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Application of a pH transmembrane gradient to optimize the entrapment efficiency and drug loading of sildenafil citrate liposomes

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Introduction and aims of the study

Sildenafil, first approved for erectile dysfunction, has found additional uses and pulmonary arterial hypertension (PAH) is among current therapeutic indications. Oral and intravenous formulations are only available and used in all cases. Nevertheless, pulmonary delivery is an efficient strategy for passive drug targeting, with the advantages of direct access to the target, together with reduction of side effects associated to systemic exposure. Liposomes have demonstrated their suitability for pulmonary administration in animals and humans, the challenge being to achieve liposomes showing efficient and stable drug entrapment.

The aim of the present study was to develop a solvent-free method for active loading of liposomes with sildenafil citrate (SC), based on pH transmembrane gradient.

Material and Methods

1. Preparation of liposomes

Solvent-free method

SC loaded liposomes composed of Egg L-α-phosphatidylcholine (EPC) and Cholesterol (Ch), with and without D-α-Tocopheryl polyethylene glycol 1000 succinate (Vit E TPGS), were prepared by direct sonication of the components according to a previously described method [1].

Transmembrane pH gradient

For active loading, the pH of liposome suspensions prepared with citrate buffer of pH = 3.2 were adjusted to pH = 7.0 with NaOH. The resulting suspensions were maintained for 20 h at 25 ±2ºC under mechanical agitation to facilitate the diffusion of the drug across the lipid bilayer and accumulation in the liposome core (pH = 3.2), according to the pH-dependent solubility profile of sildenafil [2].

2. Characterization of liposomes

Size and zeta potential

Hydrodynamic diameter (Dh), polydispersity index (PDI) and zeta potential were determined by dynamic light scattering (DLS). The analysis was performed at 25 °C with a scattering angle of 173° after the appropriate dilution (x100 or x1000) with Milli-Q water or buffer solution (pH= 3.2) to avoid the phenomenon of multiple scattering.

Entrapment efficiency (EE%)

Liposome suspensions were centrifuged at 14.000 rpm for 45 min at 6º C to separate the unentrapped drug in the supernatant from the drug loaded into liposomes. The amount of drug in the supernatant was determined by HLPC and the EE % was estimated from the following equation:

$$EE\ (\%) = [(Qt-Qs)/Qt] \times 100$$

Where, Qt is the total drug amount in the liposome suspension and Qs is the drug amount quantified in the supernatant.

The drug loading (mg of SC/g of lipids) was estimated from EE% and lipid amount in liposomes.

Results and Discussion

As reported previously [1-3,4], the direct sonication of components produced drug loaded liposomes without the use of organic solvents, which result in an environment-friendly approach. After applying the transmembrane pH gradient, a significant increase of EE % was achieved as shown in Figure 1 that illustrates the mechanism of active loading

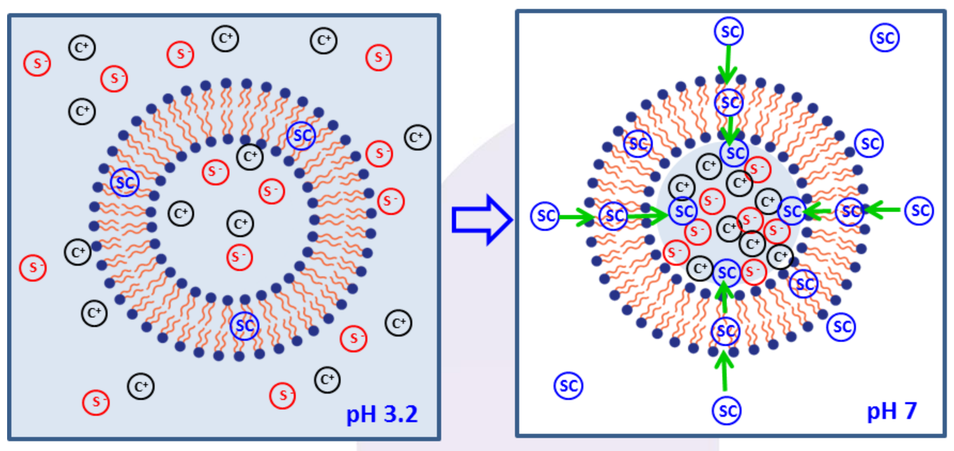

Figure 1.- Application of transmembrane pH gradient

The characteristics of the SC loaded liposomes obtained in this study are summarized in Table 1

Influence of the transmembrane pH gradient on the characteristics of the liposomes						
Liposomes		Dh (nm)	PDI	Zeta potential (mV)	EE (%)	DL (mg/g lipid)
Without Vit E TPGS	pH =6.7	-	-	-	<18%	<11
	pH = 3.2	304.3	0.413	-2.10	49.47±9.78	29.71±5.87
	pH gradient	209.7	0.537	-20.90	89.77±7.64	53.92±4.59
With Vit E TPGS	pH =6.7	-	-	-	<15%	<9
	pH =3.2	303.2	0.452	-2.05	22.67±11.32	13.62±11.00
	pH gradient	219.8	0.534	-21.30	80.30±11.03	48.23±6.62

Table 1.-Characteristics of the liposomes prepared using Milli-Q water (pH = 6.7), citrate buffer (pH= 3.2) or the transmembrane gradient (pH gradient)

Conclusions

1. SC loaded liposomes can be prepared by direct sonication of components, avoiding the use of organic solvents
2. Application of the active loading procedure, based on transmembrane pH gradient, increased the EE% of liposomes with and without Vit E TPGS to values over 80%
3. According to composition and drug loading, the liposomes obtained are suitable for SC pulmonary delivery

References

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