GOLD NANOPARTICLES USED IN IMPEDIMETRIC GENOSENSING OF DOUBLE-TAGGED PCR SAMPLES

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Double stranded DNA sequences coming from polymerase chain reaction (PCR) amplification of real samples were detected by the use of an electrochemical impedimetric genosensor previously developed in our laboratories [1]. Electrochemical Impedance Spectroscopy is a rapid developing technique for the transduction of biosensing events at the surface of an electrode [2]. Transduction principle exploits changes in interfacial resistance of charge transfer after modification of the genosensing transducer with DNA [3]. Many different protocols have been recently used in DNA detection by this technique. Some of them employ different types of nanoparticles as a way to increase the sensitivity of the method [4-6].

In the present work, an avidin bulk-modified graphite-epoxy biocomposite (Av-GEB) was employed to immobilize –onto the electrode surface– the double-tagged DNA, modified in each end with biotin and digoxigenin, respectively. Impedance spectra were recorded to detect the change in interfacial charge transfer resistance (R_{ct}), experimented by the redox marker ferri/ferrocyanide at the applied potential. A further step in the genosensing procedure was the amplification of impedimetric signal by the use of gold nanoparticles modified with Anti-Mouse IgG (whole molecule)–Gold antibody. The latter were immobilized to the digoxigenin-modified end of the amplicon by a monoclonal IgG1kappa anti-Digoxigenin antibody from mouse.

Results obtained by the comparison of R_{ct} values after each further modification of the electrode surface show a significant difference in the impedimetric signal variation between experiments and negative controls. Moreover, this difference results thoroughly amplified thanks to the procedure used for signal enhancement (see results shown in Figure 1).

The attained sensitivity plus the improved reproducibility of results confirm the validity of this method based on a universal affinity biocomposite platform coupled with the use of gold nanoparticles for signal amplification. The described strategy was used for the rapid and sensitive detection of PCR amplified samples of Salmonella spp, the most important pathogen affecting food safety.

References:

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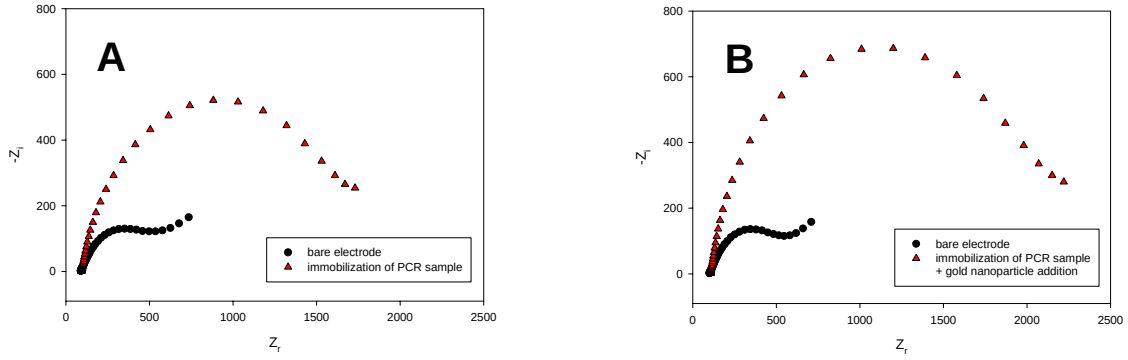

Figure 1: Comparison among Nyquist plots obtained in: A, experiment without signal amplification. B, experiment with signal amplification.