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Idebenone loaded solid lipid nanoparticles: Calorimetric studies on surfactant and drug loading effects

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ABSTRACT

In this study we prepared solid lipid nanoparticles (SLN), by the phase inversion temperature (PIT) method, using cetyl palmitate as solid lipid and three different non-ionic emulsifiers of the polyoxyethylene ethers family (ceteth-20, isoceteth-20, oleth-20). These SLN were loaded with different amount of idebenone (IDE), an antioxidant drug useful in the treatment of neurodegenerative diseases and skin oxidative damages. The differential scanning calorimetry (DSC) was employed to evaluate the effects of the different emulsifiers and the different amounts of drug loaded on the thermotropic behavior of SLN and to investigate how the drug was arranged into these nanoparticles.
The IDE seemed to be located into different regions of the SLN depending on its concentration and on the surfactant used. The results of this study suggest that the calorimetric studies performed on SLN could provide valuable information to optimize SLN design and drug release from these carriers.

1. Introduction

Oxidative stress seems to be involved in the development of several pathological conditions such as neurodegenerative diseases (Taylor et al., 2013), cancer, stroke and cardiovascular disorders (Kim et al., 2013). On the contrary, short-term oxidative stress is a physiological defense mechanism against pathogens that is finely regulated. Its imbalance leads to overproduction of reactive oxygen species (ROS), which alters the normal mechanisms of cell signaling, damaging lipids, proteins and DNA. Coenzyme Q_{10} is a biological cofactor of the electron transport chain and it is an essential antioxidant agent in mitochondrial and cell membranes. Idebenone (IDE), a synthetic analogue of coenzyme Q_{10} (Fig. 1), is a free radical quencher that is able to counteract cell and tissue damages due to ROS overproduction. It has been proved useful in the treatment of a wide range of diseases (Villalba et al., 2010) but particularly it is regarded as a life-saving drug in Friedreich Ataxia as it improves cardiomyopathy and, at higher dosages, it reduces some neurological symptoms (Meier and Buyse, 2009). When administered orally, IDE undergoes a pronounced first pass metabolism and it is rapidly converted into inactive metabolites (Bodmer et al., 2009; Kutz et al., 2009). Consequently, IDE plasma and brain levels may not be sufficient to exert pharmacological effects (Schwarz and Weisspapir, 2010).

A number of research efforts have been made to overcome the drawbacks that limit the pharmaceutical use of IDE such as its photo-instability and its low bioavailability. Several works reported the feasibility of delivering IDE or its natural parent coenzyme Q_{10} using colloidal carriers, such as liposomes (Gokce et al., 2012; Xia et al., 2009), microspheres (Ozawa et al., 1986), nanoparticles (de Amorim et al., 2010; Li and Ge, 2012; Montenegro et al., 2012a; Swarnakar et al., 2011; Yue et al., 2010), nanoemulsions (Belhaj et al., 2012) and self-emulsifying systems (Onoue et al., 2012). Thanks to their nanoparticle size and lipophilicity, the solid lipid nanoparticles (SLN) are colloidal carriers that could improve drug plasma half-life and could pass blood-brain barrier (BBB), thus enhancing drug concentration and efficacy in brain diseases (Kaur et al., 2008; Montenegro et al., 2011a).

In a previous work, by Montenegro et al., 2012b, we reported SLN loaded with IDE that were able to improve the drug uptake by a biological membrane model. In the attempt to elucidate the mechanisms involved in the improvement of IDE uptake, in this study, we have investigated SLN prepared with different primary surfactants and loaded with different amounts of IDE. The primary

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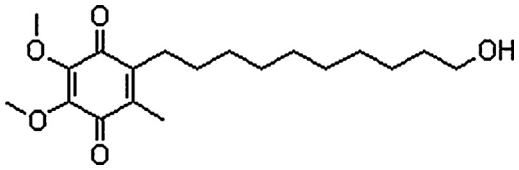

Fig. 1. Chemical structure of idebenone.

non-ionic surfactants used in this study differed for the structure of their acyl chain (isoceteth-20: branched acyl chain; ceteth-20: linear acyl chain; oleth-20: unsaturated acyl chain) while maintaining the same hydrophilic poly-ethoxylated group. These SLNs were studied by differential scanning calorimetry (DSC) to assess the influence of the surfactants and of IDE loading on both the nanoparticle thermotropic properties and on the arrangement of IDE molecules into the nanoparticles. The DSC is usually used in the SLN characterization to evaluate lipid modifications (Müller et al., 2000; Ruktanonchai et al., 2008). During cooling, lipids can crystallize in different polymorphic forms, called α (unstable), β′ (metastable) and β (stable). In thermodynamically unstable configurations, lipid molecules have higher mobility and higher capability to incorporate drugs. Stabilizers such as surfactants may affect the crystallinity and the polymorphic transitions of lipids in nanoparticles. Therefore, the study of these effects may allow a better understanding of the lipid/surfactant interactions (Kovacevic et al., 2011). In addition, the stabilizers may affect drug distribution within the lipid nanoparticles that plays a crucial role for drug targeting and release pattern. Drug attachment to the SLN surface could provide targeting to a specific biological structure, and a rapid release; instead, when the drug is in the SLN core, a prolonged release might be more likely.

2. Materials and methods

2.1. Materials

Polyoxyethylene-20-cetyl ether (Brij 58®, ceteth-20) was supplied by Fluka (Milan, Italy). Polyoxyethylene-20-isohexadecyl ether (Arlasolve 200 L®, isoceteth-20) was a kind gift from Bregaglio (Milan, Italy). Polyoxyethylene-20-oleyl ether (Brij 98®, oleth-20), was bought from Sigma–Aldrich (Milan, Italy). Glyceryl oleate (Tegin O®, GO) was obtained from Th. Goldschmidt Ag (Milan, Italy). Cetyl palmitate (Cutina CP®, CP) was a kind gift from Basf (Dusseldorf, Germany). Idebenone (IDE) was a kind gift from Wyeth Lederle (Catania, Italy). Methylchloroisothiazolinone and methylisothiazolinone (Kathon CG®), and imidazolidinyl urea were kindly supplied by Sinerga (Milan, Italy).

Table 1
Composition (% w/w) of unloaded and IDE-loaded SLN prepared by the phase inversion temperature (PIT) method.

SLN	Ceteth	Isoceteth	Oleth	GO	CP	IDE	Water[a]
A	–	10.6	–	3.5	7.0	–	q b 100
A1	–	10.6	–	3.5	7.0	0.5	q b 100
A2	–	10.6	–	3.5	7.0	0.7	q b 100
B	8.7	–	–	4.4	7.0	–	q b 100
B1	8.7	–	–	4.4	7.0	0.5	q b 100
B2	8.7	–	–	4.4	7.0	0.7	q b 100
B3	8.7	–	–	4.4	7.0	1.1	q b 100
C	–	–	8.7	4.4	7.0	–	q b 100
C1	–	–	8.7	4.4	7.0	0.5	q b 100
C2	–	–	8.7	4.4	7.0	0.7	q b 100
C3	–	–	8.7	4.4	7.0	1.1	q b 100

[a] Water containing 0.35% w/w imidazolidinyl urea and 0.05% w/w Kathon CG.

Table 2
Characterization of unloaded and IDE-loaded SLN: phase inversion temperature values (PIT), particle size (size ± S.D.), and polydispersity index ± S.D. (PDI ± S.D.) 24 h after their preparation.

SLN	PIT (°C)	Size ± S.D. (nm)	PDI ± S.D.
A	80	40.5 ± 0.6	0.310 ± 0.012
A1	80	42.5 ± 0.6	0.291 ± 0.011
A2	80	45.4 ± 2.0	0.233 ± 0.027
B	80	50.8 ± 4.5	0.331 ± 0.022
B1	80	48.7 ± 0.9	0.323 ± 0.019
B2	80	45.3 ± 1.1	0.289 ± 0.084
B3	81	29.9 ± 0.2	0.156 ± 0.017
C	84	37.1 ± 0.8	0.231 ± 0.015
C1	85	34.8 ± 0.1	0.161 ± 0.020
C2	84	36.1 ± 0.3	0.177 ± 0.123
C3	84	33.3 ± 0.1	0.140 ± 0.013

2.2. Preparation of SLN

The composition of unloaded and IDE-loaded SLN is reported in Table 1. All the SLN were prepared using the phase inversion temperature (PIT) method, as previously described (Montenegro et al., 2011a). Briefly, the aqueous phase and the oil phase (cetyl palmitate, the selected emulsifiers and different percentages w/w of IDE for IDE-loaded SLN) were separately heated at ~90 °C; then the aqueous phase was added drop by drop to the oil phase, under agitation. At the phase inversion temperature, the turbid mixture turned clear. The resulting mixture was cooled to room temperature under slow and continuous stirring. The PIT values were determined using a conductivity meter mod. 525 (Crison, Modena, Italy) which measured an electric conductivity change at the phase inversion from a W/O to an O/W system. Distilled water contained 0.35% w/w imidazolidinyl urea and 0.05% w/w methylchloroisothiazolinone and methylisothiazolinone as preservatives. No degradation of IDE occurred under these conditions, as confirmed by the TLC analyses.

2.3. Transmission electron microscopy (TEM)

Negative-staining electron microscopy was performed by placing 5 μl of SLN dispersions on a 200-mesh formvar copper grid (TAAB Laboratories Equipment, Berks, UK), and allowing them to be adsorbed. Then the surplus was removed by filter paper and a drop of 2% (w/v) aqueous solution of uranyl acetate was added over 2 min. After the removal of the surplus, the sample was dried at room temperature before imaging the SLN with a transmission electron microscope (model JEM 2010, Jeol, Peabody, MA, USA) operating at an acceleration voltage of 200 kV.

2.4. Photon correlation spectroscopy (PCS)

The SLN particle sizes were determined at room temperature using a Zetasizer Nano ZS 90 (Malvern Instruments, Malvern, UK), by scattering light at 90°. The instrument performed particle sizing by means of a 4 mW laser diode operating at 670 nm. The values of the mean diameter and polydispersity index were the averages of results obtained for three replicates of two separate preparations.

2.5. Stability tests

The samples of SLN were stored in airtight jars, and then kept in the dark at room temperature and at 37 °C for two months, separately. Particle size and polydispersity index of the samples were measured at fixed time intervals (24 h, one week, two weeks, three weeks, one month, and two months) after their preparation.