Poster

AN ELECTROCHEMICAL NANOSTRUCTURED APTAMER BIOSENSOR-BASED SANDWICH ASSAY FOR DETECTION OF C REACTIVE PROTEIN (CRP) AND OCHRATOXINE A (OTA)

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Detection and quantification of C-reactive protein (CRP) in an easy, cheap, and fast way can improve clinical diagnostics in order to prevent serious inflammatory states [1]. The CRP reference concentration of healthy subjects is < 5 mg/l in serum and the clinical range of interest is 1-500 mg/l [2]. The few proposed biosensors for CRP detection employ antibodies as bio-recognition elements , whereas few papers are based on the use of aptamers [3-5].

We are developed,optimized and validated, an electrochemical aptamer-based nanostructured sandwich aptasensor performed on magnetic nanoparticles for detection of CRP in serum samples .

This aptasensor involves the use of screen-printed electrodes for the transduction step and the use of another physical support for the affinity reaction.

Magnetic nanoparticles are available with a wide variety of surface functional groups and size and have the possibility of reaction kinetics similar to those found in free solution. Graphite and magnetic nanoparticles represent the most commonly used beads in bioelectroanalytical systems. Magnetic nanoparticles respond to an applied magnetic field and re-disperse upon removal of the magnet. They consist of 36–40% magnetite dispersed within a copolymer matrix consisting of styrene and divinyl-benzene. Their binding capacity varies with the bead size, composition and the size of the binding ligand. There is a general consensus that the use of magnetic beads greatly improves the performance of the immunological reaction, due to an increase in the surface area, as well as the faster assay kinetics achieved because the beads are in suspension and the analytical target does not have to migrate very far

The designed aptasensor is based on the use of two surfaces, magnetic nanoparticles for immunoassay and screen-printed electrodes for electrochemical trasnduction, gaving the best analytical performances in terms of sensitivity and speed of the analysis.

After the assay, the modified magnetic beads were captured by a magnet on the surface of a graphite working electrode and the electrochemical detection was thus achieved through the addition of the AP substrate (α-naphthyl-phosphate) and α-naphthol produced during the enzymatic reaction was detected using differential pulse voltammetry (DPV).

The LOD and LOQ calculated in CRP free serum were 0.2 and 6 mg/l respectively and the average coefficient of variation (ACV) was 8 %. The LOD found was comparable with the reported by ELISA and it was much lower than the clinically useful borderline value (8 mg/l). Finally, this approach was applied to the analysis of some serum samples and it resulted as a promising tool to predict the risk of a possible disease with CRP levels

Additionally, we are going in this comunication to explain the nanostructured design developed using a selective aptamer for Ochratoxine A (OTA), but in this case the magnetic beads weren´t used as solid support. OTA-BSA conjugated was immobilized onto screen printed electrodes (SPCEs) and then the aptamer biotinylated was adden onto surface. Finally, Alkaline Phosphatase-ExtrAvidin was used as enzymatic conjugate in the electrochemical detection step.We will present this new aptasensor for OTA with good analytical performances to determine this mycotoxine in foods and beverages.

References:

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