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Influence of spin probe structure on its distribution in SLN dispersions

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Abstract

Solid lipid nanoparticles (SLN) are drug carrier system composed of biodegradable substances, which are solid at room temperature. The physico-chemical properties and structure of the incorporated compounds can affect their partitioning in SLN dispersions. In this work the influence of lipophilicity and structure of different SP on its location in SLN were studied. By electron paramagnetic resonance (EPR) measurements it was found that lipophilic SP distribute between a solid glyceride core and a soft phospholipid layer, with the more polar part (piperidine ring or methylcarboxylic groups) oriented toward the water–lipid interface. The majority of SP is located in the phospholipid layer, but the portion in the solid lipid core increases with SP lipophilicity. The hydrophilic Tempol does not incorporate into SLN.

Keywords: Solid lipid nanoparticles; Lipophilic spin probes; Location; Electron paramagnetic resonance

Solid lipid nanoparticles are an attractive carrier system for drugs. They represent aqueous colloidal dispersions of biodegradable substances, which are solid at room temperature (Müller et al., 1995). The loading capacity of SLN dispersion for poorly water-soluble drugs depends on the composition of SLN and is limited by the generally low solubilization capacity of the molten lipids for many poorly water-soluble drugs (Westesen et al., 1997). The quantitative structure activity approach uses parameters which reflect several different properties. The three most commonly are steric and electronic parameters and parameters related to partitioning (Dearden and James, 1998). Limited data are available about the drug distribution within the system and its mobility in SLN. These data are of vital interest for the design of SLN and for their evaluation as a potential drug carrier system.

In our previous work EPR spectroscopy was used to study the incorporation of model lipophilic drugs (spin-labelled derivatives of fatty acids) into SLN in order to establish their location, the entrapment efficiency and to follow the stabilization of SLN dispersion during storage. We have shown that lipophilic SP distribute between the solid glyceride core and the phospholipid layers. The distribution depends on the type of lipid matrix and on the phospholipid concentration (Ahlin et al., 1998a).

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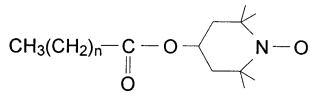
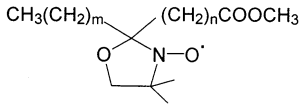


C_n-Tempo (n = 12, 14)



MeFASL (m,n)

(m,n) = (1,12), (8,5), (10,3), (4,5), (2,5)

Fig. 1. Structural formula of spin probes C_n-Tempo, MeFASL (m,n) and Tempol.

The physico-chemical properties and structure of the incorporated compound can also affect its partitioning in the SLN dispersion — in the solid lipid core, outer layer composed of phospholipids and steric stabilizers or in the water phase. In this work the influence of lipophilicity and structure of different SP on its location in SLN were studied.

SLN were composed of glyceryl tripalmitate-Dynasan®116, soy bean lecithin — (Phospholipon®80) and poloxamer 188 (Pluronic®F68). Model lipophilic compounds were spin-labeled derivatives of fatty acids with different acyl chain lengths and with the nitroxide group at different positions on the acyl chain (C_n-Tempo and MeFASL (m,n)); and the hydrophilic molecule Tempol (Fig. 1), all synthesized in our laboratory.

SLN were prepared by the melt-emulsification process. 2 ml of heated poloxamer 188 water solution (0.5 %) was added to the melted lipid (50 mg), phospholipid (20 mg) and spin probe (2×10^{-7} mol) which was prepared as a thin film on the wall of glass tubes. The mixture was stirred 8 min at 20 000 rpm at 70–80°C. After cooling to room temperature (10 min of stirring at 5000 rpm) the glycerides were solidified and solid nanoparticles were formed (Ahlin et al., 1998b).

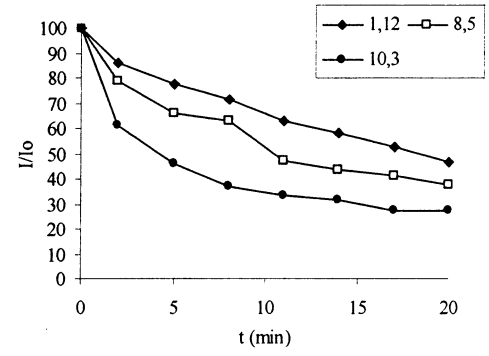
Particle size and polydispersity index (PI) were determined using photon correlation spectroscopy and the particle shape was estimated by transmission electron microscopy. SLN with an anisometrical shape and hydrodynamic radius of approximately 160 nm were obtained.

EPR spectra of SLN with incorporated spin probes were recorded on a Bruker ESP 300 X-band spectrometer at room temperature and at 80°C, i.e. above the melting point of glyceride. The location of SP in SLN was determined directly from the EPR spectra, from the reduction kinetics of the spin probe with sodium ascorbate and by computer simulation of EPR spectral line shapes.

With EPR spectra intensity measurements, determined by double integration of the EPR spectra, only a part of the total amount of spin probes was detected at room temperature. The other part becomes detectable above the melting point of

Table 1
The partition coefficient of SP (log P) calculated by Rekker, the percentage of SP incorporated into solid lipid core of SLN (%), hyperfine splitting constant (a_N), and the percentage of SP which is reduced with sodium ascorbate after 2 min (p_{asc}) (in water or well exposed to ascorbate)

Spin probe	Log P	% SP in core	a_N (mT)	p_{asc} (%)
MeFASL (1,12)	5.12	46 ± 3	1.47 ± 0.01	13.8
MeFASL (8,5)	5.12	40 ± 8	1.46 ± 0.07	21.5
MeFASL (10,3)	5.12	34 ± 5	1.46 ± 0.06	38.8
MeFASL (4,5)	3.04	29 ± 7	1.51 ± 0.06	25.4
MeFASL (2,5)	1.48	28 ± 5	1.54 ± 0.06	39
Tempol	0.62	0 ± 4	1.72 ± 0.01	100
C_{12}-Tempo	6.78	15 ± 6	1.62 ± 0.01	60
C_{14}–Tempo	7.02	46 ± 5	1.62 ± 0.01	60



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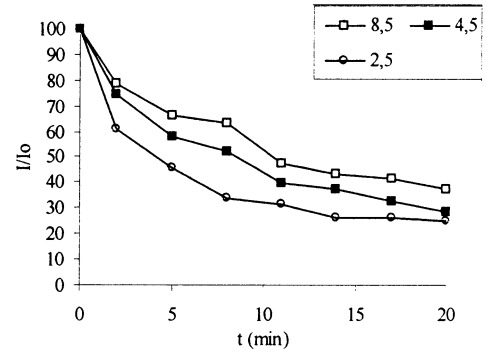

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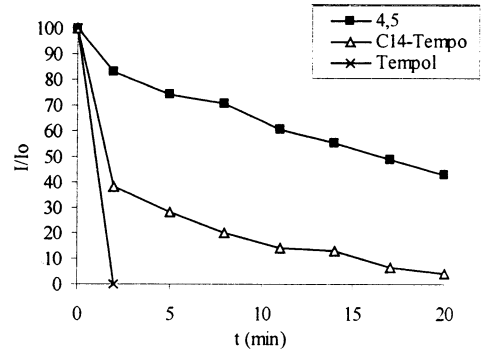

c.

Fig. 2. EPR spectra intensity decrease of SP after addition of ascorbate (0.01 mol/l, 1:1, v:v): (a) SP with different position of nitroxide group on the acyl chain; (b) SP with different lipophilicity; and (c) SP with different structure.

glycerides. We presume that at room temperature this part of SP forms highly interacting pairs or aggregates in the solid crystalline core, which are not detectable by EPR due to the strong spin exchange interaction. The portion of SP in the lipid core, i.e. the portion which was not visible by EPR at room temperature, is presented in Table 1. It increases with higher lipophilicity of SP and also for MeFASLs with the distance of the nitroxide group from the surface of SLN.

In the same table the nitrogen isotropic hyperfine splitting constant, a_N, is shown. It is a sensitive function of polarity of the SP environment and ranges from cca. 1.4 mT for a non-polar to about 1.7 mT for a highly polar environment. Values of a_N for Tempol and C_{14}-Tempo show that their nitroxide groups are in a polar environment. For the other SP a_N increases with decreasing molecules lipophilicity (smaller log P), indicating that more lipophilic SP are immersed deeper into the phospholipid layer.

Some additional data about the location of SP in SLN were obtained from the EPR spectra intensity decrease with time after the addition of sodium ascorbate. It is a reducing agent, which reduces nitroxide groups to non-paramagnetic hydroxylamine. Due to its charge it could not penetrate into the particles and could reduce only the nitroxide groups located in water or on water–lipid interfaces. It gives information about the accessibility of ascorbate to the nitroxide group of SP entrapped in SLN. Our results show that the rate of nitroxide reduction depends on the position of the nitroxide group on the acyl chain, on the lipophilicity and on the structure of SP (Fig. 2a, b, c). From Fig. 2a it can be seen that the reduction is the fastest for MeFASL (10,3), with the nitroxide group closer to the surface of SLN and decreases with the distance of the nitroxide group from the surface. Reduction rates are slower for more lipophilic molecules (Fig. 2b) and is extremely fast for hydrophilic Tempol (Fig. 2c).

For better evaluation of SP location in SLN the EPR spectra line shapes were calculated by the model in which the nitroxide group is in a fast isotropic motion regime and spins relax via the rotational motion and spin-exchange (Nordio, 1976). In general EPR spectra of SP could be fitted with two populations of SP, which belong to a different location in the SLN dispersion. The

Table 2
EPR parameters obtained from the simulated EPR spectra for different SP entrapped in SLN[a]

Spin probe	τ_1 (ns)	τ_2 (ns)	a_{N_1} (mT)	a_{N_2} (mT)	d_1 (%)	d_2 (%)
MeFASL (1,12)	0.96	–	14.71	–	1	–
MeFASL (8,5)	2.09	1.21	14.62	15.26	0.62	0.38
MeFASL (10,3)	1.69	0.13	14.88	16.11	0.99	0.01
MeFASL (4,5)	1.66	0.05	14.76	15.82	0.98	0.02
MeFASL (2,5)	1.63	0.10	14.83	15.90	0.93	0.07
Tempol	0.02	–	15.53	–	1	–
C_{12}-Tempo	1.30	0.59	15.40	16.02	0.37	0.63
C_{14}-Tempo	0.84	0.64	15.11	16.36	0.39	0.61

[a] τ, Rotational correlation time; a_N, isotropic hyperfine splitting constants; d, relative portion of one SP population (indexes 1 and 2 denotes two populations).

exceptions are Tempol and MeFASL (1,12) which could be fitted by one population (Table 2). For most of MeFASL (m,n) the majority of molecules, which are detectable by EPR at room temperature, incorporate into the phospholipid (PL) layer, while a small part (1–7%, depending on lipophilicity) stays in the water phase. However, for C_{14}-Tempo and MeFASL (8,5) the two populations are of the same order of magnitude, one well exposed to the surface of SLN, which is reduced faster by ascorbate, and the other which is, according to its a_N values and reduction kinetics, immersed deeper in the phospholipid layer.

The incorporation of drug molecules into SLN, especially the influence of lipophilicity and molecular structure, could be satisfactorily monitored by EPR spectroscopy. By applying different spin probes, the information about the position within the SLN particle is obtained too. This knowledge is of great importance for drug incorporation into SLN particles.

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