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Development of a new approach to investigating the drug transfer from colloidal carrier systems applying lipid nanosuspension-containing alginate microbeads as acceptor

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ABSTRACT

As a new approach to analyzing the release behavior of lipophilic drugs from colloidal carriers, solid trimyristin nanoparticles were incorporated into differently sized (34–1363 μm) calcium alginate hydrogel microbeads to serve as acceptor in release studies. The microbeads were prepared by electrostatic droplet generation or by a spraying method. Trimyristin nanoemulsion samples loaded with the fluorescent drug model Nile red were mixed with the nanoparticle-containing microbeads to perform transfer studies. As a result of a rather large diffusion barrier a slow transfer (24–57 min) was observed using large acceptor beads (~330–1360 μm). In contrast, Nile red transferred quickly (~1.4 min) into smaller microbeads (<50 μm). This new experimental approach applying nanoparticle-containing hydrogel particles with a size below 50 μm as acceptor systems is a promising technique to investigate the release of lipophilic substances from lipid nanoparticles under close to realistic conditions. However, there is still room for technical improvement, e.g., with regard to the water loss from microbeads that was observed during sampling by centrifugation and filtration (required to separate the small sized alginate particles) which is expected to have had some effect on the dye content determined during these experiments.

1. Introduction

Colloidal carrier systems are being intensively investigated for intravenous drug delivery, in particular for lipophilic drugs. Not only with regard to drug targeting, knowledge on the drug release behavior of such formulations is essential. Since the particles of intravenous drug delivery systems are required to be very small, the evaluation of the in vitro drug release is difficult. Pharmacopoeias describe standard setups for studying drug release from conventional dosage forms (European Pharmacopoeia, 2014), but these methods cannot be applied without modification for colloidal carriers. In drug release studies with colloidal carriers the presence of the nanoparticles often complicates the determination of free drug in the release medium. Nevertheless, several methods have been suggested in order to evaluate the drug transfer. These include sample and separate (Boyd, 2003; Cui et al., 2006; D'Souza and DeLuca, 2006; Hu et al., 2002; zur Mühlen et al., 1998), membrane-barrier (Araújo et al., 2011; D'Souza and DeLuca, 2006; Henriksen et al., 1995) and continuous flow methods (D'Souza and DeLuca, 2006; Washington, 1990). All these techniques offer the possibility to determine the drug content in a particle-free medium. However, these methods do often not appropriately reflect the in vivo drug transfer. The release rates obtained may be distorted due to, for example, filter clogging, drug binding, limited size of surface area and insufficient time resolution (Boyd, 2003; Chidambaram and Burgess, 1999; D'Souza and DeLuca, 2006; Levy and Benita, 1990; Magenheim et al., 1993; Washington, 1990). To avoid such problems in situ methods can be applied. In this way, it is possible to analyze the drug directly within the nanoparticle-containing release medium. Unfortunately, only a limited number of substances are accessible by in situ techniques (Landry et al., 1997; Rosenblatt et al., 2007; Salmela and Washington, 2014; Washington, 1990).

As a further complication, the aqueous release media usually applied in the in vitro drug release techniques described above do not represent the situation in the bloodstream well, which contains lipophilic binding sites. This is particularly relevant in cases where water does not represent a good solvent for the drug of interest. Especially in order to investigate the release of lipophilic drugs from colloidal carrier systems, the release medium should

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thus be modified. For example, in some studies albumin (Magenheim et al., 1993), surfactant (Allémann et al., 1993; Hsu et al., 2003; Hu et al., 2002) or organic solvents (Chen et al., 2001) were added to the release media to improve the solubility of the released lipophilic drugs. However, to approximate in vivo-like release conditions with special regard to intravenous administration, lipophilic components should be present in the release medium. For instance, Jenning et al. (2000) and Wissing and Müller (2002) presented an approach in which the transfer into a bulk phase of liquid triglycerides is investigated. However, due to the small interfacial area and long diffusion pathways the drug transfer is very slow in such experiments (Petersen et al., 2010). Several investigators tried to mimic more physiological conditions by applying lipophilic acceptor compartments in the nanometer and micrometer range. Thus, Shabbits et al. (2002) used multilamellar vesicles as acceptor particles and applied a centrifugation technique to separate them from the donor system. Much earlier Hellings et al. (1974) and van den Besselaar et al. (1975) performed transfer experiments applying liposomes as acceptor particles. Separation of donor and acceptor system was achieved by application of an ion exchange column technique. A disadvantage of these two techniques is the need for separation between the donor and acceptor particles. During separation, the transfer may continue. Very rapid transfer rates cannot be accurately measured in this way. Petersen et al. (2010) developed a method which does not require a separation step. They investigated the transfer from carrier particles into oil-in-water emulsion droplets using flow cytometry to detect the transferred substance. However, this method relies on fluorescence detection and is consequently not applicable for the investigation of most drugs.

The aim of this study was to develop a technique which enables the investigation of the drug transfer from lipid nanoparticle carrier systems to lipophilic acceptor particles and which (a) is easy to handle, (b) is close to physiological conditions and (c) has no restrictions regarding the drug under investigation. To address this task, a lipid nanoparticle suspension was incorporated into small Ca-alginate hydrogel microbeads which served as acceptor system. The preparation of such lipid-containing microparticles has been described earlier (Strasdat and Bunjes, 2013). A trimyristin nanoemulsion was applied as donor system. The emulsion was loaded with the fluorescent drug model Nile red which was chosen to allow for an easy and quick dye content determination in the donor system by fluorescence spectroscopy. Entrapment of the acceptor nanoparticles into hydrogel beads allows for the separation of donor and acceptor particles at different transfer time points (Fig. 1). The influence of the size of the lipid-containing hydrogel beads on the transfer of Nile red from the trimyristin lipid emulsion was investigated in order to find the most suitable conditions for such transfer experiments.

2. Material and methods

2.1. Materials

The triglyceride trimyristin (Dynasan 114) was donated by Condea Chemie, Witten, Germany, the surfactant tyloxapol and glycerol anhydrous were obtained from Sigma–Aldrich, Steinheim, Germany, and thiomersal was purchased from Caesar & Loretz, Hilden, Germany. Sodium alginate (Protanal® LF 20/40) was a kind gift of FMC BioPolymer, Girvan, Scotland. Calcium chloride anhydrous was from Roth, Karlsruhe, Germany. Nile red (9-(diethylamino)-5H-benzo[α]phenoxazin-5one) was purchased from Acros Organics, Geel, Belgium. The solvent ethanol was obtained from Sigma–Aldrich, Steinheim, Germany. All materials were used as received. Purified water was prepared by filtration and deionization (EASYpure™ LF, Barnstead, Dubuque, IA, USA).

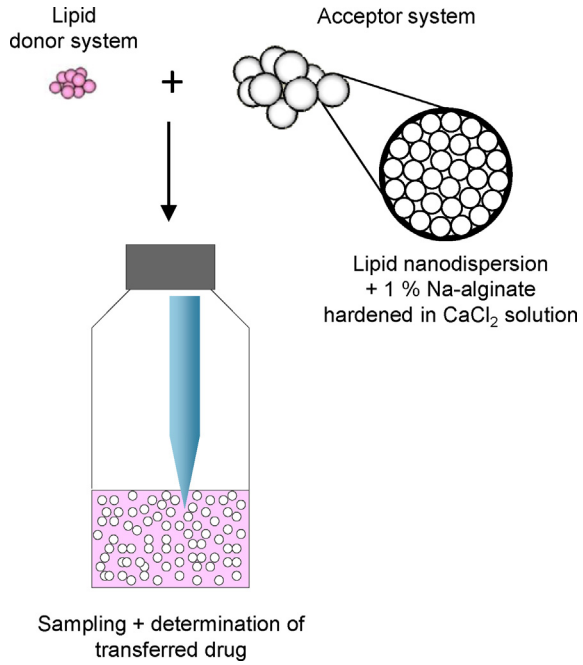

Fig. 1. Experimental approach: Trimyristin nanoemulsion droplets loaded with Nile red were mixed with nanoparticle-containing microbeads. At a given time point, the nanomeulsion was withdrawn from the vial and the content of Nile red remaining in the droplets was determined.

2.2. Methods

2.2.1. Preparation of donor and acceptor lipid nanoparticles

The lipid nanodispersions contained 10% trimyristin, 6% tyloxapol and an aqueous phase preserved with 0.005% thiomersal and isotonized with 2.25% anhydrous glycerol (w/w concentrations). A preemulsion was prepared from molten trimyristin (70 °C) and a preheated tyloxapol-containing aqueous phase (70 °C) with an Ultra-Turrax (T25, IKA, Staufen, Germany; 3 min, 17,000 rpm). The mixture was subsequently processed for 5 cycles by high-pressure homogenization (Micron Lab 40, APV Gaulin, Lübeck, Germany) at 1200 bar and 70 °C. Afterwards, the hot emulsion was filtered through a polyethersulfone filter (0.22 μm, Roth, Karlsruhe, Germany) and was allowed to cool to room temperature. Under these conditions, the nanoparticles remained in a liquid state due to supercooling. One fraction of the dispersion was stored at about 4 °C (refrigerator). At this temperature the formulation transformed into a dispersion of crystalline, platelet-like particles (Westesen, 2000; Westesen and Bunjes, 1995; Westesen et al., 2001). This lipid nanoparticle suspension served as incorporated acceptor system. The lipid nanoparticle emulsion was stored at 20 °C until it was used as donor system for the transfer studies.

For the transfer experiments the nanoemulsion was loaded with Nile red (8 μg/ml). An ethanolic dye stock solution (1 mg/ml) was evaporated in a glass vial leaving a thin film of Nile red on the glass. After addition of the lipid nanoemulsion the glass vial was shaken at room temperature for at least 24 h resulting in complete dissolution of Nile red in the emulsion.

2.2.2. Preparation of acceptor hydrogel particles

Ca-alginate beads were prepared as described earlier utilizing two different production methods (Strasdat and Bunjes, 2013). The particles were either formed by an electrostatic droplet generation technique (Bugarski et al., 1994; Poncelet et al., 1999) or by spraying using the two-fluid spray nozzle (diameter: 0.7 mm) of a BÜCHI Mini Spray Dryer B-191 (BÜCHI Labortechnik AG, Flawil, Switzerland). Briefly, in the first case, a mixture of lipid