Electrochemical Method Development using Antibody Modified Electrodes Functionalised with Gold Nanoparticles for Highly Specific and Sensitive Detection of Morphine

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Abstract

Morphine is one of the most potent opioid analgesics recommended by World Health Organization (WHO) but long-term usage can lead to addiction. Morphine is a phenolic chemical and an alkaloid originating from the poppy plant that induces central nervous system dysfunction and is poisonous in high doses so, it must be properly controlled and kept within safe ranges. Several analytical techniques such as thin layer chromatography, gas chromatography, and high-performance liquid chromatography have been employed, however these approaches are expensive and time consuming. Electrochemical sensors, in comparison, are efficient analytical devices because of their desirable qualities, such as minimal cost, portability, high sensitivity, and ease of operation for on-the-spot or mobile detection of morphine, which is electroactive in nature. Cyclic and differential pulse voltammetry modes have been explored. A screen-printed carbon electrode has been functionalised by gold nanoparticles which considerably increases the sensitivity of the sensing platform as they have huge surface area relative to their volume. Its further conjugated with antibodies for precise antibody-antigen recognition of morphine thus increasing the selectivity of the sensing platform.

Keywords: morphine; opioid; gold nanoparticles; electrochemical sensors; cyclic voltammetry; differential pulse voltammetry.

1. Introduction

Since more than a century ago, powerful analgesics known as opioids have been used to alleviate pain. However, prolonged use of opioids may lead to addiction and physical and psychological dependency (L. Y. Zhang & Liu, 2014). Neuroadaptive alterations in the CNS are linked to physical dependency, both at the molecular and cellular levels and psychological dependency (Listos et al., 2019). The emergence of typical withdrawal symptoms after ceasing drug use is caused by these alterations. One of the most significant and potent opioid analgesics is morphine. The World Health Organization (WHO) recommends it for people with cancer or moderate to severe pain (Cancer *et al.*; Gillian R. Hamilton BA and Thomas F. Baskett MB FRCSC, 2000). It is an alkaloid and phenolic substance obtained from the poppy plant that can disrupt the central nervous system and be lethal in excessive doses (Aliabadi & Rounaghi, 2019). To avoid overdose or abuse-related morphine toxication, the morphine must be precisely regulated and kept within safe limits (Chen et al., 2020).

Although there are analytical techniques with high separation efficiencies such as gas chromatography (GC) and high-performance liquid chromatography (HPLC) but their need for expensive and sophisticated instruments, qualified experts, and complex procedures can only partly address the range of detection requirements (Marigo et al., 1986). From this point on, the current objectives involve the creation of alternative techniques that can accomplish quick, economical, and ultrasensitive morphine detection. However, electrochemical sensors are a form of valuable analytical sensors for the detection of several analytes due to their advantageous characteristics, such as high sensitivity, low cost, easy miniaturisation, and

easy operation. In the following study, a new method is developed on electrochemical work station.

A chemical process in which electrons release, accept, or consume ions is the basic operating principle of electrochemical sensors (Gandhi et al., 2009). An analyte of interest and a constrained ligand engage in a chemical reaction that demonstrably affects the transduced signal in terms of electrical current or potential as analyte undergoes oxidation and reduction. The magnitude of the electrochemical signal is directly correlated with the quantity of the marker or analyte in the sample solution. On the basis of the numerous signal types, electrochemical detection methods may be divided into subtypes such potentiometry, voltammetry, amperometry, and electrochemical impedance spectroscopy. In the current study, two modes of voltammetry namely, cyclic voltammetry and differential pulse voltammetry have been explored (Aliabadi & Rounaghi, 2019; Amare & Admassie, 2012; Atta et al., 2014). Cyclic voltammetry is an electrochemical technique for measuring the current response of a redox active solution to a linearly cycled potential sweep between two or more set values (Li et al., 2009). The 'duck-shaped' plot generated by cyclic voltammetry is called a cyclic voltammogram (figure 1).

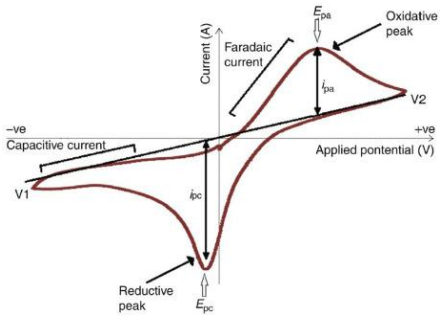


Figure 1: Standard Cyclic Voltammogram

In differential pulse voltammetry, electrochemical measurements are made as a derivative of linear sweep voltammetry or staircase voltammetry, with a series of regular voltage pulses superimposed on the potential linear sweep or stairsteps (figure 2).

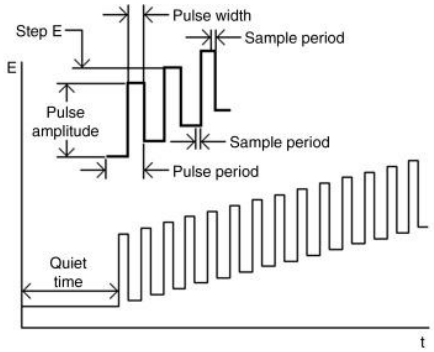


Figure 2: Standard Differential Pulse Voltammogram

Morphine has a phenolic group and a tertiary amine group which undergo oxidation and makes it an electroactive compound (Abraham et al., 2020; Li et al., 2010; Verrinder et al., 2021). In this study, morphine concentrations in liquids were measured using a screen-printed carbon electrode. The bare electrodes are not very sensitive to morphine detection. A functionalization step using gold nanoparticles and a modification technique using morphine specific antibodies have been used to enhance the electrode's electrochemical properties (Qi et al., 2005; C. Zhang et al., 2017). Because of the remarkable properties of nanomaterials,

nanotechnology is a field of study that is expanding quickly (Castañeda et al., 2007). Nanomaterials have been discovered to exhibit improved thermal, optical, magnetic, plasmonic, and catalytic capabilities as compared to bulk materials, among other physical and chemical properties. Due to these improved characteristics, nanoparticles are now being used into electrochemical sensing systems as a way to advance existing sensor technology. In particular, carbon, silver, and gold nanoparticles are used to create adaptable electrochemical platforms (Erdem et al., 2010). Specifically, gold nanoparticles (AuNPs) have well-proven benefits of simple synthesis procedures, high stability, and huge surface area (Kerman et al., 2007; Lv et al., 2019). And it is because of their exceptional electrical conductivity, catalytic activity, and biocompatibility that AuNPs have been employed in the sensor matrix to increase the sensitivity of the sensing platform.

Immunoassays and electrochemical assessments are occasionally coupled. Immunosensors are the resultant sensors (Gandhi et al., 2009; Munawar et al., 2019). Immunosensors based transducer are the analytical tools where because of the formation of antigen-antibody (Ag-Ab) complex, the electrical signal is recorded and displayed. Because of their propensity for binding small molecules, antibodies (Ab) are frequently utilised in the construction of immunosensors (Islam & Channon, 2019). The adsorption of an antibody directly onto the AuNPs can be used to create the sensors. Such super-nanostructures can provide a spectacular electrochemical response being contained on the electrode surface via precise antibody-antigen recognition that provides information about morphine concentration.

In the current study, gold nanoparticles are conjugated with biotin and then bound with streptavidin which is a protein derived from Streptomyces avidinii (Díaz-González et al., 2005; González-García et al., 2000). Also, biotin conjugated monoclonal mouse antibody specific to the target of interest i.e. morphine is used. Basically, biotin, also known as vitamin B7 is a small molecule with a ring structure that contains a carboxyl group, an amide group, and a sulfur-containing group. These chemical groups allow biotin to form strong non-covalent bonds with streptavidin through hydrogen bonding, electrostatic interactions, and hydrophobic interactions (Hernández-Santos et al., 2005; Jianxiu Wang et al., 2008; Jun Wang et al., 2003; Wu et al., 2016). The binding of biotin to streptavidin is highly specific, with a dissociation constant (Kd) of about 10^-14 M, which means that the binding is extremely tight and difficult to disrupt. This high affinity and specificity make biotin-streptavidin interaction a useful tool in many research and biotechnological applications (Erdem et al., 2010).

The following in-depth descriptions of the working theory, experimental conditions optimization, analytical performance, and real sample analysis show that the current study is here to offer profound insights into the creation of rationally designed nanoarchitectures for potential analytical applications.

2. Methods & Materials

2.1. Chemicals

Morphine, codeine, thebaine, papaverine, heroin, atropine & caffeine standard were procured from Government Opium and Alkaloid Factory (New Delhi, India). Narcotic evidences were seized from the street. The chloroauric acid (HAuCl4) and bovine serum albumin (BSA) were purchased from Merck Chemicals (Darmstadt, Hesse, Germany). Biotin was procured from HiMedia (West Thane, Maharashtra), Streptavidin and N-ethyl-N'-(3-dimethylaminopropyl) carbodiimide (EDC) were purchased from ThermoFisher Scientific (Massachusetts, United States) and biotin conjugated monoclonal morphine antibody was purchased from LS Bio (Seattle, United States) and all these were kept in refrigerator at 4°C. Ethanol is purchased from shree chalthan vibhag khand udyog sahakari mandli ltd, (Chalthan, Surat, India). Trisodium citrate, 6-mercapto-1-hexanol (MCH), N-hydroxysuccinimide (NHS), Potassium

dihydrogen phosphate (KH2PO4), Monosodium phosphate (NaH2PO4), di-Sodium hydrogen phosphate (Na2HPO4), were obtained from Merck Chemicals (Darmstadt, Hesse, Germany).

2.2. Instruments

The electrochemical assays of cyclic voltammetry (CV), and differential pulse voltammetry (DPV) were commonly performed on a multi autolab M204 by Metrohm (made in The Netherlands) at room temperature. SPCEs were purchased from DropSens Inc. (Oviedo, Spain).

2.3. Procedure

2.3.1. Synthesis of AuNP

25ml MiliQ water was taken in a round bottom flask and then, 59mg of trisodium citrate of 9mM concentration was added. It was then stirred at 100ºC in oil bath for 15 mins. Then, 1ml of gold chloride salt of 15mM concentration was added to it which was then continued to stir for next 10-15 mins until yellow turned into pink. Hence, the AuNPs with an average diameter of 20-30 nm were prepared by reducing chloroauric acid using sodium citrate with minor modifications (Khan et al., 2015).

2.3.2. Fabrication of Electrode

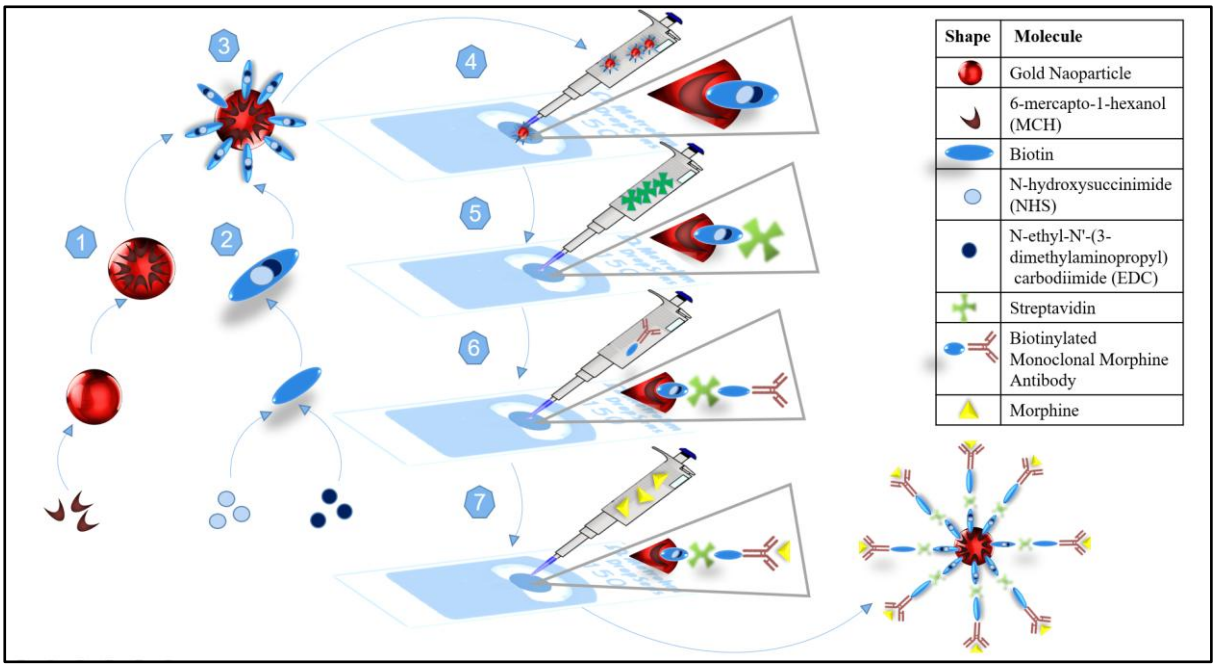


Figure 3: Stepwise fabrication of Screen-printed electrode

Step 1. Functionalisation of Gold Naoparticles:

Firstly, the surface of the gold nanoparticles is functionalised using a suitable linker molecule (figure 3). For this, the gold nanoparticles are mixed with a solution of 6-mercapto-1-hexanol (MCH) in ethanol and incubated at room temperature for 4-6 hours which resulted in their binding to the surface of the gold nanoparticles through thiol groups (-SH).

Step 2. Activation of Biotin:

Carboxylic acid group (-COOH) of biotin is activated by dissolving in a cross-linking agent such as N-hydroxysuccinimide (NHS) and N-ethyl-N'-(3-dimethylaminopropyl) carbodiimide (EDC) at room temperature for a 2-4 hours (figure 3).

Step 3. Biotinylation of Gold Nanaoparticles:
Then, added the activated biotin solution to the MCH-functionalized gold nanoparticles and incubated at room temperature for another 2-4 hours. The biotin molecules were bound to the MCH molecules on the surface of the gold nanoparticles through amide bonds (-CONH-) (figure 3).

Step 4. Drop Casting Biotinylated Gold Nanoparticles:
After that, different amount of biotinylated gold nanoparticles was drop casted by micropipette onto the working electrode area of screen printed carbon electrode (SPCE) starting from 100ul with gradual decrease of 10ul until 40ul as the result showed that below 40ul, proper binding was not taking place and it was kept for different incubation times i.e. 6 hrs, 12hrs, 18hrs, 24hrs, 32hrs and 48hrs. It was found that 24 hrs was the suitable time duration for proper incubation to happen, so biotinylated gold nanoparticles were left at room temperature for 24 hours so as to bind carbon surface of the electrode through π-π stacking interactions (figure 3). Then, unbound biotinylated gold nanoparticles were removed by rinsing the electrode surface with phosphate-buffered saline (PBS).

Step 5. Streptavidin Coating:
Streptavidin amount to be added was also optimized by starting with 100ul and gradually decreasing by 10ul until 20ul as the result showed that below 20ul, efficiency was getting impeded. And incubation time was first kept to be 3 hours and then further gradually reduced by 30 mins until 1 hour and it was found that streptavidin bound perfectly when the incubation time was 2 hours (figure 3). So, 20ul of streptavidin was drop casted and kept for 2 hours of incubation at room temperature, again followed by rinsing with PBS. To further prevent non-specific binding of other biomolecules to the streptavidin-coated electrode, the electrode is blocked by putting 40ul of 2% bovine serum albumin (BSA) for 30-60 minutes. Then, finally again rinsed the electrode with PBS to remove unbound BSA.

Step 6. Modification by Monoclonal Morphine Antibodies:
Then, the biotinylated monoclonal morphine antibodies were added to the streptavidin-coated nanoparticles after optimising its amount, starting from 100ul and gradually decreasing by 10ul until 40ul as the result showed that below 40ul, efficient binding was not taking place. The biotin on the antibody bound to the streptavidin on the nanoparticles via another biotin-streptavidin interaction since, streptavidin is a tetrameric protein that can bind up to four biotin molecules with very high affinity and specificity (figure 3). After incubating the mixture for 24 hours as during optimisation it was found it is the most appropriate duration for stable interaction and finally, the unbound components were removed by rinsing with PBS.

Step 7. Sample Analysis:
Various analyte sample solutions of concentration range varying from 3uM to 6nM were prepared by serial dilution (figure 3). 40ul of each concentration was then put on modified SPCE and electrograms were taken in both CV and DPV mode.

2.3.3. Voltammetry

Several CV & DPV scans were run until the stabilized voltammetric signal were shown. The following table shows the parameters that were used.

2.3.3.1. Cyclic Voltammetry Signatures

Cyclic Voltammetry (CV) was employed in potentiostatic mode within current range of 1mA to characterize electrochemical signatures at 0.1 V/s scan rate and step potential of 0.002 V (staircase mode). The CV scan was swept from start potential: +0.1 Vref to upper vortex potential: +0.7 Vref, whereupon it was reversed to lower vortex potential: 0.1 Vref, and then

swept back to stop potential: 0.102 Vref. Deposition potential of -1.2Vref was applied at deposition time of 5s. Several CV scans were run until the voltammetric signal stabilized, and stabilized signals are shown here.

2.3.3.2. Differential pulse Voltammetry Signatures

Differential pulse Voltammetry **(**DPV) was recorded in the potentiostatic mode within current range of 1mA. The scan was swept from potential +0.1 Vref to +0.7 Vref. Other parameters included step potential of 0.005 V, modulation amplitude of 0.025 V, modulation time of 0.05 s, interval time of 0.5s, and scan rate of 0.01 V s−1. Deposition potential and deposition time were set at 0.1Vref and 5s respectively.

2.3.3.3. Preparation of 0.1M PBS solution of pH7

Adding together 10 mL NaH_2PO_4, 40 mL of 0.2 M Na_2HPO_4 and 0.9 g NaCl and finally, the volume was made upto 100 mL with distilled H_2O and adjusted the pH to 7.

2.3.3.4. Sample preparation

Extraction: Firstly, 100mg of each sample was dispersed with 10ml of saturated aqueous sodium chloride at pH 10 (adjusted with dilute ammonia). Then, the mixture was extracted using three 20ml portions of chloroform/ isopropanol (3:1). And then organic layer was filtered through anhydrous sodium sulfate, or phase separation paper, and further, the organic solvent was evaporated to dryness. And then, took the residue of each sample and further, diluted by mixing in 10ml of 0.1M PBS of pH7.

2.3.3.5. Preparation of standard/ stock solution

Concentration range of 3uM- 6nM was prepared by serial dilution in PBS.

3. Results & Discussion

3.1. Investigation of the electrochemical behaviour of morphine at the surface of the modified electrode

(a) (b)

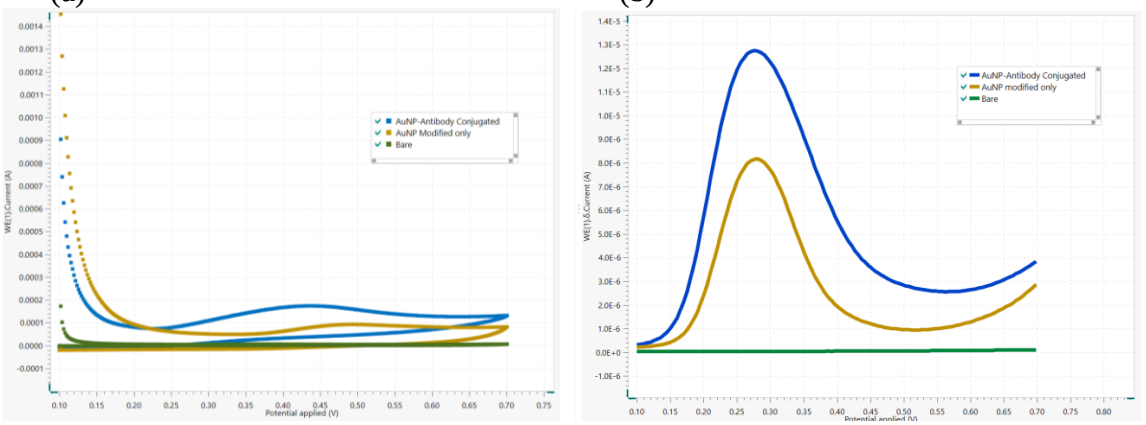


Figure 4: Comparison between bare (green), only AuNP modified (brown) and AuNP-Antibody conjugated (blue) electrode, via (a) CV & (b) DPV

Figure 4 clearly showed that the peak height increased in case of modified ones as compared to bare screen printed carbon electrode indicating that integrating gold nanoparticles (AuNPs) and antibodies onto a screen-printed electrode (SPE) enhances electrochemical sensor performance (Díaz-González et al., 2005; González-García et al., 2000; Islam & Channon, 2019). The AuNPs increase surface area, improving target analyte capture and recognition,

leading to enhanced sensitivity. Signal amplification from AuNPs boosts detectability. Antibodies confer specificity, reducing interference for selective detection. High affinity enables trace-level analyte detection. Stability allows for multiple uses without performance loss. Rapid response and cost-effectiveness make it suitable for dynamic and point-of-care applications. The versatile approach accommodates various fields, including environmental monitoring, medical diagnostics, food safety, and drug development, with customizable target detection based on chosen antibodies.

3.2. Analytical performance of electrochemical sensor

3.2.1. Linearity

The potential of a method to produce test findings that are directly proportional to the sample concentration over a specified range is known as linearity. By diluting the standard stock (figure 5) or separately weighing the sample components, the relationship can be apparent on the drug substance using the suggested methods. Visual evaluation of a plot of signals as a function of concentration or content of the analyte should be used to assess linearity (figure 6). If a linear relationship exists, the test findings should be examined using the suitable statistical techniques, such as regression analysis. Regression line data can be used to calculate estimations of the degree of linearity. It is typically stated as a variance around the regression line's slope. By using y = 2E+06x - 834.29 from the linear plot, R square value came out to be 0.99568 for CV & 0.99276 for DPV (Alquadeib, 2019; Chan, 2017; United Nations, 1998).

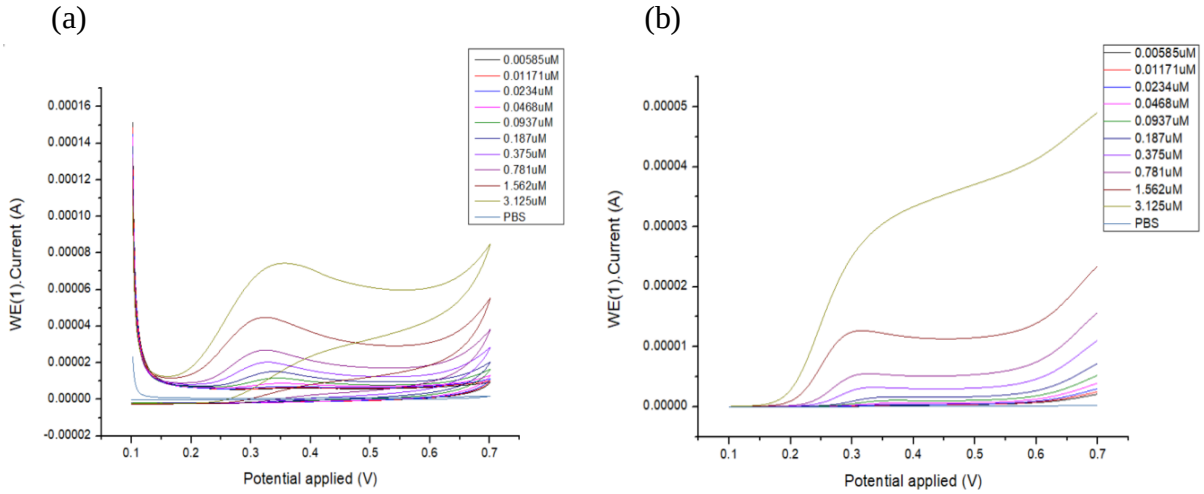
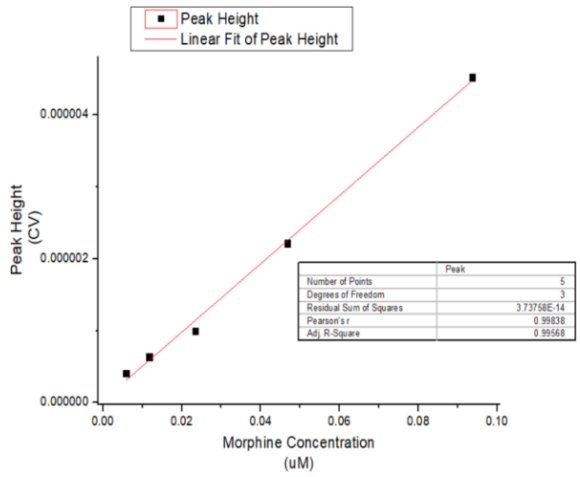


Figure 5: Overlay of various concentration of morphine from 3uM to 6nM (indicated by different colors) via (a) CV & (b) DPV

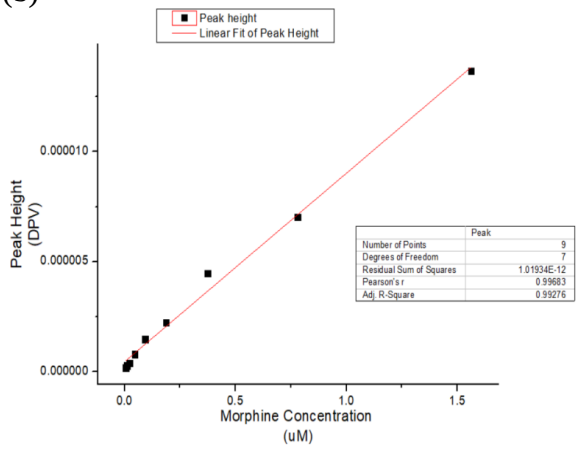
Figure 5 indicates that with the gradual decrease in concentration from 3uM, peak height was also getting reduced but it was significant till approx. 6nM concentration in both CV and DPV mode.

(a)



Concentration of Morphine in PBS (uM)	Current Height
0.00585	0.00585
4.06E-07	4.06E-07
0.01171	0.01171
6.37E-07	6.37E-07
0.0234	0.0234

(b)



Concentration of Morphine in PBS (uM)	Current Height
0.00585	0.00585
1.78E-07	1.78E-07
0.01171	0.01171
2.73E-07	2.73E-07
0.0234	0.0234
3.81E-07	3.81E-07
0.0468	0.0468
7.96E-07	7.96E-07

Figure 6: Standard calibration curve of Morphine via (a) CV & (b) DPV

3.2.2. Limit of detection & Limit of Quantification

The lowest concentration of analyte in the sample that can be detected but not absolutely quantified as an exact value is the limit of detection (LOD) of a specific analytical method (Alquadeib, 2019; Chan, 2017; Shabir, 1993; United Nations, 1998). Several methods can be used to determine the LOD. The signal to noise ratio is the foundation of the simplest strategy. By comparing measured signals from samples having known low analyte concentrations with those from blank samples, the signal to noise ratio may be calculated. The alternative method is based on the response's standard deviation and slope.

The LOD may be expressed as:

LOD = 3.3 σ / S (where, σ = the standard deviation of the response & S = the slope of the calibration curve)

The lowest amount of analyte in the sample that can be quantitatively measured with enough precision and accuracy is the Limit of Quantitation (LOQ) of a specific analytical method (Alquadeib, 2019; Chan, 2017; Shabir, 1993; United Nations, 1998). It is mostly impacted by the sensitivity of the detector and the accuracy of sample preparation. The same method used

to estimate the LOD can also be used to establish the LOQ. Based on the standard deviation of the response and the slope it is calculated by the formula:
LOQ = 10 σ / S (where, σ = the standard deviation of the response & S = the slope of the calibration curve)

(i) Cyclic Voltammetry

LOD = (3.3* 1.56E-06)/4.75E-05 = 0.108uM
LOQ = (10* 1.56E-06)/4.75E-05 = 0.328uM

Regression Statistics for CV		
Slope Value	**Standard Error**	**Adj. R Square**
4.75E-05	1.56E-06	0.99568

(ii) Differential pulse voltammetry

LOD = (3.3* 2.59E-07)/8.57E-06 = 0.0997uM
LOQ = (10* 2.59E-07)/8.57E-06 = 0.302uM

Regression Statistics for DPV		
Slope Value	**Standard Error**	**Adj. R Square**
8.57E-06	2.59E-07	0.99276

3.3. Optimization of Parameters for modified surface

The current response for all the parameters was examined for 1.562 mM morphine in 0.1 M PBS (pH 7) at the MorAb/AuNPs modified eletrode using both CV & DPV mode.

3.3.1. Effect of scan rate

The effect of scan rate on the response of morphine at the modified screen printed electrode was also examined by cyclic voltammetry (Aliabadi & Rounaghi, 2019). The results show that with increasing the scan rate from 0.1 to 0.2, 0.4, 0.7 and 1mV s−1 as shown in figure 7, the anodic peak current increases gradually.

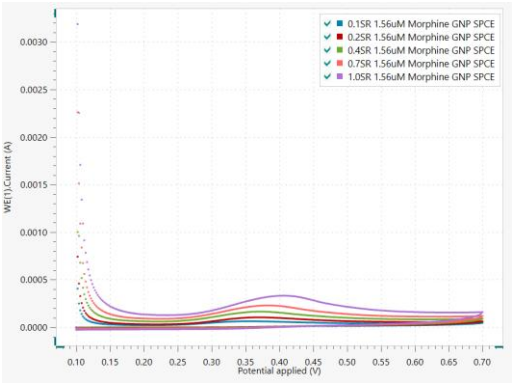


Figure 7: CV Overlay for Scan Rate

3.3.2. Effect of accumulation time

The effects of accumulation time on the current response were examined. The binding of morphine to the antibody is dependent on the accumulation time.(Radi et al., 2003). Figure 8 shows the dependence of the peak heights on accumulation time as it was changed from 5 to 10, 50, 100 and 300s.

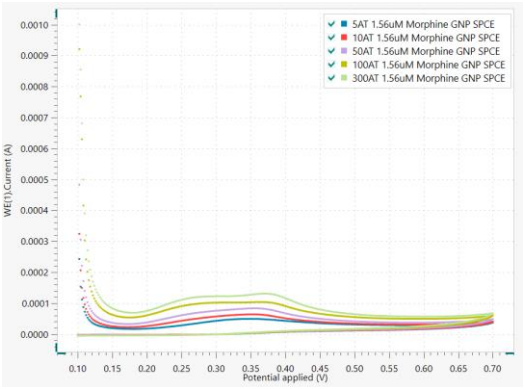


Figure 8: CV Overlay for a possible range of Accumulation time

3.3.3. Accuracy & precision

It is essential to assure that the instrument can produce a set of close-by results (Alquadeib, 2019; Chan, 2017; Shabir, 1993; United Nations, 1998). To evaluate intra-day precision, two consecutive measurements were made on the same day and the aliquots were examined on two consecutive days for inter-day precision. So, it can be observed in both CV & DPV mode that there is no much variation in intraday measurements (figure 9 & 10). However, significant variation can be observed in interday measurements suggesting that sample should be freshly prepared for better identification by voltammetry.

(a) (b)

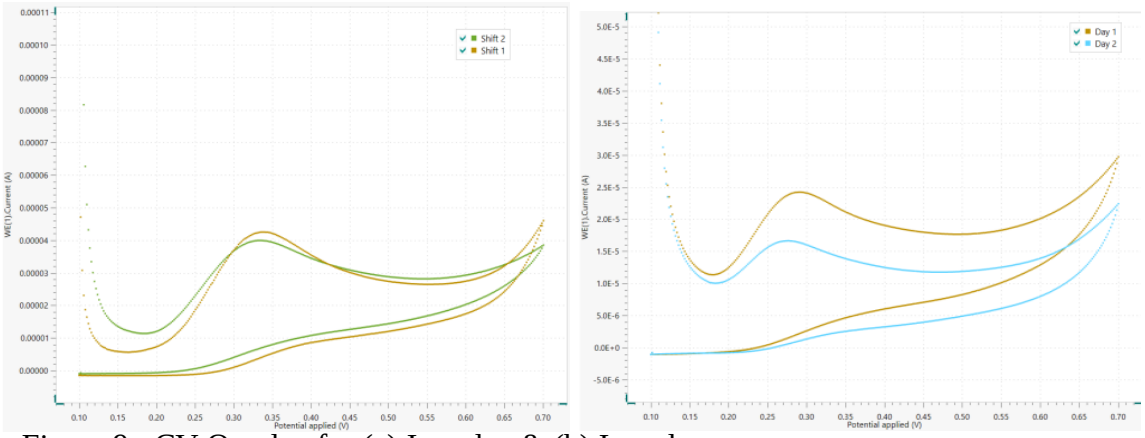


Figure 9: CV Overlay for (a) Intraday & (b) Interday measurements

(a) (b)

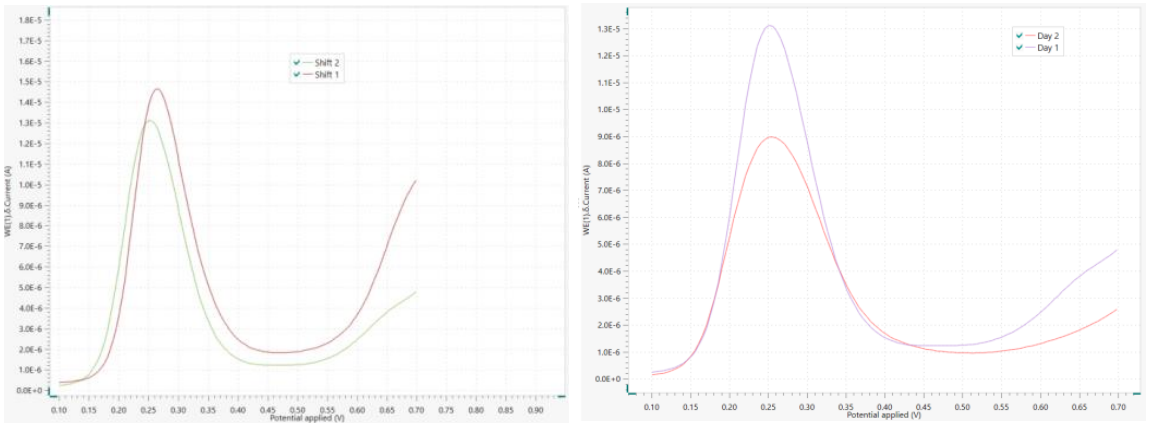


Figure 10: DPV Overlay for (a) Intraday & (b) Interday measurement

3.3.4. Selectivity & Specificity

The method's selectivity was first evaluated against morphine while also being exposed to other 4 related interferent: Codeine; papaverine; thebaine; heroin & 2 unrelated analytes: Atropine; caffeine. These molecules were selected either based on their similarity to molecular structure of morphine or the high probability of their simultaneous presence with morphine. Each interferent was first measured with a separate electrode. After that, the measurements were done for the mixture of morphine and all the interferents by following the same protocol of measuring morphine in PBS. The ability of an analytical method to quantify the analyte in the presence of other substances is known as selectivity. Sometimes, the words "specificity" and "selectivity" are used interchangeably. In general, the term "specific" denotes a method that yields a result for a single analyte only, whereas the term "selective" denotes a method that yields results for several chemical entities that may or may not be differentiated from one another (Chan, 2017). "Specificity is the ultimate of Selectivity," according to the International Union of Pure and Applied Chemistry (IUPAC). The IUPAC prefers to use the term selectivity and discourages the usage of specificity (Paithankar, 2013). Since, the peak for interferents were not at the same position and not of same height as that of morphine. Hence, the method proposed can be used selectively for morphine detection (figure 11).

(a) (b)

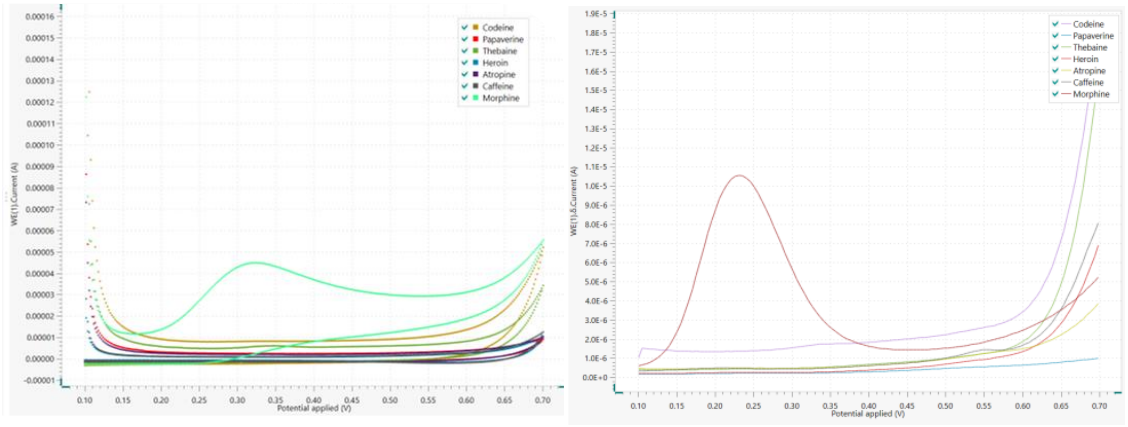


Figure 11: Voltammogram of morphine in presence of other 4 related interferent : Codeine; papaverine; thebaine; heroin & 2 unrelated analytes : Atropine ; caffeine via (a) CV & (b) DPV

3.4. Population Study

The developed method was successfully used to identify and quantify 8 different seized samples which were suspected to be having morphine (figure 12).

(a) (b)

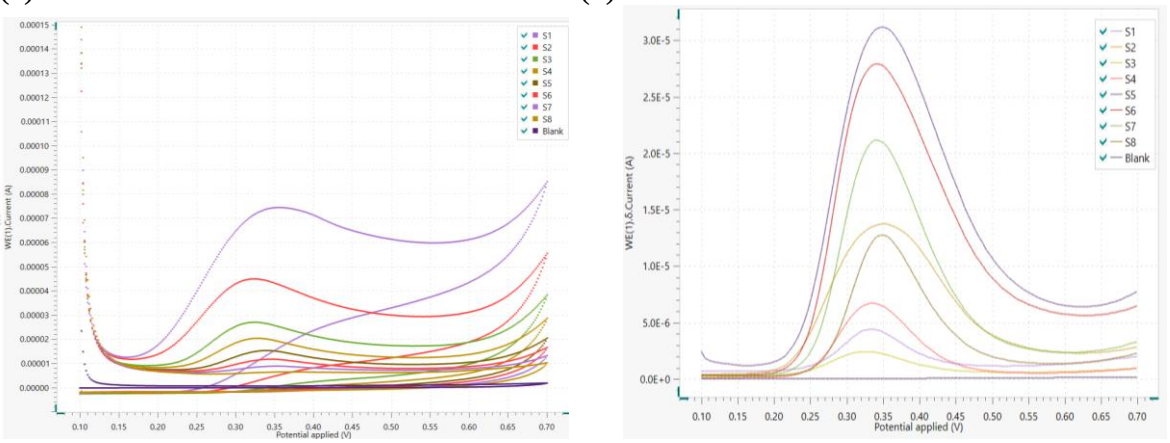


Figure 12: Voltammogram showing presence of morphine in 8 different seized samples of opium via (a) CV & (b) DPV

4. Conclusion

Electrochemistry plays a vital role in the realm of forensics, offering valuable tools for crime scene investigations and evidence analysis. Moreover, electrochemical sensors provide real-time monitoring capabilities, facilitating rapid analysis during critical investigations. With its accuracy, sensitivity, and versatility, electrochemistry has become an indispensable asset in modern forensic science, ensuring justice and safety in society.

Incorporating nanoparticles onto screen-printed electrodes (SPEs) enhances the sensitivity of electrochemical sensors through various mechanisms. Nanoparticles offer a larger surface area for target analytes to interact with, enabling higher sensitivity for detecting low concentrations of substances. They also facilitate efficient electron transfer during redox reactions, improving the sensor's response time and sensitivity. Additionally, functionalized nanoparticles can be tailored for selective recognition of specific analytes, reducing interference and enhancing sensitivity. Furthermore, the uniform deposition of nanoparticles on the electrode surface improves stability and reproducibility, ensuring reliable and accurate sensing applications. These nanoparticle-modified SPEs can be miniaturized and made portable, making them ideal for point-of-care diagnostics and on-site monitoring, catering to applications where high sensitivity and ease of use are crucial. Overall, the integration of nanoparticles on SPEs significantly improves the performance and versatility of electrochemical sensors in various fields like environmental monitoring, biomedical diagnostics, and chemical analysis.

Furthermore, the incorporation of antibodies onto screen-printed electrodes greatly enhances the selectivity of electrochemical sensors, enabling accurate and specific detection of target analytes in various applications. Antibodies are highly specific biomolecules with unique binding sites that recognize and selectively bind to specific target analytes with high affinity. Immobilizing antibodies on the electrode surface ensures close proximity to the target analytes, increasing the likelihood of interaction. When a sample is introduced to the sensor, the target analyte binds specifically to the immobilized antibodies, triggering a specific electrochemical reaction. This generates an electrical signal proportional to the amount of

bound target analyte, resulting in a high signal-to-noise ratio and improved sensitivity. The presence of immobilized antibodies reduces interference from other substances in the sample, ensuring reliable measurements. Antibody-based electrochemical sensors find applications in medical diagnostics, environmental monitoring, and food safety due to their versatility and real-time monitoring capability. Additionally, the stability of immobilized antibodies extends the sensor's shelf life, making it suitable for long-term use in both laboratory and field settings. Overall, this integration enhances the sensor's performance and applicability, making it a powerful tool for precise and reliable analytical measurements.

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Conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this article.

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