Synthesis, characterization of V₂O₅ nanoparticles and determination of catalase mimetic activity by new colorimetric method

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Abstract
This paper deals with an advanced colorimetric method used to determine the catalase mimetic activity of V₂O₅ nanoparticles by measuring the decrease in potassium permanganate concentration in a mixture containing V₂O₅ and hydrogen peroxide. The experiments were carried out in batch reactor at room temperature for 3 min at wavelength number of 525 nm. Vanadium pentoxide was synthesized by hydrothermal method (reflux) from ammonium metavanadate (NH₄VO₃) as a precursor and cetyltrimethylammonium bromide as a surfactant. The annealing of the product was carried out for 2 h, at temperatures of 250, 500 and 750 °C. In order to determine the structure and the chemical nature of the nanoparticles prepared, the characterization was carried out by X-ray diffraction and scanning electron microscopic techniques. Atomic force microscopic and thermal gravimetric investigations have shown the decomposition steps of V₂O₅ at different temperatures. UV–visible spectroscopic technique and Fourier transform spectrometry were used to further characterize the nanoparticles. Advanced colorimetric method was used to study the catalase mimetic activity of the newly synthesized vanadium pentoxide (V₂O₅) nanoparticles using hydrogen peroxide (H₂O₂) as substrate. V₂O₅ nanoparticles resulted in an increase in the catalase mimetic activity with increasing the annealing temperature of the V₂O₅ nanoparticles. The maximum activity was found at 500 °C, which subsequently decreased with further increase in the annealing temperature.

Keywords Vanadium pentoxide nanoparticles · Catalase · Hydrothermal treatment

Introduction
Hydrogen peroxide is a by-product produced inside of the cells of living organisms and has detrimental effects on them. Catalases are enzymes used by the cells to get rid of hydrogen peroxide and protecting them from its harmful impacts. However, if the concentration of H₂O₂ increases due to disease, exposure to radiation and certain chemicals and medications which surpasses the capacity of catalase, H₂O₂ begins to accumulate and cause cellular injury. Extracting the catalase enzyme from the cells and using it as a medication is costly, and furthermore, by introducing the enzyme into the human body by injection or ingestion, it will not penetrate into the cells because of its high molecular mass. Therefore, it will function outside the cells and will have a minor effect. On the other hand, the enzyme itself is costly to be stored because it requires special conditions including cooling and lighting. As a result, utilizing compounds that mimic the enzymatic function of catalase is a welcome method by the researchers.

The famous physicochemical properties of nanomaterials have attracted the attention of researchers in the fields of gas sensors [1], catalysis [2–4], energy production [5], medicine [6] and environmental protection [7]. These methods signify the importance of nanomaterials regarding the production of variety of products and better materials [8], such as cosmetics, clothing, computers, medical devices and sports...
Vanadium oxides exist in various V–O coordination geometrical forms such as VO, V₂O₅, VO₂ and V₂O₄[11], [12]. The most stable form among these is the vanadium pentoxide (V₂O₅) in the V–O system. It has been the center of industrial and applied research owing to its exceptional physical and chemical properties [12]. Various physical as well as chemical methods are applied for the preparation of vanadium pentoxide including chemical vaporization deposition [13], electrochemical deposition, laser ablation [14], precipitation [10], sol–gel synthesis [15] and hydrothermal method [16], [17]. Among the various methods available, hydrothermal method is a simple, easy, inexpensive, high-speed and eco-friendly method [17]. Several phases of V₂O₅ were identified, such as α-V₂O₅, β-V₂O₅ and γ-V₂O₅. These phases are different in their structures. Both α-V₂O₅ and γ-V₂O₅ were found as orthorhombic, while β-V₂O₅ was found in monoclinic form. The tetragonal α-V₂O₅ is the most stable among these phases, and all phases are converted into α-V₂O₅ phase at elevated temperatures [12].

Vanadium-based compounds are used as oxidation agents. The vanadium pentoxide has the highest oxidation state which makes it very important as catalysts in oxidation processes such as oxidation of SO₂ to SO₃ [18], xylene oxidation to phthalic anhydride [19] and furfural to maleic anhydride [20]. Similarly, some vanadyl complexes were synthesized and characterized for the application as insulin mimetic agents [21]. Moreover, various vanadium–chelate complexes have been reported to have insulin mimetic properties. Due to this, these complexes are very important for pharmacotherapy of diabetes [22, 23]. The physicochemical properties of the nanomaterials are utilized to mimic the catalytic activity of various enzymes such as catalases, oxidases and peroxidases [24].

Catalase is among the major enzymes with crucial antioxidant function which minimizes the oxidative stress through cellular breakdown of hydrogen peroxide, resulting in the production of oxygen and water. The importance of the catalase enzyme can be estimated from the numerous diseases associated with malfunctioning or deficiency of enzyme. These diseases include cancer, anemia, hypertension, Parkinson’s disease, diabetes mellitus, Alzheimer’s disease, schizophrenia and bipolar disorder [25].

According to their function and structure, the catalase can be categorized into three main classes. First and second categories are known as heme-containing enzymes hence known as catalase-peroxidases. These are generally found in bacteria, fungi and archaeabacteria with 120 and 340 kDa molecular mass. The third category is known as manganese catalase. It is mainly found in bacteria. It has oligomeric structures having molecular mass in the range of 170 and 210 kDa. Two manganese ions can be identified in the hydrogen peroxide decomposition reactions as given below [25, 39]:

First step: H₂O₂ + Mn⁴⁺ + Mn⁴⁺ + 2H⁺ → Mn⁵⁺ + Mn⁵⁺ + 2H₂O

Second step: H₂O₂ + Mn⁵⁺ + Mn⁵⁺ + Mn⁵⁺ + 2H⁺ + O₂

Equations 1 and 2 show the oxidation and reduction steps due to the metal (manganese) in catalase enzyme reaction. On the basis of these reactions, the use of another metal was considered to follow the degradation of H₂O₂. The motivation of the current work was to develop an advanced colorimetric method to be used for the determination of the catalase mimetic activity of pentavalent metal oxide V₂O₅ nanoparticles [26, 27]. V₂O₅ nanoparticles were prepared and used as catalase mimetic enzyme to compare the activity of the vanadia nanoparticles with that of the manganese in the above given reactions. The research concentrated on three focal points:

1. Preparing and characterization of vanadium pentoxide nanoparticles;
2. Mimetic catalase activity studies by using vanadium pentoxide nanoparticles;
3. Using an advanced colorimetric method for assessing the mimetic catalase activity depending on the reaction between potassium permanganate and hydrogen peroxide, depending on the color change of potassium permanganate.

In this research, we studied the activity/catalase mimetic activity of V₂O₅ nanoparticles by measuring the decrease in potassium permanganate concentration in a mixture containing V₂O₅ and hydrogen peroxide to determine the surface chemistry properties of the vanadia nanoparticles. V₂O₅ nanoparticles were prepared by using hydrothermal method from ammonium metavanadate as a precursor and cetyltrimethylammonium bromide (CTAB) dissolved in ethanol/water mixture solvent as a surfactant solution. The annealing of product was carried out at various temperatures (250, 500 and 750 °C) for 2 h. The characterization of the vanadia nanoparticles was carried out with different techniques to confirm the effectiveness of the synthesis of the desired nanoparticles. The catalytic activity of vanadium pentoxide
nanoparticles was studied also in the function of the annealing temperature.

**Experimental**

**Materials and methods**

Ammonium metavanadate (NH$_4$VO$_5$, 99.99%; from Sigma-Aldrich Co.), nitric acid (HNO$_3$, 99%; from Merck Chemicals), cetyltrimethylammonium bromide (CTAB, C$_{19}$H$_{42}$BrN, 99%; from Merck Chemicals) and ethanol (99.8%; from Sigma-Aldrich Co.) were used for the experiments.

**Synthesis of vanadium pentoxide nanoparticles**

Hydrothermal method was used for the preparation of vanadium pentoxide nanoparticles, and the process was initiated by dissolving 0.1 g of ammonium metavanadate and 0.1 g of CTAB in a mixture of water–ethanol (100 mL) in the ratio of 7:3, respectively. It was followed by adding nitric acid very slowly under continuous stirring until the pH was set to 2.5. The mixture was refluxed for 6 h. The orange color precipitate was washed with distilled water (10 times), then washed with ethanol and dried in oven at 90 °C for 1 h; then, it was annealed at 250, 500 and 750 °C for 2 h.

**Mimetic activity procedure**

**Determination of mimetic activity of catalase**

In order to determine the mimetic activity of catalase, an advanced colorimetric method was developed and used [28]. Standard solution of acidic KMnO$_4$ (0.00185 M) was prepared by titration with standard solution of sodium oxalate (Na$_2$C$_2$O$_4$, 0.14 M). The reaction took place between the reactants according to chemical reaction (Eq. 3):

$$2\text{KMnO}_4 + 5\text{Na}_2\text{C}_2\text{O}_4 + 8\text{H}_2\text{SO}_4 \rightarrow 2\text{MnSO}_4 + \text{K}_2\text{SO}_4 + 5\text{Na}_2\text{SO}_4 + 10\text{CO}_2 + 8\text{H}_2\text{O}$$

The molarity (M) of the KMnO$_4$ solution can be calculated by Eq. 4:

$$\text{M of KMnO}_4\text{ solution} = \frac{\text{volume of Na}_2\text{C}_2\text{O}_4\text{ used} \times \text{M of Na}_2\text{C}_2\text{O}_4}{(2/5) \text{volume of KMnO}_4}$$

In order to determine the concentration of the hydrogen peroxide (H$_2$O$_2$), unknown concentration of H$_2$O$_2$ was titrated with known concentration of acidic KMnO$_4$ solution. The molarity (M) of the H$_2$O$_2$ solution can be calculated according to chemical reaction Eq. 5:

$$2\text{KMnO}_4 + 5\text{H}_2\text{O}_2 + 3\text{H}_2\text{SO}_4 \rightarrow 2\text{MnSO}_4 + \text{K}_2\text{SO}_4 + 5\text{O}_2 + 8\text{H}_2\text{O}$$

The molarity (M) of the H$_2$O$_2$ solution can be calculated by using Eq. 6:

$$\text{M of H}_2\text{O}_2\text{ solution} = \frac{\text{volume of KMnO}_4\text{ required} \times \text{M of KMnO}_4}{(5/2) \text{volume of H}_2\text{O}_2}$$

The maximum absorbance of KMnO$_4$ solution (1 × 10$^{-5}$ mol mL$^{-1}$) was determined by using UV–visible spectrophotometry (UV–Vis) in the wavelength range of 400–700 nm. The solutions have exhibited two peaks in the UV–Vis spectrum, and the taller peak is the primary peak (λ max) at 525 nm as shown in Fig. 1a.

Standard calibration curve was prepared by using solutions having different KMnO$_4$ concentrations (0, 1, 2, 3, 4 and 5 × 10$^{-5}$ mol mL$^{-1}$) to find the relationship between the concentrations and absorbance as given in Fig. 1b.

Catalase mimetic activity was determined by using the reaction of vanadium pentoxide solution and hydrogen peroxide as depicted in chemical reaction (Eq. 7):

$$2\text{H}_2\text{O}_2 \rightarrow \text{V}_2\text{O}_5\text{O}_2 + \text{H}_2\text{O}$$
**Procedure**

The following reagents, acidic potassium permanganate solution (1.80 mmol mL\(^{-1}\)), hydrogen peroxide (2.25 mmol mL\(^{-1}\)) and vanadium pentoxide solution (0.01 mmol mL\(^{-1}\))—by dissolving the vanadium pentoxide in dimethyl sulfoxide (DMSO) solvent—were used in our work. At first, four test tubes were prepared and marked as test (T), control (C), standard (S) and blank (B). Distilled water was added (1.3, 1.5, 2.3 and 2.5 mL spontaneously) to these test tubes, respectively. 0.2 mL of vanadium pentoxide solution (0.01 mmol mL\(^{-1}\)) was added to two tubes: control and test tubes; then, those were shaken to make sure of the well mixing. 1 mL of hydrogen peroxide (2.25 mmol mL\(^{-1}\)) was added to both tubes (test and standard solution tubes); then, those were shaken well. After 3 min, 0.5 mL of potassium permanganate was added (1.80 mmol mL\(^{-1}\)) to all four tubes and those were shaken well. The absorbance at 525 nm was measured as shown in Table 1. The procedure can be explained by the following steps:

1. Distilled water (1.3 mL and 2.3 mL) was added into two test tubes labeled “T1 and C1,” respectively; then, 0.2 mL vanadium pentoxide solutions was added to both test tubes. Following this, 1 mL hydrogen peroxide solution was added into “T1” test tube. After this, all tubes were sealed and thoroughly mixed.

2. After 3 min, 0.5 mL of acidic solution of permanganate was added into both tubes “T1” and “B1”; then, all test tubes were mixed with the vortex.

3. The absorbance decrease of KMnO\(_4\) is equal to B1–T1 which was measured calorimetrically at 525 nm wavelength by using standard calibration curve (Fig. 1) where B1 and T1 are blank solution tube and standard solution tube, respectively.

4. Catalase mimetic activity was calculated according to the first-order reaction Eq. 8:

\[
\text{Catalase mimetic activity} = (2.303/t) \times \log \left( \frac{A_o}{A} \right)
\]

where \(K\) is the reaction rate of catalase mimetic reaction (i.e., reaction rate of hydrogen peroxide decomposition) [29].

\(t\) : time of reaction in seconds.

\(A_o\) : concentration of \(\text{H}_2\text{O}_2\) before the reaction which is equal to absorbance difference of \((B–S)\), where \(B\) and \(S\) are blank solution tube and standard solution tube, respectively.

\(A\) : the concentration of \(\text{H}_2\text{O}_2\) after the reaction which is equal to the absorbance difference of control solution tube (\(C\)) minus test solution tube (\(T\)) multiplied by \(5/2\).

5. The catalase mimetic activity determination was carried out according to the following steps summarized in Table 1.

**Results and discussion**

**Fourier transform infrared (FTIR) analysis**

FTIR spectra of \(\text{V}_2\text{O}_5\) samples as-prepared at 90 °C and annealed at 250, 500 and 750 °C were recorded in the wave number range of 4000 cm\(^{-1}\) and 400 cm\(^{-1}\) for functional groups and chemical bonds, and these are presented in Fig. 2. The appearance of the FTIR bands at two different wave numbers (3212 cm\(^{-1}\) and 1661 cm\(^{-1}\)) corresponds to O–H stretching and bending vibrations, respectively. The intensity of the bands decreased with increasing the annealing temperature. This clearly indicates the direct relationship between the annealing temperature and bonds. The characteristic peaks at 1020 cm\(^{-1}\), 827 cm\(^{-1}\), 615 cm\(^{-1}\) and 479 cm\(^{-1}\) correspond to...
479 cm$^{-1}$ correspond to the stretching vibration of terminal oxygen bonds (V = O), the vibration of doubly coordinated oxygen (bridging oxygen) bonds (V–O–V), and the asymmetric and symmetric stretching vibrations of triply coordinated oxygen (chain oxygen) bonds, respectively [30].

**UV–visible spectroscopic results**

The UV-Vis absorption spectra was used to record 0.001 M of V$_2$O$_5$ which dissolved in ethanol. UV–Vis optical properties in the range (200–800) nm at various temperatures (90, 250, 500 and 750 °C) showed temperature-dependent absorbance as given in Fig. 3. It can be seen that the absorption peaks of V$_2$O$_5$ “as-prepared sample” appear around 316 nm (Eg=2.55 eV). The energy gap increased to 2.63 eV when the sample was annealed at 250 °C, while it decreased to 2.48 eV when the sample was annealed at 500 °C. On the other hand, the energy gap increased again (2.56 eV) when the sample was annealed at 750 °C. On increasing the annealing temperature, the phase change of the vanadia nanoparticles occurred from amorphous to crystalline. The absorption at annealing temperature from 250 to 500 °C changed toward “blueshift,” while the absorption from 500 to 750 °C changed toward “redshift.” The literature refers to this phenomenon as the Burstein–Moss effect [31, 32].

**X-ray diffraction results**

XRD technique was used to determine the structure of the prepared nanoparticles, using CuKα radiation source ($\lambda$= 1.54050 Å). The XRD patterns of the nanoparticles powder were recorded by scanning 2θ in the range of 20–80°. The XRD patterns of the V$_2$O$_5$ nanoparticles annealed at different temperatures (90, 250, 500 and 750 °C) for 2 h are shown in Fig. 4.

The main diffraction peaks of V$_2$O$_5$ (Fig. 4 a) at 90 °C 2θ= 15.62°, 26.1°, 31.4°, 40.9°, 50.5° and 58.8°,
Fig. 5  AFM records of V$_2$O$_5$ at annealing temperatures: a 90 °C, b 250 °C, c 500 °C and d 750 °C
respectively, correspond to the characteristic diffraction of the (200), (301), (110), (310), (002) and (611) planes of the V2O5 as indexed in the JCPDS card No. 41–142.6 [30]. When V2O5 was annealed at 250 °C, the peaks (010) and (101) appear at 2θ = 20.32° and 21.74°, respectively, which are related to the alpha phase (orthorhombic structure) as shown in Fig. 4b; with increasing the annealing temperature from 250 to 750 °C, the intensity of diffraction peaks (010), (101), (301) and (002) increased, and at the same time, the intensity of diffraction peak (110) decreased until it disappeared at annealing temperature of 750 °C as shown in Fig. 4c and d.

The estimation of the mean size of ordered α-V2O5 nanoparticles can be calculated from Debye–Scherrer formula on the basis of full width at half maximum (FWHM) as per Eq. 9:

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

where \( D \) is the particle size, \( K \) is the shape factor having value 0.89, \( \lambda \) indicates wavelength of X-ray, \( \beta \) in radians shows the line broadening at half of the maximum intensity (FWHM), while \( \theta \) is known as Bragg angle.

From Debye–Scherrer equation, the mean size of the annealed V2O5 nanoparticles was found to be around 20 nm.

**Atomic force microscopic studies**

The images obtained from AFM studies at various annealing temperatures of 90, 250, 500 and 750 °C appear to be as granularity accumulation distribution chart of V2O5 as shown in Fig. 5. The average grain sizes (86.02–70.34 nm) are shown in Table 2. The results are self-explanatory since there is a decrease in grain size as the annealing temperatures are increased.

**Scanning electron microscopic studies**

Field emission scanning electronic microscopic (FE-SEM) studies were carried out on V2O5 nanoparticles to obtain information on the morphology of the prepared samples. The almost perfect shell of vanadium pentoxide nanoparticles was confirmed by FE-SEM image. FE-SEM image also indicated that high-porosity structure can be observed on the sample surface when the annealing temperature was increased. The morphology of the synthesized nanoparticles of vanadium pentoxide changed to form nanoflakes due to increasing the annealing temperature to 500 °C. The SEM image of the vanadia nanoparticles annealed at 500 °C is given in Fig. 6.

**Advanced spectrophotometric method development**

The authors developed a new spectrophotometric method to measure the catalase mimetic activity of V2O5 solution [25, 29, 38] that included the use of acidic permanganate solution. In order to calculate the reaction rate, the catalase mimetic activity of V2O5 was determined for samples as-prepared at 90 °C and after annealing at different temperatures 250–750 °C for 120 min. Catalase mimetic activity was calculated according to the first-order reaction equation \( x \). The results show that there is an increase in reaction rate \( (K) \) on increasing the annealing temperature of V2O5 and reaches a maximum at annealing temperatures of 500 and 750 °C \( (K = 3.269 \times 10^{-2} \text{ s}^{-1} \text{ and } 3.282 \times 10^{-2} \text{ s}^{-1}) \), respectively (Fig. 7); since these values are very close, the value of 3.269 \( \times 10^{-2} \text{ s}^{-1} \) was selected at annealing temperature of 500 °C as the best value. These results are summarized in Fig. 7 and Table 3.

If there is no KMnO4 consumption (the color of the solution does not change), it means that the vanadium pentoxide nanoparticles decomposed all the hydrogen peroxide and it works as catalase. If there is KMnO4 consumption, it results in color change and it means that V2O5 nanoparticles do not function as catalase and the KMnO4 had to complete the decomposition of H2O2. The catalase mimetic activity of V2O5 annealed at different temperatures (90, 250, 500, 750 °C) was studied, and it was conducted that the highest activity, on the basis of changes in permanganate color, can be obtained after annealing the V2O5 nanoparticles at temperature of 500 °C.

**Thermal gravimetric studies**

TG analytical studies were used for measuring the changes in mass during heating, and differential thermal analysis (DTA) measures the temperature difference between the sample and reference sample. In this way, the changes in the sample, either exothermic or endothermic, can be detected relative to the inert reference. Figure 8 shows the TGA, DTA and high-temperature differential scanning calorimetry (HDSC) records of sample V2O5·nH2O from room temperature to 688 °C. HDSC curves indicated that four stages of conversions can be distinguished. According to [26, 32], the samples indexed as V2O5·nH2O were found to be in monoclinic
phase, where the increase in basal plane distance corresponds to the increase in the amount of water molecules. \(\text{V}_2\text{O}_5\cdot n\text{H}_2\text{O} \ (n \approx 1.8)\) aerogel is obtained when the sample is dried at room temperature in air. If the sample is not heated above 150 °C, the dehydration remains reversible.

The first stage in Fig. 8 when the sample is heated from 100 to 217 °C indicates a decrease in sample mass of 0.7616 mg because of the removal of adsorbed water molecules between the layers, which are weakly bound [33, 34]. The second stage occurs when the same sample is heated from 217 to 285 °C. The decrease in sample mass is 1.1920 mg due to the removal of all water molecules bonded in the \(\text{V}_2\text{O}_5\) sample. These results are in agreement with the literature data [35] and relate to the conversion of CTAB to liquid. (Melting point of CTAB is 243 °C.) The third stage occurs when the sample is heated from 285 to 387 °C. This stage can be characterized with high mass decrease of 4.8544 mg. In the fourth stage heating, the vanadia nanoparticles from 387 to 688 °C induced the loss of the remaining tightly bound water and crystallization of the \(\text{V}_2\text{O}_5\) sample into dehydrated orthorhombic form [36, 37]. The fourth stage results in mass loss of 4.1744 mg. The sample remains liquid and in orthorhombic phase. The DTA curve exhibits a high decrease at 322 °C temperature. It means that there is a highly endothermic process, which can be assigned to the start of the crystallization of the \(\text{V}_2\text{O}_5\) sample to orthorhombic phase and melting of the sample. Exothermic processes can be observed between 387 and 530 °C due to the complete crystallization and melting of the \(\text{V}_2\text{O}_5\) sample, respectively.

### Table 2: Variation of grain size of \(\text{V}_2\text{O}_5\) annealed at different temperatures

| Sample (annealing temperature) | Average diameter/ nm |
|--------------------------------|----------------------|
| \(\text{V}_2\text{O}_5\cdot n\text{H}_2\text{O}\) (as-prepared) | 86.02 |
| \(\text{V}_2\text{O}_5\) at (250 °C) | 78.96 |
| \(\text{V}_2\text{O}_5\) at (500 °C) | 70.34 |
| \(\text{V}_2\text{O}_5\) at (750 °C) | 76.17 |

### Table 3: Reaction rate for H\(_2\)O\(_2\) decomposition as catalase mimetic activity of \(\text{V}_2\text{O}_5\) annealed at different temperatures (90–750 °C) for 120 min

| Annealing temperature/ °C | Reaction rate of H\(_2\)O\(_2\) decomposition as catalase mimetic activity \(K \times 10^{-3}/\text{s}^{-1}\) |
|--------------------------|-------------------------------------------------|
| 90 (as-prepared)         | 2.305                                           |
| 250                      | 2.599                                           |
| 500                      | 3.269                                           |
| 750                      | 3.282                                           |

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Fig. 6 SEM image of \(\text{V}_2\text{O}_5\) annealed at 500 °C for 120 min

Fig. 7 Catalase mimetic activity of \(\text{V}_2\text{O}_5\) annealed at 90, 250, 500 and 750 °C temperatures for 120 min
Conclusions

Advanced colorimetric method was devised and used first time to determine the catalase mimetic activity of V$_2$O$_5$ nanoparticles using potassium permanganate solution as indicator, instead of the commonly used UV–Vis method which is used by the researchers. Vanadium pentoxide nanoparticles were prepared by hydrothermal method, and the nanoparticles were annealed at different temperatures (250, 500 and 750 °C) for 120 min. The nanoparticles were characterized by using different methods such as XRD, FTIR, UV–Vis, AFM, SEM, TGA and DTA. The UV–Vis records show that there is an increase in band gap (blueshift) with increasing the annealing temperatures. The results show that the phase of V$_2$O$_5$ annealed at 500 °C is orthorhombic and has nanoflakes shape, while the average diameter of the nanoparticles was the smallest (70.34 nm) in this case. The authors were successful in elaborating and using an advanced colorimetric method to determine the catalase mimetic activity of V$_2$O$_5$ annealed at different temperatures. It was found that the annealing temperature of 500 °C results in the best catalase mimetic activity (3.269 × 10$^{-3}$ s$^{-1}$) in case of vanadia nanoparticles. Therefore, the optimum annealing temperature is 500 °C.

According to the best knowledge of the authors, this is the first time when this advance colorimetric method is used. It is very easy, accurate and not complicated, as compared with common method (UV–Vis) which depend on the measurement of decrease in absorbance at 240 nm ($\lambda_{\text{max}}$) of hydrogen peroxide. In UV method, the concentration of H$_2$O$_2$ limited to the range of 5 to 500 mmol mL$^{-1}$ at low concentration less than 5 mmol mL$^{-1}$ is undetectable, while at high concentration more than 500 mmol mL$^{-1}$ it causes mistake in measurement because of oxygen bubbles released from H$_2$O$_2$.

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