**Disposable paracetamol sensor based on electroactive molecularly imprinted polymer nanoparticles for plasma monitoring**

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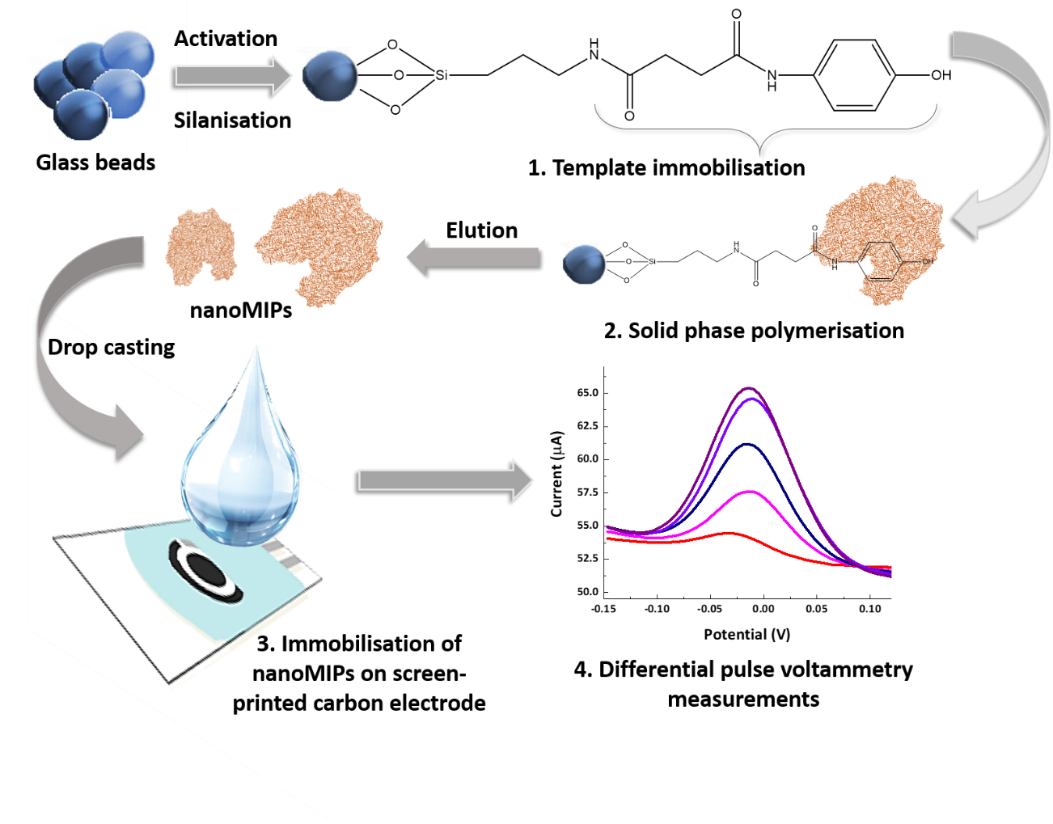
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**Abstract:** A highly sensitive disposable electrochemical sensor for paracetamol was devised using electroactive molecularly imprinted polymers nanoparticles (nanoMIPs). NanoMIPs were prepared by solid-phase synthesis and carried out with the inclusion of a redox label, which confers electroactivity to the nanoparticles. NanoMIPs were characterized by Fourier-infrared spectroscopy, dynamic light scattering and scanning electron microscopy. Sensors were fabricated by covalent attachment of nanoMIPs on screen-printed carbon electrodes and then employed for electrochemical determination of paracetamol using differential pulse voltammetry. The sensor was successfully evaluated in spiked human plasma with recoveries at 94-108%, presenting a sensitivity of 6.18 ± 0.22 µA/mM. The limit of detection and limit of quantification for the sensor were found to be 50 µM and 167 µM, respectively, in a linear concentration range between 0.1 and 1 mM. High selectivity was demonstrated, with no interference found in the presence of caffeine, procainamide or ethyl 4-aminobenzoate. The sensors exhibited high reproducibility (RSD, 4.8 %), fast response time (~8 s) and acceptable shelf life (90 days), confirming its suitability for point of care diagnostic applications.

**Keywords:** Paracetamol, molecularly imprinted polymer nanoparticles, electrochemical sensor, point-of-care diagnostics.

**Graphical abstract**

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**1. Introduction**

Paracetamol (acetaminophen, **Figure S1a**) is a popular and effective antipyretic and analgesic, widely used without prescription to treat a variety of symptoms including headache, fever, arthritis, colds and pain. Paracetamol is also considered a good alternative treatment for those with an intolerance to aspirin, and is without adverse health effects when it is taken in the recommended dose.1 The United Kingdom´s National Health Service suggests a maximum dosage for adults of 500 mg every 4 hours.2 Paracetamol poisoning becomes a UK public health risk. In the event of paracetamol overdose, it causes liver and kidney damage.3-5 In addition, around 200 deaths occur annually in the UK from belated identification of paracetamol poisoning .6,7

Drug detection is highly important in forensic analytical chemistry, but analysis of pharmaceuticals in biological fluids is challenging.1 There is a growing need for analytical methods and technologies performing rapid, simple, sensitive and accurate drug identification and quantification.8 Several techniques are currently used to determine paracetamol in biological fluids,9 including spectrophotometry, chemiluminescence, colourimetry, high-performance liquid chromatography (HPLC) and electrochemistry.10 These techniques frequently require laborious sample preparation, tedious analysis, trained personnel and expensive instrumentation (e.g. HPLC-MS). Very few hospitals are fortunate enough to have access to chromatographic facilities and automatized immunoassay systems. Therefore, only a small number of hospitals can precisely determine paracetamol levels in blood via HPLC and spectrophotometry.11 These techniques commonly involve long and time consuming analysis, which could result in late detection and paracetamol poisoning. Hence, there is a need for a simple and rapid method for early detection and monitoring of paracetamol for the prevention and diagnosis of overdose.

Herein, an alternative technology is proposed for paracetamol determination in blood using a sensor device based on molecularly imprinted polymer nanoparticles (NanoMIPs) as recognition elements. Molecularly imprinted polymers (MIP) are synthetized in the presence of a template molecule. After polymerization and removal of the template, MIP are embossed with complementary cavities and functionalities, which result in a polymer with specific recognition sites.12 MIP are additionally robust, stable and easy to produce, and are for these reasons considered efficient alternative materials to natural receptors. MIP are commonly used to recognize drugs, proteins, biological markers and agrochemicals,13 and have been successively applied for a variety of applications including drug delivery, affinity separations, assays, sensors and solid phase extraction.14

MIP can easily be applied in sensing devices. For example, electrochemical MIP micro-beads have been prepared by anchoring ferrocene into polymer (e.g. polyacrylamide) structures, resulting in the formation of electroactive artificial receptors.15 Similarly, electro-responsive nanoMIPs were functionalized with a ferrocene derivative (namely, vinylferrocene and ferrocenylmethyl methacrylate) and applied in voltammetric sensors using the solid phase synthesis method.13,16,17 Unfortunately, those sensors were not satisfactorily robust and performant to be applied in biological fluids for selective recognitions of drugs.

Electrochemical sensors are preferred for applications in the field due to their simplicity, fast response, portability, cost effectiveness and easy adaptation to drugs detection. The most common sensors in the market are the glucometers, alcoholmeters and pregnancy test. However, the key advantage of electrochemical sensors is the possibility of miniaturization, and thus smaller devices can be fabricated. Of all the electrochemical techniques, voltammetry has been the most extensively used for quantification of drugs.14,18 Applying these methods, several (bio)sensors with nanomaterials have been used for the analysis of paracetamol samples.10 Unfortunately, those sensors are typically designed to exploit the electro-oxidation of paracetamol, which may lead to cross-reactivity and low sensitivity in biological samples. There is therefore a clear necessity for a reliable sensor for point of care (PoC) testing in medical emergencies.

Generally, the Rumack-Matthew nomogram (or paracetamol toxicity nomogram)19 is used to deduce paracetamol serum concentrations in relation to time of ingestion, in order to evaluate potential overdose and instruct the clinician in the necessity of proceeding with antidote [N-Acetylcysteine](https://en.wikipedia.org/wiki/N-Acetylcysteine) (NAC) treatment.20 Usually, a plasma paracetamol concentration higher than 662 µM (100 µg/mL) at 4 hours post ingestion (revised UK treatment line), indicates the need for NAC treatment, which is necessary to prevent hepatotoxicity and liver failure.21,22

The technology presented herein could potentially help to rapidly determine a paracetamol overdose, preventing these further complications. The voltammetric sensor for paracetamol detection uses electroactive nanoMIPs, produced by introducing ferrocene monomer into the polymeric structures, which serves as an efficient transducer of electrochemical response.23-25 The preparation of electroactive nanoMIPs was successfully achieved via solid phase synthesis and radical polymerization,13 followed by covalent immobilization of the nanoMIPs on electrodes. Sensors were then tested in spiked buffer solutions and plasma samples, presenting a current response proportional to the specific paracetamol concentration, while maintaining a high sensitivity, selectivity and stability.

1. **Experimental** 
   1. **Chemical and reagents**

N-[3-(Trimethoxysilyl)propyl]ethylenediamine (DAMO), 1,2-Bis(triethoxysilyl)ethane (BTSE), ethylene glycol dimethacrylate (EGDMA), 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC), ferrocenyl-methylmethacrylate (FcMMA), itaconic acid (ITA), pentaerythritol tetrakis(3-mercaptopropionate) (PETMP), N-hydroxysuccinimide (NHS), N,N-diethyldithiocarbamic acid benzyl ester, trimethylolpropane trimethacrylate (TRIM), dimethylformamide (DMF), 4-((4-hydroxyphenyl)amino)-4-oxobutanoic acid (carboxyl-Paracetamol), human plasma, toluene, ethanol and acetone were purchased from Sigma-Aldrich (UK). Carboxyl-paracetamol, procaine amide, benzocaine and caffeine were purchased from Across (UK). Acetonitrile (ACN), sodium hydroxide (NaOH) and N,N’-diethyldithiocarbamic acid benzyl ester were purchased from TCI Europe (Belgium). All chemicals and solvents were HPLC grade. Phosphate-buffered saline (PBS) tablets (Gibco, UK), and glass beads (Spheriglass®) were purchased from Blagden Chemicals (UK). Paracetamol stock solutions were diluted in PBS buffer (5 mM, pH = 7.4).

* 1. **Materials and methods**

Dialysis tube membrane Spectra/Por 7 (regenerated Cellulose, 10 kD MWCO, 11 cm Tubing Length, 32 mm Flat-width, 20.4 mm diameter, 3.3 mL/cm) was used for purification. Attenuated total reflectance-Fourier Transform Infrared (ATR-FTIR) spectroscopy spectra was acquired using a platinum Diamond ATR accessory and INVENIO FTIR spectrometer equipped with the Bruker FM optical components. Samples were viewed on a Zeiss Sigma 500VP Scanning Electron Microscopy (SEM) and an electron high tension at 5kV. The plasma cleaner instrument (Emitech, K1050X RF Plasma Cleaner, 50 W, 13.56 MHz RF for 5 min) was used to clean and to activate the surface of the screen-printed electrodes and gold chips, the latter used for SPR analysis.

* 1. **Preparation of glass-beads@paracetamol**

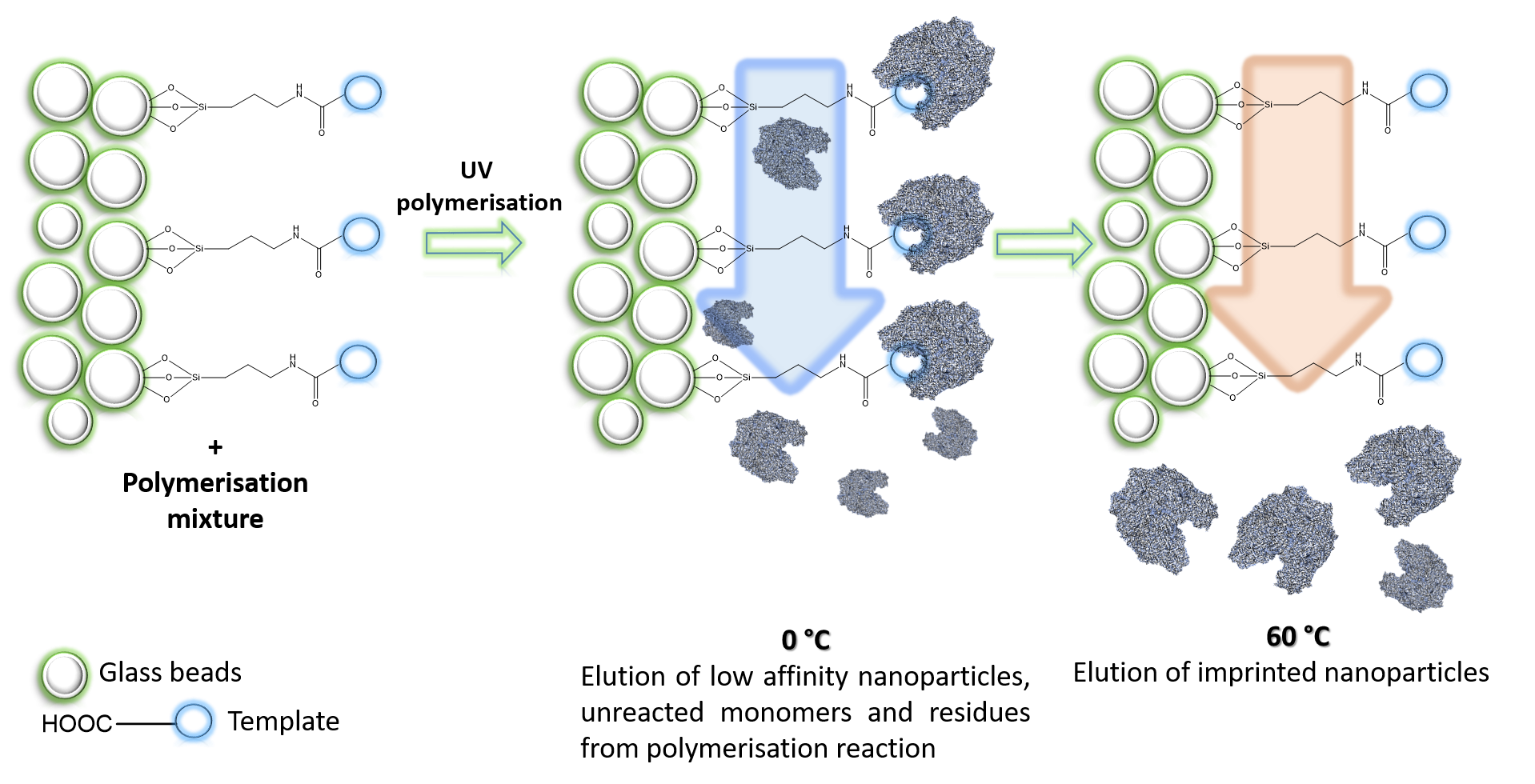
The solid-phase imprinting synthesis of the nanoMIPs was carried out as previously reported by our group.26 Briefly, 200 g of glass beads (150–200 µm) were activated by boiling in 4 M NaOH (1 mL/g of glass beads) for 15 min. Following this, the beads were washed with deionized water (3 × 300 mL), rinsed once with 20% sulphuric acid, and then washed (3 × 300 mL) with deionized water, 5 mM PBS and acetone. Finally, glass beads were dried in the oven at 70°C for 15 min followed by 30 min at 150°C.

Glass beads were silanized by reflux (4h) in a solution of 4% v/v of DAMO (100 µL/mL) and 0.24% v/v of BTSE (5 µL/mL) in dry toluene. After cooling, the beads were washed with acetone (3 × 300 mL) and dried for 15 min under vacuum and cured in the oven at 150°C for 30 min.

Carboxyl-paracetamol (4-(4-hydroxyanilino)-4-oxobutanoic acid (**Figure S1b**), an analogue of paracetamol, was immobilised as a template on the surface of the glass beads by a coupling reaction between the carboxyl group of the template and the amino group from the silanized glass beads. The immobilisation of carboxyl-paracetamol (template) was achieved by incubation of the beads (20 g) for 8h at room temperature in a solution of ACN (30 mL) containing 2.4 mM of the template (0.5 mg mL-1), 52 mM of EDC (0.10 mg mL-1) and 130 mM of NHS (0.15 mg mL-1). The beads were then washed with ultra-pure water using a solid phase extraction (SPE) cartridge fitted with a polyethylene frit (pore diameter of 20 μm), dried under vacuum and stored at 4°C until use.

* 1. **Preparation of nanoMIPs**

NanoMIP synthesis was performed using glass beads (20 g) with immobilised template and a polymerisation mixture comprising 1.7 g of ITA (594.4 mM) as functional monomer, 1.3 g of EGDMA (265.7 mM) and 1.3 g of TRIM (174.6 mM) as cross-linkers, and 0.17 g of the redox label FcMMA (27.1 mM) dissolved in 22 mL DMF. After that, 0.1 g of PETMP (9.3 mM) was added as a chain transfer agent and 0.3 g of N,N-diethyldithiocarbamic acid benzyl ester (56.9 mM) was used as an initiator. This polymerization mixture was purged with nitrogenfor 5 min and exposed to UV light (Philips lamp HB/171/A, 4×15 W) for 1.5 min to complete the polymerisation reaction. Once the polymerisation was completed, the resulting glass-beads@paracetamol@nanoMIPs were placed into a SPE cartridge and washed with DMF (3 × 30 mL) and acetonitrile (1 × 30 mL) at 0°C to remove unreacted monomers and low affinity nanoMIPs. Finally, elution of high affinity nanoMIPs was carried out with ethanol (3 × 30 mL) at 60°C as shown in **Figure 1**.



**Figure 1**. Purification of nanoMIPs by using solvent and temperature variation in an SPE cartridge.

The purification of the nanoparticles was accomplished by dialysis using a tube membrane. For that, nanoMIPs were diluted 10 fold using PBS buffer (5 mM) and sonicated for 5 min. The dialysis was performed in a dialysis system (1 L capacity) with constant water flow (20 mL/min) and completed after 4 h.

* 1. **Characterization of nanoMIPs**

The nanoMIPs were characterised by dynamic light scattering (DLS) at 25°C using a Zetasizer Nano (Nano-S) from Richmond Scientific Instruments Ltd. (Lancashire, UK). Prior to the measurements, 1 mL of nanoparticles solution was ultra-sonicated for 2 min to disrupt potential agglomerates. Digital images were collected with a Megaview III digital camera. The size of the nanoparticles was calculated using ImageJ v. 151o software.

* 1. **Surface plasmon resonance (SPR) analysis**

A Biacore 3000 SPR instrument (GE Healthcare Life Sciences, UK) was employed to evaluate the affinity of nanoMIPs towards paracetamol. The gold sensor chip was cleaned with nitrogen plasma, incubated in mercaptododecanoic acid (0.2 mg mL-1), rinsed with ethanol and dried in air. Subsequently, the nanoMIPs were covalently immobilized on the chip surface using cabodiimide coupling (0.4 mg mL-1 of EDC and 0.6 mg mL-1 of NHS). All SPR measurements were performed at 25 °C in KINJECT mode (injection volume of 100 μL and dissociation time of 120 sec), with PBS (10 mM pH 7.4) as running buffer and a flow rate of 35 µL/min. The dissociation constant (Kd) calculations were performed using BiaEvaluation v 4.1 software based on the 1:1 binding model with drifting baseline fitting.

* 1. **Preparation of the sensor**

Screen-printed carbon electrodes (3.4 x 1.0 x 0.05 cm, SPCE, Dropsens) were purchased from Metrohm (UK). Before usage, SPCE were cleaned using nitrogen plasma. The SPCE were chemically functionalised by incubation in a solution of 6% APTES in 5% ethanol for 30 min and cured at 120 °C for 30 min. NanoMIPs were immobilised on SPCE using carbodiimide cross-linker chemistry. For this, a solution (100 µL) comprising 2 mg mL-1 of nanoMIPs, 52 mM(0.1 mg mL-1) EDCand 130 mM(0.15 mg mL-1) NHS in ethanol was drop-casted on the electrodes surface and incubated for 45 min.

**2.8 Electrochemical measurements**

Electrochemical measurements were performed using a µ-Autolab Type II potentiostat/galvanostat equipped with a connector (DropSens) for screen-printed electrodes. The instrument was controlled with GPES Manager Software. The electrochemical detection of paracetamol was performed by following the nanoMIPs (labelled with FcMMA) response measured at the potential of 57 mV. Here, the nanoMIPs function as paracetamol specific receptors and electroactive reporters. All measurements were performed in PBS buffer (5 mM, pH = 7.4) using differential pulse voltammetry (DPV) in a potential window between -200 and 200 mV (scan rate 50 mV/s) and a concentration range from 0.1 to 1 mM of paracetamol. The sensor current responses were evaluated with respect to a blank (non-spiked sample). The same experimental conditions were used to evaluate the sensor responses towards possible interferences, such as caffeine, procainamide and ethyl 4-aminobenzoate. The chemical structures are shown in **Figure S1**.

**2.9 Sample preparation**

Lyophilised human plasma was dissolved in PBS buffer (10 mM, pH=7.2) and vortexed for 3 min. Subsequently, the plasma solution was centrifuged (3000 g, 5942 rpm) and the supernatant was recovered and filtered using a micro-membrane syringe filter (25 mm, 0.45 µm PTFE, 87 psi max). Spiked plasma solutions were prepared using a paracetamol intravenous solution (10 mg mL-1) in the concentration range from 0.1 to 1 mM.

1. **Results and discussion**

**3.1 Selection of the functional monomers**

NanoMIPs for specific recognition of paracetamol were previously designed using computational screening and docking of different acryl functional monomers.27 In the previous report, molecular modelling was performed in order to predict the interactions between the functional monomers and the template. The results of the screening of 25 possible functional monomers against paracetamol demonstrated that the ITA monomer formed a stable complex. The complex presented a higher binding score (-41.2 kcal/mol), six hydrogen bond interaction and the highest interaction ratio (template to functional monomer 1:5).27

* 1. **Characterisation of nanoMIPs**

The size and morphology of the nanoMIPs was assessed using DLS and SEM analysis. DLS measurements reveal an average size of the nanoMIPs of 186 ± 38 nm. SEM analysis suggest that nanoMIPs are spherical particles (**Figure 2**).

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**Figure 2**. SEM images obtained for nanoMIPs, 200 nm scale.

* 1. **FT-IR analysis**

The FT-IR analysis were carried out to evaluate the chemical composition of the nanoparticles. For that, nanoMIPs were analysed and compared before and after paracetamol treatment (500 µM) as shown in **Figure S2**. Polymer samples were washed and filtered with 5% hydrochloric acid prior to the FT-IR analysis. The FT-IR spectra display the main characteristic polymer bands in the range between 1170 and 1600 cm-1, which are related to the functional groups present in a polyacrylamide structure and are composed of the bands at 3162 cm-1 for N-H, 2910 and 730 cm-1 for C-H, 1680 cm-1 for C=O and 1350 cm-1 for C-N vibrations. The polymer treated with paracetamol presented bands at 3100 cm-1 for N-H and O-H, 3032 cm-1 for C-H, 2971 cm-1 for C-OH, 1645 cm-1 for C=O , 1562 cm-1 for C-C, 1220 cm-1 for C-N vibrations and 650 cm-1 for N-H, which are in agreement to the presence of paracetamol.28 The band at 3266 cm-1 corresponds to O-H stretching from water. To conclude, polyacrylamide signals after paracetamol treatment revealed significant differences when compare to those obtained with pure nanoMIPs, indicating the presence of paracetamol in the polymer network and also confirming the binding interaction.

* 1. **Electrochemical characterisation of the materials**

To evaluate the specificity and electroactivity of nanoMIPs, DPV responses to buffer and paracetamol were recorded and compared to the controls as shown in **Figure S3**. The DPV measurements for the SPCE/APTES/nanoMIPs displayed a specific response towards paracetamol at approximately +0.05 V (vs Ag/AgCl), whereas the SPCE and the SPCE/APTES do not shown any response for paracetamol in the same potential range (-0.2 to 0.2 V vs Ag/AgCl), see **Figure S3**. Also, nanoMIPs and controls do not show any response to PBS, as expected. Correspondingly, DPV measurements for ferrocenyl-methylmethacrylate (FcMMA) on bare SPCE revealed an oxidation peak at +0.057 V (vs Ag/AgCl) as shown in **Figure S4**. The potential window of the FcMMA signal is influenced by the electrolyte, solvent and nature of the working electrode. The voltammograms confirmed that the nanoMIPs electroactivity is conferred by the presence of FcMMA moieties in the polymer structure. Presumably, the nanoMIPs actuation is a consequence of the analyte recognition. This suggest that the analyte activates a polymer conformation change exposing the hindered FcMMA moieties in the polymer, as a result, the electron transfer of FcMMA moieties is favoured. Hence, nanoMIPs can be effectively used as selective receptors and reporters.13,17

The redox properties of the polymer are dependent on the structure and the electronic environment. Previous reports have shown that ferrocene monomers present a shift in the oxidation potential when these are incorporated into polymers.16,23 For example, pyrrole-ferrocene polymers present an oxidation peak at +0.05 V (vs. Ag/AgCl).23 Another example is the Vinyl-ferrocene monomer oxidation potential observed at +0.3V (vs. Ag/AgCl), this value diverges from the polyacrylamide derivate observed at +0.18 V (vs. Ag/AgCl).16 Therefore, the changes observed in the oxidation potential values in ferrocene polymers could be directly related to the polymer backbone structure and also to the electrode used as internal reference.23 Moreover, polymers may present potential shifts in the ferrocene oxidation potentials due to the deposition of material at the reference electrode. As a result, the ferrocene polymer response can be measured at ~ 0.0 V(vs. Ag/AgCl).23 In addition, the ferrocene oxidation potential in polymers may also shift after analyte recognition event.15 The slight shift of the FcMMa oxidation potential observed in the nanoMIPs appears to be a result of the interactions between the nanoMIPs and the analyte. Therefore, the significant advantage of the nanoMIPs is their electroactivity, thus no enzymatic reactions or electroxidation of analyte is needed.

* 1. **Optimization of the sensor: tuning sensitivity and working range**

The nanoMIPs were immobilised on SPCE and subsequently applied successfully for paracetamol detection. Sensor responses were evaluated using DPV measurements in PBS buffer (5 mM, pH = 7.4) in the potential window from -0.2 to 0.2 V and in the linear concentration range from 0.1 to 1 mM. The electrochemical determination of paracetamol was measured by following the increase of the nanoMIP’s current response at +0.05 V (vs Ag/AgCl); this response was linear and directly proportional to the paracetamol concentration (**Figure 3**).



**Figure 3**. (A) Paracetamol calibration plot recorded for nanoMIPs immobilised on SPCE in a linear concentration range from 0.1 to 1 mM. (B) DPV response from (a) PBS, (b) 0.1 (c) 0.2 (d) 0.4 (e) 0.6 (f) 0.8 and (g) 1 mM paracetamol in PBS buffer (5 mM, pH = 7.4).

The flexibility of this technology allow to regulate the characteristics and performance of the sensors by controlling the concentration of the nanoMIPs on the electrode. Consequently, the sensitivity and LOD was optimized according to requirements of the Rumack-Matthew nomogram. In order to prevent hepatotoxicity and liver failure, the sensor should work in a clinically meaningful paracetamol concentrations ranging from low concentrations (0.1 mM) to high (1 mM). Therefore, sensors working in a nano and micro molar range will saturate in real scenarios. Thus, for practical aspects and to avoid sample dilution, sensors were adjusted to the clinical range.

The sensor was optimized by varying the concentration of the nanoMIPs immobilized on the electrode from 0.2 to 2 mg mL-1. The performance of those sensor is summarized in **Figure S5** and summarized in **Table S1**. From those results, nanoMIP sensors were found to operate in a milimolar, micromolar and nanomolar range. The evidence of the nanomolar paracetamol detection is discussed in the surface plasmon resonance (SPR) analysis. The sensor fabricated at low concentration of nanoMIPs (0.2 mg mL-1) displayed a narrow working range from 0.1 to 0.8 mM and sensitivity at 6.7 µA mM-1. Sensors prepared at high concentration of nanoMIPs (2 mg mL-1) displayed a broader working range from 0.1 to 1.4 mM and higher sensitivity (9.83 µA mM-1), which is adequate for POC applications. The optimized sensor in a linear concentration range from 0.1 to 1 mM (R2 = 0.998) displayed a sensitivity of 10.15 ± 0.23 µA/mM, and the limit of detection (LOD) and limit of quantification (LOQ) were respectively calculated at 70 µM (S/N=3) and 232 µM (S/N=10) as shown in **Figure 3**.

**3.5 Selectivity of the sensor**

The sensor incorporates immobilized artificial receptors called nanoMIPs that can reversibly detect receptor-ligand interactions with a high selectivity and in a non-destructive fashion. Consequently, the recognition mechanism is quite similar to an antibody system. In that way, the key-lock analogy still applicable involving binding constants. Often, nanoMIPs present dissociation constant (Kd) values between 0.5 to 15 nM, which is considered as a high affinity receptors compared to antibodies. Typically, antibodies present Kd values are in the micromolar to nanomolar range. The specificity and selectivity of nanoMIPs towards paracetamol was evaluated and then compared to different drugs (caffeine, procainamide, and ethyl 4-aminobenzoate) by SPR analysis. The analysis was performed in a concentration range between 1 nM and 100 µM. The dissociation constant (Kd) achieved from the specific interaction between paracetamol and nanoMIPs was estimated as 3.9 nM. The low Kd represent a relatively high affinity compared to other drugs which presented no interaction as shown in **Figure 4a**. The nanoMIPs present a negligible interaction with caffeine, procainamide and ethyl 4-aminobenzoate (benzocaine) according to SPR analysis. Similar results were observed for the sensor using DPV measurements. The voltammograms showed a response only towards paracetamol and no responses were recorded for other drugs (**Figure 4b**), those results confirm the selectivity and affinity of the sensor.

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| **a** | **b** |
| **Figure 4** (a) SPR and (b) DPV responses recorded from nanoMIPs for (1) paracetamol, (2) caffeine, (3) procainamide, and (4) ethyl 4-aminobenzoate. DPV measurements were performed at 600 µM of the analyte and SPR measurements in the concentration range from 1 nM to 100 µM. | |

* 1. **Paracetamol measurements in plasma**

Voltammetric determination of paracetamol concentration was also evaluated in spiked human plasma. The DPV sensor response for paracetamol on SPCE exhibited adequate linearity (R2 = 0.996) in a concentration range between 0.1 to 1 mM in spiked human plasma. The LOD (S/N=3) and LOQ (S/N=10) were calculated as 50 and 167 µM, respectively. The sensitivity was calculated at 6.18 ± 0.22 µA/mM as shown in **Figure 5**.

**Figure 5**. (A) Paracetamol calibration plot recorded for nanoMIPs immobilised on SPCE in a linear concentration range from 0.1 to 0.8 mM. (B) DPV response from (a) PBS, (b) 0.1, (c) 0.2, (d) 0.4, (e) 0.6 and (f) 0.8 mM (g) 1 mM paracetamol in spiked human plasma.

Moreover, the sensor exhibited a good recovery (94-108%) in spiked human plasma. It was also observed that the sensor sensitivity was reduced by 39% compared to buffer spiked samples due to the matrix effect. The SPCE sensor presented great stability due to the robust attachment of the sensing polymer. The reliability of the sensor was approximately 60 (± 3) reproducible measurements per sensor. The shelf life of the sensor was found to be 6 months (92% recovery of the response). For this analysis sensors were stored at -6 (± 2) °C after being lyophilized using 1% trehalose in water.

The operability of the sensor covers the concentration range demanded according to the Rumack-Matthew nomogram. Currently, paracetamol levels in blood samples are determined after separation in plasma using an automatized analyser which requires hours to perform measurements.29 However, in emergency scenarios fast analysis is required, therefore the technology presented in this work is an excellent alternative. The sensor allows measurement of plasma paracetamol levels in ~8 min. Additional advantages of this technology over previous electrochemical sensors are the selectivity and reliability of the sensing material (nanoMIPs). The presented technology is robust and allow reproducible and accurate measurements in samples.

The nanoMIPs fabrication process involves solid phase synthesis, which is relatively easy, efficient, low cost and can be performed using an automatized process.30,31 Additionally, nanoMIPs deposition on electrodes can easily be automatized with an automatic printer. Several sensors have been reported previously for determination of paracetamol in biological samples, as shown in **Table 1**. Usually their fabrication involves lithography techniques and complex multistep synthesis. The main analytical advantage of this sensor technology over previous sensing devices is that the electrochemical measurements are not based on the traditional electro oxidation of the analyte (paracetamol), which can be affected by the presence of other compounds and the nature of the human sample (matrix effect). Alternatively, the presented technology employs as sensing element the electroactive nanoMIPs. The nanoMIPs are used as recognition element as well as reporters. Therefore, the engineering and functioning of the sensor overcomes typical issues in PoC measurements such as side reactions, deposition of by-products, cross reactivity and matrix effects.

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| **Table 1. Comparison of sensor performances in plasma** | | | | |
| *Sensor* | *Detection method* | *Linear range (µM)* | *Clinical applicability according to Rumack-Matthew nomogram* | *References* |
| ERGO/GCE | DPV | 0.005-800 | No | 32 |
| PbS-NP/CPE | DPV | 0.033-158 | No | 33 |
| MWCNT-PtNP | AdS DPV | 0.351-56.10 | No | 34 |
| AuNPs/MWCNT/GCE | DPV | 0.09-35.0 | No | 35 |
| BDDE | MPA | 1-100 | No | 36 |
| BDDE | MPA | 0.08-100 | No | 37 |
| AuNPs@Fe3O4/CPE | DPV | 0.1-70 | No | 38 |
| Paracetamol Ab/GO | SWV | 0-10 | No | 39 |
| FeS-NPs/ERGO/GCE | DPV | 5.0–300 | No | 40 |
| MWNT/CPE | DPV | 15.0–270 | No | 41 |
| NanoMIPs/SPCE | DPV | 100-1000 | Yes | This work |
| *They key advantages are the (a) applicability in real scenarios, (b) selectivity, (c) no saturation in the clinical relevant range, and (d) the direct detection method* | | | | |
| Electrochemically reduced graphene oxide (ERGO), glassy carbon electrodes (GCE), nanoparticles (NP), carbon-paste electrode (CPE), multi-walled carbon nanotubes (MWCNT), boron doped diamond electrode (BDDE). | | | | |

Also, the flexibility of this sensor technology allow to regulate the characteristics and performance of the sensors (sensitivity and linear range) by controlling the concentration of the nanoMIPs on the electrode. To summarize, the main benefits of the present sensor technology are their robustness, originating from polymeric nature of nanoMIP, the easy integration protocols, and selectivity.

1. **Conclusion**

An electrochemical sensor was successfully devised using nanoMIPs and employed for monitoring of paracetamol plasma levels. The high affinity of the nanoMIPs towards paracetamol was confirmed by SPR analysis. The sensor displays excellent performance and no cross-reactivity against interfering compounds, as shown using DPV and SPR experiments.

Sensor shown satisfactory performance in human plasma with recoveries of 94-108 % and fast measurements of ~ 8 min in a linear concentration range between 0.1 and 1 mM. These results ensure the operability according to requirements of the Rumack-Matthew nomogram in a clinically meaningful paracetamol concentrations. Thus, they key advantages are the applicability in real scenarios, high selectivity, no saturation in the clinical relevant range, and the direct detection method avoiding cross reactivity or false positives. Previous reported sensors were no capable of meet these practical requirements. To conclude, the present technology can be potentially employed for medical diagnostic, drug monitoring and PoC testing.

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