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Electrochemical biotin detection based on magnetic beads and a new magnetic flow cell for screen printed electrode

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ABSTRACT

The use of the first flow-cell for magnetic assays with an integrated magnet is reported here. The flow injection analysis system (FIA) is used for biotin determination. The reaction scheme is based on a one step competitive assay between free 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, 100 μL of the mixture is injected and after 30 s 100 μL of 3,3′,5,5′-Tetramethylbenzidine (TMB) is injected and the FIAgram is recorded applying a potential of −0.2 V. The linear range obtained is from 0.01 to 1 nM of biotin and the sensitivity is 758 nA/nM. The modification and cleaning of the electrode are performed in an easy way due to the internal magnet of the flow cell.

1. Introduction

Flow injection analysis (FIA) techniques have been employed to automate a wide variety of chemical and biochemical analyses since their invention in the middle of the seventies [1,2]. FIA techniques are classified into the basic mode (also named normal flow injection analysis) and different special modes as stopped flow [3,4] or reverse flow [5,6]. FIA is one of the most popular continuous flow techniques and its simplicity, and flexibility allow its application in various chemistries. Moreover the flexibility in the different created formats and designs helps its introduction in laboratories using low cost instrumentation but each one has to comply with the three cornerstones of the FIA: (i) injection of discrete and well defined volume of sample solution into a flowing carrier stream; (ii) reproducible and precise timing of the manipulation that the injected sample zone is subjected to in the system, from the point of the injection to the point of detection; and (iii) the creation of a concentration gradient of the injected sample, providing a transient, but reproducible readout of the recorded signal. The second generation of FIA including stopped flow injection [7], bead injection [8–10], sequential injection with lab on valve [11] has opened new perspectives with many analysis systems. The majority of these systems have in common the detection of the product before the equilibrium state is reached, which considerably reduces the analysis time. To overcome shortcomings such as contamination of the surface of the detector or the lack of sensitivity, an appealing tool is used called the bead-injection analysis [12,13]. Magnetic beads (MBs) are generally used for it high surface area per volume. The ability to accommodate higher numbers of immobilized molecules helps to improve sensitivity and consequently detection limit of the assay. Another benefit is their easy manipulation with external magnets which makes it possible to perform biological reaction events away from the electrode surface [14] and reduce the complexity for sensing application. Moreover their uses minimize the matrix effect thanks to the washing procedures and faster assay kinetics that are achieved since the beads are in suspension [15]. Many articles have been published using both the advantages of magnetic beads and screen printed electrodes [16,17]; articles about FIA and its application in pharmaceutical [18,19] and biomedical analysis can be found [20] but articles on any assay using a magnetic flow cell with an integrated magnet for screen printed electrodes have not been reported.

In this paper an electrochemical biotin assay using a flow injection system which includes for the first time a flow cell for magnetic assay with an integrated magnet is described. The present strategy is based on a competition scheme where biotin and the biotin-HRP compete for the binding sites of streptavidin, which are immobilized on the magnetic beads surface. After molecular recognition, the beads are injected into the FIA system and immobilized on the electrode surface with the help of the integrated magnet of the flow cell which is exactly situated underneath the working electrode of the screen printed electrode. After the injection of the substrate, enzymatic product reduc-

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tion current is obtained, which is inversely related to biotin concentration. The electrochemical response is measured using the amperometric technique applying a constant potential. Moreover the packing of MBs on the SPCE surface also implied that the enzyme reaction product was generated very close to the electrode surface, thus allowing the steady-state to be reached rapidly (which implies faster measurements), and minimizing diffusion limitations of the electroactive species.

2. Experimental

2.1. Chemicals

Sodium hydroxide (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 from 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) were purchased from SIGMA. 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 was daily prepared in 0.1 M phosphate buffer (PB) pH 7.2 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-CAC), were purchased from DropSens, S.L (Oviedo, Spain). The screen-printed electrodes consist of a Carbon working (4 mm diameter), carbon auxiliary and silver pseudo reference electrodes printed on an alumina substrate. An insulating layer serves to delimit the electrochemical cell and electric contacts. The magnetic flow cell (ref. CFLWCL-MAGN) was designed and purchased from DropSens, S.L (Oviedo, Spain). It is a methacrylate wall-jet Flow-Cell for FIA, with an unscrewed open–close system which allows an easy electrode replacement. This cell is designed to obtain an inlet flow perpendicular to the electrode's surface, and an outlet flow forming a 45° angle and an O-ring which limits the volume of the electrochemical cell. The integrated magnet is a cylindrical magnet which in the upper position is situated exactly above the working electrode and permits the modified magnetic beads to fix on the surface of the electrode. When the magnet is in the lower position, the magnetic beads are eliminated from the surface of the electrode.

This new magnetic flow cell is represented in Fig. 1A. The cell is one of the parts of the flow injection analysis system which is schematically represented in Fig. 1B

The FIA system used for the detection of biotin is a 12 cylinder Perimax Spetec peristaltic pump (Spetec GmbH, Germany) which allows the 0.1 M PB pH 7.2 stream to flow through the system. Desired solutions are injected by means of a six port rotary valve, Model 1106 (Omnifit Ltd., UK) equipped with a 100 μL loop.

2.3. Assay procedure

The reaction scheme is based on a one step competitive assay between free 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 for 15 min under stirring and then a washing step is performed. It consists of a resuspension of the beads in the 0.1 M PB solution at pH 7.2 and then a separation with the magnet to remove the supernatant. This operation is repeated 3 times. The scheme of the assay is reported in Fig. 2.

2.4. Electrochemical measurement

Amperometric detection was performed applying a constant potential to the modified electrode. The carrier solution (phosphate buffer, pH 7.2) was pumped at a constant flow rate (1 mL/min) until a stable baseline was recorded. Then, the flow rate was changed to 0.5 mL/min and the magnet was moved to the upper position. Finally, 30 s after the injection of 100 μL of the sample, the flow rate was changed to 1 mL/min and then 100 μL of TMB diluted 5 times (this dilution was necessary to avoid the oxidation peak due to the TMB itself) were injected into the system and the reduction current was observed and recorded 10 s after the injection of the substrate. Finally, the magnet is moved to the lower position and for 3 min at a flow rate of 3 mL/min the carrier is pumped in order to wash the electrode surface. After the washing step another analytical run can be performed.

2.5. Real sample measurement

The methodology was tested in two pharmaceuticals with a known concentration of biotin. The first one is MEDEBIOTIN tablets (pharmaceutical 1) and second is MEDEBIOTIN vials (pharmaceutical 2). Each tablet (0.072 g) contains 5 mg of biotin, and each vial of 1 mL contains 4.6 mg of biotin. So 0.01 g of the pharmaceutical 1 was dissolved in 100 mL of the buffer. Then, an aliquot of this solution was diluted 1:50,000 times. Concerning the pharmaceutical 2, the vial was diluted in 1 L of the buffer and from this solution, a second dilution was performed (1:50,000). Finally mixtures of Strep-MB/B-HRP/Pharmaceutical were prepared and left under stirring for 15 min. Then a washing step was performed and finally the amperometric measurement was performed as explained in Section 2.4. Both samples were measured in triplicate.

3. Result and discussion

3.1. Optimization of the parameters of the flow injection analysis system

3.1.1. Flow rate

3.1.1.1. Injection of the mixture. The flow rate obtained when the mixture of reaction is injected in the FIA system is very important because there must be equilibrium between the pulling power of the flow carrier and the power of magnet in the flow cell that retains the magnetic beads. To carry out this study, a mixture of Strep-MB (7×10^{7} magnetic beads per mL)/B-HRP (5×10^{-7} M), prepared as explained in Section 2.3 was injected at different flow rates of the carrier (0.5, 1, 1.5, 2 mL/min). Then TMB was injected and the reduction current was recorded applying a potential of −0.20 V and using a flow rate of 1 mL/min. The results obtained are summarized in Table 1. The analytical signal decreases when the flow rate increases because the number of Strep-MB fixed on the electrode surface is lower. At higher flow rates the pulling power of the flow carrier is higher than the capacity of the magnet to retain all magnetic beads on the electrode surface. In other words, the effect of cleaning is higher than the retention of the Strep-MB on the electrode surface and consequently, lower analytical signals are obtained. Thus, for further studies, a flow