

# Sol-Gel Derived Mn-doped TiO<sub>2</sub>-PVA nanobiocomposite for Urea Biosensor

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**Abstract:** 1% Mn-doped TiO<sub>2</sub> material has been synthesized by sol-gel citrate method. Nanostructured 1% Mn-doped TiO<sub>2</sub> (Nano-tio<sub>2</sub>)-poly vinyl alcohol (PVA) film has been fabricated on gold substrate to immobilized Urease (Urs) via physisorption method for urea biosensor. The nanobiocomposite film have been characterized by X-Ray diffraction (XRD), scanning electron microscopy (SEM), Cyclic Voltametry (CV) and electrochemical impedance spectroscopy (EIS).The fabricated urease immobilized on the material shows excellent performance of biosensor exhibits direct electron transfer between redox enzyme and the surface electrode, good stability and sensitivity which indicated porous 1% Mn doped TiO<sub>2</sub> material is attractive material for use in fabrication of biosensor, particularly enzymatic sensor.

**Keyword:** Biosensor, Urea, Nanostructured TiO<sub>2</sub>, Urease, Nanobiocomposite

## Introduction

Recent years have seen increased interest towards the development of biosensing devices. Application of nanoscale materials for electrochemical biosensors has been grown exponentially due to high sensitivity and fast response time [1-2]. Electrochemical synthesis of titanium oxide nanotubes (TiO<sub>2</sub>) in different type of electrolyte and application for biosensors application has showed great interest [3]. As sensing platform, Mn-doped TiO<sub>2</sub> nano- arrayed structured has much higher surface area to volume ratio, strength and better electron transfer characteristics than ordinary TiO<sub>2</sub> base electrode formed by surface coating process. To demonstrate usefulness of this material as biosensor platform [4]. Titanium oxide (TiO<sub>2</sub>) is frequently used as matrix to immobilized protein and enzyme for biomaterial and biosensor applications due to its chemical inertness, rigidity, and thermal stability [5-7]. Various wet chemical methods have been reported for the preparation of nano-sized TiO<sub>2</sub>. Sol-gel route has been regarded as an excellent method to synthesized nano-sized metallic Oxides [8]. Since biosensor allow a wide range of transduction technology so they have advantage over ordinary electrochemical sensor. Among them, Urea biosensor has widely been distributed in nature, and its analysis is of considerable interest in clinical and agricultural chemistry

[9-10]. The increased demand for clinical diagnostics relating to kidney and liver diseases has necessitated evolution of new methods for faster and accurate estimation of urea of desired samples including urine and blood samples. Increased urea concentration (normal level of serum is 8-20mg/dL) causes renal failure (acute or chronic), Urinary tract obstruction and dehydration. Moreover, decreased urea concentration causes hepatic failure, nephritic syndrome, Catexia (low protein and high carbohydrates diets) [11-13].

## 2. Materials and methods

**2.1. Material :** Urease (Urs), poly Vinyl alcohol (PVA), Manganese Oxide, titaniumn-butaoxide Ti(O-Bu)<sub>4</sub>, citric acid, ethanol, Na<sub>2</sub>HPO<sub>4</sub>, NaH<sub>2</sub>PO<sub>4</sub>, potassium ferricyanide [Fe(CN)<sub>6</sub>]<sup>3-/4-</sup> potassium Chloride Phosphate buffer (ph.7.0), gold substrate. All chemicals and solvents were of analytical grade. The deionized water has been used for preparation of reagents. All solution and glass wares are autoclaved prior to being used. All electrochemical experiments were carried out at room temperature.

### 2.2. Preparation of PVA-Mn doped TiO<sub>2</sub> hybrid nanobiocomposite

1% Mn-doped TiO<sub>2</sub> nanoparticles (nm) prepared by sol-gel citrate method are dispersed into 10ml of PVA (0.5mg/dL) solution in phosphate buffer of 0.05M at Ph-7.0 by under continuous stirring at room temperature. Viscous solution of PVA with uniformly dispersing 10ul of solution PVA-Mn doped TiO<sub>2</sub> composite on gold substrate by physical adsorption method and allow it to dry at room temperature for 12h. These solutions cast PVA-Mn doped TiO<sub>2</sub> hybrid nanobiocomposite films were washed repeatedly with deionized water to remove any unbound particles.

### 2.4. Immobilization of Urs onto PVA-Mn doped TiO<sub>2</sub> hybrid nanobiocomposite film

Urease enzyme used for immobilization. 10ul of enzyme solution containing Ur (10mg/ml) prepared in PBS (5mM) was immobilized onto PVA-Mn doped TiO<sub>2</sub> nanobiocomposite/gold electrode. The Urs/PVA-Mn doped TiO<sub>2</sub> nanobiocomposite/gold electrode was allowed to dry overnight under desiccated condition and finally we get Urs/PVA-Mn doped TiO<sub>2</sub> nanobiocomposite/gold electrode. This dry electrode is immersed in 50mM PBS (Ph-7.0) in order to wash out any unbound enzymes from electrode surface and stored in desiccators at 25°C when not in use.

### 3. Result and Discussion:

#### 3.1. Characterization of TiO<sub>2</sub> nanoparticles

Fig.1 [A] shows X-Ray diffraction (XRD) pattern of synthesized Mn doped TiO<sub>2</sub> nanoparticles reveals reflection planes that are consistent with Mn doped TiO<sub>2</sub> with their characteristics peak of 2θ value corresponding planes 25.41, 27.60, 37.80, 39.24, 44.13, 48.13, 54.46, 56.79. These peaks were assigned to reflections from (101A), (110R), (004A), (210R), (200A), (211R), (220R), crystal planes, which corresponds to both anatase and rutile phase of TiO<sub>2</sub> and notation 'A' and 'R' were associated with anatase and rutile. The crystalline size of TiO<sub>2</sub> calculated using Debye Scherer's formula,  $(D = \frac{K\lambda}{\beta \cos\theta})$  Where, D is crystalline size, K is scherrer constant usually taken as 0.89, λ is wavelength of X-Ray radiation (0.15418nm) for Cu-Kα, β is full width half maximum of diffraction peak measured at 2θ. The average particle size was 42 nm.

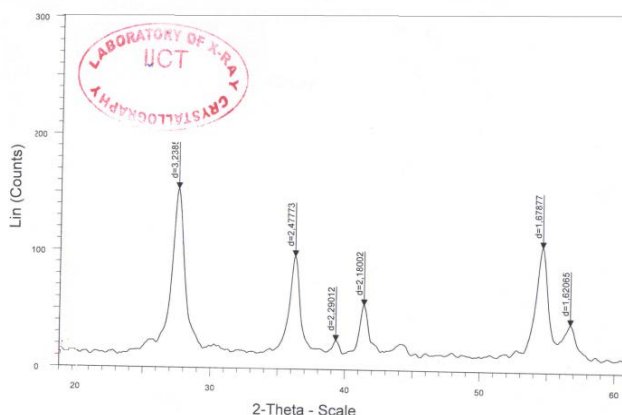


Fig.1.XRD Pattern of 1% Mn doped TiO<sub>2</sub>

#### 3.2. Characterization of PVA-Mn doped TiO<sub>2</sub> and Urs--PVA -nanobiocomposite Mn -doped TiO<sub>2</sub> gold electrode

##### 3.2.1. Optical properties

The surface morphologies of PVA-Mn doped TiO<sub>2</sub> nanobiocomposite/gold electrode and Ur/ PVA-Mn doped TiO<sub>2</sub> nanobiocomposite/gold electrode have been investigated using scanning electron microscopy (SEM, Fig.2. [A], [B]). The large surface area and porous nature of Mn-doped TiO<sub>2</sub> nanoparticles offers a good platform for anchoring urease enzyme. A SEM study reveals the globular porous PVA network which shows Mn-doped TiO<sub>2</sub> nanoparticles were embedded in the porous PVA. This may be attributed to electrostatic interaction between PVA and surface charged Mn doped TiO<sub>2</sub> nanoparticles. SEM studies show coating of nanoparticles onto PVA/gold electrode and there was perfect immobilization of Urease onto PVA-Nano Mn doped TiO<sub>2</sub>/gold electrode.

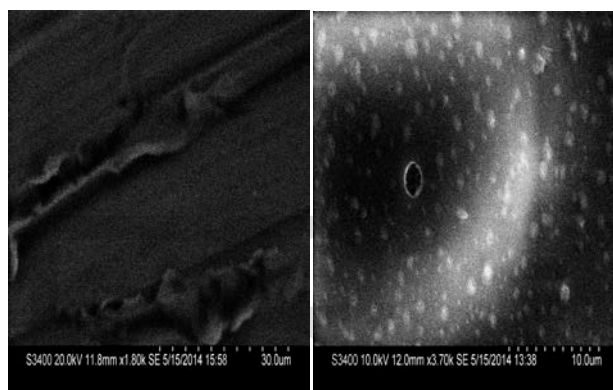


Fig.3.Scanning electron microscopy of (A) PVA-Mn doped TiO<sub>2</sub>/gold electrode (B) Urs/PVA- Mn doped TiO<sub>2</sub>/gold electrode.

##### 3.2.2. Electrochemical Study

The changes of electrode behavior after surface modification with enzyme Urs were studied by cyclic voltametry (CV) in presence of ferricyanide mediator. When electrode surface is modified by the biocatalytic material, the change in electron transfer kinetics of [Fe (CN)<sub>6</sub>]<sup>3-/4-</sup> gives indication of enzyme attachment. Fig.3. [A] Shows Cyclic voltammograms for (a) bare gold electrode (b) Mn doped TiO<sub>2</sub>-PVA/gold electrode (c) Urs/ Mn doped TiO<sub>2</sub>-PVA/gold electrode in PBS [50mM, Ph.7.0, 0.9% NaCl] containing 5mM [Fe(CN)<sub>6</sub>]<sup>3-/4-</sup> at scan rate of 50mV/s. Bielectrode increases with peak to peak separation as shown in figure.3. [A], a well-defined redox coupled of [Fe (CN)<sub>6</sub>]<sup>3-/4-</sup> is observed on the bare gold electrode (Curve a). When the Mn doped TiO<sub>2</sub>-PVA gold nanocomposite layer is deposited on gold electrode, the peak current gradually decreased (Curve b). The decreased in the peak potential after deposition of Mn-doped TiO<sub>2</sub>-PVA may be attributed to the hindrance of electron flows as a result of reduction in

electrical conductivity of nanostructured Mn-doped TiO<sub>2</sub>-PVA/gold separation between cathodic and anodic waves of redox probe.

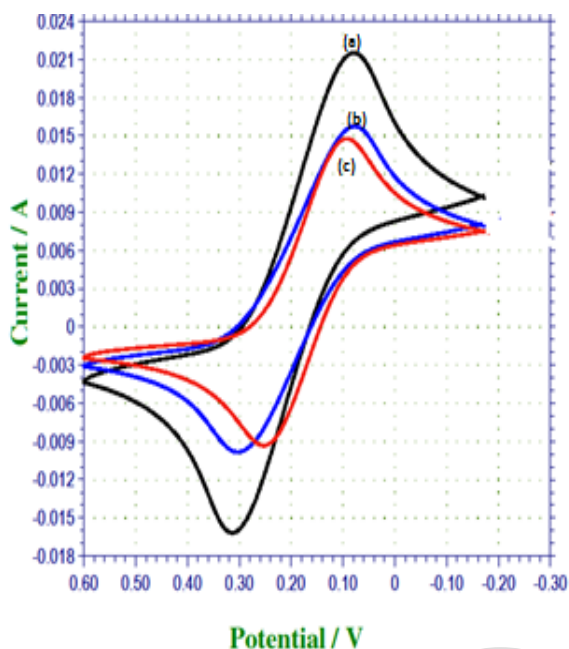


Fig.3. Cyclic Voltammetry of (a) Gold Substrate (b) PVA-Mn doped TiO<sub>2</sub> (c) Urs-PVA-Mn doped TiO<sub>2</sub>

Electrochemical impedance spectroscopy (EIS) is an effective method of probing the features of surface-modified electrode, which can provide useful information about the impedance changes of electrode surface during the fabrication process [14]. Impedance spectroscopy is an effective means probing the features of surface modified electrode. The curve of EIS includes a semicircular part and linear part. Fig presents the representative impedance spectrum of the a) gold electrode b) PVA-Mn doped TiO<sub>2</sub>/gold electrode c) Urs/PVA-Mn doped Mn doped TiO<sub>2</sub>/gold electrode in 10Mm PBS solution, ph7.0 containing [Fe(CN)<sub>6</sub>]<sup>3-</sup> (5Mm). The semicircular part of higher frequencies correspondence to electron transfer limited process, which controls the electron transfer kinetics of redox probe at electrode interface. Meanwhile, the linear part at lower frequencies corresponds to the diffusion process [15]. It can be seen that electrode exhibits lower conductivity than the Mn doped TiO<sub>2</sub> -PVA electrode and Urs/ TiO<sub>2</sub>-PVA bielectrode. It indicates the impedance of the electrode increases in presence of Mn doped TiO<sub>2</sub>-PVA electrode and Urs/ TiO<sub>2</sub>-PVA bielectrode which obstructed the electron transfer of [Fe(CN)<sub>6</sub>]<sup>3-/4</sup>. These results indicate building of Urs onto TiO<sub>2</sub> electrode. This suggests that immobilized Urs molecules strongly bind with hybrid nanobiocomposite film these were shown in fig.4. The

conductivity of the samples was measured by an AC complex impedance method, and the applied frequency range of AC is from 42Hz to 5 MHz Fig also indicate that the, conductivity values are greater at the lower frequency compare to the higher frequency.

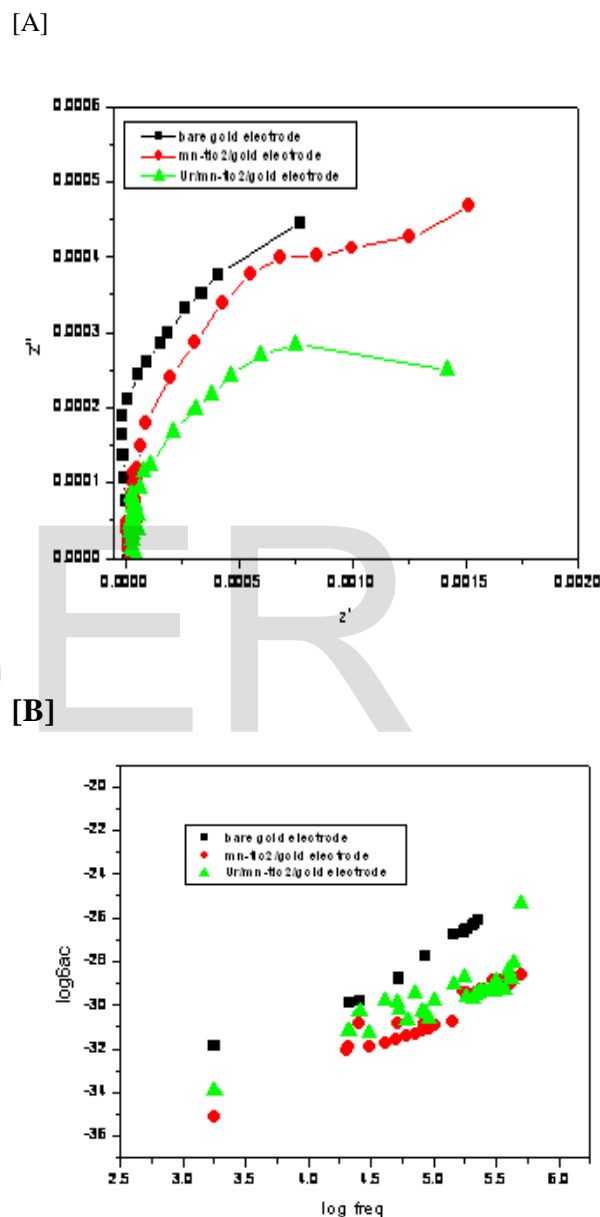


Fig.4. EIS Study of a) gold electrode b)PVA-Mn doped TiO<sub>2</sub>/gold electrode c) Urs /PVA-Mn doped TiO<sub>2</sub>/gold bielectrode.

#### 4. Conclusion

This work has developed a simple and effective method to fabricate a slack and porous nanostructured 1% Mn-doped TiO<sub>2</sub> with large specific surface. In this work, we prepared a novel gold bielectrode that show better electrochemical properties. Lower detection limit of 2mg/Dl response time 1s and sensitivity indicates enhanced enzyme affinity of Urs to urea. This is attributed to the good electrochemistry behavior of the nanocomposite as well as smaller geometry of the sensor.

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