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Clinical Batch Production **of Peptide-loaded Nanoparticles**

Modern biotech drugs, e. g. peptides and proteins, generally require a suitable delivery system to be used in therapy and be placed on the pharmaceutical market. Particulate delivery systems such as microparticles are already on the market, solid nanoparticles are under development. To test new upcoming delivery systems in vivo, qualified medium scale production units to produce clinical batches for human trials are essential. A newly developed clinical production unit for the production of peptide-loaded lipid nanoparticles is presented. The design of the system was chosen to allow qualification and validation, production capacity is up to 10 kg particle dispersion within 20 minutes.

1 *Basic design of the LAB 60-based clinical production unit for lipid nanoparticles (F – feeding container, P – product container, DD – dissolver disk).*

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Pharmaceutical biotechnology offers the perspective to design and produce new drugs tailor-made [1]. However, to exploit many of these drugs in therapy they need to be formulated into an appropriate delivery system [2]. Microparticulate formulations have been introduced to the market as delivery systems for e. g. LH/RH analogues (e. g. Decapeptyl®). A new upcoming area are solid nanoparticles (SLN) for various delivery routes, e. g. ranging from oral administration to parenteral injection.

Polymeric nanoparticles investigated for about 30 years did not lead to a breakthrough, products based on polymeric nanoparticles are practically not existing on the market. The reasons for this lack of success are manifold, a major reason is definitely the lack of suitable (and simultaneously cost-effective) production methods yielding a product of a quality acceptable by the regulatory authorities. In addition these production lines need to be able to be qualified and validated [3]. As alternative system to polymeric nanoparticles the patent protected solid lipid nanoparticles (SLN) have been developed at the beginning of the nineties [4-6]. A major advantage is the simplicity of the production technique including easy scale up.

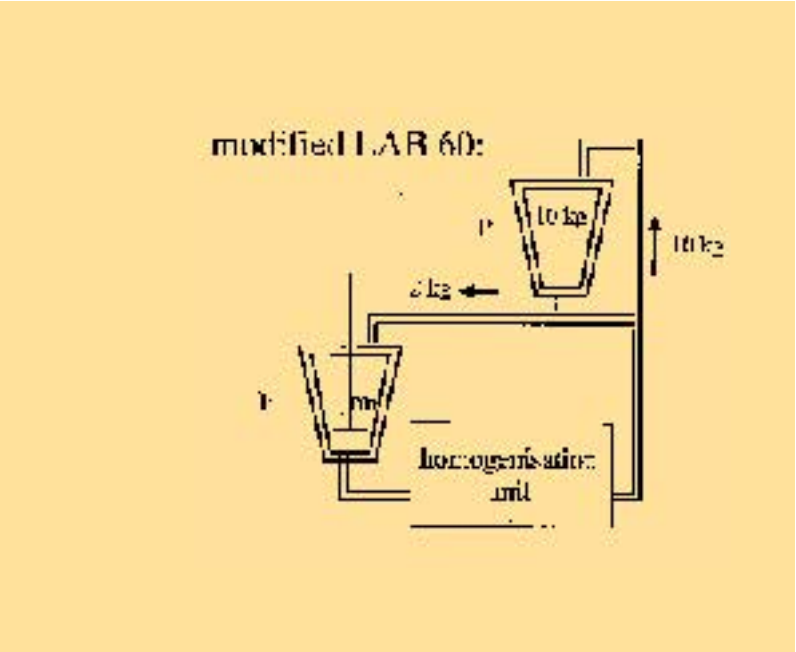
Being a new technology the first developments focussed on high sales drugs. For oral administration the peptide cyclosporine A has been formulated as SLN dispersion [7, 8]. With a total annual turnover of about 1.3 billion US$ for the existing products Sandimmun and Sandimmun optoral, this peptide drug is of high commercial interest. The basic formulation of cyclosporine SLN was developed on lab scale producing batches of 40 g particle dispersion (10% solid content). For the conductance of the first human clinical study a larger batch volume was required leading to the development of a clinic production system with a minimum capacity of 2 kg and maximum capacity of 10 kg dispersion [9]. The production system was suitable for qualification and validation [10], being prerequisites to use it for clinical batch production.

Lab-scale production of cyclosporine-loaded SLN

Preparation of cyclosporine-loaded SLN is a very simple process. As lipid matrix Imwitor 900 was used. The lipid was melted at approx. 80°C, the cyclosporine dissolved in the lipid melt and the drug-lipid solution dispersed in a hot aqueous surfactant solution by stirring. As surfactant mixture 2.5% Tagat S and 0.5% Sodium cholate were employed. The obtained coarse pre-emulsion was then passed through a high pressure homogeniser (LAB 40, APV Homogeniser GmbH, Lübeck, Germany) at 500 bar for three cycles. The homogenisation itself is performed at elevated temperature, that means the lipid is dispersed in its melted state. The LAB 40 was equipped with a temperature control jacket. The mean particle size obtained by this method is typically in the range between 100 nm to a mean diameter up to 300 nm.

Design of clinical batch production unit

To produce the larger batch sizes required for the clinical study, a Micron LAB 60 was modified and equipped with special features. The LAB 60 has a capacity of producing 60 l homogenisation product per hour. The homogenisation unit can be temperature-controlled allowing exact processing. The homogeniser LAB 60 bought from the shelf was equipped with a double-walled 10 kg feeding container and a second 10 kg double-walled product container. The connecting types were all double-walled allowing temperature control by a water flow. The basic design of the system is shown in figure 1, the original system in figure 2. It shows a photograph of the clinical batch unit. It is especially designed that it can be placed underneath a laminar flow unit. The dimensions of the production unit are 1 m x 1.2 m in square and 1.8 m in height. Therefore it can be placed underneath commercially available LAF units fixed to the ceiling of a laboratory. To make it suitable for pharmaceutical production only pharma grade materials were used, surfaces were electro-polished etc. A documentation was prepared for qualification of the unit. One production unit is placed in the company Pharmatec in Milan and available for contract production.



2 *Clinical production unit based on modified LAB 60 (for details of unit cf. figure 1).*

Production of clinical batches

Production of a 2 kg batch: The cyclosporine-containing lipid melt is dispersed in the hot aqueous surfactant solution in the feeding container using a dissolver disk, that means the coarse emulsion is directly produced inside the unit. Then the pre-emulsion is passed through the temperature-controlled homogenisation unit and circled back directly to the feeding container. It is a continuous process in a circle. The feeding container and the double-walled pipes are all heated to 80°C. Based on theoretical consideration, it takes about 15 minutes to ensure that 99.9% of the emulsion droplets have passed the homogenisation unit at least once. Production is completed after 15 minutes of circulation. The mean photon correlation spectroscopy diameter of the product is 184 nm. Laser diffractometry analysis showed that 95% of the particles were below 0.69 µm, that means the nanoparticle dispersion contains only a low amount of microparticles.

Production of a 10 kg batch: This larger batch is produced in a discontinuous way. The pre-emulsion is again prepared in the feeding container, the total volume being now 10 l (approx. 10 kg). The pre-emulsion is passed through the homogenising unit and collected in the product container. After this first cycle the product is fed back to the feeding container through a temperature-controlled double-walled pipe by gravity. The emulsion is passed a second time through the homogeniser, collected in the product container and cooled down to room temperature. To produce 10 kg dispersion by running two homogenisation cycles takes about 20 minutes.

Final formulation for the pharmaceutical market

The aqueous SLN dispersion can be used directly as a "drink suspension". Alternatively the SLN can be incorporated into solid dosage forms, e. g. tablets or capsules. The SLN dispersion can be used as granulation fluid for granulation to produce tablets or as wetting liquid for the extrusion mass for pellet production.

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