Polymun Scientific Immunbiologische Forschung GmbH

Pioneering a versatile LNP production process for mrNA vaccines, therapeutics and gene editing – Unveiling the proof of concept.

Andreas Wagner

PRC Webinar April 24th 2024

POLYMUN SCIENTIFIC

Polymun Scientific Immunbiologische Forschung GmbH

- **A PRIVATE COMPANY**
  Developing and Manufacturing Biopharmaceuticals
  and Liposomal Formulations for Human Application

  - CEO: Dr. Dietmar Katinger
  - Founded: 1992
  - Employees: 98

- regularly inspected by the Austrian regulatory authority AGES on behalf of EMA, last inspection in April 2024
- inspected by FDA in October 2013 / July 2023
- numerous audits by clients (~10 per year)

Core Activities

- ***Contract Development & Manufacturing of Biopharmaceuticals***
  for human application with focus on mammalian cell culture, process development & GMP production
- ***Contract Development & Manufacturing of LNPs and Liposomal Formulations***
  LNP & liposomal formulation development for APIs and vaccine antigens & GMP production
  - ***Formulation of mRNA and oligonucleotides in liposomes/LNPs***
    siRNA, saRNA, miRNA and mRNA formulated up to 300 g API input per batch
  - ***Liposomal adjuvants, liposomal vaccines***
    liposomal formulation of MPLA as well as other TLR4 agonists in combination with other adjuvants like saponins, CpG,..
  - ***Covid-19 mRNA vaccine collaborations with:***
    - BioNTech/Pfizer
    - CureVac
    - Imperial College London
    - Arcturus Therapeutics
- ***Research Reagents***
  manufacturing and distribution of HIV antibodies and antigens
- ***Own R&D Projects***
  funded by revenues from contract development and contract manufacturing

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At a Polymun laboratory in Klosterneuburg, Austria, the size distribution of lipid nanoparticles is measured. MARYLISE VIGNEAU FOR THE WALL STREET JOURNAL

How it started

JOURNAL OF LIPOSOME RESEARCH
Vol. 12, No. 3, pp. 259–270, 2002

THE CROSSFLOW INJECTION TECHNIQUE: AN IMPROVEMENT OF THE ETHANOL INJECTION METHOD

Andreas Wagner,[1,*] Karola Vorauer-Uhl,[1] Günther Kreismayr,[2] and Hermann Katinger[1]

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[2]Polymun Scientific, Immunbiologische Forschung GmbH, Nussdorfer Lände 11, A-1090 Vienna, Austria

ENHANCED PROTEIN LOADING INTO LIPOSOMES BY THE MULTIPLE CROSSFLOW INJECTION TECHNIQUE

Andreas Wagner,[1,*] Karola Vorauer-Uhl,[1] Günther Kreismayr,[2] and Hermann Katinger[1]

[1]Institute of Applied Microbiology, University of Agricultural Sciences, Muthgasse 18, A-1190 Vienna, Austria
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ELSEVIER

European Journal of Pharmaceutics and Biopharmaceutics 54 (2002) 213–219

European Journal of Pharmaceutics and Biopharmaceutics

www.elsevier.com/locate/ejphabio

Research paper

Liposomes produced in a pilot scale: production, purification and efficiency aspects

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[b]*Institute of Applied Microbiology, University of Agricultural Sciences, Vienna, Austria*

Received 21 January 2002; accepted in revised form 26 April 2002

GMP Production of Liposomes—A New Industrial Approach

ANDREAS WAGNER,[1] MIRKO PLATZGUMMER,[1] GÜNTHER KREISMAYR,[1] HERIBERT QUENDLER,[2] GABRIELA STIEGLER,[1] BORIS FERKO,[2] GABRIELA VECERA,[1] KAROLA VORAUER-UHL,[2] AND HERMANN KATINGER PROF[1,2]

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Review Article

Liposome Technology for Industrial Purposes

Andreas Wagner[1] and Karola Vorauer-Uhl[2]

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Received 30 June 2010; Accepted 20 October 2010

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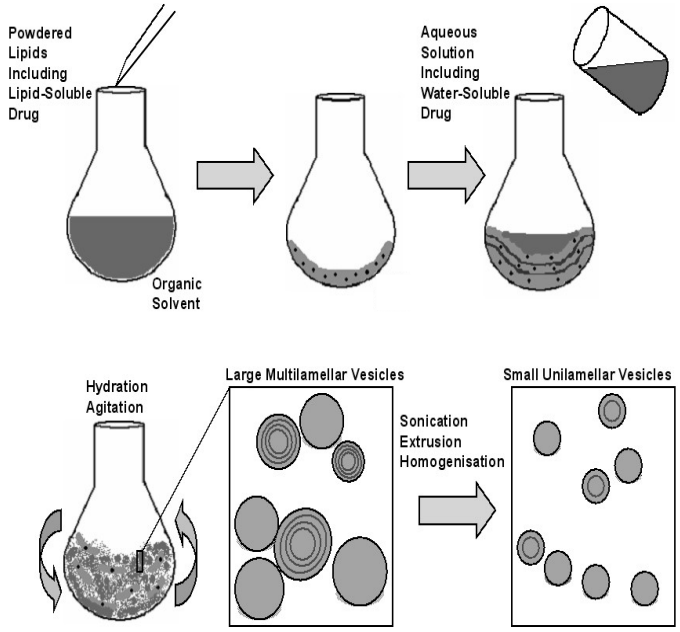
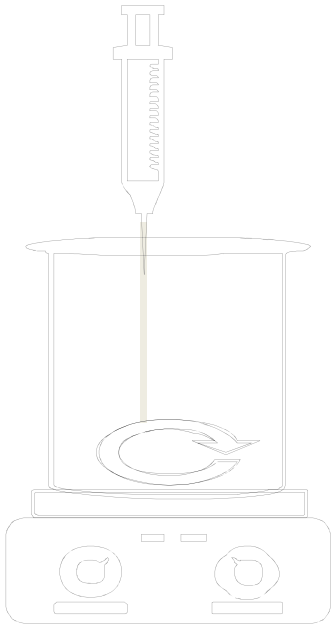
Liposome formulation processes

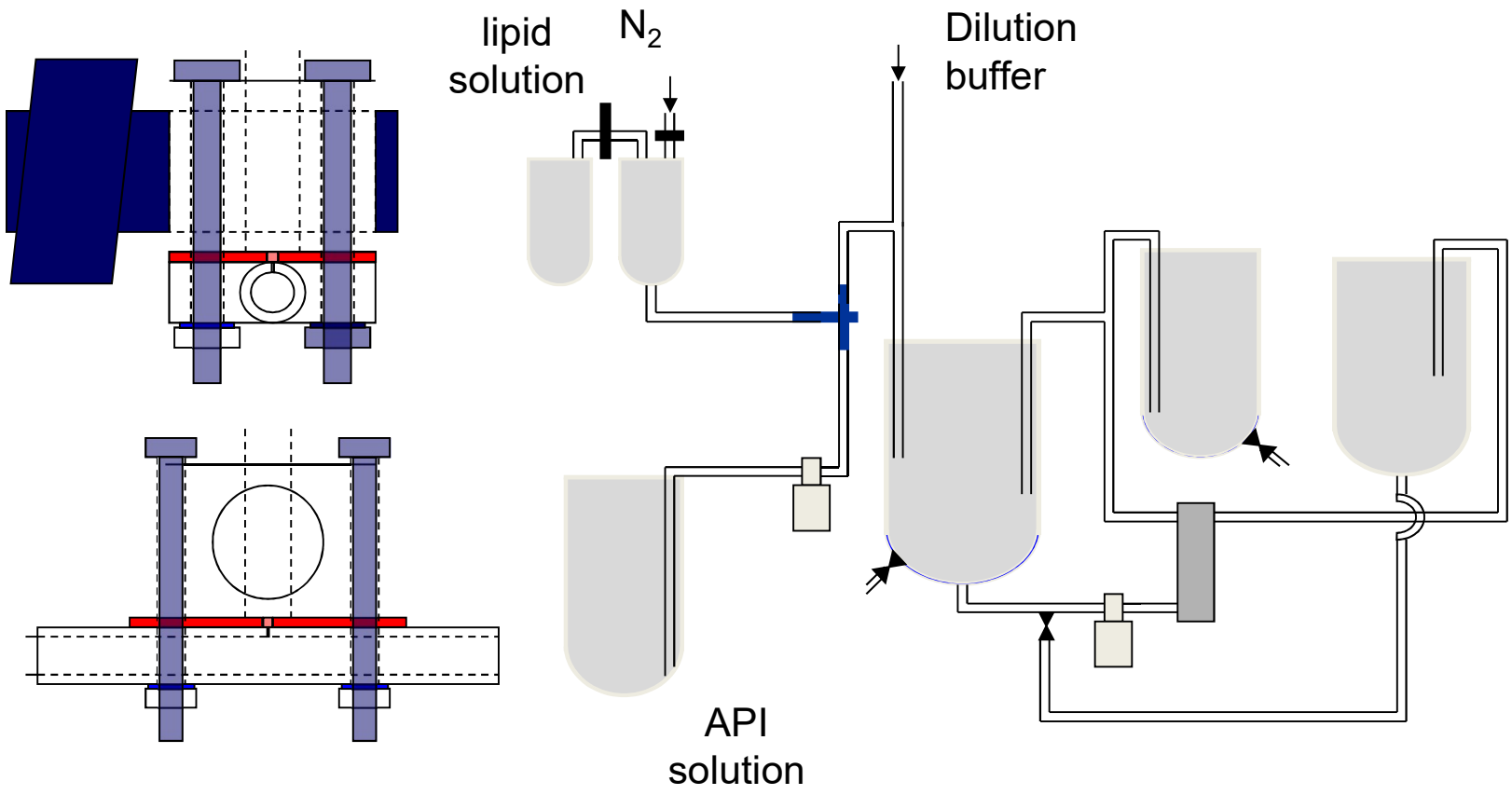
- Film method – most frequently used lab scale liposome formulation technique



- Lab scale ethanol injection method according to Batzri et al.

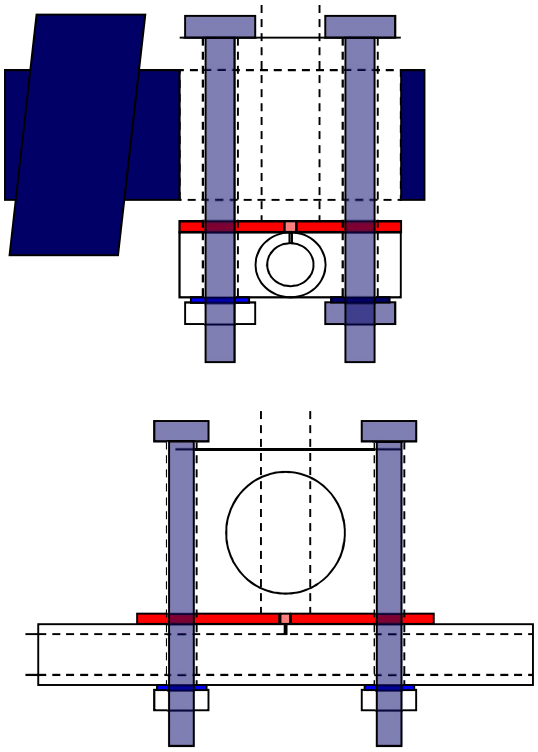


The Liposome Technology



Wagner et al., 2006, GMP Production of Liposomes - A New Industrial Approach. J Liposome Res 16(3):311-9

Critical Process Parameters



- lipid concentration in EtOH
- injection pressure/flow rate
- injection hole diameter
- lipid composition
- aqueous phase – ionic strength, pH
- flow rate aqueous phase
- process temperature
- solvent / solvent mixtures
- Ratio aqueous phase / solvent

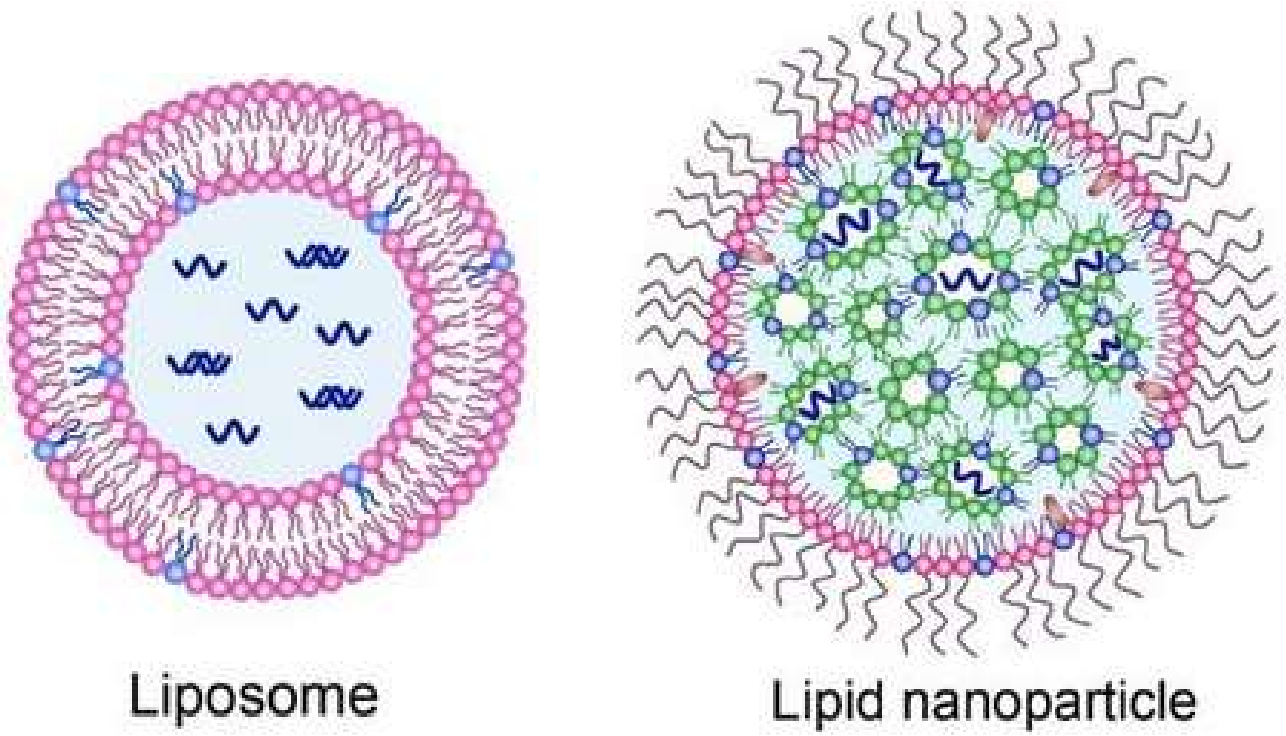
Advantages of the Polymun Technology

- Full scalability
- Aseptic process conditions
- Homogeneous, uniform vesicles
- Single step process
- Excellent batch to batch consistency
- Mild procedure - stability

Liposomal Formulation in the Lab Scale: 100 – 200 mL in seconds

Liposomal Formulation at Production Scale: 250 L in 1.5 hour

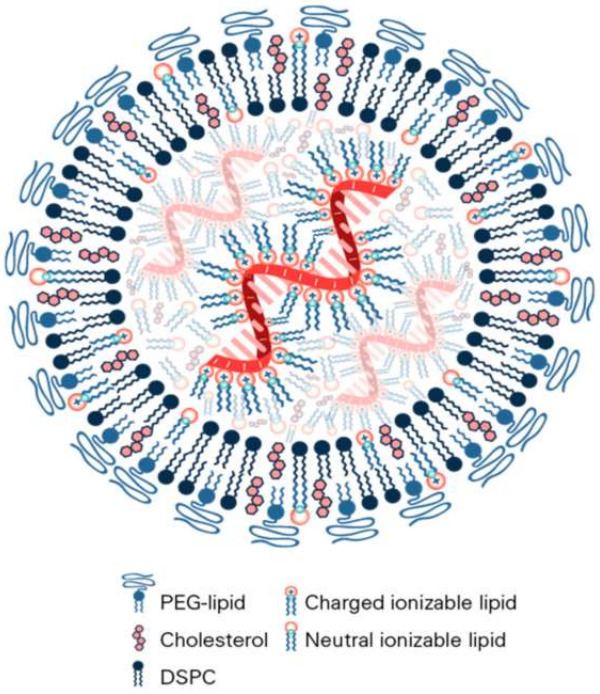
Comparison of Liposomes and Lipid-Nanoparticles

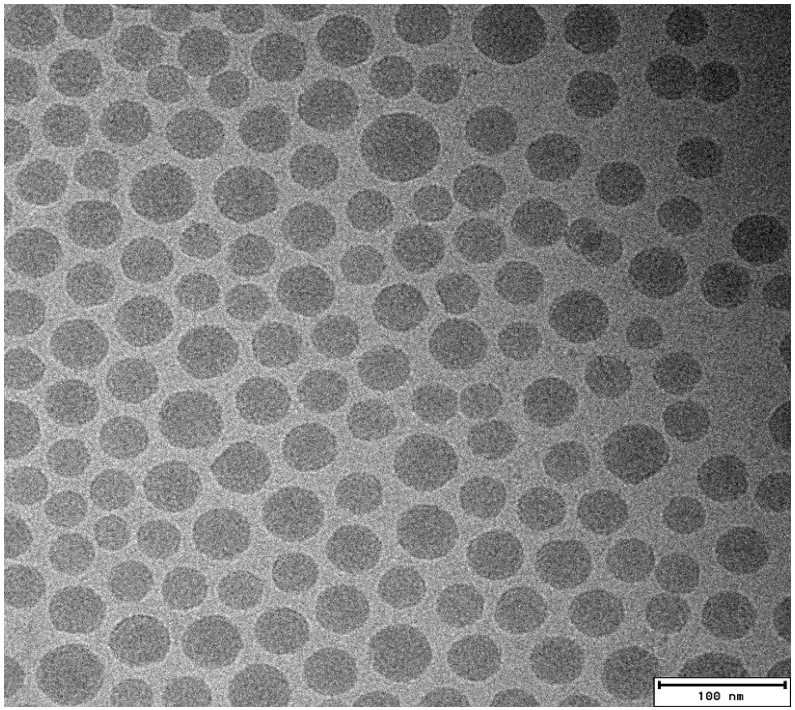


From:
Delivering the right message: Challenges and opportunities in lipid nanoparticles-mediated modified mRNA therapeutics—An innate immune system standpoint
Granot & Peer, Seminars in Immunology 2017, 34

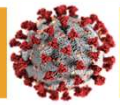
Structure of Lipid-Nanoparticle

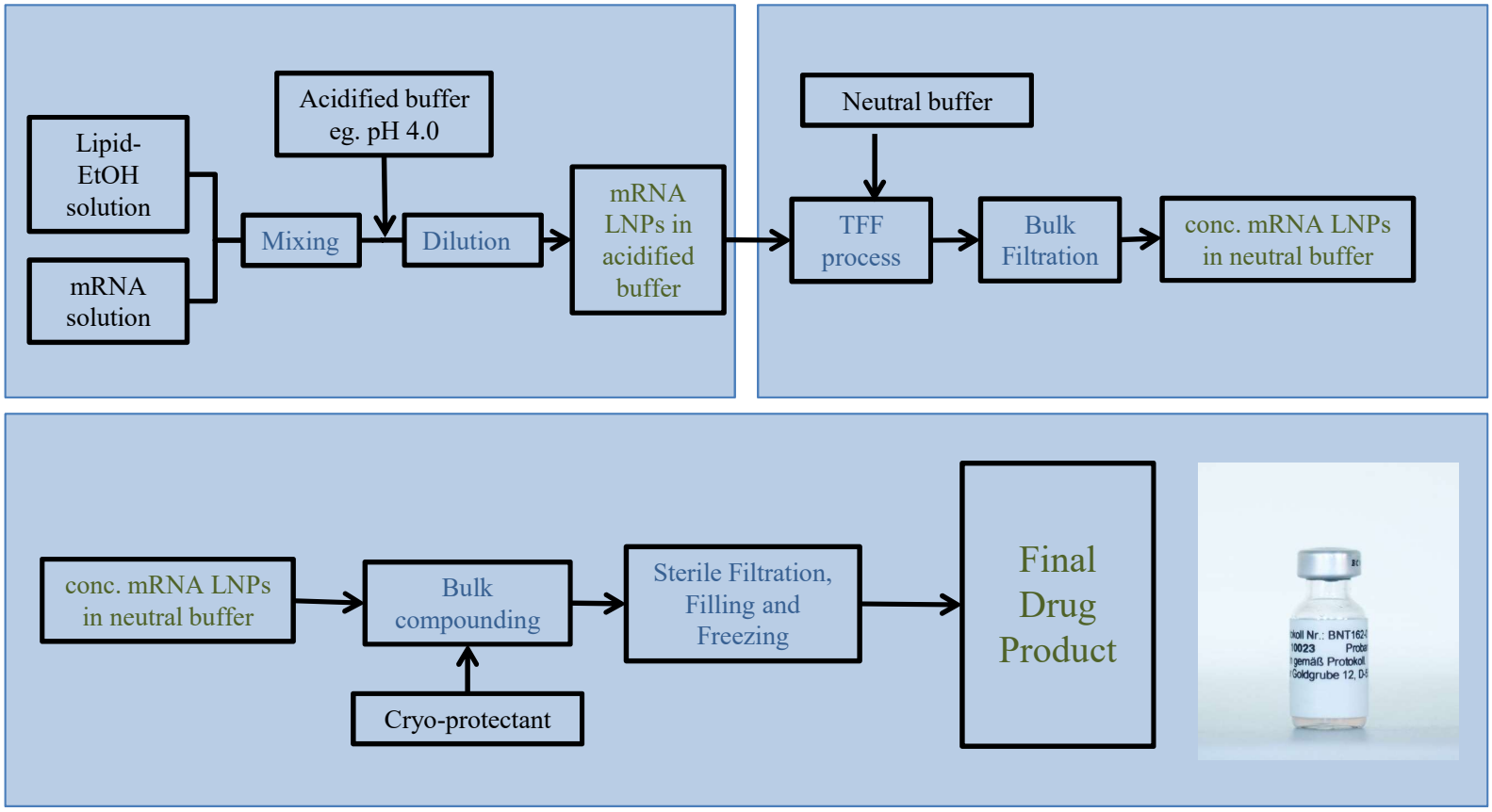
From:
Nanomaterial Delivery Systems for mRNA Vaccines
Buschmann et al., Vaccines 2021, 9, 65





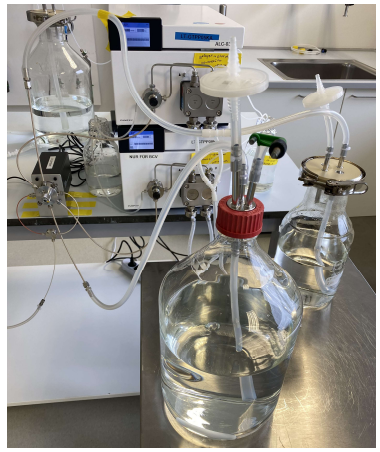
Production of mRNA LNP Vaccines

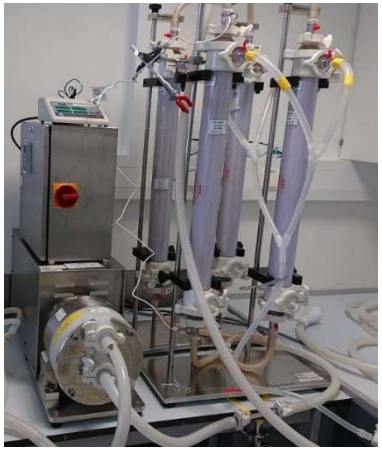




mRNA Vaccines – Achievements within < 1 year

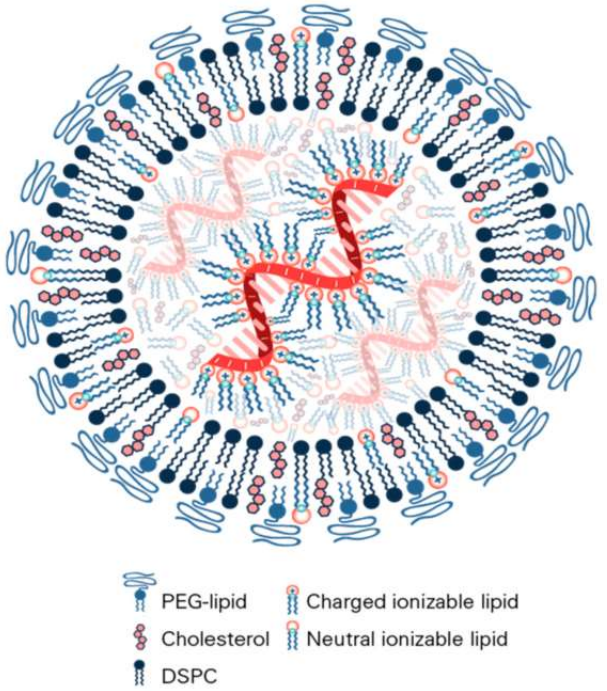
- *Process set-up, optimization and scale-up*
- *Production of 10 different vaccines for tox studies*
- *Production of 5 different vaccines to initiate clinical trials*
- *Production of 2 different vaccines for phase 3 (> 40 000 subjects)*
- *Tech transfer to BioNTech/Pfizer network*
- *Analytical method validations*
- *(multi-center) process validation*
- *Regulatory support*
- *Production of 15 million doses to be used in EUA program in late 2020 / early 2021*
- *Continued bulk DP production in 2021*





Basics for LNP formulations

* *lipid composition (ratio ionizable lipid, PEG-lipid, PC, cholesterol)*
* *drug substance type, size,*
* *ratio mRNA to ionizable lipid*
* *raw material quality/purity*
* *aqueous phase: pH, ionic strength, viscosity*
* *Ratio aqueous phase vs. solvent and solvent type*



mRNA LNP process development – critical process parameters (CPPs)

– *LNP formation step:*

** concentration of mRNA in acidified buffer and lipids in EtOH*

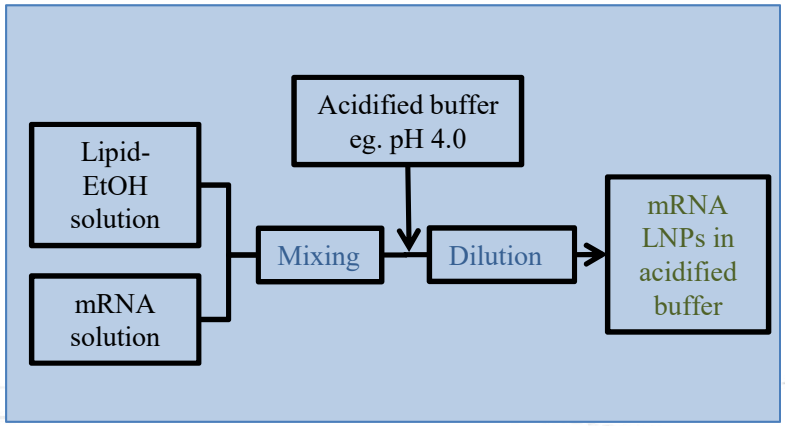
** flow rates and flow rate ratios*

** pump types – pulsation, cavitation,….*

** inline dilution: type of buffer, time of particle maturation, EtOH concentration reduction*

** aqueous phase: pH, ionic strength, viscosity*

** process temperature – impacts mRNA as well as particle quality*



CQAs:

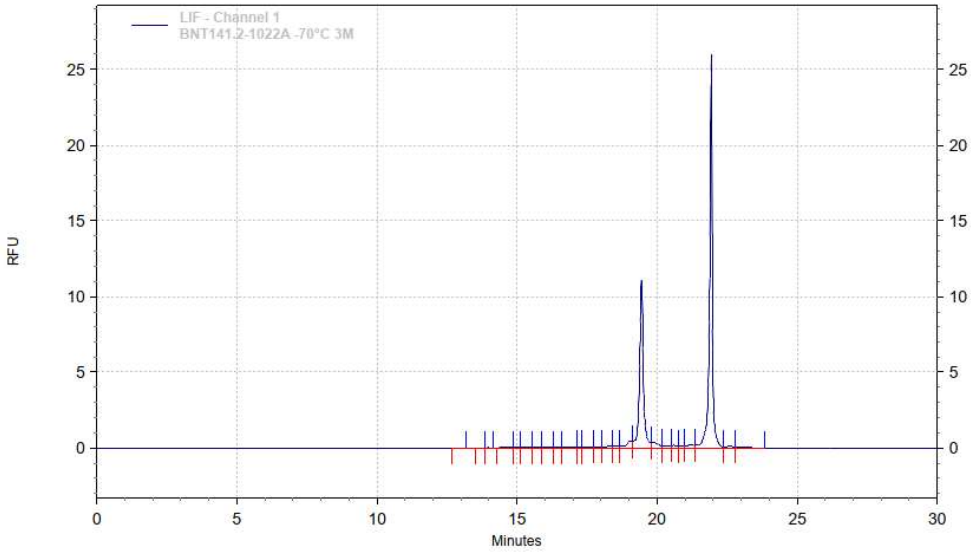
** particle size and PdI*

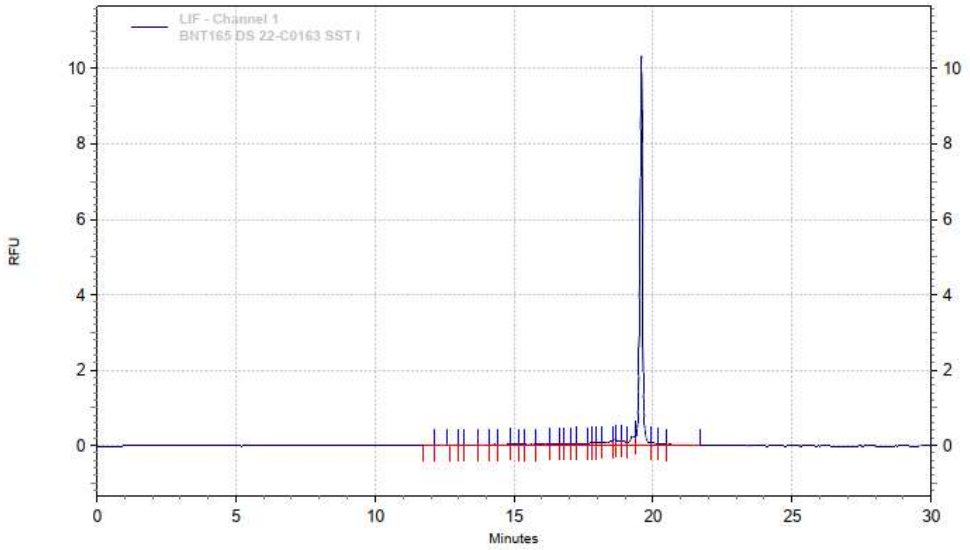
** RNA content and EE%*

Quality Control

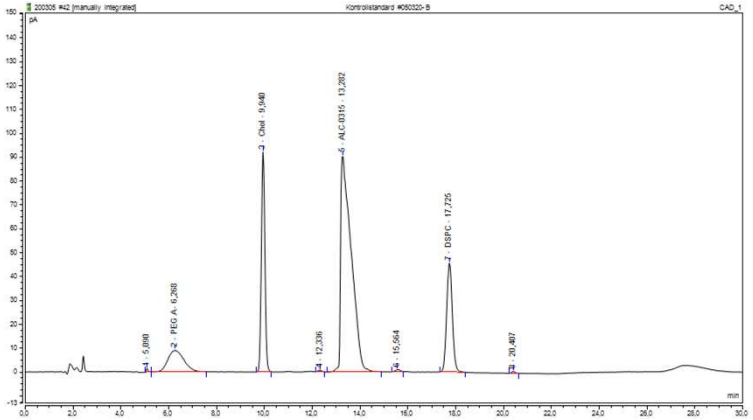
- *mRNA content and EE%*
- *mRNA identity and integrity (Capillary electrophoreses)*
- *lipid identity and quantity (HPLC CAD)*
- *LNP size / size distribution (QELS/PCS)*
- *pH*
- *Osmolality*
- *Bioburden testing*
- *Sterility testing*
- *Endotoxin testing*
- *Subvisible particles*
- *residual ethanol (GC)*

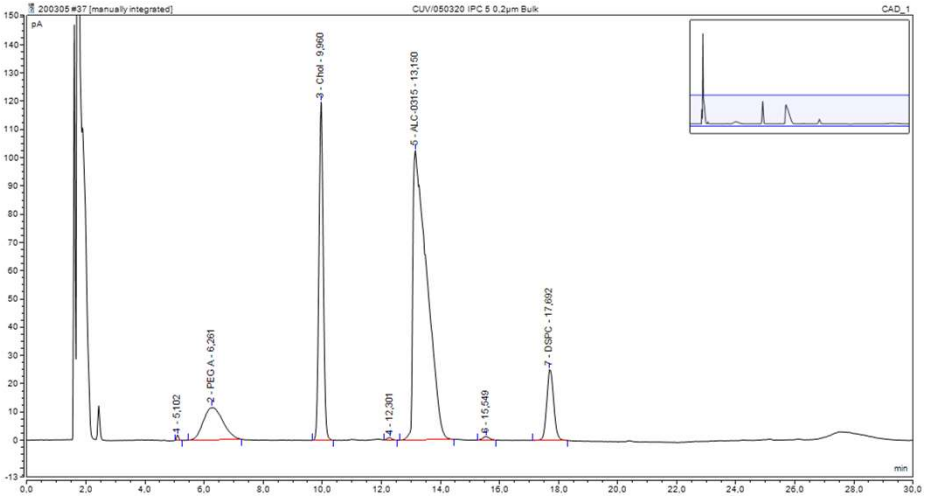
RNA Integrity by Capillary Electrophoreses

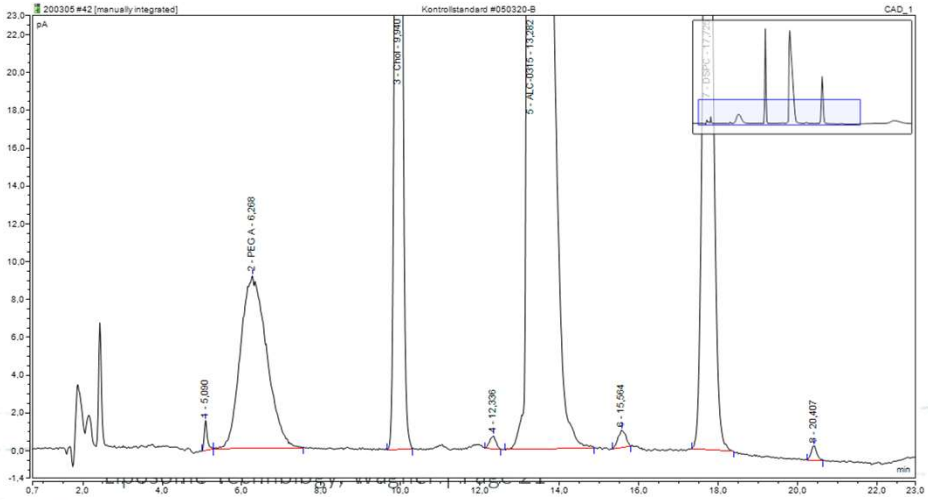




Lipid Identy and Quantity by rp-HPLC-CAD







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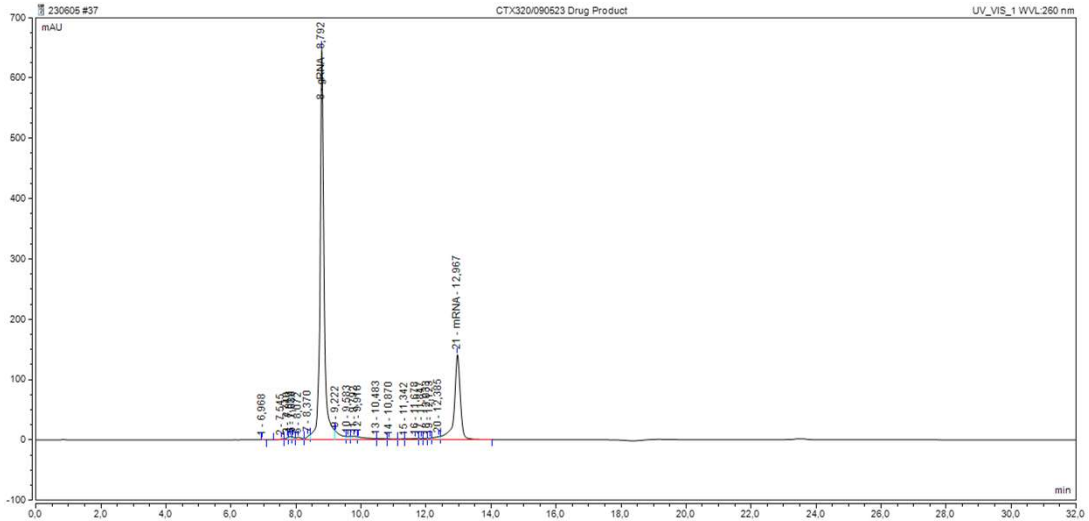
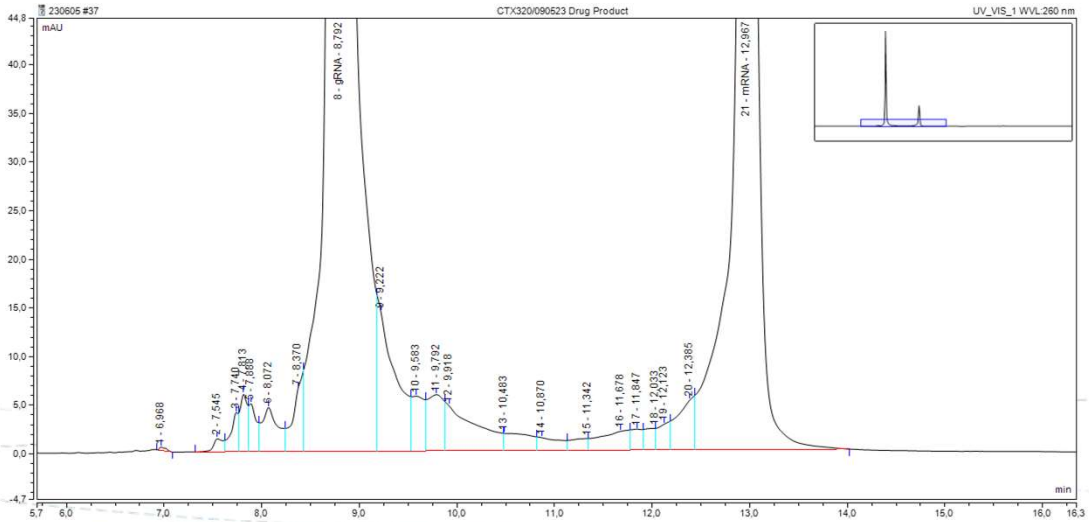
RNA – Quantity and Identity of gRNA and mRNA by IPRP





LNP processing – ongoing activities / optimization

- *LNP formation step:*
  - * *mRNA buffer optimization*
  - * *concentration of mRNA in acidified buffer and lipids in EtOH*
  - * *flow rates and flow rate ratios*
  - * *mixing unit, mixing angles*
  - * *pump types – pulsation, cavitation,….*
  - * *inline dilution: type of buffer, time of particle maturation, EtOH concentration reduction*
  - * *aqueous phase: pH, ionic strength, viscosity*
  - * *process temperature – impacts mRNA as well as particle quality*

LNP processing – ongoing activities / optimization

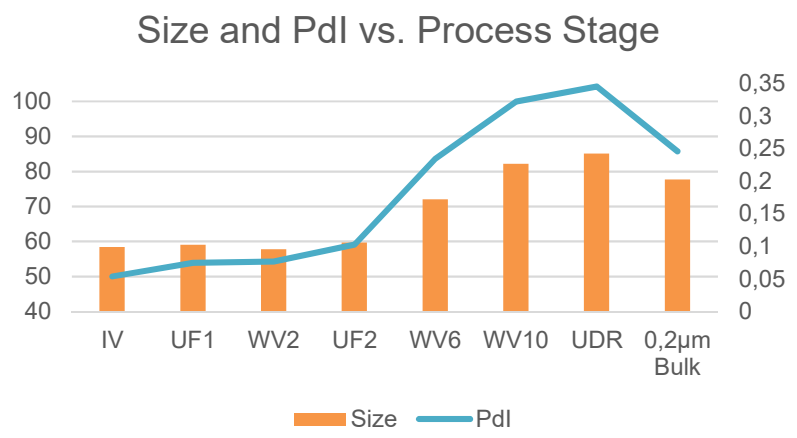
– *mRNA buffer optimization:*

** LNPs composed of ionizable lipid (pK 6 - 7)/Chol/DSPC/PEG-lipid*

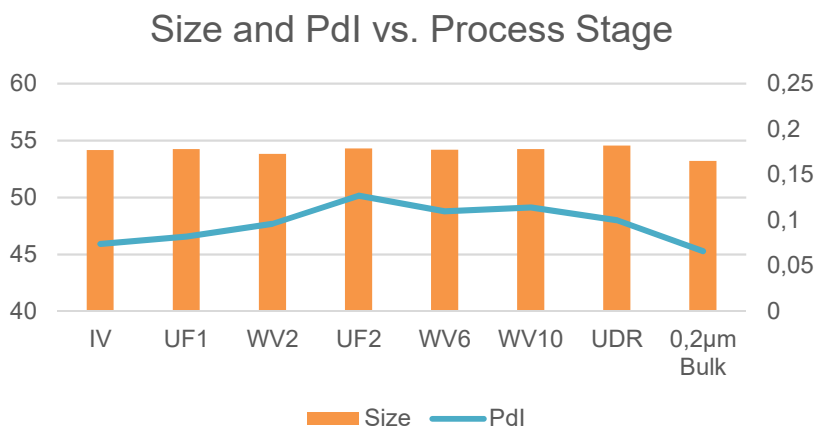
** mRNA in acetate buffer pH 4.0 vs pH 6.0*

** Exchange to PBS pH 7.4 during TFF*

formulated at pH 4.0

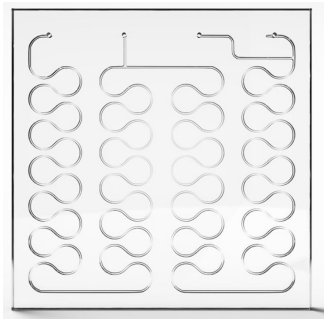
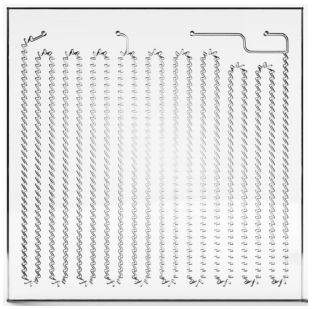
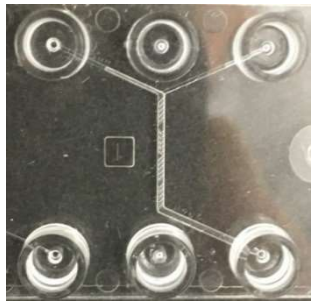


formulated at pH 6.0

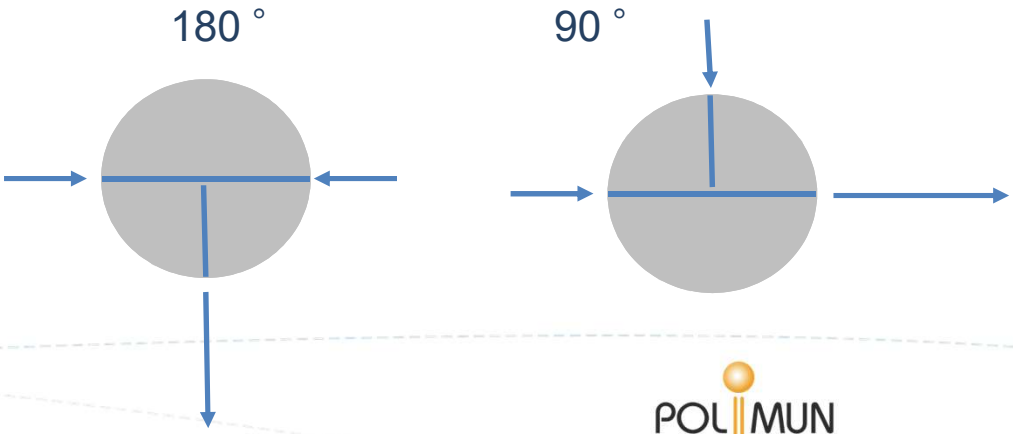


LNP processing – ongoing activities / optimization

- *Mixing unit:*
  - * microfluidic mixing
  - * T-mixer, Y-mixer, X-mixer
  - * Polymun cross-flow mixer


- *Mixing angels:*

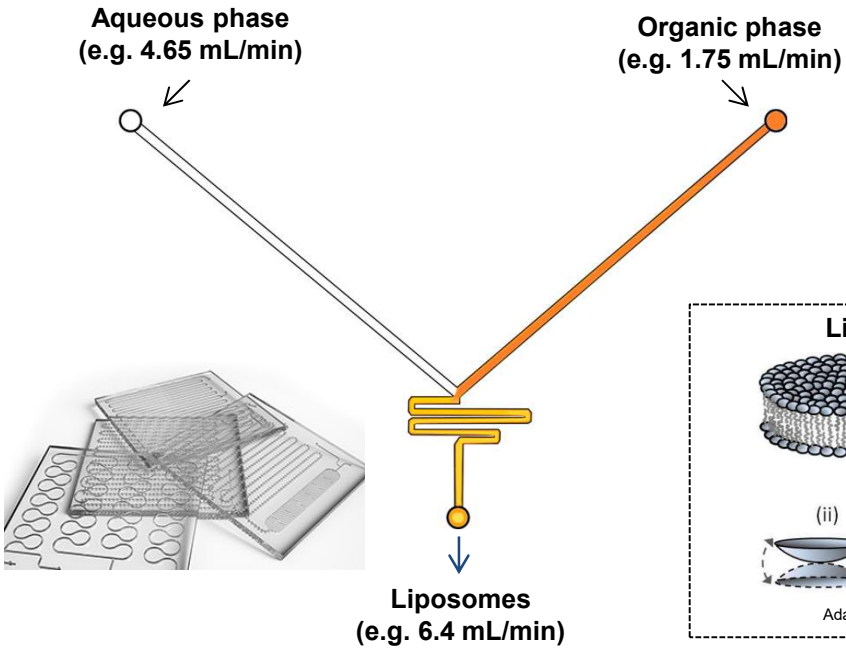


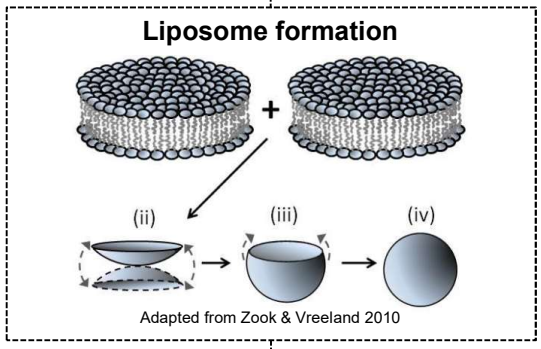
- *Pump types:*

Ethanol injection method – Laboratory Scale

Microfluidic mixing

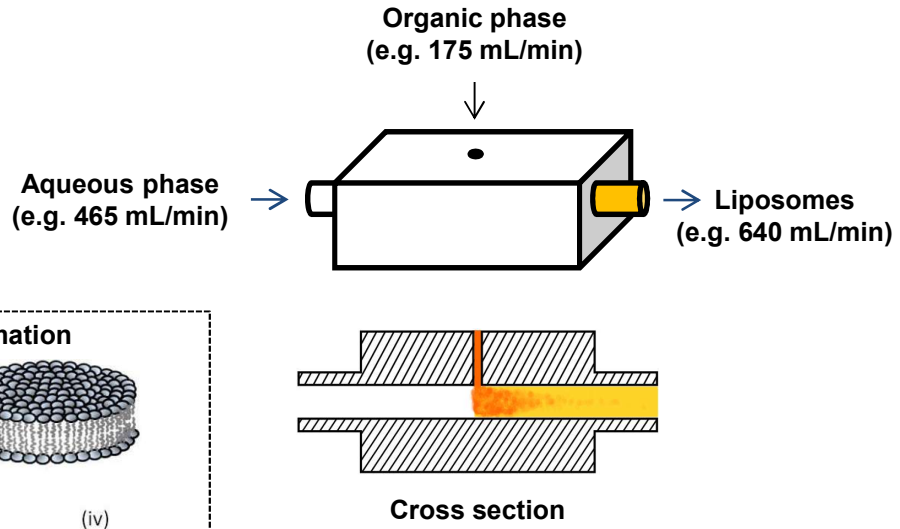
(Piston Pump driven system - mixing of both phases on a chip or within t-connectors)





Cross-flow solvent injection

(Polymun Scientific GmbH)



Processing at low mL-scale

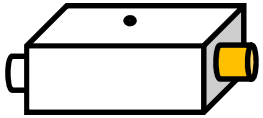
Production at mid and large scale

Study Case I – Product specifications

Formulation
- Ionizable lipid
- PC
- cholesterol
- Pegylated lipid

Target Size/PdI
<100 nm / <0.200

VS

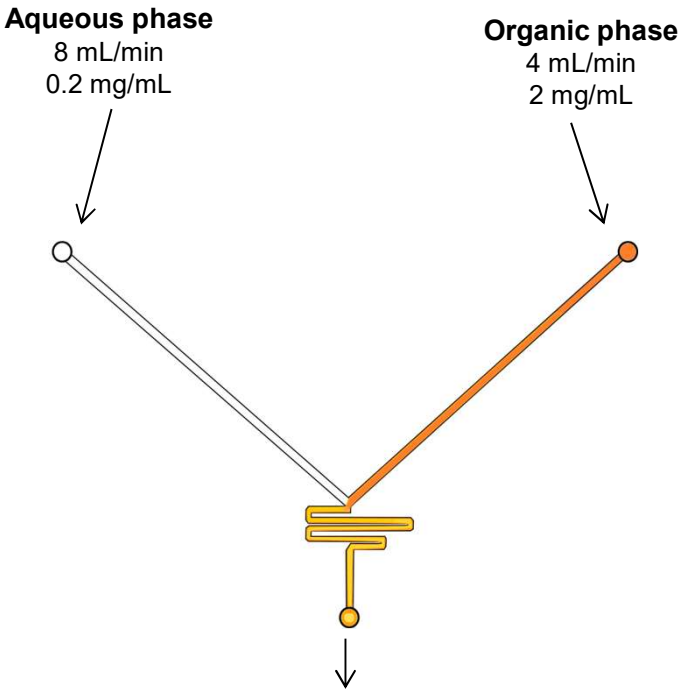


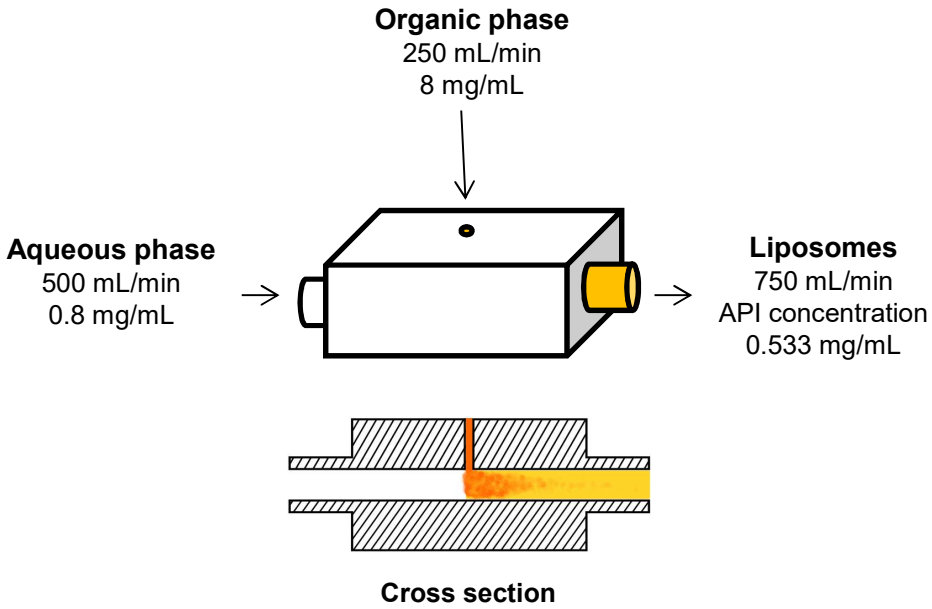
Target API concentration
5 mg/mL

Yield
>70%

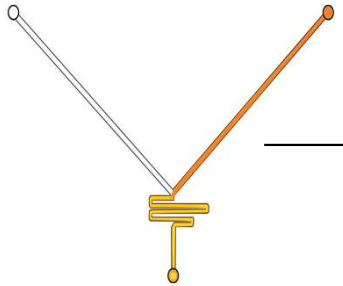
Encapsulation efficiency
>80%

Study Case I – Process parameters – Injection step





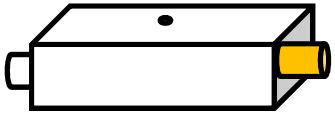
Study Case I – Process parameters – UDF



Injection step
Total flow rate: 12 mL/min
API concentration: 0.133 mg/mL
Production time for 1 Lit: 83 min.

Ultra-/dia-filtration step
Concentration factor: 38
Start volume: 1 Lit
End volume: 0.026 Lit

0.2 µm filtration

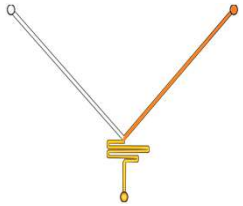


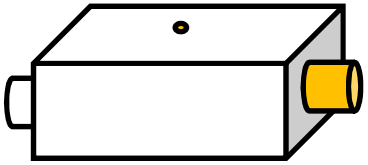
Injection step
Total flow rate: 750 mL/min
API concentration: 0.533 mg/mL
Production time for 1 Lit: 1.3 min.

Ultra-/dia-filtration step
Concentration factor: 9.4
Start volume: 1 Lit
End volume: 0.106 Lit

0.2 µm filtration

Study Case I – Product parameters – Polymun vs Microfluidics



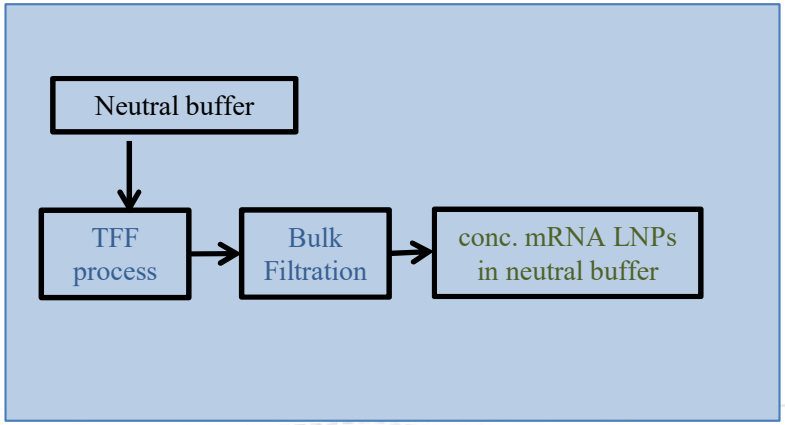


- Size: 70.79 nm
- PdI: 0.158
- Oligonucleotide yield: 75%
- Encapsulation efficiency: 100%
- Production time of 1 Lit: 83 min

- Size: 72.76 nm
- PdI: 0.104
- Oligonucleotide yield: > 98%
- Encapsulation efficiency: 100%
- Production time of 1 Lit: 1.3 min

mRNA LNP process development – critical process parameters

- *TFF process:*
  - ** loading: DP per membrane area*
  - ** shear rate, TMP, HF-length*
  - ** process temperature*
  - ** TFF sequence (ultrafiltration – concentration factor)*
    (diafiltration – number of volume exchanges)



- *bulk filtration process:*
  - ** filter type: material, cut-off*
  - ** loading: DP per filter membrane area*
  - ** flow rate, pressure*
  - ** pump/flow type*

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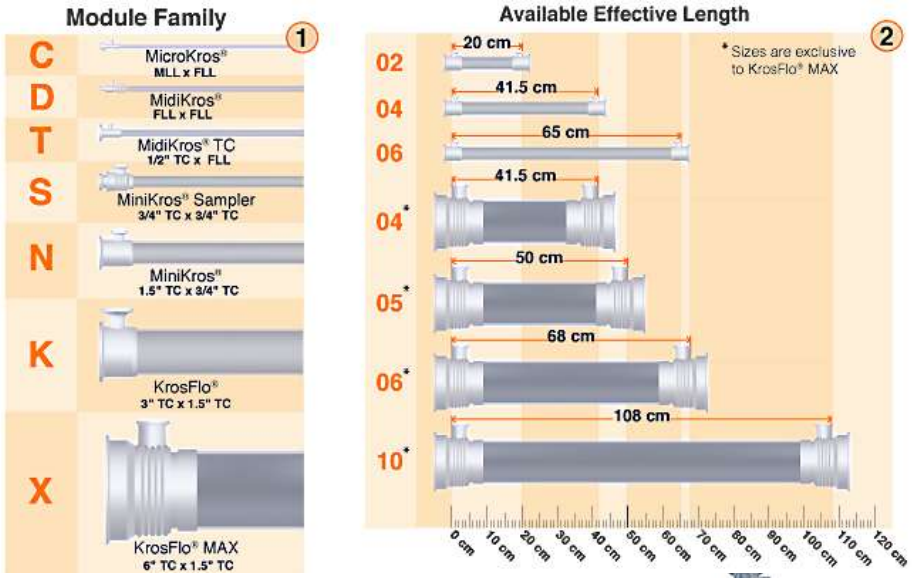
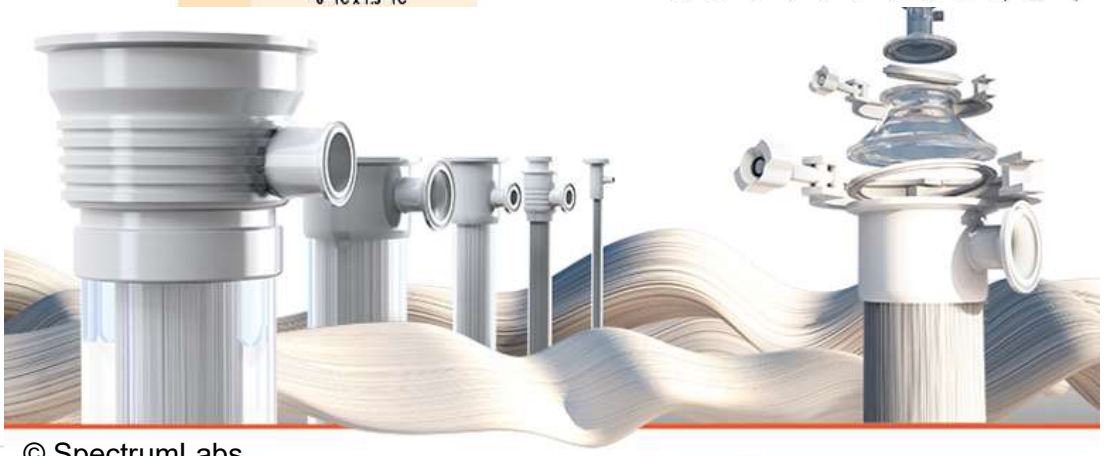
Optimization phase – Optimization of purification parameters

Hollow Fiber Modules:

- Scalable
- Different length and diameters
- Different cut-off
- Good compatibility with other equipment





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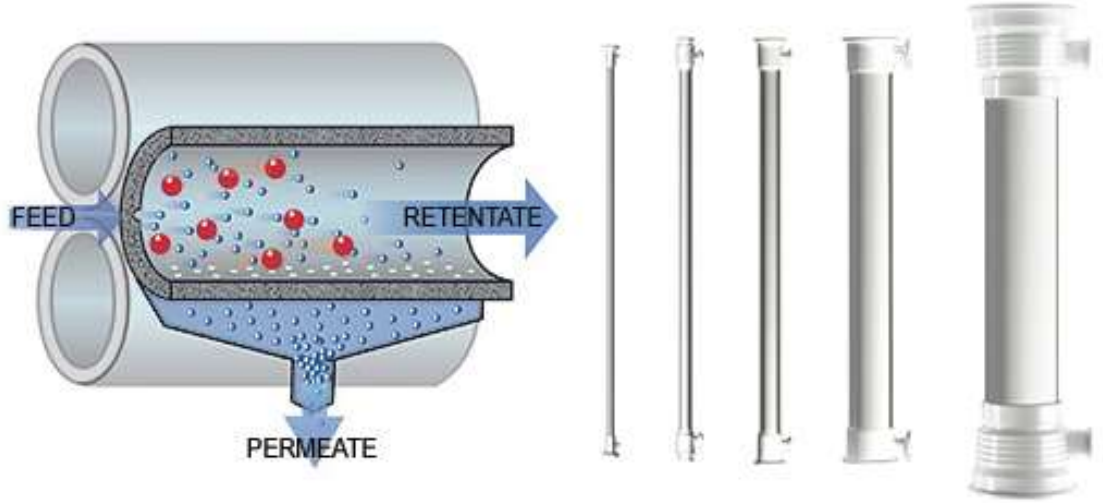
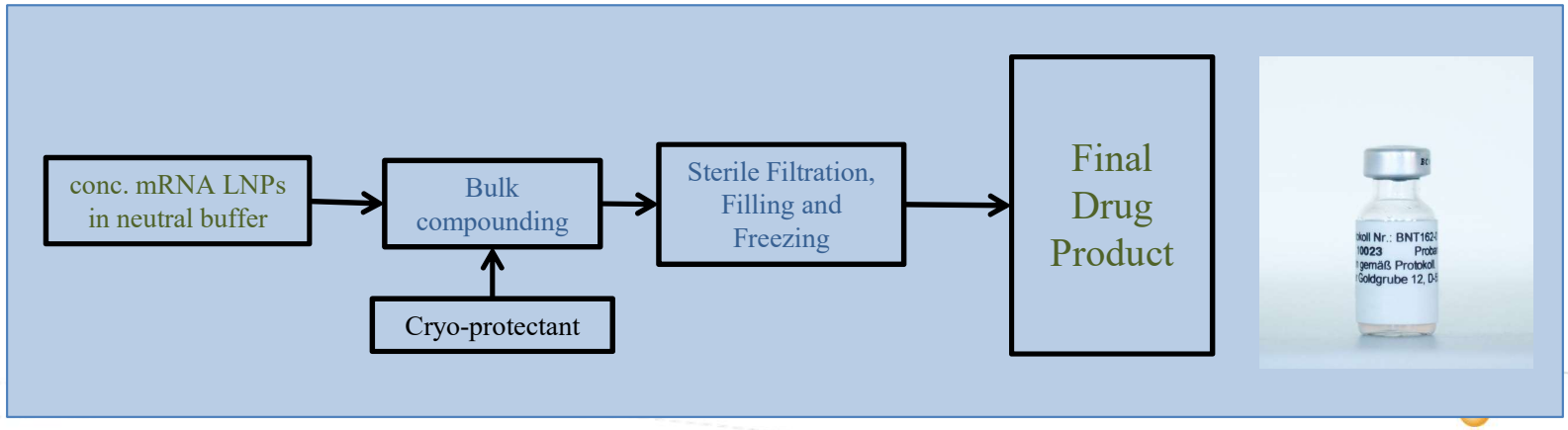
Optimization: Concentration Factor

- Constant Parameters:
  - Pump flow-rate (= constant Shear-rate)
- Variable Parameters:
  - Permeate flow
  - Concentration factor
  - Inlet pressure
- Further Process Parameters:
  - Process temperature
  - EtOH concentration at start of process
  - Buffer composition



mRNA LNP process development – critical process parameters

- *sterile filtration process and filling:*
  - ** filter type: material, cut-off*
  - ** pump type: impact on DP quality to avoid generation of particulates*
  - ** loading: DP per filter membrane area*
  - ** flow rate, pressure*
  - ** pump/flow strategy: vacuum, positive pressure, pump (type)*
  - ** primary packaging material; CCIT @- 80° C storage*
  - ** process temperature*



Optimization phase – 0.2 µm Filtration

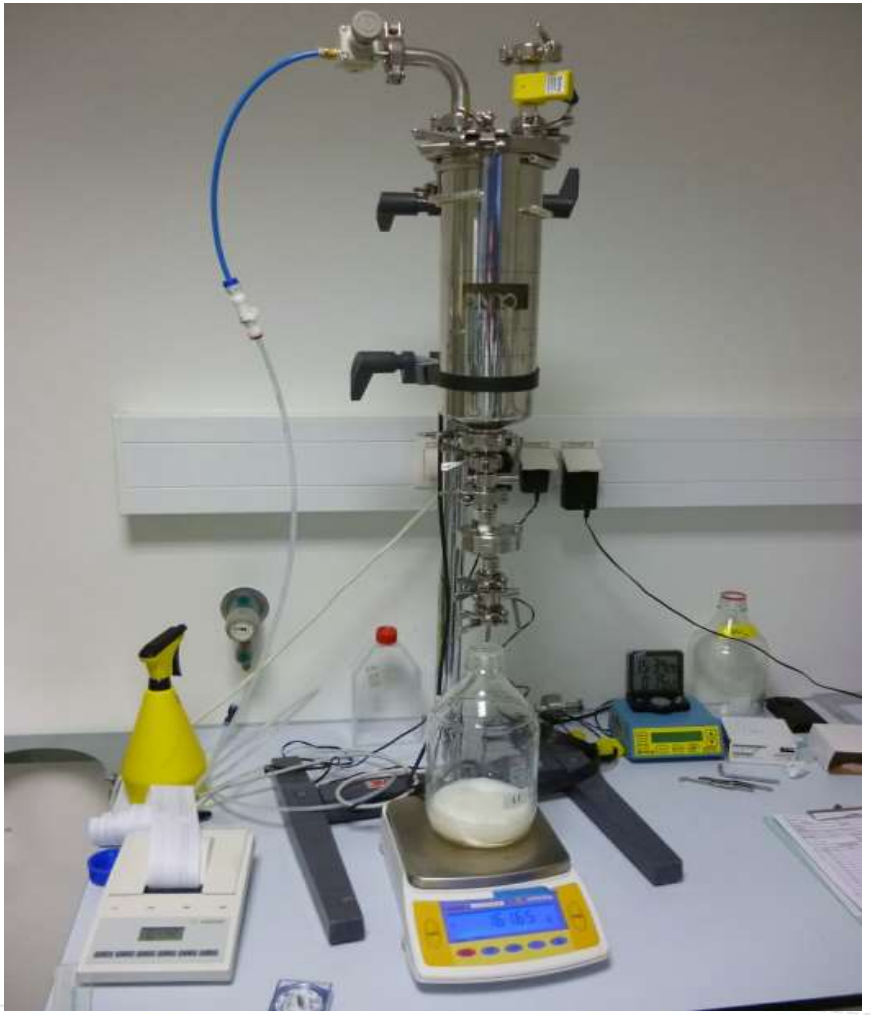
- Gradual Pore Plugging Model (V_{max})
  - Filter capacity measurement
  - Constant pressure (1 bar)
  - With 47 mm 0.2 µm filter discs

  V_{max} is the maximum volume of fluid that will pass through the filter before it is completely plugged.

  Gradual Pore Plugging Model (V_{max})

- Sterile Filter Validation

- Evaluation/Comparison of different suppliers



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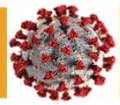
mRNA Vaccines – Scale up



- *Initial LNP formation process was designed to formulate 1 g mRNA- LNPs within 45 min*
- *Target: formulation of 1 g mRNA ≤ 1 min*
- *Scale up strategies:*

LNP formation

* *Increase of concentration of mRNA in acidified buffer and lipids in EtOH*
* *Increase of flow rates*
* *Multiple mixing lines*

TFF process

* *Increase of filter membrane area at constant shear rate*
* *Optimizing the TFF sequence*

Sterile filtration process

* *Increase of filter membrane area at constant pressure*

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Production History

- 200+ GMP productions runs for oligo formulations
- 100+ GMP productions for mRNA vaccines (incl. market product)
- Intermediate batch volume > 500 L
- mRNA input amounts > 40 g / batch
- High reproducibility
- High yields

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Thank you
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