Polydiacetylene sensor interaction with food sanitizers and surfactants

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Abstract

Polydiacetylene (PDA) vesicles are of interest as biosensors, particularly for pathogenic bacteria. As part of a food monitoring system, interaction with food sanitizers/surfactants was investigated. PDA vesicles were prepared by inkjet-printing, photopolymerized and characterized by dynamic light scattering (DLS) and UV/Vis spectroscopy. The optical response of PDA vesicles at various concentrations versus a fixed sanitizer/surfactant concentration was determined using a two variable factorial design. Sanitizer/surfactant response at various concentrations over time was also measured. Results indicated that only Vigilquat and TritonX-100 interacted with PDA vesicles giving visible colour change out of 8 sanitizers/surfactants tested. PDA vesicle concentration, sanitizer/surfactant concentration, and time all had a significant (P < 0.0001) effect on colour change. As they are highly sensitive to the presence of Vigilquat and TritonX-100, PDA sensors could be used to detect chemical residues as well as for detection of various contaminants in the food industry.

1. Introduction

Polydiacetylenes (PDAs) are conjugated polymers which consist of an alternating double bond – triple bond chain backbone. PDA vesicles are stable liposome-like bilayer polymers which exhibit unique organizational and chromatic properties. To clarify what is meant by vesicles in this paper, the PDA vesicles may be more accurately described as liposomes (hollow spheres with a bilayer wall). Also, it can be argued that PDA vesicles would be more properly called an “amphiphile” rather than a lipid, since it is not of biological origin and that liposomes are composed specifically of phospholipids rather than fatty acid derivatives. External stimuli such as heat, pH, mechanical stress and chemical solvents (de Oliveira, Soares, Fontes, de Oliveira, & Maradini Filho, 2012; Jelinek & Kulusheva, 2001; Okada, Peng, Spevak, & Charych, 1998) leads to rapid colour change from the initial intense blue colour to red. It is believed that stress induced twisting of the highly planar backbone lead to the colour change of PDA vesicles (Berman & Charych, 1999).

By attaching a ligand to the PDA vesicle, Charych, Nagy, Spevak, and Bednarski (1993) developed a biosensor for influenza virus detection. Since then, great progress has been made in developing PDA vesicle based sensors. Sensors have also been developed that can rapidly detect cations (Kolusheva, Shahal, & Jelinek, 2000), proteins (Jung, Kim, Park, & Soh, 2010), melanine (Lee, Jeong, & Kim, 2011), bacteria toxins (Ma & Cheng, 2005), lipopolysaccharides (Rangin & Basu, 2004; Wu, Zawistowski, Ehrmann, Yi, & Schmuck, 2011) and bacteria (de Oliveira et al., 2013; Nagy et al., 2008; Silbert et al., 2006).

Applications of PDA sensors for bacteria detection in apple juice (dos Santos Pires et al., 2011) and chicken (de Oliveira et al., 2015) have been explored in a laboratory setting. However, the use of such sensors for bacteria detection in food production settings is much more complex, due to interactions with environmental agents. False positives may occur due to the presence of compounds such as sanitizers and food components. Sanitation is routinely performed in food plants to minimize pathogenic and spoilage bacteria contamination. Most commonly used sanitizers (usage levels) in food plants include chlorine (200 ppm), chlorine dioxide, iodophores (25 ppm), quaternary ammonium (200 ppm), peracetic acid and ozone (Cramer, 2006). Concentrations of tested...
sanitizers were determined as usage level for direct use on food product contact surface with no-rinse, according to the Code of Federal Regulations (CFR, 2015). Additionally, electrolyzed acid water (E.W.Acid) (Huang, Hung, Hsu, Huang, & Hwang, 2008; Issa-Zacharia, Kamitani, Miwa, Muhibbula, & Iwasaki, 2011) and electrolyzed neutral water (N.E.W.) (Abadias, Usall, Oliveira, Alegre, & Viñas, 2008; Izumi, 1999) have been reported as effective in laboratories and is found in several types of cleaning compounds, ranging from heavy-duty industrial products to gentle detergents. No studies have been published reporting concerning the interaction of PDA and TritonX-100 is a commonly used detergent in laboratories and is found in several types of cleaning compounds, ranging from heavy-duty industrial products to gentle detergents. No studies have been published reporting concerning the interaction of PDA and TritonX-100.

2. Materials and methods

2.1. Sanitizers and surfactant

The seven commonly used sanitizers and one surfactant tested in this study are as follows: Clorox (the Clorox company), BTF lodo- phor sanitizer (National Chemical Inc.), AFCO 4312 Vigilquat (Alex C. Fergusson), FreshFx AL (Synergy Technologies), FreshFx LP (Synergy Technologies), TritonX-100 (Sigma-Aldrich) and Electrolyzed Water (Acid pH = 2.96, Neutral pH = 7.09 which generated from Hoshizaki water electrolyzer).

2.2. PDA vesicles preparation

PDA vesicles were prepared using modified commercial inkjet printers (Hausschild et al., 2005). Briefly, a solution of 5 mg 10, 12-pentacosadiynoic acid (PDA) (GFS chemicals, OH) in 3.3 ml isopropl alcohol was placed into a printer cartridge then the printing sequence was initiated resulting in “printing” of the PDA/isopropl alcohol solution into 10 ml of 60 °C distilled water, yielding a colourless, slightly turbid solution. The majority of isopropl alcohol in the solution was removed under vacuum by rotary evaporator (IKA RV10, NC, USA) and the solution was stored at 4 °C overnight to allow the vesicles (liposomes) to self-assemble. The resulting solution was filtered through a 0.45 μm mesh (Environ- mental Express Inc., SC) and exposure to 254 nm UV light for 1.5 min to induce polymerization of PDA vesicles. This resulted in an intense blue transparent suspension.

2.3. Characterization of PDA vesicles

Particle size distribution was measured by diluting the PDA vesicles with distilled water and analyzing by dynamic light scattering (DLS) (Malvern Zetasizer ZS). Scattering angle was fixed at 173° and temperature was fixed at 25 °C. Zeta potential test was also performed with DLS to measure the stability of the PDA vesicle solution.

2.4. Experimental design

2.4.1. Experiment 1. Effect of PDA vesicle concentration on PDA colorimetric response

The PDA vesicle concentration was measured using the optical density (O.D.). The PDA vesicle O.D. (concentrations) were set at 0.1, 0.3, 0.6, 0.8, and 1.0 in wells at 640 nm wavelength. Sanitizer/surfactant concentration in wells were set at 200 ppm (0.02%), 100 ppm (0.01%), 50 ppm (0.005%), 25 ppm (0.0025%), 10 ppm (0.001%), and 1 ppm (0.0001%) or 0.5% (5000 ppm), 0.4% (4000 ppm), 0.3% (3000 ppm), 0.2% (2000 ppm), 0.1% (1000 ppm) and 0.05% (500 ppm). PDA vesicle O.D. in wells was adjusted to 0.6 and 1.0, and 0.1% (1000 ppm) (TritonX-100). Data was collected at 6.5 h for TritonX-100 and 4.5 h for all other sanitizers/surfactant.

2.4.2. Experiment 2. Effect of sanitizer/surfactant concentration and time on PDA colorimetric response

Wavelength scans were taken at 0.5 h, 1.5 h, 2.5 h, 4.5 h for Vigilquat, and TritonX-100 with an additional reading scan at 6.5 h for TritonX-100. The 6.5 h reading for TritonX-100 was conducted since CR% did not equilibrate within 4.5 h. Sanitizer/surfactant concentrations in wells were set at 200 ppm (0.02%), 100 ppm (0.01%), 50 ppm (0.005%), 25 ppm (0.0025%), 10 ppm (0.001%), and 1 ppm (0.0001%) or 0.5% (5000 ppm), 0.4% (4000 ppm), 0.3% (3000 ppm), 0.2% (2000 ppm), 0.1% (1000 ppm) and 0.05% (500 ppm). PDA vesicle O.D. in wells was adjusted to 0.6 and 1.0.

2.4.3. Measurement of PDA colorimetric response

A 96-well plate and scanning wavelength spectrophotometer (Epoch Plate reader, Bio Tek Instruments Inc, VT) was used for analysis. It has been reported that PDA vesicle biosensors decorated with phospholipids and lysine/glycine and cholesterol, which were developed for bacteria or toxin detection, were evaluated by the optical colour change and absorbance spectrum (de Oliveira et al., 2013; Ma & Cheng, 2005). Therefore, results were evaluated by both observation of colour change with the naked eye (also digital photograph) and absorbance spectrum. Quantitative measurement of colour change was also evaluated by percentage colorimetric response (CR%). Observation of colour change is helpful for rapid detection while CR% is much more sensitive.

\[
CR\% = \left( \frac{A_{\text{blue}} - A_{\text{red}}}{A_{\text{blue}} + A_{\text{red}}} \right) \times 100
\]

In this equation, “A” represent absorbance of blue (640 nm) colour or red (520–540 nm) colour sample obtained by spectrophotometer. The subscripts “blue” and “red” represent the absorbance at the 640 nm (blue) and 520–540 nm (red) wavelengths of the sample. The indices “b” and “a” refer to the absorbance before and after addition of sanitizer/surfactant.

2.5. Statistical analysis

JMP Pro 12.0.1 software was used to perform all the statistical analysis, with the significance level assigned at P ≤ 0.05. For experiment 1, a completely randomized block design (CRBD) was used to evaluate the effect of PDA vesicle concentration and sanitizer/surfactant concentration on PDA colorimetric response. A two-way analysis of variance was used with the main factors of PDA vesicle O.D. and sanitizer/surfactant concentration. Means were separated by the least significant difference (LSD) test and regression analyses were applied to compare the effect of sanitizer/surfactant concentrations on colour change. The CRBD model for experiment 1 follows,

\[
y = \mu + P + S + P \cdot S + BLK + P \cdot BLK + S \cdot BLK + P \cdot S \cdot BLK + \varepsilon
\]

For experiment 2, a completely randomized block design was used for both AFCO 4312 Vigilquat and TritonX-100 with factors being time, sanitizer/surfactant concentration, and PDA vesicle concentration. Analysis of variance was performed with main factors of sanitizer/surfactant concentration, time and PDA vesicle O. D. Means were separated using the LSD test, and regression analyses were applied to compare the effect of sanitizer/surfactant concentrations on colour change. The CRBD model for experiment 2 follows,

\[
y = \mu + T + S + P + BLK + T \cdot S + T \cdot P + P \cdot S + T \cdot P \cdot S + \varepsilon
\]
μ = Overall mean, T = Time, S = Sanitizer/surfactant concentration, P = PDA O.D., BLK = Replication, ε = Error.

3. Results and discussion

3.1. Characterization of PDA vesicles

After polymerization, PDA vesicles showed a clear blue colour with a maximum absorbance wavelength at 640 nm. Dynamic light scattering revealed that the Z average particle size was 133 ± 16 nm in diameter and particle dispersion index (PDI) was 0.20 ± 0.03 with a Zeta potential of -33.3 ± 2.3 mV. Hauschild et al. (2005) used different lipids to form lipid vesicles with the ink-jet printing method and reported 100 nm and 130 nm in diameter. Kauffman et al. (2009) reported a bimodal particle distribution with largest particle size of 144 ± 22 nm in diameter using a more traditional sonication method. Sonication is a much cruder process compared to the inkjet method. The DLS analysis indicated that the printed PDA vesicles were in a stable dispersion solution and comparable to PDA vesicles reported in previous studies.

3.2. Effect of PDA vesicle concentration on PDA colorimetric response

Among all the sanitizers and surfactant, only Vigilquat and TritonX-100 triggered a colorimetric response with the PDA vesicles, giving a visual colour change. The absorbance spectrum of Vigilquat showed intense absorption peaks in green region (520 nm) and slight absorption in orange region (640 nm) which are visibly reflecting red and blue light, respectively (Fig. 1 (a)). Similarly, peaks in reflected red (540 nm) and blue (640 nm) were observed in absorbance spectrum of TritonX-100 (Fig. 1 (a)). All other sanitizers showed same absorbance spectrum as control which means PDA vesicle do not response to those sanitizers. As shown in Fig. 1 (b, c, d), different PDA vesicle concentrations resulted in similar absorbance spectrum but differed in absorbance intensities.

Both PDA vesicle concentration (O.D.) and sanitizer concentration had a significant (P < 0.001) effect on colour change. Regression analysis revealed a linear relationship for higher concentration of sanitizer (200 ppm)/surfactant (0.5%/5000 ppm) but a polynomial relationship for lower concentration of sanitizer (25 ppm)/surfactant (0.1%/1000 ppm) (Fig. 2 (a), (b)). CR% decreased with increasing the O.D. of PDA vesicles for both Vigilquat and TritonX-100. (Fig. 2 (a), (b)). CR% is a reflection of percentage colour change using both 520 nm and 640 nm wavelengths. In general, higher CR% indicated more colour change which is desirable for detection. At low O.D. levels (such as O.D. 0.1, O.D. 0.3), the PDA response to the sanitizer and surfactant could not be detected by the naked eye but required more sensitive instrumentation (CR% via wavelength absorbance). O.D. of 0.6 is the minimum O.D. which yielded a visible colour change. Statistical analysis revealed that colorimetric response of PDA vesicles to both Vigilquat and TritonX-100 at O.D. 0.6 and O.D. 0.8 are not significantly different from each other.

Since one goal was for rapid visual detection, O.D. 0.6 and O.D. 1.0 were used for further study (Table 1).

3.3. Effect of sanitizer/surfactant concentration and time on PDA colorimetric response

With a stepwise decrease in sanitizer/surfactant concentration, there is a concurrent reduction of the PDA colorimetric response (Table 2). At 200 ppm, Vigilquat transformed the PDA vesicle solution to a bright orange appearance, while at 10 ppm, PDA vesicles remained blue. TritonX-100 at 0.5%/5000 ppm level turned PDA vesicle from blue to a pink colour while TritonX-100 at 0.05%/500 ppm level gave a blue to purple colour tint. Changes in concentration were reflected similarly in both visual observations.

Figure 1. Absorbance spectrum of (a) PDA vesicle (O.D. 0.6) and sanitizer (TritonX-100: 0.5%, Vigilquat: 200 ppm, Clorox: 200 ppm/Iodophores: 25 ppm/FreshFx LP: 10,000 ppm/FreshFx AL: 40,000 ppm/Electrolyzed acid water/Electrolyzed neutral water/control: distilled water) at 4.5 h. (b) PDA vesicle (O.D. 0.1, 0.3, 0.6, 0.8, 1.0) and Vigilquat (200 ppm) at 4.5 h. (c) PDA vesicle (O.D. 0.1, 0.3, 0.6, 0.8, 1.0) and TritonX-100 (0.5%) at 6.5 h. (d) PDA vesicle (O.D. 0.1, 0.3, 0.6, 0.8, 1.0) and Clorox (200 ppm)/Iodophores (25 ppm)/FreshFx LP (10,000 ppm)/FreshFx AL (40,000 ppm)/Electrolyzed acid water/Electrolyzed neutral water/distilled water at 4.5 h.
Table 2
Effect of sanitizer/surfactant concentration on PDA colorimetric response of Vigilquat (4.5 h) and TritonX-100 (6.5 h).

<table>
<thead>
<tr>
<th>Sanitizer/Surfactant</th>
<th>Concentration</th>
<th>Colour Change</th>
<th>CR%</th>
<th>Standard Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vigilquat 200 ppm</td>
<td></td>
<td></td>
<td><img src="a" alt="200 ppm" /></td>
<td>91.07% A 0.0086</td>
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<tr>
<td>Vigilquat 100 ppm</td>
<td></td>
<td></td>
<td><img src="a" alt="100 ppm" /></td>
<td>74.90% B 0.0078</td>
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<tr>
<td>Vigilquat 50 ppm</td>
<td></td>
<td></td>
<td><img src="a" alt="50 ppm" /></td>
<td>44.52% C 0.0078</td>
</tr>
<tr>
<td>Vigilquat 25 ppm</td>
<td></td>
<td></td>
<td><img src="a" alt="25 ppm" /></td>
<td>20.81% D 0.0078</td>
</tr>
<tr>
<td>Vigilquat 10 ppm</td>
<td></td>
<td></td>
<td><img src="a" alt="10 ppm" /></td>
<td>8.21% E 0.0094</td>
</tr>
<tr>
<td>Vigilquat 1 ppm</td>
<td></td>
<td></td>
<td><img src="a" alt="1 ppm" /></td>
<td>0.59% F 0.0078</td>
</tr>
<tr>
<td>TritonX-100 0.50%</td>
<td></td>
<td></td>
<td><img src="a" alt="0.50%" /></td>
<td>33.68% A 0.0141</td>
</tr>
<tr>
<td>TritonX-100 0.40%</td>
<td></td>
<td></td>
<td><img src="a" alt="0.40%" /></td>
<td>26.53% B 0.0141</td>
</tr>
<tr>
<td>TritonX-100 0.30%</td>
<td></td>
<td></td>
<td><img src="a" alt="0.30%" /></td>
<td>20.14% C 0.0141</td>
</tr>
<tr>
<td>TritonX-100 0.20%</td>
<td></td>
<td></td>
<td><img src="a" alt="0.20%" /></td>
<td>14.61% D 0.0141</td>
</tr>
<tr>
<td>TritonX-100 0.10%</td>
<td></td>
<td></td>
<td><img src="a" alt="0.10%" /></td>
<td>9.14% E 0.0141</td>
</tr>
<tr>
<td>TritonX-100 0.05%</td>
<td></td>
<td></td>
<td><img src="a" alt="0.05%" /></td>
<td>6.98% F 0.0166</td>
</tr>
</tbody>
</table>

A-D Means of same sanitizer/surfactant with different letters are significantly (P ≤ 0.05) different.

*Data represent the least square mean of two replications.

Figure 2. Effect of PDA vesicle O.D. on CR% of (a) Vigilquat at 4.5 h, (b) TritonX-100 at 6.5 h.
and absorbance spectrums. The 10 ppm Vigilquat/PDA solution showed peaks at 640 nm (Fig. 3 (a)), which is consistent with the blue colour. The absorbance spectrum of 0.05% (500 ppm) TritonX-100/PDA displayed peaks at both 540 nm and 640 nm which reflects the visual purple colour (Fig. 3 (b)). Since the undecorated PDA vesicles are highly sensitive to Vigilquat and TritonX-100, PDAs may have industrial application for the detection of residues of these sanitizers/surfactants.

Statistical analysis revealed that sanitizer/surfactant concentration has significant ($P < 0.0001$) effect on colour change. Regression analysis revealed a linear relationship of PDA response with VX (Fig. 3 (c), (d)). Vigilquat also had a linear relationship with PDA vesicles between concentrations of 0 and 100 ppm. Within these concentration ranges, PDA vesicles can be used to determine concentrations of Vigilquat and TritonX-100.

Time effect on colour change was evaluated by comparing CR% at 0 h, 0.5 h, 1.5 h, 2.5 h, 4.5 h, (6.5 h, for TritonX-100 only). Colour change was observed immediately once Vigilquat contacted PDA vesicles however, holding time had a significant ($P < 0.0001$) effect on PDA CR% change. As displayed in Table 3, CR% for Vigilquat increased dramatically within 0.5 h and then the rate of increase slowed with continued holding time. Colour change of TritonX-100 was more gradual and CR% increased slowly during the holding period (Table 3).

### 4. Conclusions

Among eight commonly used sanitizer and surfactant in food industry, PDA vesicles only response to Vigilquat and TritonX-100 which indicated a selectivity of PDA vesicles. Possible reasons of this phenomenon maybe the active component in Clorox, Electrolyzed acid water and Electrolyzed neutral water is ClO which may repel negative charged PDA vesicles and therefore will not interact with PDA vesicles. Iodine and hydriodic acid in Iodophor may repel negative charged nor hydrophobic and therefore cannot trigger a colour transition. Organic acid such as citric acid, phosphoric acid, sulfuric acid and lactic acid constitute FreshFx LP and FreshFx AL. They are all hydrophilic and can lower the pH of solution but cannot twist the configuration of PDA vesicles and therefore no colour change was observed.

### Table 3

<table>
<thead>
<tr>
<th>Sanitizer/Surfactant</th>
<th>Concentration</th>
<th>0 h</th>
<th>0.5 h</th>
<th>1.5 h</th>
<th>2.5 h</th>
<th>4.5 h</th>
<th>6.5 h</th>
<th>Standard Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>TritonX-100</td>
<td>0.50%</td>
<td>0.0%</td>
<td>D</td>
<td>11.9%</td>
<td>CD</td>
<td>23.7%</td>
<td>BC</td>
<td>28.3%</td>
</tr>
<tr>
<td>TritonX-100</td>
<td>0.40%</td>
<td>0.0%</td>
<td>D</td>
<td>10.3%</td>
<td>c</td>
<td>19.1%</td>
<td>BC</td>
<td>22.8%</td>
</tr>
<tr>
<td>TritonX-100</td>
<td>0.30%</td>
<td>0.0%</td>
<td>D</td>
<td>8.8%</td>
<td>c</td>
<td>14.8%</td>
<td>BC</td>
<td>17.3%</td>
</tr>
<tr>
<td>TritonX-100</td>
<td>0.20%</td>
<td>0.0%</td>
<td>c</td>
<td>7.9%</td>
<td>c</td>
<td>12.0%</td>
<td>c</td>
<td>13.2%</td>
</tr>
<tr>
<td>TritonX-100</td>
<td>0.10%</td>
<td>0.0%</td>
<td>c</td>
<td>6.4%</td>
<td>c</td>
<td>8.4%</td>
<td>A</td>
<td>8.6%</td>
</tr>
<tr>
<td>Vigilquat</td>
<td>200 ppm</td>
<td>0.0%</td>
<td>c</td>
<td>84.7%</td>
<td>A</td>
<td>87.3%</td>
<td>A</td>
<td>90.0%</td>
</tr>
<tr>
<td>Vigilquat</td>
<td>100 ppm</td>
<td>0.0%</td>
<td>c</td>
<td>53.7%</td>
<td>A</td>
<td>61.0%</td>
<td>A</td>
<td>63.3%</td>
</tr>
<tr>
<td>Vigilquat</td>
<td>50 ppm</td>
<td>0.0%</td>
<td>c</td>
<td>32.3%</td>
<td>A</td>
<td>39.3%</td>
<td>A</td>
<td>41.0%</td>
</tr>
<tr>
<td>Vigilquat</td>
<td>25 ppm</td>
<td>0.0%</td>
<td>c</td>
<td>15.0%</td>
<td>B</td>
<td>15.3%</td>
<td>B</td>
<td>17.7%</td>
</tr>
<tr>
<td>Vigilquat</td>
<td>10 ppm</td>
<td>0.0%</td>
<td>B</td>
<td>7.0%</td>
<td>A</td>
<td>7.0%</td>
<td>A</td>
<td>7.0%</td>
</tr>
<tr>
<td>Vigilquat</td>
<td>1 ppm</td>
<td>0.0%</td>
<td>B</td>
<td>0.7%</td>
<td>A</td>
<td>0.7%</td>
<td>A</td>
<td>0.7%</td>
</tr>
</tbody>
</table>

A-D Means of CR% within same row with different letters are significantly ($P < 0.05$) different.

- Data represent the least square mean of two replications.
Sensitivity of PDA vesicles was explored in Experiment 2 with different concentration of sanitizer/surfactant. Take Vigilquat (200 ppm) for example, PDA vesicle response to Vigilquat immediately and CR% reached 80% within 0.5 h. However, for TritonX-100 (0.5%), PDA vesicle response gradually and CR% increased slowly with time. The mechanism of interaction between the PDA vesicles and Vigilquat may be due to the positively charged Vigilquat molecule attracted by negatively charged PDA vesicles interacting quickly to turn the vesicle to a bright orange colour. Additionally, the slow penetration through the hydrophilic surface and into the hydrophobic bilayer of the PDA vesicles by long hydrophobic tails of Vigilquat may also play a part in colour transition, explaining the continued slower change in colour and absorbance during the holding period. TritonX-100 is a non-ionic surfactant with a long hydrophilic polyethylene glycol tail and a short hydrophobic head group substituent. The mechanism of the TritonX-100 interaction with PDA vesicles may due to the penetration of hydrophobic head group into vesicles after a loose, non-colorimetric association of the hydrophilic tail. This is slower than with Vigilquat and mimics the slower due to the weaker forces driving the initial association.

This study addressed the influence of PDA vesicle concentration, sanitizer concentration and time on PDA colorimetric response. PDA vesicles are sensitive to Vigilquat and TritonX-100 thus these cleaning agents may interfere with the application of PDA vesicle based biosensors in food production setting. On the other hand, PDA vesicles may have application for detecting some chemical residues of food cleaning agents. Knowledge of PDA vesicle concentration, sanitizer concentration and time effect on PDA colorimetric response will be important for applying PDA vesicles in the food industry.

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