

RESEARCH ARTICLE

## Investigating the synthesis of tellurium nanoparticles and investigating its physicochemical and toxicological properties on fibroblast cells

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### ABSTRACT

In recent years, metal nanoparticles have become very important in biomedical research. Among them, tellurium nanoparticles have received less attention. However, these structures have many applications in the treatment of malignancies and infections. For this purpose, we investigated the important aspects of the synthesis of tellurium nanoparticles for biomedical knives. However, the hydrodynamic diameter of the particles was estimated to be 35 nm, while, the average size of tellurium nanoparticles was  $18.3 \pm 4.3$  nm based on the evaluation of TEM micrographs. The synthesized nanoparticles have a quasi-crystalline structure and have a broad absorption peak in the region of 550 nm. Our investigation reveals that the suitable washing procedure for synthesized metalloid nanoparticles is crucial for their biomedical properties including antioxidant activity and cytotoxic effects on mouse fibroblast cells if the synthesis of nanoparticles in the environment contains different chemicals, such an assumption can be made for other synthesized nanoparticles as well

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### INTRODUCTION

The unique features of nanoparticles, resulting from their small size and high surface-to-volume ratio due to quantum limitations, make them an attractive option for various industries. One of the most widely used categories of nanomaterials in different branches of biomedical application is metal nanoparticles [1]. This category of nanomaterials includes metals, metal oxides, and metalloids. Metal nanoparticles have various potential applications in cancer treatment and diagnosis. These tiny particles can serve as a thermotherapy agent [2], applied for radiation protection [3], increase the performance of radiotherapy [4], and phototherapy

[5], sonodynamic therapy [6], act as a carrier for anti-cancer drugs [7], or even function as an anti-cancer drug themselves. Additionally, they can modify the expression of genes in cancer cells [8]. Nanoparticles also have the potential to improve cancer diagnosis methods, including the creation of biosensors [9], and enhancing traditional imaging techniques such as X-ray [10], and magnetic field resonance imaging [11].

Tellurium is a rare element with intermediate properties between metals and nonmetals. Although it is abundant in the Earth's crust, it is rarely found in nature in its neutral form. It can exist in different oxidation states, such as -2, +4, and +6 [12]. Currently, the biological and medical properties of tellurium are not fully understood.

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Further research is needed to unlock its therapeutic potential. This element is available in the amount of 0.7 mg in the human body, compared to selenium with 13-20 mg; It is much less [13]. Two tellurium compounds with an oxidation number of IV have already shown impressive anti-corrosion properties. They inhibit IL-10, leading to the dephosphorylating of Stat3 and reduced expression of Bcl-2 [14]. The antioxidant property of tellurium nanoparticles (TeNPs) has been recently investigated and its effect on different cancerous cell lines such as human melanoma and MCF-7 cells has been evaluated [15-17].

Chitin ranks as the second most abundant natural polysaccharide, following cellulose. Chitosan, in turn, emerges as a leading natural polyaminosaccharide, derived from the N-deacetylation process of chitin [18]. Its structure consists of  $\beta$ -(1-4)-linked d-glucosamine and N-acetyl-d-glucosamine units, which are arranged randomly throughout the polymer. This unique arrangement contributes to the cationic nature of chitosan [19]. In addition, chitosan has several characteristics such as good biocompatibility biodegradability, and low sensitizing, which persuade researchers for various biomedical applications of chitosan. Chitosan has also been noted for its various biological benefits, including antioxidant, antimicrobial, and antitumor properties [20, 21].

Various synthesis strategies have been developed in recent years that couple different metal nanoparticles with chitosan as a surface coating agent, direct synthesis agent, or both. Metals such as silver, copper, or gold [22], metal oxides such as zinc, iron, and nickel [23], or semi-metals such as selenium [5] have all formed nanostructures with chitosan that have various applications in different fields. They have biomedicine. In this article, TeNPs were made with chitosan coating, and their toxic effects on healthy mouse fibroblast cells were evaluated. For the first time, we have demonstrate the importance of a suitable washing procedure for synthesized metalloids nanoparticles on their physicochemical properties and toxicological effects.

## MATERIAL AND METHODS:

### *Synthesis and characterizations*

To prepare TeNPs, the chemical reduction method was applied in an aqueous (Barnstead E-PureTM18.3 MX water) medium. For this

purpose, the chitosan (100 kDa, Merck, Germany) solution was first prepared in water (pH=5-6) (1% by weight and volume). Acetic acid (Merck, Germany) was applied to dissolve chitosan if needed. Next, an aqueous solution of potassium telluride ( $K_2TeO_3$ , 1 mM, Merck, Germany) was added to the chitosan solution and after a few minutes, 100  $\mu$ l of sodium borohydride ( $NaBH_4$ , 0.01 M, Merck, Germany) was added to the solution. Depending on the size and shape of the nanoparticles regenerated by the reducing agent, the color of the solution changes from clear to pale yellow or dark. To wash the nanoparticles in order to remove the unreacted tellurium ions outside the chitosan polymer micelles, the nanoparticles will be washed at least 5 times under a series of centrifugation and decantation processes based on our previous study [24].

The UV-Vis absorption spectrum of TeNPs is investigated with a SPEKOL 2000 double-beam UV-visible spectrophotometer (Analytik Jena, UK). To measure the surface potential or the hydrodynamic diameter of nanoparticles NANO-flex (Germany) Particle Sizer instrument was used. To evaluate the size and morphology of TeNPs, a drop of TeNPs solution was poured on the grid, and after drying, a transmission electron microscope (TEM, Zeiss LEO 906,100kV) was used. The micrographs obtained from TEM were evaluated in terms of size distribution using digital micrograph software, and their average particle size was calculated using Gaussian distribution evaluation. After drying, the synthesized TeNPs were collected on a glass slide then, the crystalline structure of the particles was analyzed with X-ray diffraction (XRD) Rigaku—Ultima IV X-ray diffractometer with CuK $\alpha$  source ( $k=1.541$  Å, 40kV, and 40 mA).

### *DPPH Radical Scavenging Analysis*

The antioxidant capacity of the TeNPs was assessed through a DPPH assay. DPPH, or 1,1-diphenyl-2-picryl-hydrazine, is a stable free radical that appears purple and changes to yellow upon neutralization. This property is leveraged to evaluate the antioxidant effectiveness of nanoparticles [24]. In the experiment, 1 ml of a 0.1 mM DPPH solution in methanol was combined with 1 ml of TeNP solution at varying concentrations (ranging from 1 to 256  $\mu$ g/ml). Absorbance readings were taken at 517 nm using an ELISA plate reader. The absorbance of the sample solutions was then used to calculate the percentage

of inhibition based on the following equation:

$$DPPH \text{ inhibition (\%)} = \frac{(\text{Ctrl. adsorbance} - \text{Sample adsorbance} *)}{\text{Ctrl. adsorbance}}$$

### Cell studies

The mouse fibroblast cell line (L929) was sourced from the Pasteur Institute in Tehran, Iran. The cells were cultured at 37°C in a 5% CO<sub>2</sub> environment, utilizing RPMI-1640 medium supplemented with 1% penicillin-streptomycin and 10% fetal bovine serum. Following three consecutive passages, the cells were equally distributed across a 96-well plate. After 24 hours, the culture medium was replaced with fresh medium containing TeNPs at specified concentrations, and the cells were then re-incubated. After another 24 hours, the cells were rinsed with phosphate-buffered saline (PBS) to remove any unbound TeNPs before proceeding to the MTT assay. The cells were subsequently treated with 100 µL of a 0.5 mg/mL MTT solution in PBS for 2-4 hours, after which the solution was replaced with 100 µL of dimethyl sulfoxide (DMSO). The absorbance in each well was then measured at a wavelength of 570 nm.

### Statistics and Software's

The data was statistically analyzed using GraphPad Prism 6 (GraphPad Software, San Diego, CA) and Origin 2015 (OriginLab Co., USA). All results are presented as mean ± SD, utilizing the specified software.

## RESULTS

Synthesis, and characterization of TeNPs with chitosan coating: TeNPs were synthesized

by chemical reduction method using sodium borohydride as a chemical reducing agent and chitosan as a surface coating agent. Depending on the amount of reducing agent applied, the absorption spectrum of the obtained nanoparticles was different, which is shown in Figure 1 for the two nanoparticles. As it is known, the nanoparticles synthesized with a lower amount of borohydride are more stable, and this nanostructure was used for subsequent evaluations.

In order to measure the surface potential or the hydrodynamic diameter of nanoparticles, TeNPs dispersed in the aqueous phase after 5 minutes of applying sonication, 3 milliliters of the nanostructure solution with the appropriate concentration was poured into the cuvette of the device, and depending on the type of test, the hydrodynamic diameter or the surface potential of the nanoparticles was studied. The hydrodynamic diameter of TeNPs was estimated (Figure 2). The hydrodynamic diameter of the particle was evaluated based on the scattering intensity, sample volume, and number of samples. Based on the scattering intensity, a diagram consisting of a broad peak with a size of 351 nm was obtained (Figure 2a).

Metal nanoparticles tend to agglomerate due to inherent flocculation and Brownian motions. For this reason, larger size peaks are also obtained in the size distribution diagram [25]. The sensitivity of the optical scattering subtraction method for size estimation, which can result in several metal nanoparticles instead of one nanoparticle, can be a factor for the presence of this broad peak in the size distribution. The evaluation of the sample size is very different in the examination of the

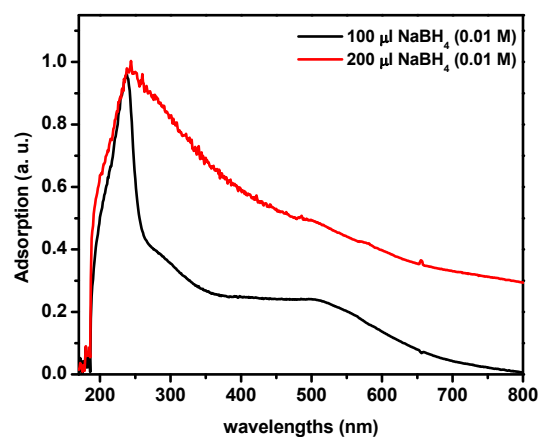


Fig. 1. Investigating the role of the amount of reducing agent used in the physical and optical properties of the synthesized tellurium nanoparticles

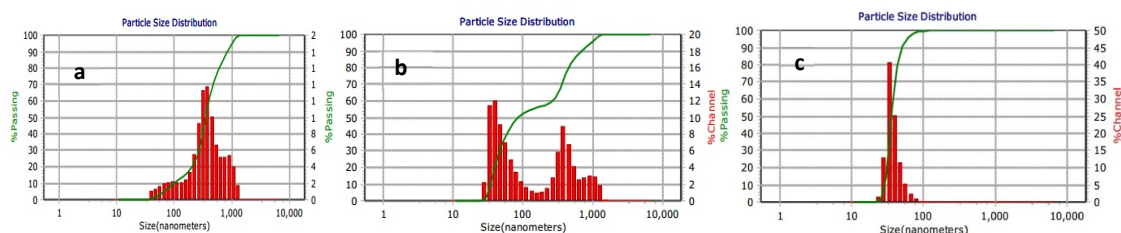


Fig. 2. Investigating the hydrodynamic diameter of tellurium nanoparticles made with lower amounts of sodium borohydride reducer. Evaluation is based on the intensity of scattering (a), based on the volume of samples (b), and based on the number of evaluated samples (c).

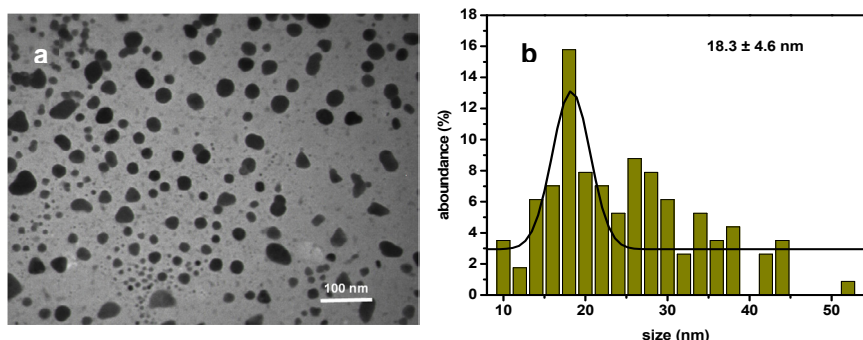


Fig. 3. TEM micrograph of synthesized chitosan-coated TeNPs and their corresponding size distribution diagram

volume and number of samples, so that in the volume diagram two peaks of 44 and 367 nm are seen and in the graph based on the number of one peak with a size of 35 nm. Therefore, it can be concluded that the hydrodynamic diameter of most of the nanoparticles is about 35 nm, which requires microscopic examination for a more accurate assessment (Figure 3).

To evaluate the exact diameter of the particles, the transmission electron microscope test was used. In the micrographs obtained from tellurium particles covered with chitosan that were reduced by sodium borohydride, there were significant points. Almost most of the nanoparticles were spherical in shape, which indicated the reduction of tellurium ions inside the spherical micelles of chitosan. Also, a small number of very fine particles were observed, which was caused by the reduction of tellurium ions in smaller amounts outside the chitosan micelle by sodium borohydride. According to this point, after the synthesis of nanoparticles, washing was done at least three times by centrifugation and decantation to remove these small particles. To calculate the average particle size, digital micrograph software was used and the data was drawn by Origin software, and the

average size obtained was calculated as  $18.3 \pm 4.6$  nm. The sample micrograph and the obtained size distribution curve are presented in Figures 4a and 4b respectively.

To assess the crystalline or amorphous nature of the nanoparticles, the X-ray diffraction (XRD) pattern was analyzed in conjunction with the JCPDS database. The most prominent peak was indexed according to card numbers 1515-47 and 2245-86, as illustrated in Figure 4.

The antioxidant properties of nanoparticles are highly dependent on their coating compounds. Proper washing can remove the remaining chemical-reducing agents in the nanoparticle solution. By examining the antioxidant properties of washed nanoparticles and comparing them with pristine nanoparticles, it was found that the washing procedure can increase the antioxidant properties of TeNPs (Figure 5).

To investigate the toxicity of nanoparticles, L929 fibroblast cells were cultured and exposed to different concentrations of TeNPs. As it is known, chitosan-coated TeNPs made by sodium borohydride have significant toxicity on fibroblast cells (Figure 6a). According to the results of the characterization of nanoparticles, especially the

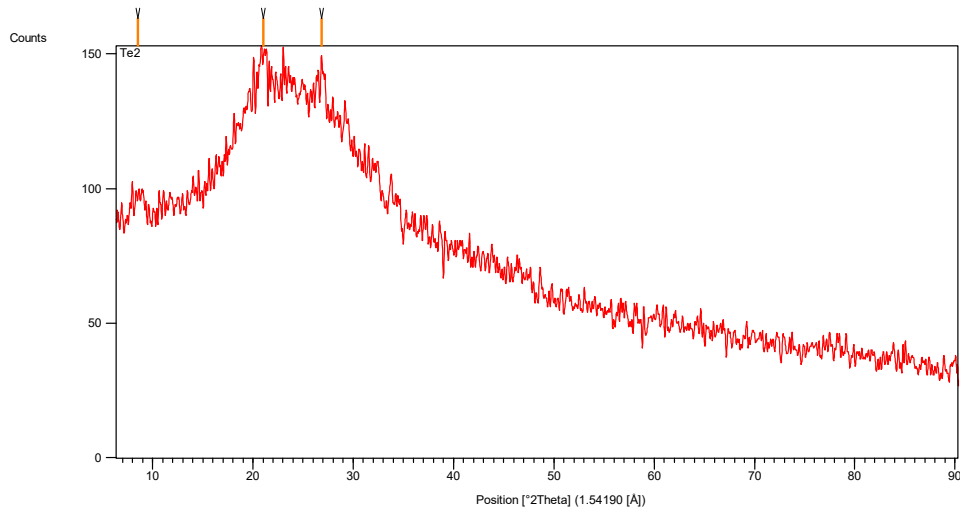


Fig. 4. Structural characteristics of chitosan-coated, NPs using X-ray diffraction (XRD).

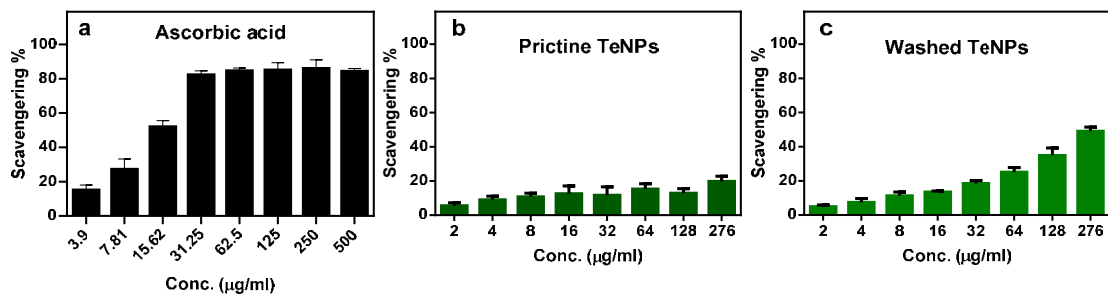


Fig. 5. Free radical scavenging evaluation of ascorbic acid as a positive control (a), pristine tellurium nanoparticles (b), and washed tellurium nanoparticles (c) using DPPH.

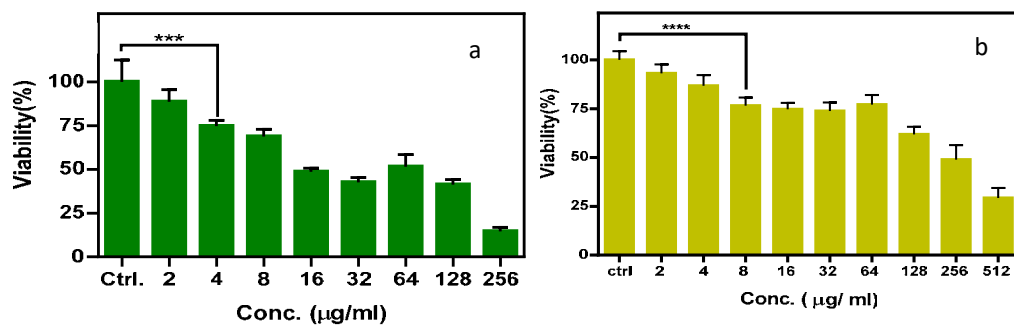


Fig. 6. Evaluation of the toxicity of tellurium nanoparticles with chitosan coating on L929 fibroblast cells by MTT method. Pristine particles (a) have significant toxicity compared to washed particles (b).

micrographs obtained from the transmission electron microscope test, the presence of very small particles in the solution of nanoparticles can be one of the important factors in causing this toxicity. Another possible cause can be sodium borohydride itself which has been used to reduce

tellurium ions, which is a highly toxic substance. Due to the presence of these two substances in the nanoparticle solution, we carefully washed the prepared particles 5 times and repeated the toxicity test, which showed a significant reduction in cytotoxicity (Figure 6b).

## DISCUSSION

This study focuses on the synthesis of TeNPs coated with chitosan and the evaluation of their toxic effects on healthy fibroblast cells. For the first time, we explored how an optimized washing process impacts the physicochemical characteristics and toxicological profile of these synthesized metalloid nanoparticles. TeNPs were synthesized using a chemical reduction method with sodium borohydride as the reducing agent and chitosan as the surface coating. The stability and properties of the nanoparticles varied based on the amount of reducing agent used, with lower borohydride concentrations producing more stable nanoparticles. Hydrodynamic diameter measurements revealed an average particle size of 35 nm, confirmed through TEM, which calculated an average size of  $18.3 \pm 4.6$  nm. XRD analysis confirmed the quasi-crystalline structure of the nanoparticles. The antioxidant properties of the nanoparticles were enhanced by removing excess unreacted materials through washing. However, initial toxicity tests on L929 fibroblast cells indicated significant toxicity, likely due to small particle presence and residual sodium borohydride. After extensive washing, cytotoxicity was significantly reduced, highlighting the importance of purification in the biocompatibility of TeNPs.

Using chemicals to synthesize metal nanoparticles is the most common and cost-effective way. Due to the high reducing power of metal ions, sodium borohydride is widely used in the synthesis of various metal nanoparticles such as gold [26], silver [27], and selenium [5]. The use of biological methods for the synthesis of metal nanoparticles is another approach that, despite many advantages such as eco-friendliness, low cost, and biocompatibility of the final product, cannot replace the usual synthesis with chemical methods [28]. Among the problems of biological methods are the low quality of synthesized nanoparticles and the slow reaction kinetics of nanoparticle synthesis [25]. In addition, the use of different washing methods to remove unreacted biological desiccant materials is necessary in many cases, just like chemical methods [8]. The presence of unreacted substances or by-products in nanoparticle solutions that have been synthesized by chemical or biological methods can cause unwanted effects such as cytotoxicity [24], particle instability [29], and so on. Also, these materials may cause false positive effects attributed to nanoparticles [27, 30].

A recent study employed a green nanotechnology approach, using extracts from citrus fruits (orange, lemon, and lime) as natural reducing and capping agents within a microwave-assisted synthesis to produce TeNPs with varied morphologies and consistent size distribution. These biosynthesized TeNPs exhibited limited cytotoxicity in human dermal fibroblasts at concentrations up to 50  $\mu\text{g}/\text{mL}$ . This eco-friendly approach diverges markedly in both methodology and applications, as these biosynthesized TeNPs showed high efficacy for antimicrobial and anticancer purposes, maintaining low toxicity in human cells without requiring additional purification. Comparison with our results highlights how different synthesis techniques and capping agents influence the functionality of TeNPs, shaping their suitability for targeted biomedical uses, whether for antibacterial, and anticancer applications or enhancing biocompatibility [15]. An alternative study employed a green synthesis route, utilizing the antioxidant gallic acid (GA) to produce spherical, monodispersed TeNPs with a hexagonal crystalline structure, averaging  $19.74 \pm 5.3$  nm. GA's dual role as both a capping and stabilizing agent in TeNP synthesis endowed the particles without affecting NIH3T3 cells or red blood cells up to concentrations of 250  $\mu\text{g}/\text{mL}$ . This comparison underscores that while our optimized washing process reduces toxicity, GA-functionalization directly improves multifunctional biological efficacy, highlighting distinct synthesis approaches in shaping TeNP safety and utility for biomedical applications [31].

Also, another study utilized lactose as a reducing agent to produce TeNPs. These TeNPs demonstrated selective toxicity favoring cancer cell lines (IC<sub>50</sub> of 7.41  $\mu\text{g}/\text{mL}$  for 4T1 cells) over non-cancerous CHO cells (IC<sub>50</sub> of 50.53  $\mu\text{g}/\text{mL}$ ). In vivo, TeNP treatment via IP and IV injections in breast cancer-bearing mice led to significant tumor inhibition and improved survival, particularly at the highest dose administered thrice weekly. This highlights TeNPs' potential in cancer treatment, while our findings underscore the importance of synthesis and purification processes for biomedical applications, especially to achieve low toxicity in healthy cells [32].

## CONCLUSION

TeNPs were made based on a chemical method with a natural chitosan coating. These nanoparticles have good spectral stability and have an average

hydrodynamic diameter of 35 nm, but their average size was about 18 nm, which is due to the difference in hydrodynamic diameter and particle size. Also, the crystal structure of the particles was shown using an X-ray diffraction spectrum. Cell studies show significant toxicity of 50% of these particles in concentrations higher than 16 micrograms/ml. It seems that this high toxicity is due to the presence of a large number of very small tellurium particles synthesized outside the chitosan micellar environment. Further, by washing the particles for several intervals, their toxicity was significantly improved.

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#### DECLARATION OF INTEREST

None

#### DATA AVAILABILITY STATEMENT

Our manuscript has no associated data.

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