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Novel Electrochemical Sensor for Highly Sensitive Detection of Adenine Based on Vanadium Pentoxide Nanofibers Modified Screen Printed Carbon Electrode

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Herein, we report a simplistic approach to prepare vanadium pentoxide nanofibers (V_2O_5 NFs) using simple hydrothermal method. The as-synthesized material was confirmed by X-ray diffraction (XRD), energy dispersive X-ray spectroscopy (EDX) techniques, scanning electron microscopy (SEM). In addition, V_2O_5 NFs was used as an electrocatalytic performance towards the electro-oxidation of Adenine. Interestingly, V_2O_5 NFs modified electrode (V_2O_5 NFs/SPCE) showed an excellent electrocatalytic activity towards Adenine with lower detection limit (0.013 µM), wide linear ranges (0.5 to 512 µM) and the higher sensitivity about 8.5333 µA μM^{-1} cm^{-2}. Furthermore, V_2O_5 NFs/SPCE was also demonstrated good recovery even in real human urine samples. This study opens a new window for the trace level identification of biological molecules in real samples.

Keywords: Vanadium pentoxide, nano-fibers, Adenine, electro-oxidation, human urine sample.

1. INTRODUCTION

Adenine is an important purine component of DNA, RNA and other biologically significant species such as Adeno-sine triphosphate (ATP) [1]. Besides, Adenine is a building block of deoxyribonucleic acids which plays a vital role in protein synthesis and storage of genetic information [2]. Normally, the purine components are biologically important which plays numerous parts such as powerful consequence on cardiovascular systems, impact of cell proliferation, effect of coronary blood

flows as bio-regulators. Fluctuations in the purine level and metabolism are moral indications for cancer, renal calculi, AIDS, anemia inter-conversion of enzymes, mental retardation, tumorigenesis, carcinoma, liver diseases [3-7] etc. Owing to these reasons, the determination of Adenine is the important significance in the biological fluids and physiology. In recent decades, abundant techniques had been used for the determination of adenine such as ion-pairing liquid chromatography [8], isotope dilution mass spectrometry [9], chemiluminescence, HPLC (high-performance liquid chromatography) [10], calorimetry [11], capillary electrophoresis [12], surface enhanced Raman scattering [13], and Spectro-photometry. However, the above-mentioned methods are having some limitations such as high cost, tedious protocol and longtime consumption. On the other hand, the electrochemical techniques are quick response, simple, least cost and highly sensitive [14,15]. Hence, we decided that the electrochemical technique is the most suitable for the determination of Adenine separately or in the occurrence of the other substances. However, the selection of suitable electrode for the determination of Adenine is an important task.

So far, various nanostructures of metal alloys, metal oxides and metal nanoparticles such as Pt-Pb, Pt-Ru, Pt-Au, RuO_2, NiO, ZnO, MnO_2, Co_3O_4, V_2O_5, Pt, Au, Cu and Ni have been used in electrochemical sensing application. Among them, metal oxides have ridiculous attention due to their enhanced electro and photochemical properties. These excellent properties of the metal oxides were utilized in electrochemical sensor, photo-catalysis, biomedical, drug delivery, energy storage applications, and biomedical diagnosis [16].

Recently, vanadium oxides are emerging as a familiar catalyst among various metal oxides. In addition, vanadium oxides are used as an electrode material in electro catalytic oxidation, electrochemical sensing and super capacitors applications due to their low cost, unique layered structure, physical and chemical properties, multiple valance states [17]. In this concern, the metal-semiconductor transition in vanadium oxides exhibits good optical and electrical properties and widely used in sensor and switching application [18,19]. The crystal structure and morphology of the electrode materials are plays main role in electrochemical performance. Hitherto, various forms of vanadium oxides were reported such as VO, VO_2, V_2O_3, and V_2O_5. Among these, V_2O_5 is thermodynamically stable and shows a good electro catalytic and electrochromic properties [20]. Therefore, we have chosen the V_2O_5 for the fabrication of electrochemical sensor to detect the adenine.

In this study, we described the synthesis of V_2O_5 NFs and fabrication of the V_2O_5 NFs modified SPCE (screen printed carbon electrode). The modified electrode exhibits an admirable electro-oxidation of Adenine. Furthermore, the V_2O_5 NFs/SPCE reveals the good sensitivity, extensive linear range and lower limit of detection.

2. EXPERIMENTAL SECTION

2.1 Materials and Methods

Ammonium metavanadate ($NH_4.VO_3$), hydrogen peroxide (H_2O_2), polyvinyl pyrrolidine (PVP), ethanol (C_2H_5OH) and other reagents were collected from Sigma-Aldrich. All other chemicals

were used as AR (analytical grade). The 0.05 M PBS (phosphate buffer solution) was used to the entire electrochemical experiments and all the standard solutions were prepared by deionized (DI) water.

The crystal structure of as-prepared V_2O_5 NFs was studied by the powder X-ray diffraction (XRD) studies were carried out at room temperature on a X'Pert PRO; PANalytical. The average diameter of the crystal was calculated by the Debye-Scherrer formula, $D = k\lambda/\beta\cos\theta$. Hence, crystallite size is denoted by D, wavelength of the radiation is λ, full width of the half maximum (FWHM) is denoted as β and the diffraction angle is θ. The surface morphology of V_2O_5 NFs was predicted by Scanning Electron Microscopy (SEM; Hitachi S-3000 H) and X-ray energy-dispersive spectrometry (EDX; JEOL 2000). Electrochemical experiments were done in a three-electrode cell system, SPCE (Zensor Research& Development, Taiwan) used as a working electrode, saturated Ag/AgCl (saturated KCl) as a reference electrode and Platinum (Pt) wire as a counter electrode by using CH Instruments (CHI 1205b and CHI 900).

2.2 Preparation of V_2O_5 nanofibers

Vanadium pentoxide nanofibers (V_2O_5 NFs) were prepared by simple hydrothermal method. Initially, 0.06 g of $NH_4.VO_3$ was dissolved in 30 mL of deionized water which containing 4 mL of H_2O_2. Subsequently, 0.2 g of polyvinyl pyrrolidine (PVP) was added to the above solution with continuous stirring. Afterwards, stirred solutions were poured into 50 mL of stainless steel autoclave and then heated for 6 h at 150 °C. Lastly, the precipitates were centrifuged and washed with DI water, and C_2H_5OH then dried in a vacuum at 60°C.

2.3 Fabrication of V_2O_5 NFs/SPCE modified electrode

The V_2O_5 NFs were prepared aforementioned procedure and the gained precipitates (5 mg/mL) were dispersed in de-ionized water through ultrasonic agitation for 20 min. The surface of SPCE was pre-cleaned by voltammetric cycling in PBS (pH 5). Subsequently, the re-dispersed V_2O_5 NFs were drop casted (5 µL) on the pre-cleaned SPCE and it was dried in an ambient condition. Finally, the V_2O_5 NFs modified SPCE was used to additional electrochemical experiments.

3. RESULT AND DISCUSSION

3.1 Characterizations of V_2O_5 nanofibers

The crystallinity of the material was studied by XRD. As shown in Fig. 1A, the diffraction peaks were locating at 2θ = 20.24, 21.65, 26.12, 32.35, 33.41, 35.87, 41.14, 45.74, 47.28, and 52.42° corresponding to the planes of (001), (101), (110), (400), (011), (310), (002), (411), (600) and (020), respectively. The XRD results demonstrate that the V_2O_5 NFs are presented in orthorhombic structure (JCPDS card no. 89.0612). In addition, all peaks were in good agreement with previously reported works and the average crystalline size were calculated by Scherer's equation from XRD is to be 58.87

nm [21]. The morphology of the V_2O_5 NFs was characterized by SEM. Fig. 1B displays that the SEM image of V_2O_5 NFs, which exhibits the fiber like structure. In addition, the elemental composition of the as-prepared material was evaluated by EDX. The EDX spectrum of V_2O_5 NFs was shown in Fig. 1C where the strong signals obtained for vanadium, and oxygen. These results confirm that the existence of those elements in the prepared material. Inset figure shows the weight percentage of the vanadium (79.65%) and oxygen (20.35%) which suggest that the as prepared nanofibers were formed without any impurities. Furthermore, the elemental mapping of the V_2O_5 NFs is shown in Fig.1(D), 1(E) and 1(F). It can be seen that the V_2O_5 NFs was composed of V and O elements.

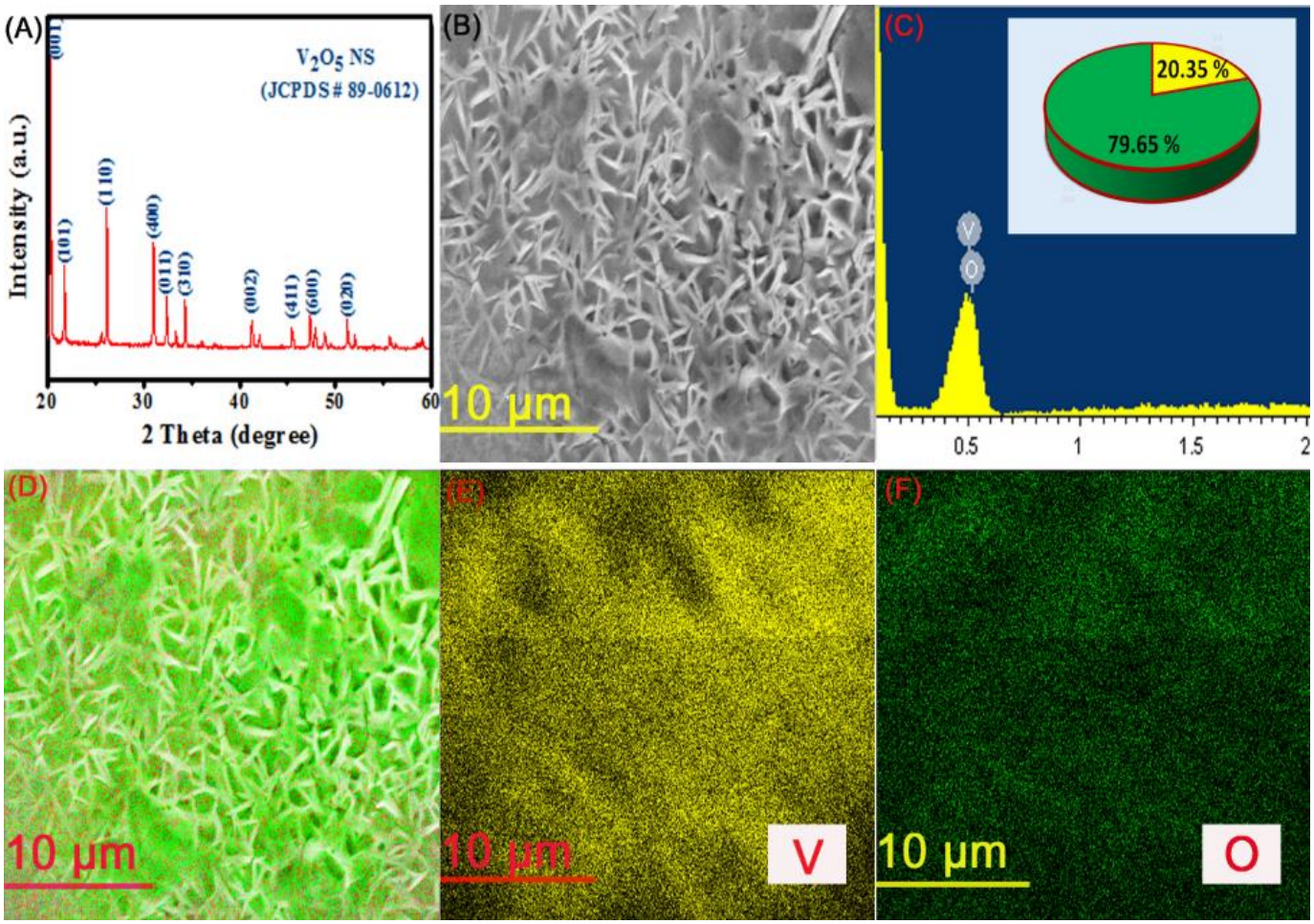


Figure 1. (A) XRD pattern, (B) SEM micrograph, (C) EDX spectrum and (D-F) EDX elemental mapping images of V_2O_5 NFs.

3.2 Electrocatalytic activity of oxidation of Adenine

The electrocatalytic activity of the V_2O_5 NFs was studied by cyclic voltammograms (CV) in 0.05 M PBS (pH 5) for the electro oxidation adenine. Fig. 2 presented the cyclic voltammograms (CVs) of unmodified-SPCE (A) then V_2O_5 NFs/SPCE (B) with 200µM of adenine at the scan rate 50 mV s^{-1}. In this study clearly exposes, the unmodified SPCE exhibited the poor electrocatalytic performance for the detection of adenine. Remarkably, an improved and irreversible anodic peak

current response was observed at V_2O_5-NFs/SPCE for the electro oxidation of adenine. These results clearly reveal that the higher electrocatalytic activity of the V_2O_5-NFs/SPCE. Moreover, the electro oxidation of adenine is an irreversible two protons and electrons involved process [22].

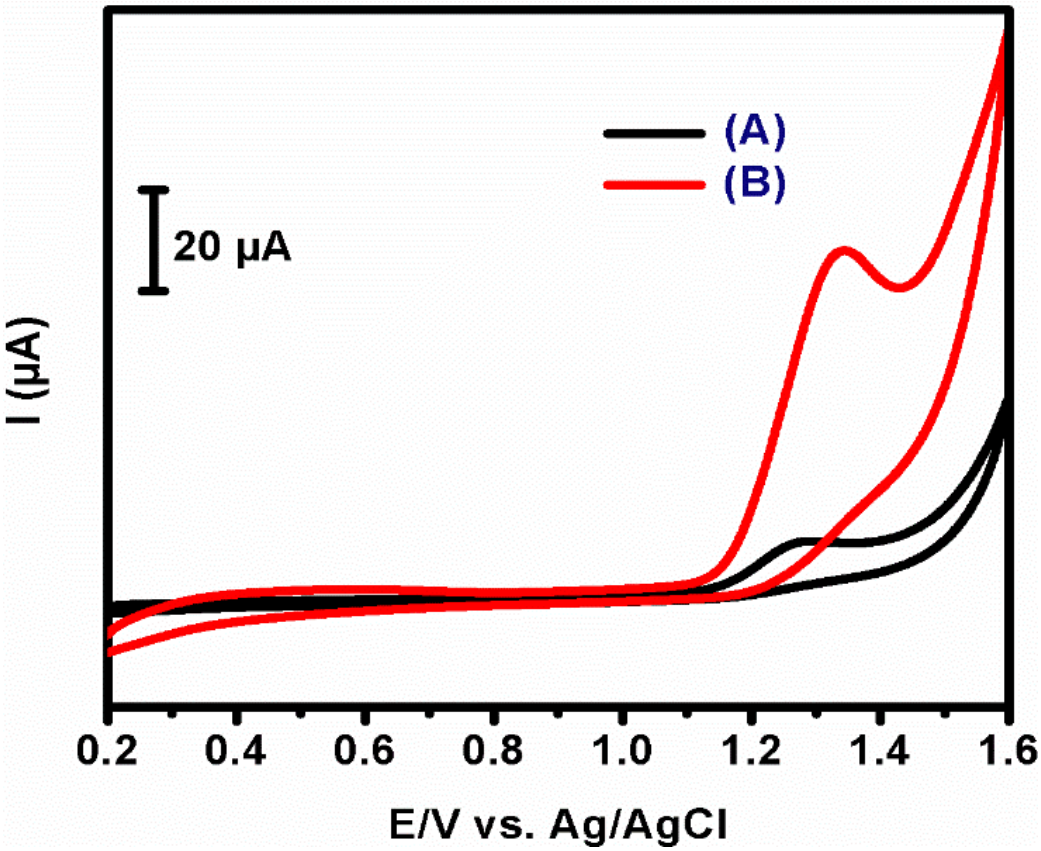


Figure 2. CV response obtainted at (A) unmodified SPCE and (B) V_2O_5- NFs /SPCE in N_2 saturated 200 µM Adenine in PBS (pH 5) at the scanning rate of 50 mV s^{-1}.

3.3 Influence of Concentration

In order to evaluate the electrocatalytic performance of the V_2O_5- NFs /SPCE, different concentration additions were performed. Fig. 3A shows the CV curves for the various additions of Adenine from 50 to 300 µM in PBS (pH 5) at the scanning rate of 50 mV s^{-1}. As can be seen, the anodic peak current (I_{pa}) response was increased with respect to different additions of adenine. Moreover, the obtained peak current values are plotted against the different concentrations of Adenine (Fig. 3B). Whereas, the I_{pa} values are linear relationship by the different concentrations of Adenine with correlation co-efficient of R^2 = 0.9963. These results are suggested that the V_2O_5 NFs/SPCE is good material for the electrochemical determination of Adenine.

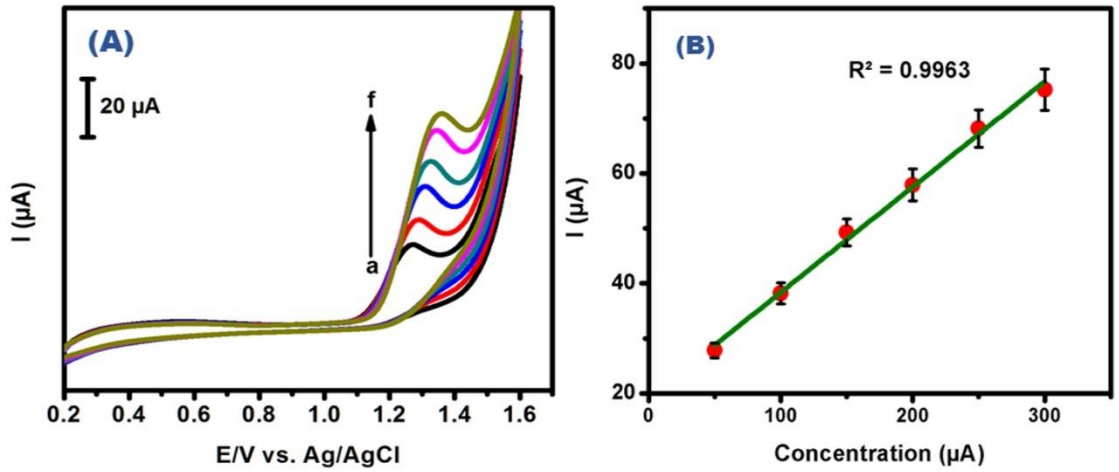


Figure 3. CVs obtained of (A) V_2O_5 NFs/SPCE various Adenine concentrations (a to f = 50 to 300 µM Adenine) and (B) linear plot of anodic peak current versus the Adenine concentrations with the 50 mV s^{-1} scanning rate in N_2 saturated PBS.

3.4 Influence of Scan rate

So as to study the influence of scan rate was verified by varying the scan rate of CV measurements, as demonstrated in Fig. 4A for V_2O_5 NFs/SPCE in N_2-saturated PBS with 200 µM Adenine. It can be seen that the I_{pa} responses were increased while increasing the scan rates from 20-200 mV s^{-1}. The circumstances that the I_{pa} shows a linear dependence with the scan rate 20-200 mV s^{-1}. Moreover, the obtained peak current values are plotted against the square root of the scan rates. As given in Fig. 4B, I_{pa} values had a direct relationship with square root of the scan rates with a regression coefficient R^2 = 0.9972. These results suggested that the electro oxidation of adenine at V_2O_5 NFs/SPCE is diffusion-controlled process [13].

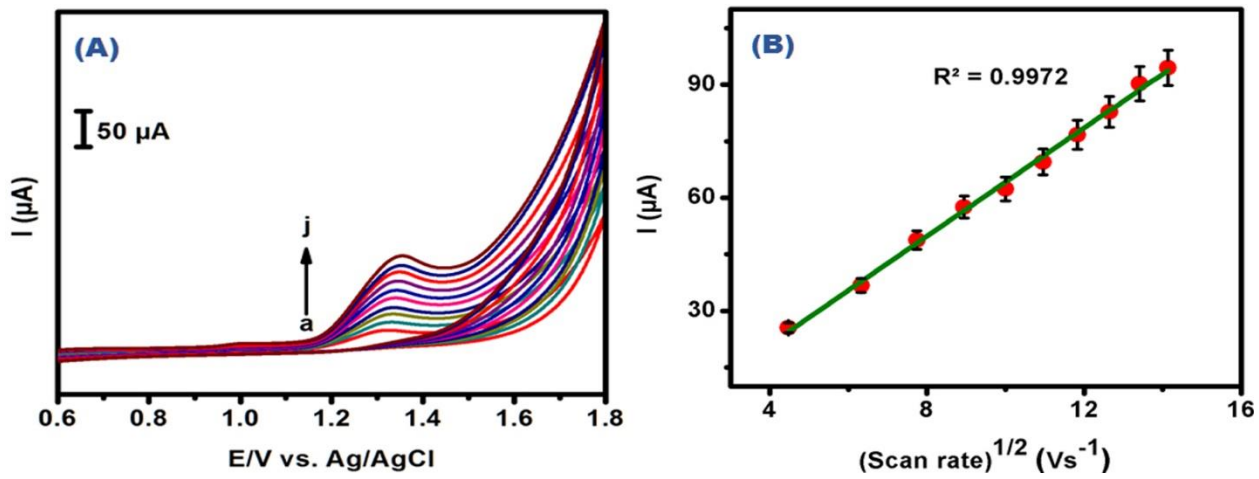


Figure 4. CVs obtained of (A) V_2O_5 NFs/SPCE various scan rates (from 20 to 200 mV s^{-1}) with 200 µM Adenine and (B) linear plot between anodic peak current versus the square root of the scan rates with N_2 saturated PBS.

3.5 Influence of pH

Generally, in the electrocatalytic experiments the pH study is very the extensive due to when the peak current and peak potential will be changed in change in the pH solutions. In order to the influence and dependence of electrolyte pH towards the electro-oxidation of Adenine at V_2O_5 NFs/SPCE was investigated by CV. This experiment was carried out in different pH values from pH 3 to 11 solutions containing 200 µM of Adenine. Fig. 5A exhibited the CV curves for the oxidation of adenine in distinctive pH values. Upon increasing the pH of the PBS, the peak potential was shifted to negative side which exposes that the protons were involved in the electro-oxidation process of Adenine. The Adenine I_{pa} response was increased when increase the pH (3-5). Afterwards, the I_{pa} response was decrease the (7 to 9). As shown Fig. 5B, maximum I_{pa} was observed at pH-5, thus we have chosen the pH 5 for the whole electrochemical experiments. In addition, the oxidation peak potential had a direct relationship with pH 3 to 11 (Fig. 5C). To the conclude, the detection of Adenine at the V2O5 NFs modified SPCE is a fully pH dependent reaction [23]. From this linear plot, the obtained slope value is 55 mV/pH. According to the Nernst equation, the obtained slope value (55 mV/pH) is equal to the theoretical value (59 mV/pH), which clearly explain that the identical number of protons and electrons were involved in the electrocatalytic oxidation of Adenine [24-28]. Furthermore, the plausible oxidation mechanism of adenine was illustrated in Scheme 1.

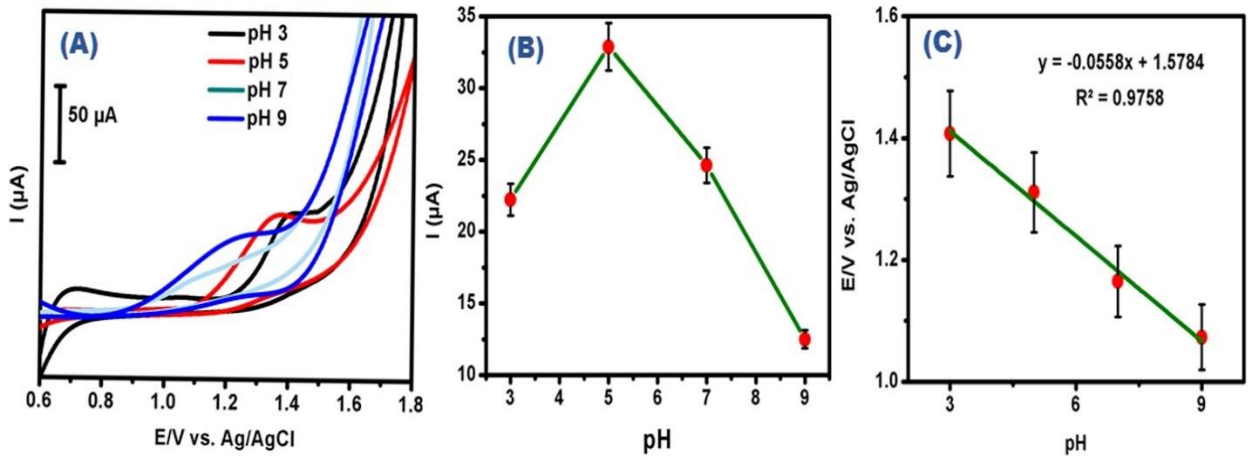
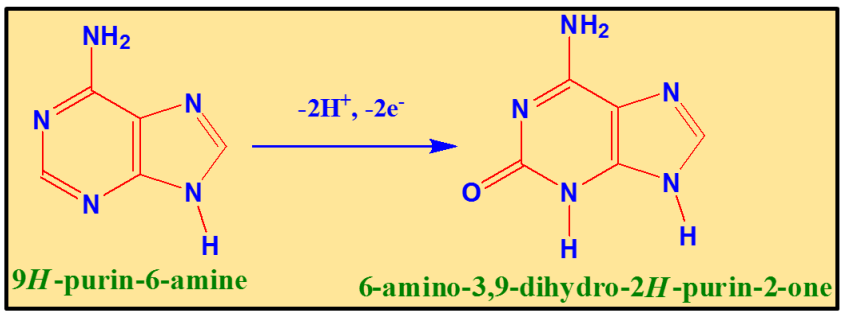


Figure 5. CVs obtained of (A) V_2O_5 NFs/SPCE of 200 µM Adenine with different pH (3 – 9) (B) Plot between I(µA) vs. pH and (B) E/V vs. Ag/AgCl (peak potential) vs. pH with 200 µM Adenine.



Scheme 1. Electrochemical oxidation mechanism of Adenine.

3.6 Accumulation time, reproducibility and stability

Fig. 6(A) reveals that the anodic peak current of Adenine was distinguished for the assorted accumulation time (from 0 to 210 s). The anodic peak current response increases with increase the accumulation time. The highest anodic peak current was noted at 150 s. Hence, the enhanced accumulation time of 150 s was accepted for the operative oxidation of Adenine. To estimate the reproducibility of the electrochemical sensor, five different electrodes were verified towards the electro oxidation of 200 µM Adenine in PBS (pH 5) at 50 mV s^{-1} scanning rate. In this result, V_2O_5 NFs/SPCE having a good reproducible capacity and it should be used to detect Adenine was shown in Fig. 6B. The CV demonstrates that the 50 consecutive cycles of the V_2O_5 NFs/SPCE without and with Adenine in 200 µM was exposed in Fig. 6C and 6D. In these result, the fabricated electrode having the excellent operational stability of the planned sensor and highly applicable for the real sample analysis.

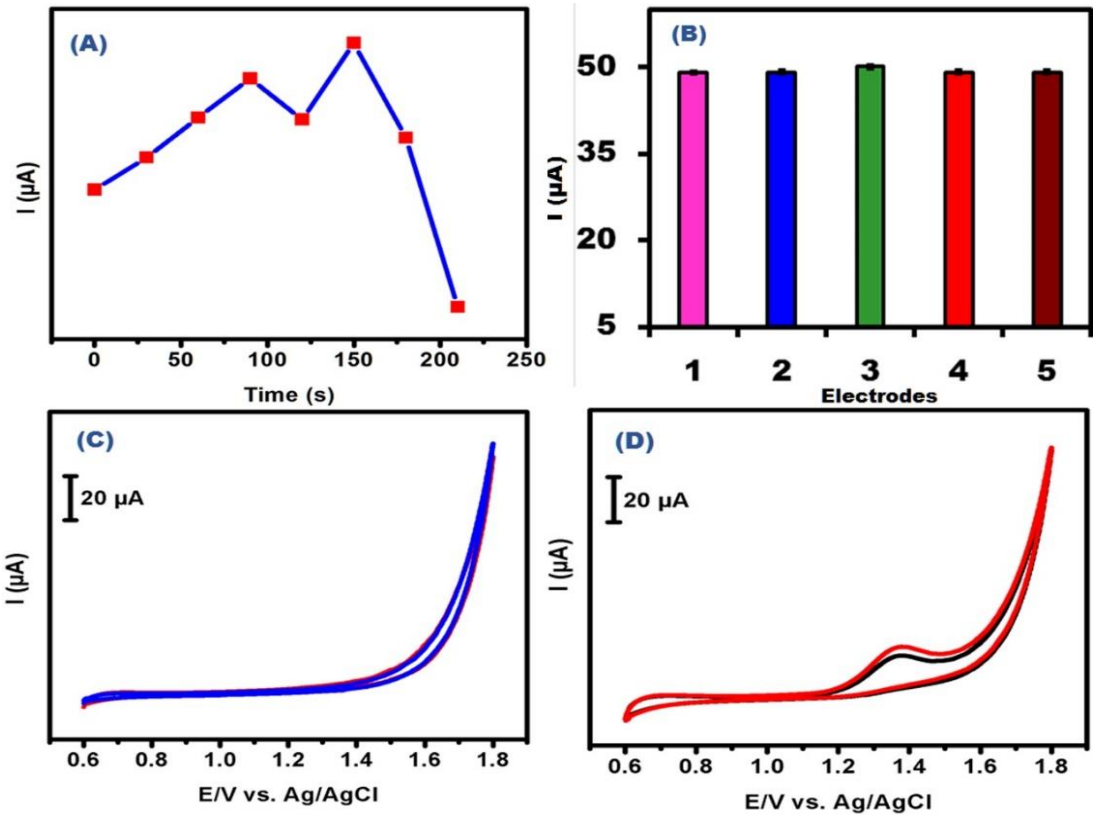


Figure 6. CV responses of (A) accumulation time (B) reproducibility (C) operational stability of 50 consecutive cycles without and (D) with Adenine of V_2O_5 NFs /SPCE at the 50 mV s^{-1} scanning rate in N_2 saturated PBS.

3.7 Sensitivity of Adenine

So as to calculate the sensitivity of the as-proposed Adenine sensor, the DPV (differential pulse voltammetry) technique was used. In Fig. 7A reveals that the DPV replies for the oxidation of Adenine at various Adenine concentrations through the under excellent experimental conditions into PBS (pH 5). The anodic peak current of Adenine was equivalent to the concentrations of Adenine. Furthermore,

the oxidation peak currents were increased linearly while increasing the Adenine concentrations as exposed in Fig. 7A. The linear regression equation y = 0.1792x + 17.04 with a correlation coefficient R^2 = 0.9983 is shown in Fig. 7B, where x is the concentration of Adenine. From the slope of the linear plot the sensitivity was calculated. The calculated linear range, sensitivity and limit of detection were 0.5 to 512 µM, 8.5333 µA μM^{-1} cm^{-2} and 0.013 µM respectively. The sensitivity can be attained by the high charge transfer of the V_2O_5 NFs/SPCE and due to its catalytic property, the electron-transfer will be effectively. The analytical studies of the V_2O_5 NFs/SPCE was compared with previously reported Adenine sensors and demonstrated in Table 1.

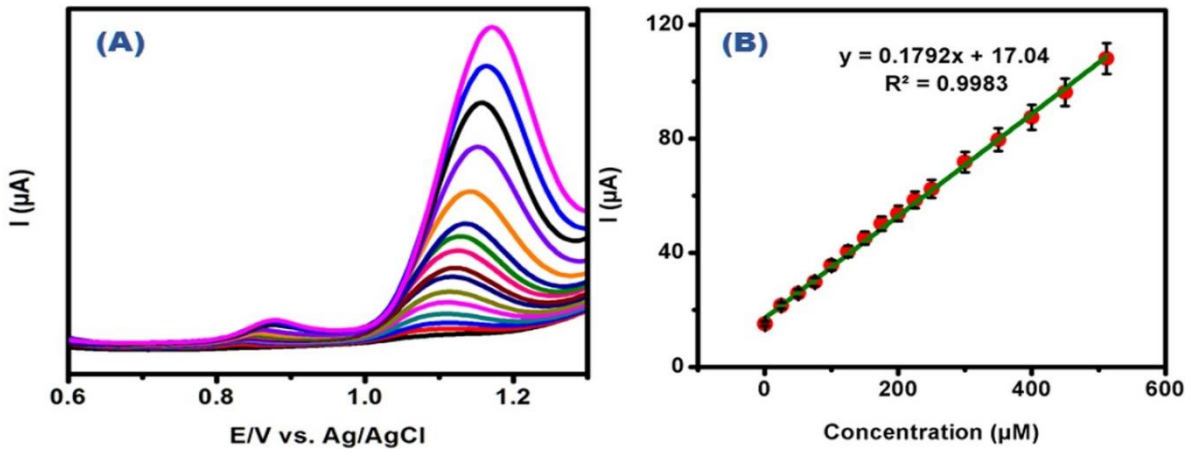


Figure 7. (A) DPVs of V_2O_5 NFs/SPCE towards increasing concentration of Adenine in PBS (pH 5) (B) Calibration curve of the peak current on the Adenine Concentrations.

Table 1. Comparison for the analytical performance of Adenine oxidation of previously reported sensors.

Electrode modifier	**Linear range (µM)**	Limit of detection (LOD) /µM	**Ref.**
GCE modified PANI/MnO_2	10 - 100	2.9	[29]
GCE modified TiO_2/GO	0.5 - 200	0.10	[30]
GCE modified CeO_2/MWCNT	5 - 35	0.02	[31]
GCE modified NF/CHT-ARGO	0.2 - 110	0.2	[32]
SPCE modified V_2O_5 NFs	0.5 - 512	0.013	This work

3.8 Real sample analysis

Furthermore, we examined the practical probability of the fabricated electrode towards the determination of Adenine in spiked human urine sample. Around, 1 mL of urine sample was diluted with 25 mL PBS (pH 5) and known concentrations of Adenine were spiked into the solution. DPV

experiments were performed using this solution and the V_2O_5 NFs/SPCE distributes sensitive and fast signals. The recovery data for each addition were obtained and the results are shown in Table 2. The obtained recovery results are quite sufficient and thus the modified electrode has greater potential in real time analysis of Adenine.

Table 2. Detection of Adenine at V_2O_5 NFs/SPCE in human urine sample.

Real sample	**Added/µM**	**Found/µM**	**Recovery/% (mean ± RSD) (n=3)**
Human urine sample	10	9.82	98.2±0.0173
	20	19.75	98.75±0.0125
	30	29.56	98.53±0.0157

4. CONCLUSION

In summary, a simple and sensitive method was developed based on V_2O_5 NFs/SPCE for the determination of Adenine. The V_2O_5 NFs was characterized by various physical characterizations such as XRD SEM, EDX and its electrocatalytic behavior was examined for the determination of Adenine. In this study, the electrode exhibited a greater linearity over the concentrations from 0.5 – 512 µM and the least limit of detection is 0.013 µM, the sensitivity is about 8.5333 µA μM^{-1} cm^{-2}. The modified electrode achieved good reproducibility and high stability towards the detection of Adenine. It was successfully revealed to detect Adenine by spiked method in human urine sample, which produces the recovery range from 98.2 -98.53 %.

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