Fabrication of Stable Bio-Compatible Mesoporous Silica and Core-Shell Silica-Magnetite Nanoparticles for Potential Application In Drug Delivery

Maneea Eizadi Sharifabad[1], Tim Mercer[2] and Tapas Sen[1,3]

[1]Centre for Materials Science, Chemistry Section, School of Forensic and Investigative Sciences
[2]School of Computing Engineering and Physical Sciences
[3]Surface Patterning Group, Institute of Nanotechnology and Bio-engineering, University of Central Lancashire, Preston, United Kingdom, Email: tsen@uclan.ac.uk

ABSTRACT

Herein, we report the fabrication and characterization of stable bio-compatible core-shell superparamagnetic iron oxide nanoparticles (SPIONs) of ~60 nm to 80 diameters and their *in vitro* drug loading / releasing study of anticancer drug Mitomycin C. This study consists of two different coatings on SPIONs, mesoporous silica shell and lipid bilayer shell. The *in vitro* drug loading and releasing studies were carried in phosphate buffer solution and determined by UV-Visible spectrophotometer and the stability study of bio-compatible core-shell nanoparticles in suspension were determined by magnetic susceptibility measurement and magnetic sedimentation study using scanning column magnetometry (SCM). The mesoporosity of the silica shell and the diameter of the particles were determined by small angle X-ray scattering (SAXS) and transmission electron microscope (TEM).

1 INTRODUCTION

Superparamagnetic iron oxide nanoparticles (SPIONs) have become increasingly attractive materials in the field of Nano / Nano-biotechnology due to their potential applications in heteogeneious catalysis [1, 2], nanomedicine such as drug delivery [3, 4], cell sorting / tissue engineering [5] and hyperthermia treatment of tumour in the presence of an external magnetic field [6]. Successful application of such magnetic nanoparticles in the areas listed above is highly dependent on various important parameters such as stability in suspension, size, morphology and surface charge for drug loaded nanoparticles and their behavior in the blood stream when injected intravenously. Gupta *et al* [7] have reported that diameter of nanoparticles ranging from 10 to 100 nm are most effective for drug delivery because they can evade reticuloendothelial system (RES) and hence their circulation time in blood can be prolonged. One of the major problems of working on such nanoparticles is their propensity to aggregate. Such small particles tend to form agglomerates to reduce the energy associated with the high surface area to volume ratio of the nanosized particles [8]. For many applications it is thus crucial to develop strategies to stabilize the magnetic nanoparticles in suspension. These strategies comprise grafting or coating with organic species, including surfactants or polymers, or coating with an inorganic layer, such as silica or carbon onto the nanoparticles. In many cases, protecting shells not only stabilize the nanoparticles, but can also be used for further functionalization.

Mesoporous structures with a network of pores and channels are very promising materials for drug delivery [9, 10] due to their large surface area, controllable surface functionality, endocytosed by cells [11] and nontoxic [9, 12] in nature. Fabrication of well defined mesoporous silica nanoparticles of mesopores in the dimension of 3-10 nm and particles diameters below 100 nm could be the best candidate for *in vivo* drug delivery. Incorporation of superparamagnetic property into such mesoporous silica materials could have an added benefit of mobilisation using external magnetic field to the targeted parts of the body.

Similarly liposomes have been well known for drug delivery for a long time [13, 14]. Liposomes can be used for coating SPIONs for the modification of surface charge and stability against aggregation in suspension. Magnetoliposomes [15, 16] are new class of nanocomposites for drug delivery as they are biocompatible and magnetic. However, most of the reports involved large size (>100 nm) liposomes where magnetite or maghemite nanoparticles have been entrapped and hence could have serious diffusional limitation towards to target cell. So it is always desirable to fabricate biocompatible SPIONs under 100 nm in diameter especially for *in vivo* applications. Recently we have reported [16] bio-compatible superparamagneic iron oxide nanoparticles (SPIONs) core and bio-compatible liposome shell for *in-vitro* drug loading and releasing study.

2 EXPERIMENTAL

2.1 Synthesis of Magnetite Cores

Bared superparamagnetic iron oxide nanoparticles (SPIONs) were synthesized following Massart method [17]

by co-precipitation of an aqueous solution of ferrous and ferric chloride in the presence of ammonium hydroxide with a slight modification which has been previously reported [18] by Sen *et al.*

2.2 Synthesis of Core-Shell Silica-Magnetite Nanoparticles

Mesoporous silica coated superparamagntic iron oxide nanoparticles were prepared using tetraethoxy silane (TEOS) as a silica souce, cetyl trimethyl ammonium bromide (CTAB) as surfactant under acidic condition in the presence of pre-fabricated SPIONs cores.

2.3 Synthesis of Core-Shell lipid bilayer-Magnetite Nanoparticles

Magnetoliposomes were prepared by dissolving 270 mg of SPC phospholipid in 1 ml chloroform within a 500 mL round bottom flask. The flask containing the phospholipid solution was attached to a rotary evaporator (Buchi Rotavapor R-114, Buchi, Switzerland) and immersed in a 35 ºC water bath. Upon evaporation of chloroform, a thin film of lipid formed on the inner wall of the flask. The film was hydrated with 27 mL of deionized water and shaken manually for 10 min and left for 2 h annealing at room temperature before bared SPIONs were added to the solution. The mixture was then placed under strong ultrasonic vibration (titanium horn) for 8 min using a Vibra Cell Sonicator (Sonics and Materials Inc., USA). The mixture was kept in ice bath during the ultrasonication.

2.4 Characterisation of core-shell nanoparticles

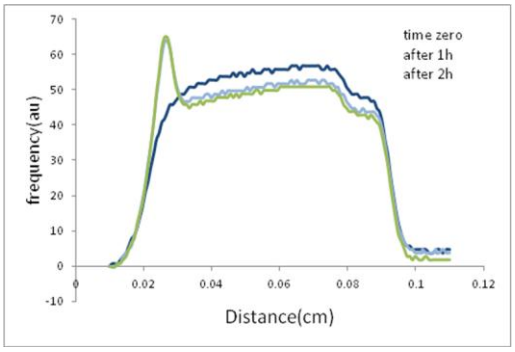
The suspension stability of core-shell magnetic materials was monitored by scanning column magnetometry (SCM). In this technique a column of suspension was scanned to obtain a magnetic profile of the column of dispersion. Comparing profiles as a function of time, the changes in settling of particles can be observed. Small angle X-ray scattering (SAXS by HECUS, Austria) was used for the determination of shell structure of mesoporous shell of core-shell silica-magnetite nanoparticles. Magnetic measurements were performed at room temperature using an in-housed vibrating sample magnetometer (VSM).

2.5 Drug Loading and Release

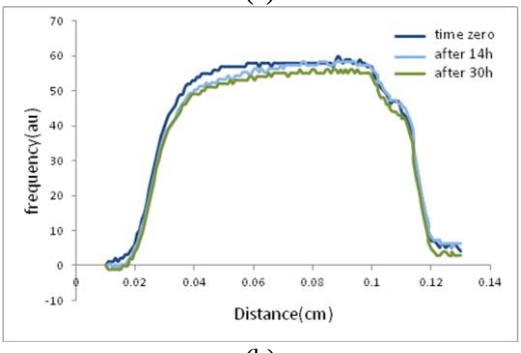
4 mg of silica coated SPIONs were placed in 1.5 mL Eppendorf tubes and incubated with 1 mL of aqueous solution of mitomycin C (MMC) having a concentration of 100 mg/ mL at 18 and 10 °C for up to 30 h. The amount of MMC loading into the nanomaterials at different time intervals were determined by measuring the UV absorption at 365 nm before and after the incubation with nanoparticles. The concentrations of MMC were determined by comparing the absorption values with a pre-established standard curve of known MMC concentrations in water. MMC-loaded nanoparticles were separated from the reaction solution by magnetic separation and washed with deionised water before being used for the release study. Release of MMC from nanomaterials was studied in PBS buffer. 4 mg of washed nanoparticles were treated with 1 mL of PBS buffer (pH = 7.1) at 37 °C under end-over-end rotation for up to 48 h. The nanoparticles were separated in different time intervals from the solution by magnetic separation and the absorbance was measured at 365 nm. The concentrations of MMC in the solution were determined by comparing the absorption value with a pre-established standard curve of known MMC concentrations in PBS buffer.

3 RESULTS AND DISCUSSION

Stability of the two samples(liposome coated magnetite and bared magnetite) have been compared using the SCM. As shown in figure 1, liposome coated magnetite showed slight change in resonance frequency over long period of time (30 hrs) comparing to bared magnetite (1 hr). This confirms that the liposome coated SPIONs were stable in suspension.



(a)



(b)

Figure 1: SCM results for (a) bare magnetite and (b) liposome coated magnetite.

SAXS data (see Figure 2) suggests that the shell structure was mesoporous in nature.

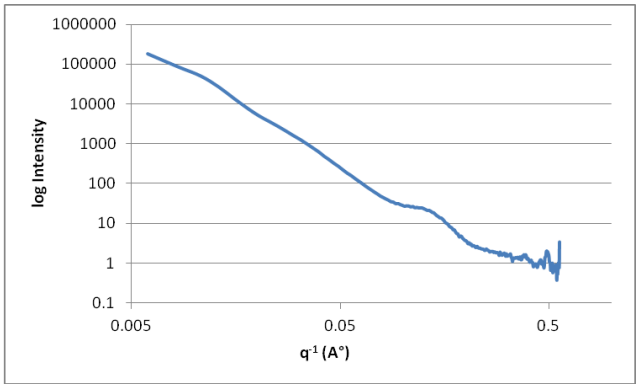


Figure 2: SAXS results for silica coated SPIONs

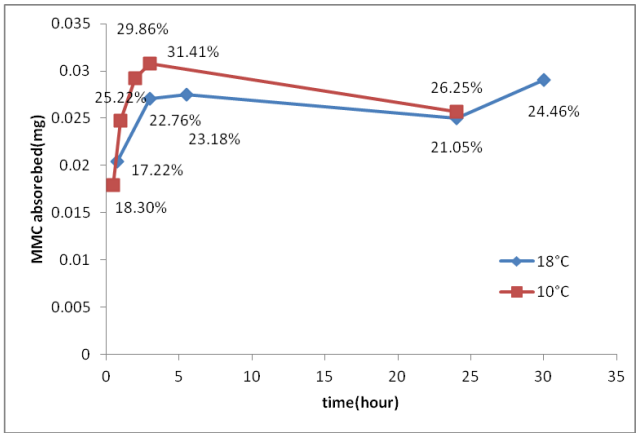


Figure 3: drug loading performance for silica coated SPIONs

Figure 3 illustrate that the uptake of MMC from the solution into the silica coated magnetite against time at two different temperatures (10°C and 18°C). It was observed that decreasing the temperature has increased the drug loading performance of the particles. The maximum loading of drug was observed after 3 hours of incubation and was around 0.03 mg (31% loading).

It was observed that after 3 hours of incubation, the amount of drug loaded into the sample was decreased due to leaking of drug back to the suspension.

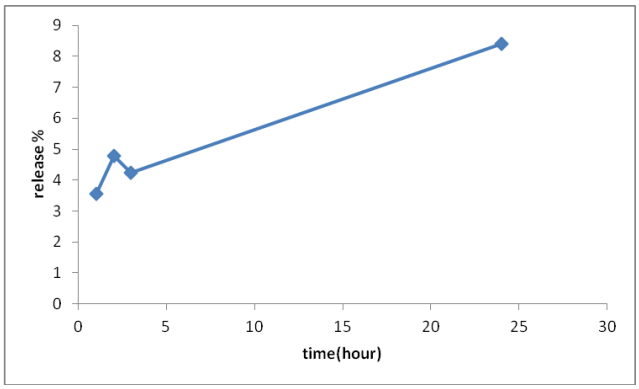


Figure 4:drug release performance for silica coated SPIONs

MMC release data in PBS buffer at body temperature (37°C) has been presented in figure 4. MMC released into the solution with a very slow rate and only around 0.0017mg (8% of the loaded MMC) after 24 hours.

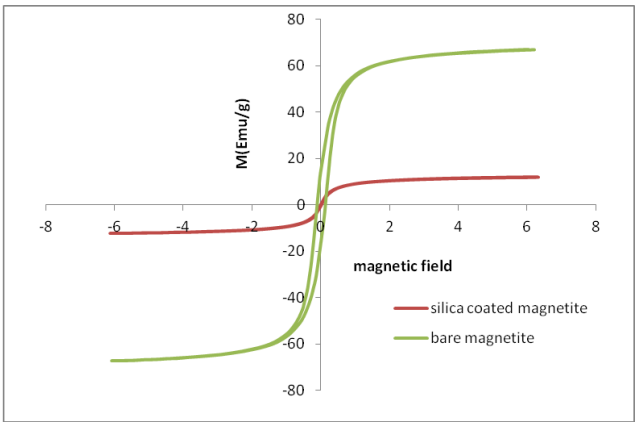


Figure 5: magnetige data for bare and silica coated magnetite

Figure 5 present the magnetic hystersis curves obtained by vibrating sample magnetometer at room temperature. Bared SPIONs exhibited high saturation magnetization of around 70 emu/g. It is observed that coating the magnetite with mesoporous silica resulted a reduction of magnetic satureation to ~15 emu/g due to silica coating.

4 CONCLUSIONS

Superparamagnetic iron oxide nanoparticles (SPIONs) have been fabricated and coated with two different materials (i) mesoporous silica and (ii) liposomes. Liposome coated nanoparticles were stable in suspension. Silica coated nanoparticles were observed to be efficient for drug loading and releasing. All materials were observed to be superparamagnetic in nature hence could be potential for targeted drug delivery using external magnetic field.

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