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Electrochemical impedance spectroscopy for monitoring of alkaline phosphatase reaction with substrate

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Abstract

Electrochemical impedance spectroscopy was compared to square wave voltammetry for monitoring the reaction between alkaline phosphatase and its substrates.

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Keywords: Impedance spectroscopy; voltammetry; alkaline phosphatase; enzymatic reaction; immunosensors

1. Introduction

The reactions of enzymatic labels are usually followed by amperometry or voltammetry. Nevertheless, electrochemical impedance spectroscopy (EIS) can often be more sensitive and convenient than other electrochemical methods [1]. We have investigated the use of EIS for the detection of alkaline phosphatase (ALP) reaction with three different substrates (1-naphthyl phosphate (1-NPP), 2-phospho-1-ascorbic acid (AAP), and hydroquinone diphosphate (HQDP)) by monitoring their electroactive products (1-naphthol (NP), ascorbic acid (AA), and hydroquinone (HQ)). The results from EIS were compared to those from SWV.

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2. Experimental

SWV and EIS were performed with PalmSens3 potentiostat using DropSens carbon screen-printed electrodes. Optimum conditions of SWV measurement were: Potential range: -0.8 V to 1.0 V (1-NPP), -0.2 V to +1.2 V (AAP), -0.5 V to +0.6 V (HQDP), Estep = 4 mV, amplitude: 0.1 V and frequency: 10 Hz. Optimum conditions of EIS measurement were: Frequency range: 10 kHz – 0.1 Hz (50 frequences), Edc = -0.2 V (1-NPP), +0.471 V (AAP), and +0.01 V (HQDP), amplitude = 0.01 V. Main chemicals used in the study were: 1-NPP, AAP, HQDP, AP, Tris buffer solution (TBS) containing 0.1 M Tris, 0.08 M KCl and 1 mM MgCl2 of pH 7.4.

3. Results

The highest SWV signal was observed for HQ while the lowest SWV signal shows NP (Fig. 1a). Similar order of the ALP products' electroactivity was observed when measurement was done by EIS (Fig. 1b). In this case the electroactive products took the role of the redox species needed for Faradaic type of EIS. The measurement in absence of electroactive product thus corresponds to the non-Faradaic EIS. This fact allowed us to obtain remarkable differences between EIS in absence and presence of electroactive product, which was the highest in case of HQDP.

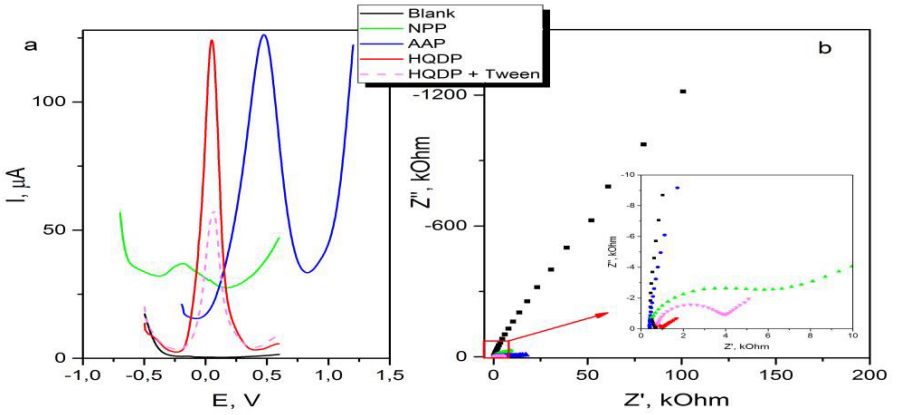

Fig. 1. (a) SWV; and (b) EIS after reaction between ALP and substrate.

4. Conclusions

This result is important for further application of EIS as a detection method in the immunoassay where various blocking agents (Tween, milk) are used to avoid non-specific interactions. These agents significantly decrease the electrochemical signal in voltammetric methods. However, EIS of HQ is not so significantly affected by blocking agent and method can still be used for immunoassay.

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