Synthesis and characterization of a highly sensitive and selective electrochemical sensor based on molecularly imprinted polymer with gold nanoparticles modified screen-printed electrode for glycerol determination in wastewater

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ABSTRACT

Glycerol is widely used as humectant in cosmetics to improve skin's smoothness and moisture. However, its level must be controlled in cosmetics at the risk of causing irritation or allergy. Therefore, determining glycerol concentration in environmental waters with more advanced, inexpensive and accurate sensing systems is of great importance. In this work, a fast, simple, portable and cheap molecular imprinted polymer (MIP) approach is used to develop an electrochemical sensor for glycerol determination. The MIP based screen-printed gold electrode (Au-SPE) is prepared by electro-polymerizing Acrylamide/Bisacrylamide (AAM/ NNMBA) and gold nanoparticles (AuNPs) in the presence of glycerol as a template. Techniques, such as cyclic voltammetry (CV), differential pulse voltammetry (DPV) and electrochemical impedance spectroscopy (EIS) are used for electrochemical measurements. Energy-dispersive X-ray spectroscopy (EDS) is utilized to characterize the chemical composition analysis. In contrast to its high response towards glycerol, the electrochemical sensor exhibits negligible responses when exposed to interfering species, such as glycolic acid, glycerol monostearate, tartaric acid, sodium citrate, ammonium sulfate, decyl-glucoside, caprylyl glucoside and glutamic acid. Under optimal experimental conditions, a detection limit (LOD) as low as 0.001 μg/mL (signal-to-noise ratio S/N=3) is calculated over a linear concentration range (20.00 to 227.81 μg/mL). Interestingly, the sensor was successfully applied to wastewater samples relating to glycerol determination with a relative standard deviation (RSD) less than 4%. Besides, the reproducibility, the working and storage stabilities of the sensor were proven. According to these outcomes, the electrochemical MIP sensor could be viable enough to detect the presence and levels of pollutants in real water samples.

Keywords: Glycerol; Electrochemical sensor; Gold nanoparticle; Molecularly imprinted polymer; Screen-printed gold electrode; Wastewater.

1. Introduction

Glycerol is an emollient, humectant, oral care agent, skin protection and skin conditioning agent. It is widely used in many products including toothpastes, creams, shower gels, hair care products and soaps [1]. One key point in favour of glycerol is that it prevents the premature loss of moisture from personal care products [2]. Furthermore, as an indicator of food quality, glycerol is an additive widely utilised to improve or preserve many food products [3]. As a sweetening agent, it is sometimes added to pharmaceuticals, and utilized as a tablet-coating agent. Glycerol concentration varies regarding the nature of the product and manufacturing process. Indeed, it typically ranges from 2 to 10% and from 1 to 2% in cosmetic and cleaning products, respectively [4]. However, after application of glycerol at concentrations around 10 g/100 mL, slight skin irritation occurs after 48 h [5]. At higher concentrations, it can cause severe skin irritation. Moreover, ingested glycerol is rapidly absorbed from the gastrointestinal tract [6]. A toxicity of waters containing glycerol concentrations over than 1 mg/mL has been reported [7]. Since the presence of glycerol-based products has become a habit in most people's lives, the identification and accurate measurement of glycerol, as a suspicious substance, have been also reported as a desideratum in assorted environmental studies [8].

A large amount of literature has been evolved for glycerol detection. Electrochemistry [9, 10], biosensor [11], spectrophotometry [12], chemical analysis [13] and enzymatic reactions have been deployed in this respect [14]. Among these techniques, traditional detection methods usually include high-performance liquid chromatography (HPLC), and chromatography-mass spectrometry [15, 16]. Although these techniques are known to be reproducible and sensitive for qualitative and quantitative analysis, their defects are unfortunately and inevitably obvious. However, these methods suffer from limitations, such as high cost, long and complex pre-treatment as well as the need for trained personnel [17]. In the case of biological analysis, the detection heavily relies on the test conditions (e.g., temperature and pH) because of the poor stability of biological materials, such as antibodies and enzymes. These later are very expensive, sometimes unavailable, and less reproducible [18].

Compared with the classical techniques, electrochemical methods are preferred thanks to their simplicity, high sensitivity, rapidity, energy saving devices, good stability, on-site monitoring, and low-cost instrumentation analysis [19]. Recently, molecularly imprinted polymers (MIPs) technology has received great attention. With regards to the context, MIPs, renowned types of electrochemical sensors, have been carefully considered to achieve the purpose of the study. The molecular imprinting strategy comprises polymerization of a functional monomer in the

presence of the template molecules and subsequent template extraction from the polymer network. This involves creation of cavities that are capable of selectively recognizing the target molecules in any matrix of interest [20, 21]. Among others, MIPs are commonly immobilised by different methods including the electropolymerization technique. Through electropolymerization conditions (e.g., applied voltage, cyclic scan, ratio/concentration of target/monomer, etc.), key advantage of this technique lies in the possibility to control the film thickness of the electrodeposited film and the deposition rate [22].

Nowadays, the combination of surface molecular assembly with nanostructures in the imprinting technology is very promising for enhancement and enlargement of conductivity and active surface area, respectively. In fact, AuNPs have attracted considerable attention in electrochemical studies because they allow a fast electron transfer between the redox probe and electrode surfaces [23]. Besides, SPEs have been identified as potential sensor substrates to develop systems of disposable MIPs [24]. For this reason, gold, one of the most stable metals, is widely used in the fabrication of SPEs thanks to its noteworthy stability [25].

In the present work, a MIP sensor for glycerol detection, through electropolymerization of AAM/ NNMBA, and AuNPs on Au-SPE, was developed. Given the wide use of glycerol and the need to control and monitor it, the aim of this study is the development of an electrochemical sensor based on MIP including AuNPs on Au-SPE for glycerol detection in wastewater. To our knowledge, this strategy has never been reported in the literature. Therefore, the new innovative electrochemical sensor presented in this work demonstrates that the combination of the polymer with AuNPs is an effective and sensitive approach compared to the functionalization with AuNPs on Au-SPE. In addition, relative to others (without inclusion of AuNPs), a greater enhancement of the conductivity as well as a larger active area are achieved by the proposed method as reported [26]. CV, DPV, and EIS were used as electrochemical characterisation techniques at each step of the sensor development process. The chemical composition analysis of the prepared sensor is characterized by EDS technique. The experimental parameters and analytical performance of the MIP sensor with regard to its linear range, LOD, LOQ, sensitivity, stability, and its interference behaviour were evaluated, optimized and compared to those of reported works in this topic. Finally, the sensor practical application was studied in environmental matrices (wastewater samples), and results were validated by using the spectrophotometry method.

2. Materials and methods

2.1. Reagents and solutions

Glycerol (99.5%), glycerol monostearate, ethanol, phosphate buffered saline (PBS), glycolic acid, tartaric acid, sodium periodate, acetylacetone, gold (III) chloride trihydrate ($HAuCl_4.3H_2O$) (99.99%), trisodium citrate, ammonium sulfate, decyl-glucoside, caprylyl glucoside, and glutamic acid were purchased from Sigma Aldrich. *N'*-methylene-bisacrylamide (NNMBA), acrylamide (AAM), *N, N, N', N'*-Tetramethyl-ethylenediamine (TEMED), ammonium persulphate (APS), potassium ferricyanide $K_3[Fe(CN)_6]$ and potassium ferrocyanide $K_4[Fe(CN)_6]$ were all obtained from Fluka. Sodium citrate was from Riedel de Haën. PBS solution (0.01 M, pH 7.0) was prepared by dissolving a PBS tablet in 200 mL of distilled water (DW). A standard solution of glycerol (1 mg in 1 mL of DW) was diluted using DW to obtain less concentrated glycerol solutions over the adopted range (20.00 to 227.81 µg/mL). All the experiments were carried out at room temperature (25°C).

2.2. Sample preparation

Domestic wastewater samples were collected from three different wastewater sites of Meknes (Morocco), and its surroundings. Prior to measurement, the samples were mixed using a microfuge and then filtered through membrane filters of 0.45 µm pore size before immediately performing the analysis. A volume of 50 µL of wastewater samples was exposed to the Au-SPE/ MIP+AuNPs sensor.

Tap water sample free of glycerol was also collected from our laboratory. Indeed, the tap water sample is aliquoted into three and then spiked with different glycerol concentrations (20.00, 30.00 and 50.00 µg/mL). After thoroughly stirring, a volume of 50 µL of each spiked aliquot was deposited onto the working electrode. The standard addition method was utilized to analyse the tap water samples to generate accurate results.

2.3. UV-Vis spectrophotometry analysis

Absorbance measurements were performed by using a UV–Vis spectrophotometer. The domestic water samples were analysed via an ANACHEM instruments UV220 spectrophotometer by using a quartz cell (1 cm path length). Each wastewater sample was pre-treated by adding 1.2 mL of a sodium periodate solution (10 mM). Furthermore, 1.2 mL of acetylacetone solution (0.2 M) was added to the previous solution. The absorbance of the obtained solution was finally read in a spectrophotometer at a wavelength of 410 nm [27].

The glycerol levels of each sample were calculated using a linear calibration curve derived from synthetic glycerol measurements.

2.4. Synthesis of AuNPs

AuNPs were prepared by using citrate reduction method. For this purpose, $HAuCl_4.3H_2O$ was dissolved in DW (0.005 g/50 mL) and the obtained solution was heated. As the solution began boiling, 0.5 mL of trisodium citrate (0.5 g/50 mL) was added in a dropwise fashion until the colour changed from yellow to deep red, indicating the formation of AuNPs. Here, trisodium citrate is used as a reducing and stabilizer agent [28].

Furthermore, the AuNPs size was measured using an optical technique named, Dynamic Light Scattering (DLS). This measurement was performed by placing 500 µL of the AuNPs solution in a plastic cuvette at 25°C. The obtained AuNPs size was 131.8 nm. The solution was left in dark until use.

2.5. Preparation of MIP and non-imprinted polymer (NIP) sensors

First, pre-treatment of the Au-SPE was performed by rinsing with ethanol and DW. Second, in a 1:1 volume ratio, AAM (11 mg/mL) and NNMBA (71 mg/mL) were mixed in a PBS buffer (0.01 M, pH 7.0). In addition, 1 mL of APS solution (13 mg/mL) and 20 µL of TEMED (5%) were added to the previous mixture to initiate and accelerate polymerization, respectively. A volume of 1 mL glycerol (1 mg/mL in DW) was also added to the previously obtained solution. The final chemical mixture is called P_1.

Electropolymerization processes were chosen to design the MIP. It was performed using CV technique over a potential range between -0.2 to 1.2 V at a scan rate of 100 mV/s for 10 cycles.

For the first sensor elaboration, a small drop (50 µL) of P_1 was electropolymerized on Au-SPE and the obtained sensor is denoted Au-SPE/ MIP.

Since AuNPs have notable electrical properties, and are expected to improve the active surface, electron transfer rate and electrode sensitivity, the effect of their inclusion on the MIP sensor preparation was investigated. Indeed, an AuNPs solution was prepared.

Then, for the second sensor fabrication, the Au-SPE was first functionalized with AuNPs (50 µL). Then, 50 µL of P_1 was electropolymerized to obtain the Au-SPE/ AuNPs/ MIP sensor. To prepare the third sensor, P_1 is mixed with 1 mL of AuNPs. A volume of 50 µL of the obtained solution is used for the electropolymerization to fabricate the Au-SPE/ MIP+AuNPs sensor.

A judicious choice of functional monomer is crucial for successful imprinting, which requires a solid binding of the template. AAM, which is hydrophilic in nature, provides multiple functional groups for hydrogen bonds. This allows it to form a solid bond with several substances. For the electropolymerization process, AAM as a functional monomer was chosen because it contains -NH_2 groups that offer a rapid and very specific interaction through hydrogen bonds with the -OH groups of glycerol [29]. In addition, AAM is stable, inexpensive and can be polymerized under mild conditions. Indeed, the functional monomer, the crosslinking agent, gold nanoparticles and the template molecule interact in solution. After the electro-polymerization step on the electrode surface, the AAM spatially forms a mesh around the glycerol template leading to a homogeneous structure. This later contains recognition sites formed after hydrogen bonds between the AAM and glycerol. This reduces the activity of non-specific bonds. Thus, a sensitive mimetic layer (AAM-Glycerol) was obtained.

Then, the glycerol extraction step was carried out with ethanol for 20 minutes at room temperature (25°C) [30]. After thoroughly washing with DW, the MIP sensor was ready for use.

As a control test, the NIP sensor was prepared in the same procedure but without adding glycerol to the electro-polymerization process.

2.6. Characterization methods

All electrochemical experiments were performed using a Potentiostat (Palmsens^3, Spain) interfaced with a computer. The MIP sensor was designed on a standard Au-SPE (DropSens, DRP-C223BT) configuration consisting of a gold working electrode (0.19 cm^2), a gold counter electrode (0.54 cm^2) and a silver reference electrode. Parameters, such as potential range from -0.4 V to 0.6 V and a scan rate of 50 mV/s were used for electrochemical characterization by CV. Differential pulse voltammograms were recorded over a potential range from -0.3 to 0.3 V and a scan rate of 100 mV/s. The EIS measurements were carried out at a frequency range of 0.1 Hz - 50 kHz. The Nyquist plots were recorded at a DC potential of 0.01 V/ref and an AC potential of 25 mV. The charge-transfer resistance (R_{ct}) was determined after a best fit with the simple Randles equivalent circuit using the implemented EIS spectrum analyzer software. All the electrochemical characterisations were performed using a solution of $[Fe(CN)_6]^{3-/4-}$ as redox probe to study the performance of the prepared sensor [31]. For this purpose, 50 µL of $[Fe(CN)_6]^{3-/4-}$ was dropped onto the surface electrode.

Moreover, the chemical composition analysis of the electrode was carried out using EDS (FEI Quanta 250).

3. Results and discussion

3.1. Electrochemical characterization of the different modified electrodes

In this study, three different sensors are developed, and their sensitivity are compared. The first sensor (Au-SPE/ MIP) was developed by a direct immobilization of the MIP. The second sensor (Au-SPE/ AuNPs/ MIP) was fabricated by first modifying the electrode surface with AuNPs and then the MIP was immobilized. The third sensor (Au-SPE/ MIP+AuNPs) was developed by immobilizing the complex of MIP+AuNPs onto the electrode surface. After the development of these three sensors, the CV method is operated in a potential range of -0.4 V to 0.6 V at 50 mV/s using a PBS buffer (supporting electrolyte at pH = 7) containing 5 mM $[Fe(CN)_6]^{3-/4-}$. Fig. 1A shows all the corresponding cyclic voltammograms.

The unmodified gold electrode (Au-SPE) shows a reversible redox signal of the $[Fe(CN)_6]^{3-/4-}$. It can be seen that the signal corresponding to Au-SPE/ MIP admits an anodic current amplitude (I_{pa}) of about 20 µA with a peak separation ($\Delta E_p = E_{pa} - E_{pc}$) of about 0.57 V. For the Au-SPE/ AuNPs/ MIP sensor, an increase of current ($I_{pa} \approx 40$ µA) with ($\Delta E_p \approx 0.45$ V) is observed. It is noted that the third sensor (Au-SPE/ MIP+AuNPs) admits a higher anodic current I_{pa} of about 60 µA with ($\Delta E_p \approx 0.27$ V) compared to those of the two previous sensors. Indeed, ΔE_p of sensors with AuNPs are lower than that without AuNPs. In other words, the reason for these lower ΔE_p values could be attributed to the presence of AuNPs. While making the sensors more sensitive, AuNPs play a key role in rate of electrons transfer. As marked, ΔE_p of Au-SPE/ MIP+AuNPs is lower than Au-SPE/ AuNPs/ MIP, suggesting a faster electron transfer of $[Fe(CN)_6]^{3-/4-}$. This suggests that for better sensitivity, the AuNPs should be mixed with the polymer complex.

Moreover, the importance of AuNPs inclusion are justified by exposing several concentrations of glycerol on the three different sensors. As shown in Fig. 1B, sensitivities of -0.05, -0.106 and -0.79 (µA/ (µg.mL^{-1})) are obtained for Au-SPE/ MIP, Au-SPE/ AuNPs/ MIP and Au-SPE/ MIP+AuNPs, respectively. As results, it is found that the sensitivity of the Au-SPE/ MIP+AuNPs sensor is 16-fold more sensitive than that of the Au-SPE/ MIP. Then, the Au-SPE/ MIP+AuNPs sensor with higher sensitivity to glycerol was selected as the appropriate sensor for all subsequent measurements in this manuscript.

Elsewhere, the active surface area was calculated by taking into consideration the anodic current peak (I_{pa}) and using the Randles-Sevcik Eq. (1) [32].

$$I_{pa} = 2.69 \cdot 10^{5} n^{3/2} A D^{1/2} C \upsilon^{1/2} \qquad Eq.\ (1)$$

Where n is number of electron transfer involved (n = 1 for the used redox probe), A is the electrode surface area (cm^2), D is the diffusion coefficient of ferrocyanide taken to be D = $7.6 \cdot 10^{-6}$ cm^2/s [33], C is the $[Fe(CN)_6]^{3-/4-}$ concentration ($5 \cdot 10^{-6}$ mol/cm^3) and υ is the scan rate (V/s).

As results, the surface area was calculated to be A = 0.02 cm^2 for Au-SPE/ MIP, A = 0.04 cm^2 for Au-SPE/ AuNPs/ MIP and A = 0.08 cm^2 for Au-SPE/ MIP+AuNPs. To summarize, the electro-kinetic analysis shows that the inclusion of AuNPs significantly improves the active area. In addition, the active area of the Au-SPE/ MIP+AuNPs sensor is higher than that corresponding to the Au-SPE/ AuNPs/ MIP sensor.

3.2. Preparation of glycerol imprinted sensors

Electropolymerization is a straight way to prepare MIPs on a conducting surface electrode. This was carried out using an electrolyte solution of PBS (pH = 7) within a potential range from -0.2 to 1.2 V at a scan rate of 100 mV/s for 10 cycles. The imprinted film formation was monitored by changes in the current amplitude per cycle. Indeed, the recorded cyclic voltammograms of the electropolymerization for MIPs and NIPs are recorded in Fig. 2.

Fig. 2A clearly shows an obvious oxidation peak at about +0.1 V, indicating that glycerol molecules were successfully imprinted into the MIP. However, as expected, non-oxidation peaks are observed during the electropolymerization in the absence of glycerol (NIP) (Fig. 2B). In the both cases, the current peaks decrease while increasing the cycle number from the 1^{st} to the 10^{th}, suggesting that the imprinted compact polymeric film was successfully deposited on the electrode surface.

During the MIP electropolymerization, hydrogen bonding occurs between the glycerol hydroxyl groups (-OH) and amine groups ($-NH_2$) of polyacrylamide. This process is capable of generating cavities in a large area because of the AuNPs' presence, which allow the sensitive recognition of glycerol. The schematic view (Schematic 1) of the mechanism is provided for better understanding of the readers.

3.3. EDS characterization

EDS technique was utilized to determine the chemical composition of the electrode surface. Indeed, Fig. 3 shows the recorded spectra of the atomic composition of the sensor during its

elaboration steps. The EDS spectra of the bare Au-SPE indicates 1.92 wt% of aluminum (Al), and a presence of gold (Au) elements in large quantities (90.74 wt%), which confirms that the electrode surface was not modified (Fig. 3A). The Au-SPE/ MIP+AuNPs spectra shows peaks of carbon (C), potassium (K), sodium (Na), phosphate (P), sulfide (S), oxygen (O) and gold (Au) in percentages of 7.77 wt%, 0.12 wt%, 5.64 wt%, 6.72 wt%, 6.16 wt%, 29.15 wt% and 40.21 wt%, respectively. This could be attributed to the presence of moieties constituting the complex (AAM/ NNMBA, glycerol, and AuNPs) (Fig. 3B). In this figure, aluminum atoms are not detected in the point of interest, demonstrating that the polymer deposit was homogeneous. Furthermore, the O content is higher, proving the presence of the glycerol molecules. Indeed, the presence of O at lower percentage (16.42 wt%) shows that most of the glycerol molecules were removed from the Au-SPE/ MIP+AuNPs matrix (Fig. 3C). These EDS spectra confirm that the electropolymerization in the presence of glycerol was well performed and indicated that MIP was successfully immobilized on the electrode surface. Therefore, from these outcomes, it could be concluded that the polymer layer deposition and extraction of the glycerol molecules were successfully done.

3.4. Electrochemical characterizations

CV technique was used to characterize the electrochemical behaviour of the bare Au-SPE, Au-SPE/ MIP+AuNPs (before template removal), Au-SPE/ MIP+AuNPs (after template removal) and Au-SPE/ NIP+AuNPs.

As expected, a pair of redox peaks was observed for Au-SPE/ MIP+AuNPs (before template removal). As shown, Fig. 4A displays a cyclic voltammogram of the redox probe, which is different in shape and magnitude compared to that corresponding to bare Au-SPE. This demonstrates that the electrode surface is well modified. However, after removal of template molecules, the voltammogram of the electrode Au-SPE/ MIP+AuNPs (after template removal) admits a highest current peak. This is because some holes become available for the electrochemical reaction of active probe to take place at the electrode surface due to removal of glycerol from the MIP film. The Au-SPE/ NIP+AuNPs exhibits an anodic current peak less than that of Au-SPE/ MIP+AuNPs (after template removal). This may be explained by the absence of glycerol (a negatively charged molecule) and the absence of holes [34].

Besides, EIS is well known to be a useful technique to study the impedance changes in electrode surfaces during the modification process [35]. The faradaic impedance measurements are in good agreement with the CV results as the diameter of semicircles (the

Nyquist diagrams) correlate with variations of the oxidation current peaks inferred by the CV technique (Fig. 4B).

These results demonstrate that the imprinted film had a stronger affinity to glycerol, which was ascribed to the specific binding sites in the MIP film.

3.5. Optimization of experimental parameters

In order to develop a sensor with high performance, important parameters, such as the number of cycles for electropolymerization, the pH value of the electrolyte, the extraction time and the incubation time have been previously optimized.

3.5.1. Cycles for electropolymerization

The thickness of the MIP film is indeed an important factor influencing the sensitivity and stability of a MIP sensor. In this study, the appropriate number of electropolymerization cycles is investigated for the fabrication of the Au-SPE/ MIP+AuNPs sensor. For this purpose, numbers of cycles ranging from 7 to 15 are used. After each of these cycle numbers, the glycerol molecules are extracted and the current peaks of the ferrocyanide generated by the sensor are shown in Fig. 5A. As seen, the sensor current peaks increase up to 10 cycles. This finding can be explained by an increasing number of imprinted sites, which facilitate the access of the ferrocyanide to the electrode surface. Nevertheless, beyond 10 cycles, a high resistance to charge transfer is formed. This makes the electrode surface compact and the number of sites no longer increases. For this reason, the current peaks of the ferrocyanide decrease. Through these observations, the highest sensitivity for the detection of glycerol is obtained for 10 cycles.

Additionally, the electropolymerization process is operated at different potential intervals and the best response is obtained at potential range from -0.2 to +1.2 V.

3.5.2. Effect of pH

The pH of the supporting electrolyte is a crucial key factor in achieving an optimized electrochemical response of the MIP sensor. Therefore, the pH of the PBS containing ferrocyanide has been studied and optimized in order to have a better charge transfer. For this purpose, pH values ranging from 5 to 10 were used. As can be seen in Fig. 5B, pH 7.0 gave a higher signal value and was chosen as an optimum pH for all characterizations in the subsequent studies.

3.5.3. Extraction and incubation time

In order to enhance the sensitivity, selectivity, and reproducibility of the electrochemical sensor, the duration of glycerol extraction step so as to create the recognition cavities are also investigated. Fig. 5C shows the variation of the current at different extraction times (from 10 min to 30 min). As shown, from 10 to 20 min, the current increases in accordance with the extraction time. After 20 min, no current variation is observed. Thus, 20 min was chosen as the best removal duration of the glycerol molecules.

Similarly, the effect of incubation time after synthetic glycerol concentrations deposit is studied using durations ranging from 10 to 50 min at room temperature (Fig. 5D). The best optimization is achieved for a period of 30 min as optimal incubation time.

3.6. MIP and NIP responses

Compared to CV, the DPV technique is more sensitive and has been extensively applied to electrochemical detection [36]. After the template removal, different synthetic concentrations of glycerol solution were subsequently utilized in order to determine the retention capability of the developed MIP sensor. Indeed, the electrochemical and synthetic-detection of glycerol was achieved by dropping 50 µL of a glycerol concentration solution onto the working electrode of the MIP sensor. After each incubation time (30 min), DPV and EIS characterizations techniques were performed using PBS containing 5 mM $[Fe(CN)_6]^{3-/4-}$ solution at room temperature.

By using the DPV technique, the obtained voltammograms are plotted in Fig. 6A. By increasing glycerol concentrations, a decrease in the current peaks of $[Fe(CN)_6]^{3-/4-}$ signal is observed. Upon increasing the concentration of glycerol, more imprinted sites were rebound with glycerol molecules at the surface of the sensor. This explains the obtained outcomes in Fig. 6A.

Moreover, a NIP test was performed to confirm the sensor responses during the MIP test. The objective was to control experiments without glycerol addition during the electropolymerization stage. Fig. 6B represents the DPV signals recorded during the detection of synthetic glycerol concentrations by the non-imprinted device. The oxidation current peaks remain almost unchanged for the different glycerol concentrations. This confirms that the absence of specific cavities able to recognize glycerol results in the lack of sensitivity of the NIP sensor.

The linear relationship between the current peaks and their corresponding concentrations is illustrated in Fig. 6C and Fig. 6D. These figures show the calibration curves of MIP and NIP sensors. The relative variation of $(I_c-I_0)/I_0$ is plotted in function of logarithmic

concentration of glycerol in a linear range of 20.00 to 227.81 µg/mL. As shown in Fig. 6, the equations expressing the relationship between the relative currents and their corresponding concentrations for MIP and NIP are y= -0.58 Log C + 0.33 and y= -0.08 Log C - 0.002, respectively. The LOD is calculated using LOD = 3 Sb/m, where Sb is the standard deviation of the y-intercept of the regression line and m is the slope of the calibration curve, based on signal-to-noise ratio (S/N=3) [37-39]. For the DPV technique, good linearity (R^2 = 0.99) is observed over the study range with analytical parameters, such as sensitivity, LOD and LOQ of 0.58 mL/µg, 0.001 µg/mL and 0.025 µg/mL, respectively.

According to EIS technique, the charge transfer resistances (R_{ct}) of the MIP sensor increase with an elevation of glycerol concentration as shown in Fig. 7A. Glycerol molecules are known to be non-conductive and negatively charged [34]. The characterization, using a negative redox probe $[Fe(CN)_6]^{3-/4-}$, therefore, involved repulsive interaction between it and the glycerol molecules. This could explain the decrease in the recorded current peaks and correspondingly the elevation in R_{ct} values while increasing glycerol concentration.

In contrast, the negligible variations in R_{ct} observed for NIP sensors, reveal the minimal, non-specific interaction existing between the polymer and glycerol (Fig. 7B).

A logarithmic linear relationship between electrochemical sensor responses and glycerol concentration is obtained using EIS. The value of $(R_c-R_0)/R_0$ was calculated for each glycerol concentration. As shown in Fig. 7C and Fig. 7D, the equations expressing the relationship between the R_{ct} and their corresponding concentration for MIP and NIP are y= 0.46 Log C - 0.33 and y= 0.09 Log C + 0.71, respectively. EIS results are in good consistency with those of DPV. The normalized data for the MIP and NIP sensors showed good linearity with R^2 of 0.99 and 0.98, respectively. The LOD and LOQ using EIS technique are calculated to be 0.11 µg/mL and 0.37 µg/mL, respectively.

A comparison of experimental data between the relevant MIP sensor and previously reported methods [8, 9, 10, 40, 41] for glycerol determination is summarized in Table 1. The proposed MIP sensor exhibits a high accuracy, a lower LOD, and a higher sensitivity towards glycerol traces detection compared with reported works. Although these works were well performed, the majority of them need expensive reagents, organic solvents for detection, and long synthetic protocols with sometime a problem of over-potential. These disadvantages significantly limit their use in wearable systems. However, the sensor developed in this work departs from all these drawbacks. Moreover, we could hardly find literature on glycerol detection in wastewater that develops a technology similar to the one described in this work with a high sensitivity, and a process as simple as that. As conclusion, with an economical

391 cost, the MIP sensor is easily developed while admitting satisfactory analytical results, such
392 as good stability, sensitivity and specificity.

393 ***3.7. Selectivity, reproducibility and stability of the MIP sensor***

394 To be practically viable, the sensor should exhibit little or nil responses towards the
395 interfering species, suspected to be present with glycerol in wastewater. Responses of the
396 developed sensor toward glycerol were first compared with those obtained in the presence of
397 the same concentrations of the interfering species including glycerol monostearate, glycolic
398 acid, tartaric acid, sodium citrate, ammonium sulfate, decyl-glucoside, caprylyl glucoside and
399 glutamic acid. The obtained results are summarized in Fig. 8. As a result, the slopes of the
400 interfering species are found significantly lower than that of glycerol, which demonstrates the
401 MIP sensor selectivity toward glycerol.
402 Second, the existence of matrix effect in the samples was studied by the mixed method [42]
403 using a ratio of glycerol and each interfering substance (1:10). By using the DPV technique,
404 the sensor responses are recorded and the relative errors ($\Delta I_m/\Delta I_o$) are calculated, where ΔI_m is
405 the normalized current when the competitor was included, while ΔI_o is the normalized current
406 without the competitor. Accordingly, Table 2 summarizes the effects of the interfering
407 species. The range of $\Delta I_m/\Delta I_o$ values comprise between 60.97% and 92.01%. Based on these
408 experimental results, it is found that 10-fold competitors did not significantly interfere with
409 the determination of glycerol.
410 In order to explore the reproducibility test, five MIP sensors were developed separately under
411 identical conditions. Responses from these sensors were investigated during exposure to a
412 glycerol concentration of 20.00 µg/mL. Indeed, the sensor responses are recorded with a
413 relative standard deviation (RSD) of 5%, indicating good reproducibility of the measurement.
414 The working and storage stabilities of the proposed sensor were also investigated. As shown
415 in Fig. 9A, for several measurement times, there is no obvious decay in the anodic current
416 peaks for successive responses of the sensor using a 20.00 µg/mL glycerol solution. A rinsing
417 step was performed after each measurement. As a result, an RSD of 4.6% is found, suggesting
418 the good working stability of the proposed sensor for glycerol detection.
419 Furthermore, the storage stability test was evaluated by measuring the MIP sensor responses
420 using DPV at 20.00 µg/mL of glycerol. The sensor was stored at 4°C in a refrigerator in PBS
421 (pH = 7.0) solution during the stability test. The results depicted in Fig. 9B indicate that after
422 60 days, the sensor response decreased to 86% compared to its initial response. This means
423 that the proposed sensor has good storage stability.

3.8. Recovery in tap water

To demonstrate the reliability and accuracy of the proposed method for the real samples application, tap water samples were analysed using the standard addition method. The tap water sample is aliquoted into three and then spiked with different glycerol concentrations (20.00, 30.00 and 50.00 µg/mL). An appropriate volume of the aliquot (50 µL) was placed in the working electrode for the determination of glycerol. After a period for incubation, the electrode was rinsed and an electrochemical characterization was performed by using DPV technique. The oxidation current peaks obtained from the sensor responses are inserted to the calibration equation (y= -0.58 Log C +0.33) in order to determine the content of glycerol. Satisfactory recoveries are obtained from 98.26 to 107.03% of glycerol concentrations with an RSD less than 3%. The results are listed in Table 3. In summary, the most obvious results of this study are that the developed sensor could be successfully applied to the detection of glycerol in tap water.

3.9. Application in real samples

As a final step, to ascertain practical applicability of the proposed method, the MIP sensor was used for glycerol analysis in wastewater samples and the experimental results are shown in Table 4. Accordingly, the obtained results from the proposed sensor by DPV technique are in good agreement with those obtained by using a spectrophotometry method as reference method. An RSD lower than 4% is found, which reveal the proposed MIP sensor reliability with respect to glycerol determination. An RSD less than 6% was also obtained by using the spectrophotometry measurements. The results clearly show that the developed sensor could be potentially used in the preparation of portable electrochemical sensors in environmental experiments.

4. Conclusions

In conclusion, the present research study proposes, for the first time, a portable electrochemical sensor employing a matrix of polyacrylamide and AuNPs coated on Au-SPE for glycerol analysis. Correspondingly, the MIP sensor shows a LOD calculated to be 0.001 and 0.11 µg/mL by DPV and EIS, respectively over a working range of 20.00 to 227.81 µg/mL. Similarly, LOQ of 0.025 µg/mL and 0.37 µg/mL are obtained by using DPV and EIS, respectively. One of the significant findings, which emerges from this study, is that the MIP sensor device is 7-fold more sensitive than NIP with a particularity of its simplicity of implementation, its portability and low cost. The developed sensor displays high sensitivity

and selectivity, short response time, good reproducibility, working and storage stabilities. Moreover, better analytical parameters are found in comparison with previously reported works. Taking into account all these outcomes, the MIP sensor well demonstrates its ability towards glycerol determination in tap water and wastewater samples. Spectrophotometry was utilized as a validation method. Of greater significance, this approach for achieving a highly sensitive and selective glycerol electrochemical sensor could be easily extended for other applications in environmental fields.

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Figure captions

Fig. 1. Electrochemical comparison in 5 mM $[Fe(CN)_6]^{3-/4-}$ in PBS buffer at pH 7.0 of: Au-SPE, Au-SPE/ MIP, Au-SPE/ AuNPs/ MIP and Au-SPE/ MIP+AuNPs: (A) Cyclic voltammograms and (B) Calibration curves.

Fig. 2. Electropolymerization of (A) MIPs electrode and (B) NIPs electrode using cyclic voltammograms.

Schematic 1. Procedure of the MIP sensor preparation.

Fig. 3. EDS results of: (A) Bare Au-SPE, (B) After electropolymerization and (C) After glycerol extraction.

Fig. 4. Electrochemical characterization in PBS buffer (pH 7.0) containing 5 mM $[Fe(CN)_6]^{3-/4-}$ of Au-SPE, Au-SPE/ MIP+AuNPs (before template removal), Au-SPE/ MIP+AuNPs (after template removal) and Au-SPE/ NIP+AuNPs: (A) Cyclic Voltammograms and (B) Nyquist plots.

Fig. 5. Optimization of factors affecting the performance of the MIP sensor in PBS buffer (pH 7.0) containing 5 mM $[Fe(CN)_6]^{3-/4-}$: (A) Effect of the cycles number, (B) pH, (C) Extraction and (D) Incubation times on the current response.

Fig. 6. Differential pulse voltammograms in 5 mM of $[Fe(CN)_6]^{3-/4-}$ obtained after glycerol detection at different concentrations on (A) MIP and (B) NIP sensors; Normalized calibration curves corresponding to (C) MIP and (D) NIP sensors.

Fig. 7. Nyquist impedance plots in 5 mM of $[Fe(CN)_6]^{3-/4-}$ for glycerol detection at different concentrations on (A) MIP and (B) NIP sensors; Normalized calibration curves of (C) MIP and (D) NIP.

Fig. 8. Sensor responses toward interfering molecules.

Fig. 9. (A) Working and (B) Storage stabilities of the MIP sensor.