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Gamma radiation synthesis of poly(PVP/Gelatin) zinc oxide/zinc sulfide nano- composite for antibacterial application

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CrossMark

THIS work focuses on the preparation of zinc oxide nanoparticles using gamma radiation within the network of PVP/ Gelatin nanogel which acts as a capping agent for the prepared nanoparticles. Particle size of the prepared composite was determined using High resolution transmission electron microscopy (HRTEM). Moreover, the selected area electron diffraction pattern (SAED) obtained from the results of HRTEM confirmed the formation of zinc oxide and zinc sulfide nanoparticles. FT-IR was used to identify and investigate the different function groups of the prepared PVP/Gelatine/ Ascorbic acid/ Glycerin embedded zinc oxide nanoparticles. Zinc nanoparticles showed a strong antibacterial activity against the growth of the two bacteria used. The effect is more efficient against Gram-positive bacteria than against Gram-negative bacteria and the most sensitive is *Staphylococcus aureus*.

Keywords: PVP/Gelatin, zinc nanoparticles, HRTEM, gram negative bacteria.

Introduction

The preparation and synthesis of polymeric materials with desirable properties, known as hybrid materials, has been one of the most important topics in recent decades (Chen, Ng, & Weil, 2020). Gamma irradiation is one of the most important natural methods for preparing and synthesizing polymeric materials, as it is a safe, effective, and low-cost method (Mohamed, Sayed, & Mahmoud, 2023). It combines and links materials together, either by crosslinking with strong covalent bonds or by forming hydrogen bonds, etc (Mohamed & Mahmoud, 2024). Gamma irradiation also plays a significant role in the preparation of nanomaterials(Salah, Abdelmaksoud, & Abdel-Wahab, 2023). Nanotechnology has revolutionized the field of biomedicine, enabling the development of nanoscale materials with unique structural, chemical, and physical properties. Among various nanomaterials, zinc oxide nanoparticles (ZnO NPs) have received significant attention due to their remarkable versatility, low cost, ease of fabrication, and multiple biological activities (Mandal et al., 2022){Anjum, 2021 #15}{Anjum, 2021 #13}. With a typical size of less than 100 nm, zinc oxide nanoparticles (ZnO NPs) possess a high surface area-to-volume ratio, which enhances their interaction with biological systems, making them particularly suitable for a wide range of biomedical applications.

ZnO nanoparticles exhibit antimicrobial, anti-inflammatory, and anticancer properties, largely due to their ability to generate reactive oxygen species (ROS) and release zinc ions (Zn^{2+}) (Rehman et al., 2023). These properties are beneficial in disrupting microbial membranes, inducing apoptosis in cancer cells, and promoting tissue regeneration (Makvandi et al., 2021). In addition to their cytotoxic effects

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against pathogens and cancer cells, zinc oxide nanoparticles are considered biocompatible and safe at low concentrations, supporting their use in clinical and therapeutic settings.

Their surfaces can be enhanced with targeting ligands, polymers, or therapeutic agents to achieve site-specific delivery and controlled release (Srinivasarao and Low, 2017).

Furthermore, zinc oxide nanoparticles are increasingly used in wound healing materials, antibacterial coatings for implants, dental composites, and tissue engineering structures (Pushpalatha et al., 2022). Their ability to promote cell adhesion, proliferation, and differentiation supports bone and soft tissue regeneration, making them valuable components in regenerative medicine. Despite these promising features, the toxicity and long-term effects of zinc oxide nanoparticles remain a subject of ongoing research (Keerthana and Kumar, 2020).

Zinc oxide nanoparticles represent a dynamic and multifunctional tool in modern biomedicine (Jiang, Pi, and Cai, 2018). Their broad spectrum of activity, tunable surface properties, and favorable biological interactions offer promising opportunities for innovation in diagnostic, therapeutic, and regenerative technologies.

In this research, a new type of composite consisting of PVP/Gelatin/ ZnO nanoparticle was prepared, and characterized with some advanced techniques to investigate the composition of the prepared nanogel, ZnO nanoparticles were also formed by gamma radiation techniques to produce a new material that is more efficient for antibacterial applications.

Material and Methods

Materials

Polyvinyl pyrrolidone (PVP), M.Wt 40000, was purchased from Universal Fine Chemicals PVT. LTD. Gelatin, El-Gomhouria Co., Egypt. Ascorbic acid ($C_6H_8O_6$), M.W. 176.13, Alpha Chemika. Zinc sulfate ($ZnSO_4 \cdot ٧H_2O$), M.Wt. 161.47 g/mol, purity 99% provided by El Gomhouria Co., Egypt. Glycerin ($C_3H_8O_3$) with M.Wt. 92.094 g/mol, purity 99.5% (spectrophotometric grade), El Gomhouria Co., Egypt.

Preparation of PVP/Gelatin/ascorbic acid/ glycerol loaded zinc nanoparticles

A detected amount of PVP (1 wt%) and Gelatin (1 wt%) was dissolved in 100 ml bidistilled water via a magnetic stirrer. After complete dissolving, ascorbic acid (1 wt%) was added followed by glycerol through a stirring process and raising the temperature to 70 C. Last but not least, 3% zinc sulfate was added. The obtained solution was exposed to gamma irradiation doses 1, 2.5, 5 kGy (dose rate 0.7 kGy/h)

Characterization

High Resolution Transmission electron microscopy (HRTEM)

High Resolution Transmission electron microscopy (HRTEM) measurements were performed with (JEOL, JEM 2100, Japan) operating at 200 kV.

Fourier transform infrared (FT-IR) spectroscopy.

The FT-IR spectra of poly(PVP/Gelatin) zinc oxide nanocomposites were carried out on an ATR-FT-IR model Bruker, Unicom infra-red spectrophotometer, Germany, at the 400–4000 cm^{-1} wavelength range.

Assessment of Antibacterial Activity of Zinc Nanoparticles

Three gram-negative pathogenic bacteria (*Escherichia coli* ATCC 8739) and one gram-positive bacterium (*Staphylococcus aureus* ATCC 6538) were used as test organisms. The antibacterial activity of two types of zinc nanoparticles (at 1 kGy, and 5 kGy) was evaluated against these bacteria by the agar disc diffusion method. Nutrient agar plates were spread with 100 µl of actively grown broth cultures of the respective test bacteria and allowed to dry for 10 minutes. Irradiated solutions of Zinc oxide nanoparticles (ZnNP1) (at irradiation dose 1 kGy) and $ZnNP_2$ (at 5 kGy) (Sameeh, Khairy and Qasim, 2025) were imposed on the plates and inoculated for 48 hours at 37 ºC, then the inhibition zone around the loaded discs was recorded.

Result and discussion

HRTEM measurement

High resolution transmission electron microscopy (HRTEM) imaging is similar to TEM imaging in that the magnifications utilized are high enough to show the lattice spacing of inorganic materials (Greer and Zhou, 2011).

The images (1a and 1b) illustrate that zinc nanoparticles are well dispersed in PVP/ Gelatin network. Also, it can be noticed that zinc nanoparticles have different geometrical shapes as hexagonal and rice like rods bundles as illustrated in images 1 (c and d). Furthermore, the size of zinc nanoparticles varies from 24 to 34 nm as shown in Fig. 1b. Hence, this size range of zinc oxide nanoparticles is a clear indication of the successful synthesis and formation for zinc oxide nanoparticles in PVP/ Gelatin network. Over and above that, the nanosize of zinc oxide particles is significant for the capping effect of PVP/ Gelatin copolymer nanogel for zinc particles and keeping it in the nanoform. Last but not least, all the above results prove the role of gamma irradiation in the reduction process for zinc ions to zinc oxide nanoparticles without using more chemicals as a reducing agent.

The shining spot rings in the selected area electron diffraction (SAED) pattern are a definite indicator and typical of polycrystalline Zinc oxide nanoparticles. The SAED pattern is illustrated in Fig. 2 (a, b, c, and d). The characteristic concentric rings associated with the crystalline structure of zinc oxide nanoparticles are visible as bright spots in the electron diffraction pattern. From SAED pattern (Fig. 2 b and d), it was found that distances between two bright spots values are 5.211, 6.611, 8.401 and 11.301/nm, which are related to (0.38 (nm) d-spacing value, 23˚ (2ϴ) XRD peak position), (0.30 (nm), 29.2° (2ϴ)), (0.24 nm, 36.2° (2ϴ)), and (0.18 (nm), 49.9° (2ϴ)) respectively. The diffraction angle at 2θ = 29.2° and 49.9° which belong to the diffraction from planes (111) and (220) are regarding to ZnS nano-crystallites wurtzite structure (JCPDS card No. 36-1450). On the other hand, the diffraction angle at 2θ = 36.2° which is related to the diffraction from plane (101), which is distinctive to the hexagonal ZnO NPs phase. Ultimately, the diffraction angle at 2θ = 23˚ which is assigned to the poly-crystalline nature between the functional groups of PVP and Gelatin.

UV–Vis spectra

Metallic nanoparticles (e.g., gold, silver) exhibit a strong absorbance peak known as the Surface Plasmon Resonance (SPR) band. The position and intensity of this peak depend on factors like nanoparticle size, shape, and the surrounding medium. The UV-Vis absorbance spectrum for the ZnO nanoparticles (Taghizadeh et al., 2020) is shown in Fig. 3.

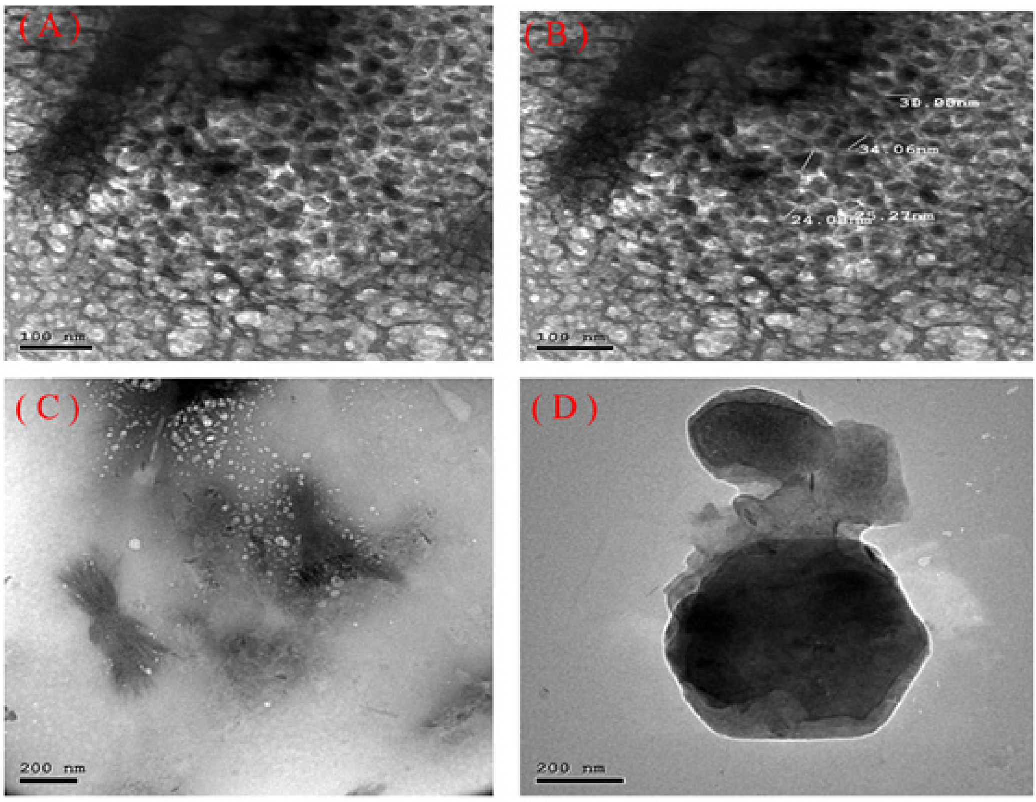

Fig. 1. HRTEM images of zinc oxide nanoparticles embedded in PVP/ Gelatin network.

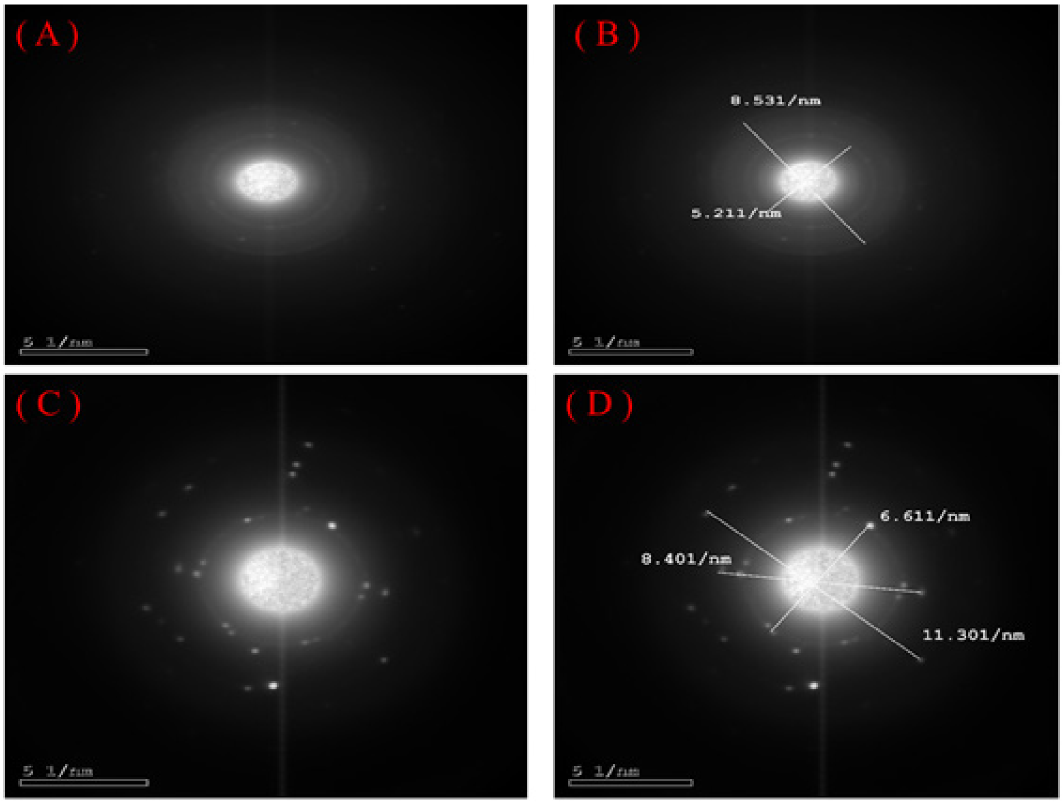

Fig. 2. Zinc oxide nanoparticles' selected area electron diffraction pattern (SAED).

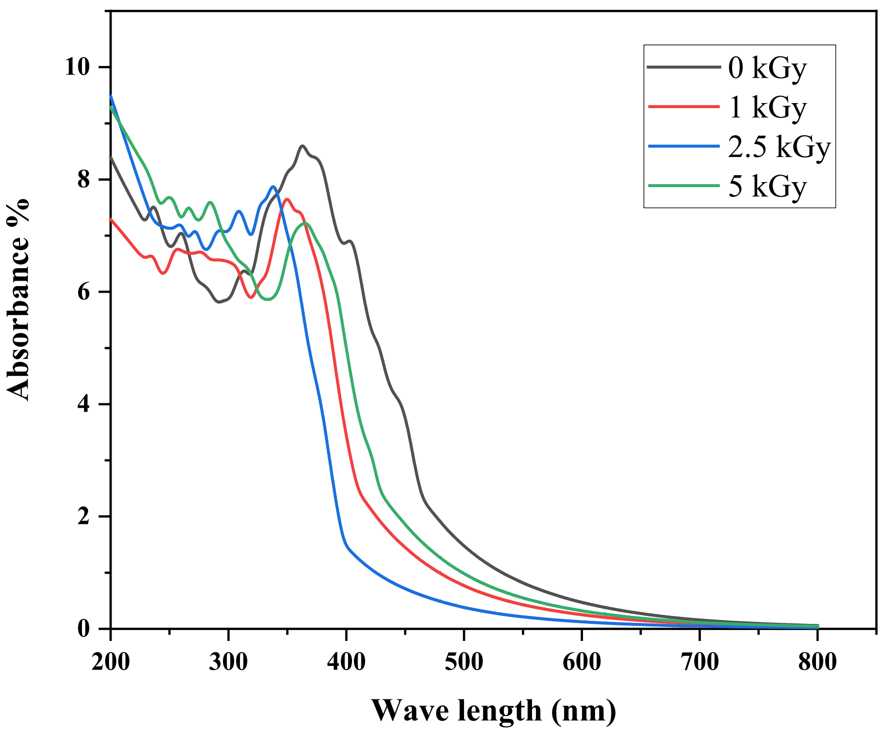

Fig. 3. The UV Absorption spectrum of zinc oxide nanoparticles capped with PVP/ Gelatine/ ascorbic acid/ Glycerol system

For metal nanoparticles like zinc, Surface Plasmon Resonance (SPR) is a common phenomenon. SPR occurs when the conduction electrons on the nanoparticle surface resonate with incident light, which typically results in characteristic absorption peaks at 366.56 nm, 362 nm, 349 nm, and 338 nm. Zinc oxide nanoparticles exhibit strong electronic transitions, particularly involving the conduction band and valence band (Salah et al., 2023).

The peaks at 366.56, 362, 349, and 338 nm might be related to transitions in the nanoparticle's electronic structure. Zinc oxide (ZnO) nanoparticles, for example, are commonly studied in the UV range, and similar peaks might arise due to electronic transitions between different energy levels of the nanoparticle. These peaks may arise from transitions within the nanoparticle's defects or surface states as well.

In conclusion, these peaks likely reflect surface plasmon resonances or electronic transitions occurring in the zinc oxide (ZnO) nanoparticles. The exact nature of the peaks (366.56, 362, 349, and 338 nm) suggests size-dependent quantum effects and defect-related transitions. The minor shifts in the peaks might also indicate the influence of surface states, the surrounding medium, or the capping agents on the nanoparticles (Helmy, Partila and Salah, 2020).

FT-IR measurement

Investigation of the fuction groups in prepared polymeric material was carried out using FT-IR instrument (Matei, Cernica, Cadar, Roman, and Schiopu, 2008). The chemical structure of the prepared PVP/Gelatin embedded with zinc oxide nanoparticles was studied using FT-IR. Tables 1 and 2 show the corresponding characteristic FTIR bands of PVP and Gelatin. From the noticed FTIR spectrum (Fig. 4), when PVP and gelatin are blended, the intensity of the characteristic IR peaks increases. Also, Broadening of N–H and O–H combined (or overlapped) band (which centered at 3262 cm-1) – Indicating increased intermolecular interactions. Moreover, a shift in Amide and C=O peaks (around 1638 cm-1) – Suggesting hydrogen bonding between PVP's carbonyl and gelatin's amino or hydroxyl groups. In addition, shift in C-O stretch, and CH2 bending confirm chemical interaction between PVP, gelatin, and zinc oxide nanoparticles.

Tables 1 and 2 provide information about the band's assignments of IR spectra for pure PVP and Gelatin.

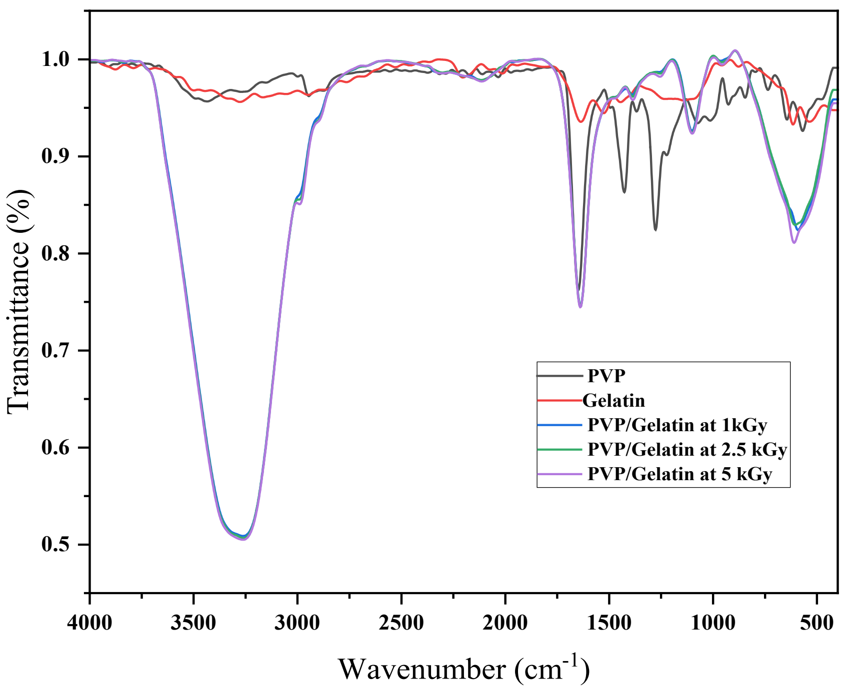

Fig. 4. FT-IR spectra of PVP, Gelatin, PVP/Gelatin/ zinc oxide nanoparticles at different gamma irradiation doses (1, 2.5, and 5 kGy).

TABLE 1. Bands declaration of IR spectra for pure PVP.

Wavelength (cm^{-1})	**Band description**
3433	OH stretching
2945.95	CH_2 asymmetric stretching
1648.5	C=O stretching
1427	CH_2 scissoring vibrations
1368	CH_2 bending
1276	CH_2 twisting or wagging
1074	C-O stretching
926	CH_2 rocking
845.8	CH_2 bending
735.6	CH_2 rocking

TABLE 2. Bands declaration of IR spectra for Gelatin

FT-IR Peak (cm^{-1})	Assignment
3876.5	OH stretching
3277.38	N-H stretching
3166.9	OH stretching
2948.4	CH2 asymmetrical stretch
1636.2	C=O stretch coupled with COO-
1526.38	NH bend coupled C-N stretch
1445.29	CH2 bend
1140.7	C-O stretch
877.17	-C-C-, Skeletal stretch
614.95	CH out of plane, skeletal stretch

Effect of PVP/Gelatin/ZnO nanoparticles on some types of pathogenic bacteria

Screening tests of the inhibition zone were performed by the well diffusion method (Ferreira, Rosa, Torres, Ferreira, and Bernardinelli, 2002). Colonies cultured overnight on an agar plate were used to make the inoculum suspension, which was then added to Mueller-Hinton broth. To inoculate Mueller-Hinton agar plates (bacteria employing nutritional agar plates), a sterile brush was submerged in the suspension. After bacteria were exposed to nanocomposites, the ZOI assay's "zones of bacterial inhibition" were identified using the disc diffusion technique. Fresh LB agar plates inoculated with bacterial cultures were placed on the surfaces of sterile discs (10 mm diameter) of Whatman No. 2 filter paper loaded with 50µL/disc of Nanocomposite PVP/Gelatin/Zn NPs of concentration 30 g/l prepared at various gamma irradiation doses (1, 2.5, and 5 kGy). As test organisms, one gram-positive bacterium (Staphylococcus aureus) and one gram-negative pathogenic bacterium (Escherichia coli) were employed. The Mcfarland scale of 105 CFU was used to compare the created inoculums to standardize them. After 24 hours of incubation at 37°C, the zone of inhibition was identified, and the diameters were precisely measured in triplicate on perti plates. After 24 hours at 37°C, the inhibitory zone surrounding each well was tested for bacteria.

ZOI measurement was used to qualitatively evaluate the bacterial inhibitory effects of the synthesized nanocomposites PVP/gelatin/Zn NPs at varying gamma irradiation dosages for both Gram-positive (+ve) (S. aureus) and Gram-negative (-ve) (E. coli) bacteria. The results of PVP/gelatin/Zn NPs' antibacterial activity are displayed in Fig. 5 (a, b) and Table 3. Gram-positive (+ve) bacteria (S. aureus) were more impacted by the concentration of scattered zinc oxide nanoparticles than Gram-negative (-ve) bacteria (E. coli).

Comparison of Inhibition Zone Diameter (cm) by PVP/Gelatin/ zinc oxide nanoparticles with different studies.

Table 4 shows the comparison of PVP/ Gelatin/ zinc oxide nanoparticles with different studies, since the obtained data indicated that the prepared composite in the present study has a higher antibacterial activity against some types of gram-positive and gram-negative bacteria.

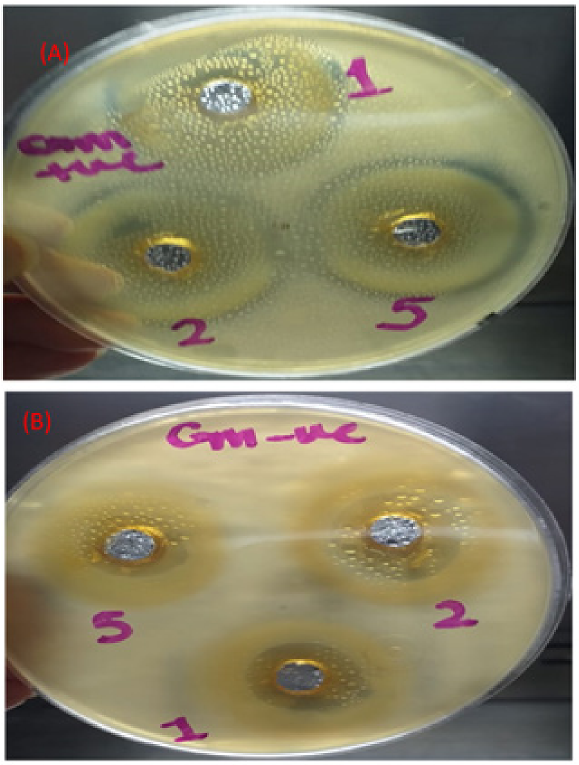

Fig. 5. Antibacterial activity of PVP/Gelatin/ zinc oxide nanoparticles, A: Gram-positive (+ve) bacteria (S. aureus), and B:Gram-negative (-ve) (E. coli).

TABLE 3 . Inhibition zone measurement ZOI of PVP/Gelatin/ zinc oxide nanoparticles in case of Gram-positive (+ve) bacteria (S. aureus), and Gram-negative (-ve) (E. coli).

		Inhibition Zone Diameter (cm)	
Sample code	Dose (kGy)	Gm(+ve) bacteria S. aureus	Gm (-ve) bacteria E. coli
1	1	3.7	3.2
2	2.5	3.4	2.8
5	5	3.5	3

TABLE 4. Comparison of Inhibition Zone Diameter (cm) by different studies.

Materials	Inhibition Zone Diameter (cm)		References
	Gm(+ve) Staph. aureus	**Gm(-ve) E.coli**	
poly(PVP/Gelatin/ zinc oxide nanoparticles)	3.6	3.1	Present work
ZnO	≥1	≥0.5	(Yousef & Danial, 2012)
ZnO nanoparticles	5	1.25	(Hoseinzadeh, Alikhani, Samarghandi, & Shirzad-Siboni, 2014)
ZnO nanoparticles	4.0	0.99	(Raghupathi, Koodali, & Manna, 2011)

Conclusion

This research, focuses on the preparation of a safe and effective nanogel composed of zinc oxide embedded within Gelatin-PVP network using gamma irradiation technology as a green technique. In this context, the most appropriate methods necessary for preparation were studied. The prepared nanogel was prepared, which is characterized by different analytical methods such as selected area electron diffraction pattern (SAED) , High resolution transmission electron microscopy (HRTEM) , FT-IR and UV-visible which indicated the structure and properties of the prepared nanogel. The results proved that using the prepared poly (PVP/Gelatin) zinc oxide nanogel as an antibacterial, has good effectiveness anti bacteria

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