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Area/Section: Chemical Science.
Type of Paper: Experimental Research.
Number of Peer Reviews: Two.
Type of Review: Peer Reviewed as per |C|O|P|E| guidance.
Indexed in: OpenAIRE.
DOI: https://doi.org/10.5281/zenodo.18368026
Google Scholar Citation: PIJBAS

How to Cite this Paper:
D'Souza, E. S. & Raril, C. (2025). Sensitive Electrochemical Detection of Hydroquinone by Designing Chitosan-Silver Nanoparticle-Pluronic F-127 Modified Glassy Carbon Electrode. *Poornaprajna International Journal of Basic & Applied Sciences (PIJBAS), 2*(2), 80-89. DOI: https://doi.org/10.5281/zenodo.18368026

Poornaprajna International Journal of Basic & Applied Sciences (PIJBAS)
A Refereed International Journal of Poornaprajna Publication, India.
ISSN: 3107-8478

Crossref DOI: https://doi.org/10.64818/PIJBAS.3107.8478.0015

Received on: 12/11/2025
Published on: 30/12/2025

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ABSTRACT

Purpose: *Hydroquinone is a well-known tyrosinase inhibitor widely used in topical formulations for the treatment of hyperpigmentation and other skin disorders; however, its use is associated with adverse effects such as exogenous ochronosis, contact dermatitis, and photosensitivity. An attempt has been made for the detection of hydroquinone using a glassy carbon electrode modified with chitosan-functionalized silver nanoparticles and non-ionic surfactants.*

Methodology: *The present work involves the fabrication of a glassy carbon electrode modified with chitosan-functionalized silver nanoparticles and non-ionic surfactants. The modified electrode is characterized for surface morphology using field emission scanning electron microscopy. The electrochemical behavior of hydroquinone is then investigated using linear sweep, cyclic, differential pulse, and square wave voltammetry. Calibration studies are performed to evaluate linearity, sensitivity, and detection limit over a defined concentration range. Finally, the developed electrode is applied to the determination of hydroquinone in pharmaceutical samples, and its reproducibility and stability are assessed.*

Analysis/Results: *The linear relationship between peak current and hydroquinone concentration in the range of 1–12 μM, along with a low detection limit of 0.039 μM, highlights the high sensitivity of the developed sensor. FESEM analysis further supports these findings by confirming uniform dispersion of silver nanoparticles within the chitosan matrix, contributing to enhanced active sites on the electrode surface. Application of the sensor to pharmaceutical (dermatological cream) samples yields satisfactory recovery values, demonstrating its practical applicability. Additionally, the good reproducibility and stability observed indicate that the developed electrode is reliable for routine analytical applications.*

Originality/Value: *The novel integration of chitosan-functionalized silver nanoparticles with non-ionic surfactants on a glassy carbon electrode to construct a highly sensitive electrochemical sensing interface for hydroquinone. Unlike conventional modified electrodes, this approach exploits the synergistic effects of biopolymer functionality, enhanced electron transfer from silver nanoparticles, and improved analytical accessibility provided by surfactants. The developed sensor thus offers a distinctive and effective platform for hydroquinone analysis with potential applications in pharmaceutical and environmental monitoring.*

Type of paper: *Experimental Research.*

Keywords: Glassy carbon electrode, Hydroquinone, Cyclic voltammetry, Differential pulse voltammetry

1. INTRODUCTION :

Hydroquinone (HQ, 1,4 dihydroxy benzene), phenolic compound, found naturally in crude oil, tobacco smoke, wood etc. and it is widely employed in cosmetics, rubber, dyes, plastics, food industries, and

pharmaceuticals (Felipe, A. G., et al (2015). [1], (Schweigert, N., et al (2001). [2], Nudelman, A., et al (1993). [3]). The presence of this substance in cosmetics, the environment, medicine etc., results in environmental contamination and harmful threats to human health because of its high toxicity and slow rate of degradation (Mingju, S., et al (2016). [4]). The absorption of HQ will lead to some diseases such as renal tube degeneration and liver function diseases (Lv, M., et al (2010). [5]). In the aquatic environment, HQ is a significant xenobiotic micro pollutant and is thought to cause cancer (Rueda, M.E., et al (2003). [6]). It is difficult to break down and extremely hazardous to both humans and animals, even at very low concentrations. They are the main issue and source of environmental pollution as a result of this. They are the main source for the cause of environmental pollution due to their widespread use in the chemical and pharmaceutical sectors. In order to detect HQ with high sensitivity, an efficient analytical approach must be developed. Different analytical techniques have been developed thus far for determining HQ, such as liquid chromatography (Lin, C.H., et al (2005). [7]), chemiluminescence Li, S.F., et al (2008) [8], gas chromatography (Moldoveanu, S. C., et al (2007). [9]), pH-based flow injection analysis (Garcia-Mesa, J. A., et al (2007). [10]), synchronous fluorescence (Pistonesi, M.F., et al (2006). [11]) and electrochemical (Qi, H. et al (2005). [12]), (Yu, J., et al (2009). [13]), (Wang, L., (2007). [14]), (Yin, H., et al (2011), 15]) methods. Compared to all other techniques electro analytical techniques offer additional benefits such as quick and accurate response time, repeatable outcomes, and simple operating procedure.

The advantages of carbon materials over other electrode materials are their chemical stability and active surface chemical energy. As a result, electrodes made of carbon have found extensive use in both analytical and commercial electrochemistry (Ma, L., et al (2011) [16]). Detection of HQ using conventional electrodes faces difficulties in determining sensitivity. Recent years have seen a significant increase in interest in various chemically modified electrodes for the determination of HQ.

Chitosan is occurring naturally (polysaccharide consisting 2-amino-2-deocy and 2-acetamido-2-deoxy-D-glucopyranose) and is harmless. It can develop a coating on the electrode surface and can have amino and hydroxyl groups in it. Chitosan has the ability to attract negatively charged compounds in the solution. Chitosan is a promising biomaterial due to its degradability, compatibility, and less toxicity (Lin, J. H., (2004) [17]). The chitosan contains distinct functional groups and holds a significant interaction between chitosan and metallic nanoparticles. In particular, the interaction of chitosan with the metal surface is by the primary amino group present in it. As a result, chitosan will form a composite with silver and other noble metals in their ionic or metallic form. The affinity of the chitosan toward silver can be explained by the formation of chemical bonds between the nitrogen atoms. Chitosan contains reactive amino and hydroxy groups, which makes it a suitable material for research.

Pluronic F-127 is a nonionic surfactant having hydrophilic and hydrophobic properties. It is a triblock polymer containing polyethylene oxide, which is hydrophilic and polypropylene oxide, which is hydrophobic. In order to enhance the electrochemical sensing capabilities, electrodes were modified with both chitosan functionalized nanoparticles and non-ionic surfactants to make use of the combining properties for the determination of HQ.

In this study, a simple, low-cost electrochemical technique has been designed based on the glassy carbon adapted with chitosan functionalized silver nanoparticles (CT-AgNPs) and a non-ionic surfactant, Pluronic F-127 (PF-127). The developed electrode system shows good conductivity and sensitivity compared to the bare electrode. The electrochemical rejoinder of HQ has been inspected using different techniques like cyclic voltammetry (CV), linear sweep voltammetry (LSV), square wave voltammetry (SWV) and differential pulse voltammetry (DPV).

2. REVIEW OF LITERATURE :

Hydroquinone (HQ) is a toxic phenolic compound widely used in cosmetics, textiles and industrial chemicals. Because of its health and environmental hazards, sensitive and selective detection of HQ in water and biological samples is essential. Conventional methods (e.g., spectrophotometry, chromatographic techniques) are often costly, time-consuming and complex. Electrochemical sensors have emerged as attractive alternatives due to high sensitivity, portability, rapid response, and real-time measurements [18]. Glassy carbon electrodes (GCEs) are a common transducer in HQ detection due to their wide potential window, low background current, and robustness**.** However, bare GCEs often suffer from poor sensitivity and selectivity when detecting HQ alone, especially in the presence of interfering phenolic isomers [19]. Modification of GCE with functional nanomaterials (e.g., metal/metal oxide

nanoparticles, carbon nanostructures) enhances electron transfer, increases surface area, and improves catalytic activity towards the HQ redox reaction. For example, MnO_2/graphene oxide and metal-nanocomposite modified GCEs have shown improved sensitivity and low detection limits for HQ [20]. Chitosan (CS) is a biodegradable, non-toxic polysaccharide derived from chitin. It has abundant amine and hydroxyl groups that facilitate film formation and chemical interaction with analytes [21]. CS facilitates the dispersion and immobilization of nanoparticles (e.g., silver) onto electrode surfaces, enhancing conductivity and stability. Chitosan also improves the adhesion of sensing layers to the electrode surface. Chitosan-based sensors have been widely used for electrochemical detection of phenolic compounds (e.g., catechol and HQ derivatives) due to enhanced electron transfer and high surface area from nan composite formulations [22]. AgNPs are well known for their excellent electrocatalytic properties, high surface-to-volume ratio, and conductivity. These properties boost the oxidation/reduction current signals of electro active analytes such as HQ [23]. In electrochemical sensors, AgNP incorporation reduces electron transfer resistance, increases peak currents, and often leads to much lower limits of detection (LOD). AgNP-based electrodes have been reported for sensitive detection of organic compounds in environmental sample [24]. Pluronic F-127 is a triblock copolymer (polyethylene oxide–polypropylene oxide–polyethylene oxide) widely used to stabilize nanoparticles and prevent aggregation due to its amphiphilic nature. While Pluronic F-127 has been studied mostly in pharmaceutical applications, its ability to disperse nanomaterials effectively can also be beneficial in forming stable sensing layers on electrodes. The hydrophilic–hydrophobic balance helps uniformly distribute AgNPs in composite matrices like chitosan on electrode surfaces, leading to improved consistency and reproducibility of electrochemical signals.

3. OBJECTIVES OF THE PAPER :

(1) To fabricate and characterize a glassy carbon electrode modified with chitosan-functionalized silver nanoparticles and non-ionic surfactants, and to study its surface morphology, conductivity, and effective surface area using FESEM.

(2) To investigate the electrochemical behaviour of hydroquinone at the modified electrode using various voltammetric techniques (LSV, CV, DPV, and SWV) in order to achieve enhanced current response, sensitivity, and analytical performance.

(3) To apply the developed electrochemical sensor for the determination of hydroquinone in pharmaceutical (dermatological cream) samples and to assess its reproducibility, stability, and reliability.

4. METHODOLOGY :

Hydroquinone, sodium borohydride ($NaBH_4$), and hydrochloric acid (HCl), Pluronic F-127 were received from Molychem, Mumbai, India. Monosodium phosphate (NaH_2PO_4), and disodium hydrogen phosphate (Na_2HPO_4), obtained from Aladdin. Chitosan and silver nitrate ($AgNO_3$) were obtained from Sigma-Aldrich. Ultra-purified water has been utilized to prepare the desired solutions. Solution of 0.1 M phosphate buffer has been developed by mixing a suitable amount of NaH_2PO_4 and Na_2HPO_4. The experiments related are carried out at room temperature.

5. EXPERIMENTAL :

5.1 Instrumentation

Voltammetric measurements such as cyclic voltammetry, linear sweep voltammetry, square wave voltammetry and differential pulse voltammetry, were performed in a predictable three-electrode configuration controlled by CHI630D electrochemical workstation. Glassy carbon electrode (3 mm diameter), GCE/CT-AgNPs, and GCE/CT-AgNPs/PF-127 were functioned as the working electrode, saturated calomel electrode (SCE) and Pt wire as a reference electrode and counter electrode. Electrochemical impedance analysis was performed by using potentiostat-AMETEK from Princeton Applied Research. pH of the buffer solution was adjusted with a pH meter from BANTE Instrument. Morphological studies were performed by using Field Emission Scanning Electron Microscope (FE-SEM), obtained from JSM-7800F. The execution of electrochemical experiments performed at room temperature.

5.2 Production of Chitosan Functionalized Silver Nanoparticles (CT-AgNPs)

The production of CT-AgNPs describes as follows. 2% of chitosan solution (47 ml) was mixed with 0.02 M solution of $AgNO_3$ (2 ml). The combination was constantly agitated at normal status temperature for 30 minutes. Following this, a freshly prepared solution (1 ml of 8 mg/ml) of sodium borohydride ($NaBH_4$) has to be added to the blend of chitosan and $AgNO_3$. The agitation has to be processed for another two hours. The chitosan- AgNPs thus developed were stored in the dark until use.

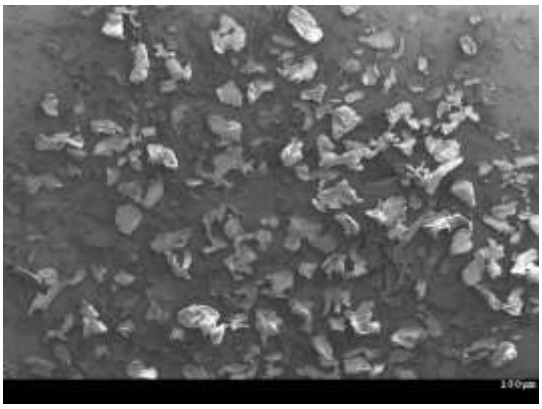
5.3 Preparation of bare electrode

GCE (2 mm in diameter) is made elegant to a shining surface using a polishing scrubber with 0.3 and 0.05µm alumina semi solid. The further sonication of the electrode was done with ethyl alcohol and pure water for the removal of any of the residual polished substance. Modified electrode is prepared by drop molding of modifier on bare glassy carbon electrodes surface.

6. RESULTS AND DISCUSSION :

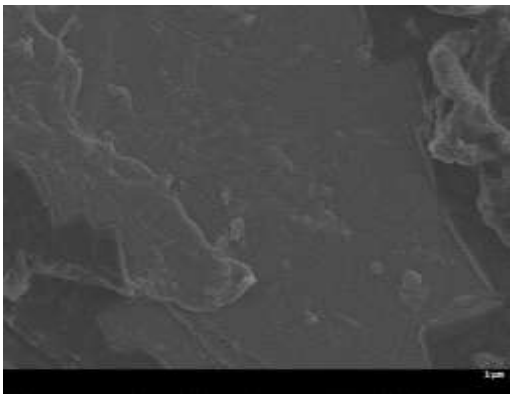
6.1 Characterization:

Surface morphological characteristics of GCE/CT, GCE/CT-AgNPs and GCE/CT-AgNPs/PF-127 were measured by Field Emission Scanning Electron Microscopy (FESEM) micrographs. It has been observed that the Figure 1a comprises of palette-like structure of chitosan. With respect to the surface of GCE/CT-AgNPs, figure 1b, reveals the smooth and compact surface layers. This confirms the deposition of CT-AgNPs on the surface of the electrode. Figure 1c reveals the spherical size particle on the surface of the GCE/CT-AgNPs, confirms the presence of PF-127.



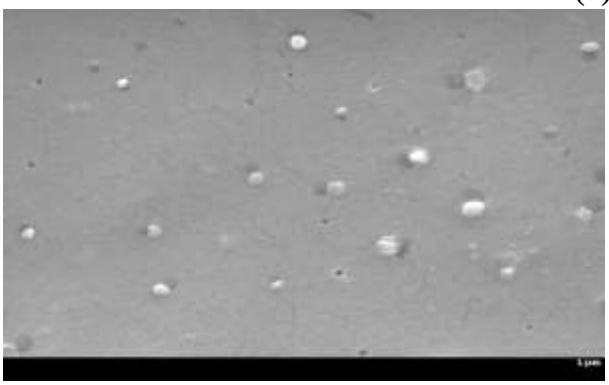
GCE/CT

(a)



GCE/CT-AgNPs

(b)



GCE/CT-AgNPs/PF-127

(c)

Fig. 1: FE-SEM micrographs of (a) GCE/CT, (b) GCE/CT-AgNPs, (c) GCE/CT-AgNPs/PF-127

6.2 Electrocatalytic Oxidation of HQ:

Electrochemical features of different developed electrodes towards the electrochemical oxidation of HQ was investigated by using different voltammetric techniques such as cyclic voltammetry (CV), linear sweep voltammetry (LSV), differential pulse voltammetry (DPV) and square wave voltammetry (SWV). Figure 2a shows the linear sweep voltammogram of HQ (100 µM) at GCE, GCE/CT-AgNPs, and GCE/CT-AgNPs/PF-127 in 0.1 M PBS of pH 7.0 in potential window from -0.6 to 0.6 V at a scan rate of 0.1 V/s. Low electrochemical response was observed for HQ at bare electrode. With the incorporation of modifier such as chitosan functionalized silver nanoparticles and Pluronic F-127 on to the glassy carbon electrode surface, electrochemical response of HQ increased. Among all electrode systems GCE/CT-AgNPs/PF-127 shows a high current response for HQ, confirms the high electrocatalytic activity. This increase in the electrochemical response is due to the increase in the surface area and conductivity of the GCE/CT-AgNPs/PF-127 electrode.

Figure 2b demonstrates the cyclic voltammograms of HQ at GCE, GCE/CT-AgNPs, and GCE/CT-AgNPs/PF-127 in 0.1 M solution of Phosphate buffer of pH 7.0 having 100 µM HQ at 0.1 V/s a scan rate. A redox behavior can be perceived for HQ at the bare electrode with an oxidation and reduction potential of 0.209 V and 0.301 V with a low voltammetric response. On the other hand, the electrochemical response of HQ at GCE/CT-AgNPs/PF-127 is observed at a potential of 0.124 V and 0.260 V with a higher current response. This result confirms that the GCE/CT-AgNPs/PF-127 catalyses the oxidation process of HQ.

The electrochemical performance of HQ at the different electrodes is also measured by the differential pulse voltammetry technique. The obtained differential pulse voltammogram in the presence of 10 µM HQ at GCE, GCE/CT-AgNPs, and GCE/CT-AgNPs/PF-127 are shown in Figure 2c. It can be observed that there is a drastic improvement in the peak current of HQ can be detected at GCE/CT-AgNPs/PF-127 as compared to the other electrode system.

Square wave voltammetry offers higher current sensitivity and good resolution than other techniques like cyclic voltammetry, linear sweep voltammetry and differential pulse voltammetry. Figure 2d shows the square wave voltammogram of HQ (10 µM) at GCE, GCE/CT-AgNPs, and GCE/CT-AgNPs/PF-127 in 0.1 M solution of Phosphate buffer of pH 7.0. It has been observed that the oxidation peak current increased with the modification. A sharp peak and substantial enrichment in the peak current clearly indicate that the adapted electrode act as an effective supporter to increase the behaviour of the electrochemical response of HQ.

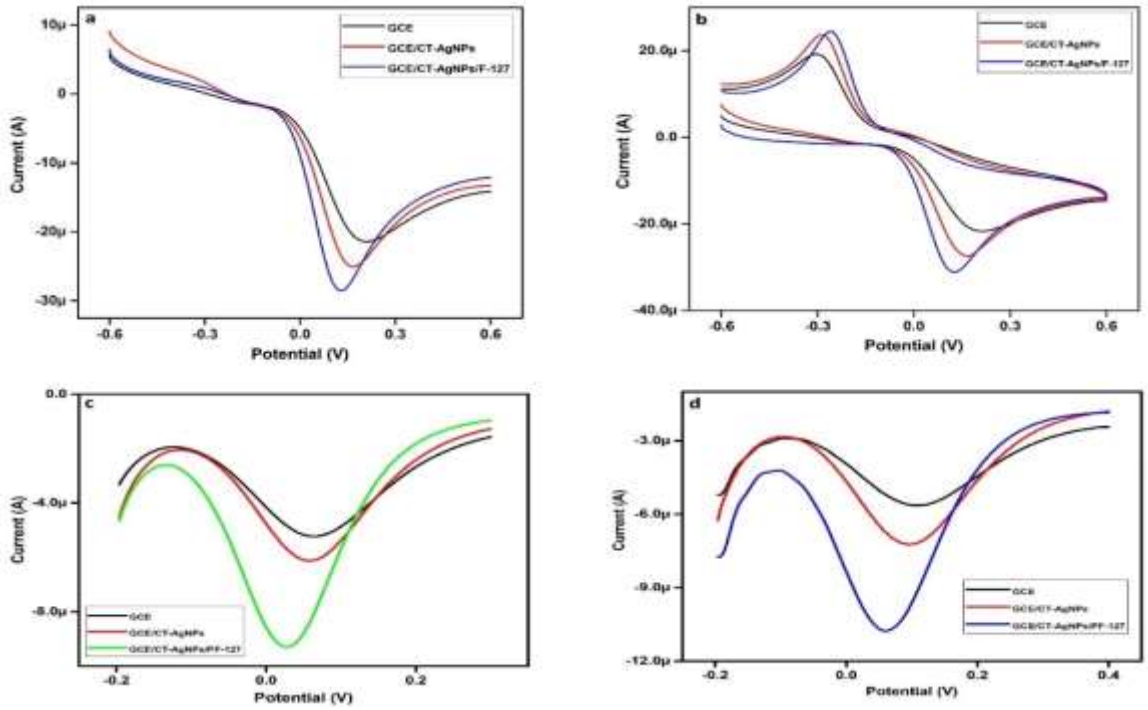


Fig. 2: LSVs (a), CVs (b) DPVs (c), SWVs (d) of HQ at GCE, GCE/CT-AgNPs, and GCE/CT-AgNPs/PF-127 in 0.1 M, pH 7.0.

6.3 pH effect on HQ :

The consequence of various pH values on the electrochemical behavior of HQ at the GCE/CT-AgNPs/PF-127 was investigated by the CV technique. Figure 3a indicates the cyclic voltammograms of HQ at GCE/CT-AgNPs/PF-127 at various pH ranges from 3.0 to 8.0 in 0.1 M PBS at the scan rate of 0.1 V/s. It can be observed that, as the pH of the supporting electrolyte was improved peak current of the redox system increased and was shifted to a less negative side, and the maximum current response was observed when the supporting electrolyte had a pH of 7.0 (Figure 3b). Both adsorption and thickness of the diffusion layer on the electrode surface have been reduced, causing in the decline of the peak response with the rise in the pH. After 7.0pH there is a decrease in the current response of HQ can be observed, this is due to the electrostatic repulsive effect of negatively charged ions, which affects the electrochemical response of HQ amongst the electrode surface and the electrolyte solution. With this, decrease in the peak current due to alkaline condition HQ undergoes autooxidation with oxygen molecules creating a brownish 2-hydroxy-p-benzoquinone compound. pH and peak potential are linearly related with a equation of regression E_{pa} (V) = 0.59 - 0.063 pH, and a coefficient of correlation, 0.99 (Figure 3c). The slope obtained is found to be 0.063 V/pH is near the theoretical value of 0.059 V/pH, which infers that an equal number of proton and electrons have taken part in the reaction of HQ at the surface of the GCE/CT-AgNPs/PF-127.

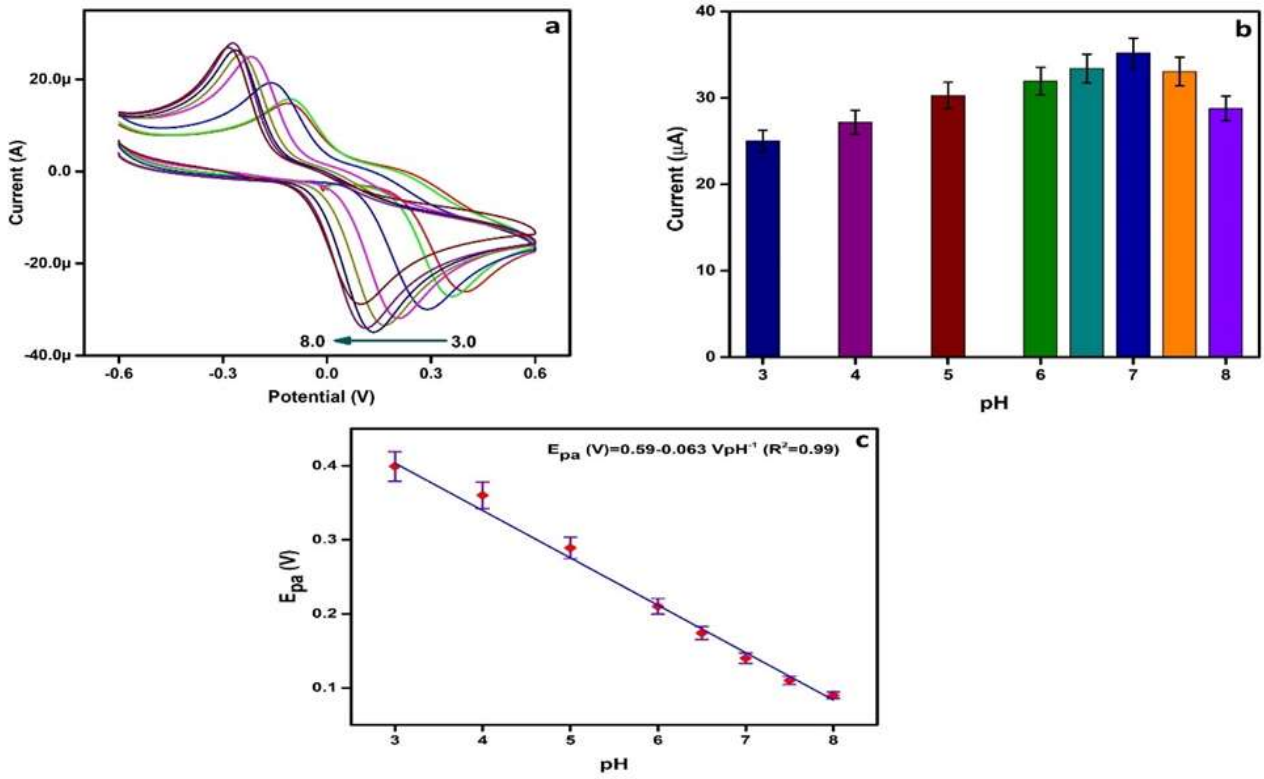


Fig. 3: (a) Cyclic voltammogram of HQ (100 µM, 0.1 M PBS) at GCE, GCE/CT-AgNPs and GCE/CT-AgNPs/PF-127 at different pH values, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0 (b) E_{pa} vs. pH (c) I_{pa} vs pH

6.4 Influence of Scan Rate:

Scan rate effect on the peak current of HQ at GCE/CT-AgNPs/PF-127 is presented in Figure 4a It can be noticed that peak current of HQ (100 µM, 0.1 M PBS, 7.0 pH) increased with the increase in the scan rate as 0.01-0.6. Square root of scan rate and peak current represents a linear relationship with the regression equation of I_{pa} (A)=-1.41-89.77 $v^{1/2}$ (V/s)$^{1/2}$, with the coefficient of correlation value 0.99 and I_{pc} (A)=-0.144+82.22 $v^{1/2}$ (V/s)$^{1/2}$ with coefficient of correlation value 0.99 (Figure 4b), which displayed that the redox reaction of HQ at GCE/CT-AgNPs/PF-127 was diffusion-controlled electrode process. In order to maintain stability and reduce the influence of background current, 0.1 V/s was preferred as the scan rate for the experiment.

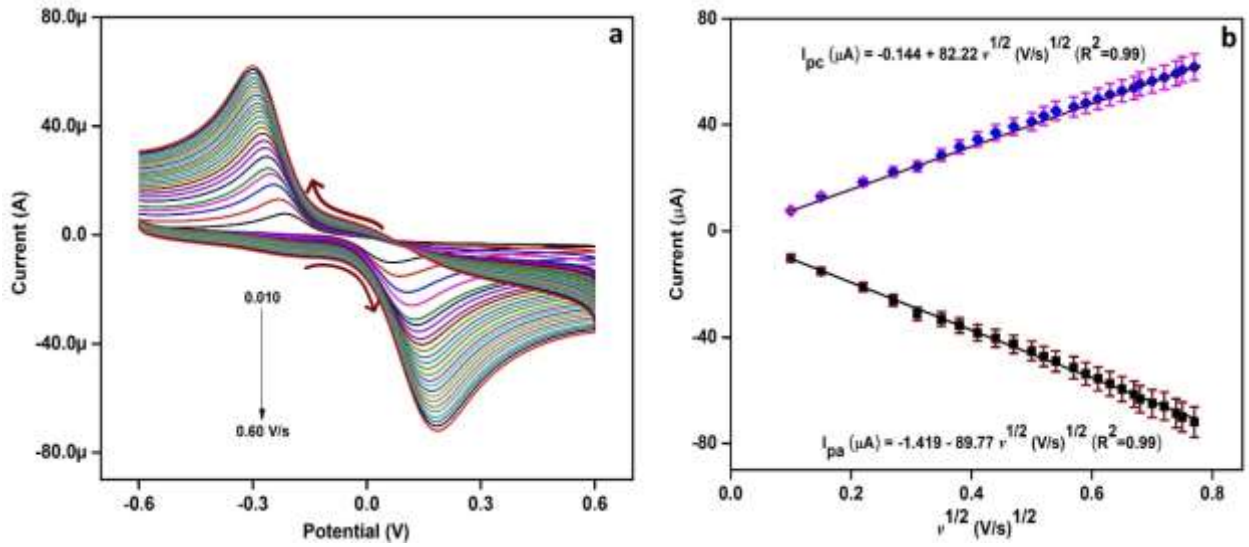


Fig. 4: (a) Cyclic voltammogram of HQ (100 µM, 0.1 M PBS) at different scan rate, (b) Peak current vs square root of scan rate

6.5 Calibration curve and limit of detection:

Beneath optimum conditions of investigation, SWV is utilized to investigate the relativeness between concentration and peak current. Figure 5a shows the square wave voltammogram of several concentration of HQ in 0.1 M PBS (pH = 7.0). The response of the oxidation current is showing a linear relationship with the concentration of HQ in the series from 1 to 12 µM with a linear regression equation of I_{pa} (A)=3.50×10^{-6}+7.75[HQ]$_{µM}$ (R^2=0.99) (Figure 5b). The detection limit (LOD=3S/M) and quantification limit (LOQ=10S/M) is determined and obtained value is 3.9 nM and 13.15 nM. The present obtained detection limit value with this system was compared with other systems. It shows that the electrochemical sensor developed for the resolution of HQ shows a lower detection limit.

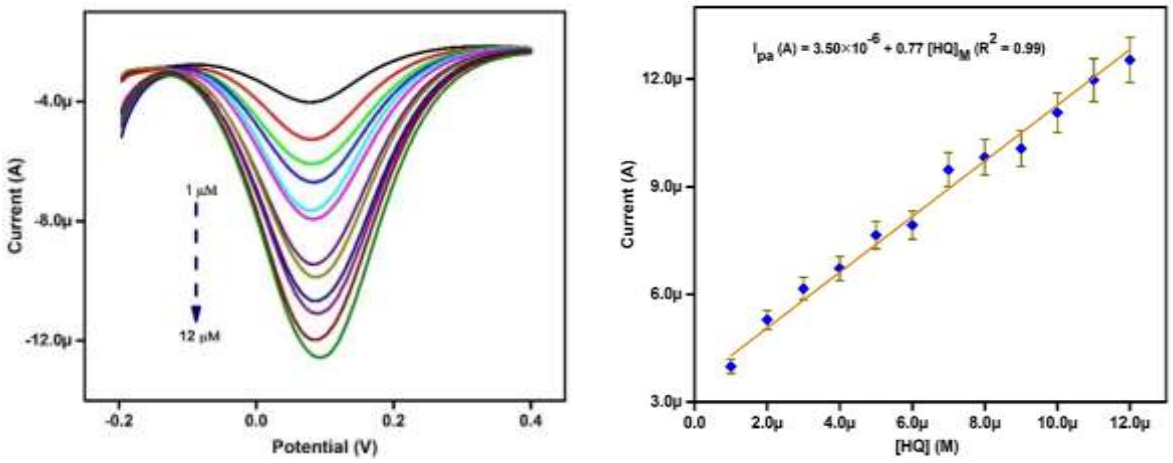


Fig. 5: (a) Square wave voltammograms of HQ at GCE/TiO_2/poly(L-lysine)/GQDs at different concentration, (b) Plot of I_{pa} vs concentration of HQ

6.6 Reproducibility, Stability and Interference Study:

To verify the reproducibility, the electrochemical response of HQ in the solution was investigated by using five independently developed GCE/CT-AgNPs/PF-127. The relative standard deviation (RSD) is observed to be 3.5%, indicating the good reproducibility of the designed GCE/CT-AgNPs/PF-127 electrode.

Short term stability of the developed GCE/CT-AgNPs/PF-127 was measured by scanning continuously for 100 cycles in the presence of 100 µM solution of HQ. It has been observed that even after 100 cycles 88.3% initial peak current is reserved, indicating that the established electrochemical sensor had a better stability.

To estimate the anti-interference of this method, solutions containing 100 µM of HQ with a number of potential interfering chemicals were evaluated. It was found that in the presence of Zn^{2+}, NH_4^+, Mn^{2+}, Mg^{2+}, K^+, Cu^{2+}, Ca^{2+}, Glucose and Bisphenol A, fluctuation in the peak current below 2%. This confirms the food anti-interference ability of the developed sensor.

6.7 Analytical Applications of the GCE/CT-AgNPs/PF-127 in Real Sample Analysis

Pharmaceutical sample (Cream) is obtained from the nearby medical shop and 1 mg of sample mixed with 50 mL of purified water. A quantity of 1.0 mL diluted substance is placed in an electrochemical cell having 0.1 PBS of pH 7.0 and then exposed to voltammetric examination at a scan rate of 0.1 V/s. Throughout the analysis, HQ was not detected in diluted pharmaceutical product. Consequently, the standard addition process was developed for HQ investigation. HQ retrieval was in the range of 95.00 % to 97.66%.

7. CONCLUSIONS :

Glassy carbon paste electrode adapted with chitosan Functionalized Silver Nanoparticle and Pluronic F-127 can be used for the determination of HQ by DPV. The extreme benefit of the electrode is trouble-free maintenance and renewal of its exterior area before a sequence of measurements. It is observed that the introduction of chitosan in glassy carbon paste electrode indicates attraction towards the determination of HQ with good reproducibility and a lower value for the detection limit. The electrode preparation is very simple, easy, is of more precise and healthier sensitivity. It is inexpensive and delivers quick response in the resolving of the concentration of HQ in solutions. The outcome of the method has revealed that the simultaneous determination of HQ in some of the pharmaceutical products is possible with greater accuracy.

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