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(54) Benævnelse: **A LIQUID MEMBRANE SUITABLE FOR WATER EXTRACTION**

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(57) Sammendrag:

The present invention relates to the industrial use of an aquaporin liquid membrane system wherein said liquid membrane comprises aquaporin water channels in a dispersion of amphiphilic molecules. Said use includes use for desalination of salt water, and, in addition, will facilitate pure water extraction from liquid aqueous media by forward osmosis.

fortsættes

Fig. 1a

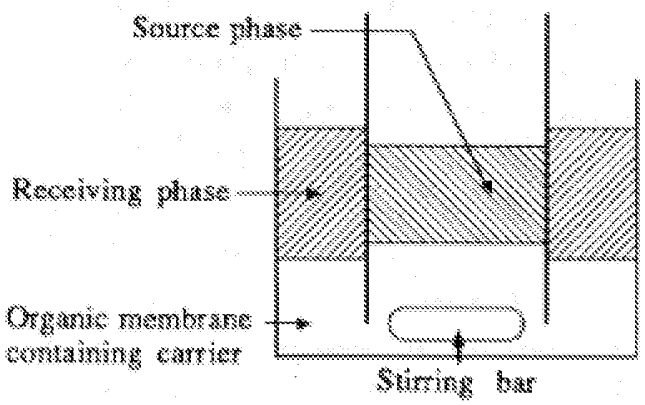


Fig. 1b

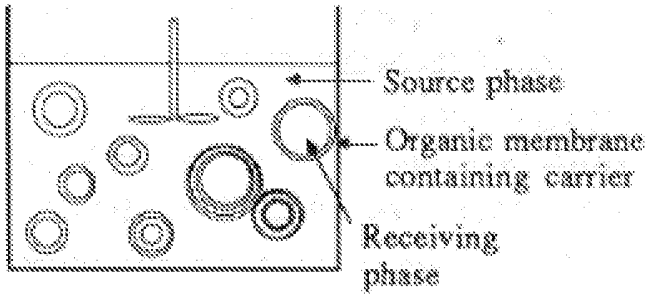
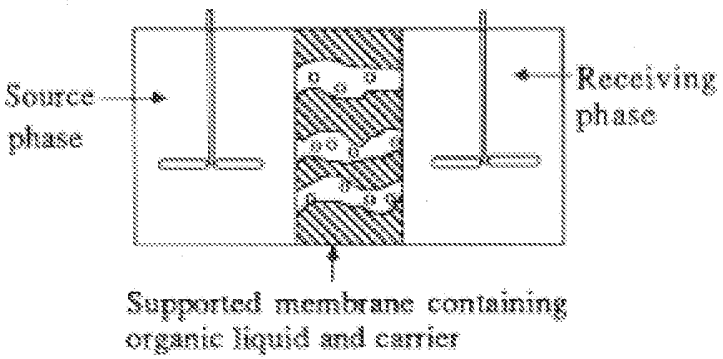


Fig. 1c



A LIQUID MEMBRANE SUITABLE FOR WATER EXTRACTION

The invention relates to the use of an aquaporin liquid membrane consisting of aquaporins in a dispersion of amphiphilic lipids, particularly for pure water extraction from aqueous liquid media, e.g. in forward osmosis applications.

BACKGROUND

Liquid membrane separation processes have been used for removal of dissolved substances such as ions from aqueous solutions, such as disclosed in US 4360448(A). This invention relates to a process for the removal of dissolved species from aqueous solutions, which comprises contacting said aqueous solution with an emulsion, said emulsion comprising an exterior phase which is characterized as being immiscible with said aqueous solution and yet permeable to said dissolved species, and an interior phase which contains a reactant, such as an ion exchange compound, capable of converting said dissolved species to a nonpermeable form. The dissolved species permeate the exterior phase, into the interior phase where they are converted into nonpermeable forms and thus retained in the interior phase of said emulsion. The aqueous solution, depleted in said dissolved species, is separated from said emulsion and the emulsion cycled for reuse. However, when multiple or unspecified ions or solutes are present in an aqueous solution or medium, such as a biological liquid it becomes increasingly complex to remove solutes by this or similar methods, since it would be necessary to device a specific reactant for each species to be removed. Another example of the use of a liquid membrane extraction process is described in (WO/1987/002380) Production of Low-Ethanol Beverages by Membrane Extraction which relates to membrane extraction systems designed to selectively remove ethanol from wine and other beverages while retaining the water and numerous other organic constituents. Thus, liquid membrane separation methods have hitherto been developed for selective removal of solutes in, e.g. aqueous liquids. Seeing a need to selectively remove or extract water from aqueous liquid sources the present inventors have devised a liquid membrane process suitable to removal or extraction of pure water from an aqueous liquid using the selective water channel known from aquaporin proteins. WO2006122566 A2 describes membranes comprising functional aquaporin channels used in filtering devices and systems for purification of water. The membrane utilizes aquaporin water transport proteins that have been reconstituted in lipid vesicles and transformed into a supported layer. Said water membrane may be used to extract excess water from aqueous substances or solutions, e.g. to obtain increased concentration of a desirable solute. The use of stabilising components for the vesicle preparations, e.g. such as squalene, squalane or any derivative thereof has not been disclosed in WO2006122566.

SUMMARY OF THE INVENTION

The present invention relates to an aquaporin liquid membrane system in the form of a bulk liquid membrane (BLM), an aquaporin containing emulsion liquid membrane (ELM), and aquaporin containing supported (immobilised) liquid membrane (SLM), and a combination thereof wherein said liquid membrane system is based on vesicles formed from amphiphilic lipids forming a bilayer wherein aquaporin water channels have been incorporated and wherein said vesicles further comprise stabilising components selected from the group consisting of squalane or squalene and derivatives thereof.

In addition, the invention relates to the use of such a membrane system for water extraction from liquid aqueous media by forward osmosis, e.g. for desalination of salt water.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1a, 1b and 1c show the general principles of three types of liquid membranes: Bulk liquid membrane (BLM), Emulsion liquid membrane (ELM), Supported liquid membrane (SLM).

Fig. 2a and 2b show hollow fibers and spiral wound separation modules in liquid membrane modules

Fig. 3 shows a principle sketch of a two Module Hollow Fiber Supported Liquid Membrane.

Fig. 4 shows a principle sketch of an apparatus and a method for concentration of aqueous solutions using the vesicles of the invention.

Fig. 5 shows a principle sketch of a method of providing a nutrient drink comprising pure drinking water through forward osmosis.

Fig. 6 shows a principle sketch of the use of the vesicles of the invention in forward osmosis – following ultrafiltration of a uniform electrolyte. This is an example of a complete application of water extraction from any aqueous solution or liquid.

Fig. 7 shows a principle sketch for the use of vesicles of the invention in pressure retarded osmosis for osmotic power production.

Fig. 8 shows the relation between liquid membraneformulation and concentration of the proteoliposome microemulsion (liquid membrane).

DETAILED DESCRIPTION OF THE INVENTION

The aquaporin containing liquid membrane system described herein may be in the form of an aquaporin containing emulsion liquid membrane (ELM) wherein said liquid membrane comprises aquaporin water channels in a dispersion of amphiphilic lipids, preferably comprising vesicles in the form of proteoliposomes, having functional aquaporins incorporated into an amphiphilic vesicle bilayer, such as a lipid bilayer and the like.

The aquaporin containing emulsion liquid membrane, or Aqp-ELM, of the invention can be used for water extraction by being mixed into or suspended in a first aqueous liquid having an osmotic pressure which is less than that of the Aqp-ELM vesicles which will selectively transport pure water molecules from said first liquid through the aquaporin water channels into the swelling vesicles. After extraction of pure water from the first liquid the vesicles can be separated, e.g. by centrifugation or filtration, and resuspended in a second aqueous liquid having an osmotic pressure that exceeds the pressure of the vesicles having extracted pure water. The extracted water will now flow from the shrinking vesicles into the second liquid as long as an osmotic gradient is present. Second aqueous liquids should preferably be separable from the product (purified) water, have low or no toxicity, and be chemically inert to the liquid membranes. Examples of second aqueous liquids (draw solutions) are mixtures of glucose and fructose that have been used for seawater desalination, and lately draw solutions based on combining ammonia and carbon dioxide gases in specific ratios highly concentrated draw solutions of thermally removable ammonium salts have been obtained. 76] J.O. (Kessler, and C.D. Moody, Drinking water from sea water by forward

osmosis. Desalination 18 (1976) 297-306., J.R. McCutcheon, R.L. McGinnis, and M. Elimelech, Desalination by a novel ammonia–carbon dioxide forward osmosis process: influence of draw and feed solution concentrations on process performance. J. Membr. Sci. 278 278 (2006) 114-123).

Aqp-ELM vesicles as described herein are able to swell and shrink in repeated cycle. Typically the vesicle volume may shrink with by up to 80% and swelling may be up to 5% or more, cf. M. Goulian et al., Biophysical Journal, Vol. 74, January 1998, pp. 328–337.

Small unilamellar phosphatidylcholine (SUV) vesicles useful in the invention having a diameter of approx. 20 nm are osmotically sensitive. Such vesicles respond to osmotic pressure by swelling or shrinking depending on the direction of the applied salt gradient. This is true for small unilamellar vesicles of egg phosphatidylcholine and dimyristoylphosphatidylcholine below and above their crystal-to-liquid crystal transition temperature. In the presence of osmotic gradients the influx and efflux of H_2O is de-coupled with the movement of ions due to the presence of aquaporins. During osmotically induced shrinking and swelling of SUV the integrity of the phospholipid bilayer is maintained to the extent that vesicles do not break, and therefore equilibration between external medium and vesicle cavity does not take place unless the membranes are containing aquaporin proteins.

Typical osmotic pressures of the first aqueous liquid or source phase is in the range of about100 mOsm to about 500 mOsm or 1000 mOsm, and typical osmotic pressures of the second aqueous liquid or receiving phase are about 100 to 1000 mOsm higher in order to obtain a suitable osmotic pressure difference. The osmolarity of sea water ranges from 2000-2400 mOsm, primarily contributed by sodium chloride. This is 8 times the osmolarity of blood plasma, i.e. 300 mOsm. The most concentrated urine our kidneys can produce ranks at 1400 mOsm, far below the level of ocean water.

Definitions

The term liquid-liquid extraction is used for a separation process using liquid membranes. In the present invention this is a liquid-water extraction, as water is extracted into a liquid membrane.

Using a liquid membrane the general term "water extraction" will be used herein together with the general term "water separation".

The term "vesicle" is used herein to specify approximately globular liquid structures of amphiphilic lipids, such as phospholipids where the the term is used interchangably with the term "proteoliposome" which can incorporate amphiphilic proteins, e.g. transmembrane proteins including aquaporins.

Amphiphilic lipids are, e.g. phospholipidsphosphoglycerides, sphingolipids, and cardiolipin, as well as mixtures thereof, e.g. phospholipids such as 1,2-dipalmitoyl-sn-phosphatidylcholine (DPPC), or mixtures of phospholipids.

Useful lipids are listed in Table 1:

Phosphatidylcholines:

1,2-dimyristoylphosphatidylcholine (DMPC)
1,2-dipalmitoylphosphatidylcholine (DPPC)
1,2-distearoylphosphatidylcholine (DSPC)
1,2-dioleoylphosphatidylcholine (DOPC)
1,2-dimyristoleoylphosphatidylcholine
1,2-dipalmitoleoylphosphatidylcholine
1,2-dipetroselinoylphosphatidylcholine
1,2-dielaidoylphosphatidylcholine
1,2-dilinoleoylphosphatidylcholine
1,2-dilinolenoylphosphatidylcholine
1,2-dieicosenoylphosphatidylcholine
1,2-diarachidonoylphosphatidylcholine
1,2-dierucoylphosphatidylcholine
1,2-dnervonoylphosphatidylcholine
1-palmitoyl-2-oleoylphosphatidylcholine (POPC)
1-palmitoyl-2-linoleoylphosphatidylcholine
1-palmitoyl-2-arachidonoylphosphatidylcholine
1-palmitoyl-2-docosahexaenoylphosphatidylcholine
1-stearoyl-2-oleoylphosphatidylcholine (SOPC)
1-stearoyl-2-linoleoylphosphatidylcholine
1-stearoyl-2-arachidonoylphosphatidylcholine
1-stearoyl-2-docosahexaenoylphosphatidylcholine
1-oleoyl-2-palmitoylphosphatidylcholine
1-oleoyl-2-stearoylphosphatidylcholine
1,2-didocosahexaenoylphosphatidylcholine

Phosphatidylethanolamines:
1,2-dimyristoylphosphatidylethanolamine (DMPE)
1,2-dipalmitoylphosphatidylethanolamine (DPPE)
1,2-distearoylphosphatidylethanolamine (DSPE)
1,2-dioleoylphosphatidylethanolamine (DOPE)
1-palmitoyl-2-oleoylphosphatidylethanolamine (POPE)
1-palmitoyl-2-linoleoylphosphatidylethanolamine
1-palmitoyl-2-arachidonoylphosphatidylethanolamine
1-palmitoyl-2-docosahexaenoylphosphatidylethanolamine
1l-stearoyl-2-oleoylphosphatidylethanolamine (SOPE)
1-stearoyl-2-linoleoylphosphatidylethanolamine
1-stearoyl-2-arachidonoylphosphatidylethanolamine
1-stearoyl-2-docosahexaenoylphosphatidylethanolamine
1,2-dielaidoylphosphatidylethanolamine
1,2-dilinoleoylphosphatidylethanolamine

1,2-dilinolenoylphosphatidylethanolamine
1,2-diarachidonoylphosphatidylethanolamine
1,2-didocosahexaenoylphosphatidylethanolamine
1,2-dipalmitoleoylphosphatidylethanolamine

Phosphatidylglycerols:
1,2-dimyristoylphosphatidylglycerol (DMPG)
1,2-dipalmitoylphosphatidylglycerol (DPPG)
1,2-distearoylphosphatidylglycerol (DSPG)
1,2-dioleoylphosphatidylglycerol (DOPG)
1-palmitoyl-2-oleoylphosphatidylglycerol (POPG)
1-palmitoyl-2-linoleoylphosphatidylglycerol
1-palmitoyl-2-arachidonoylphosphatidylglycerol
1-palmitoyl-2-docosahexaenoylphosphatidylglycerol
1-stearoyl-2-oleoylphosphatidylglycerol (SOPG)
1-stearoyl-2-linoleoylphosphatidylglycerol
1-stearoyl-2-arachidonoylphosphatidylglycerol
1-stearoyl-2-docosahexaenoylphosphatidylglycerol

Phosphatidylserines:
1-palmitoyl-2-oleoylphosphatidylserine (POPS)
1-palmitoyl-2-linoleoylphosphatidylserine
1-palmitoyl-2-arachidonoylphosphatidylserine
1-palmitoyl-2-docosahexaenoylphosphatidylserine
1-stearoyl-2-oleoylphosphatidylserine (SOPS)
1-stearoyl-2-linoleoylphosphatidylserine
1-stearoyl-2-arachidonoylphosphatidylserine
1-stearoyl-2-docosahexaenoylphosphatidylserine
1,2-dimyristoylphosphatidylserine (DMPS)
1,2-dipalmitoylphosphatidylserine (DPPS)
1,2-distearoylphosphatidylserine (DSPS)
1,2-dioleoylphosphatidylserine (DOPS)
1,2-didocosahexaenoylphosphatidylserine
1,2-dierucoylphosphatidylserine

Special lipids:
Cardiolipin
Bipolar lipids

Natural lipid extracts:
Egg yolk phosphatidylcholine

Bovine heart phosphatidylcholine
Brain phosphatidylcholine
Bovine liver phosphatidylcholine
Soybean phosphatidylcholine
E. Coli phosphatidylethanolamine
Bovine Heart phosphatidylethanolamine
Brain phosphatidylethanolamine
Bovine Liver phosphatidylethanolamine
Egg phosphatidylethanolamine
Bovine liver phosphatidylinositol
Soybean phosphatidylinositol
Brain phosphatidylserine
Soy phosphatidylserine

Polymerizable lipids:
1,2-di-10,12-tricosadiynoyl-sn-glycero-3-phosphocholine (DTPC)
1,2-di-10,12-tricosadiynoyl-sn-glycero-3-phosphoethanolamine (DTPE)
1-palmitoyl-2,10,12-tricosadiynoyl-sn-glycero-3-phosphoethanolamine (PTPE)
(DC8,9PC [1,2-bis(10,12-tricosadiynoyl)-sn-glycero-3-phosphocholine]
diPhyPC 1l,2-diphytanoyl-sn-glycero-3-phosphocholine]
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The term "aquaporin" as used herein shall mean any functional water channel, such as the tetrameric transmembrane proteins described in WO/2006/122566 "Membrane for filtering of water" and by Tamir Gonen and Thomas Walz, Quarterly Reviews of Biophysics (2006), **39**:**4**:361-396, Cambridge University Press. A preferred aquaporin protein as used herein is selected from the group consisting of Aqp 4, Aqp 1, Aqp Z, SoPIP2;1.

The terms "aqueous liquid" and "aqueous liquid media" are used herein to encompass aqueous solutions, natural water sources, waste water sources, aqueous suspensions, dispersions, emulsions and the like.

The term "forward osmosis" (FO) signifies a process where the osmotic pressure differential across a semipermeable membrane is the driving force for transport of water through the membrane. The FO process results in concentration of a feed stream and dilution of a highly concentrated stream (referred to as the draw solution), cf. Cath et al., Journal of Membrane Science, 281 (2006) 70–87.

The term "first aqueous liquid" corresponds to "feed" liquid or the source phase.

The term "second aqueous liquid" corresponds to "draw" liquid or the receiving phase, also known as stripping solution.

The term "standard form factors" usable with liquid membranes as described herein shall mean the modern industry device and apparatus standards for liquid membrane extraction equipment.

The term "liquid membrane contactor" as used herein shall mean a device or composition that will allow two liquid phase to come into contact with each other for the purpose of mass transfer between the phases, through an aquaporin bulk liquid membrane. Examples of contactors as used herein include two module hollow fiber modules, multibundle hollow fiber contactors, such as Liqui-Cel™ contactors, a Hollow fiber pertractor and a Two chamber contactor system, cf. http://sschi.chtf.stuba.sk/MembraneLab/Equipment.htm

Specific embodiments

Use of the aquaporin liquid membrane of the invention is especially advantageous in production of fresh water from desalination of saline feed solutions, such as sea water, where the specific pure water transporting and chloride rejecting properties of the aquaporin water channels offer unique process conditions. An interesting embodiment of the invention is the use of aquaporin liquid membranes (e.g. emulsion liquid membranes, supported emulsion liquid membranes, or bulk liquid membranes) in a forward osmosis process for the production of fresh water, where salt water is the feed and a CO_2/NH_3 aqueous solution is the draw solution having the advantage of easy elimination of the dissolved gases through heating to about 58 °C, cf. McGinnis and Elimelech, Desalination, 207 (2007) 370-382; and Quirin Schiermeier, "Purification with a pinch of salt", Nature, 452, 20 March 2008.

Examples of liquid membranes of the invention in the form of aquaporin proteoliposomes are illustrated in the tables below:

Table 1. Membrane surface pr. liter ELM / BLM for two different vesicle diameters

200 nm proteoliposome	400 nm proteoliposome
Max. ~500.000[3] liposomes/liter	Max. ~250.000[3] liposomes/liter
$A = 4 \pi r^2$	$A = 4 \pi r^2$
12,56 x 10^{-14} m^2 membrane surface/liposome	50,24 x 10^{-14} m^2 membrane surface/liposome
Max. ~15.700 m^2 membrane surface/liter	Max. ~7.850 m^2 membrane surface/liter
Max. transport rate/bar: ~66 liter/second (p_f ~10^{-13})	Max. transport rate/bar: ~33 liter/second (p_f ~10^{-13})

Table 2. Components pr. liter ELM / BLM for two different vesicle diameters

200 nm proteoliposome	400 proteoliposome
~34.5 g aquaporin/liter membrane (50% coverage)	~17.25 g aquaporin/liter membrane (50% coverage)
~35 g lipid/liter membrane	~17.5 g lipid/liter membrane
~0,523 liter water/liter membrane (inside)	~0,523 liter water/liter membrane (inside)
~0,4 liter other component (outside)	~0,4 liter other component (outside)

The invention is illustrated in the figures 1 to 7 which are explained in detail below:

Fig. 1a, 1b and 1c show the general principles of three types of liquid membranes: Bulk liquid membrane (BLM), Emulsion liquid membrane (ELM), Supported liquid membrane (SLM). In the case of aquaporin liquid membranes, the carrier consists of aquaporin water channels embedded into an amphiphilic

vesicle bilayer, such as a phospholipid bilayer and the like. For all three liquid membrane types, the source phase and the receiving phase will be an aqueous solution, where the receiving phase has a higher osmotic gradient than the source phase.

Fig. 2a and 2b show hollow fibers and spiral wound separation modules in liquid membrane modules. For both modules the build up works as a contactor, between a source phase and a receiving phase. The source phase and the receiving phase will both be an aqueous solution, where the receiving phase has a higher osmotic gradient than the source phase.

Fig. 3 shows a principle sketch of a two Module Hollow Fiber Supported Liquid Membrane with a bulk liquid membrane as the carrier. In the case of an aquaporin bulk liquid membrane being the carrier, the aquaporin bulk liquid membrane will extract water from the source phase through the aquaporin water channels and into the receiving phase. The source phase and the receiving phase will both be an aqueous solution, where the receiving phase has a higher osmotic gradient than the source phase.

Fig. 4 shows a principle sketch of an apparatus and a method for concentration of aqueous solutions using the vesicles of the invention. In the case of an aquaporin emulsion liquid membrane being the carrier, the aquaporin emulsion liquid membrane will extract water from the solution to be concentrated, into the aquaporin emulsion liquid membrane, hereby ending up with a concentrated solution.

Fig. 5 shows a principle sketch of a method of providing a nutrient drink comprising pure drinking water through forward osmosis using an aquaporin emulsion liquid membrane as the carrier system. As an example, the aquaporin emulsion liquid membrane will extract water from a urine solution into the aquaporin emulsion liquid membrane. The aquaporin emulsion liquid membrane and the concentrated urine solution will be phase separated, and following the aquaporin emulsion liquid membrane will be mixed with a receiving aqueous solution with a higher osmotic gradient. Water will then be extracted from the aquaporin emulsion liquid membrane into the receiving solution, and the aquaporin emulsion liquid membrane and the receiving solution will be phase separated, giving an end result of transfer of water from a urine solution to another solution, in this example being a solution of glucose and protein.

Fig. 6 shows a principle sketch of the use of the vesicles of the invention in forward osmosis – following ultrafiltration of a uniform electrolyte or degassing of dissolved gasses. This is an example of a complete application of water extraction from any aqueous solution or liquid. As an example, the aquaporin emulsion liquid membrane will extract water from a waste water solution into the aquaporin emulsion liquid membrane. The aquaporin emulsion liquid membrane and the concentrated waste water solution will be phase separated, and following the aquaporin emulsion liquid membrane will be mixed with a receiving aqueous solution with a higher osmotic gradient. Water will then be extracted from the aquaporin emulsion liquid membrane into the receiving solution, and the aquaporin emulsion liquid membrane and the receiving solution will be phase separated, giving an end result of transfer of water from a waste water solution to another solution, in this example being a solution of another electrolyte or a solution of dissolved gasses.

Fig. 7 shows a principle sketch for the use of vesicles of the invention in pressure retarded osmosis for osmotic power production. The example shows the principle sketch of a two Module Hollow Fiber Supported Liquid Membrane with a bulk liquid membrane as the carrier integrated into a pressure retarded osmosis system for osmotic power production. In the case of an aquaporin bulk liquid membrane being the carrier, the aquaporin bulk liquid membrane will extract water from the source phase through the aquaporin water channels in the aquaporin bulk liquid membrane and into the receiving phase. The source phase will in the example of pressure retarded osmosis be the brackish water/fresh water and the receiving phase will be seawater or even a brine of seawater. The extraction of water from brackish water into seawater will enable the production of osmotic power harvested through a turbine driven by a pressure gradient.

Experimental section

Example 1 Preparation of Aquaporin-BLM/ELM: Proteoliposomes

Proteoliposome preparation:
Purified SoPIP2;1 was reconstituted into vesicles by mixing with DOPC (1,2-dioleoylphosphatidylcholine) lipid vesicles (10 mg/ml) solubilized in 1% OG (detergent, octylglucoside) at a lipid-to-protein molar ratio (LPR) of 200 in Phosphate buffer (PBS) 10 mM, NaCl 150 mM, pH 7.5. The mixture was dialyzed against phosphate buffered saline buffer in a Float-A-Lyzer® G2 Dialysis Cassettes (Spectrum Laboratories Inc, CA, USA) with a molecular cut-off of 8-10.000 Mw at room temperature for 2 days with two buffer changes per day (minimum 1:1000 volume sample: volume dialysis buffer). Control vesicles were made in the same manner without protein.

Bulk liquid membrane preparation:
To SoPIP2;1 proteoliposomes prepared as described above was gently added a lipid suspension consisting of DOPC dissolved in squalene in a ratio of 1:5 proteoliposomes: lipid suspension without mixing. This was placed on end-over-end over night at 4 degree Celsius. The resulting bulk liquid membrane emulsion may be used directly or may be up-concentrated by centrifugation at 14.000 rpm for 10 min and subsequently using the middle phase of the resultant three phases solution (top phase: lipid/squalene, middle phase: protein/lipid/squalene, bottom phase: phosphate buffered saline solution). Store at 4 °C until use.

Example 2. Preparation of lipid mixture for solventless aquaporin BLM

Materials and Chemicals: phospholipids (DOPC) , glycerides (mono-oleoyl-glyceride), squalene, linoleic acid (both stored at +5 °C), pentane, labelled phospholipid (e.g. Texas Red® DHPE, Sigma Aldrich).
Equipment: vacuum dessicator, standard lab equipment, water suction flow
Required laboratory working time: 1 hour + overnight for storage

Preparation steps for lipid mixture/solution where lipid / fatty acid / squalene ratio is 1 / 6 / 35

1) dry down 10 mg lipid from chloroform stock under N_2, put under vacuum 30 min
2) add 200 µL of squalene
3) add 20 µL of linoleic acid (use Hamilton and pipette through septum)
4) whirlimix gently, preferable under flow of N_2
5) add 300 µL pentane, whirlimix, or alternative 5) If lipid-label is used, then use the chloroform-phase of the labelled lipid (normally around 50 µL) to mix the ternary component phase. Continue as below, removing the chloroform.
6a) evaporate pentane under flow of N_2, then under vaccum until pentane is gone
6b) freeze-thaw samples 5 times in ethanol and dry ice
7) degas emulsion under water suction pump
8) N_2-gas on top, put cap and parafilm on and label
9) store at -80 °C overnight.

The solvent free and solvent less lipid mixtures prepared herein are especially suitied for incorporation of amphiphilic, transmembrane proteins, such as aquaporins. The proteoliposome preparation of Example 1 can be added to the solvent less lipid mixture according to the procedure mentioned in Example 1.

The BLM preparations of Examples 1 and 2 may be used in the following applications:

a) After separating the BLM emulsion phase as shown in Fig. 8 this may be deposited on a filter unit, such as a Centriprep® filter device taking due care to completely cover the filter disk optionally using an excess volume. The filter device is now ready for use in separation of pure water from an aqueous medium providing an osmotic pressure difference or gradient is established across the filter disk with the deposited aquaporin BLM. Fig. 8 shows the relation between liquid membrane formulation and concentration of the proteoliposome microemulsion (liquid membrane) prepared as described in the experimental section Example 1. Basically the liquid membrane is formed through the successive stages: Evaporate chloroform from lipid, Redissolve lipid in buffer (PBS) + 1% OG (detergent), Extrude lipid solution to obtain vesicles, 200 nm, 21× extruding, Mix protein SoPIP2;1 in Buffer (PBS)+ 1% OG with extruded vesi cles to desired Lipid-to-protein ratio (LPR), Dialyse ≥ 48h against PBS buffer .

Example 3. Application of BLMs as prepared above

The BLM preparations of the invention can suitably be incorporated in a hollow fiber module designed for concentration driven liquid-liquid mass transfer, e. g. a Liquid-Cel extra-flow 10x28 contactor as described in section 4.21 and shown in Figure 4.1(b) in Manuel Aguilar & Jose Luis Cortina "Solvent Extraction and Liquid Membranes", CRC Press, 2008. The liquid membrane emulsion of the invention

can be incorporated in the microporous hollow fibre membranes, and using salt water as the feed fluid and a suitably concentrated draw fliud pure water can be extracted from the salt water feed.

References

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Cath et al., Journal of Membrane Science, 281 (2006) 70–87

http://sschi.chtf.stuba.sk/MembraneLab/Equipment.htm

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Quirin Schiermeier, "Purification with a pinch of salt", Nature, 452, 20 March 2008

Manuel Aguilar & Jose Luis Cortina "Solvent Extraction and Liquid Membranes", CRC Press, 2008

Patentkrav

1. Et aquaporin-væskemembransystem i form af en bulk væskemembran (BLM), en aquaporin-indeholdende emulsions- væskemembran (ELM), og en aquaporin-indeholdende understøttet (immobiliseret) væskemembrane (SLM), og en kombination deraf, hvori væskemembransystemet er baseret på vesikler dannet udfra amfifile lipider der danner et dobbeltlag, hvori aquaporin vandkanaler er blevet inkorporeret, og hvori vesiklerne ydermere omfatter stabiliserende komponenter udvalgt fra gruppen bestående af squalan eller squalen og derivater deraf.
2. Væskemembransystemet i henhold til krav 1, hvori vesiklerne er i stand til at kvælde med op til omtrent 5 volumen % og/eller krympe med op til omtrent 80 volumen %.
3. Væskemembransystemet i henhold til krav 1 eller 2, hvori aquaporinkanalerne er til stede i et forhold på 1% til omtrent 50% i forhold til vesikeloverfladearealet.
4. Væskemembransystemet i henhold til et hvilket som helst af krav 1 til 3, hvori vesiklerne har en tilnærmet diameter i området fra omtrent 100 nm til omtrent 500 nm.
5. Væskemembransystemet i henhold til et hvilket som helst af krav 1 til 4, kendetegnet ved at have en vandtransport-hastighed på mellem 20 og 100 L/s.
6. Anvendelse af væskemembransystemet i henhold til et hvilket som helst af krav 1 til 5 i udstyr til væskemembranekstraktion såsom et tomoduls hulfiber understøttet væskemembranmodul eller en flydende-celle extra-flow membrankontaktor til rentvandsekstraktion fra flydende vandige medier.
7. Anvendelse i henhold til krav 6, hvor rentvandsekstraktionen foregår i en forward osmose proces.
8. Anvendelse i henhold til krav 7, hvor der i forward osmose processen anvendes saltvand som fødeopløsningen og en vandig CO_2/NH_3- opløsning som trækopløsningen, og hvor elimination af de opløste CO_2- og NH_3-gasser udføres gennem opvarmning til omtrent 58 °C.

Fig. 1a

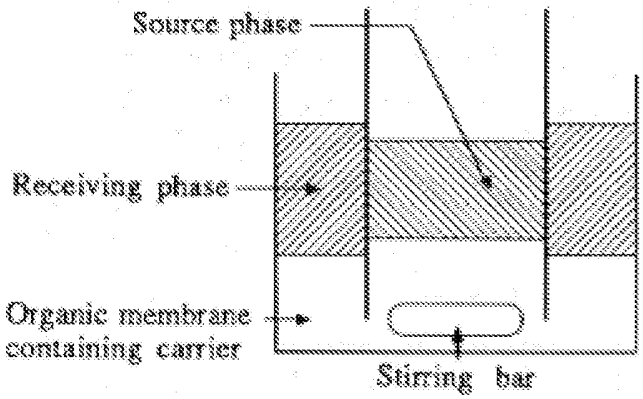


Fig. 1b

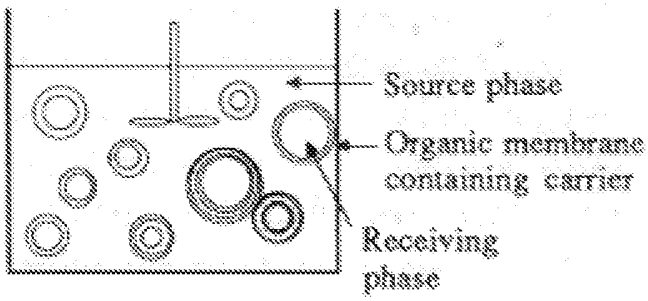


Fig. 1c

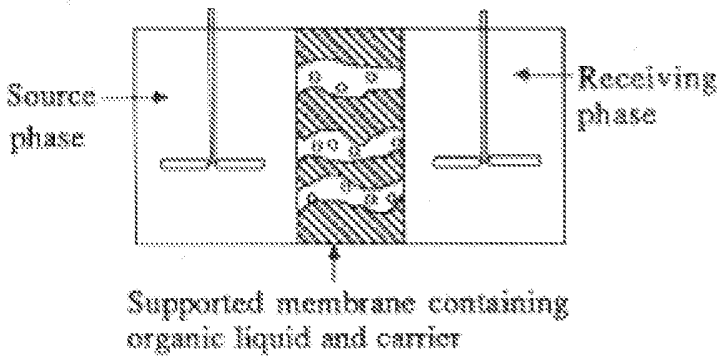


Fig. 2a

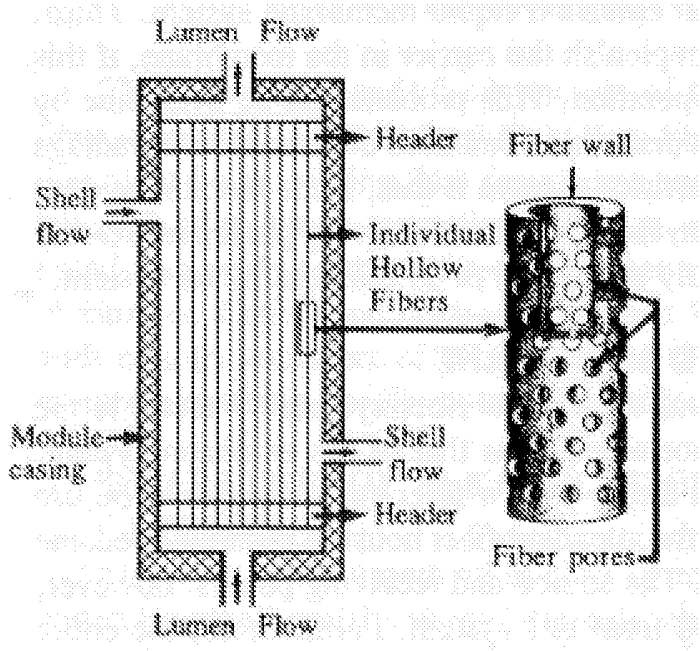

Lumen Flow
Header
Fiber wall
Shell flow
Individual Hollow Fibers
Module casing
Shell flow
Header
Fiber pores
Lumen Flow

Fig. 2b

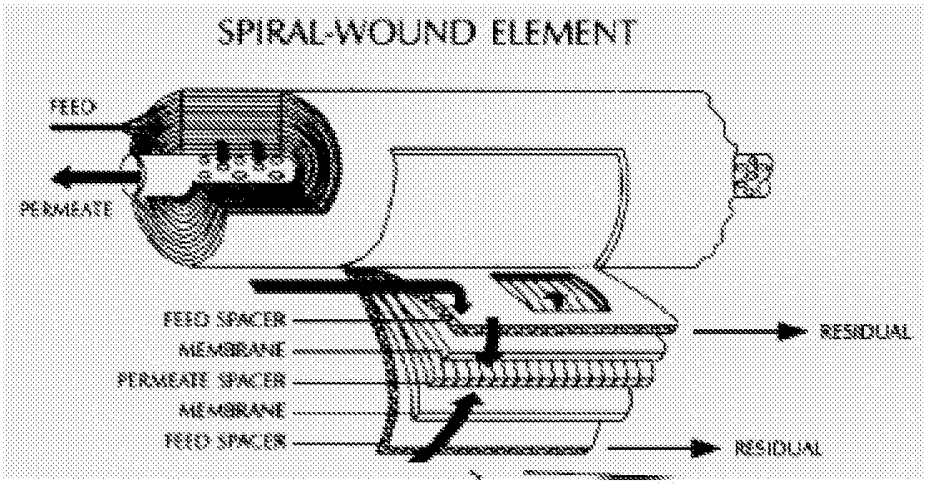

SPIRAL-WOUND ELEMENT
FEED
PERMEATE
FEED SPACER
MEMBRANE
PERMEATE SPACER
MEMBRANE
FEED SPACER
RESIDUAL
RESIDUAL

Fig. 3

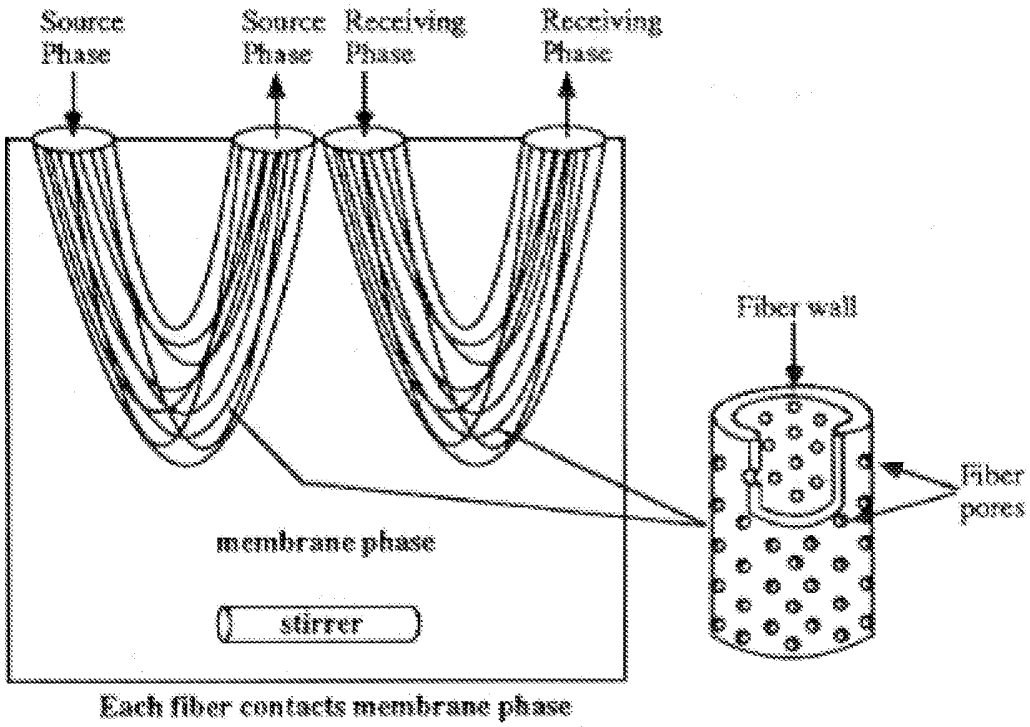
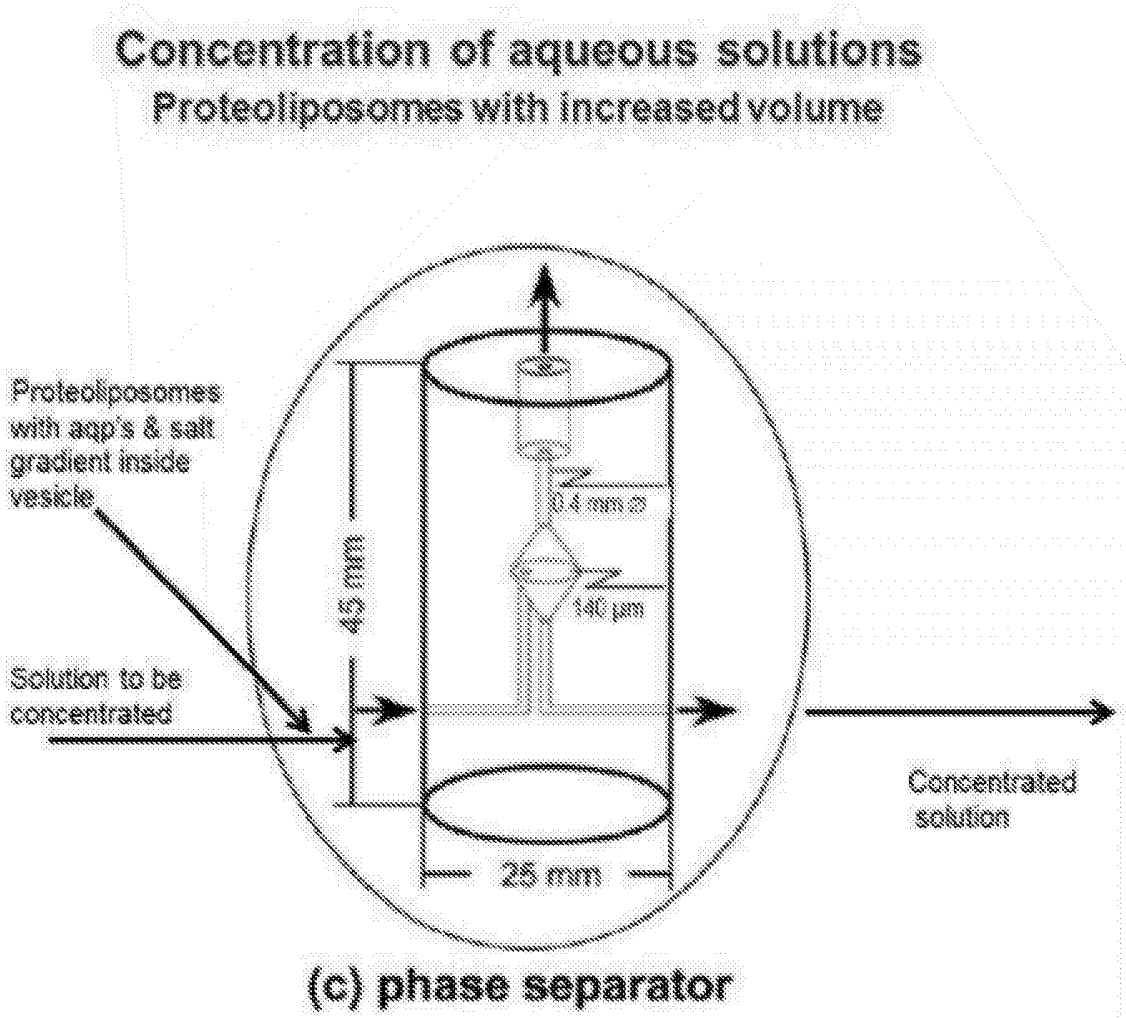

Source Phase
Source Phase
Receiving Phase
Receiving Phase
Fiber wall
Fiber pores
membrane phase
stirrer
Each fiber contacts membrane phase

Fig. 4


Concentration of aqueous solutions
Proteoliposomes with increased volume
Proteoliposomes with aqp's & salt gradient inside vesicle
Solution to be concentrated
45 mm
0.4 mm ⌀
140 µm
25 mm
Concentrated solution
(c) phase separator

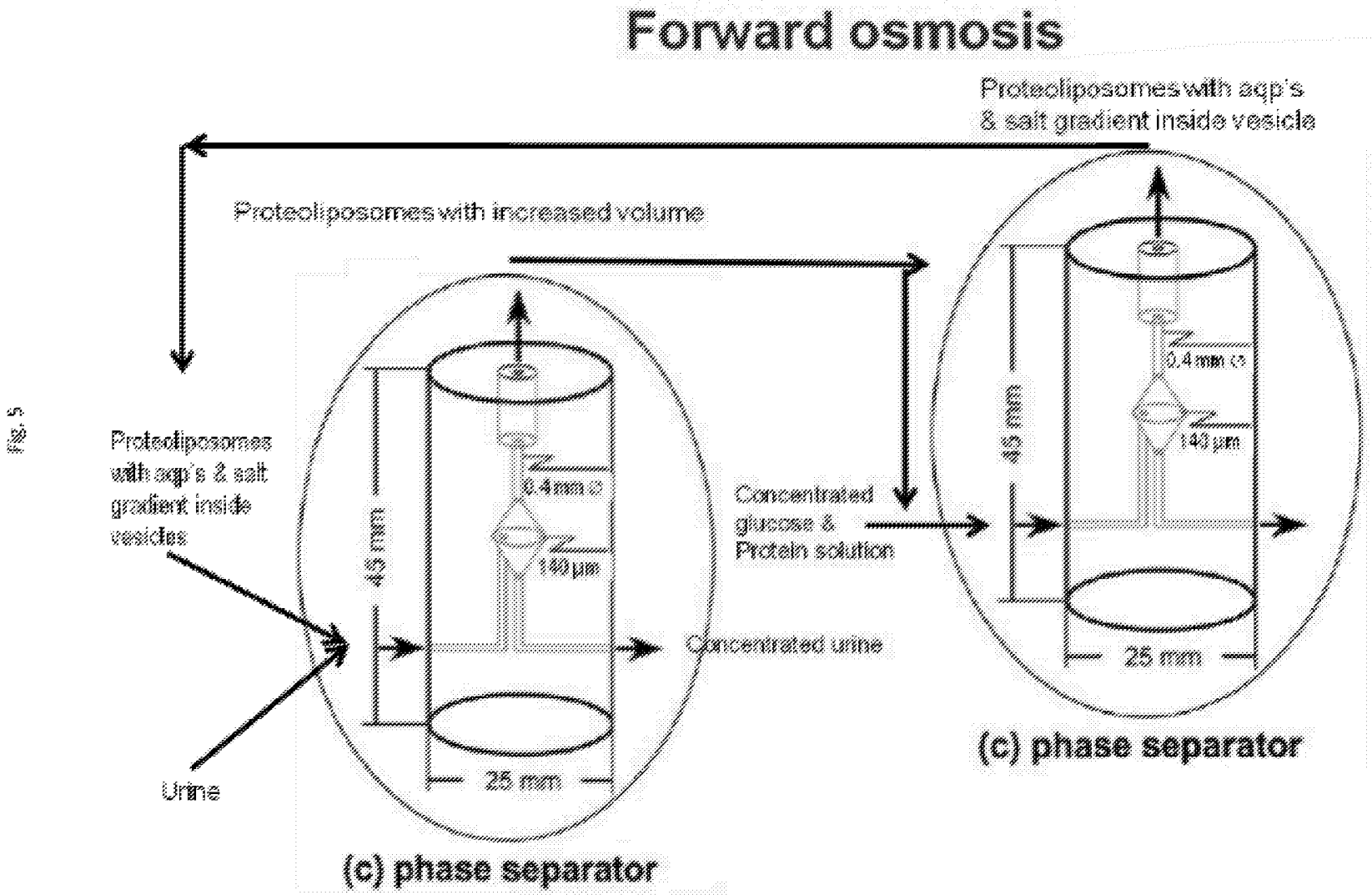

Forward osmosis
Proteoliposomes with aqp's & salt gradient inside vesicle
Proteoliposomes with increased volume
Proteoliposomes with aqp's & salt gradient inside vesicles
Urine
Concentrated urine
Concentrated glucose & Protein solution
40 mm
0.4 mm ø
140 µm
25 mm
(c) phase separator
40 mm
0.4 mm ø
140 µm
25 mm
(c) phase separator

Fig. 6

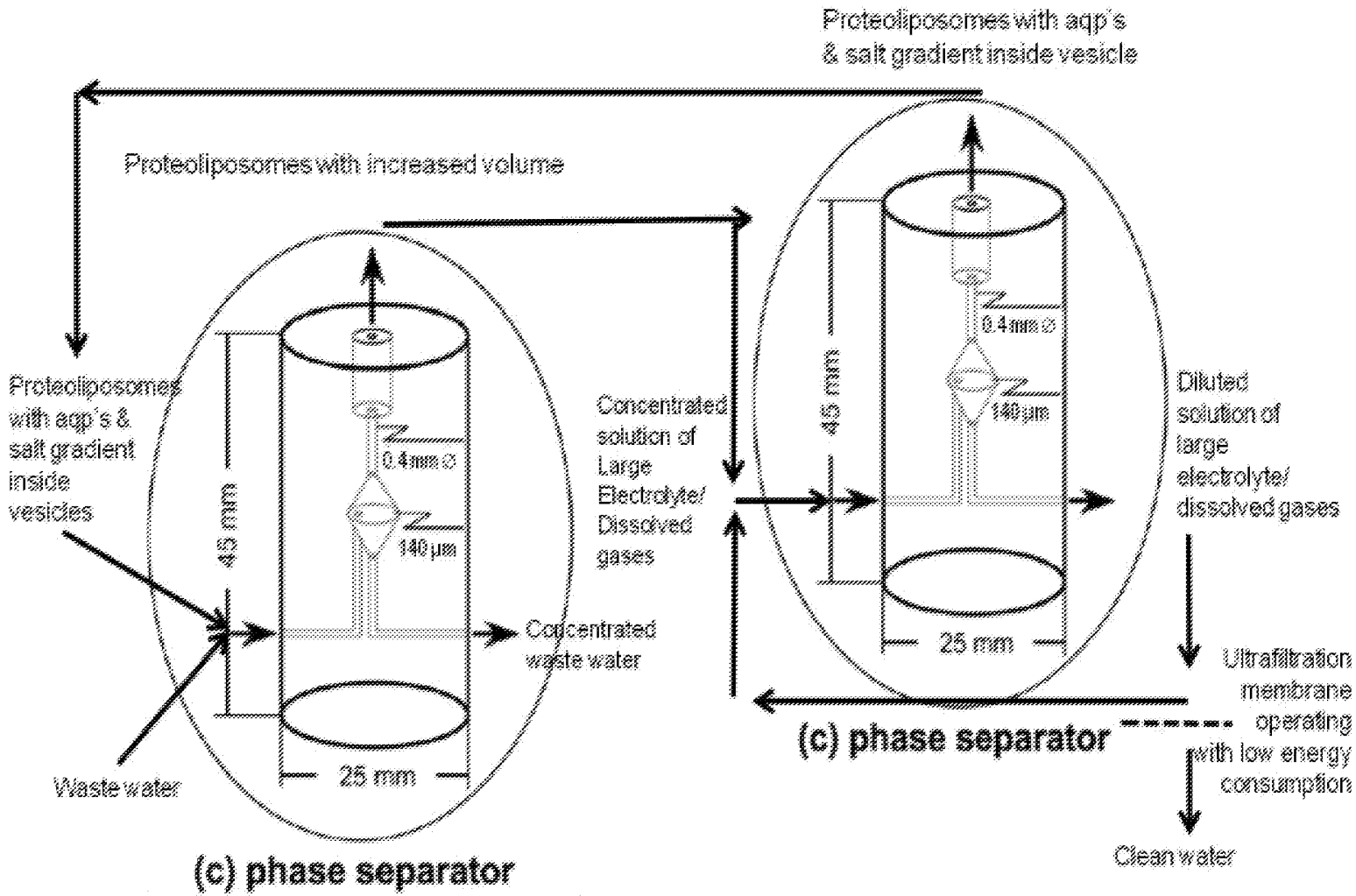

Forward osmosis – following ultrafiltration of uniform electrolyte.
Complete application of water extraction from any aqueous solution
Proteoliposomes with aqp's & salt gradient inside vesicle
Proteoliposomes with increased volume
Proteoliposomes with aqp's & salt gradient inside vesicles
Concentrated solution of Large Electrolyte/ Dissolved gases
Diluted solution of large electrolyte/ dissolved gases
Concentrated waste water
Waste water
Ultrafiltration membrane operating with low energy consumption
Clean water
45 mm
0.4mm ø
140 µm
25 mm
(c) phase separator
(c) phase separator

Fig. 7

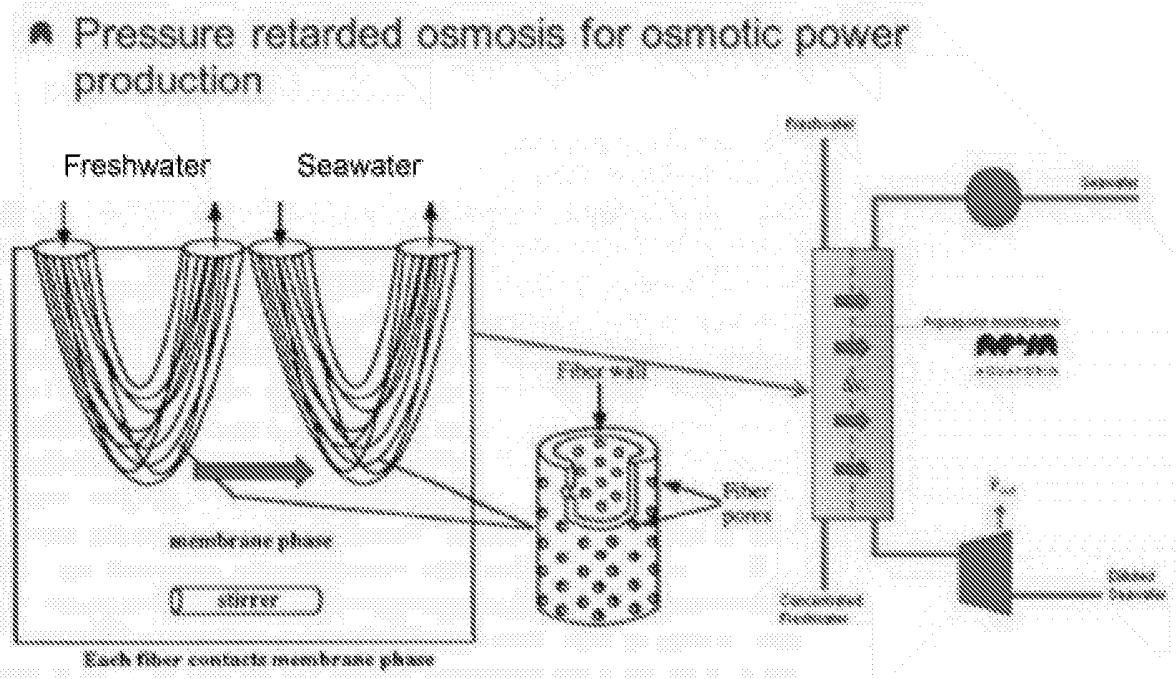
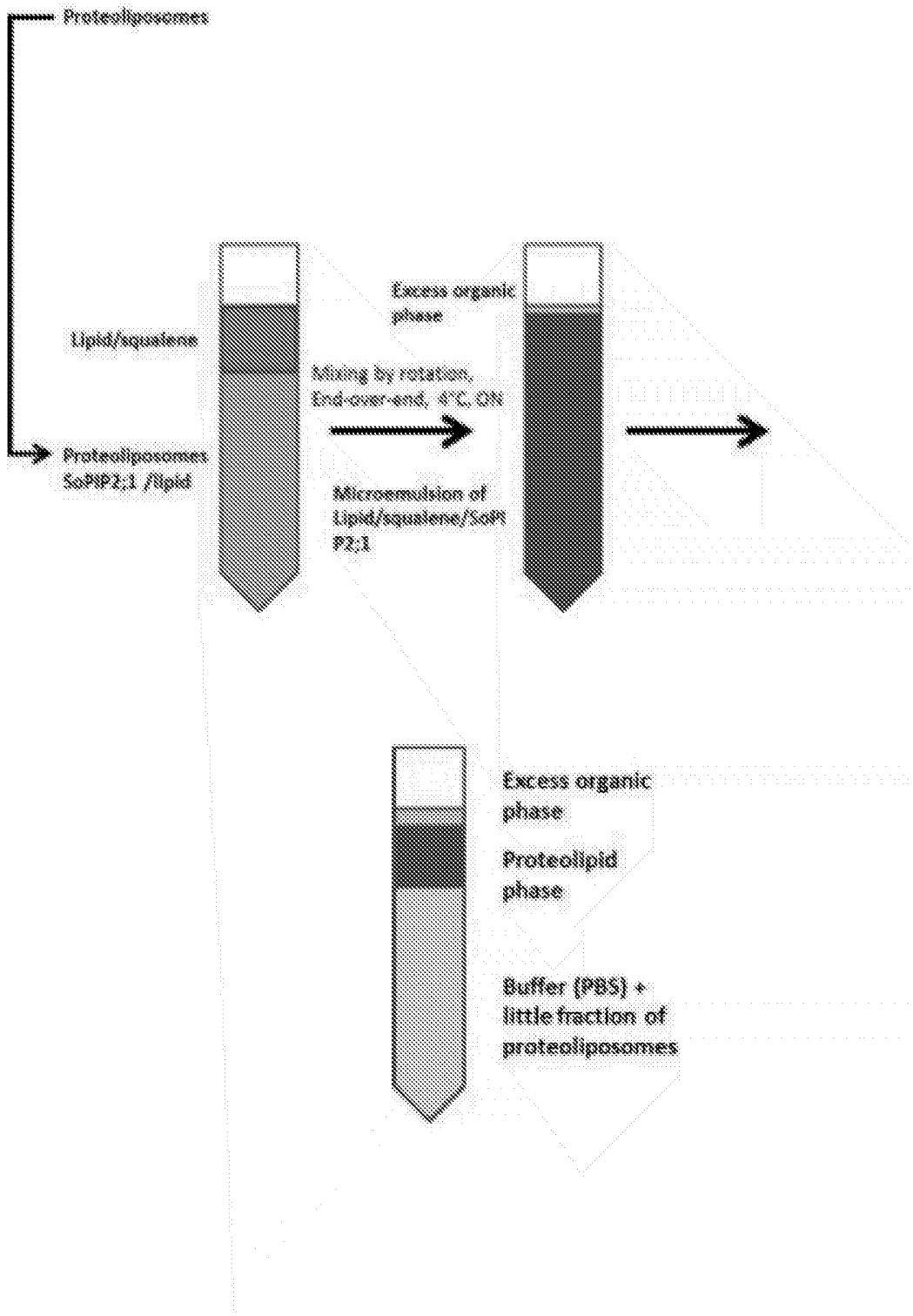

Pressure retarded osmosis for osmotic power production
Freshwater
Seawater
membrane phase
stirrer
Each fiber contacts membrane phase

Fig. 8


Proteoliposomes
Lipid/squalene
Proteoliposomes SoPIP2;1 /lipid
Mixing by rotation, End-over-end, 4°C, ON
Microemulsion of Lipid/squalene/SoPIP2;1
Excess organic phase
Excess organic phase
Proteolipid phase
Buffer (PBS) + little fraction of proteoliposomes