Synthesis and Characterization of Polyethylene Glycol-coated Mesoporous Maghemite Nanoparticles as Carriers of Anticancer Therapeutics in Inhalational Delivery Systems

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***Abstract*— The iron oxide nanoparticles with superparamagnetic characteristics are potential carriers for utilization in pulmonary drug delivery systems. However, their low surface area leads to insufficient loading capacity for encapsulation or conjugation of diagnostic and therapeutic agents. In this study, we have synthesized maghemite nanoparticles with mesoporous structure using cetyl trimethyl ammonium bromide (CTAB) as template. The synthesized nanoparticles were then coated with a thin layer of polyethylene glycol with molecular weight of 1500 Da (PEG1500) in order to endow thermosensitive characteristics to the nanoparticles and protect their cargo from uncontrolled release. The results showed that although incorporation of CTAB to the synthesis solution led to a significant increase of particle size, however the surface area was also increased from 29 ± 8 to 91 ± 17 $m^2.g^{-1}$, indicating the improved capability of nanoparticles for loading and attachment of different diagnostic and therapeutic compounds. Moreover, the onset phase transition temperature of the protective PEG1500 at 40.4 ± 1.8°C makes these nanoparticles appropriate for application under hyperthermia condition.**

Keywords— Maghemite, Surface Area, Hyperthermia, lung cancer, pulmonary delivery.

I. INTRODUCTION

Lung cancer is a common kind of malignancy with the highest mortality rate as well as a miserable dismal rate of below 5 years, causing 14% and 24% of overall cancer deaths in women and men, respectively [1]. Although intravenous administration of chemotherapeutics is the preferred treatment for lung cancers in advanced stages, the high drug dosages injected by this method result in drastic increase of systemic toxicity.

Recently, administration of chemotherapeutics via inhalation has received a growing interest to obtain localized delivery of low drug dosages with minimized systemic toxicity, higher absorption rate, and faster onset of action in the tumor cells [2]. However, direct inhalational delivery of chemotherapeutics increases their negative effect on the lung parenchyma [3]. Therefore, these therapeutics are mostly loaded on various particle-based carriers comprised of lipids, polymers, mesoporous silica, and gelatin to decrease the adverse effects of chemotherapeutics on the healthy airways and tissues surrounding the target tumor. Nevertheless, the presence of bio-barriers such as mucus, ciliated cells, and resident macrophages could considerably hamper the targeting, diffusion, and adsorption of these particle-based delivery systems [4] and result in generation of a sharp gradient in particle concentration [5]. Moreover, the regions that require to be targeted by the therapeutic compounds mostly possess inflamed or obstructed airways, which result in deviation of the inhaled particles into the unobstructed airways of the healthy regions and thus, remarkable decrease of the drug deposition in the affected areas [6].

One particular approach to overcome this matter is development of carrier systems that could be actively navigated through the respiratory system using the driving forces greater than those applied by the bio-barriers. For this aim, superparamagnetism could be an appropriate driving force for enhancing the controllability of the inhaled particles and allowing their navigation through the airways using external magnetic fields, while simultaneously improving the contrast properties in magnetic resonance (MR) images. The superparamagnetic carriers could also be utilized to obtain a synergistic effect of chemotherapy and hyperthermia when exposed to the clinical induction heating systems [7]. In such cases, these carriers could be navigated through the pulmonary system to unload their cargo in the tumor site and then, simultaneously produce a significant amount of thermal energy via induction by an external

magnetic field with appropriate intensity. Moreover, the particles could be coated by different thermosensitive agents to protect the encapsulated compounds from uncontrolled leakage inside the pulmonary system [7-9]. When the particles reach the target tumor, the thermal energy produced in the superparamagnetic core could dissociate the protective shell and facilitate release of the therapeutic load. This technique could also overcome the focusing limitation of other thermal modalities such as radiofrequency (RF) and high intensity focused ultrasound (HIFU) in treatment of lung cancer, which is mainly caused due to the intensive movement of lungs by breathing.

In spite of the intrinsic advantages of superparamagnetic nanoparticles, their limited loading capacity for encapsulation of therapeutic agents may result in insufficient drug dosage delivered to the tumor site [9]. Hence, synthesis of these nanoparticles with porous structure could be a particular solution to increase their surface area and achieve higher drug loading capacities. This research is an attempt to synthesize mesoporous maghemite nanoparticles (MMNs) as a potential carrier for inhalational delivery of anticancer drugs. Moreover, a protective polymer shell comprised of polyethylene glycol with molecular weight of 1500 Da (PEG1500) was produced as a thermosensitive capping agent to protect the encapsulated drug at physiological temperatures and allow its controlled release at hyperthermia temperature range provided by an external magnetic field within the tumor site.

II. MATERIALS AND METHODS

A. Synthesis of Nanoparticles

MMNs were synthesized by modification of the method described in our previous work [10]. In this recipe, cetyl trimethyl ammonium chloride (CTAB) was added as template compound in order to obtain nanoparticles with mesoporous structure. In a typical synthesis procedure, 1.0 ml of ferrous sulfate heptahydrate ($FeSO_4.7H_2O$; 2 M), 3.5 ml of ferric chloride ($FeCl_3$; 1 M), and 500 mg of CTAB were thoroughly mixed in 25 ml of ultrapure water, followed by addition of 25 ml ammonium hydroxide (NH_4OH; 33%) dropwise under vigorous stirring. After 2 hours, the nanoparticles were separated using a small magnet, washed with ethanol, and incubated in a methanol solution containing hydrochloric acid (HCl; 3%v/v) for 6 hours to remove the template. The particles were again magnetically separated, rinsed with ethanol, and dried in 80°C for 24 hours.

For synthesis of PEG1500-MMNs, the "graft-to" method was used according to a previously-described protocol [11]. Briefly, the surface-modified nanoparticles were firstly prepared by stirring the as-synthesized MMNs and 3-aminopropyl triethoxysilane (APTES) in toluene at 80°C for 24 hours. Then, the obtained particles were mixed with carboxyl-terminated PEG (COOH-PEG; MW 1500) in toluene at 80°C for another 24 hours. The synthesized nanoparticles were washed with ethanol and freeze-dried for 24 hours to produce PEG1500-MMNs with sufficient stability in aqueous solutions.

B. Characterizations

The chemical composition of the synthesized particles was analyzed using X-Ray diffractometry technique (XRD; Philips PW1840, the Netherlands) with Cu Kα radiation (λ=1.542 A°), step size of 0.026°, and 2Θ range from 20° to 70°. The particle morphology and size were investigated using the transmission electron microscope (TEM; LEO Libra 120 kV, Carl Zeiss AG, Germany). Dynamic light scattering (DLS; Malvern Instruments, UK) was employed to determine the size distribution of the produced particles as well as their zeta potential at pH 7.0. The surface area of the particles was also measured by Brunauer–Emmett–Teller approach (BET; ASAP2020, TRISTAR II 3020 Kr, Micromeritics Instrument Corp., USA). In order to calculate the onset phase transition temperature of PEG1500, differential scanning calorimetry (DSC; DSC820 with TSO 801RO robot, Mettler-Toledo, USA) with scan rate of 5 $^oC.min^{-1}$ was used.

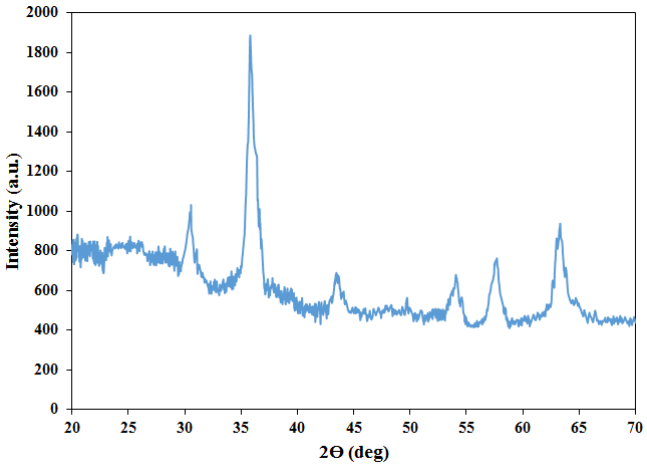

Fig.1. The XRD spectrum of the synthesized MMNs.

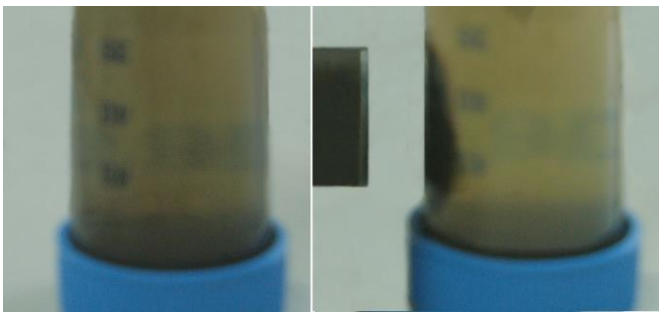

Fig.2. The effect of external magnetic field on MMNs distribution dispersed in an aqueous solution.

The BET, zeta potential, DLS, and DSC experiments were repeated for at least three times and the obtained values were reported as mean ± standard deviation (mean ± SD) using Statistical Package for Social Sciences software (SPSS; version 20, SPSS Inc., USA).

III. RESULTS AND DISCUSSION

The XRD spectrum of the synthesized MMN particles (**Fig.1**) confirm formation of the cubic maghemite phase (ICDD-JCPDS 00-004-0755), where the peaks at 2Θ of 30°, 36°, 43°, 54°, 57°, and 63° respectively correspond to the (220), (311), (400), (422), (511), and (440) planes of maghemite lattice structure.

Fig.2 shows an aqueous solution containing the synthesized particles during exposure to an external magnetic field. The superparamagnetic characteristics of the PEG1500-MMNs allow their navigation in the fluid from a remarkably long distance by a magnet with sufficient field strength. Therefore, it is assumed that fabrication of inhalational delivery systems using MMN cores could be beneficial for more controlled delivery of therapeutic agents to the deep lung regions.

The TEM images of the synthesized non-porous (MNs) and mesoporous maghemite nanoparticles (MMNs) are shown in **Fig.3**. When the particles are synthesized without using the CTAB, their diameter was mostly smaller than 100 nm. However, addition of CTAB to the reaction mixture results in a significant increase of the particles diameter to around 200 nm. When this template is removed from the particles, a mesoporous structure is obtained which could potentially result in higher loading capability of these superparamagnetic nanoparticles.

Fig.4 illustrates a schematic of the expected release mechanism from the PEG1500-MMNs at hyperthermia temperatures. When the MMN cores are exposed to an external magnetic field with sufficient field strength, the thermal energy generated in the superparamagnetic cores results in rapid melting of the protective PEG1500 shell at hyperthermia temperatures, thus providing preferred routes for drug diffusion into the target tissue.

In a preliminary study, different PEG formulations with various molecular weights were investigated and PEG1500 was determined as the optimal formulation with phase transition at hyperthermia temperature range. Although the DSC spectrum of PEG1500 shown in **Fig.5** indicated that the peak temperature of phase transition was approximately 48.3 ± 1.7°C, however the onset temperature of phase transition at 40.4 ± 1.8°C confirmed the suitability of PEG1500 for drug release under hyperthermia condition.

The particle size and zeta potential of the MNs, MMNs, and PEG1500-MMNs are given in **Table 1**. The decrease of zeta potential value in PEG1500-MMNs is probably due to the coverage of surface reactive groups, which act as anchors for PEG conjugation. Moreover, synthesis of the maghemite nanoparticles in mesoporous structure results in around three-fold increase of the surface area (**Table 1**), which could potentially enhance their loading capacity and available sites for conjugation of various compounds.

IV. CONCLUSIONS

A clinically insufficient encapsulation capacity of superparamagnetic materials is their crucial limitation which may lead to multidrug resistance of the tumor cells. In this study, we synthesized mesoporous maghemite nanoparticles

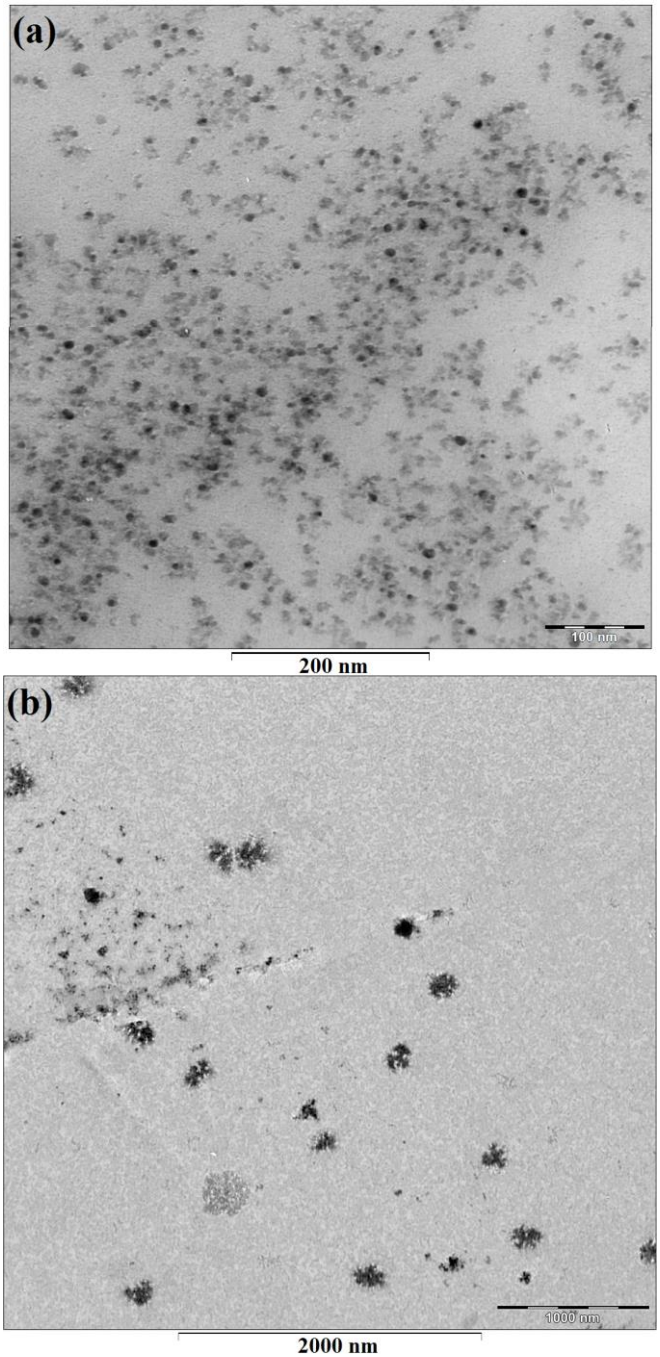

Fig.3. The TEM images of a) MNs, and b) MMNs.

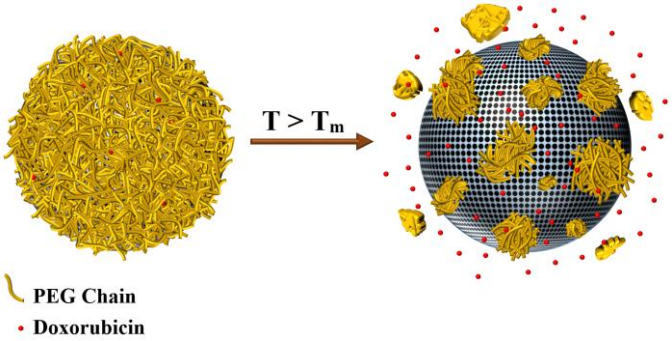

Fig.4. A schematic illustration of the suggested mechanism for thermal-induced drug release from the synthesized PEG1500-MMN carriers under hyperthermia condition. T_m: melting temperature of the polymer layer [9].

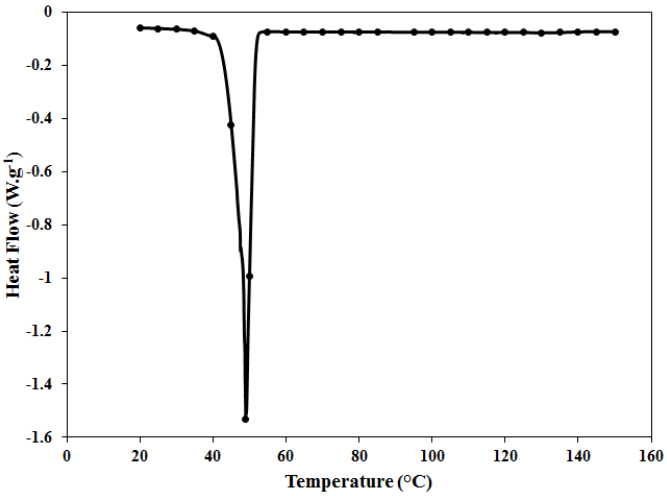

Fig.5. The DSC spectrum of PEG with molecular weight of 1500 Da.

Table 1. The physical characteristics of MNs, MMNs, and PEG1500-MMNs.

	MNs	MMNs	PEG1500-MMNs
Particle size (nm)	87±20	175± 92	179± 70
Zeta potential (mV)	-	25. 8±1.4	5.5±0.9
Surface Area ($m^2.g^{-1}$)	29±8	91±17	-

in order to obtain higher surface areas combined with intrinsic advantages of superparamagnetic behavior. Although formation of the mesoporous structure resulted in significant increase of the mean particle size, the obtained surface area was also three times higher than that of non-porous maghemite particles.

The synthesized particles were also covered by a thin layer of polyethylene glycol with molecular weight of 1500 Da and onset melting temperature 40.4 ± 1.8°C. It is expected that by inhalational delivery of these carriers and simultaneous utilization of an external magnetic field with proper strength, relatively higher ratios of localized delivery with higher efficacy at hyperthermia condition and lower systemic toxicity levels could be obtained. However, the stability tests, drug loading and release studies in complete serum, and *in-vivo* comparative studies under the induction heating condition must be carried out to determine the *in-vivo* efficiency of these newly-synthesized carriers.

REFERENCES

[1] L.A. Torre, F. Bray, R.L. Siegel, J. Ferlay, J. Lortet-Tieulent, A. Jemal, "Global cancer statistics, 2012", *CA Cancer J. Clin.*, vol. 65(2), pp. 87-108, 2015.

[2] Z. Liang, R. Ni, J. Zhou, S. Mao, "Recent advances in controlled pulmonary drug delivery", *Drug Discov. Today*, vol. 20(3), pp. 380-389, 2015.

[3] K. Darwiche, P. Zarogoulidis, N.K. Karamanos, K. Domvri, E. Chatzaki, T.C. Constantinidis, S. Kakolyris, K. Zarogoulidis, "Efficacy versus safety concerns for aerosol chemotherapy in non-small-cell lung cancer: a future dilemma for micro-oncology", *Future Oncol.*, vol. 9(4), pp. 505-525, 2013.

[4] C.A. Ruge, J. Kirch, C.-M. Lehr, "Pulmonary drug delivery: from generating aerosols to overcoming biological barriers—therapeutic possibilities and technological challenges", *Lancet Respir. Med.*, vol. 1(5), pp. 402-413, 2013.

[5] H.X. Ong, D. Traini, C.-Y. Loo, L. Sarkissian, G. Lauretani, S. Scalia, P.M. Young, "Is the cellular uptake of respiratory aerosols delivered from different devices equivalent?", *Eur. J. Pharm. Biopharm.*, vol. 93, pp. 320-327, 2015.

[6] P. Zarogoulidis, E. Chatzaki, K. Porpodis, K. Domvri, W. Hohenforst-Schmidt, E.P. Goldberg, N. Karamanos, K. Zarogoulidis, "Inhaled chemotherapy in lung cancer: future concept of nanomedicine", *Int J Nanomedicine*, vol. 7, pp. 1551-1572, 2012.

[7] A. Dabbagh, B.J.J. Abdullah, H. Abdullah, M. Hamdi, N.H.A. Kasim, "Triggering Mechanisms of Thermosensitive Nanoparticles Under Hyperthermia Condition", *J. Pharm. Sci.*, vol. 104(8), pp. 2414-2428, 2015.

[8] A. Dabbagh, B.J.J. Abdullah, N.H. Abu Kasim, H. Abdullah, M. Hamdi, "A new mechanism of thermal sensitivity for rapid drug release and low systemic toxicity in hyperthermia and thermal ablation temperature ranges", *Int. J. Hyperthermia*, vol. 31(4), pp. 375-385, 2015.

[9] A. Dabbagh, R. Mahmoodian, B.J.J. Abdullah, H. Abdullah, M. Hamdi, N.H. Abu Kasim, "Low-melting-point polymeric nanoshells for thermal-triggered drug release under hyperthermia condition", *Int. J. Hyperthermia*, vol. 31(8), pp. 920-929, 2015.

[10] A. Dabbagh, N.H. Abu Kasim, M.M. Bakri, H. Wakily, C. Ramasindarum, B.J.J. Abdullah, "Polyethylene-glycol coated maghemite nanoparticles for treatment of dental hypersensitivity", *Mater. Lett.*, vol. 121, pp. 89-92, 2014.

[11] S. Gil, E. Castro, J.F. Mano, "Synthesis and characterization of stable dicarboxylic pegylated magnetite nanoparticles", *Mater Lett*, vol. 100, pp. 266-270, 2013.