Electrochemical sensing using magnetic molecularly imprinted polymer particles previously captured by a magneto-sensor

Gerson A. Ruiz-Córdova[a,b,c], Sabir Khan[b,c], Luís Moreira Gonçalves[d,e], Maria Isabel Pividori[f], Gino Picasso[a], Maria Del Pilar T. Sotomayor[b,c,*]

[a] Laboratory of Physical Chemistry Research, Faculty of Sciences, National University of Engineering, Lima, Peru
[b] Department of Analytical Chemistry, Institute of Chemistry, UNESP – Univ Estadual Paulista, Araraquara, SP, Brazil
[c] National Institute for Alternative Technologies of Detection, Toxicological Evaluation and Removal of Micropollutants and Radioactives (INCT-DATREM), Araraquara, SP, Brazil
[d] REQUIMTE/LAQV, Faculdade de Ciências, Universidade do Porto, Porto, Portugal
[e] Departamento de Química Fundamental, Instituto de Química, Universidade de São Paulo (USP), São Paulo, SP, Brazil
[f] Universitat Autònoma de Barcelona, Barcelona, Spain

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ABSTRACT

The determination of 1-chloro-2,4-dinitrobenzene (CDNB) was used as a proof-of-concept to a simple analytical practical configuration applying magnetic molecularly imprinted particles (mag-MIPs). Mag-MIPs were captured from an emulsion by a home-made magneto-sensor (where a small magnet was entrapped by a graphite-epoxy composite) and then, this sensor, was transferred to the solution containing the analyte, where, after binding to the mag-MIPs, the analyte was directly analysed using differential pulse voltammetry (DPV) since the magneto-sensor acted as the working electrode. After optimization, a detection limit of 6.0 $\mu mol\,L^{-1}$ with a RSD of 2.7% was achieved along with suitable recoveries and selectivity. This methodology offers a different approach for electroanalytical methodologies using mag-MIPs.

1. Introduction

Since the early reports on magnetic separation technology, magnetic particles have been used as powerful and versatile options in many biotechnological applications [1], including with analytical intents, being the electrochemical biosensing an example of it [2]. The magnetic properties allow in a very simple and selective way, a quick separation due to straightforward manipulation [3].

Magnetic molecular imprinting polymers (mag-MIPs) are particles where MIPs (a biomimetic material containing gaps where the aimed analyte can selectively bind) surround a metallic core that is easily susceptible to an external magnetic field [4,5]. MIPs were idealized as a synthetic alternative to biological receptors, imitating antibodies, in some ways they can be considered as biomimetic polymers. MIPS have sites of molecular recognition for a specific molecule. Mag-MIPs combine the advantages of the magnetic beads with that of molecular imprinted polymers (such as the low cost of synthesis, less time consumed when compared with the traditional solid-phase extraction (SPE), high mechanical and chemical stability, and, most notably, a tailor-made selective recognition) [5–17].

In general, MIPs and mag-MIPs are used in chromatographic applications as an alternative to conventional adsorbents [17]. Herein, it is intended to have an electrochemical application making use of a magneto-sensor. Magneto-sensors can capture different functionalized magnetic beads making it possible to creatively develop different analytical approaches [5,8,18–20]. Despite their prominent advantages, such as good sensitivity [21] and 'user-friendliness', electrochemical techniques often suffer from poor selectivity towards the analyte species, an issue that can be solved recurring to a suitable sample pretreatment step (sample preparation) [22–29]. 1-chloro-2,4-dinitrobenzene (CDNB) is a toxic xenobiotic compound known to cause oxidative stress and cell death and a well-known allergenic [30–32], herein it was used as the proof-of-concept analyte.

2. Experimental

2.1. Reagents

All reagents used were of analytical grade.

Sodium dodecyl sulphate (SDS), CDNB, $Fe_2(SO_4)_3 \cdot 9H_2O$, $FeSO_4 \cdot 7H_2O$, $K_4[Fe(CN)_6]$, oleic acid (OA), methacrylic acid (MA), ethylene glycol dimethacrylate (EGDMA), methylene diphenyl

diisocyanate-4 (MDI), bisphenol A (BPA), phloroglucinol were acquired from Sigma-Aldrich. Glycine, Tween 20, sodium chloride, potassium chloride, boric acid, acetic acid, potassium persulfate, n-hexane, chloroform and phosphoric acid were all purchased from Merck. Methanol and tetrahydrofuran were purchased from ACS Synth.

Ultrapure water (resistivity not lower than 18.2 MΩ cm at 298 K) from a Purelab Classic water purification system was used in all the experiments.

2.2. Mag-MIPs synthesis

Magnetic nanoparticles were prepared by the co-precipitation method, using $FeSO_4 \cdot 7H_2O$ and $Fe_2(SO_4)_3 \cdot 9H_2O$ as the starting materials, and mixed with oleic acid (OA). This material was then encapsulated in a hydrophobic polymeric matrix, poly-(MA-co-EDGMA), by the mini-emulsion method. The mag-MIP selective to CDNB was synthesized over the magnetic-hydrophobic surface using the precipitation polymerization in the presence of the analyte, MDI, BPA and phloroglucinol, forming a core@shell structure. A magnetic non-imprinted polymer (mag-NIP) was prepared as a control polymer using the same procedure employed for the mag-MIP, but without the analyte.

Full details concerning the synthesis of the mag-MIPs, along with the full physicochemical characterization of these materials can be found in a recent publication [32].

2.3. Apparatus and working electrode manufacturing

Electrochemical measurements were performed using a PalmSens® 3 potentiostat. Final electrochemical measurements were performed in a Britton-Robinson buffer solution (H_3BO_3 0.04 mol L^{-1}, CH_3COOH 0.04 mol L^{-1} and H_3PO_4 0.04 mol L^{-1}). Dissolved oxygen was removed by purging with nitrogen. A platinum wire was used as the counter-electrode (CE), and the pair Ag|AgCl (in KCl, 3 mol L^{-1}) was used as the reference electrode (RE). The working electrode (WE) was home-built, a diagram of its manufacturing is shown in Fig. 1A. Graphite powder and epoxy resin were hand-mixed in a 1:4 (w/w) ratio. This mixture was thoroughly hand-mixed to ensure the uniform dispersion of the graphite powder throughout the polymer. The resulting paste was placed to a depth of 3 mm in a cylindrical PVC sleeve body (6 mm i.d.) with a copper plate as electrical contact [24]. A small neodymium magnet (3 mm i.d.) was placed into the centre of this electrode after the addition of a thin layer of composite paste in order to avoid direct contact between the magnet and the electrical connector. After filling the electrode body gap completely with the soft paste, the electrode was tightly packed. An image of the final electrode is shown in Fig. 1B. Before each use, the electrode surface was renewed by a simple polishing procedure [25].

3. Results and discussion

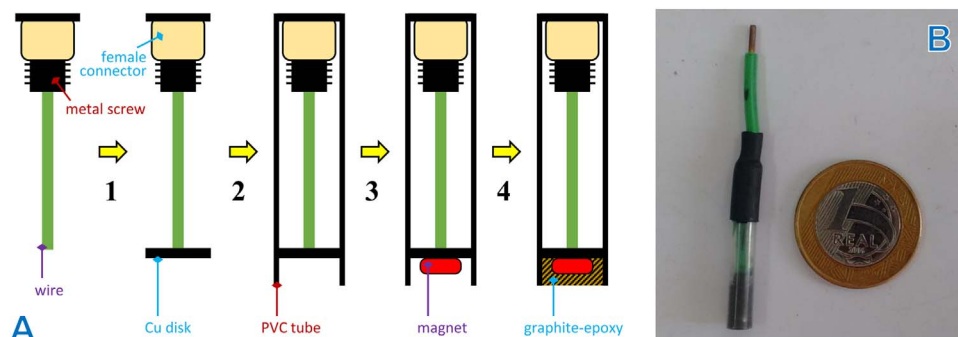The aim of this work was to experiment a different analytical methodology for mag-MIPs taking advantage of a magneto-sensor. This is schematically explained in Fig. 2. Initially, the prepared mag-MIPs were dispersed in a glycine solution, by sonication for two hours in order to obtain a reproducible homogenous suspension. Afterwards, the mag-MIPs were seized, in a 20 s period, becoming 'concentrated' in the surface of the home-made magneto-sensor [24]. Then, this magneto-sensor with a modified working surface was transferred to the electrochemical cell sample, where the analyte was captured in the analyte-sized mag-MIPs' holes and was electrochemically quantified (CDNB is electrochemically active), thus becoming the magneto-sensor a working electrode. Since the mag-MIPs were concentrated in the electrode surface and not within a carbon paste, a higher and quicker analyte binding was achieved.

As is shown in the literature, at pH of 2.0, the reduction peak of CDNB is around −0.4 V (α in Fig. 3A) [33]. Moreover, in literature it is suggested that it is a reductive cleavage of the carbon–chlorine bond [34,35]; a further mechanistic discussion can be found in literature [35]. Optimized parameters ended up being the following: potential of deposition (for 60 s) of −0.20 V, electrochemical window from −0.20 to −0.50 V, potential step of 4 mV, potential pulse of 0.12 V with a scan rate of 4 mV s^{-1} (i.e. 200 ms per pulse). The analytical methodology was also optimized concerning different times in the several steps, namely the time of dispersion (t_{dis}), i.e. the time the Mag-MIPs were shaken within the Tween solution creating the emulsion, time of collection (t_{col}), i.e. the time the magneto-sensor spent capturing the mag-MIPs into its surface and equilibration time (t_{eq}), i.e. the time the magneto-sensor was placed in the sample previous to the electrochemical stripping. Longer dispersion times originated larger currents up to certain point since an emulsion with suitable dispersion was already formed. In the same conditions ([CDNB] of 1×10^{-3} mol L^{-1}, room temperature, scan rate of 20 mV s^{-1}): for 30 min − 35 µA, 60 min − 67 µA and 120 min − 90 µA. Hence a time of 120 min was chosen. The optimization of t_{col} and t_{eq} is shown in Fig. 3B. Larger currents were obtained for shorter t_{col} and longer t_{eq}, it seems that longer capturing times are creating more than one mag-MIP layer and, therefore, not just making the analyte further apart from the electrode surface but also complicating the electron transfer. For a better understanding of the whole process, a mention to these times also appears in the schematics of Fig. 2. The temperature of analyte binding to the mag-MIPs was also optimized, and, as it is shown in Fig. 3A, a higher temperature, for the same period of time, clearly increases the signal. This is not surprising since, in general an increase in temperature may enhance reaction rates as well as favouring adsorptive processes [36].

The corresponding calibration curve (n = 7) had the following analytical parameters (Fig. 4): a r^2 of 0.983, peak current (µA) = (1.76 ± 0.10) × [CDNB] (µmol L^{-1}) - (4.5 ± 3.1), limit of detection (LOD) and quantification (LOQ) of 6.0 and 20.0 µmol L^{-1} (1.2 and 4.1 mg L^{-1}), respectively. LOD and LOQ were calculated as three and ten times the standard deviation of the intercept/slope, respectively, these are comparable to other works in literature using HPLC-UV [31]. Repeatability was evaluated comparing five calibration curves, the relative standard deviation (RSD) obtained was of 2.7%.

When comparing the mag-MIPs with the corresponding mag-NIPs

Fig. 1. A – Schematics of the manufacturing of the magneto-sensor, a copper disc connector is placed on the tip of the conducting wire (1), then everything is inserted in a PVC tube (2), then the neodymium magnet is placed (3) and, finally, a thin layer of a graphite-epoxy composite is placed (4). B – Photograph of the built magneto-sensor next to a 1 Brazilian real coin (27 mm diameter) for scaling purposes.