Experimental report

21/05/2019

Proposal: **9-13-787** **Council:** 4/2018

Title: Fabrication of polyelectrolyte decorated liposomes obtained through layer by layer deposition

Research area: Chemistry

This proposal is a new proposal

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Samples: dimethyldioctadecylammonium bromide
1,2-dioleoyl-sn-glycero-3-phosphocholine
d and h poly(4-styrenesulfonate of sodium)
d and h poly(allylamine hydrochloride)

Instrument	Requested days	Allocated days	From	To
D11	4	2	17/10/2018	19/10/2018

Abstract:

The layer-by-layer (LbL) self-assembly method has shown a wide range of applications in modern biotechnology, allowing the fabrication of polyelectrolyte microcapsules for encapsulation and release of active compounds by the coating of colloidal templates such as liposomes. These platforms present promising applications in drug delivery.

We study the fabrication of polyelectrolyte microcapsules by the LbL coating of charged liposomes with layers of oppositely charged polyelectrolytes. For this purpose, liposomes formed by mixtures with different compositions of the lipid dioleoylphosphatidylcholine (DOPC) and the charged surfactant dioctadecyl-dimethylammonium bromide (DODAB) have been used as template. The multilayers were built by the sequential adsorption of oppositely charged polyelectrolytes: anionic poly(4-styrenesulfonate sodium salt) (PSS) and cationic poly(allylamine hydrochloride. These polyelectrolytes present good properties to obtain physico-chemical insights on the process of fabrication of the capsules.

Fabrication of polyelectrolyte decorated liposomes obtained through layer by layer deposition

Eduardo Guzmán (Main Proposer), Sylvain Prevost (Local Contact), Ana Mateos-Maroto, José E. F. Rubio, Francisco Ortega, Ramón G. Rubio, Armando Maestro

Scientific Background. The Layer by Layer (LbL) methods has been widely used for the fabrication of capsules by the alternate deposition of polyelectrolytes onto colloidal templates, generally nano- or micro-particles. However, this approach for the fabrication of capsules presents a main drawback that is associated with the presence of the colloidal template which should be removed in some cases through hard chemical procedures, limiting the application of this type of methodologies in certain technological fields. A promising alternative for overcoming the aforementioned drawback is the use of unilamellar vesicles as templates which present an empty cavity in the inner region after its formation. This opens new possibilities on the fabrication of sketched vectors for encapsulation and release of active compounds with interest in cosmetics, drug delivery or food science. However, beyond the interest of the fabrication procedure and the potential application of this polyelectrolyte decorated liposomes in encapsulation of active molecules, it is interesting to characterize the physico-chemical properties and structure of the obtained supramolecular structures. The understanding of this latter aspect is especially important because the layer interpenetration is known to affect significantly to the permeability of the multilayered structure, and consequently to retention capacity of the supramolecular assembly. For deepening on the structural characterization of polyelectrolyte decorated liposomes, the use of small angle neutron scattering (SANS) combined with other ancillary techniques such as dynamic light scattering (DLS), small angle X-ray scattering (SAXS) and electron paramagnetic resonance (EPR) can provide helpful insight.

Experimental Part. Naked and polyelectrolyte decorated liposomes formed by 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) and Dimethyldioctadecylammonium bromide (DODAB) in 70:30 molar ratio were studied studied by SANS using D11 beamline at the Institut Laue-Langevin (ILL, Grenoble, France). The studied systems have been previously characterized using DLS, electrophoretic mobility and EPR. For the aim of this study, measurements of the dispersions of liposomes were performed in pure D_2O. In order to obtain the whole reflectivity patterns, six different distances between the sample and the detectors were used, giving access to the entire Q-range (0.004-3 nm^{-1}). The experimental studies can be divided in two blocks:

1. Characterization of naked liposomes obtained by extrusion methods using membranes with three different pore diameters (50 nm, 80 nm and 100 nm).
2. Study of polyelectrolyte decorated liposomes, in which the coating is formed by different number of layers (from 1 to 3) and for different polyelectrolyte sets (poly(allylamine) (PAH)-poly(4-styrene sulfonate of sodium) (PSS), poly(diallyl-dimethylammonium chloride) (PDADMAC)-PSS, poly(l-lysine) (PLL)-Poly(glutamic acid) (PGA)). These studies were performed with liposomes of about 50 nm of diameter.

The data analysis was performed using SASfit software which enables for obtaining the radius of the inner cavity of the liposomes (r_c), thickness of the lipid bilayer (t_b) and the average radius of the liposomes (r_l). Information about the polydispersity can be also obtained from the analysis of SANS results. Furthermore, information about the scattering length density profiles of the supramolecular architectures can be obtained. For polyelectrolyte decorated liposomes, the analysis of SANS data give also information on the thickness of the polyelectrolyte films (t_f). For the modelling a model considering liposomes as an inner aqueous core surrounded by a shell has been chosen.

Results. Figure 1 shows the SANS profiles obtained over the whole accessible Q-range for liposomes presenting different average size and the corresponding fitting curves obtained using a model that considers the geometrical parameters of a spherical vesicle.

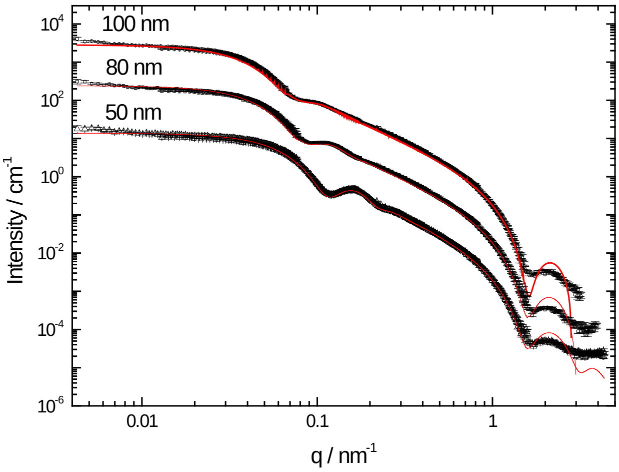

Figure 1. SANS profiles for naked liposomes obtained using membranes with different pore diameter. The symbols represent the experimental results and the lines represent de theoretical curved describing the data. Note that for the profiles corresponding to liposomes of 80 nm and 50 nm, experimental intensities were divided by a factor of 10 and 100, respectively.

The experimental curves show similar q-dependences for the scattered intensity for the liposomes independently of their size, with the only difference being found in the position and importance of the correlation peaks. Such peak for its position can be ascribed to the internal structure of the bilayer. The analysis of such aspects evidences that the decrease of the nominal size of the liposomes leads to the increase of the packing and ordering of the bilayer, making such liposomes more adequate for their use as template on the fabrication of polyelectrolyte decorated liposomes. In Table 1 are summarized the results obtained for the characterization of the naked liposomes presenting different sizes. It is worth mentioning that for all liposomes good fits were obtained with a fixed value of the scattering length density of the bilayer of $0.0489{\cdot}10^{-6}$ Å^2.

Nominal size/nm	**r_c/nm**	**t_b/nm**	**r_l/nm**
100	32.9	3.97	36.8
80	30.9	4.00	34.9
50	23.3	4.01	27.3

Table 1. Average values obtained from the analysis of the SANS profiles for naked liposomes with different nominal sizes.

On the bases of the above results, it is possible to assume that the preparation of liposomes presenting a nominal size of 50 nm leads to better results, either in relation to the efficiency of the preparation procedure or in the quality of the obtained profiles. Thus, in the following the study of the liposomes decorated with polyelectrolyte will be performed using as template liposomes presenting a nominal diameter of 50 nm.

Figure 2 shows the effect of the fabrication of polyelectrolyte film on the scattered intensity profile of liposomes presenting 50 nm. The results show clearly that the adsorption of polyelectrolyte layers onto the liposomes leads to the disappearance of the correlation peak associated with the bilayer structure in naked liposomes. This could be easily justify assuming that polyelectrolyte adsorption distort the packing of the lipid bilayer, and under certain conditions it is possible that some of the polymer segments can penetrate through the bilayer structure weakening even more the packing of the lipid structure. On the other side the reflectivity curves present a new feature (intensity fluctuation) which may be reminiscent of the appearance of a new structural characteristic be related with the adsorbed layer (about q ~ 1 nm^{-1}).Furthermore, the dependence found in the scattering curves shows important signatures of modification in the shape of the supramolecular architecture with assume probably an ellipsoidal shape.

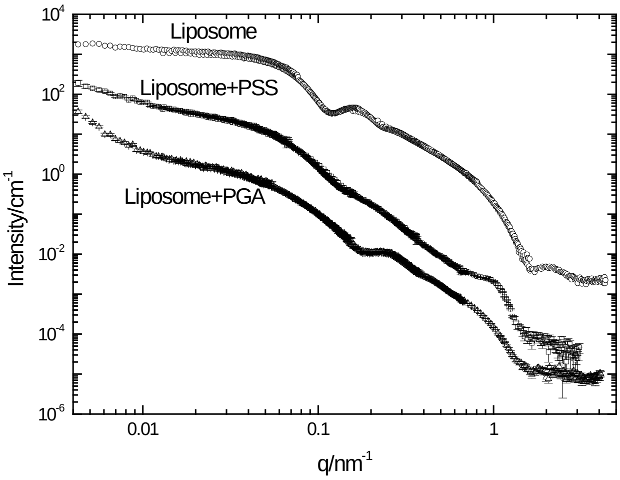

Figure 2. SANS profiles for naked liposomes and liposomes decorated with one PSS layer and one PGA layer. Notice that the intensity data for liposomes decorated with a PSS layer was divided by a factor of 10 and with a PGA layer by a factor of 100.

Deepening on the study of liposomes decorated with LbL polyelectrolyte multilayers, Figure 3 shows the results obtained for supramolecular architectures in which an increasing number of polyelectrolyte layers are adsorbed.

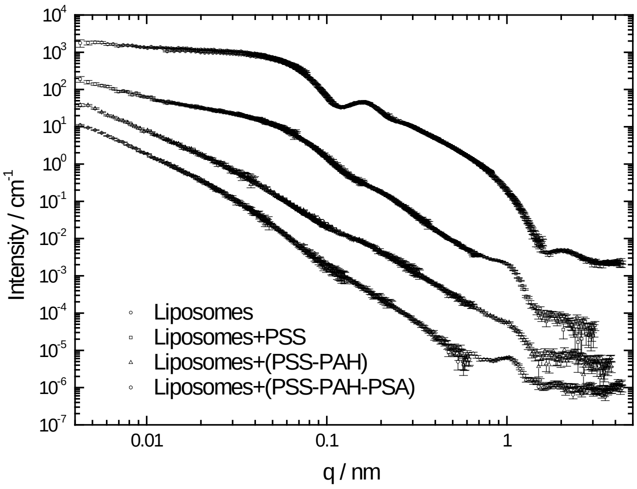

Figure 3. SANS profiles for naked liposomes and liposomes decorated with one PSS layer, a bilayer PSS-PAH and three layers PSS-PAH-PSS. Notice that the intensity data for liposomes decorated with a PSS layer was divided by a factor of 10, with PSS-PAH bilayer by a factor of 100 and with PSS-PAH-PSS by a factor 1000 The SANS profiles are shown from top to bottom with the increase of the number of polyelectrolyte layers.

The results clearly evidences that the introduction of PSS layers leads to the appearance of a correlation peak in the SANS profiles, which is could be related with the fact that the introduction of PSS layers leads to the enhancement of the stratification of the multilayer whereas the adsorption of a PAH layer leads to the formation of a more fuzzy layer, hindering the real stratification of the system. The distortion of the structure associated with the introduction of a polycation layer seems to be a general behavior independently of the type of polycation considered (data not shown) without any correlation peaks which is found when films are ended in PSS (see Figure 3).

Conclusions. The study of the structure of liposome decorated with polyelectrolyte multilayers has provide important insights related to the internal structure of the supramolecular architecture, providing interesting information about the layer organization which is of critical interest for the development of future applications of this type of systems as platforms for the encapsulation of active compounds. Therefore, the here obtained results open the gate to the standardization of the use of liposome as template in the fabrication of capsules using the LbL method.

Diffusion of the results. The results obtained in this proposal are intended to be published in a manuscript that is at the present time in preparation:

- Mateos-Maroto, E. Guzmán, J.E.F. Rubio, S. Prevost, R.G. Rubio, F. Ortega. Liposomes decorated with polyelectrolyte multilayers: formation, structure and physico-chemical preparation. In preparation (2019).