Polythiophene Gold Nanoparticles Composite Film for Application to Glucose Sensor

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ABSTRACT: Regioregular poly-3-hexylthiophene (regP3HT) and dithiobissuccinimidyl propionate (DTSP) have been used to prepare bifunctionalized gold nanoparticles (Bf AuNPs). Processable properties of regP3HT have been used to obtain regP3HT-AuNPs-DTSP film on a gold-coated glass surface and the succinimidyl group of DTSP in this regP3HT-AuNPs-DTSP/Au electrode has been utilized for covalent immobilization of glucose oxidase (GOx). The UV-visible (UV-vis), scanning electron microscopy (SEM), and Fourier transform infrared (FTIR) spectroscopic studies have been used to characterize regP3HT-AuNPs-DTSP/Au and GOx-regP3HT-AuNPs-DTSP/Au electrode, respectively. This GOx-regP3HT-AuNPs-DTSP/Au bioelectrode shows response time of 10 s, linearity from 25 to 300 mg/dL of glucose and the value of Michaelis-Menten constant as 5.85 m*M* (105.3 mg/dL).
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Key words: gold nanoparticles; regioregular poly-3-hexylthiophene; dithiobissuccinimidyl propionate; glucose biosensor

INTRODUCTION

Nanomaterials and nanocomposites have recently attracted much interest owing to their interesting multifunctional properties.[1–3] There are reports that highly ordered nanoparticle architectures in ultra-thin layers derived from nanocomposites have potential applications in biosensors,[4–7] molecular switches,[8] nanodevices,[9] and nanocircuitries.[10] It may be noted that gold nanoparticles-based composites have been predicted to offer various advantages such as tunability of physical and chemical properties with functional and structural flexibility of respective components for fabrication of various molecular electronic devices including biosensors.[11–14]

Biosensors in the area of health care have acquired much interest due to their important characteristics such as short response time, high selectivity, and stability.[15–17] Progress in the development of enzyme-based biosensor system is critically linked with the advances in materials preparation and availability of suitable immobilization procedures for desired biomolecules on a given surface.[18–20] A large number of techniques have been used for immobilization of desired biomolecules on suitable matrices. However, these methods require numerous tedious procedures including pretreatment in harsh conditions.[21,22] The thrust is now shifting towards the search of suitable matrices including conducting polymers,[21–24] nanomaterials,[25,26] and their composites[27,28] that can be utilized for the immobilization of desired biosensing molecules under mild conditions.

Gold nanoparticles-based composites have recently been reported to provide a suitable matrix for biosensing application.[29] However, it is found that gold nanoparticles and their composites can either be employed in core shell structure or assembled on an electrode surface necessitating chemical modification for binding of given biomolecules.[30–33] There is thus an urgent need for the availability of novel hybrid systems that can be utilized for binding of desired biomolecules without additional treatment. It has been reported that regioregular poly-3-hexylthiophene (regP3HT) can be used to prepare a composite for applications to solar cells, schottky devices, and biosensors etc.[34–36] This has been attributed to the

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processable behavior of regP3HT and its high affinity with gold. However, it may be remarked that this conjugated polymer does not have any functional group for immobilization, necessitating pretreatment for coupling of desired biomolecules.[21]

In search of a suitable matrix that can obviate the need of any crosslinker, we report results of our studies relating to preparation and characterization of a composite film based on gold nanoparticles functionalized by regP3HT and dithiobissuccinimidyl propionate (DTSP). It is shown that presence of gold nanoparticles helps to increase the surface area of the electrode, increased loading of glucose oxidase and it also provides biocompatible environment for biomolecules. Further, the linked DTSP molecules facilitate direct immobilization of biomolecules using succinimidyl group in very mild conditions.

EXPERIMENTAL SECTION

Materials

Hydrogen tetrachloroaurate (III) ($HAuCl_4.3H_2O$), regP3HT, DTSP, tetraoctyl ammonium bromide (TOAB), glucose oxidase (GOx) (200 U/mg), HRP (200 U/mg) were purchased from Sigma-Aldrich. Gold coated glass surfaces were prepared as reported.[37] All other chemicals were of analytical grade and were used without further purification. Deionized water (18.2*M*Ω) obtained from Millipore system was used for the preparation of aqueous solutions.

Preparation of regioregular poly-3-hexylthiophene capped gold nanoparticles

Regioregular poly-3-hexylthiophene capped gold nanoparticles have been prepared by using a two-phase reaction wherein reduction of $HAuCl_4{\cdot}3H_2O$ by sodium borohydride occurs in the presence of regP3HT.[38] Briefly, the tetraoctyl ammonium bromide (0.27 g, 0.5 mmol) (phase transfer reagent) is added to 8 mL of toluene in a 25 mL round bottomed flask and is stirred until dissolved. Then, an aqueous solution of $HAuCl_4{\cdot}3H_2O$ (3 mL, 0.1 mmol) is added and the two phase mixture thus obtained is stirred vigorously until all the tetrachloroaurate ions get transferred to the organic layer. The observed color change from yellow to colorless indicates the transfer of tetrachloroaurate ions. The reg P3HT (15 mg, 0.09 mmol) dissolved in 3 mL of toluene is added to this mixture and is stirred for about 3 h. A freshly prepared aqueous solution of sodium borohydride (2.5 mL, 10 mmol) is added slowly to the mixture with vigorous stirring. After complete addition the mixture is further stirred for overnight. The appearance of dark purple color indicates the formation of regP3HT capped gold nanoparticles. These nanoparticles are retrieved from methanol using ultracentrifugation.

Preparation of bifunctionalized gold nanoparticles (regP3HT-AuNPs-DTSP)

To prepare bifunctionalized gold nanoparticles (Bf-AuNPs), place exchange reaction has been used.[39] For this purpose, the regP3HT capped gold nanoparticles were dissolved in toluene after which 1 m*M* toluene solution of DTSP is added and is kept at 30°C for various durations (2, 4, 6, 8, 12, 24 h) of time for place exchange reaction. After completion of exchange process, the composite solution is washed with methanol and thus prepared Bf-AuNPs are further dispersed in toluene. It may be noted that replacement of regP3HT molecules by DTSP occurs due to higher affinity of sulfur atom present in DTSP molecule than that of regP3HT towards AuNPs. It has been found that about 8 h are sufficient for obtaining desired regP3HT-AuNPs-DTSP composite for uniform film formation onto desired gold-coated glass surface.

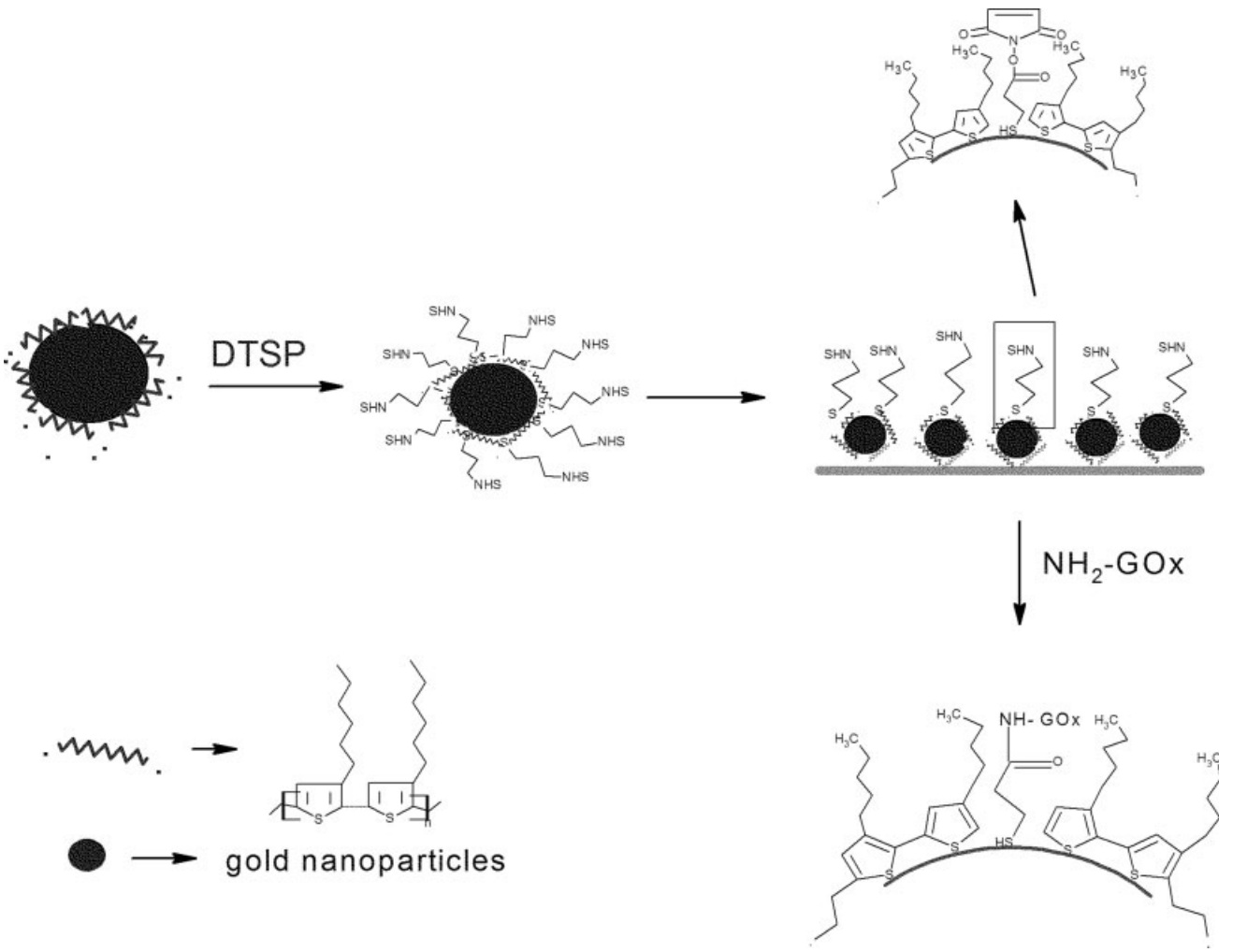
Covalent immobilization of glucose oxidase

The colloidal solution of regP3HT-AuNPs-DTSP composite prepared in toluene has been used to obtain film on pirhana cleaned gold coated glass surface (0.5 cm × 1 cm) using dip coating method. After drying, this regP3HT-DTSP-AuNPs/Au electrode is thoroughly rinsed with deionized water and is immediately utilized for covalent binding of GOx. For covalent immobilization, 20 μL of GOx solution (1 mg/mL) in PBS (50 m*M*, 0.9% NaCl) pH 7.4 is dispensed onto 0.5 cm × 1 cm of regP3HT-AuNPs-DTSP/Au film and is kept for about 4 h in a humid chamber at 4°C. It may be noted that glucose oxidase (GOx) molecules get covalently bound to the regP3HT-AuNPs-DTSP/Au electrode via amine reactive succinimidyl group of DTSP (Scheme 1). The UV-vis spectroscopy, scanning electron microscopy (SEM) and Fourier transform infrared (FTIR) spectroscopy techniques have been used for the characterization of regP3HT-AuNPs-DTSP composite, regP3HT-AuNPs-DTSP/Au electrode, and GOx-regP3HT-AuNPs-DTSP/Au bioelectrode, respectively. The fabricated GOx-regP3HT-AuNPs-DTSP/Au bioelectrode has been used for estimation of glucose in solution using UV-visible (UV-vis) studies.

RESULTS AND DISCUSSION

UV-vis studies

Figure 1 shows the UV-vis absorption spectra of (a) citrate capped gold nanoparticles (AuNPs),[28,40] (b) regP3HT, and (c) regP3HT capped AuNPs. The UV-vis spectra of regP3HT-AuNPs [Fig. 1(c)] in solution



Scheme 1 Covalent immobilization of glucose oxidase onto regP3HT-AuNPs-DTSP/Au electrode.

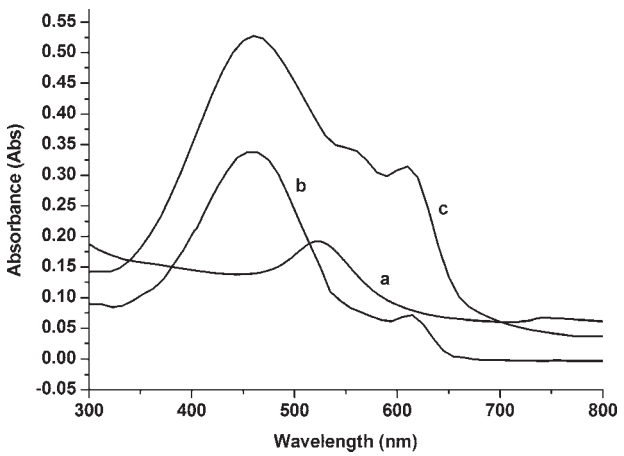


Figure 1 UV-vis absorption spectra of (a) citrate capped gold nanoparticles, (b) reg P3HT in toluene, (c) regP3HT-AuNPs in toluene.

show absorption band at 450 nm and a shoulder at 557 nm. The 450 nm peak reveals the presence of regP3HT molecules arising due to π–π^* transition of conjugated regP3HT chains. The 557 nm peak indicates the formation of regP3HT-AuNPs and is assigned to the surface plasmon of AuNPs. Further, the shift in the surface plasmon resonance of gold nanoparticles from 520 nm in UV-vis spectra of citrate caped AuNPs [Fig. 1(a)] to 557 nm in regP3HT-AuNPs [Fig. 1(c)] is ascribed to the capping of gold nanoparticles with the regP3HT moieties. The results are in agreement with literature.[38]

FTIR studies

Figure 2 shows the FTIR spectra of regP3HT-AuNPs (Fig. 2, curve a) and regP3HT-AuNPs-DTSP comp-

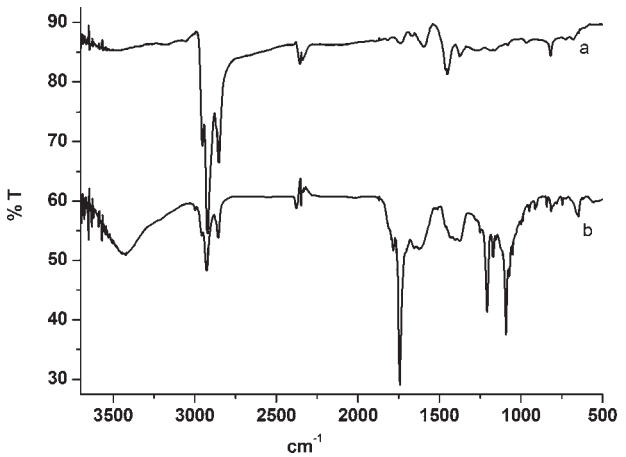


Figure 2 FTIR spectra of (a) regP3HT-AuNPs/Au electrode and (b) regP3HT-DTSP -AuNPs/Au electrode.

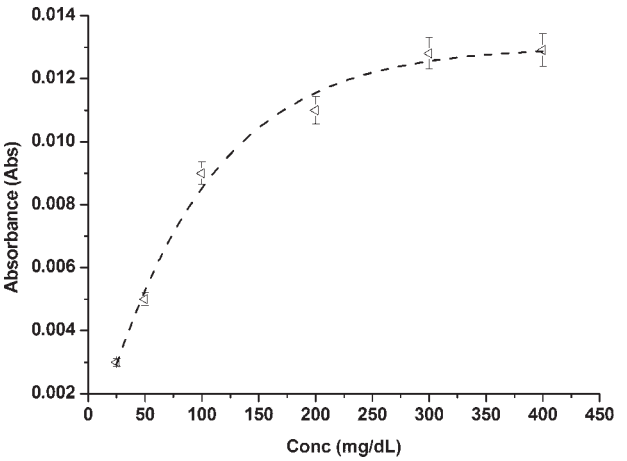


Figure 4 Photometeric response of GOx-regP3HT-AuNPs-DTSP/Au bioelectrode as a function glucose concentration.

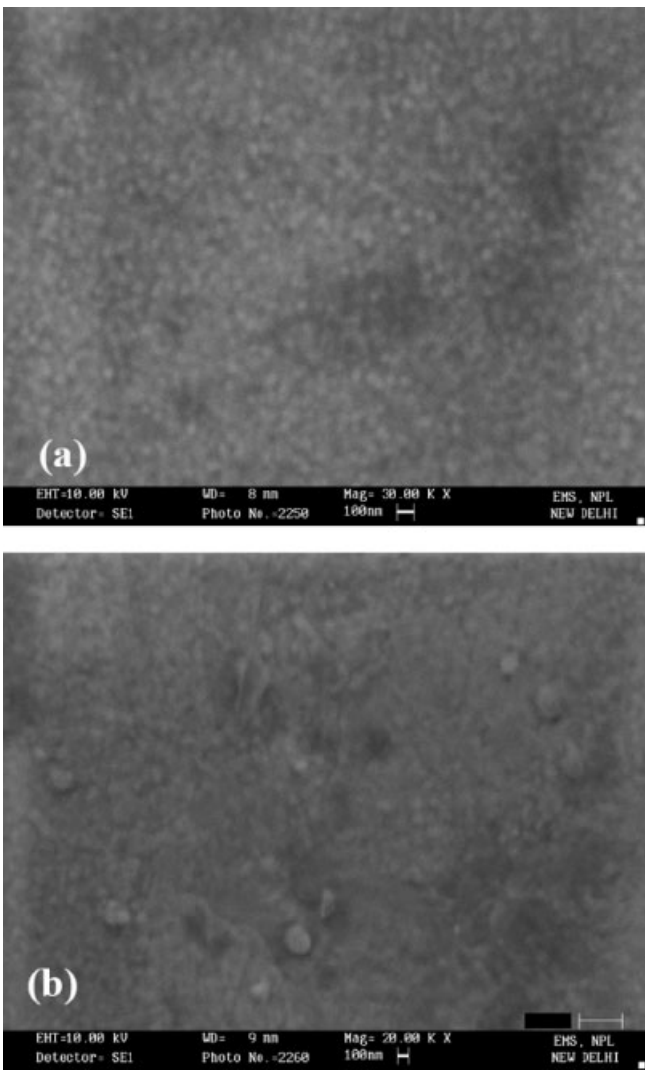


Figure 3 Scanning electron micrograph (a) regP3HT-AuNPs-DTSP/Au electrode and (b) GOx-regP3HT-AuNPs-DTSP/Au bioelectrode.

osite (Fig. 2, curve b). The peaks observed at 2924 cm^{-1} and 2853 cm^{-1} in curve a and b are due to C—H antisymmetric and symmetric stretching, respectively. The peaks appearing at 1740 cm^{-1}, 1410 cm^{-1}, 1640 cm^{-1}, and 1300 cm^{-1} (Fig. 2, curve b) are attributed to C=O, C—O, tertiary amide groups, and C—N stretching, respectively, indicating replacement of some of the regP3HT moieties with the DTSP molecules.

SEM studies

Uniformly distributed spherical structures in scanning electron micrographs of regP3HT-DTSP-AuNPs/Au electrode on gold coated glass surface [Fig. 3(a)] indicate the presence of AuNPs. Figure 3(b) reveals the morphology of the GOx-regP3HT-AuNPs-DTSP/Au bioelectrode, wherein the observed globular structure and reduced graininess could be attributed to the presence of GOx.

Photometric response of GOx-regP3HT-AuNPs-DTSP/Au bioelectrode

Figure 4 shows results of the photometric response studies of GOx-regP3HT-AuNPs-DTSP/Au bioelectrode carried out using UV-vis spectrophotometer at 500 nm. To carry out the photometric enzymatic assay GOx-regP3HT-AuNPs-DTSP/Au bioelectrode is dipped in 3 mL PBS solution (50 m*M*, 0.9% NaCl) pH 7.4, containing 20 μL horseradish peroxidase (HRP, 1 mg/mL in 50 m*M* PBS pH 7.4), 20 μL *o*-dianisidine dye (1% in deionized water), and 100 μL of glucose. The difference between the initial and final absorbance value at 500 nm after 1 min incubation of glucose is recorded and is plotted (Fig. 4) as a function of glucose concentration.

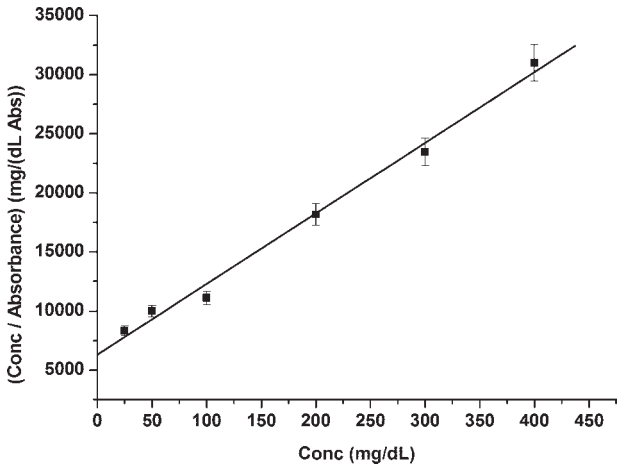


Figure 5 Hanes plot of GOx-regP3HT-AuNPs-DTSP/Au bioelectrode as a function of glucose concentration.

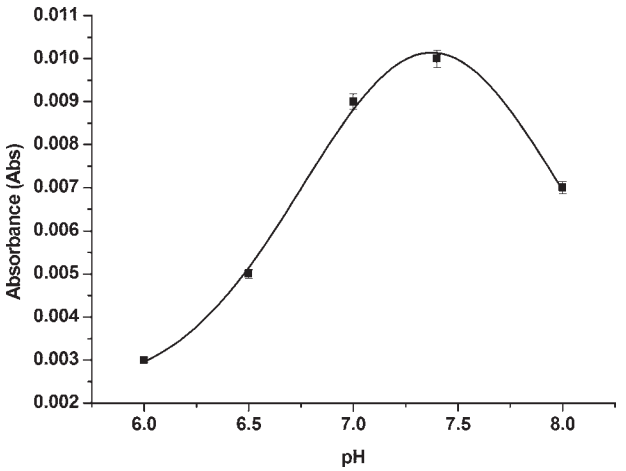


Figure 6 Absorbance response of GOx-regP3HT-AuNPs-DTSP/Au bioelectrode in PBS buffer (50 m*M*, 0.9% NaCl) of pH (i) 6.0 (ii) 6.5 (iii) 7.0 (iv) 7.4 (v) 8.

UV-vis results have been utilized for the estimation of immobilized GOx using eq. (1)

$$a_{\text{app}}^{\text{enz}}\ (\text{U cm}^{-2}) = AV/\varepsilon ts \tag{1}$$

where A is the difference in absorbance before and after incubation, V is the total volume, ε is the millimolar extinction coefficient for *o*-dianisidine (7.5 at 500 nm), t is the reaction time in minutes, and s is the surface area of the electrode.[41] The value of the immobilized GOx has been found to be 8.72×10^{-3} unit/cm^2.

Calculation of apparent Michaelis-Menten constant (K_m^{app})

The value of the apparent Michaelis-Menten constant has been estimated to evaluate the affinity of immobilized GOx for glucose using Hanes plot i.e., the graph between [Concentration] and [Concentration/Absorbance] (Fig. 5). The value of K_m^{app} obtained from the Hanes plot has been found to be 5.85 m*M* (105.3 mg/dL) and is in agreement with the value (5.85 m*M*) for free GOx in solution.[31] These results suggest that the bifunctionalized gold nanoparticles-based composite film can be used for immobilization of desired biomolecule without any loss of its activity.

Effect of pH on GOx-regP3HT-AuNPs-DTSP/Au bioelectrode

GOx modified bioelectrode has been investigated in the pH range of 6.0–8.0 at 30°C. The behavior of GOx-regP3HT-AuNPs-DTSP/Au bioelectrode at various pH (Fig. 6) indicates that the biosensing electrode is most active around pH 7.4. Thus all experiments have been carried out at pH 7.4, which is a biological pH.

Thermal stability and shelf life of GOx-regP3HT-AuNPs-DTSP/Au bioelectrode

The effect of temperature on the reaction kinetics of the immobilized GOx investigated in the range of 15–50°C. Figure 7 reveals that the photometric response increases with increasing temperature, upto ~ 40°C where after it decreases. The results indicate that GOx is partly denatured at temperature >40°C.

The shelf life of the GOx-regP3HT-AuNPs-DTSP/Au bioelectrode has been investigated using UV-visible studies for a given concentration of glucose at regular intervals of 15 days for 4 months. It has been found that immobilized GOx enzyme retains about 97% of its activity even after about four months (data not shown).

Table I shows results of these studies along with those reported in literature. It can be seen that the regP3HT-AuNPs-DTSP/Au electrode provides GOx friendly environment without any deformation in its structure during the covalent attachment.

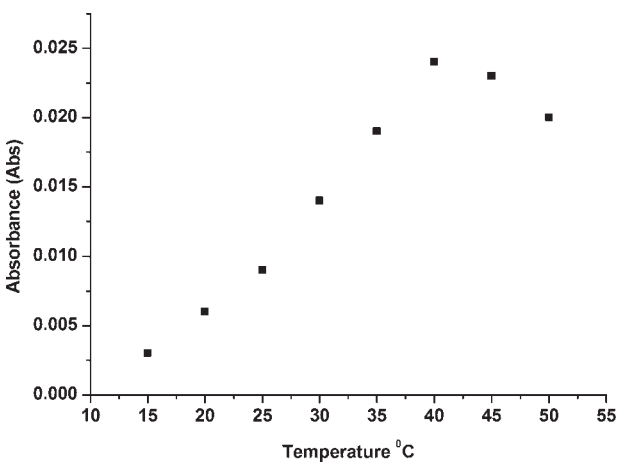


Figure 7 Effect of temperature on response of GOx-regP3HT-AuNPs-DTSP/Au bioelectrode.

TABLE I
Characteristics of GOx Modified Gold Nanoparticles Composite (GOx-regP3HT-AuNPs–DTSP/Au) Bioelectrode for Glucose Sensing Along with Those Reported in Literature

Matrix	Procedure of biomolecule immobilization	Number of steps involved in biomolecule immobilization	Characteristics	References
GOx/AuNPs/PtNPs/CNT electrode/Au	EDC-NHS (Covalent)	Six steps	L.R.−0.5–17.5 m*M*	42
Aminated silica nanoparticles (AsNPs)/glucose oxidase	Glutaraldehyde (Covalent)	Layer by layer	L.R.−8 m*M*, D.L. −9 μ*M*	43
Aminated iron oxide nanoparticles/GOx (solution)	Glutaraldehyde (Covalent)	Two	D.L.−20 m*M*	44
Gox/Au NP/Cyst/AuE	Covalent	Four	L.R.−0.01–10 m*M*	45
Thiolated gold nanoparticles/ GOx (solution)	EDC/NHS (Covalent)	Two	Shelf life 180 days, L.R. −15 m*M*	31
GOx/AuNPs/CPE	Electrostatic		L.R. −0.04 to 0.28 m*M*, D.L.−0.01 m*M*	46
MPTS/AuNps/Aue	Covalent	Three	L.R.−4 × 10^{-10}*M* to 6*M*	47
IO_4–oxidized-GOx/ GNPs-SBA-15/Au	2-aminoethanethiol (Cross-linker)	Four	L.R.−0.02 to 14 m*M*, R.T. 〉 7s	48
regP3HT-AuNPs-DTSP/	DTSP (Direct, Covalent)	Two	L.R.−2.7 to 17 m*M*, R.T.−10 s	Present work

Pt NP, platinum nanoparticles; Cyst, cystiene; CPE, corbon paste electrode; MPTS, mercapto propyl triethoxy silane; AsNPs, aminated silica nanoparticles; SBA-15, mesoporous silica-15, L.R., linear range; D.L., detection limit; R.T., response time.

CONCLUSIONS

RegP3HT and DTSP have been used to prepare bifunctionalized AuNPs by place exchange reaction. RegP3HT-AuNPs-DTSP derived electrode has been prepared by dip coating on desired gold coated glass surface. The fabricated GOx-regP3HT-AuNP-DTSP/Au bioelectrode shows response time of 10 s, linearity from 25 to 300 mg/dL and the value of Michaelis-Menten constant as 5.85 m*M* (105.3 mg/dL). It is revealed that reg P3HT-AuNPs-DTSP/Au electrode obviates the need of crosslinking chemistry and is an interesting matrix for immobilization of desired biomolecule. Efforts should also be made to undertake measurements in real samples and to test the effect of various interferents on the response of GOx-regP3HT-AuNPs-DTSP/Au bioelectrode. Besides this, attempts should be made to utilize the regP3HT-AuNPs-DTSP/Au matrix for immobilization of other biomolecules such as DNA, urease, and cholesterol oxidase etc.

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