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Quantum dots as nanolabels for breast cancer biomarker HER2-ECD analysis in human serum

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ABSTRACT

Early detection of cancer increases the possibility for an adequate and successful treatment of the disease. Therefore, in this work, a disposable electrochemical immunosensor for the front-line detection of the ExtraCellular Domain of the Human Epidermal growth factor Receptor 2 (HER2-ECD), a breast cancer biomarker, in a simple and efficient manner is presented. Bare screen-printed carbon electrodes were selected as the transducer onto which a sandwich immunoassay was developed. The affinity process was detected through the use of an electroactive label, core/shell CdSe@ZnS Quantum Dots, by differential pulse anodic stripping voltammetry in a total time assay of 2 h, with an actual hands-on time of less than 30 min. The proposed immunosensor responded linearly to HER2-ECD concentration within a wide range (10–150 ng/mL), showing acceptable precision and a limit of detection (2.1 ng/mL, corresponding to a detected amount (sample volume = 40 μL) of 1.18 fmol) which is about 7 times lower than the established cut-off value (15 ng/mL). The usefulness of the developed methodology was tested through the analysis of spiked human serum samples. The reliability of the presented biosensor for the selective screening of HER2-ECD was confirmed by analysing another breast cancer biomarker (CA15-3) and several human serum proteins.

1. Introduction

The incidence of cancer has increased considerably worldwide, and affects the general population, resulting in significant mortality rates [1,2]. Screening and early detection allow appropriate treatments according to the stage of the disease and the availability of healthcare resources. Most European countries have national cancer screening programmes for colorectal [3,4], cervical [5,6] and breast [7,8] cancer. Breast cancer is an important public health concern with considerable impact for the patients, families and society as a whole. Its incidence and prevalence reveals slight differences among developed and developing countries, with the former presenting a global reduction in mortality due to a combination of improvements in prevention, detection and treatment [1,9]. In fact, some studies indicate that mortality rates have been diminishing in places where active screening programmes (e.g. mammography) were implemented [7–10]. Therefore, new analytical tools for the point-of-care (POC) detection of this disease at the early stages, as well as during its management and follow-up, are widely demanded. Moreover, the development of portable equipment for *in situ* breast cancer analysis could also be very useful in less developed regions, remote access areas or even for patients with reduced mobility.

The guidelines established by the European Group on Tumor Markers (EGTM) on the use of breast cancer biomarkers for decision-making regarding the treatments to be administered were recently updated. Despite the large number of new biomarkers that have been reported, only three are mandatory for all patients diagnosed with invasive breast cancer: oestrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2) [11]. The latter is established as an important diagnostic biomarker and is recommended for testing since this protein is overexpressed in 15–20% of primary invasive breast cancers and is related with the most aggressive phenotypes [12,13]. HER2 is a transmembrane protein located on the cell surface and has distinct domains: an extracellular domain (ECD), an intracellular tyrosine kinase domain and a transmembrane lipophilic segment. ECD can be cleaved by metalloproteases and released into the bloodstream. Consequently, HER2 can be measured in serum, which is key for the development of quantitative analytical strategies for breast cancer detection [14,15].

Currently, the two main types of tests accepted for the evaluation of HER2 overexpression in clinical use are immunohistochemistry and *in situ* hybridisation (ISH), which are not suited for POC diagnostics [11]. Hence, a considerable effort in the development of new alternative methodologies has been carried out. The use of “lab-on-a-chip” and

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biosensor technologies are currently providing remarkable tools for POC analysis. The possibility of miniaturization, portability, fast response and low cost are some of the electrochemical biosensors' features that make them attractive for the development of new analytical devices [16,17]. Electrochemical-based approaches for the detection of HER2-positive breast cancer have already been proposed. Different biosensing strategies were reported, which include nanomaterial-based sensing- and/or detection platforms [18–24], nanoelectrode ensembles (NEEs) [25], magnetic-based immunoassays [26,27], a sandwich-type immunoassay based on nanobodies [28], sensing strategies based on affibody/antibody recognition events [29], a MIP-based sensor [30], an impedimetric biosensor based on a zwitterionic hydrogel [31], an impedimetric immunosensor based on the use of single-chain fragment variable antibody fragments [32], a cellulase-linked sandwich assay [33], an inkjet-printed electrochemical platform [34], a polycytosine DNA-based immunosensor [35], and a microfluidic device based on a nanoshearing method [36]. While all of these methods have demonstrated suitable analytical performances, their application is still limited because of extensive and laborious protocols. The use of screen-printed electrodes (SPE) as the biosensor's transducer, without any surface modification prior to the functionalization with the biorecognition element, would considerably simplify the methodology. To date, only one electrochemical immunosensor for HER2 detection without prior modification of the electrode surface has been published [37]. In this work, a sandwich assay with enzymatic labelling allowed to achieve a low detection limit (4 ng/mL). However, a total assay time of 8 h revealed to be its major disadvantage because it is incompatible with a POC sensing strategy.

Nanoparticle-based signal amplification has attracted considerable interest in the development of electrochemical methods. Distinct signal amplification methodologies can be achieved using nanomaterials. Their application as labels can greatly improve the signal transduction and simplify the detection strategy. Quantum Dots (QDs) revealed to be promising candidates as such labels, since they can be conjugated to antibodies and other proteins [38–41]. In addition, they can be synthesized with different compositions or with distinct core-shell structures, which can be useful for multiplexed sensing. The commonly used QDs are composed of a CdS or CdSe core with an external shell to provide functional groups for bioreceptor immobilization with inert and biocompatible coatings [38–40]. Comparing to laborious enzymatic methodologies, the use of QDs eliminates the need for substrate addition, which can contribute to the reduction of the analysis time by applying a straightforward process consisting of: QD dissolution to release the metal ions, electrochemical deposition (preconcentration) of the released ions and a potential (stripping) scan to detect the deposited metal. The obtained electrochemical signal can then quantitatively be related to the analyte concentration. Furthermore, working with electroactive labels also surpasses thermal instability aspects inherent to the nature of enzymes, the main difficulties in their use as labels [42,43].

To the best of our knowledge, this is the first electrochemical immunosensing strategy based on QDs as electrochemical label for *in situ* detection of HER2-ECD.

2. Material and methods

2.1. Apparatus and electrodes

Electrochemical measurements were carried out with a potentiostat/galvanostat (Autolab PGSTAT204, Metrohm Autolab) controlled by the NOVA software package v.1.10 (Metrohm Autolab). Disposable screen-printed carbon electrodes (with a 4-mm working electrode, a silver pseudoreference electrode and a carbon counter electrode, all made of conducting ink (SPCE, DRP-110)) as well the specific connector to interface the electrodes (DRP-CAC) were supplied by Metrohm DropSens.

2.2. Reagents and solutions

Rabit IgG monoclonal anti-human-HER2-ECD (clone 002) antibody (capture antibody – Ab-C), mouse IgG2a monoclonal biotinylated anti-human-HER2-ECD (clone 8B5DAC1) antibody (detection antibody – Ab-D), and a recombinant human HER2/ErBb2 protein (antigen) were obtained from Sino Biological Inc. Qdot® 655 streptavidin conjugate (QD-Strep) was purchased from Invitrogen - ThermoFisher Scientific. Bismuth ICP standard, acetic acid and sodium hydroxide were acquired from Merck. Human serum (from male AB clotted whole blood), albumin from bovine serum (BSA), nitric acid (HNO_3) and tris(hydroxymethyl)aminomethane (Tris) were obtained from Sigma-Aldrich.

Working solutions of BSA, the antibodies, antigen and QD-Strep were prepared in 0.1 M Tris-HNO_3 pH 7.2 buffer (Tris Buffer). The Bi (III) solution (1 mg/L) was prepared in acetate buffer (0.1 M, pH 4.5). Ultra-pure water was obtained from a Millipore (Simplicity 185) water purification system. Buffers and solutions were prepared daily.

The male human serum was stored at −20 °C. Serum samples (diluted 1:1 in Tris buffer) were spiked with HER2-ECD at different concentrations and analysed without further treatments. To evaluate the selectivity of the sensor, non-target proteins (possible interferents) were added to the serum solution.

2.3. Immunosensor development and detection strategy

Scheme 1 elucidates the immunosensor's construction and detection strategy according to the following optimized protocol:

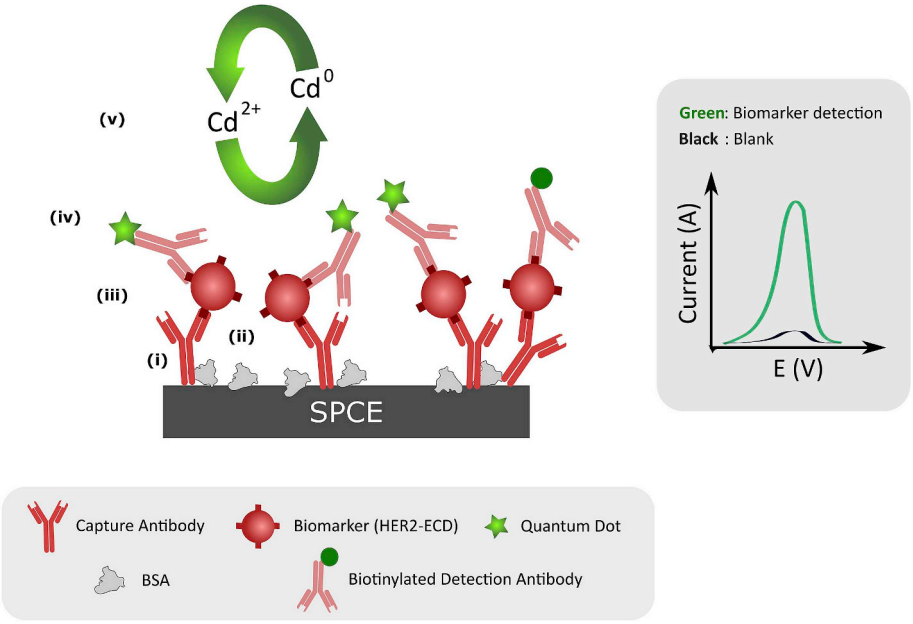
(i) the Ab-C was immobilized on the SPCE surface by drop-casting a 10-μL aliquot of a 25-μg/mL Ab-C solution, which was left overnight in a humidified chamber at 4 °C. Then, the SPCE was washed with Tris buffer before the (ii) incubation with BSA ((2% (m/V), 40 μL), for 30 min. After the free surface sites were efficiently blocked, (iii) 40 μL of a previously prepared mixture (10 min before use) containing the Ab-D (2 μg/mL), HER2-ECD and BSA 0.5% (m/V), was placed on the SPCE for 60 min. Afterwards, the washing step was repeated with Tris buffer and (iv) a 30-μL aliquot of QDs-Strep solution (5 nM, containing BSA 0.5% (m/V)) was placed on the sensor surface and left to incubate during 30 min.

Prior to the measurements, a last washing step was performed with ultra-pure water. The analytical signal was obtained by differential pulse anodic stripping voltammetry (DPASV) based on a procedure described elsewhere [44]. Briefly, (v) 5 μL of HCl (1.0 M) was added to fully cover the working electrode and to release Cd^{2+} from the QDs. This was followed by the addition of 40 μL of an acetate buffer (0.1 M, pH 4.5) containing Bi(III) (1.0 mg/L). To activate the working electrode, a constant potential of +1.00 V was applied during 60 s. Then, the released cadmium ions were pre-concentrated by applying a potential of −1.10 V for 300 s. At the same time a bismuth film was formed. Finally, the potential was swept from −1.0 V to −0.7 V to strip the cadmium into the solution, recording the analytical signal (DPV parameters: pulse amplitude: 0.05 V; step potential: 0.01 V; modulation time: 0.01 s; interval time: 0.1 s).

3. Results and discussion

3.1. Optimization of experimental conditions

Non-invasive analysis of cancer biomarkers can be performed in biological fluids such as serum. Bearing in mind the complex matrices of biological samples, it is mandatory to ensure that background interferences, due to the nonspecific adsorption of biomolecules, are minimized. Hence, the blockage of nonspecific adsorptions on the sensor platform after the modification with the Ab-C is of great importance for efficient and specific biomarker detection. The Ab-C



Scheme 1. Representation of the immunosensor construction and detection strategy.

concentration (25 µg/mL) was selected in accordance with our previous study [18] and was used throughout the work. Casein (2% m/V) and bovine serum albumin (BSA) (2% m/V) were the two tested blocking agents. When casein was used as the blocking agent no electrochemical signal was obtained. This was possibly due to an excessive blocking effect (Fig. S1 I). When BSA was used this behaviour was not verified. Nevertheless, and as can be seen in Fig. S1 (II), the difference between the blank signal and the signal obtained for the analyte was very low. Thus, BSA was added in other steps of the assay: (III) BSA 0.5% (m/V) in both the HER2-ECD and the Ab-D solutions and (IV) BSA 0.5% (m/V) in the HER2-ECD- and 1% (m/V) in the Ab-D solution. The obtained peak current intensities (i_p) shown in Fig. S1 demonstrated that alternative III efficient blocked nonspecific adsorption, leading to the highest signal-to-blank ratio.

Promising new approaches to improve the detection strategy in the development of electrochemical immunosensors have been aligned with nanotechnology through the use of nanolabels, such as QDs. Moreover, the functionalization of these nanomaterials with biomolecules such as streptavidin allows a strong binding to biotinylated antibodies. To optimize the conjugation between the biotinylated Ab-D and the QD-streptavidin conjugate (QD-Strep), different concentrations of Ab-D were tested (1, 2 and 4 µg/mL). The obtained i_p values are presented in Fig. 1, and although this value increased significantly between 1 and 2 µg/mL, only a slightly increase was observed between 2 and 4 µg/mL, which indicates that the latter Ab-D concentration is close to saturation. Therefore, 2 µg/mL was chosen as the best compromise between the Ab-D concentration and the signal-to-blank ratio.

In this work QDs were used to enhance the electrochemical detection, to simplify the methodology and to reduce the assay time. Thus, after the optimization of the surface blocking and the antibodies' concentrations, three different QD concentrations were tested: 5, 10 and 20 nM (in terms of QDs) (Fig. 2A). The i_p increased with the increase of the QD concentration both in the absence and presence of the analyte (50 ng/mL). Accordingly, 5 nM was selected because it provided the highest signal-to-blank ratio. BSA 0.5% (m/V) was added to the QDs solution to further minimize nonspecific adsorption, which also led to a much higher signal-to-blank ratio and a better precision of the results (Fig. 2B).

Regarding the optimization of the assay, several formats were evaluated: step-by-step assays (A, B, C, D) and combined assays (E, F, G). The tested alternatives were: (A) antigen 60 min, Ab-D 60 min, QD 60 min (control assay, used during optimization studies), (B) antigen 60 min, Ab-D 60 min, QD 30 min, (C) antigen 30 min, Ab-D 60 min, QD 30 min, (D) antigen 60 min, Ab-D 30 min, QD 30 min; and the combined steps, which involve the pre-incubation of reagents: (E) antigen + Ab-D 60 min, QD 60 min, (F) antigen + Ab-D 60 min, QD 30 min, (G) antigen 60 min, Ab-D + QD 60 min (Fig. 3). The alternatives B and F provided the best signal-to-blank ratios. In both assays,

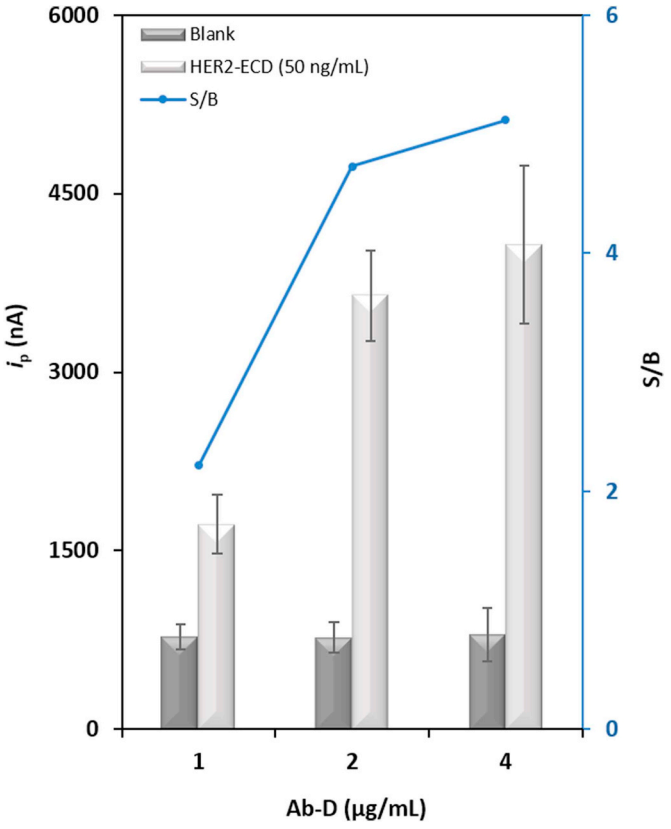


Fig. 1. Optimization of Ab-D concentration (1; 2 and 4 µg/mL). Experimental conditions: BSA (2% (m/V), Ab-C 25 µg/mL, HER2-ECD (0 and 50 ng/mL), QD 5 nM.

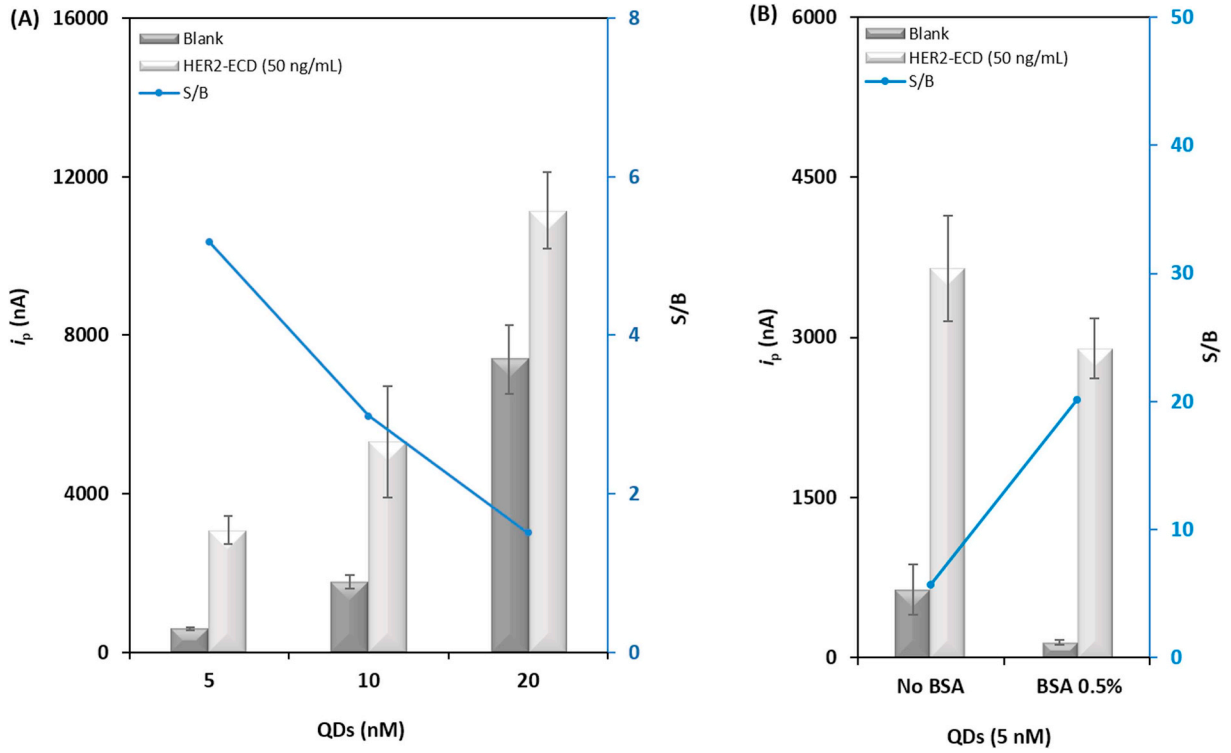


Fig. 2. (A) Optimization of QDs concentration (5; 10 and 20 nM) and (B) Current intensities obtained with QDs 5 nM in the absence and in the presence of BSA (0.5% (m/V)) in the solution. Experimental conditions: BSA (2% (m/V)), Ab-C (25 µg/mL), HER2-ECD (0 and 50 ng/mL), Ab-D (2 µg/mL).

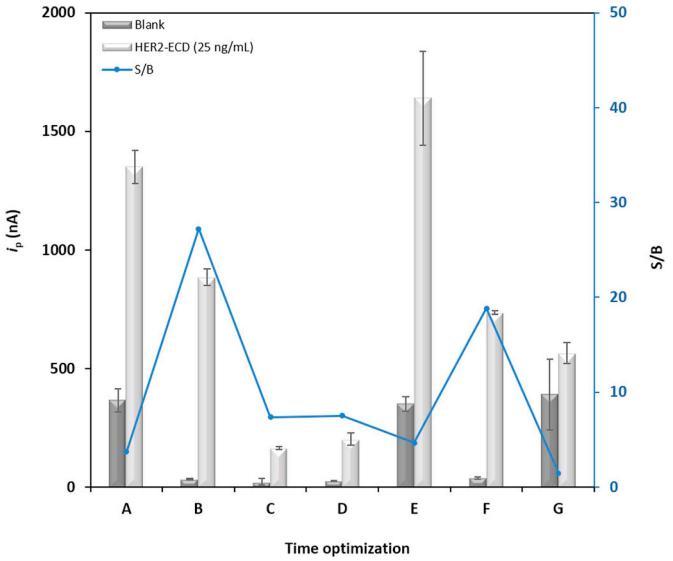


Fig. 3. Influence of the incubation time for step-by-step assay (A, B, C and D), and joining steps assay: HER2-ECD + Ab-D (E and F) and Ab-D + QDs (G). Experimental conditions: BSA (2% (m/V), Ab-C (25 µg/mL), HER2-ECD (25 ng/mL), Ab-D (2 µg/mL), QDs (5 nM + BSA 0.5% (m/V)).

the QD incubation time was 30 min, which resulted in a reduction of both the blank and analytical signal and an increase of the signal-to-blank ratio. The sensor's response to increasing concentrations of HER2-ECD was assessed for both alternatives (B and F), obtaining similar sensitivities. Therefore, alternative F was chosen to proceed with the studies since the total time assay (120 min) was 60 min shorter than the one of alternative B. The combination of two steps not only allowed the increase of the precision of the results but also led to a more user-friendly assay.

3.2. Analytical performance for HER2-ECD determination in human serum

The analytical performance of the developed immunosensor was tested using the previous optimized parameters. Initially, distinct HER2-ECD concentrations, in buffer solution, were analysed and a linear range was established between 5 and 150 ng/mL (Fig. S2). Then, male human serum samples (diluted 1:1 in Tris buffer) were spiked with HER2-ECD from 5.0 to 400 ng/mL. A linear relationship between the analyte concentration and i_p was found between 10 and 150 ng/mL, according to the following equation: i_p = (5.07 ± 0.14) [HER2-ECD] + (18.4 ± 11.8), r = 0.9989, n = 5. The corresponding calibration plot as well typical voltammograms within the linear range are presented in Fig. 4. When comparing the calibration plots constructed in buffer solution and serum, a clear matrix effect was observed; the slope of the calibration plot in serum was 5 times lower than the slope obtained with the measurements in buffer. This could be due to human serum albumin, which is the most abundant protein in human blood, and globulins, especially immunoglobulins G (IgG) [45,46]. To avoid excessive blocking of the transducer's surface, BSA was not added to the antigen - Ab-D solution. The limits of detection (LOD) and quantification (LOQ) were calculated as the concentration corresponding to 3 × S_{blank}/m for the LOD and 10 × S_{blank}/m for the LOQ (S_{blank}: standard deviation of the blank signal; m: slope of the calibration plot). The obtained limit of detection (2.1 ng/mL) was far below the cut-off value established for HER2-ECD (15 ng/mL), which corroborates the adequacy and practical utility of the developed sensor. Table 1 summarizes the figures of merit of the developed sensor.

3.3. Selectivity, stability, precision and recovery studies

The selectivity of the immunosensor was tested through the analysis of other proteins that could be found in serum. In this study, the sensor's response towards cancer antigen 15–3, another breast cancer protein (CA15-3, 50 U/mL) and cystatin C, a biomarker of kidney function (550 ng/mL) was tested. Blank and HER2-ECD (50 ng/mL) solutions

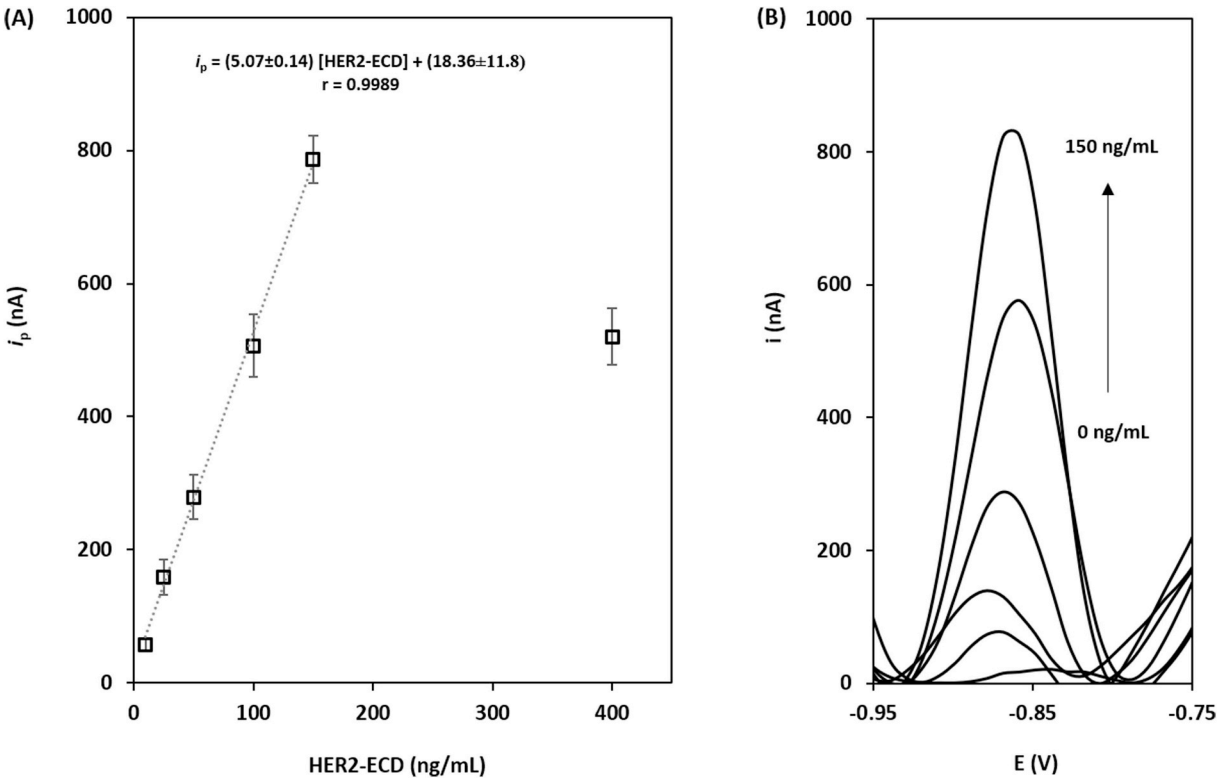


Fig. 4. (A) Calibration plot using the developed immunosensor in the presence of growing concentrations of HER2-ECD in human serum and (B) examples of typical differential pulse voltammograms obtained within the established linear range (0, 10, 25, 50, 100 and 150 ng/mL), Experimental conditions: Ab-capture: 25 µg/mL, BSA: 2% (m/V), Ab-D: 2 µg/mL, QDs: 5 nM with addition of BSA 0.5% (m/V)).

Table 1
Figures of merit of the developed biosensor for the analysis of the cancer biomarker HER2-ECD in Human Serum samples.

Figure of merit	
Concentration interval (ng/mL)	10–150
Correlation coefficient (r)	0.9989
Slope (m) (nA/(ng/mL))	5.07
Standard deviation of the slope (S_m) (nA/(ng/mL))	0.14
Intercept (b) (nA)	18.4
Standard deviation of the intercept (S_a) (nA)	11.8
Standard deviation of the linear regression ($S_{y/x}$)	16.1
Standard deviation of the method (S_{x0})	3.2
Coefficient of variation of the method (V_{x0}) (%)	5.6
Limit of detection (LOD) (ng/mL)	2.1
Limit of quantification (LOQ) (ng/mL)	7.1

were also assayed for comparison proposes. All the proteins were added to male human serum and their concentrations were chosen based on the values that can be expected in real clinical environments. The obtained i_p values are presented in Fig. 5. In comparison to the analytical signal obtained for HER2-ECD, the other proteins did not show a significant response; the obtained i_p values were very similar to the blank signal, which confirmed the sensor's selectivity towards HER2-ECD.

The stability of the immunosensor was also studied. The as-prepared sensor was stored at 4 °C and measurements were performed during a month (Fig. S3). It was concluded that the sensor was stable for up to a week. So, the sensing phase of the proposed biosensor should be prepared on a weekly basis to guarantee a sensitive analysis.

The intermediate precision and the reproducibility of the results were assessed by analyzing a 50-ng/mL HER2-ECD solution (in human serum) on the same day and on three different days, obtaining relative standard deviation (RSD) of 7.1% and 4.9%, respectively, which indicates that the optimized sensor provided precise results.

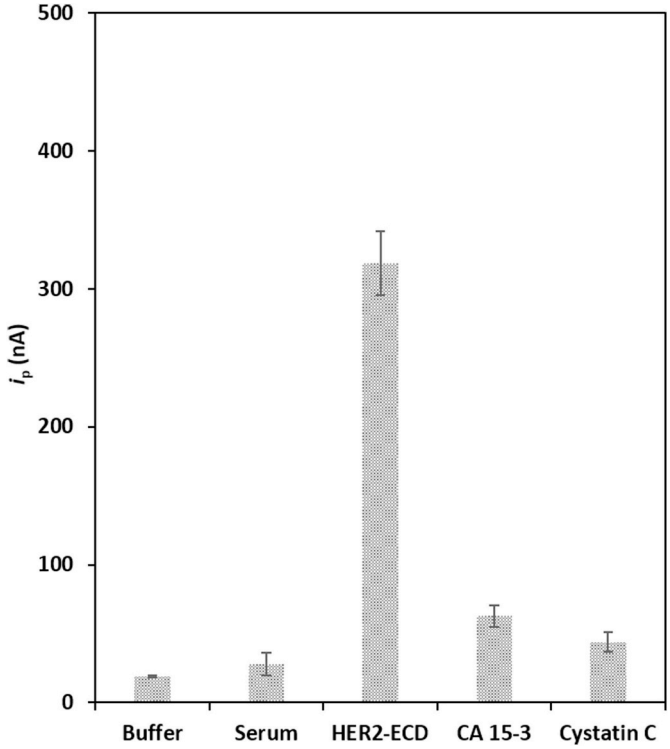


Fig. 5. Evaluation of the selectivity of the HER2-ECD sensor against other serum proteins. Experimental conditions: Ab-C: 25 µg/mL, BSA: 2% (m/V), Ab-D: 2 µg/mL, QDs: 5 nM with addition of BSA 0.5% (m/V)). Serum proteins concentration: HER2-ECD: 50 ng/mL, CA 15–3: 50 U/mL, Cystatin C: 550 ng/mL.

Table 2
Summary of experimental parameters of electrochemical immunoassay-based procedures for HER2 analysis.

Breast cancer analyte	Sensing surface				Label	Technique	Detection	Assay time	LOD	Sample	Ref
	Transducer	Transducer preparation time[a]	Modification with the biorecognition element[a]	Long-term stability							
HER2-ECD	Bare SPCE	–	~12 h	7 days	CdSe@ZnS QDs	DPV	Cadmium	2 h	2.1 ng/mL	Spiked human serum	**This study**
HER2-ECD	SPCE/MWCNT-AuNP	~15 min	~12 h	n.d.	AP	LSV	Silver	2 h 20 min	0.16 ng/mL	Spiked human serum	[18]
SK-BR-3	FTO/NFG/AgNP/PANI	> 4 h	~12 h	45 days	n.a.	DPV	$[Fe(CN)_6]^{3-/4-}$	30 min	2 cells/mL	Whole blood	[19]
HER2	GCE/Fe_3O_4-APTMS	> 3 h	2 h	10 days	Hydrazin	DPV	Silver	2 h	2.0×10^{-5} ng/mL	Patient serum	[20]
HER2	CILE-MWCNT/AuNP	> 30 h	6 h	n.d.	n.a.	EIS	$[Fe(CN)_6]^{3-/4-}$	35 min	7.2 ng/mL	Patient serum	[21]
HER2-ECD	SPCE/AuNP	~10 min	~12 h	n.d.	AP	LSV	Silver	2 h 50 min	4.4 ng/mL	Spiked human serum	[22]
HER2	AuE/AuNP-MPA-Cys	> 22 h	~12 h	3 weeks	n.a.	DPV	$[Fe(CN)_6]^{3-/4-}$	30 min	0.995 pg/mL	Patient serum	[23]
HER2	GCE/poly-DPB(AuNP)	> 4 h	~12 h	n.d.	Hydrazin	SWSV	Silver	1 h 10 min	0.037 pg/mL	Cancer cells	[24]
HER2	NEEs	n.d.	2 h	n.d.	HRP	CV	MB	7 h 10 min	40 ng/mL	Cell lysates	[25]
HER2	SPCE/MBs	~15 min	40 min	10 days	AP	DPV	1-NP	2 h 05 min	6.0 ng/mL	Patient serum	[26]
ErbB2	SPCE/MBs	~40 min	1 h	n.d.	HRP	Amperometry	HQ	1 h	26 pg/mL	Patient serum and cell lysates	[27]
HER2	SPE	1 h	1 h	3 weeks	HRP	Amperometry	HQ	22 min	1 μg/mL	Cell lysates	[28]
HER2	8 × SPE/Strep-MBs or ProtA-MBs	~15 min	~12 h 45 min	1 week	AP	DPV	1-NP	2 h 51 min 1 h 51 min	1.8, 2.6 and 3.4 ng/mL	Spiked human serum	[29]

1-NP – 1-naphthol; AgNP – silver nanoparticles; AP – alkaline phosphatase; APTMS – (3-Aminopropyl)triethoxysilane; AuE – gold electrode; AuNPs – gold nanoparticles; Cys – cysteamine; CV – cyclic voltammetry; CILE –carbon ionic liquid electrode; FTO – fluorine doped tin oxide; GCE – glassy carbon electrode; DPB – 2,5-bis(2-thienyl)-1H-pyrrole-1-(p-benzoic acid); DPV – differential pulse voltammetry; HER2 – human epidermal growth factor receptor 2; HRP – horseradish peroxidase; HQ – hydroquinone; LSV – linear sweep voltammetry; MBs – magnetic beads; MB – methylene blue; MPA – 3-mercaptopropionic acid; MWCNT – multiwalled carbon nanotube; NEEs – nanoelectrode ensembles; NFG – nitrogen-doped graphene; n.d. – no data; n.a. – not applicable; PANI – polyaniline; QDs – quantum dots; SPCE – screen-printed carbon electrode; SWASV – square wave anodic stripping voltammetry; SWV – Square wave voltammetry.

[a] Overnight incubations were considered as a 12-h period for comparison purposes.

To demonstrate the potential clinical utility of the immunosensor, recovery studies were performed using serum samples spiked with three different HER2-ECD concentrations: 25, 75 and 125 ng/mL. The average recoveries were found to be 105.6, 105.0 and 103.5% with RSDs of 5.6, 5.0 and 3.5%, respectively (n = 3). This indicates that the developed sensor provided both precise and accurate results.

3.4. Comparison with previous reported electrochemical immunosensors and immunoassays

The proposed immunosensor was compared with previously published immunosensors and immunoassays with electrochemical detection for HER2 analysis. As can be seen in Table 2, distinct configurations with comparable performances were described. The immunosensor developed in this work has a competitive assay time that is only surpassed by the label-free sensors [19,21,23] and by three works in which an enzymatic label was employed [24,27,28]. However, some of these shorter assay times are preceded by complex modifications of the electrode surface, as well as time-consuming procedures, which appears as a major drawback [19,21,23,24]. Moreover, the work developed by Patris et al. presented a good compromise between assay time and LOD, considering the complexity of the sample (cell lysates) [28]. The electrochemical magneto-immunoassay described by Eletxigerra et al. [27] demonstrated an exceptional LOD and assay time. Nevertheless, the dependence on an enzymatic substrate and the production of a substantial amount of waste, due to the volume of mediator needed for the measurement step, are the major drawbacks of the reported work. The remaining works did not improve the assay time of our assay. Accordingly, the immunosensor developed in this work presents a user-friendly straightforward 3-step assay protocol, within a highly competitive assay time, and achieving a low LOD (< reference cut-off value).

Furthermore, an additional overview of the use of metallic and carbon-based QDs for the immunosensing of different breast cancer biomarkers is summarized in Table SI 1. In some works, the QDs were used as detection label while in others they were employed in the modification of the transducer surface. Most of these works imply laborious protocols [38,40,47,48] and longer assay times [41,49]. Additionally, our immunosensor is the first one that employs QDs for the development of an electrochemical immunosensor for HER2-ECD.

4. Conclusions

A novel and efficient electrochemical immunosensor for the analysis of the breast cancer biomarker HER2-ECD, with a total time assay of 2 h, was developed. This work highlights the simplicity of the assay, with an actual hands-on-time of less than 30 min, without resorting to laborious electrode surface modifications. An electroactive QD label was employed and a limit of detection of 2.1 ng/mL was achieved, corresponding to the detection of 1.18 fmol of the analyte (sample volume = 40 µL). The analytical performance was tested in spiked human serum samples, demonstrating an excellent performance in a wide linear range with a high sensitivity, stability, precision and accuracy. The applicability and selectivity of the proposed methodology was tested and confirmed through the analysis of distinct serum proteins in human serum.

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Appendix A. Supplementary data

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