AGRICULTURAL RESEARCH FOUNDATION
INTERIM REPORT
FUNDING CYCLE 2016 – 2018

TITLE:
Development of colorimetric sensor arrays based on conjugated electrospun fibers for rapid evaluation of food quality

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SUMMARY:
The development of rapid non-destructive techniques to determine food freshness and shelf-life is significant for food safety. Conventional methods to identify microbial spoilage or chemical contamination are time-consuming and require highly trained laboratory personnel or expensive equipment. Therefore, an inexpensive and user-friendly tool is highly needed for rapid evaluation of food freshness and quality control.
Food decay is accompanied by the production of several microbiologically induced biochemical processes which generate a wide variety of volatile organic compounds (VOCs), such as sulfur compounds (i.e. hydrogen sulfide, dimethyl disulfide) and acids (lactic acid, acetic acid). An inexpensive disposable sensor will be developed in this project for rapid detection of volatile compounds as an evaluation tool for food quality, including monitoring fish and meat freshness, early detection of grain spoilage etc. Diverse VOCs can be monitored with this sensor owing to VOC-induced color changes that are composed of inkjet-printed arrays (metalloporphysrins and pH indicators) on solvatochromic electrospun fibers. The electrospinning technique allows for rapid and cost-effective fabrication of fibrous polymer membranes with large surface area. A wide range of polymers could be chosen in electrospun fibers, such as polydiacetylenes (PDAs). The stress (thermal, mechanical and ligand-receptor interaction, etc.) induced blue-to-red color transition of PDAs is well-known phenomena. Inkjet printing using a reconstructed desktop printer provides a rapid and inexpensive way of fabrication. The combination of novel PDA conjugated electrospun fibers and cost-effective inkjet-printed indicator dyes would serve as the basis for a unique and practical application of colorimetric detection of VOCs.

OBJECTIVES:
The objective of this project is to use colorimetric sensory array for odor visualization, therefore minimizing the need for extensive signal transduction hardware. The objectives during current phase (2016-2017) include the following:
1. Screening of pH indicator
2. Encapsulation of pH indicator and preparation of dye-doped sol gel
3. Electrospining of dye-doped sol gel

4. Evaluation of sensor performance on pure VOC standard

PROCEDURES:

1. Screening of pH indicators

Requirements on the pH indicators were investigated, including the ΔpKa between the indicators, and the colors and concentrations of the indicators. It has been reported that a linear response over a broad pH range can be obtained by using multiple indicators that have ΔpKa ≤1.7. Some key parameters for screening pH indicators include ΔpKa between indicators, colors of indicators, and concentrations of indicators.

Table 1. List of dyes as potential colorimetric indicators (properties of aqueous acid-base indicators at 25 °C)

Dye name	pKa	pH range and color change
Methyl Violet	0.8	0.0 (Yellow) – 1.6 (Blue)
*Cresol Red	1.0	0.2 (Red) – 1.8 (Yellow)
*Thymol Blue	1.6, 8.9	1.2 (Red) – 2.8(Yellow), 8.0 (Yellow) – 9.6(Blue)
Methyl Yellow	3.3	2.9 (Red) – 4.0 (Yellow)
Methyl Orange	3.4	3.1 (Red) – 4.4 (Yellow)
Bromophenol Blue	3.85	3.0 (Yellow) – 4.6 (Blue)
Bromocresol Green	4.7	3.8 (Yellow) – 5.4 (Blue)
Methyl Red	4.9	4.4 (Red) – 6.2 (Yellow)
*Chlorophenol Red	6.0	4.8 (Yellow) – 6.7 (Violet)
Bromocresol Purple	6.3	5.2 (Yellow) – 6.8 (Pink)
*Bromothymol Blue	7.1	6.0 (Yellow) – 7.6 (Blue)
p-Nitrophenol	7.2	5.3 (Colorless) – 7.6 (Yellow)
*Phenol Red	7.9	6.4 (Yellow) – 8.0 (Red)
m-Cresol Purple	8.3	7.4 (Yellow) – 9.0(Violet)

* pH indicators used in the dye-doped sol gel in this study.

2. Fabrication of dye-doped sol gel

The entrapment of pH indicators into sol-gel silica has widely been analyzed over the past few years. It has been demonstrated that different analytical reagents in the sol-gel derived matrix retain their functional characteristics to large extent (Zaggout, 2006). The porosity of sol-gel glasses allows small analyte molecules to diffuse into the matrix. There are three methods of indicators immobilization in the sol-gel which include impregation, covalent binding and chemical doping. In our study, encapsulation of different pH indicator was conducted and compared using two chemical doping methods

2.1 (*Method 1*) The sol–gel glass-sensing films were prepared by entrapping pH indicators in porous silica glass prepared by the sol–gel method. The recipe and procedure for film preparation are as follows: (1) 0.005–0.010 g of indicator(s), 7ml of H_2O, 3ml of methanol, 4ml of $Si(OCH_3)_4$, 0.4 ml of Triton X-100, and 0.02 ml of 1M HCl were mixed

in a 50ml beaker, (2) the solution was stirred for about 40 min at room temperature before the viscous sol–gel solution was coated onto both sides of microscope glass slides (1_ 3 inch) by dip-coating, (3) the sol–gel glass films were allowed to gel and age in air at the room temperature for 10–14 days before the measurements were made. Triton x-100 was used as a surfactant to improve the quality of coating of the sol–gel glass films on the glass slides.

2.2 (*Method 2*) In order to prepare the monolithic disk, tetraethylorthosilicate (20.8 g, 0.10 mol) in 7 ml methanol and 3.6 ml of 0.032 M HCl were stirred for 30 min to obtain a homogeneous sol solution. One ml of pH indicators (i.e. phenol red) of different concentrations (5×10^{-3}, 5×10^{-4}, 5×10^{-5}) were added to 1.5 ml of the previous solution, respectively, and transferred into glass vials (10 mm in radius) covered with parafilm having fine pores and stored at room temperature. Monolithic clear gels were formed within 7 days. After another 7 days, solid transparent crack-free disks were obtained with thickness around 0.6 mm, 7.2 mm in radius. These disks were washed several times with methanol/deionized water (5× 5 mL). The monolithic disks were allowed to dry at room temperature. Disks containing 0.0005 M SDS surfactant were prepared. A monolithic siloxane disks free from indicators were prepared in a similar method by mixing TEOS and HCl as catalyst. To evaluate the entrapment capacity of phenol red, the produced monolithic entrapped indictor of various concentrations of the doped material were washed with methanol/water solvent mixture in 1: 1 volume ratio several times. The washes were collected, diluted to the linear phenol red UV/VIS spectrophotometer calibration curve (λmax= 424 nm).

3. Fabrication of polymer membrane substrate by electrospinning.

Electrospinning technique is a simple and cost-effective technology that generates non-woven fibers with high porosity and surface area to volume ratio. The electrospinning process involves the application of a strong electrostatic field to a capillary connected with a reservoir containing a polymer solution or melt. Under the influence of the electrostatic field, a pendant droplet of the polymer solution at the capillary tip is deformed into a conical shape (Taylor cone). If the voltage surpasses a threshold value, electrostatic forces overcome the surface tension, and a fine charged jet is ejected. The jet moves towards a ground plate acting as counter electrode. Due to the extensional viscosity of the polymer solution and the presence of entanglements, the jet remains stable and does not transform into spherical droplets as expected for a liquid cylindrical thread. The solvent begins to evaporate immediately after the jet is formed. The result is the deposition of a thin polymer fiber on a substrate located above the counter electrode (Choi, Lee, Im, Kim, & Joo, 2003).

Dye-doped silica sol-gel was prepared using method mentioned previously. The silica sol gel solution was placed in a syringe and the electrode was directly connected with the solution. The fibers were collected on aluminum foil covered a squared copper plate. The distance from needle to counter electrode (tip-to-collector distance, TCD) was set up between 2cm-4cm, and the applied voltages was set at 6 kV.

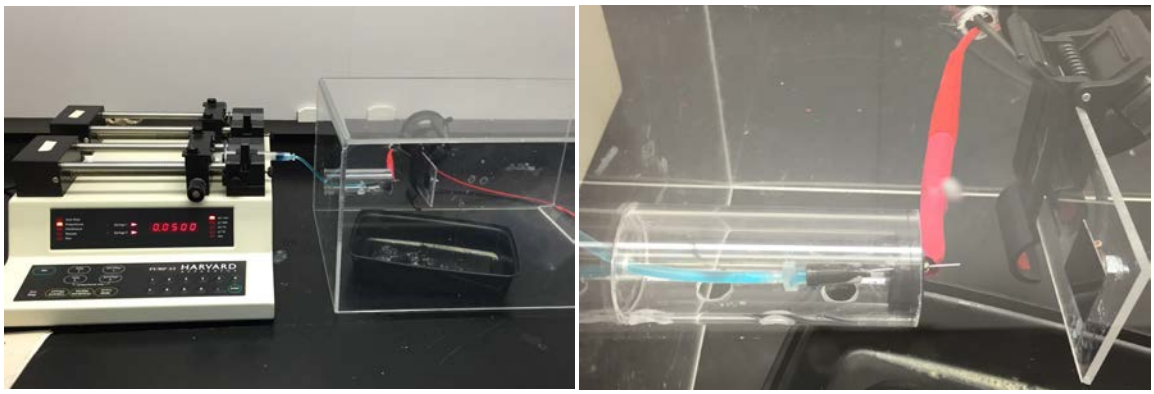

Figure 1. Set up of electrospinning devices.

Modification of electrospinning parameters (i.e voltage, solution concentration and viscosity) is still needed at this stage in order to obtain a functional and effective nanofibers from the dye-doped sol gel solution.

4. **Evaluation of sensor performance on pure VOC standard**
   To demonstrate the ability of sensor array to discriminate among analytes, different VOCs will be tested representing common organic functionalities including primary, secondary, tertiary and aromatic substituents of amines, arenes, alcohols, aldehydes, carboxylic acid, esters, hydrocarbons, ketones, phosphines, and thiols. The sensor array will be exposed to headspace of individual VOCs, and images will be taken using a digital camera before and after exposure to VOC standard. Triplicate arrays will be used for each analyte to test the reproducibility of the array response. A database of array responses will be generated in this part of study.

SIGNIFICANT ACCOMPLISHMENTS TO DATE:

We have successfully encapsulated the pH indicator into a sol-gel matrix. The entrapment of pH indicators into sol–gel silica has demonstrated that different analytical reagents in the sol–gel derived matrix retain their functional characteristics to large extent. The porosity of sol–gel glasses allows small analyte molecules to diffuse into the matrix. The organic compounds were chemically or physically immobilized into a solid support. In this work, the chemical doping methods was used for the immobilization of phenol red pH indicator. The immobilized phenol red was prepared by doping the phenol red reagent into sol–gel silica matrix. The synthetic reaction includes hydrolysis of tetraethylorthosilicate (TEOS) in presence of HCL as catalyst. Then the pH indicator (i.e. phenol red) was added in the presence or absence of sodium dodecylsulphate (SDS), to the sol–gel solution to obtain a monolithic polysiloxane solid material. The entrapment of the enzyme at the paper surface allows the sensor to be used for either lateral flow or dipstick sensing applications.

ADDITIONAL FUNDING RECEIVED DURING PROJECT TERM: N/A

FUTURE FUNDING POSSIBILITIES:

This project focuses on the development and deployment of simple and cost effective colorimetric indicators for use in applications such as detection of food spoilage. The initial

focus area applies to the large market, where these indicators can be used to determine the freshness of meat and seafood, and can help address the problems of food waste and food-borne illnesses. The broader impact/commercial potential of this project is advancement of sensing technology for health, food safety, and diagnostics, and taps into the rapidly growing global market for sensors. Upon completion of this project, future funding for this area of work will be sought from the NIH, USDA and industrial sectors.

References

Choi, S.-S., Lee, S. G., Im, S. S., Kim, S. H., & Joo, Y. L. (2003). Silica nanofibers from electrospinning/sol-gel process. Journal of Materials Science Letters, 22(12), 891-893.
Zaggout, F. R. (2006). Entrapment of phenol red pH indicator into a sol–gel matrix. Materials Letters, 60(8), 1026-1030.