International Journal of Pharmaceutics 447 (2013) 31–37

Contents lists available at SciVerse ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Pharmaceutical nanotechnology

# Dual asymmetric centrifugation as an alternative preparation method for parenteral fat emulsions in preformulation development

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ARTICLE INFO

*Article history:*
Received 14 November 2012
Received in revised form 6 February 2013
Accepted 8 February 2013
Available online 21 February 2013

*Keywords:*
Dual asymmetric centrifugation
Fat emulsions
Static light scattering
Hemolytic activity
Emulsion stability

ABSTRACT

Nanoscaled fat emulsions are well established as a drug delivery system for lipophilic drugs and for the use in parenteral nutrition. Typically, the production of nanoscaled fat emulsions requires several formulation steps, including high pressure homogenization and filtration. The applicability of dual asymmetric centrifugation as an alternative technique to produce submicron fat emulsions in a short and easy way was investigated. The emulsions could be prepared without substance loss in a closed system within 30 min. Formulations with 10% soybean oil and up to 5% emulsifier-mixture were produced. The droplet size distribution was determined by static light scattering. Stability over six months was shown by regular static light scattering measurements and determination of the zeta potential. Furthermore, hemolytic activity of the samples was investigated. With the dual asymmetric centrifugation physiological tolerable emulsions with droplets in the lower micron and submicron range could be prepared. This method could be used as a model for screening active pharmaceutical ingredients.



## 1. Introduction

Nanoscaled fat emulsions containing lecithin as emulsifier and vegetable oils as lipophilic phase have a long tradition in parenteral nutrition and as drug carriers for poorly soluble lipophilic drugs (Collins-Gold et al., 1990; Hippalgaonkar et al., 2010). For a long time they have been on the market and intensive scientific work was done on this topic (Koster et al., 1996). As the application way is intravenously, the droplet size needs to be in the nanoscale range to prevent embolism. However, the limitations in particle size for parenteral emulsions are not specified (Koster et al., 1996). In most cases parenteral fat emulsions are produced by high pressure homogenization (HPH) that is an effective, but labor-intensive procedure. In general, the preparation includes four manufacturing stages: heating up to 80 °C and stirring, primary emulsification with a high-shear mixer, high pressure homogenization and filtration of the product (Benita and Levy, 1993). The amount of material needed for one sample is respectively high. For the development of a new drug delivery system based on fat emulsions, smaller sample sizes are preferred, especially in the preformulation development. In addition a fast and easy to handle technology could help to improve and shorten the screening process for active pharmaceutical ingredients. It is the aim of the present study to explore the potential of dual asymmetric centrifugation (DAC) as an alternative technique for the use in pharmaceutical preformulation development. Furthermore, the homogenization process via dual asymmetric centrifugation is supposed to be gentle with respect to sensitive substances. Some successful attempts to prepare vesicular phospholipid gels with encapsulated proteins or siRNA were made (Tian et al., 2010; Hirsch et al., 2009; Adrian et al., 2011), but other dosage forms have not been investigated yet. The article describes the preparation and characterization of submicron fat emulsions with DAC.

## 2. Materials and methods

### 2.1. Materials

Soy lecithin containing at least 75% phosphatidylcholine (Lipoid® S 75) was kindly provided by Lipoid GmbH (Ludwigshafen, Germany). Soybean oil was purchased from Caelo GmbH (Hilden, Germany), sodium oleate from Riedel-de Haen (Seelze, Germany) and Lutrol® F 68 (Poloxamer 188) from BASF (Ludwigshafen, Germany). Double distilled water preserved with 0.02% sodium azide and charged with 2.2% glycerol or 4.5% sorbitol (Jumaa and Müller, 1999) was used. All the chemicals used were reagent-grade. Lipofundin® 10% N, which was considered as a reference emulsion, was kindly provided by B. Braun Melsungen AG (Melsungen, Germany). It contains 0.8% egg-yolk-lecithin (phosphatidylcholine

*Abbreviations:* DAC, dual asymmetric centrifugation; HPH, high pressure homogenization; SLS, static light scattering.

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http://dx.doi.org/10.1016/j.ijpharm.2013.02.033

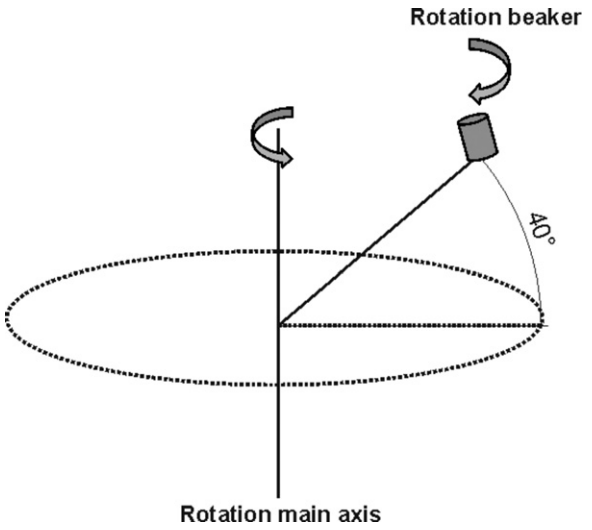


**Fig. 1.** Rotation principle of a dual asymmetric centrifuge modified after Tian et al. (2010) and Massing et al. (2008).

content ≥75%), 10% soybean oil, 2.5% glycerol, α-tocopherol, sodium oleate and water for injection (B. Braun Melsungen AG, 2007).

### 2.2. Dual asymmetric centrifugation

The SpeedMixer™ DAC 150 SP (Hauschild & Co. KG, Hamm, Germany) was used for sample preparation. With the DAC technology, samples up to 150 g can be prepared. The speed can be varied from 300 rpm to a maximum of 3500 rpm (Hauschild, 2008). In principle the rotational moves are similar to a standard centrifugation process. According to this, the sample rotates backwards around itself, which leads to an overlaid agitation of the inward and outward movement of the sample (Fig. 1) (Massing et al., 2008; Tian et al., 2010). Hence, a homogeneous mixture of the ingredients can be achieved within a short time.

### 2.3. Preparation of fat emulsions with dual asymmetric centrifugation

Comparable to the reference emulsion Lipofundin® 10% N, the formulation for the SpeedMixer™ emulsion contained soybean oil (10%), soy lecithin (up to 2.5%) and one or two additional emulsifiers (up to 2.5%). The aqueous phase was isotonized with glycerol or sorbitol (Jumaa and Müller, 1999). An amount of 0.02% sodium azide was used for microbial stability (De Vleeschauwer and Van der Meeren, 1999). In preliminary tests, the formulation procedure was investigated and the resulting optimized preparation way is presented in Fig. 2. Soybean oil and the stabilizers were heated together at 55 °C. At the same time the aqueous phase was heated separately at 55 °C and was added to the lipophilic phase sequentially. All in all, three mixing steps were accomplished and the whole mixing time did not extend 2.5 min. No improvement in particle size reduction was found for longer mixing times (see Supplementary data). For the mixing speed, two velocities were used due to better homogenization. Every experiment was done at least in triplicate and an overall sample volume of 5 ml was prepared.

### 2.4. High pressure homogenization

The oil phase and the aqueous phase were heated separately at 55 °C. The ingredients were united in a beaker and mixed with an ultra turrax (IKA, Staufen) at 14,000 rpm for 3 min to gain a coarse emulsion. Final emulsification was carried out by passing the coarse emulsion through a two stage high pressure homogenizer (Stansted Fluid Power Ltd.) three times with a pressure of 50 MPa and one time with a pressure of 25 MPa. All in all a sample volume of 50 ml was prepared.

### 2.5. Static light scattering

The samples were analyzed by a Mastersizer 2000 with a Hydro 2000 S automatic dispersion unit and submicron instrumentation (Malvern Instruments, Worcestershire, UK). The measurements were performed at room temperature with purified water as diluent. There were 5 runs for each sample with a measurement time of 10 s. The Mie theory with a refractive index of 1.473 (Malvern Instruments, 1997) and an absorption of 0.001 (Morales Chabrand et al., 2008) was used for the calculation of the particle size.

### 2.6. Zeta potential

The samples were diluted 1:50 (v/v) in 0.067 M phosphate buffer pH 7.4. For the measurements, the Zetasizer Nano ZS (Malvern Instruments) was used. Every sample passed five measurement cycles at 25 °C with a delay of 20 s between each measurement.

### 2.7. Storage stability

To investigate the long time stability, the emulsions were stored at 4–6 °C. Directly after preparation and after 3 and 6 months the droplet size distribution was determined by static light scattering (SLS) and the appearance of the emulsions was described. Some emulsions showed a creaming effect after storage which is characterized by Collins-Gold et al. (1990) as a readily reversible, slow flotation of lipid droplets on the denser aqueous phase. These samples were shaken gently before the measurement to achieve a homogeneous dispersion. Additionally, the zeta potential of the emulsions was determined directly after preparation and after 6 months.

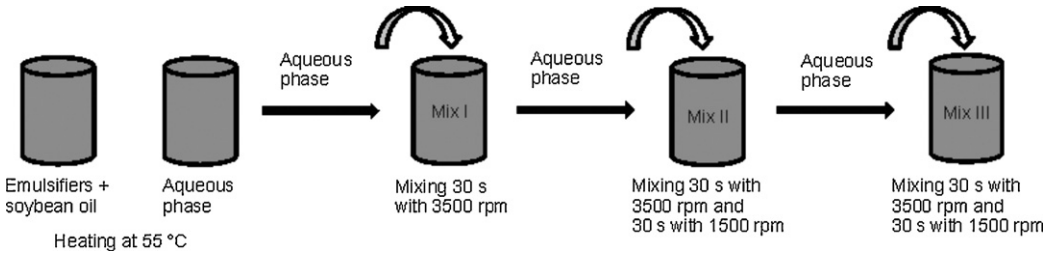


**Fig. 2.** Preparation process for fat emulsions with the DAC technique.