Cr₂O₃–TiO₂-Modified Filter Paper-Based Portable Nanosensors for Optical and Colorimetric Detection of Hydrogen Peroxide

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ABSTRACT: In the present approach, a Cr₂O₃–TiO₂-modified, portable, and biomimetic nanosensor was designed to meet the requirement of a robust and colorimetric sensing of hydrogen peroxide. Cr₂O₃–TiO₂ nanocomposites prepared via the hydrothermal method were fabricated as a transducer surface on the filter paper using the sol–gel matrix. The color on the filter paper sensor changed from green to blue upon the addition of hydrogen peroxide in the presence of TMB. This change in the color intensity was linear with the concentration of H₂O₂. RGB software was used as a color analyzing model to evaluate the optical signals. This paper-based colorimetric platform provided us with an improved analytical software was used as a color analyzing model to evaluate the optical signals. This paper-based colorimetric platform provided us with an improved analytical software was used as a color analyzing model to evaluate the optical signals. This paper-based colorimetric platform provided us with an improved analytical software was used as a color analyzing model to evaluate the optical signals. The real sample analysis and excellent anti-interference potential results proved the good analytical performance of the proposed design, providing a more promising tool for colorimetric H₂O₂ detection. Introducing Cr₂O₃–TiO₂ nanocomposite-based paper sensors, being a novel method for optical and colorimetric detection, can pave the way for the development of other sensing devices for the detection of different analytes.

1. INTRODUCTION

Hydrogen peroxide, being a reactive oxygen species, causes oxidative stress that results in protein DNA and lipid damage. It is also associated with several severe humanoid diseases including cardiovascular disorders, neurodegenerative diseases, and cancer. A number of analytical techniques such as electron spin resonance spectroscopy, chromatography, chemiluminescence, UV–visible spectrophotometry, flow injection analysis, electrochemistry, and amperometric biosensing have been applied for H₂O₂ detection, many of which involve time-consuming derivatization, high cost, and require professional operation. In contrast, sensors specifically portable and wireless devices can circumvent these shortcomings and play a significant role in the point-of-care testing of hydrogen peroxide in chemical industry, environmental protection, and also in medical applications. However, the excessive demand of such sensors leads us to focus on cost-effective, lightweight, small-sized, and field-portable platforms which should be capable of on-site monitoring. Moreover, such designs should also be user-friendly and nonenzymatic and avoid heavy electronic analytical instruments as in the case of UV–visible spectrophotometry. Therefore, a more sensitive and selective method for H₂O₂ detection is extremely desirable.

Literature study has revealed a variety of advantages of nanomaterial-based peroxidase mimetics as they are not only easy and simple to synthesize but also efficient in working due to their tunable catalytic activity along with low cost and high stability. Nanostructured titania (nTiO₂) is receiving enormous attention owing to its important applications in photocatalysis for environmental cleanup, solar cells, clean H₂ energy production, antimicrobial activity, food processing, and in glucose as well as gas sensing. Also, TiO₂ nanoparticles are preferably chosen for the synthesis of nanocomposites as they act as an efficient synergizing modulator for mimicking peroxidase activity and lower toxicity. However, TiO₂ exhibits a large energy band gap of 3.2 eV and a lower photocatalytic activity when the recombination of charge carriers occurs. These properties somehow limit its electron-transfer ability. Thus, to enhance the intrinsic peroxidase-like activity, nano-TiO₂ can be conjugated with lower-oxidation-state metallic cations such as Cr₂O₃. Cr₂O₃ nanoparticles could be prepared in different sizes using different techniques including sol–gel method, gas condensation, and microwave plasma with a variety of morphologies such as thin films, porous microspheres, nanowires, nanotubes, and so on. Among all of these synthesis processes and

Received: June 22, 2021  
Accepted: August 23, 2021  
Published: September 2, 2021
morphologies, only few research studies have been done on the synthesis of round-shaped Cr₂O₃ nanoparticles ranging from 5 to 200 nm which have the potential to be used as spraying materials having significant features such as high-temperature resistance which is a very important factor in various applications, specifically in sensing.33,34 Besides, the ionic radius of Cr³⁺ is 62 pm, approximately similar to that of Ti⁴⁺ (61 pm). Therefore, the Cr³⁺ ion can be easily incorporated into TiO₂ nanoparticles to form stable26 and enzyme mimic nano-composites for the enhancement of their biomimetic properties and large surface area.25,35 Furthermore, photogenerated electrons may be easily transferred from the conduction band of Cr₂O₃ to that of TiO₂.27,28 Thus, high-purity Cr₂O₃–TiO₂ nanocomposites were synthesized to utilize their smaller concentration for the developed sensor to avoid any potential risks imposed by its higher amounts. Additionally, nanocomposites were encapsulated in sol–gel, which plays a significant role in immobilization to gain rigidity, chemical inertness, and porosity due to the retention capability of the silica matrix.29–31 As in one of the studies, mesoporous silica was used as a template for the synthesis of CeO₂.32–34

Therefore, in the current strategy, biomimetic Cr₂O₃–TiO₂ was synthesized for the first time as a nanozyme for H₂O₂ detection using a filter paper. Filter paper-based sensors could meet the current objectives of a viable low-cost and portable device in addition to offering high sensitivity and selectivity and multiple analyte discrimination.35 Recently, filter paper has drawn much interest as a potential material for sensors and devices in analytical and clinical chemistry because of its versatility, high abundance, and low cost.36 These analytical devices can be integrated in a manner that is flexible, portable, disposable, and easy to operate. Following the invention of paper chromatography in the early 21st century, diagnostic devices based on paper began to emerge.37 Keeping in view these factors, filter paper was used in this study. Filter paper was modified as a transducer surface with Cr₂O₃–TiO₂ nanocomposites encapsulated in sol–gel. In order to reduce the cracking of the gel employed, hydroxyethyl cellulose (HEC) and bovine serum albumin (BSA) mixture was used, while 3,3′,5,5′-tetramethylbenzidine (TMB) acted as a peroxidase substrate for H₂O₂ detection. The analyte was detectable as it came into contact with the modified filter paper surface. The color of the nanocomposite changed from green to blue, indicating the presence of H₂O₂. These nanocomposites act as an optical probe for the colorimetric detection of H₂O₂ due to the excellent peroxidase mimicking activity. Furthermore, they also avoid the aggregation/disaggregation of nanoparticles, which is commonly observed in solution-based sensing.38,39 The abovementioned strategy entailed only a few minutes to detect the analyte, proving its fast analytical activity. The analysis conditions were optimized, and the performance of the proposed system was investigated using a color analyzing model (RGB software). The proposed colorimetric sensor was also employed for H₂O₂ detection in spiked samples. With respect to the traditional analytic systems, this sensor possesses simplicity, field portability, and high sensitivity along with rapid detection toward the low concentration of H₂O₂ with a superior control of the reaction conditions. Nevertheless, in our proposed sensor, the change in color is not reversible. However, it promises ready-to-use availability of the device, which is another major factor for the effectiveness of a design strategy. This ready-to-use sensor not only guarantees high speed for continuous monitoring without renewal but also removes the requirement of a trained person along with strong tenable response and easy miniaturization. These features prove that the proposed sensor not only has a good analytical performance but also offers a common and promising technique for the highly sensitive detection of hydrogen peroxide and other analytes as well.

2. RESULTS AND DISCUSSION

2.1. Characterization of Cr₂O₃–TiO₂ Nanocomposites.

To verify the successful preparation of Cr₂O₃–TiO₂ nanocomposites, scanning electron microscopy (SEM), X-ray diffraction (XRD), Raman spectroscopy, Fourier transform infrared (FTIR), UV-differential reflectance spectroscopy, photoluminescence (PL), and energy-dispersive X-ray (EDX) spectrometry were performed. SEM was employed to investigate the morphology of TiO₂ and Cr₂O₃–TiO₂ nanocomposites. SEM images show the spherical shape and aggregated nature of TiO₂, as shown in Figure 1Aa. After the incorporation of Cr₂O₃ into TiO₂, uniform morphologies were observed, expressing Cr₂O₃–TiO₂ nanocomposite formation shown in Figure 1Ab. It was observed that after Cr₂O₃ addition into the TiO₂ lattice, the growth of the crystal progressed, and therefore, the size becomes slightly bigger and the distribution of the particles gets more even, as shown in Figure 1Ab.40

However, the elemental composition was determined by an area scan using EDX, which was carried out on 5k magnification. Only C, Cr, Ti, and Si peaks were obtained, as shown in Figure 1Ba,b, and their percentage composition is given in Table 1. Then, XRD was used for structural and phase fortitude. Figure 2A illustrates the experimental XRD pattern of TiO₂ with reference to a JCPDS card no. 21-1272.41–43 XRD peaks

### Table 1. % Composition (Atomic) of Samples

| name                               | Ti %  | Cr %  | C %  |
|------------------------------------|-------|-------|------|
| TiO₂ nanoparticles                 | 19.32 | 0     | 25.48|
| Cr₂O₃–TiO₂ nanocomposites          | 26.13 | 0.10  | 7.98 |

ACS Omega 2021, 6, 23368–23377

https://doi.org/10.1021/acsomega.1c03119
of pure TiO$_2$ at a 2$\theta$ angle were recorded at 25.4° (101), 37.8° (004), 48° (200), 55.01° (211), 62.9° (204), and 75° (215). Peaks at 25.4 and 48° were very strong. However, the intensity of the characteristic XRD peak of the anatase phase at 25.4° was found to be increasing with the addition of Cr content.\textsuperscript{44,45} The incorporation of Cr$_2$O$_3$ did not show a high peak shift when compared with pure TiO$_2$ peaks. This confirms the formation of nanocomposites with the anatase phase of TiO$_2$.\textsuperscript{43} No other secondary phase was found in the sample analysis. The crystal sizes of pure TiO$_2$ and Cr$_2$O$_3$–TiO$_2$ nanocomposites were calculated as 10.447 and 14.363 nm, respectively, by using the Scherrer equation ($D = K\lambda /\beta \cos \theta$). These results show high crystallinity and confirm the nanosized TiO$_2$ powder.

Raman investigation (Figure 2B) of Cr$_2$O$_3$–TiO$_2$ nanocomposites describes active models at 142.47, 395.7, 515, and 637.8 cm$^{-1}$. Raman result was consistent with that of XRD patterns. These results confirmed that Cr$_2$O$_3$ was well incorporated in titania for nanocomposite formation.\textsuperscript{46,47} A strong E$_g$ mode at 142.47 cm$^{-1}$ was found, indicating the anatase phase formation.\textsuperscript{48}

Additionally, FTIR analysis was utilized to identify different functional groups present in Cr$_2$O$_3$–TiO$_2$ nanocomposites within the 500–4000 cm$^{-1}$ range (Figure 2C). The first region was observed between 3000 and 3500 cm$^{-1}$ and the second between 500 and 1800 cm$^{-1}$. An absorption band at 3210 cm$^{-1}$ was detected in these spectra attributed to H$_2$O adsorbed at the surface. This absorption was confirmed by the O–H
stretches of the H₂O molecule. The peak at 1626 cm⁻¹ was attributed to the bending vibration of the water molecule H−O−H. The band around 660 cm⁻¹ corresponds to Ti−O−Ti with a minor expansion. This minor expansion observed was due to the lower concentration of Cr. Cr−O−Cr represents the weak bending mode at 870 and 1047 cm⁻¹.30

UV−vis absorption measurements were explored for the optical analysis of the Cr₂O₃−TiO₂ nanocomposites. The absorption spectra in Figure 2D demonstrated the electron photoexcitation from the valence band (formed by oxide anions’ 2p orbitals) to the conduction band (formed by 3d₅g orbitals of the Ti⁴⁺ cations). Titania has a tendency to accept chromium, thus showing a red shift in the absorption edge. This shift was enhanced with a higher Cr₂O₃ concentration. The 540−800 nm range was noticed for having some other peaks.51,52 PL spectra in Figure 2E demonstrate the strong exciton−photon interaction in TiO₂ under the 488 nm laser excitation. These spectra showed that the emission intensity of pure TiO₂ decreases significantly after the addition of Cr₂O₃. The PL intensity of doped Cr³⁺ ions (3d² 4s¹) decreased due to the capturing of photogenerated holes, resulting in the formation of more stable chromium ions. Excessive addition of Cr³⁺ weakens the PL intensity, as shown in Figure 2E.52 When the quenching effect is higher, there will be a decrease in the PL intensity.53

The emission intensity of the ground level reached a higher excitation and reduced the integrated PL intensity, broadening the spectra of pure TiO₂. The relative peak intensity between the ground- and second-level emission greatly changed for pure TiO₂ and Cr₂O₃−TiO₂. The second-level emission is stronger than the ground-level emission in the Cr₂O₃−TiO₂ nanocomposite. This effect proves the formation of Cr₂O₃−TiO₂ nanocomposites.

Moreover, SEM was employed to explore the morphology and chemical composition of silica sol−gel. A porous matrix was observed in sol−gel with a micro- as well as macroporous structure with open pores, as illustrated in Figure 3a. The percentage porosity of the image was calculated using ImageJ software. The whole process was repeated 10 times, and the average percentage porosity was reflected as 9%. However, the elemental composition was determined by an area scan using EDX, which was carried out on 5k magnification. Only O and Si peaks were obtained, as shown in Figure 3b, and its percentages are given in Table 2.

**Table 2. Composition % (Atomic) in Silica Sol−Gel**

| name            | C  | O  | Si  |
|-----------------|----|----|-----|
| silica sol−gel  | 42.47% | 42.61% | 14.92% |

Figure 4. Schematic representation of the colorimetric filter paper-based assays for hydrogen peroxide detection.

The principle of a modified sol−gel-based filter paper sensor is shown in Figure 4. Cr₂O₃−TiO₂ nanocomposites due to their peroxidase mimicking activity and since they act as an optical probe for H₂O₂ detection were immobilized on a filter paper after being encapsulated in a porous matrix of gel. TMB, acting as the chromogenic peroxidase substrate, might get oxidized by H₂O₂ in the presence of Cr₂O₃−TiO₂ nanocomposites. H₂O₂ acts as a catalytic oxidizer by reducing itself, mechanism for the oxidation of TMB by OH radicals can follow the below path

\[
\text{Cr}_2\text{O}_3\text{−TiO}_2 + h\nu \text{Cr}_2\text{O}_3\text{−TiO}_2 (e^- + h^+) \\
\text{Cr}_2\text{O}_3\text{−TiO}_2 (e^−) + \text{H}_2\text{O} \rightarrow \text{OH}^+ + \text{OH}^− \\
\text{Cr}_2\text{O}_3\text{−TiO}_2 (h^+^) + \text{H}_2\text{O}_2 \rightarrow \text{OH}^− + \text{H}^+ \\
\text{OH}^+ + \text{TMB} \rightarrow \text{TMB}^*(\text{oxidized}) \\
2\text{H}^+ + 2\text{OH}^- \rightarrow 2\text{H}_2\text{O} \\
h^+ + e^- \rightarrow (e^- + h^+)^n \text{n} \text{egligible recombination reaction}
\]

https://doi.org/10.1021/acsomega.1c03119

ACS Omega 2021, 6, 23368−23377
change in the optical properties was observed in the absence of H$_2$O$_2$ or TMB. The more the concentration of H$_2$O$_2$, the higher the color intensity will be. The reason is that at higher concentration, more reactive oxygen species (i.e., hydroxyl radicals) will be produced, which accelerate the electron-transfer process. Therefore, the color intensity generally depends on the generation of reactive oxygen species (i.e., hydroxyl radicals) or the electron-transfer process. The stable blue color indicates the completion of the reaction. As can be seen from Figure 4, the presence of H$_2$O$_2$ or TMB alone did not produce any optical change on the surface of the filter paper, while an optical change was observed upon the addition of H$_2$O$_2$ + TMB.

2.3. Optimization of Experimental Parameters.

2.3.1. Gel Stability and Temperature Effect on the Filter Paper Sensor. To design a ready-to-use filter paper sensor for on-site monitoring, it was necessary to optimize the experimental conditions. Sol−gel was used with different concentrations of additives such as BSA, while HEC was used to improve the stability of the gel and to avoid cracking stress. Different volumes of BSA/HEC solutions were used from the prepared concentrations to optimize the concentration and volume effect on sol−gel. 10 μL from each of these solutions was added to sol−gel-based filter papers. These conditions were applied and observed step by step with respect to the concentrations of BSA, HEC, and volume. 0.3 mg of BSA, 2% HEC, and 10 μL from 400 μL of BSA/HEC (200 μL:200 μL) solution showed the best texture and stability of gel up to 24 h. The gel was not cracked and only got squeezed in 24 h in comparison with other samples.

For a ready-to-use on-site monitoring filter paper sensor, four filter papers were prepared using the best optimal conditions and stored at different temperatures (−4, −26, 8, and 10°C). After careful consideration at different time intervals, the sol−gel-based filter paper at 10°C was found to be the best.

2.3.2. Effect of Nanocomposites and TMB on the Filter Paper Sensor. Different concentrations of the 0.266 g Cr$_2$O$_3$−TiO$_2$ nanocomposite samples were optimized for a better colorimetric response. 6 μL of (10 mM) TMB solution and 6 μL of (1 mM) H$_2$O$_2$ solution were added to paper sensors. Filter paper colors changed from light green to blue. Figure 5a shows that the color intensity was in linear proportion with the nanocomposite concentration and 3 mg concentration provided the best colorimetric signal. Thus, this concentration was selected for further experiments. The addition of TMB and H$_2$O$_2$ changes color in 5 min and started to become more intense slowly in 20−25 min. A uniform color distribution on filter paper shows an even spreading of the immobilized mixture. In the start, the color stabilized itself which eventually started to disappear after 40 min, as shown in Figure 5b.

The influence (impact) of concentrations of TMB and H$_2$O$_2$ on the peroxidase-like activity of Cr$_2$O$_3$−TiO$_2$ nanocomposites was also studied, as shown in Figure 5c. A fixed concentration and volume as 6 μL of (1 mM) H$_2$O$_2$ was used with TMB ranging from 2 to 50 mM. After 5 min, the color started to change from green to blue with a gradual improvement in color intensity as the TMB concentration increased. At 20 min, the color reached its maximum intensity. The total time duration was 50 min in which these images were taken at different time intervals. Figure 5c inset illustrates the corresponding calibration curve between the color intensity and TMB.

Figure 5. Optimization of experimental conditions for the colorimetric response of the sol−gel-based filter paper. (a) Different concentrations of Cr$_2$O$_3$−TiO$_2$ nanoparticles: (1) 0.5, (2) 1, (3) 1.5, and (4) 3 mg; (b) effect of time; and (c) different concentrations of TMB: (1) 2, (2) 5, (3) 8, (4) 10, (5) 15, (6) 25, (7) 35, and (8) 50 mM (n = 3).
increasing the a although a higher di there was a signi observed for extremely lower initial concentrations. However, of the lower concentrations, as can be observed in the plot for carried out. Figure 6a illustrates the corresponding dose and reported nanomaterial peroxidase mimics. The lower could be lower or comparable to the values obtained with previously directly related to the (H2O2) is required to achieve the maximum catalytic response with Cr2O3 combined Cr2O3 and TiO2 has a much profound e (Km) value reveals that the synergistic catalytic effect of combined Cr2O3 and TiO2 has a much profound effect, thus increasing the affinity of the nanocomposite toward H2O2.

2.3.3. Detection of H2O2 under Optimal Conditions. Using the optimized conditions, quantitative detection of H2O2 was carried out. Figure 6a illustrates the corresponding dose and response curve between the color intensity and H2O2 concentration. Color intensity steadily increased with the increase in H2O2 concentration, and there was a significant increase in the absorption intensity after 20 μM H2O2, although a higher difference in the visual impact was not observed for extremely lower initial concentrations. However, there was a significant difference in the calculated RGB values of the lower concentrations, as can be observed in the plot for lower concentrations (Figure 6a'). The limit of detection (LOD) value calculated was calculated using the following formula.

Under optimal experimental conditions, the LOD for H2O2 was as low as 0.003 μM, while the "LOD" or detection limit is the lowest concentration level of an analyte that can be determined during an analytical run calculated by the following formula

\[ \text{LOD} = 3.3Sd/Slo \]

where Sd = standard deviation.

This LOD was based on the RGB value and it does not represent the visual LOD. The straight line graph in Figure 6b shows a good linear relationship of H2O2 with the correlation coefficient \((R^2)\) as 0.987. To measure RGB values of each concentration, a RGB color model was used. This model is based on the theory of human eye acuity of colors. Three measurements of scene intensity were taken at every pixel considering blue, red, and green components of the detected light. Moreover, in the case of sensor color, human error, that is, handling may be the reason for poor uniformity. A better relationship was observed between RGB values and selected H2O2 concentrations. This method permitted the detection of H2O2 with the linear range 0.005–100 μM in the presence of a fixed concentration of TMB.

2.3.4. Specificity against the Interfering Compounds. The most reliable and sensitive sensor is the one which responds specifically toward the targeted analyte in a complex system, although several constituents in blood can be measured by quantitation of the hydrogen peroxide generated by specific oxidase enzymes, including glucose, cholesterol, uric acid, glycerol, oxalate, and galactose. Furthermore, methods for the quantitative measurement of hydrogen peroxide have been developed as a consequence of the increasing use of oxidases in reagents. However, a highly specific fast method was still needed for the effective detection of hydrogen peroxide using easily available laboratory reagents. Thus, to evaluate specificity of the proposed sensor, ascorbic acid, isopropyl alcohol, dopamine, and uric acid were used under specific optimized experimental conditions. Modified filter paper strips were incubated using 6 μL of interfering compound solution (1 mM). After incubation, their response was observed in the presence of TMB (10 μL). Color analyzing model RGB was used to analyze the behavior of these interfering compounds. The negligible response of these compounds showed the high selectivity and specificity of the proposed sensor (Figure 7). Hence, it was concluded that our proposed sensor is highly specific toward H2O2 detection.

![Figure 6.](https://doi.org/10.1021/acsomega.1c03119)  
Figure 6. (a) Colorimetric responses of the sol–gel-based filter paper sensor to the different concentrations of H2O2: (1) 0.005, (2) 0.05, (3) 0.1, (4) 1, (5) 3 (6) 5, (7) 7, (8) 11, (9) 17, (10) 2, (11) 35, (12) 50, (13) 70, (14) 80, and (15) 100 μM; (a') magnification of colorimetric response of initial seven concentrations; and (b) linear fitting curve \((n = 3)\).  

![Figure 7.](https://doi.org/10.1021/acsomega.1c03119)  
Figure 7. Selectivity analysis of the filter paper-based assay for H2O2 detection using interfering species. HP = hydrogen peroxide, AA = ascorbic acid, IA = isopropyl alcohol, DA = dopamine, and UA = uric acid.
A colorimetric portable sensing platform for sensitive and rapid H$_2$O$_2$ detection was developed in this study. The H$_2$O$_2$ assay was performed and investigated using peroxidase mimic Cr$_2$O$_3$–TiO$_2$ nanocomposites in the presence of TMB, immobilized on a filter paper surface successfully. The best optimal conditions were selected to design this paper-based sensor. Besides, calibration curves were constructed on the basis of the RGB color analyzing model between the color intensities and TMB and H$_2$O$_2$ concentrations. The transducer surface’s portability and wireless working remove the need for special equipment and a trained person for processing. It works as a bridge for on-site analysis without the centralized labs. A comparison between previously reported strategies and this new study is made in Table 4. It further confirms the effective functionality of our proposed sensor with the lowest LOD.

3. CONCLUSIONS

A colorimetric portable sensing platform for sensitive and rapid H$_2$O$_2$ detection was developed in this study. The H$_2$O$_2$ assay was performed and investigated using peroxidase mimic Cr$_2$O$_3$–TiO$_2$ nanocomposites in the presence of TMB, immobilized on a filter paper surface successfully. The best optimal conditions were selected to design this paper-based sensor. Besides, calibration curves were constructed on the basis of the RGB color analyzing model between the color intensities and TMB and H$_2$O$_2$ concentrations. The transducer surface’s portability and wireless working remove the need for special equipment and a trained person for processing. It works as a bridge for on-site analysis without the centralized labs. A comparison between previously reported strategies and this new study is made in Table 4. It further confirms the effective functionality of our proposed sensor with the lowest LOD.

4. EXPERIMENTAL SECTION

4.1. Reagents and Materials. Cr$_2$O$_3$ (90%), isopropyl alcohol (99.5%), titanium isopropoxide (TTIP) (98%), and HEC (extra pure) (pH 6–8.5) were purchased from DAEJUNG Chemical Reagent, KOREA. Dopamine hydrochloride (99%), hydrogen peroxide (H$_2$O$_2$) (29–32% w/w), and tetramethoxysilane (TMOS) (98%) were purchased from Alfa Aesar, Germany. BSA (pH-7) was purchased from Fischer Scientific, UK. TMB (99.6%) was purchased from CHEM-IMPEX INT’L INC. Uric acid and polyethylene glycol 600 (PEG) were purchased from BDH Chemicals Ltd., England. The milk sample was purchased from the market in Lahore (Pakistan). Nanjing Sunshine Biotechnology Ltd. was contacted for purchasing the FBS sample. All chemicals were of analytical quality, need no purification, and were used as received. Distilled deionized water was used throughout the work to prepare the solutions.

4.2. Apparatus and Characterization. XRD patterns of Cr$_2$O$_3$–TiO$_2$ nanocomposites were recorded using the PANalytical Xpert powder diffractometer (Cu K$_α$ radiation) with a diffractive beam monochromator functionalized at 100 mA and 40 kV. XRD data were investigated by the Rietveld refinement method by using PANalytical high score plus software in the multiphase mode. A scanning electron microscope (LMU, Tescan Vega 3) fortified with an EDX spectrometer was used for elemental exploration and to investigate surface morphologies. The EDX spectrometer was operated at 10 kV. Raman spectra and PL measurements were carried out on a spectrometer [inVia Raman microscope (Renishaw UK, Raman and PL setup)] at 488 nm laser
excitation, with 10 s laser exposure time, and the grating rate was 1800 l/mm. FTIR spectra were recorded under the 4000–600 cm$^{-1}$ scan range at the attenuated total reflectance mode using Nicolet 6700 (Thermo Fischer Scientific, USA). A spectrometer with 8 cm$^{-1}$ resolution UV–vis LAMBDA 35 using Spectralon diffuse reflectance standard for 100% reflectance was used for the analysis of absorption spectra (double beam PerkinElmer). A spectrophotometer (UV-25, Perkin Singapore) with disposable cuvettes (10 mm, having 3 mL capacity and a bandwidth setting of 1 nm) and a scan speed of 960 nm min$^{-1}$ in the range of 400–800 nm was used for UV–vis spectrophotometry.

4.3. Synthesis of TiO$_2$ Nanoparticles. To prepare TiO$_2$ nanoparticles, 30 mL of isopropyl alcohol was added to 20 mL of TTIP. 50 mL of ethanol and 2 mL of deionized water were taken in a separate flask and mixed with the previous solution. Solution pH was maintained up to 3 by adding 1 mL of HCl. The sample was placed in a fume hood for several hours. After the formation of gel in the solution, it was placed in a drying oven at 50°C for 3–4 h. Dried TiO$_2$ nanoparticles obtained were of 6.3 g in weight.

4.4. Synthesis of Cr$_2$O$_3$–TiO$_2$ Nanocomposites. Synthesis of Cr$_2$O$_3$–TiO$_2$ nanocomposites was carried out using a constant volume (40 mL) of ethanol in a round-bottom flask with continuous magnetic stirring. 10 mL of TTIP was added dropwise up to 1 mL after every 30 s. Solution pH was checked with a digital pH meter and maintained at 2 by adding dilute HCl up to 18–20 mL. After 3 h of continuous stirring, 0.2661 g of Cr$_2$O$_3$ was added into the above solution and stirred for 1.5 h again. Then, 30 mL of H$_2$O was added, and the mixture was again set under stirring for the next 30 min. Then, the hydrothermal treatment was given to the slurry for 17 h on 180°C by placing in the hydrothermal vessel. The apparatus was cooled to room temperature, and the solvent layer was removed and centrifuged at 6000 rpm for 3–4 h. Dried TiO$_2$ nanoparticles obtained were 6.3 g in weight.

4.5. Preparation and Loading of Sol–Gel on a Filter Paper. Silica sol–gel preparation was carried out by using TMOS (14%) and deionized water (42.3%) in an Eppendorf tube. 1 mM HCl (40%) and PEG 600 (3.7%) were added to the above solution. This mixture was sonicated for 10–15 min and kept for one night at 4°C. A clear transparent gel was obtained. The surface of Whatman filter paper no. 1 was modified by using 100 μL of the synthesized gel. Standard grade Whatman filter paper no. 1 was used in the current work, possessing high-quality medium of retention and flow rate. Cellulose fiber is the main component of the filter paper and allows the liquid to penetrate within its matrix deprived of any external source.

4.6. Preparation of a Filter Paper-Based Sensor. For fabrication of a filter paper-based sensor, 3 mg of Cr$_2$O$_3$–TiO$_2$ nanocomposite powder was allowed to mix with 100 μL of sol–gel in a Petri dish. The gel and nanocomposite mixture was loaded on a small filter paper with great care, so it may have an equal surface. The diameter of the paper strip was 0.6 mm.

4.7. Colorimetric Assay of H$_2$O$_2$ and Color Intensity Measurements. In order to observe and monitor H$_2$O$_2$ via portable colorimetric assays, the above-designed filter paper sensor was used. 6 μL of (10 mM) TMB solution and then 6 μL of (1 mM) H$_2$O$_2$ solution were added to paper sensors. For color quantification and intensity measurements, filter paper images were obtained with a high-resolution smartphone camera model HUAWEI P10 lite at zoom mode with the same distance of 6 cm from each filter paper sensor. It took less than 5 min. To analyze the output optical signal and performance from these images, a color analyzing software (RGB) was inclined, which depicts the color intensity value. The calibration graph was assembled to govern the performance of this assay based on the elucidation of the employed analyzing software results of the captured filter paper images. Color analyzing software RGB comprises three additive primary colors (red, green, and blue). RGB software was preferred for colorimetric optical analysis because it is easy to use and significant as it has been employed in computers, graphic cards, and LCDs to display images. The analysis was carried out based on the color intensity of each picture.

4.8. Real Sample Preparation. The above-described assay for hydrogen peroxide detection was used for analysis in tap water, milk, and FBS. Then, a known amount of hydrogen peroxide was spiked to each real sample to achieve three selected concentrations: one near the LOD (0.01 μM) and two in the linear range (50 and 100 μM). These samples will be further employed to check the potential of the proposed sensor under the optimal conditions of sensing.

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Notes

The authors declare no competing financial interest.
ACKNOWLEDGMENTS

This work was carried out as a part of the research project through the Higher Education Commission, Pakistan National Research Program for Universities (ID no. 5432). S.J. highly acknowledges the financial support of the Higher Education Commission, Pakistan, through a financial grant under the National Research Program for Universities (ID no. 5432).

REFERENCES

(1) Qu, L.-L.; Liu, Y.-Y.; He, S.-H.; Chen, J.-Q.; Liang, Y.; Li, H.-T. Highly selective and sensitive surface enhanced Raman scattering nanosensors for detection of hydrogen peroxide in living cells. Biosens. Bioelectron. 2016, 77, 292–298.
(2) Nakashima, K.; Maki, K.; Kawaguchi, S.; Akiyama, S.; Tsukamoto, Y.; Imai, K. Peroxyxalate chemiluminescence assay of hydrogen peroxide and glucose using 2, 4, 6, 8-tetramorpholino-pyrimido [5, 4-d]-pyrimidine as a fluorescent component. Anal. Sci. 1991, 7, 709–713.
(3) Matsubara, C.; Kawamoto, N.; Takamura, K. Oso [5, 10, 15, 20-tetra (4-pyridyl) porphyrinato] titanium (IV) [IV]: an ultra-high sensitivity spectrophotometric reagent for hydrogen peroxide. Analyst 1992, 117, 1781–1784.
(4) Lin, J.-M.; Sato, K.; Yamada, M. Hydrogen peroxide chemiluminescent flow-through sensor based on the oxidation with peridate immobilized on ion-exchange resin. Microchem. J. 2001, 69, 73–80.
(5) Oungpipat, W.; Alexander, P.; Southwell-Keely, P. A reagentless amperometric biosensor for hydrogen peroxide determination based on asparagus tissue and ferrocene mediation. Anal. Chim. Acta. 1995, 309, 35–45.
(6) Gao, Z.; Ivaska, A.; Li, P.; Lui, K.; Yang, J. Electrocatalysis and flow-injection analysis of hydrogen peroxide at a chemically modified electrode. Anal. Chim. Acta 1992, 259, 211–218.
(7) Cosgrove, M.; Moody, G. J.; Thomas, J. D. R. Chemically immobilised enzyme electrodes for hydrogen peroxide determination. Analyst 1988, 113, 1811–1815.
(8) Ruzgas, T.; Gorton, L.; Emneus, J.; Marko-Varga, G. Kinetic models of horseradish peroxidase action on a graphite electrode. J. Electrochem. 1995, 391, 41–49.
(9) Vidal, J. C.; Yague, M. A.; Castillo, J. R. A chronoaerometric sensor for hydrogen peroxide based on electron transfer between immobilized horseradish peroxidase on a glassy carbon electrode and a diffusing ferrocene mediator. Sens. Actuators, B 1994, 21, 135–141.
(10) Vreeke, M.; Maidan, R.; Heller, A. Hydrogen peroxide and beta-nicotinamide adenine dinucleotide sensing amperometric electrodes based on electrical connection of horseradish peroxidase to electrodes through a three-dimensional electrode relaying polymer network. Anal. Chem. 1992, 64, 3084–3090.
(11) Popescu, I. C.; Zetterberg, G.; Gorton, L. Influence of graphite powder, additives and enzyme immobilization procedures on a mediatorless HRP-modified carbon paste electrode for amperometric flow-injection detection of H2O2. Biosens. Bioelectron. 1995, 10, 443–461.
(12) Li, J.; Tan, S. N.; Ge, H. Silica sol-gel immobilized amperometric biosensor for hydrogen peroxide. Anal. Chim. Acta 1996, 335, 137–145.
(13) Laine, D. F.; Chong, F. F. Analysis of hydrogen peroxide and an organic hydroperoxide via the electrocatalytic Fenton reaction. Microchem. J. 2009, 91, 78–81.
(14) Rakow, N. A.; Suslick, K. S. A colorimetric sensor array for odour visualization. Nature 2000, 406, 710.
(15) Martinez, A. W.; Phillips, S. T.; Whitesides, G. M.; Carrillo, E. Diagnostics for the developing world: microfluidic paper-based analytical devices. Anal. Chem. 2010, 82, 3. ACS Publications
(16) Hayat, A.; Marty, J. Disposable screen printed electrochemical sensors: Tools for environmental monitoring. Sensors 2014, 14, 10432–10453.
(17) Capitan-Vallvey, L. F.; Palma, A. J. Recent developments in handheld and portable optosensing—A review. Anal. Chim. Acta 2011, 696, 27–46.
(18) Moreau, P.; Anizon, F.; Sancelme, M.; Prudhomme, M.; Bailly, C.; Sevère, D.; Riou, J.-F.; Fabbro, D.; Meyer, T.; Aubertin, A.-M. Syntheses and Biological Activities of Rebeccamycin Analogues. Introduction of a Halogenoacetyl Substituent. J. Med. Chem. 1999, 42, 5846–5849.
(19) Liu, C.; Yang, S. Synthesis of Angstrom-Scale Anatase Titania Atomic Wires. ACS Nano 2009, 3, 1025–1031.
(20) Sisman, O.; Poli, N.; Zappa, D.; Comini, E. Synthesis of nanoporous TiO2 with the use of diluted hydrogen peroxide solution and its application in gas sensing. Coatings 2019, 9, 681.
(21) Jeong, H.; Yoo, J.; Park, S.; Lu, J.; Park, S.; Lee, J. Non-Enzymatic Glucose Biosensor Based on Highly Pure TiO2 Nanoparticles. Biosens. Bioelectron. 2021, 11, 149.
(22) Nasir, M.; Bagwasi, S.; Jiao, Y.; Chen, F.; Tian, B.; Zhang, J. Characterization and activity of the Ce and N co-doped TiO2 prepared through hydrothermal method. Chem. Eng. J. 2014, 236, 388–397.
(23) Mangrola, M.; Parmar, B.; Pillai, A.; Joshi, V. Structural, optical and electrical properties of titanium dioxide nanoparticle. Multi. Disci. Educ. Glob. Que. 2012, 1, 138–145.
(24) Ghosh, S.; Das, A. P. Modified titanium oxide (TiO2) nanocomposites and its array of applications: a review. Toxicol. Environ. Chem. 2015, 97, 491–514.
(25) Talavari, F.; Shakeri, A.; Mighani, H. Synthesis of Cr2O3/TiO2 Nanocomposite and its Application as the Blocking Layer in Solar Cells. J. Environ. Anal. Chem. 2018, 5, 2380–2391.
(26) Yang, Z.; Ren, W.; Zhu, L.; Qings, Y.; Huang, Z.; Luo, F.; Zhou, W. Electromagnetic-wave absorption property of Cr2O3–TiO2 coating with frequency selective surface. J. Alloys Compd. 2019, 803, 117.
(27) Chen, M. L.; Cho, K. Y.; Oh, W. C. Synthesis and photocatalytic behaviors of Cr 2 O 3–CNT/TiO2 composite materials under visible light. J. Math. Sci. 2010, 45, 6611–6616.
(28) Lan, S.-M.; Sin, J.-C.; Mohamed, A. R. Cr2O3 nanoparticles anchored on ZnO nanorods as active heterostructure catalysts for phenol degradation. Sains Malays. 2018, 47, 253–259.
(29) Lev, O.; Tsionsky, M.; Rabinovich, L.; Glezer, V.; Sampath, S.; Pankratov, I.; Gun, J. Organically modified sol-gel sensors. Anal. Chem. 1995, 67, 22A–30A.
(30) Dave, B. C.; Dunn, B.; Valentine, J. S.; Zink, J. I. Sol-gel encapsulation methods for Biosens. Anal. Chem. 1994, 66, 1120A–1127A.
(31) Avnir, D. Organic chemistry within ceramic matrices: doped sol-gel materials. Acc. Chem. Res. 1995, 28, 328–334.
(32) Orcel, G.; Phalippou, J.; Hench, L. Structural changes of silica aerogels during low temperature dehydration. J. Non-Cryst. Solids 1986, 88, 114–130.
(33) Orcel, G.; Hench, L. Effect of formamide additive on the chemistry of silica sol-gels: Part I: NMR of silica hydrolysis. J. Non-Cryst. Solids 1986, 79, 177–194.
(34) Rossinyol, E.; Arbiol, J.; Peiró, F.; Cornet, A.; Morante, J. R.; Tian, B.; Bo, T.; Zhao, D. Nanostructured metal oxides synthesized by hard template method for gas sensing applications. Sens. Actuators, B 2005, 109, 57–63.
(35) Pesarani, S.; Rafatmah, E.; Hamedienejad, B. An All-in-One Solid State Thin-Layer Potentiometric Sensor and Biosensor Based on Three-Dimensional Origami Paper Microfluidics. Biosens. 2021, 11, 44.
(36) Kim, J.; Ahn, D.; Sun, J.; Park, S.; Cho, Y.; Park, S.; Ha, S.; Ahn, S.; Kim, Y. A.; Park, J.-J. Vertically and Horizontally Drawing Three-Dimensional Origami Paper Microfluidics. Solid State Thin-Layer Potentiometric Sensor and Biosensor Based on Highly Pure TiO2 Nanoparticles. Biosens. Bioelectron. 2021, 11, 149.
(37) Bendicho, C.; Lavilla, I.; Pena-Pereira, F.; de la Calle, I.; Romero, V. Nanomaterial-Integrated Cellulose Platforms for Optical
Sensing of Trace Metals and Anionic Species in the Environment. 

Sensors 2021, 21, 604. 
(38) Heo, J. H.; Yi, G. S.; Lee, B. S.; Cho, H. H.; Lee, J. W.; Lee, J. H. A significant enhancement of color transition from an on–off type achromatic colorimetric nanosensor for highly sensitive multi-analyte detection with the naked eye. Nanoscale 2016, 8, 18341–18351. 
(39) Nilam, M.; Hennig, A.; Nau, W. M.; Assaf, K. I. Gold nanoparticle aggregation enables colorimetric sensing assays for enzymatic decarboxylation. Anal. Methods 2017, 9, 2784–2787. 
(40) Vidyasagar, C. C.; Muralidhara, H. B.; Naik, Y. A.; Hosamani, G.; Ilango, M. S. Effect of annealing on structural, crystallinity and optical properties of anatase Cr–TiO2 nanoparticles. Energy Environ. Focus 2015, 4, 54–63. 
(41) Antić, Z.; Krsmanović, R. M.; Nikolić, M. G.; Marinović-Cincovic, M.; Mitrić, M.; Polizzi, S.; Dramčanin, M. D. Multisite luminescence of rare earth doped TiO2 anatase nanoparticles. Mater. Chem. Phys. 2012, 135, 1064–1069. 
(42) Theivasanitti, T.; Alagar, M. Titanium dioxide (TiO2) Nanoparticles XRD Analyses: An Insight. 2013, arXiv:1307.1091. arXiv preprint. 
(43) Asemi, M.; Maleki, S.; Ghanatashoar, M. Cr-doped TiO2 2-based dye-sensitized solar cells with Cr-doped TiO 2 blocking layer. J. Sol-Gel Sci. Technol. 2017, 81, 645–651. 
(44) Ba-Abbad, M. M.; Kadhum, A. A. H.; Mohamad, A. B.; Takriff, M. S.; Sopian, K. Synthesis and catalytic activity of TiO2 nanoparticles for photochemical oxidation of concentrated chlorophenols under direct solar radiation. Int. J. Electrochem. Sci. 2012, 7, 4871–4888. 
(45) Thamaphat, K.; Limsuwan, P.; Ngotawornchai, B. Phase characterization of TiO2 powders by XRD and TEM. Kasetsart J./Nat. Sci. 2008, 42, 357–361. 
(46) Nguyen, D.-T.; Hong, S.-S. Synthesis of metal ion-doped TiO2 nanoparticles using two-phase method and their photocatalytic activity under visible light irradiation. J. Nanosci. Nanotechnol. 2016, 16, 1911–1915. 
(47) Kernazhitsky, L.; Shymansovska, V.; Gavrilko, T.; Naumov, V.; Fedorenko, L.; Kshnyakin, V.; Baran, J. Photoluminescence of Cr-doped TiO2 induced by intense UV laser excitation. J. Lumin. 2015, 166, 253–258. 
(48) Nassoko, D.; Li, Y.-F.; Li, J.-L.; Li, X.; Yu, Y. Neodymium-doped with anatase and brookite two phases: mechanism for photocatalytic activity enhancement under visible light and the role of electron. Int. J. Photoenergy 2012, 2012, 716087 DOI: 10.1155/2012/716087. 
(49) Bsiiri, N.; Zrir, M. A.; Bardaoui, A.; Bouaicha, M. Morphological, structural and ellipsometric investigations of Cr doped TiO2 thin films prepared by sol–gel and spin coating. Ceram. Interfaces 2016, 42, 10599–10607. 
(50) Dwivedi, S.; JadHAV, J.; Sharma, H.; Biswas, S. Pulsed laser deposited ferromagnetic chromium dioxide thin films for applications in spintronics. Phys. Procedia 2014, 54, 62–69. 
(51) Dubey, R. S.; Singh, S. Investigation of structural and optical properties of pure and chromium doped TiO2 nanoparticles prepared by solvothermal method. Res. Inst. Phys. 2017, 7, 1283–1288. 
(52) Li, X.; Guo, Z.; He, T. The doping mechanism of Cr into TiO 2 and its influence on the photocatalytic performance. Phys. Chem. Chem. Phys. 2013, 15, 20037–20045. 
(53) Gfroerer, T. H. Photoluminescence in analysis of surfaces and interfaces. Encyclopedia of Analytical Chemistry; Wiley, 2006. 
(54) An, Q.; Sun, C.; Li, D.; Xu, K.; Guo, J.; Wang, C. Peroxidase-like activity of Fe3O4@ carbon nanoparticles enhances ascorbic acid-induced oxidative stress and selective damage to PC-3 prostate cancer cells. ACS Appl. Mater. Interfaces 2013, 5, 13248–13257. 
(55) Kim, K. I.; Yoon, S.; Chang, J.; Lee, S.; Cho, H. H.; Jeong, S. H.; Jo, K.; Lee, J. H. Multifunctional Heterogeneous Carbon Nanotube Nanocomposites Assembled by DNA-Binding Peptide Anchors. Small 2020, 16, 1905821. 
(56) Ahmed, A.; Hayat, A.; Nawaz, M. H.; John, P.; Nasir, M. Construction of sponge-like graphitic carbon nitride and silver oxide nanocomposite probe for highly sensitive and selective turn-off fluorimetric detection of hydrogen peroxide. J. Colloid Interface Sci. 2020, 558, 230–241. 
(57) Fierke, C. A.; Hammes, G. G. Transient kinetic approaches to enzyme mechanisms. Contemporary Enzyme Kinetics and Mechanism; Academic Press: New York, 1996; p 35. 
(58) James, D. R.; Price, C. P. Interference in colorimetric reactions for measuring hydrogen peroxide. Ann. Clin. Biochem. 1984, 21, 398–404. 
(59) Hayat, A.; Barthembs, L.; Marty, J.-L. A simple colorimetric enzymatic-assay for okadaic acid detection based on the immobilization of protein phosphatase 2A in sol-gel. Appl. Biochem. Biotechnol. 2012, 166, 47–56. 

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