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Polydiacetylenes for Colorimetric Sensing

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by

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DEDICATION

This dissertation is dedicated to my mom, Kelli, and my siblings, Kelsey, Jacob, and Kennedy. Thank you for never doubting me. I am profoundly grateful to have you in my corner.

ABSTRACT OF THE DISSERTATION

Polydiacetylenes for Colorimetric Sensing

by

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Doctor of Philosophy, Graduate Program in Bioengineering
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Dr. Hideaki Tsutsui, Chairperson

Point-of-care (POC) diagnostics have emerged as a critical tool in modern healthcare and agriculture, offering the potential to revolutionize disease detection and management, particularly in resource-limited settings. By enabling rapid, on-site testing without the need for specialized laboratory equipment, POC diagnostics can significantly improve patient outcomes, reduce healthcare costs, and enhance disease surveillance capabilities. Polydiacetylenes (PDAs) are a unique class of polymers with conjugated backbones that undergo a colorimetric blue-to-red transition in response to various stimuli, making them promising candidates for simple, visual detection systems.

This dissertation explores the development and optimization of PDA-based sensors for point-of-use diagnostic applications. A systematic investigation of liposome synthesis parameters is conducted, identifying optimal conditions for uniform and responsive PDA assemblies. The effects of diacetylene monomer structure and lipid doping on sensor performance are examined, revealing that shorter alkyl chains and moderate lipid incorporation enhance sensitivity while maintaining stability.

Efforts to develop antibody-functionalized PDA sensors for detecting plant pathogens and model proteins are described, highlighting both the potential and limitations of this approach. To overcome challenges encountered with antibody-based systems, an alternative strategy using small-molecule ligands for protein detection is explored. Throughout the work, emphasis is placed on creating robust, reproducible protocols suitable for point-of-use applications.

The insights gained from this research contribute to the broader understanding of PDA-based biosensors and provide a foundation for their future development as accessible diagnostic tools for healthcare and agricultural applications.

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CHAPTER 1:

INTRODUCTION

In recent years, the field of point-of-care (POC) diagnostics has gained significant attention and importance in both healthcare and agricultural sectors. These innovative diagnostic tools offer the potential to increase access to critical testing technologies that might otherwise be inaccessible or prohibitively expensive (Nayak et al., 2017). The growing interest in POC diagnostics stems from their ability to provide rapid, on-site results, thereby streamlining diagnostic procedures and enhancing the efficiency of healthcare delivery.

The ideal POC diagnostic tool should possess several key characteristics: sensitivity, accuracy, specificity, robustness, user-friendliness, and cost-effectiveness (Heidt et al., 2020). Traditional diagnostic methods, while extensively used, often fall short in one or more of these areas. They tend to be slow, expensive, and require trained professionals to operate – limitations that pose significant challenges, especially in developing countries. These shortcomings have driven the search for alternative diagnostic approaches that can overcome these barriers and provide accessible, reliable testing in diverse settings.

The development of effective POC devices has been a complex challenge, requiring a delicate balance between simplicity, accuracy, and cost-effectiveness. The emergence of nanotechnology has made these tests feasible and allowed for complex detection methods that remain extremely user-friendly (Thwala et al., 2023). By leveraging nanoscale material, researchers have been able to create diagnostic platforms that are both highly sensitive and easy to use, even in resource-limited environments.

One promising material in the realm of nanotechnology-enabled POC diagnostics is polydiacetylene (PDA). PDAs are a class of conjugated polymers that possess unique properties making them excellent candidates for use in diagnostic applications. Their most notable feature is their chromatic response to various stimuli, which allows for the development of colorimetric sensors that can detect biological and chemical analytes with high sensitivity and specificity.

This dissertation focuses on the application of PDAs in the development of novel POC diagnostic devices. The research presented here aims to explore and expand the potential of PDA-based sensors in creating lower-cost, easy-to-use diagnostic tools that can address the limitations of traditional diagnostic methods.

Chapter 2 provides a comprehensive literature review on the structure of PDA and its current applications in diagnostics. This chapter lays the foundation for understanding the unique properties of PDAs that make them suitable for POC diagnostic applications. Chapter 3 delves into the development and characterization of antibody-functionalized PDA sensors. This approach combines the specificity of antibody-antigen interactions with the sensitive chromatic response of PDAs, potentially enabling highly specific and easily readable diagnostic tests. Chapter 4 explores the integration of PDA sensors with polyvinylidene fluoride (PVDF) membranes. This combination aims to enhance the stability and usability of PDA-based sensors, potentially leading to more robust POC devices suitable for use in diverse environmental conditions. Chapter 5 investigates the development of carbonic anhydrase inhibitor-functionalized PDA sensors. This novel approach could open up new avenues for detecting specific enzymatic activities or

inhibitors, broadening the range of conditions that can be diagnosed using PDA-based POC devices. The final chapter presents a policy analysis of POC diagnostics, examining the broader implications of these technologies on healthcare systems, regulatory frameworks, and global health initiatives. This analysis aims to contextualize the technical developments presented in the earlier chapters within the larger landscape of healthcare policy and practice.

By exploring these various aspects of PDA-based POC diagnostics, this dissertation seeks to contribute to the ongoing efforts to create more accessible, efficient, and effective diagnostic tools. The potential impact of such innovations extends beyond mere technological advancement, holding promise for improving healthcare and agricultural outcomes, particularly in resource-limited settings where traditional diagnostic methods are often unavailable or impractical.

CHAPTER 2:

POLYDIACETYLENE MATERIALS: STRUCTURE, SYNTHESIS, AND SENSING MATERIALS

2.1 INTRODUCTION

Polydiacetylenes (PDAs) are a class of conjugated polymers that have captured the attention of researchers since their discovery by Gerhard Wegner in 1969 (Wegner, 1969). PDAs are characterized by their distinctive optical properties, most notably their ability to undergo a chromatic transition, from blue to red, in response to various external stimuli. PDAs exhibit two primary optical phases: a blue phase corresponding to the polymerized state, and a red phase associated with the perturbed state, forming the basis for their application in various sensing technologies. This color change is visible to the naked eye, making PDAs excellent candidates for simple, visual detection systems.

PDAs use for detection of an analyte was first reported in 1993 when Charych et al. demonstrated their use for ligand-receptor binding detection (Charych et al., 1993). In their groundbreaking study, they showed the detection of influenza virus using sialic acid-modified PDA headgroups deposited onto Langmuir-Blodgett films. This work opened a new avenue for biosensing applications using PDA materials.

The versatility and responsiveness of PDAs have led to their exploration in numerous fields, including biosensors, chemosensors, and smart materials. Their ability to self-assemble into various structures and their ease of functionalization further expand their potential applications. In this chapter, we review the structure, synthesis, and sensing applications of PDA materials, focusing on their unique optical properties and versatility in various detection systems.

2.2 STRUCTURE

Diacetylene monomers for self-assembled materials are composed of two parts: a polar headgroup and a hydrophobic tail. The hydrophobic tail consists of three components: a fatty acid tail, a polymerizable, diacetylene moiety, and an alkyl spacer. Each of these components plays a crucial role in determining the self-assembly behavior and the properties of the resulting polymer.

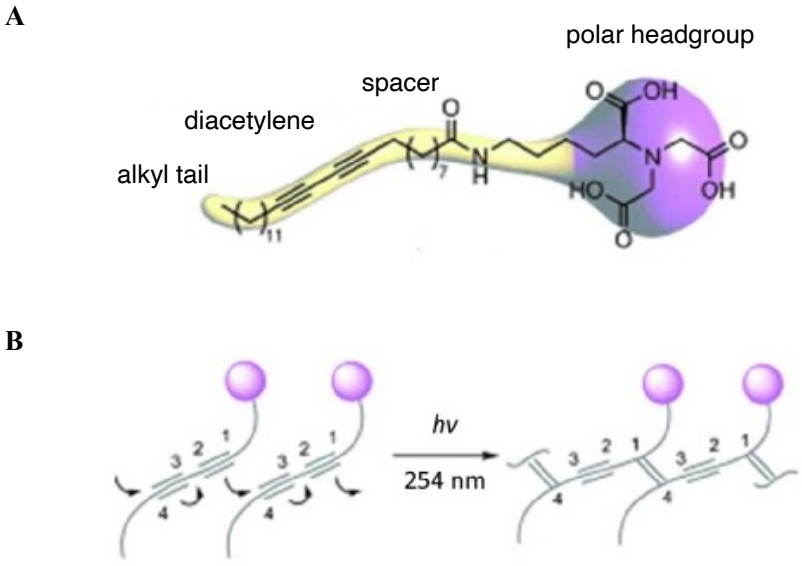

Figure 2.1. Structure of Diacetylene Monomers (A) and Polymerization (B). Reprinted with permission from (Jelinek & Ritenberg, 2013)

2.2.1 Polar Headgroup

The polar headgroup affects several important factors, including packing, inter- and intramolecular interactions, and is the often the site for functionalization. The modification of the head group can significantly affect the strength of interactions at the PDA assembly surface, influencing factors such as color transition temperature, response to external stimuli like pH and solvents, and the overall stability of the PDA structure. For example,

changing the carboxylic head group to an amine and amide group combination, resulted in unique behaviors such as multi-step color transitions in response to pH changes and a higher resistance to ethanol-induced color changes, demonstrating how head group architecture can be tailored to achieve specific sensing capabilities in PDA-based materials (Charoenthai et al., 2011).

2.2.2 Packing

Packing in PDA assemblies plays a crucial role in determining their structure and properties (Khanantong et al., 2019). The arrangement of diacetylene monomers, influenced by factors such as head group size and structure, affects the morphology of the assemblies, ranging from spherical vesicles to rod-like structures. Molecular packing, characterized by parameters like tilting angle and d-spacing, directly impacts the strength of inter- and intramolecular interactions within the PDAs. These packing-induced interactions, in turn, govern key properties of PDAs, including their thermochromic behavior, color transition reversibility, and sensitivity to various chemical stimuli.

2.2.3 Hydrogen Bonding

The headgroup's ability to form hydrogen bonds impacts the chromatic properties and stability of PDA materials. Stronger hydrogen bonding in the headgroups can increase stability against chromatic changes caused by temperature and UV light. Changes in hydrogen bonding within headgroups, such as those induced by pH changes, can also trigger chromatic shifts. Hydrogen bonding between head groups contributes to the overall

inter- and intramolecular interactions within the PDA structures. For instance, the incorporation of aromatic head groups like benzoic acid or naphthoic acid enhances hydrogen bonding, leading to reversible thermochromism and higher color transition temperatures. The architecture of the head group, such as in the case of AEPCDA with amine and amide groups separated by an ethyl spacer, can create multiple hydrogen bonding sites, increasing molecular rigidity and affecting the assembly's response to stimuli. Stronger hydrogen bonding generally results in more stable assemblies that require higher energy input (e.g., temperature) to induce color transitions. Additionally, hydrogen bonding influences the packing of PDA molecules, impacting properties such as melting behavior, phase transitions, and the formation of liquid crystalline phases. The strength and nature of hydrogen bonding also affect how PDA assemblies interact with external stimuli like solvents and pH changes, sometimes leading to unique behaviors such as multi-step color transitions or increased resistance to certain perturbations. Overall, the head group's ability to form hydrogen bonds is a key factor in determining the structural integrity, responsiveness, and functional properties of PDA assemblies.

2.2.4. Functionalization

Functionalization of the head group allows for the incorporation of specific recognition elements, enabling PDAs to detect a wide range of analytes including enzymes, proteins, DNA, microorganisms, and small molecules. For example, researchers have functionalized PDA head groups with antibodies, peptides, nucleic acids, and various chemical moieties to create sensors with high specificity for target molecules. The head

group can also be modified to alter the PDA's sensitivity to environmental stimuli such as pH, temperature, or mechanical stress. For instance, incorporating urea into the head group has been shown to increase thermal stability, while ester modifications can lower the color transition temperature. The versatility of head group functionalization makes PDAs highly adaptable for various sensing applications. Though, it should be noted, that the lipid constituent is sometimes functionalized instead of the headgroup (Kolusheva et al., 2006)

2.2.5 Spacer

The spacer, which is the distance between the headgroup and the diacetylene moiety, has multiple effects The length and nature of this spacer significantly affect the packing, stability, and sensing characteristics of PDAs. Studies have shown that altering the length of this alkyl spacer can have a profound impact on the thermochromic behavior and sensitivity of PDA sensors. For instance, shorter spacers (e.g., 5,7-derivatives) generally lead to higher sensitivity compared to longer spacers (e.g., 10,12-derivatives), as they allow for a stronger association between the recognition element in the head group and the diacetylene transducer. However, the effect can be complex, with some studies reporting that very short spacers can increase the color transition temperature due to restricted head group dynamics. The spacer also influences the molecular tilting angle within the PDA assembly, affecting the overall packing structure. Too short of a spacer may, therefore, lead to unstable PDA assemblies. Furthermore, the spacer length can impact the efficiency of topochemical polymerization and the stability of the resulting PDA. In some cases, the effect of the spacer can be dominant over the total chain length in

determining PDA properties. Understanding and manipulating this spacer region is therefore critical for optimizing PDA assemblies for specific sensing applications, allowing researchers to fine-tune the balance between sensitivity, stability, and specificity in PDA-based sensors.

2.2.6 Alkyl Tail

The alkyl tail is important in determining self-assembly and stability of PDA assemblies. The number and length of the tails largely control the range of conditions under which an amphiphile will undergo self-assembly. Different tail structures can lead to the formation of various self-assembled structures such as vesicles, tubules, or sheets. Longer alkyl tails generally increase the stability of the self-assembled structures, so may be preferred in applications such as vesicles.

2.2.7 Crystal Structure

Chen and colleagues used a new development of hyperspectral microscopy, which provides spatial resolution at the sub-crystal level combined with spectral information, to study the crystal structure of PDA (J. Chen et al., 2022). PDA samples, both in blue and red forms, contain diverse microstructures even within single crystals, with at least 10 different structural variants identified in each form. The blue-to-red transition, induced by heat in this study, follows specific patterns where certain blue structures preferentially transform into particular red structures. This structural diversity and the observed transition patterns offer new insights into the complex nature of PDA, potentially explaining issues

with reproducibility in PDA-based sensing applications. While the exact molecular structures remain unknown, differences likely stem from variations in monomer distance, carbon chain angles, or lipid phase architectures. While this is the first and only study imaging a single PDA crystal, the outcome compliments past studies using Raman spectroscopy, infrared spectroscopy (IR), X-ray diffraction, and nuclear magnetic resonance (NMR)

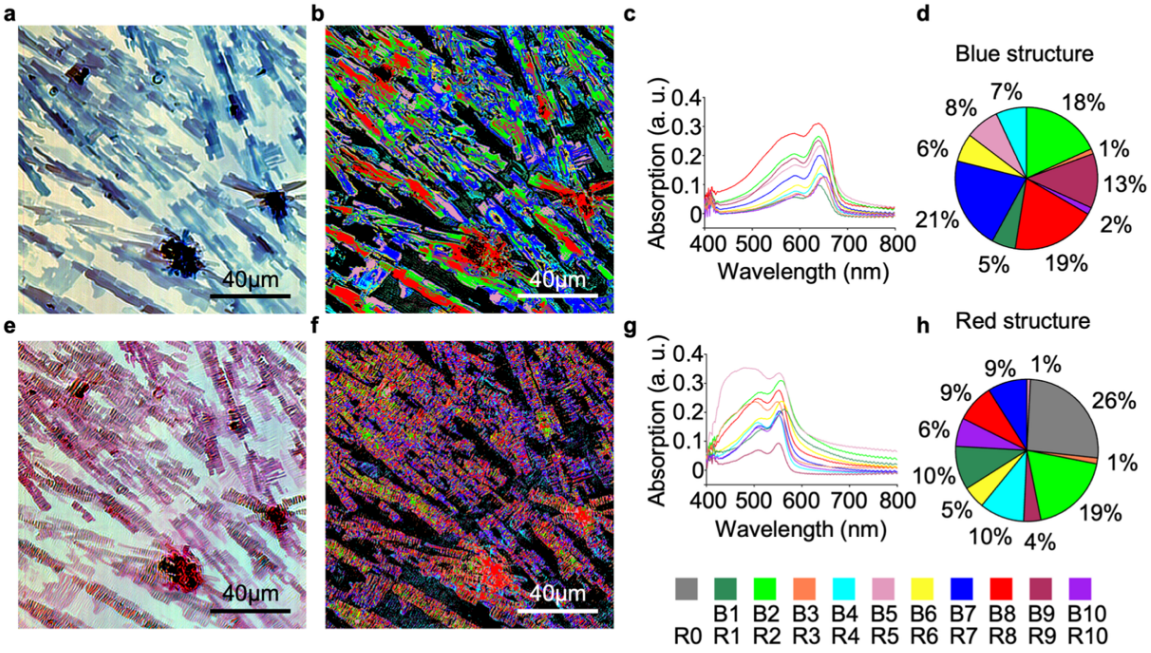

Figure 2.2. Crystal Structure of Polydiacetylene in Blue and Red Phases. Reprinted from (J. Chen et al., 2022)

2.2.8 Characterization

Several methods are commonly used to visualize and measure PDA materials. Dynamic light scattering (DLS) is frequently employed to determine the size of synthesized liposomes, which can vary with preparation methods such as sonication time. To observe

the surface and shapes of PDA materials, transmission electron microscopy (TEM) and scanning electron microscopy (SEM) are prevalent, with TEM often preferred for higher resolution imaging of nanoscale liposomes. However, the conditions for TEM and SEM may affect liposome structure. Atomic force microscopy (AFM) is used to characterize PDA liposome coatings on substrates, though it can cause color changes due to its destructive nature.

To examine the molecular structure of PDA materials, 1H nuclear magnetic resonance (NMR) is widely used to verify the synthesis of diacetylene (DA) conjugates by comparing peaks in their spectra. Fourier transform infrared spectroscopy (FTIR) is another approach used to confirm the presence of specific compounds and investigate changes in hydrogen bonding between PDA head groups, which is believed to be key to chromatic transitions in PDA materials.

2.3 SYNTHESIS

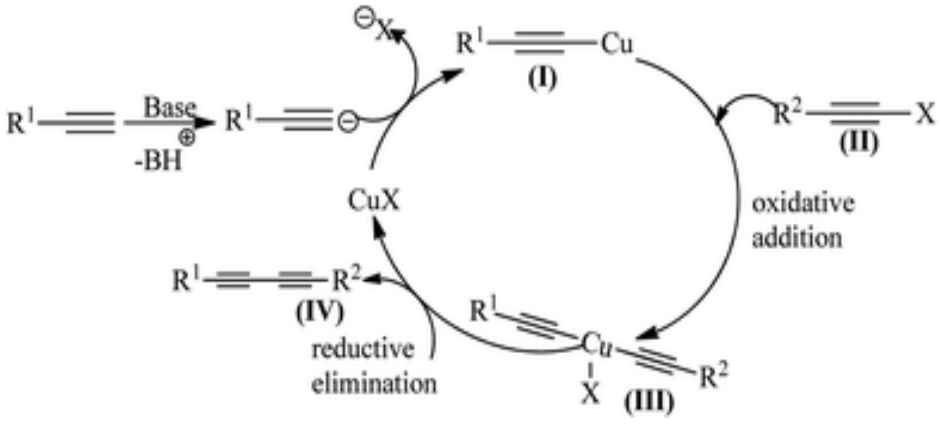

Figure 2.3. Synthesis of DA monomers via Cadiot-Chodkiewicz reaction. Reprinted from (Sindhu et al., 2015)

Diacetylene monomers, in the form of $R_1–C≡C–C≡C–R_2$, are typically prepared using the Cadiot-Chodkiewicz reaction. This reaction involves the Cu(I) catalyzed coupling of an acetylene and a halo-acetylene. The synthesis allows for the creation of a wide variety of diacetylene monomers with different structural features, enabling the tailoring of PDAs for specific applications (Radhika et al., 2019; Reppy & Pindzola, 2007; Sindhu et al., 2015). A simpler way to obtain desired DA monomers is to chemically modify the headgroups of commercially available DA. 10,12-Pentacosadiynoic acid (PCDA) and 10,12-tricosadiynoic acid (TCDA) are the two most common commercially available diacetylenes with a carboxylic acid headgroup. This modification process is straightforward due to the high reactivity of the carboxylic acid group, allowing for the development of more complex, robust, and sensitive polydiacetylene (PDA) systems.

Substituents containing carboxylic acid, amides, or sulfonic acid esters groups are particularly useful to design hydrophilic head groups of DAs that can bind to a molecular probe. Not only do they increase hydrogen bonding, which is beneficial to the self-assembly and polymerization of PDA assemblies, but they also allow for functionalization of the head group for target detection. Larger side groups are not conducive to the polymerization of DAs, as it can inhibit proper packing and, therefore, polymerization. Figure 2.4 illustrates several DA monomers and the effect of monomer structure on colorimetric shift in response to heat.

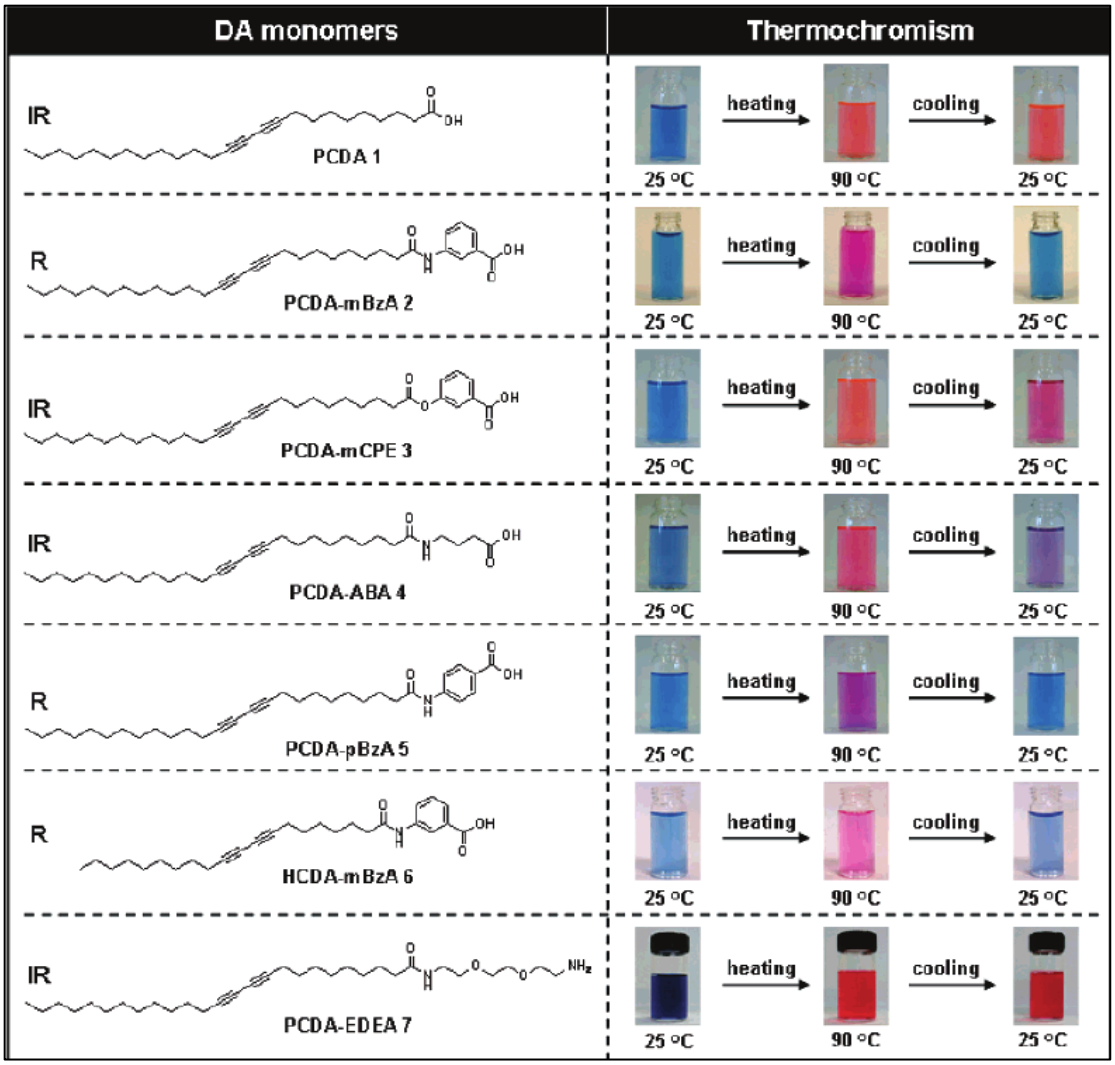

Figure 2.4 DA Monomer Structures and the Effect on Thermochromism in PDA Liposomes. Reprinted from (Zunino III et al., 2008)

2.4 POLYMERIZATION

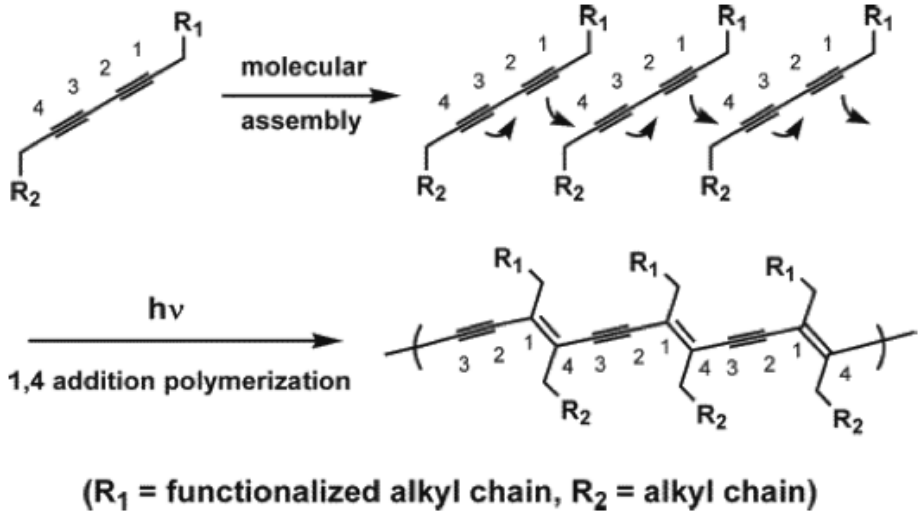

Figure 2.5 PDA Topochemical Polymerization Initiated by UV Irradiation. Reprinted from (Park et al., 2007)

The polymerization of diacetylene monomers to form polydiacetylenes occurs by 1,4-topochemical polymerization. This process requires DA monomers be self-assembled to meet specific geometrical parameters, this optimal packing orientation of DA is required to promote propagation of the liner chain through the ordered phase. The polymerization process follows a free radical mechanism and is typically initiated by UV irradiation, although other energy sources such as γ-rays (Chance & Patel, 1978) and visible light (Shusterman et al., 2009) have been shown to initiate polymerization. The absorbed energy breaks the π-bonds in the diacetylene moiety, creating a radical initiator with 8 unpaired electrons. This initiator propagates along the diacetylene backbone, causing neighboring monomers to become radicals and shift slightly, bringing carbon atoms close enough to form bonds (Figure 2.5). The polymerization terminates when the growing chain

encounters a defect or reaches an energetically stable condition. The resulting polymer backbone consists of alternating single and triple bonds (σ and π bonds).

Polymerization of DA monomers stabilizes the physical structure and increases the stability of the assembly. Polymerization results in an assembly with a conjugated backbone that is key to the unique chromic properties of PDAs. Since chemical catalysts or initiators are not required, allowing for high purity synthesis with no byproducts, which is significant when considering PDA assemblies for use as sensors.

2.5 SYNTHESIS AND PREPARATION METHODS OF PDA-BASED MATERIALS

PDAs can be synthesized and prepared in various forms, each offering unique properties and potential applications. The structure of PDA assemblies can be tailored by modifying the molecular structure of the diacetylene monomer and employing different fabrication methods. Common preparation techniques include the creation of films through Langmuir-Blodgett (LB) (Lio, Reichert et al. 1997; Tachibana, Yamanaka et al. 1999; Finney, Parikh et al. 2021), spin-coating (Ahn, Chae et al. 2003; Lu, Yang et al. 2001; (Chanakul et al., 2013), and drop-casting methods, each offering varying degrees of control over film thickness and molecular orientation. Under suitable conditions, diacetylene monomers can also be grown into crystals, typically forming needle-like or plate-like structures (Lauher, Fowler et al. 2008; Jo, Yoshikawa et al. 2005; Li and Stupp 1997). PDA tubules, cylindrical nanostructures formed through self-assembly, show promise in applications such as drug delivery and nanoscale electronics (Heo et al., 2019; Xu et al.,

2014). For aqueous applications, PDAs can be prepared as liposomes, providing a versatile platform for drug delivery and biosensing in liquid environments. Furthermore, PDAs can be incorporated into various matrices, including hydrogels (W. Chen et al., 2022; Rao et al., 2020), membranes (Sabatani et al., 2008; Volinsky et al., 2007), and nanowires (Gan et al., 2005; Zhou et al., 2007), to create composite materials with enhanced functionalities.

2.6 COLORIMETRIC SENSING

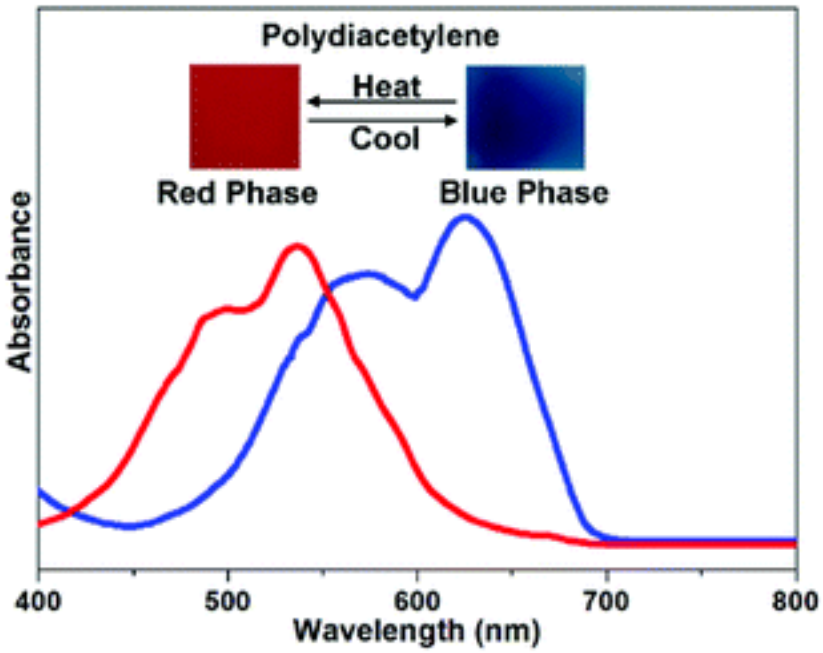

Figure 2.6 Absorbance of Blue Phase and Red Phase PDA. Reprinted From (Huo et al., 2017)

PDA materials unique optical properties make them excellent candidates for various colorimetric sensor applications. The colorimetric changes in PDAs are often visible to the naked eye, allowing for easy, equipment-free detection. These changes can also be monitored more precisely using visible spectroscopy (Figure 2.6). PDAs typically

have two distinct optical phases. The red phase corresponds to the perturbed state, with an absorbance maximum around 550 nm. The blue phase corresponds to the polymerized state, with an absorbance maximum around 650 nm. Though more stable, blue form PDA with longer effective conjugation lengths may absorb at higher wavelengths (717 nm – 848 nm), and (Kuriyama et al., 1998) (S. J. Kew & E. A. Hall, 2006b) (Wang & Hollingsworth, 1999). Shoulder peaks 500 nm and 600 nm are associated with the red and purple phase (Deckert et al., 1994). In addition to these primary phases, PDAs can also exhibit distinct purple (Deckert et al., 1994), green (Mergu et al., 2019), and yellow phases (Yoo et al., 2018), depending on the specific polymer structure and method of synthesis.

The colorimetric transitions in PDAs can be triggered by various stimuli, including: pH changes (Chanakul et al., 2014; Jannah & Kim, 2019; S. J. Kew & E. A. Hall, 2006a; Yapor et al., 2017), increased temperature (Wacharasindhu et al., 2010, Chanakul, 2013 #104, Chanakul, 2013 #104) , ionic stress (Yang et al., 2020), mechanical stress (Das et al., 2022), and in response to molecular binding events (Jannah et al., 2023) (Yang et al., 2020) (Zhou et al., 2020).

2.6.1 Mechanism of Color Change

Though the mechanism is not fully elucidated, it is suspected that PDA color change is caused by C-C bonds in the conjugated polymer backbone, which alters the π-orbital overlap and causes a significant shift in the absorption spectrum (Carpick et al., 2004). This rotation is coupled with conformational changes in the pendant side-chains. Initially, in the blue phase, the backbone is in a strained configuration due to the packing arrangement of

the side-groups. When exposed to stimuli such as heat, mechanical stress, or chemical changes, the side-chains undergo fluctuations or reconfigurations. This allows the backbone to adopt a more relaxed conformation, involving rotation about the C-C backbone bonds. Theoretical models predict that even minor rotations (just a few degrees) around C–C bonds can substantially alter π-orbital overlap, leading to significant changes in a molecule's electronic state and consequently its absorption spectrum . The relaxation of strain in the backbone, along with the associated side-chain reorganization, leads to a reduction in the effective π-conjugation length. This structural change results in a shift of the absorption spectrum, transforming the material from the blue form (640 nm) to the red form (540 nm).

2.6.2 Quantifying Color Change

The standard method to quantify the blue to red color transition is by calculating the colorimetric response (CR), first developed by (Okada et al., 1998). The CR is based on the peak absorbance of PDA when in its blue form (~640 nm) and red form (~540 nm); though the exact wavelengths of the peak absorbance varies depending on the specific PDA polymer used. It is a measure of the percentage of blue versus red PDA present in the sample. To calculate the CR, you first calculate the percent blue, PB

, given by *equation 1*:

(equation 1) $$PB = \frac{A_{blue}}{A_{blue}+A_{red}} \times 100\%$$

where the A_{blue} is the peak absorbance value at the wavelength where the absorbance maxima occurs for blue phase PDA. Likewise, A_{red} is the equivalent peak absorbance value for red phase PDA. Using the PB, the CR is calculated using *equation 2*:

(equation 2) $$CR = \frac{(PB_0 - PB_f)}{PB_0} \times 100\%$$

where PB_0 is the percent blue of a baseline, i.e. the PB_0 of a negative control, and PB_f is the percent blue of a sample. This approach is robust in that is normalizes all factors other than the color change due to PDA shifting from blue to red. According to past literature, the minimum colorimetric response discernable by naked eye is around 10%, where a blue to purple transition occurs (Su et al., 2004).

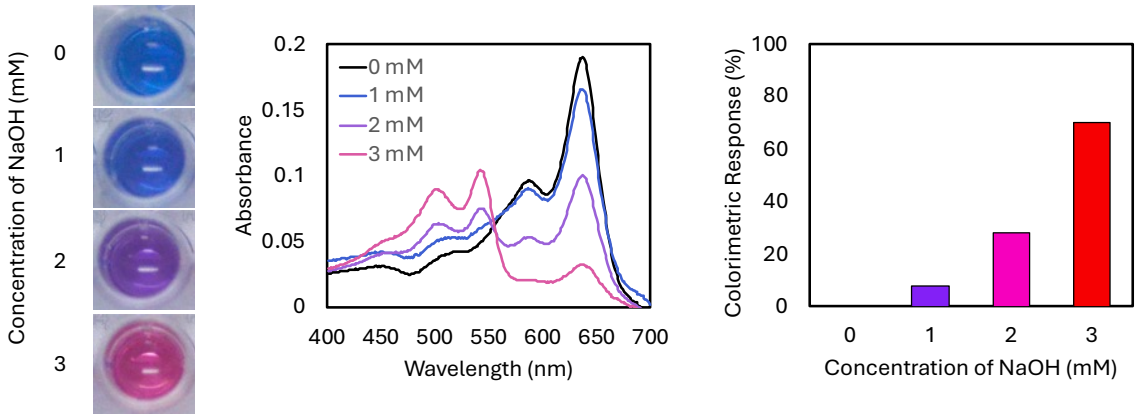

Figure 2.7 Colorimetric Response of PDA Liposomes Exposed to NaOH

2.6.3 Colorimetric Sensing in Literature

These color changing properties make PDAs suitable for a wide range of sensing applications. Colorimetric sensing for food safety applications, pathogen sensing, detecting volatile organic compounds (VOCs), and biomolecule detection.

One example used PDA assemblies for food safety applications. Weston et al. constructed a system using 5,7-hexadecadiynoic acid (HDDA) and ZnO functionalized PDA can discriminate between fresh (pH 6.8–6.0), spoiling (pH 6.0–4.5), and spoiled milk (pH 4.5–4.0) by a respective blue to purple to red color change (Weston, Kuchel, et al., 2020).

Another group, Zhou and colleagues, developed a colorimetric paper strip sensor using polydiacetylene (PDA) conjugated with aptamers specific to *Bacillus thuringiensis* spores, which changes color from blue to red in the presence of the target spores, allowing visual detection without specialized equipment (Zhou et al., 2020).

A final example, Jannah et al demonstrated diaminotriazine-functionalized polydiacetylene (PDA-DAT) sensor that can selectively detect thymine and oligothymidine through hydrogen bonding interactions, resulting in a visible blue-to-purple color change. This simple colorimetric method for sensitively detecting thymine-containing DNA has potential applications in genetic analysis and disease diagnostics (Jannah et al., 2023).

2.7 FLUORESCENCE-BASED DETECTION

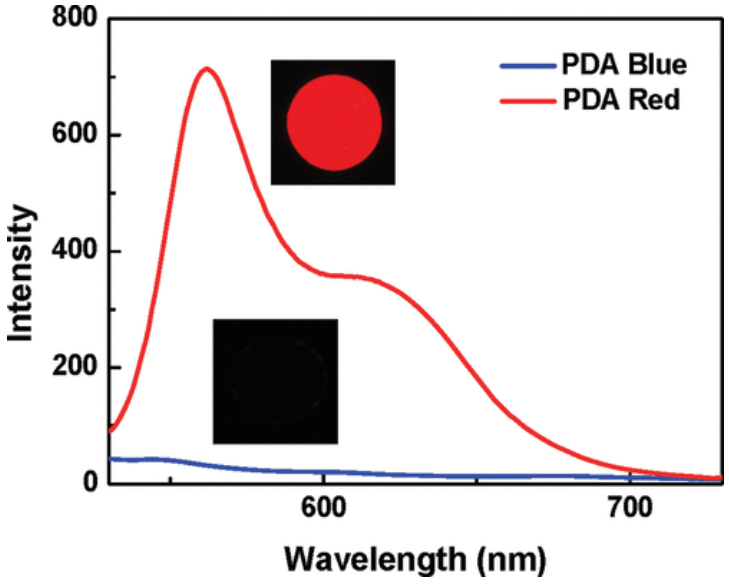

Figure 2.8 Fluorescence emission spectra of red form and blue form PDA Reprinted (adapted) with permission from (Ahn & Kim, 2008). Copyright 2008 American Chemical Society

Polydiacetylene (PDA) systems exhibit unique fluorescence properties that complement their well-known colorimetric responses. Initially discovered by Baughman and Chance in 1976, PDAs undergo a turn-on in fluorescence signal upon stimulation, transitioning from a non-fluorescent "blue phase" to a fluorescent "red phase," which has two emission peaks at 560 nm and 635 nm (Figure 2.7) (Baughman & Chance, 1976). This transition can be triggered by various stimuli such as heat, pH changes, mechanical stress, or specific molecular interactions. PDA emission can be excited either directly or through energy transfer from other emissive species. While the quantum yield of PDAs is generally low (negligible for the blue phase and 1-3% for the red phase), this fluorescent property can still be leveraged for sensing applications, especially when enhanced through strategic design.

Research by Reppy and colleagues in 2008 provided deeper insights into the fluorescence characteristics of PDA systems, particularly focusing on 10,12-pentacosadiynoic acid (PCDA) liposomes. They found that the polymerization of PCDA liposomes affects the ratio of two emission peaks (560 nm and 635 nm), with low UV doses leading to a dominant 560 nm peak and higher UV exposure increasing the 635 nm peak. Importantly, they observed that emission changes in PDA liposomes can be much greater than absorbance changes. For instance, when exposed to a base, the emission change at 640 nm was 70 times greater than the absorbance change. This significant difference between emission and absorbance changes highlights the potential advantage of using PDA fluorescence properties for signal generation, especially in cases where the blue to red color change is incomplete. To further enhance the fluorescent signal, researchers have successfully integrated additional fluorophores into PDA systems, often utilizing Fluorescence Resonance Energy Transfer (FRET) to amplify the emission signal. This combination of colorimetric and enhanced fluorescence responses makes PDA systems versatile and potentially highly sensitive platforms for various sensing applications.

2.7.1 Mechanism of Fluorescence Turn-On

The suspected mechanism of fluorescence in PDA systems is closely linked to conformational changes in the polymer backbone. When stimuli induces the blue-to-red phase transition, it's believed to cause a partial twist in the backbone, reducing the effective conjugation length. This structural change likely relaxes some of the constraints on electron delocalization, allowing for increased radiative decay pathways. The resulting red-phase

PDAs have a more localized exciton state, which is thought to be responsible for the observed increase in fluorescence quantum yield. However, the exact molecular details of this process, including potential intermediate states, are still not fully understood.

These sensing mechanisms make PDAs versatile materials for a wide range of diagnostic and analytical applications, from simple visual indicators to more complex quantitative sensing systems. For example, Shin et al. demonstrated the efficacy of imidazole-functionalized PDA in detecting iron ions, illustrating its potential in metal ion sensing (Shin et al., 2022). Beyond analytical applications, fluorescent PDAs have shown promise in bioimaging (Huang et al., 2020). Wang et al. developed aptamer-functionalized PDA liposomes specifically designed for imaging cancer cells, showcasing the potential of PDAs in targeted diagnostic applications (Wang et al., 2020). These examples underscore the versatility of PDA-based systems and their potential to advance both analytical chemistry and biomedical imaging technologies.

2.8 ELECTROCHEMICAL DETECTION

PDA generally has low conductivity and a rigid crystal microstructure, making it unsuitable for electrochemical applications (Baughman & Chance, 1978; Takami et al., 2004). However, it has been demonstrated that in certain structures, PDA assemblies can have increased conductivity (Marikhin et al., 1997). This increase in conductivity is likely due to the nanostructure having a high surface to volume ratio, which increases doping efficiency. More commonly, increased conductivity is achieved by integrating PDAs with a conductive material to form a nanocomposite.

One example of electrochemical detection, Rao embedded various PDAs in polyvinylpyrrolidone (PVP) films for capacitive vapor sensing, demonstrating that different PDA/PVP combinations undergo distinct, reversible capacitance changes when exposed to various vapors, allowing for selective vapor detection through an "artificial nose" array-based approach (Rao et al., 2019).

2.9 FUNCTIONALIZATION STRATEGIES FOR PDA SENSORS

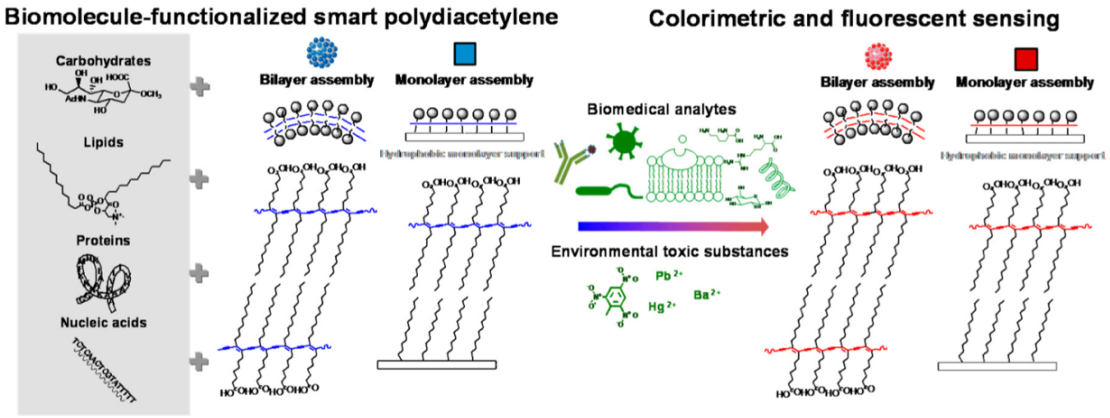

Figure 2.9 Various Molecular Probes for Biomedical and Environmental Sensing

PDA undergoes colorimetric shift and fluorescent turn-on in response to various environmental stimuli, as highlighted in Section 2.6. PDA can be functionalized such that that the colorimetric shift and fluorescent turn on occurs in response to a molecular binding event. The first demonstration by Charych and colleagues developed a method to detect influenza virus by functionalizing DA monomers with sialic acid with a triethylene glycol linker and using the functionalized monomers to create liposomes and LB-films capable of detecting influenza virus (Charych et al., 1993). Since this initial study, PDA has been

functionalized using various carbohydrates, proteins, and lipids (Cho & Jung, 2018), this section will cover common methods of functionalization.

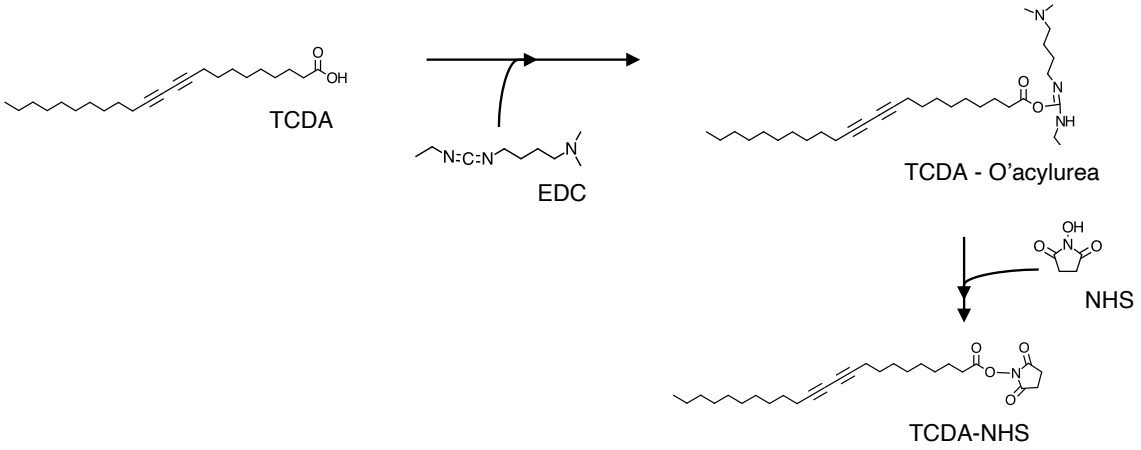

Figure 2.10 EDC-NHS Activation of a Carboxylic Acid to Crosslink to Amine Group.

Most often, detection probes are covalently attached using established EDC-NHS chemistry (Fischer, 2010). EDC-NHS chemistry is a widely used method for covalently coupling carboxylic acids to primary amines. In this reaction, EDC (1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide) activates carboxylic acid groups, forming an unstable O-acylisourea intermediate. NHS (N-Hydroxysuccinimide) then reacts with this intermediate to form a semi-stable NHS ester, which can efficiently react with primary amines to form stable amide bonds, making it ideal for conjugating proteins or other amine-containing molecules to carboxyl-containing surfaces or molecules.

EDC-NHS activates carboxyl groups on the PDA headgroups, allowing for covalent attachment of amine groups on molecular probes (i.e. antibodies) to the PDA headgroups (Figure 2.9). This conjugation step is often followed by blocking remaining

NHS-activated esters using ethanolamine or bovine serum albumin to prevent non-specific interactions.

2.9.1 Antibody Functionalization

Antibodies are biological molecules that exhibit high specificity and strong binding affinity for their target antigens, making them excellent recognition elements for biosensors. Research groups have demonstrated that antibodies can be covalently attached to PDA headgroups using EDC-NHS chemistry, and PDA liposomes or lipid bilayers will undergo a color change when exposed to the target antigen (de Oliveira et al., 2015; Jeong et al., 2017; Jeong et al., 2018; Jiang et al., 2015; Kim & Lee, 2019).

Though antibodies are highly specific, there are some disadvantages to antibody-based sensing. Antibodies must be correctly oriented to bind to their target. EDC-NHS chemistry will result in nonspecific binding to any of the numerous amine groups on the antibody. Some groups have demonstrated antibody conjugation that allows for orientation-specific binding by reacting thiol groups of reduced antibodies to maleimide-functionalized PDA headgroups and by using biotin-tagged antibodies for biotin-streptavidin binding (Pindzola et al., 2006).

2.9.2 Aptamer Functionalization

Aptamers are short single-stranded DNA or RNA oligonucleotides that can bind to specific target molecules with high affinity and selectivity. Aptamers can be used as recognition elements in biosensors due to their ability to fold into unique three-dimensional

structures that enable specific binding to various targets including proteins, small molecules, and whole cells. Aptamer-PDA sensors have been developed in various formats, including vesicles, films, and arrays, for the detection of various analytes including proteins, bacteria, and small molecules, offering versatility in sensor design and application (Jung et al., 2010; Kim et al., 2011; Wen et al., 2016; Zhong et al., 2024). Since aptamer's are easily modified, there are several routes for attaching aptamers to PDA headgroups. Common methods are similar to antibody functionalization, with many groups utilizing EDC-NHS chemistry, thiol-maleimide chemistry, and biotin-streptavidin.

2.9.3 Carbohydrate and Small Molecule Functionalization

PDA can be functionalized with various biomolecules besides antibodies and aptamers to create biosensors. Carbohydrates have been widely used, with examples including sialic acid for influenza detection, mannose for E. coli recognition, and oligosaccharides for small molecule sensing. Researchers have also incorporated glycolipids like GM1 and GT1b gangliosides into PDA assemblies to detect bacterial toxins. These carbohydrate-functionalized PDAs often mimic cell membrane interactions.

Other biomolecules used to functionalize PDA include lipids, proteins, peptides, and small molecules. Various proteins and peptides have been conjugated to PDA for specific sensing applications, such as hexokinase for glucose detection or designed peptides for TNT explosive sensing. Small molecules like biotin, folic acid, and dopamine have also been used to functionalize PDA for diverse applications including cancer cell targeting, heavy metal detection, and immobilization strategies. These various

functionalization strategies expand the range of PDA sensor applications (Cho & Jung, 2018).

2.10 APPLICATIONS OF PDAS IN POINT-OF-CARE DIAGNOSTICS

Developing point-of-care diagnostics is crucial for improving global health outcomes by enabling rapid, on-site disease detection and monitoring, particularly in resource-limited settings. These accessible diagnostic tools can lead to earlier interventions, better disease management, and reduced healthcare costs, ultimately improving patient outcomes and helping to contain the spread of infectious diseases. PDA sensors are excellent candidates for point-of-use diagnostics due to several key characteristics. PDA sensors offer a unique combination of simplicity, sensitivity, and versatility that makes them well-suited for rapid, on-site detection of various analytes. Their most striking feature is the colorimetric response - a visible blue-to-red color change that can often be detected by the naked eye, eliminating the need for complex instrumentation. This property allows for quick, qualitative assessments in resource-limited settings. Additionally, PDA sensors can be easily functionalized with a wide range of biomolecules, including aptamers, antibodies, and carbohydrates, enabling highly specific detection of diverse targets such as pathogens, toxins, and biomarkers. The self-assembly nature of PDA monomers allows for the creation of various structures like vesicles, films, and microarrays, which can be tailored to different diagnostic formats. Furthermore, PDA sensors exhibit both colorimetric and fluorescent responses, providing multiple detection modalities that can enhance sensitivity and reduce false positives. Their ability to function

in complex biological matrices, coupled with their relatively low cost and ease of production, makes PDA sensors particularly attractive for developing portable, user-friendly diagnostic devices. The recent advancements in improving PDA sensor sensitivity, such as incorporating nanoparticles or using dual-recognition elements, have further enhanced their potential for detecting low concentrations of analytes, which is crucial for early disease diagnosis. All these factors combine to make PDA sensors promising candidates for developing efficient, cost-effective, and user-friendly point-of-use diagnostic tools for various applications in healthcare, environmental monitoring, and food safety.

2.11 CHALLENGES AND LIMITATIONS OF PDA-BASED DIAGNOSTICS

Despite the many advantages of PDA-based diagnostics, there are several challenges and limitations that need to be addressed for their widespread adoption in point-of-care settings. One significant challenge is the relatively low quantum yield of PDA, which can limit the sensitivity of fluorescence-based detection methods, especially for low concentration analytes. The colorimetric response, while visually striking, may not always provide sufficient quantitative information for precise diagnostics. PDA sensors can also suffer from non-specific interactions in complex biological samples, potentially leading to false positives or reduced sensitivity. The stability of PDA assemblies in various environmental conditions, such as extreme temperatures or pH, can affect the reliability and shelf-life of PDA-based diagnostic devices. The synthesis and functionalization of PDA sensors with specific biomolecules can be time-consuming and may require

optimization for each target analyte. There are also challenges in standardizing the production and quality control of PDA sensors for consistent performance across different batches. Furthermore, while PDA sensors have shown promise in laboratory settings, their performance in real-world, point-of-care environments may vary due to factors like sample complexity and user expertise. Overcoming these limitations will require continued research into improving PDA stability, sensitivity, and specificity, as well as developing robust manufacturing processes and user-friendly designs for practical implementation in diverse diagnostic scenarios.

2.12 FUTURE PERSPECTIVES

The future of PDA-based sensors for point-of-use diagnostics is promising, with several possible applications in the future. Researchers are expected to focus on enhancing sensitivity and specificity through novel signal amplification techniques and advanced molecular recognition elements. Multiplexed detection capabilities will likely be developed, allowing for simultaneous analysis of multiple analytes. Integration with portable devices like smartphones could enable quantitative analysis and data sharing, while improvements in stability and shelf-life will create more reliable diagnostic tools. The application of PDA sensors may expand beyond healthcare into environmental monitoring, food safety, and biosecurity. Miniaturization and integration with microfluidics could lead to sophisticated lab-on-a-chip platforms. Theranostic applications combining diagnosis and treatment may emerge, and the integration of artificial intelligence could enhance diagnostic accuracy. As sustainability becomes increasingly important, the

development of eco-friendly, biodegradable sensors may gain focus. Finally, PDA sensors could play a significant role in advancing personalized medicine by enabling tailored diagnostic approaches.

CHAPTER 3:

POLYDIACETYLENE LIPOSOMES FOR SENSING APPLICATIONS

3.1 INTRODUCTION

Polydiacetylenes (PDAs) amphiphilic structure can readily self-assemble into various formats, liposomes are one of the most common formats for PDA-based sensors. PDA liposomes, also referred to as vesicles, are small, spherical structures composed of a lipid bilayer composed of DA monomers and optionally, additional lipid constituents. PDA liposomes are self-assembled in solution. Non-covalent interactions between amphiphilic DA monomers, including: hydrogen bonds, electrostatic interactions, hydrophobic/hydrophilic interactions, π-π stacking, and van der Waals (Xu et al., 2018). These interactions facilitate the natural alignment of DA monomers to form liposomes in aqueous solution.

PDA liposomes are prepared by probe sonication or extrusion through polycarbonate membranes, a detailed protocol for fabrication via sonication is provided in Section 3.2. Work by Kim and colleagues demonstrate a novel method of synthesizing PDA liposomes using a microfluidic chip (Kim et al., 2010), allowing for highly uniform PDA liposomes, though this method has not been adopted since it was first described in 2010.

Liposomes offer several advantages for sensing applications. Liposomes can be loaded with drugs or active ingredients for targeted therapeutics or imaging (Wang et al., 2020). The surface of liposomes may be functionalized with molecular probes for specific binding to target molecules (Cho & Jung, 2018).

The sensitivity and stability can be modified by varying DA monomer used, i.e. TRCDA is a shorter monomer that confers higher sensitivity, but lower stability than the slightly longer monomer PCDA. Doping with lipids or cholesterol is shown to increase

sensitivity by increasing fluidity of the PDA liposome. Though, too high of a lipid concentration (greater than 60% molar ratio) can result in liposomes that cannot maintain their structure.

Some studies have shown that PDA vesicles prepared by bulk methods, sonication or extrusion, show inconsistent fluorescence intensities, polydispersity, and/or color response in spite of being subjected to the same stress, while variance within the same batch of PDA vesicles is minimal (Beasley et al., 2020; Kim et al., 2010). This batch-to-batch inconsistency is not desirable for sensing applications. Developing a reliable method of synthesizing liposomes to decrease batch to batch variability is important for the use of PDA liposomes as sensors.

The synthesis of polydiacetylene (PDA) liposomes has been a subject of considerable interest in the scientific community, with numerous research groups contributing to the development and refinement of methodologies (Jung et al., 2008; Jung et al., 2010; Kim & Lee, 2019; Lee et al., 2009; Rangin & Basu, 2004; Reichert et al., 1995; Seo et al., 2021; Wang et al., 2020). While the core process remains consistent across publications, there is notable variability in the specific steps and parameters employed (Table 3.1). This diversity in approaches reflects the ongoing optimization efforts and the adaptability of PDA liposome synthesis to different research objectives and equipment availability. While these methodological variations are prevalent, publications often do not provide explicit reasoning for their specific choices or deviations from other established protocols. This lack of justification for differing steps can make it challenging for researchers to discern the optimal approach for their particular need. The numerous

methodologies not only highlights the versatility of PDA liposomes but also underscores the importance of careful consideration when selecting or adapting a synthesis protocol for specific research needs.

Table 3.1 PDA Liposome Synthesis

1	Dissolve PDA and lipids in chloroform (0.5 mM to 5 mM total lipid concentration), remove chloroform under thin stream of nitrogen gas
2	Let lipid bilayer dry under vacuum for 3-8 hours to remove any remaining organic solvent
3	Rehydrate lipid bilayer with aqueous solution
4	Incubate at 60 ºC for 5-25 minutes
5	Sonicate liposomes for 3-15 minutes
6	Filter solution through cellulose syringe filter
7	Let solution cool at 4 ºC for 4-8 hours
8	Polymerize under 254 nm light for 5 seconds – 5 minutes

In this chapter, we address the lack of systematic evaluation of key synthesis parameters in PDA liposome preparation by conducting a comprehensive study on four critical aspects: polymerization time, sonication duration, the impact of post-sonication filtration, and the effect of pre-sonication incubation. Our research methodically examines how variations in these parameters influence three essential properties of PDA liposomes: color intensity, which indicates polymerization efficiency; polydispersity, reflecting the uniformity of the liposome population; and colorimetric response to NaOH, demonstrating sensitivity. By analyzing these relationships, we aim to provide valuable insights into optimizing PDA liposome synthesis, enhancing reproducibility of liposome synthesis, and enabling the fine-tuning of these versatile assemblies for specific sensing and diagnostic applications.

3.2 MATERIALS AND METHODS

3.2.1 Materials

DA monomers, 10,12-Pentacosadiynoic acid (PCDA), was purchased from GFS Chemicals (Powell, OH, USA). DA monomers were dissolved in chloroform and filtered through a Corning® 0.2 µm polyether sulfone syringe filter to remove any polymerized or aggregated particles prior to use. Lipid, 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC), was purchased from Avanti Polar Lipids (Alabaster, AL, USA). All other reagents were purchased from Sigma Aldrich (St. Louis, MO, USA) and used without further purification.

3.2.2 Liposome Synthesis

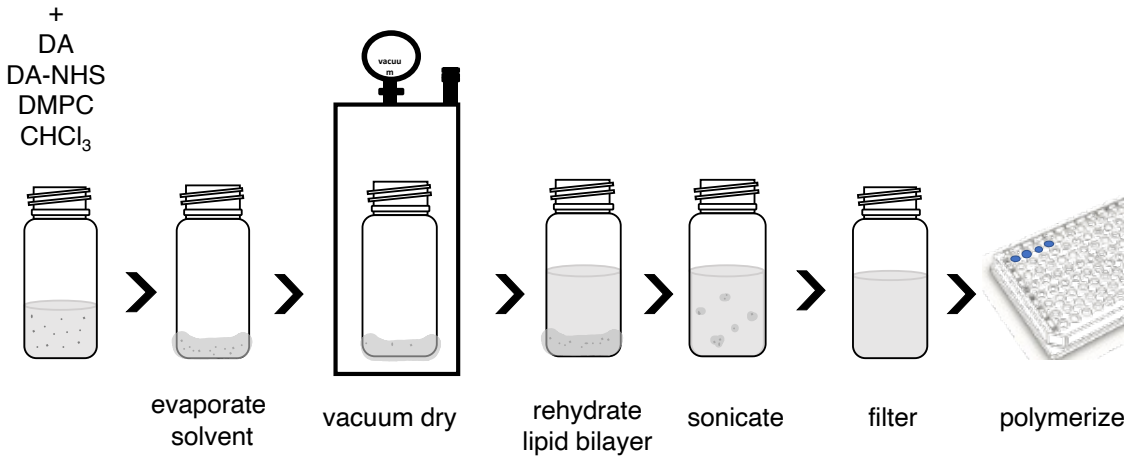

Figure 3.1 Schematic of Liposome Synthesis

Liposomes are synthesized following similar protocols that have been previously reported, a brief overview is represented above (Figure 3.1). In a 5 mL amber glass vial, PCDA, PCDA-NHS (if being used), and DMPC are fully dissolved in 1 mL chloroform.

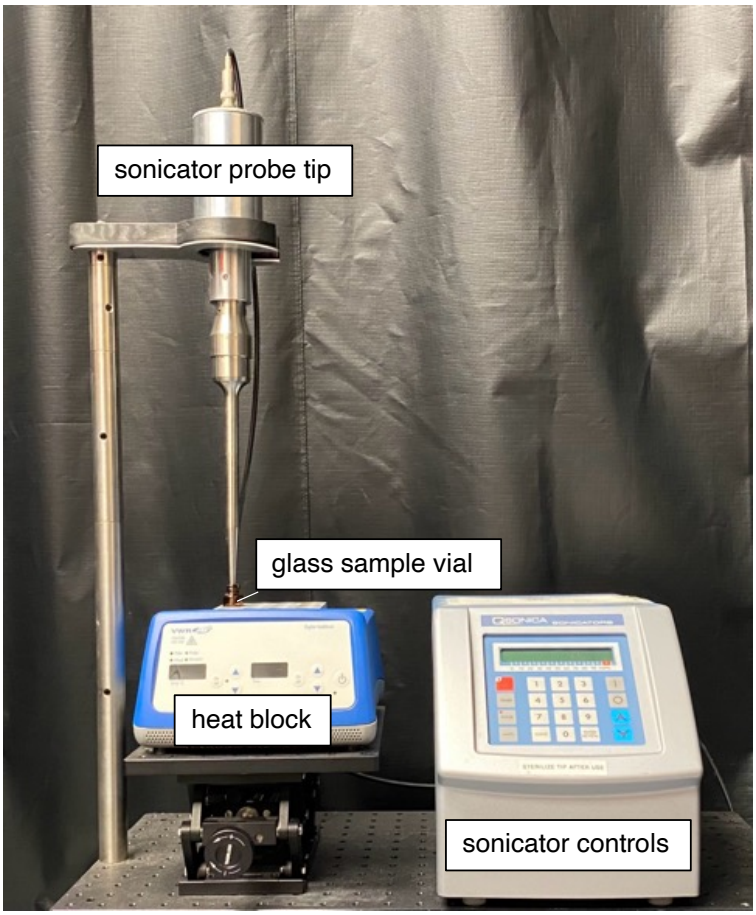

Figure 3.2 Liposome Synthesis Equipment

Chloroform is evaporated using a thin stream of nitrogen gas, leaving behind a lipid bilayer coating the glass vial. The glass vial with lipid bilayer is placed in a vacuum desiccator for 3 hours to remove any residual organic solvent. 5 mL DI water is added to rehydrate the lipid bilayer. The vial is placed in a heat block set to 60°C, the T_m of DA monomers, and is incubated prior to sonication. After incubation, the vial remains in the heat block for sonication. A QSonica Q500 Sonicator® (500 Watts, 20 kHz) probe-tip sonicator is used. A 3.2 mm probe tip is placed in the vial, submerged halfway in solution. The rehydrated lipid bilayer solution is sonicated for 15 minutes at 20% amplitude. The warm solution is

filtered through a 0.8 μm cellulose syringe filter (Corning Inc., Corning, NY, USA) to remove any aggregated particles. The solution is left to cool at room temperature, then placed in the refrigerator for 4 hours or overnight to allow the liposome backbone to stabilize. Liposome solution is aliquoted into a microwell plate (100 μL/well) and placed in a UVP Crosslinker Oven from Analytik Jena US LLC (Upland, CA, USA).

3.2.2 Characterization

Dynamic light scattering (DLS) using a Malvern Zetasizer Nano ZS90 (Malvern Panalytical, Malvern, Worcestershire, UK) analysis indicates the mean diameter of the liposomes. Absorbance value to calculate colorimetric response taken using an Agilent Cary 60 UV/Vis spectrophotometer (Agilent Technologies, La Jolla, CA, USA). Photos were taken on a Nikon D5100 digital camera.

3.3 RESULTS AND DISCUSSION

3.3.1 Filtration

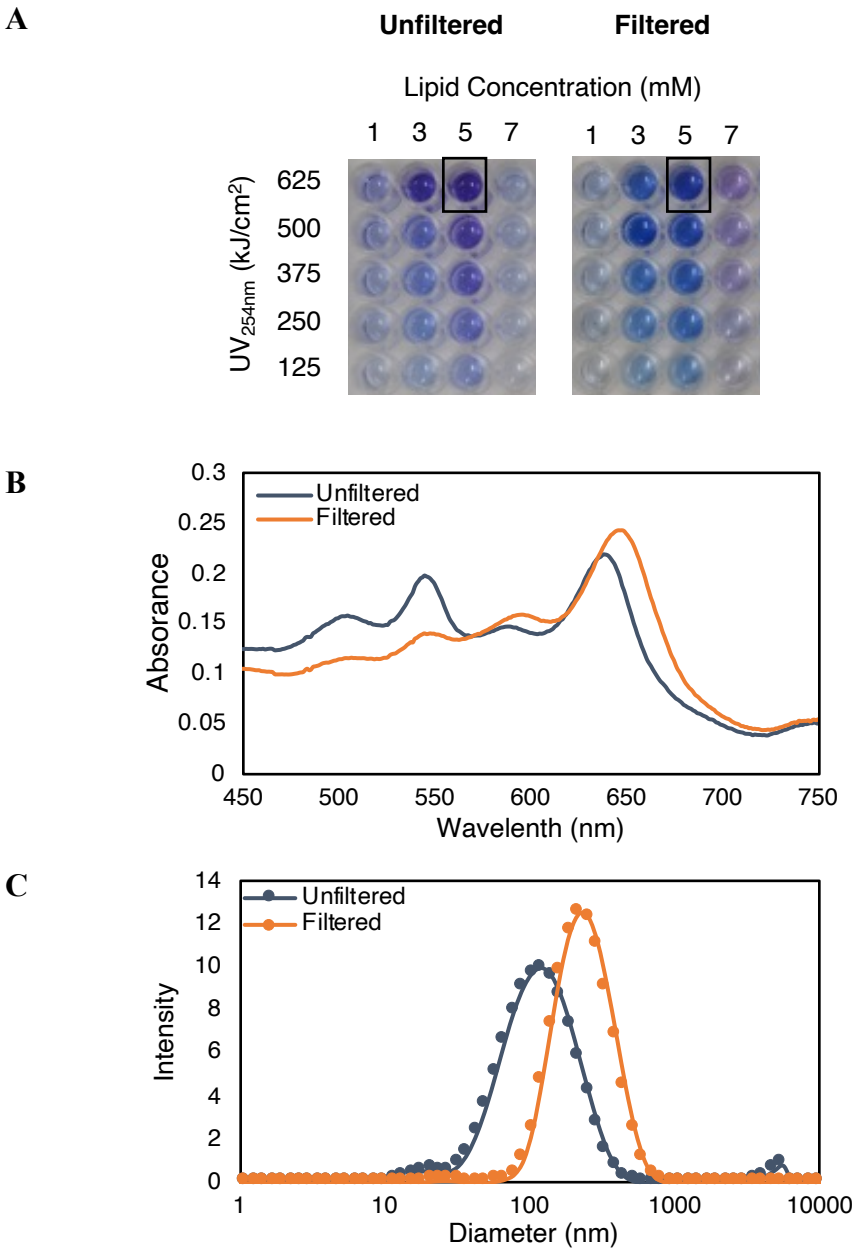

Figure 3.3 Effect of Filtration on Liposomes.
Unfiltered (left) and Filtered (right) (A). Absorbance values (B) and Size Distribution (C) of Unfiltered (blue) and Filtered (orange) liposomes of 5 mM liposomes that received 625 kJ/cm^2 of $UV_{254\ nm}$ light.

To determine the effect of filtering liposome solution post-sonication, liposomes were synthesized at 4 different concentrations: 1, 3, 5, and 7 mM. Half of the liposome solution (2.5 mL) was filtered through a 0.8 μm cellulose syringe filter and half was left unfiltered. Liposomes were polymerized under 254 nm light, at 5 different UV dosages (Figure 3.3A). The absorbance values were measured using visible spectrophotometry (Figure 3.3B) and the size distribution was measured using DLS (Figure 3.3C). Absorbance and Size was measured for all conditions, but only one was representative example is shown (5 mM, 625 kJ/cm^2 of $UV_{254\ nm}$ light).

The unfiltered samples have slightly higher absorbance values, but have peaks at 650 nm and 450 nm, indicating both red and blue form PDA present. Filtered samples have slightly lower absorbance intensities, but it does not contain any red form PDA. The filtered sample (orange line) shows a narrower size distribution with a peak shifted slightly to the right, indicating a larger average particle size.

Larger aggregates might hinder uniform UV penetration, leading to incomplete or non-uniform polymerization across the sample. Unfiltered solutions likely contain a wider range of liposome sizes, including larger aggregates. Larger structures may polymerize differently, potentially leading to variations in the conjugated backbone that result in a slight purple hue. This could result in a mixture of blue (fully polymerized) and red (partially polymerized) domains, appearing purple overall. Filtering the PDA liposome solution through a 0.8 μm syringe filter emerges as a crucial step in the synthesis process, as it promotes uniformity in liposome size, enhances polymerization consistency, and

ultimately leads to more reliable and reproducible PDA-based sensors with a clearer and more defined colorimetric response.

3.3.2 Sonication Time

Sonication is another parameter that varies greatly between publications. To determine the effect of sonication on liposomes, 3 different sonication times were tested, 5, 10, and 15 minutes. Increasing sonication time led to a decrease in liposome diameter and a decrease in standard deviation. The average diameter decreased from approximately 300 nm at 5 minutes to about 150 nm at 10 minutes, and further reduced to around 120 nm at 15 minutes of sonication. The color response of liposome solutions at different sonication times and NaOH concentrations (Figure 3.4A), was higher at NaOH concentrations. Longer sonication times generally resulted in higher color intensity, particularly at higher NaOH concentrations (Figure 3.4B).

Sonication time has an impact on PDA liposome size and color response. Longer sonication times produce smaller, more uniform, liposomes, likely due to increased energy input breaking down larger vesicles. The smaller liposome size correlates with enhanced color response to NaOH, possibly due to increased surface area and more efficient chromatic transitions. This study demonstrates that optimizing sonication time is crucial for controlling liposome size and maximizing the colorimetric sensing capabilities of PDA liposomes. The 15-minute sonication time appears to be most effective in producing uniform, responsive liposomes for potential sensing applications. These time intervals were chosen based on literature. Sonicating for less time would cause liposome solutions with

high polydispersity. Increasing sonication time beyond 15 minutes is shown to have similar effects, as the liposomes begin to break down as they are overheated.

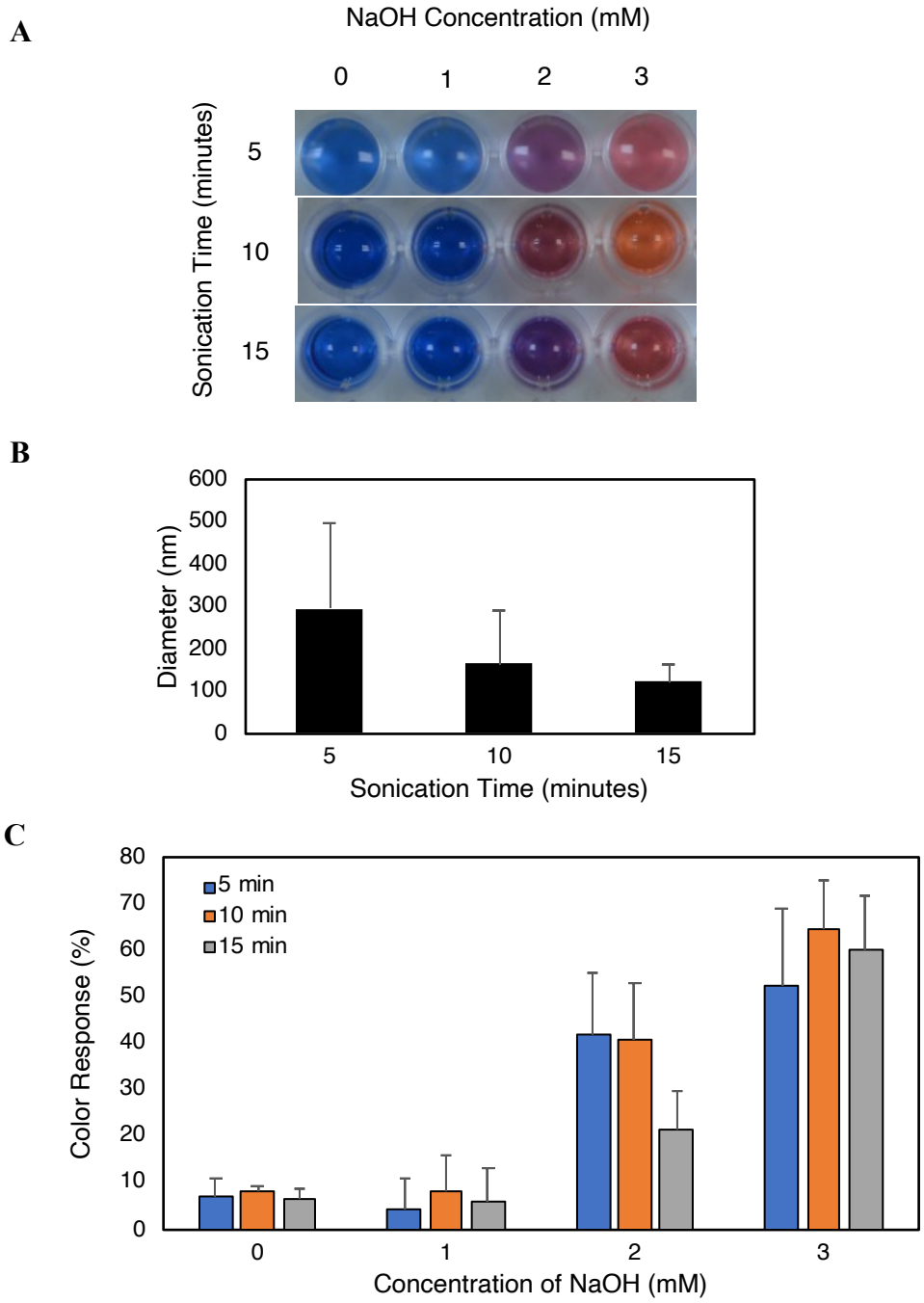

Figure 3.4 Effect of Sonication Time on Liposome Size and Sensitivity to pH Change.

3.3.3 Sensitivity

Once filtration, UV dosage, and sonication time were chosen for, another optimization step was done to ensure the best concentration was chosen to ensure adequate color intensity for a naked-eye diagnostic and maximum sensitivity (Figure 3.5).

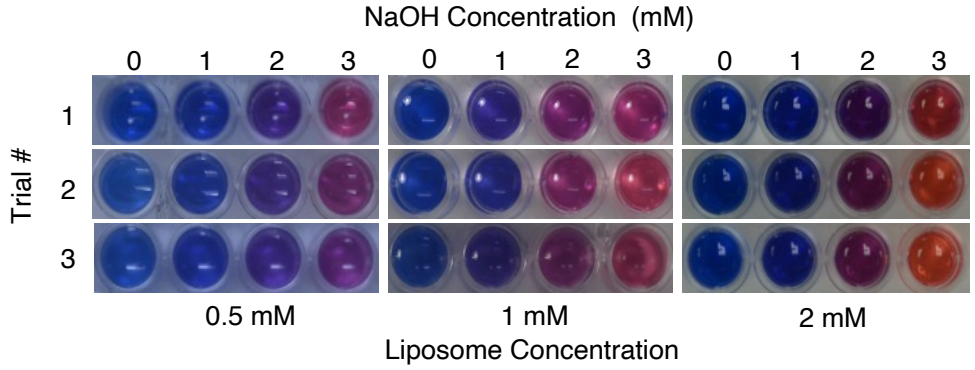

Figure 3.5 Liposome Color Response to Varying Concentrations of NaOH

The size distribution curves show a predominantly unimodal distribution for all three concentrations (0.5 mM, 1 mM, and 2 mM) with an average diameter between 100-200 nm for all conditions (Figure 3.6A). There's a slight shift towards smaller sizes as the concentration increases from 0.5 mM to 2 mM. The absorbance spectra show a clear response to increasing NaOH concentrations. At 0 mM NaOH, there's a small peak around 640 nm, characteristic of the blue phase of polydiacetylene. As NaOH concentration increases, a new peak emerges and grows around 540 nm, indicating a shift to the red phase. The intensity of the red phase peak (540 nm) increases with higher NaOH concentrations, while the blue phase peak (640 nm) diminishes (Figure 3.6B). The colorimetric response increases significantly with increasing NaOH concentration. The error bars suggest good reproducibility, especially at 1 mM Liposome concentration.

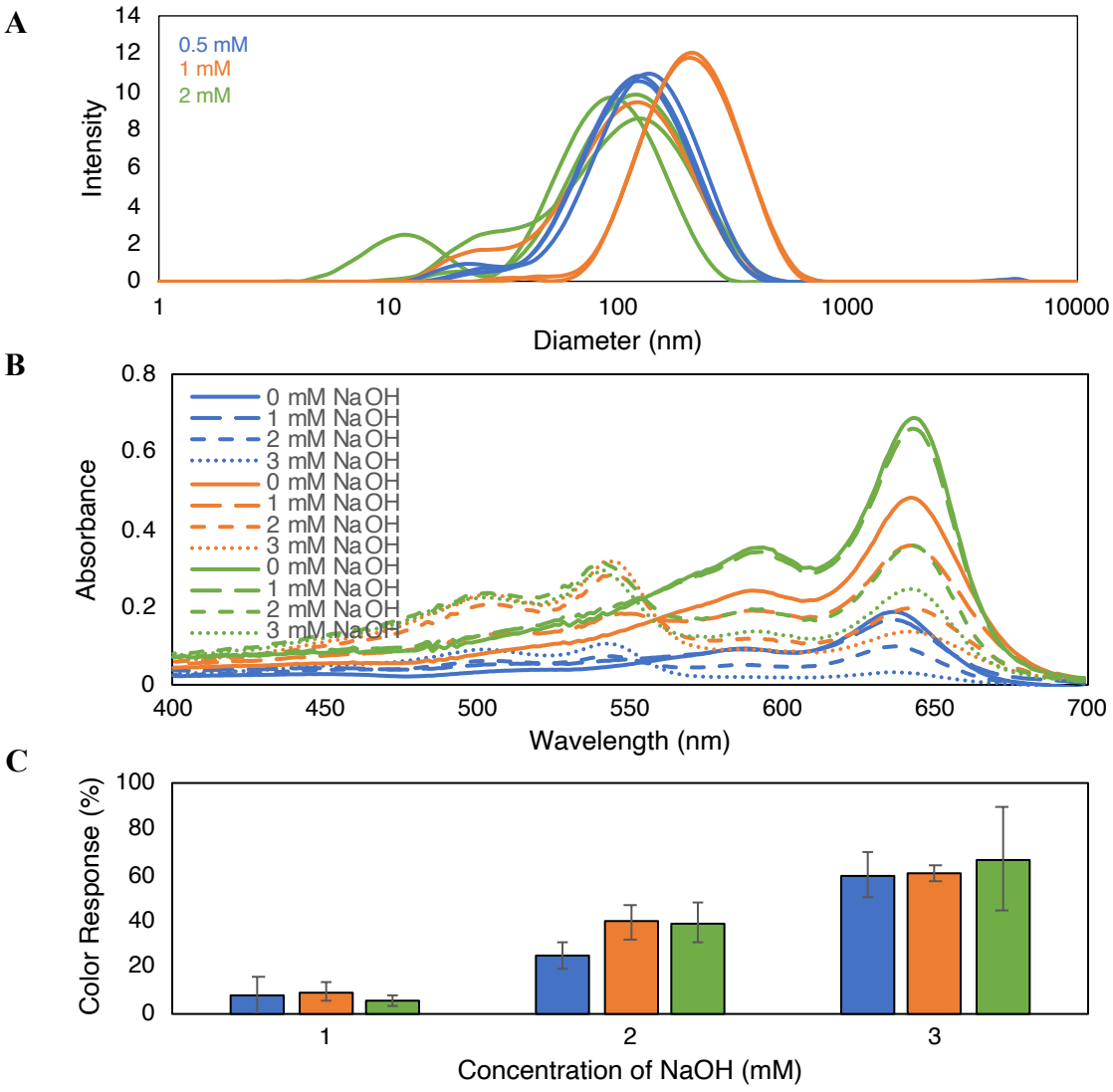

Figure 3.6 Characterization of 0.5, 1, and 2 mM Liposomes.
Size distribution (A), Absorbance (B), and Colorimetric Response (C) are used to measure repeatability and sensitivity

3.4 CONCLUSION

This study on PDA liposome synthesis has identified several important parameters that significantly influence the quality and performance of the resulting liposomes. Post-sonication filtration was found to enhance uniformity and polymerization consistency, leading to more reliable colorimetric responses. Longer sonication times, particularly 15 minutes, produced smaller, more uniform liposomes with enhanced color response to pH changes. The optimization of liposome concentration revealed that 1 mM offered a balance between color intensity and sensitivity to NaOH. These findings provide valuable insights for optimizing PDA liposome synthesis for various sensing and diagnostic applications, potentially improving reproducibility and sensitivity in future studies.

Kim and colleagues have sought to increase reproducibility via a microfluidic chip (Beasley et al., 2020; Kim et al., 2010)., this optimized protocol seeks to improve the standard synthesis of liposomes, as not all PDA researchers will have access to microfluidic devices. Another group, Beasely and colleagues, sought to decrease batch-to-batch variation through a normalization step post liposome synthesis. This method does result in normalized sensor values (Beasley et al., 2020). A normalization step may be burdensome for a point of use diagnostics additionally, it requires more liposome solution for additional tests. If liposomes contain antibodies, or other expensive probes, this normalization may increase test cost. The current study's optimized protocol for standard liposome synthesis offers a practical, cost-effective approach to improving PDA liposome reproducibility and performance, complementing existing methods and potentially broadening the accessibility of high-quality PDA-based sensors across diverse research settings.

CHAPTER 4:

EFFECT OF MONOMER LENGTH AND LIPID DOPING ON SENSITIVITY

4.1 INTRODUCTION

Polydiacetylenes (PDAs) are often used in point-of-use diagnostics due to their unique optical properties, ability to readily self-assemble into various formats, and ability to be chemically modified to optically transition from blue to red in response to various external stimuli. These advantageous properties allow PDAs to be used as label-free biosensors for naked eye detection of various analytes, including bacteria, heavy metal ions, DNA, and viruses, depending on the molecular probe utilized. Despite the many advantages of PDAs, their relatively low sensitivity has been an obstacle to their use as sensory materials. In the decades since PDA has first been studied for use in diagnostics, numerous strategies have been developed to increase sensitivity and specificity. Recently, two comprehensive reviews have been published on tuning the sensitivity of PDA ((Kim et al., 2021), (Weston, Tjandra, et al., 2020).

PDAs are demonstrably a versatile sensing platform. However, the sensitivity and specificity of PDAs is an important factor in ensuring their use outside of a laboratory setting. There are a multitude of factors that affect the sensitivity of PDA sensors, three important ones are: DA structure (alkyl chain length, head group modification, and spacer length), Lipid Doping, and PDA Format.

4.1.1 Diacetylene Monomer Structure

Most DA monomers consist of a polar head group and alkyl tail, connected via a diacetylene group. The characteristics, such as responsiveness, of PDA are based on the alkyl chain and hydrophilic groups of the diacetylene monomer that makes up the PDA membrane. Modifying the alkyl chain at the head group and tail will affect the physical and

self-assembly properties of PDA. Decreasing the length of the alkyl tail weakens intramolecular forces as hydrophobicity decreases, in turn decreasing the melting temperature of the PDA. In contrast, decreasing the alkyl length of the headgroup, hydrogen bonding increases, which increases the melting temperature of PDA. Notably, it is possible that intermolecular forces within the head group are dominant, and overtake any effects the alkyl chain lengths may have (Kim et al., 2005)

The spacer, which connects the head group and tail group, also effects PDA response. PDA diagnostics are often synthesized such that the probe is on the headgroup of the PDA. When a binding event occurs at the probe, the stimulus must be transferred from the PDA surface to the backbone. A longer spacer increases degree of freedom of the DA, decreasing the efficacy of signal transduction from PDA surface to PDA backbone. Thus, decreasing spacer length increases sensitivity of PDA (Khanantong et al., 2018; Potai et al., 2018). Importantly, decreasing the spacer too much may have negative effects on polymerization efficiency (Charoenthai et al., 2011).

4.1.2 Lipid Doping

The sensitivity of PDA can be changed by incorporating lipids and/or cholesterol. Lipids with similar properties to diacetylenes are often used, such as surfactants (Shin et al., 2015), phospholipids (Kang et al., 2012; Kim & Lee, 2019). Cholesterol is also used (Kolusheva et al., 2003; Kwon et al., 2014). Lipids are added in the lipid bi-layer formation step of synthesis, where they flow between alkyl chains of DA. Once polymerized, PDA structure becomes rigid, and lipids may form a separate domain, creating lipid “units” within the polymer, or become trapped in the alkyl tail. The lipids present in the PDA

bilayer change the degree of freedom of the DA alkyl chain and effected the structure and fluidity of the membrane. The resulting PDA membrane may be sensitive to external stimuli and may be able to achieve a lower limit of detection (Kim et al., 2021). It is important to note that formation of liposomes becomes impossible above a certain ratio of lipids, the upper limit of lipid ratios for PVDF coated membranes is not noted, though generally 40% is the maximum percentage in literature.

4.1.3 PDA Format

There are numerous PDA Formats that have been used for sensing. The size and morphology of PDA nanoparticles in aqueous solution, embedded into materials, or coating a surface or thin film, varies across PDA formats and effects the sensitivity of the sensor. Solid-phase PDA sensors has gained interest for their stability over aqueous formats. PDA has been immobilized on a variety of solid surfaces, such as glass (Volinsky et al., 2007), filter paper (Pumtang et al., 2011), PVDF membranes (Son et al., 2019). PDA-coated PVDF membranes have been used to detect various analytes incusing zinc ions (Wen et al., 2016) and pH1N1 virus (Son et al., 2019). It is simple to manufacture and inexpensive, in addition to being a stable and sensitive format for PDA sensing, making it an ideal candidate for point-of-use diagnostics.

Tuning the sensitivity of PDA sensors is crucial to further developing PDA-based diagnostics. In this study, the effect of DA monomer length and lipid doping on sensitivity in PVDF systems is determines. Two commonly used and readily commercially available monomers – 10,12-Pentacosadiynoic acid (PCDA) and 10,12-Tricosadiynoic acid

(TRCDA) are used. TRCDA is slightly shorter, with two-fewer carbons in the alkyl tail. Lipid doping has been shown in many studies to increase the sensitivity of PDAs. It is believed that the lipid increased hydrogen interactions in the headgroup. In this study, the lipid 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) is used to study the effects of lipid on sensitivity (Figure 4.1).

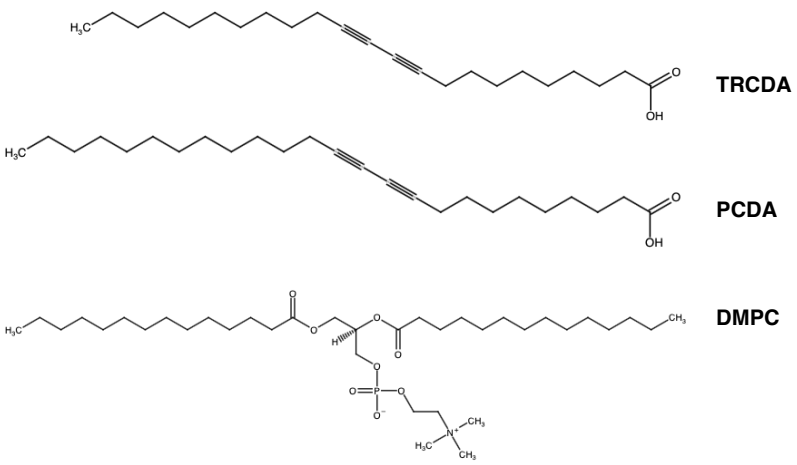

FIGURE 4.1 Structure of TRCDA, PCDA, and DMPC.

We hypothesize that the shorter TRCDA monomer will exhibit higher sensitivity due to reduced intermolecular forces, and that lipid doping will further enhance this effect by increasing membrane fluidity. However, we also anticipate potential trade-offs between sensitivity and stability that must be carefully balanced for practical applications. By elucidating the relationships between molecular structure, composition, and sensor performance in a solid-phase format, this study aims to contribute to the rational design of

more sensitive and reliable PDA-based diagnostic tools. The insights gained here may inform the development of improved sensors for a wide range of applications, from medical diagnostics to environmental monitoring.

In the following sections, we describe our experimental approach, present our findings on the effects of monomer length and lipid doping on PDA sensitivity, and discuss the implications of these results for future sensor development.

4.2 MATERIALS AND METHODS

4.2.1 Materials

DA monomers, 10,12-Pentacosadiynoic acid (PCDA) and 10,12-tricosadiynoic acid (TRCDA) were purchased from GFS Chemicals (Powell, OH, USA). DA monomers were dissolved in chloroform and filtered through a Corning® 0.2 μm polyethersulfone syringe filter to remove any polymerized particles prior to use. Lipid, 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC), was purchased from Avanti Polar Lipids (Alabaster, AL, USA). 0.45 μm pore size Poly-vinyl difluoride membrane (PVDF) was obtained from Merck Millipore Ltd. (Tullagreen Townland, Co. Cork, Ireland). All other reagents were purchased from Sigma Aldrich (St. Louis, MO, USA) and used without further purification.

4.2.3 Preparation of PVDF strips for sensitivity study

PVDF was cut into 1 cm x 1 cm squares. TRCDA or PCDA and DMPC were combined in 10 mL chloroform according to the Table 4.1 and 4.2 below.

Table 4.1 Ratios and Masses of PCDA and DMPC

% DMPC	0%	20%	40%
PCDA	11.24 mg	11.24 mg	11.24 mg
DMPC	0 mg	6.78 mg	13. 56 mg

Table 4.2. Ratios and Masses of TRCDA and DMPC

% DMPC	0%	20%	40%
TRCDA	10.40 mg	10.40 mg	10.40 mg
DMPC	0 mg	6.78 mg	13.56 mg

The DA solution was stirred at room temperature at 200 rpm. While the solution is being stirred, tweezers were used to fully submerge the PVDF test strip into solution and removed immediately (Figure 4.2). Strip sensors were dried for 4-8 hours at room temperature in the dark. Dried strip sensors were polymerized under 0.005 J/cm^2 of UV_{254nm} light using the Legacy UVP Crosslinker CL-1000 from Analytik Jena US LLC (Upland, CA, USA). After polymerization, strip sensors were stored in a cool, dark place, until ready for use.

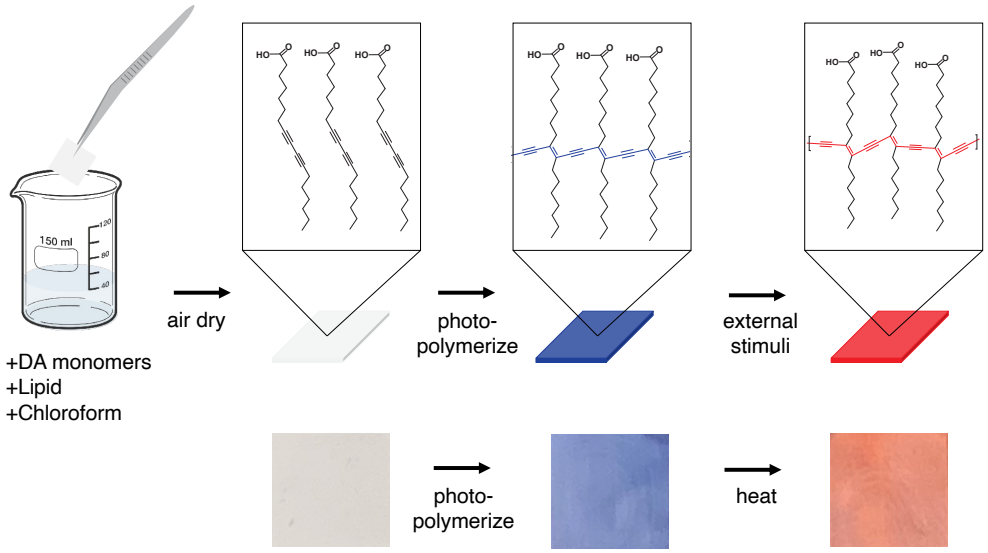

Figure 4.2. PVDF Strip Sensor Fabrication.
Illustration of strip sensor and DA alignment (Top). Representative photos of a strip sensor exposed to a temperature of 100C for 1 minute (Bottom).

4.2.4 PVDF strips for temperature sensitivity

A FD 260 Drying Oven from BINDER Inc. (Bohemia, NY, USA) was pre-heated and allowed to stabilize for approximately 5 minutes. Strip sensors were placed into a pre-heated drying oven ranging from 40-80°C for 45 seconds, then removed and photographed immediately.

4.2.5 PVDF strips pH test

pH solutions were prepared using 1M NaOH and 12 M HCl stock solutions. Polymerized DA strip sensors were submerged in pH solution for 45 seconds, removed, and air dried at room temperature in the dark for 6-8 hours before imaging for analysis.

4.2.6 PVDF strips UV test

Polymerized DA strip sensors were exposed to varying amounts of UV 254 nm light. Strip sensors were placed in Legacy UVP Crosslinker CL-1000, at a fixed distance from the bulbs. Strip sensors were exposed to varying amounts of UV_{254nm} light and photographed immediately after UV exposure.

4.2.7 Image Analysis and Colorimetric Response

DA strip sensors were photographed with a Nikon D1500 using a Digi-Slave L Ring 3200 light. Fiji was used to split the color channels and measure the average Red, Green, and Blue intensity across DA strip sensors. The average Red, Blue, and Green intensities were used to calculate the colorimetric response, based on (Pumtang et al., 2011).

First, the average Red Chromaticity, r, is calculated as an average of the total color intensities:

$$r = \frac{R}{R + G + B}$$

Where R is the average intensity of Red, G is the average Green Intensity, and B is the average Blue intensity.

Then, the Colorimetric Response can be characterized as a change in the total Red Chromaticity as compared to the un-treated sensor (i.e. negative control) and sensor with maximum blue to red transition (i.e. positive control). The Red Chromatic Shift is found by:

$$\%RCS = \frac{r_{sample} - r_0}{r_{max} - r_0} \times 100$$

Where r_{sample} is the Red Chromaticity of the sample, r_{max} is the Red Chromaticity of a positive control and r_0 is the Red Chromaticity of a negative control.

4.3 RESULTS AND DISCUSSION

4.3.1 Effect of DA Monomer Length on Sensitivity

Two DA monomers, PCDA and TRCDA, were investigated to determine the effect of DA monomer alkyl chain length on sensitivity in response to three types of external stimuli: heat, pH, and UV exposure, on PVDF sensor strips.

When exposed to temperatures of 40-80°C, TRCDA consistently showed higher sensitivity than PCDA. At 40-60°C, TRCDA was slightly more sensitive than PCDA. However, at 70°C, TRCDA was approximately 3 times more sensitive than PCDA, and at 80°C, TRCDA was approximately 5 times more sensitive (Figure 4.3A). This increased sensitivity of TRCDA can be attributed to its shorter alkyl chain length, which likely results in reduced intermolecular forces and increased molecular mobility. The shorter chains of TRCDA may also allow for easier conformational changes upon thermal stimulation, leading to more pronounced color transitions at lower temperatures compared to PCDA.

In response to changes in pH from 0 to 13, PCDA and TRCDA showed similar sensitivity until pH = 13, where TRCDA was 2 times more sensitive (Figure 4.3B). This difference at extreme pH could be due to the shorter alkyl chains of TRCDA allowing for more efficient propagation of conformational changes from the head group (where pH changes are sensed) to the conjugated backbone (S. J. Kew & E. A. H. Hall, 2006).

UV dosage showed minimal color change and minimal differences between PCDA and TRCDA (Figure 4.3C). Though high and/or prolonged dosages of UV exposure can cause blue to red color change, the UV exposure in this study had minimal effect on color transition. This suggests that the mechanism of UV-induced color change may be less dependent on alkyl chain length compared to thermal or pH-induced changes.

Across all test conditions, TRCDA was on average 1.5 times more sensitive than PCDA. This consistent trend supports the hypothesis that shorter monomers generally lead to higher sensitivity in PDA systems. The increased sensitivity likely stems from the reduced van der Waals interactions between the shorter alkyl chains, allowing for greater molecular mobility and easier propagation of stimuli-induced conformational changes throughout the polymer structure.

It's important to note that while shorter monomers are associated with higher sensitivity they may also be less stable in certain formats, like liposomes (Okada 1998), and may lead to decreased specificity. The solid support of the PVDF strips used in this study may mitigate some of these stability concerns, but further long-term stability testing would be needed to confirm this. Additionally, the effects observed here on PVDF strips may differ from those in vesicle systems, as the solid support likely influences polymer packing and response mechanisms.

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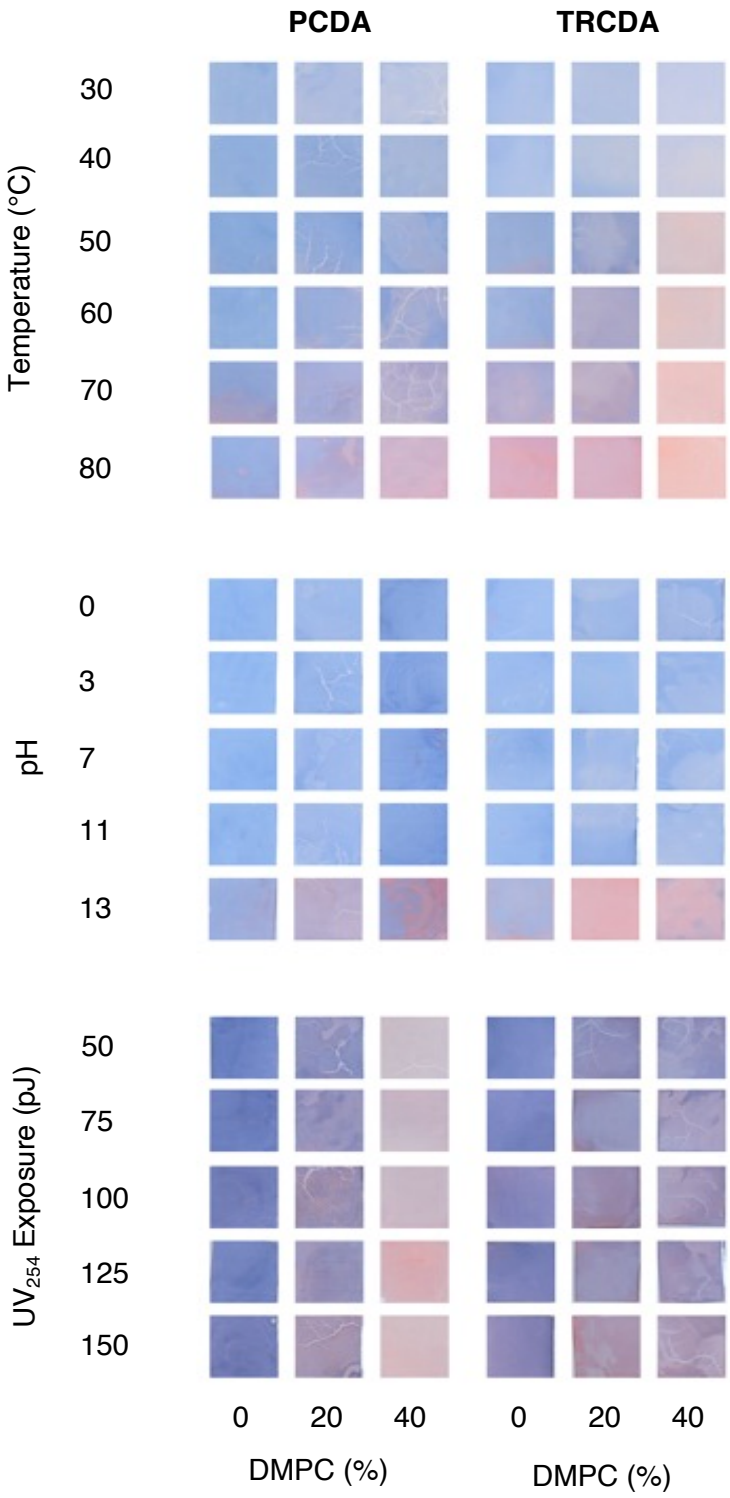

Figure 4.3. Colorimetric response of PCDA and TCDA with varied amounts of lipid to varying external stimuli: temperature, pH, and UV exposure.

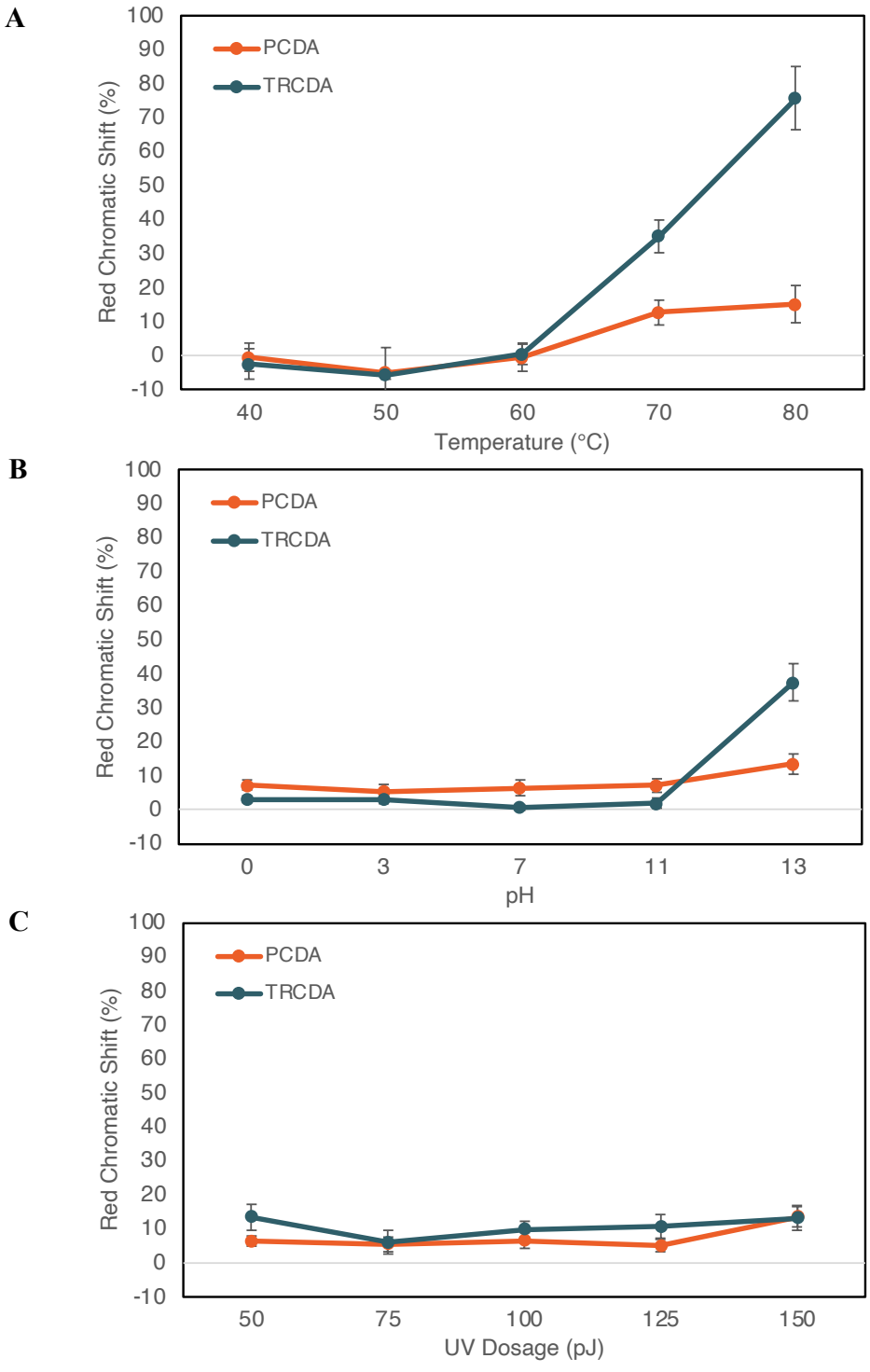

Figure 4.4. Red Colorimetric Shift of PCDA and TRCDA in Response to Change in Temperature (A), pH (B), and UV Exposure (C).

4.3.2 Effect of Lipid Doping on Sensitivity

In this study, PCDA and TRCDA were doped with phospholipid, DMPC. The concentration of DA monomer remained constant, and three different ratios of DA monomer to DMPC were studied: 100:0, 80:20, and 60:40. Sensitivity was measured in response to temperature, pH, and UV exposure.

For both PCDA and TRCDA, increasing lipid concentration generally led to increased sensitivity across all stimuli tested. This effect was particularly pronounced for TRCDA, suggesting a synergistic effect between the shorter alkyl chains and lipid doping. The lipids likely further disrupt the packing of the DA monomers, increasing overall mobility and amplifying the inherent sensitivity advantage of TRCDA.

In response to temperature changes, the maximum color transition for PCDA was 50% at 90°C, while TRCDA had a maximum transition of 90% at 90°C (Figure 4.4). The lipid-doped samples showed earlier onset of color change and steeper response curves, particularly for TRCDA. This suggests that lipid doping lowers the energy barrier for thermally-induced conformational changes in the PDA structure.

pH response showed similar trends, with PCDA reaching a maximum color transition of 55% at pH = 13, while TRCDA had a maximum transition of 85% at pH = 13 (Figure 4.5). The enhanced pH sensitivity with lipid doping may be due to increased hydration and ion accessibility in the head group region of the PDA structure.

UV exposure results were more complex, with PCDA showing a maximum color transition of 55% at UV = 150 pJ, while TRCDA had a maximum transition of 75% at UV

= 75 pJ (Figure 4.7). The non-linear response to UV exposure, especially for TRCDA, suggests competing processes may be at play. Higher UV doses may induce both color-changing conformational shifts and further polymerization or even degradation, leading to the observed variability.

While lipid doping generally increased sensitivity, it's important to note that higher lipid concentrations (40% DMPC) sometimes led to increased variability in response. This highlights the need to balance sensitivity gains with consistency and reliability in sensor performance.

The combination of shorter alkyl chains (TRCDA) and moderate lipid doping (20% DMPC) appears to offer an optimal balance of increased sensitivity, decreased variability, and maintained stability. This formulation will be used in subsequent studies, as it provides enhanced sensor performance without sacrificing reliability.

These findings underscore the complex interplay between molecular structure, composition, and sensitivity in PDA-based sensors. The ability to tune sensitivity through monomer selection and lipid doping offers a powerful tool for optimizing PDA sensors for specific applications, potentially enabling more sensitive and reliable point-of-use diagnostic devices.

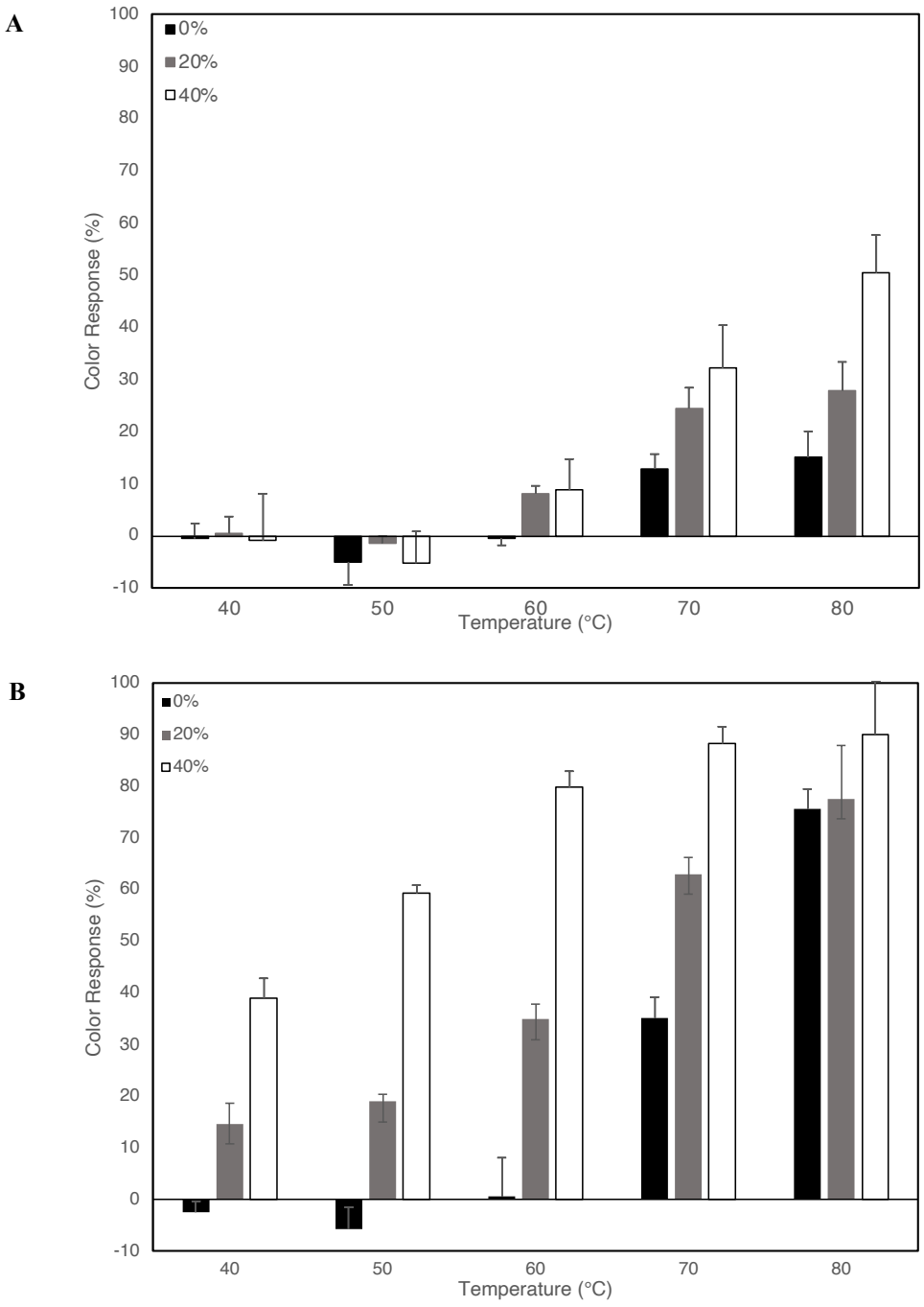

Figure 4.5. Colorimetric response of PCDA (A) and TCDA (B) with varied amounts of lipid to varying temperatures. Colorimetric response of PVDF membranes coated with PCDA doped with 0%, 20%, and 40% DMPC (A) and TCDA doped with 0%, 20%, and 40% DMPC (B). N=5.

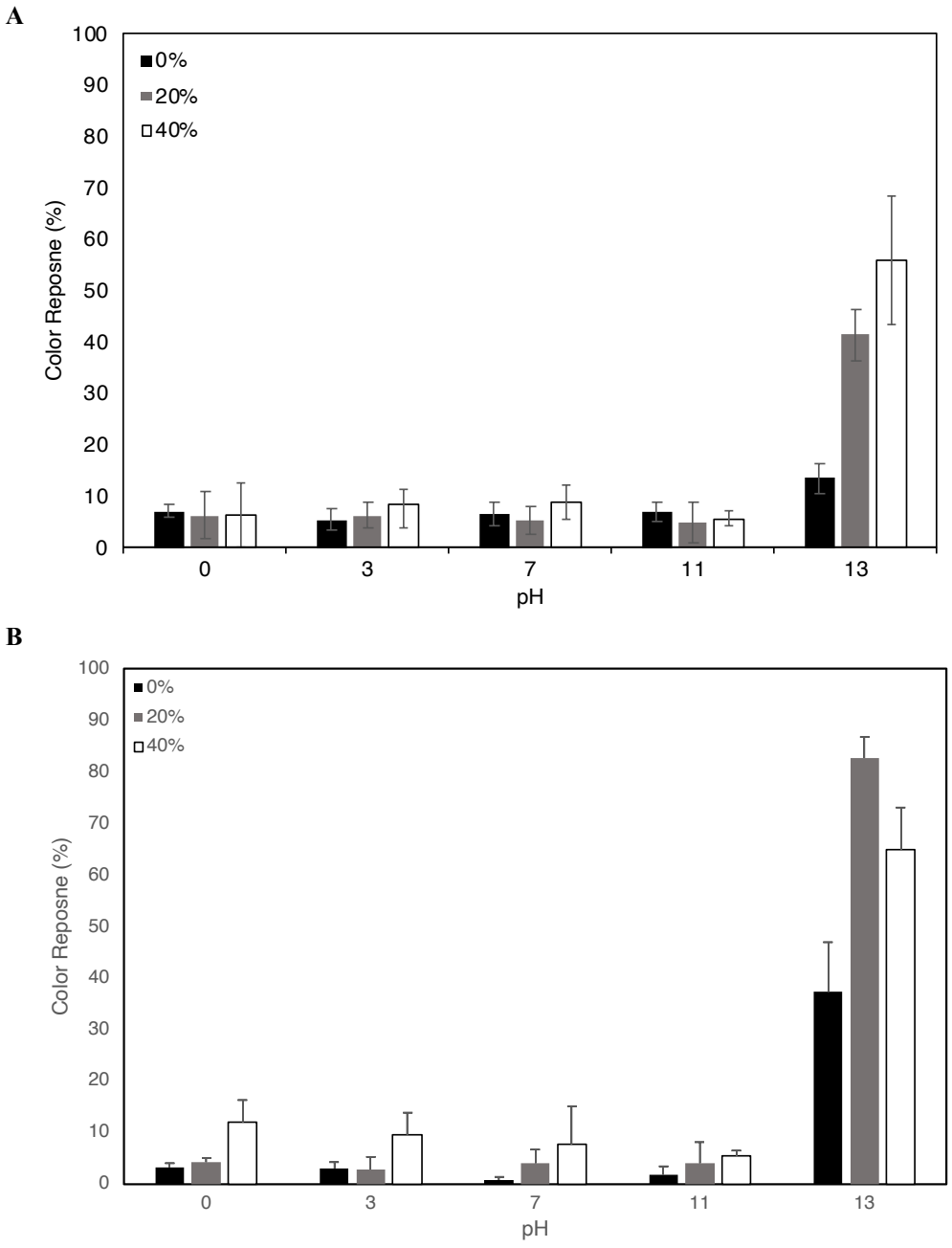

Figure 4.6. Colorimetric response of PCDA (A) and TCDA (B) with varied amounts of lipid to varying pH. Colorimetric response of PVDF membranes coated with PCDA doped with 0%, 20%, and 40% DMPC (A) and TCDA doped with 0%, 20%, and 40% DMPC (B). N=5.

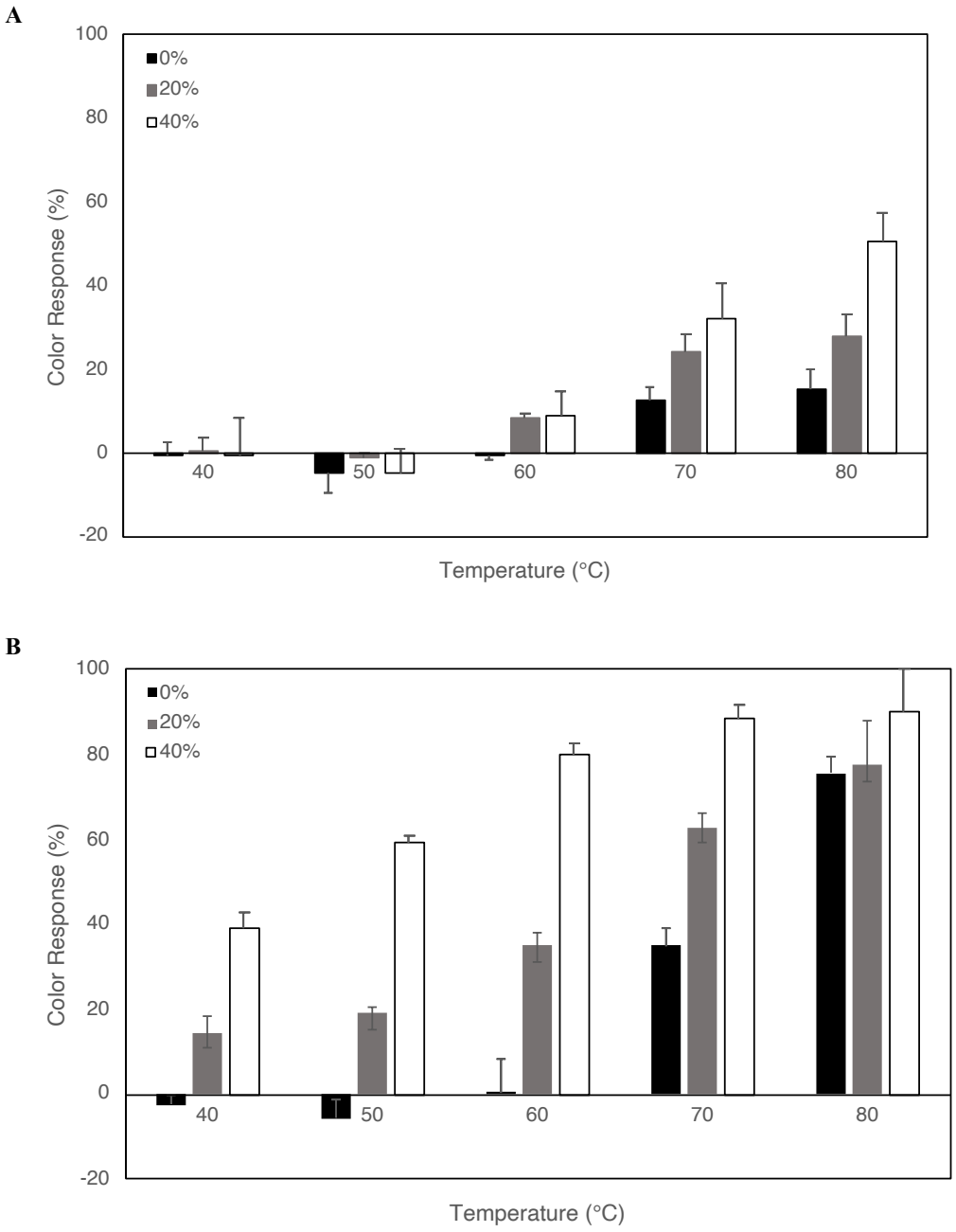

Figure 4.7. Colorimetric response of PCDA (A) and TCDA (B) with varied amounts of lipid to varying UV exposure. Colorimetric response of PVDF membranes coated with PCDA doped with 0%, 20%, and 40% DMPC (A) and TCDA doped with 0%, 20%, and 40% DMPC (B). N=5.

4.4 CONCLUSION

In this study, we observed that a shorter alkyl chain length and lipid doping increased the sensitivity of polydiacetylenes deposited onto PVDF strips. By characterizing colorimetric response to UV, heat, and pH, we were able to determine the relationship between chain length, lipid doping, and sensitivity and stability of the polymers.

Our findings indicate that TRCDA, with its shorter alkyl chain, consistently outperformed PCDA in terms of sensitivity across all tested stimuli. This increased sensitivity is likely due to reduced intermolecular forces and increased molecular mobility, allowing for easier conformational changes upon stimulation. Additionally, lipid doping further enhanced sensitivity, with the effect being more pronounced in TRCDA, suggesting a synergistic interaction between shorter alkyl chains and lipid incorporation.

Based on our results, we have identified TRCDA with 20% DMPC as the optimal formulation for further studies. This composition balances increased sensitivity with decreased variability and maintained stability. The 20% lipid concentration provides enhanced responsiveness without the increased variability observed at higher lipid concentrations. This optimized formulation holds promise for improving the performance of PDA-based sensors in various applications.

The insights gained from this study have broader implications for the design and optimization of PDA-based sensor systems beyond PVDF strips. The principles of tuning sensitivity through monomer selection and lipid doping could be applied to other PDA formats, such as vesicles or thin films, potentially leading to improved sensors for a wide range of diagnostic and environmental monitoring applications.

Future work should focus on further characterizing the selected TRCDA with 20% DMPC formulation, including long-term stability testing and investigating specificity to target analytes. Additionally, exploring the performance of this optimized formulation in real-world scenarios, such as detecting specific pathogens or environmental contaminants, would be valuable in assessing its practical utility.

CHAPTER 5:

ANTIBODY-FUNCTIONALIZED LIPOSOMES AND PDA-COATED PVDF SENSORS

5.1 INTRODUCTION

5.1.1 Antibodies for Diagnostic Applications

Antibodies are powerful tools in the development of highly specific and sensitive biosensors due to their highly specific binding affinity. This high specificity allows for the creation of sensors that can detect a wide range of biomolecules, pathogens, and even small chemical compounds with minimal cross-reactivity. Antibodies can be easily functionalized or immobilized on various sensing platforms without significant loss of their binding capabilities, making them compatible with numerous detection methods, including PDA-based sensors.

Despite their many advantages, antibodies also present some challenges when used in PDA sensor applications. Antibodies are sensitive to environmental conditions such as temperature, pH, and ionic strength, potentially leading to denaturation and loss of functionality during sensor fabrication or storage. The large size of antibodies (approximately 150 kDa) may present a limitation in sensor functionality. Molecular binding must cause distress to the PDA backbone to induce a color change. The distance from antibody binding site to PDA backbone may cause low sensitivity to molecular binding events.

5.1.2 Pantoea stewartii Detection

In this chapter, we investigate the development and application of antibody-functionalized polydiacetylene (PDA) sensors for the detection of plant pathogenic bacteria, *Pantoea stewartii*. Building upon established methodologies in the literature, we

aimed to create a sensitive and specific colorimetric biosensor using anti-*P. stewartii* antibodies conjugated to PDA vesicles.

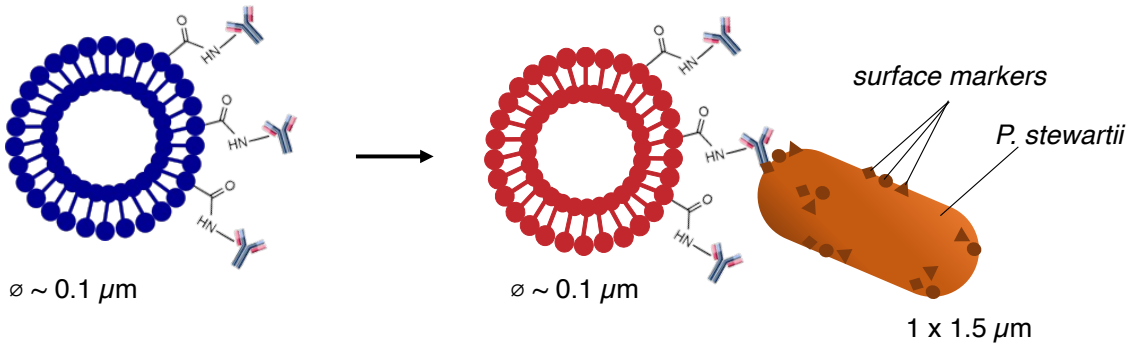

Figure 5.1 Schematic of Antibody-functionalized PDA liposomes for detection of *P. Stewartii*

Pantoea stewartii sups. *stewartii* is a gram-negative, phytopathogenic bacteria that causes Stewart's wilt disease in sweet corn (*Zea mays*) (Roper, 2011) . *P. stewartii* is vectored into plants by the corn flea beetle (*Chaetocnema pulicaria*). The bacteria colonize intercellular spaces and the xylem, where it causes water-soaked lesions and eventually leads to wilting. It is indigenous to North America and has caused significant economic impact in the past. It is not currently a threat, due to development of resistant cultivars and widespread use of insecticides (Pal et al., 2019). *P. stewartii* is ideal for laboratory studies, as it is reasonably easy to propagate and has few pathogenicity mechanisms. There are several established protocols for detection of *P. stewartii* in liquid cultures and in maize leaves in order to verify novel PDA biosensor results, specifically qPCR (Pal et al., 2019). Anti-*P. stewartii* antibodies are commercially available (Agdia Inc., Elkhart, IN), though the specific details are proprietary, the antibodies target a surface marker. Due to its ease

of culturing, commercial availability of antibodies, *P. stewartii* is a good candidate for pathogen detection.

5.1.3 Streptavidin Detection

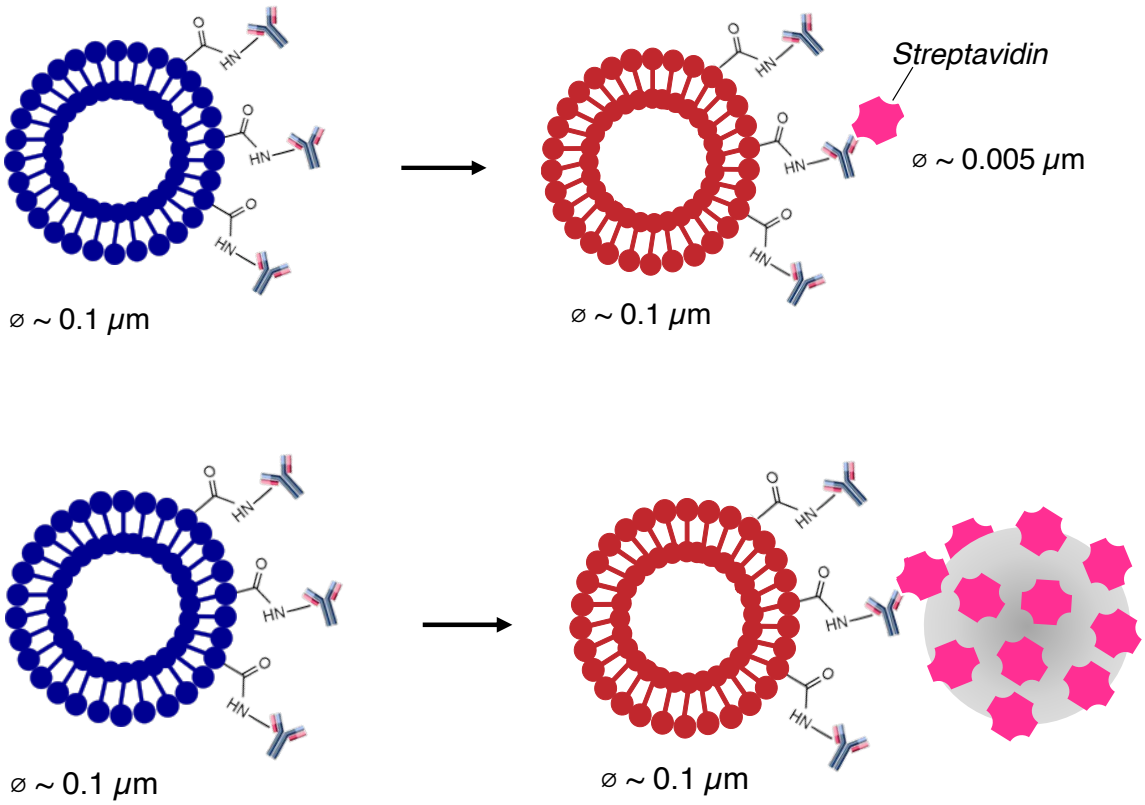

Figure 5.2 Schematic of Antibody-functionalized PDA liposomes for detection of *Streptavidin*

Since there are no standard protocols using commercially available antibodies and antigens for PDA sensor systems, we aimed to establish a model system using commercially available, cost-effective antibodies and antigens. Anti-streptavidin antibodies and streptavidin were selected as candidates for this standardized approach.

Streptavidin, a tetrameric protein derived from *Streptomyces avidinii*, is stable across diverse conditions, including extreme temperatures and pH ranges (Chaiet & Wolf, 1964). While commonly utilized for its high biotin affinity in various biotechnological and molecular biology applications such as protein purification, immunoassays, and molecular imaging, our study focuses on its interaction with anti-streptavidin antibodies to simulate antibody-antigen detection systems.

Streptavidin is a small protein, and previous literature suggests that target molecule size may influence PDA color transitions (Seo et al., 2013). To account for this and to model larger biological targets, we conducted parallel experiments using both pure streptavidin and streptavidin-coated microbeads.

This approach allows for a systematic evaluation of PDA-based detection systems, potentially offering insights into size-dependent effects on sensor performance and establishing a foundation for future optimization strategies in antibody-antigen detection platforms.

5.2 MATERIALS AND METHODS

5.2.1 Materials

DA monomers, 10,12-Pentacosadiynoic acid (PCDA), was purchased from GFS Chemicals (Powell, OH, USA). DA monomers were dissolved in chloroform and filtered through a Corning® 0.2 μm polyether sulfone syringe filter to remove any polymerized or aggregated particles prior to use. Lipid, 1,2-dimyristoyl-sn-glycero-3-phosphocholine

membranes (EMD Millipore, 0.45 μm). All other reagents were purchased from Sigma Aldrich (St. Louis, MO, USA) and used without further purification.

5.2.2 DA-NHS Synthesis

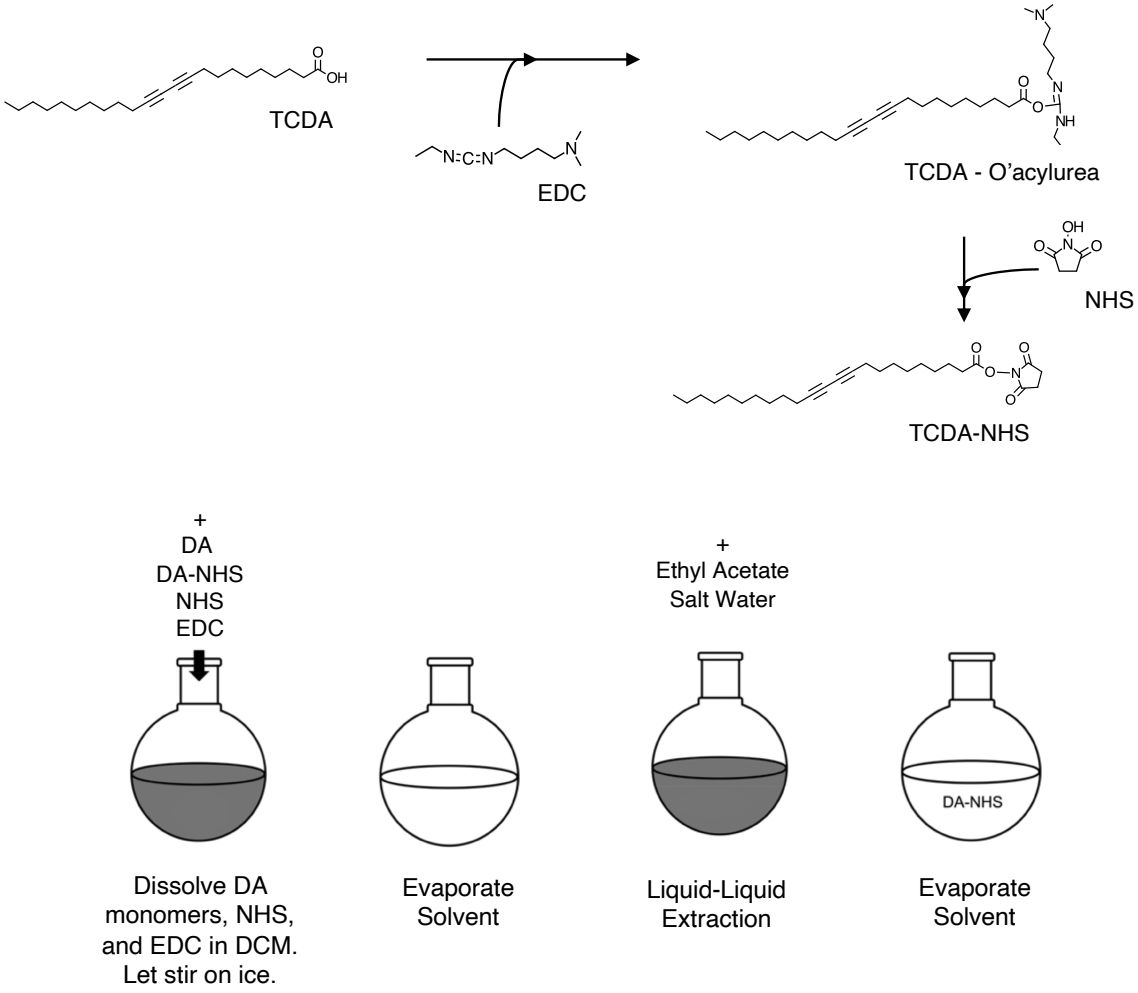

Figure 5.3 Schematic of DA-NHS Synthesis

NHS-active NHS is synthesized based on previously established protocols (Lee et al., 2008). 1.7 mmol NHS is dissolved in 10 mL dichloromethane in a round bottom glass flask with a magnetic stir bar under argon gas. Once fully dissolved, 1 mmol DA monomers are added and stirred for 5-10 minutes. The sealed round bottom flask is placed on ice. In a separate round bottom flask, dissolve 1.7 mmol EDC in 5 mL dichloromethane. Add the EDC dropwise to the DA-NHS solution. Let the solution stir overnight, covering the flask in foil to keep it from the light.

Using a rotary evaporator, remove solvent from the reaction solution. Add ethyl acetate to the round bottom flask with crude product and swirl to incorporate the product into the organic solvent. Add to a separatory funnel and add an equal volume of saturated salt water. Shake and let organic and aqueous layers separate. Remove the bottom aqueous layer with waste products and repeat the wash step 2 more times. Add sodium sulfate anhydrous to the organic layer to remove any residual water. Rotary evaporate the ethyl acetate. The final product is a white powder and is verified via ^{1}C-NMR and Mass Spectroscopy.

5.2.3 Antibody-Functionalized Liposome Synthesis

Liposome synthesis was performed according to the protocol described in Section 3.2.2. In brief, PCDA, PCDA-NHS, and DMPC were fully dissolved in 1 mL of chloroform. The solvent was then evaporated under a gentle stream of nitrogen gas, and the resulting lipid film was further dried in a vacuum desiccator for 3 hours. The dried lipid film was rehydrated with 5 mL of deionized water and incubated at 60°C for 15 minutes in

a heat block. The rehydrated lipid suspension was then sonicated using a probe tip sonicator at 20% amplitude for 15 minutes, with the probe submerged halfway into the solution. To remove any aggregates, the warm solution was filtered through a 0.8 μm cellulose syringe filter (Corning Inc., Corning, NY, USA) and allowed to cool to room temperature.

Antibodies were added to the cooled liposome solution, and then refrigerated for 4 hours or overnight to allow for the liposome backbone to stabilize and antibodies to conjugate. Subsequently, the solution as incubated with 150 μg/mL of Bovine Serum Albumin or 2.0 mM Ethanolamine for 1 hour at room temperature to block any remaining NHS sites.

100 μL aliquots of the liposome solution were dispensed into a microwell plate and subjected to UV-induced polymerization. This was achieved using a UVP Crosslinker Oven (Analytik Jena US LLC, Upland, CA, USA) with exposure to 625 kJ/cm² of UV light at 254 nm.

5.2.4 Antibody-Functionalized PVDF Strips

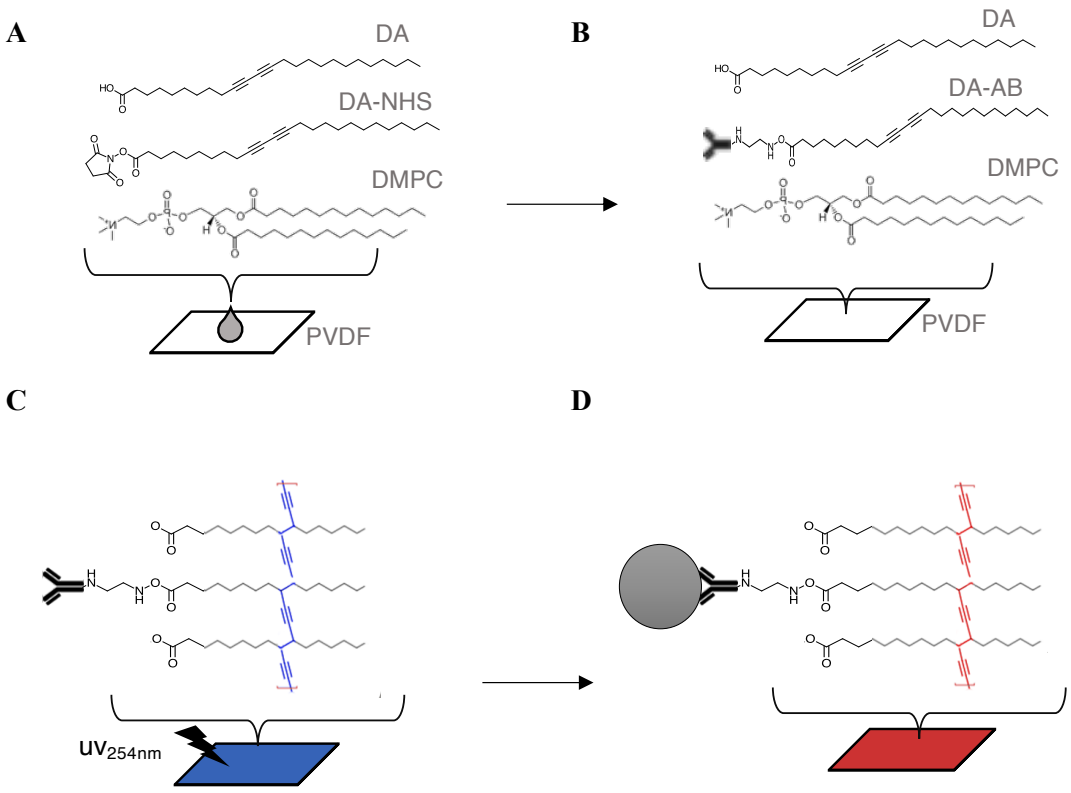

Figure 5.4 Protocol for Fabricating PVDF Sensor Membranes.
(A) DA monomers, DA-NHS monomers, and DMPC are dissolved in organic solvent. 1 cm x 1 cm PVDF squares are submerged in the chloroform mixture, immediately removed, and dried in the dark at room temperature for several hours. (B) Dried PVDF squares are incubated in 10 µg/mL antibody solution in the dark at 4°C overnight. The following day, PVDF membranes are incubated in 0.1% Bovine Serum Albumin for 1 hour to quench remaining active binding sites then allowed to dry for several hours. (C) PVDF membranes are polymerized under UV_{254nm} light for ~30 seconds. (D) PVDF sensors are incubated with the target molecule. When the antibody binds to the target, the PVDF sensor transitions from blue to red

PVDF sensor preparation is based on previously reported methods by Prainito and colleagues (Prainito et al., 2022). PVDF membranes are cut into 1 cm x 1 cm squares using a paper cutter. DA monomers, DA-NHS monomers, and lipid are dissolved in chloroform. Using tweezers, the PVDF strip in dipped in chloroform and promptly removed and left to dry, in the dark, and room temperature for 3-5 hours. Coated PVDF strips are then

submerged in antibody solution and placed in the dark, in the fridge, for four hours or up to overnight. Antibody-conjugated PVDF sensors are rinsed with DI water to remove unreacted antibodies, then incubated in 150 μg/mL BSA to block any remaining NHS active sites.

5.2.5 Characterization

Dynamic light scattering (DLS) using a Malvern Zetasizer Nano ZS90 (Malvern Panalytical, Malvern, Worcestershire, UK) analysis indicates the mean diameter of the liposomes. Absorbance value to calculate colorimetric response taken using an Agilent Cary 60 UV/Vis spectrophotometer (Agilent Technologies, La Jolla, CA, USA). Photos were taken on a Nikon D5100 digital camera. Photoshop and FIJI were used for digital colorimetric analysis.

5.3 RESULTS AND DISCUSSION

5.3.1 PCDA-NHS Synthesis

PCDA-NHS was synthesized and confirmed via TLC (3:1 Hexanes: Ethyl Acetate). Mass Spectroscopy, ^{1}H-NMR (400 MHz, CHLOROFORM-*d*) d ppm: PCDA-NHS - 0.89 (t, 3 H) 1.21 - 1.79 (m, 36 H) 2.25 (t, 4 H) 2.61 (t, 2 H) 2.84 (br. s., 4 H) (Figure 5.3)

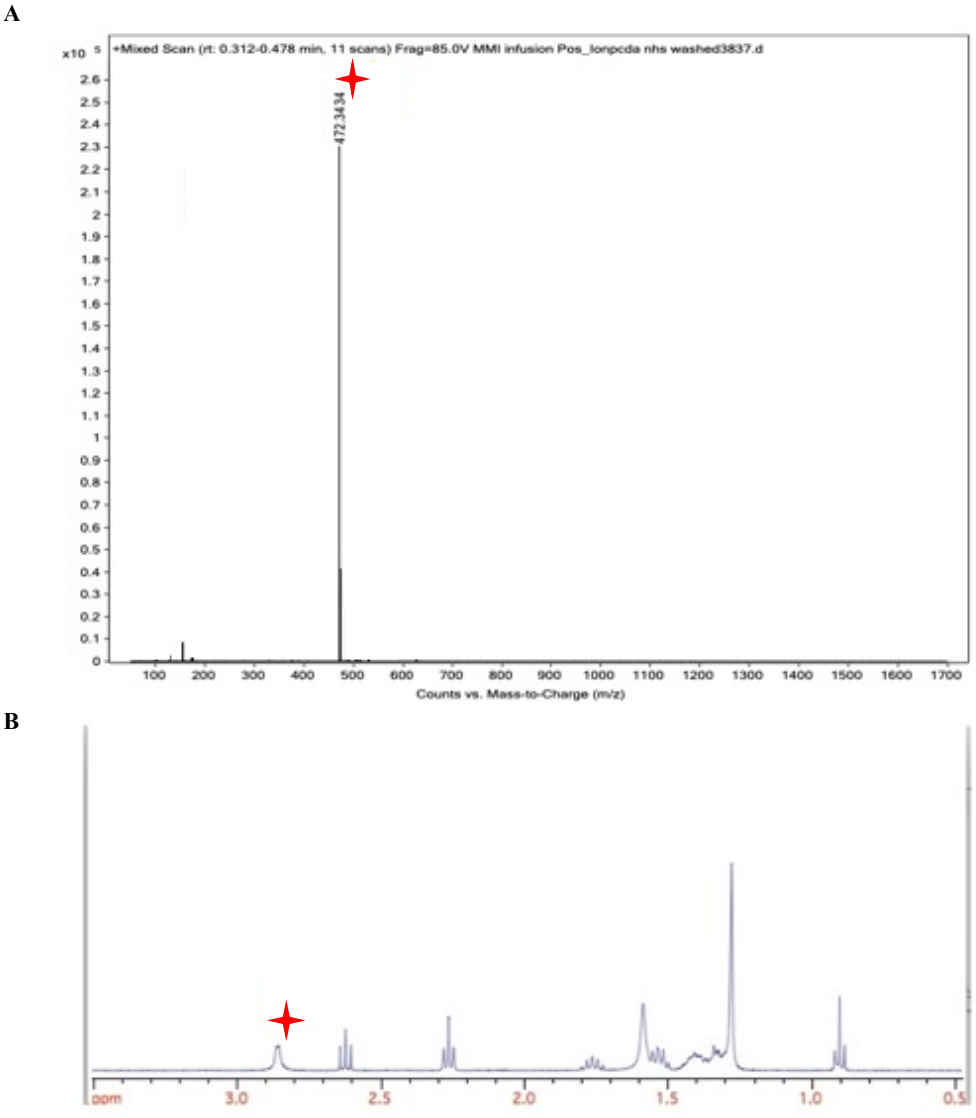

Figure 5.5 Mass Spectrocopy (A) and 1H-NMR (B) of PCDA-NHS.
The red-star represents PCDA-NHS (A) and the NHS group (B)

5.3.2 Anti-P. stewartii Antibody-functionalized Liposomes for Detection of P. stewartii

The development of antibody-conjugated PDA liposomes for detecting *P. stewartii* presents several challenges. A critical issue lies in the stability of the NHS-ester groups used for antibody conjugation. These groups are highly susceptible to hydrolysis in aqueous environments, especially under the high temperatures and turbulent conditions

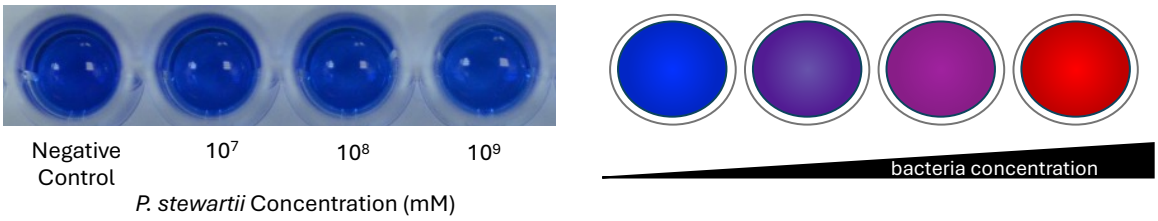

Figure 5.6 Liposomes treated with *P. stewartti* experimental results vs expected results.

employed during liposome formation. This instability may result in a significant loss of active binding sites for antibodies, potentially leaving insufficient functionalization for effective sensing. While, on the other hand, too many active binding sites can lead to over functionalization of the liposome. While initial verification of NHS-modified diacetylene monomers is typically performed using mass spectrometry or NMR, there is currently no reliable method to confirm the presence and activity of NHS groups post-liposome formation. This lack of verification extends to the antibody conjugation process, where quantifying the number and orientation of bound antibodies remains a challenge. These uncertainties in the functionalization process make it difficult to optimize the sensor's performance and troubleshoot poor sensor performance.

In this study, the effects of varying antibody concentrations, DA to DA-NHS monomer ratios, total lipid concentration, and UV dosage in liposome solutions were systematically investigated. PCDA was used as the DA monomer due to its frequent use in liposome studies for its balance of stability and sensitivity. Liposomes were also chosen to be doped with 60% DMPC, as it is shown to increase sensitivity of liposomes. Based on past literature, sensitivity is maximized by 60% while the liposome is still allowed to remain intact. Preliminary tests were conducted to select an appropriate blocking agent.

Although both BSA (Bovine Serum Albumin) and Ethanolamine are commonly used as blocking agents in literature, it was revealed by initial trials that immediate purple-phase PDA formation was caused by ethanolamine upon polymerization, even at low UV doses. Consequently, BSA was chosen as the preferred blocking agent for subsequent trials, as the desired colorimetric properties of the system were not interfered with by it.

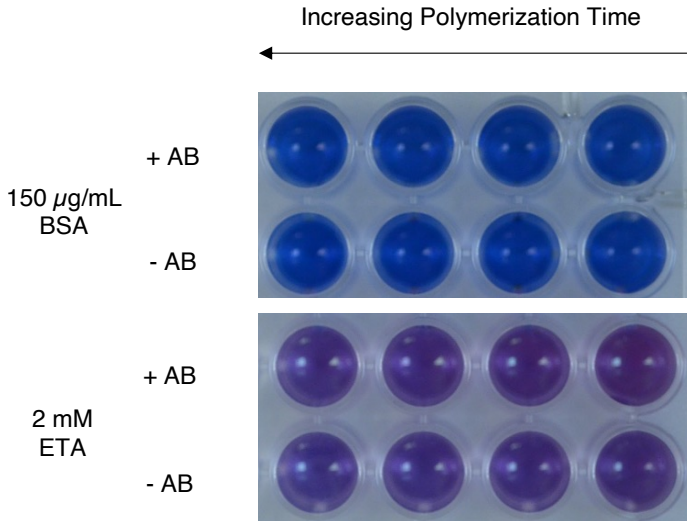

Figure 5.6 Optimization of Polymerization Time and Blocker
2 mM 3:2 PCDA:DMPC Liposomes, 98:2 PCDA:PCDA-NHS, 1 µg/mL anti-*P. stewartii* antibodies blocked with Bovine Serum Albumin (BSA) and Ethanolamine (ETA).

A range of key parameters was systematically explored. Four different liposome concentrations (0.5 mM, 1 mM, 2 mM, and 3 mM) and three ratios of DA to DA-NHS (10%, 30%, and 50%) were chosen (Figure 5.6B). Additionally, the effect of antibody concentration was examined by testing two levels: 1 µg/mL and 3 µg/mL (Figure 5.6A). The liposomes were exposed to *P. stewartii* cultures at a concentration of 1 x 10^9 CFU/mL and incubated for 4 hours at room temperature in dark conditions. Across all trials, no

discernible colorimetric shift was observed visually, and spectrophotometric measurements revealed no significant changes in absorbance values.

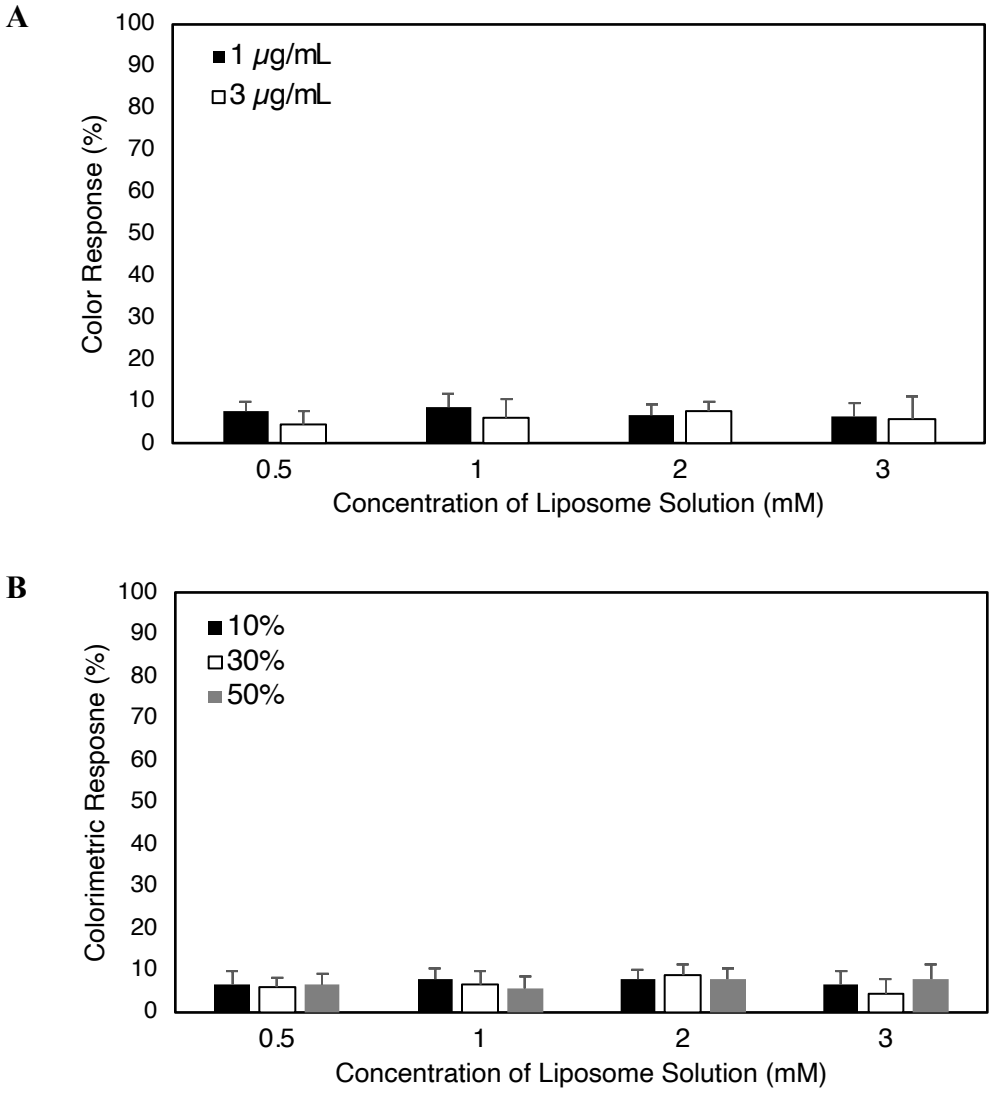

Figure 5.7 Colorimetric Response to Antibody-Functionalized Liposomes exposed to *P. Stewartii.*

To verify the efficacy of the antibodies and rule out potential issues with bacterial binding, we employed the commercially available Agdia ELISA kit as a control measure (Figure 5.8). This assay demonstrated a clear to yellow color change in the presence of bacteria, confirming that the antibodies successfully bind to the bacterial cultures utilized in our liposome studies. This indicates that the antibody-antigen interaction is not media-dependent, and functions as expected.

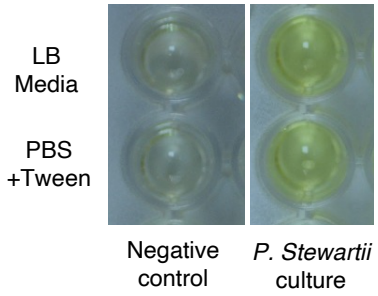

Figure 5.8 *P. Stewartii* Detection using commercially available ELISA kit.

Furthermore, to rule out any potential issues with the liposome's ability to shift colors, we exposed liposomes to heat and to NaOH as a positive control (Figure 5.9). Liposomes exposed to heat and low pH showed remarkable colorimetric response, from 75-85%

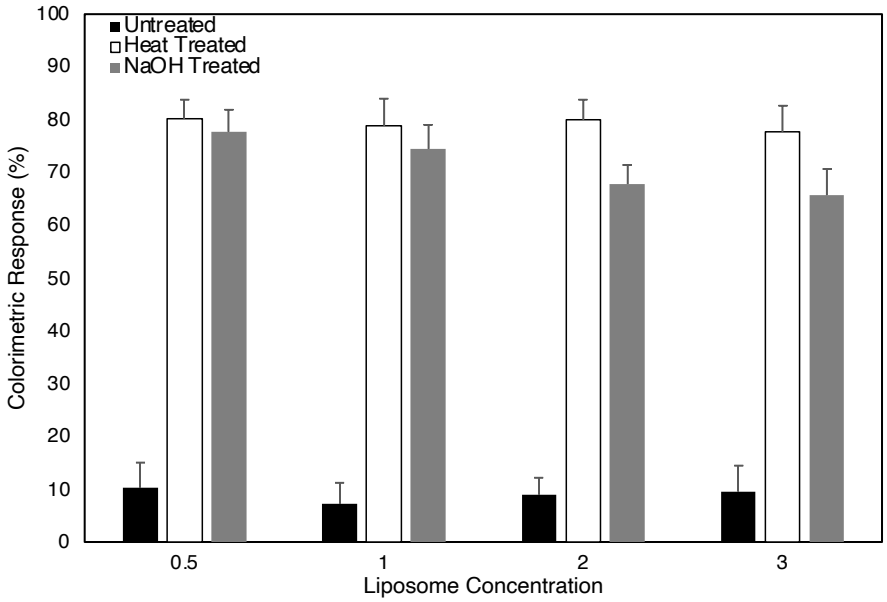

Figure 5.9 Liposome Response to Heat and Low pH

5.3.3 Anti-Streptavidin Antibody Functionalized Liposomes for Streptavidin Detection

In an effort to validate the experimental approach and establish a reliable model system, we extended our investigation to a well-characterized antigen-antibody pair: streptavidin and anti-streptavidin antibodies. This system was chosen for its robust and specific interaction, which has been widely used in various biosensing applications. Employing the same methodological framework developed for *P. stewartii* detection system, antibody concentration was the first parameter tested. For these trials, only 1 mM liposome solution was studied. PCDA was used as the DA, liposomes were doped with 60% DMPC, and 150 μg/mL BSA was used for blocking. No colorimetric change was observed (Figure 5.10)

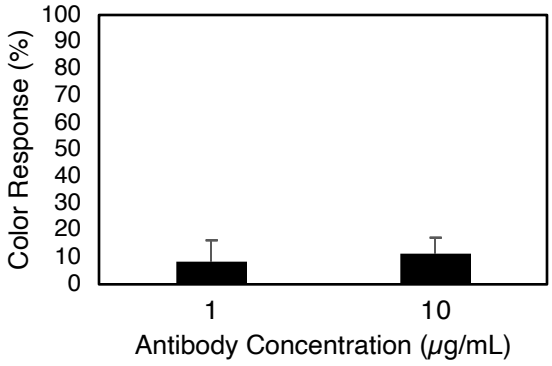

Figure 5.10 Colorimetric Response Antibody-Functionalized Liposomes exposed to *Streptavidin.*

Since streptavidin is a small protein and PDA color transition is effected by size of the target, streptavidin coated, clear, polystyrene, microspheres were utilized. Uncoated clear, polystyrene microspheres were used as a negative control. The same parameter as above was tested (Figure 5.11A) as well as 3 different amounts of PCDA-NHS (10%, 30%, and 50%) (Figure 5.11B). Across all conditions, there was no colorimetric change observed.

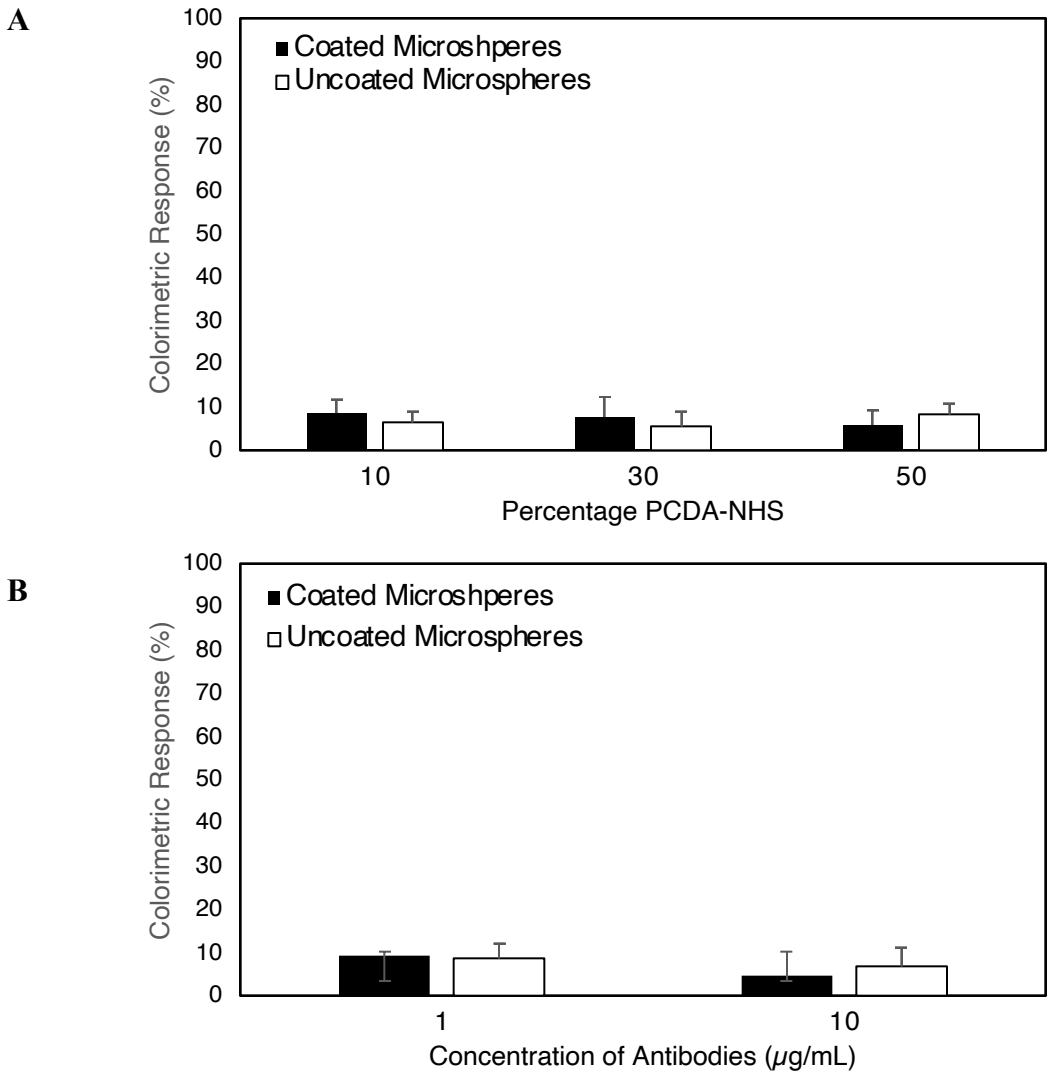

Figure 5.11 Colorimetric Response Antibody-Functionalized Liposomes exposed to Streptavidin-Coated Microspheres.

5.3.3 Anti-Streptavidin Antibody Functionalized PVDF Sensors for Streptavidin Detection

Following the challenges encountered with liposome-based sensors, an alternative approach using polyvinylidene fluoride (PVDF) membranes as a substrate for polydiacetylene (PDA) sensors was explored. PVDF membranes offer several advantages, including increased stability and ease of handling compared to liposomes. This section details the efforts to develop and optimize antibody-functionalized PVDF-PDA sensors for the detection of streptavidin, building upon the protocols established by Prainito et al. (Prainito et al., 2022). The transition to a solid support system aimed to overcome some of the limitations observed in the liposome-based sensors while maintaining the colorimetric detection capabilities of PDA.

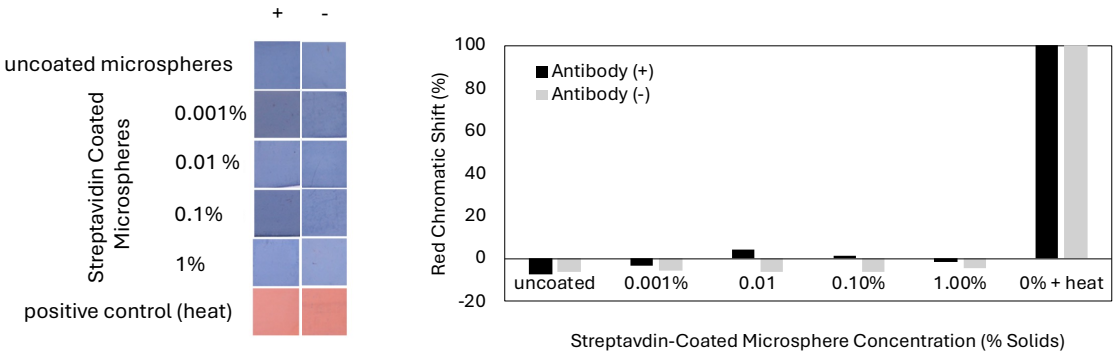

Figure 5.12 Colorimetric Response Antibody-Functionalized Liposomes exposed to Streptavidin-Coated Microspheres.

No colorimetric change was indicated in any of the test groups (5.12). These results indicate that while PVDF membranes offered practical advantages in sensor preparation, they did not resolve the fundamental challenge of achieving a detectable colorimetric response in this PDA-based biosensing system.

5.4 CONCLUSIONS

This chapter investigated the development and application of antibody-functionalized polydiacetylene (PDA) sensors for the detection of biological targets, focusing on the plant pathogen *P. stewartii* and the protein streptavidin. Two main approaches were explored: liposome-based sensors and PVDF membrane-based sensors. Despite systematic optimization of various parameters in liposome-based sensors, including antibody concentration, DA to DA-NHS ratios, total lipid concentration, and UV dosage, no significant colorimetric response was observed for *P. stewartii* or streptavidin detection. Control experiments confirmed both the efficacy of the antibodies (using ELISA) and the capability of the liposomes to undergo color transitions in response to heat and pH changes, highlighting the complexity of the challenge. In an effort to overcome the limitations of liposome-based systems, PVDF membranes were explored as an alternative substrate. While PVDF membranes offered increased ease of handling, they too failed to produce a discernible colorimetric change in streptavidin detection experiments.

While this study did not achieve the desired colorimetric detection of P. stewartii or streptavidin, it has provided valuable insights into the challenges of developing antibody-functionalized PDA sensors. The systematic approach taken and the exploration of both liposome and solid-support systems lay a foundation for future work in this field. These results underscore the complexity of biosensor development and highlight the need for continued innovation in materials and detection strategies for sensitive and specific pathogen and protein detection.

CHAPTER 6:

LIGAND-FUNCTIONALIZED PDA-COATED PVDF SENSORS FOR DETECTION OF CARBONIC ANHYDRASE

6.1 INTRODUCTION

6.1.1 Carbonic Anhydrase

Carbonic anhydrase (CA) is a ubiquitous enzyme found in various organisms, including humans, that plays a crucial role in physiological processes. CA catalyzes the rapid interconversion of carbon dioxide and water to bicarbonate and protons, a reaction essential for maintaining acid-base balance in the body. In humans, carbonic anhydrase is present in red blood cells, kidney tubules, and other tissues, where it facilitates the transport of carbon dioxide from tissues to the lungs for exhalation. Its importance extends to several bodily functions, including pH regulation, electrolyte secretion, and gas exchange. Due to its significance, carbonic anhydrase inhibitors have found medical applications in treating conditions such as glaucoma, altitude sickness, and certain types of seizures (Supuran, 2016). CA is an ideal model system for studying protein-ligand binding (Krishnamurthy et al., 2008). Its monomeric, single-chain structure is simple and stable, it is widely commercially available, relatively low cost, and well-characterized.

6.1.2 Polydiacetylene Sensor Detection of Carbonic Anhydrase

This study aims to leverage the well-characterized carbonic anhydrase (CA) system to further advance the development and understanding of polydiacetylene (PDA) sensors. By modifying a diacetylene monomer with a small ligand that acts as a CA inhibitor, we seek to create a model system that can demonstrate the potential of PDA-based biosensors for protein-ligand interactions. Carbonic anhydrase inhibitor, *4-(2-aminoethyl)benzenefulfonamide* (AEBS), will be used as the ligand and will be

functionalized on the DA headgroup. PVDF strip sensors will be fabricated with the AEBS functionalized DA monomers for the specific detection of carbonic anhydrase.

6.2 MATERIALS AND METHODS

6.2.1 Materials

DA monomers, 10,12-Pentacosadiynoic acid (PCDA), was purchased from GFS Chemicals (Powell, OH, USA). DA monomers were dissolved in chloroform and filtered through a Corning® 0.2 μm polyether sulfone syringe filter to remove any polymerized or aggregated particles prior to use. CA ligand, 4-(2-aminoethyl)benzenesulfonamide, was purchased from TCI America (Portland, OR, USA). Lipid, 1,2-dimyristoyl-sn-glycero-3-phosphocholine, was obtained from Avanti Polar Lipids (Alabaster, AL, USA). 0.45 μm PVDF membranes were purchased at EMD Millipore. All other reagents were purchased from Sigma Aldrich (St. Louis, MO, USA) and used without further purification.

6.2.2 PVDF Strip Preparation

PVDF sensor preparation is based on previously reported methods by Prainito and colleagues (Prainito et al., 2022). PVDF membranes are cut into 1 cm x 1 cm squares using a paper cutter. DA monomers, DA-NHS monomers, and lipid are dissolved in chloroform. Using tweezers, the PVDF strip in dipped in chloroform and promptly removed and left to dry, in the dark, and room temperature for 3-5 hours (Figure 6.1). Coated PVDF strips are then submerged in AEBS in DI water and placed in the dark, in the fridge, for four hours or up to overnight. AEBS-conjugated PVDF sensors are rinsed with DI water to remove

unreacted AEBS, then incubated in 150 µg/mL BSA to block any remaining NHS active sites. The PVDF strips are rinsed with DI water and left at room temperature, in the dark, to dry for 4-8 hours. Once dry, PVDF strips are placed in a UV Oven and exposed to 50 kJ of 254 nm light. The strips should be stored in the fridge, in the dark, until ready for use.

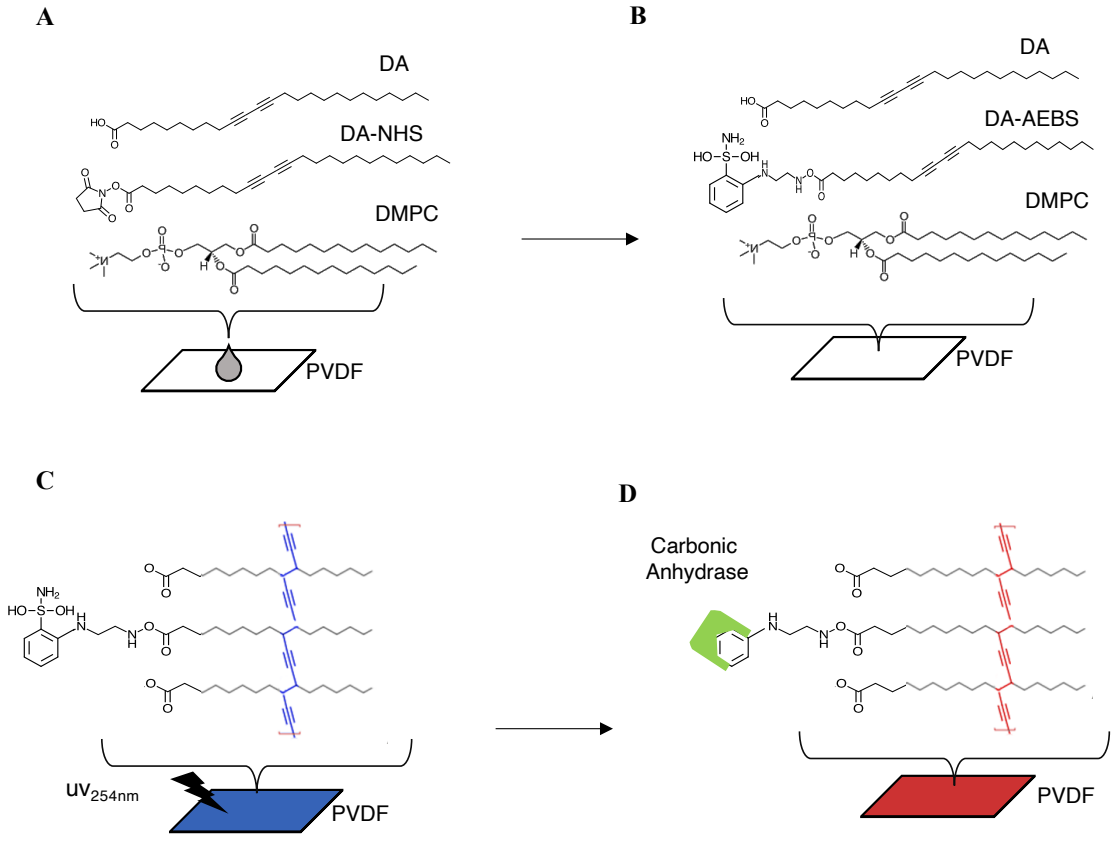

Figure 6.1 Schematic of DA-AEBS PVDF Strip Fabrication for CA detection.

6.2.3 DA-ligand Synthesis

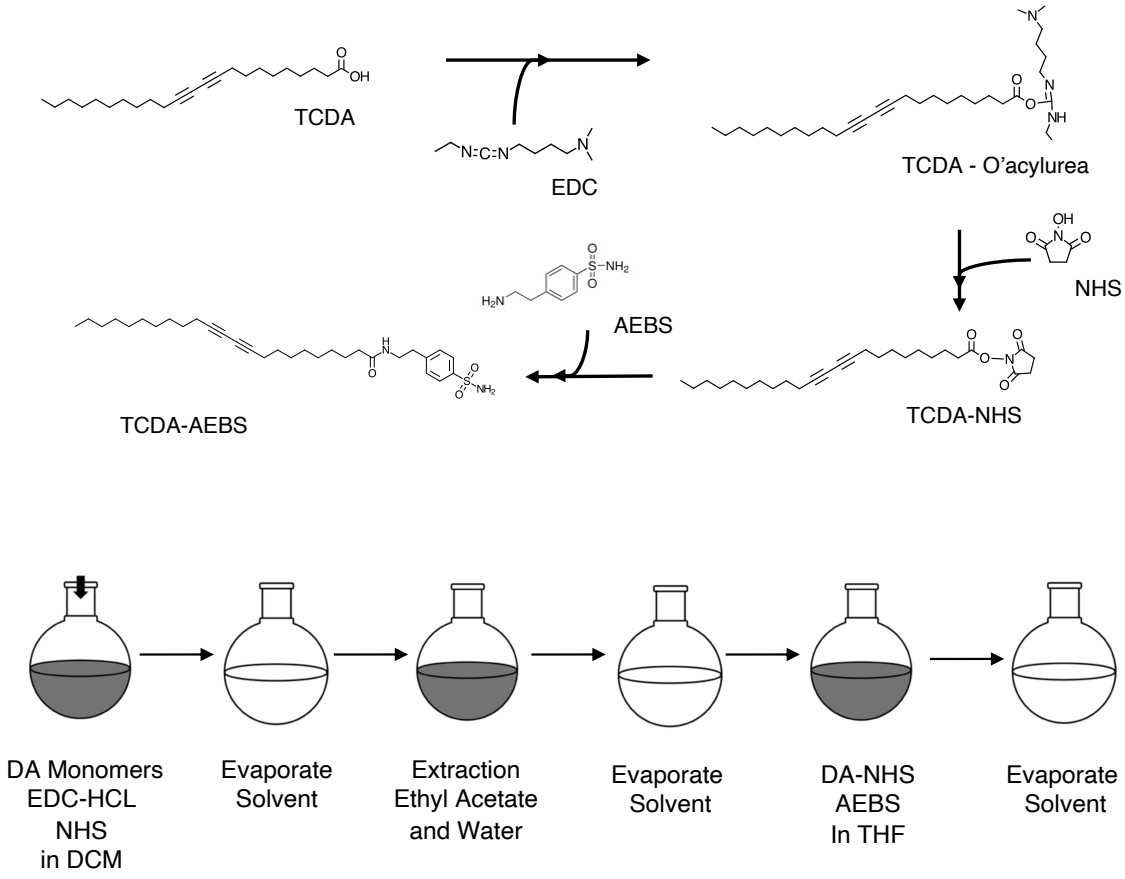

Figure 6.2 DA-AEBS Synthesis Reaction (A) and Schematic of DA-AEBS Synthesis.

Let all glassware bake in an oven set to 105 °C overnight. Remove glassware from oven, place a rubber septum on the flask. Allow to cool under argon gas, and purge air from flask. 1.7 mM NHS is dissolved in 10 mL DCM in the sealed round bottom flask, under argon gas, with a magnetic stir bar. 1.7 mM of filtered DA monomer is added to the NHS solution and allowed to stir at room temperature until both are fully dissolved. 1.7 mM of EDC-HCL is dissolved in 5 mL of DCM in a separate, sealed round bottom flask, under argon. Once dissolved, a glass syringe with a long, flexible needle, is used to add EDC-

HCL to the DA-NHS solution dropwise. The sealed flask with DA, NHS, and EDC is covered with foil, placed on an ice bath, and allowed to stir overnight.

The solvent is removed via rotary evaporation. The product is washed with water and ethyl acetate to remove unreacted starting material and waste products, namely urea. The solution is placed in a separatory funnel and the bottom, aqueous layer, is discarded. Sodium sulfate anhydrous is added to the organic layer to remove any additional water. The solvent is removed via rotary evaporation and the DA-NHS is confirmed via TLC suing 3:1 hexanes: ethyl acetate, ^{1}H-NMR, and Mass Spectroscopy.

Immediately, DA-NHS and equimolar amount of AEBS are dissolved in a sealed round-bottom flask in 10 mL THF in a sealed round bottom flask, under argon gas, with a magnetic stir bar. Let stir for 4 hours. Remove solvent via rotary evaporation. Confirm product with TLC (100% ethyl acetate) and Mass Spectroscopy.

6.3 RESULTS AND DISCUSSION

6.3.1 PVDF Strips for Carbonic Anhydrase Detection

The first step toward developing a PVDF sensor for carbonic anhydrase detection, PVDF strips were coated in DA-NHS monomers and incubated with 5 different concentrations of ligand, AEBS, and left to conjugate for 4 hours.

PVDF strips were prepared and incubated with water (negative control) and 10 µg/mL of carbonic anhydrase. Colorimetric response of the AEBS functionalized PVDF strip showed a slight color change (~30%) compared to the control condition (Figure 6.3).

While the colorimetric response is low, it indicates that carbonic anhydrase may be detected by PDA and further optimization could be used to increase sensitivity of the sensor.

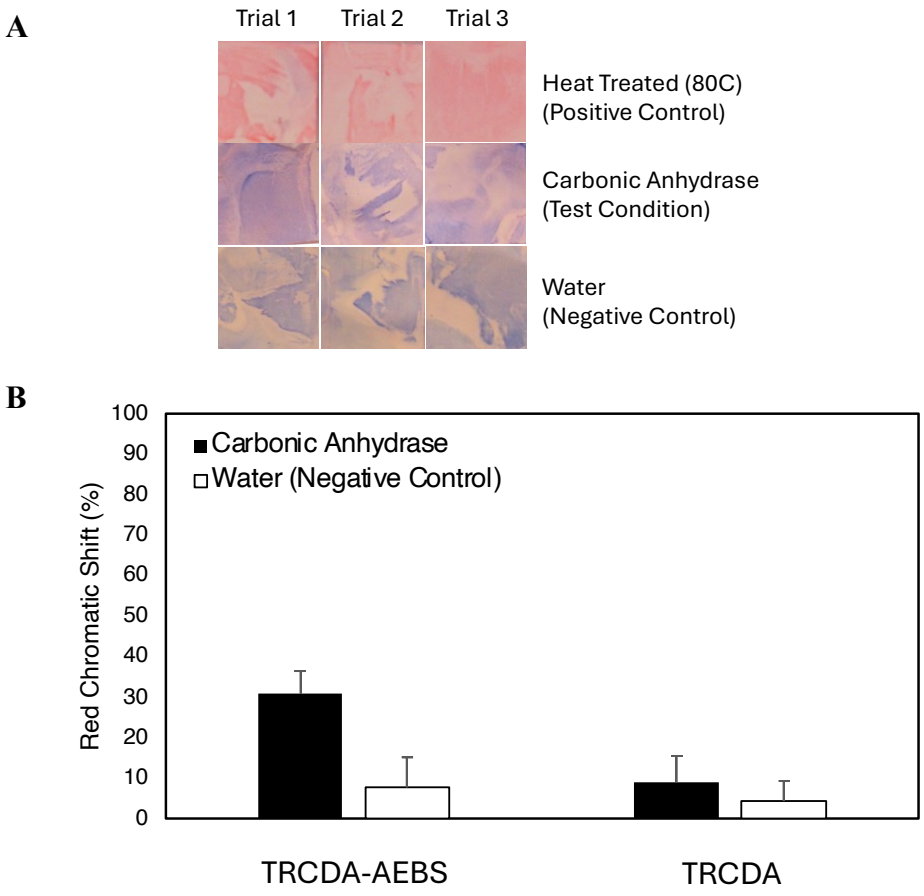

Figure 6.3 Strip Sensors (A) and Red Chromatic Shift of PCDA-AEBS Detection of Carbonic Anhydrase (B)

6.3 CONCLUSION

A PDA sensor for detecting carbonic anhydrase (CA) was developed. Carbonic anhydrase was selected as a model system due to its well-characterized nature and importance in protein-ligand binding studies. The biosensor was created by modifying diacetylene monomers with 4-(2-aminoethyl)benzenefulfonamide (AEBS), a known CA inhibitor. Although a low colorimetric response (~30%) was observed when the sensor was exposed to carbonic anhydrase, a detectable change was noted. This suggests potential for further refinement of this PDA-based biosensor for protein-ligand interactions.

CHAPTER 7:

ADVANCING ACCESS: POLICY RECOMMENDATIONS FOR WIDESPREAD IMPLEMENTATION OF POINT-OF-USE DIAGNOSTICS

7.1 EXECUTIVE SUMMARY

Point-of-use diagnostics offer significant potential to improve healthcare access and outcomes by enabling rapid, on-site testing, particularly in resource-limited settings and during public health crises. However, challenges such as reduced accuracy, higher per-test costs, and quality control issues must be addressed for successful implementation. The COVID-19 pandemic highlighted both the critical importance of rapid, accessible testing and the need for robust supply chains, quality control, and data integration systems. To maximize the benefits of point-of-use diagnostics, policymakers should develop comprehensive national strategies, establish strong regulatory frameworks, and invest in workforce training and development.

7.2 BACKGROUND

Diagnostics play a fundamental and central role across multiple critical domains, including: healthcare, agriculture, environmental monitoring, and forensics. This brief will focus on diagnostics in healthcare settings, though it should be noted that these principles could be applied across disciplines. Traditional laboratory diagnostic technologies, such as qPCR, are sensitive and specific. They detect disease early on and with high accuracy. However, they require trained personnel, expensive equipment, and turnaround time can take days.

Point-of-use diagnostics, also known as point-of-care testing, represent a paradigm shift in healthcare delivery and disease management. These rapid, portable, and user-friendly diagnostic tools enable healthcare providers to perform tests and obtain results in

real-time, often in non-traditional settings such as patients' homes, community clinics, or remote areas. The importance of point-of-use diagnostics cannot be overstated in today's global health landscape. They offer the potential to democratize access to crucial medical information, reduce diagnostic delays, and ultimately improve patient outcomes. By bringing sophisticated testing capabilities directly to the patient, these technologies bridge gaps in healthcare infrastructure, particularly in resource-limited settings. Moreover, point-of-use diagnostics play a pivotal role in managing infectious diseases, chronic conditions, and public health crises, offering rapid decision-making capabilities that can significantly impact treatment strategies and disease containment efforts.

While there are many benefits to adopting point of care testing, there are several drawbacks. One of the primary concerns is the potential for reduced accuracy compared to centralized laboratory testing, which can lead to false positives or negatives, potentially compromising patient care. This issue is exacerbated by the fact that POC tests are often performed by non-laboratory personnel who may lack specialized training in quality control and error recognition. Additionally, the cost per test for POC diagnostics is typically higher than centralized testing, which can strain healthcare budgets, especially in resource-limited settings. The portability of POC devices, while advantageous, also exposes them to environmental factors such as temperature fluctuations and humidity that can affect test performance. Quality assurance and regulatory compliance can be challenging to maintain across multiple testing sites, particularly in remote or underserved areas. These are all factors that must be considered when developing a national strategy to integrate POC diagnostics into healthcare systems.

7.3 A CASE STUDY: COVID-19

The COVID-19 pandemic provided valuable lessons about the critical role of point-of-care (POC) diagnostics in managing public health crises. One of the most significant insights was the importance of rapid, widely accessible testing in controlling disease spread. POC tests, particularly rapid antigen tests, proved instrumental in quickly identifying infected individuals, enabling prompt isolation and contact tracing. This experience highlighted the need for a robust, decentralized testing infrastructure that can be rapidly scaled up in response to emerging threats. The pandemic also underscored the importance of balancing test sensitivity with speed and accessibility. While PCR tests were more sensitive, the faster turnaround time of POC tests often made them more practical for large-scale screening and triage. Most notably, it accelerated innovation in POC diagnostics, leading to the development of new testing platforms and protocols that could have lasting impacts on disease diagnosis and management beyond COVID-19.

However, the pandemic also exposed significant challenges in the POC diagnostics field. The initial shortage of testing capacity revealed vulnerabilities in supply chains and manufacturing capabilities, emphasizing the need for diversified production and stockpiling of essential components. Quality control issues with some rapidly developed tests highlighted the importance of maintaining rigorous standards even in emergency situations. The experience also demonstrated the critical need for clear communication about test performance characteristics and appropriate use to healthcare providers and the public. Additionally, the pandemic revealed gaps in data integration and reporting systems, as many POC test results were not effectively captured in public health surveillance efforts.

This underscored the need for better digital infrastructure to collate and analyze POC test data in real-time. Lastly, the disparities in access to testing, particularly in low-resource settings and underserved communities, highlighted the ongoing need to develop affordable, easy-to-use POC diagnostics that can function reliably in diverse environments.

7.4 POLICY RECCOMENDATIONS

1. Develop national diagnostic strategies that incorporate POC testing. Countries should create comprehensive, costed national plans for implementing integrated, tiered diagnostic networks that include POC testing. These strategies should outline how POC diagnostics will be incorporated into existing healthcare systems, particularly at the primary care level.

2. Establish robust quality assurance and regulatory frameworks. Implement policies to ensure the quality, safety, and reliability of POC diagnostics. This includes developing national regulatory frameworks for device approval, quality control, and post-market surveillance.

3. Invest in workforce development and training: Create policies to expand and upskill the healthcare workforce to effectively utilize POC diagnostics. This includes updating training curricula, implementing task-shifting programs, and providing ongoing education and competency assessments for POC test operators.

CHAPTER 8:

CONCLUSION

This research has explored the development and optimization of polydiacetylene (PDA)-based sensors for colorimetric sensing. We studied the potential use of PDA liposome-based aqueous sensors and PDA-coated PVDF sensors.

A thorough investigation of liposome synthesis parameters was conducted, identifying optimal conditions for producing uniform and responsive PDA assemblies. This work has resulted in a reproducible protocol for PDA liposome preparation that can be readily adopted.

DA-coated PVDF sensors were studied. The imact of of diacetylene monomer structure and lipid doping on sensor performance on PVDF strips was determined. It was demonstrated that shorter alkyl chains in the diacetylene monomers led to increased sensitivity, likely due to reduced intermolecular forces and increased molecular mobility. Additionally, moderate lipid incorporation was found to enhance sensitivity while maintaining stability. These insights provide valuable guidance for the rational design of future PDA-based sensors, allowing for the fine-tuning of sensor properties to meet specific application requirements.

Efforts to develop antibody-functionalized PDA sensors for detecting plant pathogens and model proteins revealed both the potential and limitations of this approach. While challenges were encountered in achieving the desired sensitivity and specificity, particularly for the detection of *Pantoea stewartii*, these experiments provided crucial insights into the complexities of integrating biological recognition elements with PDA systems. The lessons learned from these studies will inform future efforts to create more effective antibody-based PDA sensors.

To address some of the limitations encountered with antibody-based systems, an alternative strategy using small-molecule ligands for protein detection was explored. This approach, exemplified by the work on carbonic anhydrase detection using inhibitor-functionalized PDAs, shows promise for expanding the range of analytes that can be detected using PDA-based sensors.

Despite progress made, several challenges remain to be addressed in future research. These include improving the sensitivity and specificity of antibody-functionalized PDA sensors, enhancing the stability of PDA sensors under various environmental conditions, expanding the range of detectable analytes, developing strategies for multiplexed detection, and addressing potential regulatory challenges associated with the implementation of novel diagnostic technologies.

Continuing to elucidate PDA interactions will allow for expansion of PDA-based sensor technology, which could play a crucial role in addressing global health and food security challenges. By providing accessible, rapid, and reliable diagnostic solutions, PDA sensors can empower healthcare workers, farmers, and individuals to make informed decisions and take timely action in managing diseases and crop health.

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