

# The optimization of liposomal formulations for molecular MR imaging

G. J. Strijkers<sup>1</sup>, W. J. Mulder<sup>1</sup>, E. Kluza<sup>1</sup>, K. Nicolay<sup>1</sup>

<sup>1</sup>Biomedical NMR, Department of Biomedical Engineering, Eindhoven University of Technology, Eindhoven, -, Netherlands

## Introduction

Molecular imaging, in which new agents are combined with traditional imaging techniques to visualize biochemical pathways at the molecular and cellular level, is one of the most challenging applications of MRI. The main challenge arises from the relative insensitivity of MR and necessitates powerful amplification strategies to detect sparse molecular targets. We have recently shown that the use of liposomes containing Gd-DTPA-lipid may provide an effective approach for molecular MRI [1-3]. The present study was aimed to optimize the liposomal preparation for use in molecular MRI. Measurements of the molar relaxivities  $r_1$  and  $r_2$  were done as a function of the content of Gd-DTPA-containing lipid and temperature at 0.47 and 1.41 T.

## Materials and Methods

Five different liposomal preparations (100 nm diameter) were prepared in HEPES buffer as described before [1], using Gd-DTPA-bis(4-hexadecyl anilide) as the MRI label (5 to 25 mol%, i.e. up to the maximum concentration of Gd-lipid that still ensures liposomal stability [1]), dipalmitoylphosphatidylcholine (DPPC) (57 to 37 mol%), cholesterol (33 mol%), and distearoyl-SN-glycero-3-phosphoethanolamine-N-[methoxy(poly(ethylene glycol))-n (n=2000)] (PEG-DSPE) (5 mol%). Relaxivity  $r_1$  and  $r_2$  were measured at 25° C at 0.41 and 1.41 T on a Bruker MiniSpec mq20 and mq60, respectively, using 5 different liposomal concentrations equivalent to 0 to 0.6 mM Gd-lipid. As a reference,  $r_1$  and  $r_2$  of Gd-DTPA (Magnevist) were determined using 6 different concentrations from 0 to 10 mM. The temperature dependence of  $r_1$  and  $r_2$  was measured at 1.41 T in a temperature range from 5 to 70° C, with steps of 5° C.

In a second series of experiments,  $r_1$  and  $r_2$  were measured as a function of temperature (range: 30-60° C; steps of 1° C), using liposomes prepared from Gd-DTPA-BSA (5 mol%), DPPC (92.5 mol%) and PEG2000-DSPE (2.5 mol%). This cholesterol-free preparation exhibits a sharp gel-to-liquid crystalline phase transition at 41° C. Gd-DTPA was again used as a reference. Gd content was measured using ICP, as described [1]. All measurements were done in duplicate.

## Results and Discussion

Table 1 shows the ionic  $r_1$  and  $r_2$  of the liposomal preparation and Gd-DTPA at 0.47 and 1.41 T, at 25° C. The ionic  $r_1$  and  $r_2$  of most liposomal formulations were higher than those of Gd-DTPA, and decreased somewhat with increasing Gd-lipid fraction. The  $r_1$  of the liposomes decreased with increasing field strength, while the  $r_2$  increased. Consequently, there was a moderate increase of the  $r_2/r_1$  ratio with field strength and Gd-lipid concentration. These data suggest that the liposomes containing 25 mol% Gd-DTPA-lipid are the most effective for use in molecular MRI, because the small increase of  $r_2/r_1$  does not outweigh the gain in relaxivity per mM of liposomes that is obtained by the higher concentration of Gd-lipid.

Next, we measured the temperature dependence of the  $r_1$  and  $r_2$  of the liposomal formulations and Gd-DTPA at 1.41 T (Figure 1). Up to circa 35° C, the liposomal  $r_1$  showed very little change with increasing temperature and started to gradually increase thereafter, while the Gd-DTPA  $r_1$  showed a monotonous decline across the entire temperature range. Similar observations were made for the  $r_2$  of both types of contrast agents. These data demonstrate that the liposomes are suitable for use at physiological temperatures and suggest that the Gd-DTPA-lipid in the inner leaflet of the liposomal bilayer becomes gradually accessible to exchange with bulk water at elevated temperature.

Similar conclusions were reached from temperature-dependent  $r_1$  and  $r_2$  measurements on liposomal formulations without cholesterol. These preparations are known to exhibit a sharp phase transition at 41° C that is accompanied by an increased permeability of water across the membrane bilayer. Relaxivities  $r_1$  (Figure 2) and  $r_2$  (not shown) displayed a relatively steep increase when approaching the phase transition temperature and a more gradual increase thereafter. This finding suggests that with increasing temperature the inner layer Gd-DTPA-lipid increasingly contributes to the relaxivities of bulk water.

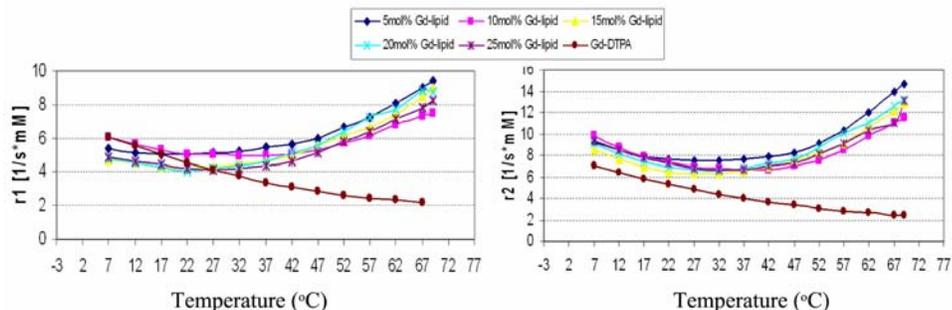
## Conclusions

This study demonstrates that liposomes with a high payload of Gd-DTPA-lipid are attractive candidates for the MRI-based detection of sparse molecular targets. The liposomes containing 25 mol% Gd-lipid are most suitable, because they exhibit the highest relaxivity per mM liposomes with acceptable  $r_2/r_1$  ratio. Further research is warranted to increase the contribution of inner monolayer lipid to the paramagnetic properties of these nano-particulate contrast agents, while maintaining favorable stability properties.

## References

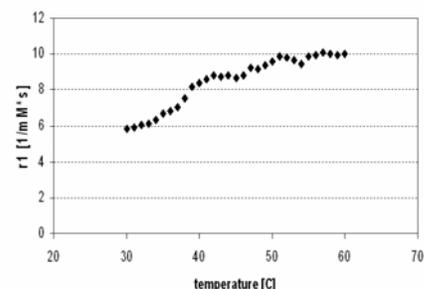
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**Figure 1:** Temperature dependence of the  $r_1$  and  $r_2$  of the liposomal formulations and Gd-DTPA.



**Table 1:** Ionic relaxivities  $r_1$  and  $r_2$  ( $\text{mM}^{-1} \text{s}^{-1}$ ).

Compound	0.47 T			1.41 T		
	$r_1$	$r_2$	$r_2/r_1$	$r_1$	$r_2$	$r_2/r_1$
Gd-DTPA	5.0	5.7	1.13	4.4	5.1	1.16
5 % Gd-lipid	6.0	6.9	1.14	5.0	7.4	1.46
10 % Gd-lipid	5.7	6.6	1.15	4.9	6.8	1.37
15 % Gd-lipid	5.4	6.3	1.16	4.2	6.7	1.59
20 % Gd-lipid	5.4	6.3	1.15	4.2	6.9	1.62
25 % Gd-lipid	5.3	6.3	1.19	4.0	6.7	1.67



**Figure 2:** Temperature dependence of  $r_1$  of the liposomal formulation without cholesterol.