

Review Article

Functional Conducting Polymers in the Application of SPR Biosensors

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In recent years, conducting polymers have emerged as one of the most promising transducers for both chemical, sensors and biosensors owing to their unique electrical, electrochemical and optical properties that can be used to convert chemical information or biointeractions into electrical or optical signals, which can easily be detected by modern techniques. Different approaches to the application of conducting polymers in chemo- or biosensing applications have been extensively studied. In order to enhance the application of conducting polymers into the area of biosensors, one approach is to introduce functional groups, including carboxylic acid, amine, sulfonate, or thiol groups, into the conducting polymer chain and to form a so-called “self-doped” or by doping with negatively charged polyelectrolytes. The functional conducting polymers have been successfully utilized to immobilize enzymes for construction of biosensors. Recently, the combination of SPR and electrochemical, known as electrochemical-surface plasmon resonance (EC-SPR), spectroscopy, has been used for in situ investigation of optical and electrical properties of conducting polymer films. Moreover, EC-SPR spectroscopy has been applied for monitoring the interaction between biomolecules and electropolymerized conjugated polymer films in biosensor and immunosensor applications. In this paper, recent development and applications on EC-SPR in biosensors will be reviewed.

1. Introduction

Conducting polymers (CPs) are materials discovered over 20 years ago, which have attracted considerable attention for their electronic conducting properties, optical properties, and chemical and biochemical properties [1–8]. These unique properties of CPs have been used in wide range of applications including battery technology, photovoltaic devices, light emitting diodes, electrochromic displays, and more recently in biological application [9–11]. Typical examples of conducting polymers such as polyaniline, poly(3,4-ethylenedioxythiophene), polypyrrole, and their derivatives have been widely studied [12, 13]. In particular for biosensor application, conducting polymers based on polypyrrole (PPy) have been extensively investigated due to their good electrical conductivity, environmental stability to air and

water, and ease of synthesis through electrochemical and chemical routes [4, 14]. Several techniques coupled to electrochemistry have been developed to study the properties of polymer films such as quartz crystal microbalance (QCM) [15], Fourier transform-infrared (FT-IR) spectroscopy [16], electron spin resonance (ESR) [17], and surface plasmon resonance (SPR) spectroscopy [18, 19]. SPR spectroscopy is the technique for monitoring the change of the refractive index at a solid-liquid interface, which has been widely used for the characterization and study of polymer films, interfaces, and kinetic processes at surfaces [20–23]. Recently, the combination of SPR and electrochemical, named electrochemical-surface plasmon resonance (EC-SPR) spectroscopy, have been used for in situ investigation of optical and electrical properties of conducting polymer films [24–26]. Moreover, EC-SPR spectroscopy has been applied for monitoring

the interaction between biomolecules and electropolymerized conjugated polymer films in biosensor and immunosensor applications [27–30]. In this paper, recent development and applications on EC-SPR in biosensors will be reviewed.

2. Electrochemical SPR for Monitoring of Conducting Polymer Thin Films

In 2003, the properties of poly(3,4-ethylenedioxythiophene) (PEDOT) ultrathin films investigated by the combination of surface plasmon resonance (SPR) and surface plasmon enhanced photoluminescence spectroscopy (SPPL) with electrochemical techniques, known as EC-SPPL, were reported by Baba and Knoll [21]. The electrochromic properties and the detecting of photoluminescence in PEDOT ultrathin films were observed. The photoluminescence of PEDOT was observed when the polymer was dedoped under the applied potential. The photoluminescence intensity was controlled by the potential and dependent on the angular position in an SPR reflectivity experiment. The SPR characterization of the PEDOT film is corresponding with the PEDOT bulk electrochromic properties obtained from UV-vis-NIR spectra. The EC-SPPL method can be acted as a sensitive tool for detection the photoluminescence from a conjugated polymer film and could have potentials for sensors and optoelectronic devices applications.

An electroactivity of polyaniline (PANI) films in neutral pH condition and their electrocatalyzed oxidation of β -nicotinamide adenine dinucleotide (NADH) were also reported by Tian et al. [23]. Self-assembled PANI multilayer films by forming with poly(anion) such as sulfonated polyaniline (SPANI), poly(acrylic acid) (PAA), poly(vinyl sulfonate) (PVS), and poly(styrene sulfonate) (PSS) were prepared using layer-by-layer (LBL) method. The combination of EC-SPR and quartz crystal microbalance (QCM) techniques was used to monitor the electrochemical behavior and catalytic ability for the oxidation of β -nicotinamide adenine dinucleotide (NADH) in neutral solution of PANI multilayer films. The self-assembled PANI multilayer films prepared by LBL method showed very good stability, reversible, and electroactive in neutral solution. Moreover, the results of the electrocatalytic activity of PANI multilayer films indicated that the PANI copolymers can electrocatalyze the oxidation of NADH in neutral solution but their potential to electrocatalyze NADH oxidation was quite different under the same condition. The catalytic ability of PANI/SPANI is better than the other assemblies under the same conditions due to both PANI and SPANI monolayers of PANI/SPANI system being electroactive, while for the other systems only the PANI layer is electroactive.

The investigation of the formation and effects of doping/dedoping processes on the optical properties associated with ultrathin polypyrrole (PPy) film using EC-SPR was studied by Damos et al. in 2006 [4]. The electropolymerization of pyrrole was performed by potentiostatic, potentiodynamic, and galvanostatic methods. The in situ EC-SPR was monitored by the nanometric films of PPy. The results showed the electropolymerization mode was essential to the stability of polymer and the reversibility of its optical

properties during the doping and dedoping processes. The changes in the electrochemical and optical properties of the thin polypyrrole films produced the changes of the SPR angle position due to the changes in the values of the real and imaginary parts of the dielectric constant for PPy films during doping/dedoping processes. The results from the QCM measurement to monitor the correlation between doping/dedoping processes and the changes in the real and imaginary parts of the dielectric constant of the polypyrrole film were consistent with the results from EC-SPR technique.

In 2009, an electrochemical surface plasmon excitation and emission light properties in poly(3-hexylthiophene) (P3HT) thin films were investigated by Kato et al. [22]. The electrochemical change of P3HT thin films was in situ measured by the attenuated total reflection (ATR) and emission light properties utilizing surface plasmon (SP) excitations. The ATR and SP emission light properties were observed for the doping-dedoping states of P3HT thin film. The ATR and SP emission light properties were remarkably changed with the electrochemical doping and dedoping. The SP emission light also decreased by decreasing the molecular luminescence of P3HT by doping. For the dedoped-state P3HT thin film, SP emission light also increased by increasing the molecular luminescence. The SP emission light excited by molecular luminescence can be controlled by controlling the doping-dedoping states.

3. SPR Biosensors Based on Functional Conducting Polymers

Conducting polymers in the area of biosensors have attracted much attraction. Biosensors based on conducting polymers were used to detect the biomolecules including glucose, hormones, neurotransmitters, antibodies, and antigens [1, 20, 31–34]. Immunosensors are biosensors based on specific antigen-antibody interactions and in which the transducer detects either directly or indirectly the immunochemical reactions [35–37]. Polypyrrole and its derivative were studied for the biosensor application [26, 34]. The EC-SPR spectroscopy can be applied not only in monitoring the electropolymerization of polymer films, but also in illustrating the properties of immunosensors [12, 38, 39].

In 2004, the label-free immunosensor for the detection of a rabbit IgG antigen by an antirabbit IgG antibody at the surface of polypyrrole (PPy) membrane was reported by Gooding et al. [13]. The investigation of the electrochemistry on a glassy carbon electrode, modified with antibody entrapped in an electrodeposited polypyrrole (PPy) membrane, was demonstrated in a flow injection apparatus (FIA). The measured signals were aroused by a change in ion flux into and out of the conducting polymer membrane when the antigen interacted with the antibody-modified polymer. The most sensitivity of the label-free immunosensors was shown by a change of the cyclic voltammograms at 0.4 V when the antigen was bound. The concentration of the antibody immobilized throughout the polymer membrane plays an important role for altering the electrode stability and has the lowest detectable concentration, but had little effect on the concentration range over which immunosensor operated.

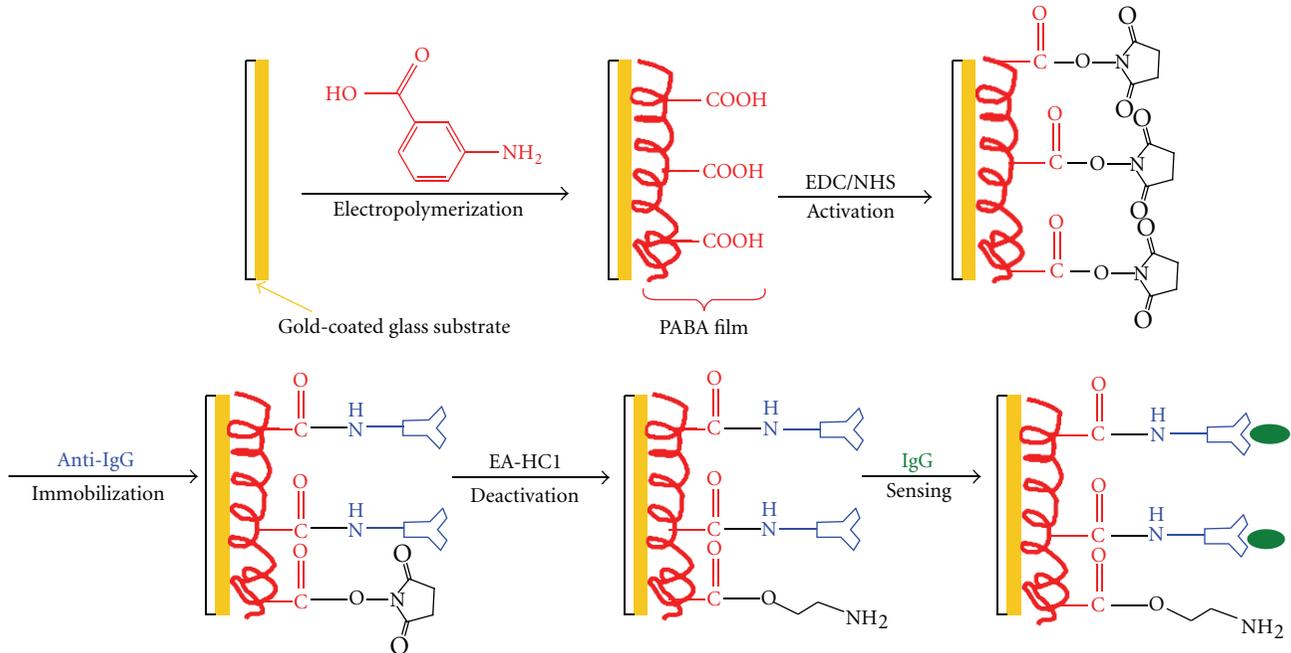


FIGURE 2: Schematic diagram of the construction of PABA-based immunosensor and sensing of human IgG [30].

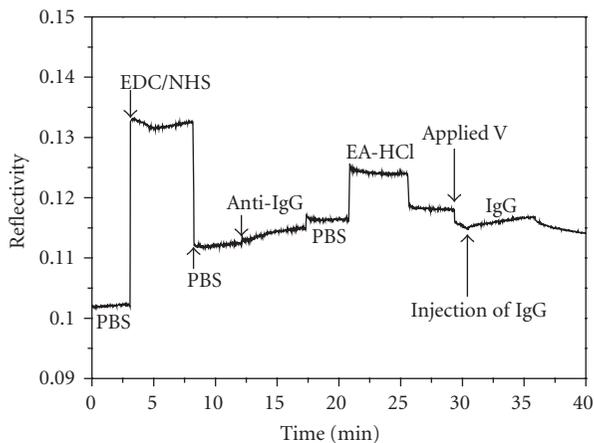


FIGURE 3: SPR response during construction of PABA-based immunosensor and sensing of human IgG ($1 \mu\text{g/mL}$) at constant applied potential of 0.4 V [30].

(EDC). This process, called activation process, led to activation of carboxylic acid to NHS ester group. After rinsing with PBS, the anti-human IgG was then immobilized to the activated surface, which led to the formation of amide bond between NHS ester group and amine group of anti-human IgG. This process is called immobilization process. The reflectivity increased during both activation and immobilization processes. A buffer solution of ethanolamine—hydrochloride (EA-HCl) was then employed to deactivate the residue of unreacted NHS ester group. After rinsing with PBS, constant potential was applied for 1 min before adding human IgG for sensing experiment. The SPR reflectivity

increased during the sensing experiment, which is corresponding to the binding of anti-human IgG and human IgG. Different concentrations of human IgG ($1, 2.5, 5, 7, 10 \mu\text{g/mL}$) were employed for sensing experiment at each constant applied potentials of open circuit, 0.4 V and -0.2 V (results were not shown). The other results showed that higher binding amount of human IgG was obtained at applied potentials of -0.2 and 0.4 V . The conducting polymer-based EC-SPR sensor could control the surface morphology and was a sensitive tool for enhancing the binding of biological molecules.

Recently, poly(pyrrole-3-carboxylic acid)- (PP3C-) based immunosensor investigated by in situ EC-SPR for monitoring the immobilization of anti-human IgG and its interaction with human IgG was reported by Janmanee et al. [39]. A pyrrole-3-carboxylic acid monomer was used for electropolymerization of PP3C film on gold surface. After the electropolymerization, SPR immunosensor was performed for the detection of human IgG, as a model analyte. The obtained PP3C-based immunosensor was employed for the detection of human IgG at various concentrations ($1\text{--}10 \mu\text{g/mL}$) with different constant potentials. The immunosensing process of the PP3C-based immunosensors was in situ monitored by EC-SPR measurement. The real-time SPR kinetic curve during the construction of PP3C-based immunosensor shows the immobilization of anti-human IgG and the binding and dissociation process of anti-human IgG/human IgG. The SPR reflectivity change increased with applied constant potentials as shown in Figure 4(a), indicating that an electrochemically controlled PP3C film by applying the constant potential could be enhanced by the binding of human IgG to anti-human IgG on PP3C film, which is supported by AFM result. Moreover,

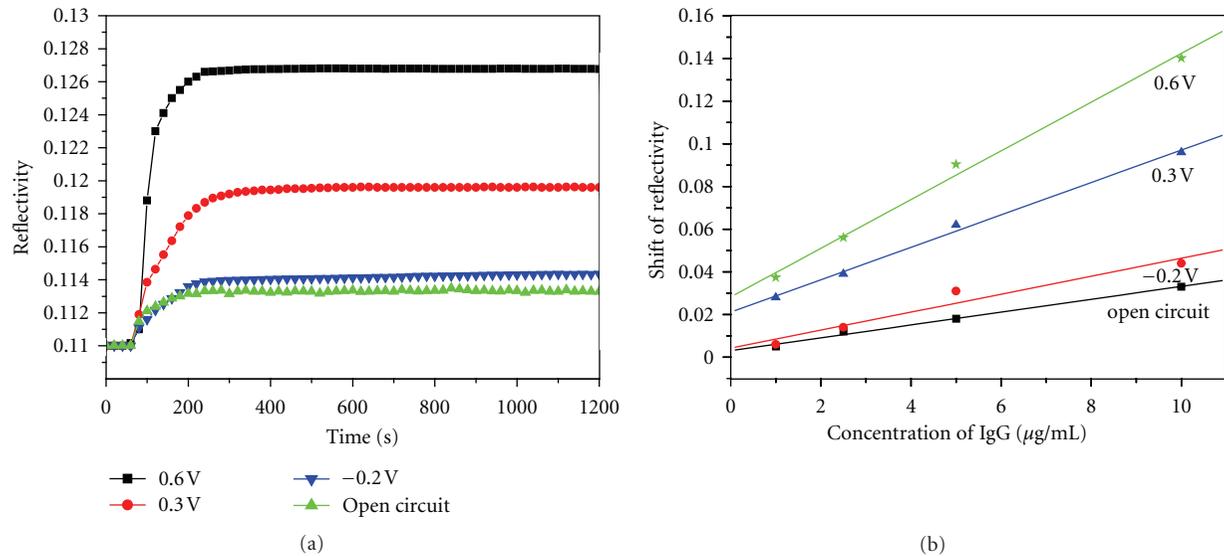


FIGURE 4: (a) SPR responses at different applied potentials for detection of human IgG ($10 \mu\text{g/mL}$) at several constant potentials, and (b) the plot of the shift of reflectivity during binding process with concentration of IgG at different constant applied potentials [39].

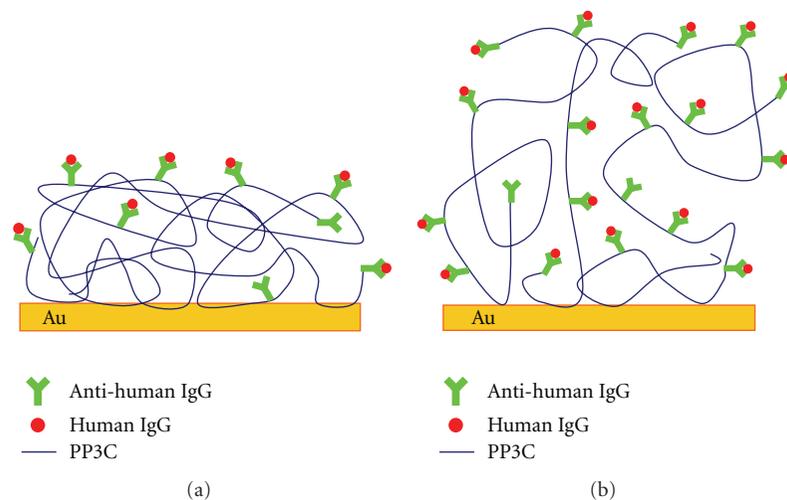


FIGURE 5: Schematic diagram of the binding process of anti-human IgG to human IgG on PP3C film (a) without applying potential and (b) with applying potential [39].

the binding amount of human IgG to anti-human IgG increased with increasing the concentration of human IgG as shown in Figure 4(b). The SPR reflectivity shifts show linear relationship with the concentrations of human IgG. The reflectivity change at applied potential of 0.6 V, showing the highest sensitivity, was about 6-times more than that without applying potential. In addition, the sensitivity signal of the PP3C-based immunosensor shows a higher efficiency for the detection of anti-human IgG/human IgG compared with the standard MUA system. Figure 5 shows a schematic diagram of the binding process of anti-human IgG/human IgG on the PP3C film with applied potential and without applied potential. The surface morphology of PP3C film was changed with applied potential. The PP3C film swelled and

the surface seemed to be rugged, leading to have more space for the binding of anti-human IgG and human IgG in the polymer chain, which leads to an increase of the sensitivity of immunosensor system.

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