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Cyclic voltammetry determination of antipsychotic drug by a screen-printed electrode modified with a nanocomposite prepared from carbon nanotubes and metals

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ABSTRACT

To achieve the proper use of clozapine (CLZ) and ensure that it is safe for patients with schizophrenia, the regular monitoring of serum CLZ levels is essential for ensuring the safe and effective use of this drug. Such monitoring enables required modifications to treatment programs to enhance effectiveness and reduce undesirable side effects. RuO_2/Au/MWCNT refers to a composite material composed of three distinct layers: ruthenium dioxide (RuO_2), gold (Au), and multi-walled carbon nanotubes (MWCNT). The RuO_2/Au/MWCNT composite is likely designed to combine the beneficial properties of each component. In order to increase the surface-specific area of the working electrode (WE), electrical conductivity, and detection efficiency of CLZ, this study offers a modified screen-printed electrode (SPE) using RuO_2/Au/MWCNT nanocomposites. The electrochemical CLZ determination was studied by using cyclic voltammetry (CV). The proposed electrochemical sensor exhibited a CV response to CLZ within a linear range from 0.5 to 20 µM, a limit of detection (LOD) of 0.05 µM, at a correlation coefficient of 0.999 and a sensitivity of 0.479 µA/µM. The resulting electrochemical sensor demonstrated wide linearity covering the therapeutic range (350-600 ng/ml) of CLZ, and this approach can be a good model for biomedical application for CLZ detection in the future.

1. INTRODUCTION

Schizophrenia is a severe and chronic mental illness that affects a person's thoughts, emotions, and behavior. A few of the symptoms that characterize schizophrenia include hallucinations, delusions, disorganized thinking and speech, a lack

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of emotion, and impaired social functioning. Typically, a combination of medicine and therapy is used to treat schizophrenia. Antipsychotic medicine, which helps with symptoms like hallucinations, delusions, and disordered thinking, is the primary method of treatment (American Psychiatric Association 1998). For patients who have not reacted well to previous antipsychotic medications, clozapine (CLZ) is frequently administered (Dickerson and Lehman 2011). The ability of CLZ to effectively manage treatment-resistant schizophrenia (TRS) is one remarkable aspect (Siskind et al. 2021), (Leon et al. 2020). However, because of potential side effects such sedation, weight gain, and hypersalivation, CLZ carries some serious dangers and requires strict monitoring. The most serious issue with CLZ, however, is agranulocytosis, a condition that can be deadly and is marked by a rapid decrease in white blood cells (Leon et al. 2020). White blood cell counts and clozapine levels must be regularly checked in blood tests while using clozapine.

High-performance liquid chromatography (HPLC) (Zhang, Terry, and Bartlett 2007), solid phase (LC-MS) (Niederländer et al. 2006), and liquid-liquid extraction (UHPLC-MS/MS) (Wohlfarth et al. 2011) are a few examples of conventional approaches for the detection of CLZ. It's important to note that because these approaches require specialists, complicated sample preparation processes, and are time-consuming. the availability and application of particular analytical techniques may vary depending on the research facility and resources available. Compare with conventional approaches, an electrochemical technique might be another approach that can be applied for CLZ detection. There are many advantages of this technique, such as high sensitivity, selectivity, and quick response times. Additionally, electrochemical technique is suitable for on-site or point-of-care testing due to their ability to be miniaturized and integrated into portable device. A screen-printed electrode (SPE) is a versatile, cost-effective electrode design that allows for easy mass production and disposable usage.

Nanomaterials (nanoparticles, nanowires, or nanotubes), molecularly imprinted polymers (MIPs), metal/metal oxide, and other material modifications are all possible for electrodes in electrochemical sensors (Majumdar, Maiyalagan, and Jiang 2019; Shah 2018). To produce synergistic effects and improve sensor performance, various modifications can be combined. Multi-walled Carbon Nanotubes (MWCNT) are a type of carbon-based nanomaterial that have several unique characteristics that make them promising for a variety of applications (Gao et al. 2012), (Gobbo, Biesinger, and Workentin 2013). In previous report, a gold nanoparticles (Au) have extremely stable and conductive electrical characteristics when applied, and they are frequently utilized in electronics and electrical applications (El-Cheick et al. 2010), (Shah 2018). Moreover, Ruthenium dioxide (RuO_2) is a metal oxide that has excellent electrical conductivity and acts as a catalyst for electrochemical processes (Aflatoonian et al. 2020; Majumdar, Maiyalagan, and Jiang 2019; Shah 2018).

In this study, novel, simple, and effective disposable sensors for CLZ detection were developed using RuO_2/Au/MWCNT nanocomposites modified on SPE. CV was used to investigate the electrochemical response of CLZ. This study aims to create a type of nanocomposite that could be a good candidate for a sensing platform for other medications through the combination of metal, metal oxides, and MWCNT.

2. MATERIALS AND METHODS

2.1 Chemicals and materials

Acetic acid (CH_3COOH) >99.0%, phosphoric acid (H_3PO_4), boric acid (H_3BO_3), ethanol ($C_2H_6O_6$), methanol (CH_3OH), gold (III) chloride trihydrate 99.9% ($HAuCl_4$), sodium hydroxide (NaOH) 98.0 %, sulfuric acid (H_2SO_4) ≥95.0%, potassium chloride (KCl) 99.0%, COOH-functionalized MWCNT (MWCNT-COOH) > 95%, ruthenium (III) chloride hydrate ($RuCl_3$·xH2O), and clozapine (European Pharmacopeia) were purchased from Sigma-Aldrich. Britton Robinson buffer solution (BRS) pH 4 to 10 was used as a supporting electrolyte in the present study. The screen-printed carbon electrode (SPE) was purchased from Quasense Co., Ltd. (Thailand). All of electrochemical measurements were performed on AutoLab PGSTAT128N system (EcoChemie B.V., Ultrecht, Netherlands).

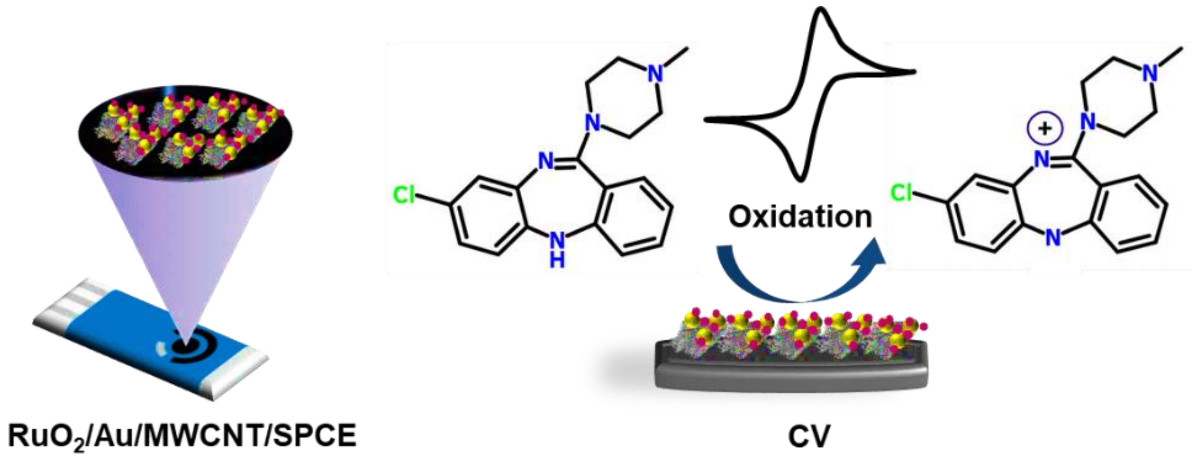

Fig. 1 The schematic of SPE modification with RuO_2/Au/MWCNT nanocomposite for CLZ determination.

2.2 Electrode fabrication

The SPE with the RuO_2/Au/MWCNT nanocomposite for CLZ determination was prepared as follows: 0.25 mg/ml of MWCNT was dispersed in a 60% v/v of ethanol using ultrasonication. Then, 3 µl of the suspension was gently applied on the surface of WE, and wait until completely dry at room temperature, resulting in the MWCNT/SPE. Next, the MWCNT/SPE was modified by electrodeposition of gold nanoparticles (Au NPs) using 1.0 mM $HAuCl_4$ solution in 0.5 M H_2SO_4, with an applied potential of −0.2 V for 4 minutes. Afterward, the electrode was rinsed with deionized water to remove unreacted gold (III) ions and dried at room temperature to obtain the Au/MWCNT/SPE. Finally, the

RuO_2/Au/MWCNT/SPE modified electrode was prepared by electrochemically depositing $RuCl_3$ onto the Au/MWCNT/SPE using differential pulse voltammetry (DPV). This was achieved by applying a potential of 1.2 V for 2 minutes in a solution containing 0.05 mM $RuCl_3$ and 30% v/v of methanol. The resulting schematic of the SPE modification with the RuO_2/Au/MWCNT nanocomposite for CLZ determination was shown in Fig. 1.

2.3 Preparation of standard CLZ

To prepare the stock solution of CLZ, 0.1 mg of CLZ powder was dissolved in methanol. From this stock solution, various concentrations of CLZ were prepared by diluting with 0.1 M BRS buffer solution containing 0.01 M KCl at pH 7. These diluted solutions were used for recording the CV data.

3. RESULTS AND DISCUSSIONS

3.1 Effect of scan rate

The scan rate and pH value were determined in order to optimized an operational condition for CLZ determination. With a 1.0 μM CLZ solution in BRS buffer at pH 7, cyclic voltammetry was applied with scan rates ranging from 10 to 100 mV/s^{-1} (10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 mV/s^{-1}). With scan rate increasing, the oxidative peak currents increasing were observed, a slightly shifted to a positive potential was found (Fig. 2 (A)). The CV showed the anodic peak currents are linear with potential scan rates in a range from 10 to 100 with a correlation coefficient of 0.9889 (Fig. 2 (B)). These results indicated that an adsorption process controls the electrochemical oxidation of CLZ on the RuO_2/Au/MWCNT/SPE.

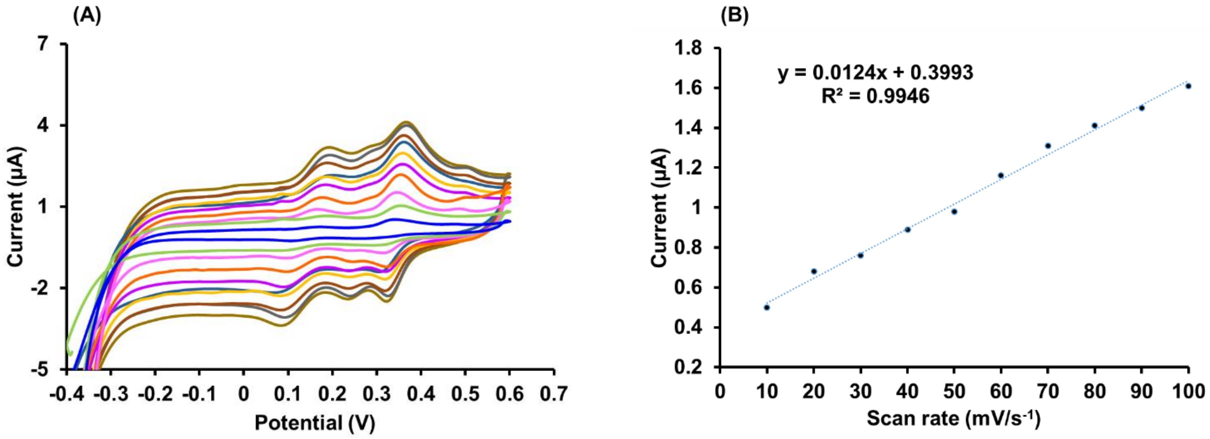

Fig. 2 (A) The CV current response of RuO_2/Au/MWCNT/SPE in BSR buffer solution at pH 7 containing 1.0 μM CLZ at various scan rates between 10 to 100 mV/s^{-1}, respectively. (B) The plot of variation of CV peak current versus different scan rates.

3.2 Effect of pH

The influence of pH on the electrochemical oxidation of CLZ on the RuO_2/Au/MWCNT/SPE modified electrode were also studied. Cyclic voltammograms were recorded for 1.0 μM CLZ in BRS buffer solution at a scan rate of 50 mV/s^{-1} at various pH values ranging from 4 to 10 (Fig. 3 (A)). In Fig. 3 (B), the current plotted against different pH values revealed that the oxidation peak current change increased (from pH 4 to 7) and then decreased for higher pH values. The maximum current response was observed at pH 7, therefore BRS of pH 7 was used for CLZ detection throughout this work.

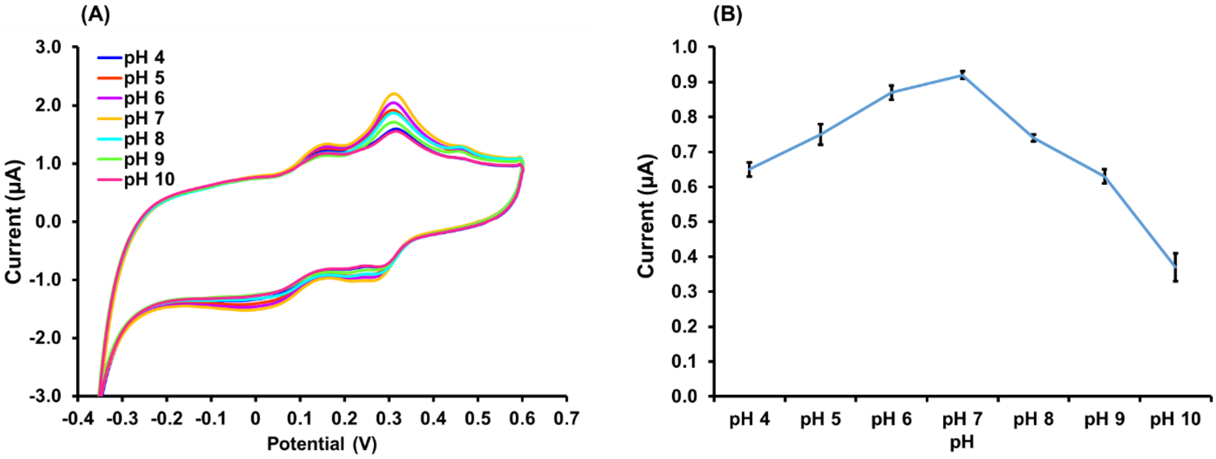

Fig. 3 (A) CV responses of RuO_2/Au/MWCNT/SPE modified electrode in BRS buffer solution at different pH value between 4 to 10. (B) Plot of current (μA) versus different pH values between 4 to10.

3.3 Determination of CLZ by RuO_2/Au/MWCNT/SPE modified electrode

The studies were carried out by CV under optimum conditions to examine the electrochemical performance of RuO_2/Au/MWCNT/SPE to CLZ. Fig. 4 (A) displayed the signal response for different concentrations of CLZ from 0.5 μM to 20 μM in 0.1 M BRS buffer solution at pH 7, with a scan rate of 50 mV/s^{-1}. As expected, the oxidation peaks current increased with the increased of analytes concentration. The resulting calibration plots (Fig. 4 (B)) show a good linear over the range from 0.5 μM to 20 μM with the corresponding linear regression equation of $y = 0.4793x + 0.306$ ($R^2 = 0.999$) and a sensitivity of 0.479 μA/μM. The limit of detection (LOD) was determined to be 0.05 μM, calculated using the ratio between 3 times the standard deviation of the blank (n = 7) and the slope from the linear range. This result demonstrated the excellent electrochemical performance of the RuO_2/Au/MWCNT/SPE for CLZ determination. Additionally, this sensor showed wide linearity and low detection limit cover the therapeutic range of 350-600 ng/ml or 1.07-1.84 μM, of CLZ (Leon et al. 2020). This result indicated the potential utility of the RuO_2/Au/MWCNT/SPE for accurate CLZ detection within the therapeutic dose range.

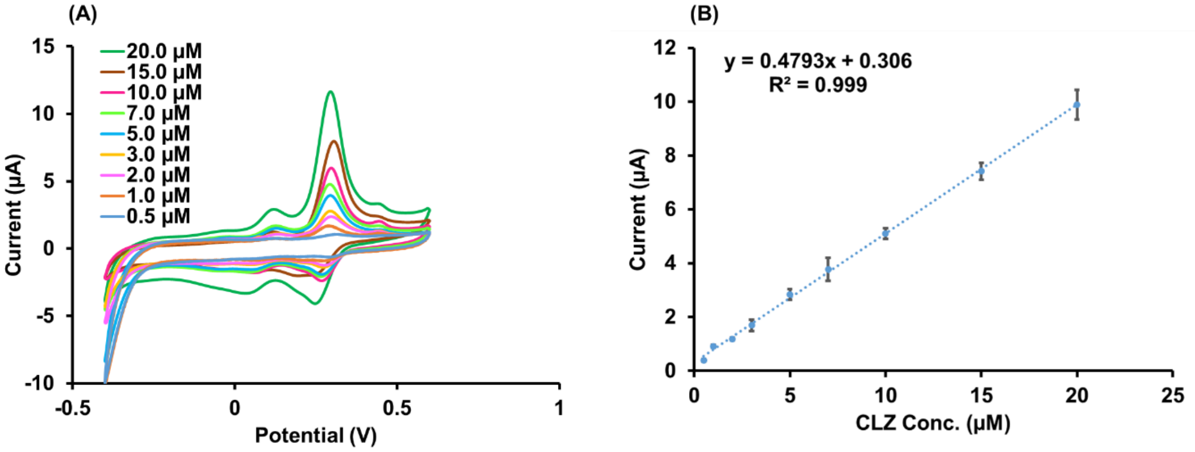

Fig. 4 (A) The CV current responses of RuO_2/Au/MWCNT/SPE modified electrode for various concentrations of CLZ from 0.5 to 20 µM (0.5, 1.0, 2.0, 3.0, 5.0, 7.0, 10.0, 15.0 and 20.0 µM) in 0.1 M BRS buffer solution, pH 7.0 at scan rates 50 mV/s^{-1} respectively. (B) The calibration plot presents the relationship between the CV peak current (µA) and the concentrations of CLZ (µM).

3. CONCLUSIONS

As a sensing platform for CLZ detection, we have introduced a RuO_2/Au/MWCNT nanocomposite that exhibits captivating potential. The result showed the effectiveness of the electrochemical sensor based on the RuO_2/Au/MWCNT/SPE for CLZ determination. This success can be attributed to the modified electrode's outstanding electrocatalytic activity, high electrochemically active surface, and other specific features such its simplicity in surface preparation and regeneration. Before this sensor could be fully utilized, more testing is required. It must be tested on real samples to confirm its accuracy and efficacy. Furthermore, the selectivity, reproducibility, and stability need to be evaluated.

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REFERENCES

Aflatoonian, Mohammad Reza, Somayeh Tajik, Bita Mohtat, Behnaz Aflatoonian, Iran Sheikh Shoaie, Hadi Beitollahi, Kaiqiang Zhang, Ho Won Jang, and Mohammadreza Shokouhimehr. (2020), “Direct Electrochemical Detection of Clozapine by RuO_2 Nanoparticles-Modified Screen-Printed Electrode.” *RSC Advances* **10** (22): 13021–28. https://doi.org/10.1039/D0RA00778A.

American Psychiatric Association, ed. (1998), "Diagnostic and Statistical Manual of Mental Disorders: DSM-IV"; *Includes ICD-9-CM Codes Effective 1. Oct. 96*. 4. ed., 7. print. Washington, DC.

Dickerson, Faith B., and Anthony F. Lehman. (2011), “Evidence-Based Psychotherapy for Schizophrenia: 2011 Update.” *Journal of Nervous & Mental Disease* **199** (8): 520–26. https://doi.org/10.1097/NMD.0b013e318225ee78.

El-Cheick, F. M., F. A. Rashwan, H. A. Mahmoud, and Mahmoud El-Rouby. (2010), “Gold Nanoparticle-Modified Glassy Carbon Electrode for Electrochemical Investigation of Aliphatic Di-Carboxylic Acids in Aqueous Media.” *Journal of Solid State Electrochemistry* **14** (8): 1425–43. https://doi.org/10.1007/s10008-009-0957-4.

Gao, Chao, Zheng Guo, Jin-Huai Liu, and Xing-Jiu Huang. (2012), “The New Age of Carbon Nanotubes: An Updated Review of Functionalized Carbon Nanotubes in Electrochemical Sensors.” *Nanoscale* **4** (6): 1948. https://doi.org/10.1039/c2nr11757f.

Gobbo, Pierangelo, Mark C. Biesinger, and Mark S. Workentin. (2013), “Facile Synthesis of Gold Nanoparticle (AuNP)–Carbon Nanotube (CNT) Hybrids through an Interfacial Michael Addition Reaction.” *Chemical Communications* **49** (27): 2831. https://doi.org/10.1039/c3cc00050h.

Leon, Jose, Georgios Schoretsanitis, John M. Kane, and Can-Jun Ruan. (2020), “Using Therapeutic Drug Monitoring to Personalize Clozapine Dosing in Asians.” *Asia-Pacific Psychiatry* **12** (2). https://doi.org/10.1111/appy.12384.

Majumdar, Dipanwita, Thandavarayan Maiyalagan, and Zhongqing Jiang. (2019), “Recent Progress in Ruthenium Oxide-Based Composites for Supercapacitor Applications.” *ChemElectroChem* **6** (17): 4343–72. https://doi.org/10.1002/celc.201900668.

Shah, Kwok Wei. (2018), “A Review on Enhancement of Phase Change Materials - A Nanomaterials Perspective.” *Energy and Buildings* 175 (September): 57–68. https://doi.org/10.1016/j.enbuild.2018.06.043.

Siskind, Dan, Meghna Sharma, Mrinal Pawar, Ella Pearson, Elias Wagner, Nicola Warren, and Steve Kisely. (2021), “Clozapine Levels as a Predictor for Therapeutic Response: A Systematic Review and Meta-analysis.” *Acta Psychiatrica Scandinavica* **144** (5): 422–32. https://doi.org/10.1111/acps.13361.

Wohlfarth, Ariane, Nicole Toepfner, Maren Hermanns-Clausen, and Volker Auwärter. (2011), “Sensitive Quantification of Clozapine and Its Main Metabolites Norclozapine and Clozapine-N-Oxide in Serum and Urine Using LC-MS/MS after Simple Liquid–Liquid Extraction Work-Up.” *Analytical and Bioanalytical Chemistry* **400** (3): 737–46. https://doi.org/10.1007/s00216-011-4831-8.