

Impact of High Molecular Weight Chitosan on Theranostic Performance of Curcumin-Loaded Magneto-Graphene Quantum Dots Nanocomposite

Baharak Divband (PhD)¹, Eman Turkey Shamkhy (PhD)², Shaimaa Sabte Mutlak (PhD)², Nahideh Gharehaghaji (PhD)^{3,4*}, Nasim Roustaei (MSc Student)^{4,5}, Sahba Mofazal (PhD Candidate)⁶

ABSTRACT

Background: The type of coating material plays an important role in the imaging and treatment properties of cancer nanotheranostics.

Objective: The aim of this study was to investigate the effect of high molecular weight chitosan coating on the theranostic properties of the curcumin-loaded magneto-graphene quantum dots ($M_{NPs}/G_{QDs}/Chit_{HMW}/Cur$) nanocomposite.

Material and Methods: In this in vitro study, $M_{NPs}/G_{QDs}/Chit_{HMW}$ nanocomposite was synthesized by the sol-gel method, loaded with anticancer drug curcumin, and characterized using different analytical techniques. Then, the curcumin loading, the drug release at pH 5.5 and pH 7.4, cytotoxicity, MRI transverse relaxivity, and fluorescence spectrum of the nanocomposite were investigated.

Results: The findings showed a curcumin loading capacity of 98%, burst drug release at pH 5.5 (79.00% after 24 h), and sustained drug release at pH 7.4 (33.14% after 24 h). Significant concentration-dependent toxicity against MCF-7 cells was observed for the drug-loaded nanocomposite, while the unloaded nanocomposite exhibited the high cytocompatibility. The imaging results showed an MRI transverse relaxivity of $215.4 \text{ mM}^{-1} \text{ S}^{-1}$, and excellent fluorescence properties.

Conclusion: Using high molecular weight chitosan coating in $M_{NPs}/G_{QDs}/Chit_{HMW}/Cur$ nanocomposite provides adequate cancer theranostic performance.

Keywords

Chitosan; Molecular Weight; Magnetic Resonance Imaging; Fluorescence; Curcumin; Nanocomposite

Introduction

Recently, theranostics have attracted increased attention because they combine diagnosis and therapy. Using theranostics containing imaging contrast agents and anticancer drugs in one carrier provides real-time imaging and monitoring of treatment outcomes. Polysaccharides are natural biomolecules widely investigated to develop theranostics because they can act as a matrix to load both imaging contrast agents and drugs simultaneously [1]. Chitosan is a cationic

¹Molecular Medicine Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

²Department of Basic Science, College of Dentistry, University of Baghdad, Iraq

³Medical Radiation Sciences Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

⁴Department of Radiology, Faculty of Allied Medical Sciences, Tabriz University of Medical Sciences, Tabriz, Iran

⁵Student Research Committee, Tabriz University of Medical Sciences, Tabriz, Iran

⁶Department of Medical Imaging Technology, Molecular Imaging, Faculty of Advanced Technologies in Medicine, Iran University of Medical Sciences, Tehran, Iran

*Corresponding author: Nahideh Gharehaghaji
Department of Radiology, Faculty of Allied Medical Sciences, Tabriz University of Medical Sciences, Tabriz, Iran
E-mail: gharehaghajin@tbzmed.ac.ir

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polysaccharide produced from deacetylated chitin [2]. Based on chitosan molecular weight, it can be classified into high, medium, and low molecular weight chitosan [3]. Chitosan-based nanomaterials have been used in the biomedical field due to their non-toxic, biocompatible, biodegradable, and antimicrobial properties [4]. Chitosan derivatives-based MRI contrast materials have great potential for diagnosing diseases [5]. The primary amino groups of chitosan make it suitable for pharmaceutical applications [2]. Besides, chitosan significantly improves the biodegradability and biocompatibility of magnetic nanocarriers [6].

The diagnostic portion of magnetic nanotheranostics can consist of MRI contrast agents solely for contrast-enhanced MRI, or in combination with other imaging modalities' contrast materials for bimodal imaging [7-9]. The nanoprobe containing magnetic and fluorescent components are highly useful for early cancer diagnosis [10]. MRI provides detailed anatomical images of soft tissues without using ionizing radiation [11], and fluorescence imaging enables high-sensitivity cancer detection or real-time cancer imaging [12]. Nanotheranostics based on iron oxide nanoparticles have been extensively investigated for cancer imaging and treatment [13]. They act as MRI contrast agents in cancer theranostics because of their superparamagnetic property, biocompatibility, and chemical stability [14]. The nanoparticles have also been used as drug carriers in cancer therapy, providing MRI-guided therapy and monitoring treatment outcome [15]. Graphene quantum dots (GQDs) are widely used in fluorescence imaging and theranostics due to their excellent water dispersibility, small size, and chemical stability [16]. Moreover, low cytotoxicity, easy functionalization, and large surface-to-volume ratio of graphene quantum dots make them appropriate for biomedical applications [17].

Curcumin (Cur) is a polyphenol derivative of *Curcuma longa* with antitumor, chemo-preventive, chemo-sensitizing, [18] antioxidant,

and anti-inflammatory properties. However, rapid metabolism, poor bioavailability and poor aqueous solubility of curcumin limit its therapeutic efficacy. Nanocarrier-based curcumin delivery improves its stability, solubility, cellular uptake, and controlled release, enhancing anticancer efficacy while reducing side effects [19].

Investigation of the diagnostic and/or therapeutic properties of the nanomaterials containing iron oxide nanoparticles, graphene derivatives, and chitosan, with curcumin or other agents was carried out in the previous studies. In one study, the hybrid chitosan-grafted graphene oxide/magnetic nanoparticle system (MNP/GO/chitosan) was designed for MRI and doxorubicin delivery. Baktash et al. investigated chitosan with different molecular weights and reported that the nanosystem incorporating high molecular weight chitosan exhibited the most favorable theranostic properties [20]. However, the MRI transverse relaxivity was relatively low due to chitosan grafting. Moreover, the fluorescence property of the nanosystem was not reported in the study. The potential of GQDs modified magnetic chitosan as doxorubicin delivery nanosystems (DOX-Fe₃O₄@CGA) for targeted synergistic photothermal-chemotherapy of hepatocellular carcinoma was investigated in another study. The results showed the excellent tumor imaging and drug encapsulation capabilities of the magnetic chitosan [21]. Notably, the MRI and fluorescence properties of the nanosystem were not investigated. Kazemi et al. explored a medium-molecular-weight chitosan-based magnetite/reduced graphene oxide nanocomposite for curcumin delivery, without assessing its imaging properties [22]. In our previous study, we investigated low molecular weight chitosan to coat iron oxide/graphene quantum dots, producing a multifunctional nanoprobe for dual-mode MRI/fluorescence imaging and 5-fluorouracil delivery [9]. However, chitosan molecular weight influences the imaging and drug delivery properties of the nanomaterial.

Herein, we synthesized a theranostic nanocomposite containing Fe_3O_4 nanoparticles as the core and high molecular weight chitosan functionalized graphene quantum dots as the coating for bimodal MRI and fluorescence imaging and curcumin delivery. The use of high molecular weight chitosan can improve the loading and release of hydrophobic curcumin due to multiple binding sites. Besides, the formation of a thicker chitosan coating may reduce the cytotoxicity of the nanomaterial in the absence of curcumin. This in vitro investigation can also benefit from the advantages of bimodal imaging and achieving sufficient MRI relaxivity based on the synthesis method.

This study aimed to investigate the effect of high molecular weight chitosan coating on the curcumin loading/release, cytotoxicity, MRI transverse relaxivity, and fluorescence spectrum of the curcumin-loaded magnetographene quantum dots nanocomposite.

Material and Methods

Materials

Iron(III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), Iron(II) chloride tetrahydrate ($FeCl_2 \cdot 4H_2O$), citric acid, chitosan (molecular weight (MW) >700 kDa), 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), N-hydroxysuccinimide (NHS), sodium hydroxide (NaOH), and curcumin were obtained from Sigma-Aldrich. We prepared all aqueous solutions using double distilled water. We used the chemicals without further purification. Also, we washed all glassware with distilled water and dried them before using.

Methods

This study had an in vitro design with the following steps:

Synthesis of $M_{NPs}/G_{QDs}/Chit_{HMW}$ nanocomposite

The preparation of the water-soluble GQDs was carried out by using a simple and

environment-friendly method, pyrolysis of citric acid (CA), in accordance with the literatures [23, 24]. In short, 2 g of CA was heated to 200 °C for 5 min, then continued up to 30 min to produce a light orange product. Subsequently, 100 mL of a 0.25 mol/L NaOH solution was added vigorously and continuously until a pH of 7. Finally, the solvent was separated and the solid product was dried and named G_{QDs} .

For preparation of magnetic G_{QDs} , 200 mL of a 0.2 g of as-prepared G_{QDs} solution was sonicated for 15 min. Then, 10 mL solution of $FeCl_3 \cdot 6H_2O$ (0.025 M) was added to G_{QDs} solution at room temperature under vigorous stirring (under a nitrogen flow). The temperature was increased to 80 °C, and $FeCl_2 \cdot 4H_2O$ (0.01 M) was added slowly, then vigorously stirred for an additional 30 min. The ammonia solution was added dropwise to adjust the pH of the solution to 10. The product was washed, dried and named M_{NPs}/G_{QDs} .

For the preparation of magnetic G_{QDs} coated with chitosan, as reported [25], high molecular weight chitosan ($Chit_{HMW}$) was dissolved in deionized water containing 2% (v/v) acetic acid and stirred at 1500 rpm for 12 h. The chitosan gel was sonicated for 10 min to eliminate bubbles. The resulting gel was added to as-prepared M_{NPs}/G_{QDs} in the presence of EDC/NHS (1:3 ratio) to prepare $M_{NPs}/G_{QDs}/Chit_{HMW}$.

Curcumin loading and in vitro release

The curcumin loading and release process was carried out according to the method described in the previous studies [7, 26]. Briefly, a suspension containing 1 g of the nanocomposite in 10 mL of distilled water was added to a curcumin solution (2 mg/mL) and stirred at room temperature for 48 h. Following incubation, the mixture was centrifuged, washed, dried, and named $M_{NPs}/G_{QDs}/Chit_{HMW}/Cur$. The encapsulation efficiency of curcumin within the nanocarrier was determined by measuring the absorbance of the supernatant using a visible spectrophotometer (Shimadzu 1700). To

evaluate the in vitro release performance, 50 mg of $M_{NPs}/G_{QDs}/Chit_{HMW}/Cur$ nanocomposite was stirred in 30 mL of phosphate-buffered saline (PBS), and placed inside a dialysis bag (12 kDa). The release experiment was carried out at 37 °C. At predetermined time intervals, 1 mL of the dialysate was withdrawn and replaced with an equal volume of the solution of fresh buffer. The release profile of curcumin was subsequently obtained as a function of time and the mean $\pm$ standard deviation (SD) were determined.

Physicochemical tests

The crystalline structure of M_{NPs}/G_{QDs} nanoparticles was assessed by a Philips X-ray diffractometer. The morphology and size of $M_{NPs}/G_{QDs}/Chit_{HMW}/Cur$ nanocomposite were analyzed by a field emission scanning electron microscope (FE-SEM) (Philips ES 30 KW). Vibrating sample magnetometry (VSM) was carried out to measure the saturation magnetization of the nanocomposite and determine its magnetic property. A VSM (model 7400) was used for magnetometry at room temperature under magnetic fields ranging from -12000 to $+12000$ G.

MTT assay

The nanocomposite cell toxicity was investigated using 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay [27]. A 96-well plate containing 3×10^8 MCF-7 cells was used for cell culturing with 200 μ L medium/well at 37 °C during 24 h. Various concentrations (50, 100, 200, and 400 μ g/mL) of $M_{NPs}/G_{QDs}/Chit_{HMW}$ and the curcumin-loaded nanocomposite were added to the culture medium and incubated for 24 h. Adding MTT solution (50 μ L) and culture medium (150 μ L) to each well was carried out to measure the cell proliferation. The cells were incubated at 37 °C with 5% CO_2 for 24 h. Then, MTT solution was removed from the wells, and 200 μ L/well of dimethyl sulfoxide and 25 μ L/well of Sorensen buffer were added. To measure

the optical absorbance, an ELISA plate reader (BioTek, Bad Friedrichshall, Germany) was used at a wavelength of 570 nm.

MRI

The samples with different iron concentrations (ranging from 0 to 0.04 mM at 0.01 mM intervals) dispersed in the agar aqueous solution were prepared. MRI of the samples was performed using a 1.5 T MRI system (MAGNETOM Amira, Siemens, Germany). The MR imaging parameters for acquiring the T2-weighted images with a multi-spin echo pulse sequence were repetition time (TR)/echo time (TE) of 3800/13-208 ms at 13 ms intervals, slice thickness of 5 mm, field of view (FOV) of 184×184 mm², and matrix of 128×128 pixels. The T2-weighted images in DICOM format were transferred to a personal computer. Sante DICOM Viewer 3D Pro 4.9.4 (64-bit) software was used to calculate the signal intensity of the samples by drawing a fixed-size region of interest at the center of the samples. Drawing the T2 curves and calculating the T2 relaxation rates ($1/T_2$) were carried out using MATLAB R 2016 software. The relaxation rates were then used to plot the $1/T_2$ versus iron concentration graph for determining the transverse relaxivity (r_2).

Fluorescence spectrometry

Fluorescence features of $M_{NPs}/G_{QDs}/Chit_{HMW}/Cur$ nanocomposite were investigated by a monochromator-based fluorescence spectrometer (Perkin Elmer LS 55, USA). The measurements were evaluated using excitation and emission wavelengths of 360 nm and 400-660 nm, respectively, at room temperature and a constant pH of 7.

Statistics

We used GraphPad Prism software version 10.6 to determine the mean $\pm$ SD of the results. A two-way analysis of variance (ANOVA) was used to assess statistical significance.

Results

Curcumin loading/release

Figure 1A shows the curcumin loading profile of the nanocomposite. Rapid absorption of 40% of the total drug into the nanocomposite was observed within 15 min. The remaining 58% was slowly absorbed (89% after 6 h and 98% after about 24 h).

The release profiles of curcumin from the nanocomposite at pH 5.5 (tumoral acidic environment) and pH 7.4 (physiological condition) are shown in Figure 1B. The curcumin release from the nanocomposite after 24 h was 79.00% and 33.14% at pH 5.5 and pH 7.4, respectively. It was increased to 89.40% after 48 h at pH 5.5. Then, the curve reached a plateau after 72 h with a curcumin release of 95.23%.

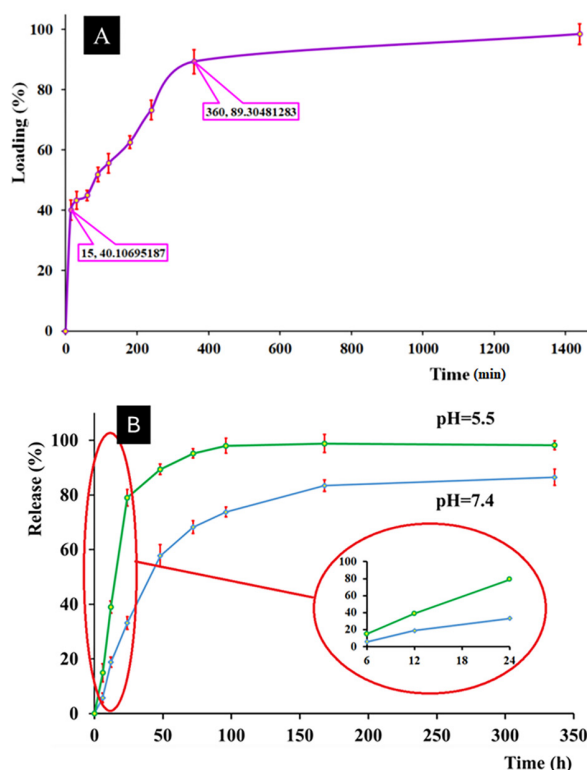


Figure 1: A) Curcumin loading profile of M_{NPs}/G_{QDs}/Chit_{HMW}/Cur nanocomposite (n=3), B) Curcumin release profiles from the nanocomposite at pH 5.5 and pH 7.4 (n=3). Inset represents the magnified view of the curcumin release after 24 h at pH 5.5 and pH 7.4

On the other hand, at pH 7.4, curcumin release from the nanocomposite was 57.73% after 48 h, and then reached a plateau after 168 h (a curcumin release of 83.42%). The results indicate a pH-dependent curcumin release pattern from the nanocomposite.

Physicochemical tests results

The X-ray diffraction (XRD) pattern of M_{NPs}/G_{QDs} nanoparticles is shown in Figure 2. The characteristic peaks, identified at about 2θ = 31°, 35°, 43°, 53°, 57° and 63°, are related to Fe₃O₄ (PDF#01-076-1849). The GQDs generally appear as weak, broad humps overlapping with the Fe₃O₄ background, making them difficult to distinguish due to the low GQDs content.

The FE-SEM image of M_{NPs}/G_{QDs}/Chit_{HMW}/Cur nanocomposite (Figure 3) shows the iron oxide nanoparticles with a spherical shape. Another notable observation was that some of the nanoparticles exhibited a nanorod structure (right region). The findings indicate that M_{NPs}/G_{QDs}/Chit_{HMW}/Cur nanocomposite was formed by the agglomeration of these spherical nanoparticles and nanorods. The agglomerated structure of the nanocomposite showed a wide range of sizes, mainly larger than 100 nm.

Based on VSM results, the relationship between the magnetization and applied magnetic field confirms the superparamagnetic property

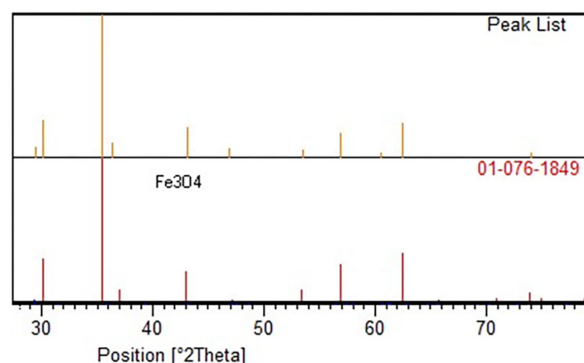


Figure 2: X-ray diffraction (XRD) pattern of M_{NPs}/G_{QDs} nanoparticles.

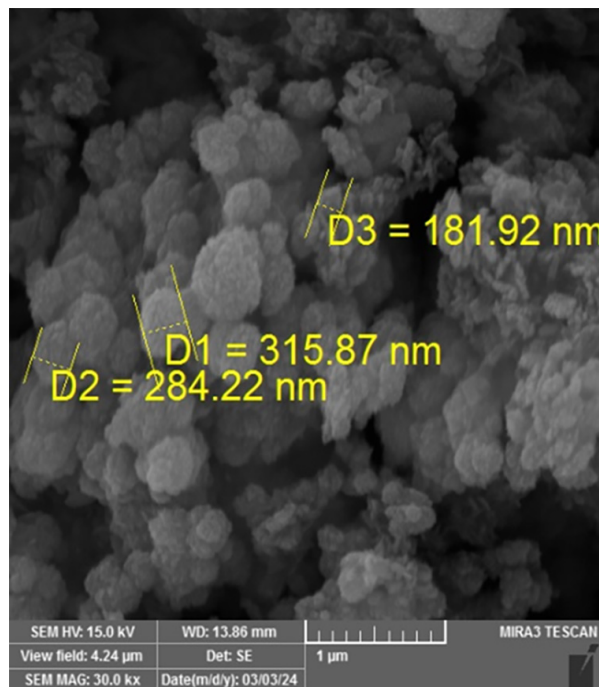


Figure 3: Field emission scanning electron microscope (FE-SEM) image of $M_{NPs}/G_{QDs}/Chit_{HMW}/Cur$ nanocomposite.

of the nanocomposite. The saturation magnetization was 20.34 emu/g (Figure 4).

Cytotoxicity

Figure 5 illustrates the MCF-7 cell viability

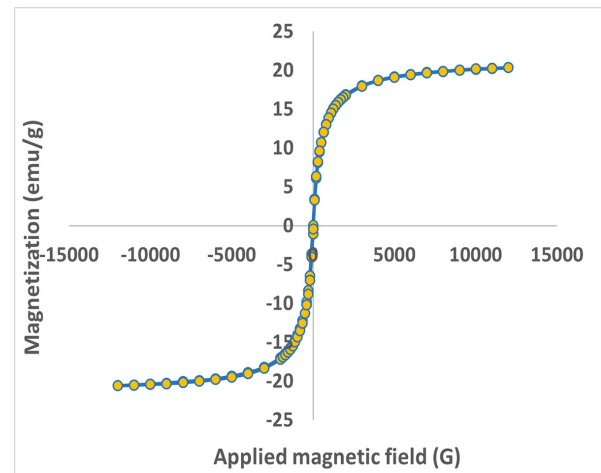


Figure 4: $M_{NPs}/G_{QDs}/Chit_{HMW}/Cur$ nanocomposite magnetization curve.

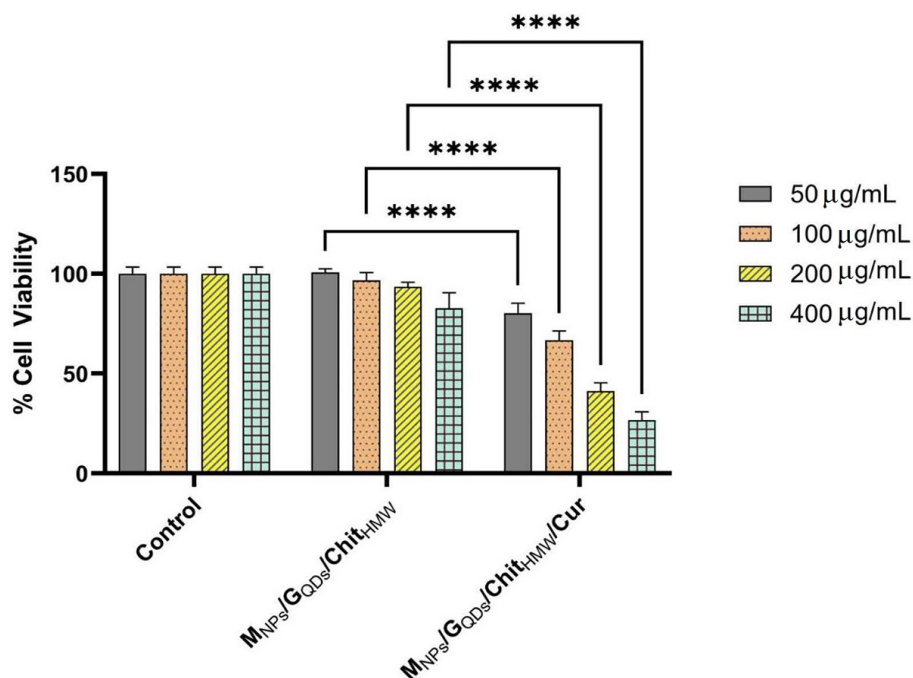


Figure 5: Cell toxicity of $M_{NPs}/G_{QDs}/Chit_{HMW}$ and $M_{NPs}/G_{QDs}/Chit_{HMW}/Cur$ nanocomposites against MCF-7 cells. Data are shown as the mean $\pm$ standard deviation ($n=4$). Level of **** $P \leq 0.0001$ shows the significant difference between the cell viability of curcumin-loaded and unloaded nanocomposites at all concentrations.

after treatment with various concentrations of $M_{NPs}/G_{QDs}/Chit_{HMW}$ and the curcumin-loaded nanocomposite. For all concentrations of $M_{NPs}/G_{QDs}/Chit_{HMW}$ nanocomposite, the cell viability was more than 80%. The concentration-dependent decrease in the cell viability was seen for the curcumin-loaded nanocomposite. Although the lowest concentration (50 $\mu\text{g/mL}$) showed the cell viability of more than 80%, MCF-7 cell viability was decreased significantly at the higher concentrations and reached 26.71% for the highest concentration (400 $\mu\text{g/mL}$).

MRI results

The signal intensity of each sample was decreased exponentially with increasing TE in the T2 curves. The lowest T2 relaxation time was related to the highest iron concentration. According to Figure 6, a linear relationship between the relaxation rate and iron concentration was seen in the relaxivity graph. The transverse relaxivity was calculated to be $215.4 \text{ mM}^{-1} \text{ S}^{-1}$.

Fluorescence spectrum

Figure 7 presents the photoluminescence (PL) excitation spectrum of $M_{NPs}/G_{QDs}/Chit_{HMW}/Cur$ nanocomposite (excitation wavelength = 400 nm, emission wavelength = 465 nm). Because the fluorescence intensity and emission peaks of GQDs and their composites vary with pH, all fluorescence measurements were performed at a constant pH of 7 [9].

Discussion

Curcumin loading/release

Based on Figure 1A, which shows the curcumin loading profile, high molecular weight of chitosan has a significant effect on the initial high loading of curcumin due to the greater availability of amino ($-\text{NH}_2$) and hydroxyl ($-\text{OH}$) functional groups for hydrogen bonding and electrostatic interactions with the drug molecules. The remaining drug is redirected

gradually to other available binding sites until the nanocomposite reaches its maximum drug-loading capacity. This is due to the greater viscosity of high molecular weight chitosan during nanoparticle preparation, which reduces drug diffusion into the external medium and consequently increases the encapsulation efficiency.

The release rate of curcumin was higher at pH 5.5 compared to pH 7.4. The inset of Figure 1B shows more than a twofold higher release at pH 5.5 during the first 24 h.

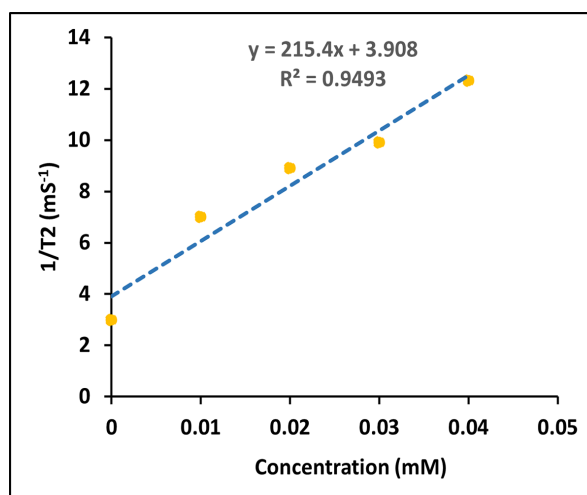


Figure 6: Transverse relaxivity graph of $M_{NPs}/G_{QDs}/Chit_{HMW}/Cur$ nanocomposite.

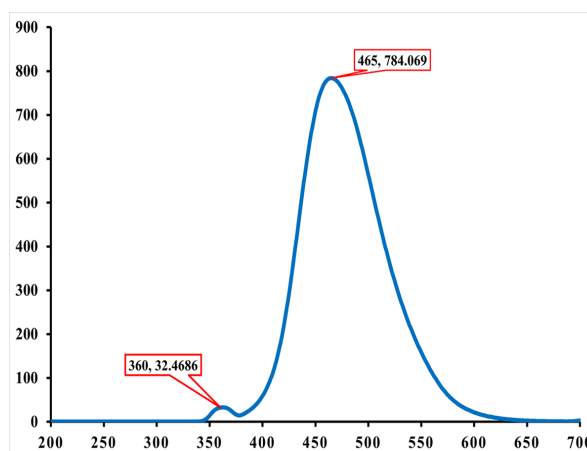


Figure 7: Fluorescence spectrum of $M_{NPs}/G_{QDs}/Chit_{HMW}/Cur$ nanocomposite.

Degradation of chitosan in response to pH 5.5 (as an acidic condition) can lead to faster drug release from the nanocomposite compared to physiological pH [28]. This pH-responsive drug release can be attributed to the protonation of the chitosan amino groups under acidic condition, which increases polymer swelling and weakens the drug-polymer interactions, leading to a significantly higher release of curcumin at pH 5.5 compared to pH 7.4. At physiological pH, the compact chitosan coating restricts drug release, thereby enabling sustained delivery and minimizing premature drug release.

Physicochemical tests

The XRD results (Figure 2) represented the successful synthesis of the magnetic nanoparticles. The characteristic Fe_3O_4 peaks remained clearly visible, indicating that the crystal structure of magnetite was preserved. The GQDs generally appear as a weak broad hump, which may overlap with the Fe_3O_4 background and be difficult to distinguish because of their low content. No additional diffraction peaks were observed, confirming the phase-pure nature of the composite. The absence of peaks corresponding to hematite (Fe_2O_3) or maghemite ($\gamma\text{-Fe}_2\text{O}_3$) indicates that no secondary iron oxide phases formed during synthesis.

As is evident from the FE-SEM image of the nanocomposite (Figure 3), the aggregation observed in these magnetic nanoparticles can be attributed to their intrinsic magnetic property and the polymeric structure of high molecular weight chitosan. Using high molecular weight chitosan significantly affected the morphology and particle size of $\text{M}_{\text{NPs}}/\text{G}_{\text{QDs}}/\text{Chit}_{\text{HMW}}/\text{Cur}$ nanocomposite. The larger particle size observed after high molecular weight chitosan coating can be attributed to the formation of a thicker chitosan layer due to its longer polymer chains and higher viscosity. The abundant amino and hydroxyl functional groups of high molecular weight chitosan also enhance the interfacial interactions, affecting the particle

growth and aggregation behavior [29].

Based on Figure 4 related to the VSM results, the magnetization versus applied magnetic field curve did not show any hysteresis loop, coercivity, or remanent magnetization, confirming the superparamagnetic property of $\text{M}_{\text{NPs}}/\text{G}_{\text{QDs}}/\text{Chit}_{\text{HMW}}/\text{Cur}$ nanocomposite. Although the presence of the non-magnetic high molecular weight chitosan as a coating material around the nanocomposite led to a decrease in the saturation magnetization (20.34 emu/g), the saturation magnetization is still sufficient to allow the nanocomposite to be directed when using an external magnetic field. The saturation magnetization of $\text{M}_{\text{NPs}}/\text{G}_{\text{QDs}}/\text{Chit}_{\text{HMW}}/\text{Cur}$ nanocomposite is higher than that of the DOX-loaded graphene quantum dots mediated magnetic chitosan (DOX- $\text{Fe}_3\text{O}_4@\text{CGA}$) [21]. However, compared to the 5-fluorouracil-loaded chitosan-coated iron oxide/graphene quantum dots (MGC-FU) nanohybrid with low molecular weight chitosan, $\text{M}_{\text{NPs}}/\text{G}_{\text{QDs}}/\text{Chit}_{\text{HMW}}/\text{Cur}$ showed a lower saturation magnetization [9].

Cell toxicity

According to the MTT results (Figure 5), $\text{M}_{\text{NPs}}/\text{G}_{\text{QDs}}/\text{Chit}_{\text{HMW}}$ nanocomposite showed a high cytocompatibility due to the presence of high molecular weight chitosan as a coating material. Even at the lowest concentration of the curcumin-loaded nanocomposite, using high molecular weight chitosan led to a high cytocompatibility. However, the cell toxicity was increased significantly at the higher concentrations of the curcumin-loaded nanocomposite.

MRI

The signal loss increasing as a function of the iron concentration indicates the negative contrast provided by the nanocomposite. The local magnetic field inhomogeneities related to the iron oxide nanoparticles enhance the water protons dephasing, shortening the T2 relaxation time. The higher the iron

concentration, the stronger the T2 shortening and signal loss. Figure 6 illustrates a linear relationship between $1/T_2$ relaxation rate and iron concentration with a high slope, which is essential for a nanomaterial to be considered as an MRI contrast agent. The transverse relaxivity was calculated $215.4 \text{ mM}^{-1} \text{ S}^{-1}$. The results confirm the potential of the nanocomposite to be used as a T2 contrast agent for MRI. Despite using high molecular weight chitosan as the nanocomposite coating, which causes limited access of the iron oxide core to the water protons, the resulting transverse relaxivity was sufficient to produce negative contrast on the MR images. The transverse relaxivity of $M_{\text{NPs}}/G_{\text{QDs}}/\text{Chit}_{\text{HMW}}/\text{Cur}$ nanocomposite is higher than that of Resovist and Endorem, as commercial negative contrast agents [30].

A comparison of the transverse relaxivity of $M_{\text{NPs}}/G_{\text{QDs}}/\text{Chit}_{\text{HMW}}/\text{Cur}$ nanocomposite with that of the doxorubicin-loaded hybrid chitosan-grafted graphene oxide/MNP nanosystem containing high molecular weight chitosan [20] shows that the former has much higher relaxivity. The differences in the relaxivities can be attributed to the variations in the size and properties of the iron oxide nanoparticles and nanosystems, drug loading, and synthesis methods. Chitosan grafting in their study may reduce the accessibility of the magnetic nanoparticles to surrounding water protons, thereby decreasing the transverse relaxivity.

The molecular weight of chitosan affects the relaxivity of the nanomaterials. The transverse relaxivity of $M_{\text{NPs}}/G_{\text{QDs}}/\text{Chit}_{\text{HMW}}/\text{Cur}$ nanocomposite is lower than that of the 5-fluorouracil-loaded chitosan-coated iron oxide/graphene quantum dots (MGC-FU) nanohybrid [9] containing low molecular weight chitosan. Although the sizes of the nanomaterials and the drugs used in these studies are different, the observed differences in transverse relaxivity may also be associated with the molecular weight of chitosan. Because of twisted and tangled chains of high molecular weight chitosan that provide a greater distance between the

iron oxide nanoparticles and the surrounding water protons, the access of the magnetic core to the water protons is limited. This leads to less T2 shortening, resulting in a lower transverse relaxivity.

Fluorescence properties

The PL excitation spectrum of the GQDs exhibits a broad excitation band in the UV region, typically extending from 250 to 400 nm, with a maximum excitation wavelength around 320–360 nm, depending on the synthesis condition. This broad excitation band is attributed to the π – π transitions of the sp^2 carbon domains and the n – π transitions associated with oxygen-containing functional groups (e.g., C=O and C–O) on the surface of the GQDs. The incorporation of the Fe_3O_4 nanoparticles may affect the excitation intensity or induce a shift in the excitation maximum due to the electronic interactions and charge transfer between the GQDs and Fe_3O_4 . Based on Figure 7, $M_{\text{NPs}}/G_{\text{QDs}}/\text{Chit}_{\text{HMW}}/\text{Cur}$ nanocomposite exhibited significant emission peaks in the visible region (400–610 nm) compared to the pristine GQDs, indicating their superior energy gap at 400 nm excitation wavelength. $M_{\text{NPs}}/G_{\text{QDs}}/\text{Chit}_{\text{HMW}}/\text{Cur}$ nanocomposite had a wide FWHM of about 89 nm. The red shift indicates that the composite can be efficiently excited at the longer wavelengths, suggesting a reduction in the effective band gap and enhanced electronic coupling between the GQDs and Fe_3O_4 . This shift is attributed to the charge transfer interactions at the $M_{\text{NPs}}/G_{\text{QDs}}$ interface, the formation of new electronic states, and the influence of the surface functional groups, which collectively modify the electronic structure of the composite. The extension of the emission band into the visible region is beneficial for applications such as bioimaging, as it enables more effective utilization of visible light.

Compared to other studies, the $\text{Fe}_3\text{O}_4@\text{CS-GQDs}$ nanocomposite exhibited an emission peak at 434 nm under excitation light of 330 nm [31]. Also, the chitosan-coated iron oxide/

graphene quantum dots (MGC) and 5-fluorouracil-loaded MGC (MGC-FU) nanohybrids showed stable emission peaks at 492 and 493 nm, respectively, under excitation light of 400 nm [9].

Conclusion

In this study, the effect of high molecular weight chitosan coating on the theranostic properties of the curcumin-loaded magnetographene quantum dots was investigated. For this purpose, the structural, magnetic, relaxometric, fluorescence, curcumin loading/release, and cytotoxic properties of the nanocomposite were evaluated. The longer polymer chains and higher viscosity of high molecular weight chitosan had an important role in forming a thicker chitosan layer and in the particle growth and agglomerated structure of the nanocomposite.

From the therapeutic point of view, due to the multiple binding sites, high molecular weight chitosan provided high loading capacity and pH-dependent curcumin release. The presence of high molecular weight chitosan coating resulted in the high cytocompatibility for the nanocomposite without curcumin loading, whereas the curcumin-loaded nanocomposite induced significant cytotoxicity toward breast cancer cells at the high concentrations.

From the diagnostic point of view, using high molecular weight chitosan coating led to decrease in the saturation magnetization and transverse relaxivity of the nanocomposite. However, the measured relaxivity was sufficiently high to qualify the superparamagnetic nanocomposite as a potential MRI T2 contrast agent. The fluorescence spectrum showed that the emission band extends into the visible region, enabling efficient utilization of visible light important for bioimaging applications.

This study was performed in vitro; therefore, future in vivo studies are essential to confirm the nanocomposite capability for biomedical applications.

Authors' Contribution

B. Divband and N. Gharehaghaji contributed to study design and data curation. B. Divband, ET. Shamkhy, and N. Gharehaghaji were responsible for methodology and validation. B. Divband, ET. Shamkhy, and SS. Mutlak performed the formal analysis. B. Divband, N. Roustaei, and N. Gharehaghaji contributed to conceptualization. S. Mofazal, N. Roustaei, and SS. Mutlak provided resources. N. Gharehaghaji was responsible for funding acquisition and supervision. All authors contributed to writing and reviewing the manuscript, and read, modified, and approved the final version of the manuscript.

Ethical Approval

This study was approved by the Research Ethics Committees of the Vice Chancellor in Research Affairs - Tabriz University of Medical Sciences (Ethical code: IR.TBZMED.VCR.REC.1401.104).

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Conflict of Interest

None

Data Availability Statement

All data supporting this article have been included as part of the main manuscript.

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