

Size Reduction of Liposomes with a novel Type of Stirred Bead Mill (Nanomill)

The potential of liposomes as carriers for drug delivery and drug targeting has frequently been reported but the production of liposomal formulations on an industrial scale remains a challenge. Consequently a novel nano-milling unit has been developed and investigated. The new nanomill fulfils the GMP standards of the pharmaceutical industry and remains entirely closed also when cleaned, sterilised and prepared for another batch.

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Introduction

Over the last years the application of liposomes in the pharmaceutical, cosmetics and food industries has elicited novel breakthroughs and products [1]; e.g. the encapsulation of cheese ripening enzymes in liposomes [2], skin care preparations for the treatment of dry skin [3] and different drug delivery systems [1]. Clinical applications in the field of drug delivery and passive targeting of solid tumors have demonstrated their potential for pharmaceutical applications. However, the production of liposomal formulations on an industrial scale remains a challenge. Ideally, an industrial production scheme should be versatile, simple, robust and cost-effective. Up to now, the large scale production of liposomes with a definite size requires high pressures and high shear rates; this entails the disadvantage of generating mechanical and thermal stress at the same time.

This research project had two major targets: The development of a novel type of nanomill dedicated to the production of liposomes and the determination of the influence of the main operating parameters necessary to form liposomes. Therefore multi-lamellar large vesicles were stressed in the novel “Concept Mill”, patent pending, (Willy A. Bachofen AG, Muttenz, Switzerland) which has been developed not only to meet the requirements of liposome production but also the requirements of the pharmaceutical industry with regard to the nanomilling of poorly water soluble drug substances.

Results

Stirred bead mills consist of a closed cooled cylindrical vessel fitted with a stirrer and filled with grinding beads–the milling chamber. The remaining volume in the milling chamber is filled with a suspension of the product to be ground, in this case with the pro-liposome solution containing multi-lamellar large vesicles. Stirrers with different geometries can be used for the agitation of the beads and the suspension. But the core of the “Concept Mill” is an entirely new process chamber, which is integrated into a milling unit ready for production on the kilo-scale.

Furthermore the milling unit fulfils the high good manufacturing practice (GMP) standards of the pharmaceutical industry and remains entirely closed, even also when the unit is being prepared for another batch. This is a world first. The design of this innovative process chamber underwent a radical change: vertically arranged, ready for gravimetric filling with grinding beads, suspension flow from bottom to top and conversely, top down unloading of grinding beads. The chamber has been optimized with regard to the



Figure 1 New reverse flow milling chamber



Figure 2 Prototype of nano-milling unit

flow conditions both in milling mode and in emptying mode. Complete emptying is ensured by the patented WAB DYN0 accelerators which generate a reverse flow inside the process chamber strong enough to carry all grinding beads out of the closed system. Furthermore, the unit is ready for cleaning and sterilization in place (CIP/SIP). The unit is designed to reduce the size of liposomes down to approximately 100 nm as well as to load them, e.g. with poorly water soluble drug substances. For this purpose experiments were conducted in circular mode with yttrium stabilized zirconium grinding beads. The beads ranged from 180 to 250 µm in diameter; this interval was found to be optimal for our purpose as it was possible to achieve a higher percentage of product liposomes smaller than 105.7 nm at a sustainable specific energy input. Approximately 65% of the 500 ml cylindrical mill chamber was packed with grinding beads and filled with the pro-liposome solution. The temperature in the double jacket mill chamber can be controlled by an external oil thermostat.

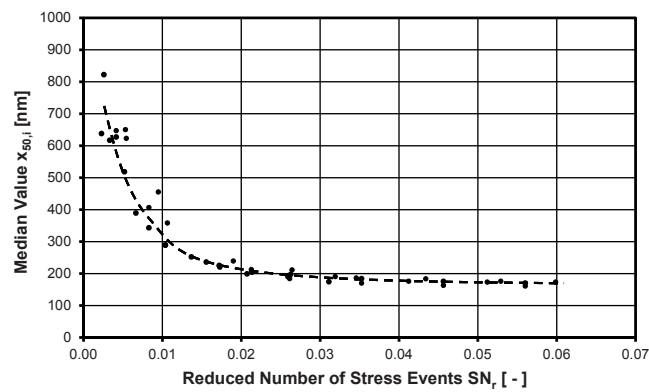


Figure 3 Comparison of liposome size reduction result vs. reduced stress number SN_r

The pro-liposome solution with a concentration of 10 mmol/l was produced by thin lipid film hydration. First ~60 % phosphatidylcholine (PC) from egg yolk and 95 % cholesterol were dissolved in a mixture of chloroform and methanol in a 2:1 ratio. The solvent was removed by evaporation under vacuum at 50°C. A thin lipid film remained on the side of the round-bottomed flask, which was then hydrated with an adequate amount of phosphate buffered saline (PBS, pH 7.4) at 50°C. The particle size of the liposomes was analyzed by dynamic light scattering using a Zetasizer Nano ZS.

The new stirred bead mill allows gentle stressing of the liposomes under controlled conditions. The size of liposomes produced correlates directly to the specific energy brought into the liposome suspension, which is the energy input into the milling chamber divided by the stressed product mass. Consequently, the size of the liposomes can be controlled by the specific energy input. This energy input is affected by the stirrer tip speed, the geometry of the stirrer, the grinding beads and the filling ratio of grinding beads [4].

The specific energy $E_{m,p,r}$ understood as an integral parameter, describes the influence of different operating parameters. It can be also defined as the product of stress energy provided by the grinding beads (SE_{GB}) and the number of stress events (SN_p).

In order to obtain the optimal set of operating parameters in case of nano-milling of inorganic materials the optimum stress energy has to be determined first: The question “*Is the energy for particle breakage high enough?*” has to be answered as the stress energy is dominant for each milling event. However, for organic materials, especially for structures like cells, another concept leads to promising results [5]: the required stress energy for cell disintegration is very low because the strength of such organic particles is weak and therefore the number of stress events is dominant. Since liposomes are more similar to cells than to inorganic crystals, a reduced number of stress events (SN_r) has been proposed to describe the result of the size reduction of liposomes:

with: SN_r [-] := reduced stress number
 ϕ_{MK} [-] := filling ratio of beads
 ϵ [-] := porosity of bead fill
 n [s⁻¹] := stirrer speed
 t [s] := time of size reduction
 x [m] := product size/diameter of liposomes
 d_{GB} [m] := diameter of grinding beads

The result of size reduction in a stirred bead mill can be fully described by the concept of the stress number (the more stressing events, the better the result), independent from the stress energy exchanged at each milling event inside the mill, fig. 3.

Conclusion and Outlook

During this study the influence of the main operating parameters necessary for the size reduction of liposomes with nanomills was determined. It has been revealed that the result of size reduction with this type of mill can be described by the concept of the stress number which, consequently, can be used to control the size reduction of soft organic particles. Furthermore this mill has been developed not only to meet the requirements of liposome production but also the requirements of the pharmaceutical industry with regard to the nano-milling of poorly water soluble drug substances. The novel nanomill was developed, built and presented at the ACHEMA 2012.

References:

- [1] Lasic D. Novel applications of liposomes. TIBTECH 1998; 16: 307-321
- [2] Sagalowicz L, Leser M. Delivery systems for liquid food products. Current Opinion in Colloid & Interface Science 2010;15 (1-2): 61-72
- [3] Patravale V, Mandawgade S. Novel cosmetic delivery systems: an application update. International Journal of Cosmetic Science 2008; 30 (1): 19-33
- [4] Kwade A (Editor). Grinding and Dispersing with Stirred Media Mills, Basic Course 2009, iPAT, TU Braunschweig
- [5] Bunge F. Mechanischer Zellaufschluss in Rührwerkskugelmöhlen, Dissertation 1992, TU Braunschweig

Research Focus Area:

Therapeutic Technologies (TT)

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Economic efficiency and benefit to society:

The novel nanomill fulfils the high GMP standards of the pharmaceutical industry and is ready for SIP/CIP as this unit remains closed for filling, bead and product emptying, or cleaning. This new milling unit allows fast batch changes and short cycle times so that production can be more efficient and cost-effective.