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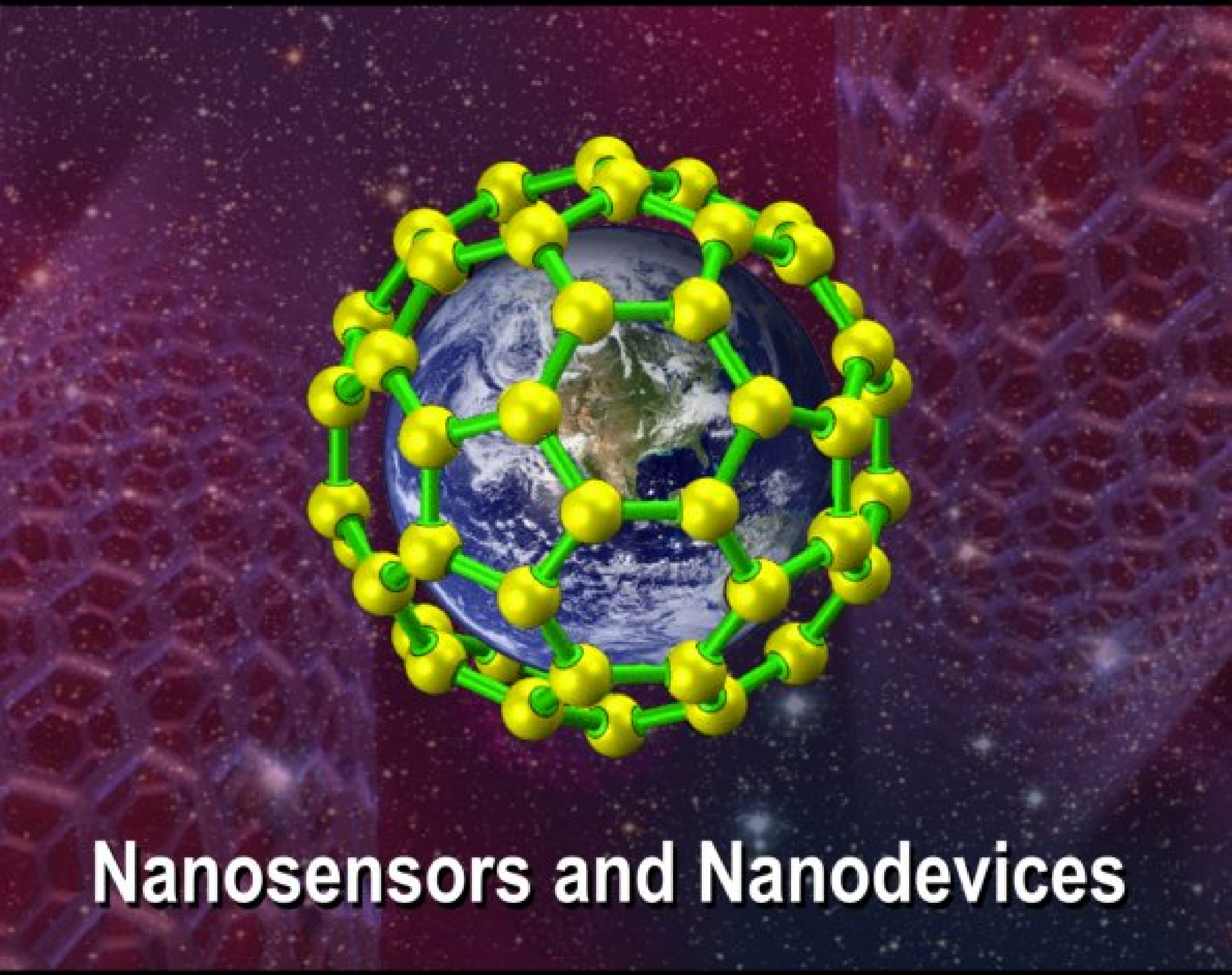

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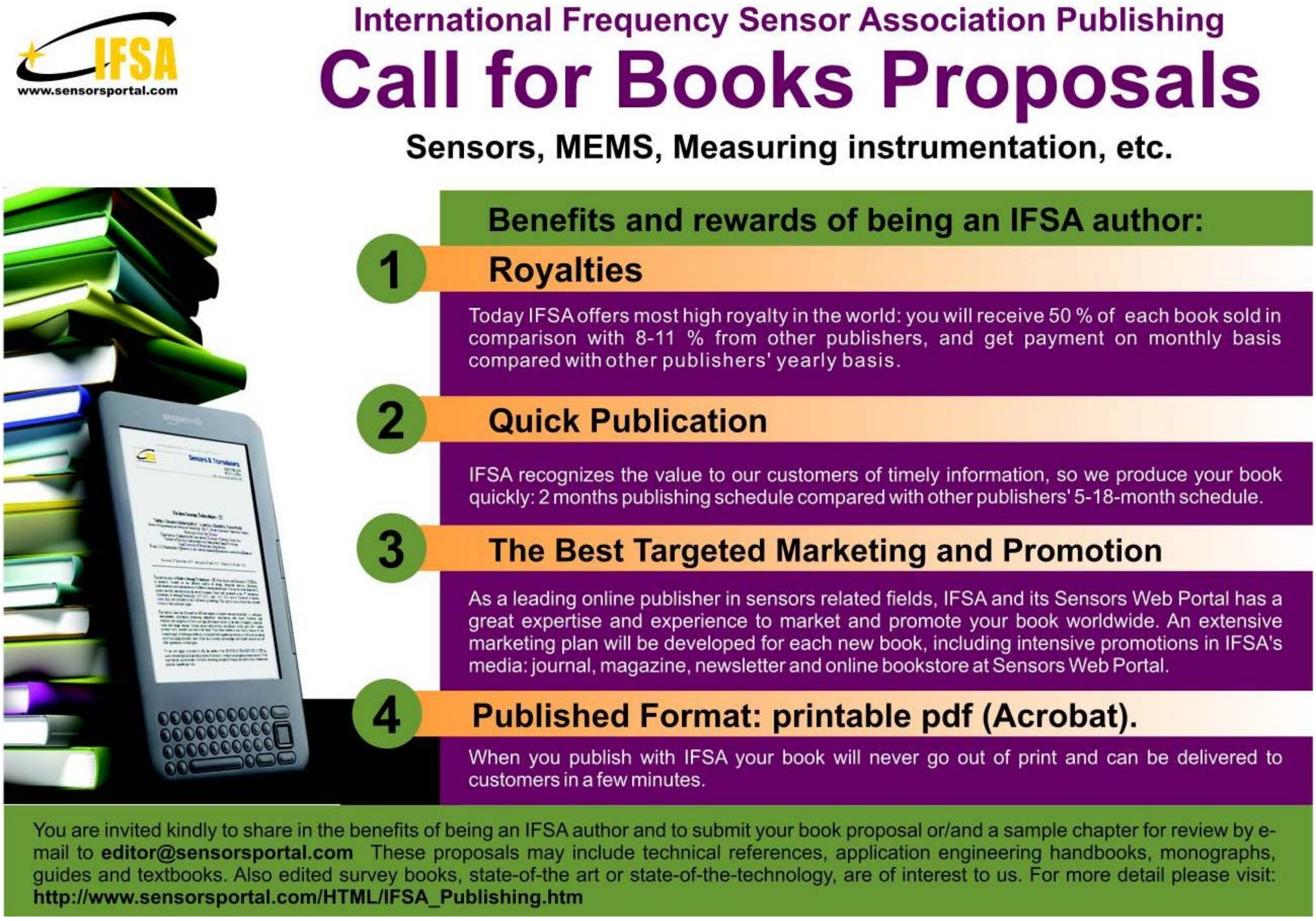

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Fabrication of Biosensors Based on Nanostructured Conducting Polyaniline (NSPANI)

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Abstract: In this study, glucose and hydrogen peroxide (H_2O_2) biosensors based on nanostructured conducting polyaniline (NSPANI) (synthesized using sodiumdodecyl sulphate (SDS) as structure directing agent) were developed. Because of the large specific surface area, excellent conductivity of NSPANI, horseradish peroxidase (HRP) and glucose oxidase (GO_x) could be easily immobilized with high loading and activity. In addition the small dimensions and the high surface-to-volume ratio of the NSCP allow the rapid transmission of electron and enhance current response. The linear dynamic range of optical glucose and H_2O_2 biosensors is 5–40 mM for glucose and 1–50 mM for H_2O_2, respectively where as the bulk PANI exhibits linearity between 5-20mM/l. The miniature optical glucose biosensor also exhibits good reproducibility. The storage stability of optical glucose and H_2O_2 biosensors is two weeks for glucose and five days for H_2O_2. The high response value of NSPANI based biosensors as compared to bulk PANI based biosensor reflects higher enzymatic affinity of GOx/NSPANI and HRP/NSPANI with glucose and H_2O_2 due to biocompatibility, active surface area and high electron communication capability of nanobiopolymer film. In conclusion, the NSPANI based biosensors proposed herein have many advantages such as a low response time, high reproducibility, high sensitivity, stable and wide dynamic range. *Copyright © 2011 IFSA.*

Keywords: Polyaniline nanoparticles, Enzyme biosensors, Glucose oxidase, Horseradish peroxidase, Glucose, Hydrogen peroxide, Spectroelectrochemistry.

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1. Introduction

Nanotechnology has recently become one of the most exciting forefront field that has impacted several areas of material science useful in solving challenging bioanalytical problems [1]. Nano structured conducting polymers (NSCPs) can be considered as building block for miniaturized nanoelectronic devices and highly sensitive chemical and biological sensors [2, 3]. Out of various polymers, nanostructured polyaniline (NSPANI) has been extensively studied for many potential applications including lightweight battery electrodes, anticorrosion coatings and sensors due to its interesting properties such as wide range of conductivity, controlled size distribution, electrochemical switching, film forming ability, bio compatible nature and environmental resistance [2, 4, 5]. PANI nanostructures have been extensively studied with respect to a wide range of applications, including sensors, analytical separations, electrorheological systems and catalyst supports.

NSCPs are synthesized by promoting the crystallization of amorphous structures along one direction by various mechanisms including template and non template synthesis [6-9]. Various synthetic routes for the preparation of Π-conjugated polymeric nanostructures have been reported [9–16]. Usual aqueous phase polymerization of aniline involves aniline and a water-soluble oxidant in the presence of a strong mineral acid. Nanostructured polyaniline (NSPANI) has been mainly prepared with the aid of template-guided polymerization within channels of microporous zeolites [10], electrodes [11], porous membranes [12] or via chemical routes in the presence of self-organized supramolecules [13-15] or stabilizers [16]. At low temperature, the liquid crystal-like arrays, formed between the inorganic-cluster and the self-assembly of organic structure directing agent (SDA), i.e. amphiphilic molecules, readily undergo reversible lyotropic transformations leading to nanostructured materials by providing a confined area for polymerization [17]. Amphiphilic molecules like sodium dodecyl benzene sulphonate (SDS) is an ideal SDA for the synthesis of ordered nano structured polymer composed of framework protonated amine [18]. Thus these amphiphilic molecules can be efficiently used as SDAs for the synthesis of various nanostructured composites.

NSPANI with high interfacial area between polyaniline and its environment has shown enhanced performances in sensor applications [4]. NSPANI has greater sensitivity and faster time response in contrast to its conventional bulk counterpart due to higher effective surface area and shorter penetration depth for target molecules [18-20]. The high surface area and porous structure [19] further allows the fast diffusion of molecules into the framework leading to their applicability as biosensors.

In the present study, we report the preparation of nanostructured conducting polyaniline by using sodium dodecylsulphate (NSPANI-SDS) as structure directing agent (SDA). Synthetic conditions for the synthesis of NSPANI-SDS are optimized with respect to concentration of structure directing agent, oxidant and monomer. UV-VIS spectroscopy, Dynamic light scattering (DLS), Cyclic voltammetry (CV), conductivity were carried out to investigate the characteristics of PANI nanoparticles. NSPANI-SDS and bulk polyaniline were investigated as transducer matrice by immobilizing glucose oxidase and horseradish peroxidase enzyme in order to evaluate its efficiency as immobilizing matrice in the fabrication of glucose and H_2O_2 biosensor. These biosensors had good performances in the determination of glucose and H_2O_2 at a low applied potential with fast response time, wide linear range, and good selectivity, stability and reproducibility.

2. Materials and Methods

2.1. Reagents

Aniline (Sigma-Aldrich), sodium dodecylsulphate (SDS) (Qualigen)**,** ammonium persulfate $(NH_4)_2S_2O_8$ (E-Merck), Sodium dodecylbenzenesulfonate (SDBS) (Sigma-Aldrich) and Camphor sulphonic acid (CSA) (Qualigen), hydrochloric acid (Qualigen) were used in the present experiment. Deionized water from a Millipore-MilliQ was used in all cases to prepare aqueous solutions. Monomer was double distilled before polymerization. Hydrogen peroxide solution was purchased from Merck. Glucose oxidase (GO_x, EC 1.1.3.4, Type X-S from Aspergilus niger 157,500 U/g solid) from sigma, horseradish peroxidase (HRP, E.C.1.11.1.7) with specific activity of 200 Umg−1, o-dianisidine(Sigma-Aldrich), glutaraldehyde was purchased from the Sigma–Aldrich (USA).

2.2. Instrumentation

The UV–vis spectrum of the PANI nanodispersions was recorded using a Shimadzou UV-1800 UV–Vis spectrophotometer. Dynamic light scattering (DLS) measurements were performed using a Malvern 4800 Autosizer employing a 7132 digital correlator. Cyclic voltammetry (CV) measurements have been conducted in 20mM $[Fe(CN)_6]^{3-/4-}$ in a three-electrodes cell consisting of Ag/AgCl as reference, platinum (Pt) as counter electrode and indium tin oxide (ITO) as a working electrode (0.25 cm^2) using Potentiostat/Glavanostat (Princeton Applied Research, model No. 273 A).

2.3. Polymerisation Procedure

In a typical synthesis, aniline was first dissolved into a dilute aqueous solution of dopants. The aniline solution was added to an aqueous solution of SDA under stirring condition. The mixture was then placed in the low temperature bath, so that the temperature was maintained at 0^0 to 5 ^{0}C. An aqueous solution of the oxidizing agent, $(NH_4)_2S_2O_8$, in ice-cold water was added to the above mixture. The polymerization was allowed to proceed for 3 to 4 h with stirring. After that the stirring was stopped and the mixture was kept under static condition for 1-3 days at 277–278 ^{0}K for polymerization to complete. The colloidal solution was precipitated using acetone. The precipitate was washed with deionized water to remove unreacted chemicals and redispersed with deionized water and thin film was fabricated on glass plate using spin coater for characterization.

2.4. Preparation of Solutions

The solutions of horseradish peroxidase (200 Uml^{-1}) and glucose oxidase and o-dianisidine were freshly prepared in phosphate buffer (PBS) (50 mM, pH 7.0) prior to being used. Stock solution of glucose and hydrogen peroxide were prepared in deionized water and stored at 4 °C.

2.5. Fabrication of Optical Biosensor

The NSPANI-SDS cast film was treated with aqueous glutaraldehyde (0.1 %) as a cross-linker. Freshly prepared enzyme solution in phosphate buffer (PBS,7 pH) was uniformly spread onto glutaraldehyde treated NSPANI-SDS film and was kept in a humid chamber for 12 h at 4 ^{0}C. The NSPANI-SDS film was immersed in 50 mM phosphate buffer solution (pH 7.0) in order to wash out unbound enzyme from the electrode surface. When not in use, the electrode was stored at 4 ^{0}C in a refrigerator.

2.6. Enzyme Response Studies

2.6.1. Glucose Biosensor

To find out the photometric response of GoX biosensor using UV-vis spectrophotometer, the film was dipped in the 3 ml PBS solution containing 25 μl HRP, 25 μl of *o*-dianisidine dye and 100 μl of substrate (glucose) and was kept for about 2 min. The difference between the initial and final absorbance value at 500 nm after 2 min incubation of substrate was recorded and plotted.

2.6.2. H_2O_2 Biosensor

To find out the photometric response of HRP biosensor using UV-vis spectrophotometer, the film was dipped in the 3 mL PBS solution containing 25 µl of *o*-dianisidine dye and 100 µl of substrate (H_2O_2) and was kept for about 2 min. The difference between the initial and final absorbance value at 620 nm was recorded and plotted against the concentration of hydrogen peroxide.

3. Results and Discussion

3.1. Effect of Concentration of Oxidizing Agent on the Properties of NSPANI

In the present investigation, the concentration of oxidizing agent was varied from 0.01 M-0.04 M for a fixed monomer and structure directing agent. All the PANI samples are characterized by UV, DLS and resistance measurements.

3.2. UV-Vis Spectra

Fig. 1 [Inset (a)] shows the UV-Vis spectra of NSPANI-SDS synthesized at various oxidant concentrations. We have studied only polaron peak in the visible region due to the more localized nature of polaron peak. It has been observed that with increase in concentration of oxidant there occurs a bathochromic shift in the polaron band up to certain limit (0.02M). Beyond that concentration there occurs a hypsochromic shift in the polaron band. This is due to the over oxidation of polymer by oxidant. This confirmed that the optimum concentration of oxidant for synthesis of NSPANI-SDS is 0.02 M.

3.3. Dynamic Light Scattering (DLS) Studies

DLS was used to determine particle size of the NSPANI-SDS synthesized using various concentration of oxidant. The sample synthesized with 0.02M oxidant appears to be the best as it contained the smallest particle size (106.2) and minimum PDI (0.430) Fig. 1[Inset(b and c)].

3.4. Resistance Measurements

Resistance measurements were used to determine conductance of the NSPANI-SDS synthesized using various concentration of oxidant. As conductance is inversely proportional to resistance. It has been found that the sample synthesized with 0.02M oxidant shows lowest resistance (8.62 kOhm) Fig. 1. Therefore it is highly conducting in nature.

From all the characterization techniques it is clear that the sample synthesized with 0.02M oxidant concentration gives the optimum results in terms of size and conductivity.

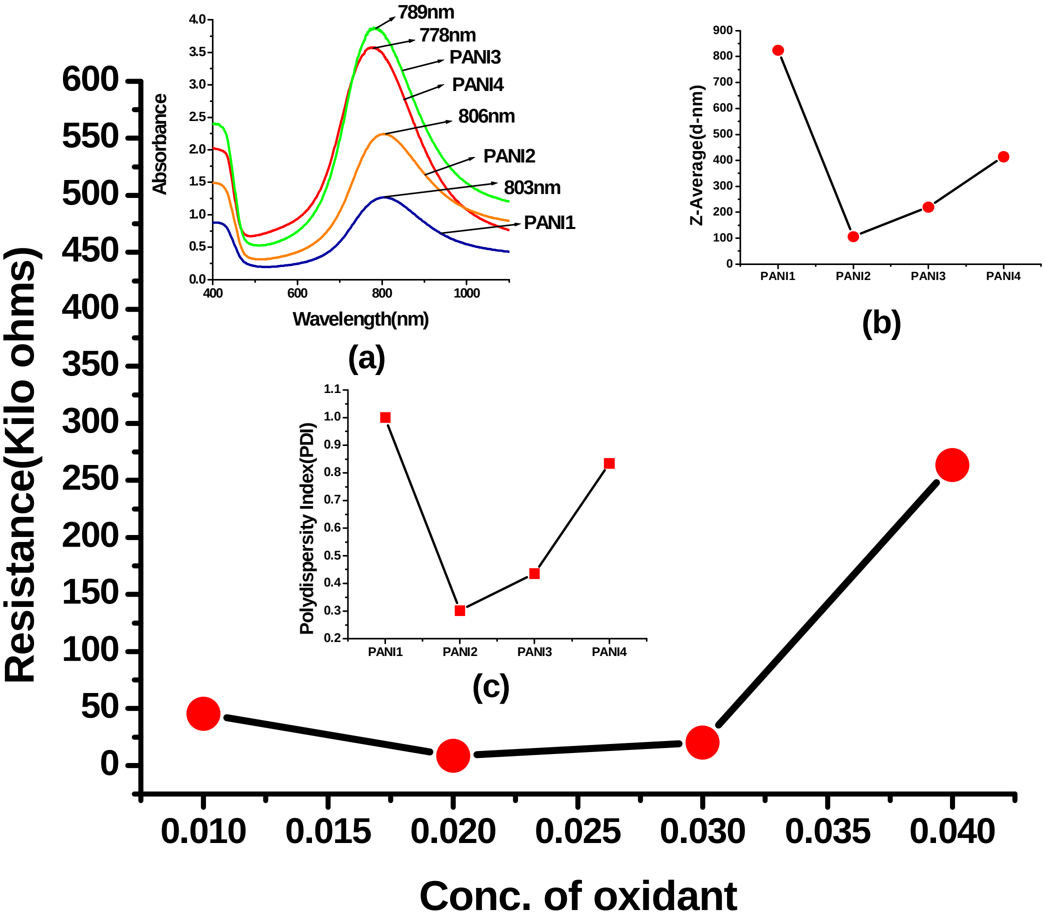


Fig. 1. Resistance measurement for NSPANI-SDS synthesized at various oxidant concentrations. Inset shows (a) UV-Vis spectra; (b) DLS (Z-av); (c) DLS(PDI) for various NSPANI-SDS.

3.5. Effect of Concentration of Structure Directing Agent on the Properties of NSPANI

The concentration of structure directing agent was varied from 0.02M-0.08M for a fixed monomer and oxidizing agent. All the PANI samples are characterized by UV, DLS and resistance measurements.

3.6. UV-Vis Spectra

Fig. 2 [Inset (a)] shows the UV-Vis spectra of NSPANI-SDS synthesized at various structure directing agent concentrations. A gradual bathochromic shift of the polaron absorption band in the visible region has been observed with increase in the concentration of SDS due to the strong complexation ability of SDS with the polymer backbone. The bathochromic shift is attributed to the nanostructure, extended π conjugation and increased conductivity.

3.7. Dynamic Light Scattering (DLS) Studies

DLS was used to determine particle size of the NSPANI-SDS synthesized using various concentrations of structure directing agent. The sample synthesized with 0.08M structure directing agent appears to be the best as it contained the smallest particle size(106.2nm) and minimum PDI(0.430) Fig. 2 [Inset(b and c)].

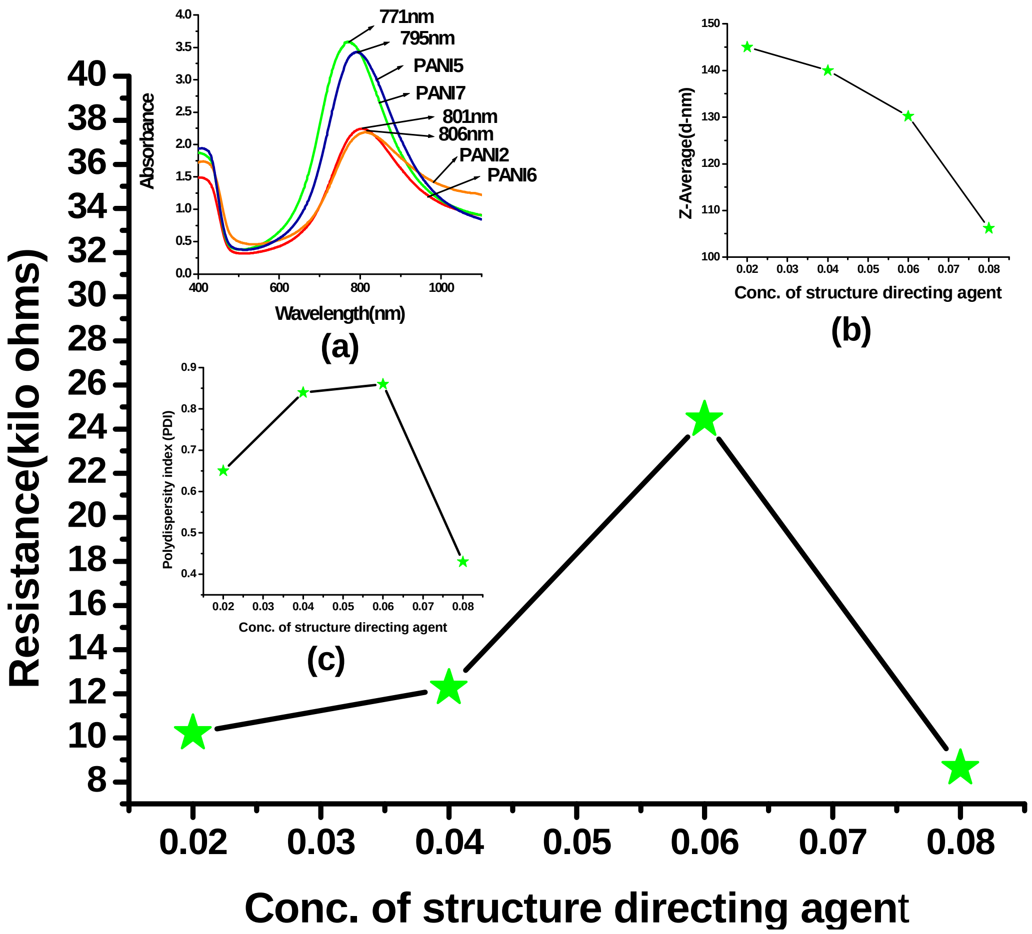


Fig. 2. Resistance measurement for NSPANI-SDS synthesized at various structure directing agent concentrations. Inset shows (a) UV-Vis spectra; (b) DLS (Z-av), and (c) DLS (PDI) for various NSPANI-SDS.

3.8. Resistance Measurements

It has been found that the sample synthesized with 0.028 M structure directing agent shows lowest resistance (8.62 kOhm) Fig. 2. Therefore it is highly conducting in nature.

From the above characterization techniques it has been concluded that smallest size, uniform size distribution and the highest conductivity are obtained when SDS concentration is 0.08 M.

3.9. Relationship between Structure Directing Agent Concentration and Size of Polymer Nanoparticles

Fig. 3 illustrates the variation of PANI nanoparticle sizes as functions of the surfactant concentration. The size of PANI nanoparticles decreased with increasing the surfactant concentration. The increment in surfactant concentration produces the larger number of micelles, resulting in the particle size reduction. Therefore diameter of PANI nanoparticle can be readily controlled by and surfactant concentration. Inset shows variation of size of micelle with concentration of structure directing agent. As the concentration of SDA increases the size of micelle first increases and then decreases. Smaller the size of micelle, smaller will be the size of nanoparticle as nanoparticle grows inside the pores of micelle.

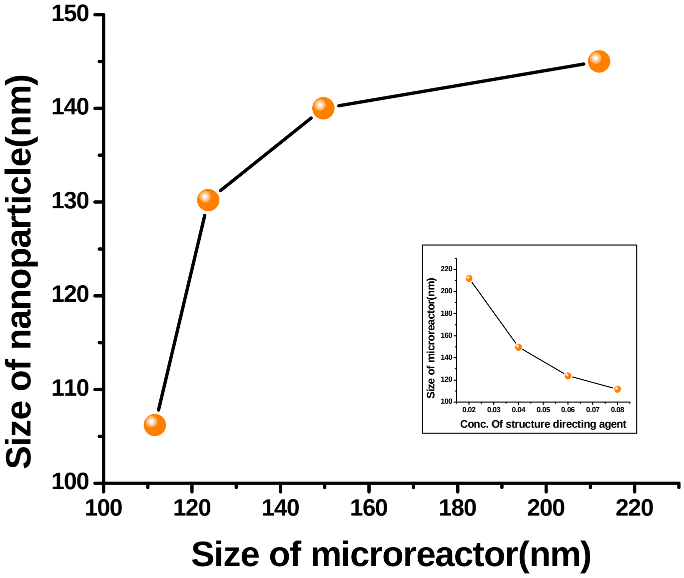


Fig. 3. Average change in particle size as a function of microreactor size (Inset shows variation of size of micelle with concentration of structure directing agent).

3.10. Effect of Concentration of Monomer on the Properties of NSPANI

The concentration of monomer was varied from 0.005M-0.02M for a fixed structure directing agent and oxidizing agent. All the PANI samples are characterized by UV, DLS and resistance measurements. The results are shown by the following Table 1.

Table 1. Characteristics features of NSPANI-SDS synthesized at various monomer concentrations.

Sample no.	Conc. of monomer	Colour of dispersion	UV-Vis spectra	DLS(Z-average)	DLS(PDI)	Resistance measurements
1	0.005	Brown	-	-	-	-
2	0.01	Brown	-	-	-	-
3	0.015	Green	790 nm	220	0.810	20.3 kΩ
4	0.02	Green	806 nm	106.2	0.430	8.62 kΩ

From the Table 1 it is clear that a particular concentration of monomer is required for polymerization to take place. When molar concentration is less than 0.015, polymerization is not completed as there is oligomer formation instead of polymer formation. Colour of the dispersion becomes brown instead of green which is the colour for nanostructured conducting polyaniline. Optimum concentration of monomer is 0.02 M for the synthesis of NSPANI-SDS as it is confirmed by UV-Vis spectra, DLS, resistance measurements.

In conclusion, by varying concentration of oxidant, structure directing agent and monomer it is revealed that smallest size and highest conductivity is obtained when molar concentration of oxidant is 0.02M, molar concentration of structure directing agent is 0.08M, molar concentration of monomer is 0.02 M respectively. Therefore we have chosen that NSPANI-SDS as best transduction matrice for biosensor application.

3.11. Cyclic Voltammetric Studies of Best Nanostructured Conducting Polyaniline

Cyclic voltammetry was the method used for electrodeposition of the nanoPANI-SDS emeraldine salt (ES) onto ITO from the aqueous dispersion of nanoparticles (Fig. 4). The potential was swept from -200 mV to +1200 mV (vs. Ag/AgCl), as polyaniline is redox active within this region at acidic pH. The CV is typical for NSPANI-ES with the main peaks A and B corresponding to the transformation of leucoemeraldine base (LB) to ES and ES to pernigraniline salt (PS), respectively. On the reverse scan, peaks B and A correspond to the conversion of PS to ES and ES to LB, respectively. The presence of a small redox peak around +350 mV (C and C) is associated with the formation of p-benzoquinone and hydroquinone as a side product upon cycling the potential to +900 mV. It was also found that the current responses increased on subsequent scans indicating that the nanoparticles aggregated/assembled on the ITO electrode surface. Shifts in peak potentials began to occur after a number of cycles. This may be the result of increased resistance of the electrode, as the film deposited becomes thicker.

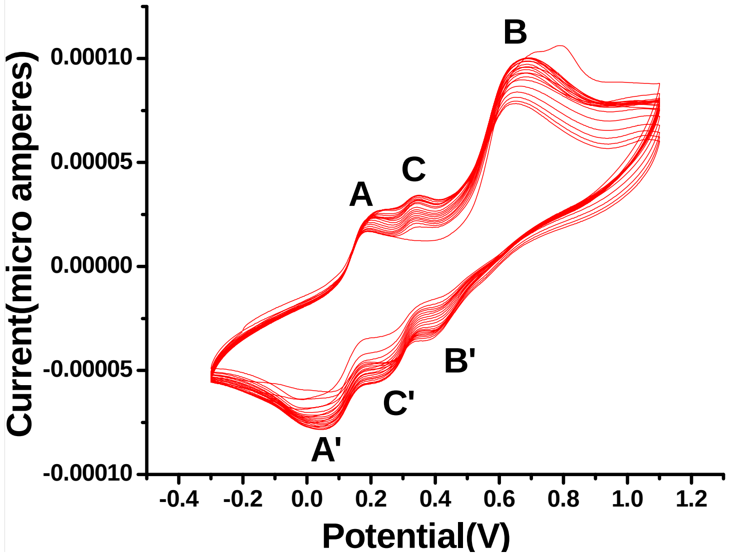


Fig. 4. Electrodeposition of the NSPANI film onto ITO electrode.

3.12. Application of Nanostructured Conducting Polyaniline to Biosensors

3.12.1. Characterization of Bioelectrode

CV of the ferricyanide system $[Fe(CN)6]^{3-/4-}$ is a convenient and valuable tool to monitor the characteristics of the surface of modified electrode. CV (Fig. 5) was conducted in 20 mM $[Fe(CN)6]^{3-/4-}$ for three different types of electrodes: bare ITO, NSPANI/ITO and GoX/NSPANI/ITO. Diffusion-controlled redox process was observed at the bare ITO (Fig. 5a). For NSPANI/ITO electrode, though there is a marked decrease in the peak current (Fig. 5b) but difference between the anodic and cathodic peak potential is also reduced. Reduction in the difference of anodic and cathodic peak potential obtained for the NSPANI/ITO (0.3 V, curve (b)) compared to that of bare ITO electrode (0.5V, curve (a)) indicates the reversibility and electrocatalytic activity of the electrode which may facilitate the electron conduction pathway between the enzyme and electrode. The decrease in the anodic current obtained for the GoX/NSPANI/ITO (Fig. 5c) as compared to that of NSPANI/ITO electrode indicates the hindrance caused by the macromolecular structure of the enzyme indicating GoX immobilization and slow redox process.

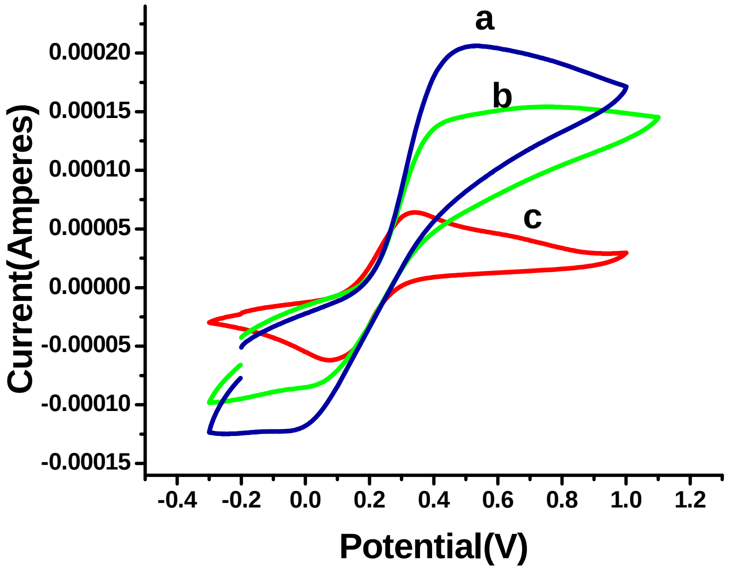
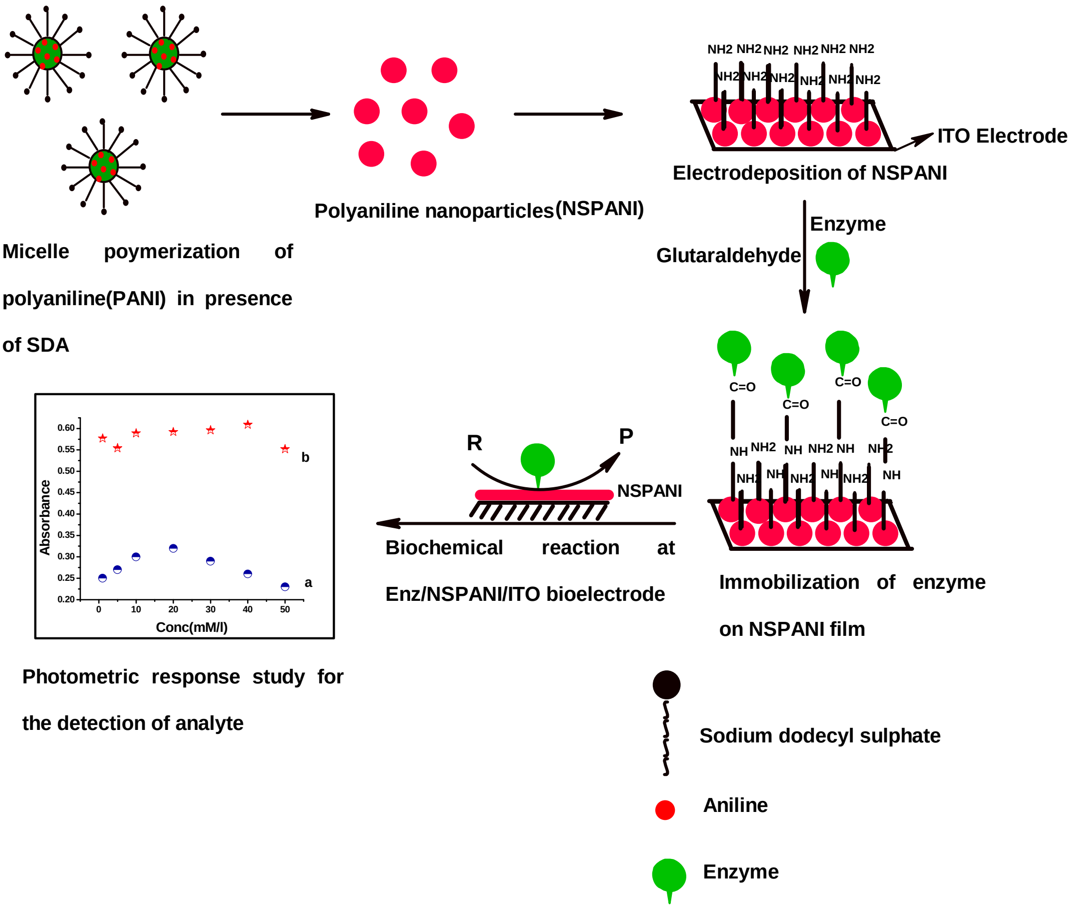


Fig. 5. Cyclic voltammogram of (a) bare electrode; (b) NSPANI/ITO; (c) GoX/NSPANI/ITO bioelectrode at 80mV/s in $[Fe(CN)6]^{3-/4-}$.

Scheme 1 represents diagrammatically the biochemical reaction taking place at Enzyme/NSPANI/ITO bioelectrode.



Scheme 1. Pictorial representation of synthesis of NSPANI, Immobilization of enzyme and biochemical reaction at Enzyme/NSPANI/ITO bioelectrode.

3.12.2. Photometric Response Studies of Glucose Biosensor

Out of various NSPANI synthesized, PANI 2 was used for biosensor fabrication along with bulk PANI as a control. The reason for using PANI 2 as biosensing matrice is that they can be precipitated and redispersed in several times. The biochemical reactions involved in the photometric measurements are shown in the following reactions (1 and 2) wherein the absorption rate is proportional to the concentration of H_2O_2 produced, which is directly proportional to the analyte (glucose) concentration.

$$\text{Glucose} + O_2 \xrightarrow{\text{GOx/NSPANI}} \text{Gluconic acid} + H_2O_2 \qquad (1)$$

$$H_2O_2 + \text{O-anisidine (red)} \longrightarrow 2\,H_2O + \underset{\text{(Orange-red colour)}}{\text{O-anisidine (oxidized)}} \qquad (2)$$

The response characteristics of GOx/NSPANI biosensors were studied as a function of glucose concentration (Fig. 6) and the value of absorbance resulting from the oxidized form of dye was found to be increasing linearly in the range of 5 mM/l to 40 mM/l where as bulk PANI exhibits linearity between 5-20mM. It was found that the GoX/NSPANI/ITO bioelectrode in the range of 1 to 40 mM for glucose concentration follows the equation [Change in absorbance = 0.578 + 0.00011 x glucose concentration (mM)] with 0.023 as standard deviation.

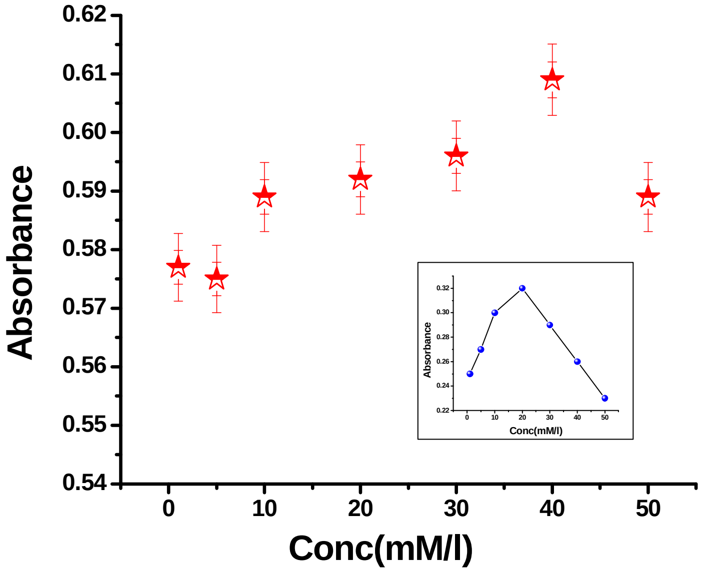


Fig. 6. Plot of optical absorbance vs. glucose concentration for GoX/bulk PANI. Inset GoX/NSPANI.

The value of apparent Michaelis–Menten constant (Km) has been estimated using the Lineweaver–Burke plot, graph between inverse of absorption and inverse of glucose concentration and shown in Table 2. The lower value of Km for GOx/NSPANI biosensor as compared to bulk PANI biosensor suggest that the NSPANI martice is facilitating the enzymatic reaction. Response time was also noted when a steady UV absorbance was observed during above biochemical reactions and is mentioned in Table 2. Minimum response is observed for GOx/NSPANI biosensors indicating high rate of electron transfer. Lower detection limit for GOx/NSPANI biosensors is 0.1 mM and upper detection limit is 60 mM.

Table 2. Optical characteristics of various glucose biosensor.

Name of the bioelectrode	Km (mM)	Linearity(mM/l)	Response time(s)	Lower detection limit(mM/l)
GoX/NSPANI	0.60	5to40	30	0.1
GoX/bulk PANI	21.0	5to 20	90	1

3.12.3. Electrochemical Response Studies of Glucose Bioelectrode

The cyclic voltammetric studies have been carried out between -200 mV and 1200 mV on bioelectrode as a function of glucose concentration (Fig. 7). It is observed that the anodic current increases with increased concentration of glucose.

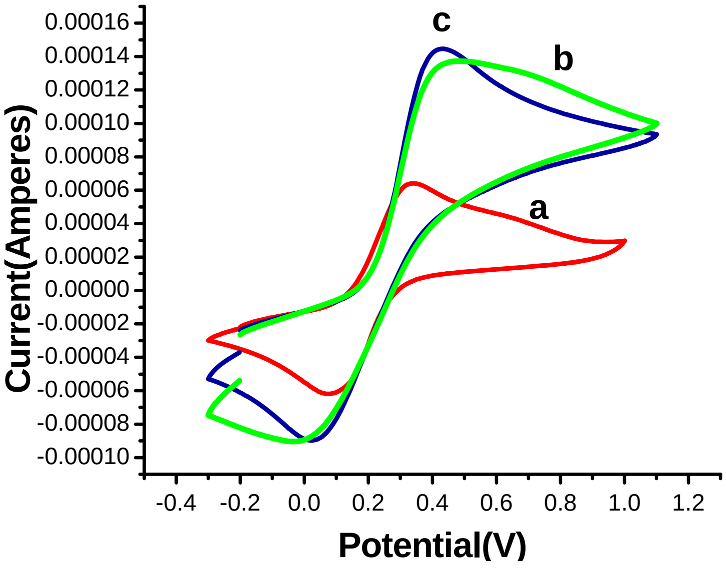


Fig. 7. Cyclic voltammetric curves obtained for bioelectrode as a function of glucose concentration.

3.12.4. Effect of pH on GoX/NSPANI/ITO Bioelectrode

Photometric studies were carried out to find the optimal pH for the bioelectrode in PBS of pH 6.0-7.8. The higher value of the absorbance obtained at pH 7.0 (Fig. 8) indicates GoX/NSPANI/ITO bioelectrode is the most active at pH 7.0. At this pH, biomolecules retain their natural structures and do not get denatured. Thus all experiments have been conducted out at a pH 7.0.

3.12.5. Effect of Interference on GoX/NSPANI/ITO Bioelectrode

The effect of interferents such as uric acid(UA), sodium ascorbate(SA) on the glucose measurement has been studied by taking the solution containing (1:1) ratio of glucose (20 mM) and interferents such as uric acid (0.1 mM), sodium ascorbate (0.05 mM). The results (Fig. 9) indicate the negligible effect of these interferants on the photometric response of GoX/NSPANI/ITO electrode.

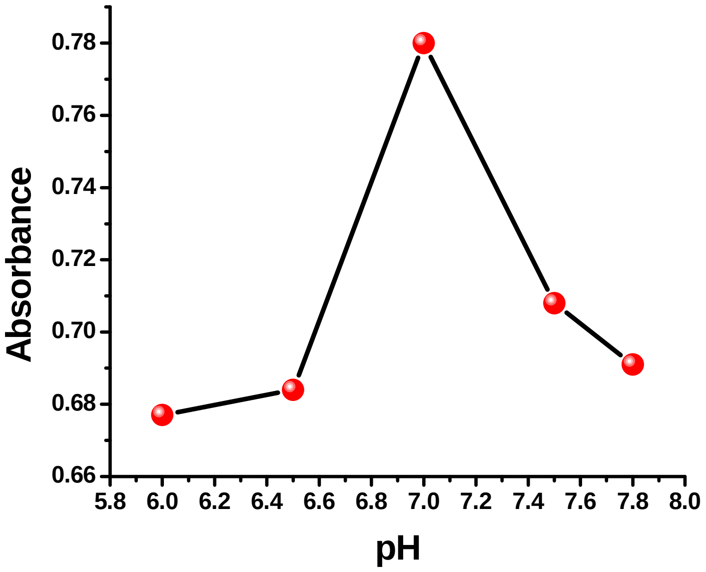


Fig. 8. Absorbance response of GoX/NSPANI/ITO bio-electrode in PBS buffer of pH (i) 6.0; (ii) 6.5; (iii) 7.0; (iv) 7.5; (v) 7.8.

Fig. 9. Effect of various interferents on the response of GoX/NSPANI/ITO Bioelectrode (Glu= glucose).

3.12.6. Stability

The stability of GoX/NSPANI/ITO biosensor has been checked by carrying out UV-vis experiments at the regular interval of a week and it has been found that GoX/NSPANI/ITO based optical biosensor retains its 90 % activity after 15 days. The loss in the activity of biosensor is not due to the denaturation of enzyme but it is due to the poor adhesion of NSPANI film on the ITO electrode.

3.12.7. Photometric Response Studies of H_2O_2 Biosensor

The enzyme that is used intensively in development of hydrogen peroxide sensors is horseradish peroxidase. Horseradish peroxidase (HRP) is hemic enzyme that contain Fe(III)-protoporphyrin as the prosthetic group. This enzyme works with hydrogen peroxide as the substrate. HRP catalyses the oxidation of substrate by hydrogen peroxide, following the general reaction (3):

$$H_2O_2 + \text{O-anisidine (red)} \xrightarrow{\text{HRP/NSPANI}} 2\,H_2O + \text{O-anisidine (oxidized)} \quad (3)$$
$$\text{(Orange-red colour)}$$

3.12.8. Effect of Analyte Concentration

The response characteristics of HRP/NSPANI biosensors were studied as a function of H_2O_2 concentration and the value of absorbance resulting from the oxidized form of dye has been found to be increasing linearly in the range of 1 mM/l to 50 mM/l where as the bulk PANI, exhibits linearity between 10 mM/L to 30 mM/L (Fig 10, Table 3). The response value for HRP/NSPANI is four times than that of bulk PANI The high response value reflects higher enzymatic affinity of HRP/NSPANI with H_2O_2 due to biocompatibility, active surface area, and high electron communication capability of nanobiopolymer film. It was found that the HRP/NSPANI/ITO bioelectrode in the range of 1 to 50 mM for hydrogen peroxide concentration follows the equation [Change in absorbance = 0.589 + 0.009 x hydrogen peroxide concentration (mM)] with 0.099 as standard deviation.

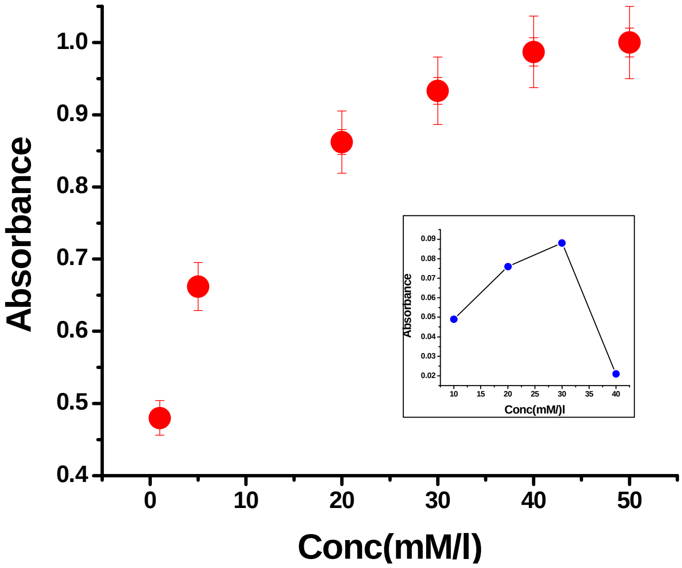


Fig. 10. Photometric response of HRP/NSPANI biosensors as a function of H_2O_2 concentration Inset HRP/bulkPANI.

Table 3. Photometric response studies of various Hydrogen Peroxide (H_2O_2) biosensor.

Name of the samples	Km(mM)	Linearity(mM/l)	Standard Deviation	Regression Coefficient
HRP/bulk PANI	21.0	10 to 30	0.37	0.99
HRP/NSPANI	1.07	1 to 50	0.03	0.99

The value of the enzyme–substrate kinetics parameter (Michaelis–Menten constant, *K*m) estimated using the Lineweaver–Burke plot reveals affinity of enzyme for desired analyte (H_2O_2). It may be noted that *K*m is dependent both on matrix and the method of immobilization of enzymes that often results in their conformational changes resulting in different values of *K*m. We have obtained value of *K*m for the HRP/NSPANI biosensor as 1.07 mM that is much lower than the bulk PANI(21.0 mM). The lower *K*m value indicates high affinity for hydrogen peroxide attributed to the immobilization of

HRP onto NSPANI for faster biochemical reaction. From the above discussion, it is obvious that NSPANI offers a more bio compatible and efficient transducer matrice for biosensor application as compared to bulk PANI.

3.12.9. Storage Stability

The stability of HRP/ NSPANI films along with bulk PANI has been found to be poor which can be explained by the degradation of PANI film by the oxidation reaction of H_2O_2.

4. Conclusions

It is clear from the above study that it is required to maintain a very specific concentration of monomer, oxidant, SDA as well as dopant in order to achieve the best NSPANI with respect to size, conductivity as well as electrocatalytic properties. With slight variation from the optimum condition of polymerization causes a drastic change in the electrochemical properties. The cast films on ITO have been utilized to fabricate a GoX/NSPANI/ITO and HRP/NSPANI/ITO bioelectrode This biosensor format has shown linearity from 1mM-50mM, fast response time (30 s) (photometric), negligible interferent effect(0.1%) and low Km value. Further studies on overall electrochemical response and stability of the sensor are needed to be carried out.

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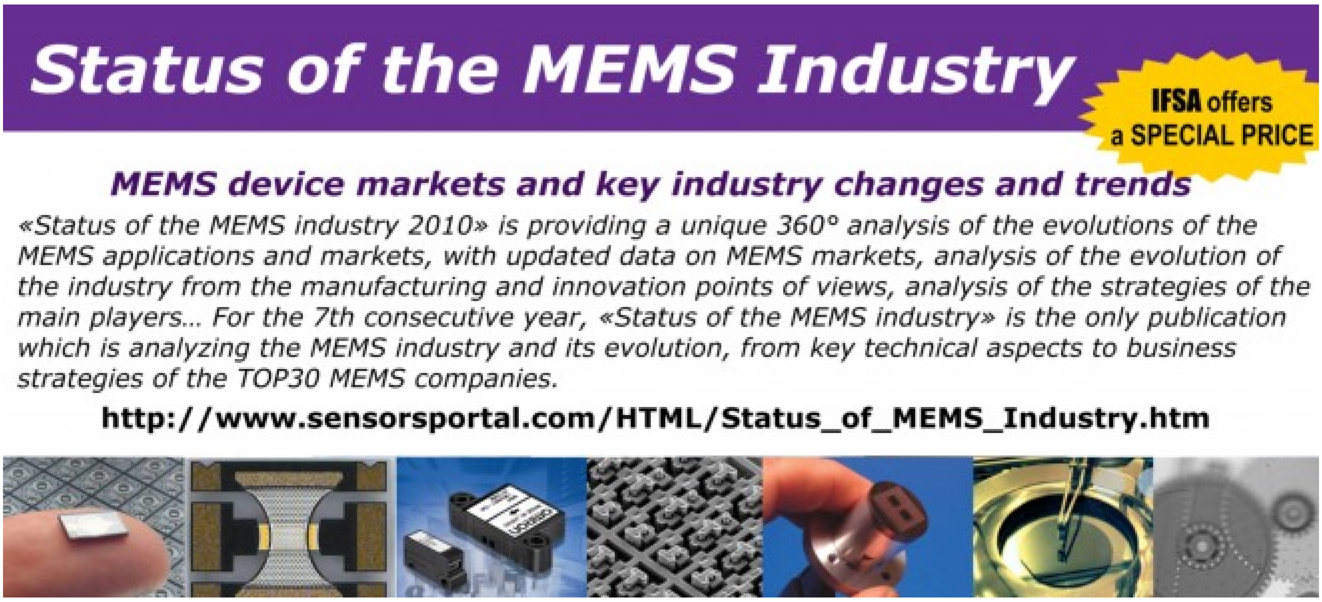

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Sensors & Transducers Journal (ISSN 1726-5479) provides an advanced forum for the science and technology of physical, chemical sensors and biosensors. It publishes state-of-the-art reviews, regular research and application specific papers, short notes, letters to Editor and sensors related books reviews as well as academic, practical and commercial information of interest to its readership. Because of it is a peer reviewed international journal, papers rapidly published in *Sensors & Transducers Journal* will receive a very high publicity. The journal is published monthly as twelve issues per year by International Frequency Sensor Association (IFSA). In additional, some special sponsored and conference issues published annually. *Sensors & Transducers Journal* is indexed and abstracted very quickly by Chemical Abstracts, IndexCopernicus Journals Master List, Open J-Gate, Google Scholar, etc. Since 2011 the journal is covered and indexed (including a Scopus, Embase, Engineering Village and Reaxys) in Elsevier products.

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Contributions are invited on all aspects of research, development and application of the science and technology of sensors, transducers and sensor instrumentations. Topics include, but are not restricted to:

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- Smart sensors and systems;
- Sensor instrumentation;
- Virtual instruments;
- Sensors interfaces, buses and networks;
- Signal processing;
- Frequency (period, duty-cycle)-to-digital converters, ADC;
- Technologies and materials;
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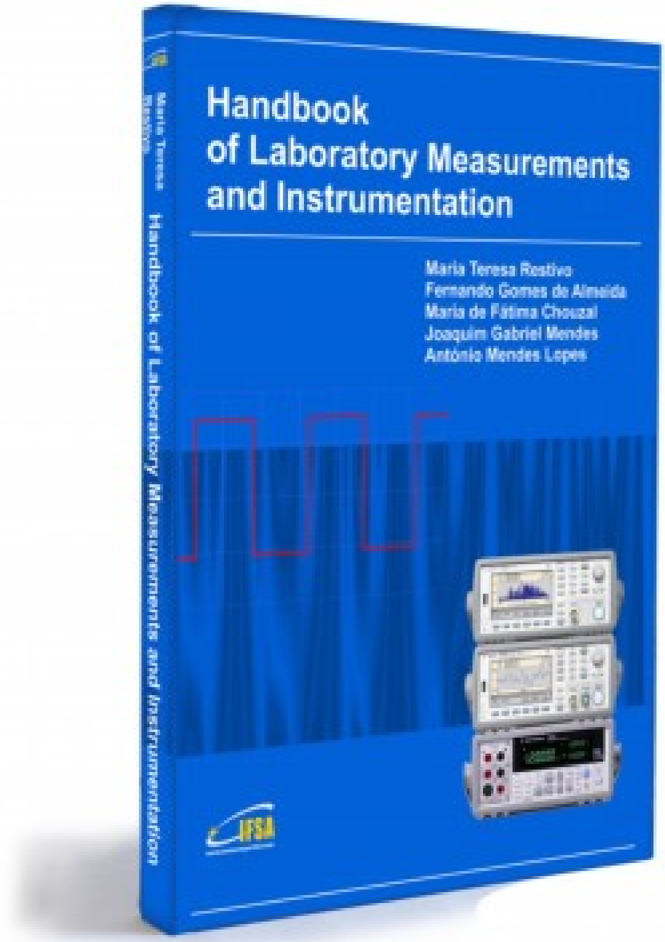

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