Technological and nutritional aspects of solid lipid nanoparticles added to o/w emulsions

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ABSTRACT
Nanoscaled carrier systems have been stated in the literature to be potentially useful in food systems as delivery systems for bioactive compounds. However, in the present paper, it will be shown that their use in food can cover two areas, i.e. technological and nutritional aspects. When used for technological reasons they may serve as encapsulants e.g. for aroma compounds, preservatives, colours or antioxidants. Technological aspects include interactions with the food matrix and the stability of the particles as well as the encapsulated compound during processing and storage.
The aim of the present study was to develop and characterise food grade solid lipid nanoparticles (SLN) that can be used to enhance the technological and the nutritional quality of a model food system. The technological aspects cover the physical and oxidative stability of an o/w emulsion, while nutritional aspects include the stability of the particles in the GIT.
The formulation and processing parameters applied allowed the preparation of SLN in the size range of 120 nm. The particle size increased only marginally upon the encapsulation of up to 5 % α-tocopherol relative to the lipid phase.
The encapsulated tocopherol could efficiently inhibit lipid oxidation in oil-in-water (o/w) emulsions. The particles were stable under simulated gastric and intestinal conditions. A food grade formulation was developed that can be used in food either for technological reasons or to enhance the nutritional value of the product.

Keywords: Solid lipid nanoparticles; α-tocopherol; Antioxidant; Nanoencapsulation

INTRODUCTION

SLN have gained increasing interest during the last decade. While their potential as delivery systems for active compounds was primarily of interest for pharmaceutical applications they are now also under investigation for nutritional benefits and / or technological applications. The solid lipid matrix is regarded to represent an effective physical barrier resulting in increased chemical stability of encapsulated compounds [1,2]. Basically, SLN can be regarded as o/w emulsions consisting of a solid lipid phase with droplets in the range of 100 nm. It has been demonstrated that the combination of the lipid phase and the emulsifiers used is of major importance for both the stability of the SLN during storage and digestion and for the stability of encapsulated compounds [3]. Formulations yielding stable SLN have been widely reported in the literature. However, since most studies have been carried out with a pharmaceutical background, they often include the use of non-food grade compounds.
The aim of the present study was to develop a formulation for the preparation of food grade SLN that can serve as carrier systems for a bioactive compound. The potential usefulness of SLN in food should be evaluated from a technological and a nutritional point of view. The quality of lipid containing food is largely dependent on the progress of lipid oxidation during storage and processing. Therefore great effort is put into the development of strategies to inhibit lipid oxidation e.g. by the use of antioxidants. In o/w emulsions the activity of antioxidants is determined by properties of the o/w interface and by interactions between antioxidants and emulsifiers. The scope of the first part of this study was to investigate how the antioxidant activity of α-tocopherol in o/w emulsions is influenced by its encapsulation in SLN. The second part focussed on the stability of SLN during simulated digestion. If SLN are added to food systems their fate after oral intake has to be known. It is of particular interest if the SLN are biodegradable under gastric and intestinal conditions, i.e. whether they may interact with or can be absorbed through the intestinal wall.

MATERIALS & METHODS

SLN were prepared by ultrasound assisted hot emulsification. An aqueous solution of S1670 (Harke, Muelheim, D) was added to a melt of glycerin tristearate (Sigma-Aldrich, Schnelldorf, D) and lecithin (Epikuron 100, Cargill, Hamburg, D). The mixture was emulsified by ultrasonic homogenization. The emulsion was added to an equal amount of an aqueous 8 % Tween 20 solution and quickly cooled to 20 °C in ice water. The final composition of the SLN suspension was: 5 % tristearin, 1.35 % S1670, 0.25 % Epikuron 100 and 4 % Tween 20.

Tocopherol loaded SLN were prepared by dissolving the appropriate amount of α-tocopherol in the hot emulsion before the addition of the Tween 20 solution and the cooling step. The encapsulation efficiency was determined by ultrafiltration using centrifugal ultrafiltration devices (Amicon 5 kDa and Amicon 300 kDa, Millipore, Schwalbach, D). The filtrate was directly analysed by rp-HPLC equipped with a DAD.

One portion of the loaded SLN was dialysed through 10 kDa dialysis membranes against 0.2 M sodium acetate buffer (pH 5.0) to remove the co-existent micellar phase. The absence of micellar bound α-tocopherol was confirmed by ultrafiltration followed by HPLC analysis of the filtrate.

Particle size distributions were determined by static light scattering (Mastersizer 2000, Malvern Instruments Ltd., UK). Differential scanning calorimetry (DSC) measurements were carried out on a TA Instruments 2920 DSC.

The antioxidant activity of encapsulated α-tocopherol was determined by adding α-tocopherol either via an ethanolic stock solution, via loaded SLN or via loaded and dialysed SLN to o/w emulsions consisting of 10 % w/w stripped rapeseed oil, 1 % w/w Tween 20 and 89 % w/w sodium acetate buffer (0.2 M, pH 5.0) to yield tocopherol concentrations of 1000 µmol/kg relative to the oil phase. The samples were kept in gas tight flasks and were stored at 25 °C in the dark. Hydroperoxide concentrations were measured over time by the ferric thiocyanate method [4].

Simulated digestion was carried out by applying gastric conditions for 1 h and intestinal conditions for 5 h at 37 °C. Gastric conditions were simulated with gastric electrolyte solution (pH 2) containing 100 mg pepsin / 100 ml electrolyte. Intestinal conditions were simulated using intestinal electrolyte containing 5 % pancreatic enzyme and bile salts (20 g/100 g fat). The pH was manually adjusted to 8.0 ± 0.2 by the addition of 1M HCl or 9 % $NaHCO_3$ solution, respectively.

RESULTS & DISCUSSION

It was possible to load the SLN with up to 5 % of tocopherol by replacing the respective amount of the lipid phase without significantly altering the particle size. Ultrafiltration results reveal that 95-98 % of the α-tocopherol is located in the SLN. The remaining fraction is solubilised by the coexistent micellar phase, which is formed by excess emulsifier present in the continuous phase and is known for its high solubilisation capacity [5]. In the aqueous phase, no α-tocopherol could be detected, which can be explained by the low water solubility of α-tocopherol. These results do not allow to draw any conclusions about the location of the α-tocopherol in the SLN. The following scenarios have been suggested: The location at the surface of the particles, the presence as an oily core in the particles or an even distribution within the particle interior [6].

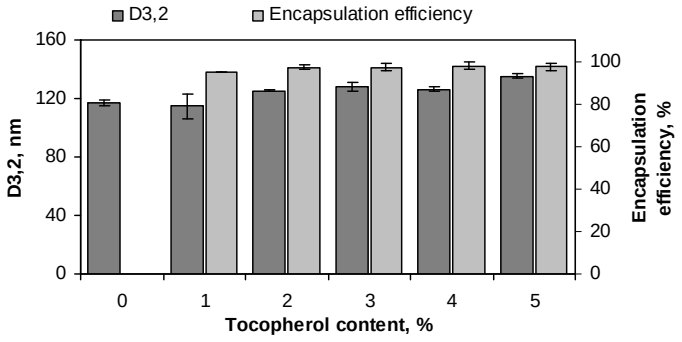

Figure 1. Sauter mean diameters of SLN with different amounts of α-Tocopherol determined by static light scattering. Tocopherol content is given as percentage relative to the fat content

For further investigations, it was necessary to determine the melting point of the lipid phase to ensure that the SLN are in the solid state throughout the experiments. The melting point of bulk tristearin is 68 °C. However, a typical property of SLN is the reduced melting point of the lipid phase compared to the bulk material. DSC measurements revealed that melting of the loaded and unloaded SLN starts at about 40 °C. It was thus confirmed that the particles remained in the solid state during the lipid oxidation experiments at 25 °C and the digestion experiments at 37 °C.

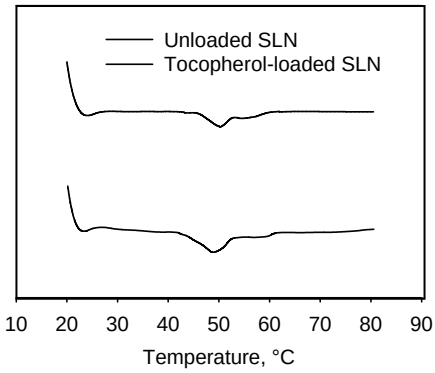

Figure 2. DSC thermographs of unloaded and loaded SLN

The impact of loaded and unloaded SLN on lipid oxidation was determined in storage experiments. Lipid hydroperoxide formation was reduced by the presence of unloaded SLN (control+SLN). A possible reason may be the increased total emulsifier concentration and thus the presence of micelles. It has been discussed that non-ionic micelles solubilise radicals and/or lipid hydroperoxides from the o/w interface thereby increasing the oxidative stability of the system [7].
The antioxidant activity of SLN encapsulated α-tocopherol in o/w emulsions was determined in comparison to free α-tocopherol. To exclude the possible impact of the micellar bound α-tocopherol on the total antioxidant activity, one set of samples contained an SLN dispersion that was dialysed to remove the co-existent micellar phase.

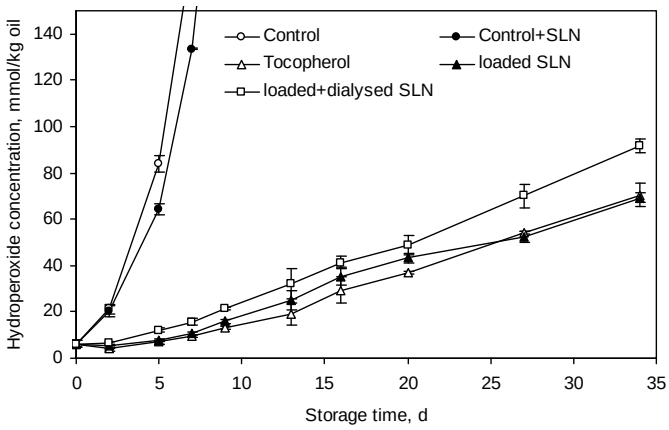

Figure 3. Progress of hydroperoxide formation in o/w emulsions

Lipid oxidation was effectively inhibited by α-tocopherol compared to the control samples. Except at day 20 the formation of hydroperoxides was similar in samples containing encapsulated or free α-tocopherol. The dialysed SLN suspension was slightly less efficient than the SLN suspension containing a micellar bound fraction of α-tocopherol. This may be attributable to two possible effects. The presence of micelles, as indicated above, could to a certain extent inhibit the formation of lipid hydroperoxides. In addition, the availability of the α-tocopherol at the o/w interface may be increased by its solubilisation into emulsifier

micelles. The o/w interface is the main reaction site for α-tocopherol with respect to the inhibition of hydroperoxide formation [8,9]. Its high activity even when it was encapsulated in SLN indicates that some transfer from SLN to the oil droplet surface occurs. Whether a release and transfer mechanism for α-tocopherol from the SLN to the interface exists or whether α-tocopherol is located at the surface of the SLN which may be in close contact to the oil droplets cannot be deduced from these results. However, the partitioning of α-tocopherol in emulsions containing tocopherol loaded SLN was determined by ultrafiltration. Since no tocopherol was detectable in the micellar pseudophase, the direct contact of SLN with oil droplets seems to be more likely. The underlying mechanisms are currently under investigation.

SLN were digested in simulated gastric and intestinal media. The particles size distribution was determined after gastric digestion for one hour and subsequent intestinal digestion for five hours. SLN were detectable in the reaction media without any prior sample preparation except dilution. The particle size distributions of the initial sample and at the end of the experiment were practically identical and contain a significant peak in the 100 nm range. These results demonstrate that the SLN were not completely digested under these conditions. Earlier studies showed that the stability at different pH values and the digestibility are largely dependent on the combination of the lipid phase and the emulsifiers used. These studies indicate that high-melting ("solid") emulsifiers increase the stability of SLN towards electrolytes and digestive enzymes, which is in accordance to the results presented here [10] .
Based on these results it has to be assumed that a significant number of SLN would reach the intestine after oral intake. Thus, interactions with the intestinal wall and the possible absorption need further investigation. The low digestibility of SLN may be useful for the encapsulation of acid labile bioactive compounds and compounds which are sensitive to pepsin. It is likely that the compound will withstand the gastric conditions and reach the intestine.

CONCLUSION

Stable SLN in the size range of 100 nm could be prepared with exclusively food grade ingredients by using a hot homogenisation technique. The encapsulation efficiency reached > 95 % when up to 5 % of the lipid phase was replaced by α-tocopherol. Encapsulated α-tocopherol was an effective antioxidant in o/w emulsions comparable to the same concentration of non-encapsulated α-tocopherol. A significant fraction of the SLN remained stable after digestion under simulated gastric and intestinal conditions. Therefore, the SLN may be suitable as delivery systems for bioactive compounds. However, the stability of the SLN in the GIT requires further investigations regarding their absorption through the intestinal wall.

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