Case Study

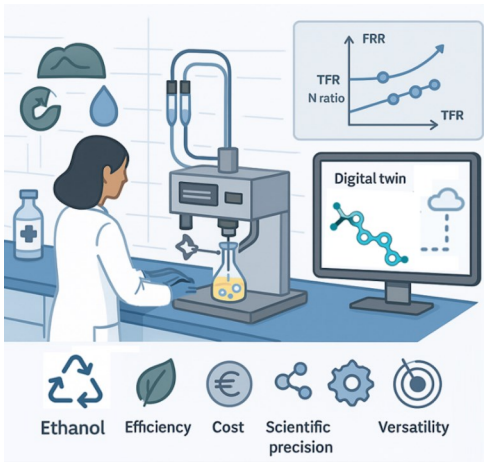
ETERNAL

GREEN SCALABLE NANOFORMULATION

CONTINUOUS LIPID-BASED ENCAPSULATION

Lipid-based drug delivery systems can encapsulate a broad spectrum of therapeutically important compounds advantageously, but the batch-wise methods by which they are conventionally made are insufficiently scalable, sustainable, and reproducible. This case study at lab–pilot scale, is aimed towards minimising solvent use and environmental footprint through ethanol recycling and process simulation via digital tools.

Traditional liposome manufacturing using sonication is difficult to scale and involves significant solvent consumption. This challenge, and the opportunity for improvement, have been addressed by developing a continuous microfluidic process for producing liposomes and lipid nanoparticles that enables solvent reduction, reproducibility, and sustainable development of pharmaceutical formulations.



The transition from batch sonication to a continuous microfluidic process is a major stride towards sustainability and scalability. Coupling solvent recycling and digitalisation (predictive particle characteristics), the foundations supporting greener, more efficient pharmaceutical manufacturing are in place.

Context

Lipid-based drug delivery systems can encapsulate a broad spectrum of compounds advantageously, but conventional batch production methods are insufficiently scalable, sustainable, and reproducible. This case study is aimed towards minimising solvent use and environmental footprint through ethanol recycling and process simulation via digital tools.

Challenge

Traditional liposome manufacturing via sonication is difficult to scale and involves significant solvent consumption. The challenge is to develop a continuous microfluidic process for producing liposomes and lipid nanoparticles that enables solvent reduction, reproducibility, and sustainable development of pharmaceutical formulations.

Innovation

Laboratorio Reig Jofre has introduced a continuous microfluidic platform to produce both diclofenac-loaded liposomes and DNA-loaded lipid nanoparticles (DNA-LNP). Design of experiments (DoE), together with digital twin simulation of particle properties, enable predictive formulation, reducing the need for physical experimentation.

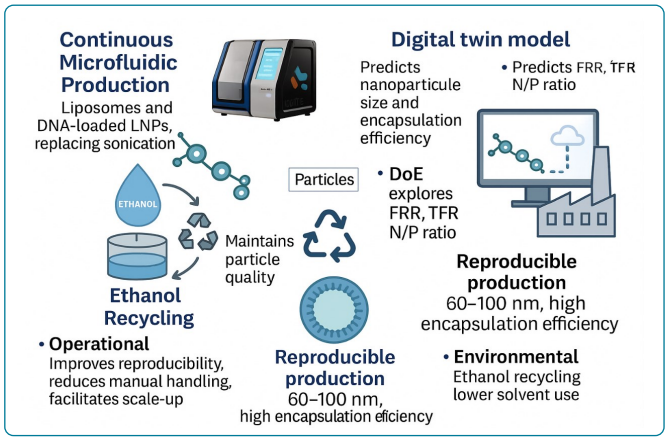
Next Steps

Final validation of the digital twin for DNA-LNP production is ongoing. In a further step Reig Jofre is exploring the use of a microjet reactor as an alternative to microfluidic chips for potential industrial scale-up. The aspiration is to offer a reproducible and predictive platform applicable to other nanoparticle-based formulations supporting widespread environmentally responsible pharmaceutical innovation.

Laboratorio Reig Jofre has introduced a continuous microfluidic platform to produce both diclofenac-loaded liposomes and DNA-loaded lipid nanoparticles (DNA-LNP) as a step towards a predictive platform widely applicable to environmentally responsible production of many other nanoparticle-based formulations.

Features and Advantages

Physical and virtual DoE using a predictive digital twin process have been used to explore how three critical process parameters - flow rate ratio (FRR), total flow rate (TFR), and N/P ratio - influence the resultant particle characteristics with reduced need for physical experiments. Reproducible nanoscale particles (60–100 nm) with narrow size distribution and high encapsulation efficiencies have been achieved. Furthermore, the new process facilitates ethanol recycling, with product particle quality maintained through successive solvent reuse cycles. Advantages include:



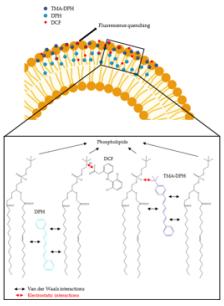
- **Operational:** Better reproducibility, reduced manual handling, easier scale-up.
- **Environmental:** Reduced solvent use and waste generation.
- **Commercial:** Shortened development time and lower material costs.
- **Technical:** Improved formulation performance.
- **Strategic:** Supporting innovation across both small molecule and gene therapies.

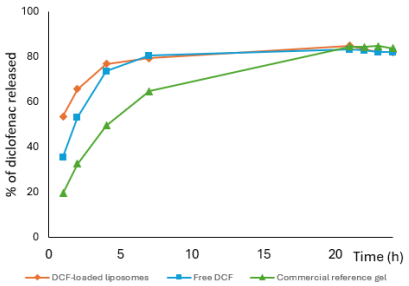
Strategic support underpinning innovation across therapeutic areas

Characterisation

Particle Size (nm)	83.43
PDI	0.145
Z-Potential (mV)	-17.93
%EE	88.8

diclofenac predominantly localizes in the external region of the lipid bilayer of SUVs

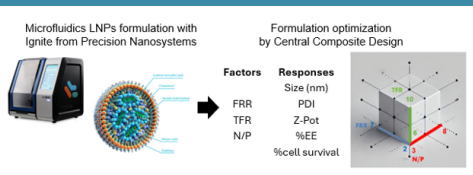


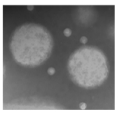


Anti-inflammatory efficacy assay:

- 70% reduction in TNF and CXCL1. Diclofenac-loaded liposomes reverse LPS inflammation.
- No reduction in IL6 or IL8 observed.

Optimisation





Responses	Significant parameters	R^2	Pred R^2
%EE	FRR×N/P, N/P, FRR^2	60%	9%
Particle size	$(N/P)^2$, FRR^2, FRR, TFR^2	75%	52%
PDI	N/P	44%	0%
Z-Potential	None	19%	0%
Cell survival	N/P^2, FRR^2	65%	26%

Optimization

FRR	TFR	N/P	Composite desirability
4.21	4.73	4.80	0.245

	%cell survivol	Z- Pot	PDI	Size (nm)	%EE
Pred	63.2	-0.47	0.067	81.8	92.3
Exp	16.2	0.45	0.065	72.6	98.5
Bias	74.4%	4.3%	3.0%	11.2%	6.7%

Results and Benefits

A continuous microfluidic process for diclofenac-loaded liposomes and DNA-loaded LNPs has been developed, replacing traditional sonication. Liposomes achieved high encapsulation efficiency (~89%) and narrow size distribution (~86 nm). For LNPs, particle sizes were between 72–82 nm and encapsulation efficiencies up to 99.5%. Digital twin development is ongoing and shows promising predictive capability. In further collaboration with ETERNAL partner MyBiotech, Reig Jofre is exploring the use of a microjet reactor as an alternative to microfluidic chips for potential industrial scale-up.

Reducing solvent consumption and experimental waste contributes to greener pharmaceutical manufacturing. Better reproducibility and scalability of nanomedicine production can accelerate the availability of effective topical and gene therapies, ultimately providing manufacturers with a sustainable, efficient development platform, and patients with more targeted, safer, and better performing treatments.

ETERNAL is contributing to the sustainable development of pharmaceutical manufacture, use and disposal, by using and promoting full life cycle approaches covering design, manufacture, use, and disposal through

- application-industry oriented R&D and scale-up;
- clear pathways to compliance;
- new scientific knowledge on the environmental fate and eco-toxicological effects of pharmaceuticals; and
- behavioural change for safe use and disposal.

Co-funded by the European Union

This project has received funding from the European Union's Horizon Europe Framework Programme (HORIZON) under grant agreement No 101057668. The work of UK-based Associated Partners has been funded by UK Research and Innovation (UKRI) under the UK government's Horizon Europe funding guarantee.

Find out more at: www.eternalproject.eu