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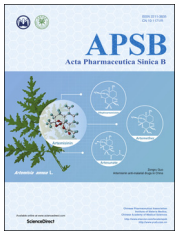

ORIGINAL ARTICLE

Comparison of drug release from liquid crystalline monoolein dispersions and solid lipid nanoparticles using a flow cytometric technique

Mohamed Z. Dawoud*, Mohamed Nasr

Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Helwan University, Cairo 11790, Egypt

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KEY WORDS

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Abstract Colloidal lipid particles such as solid lipid nanoparticles and liquid crystalline nanoparticles have great opportunities as drug carriers especially for lipophilic drugs intended for intravenous administration. In order to evaluate drug release from these nanoparticles and determine their behavior after administration, emulsion droplets were used as a lipophilic compartment to which the transfer of a model drug was measured. The detection of the model drug transferred from monoolein cubic particles and trimyristin solid lipid nanoparticles into emulsion droplets was performed using a flow cytometric technique. A higher rate and amount of porphyrin transfer from the solid lipid nanoparticles compared to the monoolein cubic particles was observed. This difference might be attributed to the formation of a highly ordered particle which leads to the expulsion of drug to the surface of the crystalline particle. Furthermore, the sponge-like structure of the monoolein cubic particles decreases the rate and amount of drug transferred. In conclusion, the flow cytometric technique is a suitable technique to study drug transfer from these carriers to large lipophilic acceptors. Monoolein cubic particles with their unique structure can be used successfully as a drug carrier with slow drug release compared with trimyristin nanoparticles.

*Correspondence to: Department of Pharmaceutics, Faculty of Pharmacy, Umm Al Qura, University, Holy Makkah, KSA. Tel.: +20 966546116057.
E-mail address: Mohameddawoud3@yahoo.com (Mohamed Z. Dawoud).

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1. Introduction

Many promising drugs are poorly water soluble and lipophilic, which can lead to difficulty in the parenteral administration of these drugs[1]. For that reason, lipid nanoparticles have arisen as an important colloidal carrier system. This importance is based on their physiological acceptance and the possibility of large-scale production by high-pressure homogenization[1]. These nanoparticles have different physical states (liquid, crystalline and liquid crystalline) and sometimes also exist in several crystal modifications[1,2] according to the storage temperature and the type of the lipid used in their preparation. Lipid nanoparticles may be in the form of a nanoemulsion or as solid nanoparticles. A major disadvantage of the lipid nanoemulsions is the difficulty in obtaining controlled drug release due to the liquid state of the carrier. For most drugs, a rapid release of the drug will be observed[3–5]. To overcome this problem, solid lipids instead of liquid oils have been used to obtain controlled drug release, because drug movement in a solid lipid should be less than in liquid oil. Solid lipid nanoparticles combine the advantages of both the lipid nanoemulsion and the controlled release, which was expected from the solid state of the lipid[6,7]. To keep this important advantage of controlling drug release upon administration, the melting point of the lipid must exceed body temperature[8].

Another important colloidal drug delivery system is particles with a cubic internal structure. Depending on the concentration of monoolein in water, monoolein dispersions form different lyotropic liquid crystalline structures[9]. By the addition of monoolein to excess water, a bicontinuous cubic phase is formed at room and body temperature. This cubic phase consists of a pair of interpenetrating but non-contacting water channels separated by a single, highly curved continuous lipid bilayer. Due to its unique structure the cubic phase can accommodate different types of drugs with different solubility[10,11]. Furthermore, cubic particles can be dispersed into nanoparticles which are termed cubosomes. Cubosomes were obtained from the cubic particles by applying high shear (*e.g.*, using high pressure homogenization or sonication) to disrupt a coarse particles of the cubic phase into small, often submicron-sized particles in the presence of surfactants like poloxamer[9,12]. The major drawback of this size-reduction process is the formation of monoolein vesicles. However, heat treatment of the homogenized dispersions results in the transformation of vesicles into cubic particles.

Many methods have been described to investigate the *in vitro* drug release of these colloidal drug delivery systems, based on (ultra)filtration or centrifugation to separate the released drug from the drug carrier particles[5,13–15]. All these methods depend on the use of simple aqueous release media which appears to be of limited suitability due to the absence of lipophilic compartments as present in the blood stream. Moreover, these lipid colloidal carriers incorporate lipophilic drugs, which have a much higher affinity to the drug carrier than to the release medium. To simulate the conditions encountered by the drug and drug carriers inside the body, the transfer from different colloidal carriers such as lipid nanoparticles into lipophilic acceptor compartments, which mimic the physiological environment, was studied. For example, release media was supplemented with albumin or unilamellar vesicles and oil/water (o/w) emulsion droplets[16–21].

In the present study, the transfer from both lipid carriers (solid lipid nanoparticles and monoolein cubic particles) into the acceptor o/w emulsion droplets was performed by using a flow cytometric technique. Through the flow cytometric technique the amount of drug in the large acceptor particles can be detected without interference from the small donor nanoparticles. Thus a separation step between the donor and acceptor was not required and the transfer mixture can be analyzed *in situ* after dilution[21]. Porphyrin was employed as a model drug to investigate the transfer behavior with comparison between the two lipid nanoparticles.

2. Materials and methods

2.1. Materials

Triglycerides trimyristin (D114, Dynasan 114) and Miglyol 812 were from Condea Chemie (D-Witten), Poloxamer 407 (Lutrol F127) was from BASF AG (D-Ludwigshafen), sodium glycocholate (SGC) and 5,10,15,20-tetrakis (4-hydroxyphenyl)-21*H*, 23*H*-porphine (porphyrin) were from Sigma-Aldrich (D-Steinheim), Lipoid S75 was from Lipoid GmbH (D-Ludwigshafen), monoolein (GMOrphic-801) from Eastman Chemical Company (Kingsport, TN), methanol was from Carl Roth GmbH (D-Karlsruhe), acetonitrile, ethanol and chloroform all from VWR International (D-Darmstadt), tetrahydrofurane (THF) was from Fisher Scientific (D-Nidderau), and Hepes and sodium chloride were from AppliChem GmbH (D-Darmstadt). Purified water was prepared by filtration and deionization/reverse osmosis (Milli RX 20, Millipore, D-Schwalbach).

2.2. Preparation of the donor monoo`lein dispersions

The monoolein dispersions were prepared from 5% amphiphile (monoolein and poloxamer) with 12% poloxamer (related to the total amphiphile amount). Molten monoolein (MO) was mixed with poloxamer 407 followed by the dropwise addition of the molten mixture to water while stirring at room temperature[22]. The resulting coarse dispersions were kept under magnetic stirring and protected from light for at least 1 day at room temperature before homogenization in a microfluidizer M-110S (Microfluidics, US-Newton) at 350 bar for 15 min at 40 °C. After homogenization, fractions of the dispersions were autoclaved at 121 °C in a laboratory autoclave (Varioklav, 65T, D-Oberschleissheim) for 15 min plus an equilibration time of 5 min. Autoclaving was used as a source of heat to improve the properties of the dispersions and convert vesicular structures, which were obtained after the homogenization process, into particles of cubic structure[22,23].

Loading of porphyrin was performed by adding 500 µL of a porphyrin stock solution in methanol (10 mg/mL) to 10 mL of the monoolein cubic particles. The samples were shaken for 3 days at 25 °C in a shaking water bath (Grant OLS 200, Cambridge, England).

2.3. Preparation of the donor trimyristin solid lipid nanoparticles

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. The preparation was done by high-pressure melt homogenization using a Microfluidizer M-110S (Microfluidics, US-Newton)[24]. 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 into 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[25].

To separate the solid trimyristin nanoparticles from the excess emulsifier S75, 5 mL samples of the nanosuspensions were subjected to ultracentrifugation (XL-80 ultracentrifuge, rotor type SW55 Ti, Beckman Coulter Inc., US-Fullerton) for 1 h at 35,000 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 surfactant-free aqueous phase and sonicated for 10 min at 60 °C.

The trimyristin nanoparticles were loaded with porphyrin (0.5 mg/mL) after the ultracentrifugation process and removal of the excess emulsifier (S75). A stock solution of porphyrin was prepared in methanol (10 mg/mL) and from this stock solution 500 µL was added to 10 mL of the nanoemulsion. The samples were mixed by shaking for 2 days at 25 °C in a shaking water bath (Grant OLS 200, Cambridge, England) followed by solidification.

2.4. *Preparation of the acceptor o/w emulsion droplets*

The acceptor o/w emulsion was composed of 5% (*w/w*) liquid medium chain triglycerides (Miglyol 812) stabilized with 3% (*w/w*) polyvinyl alcohol in an aqueous phase containing 2.25% glycerol and 0.01% thiomersal[21]. The emulsion was prepared at room temperature using an Ultra-Turrax (T8, IKA Labortechnic, Germany) for 15 min. The emulsion was stored at room temperature and used directly after preparation. For calibration of the flow cytometer, small fractions of the emulsion were loaded with different amounts of porphyrin in the same way as described for the donor lipid nanoparticles.

2.5. *Particle size and zeta potential analysis*

Particle sizes of the donor lipid nanoparticles with and without the model drug were measured by photon correlation spectroscopy (PCS) using a Zetasizer Nano ZS (Malvern Instruments Ltd., UK-Worcestershire). Ten µL of the dispersions was diluted with 1 mL of 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 min 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 sizes of the donor nanoparticles before and after ultracentrifugation, the donor monoolein dispersions, and the acceptor o/w emulsion droplets were measured with laser diffraction (LD) in combination with PIDS (polarization intensity differential scattering) using a Coulter LS 230 Particle Sizer (Beckman Coulter, D-Krefeld). Eight 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.

The zeta potential of donor nanoparticles before and after ultracentrifugation was measured after diluting 10 µL of the samples with 1 mL 10 mmol/L tris buffer using the same Malvern Zetasizer Nano series (Malvern Instruments Ltd., UK-Worcestershire). The results of three consecutive measurements each consisting of 20 runs were averaged.

2.6. *Small angle X-ray diffraction measurements of the donor monoolein dispersions*

Small angle X-ray measurements were performed to confirm the presence or absence of cubic particles in the donor monoolein/poloxamer dispersions before and after autoclaving. Small-angle X-ray diffractograms were recorded for 1–2 h in a capillary sample holder with a SWAX camera based on a Kratky collimator system (Hecus M. Braun, Optical Systems GmbH, A-Graz) with an Iso-Debyeflex 3003 60 kV generator (Seifert-FPM D-Freiberg), an X-ray tube (copper anode) FK 61-04 × 12 and equipped with 2 position sensitive detectors (PSD-50M, M. Braun, D-Garching).

2.7. *Determination of the lipid content of the donor trimyristin nanoparticles by HPLC*

HPLC analysis was carried out on the trimyristin lipid nanoparticles because these nanoparticles were subjected to an ultracentrifugation process to separate them from the excess emulsifier. The amount of trimyristin in the nanoparticles and in the supernatant layer before and after ultracentrifugation was determined using reversed phase HPLC with evaporative light scattering detection (Varex MKIII ELSD, Alltech GmbH, D-Unterhaching). The analysis was performed with a 25 cm × 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 evaporation of the mobile phase, the temperature of the detector was adjusted at 91 °C and the flow rate of nitrogen gas was 2.2 L/min. A calibration curve for trimyristin was obtained from measurements of standard solutions of trimyristin. In order to measure the amount of trimyristin in the nanoparticles, small amounts of the nanoparticle dispersions (before and after ultracentrifugation) and the supernatant layers were dissolved in acetonitrile-tetrahydrofurane 20:80 (*v/v*) to prepare 1 µL/mL samples and 100 µL aliquots of these solutions were injected into the HPLC for analysis. The amount of trimyristin in the samples was determined from the calibration curve.

2.8. *Transfer investigations by flow cytometry*

The measurements were performed in a similar way as described previously[21]. To choose the conditions for the measurements, the acceptor emulsion droplets were measured (without drug) in the flow cytometer. Different amounts of the emulsion droplets were diluted with purified water in a measurement tube and subsequently measured by flow cytometry. The optimal amount of emulsion droplets was achieved when a count rate of approximately 250 events per second was reached. The measurements were stopped after the detection of 10,000 events. The emitted fluorescence of porphyrin was detected at the photomultiplier tube number 4 (FL4) with a wavelength range of 665–685 nm. Calibration of the flow cytometer was performed by measuring the fluorescence intensity of acceptor emulsion droplets which had been loaded with defined amounts of the porphyrin. This calibration curve was used to calculate the fractions of porphyrin

transferred during the transfer experiments. Between the measurements cleaning steps were introduced to avoid mixing with residual particles from preceding samples.

The transfer of porphyrin was investigated by mixing different amounts of the loaded donor particles with 1 mL of the acceptor o/w emulsion in Eppendorf tubes followed by incubating these tubes in a water bath shaker at 37 °C (Grant OLS 200, Cambridge, England). Samples were collected at different time points after mixing and 12 µL of the transfer mixture were diluted with 1 mL purified water and subsequently measured at the flow cytometer.

2.9. *Transfer kinetics*

The curves of the percentage porphyrin transferred from both donors (solid lipid nanoparticles and monoolein cubic particles) to the acceptor emulsion droplets, determined using the flow cytometric technique, were exponentially fitted using Microcal Origin 6.0 software (OriginLab Corporation, US-Northampton) and the exponential function:

$$A_{acc} = A_{final} - A \times e^{-k \times t} \quad (1)$$

where A_{acc} is the percent amount of porphyrin transferred to the acceptor particles at time t, A_{final} is the final percent transferred amount of drug 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.

3. Results

3.1. *Preparation of the donor nanoparticles*

The solid lipid nanoparticles were prepared by high pressure homogenization which led to dispersions with PCS z-average values around or slightly above 100 nm (Table 1). Crystallization of trimyristin nanoparticles by storing them at refrigerator temperature resulted in larger particle sizes than for the corresponding emulsion formulations (stored at room temperature). This effect can be attributed to a change in particle shape during recrystallization of the nanoparticles. The transformation of the spherical emulsion droplets into platelet-shape crystals resulted in particles with a larger hydrodynamic PCS diameter[25]. The particle size of nanoparticles loaded with the drug model was similar to that of their unloaded counterparts (Table 1).

After high pressure homogenization, colloidal lipid emulsions and solid lipid nanoparticles that have been stabilized with the aid of phospholipids may contain a significant fraction of small unilamellar vesicles (SUV) which was related to the excess phospholipids[6,17,26]. Since this study is based on measuring the drug transfer from triglyceride nanoparticles (not from the vesicles), an ultracentrifugation step was carried out on the crystallized nanoparticles to separate them from excess phospholipid vesicles. Similar procedures are often used in the investigation of parenteral fat emulsions[26–28].

In order to determine the effect of the ultracentrifugation procedure on the particle size of the lipid nanoparticles, particle size of the resuspended nanoparticles was measured. Moreover, the mechanical stress upon ultracentrifugation might lead to an aggregation of the triglyceride nanoparticles. Compared to that of the non-centrifuged dispersions, the PCS z-average values of the crystalline nanoparticles had indeed increased after ultracentrifugation and redispersion but the PDI values all remained far below

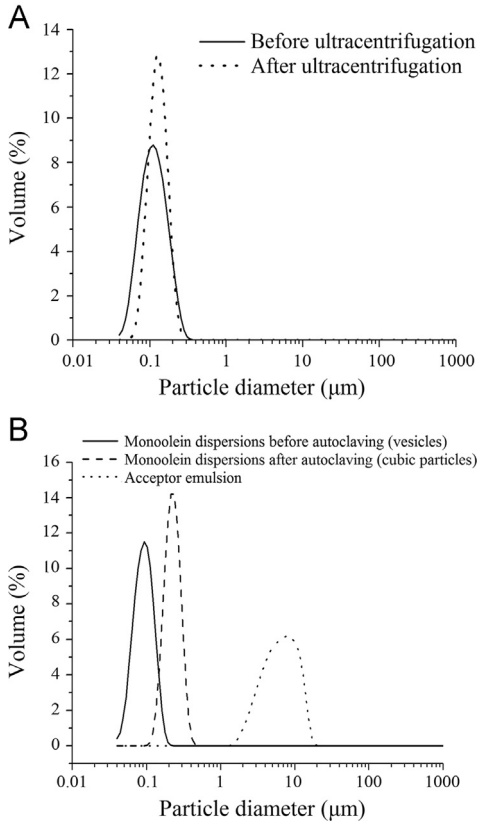

Figure 1 (A) LD-PIDS particle size distributions of the original crystalline nanoparticles (before ultracentrifugation) and the resuspended crystalline nanoparticles (pellets after ultracentrifugation). (B) LD-PIDS particle size distribution of the donor monoolein dispersions and the acceptor emulsion droplets.

Table 1 PCS z-average mean particle size and polydispersity indices (PDI) of the trimyristin nanoparticles (before and after ultracentrifugation).

Formulation	z-Average ± SD (nm)/PDI		
	Unloaded		Loaded with porphyrin
	Stored at 23 °C	Stored at 4 °C	
Nanoparticles before ultracentrifugation	115 ± 0.6/0.14	122 ± 0.7/0.16	123 ± 0.9/0.17
Nanoparticles after ultracentrifugation	N.D.	141 ± 0.4/0.11	142 ± 1.5/0.11

N.D.: not detected.

0.2, indicating an acceptable homogeneity of the particle size distributions (Table 1). A slight shift to larger particle size values was confirmed by laser diffractometry (LD) in combination with PIDS (polarization intensity differential scattering) (Fig. 1A). Zeta potential of the donor nanoparticles before and after ultracentrifugation was -60 ± 2.1 mV and -55 ± 1.8 mV, respectively. This negative zeta potential could be attributed to the presence of the anionic emulsifier SGC in these formulations. Furthermore, this high potential indicates the stability of these nanoparticles before and after the ultracentrifugation process.

Fig. 1B illustrates LD-PIDS particle size distributions of the monoolein dispersions before and after autoclaving. After homogenization, the monoolein dispersions were translucent and homogeneous with monomodal particle size distributions and mean sizes of about 100 nm. Heat treatment of the monoolein dispersions led to the aggregation and the transformation of monoolein vesicles into cubic nanoparticles (cubosomes) which had milky appearance[23,29]. The mean particle size of the dispersions after autoclaving was about 300 nm.

LD-PIDS of the emulsion droplets shows acceptor particles with a mean particle size of about 6 µm (Fig. 1B). LD-PIDS of the acceptor emulsion droplets after 4 months of storage at refrigerator temperature showed no alterations in the mean particle size, which indicates the stability of these particles.

3.2. *Small angle diffraction measurements of monoolein dispersions*

The occurrence of corresponding small angle X-ray reflections confirmed the existence of cubic particles in all autoclaved dispersions (Fig. 2). In contrast, no reflections were observed for the dispersions before the autoclaving process. According to earlier results[23], these dispersions before autoclaving contain a high ratio of monoolein vesicles and not cubic particles, which do not display small angle reflections. In all cases, the small angle X-ray reflections observed were characteristic of a P-type cubic phase. Since the comparison was intended to be carried out between the solid lipid nanoparticles and the cubic particles, the transfer experiments were performed from the donor monoolein dispersions after the autoclaving process (monoolein cubic particles).

3.3. *High performance liquid chromatography of the donor particles*

Since the ultracentrifugation process may lead to a loss of triglyceride from the formulations, a measurement of the real triglyceride content in the nanoparticle before and after ultracentrifugation was determined by HPLC. Only 77% of the original trimyristin content remained in the re-dispersed nanoparticle suspensions after ultracentrifugation. About 20% of the trimyristin was lost into the supernatant during the ultracentrifugation process.

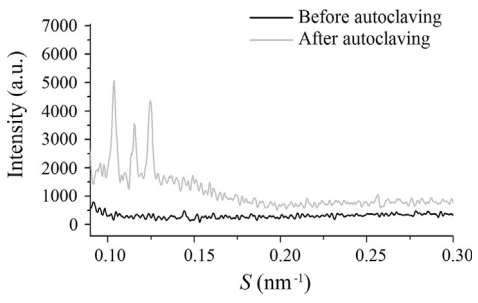

Figure 2 Small angle X-ray diffractograms of monoolein/poloxamer dispersions prepared with 5% amphiphile (monoolein and poloxamer).

3.4. *Investigation of porphyrin transfer*

The flow cytometric technique was used to monitor the drug transfer from the donor monoolein cubic particles and the trimyristin solid lipid nanoparticles to the acceptor emulsion as the particle size of the acceptors (about 6 µm) was large enough to be recognized by the flow cytometer[21]. Furthermore, the lower size detection limit of the flow cytometry device (0.5 µm) indicates that detection of the donor nanoparticles (solid lipid nanoparticles and cubic particles) with a z-average diameter less than 0.4 µm will not be possible and thus these small particles will not interfere with the measurements of the large acceptor particles[21].

The drug transfer from the trimyristin solid lipid nanoparticles to the acceptor emulsion was very rapid and equilibrium was obtained after about 5 min with the different lipid molar ratios (Fig. 3A, Table 2). In contrast to donor trimyristin solid lipid nanoparticles, the transfer from the donor monoolein cubic particles was moderate and equilibrium was obtained after about 6.5 h with the different lipid molar ratios (Fig. 3B, Table 2).

Fig. 3A and B shows that the final amount of porphyrin transferred from both donors to the acceptor emulsion droplets was much lower than the theoretical equilibrium values. Assuming an equal porphyrin distribution between the donor and acceptor, about 99% of the porphyrin was expected in the different acceptors at a molar ratio of 1:100 between the donor and acceptor and about 96% at a molar ratio of 1:25. However, the experimentally determined amount of transferred porphyrin ranged only between 35% and 70% (Table 2).

As expected the final amount transferred was increased by increasing the acceptor-to-donor ratio from 1:25 to 1:100 and this was observed with both donors.

4. Discussion

Solid lipid nanoparticles are at the forefront of colloidal carrier systems with potential in the delivery of many drugs. Due to their unique structure and ability to incorporate lipophilic and hydrophilic drugs, colloidal particles of cubic structure monoolein (cubosomes) have a great and growing importance in the field of drug delivery. As a result of the importance of these colloidal drug carriers, it is of great value to evaluate their drug release behavior.

In order to avoid the methodological problems that were observed with the conventional release methods (such as sample and separate method and dialysis-based assay), transfer experiments from both donors to lipophilic acceptor emulsion droplets were used instead of the conventional release techniques. These acceptor droplets are intended to mimic lipophilic compounds present in the blood, *e.g.*, lipoproteins.

Flow cytometry is mainly used for cell studies but it has also been used for the characterization and quantification of lipid particles like liposomes and emulsion droplets[30-34]. Recently, it has been used to investigate drug transfer between lipid particles[18,21]. Since it does not require a separation step between the donor and acceptor particles, the flow cytometric method is characterized by a very good time resolution and can detect large acceptor particles, which have a particle size of more than 1 µm[18,31,33,35]. Moreover, the high time-resolution of this

technique allowed the study of the very rapid transfer and many data points in the rising part of the transfer curves were obtained. Despite this advantage of the flow cytometric technique, it requires the use of fluorescent substances as drug models. In addition, one of the two populations (donor and acceptor) should have large particles to be detected by the flow cytometer.

A higher rate and amount of porphyrin transfer from the crystalline solid lipid nanoparticles compared to the transfer from monoolein cubic particles was observed. This higher drug transfer might be attributed to the formation of highly ordered structured particles in the β modification upon crystallization, which leaves little space for the drug molecules, and so leads to the expulsion of drug to the surface of the crystalline particles[36-39]. The presence of the drug molecules at the surface of the crystalline solid lipid nanoparticles facilitates transfer and decreases the time for transfer to the acceptor emulsion droplets.

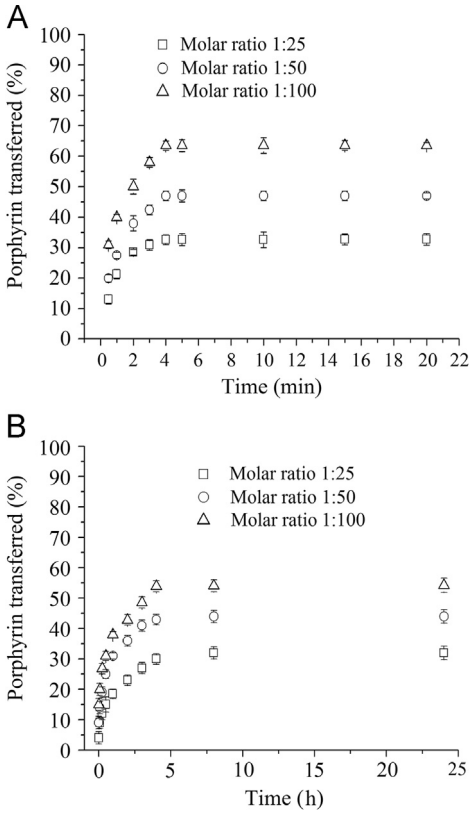

Figure 3 (A) Percentage porphyrin transferred from the donor resuspended crystalline nanoparticles to the acceptor emulsion droplets with different molar ratios using the flow cytometric technique and (B) from the donor liquid crystalline monoolein/poloxamer cubic particles to the acceptor emulsion droplets with different molar ratios using the flow cytometric technique.

Furthermore, this difference in the transfer course (rate and amount) could be related to the sponge-like structure of the monoolein cubic particles, which incorporate the drug in a three-dimensional network and thus decreases the rate and amount of drug transferred from this structure. These results are in agreement with previous observations[40], indicating that cubic particles should be quite useful for rapid uptake because they can rapidly absorb pollutants (*e.g.*, for water treatment or cosmetic skin protection) and retain an amount determined by the solute partition coefficient. These observations supported the high affinity of the lipophilic porphyrin for the donor monoolein cubic particles and consequently the lower amount and rate of transfer that was observed from such donor particles.

Another explanation of the slow release from cubic particles in comparison with the solid lipid nanoparticles is the unique structure of the cubic particles, which have a great specific bilayer/water interfacial area (500–600 m^2/g lipid)[41]. As mentioned before[42], the interfacial area (lipid/water interface) plays an important role in the transfer of porphyrin to the different acceptor particles. This great interfacial area of the cubic particles leads to the mobility of the drug molecules within the bilayer and decreases diffusion out of this unique structure.

As observed with both donors, the transfer stopped at concentrations far below an equal distribution between the donor and acceptor. Wiehe and his coworkers[42] reported that the transfer of temoporfin, which has a similar structure to porphyrin, was limited only to the interface as a result of this amphiphilicity. As a result of this amphiphilicity and the limited size of the large acceptor particles, saturation might occur and transfer might therefore stop at a lower level than was previously assumed[21].

For both donors, an increase in the acceptor-to-donor ratio led to an increase in the final amount of porphyrin transferred. This observation was not unexpected and can be explained by the increase in the number of the acceptor particles relative to the donor particles.

5. Conclusions

Colloidal particles from monoolein dispersions known as cubosomes can be successfully used as a drug carrier with slow drug release compared to trimyristin solid lipid nanoparticles. Therefore, it is better to use monoolein cubic particles rather than trimyristin solid lipid particles when sustained drug release is required. Compared to commonly applied release methods, the transfer to a lipophilic acceptor compartment is better than the commonly applied release methods. Moreover, flow cytometry is a suitable technique to study the transfer of porphyrin from the

Table 2 Kinetic parameters derived from fitting to the transfer curves of porphyrin from the different donors to the acceptor emulsion droplets obtained by the flow cytometric technique assuming transfer kinetics according to equation (1).

Donor	Molar ratio	Transfer rate constant K (min^{-1})	Final percentage transferred (%)	Equilibrium time	R^2 for fitting
Solid lipid nanoparticles	1:25	0.95 ± 0.03	33 ± 1.1	4.4 min	0.983
	1:50	0.85 ± 0.04	47 ± 0.9	5.4 min	0.985
	1:100	0.8 ± 0.07	65 ± 1.5	5.5 min	0.987
Monoolein cubic particles	1:25	0.017 ± 0.005	32 ± 1.9	7 h	0.988
	1:50	0.019 ± 0.008	44 ± 1.2	6.5 h	0.992
	1:100	0.021 ± 0.005	54 ± 1.3	6 h	0.987

different drug carriers to the lipophilic acceptor as it mimics lipid compartments in the body.

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