Magneto-liposomes: Stability of magnetic nanoparticles in suspension for drug delivery

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ABSTRACT

Superparamagnetic iron oxide nanoparticles (SPIONs) of diameter 30-50 nm have been synthesized and stabilized in suspension by one-pot fabrication of magneto-liposomes of diameter around 80nm. Magneto-liposomes have been prepared by mixing with optimum ratio of prefabricated SPIONs and multi lamellar vesicles (MLVs) under strong ultrasonic vibration. Magneto-liposome nanocomposites are stable in aqueous suspension at physiological pH (~7) for days. Surfaces of magneto-liposomes have been characterized by measuring zeta potential at physiological pH and Salmon sperm DNA binding/elution experiments. Stable magneto-liposome exhibited nearly neutral surfaces whereas bared SPIONs exhibited positive and MLVs exhibited negative surface charge at physiological pH (~7). SPIONs have been uniformly coated with liposomes evidenced from high binding and elution of Salmon sperm DNA unlike to bared SPIONs. Pro-drug (Mitomycin-C) entrapment into stable magneto-liposomes at physiological pH (~7) is under study and the results will be presented in the conference (NSTI 2010).

Keywords: SPIONs, liposome, magneto-liposomes, surface charge, drug loading

1 INTRODUCTION

Superparamagnetic iron oxide nanoparticles (SPIONs) have become increasingly important materials for the quick, easy, sensitive and reliable separation of specific bio-molecules and for magnetic hyperthermia agents in medical diagnostics and therapeutics [1-4]. The surface properties and the interactions between nanoparticles in suspension are not well understood. One of the major problems of working on such nanoparticles is the aggregation. Surface modification of such nanoparticles in suspension raised an important question "Does the self-assembled coating of magnetic nanoparticles cover individual particles or agglomerates?"[5]. Recently Sen *et al* [6] have reported the stability of magnetic nanoparticles using commercial dispersing agents, however, they lacked bio-compatibility for *in vivo* purposes such as drug delivery, magnetic hyperthermia and magnetic contrasting.

Liposomes (vesicles formed by amphilphilic phospholipid molecules in water) are well known for drug delivery for long times [7-9] and several review papers have been published [10-12]. Drugs can be entrapped either in the inner aqueous phase or in the lipid bilayers, depending on their hydrophobicity/hydrophilicity ratio. Similarly SPIONs are also well known in the field of drug delivery [13]. The advantage of using SPION as a drug carrier is that they can be transported through the vascular system and can be concentrated at a particular point of the body with the aid of magnetic field [14]. Nanoparticles (diameter < 100nm) based drug carrier are promising candidate for drug delivery as they can diffuse through cell membrane. Size, morphology and surface charge are three important parameters of drug loaded nanoparticles and their behaviour in the blood stream when injected intravenously. Gupta *et al* [15] 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). Recently, Gabizon *et al* [16] have reported that incorporating polyethylene glycol (PEG) to liposome bilayer results in inhibition of liposome uptake by the reticulo-endothelial system and significant prolongation of liposome residence time in the blood stream. Fabrication of bio-compatible SPIONs of diameter 10-100 nm with specific surface charge and hydrophilicity is a challenge for drug delivery.

Sasaki *et al* [17] reported the entrapment of pro-drug Mitomycin-C in liposome and Tokunaga *et al* [18] have reported the release in to the blood stream through intravenous injection. Mitomycin-C is a potent antibiotic-antineoplastic drug for ocular surgery, however, suffers due to ocular toxicity. Chetoni *et al* [19] have reported that liposomal preparation containing Mitomycin-C was capable of reducing the corneal healing rate and drug toxicity of a corneal lesion in a rabbit model. Recently, Zalipsky *et al* [20] have reported that Mitomycin-C loaded STEALTH liposome (SL) has enhanced antitumor activity compared to pure Mitomycin-C or pure Doxorubicin or Doxorubicin loaded SL.

Liposome coated magnetic nanoparticles (magneto-liposomes) are new class of nanocomposites for drug delivery [21-24]. Herein, we report the one-pot fabrication of stable magneto-liposomes and their surface

characteristics at physiological pH (~7) along with the preliminary data on entrapment of Mitomycin-C *in vitro*.

2 EXPERIMENTAL

Bared SPIONs were synthesized following Massart method [25] by co-precipitation of an aqueous solution of ferrous and ferric chloride in the presence of ammonium hydroxide.

Multilamellar liposomes (MLVs) solution was prepared by mixing 270mg of phospholipid (SPC) in 1ml chloroform in a 500mL round bottom flask. The flask containing the phospholipid solution was attached to a rotator evaporator placed on 35^0C water bath. Upon evaporation, a thin film of lipid was observed to be formed in the round bottom flask. The flask containing lipid film was flashed with nitrogen gas in order to remove chloroform residue. The film was dissolved in 27mL of deionized water and stirred manually for 10 min and annealed for 2 hr at room temperature before use for the fabrication of magneto-liposomes.

Magneto-liposomes suspensions were prepared by mixing SPIONs of various amount (0.25mg/mL to 15mg/mL) in 7mL MLVs and placed under strong ultrasonic vibration (titanium horn) for 2, 4, 6 and 8 min.

Bared SPIONs were characterized by X-ray diffraction, magnetometry, scanning and transmission electron microscopy. All materials (bared SPIONs and magneto-liposomes) were characterized by laser particle size analyzer (Nanosizer) and salmon sperm binding and elution experiment [26].

3 RESULTS AND DISCUSSION

The synthesized iron oxide materials exhibited X-ray diffraction pattern of multiple peaks within the 2θ range 30 to 75^0 (data not shown). Indexing peaks corresponding to the fingerprint peaks (220, 311, 400, 440) of pure magnetite (Fe_3O_4). Figure 1 presents the magnetic data of the materials at room temperature. Almost closed loop with negligible coercivity was observed indicating the superparamagnetic nature of the iron oxide materials (SPIONs).

Figure 2 presents the particle size distribution of the bared iron oxide nanoparticles (SPIONs), multilamellar vesicles (MLVs), small unilamellar vesicles (SLVs) and magneto-liposomes (liposome coated SPIONs). Bared SPIONs in suspension exhibited a mean diameter of 60nm whereas MLVs exhibited a bimodal size distribution of sizes around 300nm and 1000nm. SLVs exhibited the smallest size (30nm); whereas SPION coated liposomes (magntoliposomes) was observed to be around 80nm in diameter. The mean size distribution of magneto-liposomes positioned in between bared SPIONs and MLVs with unimodal distribution indicating that a thin layer (~10nm) of phospholipids was formed around SPIONs as a shell.

Electron micrographs (Figure 3) exhibited that bared superparamagnetic iron oxide materials (SPIONs) were 30-50nm in diameter. The size of magneto-liposomes can be characterized by Cryo-TEM, however, currently we don't have the facility to obtain such information.

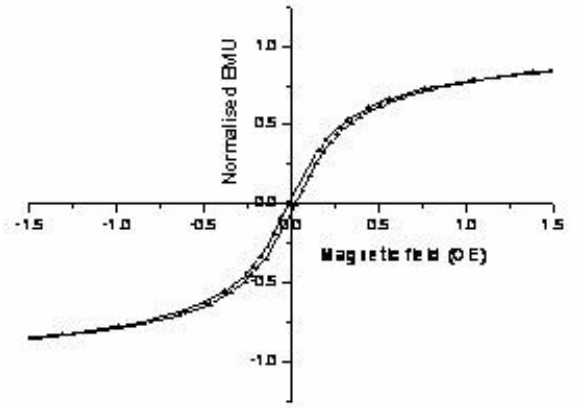

Figure 1 Magnetic susceptibility data of iron oxide materials

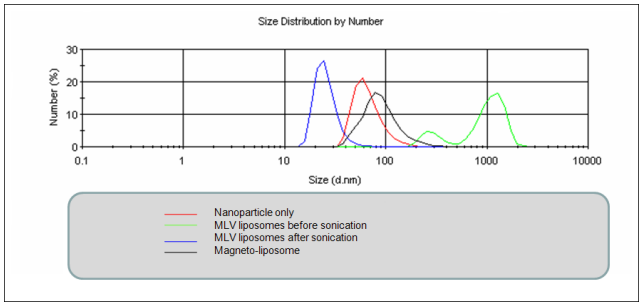

Figure 2 Particle size distributions of liposome, SPIONs and magneto-liposomes

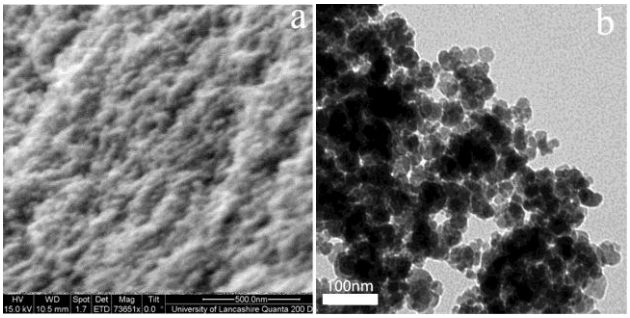

Figure 3 Scanning electron micrograph (a) and transmission electron micrograph (b) of bared SPIONs.

Based on the size of the MLVs, SUVs, SPIONs and magneto-liposomes, formation mechanism of vesicles, SUVs and magneto-liposomes are presented graphically in figure 4. The thickness of phospholipids bilayers can be considered as 8 nm based on the head to tail distance (~4nm) of a typical phospholipid molecule (see figure 4a). The diameter of MLV varies from few hundred nm to few microns (see figure 2) but upon ultrasonication, MLVs can

produce LUV or SUV of smaller in diameter (see figure 4b). Based on the hydrodynamic diameter of SPIONs (~60nm), the minimum size of magneto-liposomes can be around 76nm with SPIONs core and single bilayer shell. The size of our magneto-liposomes (~80nm) supports the formation of single bilayer phospholipid coated SPIONs (see figure 4c).

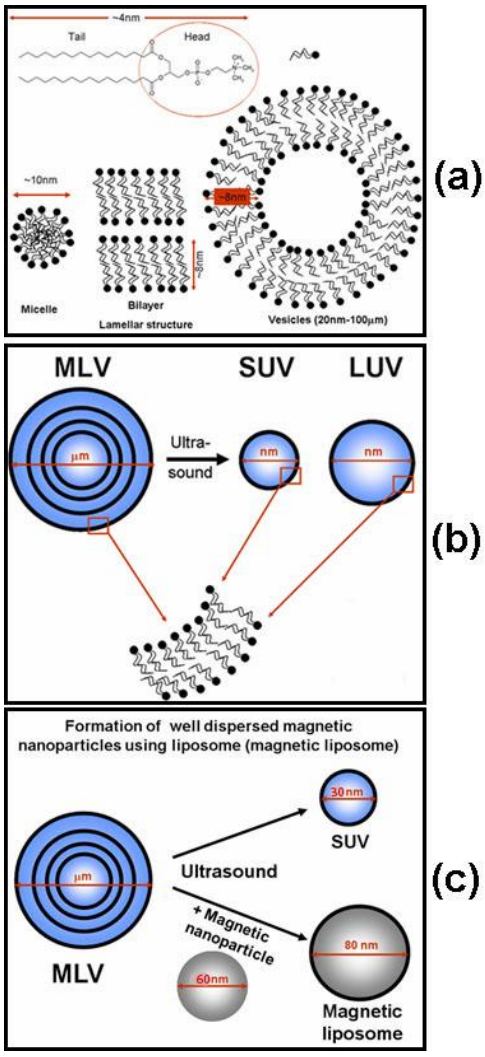

Figure 4 Graphical representation of the formation of liposomes using phospholipids (a), small (SUV) and large (LUV) unilamellar vesicles from multilamellar vesicles (b) and magneto-liposomes (c)

The suspension stability of SPIONs and magneto-liposomes prepared using a mixture of SPIONs (0.5mg/mL to 15mg/mL) in prefabricated MLV solution has been presented in figure 5. Bared SPIONs are unstable in suspension with (Figs.5B and 5C) or without ultrasonication (Fig. 5A). Magneto-liposomes with increasing concentration of SPIONs in suspension increased the suspension stability (Fig.5G). Further increase of SPIONs concentration (> 10 mg/mL) in MLV solution produced an unstable magneto-liposomes suspension (Fig.5H). Ultrasonication time had an effect on the stability of magneto-liposomes (data not shown) and the optimum time was observed to be 8 min for the formation of stable magneto-liposomess.

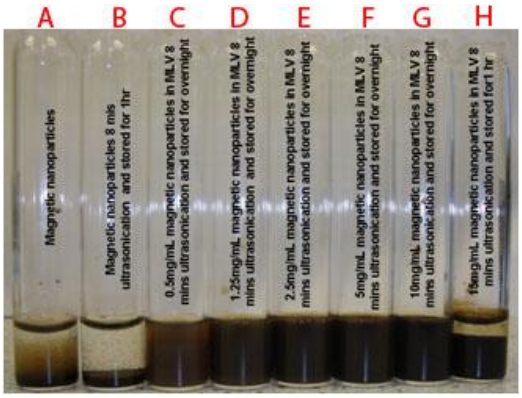

Figure 5 Pictorial presentation of bared SPIONs (A), bared SPIONs after ultrasonication batch SS068(B,C), magneto-liposomes of various batches SS067 (C), SS065 (D), SS063 (E), SS069 (F), SS070(G), SS073(H).

The surface characteristics of bared SPIONs and magneto-liposomes have been studied by Salmon sperm DNA binding/elution experiment and zeta potential measurements (see figure 6). The binding of DNA at physiological pH (~7) under high salt concentration (NaCl) was observed to be quite high in all materials (Fig. 6a), however, elution of DNA from the surface was observed to be different (see Figs. 6a and 6b). The elution of DNA passes through a maxima with increasing concentration of SPIONs during the fabrication of magneto-liposomes. The best elution results were observed with 5 and 10mg/mL of magnetite in magneto-liposomes suspensions. This is a direct indication of a different surface of magneto-liposomes compared with bared SPIONs. Bared SPIONs were reported [26] to be good in binding DNA and poor in elution from the surface.

Zeta potential measurement (Fig. 6c) indicates that SPIONs were positively charged at physiological pH (~7) whereas MLV or SUVs were negatively charged. Magneto-liposomes containing a range of concentration of SPIONs exhibited a systematic change in their zeta potential values. The zeta potential data indicates that increasing the concentration of SPIONs in magneto-liposomes suspension, the surface charge cross over took place from negative to positive values.

In conclusion, a series of stable magneto-liposomes were synthesized in suspension at physiological pH (~7) by simple one-pot method under strong ultrasonication. The size of the magneto-liposomes was measured to be around 80 nm and they were observed to be stable in suspension for days. The drug entrapment into bared SPIONs and magneto-liposomes at physiological pH (~7) is under study. The initial results indicate that entrapment of Mitomycin-C is found to be related to the stability of the magneto-

liposomes in suspension. Detailed data will be presented in NSTI 2010 conference followed by a full manuscript publication in a relevant journal.

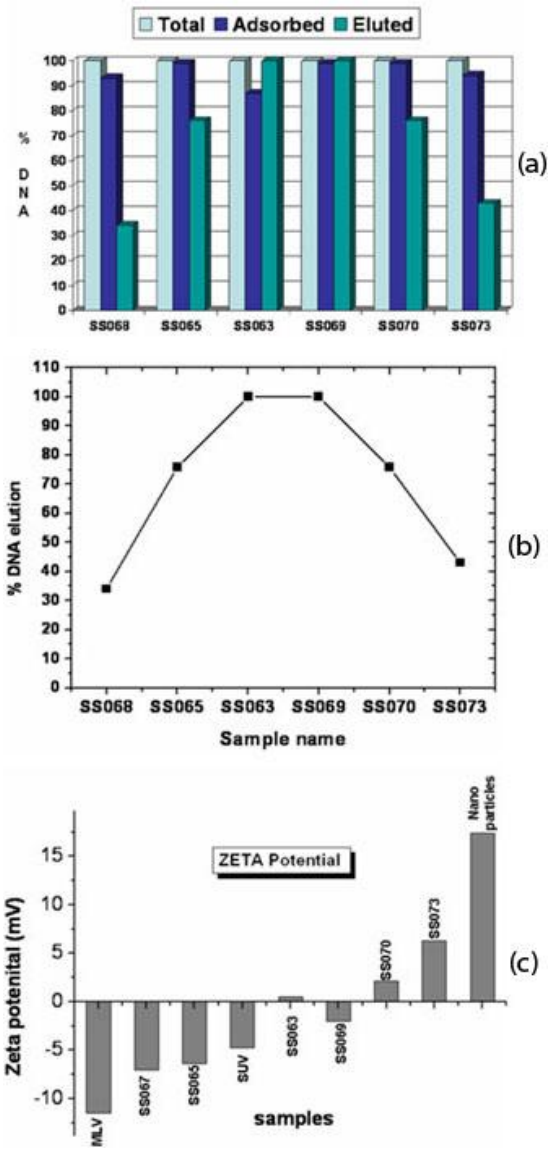

Figure 6 DNA Salmon sperm binding and elution results (a and b) and zeta potential (c) of bared SPIONs, MLVs, SUVs and magneto-liposomes.

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