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A proposed mechanism to induce multi-layer polydiacetylene-coated filter color response to bacteria

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ABSTRACT

The purpose of this study was to develop a multi-layer diacetylene (PDA)-coated filter and to verify the proposed color response mechanism of PDA-coated filters to bacteria. Unlike other reports which immobilize PDA liposomes on solid support or create monolayer Langmuir films, multi-layer PDA-coated filters were generated by directly evaporating organic solvents. Different from incorporating functional headgroups or inserting phospholipids into PDA, we reported that bacterial growth can trigger the color change of PDA sensors without any modification or phospholipid insertion. The mechanism that pH change from bacteria metabolites lead to color change of PDA filter was proposed and verified by carefully designed dextrose-free medium with phenol red. Further, culturing Salmonella Typhimurium, E. coli, L. innocua and M. luteus with PDA-coated filters on phenol red agar in absence of dextrose verified the potential of applying PDA-coated filter for bacterial detection, specifically amine-producing bacteria. Thus, PDA-coated filters may be a useful tool for food safety and shelf life applications.

1. Introduction

Polydiacetylenes (PDAs) are highly conjugated polymers assembled from monomers containing two conjugated diacetylenes (DAs). DAs undergo photopolymerization via a 1,4-addition to form a repeating double-single-triple bond pattern along the carbon backbone. The highly-conjugated material takes on an intense blue appearance and has rather strict geometrical arrangement [1]. Stimuli such as heat [2], pH change [3], mechanical stress [4] and chemical solvents [5] have been reported to lead to an obvious color change of PDAs from blue to a red/pink color. This color change can be observed easily by the naked eye. It has been widely accepted that the color change of PDA is highly related to PDA geometry and comes from the conformational change of the PDA backbone [6]. Considering the chromatic properties of PDAs, it serves well in applications for visual detection of biological or chemical substances.

Liquid-based and substrate-based forms are typical forms of PDA-based sensors. A well-known example of liquid-based PDA is self-assembled liposomes or so-called vesicles. Self-assembled, non-spherical structures such as flat sheets [7], tubules [8,9], helices [10], and ribbons [11,12] have also been observed when supplemented with phospholipids or modified with headgroups. Examples of substrate-based forms are self-assembled monolayers [13], multi-layer coatings on surfaces [14], fibers [15], nanotubes [16], and liposome-immobilized on a solid support [17]. Currently, most reported PDA-based sensors for biological application were in liquid-based form especially liposomes [18,19] due to the relative ease of PDA modification. Reported substrate-based PDA sensors for biological application have focused on immobilizing liposome on glass [20] or embedded in semi-solid substrates such as agar [20–22] or sodium alginate [23]. Substrate-based sensors such as paper-based PDA sensors are mostly reported for chemical detection [14,24] but seldom reported for biological applications. In this study, we generated a substrate-based PDA sensor, multi-layer PDA on filter by directly evaporating an organic solvent containing PDA followed by photopolymerization.

Development of PDA-based biosensors focus on incorporating functional headgroups to enhance sensor specificity and sensitivity. Target biomolecules or chemical species interact with the incorporated functional headgroups that change the conformation of the PDA backbone and therefore trigger color change of the biosensor. Nagy et al. [25] reported incorporating Gal-α1,4-Gal functional headgroups into PDA nanoparticles for detection of Shiga-like toxin-producing E. coli O157:
HunterLab UltraScan® Pro dual-beam spectrophotometer was used to multi-layer blue color poly-PCDA coated membrane 2 min followed by exposure to UV at 254 nm for 1 min to obtain the “eliminate aggregates then stored at 4 °C until use. The PCDA stock solution of dextrose supplement was used as a direct indicator to monitor pH change due to bacterial growth. Dextrose at a 5% concentration was used to supply phenol red broth with dextrose. S. Typhimurium, E. coli, L. innocua and M. luteus were cultured in phenol red broth without and with dextrose at 37 °C with mixing at 60 rpm for 16 h then centrifuged at 3000 g for 5 min to harvest the suspension. The pH of suspension was measured and recorded. PDA-coated filter discs were added to suspensions and kept at 37 °C with mixing at 60 rpm for 2 h. PDA-coated discs were then taken from metabolite-supernatant and photos were taken using iPhone 6 s in Samtian photo box (LED light source at maximum) to record the color change of PDA-coated filter discs.

2.4. Effect of metabolites on pH and color response of PDA-coated filters

Furthermore, the pH of another set metabolite-supernatant was adjusted to 7 with HCl (0.1 N) for phenol red broth without dextrose and adjusted to pH = 8 with NaOH (0.1 N) for phenol red broth with dextrose to investigate pH effect on PDA-coated filter discs. PDA-coated filter discs were then added to metabolite-supernatants and kept at 37 °C with mixing at 60 rpm for 2 h. PDA-coated discs were then taken from metabolite-supernatant and photos were taken using iPhone 6 s in Samtian photo box to record the color changes of PDA-coated filter discs.

2.5. Application of PDA-coated filters for bacteria detection

Phenol red in the absence of dextrose was used to culture S. Typhimurium, E. coli, L. innocua and M. luteus. The pH of phenol red agar without dextrose was adjusted to pH 7.2 before autoclaving. Salmonella Typhimurium and E. coli were cultured in TSB overnight for 16 h then centrifuged at 3000 g for 5 min and washed twice with phosphate buffered saline (PBS) and then adjusted to ~108 cells/ml by diluting with phosphate buffer. Cell concentration was estimated by light transmission at 600 nm adjusting O.D. = 0.5 and verified by the plate count method. One ml of 108 cells/ml was added to 99 ml of PBS followed by filtering through PDA-coated filters. After filtration, PDA-coated filters were transferred to phenol red agar and incubated at 37 °C for 22 h after which plates were photographed.

3. Results and discussion

3.1. Characterization of PDA-coated filters

PDA-coated filters displayed blue color with a relatively uniform coating based on visual inspection. Reflectance to transmittance spectrum of PDA-coated filters quantified color of PDA-coated filters as shown in Fig. 2 and can be used as a measurement of reproducibility of PDA-coated filters. Average CIE L’ a’ b’ values for all filters used in the experiment were L* = 51.53 ± 3.95, a* = 1.36 ± 1.48, b* =

![Fig. 1. Scheme of coating PCDA on filters and small discs cut from larger filter.](image-url)
30.83 ± 1.34 with Chroma calculated as 30.8 ± 2.0. The amount of PDA coated on the filters was difficult to quantify because of the coating method; however, the reflectance to transmittance spectrum and average CIEL*a*b* values of PDA-coated filters provided a measurement for estimating the consistency of PDA-coatings.

3.2. Effect of dextrose supplement on bacteria metabolites and color response of PDA-coated filters

Phenol red indicates the pH change of the growth medium due to bacteria metabolites, changing color from orange to magenta (alkaline) or yellow (acid) as shown in Fig. 3 Metabolite-supernatant. Culturing bacteria in broth overnight and then utilizing metabolite-supernatant without cells for testing ensured harvesting all metabolites from bacteria growth as well as eliminated the possibility of PDA-coated filter interacted directly with bacterial cells. Control group of non-inoculated phenol red broth and phenol red broth with dextrose did not trigger color transition of PDA-coated filter discs (Fig. 3 Control). This result indicated that the medium itself did not trigger the color transition of PDA-coated filter discs. Moreover, the color change of PDA-coated filter discs from blue to pink was observed after being treated with metabolite-supernatant of phenol red without dextrose but not metabolite-supernatant of phenol red with dextrose (Fig. 3 PDA-coated filter discs). *L. innocua* cultured in phenol red broth without dextrose is an exception and can be explained by *L. innocua* cannot grow without dextrose supplement in phenol red broth and therefore no

![Fig. 2.](image-url) a. Reflectance to transmittance spectrum of PDA-coated filters. b. PDA-coated filter. c. PDA-coated filter discs.

![Fig. 3.](image-url) Effect of dextrose on bacteria metabolite and color response of PDA-coated filter discs. Columns with the same label originated from the same sample. (P = Phenol red broth, PD = Phenol red broth with dextrose; + indicated color change, − indicated no color change.) (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
metabolites were produced in supernatant. These observations revealed that bacteria metabolites from growing on phenol red broth without dextrose triggered the color change of PDA-coated filter discs while metabolites which come from growing on phenol red broth with dextrose supplement cannot trigger color change of PDA-coated filter discs. Comparing PDA-coated filter discs incubated in phenol red broth with and without dextrose supplement, we observed that bacteria produce different metabolites during growth when in supply or in absent of dextrose. Those metabolites vary pH of supernatant and this pH change may play an important part in color transition of PDA-coated filter discs.

![Fig. 4. Effect of metabolite-supernatant pH adjustment on color response of PDA-coated filter discs. (n = 2) (a) pH adjustment for phenol red supernatant with dextrose from acid to pH = 8 (b) pH adjustment for phenol red supernatant without dextrose from alkaline to pH = 7. Rows with the same label originated from the same sample. (PD = Phenol red supernatant with dextrose, PD.A = Phenol red supernatant with dextrose adjusted, P = Phenol red supernatant, PA = Phenol red supernatant adjusted; + indicated color change, − indicated no color change). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)](image-url)
3.3. Effect of pH on color response of PDA-coated filters

Metabolites due to bacterial growth increased the pH of phenol red broth without dextrose and decreased the pH of phenol red broth with dextrose. To investigate pH effect on color response of PDA-coated filter discs, metabolite-suspension pH was adjusted from acid to alkaline and from alkaline to neutral. Color change of PDA-coated filter disc was observed when adjust the metabolite-solution pH in phenol red broth with dextrose from acidic to pH 8 (Fig. 4, P.A column). This phenomenon indicated that pH can trigger color change of PDA-coated filter discs. Adjustment of the metabolite-solution pH in phenol red broth from alkaline pH to pH 7 did not trigger a color change in PDA-coated filter discs (Fig. 4, P.A column). This observation eliminated the possibility of other metabolites which cannot change pH of supernatant trigger color response of PDA-coated filters since these metabolites were maintained in supernatant when adjusting pH to neutral but color change of PDA-coated filter was not observed. In summary, these observations supported the theory that bacteria metabolites which can increase the pH of supernatant induce color change of PDA-coated filter and implied that PDA-coated filter may be applied for amine producing bacteria detection.

3.4. Mechanism for color change of PDA-coated filters

Microorganisms frequently change the pH of their habitat by producing acidic or basic metabolic waste products [28]. With dextrose supplementation, microorganisms can catabolize carbohydrates into pyruvate and similar intermediates and then enter the tricarboxylic acid (TCA) cycle pathway to produce energy [28]. Metabolites from carbohydrate are acidic which decrease the microbial habitat pH. In absence of dextrose supplement, microorganisms utilize proteins, peptides or amino acids as an energy source. Decarboxylation of amino acid will produce CO₂ and amine products which increases the microbial habitat pH. It has been reported that Salmonella and Escherichia spp. are capable of amine production [29,30]. This pH change caused by bacteria growth was verified in this experiment by culturing Salmonella Typhimurium, E. coli, L. innocua and M. luteus in phenol red broth with or without dextrose supplement. The mechanism of bacteria metabolites increase environment pH and therefore lead to color change of PDA-coated filter was proposed based on pH adjustment observations and analysis. The proposed mechanism was further supported by the fact that it has been well known that alkaline pH triggers the color change of PDA liposome and PDA monolayers. Possible explanation is that alkaline pH results in the ionization of the carboxyl group of PDA in PDA coated filters and the consequences of ionization are breakdown of hydrogen-bonding network in PDA and electrostatic repulsion between adjacent carboxylate groups [12,31]. Stress created from electrostatic repulsion accumulates in the PDA backbone and eventually twists the PDA backbone. This twist of the PDA backbone causes the color change of multi-layer PDA coated filters from blue to pink.

3.5. Application of PDA-coated filters for bacteria detection

To investigate the possibility of applying PDA-coated filters for bacteria detection, S. Typhimurium, E. coli, L. innocua and M. luteus were filtered through PDA-coated filters and cultured on phenol red agar without dextrose. Fig. 5 shows the top of the plate which is the PDA-coated filter and the bottom of the plate which is the phenol red agar. Consistent with growing bacteria in broth, bacteria changed the color of PDA coated filters to pink, while the phenol red indicator was turned from orange to magenta (Fig. 5P column) which indicated an increase of medium pH. Time difference for obvious color change was observed for Salmonella Typhimurium in 7 h and E. coli, M. luteus in 10 h. Colonies

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Fig. 5. Color response of PDA-coated filters and phenol red indicator to bacteria growth. Columns with the same label originated from the same agar plate (Top = top view of plates showing the PDA filter. Bottom = bottom view of plates showing the phenol red agar without dextrose. P.C = Control group of phenol red without dextrose, P = Phenol red without dextrose). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
of *M. luteus* formed are yellow and therefore color change of PDA-coated filter for *M. luteus* is not as obvious as *S. Typhimurium* and *E. coli*. Colors of *L. innocua* was not observed on PDA-coated filter while phenol red did not change color. This observation means that *L. innocua* cannot grow on phenol red without dextrose medium and therefore did not change color of PDA-coated filters. The color of PDA-coated filter and phenol red indicator of control group remained unchanged during 22 h culturing, which eliminated other possible reasons for color change of PDA-coated filters such as temperature, PBS buffer, and environment (Fig. 5 P·C and PD.C column). These observations verified that combining with dextrose absence medium, PDA-coated multilayer filter can be applied for *Salmonella Typhimurium* and *E. coli* detection.

### 4. Conclusions

In this study, coating of PDA on the surface of membrane filters was achieved by evaporation of organic solvent. Degree of coating was quantified by measuring color of PDA-coated filter using a spectrophotometer which at the same time ensured only PDA-coated filters with good reproducibility utilized in this experiment. Color change of PDA-coated filters from blue to pink was observed concurrent with the alkaline pH induced from bacteria metabolism. Adjustment of supernatant pH verified that alkaline pH from bacteria metabolites leads to color change of PDA-coated filters. The mechanism of color change of PDA-coated filter to cultured bacteria was proposed and the potential of applying PDA-coated filter for bacteria detection was supported by testing on *Salmonella Typhimurium*, *E. coli*, *L. innocua* and *M. luteus* on phenol red without dextrose agar. Advantages of using this strategy include concentrating bacteria in sampling, visual detection and the potential to quantitate bacteria on the filter.

### Credit roles

Yuexue Zhang, Investigation, Methodology, Validation, Visualization, Writing - original draft; Writing - review & editing. Paul L. Dawson, Investigation, Methodology, Validation, Writing - original draft; Writing - review & editing. Project administration; Resources, Supervision Tzuenn-Rong Tzeng, Methodology, Writing - review & editing Timothy W. Hanks, Methodology, Validation, Writing - original draft; Writing - review & editing. Julie K. Northcutt, Methodology, Validation, Writing - original draft; Julie K. Northcutt, Methodology, Validation, Writing - review & editing. William T. Pennington, Methodology, Validation, Writing - original draft; Writing - review & editing.

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### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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### References


Paul L. Dawson is a Professor in the Department of Food, Nutrition and Packaging Sciences that studies ways to improve food safety and quality. His research has encompassed many food products from the farm, food production facilities and in the laboratory. He works directly with the industry through the Cooperative Extension Service and funded research projects. Paul grew up in Salisbury, MD completing a B.S. in 1977 at Salisbury University. After two years of post-baccalaureate work, Perdue Inc. employed him for 1.5 years in the Products Research Area. Paul then obtained a MS degree at University of Florida in Poultry Science studying processing effects on poultry meat quality with Dr. Doug Janky. Paul then attended North Carolina State University completing his Food Science doctorate in 1989 studying various treatments on the lipid fractions of mechanically deboned poultry meat under the guidance of Dr. Brian Sheldon. After a two-year post-doctorate at NC State treatments to reduce microbial growth on carcasses and eggs, Paul came to Clemson University’s Food Science Department and was promoted to Full Professor in 2001. Dawson has authored/coauthored 113 refereed scientific publications, 18 technical book chapters, two books and has over 250 popular media interviews in the field of food microbiology, antimicrobial active packaging and food quality.

Julie K. Northcutt is a Professor in the Department of Food, Nutrition and Packaging Sciences at Clemson University, where she has worked since 2008. From 2014 to 2018, Dr. Northcutt served as the Extension Program Team Leader for the Food Safety and Nutrition Program (43 County Agents, 5 Extension Associates and 4 Faculty Specialists). Prior to joining the faculty at Clemson, she was a Research Food Technologist promoted to Lead Scientist for the United States Department of Agriculture (2001–2008). While working at the USDA, Dr. Northcutt led a team of research scientists working on pathogen reduction strategies during commercial processing of food. From 1995 to 2001, Dr. Northcutt was an Assistant Professor, promoted to Associate Professor at the University of Georgia. Her research interest includes food microbiology (Salmonella, Listeria, generic Escherichia coli and Campylobacter spp.) and food chemistry (quality, shelf-life and product formulation), especially as they relate to commercial food manufacturing. Dr. Northcutt received her PhD. in Food Technology with a minor in Biochemistry from North Carolina State University. She received a Master of Science degree in Food Science and Human Nutrition, and a Bachelor of Science degree in Biochemistry from Clemson University.

Timothy W. Hanks received a B.S. in Chemistry from the South Dakota School of Mines and Technology and a PhD in Organic Chemistry from Montana State University. After working as a Postdoctoral Research Associate at the University of Minnesota, he moved to South Carolina, joining the Chemistry Department at Furman University in 1990. He is currently Professor of Chemistry and Department Chair at Furman and also an Adjunct Professor in the Chemistry Department at Clemson University. He has held positions as a Visiting Professor in the Clemson School of Materials Science, and Visiting Scientist at Décembre du Recherche Fondamentale sur la Matière Condensée, Commissariat à l’Energie Atomique in Grenoble, France. In 2011 he spent six months as a Fulbright Senior Scholar and Visiting Scientist at the Intelligent Polymer Research Institute at the University of Wollongong, Australia.

William T. Pennington is an Alumni Distinguished Professor and Chair of the Department of Chemistry at Clemson University. He received a B.A. degree in chemistry from Hendrix College in 1977, a Ph.D. in inorganic chemistry in 1983 from the University of Arkansas, with A.W. Cordes, and was a postdoctoral research associate with I.C. Paul and D.Y. Curtin at the University of Illinois for two years after graduate school. Before joining the faculty at Clemson, he worked for two years at Molecular Structure Corporation. His two main areas of research involve the study and application of chromic polydiacetylenes chemico- and biosensors, and the investigation of halogen bonding as a reliable solid-state synthon for crystal design applications.

Yueyuan Zhang received her Master of Science in Food Science from Clemson University in 2014. Currently, she is a Ph.D. candidate in Department of Food, Nutrition, and Packaging Sciences under supervision of Dr. Paul Dawson at Clemson University, SC, USA. Her research focuses on developing polydiacetylene-based biosensors for bacteria detection.