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Omega-3 fatty acids-loaded lipid nanoparticles for patient-convenient oral bioavailability enhancement

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Omega-3 fatty acids are commonly used as food supplements not only for their positive effects on the blood lipid profile but also for their cardioprotective properties. The majority of the commercially available products is made out of fish oil. Apart from the unpleasant side effects, up to 10 capsules per day have to be taken by the patients. This article describes the development and characterisation of an alternative lipid nanoparticle delivery system, which has the potential to reduce side effects and enhance bioavailability.

1. Introduction

The nutritional status of cancer patients is important; maintaining them in a good nutritional condition will support their battle against the disease (Chapkin et al. 2007; Dupertuis et al. 2007). Therefore various nutrition supplements for cancer patients are on the market, e.g. NT24 [Orcapharm, Pensberg/Germany]. The additional oral nutrition supply improves the health condition and prolongs the time until the necessity of parenteral nutrition.

Omega-3 fatty acids are important for the body because of various aspects:

- Synthesis by the body of omega-3 fatty acids eicosapentaenic acid (EPA) and docosahexanic acid (DHA) from the essential alpha-linoleic acid is very limited. Nevertheless, EPA and DHA are considered the most valuable omega-3 fatty acids: providing them with the nutrition is recommended (Harris, 2004; Hansen & Harris, 2007).
- Omega-3 fatty acids are known to be cardioprotective (Lemaitre et al. 2003; Hansen and Harris 2007) and have a positive effect both on the blood lipid profile (Anil 2007) and on inflammatory processes (Kelley et al. 1999; Belluzzi 2004).

There are a number of products on the market for additional supply with omega-3 fatty acids by oral administration. In general these products are soft gelatine capsules filled with fish oil. Most of the commercial products contain 500 mg of fish oil per capsule with an omega-3 fatty acid content of approximately 20–25% [Lipiscor®, Sanum-Kehlbeck, Hoya, Germany], [Ameu®, Lichtwer Pharma, Germany], [Eicosan®, Stada, Germany] (Lichtwer Pharma GmbH 2007). Disadvantage of this dosage form is that the patients actually need to swallow 3–6 capsules. Such a high number of capsules is necessary to meet the required dose considering the oral bioavailability of the omega-3 fatty acids. Cansell et al. have found a 72 ± 6% oral bioavailability in a rat model, which was improved to 98% using a very diluted liposome suspension (Cansell et al. 2003). Increasing the oral bioavailability without enlarging the volume of the dosage form decreases the total dose to be administered, which is patient-friendly.

In special conditions, e.g. for the treatment of high triglyceride blood levels, the dose is even higher; in this case 10–20 capsules per day (which is equivalent to 1200–2400 mg omega-3 fatty acids) may have to be administered. At a certain stage, especially cancer patients develop the problem of swallowing capsules and tablets. Therefore a liquid or semi-solid dosage form will be definitely more appropriate for such patients. In addition, such a dosage form would be very convenient for elderly patients.

The soft gelatine capsules disintegrate in the stomach releasing the fish oil. Reported undesired side effects are a fishy odour in the breath and undesired regurgitation leaving an unpleasant fishy taste in the mouth (Lichtwer Pharma GmbH, 2007). Enteric-coated fish oil capsules have also been developed for studies but are not commercially available (Belluzzi et al. 1996).

Based on these considerations, a novel semi-solid, pastelike formulation was developed. It consists of a highly concentrated lipid nanoparticle dispersion (70% w/w). This paste can be administered via a teaspoon and swallowed directly; alternatively the paste can be dispersed in soft drinks or water, avoiding swallowing problems with solid dosage forms. The omega-3 fatty acids were incorporated in particles with a solid matrix, nanostructured lipid carriers (NLC®) (Müller et al. 2000). The solid matrix has a taste masking effect minimizing odour and taste problems. This report describes the development and characterization of this semi-solid oral formulation.

2. Investigations, results and discussion

2.1. Rationale of development

The price of omega-3 fatty acids as a pure substance is fairly high, for example 340 Euro for 1 gram of purified docosahexaenoic acid. Therefore fish oils with a high percentage of unsaturated omega-3 fatty acids and plants oils (like flax seed oil) are used as nutrition supplements. The fish oil used for preparation of the nanostructured lipid carriers (NLC®) had a relatively high content of 38% omega-3 fatty acids (Schmitt). For chemical stabilisation, inclusion of chemically labile compounds in a solid matrix can be protective. Therefore creation of a solid particle matrix in form of NLC® was chosen to stabilise the fatty acids. In addition, a solid matrix can mask unpleasant taste and smell to a certain extent. Further reduction of the unpleasant smell was achieved by the solid-in-water dispersion system since the aqueous phase may additionally act as a barrier with the external environment. The fish oil-loaded NLC® are surrounded by a water phase, which has a very low solubility for the lipophilic compounds, *thus* further reduces evaporation. This is an old principal used in many pharmacopoeiae for taste masking, e.g. by producing oleum jecoris emulsions.

NLC® are prepared by mixing a solid lipid with a liquid lipid (oil). In this case the oil compound is the fish oil. Admixing of the oil reduces the melting point of the solid lipid. To minimize unpleasant side effects from the stomach, the NLC® should be solid at body temperature to slow down the degradation, that means degradation should mainly take place in the gut. Therefore a number of lipids was screened. The mixture should still melt above 40 °C but nevertheless contain the highest possible percentage of fish oil. This should minimise the total amount of NLC® to be administered in a single dose. It was found that a lipid blend containing 20% Dynasan 118 and 80% fish oil was still solid at 40 °C.

To promote absorption, the NLC® paste should be dispersible easily to yield a fine NLC® suspension taking into account that aggregation reduces the bioavailability. The gut contains many electrolytes able to destabilise dispersions due to zeta potential reduction. Therefore a combination of electrostatic and steric stabilisation was chosen to minimize aggregation. Sodium dodecyl sulfate (SDS) is well known as an efficient dispersing agent; in addition it creates highly negative zeta potentials after adsorption to the surface (Lucks et al. 1990). As steric stabilisers TPGS and PVP were also selected. Both SDS and TPGS are known absorption enhancers. TPGS was used primarily as steric stabilizer. It is also known as absorption enhancer mainly due to inhibition of P-glycoprotein (Rege et al. 2002). The SDS was used in the first formulation attempts because it is an efficient surfactant for dispersing oils. In addition it was a challenge to produce a 70% concentrated NLC® suspension, which is fine in size and not too viscous. Generally, the SDS concentration in pharmaceutical preparations is up to 2%, whereas 3% were used in this formulation approach. However, we have also shown that is was possible to replace SDS by Poloxamer 188, which can be used also at higher concentrations (data not shown).

The solid matrix of the NLC® is chemically protective but additionally Vitamin E can be added as anti-oxidant. It is also frequently used in commercial fish oil soft gelatine capsules (e.g. Ameu®).

2.2. Particle size and charge

PCS covers a size range of approximately 3 nm – 3 µm, thus it was employed to determine the size of the bulk population. The PCS diameter was 243 nm and the polydisperity index 0.064. Polydispersity indices around 0.1 indicate a relatively narrow particle size distribution. The NLC® with 243 nm represent a highly dispersed ultra fine system compared to orally administered oils. Once administered, an oil is emulsified by the surfactants present in the gut and dispersed by gut movement resulting in oil droplets typically in the size of 1–50 µm (Patton and Carey 1979; Armand et al. 1996). The degree of dispersity affects bioavailability as known from the first cyclosporin A product Sandimmu (Meinzer et al. 1998). With decreasing droplet/particle size, the absorption increases.

Laser diffractometry (LD) was performed to assess the potential presence of particles in the low micrometer range (measuring range of LD: 0.04–2000 µm). The LD yields a volume distribution, thus is very sensitive to detect even low amounts of particles larger than the bulk population. The 50% diameter was 0.313 µm, 90% diameter 0.950 µm and 99% diameter 1.240 µm. This indicates a product with particles almost completely in the nanometer range with a low amount of micrometer particles. Figure 1 shows the LD size distribution curve.

The zeta potential measurement is a tool to foresee the physical stability of colloidal suspensions. Stability is

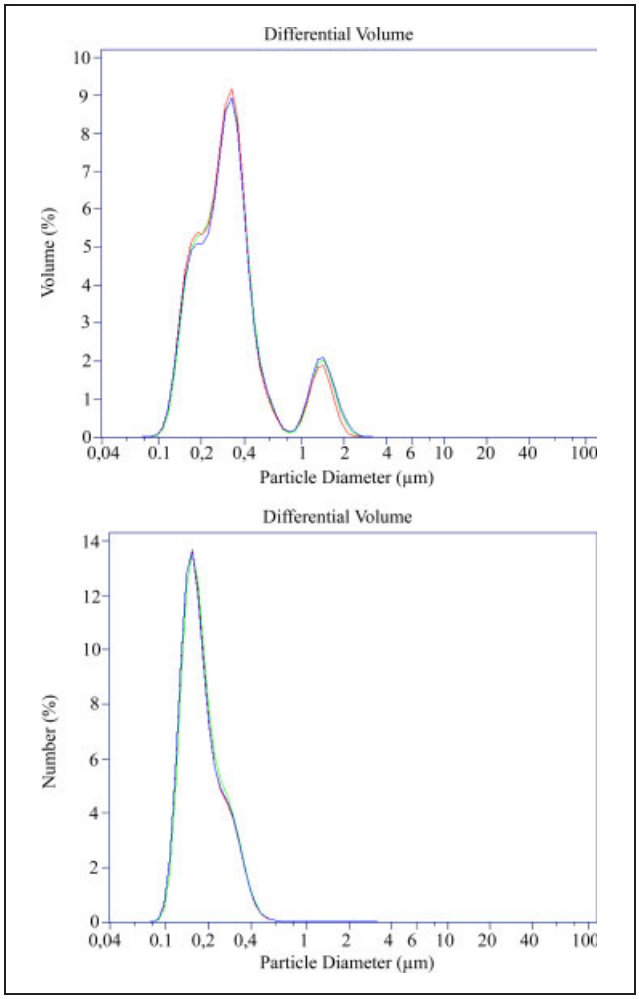

Fig. 1: LD size analysis of fish oil-loaded NLC® paste: Volume distribution curve (upper) and transfer to the number distribution curve (lower)

higher in case of high electrostatic repulsion. The measurements in ultrapure water (conductivity adjusted to 50 µs/cm) yielded a value of −20 mV. This is a characteristic value for combined electrostatic (SDS) and steric stabilisation (TPGS, PVP). In addition, zeta potential measurements were performed in the original dispersion medium (water with 3.0% SDS, 1.0% TPGS and 0.1% PVP). Measurement in the original dispersion medium provides information about the stability in the original dispersion. The high electrolyte concentration leads to compression of the diffuse double layer and subsequently to a reduction in the measured zeta potential. The zeta potential of −15 mV is relatively low but the rather high viscosity of the system additionally stabilises the dispersion. Furthermore, the high fraction of inner phase (70%) promotes the formation of a pearl-like particle network also stabilising the system. This is well described for highly concentrated NLC® dispersions. Considering the SDS adsorption onto the NLC® surface (electrostatic stabilisation), the additional steric barrier by TPGS and PVP in combination with the pearl network structure, the NLC® pastes are predicted to be physically stable.

2.3. Crystalline status

The NLC® paste was analysed by differential scanning calorimetry (DSC) and Fig. 2 shows the DSC graph. Compared to lipid Dynasan 118 bulk material, the peak onset decreased from 72.7 °C to 55.1 °C (Fig. 3).

Dynasan 118 as a triacylglycerol can crystallise in three different modifications: α, β′ and β (most thermodynamically stable). Running a DSC with two cycles reveals the three modifications. The heating curve of the first cycle shows the β modification with a peak maximum at 72.7 °C whereas cooling leads to cristallisation into the α modification (51.7 °C). Furthermore, the second cycle shows the β′ modification with its melting point of 62.9 °C.

The melting enthalpy of the lipid blend in NLC® (fish-oil + Dynasan 118) was 41.47 J/g, compared to 201.04 J/g of the bulk lipid. Taking into account that only 20% of the NLC® consists of Dynasan, the melting enthalpy increased to 207.35 J/g, which means that all the Dynasan in the particles is of crystalline status.

Cooling of the melted lipid dispersion lead to a delay in recrystallisation with a temperature shift from 61.1 °C to 41.4 °C (peak maximum). In addition, the melting enthalpy was distinctly reduced (6.26 J/g) indicating a delayed solidification process and increased α modification of the Dynasan.

The recorded x-ray diffraction spectrum of the bulk material Dynasan 118 (Fig. 4) shows the three expected peaks

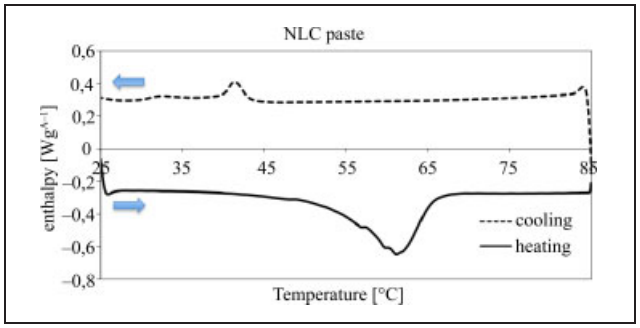

Fig. 2: DSC graph of the highly viscous 70% lipid nanoparticle dispersion (=NLC® paste), heating from 25 °C to 75 °C and subsequent cooling to 25 °C

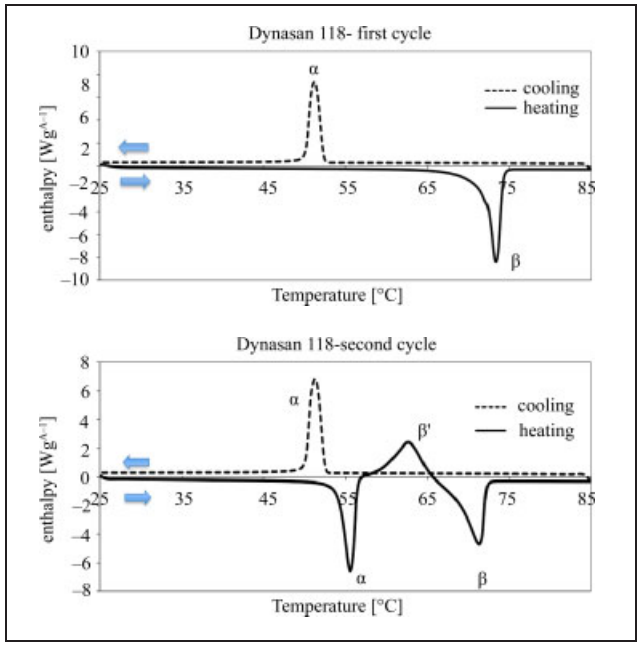

Fig. 3: Two-cycle DSC graph of Dynasan 118 bulk material revealing the three different crystalline modifications of the lipid

for triacylglycerols representing the three modifications of the lipid: 16.4 (2θ) for the α modification, 19.3 (2θ) for β and 23.1 (2θ) and 24.1 (2θ) for the β′ modification (Hagemann 1988).

The fish oil spectrum shows no significant peaks, which is also expected for a liquid.

The x-ray diffraction pattern of the paste exhibit mainly the peaks of the β′ and β modifications of Dynasan 118. The peaks indicate the solid, crystalline character of the particles (Fig. 5).

2.4. Re-dispersion properties

From the concept the fish oil NLC® paste can be dosed via a spoon and swallowed directly. For this, the paste

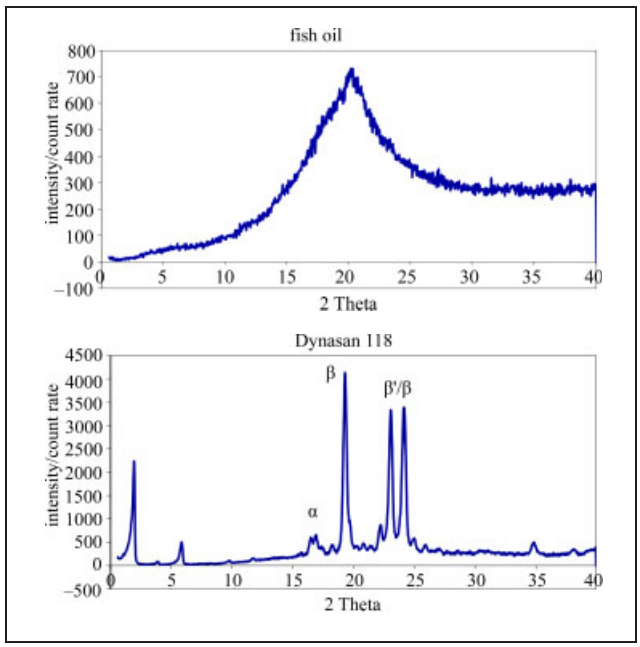

Fig. 4: X-ray diffraction pattern of the bulk materials fish oil and Dynasan 118 (explanations in the text)

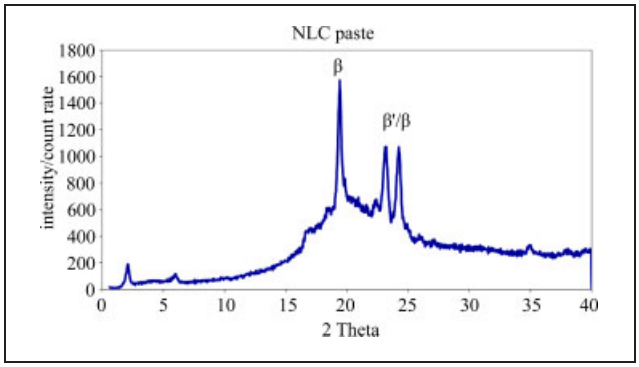

Fig. 5: X-ray diffraction pattern of the NLC® paste with the β′ and β modification peaks of Dynasan 118

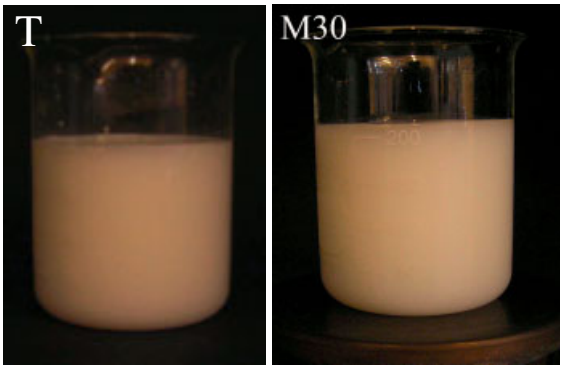

Fig. 6: Appearance after 10 seconds of stirring with a teaspoon (T) and after magnetic stirring at 50 rpm for 30 s (M30) following administration of 5 cm NLC® paste (squeezed out of a tube) in 200 ml water

requires appropriate flavouring. Alternatively the non-flavoured paste can be dispersed for example in soft drinks. In both cases the paste should re-disperse easily, either in the gut fluids or in water. A 500 mg capsule of fish oil (Ameu®) contains approximately 125 mg of omega-3 fatty acids. Considering the higher omega-3 fatty acid content in the fish oil used for preparation of the NLC® paste, despite addition of Dynasan 118 and water, 500 mg paste will contain approximately 110 mg omega-3 fatty acids. That means for a dose of approximately 500 mg omega-3 fatty acids a total of about 2.5g NLC® paste should be taken (equals 3–4 capsules of the commercial product).

The two grams of paste represent approximately one level teaspoon or alternatively an approximately 5 cm long strand squeezed out of a standard tube. To test the redispersibility, 5 cm paste were given into 200 ml of water (approximately equivalent to a typical volume of a soft drink). The paste was either dispersed by manual stirring with a spoon (Fig. 6 left) or alternatively with a slow moving magnetic stirrer to imitate gut forces (Fig. 6 right).

The formulation is well dispersible by spoon stirring and shows also good redispersibility when applying weak forces. To admix the NLC® paste to soft drinks instead of using a spoon, a tiny eggbeater can be used which eases even further dispersion.

2.5. Absorption mechanism

It is well documented in the literature that the presence of lipids can promote the absorption of drugs (Charman et al. 1997; Porter and Charman, 2001). To obtain maximum effect, the drug or active needs to be closely associated with the lipid, that means preferentially the active

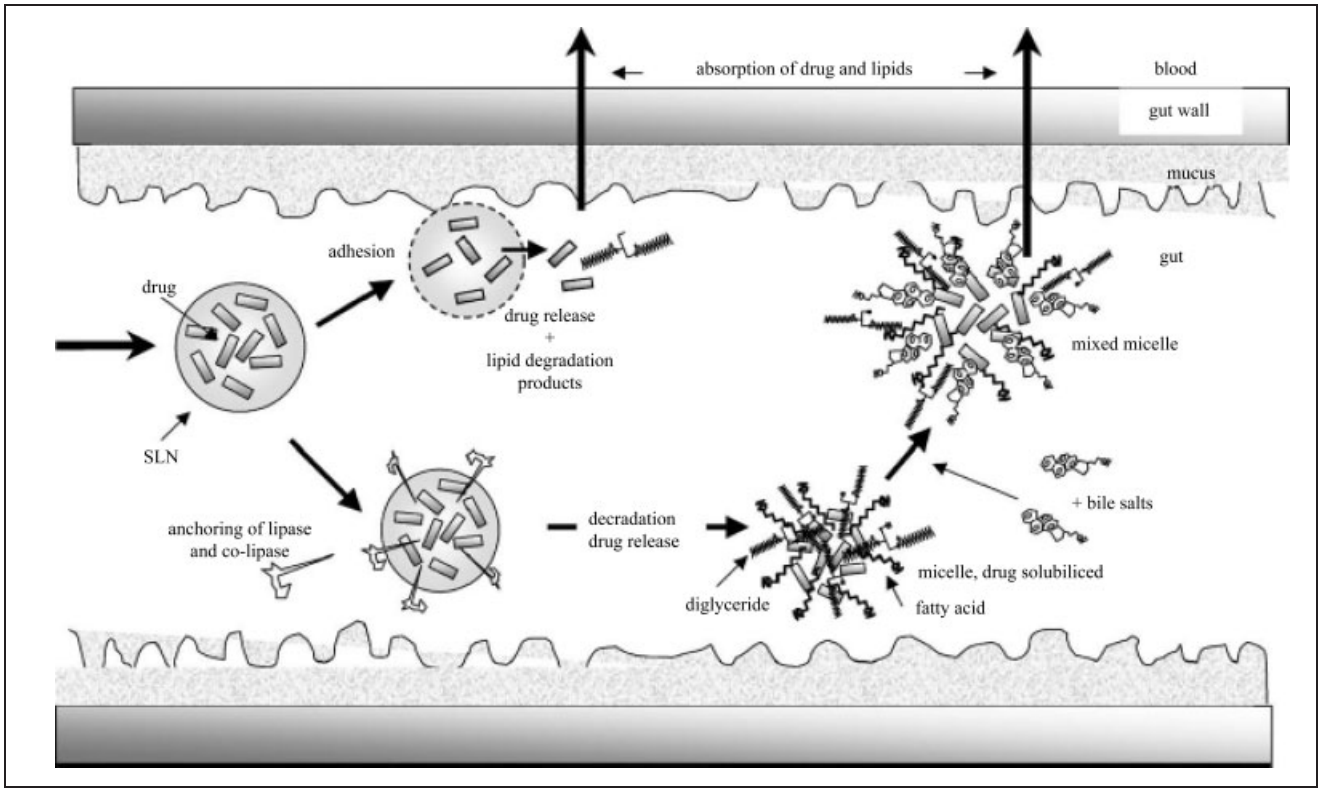

Fig. 7: Degradation of drug loaded lipid nanoparticles (SLN) in the gut and absorption mechanism by formation of micelles and subsequently mixed micelles with bile salts (reprinted from Journal of Biotechnology (113), Müller RH, Keck CM (2004), Challenges and solutions for the delivery of biotech drugs – a review of drug nanocrystal technology and lipid nanoparticles. J Biotechnol 113. 151–170, Copyright with permission from Elsevier)

to be absorbed should be incorporated or dissolved in the lipid.

It was also found that the length of the fatty acid chains has an effect on the absorption. Fatty acids with a chain length of C14 to C18 promote absorption by lymphatic uptake (Porter and Charman, 2001). In addition, it is beneficial if the lipid is presented as finely dispersed as possible. The variations in bioavailability of the first cyclosporin A product, Sandimmune®, were explained by individual variation in surface active bile salts: indeed, less bile salts, less effective dispersion of the oil, less bioavailability and *vice versa*. Based on these considerations, the omega-3 fatty acids were incorporated into the solid matrix (close association). The triglyceride chosen (Dynasan 118 = glyceryl tristearate) contains C18 fatty acids and the size of the particles was as small as possible, that means in the nanometre range. Fulfilling all these three requirements should provide an optimal formulation, whereas of course the bioavailability enhancement has to be investigated in *in vivo* studies, which are currently in preparation.

It could be shown that there is no major difference in the mechanism of degradation between oil droplets and solid lipid particles. The solid lipid particles are degraded by lipases, the degradation velocity is a function of the chemical composition of the particle matrix and the stabilisers used (Müller and Olbrich 1999; Olbrich et al. 2002).

Figure 7 shows a model of the degradation and the subsequent absorption promoting mechanism. The lipid nanoparticles enter the gut, lipase adsorbs onto the particle surface whereas the adsorption is promoted by the presence of co-lipase. Enzymatic degradation of the lipid particles leads to the formation of surface-active di- and monoglycerides forming micelles. In case of drug-loaded lipid nanoparticles, these micelles contain solubilised drug. In case of the fish oil-loaded NLC® they consist of glycerides with various fatty acids including the omega-3 fatty acids. In the next step mixed micelles will be formed with bile salts leading finally to the lipid absorption.

2.6. Flavouring and colouring

Dilution of the fish oil in the solid particle matrix and surrounding the particles by a poorly diffusible medium (water) for the lipophilic fish oil components could not completely eliminate the unpleasant smell: A slight fishy smell remained which could irritate sensitive noses. Therefore various flavours were tested for masking: Orange, lemon, strawberry and mango. The flavours were applied in different concentrations. The most efficient one was orange flavour in a concentration of 1.25%. The flavour was incorporated by adding it to the molten lipid blend just prior to homogenisation. Evaporation of the volatile flavour does not seem to be a problem because the processing time is relatively short. In addition, at large scale production, homogenization will take place in closed containers avoiding any relevant flavour loss.

A colour appropriate to the flavour was chosen for colouring the product: Food colour yellow ZLT3 in a concentration of 0.24% and 0.013% food colour red ZLT2.

2.7. Conclusions

The fish oil-loaded NLC® paste possesses an ultra fine particle size, which is favourable for absorption in the gut. It is easily dispersible in fluid media. The formulation represents an alternative for persons, especially cancer patients, who have problems swallowing solid dosage forms. Encapsulation in the particle matrix and formulation as O/W system minimized undesired odour and taste.

3. Experimental

3.1. Materials

Dynasan 118 (glyceryl tristearate) used as matrix lipid for the production of NLC® was obtained from Condea (Witten, Germany). Sodium dodecyl sulfate (SDS) and polyvinyl pyrrolidon were purchased from Fluka Chemie GmbH (Buchs, Switzerland) and from Merck (Darmstadt, Germany), respectively. Tween 80 was bought from Uniqema (Eversberg, Belgium), whereas TPGS (D-alpha tocopheryl polyethylene glycol 1000 succinate) was a kind gift from Eastman (Anglesey, U.K.). All excipients were used as received. The water used was produced by a MilliQ system (Millipore, Billerica, MA, United States). The fish oil was obtained as a gift from Pharmacol GmbH (Berlin, Germany). It contained 38% omega-3 fatty acids including 19% EPA (eicosapentaenoic acid) and 13% DHA (docosahexaenoic acid) according to its analysis certificate.

For taste masking, aroma concentrates (lemon, orange, mango and strawberry flavour) from Symrise (Holzminden, Germany) were used which were a kind gift of the company. For colouring, Sicovit food colours (BASF AG, Ludwigshafen, Germany) were used.

3.2. Methods

The NLC® were prepared by high pressure homogenisation. The solid lipid Dynasan 118 was molten at 70 °C and the fish oil was then added. Both lipids where mixed at 70 °C at a ratio of 20% Dynasan 118 and 80% fish oil. Then a stabilizer solution containing 3.0% (w/w) SDS, 0.1% polyvinylpyrrolidon (PVP), 0.1% Tween 80 and 1% vitamin E TPGS at equivalent temperature was added under stirring (30% stabiliser solution, 70% oil phase). Alternatively a SDS-free stabiliser mixture containing Poloxamer 188 was used. Stirring (9000 rpm) was performed for 30 s using an Ultra-Turrax with a T-25 head (IKA Janke und Kunkel, Stauffen, Germany.) The obtained pre-emulsion was then homogenized using a Micron LAB 40 (APV Deutschland GmbH, Unna, Germany) at 500 bar and one homogenization cycle at 70 °C. The LAB 40 was equipped with a temperature control jacket; the temperature controlling fluid (water) was heated to 85 °C.

For determination of the bulk population, photon correlation spectroscopy (PCS) was applied using a Malvern Zetasizer 4 (Malvern Instruments, Malvern, UK). PCS yields a mean diameter (z-average) and a polydispersity index (PI) as a measure for the width of the size distribution. A PI of 0 is theoretically indicating a monodisperse population, 0.10–0.20 indicates a relatively narrow distribution and values > 0.50 indicate a very broad distribution. For the detection of microparticles, a Coulter LS 230 laser diffractometry (LD) was applied (Beckmann-Coulter, Krefeld, Germany). LD analysis was performed applying the Mie-theory, the real refractive index was 1.456 and the imaginary refractive index 0.001. The values were previously assessed to be valid for lipid nanoparticle dispersions (unpublished data). There are slight variations in the indices depending on the nature of matrix lipid and stabilizer used (Keck, 2006) but for the envisaged development of an oral formulation (not an intravenous formulation) these effects can be neglected.

Zeta potential was also determined using the Zetasizer 4. The measurements were performed in two different media: MilliQ water (with a conductivity adjusted to 50 μS/cm by adding sodium chloride solution) and the original dispersion medium. The pH was in the range of 5.8–6.3 during all measurements. Applied field strength was 20 V/cm. The obtained electrophoretic mobility was converted to the zeta potential by applying the Helmholtz-Smoluchowski equation.

Differential scanning calorimetry (DSC) was performed using a Mettler Toledo DSC 821e (Mettler Toledo, Gießen, Germany). The heating rate was 5 K/min. The peaks were analysed using the provided "Star" software by Mettler Toledo.

X-ray analysis was used to determine the crystalline status of the lipid nanoparticles, in addition to the DSC measurements. Diffraction patterns were measured using a Philips X-ray generator PW 1830 equipped with a copper cathode ($\lambda = 1.5418$ Å, 40 kV, 20 mA) coupled to a computer-interfaced Philips PW 1710 diffractometer control unit. The scattered radiation was measured with a vertical goniometer (Philips PW 1820) (Philips Industrial & Electro-Acoustic Systems Division, Almelo, The Netherlands). The system is a powder diffractometer – it can only analyse particles in suspensions if the viscosity of the dispersion medium is sufficiently enhanced. For analysis of highly fluid suspensions a viscosity enhancer is added (e.g. xanthan gum). In the case of the paste this was not necessary because it had already a sufficiently high viscosity.

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