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Synthesis of nano structured polyaniline with ionic and nonionic polymers based enzymatic application of glucose biosensing

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Abstract— **Polyaniline (PANI) a conducting polymer solid material stable at room temperature. The nature of aniline monomer in yellowish colour remains in gel form. The monomer aniline particle remains separate at this gel form. Now aniline polymerized in presence of sodiumdodecylsulfate (SDS) an ionic surfactant. It entrapped to the aniline monomer so that the size of nanoparticles got stable manner .A micelles formation takes place and get swellings, to prevent this formation we have to mixed ammoniumpersulfate (APS) .It does oxidized to the polymerized aniline with sodiumdodecylsulfate solution. The formation of nano particles are in spherical in morphological manner at very low monomer concentration. Non-ionic polymer Pluronic® F-123 is compositionally synthesized. Now prepared materials are characterized using UV visible spectroscopy, Fourier transform infrared spectroscopy, XR-D, Scanning electron microscopy, Atomic force microscopy.. These experiments are provides strong base to identify the characteristics of nanomaterials ,so that transferring of electrons have fast and provide convenience to measure quickly glucose with glucose biosensor.**

Index Terms— **concentration activity, Amperometry, Cyclic voltammetry, Ag/AgCl and Pt. electrodes, H2O2 electro chemical activity, Glucose Oxidase (Enzyme).**

I. INTRODUCTION

Sensor is a device which responds to the physical parameters in form of sensitivity. Biosensor combines the sensitivity of electro analytical methods of the biological components [1]. These component senses its catalytic activity which produces the electrical signals i.e. recognizes by electrodes like, transducer which is proportional to the analytic concentration. The sensor has a recognition component that acknowledge to a particular group of analytes. Another main component of biosensor is transducer or detector electrodes, which is complexes with analytes, called Bioreceptor [2].

The glucose biosensor is based on the nature of bio-recognition which is called bio catalytic device. A bio-catalytic device allows enzymes, cells, and group of cells i.e. tissues. This recognises the target analytes. Conducting polymer polyaniline (PANI) and its group of surfactants are used as analyte product i.e. detected. In human body system enzymes are work as a catalyst which dissociates to the glucose in glycogen. This provides internal energy to the cells and tissue and whole immune system. The chemical reaction of catalyst enzyme work as a biological recognition element and biological components works as an antigen [3]. Mainly electrocatalytic glucose biosensor is depending on the glucose oxidase (GOx) enzyme, which catalyzes glucose in gluconolactone in form of oxidation [4].
Advanced materials are part of the organic compounds [5]. Organic compound used with the polymers as conducting polymer materials. Basically polymers are insulator, at this level we have to create a mechanism that they starts conduct, called doping, such type of materials are called conducting polymers and behaves as a semiconductor materials [6-7]. In advanced recently such materials has been invented that can be undertake according to the application, means these materials are case sensitive ,called smart materials. Conducting polymers have a specific band gap due to that reason they have a property of conduction of electron [8]. In case of conducting polymers there are three types of mechanisms are found i.e. polaron, bipolaron, and solitons [8-9]. These are the structures which are responsible for conduction.

Conducting polymers used in analytical application like biological parameters. CP's (conducting polymers) works as electrochemical transducer that, converts biological contents in to electrical signals. Conducting polymer (PANI) is most prominent materials which is used in biological application such as biosensors [10]. Because it has greater stability and less specific gravity compare to other conducting polymers like: polypyrrole, polyacetylene, polythiophene ect. and polyaniline is most biocompatible material [11]. Polyaniline (PANI) exists in different structures forms as per chemically and physically. These localized states are: Leucoemeraldine (C6H6NH)n, Emeraldine(Green for emeraldine salt and blue for emeraldine base).Among structures most mutual is greenish protonated polyaniline (emeraldine salt most conductive) has higher conductivity compare to the, polyaniline (emeraldine base i.e. non conductive) in bluish colour and per nigraniline blue violet colour [12].

Aniline (Molecular wt. 93) is colourless liquid, which gradually turns yellow or brown. It soluble in organic compound and water [13]. Aniline in solution adsorbs strongly to colloidal organic materials, which raises its solubility and dynamic movement of nanoparticles in to prescribed solution [14]. Adsorption depends upon pH of

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solution [15]. The presence of surfactant increases the ability of conductivity but reduced the electrical stability of polyaniline because polyaniline has high surface area which entrapped by surfactant and affects the electrical stability [16-17]. Surfactants are main gradient component in polymerization of aniline to enhance the property of polymers with respect to conductivity, solubility in to the organic solvents [16].

There are different-different methods for polymerization of monomers like:

Seeding polymerization, templets methods, electrochemical synthesis, and vibrational irradiation. In these methods important term is organic acids which forms micelles upon the aniline, after polymerized .In this case micro level clusters are formed like nano fibers [18]. Surfactant increases the diameter of the nanostructure particles [19]. Polyaniline makes a aques interface with nano surfactant entrapped fibers, which was generated from surfactant and co-dissolved in to the solution. This technique is called interfacial polymerization [16-19]. At this level, after interfacial polymerization we have to dissolve the Ammonium per Sulfate (APS).in deionized water for aques solution. In this method the genesis of nano fibers become very low quantity, because they get oxidized [20].

Polyaniline prepared using soft template method as well as hard template methods. Soft template method based materials can be easily washed and prepared. But hard template based materials we have to use external catalyst like metallic materials to enhance the rate of reaction [21]. The effective method to raise the surface to volume to ratio is to induct mesophase by using wetting agent, which form a super molecule gathering [22]. The mixed surface active agent is better method to ascendance the sizing of nanoparticles over wetting agent /coemulsion [23].The particle emergence in mixed microemulsion arrangement is quick complicated, which affects directly the size of particle and surface activity [24].Conducting polymers shows qualitative response as sensor for functional group like: alcohol evaporation such as alkenols [24-25]. Polyaniline (PANI) and its alternative derived function such as poly(o-toluidine),poly(o-anisidine),poly(N-methylaniline),poly(o-N-Ethylaniline),poly(2,3-dimethylaniline)and poly(diphenylamine) were found sensitive to alkanol [26].

To find out the concentration of glucose in our body system as well as in several aspects like nutrient, micro-organism and in the field of aesulapian/medicine [27-28]. The invention of glucose biosensor is crucial due to preponderance of heart valves diseases is an important threat. There are several methods to methods to recognize glucose like: electrochemiluminisence, capacitive detection, fluorescence detection, but highly efficient in form of current amperometry is best to determine to the glucose [29].Now I have to describe that there are methods to find out the mesostructured PANI at nanostructure level .we have used ionic materials like sodium dodecylsulfate as surfactant containing as self mixed property in form of mesostructured. Again there is non-ionic surfactant polymer as Pluronic -F123 (EO20PO70EO20), Brij-35(Polyoxyethylene lauryl ether).Among the surfactant when we made the following materials like: PANI-SDS-F123, PANI-SDS, and PANI-SDS-Brij-35 among the materials PANI-SDS-F123 was shows higher amperometric current so we refers PANI-SDS-F123 at nano structure level. Because transferring of electron is higher in this material so it w shows higher response .We has various characterizations up to nano level.

II. MATERIALS AND METHODS

A. *Materals used in synthesis*

Chemical used in above references to synthesis the PANI nano-structrued particles with the various compositions as above defined. In this way we have to use following materials:

Aniline monomer in liquid form as light yellow or brown colours which stored in dark place. Hydrogen Peroxide (30%) GR ,H2O2 ,Mol. Wt. is 34.01 g per mol. HCL and Sodium dodecylsulfate (SDS).Polyoxyethylene lauryl ether called Brij® 35 Mol. Wt.1199.56 purchased from ACROS Organics. Sodium phosphate dia basic AR (Anhydrous) Mol. Wt. 141.96 (Na2HPO4), Ammonium per sulphate (APS), 98% ACS reagent, Mol.wt. 228.19 gm per mol, H8N2O8S2, Glutaraldehyde (25% aques solution C5H8O2, Mol.wt. 100.12 gm per mol), Pluronic ® F123, polyethylene glycol (PEG) Mol.wt. is 4000 gm per mol., Glucose Oxidase (Gox Type 2-s Aspergillus niger),Pluronic ® F123 it is a polymeric nonionic reagent, Deionized water from Bio-age system at resistivity 18.3 MΩ per cm was used in all cases to prepare aques and chemical molar solutions, purchased from *SIGMA ALDRICH*.

B. *Synthesis methods of nanoparticles*

The mainstream PANI and its various compounds are synthesized using ionic and non-ionic polymeric surfactant as a structure directing agent, for example sodium dodecyl sulphate, dodecyl benzene sulphonate (SDBS) and camphor sulphonic sulphate. Below given table shows the various chemical compositions as concentration variation activity.

Chemical Composition	**Aniline Concentration (M) gm**	**SDS Concentration (M) gm**	**Pluronic F123**	**pH value**	**Relative Viscosity**
PANI-SDS	1.8 gm	3.0 gm	-	0.816	1.16
PANI-SDS -F123(a)	2.0 gm	3.0 gm	1.5 gm	0.579	1.01
PANI-SDS -F123(b)	2.5 gm	3.0 gm	1.5 gm	0.52	1.03
PANI-SDS -F123(c)	1.8 gm	3.0 gm	2.0 gm	0.67	1.41
PANI-SDS -F123(d)	1.8 gm	3.0 gm	2.5 gm	0.84	1.71

Now we have to describe solution PANI-SDS and for remaining all solutions procedure are same. Take the commercially available 12M HCL; dissolve 1.5 gm in 120 ml deionized water and make aques solution of it. Now take

2.0 gm aniline monomer in liquid form and dissolved in to 40 ml aques solution of HCL and stirrer up to 1 hr. Now take 3.0 gm ionic polymeric surfactant sodium dodecyl sulphate (SDS) and mixed in to the remaining in 80 ml aques solution of HCL and stirrer up to 1 hr for completely polymerization of aniline and becomes homogeneous solution. In this manner surfactant get started micelles formation and air get entrapped by surfactant and aniline starts polymerization .It remains stable form due to structure directing agent SDS and reduce the size of nano-surfactant.

Now we have to make a provision of temperature for oxidation at 0-3 degree centigrade, using ice tub and keep PANI solution in it and start to stirrer. In this way to make conductive materials this solution we have to employ under oxidation reaction after mixing ammonium per sulphate (mixed APS 9.0 gm in deionized water and make aques solution ammonium per sulphate). Dissolving of APS should be slowly in surfactant solution. APS reduces the concentration of surfactant in form of oxidation and enhances the conductivity of green emeraldine base. This solution has to remain static up to 3 days. In this way prepared solution is PANI-SDS emeraldine base Now similarly we ca make solution of PANI-SDS-F123 (a) in non-ionic polymeric surfactant. For this we have to proceed as following manner:

Prepare 1.5 gm, 12 M commercially available HCL solution in 120 ml deionized water, from which 40 ml solution stirrer up to 1 hr with 2.0 gm aniline monomer. Prepare a homogeneous solution of SDS and F-123 for this we have to use, 3.0 gm SDS and 1.5 gm Pluronic ® F-123 and stirred up to 0.5 hr up to complete polymerization and become completely dissolved. After complete polymerization we have to mixed oxidizing agent APS drop wise slowly (APS solution prepare using 100 ml deionized water stirred with 9.0 gm APS). This whole arrangement should be at 0 degree to 4 degree centigrade temperature. After complete mixing of APS we have to leave this solution static up to 3 days at 4 to 5 degree centigrade temperature. In this way it will completely polymerized and become dark greenish colour. This solution does filter and dry in vacuum oven for 48 hr at 40 degree centigrade temperature.

C. *Activity of nano particles*

I this way we have prepared different composition in fixed proportion and molar ratio. When we increase the concentration of aniline than resultant solution become dark greenish colour, and when increases the concentration of Pluronic F-123 then solution turned in greenish colour with nano fibers.

When concentrations of non-ionic polymeric surfactant increases then small nano structured fibers are arises. But at lower concentration of Pluronic F123 these are completely oxidized b APS and gives fine dark greenish powder after dry. This powder get illumination under sun light , because conductive nano particles when gets start to scattering motion then an energy is liberated and they gets illuminated. Every scattered particle are arranged in symmetrically and shows elastic motion. So in case of conductivity what is nearly band edge absorption after UV Visible spectroscopy can be determine most probable band gap.

III. CHARACTERIZATION OF MATERIALS

A. *X-Ray diffraction pattern*

Materials characterizations recorded using material analytical equipments like X Ray Diffraction (X-RD), PAN Analytical expert PRO Diffractometer using Cu Kα radiation (λ=0.1541 nm,40 kv,21 mA). The detector moved step by step from 4° to 45° at the speed of 2°/min.ad X-ray powder placed under 40 kW at 21mA.

The X-Ray diffraction model of polyaniline at various concentrations were represented in Fig……….as expressed in elemental analysis. The characteristics design graph of organic compound which doped in polyaniline has been obtained some different but not more different. All of the profile were same but differ in corresponding intensities peaks [30]. There are two types of angles in X-RD pattern, wide as well as low angle [31-32]. The wide angle is attribute continues along to the polymer long chain 2θ=…... while the maximum peak where the centered peak was 2θ= ……………which causes by continuously perpendicular to the polymer chain [31]. Low angle area graph is measured in the range of 1° to 5°.We know that polyaniline is porous in nature so X-RD shows its nature not a sharp peaks but a curvature type graph smoothly lines we get which clearly shows that resultant material is polymer. Materials which are mixed with highly polymeric materials (PANI-SDS-F123) like F-123 surfactant, this covers large surface to volume ratio, so in this case low as well as wide angle were calculated. But in case of with out polymeric materials (PANI-SDS) there is not possible to calculate the low angle.

B. *Fourier Transform Infrared Radiation Spectrum*

In general principles of Infrared spectroscopy including fourier transform infrared spectroscopy (FTIR) technique measure the absorption of radiation by high frequency (i.e. optical branch) phonon vibrations and they are also sensitive to the presence of particular chemical groups such as hydroxyl (-OH),Methyl (-CH3),Imido(-NH),and Amido(-NH2),Each of these groups absorbs infrared radiation at a characteristics frequency and the actual frequency of absorption varies somewhat with the environment .We discuss some result based on work of FTIR ,PerkinElmer ,Spectrum65,Model no.SP-65 [33].

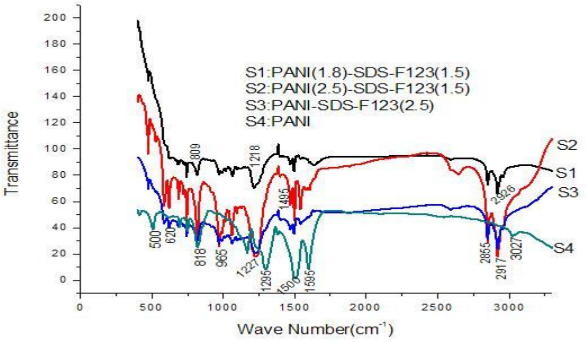


Fig: (1) FTIR spectra of PANI-SDS-F123 samples and study.

FTIR spectra of conventional PANI and its samples with different concentrations have been shown in above spectrum. Fig: 1 shows the polymeric compound of PANI-SDS-F123 with different concentration of F123 from 1.5wt% for spectrum S1, 2.0 wt% for spectrum S2 and 2.5wt% for spectrum S3 and spectrum S4 for PANI. Above spectrum shows bond stretching at various peaks of spectrum. These peaks show different–different compositions at varies concentrations of polymeric surfactant in Fig 1 (a). It shows that high bond energy in C-C shows at wave number of 1462 cm^{-1} to 1545cm^{-1} and C=C stretching at approximate 1500cm^{-1}.This stretching of bonds and vibrations on benzene ring and its conjugate elements gets vibration of quinoid ring and benzene ring. This shows the emeraldine salt (ES) of polyaniline.PANI-SDS-F123 shows C-C stretching overall ranging 1500cm^{-1} to 1545cm^{-1} for (ES) emeraldine salt Fig: 1. Except that remaining peaks came on different wave numbers. The absorptions at alternate wave number like 1220,1500,1462,1063cm^{-1}[34].Fig 2 shows more concentration of aniline monomer with structure direct agent (SDA) compound is PANI-SDS.The peak at 1225cm^{-1} show C-N stretching more absorbance compare to PANI-SDS-F123 polymer.

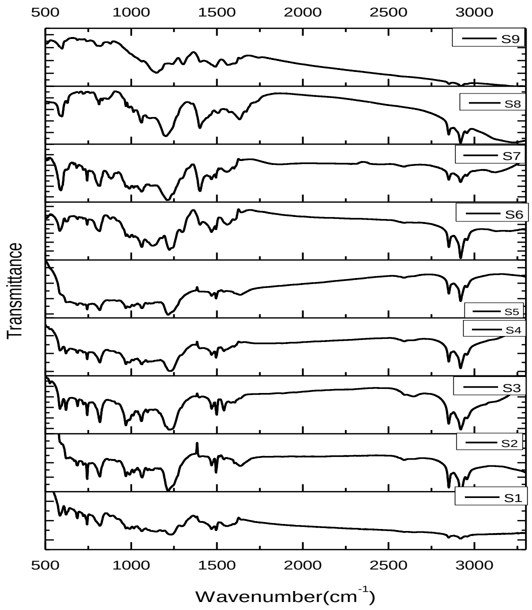


Fig (2) FTIR spectra of PANI-SDS-F123 with PANI and SDS

S1	---	*PANI-SDS*
S2	*1:0.2*	*PANI-SDS-F123*
S3	*1:0.6*	*PANI-SDS-F123*
S4	*1:0.7*	*PANI-SDS-F123*
S5	*1:0.9*	*PANI-SDS-F123*
S6	*1:1*	*PANI-SDS-F123*
S7	*1:3*	*PANI-SDS-F123*
S8	*1:4*	*PANI-SDS-F123*
S9	*6:1*	*PANI-SDS-F123*

The exact wave number at 1220 cm^{-1} shows C-N stretching of benzene ring. There are three peaks of C-N group matching in which one is sharp and one is round shape at exact 1220 cm-1 wave number. These indicate that mixed compositions like polymeric surfactant F123 mixed in varies concentration increases sharpness in spectrum because of nature of emeraldine base increases [35]. The range from 743 to 813 cm^{-1} shows mixed doping of the moiety compounds like high surface area directing agent SDS and F123,and make a dark greenish salt in meso structured form. All samples were in emeraldine oxidation state and conform that pure greenish after stabilized polymerization and purplish colour before oxidation starts [36]. Some peaks are more sharp means highly intense at 1500cm^{-1} and 2900cm^{-1}.It means a complete polymerization with regular arrangement of polymerized material which have high energy in form of absorbance that shows highly intense peak [37].

The compound materials PANI-SDS-F123 is compared with the standard PANI and surfactant SDS, stretching of sulfate ion with –S=O is 1213-1232 cm^{-1} standard. But in this case here 1218 cm^{-1} showing peak is S=O sulfonate stretch. In case of SDS there are –CH- ,-O-CH2- and –O-SO3- bonds are available so every bond has specific peak and bond stretching respectively. Here some peaks are highly sharp which shows that corresponding intensity of the functional group. In case of aeromatic compound meta –CH- strongly stretching shows at 809 to 818 cm^{-1} and –CH- band para shows at 850 cm^{-1}.-SO- stretching shows at Fig: 2 spectrums S9 at 809 to 1069cm^{-1}. In the sample S6 and S7concentration of F123 increases the –CH- started to show weakly stretching at 2850 cm^{-1} and at reducing the concentration of F123 it started to show –CH- stretching at 2950 cm^{-1}. Again we can say that to increase the concentration of aniline monomer it started to stretch at various points like Ortho, Meta and para of the –CH- and –NH- groups. At 2950-3100 cm^{-1}-NH- shows the primary absorption because PANI (6:1) S9 due to high concentration of aniline monomer –CH- strongly stretch i.e. completely disappeared. This is the reason that a sharp comes at 2850-2900 cm^{-1}. Here all vibrational frequencies have been shows of exact composition of PANI-SDS-F123 materials.

C. UV-VIS Absorption spectrum

UV-VIS absorption spectrum mainly used to identify to the band edge absorbance. The instrument is PERKIN ELMER Lantrisant, CF728YW UK LAMBDA 750 Part no. L6020041.Polyaniline haves π-$\pi *$electronic transition in to benzenoid segment and σ bond also. In PANI conductivity mainly causes via $\pi - \pi *$ electronic cloud. So at this level first absorbance band comes by benzenoid segment and other two are due to dominating part like F123 concentration and formation of corresponding polaron at polyaniline after polymerization [39]. Different concentration containing PANI-SDS-F123 shows characteristics absorbance at 400-441,600-640,721-841nm wavelengths. As the concentration of monomer increases corresponding peak increases, maximum peak gets at 841nm and minimum peak gets at 400 nm.

The overall specturm not long falling,because absorption specturm showing that received peaks are shifted from 40 nm wavelength. This shifteing of wavelength is due to band locating of the polaron aroungd the 721-841 nm. At this range shows the formation of band polaron due to F123.PANI shows shifting due to small surfactant SDS micellers at 400-441 and 600-640 nm. After finding the absorption of particular functional group at specific wavelength.In this way we can find out band edge of our semiconductor materials.Band edge depends on the absorption coefficient (α) and related energy $(\alpha h\nu)^2$ of the materials i.e. absorbed [38].

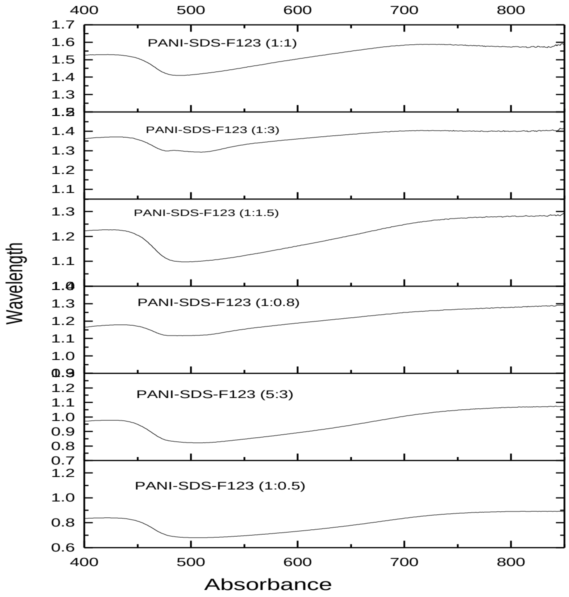
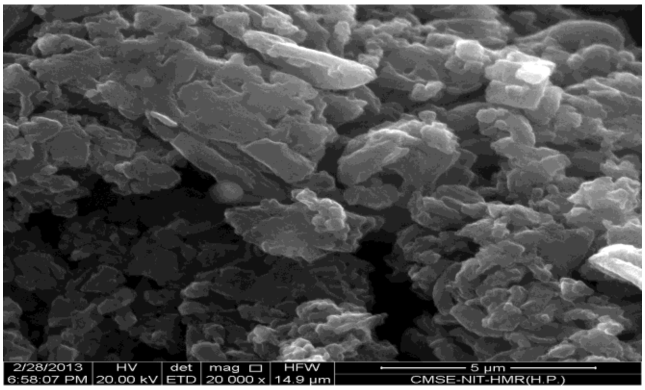


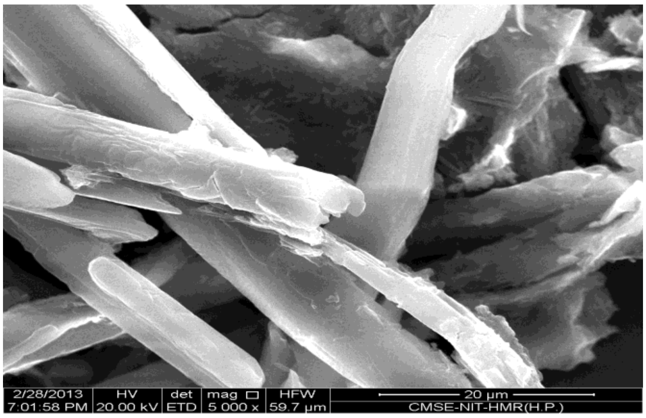
Fig (3) UV Vis absorption spectra for PANI-SDS-F123 synthesized at different aniline and F123 concentrations

D. Scanning Electron Microscopy (SEM) Images

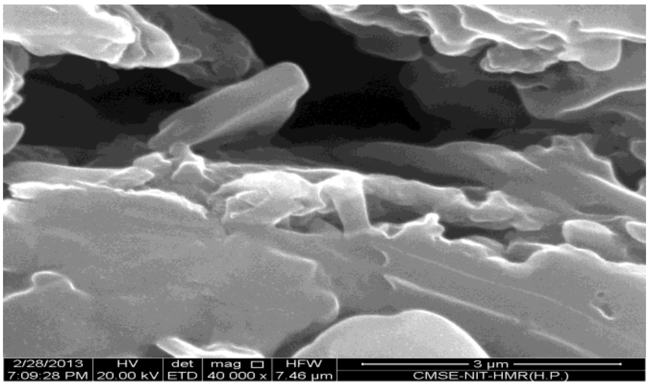
At various concentrations PANI-SDS-F123 materials have been prepared and corresponding SEM images also we have get. The low concentration of SDS as micelle directing agent which polymerized aniline in to nano rods of PANI.



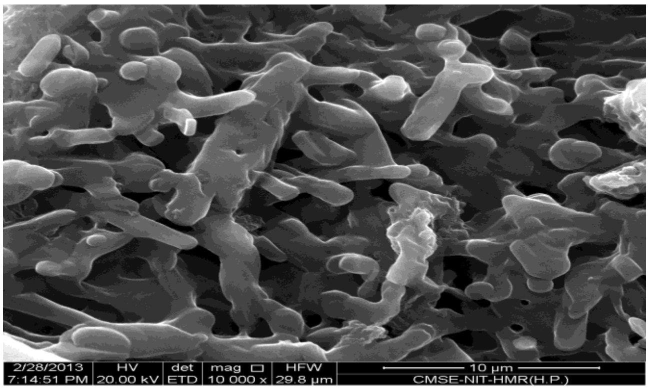
PANI-SDS



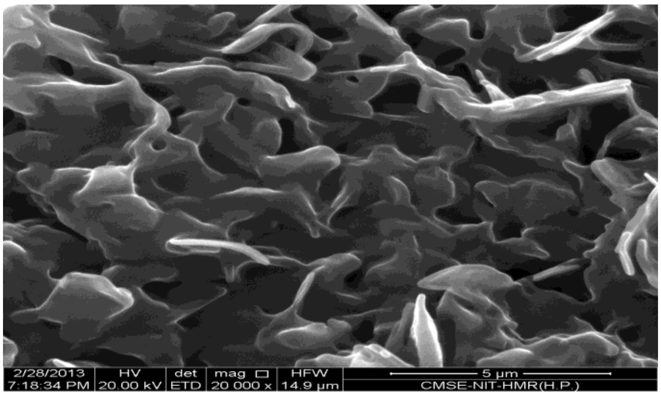
PANI-SDS-F123 (1:0.5)



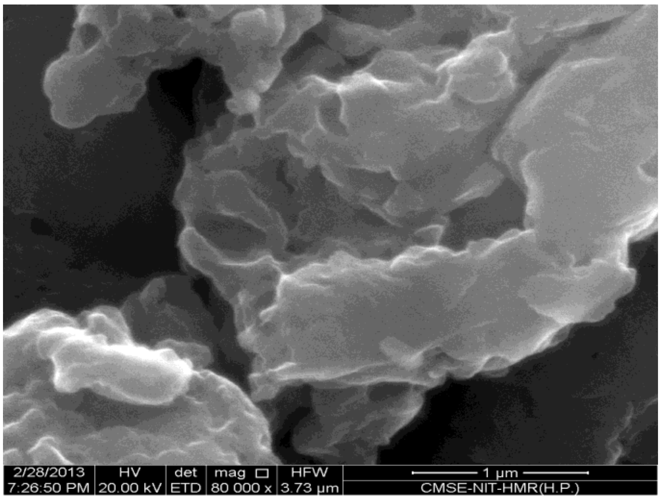
PANI-SDS-F123 (1:0.6)



PANI-SDS-F123 (1:0.8)



PANI-SDS-F123 (1:1.5)



PANI-SDS-F123 (1:3)

Here ratio of sodium dodecyl sulfate (SDS) materials is kept constant through out chemical synthesis of PANI.Where as the changing the concentration of the surfactant (SDS) and co-polymer surfactant F123 in fixed proportion respectively. Here showing SEM images are porous and rod type in nature. This is suitable for involving of enzyme. The porous matrix increases sensitivity of glucose biosensor. So it can enclose the Bioreceptor with enzyme easily for longer time. The scanning electron microscopy (SEM) pictured above.SEM model no. i.e. FEI-QUANTA 450 FES we have used. All pictures bearing the PANI nanofibers and particles at micro level. Above pictures shows uniform distribution of PANI particles where shapes like small leaves. The small nano fibers with 3 µm and some nano fibers 2.5µm diameters. The 1:3 proportion haves 500 nm diameter of nano dendrites. Upper age of size of PANI respond to ~200-500 nm diameters of nanoparticles and length between the two dendrites is 1-3 µm. At higher magnifications, the resolution of image is distorted in last image. It means further we can't resolve to this picture. Here various concentrations varies composition has prepared. So according to change of concentration of monomers as well as surfactant and co-surfactant than affected structure and size of nanofibers. As per concentration of co-surfactant are increases then agglomerate started of PANI and its compositional materials. This effect we can see in 1:0.5 to 1:3 proportion type SEM images [40].

E. A.C. Conductivity Measurement

The electrical conductivity measurement of PANI-SDS-F123 composite material is measured using the LCR meter model no. WAYNE KERR 6520 A [41]. The frequency range of this instrument is 100 Hz-15 MHz's During measurement of a.c. conductivity we have find that increasing order of the conductivity with frequency i.e. ω. There are polaron, bi-polaron, solitons are moves in overall materials surface structure in chain formation so that this phenomenon creates a conductivity at the frequency. So electrical conductivity σac of PANI-SDS-F123 composite shows higher response beyond 1MHz [42].This measurement provides some parameter like, capacitance(C), dissipation factor (D), resistance(R).

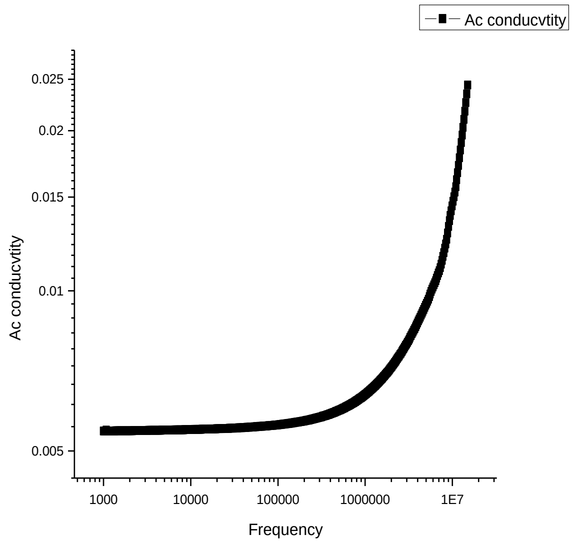


Fig 5 A.C. conductivity changes with the frequency at log scale

The a.c. conductivity we have found out using a relation of under following [42].

$$\Sigma\mathrm{total}\ (\sigma) = \Sigma\mathrm{ac}\ (\sigma) + \Sigma\mathrm{dc}\ (\sigma) \qquad \ldots (1)$$

$$\text{Where } \Sigma\mathrm{ac}\ (\sigma) = 2\pi f\ \varepsilon 0\ \acute{\varepsilon}\ \tan(\delta) \qquad \ldots. (2)$$

Here tan (δ) = loss factor
Cp = Capacitance factor
f= Alternating current frequency
ε0=Permittivity of free space

We have made a small spherical pallet using palletization pressing machine at 150 lb pressure. This pallet has 13.12 mm diameter (D) and thickness (d) is 1.60 mm. Here total conductivity is a.c. as well as d.c. In this case d.c.conductivity is not considered due to a.c. frequency dependent measurement. So a.c. conductivity we have obtained using dielectric constant i.e. έ.

$$\text{Where } \acute{\varepsilon} = (Cp \times d) / (\varepsilon 0 \times A) \qquad \ldots (3)$$

$$\text{And Area of pallet is } = \frac{\pi D^2}{4} \text{ so from here } \frac{d}{A} = 11.84$$

In this way we have find out overall a.c. conductivity and drawn graph in origin then we see that above 1MHz frequency highly increasing the conductivity. Similarly we can draw the relation between dielectric constant and frequency. Which shows that έ decreases w.r.t. the applied frequency.

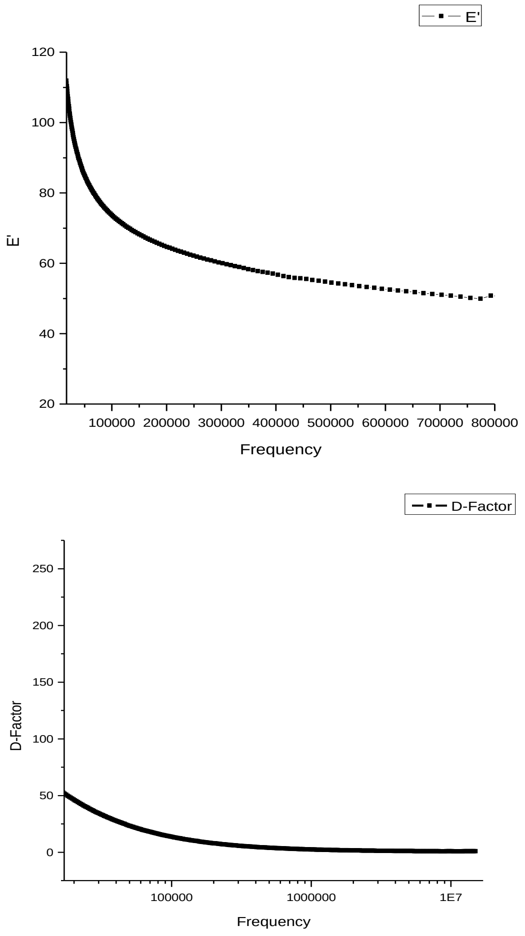


Fig 6 Changes of dielectric constant and loss factor w.r.t. a.c. frequency at room temperature

As the doping gradient is increases then conductivity is increases up a finite level [43]. But concentration of monomer is increases then conductivity is decreases in case of glucose sensing. Because polymerized particles are inter cross linked and agglomerate on the surface of secondary surfactant like F123. Due to this reason the transferring of conjugated ions decreases and conductivity decreases. But at 1:1 ratio of Aniline: F123 shows higher conductivity. Here showing characterization of loss factor and relative dielectric constant w.r.t. frequency. In both cases έ and tan(δ) decreases as the frequency increases. Fig 5 shows sudden increases in conductivity. So such type of materials can be employed in electrical and electronic application like, micro-strip antennas and in solid state devices.

F. Glucose Sensing Characteristics

Amperometric, cyclic voltammeter (CV) measurement has to perform using (CH-Instrument, USA) in a conventional three electrode cell [44].

a) CV Characteristics with H2O2

H2O2 sensing and glucose sensing was performed using CV and continuous amperometry. The electrochemical cell was formed by a three electrode arrangement using Ag/AgCl as the referenced electrode (KCl salt bridge), polymer coated on Pt disk as the working electrode and Pt foil as the counter electrode. In this procedure the sensing study of H2O2 was performed using PANI-SDS-F123 samples. This result shows that PANI-SDS-F123 electrode on successive addition of H2O2 in to 0.1 M Phosphate buffer solution (PBS) (pH~7.0) exhibits higher current than PANI for same concentration of H2O2 as shown in fig7 below.

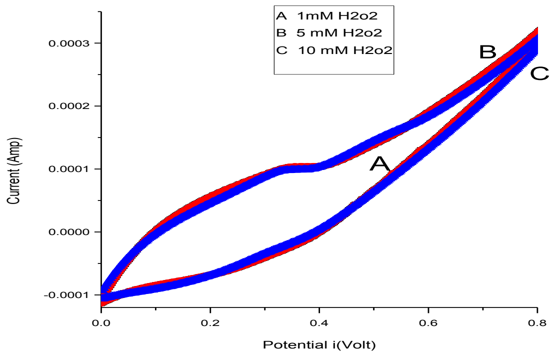


Fig: 7 Cyclic Voltammogram of PANI-SDS-F123

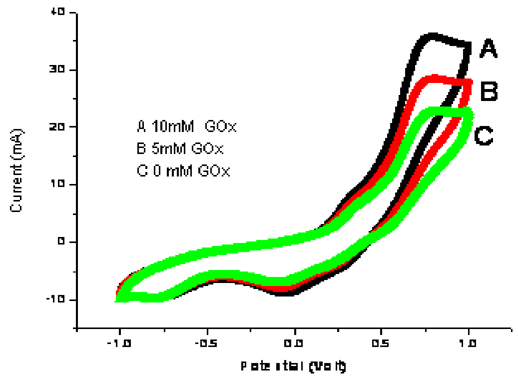


Fig: 8 CVs of planer Pt/PANI-SDS-F123/GOx immobilized electrodes

PANI-SDS-F123 shows electrocatalytic ability in the reduction and oxidation of H2O2 in various concentrations of H2O2 PBS solution [45].

b) CV response with Glucose concentration

Cyclic voltammetry measurements were performed in a three electrode system. The working electrode is Pt disk is fabricated with polymeric materials and counter electrode is Pt. foil and reference electrode is Ag/AgCl/KCl salt. Potential scanned from -0.2-1.0 V with scan rate of 50mV/sec. All three electrodes were to a common potentiostat that control that controls the impedance measurements and immersed in to a conducting solution such as PBS. Fig 8 shows cyclic voltammogram (CVs) of a planer Pt/PANI-SDS-F123/GOx fabricated electrodes in the presence of (enzyme) glucose oxidase. As shown in fig 8 the electrode current in the presence (40mM) of GOx is less than the current in the absence (0 mM) of GOx. In the case of GOx immobilization, the current of the CV is decreased in the absence of the substrate, because of surface of the electrode is covered by GOx [46].

G. *Amperometry H2O2 sensing*

Same quantity of PANI-SDS-F123 i.e. used in CV proceeded in following manner.

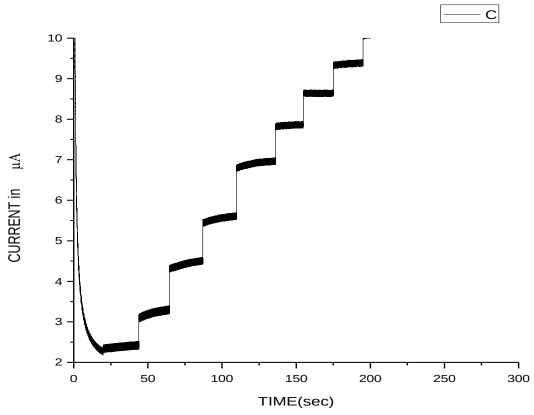


Fig: 9 Amperometric response of H2O2

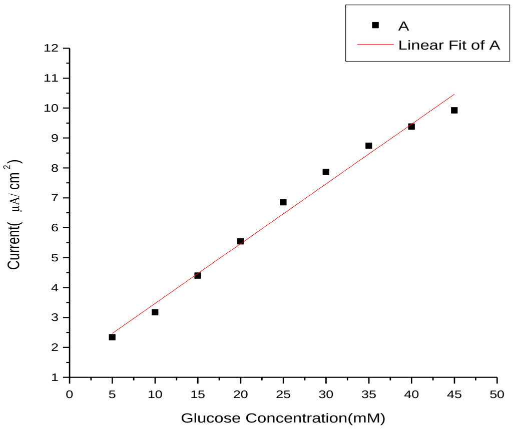
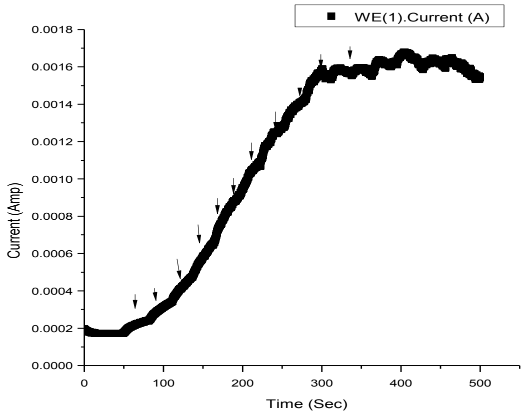


Fig: 10 Calibration curve of H2O2 sensing of electrodes

Amperometric response of the PANI-SDS-F123 and fabricated electrodes from polymer of successive addition of H2O2 and calibration curve for the biosensor response to H2O2 has shows in above figure 9.As per H2O2 added the response of sensing is step wise increasing in reverse order. It doesn’t matter that calibration curve remains in forward order or reverse order beside the step function is increasing order.

H. *Amperometry glucose Sensing*

The current measurements are does by the Amperometric measurements. This technique is based on the current response means oxidation reaction of analytes and time in seconds. It means Amperometric response is based on the current and time characteristics. In experimental part we have to prepare a D-glucose (Grape Sugar) solution and keep it stable up to 24 hr. After that this solution employed under PANI-SDS-F123 and Glucose Oxidase fabricated electrodes at fixed applied potential. Characteristics of current versus time obtained a linear step function. During measurement we have to added 1mM glucose concentration in to the solution at 25 second interval. At last we obtained a linear step sized curved means as concentration added then oxidation reaction takes place and current rises up to fixed amplitude and after that it remains constant up to complete oxidation. It means current response is based on the formation of H2O2 during the oxidation reaction at PANI-SDS-F123 and GODx immobilized electrodes .The electrode oxidation reaction as followed reaction [47].



1mM H2O2 added at every 20 second

Fig: 11 Amperometric Response of Glucose Concentration

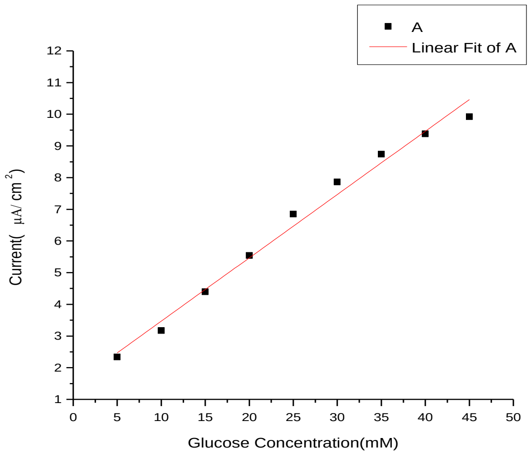


Fig 12 Calibration curves of enzyme electrodes for glucose

D-Glucose + O2 GOx Gluconic acid + H2O2 $\longrightarrow$

H2O2 O2 + $2H^{+}$ + $2e^{-}$ $\longrightarrow$

Where $E^{0} = 0.60\ V\ vs$ Ag/AgCl Standards KCl and GODx represents enzyme Glucose Oxidase in oxidized state and reduced state of enzyme, respectively. The reoxidize current of H2O2 is detected at the electrode in order to determine the glucose concentration. It exhibits a good linearity for sensing glucose in the range 1 mM to 45 mM.

a) *Amperometric Linear Response*

Amperometric response obtained from the figure 10, from which it is clearly seen that the enzyme electrode produces a linear steady state amperometric response up to 50 mM successive addition of glucose concentration [48]. The existence of this linear relationship between the current and concentration of glucose is important for the accurate determination of glucose levels in human body which lies with in the narrow range [49]. Here linear sensing of electrodes analyte PANI-SDS-F123 is 0.199983 $\frac{\mu A}{cm*cm*mM}$ and error of sensing is 0.00871 $\frac{\mu A}{cm*cm*mM}$ ect.

Sensitivity	Limit of Detection (LOD)	Standard Deviation	Materials
2.8284	19.2901	18.18679	PANI-SDS-F123 with Glucose
14.9994	5.014	25.0810	PANI-SDS-F123 with H_2O_2
3.7609	13.3509	16.7372	PANI-SDS-PEG with H_2O_2
7.6716	5.6575	14.4674	PANI-SDS with H_2O_2

Table Limit of detection of sensitivity

I. Effect of pH value of Analytes and GOx Electrodes

The pH of solution has various effects of the structure and activity of enzymes. Changes in pH may not affect only shape of an enzyme but it may also change the shape of charge properties of the substrate so that either the substrate can not bind to the active site, means can not undergo catalysis [50]. The effect of pH on the behavior of the enzyme electrodes was studied with 0.1 M phosphate and acetate buffer solutions containing 1-45 mM glucose. The steady state current at 0.43 V, as a function of the pH value is shown in Fig 8. The electrochemical responses were quite good at pH ranging from 4.0-8.0, and maximum current occurred at about pH~7. During the experimental work it is found that dependent of pH solubilized PANI-SDS-F123/GOx reactant gives maximum current response at pH~7.0-7.2 [51].

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