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Transfer of A Lipophilic Drug Model from Lipid Nanoparticle Carriers to A Lipophilic Acceptor Compartment

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ABSTRACT

Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) are attracting increasing attention as colloidal drug carriers for intravenous application. In order to obtain information on their potential in-vivo performance, a simple and effective in-vitro assay that mimics the environment encountered by the drug upon administration is required. In this study, unilamellar vesicles as a lipophilic acceptor compartment were used for such investigations. Trimyristin (D114) and Miglyol oil were chosen as the solid and liquid lipid respectively and porphyrin was used as a model lipophilic drug. Properties of these lipid nanoparticles such as particle size and its distribution, zeta potential and entrapment efficiency were investigated. The determination of the transferred drug to the acceptor particles was performed after the separation between the donor and acceptor particles on an ion exchange column. As a result, the entrapment efficiency was improved by adding liquid lipid to the solid lipid. On the other hand, both population donor and acceptor were effectively separated using ion exchange columns. Higher amount of porphyrin was transferred from the donor NLC to the acceptor in comparison with SLN. On the contrary, the transfer rate constant from the donor SLN was slightly higher than from NLC. These results indicate that the Ion exchange column is a suitable technique to study the transfer of lipophilic drug models but one of the two populations donor or acceptor should be charged. In addition, NLC could be used as a colloidal lipid carrier with improved drug loading capacity.

Keywords: Solid lipid nanoparticles, Nanostructure lipid carriers, Drug transfer, Ion-exchange columns

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INTRODUCTION

Nanoparticles made from solid lipids are attracting increasing attention as colloidal drug carriers for intravenous application. The nanoparticles are in the submicron size range and they are composed of physiological lipids. They are stabilized with non-toxic surfactants like poloxamer and lecithin. Due to the production by high-pressure homogenization, they can be produced on large industrial scale. In addition, this production method avoids the use of organic solvents.

There are some advantages that can be theoretically deduced from the solid state of the dispersed lipid phase compared to the liquid (emulsions) or liquid crystalline state (liposomes, cubosomes) of lipidic carriers, such as: improved physical stability and sustained drug release. However, solid lipid nanoparticles (SLN) based on one lipid component exhibit limited drug payloads and may result in potential drug expulsion from the crystal lattice due to polymorphic transitions of the lipid crystals or a reduction in their number of imperfections [1]. To overcome this drawback, nanostructured lipid carriers (NLC) have been developed based on a mixture of solid and liquid lipid (oil), which leads to an imperfect matrix structure [2]. Liquid lipids usually show a higher solubility for drugs than solid lipids [3]. The aim of NLC-formulation is to create particles in which the oil is incorporated into the core of a solid lipid. This would lead to a higher loading capacity and controlled drug release as the drug is dissolved in the oil and simultaneously encapsulated in the solid lipid. According to this model, The NLC would provide a high incorporation capacity (due to the liquid lipid) and control of drug release (due to the encapsulating solid lipid). To evaluate the drug release behaviour of such nanoparticles, many methods have been described to investigate the in vitro drug release of colloidal drug delivery systems such as sample and separate method [4-7], dialysis based assay [8-10] and continuous-flow method [11-12]. These common release methods appear to be of limited suitability to determine the in-vivo performance of these colloidal carriers and this is due to the use of aqueous release media, e.g. buffer solutions. To find more realistic release conditions with a special regard to intravenous administration, the transfer from these colloidal lipid nanoparticles carrier systems (SLN and NLC) into lipophilic acceptor compartment was measured by using ion exchange column technique, which is unlike the common techniques that measure the drug release from such carrier systems.

The ion-exchange mini-column model is an in vitro system for measuring the transfer of lipophilic drug molecules from the colloidal carrier systems to model membranes mimicking other membranous binding places in the body. This ion-exchange mini-column retains negatively

charged donor particles, allows only the neutral acceptor particles to be eluted [13]. Neutral liposomes (mostly PC-liposomes) are used as acceptor medium at excess in relation to the donor particles [13]

MATERIALS AND METHODS

Materials

The triglyceride trimyristin (D114, Dynasan 114) was a gift of Condea Chemie, D-Witten, medium chain triglyceride Miglyol 812 (Condea, Germany), sodium glycocholate (SGC), cholesterol, 5,10,15,20-tetrakis (4-hydroxyphenyl)-21H, 23H-porphine, phosphoric acid, sucrose and Trizma 7.4 pre-set crystals were from Sigma-Aldrich (D-Steinheim), diethylaminoethyl (DEAE) Sepharose CL-6B Amersham Biosciences AB (S-Uppsala), egg phosphatidyl choline (EPC) and lipoid S75 both from (Lipoid GMBH, D- Ludwigshafen), glycerol (Solvay, GmbH, D-Rheinberg), sodium azide (Sigma-Aldrich, D-Seelze), thiomersal (Caesar and Loretz, D-Hilden), methanol, tris and scintillation cocktail were from (Carl Roth-GmbH-Karlsruhe), acetonitrile, isopropyl alcohol and chloroform all from VWR International (D-Darmstadt), tetrahydrofurane (THF) was from Fisher Scientific (D-Nidderau), sodium chloride (AppliChem GmbH-Darmstadt), Purified water was prepared by filtration and deionization/reverse osmosis GE Healthcare (Amersham, radiochemical, Buckinghamshire, UK).

Methods

Preparation of donor solid lipid nanoparticles (SLN)

The dispersions were prepared from 5 % (w/w) trimyristin stabilized with 1.8 % (w/w) Lipoid S75 and 0.45 % (w/w) sodium glycocholate (SGC) in an aqueous phase containing 2.25 % glycerol for isotonization and 0.01 % thiomersal for preservation [14-15]. The preparation was done by high-pressure melt homogenization using a Microfluidizer M-110S (Microfluidics, US-Newton). S75 and SGC were dispersed/dissolved in the aqueous phase by magnetic stirring overnight. The matrix lipid and the surfactant-containing aqueous phase were heated to 70 °C. After melting of the triglyceride, the aqueous phase was poured to the molten lipid and the mixture was pre-homogenized for one minute (Ultra-Turrax T8, IKA Labortechnic, Germany). This crude emulsion was transferred to the warm (70°C) high-pressure homogenizer and treated for 5 min at 500 bar. The resulting hot colloidal emulsion was allowed to cool to room temperature. Under these conditions the matrix lipid remains in its liquid state due to supercooling [14]. Crystallization of the solid lipid was done by storage of the dispersion at refrigerator temperature (2-8 °C). Ultracentrifugation (SW 55 Ti, Beckman Coulter) of the crystalline dispersions to separate the SLN from the liposomes (due to excess emulsifier S75)

was carried out. 5 ml samples were subjected to ultracentrifugation for 1 hour at 35000 rpm and 15°C. After removing the aqueous supernatant containing the excess emulsifiers, the pellet was scraped from the tube bottom resuspended in 5 ml of aqueous phase and sonicated for 10 minutes. The aqueous supernatants containing the excess emulsifiers were subjected to a second ultracentrifugation cycle for 1 hour at 45000 rpm and 15°C and the pellets were treated as before and added to the pellets obtained from the first ultracentrifugation cycle.

Preparation of donor nanostructured lipid carriers (NLC)

The drug free NLC were prepared by hot high-pressure homogenization method. Briefly, three formulations of NLC (NLC-10, NLC-20 and NLC-30) from D114 as a solid lipid and medium chain triglyceride miglyol 812 as a liquid lipid (9, 8 and 7% w/w according to the total formulation of D114 and 1, 2 and 3% w/w miglyol oil) were prepared. The lipid phase (D114, miglyol oil and 2.4% w/w S75) was melted at 70°C to obtain a clear lipid. At the same time, an aqueous surfactant solution (0.6% w/w SGC) has been prepared and heated to the same temperature. The hot aqueous phase was poured to the molten lipid and the mixture was pre-homogenized for one minute (Ultra-Turrax T8, IKA Labortechnic, and Germany). This crude emulsion was transferred to the 70 °C warm high-pressure homogenizer and treated for 5 min at 550 bar. The hot colloidal emulsion was allowed to cool to room temperature. Crystallization and ultracentrifugation were done as described before with the SLN. Porphyrin (0.5 mg/ml of the formulation) was added to the original samples (SLN and NLC) before solidification and ultracentrifugation. Stock solution from porphyrin was prepared by using methanol (10 mg/ml) and from this stock solution 500 µl was added to 10 ml of the original samples. Shaking of the samples was done for 3 days at 25°C with a speed 100 shakes per minute in a shaker water bath (Grant OLS 200, Cambridge, England) then solidification and ultracentrifugation were carried out as described before.

Determination of entrapment efficiency

The amount of encapsulated porphyrin was calculated by the difference between the total amount used to prepare the systems and the amount that remained in the supernatant layer applying Equation [1]:

$$\mathbf{E.E.} = \frac{\text{Total amount of porphyrin} - \text{the amount in the supernatant}}{\text{Total amount of porphyrin}} \times 100\ \% \qquad [1]$$

The amount of porphyrin in the supernatant layer was determined after diluting the samples to 5 ml with a mixture of acetonitrile-tetrahydrofurane 20:80 (v/v) and measuring the UV absorbance at 421 nm.

Preparation of the acceptor unilamellar vesicles

Acceptor unilamellar vesicles were prepared using EPC and cholesterol in a molar ratio of 8:2 [16-17]. EPC stock solution was prepared by dissolving 1.52 g in 20 ml chloroform and cholesterol stock solution was also prepared by dissolving 0.194 g in 20 ml chloroform. The two lipids (1 ml of each stock lipid solution) were mixed in a small bottom flask and dried to thin film under vacuum 200 mbar for 2 hours and then at 30 mbar for 1 hour (Büchi Rotavapor R-114, D-Essen). The resulting thin film was hydrated for 10 minutes with 1 ml tris buffer saline (10 mM tris, 140 mM NaCl, pH 7.4) under vortexing. The resulting lipid suspension was extruded through 200 nm membrane followed by extrusion through 100 nm polycarbonate membranes using Liposofast extruder (Avestin Europe GmbH, D-Mannheim). The acceptor vesicles were stored at refrigerator temperature.

Particle size and zeta potential analysis

Particle sizes of the donor lipid nanoparticles with and without porphyrin and the acceptor unilamellar vesicles were measured by photon correlation spectroscopy (PCS) using a Zetasizer Nano ZS (Malvern Instruments Ltd., UK-Worcestershire). The dispersions were diluted with filtered demineralized water and measured at 25 °C at a scattering angle of 173 °. The results of three consecutive measurements of 5 min duration performed after 5 minutes of equilibration were averaged. The results are given as the z-average diameter and the polydispersity index (PDI, measure for the relative width of the particle size distribution). The particle size of the donor particles was measured with laser diffraction (LD) in combination with PIDS (polarization intensity differential scattering) using a Coulter LS 230 Particle Sizer (Beckman Coulter, D-Krefeld,). 8 consecutive measurements of 90 s were averaged. The applied evaluation model used the Mie theory with a refractive index of 1.332 for water and 1.45 for the sample. The volume distributions of the samples were calculated and the results are given as the mean particle sizes.

Zeta potential of the donor and acceptor particles was measured after diluting the samples with 10 mM tris buffer using the same Malvern Zetasizer Nano series (Malvern Instruments Ltd., UK-Worcestershire). The results of three consecutive measurements each consists of 20 runs were averaged.

Determination of the lipid content of the donor lipid nanoparticles by HPLC

The amount of trimyristin in the nanoparticles (SLN and NLC) after ultracentrifugation (resuspended nanoparticles) was determined using reversed phase HPLC with evaporative light scattering detection (Varex MKIII ELSD, Alltech GmbH, D-Unterhaching) [18]. The analysis was

performed with a 25 cm x 3 mm LiChrocart column packed with LiChrospher 100-5 RP 18 (Merck KgaA, D-Darmstadt) in a System Gold 126 HPLC (Beckman Coulter GmbH, D-Krefeld). Acetonitrile-tetrahydrofurane 55:45 (v/v) was used as the mobile phase and the isocratic flow rate was set at 1 ml/min. For the evaporation of the mobile phase, the temperature of the detector was adjusted at 91°C and the pressure was 2.2 L/min. A calibration curve for trimyristin was obtained from measurements of standard solutions of trimyristin. To determine the amount of trimyristin in the nanoparticles, small amounts of the nanoparticle dispersions were dissolved in acetonitrile-tetrahydrofurane 20:80 (v/v) to prepare 1µl/ml samples and 100 µl of these solutions were injected into the HPLC for analysis. The amount of trimyristin in the samples was determined from the calibration curve.

For the determination of the Miglyol content in the donor NLC, a reversed-phase HPLC (Beckman Coulter, LC-125) method has been developed utilizing a C18 (150 mm X 4.6 mm, 5 µm particle size) column with acetonitrile and water as eluent [19]. The flow rate was 1 ml/min and 50-µl-injection volume was used. The injection was carried out using an autosampler (Beckman Coulter, 507e). The eluent was monitored using a UV detector (Beckman Coulter, LC-168) at 220 nm. The eluent composed of acetonitrile and 0.1% phosphoric acid (99:1 v/v).

Stock solution of Miglyol oil was prepared by dissolving 1.0164 g Miglyol oil in 100 ml (10.164 mg/ml) isopropyl alcohol and 0.1% phosphoric acid (70:30 v/v). Different volumes from this stock solution were taken and diluted with the same solvent mixture to 10 ml to prepare the standard solutions. These standard solutions were eluted with a mobile phase composed of acetonitrile and 0.1% phosphoric acid (99:1 v/v). The flow rate was 1 ml/min and 50-µl-injection volume was used. The eluent was monitored using a UV detector at 220 nm. The sum of the area under the curves (AUC) of the four peaks was calculated. The Miglyol content in the donor NLC was determined by dissolving 50µl of the NLC in a 450-µl mixture of isopropyl alcohol and 0.1% phosphoric acid (70:30 v/v). 100 µl from each sample was injected and the AUC was determined. The amount of the oil in each sample was calculated using the HPLC calibration curve.

Preparation of the ion exchange column

A total of 50 ml of DEAE-Sepharose CL-6B was washed twice with a 3-fold excess of tris buffer saline (10 mM Tris, 140 mM NaCl, pH 7.4). After each washing the tris buffer was carefully decanted off and finally the gel was washed with a 3-fold excess of sucrose buffer (290 mM sucrose, 10 mM Trizma 7.4 pre-set crystals, 0.02% sodium azide, pH 7.4) and then diluted 1:1 (v/v) with sucrose buffer, which was also used for the elution of the columns [17]. The column

length is 5 cm with an inner diameter of 0.5 cm. Some glass wool was placed at the bottom of the columns. About 1 ml ion exchange suspension (DEAE-Sepharose) was filled in the column and the column was eluted with 2 ml sucrose buffer for packing and the eluate was discarded. The columns were lipid saturated (to reduce non-specific adsorption and improve recovery of acceptor particles) by applying 20 µl of the acceptor unilamellar vesicles and eluting with 1.5 ml sucrose buffer [13]. This eluate was also discarded. In all experiments, the elution of the columns was done using 1.5 ml sucrose buffer.

Porphyrin transfer to the acceptor unilamellar vesicles

All the transfer experiments were carried out on the resuspended nanoparticles (after ultracentrifugation). The transfer experiments to the acceptor unilamellar vesicles were carried out with lipid molar ratios 1:25 and 1:100 with. Different amount of the donor particles was added to Eppendorf tubes containing different amount of unilamellar vesicles and sucrose buffer. The Eppendorf tubes were incubated for the intended times at 37°C. At appropriate time intervals 200 µl of the incubation mixture was placed on the ion exchange columns. The eluate was dissolved with a mixture of acetonitrile-tetrahydrofurane 20:80 (v/v) and the UV absorbance was measured at 421 nm

Transfer kinetics

The transfer curves of the percental transferred amount of porphyrin to the acceptor unilamellar vesicles were exponentially fitted using Microcal Origin 6.0 software (OriginLab Corporation, US-Northampton) and the exponential function:

$$A_{acc} = A_{final} - A \times e^{-k \times t} \qquad [2]$$

A_{acc} is the percental amount of porphyrin transferred to the acceptor particles at time t, A_{final} is the final percental transferred amount and marks the height of the plateau, A is a pre-exponential coefficient and k is the rate constant of the transfer. The equilibrium time was determined by calculating the time required to reach 99% of the equilibrium amount.

RESULTS AND DISCUSSION

Particle sizes and zeta potential

Lipid nanoparticles were prepared by using lipoid S75 and SGC to obtain stable and negatively charge nanoparticles, which can be separated from the neutral acceptors on the ion exchange column [14]. In the preparation of the NLC, the total amount of lipid phase (trimyristin and Miglyol 812) was kept constant being 10% with regard to the total formulation, while the ratio between solid lipid (trimyristin) and oil (Miglyol) varied, i.e. from a percentage of 10% to 30% Miglyol

oil in the particles. After production, it was found that the particle size of the drug loaded NLC dispersions was the same as the drug free dispersions (NLC-30 particle size was 90 nm at 23°C, while at 4°C it was 100 nm and zeta potential was –53 mV but more investigation on the particle size with the drug and after centrifugation could not be done due to the difficulty in the separation of the NLC from the excess S75 liposomes). According to figure 1, it could be deduced that the oil loading did not influence the particle size of the NLC as reported before [20] and the presence of porphyrin did not affect the particle size of the NLC. As a result, all the NLC formulations stored at 4°C (crystallized form) showed a higher particle size determined by PCS than those stored at 23°C (liquid form or nanoemulsion form). This might be due to the characteristic anisometric shape of NLC that leads to different diffusion coefficient (D) values between spherical nanoemulsion droplets and NLC, as previously described [21]. This phenomenon directly affected the mean particle size computed by Stokes–Einstein equation ($D= KT/3\pi\eta d$ where D is the diffusion coefficient, k is the Boltzmann constant, T is the absolute temperature, η is the viscosity of the dispersing liquid, and d is the particle size diameter), which was used to calculate the mean particle size by PCS. Comparing in the same sample volume the D values of the spherical nanoemulsion and the anisometric NLC, the former ones were higher than the latter. With respect to the Stokes–Einstein equation, the higher the D values, the smaller the particle size was.

It was reported earlier [22] that colloidal emulsions and lipid nanoparticles that were prepared by the homogenization process and phospholipids as emulsifier contain a significant amount of small unilamellar vesicles (SUV) due to the excess emulsifier (phospholipid) used. The transfer was intended to be measured only from the lipid nanoparticles to the acceptor unilamellar vesicles. According to this requirement, it was important to separate the donor nanoparticles from the excess phospholipid. So after crystallization of the lipid nanoparticles, the formulations were treated by ultracentrifugation in an attempt to remove excess phospholipid into the supernatant [23-25].

By comparing figure 1 and 2, it could be observed that there was no difference in the particle size of the crystalline NLC before (original NLC 4°C) and after (resuspended NLC) ultracentrifugation. To ensure that there was no increase in the particle size to µm size range after ultracentrifugation, laser diffraction measurements were carried out. Figure 3 (a and b) illustrates that there were no differences in the particle size before and after ultracentrifugation and there were no particles in the µm size range with both formulations. The data obtained from LD measurements confirm the data from PCS measurements. By analyzing the zeta potential for

both formulations (NLC_{10} and NLC_{20} before and after ultracentrifugation) it could be seen that both formulations have a high zeta potential around –50 mV before ultracentrifugation and around –40 mV after ultracentrifugation figure 4. This high zeta potential of both formulations could be explained due to the presence of SGC (anionic surfactant) and S75 as reported before [26]. In spite of the neutral charge of Lipoid S75, the negative potential can be attributed to the presence of fatty acids in its composition [26]. From Figure 4 it could also be seen that the zeta potential of both formulations after ultracentrifugation (NLC only without excess S75 liposomes) was lower than before ultracentrifugation (NLC and mixed vesicles) and this was referred to the loss of the mixed vesicles obtained from SGC and S75 with the high negative zeta potential.

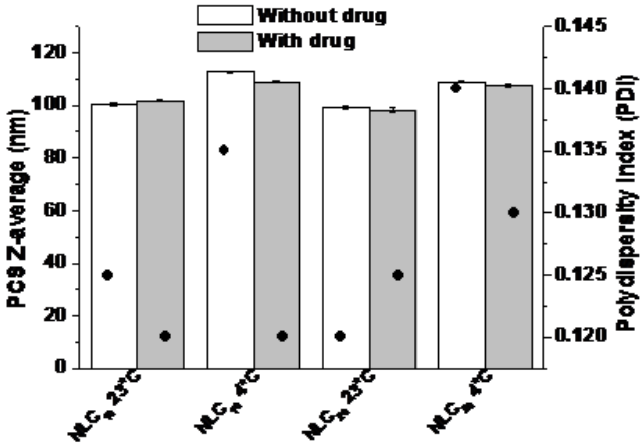

Figure 1: PCS z-average mean particle size (bars) and polydispersity indices (PDI, circles) of NLC_{10} and NLC_{20} with and without the drug stored at room (23°C) and refrigerator temperature (4°C).

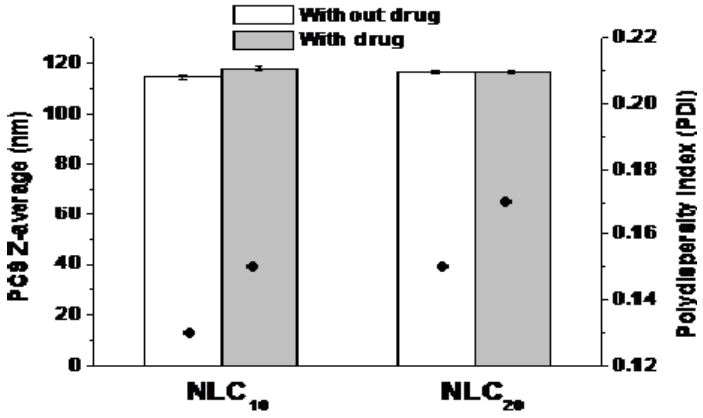

Figure 2: PCS z-average mean particle size (bars) and polydispersity indices (PDI, circles) of NLC_{10} and NLC_{20} with and without the drug after ultracentrifugation (resuspended nanoparticles).

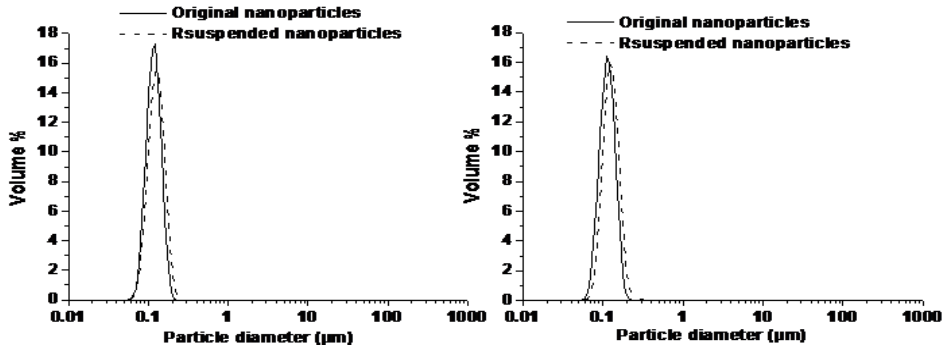

Figure 3: LD-PIDS particle size distributions of the NLC_{10} and NLC_{20} before (original nanoparticles) and after (resuspended nanoparticles) ultracentrifugation, a) NLC_{10}; b) NLC_{20}

Particles size of the unloaded SLN before ultracentrifugation was about 125 ± 0.7 nm and after ultracentrifugation the particle size slightly increased to 141 ± 0.9 nm and the polydispersity indices (PDI) for particles before and after ultracentrifugation was less than 0.15 which indicates the homogeneity of the nanoparticles. After the addition of porphyrin, particle sizes of the dispersions before and after ultracentrifugation were about 127 ± 1.1 and 142 ± 1.5 nm respectively with also polydispersity indices (PDI) lower than 0.15. Zeta potential of SLN before and after the ultracentrifugation was about – 50 mV which indicates the suitability of these particles to be retained on the ion exchange column. The z-average particle size of the unilamellar vesicles from EPC and cholesterol that had been extruded through a 100 nm membrane filter was 151.3 ± 1.2 nm (PDI 0.07). The zeta potential was –5.34 ± 1.1 mV and thus close to neutrality. EPC and cholesterol were chosen for the acceptor unilamellar vesicles, since they represent an unsaturated and uncharged bilayer that is similar to many physiological membranes.

Entrapment efficiency

The entrapment efficiency of SLN and NLC (which were prepared from different concentrations of Miglyol oil) was investigated. The effect of the liquid lipid Miglyol was demonstrated in the entrapment efficiency where the entrapment efficiency of the NLC (63% ± 2.1 and 74% ± 1.9 for NLC_{10} and NLC_{20} respectively) was higher than SLN (55% ± 2.3). It is clear that the drug entrapment efficiencies of NLC were enhanced with increasing the liquid lipid content from 10% to 20%.

Although SLN have a lot of advantages, some limitations have been reported for these nanoparticles such as, limited drug loading capacity and the formation of highly ordered structure β modifications upon crystallization, which leaves a little space for the drug molecules,

and so leads to the expulsion of drug to the surface of the crystalline particles [27-30]. Due to this highly ordered structure of the SLN, the entrapment efficiency of the NLC was higher than SLN. In addition, the entrapment efficiency increased by increasing the liquid lipid concentration in the NLC and this could be attributed to the fact that the solubility of active ingredients in oils is generally much higher than in solid lipids. For that reason, the higher loading capacity could be achieved by increasing the concentration of the oil. On the other hand drug expulsion during storage time is also minimized in case of NLC. Admixture of liquid with solid lipids leads to the creation of a less ordered inner structure. Thus, the drug molecules can be accommodated in between solid lipid and liquid lipid.

Determination of the lipid content by HPLC

Since the ultracentrifugation process may lead to a loss of triglyceride and oil from the formulations, a determination of the real lipid content in the nanoparticles formulations was performed. Determination of the lipid content in the lipid nanoparticles was carried out by HPLC. The HPLC analysis of D114 showed a distinct peak at about 2 minutes. The plot of the peak area versus sample concentration in double logarithmic coordinates gave a linear correlation, which showed a good correlation coefficient (R^2) = 0.999.

The HPLC analysis of Miglyol oil showed 4 distinct peaks at retention times 6.5, 9.1, 13.5 and 20 minutes. The lipid (Miglyol and D114) concentrations were determined from these calibration curves and listed in table 1.

Table 1: Concentration of the matrix lipid (D114 and Miglyol) in the resuspended nanoparticles after ultracentrifugation of NLC and SLN determined by HPLC

Formula		Concentration of lipid (mg/ml)	% of the lipid in relation to the original
NLC_{10}	D114	63 ± 0.4	70
	Miglyol	7.3 ± 0.12	73
NLC_{20}	D114	57.6 ± 0.25	72
	Miglyol	15 ± 0.19	75
SLN	D114	39 ± 0.18	78

Porphyrin transfer to the acceptor unilamellar vesicles

The drug transfer from the colloidal lipid nanoparticles to the different acceptors, which mimics the different membranes in the body, is of great importance. Ion exchange column technique was used to measure the drug transfer from the lipid nanoparticles to different lipophilic acceptors that mimic the different membranes in the body. This technique was first presented by Hellings and co-workers [31] and was afterwards modified by van den Besselar and co-workers [32]. This assay employs two populations, negatively charged donor and neutral acceptor. Separation

between the two populations was done on anion exchange columns, which allow the neutral acceptor to be eluted. According to this overview, this ion exchange technique requires the use of charged particles (donor or acceptor) to separate easily the two populations and both particles should posses a small size (in the nanometer size range) to avoid the blockage of the column.

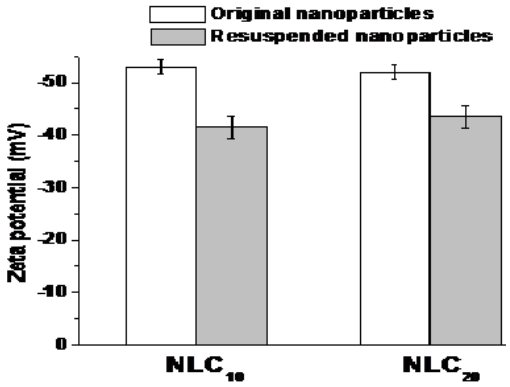

Figure 4: Zeta potential of NLC_{10} and NLC_{20} before (original nanoparticles) and after (resuspended nanoparticles) ultracentrifugation

Figures (5 and 6) and table 2 show the transfer of porphyrin from the donor SLN and NLC to the acceptor unilamellar vesicles. It could be observed from these figures that the initial drug transferred from the SLN with the different lipid molar ratios was higher than the initial amount transferred from NLC and this might be attributed to the expulsion of the drug into the aqueous phase or, alternatively, might be enriched on the surface of the particles [28-29]. This would make the drug easily available for release. On the contrary, the final percent transferred or the equilibrium amount was nearly the same or higher in case of NLC than SLN.

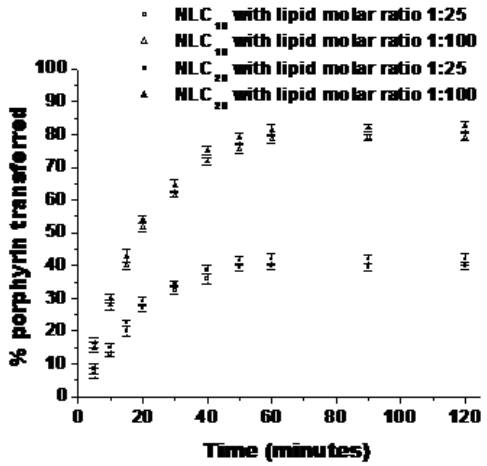

Figure 5. Percentage porphyrin transferred from the donor NLC to the acceptor unilamellar vesicles as determined by the ion-exchange column technique at different time intervals with lipid molar ratios 1:25 and 1:100.

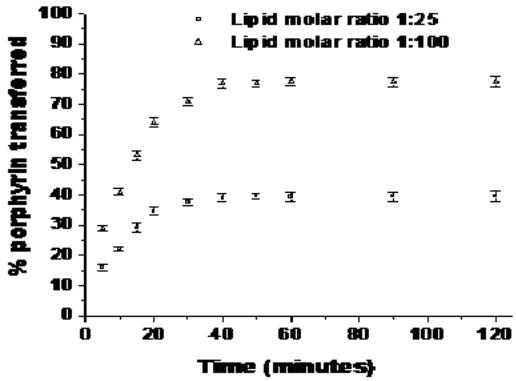

Figure 6. Percentage porphyrin transferred from the donor SLN to the acceptor unilamellar vesicles as determined by the ion-exchange column technique at different time intervals with lipid molar ratios 1:25 and 1:100.

Although the final amount transferred in case of NLC was slightly higher than SLN, the transfer rate constant from SLN was higher than NLC and this could be attributed to the higher initial amount transferred from SLN in comparison to NLC. Thus the time needed to reach the equilibrium in case of the donor SLN was lower than the time needed with the donor NLC (with the same lipid molar ratio). The easier drug diffusion through the liquid lipid could explain the higher amount of porphyrin transferred from NLC_{20} (20% Miglyol oil) in comparison to NLC_{10} (10% Miglyol oil).

Table 2: Kinetic parameters derived from fits to the transfer curves of porphyrin from the different lipid nanoparticles to the acceptor unilamellar vesicles

Donor	Molar ratio	Transfer rate constant K (min^{-1})	Final % transferred	Equilibrium time (minutes)	R^2 for fitting
NLC_{10}	1:25	0.058 ± 0.005	40.7 ± 0.7	65	0.997
	1:100	0.053 ± 0.003	80.7 ± 1.0	70	0.993
NLC_{20}	1:25	0.059 ± 0.004	42.8 ± 0.6	69	0.995
	1:100	0.054 ± 0.003	83.8 ± 0.9	73	0.998
SLN	1:25	0.08 ± 0.007	39 ± 0.5	45	0.991
	1:100	0.07 ± 0.006	78 ± 0.9	50	0.993

As expected, increasing the acceptor to donor ratio from 1:25 to 1:100 led to an increase in the final percent of drug transferred and this may be attributed to the increase in the number of the acceptor particles relative to the donor particles. This increase in the number of the acceptor particles will lead to an increase in the accessible surface available for drug transfer.

In addition to the similar equilibrium values that were observed with the SLN and NLC, another similarity was observed. The final percent of drug transferred from both SLN and NLC with different lipid molar ratios was much lower than the expected values (theoretical values) as seen from table 2. Assuming an equal porphyrin distribution between the donor and acceptor, about

99% of the porphyrin was expected in the acceptor unilamellar vesicles with a molar ratio of 1:100 between the donor and acceptor and about 96% of the porphyrin was expected in the acceptor particles with a molar ratio 1:25.

This low equilibrium values could be attributed to the drug location at the outer interface of the acceptor particles [33], which means that the inner interface was not included in the transfer. This decreased the available surface for the drug transfer. Additionally, the acceptor unilamellar vesicles was prepared from EPC with cholesterol, which increases the rigidity of the bilayer [34] and occupies a part from the accessible outer surface and so decreases the rate and amount of drug transfer to the acceptor particles.

CONCLUSION

Compared to commonly applied release methods, the use of the lipophilic acceptor particles is a better approach to the conditions in blood. Ion exchange column technique can be used successfully to measure and differentiate between the drug transferred from both the negatively charged SLN and NLC to the neutral acceptors. NLC obtained in this work could be exploited as a carrier with improved entrapment efficiency in comparison with SLN.

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