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RESEARCH ARTICLE

Physicochemical properties of lipid nanoparticles: Effect of lipid and surfactant composition

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Abstract

Understanding the effect of lipid and surfactant composition on particle size and colloidal stability plays a pivotal role in designing lipid nanoparticles (LN) for drug delivery. With respect to our long-term goal, LN for brain delivery, formulations containing lipids and surfactants suitable for intravenous (i.v.) application were selected for the current formulation screening study. LN were prepared by hot high pressure homogenization (HPH) and were characterized during 1 year in terms of macroscopic appearance, particle size by photon correlation spectroscopy (PCS) and optical single particle sizing (OSPS), zeta potential (ZP), as well as physical state and polymorphism by differential scanning calorimetry (DSC). The LN dispersions showed a wide variability in macroscopic appearance, mean size and colloidal stability. Influence factors were the type and concentration of both, the lipid and surfactant component used. The most promising LN showed a small mean size (< 200 nm), a low polydispersity index (PI), (< 0.25) absence of particles in the several-micron range, and a slightly negative ZP (> −12 mV); DSC revealed that some represented supercooled liquids; such LN may be stable at room temperature for at least 1 year. The obtained results are regarded helpful for defining the design space for LN delivery systems, i.e., identifying possible designs and design parameters within the given HPH technology to be applied during future formulation development studies.

Keywords: Cutina (CP); Dynasan 114 (D14) and 116 (D16); Precirol ATO5 (POL5); witepsol E85 (WE85); polysorbates; poloxamers; photon correlation spectroscopy (PCS); optical single particle sizing (OSPS); differential scanning calorimetry (DSC)

Introduction

Colloidal stability is a demanding requirement for a nanoparticulate system intended for intravenous (i.v.) delivery, because particles may aggregate over time leading to large particles, thus creating a potential safety concern[1]. Consequently, determination of particle size and particle size stability are regarded essential for the development of a new drug carrier system.

Lipid nanoparticles (LN) are colloidal drug delivery systems with a mean size in the range between 50–1000 nm[2], with a matrix composed of lipids, dispersed in an aqueous medium and stabilized by surfactants. LN combines the advantages of traditional colloidal carriers such as liposomes, polymeric nanoparticles and emulsions, but avoids or minimizes the drawbacks associated with these[3]. The use of lipids and/or excipients of physiological origin or accepted status is an exceptional advantage since it decreases the risk of acute and chronic toxicity[3]. Good tolerability depends firstly on the added surfactant and secondly on the lipid composition. In formulation of parenteral LN, surfactants that are generally recognized as safe (GRAS) by US Food and Drug Administration, should be preferred, e.g., polysorbates and poloxamers[4,5]. Moreover, LN are biodegradable and biocompatible, and able to incorporate both lipophilic and hydrophilic drugs in considerable amounts within the lipid matrix.

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The particle size of LN represents a key role of their *in vitro* behavior as well as of their distribution *in vivo* performance and success of site-specific drug delivery[6,7]. One outstanding example of LN targeting to specific organs is the brain delivery. The uptake of LN by the brain might be explained by adsorption of blood proteins such as apolipoproteins on particle surfaces mediating the adherence to endothelial cells of the blood–brain barrier (BBB)[8]. Different surfactants have been investigated for use in brain delivery. Generally, they should show very low toxicity to be used in i.v. formulations[1,4,5] and also some tendency to enhance the brain delivery of drugs encapsulated in nanoparticles[9–11]. Polysorbates and poloxamers may form a surface coating of nanoparticles and therefore increase BBB permeability properties of these systems[12]. Furthermore, promising surfactants suitable to deliver drugs to the brain may also inhibit the efflux function of P-glycoprotein, such as polysorbates and poloxamers[9].

LN are easily produced in large scale production by high pressure homogenization (HPH) which is used, e.g., for parenteral fat emulsions or liposomes[2,13,14]. LN size may be adjusted by varying process parameters such as homogenization pressure and cycle number, but, is expected to depend on formulation parameters such as surfactant and lipid concentration. LN colloidal stability is also expected to be related to the composition, i.e., choice of components and their chemical structure. As such, lipids and surfactants have to be carefully chosen to develop proper delivery systems preventing particle size changes during processing and storage.

For the present work, triglycerides with increasing length of the chain of the fatty acid (Dynasan 114 (D14) and Dynasan 116 (D16)) were chosen. In addition, Precirol ATO5 (POL5), a lipid composed of mono- (8–17%), di- (≈54%) and triglycerides (≈30%) of palmitostearic acid (C16–C18 fatty acid), with the diester fraction being predominant, and having a very different hydroxyl number, was also used. Witepsol E85 (WE85) was also included as a lipid with a high emulsifying capacity. An overview of the lipids used and their relevant properties is given in Table 1.

The main task of the surfactants is to stabilize LN in the colloidal state in order to avoid particle size growth during storage. Furthermore, the surfactants may have an influence on recrystallization and polymorphic transitions of the LN matrix[15–17]. The type of surfactant and its concentration may thus affect physical stability of the lipid matrix.

For the present study, nonionic and strongly hydrophilic surfactants with HLB values in the range 14.9 (polysorbates) to more than 24 (poloxamers) were selected. An overview of the surfactants used and their relevant properties is given in Table 2. With respect to brain delivery surfactants were preferred that are acknowledged pharmaceutical excipients, and known as sensitizers of drug-resistant cancers to anti-cancer drugs and/or transport-enhancers of drugs across the BBB[18–20].

The main tasks of LN with regard to parenteral administration are good physical stability, protection of incorporated drugs from degradation, controlled drug release, good tolerability and site-specific targeting. Good colloidal stability of LN may be reached by means of: (i) small particle sizes in conjunction with a narrow (monomodal) size distribution, and (ii) avoidance of particle aggregation and/or substantial particle growth during long-term storage. Shelf life of optimized LN should preferentially last for more than 1 year[4]. In fact, LN stable for more than 3 years have been reported[21].

LN intended for i.v. administration should preferentially have a submicron size and be sufficiently stable since any larger particle present in the circulation may result in lung embolism[22]. With a size below 1 µm, LN formulations can be used for systemic distribution with a minimized risk of blood clotting and aggregation leading to embolism. The exact size at which this phenomenon becomes important is extensively discussed and the Pharmacopoeia limits on particulate contaminants in

Table 1. Main properties of the lipids and wax used in the preparation of the lipid nanoparticles (LN) (manufacturer information).

Lipids/wax	Constitution	Melting range (°C)	HLB	OH	MW
CP	Palmitic acid cetyl ester	43–54	—	<20	480
D14	Trimyristate acylglycerols	55–60	—	<10	723
D16	Tripalmitate acylglycerols	61–68	—	<10	807
POL5	Palmitostearate acylglycerols	52–60	2	90–110	633
WE85	Hydrogenated Coco-acylglycerols	42–48	—	5–15	720

CP, cetyl palmitate; D14, Dynasan 114; D16, Dynasan 116; POL5, Precirol ATO5; WE85, witepsol E85.

Table 2. Main properties of the surfactants used in the preparation of the lipid nanoparticles (LN) (manufacturer information).

Surfactant	Other name	Composition	Balance primarily	HLB
P20	Tween®20;	Lauric acid, ≥40%	Myristic, palmitic and stearic acids	16.7
P40	Tween®40;	Palmitic acid, ~90%	Stearic acid	15.6
P60	Tween®60;	Stearic acid, ~50%	Palmitic acid	14.9
P80	Tween®80	Oleic acid, ~70%	Linoleic, palmitic, and stearic acids	15
PL188	Pluronic®F68	Block copolymer of ethylene glycol and propylene glycol	—	>24
PL407	Pluronic®F127		—	18–23

P20, polysorbate 20; P40, polysorbate 40; P60, polysorbate 60p; P80, polysorbate 80; PL188, poloxamer 188; PL407, poloxamer 407.

parenteral formulations are not applicable to particulate formulations. Nevertheless, 5 µm is commonly accepted as an upper limit[22,23]. For LN sizes much smaller than this limit (<400 nm) were produced, but there are indications in literature that micron-range contaminants in submicron particle dispersions are hardly detected by photon correlation spectroscopy (PCS)[24]. To overcome this lack of information in the micron size region optical single particle size (OSPS) measurements can be used[25].

The aim of this work was to gain insight into the effect of both surfactant and lipid on colloidal stability of nanoparticles in order to derive hints for future design studies on LN produced by HPH, with the final goal to develop stable formulations with a mean size below 200 nm and low PI with the lowest possible amount of surfactants. Since the LN formulations are intended for brain targeting upon i.v. administration, absence of larger particles (micro-size range) and agglomerates should always be confirmed.

Materials and methods

Materials

The chosen glycerides: D14 (trimyristin), D16 (tripalmitin), WE85 were gifts from Sasol Germany GmbH, (Witten, Germany). Precirol® ATO 5 (POL5) (palmitostearate) was a gift from Gattefossé SA, (St Priest, France). The wax cutina (CP) (cetyl palmitate) was supplied by Apotekproduksjon AS (Oslo, Norway). The surfactants selected: polysorbate 20 (P20), 40 (P40), 60 (P60) and 80 (P80) were supplied by Merck (KgaA, Darmstadt, Germany). Poloxamer 188 and 407 (PL188 and PL407) was provided by BASF (Ludwigshafen, Germany). Organic solvents (acetonitrile, triethylamine and acetic acid) for high performance liquid chromatography (HPLC) were purchased from Merck KgaA, (Darmstadt, Germany). Purified water was of MilliQ®-quality.

Formulations

Formulations containing the lipids CP, D14, D16, POL5 and WE85 and with the surfactants P20, P40, P60, P80, PL188 and PL407 were prepared at different concentrations ranging between 5–15% of lipid and 0.8–2% surfactant. One lipid and one surfactant were combined at the time.

A unique code is used for designating the quantitative composition of the various formulations; it consists of an abbreviation for the lipid type and a subscript for the lipid concentration in percent plus an abbreviation for the surfactant with a subscript for the surfactant concentration. $CP_5PL407_{0.8}$ for example means CP for cetyl palmitate, subscript 5 for 5% lipid, PL407 for poloxamer 407, subscript 0.8 for 0.8% surfactant.

Methods

Production of lipid nanoparticles

For the production of LN, the lipid phase was melted at approximately 5–10°C above its melting point followed by dispersion in an aqueous surfactant solution heated at the same temperature. A hot pre-emulsion was formed by high speed stirring during 1 min in an ultra-turrax T25 at 8000 rpm. The produced hot pre-emulsion was then processed three cycles at 50 megapascal (MPa) in a temperature controlled MicronLab 40 high pressure homogenizer (APV Deutschland GmbH, Unna, Germany). The obtained hot oil-in-water nanoemulsion was cooled down to room temperature allowing the inner oil phase to solidify and forming LN dispersed in an aqueous phase.

Assessment of particle size and size distribution

The submicron particle size of LN were analyzed by PCS (Nicomp model 370, PSS-Nicomp Santa Barbara, CA) as described by Frantzen et al[24]. In all the measurements χ^2 was smaller than three, indicating a monomodal (Gaussian) distribution; it was made sure, that the amount of data collected was higher than 1000 K in the first channel and the baseline adjustment was smaller than 0.03%. The refractive index entered was that of water (1.333), which also was the solvent used to dilute the samples until a count rate of 250–350 kilohertz (kHz) had been reached. This dilution is required to eliminate multiple scattering.

In order to complement particle size measurements, additional OSPS was used to detect any content of particles in the micrometer range or aggregates of LN. The ranges of measurement of the Accusizer 780 (PSS-Nicomp, Santa Barbara, CA) are rather wide, from 500 nm up to 400 µm. This equipment combines single particle light extinction measurement with light scattering. Samples were step-wise diluted with MilliQ filtered water until consecutive dilutions yielded a linear drop in total particle counts. OSPS yields a number-weighted distribution, meaning that a diameter of 95% (OSPS 95%) reflects the fact that 95% of the count of particles is below the given size. A diameter OSPS 95% of 1 µm means that 95% of all particles have a diameter below 1 µm. Characterization parameters were the diameters OSPS 95% and 99%.

LN stability was accessed by measuring the mean particle diameter (nano and micro range) and PI of LN immediately after production and after storage at room temperature for 1 year.

Zeta potential

The electrophoretic mobility (zeta potential (ZP)) was measured by combining laser Doppler velocimetry and phase analysis light scattering (PALS) using a Zetasizer Nano ZS (Malvern, Worcestershire, UK). The samples were diluted with MilliQ-water having a conductivity adjusted to 50 µS/cm by dropwise addition of 0.9% (m/v) NaCl solution.

Differential scanning calorimetry analysis

Differential scanning calorimetry (DSC) analysis was carried out using a Perkin-Elmer Pyris 1 DSC differential scanning calorimeter (Perkin-Elmer Analytical

Instruments, Norwalk, CT) and Pyris software for Windows NT. DSC analyses were performed on bulk lipids and LN on the day of production and 1 year after the production. In this way, the samples were weighed directly in aluminum pans and scanned between 25°C and 85°C at a heating rate of 5°C/min under nitrogen gas. The degree of crystallinity or recrystallization indices (RI) were determined as follows:

$$\mathrm{RI}(\%) = \frac{\mathrm{Enthalpy\,LN}\left[\frac{\mathrm{J}}{\mathrm{g}}\right]}{\mathrm{Enthalpy\,bulk\,material}\left[\frac{\mathrm{J}}{\mathrm{g}}\right] \times \mathrm{Concentration\,lipid\,phase}(\%)} \times 100$$

Results

LN were first prepared using all combinations of the five lipids and six surfactants at fixed concentrations (5% lipid and 2% surfactant). Then, one selected lipid (D14) was combined with all surfactants at selected lipid and surfactant concentrations. All LN were formulated without preservatives in order to minimize the influence of composition parameters.

Right after production, the macroscopic appearance of all dispersions was similar to milk, of low viscosity and of white color. To assess the physicochemical stability, all LN formulations were stored at room temperature under ambient storage conditions.

Upon screening the lipids and surfactant combinations, only those not developing gels or particles in the size range to be seen by a naked eye were selected for further studies. Tendency for LN gelation was analyzed upon cooling down to the room temperature, after 1 week, 1 month and 1 year of storage.

Particle size and size distribution

All formulations with acceptable macroscopic appearance were analyzed in terms of mean size. Formulations with a mean size in the size range between 80 nm (mostly combination of low content of lipid (5%) and high content of surfactant (2%)) and 400 nm (mostly combination of high content of lipid (15%) and low content of surfactant (0.8%)) were obtained. PI-values were typically below 0.25.

The mean sizes and PIs of LN in the concentration most suitable to produce small particles (5% lipid and 2% surfactant) are presented in Figure 1. All formulations depicted a mean size <185 nm and a PI <0.23.

The formulations made with CP, D14 and WE85 were effectively stabilized by all the surfactants tested. When using POL5 as lipid matrix, the PL188 was not able to produce stable LN, whereas for D16 similar conclusions were drawn based LN the surfactants P40 and P60.

The most favorable combination for 5% lipid/2% surfactant on the day of production were found to be D14 in combination with P20, P40, P60 and P80 and WE85 in combination with P60 and P80 because they showed the smallest mean particle sizes.

In the second step the influence of lipid and surfactant concentration was investigated. One example (D14) is presented in Figure 2. All the different surfactants at three different concentrations were combined with the selected lipid (D14) at three different concentrations.

Figure 2 depicts the mean sizes and PIs of this sample set at the day of production. In this figure it can be seen

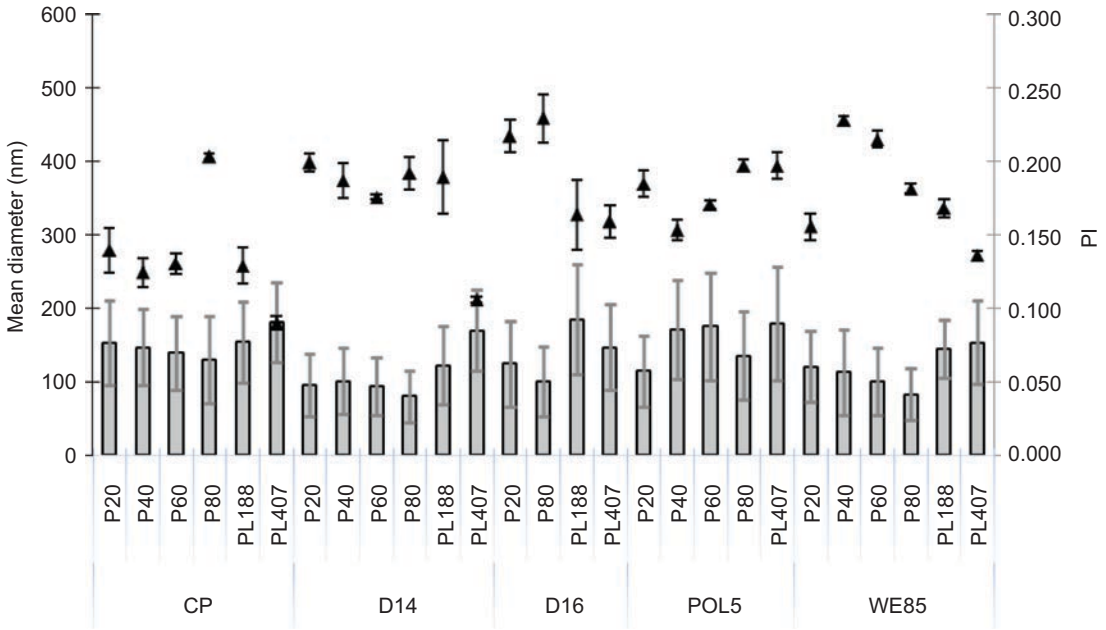

Figure 1. Photon correlation spectroscopy (PCS)-data: mean diameters (mean ± SD) and PI (mean ± SD) on production day of five parallels. Given are the formulations of cetyl palmitate (CP), Dynasan 114 (D14), Dynasan 116 (D16), Precirol ATO5 (POL5) and witepsol E85 (WE85) with 5% of lipid and 2% of the surfactants polysorbate 20 (P20), 40 (P40), 60 (P60), 80 (P80), poloxamer 188 (PL188) and 407 (PL407) (particle size given as volume-weighted mean).

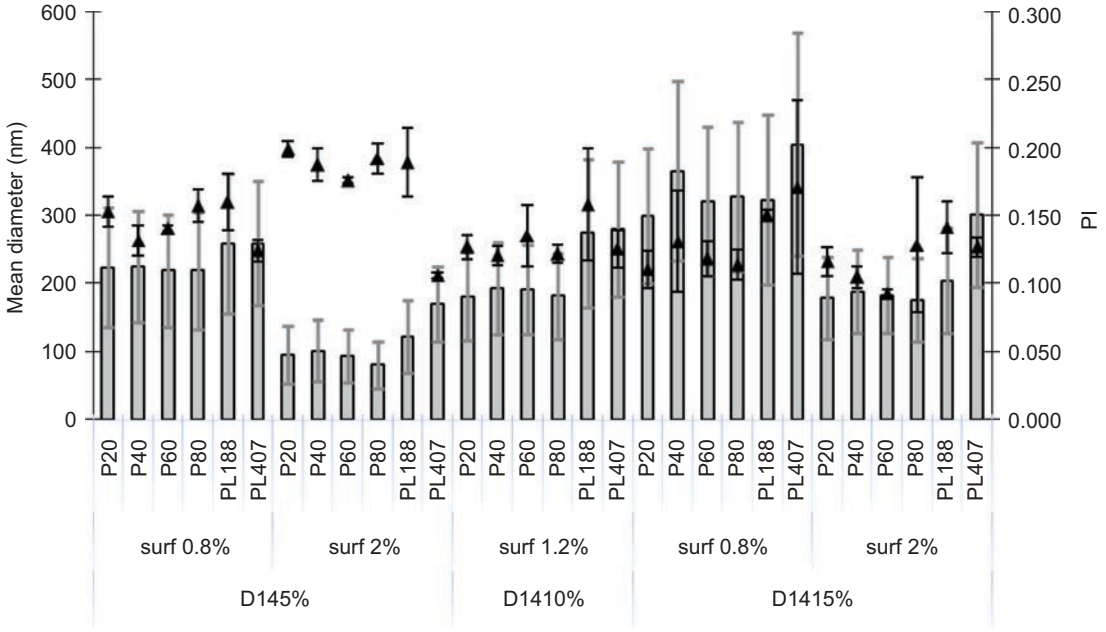

Figure 2. Photon correlation spectroscopy (PCS)-data: mean diameter (mean ± SD) and PI (mean ± SD) on production day of five parallels. Given are the formulations of Dynasan 114 (D14) based lipid nanoparticles (LN) with 5, 10 and 15% of lipid and 0.8, 1.2 and 2% of the surfactants polysorbate 20 (P20), 40 (P40), 60 (P60), 80 (P80), poloxamer 188 (PL188) and 407 (PL407) (particle size given as volume-weighted mean).

that LN with a mean size between 80 nm ($D14_5P80_2$) and 400 nm ($D14_{15}PL407_{0.8}$) and a PI from 0.09 ($D14_{15}P60_2$) to 0.20 ($D14_5P20_2$) were produced. Irrespective of type of surfactant the combination 5% lipid and 2% surfactant appeared to give the smallest mean particle sizes. Among the different surfactants, the surfactants that were more efficient in the production of D14-LN with small sizes were the polysorbates.

All the formulations were checked for changes in macroscopic appearance 1 year after production. About 40% of the formulations showed macroscopic phase separation, detectable by the naked eye. For the remaining formulations the mean particle sizes and PIs were determined. Results for the stable D14-formulations are presented in Figure 3. They depicted a mean particle size very close to the corresponding values right after production (the difference was generally smaller than 10 nm).

A typical particle size distribution plot (relative number of particles at a given size in comparison to total number of counted particles within the measuring range of the instrument) is presented in Figure 4.

The quantitative and qualitative compositions of lipids and surfactants responsible for LN <200 nm were selected for further studies since they have a mean size more suitable for brain delivery. The OSPS instrument used here allows the measurement of particles in the size range between 0.5 µm and 400 µm being highly sensitive to detect a few larger particles even if numerous smaller particles are present. (Figure 5)

No particles of 5 µm or larger were detected in the LN dispersions in the OSPS analysis (data not shown). The diameter at 95% and 99% of the total particle count are compared for three different lipids in combination with the four polysorbates. Depending on the surfactant used, CP-LN revealed OSPS 99% sizes between 1.49 µm and 1.88 µm, D14-LN had OSPS 99% sizes between 1.37 µm and 1.52 µm and WE85-LN showed sizes between 0.96 µm and 1.96 µm. Among the various formulations the D14 appeared to contain the least amount of larger particles.

Zeta potential

The ZP of the lipid D14 combined with all surfactants is shown in Table 3. Only one lipid is reported since the ZP values obtained with the other tested lipids and surfactants were very similar.

All the formulations showed slightly negative ZP values between –3.16 ($D14_5PL407_2$) and –11.50 mV ($D14_5P60_2$).

Differential scanning calorimetry

The DSC-results obtained for each type of LN are depicted in Table 4. Since the thermograms were quite similar only one formulation per lipid is given providing an example for each lipid.

In general, the onset temperature and the melting peak of the LN were approximately 2–5°C lower than those obtained for the bulk materials. Nevertheless, for D14 and D16 the recrystallization of the molten lipids was delayed by about 20°C. The crystallization temperatures of LN are distinctly below those of the melting transition for WE85-LN and decrease slightly with particle size (smaller than 2°C) within a certain series of LN dispersions (data not shown).

In this study, the RI of the LN was always below the crystallinity of the bulk material and increased with

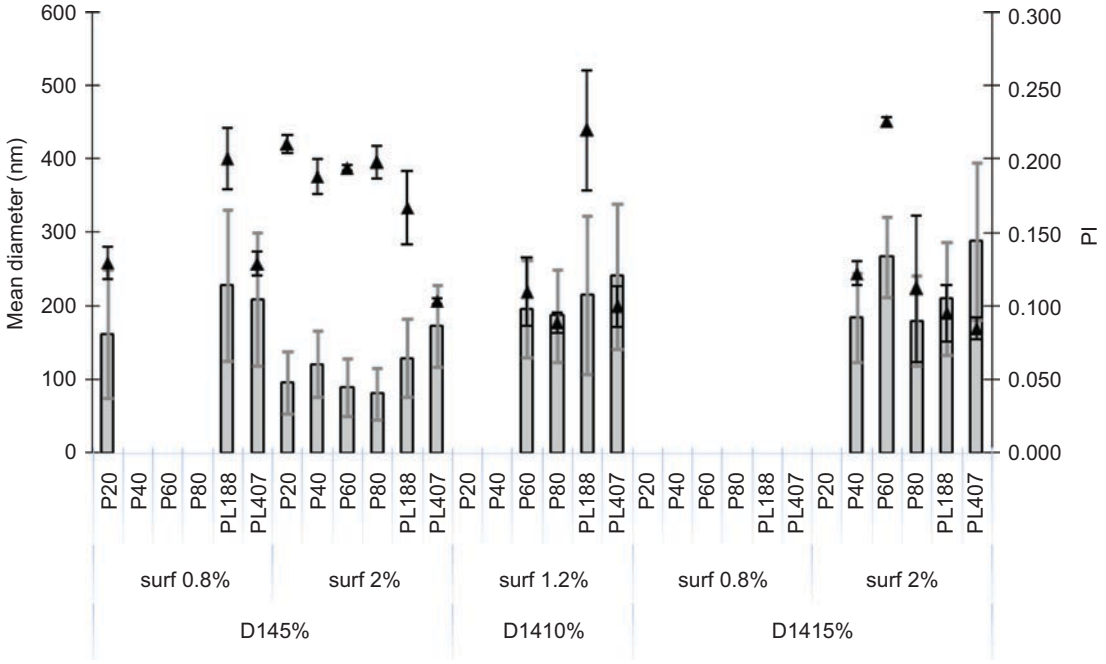

Figure 3. Photon correlation spectroscopy (PCS)-data: mean diameter (mean ± SD) and PI (mean ± SD) 1 year after production of five parallels. Given are the formulations of Dynasan 114 (D14) based lipid nanoparticles (LN) with 5, 10 and 15% of lipid and 0.8, 1.2 and 2% of the surfactants polysorbate 20 (P20), 40 (P40), 60 (P60), 80 (P80), poloxamer 188 (PL188) and 407 (PL407) (particle size given as volume-weighted mean). Absence of results means that phase separation occurred.

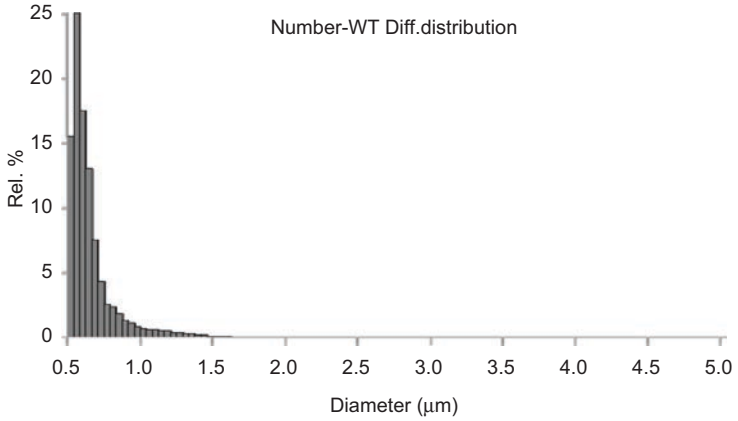

Figure 4. Typical diameter of relative distribution of lipid nanoparticles (LN) (particle size obtained as a measure of number distribution).

increasing storage time for all formulations (apart from $POL5_5P60_2$). The degree of crystallinity of the investigated LN was found between 1% ($D14_5P80_2$) and 87% ($POL5_5P60_2$) (Table 4). One year after production, the RI slightly increased for CP, D14, D16 based LN and increased significantly from 11 to 77% for WE85-LN. This increase $WE85_5P60_{0.8}$ RI was followed by an increase in the size from 139 nm (production day) to 222 nm (1 year after production). For $POL5_5P60_2$ a slight decrease of the RI was detected (from 87.2% to 86.7%) and was followed by a decrease in the size from 175 nm (production day) to 132 nm (1 year after production).

Supercooled melts were obtained with D14 lipid (ΔH ~2 J/g) whereas liquid crystalline particles were obtained with WE85 lipid (ΔH ~17 J/g). With D16, POL5 and CP lipids crystalline particles (ΔH between 138 and 160 J/g) were obtained.

DSC thermograms (Figure 6) of CP-LN on day production and 1 year after production were very similar. Although, they revealed that during storage of CP-LN, the shoulder corresponding to the α-form became slightly smaller. No peaks were observed on D14-LN DSC thermograms on day production and one year after storage. DSC thermograms of D16-LN revealed on the day production two peaks one corresponding to the α -form (55°C) and other to the β-form (62°C). One year after D16-LN production the thermograms were very similar. With regard to POL5-LN dispersion during storage, the two peaks corresponding to the α- (52–54°C) and β-form (57–60°C) tends to melt in just one peak with an onset and a maximum of

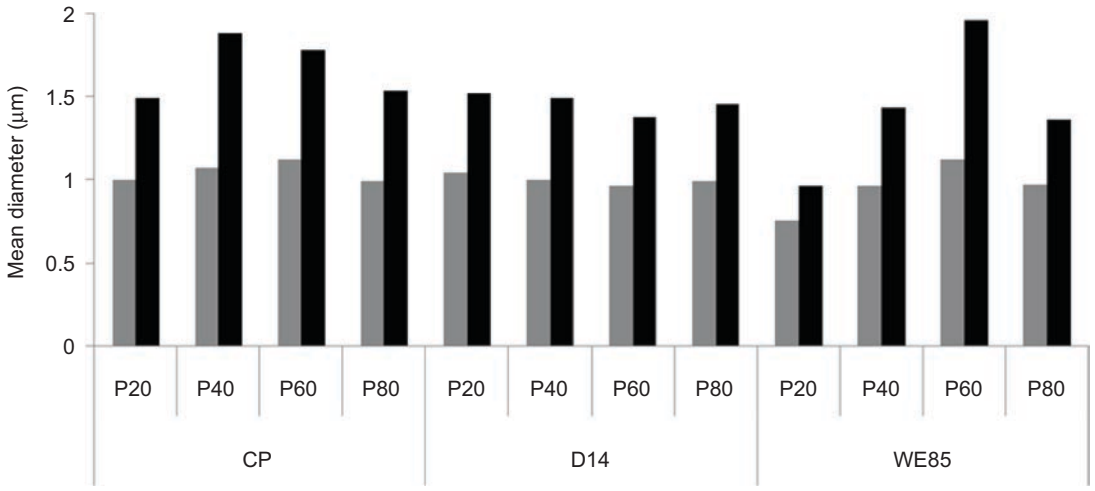

Figure 5. Optical single particle sizing (OSPS) 95% (■) and 99% (■) data of lipid nanoparticles (LN) based on cetyl palmitate (CP), Dynasan 114 (D14) and witepsol E85 (WE85) (5%lipid/0.8% surfactant) on day production (particle size obtained as a measure of number distribution).

Table 3. Zeta potential (ZP), size (mean ± SD) and PI (mean ± SD) of Dynasan 114 (D14) based lipid nanoparticles (LN) with 5% of lipid and 2% of surfactant.

Formulations	ZP ± SD (mV)	Size ± SD (nm) Day 0	PI ± SD Day 0	size ± SD (nm) 1 year	PI ± SD 1 year
$D14_5P20_2$	−8.9 ± 0.6	95.4 ± 42.6	0.20 ± 0.01	96.4 ± 43.7	0.21 ± 0.04
$D14_5P40_2$	−10.5 ± 1.1	101.4 ± 44.7	0.19 ± 0.01	121.2 ± 40.3	0.19 ± 0.02
$D14_5P60_2$	−11.5 ± 0.4	93.7 ± 39.3	0.18 ± 0.00	89.9 ± 39.6	0.19 ± 0.00
$D14_5P80_2$	−8.41 ± 0.3	80.4 ± 35.2	0.19 ± 0.01	81.0 ± 39.6	0.20 ± 0.01
$D14_5PL188_2$	−3.97 ± 0.2	122.4 ± 53.1	0.19 ± 0.02	129.3 ± 52.8	0.17 ± 0.01
$D14_5PL407_2$	−3.16 ± 0.2	170.2 ± 55.4	0.11 ± 0.00	172.4 ± 55.4	0.10 ± 0.01

53.2°C and 56.7°C, respectively. On the production day a very small melting peak of the WE85-LN was detected comparing to the bulk, this peak will although increase during storage.

Discussion

Particle size

All formulations present in Figure 1 show a mean size <185 nm and a PI <0.23, which are promising results for i.v. administration according to literature[4,23,26].

Especially the LN produced with 5% of lipid and 2% of surfactant were found quite stable even after 1 year of storage without any indications of phase separation and with mean sizes and PI-values almost identical to the ones at the day of production. For this reason, this concentration of lipid and surfactant was chosen for further studies.

It is evident from Figures 1–3 that for all the different formulations the smallest sizes were achieved with 5% of lipid and 2% of surfactant and the highest with 15% of lipid and 0.8% of surfactant. Generally, by the analysis of the graphs was found that the smallest size for the lipids CP, D14, D16 and WE85 was reached when the formulations were stabilized with P80.

The mean diameter of 80–400 nm as measured by PCS is well below the size of the smallest blood capillaries in the range of 5–6 µm. This appears to indicate that LN may be injected intravenously and used to target drugs to particular organs. The liver and spleen are the organs responsible for clearing, from the circulation, all i.v. injected and colloidal particulates, such as LN[4]. PCS may, however, be inappropriate to study particles that are much larger than the majority of the preparation. Therefore OSPS was used to measure selected formulations.

In OSPS analysis no particles larger than 5 µm were detected (data not shown). This is very important since the LN are intended for i.v. administration.

Both for the micro and nano range size, in the midst of the various formulations the D14 appeared to contain the smallest mean size and the least amount of larger particles.

The lipids with the most pronounced tendency to form homogeneous liquid LN dispersions at the studied concentrations and under the chosen preparation conditions were CP, D14 and WE85. Possibly, increasing amounts of partial glycerides like monoglycerides are responsible for the physical destabilization. The amount of monoglycerides is approximately 8–17% for POL5. The diglycerides amount is around 4% for D14, 3% for D16, 10% for WE85 and 54% POL5. The amount of triglycerides is about 95% for D14, 96% for D16, 89% for WE85 and ≈30% for POL5.

Furthermore, the different contents of emulsifying monoglycerides and diglycerides (e. g., quantified by hydroxyl (OH) number) might lead to different contents of water in the LN lipid matrix, which could potentially

Table 4. Enthalpy, peak onset and maximum and RI of the lipid bulk and the LN on day production and 1 year after.

Formulation		Enthalpy J/g	Melting of lipid (°C)		RI (%)	Size ± SD (nm)
			Peak onset	Peak maximum		
Bulk CP		190.75	51.37	53.52	100	—
$CP_{10}P80_{1.2}$	d0	159.66	47.59	50.65	83.7	193.1 ± 60.3
	1 year	164.33	47.81	50.49	86.1	198.2 ± 68.6
Bulk D14		190.30	57.39	59.29	100	—
$D14_5P80_2$	d0	2.07	55.44	57.23	1.1	80.4 ± 35.2
	1 year	3.11	55.19	57.10	1.6	81.0 ± 36.0
Bulk D16		199.21	65.38	67.75	100	—
$D16_5P80_2$	d0	141.26	59.49	62.22	70.7	100.6 ± 48.1
	1 year	159.42	59.76	62.38	79.8	102.2 ± 47.6
Bulk POL5		158.48	52.85	59.51	100	
$POL5_5P60_2$	d0	138.12	52.05	57.48	87.2	175.4 ± 72.4
	1 year	137.34	53.21	56.67	86.7	136.2 ± 60.8
Bulk WE85		156.02	45.33	47.74	100	—
$WE85_5P60_{0.8}$	d0	16.90	38.10	42.09	10.8	139.1 ± 60.9
	1 year	120.34	36.44	42.48	77.1	222.1 ± 115.6

CP, cetyl palmitate; D14, Dynasan 114; D16, Dynasan 116; POL5, Precirol ATO5; WE85, witepsol E85.

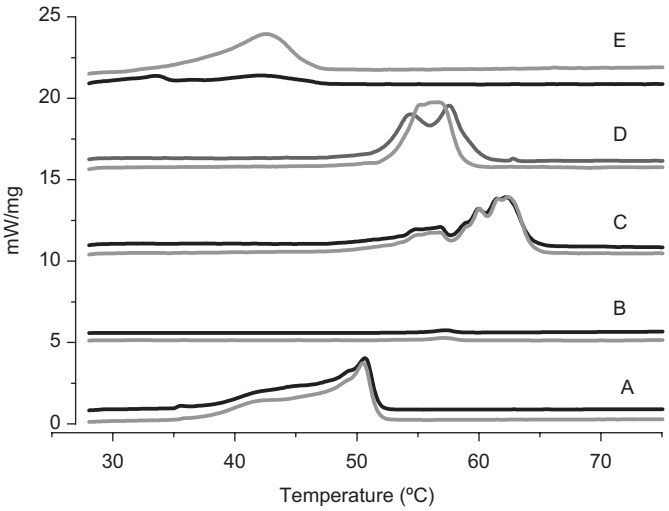

Figure 6. DSC thermograms of lipid nanoparticles (LN) based $CP_{10}P80_{1.2}$ (A), $D14_5P80_2$ (B), $D16_5P80_2$ (C), $POL5_5P60_2$ (D) and $WE85_5P60_{0.8}$ (E) on day production (black) and 1 year after production (grey).

also destabilize particle. Among the lipids, the OH number varies from 5 to 240 and increases in the follow order: D14 ≈ D16 < WE85 < CP < POL5. According to literature, the majority of drugs LN composed of glycerides reveal better encapsulation efficiency than waxes. On the other hand the physical stability is reported to be better for the waxes rather than glycerides[27].

Zeta potential

In terms of electrostatical repulsion, ZP is recommended to range in the magnitude of at least ± 30 mV in order to make physically stable aqueous nanoparticle dispersions[28]. Nonetheless, ZP is not the only parameter that influences the stability of the LN dispersions. Even with a ZP lower than 30 or high than –30 mV LN formulations could be stable for a long time, if enough and appropriate surfactants are used, providing steric stability instead. All the formulations presented in Table 3, though the little negative ZP revealed, are stable formulations in terms of mean size and PI for at least 1 year. This is due to the use of nonionic surfactants. Poloxamers were generally responsible for less negative ZP than the polysorbates. In spite of the small ZP values also those formulations were stable during storage for at least 1 year.

In general, carriers with neutral or anionic charge are reported to result in good *in vivo* brain targeting[29,30]. According to the literature the small negative ZP values of the LN produced should be good indicatives for targeted brain delivery.

Differential scanning calorimetry

The thermograms of each lipid were quite similar due to the fact that both the melting and crystallization properties of the LN were mainly governed by the hard fat component than by the surfactant[31,32].

The decrease of the onset temperature and the melting peak of the LN compared to the bulk materials (2–5°C) can be explained by the small particle sizes of the colloidal dispersion, their high specific surface area and the presence of a surfactant[33–35].

The reduction in crystallinity is due to the partial formation of lower energy lipid forms. In addition, surfactants distributed in the melted lipid phase can distort crystallization, resulting in a lower melting enthalpy.

Although crystalline lipids are used for the production of LN dispersions, the lipid particles are not necessarily present in the solid state after processing[36]. Consequently, reassuring the physical state of the matrix lipid is of utmost relevance for the development of nanoparticles based on solid lipids. In most cases, the required state of the particles will be solid or partially solid[1]. For certain applications, however, the state of a supercooled liquid melt may be expected, for example to achieve particular carrier properties[37], or to increase the bioavailability of very lipophilic drugs[38]. LN are characterized by the absence (supercooled melts) or presence (in the case of crystalline or liquid crystalline particles) of characteristic thermal transitions in the DSC heating curve which allow describing their physical state[31]. Supercooled melts were obtained with D14 lipid whereas liquid crystalline particles were produced with WE85 lipid. With D16, POL5 and CP lipids crystalline particles were obtained.

The heating curves (data not shown) and the melting enthalpies, of the D14-LN stored at room temperature, indicate that the supercooled D14-LN were stable in terms of size during at least 1 year for all the surfactants in concentrations of 2% surfactant and 5% lipid.

Conclusions

In the present study, the type of lipid, the type of surfactant as well as both lipid and surfactant concentration were identified to influence initial mean particle size and polydispersity as well as colloidal stability of the LN dispersions. Although for each of the five lipids studied here at least one combination with a surfactant could be found to produce stable formulations fulfilling our aim of a mean particle size <200 nm and a PI <0.25 considerable differences were seen between the different lipids. In fact, all combinations of CP, D14 and WE85 with any of the six surfactants in this study yielded such small and homogeneous particles. For all lipids, the combination of 5% lipid/2% surfactant was the formulation providing LN with the lowest mean particle size. At the same time did the 5% lipid/2% surfactant formulations with D14 show the best physical stability; no phase separation occurred and the mean particle sizes were found virtually unchanged after 1 year of storage. The DSC-results are in agreement with this finding: D14-LN, in contrast to all other LN had not recrystallized right after production and did not recrystallize during storage, which may be one important factor to explain the small particle sizes and storage stability. The only other LN with a low degree of crystallinity ($WE85_5P60_{0.8}$) after production recrystallized within 1 year. This recrystallization was accompanied by increases in size.

Although, in case other amounts of lipid and surfactant are required or other mean sizes are suitable, for instance to decrease toxicity or improve drug entrapment, the achievements of this work may be a good starting point to select the most promising combination of lipid and surfactant and their concentrations.

The qualitative and quantitative composition of lipids and surfactants producing LN with mean size below 200 nm, absence of microparticles and stable for at least 1 year are being used for further studies since these LN are a promising drug delivery system for brain delivery. Optimized wax and glyceride based LN can be characterized by excellent particle size distribution and physical long-term stability

The obtained results may be looked at in terms of defining the design space for LN delivery systems, i.e., identifying possible designs and design parameters within the given HPH technology. They should be applied during future formulation development studies, where inter-dependencies between the influence factors identified here may be studied in a multi-variate analysis approach.

Declaration of interest

The authors report no declarations of interest.

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