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Article

Superparamagnetic Graphene–Based Anticancer Drug Carrier for Targeted Drug Delivery

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Abstract: A superparamagnetic graphene oxide based drug carrier Fe₃O₄@SiO₂/N-rGO was prepared through surface modification, material doping, and composite methods. This carrier has a large number of active sites, suitable for loading anticancer drugs, It can increase the drug load. Currently, the widely used drug in clinical use is epirubicin hydrochloride (EPI). The graphene based complex has a pH sensitive property and exhibits a pH sensitive property at pH=4.3. The release efficiency (RE) is 77.4%, making it suitable for drug release in cancerous acidic liquids. The physicochemical properties of drug carriers were studied using X-ray diffraction, Raman spectroscopy, and Fourier transform infrared spectroscopy. Electrochemical signals confirm that the anticancer drug EPI acts on the graphene plane through hydrogen bond interactions and $\pi - \pi$ stacking.

Keywords: drug carrier; Fe₃O₄@SiO₂/N-rGO; targeted drug delivery

1. Introduction

As populations age and socioeconomic development accelerates, cancer has been a major cause of reduced life expectancy in many countries[1]. Compared with traditional treatment, various therapeutic methods have been researched and developed, such as chemodynamic therapy (CDT) [2].

Magnetic iron oxide (Fe₃O₄) has a wide range of raw materials, low cost, and is easy to surface modify[3]. Superparamagnetic Fe₃O₄ nanoparticles have biocompatibility and low cytotoxicity. Fe₃O₄ nanoparticles are prepared by solvothermal method[4], and the combined method of surface charge and steric resistance is used to improve the stability of Fe₃O₄[5]. Silica has a mesoporous structure and a surface containing silanol groups[6]. The magnetic iron oxide coated on the outer layer has the ability to provide pharmaceutical active sites. The surface modification of silicon layer can effectively change the surface charge, functional property, and reactivity, and improve the stability and dispersion of particles[7].

Graphene is a two-dimensional sp² hybrid carbon sheet with excellent physical and chemical properties[8], such as large surface area, easy functionalization, and rich functional groups. Graphene has various functional groups on its surface, making it easy to carry out surface modification[9], and has a large specific surface area, which is conducive to the uniform dispersion of the anticancer drug EPI on the graphene material. Reduced graphene oxide (rGO) has been used as a nano carrier for gene or drug delivery in cancer treatment. The interaction of curcumin Fe₃O₄/rGO complex[10] with human serum albumin (HSA). It has been proved that Fe₃O₄/rGO not only has low toxicity to HSA, but also has an enhancing effect[11]. Polyethyleneglycolated and functionalized magnetic graphene oxide (MG-NH₂-PEG) complexes are used as nanocarriers[12]. Polyethyleneglycols reduce biological toxicity and use $\pi - \pi$ interactions to load doxorubicin (DOX) onto MG-NH₂-PEG. MG-NH₂-PEG

has excellent superparamagnetism and can be used as targeted drug carriers[13]. At low temperature, free polymer graphene aerogel nanoparticles (GA NPs) were synthesized using graphene oxide sheets[14], which were loaded with ionized doxorubicin hydrochloride (DOX), showing high pH sensitivity[15].

Currently, the main drug delivery targets used are 5-FU[16], paclitaxel[17], and DOX[18]. EPI is a new generation of anthracycline anticancer drugs with few toxic and side effects, but still has cardiac toxicity[19]. Targeted drug delivery systems are used to deliver EPI to patient sites, reducing the release of non patient sites, improving drug efficiency, and reducing damage to the human body[20].

In this paper, a superparamagnetic $\text{Fe}_3\text{O}_4/\text{SiO}_2/\text{N-rGO}$ drug carrier with a saturation magnetization of 40.9 emu g^{-1} was prepared, which can respond to changes in external magnetic fields and achieve magnetic targeted drug delivery. Using EPI as a targeted drug, due to the high specific surface area of graphene, there are many functional groups on the pore surface, $\text{Fe}_3\text{O}_4/\text{SiO}_2$ dispersed on the folded surface of graphene, it provides active sites for drug loading, achieving the effect of adsorbing drugs through hydrogen bonds and electrostatic adsorption, increasing the drug loading amount.

2. Materials and Methods

2.1. Experimental Materials

Chemicals $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, N,N-dimethylformamide (DMF), glucose, urea, and anhydrous ethanol were purchased from Aladdin Chemical Co. Conductive carbon black, hydrogen peroxide, concentrated sulfuric acid, nitric acid, and potassium permanganate were purchased from China National Pharmaceutical Group Chemical Reagent Co. Epiamphenicol hydrochloride (EPI) was obtained from Sigma Aldrich.

2.2. Synthesis of Magnetic (Fe_3O_4) Nanoparticles

In the synthesis of superparamagnetic Fe_3O_4 nanoparticles, a transparent yellow solution was first formed by sonicating a DMF solution of ferric chloride hexahydrate (200 mg) and urea (300 mg) containing glucose (500 mg) for 30 min. The solution was sealed in a stainless steel autoclave lined with polytetrafluoroethylene and heated at 200°C for 8 h. The solution was washed with deionized water and anhydrous ethanol, washed several times by centrifugation and dried to obtain powder samples. Finally, the powder samples were annealed at 450°C for 2h in an argon atmosphere.

2.3. Synthesis of $\text{Fe}_3\text{O}_4/\text{SiO}_2$

Fe_3O_4 (500 mg) and PVP (1 g) were dispersed in deionized water (60 mL), anhydrous ethanol (90 mL) and ammonia (4 mL) and sonicated for 30 min. followed by the addition of TEOS (1.6 mL) and mechanical stirring for 1 h. The $\text{Fe}_3\text{O}_4/\text{SiO}_2$ nanocomposite was separated by centrifugation and washed with deionized water, followed by ethanol to remove The excess NH_3 in the solution was then removed with ethanol.

2.4. Synthesis of Graphene Oxide

Graphene oxide was prepared via modified Hummer's method. First, graphite powder (2 g) and concentrated sulfuric acid (200 mL) were stirred magnetically at 45°C for 40 min, followed by potassium permanganate (2 g) and concentrated nitric acid (20 mL) for 20 min. Subsequently, the temperature of the solution was increased to 60°C and stirred for 8 h to obtain a brownish solution. The solution was diluted and hydrogen peroxide (20mL) was added dropwise, and the color of the solution changed to golden yellow. The solution was repeatedly washed and diluted by deionized water until the solution became neutral. After centrifugation, freeze-drying (12h) was performed to obtain graphene oxide powder (GO).

2.5. Preparation of $\text{Fe}_3\text{O}_4@\text{SiO}_2/\text{N-rGO}$

$\text{Fe}_3\text{O}_4@\text{SiO}_2$ (40 mg) and GO (60 mg) were dissolved in deionized water (60 mL) and sonicated for 30 min. The solution was sealed in a stainless steel autoclave lined with PTFE and heated at 200 °C for 2 h. The solution was centrifuged and freeze-dried (12 h) to obtain a superparamagnetic $\text{Fe}_3\text{O}_4@\text{SiO}_2/\text{rGO}$ drug carrier. After ultrasonic treatment, ammonium carbonate (100 mg) was added to obtain $\text{Fe}_3\text{O}_4@\text{SiO}_2/\text{N-rGO}$ by heat treatment and freeze drying.

2.6. Drug Loading Preparation of EPI-Loaded $\text{Fe}_3\text{O}_4@\text{SiO}_2/\text{rGO}$

First, the superparamagnetic $\text{Fe}_3\text{O}_4@\text{SiO}_2/\text{rGO}$ (10 mg) was dissolved in phosphate buffer solution (PBS, pH 7.2, 10 mL) containing different concentrations of EPI and sonicated for 30 min. After that, the solution was magnetically stirred for 30 min to mix the EPI with the drug carriers. The supernatant was collected for UV-Vis analysis to determine the free EPI concentration in the supernatant. The loading efficiency (LE) and loading capacity (LC) were determined as:

$$\text{Loading efficiency (LE)} = \frac{W_{\text{EPI}} - C_{\text{supernatant}} V_{\text{supernatant}}}{W_{\text{EPI}}} \quad (1)$$

$$\text{Loading capacity (LC)} = \frac{W_{\text{EPI}} - C_{\text{supernatant}} V_{\text{supernatant}}}{W_{\text{drug carrier}}} \quad (2)$$

where W_{EPI} is the amount of initial anticancer drug EPI in phosphate buffer, $C_{\text{supernatant}}$ and $V_{\text{supernatant}}$ are the concentration and volume of EPI in the supernatant, and $W_{\text{drug carrier}}$ is the amount of $\text{Fe}_3\text{O}_4@\text{SiO}_2/\text{rGO}$ drug carrier.

2.7. Drug Release

EPI release experiments were performed in an incubator at a constant temperature of 37°C to simulate human body temperature. $\text{Fe}_3\text{O}_4@\text{SiO}_2/\text{rGO}$ -EPI (20 mg) drug carriers were dispersed in phosphate buffer (pH=7.2) and acetate buffer (pH=4.3). The supernatant was collected at predetermined time intervals and UV-Vis analysis was performed to determine the free EPI concentration in the supernatant. The loading efficiency (LE) and loading capacity (LC) were determined as:

$$\text{Release efficiency (Re)} = \frac{\sum C_{\text{release}} V_{\text{release}}}{W_{\text{EPI}} - C_{\text{supernatant}} V_{\text{supernatant}}} \quad (3)$$

where W_{EPI} is the amount of initial anticancer drug EPI in phosphate buffer, $C_{\text{supernatant}}$ and $V_{\text{supernatant}}$ are the concentration and volume of EPI in the supernatant, C_{release} and V_{release} are the concentration and volume of EPI released from the drug carrier in the supernatant.

The surface morphology of nanocomposites was characterized using field emission scanning electron microscopy (JSM-IT300) from Zeiss GmbH in Germany and energy dispersive X-ray spectroscopy (EDS) from Kratos Axis GmbH in the UK. The crystal structure of nanocomposites was analyzed by X-ray diffraction (XRD) using the Shimadzu X-ray diffractometer 7000 from Shimadzu Co., Ltd. of Japan. Using the HR800 Raman microscope from Jobin Yvon GmbH, France λ = Raman spectroscopy was performed at 488 nm excitation wavelength. All cyclic voltammetric (CV) measurements were performed using the CHI660D electrochemical workstation of Shanghai Chenhua Instrument Co., Ltd. The magnetic properties of nanocomposites were analyzed using the American LakeShore 7404 vibrating sample magnetometer (VSM). The zeta potentials of different samples were characterized using Litesizer 500 from Anton Parr GmbH, Austria. Finally, a Lambda 650S ultraviolet spectrophotometer from Perkin Elmer Co., Ltd. in the United States was used for quantitative analysis of the anticancer drug EPI.

3. Results and Discussion

3.1. Structural Characterization

Figure 1 depicts the schematic process of formation mechanism for magnetic drug carrier. In this experiment, $\text{Fe}_3\text{O}_4@\text{SiO}_2$ nanocomposites were prepared by hydrothermal method and their structure and crystallographic surfaces were characterized by XRD in order to further determine a better understanding of the formation of $\text{Fe}_3\text{O}_4@\text{SiO}_2/\text{rGO}$ nanocomposites. To characterize the formation of Fe_3O_4 magnetic nanomaterials, XRD (Figure 2) was used to determine their structure and crystallographic planes. The diffraction peaks appear at $2\theta = 18.4^\circ, 30.34^\circ, 35.7^\circ, 43.2^\circ, 53.5^\circ, 57.4^\circ$, and 62.8° , corresponding to the crystallographic planes (1 1 1), (2 2 0), (3 1 1), (4 0 0), (4 2 2), (5 1 1), and (4 4 0). The XRD spectrum of Fe_3O_4 referring to the JCPDS database (File No. 19–0629)[21], which shows the formation of Fe_3O_4 with a cubic anti-spinel structure. In addition, the formation of high-purity Fe_3O_4 can also be observed because there are no peaks of other phases in the XRD pattern. In Figure 2, the XRD diffraction pattern of $\text{Fe}_3\text{O}_4@\text{SiO}_2$ shows a diffraction peak of 22.8° , which is the amorphous diffraction peak of SiO_2 [22].

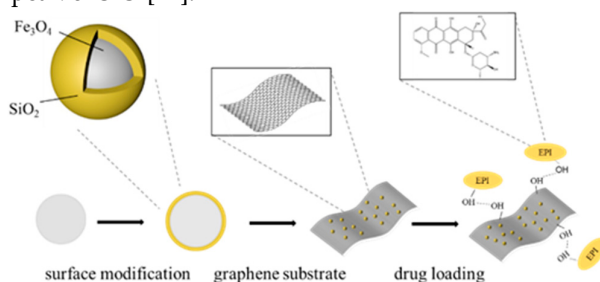


Figure 1. The schematic processes of $\text{Fe}_3\text{O}_4@\text{SiO}_2/\text{N-rGO-EPI}$.

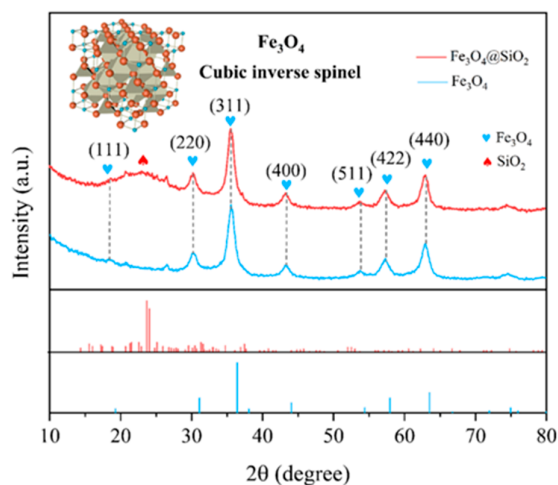


Figure 2. XRD diffraction patterns of Fe_3O_4 and $\text{Fe}_3\text{O}_4@\text{SiO}_2$.

To further determine the formation of $\text{Fe}_3\text{O}_4@\text{SiO}_2$ composites, the characteristic peaks of the composites were determined using Fourier infrared spectroscopy. The infrared spectra of $\text{Fe}_3\text{O}_4@\text{SiO}_2$ (Figure 3) were used to study its chemical bonding species, and the symmetric stretching vibration peaks of Si–O appeared at 467 cm^{-1} and 796 cm^{-1} , the peak at 574 cm^{-1} was the Fe–O vibrational absorption peak, the peak at 945 cm^{-1} belonged to the bending vibration absorption peak of Si–OH, and the broad and strong absorption band at 1095 cm^{-1} was the Si–O–Si. The peaks at 1625 cm^{-1} and 3413 cm^{-1} are the bending vibration peak and the antisymmetric stretching vibration peak of H–O–H of structured water, respectively. Among them, the absorption peak at 945 cm^{-1} in Figure 2 indicates the presence of silica hydroxyl group (Si–OH) on the surface of SiO_2 coated with Fe_3O_4 , which is beneficial for the subsequent loading of anticancer drugs in combination with the mesoporous structure and large specific surface area of SiO_2 .

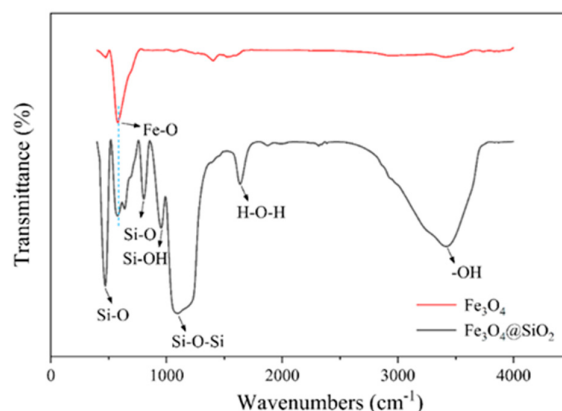


Figure 3. FTIR spectroscopy of Fe_3O_4 and $\text{Fe}_3\text{O}_4@\text{SiO}_2$.

The surface morphological characteristics of Fe_3O_4 and $\text{Fe}_3\text{O}_4@\text{SiO}_2$ were characterized by SEM, and the surface morphology is shown in Figure 4. The magnetic Fe_3O_4 has a nanosphere structure with a rough and obvious granular surface. there is a certain agglomeration between the Fe_3O_4 particles, forming nanoclusters with large size and uneven distribution between the particles, which affects the magnetic iron oxide properties. In contrast, the surface of $\text{Fe}_3\text{O}_4@\text{SiO}_2$ (Figure 4d) is smoother and has a uniform nanosphere structure. More importantly, the encapsulated SiO_2 can play a role in magnetic iron oxide to alleviate the agglomeration problem of nanoparticles and make the magnetic iron oxide uniformly dispersed, while increasing the specific surface area of magnetic iron oxide, which is beneficial to the loading of anti-cancer drugs.

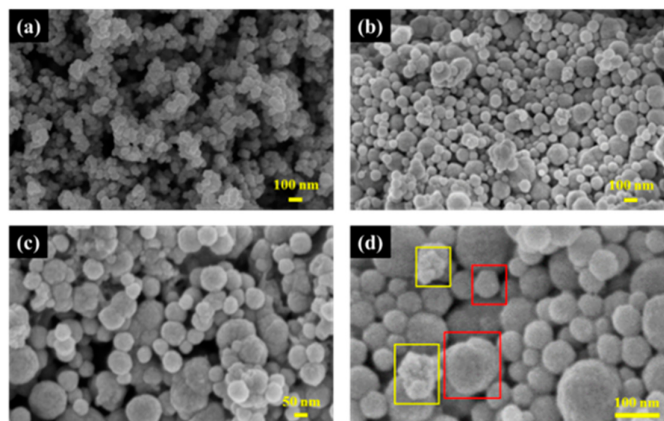


Figure 4. SEM image of Fe_3O_4 (a) and $\text{Fe}_3\text{O}_4@\text{SiO}_2$ (b–d).

The structure of graphene oxide is transparent muslin-like with shallow surface folds, while pure graphene and nitrogen-doped graphene still have the characteristics of light and transparent with significantly deeper surface folds and more bulges. The morphological characterization of the magnetic carrier composite formed after hydrothermal treatment with $\text{Fe}_3\text{O}_4@\text{SiO}_2$ is shown in Figure 5d. The $\text{Fe}_3\text{O}_4@\text{SiO}_2$ nanospheres are uniformly dispersed on the surface and folds of nitrogen-doped graphene without extensive agglomeration, indicating that graphene can overcome the agglomeration problem of nanoparticles.

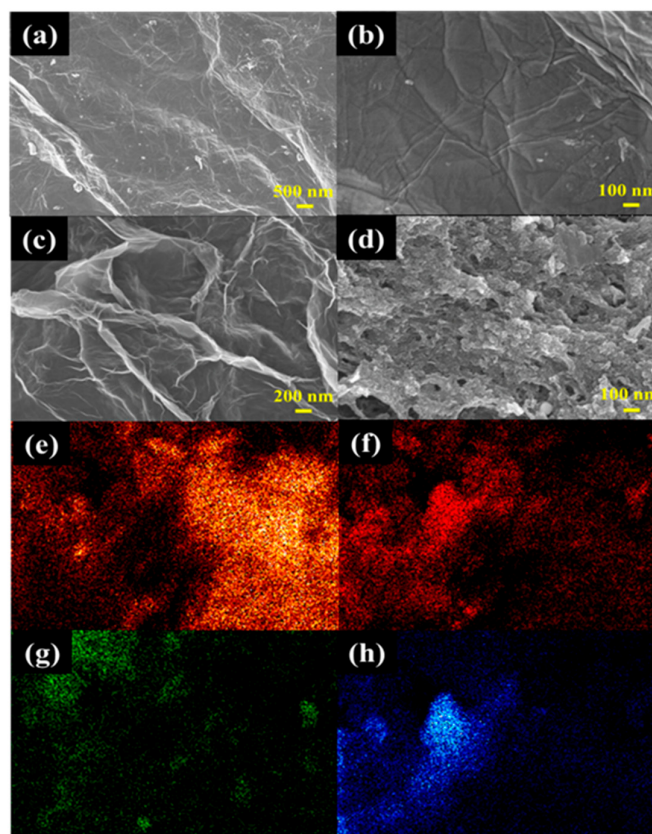


Figure 5. SEM images of GO (a–b), N-rGO (c) and (d) $\text{Fe}_3\text{O}_4/\text{SiO}_2/\text{N-rGO}$. Mapping images of (e–h) C, O, Fe and Si.

The TEM images in Figure 6 show that $\text{Fe}_3\text{O}_4/\text{SiO}_2/\text{N-rGO}$ retains a hollow structure and a shell of exceptionally thin SiO_2 after calcination treatment. HRTEM was used to further characterise the details of the hollow composites as shown in Figure 6(c, d). Folded few-layer graphene can be clearly seen on the surface of $\text{Fe}_3\text{O}_4/\text{SiO}_2/\text{N-rGO}$ as shown in Figure 6(a, b). Large $\text{Fe}_3\text{O}_4/\text{SiO}_2$ nanostructures are present on the surface of the carbon material.

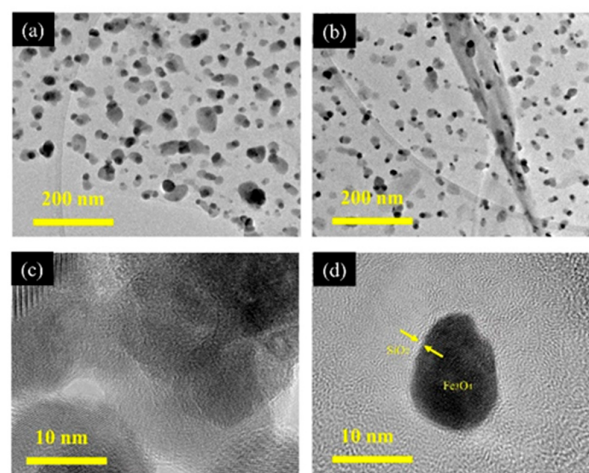


Figure 6. Encapsulation of $\text{Fe}_3\text{O}_4/\text{SiO}_2/\text{N-rGO}$ core-shell nanoparticles formed. (a and b) TEM images of the as-prepared $\text{Fe}_3\text{O}_4/\text{SiO}_2/\text{N-rGO}$ nanoparticles. (c and d) High-resolution TEM image of the $\text{Fe}_3\text{O}_4/\text{SiO}_2/\text{N-rGO}$ nanoparticles.

The Raman spectra of the materials can also be used to characterize the layers, defects and crystal structure of rGO. the Raman spectrum of the $\text{Fe}_3\text{O}_4/\text{SiO}_2/\text{N-rGO}$ composite (Figure 7) shows several well-characterized peaks. The D peak at 1348.7 cm^{-1} is caused by the reduced symmetry of carbon atom arrangement due to the finite size effect or defects of SP^3 carbon. the G peak at 1591.4 cm^{-1} reflects the regular arrangement of SP^2 carbon and is the main characteristic peak of graphene carbon, reflecting the regular arrangement of SP^2 carbon. The degree of disorder in the crystal structure is reflected in the relative intensity of the D-band. The relative intensity of the D-band reflects the degree of disorder in the crystal structure. The D/G strength ratio of the magnetic carrier composite material is 1.18. As can be seen from the figure, the relative intensity of the D peak in the Raman pattern of graphite is significantly lower than that of $\text{Fe}_3\text{O}_4/\text{SiO}_2/\text{N-rGO}$, which also proves that the crystalline structure of graphite is more ordered, which also indicates that it has not been oxidized at this time.

We can find that the D peak of $\text{Fe}_3\text{O}_4/\text{SiO}_2/\text{N-rGO}$ is higher than that of graphite, and a strong D peak indicates that there are many defects, and more functional groups on the surface are conducive to drug loading. This also indicates that the $\text{Fe}_3\text{O}_4/\text{SiO}_2/\text{N-rGO}$ has more oxygen-containing functional groups, which is conducive to the next step of drug loading.

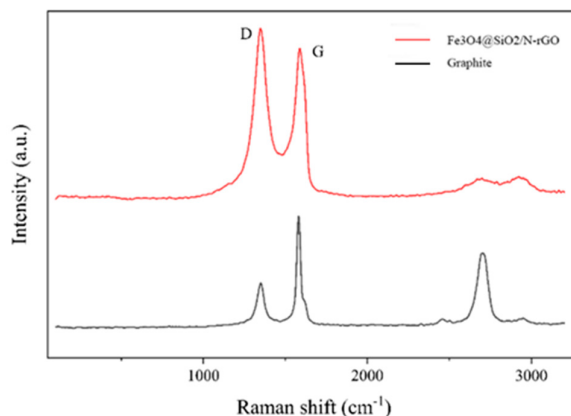


Figure 7. Raman spectra of $\text{Fe}_3\text{O}_4/\text{SiO}_2/\text{N-rGO}$ and graphite.

Zeta potential, also known as surface potential, is a characterization of the number of charges carried on the surface of the particles and can effectively characterize the stability of the material dispersion system. Initially, the absolute value of the potential of magnetic iron oxide is small, indicating that it has a high inter-particle attraction and tends to inter-particle agglomeration, which is prone to the typical agglomeration problem of nanoparticles when analyzed with SEM morphology (Figure 4a); during the subsequent surface modification process, the potential of $\text{Fe}_3\text{O}_4/\text{SiO}_2$ nanospheres increases significantly, the electrostatic repulsion between particles increases, and the system is more physically stable. $\text{Fe}_3\text{O}_4/\text{SiO}_2/\text{N-rGO}$ has a slightly decreased potential value but still maintains the system stability.

3.1. Performance of Drug Carrier

For nanomagnetic targeted drug delivery carriers, they need to have to be well dispersed in the physiological environment and able to respond to external magnetic fields for targeted drug delivery. Figure 9 shows a typical magnetization loop indicating that $\text{Fe}_3\text{O}_4/\text{SiO}_2/\text{N-rGO}$ has superparamagnetic properties. The saturation magnetization (M_s) of Fe_3O_4 is 98.3 emu g^{-1} , which decreases with silicon layer cladding and graphene loading to 81.9 and 40.9 emu g^{-1} for $\text{Fe}_3\text{O}_4/\text{SiO}_2$ and $\text{Fe}_3\text{O}_4/\text{SiO}_2/\text{N-rGO}$. However, the magnetic properties of $\text{Fe}_3\text{O}_4/\text{SiO}_2/\text{N-rGO}$, although decreased, still responded when an external magnetic field was applied, as shown in Figure 9 (inset). Initially, it shows the good dispersion of $\text{Fe}_3\text{O}_4/\text{SiO}_2/\text{N-rGO}$ in an aqueous solution in the absence of a external magnetic field. When a permanent magnet is placed, the magnetic composite is immediately attracted to the wall of the sample vial next to the external magnetic field.

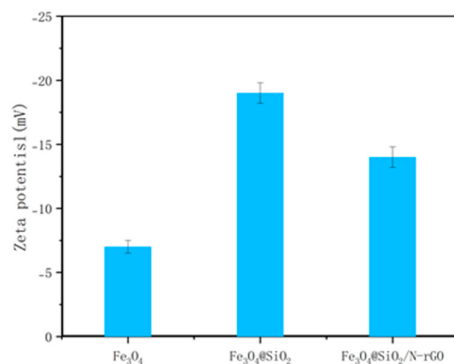


Figure 8. Zeta potential of Fe_3O_4 , $\text{Fe}_3\text{O}_4@\text{SiO}_2$ and $\text{Fe}_3\text{O}_4@\text{SiO}_2/\text{N-rGO}$.

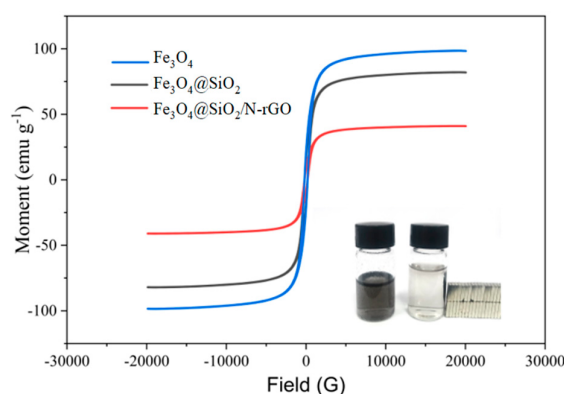


Figure 9. VSM of Fe_3O_4 , $\text{Fe}_3\text{O}_4@\text{SiO}_2$ and $\text{Fe}_3\text{O}_4@\text{SiO}_2/\text{N-rGO}$

The loading of the drug on the magnetic carrier was studied by electrochemical cyclic voltammetry. The graph (Figure 10) shows the changes in the electrochemical signal of the carriers before and after drug loading. The drug is loaded on the carriers in two ways: one is loaded onto the silicon layer surface of magnetic nanospheres by electrostatic adsorption; the other is loaded onto nitrogen-doped graphene by hydrogen bonding interactions and π - π stacking. $\text{Fe}_3\text{O}_4@\text{SiO}_2/\text{N-rGO}$ -EPI shows a higher current response, indicating that the drug can be loaded onto the graphene surface. Graphene has excellent electrical conductivity with the presence of off-domain electrons on the surface, and when the drug is loaded onto graphene via hydrogen bonding or π - π stacking, an effective electron transfer between the drug and graphene takes place, which in turn enhances the electrical conductivity. If the loading is only onto the silicon layer but not onto the graphene surface, no enhancement of the current response is observed and electron transfer is inhibited. Studying the drug loading will facilitate the subsequent drug loading and the study of pH sensitivity during drug release.

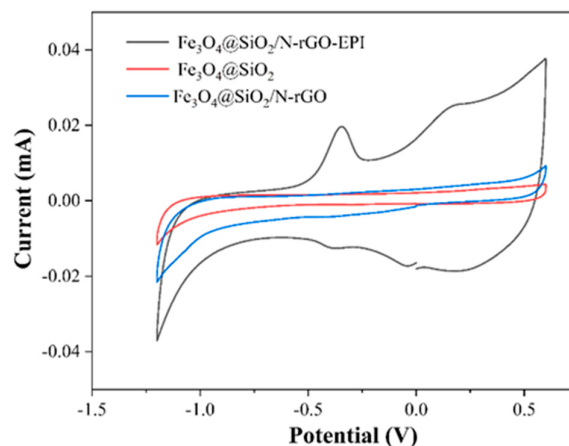


Figure 10. CV profile of $\text{Fe}_3\text{O}_4/\text{SiO}_2$, $\text{Fe}_3\text{O}_4/\text{SiO}_2/\text{N-rGO}$ and $\text{Fe}_3\text{O}_4/\text{SiO}_2/\text{N-rGO-EPI}$.

In drug delivery systems, the drug loading and release of drug carriers are the key factors for their use as drug carriers. $\text{Fe}_3\text{O}_4/\text{SiO}_2/\text{N-rGO}$ magnetic nanocomposites are suitable as magnetic targeting drug carriers in biomedical fields. The drug loaded on the carrier in this study is EPI, which causes myelosuppression, induces heart failure, and many other conditions, and thus needs to be strictly controlled in small doses for treatment. The loading efficiency and drug loading of the magnetic carrier loaded with EPI are shown in Figure 11a. The lower the concentration of EPI, the higher the $\text{Fe}_3\text{O}_4/\text{SiO}_2/\text{N-rGO}$ load efficiency. The load capacity of $\text{Fe}_3\text{O}_4/\text{SiO}_2/\text{N-rGO}$ is 47.1%, which is much greater than $\text{Fe}_3\text{O}_4/\text{SiO}_2/\text{rGO}$, and the loading capacity is increased with the increase of the initial anti-cancer drug concentration. The silicon layer encapsulated on the outside of the magnetic iron oxide has a mesoporous structure and contains silicon hydroxyl groups on its surface. The graphene substrate also has a large number of functional groups such as hydroxyl and carboxyl groups, which can bind to the anticancer drug through π - π stacking and hydrogen bonding interactions. In addition, the surface of graphene is not smooth, and this folded structure has more spatial advantages than graphene oxide in the three-dimensional structure. With the increase of EPI concentration, a large number of drug molecules with hydroxyl groups and carbon six-membered rings will more fully form intermolecular forces with the functional groups on the carrier surface, and its loading will be larger. On the contrary, the loading efficiency decreases, and the loading of the drug at the relatively easy binding sites on the surface will prevent further loading.

Drug loading has been one of the challenges in the field of drug delivery systems. The lack of targeting of anticancer drugs by direct injection and the high burden on the patient's body as they flow through the bloodstream after injection. In addition, anti-cancer drugs are expensive, and increasing the effective drug delivery rate can largely alleviate the financial pressure on patients' families. In the case of externally applied magnetic field, $\text{Fe}_3\text{O}_4/\text{SiO}_2/\text{N-rGO}$ nanocomposites possess a magnetic field response mechanism and are able to use surface modification to load a large amount of drugs and direct them to a designated location for drug release. This drug carrier can reduce the drug dose and the drug delivery rate will be greatly improved, which has a great potential as a drug carrier for cancer treatment.

To investigate the environmental conditions and extent of drug release, the release profiles of magnetic drug carriers were plotted in different pH liquid environments. In this study, it was found that the carriers have a pH response mechanism and the drug release in an acidic environment is more different from a neutral liquid environment.

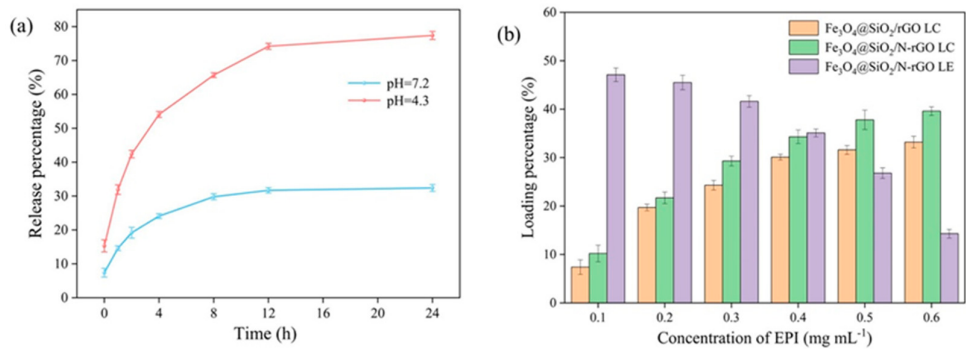


Figure 11. Release capacity of Fe₃O₄@SiO₂/N-rGO at a pH of 7.2 and 4.3 (a). Loading capacity of Fe₃O₄@SiO₂/rGO and Fe₃O₄@SiO₂/N-rGO. Loading efficiency of Fe₃O₄@SiO₂/N-rGO (b).

EPI of Fe₃O₄@SiO₂/N-rGO is released in phosphate buffer solutions with pH=7.2 and pH=4.3. The pH value of healthy cells in the human body is about 7.2. Emami et al.[23] explored the effect of CS/PVP/ α - Fe₂O₃/Dox at pH=5.4 and pH=7.4 nanoparticles on the release curve of doxorubicin. At pH=5.4, nanocomposites release more drugs. From the curves in the Figure 10a, the release rate of the anticancer drug EPI was faster in 4 h, and then tended to level off with the increase of release time, and the amount of drug released from the carrier was significantly higher under acidic conditions. The amino and hydroxyl groups contained in the drug and the functional groups of the carrier formed intermolecular forces through hydrogen bonding and π - π stacking. When the drug-loaded carriers are in an acidic environment, the hydrogen bonds formed between the functional groups will be broken faster and the intermolecular forces will be weakened. In addition, a large number of hydrogenions in the solution react with amino groups, and the protonation of amino groups will weaken the combination of drug molecules and carriers, so that the magnetic carrier can achieve the purpose of drug release.

Finally, an external magnetic field was applied to the magnetic drug carrier during the release process. At 39 °C, the EV secretion of breast cancer increased with the increase of temperature[24], so the solution temperature increased by 2 °C, which was conducive to killing the virus, indicating that the magnetic carrier had a certain impact on magnetothermal therapy[25]. The heat transfer caused by temperature rise has a positive impact on drug release from the carrier.

Table 1 shows that the performance of Fe₃O₄@SiO₂/N-rGO compared with magnetic drug carriers reported in other literatures. In the research of targeted drug delivery, the drugs involved as drug delivery objects are 5-FU, paclitaxel, and DOX. Compared with these drugs, Epirubicin (EPI) is a new generation of anthracycline anticancer drugs, and its toxic side effects have been reduced. EPI is one of the most widely used anthracycline antitumor chemotherapy drugs in clinical practice. Currently, EPI is widely used in domestic clinical practice, but there are few researches on targeted delivery and lack of scientific data. It has been pointed out that although the toxic side effects of EPI have been reduced, it may still lead to severe bone marrow suppression and certain cardiotoxicity. Therefore, it is necessary to deliver the drug to the patient site through magnetic targeting drug carriers to improve the effective drug delivery rate and reduce the damage to the human body.

Table 1. Comparison of the performance of magnetic drug carrier.

Carrier name	Surface modification	Substrate	Ms / emu g ⁻¹	Drug	LC/%	LE/%	RE/%	References
MC	— —	Cellulose	34.8	5-FU	12.0	62.5	93	[26]
MGO	— —	GO	32.5	PAC	19.4	95.8	67.6	[27]

GO-PVP-Fe3O4	— —	GO-PVP	— —	QSR	62.8	— —	35.4	[28]
Fe3O4@Au@SiO2	Au/SiO2	— —	18.3	VP16	74.3	— —	67.3	[29]
Fe3O4@mSiO2-P(EO-co-LLA)	mSiO2-P(EO-co-LLA)	— —	48.7	DOX	6.8	— —	92.7	[30]
Fe3O4@SiO2/N-rGO	SiO2	N-rGO	40.9	EPI	39.6	47.1	77.4	This work

4. Discussion

In this paper, superparamagnetism was prepared by solvothermal method Fe₃O₄@SiO₂/N-rGO nano drug carriers. The design idea is to continuously increase the loading capacity of drug carriers through the dual methods of surface modification of magnetic cores and substrate composite. The carrier has superparamagnetism and can respond to changes in external magnetic fields to achieve magnetic targeted drug delivery. Its application mode is to guide magnetic targeted particles through magnetic field changes in vitro, enrich them in cancer lesions, and centrally release drugs to increase the effective drug delivery rate. However, the responsibility in the field of biomedicine is significant, and the human environment is more complex and volatile. Therefore, larger experiments and more rigorous scientific research methods are required to be applied in the field of targeted delivery.

In addition, drug carriers have targeted drug delivery capabilities and acid pH sensitivity. In patients, cancer cells convert body fluids from neutral to acidic to promote reproduction and metastasis. Drug carriers utilize this condition to promote drug release in acidic environments. therefore Fe₃O₄@SiO₂/N-rGO can be used as a magnetic targeted drug carrier for drug delivery systems and cancer combined therapy systems. Finally, at this stage, the research direction of drug carriers has developed towards multifunctional and multidisciplinary directions. During the application of external magnetic fields, magnetic carriers exhibit a magnetic therapy effect of heat transfer, which is worthy of further research in future work.

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