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Enzyme integrated silicate–Pt nanoparticle architecture: A versatile biosensing platform

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ABSTRACT

A novel 3-D nanoarchitectured platform based on Pt nanoparticles (nPts) is developed for the sensing of sub-nanomolar levels of hydrogen peroxide and for the fabrication of amperometric biosensor for uric acid, cholesterol and glucose. The nPts have been immobilized on the thiol functional group containing sol–gel silicate 3-D network derived from 3-mercaptopropyltrimethoxysilane (MPTS). The nanoparticles on the 3-D architecture have size distribution between 7 and 10 nm. The nPts on the platform efficiently catalyze the oxidation of H_2O_2 at the potential of +0.45 V in the absence of enzymes and redox mediators. This nanoarchitectured platform is highly sensitive and can detect H_2O_2 at sub-nanomolar levels (0.1 nM) in neutral solution. The nanoarchitectured platform does not suffer from interference due to other common easily oxidizable interfering agents. Excellent reproducibility, long-term storage and operational stability are observed. This platform is used to determine H_2O_2 concentration in rainwater and for the fabrication of biosensors. Amperometric oxidase-based biosensing platforms are developed by integrating the enzymes and nPts with the silicate network for the sensing of uric acid cholesterol and glucose. The enzyme encapsulated 3-D architecture retains the enzymatic activity and efficiently detects enzymatically generated H_2O_2 without any interference. These biosensors are stable and show excellent sensitivity and fast response time. A linear response was obtained for a wide concentration range of all analytes. The practical utilization of the biosensor for the measurement of uric acid, cholesterol and glucose in serum sample is demonstrated. The biological sample analysis was validated with clinical laboratory measurements.

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1. Introduction

Tailoring of electrochemical interfaces with nanomaterial has gained enormous interest in the field of electrocatalysis and in the development of electrochemical sensors/biosensors (Zhong and Maye, 2001; Katz and Willner, 2004; Shipway et al., 2000). Highly dispersed metal particles are known to exhibit exceptional catalytic activity toward several reactions (Zhong and Maye, 2001; Narayanan and El-Sayed, 2004). The catalytic performance of these nanostructured particles is sensitive to their size, surface structure, coverage and catalyst support (Yan et al., 2009). Nanometer-sized Au and Pt particles have attracted special attention in the development of amperometric biosensors, biofuel cells, bioreactors, etc. (Shipway et al., 2000; Yan et al., 2009; Kim et al., 2008). The unique properties of nanoparticles offer excellent prospects for designing miniaturized biosensing devices with exceptional performance and the ability to tailor the properties of the nanostructured particles opens a way for improving the performance of nanomaterial-based biosensors. Although Au nanoparticles have been extensively used in the design of chemical and biochemical sensors in the past two decades (Katz and Willner, 2004; Daniel and Astruc, 2004; Pingarron et al., 2008; Oyama, 2010), Pt nanoparticles have only recently received much attention in the development of electrochemical sensors (Hrapovic et al., 2004; Polsky et al., 2006; Yang et al., 2006; Gong et al., 2010; Ai et al., 2009; Wen et al., 2009; Ahmad et al., 2010; Chen and Holt-Hindle, 2010).

Development of sensitive methods for the sensing of H_2O_2 is of great interest in many different areas (You et al., 2003; Karam and Halaoui, 2008; Sanford et al., 2010; Li et al., 2009). H_2O_2 is the product of the reaction catalyzed by a large number of oxidase enzymes. The operating principle of the oxidase-based amperometric biosensor involves the determination of enzymatically generated H_2O_2. A highly sensitive and selective transducer is very essential for the precise monitoring of H_2O_2 generated during the enzymatic reaction. Moreover, H_2O_2 is the most valuable marker for oxidative stress; oxidative damages resulting from the cellular imbalance of H_2O_2 and other reactive oxygen species generated from H_2O_2 are connected to aging and severe human diseases like cancer and cardiovascular disorders (Lazrus et al., 1985; Yorek, 2003). Furthermore, it is recognized that H_2O_2 has an important role as an

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oxidant in the conversion of dissolved SO_2 to H_2SO_4, causing acidification of the aqueous phase of the troposphere (Rodrigo and Rivera, 2002). The concentration of H_2O_2 in natural water ranges from sub-nanomolar to micromolar level (Gunz and Hoffmann, 1990).

The carbon paste, carbon nanotube, redox mediator, peroxidase enzyme, etc. based electrodes have been used for H_2O_2 sensing (King et al., 2007; Karyakin et al., 2004; Kong et al., 2010; Ruzgas et al., 1996; Lindgren et al., 2000; Jia et al., 2002; Wang et al., 1999; Peng et al., 2009; Chen et al., 2009). The mediator-modified electrodes are generally unstable, probably due to the leaching of mediator from the electrode surface. The unmodified polycrystalline Pt electrode has also been widely used for the detection of H_2O_2 (Hall et al., 1998; Han et al., 2006; Prabhu et al., 1981; Zhang and Wilson, 1993). However, deactivation of the electrode surface is one of the problems with the Pt-based electrodes (You et al., 2003). Therefore, several attempts have been made to improve the performance of Pt based electrodes (Evans et al., 2002; Sandison et al., 2002; Kicela and Daniele, 2006; Karam and Halaoui, 2008; Li et al., 2009; Chakraborty and Raj, 2009).

It has been demonstrated both theoretically and experimentally that the electrochemical platforms based on microelectrode arrays and nanoelectrode ensembles show significantly low detection limits with respect to the analogous macrosized electrode counterpart (Reller et al., 1984; Penner and Martin, 1987; Ugo et al., 2002). The low detection limit achieved with such platform is due to the enhancement of the signal-to-noise (S/N) ratio. Our group is interested in the development of electrochemical sensors based on nanomaterials and self-assemblies for the sensing of biomolecules (Jena and Raj, 2006a,b; Raj and Behera, 2005; Chakraborty and Raj, 2007). In continuation of our earlier efforts, herein we describe the development of a highly sensitive nanoarchitecture based on nPts for the sensing of H_2O_2 and biosensing of clinical analytes such as uric acid, cholesterol and glucose. The electrocatalytic activity of surface confined Pt particles was combined with the enzymatic activity of oxidase enzymes to develop the novel nanoarchitectured biosensor. The biosensor is constructed by integrating both nPts and oxidase enzyme with the sol–gel derived silicate network. The analytical performance of the nanoarchitectured biosensing platform with respect to sensitivity, selectivity, limit of detection and response time is described.

2. Materials and methods

2.1. Materials

H_2PtCl_6, MPTS, glucose oxidase (GOx), uricase (UOx), cholesterol oxidase (ChOx) and cholesterol esterase (ChEs) were obtained from Sigma–Aldrich and used as received. GOx (from Aspergillus Niger, 100 units/mg), UOx (from Candida sp., 5.3 units/mg), ChOx (from Streptomyces sp. 25 units/mg) and ChEs (from Pseudomonas sp. 10,000 units/g) were obtained as lyophilized powder. Hydrogen peroxide (30%) was obtained from Merck Ltd. All other chemicals (Merck Ltd.) were of analytical grade unless mentioned otherwise. All the solutions were prepared with Milli-Q (18 MΩ cm) deionized water.

2.2. Instrumentation

Electrochemical measurements were performed using a two-compartment, three-electrode cell with a polycrystalline Au working electrode, a Pt wire auxiliary electrode and Ag/AgCl (3 M NaCl) reference electrode. Cyclic voltammograms were recorded using a computer controlled CHI643B electrochemical analyzer (CH Instruments, Austin, TX). All the electrochemical experiments were carried out under an argon atmosphere. Transmission electron microscope (TEM) images of nPts were obtained using a transmission electron microscope (JEOL JEM 2010 Electron Microscope) operating at 200 kV. Scanning electron microscopic measurements were performed with a JEOL JEM 6700F field emission scanning electron microscope (FESEM). An Oxford ISIS-300 microanalytical system attached to the JEOL JMS-5800 scanning electron microscope was used for the EDS measurement.

2.3. Synthesis of Pt nanoparticles

Colloidal nPts were synthesized as previously described (Jena and Raj, 2008). The size and shape of nPts were examined TEM measurements. The nPts have near-spherical shape with an average size of 9 ± 1 nm (Supporting Information).

2.4. Preparation of MPTS sol-enzyme biocomposite

The MPTS sol was prepared by mixing MPTS, HCl and ethanol (Jena and Raj, 2006a,b). For the fabrication of the biosensor, the MPTS sol-enzyme biocomposite was prepared by the following procedure: first the MPTS sol was prepared by taking 24 μL MPTS and 10 μL HCl (0.1 M) in 2 mL water and stirring the mixture vigorously for 30 min. Then 0.5 mL MPTS sol was mixed with 0.5 mL of enzyme solution (GOx or UOx, 20 mg/mL in 5 mM phosphate buffer solution of pH 8) and stirred for 2–3 min for the encapsulation of enzymes into the silicate network. For the fabrication of the cholesterol biosensor, cholesterol esterase (10 mg/mL) and cholesterol oxidase (20 mg/mL) were mixed with MPTS sol as previously described. The resulting sol–gel biocomposite was stored at 4 °C.

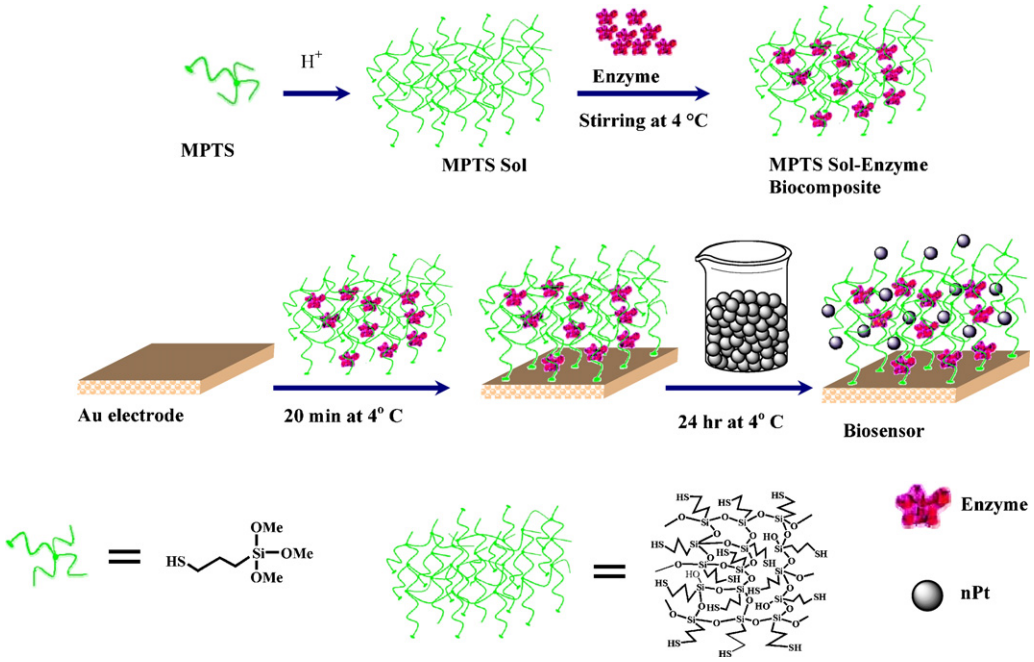
2.5. Self-assembling of nPts on sol–gel network

The nPts were self-assembled on the MPTS network and characterized according to our previous methods (Jena and Raj, 2008). Briefly, an electrochemically cleaned polycrystalline Au electrode (geometrical surface area 0.02 cm^2) was soaked in 0.5 mL MPTS sol for 10 min. The MPTS sol is known to chemisorb on the polycrystalline Au electrode surface and exists as 3-D silicate network. Then the nPts are chemisorbed on the thiol groups of the silicate network by soaking the MPTS modified electrode in colloidal nPts for 24 h. Hereafter, the MPTS sol modified and nPts self-assembled electrodes will be referred as MPTS and nPt electrodes, respectively. For FESEM measurements, gold-coated cover slips were used instead of polycrystalline Au electrode.

2.6. Fabrication of biosensor

The nanoarchitectured biosensor was fabricated according to Scheme 1. The cleaned polycrystalline Au electrode was first soaked in MPTS sol-enzyme biocomposite for 20 min for the spontaneous adsorption of enzyme encapsulated sol–gel network on to the electrode surface. The nPts were then self-assembled on the free thiol groups of the enzyme encapsulated sol–gel network at 4 °C by soaking the enzyme encapsulated silicate network modified electrode.

Phosphate buffer solution (PBS) of pH 7.2 was prepared from NaH_2PO_4 and Na_2HPO_4 and used as a supporting electrolyte in all experiments. Cholesterol stearate solution was prepared in 1% NaCl solution using the surfactant Triton-X 100 (0.2%, v/v). Freshly prepared solutions were used in all the experiments. The amperometric measurements of H_2O_2, glucose and cholesterol were performed at pH 7.2 whereas the uric acid measurements were carried out at pH 8.5. The supporting electrolyte solution was stirred at the rate of 200 rpm during the amperometric measurements. All the experiments were repeated at least 3 times and reproducible results were obtained. The calibration plots for the sensing of H_2O_2,



Scheme 1. Scheme illustrating the fabrication of a nanoarchitectured biosensor.

glucose, uric acid and cholesterol were generated from three independent measurements.

2.7. Real sample analysis

Filtered blood serum samples were collected from B.C. Roy Technology Hospital, Indian Institute of Technology, Kharagpur; the samples were stored in the dark at 4 °C. An aliquot of 5 μL of the serum sample was injected into 5 mL supporting electrolyte solution (PBS, pH 7.2) and the amperometric current was measured after attaining the steady state. Rainwater was collected at the Indian Institute of Technology campus and was analyzed immediately after collection.

3. Results and discussion

3.1. Characterization of nPts on the sol–gel network

Fig. 1 is the FESEM images of nPts on MPTS network and nanoarchitectured biosensor. The nPts have an average size of 9 ± 1 nm and have near-spherical shape. The inter-particle distance is very small and the nanoparticles are closely packed. The nanoparticle-modified network can be considered as a nanoparticle ensemble. The binding of nPts was confirmed by FTIR, XPS and electrochemical measurements according to the previously reported procedures (Jena and Raj, 2008). FESEM image of the integrated enzyme-nanoparticle assembly shows bright-island like aggregate structures for the enzyme (Fig. 1b). The existence of both nanoparticles and enzyme throughout the network would favor access to substrate. The surface area of electrochemically accessible nPts on the silicate network was calculated from the voltammogram obtained in 0.5 M H_2SO_4. The charge consumed during hydrogen adsorption or desorption was used to calculate the surface area (Trasatti and Petrii, 1991) and it was calculated to be 0.056 ± 0.01 cm^2.

3.2. Electrochemical oxidation of H_2O_2

The main objective of the present investigation is to develop a highly sensitive H_2O_2 sensing platform and to fabricate oxidase biosensors using the nanoarchitectured electrode. First we have investigated the electrocatalytic property of nPts on the sol–gel network toward oxidation of H_2O_2. It is well known that the direct oxidation of H_2O_2 on bare electrode requires a high overpotential. On the bulk Pt electrode, oxidation of H_2O_2 occurs at potentials >0.5 V and the voltammetric response is ill-defined and not repro-

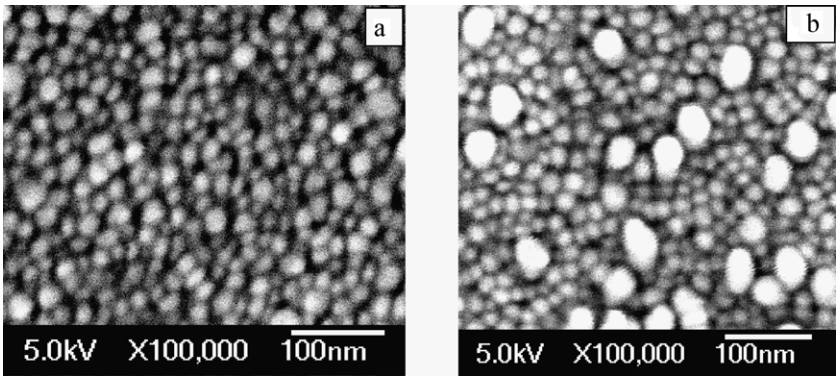


Fig. 1. FESEM images obtained for (a) nPts on the silicate network and (b) nanoarchitectured glucose biosensor.

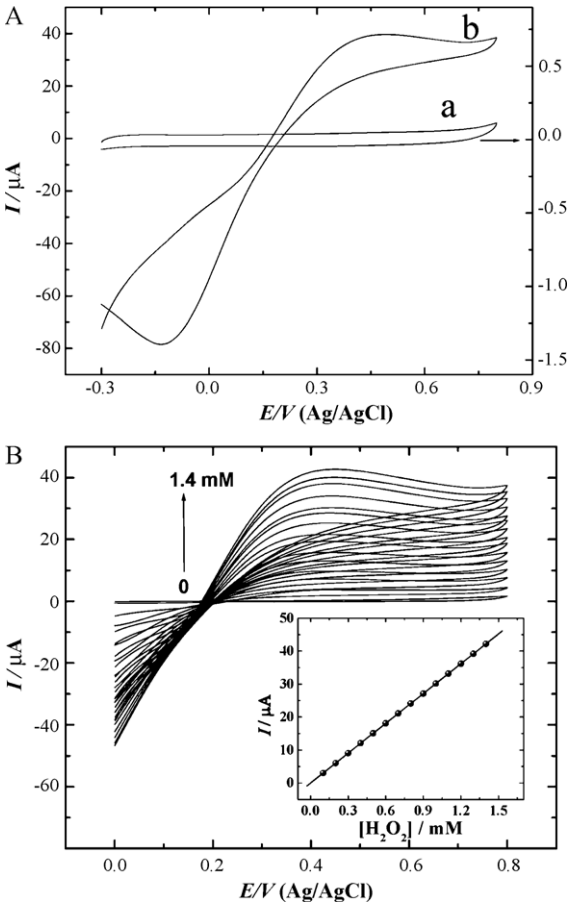


Fig. 2. (A) Cyclic voltammograms obtained for the nanoarchitectured platform in the absence (a) and presence (b) of H_2O_2 in 0.1 M PBS. (B) Voltammetric response at different concentrations of H_2O_2. Each addition increased the overall H_2O_2 concentration by 0.1 mM. Scan rate: 50 mV/s. Corresponding calibration plot is shown as the inset.

ducible; gradual changes in the voltammetric peak position and peak height were noticed in subsequent voltage sweeps. On the other hand, the MPTS electrode does not show any characteristic voltammogram for H_2O_2; the silicate network on the electrode surface impedes the permeation of H_2O_2. However, as shown in Fig. 2A, the nPt electrode displays a well-defined voltammogram for H_2O_2 and the oxidation peak appears at ~0.45 V. A significant decrease in the overpotential and enhancement in the peak current with respect to bulk Pt electrode were observed. Such a decrease in the overpotential is ascribed to the high catalytic activity of the surface confined nPts. The nPts facilitate the electron transfer and decrease the overpotential for the oxidation of H_2O_2. The voltammetric response is very stable and the peak position and peak height did not change upon subsequent voltage sweeps. A gradual increase in the peak current during each addition of H_2O_2 was observed (Fig. 2B). The peak current linearly increase with increase in concentration of H_2O_2, and the calibration plot (Fig. 2B, inset) is linear upto 1.4 mM.

The analytical performance of the electrode was further examined by recording the amperometric response at different concentrations of H_2O_2. Fig. 3 displays the amperometric trace obtained for the oxidation of H_2O_2 on the nPt electrode. The electrode was polarized at +0.45 V and aliquots of H_2O_2 were injected into a stirred supporting electrolyte solution. A fast and stable response was obtained upon each injection; the response time was 2 s. The sensitivity and the experimental limit of detection

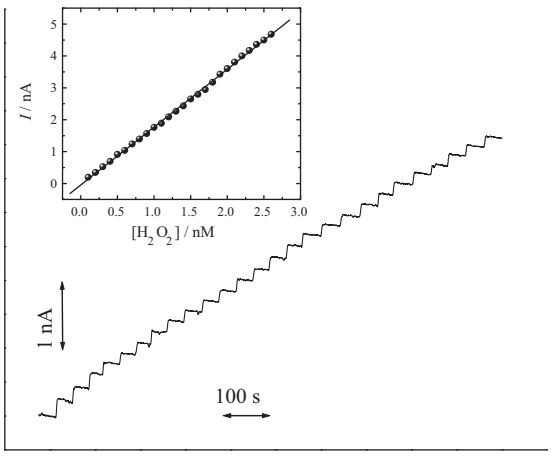


Fig. 3. Amperometric *i–t* curve for the detection of H_2O_2 with the nanoarchitectured platform. H_2O_2 (0.1 nM) was injected into the stirred 0.1 M PBS at regular time intervals. The electrode was polarized at +0.45 V. Inset shows the corresponding calibration plot.

(S/N = 9) were found to be 1.82 ± 0.01 nA/nM and 0.1 nM, respectively. Interestingly, this is the first time such a low detection limit has been achieved in the absence of any enzyme or redox mediator. The 3D nanoarchitecture design of the electrode surface allows the facile access of the reactant to the catalyst site and the rapid removal of the product by diffusion to solution from the film.

3.3. Measurement of H_2O_2 in rainwater

The analytical utility of the present electrode was demonstrated by measuring the concentration of H_2O_2 in rainwater. Four samples were collected and analyzed amperometrically. A stable amperometric response was obtained upon each injection of rainwater (Supporting Information). Addition of standard H_2O_2 solution resulted in a linear relationship between the measured amperometric current and the concentration of H_2O_2. The total concentration of H_2O_2 in the rainwater was in the range of 150–170 nM (Table 1S, Supporting Information). The recovery efficiencies for the spiked H_2O_2 sample were 92–94%. The concentration of H_2O_2 in rainwater was close to values previously reported (Marle and Greenway, 2005). However, it should be noted that the concentration of H_2O_2 in rainwater depends on various factors (Deng and Zuo, 1999) such as season, place of collection and air pollutants. Therefore, the concentration of H_2O_2 in multiple rainwater samples need not be the same.

3.4. Selectivity

The elimination of interferences due to easily oxidizable compounds present in the physiological system is a real challenge in the voltammetric detection of H_2O_2. Ascorbic acid, uric acid and paracetamol are the major interfering analytes present in the physiological system. To examine the performance of nanoarchitectured electrode in the presence of interferents, amperometric response of the electrode toward H_2O_2 was recorded in the presence of ascorbic acid, uric acid and paracetamol. The amperometric response of the nPt electrode was first registered by injecting H_2O_2 (30 μM) into a stirred supporting electrolyte and aliquots of ascorbic acid, uric acid and paracetamol (0.1 mM each) were injected subsequently at regular time intervals. Interestingly, no observable change in the steady state current for H_2O_2 was noticed upon the addition of each interferent, indicating that the present electrode can be success-

fully used for the measurement of H_2O_2 in the presence of common interferents (Supporting Information).

3.5. Stability and reproducibility

The long-term storage and operational stability of the sensor is essential for continuous and reliable monitoring of H_2O_2. The stability of the present H_2O_2 sensor was evaluated by using the same transducer for 20 repetitive measurements in a supporting electrolyte solution containing 5 μM of H_2O_2. This electrode was subjected to another 20 repetitive measurements after a period of 24 h. No change in the peak potential and peak current for the oxidation of H_2O_2 was observed in both sets of experiment. The coefficient of variation in the peak current between both sets of experiments was calculated to be 0.12%, confirming that the electrode is stable and does not undergo deactivation; the sensor can be successfully used for repeated measurements.

To further ascertain the operational stability of the present sensor, the amperometric response of the sensor was recorded in a supporting electrolyte solution containing 50 μM of H_2O_2 while polarizing the electrode at +0.45 V. A stable amperometric response was obtained over long time duration (Supporting Information). Furthermore, the long-term storage stability of the sensor was evaluated by measuring the electrode response for a period of 15 days. The amperometric response of the electrode was recorded every day and the electrode was stored in PBS at pH 7.2 between measurements. No change in the peak current or peak potential was observed over a period of 11-day (Supporting Information). Only 9% decrease in the peak current was detected after 15 days, demonstrating that the transducer is highly stable and superior to existing H_2O_2 transducers. The reproducibility of the results was examined with four different electrodes and identical results were obtained with all the electrodes.

Comparison of the analytical performance of the present electrode with other electrodes based on Pt nanoparticles (Hrapovic et al., 2004; Ai et al., 2009; Xiao et al., 2009) mesoporous Pt microelectrodes (Evans et al., 2002), Prussian Blue (Karyakin et al., 2004), and CNT-based electrodes (Hrapovic et al., 2004; Tsai et al., 2005) reveals the present electrode exhibits excellent sensitivity, a low detection limit and superior stability. The mesoporous Pt electrode exhibits a detection limit in the micromolar range (Evans et al., 2002), whereas the singlewall CNT/Pt nanoparticle electrode (Hrapovic et al., 2004) shows a detection limit of 25 nM (S/N = 3) at +0.55 V, which is 100 mV more positive than our electrode. In the case of CNT/Pt nanoparticle-based electrode, high sensitivity and reproducibility were obtained only at low concentrations of H_2O_2. The Prussian blue-based electrode could monitor 10 nM of H_2O_2 level under hydrodynamic condition (Karyakin et al., 2004). However, the long-term stability of the electrode was not evaluated. On the CNT modified electrodes, oxidation of H_2O_2 occurred at a more positive potential (>0.75 V) (Tsai et al., 2005). The polyacrylate capped Pt nanoparticle on a polyelectrolyte modified electrode has been recently used for sensing H_2O_2 (Karam and Halaoui, 2008). This electrode could detect as low as 42 nM of H_2O_2 at a potential of +0.6 V, which is 150 mV more positive than our electrode. Recently, Peng et al. (2009) achieved a detection limit of 0.64 nM at +0.5 V, which is still 50 mV more positive than the present system. This method required a mediator and horseradish peroxidase enzyme. The carbon fiber-based microelectrode has been recently used for the detection of H_2O_2 (Sanford et al., 2010) and it could detect 2 μM of H_2O_2. The performance of the present nanoarchitectured electrode is superior to existing sensors in terms of stability, sensitivity, selectivity and detection potential. The excellent performance of the platform can be ascribed to the large surface area and high catalytic activity of the particles.

3.6. Biosensing of glucose, uric acid and cholesterol

Because the nanoarchitectured platform shows excellent sensitivity, selectivity and high stability toward H_2O_2, this electrode was then used for the development of oxidase based amperometric biosensors. The oxidase enzymes catalyze the oxidation of variety of analytes in the presence of oxygen; H_2O_2 is generated during these enzymatic reactions. Since the amount of enzymatically generated H_2O_2 is proportional to the analyte concentration, the H_2O_2-sensitive electrodes can be used for the measurement of analyte present in the biosamples. In the present investigation, the nanoarchitectured platform was successfully employed for the amperometric sensing of uric acid, cholesterol and glucose using the corresponding oxidase enzymes. The biosensor was fabricated by integrating the oxidase enzyme and nPts with the silicate network (Scheme 1) as described earlier. In the case of cholesterol, the enzymes cholesterol esterase and cholesterol oxidase were used; cholesterol esterase hydrolyzes cholesterol ester into cholesterol and cholesterol oxidase converts the enzymatically generated cholesterol to 4-cholesten-3-one. The enzyme encapsulated into the network efficiently catalyzes the oxidation of glucose/uric acid/cholesterol in the presence of oxygen. Enzymatically generated H_2O_2 has been successfully detected by nPts on the biosensor. The amperometric responses for the biosensing of uric acid, cholesterol ester and glucose using the integrated biosensor are shown in Fig. 4 and Figure 6S (Supporting Information). Current was not observed in the absence of oxygen or the substrate, confirming that the observed response is due to the oxidation of enzymatically generated H_2O_2.

For the practical application of any biosensor a fast response time is essential. The response time of the present sensor was 2 s. The facile access of the nanoarchitecture to the reactant from solution and the fast diffusion of product to solution resulted in a fast response. The sensor linearly responded to glucose/uric acid/cholesterol at lower concentrations and a saturation response was reached at higher concentrations, as expected for a Michaelis–Menten type process. The linear Lineweaver–Burk plot of $1/i_{cat}$ vs. $1/C_s$ was used as a diagnostic of kinetic control of the amperometric response and the apparent Michaelis–Menten constant (K_M^{app}) for glucose, uric acid and cholesterol was calculated to be 28 mM, 300 μM and 16 mM, respectively. These values are in close agreement with the values reported in the literature (Li and Lin, 2007; Tan et al., 2005; Zhang et al., 2007). The sensitivity of the biosensor toward glucose, uric acid and cholesterol was obtained from the slope of the calibration plot and was found to be 0.85 ± 0.01 μA/mM, 9.43 ± 0.01 nA/μM and 0.0123 ± 0.003 μA/mM, respectively. Note that the difference in the measured sensitivity of the biosensor toward glucose, uric acid and cholesterol is probably due to different amounts of enzyme loaded into the sol–gel network. Regardless of enzyme loading, the biosensor is highly sensitive and stable with detection limits as low as 10 nM (S/N = 6) of glucose, 100 nM (S/N = 5) of uric acid and 0.5 μM (S/N = 7) of cholesterol.

3.7. Real sample analysis

The practical application of the present transducer was examined by measuring the concentration of glucose, uric acid and cholesterol in biological samples. Filtered blood serum samples were collected and used for amperometric measurements with the nanoparticle decorated enzyme electrode. A fast and stable amperometric response was obtained upon the injection of biological sample and the results are summarized in Table 1. To authenticate the validity of the result, the samples were spiked with a known concentration of each analyte. The recoveries for the spiked analytes were excellent (94–99%). To further con-

Table 1
Determination of uric acid, glucose and cholesterol in serum sample using the nanoarchitectured biosensor.[a]

Parameter	UA		Glucose		Cholesterol	
	Serum 1	Serum 2	Serum 1	Serum 2	Serum 1	Serum 2
Original value (μM)	0.33	0.23	5.77	13.1	1.53	1.62
Total concentration (mg/dL)	6.15	4.05	93.8	303	199.6	211
Total concentration by colorimetric method[b] (mg/dL)	6.25	4.25	92	282	208	248

[a] Three independent amperometric measurements were made with each serum samples.
[b] Performed in the clinical laboratory.

firm the validity of the results, the concentration of glucose, uric acid and cholesterol in the serum samples was measured by the conventional colorimetric method used in clinical laboratories. Interestingly, the values obtained with our electrode were in close agreement with those obtained from the clinical laboratory, validating the excellent performance of the nanoarchitectured transducer.

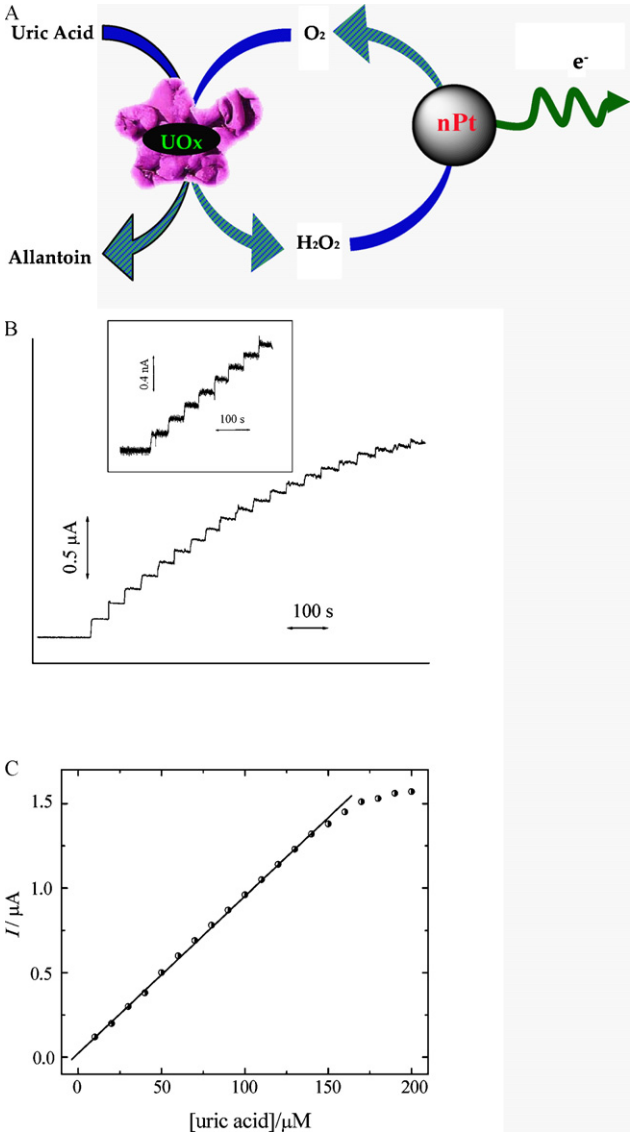


Fig. 4. (A) Scheme illustrating the biosensing of uric acid with the nanoarchitectured biosensor. (B) Amperometric response obtained for the biosensing of uric acid with the nanoarchitectured biosensor. 10 μM of uric acid was injected into stirred 5 mM PBS at regular time intervals. Electrode potential: 0.46 V. (C) Corresponding calibration plots. Amperometric response obtained for the biosensing at nanomolar level of uric acid (100 nM) is shown as the inset in (B).

4. Conclusions

A highly sensitive nanoarchitectured amperometric sensor based on nPts for the sensing of H_2O_2 in the sub-nanomolar range has been developed for the first time. The nPts efficiently catalyze the oxidation of H_2O_2 at less positive potentials in neutral pH without interference from common analyte interferents. This nanoarchitectured electrode is highly stable and does not undergo deactivation. The catalytic activity of the nPts is combined with the enzymatic activity of GOx, UOx and ChEs/ChOx to develop amperometric biosensors for glucose, uric acid and cholesterol. The oxidase-based enzyme was encapsulated into the silicate network and the encapsulated enzyme retained its enzymatic activity. The nanoarchitectured biosensor exhibits a fast and stable response. The high sensitivity of the sensor can be explained by considering the possible ensemble behavior of the transducer. The biosensor has been successfully used for the sensing of glucose, uric acid and cholesterol in blood serum samples.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2010.11.046.

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