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Impedimetric immunosensor using avidin–biotin for antibody immobilization

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Abstract

The potentialities of an electrodeposited biotinylated polypyrrole film as an immobilisation matrix for the fabrication of impedimetric immunosensors are described. Biotinylated antibody (anti-human IgG), used as a model system, was attached to free biotin groups on the electrogenerated polypyrrole film using avidin as a coupling reagent. This immobilization method allows to obtain a highly reproducible and stable device. The resulting immunosensor has a linear dynamic range of 10–80 ng ml^{-1} of antigen and a detection limit of 10 pg ml^{-1}. Furthermore, this immunosensor exhibited minor loss in response after two regeneration steps. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Electropolymerization; Biotinylated polypyrrole; Impedance spectroscopy; Immunosensor

1. Introduction

During the past few years, immunosensors [1] have been the subject of increasing interest because of their potential application as an immunoassay tools in several fields such as clinical diagnostics and environmental control. Owing to their low cost, small size, possible use in vivo and also their short response time, these devices can compete seriously with classical immunoassays. However, the immobilization of the molecular receptor on the transducer is of key importance for the control and the improvement of the performance of such a sensor. On the other hand, in the last 40 years, considerable attention has been focused on conducting polymers because of their unusual electronic properties and their great potential for biomolecules immobilization. Several immobilization procedures [2] were used to ensure the coupling of immune species onto conducting polymers, they range from the entrapment within the polymer film to the electrostatic binding.

In the present study, the immobilization of the antibody (anti-human IgG) on a bare gold electrode was achieved by taking advantage of the strong bond formed between avidin and biotin. In fact, a biotin polypyrrole was electrodeposited onto gold electrode and used for the immobilization of the antibody. This immobilization method was already used by other authors [3] to ensure the attachment of glucose oxidase to a glassy carbon electrode. The characteristics of the derivative immunosensor were performed by electrochemical impedance spectroscopy.

2. Experimental

2.1. Reagents

The antibody used was a polyclonal anti-human IgG developed in goat (γ-chain specific). Biotin conjugate was purchased from Sigma. The antigen was a Human IgG reagent grade, from Serum, purchased from Sigma. Bovine serum albumin (BSA; Sigma) was used. The biotinylated pyrrole was synthesized as described in Refs. [4,5].

Measurements were performed in a solution consisting of a 10 mM phosphate buffer, 2.7 mM potassium chloride and 137 mM sodium chloride. The pH solution at 25 °C was 7.4.

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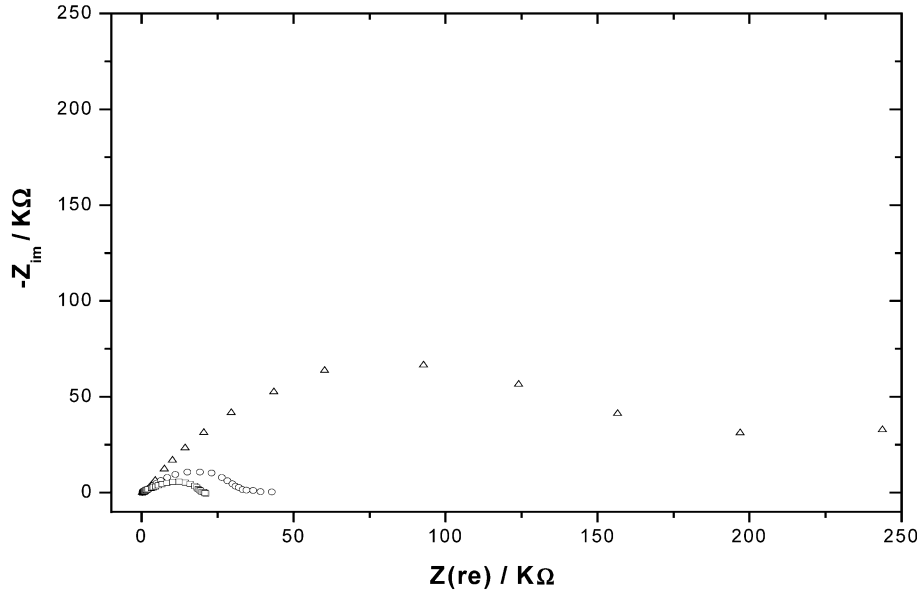

Fig. 1. Complex plane impedance plots for a bare gold electrode after different modification steps: □, after electrodeposition of the biotin polypyrrole film; ○, after immobilization of avidin; △, after immobilization of the antibody.

2.2. Electrode preparation

The electrogeneration of the biotinylated polypyrrole film on cleaned gold electrodes were performed by electrooxidation of the monomer (2 mM) at 0.85 V/Ag|Ag^+ in acetonitrile solution containing 0.1 M TBAP (tetrabutylammonium perchlorate) as a supporting electrolyte. The specific binding of avidin to polymer biotin sites was achieved via the incubation of the subsequent functionalized electrodes in a 2 mg ml^{-1} avidin solution, for 30 min at 5 °C. The obtained electrodes were carefully rinsed with PBS, then incubated in a solution of biotinylated antibody (anti-human

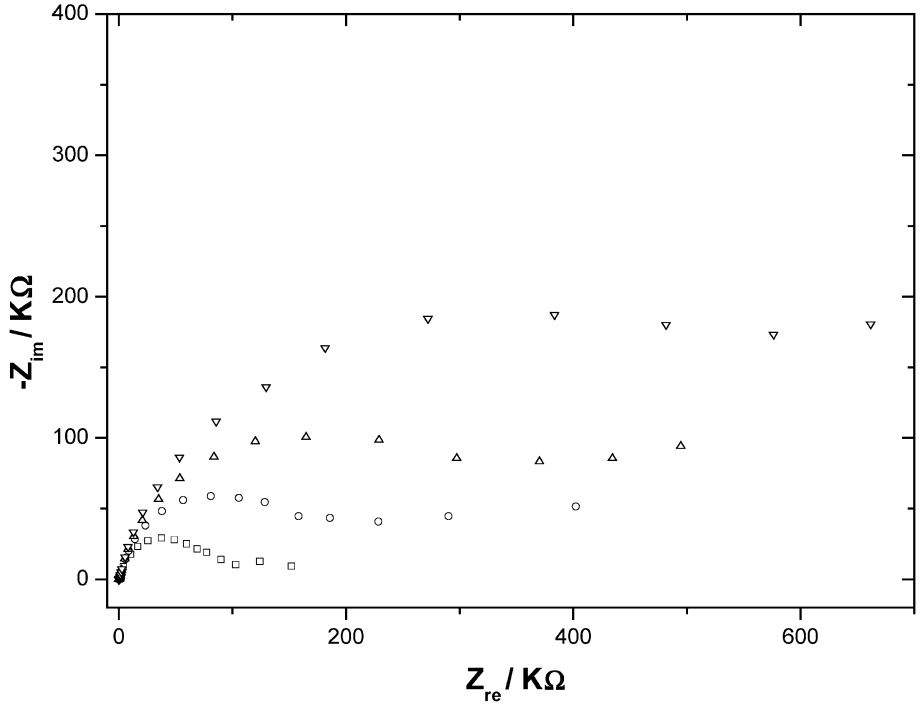

Fig. 2. Complex plane impedance plots for an antibody modified electrode at a potential of − 1.4 V vs. SCE and A.C. signal of 5 mV, in PBS, □: without antigen injection; after antigen injection at different concentrations: ○, 10 ng ml^{-1}; △, 50 ng ml^{-1}; ▽, 100 ng ml^{-1}.

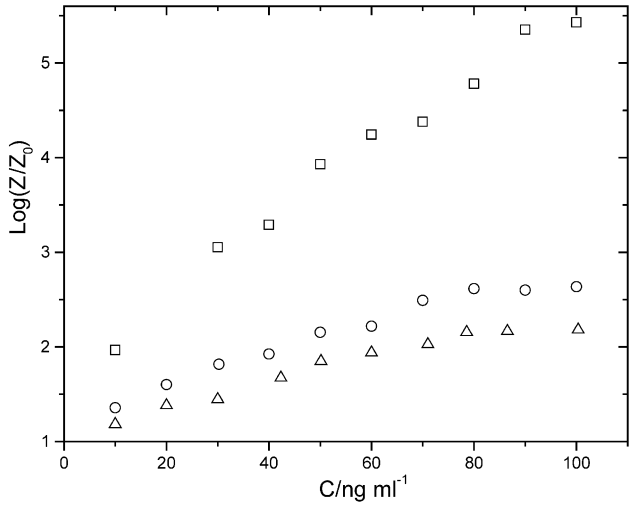

Fig. 3. Response curve of the sensor, applied potential − 1.4 V vs. SCE, frequency 0.04 Hz, 0.01 M PBS (pH 7.2). ○: first use; □: after 1st regeneration; △: after a 2nd regeneration.

IgG) at a concentration of 1 mg ml^{-1} during 12 h at 5 °C. These electrodes were then thoroughly rinsed with PBS to remove the weakly adsorbed antibodies.

3. Results and discussion

In order to characterise the behaviour of the sensing layer and to optimise the procedure for the measurements, impedance measurements have been carried out during the various steps of the antibody immobilization process (Fig. 1) It appears that the major changes in the impedance character occurred at low frequency. Therefore, a frequency of 40 mHz was chosen for concentration-dependent impedance measurements.

As shown in Fig. 2, the second semi-circle diameter in the Nyquist plot seems to increase with increasing the antigen concentration, especially at low frequency. In order to obtain a calibration curve of the sensor we have plotted the variation of $\log(|Z|/|Z_0|)$ versus the values of the antigen concentration at frequency of 0.04 Hz (cf. Fig. 3). In the above formula, $|Z_0|$ is the modulus of the impedance of the antibody-modified electrode before the injection of antigens and $|Z|$ is that of the subsequent electrode after each antigen addition. The immunosensor had a linear dynamic range from 10 to 80 ng ml^{-1} and a detection limit of about 10 pg ml^{-1}.

In order to split the antibody–antigen complex and to regenerate the sensor, acidic buffer (pH 2.3) was applied for 3 min. Then, the electrode was washed with PBS. As unexpected, the sensitivity of the sensor increased after the first regeneration, such surprising result was already found by other authors using classical immunosensing tests (ELISA) [6]. This phenomena can be due, in part, to the improvement of the accessibility of the binding sites of the antibody after a short-time acidic washing. Whereas, repeated acidic washing of the sensing layer may reduce the activity of the antibody, which can explain the decrease of the sensitivity of the sensor after the second regeneration.

4. Conclusion

Biotin–avidin system has been used to immobilize biotinylated antibodies on gold electrode functionalized by electropolymerized biotinylated polypyrrole, in order to conceive an impedimetric immunosensor. It appears that the subsequent specific immune complexes anti-human IgG–human IgG can be monitored by impedance spectroscopy. Thus, the sensor, exhibits a dynamic range 10–90 ng ml^{-1} and a detection limit of 10 pg ml^{-1}. Furthermore, the regeneration of the sensing layer was successfully reached using an acidic solution.

References

[1] E. Gizeli, C.R. Lowe, Immunosensors, Curr. Opin. Biotechnol. 7 (1996) 66–71.
[2] S. Cosnier, Biomolecule immobilization on electrode surfaces by entrapment or attachment to electrochemically polymerized films. A review, Biosens. Bioelectron. 14 (1999) 443–456.
[3] S. Cosnier, M. Stoytcheva, A. Senillou, H. Perrot, R.P.M. Furriel, F.A. Leone, Biotinylated conducting polypyrrole for the spatially controlled construction of an amperometric biosensor, Anal. Chem. 71 (1999) 3692–3697.
[4] S. Cosnier, B. Galland, C. Gondran, A. Lepellec, Electrogeneration of biotinylated functionalized polypyrroles for the simple immobilization of enzymes, Electroanalysis 10 (1998) 808–813.
[5] S. Cosnier, A. Lepellec, Poly(pyrrole-biotin): a new polymer for biomolecule grafting on electrode surfaces, Electrochim. Acta 44 (1999) 1833–1836.
[6] V. Billard, C. Martelet, P. Binder, J. Therasse, Toxin detection using capacitive measurements on immunospecies grafted onto a semiconductor substrate, Anal. Chim. Acta 249 (1991) 367–372.