

Green synthesis of ZnO NPs from Melon by-products (*Cucumis Melo* L.) extract: antioxidant activity and total phenolic content

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Abstract

In this study, zinc oxide nanoparticles (ZnO NPs) were synthesized using melon by-products (*Cucumis Melo* L.) extract. Nanoparticles were characterized using different techniques such as X-ray Diffraction (XRD), Fourier Transform Infrared (FTIR), Scanning Electron Microscopy (SEM), Energy Dispersive X-Ray Diffraction (EDX), Transmission Electron Microscopy (TEM), Ultraviolet-Visible Spectrophotometry (UV-Vis), Ultraviolet-Visible Diffuse Reflection Spectroscopy (UV-DRS), Dynamic light scattering (DLS) analysis and Zetasizer. SEM and TEM analyzes were used to show that the material is relatively spherical in shape and nanosized. It is shown in the UV absorption peaks that ZnO NPs peak at 368 nm. XRD and additionally EDX confirmed the purity of the material, and FTIR spectra confirmed the functional group of ZnO NPs. Melon byproducts were evaluated for total phenolic and DPPH activity with different solvent concentrations. ZnO NPs synthesized using melon by-product extract rich in polyphenols were also evaluated in terms of total phenolic and DPPH activity. Nanoparticles exhibited antioxidant activity. It was understood that secondary metabolites from the extract were used as capping agents.

Introduction

Small particles with a diameter of between one and one hundred nanometers, known as nanoparticles, are used in many ways [1]. Since nanoparticles have extremely different physical, chemical, and biological properties from bulk materials, including a high surface area, material science has seen a wide range of applications of nanomaterial-based technologies in recent decades [2]. It is possible to produce metal and metal oxide nanoparticles, which have uses in biotechnology, medicine, and numerous other industrial fields [3]. Because of the many uses for these materials, methodologies and procedures for synthesizing and characterizing nanoparticles are essential [4].

Zinc oxide nanoparticles (ZnO NPs) are the most interesting of these metal oxides because they are safe, easy to make, and affordable to produce [5]. Zinc oxide is now classified as GRAS (generally recognized as safe) metal oxide by the U.S. Food and Drug Administration (U.S. FDA) [6]. ZnO is a large exciton binding energy (60 meV) semiconductor with a wide band gap around 3.37 eV [7]. ZnO NP is a specialty material having products in the semiconductor, piezoelectric, and pyroelectric domains [8]. It has a wide range of uses in UV light emitters, chemical sensors, spin electronics, transparent electronics, personal care items, and specially blended paints and coatings [9–14].

Generally, three methods are used for producing nanomaterials: chemical, biological, and physical. For many years in the recent past, the physical and chemical routes of nanomaterial manufacturing have confirmed detrimental hazardous consequences on humans. These chemically induced processes take less time to complete, however the chemicals involved are hazardous and not environmentally friendly [15]. To produce nanoparticles, biological synthesis pathways have an advantage over chemical and physical synthesis techniques for the reasons mentioned above [16]. The biosynthetic pathway is a green, safe, and biocompatible method for producing nanoparticles for use in biomedical applications using plants and microorganisms. Fungi, algae, bacteria, and plants can also be used to perform out this

synthesis. A variety of nanoparticles have been synthesized using plant components, including leaves, fruits, roots, stems, and seeds, since their extract contains phytochemicals that function as reducing and stabilizing agents [17,18].

Melon (*Cucumis melo* L.) belongs to the Cucurbitaceae family, like watermelon and pumpkin, and has worldwide economic importance [19]. It has high antioxidant activity due to its phenolic content and has been identified as an important source of phytochemicals with potential health benefits. Melon is rich in important vitamins and is also a good source of pro-vitamin A. Industrial processing of melon and separation of the desired part of the fruit involves the production of by-products consisting of large quantities of peel and seeds [20,21].

The aim of this study is to synthesize ZnO NPs for the first time with an environmentally friendly method using the seeds and peel of melon fruit. In the first stage of this study, the Soxhlet extraction method was used to obtain polyphenol-rich extract from the peel and seeds of the melon fruit, which has a great industrial value, and water-ethanol were used as extraction solvents for cleaner technologies. This has been considered an environmentally friendly approach. In the second stage, it was aimed to use the extract obtained from melon by-products as a reducing, capping and stabilizing agent in the synthesis of ZnO NPs with the green synthesis. ZnO NPs were characterized by using of FTIR, XRD, SEM-EDX, TEM, UV-Vis, UV-DRS, DLS and Zetasizer. The antioxidant capacity content of the extract and ZnO NPs, 2,2-diphenyl-1-picrylhydrazyl (DPPH), and the amount of total phenolic content (TPM) were determined spectrophotometrically using the Folin-Ciocalteu method. This nanomaterial, synthesized with an environmentally friendly method, is intended to be used in biomedical applications and to contribute to the world of science.

Materials and methods

Chemicals and reagents

The melon sample was purchased from the Turkey local market in August 2023 (type Kırkağaç melon) and stored at -25°C until extraction studies. Within the scope of this study, ethanol ($\geq 99.9\%$) (EtOH) used during the extraction process was obtained from Merck (Darmstadt, Germany). Used in the spectrophotometric analysis of plants and nanoparticles, sodium carbonate ($\geq 99.0\%$) (Na_2CO_3), sodium hydroxide (NaOH), (, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 6-hydroxy-2,5, 7,8-tetramethylchroman-2-carboxylic acid (Trolox), Folin-ciocalteu reagent, gallic acid monohydrate ($\geq 98.0\%$) from Sigma-Aldrich (St. Louis, MO, USA), and methanol ($\geq 99.9\%$) (MeOH) was obtained from Merck (Darmstadt, Germany). Zinc oxide hexahydrate ($\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$) used for nanoparticle synthesis was obtained from Merck (Darmstadt, Germany). Deionized water was supplied using the Millipore, Milli-Q pure water system.

Preparation of melon (*cucumis melo*) aqueous extract

A procedure like the studies of Ciğeroğlu et al. (2021) was applied to obtain the extract of melon by-products [22]. The peel and seed parts of the melon purchased from the local market in Istanbul, Turkey

were separated from the fruit and washed in 3 stages. It was washed twice with clean drinking water in the first stage, in the second stage by keeping it in 1% (w/w) salt water for 3–5 minutes to remove microbes, and in the third stage with deionized water. It was dried in an oven at 60°C overnight. Dried fruit wastes were stored in a cool, dry, and dark storage environment. Before extraction, the peel and seeds were finely ground using a home mixer. 5 grams of ground melon by-products (2.5 grams peels and 2.5 grams seeds) were placed in an extraction cartridge, extracted with 100 mL of water and different concentrations of ethanol in the Soxhlet apparatus. Extraction was performed through three siphons at 80°C to reach equilibrium. Each flush took about 4 hours. The resulting extract was filtered. The extract was then stored at + 4°C until used in nanoparticle synthesis.

Green synthesis of ZnO NPs

The biosynthesis of ZnO NPs was performed according to previous studies with some modifications [23–25]. 14.73 mL of extract was added dropwise to 50 mL of 0.078 M $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ under stirring. The solution was then kept under stirring at 70°C for 2 hours until color change occurred. The pH of the reaction was adjusted to 10.77. Then, it was allowed to cool at room temperature and the supernatant was separated. The nanoparticle solution was centrifuged at 4000 rpm for 15 min and a solid precipitate was obtained. After washing with distilled water, the solid product was centrifuged twice at 4000 rpm for 15 min, then washed again with ethanol. It was dried at 80°C for 24 hours. A pale white solid was obtained. The resulting solid was pulverized in a mortar and stored at + 4°C for characterization and spectrophotometric studies. The steps of synthesis are shown in Fig. 1.

Figure 1. Schematic representation of the synthesis of ZnO NPs.

Characterization of ZnO NPs

XRD analysis (BRUKER D8 DISCOVER XRD) was performed at a voltage of 40 kV and a current of 40 mA with Cu K (α) radiation to determine the crystallinity and purity of the obtained ZnO NPs. To determine the functional groups, present in the synthesized nanoparticles, ZnO nanoparticles were analyzed by FTIR spectroscopy (Perkinelmer Spectrum Two FT-IR). Particle shape and surface properties of ZnO NPs were investigated with scanning electron microscopy (SEM, JEOL JSM-5600LV). The elemental structures of the ZnO NPs were analyzed using an energy dispersive spectrometer (EDX, JEOL JSM-5600LV) to determine the elemental components of the various NPs. Shape analysis was performed by TEM with closer magnification (TEM, JEOL JEM-1220). Formation of zinc nanoparticles and band gap examination, λ_{max} was confirmed with a UV-Visible spectrophotometer (PG Instruments T80 Plus UV/VIS) and UV-Vis DRS spectrophotometer (PG Instruments T92 + spectrophotometer). Dynamic light scattering (DLS) analysis was used to verify particle size distribution (Brookhaven 90 +). The determination of the effective electrical charge on the surface of the synthesized ZnO NPs was carried out on the Zetasizer (Malvern Nano ZS) device.

Determination of antioxidant activity for scavenging the free radicals by DPPH method

Scavenging activity of the extract and nanoparticles was identified by applying DPPH spectrophotometric assay at 517 nm. It was made by dilution of 0.0079 g DPPH in 40 mL of 80%, v/v methanol solution. After adding 3000 μ L of diluted DPPH solution and 600 μ L of methanol (80%, v/v) to 100 μ L of sample solution, the mixture was allowed to stand in the dark for 30 min. Trolox (6-hydroxy-2,5,7,8-teramethyl chroman-2-carboxylic acid) calibration curves were created using the proper solvents, and the results are given as milligrams of Trolox equivalent antioxidant capacity (mg-TEAC / g-DM) per gram of dry matter [26]. The percentage of DPPH scavenging effect was calculated using the following equation:

$$\text{DPPH scavenging effect (\%)} = \frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}} \times 100 \quad (1)$$

A_{control} represents the absorbance of the diluted DPPH solution, and A_{sample} represents the absorbance of the sample [27].

Determination of total phenolic content

The Folin-Ciocalteu method was used to determine total phenolic content of extracts and nanoparticles. In this assay, 20 μ L of the sample was mixed with 380 μ L distilled water, 2000 μ L Folin-Ciocalteu (reagent) solution and 1600 μ L Na_2CO_3 in plastic cuvette. The reaction was occurred in the dark at room temperature for 30 min. Then, the absorbance was examined by UV-spectrophotometer device at 765 nm. Calibration curves were prepared for pure gallic acid with appropriate solvents, the results are expressed as milligrams of gallic acid per gram of dry matter (mg-GAE / g-DM) [28].

Results and discussion

X-ray Diffraction (XRD) Analysis

XRD was confirmed crystalline structure of ZnO nanoparticles, used for structural identification and determining size in synthesis. The diffraction pattern of synthesized ZnO NPs as shown in Fig. 2. The diffraction peaks observed in 2 degrees = 31.77°, 34.42°, 36.26°, 47.54°, 56.60°, 62.85°, 66.38°, 67.95°, 69.09°, 72.56°, and 76.97° which are attributed to (100), (002), (101), (102), (110), (103), (200), (112), (201), (004) and (202) facets with JCPDS card 01-089-7102 [29]. The JCPDS file confirmed the hexagonal phase of ZnO NPs with P63mc (186) space group, and the absence of any distinctive peaks other than the ZnO peaks indicates the high purity of the synthesized nanomaterial [30]. The average crystallite size of ZnO NPs was calculated 21.8 nm using the Debye-Scherrer equation:

$$D = \frac{K\lambda}{\beta \cos \theta}$$

2

where D is the crystal size (nm), K is the Debye-Scherrer constant number (0.94), β is the full-width half maxima (0.427), θ is the Bragg angle, and λ is the X-ray wavelength (1.54 Å) [31].

Fourier Transform Infrared (FTIR) Analysis

Functional groups of green synthesized ZnO NPs were identified using FTIR spectroscopy (Fig. 3). O-H stretching appears in the spectrum as a very broad band extending to 3340.20 cm^{-1} and can be associated with phenolic and alcohol compounds [25][32]. The peak at 906.91 cm^{-1} corresponds to the stretching vibration of the primary amine's C-N bond or the primary alcohol's C-O bond. The peak at 1575.07 cm^{-1} corresponds to the vibration modes of aromatic nitro compounds and alkyl. The peaks at 1392.16 and 1032.92 cm^{-1} are due to in-plane bending or vibrations of the primary and secondary alcohols [33]. The generation of ZnO NPs is confirmed by the obvious absorption peak at 412.20 cm^{-1} , which corresponds to the Zn-O stretching band [34].

Scanning Electron Microscopic (SEM) and Energy Dispersive XRay Diffractive (EDX) Analysis

The scanning electron microscope was used for the morphological analysis of green synthesized ZnO NPs (shape and size). The SEM image is homogenous from biomolecules found in the extract and allowing them to separate them; It has shown that it is relatively globally shaped but not in a defined geometry (Fig. 4(a, b, c, d)). In Fig. 4e, the presence of Zn and O with weight percentages of 76.66 and 23.34, respectively, confirmed the amount of ZnO NPs.

Transmission Electron Microscopy (TEM) Analysis

TEM analysis was used for in-depth examination of green synthesized ZnO NPs. Figure 5 was also examined 200 nm and 50 nm scales. The existence of a nanomaterial with disorder and relatively spherical form has been confirmed (Fig. 5b). The findings matched the spherical findings in the SEM analysis.

Ultraviolet–Visible Spectrophotometer (UV-Vis) and Ultraviolet–Visible Diffuse Reflectance Spectroscopy (UV-DRS) Analysis

Nanoparticles were characterized using UV-Vis. In Fig. 6A, spectra showed an absorbance peak at 368 nm, indicating that the synthesized ZnO NPs were successfully formed. The result obtained has been shown to be like the studies [25][34]. In Fig. 6B, the absorbance curve versus wavelength was drawn using UV-DRS analysis and BaSO_4 as the solid sample reference, and this curve was used for direct band gap (eV) measurement. Figure 6C shows the Kubelka-Munk plot of ZnO NPs. The calculated band gaps of ZnO NPs were found to be 3.21 eV.

Dynamic light scattering (DLS) analysis

Figure 7 shows the particle size analysis of green synthesized ZnO NPs. The size distribution ranged from 63.1 nm to 245.6 nm and the average particle size was measured to be 135.6 nm. The polydispersity index of these nanoparticles, which were distributed in small sizes in a narrow area, was also found to be 0.206.

Zeta potential analysis

The zeta potential of ZnO NPs was examined to get exact measurements of the net electrical charge of the NPs present in the solution. In Fig. 8, ζ -potential analysis shows that ZnO NPs exhibit negative charge at -20.5 ± 3.91 mV. The finding obtained is compatible with the studies [35]. It is seen that the obtained value provides good distribution and is stable; It can be said that melon extract contains functional groups that affect the capping and maintaining service of nanoparticles.

Determination of antioxidant activity and total phenolic content of extract

Melon seeds and peel extracts were evaluated in terms of antioxidant activity, DPPH radical scavenging effect and total phenolic substance according to different solvent concentrations. In Table 1 shows that while higher results were obtained in terms of both TPM and antioxidant activity with 50% ethanol water solvent, results close to 50% ethanol water solvent were obtained in 100% pure water and the lowest results were obtained at 100% ethanol concentration. Previously studies have revealed that alcohol and water mixtures are more effective than single-component solvents in the extraction of phenolic compounds [36,37]. This can be attributed to the fact that water acts as a swelling agent for the cells, increasing the contact surface between the plant matrix and the solvent used and thus increasing the amount of phenolic compounds. It can be explained that when the ethanol concentration exceeds a certain value, it causes denaturation of proteins and thus prevents the dissolution of polyphenols, thus reducing the TPM value [38]. TPM value is also related to antioxidant capacity. Considering the results obtained, 50% ethanol water was used as the extraction solvent in obtaining the extract used in nanoparticle synthesis. To evaluate both by-products, extraction was performed with equal proportions of peel and seeds.

Solvent (% v/v)	TPM (mg-GAE/ g-DM)		DPPH (mg-TEAC / g-DM)		DPPH Scavenging Effect (%)	
	Melon Seed	Melon Peel	Melon Seed	Melon Peel	Melon Seed	Melon Peel
100% Ethanol	0.373 ± 0.003	7.279 ± 0.003	8.697 ± 0.002	9.102 ± 0.002	30.8	48.75
100% Water	11.081 ± 0.007	15.431 ± 0.002	7.093 ± 0.001	7.333 ± 0.000	83.24	87.23
50% Ethanol/Water	12.631 ± 0.004	19.800 ± 0.002	2.574 ± 0.002	4.063 ± 0.002	84.55	87.29

* Data are given as the mean (n = 3) ± standard deviation.

Table 1. Antioxidant activity and total phenolic content of extract.

Determination of antioxidant activity and total phenolic content of ZnO NPs

The synthesized nanoparticles were found to have 12.61 mg-GAE / g-DM total phenolic content, 5.04 mg-TEAC / g-DM antioxidant activity and 60% DPPH radical scavenging effect. Considering the synthesis mechanism, they donate electrons from hydroxyl groups to Zn^{2+} ion to form phenolic and antioxidant metallic Zn contained in rosehip seed extract. Metallic Zn is known to react with oxygen to form ZnO NPs. These molecules contained in the extract served as stabilizing agents for the synthesized ZnO NPs. This shows that these secondary metabolites in the plant are used in the synthesis of ZnO NPs [39,40]. In the literature, there are differences in the antioxidant activities of ZnO NPs synthesized with various plant extracts [41,42]. This can be attributed to the fact that the nanoparticles, which vary depending on the plant extract used in the synthesis and the synthesis conditions, have different sizes and thus different specific surfaces. Previous studies have reported that nanoparticles show higher radical scavenging activity as the nanoparticle size decreases [43].

Conclusion

In this study, an extract with high total phenolic and antioxidant activity was obtained from melon by-products using an environmentally friendly method and solvents. According to the characterization findings obtained in the synthesis of ZnO NPs, nanoparticles were successfully obtained with high purity and under critical size. In addition, it has been determined that the extract acts as a reducing, stabilizing and capping agent in the synthesis process of secondary metabolites. It is thought that the antioxidant properties of the synthesized nanoparticles, their particle size being nanosized and the use of extract rich in phenolic content play an important role. It is expected that ZnO NPs obtained by an environmentally friendly and inexpensive method will be used for industrial and biomedical applications in subsequent research and will contribute to science.

Declarations

Data availability

All data generated or analysed during this study are included in this published article.

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Author contributions

RNB and IK supervising, designing, methodology, investigation, review, writing and editing, YSK, TA, and RNB performed the experiments. Each author reviewed the manuscript.

Competing interests

The authors declare no competing interests.

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Figures

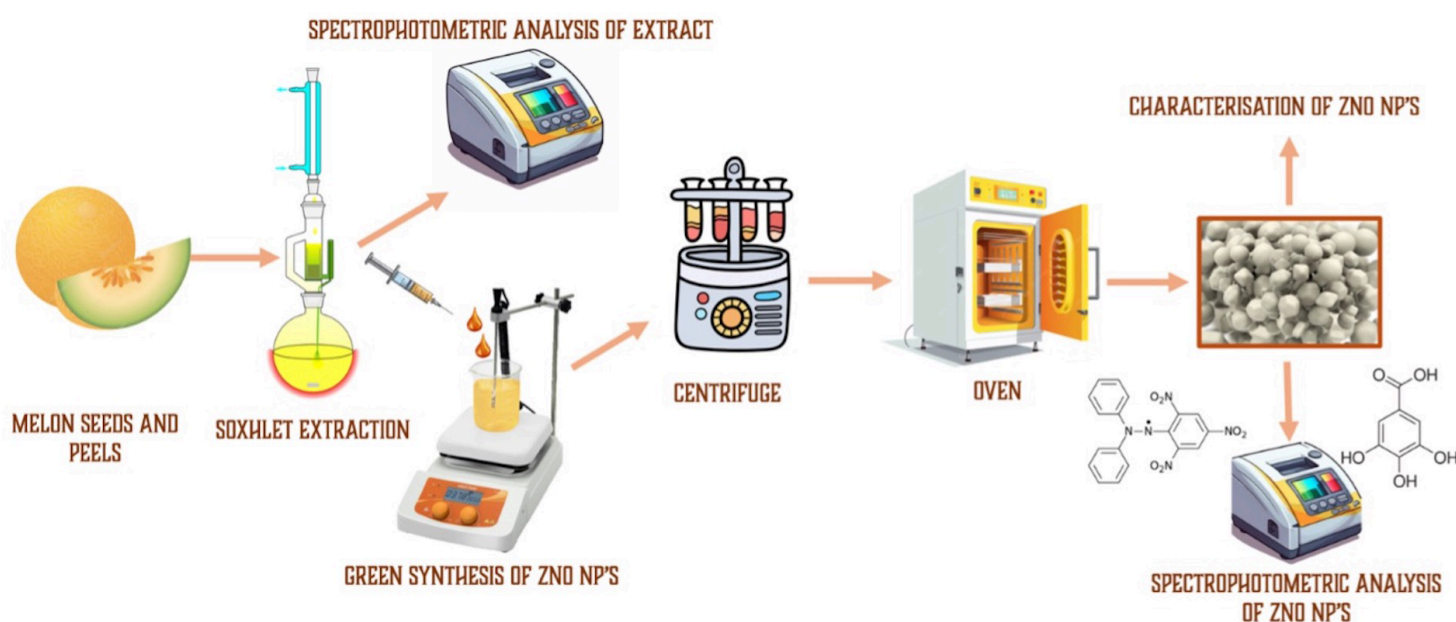


Figure 1

Schematic representation of the synthesis of ZnO NPs.

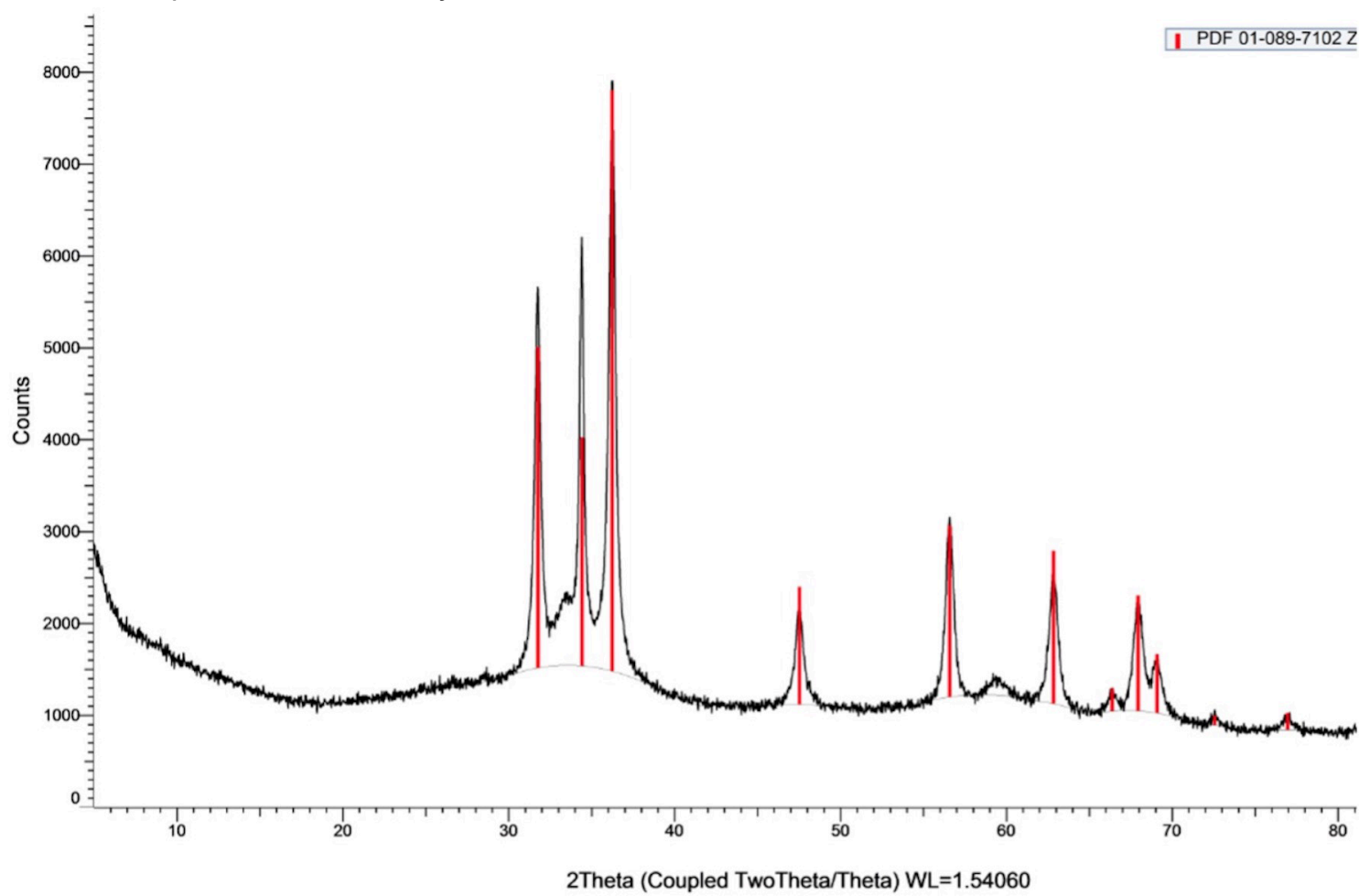


Figure 2

XRD pattern of ZnO NPs (green synthesized from Cucumis Melo L. extract).

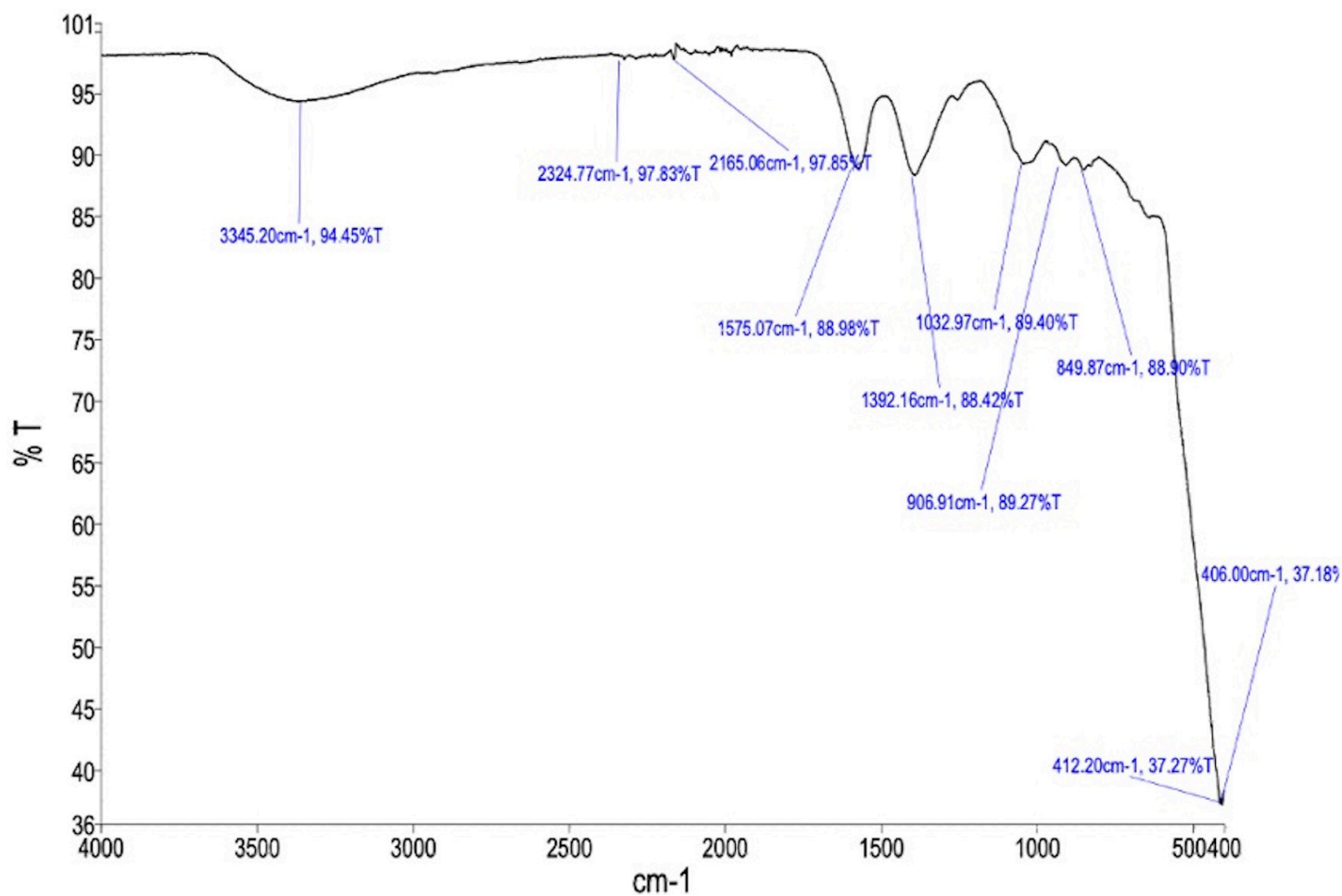


Figure 3

FTIR spectra of ZnO NPs.

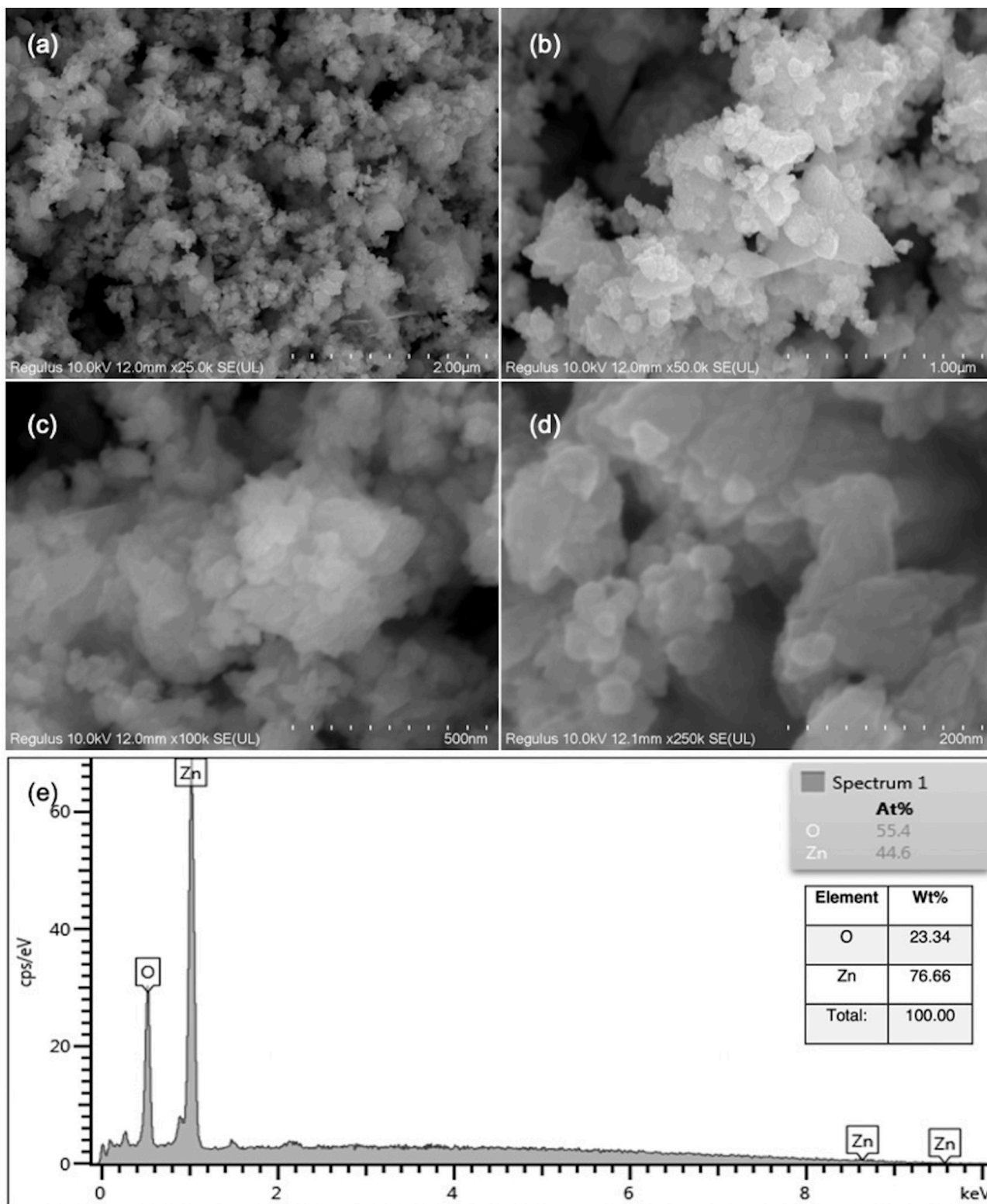


Figure 4

SEM images and EDX spectra (e) of the ZnO NPs.

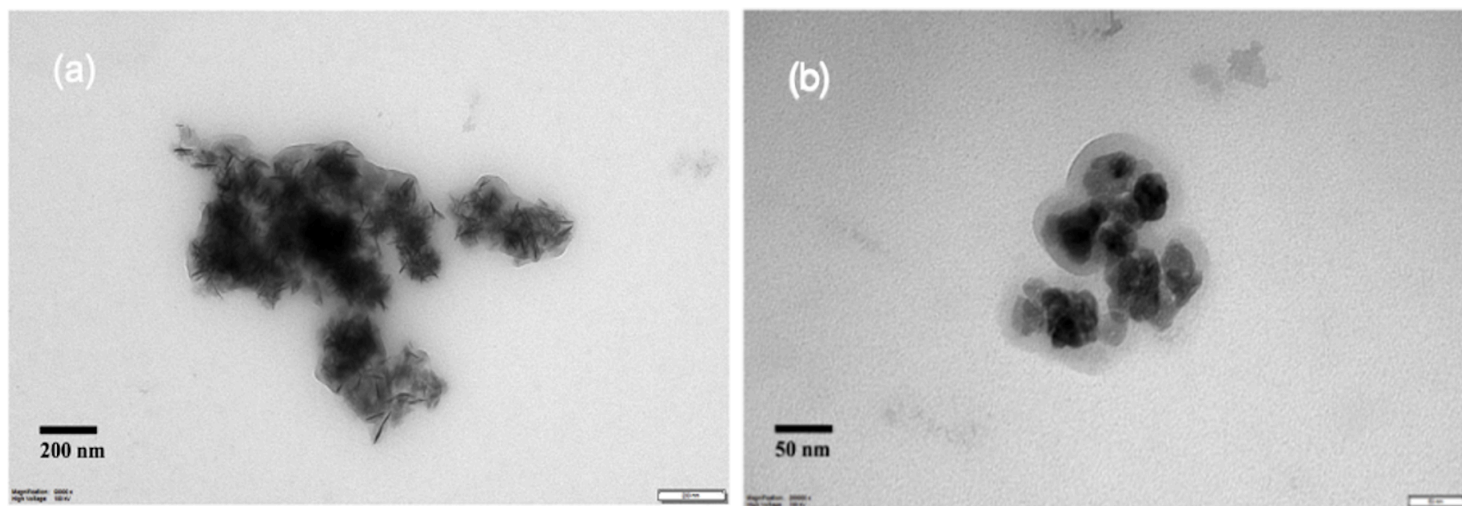


Figure 5

TEM images of the ZnO NPs.

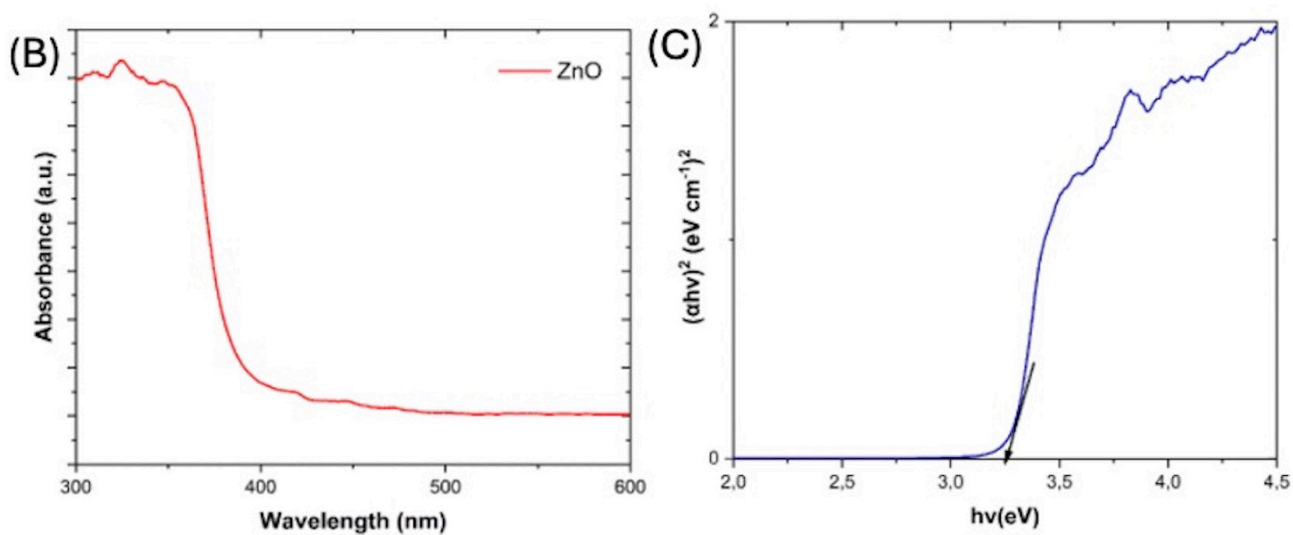
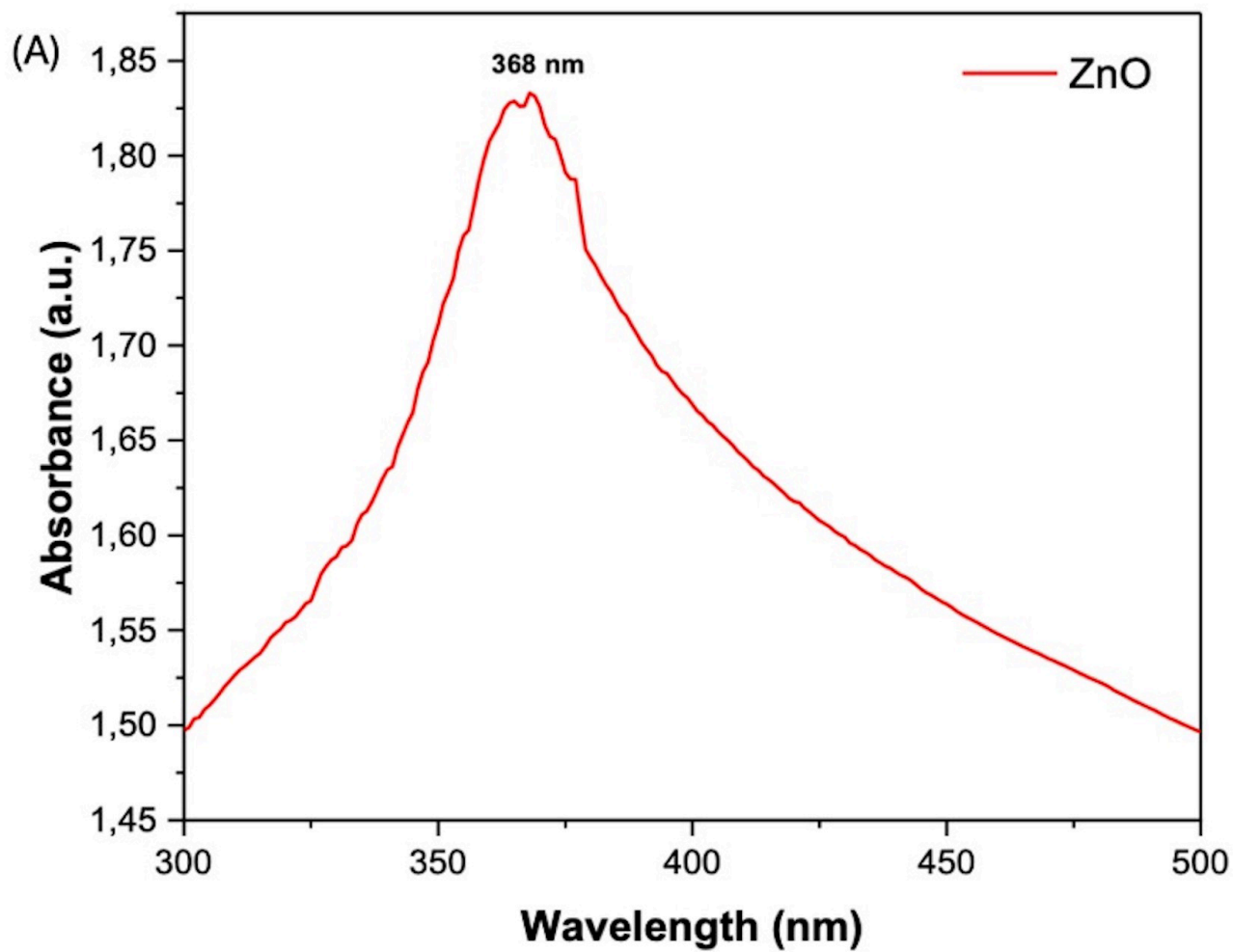


Figure 6

(A) UV-Vis and (B) UV-DRS spectra, and (C) Tauc plot of ZnO NPs.

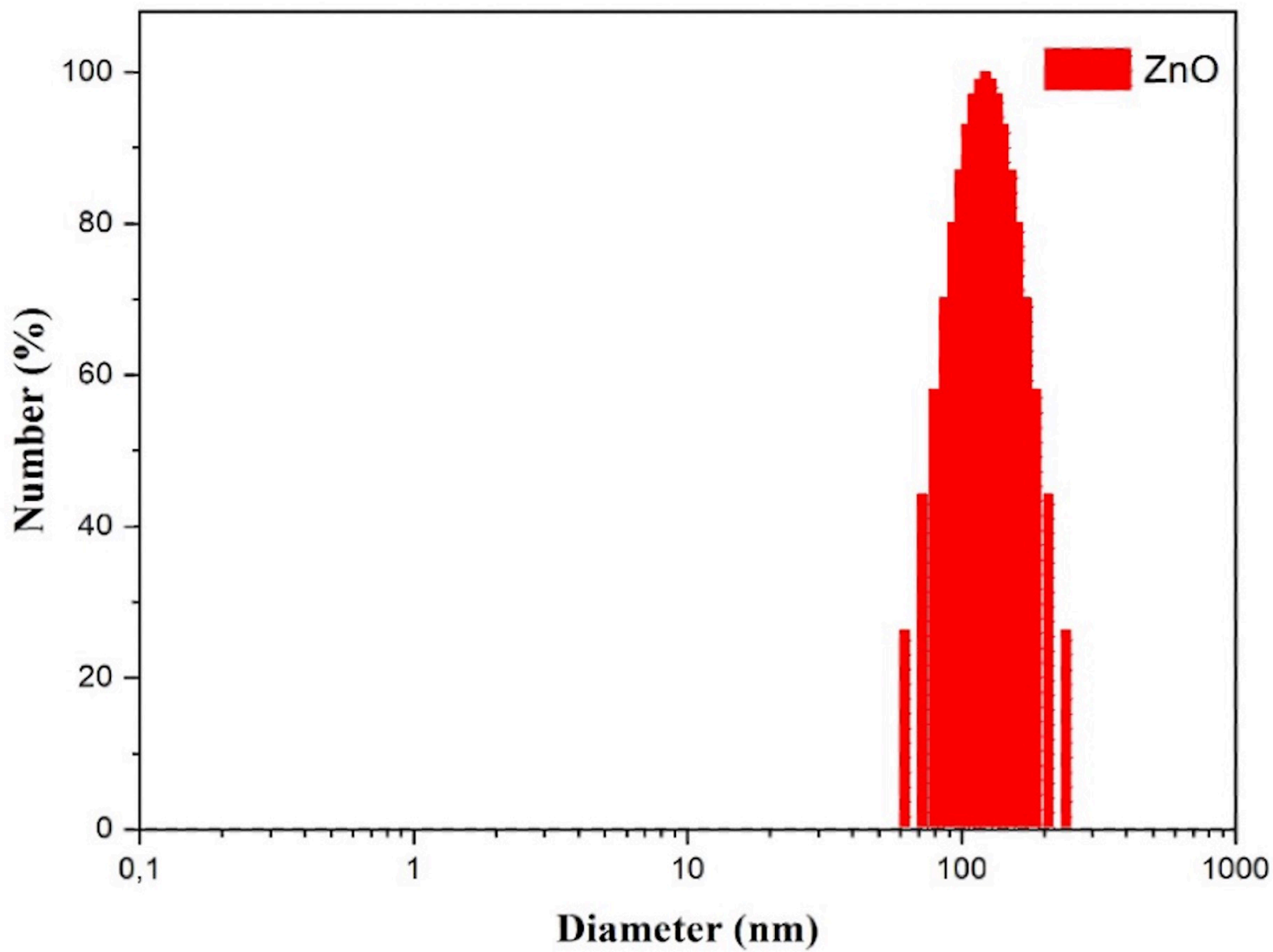


Figure 7

The particle size distribution of ZnO NPs.

Results

	Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV): -20,5	Peak 1: -20,5	100,0	3,91
Zeta Deviation (mV): 3,91	Peak 2: 0,00	0,0	0,00
Conductivity (mS/cm): 0,0475	Peak 3: 0,00	0,0	0,00
Result quality	Good		

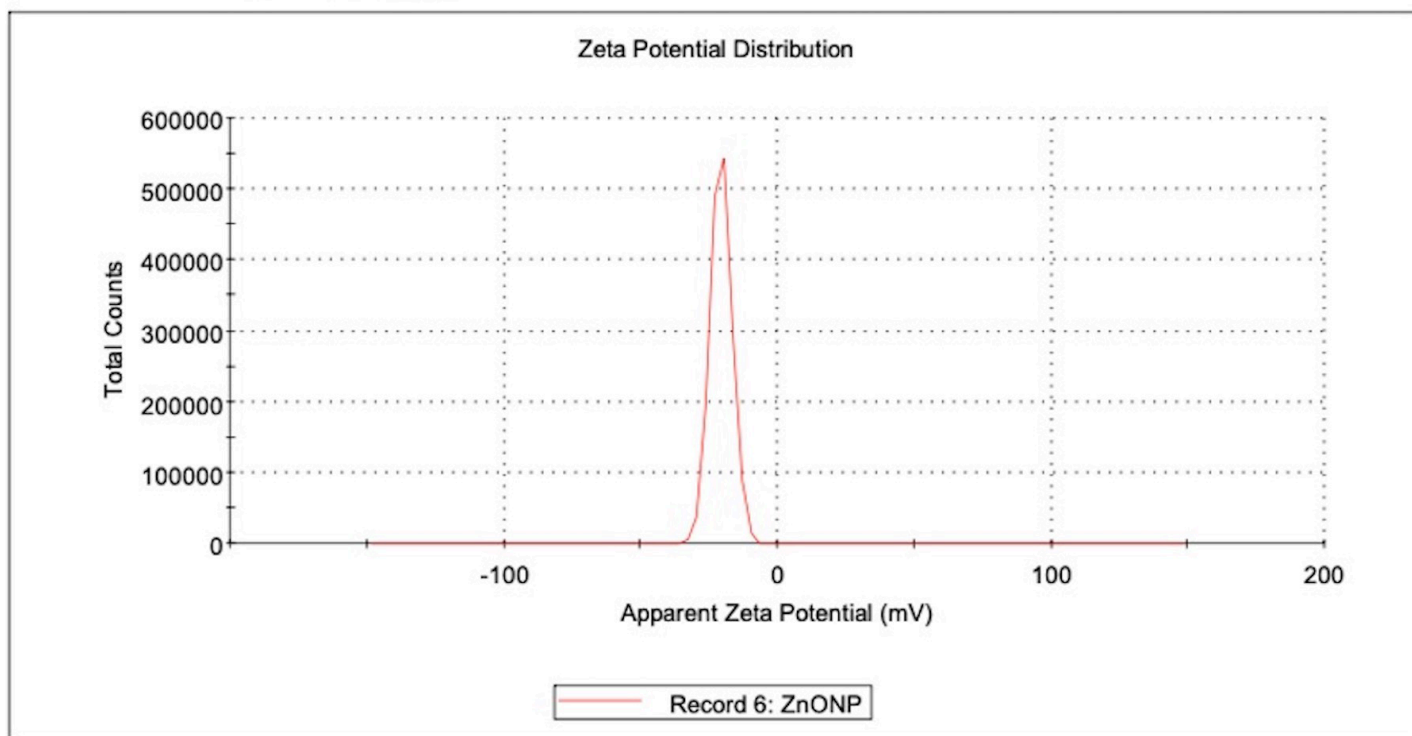


Figure 8

Average zeta potential of synthesized ZnO NPs.