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Electrochemically active biofilm assisted synthesis of Ag@CeO₂ nanocomposites for antimicrobial activity, photocatalysis and photoelectrodes

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A B S T R A C T

Ag@CeO₂ nanocomposites were synthesized by a biogenic and green approach using electrochemically active biofilms (EABs) as a reducing tool. The as-synthesized Ag@CeO₂ nanocomposites were characterized and used in antimicrobial, visible light photocatalytic and photoelectrode studies. The Ag@CeO₂ nanocomposites showed effective and efficient bactericidal activities and survival test against Escherichia coli O157:H7, and Pseudomonas aeruginosa. The as-synthesized Ag@CeO₂ nanocomposites also exhibited enhanced visible light photocatalytic degradation of 4-nitrophenol and methylene blue than pure CeO₂. A photocatalytic investigation showed that the Ag@CeO₂ nanocomposites possessed excellent visible light photocatalytic activities compared to pure CeO₂. Electrochemical impedance spectroscopy and photocurrent measurements showed that the as-synthesized Ag@CeO₂ nanocomposites exhibited excellent and enhanced responses to visible light irradiation. These results suggest that the AgNPs anchored at CeO₂ induced visible light photocactivity by decreasing the recombination of photogenerated electrons and holes, and extending the response of pure CeO₂ to visible light. Overall, as-synthesized Ag@CeO₂ nanocomposites are smart materials that can be used for a range of applications, such as antimicrobial activity, visible light photocatalysis and photoelectrode.

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1. Introduction

The demand for efficient and smart materials that can be used for multipurpose applications, such as visible light photocatalysis, photoelectrode and medicine is increasing. Metal oxide nanostructures, such as CeO₂, serving as supporting materials, have attracted increasing interest in catalysis because of their unique structural properties, such as high surface area, uniform pore distribution and rigid framework [1–6]. Li et al. [7] recently designed a unique mesoporous multicomponent nanocomposite catalyst using thermally-stable noble-metal and oxide nanoparticles. Sophisticated and supported heterogeneous catalysts have also been produced by controlling the size and shape of the active metal species, and by selecting and changing the composition of the support [8].

Fine-tuning of the interfacial interactions between the active metal species and supports are the most important factors through which high catalytic performance can be achieved. Because of the many intrinsic advantages of heterogeneous catalysts over homogeneous ones, such as their durability at high temperatures and reusability, the fine-tuning of metal–metal oxide interactions in heterogeneous catalysts is an emerging and challenging objective [9]. Interfacial interactions in noble metal-supported catalysts determine the catalytic processes of many fundamental chemical reactions [8,10]. To strengthen the interfacial interactions between the noble metals and support, supported catalysts prepared by impregnation or combustion methods need to undergo high-temperature sintering (500 °C) for activation [11]. During the sintering process, unavoidable aggregation of the noble metals and oxide supports occurs, which can lead to a significant decrease in catalytic activity. Such problems can be avoided by anchoring very small noble metal nanoparticles to the support under mild conditions, which might give rise to high catalytic performance because of the strengthened interfacial interactions and high surface area. Unfortunately, most methods generally used are complicated, and the sizes of the metal nanoparticles cannot be controlled easily to increase the interfacial interactions for enhanced catalytic performance [11,12]. Some of the studies have deposited the small metal nanoparticles on the surface of a support but those that did involve complicated steps or harmful materials [13–16].

The present study extended the biogenic protocol to synthesize Ag@CeO₂ nanocomposites using electrochemically active biofilms...
(EABs), which is novel, green and does not involve toxic chemicals [17–21]. The main aim of this study is to enhance the visible light response of P-CeO₂ by anchoring the silver nanoparticles (AgNPs). AgNPs were anchored to CeO₂, which is quite small than the conventional noble metal-supported structures [12,14,22]. The interfacial interactions between AgNPs and CeO₂ nanoparticles could be adjusted conveniently in a simple manner. The synergistic effects of AgNPs and CeO₂ would become enhanced, yielding increase in the visible light catalytic activity and stability of the as-synthesized Ag@CeO₂ nanocomposites. The as-synthesized Ag@CeO₂ nanocomposites were assessed for their antimicrobial activity, visible light photocatalysis and photoelectrodes, and showed an excellent response in each application. The significance of these findings is twofold: First, by supporting nanosized AgNPs, it is possible to induce a visible light response to CeO₂, which otherwise is inactive under visible light irradiation. Second, anchoring the small size AgNPs on CeO₂ keeps them dispersed and prevents aggregation, which helps retain and maximize their antimicrobial and photo activities.

2. Experimental section

2.1. Materials

Ce-oxide (nano-CeO₂), silver nitrate (AgNO₃, 99%) and methylene blue (MB) were purchased from Sigma–Aldrich. 4-Nitrophenol (4-NP), sodium acetate, sodium phosphate, sodium sulfate and sodium chloride were obtained from Duksan Pure Chemicals Co. Ltd. South Korea. Ethyl cellulose and α-terpineol were supplied by KANTO Chemical Co., Japan, and fluorine-doped transparent conducting oxide glass (FTO; F-doped SnO₂) was acquired from Pilkington, USA. All other chemicals were of analytical grade and used as received. The bacterial culture medium was purchased from Becton Dickinson and Company (NJ, USA). All solutions were prepared with deionized (DI) water obtained from a PURE ROUP 30 water purification system.

2.2. Methods

The diffuse absorbance/reflectance UV–Vis spectra (DRS) of the powder Ag@CeO₂ nanocomposites and pure CeO₂ (P-CeO₂) samples were obtained using an ultraviolet–visible–near infrared (UV–VIS–NIR) double beam spectrophotometer (VARIAN, Cary 5000, USA) equipped with a diffuse reflectance accessory. A given amount of the Ag@CeO₂ nanocomposites and P-CeO₂ powder was pressed uniformly in the sample holder, which was then placed at the integrating sphere for the absorbance/reflectance measurements. X-ray diffraction (XRD, PANalytical, XPert-PRO MPD) was performed using Cu Kα radiation (λ = 0.15405 nm). The microstructure was examined by field emission transmission electron microscopy (FE-TEM, Tecnai G² F20, FEI, USA) operating at an accelerating voltage of 200 kV. Selected-area electron diffraction (SAED) and a high angle annular dark field (HAADF) were carried out by TEM. Quantitative analysis was performed by energy dispersive spectrometry (EDS) attached to the TEM. X-ray photoelectron spectroscopy (XPS, ESCALAB 250 XPS System, Thermo Fisher Scientific U.K.) was conducted using the following X-ray source: monochromated Al Kα, hν = 1486.6 eV, X-ray energy: 15 kV, 150 W and spot size: 500 μm, take-off angle: 90°, pass energy: 20 eV, BE resolution: 0.6 eV (calibrated by Ag 3d₅/₂) at Korea Basic Science Institute, (KBSI) South Korea. XPS fitting was done using “AVANTAGE” software by Shirley subtraction and shape of the peaks used for the deconvolution was Gaussian–Lorentzian shapes. Sensitivity factor used for Ce 3d₅/₂ and Ag 3d₅/₂ was 10.66 and 30.5 respectively.

The photocatalytic degradation and photoelectrochemical experiments (Electrochemical impedance spectroscopy (EIS) and linear scan voltammetry (LSV)) were conducted using a 400 W lamp (3 M, USA). The EIS and LSV measurements were taken using a potentiostat (Versa STAT 3, Princeton Research, USA) using a standard three-electrode system. Ag/AgCl (saturated with KCl), a Pt gauge and FTO glass coated with 1 mM and 3 mM of the as-synthesized Ag@CeO₂ nanocomposites, and P-CeO₂ samples were used as the reference, counter and working photoelectrodes, respectively. The experiments were performed in a 0.2 M Na₂SO₄ solution, as the electrolyte, at room temperature. The projection area of each photoelectrode was 1 cm². The experimental conditions for EIS were 0.0 V and the frequency ranged from 1 to 10⁴ Hz. The working electrodes for the photoelectrochemical measurements were prepared as follows. One hundred milligrams of each sample was suspended thoroughly by adding ethyl cellulose as a binder and α-terpineol as a solvent for the paste. The paste was later coated on a fluorine-doped tin oxide glass electrode using the doctor-blade method.

2.3. Development of electrochemically active biofilms

The EABs on carbon paper were prepared using the procedure reported elsewhere [17–21]. Briefly, 0.2 g sodium acetate was added as a substrate to a 200 mL of mineral salt medium [23]. This was followed by the addition of 10 mL of anaerobic sludge (Biogas plant in Paju, Republic of South Korea) and sparging with N₂ gas for 5 min to create an anaerobic environment. Finally, carbon paper (2.5 cm × 4.5 cm) was dipped into the mixture and the bottle was sealed. All media, including the bacterial inoculum, were changed every two days under strict anaerobic conditions. The process was repeated for two weeks. The EABs formed on the carbon paper were confirmed using a microbial fuel cell by obtaining the appropriate voltage. The living EABs formed on the carbon paper specimens were used to synthesize the Ag@CeO₂ nanocomposites.

2.4. Electrochemically active biofilm mediated synthesis of Ag@CeO₂ nanocomposites

Two sets of 200 mL, 5 mM CeO₂ aqueous suspensions were prepared. Subsequently, 0.2 g sodium acetate was added to each suspension as an electron donor. Either a 1 mM or a 3 mM AgNO₃ aqueous solution was added to the above reaction mixtures. The reaction mixtures were sparged with N₂ gas for 5 min to maintain the anaerobic environment. The EAB was hung in each reaction bottle, and the systems were sealed and left for magnetic stirring at 30 °C. The reaction mixture was stirred for a further ~8 h to complete the reaction. In each case, the initial white color began changing to a light brown color within 30 min. Finally, grayish-brown and blackish-brown precipitates were obtained in the 1 mM and 3 mM AgNO₃ cases, respectively. The reaction mixtures were centrifuged, and the powdered Ag@CeO₂ nanocomposites were isolated for further characterization and applications. The obtained supernatant was tested using an UV–vis spectrophotometer to determine the quantity of AgNPs remaining in the supernatant.

Two controlled syntheses were performed to confirm the role of the EAB and sodium acetate. Two 5 mM CeO₂ aqueous suspensions (200 mL) were prepared. In the first controlled synthesis, an aqueous solution containing 0.2 g sodium acetate and 1 mM AgNO₃ was added. In the second controlled synthesis, only a 1 mM AgNO₃ aqueous solution was added. Both reaction mixtures were sparged with N₂ gas for 5 min to maintain the anaerobic conditions. The EAB was hung in the second controlled synthesis only. Both systems were sealed and stirred with a magnetic stirrer at 30 °C. No
changes were observed, even after 48 h. This confirmed that both the EAB and sodium acetate are essential to complete the reaction.

2.5. Antimicrobial activity of the Ag@CeO2 nanocomposites

The antimicrobial activity test was performed using the drop assay method to test the antibacterial activities of the P-CeO2, and the as-synthesized 1 mM and 3 mM Ag@CeO2 nanocomposites under normal laboratory condition. Two pathogenic bacteria, *Escherichia coli* O157:H7 (ATCC43895) [24], and *Pseudomonas aeruginosa* PA01 [25] of the sequenced strains were used. All experiments were conducted at 37 °C and Luria–Bertani (LB) medium [26] was used to culture *E. coli*, and *P. aeruginosa*. Two pathogenic bacterial strains were initially streaked from −80 °C glycerol stock on an LB plate, and a fresh single colony was then inoculated in LB (25 mL) in 250 mL flasks and cultured at 37 °C and 250 rpm. Overnight cultures were re-inoculated in LB medium at a 1:100 dilution. 0.1 mL of the overnight culture was spread on a LB agar plate and incubated at 37 °C for 24 h with 5 μL of P-CeO2, and the as-synthesized 1 mM and 3 mM Ag@CeO2 nanocomposites, which were dispersed in water. The final concentrations of 1 mg/mL were used to detect the bactericidal effect. The plates were incubated at 37 °C for 18 h and the antibacterial activities of the samples were evaluated by measuring a clear zone, where the inhibition of bacterial growth was observed. All experiments were performed using at least two independent cultures.

Survival test was performed on two pathogenic bacteria such as *E. coli* O157:H7 (ATCC43895) and *P. aeruginosa* PA01 against P-CeO2, 1 mM Ag@CeO2, and 3 mM Ag@CeO2. The overnight grown seed culture of pathogenic bacteria was inoculated in 2 mL LB and incubated for 4 h until the OD600 reached ~1.0. The stock solutions of each chemical were prepared in water. The cultured cells (1 mL) of each bacterium were separately mixed with P-CeO2, 1 mM Ag@CeO2, and 3 mM Ag@CeO2 by vortexing at final concentration of 3 mg/mL. The mixed cells were incubated at 37 °C for 1 h under shaking condition (250 rpm). After incubation, cells were serially diluted in phosphate buffer solution (PBS) and spread plated on LB-agar plates. These plates were incubated at 37 °C for 24 h. Total number of bacterial colonies appeared on plates were counted. The experiments were performed using two independent cultures.

2.6. Photocatalytic degradation of 4-NP and MB by Ag@CeO2 nanocomposites under visible light irradiation

The photocatalytic activities of the as-synthesized 1 mM and 3 mM Ag@CeO2 nanocomposites and P-CeO2 samples were tested for the degradation of 4-NP and MB under visible light irradiation. The photocatalytic activities of the 1 mM and 3 mM Ag@CeO2 nanocomposites and P-CeO2 were measured by the photodecomposition of 4-NP and MB at a concentration of 5 mg/L and 10 mg/L, respectively. The rate of 4-NP and MB degradation was calculated using the decrease in the absorbance of the degraded solutions [13,27]. For photodecomposition, 2 mg of each photocatalyst was suspended in a 20 mL of aqueous solutions of 4-NP and MB. Each solution was sonicated for 5 min in the dark. The solutions were stirred in the dark for 30 min to complete the adsorption and desorption equilibrium of the specific substrate on the Ag@CeO2 nanocomposites and P-CeO2. Visible light irradiation of the solutions was performed using a 400 W lamp (λ > 400 nm). The two sets of experiments were observed for 5 h and 6 h in the 4-NP and MB degradation experiment, respectively. The rate of dye degradation was monitored by taking 1.7 mL of the samples from each set every 1 h, centrifuging to remove the catalyst and recording the UV–vis spectrum. Each experiment was performed in triplicate to ensure that the photocatalytic activities of the Ag@CeO2 nanocomposites and P-CeO2.

For the control experiment, P-CeO2 was taken as the reference photocatalyst. Normally, the photocatalytic activity of any new material was compared with that of P-CeO2 as a standard. The second control experiment was performed without a catalyst (i.e. P-CeO2 and Ag@CeO2) to check the degradation of 4-NP and MB under visible light irradiation under similar conditions. To check the adsorption of dyes on the catalyst third control experiment was performed in the dark condition under similar conditions.

2.7. Ag@CeO2 nanocomposites as photoelectrodes for EIS and photocurrent

The photoelectrode response of the as-synthesized 1 mM and 3 mM Ag@CeO2 nanocomposites and P-CeO2 samples were examined by photoelectrochemical experiments, such as EIS and LSV, which were carried out under ambient conditions in the dark and under visible light irradiation in a 50 mL, 0.2 M Na2SO4 aqueous solution at room temperature. For each electrode, EIS was first performed in dark conditions and later under visible light irradiation (λ > 400 nm) at 0.0 V and with a frequency ranging from 1 to 103 Hz. The photocurrent response was obtained by LSV in dark and under visible light irradiation at a scan rate of 50 mV/s over the potential range of −1.0 to 1.0 V.

2.8. Stability and reusability of the Ag@CeO2 nanocomposites

A preliminary test for stability was performed by suspending the Ag@CeO2 nanocomposites in water and sonicing them for 1 h. The level of AgNPs leaching in the solution was examined by UV–vis spectrophotometry to confirm the stability of the Ag@CeO2 nanocomposites and their possible use as a catalyst. The reusability of the Ag@CeO2 nanocomposite was tested after centrifuging the catalyst from the 4-NP and MB solution. The isolated catalyst from first and second run were washed with DI water, dried in an oven at 90 °C, and reused for a second and third run to check their catalytic activity with a 4-NP and MB solution under the same conditions.

3. Results and discussion

3.1. Mechanism of biogenic synthesis of Ag@CeO2 nanocomposites

The EABs are well known in microbial fuel cells and the bio-inspired synthesis of nanomaterials [17–21,27–30]. EAB biologically decomposes the carbon source (sodium acetate) as shown by the equation CH3COO− + 4H2O + EABs → 8e− + 9H+ + 2HCO3− [29,30]. This study extended this biogenic and green approach to the synthesis of Ag@CeO2 nanocomposites for antimicrobial, photocatalysis and photoelectrode studies. Scheme 1 shows the proposed synthesis of the Ag@CeO2 nanocomposite in the presence of EAB and sodium acetate (carbon source) in water at 30 °C. The electrons produced by EAB, after biologically decomposing the acetate are consumed in the simultaneous reduction of Ag+ to Ag0 and the
anchoring or adsorption of AgNPs at the CeO₂ surface [15,20,21,28,29]. Supernatant analysis by UV–vis spectrophotometry did not show any absorbance in the 350–500 nm range (Fig. S1). This confirms that all the AgNPs had adsorbed or anchored at the CeO₂ surface. The product obtained in this synthesis is quite pure and free of impurities because the biofilm was supported on carbon paper. Moreover, the control experiments clearly showed the role of EAB and sodium acetate in the synthesis. The proposed approach is quite novel, and does not involve any harmful chemicals.

3.2. Characterization of the Ag@CeO₂ nanocomposites

3.2.1. UV–vis spectroscopy

Fig. 1 shows the UV–vis absorption spectra of P-CeO₂, and the 1 mM and 3 mM Ag@CeO₂ nanocomposites. Fig. S2 shows their diffuse reflectance spectra and color of the samples. The UV–vis absorption spectrum of P-CeO₂ showed a strong absorption peak at ~316 nm with no other absorption peaks in the visible region. In contrast, the 1 mM and 3 mM Ag@CeO₂ nanocomposites showed broad absorption in the visible region due to the surface plasmon resonance (SPR) effect of the AgNPs (Fig. 1). The surface plasmon absorption provided the evidence that AgNPs exist in the nanocomposites. Significant absorption was observed in the visible region, ~400–500 nm, because of the anchoring of AgNPs to the surface of the CeO₂ nanoparticles. In case of 1 mM and 3 mM Ag@CeO₂ nanocomposites the absorption in the visible region showed a red shift, and the absorption increased with increasing Ag content. In comparison to P-CeO₂ the absorption for Ag@CeO₂ nanocomposites is red shifted whereas after increase in the concentration of Ag content from 1 mM to 3 mM the absorption is blue shifted. The red shift is attributed to the visible light absorption of Ag@CeO₂ nanocomposites whereas the blue shift in the absorption of the 3 mM Ag@CeO₂ could be attributed to the decrease in the particles size of AgNPs at the surface of CeO₂. The SPR absorption band of the AgNPs can help to improve the absorption in visible light, and enhance the absorption intensity of the Ag@CeO₂ nanocomposites [22]. The maximum absorption and shifts in the visible region could be because of the interaction between AgNPs and CeO₂ nanoparticles and the difference in their work function [22,31].

3.2.2. Transmission electron microscopy

Fig. S3 presents a TEM image of the P-CeO₂ nanoparticles, which are in the range of 20–25 nm. Fig. 2 presents HR-TEM images and SAED patterns of P-CeO₂ and the 1 mM and 3 mM Ag@CeO₂ nanocomposites. The dark spots in Fig. 2(b and c) revealed the presence of AgNPs on the surface of CeO₂, which are in the range of 3–7 nm. The overall sizes of the Ag@CeO₂ nanocomposites were in the range of 25–30 nm. The SAED patterns (Fig. 2 insets) of the 1 mM and 3 mM Ag@CeO₂ nanocomposites clearly showed well-resolved lattice fringes and diffraction cycles, indicating the highly crystalline nature of the Ag@CeO₂ nanocomposites, which is in good agreement with DRS (Fig. 1) and XRD (Fig. 3). Figs. S4 and S5 show

![Fig. 1. UV-vis diffuse absorbance spectra of P-CeO₂, and the 1 mM and 3 mM Ag@CeO₂ nanocomposites.](image1)

![Fig. 2. HR-TEM images of (a) P-CeO₂, (b) 1 mM Ag@CeO₂ nanocomposite, and (c) 3 mM Ag@CeO₂ nanocomposite. The inset shows the respective SAED pattern.](image2)
the HAADF image and EDX spectrum of the 1 mM Ag@CeO₂ nanocomposite, respectively. Figs. S6 and S7 show a HAADF image and EDX spectrum of the 3 mM Ag@CeO₂ nanocomposites, respectively, corresponding to the O (K), Ag (K) and Ce (K) lines. The EDX elemental spectra are also supported by the elemental composition, as listed in Tables S1 and S2 for the 1 mM and 3 mM Ag@CeO₂ nanocomposites, respectively. This further confirms the presence of AgNPs at the CeO₂ surface. EDX revealed the 3 mM Ag@CeO₂ nanocomposite to have a higher silver content than the 1 mM Ag@CeO₂ nanocomposite, which is in agreement with DRS and XRD results.

3.2.3. X-ray diffraction

The phase and structure of the as-synthesized samples were examined by XRD. Fig. 3 shows the XRD patterns, which confirms the formation of metallic AgNPs at the surface of CeO₂ as well as the overall formation of Ag@CeO₂ nanocomposites and their crystallinity. The prominent XRD peaks confirmed that the main body was CeO₂. The XRD peaks at 28.54°, 33.07°, 47.48°, 56.33°, 59.08°, 69.4°, 76.69° and 79.06° 2θ were assigned to the (111), (200), (220), (311), (222), (400), (331), and (420) planes, respectively, of the face centered cubic fluorite structure of CeO₂ [JCPDS File No. 81-0792]. When the Ag concentration was lower i.e. 1 mM, only the face centered cubic fluorite structure of CeO₂ was observed in the XRD pattern. On the other hand, when the Ag concentration was increased to 3 mM, the Ag crystalline phase began to appear with a face-centered cubic phase structure. In the 3 mM Ag@CeO₂ nanocomposite, the (111), (200), (220), and (311) peaks for face-centered cubic (fcc) crystalline silver were detected at 38.15°, 44.21°, and 64.38° 2θ, respectively (JCPDS File No. 89-3722) [22]. The peak at 38.15° 2θ is the characteristic peak for Ag metal, which confirmed the deposition of Ag on the CeO₂ surface. The intensity of the silver peaks increased with increasing amount of AgNPs from 1 mM to 3 mM, which confirmed the anchoring of AgNPs to the CeO₂ surface. This is in accordance with the UV–vis spectrum (Fig. 1).

The mean crystallite size of P-CeO₂, and the 1 mM and 3 mM Ag@CeO₂ nanocomposites were calculated using the Scherrer’s formula, \[ D = \frac{k\lambda}{βcosθ} \]
where \( k \) is the shape factor and has a typical value of ~0.9, \( \lambda \) is the wavelength (Cu Kα = 0.15405 nm), \( β \) is the full width at half maximum of the most intense peak (in radians) and \( θ \) is the peak position [32]. Using this equation, the calculated crystallite sizes of P-CeO₂, and the 1 mM and 3 mM Ag@CeO₂ nanocomposites were 25.79 nm, 26.09 nm and 26.78 nm, respectively. The likely reason for the increase of overall size of the Ag@CeO₂ nanocomposites could be the anchoring of AgNPs to the CeO₂ surface [21,32]. In the case of the 1 mM and 3 mM Ag@CeO₂ nanocomposites, sharpening of the diffraction peaks and a slight shift to lower θ values (Fig. 3) were observed. Since anchoring of AgNPs at CeO₂ may cause some stress on the CeO₂ crystals [32], therefore, caused stress seems responsible for minor shift in the XRD peak towards lower angle which may leads to the expansion of the crystalline lattice [32]. In the present case too, stress appears to be responsible for the peak shift [21,32].

3.2.4. XPS analysis

The surface chemical composition and chemical states of P-CeO₂ and Ag@CeO₂ were examined by XPS. Fig. S8(a) presents the XP survey spectra of the P-CeO₂ and Ag@CeO₂ nanocomposites, showing that the material contains only Ce, Ag, O and C with no other impurity elements. The carbon peak in Fig. S8(b) (C 1s = 284.8 eV) was assigned to residual carbon from the sample and hydrocarbons from the XPS instrument, whereas Fig. S8(c) presents the O 1s spectra of P-CeO₂ and Ag@CeO₂, in which the peak at ~531.39 eV was assigned to the lattice oxygen present in CeO₂, whereas the peaks at 531.94 eV could be assigned to surface hydroxyl groups within the CeO₂ matrix [10]. The presence of surface hydroxyl groups facilitates the trapping of photoinduced electrons and holes, thereby enhancing the photocatalytic degradation process [5,21].

The XP spectra of Ce 3d, and Ag 3d (Fig. 4a, and b) showed that the binding energies of these electrons in the case of Ag@CeO₂ were slightly different (Tables S3–S5) from those of P-CeO₂, suggesting a strong interaction between the Ag and CeO₂ nanoparticles [10]. Fig. 4a shows the Ce 3d spectra with specific peaks at 898.94 eV for 3d₅/₂ and 916.43 eV for 3d₃/₂, indicating the oxidation state of Ce⁴⁺ in the form of CeO₂ in the materials [10]. Fig. 4b shows the core level Ag 3d spectra of the AgNPs in Ag@CeO₂ nanocomposites, showing two distinct individual peaks for Ag 3d₅/₂ and Ag 3d₃/₂ at 367.5 eV and 373.6 eV, respectively, with ~6 eV splitting between the two peaks, which is evidence of the reduction of Ag ions by EAB to produce metallic silver [10,33,34]. This is attributed to the complete reduction of Ag⁶⁺ to Ag⁰ [34]. Moreover, the binding energy (BE) of Ag 3d₅/₂ for the Ag@CeO₂ shifted to a lower binding energy (Tables S3–S5) compared to the corresponding value of the synthesized pure metallic Ag (the BE of Ag⁰ is approximately 368.2 eV). This is attributed to the anchoring of AgNPs to the CeO₂ surface [28,35]. Therefore, a new Fermi level energy in Ag@CeO₂ is formed, indicating a strong interaction between Ag and the CeO₂ nanoparticles [35]. Once the AgNPs are deposited on the surface of the metal oxides, electron transfer occurs from Ag to the CeO₂ because the work function of silver (4.2 eV) is smaller than that of CeO₂ (4.7 eV) [22,31,33,35,36,46]. The atomic % (Fig. 4b) of Ag 3d₅/₂ found in the 1 mM and 3 mM Ag@CeO₂ nanocomposites was 0.36, and 0.54 respectively which is in agreement with the EDX.

4. Applications of Ag@CeO₂ nanocomposites

4.1. Antimicrobial activities of the Ag@CeO₂ nanocomposites

Silver has extensive antibacterial activity and relatively high safety [37–41]. When silver is supported on an inorganic carrier and is released slowly from the surface, it acts as an inorganic disinfectant with superior safety, durability and heat resistance [40–42]. Therefore, an antibacterial test against enterohemorrhagic E. coli O157:H7, and multi-drug resistant P. aeruginosa PAO1 was performed to compare the bacterial colonies on agar plates in the presence of P-CeO₂, and the as-synthesized 1 mM and 3 mM Ag@CeO₂ nanocomposites. The growth of the bacteria in the presence of the 1 mM and 3 mM Ag@CeO₂ nanocomposites was...
inhibited (Fig. 5(I)), suggesting that the 1 mM and 3 mM Ag@CeO2 nanocomposites have good antibacterial properties. Table 1 lists the zone of inhibition (Fig. 5(I)) shown by the as-synthesized 1 mM Ag@CeO2 nanocomposites and 3 mM Ag@CeO2 nanocomposites against E. coli, and P. aeruginosa. The as-synthesized 1 mM and 3 mM Ag@CeO2 nanocomposites showed better bactericidal activity than P-CeO2. In addition, the 3 mM Ag@CeO2 nanocomposites showed much better bacterial inhibition than the 1 mM Ag@CeO2 nanocomposites, P-CeO2 and other studies [43]. This suggests that the antibacterial properties of the Ag@CeO2 nanocomposites increase with increasing AgNPs concentration.

The survival rate of E. coli O157:H7 and P. aeruginosa against P-CeO2 and 1 mM and 3 mM Ag@CeO2 is shown in Fig. 5(II). P-CeO2 did not show bactericidal effect on the survival of E. coli O157:H7 and P. aeruginosa PA01, whereas 1 mM Ag@CeO2 and 3 mM Ag@CeO2 reduced the survival of these pathogenic bacteria dramatically. The bactericidal effect of both 1 mM Ag@CeO2 and 3 mM Ag@CeO2 seems working on two tested pathogenic bacteria well with 1 h exposure. These results indicate that the synthesized nanocomposites show high antibacterial activity as compared to the P-CeO2 and pervious reports [44,45].

The enhanced antibacterial activity might be due to Ag ions eluted from the Ag@CeO2 nanocomposites, which could be absorbed on the surface of the bacterial cells, damaging the cell membrane and solidifying the protein structure. This will lead to a distortion of the bacterial cell shape, which gives rise to the leakage of intracellular constituents, resulting in the inhibition and death of the bacteria [42,45,46].

4.2. Photocatalytic activities of the Ag@CeO2 nanocomposites

The photocatalytic activities of P-CeO2, and the 1 mM and 3 mM Ag@CeO2 photocatalyst for the degradation of 4-NP and MB under visible light (λ > 400 nm) irradiation were examined [10,12]. 4-NP is a colorless aromatic compound, whereas MB is a colored dye. Both the 1 mM and 3 mM Ag@CeO2 photocatalysts showed better degradation than P-CeO2 under visible light irradiation. The degradation was estimated from the decrease in the absorption intensity of 4-NP and MB at fixed wavelengths, λmax = 400 and 665 nm, respectively, during the course of the visible light photocatalytic degradation reaction. The degradation was calculated from C/C0, where C0 is the initial concentration and C is the concentration after photo-irradiation (Fig. 6). Fig. S10 shows that there is almost no adsorption of 4-NP and MB on the photocatalyst in the dark condition.

The Ag@CeO2 photocatalyst showed better catalytic activity than the P-CeO2. The likely reasons for the enhanced photocatalytic activities compared to P-CeO2 are the surface modification by AgNPs in the 1 mM and 3 mM Ag@CeO2 photocatalyst [21]. The explanation for the variation in the photocatalytic activities of P-CeO2, and the 1 mM and 3 mM Ag@CeO2 photocatalyst is also supported by DRS (Figs. S2 and 1). These results suggest that the visible light photocatalytic activities of the 1 mM and 3 mM Ag@CeO2 photocatalyst were improved considerably. On the other hand, the 3 mM Ag@CeO2 photocatalyst showed the maximum photocatalytic activities to degrade 4-NP and MB. This suggests that in the 3 mM Ag@CeO2 photocatalyst, the increased amount of

![Fig. 4. Fitted XP spectra of P-CeO2, 1 mM and 3 mM Ag@CeO2 nanocomposites for (a) Ce 3d peaks, and (b) Ag 3d peaks.](image)

![Fig. 5. (I) Antimicrobial activities for (a) E. coli O157:H7, and (b) P. aeruginosa, and (II) survival rate for (a) E. coli O157:H7, (b) P. aeruginosa against the P-CeO2, 1 mM and 3 mM Ag@CeO2 nanocomposites.](image)
AgNPs induced the visible light photocatalytic degradation of 4-NP and MB by decreasing the recombination of the photogenerated electrons and holes as well as extending the response of P-CeO₂ to visible light [21,31,46,47]. The photocatalytic activities shown by these nanocomposites were better than those reported for other nanomaterials [2,13,21,48]. Fig. 7 shows the proposed mechanism for the visible light activities of the Ag@CeO₂ photocatalysts which is supported by other reports [8].

When two materials with different work functions such as Ag ($W_f = 4.2$ eV) and CeO₂ ($W_f = 4.7$ eV) were in contact with each other, Schottky barrier will be formed [35,36]. As shown in Fig. 7 the Fermi energy level of Ag ($F_m$) is higher than that of CeO₂ ($F_s$) because of the larger work function of CeO₂. This leads the transfer of electrons from Fermi level of Ag ($F_m$) to Fermi level of CeO₂ ($F_s$), until the two levels attain equilibrium and form the new Fermi energy level ($F_{ms}$). The Fermi levels of Ag and CeO₂ were adjusted to the same value during the formation of the composite and having more free electrons above the new Fermi level ($F_{ms}$). When visible light is irradiated, the equilibrated Fermi level ($F_{ms}$) electrons are excited to CeO₂ conduction band by surface plasmon resonance phenomenon [35,36].

In general, photogenerated electrons can react with oxygen molecules adsorbed on the surface of the Ag@CeO₂ photocatalyst to yield free radicals such as $\cdot O_2^-$ and $\cdot HO$. The presence of extra hydroxyl species delays the recombination through hole trapping at AgNPs which helps to retard the recombination of photogenerated electron hole pairs [21,35]. Therefore, the Ag@CeO₂ photocatalyst exhibits superior visible light photocatalytic activity to P-CeO₂. In addition, the photocatalytic activity has a direct correlation with the rate of formation of reactive free radicals, i.e. the faster formation of free radicals leads to higher photocatalytic activity of the catalyst. These results suggest that the AgNPs at the surface of CeO₂ helps decrease the recombination of photogenerated electrons and holes, increase the rate of formation of $\cdot O_2^-$ and $\cdot HO$ radicals, and facilitate the degradation of 4-NP and MB [21,35].

The first control experiment suggests that Ag@CeO₂ nanocomposites have much better visible light photocatalytic activities than P-CeO₂. The second control experiment showed that without the catalysts (i.e. P-CeO₂ and Ag@CeO₂) no degradation of 4-NP and MB occurred under visible light irradiation (Fig. S9). The third control experiment showed that there is no adsorption of dyes on the catalyst in the dark condition (Fig. S10).

4.3. Stability and reusability of the Ag@CeO₂ nanocomposite

The stability of the Ag@CeO₂ nanocomposite was evaluated by sonication the aqueous suspension of Ag@CeO₂ nanocomposites for one hour. The solution was later analyzed for any leached silver using an UV–vis spectrophotometer (Fig. S11). The analysis did not show any absorbance for silver. This confirms that the Ag@CeO₂ nanocomposite was quite stable. The reusability of the Ag@CeO₂ nanocomposite was tested by centrifuging the catalyst from the dye solutions, washing with DI water and drying in an air oven at 90 °C. The reused catalyst showed almost same response for second and third run to that of the fresh catalyst (Fig. 6) which highlights the reusability of the Ag@CeO₂ nanocomposites.

4.4. Ag@CeO₂ nanocomposites as photoelectrodes

The photoelectrochemical activity of the nanomaterials can be determined by both the light-harvesting capacity and the separation of electron–hole pairs [48]. EIS was used to examine the electrochemical properties of the photoelectrodes in the dark and under visible light irradiation. EIS was performed on P-CeO₂, and the 1 mM and 3 mM Ag@CeO₂ photoelectrodes to examine the charge transfer resistance and separation efficiency between the photogenerated electrons and holes because the charge separation efficiency of the photogenerated electrons and holes is a critical factor for the photoactivity of photoelectrodes [49,50]. Fig. 8(a) shows the typical EIS Nyquist plots of P-CeO₂, and the 1 mM and 3 mM Ag@CeO₂ photoelectrodes in the dark and under visible light irradiation. For all photoelectrodes, the resistance under light irradiation was much lower than that in the dark because of the higher charge carrier densities induced by photo-excitation. The arc radius of the EIS spectra reflects the interface layer resistance at the surface of the electrode [50]. A smaller arc radius indicates higher charge transfer efficiency [49]. The arc radii of the 1 mM and 3 mM Ag@CeO₂ photoelectrodes were smaller than that of P-CeO₂ in the dark and under visible light irradiation. This suggests...
that the 1 mM and 3 mM Ag@CeO₂ photoelectrodes have lower resistance than P-CeO₂, which can accelerate the interfacial charge-transfer process. The arc radii of the 3 mM Ag@CeO₂ photoelectrode were smaller than those of the 1 mM Ag@CeO₂ photoelectrodes in the dark and under visible light irradiation, respectively. The 3 mM Ag@CeO₂ photoelectrode had the lowest resistance in both the dark and under visible light irradiation, confirming that the 3 mM Ag@CeO₂ photoelectrodes could better facilitate electron transfer from the photoelectrode to the electrolyte. This can explain the best photo-response and stability. This suggests that the 3 mM Ag@CeO₂ photoelectrodes have the lowest charge transfer resistance and are most suitable for photocatalytic applications [49–51]. These observations confirm that the 3 mM Ag@CeO₂ photoelectrodes can be used effectively as a photocatalyst and as a photoelectrode material.

To obtain the photocurrent response of P-CeO₂, and the 1 mM and 3 mM Ag@CeO₂ photoelectrodes (Fig. 8(b)), LSV over the potential range of −1.0 to 1.0 V were recorded in the dark and under visible light irradiation [52–54]. All the samples showed a drastic increase in the photocurrent at an onset potential of −0.30 V vs. Ag/AgCl, and the photocurrent increases linearly until a potential of ~1.0 V vs. Ag/AgCl. Under visible light irradiation, the 1 mM and 3 mM Ag@CeO₂ exhibited an enhanced photocurrent response compared to P-CeO₂ but the highest photocurrent was recorded for 3 mM Ag@CeO₂ under visible light irradiation. The enhanced photocurrent for the 1 mM and 3 mM Ag@CeO₂ can be attributed to the improvement of visible light harvesting, which is due to efficient electron transfer and greater surface area achieved by the anchoring of AgNPs at the CeO₂ surface [49,52]. Under visible light irradiation, photocurrent shown by the 1 mM and 3 mM Ag@CeO₂ electrodes were approximately 3.1 and 4.5 times higher, respectively, than those of the P-CeO₂ electrode, which suggests that the separation efficiency of photo-induced electrons and holes was improved by an electronic interaction between AgNPs and CeO₂.

5. Conclusions

This study proposed a biogenic approach to synthesize Ag@CeO₂ nanocomposites, in which nanosized Ag could (i) enhance the antibacterial activity, (ii) extend the response of the nanocomposite to visible light, and (iii) improve the rapid transfer of electrons and inhibit charge recombination. The antimicrobial experiments showed that the 1 mM and 3 mM Ag@CeO₂ nanocomposites have considerable bactericidal activity due to the synergistic effect between the AgNPs and CeO₂ nanoparticles. The visible light photocatalysis and photoelectrochemical experiments (EIS and LSV) confirmed that the Ag@CeO₂ nanocomposites could be a very smart material that can be used for a range of applications. This paper reports an approach to synthesize novel and smart materials for multiple uses.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jcis.2014.06.026.

References


Fig. 8. (a) EIS Nyquist plots, and (b) LSV curves for the photocurrent response of the P-CeO₂, 1 mM and 3 mM Ag@CeO₂ photoelectrodes in dark and under visible light irradiation.