Facile synthesis of vanadia nanoparticles and assessment of antibacterial activity and cytotoxicity

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Vanadia (Vanadium oxide, $V_2O_5$) nanoparticles are being widely explored in a variety of fields including sensing, solar cells and batteries. In contrast to the current techniques that utilize chemical approaches for the synthesis of vanadium oxide nanoparticles, we present a facile technique wherein vanadium oxide was prepared from ammonium metavandate ($NH_4VO_3$). Sonication yielded nanoparticles of $\sim$300 nm size as confirmed by electron microscopy and dynamic light scattering. The crystalline and chemical nature of the particles was confirmed by X-ray diffraction, CNH analysis and X-ray photoelectron spectroscopy.

We assessed the antibacterial activity and cytotoxicity of these vanadia nanoparticles. Results show that vanadium oxide nanoparticles have excellent antibacterial properties against *Escherichia coli* which is attributed to the reactive oxygen species (ROS) generated within the cells. The particles, however, also showed cytotoxicity in HeLa cells with the generation of intracellular ROS. Thus, this study presents a physical approach for preparation of vanadium oxide nanoparticles and provides new insight into their potential environmental impact.

**Keywords:** Metal oxide nanoparticles, Vanadium oxide, Antibacterial activity, Cytotoxicity, Nanotoxicology

**Introduction**

The advent of nanotechnology has undoubtedly opened several exciting opportunities in various fields of science and technology. Nanoparticles are finding enhanced application in numerous industries such as medicine, polymers, energy, electronics and many other fields due to their extraordinary chemical, physical, electrical and optical properties. Recent advancement has witnessed an enhanced interest in vanadium pentoxide ($V_2O_5$) as semiconductors for gas sensing, batteries and solar cells. Drop in the resistivity of n-type $V_2O_5$ upon adsorption of organic gases like ammonia, ethanol and amine groups has been employed for the detection of such gases. Andre *et al.* in their study have shown the excellent peroxidase catalysing activity of $V_2O_5$ nanowire on $H_2O_2$ substrate. Utilizing the same peroxidase-catalysing activity, Han *et al.* have demonstrated its use in glucose detection.

Metal oxide nanoparticles have been proven to be bacteriostatic and bactericidal agents. They can be used to prepare antifouling surfaces and as suspension for waste water treatment. Among metal oxide nanoparticles, CuO, ZnO, MgO, FeO/Fe$_2$O$_3$, TiO$_2$, CeO$_2$, Al$_2$O$_3$ are widely used and thus their antibacterial mechanisms have been widely studied. Although metal nanoparticles owe their antibacterial activity to the release of soluble ions, the mechanism of metal oxide nanoparticles can differ significantly. CuO kills bacteria by generating reactive oxygen species (ROS) and damaging the genetic material. Similarly, Raghupati *et al.* have ruled out a significant role of $Zn^{2+}$ ion in the antibacterial activity of ZnO. ZnO particles apparently accumulate within the bacterial cell increasing the permeability of cells and causing disruption of the cell wall. These accumulated ZnO nanoparticles generate ROS, predominantly $H_2O_2$ which leads to cell death. The antibacterial activity of ZnO nanoparticles is enhanced in the presence of natural light and UV irradiation. Kumar *et al.* have reported the mutagenesis of bacterial cells upon interaction with the internalized nanoparticles. Al$_2$O$_3$, TiO$_2$ and MgO also show antibacterial effect through ROS generation and membrane disruption. The antibacterial activity and mechanism of these nanoparticles are also strain specific, requiring different concentration of nanoparticles for different bacterial strains. Wang *et al.* in their study have compared the antibacterial properties of nanoparticles with their respective bulk particles and have reported the enhanced toxicity of nanoparticles. The metal ions released from these nanoparticles have better solubility in the suspension causing cellular toxicity. The dissolved metal ions alter the pH and conductivity of the system; inactivating the bacteria or further killing them by enzyme inactivation. In addition, studies have shown that stability and dispersion of nanoparticles plays active role in killing bacteria. Decorating silver nanoparticles on clay platelets and carbon nanotubes (CNT) improved the stability and dispersion of silver nanoparticles resulting in enhanced antibacterial activity. Furthermore, nanohybrid systems of silver and CNT have shown significantly high antibacterial property that is attributed to the synergetic effect of CNT and silver nanoparticles.
With the widespread use of nanoparticles for various industrial and consumer application, there is rapidly growing concern regarding the potential adverse effect of nanoparticles on the environment and public health.\textsuperscript{34,35} The growing use of V$_2$O$_5$ nanoparticles could affect human health and the environment.\textsuperscript{36} Many counties have set a threshold limit for the exposure of V$_2$O$_5$ in air and water which ranges from 0.05 to 0.5 mg/m$^3$.\textsuperscript{37} However, studies have shown that the toxicity of metal oxide nanoparticles are shape-and-size-dependent.\textsuperscript{38-42} Smaller nanoparticles are more potent in penetrating the cell membrane and hence are more toxic. Although bulk V$_2$O$_5$ has been reported to be toxic and a tumour promoter,\textsuperscript{43,44} there are few reports on the toxicity of V$_2$O$_5$ nanoparticles. Vermek et al. have reported that the antioxidant nature of vanadium oxide nanowires protects cells from elevated ROS levels. Suppression of ROS-mitigated damages like protein oxidation, lipid peroxidation and DNA damages were simultaneously reported.\textsuperscript{46} On the contrary, Leon et al. have shown treatment of human osteosarcoma derived MG-63 cells with an oxidovanadium compound resulted in dose-dependent cytotoxicity.\textsuperscript{47} In another study using a mouse model, inhalation of V$_2$O$_5$ nanoparticles induced varied responses in different organs. Overall, V$_2$O$_5$ nanoparticles showed oxidative stress along with inflammation and DNA damage.\textsuperscript{48} It was suggested that V$_2$O$_5$ nanoparticles may be a possible carcinogen as further demonstrated by Rondini et al. showing tumour-inducing property of V$_2$O$_5$ particles.\textsuperscript{49,50} As limited literature is available on the biological effects of V$_2$O$_5$ nanoparticles, there is a need for continued research in this field owing to the burgeoning use of these nanoparticles.

The objective of the present study was to develop a facile synthesis route for vanadium pentoxide nanoparticles by mechanical method that is more amenable for scaling than the current chemical routes. We have studied the effect of the synthesized V$_2$O$_5$ nanoparticles on bacterial cell viability. The response of human cells to the nanoparticles was also studied.

Materials and methods

Synthesis and characterization of nanoparticles

Ammonium metavanadate (NH$_4$VO$_3$, SRL Pvt. Ltd., India) was used for the synthesis of V$_2$O$_5$ particles. The crystalline nature and morphology of NH$_4$VO$_3$ powder was characterized using X-ray diffraction (XRD, XPERTPro, PANalytical, UK) in the 2$\theta$ range of 10°–40° at a scan rate of 1°/min and scanning electron microscopy (SEM, FEI Sirion, USA). Four grams of NH$_4$VO$_3$ in a silicon crucible were heated at 400 °C for 3 h in a high-temperature furnace resulting in the formation of yellow V$_2$O$_5$ powder. Elemental analysis of bulk NH$_4$VO$_3$ and synthesized V$_2$O$_5$ powder was determined by CNH analysis (LECO TrueSpec). V$_2$O$_5$ powder was characterized through XRD in the 2$\theta$ range of 10°–40° at a scan rate of 1°/min. X-ray photoelectron spectroscopy (XPS, PHI 1257, PerkinElmer, USA) was performed on V$_2$O$_5$ powder placed on a carbon tape to analyse the elemental composition in the bulk V$_2$O$_5$ powder. SEM coupled with energy dispersive X-ray analysis (EDAX) was used for imaging and elemental analysis of the synthesized V$_2$O$_5$ particles.

As-synthesized bulk V$_2$O$_5$ powder was dispersed in distilled water by bath sonication (S.V. Scientific) for 2 h and then probe sonicated (UP400S, Hielscher) for 2 h to prepare V$_2$O$_5$ nanoparticles. The dispersed nanoparticles were further lyophilized (Labconco) to obtain the nanoparticles in dry powder form as needed. SEM and EDAX were performed to confirm the elements present in the nanoparticles. For transmission electron microscopy (TEM, Tenacity G2 F30 S-TWIN), a drop of highly diluted nanoparticle dispersion was dried onto a Cu grid and imaged to assess the size and shape of the nanoparticles. The size of particles was confirmed using dynamic light scattering (DLS, Zetasizer Nano ZS90, UK). Synthesized bulk V$_2$O$_5$ powder was first dispersed in water by bath sonication. Dispersed V$_2$O$_5$ particles size was reduced using well-established ultrasound probe sonication. V$_2$O$_5$ particles were probe sonicated for 30, 60, 90 and 120 min. At these different time intervals V$_2$O$_5$ particles size was analysed using DLS. Surface charge of the V$_2$O$_5$ particles after 120 min of sonication was evaluated using zeta potential analyzer (Zetasizer Nano ZS90, UK). The surface area of NH$_4$VO$_3$ and synthesized V2O5 nanoparticles were determined by Brunauer–Emmett–Teller (BET) method (Quantachrome). To assess the stability of the aqueous dispersion of the particles the suspensions at different concentration were left undisturbed at 25 °C for 3 days.

Antibacterial studies

Antibacterial activities of the synthesized nanoparticles were assessed by spreading Escherichia coli (ATCC 25922) bacterial cells on agar-coated plates containing suspended V$_2$O$_5$ nanoparticles. Nutrient agar (Himedia) was dissolved in distilled water containing dispersed V$_2$O$_5$ nanoparticles at 0.3, 0.5 and 1.0 mg/ml concentration, autoclaved and plated in polystyrene petri plates. About 0.1 ml of E. coli in the exponential phase (OD$_{600}$ = 0.5) was spread onto these plates and incubated for 12 h at 37 °C. The growth of colonies on these plates was compared. Three independent plates were used for each sample. Independently, the zone of inhibition was assessed to measure the antibacterial activity of the V$_2$O$_5$ nanoparticles along with that of NH$_4$VO$_3$ and doxycycline (Sigma) as controls. About 0.1 ml of E. coli was spread onto agar plates. Three milligrams of lyophilized V$_2$O$_5$ nanoparticles, NH$_4$VO$_3$ and doxycycline powders were placed at three different regions over the bacteria spread plate and incubated for 24 h at 37 °C to assess the formation of a zone of inhibition.

Intracellular ROS production by the V$_2$O$_5$ nanoparticles were determined using the 2,7–dichlorofluorescin diacetate dye (DCFH$_2$-DA, Sigma Aldrich) in accordance with the reported literature with slight modification.\textsuperscript{49,50} Bacterial cells were cultured in sterile Luria broth (LB, Himedia) treated with the dye (30 μg/ml) at 200 rpm and 37 °C in the dark. E. coli cell pellets were collected by centrifuging at 3000 rpm for 3 min. Bacterial pellets were resuspended in PBS with V$_2$O$_5$ nanoparticles at different concentration as above. The bacterial suspension was incubated for 30 min in dark. After incubation, fluorescence images of bacteria were taken using an epi-fluorescence microscope (Olympus IX-71) in the green channel. All the fluorescence images were taken at same magnification (40X) and camera exposure time (600 ms). Bacterial suspension in PBS without V$_2$O$_5$ nanoparticles was used as the control.

Cytotoxicity study

Cytotoxicity of V$_2$O$_5$ nanoparticles was tested using human HeLa cells (ATCC). 5 × 10$^4$ cells per well were seeded in a 96-well plate in complete culture medium prepared using...
Dulbecco’s modified Eagle’s medium (DMEM, Invitrogen, USA) supplemented with 10% foetal bovine serum (FBS, Invitrogen, USA) and 1% antibiotic mixture of penicillin–streptomycin (Sigma). After cells reached 70–80% confluency, culture media was replaced with media containing V\textsubscript{2}O\textsubscript{5} nanoparticles at concentration of 0.3, 0.5 and 1.0 mg/ml. Medium without V\textsubscript{2}O\textsubscript{5} nanoparticles was used as the control. DNA quantification was performed using Picogreen reagent kit (Invitrogen, USA) at days 1 and 3 after adding V\textsubscript{2}O\textsubscript{5} nanoparticles, as reported previously.\textsuperscript{50} Effect of nanoparticles on cellular morphology was studied using a bright-field microscope (Olympus 1X–71). The cytotoxicity of NH\textsubscript{4}VO\textsubscript{3} nanoparticles at concentration of 0.3, 0.5 and 1.0 mg/ml was also studied as above.

Intracellular ROS generated by the V\textsubscript{2}O\textsubscript{5} nanoparticles in the HeLa cells were analysed using the DCFH\textsubscript{2}-DA dye at days 1 and 3 after adding V\textsubscript{2}O\textsubscript{5} nanoparticles. About 0.1 ml of ROS dye was added to each well and incubated for 30 min. After incubation, HeLa cells were imaged using an epi-fluorescence microscope at 4X magnification and fixed exposure time of 200 ms.

**Statistical analysis**

Statistical differences between the samples were analysed using one-way ANOVA (analysis of variance) with Tukey’s test for multiple comparisons. Differences were considered statistically significant for \( p < 0.05 \) and indicated by symbols in the figure.

**Results and discussion**

**Synthesis and characterization of nanoparticles**

V\textsubscript{2}O\textsubscript{5} nanoparticles are typically synthesized through various chemical routes such as sol–gel techniques,\textsuperscript{51,52} solvothermal methods\textsuperscript{53,54} and hydrothermal methods.\textsuperscript{10,45} Though these techniques yield different shape- and size-specific nanoparticles, reproducibility is a significant challenge owing to the large number of reaction parameters that govern these reactions.\textsuperscript{53}

The duration of these reactions is typically more than a day and the yield is poor. Limitations of scaling of such laboratory synthesis for industrial production adds to the complexity associated with such procedures. In this study, we propose a facile route for synthesis of V\textsubscript{2}O\textsubscript{5} nanoparticles using NH\textsubscript{4}VO\textsubscript{3} as a precursor and using mechanical refinement as a strategy for the formation of the nanoparticles. In contrast to the chemical techniques, this technique involves fewer processing parameters and is substantially faster than the chemical methods. Thus, this method is amenable for scaling to produce nanoparticles industrially.

NH\textsubscript{4}VO\textsubscript{3} is one of the chief precursors for the production of V\textsubscript{2}O\textsubscript{5}. Upon roasting, NH\textsubscript{4}VO\textsubscript{3} decomposes into ammonia and vanadium pentoxide\textsuperscript{56} as shown by reaction (1).

\[
2\text{NH}_4\text{VO}_3 \leftrightarrow \text{V}_2\text{O}_5 + 2\text{NH}_3 + \text{H}_2\text{O} \quad \text{(1)}
\]

Thermal decomposition of NH\textsubscript{4}VO\textsubscript{3} to V\textsubscript{2}O\textsubscript{5} is reversible and endothermic. Brown \textit{et al.} studied the decomposition of NH\textsubscript{4}VO\textsubscript{3} in different environment.\textsuperscript{55} They demonstrated that during thermal reduction of NH\textsubscript{4}VO\textsubscript{3}, the intermediate products depend on the decomposition environment. In inert nitrogen and in oxidizing environment, the reaction proceeds with the decomposition of NH\textsubscript{4}VO\textsubscript{3} to (NH\textsubscript{4})\textsubscript{2}V\textsubscript{4}O\textsubscript{11}, further decomposes to NH\textsubscript{4}V\textsubscript{3}O\textsubscript{8}, V\textsubscript{2}O\textsubscript{5} in the temperature range of 180–210 °C and finally NH\textsubscript{4}V\textsubscript{3}O\textsubscript{8} decomposes to V\textsubscript{2}O\textsubscript{5} between 270 and 300 °C.\textsuperscript{55} In another similar study, thermal decomposition of NH\textsubscript{4}VO\textsubscript{3} proceeded in three steps to form V\textsubscript{2}O\textsubscript{5} in similar manner as proposed by Brown \textit{et al.}\textsuperscript{55,56}

Thus, the conversion of white coloured NH\textsubscript{4}VO\textsubscript{3} upon thermal decomposition at 400 °C into yellow colour V\textsubscript{2}O\textsubscript{5} (Fig. 1) indicates the formation of vanadium pentoxide.

| Compound       | Carbon (%) | Nitrogen (%) | Hydrogen (%) | Surface Area (m\textsuperscript{2}/g) |
|----------------|------------|--------------|--------------|---------------------------------------|
| NH\textsubscript{4}VO\textsubscript{3} | 0.0        | 11.9         | 3.4          | 0.92 ± 0.06                           |
| V\textsubscript{2}O\textsubscript{5} | 0.0        | 0.0          | 0.0          | 8.18 ± 0.24                           |

Table 1 Elemental composition and surface area of NH\textsubscript{4}VO\textsubscript{3} and V\textsubscript{2}O\textsubscript{5} particles determined by CNH and BET analysis

Intramolecular 

V\textsubscript{2}O\textsubscript{5} and NH\textsubscript{4}VO\textsubscript{3} nanoparticles are shown in Table 1. NH\textsubscript{4}VO\textsubscript{3} showed 11.9, 3.4 and 0.0% of N, H and C, respectively, consistent with the theoretical values. After thermal decomposition of NH\textsubscript{4}VO\textsubscript{3} to V\textsubscript{2}O\textsubscript{5}, elemental N and H groups were absent (Table 1). These results suggested complete conversion of NH\textsubscript{4}VO\textsubscript{3} to V\textsubscript{2}O\textsubscript{5} with no traces of elemental nitrogen and hydrogen groups. The XRD pattern of NH\textsubscript{4}VO\textsubscript{3} particles (Fig. 2a) can be indexed to orthorhombic crystalline structure with lattice parameter \( a = 4.9, b = 11.7 \) and \( c = 5.8 \) Å and space group of Pbcm (JCPDS 00-009-0411).\textsuperscript{57} Figure 2a inset displays SEM micrograph of crystalline NH\textsubscript{4}VO\textsubscript{3} particles. After thermal reduction of NH\textsubscript{4}VO\textsubscript{3} to V\textsubscript{2}O\textsubscript{5}, XRD pattern of V\textsubscript{2}O\textsubscript{5} (Fig. 2b) shows different pattern with respect to NH\textsubscript{4}VO\textsubscript{3}.  

1 Digital photograph of ammonium metavanadate (NH\textsubscript{4}VO\textsubscript{3}) and synthesized vanadium pentoxide (V\textsubscript{2}O\textsubscript{5})
The XPS plot showed the 2p vanadium peak at 519.4 eV and the 1s oxygen peak at 533 eV. These XPS results are in agreement with earlier reports. XPS data thus confirmed the complete conversion of NH₄VO₃ to V₂O₅. Furthermore, we evaluated the oxidation state of vanadium in the synthesized V₂O₅ using XPS spectra. Studies have shown that based on the binding energy difference between oxygen 1s and vanadium V₂p₃/₂, one can measure an average oxidation state.

XRD pattern of V₂O₅ was indexed to orthorhombic system with lattice constant a = 11.5, b = 4.3 and c = 3.5 Å and space group of Pmn2(1) (JCPDS 01–089–0611). Presence of distinct V₂O₅ XRD peaks (Fig. 2b) confirmed the formation of V₂O₅ particles. NH₄VO₃ is reported to be toxic. Thus, we used XPS analysis to further confirm the complete conversion of NH₄VO₃ to vanadium oxide. The XPS result showed no trace of the 1s nitrogen peak at 400 eV (Fig. 2c and inset).
of vanadium (V$^{V}$). For a given VO compound, the linear relation for V$^{V}$ is given by $V^{V} = 13.82 - 0.68(E_{b}(O1s) - E_{b}(V2p_{3/2}))$.[56,66] Figure 2d revealed binding energy difference of 13.6 eV between oxygen 1s and vanadium V2p$_{3/2}$. Based on this above-mentioned relationship, synthesized V$_2$O$_5$ showed V$^{V}$ ≈4.6. However, deconvolution is considered as more appropriate to evaluate oxidation states in a given compound. The deconvolution of V2p$_{3/2}$ peak of V$_2$O$_5$ (corrected based on the 284.5 eV C 1s reference) is shown in Fig. 2e. Deconvolution of V2p$_{3/2}$ showed only two peaks at 516.7 and 517.9 eV which may be attributed to V$^{V+}$ and V$^{V+}$ oxidation state of vanadium.[61] Based on the area under curve for V$^{V+}$ and V$^{V+}$, V$_2$O$_5$ showed the presence of 37% and 63% of vanadium in V$^{V+}$ and V$^{V+}$ oxidation state. The EDAX analysis of synthesized V$_2$O$_5$ particles (inset SEM micrograph, Fig. 2f) shows the presence of only oxygen and vanadium elemental peaks, whereas elemental gold (Au) peak arises due to gold sputtering. Thus, results from CNH, XRD, XPS and EDAX confirm the synthesis of V$_2$O$_5$ from NH$_4$VO$_3$.

The size of the synthesized bulk particles was reduced through sonication to nanometer range. The size of the particle as expected decreased with the increase in sonication time. XRD pattern of the sonicated V$_2$O$_5$ nanoparticles showed broadening of the peaks (Fig. 3a) in comparison to XRD peaks (Fig. 2b) of synthesized V$_2$O$_5$ bulk particles, suggesting a decrease in average particle size.[62] SEM micrograph (Fig. 3b) of the sonicated nanoparticles showed that the particles were equiaxed but were irregularly shaped. This shape may be attributed to the mechanical processing route of synthesis in contrast to chemical methods where nanorod-like structures are reported.[3,4] TEM characterization of the particle further confirmed the particle were equiaxed and were irregularly shaped (Fig. 3c). The hydrodynamic size of the particles was measured by DLS after the different time intervals of probe sonication at 30, 60, 90 and 120 min. The corresponding mean size was determined to be 429, 378, 319 and 285 nm, respectively (Fig. 4a). With increasing sonication time, the size of the particles progressively decreased. DLS results showed particles size reduced as the sonication time increased. The surface charge of nanoparticles plays an important role in determining the stability of the dispersion and also in the cellular binding and uptake events. The zeta potential was measured to determine the surface charge. Figure 4b shows that the surface of the V$_2$O$_5$ particles in aqueous dispersion were negatively charged bearing a net potential of −49.2 mV. Most oxides in aqueous solution exhibit a negative charge as is observed here for the V$_2$O$_5$ nanoparticles. For all further studies, the nanoparticles prepared by 120 min sonication were used. BET surface area of NH$_4$VO$_3$ and V$_2$O$_5$ nanoparticles are listed in Table 1. Increase in surface area of V$_2$O$_5$ particles in comparison to NH$_4$VO$_3$ suggests decrease in particles size for V$_2$O$_5$. Moreover, based on BET surface area ($S_{BET}$), average particle size in microns ($D_{BET}$) can be calculated using equation $D_{BET} = 6/(S_{BET} \rho_{BET})$, where $\rho_{BET}$ is the density of particle ($\rho_{V2O5} = 3.4$ g/cm$^3$; $\rho_{NH4VO3} = 2.3$ g/cm$^3$). Based on the above equation, average particle size of V$_2$O$_5$ and NH$_4$VO$_3$ was 0.22 and 2.80 μm. Average particle size of V$_2$O$_5$ particles obtained based on BET surface area was in close correlation with particle size obtained by DLS (0.28 μm).

Figure 5 shows the photographs of dispersed V$_2$O$_5$ particles in water before and after settling. After day 1 and day 3, 0.3 mg/ml V$_2$O$_5$ suspension showed good stability without any agglomeration and settling of the particles. On the other hand, the higher concentration V$_2$O$_5$ particles (0.5 and 1 mg/ml) were observed to be partially stable. The solution maintained yellowish transparent colour suggesting dispersed V$_2$O$_5$ nanoparticles, but over time a small fraction of agglomerated particles was found settled at the bottom as indicated by the arrow (Fig. 5). Interestingly, for 1 mg/ml V$_2$O$_5$ solution, colour intensity decreased with time indicating increased particle agglomeration and settling over time. These results suggest that the amount of settled agglomerated particles increases with V$_2$O$_5$ concentration and time. Nanoparticles have high specific surface area and in order to minimize their surface energy particles tend to increase size by weakly binding with neighbouring nanoparticles. As a result, with the increase
in V2O5 nanoparticle concentration, the particles tended to agglomerate eventually causing sedimentation.

Antibacterial studies
To assess the potential use of the nanoparticles for removal of bacteria in waste water, we have studied the antibacterial effect of V2O5 nanoparticles. When bacterial cells were cultured on agar containing the nanoparticles, a marked decrease in the bacterial colonies was observed with the increase in particle concentration. Fewer E coli colonies could be seen for the 0.3 mg/ml and 0.5 mg/ml plates than the control plate with no nanoparticles. In the case of 1.0 mg/ml particles, essentially no colonies could be seen (Fig. 6a). We also conducted the zone of inhibition test for the V2O5 nanoparticles and compared with that of NH4VO3 and standard antibiotic doxycycline. The results showed a distinct zone of inhibition (in Fig. 6b shown by arrows) for all three samples. The diameter of the zone of inhibition for doxycycline, NH4VO3 and V2O5 nanoparticles was ≈32 mm, ≈19 mm and ≈13 mm, respectively. In contrast to a known antibiotic doxycycline, NH4VO3 showed 59% inhibition of bacterial growth, whereas V2O5 nanoparticles showed 40% inhibition. Higher inhibition of bacterial growth for NH4VO3 can be attributed to the synergistic effect of ammonia and vanadium. All these results demonstrate the bactericidal activity of as-synthesized V2O5 nanoparticles and the vanadium-based compound (NH4VO3).

Interaction of nanoparticles with the bacterial cell is important for the antibacterial activity. Metal oxide nanoparticles have been reported to produce ROS which kills bacteria. To determine the presence of intracellular ROS, we used the DCFH2-DA dye. DCFH2-DA is catalysed by esterase present in cells to DCDF-H2 which upon further oxidation in the presence of ROS gives green fluorescence. Figure 6c presents fluorescence micrographs of bacterial cells with intracellular ROS. As is seen from the images, E coli exposed to V2O5 nanoparticles showed more intense green fluorescence intensity in comparison to the control (without exposure to V2O5). The presence of ROS may lead to cell death as reported in case of other metal and metal oxide nanoparticles, and is a likely mechanism through which V2O5 nanoparticles kill bacteria. Natalio et al. have shown that V2O5 nanowires in the presence of bromine and H2O2 produce hypobromous acid and singlet oxygen which impart oxidative stress and kill bacteria. Few studies have also showed strong antibacterial property of vanadate (V5+) and vanayl (V4+) compounds. Interestingly,
Cytotoxicity studies

Towards assessing the environmental impact of these nanoparticles, the cytotoxicity of the suspended particles was evaluated in vitro. Bulk vanadium pentoxide is reported to be toxic to humans. Davalos et al. reported the production of ROS and nitric oxide in the endothelial cells with simultaneous change in cell morphology and apoptotic cell death caused by vanadium pentoxide particles. Some studies reported that the V2O5 promotes tumour growth. On the other hand, V2O5 nanowires are shown to act as a scavenger of ROS and shield against oxidative stress in cells. In this study, the proliferation of HeLa cells, a widely used model for cytotoxicity, when exposed to the nanoparticle was assessed. DNA content was taken as a measure of cell number. The results in Fig. 7a reveal that there were no significant differences in cell numbers after 1 day of exposure to V2O5 suspension.

Both vanadium compounds showed potent bactericidal activity even for penicillin resistance strains. Bacterial cells when treated with vanadium compound in suspension for 30 min, showed increased efflux of potassium ions from cells. These studies suggested that vanadium compounds also affect transport channels on bacterial cell membrane. Currently, the mechanism for bactericidal property of vanadium compounds is not well understood. However, few studies suggest that the oxidative stress generation and damage to the membrane transport channels by vanadium compounds are main cause for bactericidal activity.

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Micrographs (Fig. 7b) reveal that cells exposed to V2O5 nanoparticles exhibit circular morphology. Circular morphology was more pronounced for cells at higher nanoparticle concentration. After 3 days, the cell number increased markedly to more than double than that at 1 day in the control without V2O5 nanoparticles. In contrast, cell numbers in the nanoparticle-containing wells decreased from day 1 to day 3, and were significantly lower than the control at day 3. Furthermore, at day 3, HeLa cells in contact with V2O5 nanoparticles were circular in morphology suggesting cells were either under stress or non-viable due to exposure to the V2O5 nanoparticles (Fig. 7b). Interestingly after day 1, V2O5 nanoparticles in 1 mg/ml concentration started to agglomerate in media (Fig. 7b, indicated by arrow) limiting the exposure of nanoparticles to cells. As a result cells in higher V2O5 concentration may not be effectively exposed to high V2O5 particles. Thus, after day 1, HeLa cell numbers in higher V2O5 concentration showed decrease in number similar to low concentrated V2O5 solution. Studied showed both well dispersed and agglomerated TiO2 nanoparticles in media showed toxic effect on human lung epithelial cell (A549). However, for agglomerated nanoparticles, time- and concentration-dependent cellular toxicity was not observed. It was attributed to limited uptake and interaction between agglomerated nanoparticles and cells.

Similarly, our results also demonstrated that well-dispersed V2O5 nanoparticles at low concentration (0.3 mg/ml) (Fig. 5) showed similar toxicity to high concentrated (0.5 and 1 mg/ml) agglomerated V2O5 nanoparticles.

For comparison, we also studied toxicity of NH4VO3 on HeLa cells. Figure S1a showed a decrease in DNA content for HeLa cells exposed to different concentration of NH4VO3 At day 1, HeLa cells exposed to NH4VO3 showed significantly less DNA in comparison to control sample. Further from day 1 to day 3, the amount of DNA decreased significantly suggesting significant cytotoxicity of NH4VO3. Results showed that DNA content decreased significantly with increase in concentration and exposure time. Figure S1b shows micrograph of HeLa cells exposed to different NH4VO3 concentration. At day 1, in comparison to the control, cells exhibit circular morphology upon treatment with NH4VO3 solution. More pronounced round circular morphology was seen with the increase in concentration and exposure time (day 1 to day 3). NH4VO3-treated HeLa cells showed similar circular morphology to that of V2O5 nanoparticles exposed cells (Fig. 7b). It indicated that both NH4VO3 and V2O5 nanoparticles are toxic to cells. The degree of cytotoxicity of soluble NH4VO3 was more in comparison to V2O5 nanoparticles. One major cause of such a difference is that NH4VO3 is much more soluble and stable in water in comparison to the synthesized V2O5 nanoparticles. Also, reports have shown that NH4VO3 disturbs cell detoxication mechanism against oxidative stress generation similar to that of V2O5. In addition, when mice were treated with boiled NH4VO3 solution showed reduction in mortality rate compared to pristine NH4VO3 solution. It indicated presence of ammonium and pentavalent vanadium in NH4VO3 showed lethal toxicity due to synergistic effect.

Intracellular ROS accumulation upon V2O5 exposure was examined by fluorescence microscopy at day 1 and day 3. Figure 8 shows fluorescence image of HeLa cells treated with the dye to measure ROS. Cells with intracellular ROS appear bright fluorescent green. Cells incubated with V2O5 nanoparticles showed bright green fluorescence intensity suggesting intracellular production of ROS. Also, cells treated with V2O5 nanoparticles showed circular morphology as seen
by bright-field microscope images. At day 3, control cell sample show negligible amount of green fluorescence, whereas cells in contact with $V_2O_5$ nanoparticles showed bright green fluorescence.

Hence, the results from cell morphology analysis and ROS study suggest the cell could be under high oxidative stress which ultimately leads to its morphological change and ROS generation upon contact with $V_2O_5$ nanoparticles as reported by its bulk counterparts. Zhang et al. demonstrated that when human lung epithelial cell line (A549) were exposed to vanadate particles resulted in cell cycle arrest and ROS generation. On exposure to vanadate, A549 cells showed hydroxyl-free radical and hydrogen peroxide ($H_2O_2$) generation which resulted in the reduction of molecular oxygen.
Cytotoxicity test of the V$_2$O$_5$ nanoparticles on HeLa cells. a DNA quantification after one and three days of incubation with the nanoparticles at various concentration; and b Cell morphology after days 1 and 3, (scale bar = 100 μm). Statistically significant difference ($p < 0.05$) compared to control was indicated by *.
Although Capella et al. reported that vanadate ($\text{V}^{5+}$) inside cells is reduced to vanadyl ($\text{V}^{4+}$) in a process dependent on NADPH. Formed vanadyl react with $\text{H}_2\text{O}_2$ generating ROS inside the cells. Some studies have also shown that cells exposed to vanadium inhibit glycolysis enzymes, like hexokinase, affecting metabolic activity of cells.

Thus, the results here suggest that $\text{V}_2\text{O}_5$ nanoparticles are toxic and induce cell death through ROS, DNA damage and affecting metabolic activity. Similar observations have been reported for many other metal oxide nanoparticles such as $\text{ZnO}$ and $\text{TiO}_2$. Toxicity of metallic oxide nanoparticles are shown to be dependent on mainly size, shape and surface charge. Taken together, the results of this study suggest that the $\text{V}_2\text{O}_5$ nanoparticles prepared here exhibit good bactericidal activity, which could be further explored for use as coating to prepare antibiofouling surfaces and for the treatment of bacterial suspensions such as in waste water. However, given the cytotoxicity, the use for biomedical applications in vivo is not recommended. Furthermore, the final disposal of the particles is critical to minimize significant accumulation in the environment as it may pose a health hazard.

**Conclusion**

A simple technique for the synthesis of vanadium oxide nanoparticles that is more amenable to scaling in contrast to the chemical routes was developed. We demonstrate that these nanoparticles exhibit antibacterial activity. We also observed that the vanadium oxide nanoparticles induce cytotoxicity. This study provides new insight into the applications and environmental effects of vanadium oxide nanoparticles.
Supplementary material

The supplementary material for this paper is available online at http://dx.doi.org/10.1080/10667857.2016.1147130.

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