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Non-enzymatic electrochemical sensing of glucose and hydrogen peroxide using a bis(acetylacetonato)oxovanadium(IV) complex modified gold electrode†

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A non-enzymatic electrochemical sensor, bis(acetylacetonato)oxovanadium(IV) complex, $[VO(acac)_2]$, fabricated on a self-assembled 4-(pyridine-4′-amido)thiophenol (PATP) monolayer modified gold electrode, was developed for the detection of glucose and hydrogen peroxide (H_2O_2) at neutral pH. The modified electrode was characterized by electrochemical and microscopic techniques. The non-enzymatic sensor exhibited a remarkable catalytic performance for glucose oxidation and H_2O_2 reduction. Chronoamperometry was used for the electrochemical determination of glucose and H_2O_2. The non-enzymatic sensing of glucose was realized with a linear response range from 0.001 to 0.5 mM with a detection limit of 0.1 μM (S/N = 3). The sensor also has a good performance for the electrocatalytic reduction of H_2O_2 with a linear response range from 0.02 to 0.9 mM with a detection limit of 0.03 μM (S/N = 3). In addition, $[VO(acac)_2]$–PATP–Au showed a good selectivity for glucose and H_2O_2 detection in the presence of potential interfering agents such as ascorbic acid, uric acid, L-dopa, L-cysteine and different ions like Na^+, K^+, Cl^- *etc.* The kinetic parameters such as the electron transfer coefficient and the catalytic reaction rate constant were also determined for glucose and H_2O_2. Finally, the modified electrode was used to achieve quantitative detection of glucose and H_2O_2 in blood and milk, respectively for practical applications.

Introduction

Green plants produce glucose by the reduction of carbon dioxide and the metabolic oxidation of glucose sustains all living beings.[1] Glucose is transported to cells *via* insulin in the bloodstream. The human body maintains blood glucose levels at a concentration of 4–8 mM (70–120 mg dL^{-1}).[2] An abnormal blood sugar level causes diabetes which represents a leading cause of several complications for human health like complications to the retina, circulatory system, kidneys *etc.*[3] To manage the blood glucose level patients need to monitor the blood glucose level on a regular basis. On the other hand, hydrogen peroxide (H_2O_2) is a simple but very important molecule in nature, and is extensively used as an oxidizing agent in the food and chemical industries.[4] Moreover, H_2O_2 is one of the most important markers for oxidative stress and also acts as a precursor in the formation of highly reactive and potentially harmful hydroxyl radicals.[5,6] Therefore, the accurate determination of glucose and H_2O_2 is of practical importance. Several analytical techniques have been carried out for the determination of glucose and H_2O_2 *viz.*, titrimetry, spectrometry, fluorometry, chemiluminescence and electrochemical methods.[7–10] Amongst them, the electrochemical approach is promising because of its higher sensitivity and selectivity, lower detection limit, faster response time, better long term stability and chip.[11] For the detection of glucose and H_2O_2 both enzymatic[12,13] and non-enzymatic[14,15] sensor have been developed. First, second and third generation enzymatic glucose sensors has been developed due to overcome the disadvantages. The third generation sensor still in their infancy, yet some of them based on nano-mesoporous electrode surface show some promise.[16] There are still some disadvantages of enzyme-based determination. Examples include complicated enzyme immobilization, critical operating conditions *viz.* optimum temperature and pH, chemical instability, poor reproducibility and high cost.[17] To solve these problems, fourth generation enzyme-free sensors have been developed for glucose oxidation and H_2O_2 reduction. In general, these electroactive analytes can be oxidized or reduced directly at ordinary solid electrodes. However, owing to their high over-potential, slow electrode kinetics and poor measurement stability caused by poisoning from the intermediate products restricts the performance of this electrodes.[18] Therefore; current efforts have mainly focused on discovering new materials with high catalytic activity and good stability in order to construct non-enzymatic sensors. The fabrication of a wide variety of nanomaterials have been introduced for the selective and sensitive detection of glucose[19,20] as well as H_2O_2.[21,22] On the other hand very limited numbers of

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metal complexes have been used so far for the electrochemical sensing of glucose and H_2O_2. Complexes with reversible redox capabilities such as cobalt phthalocyanine,[23] nickel curcumin,[24] nickel porphyrine,[25] copper hexacyanoferrate[26] have been used for the effective electrocatalytic sensing for glucose whereas cobalt tetrasulfophthalocyanine,[27] cobalt tetraruthenated porphyrin,[28] cobaltoxyhydroxide,[22] DNA–Cu^{2+} complex[29] for H_2O_2 sensing.

Instead of cobalt, nickel and copper containing complexes no other earth abundant transition metal complexes have been reported for the electrochemical sensing of glucose and H_2O_2. Among first d-block transition-metal series, vanadium has critical roles in various chemical and biological processes.[30] Presently the catalytic role of vanadium in higher oxidation states (IV and V) has received much attention after the discovery of vanadium dependent enzymes such as vanadiumiron nitrogenase of *Azotobacter vinelandii* and vanadium haloperoxidases in marine algae.[31] Several oxovanadium and dioxovanadium complexes acts as functional models of haloperoxidases and catalyze oxyhalogenation of various aromatic substrates.[32] Instead of these, oxovanadium complexes have also been used to catalyze several reactions such as the oxidation of olefins, alcohols,[33] aldehydes,[34] tertiary amine,[35] thiols,[36] hydrogen peroxide,[37] epoxidation[38] and oxidative coupling reaction.[39] Apart from their role as catalyst, there is a widespread interest on the biological chemistry of vanadium compounds because of its perceived potential for the development as a pharmacologic agent for the treatment of diabetes mellitus.[40] Extensive literature review shows that among large number of oxovanadium compounds, only bis(acetylacetonato)oxovanadium, $[VO(acac)_2]$, exhibits the greatest capacity to enhance insulin receptor kinase activity in cells associated with a significant decrease in plasma glucose concentration.[41] Posner and co-workers showed that vanadate reacted with H_2O_2 stimulated the phosphorylation of the insulin receptor in endosomes with efficiency comparable to that of insulin.[42] Makinen and Brady showed that $[VO(acac)_2]$ stimulate the uptake of glucose by serum-starved 3T3-L1 adipocytes in the presence of bovine serum albumin.[43]

These rich catalytic and pharmacological properties of oxovanadium complexes encourage us to prepare the $[VO(acac)_2]$ complex modified gold electrode and study the non-enzymatic electrochemical sensing behaviour for glucose and hydrogen peroxide. To the best of our knowledge, this is the first report of sensing both glucose and hydrogen peroxide by the same metal complex modified electrode at neutral pH. The oxovanadium(IV) complex modified gold electrode shows excellent electrocatalytic activity and exhibit notable sensing performance towards glucose and H_2O_2. The kinetics of glucose oxidation and hydrogen peroxide reduction was also examined in detail. More importantly, we demonstrate successfully its application for the quantitative detection of glucose in human blood sample and H_2O_2 in processed milk.

Experimental

Chemicals and reagents

4-Aminothiophenol, isonicotinic acid, $[VO(acac)_2]$, D-(+)-glucose and hydrogen peroxide (30 wt% in H_2O) were procured from Sigma Aldrich, India, $K_4[Fe(CN)_6]_3 \cdot H_2O$ were purchased from Merck (India). All the reagents and solvents were analytical grade and were used without further purification. 0.1 M phosphate buffer solution (PBS) was prepared by mixing 0.1 M $NaClO_4$ and 0.01 M H_3PO_4 and the pH's were adjusted by the addition of 0.11 M NaOH using Smalley's method.[44] Double distilled water was used throughout the course of the experiment.

Apparatus and instrumentations

Electrochemical measurements were performed on a CHI 660C Electrochemical workstation (CH Instrument, USA). A three electrode system was employed with gold or modified gold electrode as working electrode (2 mm diameter, 0.031 cm^2 area), Pt wire as a counter electrode and Ag/AgCl (3 M KCl) as reference electrode. All experiments were performed at ambient temperature and inert atmosphere. The field emission scanning electron microscopy (FE-SEM) images were obtained using FE SEM, FEI INSPECT F50 operated at an acceleration voltage of 20 kV. pH measurement of solutions were carried out on a pH meter (Macro Scientific Works (Regd), New Delhi).

Construction of $[VO(acac)_2]$–PATP modified gold electrode

A gold electrode was polished with wet α-alumina (0.5 μm) on a flat polishing pad for 10 minutes and rinsed several times with doubly distilled water. The cleanliness of the gold electrode surface was ascertained by recording the repetitive cyclic voltammograms in 0.5 M H_2SO_4 between −0.2 and +1.5 V *versus* Ag/AgCl with 0.1 V s^{-1} scan rate until a steady characteristic gold oxide cyclic voltammogram was obtained.[45] The electrode was then rinsed with doubly distilled water and immersed in 1.0 mM ethanolic solution of 4-aminothiophenol (4-ATP) for 24 hours. The 4-ATP was self-assembled over the gold electrode surface *via* gold–sulfur interaction and the modified electrode 4-ATP–Au was thoroughly washed with double distilled water. Thereafter, the modified gold electrode was dipped into 1.0 mM isonicotinic acid solution for 4 hours under stirring condition and 4-(pyridine-4′-amido)thiophenol modified gold electrode (PATP–Au) was formed. After washed with double distilled water PATP–Au electrode was immersed into an ethanolic solution of 1.0 mM $[VO(acac)_2]$ and stirred for 2 hours so that the pyridine nitrogen of PATP–Au was able to form adduct with the vacant coordination site of vanadium in $[VO(acac)_2]$. The finally modified electrode $[VO(acac)_2]$–PATP–Au was washed thoroughly with distilled water and dried in air for further use.

Results and discussion

Characterization of modified gold electrode

The step wise modification and surface morphology of the bare gold electrode was characterised by FE-SEM. $[VO(acac)_2]$–PATP modified gold electrode shows very rough and porous surface which is favourable for the electrocatalytic activity. Elemental mapping images confirms the immobilization of $[VO(acac)_2]$ over self-assembled monolayer 4-PATP modified gold electrode.[46] The modification process was also monitored by cyclic

voltammetry and electrochemical impedance spectroscopy using $[Fe(CN)_6]^{3-/4-}$ as redox probe in 0.1 M PBS solution at pH 7.0. The cyclic voltammogram of 0.5 mM $[Fe(CN)_6]^{4-}$ exhibits an electrochemically reversible redox couple on bare electrode. After modification the gold electrode with 4-aminothiophenol, the cyclic voltammogram of $[Fe(CN)_6]^{4-}$ exhibit an irreversible couple with low current height than bare gold electrode. The current height decreased even more when 4-(pyridine-4′-amido) thiophenol (PATP) modified Au was used as working electrode. These CV results indicated that the electronic communication between gold and $[Fe(CN)_6]^{4-}$ is blocked due to PATP film formation.[46] In the Nyquist plot, the diameter of the semi-circle increases gradually when stepwise modification on the gold electrode surface was carried out. The observed trend is due to the fact that the modified electrode blocked the electron transfer for the redox reaction of $[Fe(CN)_6]^{4-}$. Electrochemical impedance measurement supports the CV results. The fabrication of $[VO(acac)_2]$ over PATP–Au electrode was confirmed by taking a comparative cyclic voltammogram for PATP–Au and $[VO(acac)_2]$–PATP–Au in 0.1 M PBS buffer at pH 7.0. A quasir-eversible $[V^{V}O(acac)_2]^{+}/[V^{IV}O(acac)_2]$ redox couple at +0.33 V supports the fabrication of $[VO(acac)_2]$–4-PATP–Au electrode.[46]

Electrocatalytic oxidation of glucose and reduction of H_2O_2

Fig. 1 and 2 shows the cyclic voltammograms (CV) of 0.1 mM glucose and 0.5 mM hydrogen peroxide, respectively in 0.1 M PBS at pH 7.0 using bare Au, PATP–Au and $[VO(acac)_2]$–PATP–Au electrodes. An irreversible oxidation of glucose occurred at +0.65 V by the $[VO(acac)_2]$–PATP–Au electrode with large increase of current whereas no such prominent peak was observed with bare and PATP–Au electrode (Fig. 1). In absence of glucose no such oxidation peak was observed at $[VO(acac)_2]$–PATP–Au electrode (Fig. S1†). This behaviour indicate the electrocatalytic activity of $[VO(acac)_2]$–PATP modified gold electrode towards glucose oxidation. DPV experiment gives a prominent glucose oxidation peak only at $[VO(acac)_2]$–PATP–Au electrode under similar condition and supports the results obtained by CV (Fig. S2†). The catalytic pathway of glucose oxidation can be describe on assuming that the electrochemical process is

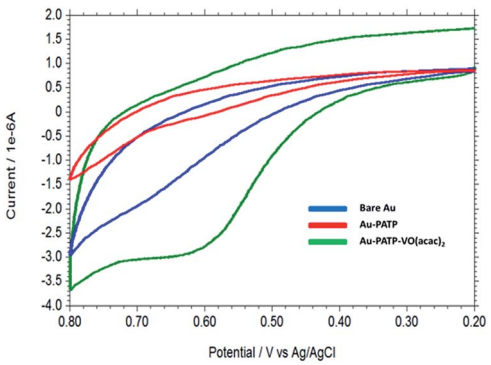

Fig. 1 Cyclic voltammograms obtained with bare, PATP and $[VO(acac)_2]$–PATP modified gold electrode in 0.1 mM glucose in 0.1 M PBS solution (pH 7.0).

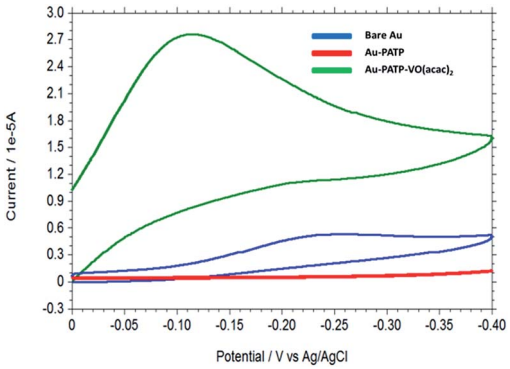

Fig. 2 Cyclic voltammograms obtained with bare, PATP and $[VO(acac)_2]$–PATP modified gold electrode in 0.5 mM hydrogen peroxide in 0.1 M PBS solution (pH 7.0).

initiated by the non-covalent interaction of glucose with surface bound $[VO(acac)_2]$. During anodic scan $[V^{IV}O(acac)_2]^0$ is oxidized to the catalytically active $[V^{V}O(acac)_2]^+$ complex over PATP modified gold electrode.

$$[V^{IV}O(acac)_2]^0 \rightarrow [V^{V}O(acac)_2]^+ + e^-. \quad (a)$$

Once $[V^{V}O(acac)_2]^+$ is formed, glucose is oxidized on the modified electrode surface *via* the following reactions.

$$[V^{V}O(acac)_2]^+ + \text{glucose} \rightarrow \text{intermediate} + [V^{IV}O(acac)_2]^0 \quad (b)$$

$$[V^{V}O(acac)_2]^+ + \text{intermediate} \rightarrow \text{gluconolactone} + [V^{IV}O(acac)_2]^0. \quad (c)$$

The cyclic voltammogram of H_2O_2 in 0.1 M PBS (pH 7.0) shows a cathodic response at around −0.24 V at bare gold electrode. When the gold electrode was modified by 4-(pyridine-4′-amido)thiophenol, no current response was observed. After modification with $[VO(acac)_2]$, a sharp peak was observed around −0.11 V with sufficiently high current response during the cathodic scan. In absence of H_2O_2 no such reduction peak was obtained at $[VO(acac)_2]$–PATP–Au electrode under similar condition (Fig. S3†). Through these observations it was clear that oxovanadium(IV) complex exhibit enhanced electrocatalytic efficiency by their adhesion on the PATP–Au electrode. This is rationalized by a high ability of the $[VO(acac)_2]$ to transfer electrons involved in the catalytic reaction and sense the presence of H_2O_2 electrochemically. A common two electron redox mechanism is proposed for hydrogen peroxide reduction at the $[VO(acac)_2]$–PATP–Au electrode surface in which a substantial interaction with H_2O_2 and vanadium promotes the electron transfer and is shown by the following reactions.

$$[V^{IV}O(acac)_2]^0 + H_2O_2 \rightarrow H_2O + [V^{V}O(acac)_2]^+ \quad (d)$$

$$[V^{V}O(acac)_2]^+ + e^- \rightarrow [V^{IV}O(acac)_2]^+ \quad (e)$$

Electrochemical impedance spectroscopy was also carried out for glucose and H_2O_2 using bare and modified gold

electrodes. The diameter of the semicircle observed in the Nyquist plot corresponds to charge transfer resistance, R_{ct}; the smaller the semi-circle, faster is the charge transfer. Fig. S4 and S5† shows that the diameter of the semicircle (R_{ct}) changes upon modification of gold electrode surface. The R_{ct} values for 0.1 mM glucose oxidation and 0.5 mM H_2O_2 reduction at $[VO(acac)_2]$–4-PATP–Au electrode in 0.1 M PBS at pH 7.0 were 1.3×10^4 Ω and 1.2×10^5 Ω respectively, which were quite smaller than the R_{ct} obtained at PATP modified and bare gold electrode. The observed results are due to the fact that the $[VO(acac)_2]$-modified electrode ease the electron transfer rate for the oxidation of glucose and reduction of H_2O_2 where as the 4-PATP modified gold electrode blocked the electron transfer. Electrochemical impedance measurements clearly indicate that $[VO(acac)_2]$–4-PATP modified gold electrode has lower resistance as compared to bare or 4-PATP modified gold electrodes. This study supports the CV results and reveals that the $[VO(acac)_2]$–4-PATP–Au electrode is an efficient electrocatalyst for the oxidation of glucose and reduction of hydrogen peroxide.

Determination of glucose and H_2O_2

Based on optimized conditions, determination of glucose and H_2O_2 were performed using chronoamperometry. Fig. S6† shows the chronoamperometry curves of glucose with different concentration. The oxidation peak current of glucose was linear with its concentration in the range of 0.1–0.5 mM (Fig. 3a). The regression equation was $I = 3.717C + 1.646$ ($R^2 = 0.99$), with a detection limit of 0.1 μM (S/N = 3). Exactly same detection limit was obtained in the lower concentration range of glucose (1.0 μM to 5.0 μM) (Fig. S8†). The detection limit was further confirmed by differential pulse voltammetry (Fig. S10†). Fig. S7 and S9† shows the chronoamperogram response for H_2O_2 in the concentration range of 0.5–0.9 mM and 20–40 μM. The current was linearly proportional to its higher concentration range with a linear regression equation $I = 9.81C + 18.61$ ($R^2 = 0.99$) (Fig. 3b) and in the low concentration range with a linear regression equation $I = 9.81C + 0.02$ ($R^2 = 0.99$) (Fig. S9b†). The detection limit for H_2O_2 was 0.03 μM (S/N = 3). The detection limit was further confirmed using CV results (Fig. S11†). Table S1† shows a comparison of the proposed electrochemical method and other modified electrodes reported for the electrocatalytic oxidation of glucose and reduction of H_2O_2. It can be seen that the detection limit obtained in the present system are comparable with some reported metal complex modified electrodes and quite better than the metal nanoparticle/nanocomposite modified electrodes.

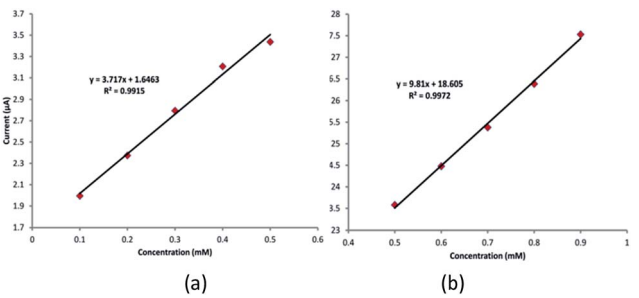

Fig. 3 (a) Plot of resulting current in chronoamperometry at 30 seconds *versus* concentration of glucose (0.1–0.5 mM). (b) Plot of resulting current in chronoamperometry at 30 seconds *versus* concentration of H_2O_2 (0.5–0.9 mM).

Effect of scan rate and kinetic analysis for glucose oxidation and hydrogen peroxide reduction

The influence of the scan rate on the electrocatalytic oxidation of glucose (Fig. S12a†) and the reduction of H_2O_2 (Fig. S13a†) at $[VO(acac)_2]$–4-PATP–Au were investigated using cyclic voltammetry. The results showed that on increasing the scan rates the oxidation peak potential of glucose and the reduction potential of hydrogen peroxide shifts to more positive and more negative values, respectively, confirming the kinetic limitation of the electrochemical reaction.[27] Moreover, a plot of scan rate–normalized current ($I_{pa}/\nu^{1/2}$) *versus* scan rate (Fig. S14†) shows a shape typical of EC catalytic process for glucose oxidation.[47] In addition, a plot of the peak current (I_{pa}) *versus* the square root of the scan rate ($\sqrt{\nu}$) (Fig. S12b†) in the range of 50–100 mV s^{-1} was found to be linear following the linear regression equation I_{pa} (μA) $= 0.3392\nu$ (mV s^{-1}) $- 1.1978$ ($R^2 = 0.9945$), revealing that the electrooxidation reaction of glucose at $[VO(acac)_2]$–4-PATP–Au electrode was followed diffusion controlled electron transfer process. The diffusion coefficient (D) for glucose was 9.5×10^{-6} cm^2 s^{-1} and is calculated using the plot (I_{pa} *vs.* $\sqrt{\nu}$) and Randles–Sevcik equation[48]

$$I_p = 2.69 \times 10^5 n^{3/2} A D^{1/2} C \nu^{1/2} \quad (1)$$

where, I_p is the peak current, n is the number of electrons transferred, A is the electrode area, C is the concentration of electroactive species, and ν is the scan rate, considering a temperature of 298 K. The electron transfer coefficient for the totally irreversible oxidation of glucose at $[VO(acaca)_2]$–4-PATP–Au can be determined from equation[48]

$$E_p - E_{p/2} = 1.857RT/\alpha F = 47.7/\alpha \text{ mV} \quad (2)$$

where E_p and $E_{p/2}$ represent the peak potential and the half-height peak potential, respectively in cyclic voltammetry experiment where R, T and F have their usual meaning. For glucose oxidation, $E_p - E_{p/2} = 38$ mV, hence electron transfer coefficient (α) is calculated to be 0.63.

The standard heterogeneous rate constant (k_s) for the irreversible oxidation of glucose at $[VO(acac)_2]$–4-PATP–Au electrode was calculated by using the Velasco equation[49]

$$k_s = 1.11 D^{1/2} (E_p - E_{p/2})^{-1/2} \nu^{1/2} \quad (3)$$

The estimated k_s values for totally irreversible oxidation of glucose at $[VO(acac)_2]$ modified electrodes was found to be 5.5×10^{-3} cm s^{-1}. The observed higher k_s value for glucose at the modified electrode indicates that the oxidation of glucose was faster at the $[VO(acac)_2]$–PATP modified gold electrode. The kinetic parameters for the reduction of H_2O_2 were also

calculated (as described for glucose): $n = 2$, $\alpha = 0.69$, $D = 10.6 \times 10^{-6}$ cm^2 s^{-1} and $k = 3.3 \times 10^{-3}$ cm s^{-1}.

Effect of accumulation potential and time

The effect of accumulation time and potential on the oxidation behavior of glucose and reduction of H_2O_2 at [VO(acac)$_2$]–PATP–Au electrode was investigated. Fig. S15† shows that the oxidation peak current of glucose and H_2O_2 which were remaining constant with increasing accumulation time from 0 to 300 s. Therefore the accumulation time of 60 s was chosen as the optimum time for further study in both cases. In addition, the influence of accumulation potential on the peak current was examined over the potential range 0.0 to 6.0 V for glucose and 0.0 to −0.5 V for H_2O_2 (Fig. S16†). The peak current for glucose was decreased by changing accumulation potential to more positive value and is due to the oxidation of glucose during the accumulation step at potential higher than that 0.35 V (Fig. S16a†) where as in case of H_2O_2, by changing accumulation potential to more negative value and is due to the reduction of H_2O_2 during the accumulation step at potential lower than that −0.02 V (Fig. S16b†). In fact, the maximum observed current were equal to those observed for open circuit accumulation.

Effect of pH

The effect of pH on the electrooxidation of glucose and H_2O_2 ware also investigated in the range of pH 5.0–10.0. As shown in Fig. S17† the oxidation peak potential of glucose were pH dependent and was shifted towards more negative potential with increments in solution pH following the linear regression equation of E_{pa} (V) = −0.0625 pH + 1.094 (R^2 = 0.9957). The slope of 62.5 mV pH^{-1} indicated that equal numbers of protons and electrons were involved in the electrode reaction process.[50] Similarly, in Fig. S18† the reduction peak potential of H_2O_2 were also pH dependent and that they shifted toward more positive potential with increments in solution pH following the linear regression equation of E_{pa} (V) = −0.026 pH + 0.068 (R^2 = 0.976). Investigation of the influence of pH on the peak current of glucose and H_2O_2 at the modified electrode revealed that peak current of glucose and hydrogen peroxide reached a maximum at pH 7.0 and then decreased by increasing pH of the solution (Fig. S19a and b†).

Reproducibility, sensitivity and stability

A reproducible and long-term stable electrochemical sensor is highly desirable for the practical application and commercialization. The reproducibility of the [VO(acac)$_2$]–PATP–Au electrode was examined by 10 repetitive measurements for glucose and H_2O_2 in 0.1 M PBS solution. The results showed that the anodic peak current for glucose and cathodic peak current for hydrogen peroxide remains same with a relative standard deviation (RSD) of 0.2 and 0.3%, respectively, indicating that the modified electrode has a good reproducibility. The modified electrode was highly sensitive towards glucose and H_2O_2 and the sensitivity was 120.24 μA cm^{-2} mM^{-1} and 326.66 μA cm^{-2} mM^{-1} for glucose and hydrogen peroxide, respectively. To further explore the long-term stability, measurements were made with five days intervals (when not in use, the sensor was stored at room temperature using a rubber cap). The sensor retained 100% of its original current response after 20 days both for glucose (1.0 mM) and H_2O_2 (0.5 mM) in 0.1 M PBS at [VO(acac)$_2$]–PATP–Au (Fig. S20†).

Interference study

In the electrochemical detection of glucose and H_2O_2, the elimination of interferences is a real challenge. Ascorbic acid, uric acid, citric acid, levodopa, cysteine, and different common ions are the major potential interfering agents in the physiological system. In the present study 0.1 mM glucose in presence of 10 fold excess interferents were used at +0.65 V. The resulting amperograms are shown in Fig. 4. There is no obvious current response observed with the addition of these interfering substances, however, an obvious current response with the addition of glucose was appeared. In addition, the influence of those co-existing electroactive species in the amperometric determination of H_2O_2 was also studied. The working potential was held at −0.11 V. The amperogram (Fig. 5) shows that all the potential interferents mentioned did not affect the sensor selectivity for H_2O_2. These results suggest that the interfering effect caused by these electroactive species is quite negligible, indicating the highly selective detection of glucose and H_2O_2 at the oxovanadium complex modified electrode.

Real sample analysis

To testify the feasibility of [VO(acac)$_2$]–PATP–Au in real sample analysis, human blood sample (after fasting) was taken for glucose determination whereas processed milk was chosen for the determination of H_2O_2. Before testing, the blood and milk samples were half diluted by 0.1 M phosphate buffer solution.

Fig. S21† shows the overlaid DPV of blood sample solution in PBS (pH 7.6) and after addition of standard glucose solution in blood sample solution. The DPV of blood sample clearly shows the oxidation of glucose at +0.56 V. The content of glucose in

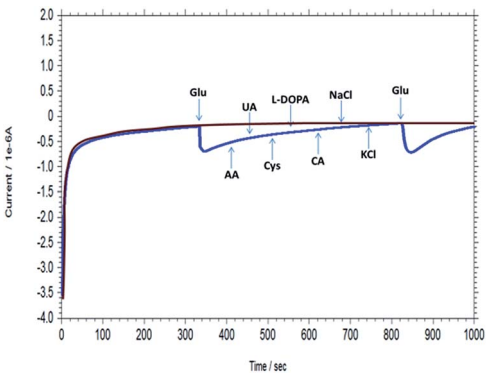

Fig. 4 Amperometric response at [VO(acac)$_2$]–4PATP–Au electrode with an applied potential of +0.65 V on subsequent addition of 0.1 mM glucose, 1.0 mM AA, 1.0 mM UA, 1.0 mM Cys, 1.0 mM L-dopa, 1.0 mM CA, 1.0 mM NaCl, 1.0 mM KCl, 0.1 mM glucose under stirring condition (supporting electrolyte: 0.1 M PBS (pH 7.0), brown curve shows background current).

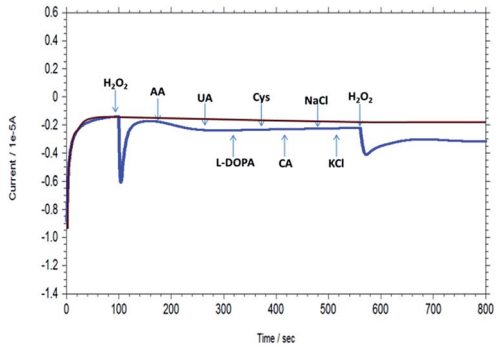

Fig. 5 Amperometric response at $[VO(acac)_2]$–4PATP–Au with an applied potential of −0.11 V on subsequent addition of 0.1 mM H_2O_2, 1.0 mM AA, 1.0 mM UA, 1.0 mM L-dopa, 1.0 mM Cys, 1.0 mM CA, 1.0 mM NaCl, 1.0 mM KCl, 0.05 mM H_2O_2 under stirring condition (supporting electrolyte: 0.1 M PBS (pH 7.0), brown curve shows background current).

blood sample (5.01 mM = 90.258 mg dL^{-1}) was calculated using the standard addition method and the direct interpolation of the linear regression (RSD = 2.24%). A normal fasting (no food for eight hours) blood sugar level is between 70 and 99 mg dL^{-1} and by comparing our result was in the similar range. The accuracy of the method was also verified by recovery studied adding standard glucose solution to the real sample and 100.2% recoveries were obtained. The H_2O_2 concentration in the milk sample was determined as 0.91 μM (= 0.003 mg dL^{-1}), using a standard addition method (RSD = 2.16%), with the recovery of 101.0%. The results are summarized in Table S2.† The results indicate that the modified electrode can effectively detect glucose in human blood and hydrogen peroxide in processed milk.

Conclusions

A unique non-enzymatic electrochemical sensor $[VO(acac)_2]$–PATP–Au was developed and used for the detection of glucose and hydrogen peroxide in pure, presence of interferents and real sample. The modified electrode was characterized by microscopic and electrochemical techniques. Cyclic voltammetry, differential pulse voltammetry, electrochemical impedance spectroscopy, amperometry, chronoamperomery was used for sensing, quantification and determination of kinetic parameters. Till date very limited number of transition metal complex modified electrode has been used for non-enzymatic sensing of glucose and hydrogen peroxide. The novelty of our work is that the same oxovanadium complex modified electrode can detect both glucose as well as hydrogen peroxide. Only few nanoparticles modified electrode has been reported those are able to detect both glucose and hydrogen peroxide. But their preparation process, stability, detection limit, cost are not so impressive. The advantage of our system are easy to prepare, have good selectivity, sensitivity, stability, reproducibility, low detection limit and most importantly cheap than the earlier reported systems. The sensor was efficiently detected glucose in blood sample and hydrogen peroxide in processed milk with good recovery. The new non-enzymatic sensor can be useful for clinical diagnosis and food industry in near future.

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