

# Layer-by-Layer Deposition of Chitosan/Polydiacetylene Vesicles for Convenient Preparation of Colorimetric Sensing Film

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## ABSTRACT

Photopolymerized vesicles of poly(10,12-pentacosadiynoic acid) (PPCDA) were successfully assembled into polyelectrolyte multilayer (PEM) thin films with polycationic chitosan by using a layer-by-layer deposition method. The linear relationship between the increase in absorbance at 639 nm and the number of deposited layers of chitosan and PPCDA vesicles was obtained. The stability of the vesicles was greatly enhanced when deposited into the PEM films. When assembled into PEM films, the desired colorimetric responses of the PPCDA vesicles to solvent (ethanol), pH and temperature were preserved with slight shifts in the color transition points. The shifts in transition points of the PPCDA vesicles assembled in PEM films from those of the vesicles dispersed in aqueous media is likely due to the highly electrostatic environment in the PEM films.

**Keywords:** Polydiacetylene Vesicles, Polyelectrolyte multilayers, Sensor, Colorimetry

## 1. INTRODUCTION

Polydiacetylenes (PDAs) [1] are an interesting class of conjugated polymers which exhibit dramatic color change upon exposure to various stimuli including light [2-4], heat [5-9], mechanical stress [10-12], solvent environment [13-14] and binding of specific chemical/biological agents [15-17]. PDAs have thus been materials of choices for colorimetric sensing applications such as thermal [18-19], and biological sensors [20-21]. PDAs can be prepared from topochemical polymerization of monomeric diacetylenes in numerous forms: bulk solids [22-24], self-assembled films [25-26] and vesicles suspended in liquids [27-28]. Nanospherical vesicles or liposomes of PDAs homogeneously dispersed in aqueous media have been successfully used as colorimetric sensors [29-34].

The immobilization of uniformly distributed PDA vesicles into thin films should facilitate the storage stability and applicable expediency of the vesicles. Despite extensive research on PDA vesicles to be used as colorimetric sensors, reports on the fabrication of films containing PDA vesicles are scarce. Recently, monomeric diacetylene vesicles were covalently fixed onto a modified

glass substrate followed by photopolymerization to form blue phase PDAs deposited as a monolayer film [35]. This approach is useful when monolayer deposition is desired but the fabrication of multilayer film with visible color can be quite difficult. Transfer of self-assembled PDA Langmuir-Blodgett films to flat substrates is somewhat simple [36]. The Langmuir-Blodgett technique, relying mainly on the hydrophobic and hydrophilic interaction, is however not suitable for the assembly of PDA vesicles which have spherical hydrophilic surface. The polyelectrolyte multilayers (PEM) technique was developed as an alternative method for the preparation of multilayer thin films [37-43]. The PEM method has proven to be very simple and efficient to immobilize polyelectrolyte compounds onto a wide range of substrates. Early attempts to use the layer-by-layer technique to assemble diacetylene monomers or polydiacetylene chains with polyallylamine (PAH) resulted in thin films with a red color which excluded them from being used as sensor [44]. The PEM technique has recently been used successfully for deposition of unpolymerized vesicles on flat substrates that the spherical structure of vesicles being intact [45-47]. The aim of this paper is to report our development of PEM technique as a method for immobilization of PDA vesicles into thin films for colorimetric sensing.

## 2. EXPERIMENTAL

### 2.1 Materials

The diacetylenic monomer, 10,12-Pentacosadiynoic acid (97%), was purchased from Fluka, USA. Poly(diallyldimethylammonium chloride) (PDADMAC)  $M_w = 200,000-350,000$  was purchased from Aldrich USA. Chitosan ( $M_w \sim 8.0 \times 10^5$ , %DD  $\sim 84$ ) was purchased from Seafresh Chitosan (Lab) Company, Thailand. Diethylether, analytical grade, was purchased from Lab-Scan, Thailand. De-ionized milliQ water with a resistance of 18.1 M $\Omega$  was used in all experiments. All solvents and chemicals were used as received without further purification, unless specified otherwise.

## 2.2 Preparation of PPCDA vesicles

The diacetylene monomer, 10,12 pentacosadiynoic acid (PCDA), was dissolved in diethylether and filtered to remove any contaminating polymerized materials. The filtrate was dried under rotary evaporator to produce thin film of lipid monomer covering inside of the flask. Deionized water was added to provide a 1 mM aqueous lipid suspension. The suspension was sonicated at 75-80 °C for 30 minutes. The vesicle suspension was allowed to cool down to room temperature and then kept at 4 °C overnight. The vesicle suspension was irradiated by UV irradiation for 5 minutes to initiate the topopolymerization. Following the polymerization, the solution was filtered through 0.45 μm PTFE syringe filter to remove the undesired lipid aggregates. The resulting filtrate appeared as a transparent deep blue solution of (PPCDA) vesicles.

## 2.3 Preparation of polycationic solutions

Chitosan powder (0.5 g) was dissolved in aqueous acetic acid solution (2% w/w, 40 mL). The mixture was stirred overnight to allow complete dissolution. The solution was diluted by water to make the total volume of 500 mL. The solution was filtered and the pH was adjusted to 4 by 0.1 M HCl prior to use. PDADMAC were dissolved in distilled water to give the final repeating unit concentration of 10 mM. The pH was adjusted to 2.8 with 0.1 M HCl. All polycationic solutions were prepared freshly for each deposition experiment.

## 2.4 Layer-by-layer deposition of the vesicles

A chemically cleaned glass slide substrate was dipped in the polycationic solution (0.1% w/w for chitosan, 10 mM for PDADMAC) for 5 minutes. After three rinsing steps with deionized water, the glass substrate was immersed in the polydiacetylene vesicle solution (0.8 mM of repeating unit) for 5 minutes. The deposition was carried out by repeating adsorption cycles of dipping in polycation, rinsing and dipping in PPCDA vesicles. UV-Vis spectrophotometer (Varian Cary 100 Bio) was employed to confirm the layer-by-layer deposition process by monitoring the increase of absorbance at 639 nm as a function of the numbers of layers deposited.

## 2.5 Evaluation of the colorimetric responses

The colorimetric response (%CR) was used to quantitatively evaluate the blue-to-red transition. The %CR is  $100 \times (PB_0 - PB) / PB_0$ , where PB is the percent blue calculated from  $A_{\text{blue}} / (A_{\text{blue}} + A_{\text{red}})$ . The  $A_{\text{blue}}$  and  $A_{\text{red}}$  were the absorbance of the blue and red phases of PPCDA measured at  $\lambda_{\text{max}}$  around 640 nm and 540 nm, respectively. The initial percent blue ( $PB_0$ ) was determined on the samples before exposure to the stimulus. The PEM thin films on glass

slides as well as the vesicle solution were evaluated for two sensing parameters: ethanol content and temperature. The completeness of color transition was determined as the first saturation point in the plot of %CR against each sensing parameter. *Ethanol sensing:* The PEM film on glass slide or the vesicle solution was introduced into a quartz cuvet filled with distilled water and allowed to stand for 5 minutes before the spectrum was taken to obtain  $PB_0$ . The procedure was repeated after replacing water by the aqueous ethanol solution starting from lower varying to higher content of ethanol until the color transition from blue to red was completed. *Temperature sensing:* The PEM film on glass slide or the vesicle solution was introduced into a quartz cuvet filled with distilled water of which the temperature was controlled at 20 °C. The system was allowed to stand for thermal equilibrium for 10 min before the spectrum was taken to obtain  $PB_0$ . The procedure was repeated with increasing temperature until the color transition from blue to red was completed.

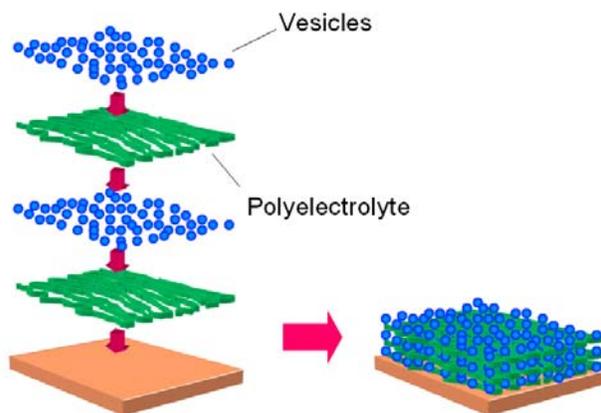


Figure 1. Illustration of chitosan/PPCDA vesicles assembly on glass substrate

## 3. RESULTS AND DISCUSSION

To obtain multilayer films containing polydiacetylene vesicles, two of polycationic polyelectrolytes, chitosan, and poly(diallyldimethylammonium chloride) (PDADMAC) were assembled with poly(10,12-pentacosadiynoic acid) (PPCDA) vesicles on clean glass substrates. Figure 1 illustrates the idealized assembly of alternating layers of polycationic polymer chains/negatively charged PPCDA vesicles.

The PEM films prepared from the assembly between PDADMAC with PPCDA vesicles gave films with a red color while the films obtained from the assembly using chitosan remained in blue color. We hypothesize that the densely localized charge nature of PDADMAC brought about deformation of the vesicles leading to the detrimental red color whereas the delocalizable charges on the dynamically protonated chitosan polymeric chain in acidic

solution did not cause the deformation of the vesicles upon deposition. Since the preservation of the characteristic blue phase of the polydiacetylene vesicles is the most critical point for sensing applications, further work was limited to the use of chitosan as polycationic polymers for the layer-by-layer assembly.

As a result of the film growth, an optically uniform thin film of increasing color intensity was obtained on glass slide substrate. A UV-Vis spectrophotometer was employed to confirm the layer-by-layer deposition process between chitosan and the PPCDA vesicles by monitoring the increase of the absorbance at 639 nm as a function of the numbers of the layers deposited. A linear relationship between the number of the layers and the absorbance was obtained (Fig. 2) indicating a well defined layer-by-layer deposition system. Unlike the vesicles dispersed in water, the blue phase chitosan/PPCDA film can be kept indefinitely in water without significant change in their color. This result suggested that immobilization of the PPCDA vesicles into the PEM films with polycationic chitosan could increase the vesicle stability and thus extended their shelf life [48].

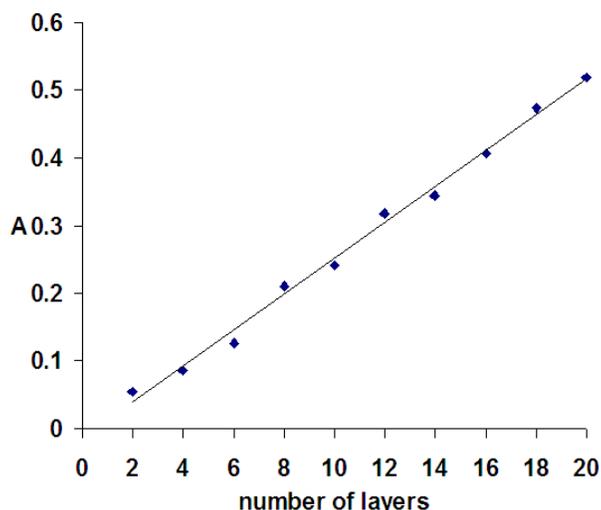


Figure. 2. UV-Vis absorbance at 639 nm of a Chitosan / PPCDA vesicles film, prepared from layer-by-layer deposition, as a function of the number of the layers.

PPCDA vesicles in aqueous media were known to change the color from blue to red when exposed to a more hydrophobic solvent such as ethanol. Our first goal was to investigate whether the vesicles embedded in the PEM film would have a similar solvatochromic response to the vesicles suspended in the aqueous media and could therefore still be used as an ethanol sensor. Immersing the PEM films into the aqueous solution containing various percentages of ethanol resulted in an increase of %CR and an irreversible color switch from blue to red. The color transitions for the chitosan/PPCDA films were completed when the aqueous solution contained 62% (w/w) ethanol, (Fig. 3a). The color change of the PPCDA vesicles

dispersed in the aqueous media was however completed at significantly lower ethanol content (47% w/w). The shift in the response of the vesicles in the PEM films comparing to the vesicles dispersed in the aqueous media is probably due to the electrostatic nature of the PEM films which tends to repel less hydrophilic solvents [49-51]. The rapid change in %CR of the PEM films agrees well with the sharp color change easily observed by eyes.

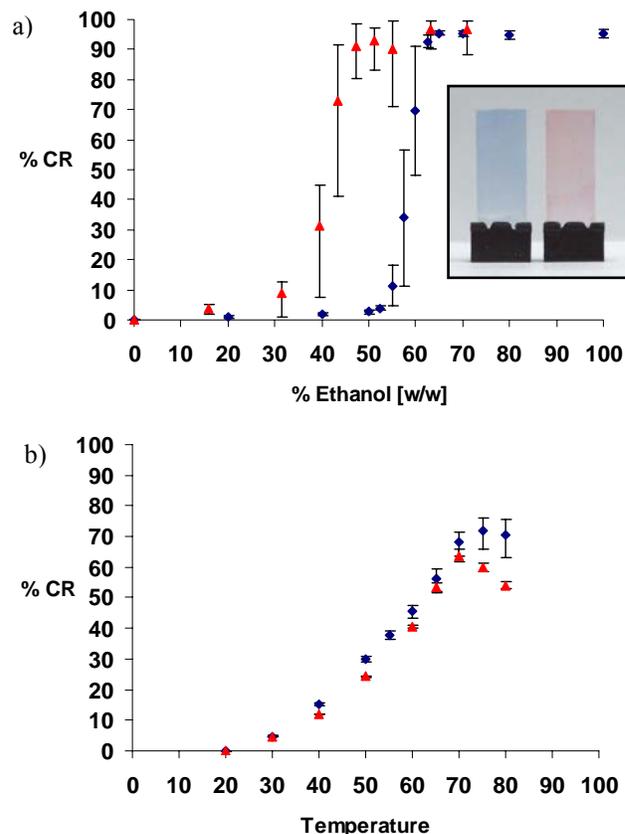


Figure 3. a) The change of the colorimetric response (%CR) as a function of EtOH content b) The change of the colorimetric response (%CR) as a function of temperature (a) PPCDA vesicles dispersed in water (▲), (b) chitosan/PPCDA vesicle PEM film (◆)

The thermochromic property of the immobilized PPCDA vesicles in the PEM films was studied by variable temperature UV-Vis spectrometer. The %CR of the PEM films and the vesicles dispersed in aqueous media increased almost linearly with the temperature in the range of 25-70 °C and the color transition was completed around 70 °C (Fig. 3b).

#### 4. CONCLUSIONS

This article has demonstrated that the layer-by-layer deposition was simple and effective method for immobilization of polydiacetylene vesicles into thin films with colorimetric sensing for the change in the surrounding

conditions i.e. solvent and temperature. The stability of the vesicles can be greatly enhanced in the form of PEM films. The abrupt change of the color of the prepared films from blue to red upon increasing ethanol content in water, and temperature suggests potential applications of the films as visible Yes/No logic sensors for food and beverage products.

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