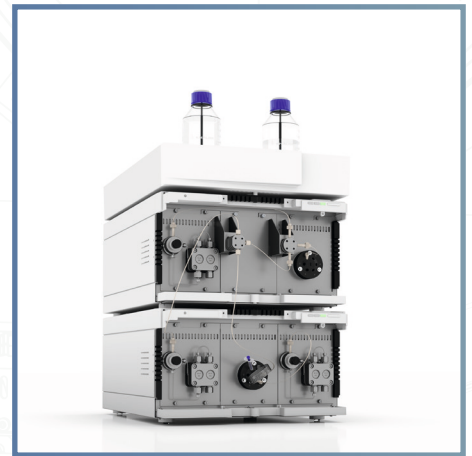


Science with Passion



Systems for lipid nanoparticle (LNP) production

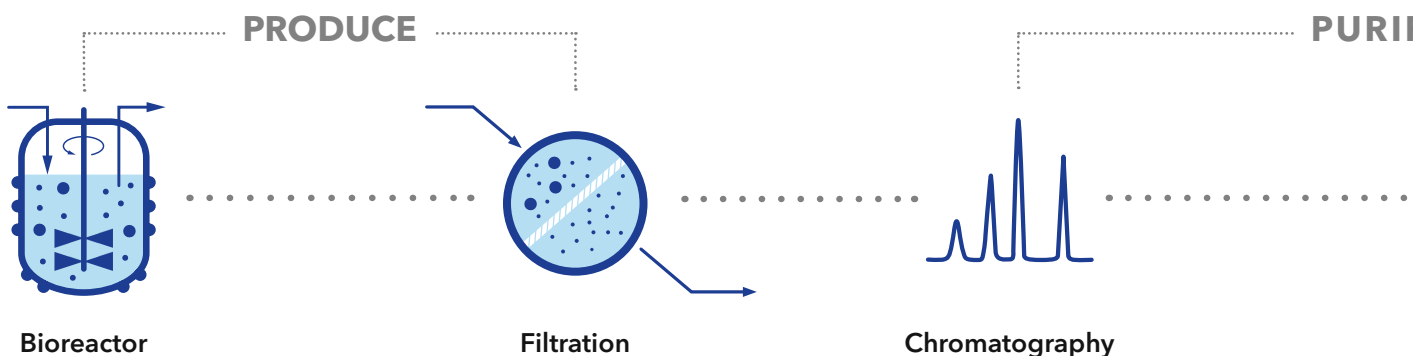


Impingement Jets Mixing units for high flow production of nanoparticles (LNP, microemulsions, etc.)

PANDEMIC PROVEN

think **LNP**. think **KNAUER**.

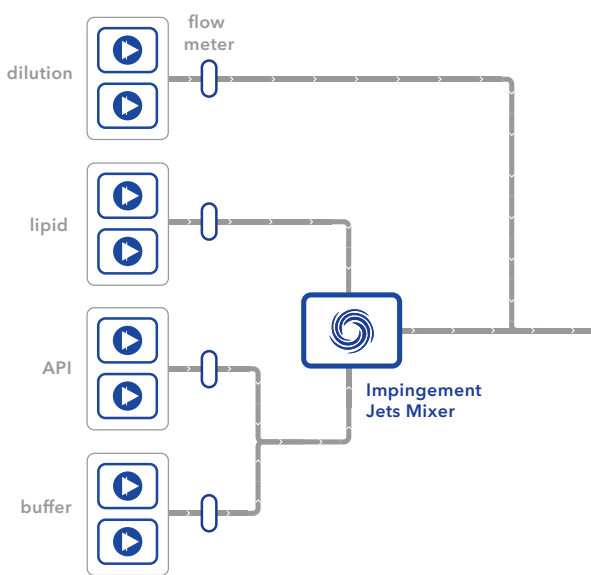
Process overview



Customized IJM NanoProducer for lipid nanoparticle production

Systems for lipid nanoparticle (LNP) production and formulation

The number of parallel units depends on the application. Also, the number of pumps, flow meters, jets mixers and pipes can be adapted to the required conditions and scale. All IJM NanoProducers are built in a stainless steel frame on casters and are suitable for CIP cleaning procedures in pharmaceutical production. The IJM DoubleCore NanoProducer contains two parallel mixing units. Each unit consists of four pumps. Concentrated Active Pharmaceutical Ingredient (API) solution is diluted first and then mixed with the organic phase. Finally, the mixture is quenched in a third mixer. Both units can be operated independently. The IJM NanoProducer can be configured with up to eight parallel mixing units depending on the customer requirements. Each unit consists of two pumps to deliver lipid and API streams, two flow meters for flow control and one jet mixer. Depending on the configuration, the predilution of the API and quenching can be performed for the combined flow of all units in one process step outside the Impingement Jets Mixing (IJM) NanoProducer.



Flow scheme for example for mixing of lipids and API

LOOKING FOR LIQUID CHROMATOGRAPHY?



As experts in liquid chromatography KNAUER offers a large variety of systems, solutions and applications for **analytical HPLC**, **preparative HPLC** and **FPLC**.

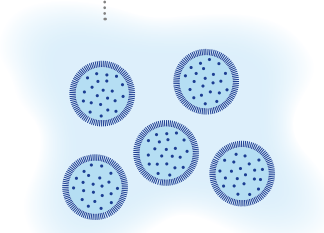
Find out more: www.knauer.net

RIFY

FORMULATE AND FILL



Concentration and sterile filtration



LNP Formulation with Impingement Jets Mixing Technology (IJM) by KNAUER



Drug product (e.g. lipid nanoparticles carrying mRNA)

The technology

Impingement Jets Mixing Technology (IJM)

enables the formulation and production of high-quality lipid nanoparticles carrying API (e.g. for **mRNA vaccines**). The encapsulation process of active pharmaceutical ingredients with lipids is based on Impingement Jets Mixing Technology, where two streams collide at high velocity in a jet mixing chamber. One of the streams contains the lipids in organic solvents and the other stream the API in water.

The mixing at high velocity reduces the solubility of the lipids so that homogenous nanoparticles are formed. The quality of nanoparticles depends on the streams' flow stability, the mixer geometry and the fluid velocities. As a final step, the mixture is quenched to stop particle growth.

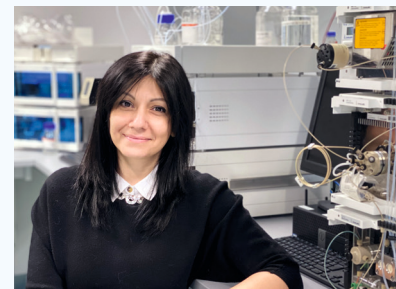
System for LNP formulation consisting of:

- KNAUER high-pressure dosing pumps 80P
- Coriolis flow meters
- KNAUER IJM mixers
- Inlet manifolds for lipid/ethanol mixture and API/buffer mixture
- Outlet manifold for LNP solution
- Back-seal washing system
- Frame (stainless steel system 1.4301)
- Installation & familiarization by KNAUER
- A customized technical documentation which is order- and article-related
- Customer Service before, during and after the set up
- Capillary customization and labeling
- FAT (Factory Acceptance Test at KNAUER Berlin)
- SAT (Site Acceptance Test at customer site)

WHAT THE EXPERT SAYS

"I am delighted that we have been able to successfully deliver all our systems for vaccine production on time. It makes our team proud that we are helping to combat COVID-19 in this way."

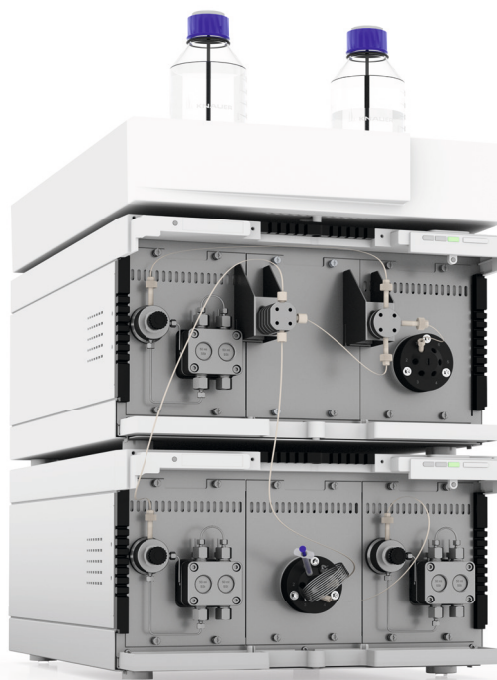
Lilit Avagyan, Senior Business Development Manager



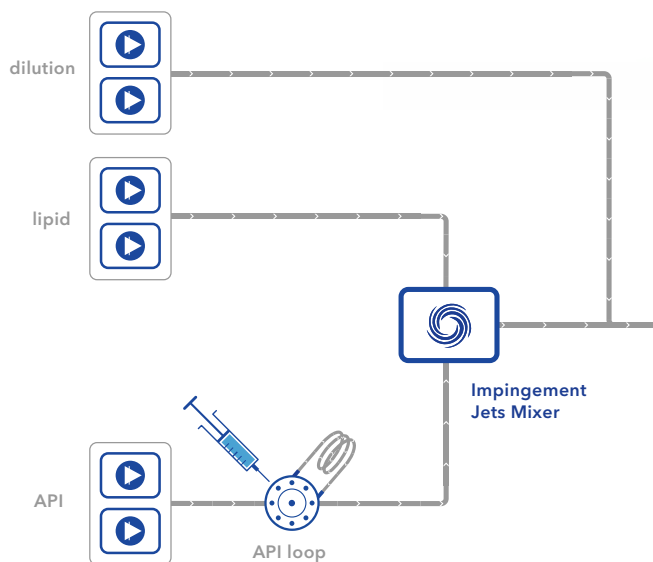
The IJM NanoScaler: a benchtop system for lipid nanoparticle formulation

KNAUER's new benchtop IJM NanoScaler system is designed for lab-scale lipid nanoparticle formulation - allowing scientists to screen for optimal process parameters to formulate API-containing lipid nanoparticles.

The IJM NanoScaler is made for research and development as well as pre-clinical and small-scale production of lipid nanoparticles. Equipped with our lipid nanoparticle producing Impingement Jets Mixing (IJM) technology the NanoScaler allows researchers to optimize encapsulation conditions for their API-based therapy before scaling up the process to run on a larger IJM NanoProducer unit.



 IJM NanoScaler



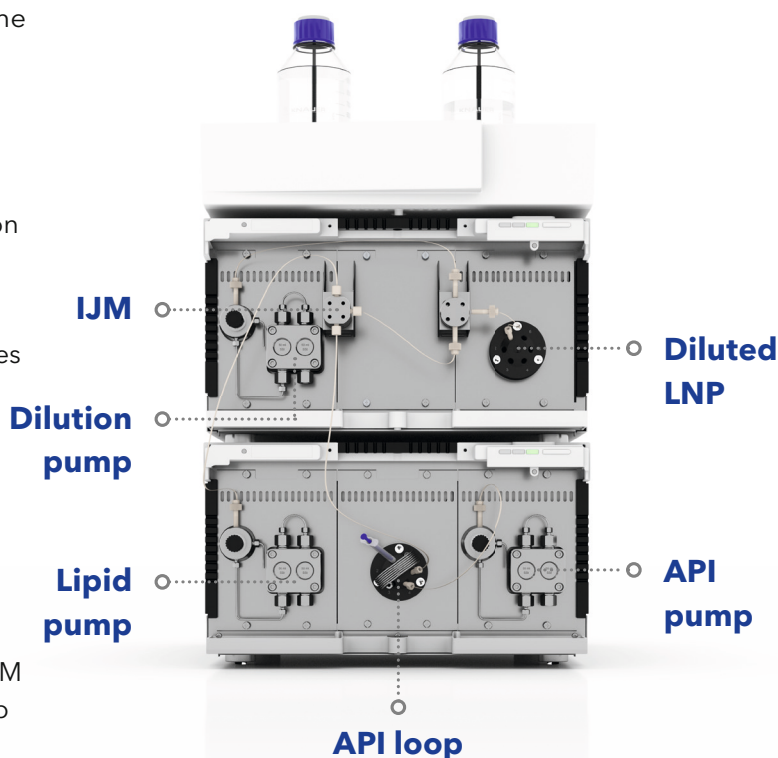
Flow scheme of the NanoScaler

The NanoScaler is made for low sample consumption so as not to waste valuable API. Its small footprint means it can comfortably sit on the benchtop of a research and development lab. The system comes with five different KNAUER Impingement Jets Mixers to help researchers determine which conditions result in the optimum level of API encapsulation. These active pharmaceutical ingredients include complex or delicate API like RNA, mRNA, siRNA, and DNA-based molecules, or products that need specific entry into target cells. In addition researchers can use their own custom mixing unit and benefit from the flexibility of the system set-up. The formulation process can be remotely controlled by software; ran from a conventional PC or laptop, or even a tablet.

LNP formulations for pre-clinical research and production

The NanoScaler can be used to produce lipid-encapsulated nucleotides at **a range from 1 ml to hundreds of milliliters** meaning the system is perfect for:

- **Proof of concept testing and process optimization** - pure nucleotides are an expensive resource. Carry out proof of concept testing and process optimization at the 1 000 µl scale to avoid wastage.
- **Pre-clinical trials** - Pre-clinical testing can be carried out using 100 ml of nucleotides such as, for *in vitro* studies to determine the tolerability, efficacy and potential toxicity of the mRNA therapy of interest.
- **Small scale production** - Once proof-of-concept and safety testing have been successfully conducted, production of LNP encapsulated API's can even be carried out at up to 70 ml/min with the IJM NanoScaler or using KNAUER's scaled-up IJM NanoProducer Units.



WHAT THE CUSTOMER SAYS

"Having already a robust LNP production process in place, we were looking out for alternative techniques to support our scale-up activities. The **Impingement Jets Mixing (IJM)** equipment provided by Knauer was the right fit for us! Even from early runs we were able to produce **LNPs of high quality** with a high encapsulation efficiency and low PDI values. We will further build on this and expand our knowledge into GMP production to further support our projects and partners by delivering high quality LNP production."



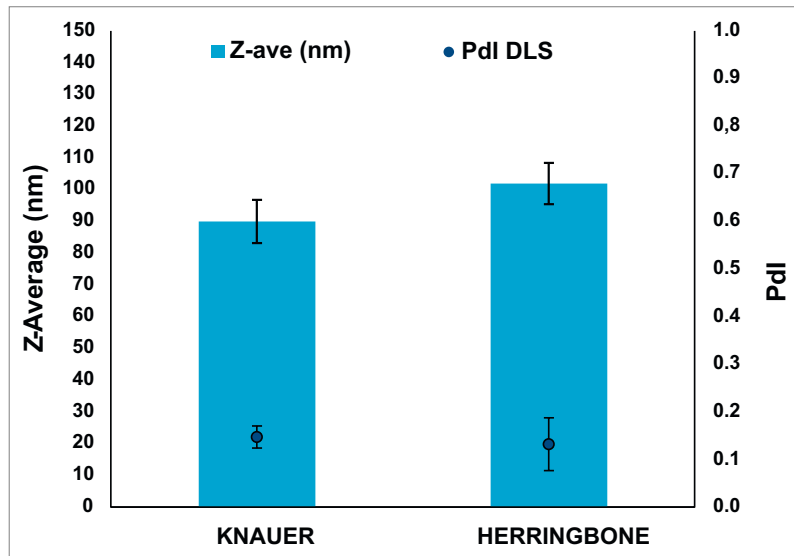
Iris Bombeke
Research Associate Drug
Product Development
eTheRNA, Ghent, Belgium

Formulate LNPs of adequate size and polydispersity index (Pdl)

Curapath develops and optimizes LNP formulations with the IJM NanoScaler during preclinical R&D. The NanoScaler is able to encapsulate mRNA and pDNA into LNPs with high reproducibility and efficiency. The performance of the NanoScaler for

formulating LNPs matched that of the traditional Herringbone. In addition, the NanoScaler offers flexibility and control that enable the user to tune the size of LNPs by increasing or decreasing the total flow rate.

IJM vs. Microfluidics (mRNA)



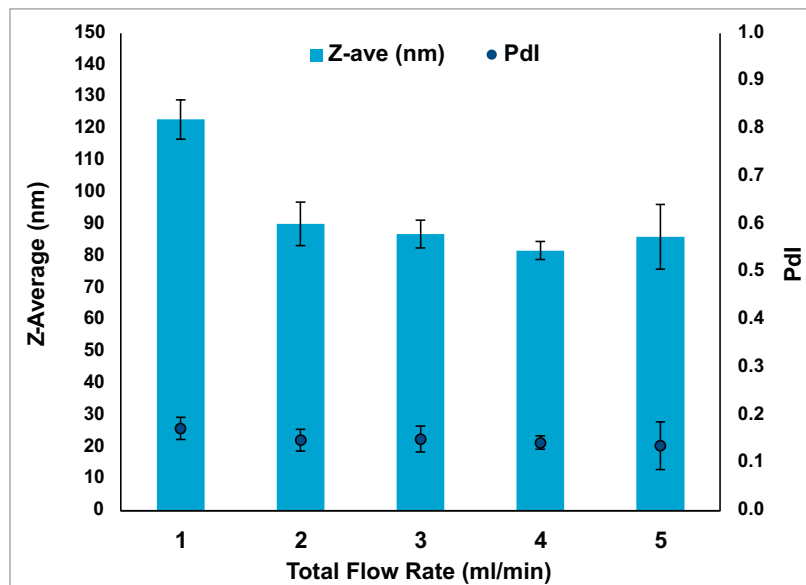
The Knauer IJM is able to form LNPs with similar characteristics as microfluidic chip with herringbone pattern. Additionally, LNPs formulated with IJM have higher reproducibility compared with Herringbone.

Size can be tuned by varying the total flow rate. By increasing the total flow rate, the size of the LNP decreases.

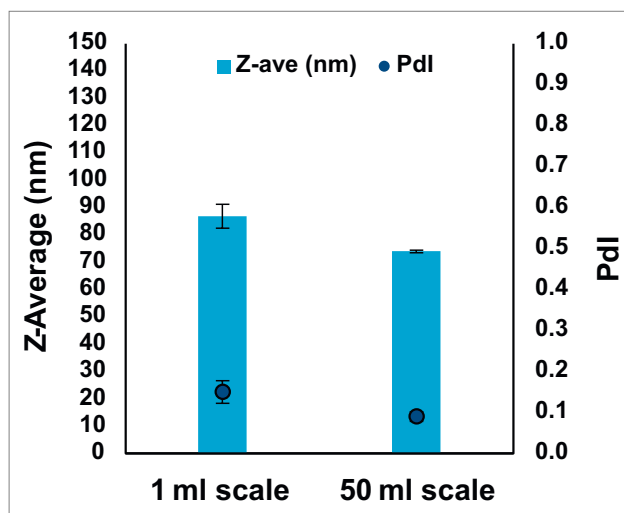
The ratio between organic and aqueous phase is 1:3 in all cases.

Furthermore, the encapsulation efficiency obtained is greater than 93 % in all the formulations.

Different flow rates



Scale-up from R&D to preclinical Studies



The IJM NanoScaler enables the formulation of batches not only for R&D purposes but also for preclinical studies with similarly adequate characteristics.

The use of the NanoScaler for production of LNPs is also facilitated by the availability of variously sized IJM mixers.

Which formulation parameters can be tested using the NanoScaler?

The IJM NanoScaler enables the scouting of several key process parameters during the lipid nanoparticle encapsulation stage.

Mixing unit - The five different Impingement Jets Mixer geometries, plus the opportunity to integrate a custom mixing unit, allow determination of the process conditions required to achieve maximum efficiency, optimal particle size and homogeneous size distribution.

Mixing flow rate and ratio - Determine how the specific physicochemical properties of the lipid nanoparticle are impacted by the mixing flow - find out which flow rates and ratio provide optimum lipid nanoparticle encapsulation.

Lipid formulation - Testing of new lipid formulations, for example lipids with embedded antibodies for targeted medicines, is possible using the NanoScaler. Determine the conditions required to ensure lipid nanoparticle encapsulation takes place before producing the therapy at scale for trials.

Dilution protocols - The liquid flow path of the NanoScaler can be configured to either include or bypass the dilution step following lipid nanoparticle formation - allowing scientists to produce formulations at the exact concentration required.

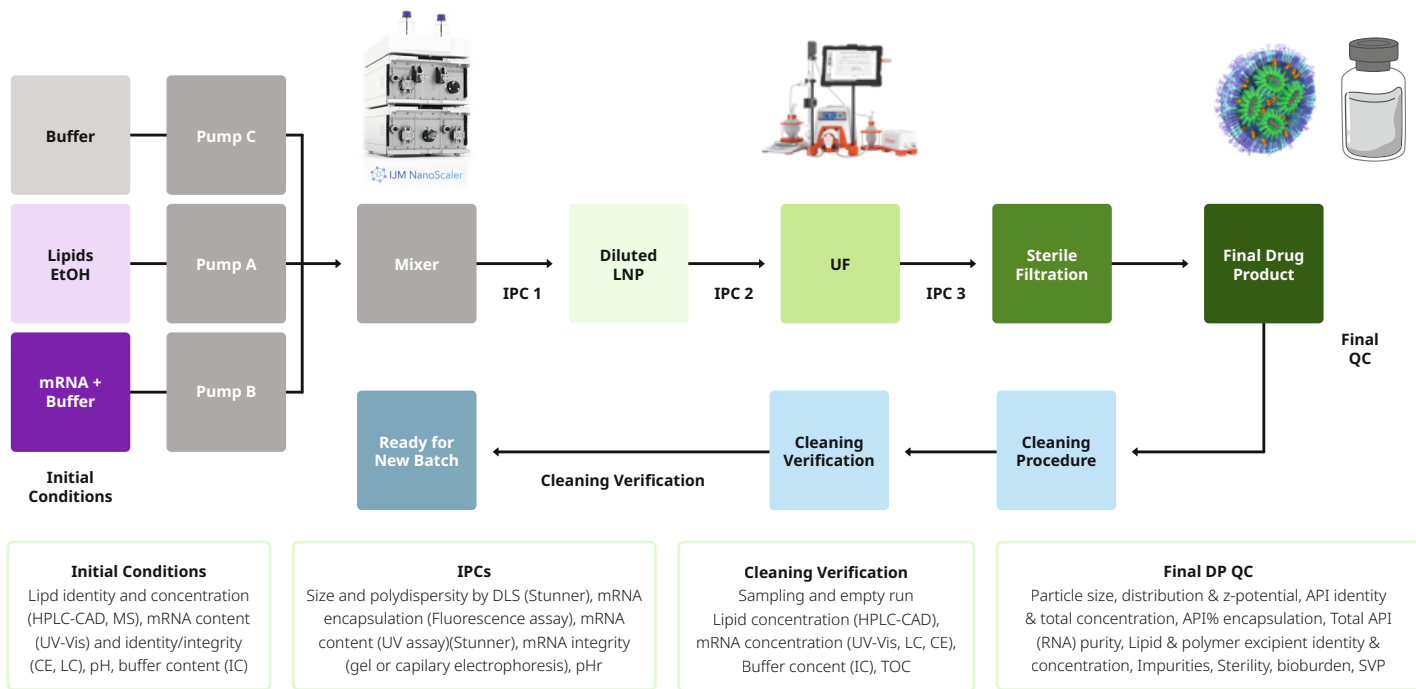


KNAUER Application Notes
For more information on the KNAUER AppNotes visit:
www.knauer.net/lnp-formulation





CURAPATH LNP Formulation and Analytical Services



End-to-End Analytical Services

From R&D to large-scale manufacturing, Curapath Services and Knauer IJM Technology can cover all your LNP formulation needs. Curapath develops, qualifies, and validates fit-for-purpose assays. We are equipped to address and resolve your most challenging analytical problems

R&D/Development

- Polymer And Lipid Custom Synthesis
- (Bio)Conjugation
- Nanoparticle Fast Formulation Screening
- BioAnalytical Assays

Pre-clinical Development

- Novel Functional Excipient and Drug Substance Process Development
- Lipid and Polymer Nanoparticle Formulation Development
- Method Development & QC

Clinical Supply

- IND/IMPD Enabling Package
- Clinical Trial Material Supply
- Aseptic F&F
- Method Development & QC
- Regulatory and CMC-IND filling support

Commercial Manufacturing

- Clinical Trial Material Supply
- Process Validation and Licensure
- Commercial Supply

Seamless transfer from alternative mixing technologies. Smooth process development and direct scale-up within vertically integrated infrastructure, leading to excellent encapsulation efficiency and cleanability.

We test and validate at every step of the process whether it's Lipid nanoparticle formulation or any of your drug delivery needs.

Lipid and Polymer Nanoparticle Characterization (Drug Product, Aseptic F&F)

- Particle size, distribution & z-potential
- Total API (RNA) purity
- API identity & total concentration
- Lipid and polymer excipient identity & concentration
- API % encapsulation

Polymer & Lipid Excipients (Functional Excipient, Bulk Solid Release)

- Lipid & polymer identity and purity
- Mw & DP by end group analysis
- Polymer Mw, polydispersity and degree of polymerization
- Impurities (elemental, process and degradation related)
- Residual solvents
- Enantiomeric excess
- Water content



Based in Berlin, KNAUER is a medium-sized, owner-managed company that has been serving the sciences since 1962. We develop and manufacture scientific instruments of superior quality for liquid chromatography. The range includes systems and



Worldwide partner in science since 1962

components for analytical HPLC/UHPLC, preparative HPLC, fast protein liquid chromatography (FPLC), multi-column chromatography/simulated moving bed (SMB), osmometry and Skids for the production of lipid nanoparticles (LNP).

Independent and family owned



It all started with a soldering iron, a jigsaw and an ingenious idea for a highly accurate electronic thermometer.

Chemist Dr.-Ing. Herbert Knauer founded the company together with his wife Roswitha in 1962. Both are still active as advisers to this day. The couple's daughter, Alexandra Knauer, is managing



director and owner of the company since the year 2000. As of April 2021, she is leading KNAUER together with Sales Director Carsten Losch.

Today, KNAUER is an established company with 180 employees that successfully develops, manufactures and markets chromatography instruments worldwide.

think **LNP.** think **KNAUER.**

IMPINGEMENT JETS MIXING UNITS BY KNAUER

IJM NanoScaler

IJM SingleCore NanoProducer



Number of impingement jets mixers	5, IJM 1-5 manual selection	1
Number of pumps	3	4
Number of flow meters	on request	4
Number of valves	2	1
Process connection inlet**	1/8" OD, 2.1 mm ID FEP tubing (UNF 1/4-28 thread, flat bottom)	1/2" Tri-clamp (4 inlets)
Process connection outlet**	1/16" OD, 0.5 mm ID PEEK tubing (UNF 10-32, thread coned)	1/2" Tri-clamp (1 outlet)
Volumetric flow rate	up to 50 ml/min****	up to 1 l/min
Volumetric flow rate	up to 3 l/h****	up to 60 l/h
Maximum operating pressure***	140 bar (2030 psi)	95 bar (1378 psi)
Maximum flow rate single pump	30 ml/min	–
Liquid temperature range	4–60 °C (39.2–140 °F)	4–60 °C (39.2–140 °F)
Wetted materials	stainless steel, PEEK, titanium, FFKM, PTFE (GFP 55), aluminum oxide, ruby, sapphire, ETFE, PETP, zirconium oxide, Hastelloy, FEP	stainless steel, PEEK, titanium, FFKM, PTFE (GFP 55), aluminum oxide, ruby, sapphire, EPDM, zirconium oxide, POM, hastelloy
Software	CDS optional available 21 CFR part 11 and Gamp 5 compliant	CDS, 21 CFR part 11 and GAMP 5 compliant
Interfaces	LAN, pin header connectors	pump: LAN, RS-232, pin header connectors; flow meter: RS-232 + FLOW-BUS™; valve drive: LAN
Power supply	docking station (including pumps and valves): 100-240 VAC single phase, 50/60 Hz; Network switch: 100-240 VAC single phase, 50/60 Hz, 12V DC Power supply	pump: 100-240 VAC single phase, 50/60 Hz; flow meter: 115/230 VAC single phase, 50/60 Hz 24V DC Power supply; valve drive: 115/230 VAC single phase, 50/60 Hz 24V DC Power supply; PC: 100-240 VAC single phase, 50/60 Hz; monitor: 100-240 VAC single phase, 50/60 Hz; network switch: 100-240 VAC single phase, 50/60 Hz, 12 V DC Power supply
Power consumption (per device)	docking station (including pumps and valves): maximum 130 W; network switch: 50 W	pump: maximum 320 W; flow meter: 3 W; valve drive: 65 W; PC: 1000 W; monitor: 380 W; network switch: 50 W
Ambient conditions	temperature range: 4–40 °C; 39.2–104 °F; below 90 % humidity (non-condensing)	temperature range: 4–40 °C; 39.2–104 °F; below 90 % humidity (non-condensing)
Dimensions (W x H x D)	361 x 501 x 603 mm	900 x 915 x 700 mm
Net weight (approx.)	35 kg	150 kg
API-predilution and quenching	only quenching	Yes
Customized technical documentation, order-and article-related	on request	Yes
Factory acceptance test	Yes	Yes
Site acceptance test	on request	Yes
Purchase order lead time	about 3 months	about 4 months

*design of the skid on customer requirements **different connections on request ***depends on specific pump configuration and application

**** depends on IJM, in production mode up to 70 ml/min

IJM DuoCore NanoProducer

Customized NanoProducers



Number of impingement jets mixers	2	up to 8
Number of pumps	8	up to 16
Number of flow meters	8	up to 16
Number of valves	2	–
Process connection inlet**	1/2" Tri-clamp (6 inlets)	Sanitary Clamp Connector (2 inlets)
Process connection outlet**	1/2" Tri-clamp (2 outlets)	Sanitary Clamp Connector (1 outlet)
Volumetric flow rate	up to 2 l/min	depending on configuration
Volumetric flow rate	up to 120 l/h	depending on configuration
Maximum operating pressure***	95 bar (1378 psi)	50-70 bar (725-1015 psi)
Liquid temperature range	4–60 °C (39.2-140 °F)	4–60 °C (39.2-140 °F)
Wetted materials	stainless steel, PEEK, titanium, FFKM, PTFE (GFP 55), aluminum oxide, ruby, sapphire, EPDM, zirconium oxide, POM, hastelloy	stainless steel, PEEK, titanium, FFKM, PTFE (GFP 55), aluminum oxide, ruby, sapphire, EPDM, zirconium oxide, POM, hastelloy
Software	CDS, 21 CFR part 11 and GAMP 5 compliant	CDS, 21 CFR part 11 and GAMP 5 compliant
Interfaces	pump: LAN, RS-232, pin header connectors; flow meter: RS-232 + FLOW-BUS™; valve drive: LAN	pump: LAN, RS-232, pin header connectors; flow meter: RS-232 + FLOW-BUS™
Power supply	pump: 100-240 VAC single phase, 50/60 Hz; flow meter: 115/230 VAC single phase, 50/60 Hz 24V DC Power supply; valve drive: 115/230 VAC single phase, 50/60 Hz 24V DC Power supply; PC: 100-240 VAC single phase, 50/60 Hz; monitor: 100-240 VAC single phase, 50/60 Hz; network switch: 100-240 VAC single phase, 50/60 Hz, 12 V DC Power supply	pump: 100-240 VAC single phase, 50/60 Hz; flow meter: 115/230 VAC single phase; depending on configuration
Power consumption (per device)	pump: maximum 320 W; flow meter: 3 W; PC: 1000 W; monitor: 380 W; network switch: 50 W	pump: maximum 320 W; flow meter: 3 W; PC: 1000 W; monitor: 380 W; network switch: 50 W
Ambient conditions	temperature range: 4-40 °C; 39.2-104 °F; below 90 % humidity (non-condensing)	temperature range: 4-40 °C; 39.2-104 °F; below 90 % humidity (non-condensing)
Dimensions (W x H x D)	1000 x 1290 x 700 mm	depending on configuration
Net weight (approx.)	250 kg	depending on configuration
API-predilution and quenching	Yes	depending on configuration
Customized technical documentation, order-and article-related	Yes	Yes
Factory acceptance test	Yes	Yes
Site acceptance test	Yes	Yes
Purchase order lead time	about 3 months	minimum 6 months, depending on customer requirements

Depending on the process the end user must consider the legal situation of patents and intellectual property. KNAUER is designing the customized hardware solution on customer's request, process and chemistry are not included. Technical information are subject to change.

KNAUER is the proud winner of the
German Innovation Award 2022 in the
category of medium-sized businesses.

**(U)HPLC • FPLC • SMB • Osmometry and
units for the production of lipid nanoparticles (LNP)**



Innovation

Own hardware and
software development



Customized solutions

Pumps, detectors, valves and
systems adapted to your needs



Made in Germany

Independent and family-
owned since 1962

think **LC.** think **KNAUER.**

KNAUER Wissenschaftliche Geräte GmbH

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info@knauer.net • www.knauer.net

