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Study on preparation of Fe_3O_4@lapatinib nanoparticles for application as a targeted drug delivery system in the treatment of breast cancer

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Abstract: Systems for targeted drug delivery in the treatment of cancer using nanoparticles are currently of interest to many domestic and foreign scientists. In this work, for application as a target drug delivery system in the treatment of breast cancer Fe_3O_4@lapatinib nanoparticles were prepared by coprecipitation of solutions of Fe^{2+} and Fe^{3+} salts in alkaline, followed by purification in a magnetic separation system. Physicochemical properties of nanoparticles were studied and determined by XRD, TEM, DLS, HPLC, VSM, AAS and zeta potential methods. The results showed that the size of Fe_3O_4@lapatinib nanoparticles is in the range of 10 to 40 nm, hydrodynamic diameter is 27.4 ± 0.6 nm, saturation magnetization is 39.1 ± 3.1 emu/g, Fe content is 16.35 ± 0.97 mg/ml, lapatinib content is 8.08 ± 0.06 mg/ml, and the zeta potential is -16.2 ± 1.3 mV. Thus, the obtained Fe_3O_4@lapatinib nanoparticles are fully suitable for targeted drug delivery in the breast cance treatment.

Keywords: *Fe_3O_4@lapatinib nanoparticles, co-precipitation, breast cancer, targeted drug delivery*.

I. INTRODUCTION

In recent years, the rate of cancer in the world has been increasing rapidly and alarmingly, the number of cases and deaths globally increases very high every year. In particular, breast cancer in women becomes the third cancer after lung and liver cancer, especially due to its high prevalence in low- and middle-income countries. It is also the third leading cause of death globally [1]. In the future, if women do not have an early cancer screening orientation, this number will increase even more. To treat cancer in general and breast cancer in particular, a number of methods can be used such as surgery, chemotherapy, radiation therapy, endocrine, immunological and targeted therapy [2]. Depending on the stage of the disease and the specific patient's condition, one or a combination of the above treatment methods is applied. However, due to late detection, the effectiveness of diagnosis and treatment is still limited.

Lapatinib is considered a specific targeted therapy for breast cancer, with molecular formula $C_{29}H_{26}ClFN_4O_4S$, is a small molecule kinase inhibitor that targets epidermal growth factor receptor and growth factor receptor type 2 (HER2), has been approved by the FDA for the treatment of patients with advanced breast cancer or metastatic tumours in HER2-overexpressing and previously treated patients with chemotherapy, which are anthracycline, taxane and trastuzumab [3].There is no denying the effectiveness of

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lapatinib treatment in the targeted treatment of breast cancer, but the drug often causes side effects on healthy cells. In addition, after a period of treatment, the patient may develop drug resistance, lose response to the drug and the drug is easily eliminated.

To overcome the above disadvantages, the use of nanoparticles as a target guide system is a matter of great interest and a high potential method in the world and Vietnam in the current diagnosis and treatment of breast cancer. In particular, the Fe_3O_4@lapatinib-^{153}Sm radioactive nanoparticle is a potential candidate with outstanding advantages such as duality, nanometer size and binding to the small molecule drug that targets breast cancer lapatinib, so the transmission of guidance. The target will be easier, causing fewerside effects and more effective treatment. Realizing the potential of Fe_3O_4@lapatinib-^{153}Sm nanoparticles, this study aims to synthesize Fe_3O_4@lapatinib, transmission system, and investigate the physicochemical properties of the material to serve as a basis for labelling with radioisotopes ^{153}Sm.

II. CONTENT

A. Material and methods

The materials of the study are Fe_3O_4 nanoparticles coated with lapatinib. $FeCl_3.6H_2O$ (99.8%, Fisher Scientific PA, USA); $FeCl_2.4H_2O$ (99.8%, Fisher Scientific PA, USA); lapatinib (99.6%, MedChemExpress, USA); NH_4OH (25%, Sigma Aldrich PA, USA); DMSO (99.9%, Sigma Aldrich PA, USA); HCl (36.5%, Sigma Aldrich PA, USA); ACN (99.8%, Merck, Germany); distilled water.

Preparation of Fe_3O_4@lapatinib nanoparticles

The synthesis of Fe_3O_4 nanoparticles occurs when the Fe^{2+} và Fe^{3+} salts react with the base solution in a pH environment of about 9-14. Equation 1 represents the general reaction of the whole synthesis.

$$2Fe^{3+} + Fe^{2+} + 8OH^- \rightarrow Fe_3O_4 + 4H_2O \quad (1)$$

In the presence of oxygen, Fe_3O_4 is oxidized to form iron(III) according to Equation 2.

$$2Fe_3O_4 + 1/2\ O_2 \rightarrow 3Fe_2O_3 \quad (2)$$

In the synthesis process, it is necessary to add antioxidants, often inert nitrogen gas is added to the reaction system to remove oxygen [4]. Fe_3O_4 nanoparticles (also known as magnetite) after forming will disperse in an aqueous environment containing lapatinib as a stabilizer [5, 6] and then Fe_3O_4 will adsorb lapatinib in an aqueous medium to form Fe_3O_4@lapatinib nanoparticles [7].

During the synthesis, the factors affecting the size of the nanoparticles were investigated at an appropriate range to find the optimal conditions (Table I).

Table I. Research factors

Factors	**Range**				
Content of lapatinib (mg/mL)	5	10	20	30	40
Concentration o NH_4OH (%)	10	20	25		
Stirring time (min)	10	30	45	60	
Stirring speed (rpm)	500	600	800	1000	

Fe_3O_4@lapatinib nanoparticles then were purified by magnet separation and washed several times by distilled water to pH = 7. The synthesis efficiency was calculated based on Formula 3.

$$H = \frac{\text{Mass of purified } Fe_3O_4}{\text{Mass of initial } Fe_3O_4} \text{ x } 100\% \quad (3)$$

Iron content in washing water was analyzed by F-AAS method to determine iron impurities.

X-ray diffraction method(XRD)

The Fe_3O_4@lapatinib nanoparticles were ground into powder by hand mill or ultrafine ball mill, then passed through a 90 μm sieve and then dried under the sieve to bring the moisture content of the sample to less than 3%. Then, the powder sample was put into the mold, using a glass piece to press the powder firmly down the mold, while brushing the top surface of the mold containing the sample. The molds containing samples was put into the sample chamber of the device (D2 Phaser, Germany), seting up the program and sample measurement parameters, the samples were analyzed and analytical data were collected on the DIFFRAC MEASUREMENT software. Comparing the spectral peak data of the analyzed sample with the spectrum of the standard to determine the phase composition.

Transmission electron microscopy (TEM) and dynamic light scattering (DLS) methods

Fe_3O_4@lapatinib nanoparticles were dispersed in SPAN 80 solvent (0.01%) and sonicated for 30 minutes. Then, drop a drop of sample solution onto a copper grid covered with carbon film. Allow to dry naturally in air and observe under TEM system (JEM-1400 JEOL, Japan).

Dynamic light scattering (DLS) method was also used to determine the size and dispersibility of Fe_3O_4@lapatinib nanoparticles in solution.

High performance liquid chromatography (HPLC)

The analysis of lapatinib content in the Fe_3O_4@lapatinib nanoparticles were performed by an HPLC system (LC-20AD$_{XR}$ Shimadzu) combined with a detector UV/Vis (SPD-20A Prominece UV/Vis). The analytical parameters are presented in Table II.

Table II. Analytical parameters for determination of lapatinib content on HPLC system

Stationary phase	InertSustain C18 column (5 μm, 4.6×250 mm, GL Sciences)
Mobile phase	ACN/H_2O (65/35 v/v) filtered through 0.22 μm filter
Flow rate	0.5 mL/min
Elution mode	Gradient
Wavelength	310 nm
Injection volume	10 μL
Column temperature	40°C

The lapatinib calibration curve was built from a series of standard solutions with the corresponding lapatinib content of 25; 50; 75; 125; 250 mg/mL diluted in dimethyl sulfoxide (DMSO).

Fe_3O_4@lapatinib nanoparticles were dissolved in concentrated DMSO solution and then placed in an ultrasonic bath for 30 minutes to separate lapatinib and Fe_3O_4. Using a magnet to separate Fe_3O_4 nanoparticles, aspirate the filtrate and filter it through a 0.22 μm filter and determine the lapatinib content in the sample.

Atomic absorption spectroscopy (AAS)

Flame atomic absorption spectroscopy method (F-AAS) using a mixture of air and acetylene is applied to determine the Fe content, thereby determining the mass concentration of Fe_3O_4 in the product Fe_3O_4@lapatinib. The Fe calibration curve was

constructed over the concentration range of 0; 0.1; 0.2; 0.5; 1; 2; 5 ppm.

The solution containing Fe_3O_4@lapatinib nanoparticles was acidified with 37% HCl solution, then concentrated to about 1 mL and then titrated to 10 mL to determine the concentration of iron in the sample, from which the mass concentration of Fe_3O_4 can be calculated with the following formula:

$$C_{Fe_3O_4} = \frac{C_{Fe}}{\%Fe(Fe_3O_4)} (mg/mL) \quad (4)$$

In addition, the F-AAS method is also used to determine the iron content in the washing water. Proceed similarly for the solution sample containing Fe_3O_4@lapatinib nanoparticles.

Vibration sample magnetometer (VSM)

Magnetic saturation (M_s) and coercivity (H_c) of Fe_3O_4@lapatinib nanoparticles were measured by a vibrating sample magnetometer VSM at room temperature, in the highest magnetic field of 11 kOe.

Method of measuring zeta potential

The charge of Fe_3O_4@lapatinib nanoparticles was determined by measuring the zeta potential of the solution containing the prepared nanoparticles on the Zetasizer nano device.

B. Results

Preparation of Fe_3O_4@lapatinib nanoparticles

After investigating the optimal conditions, the synthesis procedure is summarized in Fig. 1.

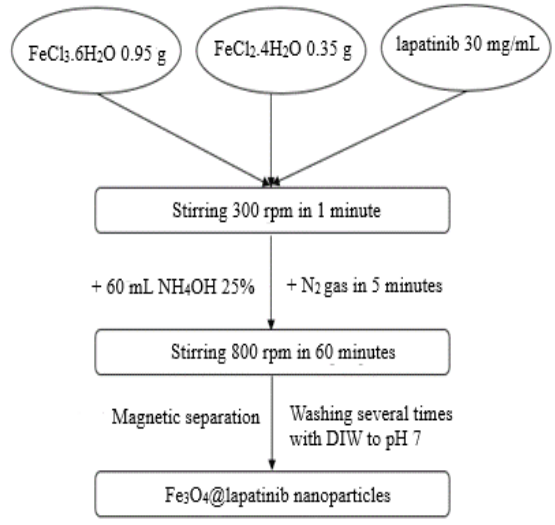

Fig. 1. Optimal process for Fe_3O_4@lapatinib nanoparticles synthesis

The purification of Fe_3O_4@lapatinib nanoparticles are determined by the content of Fe in washing solution (Table III).

Table III. The content of Fe in washing solution

STT	Samples	Content of Fe (mg/L)
1	D1	Not detected
2	D2	Not detected
3	D3	Not detected

The results show that samples are iron-free. The content of Fe in samples was lower than the LOQ value (LOQ = 0.1 mg/L), indicating that the Fe_3O_4@lapatinib nanoparticles were completely separated from iron impurities and the whole Fe_3O_4 nanoparticles were in the solution containing nanoparticles.

Based on the optimized synthesis process, the mass of unrefined obtained nanoparticles after drying was 0.557 g. After purifying and drying, the obtained nanoparticle weight was 0.532 g. Thus, the efficiency of Fe_3O_4@lapatinib nanoparticle purification is about 95.5%.

Results of determining phase composition of Fe_3O_4@lapatinib nanoparticles

X-ray diffraction patterns of Fe_3O_4@lapatinib nanoparticles. The results show that samples contain 11 characteristic diffraction peaks of Fe_3O_4 with hkl values at the planes (111), (220), (311), (222), (400), (422), (511), (440), (531), (620) and (533). This result is consistent with the diffraction peaks of the Fe_3O_4 standard sample (PDF 00-001-1111 Fe_3O_4 Magnetite) and demonstrates that the Fe_3O_4 nanoparticle has an inverted spinel structure. In the diagram there are not the characteristic peaks of Fe_2O_3 because the synthesis is carried out in a N_2 gas environment, so the possibility of the product being oxidized to Fe_2O_3 is very low, the product obtained only consists of the Fe_3O_4 phase.

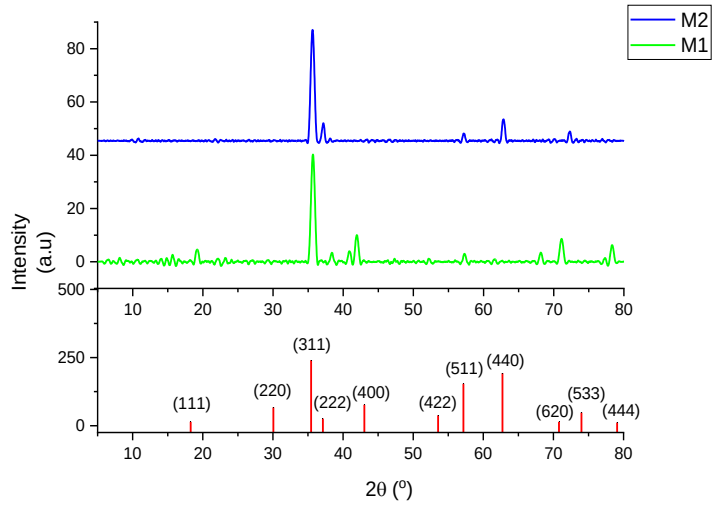

Fig. 2. X-ray diffraction (XRD) pattern of synthesized Fe_3O_4@lapatinib nanoparticles

Results of determining the size and shape of Fe_3O_4@lapatinib nanoparticles

The nanoparticle size measured by TEM ranges from 10-40 nm (Fig. 3). In solution, the average hydrodynamic size of the nanoparticles is about 27.4 ± 0.6 nm (Table IV). The image shows that the Fe_3O_4@lapatinib nanoparticles are spherical in shape and have a fairly uniform size.

Table IV. Particle sizes of Fe_3O_4@lapatinib nanoparticles (DLS)

No.	Samples	Sizes of nanoparticles (nm)
1	T1	27.1
2	T2	28.1
3	T3	27.1

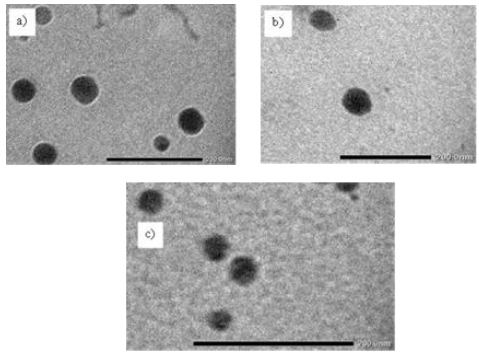

Fig. 3. TEM images of samples in 200 nm scale bar

Results of determining lapatinib content coated with Fe_3O_4 nanoparticles

Analysis results of lapatinib by HPLC method showed that the retention time of lapatinib was about 7.3 minutes (Fig. 4).

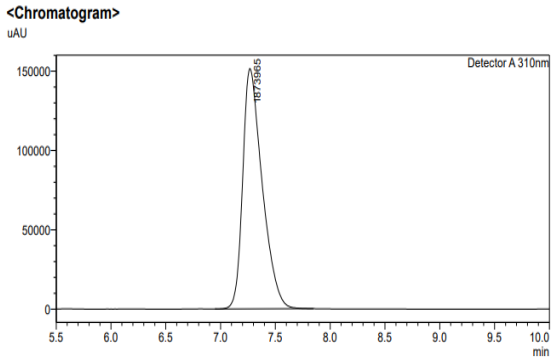

Fig. 4. The chromatogram of lapatinib

The lapatinib calibration curve, constructed over the concentration range of 25; 50; 75; 125; 250 mg/mL, had a coefficient of R^2 = 0.9963 (Fig. 5).

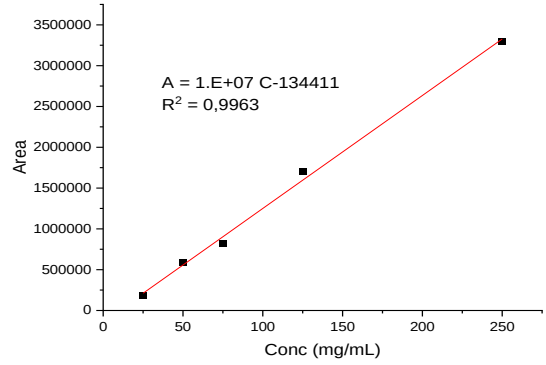

Fig. 5. Calibration curve of lapatinib

Based on the calibration curve, the concentration of lapatinib coated with Fe_3O_4 nanoparticles was determined according to the optimal synthesis process is 8.08 ± 0.06 mg/mL.

Results of determining the concentration of Fe_3O_4

The result of calibration curve (0.2; 0.5; 1.0; 2.0 ppm) constructedby F-AAS method is showed in the Fig. 6.

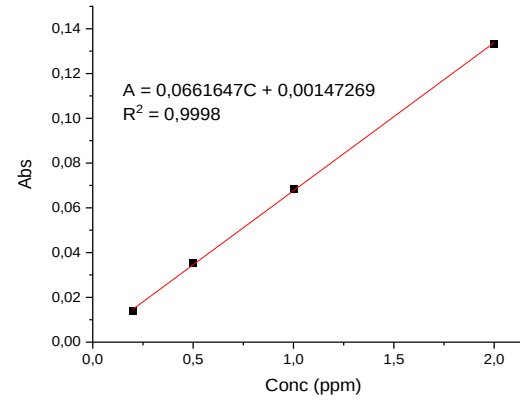

Fig. 6. Calibration curve of Fe

The average Fe content of all samples was 16.35 ± 0.97 mg/mL, corresponding to the average Fe_3O_4 content of 22.71 ± 1.34 mg/mL (Table V).

Table V. Concentration of Fe_3O_4

Samples	Content of Fe (mg/mL)	Concentration of Fe_3O_4 (mg/mL)
A1	15.97	22.18
A2	15.63	21.71
A3	17.45	24.24

Results of determining the saturation magnetization of Fe_3O_4@lapatinib nanoparticles

The results of determining the saturation magnetization and the coercive force of Fe_3O_4@lapatinib nanoparticles (Fig. 7) show that the average value of saturation

magnetization of Fe_3O_4 nanoparticles is 39.1 ± 3.1 (emu/g). If the external magnetic field increases, the magnetization will increase and reach the saturation magnet (M_S). At the point of zero magnetization, the corresponding magnetic field at this point is called the coercive force (H_C).The results also show that the hysteresis curve of samples is quite narrow, the coercive force is very small, when the magnetic field is removed, the magnetization of the samples also almost disappears.

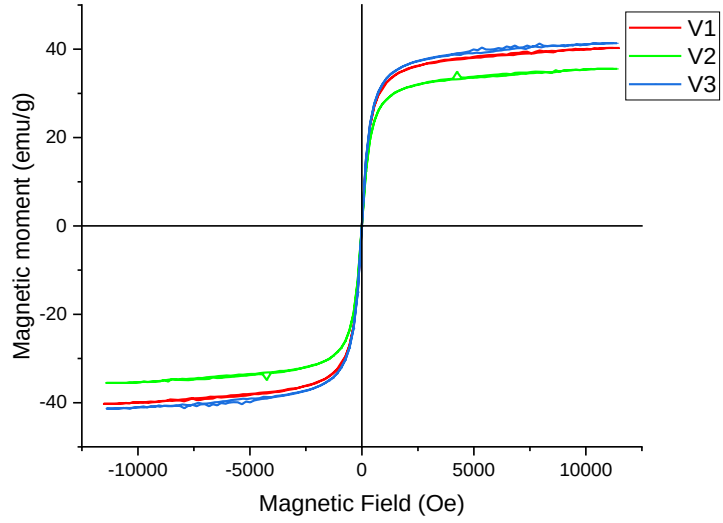

Fig. 7. Magnetization curve of Fe_3O_4@lapatinib

Results of determining the zeta potential

The average zeta potential value of Fe_3O_4@lapatinib nanoparticles is -16.2 ± 1.3 (mV).

C. Discussion

By co-precipitation method, the results showed that the obtained product was single-phase, consisting of only Fe_3O_4, since an inert gas is added during the synthesis, the products almost did not oxidize to Fe_2O_3 [8]. The size of Fe_3O_4@lapatinib nanoparticles was in the range of 10-40 nm, while in solution the size was less than 30 nm. This is the right size for building a targeted drug delivery system to destroy breast cancer tumors. Compared to the optimal size range for cancer treatment of 10 to 100 nm, this size is large enough to penetrate cancerous tumors without being rapidly eliminated from capillaries, as well as for renal filtration, leading in poor drug storage and low treatment efficacy, but also small enough to penetrate into cancerous tumors, avoiding blockage due to the large particle size and preventing the elimination of mononuclear phagocytosis in the spleen and liver [9-12]. In addition, Fe_3O_4 nanoparticles ranging in size from 15 to 30 nm will exhibit internal superparamagnetic properties, which will allow them to be transported and stored in certain places supported by external magnetic fields [13].

The Fe content of the obtained nanoparticles is in the range of 16.35 mg/mL, within the optimal concentration range of magnetic materials of 10-80 mg/mL. The content of Fe in the nanoparticles does not exceed the allowable Fe content in commercial products Ferumoxtran–10 (about 20 mg Fe/mL). The nanoparticle-coated lapatinib content had an average value of about 8.03 mg/mL, equivalent to about 28.83% by dry

particle weight. This content is in the best range for dispersion medium when synthesizing nanoparticles (10-30%) [12, 14].

Based on the hysteresis curve, the obtained nanoparticle belongs to a soft magnetic material, exhibiting superparamagnetic properties. The main characteristics of this material are high permeability, low hysteresis loss, small demagnetization field strength, and large residual magnetic induction [15]. The zeta potential value shows that the nanoparticles are almost electrically neutral, and the stability of the nanoparticles in solution is relatively good [16]. The results are quite similar to Kędzierska's study in 2021 [17].

III. CONCLUSION

In this study Fe_3O_4@lapatinib nanoparticles were prepared by co-precipitation method with high purification efficiency of 95.5%. The results showed that the size of Fe_3O_4@lapatinib nanoparticles is in the range of 10 to 40 nm, hydrodynamic diameter is of 27.4 ± 0.6 nm, saturation magnetization is 39.1 ± 3.1 emu/g, functional Fe content is 16.35 ± 0.97 mg/ml, lapatinib content is 8.08 ± 0.06 mg/ml, and the zeta potential is -16.2 ± 1.3 mV. Thus, the obtained Fe_3O_4@lapatinib nanoparticle is fully suitable for targeted drug delivery in the breast cancer treatment.

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