

Article

Molecularly Imprinted Electrochemical Sensor Electrodes Based on Poly-Pyrrole for Sensitive Detection of Morphine in Wastewater

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Abstract

Morphine is an opioid extracted from the poppy plant and highly effective for moderate to severe pain management. Development of techniques to measure the concentration of this highly addictive drug in various matrices is very important. This work was aimed at the development of a sensitive electrochemical method for detection of morphine in wastewater. Molecularly imprinted (MIP) electrodes were made by the electro-polymerization process using pyrrole as a monomer. Electro-polymerization was performed on glassy carbon electrodes in the presence of morphine before the extraction of the entrapped morphine molecules. Various techniques were employed to monitor the polymerization and response of the fabricated electrodes toward morphine. These techniques included Fourier transform infrared spectroscopy (FTIR), cyclic voltammetry (CV), square wave voltammetry (SWV), and electrochemical impedance spectroscopy (EIS). The morphine concentration was determined using SWV and CV by measuring the change in the redox peak current of $[\text{Fe}(\text{CN})_6]^{-3/-4}$. These MIP electrode sensors were used to analyze morphine concentrations between 0 and 80.0 nM solution. The SWV showed a wider linear response region than CV. The detection limit using SWV was found to be 1.9 nM, while using CV, the detection limit was 2.75 nM. This MIP electrode sensor exhibited specificity when other closely related molecules were included and hence has potential as a cheap alternative technique for analysis of morphine.



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1. Introduction

Opioid dependence and overdose have contributed to the dramatic rise of fatalities in the world. Morphine, one of the opioids commonly used as analgesics for severe pain, is used following surgery [1] and is highly effective for moderate to severe pain management. It is an alkaloid and a naturally occurring phenanthrene derivative directly extracted from the poppy plant. Morphine overuse causes addiction and can also promote breast tumor growth [2]. Heroin, a potent synthetic opiate analgesic, is obtained from morphine and is one of the most abused opioids. Morphine is the parent compound in the synthesis of other opioids, including hydromorphone and oxycodone drugs. There is renewed effort toward developing and improving methods to screen blood and urine samples for opioids. Rapid analyses of opiates in biological specimens in illicit samples and in other matrices, including wastewater, are still a great challenge. Therefore, devices/tools that enable rapid measurements of these opioids are needed.

Morphine can be found in free form or conjugated to glucuronide, forming active morphine-6-glucuronide. Another metabolite of morphine is the inactive morphine-3-glucuronide [3]. Metabolism of morphine in vivo proceeds through glucuronidation of the 6-OH alcoholic group and the 3-OH phenolic group. The molecular structure of morphine is shown in Figure 1. Several analytical methodologies have been described for the quantification of morphine on its own or in combination with other metabolites. Common methods for morphine quantification include HPLC [4], gas chromatography mass spectrometry [5], morphine-specific immunosorbents coupled to HPLC [6], high-performance liquid chromatography–tandem mass spectrometry (HPLC/MS-MS) [7], chemiluminescence [8], and surface plasmon resonance [9], among other techniques. HPLC combined with electrochemical detection (HPLC–EC) is a sensitive and selective method achieving a sensitivity of less than 50 pg/mL [10]. Although chromatography-based techniques are the traditional methods of opioid analyses, long analysis times make them quite expensive. Compared to chromatographic techniques, electrochemistry-based methods present significant advantages in terms of cost, simplicity, and ease of operation. Searching for cheap, stable, and sensitive alternatives for rapid opioid detection is very important. Morphine in serum or plasma is particularly difficult to measure due to the fact that the molecule itself is relatively hydrophobic [11]. Sensing platforms that use nanomaterials such as gold and silver for measuring morphine and other related opioids have been developed [12,13]. More importantly, biologically based chemical receptors such as immunoassays, which utilize antibody–antigen affinity for detecting biological samples, are other popular methods for morphine detection [14]. Molecular imprinting techniques in combination with electrochemical detection are powerful tools for morphine detection [15,16]. Morphine molecular imprints mimic binding activity to specific receptors. These molecular imprints, also known as plastic antibodies, are polymers capable of specific binding, akin to the “lock-and-key” mechanism. Therefore, these molecular imprints are cost-effective alternatives to biomolecule-based recognition techniques. In addition, they are easy to prepare and have a high affinity for targeted molecules and hence possess superior characteristics as compared to antibodies or enzymes. This is especially so if the measurements are to be carried out at extreme temperatures or other variable chemical conditions [17]. Non-specific binding is usually a drawback for molecular printing technology. However, the extent of nonspecific binding is determined by the quality of MIP, which is in turn determined by the kind of polymer used in imprinting. Molecular imprints are formed through polymerization in the presence of target molecules. The target molecule is thoroughly mixed with monomer followed by copolymerization using either a chemical method or electro-polymerization [18,19]. The target molecule is then extracted from the polymer by dissolving the trapped target molecule in a suitable solvent. Extraction of the target molecules/template results in the formation of three-dimensional cavities within a polymeric matrix. The three-dimensional cavities have the size, shape, and functional group orientation of the target molecule. Therefore, the target molecules can selectively occupy the cavity space. Pyrrole used here is a monomer easily electro-polymerized in acidic solution. Pyrrole is therefore suitable as a molecular mold to trap other hydrophobic target molecules. The advantage of using conducting polymers like poly-pyrrole is the fact that it is easy to monitor and control the deposition of the monomer, providing a uniform growth [20] and hence easy to control target entrapment. Imprinted electrodes based on pyrrole monomers have been developed for recognition and separation of chiral phenylalanine [21] and methyl orange [22]. Electro-polymerized pyrrole has been used for fabrication of other sensors, including DNA sensors [23], teriflunomide [24], ammonia gas [25], human IgG [26], catechol [27], ascorbic acid (AA), and uric acid (UA) [28], among other analytes. Morphine is easily oxidized on modified carbon electrodes. Using Ag/AgCl

as a reference electrode, the oxidation potential of morphine was 0.75 V [29]. The oxidation peak current can easily interfere with the desired peak current signals while using some redox probes with close redox potentials. $[\text{Fe}(\text{CN})_6]^{-3/-4}$ redox probe possesses much lower oxidation and reduction potentials compared to morphine and therefore can be used as a reporter molecule. Morphine competes with the redox probe for the imprinted recognition sites. A decrease in peak current was obtained when the sensor was immersed in morphine-containing solution. The magnitude of the current decrease was a function of the concentration of morphine in the solution. This is the first report of pyrrole monomers being used to make imprinted electrodes for morphine detection. The analytical performance of this MIP sensor in its application to the quantitative analysis of morphine in wastewater samples was assessed.

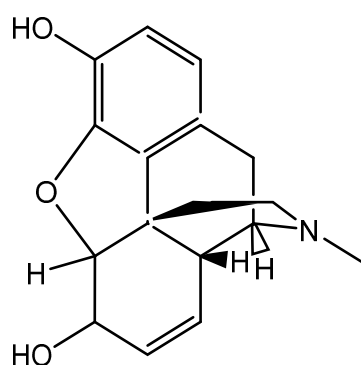


Figure 1. Molecular structure of morphine.

2. Materials and Methods

2.1. Chemicals

Morphine standard was purchased from VWR (Radnor, PA, USA), while pyrrole was purchased from Sigma-Aldrich (St. Louis, MO, USA). Potassium ferricyanide, potassium ferrocyanide, sodium acetate, acetic acid, and sulfuric acid were also purchased from VWR. Wastewater samples were obtained from the local municipality. All other aqueous solutions used here were prepared using deionized water.

2.2. Electrochemical Apparatus

Electrochemistry was conducted using an electrochemical workstation (CHI 660c, Austin, TX, USA). This instrument was computer controlled and had its ohmic drop (IR) 98% compensated. Three techniques were carried out using this equipment, namely, cyclic voltammetry (CV), square wave voltammetry (SWV), and electrochemical impedance spectrometry (EIS). Glassy carbon (diameter of 3 mm) was used as a working electrode, while Ag/AgCl was used as a reference electrode. A platinum electrode was used as an auxiliary/counter electrode. The geometric area of the working electrode was 0.07 cm². The area was sufficient as a platform where imprinting using polymers was carried out. All three electrodes were purchased from Bioanalytical Systems Inc. (West Lafayette, IN, USA).

2.3. Preparation of MIP Electrode Sensor

For proper adherence of the imprint on the glassy carbon electrodes, the electrodes were thoroughly polished using standard cleaning procedures. The cleaning procedures included ablating the electrode using a 1 µm diamond paste. The particles generated after using diamond paste were removed by ultrasonication in ethanol for one minute. The electrode was then ultrasonicated in distilled water for another minute before drying in air. Polishing using alumina was carried out as a final cleaning procedure. The alumina

particles on the carbon electrodes were removed by washing with water. The clean electrode was used as a platform to make MIP sensor electrodes. A mixture of 1.0 mL of 2 µg/mL of morphine was mixed with 1.0 mL of 3.0 mM pyrrole. Moreover, 0.5 mL of 0.1 M H₂SO₄ was also added, and the mixture was thoroughly stirred. The solution was kept refrigerated until ready for use in MIP fabrication.

The cyclic voltammetry technique was used to polymerize the pyrrole and to trap morphine using the solution mixture made using the procedure above. The electrode potential was stepped from 0.0 V to 1.0 V (vs. Ag/AgCl-saturated KCl) potential window. The cyclic voltammogram waves were obtained at varying scan rates from 30 to 100 mV/s. The thickness of the polymer of the glassy carbon depended on the number of cycles, pyrrole monomer concentration, aggregate voltage range, and template morphine concentration.

After electro-polymerization, the electrode with morphine cross-linked in the poly-pyrrole polymer matrix was rinsed with deionized water to remove any morphine not properly entrapped or loosely hanging on the electrode. The electrode was then placed in a vial containing pure methanol solution for 30 min to extract the morphine trapped in the poly-pyrrole polymer network. Morphine dissolves easily in methanol; therefore, methanol is a good solvent for morphine extraction and does not interfere with analysis. Removal of the template creates cavities with a complementary size and shape of morphine. This electrode was referred to as the MIP electrode. To make a comparison, another electrode was made following the same procedures, only omitting morphine during the polymerization step. The electrode made was the non-imprinted polymer (NIP) electrode. The pictorial representation of MIP sensor electrode fabrication is shown in Figure 2.

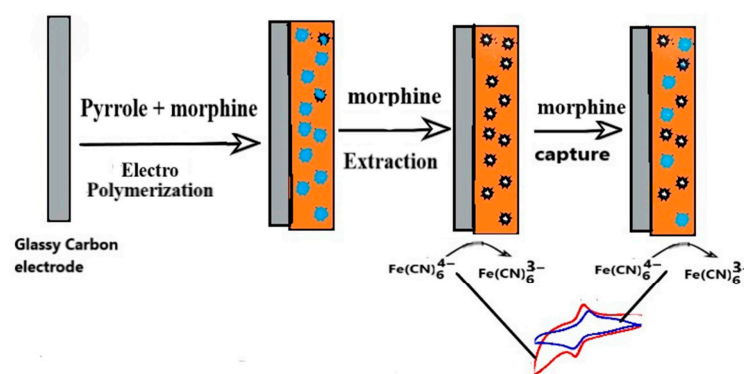


Figure 2. Pictorial representation of morphine detection using imprinted electrodes.

3. Results

3.1. Electrochemical Characterization of the Imprinted Sensor Electrodes

Initially, the optimum number of cyclic voltammogram polymerization cycles was established. The thickness of the film depended on these cycles. [Fe(CN)₆]^{−3/−4} in 0.5 M acetate buffer solution at pH 5 was used to probe the electrochemical behavior of the crosslinked polymer electrodes. The concentration of the [Fe(CN)₆]^{−3/−4} probe solution was 0.5 mM. The electro-polymerization of pyrrole monomer on glassy carbon is shown in Figure 3A. Cyclic voltammograms show layered growth of the poly-pyrrole polymer during the electro-polymerization step. Figure 3B shows typical peak currents obtained from cyclic voltammograms and obtained using the [Fe(CN)₆]^{−3/−4} redox probe. These peak currents were plotted as a function of the number of cyclic scans. Poly-pyrrole is a conductive polymer, and hence the peak current continuously increased with the number of scans until the thickness of the polymer started to affect the [Fe(CN)₆]^{−3/−4} solution electron transfer process, in which case the current started to plateau. Electro-polymerization of pyrrole was carried out using a 0.0 to 1.0 V potential window.

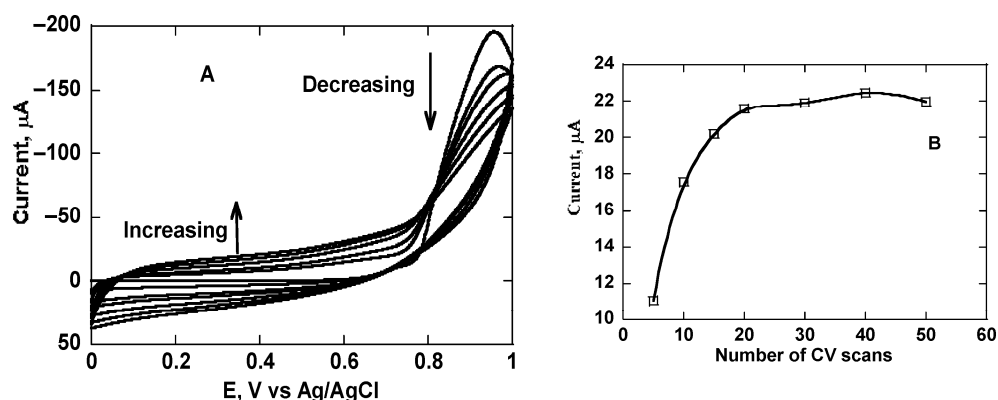


Figure 3. (A) Cyclic voltammograms showing the electro-polymerization of pyrrole monomer on a glassy carbon electrode. (B) Peak current from cyclic voltammogram obtained using $[\text{Fe}(\text{CN})_6]^{-3/-4}$ redox probe and plotted as a function of the number of voltammogram cycles.

The thickness of the poly-pyrrole layer was estimated using Faraday's law as follows:

$$l = \frac{qM}{An\rho F}$$

where l represented the thickness of the layer, and q is the charge in coulombs resulting from pyrrole electrooxidation and, hence, pyrrole electro-polymerization. This charge was obtained by cyclic voltammogram integration to obtain the area under the curve and again dividing the area with the scan rate (0.1 V/S). M is the molar mass of pyrrole (67.09 g/mol), A is the electrode geometric area (0.07 cm²), ρ is the density of pyrrole (1.5 g/cm³), and F is the Faraday constant (96,485 coulombs/mol). Using a 0.1 V/S scan rate, the film thickness for the first cyclic voltammogram was estimated to be about 4 nm. Each subsequent voltammogram added another layer of polymer, albeit of smaller thickness, as observed from the reduced charge.

From the voltammograms obtained, it was clear two regions emerged, between 0.0 and 0.8 V when the oxidation current increased and between 0.8 and 1.0 V when the oxidation current decreased. It can also be observed that changes in current were negligible after 20 cycles. The pyrrole monomer was maintained at 3.0 mM concentration during polymerization. It was important to have proper thickness of the polymer to support a three-dimensional molecular imprint. To ensure this happens, we needed to manipulate both the concentration of the monomer as well as the number of voltammetric scans. A very thin film would not effectively support a three-dimensional imprint, while a very thick film would present difficulties in both the template extraction as well as during the rebinding step. Therefore, we needed to strike a balance between the two. Using 3.0 mM of the pyrrole monomer and 5 to 15 cyclic voltammograms were deemed optimal conditions for electrochemical polymerization.

3.2. FTIR Spectroscopy

The extraction of morphine from the poly-pyrrole polymer was followed using FTIR. Morphine has well-known stretching and vibrational spectroscopy signatures. A large O-H stretching band is observed at between 3300 and 3500 cm⁻¹. Pristine morphine also has prominent C-H stretching vibrational bands at about 2800–3000 cm⁻¹. Other stretching vibrational modes include the C-O stretching observed at 1700 cm⁻¹. FTIR spectroscopy for morphine trapped in poly-pyrrole is shown in Figure 4. The O-H stretch of morphine was quite broad while trapped in poly-pyrrole, as the peak occurred at 3000 to 3500 cm⁻¹. The carbon double bond vibrational bands of poly-pyrrole are found way below 2000 cm⁻¹ and are, therefore, quite easy to distinguish from those arising from morphine. After extraction

of morphine from the poly-pyrrole, the O-H stretching vibrational band disappeared from the spectra. This meant the extraction of morphine was successful.

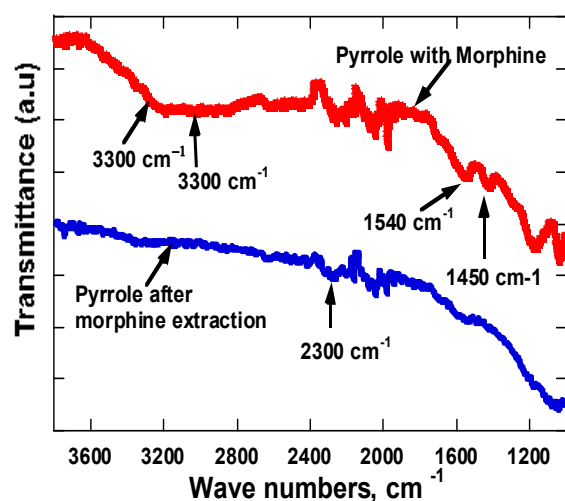


Figure 4. FTIR spectra of poly-pyrrole before and after morphine extraction.

3.3. Electrochemical Impedance Spectroscopy (EIS)

The thickness of the polymer was also monitored using electrochemical impedance spectroscopy (EIS). Change in film thickness results in change in the electron transfer resistance of the electrode and in ESI of the surfaces [30,31]. The electron transfer resistance (R_{et}), which depends on the dielectric and insulating features of the electrode/electrolyte interface. The EIS technique was carried out in 0.5 mM $[\text{Fe}(\text{CN})_6]^{-3}/[\text{Fe}(\text{CN})_6]^{-4}$ dissolved in pH 5.0 acetate buffer. The Nyquist plot (Z' versus Z'') obtained at a frequency scan ranging from 100,000 to 1 Hz is shown in Figure 5. Clearly, increasing layers on the polymer film resulted in a disruption of the semicircle. The diameter of the semicircle on the Nyquist diagram does not necessarily change significantly. This region primarily reflects the interfacial electron transfer resistance (R_{ct}). The R_{ct} controls the electron transfer kinetics of $[\text{Fe}(\text{CN})_6]^{-3/-4}$ at the electrode surface. We observed a drastic change in mass transfer control with an increasing number of cycles. It appears the mass transfer limitations are more pronounced at lower frequencies than at higher frequencies. It is also reasonable to assume that transfer kinetics are slower on the thicker electrode compared to a less thick one or with a smaller number of cyclic voltammogram scans on the glassy carbon electrode.

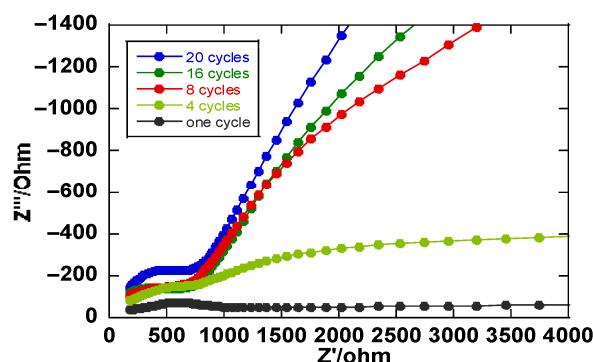


Figure 5. Electrochemical impedance spectroscopy of different layers of poly-pyrrole obtained from different cyclic voltammogram cycles.

3.4. Analysis of Morphine and Calibration Methods on MIP

Calibration plots were made using optimized MIP electrode sensors. The thickness of the MIP films was estimated using the cyclic voltammogram curves. Several morphine

concentrations were analyzed in different film thicknesses. Methanol was used to make a nanomolar range of morphine solutions. The MIP electrodes were incubated in morphine solutions for a specific amount of time and then removed from the morphine solution, rinsed with water, and then analyzed in a 0.5 mM $[\text{Fe}(\text{CN})_6]^{-3/-4}$ probe solution. The current obtained using the MIP electrodes in the $[\text{Fe}(\text{CN})_6]^{-3/-4}$ probe solution was noted. Again, the same electrode was rinsed and placed in another morphine solution with a different concentration, and again the process with the probe was repeated. Figure S1 shows two MIP electrodes made using 5 cyclic voltammogram scans and another made using 20 cyclic voltammogram scans. It is clear that five scans were not enough to generate enough film thickness to bring out a proper morphine response. The sensitivity of the MIP electrodes for the 5 cyclic voltammograms was much lower than that obtained using 20 voltammograms (Figure S1). The MIP electrode had a limited linear range as compared to the twenty 20 voltammogram scans, which had an extended linear range.

We, therefore, used the 20-cycle voltammogram to make final calibration plots for monitoring the response of the morphine in wastewater. We also made NIP and repeated the same experiments. The rebinding time of morphine to the imprints was optimized before making the calibration plots. To optimize the rebinding time, a typical freshly made MIP electrode was immersed in a morphine solution to allow rebinding. The time of rebinding was varied each time, noting the peak current from the probe solution. Figure S2 shows a plot of the peak current of the MIP electrode as a function of the rebinding time using 20 nM morphine. The current started to plateau after 15 min. However, the highest rate of rebinding was at 5 min. Based on this result and also from our prior experiment [32], a 5 min incubation was used. Morphine rebinding was carried out at ambient temperatures. Every time the MIP electrode was removed from the morphine solution, it was thoroughly rinsed first with water to remove morphine not properly bound to the three-dimensional pocket on the electrode surface. Cyclic voltammograms as well as square wave voltammograms (SWV) of the MIP electrode were obtained. Both cyclic and square wave voltammetric techniques were used, and their sensitivities were compared. As noted earlier, both MIP and NIP electrodes were placed in solutions containing different concentrations of morphine and analyzed in 0.5 mM $[\text{Fe}(\text{CN})_6]^{-3/-4}$. Figure 6A shows SWV of imprinted electrodes in various concentrations of morphine. A large peak current was obtained using the MIP electrode before incubation in morphine. However, after incubation in morphine solution, the peak current of the MIP electrode gradually reduced as the concentration of morphine was increased. This implied that the morphine was responsible for the decrease in current through occupying the three-dimensional imprints, hence, blocking the efficient electron transfer of the probe solution.

Both the SWV and CV experiments showed the same trend, with the highest current with a zero-morphine concentration, while the highest morphine concentration resulted in the lowest current. Figure 6A shows CV plots taken at different morphine concentrations. Figure 6B shows a plot of peak current from CV plotted against the concentration of morphine. The calibration plot yielded the equation $Y(\text{Amps}) = 25.44 - 0.248X \times 10^{-6}$ [morphine] with $R^2 = 0.99$. Figure 6C shows SWV of MIP electrodes in increasing concentrations of morphine. Different SWV plots are shown. Figure 6D is the plot of current obtained using SWV and plotted as a function of morphine concentration. The calibration plot obtained has a more expanded linear region as compared to the CV plot. The calibration plot is linear from zero to 40 nM with the equation $(Y(\text{amps}) = -27.3 - 0.30 \times 10^{-6} [\text{morphine}]$, with $R^2 = 0.97$. The limit of detection ($\text{LOD} = 3\sigma/m$) for morphine with cyclic voltammetry detection was 2.75 nM, while that obtained with SWV was 1.9 nM. Table 1 shows comparisons of different methods for the detection of morphine. Other than chromatographic methods, this work performed using pyrrole imprints shows much superior performance.

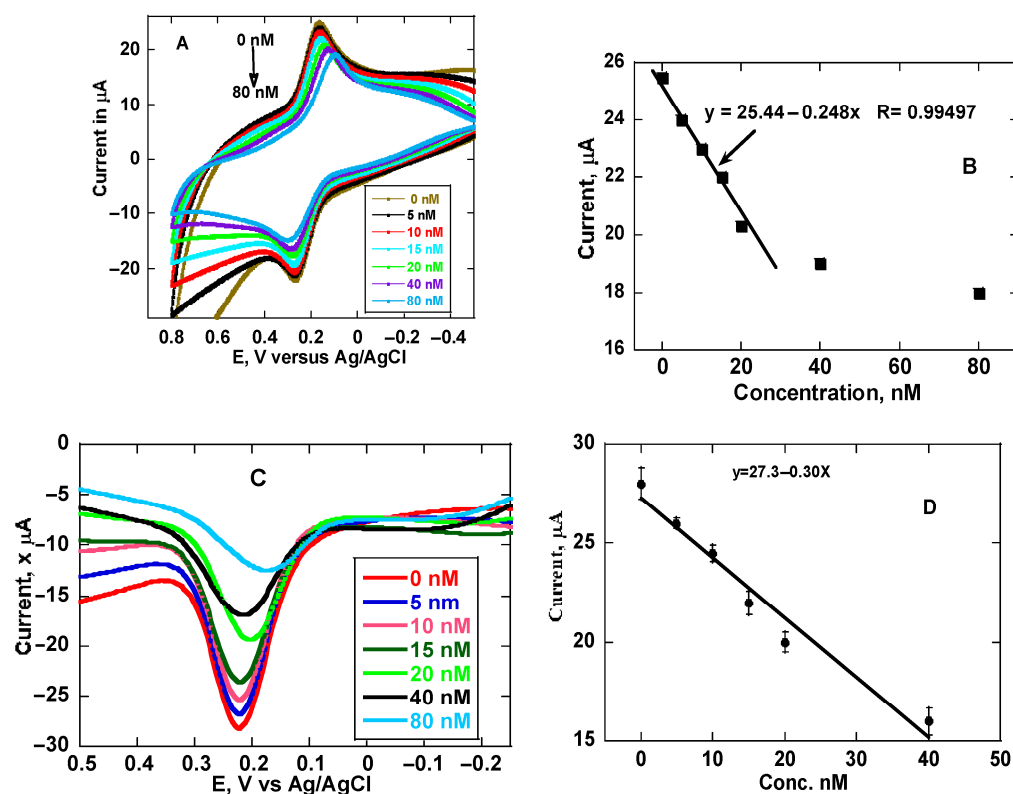


Figure 6. MIP electrodes analyzed in 0.5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ redox probe (A) Cyclic voltammograms of MIP electrodes in different concentrations of morphine solution. (B) Calibration plot of current as a function of morphine concentration using MIP electrode obtained from cyclic voltammograms. (C) Square wave voltammograms of MIP electrode in different morphine concentrations. (D) Calibration plot of SWV peak current as a function of morphine concentration.

Table 1. Comparisons of different morphine detection methods.

Electrode-Based			
Modified Electrodes	Linear Range	Detection Limit	Reference
Graphene/ Co_3O_4 (Gr/ Co_3O_4)	0.5–100.0 μM	80 nM	[33,34]
Polymer/SWCNT	0.5–10 μM	0.48 μM	[35]
Carbon Paste GNP	1.0×10^{-6} – 18.0×10^{-4} M	3.507×10^{-9} M	[36]
Poly-pyrrole polymer imprint	0–20 nM and 0 to 40 nM	2.75 and 1.9 nM	This work
Other methods			
UHPLC-MS/MS	0.025–12.5 ng/mg	20.27 pg/mg	[37]
(LC-MS/MS) with CNTs extraction	25–2000 ng/mL	2.0 ng/mL	[38]
DRI immunoassay	0 to 1466 $\mu\text{g/L}$	300 $\mu\text{g/L}$	[39]
Electrokinetic chromatography	0.5–50 ng/mg	200 pg/mg	[40]

Acetaminophen, a common painkiller sold over the counter, was used as a control to test the effectiveness of the MIP sensors. Figure S3 shows voltammograms of MIP electrodes incubated in acetaminophen. Both CV and SWV show that increasing the concentration of this drug does not result in corresponding changes in the peak currents. The small changes observed do not follow a particular trend. Figure 7A shows a comparison of acetaminophen and morphine analyses using morphine MIP electrodes. The plots were made using currents

obtained using the same concentrations of morphine and acetaminophen. The presence of acetaminophen showed statistically insignificant changes in current over the whole range of the concentration (0 to 80 nM). This indicated the imprinted polymer was specific towards morphine.

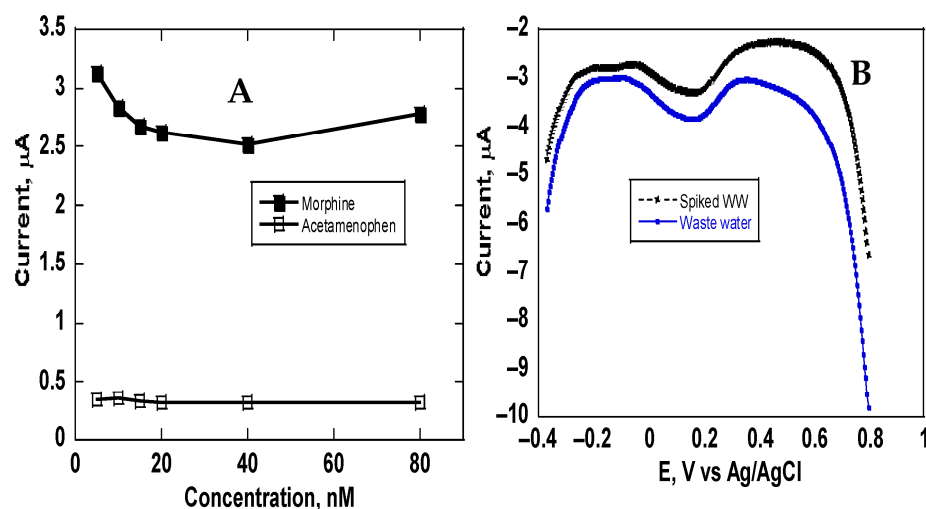


Figure 7. (A) Current as a function of the concentration of morphine and acetaminophen using IMP electrodes. (B) SWV of morphine using MIP electrodes in a wastewater sample compared with wastewater spiked with 20 nM morphine.

The most common interferants during the analysis of morphine in wastewater or in the blood include oxycodone or other metabolites such as noroxycodone and oxymorphone. Previously, analysis of oxycodone in the presence of morphine was found to not significantly affect the outcome of the results [32]. Other types of drugs present in wastewater are likely to interfere with the detection of morphine. One such drug is acetaminophen, which is an over-the-counter drug mainly used as a painkiller and a common fever reducer. The selectivity of the MIP sensor for morphine was determined by evaluating its response to acetaminophen. The structures of morphine and acetaminophen are slightly different, with acetaminophen being a slightly smaller molecule than morphine and hence easily moving in and out of the prints. As shown in Figure 7A, the current changes obtained with morphine are much higher compared to those from acetaminophen, indicating that the sensor is selective towards morphine.

Having established the specificity of this sensor toward morphine, we wanted to use the new MIP sensor electrodes to test a real field sample. To do this, wastewater from a nearby city was collected, filtered, and kept refrigerated until needed. The wastewater sample was split into two, and one of the samples was spiked with morphine and the other with distilled water. The volume of the two samples was essentially the same. MIP electrodes were analyzed in the two solutions by immersing the electrodes in these solutions for 10 min, rinsing with water, and then analyzing in 0.5 mM $[\text{Fe}(\text{CN})_6]^{-3/-4}$ probe solution as usual. Figure 7B shows the SWV of the imprinted electrodes comparing the two wastewater samples. Spiking wastewater with morphine resulted in a decreased current due to more blocking effect than the un-spiked one.

4. Discussion

Oxidation of morphine on the electrode surface is mainly dependent on the pH of the buffer used as well as the type of materials used on the electrode surface. Oxidation of phenolic groups present in morphine is responsible for its electroactivity at pH 1. The oxidation potential using Ag/AgCl as a reference electrode and glassy carbon as a working

electrode is 0.7 V with a buffer of pH 1. The oxidation potential is 0.5 V at pH 5 [41]. In the current MIP sensor electrode, direct oxidation of morphine can interfere with the measurement of morphine. A glassy carbon electrode was used as a platform where the imprints were made and analyzed. Direct oxidation of morphine using a bare glassy carbon electrode shows a quite distinguishable peak at 25 μ M morphine concentration [42]. This means the peak for morphine oxidation in the nanomolar range would be negligible using a glassy carbon electrode. $[\text{Fe}(\text{CN})_6]^{-3}/[\text{Fe}(\text{CN})_6]^{-4}$ was used as a reporter probe with an oxidation potential of less than 0.4 V on glassy carbon. Therefore, the peak for direct oxidation of morphine was avoided even at higher nanomolar range solutions. The thickness of the polymer was important in enhancing the detection of morphine. A three-dimensional polymer was needed to support imprint formation while at the same time allowing morphine molecules to easily rebind. We used both 20 CV scans and 5 CV scans with 3.0 mM of pyrrole to make polymer electrodes. The MIP electrodes and the NIP electrodes were compared, and the results indicated that morphine was clearly specifically included in the prints. As shown in Figure S4, the NIP electrodes showed a complete block of electron transfer. The blocking of electron transfer using MIP electrodes showed a time-dependent reaction when the electrode was placed in morphine solution at different times. From the plot of peak current as a function of time, it was clear the current started to plateau after 15 min and was almost completely blocked at 30 min when the current change was minimal. We have no reason to believe that morphine molecules are detached from the imprints in an equilibrium manner. The steepest slope of the morphine binding curve was at 5 min. We also note that these MIP electrodes were more sensitive while analyzing lower morphine concentrations, indicating that rebinding was more efficient. This was probably due to higher availability of binding sites, hence faster rebinding. Figure S5 shows calibration plots for both MIP and NIP sensor electrodes. Again, we have MIP electrodes showing higher current change across all the concentrations used.

We note that these MIP electrodes have much lower detection limits (2.75 nM for CV and 1.9 nM for SWV), which is much lower than other electrode-based sensors for morphine. Moreover, this is the first time a pyrrole monomer has ever been used to construct an imprint for morphine. This sensor has potential in the analysis of morphine in wastewater. There was no morphine found in wastewater samples analyzed, probably due to low concentration or because of dilution from point sources. Sensors based on chromatographic methods can detect nanomolar range and below and are in general preferred in the kind of samples used here. However, these are more complicated and are not cost-effective. Obviously, the performance of this MIP sensor for morphine shows very strong binding affinity toward morphine. Both SWV and CV techniques show this strong correlation between the peak currents and the concentration of morphine and, hence, have potential in quick analysis of samples. The ease of fabrication combined with its specificity toward morphine places it at the top compared to others found in the literature. The proposed strategy has universal significance in the fabrication of MIP electrode sensors for opioids and other related molecules.

5. Conclusions

In this contribution, MIP electrode sensors were prepared and used for analysis of morphine, a commonly abused drug. These MIP sensor electrodes were prepared using pyrrole monomer electro-polymerized by applying a CV potential. By controlling the number of cyclic scans, a polymer of 4 nm thickness was obtained. The optimized polymer thickness supported a three-dimensional receptor-type binding pocket imprint for morphine. The efficiency of the detection of morphine was based on the rebinding performance. Results show detection of morphine with appreciable figures of merit. The

linear range was 0 to 20 nM with a limit of detection of 2.75 nM using CV, while the linear range was 0 to 40 nM with an LOD of 1.9 nM using SWV. We find these results significant in that a cheap MIP electrode sensor can rapidly respond linearly to morphine in wastewater samples. The response of the MIP electrode was not significantly affected by the presence of interfering molecules. The proposed strategy has universal significance in the fabrication of MIP electrode sensors for opioids and other related molecules.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/chemosensors13080284/s1>. Figure S1: Calibration using SWV using 5 and 20 scans title; Figure S2: Time-dependence plot of morphine rebinding on an imprinted electrode monitored as a function of time; Figure S3: In this study, 0.5 mM $[\text{Fe}(\text{CN})_6]^{-3/-4}$ probe solution using NIP electrodes incubated in different morphine solutions using (a) cyclic voltammograms and (b) SWV. Figure S4: SWV for MIP electrode incubated in 10 nM morphine obtained in 0.5 mM $[\text{Fe}(\text{CN})_6]^{-3/-4}$ probe solution using MIP and NIP electrodes. Figure S5: Calibration plots for MIP and NIP electrodes obtained using SWV.

Author Contributions: Conceptualization, methodology, validation, and original draft preparation were performed by A.M. Formal analysis, investigation, and data curation were performed by P.C. Review and editing, visualization, supervision, and project administration were performed by A.M. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflicts of interest.

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