ABSTRACT
Multifunctional Zirconia Nanorods performing photocatalysis, anti-bacterial and anti-fungal activities are presented in this article. Tetragonal Zirconia is synthesized by the simple co-precipitation method. The synthesized Zirconia is characterized by various characterization methods such as XRD, SEM, EDX, UV-Vis, PL, VSM, and TG/DTA analysis. Exploration of powder XRD pattern indicates tetragonal phase. SEM image illustrates rod-shaped morphology. UV-Vis spectra reveal that the synthesized catalyst has a wide bandgap of about 4.6 eV. The emission peaks in the PL spectra reveal the presence of oxygen vacancies in the sample. Room Temperature Ferromagnetism (RTFM) is confirmed from VSM measurements. The performance of Zirconia nanorods in various applications such as photocatalysis, anti-bacterial and anti-fungal activities has been analyzed. t-ZrO₂ photocatalyst degrades methylene blue dye with 80% removal efficiency in 180 minutes under UV light irradiation. t-ZrO₂ obtained a 28mm inhibition zone against Staphaureus for anti-bacterial assessment while Amikacin has 15mm inhibition and obtained 25mm inhibition zone against Candida Albicans for anti-fungal assessment while Nystatin has 20mm inhibition. t-ZrO₂ shows a superior inhibiting effect against both gram-positive and gram-negative bacterial pathogens. Owing to its high surface area it exhibits the greatest inhibiting effect against fungal strain.

Keywords: Co-precipitation, Tetragonal, Room Temperature Ferromagnetism.
sized metal oxides such as titanium oxide, tin oxide, iron oxide have been explored for photocatalysis which may disintegrate excess dyes. Among the various photocatalysts, ZrO$_2$ is an optimistic material to perform photocatalysis due to its high negative CB potential, acid-base property, oxidation-reduction ability, and cost-effectiveness [5-8]. Only a few previous reports show the photocatalytic degradation of pollutants using pure ZrO$_2$ nanoparticles [38,39].

Nano-sized materials have antimicrobial properties along with physical and chemical properties [9]. Nano zirconia has high surface energy and has good chemical reactivity that causes superior anti-bacterial and anti-fungal properties [10, 11]. Previous studies about nano zirconia exhibit the anti-bacterial and anti-fungal consequences on C. Albicans and Aspergillus niger [12]. Active oxygen species formed from nano zirconia may improve the anti-bacterial activity due to the interruption of the cell membrane of microorganisms with nano zirconia. Higher permeability of the cell membrane results in the accumulation of nano zirconia in the cell membrane and cytoplasmic region of the cells [13]. Nano-sized ZrO$_2$ interferes with cell function and deforms the fungal hypha. Thus it exhibits a superior inhibiting effect against the growth of fungal strains [14,15]. When the particle size is reduced, the surface area-to-volume ratio increases. Due to their high surface-area-to-volume ratio and unique physical and chemical properties, nano zirconia has generated anti-microbial agents against bacterial and fungal strains.

Different physical and chemical techniques have been used for the synthesis of ZrO$_2$ nanoparticles including molten salt synthesis, sol-gel synthesis, hydrothermal synthesis, and co-precipitation methods [16,17]. Among these techniques co-precipitation is an attractive method on account of the production of smaller size materials with good quality, the process of simplicity, narrow particle size distribution, less agglomeration, and cost-effectiveness [18,19]. Present work focuses on the synthesis of tetragonal zirconia by simple co-precipitation method and makes it perform a variety of functions including photocatalysis, antibacterial and antifungal effects. Many researchers have reported the synthesis of monoclinic phase ZrO$_2$. We found few methods that deal with the degradation of MB dye and antimicrobial studies with tetragonal ZrO$_2$. Of particular interest to this issue, facile synthesis and characterization of tetragonal ZrO$_2$ are performed for the analysis of the degradation of MB dye and antimicrobial activities.

**EXPERIMENTAL**

**Materials and preparation**

Zirconium oxychloride, sodium hydroxide, and distilled water are the starting materials used for the preparation of ZrO$_2$ nanoparticles. Nano rods of pure ZrO$_2$ are prepared by the simple co-precipitation method. An aqueous solution of Zirconium oxychloride and sodium hydroxide are taken in the ratio of 0.5:2 M to maintain the pH 12. The aqueous solution of Zirconium oxychloride is stirred at 60°C using a magnetic stirrer. NaOH solution is added drop by drop till the pH value reaches 12 and stirred constantly for 2 hours at 60°C. The obtained precipitate is filtered and then washed with distilled water repeatedly and then finally with acetone to remove impurities. Thereafter the precipitate is dried at 150°C by using a hot air oven. After drying, the obtained precipitate is ground by using mortar and pestle to get a fine powder. This fine powder is calcined at 500°C by utilizing a muffle furnace to get a nanostructured Zirconia.

**Catalyst characterization**

The structural property of synthesized nano zirconia including crystallite size, phase identification has been confirmed using XPERT-PRO diffractometer in the diffraction angle 2θ range from 10º to 80 º. UV VIS spectrums for nano ZrO$_2$ have been recorded in the wavelength range of 100 to 1100nm using Perkin Elmer Lambda 35 spectrophotometer. The photoluminescence analysis is carried out by Fluorescence Spectrophotometer (Cary Eclipse) with an exciting wavelength of 270nm. The structural morphology and the chemical state are noticed with Scanning Electron Microscope (SEM) and energy dispersed X-ray (EDX) using EV018 (CARL ZEISS) and Quantax 200 with X Flash 6130. Lakeshore Vibrating Sample Magnetometer (VSM) measurements are recorded for the study of the magnetic properties of the prepared nanoparticles. Thermogravimetric (TG) and Differential Thermal Analysis (DTA) measurements are recorded using an exstar-6300 model thermal analyzer for the study of thermal characteristics of ZrO$_2$ nanoparticles under Nitrogen gas atmosphere.

Photocatalytic activity of synthesized ZrO$_2$
nanoparticles annealed at 500°C on the degradation of methylene blue (MB) is analyzed. An aqueous solution of methylene blue (0.1M, 50ml) was taken and 0.2g of photocatalyst is suspended in that solution. The experiment is done under UV light irradiation. The solution was exposed to a halogen lamp of 50W with continuous stirring. For every 60min, 4ml of dye solution was taken from the system and the dye removal efficiency is analyzed by UV-Vis spectrometer.

Kirby-Bauer test (KB test)

For the bacterial inhibition assay, the KB test otherwise known as disc-diffusion antibiotic sensitivity test was used. Nutriment agar media of pH 7.2 is prepared and inoculated with the experimenting organism for the growth of bacteria. A suspension of gram-positive and gram-negative bacteria is sprayed over the total area of antibiotic discs. Amikacin was used as a reference antibiotic which is also sprayed alongside the disc. The plate is then under incubation at 35ºc for 16 hours [20]. Zones of bacterial inhibition develop in and around the sample. After incubation, the diameter of the inhibition zones is measured. Further antifungal activities of Zirconia rods were also determined against Candida Albicans and Candida parapsilosis fungal strains by agar diffusion method, for which nystatin was used as reference antifungal. Zone of inhibition is the region in which the bacterial growth is terminated due to bacteriostatic consequence of the compound and it evaluates the inhibitory effect of the compound concerning a specific microorganism [21].

RESULTS AND DISCUSSION

Crystallographic analysis of Nano zirconia

The wide bandgap Zirconia nanorods are synthesized using the co-precipitation method and annealed at 500°C. The XRD pattern is used to identify the phase of synthesized nanoparticles and it is shown in Fig. 1. It confirms the pure tetragonal phase (JCPDS-50-1089) and it is indexed with standard peaks. The peaks are indexed as follows: 30.24° (011), 34.97° (002), 35.31° (110), 50.59° (112), 59.92° (013), 63.04° (202) and 74.34° (220). Also, no impurity peaks are identified. The diffraction pattern shows sharp and well-defined peaks which indicate the highly crystalline nature as well as purity of the sample [22]. From Full-Width Half Maximum (FWHM) of reflections of tetragonal zirconia, the average crystallite size (D) is calculated using Scherrer’s formula,

\[ D = \frac{0.9 \lambda}{\beta \cos \theta} \]

where, ‘\( \lambda \)’ is the wavelength of the X-rays; ‘\( \theta \)’ is the Bragg’s diffraction angle, and ‘\( \beta \)’ is the full width at half maximum (FWHM) of the diffraction peaks (in radians).

The calculated average crystallite size of the sample is found to be 29.74nm. The obtained crystallite size is small due to the presence of oxygen vacancies at the boundaries and the surface of grains. The existence of oxygen vacancies may stop the growth of nanoparticles and make a stress field. The dislocation density \( (\delta) \) is the length of dislocation per unit volume which depends on the crystallite size (D) and it is...
calculated using the relation, \( \delta = \frac{1}{\pi} \). The obtained dislocation density value is \(1.23 \times 10^{15}\) lines/m\(^2\). During the deformation, the dislocation density increases beyond the elastic limit. The stacking fault probability is calculated using the formula \( SF = \frac{n \tan \theta}{\pi} \). Stacking disorder in the structure can be investigated using the SF value. It is used to localize the distribution of stacking faults. The obtained SF value is 0.486. The microstrain produced due to the dislocations in the nanoparticles is calculated using the relation, \( \varepsilon = \frac{\beta \sin \theta}{2\tan \theta} \), where \( \beta \) is the FWHM and \( \theta \) is the diffraction angle. The obtained microstrain value is \(1.26 \times 10^{-3}\) which signposts better quality of deposited nanostructures. Microstrain decrease with an increase in hydrothermal treatment time and an increase in particle size. The increase of crystallite size and decrease of microstrain leads to the growth of particle size.

Morphology and chemical state of nano zirconia

The morphology of the synthesized \( \text{ZrO}_2 \) nanoparticle is analyzed with scanning electron microscopy (SEM). The SEM micrographs of t-\( \text{ZrO}_2 \) with different magnifications are shown in Fig. 2. It is observed that the morphology of the synthesized samples is rod shape. The obtained particle has a nano-sized structure and well-defined grains. Fig. 2 clearly shows that the rod-shaped zirconia nanoparticles are in uniform size and smooth surface. The EDX characterization dictates the elemental composition of the prepared \( \text{ZrO}_2 \) nanoparticles. The EDX characterization spectrum is shown in Fig. 3. A high intense peak is identified for Zirconium (Zr) and Oxygen (O).
elements.

**Optical Investigation**

The Optical absorbance and transmittance spectrum of ZrO₂ at 500°C annealing temperature are shown in Figs. 4 and Fig. 5. The strong absorption peak for ZrO₂ occurs at 235 nm and 371 nm which is in the UV region. It is because of the excitation of an electron from the valence band to the conduction band due to the presence of conjugated pi-bonding systems and the surface defect states. The excitations of electrons due to the transition of (O²⁻ → Zr⁺⁴) cause the absorption peak in the absorption band [29]. There is a transition between the 2p energy state of O which is present in the valence band and the 4d (x²-y², z²) energy state of Zr which is present in the conduction band. In the visible region, there is no distinctive feature for d-d transition. It is due to the configuration of d0 in Zr⁺⁴ ions. Compared to the optical band gap for bulk ZrO₂ in the literature [30], the obtained absorption peak has lower energy. The reduction of nanoparticle size causes changes in the bandgap of the particle. The scattering centers and mechanical stress can be affected because of the variation in the band gap of the nanoparticle. The bandgap energy of the prepared sample is calculated by using Tauc relation,

\[(\alpha h\vartheta)^n = A(h\vartheta - E_g)\],

(1)

where \(h\vartheta\) is the energy of the photon, \(E_g\) is the bandgap energy, and \(A\) is the proportionality constant. \(n\) takes the value \(\frac{1}{2}\), for direct allowed transitions and \(\alpha\) is the absorption coefficient [23]. The absorption coefficient \(\alpha\) is determined.
using the formula,

\[
\alpha = \frac{2.303 \log \left( \frac{1}{T} \right)}{t}
\]

Here, ‘T’ represents transmittance and ‘t’ represents the thickness of the sample [1]. The Tauc plot is drawn to determine the bandgap of ZrO\textsubscript{2} and it is shown in Fig. 6. It is identified that, the variation of \((\omega \alpha)^2\) with respect to \(h\omega\) is linear which reveals the transition that is directly allowed. The bandgap energy of the ZrO\textsubscript{2} nanoparticle found to be 4.6 eV.

**oluminescence analysis**

Photoluminescence investigations provide a study on electrical characterization and discrete electronic states. PL emission spectra can be used for the analysis of surface, interface, and impurity levels of nanoparticles [33]. The PL analysis offers fine points on the effect of the transfer of charge and the recombination of electron and hole-pair on the photocatalytic nanoparticle. The luminescence of ZrO\textsubscript{2} occurs due to the transition of electrons from the valence band to the conduction band while a new energy level is formed at the surface of the ZrO\textsubscript{2} nanoparticles [31]. Fig. 7 shows the PL emission spectra of ZrO\textsubscript{2} nanoparticles with an excitation wavelength of 270 nm. It is observed that the emission peaks obtained at 367 nm in the UV region and 419 nm, 485 nm, 542 nm are in the visible region. The peak centered in the UV region is due to the near band edge transition because of
the free excitons recombination. The peak centered in the visible region is due to the occupation of electrons in the mid-band gap trap states such as oxygen vacancies and surface defects [32]. Thus the photoluminescence analysis elucidates that the luminescence of ZrO₂ nanoparticles is due to the oxygen surface defects and vacancies.

Magnetic Properties

The magnetic properties of ZrO₂ nanoparticles are analyzed using Vibrating Sample Magnetometer (VSM) at room temperature with a maximum field of 15000 Oe. Fig. 8 shows the obtained M-H loop. The parameters of the M-H loop such as coercivity (Hci), saturation magnetization (Ms), and remanence magnetization (Mr) are observed from the loop.

The observed value of coercivity (Hci) is 2535.6 Oe, saturation magnetization (Ms) is 586.89 × 10⁻⁶ emu/gm and remanence magnetization (Mr) is 53.525 × 10⁻⁶ emu. The hysteresis loop shows perfect Room Temperature Ferromagnetism (RTFM). A similar M-H loop has been observed earlier for ferromagnetism [34,35]. Since the coercivity of the prepared ZrO₂ nanoparticle is high, it is observed that the ZrO₂ nanoparticle is a hard ferromagnetic material. It requires more magnetic fields to demagnetize the material. In the absence of a magnetic field for a certain duration, there will be a magnetic effect in the material and it is called permanent magnetic materials. It is widely used in different fields such as telecommunication, data processing, electronics, and instrumentation. The occurrence of RTFM is due to the presence of oxygen vacancies and surface defects as a result of the large surface energy volume ratio for nano-sized particles. The interface amongst Zr ions is due to this oxygen vacancy. PL measurements reveal the confirmation of oxygen vacancies. The tetrahedral position is occupied by oxygen ions of tetragonal ZrO₂. Due to this a model of defect structure is created. For charge compensation, two Zr³⁺ ions are formed for one oxygen vacancy [30].

Thermal analysis

The thermal behavior of ZrO₂ nanoparticles is analyzed using Thermogravimetric (TG) and Differential Thermal Analysis (DTA) studies under Nitrogen gas atmosphere. Fig. 9 shows the TG / DTA curve of ZrO₂ nanoparticles.

It is found that there is an abrupt reduction of weight loss at 70°C and there is a gradual weight loss with respect to an increase of temperature after 500°C. This abrupt reduction of loss is owing to the defeat of moisture in the synthesized material [35]. The DTA graph shows the endothermic peak at 405°C in which maximum heat is absorbed in the synthesized material. The weight loss at 70°C may be due to the trapped moisture and acetate in the sample and the weight loss after 500°C may be due to the organic residues.

Photocatalytic Activity

The photocatalytic performance of the prepared catalyst ZrO₂ has experimented with the degradation of methylene blue (MB) organic dye. An aqueous solution of methylene blue (0.1M, 50ml) is taken and 0.2g of photocatalyst zirconia is added. The experiment is done under UV light irradiation. The solution is exposed to a halogen
Photodegradation mechanism

The electrons from the valence band move to the conduction band. Under UV light irradiation due to this movement of electrons, holes are generated in the valence band and free electrons are generated in the conduction band. H₂O and O₂ from the moisture react with holes in the valance band and electrons in the conduction band, thus produces hydroxyl radical and superoxide radical [36]. These radicals react with the MB organic dye and provide degradation products. The degradation mechanism is shown in Fig. 10.

The following equations provide the detail of the degradation mechanism.

\[ \text{ZrO}_2 + h\nu \rightarrow \text{ZrO}_2 \left( e_{\text{CB}}^- + h_{\text{VB}}^+ \right) \]

\[ \text{ZrO}_2(e_{\text{CB}}^-) + \text{O}_2 \rightarrow \text{ZrO}_2 + \text{O}_2 \]

\[ \text{H}_2\text{O} \rightarrow \text{H}^+ + \text{OH}^- \]

\[ \cdot \text{O}_2 + \text{H}^- \rightarrow \cdot \text{HO}_2 \]

\[ \text{ZrO}_2(e_{\text{CB}}^-) + \cdot \text{HO}_2 + \text{H}^+ \rightarrow \text{H}_2\text{O}_2 \]

\[ \text{ZrO}_2 \left( h_{\text{VB}}^+ \right) + \text{Dye} \rightarrow \text{Degradation products} \]

\[ \text{HO}_2^- + \text{H}^+ \rightarrow \text{H}_2\text{O}_2 \]
\[ HO_2^- + e^- \rightarrow HO_2^- \]

Hydroxyl radical (\( ^\cdot OH \)) and superoxide radical (\( ^\cdot O_2^- \)) are involved in the degradation of MB organic dye.

**Photocatalytic degradation of MB**

The optical absorbance spectra of the degradation of methylene blue (MB) dye using Zirconia are shown in Fig. 11. From the absorbance spectrum, the strong peak is identified at 663.25 nm which is the absorption wavelength of methylene blue (MB) dye. The following equation provides the removal efficiency (E) of MB degradation,

\[ E = \frac{C_o - C}{C_o} \times 100 \% \quad (3) \]

where \( C_o \) is the initial concentration of dye and \( C \) is the concentration of MB after UV irradiation. It is observed that 53\% of methylene blue (MB) dye is degraded after 1 hour and 80\% of methylene blue (MB) dye is degraded after 3 hours. This is mainly due to the high crystallinity nature of t-ZrO\(_2\), prepared at 500\(^{\circ}\)C, small crystallite size, and well-defined morphology and surface properties.

The normalized residual concentration of MB dye is estimated using \( \frac{C_t}{C_0} = \frac{A_t}{A_0} \), where \( C_t \) and \( C_0 \) are the initial and residual concentrations of MB dye [25]. \( A_t \) and \( A_0 \) are the absorbance intensity at time \( t \) and at time \( t = 0 \) obtained from the UV-absorbance spectrum.

The growth of the rod-shaped particle is greater in one direction with respect to the growth of the particle in other dimensions [26]. Since the morphology of the prepared nanoparticle is rod shape, the surface area of the particle is high. The high surface area provides more active sites for the reaction of photodegradation [27]. XRD analysis clearly shows that the high crystalline nature of the prepared catalyst thus produces active oxygen sites which may act as scattering centers for the combination of electron-hole pairs. Due to this property, the efficiency of the degradation of MB is high for zirconia even though it has a wide bandgap. The decomposition of MB dye by ZrO\(_2\) with respect to time is shown in Fig. 12. The concentrations of MB dye with and without adding ZrO\(_2\) nanoparticles are shown in the graph. We find that the photocatalytic activity of the prepared ZrO\(_2\) nanoparticles provides better results when compared to the previously reported results [11, 37]. The removal percentage of MB dye with different catalysts is presented in Table 1.

**Anti-bacterial and Anti-fungal assessment**

Synthesized nano zirconia rods are screened for their antibacterial activity against gram-negative pathogens such as E.coli, pseudomonas aeruginosa, and gram-positive pathogens such as Bacillus cereus, Staph aureus. Also, the antifungal activity against Candida albicans and Candida parapsilosis is studied by the Kirby-Bauer test. The antimicrobial activity of nanoparticles is related to the electromagnetic attraction between the positively charged nanoparticles and negatively charged microbes. During this attraction, microbes...
get oxidized and destroyed [35].

The results obtained for the antibacterial and antifungal activities are summarized in Table 2. Fig. 13 (a-d) shows the inhibition zones of antibacterial and Fig. 13 (e,f) shows the inhibition zones of antifungal activity. These results reveal that the prepared Zirconia holds superior antimicrobial activity. ZrO$_2$ nanoparticle has reactive oxygen species and it behaves well as an antibacterial agent. Based on XRD and SEM analysis, Zirconia is chosen for antibacterial screening. Small-sized zirconia nanoparticles are allowed to attach to the cell wall of bacteria and they easily penetrate it, which may improve their anti-bacterial activity.

It is observed that the synthesized ZrO$_2$ nanoparticles obtain better inhibition against *Pseudomonas aeruginosa* in gram-negative and *Staphaureus* in gram-positive. The inhibition capability of nano zirconia rods is compared with Amikacin. The results reveal that ZrO$_2$ nanoparticles act better antibacterial activity against E.coli in gram-negative and both *Bacillus cereus* and *Staphaureus* in gram-positive. Zirconia exhibits a greater potential in killing the bacterial strains due to its high surface area. The reason for the superior effect against bacterial strains of ZrO$_2$ nanoparticles may be the active oxygen species that make the particle accumulate or deposit on the bacterial cells to prevent the growth. The growth of the bacterial cell can be struck by the accumulation of nanoparticles in the bacterial membrane and cytoplasmic region of the cells. The accumulation
Fig. 13. Inhibition zones of bacterial and fungal strains

Fig. 14. Antibacterial and Antifungal activity of Zirconia
The antifungal images of Zirconia nanoparticles shown in Fig. 13(c,f) reveal better results against antifungal strains such as Candida albicans and Candida parapsilosis. The inhibition capability of nano zirconia rods is compared with Nystatin. Fig. 14 shows the performance of ZrO₂ for antimicrobial activities in comparison with the antimicrobial agents. A comparison of the antifungal activity data of the test compound against fungal strains indicates that the test compound is more active against Nystatin to kill both the Candida albicans and Candida parapsilosis. Generally, the chemical reactivity of nanocrystallines depends on their shapes, atomic arrangement of the surface, and surface energy [9]. The surface energy is an important parameter of the nanoparticle to define the antibacterial and antifungal activity. Since the prepared nano Zirconia has rod shape morphology, it has a high surface area and high surface energy. Due to this high surface energy, the prepared nano has better antimicrobial activity.

CONCLUSION

Tetragonal zirconia has been synthesized by the simple co-precipitation method. The synthesized Zirconia has been characterized by various characterization methods such as XRD, SEM, EDX, UV-Vis, PL, VSM, and TG/DTA analysis. The XRD analysis indicates that the average crystallite size is 29.74nm and SEM analysis depicts rod-shaped morphology. The Ferromagnetic behavior is found using VSM measurements. The performance of Zirconia nanorods in various applications such as photocatalysis, anti-bacterial and anti-fungal activities has been analyzed. t-ZrO₂ photocatalyst degrades methylene blue dye with 80% removal efficiency in 180 minutes. The antibacterial activity of the synthesized Zirconia has experimented against Escherichia coli, Pseudomonas aeruginosa, Bacillus cereus, and Staphylococcus. The antifungal activity of the synthesized Zirconia has experimented against Candida albicans and Candida parapsilosis using the Kirby-Bauer test. t-ZrO₂ shows a superior inhibiting effect against both gram-positive and gram-negative bacterial pathogens owing to its high surface area, it exhibits the greatest inhibiting effect against fungal strain.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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