# Electrochemical sensor for the rapid detection of *Pseudomonas aeruginosa* siderophore based on a nanocomposite platform

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## ARTICLE INFO

## ABSTRACT

The rapid and accurate detection of pathogenic agents represents a milestone for precocious diagnosis and personalized therapy in nosocomial infections. The issue can be addressed by the rational identification of certain bacteria markers, such as siderophores, that are highly involved in their metabolism and also in their interactions with the host. Developing sensors with a high sensitivity and selectivity towards this type of compounds could be an important starting point in the direction of a rapid differential diagnosis of bacterial infections. Herein we report the elaboration of a nanohybrid sensing platform based on graphene, polypyrrole and gold for the detection of pyoverdine, a marker for *Pseudomonas aeruginosa*. The composite material showed a catalytic effect towards the electronic transfer rate and enhanced active surface area, displaying a limit of detection of $0.33\,\mu M$ and a linear range of $1$–$100\,\mu M$ being obtained. The sensor was tested in the presence of common interfering molecules, found in hospital environments or in biological samples and excellent recovery rates were obtained. The selectivity of the sensor was also proved to be successful when testing on real samples such as human serum, saliva and tap water.

## 1. Introduction

A major problem of nowadays healthcare system is represented by the hospital-acquired/associated infections that increase hospitalization time and therapy costs and that are associated with a higher mortality rate [1]. Infections can be caused by several organisms, in almost 90% of the cases having a bacterial ethiology, among the pathogens being enlisted *Pseudomonas aeruginosa* (*P. aeruginosa*), *Staphylococcus aureus*, *Escherichia coli* and enterococci. Moreover, linked to the abusive use of broad-spectrum antibiotics, this issue has become a major biomedical problem, with massive economic and social implications. The prevalence of nosocomial infections has a wide variability depending on the type of health system, the available financing, the implementation of prevention and control procedures, as well as the management of existing cases. According to the World Health Organization the prevalence of healthcare-associated infections was under 10% for developed countries, while in medium and underdeveloped regions, it was significantly higher due to the lack of rapid and simple identification

methods available for epidemiologists [2].

A key point in the development of rapid tests for bacteria identification is defined by the interaction of pathogenic agents with the human host and their capacity of expressing/generating virulence factors causing the development of a specific disease [3,4]. Microorganisms have developed highly efficient iron-acquisition systems, known as siderophores, iron chelators with low molecular weight that could be exploited as biomarkers [5–7]. Siderophores are nontoxic compounds, commercially available at low costs, and their rapid and sensitive detection could be a starting point for early diagnosis and therapy management. To our knowledge, the development of electrochemical sensors on this topic is only at its beginnings, but has a valuable potential, the alternatives being represented by complicated, expensive and time-consuming methods such as mass spectrometry, Raman spectroscopy, fast Fourier transform (FFT) analysis, nanoparticle-assisted microextraction or proteomic approaches [8–11]. The association of different types of nanomaterials such as graphene, conductive polymers and noble metals, is a highly studied approach due to the excellent

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analytical properties of the nanohybrid platforms such as enhanced active surface combined with a higher electron transfer rate suitable for the detection of various biomedical compounds [12–15].

Herein we report the development of a novel disposable sensor for the detection of pyoverdine (Pyo), the siderophore of *P. aeruginosa*. The elaboration strategy of the nanohybrid platform was based on the properties generated by the association of nanomaterials: graphene, carboxylic polypyrrole (Ppy-COOH) and gold nanoparticles (Au NPs) oriented towards the target molecule. The protocol consisted of the deposition of Ppy-COOH by multipulse amperometry on a graphene-based screen printed electrode (G-SPE), followed by the electrochemical generation of Au NPs. The result was the sensitive detection of Pyo in real samples and in the presence of common interferents.

## 2. Experimental section

### 2.1. Reagents

All the chemicals were of analytical grade and were ordered from Sigma Aldrich (acetylsalicylic acid, ʟ-ascorbic acid, β-nicotinamide adenine dinucleotide phosphate reduced tetrasodium salt (NADH) and glucose, pyoverdine, pyrrole-3-carboxylic acid, $LiClO_4$, $K_4[Fe(CN)_6]$, $K_3[Fe(CN)_6]$), Fluka Chemie GmbH (uric acid, $HAuCl_4$) and Merck ($Na_2HPO_4$, $NaH_2PO_4$, KCl, NaCl). All the solutions were prepared with Milli-Q ultrapure water ($18\,M\Omega\,cm^{-1}$).

### 2.2. Platform generation and characterization

The electrochemical experiments were performed on carbon-based screen printed electrodes purchased from DropSens modified with graphite/graphene as the working electrode, carbon as the auxiliary electrode and Ag as the pseudoreference electrode. The electrochemical data were collected with Autolab PGSTAT30 and PGSTAT302N. The scanning electron microscopy (SEM) images for the surface characterization were registered on SU8230 SEM (Hitachi, Japan) at an accelerating voltage of 30 kV, 10 mA extraction current and a working distance of 11 mm from multiple points on the surface. EDS analyses and mapping were recorded using Oxford Instruments X-ray detector and AZtec software.

The sensing platform was developed in two steps, which are further explained. Firstly, generation of the Ppy-COOH from a 0.1 M $LiClO_4$ containing 25 mM of Py-COOH monomer solution was performed, using multipulse amperometry. This method consisted of alternating the potential impulses as follows: 0.8 V for 10 s, respectively 0 V for 1 s, for an overall time of 165 s. The second step was represented by the generation of Au NPs by cyclic voltammetry (CV) from 1.2 mM $HAuCl_4$ in 0.5 M $H_2SO_4$ solution. The potential was scanned 10 times from $-0.2$ V to 1.2 V vs. Ag/AgCl with a scan rate of $0.1\,Vs^{-1}$. The obtained sensor was denominated G-SPE/Ppy-COOH/AuNPs.

The electrochemical characterization of the sensor was performed by CV by scanning the potential two times from $-0.5$ V to 0.8 V vs. Ag/AgCl, with a scan rate of $0.05\,Vs^{-1}$ in 10 mM $[Fe(CN)_6]^{3-/4-}$ in 0.02 M PBS pH 7.4. The electrochemical impedance spectroscopy (EIS) experiments were performed in 10 mM $[Fe(CN)6]^{3-/4-}$ in 0.02 M PBS pH 7.4.

The analytical performances of the sensor were evaluated in the presence of the target analyte by differential pulse voltammetry (DPV) in 0.02 M PBS pH 7.4 containing different concentrations of Pyo and/or interferents. The same protocol was applied for the quantification of Pyo in real samples, such as normal human serum (Thermo Scientific), human saliva (after dilution 1:100 with the buffer solution and filtration through 0.2 μm pore diameter filter, Phenex) and tap water after a 1:1 dilution with buffer. The human saliva came from healthy volunteers.

## 3. Results and discussions

### 3.1. Elaboration of the sensor

The deposition of Ppy-COOH on the graphene layer was performed by multipulse amperometry according to a previously developed protocol [16,17]. Instead of Py monomer, Py-COOH was employed, the carboxylic functionality having an important role in diminution of interferences with negatively charged compounds when working at physiological pH values. Moreover, the deposition of the polymer on the graphene sheets stabilized the composite structure and yielded a hybrid surface for the decoration with Au NPS. After the optimization, the monomer concentration was settled at 25 mM.

### 3.2. Electrochemical characterization

The configuration was tested by CV in 10 mM $[Fe(CN)_6]^{3-/4-}$ in 0.02 M PBS pH 7.4 in order to evaluate the electrocatalytic properties of the nanocomposite material and to confirm the catalytic effect of the compounds, as stated in the literature [18,19]. As can be seen from Fig. 1A, the G-SPE presented an oxidation peak at 0.1 V vs. Ag/AgCl and a reduction one at $-0.05$ V vs. Ag/AgCl (Fig.1A, black curve (a)). After the deposition of the Ppy-COOH film, the intensity of both oxidation and reduction peaks slightly decreased and the oxidation one registered an anodic shift (Fig.1A, red curve (b)), suggesting that the electron transfer rate between the graphene substrate and the analyte was affected by the presence of the polymeric layer. The CV studies showed that the intensity of the oxidation peak was higher after the deposition of the AuNPs in both cases: on the graphene surface (Fig.1A, green curve (c))b and on the graphene/polypyrrole surface (Fig. 1A, blue curve (d)), but the best results were obtained in the last case. As expected, after the subsequent deposition of Au NPs, the signal increased significantly and the oxidation/reduction peaks registered an anodic/cathodic shift, determined by the catalytic properties of the metallic NPs that contributed to the increase of the electron density and transfer towards the graphene surface. Moreover, the deposition of Ppy-COOH also acted as a substrate for the decoration with Au NPs, generating a composite material with an enhanced active surface area and electron density. The results were in agreement with the experimental EIS data (Fig. 1B), the variation of the charge transfer resistance ($R_{ct}$) followed the same pattern as described above. Thus, the deposition of the polymeric film on the graphene layer showed the increase of the $R_{ct}$ value from 24.32 Ω (Fig. 1B, black plot (a)) to 275.98 Ω (Fig. 1B, red plot (b)), while the subsequent electrochemical deposition of AuNPs on the composite material (graphene/polypyrrole) determined an important decrease (more than 22 times fold) of the $R_{ct}$ to 12.21 Ω (Fig. 1B, blue plot, (d)). The same behaviour was observed when AuNPs were deposited directly on the working surface, the decrease being from 12.32 Ω to 10.56 Ω (Fig. 1B, green plot, (c)), substantially lower than the one observed on the composite platform. The fact could be explained by the increase in the quantity of the deposited AuNPs closely related to the enhanced surface area of the graphene/polymeric platform.

### 3.3. Evaluation of the nanohybrid platform topography

The G-SPE showed the typical nanoflake surface of a graphene screen printed electrode while the G-SPE/Ppy-COOH surface had a smooth sheet-like structure attached in a thin wrinkled film to the G-SPE base. The G-SPE/Ppy-COOH/AuNPs morphology revealed the Au NPs attached to and dispersed on the polymeric surface in a uniform distribution (Fig. 1C).

### 3.4. Analytical performance of the Pyo sensor

After the optimization step, the potential was scanned from $-0.3$ V