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Electrochemical biotin determination based on a screen printed carbon electrode array and magnetic beads

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ABSTRACT

A biotin assay using magnetic beads (1 μm) and a re-usable 8-chanel screen printed electrochemical array is here reported. The reaction scheme is based on a one step competitive assay between biotin and biotin labeled with horseradish peroxidase (B-HRP). The mixture of magnetic beads modified with streptavidin (Strep-MB), biotin and B-HRP is left 15 min under stirring and then a washing step is performed. After that, 20 μL of the mixture is dropped twice on the electrodes and left 2 min. Then, the drop is removed and 20 μL of 3,3′,5,5′-tetramethylbenzidine (TMB) is deposited on the electrode surface. After 20 min a potential of −0.2 V is applied during 60 s, and 8 analytical signals are obtained. After washing with 0.1 M phosphate buffer the screen printed carbon electrode array can be use again. The linear range obtained is between 0.1 and 250 nM of biotin and have a sensitivity of 10^{-2} μA nM^{-1}.

1. Introduction

One of the main objectives for an analytical chemist is the development of methods which allows the rapid, selective and sensitive determination of the target analyte. Among the various possible combinations of techniques, the one involving electrochemical detection has been prominently used in the analysis of different substances. Electrochemical techniques provide important advantages such as low detection limit, relative simplicity, low cost of equipment, automatic on-line and portable options. These advantages have been increased with the advent of the screen printed technology. In this perspective the use of screen printed electrode (SPEs) which are characterized by low cost fabrication, versatility in it design [1–3] and the possibility of mass production seem much better suited [4,5]. Screen printing technology involves the printing of various inks on a planar ceramic or plastic support [6–8]. A wide range of inks may be used to mass produce low cost electrodes. Carbon ink formulations are commonly used for printing working electrodes and its attractive properties offer the advantage of a lower residual current leading to higher signal/noise responses and correspondingly lower detection limit [9,10]. Moreover the planar structure of the SPE allows the easy modification of the surface and the miniaturized dimensions reduces drastically the consumption of the different reagent (only few microliters). It has to be highlighted that the SPEs are not necessary used as an immobilization support of the recognition element. The immobilization of the recognition element directly on the surface electrode may affect the properties of the sensing surface itself or worst poison the electrode surface through non-specific adsorptions. To overcome this drawback, the recognition element could be immobilized on a separate support (magnetic beads) and only the end of the immunological chain is placed onto the surface of an unmodified electrode for the measurement of the enzymatic product [11,12]. In these cases the electrode only acts as a measuring device. Magnetic beads (MBs) are particles constituted from a dispersion of magnetic material (Fe_2O_3 and Fe_3O_4) and then covered with a thin shell of polymer. MBs can be easily functionalized with different linkage group such as streptavidin [13–15], tosyl groups [16–19], amino groups [20–22], antibodies or proteins [23–26]. The only problem represented by the use of the MBs is that the recognition element is not in direct contact with the surface electrode. To avoid this and to achieve greater sensitivity, it has been proposed the use of magnetized electrode surface through either the inclusion in the electrode material of magnetic particles [27] or through the placement of small magnets below the surface of the working electrode [28,29]. This allows concentrating the MBs on the electrode surface for the final measurement. In the case of the electrochemical assay it is essential that the enzymatic product is electroactive so that it can be easily measured through voltammetric or amperometric technique. To this end several efforts have been devoted to find optimal enzymatic substrates which could achieve the required sensitivity [30,31].

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HRP enzyme label offers more options in terms of electrochemical methods and substrates. However the great majority of examples reported in the literature [32–36] are based on the use of a mixture of H_2O_2 and 3,3′,5,5′-tetramethyl benzidin (TMB) in a way similar to that used for optical ELISA [37]. The produced oxidized TMB is usually measured through amperometric reduction at a fixed potential in a range between −0.2 and +0.1 V depending on the type of electrode used, the reference electrode and the specific conditions of the assay (pH, buffer used). Different articles have been published using both technology but it is always reported that the different screen printed electrode a single use and the electrodes are qualified as disposable systems [38,39].

Coupling the versatility of the design offered by the screen printed technology and the benefit of the MBs, here it is described an assay for biotin determination using an 8 channel screen printed array electrode and its magnetic support. Moreover, the method described here allows the re use of the electrode after a very easy washing step which is not common in the literature using this kind of technology.

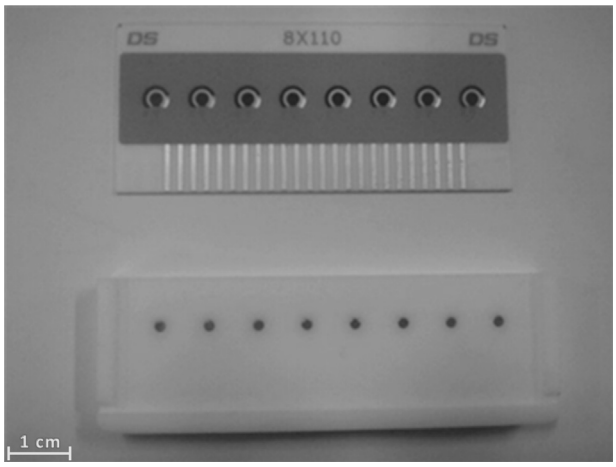

Fig. 1. Image of the screen printed carbon electrode array and it magnetic support.

2. Experimental

2.1. Chemicals

Sodium hydroxide (ref. 1.064.1000) was delivered by MERCK (Spain), magnetic beads of 1 μm diameter modified with streptavidin (Dynabeads® MyOne™ Streptavidin C1) (Strep-MB) (ref. 650.01) were purchased by INVITROGEN, Biotinylated Horseradish Peroxidase (HRP-B) (ref. 29139) was supplied by THERMO SCIENTIFIC and 3,3′,5,5′-tetramethylbenzidine (TMB) (ref. T0440), and Biotin (ref. B4501) was purchased by SIGMA. Phosphate buffer 0.1 M solution of pH 7.2. All chemicals were of analytical reagent grade, and the Milli-Q water used was obtained from a Millipore Direct-Q™ 5 purification system. Stock solution of 5.10^{-6} M of B-HRP, 5.10^{-3} M of Biotin and 7.10^{8} magnetic beads per mL were daily prepared in 0.1 M phosphate buffer and stored at 4 °C in a refrigerator.

2.2. Apparatus and electrodes

Chronoamperometric measurements were performed using an ECO Chemie μAutolab type II potentiostat interfaced with a Pentium 166 computer system and controlled by the Autolab GPES software version 4.8 for Windows 98. All measurements were carried out at room temperature. Screen-Printed Carbon Electrodes (ref DRP-110), an edge connector (ref. DRP-DSC), 8X Screen-printed Carbon electrodes (ref. DRP-8X110), a cable connector for the screen printed carbon electrode array (ref. DRP-CAST8X), a magnetic support for screen printed electrodes (ref. DRP-MAGNET) and for screen printed carbon electrode array (ref. DRP-MAGNET8X) were purchased from DropSens, S.L (Oviedo, Spain). The Screen-printed carbon electrode array is formed by eight 3-electrode electrochemical cells with carbon based working electrode (2.56 mm diameter), carbon auxiliary and silver pseudo reference electrode on an alumina substrate. An insulating layer serves to delimit the electrochemical cell and electric contacts. In Fig. 1 it can be seen the screen printed carbon electrode array with the magnetic support. The single Screen-printed carbon electrode consists in a larger carbon working electrode (4 mm), the carbon auxiliary and the silver pseudo reference electrodes are also printed on an alumina substrate.

2.3. Assay scheme

The reaction scheme is based on a one step competitive assay between free biotin and biotin labeled with horseradish peroxidase (B-HRP). Biotin was incubated with Strep-MB and B-HRP during 15 min. After the incubation stage, magnetic separation and the washing were performed. It consists in a re-suspension of the beads in a 0.1 M phosphate buffer solution pH 7.2, and then a separation with the magnet to remove the supernatant. This operation is realized 3 times (Fig. 2).

2.4. Electrochemical measurement

First the screen printed carbon electrode array is positioned on the magnetic support, then an aliquot of 20 μL of the solution prepared as explained on Section 2.3, is deposited on the electrode surface, and after 2 min, the drop is carefully removed with a micropipette and so, the magnetic beads are immobilized on the working electrode. This step is repeated two times. Then, 20 μL of TMB is added and after 20 min, a potential of −0.20 V is applied during 60 s. A typical ampereogram is reported in Fig. 3. So, the TMB is enzymatically oxidized and then applying −0.2 V is electrochemically reduced. The reduction current obtained is inversely related to biotin concentration. Finally the electrode is removed of the magnetic support and washed with the 0.1 M phosphate buffer pH 7.2 and can be used again.

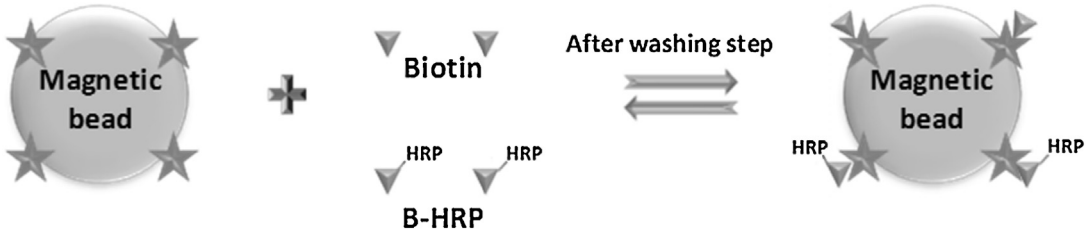

Fig. 2. Scheme of the competitive assay.