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Pharmaceuticals removal by synergistic adsorption and S-scheme photocatalysis using nano-CeO_2 coupled Fe_3O_4 on a CTAB matrix and investigation of the nanocomposite's antibacterial and anti-biofilm activities: Intrinsic degradation mechanism

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Text S1. Materials and Instrumentation

Ammonium hydroxide (98%), cerium sulfate ($Ce(SO_4)_2$, 99%), CTAB, ferrous chloride ($FeCl_2.4H_2O$, 98%) and ferric chloride ($FeCl_3.6H_2O$, 98%) were procured from Merck (Darmstadt, Germany). *Staphylococcus aureus* (MTCC 3160) and *Pseudomonas aeruginosa* (MTCC 1688) lyophilized cultures were procured from MTCC, Chandigarh.

The Fe_3O_4-CeO_2-CTAB nanocomposite (NCs) morphology was studied using a high-resolution transmission electron microscopy (HRTEM) (Jeol JEM-2100F, Japan). Selected area electron diffraction (SAED) crystallographic experiment was performed along with TEM analysis. Energy-dispersive X-ray spectroscopy (EDAX) was used to detect the presence of iron, cerium and oxygen. The prepared particles were analyzed by X-ray powder diffraction (XRD). Here, the spectra was recorded in XRD grid coated with the NCs using Bruker D8 Advance (Germany) (40 KV) to identify phase, crystallite size and lattice parameter. The stability of the prepared NCs is based on zeta potential that was determined using zeta sizer (Malvern instruments, U.K.). The particle stability was studied by recording the particle size and zeta potential for 72 h. The NCs effective size distribution was recorded by Malvern particle size analyzer. The nitrogen-adsorption/desorption studies were performed using BET analyzer. The pore volume, pore size and surface area of NCs were calculated using the Quantachrome Instruments, Autosorb IQ series. The magnetic behavior of prepared particles was characterized via a vibrating sample magnetometer (VSM, Quantum Design, PAR 155, USA) at room temperature. Thermo Fisher Scientific Nicolet IS50 machine was used to perform Fourier-transform infrared spectroscopy (FTIR). The NPs thermal stability was verified using simultaneous thermo-gravimetric analysis (TGA) and differential thermal analysis (DTA) (NETZSCH, Germany). X-ray photoelectron spectra (XPS) were performed with monochromatized Al Kα excitation for NPs to find chemical states using Physical Electronics (PHI 5000 VersaProbe III).

Text 2. Adsorption, photocatalysis and antimicrobial studies

Influence of environmental parameters on adsorption

The influence of pH on tetracycline adsorption was studied by changing experimental pH from 4 to 9. HCl and NaOH of 0.1 N were used for pH adjusting. Fe_3O_4-CeO_2-CTAB NCs (5 mg) was interacted with tetracycline (10 mg/L) in an orbital shaker (150 rpm, 4h). Thereafter, it was centrifuged and supernatant was collected. The absorbance at λ_{max} was recorded to estimate

leftover tetracycline amount by an UV-visible spectrophotometer. The effect of saline concentration on tetracycline adsorption was investigated with NaCl concentrations from 0.01 mM to 1 M. Fe_3O_4-CeO_2-CTAB NCs from 1 - 10 mg were made to interact with tetracycline (100 mg/L) to determine the influence of adsorbent dosage on adsorption.

Adsorption kinetics

The adsorption kinetics test was performed by interacting Fe_3O_4-CeO_2-CTAB NCs (5 mg) with tetracycline (250 mg/L). A small aliquot was removed from the sample at different intervals of time, centrifuged. The supernatant was used to record the absorbance. The calculation to find the adsorption amount was made using the equation:

$$q_t = \frac{(C_0 - C_t)V}{W} \quad (1)$$

where q_t is the adsorbed tetracycline amount at time t at equilibrium state, C_0 (mg/L) is the initial concentration of tetracycline, C_t (mg/L) is the tetracycline left at time t, W (mg) is the weight of NPs and V is the solution volume in L.

Stability and Scavenger study

The reusing capacity of Fe_3O_4-CeO_2-CTAB NCs was estimated for consecutive six cycle's performance. At the end of each cycle, a magnet was used to collect the dispersed NCs. Then, NCs were re-dispersed in fresh tetracycline solution and the experiment was repeated. Further, the XRD and XPS spectra of the reused NCs were analyzed in order to study the photo-corrosion and stability.

Free radicals involved in photocatalytic degradation of tetracycline using Fe_3O_4-CeO_2-CTAB NCs were identified by scavenger assay. Here, active species trapping agents such as $AgNO_3$ (1 mM), EDTA (1 mM), benzoquinone (BZQ) (1 mM) and isopropyl alcohol (IPA) (0.2 mL alcohol/20 mL tetracycline) were added into reaction mixture to trap positive and negative charge (h^+ / e^-), superoxide ($\cdot O_2^-$) and hydroxyl ($\cdot$OH) radical.

Lipid peroxidation

Lipid peroxidation assessment of *S. aureus* and *P. aeruginosa* due to Fe_3O_4-CeO_2-CTAB nanocomposites exposure was performed by the technique described by Bar-Or et al. [17] with slight modification. Cells exposed to various NCs concentration (0.1 to 100 mg/L) were analyzed by thiobarbituric acid (TBA) assay. Here, the cells exposed to NCs were mixed with phosphate buffer (pH 7.4, 20 mM) and $CuCl_2$ (0.01 mM) and incubated for 15 min. Thiobarbituric acid

(0.5% TBA) in trichloroacetic acid (TCA, 10%) was mixed and incubated in water bath (100 ^{o}C, 15 min). Then, the solution was immediately kept in cooling ice bath. The mixture was centrifugation at 9,000 × g (10 min). Supernatant was taken and the amount of thiobarbituric acid-reactive species (TBARS) present was measured with absorbance at 532 nm. Control bacterial cells were maintained without exposure to NCs. The samples were compared with control to understand the TBARS formed due to the exposure by nanocomposites.

ROS production

Oxidative stress is caused due to the imbalance in the amount of radicals and antioxidants formed in a cell. The excess reactive oxygen species (ROS) formation in cells can be induced by the exposure to NCs. The oxidative stress is mainly evaluated by the measurement of cellular ROS formation using 2,7-dichlorodihydrofluorescein diacetate acetyl ester (H_2DCF-DA). Deacetylated H_2DCF-DA by cellular esterase activity results in the formation of 2,7-dichlorodihydrofluorescein (H_2DCF), a non-fluorescent product. The H2DCF oxidation results in a fluorescent 2,7-dichlorofluorescein (DCF) in the presence of ROS. Phosphate buffer saline (PBS) was used to wash the cells interacted with nanocomposites and incubated in the presence of H2DCF DA (80 μM) (37°C, 30 min). A fluorescence spectrophotometer (Elico, India) was used to record fluorescence intensity (excitation λ = 480 nm and emission λ = 530 nm). Bacterial cells without the exposure of nanocomposites were used as a control.

Enzyme assay

The antioxidant enzymes, including superoxide dimutase (SOD), glutathione reductase and catalase, were responsible for oxidative status maintenance in a cell. The enzymes were evaluated with different methods. Here, SOD was evaluated considering its ability to prevent the nitro blue tetrazolium (NBT) reduction by superoxide [18]. Enzyme activity of catalase was determined by measuring disappearance of exogenous H_2O_2 by using an UV-visible spectrophotometer [19]. The glutathione reductase activity was measured as described. Oxidized glutathione (500 μL, 2 mM) and cell lysate (0.1 mL) were the assay buffer contents. Then, NADPH (50 μL, 2 mM) was augmented and A340 was measured for 2 min. The reduced glutathione (GSH) oxidation by 5,5-dithiobis (2-nitrobenzoic acid) (DTNB), to form 5-thio (2-nitrobenzoicacid), a yellow by-product, is used to measure GSH by recording absorbance at 412 nm by the method described by Moron and Kepeierre [20].

Figure S1. Half-life period of tetracycline by (a) Fe_3O_4 and (b) CeO_2.