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Small scale liposome extruder

Present invention relates to an apparatus for extruding liposomes and a process for producing liposomes by means of that apparatus.

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Liposomes are essentially spherical structures having a diameter amounting from 25nm to 1µm. They comprise one or more concentric lipid bilayers that enclose an aqueous interior, the so-called lipid vesicle. Liposomes are produced by dispersing lipids in aqueous solution. Suitable lipids are in particular phosphatidylcholines (lecithins), phosphatidylethanolamines or phosphatidylserine (cephalins) Liposomes are used as carriers for pharmaceutical, cosmetic or nutraceutical active substances that are selectively enriched in certain organs and cell groups.

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An overview of processes to prepare liposomes is given by Guo et al:

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Guo, P., Huang, J., Zhao, Y., Martin, C. R., Zare, R. N., Moses, M. A.: Nanomaterial Preparation by Extrusion through Nanoporous Membranes. Small 2018, 14, 1703493. DOI: 10.1002/smll.201703493

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One crucial characteristic of liposomes is their size distribution. In particular, liposomes for use in pharmaceutical, cosmetic or nutraceutical applications need to have a size distribution within a dense range. This means, that actual size of an individual liposome shall not deviate significantly from a specified average. For that reason, defining a proper size is an important step during preparation of liposomes.

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A common approach for sizing liposomes is extrusion. According to this, a feed dispersion comprising liposome precursors dispersed in a liquid dispersion medium is prepared first. This feed dispersion is subjected to a fluid pressure. The pressurized feed dispersion is subsequently extruded through a porous filter element having a defined pore size. During extrusion, the size of the precursor liposomes is reduced. Beside pore diameter, energy input is a crucial parameter.

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Liposomes are fluidic systems. They can pass e.g. a 100 nm membrane and form particles at a diameter of 250 nm afterwards. For this reason, there are generally more than one pass through the extruder is required for sizing Such liposome extrusion process is commonly understood as a pure physical operation without chemical interaction. Thus, the chemical composition of target liposomes is already given by liposome precursors.

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In commercial liposome extrusion processes, dense polycarbonate plates are used as filter elements. The porosity of these items usually amounts from 100 nm to 5 µm.

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The size of precursor liposomes is typically in the range from 50 nm to 10 µm. To effect extrusion of precursor liposomes through dense filter elements, elevated pressures are necessary. Mid

pressure processes are working with fluid pressures ranging from $20 \cdot 10^5$ Pa to $40 \cdot 10^5$ Pa (20 to 40 bar), while high pressure processes achieving nominal fluid pressures amounting from $100 \cdot 10^5$ Pa to $200 \cdot 10^5$ Pa (100 to 200 bar).

- 5 Such a filter holder for extrusion of liposomes extrusion is described in WO 2021/207841 A1, including a housing having an inlet configured to receive a material to be extruded and an outlet, and a filter support member disposed within the housing between the inlet and the outlet. The filter support member includes an upstream side having a filter support surface configured to support a membrane filter assembly, a downstream side opposite the upstream side, and a plurality of
- 10 passages extending through the filter support member from the filter support face to the downstream side.

- The filter holder also includes an outlet cavity in fluid communication with the outlet, and the filter holder is configured such that the material to be extruded flows through the membrane filter
- 15 assembly and into the outlet cavity via the plurality of passages before being discharged through the outlet. A comparable liposome extruder is known from Figure 4 and related description of WO 01/05373 A1.

- An apparatus for performing such liposome extruding process is called liposome extruder.
- 20 Essential compounds of a liposome extruder are a vessel to which the liquid dispersion of the precursor is fed to means for applying a fluid pressure on that precursor contained in the vessel and the porous filter element, through which the precursor is extruded out of the vessel.

- For bearing elevated pressures, mechanical design of liposome extruders must be tough enough.
- 25 For reasons, extruder design must be able to contain an inner pressure even higher than extrusion pressure. Due to common rules on pressure vessels, the extruder is preferably designed to stand an increased inner pressure, which is higher by a factor between 1.3 and 1.7 than the nominal operational pressure. Thus, liposome extruders need to be designed very heavy to keep such high pressures.

- 30 Examples of liposome extruders are known from WO 2021207841 A1 (entire document) or from Fig. 4 of WO 0105373 A1. Both items are equipped with a pressure chamber composed of two hemispheres, namely a fixed socket and a ring segment which is releasably coupled with the socket. The pressure chamber has a one-way inlet defined by a backpressure-valve and an outlet
- 35 which is defined by a filter element. The precursor feed is introduced through the inlet into the pressure chamber. In the pressure chamber, the feed is subjected to fluid pressure. The latter drives precursor mixture through the filter element out of the pressure chamber to facilitate extrusion.

In simplified operation, liposome sizing processes utilizing such extrusion apparatuses run until filter element loses penetrability due to clogging. Then, apparatus is depressurized, ring segment and socket are released from each other, cap is removed, filter element with reduced penetrability is replaced by a fresh filter, cap and socket are recoupled with each other, apparatus is
5 repressurized and liposome extrusion is continued.

Liposome formulations for pharmaceutical, cosmetic or nutraceutical applications are subject to diligent product development. In course of this, many tests or evaluation trails of extrusions need to be performed for optimizing formulation and process parameters. Thus, individual extrusion time of
10 a particular formulation at a particular set of process parameters is much shorter than in industrial scale production. Since extruder needs to be cleaned and filter element needs to be exchanged after every trial, the frequency of depressurizing, exchanging filter element and repressurizing is significantly higher than in industrial scale extrusion. For pharmaceutical application when handling high potent drugs (like e.g. the anti-cancer drug doxorubicin) it is also necessary to conduct the
15 extrusion under secondary containment to avoid contamination of production personal. Changing filter elements in conventional extruders under secondary containment is a time consuming and difficult procedure with a risk of spilling events.

Utilizing production extruders for trials is inefficient as the heaviness of the high-pressure chamber
20 requires a cumbersome handling: The time needed for removing heavy cap of pressure chamber is too long compared with extrusion time.

Considering this, there is a need to provide an apparatus for extruding liposomes in small scale during product development. In particular, the time required for depressurizing chamber, removing,
25 and replacing filter element and repressurizing shall be minimized. The production capacity of intended apparatus, however, shall be lower than those of extruders used in commercial production of liposomes.

This object is solved by an apparatus for extruding liposomes comprising the following
30 components:

- means for applying a hydraulic pressure P on a liquid medium, wherein said hydraulic pressure P amounting from $20 \cdot 10^5$ Pa to $200 \cdot 10^5$ Pa;
- a frame comprising
 - 35 a. vertical lifting unit (8);
 - b. feed line in fluid connection with a tempering chamber located in a housing, wherein the housing comprises protrusion extending parallel to a central axis (A), wherein said housing is mounted on said frame;
 - c. a pressure chamber comprising an outer cartridge, the protrusion of the
40 housing, a feed orifice, an inner collar for mounting at least a filter element,

wherein

- the cartridge is linked to an outlet line and mounted on a socket movable in direction of the central axis (A) and being releasably coupled for an overlapping range with the protrusion, wherein said socket is vertically guided by said vertical lifting unit, wherein said pressure chamber is designed for bearing said hydraulic pressure P

- at least one porous filter element defining an outlet of said pressure chamber and/or the cartridge, wherein said filter element comprises open pores having a diameter D , wherein said pore diameter D amounting from $80 \cdot 10^{-9}$ m to $50 \cdot 10^{-6}$ m or from $100 \cdot 10^{-9}$ m to $5 \cdot 10^{-6}$ m;
- a lifting mechanism as part of the lifting unit designed for elevating and lowering the socket;
- wherein said filter element is contained in a (filter) cartridge which is removably mounted to the socket;
- and wherein said lifting mechanism comprises at least one rack and pinion gear, wherein the pinion is pivot-mounted in said lifting unit which is mounted in said frame and the rack is vertically guided.

The pressure chamber and the feed line are preferably designed for bearing an inner hydraulic pressure P_b calculated by $P_b = S \cdot P$, with S being a reasonable security factor as defined in commonly known handbooks and regulation. Therein S is preferably selected from the range extending from 1.1 to 2.5, most preferably from 1.3 to 1.7.

According to a preferred embodiment, the frame comprising downside the vertical lifting unit, and upside the feed line in fluid connection with a housing comprising a tempering chamber located in the top of a housing, wherein the housing comprises downside the protrusion extending parallel to a central axis A, wherein said housing is mounted on said frame.

The feed line is preferably in fluid connection with a housing comprising a (inner) tempering chamber located in the top or top section of a housing.

Preferably, the outlet line leads the extruded liposomes (product) from the pressure chamber downstream to additional treatment units, such as, but not limited to, e.g. a collection vessel, control unit, purification station, confectionery station.

According to a preferred embodiment, the cartridge is linked to an outlet line and mounted on a socket linear, preferably vertically, movable in direction of the central axis (A).

The pressure chamber, in closed position, preferably comprising the outer cartridge, the inner protrusion of the housing, the feed orifice leading into the protrusion with its outlet orifice on the first

side of the filter element, cartridge with the inner collar for mounting the at least one filter element and the outlet orifice on the opposite side of the at least one filter element. Thus, outlet orifice of the protrusion and (final) outlet orifice of the pressure chamber are on opposite sides of the pressure chamber and/or the filter element. As an improved embodiment, at least one gasket
5 element is located on the inner collar and/or on the filter element, wherein the gasket element might be a sealing ring. Gasket element and filter element are preferably removable from the cartridge. An outlet orifice is at the free end of the protrusion in an open (lowered) position of the pressure chamber, and in a closed (lifted) position, the outlet orifice of the protrusion inside and/or part of the pressure chamber.

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A first preferable key element of inventive apparatus is a filter cartridge accommodating the filter element. The filter cartridge is designed as a long-lasting, reusable component, while actual filter element is intended for single use. Thus, for replacing the filter element, entire filter cartridge is removed from the pressure chamber and then the filter element is taken out of the filter cartridge.
15 Filter cartridge is cleaned and equipped with a fresh filter element again. As filter cartridge is compared to entire pressure chamber relatively small, filter cartridge is light and can be handled by ease: Removing worn filter element, cleaning cartridge, and inserting fresh filter element can be performed aside from installation site of apparatus on a workbench with optimized ergonomics.

20 A second key feature of inventive small scale liposome extruder is that filter cartridge is removably mounted to the socket, while the socket can be lifted and lowered by the lifting mechanism. In lifted position, socket is fixed to unmovable ring element. Basically, the protrusion and the socket defining the pressure chamber, whereby the surrounding, stabilising ring element ensures the suitability for the intended high-pressure use.

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By means of said lifting mechanism, pressure chamber can be opened quickly and with less exertion by lowering the socket. Once the socket is lowered, the cartridge can be removed from the socket for replacing the filter element as outlined above. Thanks to this design, the weight of the socket is completely borne by the lifting mechanism. Since the cartridge can be dimensioned light
30 compared to the weight of the socket, cartridge can be handled manually.

Thus, a basic principle of inventive apparatus is separation of bearing pressure load and bearing filter element: The pressure load is borne by the ring element and the socket which are both attached to the fixed frame. Both parts do not need to be moved manually. The only part that is
35 handled manually is the filter cartridge substantially designed for containing the filter element and at least one gasket. Due to its single function, filter cartridge can be technically designed lightweight without notable restrictions to pressure security.

According to a preferred embodiment, the channel of the ring element, the protrusion and the cartridge having a round, circular cross section. Preferably a round, circular cross section, transvers to the (vertical) axis (A.)

- 5 Inventive apparatus may be operated a broad pressure range from $20 \cdot 10^5$ Pa to $200 \cdot 10^5$ Pa. This covers mid pressure processes working with fluid pressures ranging from $20 \cdot 10^5$ Pa to $40 \cdot 10^5$ Pa (20 to 40 bar) and high-pressure processes achieving nominal fluid pressures amounting from $100 \cdot 10^5$ Pa to $200 \cdot 10^5$ Pa (100 to 200 bar) as well. Intermediate pressures, for instance between $60 \cdot 10^5$ Pa and $70 \cdot 10^5$ Pa, are operable also.

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According to a preferred embodiment, apparatus further comprising a lever for pivoting said pinion between a first angle (end-)position and a second angle position relatively to the frame, wherein in said first angle position, the cartridge on the socket is approximated to the protrusion and wherein in said second angle (end-)position the cartridge on the socket is lowered. The levers are realising a quick opening and closing of the extruder, mainly socket and pressure chamber, for using a filter element or replacing of worn filter element. Preferably, in said first angle position filter cartridge is plugged into the ring element (closed position).

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- 20 The first angle (end-)position correlates with the closed pressure chamber and closed socket, and the second angle (end-)position correlates with the open pressure chamber. However, due to the gearing mechanism used as lifting mechanism, or to activate the lifting mechanism, the first and second angle position is not limited and can be used vice versa or might have any useful angle position. The first and second angle position preferably include an angle of 10° to 130° ($\pm 60^\circ$), most preferably of 20° to 120° ($\pm 50^\circ$), or less, wherein the mid-position is preferably the
- 25 horizontal position.

- One preferred option for locking the pressure chamber is to design apparatus in a way such that in said first angle position vertical lifting unit is fixable to the frame such that flux of force between socket and ring element is transferred at least partly over vertical lifting unit and frame. Alternative locking mechanism may be designed as well.
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- According to another preferred embodiment, a ring element is mounted concentrically to the protrusion having an inner channel parallel to axis (A) and form-locking to serve as a guiding channel for the cartridge, wherein the ring element is protruding from the protrusion in direction of the axis A.
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- According to another preferred embodiment, the ring element is mounted concentrically to the protrusion on either the bottom of the housing, or a portion or area of the frame. The ring section can be a segment of the housing located at the bottom side and extending in an assembled stage in direction of the vertical lifting unit and being made as a monolithic segment of the housing and/or the bottom segment of the housing or a segment permanently welded on the housing and/or a
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bottom segment of it. Alternatively, the ring section can be a removable element, being e.g. screwed to the housing and/or a bottom segment of it. The mounting of the protrusion can be analogous to the ring element as stated, however, according to a preferred embodiment, the protrusion is a monolithic part of the housing and/or the bottom segment of the housing and/or is welded there to.

In case the ring element is mounted at the bottom side of a frame (1,) preferably a head plate of it, advantageous the ring element and the housing are fixed on said frame and/or the head plate at vis-à-vis to each other on opposite sides. The protrusion might extend through a cut-out of the frame and protrudes into the inner channel of the concentrically and radially outer ring element.

According to a preferred embodiment, filter element is a plain plate made of a porous material selected from the group consisting of polycarbonate, sintered metal, and metal foil, preferably of circular shape.

Shape of filter element is preferably flat and circular (disk shape). Typical disc diameter of filter element may be between 20 mm and 50 mm, for instance 25 mm. The pore diameter D of the filter element shall amount from $80 \cdot 10^{-9}$ m to $50 \cdot 10^{-6}$ m or from $100 \cdot 10^{-9}$ m to $5 \cdot 10^{-6}$ m. For instance, a pore diameter of $0.1 \mu\text{m}$ may be used. Pore size is given by vendor of filter element and may be verified by optical means, or by transmission electron microscopy (TEM) or by scanning electron microscope (SEM).

Inventive apparatus works with commercially available filter elements. For example, Whatman Nuclepore polycarbonate hydrophilic membranes may be used as filter element. The latter are obtainable from Cytiva Europe GmbH, Freiburg, Germany.

According to a further preferred design concept, the rack may be integrated into the lifting unit mechanically guided by a vertical lifting unit. This helps to reduce the weight of the overall apparatus.

According to another preferred embodiment, the (tempering) chamber having feed line, and wherein at least one (first) segment of the feed line crossing the tempering chamber. Preferably, the tempering chamber is at least connected to one feed line and one outlet line for a tempering fluid, and or connected to a pumping station and/or heat exchange station for the tempering fluid. As an alternative embodiment, an electrical heating device is located inside the tempering chamber, in thermal communication with the feed line and/or at least a segment of it.

According to an improved version of this preferred embodiment, at least three segments of the feed line crossing the tempering chamber, wherein

- the first segment is leading to a ring tube,

- a second segment of the feed line is defined by the ring tube, and
- a third segment is leading from the outlet of the ring tube to the feed orifice of the pressure chamber.

5 The term "crossing" means, the feed line of segments of are somehow inside the tempering chamber, leading from one feed place to the pressure chamber. However, the term "crossing" shall not be understood limiting the path, the direction, or the orientation. The feed line or a segment of it can be placed or mounted at the inner wall of the housing, at a separate carrier element and/or self-carrying by bridging at least a partial inner volume of the tempering chamber.

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According to another preferred embodiment, a venting line is connected to the feed line and/or a segment of the feed line upstream to the pressure chamber, preferably in direct neighbouring the feed orifice of the pressure chamber. This enables the release of inner gas of the feed line when filling the apparatus from the feed line prior to production and safely releasing the entire captioned gas over the venting line and venting outlet.

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Present apparatus is designed for liposome extrusion at small scale for product development, namely sizing of liposome precursors to target liposomes in a dimension of 50 nm to 300 nm, preferred 80 nm to 150 nm.

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Hence, another object of present invention is a process for preparing target liposomes from liposome precursors by employing inventive apparatus. Such process encompasses the following steps:

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- a) providing inventive apparatus for extruding liposomes, wherein said apparatus comprises at least one filter element;
- b) providing a feed dispersion comprising liposome precursors dispersed in a liquid dispersion medium;
- c) applying a hydraulic pressure P amounting from $20 \cdot 10^5$ Pa to $200 \cdot 10^5$ Pa on said feed dispersion to obtain a pressurized feed dispersion;
- d) extruding said pressurized feed dispersion through said filter element to obtain an extruded dispersion comprising target liposomes dispersed in dispersion medium;
- e) optionally: recovery of target liposomes from extruded dispersion.

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35 Preferably, inventive process is performed until reaching a defined clogging degree of the filter element. After that, apparatus is depressurized, ring element and socket carrying the filter cartridge are released from each other. The filter cartridge containing the filter element with reduced penetrability is replaced by a fresh filter cartridge containing a fresh filter element, socket is lifted. Thus, socket with the cartridge is recoupled to the protrusion and the ring element, apparatus is repressurized and process is continued. This process is easily accomplished by moving the levers.

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Inventive apparatus is intended for small scale production of liposomes. Optimally, the volume of the feed dispersion comprising the precursor shall be between 0.1 l and 10 l or from 100ml to 2000ml.

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In particular, the inventive apparatus is used for purpose of product development in field of liposomes.

Even though the different versions of the apparatus are also named as "design". This means exclusively a "technical design" or "constructional design" as a version of a technical embodiment, having a defined number of technical features. The sole outer appearance or an artistic design is not meant.

Further benefits of present apparatus and its intended use in inventive process will become apparent by following description of an embodiment. For this purpose, it shows:

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Fig. 1: Apparatus, closed, perspective view from the top;

Fig. 2: Apparatus, closed, perspective view from the bottom;

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Fig. 3: Apparatus, closed, front view;

Fig. 4: Apparatus, closed and latched, sectional side view;

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Fig. 5: Apparatus, opened, front view;

Fig. 6: Apparatus, opened, sectional side view;

Fig. 7: Apparatus, opened, sectional perspective view.

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The same embodiment of an inventive apparatus 100 is shown in all Figures.

Apparatus 100 comprises a frame 1, which can be fixed to the ground at installation site. Essential element of the apparatus is a housing 32 enclosing a tempering chamber 30 comprising a protrusion 16, a pressure chamber 2 that is composed of a cartridge 12 and the protrusion 16. The protrusion 16 is a monolithic part of the housing 32 and the ring element 3 is fixed to the frame 1, while socket 4 is fixed to a lifting unit with a lifting mechanism 5.

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As shown in the figure 1, the apparatus 100 comprises a frame 1, a having a top plate 1.1, a bottom plate 1.2 and several side elements 1.3, a housing 32 of a tempering chamber 30 located

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on top of the head plate 1.1, a lifting unit 8 with a lifting mechanism 5 placed on the bottom plate 1.2 carrying the socket 4 and the cartridge 12. A ring element 3 is mounted on the lower side of the head plate 1.1, technically designed to carry at least one filter element 14.

5 A feed line 17 leading into the tempering chamber 30 inside the housing 32. The housing 32 comprises a mid-segment 34, a head segment 36 and a bottom segment 38. A feed line 24 for tempering fluid is connected to the mid-segment 34 and the tempering chamber 30. Sensors 40, 42 are placed at relevant places for measuring fluids and monitoring conditions at the apparatus 100. The data line and/or power wire is indicated with the reference number 44. A pressure sensor 40 is placed in correspondence with the venting line 22 and temperature sensors 42 are placed in the feed line 24 of the tempering fluid and in the ring section 3. In the edge element of outlet line 19, a combined sensor 40, 42 is integrated for measuring pressure and temperature in the outlet line 19 (Fig.2) of thermal fluid which is connected to external tempering unit. The outline of the tempering fluid is not shown in the drawings.

15 The feed line 17 is in fluid communication with a venting line 22 leading to a venting outlet 24 to release captured gas volume out of the feed line 17 or the inner segments 17.1, 18. 17.2 of it.

Lifting unit 8 with lifting mechanism 5 is realized by a rack and pinion gear having two racks 6 and 20 two pinions 7 arranged symmetrically, the socket 4 and the cartridge 12 is vertically moved by the lifting mechanism 5 towards the protrusion 16, and thus, closing or forming the pressure chamber 2. The rack 6 is guided vertically by a lifting unit provided by the lifting unit 8 in frame 1, while pinions 7 are pivotally mounted in the frame 1. Each pinion 7 is provided with a lever 9 for pivoting the pinion 7 by turning the lever 9. As each rack 6 cogs with its counterpart pinion 7, turning the levers 9 effects linear movement of the racks 6 along the vertical guideway. Levers 9, pinions 7 and racks 6 are sized in a manner that lever 9 can be turned by hand between a first position and a second position. The angle between both positions is approximately 90°.

30 In the first position, which is shown in Figures 1, 2, 3 and 4, lever 9 are directed to the ground. In this first position, racks 6 are elevated such that socket 4 – that is attached to the top end of the racks 6 – is approximated to the ring element 3. Hence, in first position ring element 3 and socket 4 forming the pressure chamber 2 in a closed status, see in particular sectional side view of Fig. 4.

35 In the second position, which is shown in Figures 5, 6 and 7, pressure chamber 2 is in an opened status. In open status, racks 6 are lowered such that socket 4 is distant to ring element 3. Levers 9 are directed upwards.

40 By turning levers 7 between first and second position, pressure chamber 2 can be opened or closed, respectively. Due to gravity, opening is little lighter than closing. To maintain closed status, a latch 10 (not shown) can be plugged into lifting unit 8 to fix the racks 6 in lifted position (Figure 4

only). In latched position, flux of force between ring element 3 and socket 4 in vertical direction is closed via rack 6, lifting unit 8, latch 10 (not shown) and frame 1. Thus, in closed position, pressure chamber 2 composed of ring element 3 and socket 4 can bear an inner pressure.

- 5 On its upper side, socket 4 is provided with a receptacle 11, in which a cartridge 12 is releasably situated (see Figures 4). Cartridge 12 is designed as a relatively light hollow cylinder having an inner collar 13 bearing a filter element 14. Said filter element 14 consists of a plain porous polycarbonate plate having a porosity in the range of 100 nm to 5 μ m, suitable for sizing liposomes by extrusion through that filter element 14. Filter element 14 is intended as a throw-away item,
- 10 while cartridge 12 is designed long-lasting. Outer diameter and length of cartridge 14 is designed to fit into a central ring channel 15 extending upwards from the bottom side of ring element 3. Vice-versa, a cylindrical protrusion 16 extending downwards from the ring element 2 having an outer diameter and a length designed for fitting into the cartridge 12. In closed status front face of protrusion 16 rests on upper side of filter element 14 (Fig. 4) clamping the filter element 14 onto
- 15 cartridge's collar 13. Optionally, a gasket (not shown) is placed between protrusion 16 and filter element 14.

- Extrusion of liposomes is performed in closed status (Fig. 4). In closed position, socket is approximated to the ring element 3 and fixed in this position by the latch 10 (not shown). Cartridge
- 20 12 is received in ring channel 15, protrusion 16 extends into cartridge 12. Hence, in closed position cartridge 12 is enclosed completely by ring element 3 and socket 4. Filter element 14 is clamped between protrusion 16 and collar 13.

- During extrusion, the pressure chamber 2 is closed as shown in figure 4. A dispersion comprising a
- 25 liquid dispersion medium and therein liposome precursors of enlarged particle size are pumped via feed line 17 into the pressure chamber 2. The feed line 17 leads into the tempering chamber 30 and comprises three sections when passing through the tempering chamber 30 filled with a tempering fluid flowing through chamber 30 and being in communication with an outer pumping and heat exchange unit not shown in the figures.

- 30 The first section 17.1, partially symbolised by a dotted line, leads from the head plate 1.1 to the ring tube 18, which defines the second segment. The ring tube 18 is a capillary line warrants a defined limitation to the volume flow and provides the needed surface for the heat exchange inside the tempering chamber 30. The third section 17.2 is the connecting line between the outlet of the ring
- 35 tube 18 and the feed orifice 2.1 of the pressure chamber 2. The filter cartridge 12, which carries the outlet line 19 starting with outlet orifice 12.1, is inserted into the central channel 15 of the outer ring element 3 and an annular surface 16.1 (fig. 6) of the protrusion 16 is in contact with the filter element 14 and/or at least one gasket. Radially outside, the upper portion of the cartridge 12 protrudes the protrusion 16 concentrically in direction of axis A with the length 16.2.

The typical hydraulic pressure of the dispersion fed to the feed line 17 and/or in the pressure chamber 2 is in range of $20 \cdot 10^5$ Pa up to $200 \cdot 10^5$ Pa. Means for applying hydraulic pressure on dispersion and pumping it are not shown in the drawings. On its way to through the pressure chamber, liquid dispersion may be heated by means of a ring tube 18 surrounded by a tempering fluid. According to an alternative not shown, an electrical heating device is in thermal communication with the feed line 17 or a segment of it.

Feed line 17 ends on the front face of protrusion 16 defined by the feed orifice 2.1. Hence, filter element 14 is subjected to precursor dispersion exiting feed line 17 at high hydraulic pressure. The pressure is substantially born by ring element 3 and socket 4 enclosing cartridge 12. As cartridge is supported by thick-walled ring element 3 and socket 4 to all sides, it can be designed in a lightweight.

As the filter element 14 is porous, while ring element 3 and socket 4 are tight, precursor dispersion is forced to percolate filter element 14 through its pores (extrusion). During percolating, particle size of liposome precursors is reduced on target size defined by pore size of filter element 14.

After extruding through filter element 14, Liposomes of target size are still dispersed in dispersion medium and withdrawn from apparatus 100 via an outlet line 19.

When filter element 14 is worn after running an extrusion trial, apparatus 100 is depressurized, latch 10 (fig. 4) is removed and levers 9 are turned upward. Accordingly, socket 4 is lowered such that cartridge 12 is accessible for operator's hands (Figure 5). Thus, operator can remove connection of outlet line 19 and of filter cartridge 12 from socket 4 and carry it to his or her workbench for cleaning and replacing filter element 14 there. Meanwhile feed line 17 and outlet line 19 can be rinsed.

After that, old cartridge 12 with fresh filter element 14 is placed in receptacle 11 of socket 4 and pressure chamber 2 is closed by turning the levers 9. Vertical lifting unit 8 is locked by plugging latch 10. Apparatus 100 is repressurized and next extrusion trail is about to start.

The figure shows several edge elements connected to lines, e.g. lines 17, 19 or 22. These edge elements might be valves, preferable controllable valves. Alternatively, these lines are connected to valve elements, preferable mounted outside the apparatus.

It is clear to the skilled person, the apparatus as described herein as a preferred orientation with the central axis A vertically oriented, mainly with regard to ease access and maintenance. However, the feed flow inside, e.g. the feed line 17, its segments, the pressure chamber 2 and the outlet line 19 does not afford a vertical orientation. Thus, any description related to the vertical orientation shall not be understood limiting the invention and is meant for descriptive explanation

only, provided there is no specific description stated and/or – clear to the skilled person - a technical obligatory detail or dependency is concerned.

References

	100	Apparatus
5	1	Frame
	1.1	head plate
	1.2	bottom plate
	1.3	side elements
	2	Pressure chamber
10	2.1	feed orifice (of 2)
	2.2	outlet orifice (of 16)
	3	Ring element
	4	Socket
	5	Mechanical unit with lifting mechanism or short lifting mechanism
15	6	Rack
	7	Pinion
	8	Lifting unit, vertical
	9	Lever
20	10	Latch (not show in Figures)
	11	Receptacle
	12	Cartridge
	12.1	outlet orifice (of 12)
	13	Collar
25	14	Filter element
	15	Channel (of 3)
	16	Protrusion
	16.1	Annular surface
	16.2	Length
30	17	Feed line (educt)
	17.1, 17.2	Feed line segment
	18	Ring tube (also named as capillary ring tube)
	19	Outlet line (product)
35	20	venting outlet
	22	venting line
	24	feed line (tempering fluid)
	30	chamber, tempering
40	32	housing (of 30)

- 34 mid-segment
- 36 head segment
- 38 bottom segment
- 5 40 Sensor, pressure
- 42 Sensor, temperature
- 44 Power wire / data line

Claims

1. Apparatus (100) for extruding liposomes, comprising the following components:
- means for applying a hydraulic pressure P on a liquid medium, wherein said hydraulic pressure P amounting from $20 \cdot 10^5$ Pa to $200 \cdot 10^5$ Pa;
 - a frame (1) comprising
 - a. vertical lifting unit (8);
 - b. feed line (17) in fluid connection with a tempering chamber (30) located in the a housing (32), wherein the housing (32) comprises a protrusion (16) extending parallel to a central axis (A), wherein said housing (32) is mounted on said frame (1);
 - c. a pressure chamber (2) comprising an outer cartridge (12), the protrusion (16) of the housing (32), a feed orifice (2.1), an inner collar (13) for mounting at least a filter element (14) at the outlet orifice, wherein
 - the cartridge (12) is linked to an outlet line (19) and mounted on a socket (4) movable in direction of the central axis (A) and being releasably coupled for an overlapping range (16.1) with the protrusion (16), wherein said socket (4) is vertically guided by said vertical lifting unit (8), wherein said pressure chamber (2) is designed for bearing said hydraulic pressure P
 - at least one porous filter element (14) defining an outlet of said pressure chamber (2) and/or cartridge (12), wherein said filter element (14) comprises open pores having a diameter D , wherein said pore diameter D amounting from $80 \cdot 10^{-9}$ m to $50 \cdot 10^{-6}$ m or from $100 \cdot 10^{-9}$ m to $5 \cdot 10^{-6}$ m;
 - a lifting mechanism (5) as part of the lifting unit (8) designed for elevating and lowering the socket (4);
 - wherein said filter element (14) is contained in a (filter) cartridge (12) which is removably mounted to the socket (4);
 - and wherein said lifting mechanism (5) comprises at least one rack (6) and pinion (7) gear, wherein the pinion (7) is pivot-mounted in said lifting unit (8) which is mounted in said frame (1) and the rack (6) is vertically guided.
2. Apparatus (100) according to claim 1, further comprising a lever (9) for pivoting said pinion (7) between a first angle position and a second angle position relatively to the frame (1), wherein in said first angle position, the cartridge (12) on the socket (4) is approximated to the protrusion (16) and wherein in said second angle position the cartridge (12) on the socket (4) is lowered.
3. Apparatus according to claim 1 or 2, wherein in a ring segment (3) is mounted concentrically to the protrusion (16) having an inner channel 15 parallel to axis (A) and

form-locking to serve as a guiding channel for the cartridge (12), wherein the ring segment (3) is protruding from the protrusion (16).

- 5 4. Apparatus according to claim 3, wherein the ring segment 3 is mounted concentrically to the protrusion (16) on
 - i) the bottom of the housing (32) or
 - ii) a portion of the frame (1).
- 10 5. Apparatus according to claim 2 or 3, wherein in said first angle position filter cartridge (12) is plugged into the ring segment (3).
- 15 6. Apparatus according to claim 2 or 3, wherein in said first angle position vertical lifting unit (8) is fixable to the frame (1) such that flux of force between socket (4) and ring segment (3) is transferred at least partly over vertical lifting unit (8) and frame (1).
7. Apparatus (100) according to at least one of claims 1 to 6, characterized in that said filter (14) element is a plain plate made of a porous material selected from the group consisting of polycarbonate, sintered metal, and metal foil, preferably of circular shape.
- 20 8. Apparatus according to at least one of claim 1 to 7, wherein the rack (6) is integrated into said vertical lifting unit (8).
- 25 9. Apparatus according to at least one of claim 1 to 8, wherein the chamber (30) having feed line (24), and wherein at least one (first) segment (17.1) of the feed line (17) crossing the tempering chamber (30).
- 30 10. Apparatus according to claim 9, wherein at least three segments of the feed line (17) crossing the tempering chamber (30), wherein
 - the first segment (17.1) is leading to a ring tube (18),
 - a second segment of the feed line (17) is defined by the ring tube (18), and
 - a third segment (17.2) is leading from the outlet of the ring tube (18) to the feed orifice (2.1) of the pressure chamber (2).
- 35 11. Apparatus according to at least one of claim 1 to 10, comprises a venting line (22) connected to the feed line (17) and/or a segment of the feed line upstream to the pressure chamber (2), preferably in direct neighbouring the feed orifice (2.1) of the pressure chamber (2).

12. Process for preparing target liposomes from liposome precursors, with the following steps:
- a) providing an apparatus (100) for extruding liposomes, wherein said apparatus (100) comprises at least one filter element (14);
 - 5 b) providing a feed dispersion comprising liposome precursors dispersed in a liquid dispersion medium;
 - c) applying a hydraulic pressure P amounting from $20 \cdot 10^5$ Pa to $200 \cdot 10^5$ Pa on said feed dispersion to obtain a pressurized feed dispersion;
 - d) extruding said pressurized feed dispersion through said filter element (14) to obtain an
10 extruded dispersion comprising target liposomes dispersed in dispersion medium;
 - e) optionally: recovery of target liposomes from extruded dispersion;
characterized in
that an apparatus (100) for extruding liposomes according to at least one of claims 1 to 11
is provided.
 - 15
13. Process according to claim 12, whereby the process is performed until reaching a defined clogging degree of said filter element (14), characterized in that after reaching defined clogging degree apparatus (100) is depressurized, ring segment (3) and socket (4) are released from each other, ring segment (3) is elevated, filter cartridge (12) containing filter
20 element (14) with reduced penetrability is replaced by a fresh filter cartridge (12) containing a fresh filter element (14), ring segment (3) is lowered, socket (4) and ring segment (3) are recoupled with each other, apparatus (100) is repressurized and process is continued.
14. Process according to claim 12 or 13, whereby the volume of provided feed dispersion is
25 between 0.1 l and 10 l or between 100ml and 2000ml.
15. Process according to claim 12 to 14, performed for purpose of product development.