Carbon Paste Electrode Modified by Dibenzo-18-crown-6 for the Determination of Paracetamol Using Differential Pulse Voltammetry Technique

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Abstract: *The fabrication, optimization and validation of measurement using a modified carbon paste electrode (CPE) with dibenzo-18-crown-6 were carried out for the determination of paracetamol in commercial products. The pH, dibenzo-18-crown-6 concentration in carbon paste, and scan rate parameters were optimized. Validation of the measurement was observed from linear concentration range, LoD, LoQ, precision, and percentage of the recovery. The result showed that the optimum pH was at 2, the optimum concentration of dibenzo-18-crown-6 in carbon paste was 0.6%, optimum scan rates were 35 mV/s for CPE, and increased to 50 mV/s using modified CPE. The linear concentration range for CPE was obtained at 10-300 μM with LoD and LoQ of 28.88 and 96.28 μM, respectively. Meanwhile, CPE modified with dibenzo-18-crown-6 gave wider linear concentration range at 1–700 μM with LoD and LoQ of 52.36 and 174.53 μM, respectively. The CPE and modified CPE had good precision, with Horwitz ratio values of less than two. The percentage of recovery for two samples with three replicates measurements was obtained (89.81 ± 1.38)% and (108.02 ± 0.42)% for samples A and B, respectively. Dibenzo-18-crown-6 modified CPE was used for the determination of paracetamol in both samples yielding 97–98% compared with the paracetamol composition on its labels.*

Keywords: *carbon paste electrode; dibenzo-18-crown-6; differential pulse voltammetry; paracetamol*

■ INTRODUCTION

The methods used in drug analysis are very diverse. Various methods for determination of drugs in biological samples (urine, blood, and sweat) and pharmaceutical formulations that can be used include UV spectrophotometry, High-Performance Thin Layer Chromatography (HPTLC) Spectrophotodensitometry, High-Performance Liquid Chromatography (HPLC), Liquid Chromatography-Mass Spectrometry (LC-MS), Fourier Transform Infra-Red (FTIR), and voltammetry methods [1-6]. Voltammetry is a method in electroanalytical chemistry based on the oxidation-reduction reaction of electroactive species on the surface of the electrode. The resulting current response is proportional to the concentration of the analyte in the solution [7]. Various techniques of the voltammetry method can be used to determine drugs' content in a sample, including differential pulse voltammetry (DPV).

The performance of the voltammetry method is greatly influenced by the working electrode materials. The most used electrode materials are mercury (Hg), carbon (C), or precious metals such as platinum (Pt) and gold (Au). Carbon-based electrodes are currently widely developed in the electroanalysis field because they have several advantages, i.e., wide potential range, low background current, cheap, inert, and suitably used in various sensors. One type of carbon-based electrode is the carbon paste electrode. The electrode is easily made at a low cost, easy occur exchanges electrons on its surface and can be modified with various kinds of materials [8]. The previous research showed many compounds could be used as a modifier in carbon paste

electrodes in the determination of drugs, including anthraquinone, nanoparticles ferrocene, poly-*L*-leucine, zirconium oxide (ZrO_2), and crown ether [9-13]. The use of crown ether in the modification of carbon paste can speed up the electron transfer rate due to the adsorption process or form of complex compounds. Crown ether is one of the macrocyclic whose oxygen atoms as donor electrons in the formation of complex compounds. Crown ether can form a stable complex compound with alkali metal ions and protonated amine in the gas and solution phase [14]. It shows crown ether is widely used as a modifier in the determination of drugs in pharmaceutical formulations, including paracetamol.

Determination of paracetamol can be carried out by the voltammetry method because paracetamol can be oxidized through the transfer process of two electrons and two protons to form an unstable oxidized product of *N*-acetyl-p-quinone imine (NAPQI) [15]. The oxidation process of paracetamol is affected by pH, where the oxidation reaction of paracetamol produces an H^+ ion. If the amount of H^+ formed increase, then the pH of the solution decrease and the product of the reaction will turn back and becomes reactant. Therefore, a buffer solution is needed to retain the reaction product.

This method shall be optimized and validated to determine the performance of the modified working electrode in the identification of the analyte. The validation method gives proof to the measured parameters by testing objectively in a laboratory and has fulfilled requirements that have been determined according to the intended use [16]. In this research, the modification of the carbon paste electrode with crown ether for the determination of paracetamol was carried out by the DPV technique. Carbon paste was modified with crown ether to produce electrodes having high sensitivity or low detection limit in the determination of paracetamol in commercial samples.

■ EXPERIMENTAL SECTION

Materials

All of the reagents were analytical grade without further purification. Paracetamol, KCl, graphite powder (dark grayish black powder, particle size pass 45 μm), crown ether (dibenzo-18-crown-6), NaOH, and liquid paraffin were purchased from Wako. $K_3Fe(CN)_6$, $K_4Fe(CN)_6{\cdot}3H_2O$, acetone, H_3PO_4 85%, CH_3COOH 100%, and H_3BO_3 were obtained from Merck. On the other hand, HCl 37% was produced from Mallinckrodt while filter paper and double distilled water were purchased from Otsuka. Samples A and B were commercial paracetamol tablets of different brands, bought from a chemist.

Instrumentation

Potentiostat Ingsens 1030, 12 V DC adapter, Ag/AgCl reference electrode (0.1 M KCl), and platinum coil electrode with a diameter 0.5 mm (Nilaco) were used as the counter electrode. Besides, analytical balance (Ohaus PX224), magnetic stirrer (Thermoline), copper wire diameter 1.0 mm (Nilaco), Teflon tube with inner diameter 2.0 mm, pH meter (WalkLAB HP900), agate mortar, and laboratory glass equipment were used in this work.

Procedure

Several conditions can affect the electrochemical currents, such as pH, and the concentration of the modifier. All the measurements were recorded using a potentiostat Ingsens 1030 with three electrodes system by DPV technique at room temperature.

pH optimization

Copper wire (Cu) 7 cm in length was inserted into the Teflon tube as a working electrode body, with around ±5 mm space at the bottom of the tube for inserting carbon paste. A 100 mg graphite and 35 μL liquid paraffin were mixed gently in an agate mortar until a homogeneous carbon paste formed. Carbon paste was inserted into the space at the bottom of the working electrode (CPE). The surface of CPE was smoothed by polishing on wax paper. Determination of optimum pH was carried out by measuring the current of paracetamol standard solution 1.0 mM, dissolved in Britton Robinson buffer solution in various pH. The pH of 0.1 M Britton Robinson buffer solution was adjusted to 1.0; 1.5; 2.0; 2.5; 3.0; 4.0; 5.0; 6.0; 7.0; and 8.0 by adding HCl 0.1 M or NaOH 0.1 M. Measurement current for each solution

was conducted using a potentiostat with three electrodes system, i.e., CPE as working electrode, Ag/AgCl as reference electrode, and Pt coil as a counter electrode by differential pulse voltammetry technique.

Crown ether concentration optimization

Graphite powder and liquid paraffin were mixed with various concentrations of crown ether (dibenzo-18-crown-6), i.e., 0.0, 0.2, 0.4, 0.5, 0.6, 0.7, 0.8, 1.2, 1.6, and 2.2% from the total mass of graphite and dibenzo-18-crown-6. The modified carbon paste was inserted into the working electrode body, and the surface was smoothed. CPE modified by dibenzo-18-crown-6 was dipped in a voltammeter cell containing 10.0 mL of paracetamol 1.0 mM at optimum pH, then connected to a potentiostat for measuring peak current at a potential range of 0.0–1.2 V with scan rate 50 mV/s.

Scan rate optimization

The peak current of a standard solution of paracetamol 1.0 mM was measured with various scan rates, starting from 20, 25, 30, 35, 40, 45, 50, 55, to 60 mV/s at potential windows between 0.0–1.2 V. Measurement peak current using CPE and modified CPE with dibenzo-18-crown-6 was conducted at optimum concentration.

Determination of linear concentration range

The stock solution of paracetamol was diluted in the range concentration of 1–950 µM and then the electrochemical current was measured using CPE and modified CPE with dibenzo-18-crown-6 at pH and scan rate optimum with three repetitions. The current measured on the y-axis is plotted with the concentration solution on the x-axis, to obtain the linear regression equation $y = a + bx$ with R^2 close to 1.00.

Determination of limit of detection (LoD) and limit of quantitation (LoQ)

LoD and LoQ values were calculated with the following Eq. (1–4):

$$S_{y/x} = \sqrt{\frac{\sum\left(y_i - \hat{y}_i\right)^2}{n-2}} \quad (1)$$

$$SB = \frac{S_{y/x}}{\sum\left(x_i - \bar{x}\right)^2} \quad (2)$$

$$Y\ LoD = Y_B + 3S_B \quad (3)$$

$$Y\ LoQ = Y_B + 10S_B \quad (4)$$

where $S_{y/x}$ = standard deviation to the linear line, S_B = standard deviation of slope, and Y_B = response of blank solution. The values of LoD and LoQ were calculated using Eq. (3) and (4) [16].

Precision

The peak current of paracetamol 1.0 mM was measured using a different modified working electrode in the same composition, with 10 replicates. Measurements were carried out on different days for five days. The standard deviation (SD), relative standard deviation (RSD), and coefficient variance (CV) values were calculated. CV value compared with CV Horwitz to obtain the Horwitz ratio. Precision can be accepted if the Horwitz ratio is less than two [17].

Recovery

The measurements were carried out by measuring the electrochemical current of the sample and adding a known concentration of standard solution into the sample solution as a matrix. The percentage of recovery was calculated by Eq. (5):

$$\%R = \frac{CF - CU}{CA} \times 100 \quad (5)$$

where CF = concentration of sample and standard, CU = concentration of sample without adding standard, and CA = concentration of standard. The acceptable recovery value for analyte measurement with concentrations of g/L is in the range of 80 to 110% [18].

Samples preparation

Three tablets for each sample were weighed and crushed in a mortar, then dissolved with Britton-Robinson buffer at optimum pH. The mixture was filtered through a filter paper into a 50.0 mL flask to eliminate possible interference and diluted until the mark. The standard addition method was used for the analysis of two different samples containing paracetamol. A 2.0 mL was pipetted and transferred into a 10.0 mL flask, added standard paracetamol solution was in various volumes, and then diluted up to 10.0 mL by adding buffer solution. A blank solution was prepared in the same way without a standard solution.

■ RESULTS AND DISCUSSION

pH Optimization

For the oxidation reaction of paracetamol, DPV was used. The voltammograms were recorded in the potential from 0 to 750 mV. Fig. 1 shows the voltammograms of paracetamol 1 mM at a diverse buffer medium. The most intense anodic peak current was obtained at pH 2.0, while at pH below and above 2.0, the anodic peak current decreased. It indicated that the equilibrium of the reversible redox reaction of paracetamol to *N*-acetyl-*p*-quinone imine occurs at pH 2. The anodic peak potentials were shifted in a negative direction when pH values increased. This study showed that the electron transfer process of paracetamol was dependent on pH. Fig. 2 shows the oxidation reaction of paracetamol occurs in an acidic environment, producing an *N*-acetyl-*p*-quinone imine compound and two protons [19].

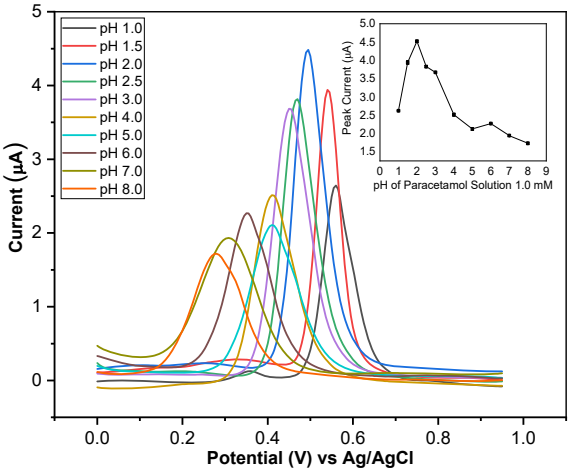


Fig 1. Differential pulse voltammogram of paracetamol 1 mM in various pH, and plot of pH vs its anodic peak current

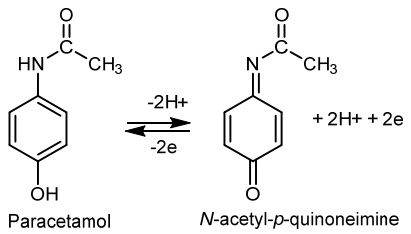


Fig 2. Oxidation reaction of paracetamol in an acidic environment

Crown Ether Concentration Optimization

DPV response of electrochemical oxidation of paracetamol in the presence of modifier dibenzo-18-crown-6 in carbon paste can be seen in Fig. 3. The anodic peak current for carbon paste without a modifier is very low at 1.021 μA. When the modifier was added, the anodic peak current increased up to the concentration of modifier 0.6% at 2.290 μA and then decreased. This shows that the modifier of dibenzo-18-crown-6 can improve the performance of the carbon paste electrode; the rate of electron transfer is faster compared to without the presence of a modifier. All peak currents at different modifier concentrations appeared at the same peak potential around 0.6 V. This is due to two benzene rings in dibenzo-18-crown-6 structure can reduce the polarity of crown ether and easily solvated with paraffin in carbon paste [20]. Dibenzo-18-crown-6 in carbon paste electrode can form a complex compound with a proton in paracetamol with oxygen atoms from the modifier, as shown in Fig. 4.

Scan Rate Optimization

The change in scan rates gives the different anodic peak currents. It was found that the optimum scan rates of paracetamol solution 1.0 mM using CPE were found at 35 mV/s and increased to 50 mV/s using CPE modified by dibenzo-18-crown-6. Moreover, the peak current was

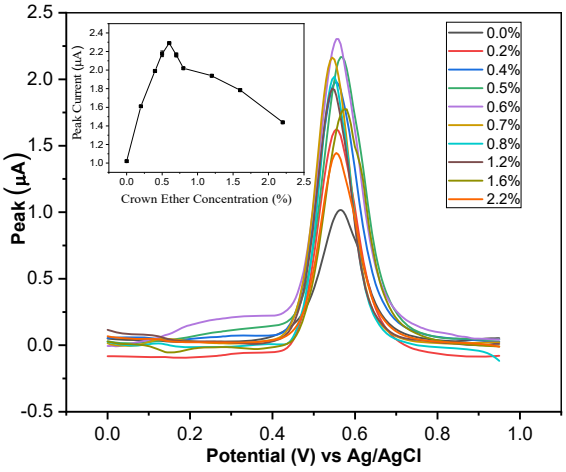


Fig 3. Differential pulse voltammograms in the optimization of dibenzo-18-crown-6 concentration

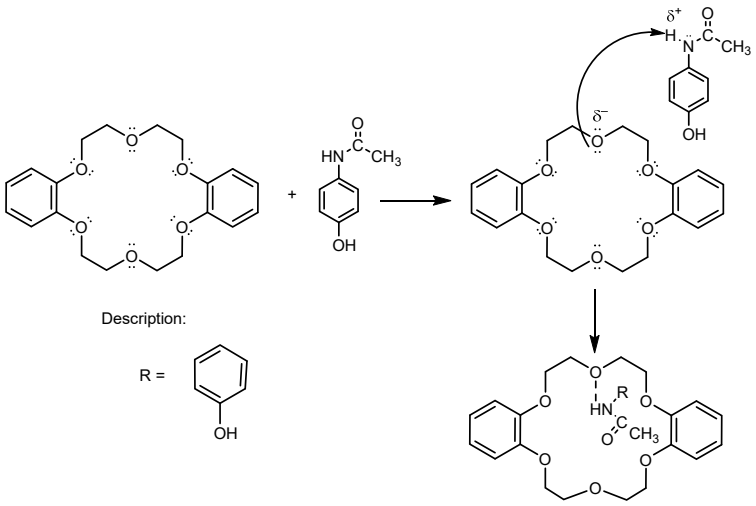


Fig 4. Reaction mechanism of dibenzo-18-crown-6 with paracetamol to form complex compound

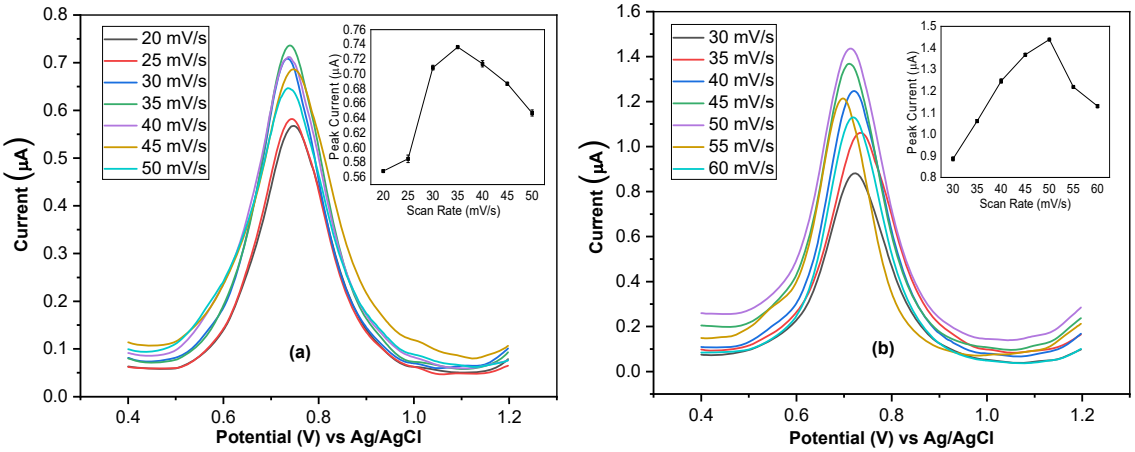


Fig 5. Differential pulse voltammograms of paracetamol 1.0 mM using CPE (a) and CPE modified by dibenzo-18-crown-6 (b) and plot between variation of scan rates and peak current (inset)

obtained at 0.736 µA and went up to 1.4391 µA by CPE and modified CPE, respectively. The result shows the addition of a modifier in carbon paste, the electron transfer rate of paracetamol at the surface of the working electrode becomes faster with a higher anodic peak current. Fig. 5 showed differential pulse voltammograms of paracetamol 1.0 mM in various scan rates.

Linear Concentration Range

Determination of linear concentration ranged using CPE and modified CPE with dibenzo-18-crown-6 in the measurement of anodic peak current was carried out in the concentration range of paracetamol 1–950 µM at optimum scan rate. Fig. 6(a-b) shows the voltammogram of peak current. Fig. 6(c) shows the linear regression equation on the concentration range of 10–300 µM using CPE was y = 0.0806 + 0.0051x with the correlation coefficient (R^2) value was 0.9947 and the linear regression equation on the concentration range of 1–700 µM using CPE modified by dibenzo-18-crown-6 was y = 0.1126 + 0.0062x with the R^2 value was 0.9951. The result displayed the addition of dibenzo-18-crown-6 in carbon paste increased the anodic peak current response with better linearity and sensitivity.

Detection Limit (LoD) and Quantitation Limit (LoQ)

LoD and LoQ values were determined based on the data from a linear concentration range of 10–300 µM for the measurement using CPE and 1–700 µM for the

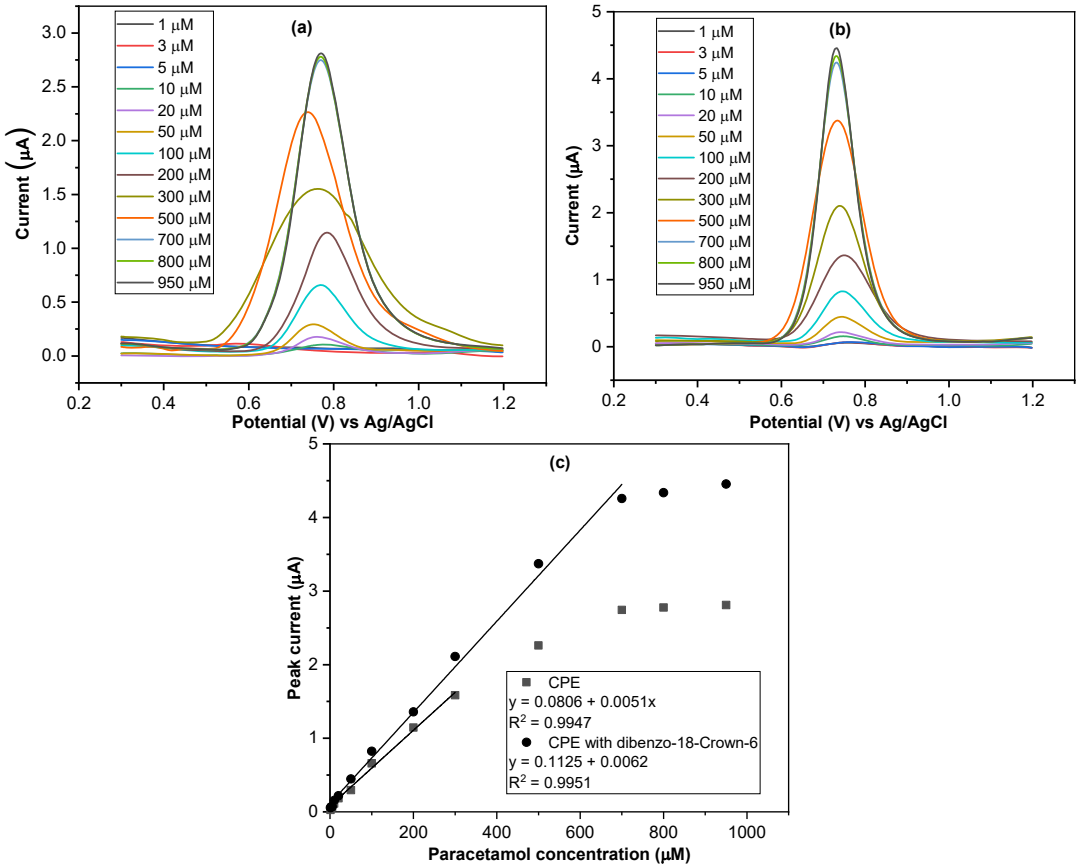


Fig 6. Differential pulse voltammograms of paracetamol with various concentration using CPE (a) and modified CPE with dibenzo-18-crown-6 (b), and linear regression line of paracetamol 10–300 µM by CPE and 1–700 µM by modified CPE with dibenzo-18-crown-6 (c)

measurement using CPE modified by dibenzo-18-crown-6. The values of LoD and LoQ for the measurement paracetamol using CPE were 28.88 and 96.28 µM, respectively, while for modified CPE were 52.36 and 174.53 µM, respectively. Dibenzo-18-crown-6 in carbon paste can increase the sensitivity of the measurement. Although the peak current of modified CPE was larger than CPE at the same concentration, the LoD and LoQ values of modified CPE were higher than CPE. This may be due to the large surface area of the working electrode, about 0.0314 cm^2. The large surface area causes high noise so the signal and noise ratio becomes larger, and the standard error is large, thereby increasing the LoD and LoQ values.

Precision

The precision can be determined by repeated measurement of the peak current of paracetamol in different concentrations using the same composition of working electrodes on different days. According to [17], a precise measurement has been established if the Horwitz ratio is less than two. Fig. 7(a) shows the Horwitz ratio value for paracetamol 50 µM by CPE, and CPE modified with dibenzo-18-crown-6 and CPE were 0.3354 and 0.2835, respectively. Fig. 7(b) shows the Horwitz ratio for paracetamol 100 µM was 0.2233 by CPE and 0.2196 by modified CPE. Both concentrations were conducted ten times. The precision of measurement standard paracetamol solution with CPE modified by dibenzo-18-crown-6 is smaller than CPE in paracetamol 50 and 100 µM. It shows the presence of dibenzo-18-crown-6 in carbon paste can increase the precision of the measurement.

Percent of Recovery

Percent of recovery was determined to find out the

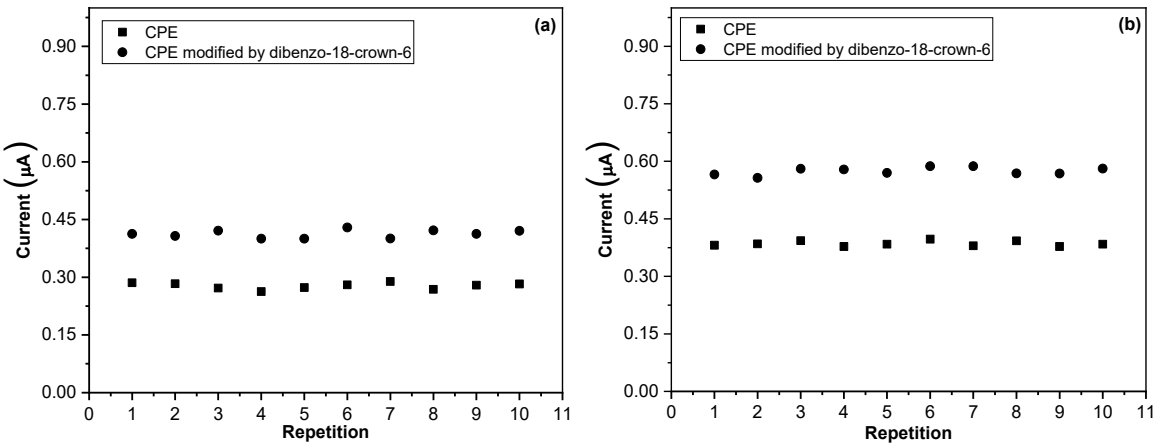


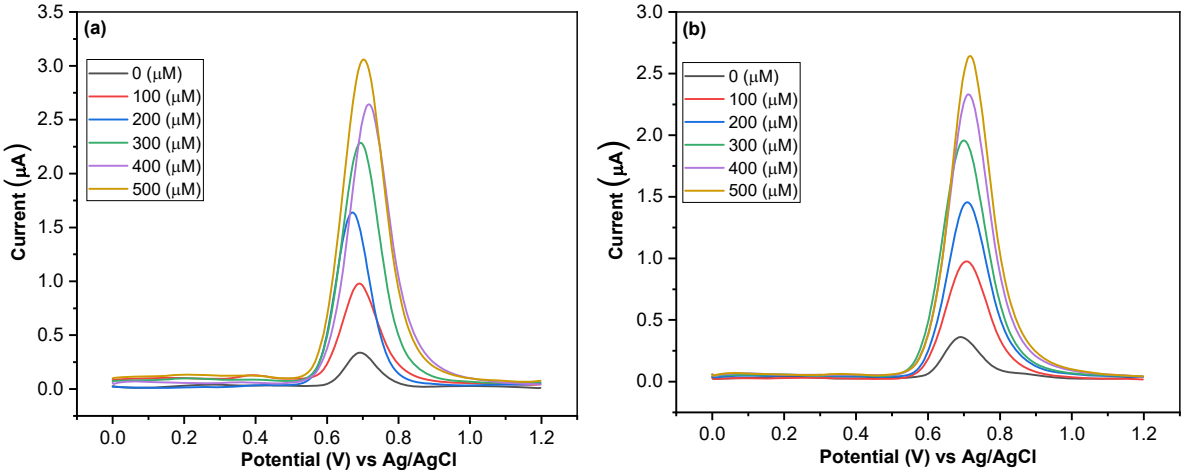
Fig 7. The peak current of ten times paracetamol measurement with concentration of 50 μM (a) and 100 μM (b)

accuracy of measurement**.** The percent recovery values were (89.81 ± 1.38)% in sample A and (108.02 ± 0.42)% in sample B. The acceptable recovery is in the range of 80–110% [18]. Based on the results of recovery obtained for samples A and B, measurements have acceptable values by DPV technique using CPE modified by dibenzo-18-crown-6. Sample B has a recovery of more than 100% because the tablet contains paracetamol and caffeine. The presence of caffeine in the solution can produce electrochemical currents that interfere with paracetamol measurement using carbon material as a working electrode due to the overlapping signal with the oxidation of the background medium, resulting in low reproducible analysis.

Determination Paracetamol in Sample

To illustrate the reliability of the results obtained, two commercial samples (A and B) were prepared, and measurements were conducted by the standard addition method. Sample A contains 500 mg paracetamol, and sample B contains 500 mg paracetamol and 65 mg caffeine. The regression line equations were found at $y = 0.3729 + 0.0063x$ with the value of $R^2 = 0.9962$ for sample A, meanwhile for sample B was obtained regression line equation $y = 0.3232 + 0.0055x$ with a value of $R^2 = 0.9986$. The voltammogram of the paracetamol standard solution and samples and the plot of paracetamol concentration vs its peak current can be seen in Fig. 8.

Paracetamol in sample A and sample B were calculated and found to be 493.2637 and 489.7067 mg/tablet, respectively. Both samples have a good agreement with the labeled value and result of measurement, which are listed in Table 1.



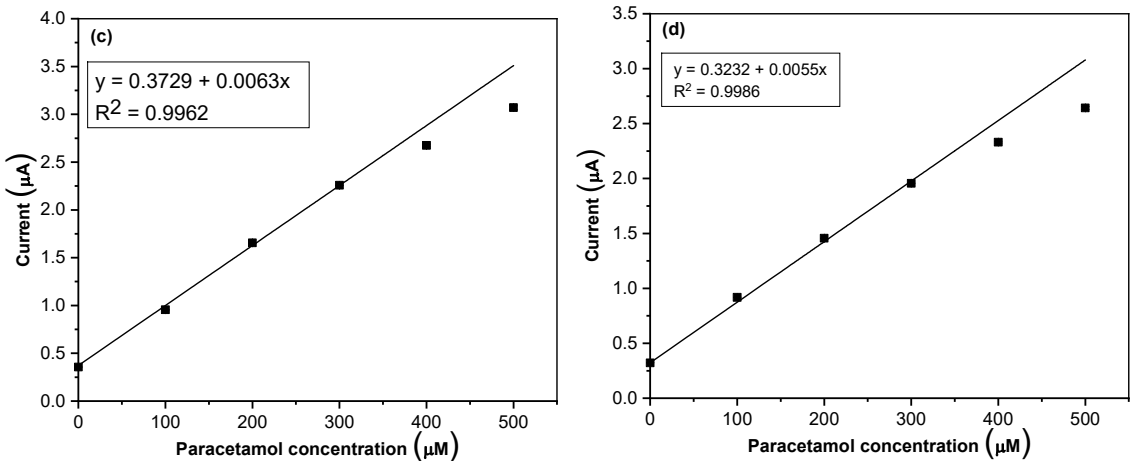


Fig 8. Voltammogram of measurement sample A (a) and sample B (b), and the plot of concentration paracetamol standard in the presence of sample A (c) and sample B (d) using DPV technique with CPE modified by dibenzo-18-crown-6

Table 1. Comparison of paracetamol mass measurement results with paracetamol mass on its labels

Sample	Paracetamol mass on its label (mg/tablet)	Paracetamol mass obtained by proposed method (mg/tablet)	Agreement (%)
A	500	493.3	98.65
B	500	489.7	97.94

■ CONCLUSION

The differential pulse voltammetry technique was applied to the study of paracetamol using CPE modified by dibenzo-18-crown-6. The highest peak current response was found at pH 2 in Britton-Robinson buffer 0.1 M, with 0.6% of modifier in carbon paste. The results of validity measurement were better using modified CPE compared with CPE. The application of CPE modified by dibenzo-18-crown-6 in the determination of paracetamol in commercial samples had a good agreement with its labeled.

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■ AUTHOR CONTRIBUTIONS

Ni Ketut Shinta Mas Methaninditya conducted the experiment. Irdhawati and Anak Agung Bawa Putra supervised, wrote and revised the manuscript. All authors agreed to the final version of this manuscript.

■ REFERENCES

[1] Tulandi, G.P., Sri, S., and Widya, A.L., 2015, Validation of analytical methods for determination of paracetamol levels in tablets by ultraviolet spectrophotometry (Indonesian), *Pharmacon*, 4 (4), 168–178.

[2] Susilawan, I.P.N.A., Siaka, I.M., and Parwata, I.M.O.A., 2018, Validation of chemical analysis methods of paracetamol and phenylbutason in traditional medicinal products with HPTLC-spectrophotodensitometry (Indonesian), *Cakra Kimia*, 7 (1), 1–11.

[3] Sarmento, Z.L.C., Rangdi, O.S.G., Olivia, S.G.R., Sena, B.M.C.D., and Dewi, K.N.M., 2020, Determination of paracetamol and caffeine levels using high performance liquid chromatography (HPLC) method (Indonesian), *Chakra Kimia*, 8 (2), 99–104.

[4] Tanuja, S.B., Kumara Swamy, B.E., and Pai, K.V., 2017, Electrochemical determination of paracetamol in presence of folic acid at nevirapine modified carbon paste electrode: A cyclic

voltammetric study, *J. Electroanal. Chem.*, 798, 17–23.

[5] Damanik, A.N.T., 2019, Determination of paracetamol levels in tablets with trade names and generics by Fourier transform infra red (FTIR) spectrophotometry (Indonesian), *Thesis*, Pharmacy Study Program, University of North Sumatra, Medan.

[6] Ayalew, M.E., and Ayitegeb, D.Y., 2021, Differential pulse voltammetric determination of paracetamol using activated glassy carbon electrode, *Am. J. Phys. Chem.*, 10 (2), 16–24.

[7] Wang, J., 2001, *Analytical Electrochemistry*, 2nd Ed., Wiley-VCH, New York.

[8] Wachid, M.R., and Setiarso, P., 2014, Preparation of bentonite modified carbon paste electrodes for the analysis of copper (II) metal with lead (II) and mercury (II) interfering ions by cyclic voltammetry stripping (Indonesian), *UJChem*, 3 (3), 93–103.

[9] Amare, M., and Teklay, W., 2019, Voltammetric determination of paracetamol in pharmaceutical tablet samples using anthraquinone modified carbon paste electrode, *Cogent Chem.*, 5 (1), 1576349.

[10] Salem, W.M., 2019, Sensitive determination of paracetamol using ferrocene nanoparticles by chitosan-functionalized-modified carbon paste electrode, *Egypt. J. Chem.*, 62 (4), 1079–1090.

[11] Naik, T.S.S.K., Swamy, B.E.K., Ramamurthy, P.C., and Chetankumar, K., 2020, Poly(*L*-leucine) modified carbon paste electrode as an electrochemical sensor for the detection of paracetamol in presence of folic acid, *Mater. Sci. Energy Technol.*, 3 (1), 626–632.

[12] Matt, S.B., Raghavendra, S., Shivanna, M., Sidlinganahalli, M., and Siddalingappa, D.M., 2021, Electrochemical detection of paracetamol by voltammetry techniques using pure zirconium oxide nanoparticle based modified carbon paste electrode, *J. Inorg. Organomet. Polym. Mater.*, 31 (2), 511–519.

[13] Irdhawati, I., Manurung, M., and Septiawan, K.A., 2015, Voltammetric dopamine detection using crown ether modified carbon paste electrodes (dibenzo-18-crown-6) (Indonesian), *J. Kim. Mulawarman*, 12 (2), 68–74.

[14] Melson, G.A., 1979, *Coordination Chemistry of Macrocyclic Compounds*, Plenum Press, New York.

[15] Sadok, I., and Tyszczuk-Rotko, K., 2015, New, simple and sensitive voltammetric procedure for determination of paracetamol in pharmaceutical formulations, *Insights Anal. Electrochem.*, 1, 1.

[16] Miller, J.N., and Miller, J.C., 2010, *Statistics and Chemometrics for Analytical Chemistry*, 6th Ed., Prentice Hall, Essex, England.

[17] Horwitz, W., and Albert, R.J., 2006, The Horwitz ratio (HorRat): A useful index of method performance with respect to precision, *J. AOAC Int.*, 89 (4), 1095–1109.

[18] AOAC International, 1998, *Peer-Verified Methods Program Manual Policies and Procedures*, Association of Official Analytical Chemists, Gaithersburg, MD, USA.

[19] Mulyasuryani, A., Tjahjanto, R.T., and Andawiyah, R., 2019, Simultaneous voltammetric detection of acetaminophen and caffein base on cassava starch-Fe_3O_4 nanoparticles modified glassy carbon electrode, *Chemosensors*, 7 (4), 49.

[20] Irdhawati, I., Manurung, M., and Maulinasari, A., 2015, Modification of carbon paste electrode with crown ether (dibenzo-18-crown-6) for ascorbic acid analysis by differential pulse voltammetry, *ALCHEMY Jurnal Penelitian Kimia*, 11 (2), 175–191.