Full Paper

Electrochemical Determination of Quercetin at β–Cyclodextrin Modified Chemical Sensor: A Voltammetric Study

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Abstract- The electrochemical behaviour of quercetin has been studied at carbon paste electrode (CPE) and β-cyclodextrin modified carbon paste electrode (β-CDMCPE) by employing cyclic voltammetry (CV) and Differential pulse voltammetry (DPV). The β-CDMCPE showed a considerable electrocatalytic effect towards the oxidation peak of quercetin, in Britton- Robinson (BR) buffer pH 2.5, (0.04 M) under, appropriate environment. The anodic peak current is linear to quercetin concentration in the range of 6×10^{-6} M to 2×10^{-5} M with detection limited of 3×10^{-7} M and the limit of quantification of 1.2×10^{-6} M respectively . The electro catalytic oxidation of quercetin at β-CDMCPE was compared with CPE. The electrochemical process was reversible and adsorption controlled. The interfacial electron transfer phenomenon of quercetin at the modified electrode was deliberated by using electrochemical impedance spectroscopy (EIS), the studies showed that β-CDMCPE was well eminent compared with bare CPE. The electrochemical sensor showed excellent sensitivity towards the determination of quercetin.

1. INTRODUCTION

Quercetin and some interrelated flavonoids naturally occurs in flowers, buds, roots, bark, steams of flosophorae species plants and chinese herbal medicine plants. Quercetin mainly make out in plants as glycosides. The molecular structure of quercetin is shown in Scheme 1.
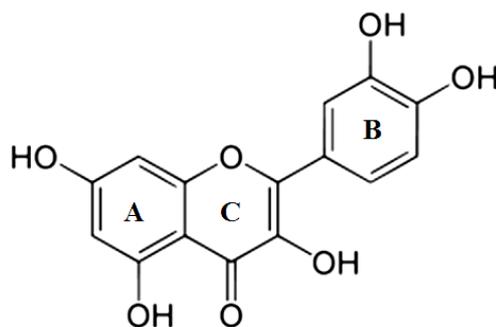
Scheme 1. Molecular structure of quercetin

Quercetin has anti cancer, anti allergic, anti inflammatory and antiviral activities [1-5]. Flavonoids has antioxidant properties, which is mainly bring into being in fruits, soyabeen, olive oils, red wine and tea [6]. The antioxidant activity mechanism of quercetin flavonoid appears to be acquiring the ability of phenols to refused radicals by electron conveying process by which phenols are converted into phenoxyl radicals. The phenolates were described and are being more easily oxidised than phenols [7]. Various analytical techniques have been proposed for the determination of quercetin, such as HPLC [8], UV absorption detector [9], Spectrophotometry [10], Mass spectrometry and electrochemical methods [11-13]. From the techniques mentioned above the electrochemical methods are of more advantage, because they are simple sensitive, selective, economical, and efficient method. Nevertheless electrochemical detectors are of attractive, unconventional method for the detection of electro active species. Thus the electrochemical methods such as cyclic voltammetry (CV), Differential pulse voltammetry (DPV), and electrochemical impendence spectroscopy (EIS), have been intensively used for the determination of quercetin and other flavonoids [14–20].

Good and novel methods are introduced to modify the electrode surface to construct new electrochemical sensors [21–24] and electro catalytic materials [25]. It has been reported that the electrochemical modified electrodes were successfully applied to study the characteristics and determination of several organic, inorganic and biological species [26–30].

Quercetin has two different benzene rings, ring A contains resorcinol, ring B contains cetechol and it has also a hydroxy group at position 3 in ring C. The quercetin rings of A and

B are not coupled and ionization of the OH groups of one ring system should not influence the ionization of the other ring OH group, they are independent [31]. It was strong-willed by the spectroscopic methods, that the predominance of the ring A on spectral properties of the radicals from ring B is miserable in flavonoids, where ring C is completely saturated [32]. The theme of our work was to develop an electrochemical sensor with high sensitivity and selectivity for the determination of quercetin. Flavonoids are less soluble in water, hence much attention has been directed towards the inclusion capability of β-CD as an electrode modifier in order to facilitate the capture of flavonoids having electroactive hydroxyl group such as quercetin, β-CD has bifunctional character due to its hydrophobic cavity and hydrophilic outer surface. A β-CD incorporated modified carbon paste electrode was more efficient to determine the electro active species such as quercetin than compare with bare carbon paste electrode .

2. EXPERIMENTAL

2.1. MATERIAL

A CHI 660D electrochemical work station (CH Instrument, Austin, USA.) was used for the electrochemical measurements, which was controlled by a personal computer. A convention three electrode system was employed, including a modified carbon paste working electrode; saturated calomel electrode (SCE) as reference electrode and glassy carbon rod electrode was used as counter electrode. The pH values were measured with Elico U 120 pH meter and a combined pH CL 51 B electrode.

2.2. Reagents

Quercetin was purchased from Sigma Aldrich company, β-cyclodextrin, sodium hydroxide from S.D Fine chem. Limited, Mumbai., Boric acid, acetic acid from Merck pvt limited, Mumbai, and ortho phosphoric acid from thermo fisher scientific pvt limited Mumbai., used without further any purification. The stock solution of quercetin was prepared 5×10^{-4} M. The working solutions were prepared through diluting the stock solution with BR buffer solution and double distilled water. Graphite powder of 60mm particle size was purchased from Loba chemie and silicon oil from S.D.Fine were used for the preparation of CPE. All reagents are analytical grade.

2.3. Preparation of CPE

The CPE was prepared by hand grinding of 85% graphite power and 15% of silicon oil using agate mortar for 30-40 min to get homogeneous paste. This carbon paste was incorporated in to 2 mm diameter of Teflon tube electrode and electrical contact was made

through a copper wire through the tube. Before measurement of modified electrode smoothed on the piece of soft filter paper [33, 34].

2.4. Preparation of modified electrode

β-CDMCPE was prepared by grinding the 20 mg of β-CD with 85% of graphite power and 15% of silicon oil in an agate mortar by hand mixing for about 30 min to get homogeneous mixture of β-CDMCPE. This paste was packed into the cavity of electrode and then smoothed on the soft filter paper.

2.5. Analytical procedure

The electrode was dipped in 0.04 M BR Buffer (pH 2.5) solution containing quercetin of desired concentration. Electrolytic solution   is stirred about 5 min, then recorded the CV and DPV plots from 0.0 to +0.7 V at the potential scan rate 100 mV s^{-1}. All experiments were carried out at room temperature (25±1) $^{\circ}$C

3.RESULT AND DISCUSSION

### 3.1. Electrochemical characterization of β-CDMCPE

The developed β-CDMCPE was characterised by employing CV studies towards 1mM potassium ferricyanide $K_3[Fe(CN)_6]$ in the potential range from -0.2 to +0.8 V at 100 mV s^{-1} scan rate in 1 M KCl solution as supporting electrolyte. The voltammogram of 1 M $K_3[Fe(CN)_6]$ at both bare CPE and β-CDMCPE, shows reversible cycles Fig. 1. The β-CDMCPE was shown very good replication in comparison to bare CPE having cathodic peak (E_{Pa}) potential 180 mV and anodic peak (E_{Pa}) 240 mV with lesser sensitivity (dashed line). Later modification of electrode with 20 mg of β-CDMCPE shows a good advancement and electrochemical enhancement of both anodic and cathodic peak current, that was shown with the solid line.

3.2. Electro catalytic response of quercetin at β-CDMCPE

Cetechol oxidizable group of quercetin can be easily identified by electrochemical methods. The Fig. 2 shows voltammograms of quercetin in BR (0.04 M) buffer solution of pH 2.5. The cyclic voltammogram for 1×10^{-5} M quercetin at bare CPE (dashed line) with anodic peak (E_{pa}) at 426 mV and cathodic peak potential (E_{pc}) at 398 with ΔE_p of 28 mV. However a pair of peaks was obtained at β-CDMCPE with a strong enhancement in both anodic peak and cathodic peak currents, with anodic peak potential at 410 mV and cathodic peak potential at 395 mV with ΔE_p value equal to 15 mV, suggesting efficient electron transfer behaviour. The β-CDMCPE has shown very good improvement of current signal for

quercetin, the enhancement of peak current at the β-CDMCPE was due to the complexation reaction and the, mechanism was shown in Scheme 2.
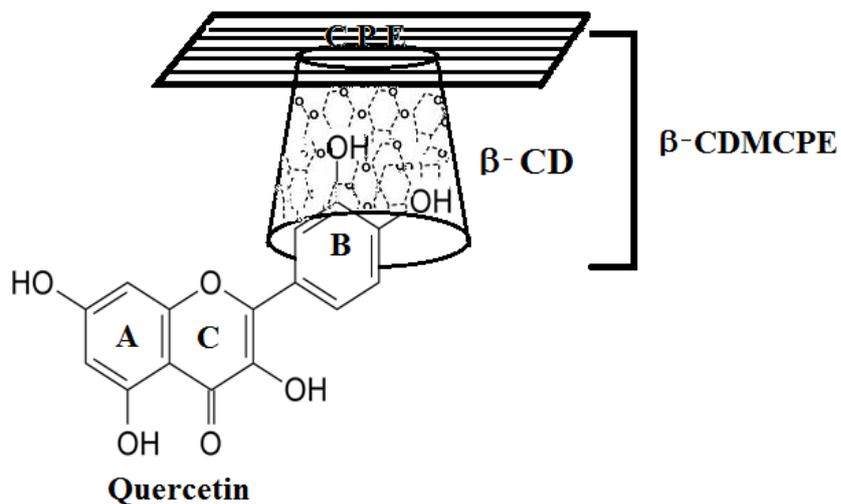
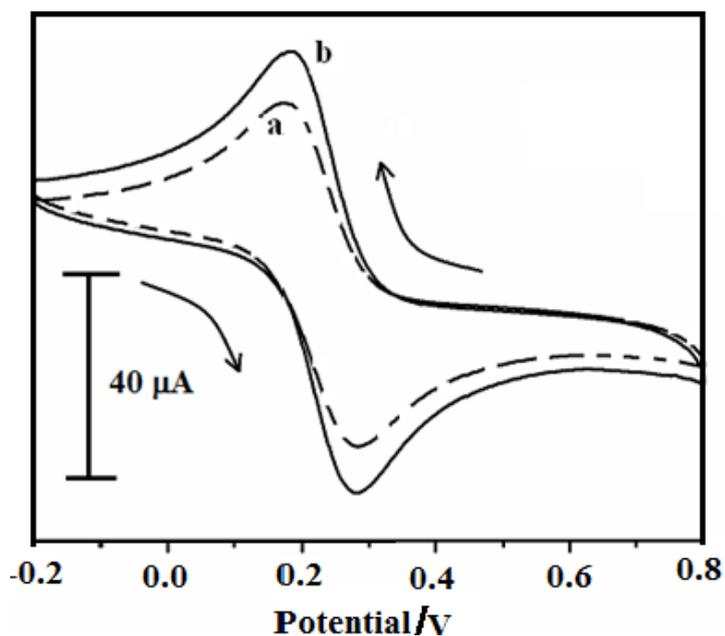**Scheme 2.** The mechanism of the β-CDMCPE grafted on quercetin

Fig. 1. Cyclic voltammograms in 1 M potassium ferricyanide and KCl solution (a) CPE (dashed line) (b) β-CDMCPE (solid line)

3.3. Effect of β-CDMCPE on peak current

The electrochemical response of β-CDMCPE, towards quercetin was studied by constructing a calibration plot and was shown in Fig. 3. The modified CPE was prepared in different ratios by adding different amount of β-CD in milligrams. The amount of β-CD was varied from 2 to 35 mg in CPE, and the electrochemical study was carried out for 1×10^{-5} M quercetin. It was noticed that there was a linear increment in the peak current up to 20 mg. On further increase in β-CD amount the peak current was decreased gradually. Hence the experiment was carried out by taking 20 mg of β-CD.
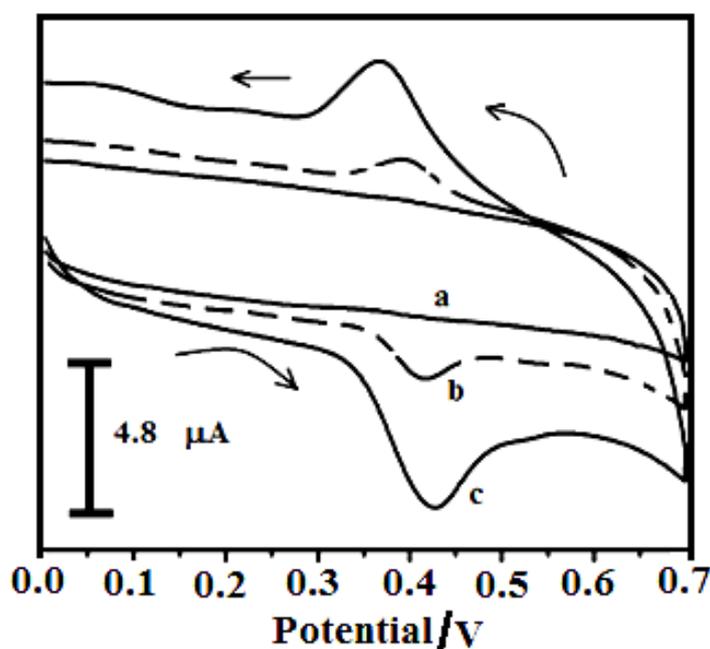
Fig. 2. Cyclic voltammograms of quercetin in 0.04 M BR buffer solution pH 2.5. (a) Blank run with CPE (b) CPE (c) β- CDMCPE

3.4. Effect of pH

The effect of pH was studied from 2.5 to 6.5 on the electro catalytic activity of quercetin at β-CDMCPE and was shown in the Fig. 4A. However the best shape of voltammogram of oxidizable peak was obtained at pH 2.5, suggesting, it is the most favorable value. The peak current obtained for the quercetin was dependent on pH and the result was shown in Fig. 4B. When the pH of the solution was raised, the peak current decreases and achieves the plateau; this is due to the deprotonation of quercetin and availability of very small proton concentration in the solution. From the Fig. 4C, it was noticed that as the pH of the electrolyte increases the corresponding electrode potential shifted towards the negative direction, suggesting the decrease in the electrocatalytic activity of β-CDMCPE. From the

plot of E_{pa} *vs.* pH, the slope was found to be 0.0533 V/pH, which was close to the theoretical value of 0.059 V/pH for the one electron and one proton process, which is in agreement with literature reported [35, 36]. The electrochemical reaction of quercetin was shown Scheme 3.
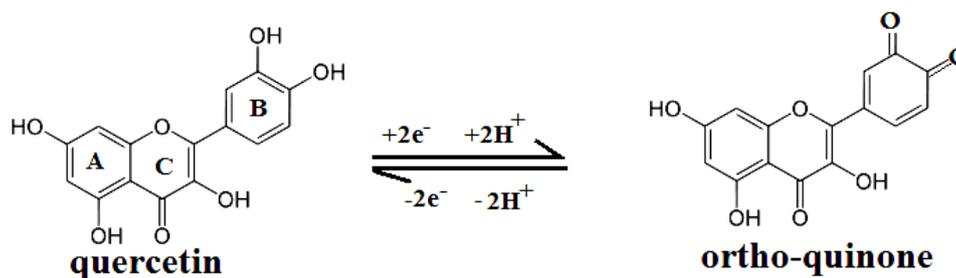
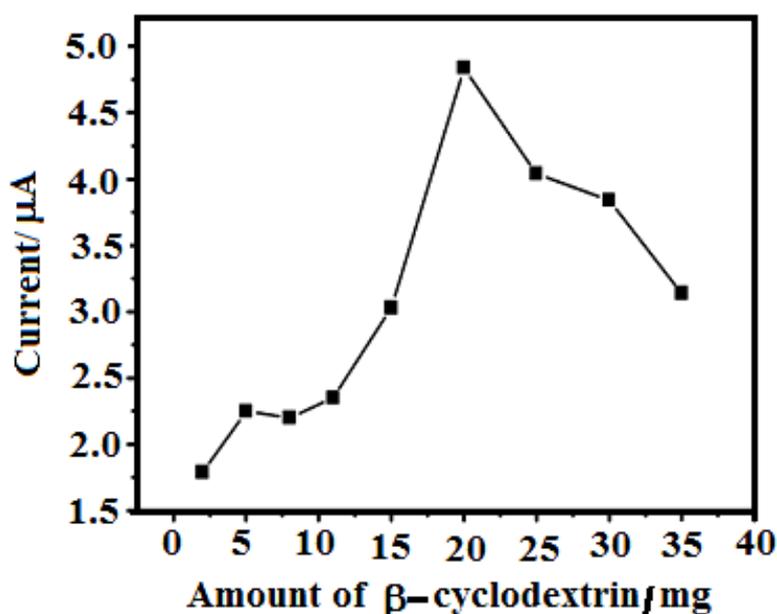**Scheme 3.** The electrochemical oxidation reaction of quercetin

Fig. 3. Calibration plot of effect of β-cyclodextrin modifier on quercetin. I_p *vs.* Amount of β-cyclodextrin

3.5. Effect of scan rate

The effect of scan rate on the peak current at β-CDMCPE in 0.04 M BR buffer was studied by cyclic voltammetry in the presence of 1×10^{-5} M quercetin Fig. 5A. The anodic peak current increase linearly with scan rate (*v*) form the range of 50 mV s^{-1} to 150 mV s^{-1} (correlation coefficient r=0.99121). When the scan rate was increases, it was found the I_{pa} values were increased gradually and anodic peak potential shifted towards positive direction.

The relationship of the oxidization peak currents with the scan rate was constructed and result was shown in Fig. 5B, which indicates the process was adsorption controlled occurring with quercetin at the β-CDMCPE.
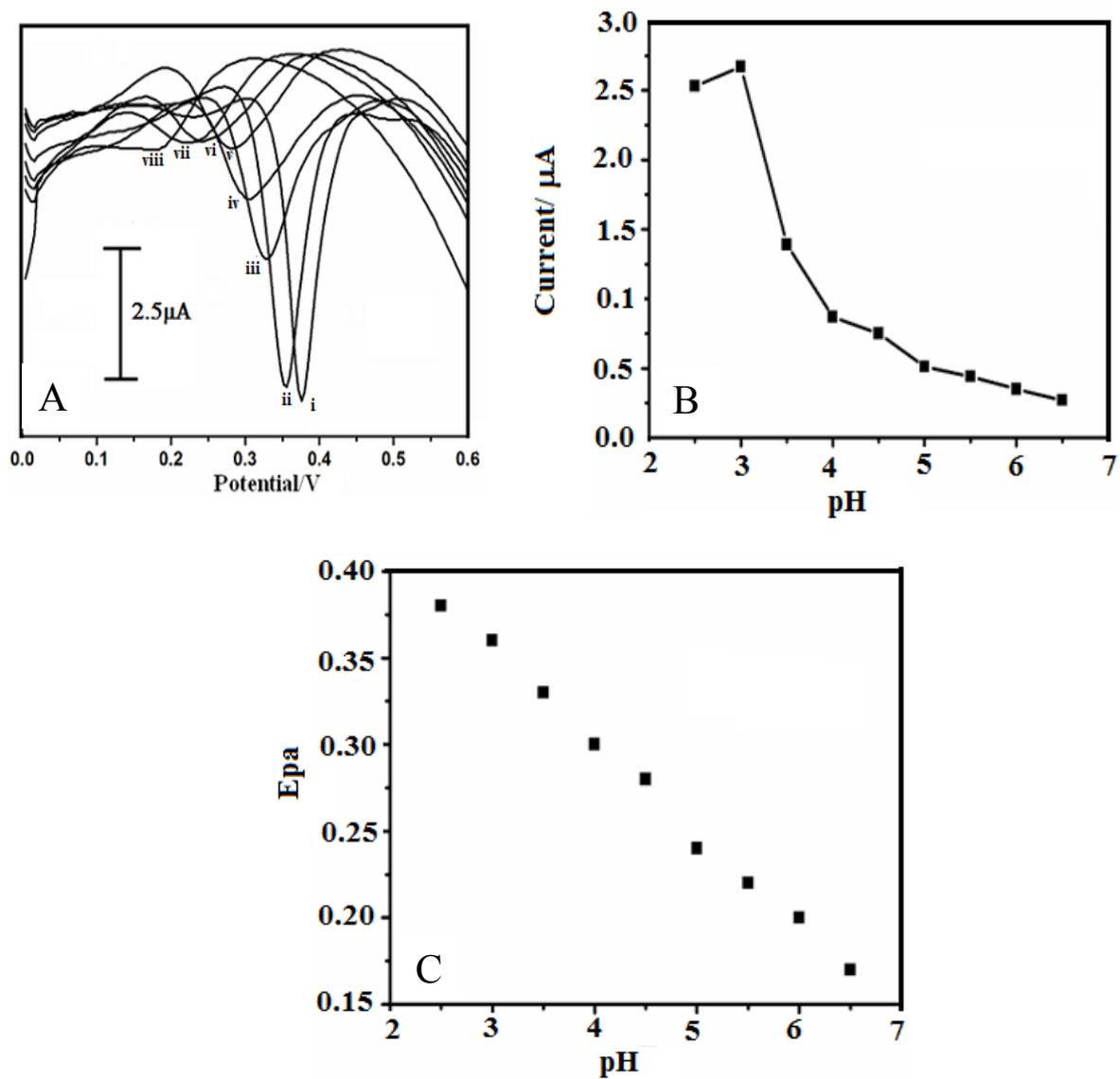

Fig. 4. (A) Differential pulse voltammograms of quercetin in BR buffer at pH (i) 2.5 (ii) 3.0 (iii) 3.5 (iv) 4.0 (v) 4.5 (vi) 5.0 (vii) 5.5 (viii) 6.5 (B) plot of I_p *vs.* pH (C) plot of E_p *vs.* pH
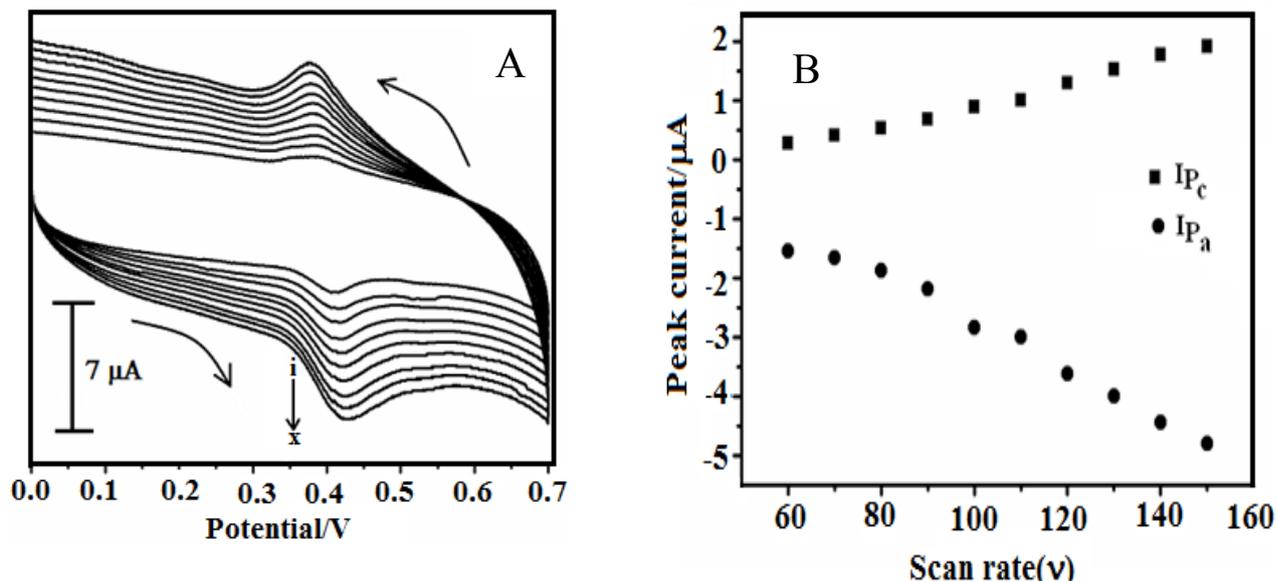
Fig. 5. (A) Cyclic voltammogram of 1×10^{-5}M quercetin in BR buffer pH 2.5 at scan rate of (i) 60 mV s^{-1} (ii) 70 mV s^{-1} (iii) 80 mV s^{-1} (iv) 90 mV s^{-1} (v) 100 mV s^{-1} (vi)110 mV s^{-1} (vii)120 mV s^{-1} (viii) 130 mV s^{-1} (ix) 140 mV s^{-1} (x) 150 mV s^{-1}. (B) Plot of I$_{pa}$ & I$_{pc}$ *vs.* Scan rate

3.6. Effect of concentration of quercetin

The effect of quercetin concentration was studied by employing CV and DPV in the concentration range from 1×10^{-5} M to 8×10^{-5} M and was shown in Fig. 6A. The diagram shows that the I$_{pa}$ and Ipc increased significantly. DPV was more sensitive than CV and varied from 1×10^{-7} M to 13×10^{-6} M of quercetin concentration and the results were shown in Fig. 6B. A linear relationship of quercetin was found from the range of 7×10^{-7} M to 10.89×10^{-6} M in Fig. 6C. The limit of detection for quercetin in lower range was found to be 3×10^{-7}M and quantification limit was 11.79×10^{-7} M, the detection limit and quantification limit was calculated by using formulas LOD=3S/M and LOQ=(10 S)/M, [37-39], were "S" is the standard deviation and "M" is the slop value obtained from the calibration plots .

3.7. EIS studies on quercetin

Electrochemical alternating current impedance technique was useful tool for studying the interfacial properties of chemically modified electrodes [40, 41]. EIS was used to determine the nature of quercetin interaction at β-CD. EIS studies for the CPE and β-CDMCPE was studied by altering the AC frequency between 10^{-5} to 10^{5} Hz with an equilibrium potential of 0.38 V corresponding to the electrochemical reaction of quercetin in BR buffer (pH 2.5). Fig. 7 shows a distinctive impedance diagram presented in the form of Nyquist plot at CPE with and without β-CD. From the comparison it was clear, that the impedance response of

quercetin was found to be different after the addition of β-CD. On the other hand in the absence of β-CD the impedance spectra include a semicircle with larger diameter, indicating larger electron transfer resistance. However after the addition of β-CD the diameter of semicircle squandered markedly. Thus the charge transfer resistance of electro oxidation of quercetin decreases greatly and charge transfer rate is enhanced by adding β-CD. To analyse the data obtained from EIS spectra, a simple equivalent circuit inset of Fig. 7 was used to fit the experimental result. Where 'Rs' is the electrolyte resistance, 'Rp' is the polarization resistance, 'Ret' is the charge transfer resistance, 'Cf_1' and 'Cf_2' are the double layer capacitance and 'Q' is the constant phase element.
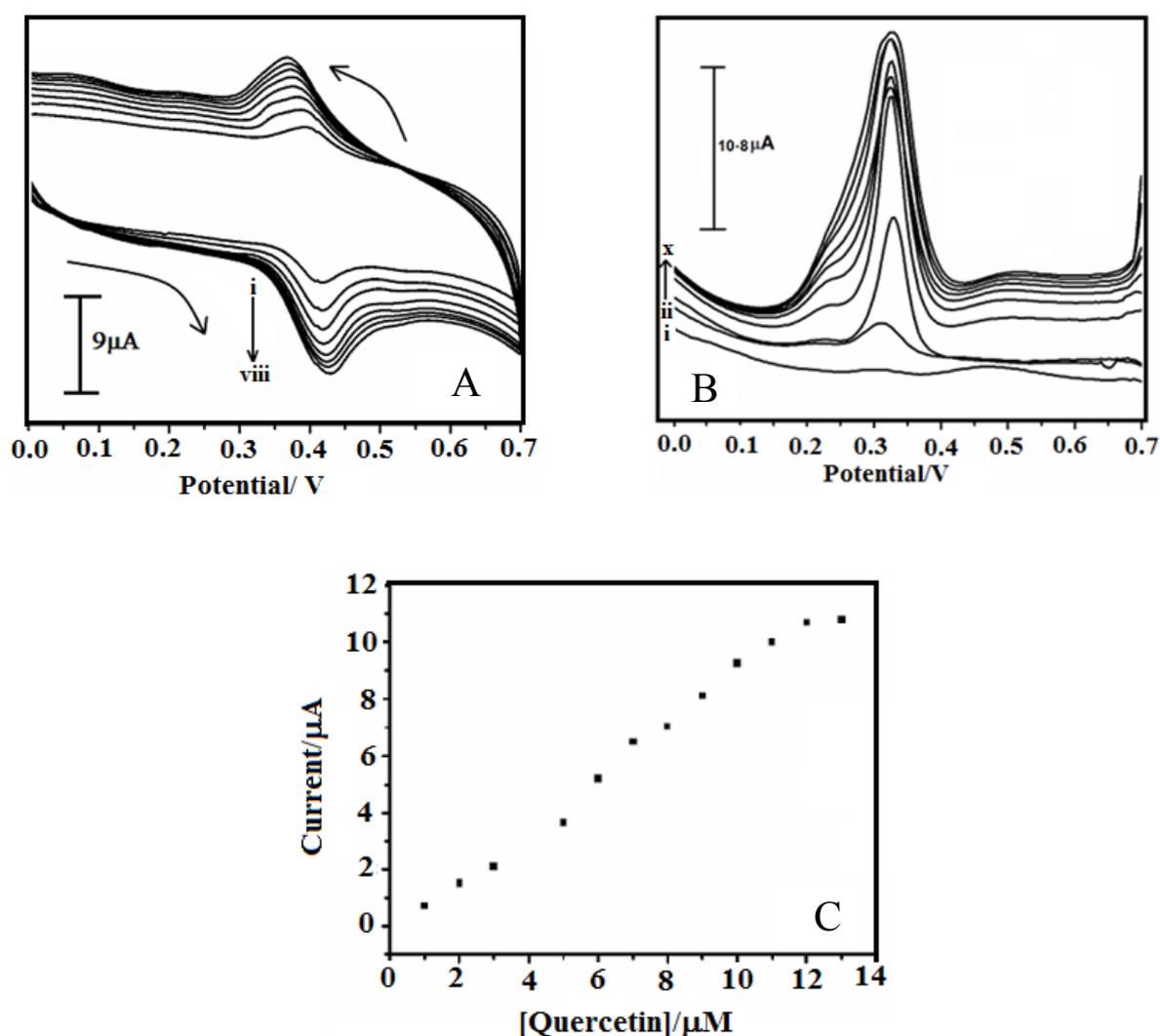

Fig. 6. (A) Cyclic voltammograms quercetin at (i) 1×10^{-5} M (ii) 2×10^{-5} M (iii) 3×10^{-5} M (iv) 4×10^{-5} M (v) 5×10^{-5} M (vi) 6×10^{-5} M (vii) 7×10^{-5} M (viii) 8×10^{-5} M (B) Differential pulse voltammograms of quercetin at (i) blank run (ii) 6×10^{-6} M (iii) 7×10^{-6} M (iv) 8×10^{-6} M (v) 9×10^{-6} M (vi) 10×10^{-6} M (vii) 11×10^{-6} M (viii) 12×10^{-6} M (ix) 13×10^{-6} M and (x) 1×10^{-7} M (C) plot of I_p *vs*. Quercetin concentration
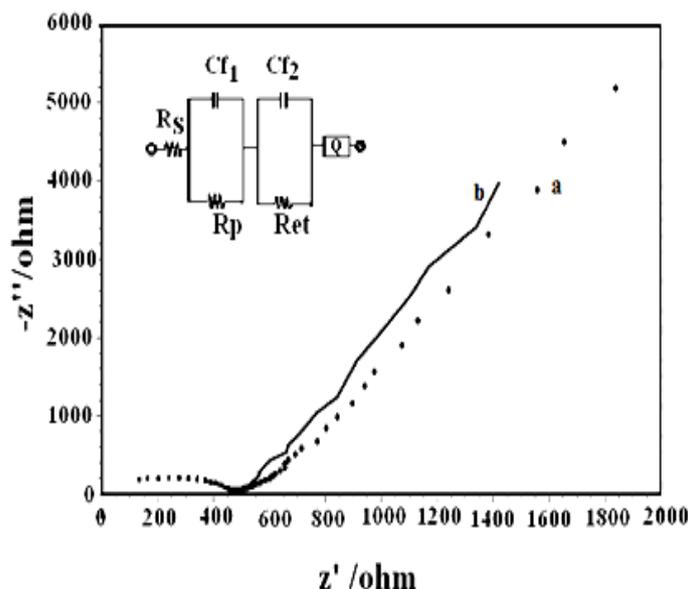
Fig. 7. EIS spectrum of quercetin (a) CPE (b) β-CDMCPE

4. CONCLUSION

In this paper, the β-CDMCPE was used to investigate the electrochemical oxidation behaviour and electrocatalytic activity of quercetin. The electrochemical parameters of quercetin on β-CDMCPE were investigated by using CV and DPV in a sensitive and selective manner. The electrode was working gently and enhances electrochemical responsibility under optimized conditions. The β-CDMCPE showed good electrochemical character with higher sensitivity in comparison with bare CPE.

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