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Stability and sensitivity of polydiacetylene vesicles to detect *Salmonella*

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ABSTRACT

Antibody incorporation in polydiacetylene (PDA) liposome was used to specifically detect *Salmonella*. Modifying physical properties of PDA liposome, such as size, surface charge, and packing of lipids, could affect the sensitivity and the stability of it. Therefore, the main goal of this study was to evaluate four factors simultaneously in PDA/antibody vesicles to detect *Salmonella*. Raman spectra were carried out to confirm covalent binding between antibody and PDA. The work followed through concomitant study of incubation temperature, incubation time, pH and antibody concentration allowing the development of some models that explain those variables related to colorimetric response, size and zeta potential. The smallest particle size (208 nm) showed the highest colorimetric response (90%), indicating PDA size enhanced sensitivity. Besides that, there is a relation between size and zeta potential: while particle size improved (from 300 nm to 700 nm), zeta potential enlarged too (from −30 to −20). This effect could be explained by PDA aggregation over time, by high temperature effect or by *Salmonella* presence. The study of zeta potential helped to understand that the interaction between *Salmonella* and antibody was through affinity between them. Therefore, understanding the parameters is important to improve PDA biosensors to apply in food industry and laboratory areas.

1. Introduction

Conjugated polymers have been broadly explored as colorimetric sensing materials due to their attractive optical and electrical properties. These properties primarily result from an extensive delocalized π-system and intrinsic conformational restrictions within the polymer chain [1].

Among the conjugated polymers reported to date, PDAs are one of particular interest because they can change color from blue to red in response to external stimuli and can be auto-arranged in liposome assembly [2–8]; these characteristics could be manipulated by lipid insertion modifying biointerfacial interactions and it could be utilized in sensing technologies to improve biosensors [9,10]. The polydiacety

In general, the insertion of lipids into PDA liposome has advantages in modifying PDA physical properties such as size [9], surface charge, and lipid packaging [11] which, in turn, affect sensitivity and stability of PDA liposome biosensor [12–14]. It has been revealed that a PDA liposome of small size appears to improve sensitivity by compartmentalization of receptor sites [15,16]. In parallel, weak lipids packaging (or high membrane fluidity) in PDA liposome enhances sensitivity by making easy distortion of π-conjugated chain of PDA [11–14,17]. Seeking stability, an appropriate level of surface charge of liposome can also introduce electrostatic repulsion providing resistivity against aggregation and coalescence, maintaining PDA vesicles size and consequently the sensitivity [18].

Another important factor for biosensor development is specificity, which can be obtained by incorporation of a receptor in the PDA system [1]. PDAs modified with specific receptors, for example antibodies that have been used to detect bacteria or a virus. The color transition of these PDA materials upon exposure to the stimuli can be observed by the naked eye making it simple to utilize them [7,8,14].

The dramatic color transition of PDA vesicles when exposed to external stimuli results from segmental reorientation within the organized structure. The change of polymer conformation causes the increase of HOMO–LUMO energy gap. Therefore, the absorption spectrum of perturbed PDA vesicles shifts to the higher energy region. The color of PDA vesicles can change from blue to purple,

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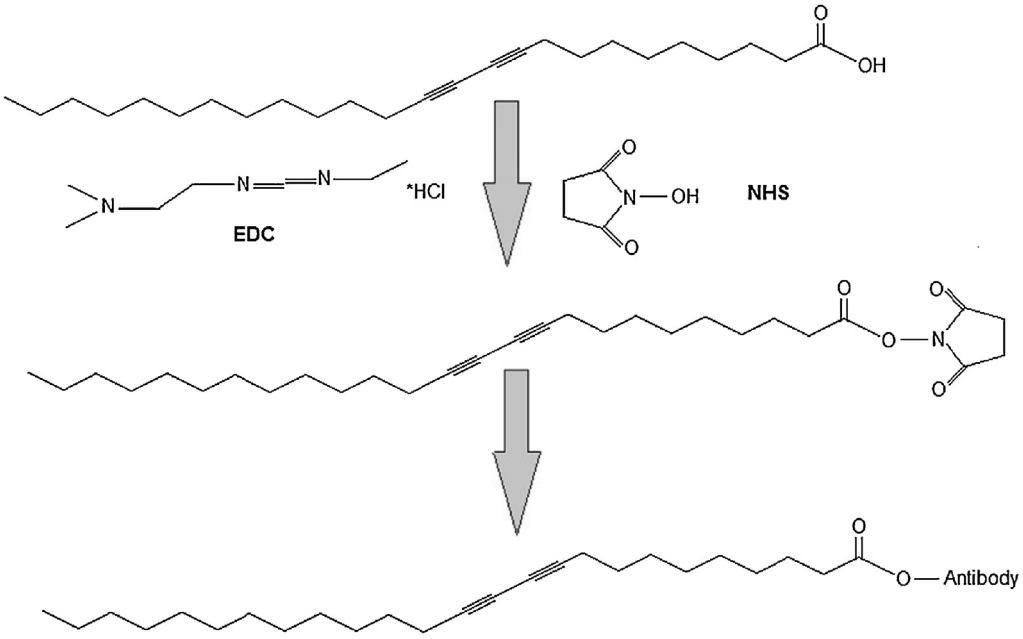

Fig. 1. Schematic illustration of the chromatic immunoassay based on polydiacetylene vesicle. The carboxylic acid units of PCDA were converted into the corresponding succinimidyl active ester by treatment with excess *N*-hydroxysuccinimide (NHS) and ethylcarbodiimide (EDC) in aqueous solution. The activated vesicles were covalently attached with antibodies via peptide bond. The vesicle-antibody conjugates were photopolymerized under UV radiation became conjugaded polymer (PDA) and assumed a blue color solution.

red, or orange depending on the extent of local perturbation [19]. The energy measurements associated with colorimetric transition support the hypothesis that such phenomena occurred due to conformational changes associated with the functional group rotation around the simple carbon–carbon bond present in PDA chains [5]. When the backbones of PDA conjugated polymer chains are perturbed, the delocalized π-network induces changes in electronic absorption and emission properties.

Despite efforts in the food science and technology fields to detect pathogens of interest, the concomitant study of such main factors help to understand the sensitivity, specificity and stability of PDA system. Therefore, the goal of our work was to study the influence of the factors pH, incubation temperature, incubation time and antibody concentration simultaneously in PDA vesicles to detect *Salmonella* in a culture medium and how these factors affect the size, surface charge and mainly the colorimetric response of it. Moreover, the study helps to understand and manipulate the factors that influence the biosensor; as well as apply this study in laboratory analysis or food packaging, which requires conditions closer to the point of colorimetric transition to be more sensitive.

2. Material and methods

2.1. Materials

All chemicals of high analytical grade were used without further purification. The 10,12-pentacosadiynoic acid (PCDA), N-hydroxysuccinimide (NHS), ethyl(dimethylaminopropyl) carbodiimide (EDC), cholesterol (CHO), NaOH ACS reagent 97.0% and HCl ACS reagent 37.0% were obtained from Sigma–Aldrich (São Paulo, SP, Brazil) HPLC-grade chloroform and HPLC-grade dimethylsulfoxide (DMSO) were purchased from Merck® (Germany). Sphingomyelin (SPH) from chicken egg yolks was obtained from Fluka (Germany). Milli-Q (>18 MΩ) water was used as solvent. *Salmonella* monoclonal antibody IgG 6321 was purchased from Klone (Campinas, SP, Brazil). *Salmonella* Choleraesuis ATCC 10708 was used in this study. The stock culture was maintained at −80 °C in tryptic soy broth (TSB) (DIFCO®, Becton, Dickinson and Company, Franklin Lakes, NJ, USA) with 10% (v/v) glycerol. Stock culture was activated twice in TSB and was incubated at (37 ± 2) °C for 24 h. Then it was used in step below [24].

2.2. Vesicle preparation

PCDA vesicles were prepared as described elsewhere [8]. The PCDA solution (1 mM) was dissolved in 2 mL DMSO and heated at 80 °C for 15 min. In a separate flask, SPH/CHO (1 mM) was dissolved in chloroform, and after that the solvent was removed under nitrogen flow forming a thin film in the background. After cooling to room temperature (25 °C) both solutions were mixed and brought to a 10 mL volume with Milli-Q water. Vesicle suspensions were sonicated for 5 min at 70 °C using a probe sonicator (Unique Vira Cell, Model VCX 130, No. R2D091109) at a power of 400 W and then filtered through a 0.25 μm PVDF filter. The filtrate was cooled to 4 °C overnight. The next step was antibody incorporation.

2.3. Antibody incorporation

The antibody conjugates were prepared according to Dong et al. with further discussed modifications [20]. Free carboxylic acid of conjugated PCDA was activated by a reaction with NHS and EDC at a molar ratio of 1:1.8 for 20 min as illustrated in Fig. 1. The *Salmonella* antibody was added in 1 mL of PCDA vesicles in the quantity described in experimental design (Item 2.5), and it was allowed to react for 3 h at 4 °C on a shaker. The vesicle was cooled to 4 °C overnight. Followed by PCDA vesicle exposure in 254 nm UV light (Prodicil, 110 V) for 15 min became PDA vesicles due to polymerization that happened, and then it was stored in a refrigerator until the time of use.

2.4. Raman

Spectra were recorded with a resolution of 1 cm^{-1} on "InVia Renishaw Micro-Raman" spectrometer with a 514.5 nm of laser