Characterization of SLN/Liposomes hybrid nanoparticles for the co-delivery of two anti-tubercular drugs: focus on SANS analysis

Eleonora Truzzi,[a] Fiorella Meneghetti,[b] Matteo Mori,[b] Luca Costantino,[a] Valentina Iannuccelli,[a] Eleonora Maretti,[a] Fabio Domenici,[c] Carlo Castellano,[d] Angela Capocefalo,[e] Eliana Leo [a]

[a] Department of Life Sciences, University of Modena and Reggio Emilia, via Campi 103, 41125, Modena, Italy; [b] Department of Pharmaceutical Science, University of Milan, Via L. Mangiagalli 25, 20133 Milano, Italy. [c] Department of Chemical Science and Technology, University of Rome Tor Vergata, via della Ricerca Scientifica, 00133, Roma, Italy; [d] Department of Chemistry, University of Milan, via Golgi 19, 20133, Milano, Italy; [e] Department of Physics, Sapienza University of Rome, P.le Aldo Moro 5, 00185, Roma, Italy;

Email of presenting authors: eleonora.truzzi@unimore.it and eliana.leo@unimore.it

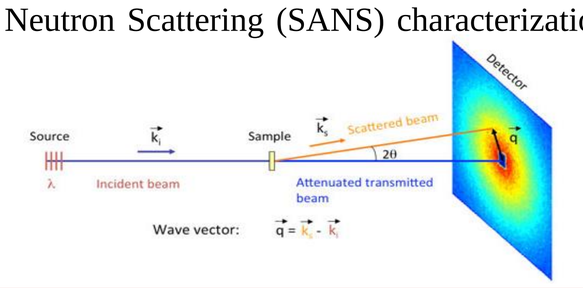
Background & Aim

Respiratory diseases constitute five of the 30 most common causes of death worldwide. Pulmonary administration of therapeutic active compounds represents the best strategy to overcome side effects related to drugs and to directly act at the target site. Lipid-based nanoparticles, such as Solid Lipid Nanoparticles (SLN) and Liposomes have been demonstrated to be efficient and biocompatible drug delivery systems able to confer on drugs suitable properties for a inhalation therapy. Unfortunately, SLNs are characterized by a low drug loading capacity, in particular when hydrophilic drugs are concerned, which leads to difficulties in retaining drugs. In the present work, Hybrid liposomes, made up of SLNs embedded in liposomes, were designed to obtain a new nanocarrier formulation with improved physicochemical properties: in particular, the lipid bilayer of liposomes was predicted to improve drug retaining and release and the efficiency of intracellular delivery of the resulting hybrid system. Rifampicin (RIF) and Isoniazid (INH), two first-line antitubercular drugs, were selected here as models for hydrophobic and hydrophilic drug, respectively.

Methods

SLNs were prepared by melt-emulsification technique using Compritol as lipid matrix. Liposomes and Hybrid liposomes were formulated with the reverse phase evaporation method, using soy lecithin and cholesterol. For the formulation of the Hybrid liposomes, SLNs suspension was used instead of water. All the samples were co-loaded with 15 mg of RIF and INH and D_2O was used instead of H_2O, in order to apply the Small-angle Neutron Scattering (SANS) characterization method.

SANS is a technique that exploits the neutron scattering to analyze without manipulations colloidal systems. Respect to vesicle carriers, with SANS it is possible to determine: :the core radius (R1), the total radius (R2) the shell thickness (ts), the shell scattering length density (SLD, ρ2), the water scattering length density (SLD, ρ1). The thickness of the shell was calculated from the differences between the two radii (ts = R2-R1) (Truzzi et al.).



Results

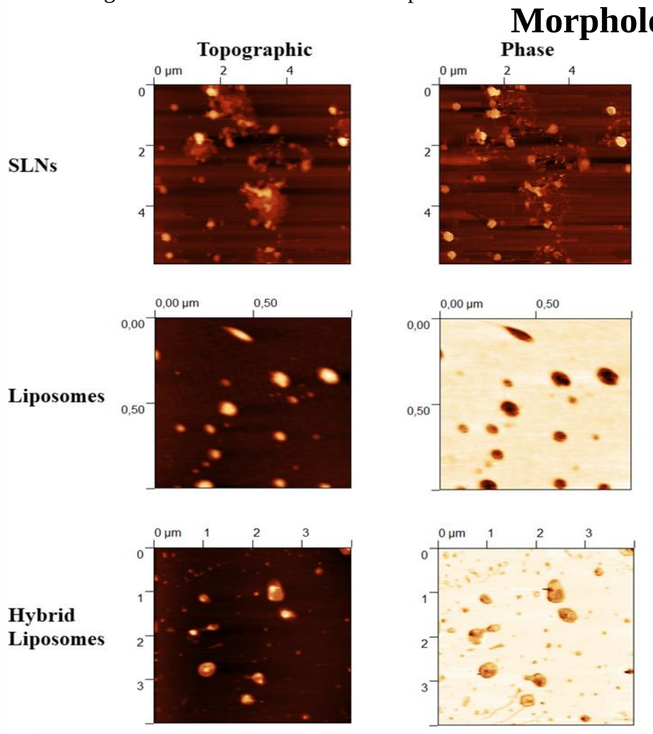
Dynamic light scattering and drug loading analyses

Sample	Size	PDI	RIF EE%	RIF DL%	INH EE%	INH DL%
Unloaded SLNs	122 ± 2	0.23 ± 0.01	/	/	/	/
Co-loaded SLN	135 ± 3	0.25 ± 0.05	61.7 ± 3.92	5.74 ± 0.69	31.27 ± 0.64	2.49 ± 0.07
Unloaded liposomes	300 ± 6	0.49 ± 0.02	/	/	/	/
Co-loaded liposomes	310 ± 6	0.45 ± 0.05	62.1 ± 4.9	8.1 ± 0.7	46.9 ± 6.5	6.1 ± 0.8
Hybrid liposomes	343 ± 6	0.33 ± 0.04	/	/	/	/
Co-loaded hybrid liposomes	350 ± 9	0.30 ± 0.05	91.43 ± 0.15 *	2.7 ± 0.1	100 ± 0.34 *	1.94 ± 0.01

Particle size, polydispersity index (PDI), Encapsulation Efficiency (EE%) and Drug Loading (DL%) of unloaded, co-loaded SLNs, Liposomes and Hybrid liposomes (mean ± standard deviation). * The EE% was calculated considering the actual loading of SLNs added to formulate the hybrid systems. DL% was calculated knowing the exact weight of SLNs in 3 mL of SLN suspension added.

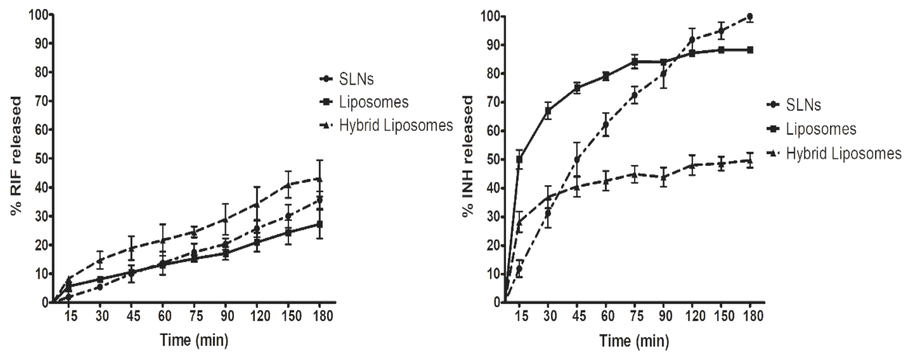
- Hybrid liposomes exhibited the biggest size. Slight differences in dimensions could be appreciated between liposomes and hybrid liposomes.
- SLNs demonstrated a good homogeneity; on the contrary, liposomes displayed a PDI higher than 0.3, indicating a multimodal particle distribution. This irregular homogeneity seemed to decrease with SLN embedding into liposomal bilayer, since hybrid liposome PDI was about 0.3.
- The encapsulation of the lipophilic drug RIF in SLNs and liposomes resulted the same, while for the hydrophilic drug INH appeared significantly lower in the case of SLNs. For hybrid liposomes, since were prepared by adding the co-loaded SLNs, the obtained EE% revealed that the drugs were retained by SLNs during the formulation, with a tiny leak of RIF (about 9%).

Morphological analysis



The images were obtained by recording both topographic and phase signal in atomic force microscopy (AFM). Phase imaging provides complementary information to the topographic image, revealing the variations in the surface properties of nanocarriers, such as viscosity, elasticity and viscoelasticity. For this reason, it is useful to differentiate materials which contain soft (dark) and rigid (bright) domains. Concerning unloaded hybrid liposomes, the analysis of the sample showed few spots corresponding to liposomes and big structures, which seemed to contain other smaller particles.

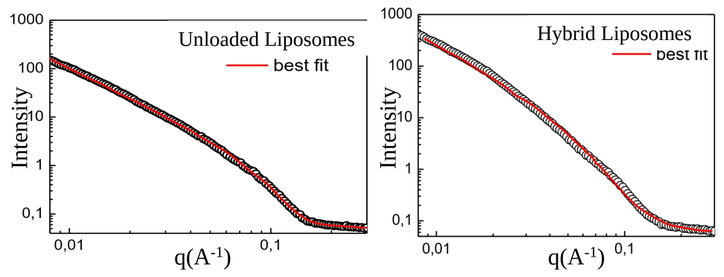
In vitro drug release



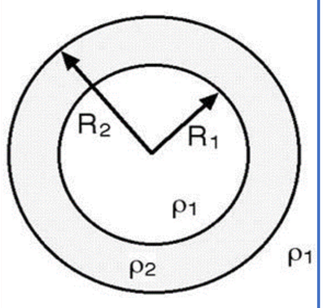
All the compared nanocarriers exhibited a comparable RIF release. In the case of the hydrophilic drug INH, SLNs demonstrated to poorly retain INH which was completely and constantly release in 3 hours. On the contrary, Hybrid liposomes in 3 hours were able to retain around the 50% of the drug. Liposomes displayed a high burst effect, releasing the 50% of INH in the first 15 min, followed by a slow and controlled release over time.

SANS analysis

	ρ_2 [$\cdot 10^{-6}$ Å^{-2}]	ρ1 [$\cdot 10^{-6}$ Å^{-2}]	R_{core} [nm]	PD(R_{core})	t [nm]	PD(t)
Unloaded liposomes	2.4	6.4	384.09 ± 0.07	0.3	3.39 ± 0.02	0.3
Co-loaded liposomes	2.6	6.4	399.14 ± 0.11	0.3	3.45 ± 0.01	0.2
Unloaded hybrid liposomes	2.4	6.4	449.24±0.07	0.3	4.00±0.02	0.6
Co-loaded hybrid liposomes	2.5	6.4	402.01±0.07	0.3	3.52±0.01	0.7



SANS scattering curves and best-fit solid line.



The experimental scattering curves from SANS analysis were fitted with the best-fit solid line. From the equation of the best-fit the information depicted in the above table were obtained.

- No Bragg's peaks were evident in the scattering curves, demonstrating the unilamellarity of both liposomes and hybrid liposomes. This result is due to the presence of negative phospholipids in lecithin, which induced electrostatic repulsion preventing the formation of further layers.
- Co-loaded liposomes showed a concomitant slight increase of the lipid shell scattering length density (SLD, ρ2) and of the membrane thickness t, compared to the unloaded ones. This result is compatible with the presence of the lipophilic RIF, which determines a rearrangement of the lipids in the single bilayer.
- The dimensions of the unloaded Hybrid liposomes were greater (~65 nm) than those of the corresponding unloaded liposome, suggesting the presence of the SLN in the liposome core. An increase of the SLD, ρ2 of the lipid shell was observed in the presence of INH and RIF
- The dimension of the unloaded hybrid system decreased of about 50 nm after the co-loading. As observed in our previous paper, the co-loading determines a stabilizing effect, coherent with the liposome radius decrease (Truzzi et al.).

Conclusions

The results reported here suggested the formation of Hybrid liposomes, as an alternative drug delivery system for the co-administration of lipophilic and hydrophilic drugs. Hybrid liposomes demonstrated to overcome the drawbacks of SLN, by achieving a controlled and long-lasting release of both the drugs. The preliminary SANS analysis provided unique information regarding the occurrence of the Hybrid carrier, highlighting drugs/lamellae interactions. SANS scattering curves will be further analyzed in order to obtain additional information regarding Hybrid liposomes structure.

Truzzi et al., J. Colloid Interface Sci. 541 (2019) 399–406