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Contribution of the cashew gum (*Anacardium occidentale* L.) for development of layer-by-layer films with potential application in nanobiomedical devices☆

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ABSTRACT

The search for bioactive molecules to be employed as recognition elements in biosensors has stimulated researchers to pore over the rich Brazilian biodiversity. In this sense, we introduce the use of natural cashew gum (*Anacardium occidentale* L.) as an active biomaterial to be used in the form of layer-by-layer films, in conjunction with phthalocyanines, which were tested as electrochemical sensors for dopamine detection. We investigated the effects of chemical composition of cashew gum from two different regions of Brazil (Piauí and Ceará states) on the physico-chemical characteristics of these nanostructures. The morphology of the nanostructures containing cashew gum was studied by atomic force microscopy which indicates that smooth films punctuated by globular features were formed that showed low roughness values. The results indicate that, independent of the origin, cashew gum stands out as an excellent film forming material with potential application in nanobiomedical devices as electrochemical sensors.

1. Introduction

The development of electrochemical biosensors has been a focal subject in biosciences and biotechnologies [1,2]. Recent developments in chemistry, physics, biochemistry and molecular biology have led to the creation of biosensors for a large range of biological recognition elements with improved selectivity and assay sensitivity [3–7].

Different kinds of materials have been used as transducer elements in biosensors, and many of them have been built from the layer-by-layer (LbL) technique that was originally developed by Decher and Hong [8]. This is an assembly method based on electrostatic alternated adsorption of oppositely charged polyions. Due to its versatility and simplicity, it has been applicable to assemble a wide variety of materials including carbohydrates, proteins, nanoparticules, dyes, DNA and natural gums [9–13]. LbL films exhibit nanometric thickness and hence the organization of the material occurs at the molecular level: what makes their combination presents different and new physical and chemical properties of the materials alone [8,12,13].

In this work, we studied the contribution of a cashew tree gum, denoted also as cashew gum (CG), which is an exudate from *Anacardium occidentale* trees, for the development of LbL films with potential application in nanobiomedical devices. This gum is a branched acidic heteropolysaccharide containing galactose as major component and also glucose, arabinose, rhamnose and glucuronic acid [14,15]. CG presents low viscosity, comparable in many aspects to gum arabic, which is an important material in the food, cosmetic and pharmaceutical industries [16].

Cashew tree culture is very important in countries including Brazil, India, Mozambique, Tanzania and Kenya. Brazil has more than 710,000 ha of area planted with the cashew nut tree [17] and the average production of gum/tree/year is 700 g, with a potential annual CG production being greater than 38,000 tons [18].

The technological interest in the cashew gum and other natural gums, which has been proven to present similar rheological characteristics and industrial applications to many synthetic polymers, comes mainly from its biodegradability and mechanical properties [19].

Another type of material well-studied for the LbL films formation are metallic phthalocyanines (MTsPc), that stand out for being non-toxic semiconducting materials with well-defined electrochemical

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activity [20]. MTsPc are macrocyclic molecules bearing a central atom with high electronic density and reactivity. Phthalocyanines may undergo chemical functionalization upon reaction via the central atom or the macrocycle with specific ligands, which make them candidate materials for sensors, catalysts and optoelectronic and optical storage devices [20,21]. Most non-substituted phthalocyanines are poorly soluble, and therefore film fabrication has relied on vapor deposition techniques. To overcome this difficulty, MTsPc with appropriate chemical groups have been synthesized, allowing for the fabrication of Langmuir–Blodgett (LB) and LbL films for nanocomposites devices and biosensors [22,23].

The development of voltammetric sensors and LbL films for neurotransmitter detection in the extra cellular fluid of the central nervous system has received much attention in the past few decades [24]. Electrochemical methods have several advantages over other transduction methods for sensing of neurotransmitters in living organisms [25].

In this study, LbL ultrathin films were constructed with CG and MTsPc. Since MTsPc and CG are polyanions, the multilayer deposition was carried out in a tetralayer fashion with a conventional cationic polyelectrolyte, poly(allylaminehydrochloride) (PAH), which was interposed between the polyanionic layers, forming the nanostructures $(PAH/CG/PAH/MTsPc)_n$, where n refers to the number of tetralayers deposited. The chemical structures of these materials are depicted in Fig. 1.

Some characteristics of natural gums, including composition, molar mass and impurities depend on the origin of the plant [14]. To evaluate the influence of the CG from different regions (Ceará state or Piauí state) in the characteristics of the LbL ultrathin films, UV–VIS spectroscopy and electrochemical experiments were conducted. We also investigated the gum composition using gel permeation chromatography (GPC) and the ability of the LbL film to act as modified electrodes for dopamine (DA) detection. In addition, atomic force microscopy (AFM) was used to study the influence of the number of multilayer depositions and the different origins of the CG on the morphology of the nanostructured films.

2. Experimental

2.1. Materials

Crude samples from CG were collected from cultivated trees at Fortaleza, Ceará, Brazil (which we denominate as CGCE) and from native trees at Ilha Grande de Santa Isabel, Piauí, Brazil (which we denominate as CGPI). They were purified as a sodium salt using the method previously described [26]. Nodules free of bark were selected and dissolved in ultrapure water at room temperature to give a 5% (w/v) solution. The solution pH was adjusted to approximately 7.0 by addition of diluted aqueous NaOH (0.05 mol/L). The clear solution was successively filtered through sintered glass (coarse grade) and the polysaccharide was precipitated with ethanol (ratio 80:20 alcohol:CG). After the purification steps, 0.5 g of cashew gum was solubilized in 100 mL of ultrapure water under stirring for 12 h and filtered through sintered glass under vacuum.

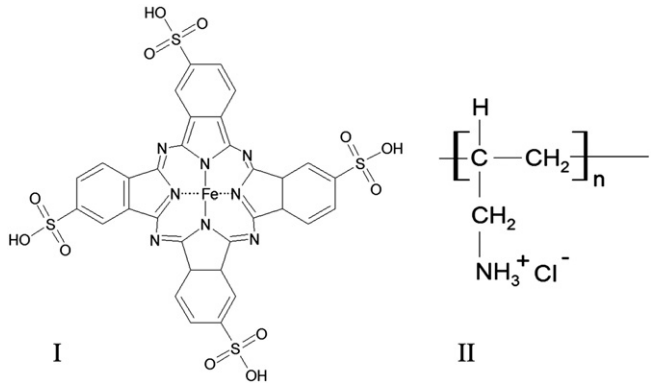

Fig. 1. Chemical structures of others polyions. (**I**) polyanion: FeTsPc and (**II**) polycation: PAH.

Xylose and mannose monosaccharides were purchased from Jansen Chimica, glucose, rhamnose and glucuronic acid were purchased from Sigma. The origin of ribose and galacturonic acid was Lancaster synthesis. Arabinose and galactose were supplied by Biochemical and FSA Laboratory, respectively.

NiTsPc, FeTsPc and PAH were purchased from Aldrich Co. and used without further purification. The aqueous solutions of these three reagents were prepared at a concentration of 0.5 mg/mL with HCl at pH 2.5. DA was also purchased from Aldrich Co. and prepared as a 10^{-3} mol/L concentration solution.

2.2. Gel permeation chromatography (GPC)

The peak molar mass was determined by GPC with a Shimadzu LC-10 AD with a refractive index detector RID-6A at room temperature using a PolySep-GFC-P linear column (7.8×300 mm) and a PolySep-GFC-P pre-column (7.8×35 mm), flow rate of 0.5 mL/min, polysaccharide concentration of 0.1% (w/v), water as the solvent and $NaNO_3$ 0.1 mol/L as the eluent and the sample volume was 50 μL.

Pullulan samples (Shodex Denko) of average molar mass (MM) of 5.9×10^3, 1.18×10^4, 4.73×10^4, 2.12×10^5, and 7.88×10^5 g/mol were used as standards. The calibration plot (log MM *versus* V_e) presents a linear dependence with correlation coefficient 0.9975. The obtained equation was $\log MM = 16.37 - 1.209V_e$ where V_e is the elution volume in mL.

2.3. High performance liquid chromatography (HPLC)

HPLC with refraction index detection was used to analyze hydrolysates and for the quantification of the monomers. Cashew gum aqueous solutions (30 mg/2 mL) were hydrolyzed with 2 mL of trifluoracetic acid (TFA) 4 mol/L at 100 °C for 4 h. To eliminate the excess of acid, methanol (MeOH) was added (8×2 mL) and the solution was concentrated by heating at 60 °C after each alcohol addition. An HPLC Shimadzu LC-10 AD, with a Phenomenex column Rezex 8 μ 8% H. Org. Acid (7.8×300 mm) and a PolySep-GFC-P pre-column (7.8×35 mm), flow 0.5 mL/min and 8.0 mmol/L H_2SO_4 as eluent was used to analyze the hydrolyzed material. A differential refractometer RID-6A was employed as the detector. The monosaccharides xylose, mannose, glucose, galactose, rhamnose, arabinose, glucuronic and galacturonic acids were also injected as standards. All HPLC analyses were average value of two determinations.

2.4. Multilayer deposition

LbL films containing natural cashew gum (0.5%) were adsorbed on glass or glass covered with indium tin oxide (ITO) slides. All slides were cleaned using hydrogen peroxide as described by Kern [27]. This treatment was followed by intensive rinsing with ultrapure water and the multilayer films were manually prepared in a tetralayer fashion (Fig. 2) in the sequence: $(PAH/CG/PAH/MTsPc)_n$ where M is Ni or Fe and n is the number of tetralayers deposited. Multilayer films with $n=1$ to 10 were obtained by immersing the substrate into the polycationic (PAH) and polyanionic (NiTsPc, FeTsPc or CG) solutions with immersion times of 5 min for each polyelectrolyte. After deposition, the substrates were rinsed in a washing solution (HCl pH 2.5) and dried under a nitrogen flow. The growth of the multilayers was monitored with UV–VIS spectroscopy by collecting the absorbance spectrum after each deposition step, using a Hitachi U-3000 spectrophotometer.