Ionic-Liquid-Stabilized TiO₂ Nanostructures: A Platform for Detection of Hydrogen Peroxide

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ABSTRACT: Hydrogen peroxide (H₂O₂) acts as a signaling molecule to direct different biological processes. However, its excess amount results in oxidative stress, which causes the onset of different types of cancers. TiO₂ nanostructure was synthesized by a facile hydrothermal method. The prepared material was characterized by FTIR spectroscopy, XRD, SEM, EDX, TGA, and Raman spectroscopy, which confirmed the formation of nanostructured material. Subsequently, the prepared nanoparticles (NPs) were capped with 1-H-3-methylimidazolium acetate ionic liquid (IL) to achieve its deagglomeration and functionalization. A new colorimetric sensing probe was prepared for the detection of H₂O₂ based on ionic liquid-capped TiO₂ nanoparticles (TiO₂/IL) and 3,3′,5,5′-tetramethylbenzidine (TMB) dye, which acts as an oxidative chromogenic substrate. H₂O₂ reacts with TMB, in the presence of ionic liquid-coated TiO₂ NPs, to form a blue-green product. The color was visualized with the naked eye, and the colorimetric change was confirmed by a UV−vis spectrophotometer. To obtain the best response of the synthesized sensor, different parameters (time, pH, concentrations, loading of nanomaterials) were optimized. It showed a low limit of detection 8.61 × 10⁻⁸ M, a high sensitivity of 2.86 × 10⁻⁷ M, and a wide linear range of 1 × 10⁻⁹−3.6 × 10⁻⁷ M, with a regression coefficient (R²) value of 0.999. The proposed sensor showed a short incubation time of 4 min. The sensing probe did not show any interference from the coexisting species. The TiO₂/IL sensor was effectively used for finding H₂O₂ in the urine samples of cancer patients.

1. INTRODUCTION

Hydrogen peroxide (H₂O₂) is one of the important analytes in the medical community due to its high reactivity.¹ H₂O₂ is the byproduct of almost all oxidative metabolic reactions in living organisms. Naturally, existing peroxidases assume a key role in the catalytic degradation of H₂O₂ in biological systems.² They regulate the concentration of H₂O₂ and require ambient conditions (temperature, pH) for their working.³ However, the higher concentration of H₂O₂ in the body creates oxidative stress-inducing protein and DNA damage. Ultimately, this DNA damage also increases our susceptibility toward the onset of different types of cancers. The human body can be affected by more than 100 types of cancers.⁴ Therefore, it is essentially imperative to monitor the concentration of H₂O₂ in the body.⁵

Over the past decades, various techniques have been utilized for H₂O₂ detection, such as chromatographic,⁶ spectrophotometric,⁷ chemiluminescence,⁸ and electrochemical methods.⁹ Nevertheless, some of these techniques are hazardous to living cells, making them unsuitable for in situ sensing of H₂O₂ in biological samples. Some of these methods are time-consuming, expensive, and complex, thus limiting their use in resource-limited laboratories.¹⁰ The detection of H₂O₂ via the colorimetric biosensor method is a very rapid, simple, low-cost, highly selective, and sensitive technique compared to the aforementioned techniques.

NPs play an important role in the development of different types of biosensors.¹¹ Nanostructures show exceptional features because of their nanoeffects like the mini size effect, larger surface-to-volume ratio, quantum effect, macro-quantum tunneling effect, and surface plasmon resonance (SPR). Various materials including metal oxides, sulfide, and selenide nanomaterials, such as polymer-coated CeO₂ NPs,¹² CuO...
NP	extsubscript{25},	extsuperscript{13} CoO	extsubscript{2}, NP	extsubscript{14}, V	extsubscript{2}O	extsubscript{3}, nanowires,	extsuperscript{15} BiFeO	extsubscript{3} NP	extsubscript{16} CoFe	extsubscript{2}O NP	extsubscript{17} and sheet-like FeS NP	extsubscript{18} have been used as peroxidase mimics. However, novel ionic liquid-capped TiO	extsubscript{2} NPs are recyclable, highly stable, and highly efficient, which give excellent sensing properties and catalytic activity that shows strong potential to replace expensive noble-metal NPs for biosensing.

In the class of metal oxides, TiO	extsubscript{2} nanostructures are most commonly used in industrial products, such as cosmetics, sunscreens, food products, paints, and drugs as well as in medical diagnosis.	extsuperscript{19,20} TiO	extsubscript{2} nanostructures are well-known semiconducting materials as well as photocatalysts.	extsuperscript{21} TiO	extsubscript{2} photocatalysts exhibit bactericidal activity	extsuperscript{22–25} and degradation of chemical pollutants such as superoxide, H	extsubscript{2}O	extsubscript{2}, etc.	extsuperscript{26} The nanomaterials based on TiO	extsubscript{2} are used commonly on priority bases in the field of energy due to their high band gap (2.8). Ionic liquids (ILs) are considered magical chemicals because of their wide temperature stability, negligible vapor pressure, and tunable properties through appropriate modification of cation and anion.	extsuperscript{27,28} The capping of NPs with ionic liquid enhances their catalytic activity manifold. Among ILs, 1-H-3-methylimidazolium acetate has a lot of uses, reported, in diagnosis.	extsuperscript{29}

In the present study, we synthesized TiO	extsubscript{2} NPs by the hydrothermal method and characterized by various analytical techniques such as FTIR, XRD, SEM, TGA, and Raman spectroscopy. The prepared NPs were dispersed in liquid ionic solution to enhance their sensing activities. The association of the ionic liquid with NPs improves both the sensing and catalytic properties of the system.	extsuperscript{30,31} For H	extsubscript{2}O	extsubscript{2} detection, a new simple, rapid, highly sensitive, and selective method is being reported, in diagnosis.	extsuperscript{29} The detection of H	extsubscript{2}O	extsubscript{2} was evaluated by colorimetric changes, in which 3,3′,5,5′-tetramethylbenzidine (TMB) was oxidized by H	extsubscript{2}O	extsubscript{2} to form a blue-green product. Capped NPs (25 μL) were taken in an Eppendorf tube, and 190 μL of TMB (14 mM) was added to the solution, and finally 550 μL of pH 7 phosphate buffer was added. Subsequently, 90 μL of H	extsubscript{2}O	extsubscript{2} (3.6 × 10^{-7} M) was added to the reaction solution and incubated for 4 min under the optimal temperature condition to detect optical changes. The resulting solution was subjected to a UV–vis spectrophotometer to record the absorption spectrum. To obtain ideal performance, some experimental parameters have been optimized, such as response time, pH, amount of capped NPs, and concentration of TMB solution.

2. EXPERIMENTAL SECTION

2.1. Chemicals and Reagent. Oxalic acid (98%), hydrogen fluoride (99.9%), HClO	extsubscript{4} (70%), NaOH (97.0%), sodium sulfate (99.0%), 3,3′,5,5′-tetramethylbenzidine (TMB), 1-methylimidazole (C\textsubscript{6}H\textsubscript{4}N\textsubscript{2}) (99%), and acetic acid (99.8%) were obtained from Sigma-Aldrich. H	extsubscript{2}O	extsubscript{2} (35%) was obtained from Merck KGaA (https://www.merckgroup.com/en). PBS of different pHs was obtained from BioWorld. All of these chemicals were found in pure form and were used without further purification. Solutions were prepared in deionized water obtained from an Elga Purelab Ultra water deionizer.

2.2. Instrumentation. The formation of the synthesized TiO	extsubscript{2} NPs was confirmed by the FT-IR spectral data. The FT-IR spectrometer used was from Agilent Technologies Danbury, Conn. The range chosen for getting FTIR spectra of the samples was 4000–600 cm\textsuperscript{-1}. The morphology and the size of the NPs were studied through a coupled scanning electron microscopy and energy-dispersive X-ray spectroscopy (SEM-EDS) on a TESCAN VEGA (LMU) SEM with INCAx-act (Oxford Instruments) EDS attachment operating at 20 kV. The analysis and phase identification of the synthesized NPs was carried out by X-ray powder diffraction (XRD; PAN analytical, X’pert Powder). The Raman spectra of the prepared NPs were recorded by employing a convenient Raman instrument (i-Raman, Bwtek, Inc.) connected with a microscope (20×). The thermal stability of the synthesized NPs was assessed via Pyris-1, V-3.81 PerkinElmer Thermal gravimetric analysis (TGA), under nitrogen atmosphere having a temperature range of 40–800 °C with 10 °C min\textsuperscript{-1} heating rate. UV–vis spectra of both the synthesized TiO	extsubscript{2} NPs and experimental samples were recorded on a UV–vis spectrophotometer (Shimadzu, UV, 1800, Japan).

2.3. Synthesis of TiO	extsubscript{2}. To prepare TiO	extsubscript{2} NPs, the titanium plate is subjected to hydrothermal treatment and pretreated with an oxalic acid solution to remove the oxides on the surface. The hydrothermal reaction medium is prepared to utilize 110 mL of Milli-Q water, eosin dye, and 50 mL of isopropanol. The pH of the medium was balanced at 2.62 by adding 25 mL of NaOH solution having 0.1 M concentration and 60 mL of HF solution having 0.1 M solutions. The synthesized solution was hydrothermally treated at a temperature of 180 °C for 3 h. After that, TiO	extsubscript{2} NPs were calcined at a temperature of 600 °C for 2 h to obtain a fluorine-free surface.

2.4. Synthesis of Ionic Liquid. Preparation of 1-H-3-methylimidazolium acetate ionic liquid was done using the modified protocol previously reported by our group.

2.5. Capping of TiO	extsubscript{2} NPs with Ionic Liquid. TiO	extsubscript{2} NPs were modified with ionic liquid as follows. First of all, TiO	extsubscript{2} NPs (6 mg) was added to 1 mL of ionic liquid. The mixture was macerated thoroughly in a mortar for around 30 min. As a result, a reddish dark mixture was obtained and stored in an Eppendorf tube for further use.

2.6. Colorimetric Sensing of H	extsubscript{2}O	extsubscript{2}. The detection of H	extsubscript{2}O	extsubscript{2} was evaluated by colorimetric changes, in which 3,3′,5,5′-tetramethylbenzidine (TMB) was oxidized by H	extsubscript{2}O	extsubscript{2} to form a blue-green product. Capped NPs (25 μL) were taken in an Eppendorf tube, and 190 μL of TMB (14 mM) was added to the solution, and finally 550 μL of pH 7 phosphate buffer was added. Subsequently, 90 μL of H	extsubscript{2}O	extsubscript{2} (3.6 × 10^{-7} M) was added to the reaction solution and incubated for 4 min under the optimal temperature condition to detect optical changes. The resulting solution was subjected to a UV–vis spectrophotometer to record the absorption spectrum. To obtain ideal performance, some experimental parameters have been optimized, such as response time, pH, amount of capped NPs, and concentration of TMB solution.

3. RESULTS AND DISCUSSION

The synthesized material TiO	extsubscript{2} NPs were characterized by FTIR spectroscopy as shown in Figure 1. A peak appears in the range of 1800–1600 cm\textsuperscript{-1}, corresponding to stretching vibrations of absorbed carbonyl (background peak of absorbed carbon dioxide). The stretching vibrations of Ti–O–Ti and Ti–O were confirmed by the peak at 780 cm\textsuperscript{-1}, which is almost the same as reported.

Figure 2 shows the XRD pattern of the TiO	extsubscript{2} NPs calcined at 600 °C. From XRD studies, it is to confirm that the materials synthesized are in the rutile TiO	extsubscript{2} phase. The crystal structures are in complete agreement with the corresponding reported JCPDS database Card-No. 21-1272. The diffraction peak of the NPs was recorded at a 2θ value of 25.8 confirms its rutile phase. The diffraction angles (2θ) of 25.35, 37.75, 48.11,
62.71, and 75.008 correspond to the (110), (121), (111), (210), and (211) crystal faces of rutile.\textsuperscript{34}

Nanoparticles size: The average crystalline size of TiO\textsubscript{2} was estimated using the Scherrer equation.

\[
\frac{\lambda \beta}{2D} = \frac{1}{4} K \cos \theta
\]

where \(D\) is the crystal size of the catalyst, \(\lambda\) is the X-ray wavelength, \(\beta\) is the full width at half-maximum (FWHM) of the diffraction peak (radian), \(k\) is the coefficient (0.89), and \(\theta\) is the diffraction angle at the peak maximum.

The average crystalline size of rutile phase NPs is 43.88 nm.

The surface morphology, i.e., particle size and shape of the prepared TiO\textsubscript{2} NPs calcined at 600 °C was characterized using the cross-sectional SEM image as shown in Figure 3. SEM image confirmed that the prepared TiO\textsubscript{2} NPs are in a crystalline state and they have a strong tendency to agglomerate. These trends are very consistent with the reported literature.\textsuperscript{35}

Using EDX analysis, the chemical composition of TiO\textsubscript{2} NPs has been examined as shown in Table 1 and Figure 4. This confirmed the existence of Ti and O in the NPs samples. The Ti and O contents by EDX analysis were found to be 29.84 and 70.16 by weight.\textsuperscript{36}

Using Raman spectroscopy, the crystalline phases of TiO\textsubscript{2} NPs were identified. Figure 6 shows the Raman spectrum of TiO\textsubscript{2} NPs prepared at different ratios calcined at 400 °C. The main characteristic Raman bands of the rutile crystal phase were observed at 167, 399, 515, 519, and 638 cm\(^{-1}\) in all samples calcined at 400 °C, which is completely consistent with earlier work.\textsuperscript{38} For the photocatalyst, a sharp peak was observed near 638 cm\(^{-1}\), indicating that the NPs have higher crystallinity. This can minimize charge recombination during the photoreaction process.\textsuperscript{39} The samples also show a fraction of the rutile phase. The sample also showed part of the rutile phase. After the two samples were calcined at 400 °C, a faint rutile peak was observed at 399 cm\(^{-1}\). The higher calcination temperature results in the anatase phase of TiO\textsubscript{2} NPs.\textsuperscript{40} The presence of glycerol has no obvious effect on the crystal phase formation of TiO\textsubscript{2} NPs. However, with increasing the concentration of water in the system, the crystallinity also increases.

### 3.1. Colorimetric Detection of H\(_2\)O\(_2\)

A simple and selective colorimetric method based on the ionic liquid-coated TiO\textsubscript{2} NPs was used for the detection of H\(_2\)O\(_2\). The optical sensing and UV–vis absorption spectra are shown in Figure 7I. The sensor system effectively detects H\(_2\)O\(_2\) by achieving blue-green products of colorless TMB (oxidative substrate) upon the addition of H\(_2\)O\(_2\). Also, the adsorption of H\(_2\)O\(_2\) on the surface of NPs generates OH radicals, which are subsequently involved in the oxidation of TMB to give blue-green products. In Figure 7II, it can be seen that when only ionic liquid (A) and only TiO\textsubscript{2} (B) were used, the colorimetric change was very low. However, when ionic-liquid-coated TiO\textsubscript{2} (C) was used, a clearly visible colorimetric change can be observed. The UV–vis spectra confirm the change.

### 3.2. Proposed Mechanism of H\(_2\)O\(_2\) Sensing

The peroxidase-like activity of ionic liquid-coated TiO\textsubscript{2} NPs was confirmed from the catalytic oxidation of 3,3′,5,5′-tetramethylbenzidine (TMB) by H\(_2\)O\(_2\) to form a blue-green product.
The reaction was also monitored using a spectrophotometer, which shows a broad peak at 653 nm for oxidized TMB. The proposed mechanism is followed by the reaction: IL-coated TiO₂ NPs absorb photon (light) whose energy is equal to its band gap energy. Due to this absorption of photon, excitation of electron takes place from the valence band to the conduction band to yield electrons and h⁺ pair. H₂O₂ having a strong oxidation ability scavenges the excited electron to prevent the recombination of the electron and h⁺ and generates OH radical and OH⁻ ions. Moreover, H₂O₂ adsorbed on the surface of TiO₂ NPs to form a peroxo complex between Ti and H₂O₂ that enhances the absorption capability of TiO₂ NPs under visible light. As a result, more OH radicals are formed, which leads to increasing TMB oxidation to form blue-green products (Scheme 1).

### 3.3. Optimization of Parameters

#### 3.3.1. Optimization of Capped NPs

Protic ionic liquids have a very versatile role in enhancing the catalytic activity as well as stabilization of metal oxide NPs because it prevents NPs from undesirable agglomeration by providing steric and electrostatic stabilization. Previously, TiO₂ NPs were doped with nitrogen and used as a colorimetric sensing platform for H₂O₂ detection. Here, we have successfully synthesized TiO₂ through an alternate method giving a different nanopore size and without any doping. Moreover, in the reported work, optimization results provided were not comprehensive and lacked necessary details. The reported work also did not provide any real sample analysis, which is a very vital step for testing the applications of a fabricated biosensor. In the current work, we have provided...
comprehensive optimizations and application for the proposed simple TiO₂-based biosensor. To prepare a more efficient catalyst to the characteristics of different reaction systems, ionic liquid design ability has become the latest research hotspot. A significant role of ionic liquids in enhancing catalytic power, i.e., stabilization of NPs, is possibly connected with their good dispersion and solvation power, electrostatic attraction, π−π stretching, weak interaction with substrate and product, and involvement of cationic part of ILs containing acidic proton, thereby facilitating the decomposition of H₂O₂ to generate OH radicals in the oxidation of chromogenic substrate TMB. In our research work, the ionic liquid is chosen as a stabilizing agent. The amount of capped TiO₂ NPs was optimized in microliters by changing its amount in the range of 10−40 μL. The best colorimetric change and the highest peak were obtained when 25 μL of capped NPs was used as shown in Figure 8. Using a small amount of capping agent, there are not enough OH radicals to oxidize the whole TMB. It was seen that increasing the amount of capped NPs helps in accelerating TMB oxidation and color change gradually. However, as the concentration of the capping agent is increased further, the change in color starts to disappear. This indicates that when the concentration of the capped amount is very high, the excess amount will be dispersed in the reaction medium and agglomeration will not occur, which indicates that the reaction is incomplete, which is consistent with the reported literature.

3.3.2. Optimization of TMB Concentration. Figure 9 shows the effect of TMB concentration on biosensor activity. By increasing the concentration of the TMB solution, the absorbance increased quickly till point D as shown in Figure 9. At a concentration of 14 mM (190 μL), the maximum colorimetric change took place. When the concentration of TMB solution was increased further, the growth rate slows down and the reaction mixture appeared in the precipitate because the available TiO₂ molecule gets utilized in the oxidation of TMB. Therefore, 14 mM was selected as the optimum TMB concentration to produce a noticeable colorimetric change.

3.3.3. Optimization of pH. In the biosensor system, the pH of the solution is a key factor. It increases or decreases the efficiency of the biosensor. To find the optimal pH of the sensor we proposed, the response of the sensor was analyzed in the pH range of 3−11. HCl and NaOH were used for variation in the pH, where the best response was obtained at pH 7.2. At pH 7.2, the sensing time was reduced to 4 min and the color of the mixture completely changed to blue-green, as shown in Figure 10. Therefore, 7.2 pH was chosen as the optimum pH for further experimental work. It is concluded that our sensor works favorably around physiological pH, which is in complete agreement with the available literature.

3.3.4. Optimization of Time. The impact of time on sensor response was examined at various time intervals 1−8 min. Figure 11 shows that the excellent response was observed at 4 min because all of the available TiO₂ is utilized during this time and reached the maximum response. After 4 min, no further change was observed. Hence, we selected 4 min as the
optimum time for our proposed sensor and all other experiments were conducted at this time.

3.4. Colorimetric Determination of H$_2$O$_2$. Under the optimum experimental conditions, a rapid and simple colorimetric method based on TiO$_2$ NPs coated with ionic liquid was used for H$_2$O$_2$ detection. Keeping in mind the color change for the quantitative assay of H$_2$O$_2$, a sensitive and selective colorimetric method has been used based on the relationship between H$_2$O$_2$ concentration and absorbance intensity at 652 nm. For the detection of H$_2$O$_2$, the sensitivity of the developed sensor was investigated with different concentrations of H$_2$O$_2$. Figure 12 shows the response of colorimetric biosensors toward various H$_2$O$_2$ concentrations. The sensor response and peak intensity were low at a lower concentration of H$_2$O$_2$ and they increased linearly by increasing its concentration. This technique enabled H$_2$O$_2$ detection with a linear range of $1 \times 10^{-9}$–$3.60 \times 10^{-7}$ M with an $R^2$ value of 0.999. The limit of quantification (LOQ) and limit of detection (LOD) were calculated as $2.86 \times 10^{-7}$ and $8.61 \times 10^{-8}$ M, respectively. An effective NP-based sensor shows a linear response to minor changes in analyte concentration.

Table 2 presents a comparison of the proposed work with the previous studies reported in the literature, which gives a proof of concept that our designed sensor can work at higher as well as lower concentrations. These observations were also compared with the previous study for H$_2$O$_2$ detection based on double molecular recognition, and the results were comparable to our proposed sensor.

3.5. Selectivity Study of the Sensor. The selectivity of the proposed sensor was analyzed with potential interfering species including, ascorbic acid, folic acid, urea, potassium ions, calcium ions, dopamine, and methanol as depicted in Figure 13. Interference studies of the developed sensor play a key role in its productivity having diverse biomedical applications toward clinical diagnoses. Urine has a wide range of potentially interfering species; hence, urine presents huge challenges to various analytical approaches for H$_2$O$_2$ detection. These challenges are not only restricted to the detection limit and sensitivity of biosensors but more essentially to the selectivity of the sensor. Selective detection of H$_2$O$_2$ by the proposed strategy was performed by taking into consideration the coexisting biomolecules and ions in urine. Compared to H$_2$O$_2$, the absorbance values for interfering species such as ascorbic acid, folic acid, urea, uric acid, potassium, calcium ions, dopamine, and methanol were very small. The absorption value observed at 652 nm increases manyfold by adding H$_2$O$_2$. This analytical platform shows high selectivity toward H$_2$O$_2$ in the presence of other coexisting species and does not affect the response even in the presence of twofold of these interfering species.
species. All of the experiments were conducted in the presence of $3.6 \times 10^{-7}$ M $\text{H}_2\text{O}_2$ and the double concentration of interfering species. The results of selectivity are in good agreement with the reported literature. Furthermore, the stability of the developed sensor was assessed by measuring the response with $\text{H}_2\text{O}_2$ after 5 months; there was no remarkable difference observed in sensitivity and selectivity of the sensor. This experiment confirms that our sensor is highly stable and reproducible.

### 3.6. Real Sample Analysis

The proposed colorimetric method was applied to monitor $\text{H}_2\text{O}_2$ in urine samples of cancer patients. In the previous work reported for $\text{H}_2\text{O}_2$ sensing, no real sample analysis was performed. By adding different amounts of $\text{H}_2\text{O}_2$ to the samples, the recoveries and quantitative results of the proposed method are shown in Table 2. The standard solution of $\text{H}_2\text{O}_2$ was spiked with different ratios such as 59, 168, 247, and 329 nm to the urine samples of cancer patients obtained from IRNUM hospital Peshawar, KP, Pakistan (Table 3). The present amount of $\text{H}_2\text{O}_2$ in urine samples of cancer patients is determined from an already established calibration plot, using different concentrations of $\text{H}_2\text{O}_2$ under the same optimized conditions generated at 652 nm. The obtained results are summarized in Table 3 using the percentage recovery formula as shown in Figure 14.

### 4. CONCLUSIONS

Ionic-liquid-capped TiO$_2$ NPs were synthesized and characterized by various analytical techniques such as FTIR, XRD, SEM, TGA, and Raman spectroscopy. The characteristic peaks related to TiO$_2$ have been identified by FTIR, Raman, and XRD analyses. A strong tendency to achieve agglomeration and round morphology of the materials was observed by SEM analysis. The weight loss that occurred at 615°C corresponds to the thermal decomposition of residual organic groups in the as-synthesized TiO$_2$. The ionic liquid utilized not only provides stabilization to NPs but also enhances the conductivity and enzyme mimic properties of the NPs. The proposed colorimetric sensor TiO$_2$/IL exhibits a low limit of detection of $8.61 \times 10^{-8}$ M, a high sensitivity of $2.86 \times 10^{-7}$ M, and a wide linear range of $1 \times 10^{-9}$ to $3.6 \times 10^{-7}$ M. The sensor showed a short incubation time of 4 min. The proposed sensor did not show any interference in results from the coexisting species present in the urine sample. The sensing probe was effectively applied for the determination of $\text{H}_2\text{O}_2$ in cancer patients’ urine samples.

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Table 2. Comparison of Colorimetric Biosensors for the Detection of $\text{H}_2\text{O}_2$ with Some Recently Reported Studies

| s. no. | materials used | method applied | limit of detection ($\mu$M) | linear range ($\mu$M) | references |
|-------|----------------|----------------|-----------------------------|----------------------|------------|
| 1     | RhNPs          | colorimetric   | 0.75                         | 5—125                | 49         |
| 2     | AgNPs/GQDs     | colorimetric   | 0.162                       | 0.5—50               | 41         |
| 3     | Fe$_3$S$_2$-MNPs | colorimetric  | 0.16                        | 2—100                | 50         |
| 4     | Fe$_6$(MoO$_4$)$_3$-F | colorimetric | 0.7                         | 1—30                 | 51         |
| 5     | NiFe LDH       | colorimetric   | 4.4                         | 10—500               | 52         |
| 6     | β-CD/Ca-NCSa   | colorimetric   | 0.2                         | 0.02—10              | 53         |
| 7     | NiO NPs        | colorimetric   | 8                           | 20—100               | 32         |
| 8     | CeO$_2$ NPs    | colorimetric   | 0.5                         | 0.6—1.5              | 54         |
| 9     | [Pyr]Ac-NiO    | colorimetric   | 120                         | 400—4000             | 46         |
| 10    | TiO$_2$ NPs    | colorimetric   | 0.086                       | 0.001—0.36           | present work |

Table 3. Recovery Tests for $\text{H}_2\text{O}_2$ Analysis in Cancer Patients’ Urine Samples Using the Proposed Assay ($n=3$)

| samples | detected (nm) | $\text{H}_2\text{O}_2$ added (nm) | $\text{H}_2\text{O}_2$ found (nm) | recovery (%) | RSD (%) |
|---------|--------------|----------------------------------|---------------------------------|--------------|---------|
| 1       | 1            | 59                               | 60                              | 101.69       | 0.838   |
| 2       | 2            | 168                              | 170                             | 101.19       | 1.009   |
| 3       | 3            | 247                              | 250                             | 101.21       | 0.459   |
| 4       | 1            | 329                              | 330                             | 100.30       | 0.923   |

**Table 2** using the percentage recovery formula as shown in Figure 14.

Figure 13. Interfering study of $\text{H}_2\text{O}_2$ with other analytes (ascorbic acid, folic acid, urea, uric acid, potassium, calcium ions, dopamine, and methanol).

Figure 14. UV−vis spectra of the real samples.
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Notes
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