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**ORIGINAL ARTICLE** **OPEN ACCESS**

# Lawsone (2-Hydroxy-1,4-Naphthoquinone) Encapsulated Cetyltrimethyl ammonium Bromide Modified Platinum Electrode for Detection of Vitamin C (Ascorbic Acid) And Distinguishing Ascorbic Acid from Glucose

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**ABSTRACT**
*Designing of modified electrodes for the detection of molecules and ions is a challenging problem which has good analytical applications in environmental chemistry, medicinal chemistry etc. Quinones constitute an important class of naturally occurring compounds ubiquitous in nature and that are found in plants, fungi, and bacteria. 2-Hydroxy-1,4-naphthoquinone (HNQ; Lawsone) is the principal natural dye contained at 1.0-1.4% (high proportion) in the leaves of Henna (Lawsonia inermis). In this paper we report that platinum working electrode when modified with lawsone (extracted from Lawsonia inermis) encapsulated in Cetyltrimethyl ammonium bromide acts as voltammetric sensor for Vitamin C (Ascorbic Acid). Addition of glucose solution in electrolytic medium gives a peak in opposite direction therefore distinguishing sugar from ascorbic acid.*
***Keywords:*** *Lawsonia inermis, plant extract, lawsone , Cetyltrimethyl ammonium bromide, Platinum electrode, Vitamin C (Ascorbic Acid), sugar.*

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## INTRODUCTION

Detection and determination of small biomolecules and metal ions are of great importance for diagnosis of a number of diseases and understanding environmental pollution leading to extensive research on development of chemical sensors. Chemical sensors have a chemical or molecular target to be measured. For analysis of small biomolecules and metal ions electrochemical methods are widely used because of low cost, high dynamic range, easy *in situ* application and easy to handle [1-8].

Quinones constitute an important class of naturally occurring compounds ubiquitous in nature and that are found in plants, fungi, and bacteria. 2-Hydroxy-1,4-naphthoquinone (HNQ; Lawsone) is the principal natural dye contained at 1.0-1.4% (high proportion)in the leaves of Henna(*Lawsonia inermis*) [9-10]. The yellow pigment lawsone is contained in the leaves of the tropical bush henna. It has been used for more than 4000 years not only as a hair dye, but also as a body paint and tattoo dye. When henna is applied in a form of paste onto hair, skin or nail, it imparts a reddish-brown coloration lasting for up to twelve weeks. The green leaves paste has been used to prevent various infections such as ulcers, constipating, anemia, small pox and inflammation from ancient time.

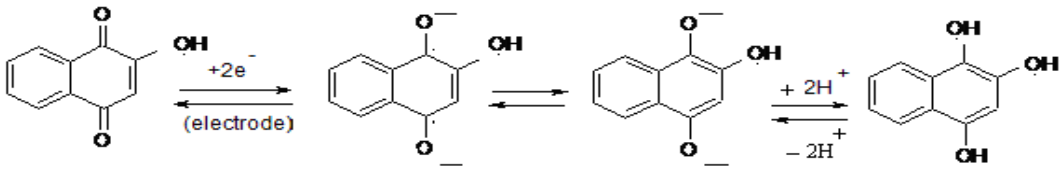
Lawsone is non-ionic moiety and has a hydroxyl group in position two. Upon deprotonation of this group, the corresponding anion presents resonance structures (o-quinone character) which is relevant to the study of biological systems [11-12].

Naphthoquinones have many physiological roles, because some members of this group as ubiquinone, plastoquinone and K vitamins are key functional constituents of biochemical systems.

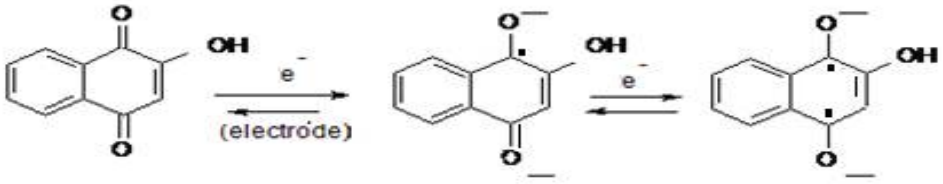
Hydroxy naphthoquinone and its derivatives have strong coordination ability towards metal ions and act as redox active ligands [11]. Kuwar and hisco-workers used lawsone in the synthesis of azo dye and prepared a colorimetric sensor for the detection of copper (II) and iron (III) ions by using fluorescence spectroscopy [12].

It is reported that Lawsone shows well-defined cyclic voltammogram due to the reversible conversion of the two ketone groups into hydroxyls [13]. The electrochemical activity (redox activity) of lawsone is due

to rapid reversible redox equilibrium between oxidized quinone form and reduced hydroquinone form. It is a two-electron redox process as shown in the scheme below:




Scheme 1: Two-electron reduction accompanied by proton transfer
Or, it is a successive one-electron redox process without proton transfer




Scheme 2: Successive one-electron reduction without proton transfer
Ascorbic acid (Vitamin C), a water-soluble vitamin, is an extremely important substance which plays a unique redox and electrochemical role. It is a well-known bioactive reducing agent[14-15]. It is used on a wide scale as an antioxidant agent in foods and drinks; it is also important for therapeutic purposes and biological mechanism. Therefore, recent advances in the food and pharmaceutical industries and the need for nutritional assessments necessitate the development of a selective, simple and accurate method to determine ascorbic acid.
Due to its selectivity and sensitivity an electrochemical method to determine ascorbic acid has been of considerable interest, especially in bioelectrochemistry [16]. Direct electrochemical detection of vitamin C is difficult as its voltammogram at a bare electrode is ill defined.
Vitamin C is unstable, undergoing oxidation, especially in aerobic conditions, alkaline media, and at exposure to light. Vitamin C is easily oxidized chemically or electrochemically to L-dehydroascorbic acid. Oxidation of vitamin C involves two consecutive one electron transfer process to form dehydroascorbic acid immediately followed by an irreversible hydration to give the final product 2,3-diketogulonic acid [17].
The electro-inactive oxidized product adsorbs on the electrode surface thereby polluting the electrode surface and a high potential is required for oxidation. The technique of self-assembled monolayer film on electrode helps in lowering the redox potential of vitamin C with a well-defined voltammogram. This has recently been investigated for the electrochemical detection of ascorbic acid [18].
Although the electrochemical oxidation of L-ascorbic acid has been thoroughly investigated at mercury electrodes [19], only a few studies have been carried out for this substance at a Pt electrode.
Carbohydrate's detection has assumed relevant importance in different areas, especially in the food and beverage industry. Among of them, molecules such as glucose, fructose, maltose and lactose are considered reducing glucoses. For the detection of these kind of glucoses, different and successful enzymatic sensors have been widely developed [20], which are characterized by high sensitivity, specificity and selectivity. However, these sensors show the known disadvantages related to the use of enzymes, such as the chemical and thermal instability. Therefore, as an alternative, different strategies with non-enzymatic sensors based on metals have been reported and exploited. Metals such as nickel, copper, platinum and gold are among the most widely studied [21].Quantitative analysis of glucose is essential in clinical chemistry [22].
In this work we report that platinum working electrode when modified with lawsone (extracted from *Lawsonia inermis*) encapsulated in cetyltrimethyl ammonium bromide acts as voltammetric sensor for Ascorbic acid (vitamin C). Addition of glucose solution in electrolytic medium gives a peak in opposite direction therefore distinguishing sugar from ascorbic acid.

## MATERIAL AND METHODS

All the electrochemical measurements were carried out under nitrogen environment on a CHI 660D CH Instrument electrochemical analyser, consisting of a three-electrode system: a surface modified working electrode (WE), Ag/AgCl (3 M NaCl) as the reference electrode (RE) and a Pt wire as the auxiliary electrode (AE).

Cetyltrimethylammonium bromide(CTAB), Tetrabutylammonium perchlorate (TBAP), Glucose, Ascorbic acid, HCl, KCl, NaCl, are purchased from Merck and employed as received.
All the electrochemical experiments were done using CHI660D Electrochemical Analyser.

**EXTRACTION OF LAWSONE (L) FROM *LAWSONIA INERMIS* (HENNA)**

The fresh leaves of *Lawsonia inermis* (henna plant) were dried at room temperature for 7 days and then crushed into powder form. *Lawsonia inermis* powder was mixed with ethanol and left for a week. Then the mixture was filtered and Lawsone was extracted by means of the rotary evaporator. The residue left is used for further experiments. *Lawsonia inermis* powder is mixed with absolute alcohol and stirred for 10 minutes. After filtration the filtrate is put under UV lamp and found red in color.

Fig. 1: **L**(in absolute alcohol) under visible light and UV light.

**PREPARATION OF L@CTAB/PT ELECTRODE**

**L** incorporated CTAB (L@CTAB) film on Pt electrode is prepared by dissolving 0.1g CTAB, 0.1g TBAP as supporting electrolyte and 0.5g extracted **L** in 10 mL absolute alcohol. Then the mixture was stirred for 1 hour and the suspension was then filtered. 40 µL of the solution was then placed on the tip of a pre-cleaned Pt electrode surface using Hamilton micro syringe. The solvent was allowed to evaporate resulting in the formation of **L** encapsulated CTAB film on the electrode surface. The fabricated electrode will be designated as Pt/L@CTAB working electrode. Cyclic voltammograms were recorded by immersing the tip of this modified electrode in 0.1 M KCl aqueous solution.

**PREPARATION OF VITAMIN C SOLUTION**

0.1 M ascorbic acid solution is prepared by dissolving 0.176 g ascorbic acid in 10 mL distilled water.

**PREPARATION OF GLUCOSE SOLUTION**

0.1 M glucose solution is prepared by dissolving 0.18 g glucose in 10 mL distilled water.

**RESULTS AND DISCUSSION**

Figure 2 shows thecyclic voltammogram (CV) of aqueous henna solution at pH 2.04 at Pt working electrode.

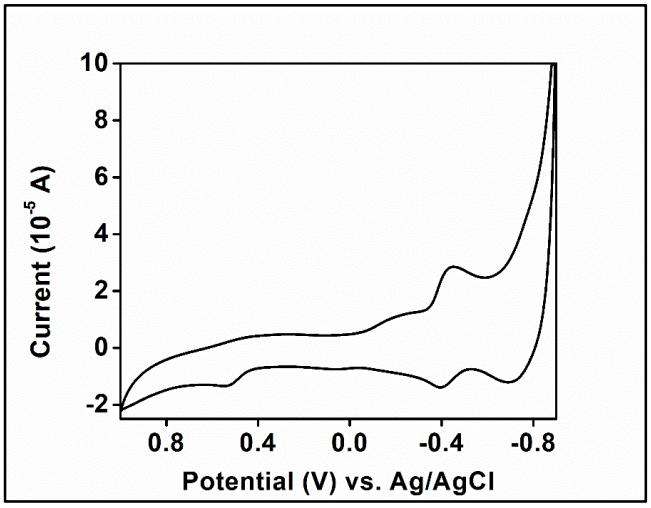



Fig. 2: CV of aqueous henna solution at pH 2.04 at Pt working electrode. AE: Pt wire, RE: Ag/AgCl (3 M NaCl), SE: $NaNO_3$. Scan rate: 0.05 $Vs^{-1}$, $\Delta E$ = -0.06 V, $E_{1/2}$ = -0.422 V.

Figure 3 shows the square wave voltammetry (SWV) of aq. henna solution at pH 2.04 at Pt working electrode.

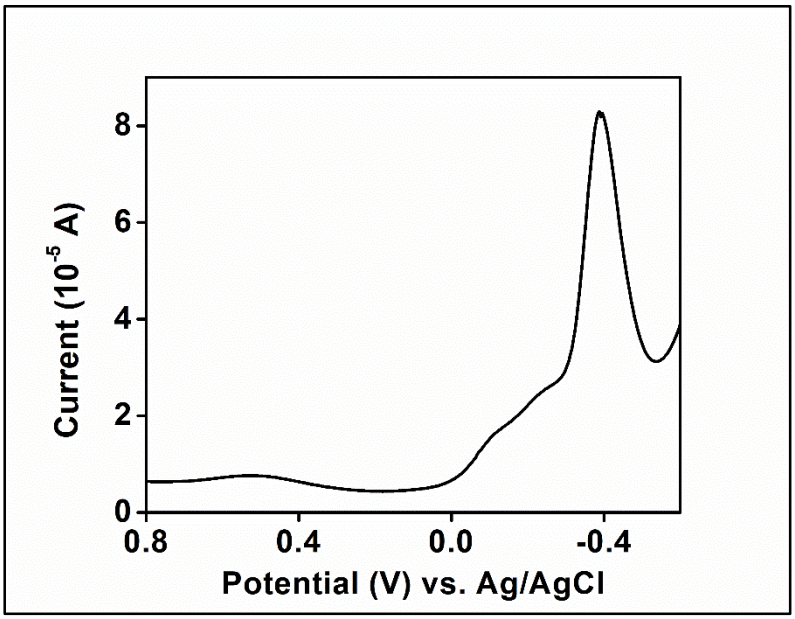



Fig. 3: SWV of aqueous henna solution at pH 2.04 at Pt working electrode. AE: Pt wire, RE: Ag/AgCl (3 M NaCl), SE: $NaNO_3$. Scan rate: 0.05 $Vs^{-1}$

Cyclic voltammetric experiments of aqueous henna solutionare done at different pH (2.74, 2.70,2.66, 2.64, 2.60,2.57,2.54) atmodified Pt working electrode. The cathodic and anodic peak currents are found to increase with decreasing pH which indicates that the redox process becomes faster on decreasing pH (Figures 4,5,6).

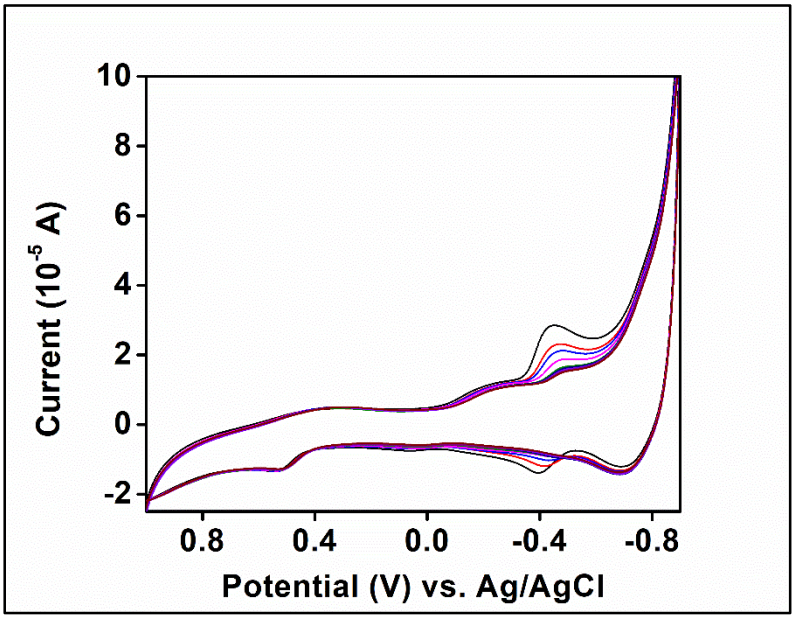



Fig. 4: Overlay of cyclic voltammogram of aqueous henna solution at Pt working electrode at different pH (3.48, 3.18, 3.00, 2.88, 2.78, 2.71, 2.49, 2.32, 2.20, 2.04). AE: Pt wire, RE: Ag/AgCl (3 M NaCl), SE: $NaNO_3$. Scan rate: 0.05 $Vs^{-1}$. $\Delta E$ = -0.06 V, $E_{1/2}$ = -0.422 V.

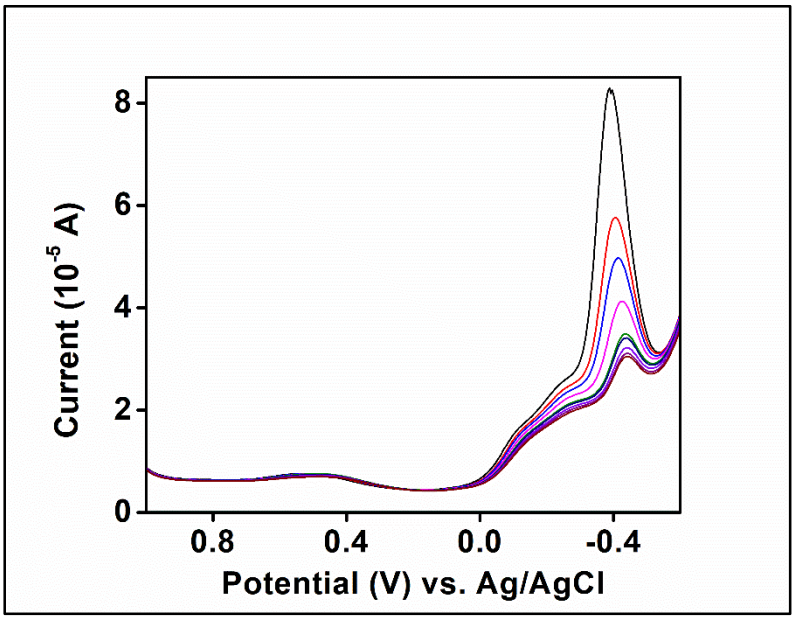



Fig. 5: Overlay of square wave voltammogram of aqueous henna solution at Pt working electrode at different pH (3.48, 3.18, 3.00, 2.88, 2.78, 2.71, 2.49, 2.32, 2.20, 2.04). AE: Pt wire, RE: Ag/AgCl (3 M NaCl), SE: $NaNO_3$. $E_{1/2}$ = -0.389 V.

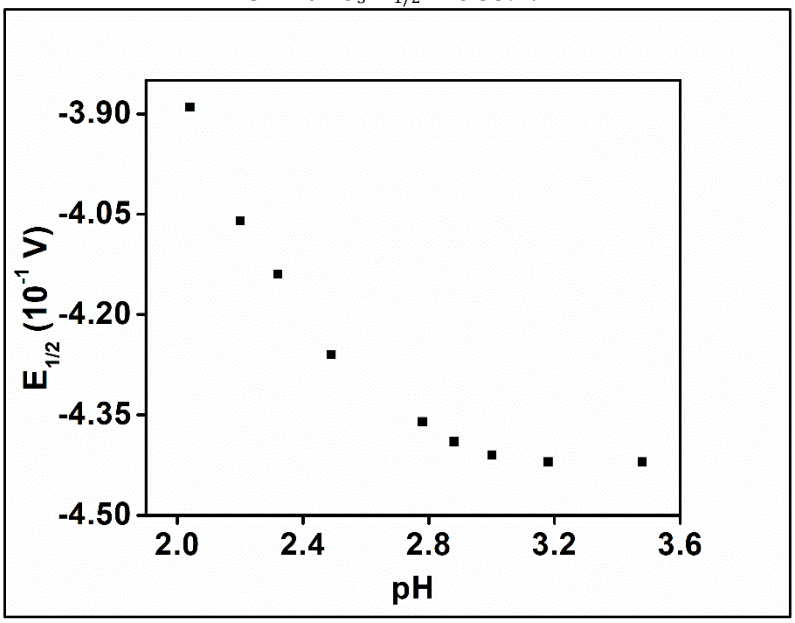



Fig. 6: Redox potential vs. pH plot of aqueous henna solution at Pt working electrode at different pH (3.48, 3.18, 3.00, 2.88, 2.78, 2.71, 2.49, 2.32, 2.20, 2.04). AE: Pt wire, RE: Ag/AgCl (3 M NaCl), SE: $NaNO_3$

Figure7 shows the cyclic voltammograms of Pt/L@CTAB electrode in water in presence of 0.1 M KCl as supporting electrolyte modified Pt working electrode at pH 2.42 at different scan rates from 0.01 $Vs^{-1}$ to 0.1V $s^{-1}$versus Ag-AgCl (3 M NaCl) as reference electrode. Inset figure shows that with the increasing scan rates both the cathodic and anodic currents of the reversible redox couple increase. The cathodic and anodic currents are found to increase linearly with scan rates which is a characteristic of surface adsorption and supports the formation of film on electrode surface.

Figure 8 shows the square wave voltammograms of Pt/L@CTAB modified electrode in water in presence of 0.1 M KCl as supporting electrolyte having redox potential value at -0.314 V.

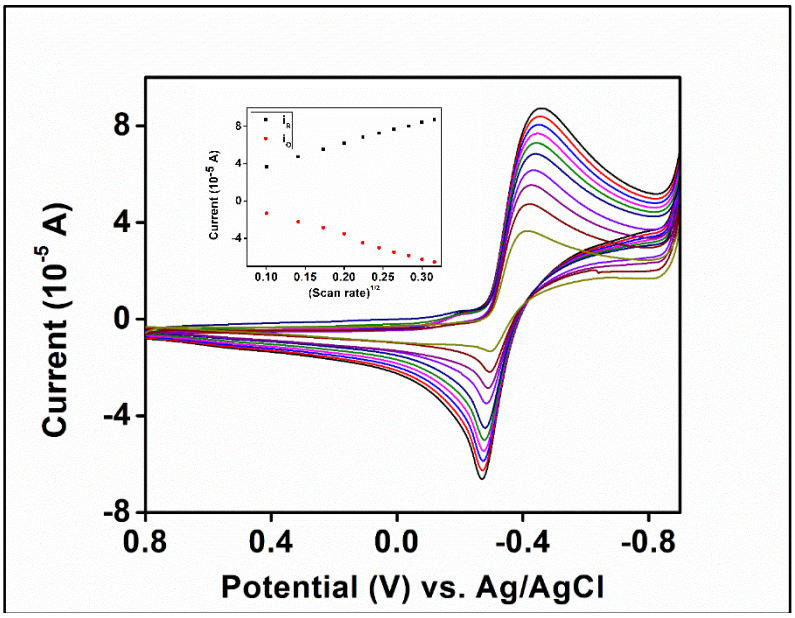



Fig. 7: Cyclic voltammograms of 0.1 M KCL at L@CTAB/TBAP modified Pt working electrode at pH 2.42 at different scan rates from 0.01 $Vs^{-1}$ to 0.1V $s^{-1}$. AE: Pt wire, RE: Ag/AgCl (3 M NaCl). Inset: The cathodic and anodic currents versus square root of scan rate linear plot.

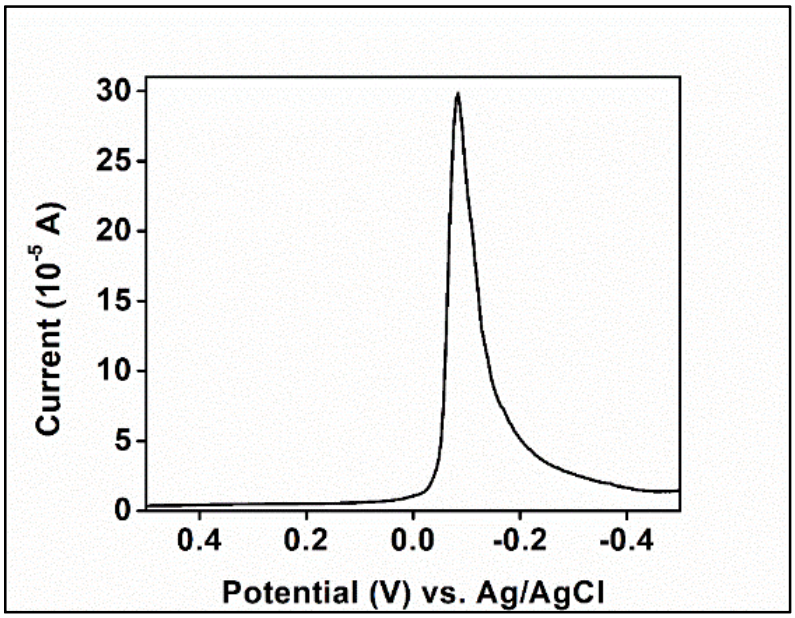



Fig. 8: Square wave voltammograms of 0.1 M KCL at L@CTAB/TBAP modified Pt working electrode at pH 2.42 at scan rate0.1 V $s^{-1}$.

Diffusion coefficients of Pt/L@CTAB electrode have been calculated using Cottrell equation. The diffusion coefficient values of lawsone inside film are $10^3$ times higher than the diffusion coefficient of lawsone in micellar medium [23]. This is due to the fact that inside the film lawsone is closer to the electrode surface compared to micellar medium.

In case of Lawsone encapsulated CTAB film modified Pt electrode, the mid-point potential and peak currents of the $HNQ^{\cdot}/HNQ^{-}$ couple were found to remain unaffected by Vitamin C concentration and a new irreversible cathodic peak appears at -0.94 V when the vitamin C concentration is 0.01M. (Figure 9, scheme1).

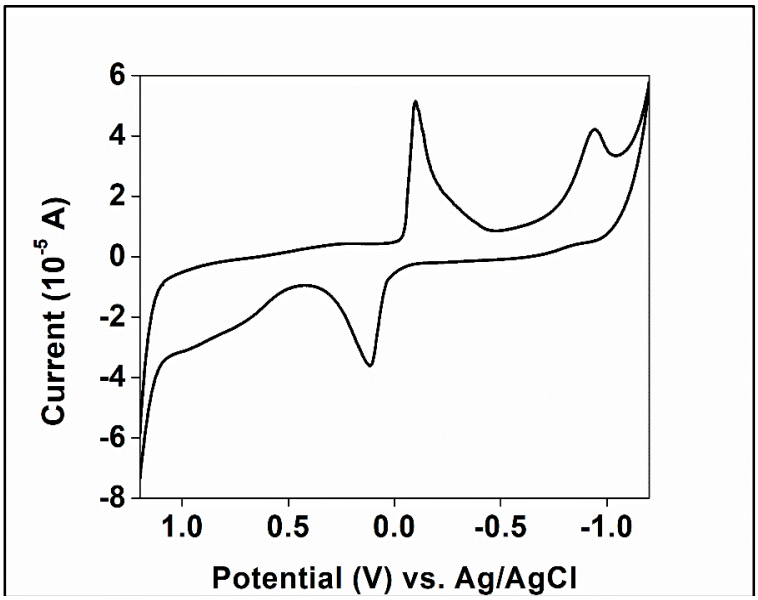



Fig. 9: Cyclic voltammogram of 0.01 M aqueous Vitamin Cat Pt/L@/CTAB. AE: Pt wire, RE: Ag/AgCl (3 M NaCl), SE: KCL, Scan rate 0.05 $Vs^{-1}$

The probable explanation for the irreversible cathodic peak inside CTAB film when Vitamin C in solution is that HNQ· free radical can easily accept electrons from ascorbate anion by oxidizing it to dehydroascorbic acid followed by hydrolysis into 1,2-diketogulonic acid [15].

It is very interesting that when we replace Vitamin C by glucose we see that when glucose concentration in water is 0.012 M, the mid-point potential and peak currents of the $HNQ^{\cdot}/HNQ^{-}$ couple were found to remain unaffected and the new irreversible peak appears at - 0.844 V (Figure 10).

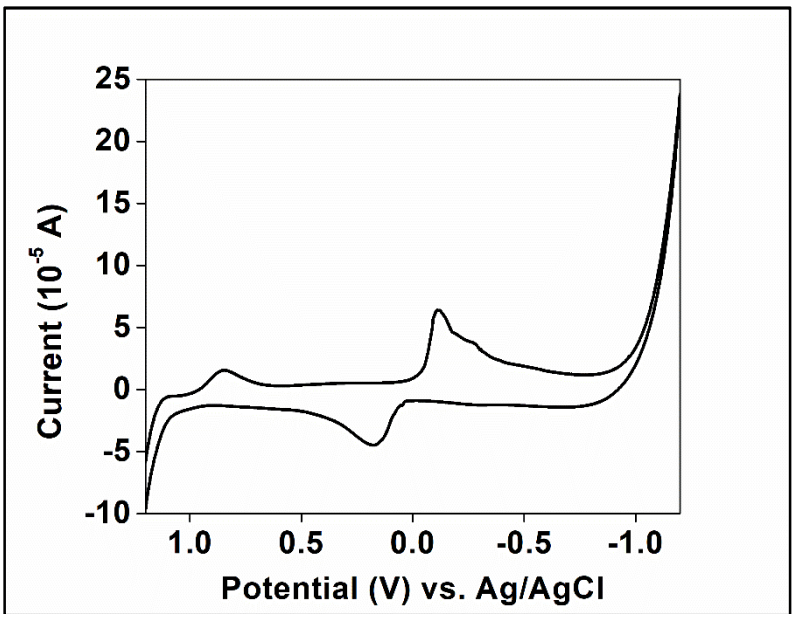



Fig. 10: Cyclic voltammogram of 0.012 M aqueous glucoseat Pt/L@/CTAB. AE: Pt wire, RE: Ag/AgCl (3 M NaCl), SE: KCL, Scan rate 0.05 $Vs^{-1}$

Figure 11 shows the comparison of cyclic voltammogramsof 0.01 M Vitamin C and 0.012 M glucose at Pt/L@/CTAB.

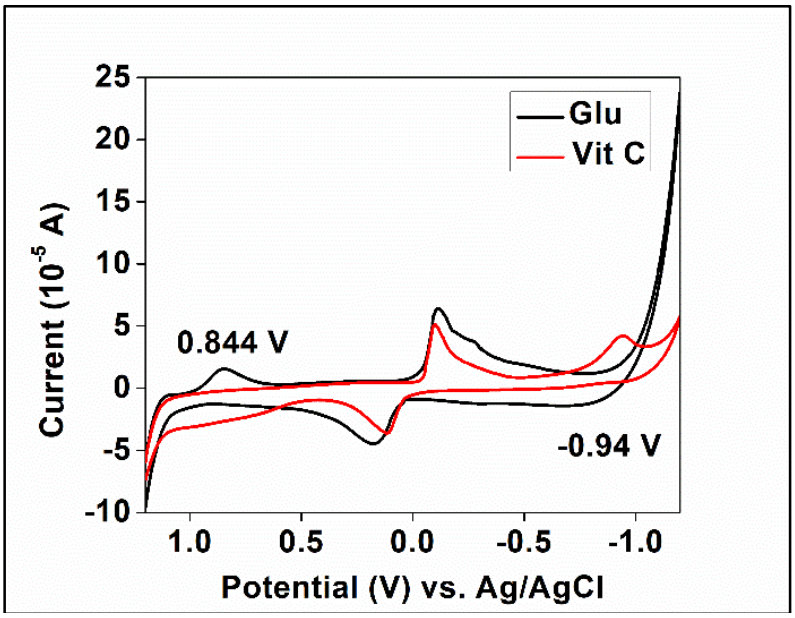
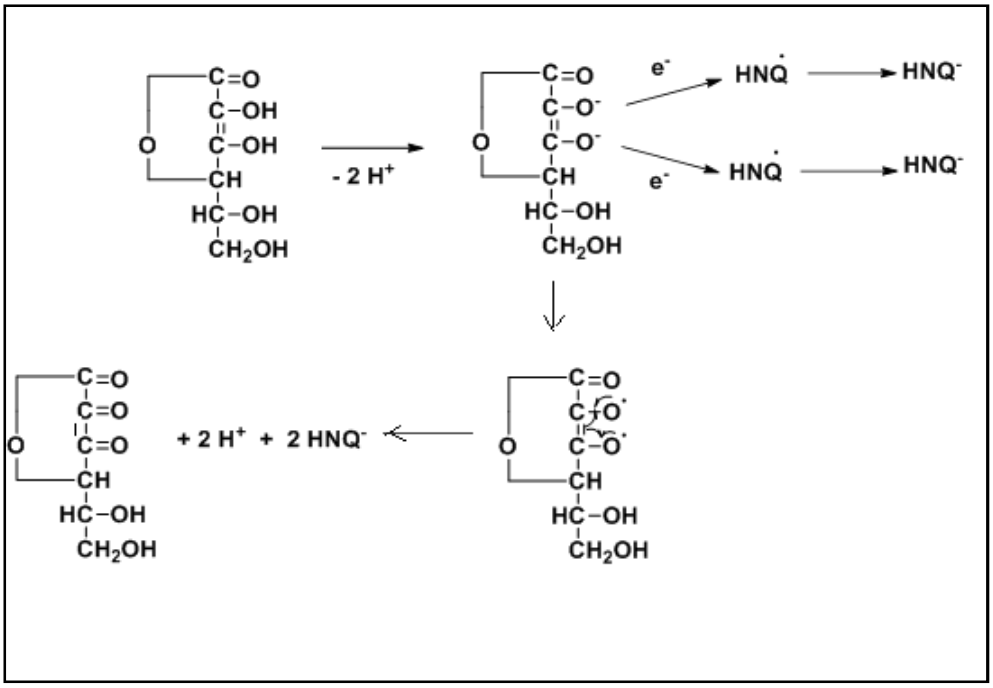



Fig. 11: Comparison of cyclic voltammograms of 0.01 M Vitamin C and 0.012 M glucose at Pt/L@/CTAB. AE: Pt wire, RE: Ag/AgCl (3 M NaCl), SE: KCL, Scan rate 0.05 $Vs^{-1}$

Scheme 1:




**CONCLUSION**

1. Cyclic voltammogram of aqueous henna solution at pH 2.04 at Pt working electrode. AE: Pt wire, as reference electrode( RE): Ag/AgCl (3 M NaCl), supporting electrolyte (SE): $NaNO_3$. Scan rate: 0.05 $Vs^{-1}$, $\Delta E$ = -0.06 V, $E_{1/2}$ = -0.422 V.
2. Cyclic voltammetric experiments are done at different pH (2.74, 2.70 , 2.66, 2.64, 2.60,2.57,2.54) of aqueous henna solution at modified Pt working electrode. The cathodic and anodic peak currents are found to increase with decreasing pH which indicates that the redox process becomes faster on decreasing pH.
3. L@CTAB/TBAP/Pt modified electrode in water in presence of 0.1 M KCl as supporting electrolyte at scan rate 0.05 $Vs^{-1}$ versus Ag-AgCl (3 M NaCl) as reference electrode give a well defned quasi-reversible cyclic voltammograms. A redox couple is obtained with redox potential $E_{1/2}$ = -0.348 V is observed with $\Delta E$ = -0.110 V.

4. The cyclic voltammogram of L@Pt/CTAB electrode in 0.1 M KCl at different added concentration of Vitamin C , the mid-point potential and peak currents of the $HNQ^{\cdot}/HNQ^{-}$ couple were found to remain unaffected by Vitamin C concentration and a new irreversible cathodic peak appears at -0.94 V when the vitamin C concentration is 0.01M.
5. It is very interesting that when we replace vitamin C by sugar we see that when sugar concentration in water is 0.012 M, the mid-point potential and peak currents of the $HNQ^{\cdot}/HNQ^{-}$ couple were found to remain unaffected and a new irreversible peak appears at 0.844 V.

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