



Immunomagnetic bead-based bioassay for the voltammetric analysis of the breast cancer biomarker HER2-ECD and tumour cells using quantum dots as detection labels

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Abstract

An electrochemical magnetic immunosensing strategy was developed for the determination of HER2-ECD, a breast cancer biomarker, and breast cancer cells in human serum. A sandwich assay was performed on carboxylic acid-functionalized magnetic beads (MBs) using a screen-printed carbon electrode (SPCE) as transducer surface. The affinity process was detected using electroactive labels; core/shell streptavidin-modified CdSe@ZnS Quantum Dots (QDs). Cd²⁺ ions, released from the QDs, were determined by differential pulse anodic stripping voltammetry (DPASV). An assay time of 90 min, with an actual hands-on time of about 20 min, a linear range between 0.50–50 ng·mL⁻¹ of HER2-ECD and a limit of detection of 0.29 ng·mL⁻¹ were achieved. Analysis of live breast cancer cells was also performed using the optimized assay. Breast cancer cell lines SK-BR-3 (a HER2-positive cell line), MDA-MB-231 (a HER2-negative cell line) and MCF-7 (a cell line with low HER2 expression) were tested. The selectivity of the assay towards SK-BR-3 cells was confirmed. A concentration-dependent signal that was 12.5× higher than the signal obtained for the HER2-negative cells (MDA-MB-231) and a limit of detection of 2 cells·mL⁻¹ was obtained.

Keywords Breast cancer · Cancer cells · Electrochemical immunoassay · HER2-ECD · Magnetic beads · Quantum dots

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Introduction

Cancer screening programs are implemented at the national level to reduce mortality rates [1]. Nevertheless, the limited sensitivity and the lack of specific results in the traditional diagnosis can significantly lead to unfavourable clinical outcomes [2]. The absence of non-invasive methodologies for accurate determination of carcinogenic biomarkers in the bloodstream is a real concern. Human epidermal growth factor receptor 2 (HER2) is a cancer biomarker overexpressed in primary cancer cases [3]. The tumour expression is dichotomized into HER2-positive and HER2-negative groups [4]. In this field, nanotechnological devices can be used to measure the protein expression in biological fluids (serum, plasma) from screening to follow-up. The assessment of the concentration of the extracellular domain of HER2 (HER2-ECD) in serum samples is a prominent alternative since this domain can be cleaved from the surface of cancer cells by matrix metalloproteases and released into the bloodstream [5]. In addition, the

assessment of HER2-positive circulating tumour cells (CTCs) is fundamental for patients that can benefit from personalized therapies and adjuvant treatment [6].

Electrochemical strategies for the determination of HER2 in human serum have been reported using distinct and elaborate electrode surface modifications based on sumptuous structures (nano, micro- and/or magnetic-based materials) to improve the transducer's performance [7]. Nevertheless, the synthesis of these materials and their functionalization require extensive and time-consuming procedures. In contrast, less innovation has been focused on the detection strategy. Distinct probes or labels to amplify the electrochemical signal are required since the redox pair $[\text{Fe}(\text{CN})_6]^{3-/4-}$ is still one of the most reported [8–12]. Although silver [13–16], 1-Naphtol (1-NP) [17], 3,3',5,5'-Tetramethyl[1,1'-biphenyl]-4,4'-diamine (TMB) [18] and molybdate [19, 20] were employed for the determination of HER2, the use of nanomaterials has not extensively been studied.

Nanoparticle-based signal amplification has attracted considerable attention in immunosensing strategies, since higher sensitivity and lower limits of detection can be achieved [21]. Quantum dots (QDs) are crystalline nanoparticles that revealed to be promising candidates as electrochemical detection labels in breast cancer assays due to the good electroactivity of the employed metals [22–24]. QDs can be analysed by stripping voltammetry after preconcentration of the metal on the electrode surface. The obtained signal can then be related to the analyte concentration [25, 26].

Bioassays using QDs as semiconductor nanolabels and magnetic particles as a platform for the targeted immobilization of antibodies constitute a versatile solution for the analysis of biomarkers in clinical practice. In the present work a magnetic immunoassay was developed using carboxylic acid-functionalized magnetic beads (COOH-MBs) onto which capture antibodies were covalently bound, allowing their oriented immobilization. This was followed by the addition of a solution containing the analyte (HER2-ECD or cancer cells) and a biotinylated detection antibody. Then streptavidin-coated core/shell CdSe@ZnS QDs were linked to the previously formed immunocomplex, and the determination was performed by differential pulse anodic stripping voltammetry (DPASV) on a screen-printed carbon electrode (SPCE). This is the first electrochemical magnetic bioassay for the analysis of HER2-ECD using a MB platform and core/shell CdSe@ZnS QDs as electroactive labels. The electrochemical analysis of HER2-positive (SK-BR-3), HER2-negative (MDA-MB-231) and low-expression HER2 cancer cells (MCF-7) using the present methodology is also new.

Experimental

Apparatus

The SEM images were obtained using FEI Quanta 400FEG ESEM/EDAX Genesis X4 M equipment at the “Centro de Materiais da Universidade do Porto (CEMUP)”. Cancer cells were imaged by a Nikon TMS microscope and counted using an automated cell counter (Countess™, ThermoFisher Scientific - Invitrogen, USA, www.thermofisher.com/invitrogen). Screen-printed carbon electrodes (with a 4-mm working electrode (WE) (electroactive area: 0.079 cm^2), a silver pseudoreference electrode and a carbon counter electrode (SPCE, DRP-110)) and the specific connector (DRP-CAC) were supplied by Metrohm DropSens (Spain, www.dropsens.com). Electrochemical measurements were carried out with a potentiostat/galvanostat (Autolab PGSTAT204, Metrohm Autolab, The Netherlands, www.metrohm-autolab.com) controlled by the NOVA software package v.1.10 (Metrohm Autolab). Magnetic separations were carried out using a DynaMag™-2 magnet (ThermoFisher Scientific, USA, www.thermofisher.com). Ultra-pure water (resistivity = $18.2 \text{ M}\Omega\cdot\text{cm}$) used throughout the work was obtained from a Millipore water purification system (Simplicity 185) (Germany, www.merckmillipore.com).

Reagents and solutions

The chemical and biological reagents are indicated in the Electronic Supporting Material (ESM, Section S1). Buffers and working solutions of the antibodies, antigens and QD-Strep were prepared daily. The following buffers were used: MES buffer (0.1 M, pH 6) for the ECD/NHS (200 mM/50 mM) and the capture antibody (Ab-C, various concentrations) solutions, PBS buffer (0.02 M, pH 8) for the EA (1 M) solution, Tris buffer (0.1 M, pH 7.4) for the QD-strep (various concentrations), containing BSA (0.5% m/V), solutions, and acetate buffer (0.1 M, pH 4.5) to prepare the Bi(III) ($1.0 \text{ mg}\cdot\text{L}^{-1}$) solution. Tween 20 (0.01%) was added to the distinct buffers to be used in the washing steps.

Cell culture and CTC analysis

Breast cancer cell lines MDA-MB-231, MCF-7 and SK-BR-3 were cultured in RPMI medium supplemented with 10 and 15% inactivated FBS, respectively, and 1% antibiotic/antimycotic solution. Cells were incubated at 37°C in a humidified atmosphere of 5% CO_2 -95% air. The culture medium was changed every 2–3 days, and the culture was split every 7 days. For analysis, cells were seeded on 21-cm^2 plastic cell culture dishes (TPP@,

Trasadingen) and were used after 2–5 days in culture (90–100% confluence). On the day of the experiment, the cells were harvested with Trypsin-EDTA 0.25% and counted using the automated cell counter. A trypan-blue exclusion assay was performed to confirm cell viability, which was between 87 and 96% for all the cell lines. The distinct solutions of the cells (1×10^2 – 1×10^5 cells·mL⁻¹) were prepared in human serum and analysed using the optimized immunoassay.

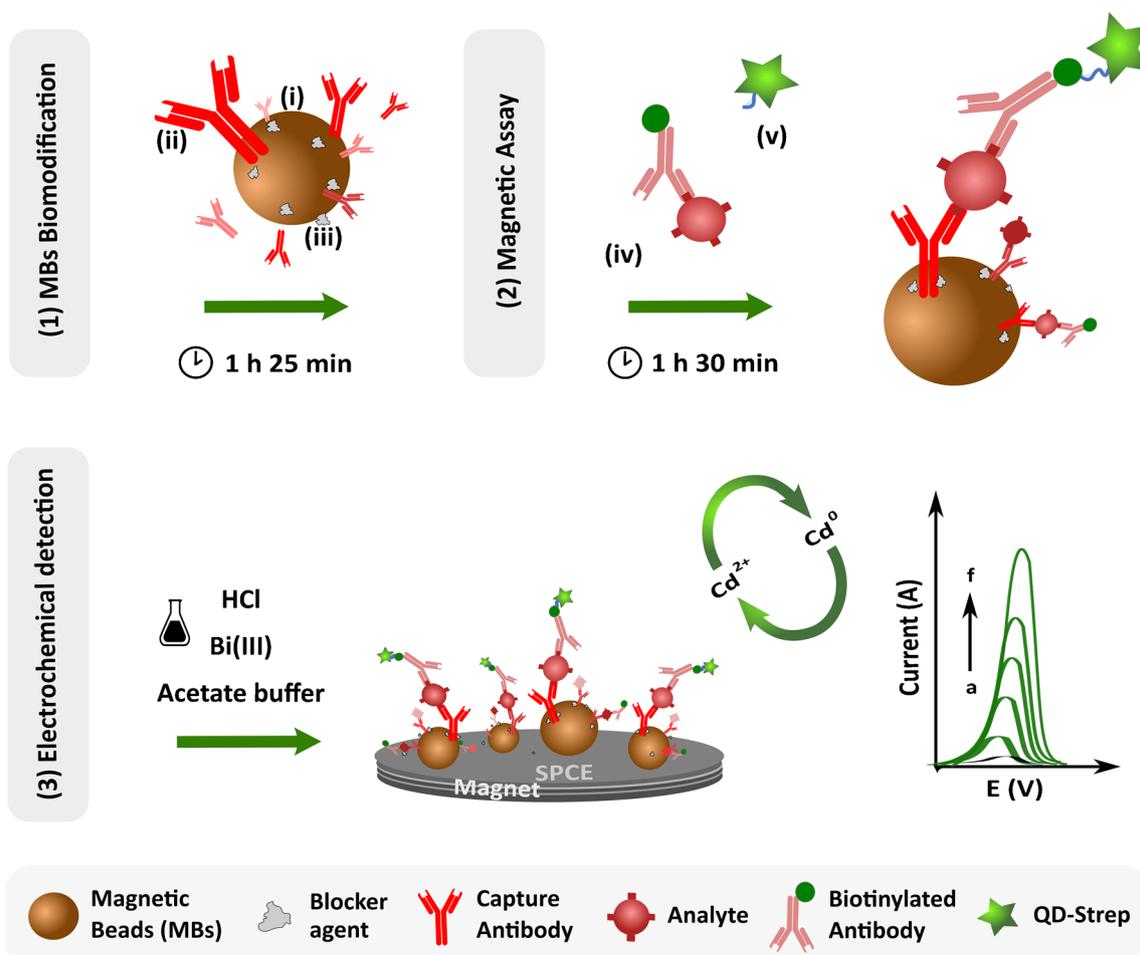
Electrochemical immunomagnetic bioassay for the analysis of HER2-ECD and living cells

A schematic representation of the sandwich immunoassay (Scheme 1) elucidates the distinct steps of the experimental procedure (note that steps (i) to (v) were performed in 0.5-mL

microtubes). All the steps of the assay were performed protected from light at room temperature using vortex stirring (950 rpm). Magnetic separations were carried out using the DynaMag™-2 magnet.

Prior to the biomodification, the MBs stock solution was washed with MES-T for 2 min.

- (1) The biomodification consisted of the following steps: (i) a 1.5- μ L aliquot of the MBs solution (containing 7.5 μ g of MBs) was resuspended in the EDC/NHS solution (100 μ L, 15 min) and (ii) incubated with Ab-C (25 μ g·mL⁻¹, 100 μ L, 1 h). After a washing step with MES-T and PBS-T (2 min each), the particles' free surfaces were (iii) blocked with EA (100 μ L, 10 min). The biomodified particles were resuspended in 100 μ L PBS-T and kept at 4 °C until use.



Scheme 1 Schematic representation of the magnetic immunoassay. **1** The MBs are first biomodified (i activation with EDC/NHS, ii addition of capture antibody, iii blocking with EA). **2** The magnetic assay is performed through the addition of iv a mixture containing the antigen (HER2-ECD or cancer cells) and the biotinylated antibody, followed by

the addition of v a QD-strep solution. The magnetic beads are magnetically attracted to the working electrode. **3** Electrochemical determination of the released Cd²⁺ is performed. Voltammograms of increasing HER2-ECD concentrations are exemplified.

- (2) The magnetic immunoassay consisted of the following:
- (iv) The analyte solution (protein biomarker or cancer cells) was previously mixed with Ab-D ($2.0 \mu\text{g}\cdot\text{mL}^{-1}$) in human serum (10 min before use). The prepared mixture was then added to the biomodified MBs ($100 \mu\text{L}$, 1 h). Afterwards, a washing step with Tris-T was performed and (v) the MBs were incubated with a QD-strep solution (5.0 nM , $70 \mu\text{L}$, 30 min).
- (3) Electrochemical analysis: prior to the measurements, a washing step was performed with ultra-pure water. Then, a $10\text{-}\mu\text{L}$ aliquot of the final solution was placed on the WE of an SPCE under which a magnet ($d = 4 \text{ mm}$) was placed. The SPCE was totally dried with a nitrogen flow, and $5 \mu\text{L}$ of the HCl (1 M) and $40 \mu\text{L}$ of the Bi(III) ($1.0 \text{ mg}\cdot\text{L}^{-1}$) solutions were added to the electrode surface to release Cd^{2+} from the QDs. DPASV voltammograms were recorded using the following conditions: constant potential of $+1.00 \text{ V}$ during 60 s, followed by a potential of -1.10 V for 300 s (in this phase the released cadmium ions were pre-concentrated and a bismuth film was formed). The potential was swept from -1.00 V to -0.70 V to strip the cadmium into the solution, recording the electrochemical signal. (DPV parameters, pulse amplitude, 0.05 V ; step potential, 0.01 V ; modulation time, 0.01 s ; interval time, 0.1 s).

Results and discussion

Electrochemical behaviour of the quantum dots on distinctly modified SPCE surfaces

To elaborate the immunomagnetic assay, prior measurements were performed to study the electrochemical behaviour of cadmium (released from the QDs) using DPASV. In the tested procedure, cadmium was deposited simultaneously with a bismuth film which is considered an adequate alternative to the traditional mercury film [27]. Distinct carboxylic acid-functionalized materials were tested using an SPCE as transducer: multi-walled carbon nanotubes (HOOC-MWCNT, $1 \mu\text{g}\cdot\text{mL}^{-1}$), PEG gold-coated magnetic nanoparticles (HOOC- Fe_3O_4 @AuNP, $30 \mu\text{g}$) and magnetic beads (HOOC-MBs, $30 \mu\text{g}$). The sensing platforms were employed to analyse a $50\text{-ng}\cdot\text{mL}^{-1}$ HER2-ECD solution, and the obtained signals were compared with the ones obtained with a bare SPCE, using concentrations of Ab-C $25 \mu\text{g}\cdot\text{mL}^{-1}$, Ab-D $2.0 \mu\text{g}\cdot\text{mL}^{-1}$, QD 10 nM , HCl 1 M and Bi(III) $1.0 \text{ mg}\cdot\text{L}^{-1}$. The respective SEM images and obtained voltammograms are presented in Fig. 1. The functionalization of nano- and micromaterials with carboxylic acid groups commonly

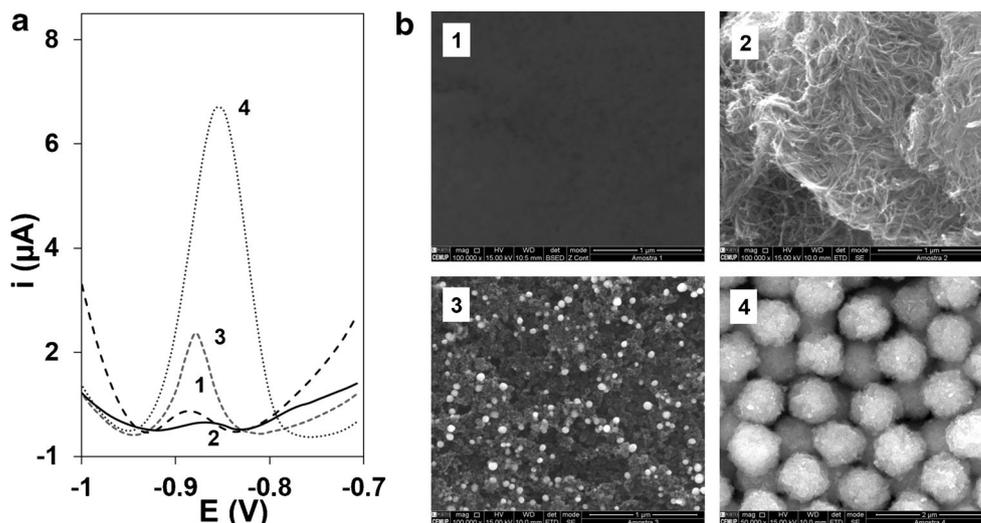
leads to an efficient and well-organized attachment of the antibody, allowing the construction of a stable and functional immunoassay [7, 28]. However, when the SPCE is modified with MWCNTs, the obtained peak current intensity was considerably lower than for the unmodified (bare) electrode, as can be seen in Fig. 1a (1 and 2). Despite the wide application of carbon-based nanomaterials in the construction of electrochemical immunosensors, in this work, the modification of the transducer with MWCNT proved to be inappropriate. As described in the literature, the electrode modification with carbon nanomaterials leads to higher adsorption of proteins on the transducer's surface, increasing the effect of the electrical double layer [29]. On the other hand, the use of both magnetic nano- and microparticles leads to an increase in the current intensity. This is probably due to an efficient immobilization of the capture antibodies on these particles, as well as the specific orientation, which is not as effective on the SPCE-MWCNT surface. Although other studies report the construction of interesting magnetic assays using core/shell nanoparticles, in the present work, the electrochemical signal is noticeably higher when (micro-sized) MBs were used (Fig. 1a (3 and 4, respectively)). In fact, the SEM images (Fig. 1b (3 and 4)) support the results since it is observed that the MBs are arranged in an organized layer that can contribute to a more sensitive method. In contrast, the core/shell nanoparticles are not as homogeneous in size and are dispersed on the electrode's surface.

Thus, considering that the MBs are the best sensing surface, further studies were carried out to evaluate the possibility of improving the detection strategy. The electrochemical response of the QDs was verified by adding HCl and Bi(III) in four different strategies (ESM, Section S2). The best results were obtained by placing the biomodified-MBs on the WE followed by the addition of the HCl and Bi(III) solutions.

Optimization of the immunosensing strategy

The following experimental parameters that affect the immunoassay's performance were optimized: (a) assay format, (b) QD concentration, (c) MB amount and (d) Ab concentrations. Respective text and figures regarding the optimizations are included in the ESM (Section S3). The parameters selected to test the immunomagnetic assay for the analysis of HER2-ECD and cancer cells in human serum are presented in Table S1. In short, the following experimental conditions were found to give the best results: MBs $7.5 \mu\text{g}$, Ab-C $25 \mu\text{g}\cdot\text{mL}^{-1}$, Ab-D $2.0 \mu\text{g}\cdot\text{mL}^{-1}$ and QDs 5.0 nM , using a previously prepared mixture of the antigen and Ab-D (Format B). Under these conditions, the average amount of antibody per MB was 0.188 pg .

Fig. 1 **a** Study of the electrochemical behaviour of cadmium (released from a 10-nM QD solution) with distinct nano- and micromaterials used on SPCEs. **b** Respective SEM images: 1 – bare SPCE; 2 – SPCE-MWCNT; 3 – SPCE-Fe₃O₄@Au nanoparticles; 4 – SPCE-MBs



Analytical performance and stability of the modified magnetic beads

The optimized method was used to analyse the cancer biomarker in human serum. The precision of the results was evaluated using a 50-ng·mL⁻¹ HER2-ECD solution. Relative standard deviations (RSD) for the repeatability and reproducibility ($n = 3$) were 2.7 and 3.1%, respectively, demonstrating that the immunomagnetic assay provides precise results.

For calibration purposes, HER2-ECD solutions with increasing concentrations were prepared in human serum (0.50, 1.0, 2.5, 5.0, 10, 25, 50 and 100 ng·mL⁻¹) and analysed with the magnetic immunoassay. The linear range was established between 0.50 and 50 ng·mL⁻¹ ($i_p = 0.75 \pm 0.03$ [HER2-ECD] + 4.99 ± 0.58, $r = 0.997$) with a sensitivity of 9.5 µA·mL·ng⁻¹·cm⁻². The calibration plot and examples of differential pulse anodic stripping voltammograms in the linear range are shown in Fig. 2. Limits of detection (LOD = 3 s/m) and quantification (LOQ = 10s/m) were calculated from the calibration straight: “s” is the standard deviation of the blank and “m” is the slope. The values obtained were LOD = 0.29 ng·mL⁻¹ and LOQ = 0.96 ng·mL⁻¹. The obtained LOD was more than 50× lower than the cut-off value (15 ng·mL⁻¹) established for HER2-ECD. The coefficient of variation of the method (V_{x0}) was 1.27%, demonstrating that the precision of the method is adequate ($V_{x0} < 5\%$). Additional figures of merit of the magnetic immunoassay are indicated in Table S2.

To assess the selectivity of the bioassay, other cancer protein biomarkers were tested, including breast cancer antigen 15–3 (CA15–3, 100 U·mL⁻¹), liver cancer biomarker alpha-Fetoprotein (AFP, 1 µg·mL⁻¹) and a possible serum interferent, human serum albumin (HSA, 100 mg·mL⁻¹). Concentrations were chosen based on values that

can be expected in real situations. The results demonstrated the high selectivity of the assay for HER2-ECD since the signals of the tested interferents are similar to the blank signal (Fig. 3a).

To assess the accuracy of the assay’s results both recovery experiments and the use of a reference method (ELISA)

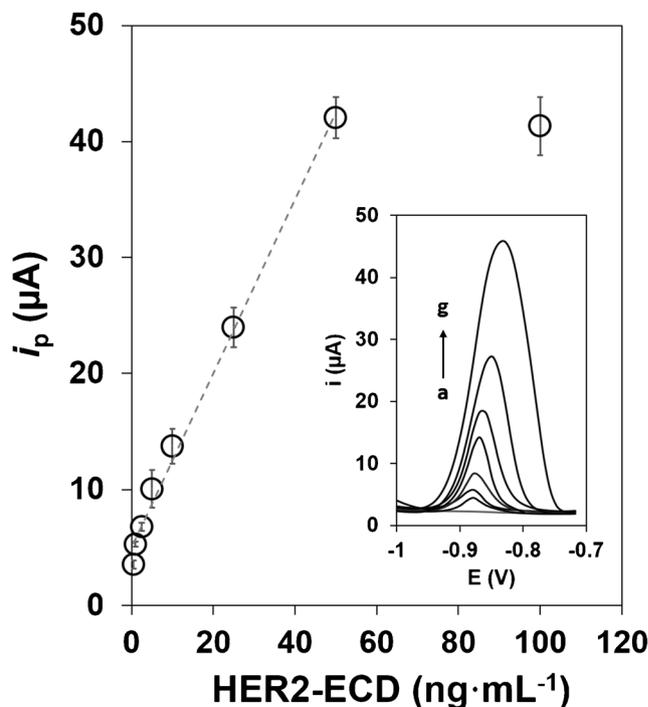
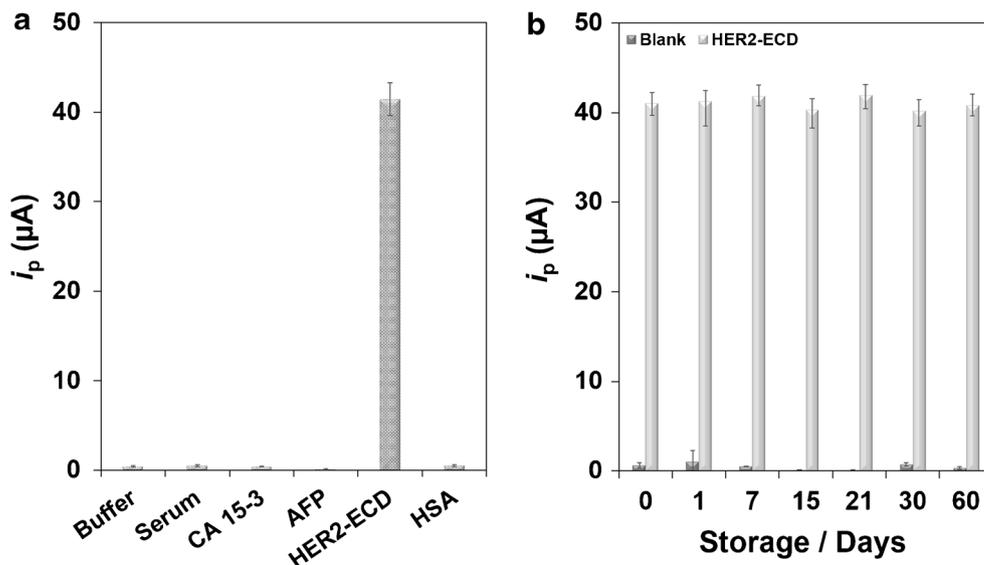


Fig. 2 Calibration plot for the analysis of HER2-ECD in human serum (i_p vs. HER2-ECD concentration) using the magnetic immunoassay. Inset: Differential pulse anodic stripping voltammograms of [HER2-ECD] (a–g); 0, 0.50, 1.0, 2.5, 5.0, 10, 25 and 50 ng·mL⁻¹. (Ab-C 25 µg·mL⁻¹; Ab-D 2.0 µg·mL⁻¹; QDs 5.0 nM. DPASV parameters: preconcentration +1.00 V for 60 s and –1.10 V for 300 s; potential scan –1.00 V to –0.70 V; pulse amplitude 0.05 V; step potential 0.01 V; modulation time 0.01 s; interval time 0.1 s)

Fig. 3 **a** Responses for HER2-ECD, buffer, serum and possible interferents (CA15-3 100 U·mL⁻¹; AFP 1 μg·mL⁻¹ and HSA 100 mg·mL⁻¹). **b** Storage stability of the modified MBs, [HER2-ECD] 0 and 50 ng·mL⁻¹



were performed. For the recovery tests non-diluted human serum was spiked with distinct HER2-ECD concentrations (1.0, 2.5, 10, 20 and 50 ng·mL⁻¹). Recoveries between 99.8 and 108.0% were obtained (Table 1), indicating that the assay provides accurate results and can successfully be applied to real samples. Experimental specifications of the ELISA are provided in the ESM (Section S.1.2). Spiked serum samples with HER2-ECD concentrations between 0.5 and 50 ng·mL⁻¹ were analysed. A 4-fold dilution was required for the ELISA to ensure that the HER2-ECD was within the linear range. The electrochemical assay did not require any dilution. Table 1 shows the obtained results. The relative deviations were below 10% for concentrations below the cut-off value, which confirms the accuracy of the results. Although the use of the ELISA kit allows the determination of very low concentrations of the biomarker with an elevated precision, a serial dilution is required

since direct analysis leads to values outside the range of concentrations in real cases. Furthermore, the ELISA has a very narrow linear range (Fig. S1) and much longer assay (4 h 45 min) and hands-on times (1 h 20 min) than the assay described in this work (1 h 30 min and 20 min, respectively). The results demonstrate that the performance of the immunomagnetic assay can be compared to that of the ELISA, and it provides an important diagnostic tool for cancer biomarker analysis. Because of the huge importance of screening and early diagnosis, the new analytical methodology can be adequate for patient follow-up.

In addition, the stability of the MBs was also studied. The biomodified MBs were stored at 4 °C in PBS-T, and the signal was monitored during several weeks, using 0 and 50 ng·mL⁻¹ HER2-ECD solutions. No significant differences in the analytical signals were observed for at least 60 days; the signal was 99.5% of the initial signal (Fig. 3b).

Table 1 Recovery values (%), relative standard deviations (RSD, %) and relative deviations (%) for the electrochemical and ELISA analysis of HER2-ECD in human serum: 1.0, 2.5, 10, 20 and 50 ng·mL⁻¹.

Technique	Added (ng·mL ⁻¹)	Found (ng·mL ⁻¹)	Recovery (%)	RSD (%)	Relative deviation (%)	Label	Platform	Assay time	Hands-on time
Electrochemistry	1.0	1.01	101.4	0.49	*	QDs	Single-use SPCE	1 h 30 min	20 min
	2.5	2.70	108.0	0.46	0.75				
	10	10.52	105.2	0.95	-8.36				
	20	20.0	99.8	0.67	-18.57				
	50	50.9	101.8	2.44	*				
ELISA	2.5	2.68	107.2	0.01		TMB	96-well plate	4 h 45 min	1 h 20 min
	10	11.48	114.8	0.01					
	20	24.5	122.5	0.02					

*Human HER2 ELISA Kit absorbance values for 1 ng·mL⁻¹ and 50 ng·mL⁻¹ concentrations are outside of the calibration range

Analysis of live breast cancer cells

The electrochemical magnetic immunoassay was also used to detect live breast cancer cell-lines: the HER2-positive SK-BR-3, the HER2-negative MDA-MB-231 and the low HER2-expression MCF-7 cell lines. The selectivity of the assay towards the SK-BR-3 cell line was assessed by comparing the signals obtained with the ones obtained for the MDA-MB-231 and MCF-7 cell lines. In Fig. 4a can be observed that SK-BR-3 provided a concentration-dependent signal that was $12.5\times$ higher than the signal obtained for HER2-negative cells MDA-MB-231 and $4\times$ higher than the signal obtained for the MCF-7 cells, confirming the selectivity of the assay for HER2-positive cells. So, the SK-BR-3 cells that overexpress HER2 can easily be captured by the Ab-C. This explains the higher analytical signal because of the higher amount of cells that were captured. The calibration straight for SK-BR-3 cells was established in the range between 1×10^2 – 5×10^3 cells·mL⁻¹ ($i_p = 0.15 \pm 0.02 \log[\text{SK-BR-3}] - 0.20 \pm 0.06$, $r = 0.983$) (Fig. 4b), obtaining a limit of detection of 2 cells·mL⁻¹. Additional figures of merit of the magnetic immunoassay for the analysis of SK-BR-3 are indicated in Table S2.

The magnetic immunoassay was compared with other electrochemical bioassays for the determination of HER-positive cancer cells [30–35] (Table 2). Although an electrochemical nanobiosensor for the detection of SK-BR-3 cell line in a 30-min test has been described in the literature [31], the time required for both the preparation of the transducer and for the modification with the biorecognition element is high (>

16 h), and the use of costly materials leads to an expensive analysis. Comparatively, the present work reports a simplified experimental procedure with a total time for both the sensing surface preparation and the assay of less than 3 h.

One of the limitations of the assay is the use of a heavy metal for detection purposes. Therefore, we minimized the used quantities: 70 μL of a 5.0-nM solution, so only 0.35 pmol of QDs are used in each assay. The generated waste can selectively be collected and sent to a certified entity operating in accordance with European standards regarding waste management. A way of replacing the present strategy by a non-polluting material is by using, for example, enzymes. However, enzymes can lose their bioactivity and the method includes an additional step (the addition of the enzymatic substrate). This leads to a longer assay time. The assay can be optimized further by using a detection antibody that is directly labelled with the QD. Like this one of the steps of the assay can be eliminated, reducing the total assay- and hands-on times.

Conclusion

Determination of HER2-ECD and cancer cells in biological samples is critical for an efficient diagnosis and follow-up of (eventually) HER2-positive breast cancer. With an assay time of 90 min, a sensitive immunomagnetic assay was developed using MBs and SPCE as transducer surface and core/shell CdSe@ZnS QDs as electroactive labels. The Cd²⁺ ions, released through acid dissolution of the QDs, were analysed by DPASV. Human serum samples were used to test the sensor's

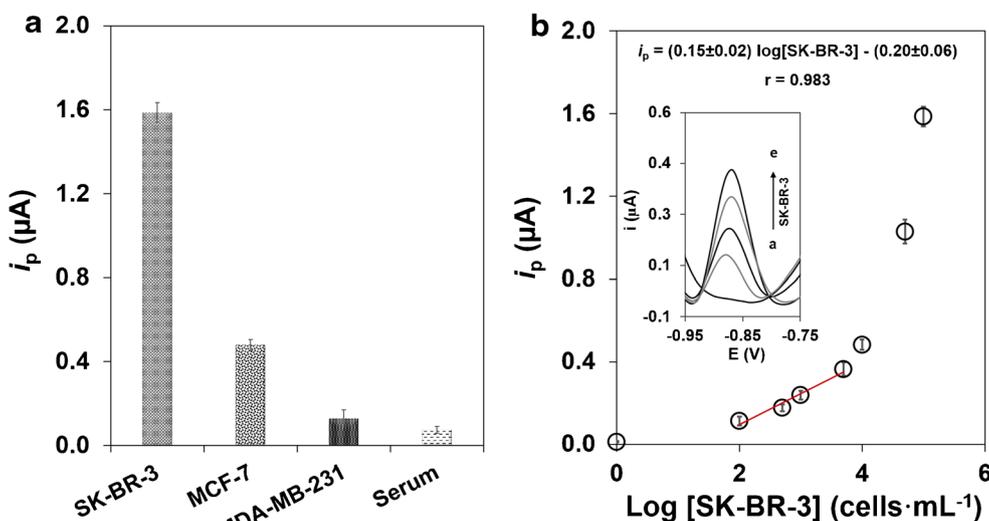


Fig. 4 Results obtained for cancer cell analysis. **a** Selectivity study of the magnetic immunoassay in the presence of 1×10^5 cells·mL⁻¹ for SK-BR-3, MDA-MB-231 and MCF-7 cells. **b** Results obtained for distinct concentrations of HER2-positive cancer cells SK-BR-3 in human serum. Inset: Differential pulse anodic stripping voltammograms of [SK-BR-3]

(a–e) $0, 1 \times 10^2, 5 \times 10^2, 1 \times 10^3$ and 5×10^3 cells·mL⁻¹. DPASV parameters: preconcentration +1.00 V for 60 s and –1.10 V for 300 s, potential scan –1.00 V to –0.70 V, pulse amplitude 0.05 V, step potential 0.01 V, modulation time 0.01 s, interval time 0.1 s

Table 2 Summary of experimental parameters of electrochemical bioassay-based procedures for HER2-expressing breast cancer cell lines

HER2 ⁺ Cell type	Sensing surface		Sample		Detection strategy		LOD (cells/ mL)	Selectivity	Ref			
	Transducer	Transducer preparation time*	Modification with the biorecognition element*	Long-term stability	Label	Technique				Detection	Assay time	
SK-BR-3	SPCE/MBs	15 min	1 h	60 days	Human serum	CdSe@ ZnS QDs	DPV	Cadmium	1 h 30 min	2	MDA-MB-231, MCF-7	This
SK-BR-3	SPCE/MBs	15 min	1 h	60 days	Human serum	AP	LSV	Silver	1 h 50 min	3	MDA-MB-231	[30]
SK-BR-3	FTO/NFG/AgNP/PANI	> 4 h	12 h	45 days	Whole blood	n.a.	DPV	[Fe(CN) ₆] ^{3-/4-}	30 min	2	ZR-75-1, MDA-MB-231, SW-742, MCF-10A	[31]
SK-BR-3	GCE/poly-DPB-(AuNP)	> 4 h	12 h	n.d.	Human serum	Hydrazin	SWSV	Silver	1 h 10 min	26	MCF10, MCF7, Hela	[32]
SK-BR-3	NEEs	n.a.	2 h	n.d.	Buffer	HRP	CV	MB	6 h	n.d.	MCF-7	[33]
BT-474	SPCE/E	n.a.	20 min	n.d.	Human serum	n.a.	DPV	[Fe(CN) ₆] ^{3-/4-}	2 h	n.d.	MDA-MB-231	[34]
**MCF-7	AuE/MnFePBA@Au	5 h	2 h	15 days	Human serum	n.a.	EIS	[Fe(CN) ₆] ^{3-/4-}	1 h	36	L929	[35]

*Overnight incubations were considered as a 12-h period for comparison purposes

**SK-BR-3 and BT-474 naturally overexpresses HER2; however, MCF-7 is considered a low expressor

AgNP – silver nanoparticles; AP – alkaline phosphatase; AuE – gold electrode; AuNP – gold nanoparticles; CV – cyclic voltammetry; DPB – 2,5-bis(2-thienyl)-1H-pyrrole-1-(p-benzoic acid); DPV – differential pulse voltammetry; EIS – electrochemical impedance spectroscopy; FTO – fluorine doped tin oxide electrode; GCE – glassy carbon electrode; HRP – horseradish peroxidase; LSV – linear sweep voltammetry; MB – methylene blue; MBS – magnetic beads; MnFePBA – MnFe Prussian blue analogue; n.a. – not applicable; n.d. – no data; NFG – nitrogen-doped graphene; NEEs – nanoelectrode ensembles; PANI – polyaniline; QDs – quantum dots; SPCE – screen-printed carbon electrode; SPCE-E – Extraavidin-modified screen-printed carbon electrodes; SWSV – square wave stripping voltammetry

applicability, and the selectivity was confirmed through the analysis of other cancer biomarkers and possible serum interferents. The immunomagnetic assay was further successfully applied to the determination of the HER2-positive SK-BR-3 breast cancer cell line and of HER2-negative (MDA-MB-231) and low-expression HER2 (MCF-7) breast cancer cell lines. The SK-BR-3 cell line provided a concentration-dependent signal that was more than 12.5× higher than the signal obtained for the other cells. The bioassay proved to be a fast, reliable and specific analytical tool for measuring tumour markers in cancer patients' serum.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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