

Study of the Structural and Magnetic Properties of CoFe_2O_4 Nanoparticles for Therapeutic Agent for Cancer Therapy

M. Khairul Islam, M. Nasir Uddin Khan and M. Shahinuzzaman

Abstract— The cobalt ferrite (CoFe_2O_4) nanoparticles were synthesized by chemical co-precipitation technique and characterized by X-ray diffraction (XRD), energy dispersive X-ray spectroscopy (EDX), transmission electron microscopy (TEM), vibrating sample magnetometer (VSM), and Mössbauer spectroscopy. The CoFe_2O_4 nanoparticles coated with biocompatible chitosan at different concentrations to produce homogeneous suspensions were also characterized by dynamic light scattering (DLS) and hyperthermia set-up. XRD data of the sample explored the particle size near about 7 nm in as-dried condition. TEM micrograph of bare CoFe_2O_4 nanoparticles provided the particle size nearly too about 8 nm which is in good agreement with the XRD data. This particle size after coating with chitosan became 14 nm. EDX results of this sample confirmed its nano dimension with spinel structure. VSM results of the sample in as-dried condition showed the ferromagnetic character which has been beyond proved by Mössbauer spectroscopy. The hydrodynamic diameter (H_d) and polydispersity index (PDI) of the chitosan-coated samples also provided promising results. The samples were tested for their induction heating properties with an RF magnetic field of 20 mT and a frequency of 342 kHz. We also studied therapeutic efficiency in-vitro on 9L gliosarcoma cancer cells, which revealed > 98% of mortality through hyperthermia protocol with the same RF field and frequency using chitosan-coated CoFe_2O_4 nanoparticles.

Index Terms— CoFe_2O_4 nanoparticles, Co-precipitation technique, Chitosan (CS), XRD, EDX, TEM, VSM, Mössbauer, DLS, and Hyperthermia.

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I. INTRODUCTION

THE toxicity, magnetization, controlled anisotropy and the efficacy of the induction heating capabilities of cobalt ferrite nanoparticle is quite high even though its toxicity should be lower value. The required dose of nanoparticles for any biomedical application might be reduced by the order of their toxicity level. i. e. The nanoparticles might be toxic if they are used in large quantities [1, 2]. The possibility of reducing the dose of nanoparticles is very important for any biomedical purpose. Toxicity of CoFe_2O_4 can be taken care of by appropriate coating and/or encapsulation which can be used in a controlled fashion. For example, the induction heating capability of zinc ferrite is much lower whereas cobalt ferrite is very effective in induction heating capability. Therefore, cobalt can be used in biological applications. We might minimize the toxicity of cobalt ferrite nanoparticle using biocompatible coating materials and its magnetic properties can be enhanced which would immensely enhance hyperthermia application. Because of its high efficacy as a thermo-therapeutic application of cancer the scientists were driven by the motivation to overcome the toxicity issues by the surface coating or encapsulation. It is also the basic principle that an agent with high efficacy might be administered in a controlled way than the agent with moderate or less efficiency. Again the use of the agent would depend on its application which would be different in different cases. Therefore, for the last few years nanoferrite based agent for the application of biomedicine is the subservient of exquisite research [3–15]. Again, a higher magnetic moment coupled with controlled anisotropy of CoFe_2O_4 nanoparticles gives rise to the enhanced specific loss power (SLP) for induction heating which is useful for the thermo-therapeutic application for cancer treatment.

Since biocompatibility is the larger issue of ferrite nanoparticle especially CoFe_2O_4 nanoparticle surface modification of the CoFe_2O_4 nanoparticles using different coating and encapsulation was focused by many researchers. The objective was to prepare a stable suspension so that it is injectable into the

animal, make them tissue-specific and biocompatible [16, 17]. In this text, $CoFe_2O_4$ nanoparticles were synthesized by chemical co-precipitation technique to develop nanometer scaled magnetic nanoparticles and coat the chitosan to achieve greater biocompatibility.

Therefore, chitosan is known as a biocompatible and biodegradable agent that can reduce the toxicity effect of the nanoparticles in the blood stream. The nanoparticles will be subjected to the structural characterization and then attested for induction heating efficacy by measuring specific loss power (SLP).

II. EXPERIMENTAL

$CoFe_2O_4$ nanoparticles have been synthesized by chemical co-precipitation technique using a molar stoichiometric ratio of cobalt and iron salts. In this text, $CoCl_2 \cdot 4H_2O$ and $FeCl_3$ salts were used to prepare the $CoFe_2O_4$ nanoparticles. $NaOH$ was used as the reaction agent. Cobalt and iron chloride salts were dissolved homogeneously in the distilled water with stirring at 500 rpm for 15 min. We added 8 M of $NaOH$ solution dropwise into the salts solution with continuous stirring at the 600 rpm. Extra $NaOH$ was added in the solution to maintain the pH in the range of 11-13 and allowed to complete the reaction for 30–40 min. We heated the cobalt ferrite nanoparticles at 90°C for 60 minutes to accomplish their ferritization reaction. The precipitation was collected through centrifugation at 8000 rpm for 20 min and 10 times washed out with distilled water. The complete extraction of the solvent was confirmed by the $AgNO_3$ test. The coating solution of low molecular weight of chitosan (SIGMA ALDRICH) with Brookfield viscosity 20 cps was prepared by adding 0.4 gm of chitosan, 40 ml water and the required amount of acetic acid solution. The chitosan solution was added with $CoFe_2O_4$ nanoparticles into the falcon tube under sonication and vortex due to homogeneous mixing for a stable suspension.

Structural characterization has been performed by X-ray diffraction (XRD), Model: PW 3040-X' Pert PRO PANalytical, Netherland; energy-dispersive X-ray spectroscopy (EDX), Model: S50 QLD9111, FEI, Netherland; transmission electron microscopy (TEM), Model: F-30 Tecnai, FEI, U.S.A; dynamic light scattering (DLS), Model: Nanoplus-1; Zeta/nano Particle Analyzer; U.S.A. XRD was carried out using the Rigaku $Mo K\alpha$ radiation source (where, $\lambda = 0.7107 \text{ \AA}$). Particle size and lattice parameters were determined from the 311 broadening peak by XRD patterns of $CoFe_2O_4$ nanoparticles in the as-dried condition. A Tecnai F-30 TEM was used to perform the morphological observation of the sample with the 200 kV operating voltage. The required amount of sample in liquid form was prepared and EDX measurement was performed by using X-band Bruker EPR spectrometer which has given the atomic percentage and crystalline formation of nanoparticles. The field dependence magnetic property of the sample

was also performed using a vibrating sample magnetometer (VSM), Model: EV9; NETZSCH Micro Sense; U.S.A which was further confirmed by Mössbauer spectroscopy, Model: MS4; U.S.A.

In order to confirm the surface modification by chitosan to investigate the DLS measurement and induction heating properties of $CoFe_2O_4$ nanoparticles, the respective solution was prepared. The required amount of suspension 20 mg/ml initial concentration was prepared by mixing of as-dried sample, chitosan solution, and water in an eppendorf tube and sonicated for 45 days and take rest this sample another 45 days owing to complete well coating and more stable solution. Actually, the chitosan-coated sample was taken under continuous sonication for 7 days by ultrasonic sonicator and vortex to perform homogeneous mixing. After that, this sample was taken in rest for 7 days to stable and highly disperse in the solution. In this way, this sample was repeatedly taken in sonication and in rest 45 days with confirming a well coating condition. Otherwise, this sample might be settled out at the bottom of the eppendorf tube which means the coating is not good and provides an unstable solution. It is noted that well coated, more stable, and highly disperse solution is desired for biomedical application. The well-coated nanoparticles reduced their cytotoxic effect. The size and shape effect of nanoparticles depend on the sonication time [18]. Therefore, the coated sample was produced to several concentrations such as 1 mg/ml, 2 mg/ml, and 4 mg/ml in three different eppendorf tubes and their hydrodynamic diameter and polydispersity index measurement have been carried out by DLS using zeta potential electrophoretic mobility technique with the +ve and -ve changing of particle diameter. Helium-Neon LASER source was used in DLS measurement where the wavelength, $\lambda = 6328 \text{ \AA}$. The understanding of hydrodynamic diameter and polydispersity index is very important for biomedical issues as well as hyperthermia applications. After that, induction heating properties were measured by hyperthermia set-up, Model: 5060LI EASY Head, Ambrell, U.S.A. The hyperthermia set-up consisting of 8 turns with a 4 mm diameter of induction coil using 200 A current, 20 mT applied field, 342 kHz frequency, and 1.5 kW applied power during the measurement.

Temperature profiles of chitosan-coated $CoFe_2O_4$ nanoparticles solution have been explored as a function of different coating concentrations and different time intervals carried out by the induction heating unit. We first examined cytotoxicity study of the sample in order to perform necrosis of 9L gliosarcoma cancer cells by magnetic particle hyperthermia. The 9L rat gliosarcoma cell line was cultured in DMEM with containing 10% FBS, (1:1 $\mu\text{g/ml}$ penicillin and streptomycin) at 37°C in a 5% CO_2 incubator for the sake of investigating the

hyperthermia effect on the cancer cells. After 80% confluence, cells were harvested and counted. We transferred 2×10^6 cells per condition in an eppendorf tube and incubated with 2 mg/ml of chitosan-coated nanoparticles which were suspended in the PBS for 24 hours, incubation at 37°C in a $5\% \text{ CO}_2$ incubator. Then the cells were subjected to an alternating magnetic field at 20 mT and frequency of 342 kHz for about 30 min and determined the hyperthermia effects on the cell viability by hemocytometer, Model: Nuaire, U.S.A. The cell viability was performed by the trypan blue exclusion test. The cells with control particle were incubated in the DMEM media followed by the exposure to RF induction heating for 30 min whereas the cells were treated with chitosan-coated nanoparticles at the 2 mg/ml concentration under RF induction heating for the same period of time.

III. RESULTS AND DISCUSSION

X-ray diffraction pattern for CoFe_2O_4 nanoparticles synthesized by chemical co-precipitation technique is presented in Figure 1(a) which gave information about phases, particle size, and lattice parameter. All Bragg reflections have been indexed as (220), (311), (400), (420), (511), (440) and (620) which confirmed the formation of well-defined single-phase cubic spinel structure. The broadening peak indicates the nanodimension of CoFe_2O_4 nanoparticles and the all peaks in the XRD pattern well matched with the previous reported XRD data of the standard spinel structure of the sample. The average particle size was determined from the full-width half maxima (FWHM) of the broadening peak (311) using the Scherrer's formula. The particle size for the sample was obtained as nearly 7 nm in the as-dried condition. The lattice parameter can also be determined from the XRD data using the Bragg's law and Nelson-Riley function, which was found to be 8.35 \AA . Figure 1(b) shows the EDX spectrum of CoFe_2O_4 nanoparticles in the as-dried condition with liquid form. It is observed that the atomic percent of Co, Fe, and O are 4.82, 12.49, and 24.52 respectively. The other minor peaks would be due to the contamination of synthesis nanoparticles.

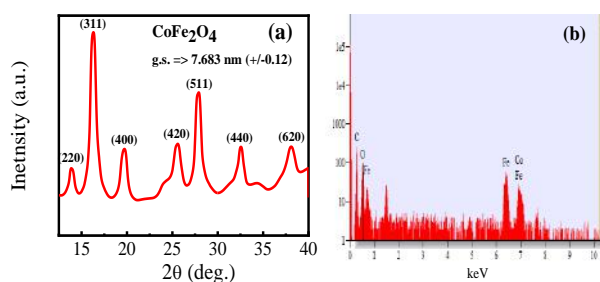


Figure 1: (a) XRD pattern of CoFe_2O_4 nanoparticles in the as-dried condition at the room temperature and (b) EDX spectrum of CoFe_2O_4 nanoparticles in the as-dried condition with liquid form.

Figure 2(a) and 2(b) show TEM micrographs of bare and chitosan-coated CoFe_2O_4 nanoparticles. It is observed from the TEM micrographs that most of the particles appear spherical in shape. The TEM micrograph of cobalt ferrite nanoparticle in the bare state provided the particle size nearly 8 nm and this particle size after coating with chitosan became 14 nm . The particle sizes in the bare state seem to be in fair agreement with the XRD data.

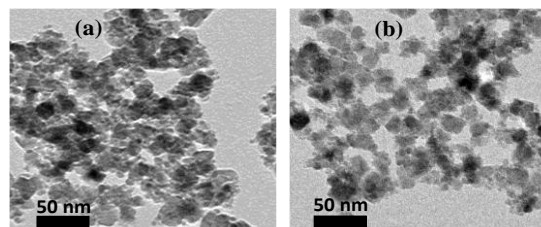


Figure 2: TEM micrographs in (a) Bare and (b) Chitosan-coated CoFe_2O_4 nanoparticles.

Figure 3(a) represents the M-H curve of CoFe_2O_4 nanoparticles in the as-dried condition by a maximum 5 Tesla applied magnetic field. It can be observed that the maximum magnetization of CoFe_2O_4 in as dried condition is 65 emu g^{-1} at 5 Tesla , which has not been saturated at such high applied field. The remanence and coercivity are 7.4 emu g^{-1} and 100 Oe respectively. Magnetocrystalline anisotropy K_1 of CoFe_2O_4 is $1.89 \times 10^6 \text{ erg cm}^{-3}$ [19]. The higher values of K_1 of cobalt based ferrites generally increase to the area in the hysteresis loop during the larger particle size.

However, in this experiment, the M-H curve of the sample shows small remanence and coercivity because of the monodomain nature of the particles where magnetocrystalline anisotropy was largely averaged out.

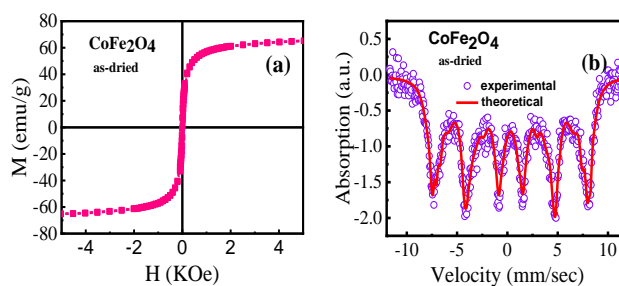


Figure 3: (a) MH hysteresis loop and (b) Mössbauer spectroscopy of CoFe_2O_4 nanoparticles in the as-dried condition at room temperature.

Figure 3(b) shows the Mössbauer spectrum of CoFe_2O_4 in as-dried conditions. It can be observed in this figure that the sample exhibits a clear sextet pattern and confirms the ferromagnetic nature of the sample [20, 21].

Table 1: Hyperfine parameters of CoFe_2O_4 nanoparticles taken by Mössbauer spectroscopy at the room temperature:

Composition	Position of Fe^{3+}	Isomer shift (δ) mm/sec	Quadrupole splitting (ΔE_Q) mm/sec	Hyperfine field (H_{hf}) KG	Area	Site Occupancy	
						B (%)	A (%)
As-dried CoFe_2O_4	B	0.257	0.000	479.955	0.324	45	55
	A	0.375	0.544	514.325	0.135		
	B	0.368	0.000	439.113	0.121		
	A	0.500	0.344	476.341	0.056		
	A	0.336	0.530	386.722	0.369		

Both theoretical and experimental data perfectly matched to each other and provides the ferromagnetic character. It has been found from the literature that for magnetic particle hyperthermia of the nanoparticles which is in a weakly ferromagnetic state [22]. The hyperfine parameters of Mössbauer spectrum were observed and recorded in Table 1. The occupancy of iron species is 45 % on B sites i.e. in octahedral position and 55 % on A sites i.e. in tetrahedral position.

The hydrodynamic diameter (H_d) and polydispersity index (PDI) of CoFe_2O_4 nanoparticles at 25°C, 37°C and 45 °C are presented in Figure 4(a)-(c). In each figure, we found the repeatability is satisfactory.

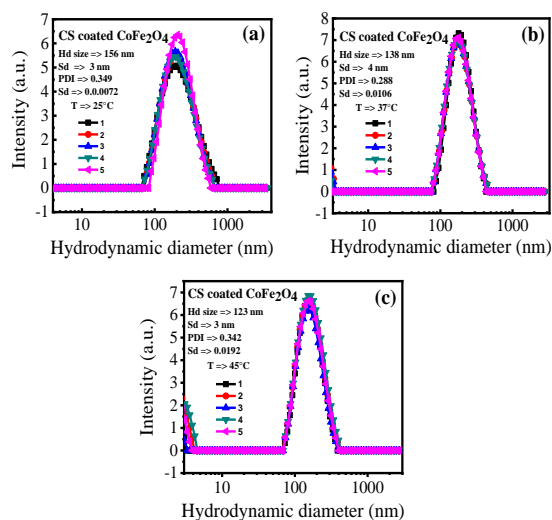


Figure 4: Hydrodynamic size distribution of: (a) Chitosan-coated CoFe_2O_4 of core diameter 156 nm and PDI 0.302 at 25°C (b) Chitosan-coated CoFe_2O_4 of core diameter 138 nm and PDI 0.288 at 37°C (c) Chitosan-coated CoFe_2O_4 of core diameter 123 nm and PDI 0.310 at 45°C, the physiological temperature inside the cuboids containing the sample used for DLS measurement are demonstrated.

The H_d of the nanoparticle is very important to know before using it as a hyperthermia agent. It reveals information about the diameter of the inorganic core along with the attached dragging fluid while coated nanoparticle is in the Brownian motion [23]. We get information only about the organic core during the estimation of particle size using XRD, TEM in the absence of a hydration layer. But for better understanding during in-vivo study of the nanoparticles with considering their biological significance, the measurement of H_d is crucial and this value should be less than 250 nm [24]. The PDI is another important parameter. Generally, the lower PDI is a prerequisite for biomedical purposes and its normal range should be 0.300 [25].

It can be seen from those figures that the average H_d and PDI slightly decrease with increasing the temperature from 25°C to 45°C because of the lowering down the pH of the solution. The change of H_d and PDI with five times the repetition of the sample at 25°C and 37°C is slightly higher than that of values at 45°C during the DLS measurement. Therefore, the H_d and PDI of the sample at 45°C is more stable than other temperature ranges. The average H_d and PDI of the chitosan-coated sample at 25°C, 37°C, 45°C are 156 nm, 138 nm, 123 nm, and 0.302, 0.288, 0.310 respectively. The H_d and PDI of chitosan-coated samples are shown in Table 2 which is satisfactory for biomedical applications.

Table 2: Variation of H_d and PDI for CoFe_2O_4 nanoparticles at different temperature:

Composition	Temperature (°C)	Hydrodynamic diameter (Hd)		Polydispersity index (PDI)	
		Hd (nm)	Sd (nm)	PDI	Sd
CS-coated CoFe_2O_4	25	156	3	0.302	0.0072
	37	138	4	0.288	0.0106
	45	123	3	0.310	0.0192

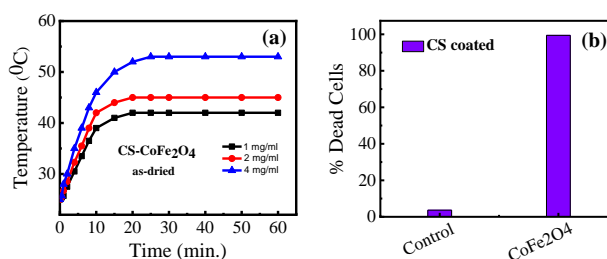


Figure 5: (a) RF induction heating properties of the chitosan-coated CoFe_2O_4 at different concentrations (b) Necrosis of 9L tumor cells by RF induction heating for 30 min without nanoparticle (control) and with chitosan-coated CoFe_2O_4 nanoparticle at the 2 mg/ml concentration in PBS by applying ($H = 20 \text{ mT}$ and $f = 342 \text{ kHz}$).

Figure 5(a) shows the induction heating properties of chitosan-coated CoFe_2O_4 nanoferrite solutions showed the temperatures are increasing as a function of time and nanoparticle concentrations. It can also be observed from these figures that the temperatures rose in the solutions of chitosan-coated solution of concentrations 1 mg/ml, 2 mg/ml, and 4 mg/ml are 44 °C, 49 °C and 53 °C respectively. Here lower ranges of temperatures (up to 46 °C) are more suitable for hyperthermia application. Therefore, solution concentration of 1 mg/ml and 2 mg/ml which attained 44 °C and 49 °C temperature suggested being the most suitable for hyperthermia application. The temperature at a concentration of 4 mg/ml is too high which is not suitable for hyperthermia application. The concentration of 2 mg/ml is better for therapeutic application of cancer therapy. These results suggested that these samples have suitability for hyperthermia application in cancer therapy. The hyperthermia results with specific loss power (SLP) are listed in Table 3.

Table 3: Hyperthermia results of CoFe_2O_4 nanoparticles at different concentration:

Composition	Concentration (mg/ml)	Temperature (°C)	Specific loss power (SLP)
CS-coated CoFe_2O_4	1	44	141
	2	49	136
	4	53	100

Figure 5(b) shows the necrosis of 9L rat gliosarcoma cells under induction heating in the presence of chitosan-coated CoFe_2O_4 nanoparticles at the 2 mg/ml concentration which were estimated by the viability assay in PBS where field amplitude $H = 20 \text{ mT}$, frequency $f = 342 \text{ kHz}$ was used. It was obtained that the cells were viable under induction heating by applying the same RF field with control particle. It can be observed from Figure 5(b) that the high mortality of cancer cells nearly about 99% showed by the chitosan-coated nanoparticle. It might be assumed from this preliminary observation that the nanoparticles were attached by the cells.

It is very interesting to note that this experiment may be more useful for the effective killing of cancer cells with remaining the normal cells. This statement also arose from a preliminary experiment that we injected the nanoparticles in chicken meat, which was subjected to the RF induction heating. The heating effect was observed only where nanoparticles were deposited. The immediate vicinity of the chicken meat remained unaffected. Thermal therapy of cancer namely hyperthermia is still at its infancy though significant research has been carried out in this direction along with clinical trial for localized tumors. Considerable research is still necessary for its successful implementation.

IV. CONCLUSION

It was found that the XRD pattern and TEM micrographs of CoFe_2O_4 nanoparticles confirm their single-phase spinel structure and nanodimension. It was also found that the EDX results provide the single crystalline in nature which implies the high-quality uniform CoFe_2O_4 nanoparticles. It was noticed from the magnetic study that these nanoparticles have satisfactory saturation magnetization with minimum remanence and coercivity. These samples also confirm their ferromagnetic in nature. It was also observed that induction heating profiles of these samples are good for hyperthermia application. It is interesting to note that this work demonstrates promising results in support of biocompatible chitosan which is a potential therapeutic agent for cancer therapy.

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