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Mechanistic study of visible light driven photocatalytic degradation of EDC 17αethinyl estradiol and azo dye acid black– 52: phytotoxicity assessment of intermediates

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The present study evaluated the phytotoxicity of degraded intermediates of an endocrine disrupting compound (EDC) 17a-ethinyl estradiol (EE2) and toxic azo dye Acid Black - 52 (AB-52) in photocatalytic degradation. A novel bimetallic (silver and zirconium) doped TiO₂ nanoparticles was synthesized for utilization in degrading these pollutants under visible light. The degradation pathway of the pollutants during photocatalytic activity was investigated by LC-MS analysis. Further, to understand the toxicity of intermediate compounds compared with the pollutants, phytotoxicity assessment was investigated on two different seeds V. radiata, and P. vulgaris. Seeds treated with 100 ppm concentration of AB-52 showed low germination percentage in V. radiata (30 %), and P. vulgaris (40 %). Similarly, seeds treated with EE2 also showed less germination in V. radiata (40 %), and P. vulgaris (50 %) compared to intermediate compounds (100 % germination) and revealed less toxic nature of degraded metabolites compared to EE2 and AB-52. Further, active radical scavenging experiments were carried out to understand the main species involved in the photocatalytic degradation process. Photoluminescence study and reactive oxygen species generation results suggested that the efficient charge carrier separation took place during the irradiation. Thus, the present work proves the ability of effective multifunctional nanomaterials to not only to degrade the hazardous pollutants but also to detoxify them.

Introduction

The major cause for progressive and increased levels of pharmaceutical contaminants in water is due to their large production and utilization all over the world (ca. 4000 types in the order of 100-200,000 tons/year)^{1,2}. Most of the drugs that are prescribed and consumed by humans and animals are later excreted and finally end in wastewater treatment plants (WWTPs). Therefore, it is of world interest to minimize these pollutants since they can be very toxic to the ecosystems even at very low concentrations (ppb). Many of these pharmaceutical ingredients (APIs) are intentionally made to be stable in which to persist biologically active with the aim of being effective in health care. Therefore, the respite of these drugs is very difficult to treat by conventional processes

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applied in WWTPs, because only a fraction of them are being destroyed³. As a result, higher levels of these emerging pollutants have been detected in aquatic environments and even in drinking water during the last years. US EPA pronouncements define these emerging contaminants as chemical compounds which are not modulated and whose effect on the environment and human health is poorly understood yet⁴.

The widespread presence of PPCPs in the environment has been extensively reported in order to increase global cognizance and pertain^{5,6}. Among PPCPs, 17α -ethinylestradiol (EE2), the synthetic estrogen and endocrine-disrupting chemical (EDC) is ranked in the top 100 of priority PPCPs'. EE2 is a primary constituent in contraceptive pills and postmenopausal hormonal appurtenances and is more fractious than normal estrogen by means of its removal during water treatment⁸. This EE2 is often found in wastewater treatment plants (WWTPs) and is discharged into receiving waters due to incomplete removal during the treatment process⁹. Although it is present in trace amounts (parts-perbillion level), EE2 can induce the feminization of male fish and falsify the reproductive potential of the fish population¹⁰. Due to the adverse effects of EE2 on ecosystems, human health and drinking water safety, and the ineffective EE2 removal from WWTPs, it is essential to explore effective techniques for EE2 removal within WWTPs.

Synthetic organic (azo) dyes are been utilized extensively in textile, cosmetic, paper, drug and food processing industries. These synthetic colorants could not easily be degradable by biological treatment methods, due to their complex structure and high refractoriness. Majority of these dyes are highly carcinogenic, harmful and have capacity to cut down the light penetration in the aqueous systems; thus causing a negative effect on the photosynthesis, apart from affecting human health¹¹. The dye containing effluents are determined by their fluctuating pH, suspended particles, high oxygen demand, non-biodegradability and resistance to oxidation, moreover formal water decontamination methods are often chemically, energetically and operationally suitable only for large systems^{12,13} thereby demanding development of new materials for effective dye removal.

The aim of the present study is to evaluate the potential of metal doped TiO₂ nanoparticles to degrade hazardous toxicants 17 α -ethinyl estradiol and Acid Black–52. The transformation pathway and intermediate compounds have been identified through LCMS analysis during degradation process. Phytotoxicity assessment upon two common plant seeds *Vigna radiata* and *Phaseolus vulgaris* indicated less toxic nature of the degraded metabolites compared to toxicants 17 α -ethinyl estradiol and acid black–52.

Materials and methods Materials

Titanium (IV) isopropoxide, Terepthalic acid (TA), Nitro blue tetrazolium chloride (NBT) and 17α -ethinyl estradiol (EE2) from sigma aldrich, Acid black-52 dye from local textile industry, Hydrazine hydrate, Zirconyl nitrate [ZrO(NO₃)₂], isopropanol, potassium iodide (KI), EDTA, potassium dichromate (K₂Cr₂O₇) ascorbic acid, ethanol and Tween 20 from Alibaba chemicals, China were used in the present study

Synthesis of metal doped TiO₂ nanoparticles

TiO₂ nanoparticles are prepared using the following method; a mixture of 5 mL of titanium (IV) isopropoxide in 50 mL isopropanol was added drop wise to 200 mL of distilled water maintained at pH 1.5 while the solution was continuously stirred. This TiO_2 sol was dried at 100 $^{\circ}C$ for 24 h, and then calcined at 450° C for 4 h to obtain the nanoparticles. Ag/TiO₂ and Zr/Ag-TiO₂ nanoparticles were prepared by the above method with few modifications. For Ag-TiO₂ nanoparticles preparation, required amount of aqueous solutions of AgNO₃ (0.2-0.8 mol %), while for Zr/Ag-TiO₂ nanoparticles required amounts of aqueous solutions of both AgNO₃ and ZrO(NO₃)₂ (0.2-0.8 mol %) were added drop wise to the TiO₂ sol with continuous stirring for 45 min. A small aliquot of distilled water, 0.05 M hydrazine hydrate and 5 mL of tween 20 were added to all the above solutions with continuous stirring for additional 30 min. The resultant sol was sonicated at 80 MHz for 90 min and then dried at 100° C in a hot air oven for 24 h to get the dry gel. The gel was then calcinated at 450^c C to obtain required nanoparticle powders.

Characterization of nanoparticles

Powder XRD crystallogram was recorded using X-ray BRUKER D8 Advance X-ray diffractometer with Cu Ka source $(\lambda = 1.5406 \text{ A}^{\circ})$. The crystalline phase of the nanoparticles was identified by comparing the major peak positions with standard JCPDS files. JEOL JEM 2100 High Resolution Transmission Electron Microscope (HRTEM) was used for imaging, SAED pattern and energy dispersive X-ray pattern with an accelerating voltage of 200 KV at different magnification. Diffuse reflectance spectra were recorded using V-670 UV–Vis IASCO spectrophotometer. The photoluminescence (PL) spectra were obtained using HITACHI F-7000 fluorescence spectrophotometer. XPS data was acquired using Kratos Axis Ultra 165 Spectrometer with a monochromated Al K α X-ray source (h α = 1486.6 eV).

Photocatalytic degradation of EE2 and AB-52

All photodegradation experiments were carried out using a multitube photoreactor (Fig. 1). The photoreactor consists of a large borosil tube at the middle of the reactor facilitated with water inlet and outlet in order to avoid temperature fluctuation during degradation process. Surrounded by the central tube six tubes (50 mL) were equipped with air pump to maintain constant mixing of catalyst with the effluent solution. A 300 W Xe lamp ($\lambda \ge 400$ nm) was utilized for photodegradation experiments. This multitube reactor has been utilized for simultaneous degradation of both EE2 and AB-52. Initially the required quantity of EE2 and catalyst was taken in a glass beaker, and then stirred in the dark for 1 h to ensure absorption–desorption equilibrium^{14,15}. The leftover concentration of EE2 was determined using High Performance Liquid Chromatography (HPLC) technique. Agilent 1260 series with Eclipse XDBC18 (5 mm) reverse phase column (4.6 x 150 mm) was used for the separation. The fluorescence detector with an excitation wavelength of 280 nm and an emission wavelength of 310 nm was used in this study¹⁶. The mobile phase used consisted of a mixture of water and methanol (30:70 v/v), with injection volume of 1 mL min $^{\text{-}1}$ for 6 minutes. The peak was observed at 4.5 minutes.

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Similarly, in the case of AB-52 50 mL of the dye solution containing appropriate quantity of the catalyst suspensions was used. AB-52 solutions (25 mg/L) containing 50 mg of photocatalyst samples were put in a sealed glass beaker and first ultrasonicated, and then stirred in the dark for 1 h to ensure absorption–desorption equilibrium. Afterwards it was irradiated by visible light in the reactor. 2 mL of samples were taken out at regular time intervals and separated through centrifugation. The supernatants were analyzed by recording variations of the absorption band maximum in the UV–vis spectra of the dye molecule by using JASCO V–670 UV–Vis spectrophotometer.

Fig. 1

Analytical Methods

HPLC-MS analysis for the EE2 degraded metabolites was carried out using Thermo Finnigan Surveyor-Thermo LCQ Deca XP MAX with a BDS HYPERSIL C-18 (4.6 × 250 mm, 5.0 μ m) HPLC column; while Agilent 1260 series with Eclipse XDBC18 (5 mm) reverse phase column (4.6 x 150 mm) was used for the reverse-phase separation of AB-52 degraded sample. The mobile phase used was a mixture of methanol and water (40:60 v/v) at a flow rate of 0.2 mL min⁻¹ for 60 min in the case of Acid Black-52, while a mixture of methanol and water at 30:70 (v/v) was used for EE2 about 60 min. The mass spectra were obtained using Electro Spray Ionization (ESI) under the flow of Helium gas at 1 mL min⁻¹ approximately and the fragment voltage was of 16 V.

Active species scavenging experiments

To identify the main active species involved in photocatalytic reaction and degradation process, a series of scavenging experiments were conducted. Ascorbic acid as $O_2 \bullet -$ scavenger¹⁷, EDTA as h^+ scavenger¹⁸, potassium dichromate as e^- scavenger¹⁹, isopropanol as $\bullet OH$ scavenger²⁰, and potassium iodide (KI) as both $\bullet OH$ and h^+ scavenger^{17,18} were used during the photocatalytic reaction experiments.

Active species quantification experiments

To investigate the main active species, hydroxyl radicals (•OH) and superoxide radical (•O₂-) produced during the photoreaction process Terepthalic acid (TA) and Nitro blue tetrazolium (NBT) were utilized respectively. To quantify the production of •OH radicals, 50 mg of the photocatalyst was added to a 50 ml mixture of TA solution (3 mmol) and NaOH (10 mmol). The production of •OH radicals under visible light irradiation was investigated by HITACHI F-7000 Fluorescence Spectrophotometer. TA readily reacts with •OH hydroxyl radicals to generate 2-hydroxyterephthalic acid (TAOH) which emits fluorescence around 426 nm on excitation of its own 312 nm absorption band (Scheme 1). The increase in photoluminescent intensity of TAOH with time should be directly proportional to the •OH radicals generation. Similarly, NBT (2 x 10^{-4} M) was used to detect the amount of $\bullet O_2$ radicals generation from 50 mg of catalyst. The amount of •O₂- generated during the photocatalytic reaction was monitored through the evolution of NBT, having an absorption maximum at 259 nm. NBT can be particularly reduced by photogenerated •O₂- by forming an insoluble purple formazan compounds in the aqueous solutions (Scheme 2). The generation of $\bullet O_2$ - was quantitatively analyzed by finding the

decrease of NBT concentration in the supernatant solutions with UV–Vis spectrophotometer (Thermo Scientific Orion aqua mate 8000).

Scheme 1

Scheme 2

Phytotoxicity test

The phytotoxicity assessment test was carried out with seeds of V. radiata and P. vulgaris using 100 ppm concentration of AB-52, EE2 and their corresponding degraded metabolites. The ethyl acetate extracted metabolites of AB-52 and EE2 were dried and dissolved in 10 mL distilled water to make a final concentration of 100 ppm. The systematic methodology was based on the prescribed guidelines^{21,22} with few changes. The seeds (10 each) were sterilized in 10 % mercuric chloride solution for 10 min and rinsed thoroughly with distilled water and used for interaction with dye, EE2 and corresponding degraded metabolites. Interaction was carried out in 100 mL Erlenmeyer flasks containing 100 ppm of pollutantsand their respective degraded metabolites. Seeds in distilled water were included alongside as control. Interacted seeds were placed on wet cotton in Petri dish and incubated at $25 \pm 1^{\circ}$ C in dark for 24 h. Germinated seeds were selected for toxicity study. The assay was carried out in standard beakers with 30 mL of 1.5 % agar media (autoclaved) containing 100 ppm of AB-52, EE2 and degraded metabolites. The beakers with agar were immediately hardened in freezer. The germinated seedlings were placed just above the surface of the agar in each beaker and incubated at 25 \pm 1° C in dark. Control was included with seedlings on agar. After 5 days the plants were separated from agar media and root and shoot length was measured. All the experiments were carried out in triplicate and average data was produced in the present study.

Results and discussion

Characterization

UV–Vis absorption spectra (DRS mode) and photoluminescence spectra (PL) of the photocatalyst were analyzed in order to understand the mechanism involved for effective visible light absorption. UV-Vis absorption spectra of pure TiO₂, Ag-TiO₂ and Zr/Ag-TiO₂ are shown in Fig. S1 (a). A well defined band edge in the UV region of 300-350 nm observed could be ascribed to the photo-excitation occurring from valence band to conduction band. The extrapolation of Kubelka Munk plot of hu vs $(\alpha hu)^2$ was applied to get optical energy band gap values (Eg) as shown in Fig. S1 (b). From the figure it showed that the energy band gap values for the pure TiO₂, Ag-TiO₂ and Zr/Ag-TiO₂ nanoparticles were found to be approximately 3.18 eV, 3.08 eV and 2.87 eV, respectively. It was noticed that the doping of TiO₂ with two transition metals (silver and zirconium) into its lattice could be accompanied with a decrease in the energy band gap and an increase in the wavelength (red shift). These results indicated that Zr/Ag-TiO₂ nanoparticles have greater possibility to exhibit a higher photo-catalytic activity in the visible region. Fig. S1 (c) shows the photoluminescence spectra of the prepared composites. The PL spectrum of the undoped TiO₂ is also exhibited for comparison. All spectra show near band gap emission (NBE) and blue or deep level emission. The PL spectra suggest quelling of NBE on doping TiO₂ with silver and zirconium. This Published on 01 September 2016. Downloaded by Northern Illinois University on 02/09/2016 07:08:24

may be due to the suppression of recombination of the photogenerated electron-hole pairs on doped TiO_2 .

Fig. 2 shows the X-ray diffraction pattern of pure TiO₂, Ag-TiO₂ and Zr/Ag-TiO₂ nanoparticles. The main diffraction peaks observed in the patterns correspond to the TiO₂ anatase crystalline phase (JCPDS 21-1272). The diffraction peaks corresponding to metallic zirconium or silver have not been observed which suggested their doping did not affect the phase crystallinity. Furthermore, the representing oxide compounds (Zr_xO_y or Ag_xO_y) are not observed either. It indicated that zirconium and silver nanoparticles are highly disseminated in TiO₂ crystal lattice. These doped metals did not show any phase changes suggesting that aggregates might have been formed on the crystal borders and on the surface of the photocatalyst, thus elevating visible light absorption, as discussed earlier.

Fig. 2

Fig. S2 (a), and S3 (a), shows TEM images of Ag-TiO₂ & Zr/Ag-TiO₂ with uniform nano-crystalline structures and consistent grain size, as indicated by the XRD data. From the images, it could be understood that the uniformly distributed particles range from 10 to 17 nm and 7 to 15 nm, for Ag-TiO₂ & Zr/Ag-TiO₂ respectively. The SAED patterns of these samples are shown in Fig. S2 (b) & S3 (b), in which the dark rings on the right correspond to the standard polycrystalline diffraction rings for the anatase phase (indexed). Evidently, no signals of diffraction rings associated to other phases were observed. The EDX profile [Fig. S2 (d) & S2 (d)], revealed the composition of the elements present in the prepared samples. In the case of Ag-TiO₂ sample the Weight % of Ag, Ti, and O was found to be 25.09, 24.73, and 50.18. Similarly, in Zr/Ag-TiO₂ the weight % of Zr, Ag, Ti and O was found to be 20.08, 19.19, 19.87 and 40.86 respectively. The stoichiometry of both $AgTiO_2$ (1:1:2) and ZrAgTiO₂ (1:1:1:2) was confirmed by this measurement. HR-TEM images were [Fig. S2 (c) & S3 (c)] used to obtain evident microstructure information in order to accurately analyze the single grains and grain boundaries.

In order to confirm the surface composition and chemical state of the Zr/Ag-TiO₂ powders prepared (0.8 mole %), XPS analysis was carried out on Kratos Axis Ultra 165 Spectrometer with Al Ka radiation. The discernible XPS scans were obtained for Ag 3d, Zr 3d, Ti 2p and O 1s to differentiate whether dopant atoms interwove into crystal lattice of TiO₂ or formed different compounds with Ti, O, and dopant elements. Fig. 3 (a) shows XPS spectrum of Ag at 3d core levels. The Ag $3d_{5/2}$ and Ag $3d_{3/2}$ core level binding energies appear at 368.1 and 374.1 eV, respectively are in good agreement with bulk silver metallic values^{23,24}. The Zr 3d core level peaks observed at 182.4 and 184.6 eV are corresponding to Zr $3d_{5/2}$ and Zr $3d_{3/2}$, respectively [Fig. 3 (b)], which are attributed to the +4 oxidation state of zirconium²⁵. The binding energies of Ti 2p [Fig. 3 (c)], photoelectron peaks at 459.2 and 464.9 eV corresponds to Ti $2p_{3/2}$ and Ti $2p_{1/2}$ lines, respectively 26,27 These values indicate that titanium is present in the +4 oxidation state. Fig. 3 (d) shows the broad spectrum of Zr/Ag-TiO₂ nanocomposite. These results indicated that in Ag/Zr-TiO₂ nanocomposite silver, titanium and zirconium metal ions are present in their highest oxidation state. The existence of Ag and Zr in broad XPS spectrum and detection of no new compound between Ag, Zr, O, and Ti atoms other than TiO₂,

Fig. 3

The N₂ adsorption-desorption isotherms and pore size distributions of the prepared materials are showed in Fig. 4. According to IUPAC, these isotherms are considered as Type IV, with characteristic TiO₂ mesoporous materials. The specific surface area of pure TiO₂, Ag-TiO₂ and Zr/Ag-TiO₂ were observed to be 144.47, 147.83 and 151.44 m²/g respectively. The pores size distributions (inset in Fig. 4) of the samples are about 14, 12 nm and 10 nm for TiO₂, Ag-TiO₂ and Zr/Ag-TiO₂ respectively. The obtained results indicated that the surface area of photocatalyst may slightly enhance the photo-activity, because Zr and Ag doped TiO₂ with a large surface area has more active sites so the organic molecules can be adsorbed in large quantities onto the TiO₂ surface, and porosity facilitates pollutant access, adsorption, and decomposition^{28,29}. According to these results Zr/Ag-TiO₂ was used for further photocatalytic tests.

Fig. 4

Photocatalytic degradation of EE2 and AB-52

To understand the photocatalytic activity of the $Zr/Ag-TiO_2$ nanocomposite sample, the kinetic behaviour was investigated further using 17 α -ethinyl estradiol (5 ppm) and acid black–52 (25 ppm). The photocatalytic degradation reaction kinetics of EE2 and AB-52 can be described by a Langmuir–Hinshelwood model. This indicated that the reactions occurred at a solid liquid interface and the rate constant can be calculated from the following equation.

$$n [C_0/C_t] = k_r K_t = K_{apr} t$$
 -----(1)

The plot of ln (C_o/C_t) versus the irradiation time interval with various photocatalysts for 5 h under visible light irradiation is shown in Fig. 5. The results revealed that degradation of both EE2 (Fig. 5 a) and AB-52 (Fig. 5 b) follows first-order kinetics because the regression coefficients (R^2) are all above 0.8856. Evidently, among the tested photocatalysts for degradation of EE2 and AB-52, Zr/Ag-TiO₂ nanocomposite (50 mg) exhibits best performance with rate constants k= 0.1617 min⁻¹ and k=0.1967 min⁻¹ respectively (Table 1).

Fig. 5

To further investigate the possible intermediates and pathway during the degradation process by $Zr/Ag-TiO_2$ nanocomposite LC–MS analysis was carried for both EE2 and AB-52 samples. The degradation of EE2 and AB-52 was initially confirmed by HPLC analysis. The HPLC analysis report of both the samples is shown in Fig. S4 & S5. At 0 h EE2 showed a single peak at a retention time of 1.54 min, after a period of 5 h as the photo degradation proceeded, the HPLC chromatogram of the degraded sample showed the presence of new peaks at 1.27 and 1.90 min respectively. Similarly, at 0 h AB-52 showed a single dye peak at a retention time of 1.47 min, while the degradation proceeded the sample showed new peaks at 1.42 and 1.54 min respectively. All HPLC profiles indicate the fragmentation of both the organic molecules into various compounds.

The LC–MS analysis of EE2 and AB-52 and its degraded product confirms the photocatalytic degradation of both

organic samples by Zr/Ag-TiO₂ nanoparticles under visible light irradiation after 5 h. LC-MS analysis of the AB-52 dye degraded sample demonstrated the presence of compounds with molecular weight of 327.3, 284.2 and 260.0 which could be interpreted as (M-4), (M-3) and (M+) peaks of structure A, B and C (Fig. 6 & S6). LC-MS analysis of dye degraded product also showed (M+1), (M+1), (M-3), (M-1), (M+2), (M+1), (M+1) and (M+1) peaks with molecular mass of 222.2, 190.2, 175.2, 316.4, 158.2, 134.1, 159.1 and 174.1 which represents the structures D, E, F, G, H, I, J and K (Fig. 6 & S6). From the degradation pathway it indicates that the dye molecule has been degraded into various compounds after irradiation by visible light for 5 h.

Fig. 6

Fig. 7

Similarly, LC-MS pattern of the EE2 degraded sample showed the presence of compounds with molecular weight of 284.9, 238.1 and 222.3 which could be interpreted as (M+3), (M+2) and (M+1) peaks of structure A, B and C (Fig. 7 & S7). The degraded sample also showed (M-2), (M+2), (M-1), (M+2) (M-3) and (M+2) peaks with molecular mass of 214.2, 206.2, 178.2, 120.1 103.1 and 82.2 which represents the structures D, E, F, G, H and I (Fig. 7 & S7). From the degradation pathway it indicates that the in 5 h irradiation under visible light EE2 molecule has been degraded into various compounds.

Phytotoxicity

The release of the untreated dyeing effluents and EDCs not only cause severe environmental and health hazards but also has direct impact on the soil affecting its fertility. Hence, it is mandatory to assess the phytotoxicity of the dye, EDC and degraded metabolites. The effect of AB-52, EE2 and their corresponding degraded metabolites at 100 ppm concentration on seeds of V. radiata and P. vulgaris was evaluated. The seeds treated with 100 ppm concentration of AB-52 showed low germination percentage in V. radiata (30 %), and P. vulgaris (40 %), similarly, seeds treated with EE2 also showed less germination in V. radiata (40 %), and P. vulgaris (50 %). In contrast, seeds treated with 100 ppm concentration of degraded metabolites and distilled water exhibited 100% germination rate in all two seeds. The root and shoot length were found to be significantly affected with AB-52 and EE2 compared to their corresponding metabolites after degradation and with distilled water (Table 2) (Fig. 8). The results of phytotoxicity study indicate the ability of the co doped nanoparticles to not only degrade AB-52 and EE2 but also to detoxify it.

Fig. 8

Active species scavenging experiment

Different scavenging experiments have been carried out to investigate the main active species for the photocatalytic degradation process of both EE2 and AB-52. Ascorbic acid was used as $O_2 \bullet -$ scavenger, EDTA as h^+ scavenger, $K_2 Cr_2 O_7$ as $e^$ scavenger, isopropylalcohol as $\bullet OH$ scavenger, and potassium iodide (KI) was used as both $\bullet OH$ and h^+ scavenger (Fig. 9 a & b). After the addition of the scavengers to each reaction solution, photocatalytic activity was decreased in the order isopropanol > ascorbic acid > EDTA > potassium iodide > potassium dichromate > no scavenger. These results suggested that the degradation of EE2 and AB-52 was most intervened with the addition of isopropyl alcohol and ascorbic acid, which shows that •OH and O_2 •– are the primary species in the photocatalytic degradation process. Further, the addition of EDTA which scavenges h⁺ and K₂Cr₂O₇ as e⁻ scavenger showed no significant decrease in the photoctalytic degradation process, implying the hydroxyl and superoxide radicals are the primary species responsible for efficient degradation process.

Fig. 9

Active species quantification

Further to quantify $\bullet OH$ and $O_2 \bullet -$ radicals probing techniques have been carried out using NBT and TA. Quantification experiments of •O₂- production have been done through the transformation of NBT (detection agent of • O_2 -) during the photocatalytic reaction³⁰ (Scheme 1). Fig. 10 (d) showed the kinetic transformation percentage of NBT at different time intervals. It could be seen that Zr/Ag-TiO2 nanoparticles showed higher transformation percentage of NBT than TiO₂ indicating that the generation of •O2- increased with increase in time [Fig. 10 (c)]. This could be attributed to the lower band gap energy of Zr/Ag-TiO₂ (2.87 eV) as shown in Tauc plot (Fig. S1 (b)), and also to the lower recombination rate of the photogenerated electron-hole pairs on doping described by PL intensities (Fig. S1 (c)). During the photoexcitation of the catalyst, most of electrons generated remain in the CB (conduction band) to react with O₂ to generate more $\bullet O_2$ -. It was proved by the highest transformation concentration of NBT of Zr/Ag-TiO₂ as shown in Fig. 10 (d). Due to smaller band gap, the photogenerated electrons in VB (valence band) can easily transfer to the CB of Zr/Ag-TiO₂, resulting in more effective charge separation and reducing the recombination of electron-hole pairs, which finally leads to more photogenerated electrons for reaction with O_2 to produce O_2 - and take part in decomposition of EE2 and AB-52, which is in good agreement with the active scavenging experiments (Fig. 9).

Fig. 10

To further quantify •OH radicals, the terephthalic acid photoluminescence probing technique (TA-PL) was employed since TA could readily react with •OH radicals to form a highly fluorescent 2-hydroxyterephthalic acid (TAOH)³¹⁻²³ (Scheme 2). The TAOH concentration was increased with increase in time over Zr/Ag-TiO₂ nanoparticles as expected compared to other prepared nanoparticles [Fig. 10 (b)]. This could be attributed to the easy charge transfer, which results in the improvement of holes on the VB of Zr/Ag-TiO₂ to produce more •OH, resulting in the highest PL peak of Zr/Ag-TiO₂ with respect to time. Fig. 10 (a) shows the concentration of •OH radicals with respect to time. Based on the results of NBT transformation and TA-PL results, it can be concluded that $\bullet O_2$ - and $\bullet OH$ radicals play a major role in the photodegradation of both the pollutants EE2 and AB-52 over Zr/Ag-TiO₂ nanoparticles which are in linearly correlated with the concentration of reactive organic species during visible light photodegradation

Mechanism of degradation

The possible photocatalytic mechanism was aimed to understand the enhanced visible activity of the as-prepared $Zr/Ag-TiO_2$ catalyst. After doping of Zr and Ag, the band gap of

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TiO₂ was narrowed from 2.87 eV. Thus, the visible light can be absorbed by Zr/Ag-TiO₂ catalysts with improved efficiency. The electrons in the valence band of Zr/Ag-TiO₂ could be excited to the conduction band with ease and a large amount of electron–hole pairs is then generated. It is well demonstrated that 'Ag' traps the electrons from CB of TiO₂. The doping of 'Zr' also suppresses the electron hole recombination by electron trapping³⁴. Subsequently, with a suitable doping concentration, the doped Zr and Ag species in the lattice structure of TiO₂ served to ameliorate the electronic conductivity, which significantly suppressed the recombination of photogenerated electron–hole pairs and lead to higher energy efficiency.

During the photodegradation process, the electrons from the valance band of $Zr/Ag-TiO_2$ can migrate easily to the conduction band by absorbing visible light, leaving holes in valence band Eq (2). These photogenerated electrons from the conduction band scavenged by surrounding oxygen absorbed on the surface of the product and convert them into superoxide anion radicals ($\bullet O_2$ -), which are responsible for the photodegradation of both the organic pollutants (Eq. 3). Similarly, the photogenerated holes in the VB of TiO₂ readily oxidize H_2O and OH^- to form •OH radicals Eq. (4) and (5), which are further involved in the photodegradation of EE2 and AB-52. Finally, the formed hydroxyl (•OH) and superoxide $(\bullet O_2-)$, radicals are highly reactive oxidizing species that will react with the EE2 and AB-52 on the catalyst surface to form the degradation products Eq. (6), the proposed mechanism is expressed as follows:

$Zr/Ag-TiO_2(eCB^-) + O_2 \longrightarrow Zr/Ag-TiO_2 + \bullet O_2$ (3)
$Zr/Ag-TiO_2$ (hVB +) + H ₂ O \rightarrow $Zr/Ag-TiO_2$ + •OH + H+ (4)
$Zr/Ag-TiO_2$ (hVB +) + OH ⁻ \longrightarrow $Zr/Ag-TiO_2$ + •OH (5)
EE2 or AB-52 + $\bullet O_2$ - + $\bullet OH \longrightarrow$ Degradation product (6)

Zr/Ag-TiO₂ + hv _____ Zr/Ag-TiO₂ (eCB⁻ + hVB +) ------ (2)

Conclusion

TiO₂ nanoparticles co-doped with silver and zirconium was prepared for effective utilization in degrading two toxic organic pollutants 17α-ethinyl estradiol and Acid Black-52. A simple multi-tube photoreactor set up was developed and utilized for degradation experiments. Recombination of photogenerated electron-hole pairs has been significantly suppressed by doping impurities into TiO₂ crystal lattice. The degradation pathway and possible intermediate compounds during degradation were investigated by LC-MS analysis. In order to evaluate the phytotoxicity of intermediate products during photodegradation process two different seeds V. radiata and P. vulgaris were utilized. The toxicity analysis suggested that degraded metabolites showed less toxic nature compared to normal EE2 and AB-52. We believe that the present work furnishes the ability of multifunctional nanomaterials to not only degrade the hazardous pollutants but also detoxify them during photo catalysis.

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Fig. 1 Photocatalytic reactor set up for the degradation experiments. The set up consists of (1) 300 W Hg lamp (2) Glass tubes (50 mL) (3) Air pump (4) Water inlet (5) Water outlet (6) Air pump compressor (7) AC (8) Top lid (9) Bottom lid (10) Fan (11) Substrate for whole reactor (12) Borosilicate condenser



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Fig. 2 X-ray diffraction pattern of synthesized nanoparticles





Fig. 3 XPS spectra of Ag 3d (a) Zr 3d (b) Ti 2p (c) and broad spectrum (d) of Zr/Ag-TiO₂ nanocomposite



Fig. 4 N₂ adsorption–desorption isotherms of as prepared composites (pore size distribution is the inset).







Fig. 6 Plausible degradation pattern of AB-52 under visible light irradiation using Zr/Ag-TiO₂ (50 mg)





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Fig. 8 Phytotoxicity assessment of AB-52, EE2 and their corresponding metabolites



Fig. 9 Active species scavenging experiments using Zr/Ag-TiO₂ during EE2 (a) and AB-52 (b) degradation



Fig. 10 Concentration of •OH (a) and $\bullet O_2$ -(c) radicals; formation of TAOH (b) and NBT transformation to formazan products (d) at different time intervals

Scheme 1. Reaction pathway between NBT and superoxide radicals with the formation of formazan



Scheme 2. Reaction pathway between terepthalic acid and hydroxyl radical with the formation of fluorescent 2-hydroxy terepthalic acid



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Sample	EE2		AB-52		
	k (min- ¹)	R^2	k (min- ¹)	R^2	
No Catalyst	0.0009	0.8351	0.0005	0.9233	
TiO_2 alone	0.0165	0.8981	0.0083	0.9541	
Ag-TiO ₂	0.0885	0.9914	0.1455	0.9690	
ZrAgTiO ₂ (25 mg)	0.1482	0.9860	0.1601	0.9799	
ZrAgTiO ₂ (50 mg)	0.1617	0.9882	0.1967	0.9918	

Table 1. Rate constant (k) and linear correlation coefficient (R²) of the first-order kinetic reaction for EE2 and AB-52 degradation

Table 2. Phytotoxicity assessment of AB-52, EE2 and their corresponding extracted intermediates

	Vigna radiata			Phaseolus vulgaris		
	Germination (%)	Shoot (cm)	Root (cm)	Germination (%)	Shoot (cm)	Root (cm)
Water	100	10.27±0.57	4.67±0.63	100	6.65±1.31	2.47±0.54
AB-52	30	2.12±0.15	0.43±0.15	30	2.76±0.76	0.66±0.15
AB-52 metabolites	100	6.45±1.21	1.15±0.24	100	4.45±0.42	1.31±0.25
EE2	40	3.30±0.31	0.72±0.17	50	3.21±0.83	0.92±0.17
EE2 metabolites	100	5.15±0.51	1.92±0.24	100	4.65±0.40	1.43±0.25

