CO-PRECIPITATION MICROWAVE-ASSISTED SYNTHESIS OF Fe_3O_4 NANOPARTICLES FOR DRUG DELIVERY SYSTEM

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ABSTRACT

In this study, Fe_3O_4 magnetic nanoparticles were prepared by the co-precipitation of Fe^{3+} and Fe^{2+} with the assistant of microwave (MW) irradiation. The crystal and magnetic properties of the Fe_3O_4 particles obtained from different conditions of microwave reaction were examined. The prepared magnetic nanoparticles were characterized by Fourier transform infrared spectroscopy (FT-IR), transmission and scanning electron microscopy (TEM & SEM), X-ray powder diffraction (XRD), vibrating sample magnetometer (VSM) and Dynamic Light Scattering (DLS). After that, the optimized Fe_3O_4 magnetic nanoparticles (size of 10-15 nm, the saturation magnetization of 69 emu/g, and zeta potential of 40.1 mV) were surface modified by alginate polymer and attached with a cancer drug Doxorubicin. The magnetic inductive heating effects and anticancer activities of the nano drug system showed that the synthesis of Fe_3O_4 with microwave-assistant maintains both physical and biological properties of the material.

Keywords: *microwave assisted synthesis; coprecipitation; Fe_3O_4 nanoparticles; drug delivery system;, Doxorubicin*

INTRODUCTION

Fe_3O_4 nanoparticles have been extensively investigated for their applications in biomedicine as well as other fields. There are a variety of methods for Fe_3O_4 preparation reported in the literature to prepare Fe_3O_4 nanoparticles such as co-precipitation [1], high-power milling [2], sol-gel [3] or microfluid engineering [4]. The help of microwave in the synthesis of the Fe_3O_4 nanoparticles have been used in different methods due to many advantages of this technique.

Microwave device helps to reduce reaction time [5], improve reaction yield because microwave more effectively distributes heat energy in the reaction system than in conventional condition [6]. Especially, microwave-assisted synthesis allows to prepare nanoparticles in large scale and in more environmentally friendly processes [7].

The authors of the publication [8] synthesize nano Fe_3O_4 by the co-precipitation of Fe (III) and Fe (II) mixture (at the molar ratio of 1.75:1) with the amoniac solution and aging by microwave with frequency of 2.45 GHz. The results showed that the use of microwave allowed to reduce reaction time from 1 week to 2 h. In addition, the obtain Fe_3O_4 nanoparticles have more complete crystallization and smaller size than Fe_3O_4 nanoparticles prepared without microwave assistant.

Using the same method, the research group [9] combined 0.02 M Fe (II) and 0.04 M Fe (III) solution with Na_2CO_3 base in a pressure bearing container. The container was put in a microwave oven and set the reaction temperature at 60^{o}C (with the microwave power of 50 or 300 W) and the reaction time of 10 or 60 minutes. The research results revealed that adjustment of reaction conditions help to control the particle size, magnetic properties as well as interactions between the particles with core-shell structure.

Using microwave device with frequency of 2.45 GHz and maximum power of 1000 W,

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nano Fe_3O_4 were synthesized by hydrothermal method at 150^oC in 10 minutes and aging at the same temperature in 2 h. The obtained Fe_3O_4 are hexagonal with the average size of 48 nm and synthesis yield of 90% [10].

Nano Fe_3O_4 was also prepared by reduced Fe (III) with hydrazine (N_2H_4) in microwave system in 10 mins at temperature of 100 ± 5 ^oC, maximum microwave power of 300 W and maximum pressure of 250 psi. The particles had the size of 30-50 nm and saturation magnetization of 70 emu/g [11]. N_2H_4 played the role of both a reductant and a basic environment, thus, the amount of N_2H_4 affected much on the crystal structure of obtained Fe_3O_4 [12].

Therefore, microwave technique can be applied in many procedures of nano Fe_3O_4 preparation. In this study, we have synthesized Fe_3O_4 nanoparticles by co-precipitation method with the assistant of a microwave device. The prepared Fe_3O_4 were compared to that synthesized by conventional coprecipitation.

MATERIAL AND METHODS

Materials

All the chemicals used were of reagent grade. Ferric chloride hexa-hydrate ($FeCl_3.6H_2O$), ferrous chloride tetrahydrate ($FeCl_2.4H_2O$), sodium hydroxide (NaOH), ammonia (NH_3), hydrochloric acid (HCl), were purchased from Aldrich and used without further purification. Alginate (Alg) with molecular weight of 40,000 Da, Doxorubicin hydrochloride (DOX), propylcarbodiimide (EDC), N-hydroxysuccinimide (NHS) and triethylamine were obtained from Sigma-Aldrich. All solvents used are HPLC grade, which include dichloromethane (DCM), ethanol from Aldrich. Distilled water was used throughout all experiments.

Preparation of superparamagnetic iron oxide nanoparticles

Magnetic nanoparticles (Fe_3O_4) were synthesized by modified co-precipitation of Fe^{3+} and Fe^{2+} [13] with the assistance of a microwave reactor. Briefly, a mixture of iron (III) chloride hexahydrate and iron (II) chloride tetrahydrate (molecular ratio 2:1) was dissolved by a dilute HCl solution in a three necked round bottom flask. The flask was put in a microwave reactor (Sineo Uwave 1000) and the solution was magnetically stirred at 600 rpm under nitrogen atmosphere. The reaction temperature and reaction time was set according to Table 1. Then a dilute ammonium hydroxide solution was added until a total black solution formed with the microwave conditions. Fe_3O_4 nanoparticles was then collected by a magnet and washed three times by double distilled water.

Table 1. *Microwave synthesis conditions of Fe_3O_4 nanoparticles*

Sample	Temp. (^oC)	Reaction time (min)	Stirring speed (rpm)
M1	50	5	600
M2	50	15	600
M3	50	25	600
M4	70	5	600
M5	70	15	600
M6	70	25	600
M7	90	5	600
M8	90	15	600
M9	90	25	600
M10	70	15	300
M11	70	15	900

Preparation of Doxorubicin loading magnetic nanoparticles

Doxorubicin was encapsulated into the optimized Fe_3O_4 nanoparticles in a procedure published elsewhere [14]. Firstly, an aliquot of 15 ml of Fe_3O_4 fluid (5 mg/ml) was added dropwise to 10 ml of 4 mg/ml Alginate solution. The mixture was then ultrasonically vibrated for 1 h and stirred for 24 h to form coated magnetic nanoparticles (assigned as FA). DOX loaded nanoparticles were prepared by an emulsion solvent evaporation method. DOX.HCl was dissolved in dichloromethane (15 ml) and then deprotonated by the addition of triethylamine (1.5 ml). The dichloromethane solution of DOX was stirred in a closed flask for 6 h and then added dropwise into the water solution of FA nanoparticles under vigorous stirring. The mixture was stirred for 24 h in the closed flask and then dichloromethane was evaporated under vacuum pressure to obtain DOX loading nanoparticles called FAD. The obtained mixture was magnetic decanted to remove free DOX. The red transparent supernatant was collected to estimate the excess DOX.

Characterization methods

Phase structure of materials was determined by X-ray diffraction (SIEMENS-D5000). Magnetic property was measured in a vibrating sample magnetometer VSM (homemade). Molecular structure of materials was characterized by Fourier transform infrared spectroscopy (FTIR, SHIMADZU spectrophotometer) using KBr pellets in the wave number region of 400–4000 cm^{-1}. Surface morphology of materials was investigated by field emission scanning electron microscopy (Fe-SEM) on a Hitachi S-4800 system. Size distribution was measured by dynamic light scattering (DLS) method in a Nano Zetasizer, Malvern UK.

Magnetic inductive heating experiments

All the magnetic induction hyperthermia (MIH) experiments were carried out on the set up with the use of a commercial generator (RDO HFI 5 kW) providing an alternating magnetic field of amplitude of 80 Oe, and frequency of 178 kHz. The sample temperature was measured by submerging the temperature probe of an optical thermometer (Opsens) directly to the solution. For characterization of the heating performance, ferrofluid samples of various particle concentrations (diluted in water) were prepared and kept in a round-bottom-shaped glass holder, so that the temperature sensor was imbedded directly in them.

The specific absorption rate (SAR) and intrinsic loss power (ILP) of the samples were calculated using the formulas [15, 16]:

$$SAR = \frac{m_{sample}}{m_{Fe_3O_4}} \cdot C \cdot \frac{\Delta T}{\Delta t} \text{ and } ILP = \frac{SAR}{H^2 \cdot f}$$

In which C is the specific heat of the medium (C = 4.18 J g^{-1} $^oC^{-1}$), $\Delta T/\Delta t$ is the maximum slope of the time-temperature curve, m_{sample} and m_{Fe3O4} are the mass of sample and Fe_3O_4 present in the sample, respectively. H and f are applied magnetic field strength and frequency, respectively.

Cell culture and cytotoxicity study

Cytotoxicity of Doxorubicin FAD were determined by XTT cell proliferation assay. Hep-G2, LU-1, RD, FL and Vero cells were treated with FAD and free DOX (at various DOX concentrations and the highest DOX doses are 25 μg ml^{-1}) in 96-well plates. After 48 h of incubation, cell viability profiles and IC_{50} (half maximal inhibitory concentration) values were determined for each particular cell type.

RESULTS AND DISCUSSION

To optimize the microwave-assisted synthesis of Fe_3O_4 nanoparticles, different reaction conditions have been investigated to determine the sample with the best crystal structure and magnetic properties.

Magnetic Properties

Magnetic properties of the samples are shown in Table 2. The rememnance M_r and coercive field H_c of the samples are close to zero prove that the samples are superparamagnetic. Microwave irratiation did not change this property of the material. The highest magnetization of ~69 emu/g belongs to M5. This value is almost unchanged compared to Fe_3O_4 synthesized by conventional coprecipitation (70.5 emu/g).

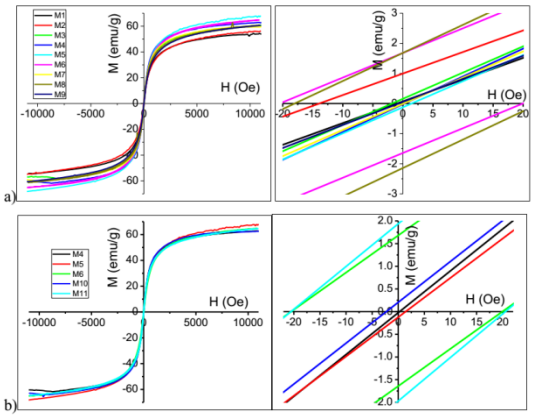

Figure 1. *Hysteresis loops of M1-M11*

Among the samples prepared at the same temperature (M1-M3, M4-M6, M7-M9), it can be seen that samples with the reaction time of 15 mins (M2, M5, M8) have higher saturation magnetization than those with reaction time of 5 of 25 mins. Howerver, the difference is quite small. Saturation magnetization of the samples are more dependent on the temperature reaction. When the temperature increases from 50 to 70°C, M_s increases. Higher temperature 90°C, however, decrease the M_s. This fact can be explained by the difference in particle size resulted from the different temperature. When increasing temperature, particles get bigger, then their Ms will increase. [17]. But when the temperature reach closely to the boiling point of the water temperature (90°C), the solvent evaporation may influence on the crystal completion, that in turns decrease the sample magnetization.

The stirring speed seems not to play an important role on magnetic properties of the samples (M5, M10 and M11). From Table 2, one can note that M5 (synthesized at 70°C, 15 mins and stirring speed of 600 rpm) is the best sample in term of magnetic properties.

X-ray diffraction diagrams

Figure 2 shows the XRD patterns of M2, M5 and M8. The peaks appear on M2 XRD diagram are not clear implying that the crystalline degree of Fe_3O_4 in this sample is not good. This result is in agreement with the low M_s of M2 that was prepared at low temperature. The characteristic diffraction peaks of M5 appears at the typical positions of Fe_3O_4 ((200) - 30.5°; (311) - 35.5°, (400) - 43°, (422) -53.3°, (511) - 57.5° , (440) - 62.5°) without any strange peaks. Thus, the crystalline structure of M5 is the ferrit spinel structure. In sample M8, besides above peaks of Fe_3O_4, there is the appearance of other peaks that may be a result of the sample oxidation at high synthesis temperature. These results confirm the best crystalline structure of M5, in accordance with its highest saturation magnetization.

Table 1. *Magnetic parameters of microwave-assisted synthesized Fe_3O_4*

Sample	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10	M11
M_s (emu/g)	53.9	56.2	56.7	63.0	69.0	64.6	59.6	60.6	60.7	62.7	64.5
H_c (Oe)	~2.5	~14	~4	~2.5	~0	~20	~0	~18	~2	~2	~21
M_r (emu/g)	~0.5	~1.0	~0.2	~0.4	~0	~1.7	~0	~2	~0.1	~0.2	~1.9

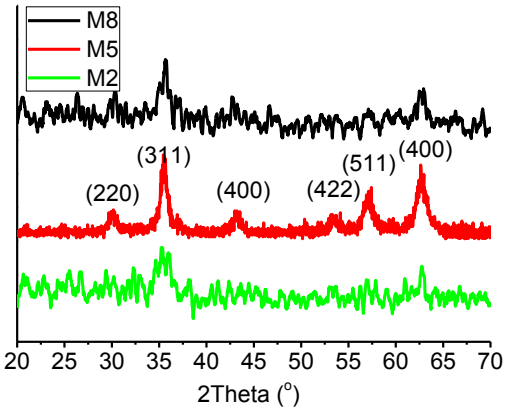

Figure 2. *XRD diagrams of M2, M5 and M8*

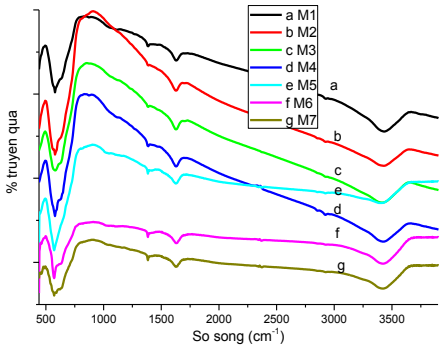

Figure 3. *FTIR spectra*

FTIR spectra

Figure 3 shows the FTIR spectra of microwave-assisted synthesized Fe_3O_4 nanoparticles. All the samples exhibit absorption peak typical for Fe-O bond at 570 cm^{-1}. However, the peak at the wavenumber of 630 cm^{-1} matching with the presence of Fe_2O_3 impurity [18] appear in the FTIR spectra of many samples except for M5. Therefore, through the survey from the mentioned features, M5 was chosen for further investigation.

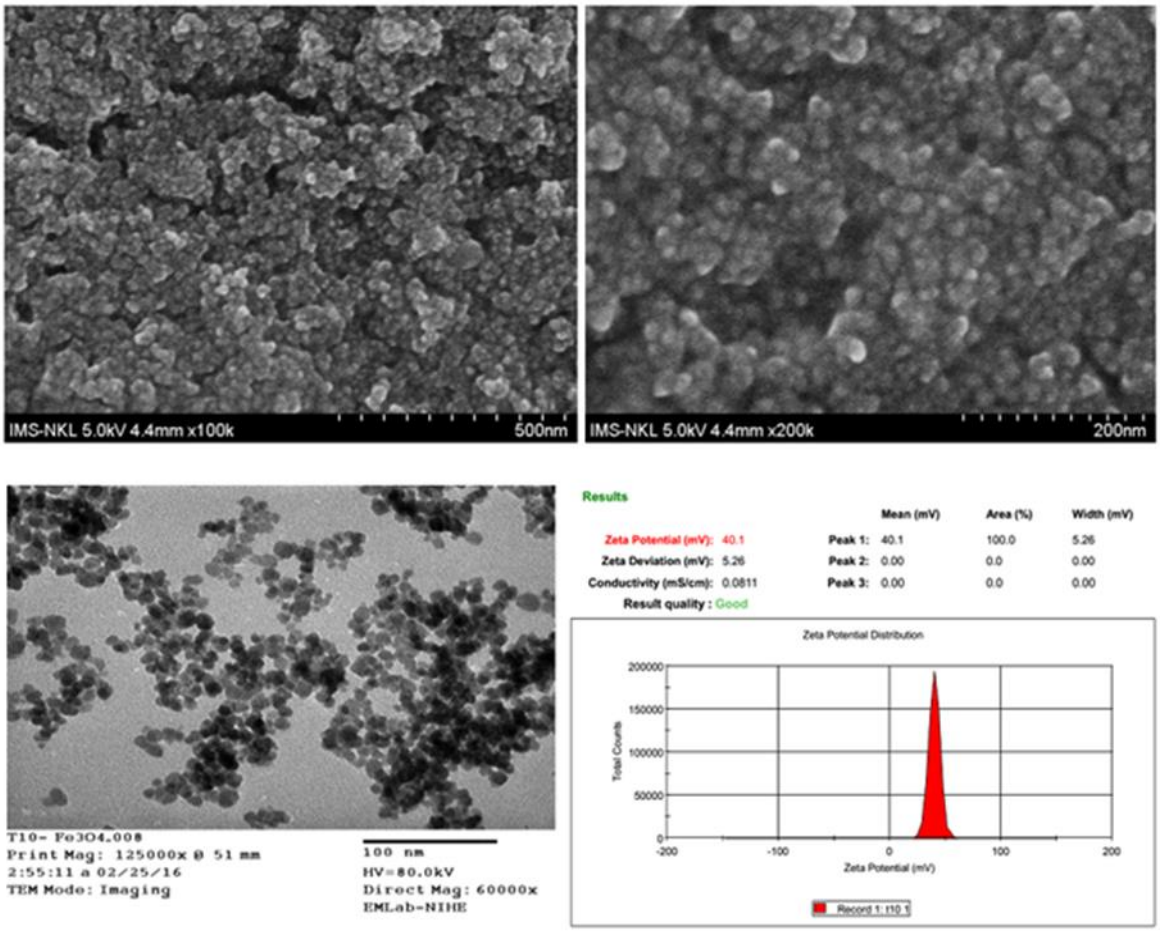

Figure 4. *SEM, TEM images and DLS spectrum of M5*

Size and size distribution

SEM, TEM and DLS spectrum of M5 are presented in Figure 4. The Fe_3O_4 nanoparticles have the spherical shape with the size of 10-15 nm. The Fe_3O_4 M5 particles can be easily dispersed in water to obtain stable solution (Zeta potiental of 40.1 mV). Therefore, microwave-assisted synthesized Fe_3O_4 is suitable to be used in biomedical applications.

Drug delivery system based on Fe_3O_4 prepared with microwave assistant

Both FA and FAD have high stability presenting in highly negative Zeta potential (-39 and -32 mV respectively). The inductive heating curves of FA and FAD shows similar trends to those of our previous drug delivery systems prepared by conventional coprecipitation [14]. The parameters of heating processes are shown in Table 2 and Figure 5.

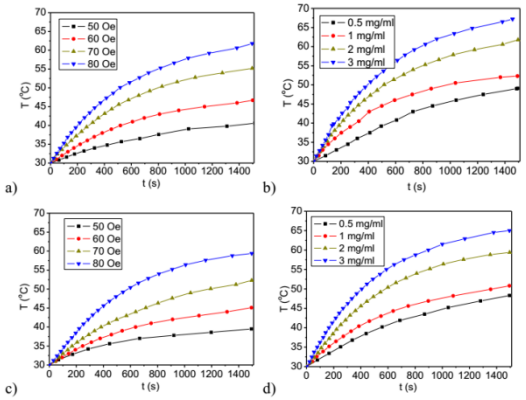

Figure 5. *Inductive heating curves of FA (a and b) and FAD (c,d) at different magnetic fields (a, c) and concentration (c,d)*

Table 2. *Saturation temperature (°C) of FA và FAD inductive heating*

Sample	Magnetic field H (Oe) (Fe_3O_4 concentration of 2 mg/ml)				Fe_3O_4 concentration (mg/ml) (magnetic field of 80 Oe)			
	50	60	70	80	0.5	1	2	3
FA	40.6	46.7	55.2	61.8	49.1	52.3	61.8	67.2
FAD	39.5	45.1	52.3	59.4	48.3	50.8	59.4	65.0

The comparision of IC_{50} values of FAD and FA4D (conventionally synthesized drug delivery system) on table 3. FAD caused similar impact on Hep G2, LU-1 and Vero to FA4D. This reveals that the use of the microwave synthesis of the Fe_3O_4 nanoparticles does not only meet the material requirement but also retain the biological interaction of drug delivery system. This phenomenon can be explained by the fact that the Fe_3O_4 preparation using microwave assistant still be the precipitation of Fe^{2+} and Fe^{3+} (molar ratio 1: 2) in basic environment. The priority of this technique is simple operation, reduce reaction time response. Special, this technique allows to synthesize nano Fe_3O_4 in large scale that is suitable for application in reality.

Table 3. *IC_{50} of FAD and FA4D*

Cell lines	Hep-G2	LU-1	RD	FL	Vero	HeLa
Dox[1]	**0.21**	**0.39**	0.11	0.16	**1.30**	-
Dox[2]	**0.18**	**0.35**	-	-	**1.34**	0.25
FA4D (Ref. [14])	**0.72**	**0.96**	0.60	1.20	**1.41**	-
FAD	**0.67**	**1.02**	-	-	**1.43**	0.81

[1]*Control sample in experiment determing IC50 of FA4D*
[2]*Control sample in experiment determing IC50 of FA4D*

CONCLUSION

In conclusion, the Fe_3O_4 nanoparticles have been successfully prepared with the assistant of microwave technique. The synthesis conditions were optimized as 70°C, 15 mins and 600 rpm at the M5 sample. This sample has uniform size about 10-15 nm, highly disperses in water. The drug delivery system FAD based on M5 express good hyperthermia effect and cytotoxicity on cancer cell lines. The results reveal that microwave assisted synthesis of Fe_3O_4 nanoparticles can be used in drug delivery systems for biomedical application.

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TÓM TẮT

TỔNG HỢP NANO Fe_3O_4 BẰNG PHƯƠNG PHÁP ĐỒNG KẾT TỦA SỬ DỤNG KĨ THUẬT VI SÓNG DÙNG CHO HỆ DẪN THUỐC

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Trong nghiên cứu này, hạt nano từ tính Fe_3O_4 được tổng hợp bằng phương pháp đồng kết tủa từ ion Fe^{3+} và Fe^{2+} với sự trợ giúp của kĩ thuật vi sóng. Cấu trúc tinh thể và tính chất từ của các mẫu Fe_3O_4 tổng hợp trong các điều kiện phản ứng vi sóng khác nhau được nghiên cứu để tìm điều kiện tối ưu. Các mẫu Fe_3O_4 được xác định đặc trưng bằng phương pháp phổ hồng ngoại (FTIR), hiển vi điện tử quét phát xạ trường (FeSEM), hiển vi điện tử truyền qua (TEM), nhiễu xạ tia X (XRD), từ kế mẫu rung (VSM) và tán xạ ánh sáng động (DLS). Mẫu hạt nano Fe_3O_4 đã được tối ưu hóa (kích thước 10-15 nm, từ độ bão hòa 69 emu/g và thế Zeta 40,1 mV) được biến đổi bề mặt bằng polime alginate và mang thuốc chống ung thư Doxorubicin. Hiệu ứng đốt nóng cảm ứng và tác động trên các dòng tế bào ung thư cho thấy quá trình tổng hợp Fe_3O_4 với kĩ thuật vi sóng duy trì tốt các tính chất vật lí cũng như sinh học của vật liệ này.

Từ khóa: *tổng hợp có kĩ thuật vi sóng, đồng kết tủa, nano Fe_3O_4, hệ dẫn thuốc, Doxorubicin*

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