



NON ENZYMATIC ELECTROCHEMICAL DETECTION OF GLUCOSE AT RICE STARCH-NANOPARTICLES MODIFIED ELECTRODE

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ABSTRACT

The need for a more rapid, reliable, specific, and sensitive non enzymatic method of detecting of glucose, at low cost is the focus of this work. In this paper, nanoparticles obtained from rice starch (RSNP) have been used to fabricate an electrode for electrochemical detection of glucose. The voltammetric behaviour of glucose on RSNP electrode was investigated by cyclic voltammetry and differential pulse voltammetry. The oxidation peak currents showed a linear range from 10^{-2} to 10^{-6} M glucose with correlation coefficient ($R^2 = 0.99753$). The biosensor shows a short response time of 7s and lower detection limit of $0.1 \mu\text{M}$. The RSNP modified electrode in the determination of glucose in acidic solution demonstrated that it has the potential to speed up the detection, increase specificity and sensitivity throughout the analysis.

KEY WORDS: Rice starchnanoparticles, Differential pulse voltammetry, Cyclic voltammetry, nonenzymatic, glucose



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INTRODUCTION

Enzymatic biosensors based on glucose oxidase have attracted much attention since 1960 for their high sensitivity and selectivity^[1-7]. But, due to the lack of stability originated from the intrinsic nature of enzyme, which can easily be affected by temperature, pH value, humidity, and toxic chemicals^[8,9], non enzymatic glucose biosensors were developed^[10-15]. Today nanotechnologies significantly influence all aspects of our life. Employment of nanotechnologies and nanomaterials opens new perspectives in electronics, chemical industry, energetics, biology and medicine, etc^[16,17]. Carbon nanotubes (CNTs) have good electrical conductivity, high electro-catalytic effect, strong adsorptive ability and because of these unique properties CNTs have been utilized and in the fabrication of electrochemical sensors^[18-21]. Now a days different forms of carbon nanoparticles^[22] like nanowires, nanopores, nanoclusters and grapheme etc have been extensively explored to develop glucose biosensors with high sensitivity, fast response time and stability via electrochemical studies. In this paper, an electrode has been fabricated using nanoparticles obtained from pyrolysis of rice starch (RSNP) for the detection of glucose. Differential pulse voltammetry (DPV) and cyclic voltammetry studies have been carried out for the detection of glucose on modified RSNP electrode and the results were compared with the commercially available screen printed carbon nanotube electrode (std-CNT). RSNP modified electrode showed high sensitivity, low potential and fast response to the detection of glucose, which may be a promising candidate for non-enzymatic glucose sensing as well as other biosensors.

MATERIALS AND METHODS

D(+)-glucose (97%) and H₂SO₄ were purchased from Sigma. All the other reagents used, were of analytical grade. All solutions were prepared

with deionized water. Electrochemical studies were performed using a minipotentiostat, which consisted a hand-held USB powered instrument, connected to a PC by means of an USB cable, for use with electrochemical cells.

(i) *Preparation, functionalization and characterization of RSNPs*

RSNPs were prepared by our own reported method^[23]. Carbon soot was collected by pyrolysing rice starch under inert atmosphere which was purified and then functionalized with dilute nitric acid. The shining black solid was washed with water and dried to yield the solid RSNPs. It was then characterized by scanning electron microscopy (SEM), transmission electron microscopy (TEM) and p-XRD.

(ii) *Fabrication and standardization of RSNP electrode*

The electrode was fabricated on an insulating Teflon material with three silver wires of length (40cm) and width (1mm). RSNPs was dispersed in polystyrene solution made in chloroform (9:1 ratio), sonicated for few minutes and mechanically mixed at 160°C. A drop of the slurry was deposited as a very fine thin film on the Teflon substrate covering two silver wires, serving as working and counter electrodes. The third silver wire was used as a reference electrode. Cyclic voltametric was performed with RSNP towards 5 X 10⁻⁴ (M) potassium ferricyanide in 0.1M H₂SO₄ at 100 mV/s scan rate and was compared with std-CNT electrode. (Fig1). For RSNP electrode the peak potential separation (ΔE_p) is 0.75V and the ratio of the cathodic current over the anodic one is close to 1. This result is in good agreement with the Nernst equation. For non-enzymatic glucose detection, DPV and CV studies with various concentrations of glucose solutions were performed using H₂SO₄ as supporting electrolyte on fabricated RSN electrode.

Standardization curve of RSNP

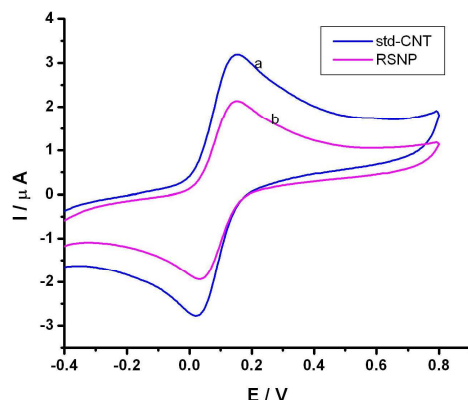


Figure 1

- a) CV of 5×10^{-4} (M) $[K_3Fe(CN)_6]$ on the std-CNT
 b) CV of 5×10^{-4} (M) $[K_3Fe(CN)_6]$ on the RSNP

(iii) Non-enzymatic Glucose detection with fabricated RSNP s electrode

RESULTS

1. Characterizations of RSNPs

The SEM images of RSNPs are shown in Fig 2 (a) and (b) which indicate the formation of nanoparticles in cubes and bricks shape. The graphitic structure of the RSNPs is confirmed

by the characteristic peaks of XRD, at around 25° and 44° which are assigned for (0 0 2) and (1 0 0) reflections respectively for graphitic carbon (Fig 3)

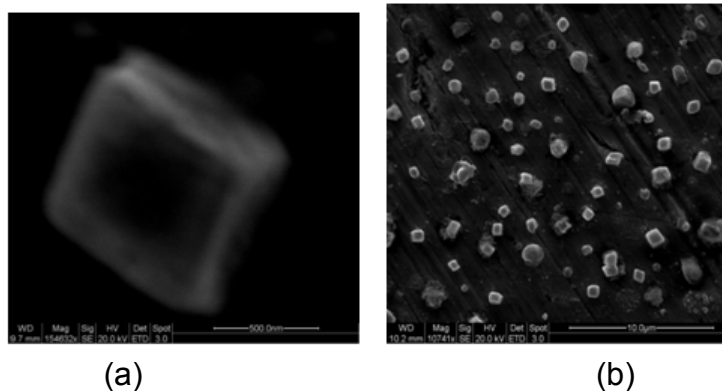


Figure 2(a), (b)
SEM images of RSNP

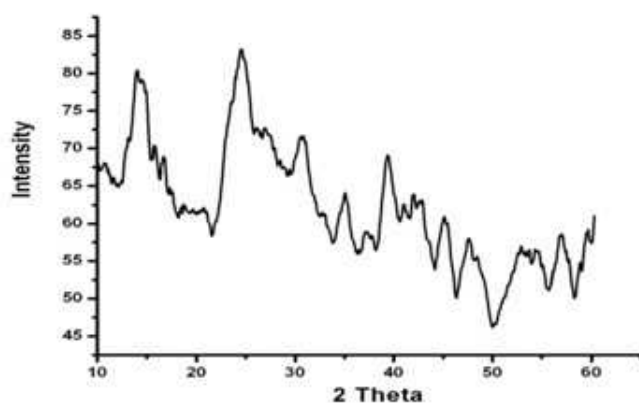


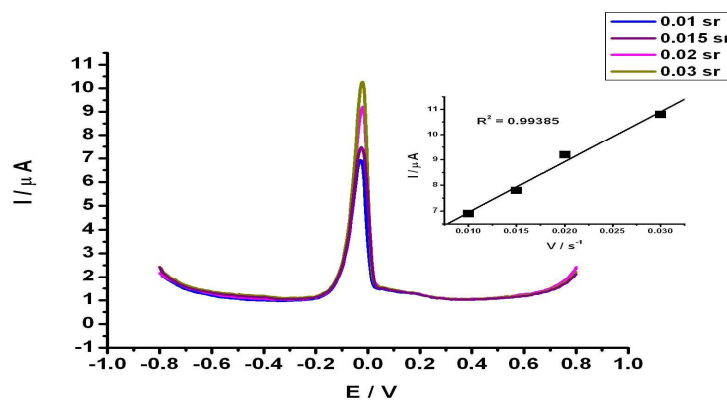
Figure 3
XRD pattern shows intense peaks at around 25° & 44°

2. Effect of Scan Rate on the Peak Current and Peak Potential of glucose at RSNP Modified Electrode

The influence of the scan rate on the electrochemistry of glucose at the RSNP modified electrode was investigated by DPV and CV techniques. Fig 4 (a) and 5 display the overlaps of voltograms of 10^{-4} M glucose at various scan rates on RSNP electrode. The current vs scan rate plot shown in the inset exhibits a linear relationship (with $R^2 = 0.99385$ for DPV and $R^2 = 0.99552$ for CV). The influence of experimental times scale manifested as scan rate on the system was investigated within the range of 0.01 Vs^{-1} to 0.03 Vs^{-1} for DPV and 50 mVs^{-1} to 150 mVs^{-1} for CV analysis. The linearity was reasonably

evident over the entire range of scan rates studied. In DPV technique potential required for direct oxidation of glucose on RSNP electrode is found to be -0.02 V and in CV the potential is in between -0.04 V to -0.02 V . The shifting of potentials may be due to the adsorption of glucose on electrode surface. A similar type of response was obtained when DPV of RSNP were compared with screen printed std-CNT electrode (with $R^2 = 0.99403$) (Fig 4 b). On std-CNT electrode, -0.03 V was required for the direct oxidation of glucose in acidic medium.

a) DPVs of 10^{-4} M glucose at various scan rates on RSNP electrode



b) DPVs of 10^{-4} M glucose at various scan rates on screen printed std- CNT electrode

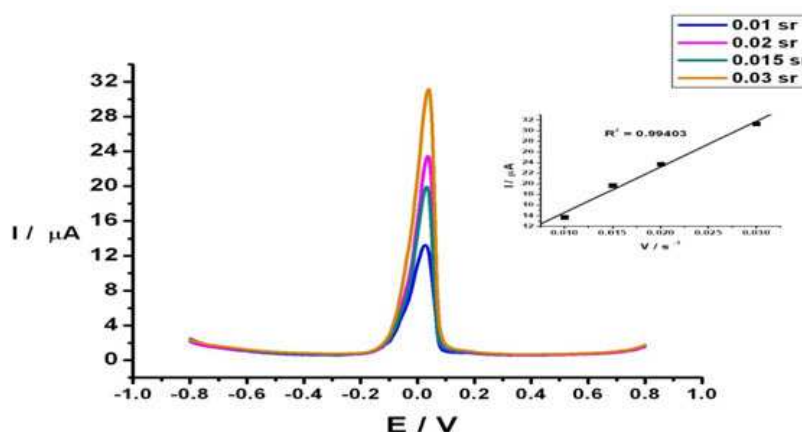


Figure 4

(a) DPVs of 10^{-4} M glucose at various scan rates starting from 0.01 V/s^{-1} to 0.03 V/s^{-1} at -0.02 V on RSNP electrode. The figure in inset shows linear relationship between current and scan rate and the value of regression constant is 0.99385

(b) DPVs of 10^{-4} M glucose at various scan rates (0.01 V/s^{-1} to 0.03 V/s^{-1}) at -0.03 V on screen printed std- CNT electrode. The inset shows linear relationship between current and potential and the value of regression constant is 0.99403

CVs of 10^{-4} M glucose at various scan rates on RSNP electrode

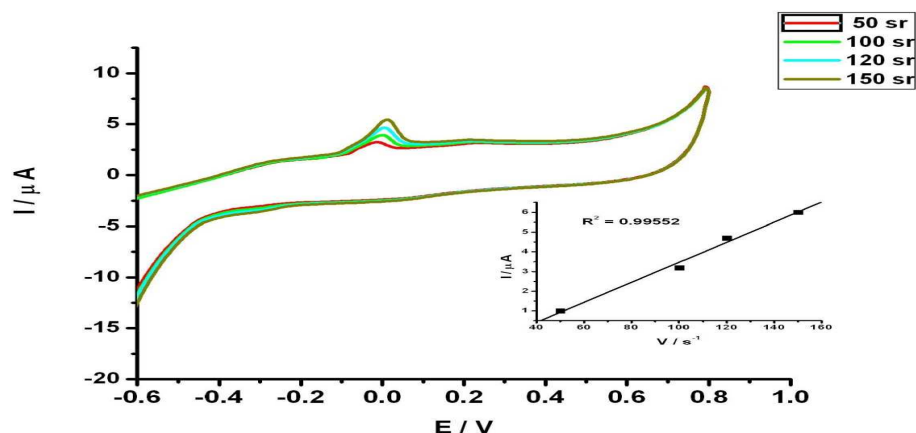


Figure 5

CVs of 10^{-4} M glucose at various scan rates (50 mV/s^{-1} to 150 mV/s^{-1}) at -0.02 V to -0.04 V on RSNP electrode. The resulting data was processed and plotted as a scatter plot (current vs scan rate) with linear regression analysis and the value of regression constant is 0.99552. In all situations it was found that the peak currents exhibited a linear relationship with the scan rate, a feature which is characteristic of surface-bound processes as outlined in the analytic theory proposed by Laviron [24-27].

3. Effect of time interval on the Peak Current and Peak Potential of glucose at RSNP Modified Electrode

Fig 6, 7 show the DPV and CV plots of 10^{-4} M glucose at various time intervals. Good linearity ($R^2 = 0.99605$ and $R^2 = 0.99172$) between current and time interval was observed. For DPV the standard deviation is 0.27104 and for CV 0.51778. The linearity was reasonably

evident over the entire range of time interval studied, 0 min to 15 min for DPV and 0 min to 14 min for CV. However studies conducted over a more extended time interval range (up to 18min) indicated a breakdown in the direct proportionality between peak current and time. This observation may also be due to adsorption of glucose on the electrode surface.

DPVs of 10^{-4} M glucose at various time interval

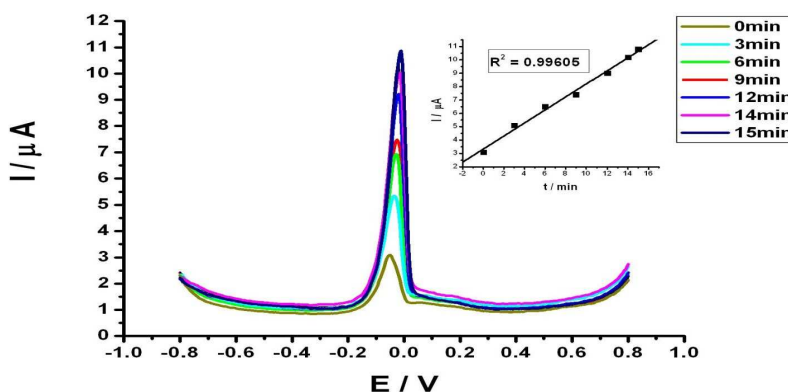


Figure 6

DPVs of 10^{-4} M glucose at various time interval at 0.015V/s^{-1} scan rate & at -0.02 V. The figure in inset shows linear relationship between current and time and the value of regression constant is 0.99605

CVs of 10^{-4} M glucose at various time interval

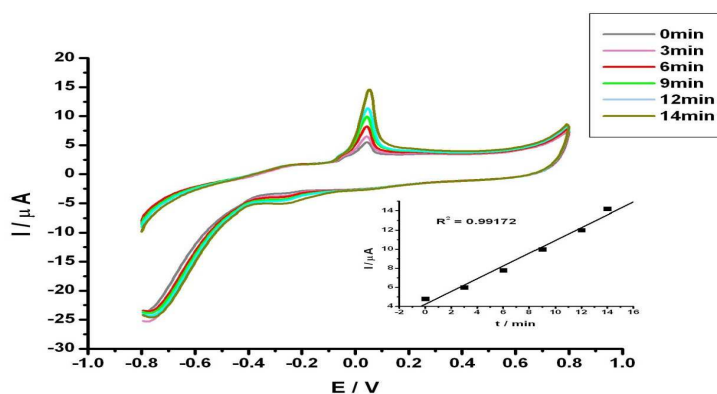


Figure 7

CVs of 10^{-4} M glucose at various time interval at 100mV/s^{-1} scan rate & at -0.01 V. The inset is a plot of the current against the time interval. The inset shows the linear relationship ($R^2 = 0.99172$) between peak current and the time interval value.

4. Differential Pulse Voltametric studies of glucose at RSNP Modified Electrode at $0.015V/s^{-1}$ with various concentrations

The results obtained for a series of DPV measurements of glucose conducted on RSNP modified electrode in H_2SO_4 , over a range of concentrations are presented in Fig 8 (a,b). Different concentration increases are used in

the two representative calibrations including labeled concentration increases from a) 0.001 M to 0.01M , b) 1 μM to 100 μM . The modified electrode responded to glucose sensor with linear dependence ($R^2 = 0.99091$ for 0.001 M to 0.01M , $R^2 = 0.99558$ for 1 μM to 100 μM ,) in the glucose concentration range of 10^{-2} to 10^{-6} M.

Calibration curves of Glucose concentrations

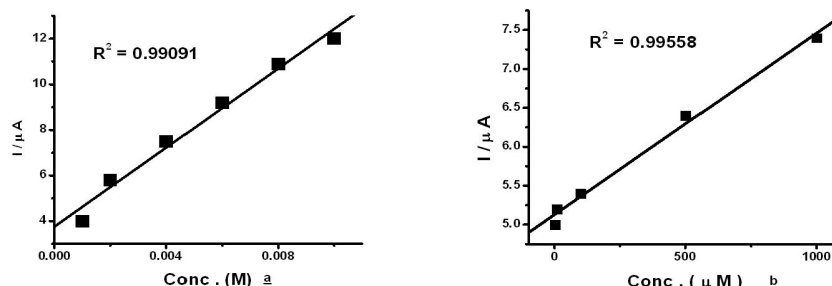


Figure 8

(a) Calibration curves of Glucose concentrations 0.001M to 0.01M at $0.015V/s^{-1}$. The value of regression constant is 0.99091

(b) Calibration curves of Glucose concentrations 1 μM to 1000 μM at $0.015V/s^{-1}$. The value of regression constant is 0.99558

The above figures shows a good linearity response over the range of glucose concentration 10^{-2} to 10^{-6} M with current. This wide linear range indicates that the RSNP modified electrode surface has a high capacity for quantitative determination of glucose^[28]. It is further interesting to note that inspite of non-tubular dimensions, the graphitic nature of carboxylated RSNP showed good performance in glucose oxidation. From these studies it can be concluded that RSNP electrode acts similar as std-CNT electrode in electron transfer.

CONCLUSION

Fabrication of rice starch-nanoparticles modified electrode was done for non enzymatic detection of glucose via electrochemical reactions. In this paper, we have done a detail study on the electrochemical behaviour of nanoparticles obtained from pyrolysis of rice starch, which showed well defined voltammetric responses towards glucose detection. RSNP based glucose biosensor

shows linearity from the concentration range of 10^{-2} M up to 10^{-6} M with lower detection limit of 1 μM and response time of 7s. It has been found that the linearity of concentration vs peak current ($R^2 = 0.99657$) is highly satisfactory in case of glucose detection and determination, which indicates that RSNP modified electrode can be utilized for other biosensors also.

ACKNOWLEDGEMENT

Authors are grateful to the Director, NIT Agartala to allow for publishing the results. Authors are also thankful to CPRI, Bangalore for financial support.

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