

Green Synthesis of Zinc Oxide Nanoparticles from (*Punica granatum L*) Pomegranate Aqueous Peel Extract

Wassan Mohammed Husain*, Jawad Kadhim Araak and Orooba M.S. Ibrahim

Department of Physiology, Biochemistry and Pharmacology, College of Veterinary Medicine, University of Baghdad, Iraq

ABSTRACT

The biosynthesis of Zinc oxide nanoparticles (ZnONPs) was done under alkaline conditions (pH 12) and temperature ≥ 80 °C. After mixing 10% of aqueous pomegranate peel extract (PPE) with 0.1 molar of zinc nitrate [Zn (NO₃)₂.6H₂O], the mixture was stirred at 70-80°C for 15 minutes and then left at room temperature overnight. Then, the mixture was separated and centrifuged at 4000 rpm for 10 minutes. This process was repeated three times and the precipitate was collected and dried for using. ZnONPs were characterized by visual observation of color changes. The estimated duration of synthesis was compared to the weight of ZnONPs using the UV-visible analysis, Fourier transform infrared (FT-IR), X-ray diffraction (XRD) and Scanning electron microscope (SEM). The results of this line investigated the proper conditions for ZnONPs synthesis. The result of FT-IR showed a functional group related to Zn-O at 613.38 to 580.59 cm⁻¹, whereas the result of X-RD at 2 theta diagnosed the type of oxide formation as ZnO and determined that the particle size was in a range of 20-30 nm. Finally, SEM image showed crystals shape of ZnO nanoparticles. In conclusion, PPE acts as a reducing agent used in synthesis of ZnO in Nano-scale size via easy and simple method.

Keywords: Green synthesis, ZnONPs, Pomegranate, *Punica granatum L*, Aqueous extract

Introduction

Zinc oxide (ZnO) is a type of inorganic metal oxide available for a wide range of nanostructures (1). Zinc oxide nanoparticles (ZnONPs) are primarily used in sunscreen and cosmetic products (2, 3). Detailed information about the amount of ZnONPs is not reviewed in the literature. However, the wide utilization of ZnONPs can elevate the health hazard of people who are exposed to ZnONPs. Despite the fact that ZnONPs are principally used as cosmetics, ZnONPs cannot be absorbed through the skin layer (4).

This NP may be accidentally ingested from sunscreen or lipstick, as well as, directly swallowed via food packaging or by medication delivery (5). Pomegranate (*Punica granatum L*) fruit consists of various types of constituents in various parts like seeds, arils and peels. The pomegranate fruit covered by peels “around 60% of the fruit” has various types of ingredients that play therapeutic role in the health management via the modulation of different biological activities (6). The *Punica granatum* peel is a rich source of flavonoids, tannins, and many phenolic compounds. Pomegranate seeds, peels and fruits play a role in diseases cure through modulation of biological activities. Investigators pointed that extract of pomegranate has scavenger activity against free radicals (7).

Developing green routes for synthesis of ZnONPs are important methods and still a challenge for materials researchers. Recently, plant extracts have been suggested as properly eco- friendly, different to chemicals in synthesis of ZnONPs (8). Using plants in order to synthesize NPs can potentially

*Correspondence: wsm_m_82@yahoo.com, Department of Physiology, Biochemistry and Pharmacology, College of Veterinary Medicine, University of Baghdad, Iraq. Received: 30 October 2019, Accepted: 2 December 2019, Published: 28 December 2019.

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eliminate the problem of toxicity of chemical methods and make these NPs more biocompatible than those prepared via chemical ways (9). Alternative types of plant extracts have been reported in the literature for green synthesis of ZnONPs, such as *Aloe barbadensis* Miller leaf (10), *Hibiscus rosa-sinensis* (11), *Camelia sinesis* (12) and *Azadirachia indica* (13)

The current study aims to synthesize ZnONPs through green method by using aqueous pomegranate peel extract (PPE).

Materials and Methods

Preparation of ZnONPs

Extraction of Pomegranate Peel

Pomegranate (*Punica granatum L*) fruit was brought from a market in Baghdad city, and washed with distilled water. The middle parts between seed and rind (Peel) were collected and dried under sun light, grinded by blender until it got powder and kept at 4°C until use (14). Ten grams of pomegranate peel dry powder were mixed with 100 ml of deionized distilled water in 250 ml conical flask, the obtained mixture was stirred at 60 -70°C for 15 minutes, and then left at room temperature overnight. The solution was separated by filter paper and centrifuged at 4000 rpm for 10 minutes. This process was repeated three times, then the solution was collected in a sterile container. Aqueous extraction was kept at 4°C until use (15).

Green Synthesis of Zinc Oxide Nanoparticles

A 2.97 g of Zinc nitrate crystal $[Zn(NO_3)_2 \cdot 6H_2O]$ were dissolved in 100 ml of deionized distilled water in 250 ml conical flask and stirred magnetically at 80°C for 20 minutes. The aqueous extract of pomegranate peel was added dropwise under stirring. As soon as the aqueous extract became in contact with zinc ions, the solution color spontaneously changed (8, 15).

The pH of mixture was adjusted to 12 or more by potassium hydroxide (KOH). The precipitate was separated by centrifuge at 4000 rpm for 15 minutes, and the pellets were collected and washed twice with ethyl alcohol followed by deionized distilled water. The pellets were dried at 60 °C after completing the conversion of ZnONPs.

The process of pellets separation by centrifuge was done at a time from 1 to 8 hours in order to estimate

the effect of time on the synthesized amount of ZnONPs (16).

Characterizations of ZnONPs

UV-vis spectrum

A 0.2 gm from ZnONPs sample was taken and the absorption was measured by scanning spectrophotometer using a wave length ranged from 200 to 1100 nm with 0.5 intervals. The peak increased positively with increasing the reaction time of ZnONPs synthesis (17). The test was done in Pharmacy Collage, AL-Muthana University .

Scanning electron microscopy (SEM) analysis of synthesized zinc oxide nanoparticles was done in Science College, AL-Basra University. SEM–Tescan VegaII, Czech, was used under following condition: signal A=SE2, EHT= 1.00KV, and WD= 2.9 mm.

X-ray diffraction (XRD) was used to examine zinc oxide nanoparticles synthesized from PPE, which was performed by using a X-ray diffractometer (6000/ shemadzu, Japan). The radiation used was Cu K α 1, 1.5406Å, 40.0 KV voltage and the current was 30.0 MA. This test was done in the central lab of Ibn-Al-Haytham College, University of Baghdad.

Fourier transform infrared spectroscopy (FT-IR) was used for the chemical analysis of biomedical samples (18) and detection of the functional groups present in the sample (19). Measurement was done by using FT-IR apparatus in Pharmacy College of Al-Muthana University. A wavelength of light from 400- 4000 cm was used to measure IR.

Results and Discussion

Biosynthesis of zinc oxide nanoparticles (ZnONPs) from pomegranate peel extract (PPE) in the present study was identified by clearly changing the color and precipitate formation as shown in Figure 1.

The color changed from white to pale yellow within a few minutes after mixing with PPE, and the spontaneous formation of pale white precipitate occurred until 8 hours of reaction at the room temperature.

The reduction rate and ZnONPs formation could be increased with incubation time. The weight of ZnONPs powder after centrifugation, washing, and drying is shown in Figure 2. The weight (gm) of pellet changed with incubation time (hour). The weight of ZnONPs powder synthesized showed a

marked elevation with the time of synthesis. Change in color indicated the start of reduction of zinc ion, and the precipitate appearance indicated complete synthesis of ZnONPs.

Bior-reduction included reducing metal ions to metal nanoparticles with the assistance of phytochemicals such as polyphenolic, polysaccharides, alkaloids, vitamins and amino acids (20).

Flavonoids act as reducing factors for metal ions. However, the functional groups of flavonoids are responsible for the nanoparticle formation. The metal ions reduction result from the transition of flavonoids from enol to the keto in order to synthesize nanoparticles (21).

Several studies have confirmed that flavonoids can act as a chelating composite, for example, quercetin, which can chelate in three situations including the carbonyl, hydroxyls and catechol groups (21). That help in understanding the role of flavonoids at the start of nanoparticles formation, aggregation, and bio-reduction steps (22).

Figure 3 illustrated the UV-visible spectra of the prepared ZnONPs from PPE. UV-visible spectroscopy is usually proceeded to confirm the synthesis of ZnONPs. Because the presence of Surface Plasmon Resonance (SPR) effect, conducting electrons start vibrating at a certain wavelength range, the test was done between wavelengths from 200 to 1100. Peaks were obtained at 210.88 nm, 280.1 nm and 369.12 nm wavelength after 8 hours of ZnONPs synthesis.

Conducting electrons beginning oscillating at a specific wavelength range resulted from surface plasmon resonance (SPR) effect (15). The peak obtained at 369.12 nm clearly illustrates the presence of ZnONPs in the mixture (23).

The value and nature of the band gap of ZnONPs was determined by using the essential absorption, which agree to electron excitation from valence band to conduction band. The absorption spectra also detected the excitonic absorption peak of the sample (23).

FT-IR spectrum for dried, powdered ZnONPs was obtained in the range of 500- 4000 cm^{-1} . The chromatographic image of FT-IR for ZnONPs in the present study is shown in Figure 4. The FT-IR spectra showed various peaks of 3350.97, 1570.11, 1425.44, 1350.22, 1232.55, 1027.39, 675.11, 648.10, 613.38, and 580.59 cm^{-1} .

According to Figure 4, several bands indicate the presence of OH, C-C, C=O, C-H and C-N. The FT-IR spectrum illustrated strong bands at 3350 cm^{-1}

that are attributed to O-H Stretch, this indicates that polyphenol groups are present (24), whereas the band at 1570 cm^{-1} was assigned to the methylene vibration from protein.

The middle peak is displayed at 1425 cm^{-1} , which is related to the presence of C-C stretching in aromatic groups (19). C-N stretch of the aromatic amines and the carboxylic acid give rise to a band at 1350 cm^{-1} . The band in 1232 cm^{-1} is due to C-N stretching vibrations of amine, while the band in 1027 cm^{-1} can be related to the presence of C-O stretching vibrations of alcohols (8).

The band absorbed at 613 cm^{-1} and 580 cm^{-1} was linked to ZnO, this pointer is the characteristic Zn-O bond, which indicates that the material is Zinc Oxide (8, 9).

The section among 500 and 900 cm^{-1} is related to metal oxygen, whereas the peaks at 1634.00 and 620.93 cm^{-1} probably stand for ZnO stretching and vibration. The FTIR spectrum illustrates that the O-H stretching act a major role through formation of nanoparticles (19). Polyphenols which can be seen at 3200-3500 cm^{-1} act as the main stabilizing agents for nanomaterials.

It can be seen from different other peaks that ZnONPs are surrounded by proteins and other metabolites. Carbonyl groups consisted of amine acid and proteins have a high affinity to form bonds with metal ions (24). Figure 5 demonstrated XRD Spectra of ZnONPs synthesized by using PPE.

Diffraction peaks were recorded at theta 2 values of different peaks 36.162°, 34.322°, 31.683°, 56.481°, 62.726°, 66.24°, 67.82° and 76.83° corresponded to hkl values of 100, 002, 101, 102, 110, 103, 200, 112 and 202 crystal planes. The sizes of ZnONPs were calculated by using Debye-Scherrer equation $D = 0.89 \lambda / \beta \cos \theta$.

Where 0.89= Scherrer's constant; λ = X-ray wavelength (1.5406 Å), β =FWHM (Full Width at Half Maximum) of the peak located at 2θ and θ =Bragg's angle of diffraction.

The particle size was found to be 20-30 nm and the material examined was zinc oxide according to PDF: 01-075-1526.

The peak position with two theta values and hkl planes, are in agreement with those of ZnO obtained from the International Center of Diffraction Data card (JCPDS-36-1451), from hexagonal phase of ZnO and the formation of a crystalline structure (8, 23,19).

Whereas, no diffraction peaks were detected on other phases indicating the purity of ZnO

nanoparticles (25). In the XRD patterns, the 101 plane matching to 36.16° was found to be clear and abundant; this indicates the preferential growth of the crystallites.

The result of XRD indicated that using PPE as a green synthesis reductant allows to synthesize polycrystalline ZnONPs without the presence of another crystalline phase and without amorphous material, which makes this material similar to ZnONPs obtained by other plants (9).

When investigating earlier reports, our results agreed with Vanathi et al. (2014) who synthesized the nanoparticles using *Eichhornia crassipes* leaf extract (26), while Narendhran and Sivaraj (27) created zinc nanoparticles using the *Lantana aculeate* leaf extract (27).

SEM analysis was performed to visualize the morphological shape and size of ZnONPs. Figure 6 (a and b) illustrates SEM images that were seen in different magnification ranges (100-200 nm), which demonstrated the presence of crystal shapes with a mean average diameter of 42.87 nm for ZnONPs formed from PPE. Green synthesis of ZnONPs from PPE appeared as self-aggregated in a close packed periodic array of crystal shape.

Figure 6 shows different particle sizes of nanoparticles when compared with the result obtained from XRD analysis, in which particle sizes were calculated by using the Debye–Scherrer equation.

These changes observed in nanoparticles shape may be due to the multiple sample were not uniform in their size, thus only the particles on the surface of the sample could be measured by SEM.

On the other hand, the presence of negative charges on the nanoparticles surface contributed with the stability of nanoparticles, and the soluble state act to prevent their accumulation by increasing electrostatic repulsion between particles (28).

However, many previous studies showed that this quantity of negative charges was not acceptable to stabilize nanoparticles sustainability, so that the remaining nanoparticles in soluble state can lead to more accumulation and increase particles size (29).

In conclusion, Pomegranate aqueous peel extract (PPE) acts as reducing agent used in synthesis of ZnO in Nano-scale size via easy and simple method.



Figure 1. The final form of precipitation after 8 hours from mixing of zinc nitrate with PPE according to steps of green synthesis of ZnONPs from PPE

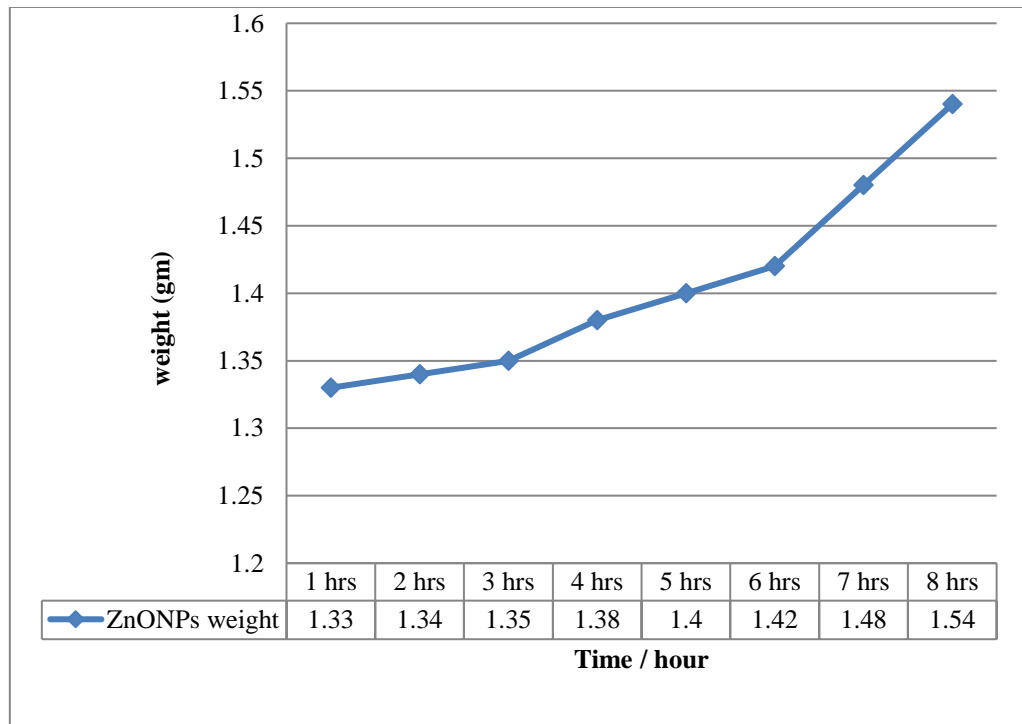


Figure 2. Weight of ZnONPs synthesized by PPE

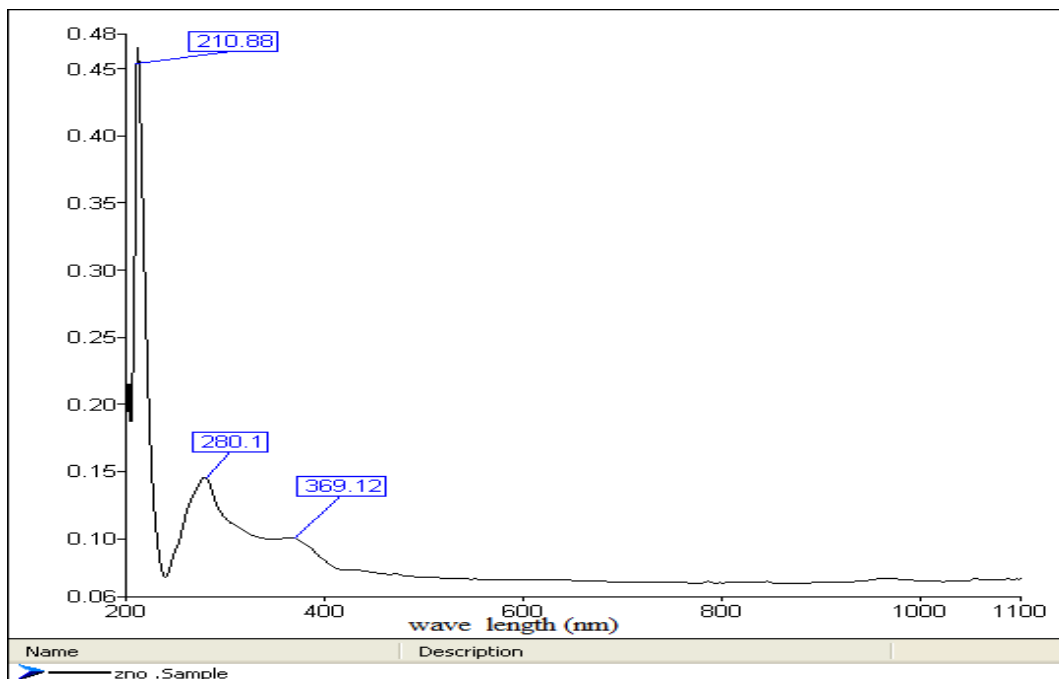


Figure 3. UV-visible absorption spectroscopy of ZnONPs synthesis shows SPR effect at 369.12 nm

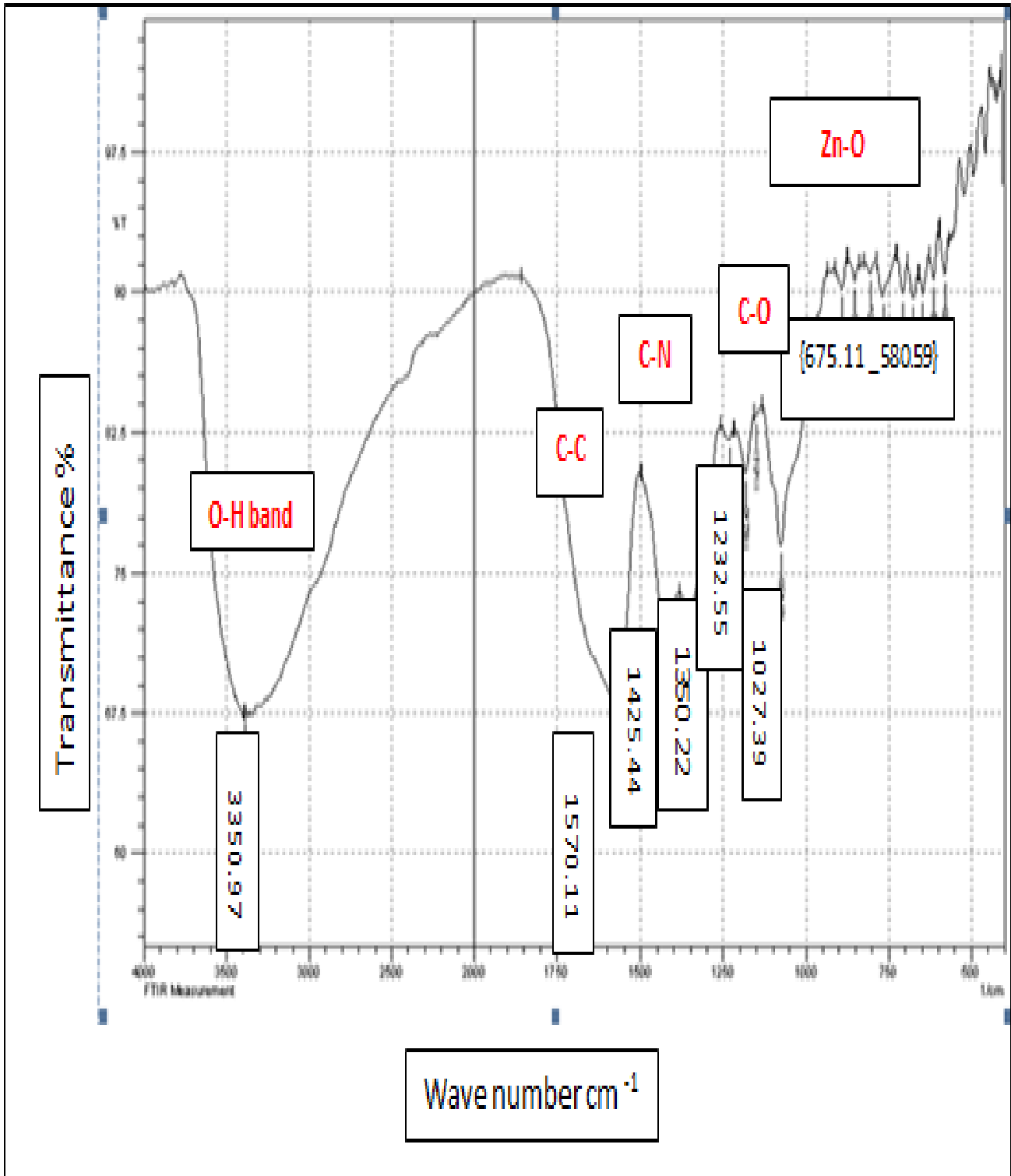


Figure 4. FT-IR Spectrogram for the ZnONPs synthesized by PPE

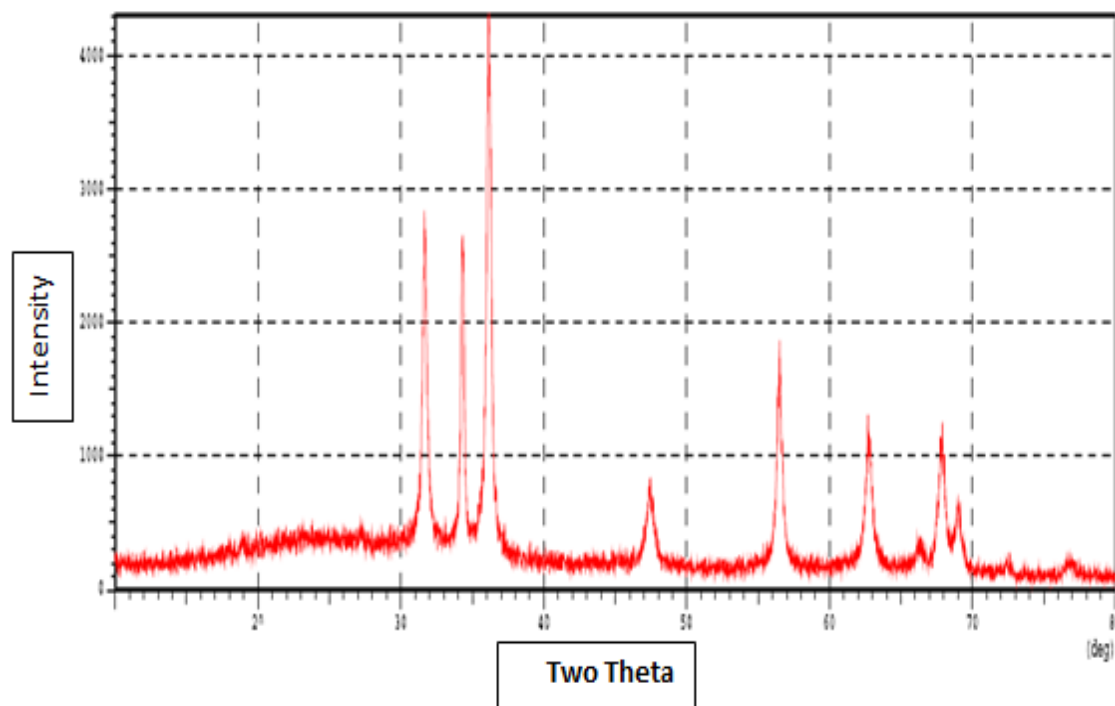


Figure 5. X-ray diffractogram of ZnONPs

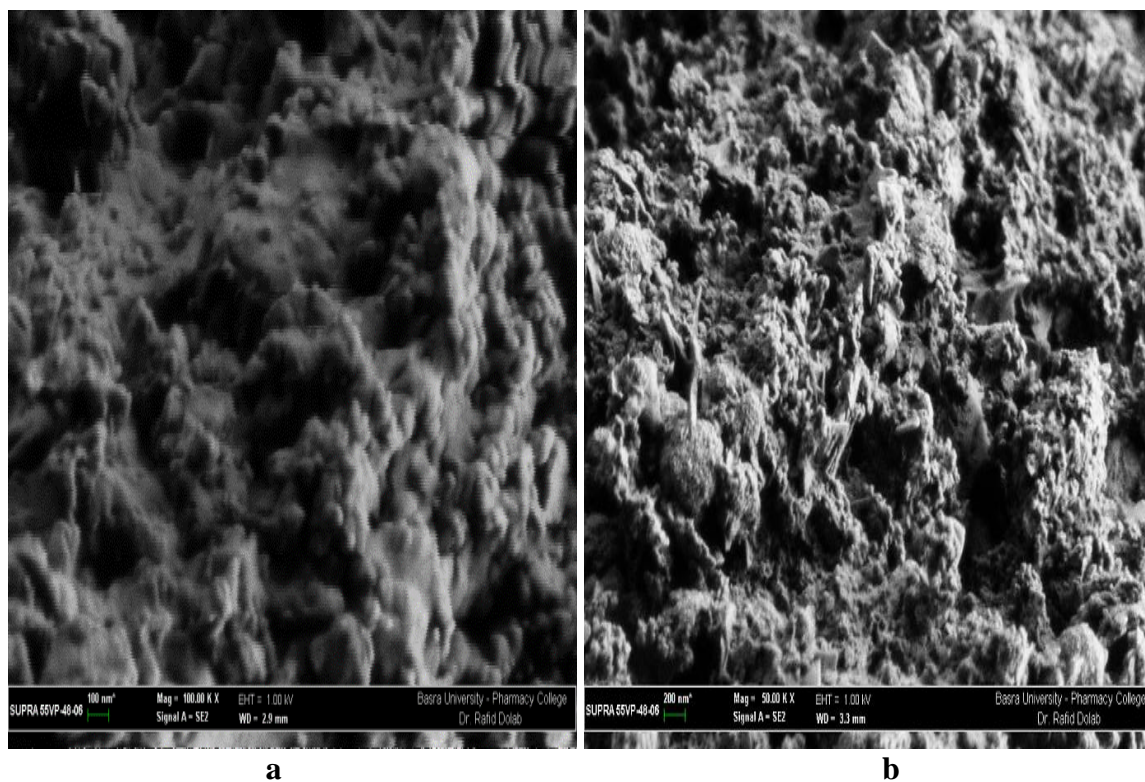


Figure 6. SEM image for ZnONPs synthesized by PPE. a)100 nm, b) 200 nm

Conflict of Interest

The authors declare that there is no conflict of interest.

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التصنيع الحيوي لجسيمات الزنك النانوية بواسطة المستخلص المائي للربان

اوسن محمد حسين،² جواد كاظم عراق،³ عروبه محمد سعيد

فرع الفلسفة والادوية والكيمياء الحياتية/كلية الطب البيطري-جامعة بغداد-العراق

E-mail: wsm_m_82@yahoo.com

الخلاصة

التصنيع الحيوي وتحضير للجسيمات النانوية لأكسيد الزنك في ظروف قلوية (اس هايدروجيني 12) وبدرجة حرارة تساوي أو تزيد عن (80 درجة مئوية). إذ تم خلط 10% من مستخلص لب الربان مع 0.1 مولاري من نترات الزنك الثقيل المستمر وبدرجه حراره 70-80 درجه مئوية لمدة 15 دقيقه، بعدها ترك بدرجه حراره الغرفه وفصل الناتج بواسطه جهاز الطرد المركزي (4000 دوره في الدقيقه) لمدة 10 دقائق، كررت العمليه ثلاث مرات بعدها تم جمع وتجفيف الراسب للاستخدام. تم توصيف جسيمات الزنك النانوية من خلال ملاحظة التغير اللوني، حساب فترة التصنيع مقارنة بالوزن الناتج من جسيمات الزنك النانوية، تحليل الأشعة فوق البنفسجية المرئية، استخدام جهاز تحويل طيف الأشعة تحت الحمراء، حيود الأشعة السينية و مسح المجهر الإلكتروني. بينت نتائج هذا المحور الشروط الصحيحة لتكوين الجسيمات النانوية لأكسيد الزنك. بينت نتائج فحص تحويل طيف الأشعة تحت الحمراء (FT-IR) ان المجموعه الفعاله المتعلقة بأوكسيد الزنك كانت ضمن (cm- 1613.38 to 580.59)، في حين اظهر فحص حيود الأشعة السينية عند زاوية (2 ثيتا) نوع الاوكسيد المتكون وحجم الجسيمات حيث كان بحدود 20-30 نانوميتر. اخيرا فقد اظهر مسح المجهر الإلكتروني الشكل الخماسي لجسيمات اوكسيد الزنك النانوية.

الكلمات المفتاحية: اوكسيد الزنك النانوي، لب الربان، المستخلص المائي