A simple and low-cost purification method for microbial-free water using zinc oxide nanoparticles

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Abstract. Availability of standard quality drinking water is very essential for all living-beings. Availability of drinkable water has become a biggest challenge for the whole world. Water is a vital necessity for living-beings. Contamination of water has caused the millions of deaths in every year. Therefore, it is dire need to develop and explore low cost and simple methods to obtain quality drinking water. Zinc oxide (ZnO) is good reactive agent that had used to immobilize the toxic substance and destroyed the virus from contaminated water. Zinc oxide nanoparticles (ZnO-NPs) were prepared by using zinc sulfate heptahydrate (ZnSO₄·7H₂O) and sodium hydroxide (NaOH). As prepared zinc oxide nanoparticles (ZnO-NPs) were characterized by using different techniques such as X-ray diffraction (XRD), scanning electron microscopy (SEM) and UV-Vis spectroscopy. From XRD results, average crystallite size of prepared ZnO-NPs was calculated using Scherrer’s equation and found to be 29 nm. SEM analysis showed that as prepared NPs has mixed morphology having hexagon and rod-like shapes. UV-Vis analysis showed that maximum absorbance range between 250 nm to 350 nm. After the characterization, zinc oxide nanoparticles (ZnO-NPs) were employed for purification of water. The treated water was then investigated by finding electrical conductivity (EC), analysis of pH, total dissolved solids (TDS), atomic absorption spectroscopy (AAS) for the study of heavy metals and colony forming units (CFU) for microbial count such as bacteria, virus and protozoa etc. It was found that water treated with ZnO killed 100 % of microorganism as compared to water without treatment of ZnO (63%).

1. Introduction

Sustainable Pure water is essential for all living-beings. In developing countries, purified water has become a major problem. Water is a vital necessity for every life. Water has been used in daily routines work involving in cooking, drinking, washing, cleaning and agriculture [1]. Pakistan is a semi-parched area, and here the resources of purified water are shorted. In difficult situation, all living-beings have affected from lake of water, whose health has depended on pure water. Unhygienic water can cause infectious diseases as cholera, jaundice, paratyphoid fever, typhoid, dysentery, amoebiasis, and malaria [2]. Water pollution also caused by industrialization and environmental pollution. Residual dyes have a strong role played to contaminate water [3]. Dyes have produced by different sources as textile industries, pharmaceutical industries, paper and pulp industries, dye and dye intermediate industries and craft bleaching industries, etc. Available water distillation apparatus was in poor condition. High cost required to distill the water. Low cost apparatus was caused to contaminate the harmful substances, toxicity and pollution in water [4]. Therefore, many researchers have adopted
different approaches including photocatalysis [Titanium dioxide (TiO$_2$), Zinc oxide (ZnO), Iron (III) oxide (Fe$_2$O$_3$), zirconia (ZrO$_2$), vanadium (V) oxide (V$_2$O$_5$), niobium pentoxide (Nb$_2$O$_5$) and tungsten trioxide (WO$_3$)] to purify water with efficiently. Among these, zinc oxide (ZnO) is most efficient and friendly candidate in environment to reduce the severe problem of water pollution because ZnO has unique properties such as strong oxidation, wide band gap (3.37 eV) near-UV spectral region, highly photocatalytic [5]. Zinc oxide nanoparticles (ZnO-NPs) are also well-known to destroy germs from water to make it drinkable water [6]. Bacteria are composed with cell membrane, cell wall and cytoplasm. The cell membrane occupies inside the cell wall. Cell wall has characterized by a homogeneous peptidoglycan layer. The function of cell wall organizes the osmotic-pressure of the cytoplasm and controls cell shape [7]. There are two types of bacteria namely Gram-positive bacteria and Gram-negative bacteria. The Gram-positive bacteria has consisted of one cytoplasmic membrane (have multilayers of peptidoglycan polymer) and cell wall which having thickness about 20 nm to 80 nm. Meanwhile, the Gram negative bacteria have composed with two cell membranes and plasma membrane which has consisted of thin layer of peptidoglycan and thickness about 7nm to 8nm. ZnO-NPs have comparable size with peptidoglycan, means that ZnO-NPs can be passed easily through the peptidoglycan to destroy the bacteria. Cytoplasm has consisted on fluid like as jelly, which has filled in all components of the cell excluding nucleus. Organisms have functions included the growth, reproduction, anabolism and metabolism [8]. Cytoplasm has contained amino acids, ion, salts, proteins, starches and water about 80%. Composition of cytoplasm leads to good electrical conductivity. Bacterial-cell wall has contained negative charge. Typically, bacterial cell structure has been shown in figure 1.

![Bacterial cell structure](image)

**Figure 1.** Bacterial cell structure.

There are several mechanisms for killing bacteria by using ZnO-NPs were conducted in previous studies. One of these mechanisms involved the direct contact of ZnO-NPs with cell walls, Reactive Oxygen Species (ROS) formation, liberation of antimicrobial ions mainly Zn, resulting in destruction of bacterial cell integrity. The property of anti-bacterial activity of ZnO-NPs has distinct for different medium such as if zinc (Zn) has reacted with species, which may cause to change toxicity mechanisms corresponding to the physico-chemical characteristics of ZnO-NPs [9].

ZnO possessed highly absorption factor to the ultra-visible light (UV). Highly absorption factor has been caused to increase conductivity dramatically, which leads to enhance the efficiency of ZnO to interact with bacteria. The morphology of ZnO has most efficient response to inactivate the microorganisms. Rod shaped nanoparticles can be easily penetrating the bacterial cell wall and distort the peptidoglycan layer than the spherical shaped nanoparticles [10]. Zinc oxide has strong Annealing property Oxygen annealing (increase the oxygen atoms on the surface of ZnO) has been great effect to increase the antibacterial efficiency involving reactive oxygen spices (ROS), which provides an intensive strength for prohibit the bacteria. The size of particle has been much smaller then it provides
efficient results to kill the bacteria, due to small particles have large surface area. Minimum Inhibitory Concentration (MIC) has been used to inhibit the bacterial growth and within perform of colony forming unit (CFU). Surface defect of ZnO has many corners for potential contact surface’s sites [11]. In this research work, ZnO nanostructures were prepared by co-precipitation route and these as prepared samples were employed to contaminated water to get microbial-free drinking water. XRD, SEM, UV-vis spectroscopy were used to characterize as prepared ZnO-NPs whereas water analysis was done by using different techniques such as electrical conductivity, total dissolved solvent (TDS) and colony forming unit (CFU).

2. Experimental process

2.1. Chemicals and synthesis
All chemicals used throughout the research were of analytical regent grade and purchased from Sigma-Aldrich. For the preparation of zinc oxide nanoparticles schematic diagram is shown in figure 2. Zinc sulfate heptahydrate (ZnSO$_4$.7H$_2$O) and sodium hydroxide (NaOH) were used in this experiment. In order to aqueous solution of zinc sulfate, sodium hydroxide solution in a molar ratio of 1:2 was added slowly drop wise with vigorous stirring and the stirring was continued for 12 hours [12]. The obtained precipitate was filtered and washed three times thoroughly with deionized water. The precipitate was let to completely filtered and dried in an oven at 100 °C. The dried sample was grinded with agate pestle and mortar to obtain fine powder. The obtained powder was calcined at temperature 700 °C for 2 hours by using the muffle furnace. The as prepared ZnO-NPs were characterized using XRD, SEM and UV-vis spectroscopy. These prepared samples then employed to treat water for its purification. In this experiment the three samples were collected from different places such as home, field and factory. Sample (1, 2, 3) indicated uncoated and without sunlight samples. Sample (A, B, C) indicated uncoated and under sunlight samples and sample (A’, B’, C’) was indicated coated and under sunlight. ZnO-NPs were prepared by co-precipitation and water purified method that was shown in figure 2.

![Figure 2. Co-precipitation method for synthesis of ZnO nanoparticles.](image-url)
3. Characterization

The prepared samples were characterized by using different techniques such as X-ray diffraction (XRD), Scanning electron microscopy (SEM) and UV-vis spectroscopy for their structural, morphological and optical properties respectively. XRD was performed through XRD system Model (PANalytical) with CuKα (λ=1.54 Å) radiation. The particle size was calculated from broadening of line by using Scherrer’s formula as given by equation (1).

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

(1)

The values of lattice parameters for all the samples were calculated by using equation (2).

\[
\frac{1}{d^2} = \frac{4}{3} \left( \frac{h^2 + hk + k^2}{a^2} \right) + \frac{l^2}{c^2}
\]  

(2)

The volume of unit cell was calculated by using equation (3).

\[
V_{cell} = a^2c \sin 60
\]  

(3)

The density of prepared sample was calculated by using equation (4).

\[
D = \frac{ZM}{N_A V_{cell}}
\]  

(4)

Where \( D \) is the crystal size, \( \beta \) is the line width (FWHM), \( \theta \) is the diffraction angle and the \( \lambda \) is the wavelength of X-ray radiation. \( Z \) is the number of molecules per unit cell, \( M \) is the molar mass of the Zinc Oxide, \( N_A \) is Avogadro number, \( V_{cell} \) is the volume of the unit cell. The scanning electron microscope (SEM) studies were performed by using SEM Model (JEOL- JSM 5910) to study the morphology of the prepared nanoparticles. UV-vis spectroscopy was performed with UV-Vis spectrophotometer of Model (PG- T80).

4. Results and discussion

4.1. Structural analysis

4.1.1. XRD Analysis. The XRD pattern of ZnO nanoparticles prepared at 700 °C. Diffraction peaks has shown of ZnO in figure 4 that provides information particle size, volume and density. The diffraction peaks are good crystalline nature of ZnO, which will be seen in figure 3. The shown XRD pattern confirms the formation of nanoparticles. The ZnO nanoparticles are observed to be highly crystalline. Peaks at 2θ of 31.80°, 32.66°, 34.47°, 36.28°, 47.58°, 56.64°, 62.93°, 68.00°, were referred to XRD planes (100), (002), (101), (102), (110), (103), (200) and (112), that shown in table 1. The XRD results showed that zinc oxide has the hexagonal wurtzite type structure. The average crystallite size of prepared ZnO nanoparticles was calculated by using Scherrer’s relation and found to be 29 nm. The values of lattice parameters for all the samples were calculated by using equation 2 and found to be in accordance with the standard values. These parameters are matching with the reported values (JCPDS file No. 36-1451).The volume of unit cell was calculated is 47.604 (Å)³, by using equation 3. There is no significant effect of annealing temperature on the volume of unit cell. The X-ray density (\( D \)) of prepared sample was found to be 5.0 g/cm³ that was calculated by using equation (4).
4.1.2 SEM analysis. SEM micrographs of the synthesized zinc oxide nanoparticles is shown in figure 4. It appears that the particles were agglomerated, clusters and hexagon along with some rod like nanostructures.

![SEM micrograph of ZnO-NPs.](image)

Figure 4. SEM micrograph of ZnO-NPs.

4.1.3 UV-Vis Spectroscopy. UV-Visible spectrometer is worked on the principle of Beer-lambert law. The concentration of ZnO (0.01 g) was dissolved in ethanol (30 ml). The UV-Visible transition of ZnO had maximum wavelength in range between 250 nm to 350 nm, which had shown in figure 5. UV-vis spectrum depends on different parameters such as method of synthesis, particle size and shape etc. This result has indicated that ZnO nanostructure had excellent UV-absorbing capability [13].
4.2. Water analysis

In order to employ ZnO to kill bacteria, different samples of water from three different places were collected and gave those different named such as sample 1, sample 2 and sample 3. All the water samples were packed in three different containers and tested in laboratory. Different tests were conducted including pH, electrical conductivity (EC), total dissolved solids (TDS) and colony forming units (CFU) for microbial count such as bacteria, virus and protozoa etc. Again three water samples (sample A, sample B and sample C) had taken from the same place where they had already collected. All water samples (sample A, sample B and sample C) had placed under the sunlight for 6 hours daily, for four days (24 hours) and tested. Three containers had coated by zinc oxide nanoparticles. Again, water samples (sample A', sample B' and sample C') had taken at the same place from where they had already collected. Sample A', sample B' and sample C' had put into the coated three containers. Finally, all ZnO-NPs coated water samples had placed under the sunlight for 6 hours daily, for four days (24 hours). Therefore, water had purified by using ZnO nanoparticles.

Overall results of all three samples, which had collected from home, field and factory, and three uncoated samples had represented by sample 1, sample 2 and sample 3, respectively. Under sunlight, the samples were named as sample A, sample B and sample C. Coated sample with ZnO-NP’s had indicated by sample A', sample B' and sample C'. From table 1, which had clearly conducted the results that coated water’s sample had more efficient results than uncoated and under sunlight’s samples. Comparison of all results of samples are shown in table 1.

| Sources | Sample name | pH   | Electrical conductivity (µS/cm) | Total dissolved solvents (meq/L) | Colony forming units (CFu/ml) |
|---------|-------------|------|--------------------------------|----------------------------------|-------------------------------|
| Home    | Sample 1    | 6.96 | 1207                           | 12.07                            | 2.80E+08                      |
|         | Sample A    | 6.3  | 990                            | 9.9                              | 2.50E+07                      |
|         | Sample A'   | 6.68 | 917                            | 9.17                             | 1.50E+07                      |
| Field   | Sample 2    | 7.14 | 1790                           | 17.9                             | 3.00E+09                      |
|         | Sample B    | 7    | 1044                           | 10.44                            | 1.76E+08                      |
|         | Sample B'   | 7.02 | 895                            | 10                               | 2.87E+07                      |
| Factory | Sample 3    | 6.84 | 5100                           | 510                              | 2.61E+08                      |
|         | Sample C    | 7.4  | 4480                           | 44.8                             | 1.20E+08                      |
|         | Sample C'   | 6.98 | 580                            | 5.8                              | 1.90E+07                      |
4.2.1. Percentage of CFU of uncoated and coated water samples under sunlight. The results of water treatment have been compared with uncoated water samples under the sunlight. The results have revealed that treated water has provided pure water. The results predicted that how many microbes were killed by using the zinc oxide nanoparticles (ZnO-NPs). The results of colony forming units (cfu/ml) killed in the coated and uncoated samples under the sunlight are given in the table 2.

Table 2. Cfu/ml killed in the uncoated and coated samples under sunlight.

| Sample name | Cfu/ml killed in the uncoated samples under sunlight | Cfu/ml killed in the coated samples under sunlight |
|-------------|-----------------------------------------------------|--------------------------------------------------|
| Home        | 1.5x10^7                                            | 2.5x10^7                                         |
| Field       | 1.76x10^5                                           | 2.87x10^7                                       |
| Factory     | 1.2x10^8                                            | 1.9x10^7                                         |

The percentage of Cfu/ml killed in the uncoated and coated samples under the sunlight is represented by in table 3.

Table 3. Percentage of cfu/ml killed in the uncoated and coated samples under sunlight.

| Sample name | Cfu/ml killed in the uncoated samples under sunlight % | Cfu/ml killed in the coated samples under sunlight % |
|-------------|--------------------------------------------------------|-----------------------------------------------------|
| Home        | 60%                                                   | 100%                                                |
| Field       | 61%                                                   | 100%                                                |
| Factory     | 63%                                                   | 100%                                                |

The Cfu/ml killed under sunlight, in the uncoated samples and coated samples of water named home, field and factory is shown in figure 6.

Figure 6. Cfu/ml killed under sunlight, in the uncoated and coated water samples.

Moreover, result was conducted that the microorganisms killed under the sunlight, in zinc oxide nanoparticles (ZnO-NPs) coated samples were 100% and in the uncoated samples were 63%. Finally, by coating zinc oxide nanoparticles (ZnO-NPs), all microorganisms were killed successfully and the water became totally pure form microorganisms, because all results are in the range of pure water shown above in the table 1.
5. Conclusion
Aim of this research was to synthesize and characterize the zinc oxide nanoparticles (ZnO-NPs) and its application for water purification. XRD technique was used to determine the crystalline size, density and volume of zinc oxide nanoparticles (ZnO-NPs) was 29 nm, 5.0 g/cm$^3$ and 47.604 (Å)$^3$, respectively. SEM technique determined that ZnO-NPs have hexagon with rod like shape. ZnO-NPs have shown highest UV-Visible absorbance range 320 nm to 380 nm, which has caused to antibacterial. Water had purified by coating zinc oxide nanoparticles (ZnO-NPs) inside the recycle plastic bottles of water and placed under the sunlight for 6 hours daily, for four days (24 hours). Finally, concluded that by coating zinc oxide nanoparticles (ZnO-NPs) under the activity of sunlight killed microbes effectively and made contaminated water as microbial-free water.

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