



Short Communication

Selective covalent immobilization of catechol on activated carbon electrodes

Annamalai Senthil Kumar^{a,*}, Sundaram Sornambikai^a, Prakasam Gayathri^a, Jyh-Myng Zen^b^a Department of Chemistry, Vellore Institute of Technology University, Vellore 632 014, India^b Department of Chemistry, National Chung Hsing University, Taichung 402, Taiwan

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ABSTRACT

Here in, we are reporting simple, selective and direct immobilization of a biological redox mediator, catechol (1,2-dihydroxy phenol, CA), on glassy carbon electrode (GCE) and screen-printed carbon electrode (SPCE) surfaces through electrochemical oxidation procedures in aqueous solutions without any other external matrix. The CA immobilized activated carbon electrodes (GCE⁺-CA_{ads} and SPCE⁺-CA_{ads}) have shown well-defined and stable surface confined redox behavior centered (E^0) at ~ 0.05 V vs Ag/AgCl and peak-to-peak separation (ΔE_p) of ~ 20 mV in pH 7 PBS. This observation is selective for CA on the carbon surfaces. Control experiments with other hydroxyl phenols including, Dopamine and Resorcinol failed to show any such adsorption or immobilization on the carbon surfaces. XPS and ATIR/FTIR characterization implies covalent immobilization of the C-4 of the CA through carboxylic functional group of the activated surfaces.

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1. Introduction

Immobilization of redox mediators directly on a carbon surface is an elegant way to tailor the electrode surface suitable for development of new chemically modified electrodes (CMEs) and further to selective electrocatalysis and electrochemical sensor applications. Catechol (CA, 1,2-dihydroxy phenol) is an efficient biological electron-transfer mediator which is considered to be an excellent candidate for electrocatalytic oxidation of bio-, pharmaceutical- and industrial-chemicals, such as Nicotinamide adenine dinucleotide (NADH) [1,2], pencillamine [3], methimazole [4] and hydrazine [5,6] at low working potentials in neutral pH's. Hence, CA and some of its derivatives were successfully immobilized onto the carbon electrode surfaces [1,2,5,7]. Unfortunately, existing immobilization procedures have required either indirect or complicated surface modification procedures. For instance, Dopamine's (DA) amino functional group or 3,4-dihydroxybenzylamine was deliberately coupled with carbonyl functionalized pyrolytic graphite electrode by radio frequency treatment for ~ 5 min in presence of O₂ to form an amide linkage in dicyclohexylcarbodiimide solvent with a reaction time of four days [1,2]. These electrodes have shown a well defined surface adsorption controlled redox peak at a standard electrode potential (E^0) of ~ 0.2 V vs Ag/AgCl for the adsorbed moiety. Following this, McCreery and coworkers reported DA adsorbed carbon surfaces (fractured) with a redox peak at $E^0 \sim 0.2$ V vs Ag/AgCl [8]. Nagaoka and Yoshino reported another

methodology to immobilize CA on glassy carbon electrode (GCE) directly [7]. In this method, GCE was first pre-anodized at 1.8 V vs Ag/AgCl for 5 min in a pH 7 buffer initially followed by spreading of 1 μ L of 25 mM CA over the surface and drying in hot air for 5 min and finally cathodization was performed at -1.5 V vs Ag/AgCl for ~ 1 min. Meanwhile, self-assembled monolayers consisting of a thiol functional groups linked CA were also reported ($E^0 \sim 0.2$ V vs Ag/AgCl) [9,10].

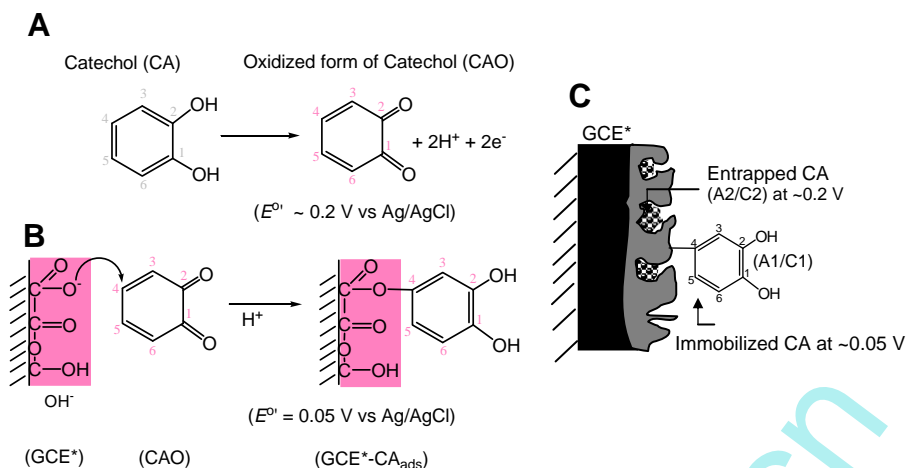
In this work, we are reporting a simple, selective and direct immobilization technique for CA on various carbon surfaces, such as glassy carbon (GCE) and Screen-printed carbon electrode (SPCE), simply by controlled pretreatment of the surface in aqueous solutions, followed by continuous cyclic voltammetric (CV) scans in the CA solution. Such procedures lead to strong adsorption (immobilization) of CA on the carbon surfaces, which showed two distinct E^0 values at ~ 0.05 and ~ 0.20 V vs Ag/AgCl similar to Nagaoka and Yoshino's report [7] (Scheme 1). This immobilization method is selective for CA alone. Other CA derivatives including DA and Resorcinol (Re) failed to show any such adsorption or immobilization on the carbon surfaces. Characterization by X-ray photoelectron spectroscopy (XPS) and Attenuated total internal reflection (ATIR) spectroscopy (ATIR)/FTIR implies that the immobilization is through covalent linkage mechanism.

2. Experimental

Catechol, Dopamine, Resorcinol and Sodium hydroxide were purchased from SD fine chemicals, India. Other chemicals used in this work were all of ACS-certified reagent grade and used without

* Corresponding author. Tel.: +91 4162202754.

E-mail address: askumarchem@yahoo.com (A.S. Kumar).



Scheme 1. (A) Solution phase Catechol (CA) redox mechanism. (B) Possible mechanism for the strong adsorption (immobilization) of CA on an activated carbon electrode (GCE*). (C) Cartoon for different adsorbed CA's and its specific redox potential values (vs Ag/AgCl). Immobilized CA = GCE*-CA_{ads}.

further purification. Screen-printed carbon electrodes (SPCE) were obtained from Zensor R&D (Taichung, Taiwan). A manual screen-printer was used to produce the disposable SPCEs [11]. A stencil with a structure of five continuous electrodes block was used to screen-print the conducting carbon (Acheson, Japan) on a flexible polypropylene film of dimension 50 mm × 70 mm as a base electrode system. After coating the carbon layers, the unit was cured under UV irradiation at an intensity of 1.85 mW/cm² for 2 h. Finally, an insulator layer was printed over the SPCE, there by leaving the working area of 0.0707 cm² surface for the application purpose. Aqueous solutions were prepared using deionized and alkaline KMnO₄ distilled water (designated as DD water). Unless otherwise stated, pH 7 phosphate buffer solution (PBS) of $I = 0.1 \text{ M}$ was used as supporting electrolyte in this study. *Caution!* Extensive activation procedures may damage the GCE.

Voltammetric measurements were all carried out with CHI Model 660C electrochemical workstation (USA). The three-electrode system consists of GCE, SPCE or its CME's as working electrode (0.0707 cm²), Ag/AgCl (3 M KCl) as reference electrode and platinum wire as the auxiliary electrode. The BAS polishing kit was used to polish the GCE. Prior to the electrochemical studies, GCE surface was cleaned by mechanical polishing with 1 μm alumina powder in the BAS polishing kit, wiped with acetone, washed with copious amount of DD water and then sonicated. Activated GCE (GCE*) and SPCE (SPCE*) were prepared either by continuous cycling ($n = 20$) of the electrodes in a potential window of -0.2 to 1.0 V vs Ag/AgCl by cyclic voltammetry (CV) at a scan rate of 50 mV/s in 0.5 M NaOH solution or by performing pre-anodization at an applied potential of 2 V vs Ag/AgCl for 60 s with 0.05 M H₂SO₄, 0.1 M KNO₃ and pH 7 PBS systems. Typical catechol adsorbed GCE* (GCE*-CA_{ads}) and SPCE* (SPCE*-CA_{ads}) were prepared by continuous CV of the respective GCE* and SPCE* in presence of 0.5–1 mM of catechol dissolved in pH 7 PBS at a scan rate (ν) = 20 mV/s ($n = 20$) in the potential window of -0.4 – 1.0 V vs Ag/AgCl . Finally, catechol adsorption behavior of the electrodes; GCE*-CA_{ads} and SPCE*-CA_{ads} were monitored by doing continuous CV in pH 7 PBS at $\nu = 50 \text{ mV/s}$ ($n = 20$) in the potential window of -0.4 – 1.0 V vs Ag/AgCl . There will be considerable decrease in the CA adsorption peak current up to 10th scan, after that the values tends to plateau (Fig. S2). It is expected that there are several irreversible bond breaking and forming steps involved within the GCE*-CA_{ads} systems during the CV potential cycling, which further may leads to large decrease in the adsorption signals initially, but the process become reversible after 10th scans and hence the plateau signals. Hence, 10th scan of CV responses of the above elec-

trodes were uniformly taken for further comparison. Determination of surface coverage, Γ_{CA} (mol cm⁻²) of catechol was performed by integrating the anodic peak area (Q_a) of cyclic voltammograms measured at $\nu = 50$ (or) 100 mV/s, and taking $\Gamma_{\text{CA}} = Q_a/nFA$, where $n = 2$ and A is the geometrical surface area of the working electrode.

Due to practical difficulty with GCE, sample characterization experiments were all performed with SPCE modified systems. XPS was investigated using Omicron ESCA spectrometer (Germany) with a monochromatic Al K α X-ray source. All spectrum were calibrated by the C 1s spectrum at a binding energy = 284.6 eV.

Attenuated total internal reflection (ATR)/FTIR spectroscopy measurements for SPCE, SPCE*, SPCE*-CA_{ads} were performed by using Jasco FT/IR-460 PLUS spectrometer equipped with an attenuated total reflectance (ATR) cell.

3. Results and discussions

Fig. 1A shows CV responses of the 0.5 M NaOH activated GCE (GCE*) in 500 μM of catechol (i.e., GCE*/CA) containing pH 7 PBS at different scan rates (ν). Well defined redox peaks centered (E°) at 0.050 (A1/C1) and 0.25 V vs Ag/AgCl (A2/C2) were observed. Calculated ΔE_p values were 13 and 53 mV for A1/C1 and A2/C2, respectively at $\nu = 50 \text{ mV/s}$, which indicates two different electron-transfer behaviors on the interface. Fig. 1B shows double logarithmic plots of i_{pa} vs ν for the respective anodic peaks. The obtained slope values ($\partial \log(i_{\text{pa}})/\partial \log(\nu)$) are 0.94 and 0.43 for A1 and A2, respectively, which is closer to 1 and 0.5 that indicates specific adsorption- and diffusion-controlled electron-transfer mechanisms for the respective redox process [12,13]. Note that these observations are true on the activated screen-printed carbon electrode (SPCE*) surface also (Fig. 1C(a)). Control experiments with unmodified screen-printed carbon electrode, SPCE (SPCE/CA) and GCE (i.e., GCE/CA) systems rather to show two redox peaks, only one redox peak response centered (E°) at 0.25 V vs Ag/AgCl (A2/C2) with large ΔE_p values of 450 mV on the electrodes were observed (Fig. 1C(b and c)). In order to verify the adsorption characteristics, the CA exposed GCE* was transferred to a blank pH 7 PBS and then CV experiment was performed in the same conditions as before. Interestingly, as can be seen in Fig. 1D, 32% of the A1/C1 peak was found to hold onto the surface ($\Gamma_{\text{CA}} = 1.2 \times 10^{-10} \text{ mol cm}^{-2}$ of A1); whereas 99% of the A2/C2 response gets reduced ($\Gamma_{\text{CA}} = 0.09 \times 10^{-10} \text{ mol cm}^{-2}$ of A2). Meanwhile, unactivat-

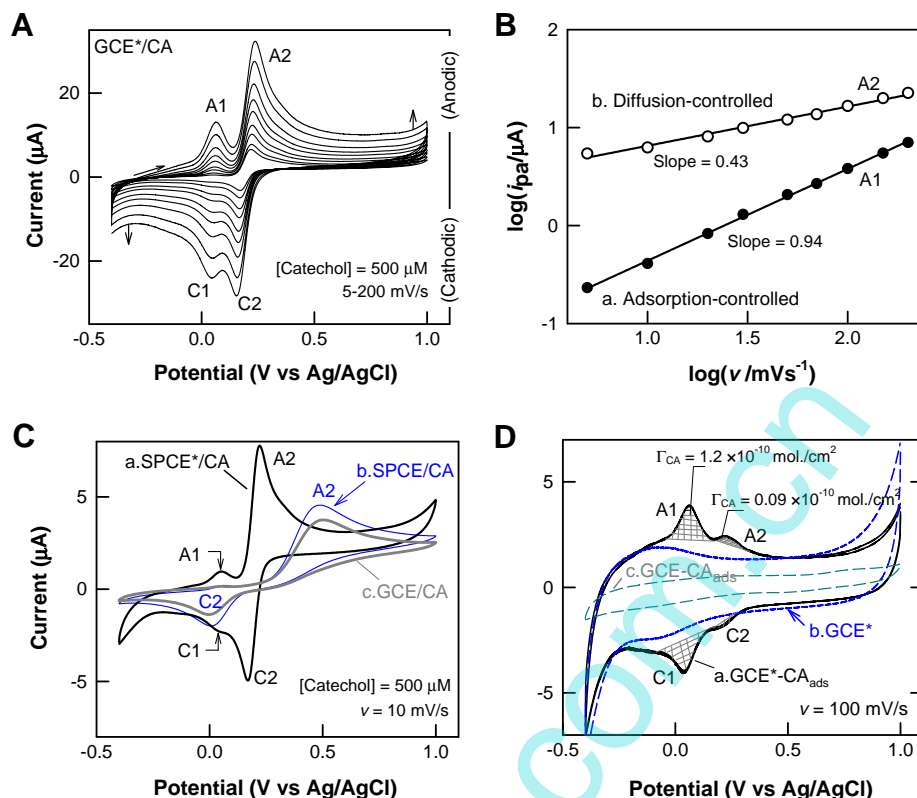


Fig. 1. (A) CV responses of 500 μM catechol (CA) on GCE* in pH 7 PBS at different scan rates (ν). (B) Double logarithmic plots of anodic peak current (i_{pa}) vs ν for the different CA's redox processes. (C) Comparative CV responses of SPCE*, SPCE and GCE in catechol dissolved pH 7 PBS (a-c). (D) Comparative CV responses of GCE*-CA_{ads}, GCE*, and GCE-CA_{ads} in pH 7 PBS (a-c).

ed GCE failed to show any such CA adsorption behavior (GCE-CA_{ads} in Fig. 1D(c)). This observation indicates strong (immobilization, GCE*-CA_{ads}) and weak adsorptions of CA on GCE* (Scheme 1). Again, similar qualitative pattern was observed on the SPCE* surface (Fig. S1). Square wave voltammetry (SWV) of the electrode shows better resolved A1/C1 and A2/C2 peaks (inset Fig. S1). This adsorption behavior is analogous to the observation of Nagaoka and Yoshino [7], yet with a much simple pretreatment procedure. Other carbon pretreatment procedures hardly resulted in such an unusual CA adsorption characteristic and sometimes even lead to polymerization of CA to cause electrode poisoning [14].

Further, other activation procedures like pre-anodization of GCE at 2 V for 60 s in 0.05 M H₂SO₄, 0.1 M KNO₃ and pH 7 PBS followed by the CV experiment with CA also resulted in qualitatively similar adsorption patterns but at different concentrations (Fig. S2 and Table 1), which may be due to the generation of different active surface functional groups to different extent by the pretreatment procedures. Dekanski et al. reported that anodic polarization of the carbon surface (GCE) in alkaline or acidic conditions resulted to marked increase in the carbon-oxygen functional groups (C_{ox}),

such as phenolic (>C-OH), carboxyl (-C(=O)O-) and carbonyl (>C=O) species [15]. Calculated ratio between C_{ox} and graphitic carbon, C_g (i.e., C_{ox}/C_g) by XPS are 0.39, 0.52 and 0.71 respectively for the mechanically polished and alkaline and acidic polarized electrodes. Meanwhile, Premkumar and Khoo noticed that KNO₃ based surface activation procedure was effective to generate oxygen rich carbon sites in turn to preferential immobilization of Ruthenium-bipyridyl complex [16]. Hence, the higher Γ_{CA} values of 3.0 and 2.5 $\times 10^{-10}$ mol cm⁻² (A1 peaks, Table 1) with the GCE*-CA_{ads} systems by KNO₃ and 0.05 M H₂SO₄ based activation procedures in this work might be due to the generation of more amount of carbon-oxygen surface functional groups and in turn to rich CA adsorption.

High resolution XPS was next performed to characterize one of the activated electrode, SPCE* (0.5 M NaOH activated) and its SPCE*-CA_{ads} surfaces (Fig. 2). Binding energy values were referred from standard literatures [15,17,18]. An obvious change in XPS response especially with O 1s core energy spectrum was observed at the SPCE*, presumably due to enhancement of oxygen functional groups such as carbonyl; >C=O (531.9 eV), carboxyl; -C(=O)O-

Table 1
Effect of activation procedures on the adsorption of CA on GCE* (GCE*-CA_{ads}).

	Activation procedures ^a	$E^{o'b}$ (mV)		ΔE_p (mV)		$\Gamma_{CA} \times 10^{-10}$ (mol cm ⁻²)	
		A1/C1	A2/C2	A1/C1	A2/C2	A1/C1	A2/C2
1	E-cycle at -0.4–1 V in 0.5 M NaOH ($\nu = 50$ mV/s, $n = 20$)	50	250	13	53	1.2	0.09
2	2 V vs Ag/AgCl for 60 s in pH 7 PBS	55	260	25	52	2.7	0.01
3	2 V vs Ag/AgCl for 60 s in 0.1 M KNO ₃	70	280	12	10	3.0	0.13
4	2 V vs Ag/AgCl for 60 s in 0.05 M H ₂ SO ₄	65	256	15	50	2.5	0.19

^a Measured after stabilization by continuous CV ($n = 10$) at $\nu = 50$ mV/s.

^b Potentials were referenced with respect to Ag/AgCl reference electrode.

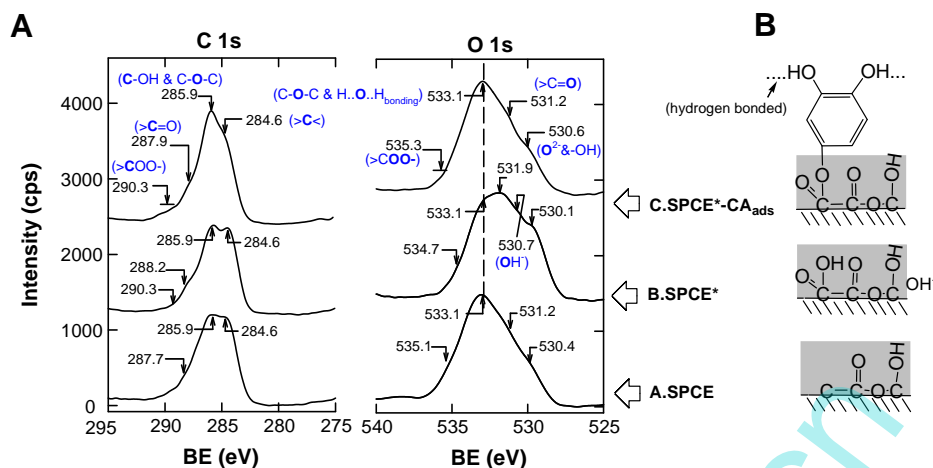


Fig. 2. (A) High resolution XPS responses for the population of C 1s and O 1s core energy levels and (B) possible structures for its corresponding chemically modified electrodes.

(O 1s = 534.7 and C 1s = 288.2–290.3 eV) together with >C–OH (530.1 eV) and C–O–C/H–O–H_{bonding} (533.1 eV). This electrode was found to be quite active towards the CA adsorption to form the SPCE*–CA_{ads} system. The C 1s core energy level of the SPCE*–CA_{ads} showed major increase in >C–OH (C 1s = 285.9 eV and O 1s = 533.1 eV (possibly with hydrogen bonding)) and carboxyl, –C(=O)O– (C 1s = 290.3 and O 1s = 535.3 eV) functional groups. These groups might originate from CA's –OH and covalently bonded carbon functional groups respectively, as given in the Fig. 2B. Besides, based on a previous reported mechanism of CA immobilization on gold surface [9,10], we propose a mechanism in terms of coupling of –C(=O)O– functional group from the SPCE* with the oxidized form of the CA (CAO = *ortho*-quinone) along with protons (liberated from CA oxidation), to form –C(=O)O–CA linkage (the C-4 of the CAO might be involved in the coupling reaction) as in the Scheme 1C. The cartoon illustrates the two different types of adsorbed CA species present in the activated carbon surface. As seen in the Scheme 1C, the A2/C2 redox peak at ~0.2 V vs Ag/AgCl is due to physically entrapped CA within the pores of the carbon surface and the redox peak at ~0 V vs Ag/AgCl is responsible for the CA immobilization through the covalent bonding. Note that the possibility for coupling of CA's –OH functional group with the GCE's –COOH was ruled out since deactivation of the CA's redox behavior [14,15] was not observed in this work (Fig. 1D).

In order to further confirm the above results, some of the CA derivatives, DA which is substituted at C-4 in the aromatic ring and Re that has 1,3-dihydroxy groups respectively were subjected to covalent immobilization similar to the CA adsorption studies (Fig. S3). Interestingly, no such adsorption or immobilization CV peaks were noticed with the DA and Re (Fig. S3). Absence of any CAO (*ortho*-quinone) like structure formation is the possible reason for the failure of any immobilization step with the Re case. In the case of DA, since it exists as cationic form (the terminal amino group as –NH₃⁺, pKa = 8.87 [19]) in a neutral solution of pH 7, it may preferentially adsorb to negatively charged surface functional groups through electrostatic attractions, which when transferred to the blank solution gets leached out from the surface. Hence, DA did not show immobilization peak in CV unlike the CA_{ads} in this work.

ATR/FTIR spectrum of SPCE, SPCE* and SPCE*–CA_{ads} were presented in Fig. 3. A significant IR band at 3199 cm⁻¹ having a width of ~500 cm⁻¹ was specifically observed with the SPCE*–CA_{ads} sample due to the stretching vibrations (ν) of CA–OH with strong intermolecular hydrogen bondings [20]. In further, sharp peaks at 2919,

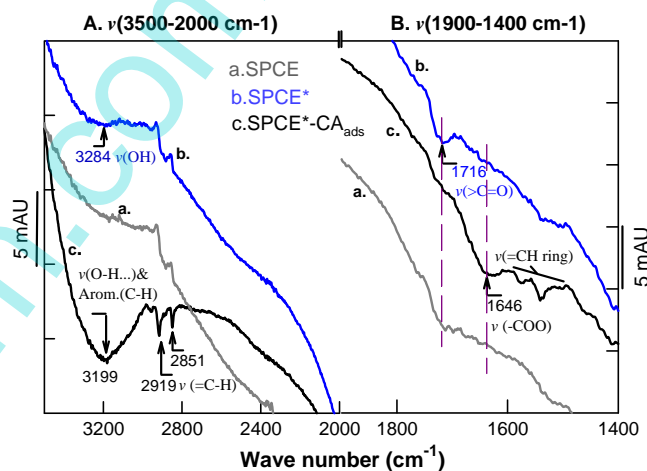


Fig. 3. Typical ATR/FTIR response of the SPCE, SPCE* and SPCE*–CA_{ads}. SPCE* was prepared by 0.5 M NaOH activation procedure.

2851, 1540 and 1578 cm⁻¹ were noticed due to the aromatic –HC = CH– functional group as observed with phenolic compounds at ~2900 cm⁻¹ [20]. Generation of the carbonyl functional group on the SPCE* was observed at $\nu = 1716$ cm⁻¹ as in the alkyl ketonic compounds [20]. Interestingly, after CA immobilization, a new peak was specifically generated at 1646 cm⁻¹ with the SPCE*–CA_{ads} system. Two possible species, carboxylate and *ortho*-quinone (from CAO, Scheme 1) are responsible for the 1646 cm⁻¹ peak [20]. Nevertheless, if *ortho*-quinone group is immobilized on the activated carbon surface, there will be absence of hydroxyl groups; whereas, a well defined hydroxyl group is identified in the higher wave-number region (3199 cm⁻¹), which in turn proves that the peak at 1646 cm⁻¹ is solely due to the formation of carboxylate group as proposed in the Scheme 1 and from the XPS analysis. Note that the *ortho*-quinone is a less stable intermediate species of short life [21] and is highly difficult to trap it on the surface.

Fig. S4 denotes three-dimensional (A–C) and two dimensional (D) AFM images of SPCE, SPCE* and SPCE*–CA_{ads}. The unmodified carbon electrode (SPCE) exhibited relatively smooth surface, whereas the activated (SPCE*) and CA adsorbed electrode (SPCE*–CA_{ads}) surfaces showed rough and fine edged surface topographies (Fig. S4D), which may be due to the surface pretreatment and the adsorption of CA on the surface respectively. Fine edged 2D topog-

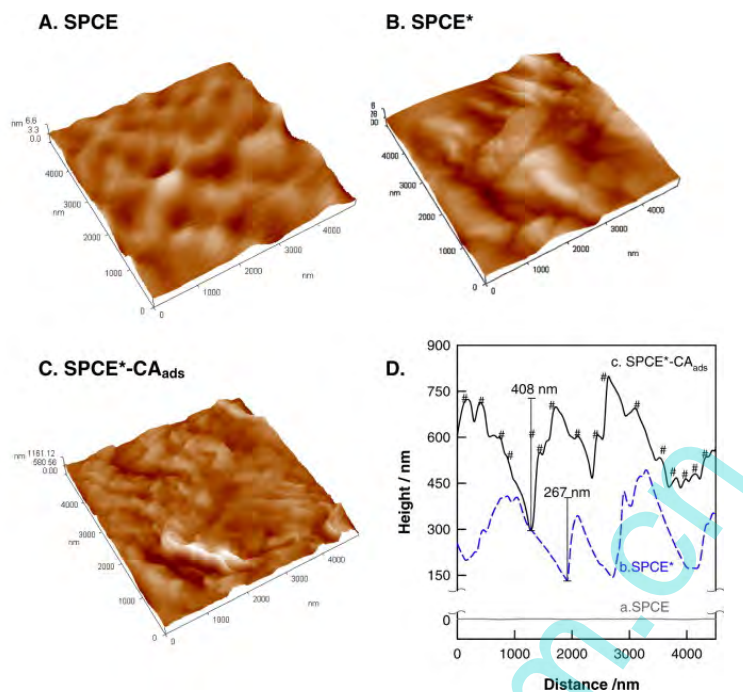


Fig. S4. AFM morphologies for the various SPCE modified electrodes (A–C) and 2D graphical representation taken at 4000 nm of y-axis uniformly (D). The # marked position in the (D) may indicate the spots where the CA was immobilized on the activated surface.

raphy sites observed in the SPCE*–CA_{ads}, are the spots where the CA_{ads} might covalently immobilize on the activated surfaces.

4. Conclusion

In conclusion, electrochemically oxidized GCE and SPCE surfaces were found to be selective for direct immobilization of Catechol (CA) in pH 7 PBS. The CA adsorbed electrodes showed a specific surface confined redox peak at ~ 0.0 V vs Ag/AgCl, which was distinctly different from the solution phase redox behavior occurred at ~ 0.20 V vs Ag/AgCl on unactivated electrodes in pH 7 PBS. XPS and ATR/FTIR characterization provided useful supporting information regarding the surface functionalities and topology of the activated- and CA adsorbed- carbon surfaces. Since the activation procedure is simple and selective to immobilize the CA, it is highly useful to construct a variety of electrochemical and biochemical sensors, especially using fixed potential flow-injection analysis coupled electrochemical detector, where the initial instability problems noticed with potential-cycling studies could be overcome [22]. Further work is in progress.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jelechem.2009.12.016.

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