

Original Research Article**CHARACTERIZATIONS OF DRUG RELEASE FROM LIQUID CRYSTALLINE MONOOLEIN CUBIC PARTICLES AND MONOOLEIN VESICLES****Mohamed Dawoud**

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ABSTRACT

Monoolein dispersions are of great importance as a colloidal drug delivery system due to its unique structure. Thus it was important to measure the drug release from such carrier system. Conventional release methods showed many drawbacks. In an attempt to avoid these drawbacks, the transfer of a lipophilic drug model porphyrin from donor monoolein dispersions into acceptor multilamellar vesicles (MLV) was carried out. The measurement of porphyrin transferred was performed after the separation between the donor and acceptor using a centrifugation technique or directly without separation using a flow cytometric technique. The transfer rate and amount of porphyrin from the donor monoolein vesicles to the acceptor MLV was higher than from monoolein cubic particles. This difference in the rate and amount transferred might be attributed to the sponge like structure of the cubic particles. In addition, the cubic bicontinuous phase has a unique structure with a high specific bilayer/water interfacial area. Additionally, transfer experiments are better than the conventional release methods from the point of similarity to the conditions in the blood. In conclusion, the centrifugation and flow cytometric techniques are suitable techniques to study the transfer from the donor monoolein dispersions to the lipophilic acceptor MLV.

Keywords: Monoolein cubic particles, monoolein vesicles, Drug transfer, multilamellar vesicles, centrifugation, flow cytometric

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Running Title: liquid crystalline monoolein dispersions

INTRODUCTION

Many promising low molecular weight drugs are poorly water soluble and **are** more or less lipophilic. This can result in serious delivery challenges, in particular if the substances need to be administered parenterally (1). For lipophilic drugs, lipid nanoparticles have developed into one of the most important carrier systems due to their advantageous toxicological profile and the possibility of large-scale production by high-pressure homogenization (1). Such nanoparticles may exist in different physical states (liquid, crystalline, liquid crystalline) and sometimes also in several crystal modifications (1, 2).

Nowadays, one of the most important colloidal drug delivery system is particles with cubic internal structure. Mixtures between monoolein and water will form different lyotropic liquid crystalline structures depending on both concentration and temperature (3). The bicontinuous cubic phase is formed at room and body temperature in the presence of excess water. This cubic structure is formed of interpenetrating but non-contacting water channels separated by a lipid bilayer. This bilayer structure extends in three dimensions with a high specific bilayer/water interfacial area (4). Due to its unique structure the cubic phase can accommodate different types of drugs (5, 6). 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 (3, 7). These nanoparticles were used as a carrier for certain drugs as in case of somatostatin, insulin and cyclosporine (8-10). The major disadvantage of this size reduction process was 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 and other colloidal drug delivery systems, based on (ultra)filtration or centrifugation to separate the released drug from the drug carrier particles (11-14). Especially in the context of the intravenous administration of nanoparticles loaded with very lipophilic drug substances, the use of simple aqueous release media appears to be of limited suitability due to the absence of lipophilic compartments as present in the bloodstream. Moreover, such drugs have a much higher affinity to the drug carrier than to the release medium under these conditions. Binding of some drugs to the filter material and the blockage of the applied filter by the colloidal particles are considered as methodological problems which resulted in inaccurate measurements of the drug release.

As an approach to more realistic release conditions, the transfer from different colloidal carriers such as lipid nanoparticles into lipophilic acceptor compartments, which mimic the physiological environment encountered by the drug more closely, can be studied. For example, release media were supplemented with albumin or unilamellar vesicles and o/w emulsion droplets were used as acceptor particles for such investigations (15-19).

The aim of this work was focused on overcoming the problems that were observed with the conventional techniques and finding more realistic release conditions with special attention to intravenous administration. In the present study, the detection of substances transferred from monoolein dispersions in the form of vesicles or cubic particles into the acceptor multilamellar vesicles was performed by using centrifugation and flow cytometric techniques. The centrifugation technique utilized a centrifugation process to separate MLV from the monoolein dispersions (vesicles or cubic particles). The flow cytometric technique detected the amount of drug transferred to the large acceptor particles (not 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(19). Porphyrin was employed as a model substance to investigate the transfer behavior and comparing between the two techniques in measuring the transfer from the donor monoolein colloidal dispersions. Furthermore these two techniques were utilized to compare between the drug release from the different monoolein dispersions (monoolein vesicles and monoolein cubic particles).

MATERIALS AND METHODS

Materials

Poloxamer 407 (Lutrol F127) was from BASF AG (D-Ludwigshafen), cholesterol, sucrose and 5,10,15,20-tetrakis (4-hydroxyphenyl)-21H, 23H-porphine (porphyrin) were from Sigma-Aldrich (D-Steinheim), egg phosphatidyl choline (EPC) was obtained from Lipoid GmbH (D- Ludwigshafen), monoolein (GMOOrphic-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).

Preparation of the donor monoolein dispersions

The monoolein dispersions were prepared from 5% amphiphile concentration (monoolein + poloxamer) with 12% poloxamer (related to the total amphiphile amount). Different amounts of molten monoolein (MO) were mixed with poloxamer 407 followed by the dropwise addition of the molten mixture to water under stirring at room temperature (20). The resulting coarse dispersions were kept under magnetic stirring and protected from light for at least about 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 (20, 21).

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 dispersions (vesicles and cubic particles). The samples were shaken for 3 days at 25°C in a shaking water bath (Grant OLS 200, Cambridge, England).

Preparation of the acceptor multilamellar vesicles (MLV)

MLV liposomes were prepared as described before (22). MLV were prepared by adding 1 ml EPC chloroform solution (76 mg) to 1 ml cholesterol chloroform (9.68 mg) in a small round bottom flask. The mixture was dried to a thin film under vacuum (200 mbar for 2 hrs to remove organic solvent and then 30 mbar for 1 hr). For the complete removal of chloroform, the lipid film was rinsed with nitrogen followed by hydrating the lipid film with 1 ml warm 300 mM sucrose solution under vortexing to yield MLV liposomes, which were transferred to a plastic Eppendorf tube. The mixture was centrifuged at 1600 xg for 10 min. The centrifugation process separated the MLV in supernatant layer with the sucrose solution below. An 18G needle syringe was used to pierce the Eppendorf tube and to withdraw the sucrose solution. After that the MLV supernatant was mixed with 0.5 ml of HBS pH 7.4 (20 mM Hepes, 150 mM NaCl adjusted to pH 7.4 with 1 M NaOH). This mixture was vortexed and centrifuged at 1600 xg for 10 min where the MLV appeared as a pellet. The HBS supernatant was decanted and the pellet was washed twice with 0.5 ml HBS. The pellet was finally resuspended in fresh HBS (1 ml) and stored at refrigerator temperature.

Particle size analysis

The particle sizes of the donor monoolein dispersions and the acceptor MLV 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). 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.

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

To be sure from the presence of cubic particles in the donor monoolein/poloxamer dispersions after autoclaving, small-angle X-ray diffractograms were performed 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-Debye flex 3003 60 kV generator (Seifert-FPM D-Freiberg), an X-ray tube (copper anode) FK 61-04 × 12 and equipped with two position sensitive detectors (PSD-50M, M. Braun, D-Garching).

Transfer of porphyrin to the acceptor multilamellar vesicles (MLV)

Transfer experiments using centrifugation technique

The transfer experiments from the donor monoolein dispersions (vesicles and cubic particles) to the acceptor MLV particles were performed with lipid molar ratios 1:25 and 1:100. The donor monoolein dispersions were added to 600 µl of the acceptor MLV and different amounts of HBS (the total volume was 1 ml) in Eppendorf tubes. The samples were incubated in a shaking water bath at 37 °C. Samples were taken at different intervals, vortexed and centrifuged (3MK centrifuge, Sigma, D-Osterode) at 5300 rpm (1600 x g) for 10 min to separate the monoolein dispersion in the supernatant layer from the pellet MLV liposomes. Decantation was carried out to collect the donor monoolein dispersions followed by measuring the UV absorbance at 421 nm after dilution with a mixture of acetonitrile-tetrahydrofurane 20:80 (v/v) to 5 ml.

The MLV containing pellet was washed twice with 250 µl HBS, vortexed and centrifuged (the centrifugation time for each washing was 10 min). The first and second washings were combined and the absorbance was measured at 421 nm after dilution with the same solvent mixture to 5 ml. To obtain an overall supernatant amount, the amount of drug detected in the supernatant and washes was combined and from which the percentage drug retained in the donor monoolein dispersion was determined.

$$\text{The percent drug retained} = \frac{\text{Amount of drug in supernatant}}{\text{total amount of drug in the donor at tim 0}} \times 100 \quad \text{Eq. 1.}$$

The amount of the drug in the MLV was determined after dissolving the MLV pellets in ethanol, diluting to 5 ml and measuring UV absorbance at 421 nm. The porphyrin recovery was calculated by combining the percentage of drug transferred and retained.

$$\text{The percent drug transferred} = \frac{\text{Amount of drug in MLV pellets}}{\text{total amount of drug in the donor at tim 0}} \times 100 \quad \text{Eq. 2.}$$

Transfer experiments using flow cytometric technique

In order to determine the conditions for the fluorescence measurements, the acceptor particles were measured in the flow cytometer. Different amounts of the acceptor MLV were diluted with purified water in a measurement tube and subsequently measured by flow cytometry. The correct amount of the acceptors was determined 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 measured 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 samples, which had been loaded with defined amounts of porphyrin. The percentage of porphyrin transferred was calculated from these calibration curves. Cleaning steps were carried out between the measurements to avoid mixing with residual particles of preceding samples.

For the comparison between the two techniques, investigations of porphyrin transfer from the donor monoolein dispersions to the acceptor MLV were performed also with lipid molar ratios 1:25 and 1:100. The transfer experiments were carried out by mixing different amounts of the loaded donor monoolein dispersions with 600 µl of the acceptor MLV in Eppendorf tubes. The tubes were subsequently incubated in a water bath shaker at 37°C (Grant OLS 200, Cambridge, England). Samples were collected at different time intervals followed by the dilution of 12 µl of the transfer mixture with 1 ml purified water and subsequently measured at the flow cytometer.

Transfer kinetics

The percentage porphyrin transferred to the acceptor MLV using the centrifugation and flow cytometric techniques were exponentially fitted using Microcal Origin 6.0 software (OriginLab Corporation, US-Northampton) and the exponential function:

$$A_{acc} = A_{final} - A \times e^{-k \times t} \quad \text{Eq. 3.}$$

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

Particle size analysis

Figure 1 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. As reported before heat treatment led to the aggregation and the transformation of monoolein vesicles into cubic nanoparticles (cubosomes) which had milky appearance (21, 23). The mean particle size of the dispersions after autoclaving was about 320 nm.

The acceptor multilamellar vesicles were prepared from egg phosphatidyl choline (EPC) and cholesterol. These two lipids were selected to prepare MLV which resemble the cell membranes and other lipid compartments in the body. Particle size of the MLV liposomes was about 10 μm (figure 1). Due to the large particle size of the acceptor particles, LD-PIDS measurements of these particles were performed after 4 months of storage at refrigerator temperature to determine their stability. The results showed no alterations in the mean particle size, which indicates the stability of these particles.

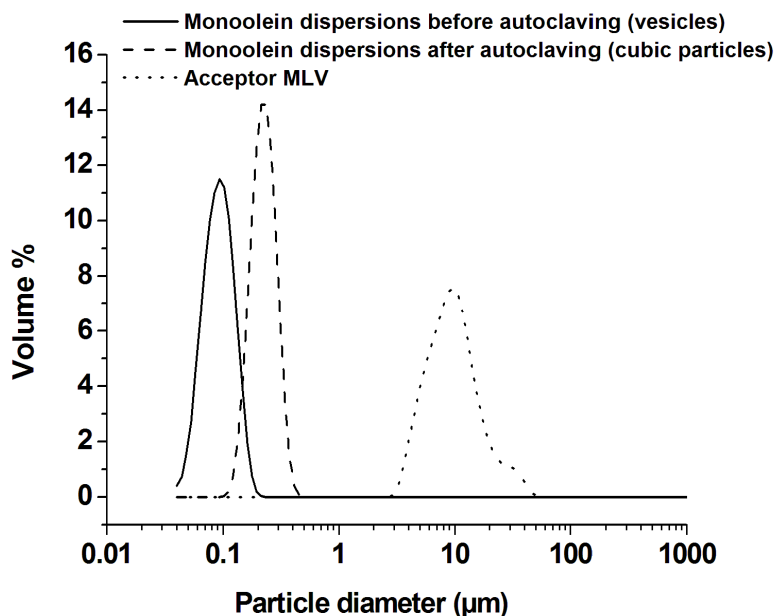


Figure 1. LD-PIDS particle size distribution of the donor monoolein dispersions and the acceptor MLV.

Small angle diffraction measurements of monoolein dispersions

The presence of cubic particles **was** proved by the existence of small angle X-ray reflections while in case of the absences of these cubic particles no reflections **were** observed. All the autoclaved samples showed small angle X-ray reflections. In contrast, the nonautoclaved dispersions did not show any small angle X-ray reflections as seen from figure 2. According to earlier results (21), these nonautoclaved dispersions contain

predominantly vesicular particles, which do not display any small angle reflections. In all cases, the small angle X-ray reflections observed were characteristic of a P-type cubic phase.

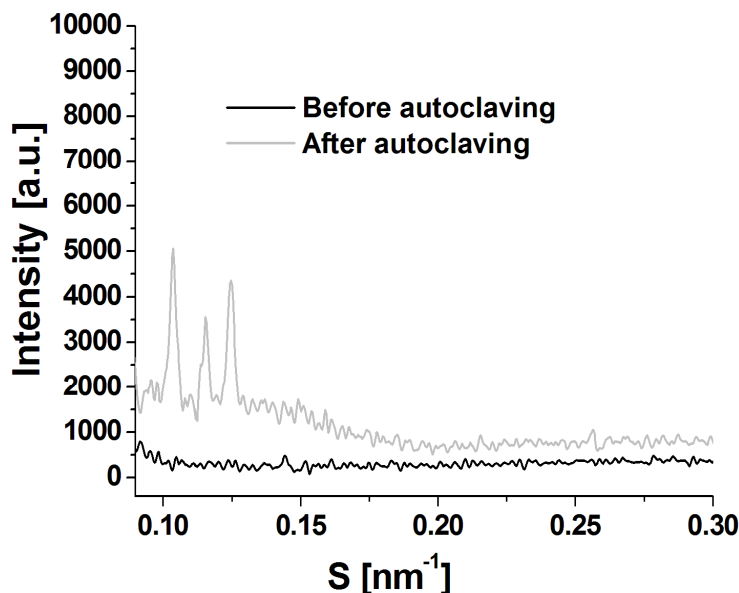


Figure 2. Small angle X-ray diffractograms of monoolein/poloxamer dispersions prepared with 5% amphiphile (monoolein and poloxamer).

Transfer of porphyrin to the acceptor multilamellar vesicles (MLV)

Centrifugation technique

The transfer experiments from the monoolein/poloxamer dispersions (vesicles and cubic particles) to the acceptor MLV using the centrifugation technique were carried out with two molar ratios 1:25 and 1:100. For both donor:acceptor ratios, the rate of drug transfer and the final percent transferred from the donor monoolein vesicles were higher than from the donor monoolein cubic particles (table 1). Due to the higher rate of transfer from the monoolein vesicles in comparison to the monoolein cubic particles, the equilibrium was obtained with the donor vesicles faster than with the cubic particles as illustrated in table 1.

Figures 3 and 4 show that the final amount of porphyrin transferred from the donor monoolein vesicles and cubic particles to the acceptor MLV at both donor: acceptor molar ratios was much lower than the theoretical values. Considering an equal porphyrin distribution between the donor and acceptor, about 99% of the porphyrin was expected in the acceptor MLV at a molar ratio of 1:100 between the donor and acceptor and about 96% of the porphyrin was expected in the acceptor particles used at a molar ratio of 1:25.

As expected the final amount transferred increased by increasing the acceptor to donor ratio from 1:25 to 1:100 and this was observed with both donors (monoolein vesicles and cubic particles).

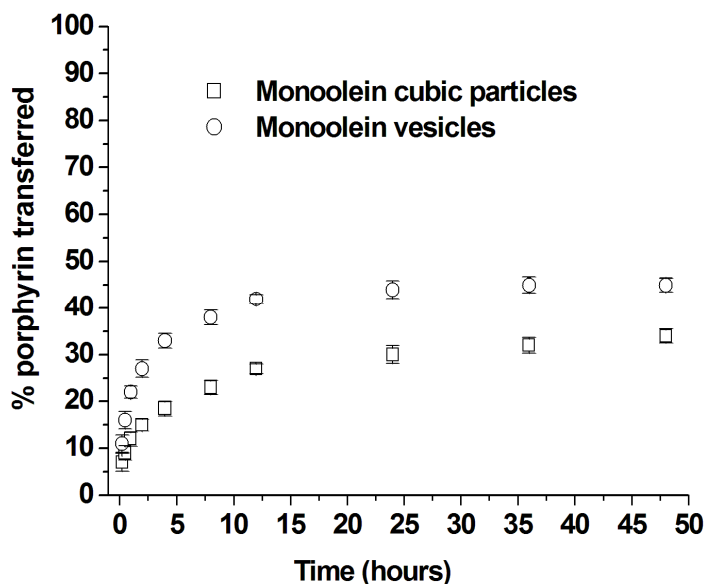


Figure 3. Percentage porphyrin transferred from the donor monoolein/poloxamer dispersions to the acceptor MLV with a molar ratio of 1:25 using the centrifugation technique ($n=3$).

Flow cytometric technique

The same transfer experiments from the donor monoolein dispersions to the acceptor MLV were carried out with the same lipid molar ratios but by using the flow cytometric technique instead of the centrifugation technique. The final percentage transferred using this technique was nearly the same as observed from the centrifugation technique and as illustrated in table 1 and figures 5 and 6. Contrary to the final percentage transferred, the transfer rate constant using the flow cytometric technique was slightly lower than the transfer rate constant obtained with the centrifugation technique (table 1). A lower final percent of porphyrin transferred (less than the theoretical values) was also obtained with the flow cytometric technique. Furthermore, the higher transferred amount that was obtained by increasing the lipid molar ratios with the centrifugation technique was also observed with the flow cytometric technique.

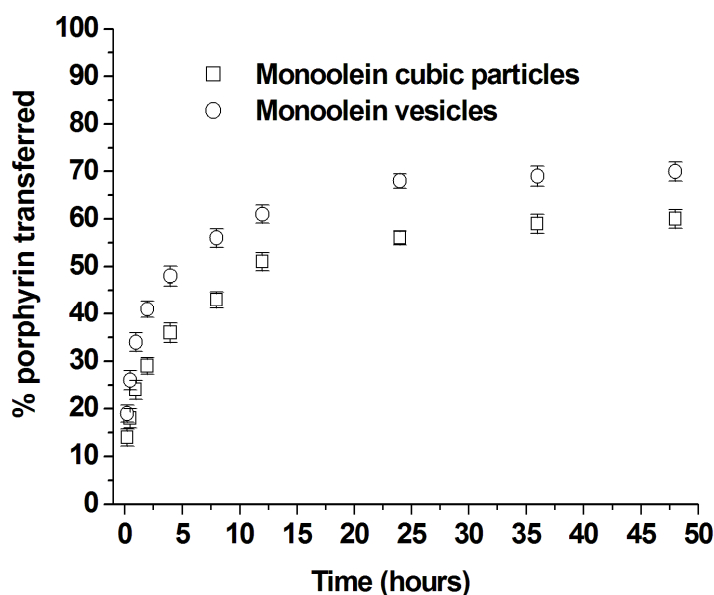


Figure 4. Percentage porphyrin transferred from the donor monoolein/poloxamer dispersions to the acceptor MLV with a molar ratio of 1:100 using the centrifugation technique ($n=3$).

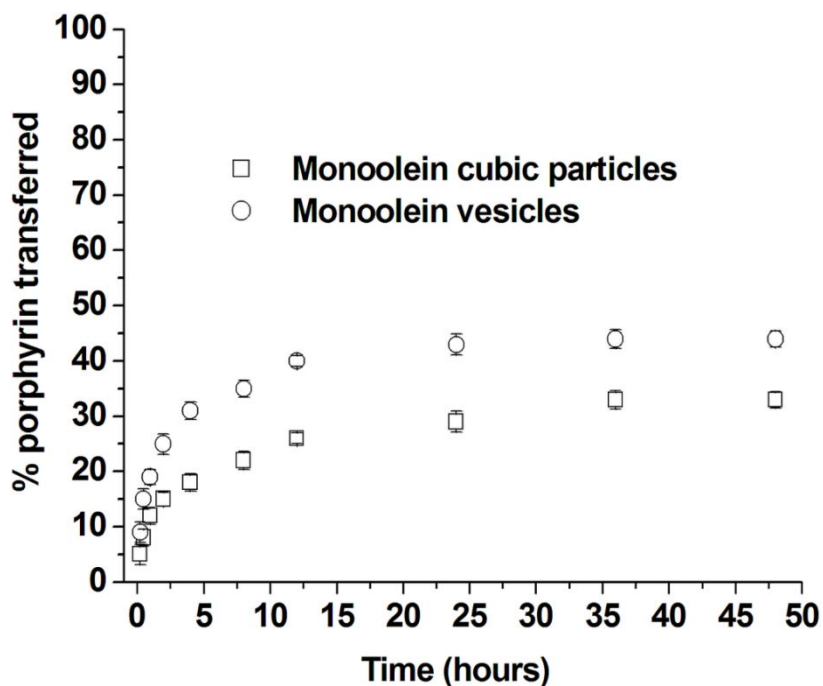


Figure 5. Percentage porphyrin transferred from the donor monoolein/poloxamer dispersions to the acceptor MLV with a molar ratio of 1:25 using the flow cytometric technique ($n=3$).

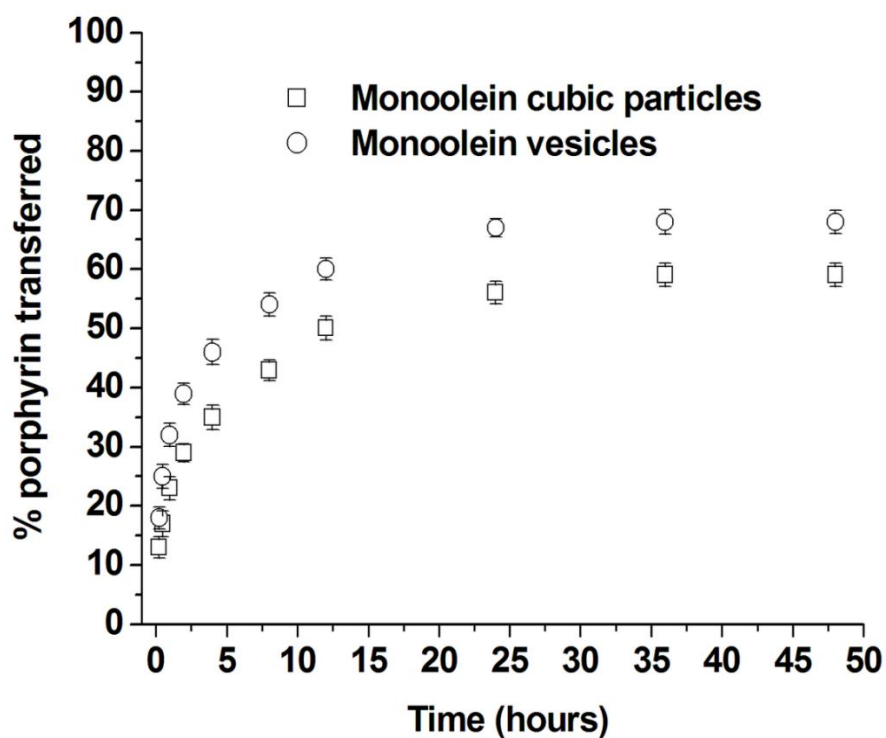


Figure 6. Percentage porphyrin transferred from the donor monoolein/poloxamer dispersions to the acceptor MLV with a molar ratio of 1:100 using the flow cytometric technique ($n=3$).

Table 1: Kinetic parameters derived from fits to the transfer curves of porphyrin from the donor monoolein dispersions to the acceptor MLV assuming transfer kinetics according to equation [3]

Measurement technique	Donor	Molar ratios	Transfer rate constant K (hr ⁻¹)	Final % transferred	Equilibrium time (h)	R ² for fitting
Centrifugation	Monoolein vesicles	1:25	0.26 ± 0.043	44.2 ± 1	26	0.983
		1:100	0.25 ± 0.038	68.5 ± 1.9	27	0.985
Centrifugation	Monoolein cubic particles	1:25	0.14 ± 0.017	32.5 ± 1.8	36	0.987
		1:100	0.16 ± 0.018	58.5 ± 1.4	40	0.988
Flow cytometric	Monoolein vesicles	1:25	0.22 ± 0.031	45.2 ± 1.7	25.5	0.991
		1:100	0.21 ± 0.025	67.5 ± 1.8	26	0.993
Flow cytometric	Monoolein cubic particles	1:25	0.12 ± 0.011	31.5 ± 1.9	36	0.987
		1:100	0.13 ± 0.021	57.5 ± 1.7	40	0.985

DISCUSSION

In order to avoid the methodological problems that were observed with the conventional release methods, transfer experiments from the donor monoolein dispersions to lipophilic acceptor MLV were used instead of these conventional release techniques. The lipophilic acceptor MLV are intended to mimic lipophilic compounds present in the blood, e.g. lipoproteins or cellular structures and thereby present a compartment in which the released lipophilic substance is soluble.

The detection of the substances transferred from the monoolein dispersions into the acceptor MLV was performed using two techniques. In case of the centrifugation technique one of the two populations (donor or acceptor) should be in the liquid state while the other should be in the crystalline form to be easily separated from each other. The acceptor MLV was prepared with a highly concentrated sucrose solution in order to be easily separated as pellet after centrifugation. The use of egg phosphatidyl choline and cholesterol in the preparation of the acceptor MLV was to resemble many physiological membranes. As an advantage with the flow cytometric technique, it does not require a separation step between the donor and acceptor particles and thus it showed an easier methodological process than the centrifugation technique. Although this advantage of the flow cytometric technique it requires the use of fluorescent substances as drug model and large acceptor particles to be detected by a flow cytometer. On the other hand, the donor particles should have a small particle size (less than 0.5 µm) in order to avoid the interference with the acceptor MLV during the measurements.

The differences in the transfer course (rate and amount) between the donor monoolein vesicles and monoolein cubic particles could be attributed to the sponge like structure of the monoolein cubic particles which decreases the rate and amount of drug transferred from this structure. These results are in agreement with previous observations (24), which indicate that cubic particles should be quite useful for a 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 to the donor monoolein cubic particles and consequently the lower amount and rate of transfer that were observed from such donor particles. Further explanation to this high affinity to the donor cubic particles is the unique structure of these donor particles with a high specific bilayer/water interfacial area (500-600 m²/g lipid) (4). As mentioned before (25), the interfacial area (lipid/water interface) plays an important role in the transfer of porphyrin to the different acceptor particles. This high bilayer/water interfacial area of the cubic particles decreases the diffusion of the drug molecules outside the donor cubic particles and subsequently a slow rate of drug transfer was observed.

Recently it was reported that the transfer of temoporfin, which is a porphyrin structure drug molecule, was limited only to the interface of the acceptor liposomes (were not entrapped in the vesicles bilayer) (16, 25) which means that after saturation of this interface the transfer stopped at low values. These recent findings might explain the low equilibrium values, which were observed in the transfer of porphyrin from both donor particles (monoolein vesicles and cubic particles), to the acceptor MLV. Furthermore, the acceptor MLV had a large particle size which will be saturated with small amount and thus probably the transfer stopped at a low level. Additionally, the acceptor MLV were prepared from EPC with the addition of cholesterol, which increases the rigidity of the bilayer (26) and occupies a part of the accessible outer surface and so decreased the amount of drug transfer to the acceptor liposomes. 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, which in turn increases the accessible surface available for drug transfer.

Finally both techniques nearly showed the same results of transfer behaviour with a slight exception of the transfer rate which was lower with the flow cytometric technique in comparison with the centrifugation technique. This slight difference in the transfer rate constant could be attributed to the separation step which was essential with the centrifugation technique. This separation took time and during this time the transfer can continue which led to an overestimation of the transfer rate constant. This overestimation was overcome with the flow cytometric technique. Also the flow cytometric methodology was easier than the centrifugation due to the absence of this separation step.

CONCLUSIONS

Compared to the conventional release methods, the transfer to a lipophilic acceptor compartment as multilamellar vesicles is better than the commonly applied release methods relative to the conditions in the blood. Both techniques are suitable techniques to study the drug release from monoolein dispersions. Additionally these techniques avoid the methodological problems which were observed with the conventional release methods. Monoolein dispersions containing cubic particles or vesicles can very successfully be used as a drug carrier.

Declaration of no conflict of interest

There are no conflicts of interest.

REFERENCES

1. Bunjes H. Lipid nanoparticles for the delivery of poorly water-soluble drugs. *J Pharm Pharmacol*. 2010;62(11):1637-45.
2. Bunjes H, Kuntsche J. Lipid nanoparticles based on liquid crystalline phases. in: Amiji MM, Torchilin, V. P. (Eds.), editor. *Pan Stanford*, Singapore 2011.

3. Larsson K. Cubic Lipid-Water Phases - Structures and Biomembrane Aspects. *J Phys Chem.* 1989;93(21):7304-14.
4. Engström S, et al. Cubic phases for studies of drug partition into lipid bilayers. *Eur J Pharm Sci.* 1999;8(4):243-54.
5. Helledi LS, Schubert L. Release kinetics of acyclovir from a suspension of acyclovir incorporated in a cubic phase delivery system. *Drug Dev Ind Pharm.* 2001;27(10):1073-81.
6. Engström S, et al. A cubosome formulation for intravenous administration of somatostatin. *Proc Int Symp Control Rel Bioact Mater.* 1996;23:382-3.
7. Gustafsson J, et al. Submicron particles of reversed lipid phases in water stabilized by a nonionic amphiphilic polymer. *Langmuir.* 1997;13(26):6964-71.
8. Drummond CJ, Fong C. Surfactant self-assembly objects as novel drug delivery vehicles. *Curr Opin Colloid Interface Sci.* 1999;4(6):449-56.
9. Gustafsson J, et al. Cubic lipid-water phase dispersed into submicron particles. *Langmuir.* 1996;12(20):4611-3.
10. Spicer PT, et al. Novel process for producing cubic liquid crystalline nanoparticles (cubosomes). *Langmuir.* 2001;17(19):5748-56.
11. Boyd BJ. Characterisation of drug release from cubosomes using the pressure ultrafiltration method. *Int J Pharm.* 2003;260(2):239-47.
12. Magenheimer B, et al. A new in-vitro technique for the evaluation of drug-release profile from colloidal carriers - ultrafiltration technique at low-pressure. *Int J Pharm.* 1993;94(1-3):115-23.
13. Washington C. Drug release from microdisperse systems: a critical review. *Int J Pharm.* 1990;58(1):1-12.
14. zur Mühlen A, Mehnert W. Drug release and release mechanism of prednisolone loaded solid lipid nanoparticles. *Pharmazie.* 1998;53(8):552-5.
15. Dawoud M. Investigations on the transfer of porphyrin from o/w emulsion droplets to liposomes with two different methods. *Drug Dev Ind Pharm.* 2015;41(1):156-62.
16. Dawoud M, Hashem FM. Comparative study on the suitability of two techniques for measuring the transfer of lipophilic drug models from lipid nanoparticles to lipophilic acceptors. *AAPS PharmSciTech.* 2014;15(6):1551-61.
17. Fahr A, Liu X. Utilization of liposomes for studying drug transfer and uptake. *Methods Mol Biol.* 2010;606:1-10.
18. Fahr A, et al. Transfer of lipophilic drugs between liposomal membranes and biological interfaces: Consequences for drug delivery. *Eur J Pharm Sci.* 2005;26(3-4):251-65.
19. Petersen S, et al. Flow cytometry as a new approach to investigate drug transfer between lipid particles. *Mol Pharmaceutics.* 2010;7(2):350-63.
20. Barauskas J, et al. Cubic phase nanoparticles (Cubosome): Principles for controlling size, structure, and stability. *Langmuir.* 2005;21(6):2569-77.
21. Wörle G, et al. Transformation of vesicular into cubic nanoparticles by autoclaving of aqueous monoolein/poloxamer dispersions. *Eur J Pharm Sci.* 2006;27(1):44-53.
22. Shabbits JA, et al. Development of an in vitro drug release assay that accurately predicts in vivo drug retention for liposome-based delivery systems. *J Control Release.* 2002;84(3):161-70.
23. Wörle G, et al. Influence of autoclaving on the ultrastructure of aqueous monoolein/poloxamer dispersions. *Eur J Pharm Sci.* 2004;23:S45-S.
24. Ribier A, Biatry B, inventors; (L'Oreal, Fr), assignee. Oily phase in an aqueous phase dispersion stabilized by cubic gel particles and Method of Making 2001.

25. Wiehe A, et al. Lead structures for applications in photodynamic therapy. Part 1: Synthesis and variation of m-THPC (Temoporfin) related amphiphilic A(2)BC-type porphyrins. *Tetrahedron*. 2005;61(23):5535-64.
26. Liu XY, et al. Effect of liposome type and membrane fluidity on drug-membrane partitioning analyzed by immobilized liposome chromatography. *J Chromatogr A*. 2001;913(1-2):123-31.