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## Electrochemical investigation of paracetamol at Congo red modified carbon paste electrode: a voltammetric study

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### Abstract

*This work illustrates the modification of carbon paste electrode (CPE) by electropolymerization of Congo red. The poly(Congo red) modified CPE (Congo red/MCPE) was used to study the electrochemical behaviour of paracetamol (PA) by cyclic voltammetry. Compared to bare CPE, Congo red/MCPE exhibited better electrocatalytic activity towards the oxidation of PA, with an enhanced oxidation peak current in phosphate buffer solution at neutral pH and a sweep rate of  $100 \text{ mV s}^{-1}$ . The effect of analyte concentration changes at Congo red/MCPE was studied by varying the concentration of PA and the limit of detection was found to be  $1.1 \mu\text{M}$ . The proposed approach was successfully applied to the simultaneous measurement of paracetamol and dopamine, with the modified electrode exhibiting relatively high sensitivity, selectivity, and stability. The applicability of the developed method to determine the drug in pharmaceutical samples (tablets) is also illustrated.*

### Keywords

Diazo dye; electro-polymerization; acetaminophen medicine; electrochemical sensor; voltammetry techniques

### Introduction

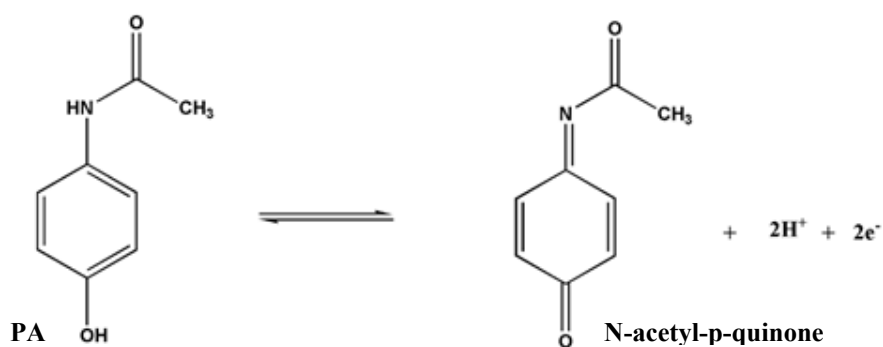
Drug monitoring and analysis are essential and can play an important role in drug quality control. In this process, it is necessary to examine the constituents present in the drug and determine the toxicity and side effects [1]. Patients are frequently treated with multiple medications of varying sorts simultaneously. The need for drug monitoring over a short period creates an opportunity to utilise several analytical techniques [2,3]. Paracetamol (PA) was first used in medicine by Von Mering in 1893 as an analgesic and antipyretic. PA is effective in reducing pain and it also helps in reducing the temperature and is used as a full drug for all ages [4-6]. Paracetamol has been used worldwide to treat symptoms of pain related to headaches, backaches, arthritis, and postoperative pain [7,8], as well as to reduce symptoms of cough, cold, and fever. The analgesic-antipyretic effect of PA is

similar to that of aspirin, but PA is normally preferred, especially for patients who are sensitive to aspirin [9,10]. Drug analysis in biological systems provides valuable information in pharmacokinetic studies and clinical diagnosis. The literature surveys revealed several already developed techniques for determining PA in different samples. To date, a variety of analytical methods such as UV/Vis spectrophotometry [11], fluorimetry [12], high-performance liquid chromatography (HPLC) [13], chemiluminescence [14], and capillary electrophoresis (CE) [15], have been developed for the detection of PA in biological fluids and tablets. However, these techniques are not convenient for routine analysis due to their tedious extraction process. Compared with other methods, electrochemical methods offer practical advantages such as simplicity of operation, high sensitivity, wide linear range, low instrumental costs, high miniaturization possibilities, rapidity and precision for quantification of essential compounds from a biological point of view [16-18]. Therefore, electrochemical methods, such as voltammetry, have drawn attention in the investigation of the redox behaviour of many molecules on suitable electrodes [19-25].

Dopamine (3, 4-dihydroxyphenylethylamine, DA) is an important neurotransmitter of the catecholamine group which exists in the mammalian central nervous system and is well characterized by its electrochemical activity [26]. DA breaks down into other compounds, which are then eliminated from the body through urine. A urine test can be used to measure the level of catecholamines in the body. The main difficulty with the electrochemical detection of DA in brain fluids is the coexistence of many interfering compounds. PA relieves pain in the central nervous system, and its concentration can be high. In antagonistic, the physiological levels of DA are below  $200 \mu\text{mol L}^{-1}$ . Thus, researchers continue to develop biosensors for the selective detection of paracetamol (PA) and dopamine (DA), considering measurements of PA concentration in the presence of DA to be very important. A literature survey reveals many electrochemical methods useful for the simultaneous determination of PA and DA [21,26-30].

Several modified electrodes have been developed to enhance the sensitivity and selectivity of biomolecule detection. Among them, chemically modified electrodes coated with dyes have emerged as effective platforms for detecting various biomolecules. In particular, polymer-modified electrodes (PMEs) offer distinct advantages, including high chemical stability, excellent sensitivity and selectivity, as well as strong adhesion of the polymer film to the electrode surface [31]. There are several redox dyes that can act as synthetic electron donors, and they can easily be polymerized from electrolytic aqueous solutions to form a uniform redox dynamic layer [32-34]. Electropolymerization techniques have been employed to incorporate a wide range of molecules onto electrode surfaces, enabling the selective and sensitive detection of drugs along with bioactive molecules [35,36].

The present work explores Congo red dye, which was electropolymerized onto the surface of a carbon paste electrode, resulting in a stable working electrode, the Congo red-modified carbon paste electrode (Congo red/MCPE). The electrocatalytic activity of this modified electrode towards the oxidation of paracetamol is tested for its sensitivity, selectivity and stability. In addition, the practical applicability of the sensor has been tested by determining PA in tablets. The mechanism for paracetamol electro-oxidation involves two electrons and two protons to generate N-acetyl-p-quinone imine, as shown in Scheme 1.



**Scheme 1.** Oxidation mechanism of PA

## Experimental

### Materials and methods

A CH660c electrochemical workstation (CH instrument-660c) was used for carrying out the electrochemical experiments. Cyclic and differential voltammetry measurements were performed using a typical three-electrode cell arrangement. The bare carbon paste electrode (bare CPE:  $0.0301\text{ cm}^2$ ) and the electropolymerized Congo red-modified carbon paste electrode (Congo red/MPCE:  $0.0452\text{ cm}^2$ ) were employed as working electrodes, with a standard calomel electrode (SCE) serving as the reference electrode and a platinum wire acting as the auxiliary electrode. The experiments utilized either an unmodified, *i.e.*, bare carbon paste electrode (CPE) or CPE electropolymerized with Congo red as the working electrode. A platinum wire functioned as the counter electrode, while a saturated calomel electrode (SCE) served as the reference. The oxidation potentials of all analytes were measured against an SCE electrode at a  $25 \pm 0.5^\circ\text{C}$  ambient temperature. For all CV recordings, the initial potential was scanned in a positive direction, denoted by arrows in all graphs.

The bare CPE was prepared according to the reported procedure [37]. A diazo dye called Congo red (CR) was electropolymerized using the CV method within the potential range of  $-0.2$  to  $1.5\text{ V}$  vs. SCE at a sweep rate of  $100\text{ mV s}^{-1}$  for 20 cycles. The electrochemical cell contained  $0.1\text{ M}$  sodium hydroxide (NaOH) as a supporting electrolyte and  $1\text{ mM}$  Congo red as a monomer solution.

### Chemicals and reagents

Paracetamol and dopamine hydrochloride were purchased from Himedia, with a molecular weight of 151.64 and 189.64, respectively. Sigma Ltd., India, supplied Congo red, with a molecular weight of 696.66. Graphite powder with an average particle size of  $50\text{ }\mu\text{m}$  was obtained from Merck Life Science Private Limited, Bangalore, India, while silicon oil for creating a CPE was provided by Himedia Laboratories Private Limited, Maharashtra, India. The above-mentioned chemicals were all analytical grades and were used precisely as supplied. Several solutions were prepared for the experiment. These include a  $25 \times 10^{-4}\text{ M}$  solution of PA in demineralized water, a  $2.5\text{ mM}$  solution of DA in  $0.1\text{ M}$  perchloric acid ( $\text{HClO}_4$ ), and a  $25\text{ mM}$  solution of CR in demineralized water. To prepare the phosphate buffer (PB) solution ( $0.2\text{ M}$  pH 7.0) (Merck Life Science Private Limited, Bangalore, India), the  $27.59\text{ g l}^{-1}$  proper amount of  $0.2\text{ M}$  aqueous sodium dihydrogen phosphate monohydrate and  $36.5\text{ g l}^{-1}$   $0.2\text{ M}$  aqueous disodium hydrogen phosphate were combined. Demineralized water was utilized to prepare a solution of  $0.1\text{ M}$  sodium hydroxide (NaOH) as a supporting electrolyte for polymerization. The bare CPE was prepared according to the reported procedure [36]. A diazo dye called Congo red (CR) is electropolymerized using the CV method within the potential range of  $-0.2$  to  $1.5\text{ V}$  vs. SCE at a sweep rate of  $100\text{ mV s}^{-1}$  for 20 cycles. The electrochemical cell contains  $0.1\text{ M}$  sodium hydroxide (NaOH) as the supporting electrolyte and  $1\text{ mM}$  Congo red as the monomer solution.

## Results and discussion

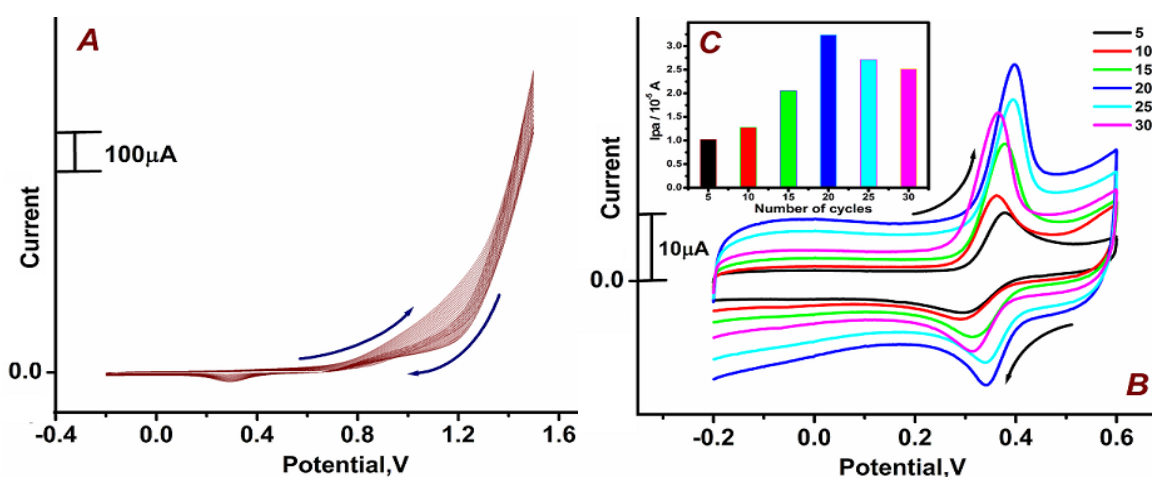
### Optimization condition for the working electrode

The small cathodic peak at 0.277 V that was observed in the successive potential cycles in Figure 1A illustrates the reduction of Congo red monomer [38]. During the process of polymerization, there is an increase in the current response, which indicates the development of a polymeric layer and the improvement of conductivity on the surface of the CPE [39,40]. After every measurement, demineralized water was used to rinse the modified electrode before it was used for further electrochemical investigation. The thickness of the polymer layer has a major impact on the modified electrode's electrocatalytic activity performance. The film thickness can be efficiently controlled by varying the number of potential cycles (5 to 30) utilized in the polymerization process. Figure 1B shows the CVs at Congo red/MCPE formed by different numbers of potential cycles at a sweep rate of 100 mV s<sup>-1</sup> to see the effect of polymer thickness on the response of PA (0.1 mM) in 0.2 M PB (pH 7.0). As illustrated in Figure 1C, the peak current of PA oxidation increases when the number of cycles is increased from 5 to 20, and then gradually decreases as the number of cycles is further increased. The graph shows that the maximum current signal of PA oxidation appears after 20 cycles. As a result, 20 cycles of Congo red polymerization at CPE were chosen as the optimum number of polymerization cycles for forming Congo red/MPCE used in further experiments.

An approximate surface concentration of the Congo red layer formed on the surface of the carbon paste electrode (CPE) was calculated by the following Equation (1) [41]:

$$I_{pa} = n^2 F^2 A \Gamma \nu / 4RT \quad (1)$$

Here,  $\Gamma$  / M cm<sup>-2</sup> is the surface coverage concentration,  $I_{pa}$  is the anodic peak current,  $\nu$  is the sweep rate,  $A$  is the geometric surface area of the electrode,  $n$  is the number of electrons involved in the reaction, and  $R$ ,  $F$ , and  $T$  have their scientific significance. The surface concentration of poly (Congo red) was determined to be 0.029 nM cm<sup>-2</sup>.

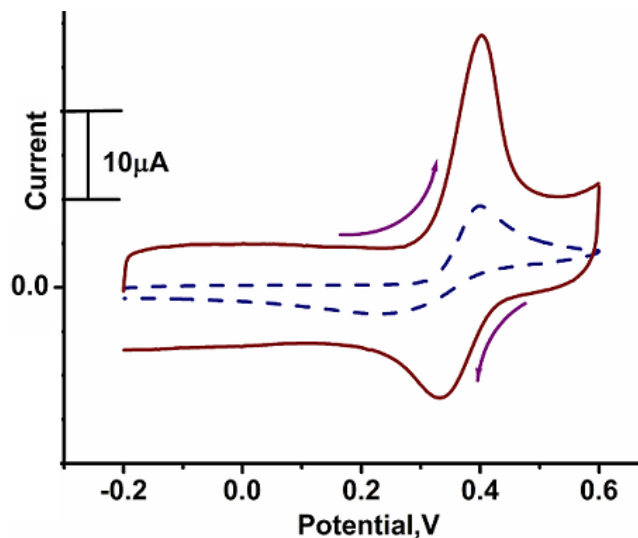


**Figure 1.** (A) CVs for the electrochemical polymerization of 1 mM of Congo red on CPE in 0.1 M NaOH for 30 cycles with the sweep rate of 100 mV s<sup>-1</sup>; (B) influence of the number of electro-polymerization cycles on the cyclic voltammetric response of 0.1 mM PA at Congo red/MCPE in 0.2 M PBS of pH 7.0; inset (C) anodic peak current of PA versus number of electro-polymerization cycles

### Electrochemical response of paracetamol at Congo red/MCPE

The electrochemical response of paracetamol (0.1 mM) at the bare CPE (dashed line) and Congo red/MCPE (solid line) was studied in neutral pH (7.0), PB solution 0.2 M as a supporting electrolyte, with a sweep rate of 100 mV s<sup>-1</sup>, as shown in Figure 2. On the bare CPE, PA shows an irreversible behaviour with relatively weak redox peak current. For Congo red/MCPE, the redox peak currents

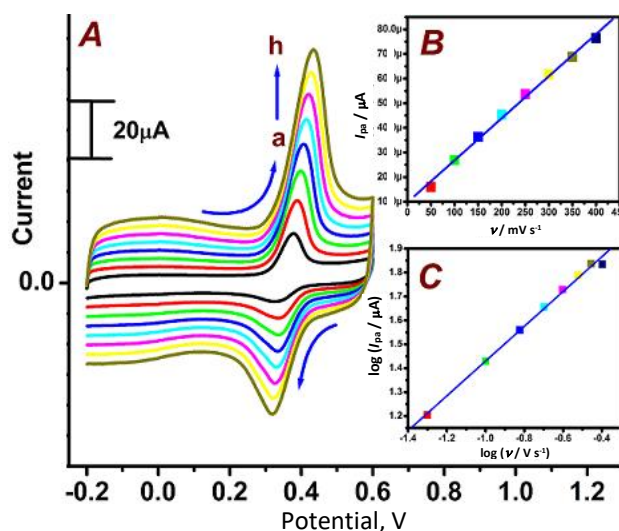
are significantly higher compared to those of bare CPE. The increased sensitivity could be attributed to an improved electron transfer process and an increased surface area of the Congo red/MCPE. The electropolymerization of Congo red dye at a bare carbon paste electrode improves the electrochemical reaction by decreasing the oxidation over-potential of paracetamol, which suggests a quasi-reversible redox performance of the PA on the surface of Congo red/MCPE [18,41]. The electrochemical reactivity of paracetamol is remarkably enhanced at Congo red/MCPE.



**Figure 2.** CVs of 0.1 mM PA in 0.2 M PB, pH 7.0, at bare CPE (dashed line) and Congo red/MCPE (solid line) at the sweep rate of  $100 \text{ mV s}^{-1}$

#### Effect of sweep rate

Sweep rate variation serves as a key tool for probing the reaction mechanism and understanding the electrode kinetics process. The peak current is directly proportional to the sweep rate, as per Randles-Ševčík's equation. The cyclic voltammograms of PA at Congo red/MCPE for 0.1 mM PA in 0.2 M phosphate buffer solution with scan rates ranging from 50 to  $400 \text{ mV s}^{-1}$  at pH 7.0 are shown in Figure 3A. The redox peak current increases as the sweep rate increases, while the oxidation peak potential moves to the positive side, and the reduction peak potential shifts to the negative side. The plots of  $I_{pa}$  versus sweep rate (Figure 3B) and  $\log I_{pa}$  versus  $\log$  of sweep rate (Figure 3C) show good linearity.



**Figure 3.** (A) CVs of 0.1 mM PA in neutral 0.2 M PB, pH (7.0) at Congo red/MCPE at different sweep rates (a to h - 50 to  $400 \text{ mV s}^{-1}$ ). Inset (B) graph of the anodic peak current of PA versus sweep rate, inset (C) graph of the log of anodic peak current of PA versus log of sweep rate

The linear regression equations for both plots were found to be  $\log I_{pa} = 0.727 \log \nu + 2.157$  ( $R^2 = 0.9978$ ) and  $I_{pa} = 0.170 \nu + 9.742$  ( $R^2 = 0.9979$ ). The slope of  $\log I_{pa}$  versus  $\log \nu$  is 0.727, which is closer to the theoretical value of 0.5 than 1.0, indicating a diffusion-controlled process [18]. From Figure 3, the obtained  $\Delta E_p$  values were used to find the heterogeneous rate constant ( $k^\circ$ ) using Equation (2). The  $k^\circ$  values for PA oxidation were obtained and recorded in Table 1 in the range of sweep rate 50 to 400  $\text{mV s}^{-1}$ .

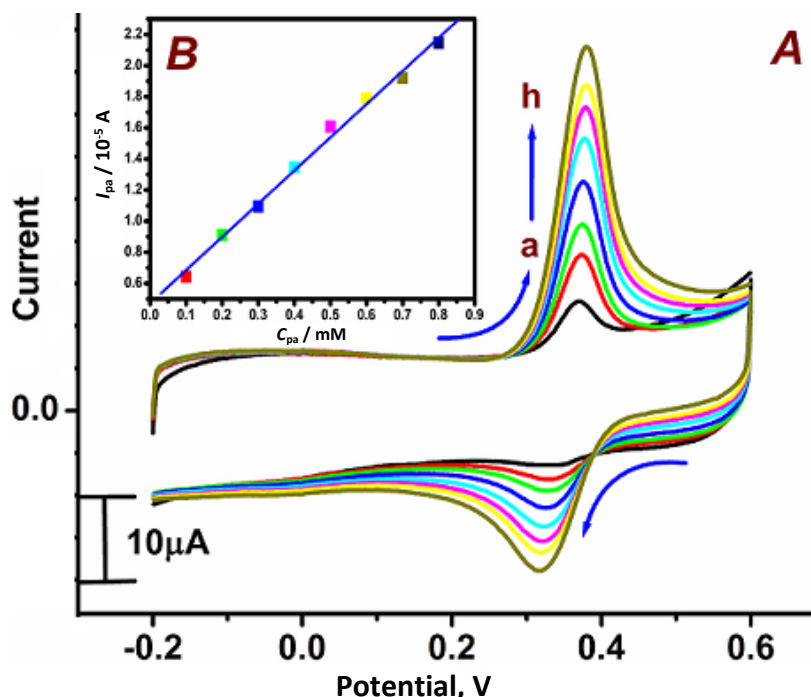
$$\Delta E_p = 201.39 \log (\nu / k^\circ) - 301.78 \quad (2)$$

**Table 1.** Electrochemical parameters of PA at different sweep rates

| $\nu / \text{mV s}^{-1}$ | $\Delta E_p / \text{mV}$ | $k^\circ / \text{s}$ |
|--------------------------|--------------------------|----------------------|
| 50                       | 53                       | 0.864                |
| 100                      | 55                       | 1.694                |
| 150                      | 63                       | 2.312                |
| 200                      | 71                       | 2.818                |
| 250                      | 85                       | 2.999                |
| 300                      | 96                       | 3.176                |
| 350                      | 105                      | 3.341                |
| 400                      | 114                      | 3.443                |

#### Effect of paracetamol concentration

The voltammetric analysis of PA was performed at the Congo red/MCPE using cyclic voltammetry in PB (0.2M, pH 7.0). Figure 4A illustrates the cyclic voltammograms (CVs) for different PA concentrations. By increasing the concentration of PA from 0.1 to 0.8 mM, the oxidation and reduction peak currents also increase, while  $E_{pa}$  moves in a more positive direction and  $E_{pc}$  in a more negative direction. The graph of  $I_{pa}$  versus concentration of PA is plotted in Figure 4B. The linear regression equation is  $I_{pa} = 2.132 \times 10^{-5} + 0.4724C$  [ $R^2 = 0.9969$ ]. The calculated detection limit ( $S/N = 3$ ) was 1.1  $\mu\text{M}$ . Table 2 shows that the proposed electrode has a lower detection limit for PA compared to some previous investigations [39,40,42-46].



**Figure 4.** (A) CVs of PA in neutral 0.2 M PB, pH (7.0) at Congo red/MCPE at the sweep rate of 100  $\text{mV s}^{-1}$  with different PA concentrations (a to h - 0.1 to 0.8 mM); (B) graph of anodic peak current versus concentration of PA

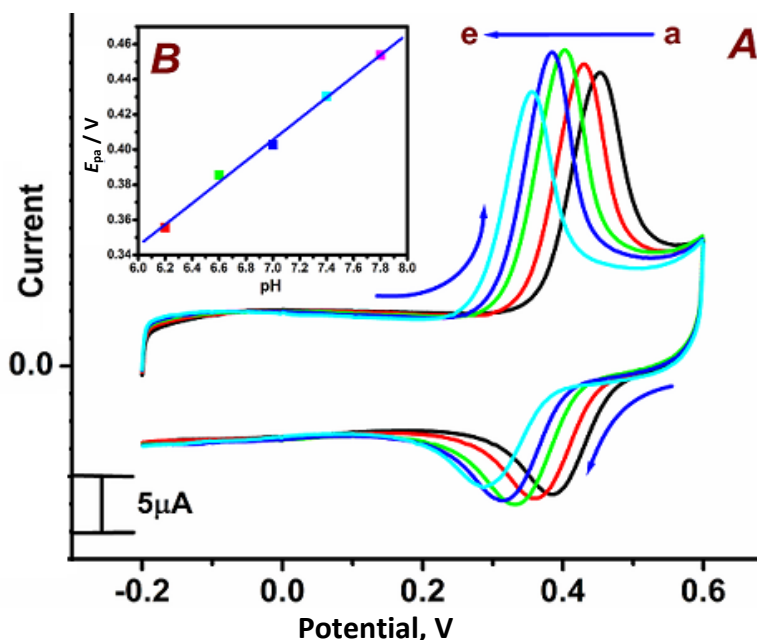


**Table 2.** Assessment for LOD values with some previous PA sensors

| Electrode     | Modifier   | pH  | LOD, $\mu\text{M}$ | Technique | Ref.      |
|---------------|--|-----|--------------------|-----------|-----------|
| Glassy carbon | C <sub>60</sub> /GCE   | 7.2 | 50                 | DPV       | [42]      |
| Carbon paste  | N-(3,4-dihydroxyphenethyl)-3,5-dinitrobenzamide modified MWCNT | 7.0 | 10                 | DPV       | [43]      |
| Glassy carbon | Polyphenol-oxidase   | 7.4 | 21                 | DPV       | [44]      |
| Glassy carbon | BDD electrode  | 8.0 | 10                 | CV        | [45]      |
| Glassy carbon | Cu-poly-TTCA   | 7.0 | 05                 | CV        | [46]      |
| Carbon paste  | Poly (naphthol green B)  | 7.0 | 1.6                | CV        | [39]      |
| Carbon paste  | Poly (rhodamine B)   | 7.4 | 1.8                | CV        | [40]      |
| Carbon paste  | Congo red/MCPE   | 7.0 | 1.1                | CV        | This work |

### Effect of pH

The pH of the buffer solution has a major impact on the electrocatalytic oxidation of PA at the Congo red/MCPE, affecting both peak potentials and peak currents. The influence of pH on the oxidation of PA at Congo red/MCPE was examined at a sweep rate of  $100 \text{ mV s}^{-1}$  throughout a pH range of 6.2 to 7.8. CVs recorded for the oxidation of 0.1 mM PA at Congo red/MCPE at different pH values are shown in Figure 5A. The oxidation peak potential ( $E_{pa}$ ) shifts to a more negative potential as pH increases. The plot of oxidation peak potential versus pH is shown in the inset of Figure 5B. Based on the linear regression equation  $E_{pa} = -0.060 \text{ pH} - 0.0164$ , with a slope of  $60 \text{ mV pH}^{-1}$  ( $R^2 = 0.9977$ ), the resulting graph has good linearity. This behaviour almost perfectly complies with the Nernst equation for two-electron and two-proton transfer processes [47] and is in accordance with Scheme 1.

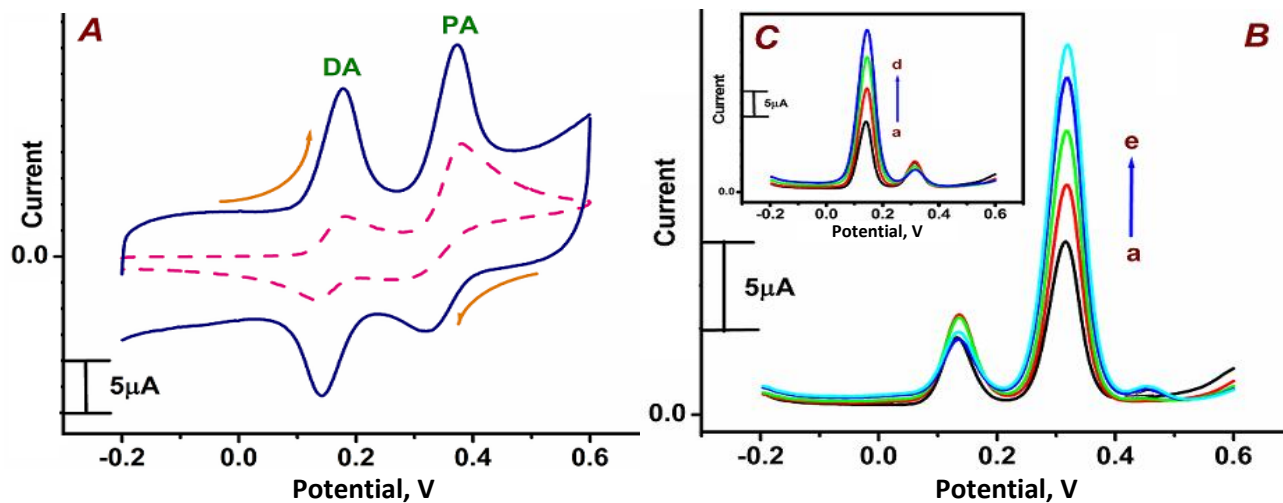


**Figure 5.** (A) CVs of the Congo red/MCPE in 0.2M PB solution containing 0.1mM PA at different pH values (a-e; 6.2 to 7.8) at the sweep rate of  $100 \text{ mV/s}$ ; (B) graph of anodic peak potential versus pH of PB

### Simultaneous determination of PA and DA

Since both PA and DA are electrochemically active substances, they frequently coexist in the extracellular fluids of the central nervous system and play important roles there [48]. A prior study [49] has evidenced that PA and DA can have an impact on each other. Indeed, prolonged usage of PA can lead to a reduction in DA concentration in organisms. Therefore, detecting PA and DA simultaneously would be very convenient for practical application [50,51]. The CV response at both bare CPE and Congo red/MCPE for the binary mixture of 0.1 mM PA and 0.1 mM DA in PB (0.2 M,

pH 7.0) at a sweep rate of  $100 \text{ mV s}^{-1}$  is illustrated in Figure 6A. At the bare CPE (dashed line), PA and DA showed small oxidation peaks at oxidation potentials of 379 and 181 mV, respectively. On the other hand, two distinct oxidation peaks at Congo red/MCPE (solid line) were found at 373 and 177 mV, respectively. The peak-to-peak separation of 196 mV is sufficient for simultaneous investigation; therefore, the modified electrode Congo red/MCPE could serve as an effective electrochemical sensor to determine the concentrations of PA and DA in the binary mixture. Concurrently, the DPV technique was used to perform an interference study on a mixture of samples that contained PA and DA at the same concentration. The Congo red/MCPE was used to measure the amounts of PA and DA in their mixtures. The concentration of one analyte was varied in this investigation, whereas the concentration of the other analyte was constant. The PA concentration increased from 0.1 to 0.5 mM in Figure 6B, while the 0.1 mM DA concentration remained unchanged. There was no change in the DA peak potential or peak current. As shown in Figure 6C, the oxidation peak current of DA amplified linearly from 0.1 to 0.4 mM while maintaining a constant PA concentration of 0.1 mM. Therefore, the developed electrode determines only one bioactive molecule even in the presence of the same concentration of another compound. This result shows that PA and DA existed independently in their mixture of samples.



**Figure 6.** (A) CVs for simultaneous determination of 0.1 mM PA and 0.1 mM DA at bare CPE (dashed line) and Congo red/MCPE (solid line) in 0.2 M PB, pH 7.0, at sweep rate of  $100 \text{ mV s}^{-1}$ ; (B) DPVs of (a) 0.1 (b) 0.2, (c) 0.3 and (d) 0.4 mM PA in the presence of 0.1 mM DA at Congo red/MCPE in 0.2 M PB of pH 7.0; (C) DPVs of (a) 0.1 (b) 0.2, (c) 0.3 and (d) 0.4 mM DA in the presence of 0.1 mM PA at Congo red/MCPE in 0.2 M PB, pH 7.0

**Analysis of real samples**

The Congo red/MCPE was used to determine the presence of PA in a commercial tablet [DOLO-650, Micro Lab Limited (the indicated amount of PA is 650 mg)]. Table 3 shows the obtained results. The recovery results indicate that the suggested technique might be effectively applied to identify PA in commercial tablets, with recovery rates ranging from 94.67 to 102.63 %. All experiments were repeated three times with Congo red/MCPE.

**Table 3.** Detection of PA in tablet (Dolo 650 mg) real sample (n=3)

| Amount added, $\mu\text{M}$ | Amount found, $\mu\text{M}$ | Recovery, % |
|-----------------------------|-----------------------------|-------------|
| 10                          | 9.46                        | 94.67       |
| 20                          | 20.52                       | 102.63      |
| 30                          | 29.21                       | 97.37       |



## Conclusion

In the present work, a stable polymeric film was successfully formed by electropolymerizing Congo red dye onto the surface of a carbon paste electrode, resulting in the formation of Congo red/MCPE. The electrochemically polymerized sensor exhibited excellent electrocatalytic activity toward the redox responses of paracetamol, with a low detection limit. Furthermore, the fabricated sensor was effectively employed for the simultaneous detection of PA and DA in a binary mixture, indicating good selectivity and sensitivity. The proposed method was also successfully applied for the determination of PA in commercial tablet formulations. Owing to its low cost, ease of preparation, and promising electrochemical performance, the Congo red/MCPE sensor holds significant potential for future sensor development.

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