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Optical and structural properties of protein/gold hybrid bio-nanofilms prepared by layer-by-layer method

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ABSTRACT

Lysozyme/gold thin layers were prepared by layer-by-layer (LbL) self-assembly method. The build-up of the films was followed by UV–vis-absorbance spectra, quartz crystal microbalance (QCM) and surface plasmon resonance (SPR) techniques. The structural property of films was examined by X-ray diffraction (XRD) measurements, while their morphology was studied by scanning electron microscopy (SEM) and atomic force microscopy (AFM). It was found that gold nanoparticles (NPs) had cubic crystalline structure, the primary particles form aggregates in the thin layer due to the presence of lysozyme molecules. The UV–vis measurements prove change in particle size while the colour of the film changes from wine-red to blue. The layer thickness of films was determined using the above methods and the loose, porous structure of the films explains the difference in the results. The vapour adsorption property of hybrid layers was also studied by QCM using different saturated vapours and ammonia gas. The lysozyme/Au films were most sensitive for ammonia gas among the tested gases/vapours due to the strongest interaction between the functional groups of the protein.

1. Introduction

Gold nanoparticles in the colloid size range enjoy popularity as a consequence of their widespread use in catalysis [1,2], photoelectron [3], biosensors [4,5], biomedicine and nanotechnology [6]. Nanoparticles in variable size and shape with nearly monodisperse dimensions can be produced by colloid chemical synthesis methods. The first replicable method has been developed by Turkevich, who prepared gold nanoparticles from gold-chloride with Na-citrate as a reductive agent [7]. Saraiva and Oliveira have pointed the fact that the concentration of reactants, the addition rate of citrate and the reaction temperature have significant effects on the size of forming gold nanoparticles [8]. He et al. have prepared sols with various particle size by changing the gold:citrate ratio. They have demonstrated that the colour of the sol changes with the particle size [9]. Majzik et al. have prepared gold NPs and nanorods that were stabilized and reduced by citrate reproducible in aqueous dispersions and on functionalized glass surface, respectively [10]. The formation kinetics of gold nanoparticles and nanorods have been studied and also the influence of modifications with cysteine and glutathione on the surface of Au nanoparticles. Gold ion reduction can be replaced by the application of polyelectrolyte. Gold containing sols with a particle diameter of 12 nm were prepared in the presence of Poly(diallyl-dimethyl-ammonium-chloride) (PDDA) [11], and 25 nm particles in poly(ethylene-imine) (PEI) [12]. Furthermore, stabile sols can be prepared by acids like ascorbic acid [13] and 3-thiophenemalonic acid [14].

Various methods can be found in the literature for the preparation of thin films like magnetron sputtering [15,16], chemical vapour deposition [17], spin-coating [18] and Langmuir–Blodgett (LB) [19–24] technique. The layer-by-layer (LbL) method is based on self-assembly, that is perfectly applicable for the preparation of thin films. The LbL method is based on taking advantage of attractive interaction where on some sort of coating components of A and B with opposite charges are alternatively applied. During one deposition cycle, a film is immersed into component A. After the excess is removed by rinsing the thin film, the electrically charged components of A stay on the film making it possible for the oppositely charged component to bind onto the surface of the film. An $(A/B)_n$ hybrid film structure is obtained by repeating this action, where n is the number of deposition cycles [25].

Thin films of several composition can be generated by the above mentioned technique, e.g. polyelectrolyte/clay [26–28], polyelectrolyte/polyelectrolyte [29–30], polyelectrolyte/semiconductive nanoparticle [31–34], polyelectrolyte/layered double hydroxide [35,36], clay/peptide [37] and semiconductor/silica [38] layers, etc. Few publications specialize on the characterization of thin films

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from gold nanoparticles by LbL technique. There are some examples however for the preparation and characterization of films composed of gold/zinc sulphide [39], gold/polyelectrolyte [40,41] and gold/biomolecule [42,43].

The creation of thin films from gold nanoparticles and lysozyme by LbL method and interaction of gold nanoparticles and lysozyme has been studied in this presentation. The study has been carried out in a quasi 2D system by applying light absorption, QCM and SPR techniques and furthermore the structure (XRD) and morphological properties (SEM, AFM) of thin films have been determined.

2. Materials and methods

2.1. Preparation of citrate stabilized gold sol

Au nanoparticles were prepared by Turkevich method [7]. The mixture of 0.4 cm^3 of $HAuCl_4$ solution (c = 0.05 mol/dm^3, $HAuCl_4 \cdot 3H_2O$, Sigma) and 13.85 cm^3 of MQ water was heated until it boils under vigorous stirring, 5.75 cm^3 of sodium citrate dihydrate solution (c = 0.034 mol/dm^3, $C_6H_5O_7Na_3 \cdot 2H_2O$, Aldrich) was added and kept stirring for the next 30 min. The colour of the solution would change until setting on wine-red. The sodium citrate acts as a reducing and stabilizing agent. The concentration of the gold sol is 0.02 g/100 cm^3 while its pH is 6.2 and the zeta-potential of the gold nanoparticles is −27.32 mV.

2.2. Preparation of lysozyme/gold nanofilms

Lysozyme/gold hybrid layers were prepared by layer-by-layer self-assembly method from the aquatic solution of lysozyme (pH 6.2, c = 0.001 g/100 cm^3, from chicken egg white, Sigma) and gold sol (pH 6.2, c = 0.02 g/100 cm^3) on the surface of glass substrates (Menzel Superfrost microscope slides) and on QCM chrome/gold electrode surface using 5 MHz crystals. A previously cleaned (immersion in piranha solution for 3 min) substrate was first immersed into the lysozyme solution for 10 min followed by rinsing with distilled water and dried with nitrogen flow to obtain a lysozyme layer. Then the substrate was dipped into the gold sol for 10 min, after which it was again rinsed and dried. These steps were repeated n times – where n is the number of the deposition cycles, i.e. bilayer number –, to obtain (lysozyme/gold)$_n$ hybrid nanostructures (n = 10, 20, 30).

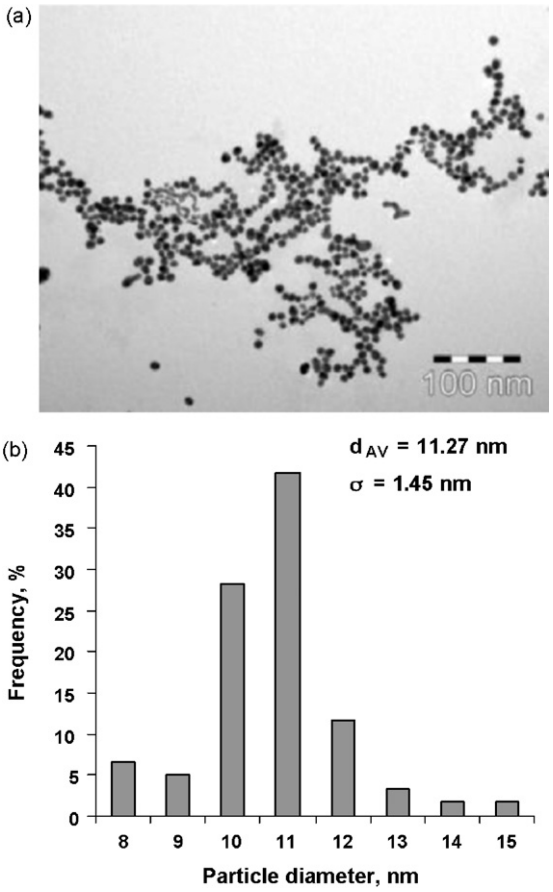

Fig. 2. (a) TEM image of the gold nanoparticles. (b) Size distribution of the gold particles determined from the TEM image.

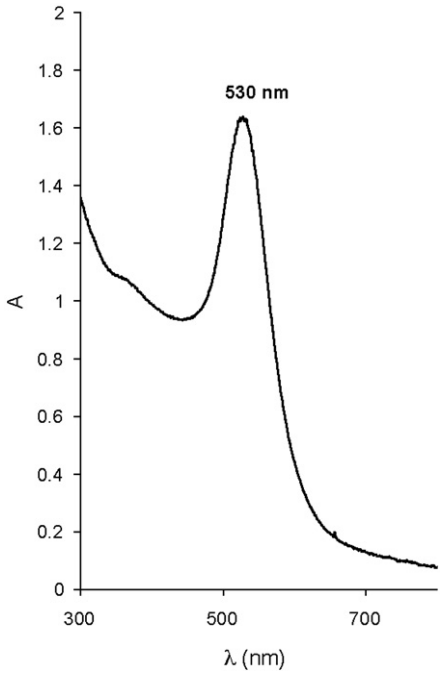

Fig. 1. UV–vis-absorbance spectrum of the gold particles.

2.3. Methods

The UV–vis absorption spectra of the gold sol and the nanolayers were measured by a Micropack Nanocalc spectrophotometer, λ = 350–850 nm range. Quartz crystal microbalance (QCM) measurements were carried out by a Stanford Research System QCM 200 quartz crystal microbalance on a chrome/gold electrode (5 MHz) in a special measuring cell, under nitrogen atmosphere at 25.0 °C. The SPR measurements were used for the investiga-

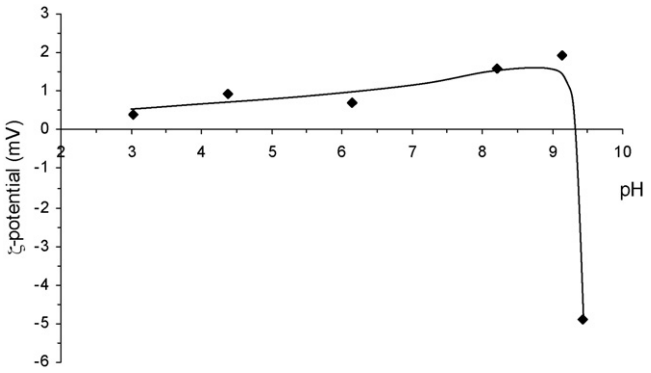

Fig. 3. Zeta-potential of lysozyme solution at various pH.