**Introduction**

Liposomes are a very attractive tool increasingly used as an in vivo delivery vector for therapies such as siRNA (1). This is primarily because they can be used to encapsulate the therapy and hence, protect it in vivo from enzymes and unspecific uptake/degradation in the blood (2). They also have a biocompatible nature and an inherent versatility due to the extensive range of lipid formulations possible, which adds to their increased usage. Liposomes can be formulated to increase blood circulation time by the addition of a pegylated lipid (3), targeted to a specific organ by the addition of a targeting ligand (4) and contain contrast agents such as optical labels and MRI for histology and tracking (5). In this study the effect of using the versatile liposome platform to encapsulate siRNA is characterised in terms of stability and the ability to act as a MR contrast agent.

**Methods**

**Liposome formulation:** Cationic liposomes were formulated consisting of Gd.DOTA.DSA:DOPC:CDAN:DSPE_PEG2000:DOPE-Rhodamine with a molar ratio of 30:31:1:7:5:0.5 mol % respectively. Liposomes were made by mixing the correct ratio of lipid stock solutions and evaporating to produce a thin film which was subsequently hydrated with 4mM HEPES buffer to give a total lipid concentration of 2.88mg ml\(^{-1}\). Liposomes were then sonicated for approximately an hour to create as small a liposome as possible, sizing the liposomes was performed using photon correlation spectroscopy (PCS) over a period of 17 days. Liposomes for encapsulating siRNA were made as liposomes only, but had the appropriate amount of siRNA (to give 0.24mg ml\(^{-1}\)) and at w/w ratio of 1:12 siRNA:lipid) added dropwise whilst vortexing.

**Percentage encapsulation:** The amount of siRNA encapsulated into the liposomes was measured on multiple occasions over 10 days, using the nucleotide binding stain propidium iodide, with the excitation and emission wavelengths set at 535 and 617 nm respectively. Equation 1 was used to calculate EP (encapsulation percentage), from \(S\) (siRNA alone), \(L_s\) (liposomal encapsulated siRNA) and \(L\) (liposome only).

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EP = \frac{S - (L_s - L)}{S} \times 100 \tag{1}
\]

**Relaxivity \(r_1\) determination:** The relaxivity value \(r_1\) for both liposomes only and siRNA-liposomes was determined using a spin echo saturation recovery method on a Varian Inova 4.7T system, with the following parameters: TR = 100, 300, 500, 700, 1000, 3000, 5000, 7000, 10000 and 15000 ms, TE = 11 ms, FOV = 70x30 mm\(^2\), matrix = 256x128, 5 coronal slices, 2 mm thick and 4 averages.

**Results**

The liposomes alone were found not to change in size over the period of 17 days as determined PCS. The encapsulation of siRNA into the liposomes caused a very slight increase in size from 99 ± 21.3 nm to 102.3 ± 21 nm. Figure 1 shows the encapsulation percentage over the 10 days, with a peak at day one at 76% and then followed by a decrease in the amount of siRNA encapsulated as time progresses. After approximately 5 days the encapsulated percentage drops below 50%. Figure 2 shows the plot of \(1/T_1\) vs concentration of gadolinium used to calculate \(r_1\), control liposome was determined at 3.9 mM \(s^{-1}\) and 4.069 mM \(s^{-1}\) for siRNA-liposome. Figure 3 shows a signal intensity image with the same dilutions for both liposome alone (Top) and siRNA-liposome (Bottom), showing there is no discernable difference between the two in intensity and T1 values (data not shown).

![Figure 1. Encapsulation percentage of siRNA into the liposomes for 10 days.](image)

![Figure 2. \(r_1\) calculation for siRNA-liposome.](image)

![Figure 3. Signal intensities from: Top L – R dilution series of liposomes alone. Bottom L – R the same dilutions, but for siRNA-liposomes.](image)

**Discussion**

Liposomes alone appear stable when stored at 4°C for at least 17 days. The encapsulation of siRNA causes a very slight increase in size, but not one significant enough to change the biodistribution properties of the liposomes. The siRNA appears to start leaking out of the liposomes after 24 hours and continues to do so up until day 8. The \(r_1\) values calculated for liposome alone and siRNA-liposome are virtually identical showing that the siRNA encapsulation has no effect on the ability of the liposome to act as a MR contrast agent, which can also be seen from Figure 3, where there is no obvious differences in signal between the two. This study shows that the encapsulation of siRNA into liposomes remains stable for 5 days and therefore experiments should be undertaken within this time period. This might also have consequences when using siRNA-liposomes in vivo. The encapsulation appears to have no tangible effect on the liposomes to act as a contrast agent for MRI and hence the testing of liposomes in vitro can be performed without the expensive and wasteful use of siRNA.

**References**