PO-EA-07: EVALUATION OF TRANSDUCTION STRATEGIES USING ENZYME OR QUANTUM DOTS AS LABELS IN THE DEVELOPMENT OF MAGNETOGENOSENSORS FOR THE DETERMINATION OF A TRANSCRIPTION FACTOR RELATED TO CANCER

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The "Electroanálisis and (Bio)sensores electroquímicos" (GEBE) research group, based in the Universidad Complutense de Madrid (UCM), and leaded by Prof. José M. Pingarrón, is engaged, among other objectives, in the development of simple and reliable electrochemical platforms, easily adapted to a broad range of settings, for routine determinations of clinical biomarkers at different molecular levels. The methodologies under study are mainly assisted by the use of superparamagnetic iron oxide microparticles (MBs) functionalized with specific bioreceptors in appropriate assay formats. These MBs allow efficient, rapid, and easy capturing of specific target molecules from scarcely treated complex samples. Moreover, the resulting magnetic bioconjugates are easily coupled with disposable sensors to perform the electrochemical transduction (**Figure 1**).

Figure 1. Efficient isolation of target biomolecules at functionalized MBs and coupling with screen-printed carbon electrodes (SPCEs) both for detection of biochemical reactions at the MBs (1) and of species released to the solution (2).

Several transduction systems can be used in the electrochemical amplification and quantitation protocols. Searching for simple methodologies also with multiplexing capabilities is of great interest in the clinical field to improve diagnosis reliability using tools compatible with the *point-of-care* (POC) technology. In this sense, quantum dots (QDs) have emerged as promising labels in both optical and electrochemical bioassays. Thus, different metal composition QDs can be attached to either detector antibodies or oligonucleotides, and after the biochemical recognition of the target analytes, digested in acidic media to determine the cationic metals released to the solution by anodic stripping voltammetry (ASV).

The genes of the sex determining region Y-BOX (SOX) encrypt a family of high mobility group (HMG) domain from the transcription factor (TF) family, which permits highly specific DNA binding. Around 20 genes of the SOX family have been defined and classified depending on their protein specificity. Among them, *SOX-2*, which is closely related to the early embryonic development, neural and sexual differentiation, and constitutes a necessary factor for induced cellular reprogramming, has been demonstrated to be amplified and overexpressed in various malignant tumours. In fact, its expression acts as a prognostic factor in various types of tumours (breast, colorectal, gastric and lung cancer and glioblastoma), and as a link between malignancy and stemness. Moreover, it shows as an amplified gene in oral squamous cell carcinomas, and has been established as one of the hallmark participants throughout the developmental process in cancer.

The main objective of this work relied on the comparison of Cd-containing QDs and the widely employed hydroquinone/horseradish peroxidase (HQ/HRP) system as electrochemical labels within the transduction systems used in the amplification and quantitation protocols coupled with MBs-based genosensors. Thus, the detection of the hybridization process between a synthetic specific biotinylated capture DNA probe immobilized onto streptavidin-functionalized MBs and a complementary biotinylated sequence including a specific fragment of the *SOX-2* gene was accomplished. **Figure 2** shows a scheme of the employed configurations. The biotinylated synthetic target DNA was labelled either with HRP to follow the hybridization event by amperometry using the H_2O_2/HQ system or with streptavidin-modified CdSe/ZnS QDs followed by their square wave anodic stripping voltammetric (SWASV) detection upon acid dissolution of the QDs.

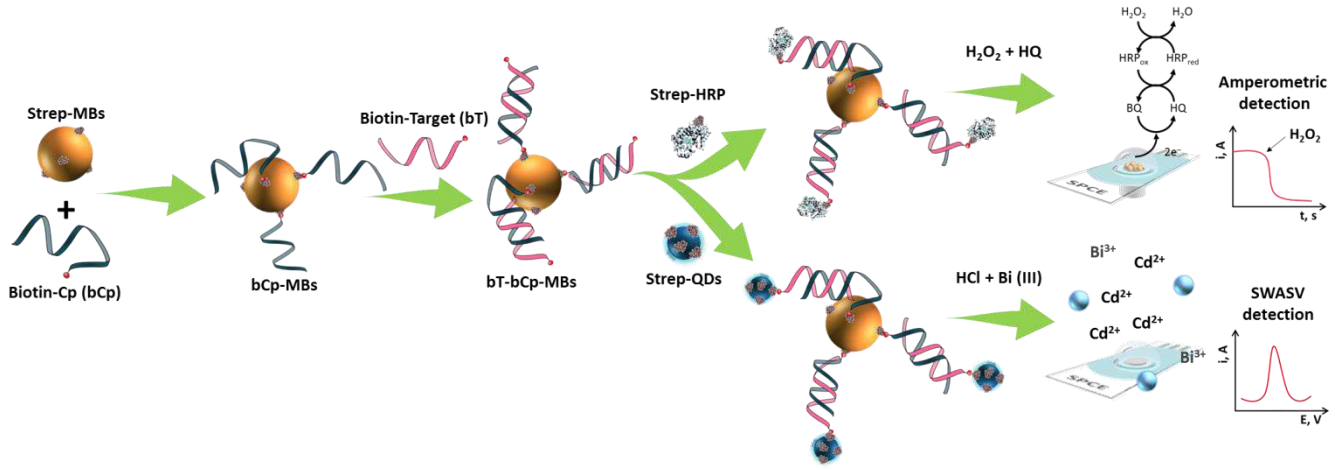

Figure 2. Scheme showing the electrochemical DNA biosensing platforms' configurations used in this work for targeting a specific region of the *SOX-2* gene.

Table 1 summarizes the analytical characteristics obtained for each configuration under the optimized experimental conditions. As can be seen, although both configurations show similar characteristics in terms of reproducibility, stability and analysis time, lower limits of detection (LOD) and quantification (LQ) were obtained with the amperometric magnetogenosensor involving enzymatic amplification.

Under optimal conditions, this amperometric magnetogenosensor exhibited a wide linear concentration range (2.5 pM –1 nM) and a LOD as low as 0.8 pM for the synthetic *SOX-2* specific target DNA. Furthermore, it showed acceptable storage stability, with the capture probe-modified MBs being stable for at least 10 days, and high reproducibility between the amperometric responses provided by different magnetogenosensors prepared in the same manner.

Table 1. Analytical characteristics obtained with each configuration evaluated.

Parameter	Voltammetric detection using QDs as labels	Amperometric detection using enzymatic labels
Linear range, nM (R^2)	0.5 – 10 (0.9971)	0.0025 – 1 (0.9992)
Slope, A nM^{-1}	$(9.7 \pm 0.5) \times 10^{-6}$	$(1.38 \pm 0.02) \times 10^{-5}$
Intercept, A	$(2.2 \pm 0.2) \times 10^{-5}$	$(4 \pm 7) \times 10^{-8}$
LQ, nM	0.61	2.7×10^{-3}
LOD, nM	0.18	8.0×10^{-4}
RSD, %	$3.4_{n = 10}$	$2.8_{n = 10}$
Stability, days	10 (4 ºC)	10 (4 ºC)
Analysis time, min	73	83

Regarding selectivity, the implemented DNA amperometric platforms gave responses only slightly higher than that measured in the absence of target DNA both for fully non-complementary and 2-mismatch oligonucleotide sequences, thus confirming the good selectivity of the developed platform.

The practical applicability of the developed amperometric magnetogenosensor using enzyme label was evaluated by the direct determination of the synthetic target DNA directly in complex non-invasive biological samples such as saliva. Although a significant matrix effect was observed in this matrix, it is important to mention that despite the heterogeneity and complexity of this biological sample, there were no statistical differences between the calibration plots constructed using saliva from different individuals, which implied the possibility of using a single calibration plot for all saliva samples analyzed. The results obtained in the analysis of spiked samples confirmed the reliability of the methodology to accurate determine low nM concentrations of the synthetic target DNA following a simple working protocol. Studies to improve the performance and applicability of these magnetogenosensors are in progress.

The acceptable sensitivity and selectivity together with the simplicity, easy translation to the determination of other nucleic acids and ability for multiplexing in reasonably rapid assays, even with complex biological samples, position these new methodologies as promising tools for high-throughput, multi-DNAs bioanalysis, of great interest in cancer research and clinical diagnosis.