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# Evaluation of the drug loading capacity of different lipid nanoparticle dispersions by passive drug loading

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ABSTRACT

When using lipid nanoparticles as drug carrier system it is important to know how much drug can be loaded to the nanoparticles. The mainly used drug loading procedure is an empirical approach dissolving the drug in the liquid lipid during preparation of the nanoparticles. This approach does not necessarily lead to the truly loadable amount, as the lipid can, e.g. be overloaded, in particular when it is processed in the heat. In this work, a different procedure, passive drug loading, was evaluated to determine the drug loading capacity of various lipid nanoparticles (supercooled trimyristin emulsion droplets, solid trimyristin nanoparticles, tristearin nanoparticles in the α-modification and cholesteryl myristate nanoparticles in the supercooled smectic as well as in the crystalline state). The nanoparticle dispersions were exposed to eight different model drug compounds (betamethasone-17-valerate, carbamazepine, diazepam, flufenamic acid, griseofulvin, ibuprofen, retinyl palmitate, ubidecarenone) in the bulk state, which varied in partition coefficient and aqueous solubility, and equilibrated over time. The passive loading procedure had no relevant impact on the particle sizes or the physicochemical state of the nanoparticles. The loadable drug amount differed distinctly for the different model compounds and also between the different types of lipid nanoparticles. For most compounds, the loaded amount was much higher than the aqueous solubility. Trimyristin-based dispersions generally had the highest loading capacity, the emulsion usually being equal or superior to the solid trimyristin nanoparticles. For betamethasone-17-valerate, however, solid lipid nanoparticles exhibited by far the highest drug load. The extremely lipophilic model drugs retinyl palmitate and ubidecarenone could not be loaded with the passive approach.



## 1. Introduction

Colloidal dispersions of lipid particles are under intensive investigation as drug carrier systems for poorly water-soluble drugs, in particular with regard to their intravenous administration. Such dispersions can be based on a broad variety of lipid compositions and particle structures such as emulsions of liquid oils, suspensions of crystalline glycerides or fatty acids (solid lipid nanoparticles), dispersions of thermotropic or lyotropic liquid crystalline phases, mixed micelles or liposomes. Some of these carrier systems have already been introduced to the pharmaceutical market [1–3]. Besides the general suitability of a potential drug carrier system for the intended way of administration (e.g., physiological compatibility of its ingredients, appropriate particle size distribution, chemical and physical stability) its ability to solubilize a sufficient amount of drug is an important prerequisite for its successful use as drug delivery system. Hitherto, there is, however, only little systematic knowledge on the solubilization capacity of the different types of colloidal lipid carrier systems. Thus, identifying a suitable carrier system for a given drug is still very much based on empirical approaches. In general, this requires to test a range of carrier systems with different drug loads in order to determine their solubilization capacity for the drug of interest.

The commonly used technique for loading colloidal lipid dispersions with poorly soluble drugs is to process the drug with the lipids. During the preparation of colloidal lipid emulsions or solid lipid nanoparticles the drug is usually dissolved in the (molten) lipid prior to particle formation, e.g., by high pressure homogenization. If, for some reason, the solubility limit of the drug is exceeded during manufacturing of the dispersion, the drug will later precipitate in the dispersion. In particular when the systems are prepared at elevated temperature, the solubility in the lipid phase may be overestimated. In solid lipid nanoparticles, the crystalline nature of the solid particle core may distinctly reduce the drug

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incorporation capacity compared to the liquid state of the lipid [4]. Moreover, the drug partitions between the lipid and the aqueous phase. If the solubility limit in the aqueous phase is exceeded as a result of the partitioning process the drug will precipitate even if the lipid phase could, in principle, still incorporate more drug [5]. As a complication, supersaturation of the aqueous phase may occur leading to crystallization of the drug from the aqueous phase only after a certain time of storage. A delay of precipitation processes may also be caused by slowly proceeding solid state transitions within the crystalline core of solid lipid nanoparticles leading to drug expulsion [4].

In addition to phenomena of delayed drug crystallization, detection of crystallized drug in colloidal dispersions may become a problem when drug crystals are hard to detect, e.g., by light microscopy, due to their shape (e.g. in the case of long, thin needles), a very small size or a low number of crystals in the overall sample. Taken together, these phenomena can make it very difficult to determine the true loading capacity of colloidal lipid dispersions. Moreover, the method is rather time and material consuming since samples with different drug concentrations may have to be prepared and investigated in order to find the solubilization limit of the respective system. This may be a serious obstacle when the amount of available drug is very low as it is usually the case in early pharmaceutical development [6,7].

With respect to the development of carrier systems for new drugs it would thus be very helpful to find a more efficient way to reliably determine the drug loading capacity of the respective type of nanoparticles. One possibility into this direction might be the addition of drug crystals to the dispersions and to observe the amount of dissolved drug as described in a study by Sznitowska et al. [8] who investigated the solubilizing effect of submicron emulsions for different drugs as well as a possible destabilization potential of the drug loading process.

The aim of our study was to extend these investigations by incubating different types of colloidal lipid dispersions with drug crystals as a passive way of drug loading. This way, it should be possible to prevent overloading of the dispersions since the solubilized drug is always in equilibrium with its crystalline counterpart. For these investigations, five kinds of colloidal lipid dispersions were selected to study the effect of properties such as the inner structure, shape, and the type of matrix lipid of the particles on the drug loading capacity. We investigated melt-homogenized, tyloxapol-stabilized trimyristin dispersions, which can either form a suspension of solid lipid nanoparticles or a nanoemulsion of supercooled droplets depending on storage conditions [9]. This allows to directly evaluate the impact of the physical state of the nanoparticles (i.e. crystalline solid vs. liquid) on their drug incorporation capacity [4]. Tristearin nanoparticles stabilized with poly (vinyl alcohol) were used as a further type of solid lipid nanoparticles since these particles can be kept in the metastable α-modification for a certain time after preparation [10]. Tristearin nanoparticles in the α-form exhibit a different inner structure and a different shape compared to the aforementioned tyloxapol-stabilized solid trimyristin nanoparticles, which rapidly transform into the stable β-modification [9]. The α-form of triglycerides is less tightly packed than the stable $\beta$-modification and there are indications that it thus might incorporate drugs more easily [4,11]. Moreover, α-form tristearin nanoparticles stabilized with poly(vinyl alcohol) are spherical whereas triglyceride nanoparticles in the $\beta$-form usually have a platelet-like shape [10,12,13]. As a fourth type of dispersion, cholesteryl myristate nanoparticles in the supercooled smectic liquid-crystalline state were investigated. In the liquid-crystalline state, the molecules display a certain degree of order but are still mobile; the particle matrix is much more viscous than in a conventional emulsion. Supercoooled smectic nanoparticles were developed with the aim to combine postulated advantages of solid lipid nanoparticles (in particular, better control over drug release) with the often observed higher solubilization capacity of lipid emulsions [14]. The drug load in crystallized cholesteryl myristate nanoparticles was also studied for comparison.

Eight poorly water-soluble model drugs (Fig. 1), which differ in structure, water solubility and partition coefficient, were chosen for our studies. Besides the determination of the drug loading capacity, the time-course of the drug loading process, drug solubility in the aqueous phase and the influence of drug loading on the particle size of the dispersions (as an indication for the physical stability) were investigated. In order to detect potential differences that might result from different drug loading procedures the drug loading capacity of trimyristin nanoemulsion and – suspension and the α-form tristearin nanoparticles was also determined with two of the drugs using the direct drug loading procedure (i.e. addition of the drug prior to preparation of the dispersion).

## 2. Materials and methods

### 2.1. Materials

As matrix lipids for the dispersions, tristearin (Dynasan 118, Hüls/Condea, Witten, Germany), trimyristin (Dynasan 114, Hüls/Condea, Witten Germany) or cholesteryl myristate (Sigma, USA) were used. The dispersions were either stabilized with poly(vinyl alcohol) (PVA, Mowiol 3-83, Clariant, Frankfurt/Main, Germany) or with tyloxapol (Sigma, Seelze, Germany). The aqueous phase contained sodium azide (Sigma, Seelze, Germany) and glycerol (Caelo, Hilden, Germany). For pH adjustment of the aqueous phase acetic acid (100 % DAB, Carl Roth, Karlsruhe, Germany) and sodium hydroxide (Carl Roth, Karlsruhe, Germany) were used.

Drugs for passive loading were betamethasone-17-valerate (Bmv, Fagron, Barsbüttel, Germany), carbamazepine (Cmz, Novartis, Basel, Switzerland), diazepam (Dzp, Synopharm, Barsbüttel, Germany), flufenamic acid (Ffa, Lindopharm, Hilden, Germany), griseofulvin (Gsf, Euro OTC Pharma GmbH, Bönen, Germany), ibuprofen (Ibu, Bayer, Germany), retinyl palmitate (Rtp, Sigma, Seelze, Germany) and ubidecarenone (Q10, Kyowa Hakko Kogyo, Tokyo, Japan).

Acetonitrile (ACN) for HPLC gradient grade (Fisher Scientific and VWR) and tetrahydrofuran (THF) for HPLC (Fisher Scientific and VWR) were used as solvents for high performance liquid chromatography. Purified water was prepared by filtration and deionization/reverse osmosis (Milli RX 20, Millipore, Schwalbach, Germany). All substances were used as received.

### 2.2. Methods

#### 2.2.1. Preparation of the dispersions

The dispersions were prepared by melt homogenization with a lipid content of 10% (w/w). The compositions of the different dispersions and the processing parameters are shown in Table 1. All dispersions were prepared with two aqueous phases differing in pH: either unbuffered or with pH 4.5 (sodium acetate buffer 25 mM), respectively (pH measurement with pH electrode Pt Inlab 415, Mettler Toledo, Germany). The aqueous phase contained 0.05% (w/w) sodium azide as preservative and 2.25% (w/w) glycerol for isotonization. The lipid was heated to about 10 °C above its melting point. In case of the conventionally loaded dispersions the drug was added to the molten lipid. The emulsifier was dissolved in the aqueous phase and the aqueous phase was heated to the same temperature as the molten lipid. Both phases were combined and pre-homogenized with an ultraturrax (Ultra-Turrax T8, Ika-Werke, Staufen, Germany) for 1 min. High pressure