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Surface plasmon resonance immunosensor for bacteria detection

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ABSTRACT

This work describes an approach for the development of two bacteria biosensors based on surface plasmon resonance (SPR) technique. The first biosensor was based on functionalized gold substrate and the second one on immobilized gold nanoparticles. For the first biosensor, the gold substrate was functionalized with acid–thiol using the self-assembled monolayer technique, while the second one was functionalized with gold nanoparticles immobilized on modified gold substrate. A polyclonal anti-*Escherichia coli* antibody was immobilized for specific (*E. coli*) and non-specific (*Lactobacillus*) bacteria detection. Detection limit with a good reproducibility of 10^4 and 10^3 cfu mL^{-1} of *E. coli* bacteria has been obtained for the first biosensor and for the second one respectively. A refractive index variation below 5×10^{-3} due to bacteria adsorption is able to be detected. The refractive index of the multilayer structure and of the *E. coli* bacteria layer was estimated with a modeling software.

1. Introduction

The development of a rapid method for the detection of pathogenic microorganisms remains a challenge and an issue for ensuring food and environment safety. The food industry needs rapid methods for the detection of pathogenic bacteria. Biosensors are needed for the detection of disease-causing agents in food and water to ensure continued security of the nation's food supply. Amperometric, piezoelectric (PZ) and impedimetric biosensors have been developed for the detection of pathogenic bacteria in food and water [1–3]. Surface plasmon resonance (SPR) phenomenon was used for biosensor applications [1–4]. SPR wave is an electromagnetic wave propagating along a metal/dielectric interface. For SPR biosensor, most recent works are based on the use of the self-assembled techniques [5–7]. For the increase of the sensitivity of the developed biosensors, one proposed solution is the use of gold nanoparticles that have gained an increasing interest due to their special features, such as unusual optical, electronic properties and easy functionalization [8–10].

The goal of this proposed work is to develop an immuno-optical biosensor for rapid detection of two bacteria, one gram negative (*Escherichia coli*) and one gram positive (*Lactobacillus*) with low detection limit. For the increase of the sensitivity, gold nanoparticles was functionalized and immobilized on gold substrates. The results indicate that gold nanoparticles increase the surface area to volume ratio which induces an increased sensitivity and an improved detection limit relative to bulk gold. A modeling software was used for refractive index and thickness determination.

2. Experimental

2.1. Reagents and apparatus

The used antibody is "goat polyclonal IgG anti-*E. coli* (ab13627)" purchased from Abcam (UK, United Kingdom). A phosphate buffer solution (PBS) of 5 μg mL^{-1} of antibody with pH = 7.2 was prepared. The used bacteria were *E. coli* K12 (gram negative) and *Lactobacillus fermentium* (gram positive) diluted in PBS (pH = 7.2) and concentration stability over time has been controlled with OD (optical density) measurement. All other materials, including amine-thiol (cysteamine), acid–thiol (16-mercaptohexadecanoic acid) (Sigma–Aldrich), 1-ethyl-3-(3-(dimethylamino)-propyl) carbodiimide (EDC) (Aldrich), *N*-hydroxy succinimide (NHS) (Aldrich) and glutaraldehyde (GA) (Aldrich) were used as supplied. The buffer solution used was a (PBS) containing 140 mM NaCl, 2.7 mM KCl, 0.1 mM Na_2HPO_4, 1.8 mM KH_2PO_4, adjusted at pH 7. All reagents were of analytical grade and ultrapure water (resistance $\geq$ 18.2 MΩ cm^{-1}) produced using a Millipore Milli-Q system was used throughout.

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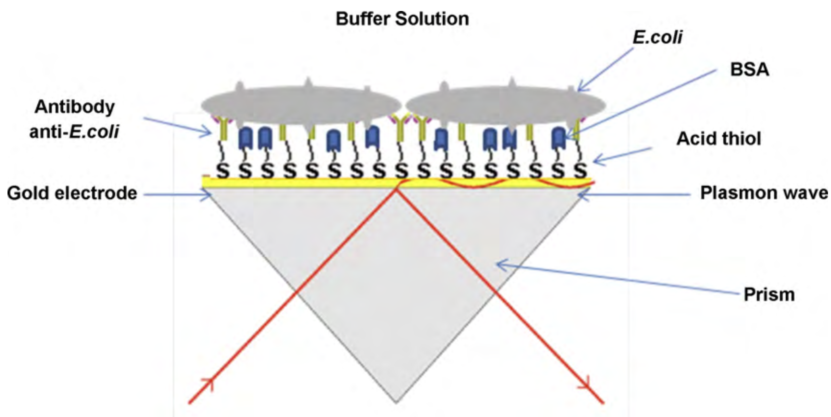

Fig. 1. SPR bacteria biosensor.

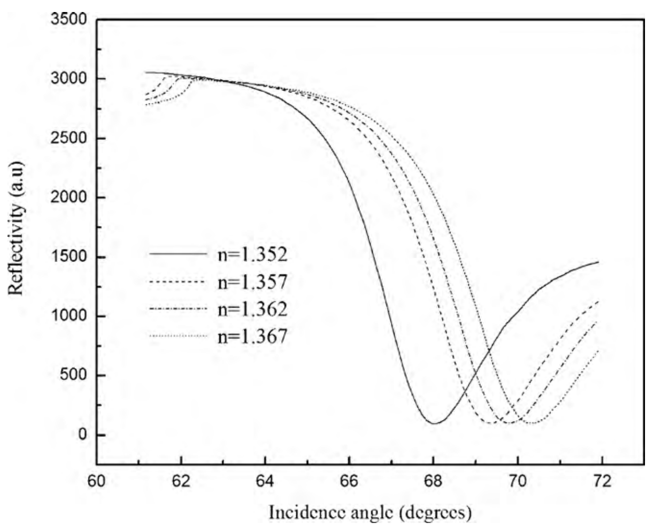

Fig. 2. SPR calibration curves for the gold substrates with different refractive index of the external medium.

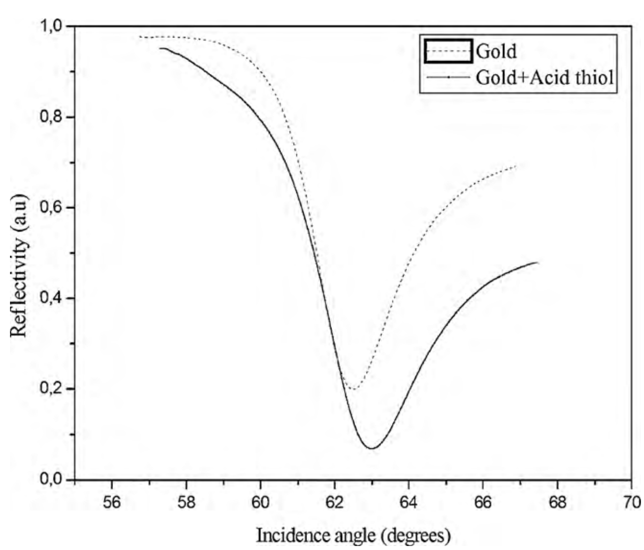

Fig. 3. Normalized reflectivity for gold and gold treated with acid–thiol as a function of the incidence angle.

2.2. Gold cleaning and functionalization

The gold substrates were cleaned in ethanol solution for 20 min. The substrates were immersed in a solution of 1 mM 16-mercaptoundecanoic acid (thiol acid) for 12 h for the obtaining of a dense self-assembled monolayer (SAM). The gold substrates were then rinsed with ethanol for removing the non-adsorbed acid–thiol. The terminal carboxylic group was converted to an active NHS

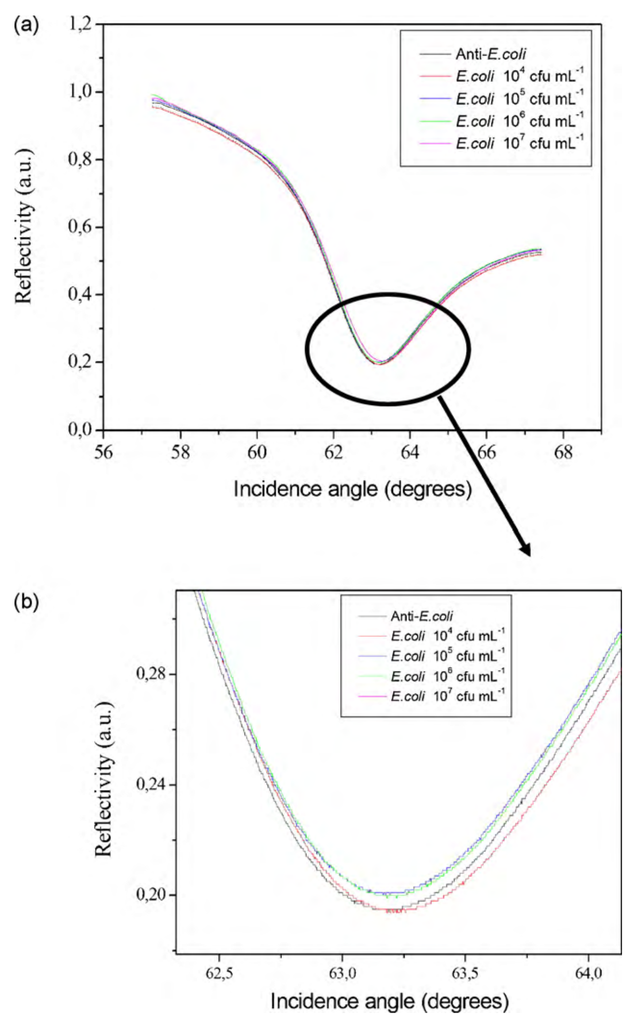

Fig. 4. Normalized reflectivity of biofunctionalized gold with immobilized antibody and with different concentrations of *E. coli* as a function of incidence angle.