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Abstract

Indium–DTPA–tagged liposomes were studied in the present work as carriers of *in vivo* ^{212}Pb / ^{212}Bi generator to be used in targeted alpha therapy. The liposomal uptake of ^{212}Pb, into preformed liposomes, was investigated using different lipophilic chelate (DCP, 2,3-dimercapto-1-propanol (BAL), sodium acetate, and A23187), as a function of various parameters (temperature, concentrations of lipids, Pb, DTPA,…) with ^{212}Pb as a tracer. Different formulations of liposomes were tested to evaluate the radiolabeling efficiency. No complexing agent was necessary for the passage of Pb^{2+} through the membrane. It occurs naturally *via* a partial permeability of the lipid bilayer which increases with the temperature. A complexing agent (DTPA) appears necessary to concentrate Pb in the internal compartment of the liposomes. Conditions were found (T = 65°C, internal DTPA concentration of 0.025 M, pH 7.4, …) yielding a high and rapid uptake of ^{212}Pb in liposomes. The protocol established provides a novel method for the efficient entrapment of about 2-3 Pb atoms per liposome with a yield of 75% in conditions relevant for nuclear medicine.

Introduction

^{212}Bi is a potentially interesting α-emitting radionuclide for targeted alpha therapy [1]. The principle is based on the stable binding of alpha emitting radionuclides to disease selective carrier molecules, such as antibodies or peptides [2]. The challenge is to deliver the radioactive atoms to the target with the objective to find the right balance between toxicity and anti-tumor effect.

Considering its short period ($t_{1/2}$ = 60.6 min), ^{212}Bi is limited to situations where the labeled carrier molecule rapidly reaches its target. To expand the range of applications, an interesting method is to use its parent, ^{212}Pb ($t_{1/2}$ = 10.6 h), which will generate *in vivo* ^{212}Bi. Data in the literature show that the classical chelation approach, used to bind the radionuclides to the carrier molecules, does not work [3]. Although the chelating agent used (DOTA) is known to form strong complexes with both Bi and Pb, a significant part of Bi escapes from the carrier molecule as a result of the radioactive transformation ^{212}Pb / ^{212}Bi and the formation of highly ionized daughter atoms after the Auger electrons emission [3].

An interesting alternative is to use liposomes [4]. Once ^{212}Pb is encapsulated in its internal compartment, the phospholipidic membrane prevents Bi release provided that the liposome size is large enough (~ 100 nm). This approach is studied in the present paper with liposomes designed to treat residual cancer diseases with a two step pretargeting strategy (Affinity Enhancement System) [5,6]. These liposomes present at their surface polyethylene glycol (PEG) to prevent fast elimination and DTPA chelating agents that are recognized, once complexed with indium, by the pretargeted bispecific anti-tumor x anti-indium-DTPA antibody.

Active encapsulation of ^{212}Pb was studied as a function of different parameters (pH, nature of the chelating agent used for the active encapsulation, temperature, metal ion concentration, liposome concentration, concentration of encapsulated DTPA) with the objective to optimize

the labeling protocol in terms of time, encapsulation yield and specific activity. To help the comprehension of the encapsulation process, different liposome formulations were tested (Table 1)

Material and methods

Reagents

All reagents were of analytical grade. All solutions were prepared using Milli-Q water and all experiments were conducted in air-conditioned laboratories (21 ± 2°C), except otherwise indicated. Liposomes of various compositions were used (Table 1). The various constituents were mixed in chloroform / methanol (9/1 v/v) mixture in a 10 mL round bottom flask. A lipid film was obtained by evaporation of the solvent in a rotary evaporator. The hydration of the lipid film was performed by addition of 1 mL of the aqueous phase. After a treatment by ultrasounds for 2 min, liposomes were calibrated in size by extrusion on polycarbonate membranes to obtain an average diameter of 100 nm [7]. The size was systematically checked by dynamic light scattering with the Autosizer 4700 apparatus (Malvern Instrument SA) [8].

Liposomes prepared in the presence of DTPA were further purified to remove non encapsulated DTPA by membrane filtration (Centrisat, 20 kDa). The purification step was monitored by UV–spectrophotometry *via* the analysis of the Bi–DTPA complex (ε[Bi–DTPA]$^{2-}$ = 8758 $cm^{-1}.mol^{-1}.L$ (λ = 278 nm)). The Bi–DTPA complexation constant (log K = 30.3, I = 0) is high enough to allow a quantitative complexation of DTPA [9]. The liposomes were stored at 4°C and the stock solution was never older than 3 months. The liposome concentration is given as the content of lipid in mol/L and was determined using a calibration curve of measured organic carbon content as a function of lipid concentration. The number of liposomes per unit volume, calculated at about 10^{14} vesicles per mL (0.02 M of lipids), was estimated from the formula given in [10].

450 kBq of ^{228}Th was provided by AREVA in 2006. A ^{228}Th / ^{212}Pb generator was prepared according to Narbutt & Bilewicz [11] using DOWEX-50x8 cation exchanger. ^{228}Th was adsorbed on the resin in 0.01 M HCl, while ^{212}Pb was stripped from the column using 1 M HCl. With time, some problems of purity were observed, probably due to radiation damage of the resin. Another purification step was then necessary and was performed using Chelex–100 [12]. Pb sorption was done at pH 5 in an acetate pH buffer solution [13]. After washing (about 30 times the void volume), ^{212}Pb was desorbed from the column with 5 M HNO_3. The solution was then evaporated to dryness and the residue dissolved with 5 mM HCl to obtain a stock solution of 20 kBq/mL.

Experimental methodologies

Encapsulation studies. Experiments were performed in 1.5 mL glass tubes. A holder in aluminium controlled by a heater was specifically designed to allow working at different temperature (25 – 150°C). Liposomes were first equilibrated in the labeling medium for 1 h before the lead addition (^{212}Pb ~600 Bq (10^{-13} M) and 10^{-9} M of natural Pb in 0.4 mL). ^{212}Pb was only use as a tracer. For some experiments, natural Pb was added to reproduce conditions relevant in nuclear medicine (~100 MBq/L or 10^{-7} mol/L). Preliminary experiments (lipid concentration of 1 mM, pH 7.4, 0.1 M NaCl, 0.025 M of encapsulated DTPA, T=65°C) showed that the encapsulation kinetic was rapid, a stable encapsulation yield being obtained after about 1 hour. This time was fixed for all the experiments.

Complexation studies between In and the liposomes. They were performed in dialysis bags (Microcon Millipore, 3 kDa) composed of two compartments of 0.5 mL (compartment 1) and 1 mL (compartment 2). Once the compartments filled with the indium solution, liposomes (10^{-4} M) were added in compartment 1. The tubes were then shaken and kinetic studies

showed that 24 hours of contact were necessary to reach equilibrium conditions. Preliminary results showed a strong adsorption of liposomes at the surface of the dialysis tubes. The concentration of indium in compartment 1 at equilibrium was thus calculated according to the relation:

$$[In]_1 = \frac{1.5x[In]_{total} - 1x[In]_2}{0.5}$$

Where $[In]_{total,}$ $[In]_1$ and $[In]_2$ are the total concentration of indium added in the system and the indium concentrations in compartments 1 and 2, respectively.

Chelex–100. It was used to monitor ^{212}Pb encapsulation, to purify ^{212}Pb solution and to label the liposomes with indium. Chelex–100 was first washed according to Biesuz et al [13] with 5 M HNO_3. About 7 g of Chelex–100 was loaded on 1.5x8 cm polypropylene columns and pre-equilibrated with the medium of interest. 0.5 mL of the solution was then injected at the top of the column and the elution was made at a speed of 2 mL/min. It was checked that the liposomes were stripped from the column without significant retention (< 2 %).

Water / octanol extraction: 2 mL of the organic phase was pre-equilibrated with the aqueous medium (2 mL) before ^{212}Pb addition. After 12 hours of stirring, both phases were separated and samples were withdrawn for activity measurements.

Analytical tools

UV-spectra were recorded on a UV–visible UV–2401 PC spectrophotometer from Shimadzu. Organic carbon analyses were done on a TOC–V_{CSH} apparatus supplied by Shimadzu. ^{212}Pb purity was verified by gamma ray spectroscopy with a high purity germanium (HPGe) detector from Canberra. ^{212}Pb activities in the encapsulation experiments were measured by liquid scintillation counting using a Packard 2550 TR/AB Liquid Scintillation analyzer with

the Ultima Gold AB scintillation liquid. Indium analysis was performed by ICP–MS using a PQ Excell apparatus provided by VG Elemental (sensibility limit of 0.1 ppb).

Quantitative analysis

The PHREEQC program [14] was used to simulate the reactions occurring in the studied systems. An input file describing the experimental conditions (medium composition, pH, E and temperature) is created and the species distribution at equilibrium was calculated using the thermodynamic database Llnl (Lawrence Livermore National Laboratory). All the equilibrium constants in the database were extrapolated at zero ionic strength using the Truncated Davies equation [15].

Results and discussion

Pre–labeling of DTPA by In

The objective was to complex indium with DTPA–tagged liposomes, the indium–DTPA complex being the bispecific antibody recognition site for pretargeting. This step was performed prior to ^{212}Pb encapsulation to avoid any complexation between ^{212}Pb and DTPA ligands which would limit the encapsulation yield. The complexation isotherm measured as a function of indium concentration is reported in figure 1. Under the studied experimental conditions, the DTPA–tagged liposome saturation starts in the presence of indium concentrations above 10^{-4} M. This is translated by a decrease in indium complexation. The calculation of data by a Langmuir–type model [16] gives a number of interacting sites of $2.5.10^{-4}$ M (dashed line in figure 1), whereas a value of 10^{-6} M was predicted based on the liposome composition (solid line in figure 1). This result indicates the presence of other binding sites at the liposome surface. These sites may be attributed to deprotonated phospholipides P-OH (about 47 for 1 DTPA) and neutral NH_2 or C=O functional groups. In

the following, these latter sites and DTPA–tagged liposomes will be designed as non–specific and specific sites, respectively. It was necessary to find a method to remove non–specifically bound indium (while leaving indium bound to specific sites) as it could compete with ^{212}Pb encapsulation or may be toxic *in vivo*. The method was defined in conditions where indium was in excess (100 for 1 DTPA) and is based on the well–known strong kinetic stability of DTPA complexes with trivalent metal ions [17], i.e. once complexed, the kinetic of de-complexation is very slow. The solution ([lipids] =10^{-2} M, [In] = $1.5.10^{-2}$ M in 0.1 M NaCl), after 20h equilibrium, was passed through a Chelex–100 column at a flow rate of 2 mL/min allowing an efficient retention of free indium and indium bound to the non–specific sites while indium complexed with DTPA–tagged liposomes was eluted from the column. Conditions were obtained where indium concentration measured in the eluted solution corresponded, within experimental errors, to the concentration of DTPA in the DTPA–tagged liposome preparation. We showed as well that another passage of the "purified" solution through the Chelex–100 column did not lead to any significant further indium release. The two experimental observations indicate that the purification process is optimum and lead to indium–DTPA labeled liposomes.

Active encapsulation

According to literature data [4], active encapsulation of metal ions should be obtained by the formation of a lipophilic complex which crosses the membrane of the liposome and by trapping them in the aqueous internal liposome compartment by forming a charged complex with strong chelating agent (figure 2). ^{212}Pb presenting an affinity for the non-specific sites, it was necessary to define a methodology allowing us to distinguish the encapsulated lead with the one adsorbed at the external surface of the liposomes. The same method as that used to prepare indium–labeled liposomes was used. In a dynamic system, we make the hypothesis

that the lead adsorbed at the liposome surface is quickly adsorbed by the sites of Chelex–100, while the incorporated Pb is eluted with the liposomes. This is indirectly shown in Table 1 where 97% of Pb is not eluted in conditions where the encapsulation is supposed negligible (liposome A, ambient temperature). The method must also retain lead in the solution and complexed to the ligand used for the encapsulation. This was checked for two temperatures in labeling solution containing no liposomes (Table 2): if one excepts the system containing A23187, Pb is retained by Chelex–100. For A23187, about 12 % passed through the column showing the presence of a relatively kinetically-stable Pb–A23187 complex. In the presence of liposomes, this amount was systematically subtracted from the content of ^{212}Pb eluted to get the percentage of encapsulated lead.

Method optimization

Several parameters can affect the encapsulation [21, 22, 23]: the pH of the external and internal compartments, the nature and concentration of the ligands used for the encapsulation, and the temperature of the experiment. DTPA was selected as the encapsulated ligand used to trap ^{212}Pb in the internal compartment of the liposome [21]. The labeling pH was fixed by default to 7.4 based on the work of Tilcok et al [21]. All other parameters were varied to optimize the labeling protocol in terms of time, yield and specific activity.

Temperature effect. The temperature plays a crucial role in the encapsulation of radionuclides in liposomes [22]. This is illustrated in figure 3. Below 55°C, the encapsulation yield is weak with less than 10% of lead encapsulated. It increases above 55°C to reach a maximum value of 35 % for T = 70°C. This is related to the phase transition temperature (Tm) of the lipids which occurs at 74°C for our liposomes [24]. This results in a decrease of the phospholipidic thickness and the membrane permeability which facilitate the encapsulation of ^{212}Pb. The

more the temperature is close to the phase transition temperature, the more the encapsulation is facilitated. However a decrease of encapsulation is observed above 80°C. At 85°C, the temperature is well above the Tm of the lipids; the bilayer becomes disorganized (rotation, flip-flop …) and much more fluid [8]. As a result, the molecules to encapsulate enter in the inner compartment of the liposomes, and come out just as easily. This may explain the decrease of encapsulation.

DTPA Effect. According to Tilcock et al [21], DTPA has a crucial role to play, i.e. due to its high complexation constant for Pb, it complexes Pb and form an anionic complex which remains in the internal compartment. The question raised here is whether the internal DTPA moieties of the DTPA–tagged liposomes could play this role without encapsulation of free DTPA. To assess this possibility, the encapsulation was studied for different composition of liposomes and the results are reported in Table 1.

Without encapsulated DTPA (liposome A), a significant encapsulation was observed at 65°C (24.6 %) while no encapsulation occurs at ambient temperature. This result indicates that the encapsulation is done naturally because of the dynamic of the surface which makes the organic phospholipidic bilayer temporary permeable. However, a higher encapsulation was observed in the presence of internal free DTPA (liposome B). DTPA is therefore an important driving force. This is notably confirmed at ambient temperature with a significant encapsulation (69.7 %) whereas no encapsulation was observed without internal DTPA. The presence of surface DTPA in addition to free internal DTPA does not improve the yield at 65°C. This is coherent with the fact that surface DTPA (about 700 par liposome) is much lower than internal free DTPA (about 12000 per liposome). However, a significant difference is observed at ambient temperature between liposomes B and D (69.7 *vs* 12.9). We can imagine that bound DTPA hampers the encapsulation at ambient temperature for sterical

reasons. Finally, in agreement with the above considerations, the presence of surface DTPA (liposome C) allows a higher encapsulation at 65°C than liposome A. The encapsulation yield is however increased in the presence of internal free DTPA. In the following, internal DTPA (0.025 M) was systematically added. A higher value was shown to induce a precipitation during liposome preparation.

Nature of X. The encapsulation is for a part linked to the lipophilicity of the ligand used for the encapsulation [23]. Different compounds were tested at ambient and 65°C (figure 2). An experiment was done without ligand, for comparison. When the equilibrium constants are known, Pb speciation in solution was calculated at 25°C to help the understanding of the experimental data. The results are given in Table 2. A23187 was chosen because it is used for the efficient encapsulation of trivalent metal ions [19]. However, the percentage of ^{212}Pb encapsulated is weak and amount to nearly 15%. This may be explained by the strong complexation (Table I) allowing DTPA not to compete with A23187 for Pb^{2+} in the internal volume. This observation may also be explained by the kinetic stability of the Pb–A23187 complex formed (log K = 6.49) [20], as already discussed in the part ”active encapsulation” which does not allow DTPA to trap ^{212}Pb in the internal compartment. Thus, it cannot be concentrated in the internal compartment and its concentration should not be higher than the one in the external compartment; considering the ratio between internal and external compartments of 0.06, an encapsulation yield around 6 % is expected, in agreement with the weak value experimentally measured. Finally, a last possibility may be related to the charge + of the complex which is predicted based on literature data [20]. The yield of encapsulation appears much higher and similar for BAL, acetate and DCP, the two later forming neutral complex with Pb^{2+}. Surprisingly, it equals, within experimental errors, with the one obtained without ligand. This result indicates that the ligand has no effect and that the passage of ^{212}Pb across the membrane does not occur *via* the diffusion of the neutral complex PbX.

To assess the latter assumption, the system containing acetate was better studied in the model water / octanol system where octanol simulate the liposome membrane [25]. If a diffusion process occurs, the complex must be partially soluble in octanol. The acetate concentration was varied to change the concentration of $PbAc_2$ in the aqueous solution from 0% ($[Ac]_{tot} = 10^{-6}$ M) to 9.3% ($[Ac]_{tot} = 0.1$ M). We then expect an increase in lead extraction in the organic phase when acetate concentration increases. However, the result showed that about 2% of lead was extracted in the presence or absence of acetate, and irrespective of acetate concentration. A similar study was performed in the presence of liposomes for different acetate concentrations. Here also, a constant encapsulation was observed, i.e. 65 ± 5 (lipid concentration of $2.5.10^{-3}$ M, lead concentration of 10^{-9} M, T = 65°C, pH = 7.4), for all acetate concentration studied (10^{-5} – 0.1 M). The result, in complement to those got from the DTPA study, confirm that the ligand has no role in the encapsulation. This latter may be explained by a physical encapsulation arising from the dynamic of the membrane, which makes it partially permeable, and notably at 65°C. In the following, no ligand was used for ^{212}Pb encapsulation.

Specific activity. Both the number of lead encapsulated (and specific activity) and the encapsulation yield are key parameters for the definition of the labeling protocol. In the optimized conditions (pH = 7.4, internal DTPA concentration of $2.5.10^{-2}$ M, T = 65°C), the encapsulation was studied as a function of liposome and lead concentrations. The results are reported in figure 4. On the one hand, for one given lead concentration, the more the lipid concentration, the more the encapsulation yield, the less the number of lead in the internal compartment. On the other hand, for one given liposome concentration, the more the lead concentration, the more the number of lead encapsulated while no effect on the encapsulation yield was observed. For a typical application in targeted radionuclide therapy, activities of several hundred MBq are necessary corresponding to concentration of ^{212}Pb around 10^{-7} M.

Under such conditions, by fixing the lipid concentration to $2.5.10^{-3}$ M, a concentration typically got from the production protocol, an encapsulation yield of about 75 % can be obtained using the optimized protocol with a mean value of 2–3 lead atoms per liposome.

Encapsulation in the In–labeled–PEG–liposomes

The methodology previously optimized was tested on PEG–liposomes using the two steps approach. Preliminary experiments showed that lead interaction with PEG chains, if it occurs, does not interfere with the determination of the encapsulation yield, i.e. lead mixed with PEG chains is retained in the Chelex–100 column. Encapsulation yields of 52 and 78.5 % were obtained for a lipid concentration of 10^{-3} M at ambient temperature and 65°C, respectively. These values appear higher than those obtained with the non–PEG–liposomes, i.e. 5 and 44 %. This shows that the PEG chains, in addition to make the liposomes stealth, facilitate ^{212}Pb encapsulation. The value at 65°C appears in close agreement with the one reported by Henriksen et al [4] obtained for related PEG–liposomes under similar conditions (80 %). This result indicates that the indium–DTPA complexes at the liposome surface do not hamper the labeling efficiency.

Conclusions

A two–step preparation process is proposed for indium–tagged liposomes loaded with ^{212}Pb: the first step involves the labeling of surface–DTPA liposomes with indium while the other one corresponds to ^{212}Pb encapsulation. Several parameters were tested to optimize the encapsulation (temperature, concentrations of lipids, Pb, and DTPA, and different lipophilic chelate…). The results indicate that the origin of the encapsulation is related to the dynamics of the surface, which makes the membrane partially permeable. The process is optimum at 65°C. No ligand was then necessary to allow the passage of ^{212}Pb from the external to the liposome internal compartment: a similar yield was observed for all chelating agents tested

and close to the one measured without chelating agent. This indicates that a significant improvement is observed when DTPA is present in the internal compartment. By forming a strong complex with Pb, it allows a concentration of the metal ion. The optimized conditions allow the encapsulation of 2–3 lead ions with a yield of 85 % under conditions relevant for nuclear medicine application. Comparison with literature data shows that the pre–labeling with indium does not affect the encapsulation yield. Further studies are on going to check that the results are still valid in the presence of several MBq of ^{212}Pb, and that the encapsulation is stable in biological media will also be studied.

Acknowledgements

We thank AREVA for providing the batch of ^{228}Th. Financial support from the ANR VecRIT (PCV07_185075) and the European Commission (EC grant HEALTH–F2–2007–201962 to the FP7 collaborative project TARCC (Targeted Alpha-Radionuclides to Combat Cancer)) is also gratefully acknowledged.

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Table 1: Composition of liposomes and ^{212}Pb encapsulation yield.

Experimental conditions: [BAL] = 10^{-4} M, lipid concentration of $2.5.10^{-3}$ M, Pb concentration of 10^{-7} M; external medium: 0.1 M NaCl, 2.10^{-3} M HEPES (pH = 7.4).

liposome number	liposome composition	internal volume	lead encapsulated (%); T=65 ± 1°C	lead encapsulated (%); T=21 ± 2°C
A	DSPC (68%), cholesterol (30.5%), DSPE (1.5%)	0.1 M NaCl, 2.10^{-3} M HEPES (pH=7.4)	24.6 ± 8.5	3.0 ± 1.2
B	DSPC (68%), cholesterol (30.5%), DSPE (1.5%)	0.025 M DTPA, 0.1 M NaCl, 2.10^{-3} M HEPES (pH=7.4)	74.9 ± 7.6	69.7 ± 9.5
C	DSPC (68%), cholesterol (30.5%), DSPE–DTPA (1.5%)	0.1 M NaCl, 2.10^{-3} M HEPES (pH=7.4)	56.3 ± 5.3	5.6 ± 0.9
D	DSPC (68%), cholesterol (30.5%), DSPE–DTPA (1.5%)	0.025 M DTPA, 0.1 M NaCl, 2.10^{-3} M HEPES (pH=7.4)	72.7 ± 3.2	12.9 ± 1.1
E	DSPC (64%), cholesterol (29.5%), DSPE–DTPA (1.5%), PEG (5%)	0.025 M DTPA, 0.1 M NaCl, 2.10^{-3} M HEPES (pH=7.4)	78.5 ± 2.5	52.0 ± 3.1

Footnotes: BAL = 2,3–dimercapto–1–propanol; HEPES = 2–[4–(2–Hydroxyethyl)–1–piperazine]ethanesulfonic acid; DSPC = 1,2–Distearoyl–sn–glycero–3–phosphocholine ; DSPE-DTPA = 1,2–Distearoyl–sn–glycero–3–phosphoethanol amine–diethylenetriamine pentaacetic acid

Table 2: Effect of the ligand X on the encapsulation; experiments realized with liposome D for X = acetate, DCP (1,10–phenantroline–2,9–dicarboxylic acid), BAL (2,3–dimercapto–1–propanol), and A23187 (see Table 1 and figure 2).

composition of external medium	T(°C)	lead distribution in solution [a]		% of lead eluted; no liposome present [b]	Encapsulation yield (%) [c]
Pb	21 ± 2	81% Pb^{2+}; 18% $Pb(OH)^{+}$; 1% $Pb(OH)_2$	[18]	0.9 ± 0.2	9.1 ± 0.7
	65 ± 1			1.1 ± 0.2	45.7 ± 1.8
Pb-acetate	21 ± 2	99% $Pb(Ac)_3^-$; 1% $Pb(Ac)_2$	[18]	0.7 ± 0.2	5.3 ± 0.6
	65 ± 1			0.5 ± 0.2	46.7 ± 1.9
Pb-DCP	21 ± 2	100% Pb(DCP)	[19]	0.6 ± 0.2	3.9 ± 0.6
	65 ± 1			0.5 ± 0.2	36.5 ± 1.7
Pb-BAL	21 ± 2	no data available		1.2 ± 0.2	5.1 ± 0.7
	65 ± 1			0.5 ± 0.3	36.5 ± 1.6
Pb-A23187	65 ± 1	96% $Pb(A23187)^{+}$	[20]	12.0 ± 0.1	15.0 ± 1.1

[a] calculated at 25°C, [b, c] $[Pb] = 10^{-9}$ M, $[X] = 10^{-5}$ M; [c] $[Lipid] = 10^{-3}$ M

Figure 1: Complexation of indium by DTPA–tagged liposomes.

Lipid concentration = 6.10^{-5} M, pH = 7.4 (2.10^{-3} M, HEPES), 0.1 M NaCl. Squares are experimental data. The lines give the number of interacting sites 10^{-6} M and $2.5.10^{-4}$ M for the solid and dashed lines respectively.

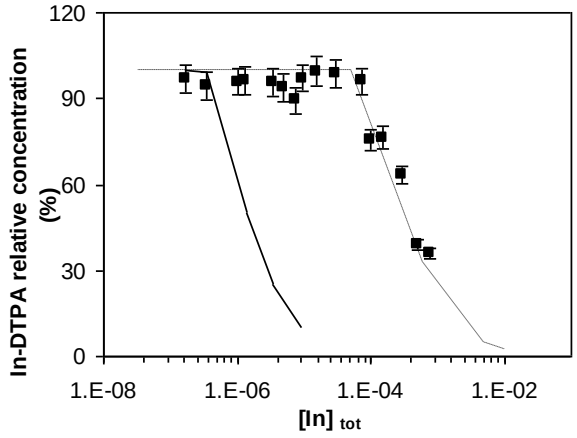
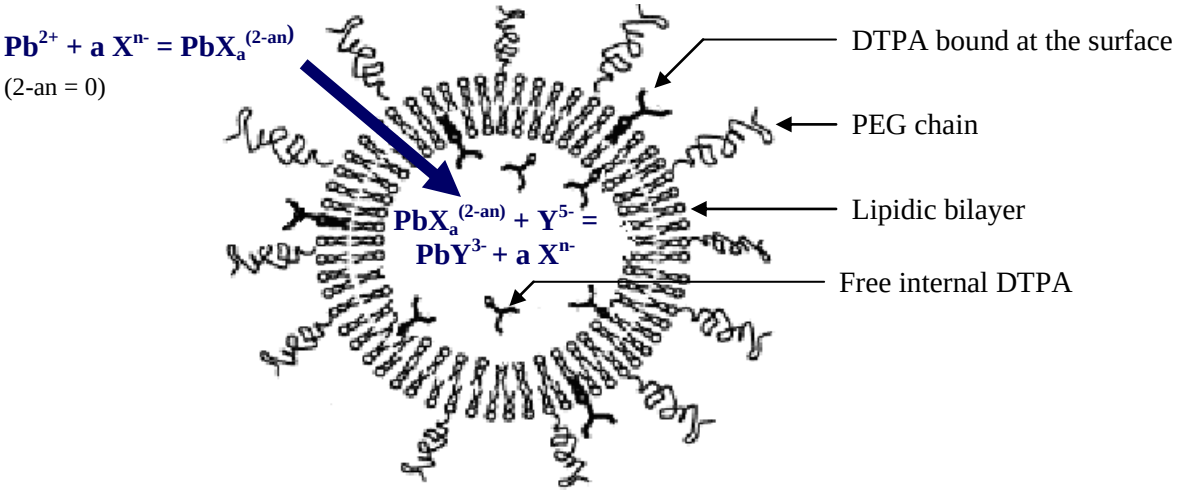
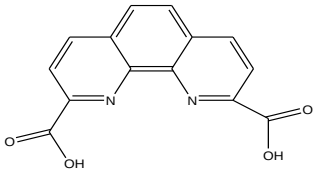


Figure 2: Scheme of the encapsulation; X

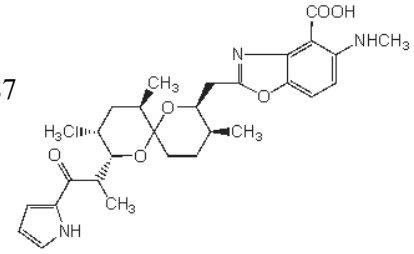


with Y = DTPA, and X =

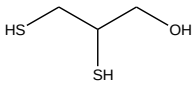
- DCP



- A23187



- BAL



- acetate

Figure 3: Effect of the temperature on ^{212}Pb encapsulation in liposomes. [Lipid] = 10^{-3} M, pH = 7.4 (2.10^{-3} M, HEPES), 0.1 M NaCl, 10^{-5} M CH_3COO^-.

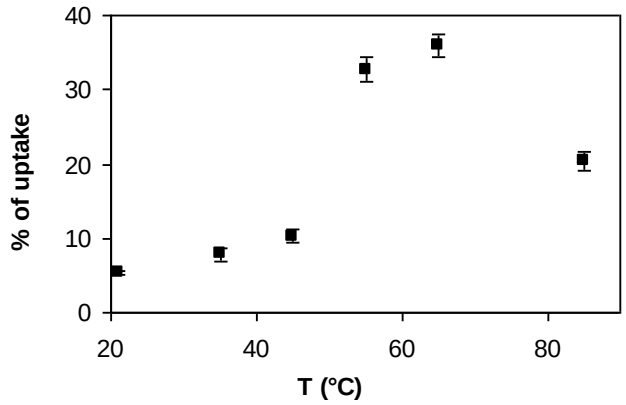


Figure 4: Study of the encapsulation as a function of liposome ([Pb] = 10^{-7} M) (A) and Pb ([lipids] = $7.5.10^{-3}$ M) concentrations (B). Experimental conditions: pH = 7.4 (2.10^{-3} M HEPES), 0.1 M NaCl, T = 65°C, $[DTPA]_{in} = 25.10^{-3}$ M. The lines are tendency curves.

(A) (B)