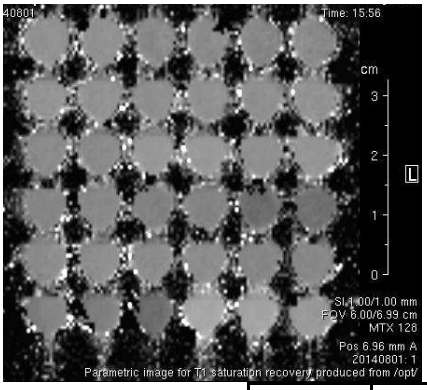
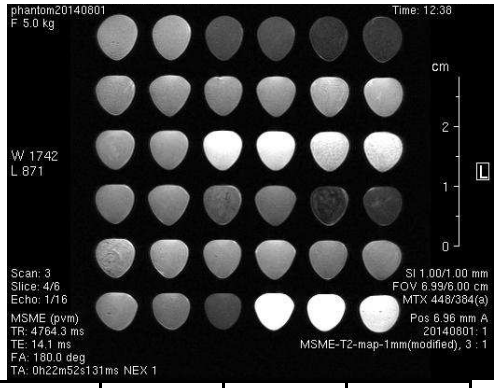
Magnetoliposomes for magnetic resonance imaging

Joan Estelrich[1,3], Josep Queralt[2], Montserrat Gallardo[1], **M. Antònia Busquets**[1,3]
[1]Departament de Fisicoquímica. [2] Departament de Fisiologia. Facultat de Farmàcia. UB. Avda Joan XXIII, s/n 08028 Barcelona
[3]Institut de Nanociència i Nanotecnologia (IN2UB)
joanestelrich@ub.edu

Abstract
The development of superparamagnetic iron oxide nanoparticles (SPIONS) has opened a new and attractive approach towards the molecular imaging of living subjects because they overcome not only many of the current limitations in diagnosis but also in the treatment and management of human diseases [1]. The combination of imaging modalities based on the use of SPIONS such as magnetic resonance imaging (MRI), optical imaging (OI) or positron emission tomography (CT) have been developed to visualize pathological situations. Among these techniques, MRI is one of the most powerful, and non-invasive modality of diagnosis due to its high soft tissue contrast, spatial resolution, and penetration depth. MRI images result from the spatial identification of hydrogen nuclei. The contrast in the images comes from local differences in spin relaxation kinetics along the longitudinal T_1 (spin-lattice) and transverse T_2 (spin-spin) relaxation times [2]. Contrast agents alter the signal intensity by selectively shortening the hydrogen relaxation times of the tissues and are used to improve the sensitivity and specificity of MRI. In particular, SPIONS have emerged as T_2 contrast agents because their enhancement of the negative contrast, thus showing darker images of the regions of interest. This contrast is strongly related to the SPIONS coating being the most widely used dextran and its derivatives. However, these coatings have raised several controversies mainly because of their weak physisorption on the nanoparticle surface and toxicology associated to the coating [3]. To overcome these drawbacks, the SPIONS can be incorporated into lipid vesicles thus obtaining magnetoliposomes (MLs). MLs have special interest because of their low cytotoxicity, enhanced versatility and target biodistribution.
To gain understanding on the magnetic relaxation processes involved in contrast generation by MLs, we seek an analysis of the impact that coating has on the relaxivity of MLs. With this purpose, we have prepared magnetite coated with polyethylene glycol (PEG) [4] and then, the resulting ferrofluid has been encapsulated into liposomes of different lipid composition in order to analyze the influence of size, physical state of the phospholipid bilayer and medium of dispersion of MLs in the MRI parameters [5].
Thus, dimyristoyl phosphatidylcholine (DMPC), dipalmytoyl phosphatidylcholine (DPPC), distearoyl phosphatidylcholine (DSPC) and dioleoyl phosphatidylcholine (DOPC) were chosen to study the effect of chain length and membrane physical state on T_1 and T_2 relaxations. In addition, ferrofluid was incorporated into DMPC:Cholesterol (CHOL) at different molar rations. Finally, the influence of size on MRI relaxivities was analyzed with non-extruded (multilamellar liposomes, MLVs) and extruded MLs (LUVs). Samples were prepared at iron concentrations ranging from 0.2 to 1.2 mM in agar (5%) fantoms as to mimic human tissues, in 8 x 6 cm wells plate (Figure 1).
MRI experiments were conducted on a 7.0 T BioSpec 70/30 horizontal animal scanner (Bruker BioSpin, Ettlingen, Germany), equipped with a 12 cm inner diameter actively shielded gradient system (400 mT/m) and a receiver/transceiver coil covering the whole mouse volume. The sample was placed in a Plexiglas holder. Tripilot scans were used for accurate positioning of the sample in the isocenter of the magnet. T1 relaxometry maps were acquired by using RARE (rapid acquisition with rapid enhancement) sequence applying 9 repetition times = 354.172, 354.172, 500, 700, 1000, 1400, 2000, 3000 and 6000 ms, echo time = 10 ms, RARE factor = 1 average, number of slices = 6 for vertical view, field of view = 69.9 x 60 mm, matrix size = 128 x 128 pixels, resulting in a spatial resolution of 0.546 × 0.469 mm in 1 mm slice thickness. For the T2 relaxometry maps, MSME (multi-slice multi-echo) sequence was used with a repetition time = 4764.346 ms with 16 echo times corresponding to 14.11, 28.21, 42.32, 56.43, 70.54, 84.64, 98.75, 112.86, 126.97, 141.07, 155.18, 169.29, 183.4, 197.5, 211.61 and 225.72 ms, 1 average, number of slices = 6 for vertical view, field of view = 69.9 x 60 mm, matrix size = 448 x 384 pixels, resulting in a spatial resolution of 0.156 x 0.156 mm in 1 mm slice thickness. Data were processed using the Paravision 5.1 software (Bruker, BioSpin, Ettlingen, Germany).

In contrast to the T_2 relaxation, T_1 relaxation depends on a fast proton exchange between the bulk water phase with slow T_1 relaxation, and protons at the surface of magnetic particle aggregates where T_1 relaxation is fast. Since residence time of protons is roughly proportional to the square of the particle size, this can explain the lower values of the MLs compared with the values described in the literature [6].





DMPC [Fe] 0,08 mM	DMPC [Fe] 0,08 mM	DMPC [Fe] 0,14 mM	DMPC [Fe] 0,14 mM	DMPC [Fe] 0,20mM	DMPC [Fe] 0,20 mM
DPPC [Fe] 0,08 mM	DPPC [Fe] 0,08 mM	DPPC [Fe] 0,14 mM	DPPC [Fe] 0,14 mM	DPPC [Fe] 0,20mM	DPPC [Fe] 0,20mM
DSPC [Fe] 0,08 mM	DSPC [Fe] 0,08 mM	DSPC [Fe] 0,14 mM	DSPC [Fe] 0,14 mM	DSPC [Fe] 0,20mM	DSPC [Fe] 0,20mM
DOPC [Fe] 0,08 mM	DOPC [Fe] 0,08 mM	DOPC [Fe] 0,14 mM	DOPC [Fe] 0,14 mM	DOPC [Fe] 0,20mM	DOPC [Fe] 0,20mM
DMPC/chol 80:20 [Fe] 0,08 mM	DMPC/chol 80:20 [Fe] 0,08 mM	DMPC/chol 90:10 [Fe] 0,08 mM	DMPC/chol 90:10 [Fe] 0,08 mM	DMPC/chol 95:5 [Fe] 0,08 mM	DMPC/chol 95:5 [Fe] 0,08 mM

Figure 1. Top: MRI Maps of T_1 (left) and T_2 (right). Below: samples of the above maps. MLVs. multilamellar magnetoliposomes with the corresponding mM iron concentration ([Fe]). DMPC: dimyristoyl phosphatidylcholine; DPPC: dipalmytoyl phosphatidylcholine; DSPC: distearoyl phosphatidylcholine, DOPC: dioleoyl phosphatidylcholine and CHOL: cholesterol. Control samples of ferrofluid (FF) were also analyzed as control (Figure not shown).

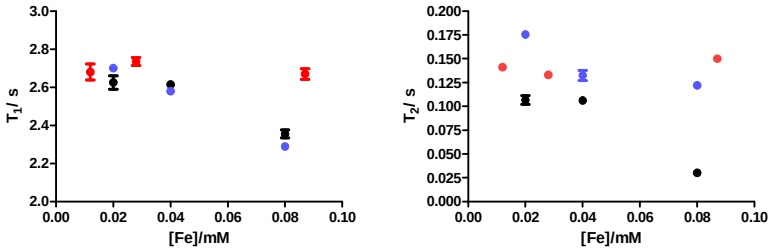


Figure 2. T_1 and T_2 relaxation times of ferrofluid (blue), large unilamellar (red) and multilamellar (black) magnetoliposomes of DMPC

Acknowledgements. The authors are grateful for the financial support given by the Spanish Ministerio de Economía y Competitividad (MINECO) to the project MAT2012-36270-C04-03.

References

[1] C. Corrot, P. Robert, JM Idee, M. Port, Adv. Drug Deliv. Rev. **58** (2006) 1471-1504.
[2] H. Fattahi, S. Laurent, F. Liu, N. Arsalani, L. Vander Elst, R.N. Muller, Nanomedicine **6** (2011) 529-544.
[3] S. Mornet, J. Portier, E. Doguet. J. Magn. Magn. Matter **293** (205) 127-134.
[4] S.García-Jimeno, J. Estelrich, Colloids Surf. A, **420** (2013) 74-81.
[5] R. Sabaté, R. Barnadas-Rodríguez, J. Callejas-Fernández, R. Hidalgo-Alvarez, J. Estelrich, Int. J. Pharm. **347** (2008) 156-162.
[6] M. Hoedenius, C. Würth, J. Jayapaul, J.E. Wong, T. Lammers, J. Gätjens, S. Arns, N. Mertens, I. Slabu, G. Ivanova, J. Bornemann, M. De Cuyper, U. Resch-Genger, F. Kiessling. Contrast Media Mol. Imaging **7** (2011) 59-67.