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Research Article

SYNTHESIS OF MICELLES GUIDED CO-FERRITE PARTICLES AND THEIR APPLICATION FOR AC MAGNETIC FIELD STIMULATED DRUG RELEASE

Debabrata Pal*[1], Debarati De[2], Arpita Das[2], Arka Chaudhuri[3], Madhuri Mandal Goswami[4]

[1]*Department of Physics, Kandi Raj College, Kandi, Murshidabad, West Bengal, India,*

[2]*CRNN division, University of Calcutta, Sector III, Block JD, Kolkata, West Bengal, India*

[3]*Department of Applied Science and Humanities, Haldia Institute of Technology, Haldia, West Bengal, India*

[4]*S. N. Bose National Centre for Basic Sciences, Sector III, Block JD, Kolkata, West Bengal, India*

**Corresponding author: deba.phy@gmail.com*

ABSTRACT

Cobalt ferrite magnetic nanoparticles of two different sizes about 250 nm and 350 nm have been prepared by coprecipitation method using oleic acid as micelles. Particles prepared in this process changes their size with change of micelles concentration. With change of oleic acid concentration, number of micelles formed changes and for higher concentration higher number of micelles and for lower concentration lower numbers of micelles are formed which function differently during growth of particles and hence helps in size change of particles by changing nucleation of the particles initially formed. Structural analysis of the particles was done by XRD measurement and morphological study was performed by SEM measurement. DC and AC magnetic property of the particles have also been studied. The particles have shown their application in drug release by stimuli responsive technique. AC magnetic field stimulated drug release event by these particles provide a direction of the promising application of these particles for better localized cancer treatment in near future.

Keywords: Micelles, Drug release, Stimuli responsive technique, Cobalt ferrite nanoparticle

1. INTRODUCTION

Recently, the area of research in magnetic nanoparticles (MNPs) with tunable size, shape, and porosity has become a subject of immense interest due to its significant applications in biomedical sciences [1, 2]. The magnetic property of the MNPs can be tuned by changing their composition, size, structure which opens up the possibility of diverse range of useful applications of the particles from magnetic resonance imaging (MRI), magnetic separation, magnetic field triggered drug delivery and release, magnetic hyperthermia therapy etc. [2-5]. Along with tunable magnetic property the MNPs are non-toxic in nature which makes them promising biocompatible material. Beside these the particles possesses chemical stability, controlled size, uniform dispersion in liquid medium and easy surface modification which are also necessary criteria for different type of biomedical applications.

The most interesting property of these MNPs is that its coercivity (H_C) has a striking dependence on their size. With the decrease of particle size they become single domains below a critical diameter (D_S) and in this size range the H_C reaches a maximum. On further decrease of particle size the H_C decreases and reaches to zero, particles become superparamagnetic. In that case the volume (V) of the single domain uniaxial particles are so small that the thermal energy becomes higher enough compared to magnetic anisotropy energy barrier KV (K= magnetic anisotropy constant) which overcomes the anisotropy forces and spontaneously reverses the magnetization of a particle from one easy direction to the other. As a result, in the absence of magnetic field the net magnetic moment of a system containing MNPs will be zero above a certain temperature known as blocking temperature (T_B) [6]. However, in the presence of a field, there will be a net alignment of magnetic moments, analogous to paramagnetic materials, except that the magnetic moment is much larger than that of a paramagnetic material. This property allows the MNPs to maintain their colloidal stability and prevent agglomeration after removal of external fields which is preferred in many biomedical applications like MRI, magnetic hyperthermia etc. [7].

Superparamagnetic or ferromagnetic nano-particles are suitable for magnetic hyperthermia therapy as they can heal the affected cell by local heating under ac magnetic field caused by hysteresis loss and eddy current [8]. Among the mentioned therapeutic treatment magnetic hyperthermia therapy has special advantage as it has less adverse side effects than the other treatment. However it is not the solution of all cases, in many cases cancer drug is required to reach at the affected site. As the anticancer drug is equally harmful for normal and cancer cell both, so the use of this drug in human body for the purpose of curing cancer only without affecting normal cell has become a great challenge before the researchers in this field [9]. Several toxic side effects of anticancer drug on healthy tissue/organ occurs during the therapy which may cause to reduce the administered dose or delay treatment or even discontinuation of the course of chemotherapy [10]. To minimize the side effect and to get a remedy of all these difficulties a controlled, tunable and targeted drug delivery technique is required for practical point of view. Recently various kind of drug delivery technique has been developed to address the issue [11]. Among them targeted drug delivery techniques has gained special interest as the drug selectively targets here only the affected region that minimizes the undesirable side effects.

Drug delivery mainly involves: placing or loading drug molecules properly onto the carrier, targeting the drug to the specific cell and control the release of the drug. Different types of carrier/vehicle such as polymer nano composite, liposome, lipid-polymer hybrid nanoparticles, MNPs etc. have been developed for drug delivery system [12]. Among them MNPs has gained special interest due to their unique magnetic property that could be utilized to serve various purpose of the magnetic field triggered targeted drug delivery process. Drug loaded MNPs can be guided to the desired site with the aid of an external dc magnetic field [13]. As the particles produce local heating under the application of ac magnetic field, the release of drug can also be controlled by magnetically induced heat treatment methodology, whereas different stimuli like electric field, heat, pH, light, enzymes, etc. [14, 15] are required for other drug delivery systems. Iron and their oxides (usually magnetite-Fe_3O_4 or maghemite γ-Fe_2O_3) with proper surface modification have been widely used as a promising MNPs in the field of biomedicine [16]. To stabilize the particles in vivo, biocompatible organic ligands such as polyethylene glycol, dextran, aminosilanes are commonly used for coating [17]. However coating with these ligands modifies the surface anisotropy of the particles which in turn modulates the magnetic property [18]. It is noteworthy to mention that the size, shape, surface modification and finally the magnetic property of the MNPs play the key role for its application in biomedical science, however it requires careful evaluation. To meet those key requirements in the aspect of biological application various kinds of wet chemical techniques to synthesise MNPs have been reported in the literature such as coprecipitation, hydothermal coprecipitation, reversed micelles coprecipitation under hydrolytic method and also in non-hydrolytic method [19-22]. Although iron oxides MNPs are still the most extensively encountered in this field, recently spinel ferrite of Co, Zn have also been proposed for this purpose as they have shown improved magnetic property compared to Fe_3O_4 or γ-Fe_2O_3[23]. It has been shown that the $CoFe_2O_4$ NPs exhibit high magneto-crystalline anisotropy, and more efficient SAR (Specific absorption rate) value variably increases of its size up to a certain limit [23, 24]. Therefore $CoFe_2O_4$ nanoparticles (NPs) with proper shape, size and surface modification may be suggested for biomedical application, although it still demands intensive study for the fulfillment of several parameters and to get the desired therapy effect.

In this work spherical $CoFe_2O_4$ NPs of two different sizes about 250 and 350 nm were prepared and various magnetic measurements were performed to study their effectiveness in hyperthermia treatment. Doxorubicin (DOX), a traditional anticancer drug of class of anthracycline family was used for the experimental purpose. The drug release profile of the drug loaded particles also been investigated under certain conditions to study their efficiency in drug delivery process.

2. EXPERIMENTAL

2.1. Material and methods

Cobalt ferrite magnetic nanoparticles (CFMNPs) were synthesized by wet chemical coprecipitation method. All the chemicals,Iron acetyl acetonate, cobalt acetate, phenyl ether, ethylene glycol, oleic acid, urea were procured from Sigma-Aldrich and used without further purification.

2.2. Synthesis of cobalt ferrite nanoparticles

3 mmol of iron acetyl acetonate and 1.5 mmol of cobalt acetate were dissolved in a mixture of 40 ml phenyl ether and 10 ml of ethylene glycol. Next 4ml oleic acid and 3

gm urea are added to this and the mixture was heated in 500 ml beaker for 1 hr at 160°C. Particles are annealed at 300 °C for 1 hr. Here two sets of particles are prepared by changing the oleic acid concentration only. In one set 4ml and in other set 2ml of oleic acid was taken keeping all other conditions unchanged. In case of 4 ml oleic acid particles formed are named as set A and in 2ml of oleic acid the prepared particles are named as set B.

2.3. Physical properties measurements of the CFMNPs

To characterize the phase and crystallinity of the synthesized particles X-ray diffraction pattern (XRD) was carried out at room temperature using Cu K_α ($\lambda = 0.154$ nm) radiation in a RigakuMiniflex II X-ray diffractometer within 2θ range from 20° to 70° under 1°/min scanning rate(at 40KV & 40 mA). Surface morphological property of the particles was studied by a scanning electron microscope (SEM, QUANTA FEG 250). A vibrating sample magnetometer (VSM, Lake Shore Model-7144) was employed to measure DC magnetic properties of the CFMNPs at room temperature up to a magnetic field of 1.6 T. The AC magnetic field dependent measurements were carried out in our own laboratory made AC hysteresis measurement setup. To study the drug release, a spectrophotometer (Shimadzu model UV-2600) with 1 cm path length of quartz cuvette was used.

2.4. Folic acid coating and drug loading of the CFMNP

For coating of CFMNPs with folic acid (FA), 10 mg of FA (purity 97%) was taken in 10 ml of distilled water and shaken well. Then 10 mg of particles were dispersed in this FA solution and the mixture was stirred overnight in dark condition. Following magnetic separation method, the FA coated particles were separated from the solution. The separated particles were washed well for several times by water to remove excess unbound FA and dried overnight.

To load the drug, 10 mg of FA coated CFMNPs were taken in 5ml of water and this 100 μL of sodium bicarbonate (1×10^{-3} M) was added to make the particles water soluble. The doxorubicin hydrochloride (Dox), purchased from Sigma Aldrich (purity of 98 - 102%), was dissolved in de-ionized water with concentration of the solution 6.897×10^{-4}M. 100 μL of this Dox solution was added to the above particles and allowed to stir for 1 hr. Then these particles are separated from solution and were gently washed with pH 7.4 PBS buffer and again dried properly.

3. RESULTS AND DISCUSSION

3.1. Structural and morphological properties

The SEM micrographs of as–synthesized two sets of samples are shown in Fig. 1 (a) and (b). It is evident from the images that the particles of both the samples are spherical in shape. The average size (diameter) of the particles in two sets (A, B) of samples is found to be about 250 nm for set A (Fig. 1 (a)) and 350 nm for set B (Fig. 1(b)). The particle size is smaller in case of higher concentration of oleic acid compared to its lower concentration. It is because at higher concentration of oleic acid the mobility of nuclear particles becomes less hence coagulation properties become also less. This causes the particles of smaller size. But in case of lower concentration of oleic acid reverse situation takes place.

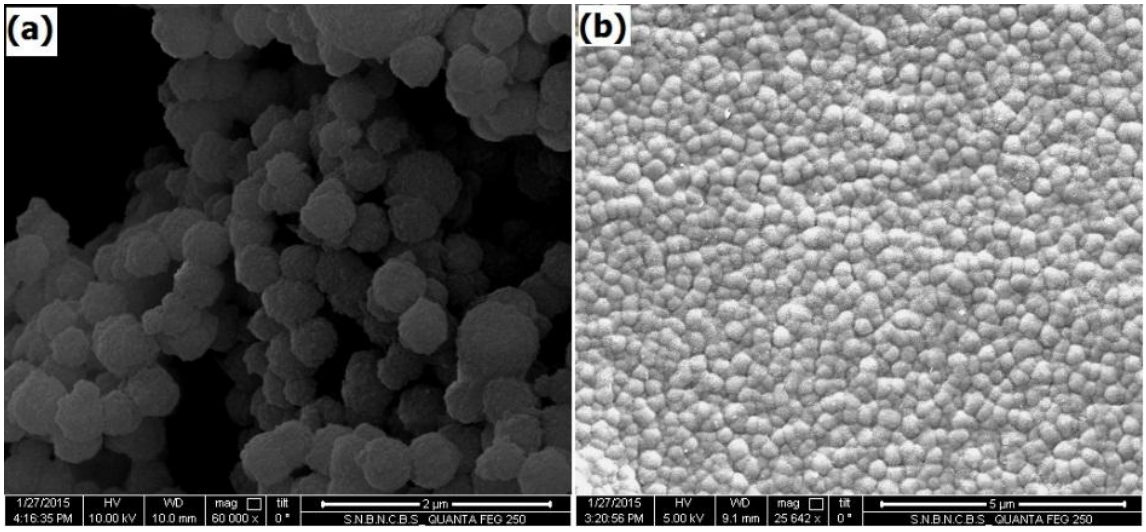

Fig. 1: SEM micrograph of the particles of size (a) 250 nm and (b) 350 nm.

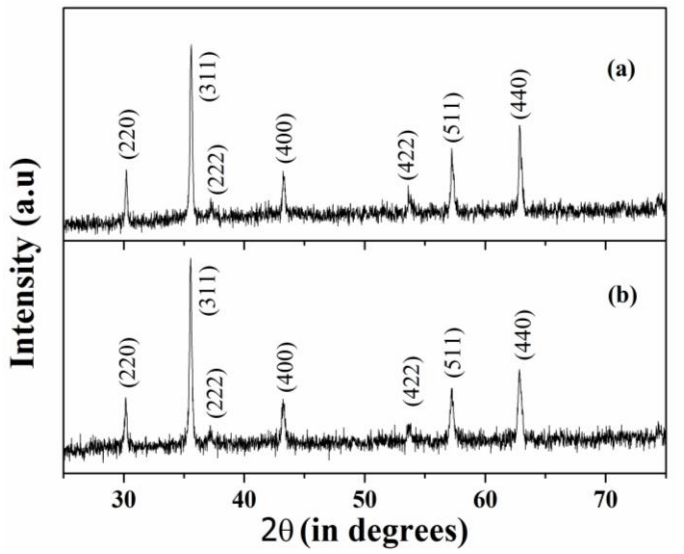

Fig. 2: X-ray diffraction pattern of the particles of size (a) 250 nm and (b) 350 nm at room temperature

The structure and crystalinity of the particles were also investigated by powder X-ray diffraction (XRD). Fig. 2 (a) and (b) show XRD pattern of the synthesized two sets (A, B) of particles of size 250 nm and 350 nm respectively at room temperate. All the diffraction peaks of the pattern are indexed and mapped with JCPDS data, which indicates a good match with the JCPDS data (card no. 22-1086) that confirms the spinel structure of $CoFe_2O_4$ for both the samples. The crystallite size (d) of the samples has been calculated using Debye Scherrer's equation ($d=0.9\lambda/(\beta\cos\theta)$). Considering the most intense peak (311) of the XRD pattern, the crystallite size (d) of the two sets of samples of size 250 nm and 350 nm was calculated and found to be about 50 nm and 60 nm respectively.

3.2. Magnetic properties of the $CoFe_2O_4$ nanoparticles

To study the static magnetic property of the as-synthesized $CoFe_2O_4$ particles, DC magnetic field dependent hysteresis curve (M-H curve) of the samples has been taken at room temperature. Both the samples of particle size 250 nm and 350 nm exhibit large hysteresis with coercivity (H_C) 1.78 KOe and 1.7 KOe respectively.

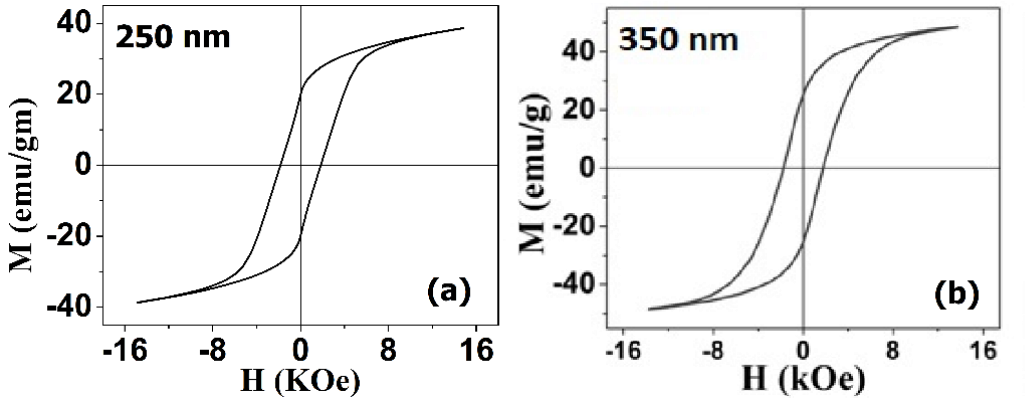

Fig. 3: DC Hysteresis loop of as-synthesized particles of size (a) 250 nm and (b) 350 nm at room temperature

Here the crystallite size of the particles (d =50 nm and 60 nm) is larger compared to the reported value of single domain particle size of $CoFe_2O_4$ (about 20 nm) [25]. Thus the particles of both the samples are multi-domain nanocrystallites. In case of multi-domain particles magnetization dominated by domain wall motion which causes the decrease of coercivity with the increase of crystallite size of the particles [6]. So the particles of diameter 250 nm show higher coercivity than the particles of diameter 350 nm. A decrease of saturation magnetization (M_S) with the decrease of particle size has been observed for the samples [Fig. 3(a) and (b)]. This behaviour may be attributed to increase in surface spin canting with the decrease of particle size.

In this work we aim to use these cobalt ferrite magnetic nanoparticles (CFMNPs) for drug release by hyperthermia technique. In this technique under AC magnetic field hysteresis loss takes place for magnetic particles which produce a thermal agitation in the particles and this stimulates release of drug molecule from the drug incorporated particles. Hence AC magnetic field plays the key role for such application. Therefore the AC magnetic property of the CFMNPs was investigated which can give an indication of their

applicability in drug delivery. This study is made as it has a crucial importance in hyperthermia application. When these CFMNPs are subjected to an AC magnetic field of frequency f, the hysteresis loss by the particles in one cycle of magnetization is given by simply integrating the area of the hysteresis loop which actually can give a quantitative measure by calculation of specific loss power (SLP) also known as SAR (specific absorption rate) value. The SLP value is obtained from the product of the area of the hysteresis loop and corresponding frequency of the applied magnetic field [9]. The higher value of SLP implies higher the amount of heat release. The SLP depends on structural and magnetic property of the particles and also on the frequency and amplitude of the magnetic field. However, SLP is an increasing function of frequency and field amplitude, the enhancement of SLP by increasing these parameters has certain limit as the induction of eddy currents in the patient's body due to high field may cause unwanted heating throughout the body. In addition to the unwanted heating effect in human body, there are some technical issues that also restrict the upper limits of this parameter [26].

To check the suitability of the particles in terms of heat release the ac hysteresis loops of the two samples were taken within a maximum magnetic field of 43 KA/m at a frequency of 50 Hz (Fig. 4 (a) and (b)). It is observed that the area of the hysteresis loop of the larger particles is little bit higher than that of the smaller particles. The power loss of the particles of size 250 nm and 350 nm was calculated from these hysteresis loops and found to be 0.052 W/g and 0.061W/g respectively.

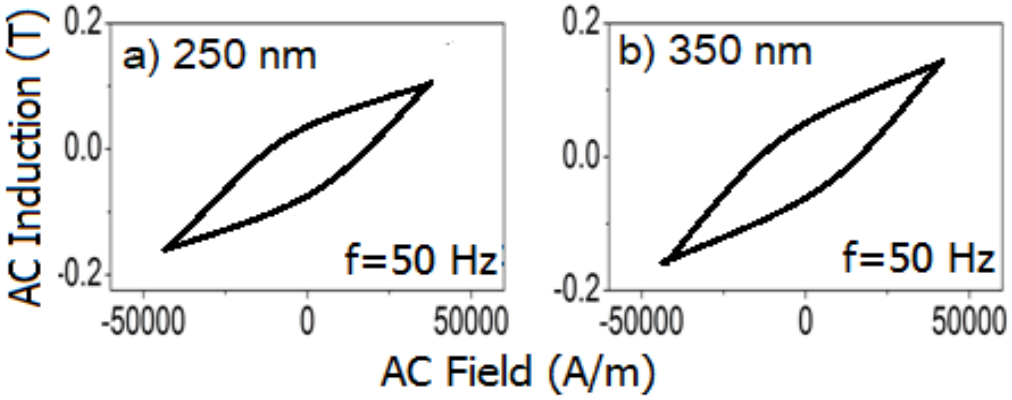

Fig. 4: Hysteresis loop under the application of AC magnetic field of frequency 50 Hz for the particles of size (a) 250 nm and (b) 350 nm.

As the particles of both the samples are multidomain in nature, magnetization reversal under the application of ac field accompanied by domain wall motion and pinning leading to hysteresis and hence power loss [26]. For the studied particles the SLP mainly contributed from hysteresis loss as N′eel or Brown relaxation and frictional loss are not applicable for the ferromagnetic multidomain solid powder system. The hysteresis loss of this particle system increases according to third power law on field amplitude and linearly with the field frequency. So for a fixed field amplitude the specific power loss varies with the square of the frequency. A parabolic nature of SLP with frequency for multidomain ferromagnetic Fe_3O_4 nanoparticles also reported in literature [9]. Therefore, a much higher SAR value that could be obtained from these particles by increasing field and/or frequency of the ac magnetic field within the permissible limit may be utilized to destroy cancer cells by hyperthermia therapy.

3.3. Drug release study

Ten mg of drug (DOX) loaded particles from each sample (of particle size 250 nm and 350 nm) were taken and they were separately dissolved in 3 ml of PBS (PH=7.4) buffer solution to perform the drug release study. With the help of UV-vis spectrometer the intensity of drug release for the two sets of particles at different interval of time has been recorded under AC magnetic field.

Percentage of drug release with time for the two samples under AC magnetic field (with a maximum field of 43 KA/m at a frequency 50 Hz) has been plotted in Fig. 5. It is evident from the graph that for both the samples drug release takes place efficiently under the AC magnetic field. Under AC magnetic field due to hysteresis loss the particles are thermally agitated and the drug molecules are detached from the particles and release of drug takes place. It is also observed that the drug release rate for

bigger particles is higher than that of smaller particles. It may be due to the fact that for bigger particles the AC hysteresis loop area is higher compare to smaller particles which in turn produce the higher heating ability of the particles and hence higher rate of drug release. Therefore the particles have the potentiality to be utilized in the AC magnetic field triggered drug release by magnetic hyperthermia technique.

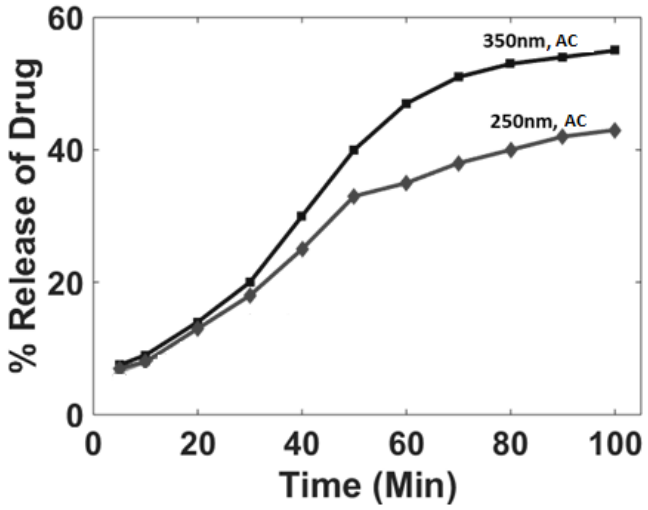

Fig. 5: Time dependent drug release curves of two samples of particle size 250 nm and 350 nm under AC magnetic field

4. CONCLUSION

Cobalt ferrite spherical magnetic nanoparticles of size about 250 nm and 350 nm have been prepared by coprecipitation method using oleic acid as micelles. Structural characterization confirms that the particles are pure phase cobalt ferrite. Drug release efficiency of the particles under the application of AC magnetic field has been studied to check their suitability in AC magnetic field triggered drug delivery. This study indicates a very good efficiency of the particles in AC magnetic field triggered drug release. Hence, the prepared particles will be useful for ac magnetic field controlled drug delivery by stimulating with the suitable magnetic field. The field can be applied from outside the body. Hence the technique will be minimally invasive and more effective.

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