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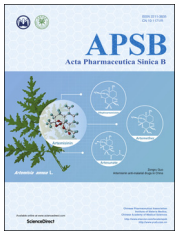

ORIGINAL ARTICLE

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

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Porphyrin

**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.



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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