



## Spray layer-by-layer films based on phospholipid vesicles aiming sensing application via e-tongue system

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### ARTICLE INFO

#### Article history:

Received 16 September 2011

Received in revised form 5 December 2011

Accepted 1 February 2012

Available online 9 February 2012

#### Keywords:

Spray layer-by-layer film

Phospholipids

Impedance spectroscopy

Xanthene

### ABSTRACT

The Layer-by-Layer (LbL) technique via spraying (spray-LbL) has been applied as new and alternative methodology to fabricate ultrathin films due to its versatility in relation to the conventional dipping-LbL method, mainly in terms of faster layer deposition and larger coated area. In this work, the possibility of immobilizing vesicles of dipalmitoyl phosphatidyl glycerol (DPPG) phospholipid onto alternating layers of the polyelectrolyte poly(allylamine hydrochloride) (PAH) using the spray-LbL method was investigated, being the results compared to the conventional dipping-LbL method. The growth of (PAH/DPPG)<sub>n</sub> spray-LbL films was systematically monitored by quartz crystal microbalance (QCM) and ultraviolet–visible (UV–vis) absorption spectroscopy, revealing a linear increase of the absorbance vs deposited layers. In relation to a possible electrostatic interaction between the groups PO<sub>4</sub><sup>-</sup> (DPPG) and NH<sub>3</sub><sup>+</sup> (PAH), it was observed through Fourier transform infrared (FTIR) absorption spectroscopy that the spectrum recorded for the spray-LbL film is basically a simple superposition of the FTIR spectra from PAH and DPPG casting films. The latter indicates a weak interaction between both materials, differently of the trend observed for (PAH/DPPG)<sub>n</sub> grown via dipping-LbL method. Atomic force microscopy (AFM) images of spray-LbL films showed evidences that the DPPG vesicles present in the aqueous dispersion are not destroyed when submitted to pressure conditions during the spray deposition. However, comparing to dipping-LbL, the DPPG vesicles do not cover completely the PAH layer for the spray-LbL film, which was further confirmed by surface-enhanced Raman scattering (SERS) measurements. Moreover, the AFM analysis showed that the spray-LbL deposition led to thicker PAH/DPPG bilayers in average than via dipping-LbL for the same concentrations of PAH solution and DPPG dispersion, which is consistent with QCM and UV–vis absorption results. PAH/DPPG films deposited by dipping- and spray-LbL techniques and also by Langmuir–Blodgett (LB) onto Pt interdigitated electrodes composing an array of sensing units (e-tongue) were applied in the detection of a xanthene derivate (eosin) in diluted solutions (10<sup>-9</sup>, 10<sup>-7</sup> and 10<sup>-6</sup> M). Despite the LB and LbL films are formed by the same materials (PAH and DPPG), it was found that their different molecular architectures play an important role on the electrical response of Pt interdigitated electrodes in impedance spectroscopy measurements. The high sensitivity reached by these sensing units was intimately related to changes in the film morphology caused by the adsorption of the eosin molecules onto the film surfaces during electrical measurements, as evidenced by micro-Raman technique.

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### 1. Introduction

Since the first report in 1992 by Decher et al. [1], the Layer-by-Layer (LbL) technique has been widely applied in the fabrication of thin films for different kinds of systems and materials [2–5]. The process is basically based on the alternately adsorption of materials with opposite charges onto a substrate by dipping it into a solution. However, the dipping-LbL methodology presents practical limitations, mainly related to long periods of time for a complete layer

adsorption and to small coating areas required for the thin film fabrication. These limitations were overtaken in 2000 by Schlenoff et al. [6], with an unconventional approach in which the solutions are alternately deposited by spraying onto a substrate instead of dipping the substrate into the solutions, forming the so called spray-LbL films. In that work [6], the polyelectrolytes poly(diallyldimethylammonium chloride) and poly(styrenesulfonic acid) (PDAD/PSS) deposited by spraying presented composition and thickness similar to those produced via dipping-LbL deposition. In 2005 Izquierdo et al. [7] reported a more detailed study on the fabrication of LbL multilayer films of poly(allylamine hydrochloride)/PSS (PAH/PSS) polyelectrolytes comparing both approaches, dipping and spraying. It became clear that spraying is a method as general as dipping; however

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much faster (a factor up to 500 times faster has been reported [7,8]) and applicable for larger coating surfaces, widening the possibility of applications. Nowadays, the spray-LbL method starts to be applied in thin film fabrication of different classes of materials such as inorganics [9], colloidal and conducting polymers [10–12], carbon nanotubes [13,14] and graphene [15], considering distinct applications. During this period, an evolution of the experimental setup has been observed [6,7] and up to date automated systems for spraying deposition are reported allowing better control of film growth [16,17].

However, the immobilization of biomolecules such as phospholipid vesicles is a difficult task due to the vesicle fragility, which might collapse due to the spraying pressure. Michel et al. [18] reported that phospholipid vesicles of palmitoyl oleoyl phosphatidyl choline (POPC) and palmitoyl oleoyl phosphatidyl glycerol (POPG), when rigidified with cholesterol and stabilized with poly-(D-lysine) (PDL), kept their shape upon spraying immobilization. The phospholipid vesicles were deposited by spraying forming two distinct layers separated by several polyelectrolyte bilayers to enhance the vesicle stability. In our work, a step forward was given by demonstrating the possibility of growing spray-LbL films containing alternated layers of the polyelectrolyte PAH and the phospholipid DPPG (dipalmitoyl phosphatidyl glycerol) in the vesicle form, leading to (PAH/DPPG)<sub>n</sub> architecture. DPPG phospholipid vesicles were prepared without addition of any other material and were deposited by spraying using a homemade device based on airbrush sprays. As proof-of-principle, the spray-LbL films were applied as transducers in sensing experiments aiming to detect trace levels of a xanthene compound (eosin) in water. Eosin was chosen due to its therapeutic importance in pharmacology applied on cancer chemotherapy [19], photodynamic therapy [20,21] and as trypanothione reductase inhibitor [22].

Three major targets were focused in this study: (i) the optimization of the growth conditions of PAH/DPPG spray-LbL films, which might be extended to other phospholipids; (ii) the comparison between both dipping and spray-LbL films in terms of molecular architecture (surface morphology, roughness, thickness, DPPG structure); and (iii) their efficiencies as transducers when applied in sensing units composing an e-tongue system based on impedance spectroscopy. The growth of the spray-LbL films was monitored using quartz crystal microbalance (QCM) and ultraviolet–visible absorption (UV–vis) spectroscopy contributing to the information related to (i). The morphology was investigated at micrometer scale via micro-Raman measurements combining chemical and morphological information, with an optical microscope coupled to a Raman spectrograph. Morphological information at nanoscale was obtained via atomic force microscopy (AFM). Fourier transform infrared absorption (FTIR) spectroscopy was performed to check possible interactions between PAH and DPPG layers. Therefore, micro-Raman, AFM and FTIR characterization techniques were applied to acquire information related to (ii). Finally, spray-LbL films of PAH/DPPG were deposited onto Pt interdigitated electrodes forming a sensing unit, which was complemented with sensing units formed by (PAH + DPPG) Langmuir–Blodgett (LB) film [23] and PAH/DPPG dipping-LbL film [23,24]. The sensor array was applied in the detection of eosin in diluted aqueous solutions, allowing a direct comparison in terms of distinct molecular architectures of the thin films (spray-LbL, dipping-LbL and LB), pointing to the information described in (iii).

## 2. Experimental section

### 2.1. Reagents and solutions

The anionic phospholipid DPPG [1,2-Dipalmitoic-*sn*-3-Glycerophospho-rac-(1-glycerol)] was purchased from Genzyme Pharmaceuticals. The cationic polyelectrolyte PAH and the xanthene eosin were acquired from Sigma–Aldrich Co. All chemicals were used without further purification. The molecular weights of DPPG, PAH and eosin

are 745;  $15 \times 10^3$  and 647 g/mol, respectively, and their molecular structures are exhibited in Fig. 1a. The spray-LbL films were fabricated from DPPG aqueous dispersion and PAH aqueous solution using ultrapure water (18.2 M $\Omega$ .cm and pH 5.6) obtained from a Milli-Q system, model Simplicity. Concentrations of 0.74 mg/mL (1.0 mM) for DPPG and of 1.0 mg/mL for PAH were prepared without any special procedure: the powder was simply added to ultrapure water at room temperature (22 °C) and the solution was gently stirred.

### 2.2. Layer-by-layer film fabrication

#### 2.2.1. Dipping-LbL films

The PAH/DPPG dipping-LbL film was grown following the experimental procedure previously reported in [24]. The substrate was immersed into distinct solutions according to the following sequence: PAH solution (3 min) → ultrapure water gently stirred to remove excess of adsorbed PAH (1 min) → DPPG dispersion (3 min) → ultrapure water to remove excess of adsorbed DPPG (1 min). After that, the first bilayer of PAH/DPPG is formed and the multilayered PAH/DPPG dipping-LbL film is grown repeating this four-step sequence.

#### 2.2.2. Spray-LbL films

The deposition of (PAH/DPPG)<sub>n</sub> spray-LbL films was made by spraying DPPG dispersion and PAH solution alternately using the experimental setup exhibited in Fig. 1b and c. It was employed a homemade device composed by airbrush spray, model BD-123A purchased from LEEpro tools with 0.3 mm of nozzle diameter and 7 mL of fluid cup capacity. For spraying deposition, it was adopted a perpendicular orientation between the spray axis and the coating surface, 30 cm apart each other. The airbrush sprays were filled with PAH solution and DPPG dispersion and sprayed with a constant pressure of 10 psi during 2 s. A valve regulator was used to control the spraying pressure from the compressor. At each deposition step, the film was rinsed with ultrapure water also sprayed for 2 s. All the parameters previously mentioned were chosen after testing different combinations involving spraying pressures (10, 20, and 30 psi), spraying times (2, 4, and 6 s), with and without rinsing steps (Fig. 2). The final setup allowed spraying the solutions forming a thin layer on the desired surface, which dries within few seconds.

The number of spraying (or dipping) bilayers and the type of substrate were chosen according to the characterization technique. For instance, the films were grown onto quartz plates for UV–vis absorption spectroscopy, onto AT-cut quartz crystal coated with Au plates for QCM, onto ZnSe for FTIR, onto glass thermally treated (600 °C for 2 h) for AFM, and onto interdigitated Pt electrodes for impedance spectroscopy. The substrates were previously cleaned using neutral detergent, being sequentially and extensively washed in distilled water, acetone (5 min sonication), chloroform (5 min sonication), and ultrapure water. This procedure was sufficient to activate the substrate surfaces avoiding the use of drastic treatments such as piranha solution (a mixture of concentrated sulfuric acid and hydrogen peroxide).

### 2.3. Characterization techniques

The growth of LbL films (spray and dipping) were monitored via UV–vis absorption spectroscopy using a Varian spectrophotometer; model Cary 50, from 190 to 1100 nm, and via adsorption mass using a quartz crystal microbalance Stanford Research Systems Inc., model QCM 200. In QCM, shifts in frequency were continuously followed with time and the mass gain ( $\Delta m$ , in grams) was obtained using the Sauerbrey equation [25]:  $\Delta F = (-2.3 \times 10^6 F_0^2 \Delta m) / A$ , where  $\Delta F$  is the frequency shift (in Hz),  $F_0$  is the initial frequency (without coating, in MHz), and  $A$  is the electrode area (in cm<sup>2</sup>). The FTIR spectra were taken using a Bruker spectrometer, model Vector 22, equipped with a DTGS detector. The morphology was characterized through

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