are applied on the surface of nylon with light initiation, as shown in Figure 2.17, it is possible to get an anti-flammable fiber because the grafted polymer has much higher percentage of N than nylon fiber, which makes nylon fiber more flame retardant. [33] The LOI (limited oxygen index) is improved from 19.9 to 27 and there are no molten drops according to this patent. So it can be used on fireman related products and accessories.

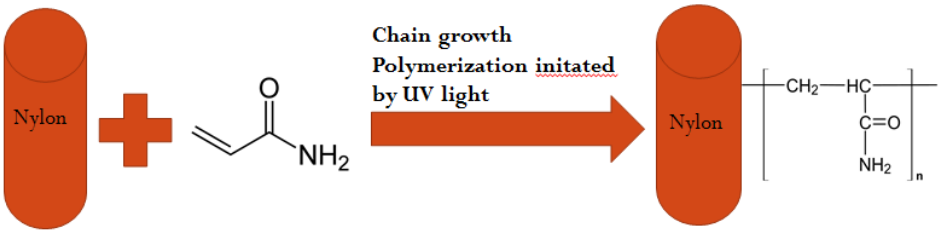



Figure 2.17. The mechanism of grafting polymer on nylon surface(*37*)

Direct polymerization on fiber surfaces has also been applied to cellulose to get various functions. Hydrophobic cellulose was achieved by gamma-irradiation-induced grafting of glycidyl methacrylate (GMA) on its surface.(*49*) The morphology change of the fiber surface is shown below. At the same time, we can see in Figure 2.19 the relative absorption of 2,4-dichlorophenoxyacetic acid by grafted cellulose decreased by 50%.

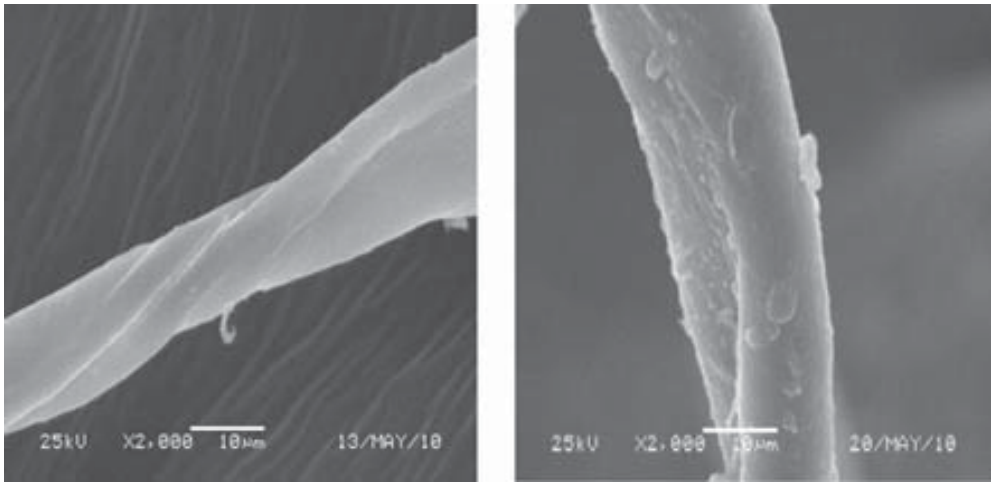



Figure 2.18. SEM picture of untreated cotton fiber (left), cotton fiber irradiated in the presence of glycidyl methacrylate and β-cyclodextrin (right).(*49*)

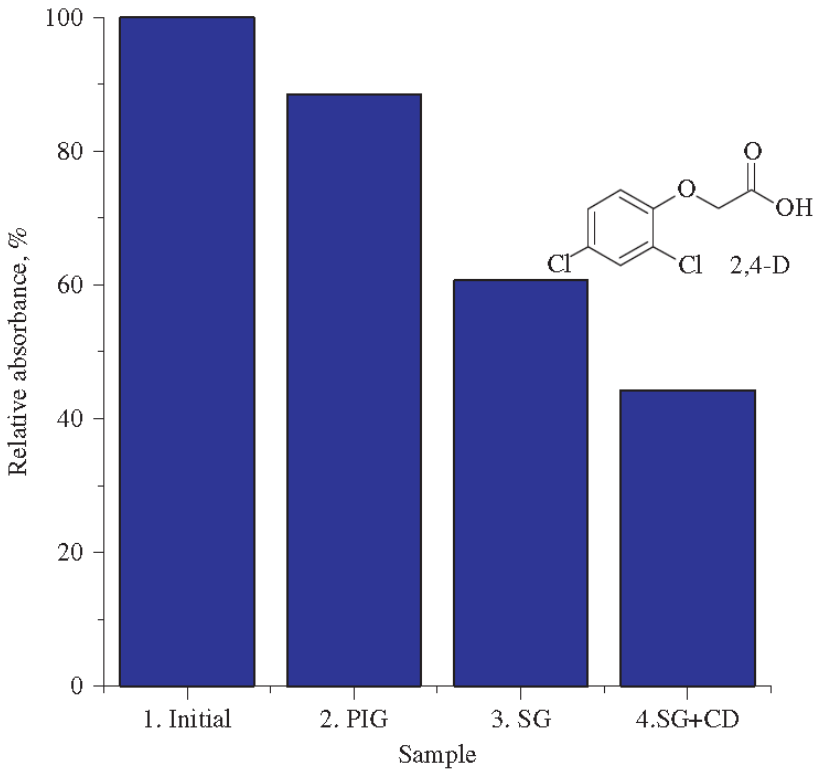



Figure 2.19. Decrease in absorbance of aqueous solutions of 2,4-dichlorophenoxyacetic acid measured by UV absorbance at 283 nm. Initial solute concentration is 0.5 mmol/(dm)$^3$. In the pre-irradiation grafting, simultaneous grafting, or simultaneous grafting plus β-cyclodextrin immobilization grafted cellulose, respectively, adsorbed less of the solute. Degree of grafting~50% and each sample had approximately the same weight. (*49*)

In recent studies of direct polymerization on fiber surfaces, we can see the fiber needs to have reactive groups that can be used as the root of the polymer. In addition, the density of reactive groups needs to be high enough to make sure they can cover the fiber surfaces completely. However, for some fibers that don't have reactive groups or don't have enough reactive chemical groups or if we want to increase the reaction rate or to make the function of

the polymer stand-out more, we need to create reactive groups by chemical pretreatment or by physical treatment, such as plasma treatment.

### 2.5.4 Chemical pretreatment plus polymerization

Chemical pretreat on nylon fibers can create more reactive sites for polymers to grow on. To obtain hydrophobic cotton, the cotton surface can be pretreated with initiators and then polymerization started. A recent paper showed the procedure by the illustration in Figure 2.20; after modifying the cotton surface by the initiator 2-bromoisobutyryl bromide (BIBB), and polymerization of methyl methacrylate, many hydrophobic ester groups were introduced onto the cotton surface. This results in an increase of the contact angle of water droplets. Figure 2.21 shows SEM pictures for non-treated cotton, initiator modified cotton and final modified cotton. (*40*) Figure 2.22 shows the relationship between CA and the polymerization time.

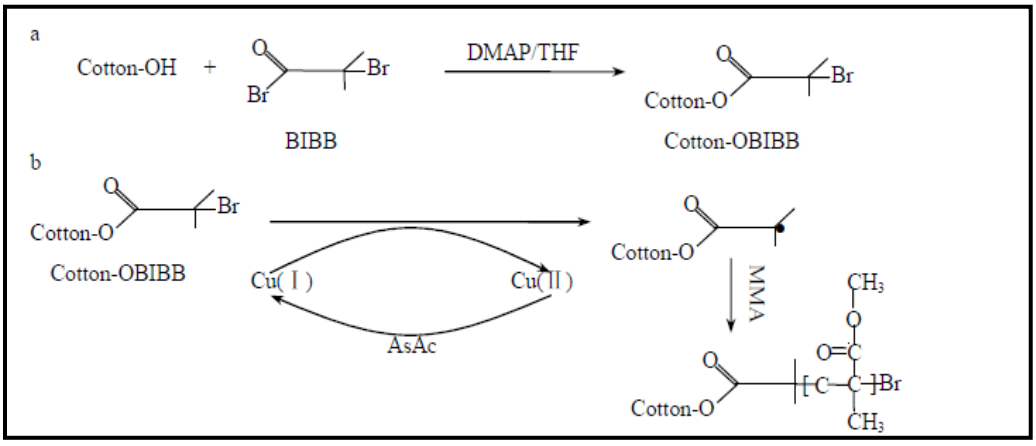



Figure 2.20. ARGET ATRP of MMA from Cotton Fibers. (*40*)

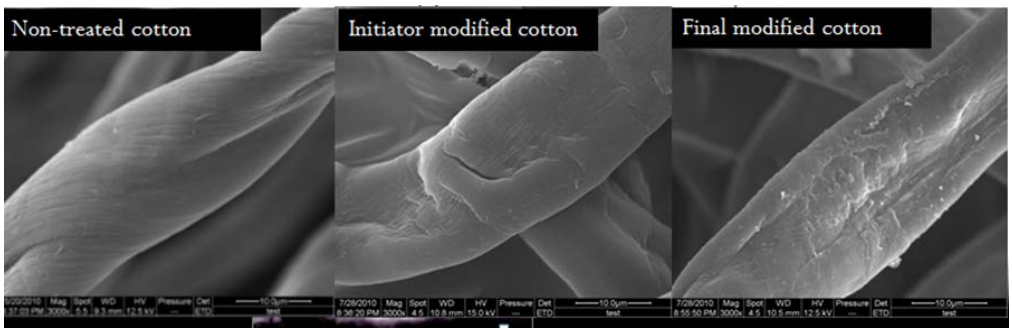



Figure 2.21. SEM of cotton fiber without treatment, initiator modified and polymer grafted. (*40*)

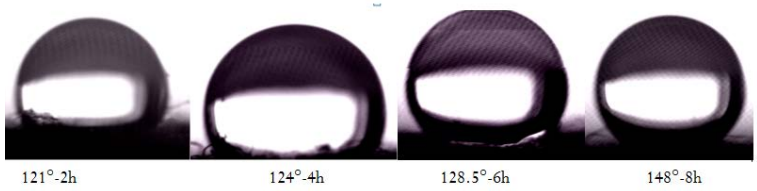



Figure 2.21. The change of contact angle along with the time of polymerization. (*40*)

Another study created more –OH groups on cellulose fiber surfaces by grafting hyperbranched poly(3-methyl-3-oxetanemethanol) (HBPO) through a surface hydroxyl group-initiated ring-opening polymerization of 3-methyl-3-oxetanemethanol (MOM). (*50*) Figure 2.23 illustrates the grafting mechanism. The content of grafted HBPO is easily adjusted by controlling feeding dosage of MOM. To verify the reactivity of hydroxyl groups in the grafted HBPO, poly (ε-caprolactone) (PCL) was further grafted from the HBPO-grafted cellulose surface.

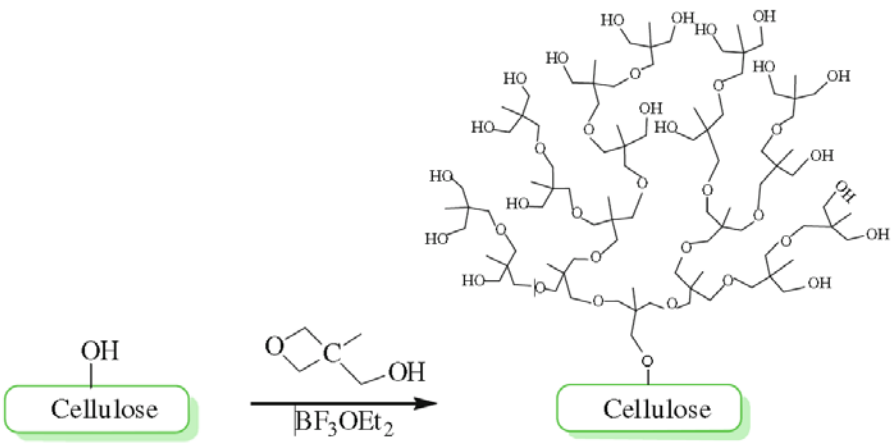



Figure 2.22. Schematic illustration of grafting multi hydroxyl hyperbranched polyether from cellulose fibers surface.(*50*)

### 2.5.5 Plasma pretreatment plus polymerization

For fibers without any active groups, such as PP and PE, it is extremely difficult for them to react with chemicals or build up polymers on their surface. In the paper written by Jie Zhao, a plasma was used to attach reactive chemical groups and then these groups were used to act as the base of further polymerization (*38*). In this article, they were attempting to protect the film from blocking or developing a protein covering, which prefers to stay together with PP, on the surface of PP in order to maintain the efficiency of the filtration membrane. Figure2.24 shows the treatment process. Through this approach, we can see water contact angles for the membranes decreased from 123° to 17° with increasing grafting density (Figure 2.25). Thus PP was made to be hydrophilic by this approach. In addition, the author of this paper also gave the relationship between grafting density and reaction conditions. We can see the grafting density trend with increasing plasma treatment time, monomer concentration,

and UV irritation time. This technology can be used in ultrafiltration membrane to increase the antifouling property of nonwoven films.

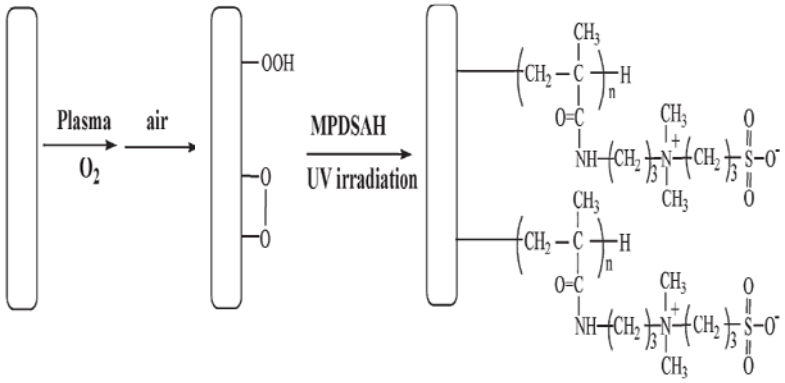



Figure 2.23. Proposed mechanism of preparation of poly(MPDSAH)-modified NWF membrane.(*38*)

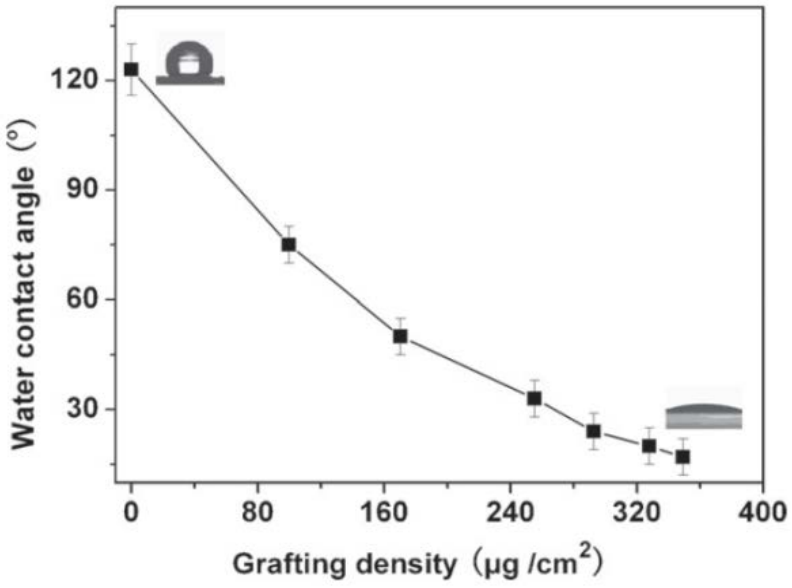



Figure 2.25. Water contact angles of membranes with different grafting densities. (*38*)

Recent studies show similar plasma plus polymerization approaches also being used on polyethylene terephthalate (PET) and polyacrylonitrile fiber.(*51*) However, plasma treatment is generally considered to have too high cost for consumers. If the cost of plasma can be reduced, this approach might be able to be applied in bulk production.

# 3. EXPERIMENTS

## 3.1 Materials

Acrylic acid (AA, anhydrous, 99%), 4-styrene sulfonic acid sodium salt hydrate (SSA), and 4-vinyl benzyl chloride (90%, VBC), were used as the three basic monomers for the polymer. Ammonium persulfate (reagent grade, 98%) and sodium metabisulfite (ACS reagent, ≥97.0%) were used as a redox catalyst system. Ferrous sulfate heptahydrate was used as a reductant before the addition of the catalysts to the system in order to capture any residual oxygen. Hydroquinone was used to terminate the polymerization. All these chemicals were purchased from Sigma Aldrich and used as received without further purification. DI water was used in all experiments discussed below. Rose Bengal, as the functional component of the final polymer, was purchased from Alfa Aesar (UK).

## 3.2 Polymerization Procedure

All reactions described in this study were carried out on a hot plate/stirrer (Corning® 4 x 5 inch top PC-220 Hot Plate/Stirrer, 120V/60Hz), which provided enough heat and stirring. A water bath and thermocouples used to control the temperature during the reaction. The reaction container used in this study was a round bottom flask (PYREX® 200ml, 24/40 standard taper joints). A nitrogen atmosphere was used in all experiments to exclude oxygen. SSA was measured using an analytical balance (Mettler-Toledo) and then delivered by Becton-Dickson 30 ml plastic syringes through 18 gauge, 6” blunt end pipetting needles with standard hub. VBC was measured by pipette (eppendorf, 200µl-1000µl) and delivered by glass syringe (10 ml). AA was measured with a graduated cylinder and delivered by glass syringe (30ml) together with a small amount of acetone to assist with solubility.

### 3.3 Synthesis of vinyl benzyl-Rose Bengal (VBRB) monomer

The synthesis of VBRB was conducted in a 125ml flask with the mixture of approximately 3.1grams of Rose Bengal and 0.51 ml of 4-vinyl benzyl chloride in 34 ml of DI water and 35 ml of acetone at the ratio of 1.0:1.05. This solvent mixture enables both Rose Bengal and 4-vinyl benzyl chloride to be soluble so that the reaction can proceed. A reflux condenser was placed on the top of the flask and cooled with chilled water to prevent loss of the solvent. A water bath at 65 ℃ was used to maintain the reaction temperature and a magnetic stir bar was used to keep the bath temperature uniform. After 3 hours reaction, the product precipitated out of solution and was washed with DI water and finally dried in a vacuum oven.

### 3.4 Copolymerization

Three different approaches were attempted in order to attach Rose Bengal onto the copolymer to get the soluble sample based on the 3 monomers and 1 dye. The first approach was to attach Rose Bengal onto poly (acrylic acid-co-vinyl benzyl chloride-co-styrene sulfonic acid after copolymerization). The second approach was to attach Rose Bengal onto poly (acrylic acid-co-vinyl benzyl chloride-co-styrene sulfonic acid) during copolymerization. The third approach was to copolymerize poly (acrylic acid-co-vinyl benzyl Rose Bengal-co-styrene sulfonic acid) after synthesis of vinyl benzyl Rose Bengal. In this project, each approach was used and then tested step-by-step. The recipe was changed gradually to optimize the final recipe. In this section, all three synthesis approaches are described in chronological order.

### 3.4.1 Attaching Rose Bengal onto poly (acrylic acid-co-vinyl benzyl chloride-co-styrene sulfonic acid)

The polymerization reaction of poly (acrylic acid-co-vinyl benzyl chloride-co-styrene sulfonic acid) was carried out at 65℃ in nitrogen atmosphere. The first polymer was copolymerized with 73 mole % acrylic acid, 25 mole % 4-styrene sulfonic acid sodium salt hydrate and 3 mole % 4-vinyl benzyl chloride. Monomers were delivered over 1.5 hours. Acrylic acid and 4-styrene sulfonic acid sodium salt hydrate were mixed in DI water and delivered together using a styrene pump. 4-vinly benzyl chloride was dissolved in 10 mL acetone and delivered by syringe pump. Initiators were ammonium persulfate and sodium monobisulfite, which were dissolved in DI water and then delivered by syringe from the beginning of the reaction to 1 hour after delivering of monomer. The polymerization reaction was quenched by hydroquinone 1.5 hours after initiator feed was finished. After the polymerization was quenched, the temperature was raised to 75 ℃ and Rose Bengal feed started. An equal number of moles of Rose Bengal as 4-vinyl benzyl chloride were delivered in DI water for 1 hour. Upon completion, the solution was neutralized by adding a 50 weight % aqueous NaOH dropwise. The addition and temperature sequence is shown in Figure 3.10.

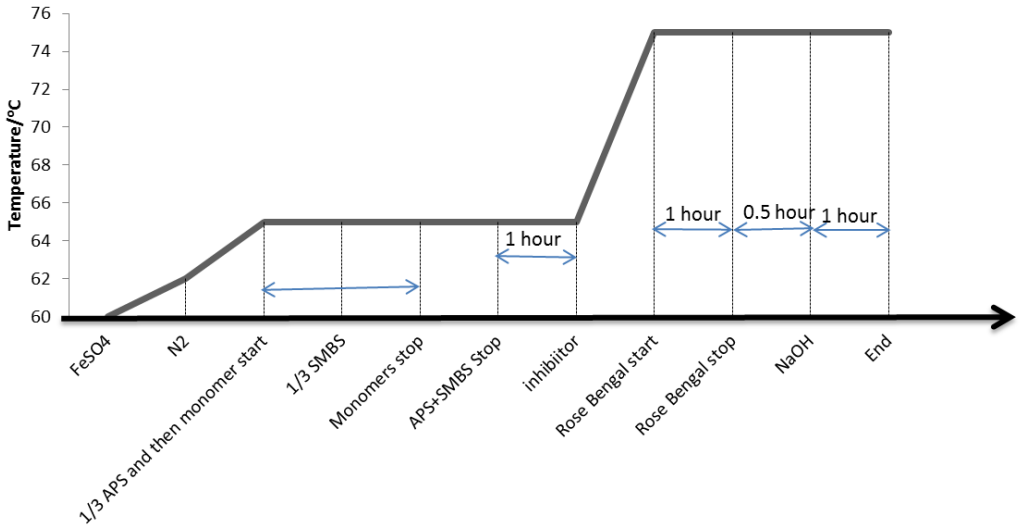



Figure 3.1 The reaction procedure used in approach 1.

The final sample was a totally clear and dark red color. The monomer ratios are shown throughout this manuscript. To assist in identifying the samples, a sample code was developed as follows: PBRB is used to identify the polymer as "polymer bound Rose Bengal. The first number after PBRB is the approach for polymerization. Number 1 means the approach 1 described above. The next two digits are the month and the final two digits are the day that the polymerization was performed. The following samples were made in same procedure but with different mole ratios. Table 3.1 shows the detailed recipe for the samples.

Table 3.1. Mole ratio of PBRB1 Samples

| *PBRB* | *1-0414* | *1-0509* | *1-0608* | *1-0724* | *1-0728* | *1-0801* |
|---|---|---|---|---|---|---|
| SSA | 25 | 25 | 25 | 25 | 25 | 25 |
| VBC | 2 | 2 | 2 | 2 | 0.8 | 0.8 |
| AA | 73 | 73 | 73 | 73 | 74.2 | 74.2 |
| RB | 2 | 2 | 1.92 | 1.92 | 0.36 | 0.36 |

### 3.4.2 Attaching Rose Bengal during copolymerization

After performing the polymerizations described above, it was recognized that the –COOH on Rose Bengal may compete with –COOH on acrylic acid when reacting with $-Cl$ on benzyl chloride. Thus, in polymerization method 2, Rose Bengal was delivered during the polymerization procedure. The temperature of the water bath was kept at 65°. The samples obtained are listed in Table 3.2.

Table 3.2. Mole ratio of PBRB2 Samples

| *PBRB* | *2-0818* | *2-0829* | *2-1024* | *2-1205* | *2-1216* | *2-1220* | *2-0102* |
|---|---|---|---|---|---|---|---|
| SSA | 25 | 97 | 25.7 | 25.5 | 25 | 24.6 | 25.7 |
| VBC | 0.8 | 3 | 0.83 | 1.6 | 3.2 | 0.8 | 0.8 |
| AA | 73 | 0 | 73 | 73 | 71.4 | 74.6 | 73.5 |
| RB | 0.36 | 1.3 | 3.67 | 0.36 | 0.36 | 0.1 | 0.05 |

### 3.4.3 Polymerization of poly(acrylic acid-co-styrene sulfonic acid-co-vinyl benzyl chloride Rose Bengal)

In a third polymerization method, vinyl benzyl Rose Bengal was polymerized with AA and SSA. However, vinyl benzyl Rose Bengal is not water soluble. This results in two challenges: the first is to dissolve the vinyl benzyl Rose Bengal monomer, 4-styrene sulfonic acid and acrylic acid in the same solvent using the least amount of organic solvent. The second is to avoid an extremely acidic pH. Therefore, the procedure was split into two steps. The first step was to polymerize vinyl benzyl Rose Bengal with acrylic acid in an acetone/water (1:1 by volume) solution medium to increase the solubility of the vinyl benzyl Rose Bengal by incorporating it into an poly(acrylic acid) oligomer. When the stirring bar began to slow due to the increasing viscosity of the oligomer solution, the solution was transferred to another reaction beaker containing SSA aqueous solution and the polymerization was continued. In order to make sure the concentration of the initiators was same, additional initiators were added to the system. The temperature of the system was kept at 65 $^{o}$C. The acetone percentage is about 17% in solvent system. Table 3 shows the mole ratio of monomers in different samples.

Table 3.3. Mole ratio of PBRB3 Samples

| *PBRB* | *3-0215* | *3-0227* | *3-0320* | *3-0322* |
|---|---|---|---|---|
| VBRB | 1 | 1 | 1 | 1 |
| AA | 7 | 70 | 140 | 280 |
| SSA | 2 | 20 | 40 | 80 |

The polymerization recipes for all the polymer samples are listed in Table 3.4. The structure of the PBRB is shown in Figure 3.2.

Table 3.4. Monomer Feed Quantities of PBRB Samples

| *PBRB* | *AA(ml)* | *SSA(g)* | *VBC(ml)* | *RB(g)* | *SMBS(g)* | *APS(g)* | *Solvent* |
|---|---|---|---|---|---|---|---|
| 1-0414 | 70.384 | 69.28 | 4.1024 | 27.5 | 0.23 | 0.23 | water |
| 1-0509 | 70.384 | 69.28 | 4.1024 | 27.5 | 0.23 | 0.23 | water |
| 1-0608 | 67 | 69.18 | 0.803 | 4.9 | 0.27 | 0.27 | water |
| 1-0724 | 11.3 | 11.6215 | 0.134 | 0.8167 | 0.1 | 0.1 | water |
| 1-0728 | 11.7 | 11.56 | 0.287 | 0.8171 | 0.06 | 0.06 | water |
| 1-0801 | 11.7 | 11.56 | 0.287 | 0.8171 | 0.05 | 0.05 | water |
| 2-0818 | 11.16 | 11.56 | 0.287 | 0.8296 | 0.05 | 0.05 | water |
| 2-0829 | N/A | 11.55 | 0.287 | 0.8202 | 0.05 | 0.05 | water |
| 2-1024 | 11.16 | 11.55 | 0.29 | 0.817 | 0.05 | 0.05 | water |
| 2-1025 | 11.161 | 11.56 | 0.574 | 0.818 | 0.05 | 0.05 | water |
| 2-1216 | 11.161 | 11.55 | 1.028 | 0.8099 | 0.05 | 0.05 | water |
| 2-1220 | 12 | 11.544 | 0.25 | 0.2083 | 0.05 | 0.05 | water |
| 2-0102 | 11.161 | 11.52 | 0.25 | 0.1012 | 0.05 | 0.05 | water |
| 3-0215 | 1.2 | 0.87 | 2.40 | | 0.01 | 0.01 | water/acetone |
| 3-0227 | 1 | 0.8373 | 0.277 | | 0.02 | 0.02 | water/acetone |
| 3-0320 | 1.2 | 0.8373 | 0.1 | | 0.02 | 0.02 | water/acetone |
| 3-0322 | 1.2 | 0.8373 | 0.05 | | 0.02 | 0.02 | water/acetone |

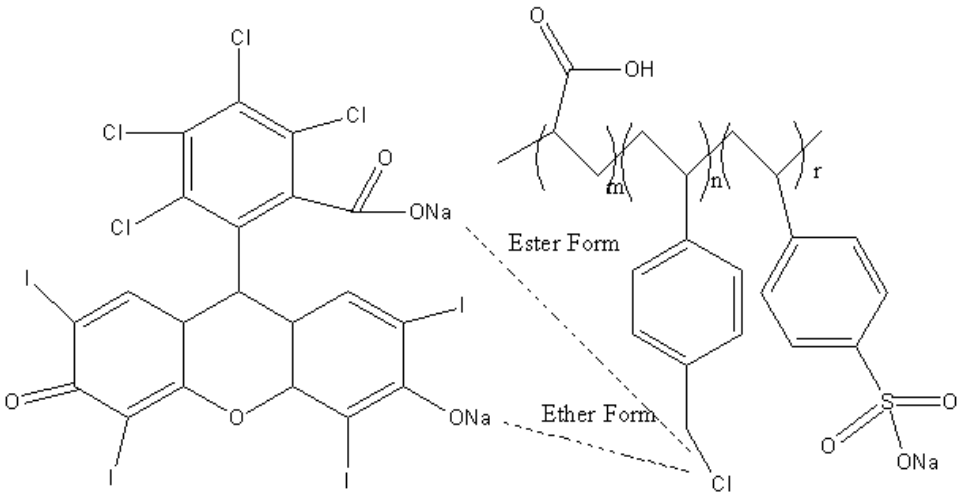



Figure 3.2 The chemical structure of PBRB

# 4. CHARACTERIZATION

## 4.1 Characterization of Final Product

The most important properties of these polymer bound dyes are the percentage of free dye, the singlet oxygen production rate, and the water solubility. Their characterization is described below. In addition, the chemical structure of vinyl benzyl Rose Bengal needs to be determined. This part will not only estimate the quality of the samples list above, but also identify the structure of the vinyl benzyl Rose Bengal, which would be very helpful for following copolymerization.

### 4.1.1 Free dye percentage evaluation

#### 4.1.1.1 Thin Layer Chromatography

TLC was conducted in order to determine if all of the Rose Bengal was attached to the polymer chain. The solvents used were acetone, methanol, and tetrahydrofuran, THF. Silica gel coated 4cm $\times$8cm elastic sheets were prepared. At 1cm from the bottom, a horizontal line was marked by pencil. The result will be shown in Figure 5.1.

#### 4.1.1.2 Solvent Separation Test

In this experiment, PBRB10414 and PBRB20608 were dried in a vacuum oven over night. The resulting solids were ground into tiny powder with a mortar and pestle followed by sand paper. A little bit of powder was measured into each of 4 vials. Next, 5 mL MEK was added to each vial. After shaking and allowing the solution to sit for 12 hours, the pink MEK solution in the vials was transferred to four 100 mL flasks by pipette. This procedure was repeated several times until the MEK in the vials was clear. The final MEK volumes are also listed in Table 5.1. Next the absorbance of each pink MEK solution was measured by

Genesys™ 10 series spectrophotometers from Thermo Electron Cooperation. In order to get more precise data, all solutions were diluted until the absorbance was between 0.05-2 and the amount of solvent required was recorded. Using the calibration curve in Figure 5.2 and data from PBRB information above, the free Rose Bengal in PBRB1 solids was determined and is shown in Table 5.1.

Similarly, we can use this approach to track the amount of free dye in each period during polymerization. During the polymerization of PBRB2, Rose Bengal was delivered before the polymerization. By tracking how much free dye is inside of the solution, the activity of –COOH in Rose Bengal and acrylic acid can be determined. In order to get free dye percentage in each step, 2mL DI water and 11 mL methyl ethyl ketone (MEK) were delivered to 10 vials as the separation solvents shown in Figure 4.1. Then 0.5 mL PBRB2 solution was taken as a sample from the polymerization system by pipette at 7 different times. Sample No. 1 is right after the delivery of Rose Bengal, next 5 samples were collected at half hour intervals during polymerization. Sample No. 7 is the final polymer sample. In order to balance the acid from the monomer, aqueous NaOH (50% by weight) was dropped into the solution in order to maintain the pH after finishing the delivery of acrylic acid.

By measuring the solution concentration, the percentage change of the free dye can be monitored during polymerization procedure. In order to dilute the solution into 0.05-2 absorption range, 1.9 mL MEK was added to 0.1 mL upper layer sample and 2.9 mL DI water was added to 0.1 mL lower layer sample 1. The result is shown in Table 5.2.

Figure 4.1. Sample pictures from PBRB20728.

#### 4.1.1.3 Dialysis Test

Dialysis membranes, made from regenerated cellulose were purchased from Fisher Scientific with the cut off molecular weight at 3500. Tubing nominal dry thickness is from 0.9 to 1.2mil (22 to 30μm). According to product instruction, the equilibrium time is around 4-6 hours. In this project, 36 hours was used in order to make sure the completed diffusion to get the same dye concentration for both sides.

The assumptions of this experiment are the concentration of free dye inside the bag equals the concentration of dye outside the bag after 36 hours and volume of the solution in the bag can be ignored. It is also assumed that any dye chemically bound to the polymer will not pass through the membrane. A 50 μL sample and 100 mL DI water were added to flask as the control group. A 50 uL sample was delivered to a 5 cm dialysis membrane tube and sealed with green clips, which were supplied with the dialysis membrane from FisherScientific. Next the sealed tube was placed in 500 mL DI water as shown in Figure 4.2. All the flasks were covered with glass caps and the entire beaker were covered with plastic film in order to

avoid evaporation. The data is shown in Table 5.3. The absorption of the control solution and the sample solutions were measured after 5 days, 12 days, and 24 days are recorded in Table 5.4, 5.5 and 5.6.

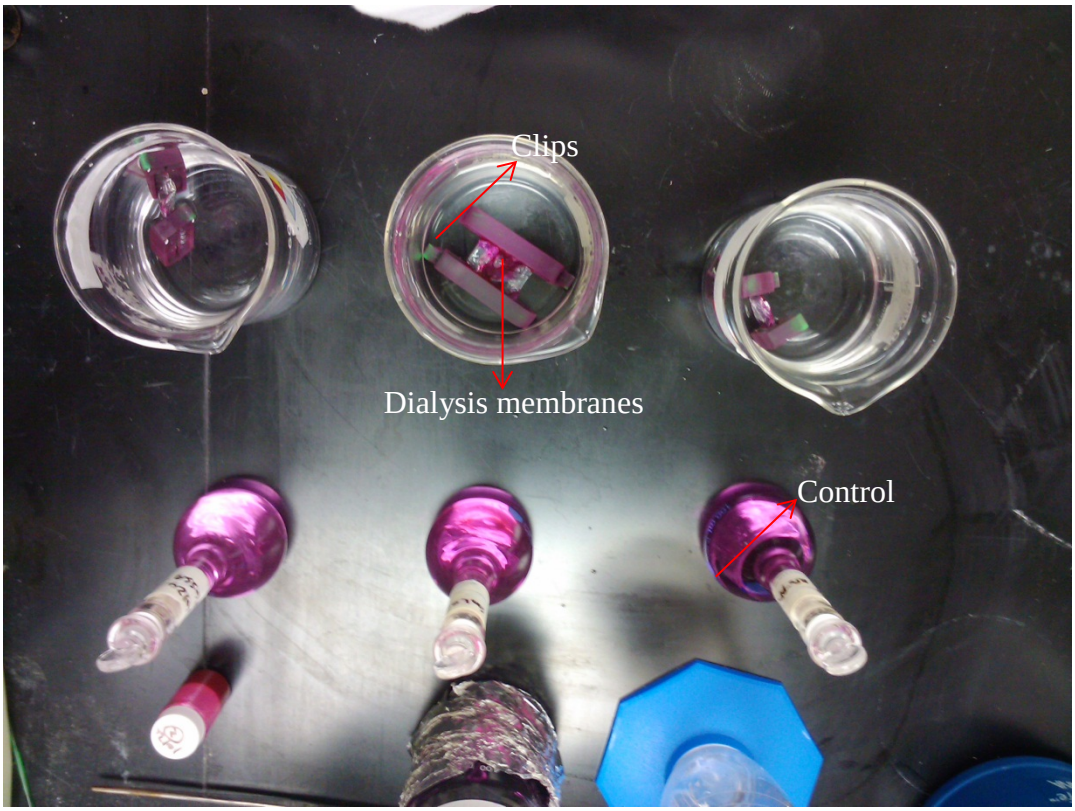



Figure 4.2. Dialysis test 1 using clips.

At the same time, the free dye of samples from different stages of the polymerization were also tested by taking  and adding1 mL sample to a dialysis  bag and another 1mL sample to a beaker containing 100 mL DI water. The dialysis membrane was sealed by a zipper cut from freezer bags and placed in beakers respectively that contain 100 mL water. The absorption of

the samples was measured after 48 hours. The same experiment was conducted using old clips which had been used many times before and new clips, respectively. The data is shown in Table 5.7.

Based on the results discussed in Chapter 5, the dialysis test was conducted using the zipper from freezer food bags rather than the supplied clips. The samples were obtained during polymerization of PBRB2-0102. Sample 1 was obtained 30 minutes after delivery of SSA and VBC. Sample 2, 3, 4, and 5 were obtained after the delivery of monomer but with different amount of NaOH. Table 5.8 shows the free dye percentage of samples from polymerization procedure of PBRB2-0102.

The final modified test is shown in Figure 4.3. 80 mL phosphate buffer (pH=7.4) was delivered to a 100 mL beaker 1. At the same time in beaker 2, 1 mL sample solution was also dropped into 80 mL buffer solutions directly. Then the bag was sealed by freezer food bag zipper placed in the buffer solution for 12 hours. Then the Rose Bengal concentration of the outside solution in beaker 1 and the concentration of the Rose Bengal in beaker 2 were measured by visible light spectroscopy. In order to satisfy the measurement range of the light spectrometer, we diluted the sample solution to obtain a suitable concentration and then calculated the real free dye according to how we diluted the polymer. So ratio of the two concentrations will reflect how much free dye was in the test sample. Table 5.9 shows the free dye percentages of representative samples from PBRB1, PBRB2 and PBRB3.


Figure 4.3. Dialysis test 3 of sample by dialysis membranes

### 4.1.2 Solubility Test

To make sure all samples were soluble in water, we need to test how dry polymers can be dissolved in water. At room temperature, dry samples were ground into powders then added to water in a glass vial (the weight of water plus vial is $W_1$, the weight of the clean vial is $W_0$) and then the vials were shaken until all the powder disappeared. When all the powder dissolved, a little more powder was added, and the vial was again shaken to dissolve the powder. This process was repeated until no more particles would dissolve. The weight of the solution plus polymer is $W_2$. So the solubility of the polymer is $s = \frac{W_2 - W_1}{W_2 - W_0} \times 100$. The solubility result of PBRB 3 is shown in Table 5.10.

### 4.1.3 Response to acidic and basic environment

1000 mL RB solution was prepared. Then small samples with different pH were obtained by adding 50% aqueous NaOH solution and 38% aqueous HCl solution. The assumption is that in the pH adjustment procedure, the concentration of Rose Bengal doesn't change. The pH can be adjusted by adding one or two drops of acidic or basic solution without a significant

change in volume. The pH values of the samples and their absorption properties are shown in Figure 5.6 and Figure 5.7.

### 4.1.4 Singlet Oxygen Production Rate Test

Singlet oxygen production rate is another of the crucial parameters for PBRB samples. The test of this rate is based on Haag's method and shown in Figure 4.4 and 4.5 (*24*). Part 1 is a lamp that offers the light source for singlet oxygen production. Part 2 is a water bath that filters out infrared light that can keep the chamber from excessive heating. Part 3 is the sample chamber that provides an air tight environment for samples. Part 4 is the probe that when kept in closed environments can detect the concentration of dissolved oxygen in the solution. Part 5 is the dissolved oxygen meter, which can output data directly to a computer.

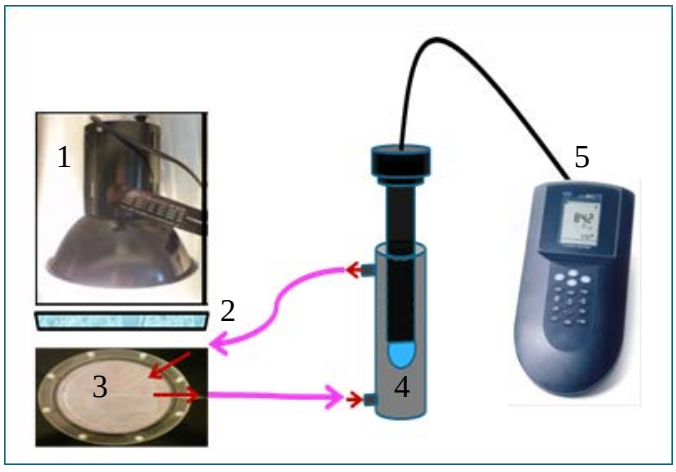



Figure 4.4. Singlet oxygen production test system.

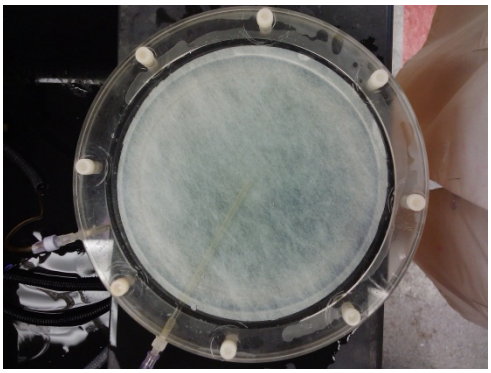


Figure 4.5. Detailed sample chamber.

300 mL of PBRB3 and Rose Bengal solution were prepared and air was bubbled through it for 5 minutes before delivering into the system by a circulating pump. Furfural alcohol, FFA, was added to the solution after bubbling air through the solution. The light was turned on when the software was ready to record data. When the concentration of dissolved oxygen decreased to an asymptotic lower point, the test was terminated and software was shut down. Then the data was transferred to the computer and plotted as in Figure 5.8. $[^3O_2]$ is the concentration of oxygen dissolved in solution. $[^1O_2]$ is the concentration of singlet oxygen in solution. [FFA] is the concentration of FFA in solution, which is a constant because we added enough FFA to the solution. [RB] is the concentration of Rose Bengal in solution which is also a constant because we assume the Rose Bengal won't have obvious self-bleaching effect in experiment. $K_0$ is the kinetic rate of FFA reaction with singlet oxygen. $K_1$ is the kinetic rate of singlet oxygen production. K is the kinetic constant of the two combined reactions. The reaction mechanism is listed as below:

$$^3O_2 + RB \underset{\times}{\overset{h\nu, k_0}{\rightleftarrows}} {}^1O_2$$

$$^1O_2 + FFA \xrightarrow{k_1} \mathrm{P}$$

So we have $$\frac{d\left[{}^3O_2\right]}{dt} = -k_0\left[{}^3O_2\right][RB] = -\frac{d\left[{}^1O_2\right]}{dt}$$

From which we get $$\left[{}^3O_2\right] = Ae^{-k_0[RB]t} = Ae^{-Kt}$$

So the concentration of dissolved oxygen has an exponential relationship with time.

## 4.2 Characterization of Vinyl Benzyl Rose Bengal

### 4.2.1 High Performance Liquid chromatography (HPLC)

HPLC was used for the analysis the VBRB product after synthesis. The HPLC experiment was conducted by Elton R. Lawrence and Brooke L. Kaznowski under the guidance of Dr. Beck for their senior design project. The liquid chromatograph was an Agilent 1200 SL with DAD (diode array detector). The flow rate was 0.5 mL per minute. Vinyl benzyl chloride Rose Bengal sample (1 mg) was dissolved in acetonitrile and DI water at a 9:1 ratio, which was found to have the best solubility characteristics after several trials at different ratios. Two samples were prepared: (1) unreacted Rose Bengal dye, and (2) vinyl benzyl-Rose Bengal in acetone, which was synthesized using the methods described above. The actual acetonitrile/water composition of each sample is listed in Table 4.1.

Table 4.1. Acetonitrile/Water composition of samples

| Sample | Chemical | Acetonitrile | Water |
|---|---|---|---|
| 1 | Rose Bengal | 2.97 mL | 0.33 mL |
| 2 | VBRB | 1.35 mL | 0.15 mL |

This chromatographic separation was done using a Waters XBridge™ Amide column with a particle size of 3.5 micrometers and 3.0 x 100 mm column length. Isocratic mobile phase composed of 20% DI water and 80% acetonitrile was used as the first trail. Then, the 40% water and 60% acetonitrile was used for the mobile phase composition. The retention time was 0.7 minutes for free Rose Bengal. Since the time was so short, 60% water and 40% acetonitrile was used as the mobile phase in order to increase retention of the Rose Bengal by increasing the percentage of polar solvent. The longer retention time means more interaction with the stationary phase. The VB-RB sample that was synthesized in acetone and water was also run at 60% water and 40% acetonitrile composition. The result is shown in Figures 5.9, 5.10 and 5.11.

### 4.2.2 Mass Spectrometry

TOF mass spectrometry was used to measure the molecular weight of vinyl benzyl Rose Bengal monomer. This part of experiment was also performed by Elton R. Lawrence and Brooke L. Kaznowski under the direction of Dr. Beck. An Agilent 6520 quadrupole time-of-flight mass spectrometer was used as the detector for the 1200SL liquid chromatograph. A Poroshell 120 EC C18 3.0 x 100 mm column containing 2.7 micrometer particles was used in this experiment. The instrument was calibrated in positive ESI (electron spray interface) with known molecular weights according to the manufacturer's recommendations. Separation was isocratic with a 50% acetonitrile (0.1% formic acid) and 50% water (0.1% formic acid). The column was then changed to an XBridge™ column that was previously used in HPLC measurement. Once the column was changed the test was performed with a solvent gradient, starting at 20% acetonitrile and increasing to 95% over 5 minutes, held for four minutes then

returned to 20% over 0.5 min. The column was equilibrated for 5 minutes between runs. The resulting separation is shown in Figure 5.12.

### 4.2.3 Fourier transform infrared spectroscopy (FTIR)

Mid-infrared FTIR was conducted using Nicolet Nexus 470 spectrometer in transmission mode. A Nicolet OMNI Germanium Crystal ATR sampling head was employed. 64 scans of data were collected and analyzed for each sample by using Omnic software. Rose Bengal was used as the sample directly. VBRB sample was washed by acetone and water and dried in vacuum oven at 30 degrees for 24 hours. The result is shown in Figure 5.15.

### 4.2.4 Response to pH

Sensitivity to pH was measured in this experiment. Acidic and neutral Rose Bengal and VBRB acetone-water solutions were prepared as described in Table 4.2. The absorption of the 4 samples was measured at range from 500-620 nm and plotted as Figure 5.16 and Figure 5.17.

Table 4.2. The preparation procedure of RB and VBRB solution

| *Sample* | *RB* | *Water* | *HCl solution* |
|---|---|---|---|
| RB A/W neutral solution | 2ml RB acetone solution | 2ml | 0 |
| RB A/W acid solution | 2ml RB acetone solution | 1ml | 1ml |
| VBRB A/W neutral solution | 2ml VBRB acetone solution | 2ml | 0 |
| VBRB A/W acid solution | 2ml VBRB acetone solution | 1ml | 1ml |

# 5. RESULTS AND DISSCUSSIONS

During the procedure of incorporating Rose Bengal into poly (acrylic acid-co-styrene sulfonic acid-co-vinyl benzyl chloride), the solubility of the polymer, its pH sensitivity, and the attachment position of Rose Bengal are important. In addition, the presence of free dye was suspected in the polymer solution. The results discussed below were obtained to determine these properties.

## 5.1 Free Dye

### 5.1.1 The investigation of free dye in final product.

From the TCL test experiments described in Chapter 4, we suspected that there might be a lot of free dye in final product as indicated in Figure 5.1. From the acetone result, we can see all Rose Bengal sample move at the same pace, which means Rose Bengal in PBRB samples is not attached to the polymer chain. From the methanol result, we can see that the PBRB sample moved slower than the free Rose Bengal sample, which can be caused by the attachment of Rose Bengal or because of the high concentration of PBRB sample. By comparing the diluted sample and the 1-0608 sample, we can conclude that the slower motion is caused by high concentration. By comparing the diluted 1-0608 sample and the free Rose Bengal, we can conclude that the there is a lot of free dye in 1-0608. From tetrahydrofuran group, the same observations were obtained. Therefore, a high percentage of free dye is suspected after TLC test. However, due to the uncertainty of the polarity of these solvents, Rose Bengal and developing sheets and the quantity of free dye, further experiments are needed to know the exact free dye percentage.

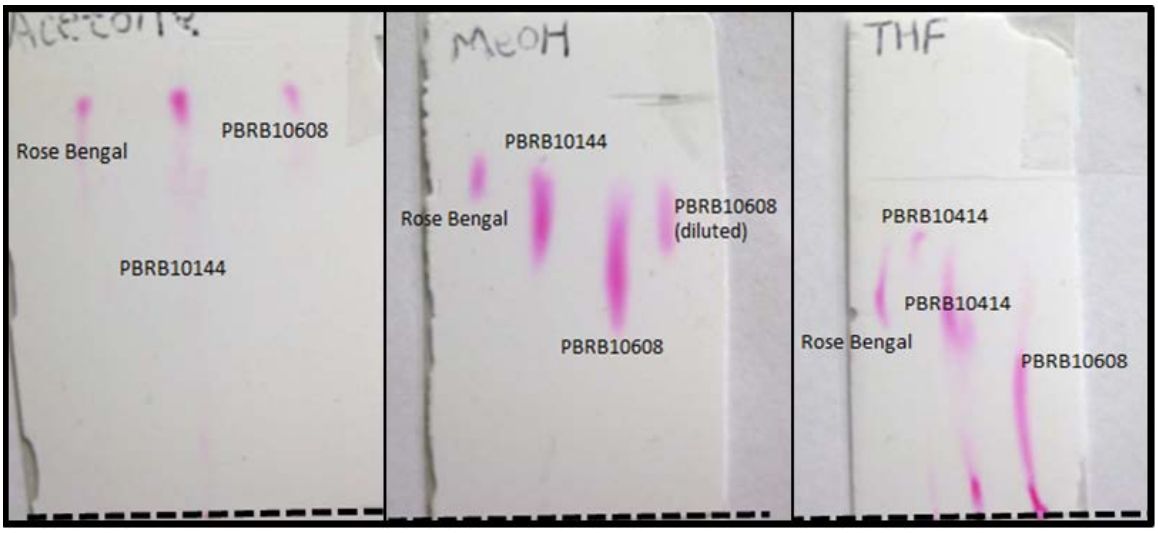



Figure 5.1. TLC results of PBRB 1 in acetone, methanol and THF solvents.

In preliminary experiments, we found free Rose Bengal totally dissolves in methyl ethyl ketone (MEK). We assume if we dissolve $m_1$ grams PBRB sample in MEK solvents, free dye would be dissolved in MEK solvent and the PBRB will not dissolve in MEK solvent because of PBRB is water-wet. According to the free dye concentration in the MEK and the summed volume described in Chapter 4, we can get the mass of free dye, $m_2$ grams. The free dye percentage can be calculated as $\frac{m_2}{m_1}\times 100\%$. The value of $m_2$ was calculated by $V_2 C_2$. The value of $C_2$ can be calculated by absorbance value of the sample $A_2$ according to the calibration curve by $C_2 = \frac{A}{69.655}$, where 69.655 is the slope of the calibration curve obtained from Figure 5.2.

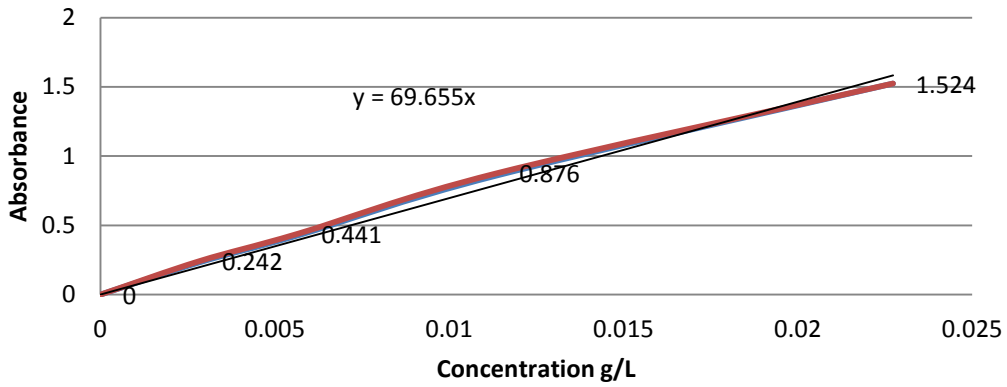



Figure 5.2. Rose Bengal MEK solution calibration curve.

In Table 5.1, $C_0$ is the diluted concentration of RB while $C_2$ is the original concentration of the sample. The sample at concentration $C_2$ is diluted to $C_0$ to bring the solution within the measurement range of the spectrometer. Table 5.1 shows that 9.46% of the dry polymer sample PBRB1-0414 is free dye and 1.81% of dry polymer sample PBRB1-0608 is free dye. However, in the polymerization recipe, we added 16.83% and 3.00% Rose Bengal, respectively, to these two samples, which means the remaining amount of Rose Bengal is attached to the polymer chain. Thus the percentage of the Rose Bengal added to the polymer that remained free in PBRB1-0414 and PBRB1-0608 is 54% and 60%, respectively. However, the free dye percentages from PRRB1-0414 and PBRB1-0608 do not seem to be consistent because we added 5.6 times more Rose Bengal during the polymerization of PRRB1-0414 than for PBRB1-0608 and the same procedure was used to make these materials. We would expect that the free dye in PBRB10414 should be higher than PBRB0608. However, since the polymer is not soluble in MEK, it is difficult to assure that

all of the free Rose Bengal in the polymer powder was extracted by MEK. Thus a better method for determining the amount of free dye is desired.

Table 5.1. Free dye percentage in PBRB samples

| *PBRB* | *1-0414* | *1-10414* | *1-0608* | *1-0608* |
|---|---|---|---|---|
| Vial # | 1 | 2 | 3 | 4 |
| Volume of solution ($V_2$, mL) | 43 | 38 | 42 | 43 |
| Absorption($A_2$) | 1.74 | 1.76 | 1.46 | 1.43 |
| Concentration($C_0$, g/L) -test sample | 0.02 | 0.03 | 0.02 | 0.02 |
| Concentration-washed off solution($C_2$, g/L) | 0.11 | 0.05 | 0.09 | 0.06 |
| Mass of free RB($m_2$, mg) | 4.82 | 1.93 | 3.96 | 2.65 |
| Mass of PBRB powder($m_1$, g) | 0.05 | 0.02 | 0.19 | 0.18 |
| Percent of free dye in solids | 9.46% | 8.71% | 2.14% | 1.48% |
| Average | 9.08% | | 1.81% | |
| Rose Bengal in solids | 16.83% | | 3.00% | |
| Free RB in all RB | 54% | | 60% | |

5.1.2 Effect of the order of addition of ingredients on the amount of free dye.

Our goal is to reduce free dye in final product. Based on the previous study, we postulated that the most likely reason for large amounts of free dye is the competition between –COOH

groups in acrylic acid and the –COOH group in Rose Bengal. When Rose Bengal tries to attach onto the vinyl benzyl group by displacing the –Cl group, –Cl groups may have already been displaced by –COOH group on acrylic acid because acrylic acid was delivered much earlier than Rose Bengal in polymerization of PRBR1 sample series. To test this hypothesis, Rose Bengal was added to the flask before the delivery of monomers and then the polymerization was initiated. At the same time, several samples were taken during the polymerization procedure in order to measure the amount of free dye throughout the polymerization. PBRB2 samples were prepared as descried in Chapter 4, method 2.

When we drop Rose Bengal to MEK and water mixture, the Rose Bengal always remains in the MEK. So we assume that when we drop PBRB samples into the MEK/water layer, the free dye will stay in the MEK layer but the reacted Rose Bengal will prefer to stay in the water layer because of the high solubility of the polymer.

In addition, to ensure that all free dye was accessible for extraction, we kept the polymer sample in water solution and extracted the free Rose Bengal with MEK. The MEK/water system results in a two phase system with MEK forming the upper layer. The samples obtained during polymerization are shown in Figure 4.1. The absorbance of each layer was measured and is shown in Table 5.2. The amount of free dye remains nearly constant throughout the reaction and is still around 60%, so polymerization method 2 did not show a significant improvement over method 1. Since according to the Beer-Lambert law, the concentration is proportional to the absorbance, the sum of the absorbances of the upper and lower layers is provided in the last column. There is a dramatic increase in the absorbance of the upper, the lower, and the sum as the reaction proceeds. Figure 5.3 shows graphically the

time evolution of the absorbance. However, all of the Rose Bengal was added prior to the polymerization, so the absorbance should remain the same or decrease over time. To reliably measure the amount of free dye, this result must be explained. After a carefully examining the polymerization procedures and Table 5.2, we postulated that the absorbance might be sensitive to the pH of the polymerization system.

Table 5.2. Table absorbance of lower, aqueous layer and the upper, MEK layer from samples taken during the polymerization.

| *Samples* | $A_{lower}$ | $A_{upper}$ | *Free Dye Percentage* | $A_{lower} + A_{upper}$ |
|---|---|---|---|---|
| 1 | 0.13 | 0.089 | 69% | 0.219 |
| 2 | 0.306 | 0.39 | 54% | 0.696 |
| 3 | 0.529 | 0.551 | 59% | 1.08 |
| 4 | 0.584 | 0.595 | 60% | 1.179 |
| 5 | 0.524 | 0.555 | 59% | 1.079 |
| 6 | 0.564 | 0.552 | 61% | 1.116 |
| 7 | 0.604 | 0.613 | 60% | 1.217 |

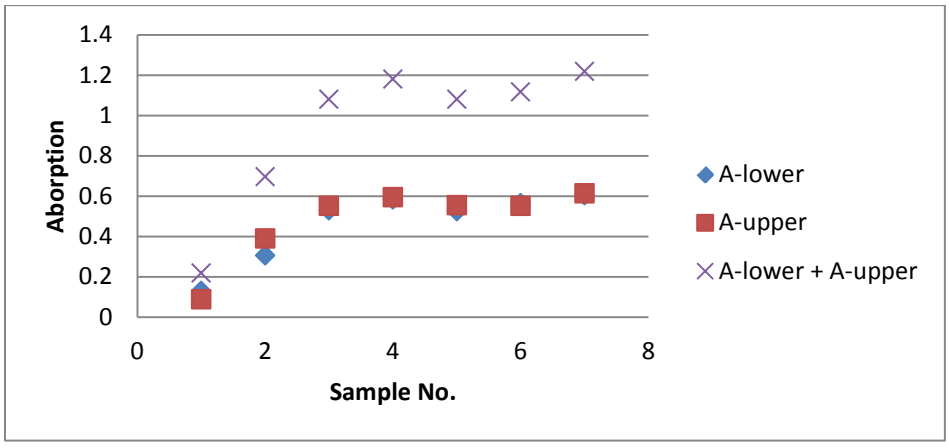



Figure 5.3. Change of the absorbance during polymerization

Since the amount of free dye was the same in polymerization methods 1 and 2, a different method is required to reduce the free dye. In the PBRB3 series, we attached Rose Bengal to vinyl benzyl chloride first to make VBRB (vinyl benzyl Rose Bengal), and then copolymerized VBRB, AA and SSA as described in the experimental section. The synthesis of VBRB was performed in acetone and water solution (1:1). Drying, grinding, and washing are necessary to remove the unattached dyes and prepare for further polymerization.

The difficulty in polymerizing VBRB, AA, and SSA is the quite different solubility properties for each of them. AA and SSA are very water soluble while VBRB is very water insoluble. At the same time, too much organic solvent will increase production costs and the environmental cost. To limit the amount of organic solvent, we initiated the polymerization of VBRB and AA in an acetone and water mixture in order to increase the solubility by forming poly(AA-co-VBRB). As the viscosity of the polymer solution began to increase, the still active polymerization system was delivered into a SSA-water solution to complete the polymerization. Again, the free dye concentration was measured on samples drawn from the final solution over time.

### 5.1.3 Measurement of free dye via dialysis

To overcome the deficiencies of our previous free dye measurements, we purchased dialysis membranes, with a 3000 g/mol cut-off molecular weight. The molecular weight for Rose Bengal is around 1000, so if it attached to the polymer, the molecular weight should be higher than 3000 except for the very shortest oligomers.

In order to evaluate the time required for diffusion of the free dye through the membrane, absorbance measurements were made after 36 hours, 5 days, 12 days and 24 days. The data is shown in Tables 5.3, 5.4, 5.5 and 5.6 and in Figure 5.4. We found that the amount of free dye changed with equilibration time.

Table 5.3. Free dye test result from dialysis test with clips after 36 hours

| *PBRB* | $A_{control}$ | $A_{washed\text{-}off}$ | *free dye percentage* |
|---|---|---|---|
| 1-0414 | 0.1494 | 0.093 | 62.25% |
| 1-0608 | 0.0462 | 0.025 | 54.11% |

Table 5.4. Free dye test result from dialysis test with clips after 5 days

| *PBRB* | $A_{control}$ | $A_{washed\text{-}off}$ | *free dye percentage* |
|---|---|---|---|
| 1-0414 | 0.2434 | 0.159 | 65.32% |
| 1-0608 | 0.0792 | 0.079 | 99.75% |

Table 5.5 Free dye test result from dialysis test with clips 12 days

| *PBRB* | $A_{control}$ | $A_{washed\text{-}off}$ | *free dye percentage* |
|---|---|---|---|
| 1-0414 | 0.1978 | 0.077 | 38.93% |
| 1-0608 | 0.0774 | 0.021 | 27.13% |

Table 5.6. Free dye test result from dialysis test with clips 24 days

| *PBRB* | $A_{control}$ | $A_{washed\text{-}off}$ | *free dye percentage* |
|---|---|---|---|
| 1-0414 | 0.1942 | 0.049 | 25.23% |
| 1-0608 | 0.0764 | 0.009 | 11.78% |

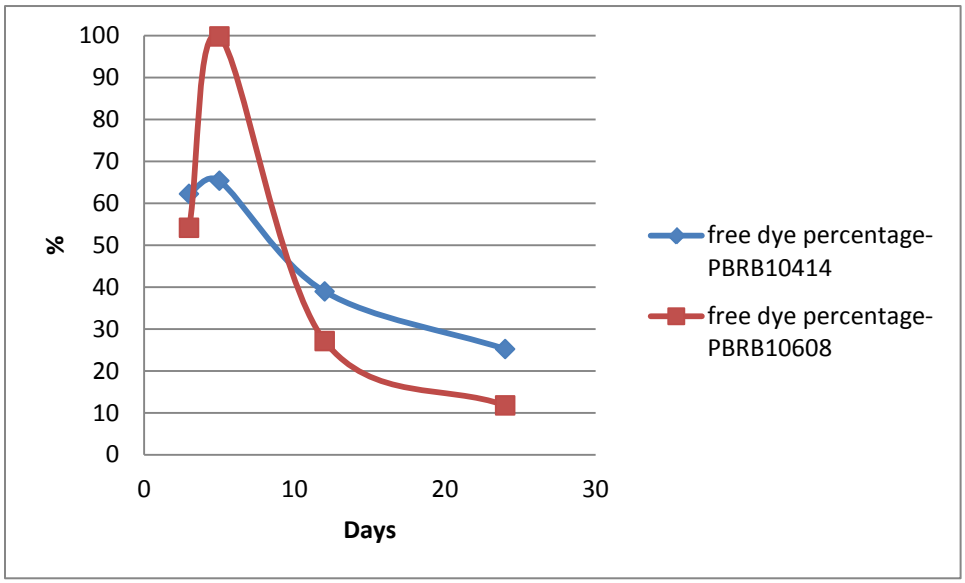



Figure 5.4. change of free dye percentage with different equilibrium time.

After excluding the possibility that Rose Bengal was photo bleaching during this time, and on observing that the dialysis clips provided by the manufacturer changed color during the test, the absorption of the dye by the clips was suspected to cause this behavior. In order to test if the clips absorption of dye caused these results, dialysis experiments on the same sample using old and new clips were conducted. From Table 5.7 it is clear that the clips we

used were affecting the experimental data. The manufacturer indicated that the clips are made of nylon, which can apparently be dyed with Rose Bengal, an acid dye. In order to exclude that factor, all the clips were replaced by the zipper portion of ziplock food bags for all subsequent experiments. These did not change color when immerses in Rose Bengal solutions.

Table 5.7. Free dye test result from dialysis test with new and old clips

| *PBRB* | *Free dye percentage using old clips* | *Free dye percentage using new clips* |
|---|---|---|
| 1-0608 | 57.18% | 27.13% |

We also suspected that the amount of free dye measured depends on sample pH. Two possibilities are that the pH can affect the cut off molecular weight of the semi permeable membrane by altering the pore size or that the dye absorbance changes with pH. By adding acid and base to Rose Bengal solution, we found the pink solution became colorless under acid pH and pinker under basic environment, as shown in Table 5.8 using freezer bag zipper for the dialysis clips. We believe the pH affects the form of Rose Bengal as shown in Figure 5.5. In acid conditions, the ring closes forming an ester group as shown in the right figure, which is a colorless form. (*52*)

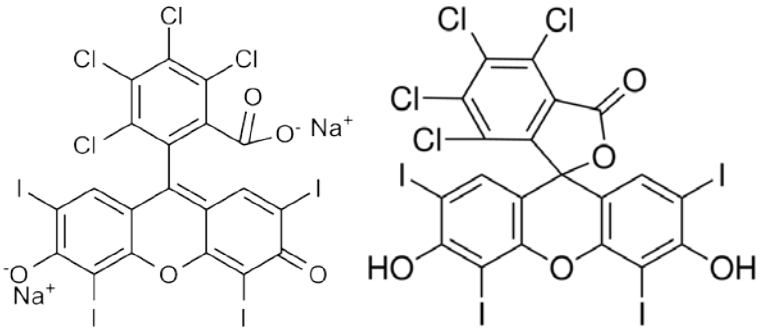


Figure 5.5. The structure of Rose Bengal in different pH solution (left basic, right acid)

Table 5.8. pH and free dye percentage of the PBRB2-0102 samples by dialysis membrane test using freezer bag zipper. Sample 1 is SSA-VBC-RB. Sample 2, 3, 4, 5 is SSA-VBC-RB-AA at different pHs.

| *2-0102* | *pH* | $A_{control}$ | *pH* | $A_{free\text{-}dye}$ | *Free Dye Percentage* |
|---|---|---|---|---|---|
| 1 | 2.87 | 0.118 | 4.09 | 0.006 | 5.08% |
| 2 | 0.46 | 0.008 | 3.13 | 0.001 | 12.50% |
| 3 | 2.63 | 0.023 | 3.61 | 0.001 | 4.35% |
| 4 | 3.1 | 0.164 | 4.08 | 0.033 | 20.12% |
| 5 | 4.27 | 0.299 | 5.27 | 0.292 | 97.66% |

Therefore, in order to obtain accurate measurement of the free dye, we need run the diffusion equilibrium in buffer solution at a constant pH. For the following experiments, we chose a buffer with pH = 7.4. Table 5.9 shows the free dye percentage of the different samples from a sample provided by LaamScience, Inc. (standard sample), PBRB1, PBRB2, and PBRB3 series. From the data, method 3, the PBRB3 approach had almost no free dye in the final product, which means nearly all of the Rose Bengal was incorporated into the polymer chain.

Table 5.9. Free dye percentage in LAAM, PBRB1, PBRB2 and PBRB3 samples by dialysis experiment at pH 7.4 and using freezer bag zippers.

| *Sample* | *Free dye percentage* |
|---|---|
| LAAM | 3% |
| PBRB1-0724 | 98% |
| PBRB1-0801 | 100% |
| PBRB2-0818 | 95% |
| PBRB2-1205 | 94% |
| PBRB2-0102 | 89% |
| PBRB3-0320 | 0% |

## 5.2 Solubility of the polymer

In the finishing procedure, a water soluble chemical can make the procedure much easier than solid chemicals because of easy application, low cost and environment friendly properties.

### 5.2.1 Determination of solubility

Solubility of the final products is determined by measuring how much dry polymer can be dissolved in water. Adding more SSA and AA makes the polymer more soluble. In Table 5.10, Sample PBRB3-0322 has better solubility than PBRB3-0320. PBRB3-0322 has double the amount of AA and SSA in the polymer compared to PBRB3-0320. So the solubility of the polymer can be improved by adding more soluble monomers. Table 5.10 shows that the best solubility we obtained was 2.1% by weight. This is higher than the concentrations

typically used for nylon surface modification. In other words, the solubility of the final product satisfied industry's production requirement.

Table 5.10. Solubility of PBRB3 samples

| *PBRB Sample* | *Solubility by weight* |
|---|---|
| 3-0320 | 1.2% |
| 3-0322 | 2.1% |

## 5.3 PH sensitivity

During the dialysis experiment, we found Rose Bengal's color is sensitive to pH of the solution. Figure 5.6 shows the effect of pH on the maximum absorption. It is obvious that at pH 2.7-3.2 range, an important shift occurred in the absorption maximum of the Rose Bengal. It is caused by the ring closure as shown on the right side of Figure 5.5. The single benzyl ring cannot share the $\pi$ electron cloud with the three aromatic rings. This causes the wavelength that we measured for the maximum absorbance to shift from the wavelength at 550 nm at neutral pH to 400 nm under strong acidic conditions. (Note: our spectrometer is limited to wavelengths greater than 400 nm so we do not know where the true maximum is.) When pH is less acidic, the dominate Rose Bengal structure is the open ring structure (left side of Figure 5.5), which is pink. In addition, as shown in Figure 5.7, the absorption at the maximum wavelength when pH is below 2.8 is much less than that when pH is higher than 3.5. The colorless Rose Bengal solution has pH lower than 2.8. The pink Rose Bengal has pH

higher than 3.5. However, all these Rose Bengal solution have same concentration. From the spectrum, we can see there is no absorbance peak in 400nm to 500nm range for solutions having low pH. The colorless Rose Bengal is believed to be the lactone structure based on Amat-Guerri's work. (*53*) The pink Rose Bengal is believed to be the quinonoid structure. Also, we think lactone structure has a very low quantum yield for producing singlet oxygen because the lactone structure destroyed the photosensitization procedure.

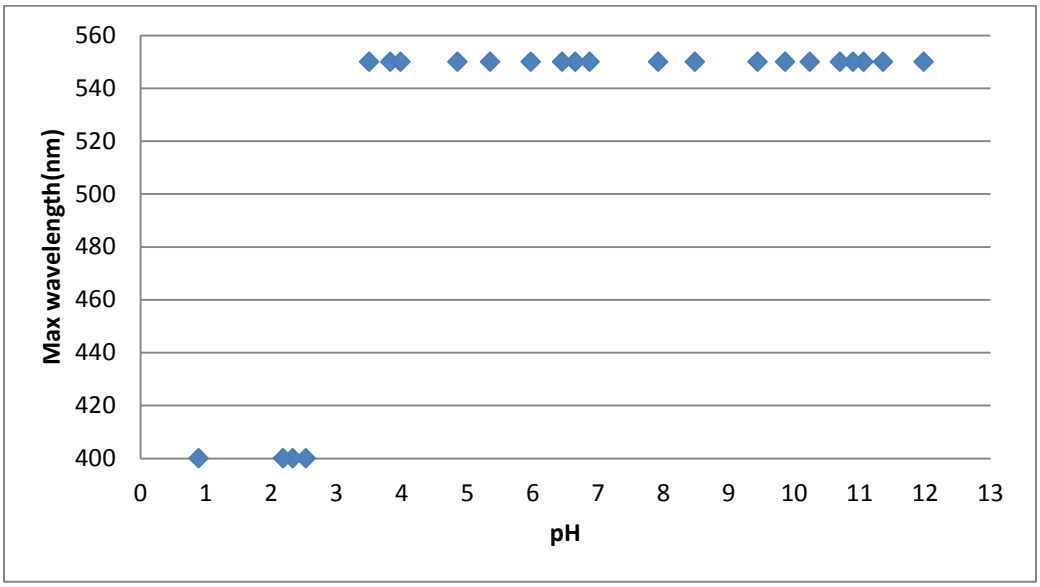



Figure 5.6. The effect of pH on the observed wavelength for maximum absorbance of RB solution.

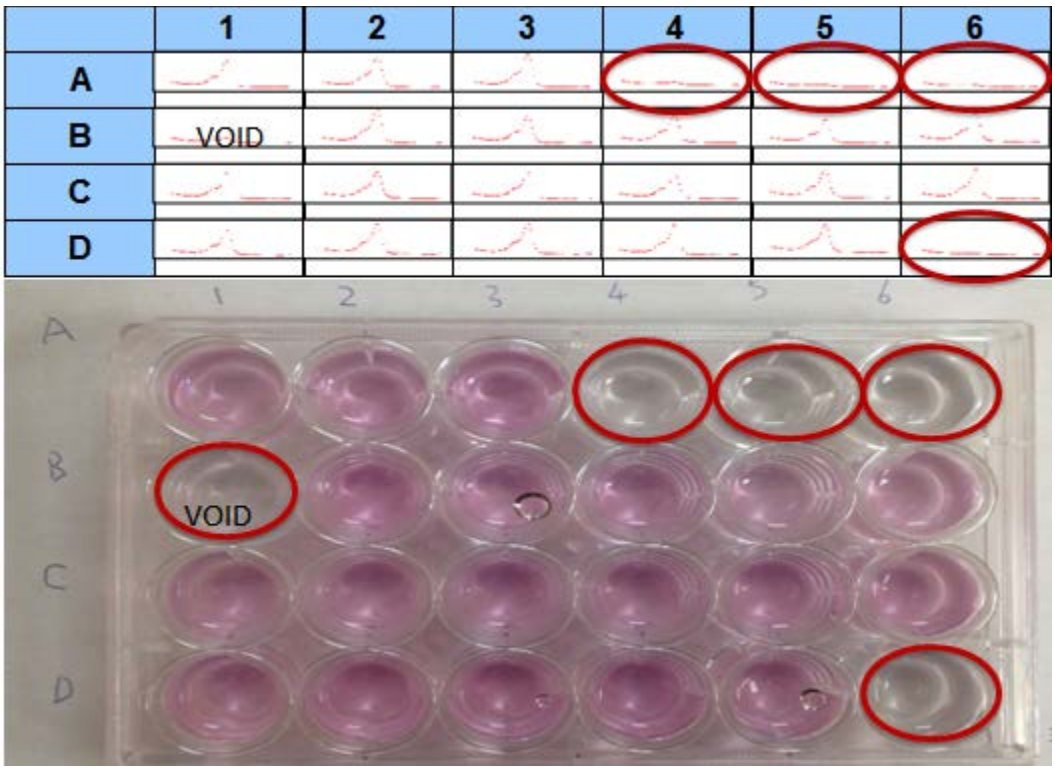



Figure 5.7. Effect of pH on absorbance of RB solution.

## 5.4 Singlet oxygen production rate

In order to make sure Rose Bengal is still active after incorporating it into the polymer chain, the singlet oxygen production was measured. The sample is Rose Bengal solution and the PBRB solution with similar concentration, the absorbance of each solution is listed in Table 5.11. Figure 5.8 shows the raw data obtained from the test. Figure 5.9 is the denervation of lines in figure 5.8. All dots in Figure 5.9 are not date but used for differ different lines. The reason why water group can consume dissolved oxygen is the probe will cost a portion of dissolved oxygen to get the concentration of dissolved oxygen. The reason why we have low decrease speed for Rose Bengal solution 2 is the

high concentration of Rose Bengal. In order to compare their efficiency more clearly, we normalized these lines by subtracting the data from water solution and then divided these data by concentration. Here we use absorbance to act as concentration for convenience. Figure 5.10 shows the efficiency of RB1, RB2 and PBRB solution. From these data, we can know the PBRB solution has higher efficiency than Rose Bengal group, which motivates us to understand the structure of VBRB in order to figure out why we have higher efficiency in PBRB solution.

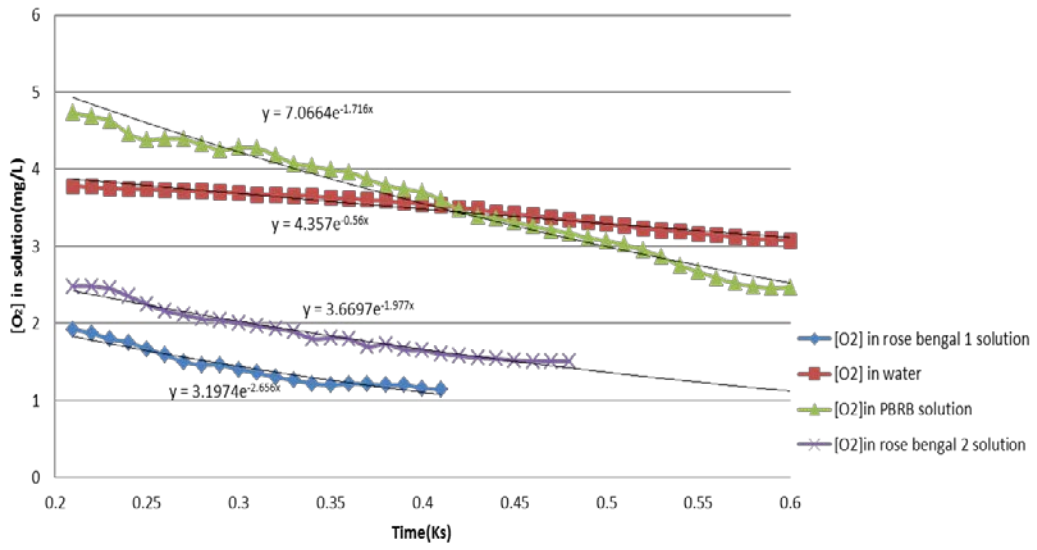



Figure 5.8. Concentration of dissolved oxygen in water, Rose Bengal, and PBRB3 solution.

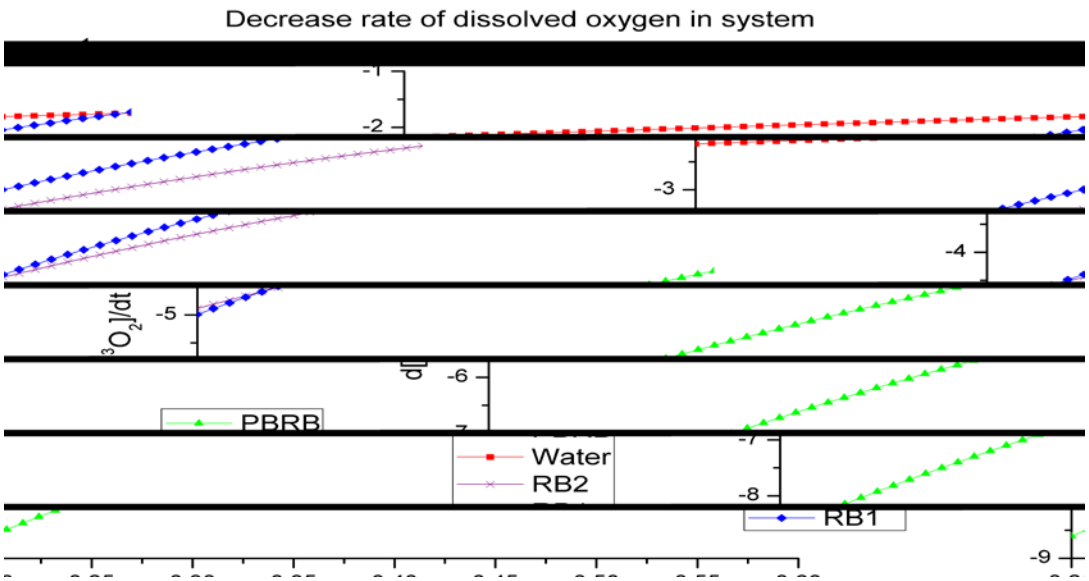



Figure 5.9. Singlet Oxygen Production Rate Along With Time.

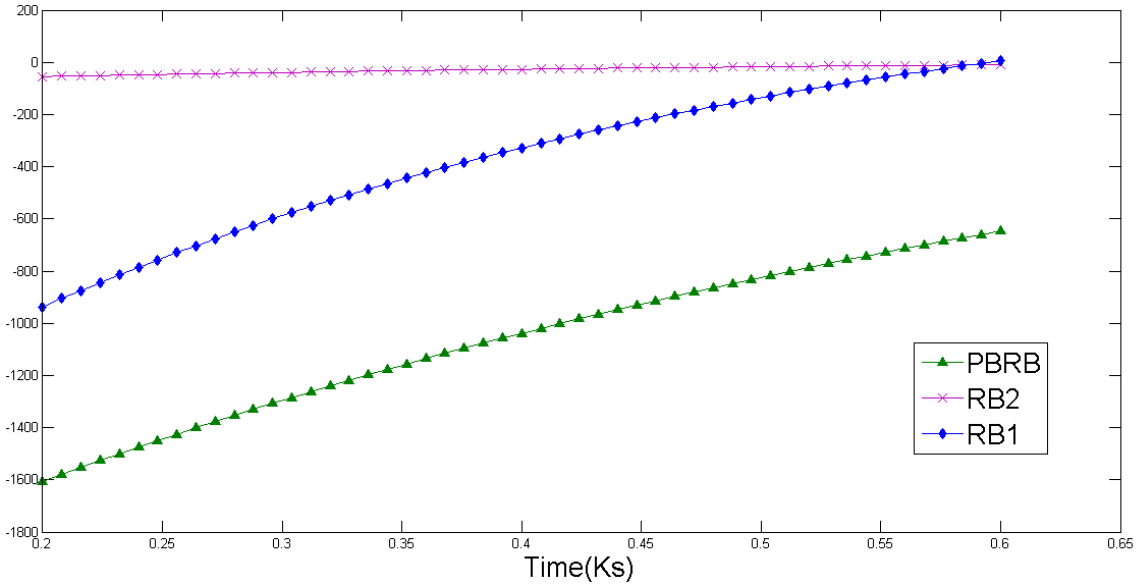



Figure 5.10. Normalized Singlet Oxygen Production Rate.

Table 5.11. Absorbance of water, Rose Bengal solutions, and PBRB3 sample

| | *Water* | *Rose Bengal1* | *Rose Bengal 2* | *PBRB3* |
|---|---|---|---|---|
| Asborbance | 0 | 0.003 | 0.05 | 0.004 |

## 5.5 Structure of vinyl benzyl Rose Bengal

Figure 5.11 shows the absorbance spectrum in 40% ACN and Figure 5.12 shows the absorbance in 80% ACN. The spectra are nearly identical. From Figure 5.13, we can see the absorbance curve of VBRB, which is also nearly the same as Rose Bengal. There is a slight change in the spectra between 200 nm and 300 nm, which will be explained later.

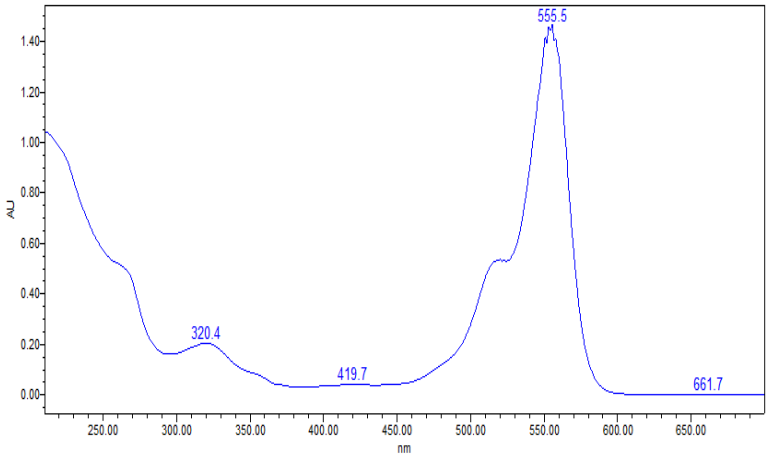



Figure 5.11. Absorbance curve of Rose Bengal using 60% ACN.

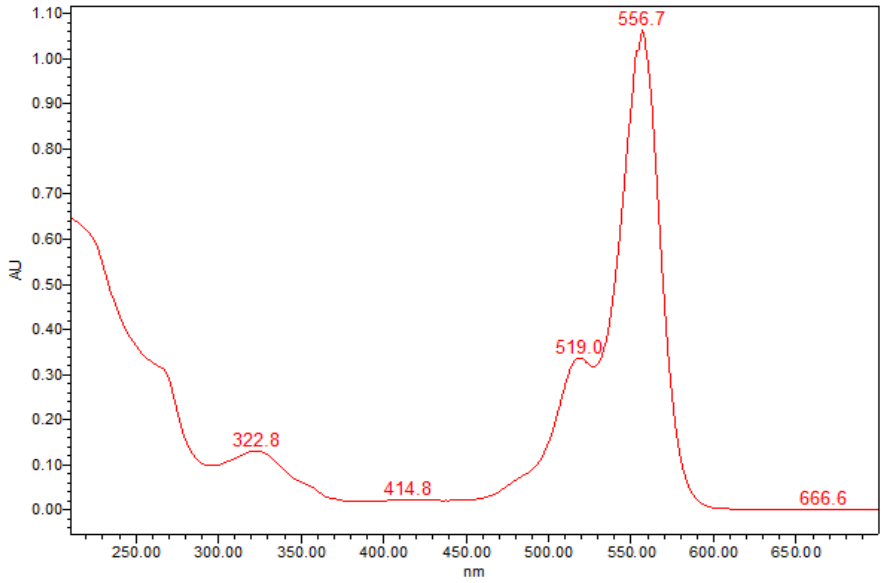



Figure 5.12. Absorbance curve of Rose Bengal using 80% ACN.

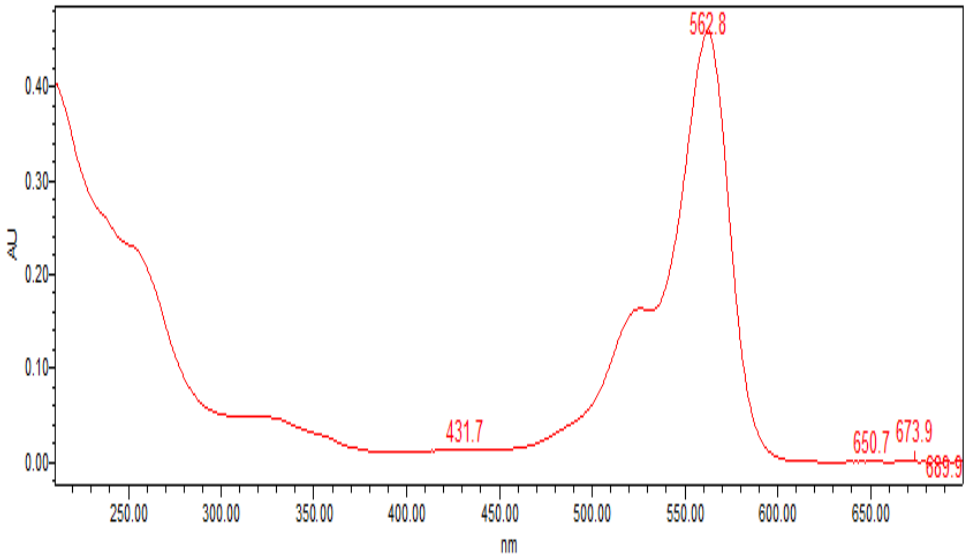



Figure 5.13. Absorbance curve of VBRB in 40% CAN

### 5.5.1 Molecular weight determination

The calculated molecular weight of VBRB should be 1088.57. By looking at the EIC (molecular weight equals 1088.57) of the sample, the extracted ion current chromatogram in Figure 5.14 was obtained, which means the VBRB we synthesized is what we expected. Also, because of the isotopes of iodine and carbon, a molecular weight distribution around 1088 was obtained, which includes 1088.57, 1089.57, 1090.57, 1090.57 and 1092.57 as shown in Figure 5.15.

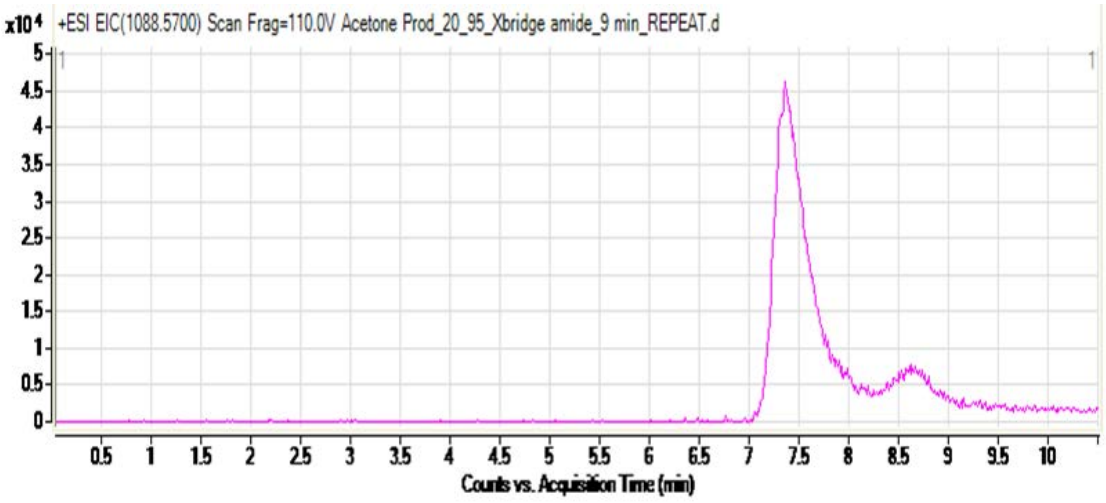



Figure 5.14. Extracted Ion Chromatogram at m/z equals1088.57 for VBRB.

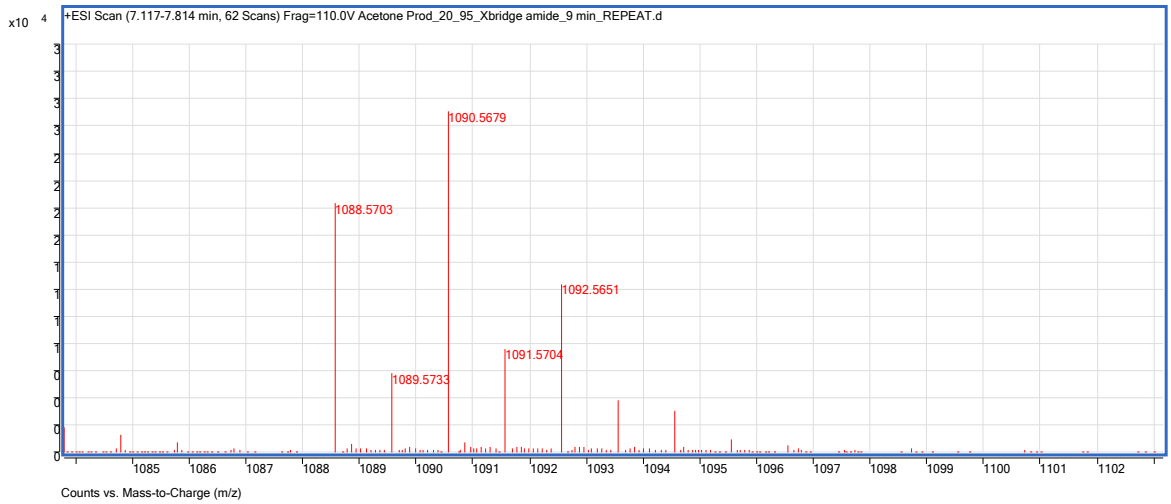



Figure 5.15. Isotopic mass Distribution around m/z equals 1088.57.

### 5.5.2 Position of attachment of Rose Bengal on vinyl benzyl chloride

There are two potential sites for attaching Rose Bengal to vinyl benzyl chloride to form VBRB, as shown in Figure 5.16. One is the ester form and the other is ether form. FTIR tests were conducted to determine which configuration of VBRB was synthesized. The spectra are shown in Figure 5.17.

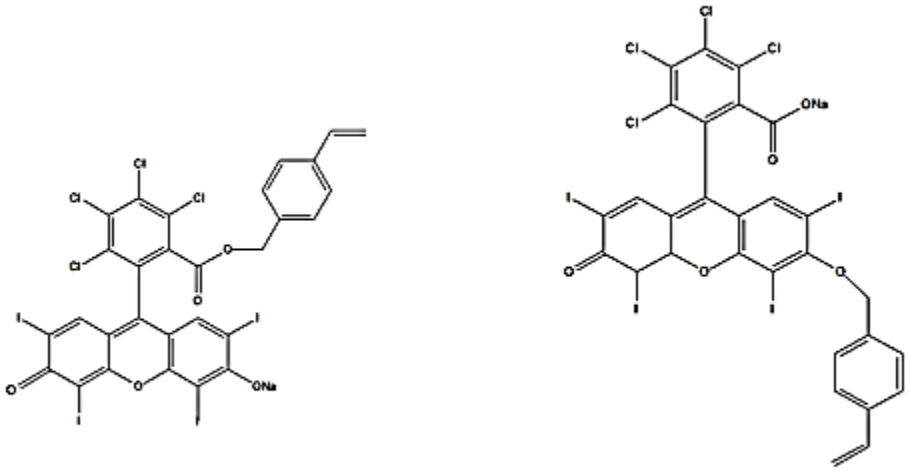


Figure 5.16. Two possible structures of VBRB (left-ester form, right-ether form).

From any differences in the spectra between VBRB and RB, we can judge the active group on Rose Bengal that the chloride group reacted with. If vinyl benzyl chloride reacts with the –COONa on Rose Bengal, then we will have unsaturated ester group absorption peak on VBRB spectrum around 1730 $cm^{-1}$. If vinyl benzyl chloride reacted with the –OH, we would get more unsaturated ethers but there should be no unsaturated ester group. If both positions are active, then we should also have unsaturated ester group absorption peakon VBRB spectrum around 1730 $cm^{-1}$. For all other peaks, VBRB spectrum should be similar with Rose Bengal spectrum.

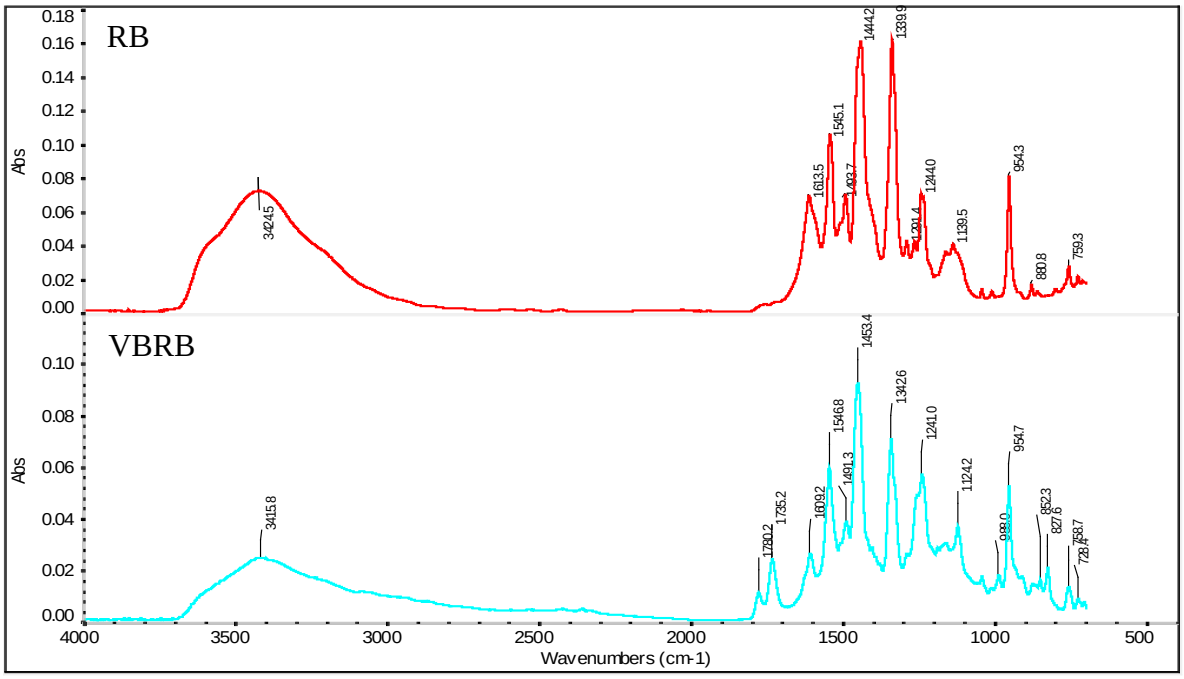



Figure 5.17. FTIR curve for Rose Bengal (upper curve) and VBRB (lower curve).

From Figure 5.17, it is clear that VBRB has a band at 1735.2 cm-1 and at 1780.2 cm-1, which indicate the presence of the unsaturated ester group and the γ-lactone group. These bands are not seen in Rose Bengal. There are several other peaks which have changed slightly from the absorbance of Rose Bengal to that of VBRB similar. γ-lactone group can be caused by the unreacted Rose Bengal or ether form VBRB. Therefore, we conclude that vinyl benzyl chloride has reacted with the –COOH on Rose Bengal, but we can't rule out the possibility that it has also reacted with the –OH group.

### 5.5.3 Percentage determination for both chemical structures

From the structure we can see the ester form should have no pH sensitivity because the carboxyl group is occupied by the benzyl group. Therefore we can take advantage of this

property and calculate the percentage of each configuration in our VBRB sample. Figure 5.18 is the Rose Bengal absorption curve in acid and neutral solution. Figure 5.19is the VBRB absorption curve in acid and neutral solution. The difference between the two curved is believed to be caused by the occupation of the carboxyl group on Rose Bengal in ester form. By a careful calculation, the percentage of ester and ether form in VBRB has been obtained and is listed in Table 5.12. The ratio of ester to ether form is 1/8, which means 12% (99.7%-87.4%=12.3%) is ester form and 88% (1-12%=88%) is in the ether form. In VBRB acid spectrum, we believe the absorbance for ester VBRB has the same wavelength as the VBRB in neutral solution based on Lamberts work.

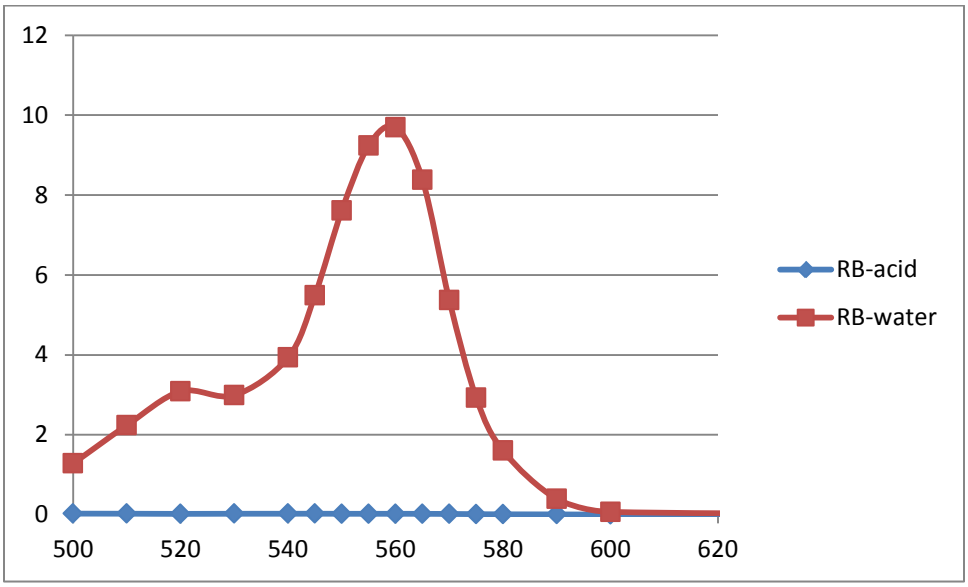



Figure 5.18 Absorption of RB solutions in acetone water solution with different pH.

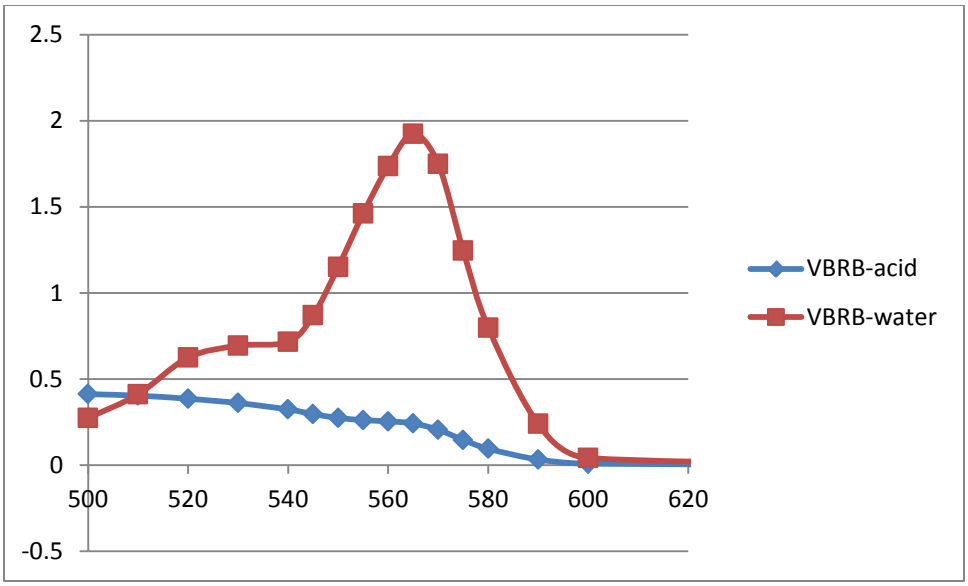



Figure 5.19. Absorption of VBRB solution in acetone water solution with different pHs.

However, we think the percentage of ester form in VBRB is less than 12% because of the absorbance in 500-500 range. If the absorbance spectrum had the same trend as the absorption of VBRB in neutral solution, shown in Figure 5.20, we could conclude that the percentage of ester form is 12%. However, we cannot positively conclude that ester group percentage is as high as 12% because of the difference in the shape of the absorbance curves.

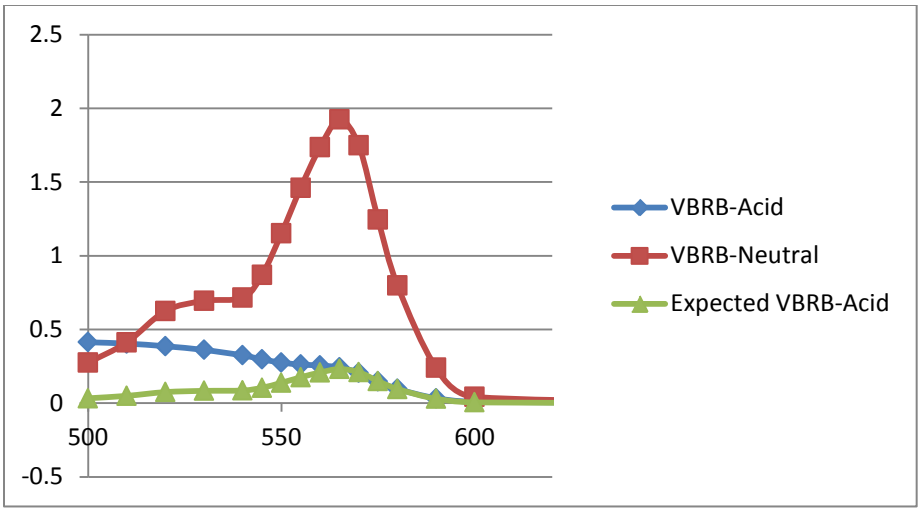



Figure 5.20. Expected absorbance spectrum for VBRB in acid solution

Table 5.12. Maximum absorption of RB and VBRB solution in acetone water solution with different pHs.

| *Samples* | $A_{555nm\text{-}H}$ | $A_{555nm\text{-}OH}$ | *Ratio of shifting* |
|---|---|---|---|
| RB A/W solution | 0.025 | 9.696 | 99.7% |
| VBRB A/W solution | 0.243 | 1.924 | 87.4% |

# 6. CONCLUSIONS

In this study, a free dye evaluation procedure was developed using dialysis membrane, freezer bag zipper and buffer solution at pH=7.4. This evaluation, we found Rose Bengal was nearly 100% attached on polymer for PBRB3 samples but not PBRB1 and PBRB2. We propose that the vinyl benzyl chloride reacted with water or –COOH from acrylic acid before it had a chance to react with Rose Bengal in procedures PBRB1 and PBRB2. Fortunately, VBRB can be synthesized first without affecting C=C groups for further polymerization. Then by copolymerizing VBRB with AA in an acetone-water solution, poly(AA-VBRB) can become water soluble. By extending the copolymerization to add SSA, we obtained a zero-free dye PBRB solution.

The solubility of PBRB copolymer can be adjusted further by increasing the mole ratio of AA and SSA. In this study, we achieved sufficient solubility to satisfy industry's needs. However, higher solubility is good for transportation and cost control in the supply chain to avoid the costly shipping of large amounts of water.

In order to prove Rose Bengal after attachment is still active in producing singlet oxygen, the singlet oxygen production rate was tested and compared with Rose Bengal solution. The efficiency of PBRB solution is higher than Rose Bengal solution. I think it is caused by the structure difference between Rose Bengal and VBRB.

The pH sensitivity of the absorbance of Rose Bengal has also been investigated in this study, which showed a absorption shift occurs in the 2.8-3.2 pH range. This is caused by a change in the structure of Rose Bengal at low acidic pH. Also, the colorless form of Rose Bengal agrees with pervious work done by Neckers. In addition, the solvent sensitivity of Rose

Bengal verified Neckers' work, which says the maximum absorbance wavelength of Rose Bengal changes with solvent.

The structure of VBRB was determined by FTIR and the pH response to acid by taking advantage of pH sensitivity of Rose Bengal. The major component (around 88%) in VBRB synthesized our lab is the ether form. Ether form still has pH sensitivity because of the unreacted –COOH, which is good for industry control. Because lactone-ether form VBRB has much less absorption than quinonoid-ether form under visible light range. We can use lactone-ether form to store PBRB if we won't use it right way, which is good for risk control. We can activate lactone form to quinonoid form by adding base because the reaction is reversible.

# 7. FURTHER STUDY

## 7.1 Performance of PBRB on fabrics

Our final application for PBRB is as a photoactive antimicrobial on fabrics. Thus the antimicrobial activity of the PBRB bonded to fabrics should be should be evaluated. There are many –COOH groups on PBRB polymer, which can used to react with –OH or $–NH_2$ fabric surfaces under appropriate conditions.

## 7.2 Further step to reduce acetone in polymerization

In PBRB3 samples, a small percent of acetone was applied in order to copolymerize AA and VBRB. However, acetone can bring extra environmental cost for industrial production. In order to reduce the amount of acetone, the combination of VBRB synthesis and copolymerization can be used.

## 7.3 Performance Improvement

One factor that can affect the antimicrobial properties is the polymer molecular weight. The higher molecular weight will offer more active –COOH sites to react with VBRB. So the ways that can increase molecular weight in copolymerization can be equally used to improve the antimicrobial ability, including decreasing the concentration of initiators and increasing the concentration of monomers, especially the percentage of VBRB.

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