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Research Article

Green Synthesis of CuO Nanoparticles Using Walnut Leaves to Remove Arsenic from Water

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A B S T R A C T

In this study, an economically viable, simple, and environmentally friendly alternative method using walnut leaves as a green source was employed for synthesis of copper oxide (CuO) nanaoparticles (NPs). The walnut leaves act as reducer and stabilizer agent in CuO nanoparticles production process. The synthesized NPs were characterized using by X-Ray Diffraction (XRD), and Transmission Electron Microscope (TEM). Based on TEM results the synthesized CuO NPs are spherical and their sizes are between 20-100 nm. Factors affecting the synthesis process of NPs such as pH, plant extract volume and cupper salt concentration were investigated and optimized. Finally, the synthesized NPs were successfully used to removal of arsenic from water samples. To measure the arsenic content in the samples, an atomic absorption spectrometer with a detection limit of 0.03 ppm was used. The final results indicate that CuO NPs removed 26–68% of arsenate and 5–89% of arsenite from the initial samples. Based on this study, green CuO NPs can be used for the removal the arsenic from drinking water. Moreover, green synthesis of these nanoparticles is an economical method and heath because the walnut leaf is available and nontoxic.

K E Y W O R D S

CuO nanoparticles, Walnut leave, Removal of Arsenic, Green chemistry.

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1. Introduction

Arsenic is an environmental pollutant and its contamination in the drinking water is considered as a serious worldwide environmental health threat. The chronic arsenic exposure is a cause of immense health distress as it accounts for the increased risk of various disorders such as cardiovascular abnormalities, diabetes mellitus, neurotoxicity, and nephrotoxicity. Moreover, few studies demonstrated the induction of carcinogenicity especially cancer of the skin, bladder, and lungs after the chronic exposure to arsenic [1]. As the permissible concentration of arsenic in drinking water is less than 0.01 mg L^{-1} [2], so it is important that use suitable methods for arsenic removal in water with more arsenic concentration. There are different methods to remove the arsenic [3-6], but applying of nanotechnology, especially nanoparticles, is one of the most effective ways. Nontoxicity and high efficiency of metal NPs, such as copper, gold, zinc, and titanium, has been notably considered [7-8]. So, there are many studies to prepare and apply the nanoparticles to different aims. Copper Oxide (CuO) nanoparticles are specified in medicine (as an antibacterial factor), biology, chemistry, etc. [9-12] and one of most applications of CuO NPs is removing of heavy metal such as arsenic from water and air [13-14]. Copper oxide nanoparticles (CuO NPs) are regularly investigated and effective adsorbent materials due to their small size, high surface area, natural abundance of starting material for synthesis, low-cost production processing and non-toxic nature [15]. Recent studies suggest that the Copper Oxide (CuO) nanoparticles effectively adsorb aqueous arsenic species under a wide range of water [16].

Pio et al. [17] combined coagulation method and adsorption (physic-chemical process) to remove the arsenic from drinking water that showed the scale of removing is 85.9% in concentration between 25-100 microgram/liter. The results show, this method is effective for the removal of total phenols, chemical oxygen demand (COD) and Escherichia coli from the water. Ding et a. [18] done another study about adsorption for As (III) and As (V) onto novel aluminum-enriched biochar derived from abandoned. Tetra Paks. Zhou et al [19] examined the adsorption kinetics of arsenate on various concentration of FeS. In this study, the adsorption kinetics followed the pseudo-second-order model and the isotherm followed by Langmuir equilibrium for different solid/solution ratios. The maximum sorption capacities were 237.45 mg g^{-1} (0.05 g L^{-1} FeS), 160.45 mg g^{-1} (0.1 g L^{-1} FeS), and 56.71 mg g^{-1} (0.2 g L^{-1} FeS), respectively. The results showed that sorption for As(V) sorption by FeS decreased with increased in solid/solution ratios. Their results showed that As(V) was reduced to As(III) or lower valences in As(V) sorption experiments. Hu et al [20] studied adsorption behavior and mechanism of different arsenic species on mesoporous $MnFe_2O_4$ magnetic nanoparticles. The obtained $MnFe_2O_4$ with large surface area and abundant hydroxyl functional groups exhibited excellent adsorption performance for As(V) and the adsorption rate was 59.45 mg g^{-1}. Also, the adsorption mechanism of different arsenic species onto $MnFe_2O_4$ nanoparticles at various pH values could be explained by surface complexation and molecular structural variations. Reddy et al [16] in a study reported a novel arsenic removal process for water using cupric oxide nanoparticles. In this study the CuO nanoparticles adsorbed with arsenic were

regenerated with a sodium hydroxide (NaOH) solution and tested again in the flow-through reactor. Three natural groundwater samples with above 0.01 mg/L of arsenic were also tested with the flow-through reactor. The results showed the arsenic adsorption process by CuO nanoparticles was kinetically rapid and followed the pseudo-second-order rate and the regenerated CuO nanoparticles were also effective in filtering arsenic from groundwater. Pezeshki et al. in a study [21], investigated the removal of arsenic using two membrane processes-nanofiltration (NF) and reverse osmosis (RO). In this study, a nanofiltration for eliminating As^{3+} and As^{5+} species by nanofiltration were as follows: concentration 20–1000 μg L^{-1}, temperature 20–37 °C, pressure 1.38–8.2 bar, pH 2–11 and concentration 20–1000 μg L^{-1}, temperature 10–40 °C, pressure 1.38–12 bar and pH 1.2–11. While the reverse osmosis operating conditions for As^{3+} are 50–750 μg L^{-1}, temperature 8–21 °C, pressure 3.1–35 bar, and pH 2–10, the working conditions for As^{5+} are 50–750 μg L^{-1}, temperature 20 °C, pressure 10–35 bar, and pH was 4.1–9. It was found that increasing the pH considerably increased the performance of the membranes in all circumstances. Rahidul Hasan in a review in 2023 provides a thorough explanation of the various well-established and cutting-edge technologies, including ion exchanges, adsorption, membrane process, electrokinetic processes, electrocoagulation, precipitation, phytoremediation, inexpensive useful methods, emerging remedial techniques, nanoparticles and nano based adsorbents that can be used to remove arsenic impurities from the drinking water. He showed that nanotechnology will be essential in supplying clean, inexpensive water to suit human needs [22].

In the current work, the impact of potential of CuO NPs synthesis by plant extract was evaluated in the removal of two different kinds of arsenic (arsenate and arsenite) in the drinking water.

2. Methods and experiments

Cupper chloride ($CuCl_2.2H_2O$), sodium arsenate ($Na_2HAsO_4.7H_2O$) and sodium arsenite ($NaAsO_2$) were purchased from Merck Company. The walnut leaves were collected from the trees in the mountainous region and then washed with distilled water and dried at room temperature away from light. The Transmission Electron Microscope (TEM) (Zeiss-EM10C-100 KV), was applied to determine the size and the shape of NPs and Atomic Absorption Spectroscopy (AAS) (model: SauantAA, GBC) to measure arsenic concentration.

2.1. Preparation of Arsenic Solutions

At first, arsenite (As(III)) and arsenate (As(IV)) solutions prepared in two different concentrations (50 and 100 μg L^{-1}). For preparation the arsenite solution, 50 μg of arsenite stock solution was dissolved in 100 mL of double distilled water and then concentrations of 50 and 100 μg L^{-1} were prepared. About arsenate, 2 mg of sodium arsenate was added to 100 mL of deionized water to prepare two different concentrations (50 and 100 μg L^{-1}).

2.2. Preparation of Extract

For extraction from walnut leaves, the leaves were washed, dried and milled in a dark room. Then, 10 gr of prepared powder was dissolved in 100 mL of deionized water. The solution was stirred by a shaker at 37°C for 30 minutes and filtered by using Whatman filter paper No. 42. Finally, to remove the suspended particle, the

extract was put in centrifuge with 1000 revolutions per min for 30 min. The extract was prepared and kept at a temperature of 4°C.

2.3. Synthesis of CuO NPs

In order to synthesis of CuO NPs, 10 mM concentration of $CuCl_2.2H_2O$ was prepared, and 4 mL of it was added to 2 mL extract of walnut at ambient temperature and the pH of the solution was adjusted at pH= 8. After a few minutes the color of the solution was changed from blue to brown, depicting synthesis CuO NPs [23].

2.4. Optimization of conditions for producing CuO NPs with the high quality

To prepared high-quality CuO NPs, some factors were altered such as pH, extract volume and copper salt concentration.

2.5. pH effect

Four solutions containing of 2 mL walnut leaf extract and 4 mL copper chloride solution (10 mM) with different pH (4, 6, 8, 10) were prepared. The pH setting was done by 0.1 M HCl and 0.1 M NaOH and then the spectrum of these solutions were recorded using a spectrophotometer (Multi-Purpose Analysis/MPANM96).

2.6. Extract volume and copper chloride concentration effect

To achieve the optimum condition for extract volume, 5 solutions containing 2, 4, 6, 8, 10 mL of extract volume were prepared, respectively. The spectrum of all mentioned solutions were compared to select the optimum extract volume.

In addition, different concentrations of copper salt (6, 8, 10, 12 mM) were examined to gain the largest amount of CuO NPs.

2.7. The surveying of CuO NPs effect in arsenic removal

To assay of CuO NPs effect for removal of arsenic specices, sodium arsenite ($NaAsO_2$) and sodium arsenate ($Na_2HAsO_4.7H_2O$) solutions with the concentrations of 50 and 100 $\mu g\ L^{-1}$, were prepared. Then, different volumes (10, 50 and 100 µL) of synthesized nanoparticles (concentration of 2.5 g/L) were added to the arsenite and arsenate solutions in polyproline tubes. In the next step, the prepared mixtures put in a dark room for two days in order to more effective reaction between CuO NPs and arsenic ions. Finally, the solutions were centrifuged with 1000 revolutions per min for 30 minutes to separate the absorbed arsenic ions on the nanoparticles from the solution. Finally, adsorbed and residual (non- adsorbed) arsenic ions were determined using atomic absorption spectroscopy.

3. Results and discussion

3.1. Structural analysis of synthesized NPs

Copper Oxide NPs were synthesized well using 4 mL of 10 mM $CuCl_2.2H_2O$ salt and 2 mL walnut leaf extract in 70ºC. The extract of walnut leaf was added as a reducing agent. The surveying of synthesized NPs spectrum by UV-Vis spectroscopy at 200-700 nm wavelength showed a broad peak at 220-230 nm (Figure 1).

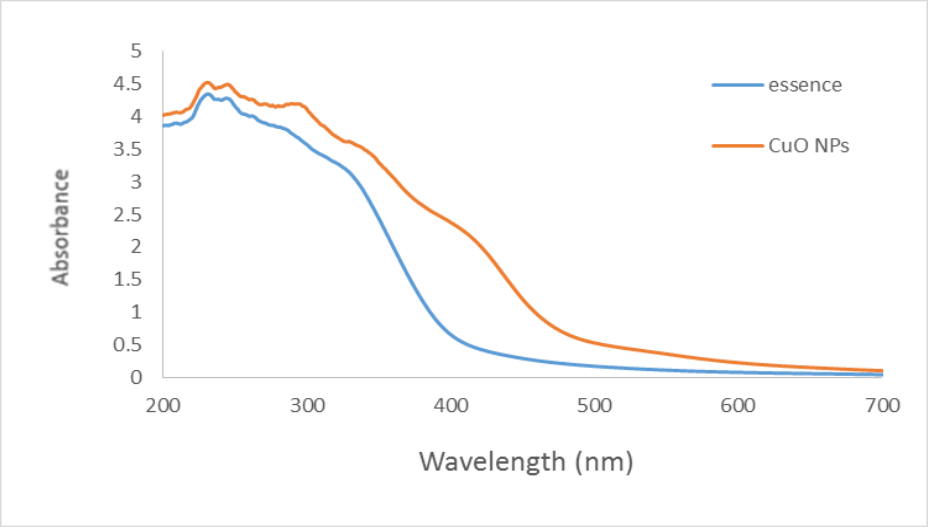


Figure 1. UV- Vis absorption spectroscopy NPs and walnut green shell extract without optimum conditions

3.2. Results of optimum pH

As the mentioned, we tested the experiments in four different pH (4, 6, 8, 10) and the spectrum (Figure 2) indicated that pH=6 solution has maximum concentration. Therefore, the pH = 6 was chosen as the optimum value and we continued the subsequent experiments with this optimal pH.

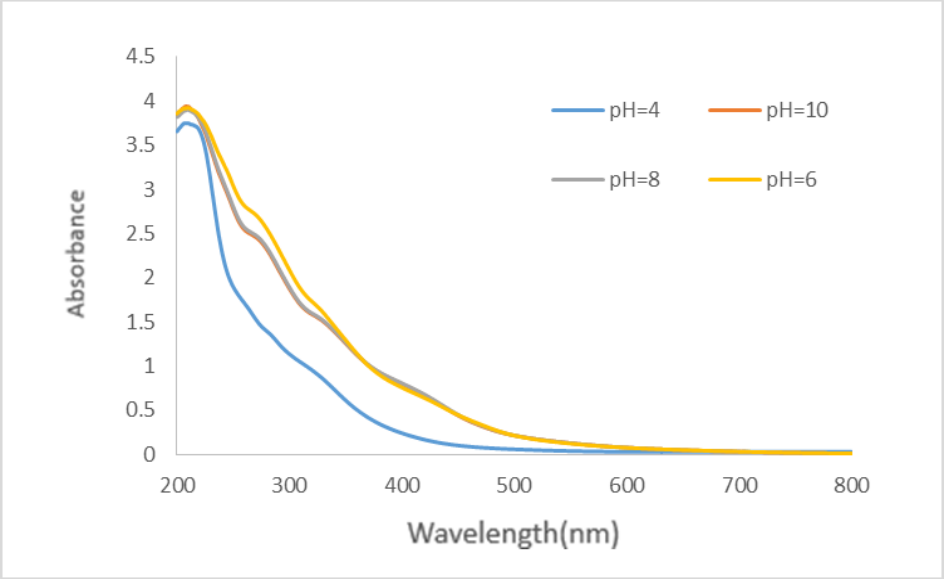


Figure 2. UV-Vis absorption spectroscopy NPs in different pH

3.3. Results of optimum essence volume

In the next step, we varied the volume of the extract while maintaining the optimal pH and a constant concentration of copper salt (10 mM). The comparison of absorption spectra of solutions containing different extract volume was performed and it was found that the highest number of nanoparticles was produced in the presence of 6 ml of extract. So, 6 mL of extract was selected as optimum volume of the extract for the next experiments (Fig. 3).

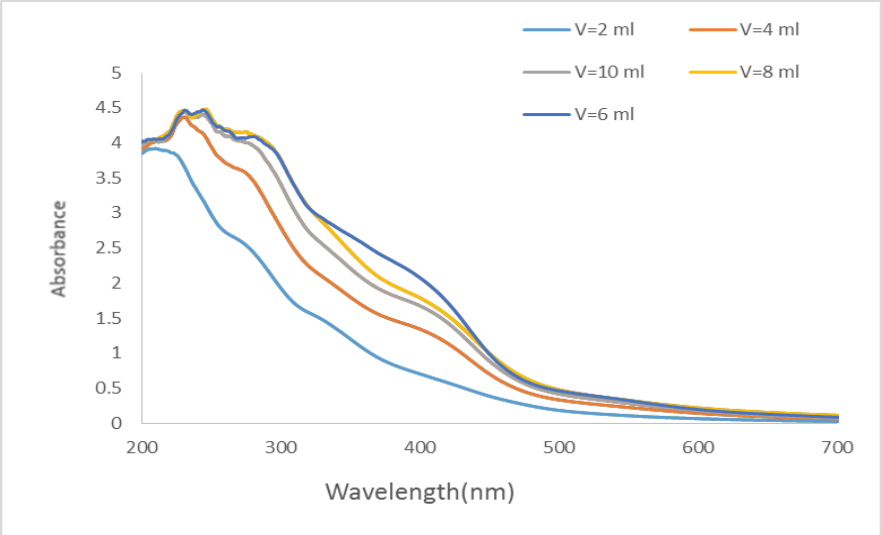


Fig 3. UV-Vis absorption spectroscopy NPs in various volumes of extract

3.4. Nanoparticle concentration effect

Four different concentrations of copper salt (in optimum pH and optimum essence volume) were prepared and their effect was studied. The highest absorption is associated with copper salt with 10 mM concentration (Fig. 4). Therefore, 10 mM was used in the next experiments.

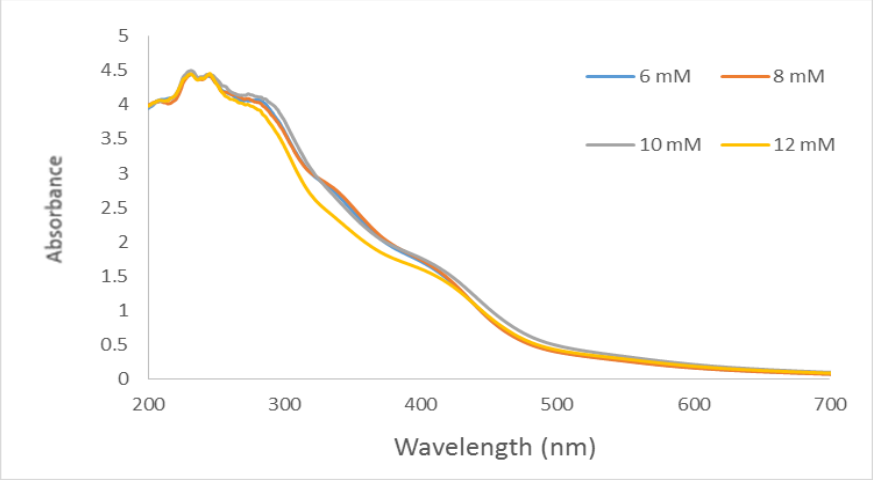


Fig 4. UV-Vis absorption spectroscopy NPs in various copper salt concentration

3.5. Characterization of the CuO NPs by TEM

In order to identify the phase of crystalline NPs and for provision of information on unit cell dimensions, x-ray diffraction ((X-ray powder diffraction) XRD (Shimadzu; Vietnam Tube: Cu (Kα = 1.54 Å) X'Pert Pro MPD (PANalytical)) was applied (Figure 5). Also, TEM test (Zeiss, EM10C, 100 KV). The result confirmed the formation of NPs with the size of between 20-100 nm (Picture 1).

The crystalline structure and phase purity of the synthesized copper oxide nanoparticles were investigated using X-ray diffraction (XRD). The raw XRD pattern exhibited a significant broad background, primarily attributed to the presence of residual organic matrices and biomolecules from the green

synthesis process (and/or the oil-based medium). To accurately identify the inorganic phase, an asymmetric least squares baseline correction was applied to the initial diagram. After rigorous baseline subtraction, the crystalline nature of the sample was clearly revealed. The diffraction peaks observed at approximately 2θ=36.27° and 38.61° are consistent with the characteristic reflections of CuO. The emergence of these peaks, despite the high amorphous background, confirms the successful formation of copper oxide nanocrystals through the green reductive pathway.

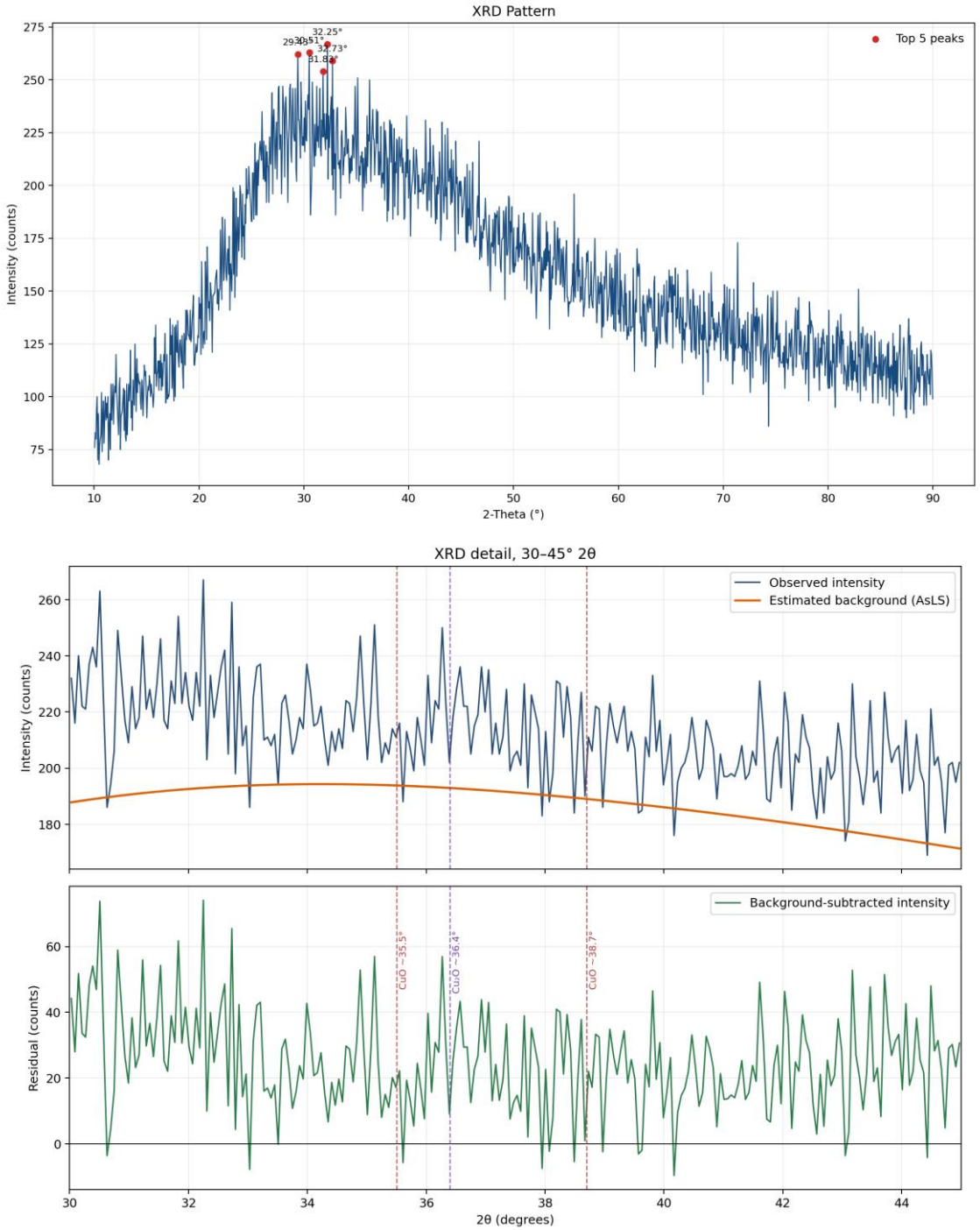
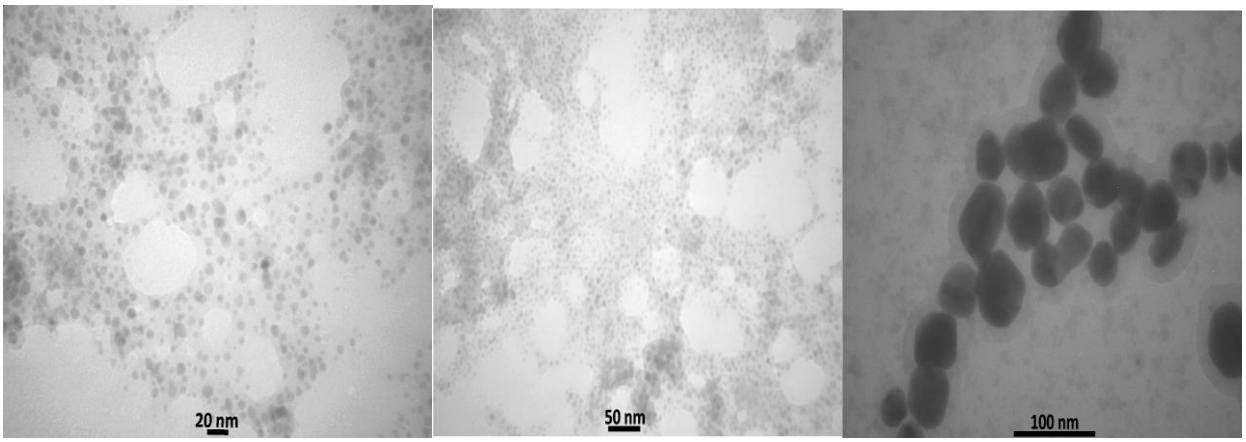


Figure 5. XRD result of synthesized NPs



Picture 1. The CuO spherical NPs pictures by TEM in different sizes

3.6. The AAS result for determining the residual arsenic in water samples

After two days, the samples were analyzed by Atomic Absorption Spectroscopy (AAS) to assay the residual and adsorbed arsenic ions. For this purpose, the samples were diluted using equal proportions, due to the relatively dark color of the nanoparticles. Subsequently, the concentration of the samples was measured using the instrument at a wavelength of 215 nm, with a detection limit of 0.03 ppm. The results show that CuO nanoparticles have effective role to delete the arsenic ions (arsenate and arsenite) but with different process. Given that the concentrations of arsenic salts in the initial solutions are known (50 and 100 ppb), the arsenic concentration measured by the instrument is considered to represent the amount of arsenic not adsorbed by the nanoparticles. Conversely, the difference between this value and the initial concentration of arsenate and arsenite salts corresponds to the amount removed As by the nanoparticles.

About sodium arsenate, at the lower volume of CuO nanoparticle (10 µL), amount of arsenic absorption by nanoparticles has been higher but sodium arsenite is more absorbed by nanoparticles with larger volume (100 µL). Therefore, it can be concluded that the removal of arsenic by CuO nanoparticles should be more favorable at a suitable volume of used nanoparticles but with same concentration. The results of AAS have been collected in Table 1.

Table 1. The adsorbed and residual arsenic concentration

Sample	The Cons. Made of As (ppb)	AAS result (Cons. Of As) (ppb)	Mean Abs.	Cons. Of Removed As by NP (ppb)	Arsenic Removal Percentage (%)
Sodium arsenate	50	50.531	0.2418	-	-
Sodium Arsenate +NP(10µL)	-	29.330	0.0563	21.201	41.95
Sodium Arsenate +NP(50µL)	-	34.946	0.0667	15.585	30.84
Sodium Arsenate +NP(100µL)	-	37.168	0.0708	13.363	26.44

Sample	The Cons. Made of As (ppb)	AAS result (Cons. Of As) (ppb)	Mean Abs.	Cons. Of Removed As by NP (ppb)	Arsenic Removal Percentage (%)
Sodium arsenate	100	98.896	0.1501	-	-
Sodium Arsenate +NP(10µL)	-	30.832	0.0849	68.064	68.82
Sodium Arsenate +NP(50µL)	-	44.855	0.0591	54.041	54.64
Sodium Arsenate +NP(100µL)	-	48.212	0.0910	50.684	51.25
Sodium Arsenite	50	51.076	0.1051	-	-
Sodium Arsenite +NP(10µL)	-	48.108	0.1016	2.968	5.81
Sodium Arsenite +NP(50µL)	-	34.441	0.0768	16.635	32.57
Sodium Arsenite +NP(100µL)	-	5.420	0.0106	45.656	89.39
Sodium Arsenite	100	99.085	0.1622	-	-
Sodium Arsenite +NP(10µL)	-	88.298	0.1940	10.787	10.89
Sodium Arsenite +NP(50µL)	-	70.032	0.1296	29.053	29.32
Sodium Arsenite +NP(100µL)	-	15.351	0.0298	83.734	84.507

Max error: 2.5733, R^2: 0.9970, R: 0.9985

4. Discussion:

Arsenic is one of the most dangerous factors in the water that caused various illness like cancer. Unfortunately, the presence of these metals is caused gastric cancer and digestive problems. In spite of many efforts for access to health water, but reports reveal because of the factories and industrial activities, heavy metals including arsenic are inseparable part in the water. One of the most important methods to remove the heavy metals are nanoparticles. On the other hand, the studies show [14, 24-25] the compounds extracted from plants and nanomaterials that extracted by the plants, are considered as a desired and practical option for remove the heavy metals. CuO NPs have recently been the important role in water purification from bacteria and arsenic [26-27]. In the present study, we investigated CuO NPs effect to removal the arsenate from drinking water. Our results demonstrated that nanoparticles with different volumes led to decrease arsenic amount through their absorption. Saif et al. [14] synthesized the copper oxide nanoparticles (CuO NPs) using leaf extract of Phyllanthus emblica that the hydrodynamic size of the CuO NPs was found to be 80 nm. The as-synthesized CuO NPs were studied for their use as an adsorbent material for the removal of As (V) from water. It was confirmed that the CuO NPs effectively removed As (V) via adsorption, and the adsorption efficiency was found to be best at high pH. The maximum adsorption capacity of

CuO for As (V) was found to be 1.17 mg g^{-1}. In other same research, McDonald e al [28] demonstrated the intrinsic abilities of copper oxide nanoparticles (CuO-NP) towards arsenic adsorption and the development of a point-of-use filter for field application. The CuO-NP were regenerated by desorbing arsenate via increasing pH above the zero point of charge. Results suggested an effective oxidation of arsenite to arsenate on the surface of CuO-NP. Naturally occurring arsenic was effectively removed by both as prepared and regenerated CuO-NP in a field demonstration of the point-of-use filter. Kumar et al. [29], prepared synthetic arsenic-contaminated water with varying concentrations of arsenic (100 to 1000 μg L^{-1}) and treated by nanosize adsorbent (copper oxide nanoparticles (CuO NPs)) that the colloidal and powder form of CuO NPs were synthesized in the laboratory by the hydrothermal technique on a large scale. It was found 30 ± 2 nm as size and spherical shape. The equilibrium adsorption of As (III) occurred at 90 min of contact time, pH 7.5, and 4 g/L adsorbent dosage. The maximum percent removal of As (III) was reached to 97.8, 94.6, 91.5, and 88.4% at an initial arsenic concentration of 100, 200, 500, and 1000 μg L^{1}, respectively.

Based on our results, synthesized CuO NPs showed high capacity to absorb the arsenic ions (both arsenate and arsenite) with concentration 50 and 100 μg L^{-1}. Of course, amount of arsenic absorption by nanoparticles in arsenic (V) and arsenic (III) has different behavior which can be due to their different chemical structure and capacity. Sodium arsenate ($Na_2HAsO_4.7H_2O$) is bigger than sodium arsenite ($NaAsO_2$), so this can be interpreted that in high volume of nanoparticles because of high concentration (density) of solution, it is not possible to properly absorb arsenic on nanoparticles. But in a dilute solution (low volume of nanoparticles: 10 μL), it is possible that nanoparticles easily absorb the arsenic (V). About sodium arsenite, it is more absorbed by nanoparticles with larger volume (100 μL). Therefore, can conclude that CuO nanoparticles have effective role for the deletion of arsenic (V) and arsenic (III) but their behavior can be different.

The surveying of CuO nanoparticles behavior in view of point their concentration and volume can be a clue for the next studies to achieve more knowledge.

5. Conclusion:

The presence of arsenic in water resources which adversely affects the health of millions of people needs to more attention to improved water quality. Although there are conventional adsorbents such as oxides of aluminum, iron, titanium, and manganese but their limitation consists of the requirement of pH adjustments, oxidation of water, and removal of competing ions, make some problems. The current study offers that copper oxide NPs (synthesizes by green chemistry) can be used for the removal the arsenic from drinking water. Moreover, green synthesis of these nanoparticles is an economical method and heath because the walnut leaf is available and nontoxic.

Informed consent

No informed consent

Authors' contributions

MSR was the main investigator, prepared the samples and performed the analyses, ABS supervised the study. MR, AG and MRM were the advisors to the study. All authors read and approved the final manuscript.

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No financial help from any authorization was received during the course of study as all the authors were self-employed.

Data availability

All data are available within the paper.

Declarations

Ethical approval: The present work received the ethical approval from Rafsanjan University of Medical Sciences as, IR.RUMS.REC.1398.115.

Competing interests: The authors declare that they have no conflicts of interest regarding the publication of this paper

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Caption to Figures

Fig.1 UV- Vis absorption spectrum of CuO NPs and walnut leaf extract.

Fig. 2 UV-Vis absorption spectrum of CuO NPs in different pH.

Fig. 3 UV-Vis absorption spectrum CuO NPs in various volumes of extract

Fig. 4 UV-Vis absorption spectrum CuO NPs in various copper salt concentrations.

Fig.5 XRD result of synthesized NPs

Pic.1 The CuO spherical NPs picture by TEM.

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مقاله پژوهشی

سنتز سبز نانوذرات اکسید مس (CuO) از برگ‌های درخت گردو برای حذف آرسنیک از آب

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چکیده

در این پژوهش، از یک روش جایگزینِ ساده، مقرون‌به‌صرفه و سازگار با محیط زیست، با استفاده از برگ گردو به عنوان یک منبع سبز، برای سنتز نانوذرات اکسید مس (CuO) بهره گرفته شد. برگ‌های گردو در فرآیند تولید نانوذرات CuO، نقش عامل احیاکننده و پایدارکننده را ایفا می‌کنند. نانوذرات سنتز شده با استفاده از روش‌های پراش پرتو ایکس (XRD) و میکروسکوپ الکترونی عبوری (TEM) مورد شناسایی و بررسی قرار گرفتند. بر اساس نتایج حاصل از TEM، نانوذرات CuO سنتز شده، کروی‌شکل بوده و اندازه‌ی آن‌ها بین ۲۰ تا ۱۰۰ نانومتر است. عوامل مؤثر بر فرآیند سنتز نانوذرات، از جمله pH، حجم عصاره گیاهی و غلظت نمک مس، بررسی و بهینه‌سازی شدند. در نهایت، نانوذرات سنتز شده با موفقیت برای حذف آرسنیک از نمونه‌های آب به کار گرفته شدند. برای اندازه‌گیری میزان آرسنیک در نمونه‌ها، از طیف‌سنج جذب اتمی با حد تشخیص 0.03 ppm استفاده شد. نتایج نهایی نشان می‌دهد که نانوذرات CuO، ۲۶ تا ۶۸ درصد آرسنات و ۵ تا ۸۹ درصد آرسنیت را از نمونه‌های اولیه حذف کرده‌اند. بر اساس این مطالعه، نانوذرات CuO سبز می‌توانند برای حذف آرسنیک از آب آشامیدنی استفاده شوند. علاوه بر این، سنتز سبز این نانوذرات یک روش اقتصادی و سالم است زیرا برگ گردو در دسترس و غیرسمی است.

واژه‌های کلیدی

نانوذرات اکسید مس (CuO)، برگ گردو، حذف آرسنیک، شیمی سبز.

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