Magnetic immunosensors for the determination of cortisol

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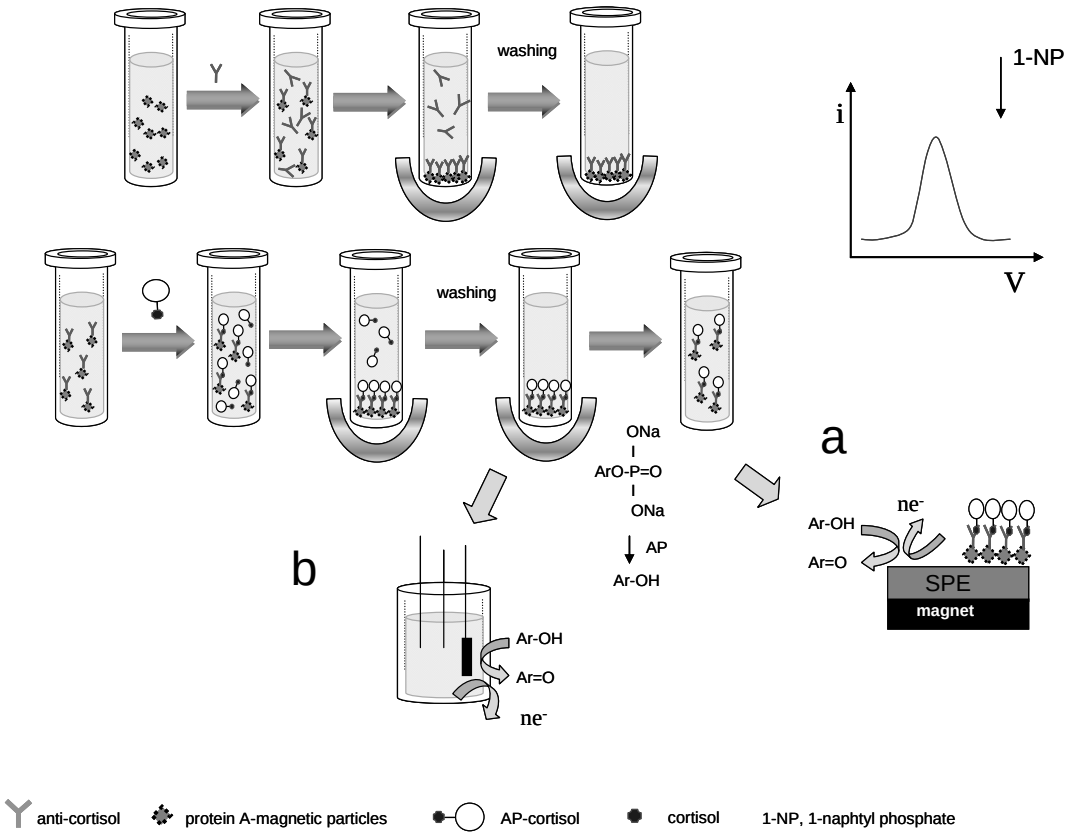
Belonging to the glucocorticoid hormones family, cortisol is an important bio-marker of stress and its detection is important in sports medicine [1]. It is well known that athletes use corticoids to improve their performance. Since 1975, the International Olympic Committee Medical Commission restricted the use of these compounds to legitimate medical purposes. However, the existing methods for detecting cortisol are limited with respect to their sensitivity, time of analysis and cost. In this context, the use of electrochemical imunosensors, because of their excellent analytical capabilities as sensitivity, reproducibility, simplicity of construction and use, and feasible miniaturization, could offer alternative advantages for doping control. Furthermore, these important advantages become more evident insofar as the immobilization of immunoreagents and the transduction event are more efficient. Thus, the preparation of bioelectrodes combining immobilization methods capable of improving stability with no significant loss of the biological activity of biomolecules, with electrochemical transducers that enhance electron transfer, constitutes a challenge in modern bioanalytical chemistry. Related to this goal, electrochemical biosensors involving the use of nanoparticles have demonstrated to possess interesting features [2].

In this work, we present two configurations of electrochemical immunosensors for cortisol based on magnetic particles and / or gold nanoparticles. In the first design (Figure 1a) a disponsable voltammetric immunosensor was developed using screen printed electrodes prepared with different materials (carbon SPE, gold nanoparticles-modified carbon SPE, and gold SPE). Competitive immunoassay involving cortisol antigen labeled with alkaline phosphatase (AP) was employed. Anti-cortisol antibody was immobilized onto protein A-magnetic particles, and the resulting conjugate was trapped with a small magnet on the surface of the screen-printed electrode. Cortisol determination was made by 1-naphtylphosphate additions, and detection of 1-naphtol using differential pulse voltammetry in the -0.15 to +0.25 V vs Ag/AgCl.

A second design, based on the preparation of an electrode surface consisted of a composite of gold nanoparticles and carbon nanotubes, using 1-n-octylpyridinium hexafluorophosphate ($OPPF_6$) or Teflon as the binding material, was also developed. The same immunoassay configuration than that described above was used. However, as shows Figure 1b, the product of the alkaline phosphatase enzyme reaction, 1-naphtol, was measured in the supernatant solution by differential pulse voltammetry at the composite electrode.

All the experimental variables involved in the assays, i.e. the amount of anticortisol immobilized or the time of incubation, and those affecting the electrochemical response (pH, cortisol-AP/cortisol ratio and composition of the electrode surface) were optimized. Calibration plots obtained for cortisol have linear ranges between 0.1 and 100 ng/mL, which cover the concentration levels required for the analysis of biological samples. Other analytical characteristics such as the limits of detection, reproducibility, and stability tests, have been evaluated.



References:

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Figures:

Scheme of the immunoassay procedure for the determination of cortisol based on the voltammetric detection of 1-naphtol: a) after immobilization of immunoconjugate onto SPE; b) by measuring in the supernatant solution at the composite electrode.