

# Effect of Temperature on Redox Potential of Au-PVA- $\text{Bi}_2\text{FeNiO}_6$ and Au-PVA- $\text{Bi}_2\text{FeNiO}_6\text{Ur}$ Electrodes

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**Abstract:** Nano crystalline  $\text{Bi}_2\text{FeO}_6$  was synthesized by using sol-gel method. The synthesized  $\text{Bi}_2\text{FeO}_6$  nanocomposite is structurally characterized by X-ray diffraction (XRD) and SEM. From the data it is found that  $\text{Bi}_2\text{FeO}_6$  having globular morphology with crystallite size 40nm.  $\text{Bi}_2\text{FeO}_6$  nanocomposite is used for preparation of Au-PVA- $\text{Bi}_2\text{FeNiO}_6$ -Urbioelectrodes. Electroactivity of the bio electrode is checked with the help of cyclic voltammetry (CV) and impedance analysis. From the CV data, it is observed that, the redox potential for  $\text{Bi}_2\text{FeO}_6$  is increases with increasing calcination temperature upto 550°C and on further heating its potential decreases.

**Keywords:** XRD, SEM, Redox potential, Electroactivity and Bioelectrode.

## Introduction

Novel electro catalytic behavior of nanostructured metal oxide, which combines characteristics of electrochemistry (e.g., simplicity, fast, high sensitivity & selectivity and low detection limit) [1]. Metal oxide nanoparticles exhibit higher ratios of surface area to volume than their bulk counterparts, so metal oxide nanoparticle modified electrochemical interfaces will provide larger electrochemically active areas and therefore probably lead to higher detection sensitivity for target molecules. Magnetic nanoparticles are promising materials in fabrication of biosensors and bioreactors. Biosensors can be fabricated by using the biological entity [2-5].

In the last decade, increased investigations with several types of iron oxides have been carried out in the field of magnetic nanoparticles, among which magnetite and maghemite is the very promising and popular candidates since its biocompatibility have already proven, however, it is a technological challenge to control size, shape, stability, and dispersibility of nanoparticles in desired solvents. Magnetic iron oxide nanoparticles have a large surface-to-volume ratio and therefore possess high surface energies. Consequently, they tend to aggregate so as to minimize the surface energies. Moreover, the naked iron oxide NPs have high chemical activity, and are easily oxidized in air (especially magnetite), generally resulting in loss of magnetism and dispersibility.[6]

Here we made an attempt to synthesize nickel doped  $\text{Bi}_2\text{FeO}_6$  nanocomposite for biosensor application. Synthesized nanocomposites are structurally characterized by X-Ray diffraction and Scanning electron microscopy. Its electroactivity is checked with the help of EIS and Cyclic voltammetry.

## Experimental

### Synthesis of $\text{Bi}_2\text{FeNiO}_6$ Nanocomposite

Nanocrystalline  $\text{Bi}_2\text{FeO}_6$  powder was synthesized by using sol-gel citrate method. Bismuth nitrate, ferric nitrate were used as a raw materials. A stoichiometric mixture of nitrates was mixed with citric acid and ethylene glycol and stirred magnetically at 80°C for 3 hrs to obtain a homogenous mixture; the solution was further heated in a pressure vessel at about 130°C for 12 hrs and subsequently kept at 350°C for 3 hrs. In order to improve the sensitivity and selectivity, nickel nitrate was added in the reaction mixture in the proper ratio. The dried powder was then calcined at various temperature in the range from 450-650°C in order to improve the crystallinity of the powder. Conductivity data suggest that the sample calcined at 550°C is serves good material for biosensor fabrication.

### Preparation of PVA- $\text{Bi}_2\text{FeNiO}_6$ hybrid Nanobio composite

$\text{Bi}_2\text{FeNiO}_6$  nanoparticles calcined at 550°C prepared using sol-gel citrate method is dispersed into 10 mL of PVA (Poly vinyl alcohol) (0.5 mg/mL) solution in distilled water under continuous stirring at room temperature. Finally, viscous solution of PVA with uniformly dispersed  $\text{Bi}_2\text{FeNiO}_6$  nanoparticles is obtained. PVA- $\text{Bi}_2\text{FeNiO}_6$  hybrid Nanobio composite films have been fabricated by uniformly dispersing solution of PVA- $\text{Bi}_2\text{FeNiO}_6$  composite onto a gold surface and allowing it to dry at room temperature for 12 hrs. These solution cast PVA-  $\text{Bi}_2\text{FeNiO}_6$  hybrid Nanobio composite films is washed repeatedly with deionized water to remove any unbound particles.

### Immobilization of Urease onto PVA- $\text{Bi}_2\text{FeNiO}_6$ hybrid Nanobio composite film

10  $\mu\text{L}$  of bio enzyme solution containing urease (10 mg/ml) [prepared in phosphate buffer (0.1 mol/l) pH-7] is immobilized onto PVA- $\text{Bi}_2\text{FeNiO}_6$  Nanobio composite/gold electrode. The peroxidase immobilized PVA- $\text{Bi}_2\text{FeNiO}_6$  nanobiocomposite/gold bio electrodes are kept undisturbed for about 12 h at 4°C. Finally, the dry bioelectrode is immersed in 50 mM PBS (pH 7.0) in order to wash out any unbound enzymes from the electrode surface. Fabrication of Au-PVA- $\text{Bi}_2\text{FeNiO}_6$ -Ur bioelectrode is shown in the form of flow chart in figure1

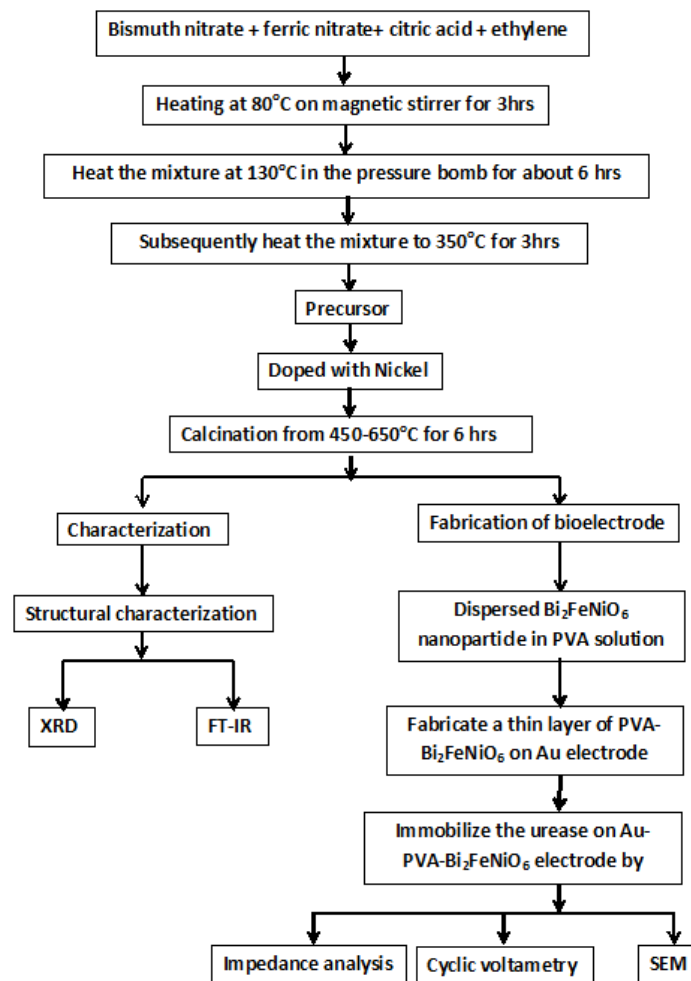


Figure 1: flow chart for the fabrication of Au-PVA-Bi<sub>2</sub>FeNiO<sub>6</sub>-Ur bioelectrode.

### Characterization

The structure and particle size of Bi<sub>2</sub>FeNiO<sub>6</sub> nanoparticles have been investigated using X-ray diffraction (XRD) studies. The surface morphological studies of nanobioelectrode before and after the immobilization of enzyme have been investigated using scanning electron microscopy. The cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS) recorded for the nanobioelectrode for the confirmation of immobilization. The electrochemical measurements have been conducted on a three-electrode cell in phosphate buffer (50 mM, pH 7.0, 0.9 % NaCl containing 50 mM [Fe (CN)<sub>6</sub>]<sup>3-/4-</sup> electrolyte.

### Result and discussion

#### Structural characterization

#### X-ray diffraction

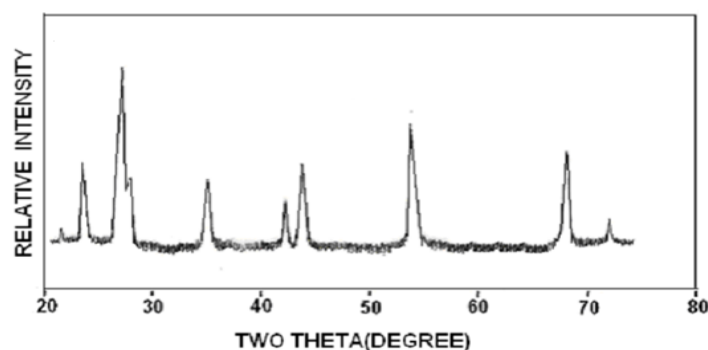
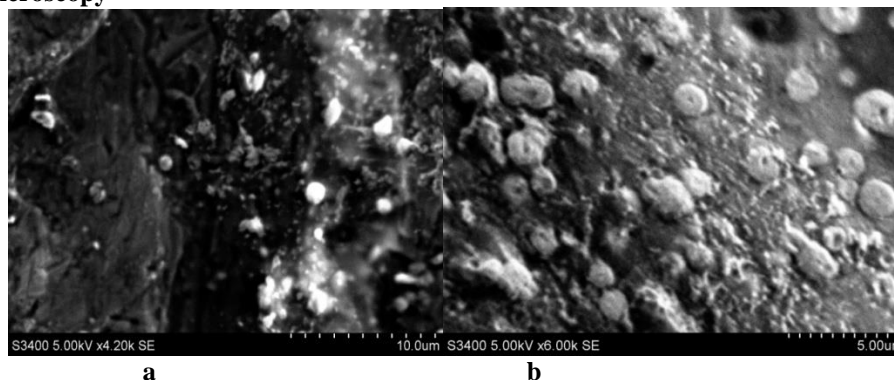


Fig 2: XRD pattern for the Bi<sub>2</sub>FeNiO<sub>6</sub> Nanocomposite

Fig2 illustrates a typical XRD spectrum of Bi<sub>2</sub>FeNiO<sub>6</sub> nanoparticles prepared by sol gel citrate method. The synthesized material was rhombohedral Bi<sub>2</sub>FeNiO<sub>6</sub>. Three major diffraction peaks were seen which can be assigned to diffraction from (001), (002) and (003) planes. This revealed that the resultant nanoparticles were Bi<sub>2</sub>FeNiO<sub>6</sub>. The average crystallite size of these samples is found to be 40 nm.

## Scanning Electron Microscopy



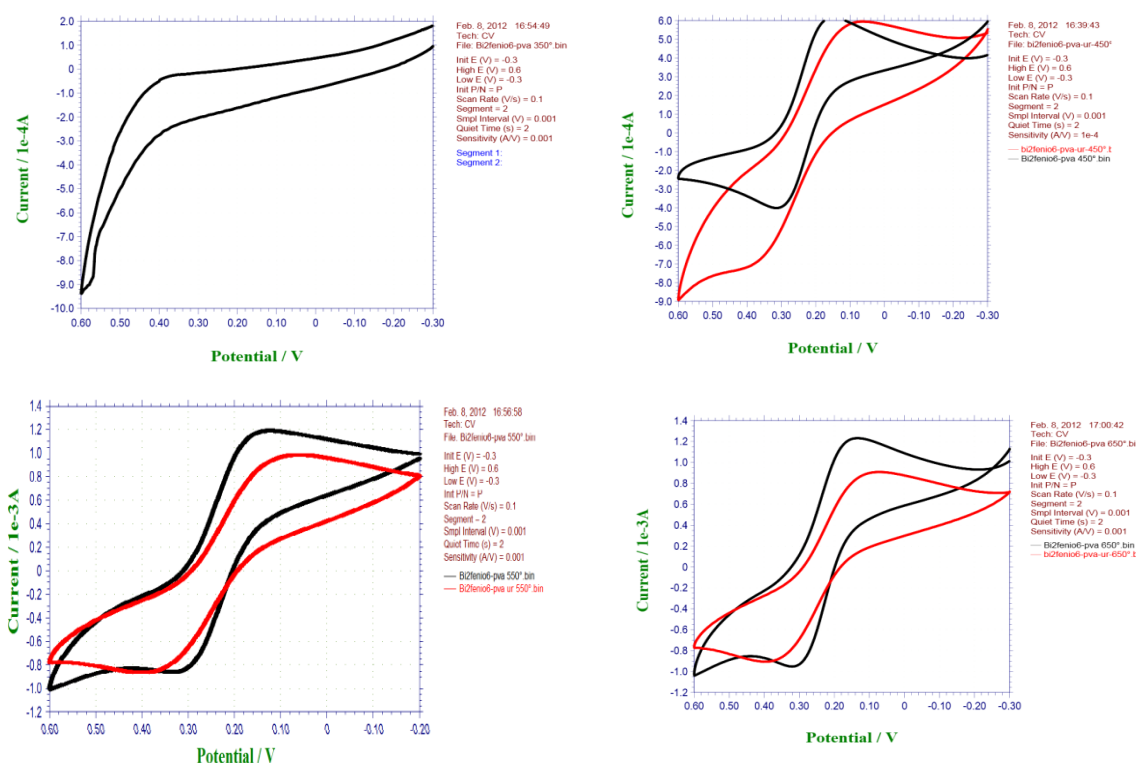
**Fig 3: SEM images of (a) PVA-  $\text{Bi}_2\text{FeNiO}_6$  Nanobio composite/gold electrode (b) urease immobilized PVA- $\text{Bi}_2\text{FeNiO}_6$  Nanobio composite/gold bio electrode**

The surface morphologies of Au-PVA- $\text{Bi}_2\text{FeNiO}_6$  electrode and urease immobilized Au-PVA- $\text{Bi}_2\text{FeNiO}_6$ -Urbio electrode have been investigated using scanning electron microscopy (SEM). SEM images (Fig.3a) shows the globular morphology of PVA- $\text{Bi}_2\text{FeNiO}_6$  Nanobio composite reveals incorporation of the  $\text{Bi}_2\text{FeNiO}_6$  nanoparticles in PVA, indicating the formation of PVA- $\text{Bi}_2\text{FeNiO}_6$  hybrid Nanobio composite. This may be attributed to electrostatic interactions between PVA and the surface charged  $\text{Bi}_2\text{FeNiO}_6$  nanoparticles.

However, after the immobilization of urease onto Au-PVA- $\text{Bi}_2\text{FeNiO}_6$  (fig 3b) electrode, the globular morphology changes to regular form. This suggests that  $\text{Bi}_2\text{FeNiO}_6$  nanoparticles provide a favourable environment for high loading of urease moieties. These results are also supported by electrochemical studies (CV).

## Electrical characterization

## Cyclic Voltammetry



**Fig 6.4. CVs of Au-PVA-  $\text{Bi}_2\text{FeNiO}_6$  & Au-PVA- $\text{Bi}_2\text{FeNiO}_6$ -Ur electrodes modified by using  $\text{Bi}_2\text{FeNiO}_6$  sample calcined at different temperature (a)  $\text{Bi}_2\text{FeNiO}_6$  sample calcined at 350°C (b)  $\text{Bi}_2\text{FeNiO}_6$  sample calcined at 450°C (c)  $\text{Bi}_2\text{FeNiO}_6$  sample calcined at 550°C (d)  $\text{Bi}_2\text{FeNiO}_6$  sample calcined at 650°C**

CV is used to investigate the interfacial changes of the electrode. Figure 4 shows the CVs of Au-PVA- $\text{Bi}_2\text{FeNiO}_6$  and Au-PVA- $\text{Bi}_2\text{FeNiO}_6$ -Ur electrodes modified by using differently calcined sample of  $\text{Bi}_2\text{FeNiO}_6$  Nanocomposite in 5 mM  $[\text{Fe}(\text{CN})_6]^{4-/3-}$  solution prepared in buffer (pH7) at a scan rate of 0.1V/s.

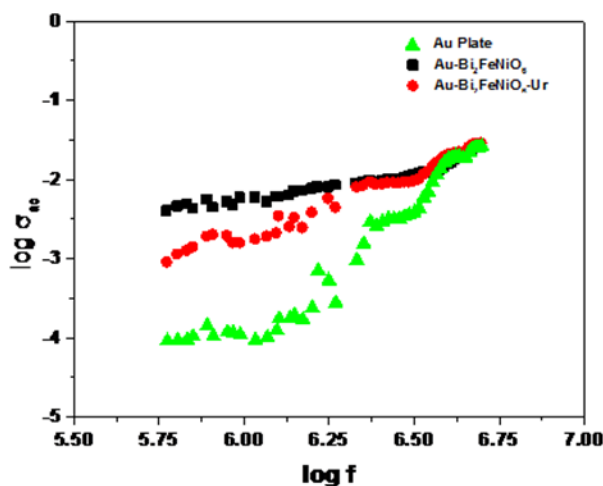
$\text{Bi}_2\text{FeNiO}_6$  specimen calcined at 350°C was not showing a definite redox curve (fig. 4a) for Au-PVA- $\text{Bi}_2\text{FeNiO}_6$ . This indicates that the film was not electro-active and cannot be used for immobilization of urease. As calcined temperature increases, the redox curves are in good agreement and show the definite curve for Au-PVA- $\text{Bi}_2\text{FeNiO}_6$  with different ip values for samples calcined at 450°C (fig 4b), 550°C (fig 4c) and 650°C (fig 4d). Indicating the films was electro-active and can be used for immobilization of urease.

From the ip values it is clear that, with increase in calcined temperature of  $\text{Bi}_2\text{FeNiO}_6$ , peak potential is also increases upto 550°C then decreases. There is slight decrease in ip values for the sample calcined at 650°C. And thus  $\text{Bi}_2\text{FeNiO}_6$  Nanocomposite calcined at 550°C is a good material for the fabrication of bioelectrode. Results were summarized as follows-

Calcination temp. Electrodes	350°C	450°C	550°C	650°C
Au-PVA-Bi <sub>2</sub> FeNiO <sub>6</sub>	No curves	Segment 1 -5.541x10 <sup>-4</sup>	Segment 1 -9.484x10 <sup>-4</sup>	Segment 1 -10.93 x10 <sup>-4</sup>
		Segment 2 5.750x10 <sup>-4</sup>	Segment 2 9.083 x10 <sup>-4</sup>	Segment 2 8.500 x10 <sup>-4</sup>
Au-PVA-Bi <sub>2</sub> FeNiO <sub>6</sub> -Ur	-	Segment 1 -6.880X10 <sup>-4</sup>	Segment 1 -8.53X10 <sup>-4</sup>	Segment 1 -8.971X10 <sup>-4</sup>
	-	Segment 2 5.892X10 <sup>-4</sup>	Segment 2 9.728X10 <sup>-4</sup>	Segment 2 8.955X10 <sup>-4</sup>

Bi<sub>2</sub>FeNiO<sub>6</sub> Nanocomposite calcined at 550°C (fig 4c) can show, a well-defined oxidation and reduction peaks of [Fe(CN)<sub>6</sub>]<sup>4-/3-</sup> (curve a) were observed for the PVA-Bi<sub>2</sub>FeNiO<sub>6</sub> Nanobio composite film deposited onto gold plate. After the urease were modified on the surface of PVA-Bi<sub>2</sub>FeNiO<sub>6</sub> Nanobio composite film deposited onto gold plate, the peak current of the modified electrode decreased (curve b) for the reason that urease could facilitate electron transfer between biomolecules and electrode surface, indicating that the urease had been successfully immobilized [7-8].

#### Impedance analysis



**Fig 5: variation of ac conductivity with frequency for (a) gold plate (b) Au-PVA- Bi<sub>2</sub>FeNiO<sub>6</sub> Nanobio composite (c) Urease immobilized Au- PVA- Bi<sub>2</sub>FeNiO<sub>6</sub> Nanobio composite**

The electrical properties of the material have been studied using a complex impedance spectroscopy (CIS) technique with an objective to understand the changes in electrical properties of the material over a wide range of frequency. 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 [9]

Fig 5 shows the variation in electrical conductivity with frequency. From figure, it is observed that conductivity values are lesser for urease immobilized PVA-Bi<sub>2</sub>FeNiO<sub>6</sub> based Nanobio composite film deposited onto gold plate compare to PVA-Bi<sub>2</sub>FeNiO<sub>6</sub> based Nanobio composite film deposited on the gold plate. Figure also indicate that the, conductivity values are greater at lower frequency compare to higher frequency.

#### Conclusion:

Bi<sub>2</sub>FeNiO<sub>6</sub> Nanocomposite was synthesized by sol-gel citrate method. The crystals of the as synthesized Bi<sub>2</sub>FeNiO<sub>6</sub> were characterized by XRD. The particle size of the Bi<sub>2</sub>FeNiO<sub>6</sub> is estimated by using XRD, which is found to be nearly 40nm.

The thin film of PVA-Bi<sub>2</sub>FeNiO<sub>6</sub> was formed on gold plate by physical adsorption. Then the urease was immobilized on the as prepared nanobioelectrode.

Urease immobilized Au-PVA-Bi<sub>2</sub>FeNiO<sub>6</sub> Nanobio electrode was studied by the SEM and electrochemical studied, which confirms the immobilization by showing different peak current for Au-PVA-Bi<sub>2</sub>FeNiO<sub>6</sub> and Au-PVA-Bi<sub>2</sub>FeNiO<sub>6</sub>-Ur nanobioelectrode. Successful immobilization is also supported by the impedance study which shows variation in electrical conductivity for Au-PVA-Bi<sub>2</sub>FeNiO<sub>6</sub> and Au-PVA-Bi<sub>2</sub>FeNiO<sub>6</sub>-Ur nanobioelectrode.

From cyclic voltametric study, it is observed that as the calcinations temperature of the Bi<sub>2</sub>FeNiO<sub>6</sub> increases ip values of their Au-PVA-Bi<sub>2</sub>FeNiO<sub>6</sub> and Au-PVA-Bi<sub>2</sub>FeNiO<sub>6</sub>-Ur nanobioelectrode is also increases upto 550°C and on further heating at 650°C it is decreases. This suggest that sintering temperature may causes the structural changes in the Bi<sub>2</sub>FeNiO<sub>6</sub> Nanocomposite, and due to structural changes of Bi<sub>2</sub>FeNiO<sub>6</sub> interaction between urease- Bi<sub>2</sub>FeNiO<sub>6</sub> shows markable changes in ip values.

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