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RESEARCH PAPER

Synthesis, Characterization, and Anticancer Evaluation of Iron Oxide/Graphene Oxide/Polyethylene Glycol/Paclitaxel Nanocomposites Against Breast Cancer Cells

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ABSTRACT

Paclitaxel, a widely used chemotherapeutic agent—particularly for breast cancer—faces limitations such as low bioavailability, rapid metabolism, degradation, and poor aqueous solubility. Known as a mitotic inhibitor, paclitaxel's effectiveness can be enhanced through nanocarrier systems. In this study, paclitaxel was delivered using iron oxide–graphene oxide–polyethylene glycol (iron oxide–GO–PEG) nanoparticles. The structural and morphological characteristics of both the unloaded and paclitaxel-loaded iron oxide–GO–PEG nanoparticles were analyzed using Fourier-transform infrared spectroscopy (FT-IR), scanning electron microscopy (SEM), X-ray diffraction (XRD), Raman spectroscopy, thermogravimetric analysis (TGA), and zeta potential measurements. The cytotoxic efficacy of free paclitaxel and paclitaxel-loaded nanoparticles was evaluated in MCF-7 human breast cancer cells using the MTT assay. Results showed that paclitaxel-loaded iron oxide–GO–PEG nanoparticles exhibited significantly higher cytotoxicity compared to free paclitaxel, indicating enhanced drug delivery efficiency. These findings suggest that iron oxide–GO–PEG nanocarriers represent a promising platform for improving paclitaxel delivery and offer potential for the development of more effective breast cancer therapies.

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INTRODUCTION

The chemical name of Paclitaxel is (2α, 4α, 5β, 7β, 10β, 13α)-4, 10-bis(acetyloxy)-13-{[(2R, 3S) - 3-(benzoylamino)-2-hydroxy-3-phenylpropanoyl] oxy} - 1, 7-dihydroxy-9-oxo-5, 20-epoxytax-11-en-2-yl benzoate [1].It is a natural compound extracted from the bark of the yew tree and used to treat many types of cancer, such as breast, lung, head and neck, ovarian, cervical, and pancreatic cancer[2]. It has been approved by the US Food and Drug A dministration. It is considered a mitotic inhibitor that targets tubulin.[3] The polyethylene glycol (PEG): It is a polymer used to improve the biocompatibility and functionalization of various nanomaterials, reduce non-specific uptake into cells and biological molecules, and improve drug transport in vivo, thus enhancing tumor targeting. It is also a biocompatible and hydrophilic polymer [4]. Most drug conjugates linked to polyethylene glycol are designed to deliver antitumor agents,

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such as cisplatin and paclitaxel chemotherapy, allowing them to reach the target tissues and cells and remain stable in the bloodstream. [5]. This study aims to determine the loading efficiency of a soluble anticancer drug(paclitaxel) on transition metal oxide nanoparticles due to their ease of preparation, drug loading methods, biocompatibility, low toxicity and surface functionality. [6]. Graphene is also called the material of the future because of its unique properties. It is one of the most studied materials in the world. It consists of carbon atoms with a hybridization of the SP^2 type. Each carbon atom is linked to three other carbon atoms to form a network resembling a honeycomb [7]. Graphene oxide (GO) is a layered carbon structure with oxygen-containing functional groups (=O, -OH, -O-, -COOH) attached to both sides of the layer as well as the edges of the plane. Due to its excellent electrical conductivity, optical transparency, excellent thermal conductivity, intrinsic elasticity, large specific surface area, large specific capacitance, chemical stability, electrochemical activity, high Young's modulus, and good biocompatibility, graphene is suitable for many applications in nanocomposites, catalysis, biomaterials, energy research, quantum physics, nanomedicine, nanoelectronics, as well as drug delivery for cell culture, bioimaging, tissue engineering, gene therapy, delivery of anticancer agents, and antibacterial applications[8]. Due to the technological importance of iron oxide nanoparticles, they have rapidly developed in the field of nanotechnology. To develop synthetic methods for producing iron oxide nanoparticles, several studies have been conducted, and iron oxides have been classified into nanoscale forms [9]. Iron oxide nanoparticles possess interesting electrical, optical, and magnetic properties. Iron oxide nanoparticles have broad applications in cancer treatment, drug delivery, tissue repair, textile manufacturing, catalysis, batteries, photo degradation, water treatment, processing, and the mechanical industry [10].

MATERIALS AND METHODS

Materials

poly ethylene glygol was purchased from india. phosphate-buffered saline (PBS) were purchased from Hi Media (India). paclitaxel was obtained from med chem express (MCE) amrica. Glycine was purchased from Media (India).

Fig. 1. Chemical formula for Polyethylene glycol [11].

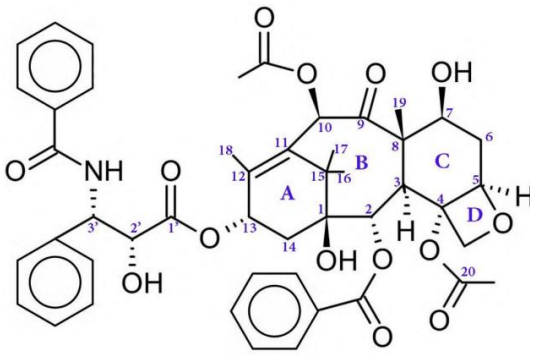

Fig. 2. Chemical formula for Paclitaxel [12].

The pH meter from OAICTON 2100-dialysis membranes were purchased from USA. dimethyl sulfoxide (DMSO) were purchased from india. dicyclohexylcarbodiamide (DCC) were purchased from india.

Instrumentation

The analysis involved various instruments uv-visible, Shimadzu, Japan. Fourier-transform infrared spectroscopy, FT-IR-8400S, Shimadzu, Japan. X-Ray Diffraction X-Ray, Siemens model D500. Scanning Electron Microscopy (SEM), ZEISS model, zeta potential (HORIBA SZ-100) and TGA (SDT Q600 V20.9 Build 20).

Methods

Synthesis of Iron oxide-GO-PEG nanocarrier

A solution containing iron oxide-GO (0.3 g) in DMSO (75 mL) mixed with PEG 200 (2.7 mL) and heated at 60 C for two days under nitrogen atmosphere. On completion of the reaction, a solution of (DCC) (0.555 g) in dimethyl sulfoxide (DMSO) (15 mL) was added to the mixture and the reaction continued for additional 24 h. The final product was a black iron oxide- GO-PEG precipitate that filtered, washed, and purified with DMSO to remove the unreacted compounds and then dried in a vacuum oven [13].

Loading of iron oxide- GO-PEG composite with Paclitaxel

Nanoparticles of 10 mg iron oxide- GO-PEG composite were immersed in paclitaxel solution [3 mg/mL, 10 ml (ethanol)]. The solution was then left to stirer for 12 hours at room temperature. The product was then washed with deionized water three times. The product was then placed in a centrifuge (5000 rpm) for 5 minutes to remove the unloaded drug. The amount of unloaded drug was then measured using UV spectrophotometry at wave length 231 nm to ascertain the percentage of paclitaxel loaded onto the iron oxide- GO-PEG composite [14].

Release (in vitro) of loaded paclitaxel

paclitaxel release was by immersing a sample of nanomaterial loaded with paclitaxel 10 mg under magnetic motor by using three pH mediums, and the pH was measured by a digital PH meter and control by using a certain buffer solution: an acidic medium (pH 2.8, glycine-

HCl buffer solution), in neutral medium (pH 7.4, phosphate-buffered saline (PBS) solution and bacic medium) pH 8, phosphate-buffered saline (PBS) solution over a period of 8 h. The amount of paclitaxel released in each case was determined by taking 5 ml aliquots of the supernatant at timed intervals. Finally, the amount of paclitaxel released was measured by applying uv-visible spectrum at 231 nm.

In vitro cytotoxicity and cell culture study

Cell culture

Breast cancer cell line (MCF7) was cultured at (Research center, at Al-Nahrain University in an {Roswell Park Memorial Institute-1640} medium (RPMI-1640)}. 1% penicillin/streptomycin solution (100X; Euroclone S.p.A.) and 10% fetal bovine serum (FBS) were added to the RPMI-1640 medium. The cells were kept in an incubator with 5% CO2 and 95% humidity at 37˚C [15,16].

Cytotoxicity assay

After culturing cells, the cytotoxic effects of paclitaxel and - iron oxide - GO -PEG-loaded paclitaxel were studied using 72-h MTT assays. 7,000cells/well were cultivated in a 96-well plate and incubated overnight to ensure cell adhesion. Cell lines (MCF7) were then exposed to increasing concentrations of compounds (6.25-100 µg/ml) three replicate wells were used for each treatment. Following incubation (72 h), the medium was removed from the plate, and 20 µl MTT solution (5 mg/ml) (Shanghai Macklin Biochemical Co., Ltd.) was added to each well and incubated for 3 h at 37˚C in the dark. To dissolve the MTT, 50 µl DMSO (Bio Basic Inc.) was added, followed by 10 min of shaking. A microplate reader (BioTek Instruments, Inc.) was used to measure the absorbance at 490 nm [17-19].

RESULTS AND DISCUSSION

The Fig. 3 The FTIR spectrum of iron oxide-GO-PEG shows the C–O of ether and

alcohol stretching bands a1089 cm^{-1} the C–O of ester at 1384.74 cm^{-1}, the C-O of ester at 1743 cm^{-1} and the C-C bonds at 1627.26 and 1465.37 cm^{-1} cm1. The stretching bands of C–H bonds were appearing at 2853.78, 2926.23, and 2856 cm1. The increased intensity of C–H and C–O stretching bands proves the effective conjugation of PEG to GO sheets, because the PEG polymer chains are full of these Bonds. In Fig. 4 the FT-IR spectra for iron oxide -GO-PEG-paclitaxel was explained,

in comparison with iron oxide -GO-PEG, the iron oxide -GO-PEG-paclitaxel showed the most characteristic bands of each though with slight shifts at 3431, 1108, and 1384 cm^{-1}. These shifts may be attributed to changes in the molecular environment or interactions between the components, indicating the successful formation of iron oxide -GO-PEG-paclitaxel nanocomposite. Fig. 5 shows the XRD pattern of iron oxide -GO -PEG. In this figure, a sharp diffraction peak at 2h = 17.5, 11.5, 20.5, 22.5, 36, 38.5, 43.5 is detected, suggesting the formation of iron oxide -GO –PEG.

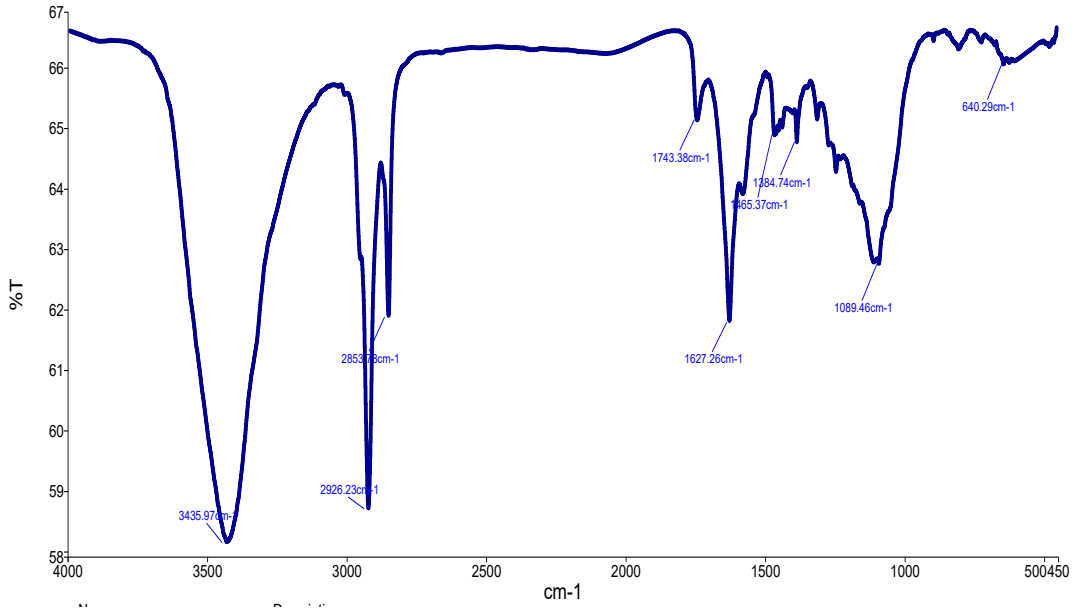

Fig. 3. FT-IR diagram of iron oxide - GO-PEG.

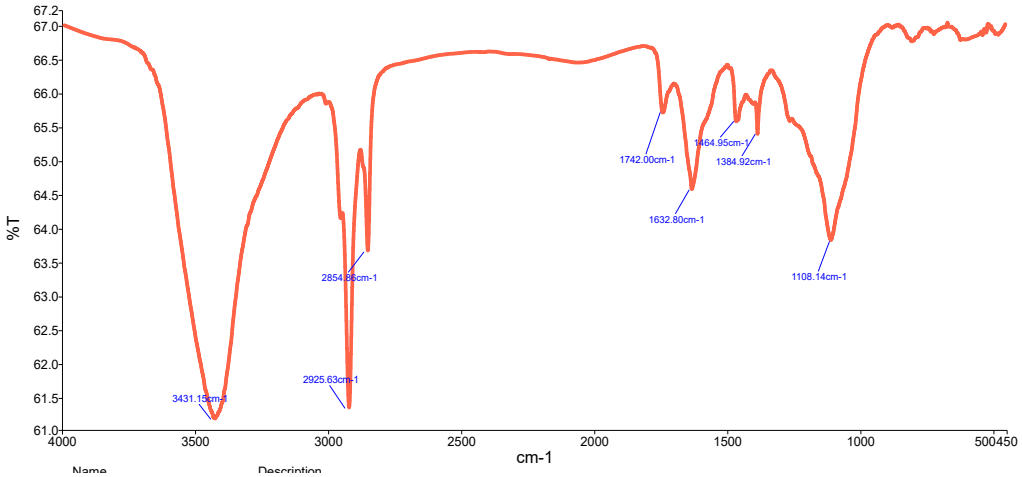

Fig. 4. FT-IR diagram of paclitaxel-loaded iron oxide - GO-PEG.

After loading paclitaxel into the iron oxide - GO-PEG nanocomposite, the XRD patterns Fig. 6 showed minimal change compared to the iron oxide - GO-PEG composite, though boarder peaks were observed. TGA curve of iron oxide - GO-PEG is shown in Fig. 7. The TGA curve represented weight

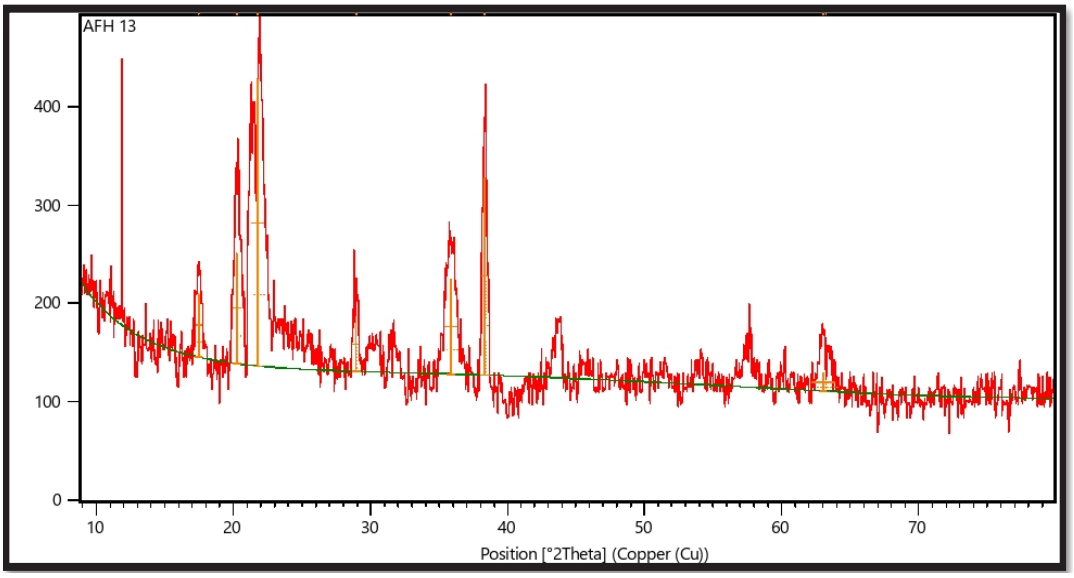

Fig. 5. XRD iron oxide - GO-PEG.

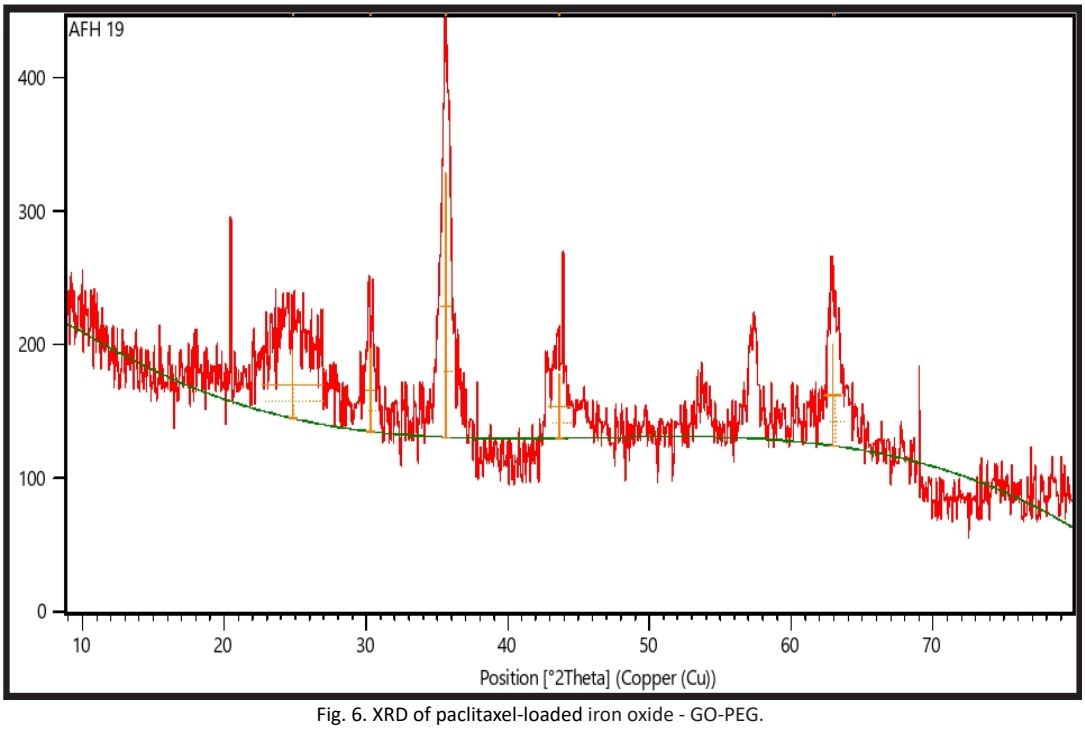

Fig. 6. XRD of paclitaxel-loaded iron oxide - GO-PEG.

loss of around 42.20 % in the sample at temperature of about 280°C and 31.73% at 370 C. This weight loss can be due to the elimination of the water molecules absorbed by the nanoparticles from the atmosphere after that sample weight is almost constant which indicates the thermal stability of the sample. TGA curve of iron oxide - GO-PEG after loading paclitaxel is shown in Fig. 8. The TGA curve represented weight loss of around 4.134% in the sample at temperature of about 150°C and

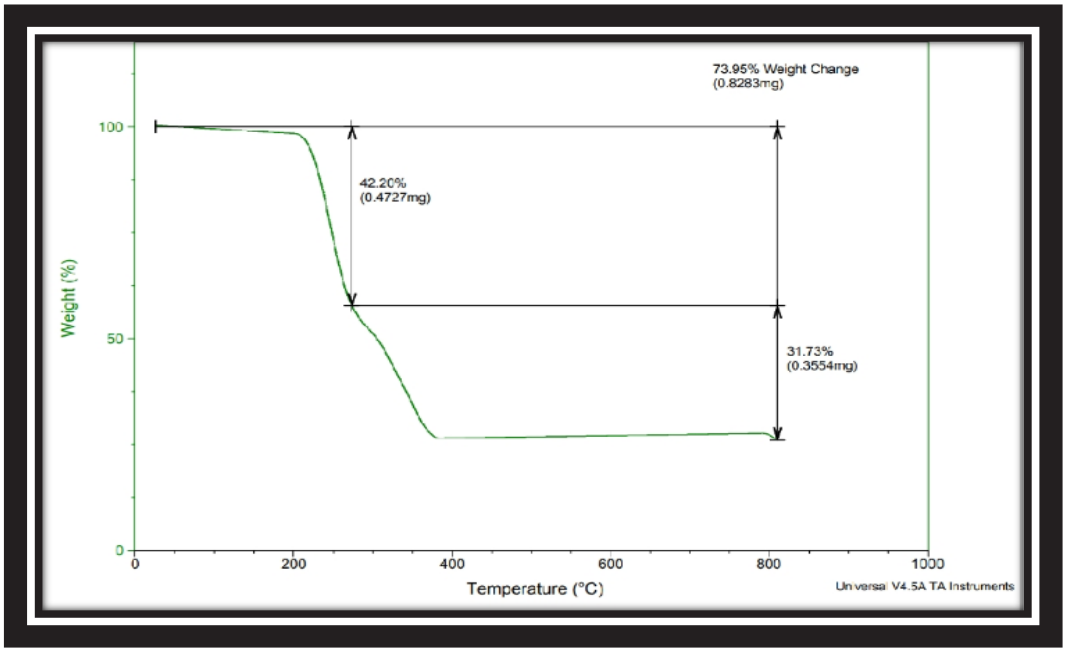

Fig. 7. TGA of iron oxide - GO-PEG.

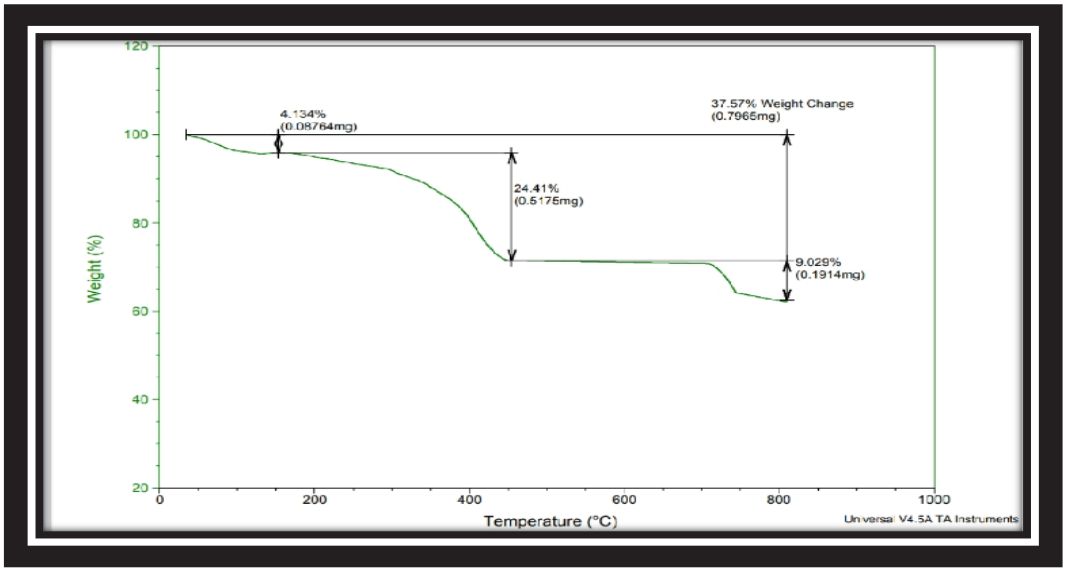

Fig. 8. TGA of paclitaxel-loaded iron oxide - GO-PEG.

24.41% at 440 C and 9.029% at 800 C. This weight loss can be due to the elimination of the water molecules absorbed by the nanoparticles from the atmosphere after that sample weight is almost constant which indicates the thermal stability of the sample [20]. Fig. 9 (SEM) shows of iron oxide - GO-PEG of the composite and reveals the homogeneous distribution of iron nanoparticles

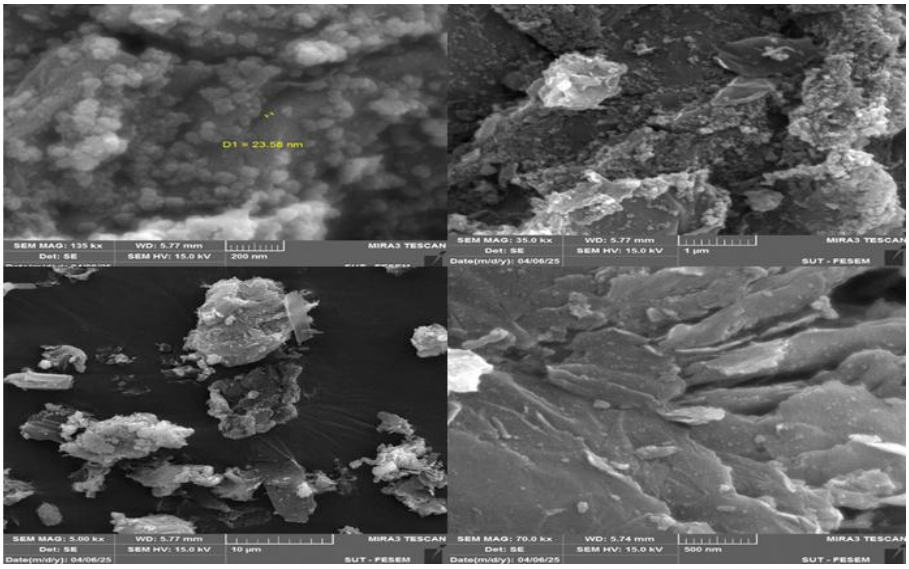

Fig. 9. SEM OF iron oxide - GO-PEG.

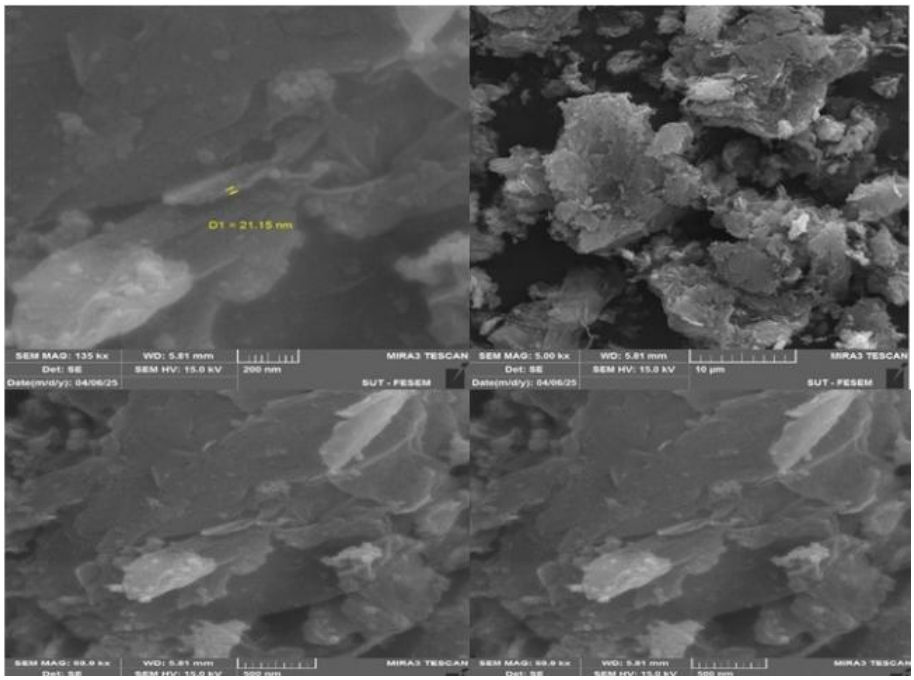

Fig. 10. SEM of paclitaxel-loaded iron oxide - GO-PEG.

and polyethylene glycol on graphene nanosheets in the interconnected graphene oxide network. Most of the nanoparticles appeared spherical. Fig. 10 shows scanning electron microscope images after loading paclitaxel onto iron oxide - GO-PEG the nanostructure. Loading the paclitaxel onto the hierarchical spherical structures resulted in structures that differed slightly in shape and size compared to the iron oxide - GO-PEG. These slight changes in size and shape are due to the successfulloading of the paclitaxel onto the surface of the nanostructure. Zeta potential for iron oxide - GO-PEG and iron oxide - GO-PEG after loading paclitaxel are -24.8 and -21.0,

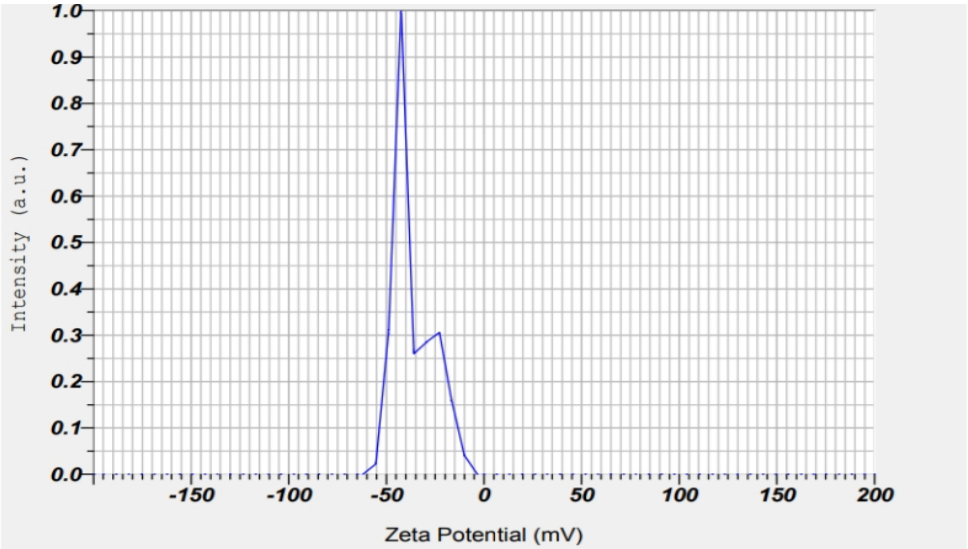

Fig. 11. Zeta potential of iron oxide - GO-PEG.

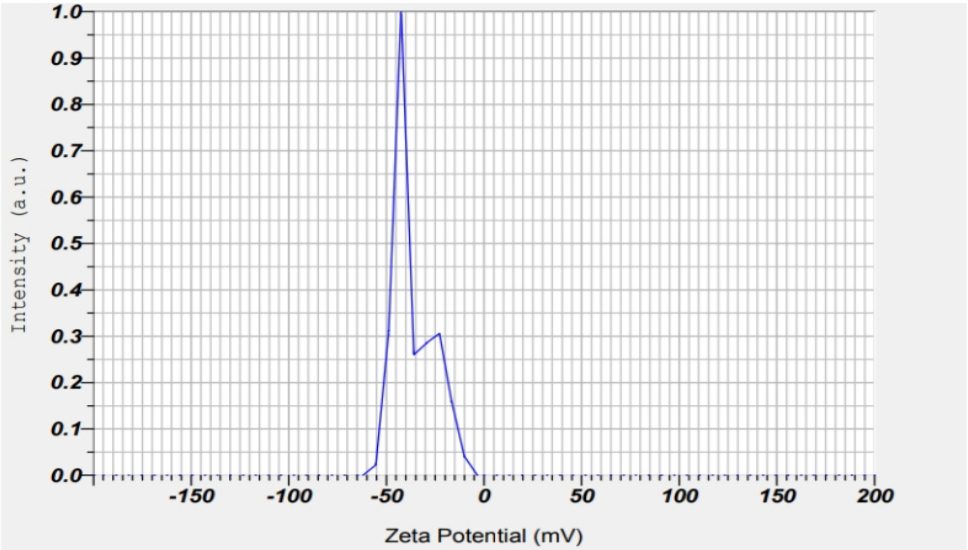

Fig. 12. Zeta potential of paclitaxel-loaded iron oxide - GO-PEG.

respectively as shown in the Fig. 11 and Fig. 12 which represents our nanocarrier has a highly negative surface charge. Therefore, it seems our compound has properly potential for using in drug delivery [21]. Fig. 13 raman spectrum show the G band is observed at 1500 cm1 and the D band is observed at 1300 cm1 and Fig. 14 raman spectrum after loading drug show the G band is observed at 1503 cm1 and the D band is observed at 1310 cm1. We did not observe any change in the bands before and after drug loading, indicating that drug loading did not alter the structural integrity of graphene [22].

Drug loading and release study in vitro

Encapsulation efficiency was 67.68%. It was demonstrated that paclitaxel release is time-dependent and greater in a pH of 2.8 which are

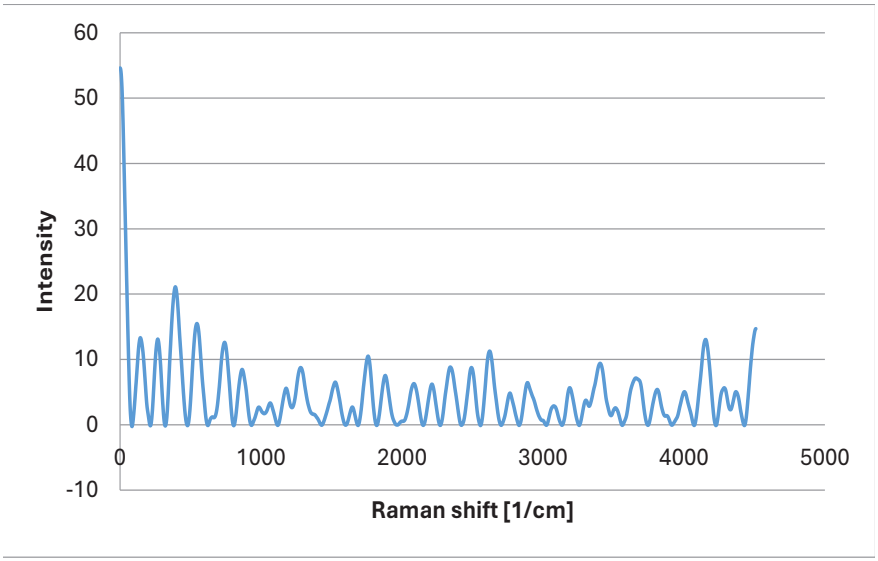

Fig. 13. Raman of iron oxide - GO-PEG.

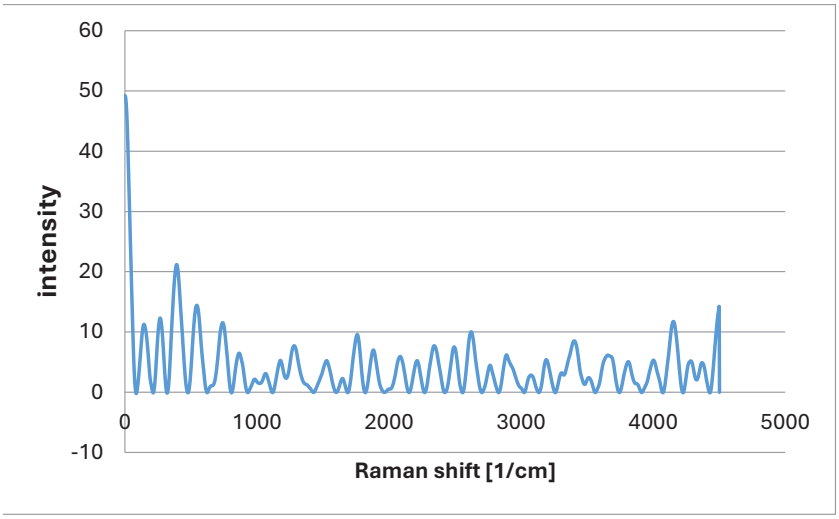

Fig. 14. Raman of paclitaxel loaded iron oxide - GO-PEG.

characteristic of an environment with cancerous cells. As can be seen from Fig. 15. at pH 7.4 the release of paclitaxel reaches 39% in 8 h, On the other hand, at pH 2.8 the total amount of paclitaxel released over 6 h was only 20%. at pH 8 the total amount of paclitaxel released over 8 h was only 26% indicating that the absorption of paclitaxel is favored at lower pH. Thus, it can clearly be concluded that the release behavior of paclitaxel from iron oxide -GO-PEG nanostructures was pH responsive.

Cell cytotoxicity (MTT assay)

to study the cytotoxic effect (MTT assay) of free paclitaxel and paclitaxel -loaded iron oxide-GO-PEG, cells of the MCF-7 human breast cancer cell line were treated with diverse concentration (6.25-100 µg/ml) of paclitaxel and paclitaxel -loaded iron oxide-GO-PEG for 72h. (Fig. 16). After 72 h treatment with paclitaxel and paclitaxel -loaded

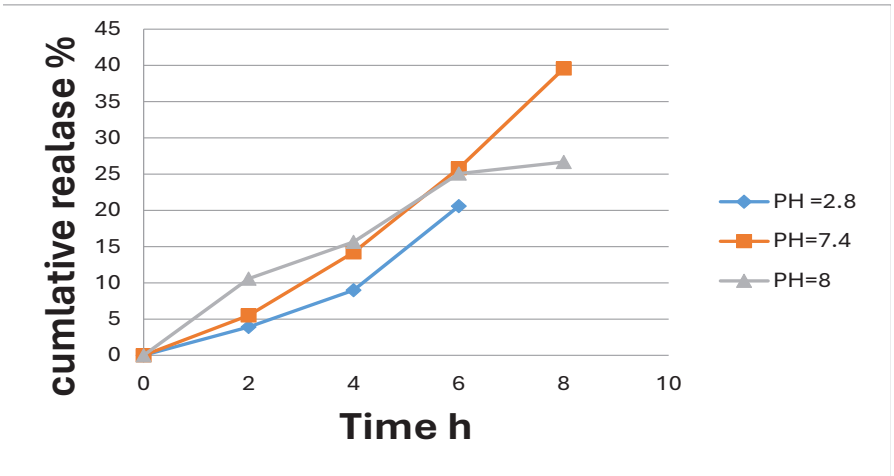

Fig. 15. Paclitaxel release for different pH.

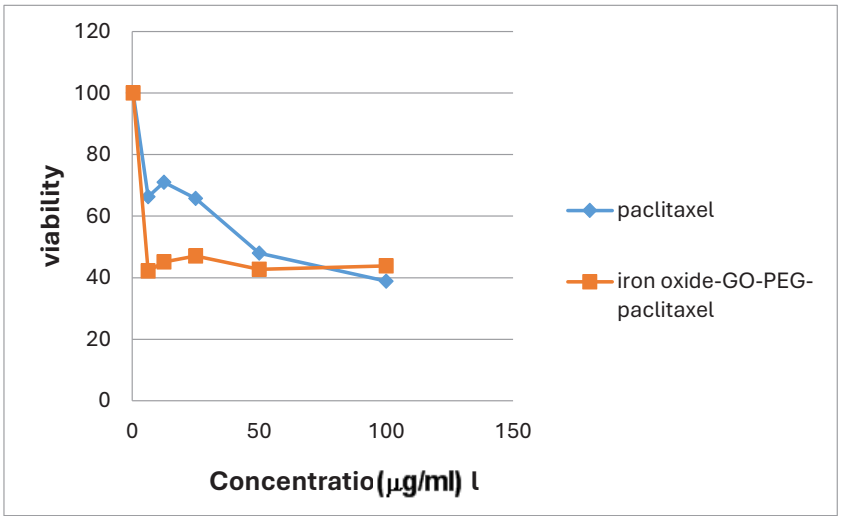

Fig. 16. MTT assay results for 72h. Cytotoxic effects of different concentrations of free paclitaxel and paclitaxel-loaded iron oxide-GO-PEG in the MCF-7 human breast cancer cell line.

iron oxide-GO-PEG nanoparticles, the IC50 values were 46.8 and 5.33 mg/ml respectively [23].

CONCLUSION

In this study, we investigate the potential of GO as an efficient system for loaded drug. Upon successful encapsulation of paclitaxel into GO, the anticipated sustained release of paclitaxel was achieved. Moreover, the present study revealed that paclitaxel-loaded iron oxide-GO -PEG nanoparticles have an inhibitory effect on the MCF-7 human breast cancer cell line to a greater degree than pure paclitaxel. This inhibition is dose and time dependent, as an analysis of our data demonstrates that iron oxide -GO -PEG particles release paclitaxel dose and time dependently. To discriminate between normal and cancerous cells.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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