Interaction of Isoniazid with unilamellar liposomes

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The ability to efficiently entrap a molecule is a parameter of crucial importance in the design of a nano-carrier. Both the loading efficiency and the release properties can be strongly influenced by the interaction between the vector and the drug to be carried.
To develop a liposome-based drug delivery system able to encapsulate Isoniazid (INH), a first line anti-Micobacterium tuberculosis-drug, we prepared charged unilamellar vesicles containing a mixture of a zwitterionic lipid (HSPC) and an anionic lipid (DPPG) at varying molar fraction. HSPC-DPPG liposomes showed a long-term stability but a low entrapment efficiency of drug.
To increase the encapsulation efficiency, a deeper understanding of the intermolecular interactions occurring in the hybrid drug-liposome system is necessary. To this aim, we carried on a systematic investigation by mean of different and complementary techniques, as Differential Scanning Calorimetry, Static Light Scattering and Langmuir monolayer technique on liposomes with different HSPC molar fraction ($X_{HS} = n_{HS}/(n_{HS}+n_{PG})$) and varying INH/lipid molar ratio ρ.
DSC results point out that INH interacts with lipid bilayer and affects the lipid arrangement, the extent of the interaction being strongly dependent on both drug amount and lipid composition. The strongest effect is found on liposomes with the largest content of the charged DPPG (X_{HS} =0.33), where drug induces peak splitting that hints at the occurrence of lipid segregation and phase separation. (Figure 1). The nature of lipid-membrane interaction at higher concentrations seems to go beyond electrostatic screening, affecting more intimately the lipid bilayer structure. This could be attributed to molecular adsorption and/or insertion within the membrane.
This scenario is confirmed by the observed variation of the gyration radii of liposomes in the presence of INH. An increase of the gyration radius is found for all the X_{HS} investigated and all temperature (Figure 2), hinting that INH accumulate on liposome surface possibly penetrating (even partially) in the bilayer.
The nature of the interaction of INH with both the lipids has been clarified by deeper analysis of Langmuir compression isotherm of one-component DPPG and HSPC monolayers deposited at air-water interface.
Our results show that INH is more surface-active with DPPG monolayers, as the relative molecular area variation increases more rapidly for increasing ρ with respect to the same quantity measured for HSPC monolayers. (Figure 3) The variation of molecular area of the monolayer in the presence of INH is the signature of INH penetration within the monolayer. Interestingly, in DPPG monolayer the drug remains inserted also at surface pressure corresponding the liposome bilayer packing.
This further confirms that INH is more affine to the anionic lipid DPPG rather than to zwitterionic one and corroborates the scenario where the lipid demixing observed in X_{HS} =0.33 liposomes is driven by the preferential binding of INH with the charged lipid component. It has to noted that this preferential interaction is expected considering that INH is positively charged at pH=7.4 [1] Furthermore, electrostatic or polar interaction of isoniazid amminic group with the anionic DPPG's and the zwitterionic HSPC's head, could lead to the drug penetration in the bilayer, inducing the formation of acqueous pore, similarly with what observed for PNAPAM dendrimers or peptidic antibiotics in cells' membrane [2,3]. This mechanism could be responsible for membrane leakage, resulting in the poor entrapment efficiency of the system.
Our result strongly point out the role of drug-bilayer interaction in the optimal design of lipid-based nanovector.

[1] Wheate, Nial J., et al. "Host–guest complexes of the antituberculosis drugs pyrazinamide and isoniazid with cucurbit [7] uril." *Journal of Inclusion Phenomena and Macrocyclic Chemistry* 68.3-4 (2010): 359-367.
[2] Chen, Xiaoyun, and Zhan Chen. "SFG studies on interactions between antimicrobial peptides and supported lipid bilayers." *Biochimica et Biophysica Acta (BBA)-Biomembranes* 1758.9 (2006): 1257-1273.
[3] Nyitrai, Gabriella, et al. "Sodium selective ion channel formation in living cell membranes by polyamidoamine dendrimer." *Biochimica et Biophysica Acta (BBA)-Biomembranes* 1828.8 (2013): 1873-1880.

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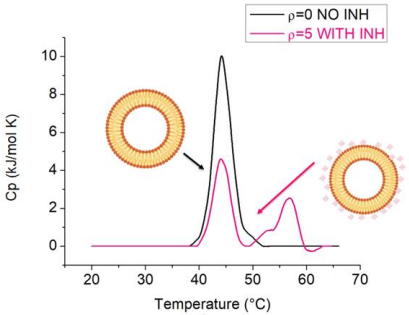

Figure 1. Isoniazid effect on excess molar heat capacity of unilamellar liposome suspensions (X_{HS} =0.33): peak splitting

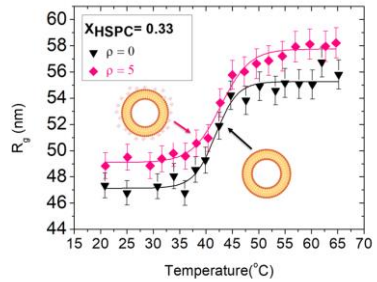

Figure 2. . Isoniazid effect on gyration radius of unilamellar liposome suspensions (X_{HS} =0.33)

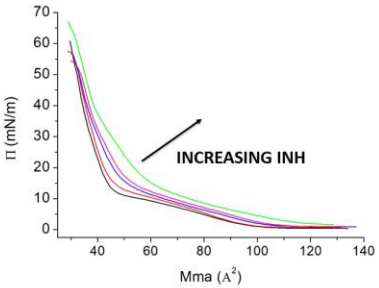

Figure 3. Surface pressure isotherms of DPPG monolayers after the injection in the subphase of different volumes of an INH solution