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PreparationSynthesis and Characterization and characterization of PEG-PEI/Fe_3O_4 nano-magnetic fluid Modified with PEG-PEI by co-precipitation method

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Abstract: PEG-PEI/Fe_3O_4 nano-magnetic fluids with different mass fractionsmass ratio of reactant were prepared by co-precipitation method. Besides The particle size analyzer, the methods of XRD, IR, VSM, and AFM were adopted to characterize the synthesized samples. Covalent bonding of PEG, PEI and Fe_3O_4 exhibits superparamagnetism. The TEM photograph shows that the particles are of stable dispersion and little aggregation, with smooth surface, spherical shape and a diameter of about 80 nm, which meets the requirements of nano-materials. When the mass fraction of PEI inin reactant is 25%, the particle size, Zeta-potential and pEGFP-C1 DNA loading efficiencyadsorption rate of DNA plasmid pEGFP-C1 are all satisfactory. In this case, PEG-PEI/ Fe_3O_4 nano-magnetic fluids can be used as gene vectors or targeted drug carriers.

Key words: Fe_3O_4; PEG; PEI; co-precipitation; nano-materials; magnetic fluids

1 Introduction

In the recent years, gene therapy gets a rapid development, and it's mainly focused on obtaining target genes and achieving high gene transfection efficiency. The type of vector used is a key to success in gene delivery[1]. Two broad approaches have commonly used been used to deliver DNA to cellsin gene transfection, namely are viral vectors and non-viral vectors, . The formerwhich has different some disadvantages, such as inducing the immune response of organism, potential viral replication, expensive production cost, not being applied repeatedly, and without targeting. regards efficiency, ease of production & safety. Though the non-viral vectors are easier to be produced and without immunogenicity and much safer, the efficiency of them are is not satisfactedsatisfied, particularly with the presence of serum protein[2]. An ideal gene delivery carrier would be a system that can safely transport the genetic materials without exhibiting any toxicity or immune responses, and can be produced on a large scale easily. According to MERDAN et al[3] and other scientists' researches, cationic polymer as the nanometric gene vector in gene therapy is one of the best non-viral vectors.

PEG-PEI/Fe_3O_4 nano-magnetic fluids prepared in this study show the advantages of good magnetic drug targeting, prolonged circulation and low toxicity. There have been lots of reports on PEG/Fe_3O_4 magnetic fluids as drug carrierscarriers[4]. PEI/Fe_3O_4 or PEI-PEG as gene vector has also been reported[5−6]. In this study, we creatively combine the advantages of PEG and PEI to prepare PEG-PEI/Fe_3O_4 nano-magnetic fluids as a new gene carriers. This can dramatically prevent non-specific uptake by the reticular- endothelial system[7] and reduce

the cytotoxicity of PEG-PEI/Fe_3O_4 magnetic fluids[8]. Up to now, there is no report on such topic. Research evidance evidence showed that the transfection efficiency of PEG-PEI was much higher than that of PEI [9]. No matter how the drug was administrated, intravenous injection or intratracheal medication, the gene expression of PEI was better than cationic liposomes [10], which enhanced the gene expression in extra pulmonary tissues and liver[11]. Coupled the PEG-PEI polymer with Fe3O4 could increase its transfection ability underBy applying magnetic field, and we can control the concentration of PEG-PEI/Fe_3O_4 in targeted tissues or cells. This would

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further decrease the harm to normal cells and minimize dosage of ~~drugs~~[drugs and obtain the standard of targeted drug carriers[9]~~12~~].

2 Experimental

2.1 Preparation of PEG-PEI/Fe_3O_4~~Fe_3O_4~~ nano-magnetic fluids and DNA adsorption on nanoparticles~~13~~

~~2.1.1 Materials and instruments~~

~~Experimental reagents: FeCl3, FeCl2, NH3·H2O, PEI(MW25000 Da, Sigma) PEG(MW 2000, Sigma), and other reagents were of analytical grade (purchased from Sinopharm Chemical Reagent Co., Ltd.), redistilled water (self-prepared).~~

~~Experimental instruments: Malvern Zetasizer 3000E (Malvern, UK); Asylum Research MFP-3D AFM Systems; LABCONCO; Ultraviolet-visible Spectrophotometer (Japan, Shimadzu); XRD (Phlips X'Pert PRO, XL-30); NICOL ET 200SXV FT-IR; VSM (Quantum Design, JDM-13);~~

~~2.1.2 Experimental methods:~~

Main reagents, $FeCl_3$, $FeCl_2$, $NH_3 \cdot H_2O$, polyethyleneimine (PEI, ~~(~~MW25000 Da~~,)~~ ~~, ~~, monomethoxy poly(ethylene glycol) (PEG~~(~~, MW 2000) were used in this experiment~~, and other reagents~~.

Main instruments included Malvern Zetasizer 3000E (Malvern, UK), Asylum Research MFP-3D AFM Systems, Freeze dryer (LABCONCO), Ultraviolet-visible spectrophotometer (Japan~~, Shimadzu~~) ~~; ~~, Supercentrifuge (BECKMAN ZK401), High speed refrigerated centrifuge (Heraeus Company) and Thermostatic circulator water bath oven.

~~In a typical procedure, 2.7 g FeCl3, 4.0 g PEG and a portion of PEI (5%-35% of the total mass of reactant) were thoroughly dissolved in 50 ml mL redistilled water respectively; then the three kinds of solution were poured into the triangular flask~~three necked flask in the order. ~~After they were shaken well, 2.0 g FeCl2 and 50 mL ammonia water (0.3 mol/L) was added into the mixed liquor and which was agitated in three necked flask with a high speed at 55℃ for 2-3 hours. The~~is whole ~~preceduree~~procedure ~~required nitrogen protection.~~

~~Added 10 ml mL NH3·H2O (1.5mol/L) was added into 40 ml mL redistilled water to prepare 50 mL ammonia water, then the ammonia water was dropped into the three necked flask drop by drop by through separating funnel within 15 min. The mixed liquor~~suspension ~~had to be~~was ~~agitated at high temperature for 2-3 h to react. Then t~~The ~~resluting~~ resulting suspension was ~~centrifugalized~~centrifugalizing at 5,000 rpm for 15 min by a centrifuge, supernatant was removed, and the sediment was suspensed suspended in redistilled water. ~~Repeated t~~This procedure was repeated for one more ~~time. Right after this, irradiation processes were conducted with an ultrasonic bath (30% of ultrasonic power rate, 30 s interval) for 6 min, then~~and then the ~~well distributed PEG-PEI/Fe3O4 nano magnetic fluids were obtained.~~

~~15 μl lL pEGFP-C1 plasmid was added to 0.5 mg PEG-PEI/Fe3O4 nano magnetic fluids, and they were mixed well by slight stirring with a uniform speed for 2 min at room temperature. Then p~~Placed it in a ~~37℃ water bath for 30 minutes to obtain the complex formation of PEG-PEI/Fe3O4 nano magnetic fluid and DNA plasmid.~~

The e~~Operational~~ xperimental ~~operational~~ sequence flowchart is shown in Fig.1.

2.2 Characterization of PEG-PEI/Fe_3O_4 nano-magnetic fluid

Malvern Zetasizer 3000E(Malvern, UK) was used to detect the Zeta potential and particle size of the complex~~ formation~~; and Asylum Research MFP-3D AFM System was used to observe the distribution and appearance~~structure~~ of the nano-particles.

After the complex was changed into fine powder by cryodesiccation, XRD (Phlips X'Pert PRO, XL-30) and FT-IR were used to analyze the functional group of PEG-PEI/Fe_3O_4 magnetic nano-particles. And the paramagnetism of the sample under certain condition was measured by VSM (Quantum Design, JDM-13).

~~2.3 Statistical analysis~~

The data were described as $x \pm S$ (x is the mean value; S is the standard deviation). The ~~–~~measurement data were analyzed with the variance analysis and the group comparison was treated with the T-test or least significant difference(LSD).

~~**Experimental data was described by X±S method; Measurement data were compared with analysis of variance and Measurement data were compared with t-test or LSD among groups.。**~~

3 Results and discussion

3.1 Particle size, Zeta potential ~~of PEG-PEI/Fe_3O_4 nano-magnetic fluid~~ and pEGFP-C1 DNA loading efficiency of PEG-PEI/Fe_3O_4 nano-magnetic fluid~~DNA adsorption on nanoparticles~~

The particle size of PEG-PEI/Fe_3O_4 nano-magnetic fluid was measured by Malvern Zetasizer 3000E (Malvern, UK). Almost all the particles are about ~~100~~ 80 nm, as shown in Fig.~~1~~1~~2~~(a). This means the PEG-PEI/Fe_3O_4 nano-magnetic fluid has typical nano-material characteristics and can be efficiently taken up ~~in~~ by cells.

The result observed with the atomic force

microscope (Asylum Research MFP-3D AFM Systems) shows that, the particles in PEG-PEI/Fe_3O_4 nano-magnetic fluid are spheroid, the surface of them is smooth, and they are dispersed evenly without adhesion,~~The morphology of nanoparticles observed by AFM was almost spherical and smooth, and the surface was homogeneous in the nano-sized range,~~ as shown in ~~Fig. 2~~Fig. ~~1~~3.

Through analyzing the result of superficial potential test of nanoparticle, on one hand, the stability of nano-suspension can be judged; on the other hand, because the charge of cell membrane is negative, the transfection system must be positive to make the transfection proceed smoothly~~Zeta potential tesing results could be used to determine the stability of the suspension. As the surface charge of the DNA delivery system was one of the critical factors affecting the transfection efficiency. So the positive zeta-potential of nanoparticles was required~~[~~14~~10]. The adsorption rate of plasmid DNA on nano-particles is one of the most direct indicators demonstrating whether nanoparticle could carry and transfect gene with high efficiency. The

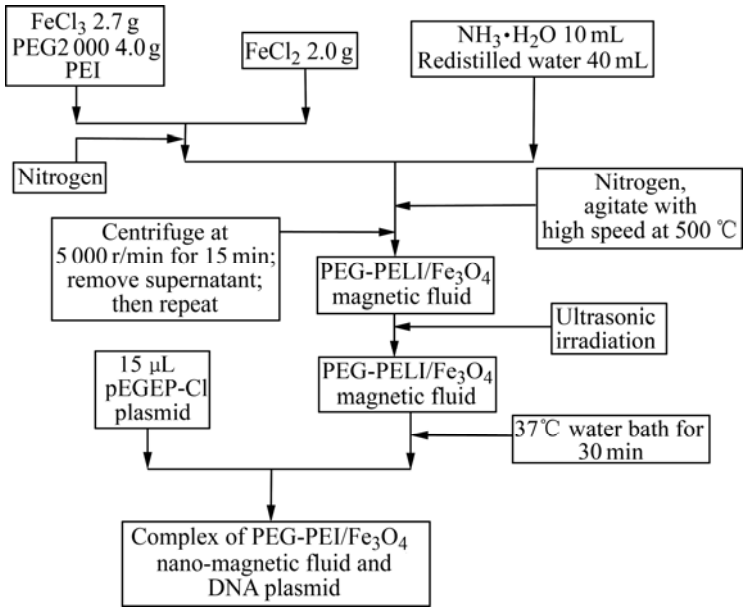


Fig.1 Experimental flowchart for preparation of PEG-PEI/Fe_3O_4 nano-magnetic fluids and DNA adsorption on nanoparticles

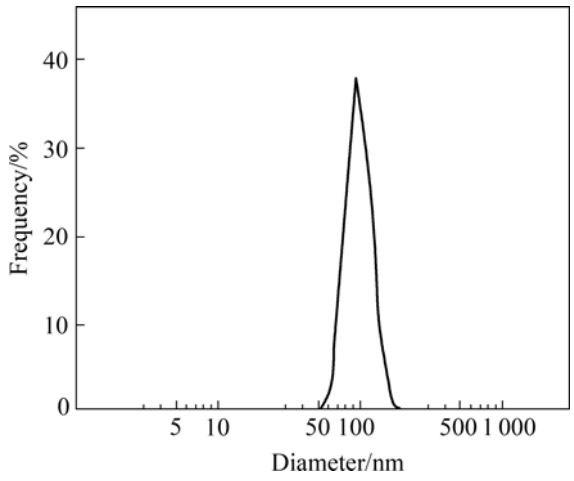


Fig.1a 2 Particle size distribution measured by Malvern Zetasizer

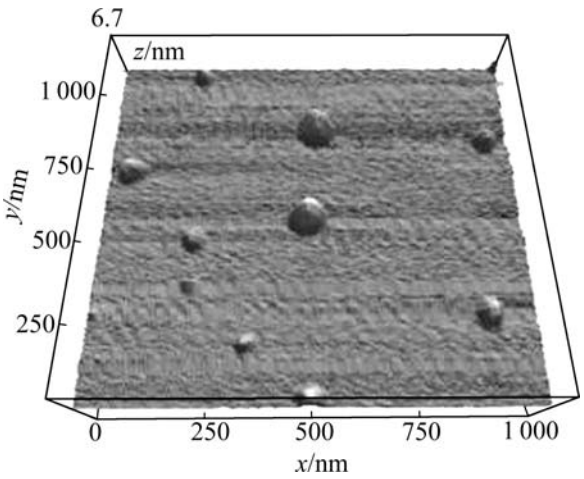


Fig.213 –AFM photograph of nanoparticles

of gene transfection efficiency.

high DNA loading efficiency is helpful to enhancing the gene transfection efficiency of nanoparticle.

Table 1 proves the effects of PEI with different mass fractions of reactantwith different weight percent on nanoparticle size, surface Zeta potential zeta potential and plasmid pEGFP-C1 DNA loading efficiencythe adsorption rate of nanoparticleDNA plasmid pEGFP-C1. As shown in Table 1, the size of nanoparticles was influenced by the amounts of PEI. The size of nanoparticles decreased as the amount of PEI in the nanoparticles was decreased. However, when the mass fraction of PEI was beyond 30%, the particle size dramatically increased into 100−200 nm. When the mass fraction of PEI was between 5% and 20%, the Zeta-potential of the resulting nanoparticles increased when the PEI concentration was increased. The surface potential was about 20 mvmV; and the pEGFP-C1 DNA loading efficiencyadsorption rate of DNA plasmid pEGFP-C1 was 40%−70%, which was not good for gene transfection. When the mass fraction of PEI was more than 25%, the surface potential would be more than 30 mvmV, and the pEGFP-C1 DNA loading efficiencyadsorption rate of DNA plasmid pEGFP-C1 could reach 90% or more, which could promote the efficiency of gene transfection.

Table 1 Effects of PEI on nanoparticle size, Zeta-potential and the pEGFP-C1 DNA loading efficiencyadsorption rate of DNA plasmid pEGFP-C1

Mass fraction of PEI/%	Particle size/nm	Zeta-potential/ mV	DNA loading efficiency/%
5	37.22±3.2	19.07±1.12	41.22±1.4
10	23.14±6.5	21.22±1.32	33.41±1.7
15	42.05±11.2	23.13±1.15	51.08±2.3
20	92.47±13.4	22.54±2.12	74.05±2.4
25	79.99±7.3	34.13±2.03	94.13±1.8
30	167.23±6.9	33.65±2.83	92.32±1.9
35	189.43±7.8	32.34±3.04	93.21±2.2

As shown in Table 1, the optimum reaction condition was obtained: mass fraction of PEI=25%, then the nano-particle size was (79.99±7.3) nm, surface potential was (34.13±2.03) mV, and the adsorption rate of DNA plasmid pEGFP-C1 was (94.13±1.8)%.

As shown in this table, The the optimum reaction condition was obtained as: weight percent of PEI=25%, then the nano-particle size was 79.99±7.3nm, surface potential was 34.13±2.03 m V, and the the adsorption rate of DNA plasmid pEGFP-C1 was 94.13±1.8%.

3.2 X-ray diffraction pattern of PEG-PEI/Fe_3O_4 nano-magnetic fluids

Fig.3 24 shows the X-ray diffraction pattern of PEG-PEI/Fe_3O_4 nano-magnetic fluid. The parameters of PEG-PEI/Fe_3O_4 nano-magnetic fluid, such as the position and value of peak were closed by to that the standard data of Fe_3O_4 alone in powder diffraction PDF card (JCPDS No.82-1533). The appearance of sample diffraction peaks at 2θ=30.16°, 35.70°, 43.33°, 53.60°, 57.10°, 62.9° corresponded to (220), (311), (400), (422), (511) and (440) crystal plane of Fe_3O_4 respectively, which indicated that the resulting particles were Fe_3O_4, with structures of cubic crystal.

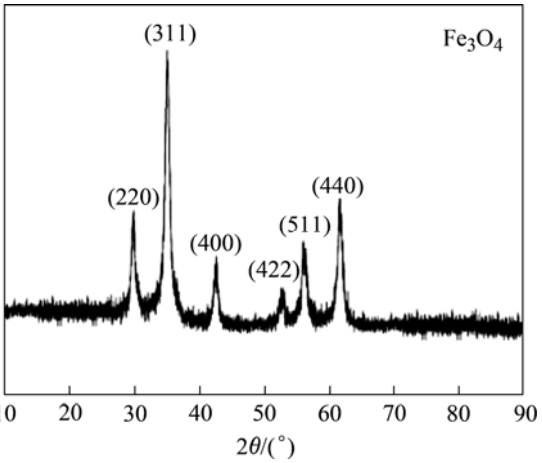


Fig.3 24 X-ray diffraction pattern of PEG-PEI/Fe_3O_4 nano-magnetic fluid

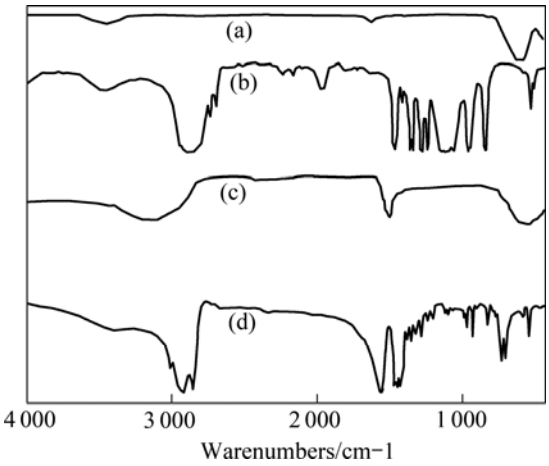


Fig.4 35 IR spectra of Fe_3O_4, PEG, PEI and PEG-PEI/Fe_3O_4: (a) Fe_3O_4; (b) PEG; (c) PEI; (d) PEG-PEI/Fe_3O_4

Up to now, magnetic fluids have been used in medicine as magnetic targetingable carriers for anti-cancer drug delivery systems. For application in biological organisms, these particles have good biocompatibility and low toxicity. The use of magnetic fluid as drug carriers aims to target drugs to a specific site through the application of a magnetic field to achieve prolonged release of high localised localized concentrations of the drug via the retention of magnetic particles in the region of interest. This can reduce the harm to healthy organs by limiting the circulation of the drug throughout the body. The magnetic particles, loaded with drug or gene, are attracted and held in the tumour region by a strong external magnetic field, which avoids non-specific uptake by the reticular-endothelial system[15 11].

PEG-PEI/Fe_3O_4 magnetic fluid mainly consists of nano sized Fe_3O_4 particles. The surface charge of Fe_3O_4 is neutralized by PEG. Effects of PEG on the resulting magnetic fluid also includes increasing dissolubility, which allows the resulting suspension to place for a long time without more agglomeration.

3.3 IR spectra of PEG-PEI/Fe_3O_4 nano-magnetic fluid

By comparing the absorption peaks of Fe_3O_4, PEG, PEI and PEG-PEI/Fe_3O_4 nano-magnetic fluid, the bond is identified among PEG, PEI and Fe_3O_4. Figs.5(a−d) show the FT-IR spectra in the region of 400−4 000 cm^{-1} of the PEG-PEI/Fe_3O_4 magnetic fluid by using infrared spectrometer (NICOL ET 200SXV FT—IR) and the KBr tablettingpellet technique.

Fig.4 35(a) shows the characteristic absorption peak of Fe_3O_4 at 576 cm^{-1}. Fig.5 (b) shows the characteristic absorption peaks of PEG at 1111 cm^{-1} (C—O—C), 3 450 cm^{-1} (OH), 844 cm^{-1} (CH_2CH_2O), 2 875 cm^{-1}, 1 465 cm^{-1}, 1 349 cm^{-1} and 1 249 cm^{-1}. Fig.5(c)43 shows the IR-spectra of PEI. The characteristic bands at 688 cm^{-1} (—NH wagging vibration), 1 637.5 cm^{-1} (NH^{2-} scissoring vibration and C—H stretching vibration), 2 357.5 cm^{-1} and 2 110 cm^{-1} (NH^+ asymmetrical stretching vibration) can be seen.

Fig. 4 35(d) shows the IR-spectra of PEG-PEI/Fe_3O_4 magnetic fluid. It had the characteristic absorption absorption peaks of PEG and PEI at 1 225.5 cm^{-1} (C — O — C), 3 470 cm^{-1} (OH), 870 cm^{-1} (CH_2CH_2O) and 1 629 cm^{-1}, 2 851.4 cm^{-1}, 1 446.2 cm^{-1}, 1 425.5 cm^{-1}, and 1 252 cm^{-1}, respectively. We could also observe an absorption peak at 1 384 cm^{-1}, which was a PEI characteristic absorption absorption peak. A weaker intensity of —OH stretching band appeared at 2 921.7 cm^{-1}, which showed an obvious red shift, indicating that the —OH radicals might interact with cationic [—CH_2—CH_2—NH_2^+—]$_n$ radicals in absorption process. This meant that a small quantity of PEI was presented absorbed on the surface of Fe_3O_4. Fig. 4(d) Compared the wide and multiplet absorption peak of hydroxyl group in PEG-PEI/Fe_3O_4 shown in Fig. 43 5(d) with singlet absorption peak of hydroxyl group in PEG, PEG showed in Fig. 4(e), the different properties were due to the hydrogen bonds [16] formed by the hydroxyl atoms in hydroxyl groups[12]. Therefore, both physical adsorption and hydrogen bonds could be found in magnetic colloid particles. Surface modification of Fe_3O_4 with PEG and PEI would prevent the aggregation of nano-particles and ensure a good stability.

PEI has been investigated extensively for use as a nonviral gene delivery vector, due to its higher transfection efficiency in polycation non-viral gene delivery vector"proton sponge" mechanism. There is one

nitrogen atom protonated in two carbon atoms of PEI molecule. Due to the different pK_a values of the primary amino groups, secondary amino groups and tertiary amino groups consist of these nitrogen atoms, PEI has the ability to capature the protons which were transfered into endosomes during their acidification at any Differing different pHs of PEI solutions do not make any significant difference in transfection efficiency conditions, namely "proton sponge" mechanism[17~~13~~].

Coupling Bonding PEIof the hydrophilic PEG with the hydrophilic PEG molecule chains resulted in ancould increase the solubility of PEG-PEI/Fe_3O_4, neutralize surface potential, decrease interaction with proteins in blood, prolong circulation in vivo and decrease toxicity[18~~14~~~~20~~16].

In vivo distribution of the resulting magnetic fluid could be affected by different amount of PEG. As reported, with the increase of modificationafter systemic administration degree of with PEGylated nanoparticles, the derease of rapid accumulation in the lung of composite injected by vein in the lung was observed. However, with increasing amounts of PEG, the lung uptake was markedly reduced[17]. The degree of modification with PEGamount of PEGylation could also affect gene transfection efficiency. With proper degree of modification with PEG (when the number of PEG segmer connected to each PEI molecule is less than 2)amount of PEGylation, the gene transfection efficiency could be enhanced. While too muchhigher degree of modification with PEG (when the number of PEG segmer connected to each PEI molecule is more than 10)PEGylation would reduce the transfection efficiency in vitro[22~~18~~]. PEG could also change the nano structure and shape of PEG-PEI/Fe_3O_4 magnetic fluid[21~~93~~],] and weaken non-specific interaction between the compositeparticles and cellular membrane to reduce the internalization uptake by cells.

3.4 Magnetism of PEG-PEI/Fe_3O_4 nano-magnetic fluid

As a magnetic targeting materials, the paramagnetismmagnetic properties property of PEG-PEI/Fe_3O_4 nano-magnetic fluid is considered as a key performance parameter. In this work, the magnetic properties of resulting magnetic fluid with different Fe_3O_4/PEI mass ratios were measured by using the VSM (Quantum Design, JDM-13). Normally, when $M_s \geqslant 40$ $A \cdot m^2 \cdot kg^{-1}$, the materials could be classified as qualified ferrimagnetic materials, which could be controlled by external magnetic field.

Fig. 5~~46~~(a) displays the magnetization curve of pure Fe_3O_4 nanoparticles, and the magnetization of Fe_3O_4 changed according to different external field strength. Fig. 5~~46~~(a) showed a typical curve for ferrimagnetic material. The initial increase of external magnetic field intensity led to rapid increase in magnetization intensity strength of Fe_3O_4. After the field intensity increased to a certain level, the increase in magnetization intensitystrength of Fe_3O_4 gradually slowed down until the saturation saturated magnetization intensitystrength(M_s) was reached (approximately when H= 6 000×79.528 A/m, M_s=60.22 $A \cdot m^2 \cdot kg^{-1}$). When the external magnetic field intensity decreased, the magnetization intensitystrength of Fe_3O_4 would decrease accordingly. When the external magnetic field intensity changed to 0, the magnetization intensity of Fe_3O_4 would change to 0 either.

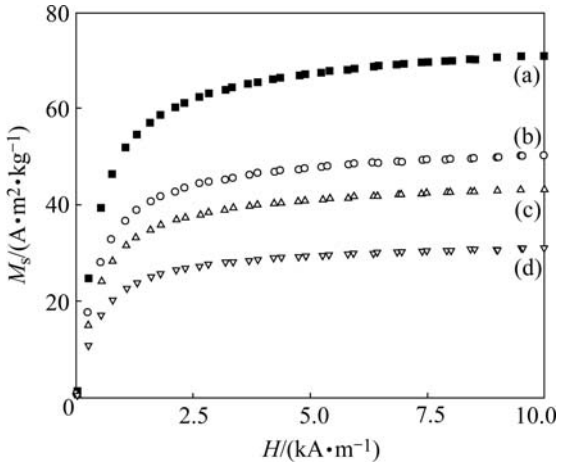
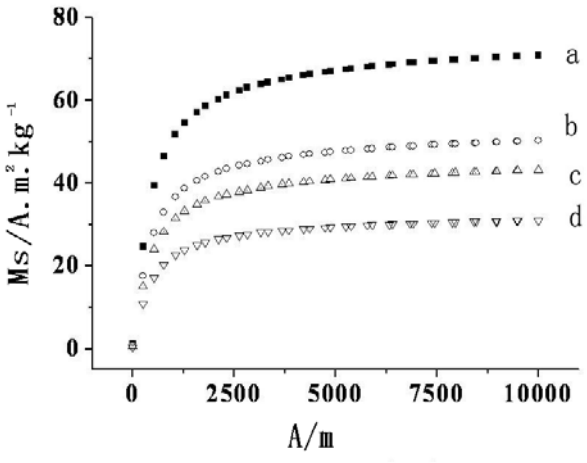


Fig. 46 Magnetization curves for pure Fe_3O_4 and PEG-PEI/Fe_3O_4 nano-magnetic fluid with different mass fraction of PEI: (a) Pure Fe_3O_4; (b) 25%; (c) 30%; (d) 35%

Ferrimagnetism was observed in PEG-PEI/Fe3O4 nano-magnetic fluid. Fig. 5 (b-d) also displayed a typical curve for of ferrimagnetic material, indicated that the PEG-PEI/Fe3O4 nano-magnetic fluid retained ferrimagnetism. When the weight percent of PEI was



a) pure Fe3O4 b) 25% c) 30% d) 35%

Fig. 5 Magnetization curves for pure Fe3O4 and

~~PEG-PEI/Fe3O4 nano-magnetic fluid with different weight percent of PEI~~

Nanotechnology is the science and technology that study~~ereating~~ movement rule and interaction of ~~functional materials, devices and~~ system~~s~~ ~~through control~~composed of matter on the nanometer length scale (1−100 nm)[~~24~~20], and performing intelligent functions and portending particular use. Ferrimagnetism was observed in PEG-PEI/Fe_3O_4 nano-magnetic fluid. Figs.6(b−d) also display typical curves of ferrimagnetic material, and indicated that the PEG-PEI/Fe_3O_4 nano-magnetic fluid retained ferrimagnetism. When the mass fraction of PEI was 25%, the saturated magnetization intensity can be obtained in H=6 000× 79.528 A/m, and M_s=60.22 A·m^2·kg^{-1}. By further increasing the amount of PEI, the saturated magnetization intensity would be decreased. PEG-PEI/Fe_3O_4 nana-magnetic fluid is a kind of stable colloidal solution, in which ferric oxide nanoparticles are well-distributed to hydrofacies, even the gravitational force, centrifugal force or magnetic force can not separate those elements. It has the magnetism of solid magnetic material and fluidity of liquid material with small particle size and strong magnetic response.

~~**Nanomaterials refer to those which have structured components with at least one dimension less than 100nm. They are a new class of materials composed by Nanoscale nanoscale objectsparticles[25]. Numerous applications exist in the medical and pharmaceutical field for nanomaterials. The therapeutic drug molecules have been either dissolved into or encapsulated into a magnetic nanosphere or conjugated on the surface of the nanosphere which made by high polymer material usually and directed to a specific target tissue. This is a novel drug delivery system[26]. Nanomaterials are notablely different from that of the same material in macro materials[27−28]. The special structure of nanomaterials are is contributed to the unique effects of nanobiomaterials, as small-dimension effect and surface (interface) effect.**~~

~~**PEG-PEI/Fe_3O_4 nano-magnetic fluid are able to condense or package DNA into small sizes so that it can be taken up by cells, across the natural biological barriersbypass or escape the cell's endocytotic pathways, deliver the DNA to the cell's nucleus, and unpackage unpacked DNA for optimal integration with chromatosome of host cell. So this polymer as gene vector offers advantages of small particle size, non-biomaterial, low toxicity, no immune response, long term gene expression, large specific surface area and high transfection efficiency[29]. The special structure of PEG-PEI/Fe_3O_4 nano-magnetic fluid as gene vector can protect the gene from being degradated or destructed by enzymes or alexin in blood plasmer or cells[30]. PEG-PEI/Fe_3O_4 nano-magnetic fluid is a kind of organic polymer, which can be produced on a large scale at a comparatively low price without the risk of recombinant virus or exhibiting any toxicity and immune responses[31].**~~

4 Conclusions

1) With ferric chloride, ferrous chloride, ammonia water, PEG and PEI as raw materials, the co-precipitation method was adopted to prepare colloid particles with size of about 80 nm and strong magnetism response. The experiment indicated that, through experimental condition optimization, when the PEG mass fraction is 25%, and ripening at 55 ℃ for 2 h, magnetic colloid particles obtained have the most superior synthesis characteristics, such as small size, positive Zeta potential and strong magnetism response. The morphology of magnetic fluid modified by PEG-PEI is spherical and smooth.~~In this study, PEG-PEI/Fe_3O_4 nano-magnetic fluids with different mass ratio are prepared by co-precipitation method. The diameter of the nano-particles are about 80 nm, with supermagnetism. When the weight percent of PEI is 25%, the results of particle size, zeta potential and adsorption rate of DNA plasmid pEGFP-C1 are all satisfactory.~~

2) ~~The methods of XRD, IR, VSM, and AFM are adopted to characterize the synthesized samples. The morphology of nanoparticles observed is almost spherical and smooth, with strong magnetism responsivenesssupermagnetism and positive zeta potential.~~

~~3)~~ High plasmid pEGFP-C1 DNA loading efficiency indicates that PEG-PEI/Fe_3O_4 nano magnetic fluid could interact strongly with phosphate framework of ~~DNA~~ DNA~~phosphate framework~~ with negative charge, promote DNA concentration and combination with itself,~~compaction~~ and ~~cellular~~ can also be easily absorbed by cellular membrane with negative charge. ~~Therefore, the PEG/PEI/Fe3O4 nano-magnetic fluid is now opening up a wide field of possible applications in medicine as targeted drug delivery systems or gene carriers.~~

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$NH_3 \cdot H_2O$ 10ml10mL
Redistilled water 40ml

$FeCl_2$ 2.0g

15 μl L pEGFP-C1 plasmid

complex Complex formation of PEG-PEI/Fe_3O_4 nano-magnetic fluid and DNA plasmid

PEG-PEI/Fe_3O_4 magnetic fluid

37℃ water bath for 30 minutes

PEG-PEI/Fe_3O_4 magnetic fluid

Ultrasonic irradiation

Centrifuge at 5,000 rpm/min, 15 min
Remove supernatant then repeat

Nitrogen

$FeCl_3$ 2.7g
PEG2000 4.0g
PEI

Nitrogen, , agitated with high speed at 55℃

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