CHEMICAL ENGINEERING TRANSACTIONS
VOL. 47, 2016
A publication of AIDIC
The Italian Association of Chemical Engineering
Online at www.aidic.it/cet
Guest Editors: Angelo Chianese, Luca Di Palma, Elisabetta Petrucci, Marco Stoller

ISBN 978-88-95608-38-9; **ISSN** 2283-9216

Magnetoliposomes: envisioning new strategies for water decontamination

Stefania Petralito[a*], Patrizia Paolicelli[a], Martina Nardoni[a], Francesca Apollonio[b], Micaela Liberti[b], Caterina Merla[d], Rosanna Pinto[d], Maria Antonietta Casadei[a], Maria Cristina Annesini[c]

[a] Department of Drug Chemistry and Technologies, Sapienza University of Rome, Rome, Italy
[b] Department of Information Engineering, Electronics and Telecommunications, Sapienza University of Rome, Rome, Italy
[c] Chemical, Material and Environmental Engineering, Sapienza University of Rome, Rome, Italy
[d] Unit of Radiation Biology and Human Health, Enea Research Center, Rome, Italy
*stefania.petralito@uniroma1.it

In this work, the inclusion of magnetic nanoparticles (MNPs) within phospholipid vesicles has been investigated as novel strategy for improving stability and reactivity of these nanoparticles and extending their potential use in the environmental field. Two phospholipids able to form liposomes characterized by different rigidity and stiffness, were used as potential carriers of MNPs. The magneto-responsive liposomes were investigated for their physicochemical and stability properties. In particular, the stability of the two systems was indirectly investigated evaluating the ability of the hybrid constructs to retain a fluorescent marker in their structure. Alterations in the permeability of the membranes were determined by the rate of the marker release from the liposomes, under both mechanical and thermal stress conditions.

1. Introduction

Over the last decades, nanotechnologies have received growing attention in different fields of fundamental and applied sciences [Daniel et al. 2004]. The reasons behind the wide interest raised by nanoparticles have to be find in the appealing and unique characteristics exhibited by nanostructured materials. In fact, both physical and chemical properties of materials change significantly, sometimes abruptly, as their size approaches the nanoscale [Liu et al. 2011, Luechinger et al. 2010, Tiwari et al. 2008]. These attractive characteristics of nanostructured materials are helping to considerably improve, even revolutionize, several research areas, finding applications in different fields from medicine to energy and environment. In the last case, nanotechnologies have been proposed as valid systems for environmental sensing and monitoring, as well as chemical degradation and remediation, among others. In this context, magnetic nanoparticles (MNPs) have proved to be effective sorbents for removal of toxic pollutants, such as heavy metals, in contaminated water [Zhang et al. 2016, Kaur et al. 2014]. Due to their high surface-to-volume ratio and magnetic properties, MNPs can adsorb on their surface various pollutants enabling their easy separation from aqueous solutions by the application of external magnetic fields [Zhang et al. 2016]. However, due to their high specific surface area nanoparticles have low energy barriers, causing them to aggregate and achieve a stabilized state. Aggregation decreases the free surface area of the nanoparticles, thereby reducing their adsorption capacity. Brownian motion of particles further contributes to reducing their effectiveness. To overcome the problems associated with aggregation, it becames extremely important to modify the surface of MNPs for good balancing of high adsorption capacity and nanoparticle stability.To this end, different surface modification approaches have been

attempted [Hu et al. 2006]. In this sense, the inclusion of MNPs within phospholipid vesicles, also known as liposomes, could represent a different and interesting approach for improving, at the same time, their stability and reactivity [Petralito et al. 2012]. The entrapment of MNPs within phospholipid vesicles generate hybrid magneto-responsive constructs (magneto-liposomes, MLs) [Spera et al. 2014, Spera et al. 2015], which may widen the potential use of MNPs within the environmental field. In fact, on the one hand, the inclusion in liposomes may prevent nanoparticles form aggregation; on the other hand, the various types of phospholipids (i.e. saturated and unsaturated) available for MLs production, offer the opportunity for novel surface modifications leading to functionalized MLs, which may open up new perspectives in the use of MNPs for environmental remediation.
Magnetoliposomes have been extensively investigated in the pharmaceutical and biomedical fields, as tools for both therapy and diagnosis [Soenen et al. 2015].
Having in mind these information, in this work, potential application of MLs for water decontamination, has been investigated. To this end, MNPs have been entrapped within phospholipid vesicles and the resulting hybrid magnetic structures have been characterized for dimensions, surface charge, morphology and entrapment efficiency. Moreover, the mechanical and physical stability of MLs have been studied as these hybrid structures could result more fragile than classical liposomes. In fact, phospholipids are in very close contact with the iron oxide surface in MLs, consequently any mechanical stress on the liposome membrane due to nanoparticles oscillations in proximity of phospholipid membrane could change the permeability of the bilayer leading to MNPs escape or even rupture of the MLs structure. Therefore, MLs should be able to preserve their physical integrity over time when exposed to a magnetic field or when subjected to a temperature increase, as fluidity and permeability of membranes increase with increasing temperature [Spera et al. 2014, Spera et al. 2015].
For these reasons, two lipid compositions, soybean phosphatidylcholine (SPC) and hydrogenated soybean phosphatidylcholine (HSPC), with different degree of unsaturation were used in order to obtain vesicles displaying a main transition temperature (T_m) lower or higher than room temperature, respectively. In fact, the thermotropic behavior and structural properties of liposomal dispersions strongly depend on the chemical properties of the fatty acids of phospholipids, which compose the bilayer membrane: the high amount of unsaturated acyl chains in SPC lecithin (predominantly oleic (18:1) and linoleic (18:2) acid) imparts a very low Tm value to the mixture. The presence of double bonds in the acyl chains led to less compact structures. For this reason, the resulting SPC liposomal membrane can be more susceptible to external perturbations, due to its liquid-phase state at room temperature. Instead, liposomal membranes formulated with saturated lipids (high purity hydrogenated soybean phosphatidylcholine, HSPC) have modest permeability due to their gel phase state at room temperature, which potentially make them less vulnerable to external perturbations. The stability of the two different hybrid constructs was investigated evaluating their ability to retain a fluorescent marker in their structure, under mechanical and thermal stress conditions.

2. Materials and Methods

2.1. Materials

Hydrogenated soybean phosphatidylcholine (HSPC) Phospholipon 90H from Lipoid GmbH and soybean phosphatidylcholine (SPC) Phospholipon 90 from Lipoid GmbH were kindly gifted by AVG Srl. Cholesterol, 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES), 5(6)-carboxyfluorescein (CF), Triton X-100 (TX-100), Sephadex G-50 and hydrochloric acid were purchased from Sigma-Aldrich. Chloroform was obtained from Merck. Bidistilled water, thiocyanatoiron, 1,2-dichloroethane and ethanol were supplied by Carlo Erba Reagents. Aqueous dispersion of 50 nm carboxymethyl-dextran coated magnetite (Fe_3O_4) nanoparticles fluidMAG-CMX (MNPs) was obtained from Chemicell GmbH.

2.2 Preparation and physicochemical characterization of liposomes

Unilamellar magnetoliposomes (MLs) were prepared using the thin lipid film hydration method followed by sequential extrusion as reported in Petralito et al. 2012. Briefly, MNPs were entrapped within the aqueous core of liposomes. Two different lipid compositions were used in order to obtain vesicles displaying a T_m lower or higher than 25°C. In particular, soybean phosphatidylcholine (PC-MLs) or mixture of hydrogenated soybean phosphatidylcholine with 20% mol/mol cholesterol (HSPC/chol-MLs) were selected to obtain T_m<4°C or T_m>50°C, respectively.
The thin film of SPC or HSPC and cholesterol was hydrated with 10 mM HEPES buffer solution (pH=7.4) containing MNPs and 20 mM CF used as a fluorescent marker for stability experiments. Plain liposomes, without MNPs, were also prepared and used as a control.
Repeated extrusion through membrane filters, having 0.4 μm and 0.2 μm pore sizes, yielded unilamellar liposomes with a narrow size distribution. Following extrusion, the unentrapped marker and MNPs were

removed by size exclusion chromatography (SEC) carried out on a Sephadex G-50 column eluted with HEPES buffer (10 mM) at pH 7.4. All liposome formulations were stored in the dark at 4°C and used within 1 week from their preparation. The hydrodynamic diameter and polydispersity index (PdI) were evaluated by dynamic light scattering (DLS) experiments. All measurements were carried out with Zetasizer Nano ZS90 (Malvern Instruments Ltd., UK) thermostatically controlled at 25°C. Phospholipid concentration was determined using the phosphorus colorimetric assay [Yoshida et al. 1980], using a double beam UV–Vis spectrophotometer Lambda 25 (Perkin Elmer, USA). The measurements were repeated before and after SEC purification of extruded samples.
MNPs content in MLs was determined using the 8.5% w/v hydrochloric acid assay described by Belikov et al. [Belikov et al. 2002]. The calibration curve was performed with standards solutions of magnetite. Measurements were repeated before and after SEC purification of extruded MLs. All data collected were used to determine the magnetite/phospholipid ratio.
The amount of CF entrapped in the inner aqueous compartment of liposomes was determined measuring the fluorescence emitted at 512 nm, after excitation at 492 nm, by purified samples pre-incubated with 10% non-ionic surfactant Triton X-100 for vesicles lysis. The measurements were carried out using a spectrofluorometer LS 50B (Perkin Elmer, USA).

2.3 AMF exposure set-up

The AMF exposure system used was the same described in details in previous works [Spera et al. 2014, Spera et al. 2015]. Briefly, it consists of two coaxial magnetic coils of square shape with a side length of 21 cm and placed 11 cm apart; coil section is square with a diameter of 2 cm and is composed of 25 cable turns. A high H field homogeneity of 99% is achieved in a volume of 10×10×6 cm^3 around center of the system where the samples were placed. The coils are connected to a signal generator (HP 3314A; Agilent Technologies, Santa Clara, CA) through a wide band amplifier (Krohn-Hite 7500; Krohn-Hite, Brockton, MA). AMF treatments were carried out with frequency of 20 kHz and intensity of 60 A/m. Both MLs and plain liposomes were exposed to AMF in continuous up to 9 hours. During the AMF treatments samples were placed in a thermostatic bath at 37.0±0.5°C placed directly inside the coil system.
MLs were also treated without AMF (sham conditions) using currents flowing in opposite directions in the two wires in order to null the resulting H field, as described in Schuderer et al. 2004. Sham samples were placed in the thermostatic bath at 37.0±0.5°C within the same coil system and located in the same position of the AMF-exposed ones.
The release of CF was calculated by monitoring the fluorescence intensity of the marker.

2.4 Measurement of CF released from liposomes

In order to have data about MLs stability, CF, which is a self-quenching hydrophilic dye, was loaded into the core of MLs. The membrane permeation and release behavior of MLs were determined fluorimetrically by monitoring CF fluorescence de-quenching at excitation and emission wavelengths of 492 and 512 nm, respectively. The release was measured both due to an applied low intensity AMF stimulation (20kHz, 60 A/m) under controlled temperature conditions (37.0±0.5°C) by the use of a thermal bath and measured by means of a thermocouple and after 1-3-6-9 h of continuous heating at selected temperature (37.0±0.5°C) in the absence of AMF.

3. Results and Discussion

Phospholipid vesicles embedding MNPs were prepared by classic thin film hydration method. Table 1 reports the results of the physicochemical characterization of plain liposomes and MNPs-loaded liposomes with two different phospholipid compositions, which are schematically depicted in figure 1. Data for magnetoliposomes refer to vesicles obtained with 2:1 NPs/Ls ratio. Indeed, different ratios between magnetic nanoparticles and lipids were attempted in order to reach optimal vesicles formation; working at a 2:1 NPs/Ls ratio, no significant interference with the vesicles formation was observed, as suggested by the percentages of phospholipids forming vesicles, which are very similar for plain and MNPs-loaded liposomes irrespective of the type of phospholipid used. In fact, the percentage of the lipid molecules recovered from the high-Tm MLs is only partially decreased respect to plain vesicles, suggesting that the hydration step of liposome preparation was only slightly influenced by the presence of the MNPs. In a similar way, no differences in hydrodynamic diameter were evidenced between the two lipid compositions containing or not MNPs. In both cases, the hybrid systems are arranged in a monomodal distribution with PdI values lower than 0.200.

Finally, the CF-entrapment reaches satisfactory values for both PC and HSPC MLs, showing that the loading capacity of the hybrid nanostructures is not limited by the co-presence of MNPs inside the vesicles (Table 1).

Table 1. Physicochemical characteristics of liposomes entrapping magnetic nanoparticles and plain liposomes. Values (±S.D.) are the mean of three determinations.

SAMPLE	SPC-MLs	SPC-Ls	HSPC/chol-MLs	HSPC/chol-Ls
Phospholipid (%)[a]	93.4	98.2	78.1	90.1
Fe_3O_4 (mg/mmol phospholipid)[b]	71.6±2.6	-	115.0±4.2	-
Fe_3O_4 entrapment efficiency (%)[c]	98.6	-	70.2	-
CF (µl/mg phospholipid)[d]	2.54±0.22	2.66±0.24	1.47±0.18	2.10±0.22
Hydrodynamic diameter (nm)[e]	207.6±2.3	166.7±2.0	235.5±6.4	242.7±9.7

[a] Phospholipid concentration as determined by Yoshida assay. [b] MNPs amount (g/mol phospholipid) determined by Belikov assay. [c] MNPs entrapment efficiency = (mean concentration after purification/mean concentration before purification)X100 determined by colorimetric assay. [d] Fluorimetric determination of CF (µl/mg phospholipid) in the final formulation. [e] Hydrodynamic diameter and size distribution of liposomes and MLs were determined by dynamic light scattering.

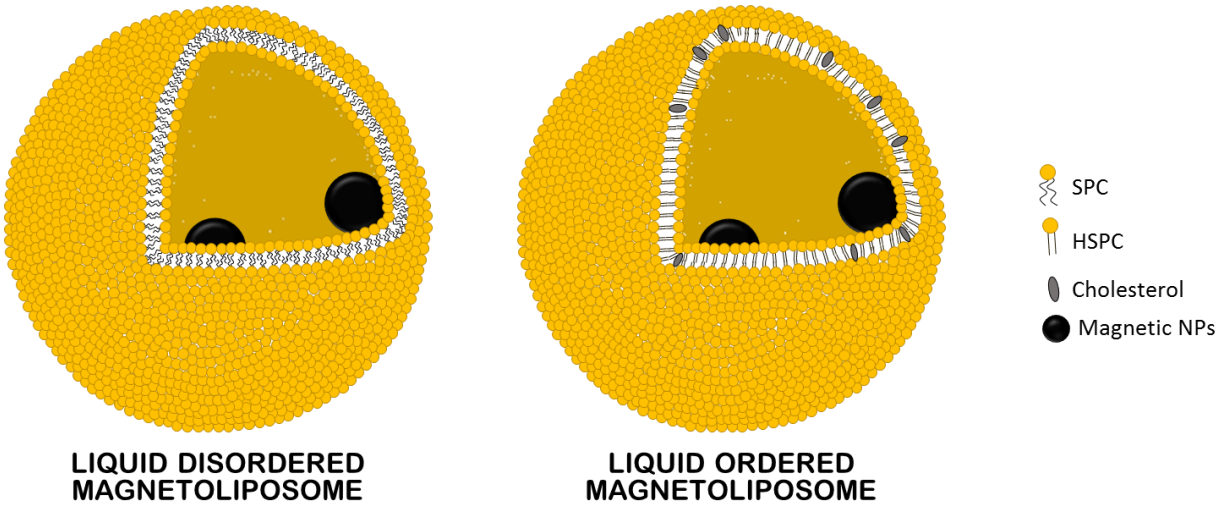

Figure 1. Schematic representation of SPC and HSPC/chol magnetoliposomes showing the different fluidity properties of the bilayers of the two hybrid constructs investigated.

In order to provide insight on the influence of the magnetic nanoparticles on the stability and permeability properties of the two liposomal constructs produced, the release behavior of the encapsulated hydrophilic marker CF was investigated under mechanical and thermal stress conditions. The application of AMF external stimulus to MLs could cause a mechanical destabilization of the vesicle membrane due to MNPs oscillation within the liposomes, which may induce the release of the dye. Therefore, the release rate of CF has been used to have indirect information about the ability of the different hybrid nanocostructs to resist to mechanical stress and avoid undesired leakage of their content.

Results reported in figure 2 show that, upon exposure to a magnetic field at 37.0°C, release from magnetoliposomes having membrane in liquid disordered (SPC)-phase is more pronounced than the leakage obtained from vesicles in liquid ordered (HSPC)-phase. The release profiles are the combination of dual effects: magnetic and thermal, which affected the release kinetics of CF in a different way. In specific, it has been observed that, in conditions of null magnetic field and 37.0±0.5°C (sham conditions), the extent of CF release is much higher from MLs characterized by disordered-state membranes, compared to the more ordered HSPC bilayer.

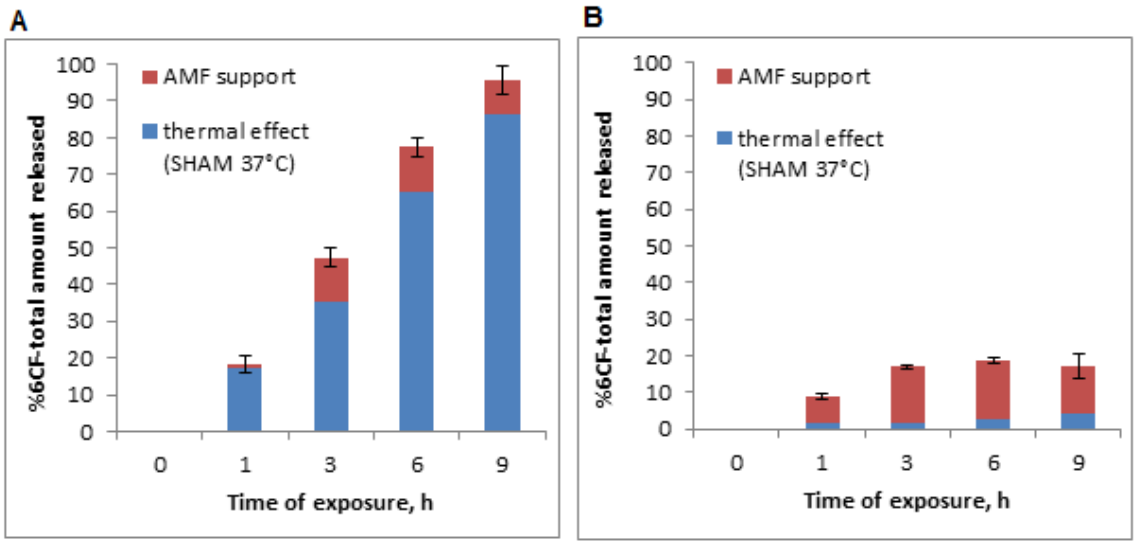

Figure 2. Extent of thermal and AMF-induced release from MLs characterized by A) disordered- (SPC) or B) ordered (HSPC)-state membranes.

These results can be explained considering that bilayers containing short or *cis*-unsaturated hydrocarbon chains are characterized by low Tm (e.g. soy phosphatidylcholine), as the double bounds hamper tight package of the hydrophobic tails, making the system more leaky than bilayers formed by saturated lipids, like HSPC. Therefore, at the same temperature, the unsaturated system will be more fluid than the respective saturated one. Consequently, when in disordered fluid state, the lateral motion of lipid molecules within the plane is much more freely, compared to liquid-ordered membrane, which results less leaky at the same temperature.

A loosely packed liquid-disordered system is obtained when low Tm lipids are used to prepare liposomes; instead, liquid-ordered domains are formed when cholesterol is included within gel-phase bilayers. The addition of cholesterol disrupts local packing orders of the saturated lipids, with a consequent increase in their diffusion coefficient and modulation of vesicle permeability. Nevertheless, results reported in figure 2B show that, even with the inclusion of cholesterol, HSPC liposomes did not become permeable and leaky under the experimental temperature conditions investigated.

External magnetic stimulation caused only a modest increase in the CF leakage compared to *release* occurring in sham conditions. The extent of AMF-induced release was quite low and almost independent from the fluidity characteristics of the bilayers. In fact, the increase in the percentage of CF released fall in the range 10-20%, with almost no differences between MLs characterized by liquid-disordered (SPC) state membrane, compared to liquid-ordered (HSPC) one.

Hence, if only magneto-mechanical effects on membrane permeability are taken into account, the stability of ordered or disordered bilayers against magnetically induced leakage suggests that both liposome structures could potentially be used as sorbents for water pollutants. The same consideration does not apply to temperature. In fact, the stability and integrity of SPC liposomes may be impaired if used in warm water. On the contrary, HSPC vesicles seems capable of withstanding both magnetic and thermal induced perturbations of the membrane. Overall, these results evidence promising and interesting features of HSPC–based magnetoliposomes for application as effective sorbents of toxic pollutants.

4. Conclusions

Phosphatidylcholine liposomes may represent a promising alternative for application of magnetic nanoparticles in water decontamination. In particular, lipids composed of saturated fatty acids seems to represent a rational choice for the formation of vesicles able to withstand both mechanical an thermal destabilization effects, thus avoiding any escape of the magnetic payload from the hybrid systems and consequent loss of effectiveness.

Acknowledgments

This work was carried out with financial support from Sapienza University of Rome (Research project: “Magnetic-responsive drug delivery systems controlled by low intensity magnetic stimuli”).

References

Belikov V.G., Kuregyan A.G., Ismailova G.K., 2002, Standardization of magnetite, Pharm. Chem. J. 36, 333–336.

Daniel M.C., Astruc D., 2004, Gold nanoparticles: assembly, supramolecular chemistry, quantum-size-related properties, and applications toward biology, catalysis, and nanotechnology, Chemical Reviews 104 (1), 293–346.

Hu J., Chen G., Lo I., 2006, Selective removal of heavy metals from industrial wastewater using maghemite nanoparticle: performance and mechanisms, J. Environ. Eng. 132 (7), 709–715.

Kaur R., Hasan A., Iqbal N., Alam S., Saini M.K., Raza S.K., 2014, Synthesis and surface engineering of magnetic nanoparticles for environmental cleanup and pesticide residue analysis: A review, J. Sep. Sci. 37, 1805–1825.

Liu J., Qiao S.Z., Hu Q.H., Lu G.Q., 2011, Magnetic nanocomposites with mesoporous structures: synthesis and applications, Small 7 (4), 425–443.

Luechinger N.A., Grass R.N., Athanassiou E.K., Stark W.J., 2010, Bottom-up fabrication of metal/metal nanocomposites from nanoparticles of immiscible metals, Chemistry of Materials 22 (1), 155–160.

Petralito S., Spera R., Memoli A., D'Inzeo G., Liberti M., Apollonio F., 2012, Preparation and characterization of lipid vesicles entrapping iron oxide nanoparticles, Asia-Pacific J. Chem. Eng. 7, 335–341.

Schuderer J., Oesch W., Felber N., Spät D., Kuster N. 2004. In vitro exposure apparatus for ELF magnetic fields, Bioelectromagnetics 25, 582–591.

Soenen S.J.H., Hodenius M., De Cuyper M., 2015, Magnetoliposomes: versatile innovative nanocolloids for use in biotechnology and biomedicine, Nanomedicine 4 (2), 177-191.

Spera R., Petralito S., Liberti M., Merla C., d'Inzeo G., Pinto R., Apollonio F., 2014, Controlled release from magnetoliposomes aqueous suspensions exposed to a low intensity magnetic field, Bioelectromagnetics 35 (4), 309-312.

Spera R., Apollonio F., Liberti M., Paffi A., Merla C., Pinto R., Petralito S., 2015, Controllable release from high-transition temperature magnetoliposomes by low-level magnetic stimulation, Colloids and Surfaces B: Biointerfaces 131, 136-140.

Tiwari D.K., Behari J., Sen P., 2008, Time and dose-dependent antimicrobial potential of Ag nanoparticles synthesized by top-down approach, Current Science 95 (5), 647–655.

Yoshida Y., Furuya E., Tagawa K., 1980, A direct colorimetric method for the determination of phospholipids with dithiocyanatoiron reagent, J. Biochem. 88, 463–468.

Zhang X., Qian J., Pan B., 2016, Fabrication of Novel Magnetic Nanoparticles of Multifunctionality for Water Decontamination, Environ. Sci. Technol. 50, 881−889.