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Ultra sensitive microfluidic immunosensor for determination of clenbuterol in bovine hair samples using electrodeposited gold nanoparticles and magnetic micro particles as bio-affinity platform

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ABSTRACT

In this article, we report the first integrated microfluidic immunosensor coupled to a screen-printed carbon electrode (SPCE) applied to determination of clenbuterol (CLB) in bovine hair samples. CLB is a member of the β_2-agonist drugs which is used in animal production and is banned in Argentine and the European Union. It represents a potential risk and has to be carefully monitored to avoid the illegal use of high amounts of this compound that could result in human food poisoning.

In order to perform the CLB detection, the SPCE was modified by gold nanoparticles (AuNPs) electrodeposition. Quantitative determination of CLB was carried out using a competitive indirect immunoassay, method based on the use of anti-CLB antibodies immobilized on magnetic micro particles. The CLB present in bovine hair samples competes immunologically with alkaline phosphatase (AP) enzyme-labeled CLB conjugate for the anti-CLB specific antibodies. Later, *p*-aminophenyl phosphate was converted to *p*-aminophenol by AP, and the electroactive product was quantified on AuNPs/SPCE at +0.1 V. The limit of detection for electrochemical method was 0.008 ng mL^{-1} and the intra- and inter-assay coefficients of variation were below 6%. This being a veterinary control tool very useful for rapid, sensitive and selective detection of CLB in an "in vitro" technique.

1. Introduction

β-adrenergic agonists have been used in meat production for their properties of enhancing growth rates of treated animal, as repartition agent in muscle. Clenbuterol (CLB) is a well known β_2-adrenergic agonist and its use in animal production is banned in Argentina (meat producing and exporting country), as in most countries including in the European Union (meat importing countries) (Kuiper et al., 1998; Mitchell and Dunnavan, 1998). However, CLB is licensed as veterinary medicine for the treatment of respiratory disease in horses, cattle and dairy cows as well as for asthmatic disease in human medicine (Zhu et al., 2011). CLB has been used illegally in animal production and accumulation of their residues in animal tissue can cause symptoms of acute poisoning in humans. Several cases of human food poisoning causing tachycardia, distal tremors, nausea, diarrhea, fever, myalgias, asthenia and hypertension have been reported (Brambilla et al., 1997; Chan, 1999). This β-agonist represents a potential risk and has to be carefully monitored to avoid the illegal use of high amounts as a growth-promoting agent in meat production. Moreover, CLB residues may adversely affect the export trade of edible products of animal origin and cause economic losses (Mersmann, 1998; Smith, 1998). Since then, it is being extensively controlled as a veterinary drug residue in food safety (Gallo et al., 2007).

Detection limits required for residue analysis of CLB in bovine hair must be within the range of ng per hair gram. Hair sampling is especially easy to perform in farm or in slaughterhouse. The great advantage of hair analysis is that the high affinity of clenbuterol for melanin prevents metabolic clearance.

In the literature several analytical methods for CLB determination have been described based on high-performance liquid chromatography (HPLC) (Liu et al., 2011) or capillary electrophoresis with electrochemical detection (Lin et al.,1997; Chen et al., 2005), liquid chromatography–mass spectrometry (LC–MS) or gas chromatography–mass spectrometry (GC–MS) (Ramos et al., 2003; Blanca et al., 2005; He et al., 2007; Chen et al., 2011), surface-enhanced Raman scattering (SERS) (Izquierdo-Lorenzo et al., 2010) and highly sensitive immunoassays enzyme-linked immunosorbent assay (ELISA) (Shelver and Smith, 2004; Ren et al., 2009). Although, these methods have high selectivity and sensitivity, they require expensive instruments, long pretreatment of samples and

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complicated testing processes. Thus, it is crucial to develop a simple, fast and portable method for CLB residues detection of illegal use.

Several electrochemical immunosensors were recently reported for CLB (Johansson and Hellenäs, 2004; He et al., 2009; Gao et al. 2011; Liua et al., 2011). Further publications have described immunobiosensor methods for screening, with immobilized antigen or antibody (Chen and Li, 2007; Traynor et al., 2003). Recently, microfluidic biosensors have attracted growing interest as quantitative method (Panini et al., 2008, Martínez et al., 2010; Fernández-Baldo et al., 2011; Pereira et al., 2011), not only because it could have high sensitivity and specificity of classical immunoassay such as ELISA that is carried out in laboratories, but also because it has a low cost and is potentially portable. Properties described for microfluidic systems, associated with screen-printed electrodes as electrochemical detection system are in accordance with the requirements of on-site screening devices, since all the equipment necessary for the electrochemical analysis is portable (Bagni et al., 2006). The main advantages of these electrodes include simplicity, versatility, modest cost, portability, reliability, small size and large scale production capability (Metters et al., 2011). Furthermore, this kind of electrode offers the possibility to perform the electrodeposition of metal nanoparticles onto its surface, as a strategy for enhancing the electrode conductivity, facilitating the electron transfer and improving the analytical sensitivity and selectivity of the immunosensor (Chikae et al., 2006).

For the electrochemistry, gold nanoparticles have great relevance, due to their good biocompatibility, excellent conducting capability and high surface/volume ratio. The introduction of gold nanoparticles into electrochemical interfaces has infused new vigor in electrochemistry (Welch and Compton, 2006; Pingarrón et al., 2008; Moreno et al., 2011).

In this work, we report the development of the first microfluidic immunosensor performed on a screen-printed carbon electrode (SPCE), which was modified by electrodeposition of gold nanoparticles (AuNPs), and characterized by scanning electron microscopy (SEM), X-ray diffraction (XRD) and cyclic voltammetry (CV). This electrochemical detection system was applied to the quantification of low concentrations of CLB present in bovine hair samples. The great advantage of hair analysis is that the high affinity of clenbuterol for melanin prevents metabolic clearance, and provides an "in vitro" piece of evidence prior to sacrifice that facilitates the action of veterinary inspectors.

2. Experimental

2.1. Reagents and solutions

All reagents used were of analytical reagent grade. CLB and $HAuCl_4$ 0.01% were purchased from Sigma Aldrich (St. Louis, MO, USA). Glutaraldehyde (GLU) (25% aqueous solution) was purchased from Merck (Darmstadt, Germany). Magnetic micro particles, amino functionalized (MMPAF) were purchased by Fluka (Buchs, Schweiz). 4-Nitrophenyl phosphatedisodium salt hexahydrate (*p*-NPP) was purchased from Fluka Chemie (Steinheim, Switzerland). Rabbit anti-clenbuterol antibody (anti-CLB Ab) and CLB-AP conjugate were supplied by Viviana G. Spotorno (Supplementary S1) obtained from National Institute of Agricultural Technology (INTA, Bs As, Argentina). All other reagents and solvents employed were of analytical grade and they were used without further purifications. All solutions were prepared with ultra-high-quality water obtained from a Barnstead Easy pure RF compact ultra-pure water system.

2.2. Instruments

Amperometric measurements were performed using the BAS LC-4C potentiostat, and the BAS 100 B/W (electrochemical analyzer Bioanalytical System, West Lafayette, IN) was used for voltammetric analysis. A syringe pump system (Baby Bee Syringe Pump, Bioanalytical Systems) was used for pumping, sample introduction, and stopping the flow. Absorbance was detected by a Bio-Rad Benchmark microplate reader (Japan) and Beckman DU 520 general UV/vis spectrophotometer. All pH measurements were made with an Orion expandable ion analyzer (Orion Research Inc., Cambridge, MA, USA) Model EA 940 equipped with a glass combination electrode (Orion Research Inc.). The structures and compositions of the nanoparticles were characterized by X-ray diffraction (XRD) using a Rigaku D-MAX IIIC diffractometer using copper radiation (k_a=0.154178 nm) and containing a nickel filter. The morphologies of the electro-synthesized nanoparticles were studied by a LEO 1450VP scanning electron microscope (SEM).

2.3. Detection unit

The main body of the sensor was made of polymethylmethacrylate (PMMA). Fig. S1 illustrates the design of the flow-through central channel (CC) of the microfluidic immunosensor. The diameter of the CC was 100 μm and the diameter of accessory channels was 80 μm. The SPCE placed at the end of the CC is made up of a graphite circular working electrode, a graphite counter electrode and an Ag pseudo-reference electrode. The present multilayer device was fabricated using commercially available 3.5 mm thick PMMA sheets. PMMA sheets were cut into plates measuring 20.0 × 45.0 mm (width × length) to form microchip substrates. The microfluidic pattern was designed and then it was sent to the laser scriber for direct machining on the PMMA substrate. Once the microfluidic channels were formed, the device was assembled by overlapping of units, which were kept in a fixed position by the action of adjusting screws at the ends of the units. All solutions and reagents were conditioned to 37 °C before the experiment, using a laboratory water bath (Vicking Mason II, Vicking SRL, Argentina).

2.4. Animals

Three Holando–Argentino steers aged of 2.5 to 3 years old and of approximately 450 kg were used in the experiment. Their phenotype is of white hair with wide regions of black hair. Two of them were treated by intravenous injection of 10 mL of a veterinary formulation containing 0.03 mg mL^{-1} of CLB according to protocol instructions (0.3 mg per animal, veterinary dose). After 38 day a second dose of 65 mL was administrated to the same animals (1.95 mg per animal, 20 times lower than an anabolic dose). The animals were grass fed and had free access to water. Every week, the hair samples were taken from the same region of the animal's back. All samples were kept at 4 °C till analysis. This experiment was performed in compliance with the relevant laws and institutional guidelines approved by the authorities of National Institute of Agricultural Technology (INTA), Argentina.

2.5. Sample preparation

Bovine hair samples were washed with SDS 1% and rinsed with water. After drying 100 mg of sample was heated with 2.5 mL of NaOH 5 M for 10 min at 95 °C. When the samples reached room temperature, 3 mL of *t*-butylmethylether were added. The mixture was stirred by vortex, incubated for 15 min in ultrasonic bath and mixed by rotation for another 15 min at room temperature. The last two steps were repeated and the organic phase was collected and evaporated to dryness (Haasnoot et al., 1998). The residue was dissolved in 0.1 mL of methanol and diluted to 1 mL with 0.01 M PBS pH 7.2. (Supplementary S2)

2.6. Immobilization of anti-CLB Ab on MMPAF

The CLB capture procedure was performed employing as bio-affinity support the MMPAF, which was obtained by the immobilization of rabbit anti-CLB Ab on MMPAF in an Eppendorf tube. 100 μL of MMPAF were washed with 1 mL of 0.01 M PBS buffer pH 7.2 three times. The pellet was suspended in 1 mL of an aqueous solution of 5% (w/w) GLU at pH 10 (0.20 M sodium carbonate buffer, pH 10) with continuous mixing for 2 h at room temperature. Then, the MMPAF were washed three times with 0.01 M PBS pH 7.2 to remove the excess of GLU. 1 mL of antibody preparation (dilution 1:100 in 0.01 M PBS pH 7.2) was coupled to the residual aldehyde groups with continuous mixing for 12 h at 4 °C. The anti-CLB Ab-MMPAF preparation was finally washed with 0.01 M PBS pH 7.2 and resuspended in 1 mL of the same buffer at 5 °C. The immobilized antibody preparation was perfectly stable for at least 1 month. In the steps previously described, the MMPAF were manipulated using an external magnet.

2.7. Preparation and modification of SPCE

An electrode pretreatment was carried out before the electrodeposition procedure (Supplementary S3). For the electrodeposition procedure of AuNPs, the SPCE was immersed into 0.01% $HAuCl_4$ solution containing 0.10 M KNO_3 (prepared in doubly distilled water, and deaerated by bubbling with nitrogen) as supporting electrolyte. After that a constant potential value of −0.2 V vs. Ag was applied for 60 s. (Mena et al., 2005; Ding et al., 2009). Then, the modified electrode (AuNPs/SPCE) was rinsed by mechanically stirring at 250 rpm for 30 s with doubly distilled water and dried carefully with pure nitrogen gas. The AuNPs/SPCE was characterized by SEM, XRD and CV.

2.8. Amperometric analysis of CLB in bovine hair samples

This method was applied for the determination of CLB in bovine hair samples from three Holando–Argentino steers. Prior to analysis of each sample, the anti-CLB Ab-MMPAF were conditioned with 0.01 M PBS pH 7.2, for 3 min. All solutions employed were injected using syringe pumps at a flow rate of 5 μL min^{-1}.

The microfluidic device was prepared by injection of anti–CLB Ab–MMPAF in the flow system for 4 min. A permanent magnet was used to attract the beads at specific area of the channel, near AuNPs/SPCE. The magnet was not moved during the experiment to keep the beads into the channel and, they were not carried away by the continuing flow. After the conditioning step, unspecific bindings were blocked by 5 min treatment at room temperature with 1% albumin in a 0.01 M PBS pH 7.2 and washed with 0.01 M PBS buffer pH 7.2 for 3 min. In a second step, the samples appropriately diluted with 0.01 M PBS pH 7.2, competed with CLB-AP conjugate (diluted 1:100 in 0.01 M PBS pH 7.2), which were injected into the PBS carrier stream for 5 min. The immunosensor was then washed free of any traces of unbound enzyme conjugate for 3 min, and finally the DEA buffer (0.1 M diethanolamine, 0.05 M KCl, 0.001 M $MgCl_2$, pH 9.6) was used to prepare the *p*-APP solution (the synthesis of *p*-APP is shown in Supplementary S4). The substrate solution (2.7×10^{-3} M *p*-APP in DEA buffer, pH 9.6) was injected into the carrier stream for 1 min, and the enzymatic product (*p*-AP) was measured on the surface of AuNPs/SPCE at +0.1 V, and the resulting anodic current was displayed on the computer monitor (Scheme 1). The electrochemical measurement procedure described above was performed on: positive controls, negative control and blank in DEA buffer pH 9.6.

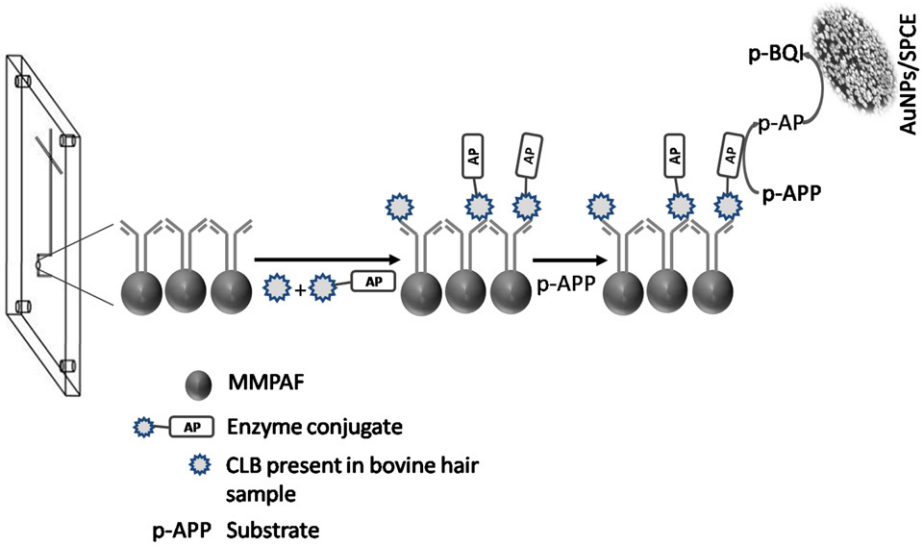
For the next analysis, the immunosensor was conditioned with desorption buffer (0.1 M glycine-HCl, pH 2) for 5 min, and then washed with 0.01 M PBS pH 7.2.With this treatment the CLB bound to the immobilized antibodies were desorbed, allowing starting with a next determination. Table S1 shows the sequence required for total analysis.

A standard curve for the amperometric analysis was produced by following our protocol with a series of standards supplied for the enzyme immunoassay test developed by Spotorno (Spotorno and Tezón, 2011), which covered the relevant range (0.01–1000 ng mL^{-1}). When not in use, the microfluidic immunosensor was stored in 0.01 M PBS pH 7.2 at 4 °C. The stock solution of *p*-APP was prepared freshly before the experiment and stored under the exclusion of light as long as the experiment lasted.

3. Results and discussion

3.1. Study of electrodeposition time and potential of AuNPs

The process of gold nanoparticles electrodeposition on the surface electrode is strongly affected by several parameters, such as the t_{edep} and E_{edep}. Both factors have been optimized to obtain



Scheme 1. Principle of immune reaction.

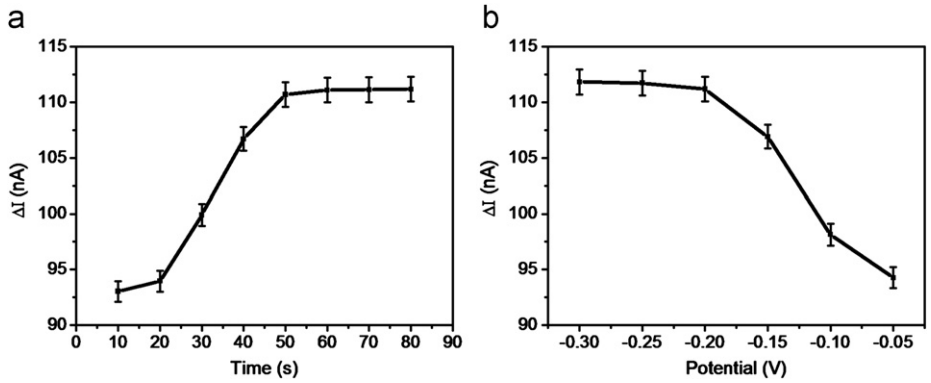


Fig. 1. (a) Study of electrodeposition time employing a standard of 6.40 ng mL^{-1} from 10 to 80 s. (b) Study of electrodeposition potential from −0.05 to −0.3 V.

the best analytical performance in our device. For the optimization of the t_{edep} the potential used was −0.2 V and the t_{edep} was evaluated in a range of 10–80 s. As Fig. 1(a) shows, the current increased by increasing the electrodeposition time up to a value of 50 s, and then the current remained constant between 60 and 80 s. Therefore, a t_{edep} of 60 s was selected as optimum. Regarding the E_{edep}, the time used was 60 s and the working electrode potential was varied between −0.05 and −0.3 V. As shown in Fig. 1(b), the current increased slowly by increasing the potential up to a value of −0.1 V, then increased rapidly between −0.1 and −0.175 V and remained constant between −0.175 and −0.3 V. Therefore, an electrodeposition potential of −0.2 V was selected as optimum. In this way, we can conclude that the values used were 60 s and −0.2 V for t_{edep} and E_{edep}, respectively, for all routine.

3.2. Characterization of AuNPs/SPCE surface

Fig. 2 shows the SEM images of unmodified SPCE 2a, and modified with electrodeposited AuNPs on the SPCE surface 2b. The diameters of these AuNPs ranged from 20 to 50 nm. The crystalline structure of the AuNPs was characterized by XRD measurement. The XRD pattern of AuNPs electrodeposition is shown in Fig. 2(c). The peak at 2θ (26.3) was from graphite and the peaks at 2θ (42.6, 54.3 and 68.9°) resulted from the AuNPs. The average size of the crystalline structure of the deposited AuNPs was calculated to approximately 30 nm according to the Scherrer formula, which states that, $t = K \times \lambda / B \times \cos\theta$. Fig. 2(d) shows the electrochemical characterization of AuNPs/SPCE. The cyclic voltammograms (CVs) of the *p*-AP system is a convenient and valuable tool to monitor the characteristics of the modified surface. The CVs of unmodified SPCE (curve *a*) and modified with electrodeposited gold nanoparticles (curve *b*) which were recorded in an aqueous solution of DEA buffer pH 9.6 and 1.0×10^{-3} M of p-AP at 100 mV s^{-1}. The potential sweep was performed from −0.5 to 0.2 V vs. Ag/AgCl. CVs well defined and characteristics of a diffusion-controlled redox process were observed at the SPCE surface. The average value of surface area for AuNPs/SPCE was 11.37 ($\pm$0.15) $\times 10^{-2}$ cm^2 according to the Randles–Sevick equation, that is $I_p = 2.69 \times 10^5 \times n^{3/2} \times AD^{1/2} \times Cv^{1/2}$. The electrodes employed in this characterization step were optimized as shown previously in Section 3.1.

3.3. Optimal conditions for immune reactions and determination of enzymatic products

The miniaturization of immunoassay systems using microfluidic devices provides several advantages over conventional techniques, such as ease of handling and high reaction efficiency (Sato et al., 2002; Lim and Zhang, 2007). Nevertheless, many factors that affect the biochemical reaction must be considered because the reaction conditions in the microfluidic system are different from those of conventional microtubes or well plate.

One of the most important parameters to consider in the optimization procedure of the microfluidic device is the flow rate. The flow rates of the sample and reagents have effect on the reaction efficiencies of the antigen–antibody complex, because unlike conventional immunoassays, samples and reagents in our system are continuously flowing through the system. The optimal flow rate was determined by analyzing a standard of 6.40 ng mL^{-1} CLB at different flow rates and evaluating the current generated during the immune reaction. As shown in Fig. 3(a), flow rates from 1 to 5 μL min^{-1} had little effect on the antigen–antibody reaction. Conversely, when the flow rate exceeded 5 μL min^{-1}, the signal was dramatically reduced. Taking into account the magnitude of the current response and analysis time for each sample, 5 μL min^{-1} was chosen for samples, reagents and washing buffer injection. Another relevant parameter is the sample size, which was evaluated in the range 2.5–30 μL (Fig. 3b). Sensitivity is almost quadruplicated in the range between 5 and 25 μL. Insignificant differences were obtained for greater sample size. A sample size of 25 μL was used, for all routine. Furthermore, we performed studies on pH range, substrate and enzyme conjugate concentration, among others (Supplementary S5 and S6).

3.4. Analytical parameters of the microfluidic immunosensor

Linearity and range of the developed method were studied by analyzing different concentrations (n=6) of the standard solution containing 0.01–1000 ng mL^{-1} of CLB on the matrix. The calibration curve was obtained by plotting ΔI (nA) versus CLB concentrations (ng mL^{-1}). A linear relation was observed between the concentration range 0.027–800 ng mL^{-1}.The data were analyzed by linear regression least-squares fit method. The calibration graph was described by the calibration equation ΔI (nA)= 143.01–35.29 log C_{CLB} with a correlation coefficient for this plot of 0.998, where ΔI is the difference between current of the blank and sample. The standard deviation (SD) for the calibration curve was 3.33. Quantifications of CLB for performing the calibration curve were directly carried out on the samples, due to a matrix effect study carried out showing that it has no influence on the quantification of CLB. The coefficient of variation (CV) for the determination of 6.40 ng mL^{-1} CLB was below 5.32% (n=6). These values demonstrate that our microfluidic immunosensor can be used to quantify the amount of CLB in unknown samples. The limit of detection (LOD) was considered as the concentration that gives a signal three times the standard deviation of the blank. The limit of quantification (LOQ) was considered as the

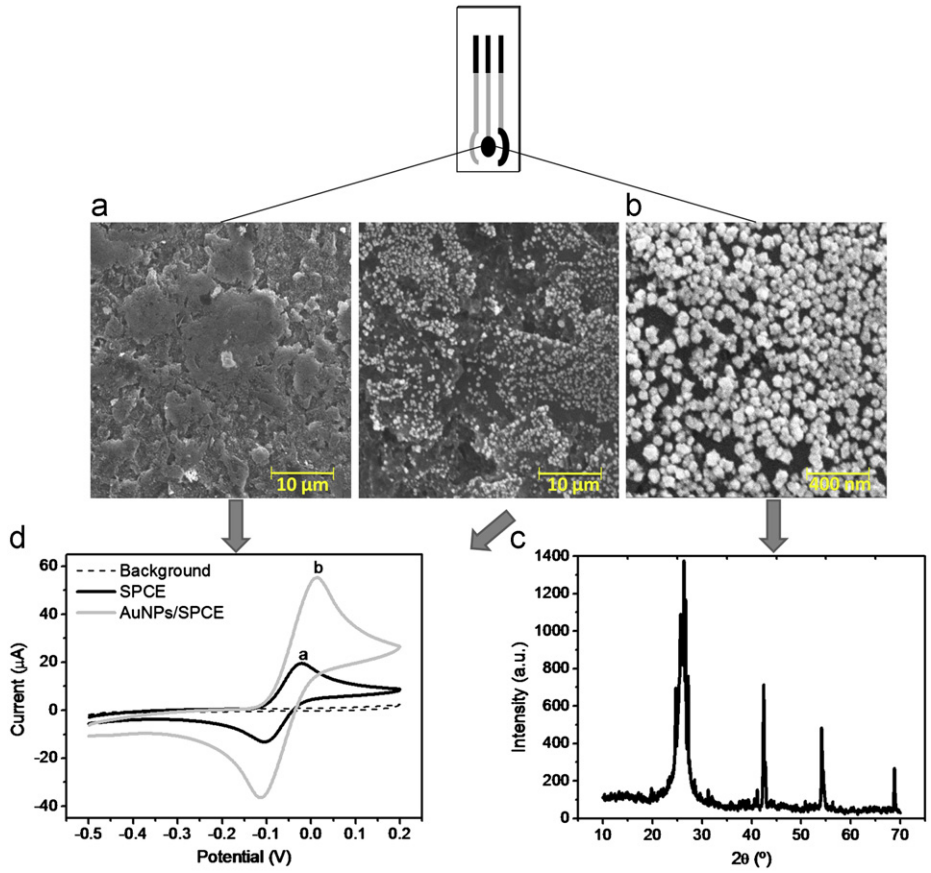


Fig. 2. (a) SEM images of unmodified SPCE. (b) Modified with electrodeposited AuNPs on the SPCE surface. The diameters of these AuNPs ranged from 20 to 50 nm. (c) XRD pattern of AuNPs/SPCE. The average size of the crystalline structure of the electrodeposited AuNPs was calculated to approximately 30 nm. (d) Cyclic voltammograms of unmodified SPCE (curve *a*), modified with electrodeposited gold nanoparticles (curve *b*), and background in an aqueous solution of DEA buffer pH 9.6 and 1.0×10^{-3} M of *p*-AP ($\nu = 100$ mV s^{-1}, $T = 25$ °C).

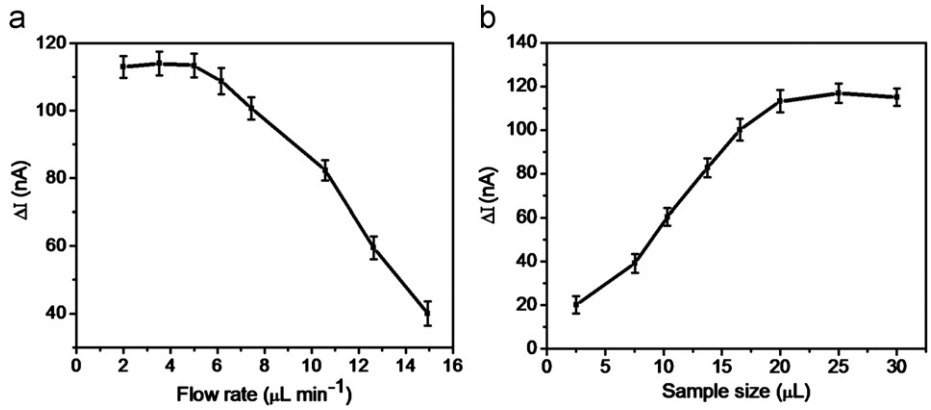


Fig. 3. (a) Study of flow rate employing a standard of 6.40 ng mL^{-1} at different flow rates from 1 to 15 μL min^{-1}. (b) Study of sample size from 2.5 to 30 μL.

concentration that gives a signal ten times the standard deviation of the blank, determined according to the IUPAC recommendations (Currie, 1995). For the electrochemical detection procedure, the LOD and LOQ were 0.008 and 0.027 ng mL^{-1}, respectively.

The precision of the electrochemical assay was checked with control CLB solutions at concentrations of 0.25, 6.40 and 160 ng mL^{-1}. The within-assay precision was tested with five measurements in the same run for each control. These series of analyses were repeated for three consecutive days in order to estimate the between-assay precision. The assay showed good precision; the CV% within-assay values were below 4% and the

Table 1
Intra- and inter-day precision and accuracy. Within-assay precision (five measurements in the same run for each control sample) and between-assay precision (five measurements for each control sample, repeated for three consecutive days).

Control (ng mL^{-1} CLB)	Within-assay		Between-assay	
	Mean	CV	Mean	CV
0.25	0.27	3.71	0.23	5.49
6.40	6.37	2.39	6.74	4.96
160	160.59	2.05	161.72	4.58

Table 2
Determination of CLB in bovine hair samples after a second dose administrated to the same animals.

Sample[a]	Day 38		Day 56		Day 65	
	MI[b]	EIA[c]	MI	EIA	MI	EIA
BH 1[d]	2.4 ± 0.11[e] CV[f] 4.58	2.5 ± 0.14 CV 5.60	2.9 ± 0.15 CV 5.17	3.0 ± 0.21 CV 5.67	16.8 ± 0.33 CV 1.96	16.4 ± 0.42 CV 2.56
BH 2	1.1 ± 0.06 CV 5.45	0.9 ± 0.05 CV 5.55	19.2 ± 0.25 CV 1.30	19.0 ± 0.31 CV 1.61	2.8 ± 0.16 CV 5.71	2.2 ± 0.10 CV 4.45
BH C	–	–	–	–	–	–

[a] ng mL^{-1}.
[b] Microfluidic immunosensor.
[c] Enzyme immunoassay.
[d] Bovine hair 1, 2 and control.
[e] Mean of five determinations ± S.D.
[f] Coefficient of variation.

between-assay values were below 6% (Table 1). Regarding the total assay time for the determination of the CLB concentration, for the proposed method, the assay time was 18 min, much less than the 90 min normally used with conventional batch well ELISA.

The electrochemical method was compared with a spectrophotometric immunoassay for the quantification of CLB in bovine hair samples. The slope obtained was reasonably close to unit, indicating a good correspondence between the two methods. Compared with the spectrophotometric immunoassay, our method showed an enhancement in the LOD, which is low enough to determine CLB in unknown samples and at very low levels. The *F*-test value for the biosensor was 0.39 (the *F*-test value is 2.26 at a 95% confidence level), suggesting that the method has a linear behavior.

In order to evaluate the analytical applicability of the proposed method it was applied to quantification of CLB in nine bovine hair samples and under the conditions previously described. These samples were previously confirmed by enzyme immunoassay. The positive samples were later analyzed by our proposed quantitative method, which revealed different concentrations of CLB in all of them. Samples of blank animal were also negative for both methods (Table 2).

In bibliography, there are published several immunosensors for determination of CLB in real samples (He et al., 2009; Liua et al., 2011; Gao et al., 2011), but our new immunosensor has significant advantages over the previously cited. One of these is that the new method is based on microfluidic technology coupled to SPCE with electrodeposited gold nanoparticles as the detection system. In addition, the achieved limit of detection is lower than that obtained by the immunosensors recently reported. Regarding to the use of bovine hair sample, the great advantage of hair analysis is that the high affinity of clenbuterol for melanin prevents metabolic clearance. Finally, our device offers the possibility of obtaining miniaturized, integrated and portable systems for on-site analysis.

4. Conclusions

In this work we have developed the first microfluidic immunosensor coupled to electrochemical detection for the quantification of CLB in bovine hair samples. The great advantage of hair analysis is that the high affinity of CLB for melanin prevents metabolic clearance. Our proposed system is coupled to SPCE with electrodeposited gold nanoparticles; this modification procedure allowed us to obtain an important increment in the sensitivity of our system. The use of magnetic micro particles as bio-affinity platform for the immobilization of antibodies not only enhances the amount of immobilized antibodies on the particle surface, but also preserves the activity of the immobilized biomolecules. This device provides a simple design with a high sensitivity and selectivity for the detection of CLB residues in low times and, represents a significant tool for an automated and portable determination for its possible application in food safety.

Acknowledgments

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bios.2012.08.020.

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