Experimental Report

Proposal: **9-13-505** **Council:** 10/2012

Title: Structural investigation of bisphosphonate self-assembling nanoparticles used in bone cancer treatment

This proposal is a new proposal

Researh Area: Soft condensed matter

Main proposer: **RISTORI Sandra**

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DE ROSA Giuseppe

Local Contact: GRILLO Isabelle

Samples: CaHPO4
1,2-diacyl-sn-glycero-3-phosphoethanolamine- N-[methoxy(polyethylene glycol)-2000] (DSPE-PEG2000)
C5H10N2O7P2 (zoledronic acid)
1,2-Dioleoyl-3-trimethylammonium-propane chloride (DOTAP)

Instrument	Req. Days	All. Days	From	To
D11	3	2	04/05/2013	06/05/2013

Abstract:

Bisphosphonates (BPs) are presently the treatment of choice for severe bone diseases due to bone resorption, such as osteoporosis, Paget's disease and bone metastases. In particular, zoledronic acid (ZOL) is a bisphosphonate able to act at micromolar concentrations. A recent study has shown that the efficiency of ZOL can be dramatically enhanced if it is administered in the form of self-assembling PEGylated nanoparticles. However, despite the high potentiality of these formulations, knowledge on their structural properties is still incomplete.
Here we propose to use SANS to assess the structure of ZOL composite nanoparticles (also containing lipids from initial PEGylated liposomes). By this means we intend to elicidate the size and internal composition for further engineering of these self-assembly aggregates with high pharmaceutical interest.

Experiment 9-13-505

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Scientific background

Zoledronic acid (ZOL) is an efficient 3rd generation bisphosphonate, able to inhibit bone resorption at micromolar concentrations. A recent study (Salzano G, Marra M, Porru M, Zappavigna S, Abbruzzese A, La Rotonda MI, Leonetti C, Caraglia M, De Rosa G. "Self-assembly nanoparticles for the delivery of bisphosphonates into tumors". Int J. Pharm,.403:292-297, 2011) has shown that the activity of ZOL is dramatically enhanced if this compound is administered in the form of self-assembling PEGylated nanoparticles (NPs). However, despite the high potentiality of these NPs, information on their structural properties is still incomplete, as it is summarized in figure 1.

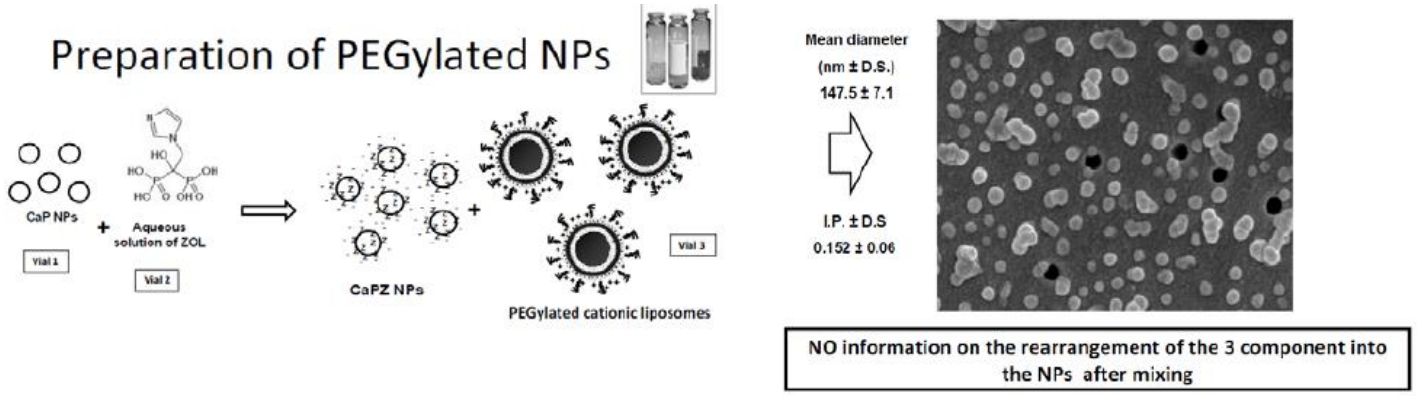


Figure 1. Scheme of the experimental steps leading to the formulations discussed in this proposal

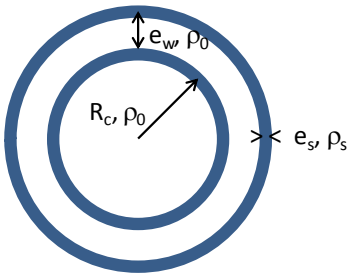
It has also been shown that adding Transferrin to this formulation further increases its antitumor ability, but no detailed information about the interaction modality between the protein and the composite nanoparticles has been gathered so far. For all these purpose SANS is a powerful and well established tool to assess the structure of nanoparticles and liposomes

Systems studied and information obtained in experiment 9-13-505

1) Non pegylated and pegylated liposomes
2) Calcium phosphate (CaP) and Calcium phosphate plus zoledronic acid (CaPZ)
3) Non pegylated and pegylated liposomes plus Z, CaP and CaPZ
4) Transferrin at 5mg/ml in PBS at pH= 7.4
5) Transferrin on pegylated liposmes (with and without CaP or CaPZ) at ratio 5:1, 50:1 and 100:1 molar ratio

The most important samples in farmaceutical application are 4) and 5), though it was necessary to investigate the structure of the composing particles to obtain a reliable set of information on the more comples systems.

The SANS diagrams of nanoparticle–liposome composites were conveniently interpreted by using a model of multilamellar vesicles, as shown scheme 1



The best fit, shown in figure 2 (right and left panels for LCaP and LCaPZ, respectively) was obtained considering only two bilayers (bi-lamellar vesicles, BLV). The fitting parameters, reported in table 1, are Rc, the inner core, e_w, the distance between the bilayers. e_s and ρ_s were fixed at 34 Å and 2.7 10^{10} cm^{-2}, while ρ_0 was set at 6.4 10^{10} cm^{-2}, as for pure D_2O. Polydispersity (PD) on the core and e_w were necessary to obtain good agrement with the experimental diagrams and were represented by a gaussian function.

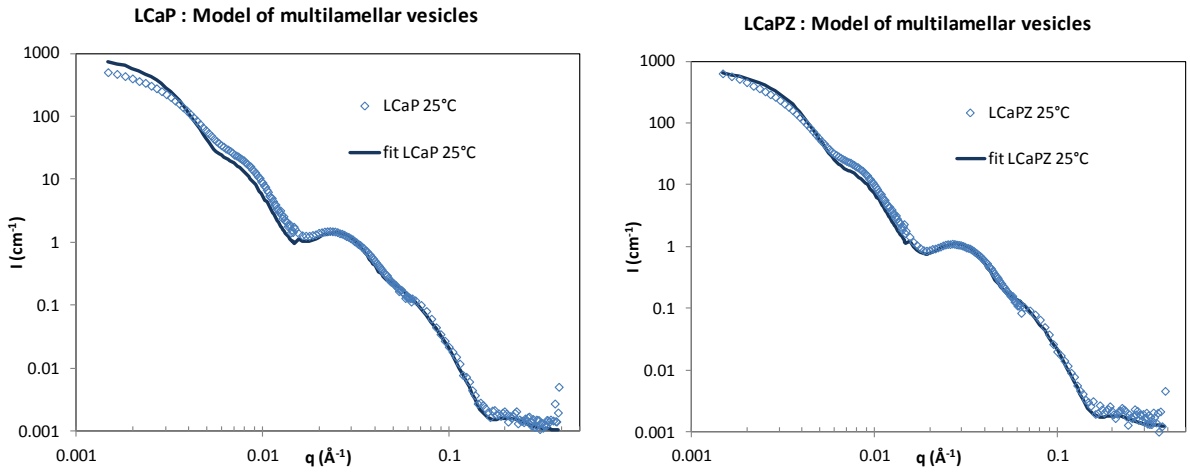


Figure 2: Fitted intensity with a model of bilayer lamellar vesicle.

Table 2: Fitted parameters for a model of bilayer lamellar vesicle

	LCaP	LCaPZ
Φ	0.0097	0.0095
R_c (Å)	310	282
e_w(Å)	161	140
e_s (Å)	38	38
PD (Rc)	0.5	0.5
PD (e_w)	0.25	0.2
PD (e_s)	0.15	0.15
ρ_0 (cm^{-2})	6.4 10^{10}	6.4 10^{10}
ρ_{shell} (cm^{-2})	2.6 10^{10}	2.6 10^{10}

However, due to time shortage, only a limited number of samples could be investigated and it was not possible to vary the ratio between nanoparticles and liposomes. This is a pitfall, since knowing the structure change induced by an excess of NPs (or of liposomes) could be useful for guiding the choice of the best pharmaceutical formulation, or at least to establish the limit within which their relative abundance can be varied.

The sample containing pegylated liposomes, CaPZ and transferrin that we could investigated were only a limited number. Figure 3 shows the comparison between the SANS diagram of composite systems and of the original pegylated liposomes, evidencing an increased structural complexity obtained by adding more components.

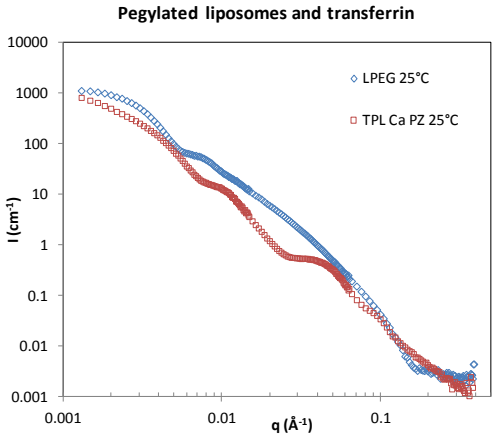


Figure 3: Scattered intensity of pegylated liposomes with transferring, CaP and zoledronic acid.

The bilamellar vescicle (BLV) model + a curve of an ellipsoid, which represents the transferrin, gave a good fitting of the experimental intensity, considering a relatively high polydispersity for the core and water thicknesses (figure 4 and table 2).

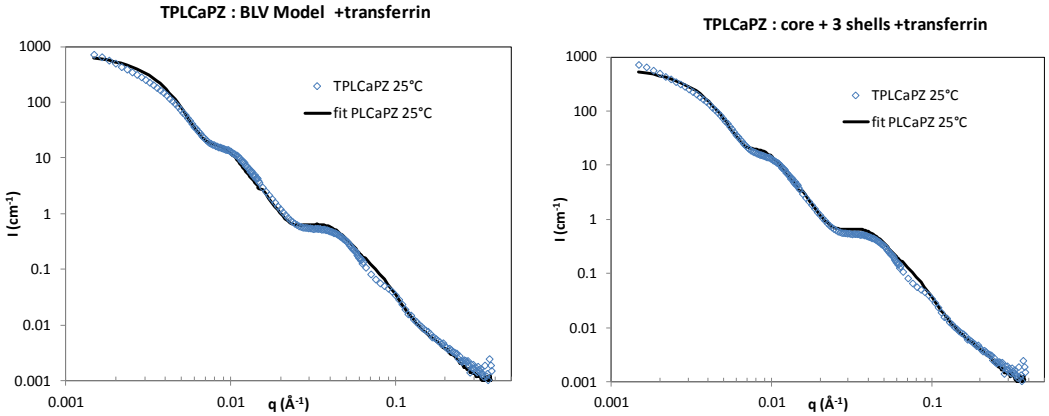


Table 2:

TPLCaPZ	BLV
Φ	0.006
R_c (Å)	289
e_w(Å)	87
e_s (Å)	39
PD (Rc)	0.4
PD (e_w)	0.4
PD (e_{shell})	0.2
ρ_0 (cm^{-2})	$6.4\ 10^{10}$
ρ_s (cm^{-2})	$2.1\ 10^{10}$

The above results are very promising. However, to give full account for the contribution brought about by different components more systems should be investigated. Unfortunately, the beamtime allocated (3 days) was considerably shorter than the beamtime requested (6 days) and a new proposal will be submitted to complete this study.