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micromachines MDPI

Lipid-based Nanoparticle Production in Micromixers

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ÉTS

Institut de recherche
Centre universitaire de santé McGill

Research Institute
McGill University Health Centre

Abstract:

Lipid-based nanoparticles have demonstrated to be a versatile vehicle for drugs, genetic material, and labels. These particles are often made of biocompatible and biodegradable materials, enabling a safe interaction with biological systems. The importance of this type of delivery vehicle has been shown recently, as the two leading vaccines are based on lipid-nanoparticles encapsulating mRNA.

Passive micromixers produce lipid nanoparticles in a reproducible and controllable way. However, micromixers suffered at the beginning of low production rate, and complicated designs which were difficult to produce and prone to clogging. In recent years, the exploration of different mixing strategies based on the use of curvilinear paths to induce centripetal forces and vortex formation at high speeds as well as the increase of the microchannel cross-sectional area while keeping laminar flow regimes has led to designs capable of producing lipid-based nanoparticles on an industrial-scale.

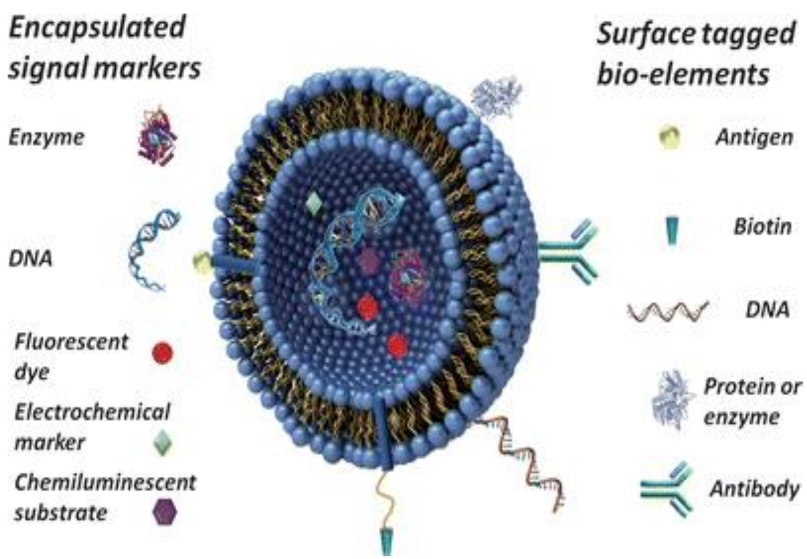
However, there are still challenges in the field which include the removal or substitution of the organic solvents that still need to be addressed.

In this presentation, we introduce a general overview of lipid nanoparticle or liposome production in micromixers, the principles of mixing using curvilinear paths, the key variables controlling lipid-based nanoparticle physicochemical characteristics and approaches that help to substitute toxic solvent residues.

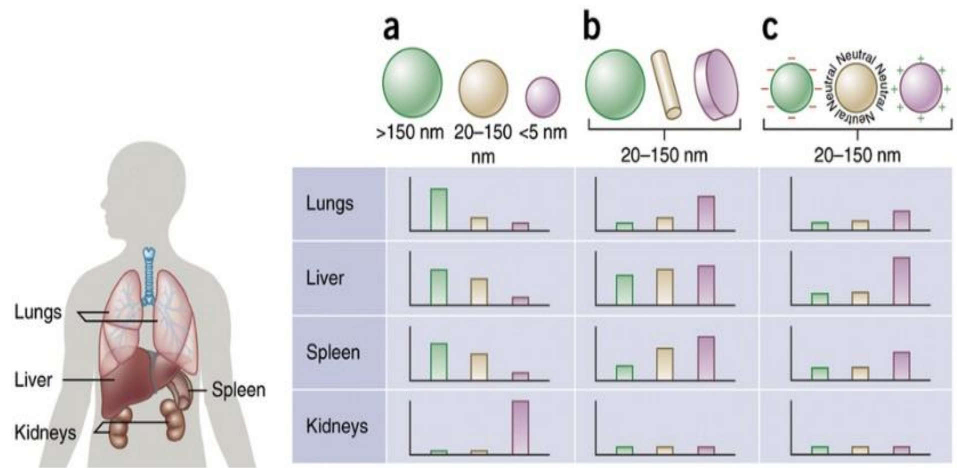
Keywords: Nanoparticle; Liposome; Microfluidic Devices; Micromixers

What are Lipid Nanoparticles?

Nanoparticles made of lipids such as liposomes.



Liu, Q.; Boyd, B. J. *Analyst* **2013,** 138, (2), 391-409)



Blanco, E.; Shen, H.; Ferrari, M. *Nat. Biotechnol.* **2015,** 33, (9), 941-51)

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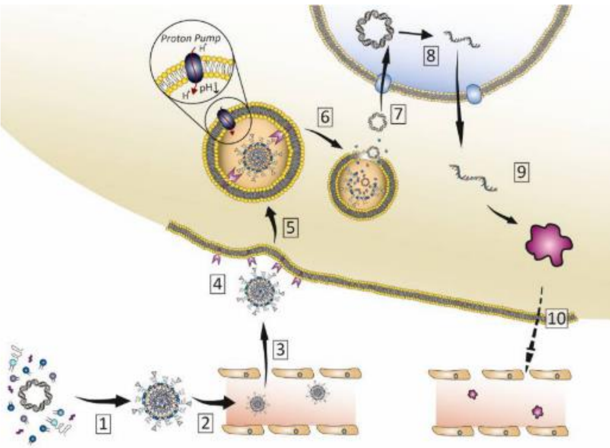
Liposome Applications

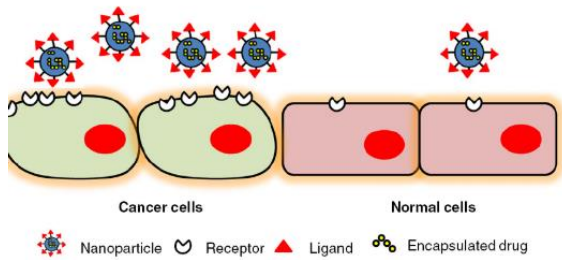
Gene Delivery



Buck, J.et al. D. *ACS Nano* **2019,** 13, (4), 3754-3782.

siRNA (Patisiran)
mRNA(BNT162b2, mRNA-1273)

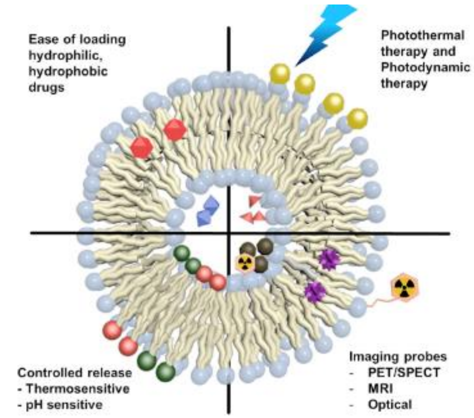
Drug Delivery



Çağdaş, M et al., *Liposomes as Potential Drug Carrier Systems for Drug Delivery*. **2014**.

Doxorubicin,
Amphotericin
among others

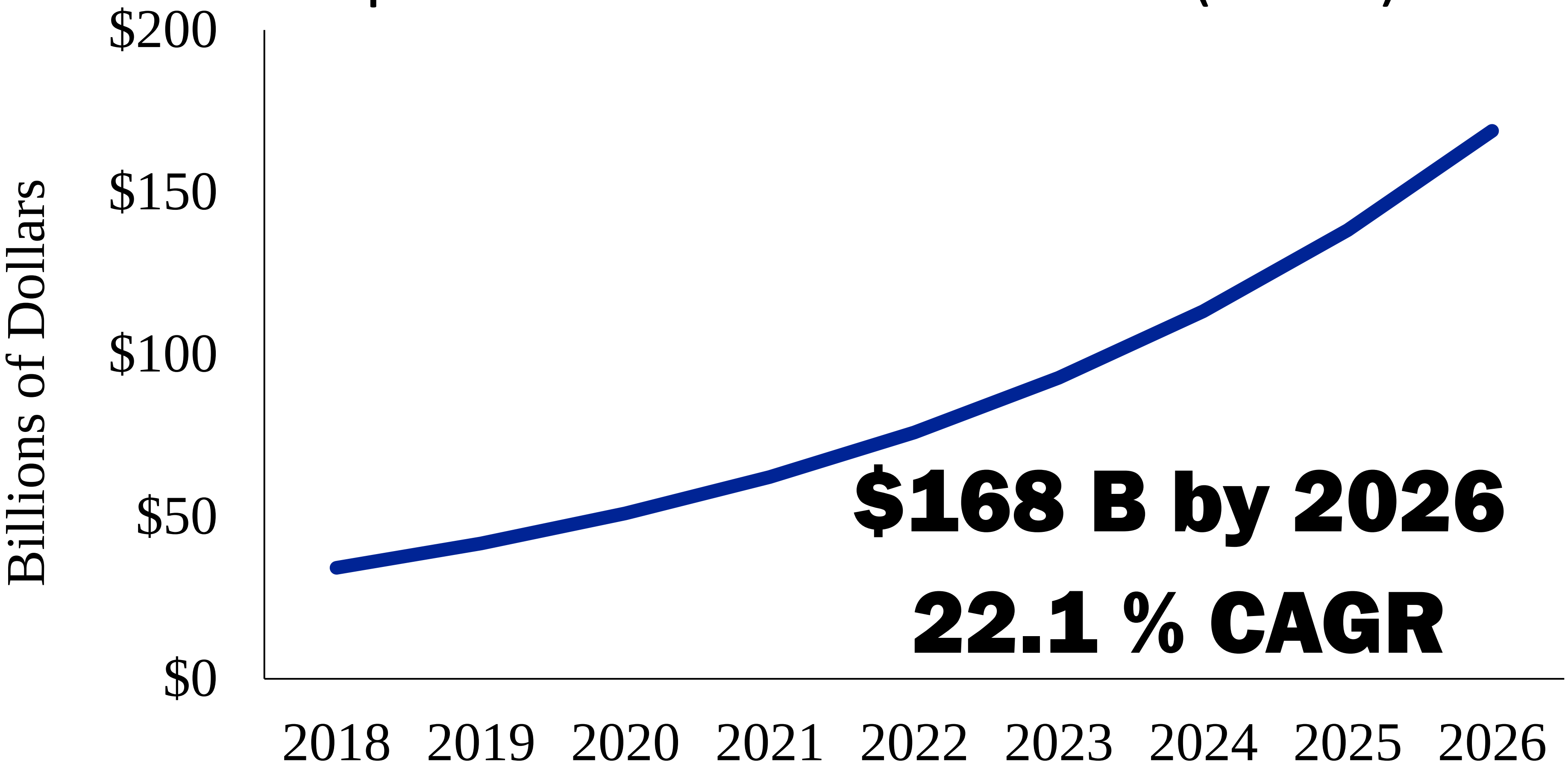
Imaging and theragnostics



Lee, W.; Im, H.-J. *Nuclear Medicine and Molecular Imaging* **2019,** 53, (4), 242-246.

Radioisotopes,
gold NP,
fluorescence dye

Nanomedicines Market
Compound Annual Growth Rate (CAGR)



(Business-Wire, 2019)

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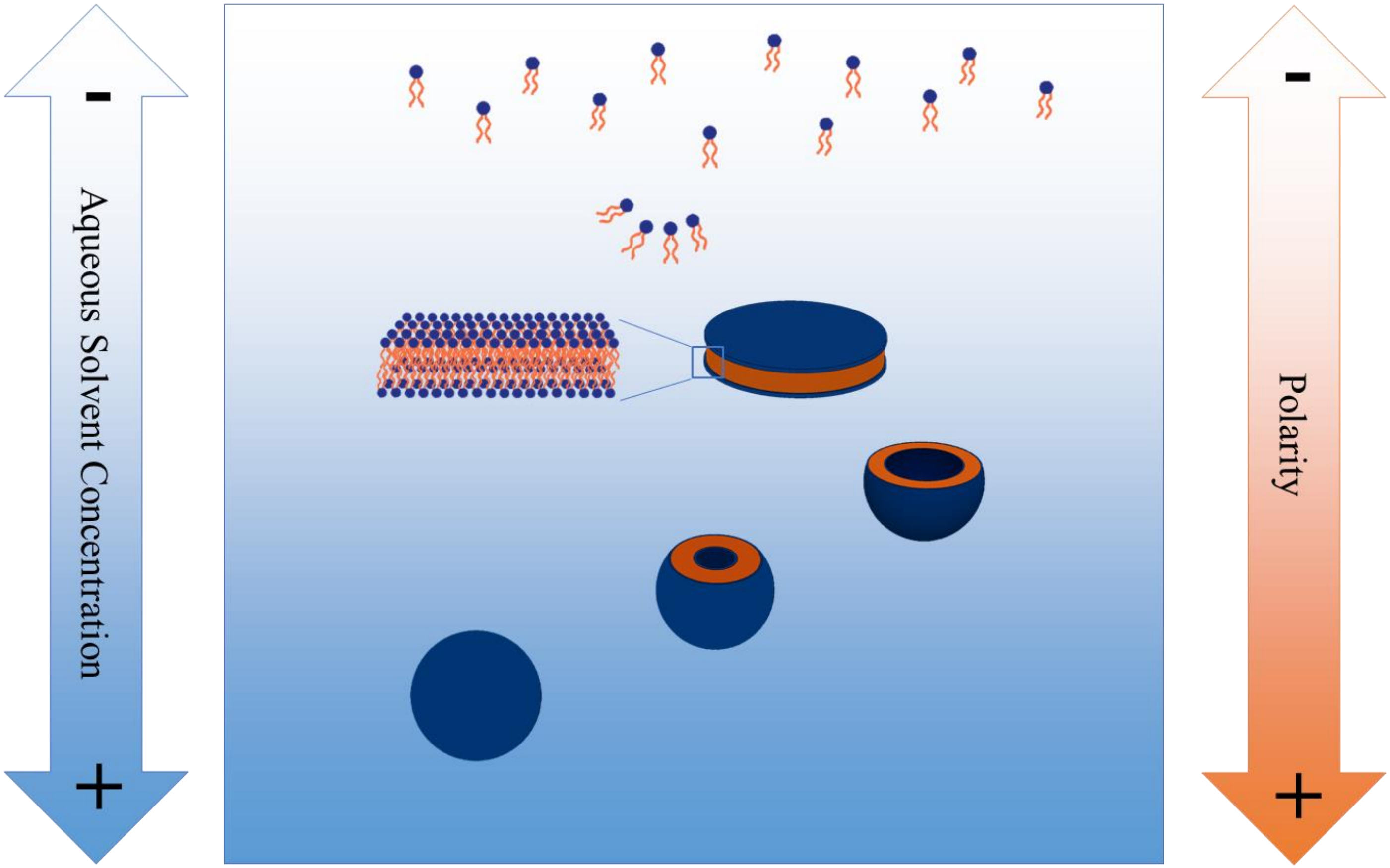
Nanoprecipitation

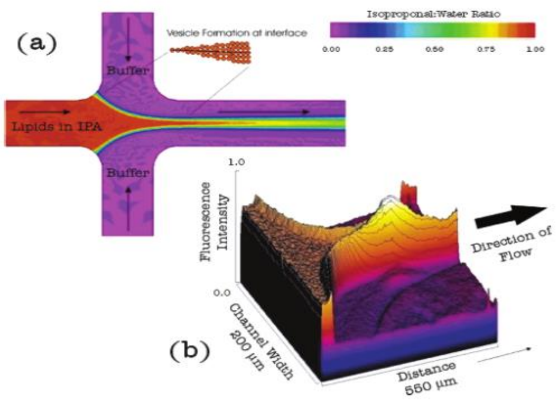
t aggregation

t self-assembly



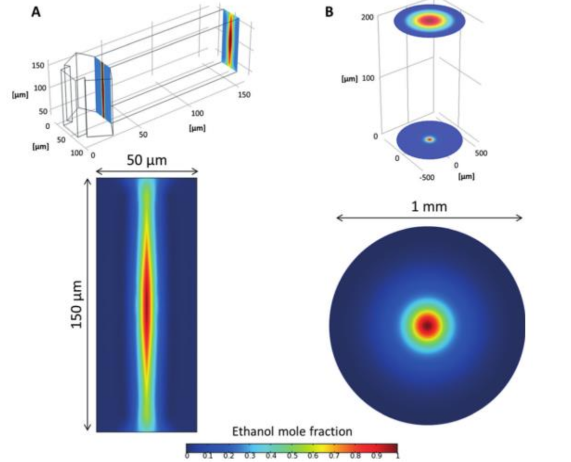
López, R. R.; et al. *Colloids Surf. B.* Biointerfaces, 2020.

How can we improve mixing? Micromixers



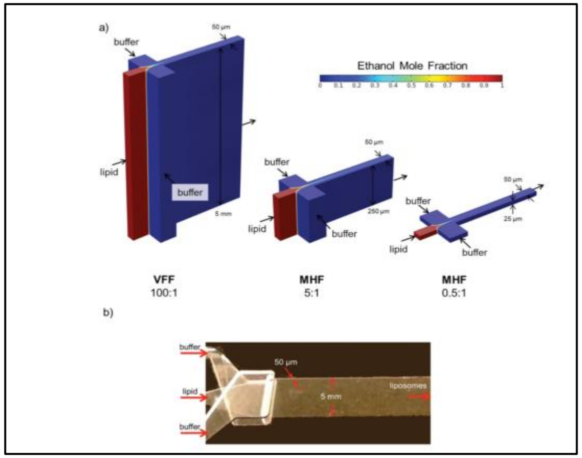
Jahn, A.; Vreeland, W. N.; Gaitan, M.; Locascio, L. E. *J. Am. Chem. Soc.* **2004,** 126, (9), 2674-2675.

MHF



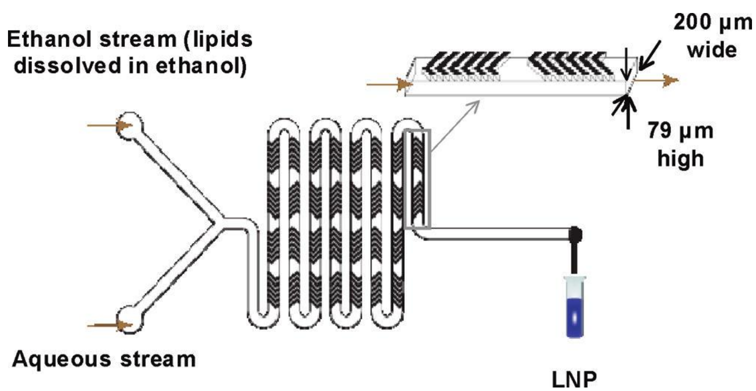
Hood, R. R.; DeVoe, D. L.; Atencia, J.; Vreeland, W. N.; Omiatek, D. M. *Lab on a Chip* **2014,** 14, (14), 2403-2409.

3D-MHF



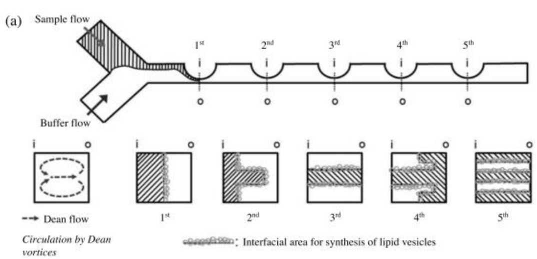
Hood, R. R.; DeVoe, D. L. *Small* **2015,** 11, (43), 5790-9.

VFF



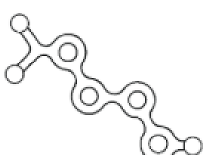
Zhigaltsev, I. V.; Belliveau, N.; Hafez, I.; Leung, A. K. K.; Huft, J.; Hansen, C.; Cullis, P. R. *Langmuir* **2012,** 28, (7), 3633-3640.

SHM



Lee, J.; Lee, M. G.; Jung, C.; Park, Y.-H.; Song, C.; Choi, M. C.; Park, H. G.; Park, J.-K. *BioChip Journal* **2013,** 7, (3), 210-217.

DEAN FLOW BASED MICROMIXER



Precision Nanosystems
Webb et al. 2020
TrM

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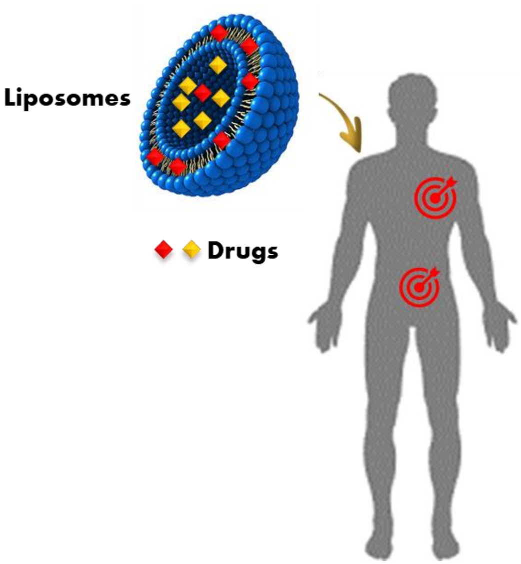
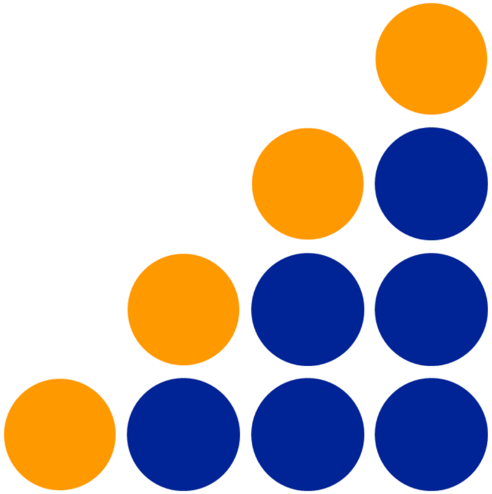
Contributions

- ***New microfluidic*** devices ***tailored*** to produce controlled-size liposomes.
- Production of liposomes in a ***size range of commercially*** available liposomal formulations
- ***Model to predict*** liposome size using microfluidic devices.
- ***Molecular factors influence*** over liposome physiochemical characteristics.
- ***Role of the organic solvent*** in liposome properties.
- ***Substitution*** of conventional organic solvents ***for Transcutol*** for liposome production.



Control of liposome properties

Reproducibility and reduction of steps (organic solvent removal)



Scalability

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General Methodology

Ideation

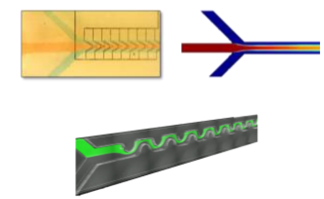
Mixing principles

Micromixer Evaluation

Numerical modeling (device evaluation)

Device fabrication and mixing imaging

Mixing Characterization

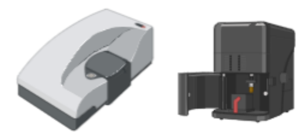


Liposome Production and Characterization

Liposomes characterization

Liposome production under several conditions

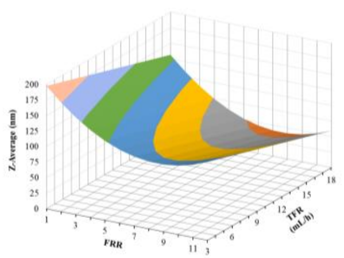
Liposome Characterization



Liposomes properties modulation

Identification and modeling of the factors controlling liposomes properties

DoE



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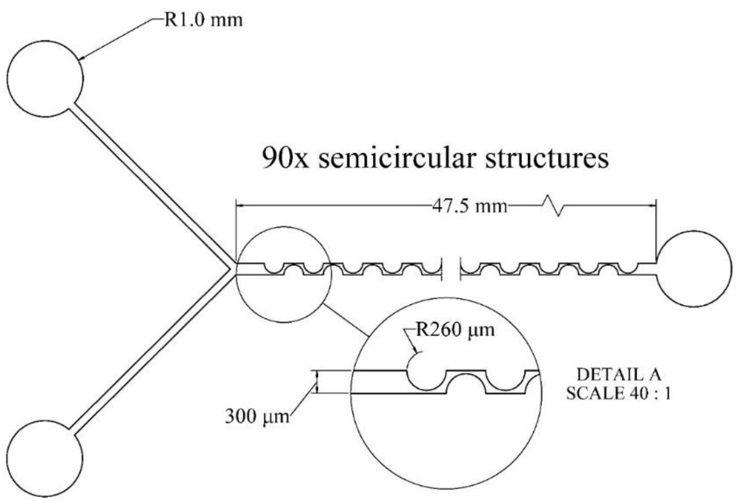
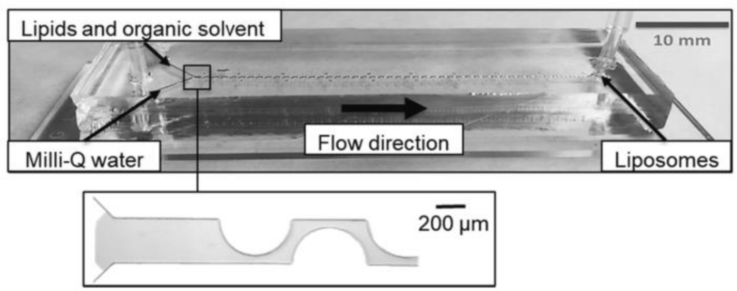
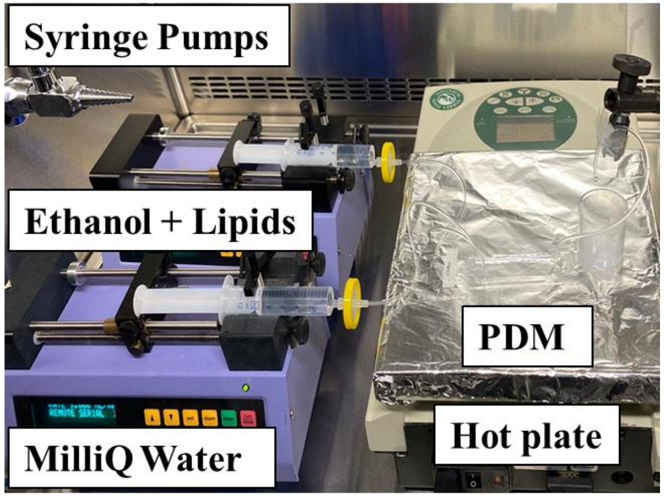
PDM-Micromixer (Periodic Disturbance Mixer)

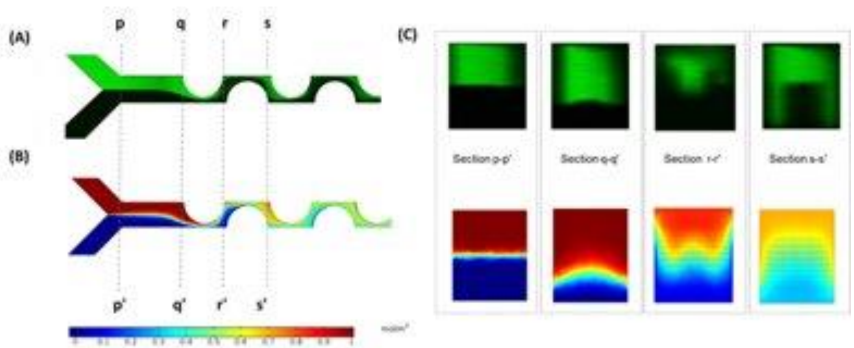




López, R. R.; et al. The Effect of Different Organic Solvents in Liposome Properties Produced in a Periodic Disturbance Mixer: Transcutol®, a potential organic solvent replacement. In *Colloids Surf. B. Biointerfaces*, 2020.

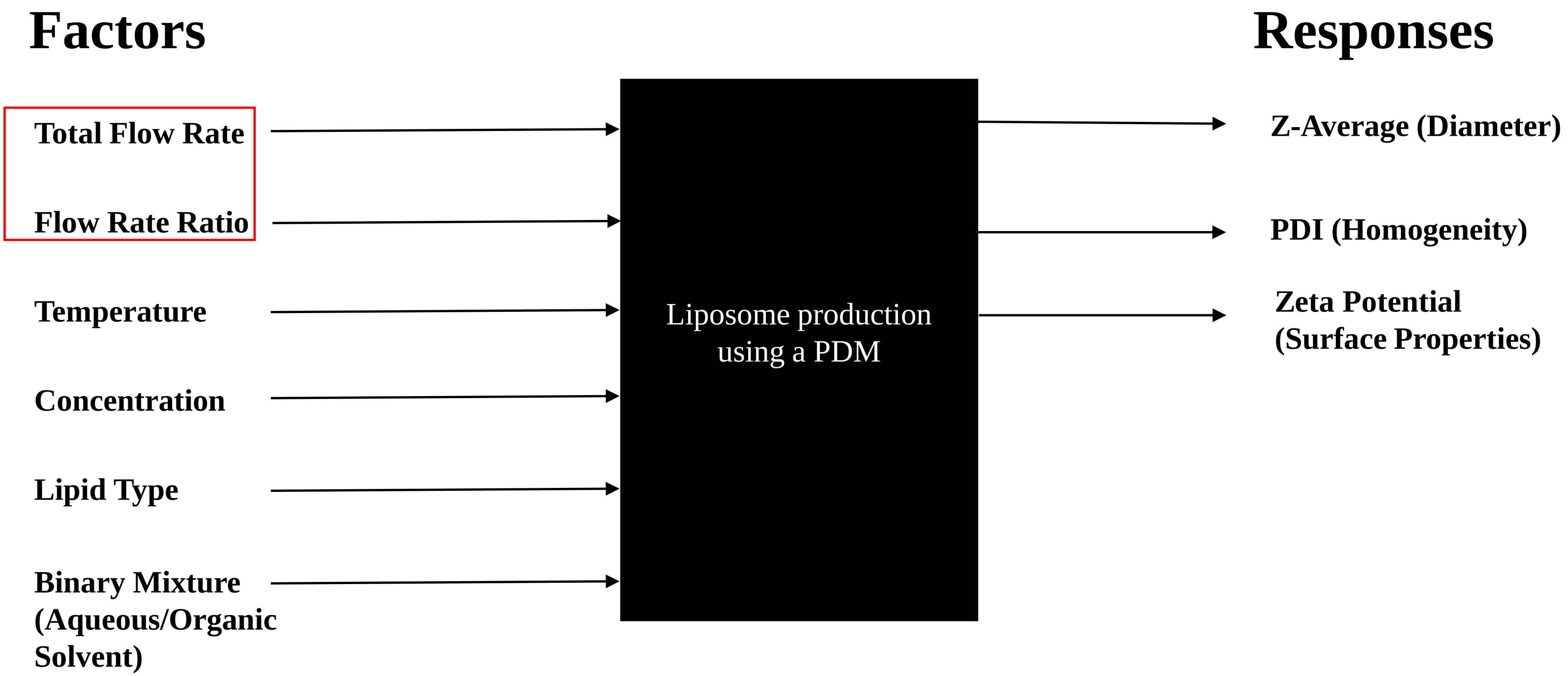


López, R. R.; et al. Lipid Fatty Acid Chain Influence over Liposome Physicochemical Characteristics Produced in a Periodically Disturbed Micromixer. In *IEEE Nano 2020*, IEEE: Montreal, 2020.



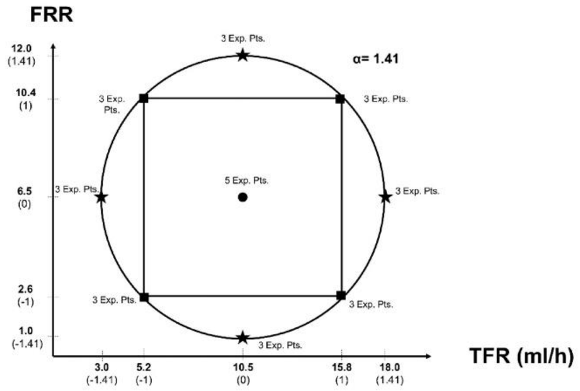
López, R. R.; et al.., Parametric Study of the Factors Influencing Liposome Physicochemical Characteristics in a Periodic Disturbance Mixer. In *Langmuir*, XX ed.; 2020; Vol. XX, p XX.

Modeling Liposome Properties



López Salazar, R. R. Controlled liposome production using micromixers based on Dean flow dynamics. École de technologie supérieure, 2020.

DoE and Surface Response Methodology



López, R. R.; et al. *Micromachines (Basel)* **2020,** 11, (3), 235.

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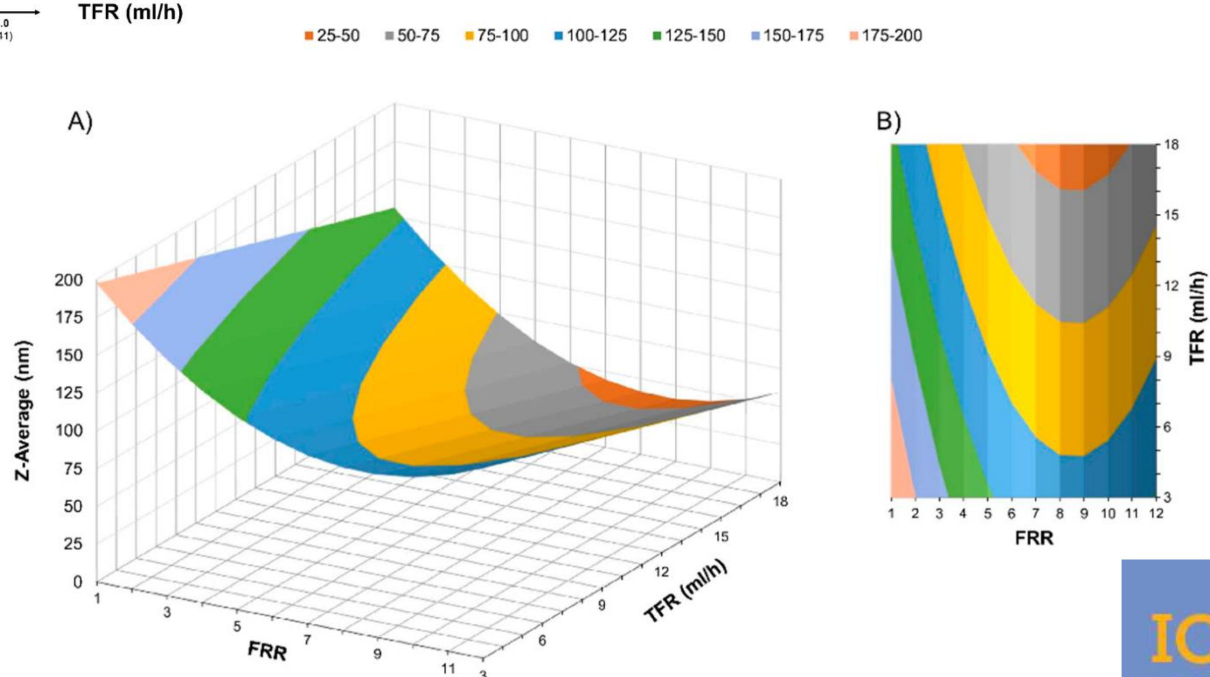
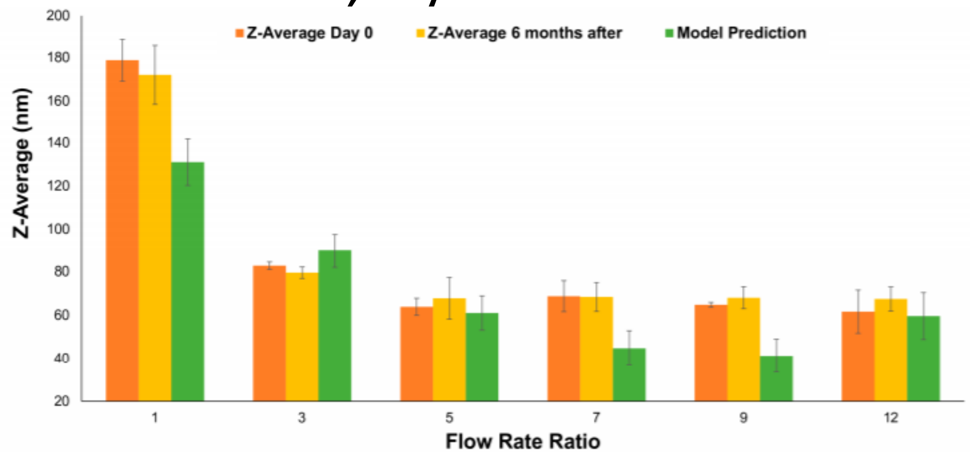
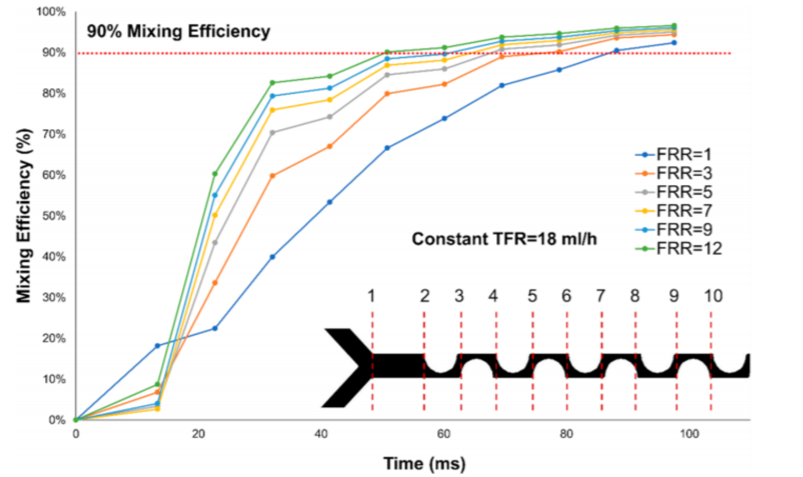


R^2	R^2 Adjusted	R^2-Predicted
78.89%	76.35%	70.20%

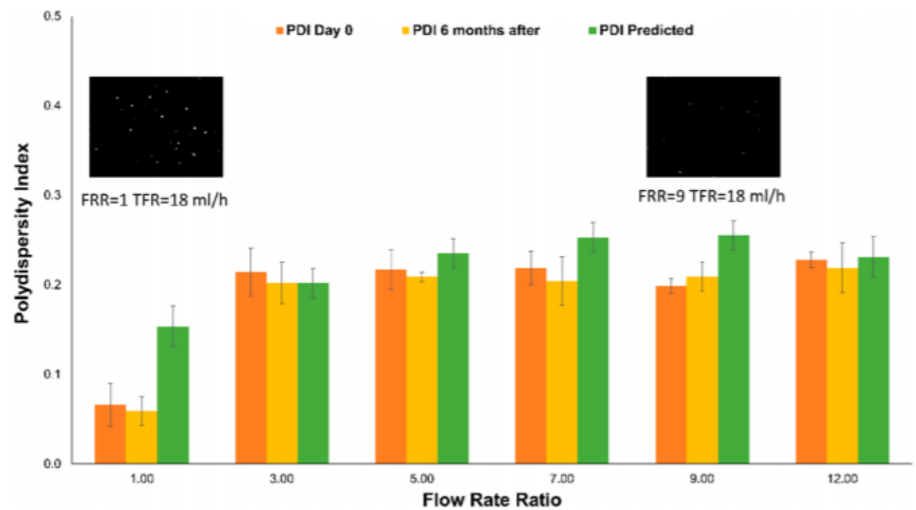
Results (Effect of FRR on size, PDI & Efficiency) Modeled, synthesized and stability (TFR= 18ml/h)



Z-average (nm) vs. FRR at a constant TFR = 18 mL/h. In orange, the Z-average immediately after production, in yellow six months after, and in green the model prediction. $n = 3$. Error bars indicate +/– 1 standard deviation (SD) for samples and SE fit for the model prediction.



Mixing efficiency at different FRRs. Each data point corresponds to a cross-section for a total of 10 data points from 1–10.



Liposomes PDI measurements immediately after production (orange), six months after (yellow), and the model prediction (green) at a constant TFR = 18 mL/h. $n = 3$, error bars indicate +/– 1 SD for samples and SE fit for the model prediction. Images are taken from videos using NTA

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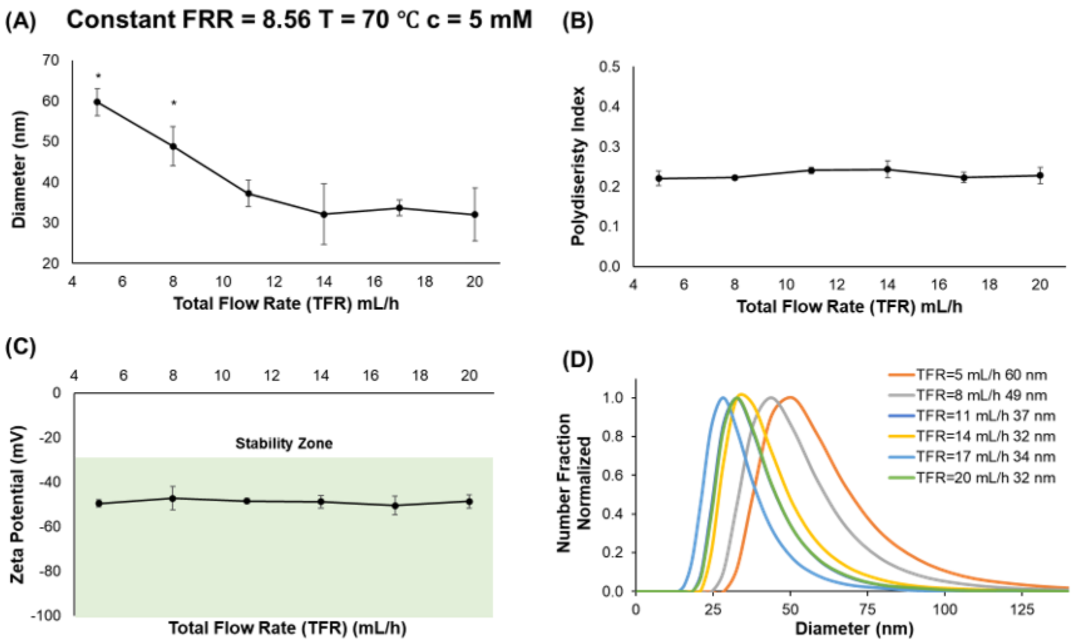
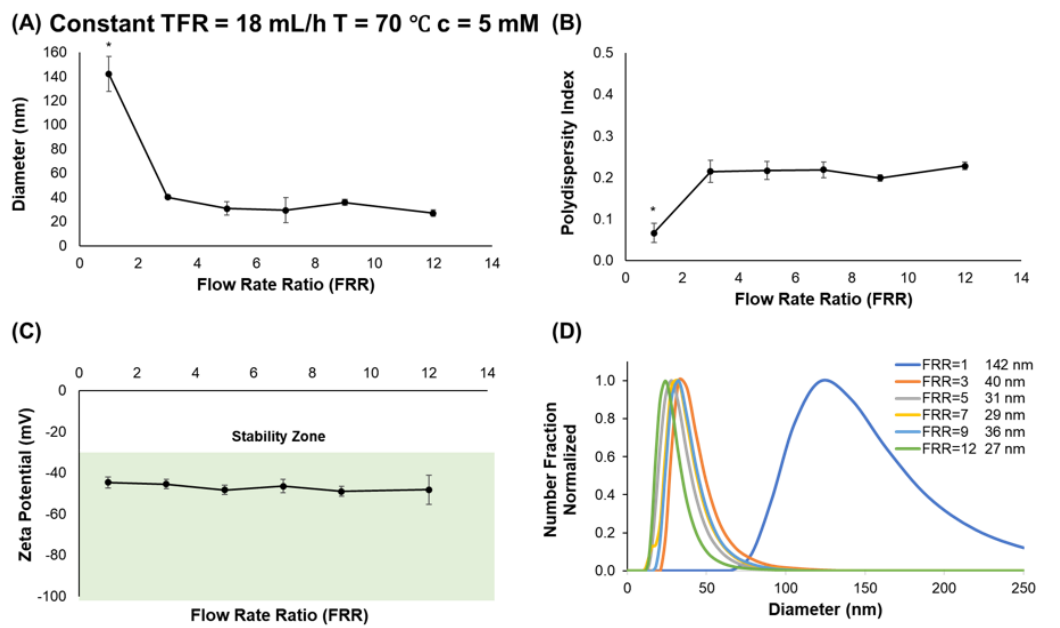
Results (Effect of FRR/TFR on size, PDI & Zeta potential)



TFR from 3 to 10 ml/h

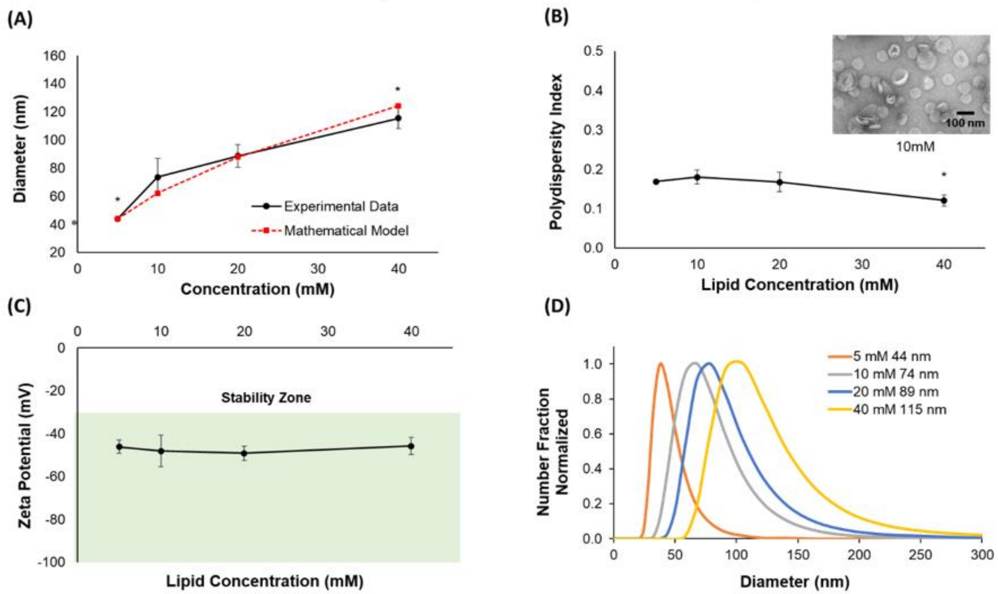
López, R. R.; et al.., Parametric Study of the Factors Influencing Liposome Physicochemical Characteristics in a Periodic Disturbance Mixer. In *Langmuir*, XX ed.; 2020; Vol. XX, p XX.



FRR from 1 to 12

Results (Effect of lipids on size, PDI & Zeta potential)

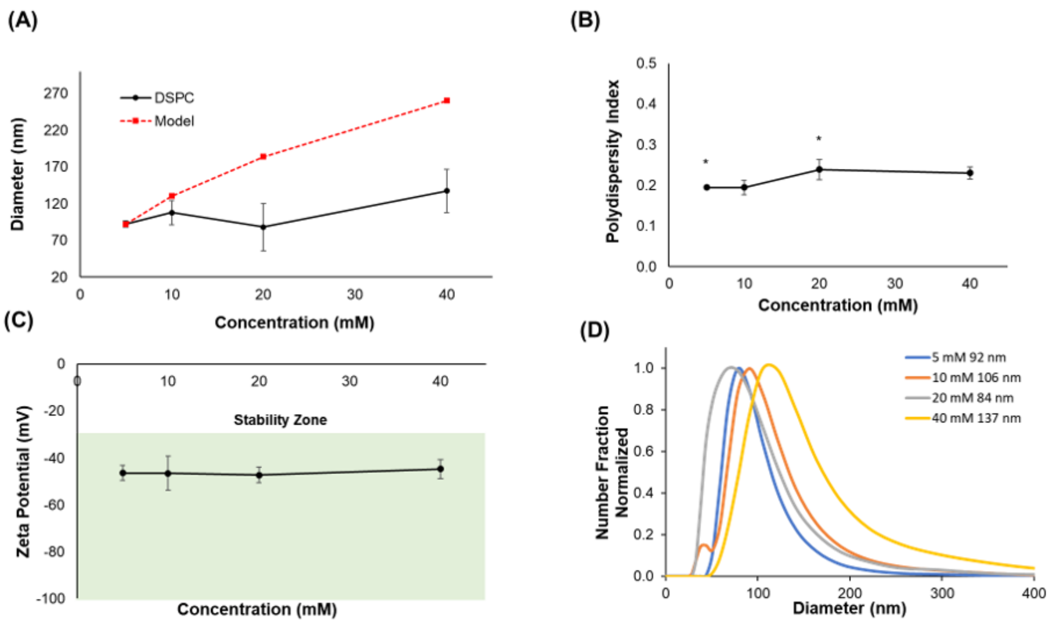
Constant FRR = 8.56, TFR = 18 mL/h T = 40 °C Main Lipid DMPC



Lipid DMPC

López, R. R.; et al.., Parametric Study of the Factors Influencing Liposome Physicochemical Characteristics in a Periodic Disturbance Mixer. In *Langmuir*, XX ed.; 2020; Vol. XX, p XX.

Constant FRR = 8.56, TFR = 18 mL/h T = 40 °C Main lipid DSPC



Lipid DSPC

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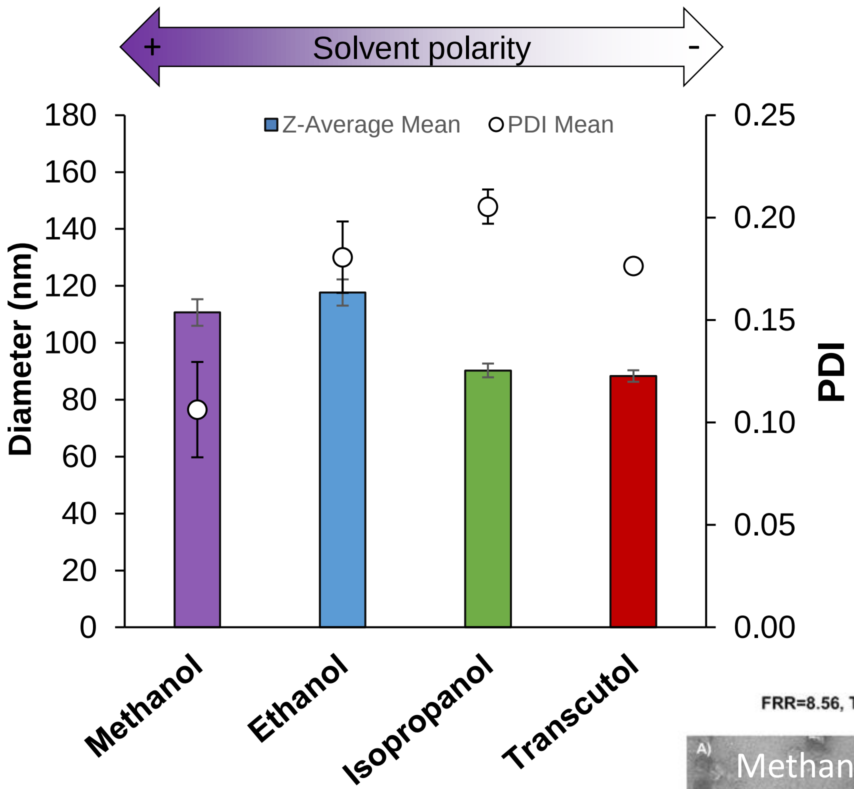
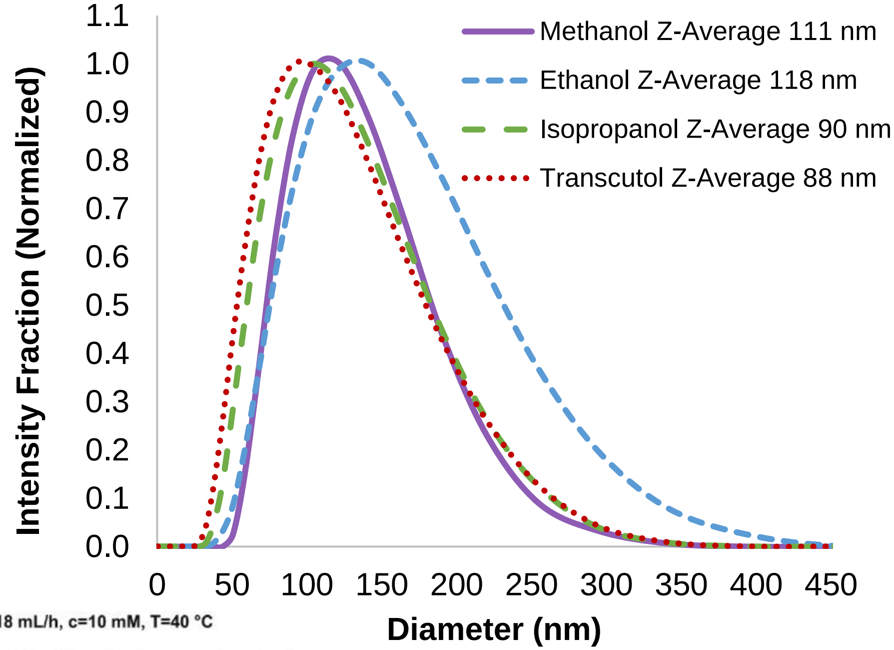
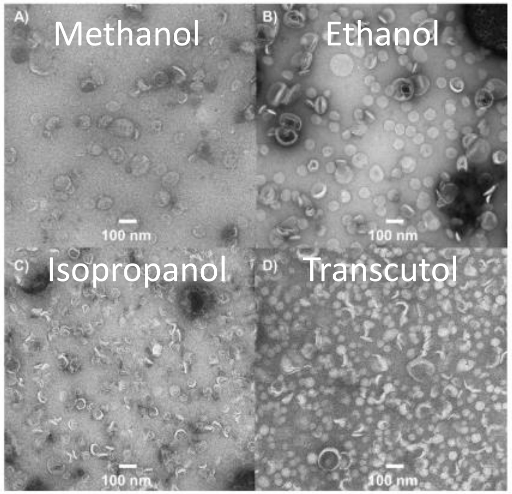
Results (Effect of different solvents)





FRR=8.56, TFR= 18 mL/h, c=10 mM, T=40 °C



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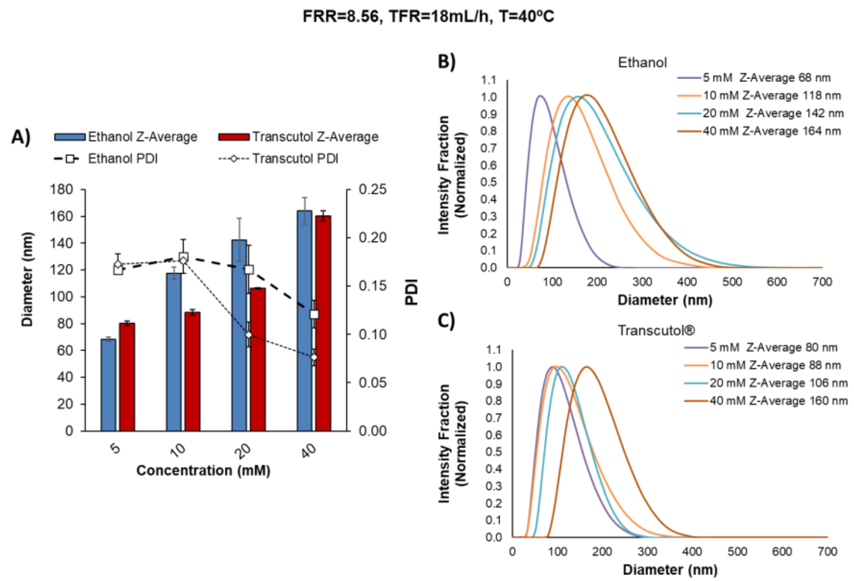
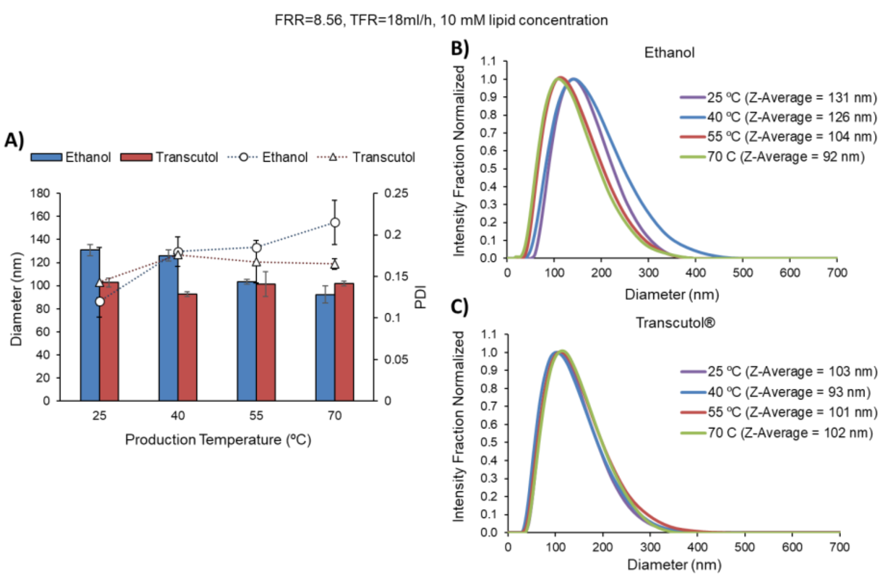
Results (Effect of different lipid concentrations and Tº)



Lipid concentration from 5mM to 40mM

López, R. R.; et al. The Effect of Different Organic Solvents in Liposome Properties Produced in a Periodic Disturbance Mixer: Transcutol®, a potential organic solvent replacement. In *Colloids Surf. B. Biointerfaces*, 2020.



Temperature from 25oC to 70oC

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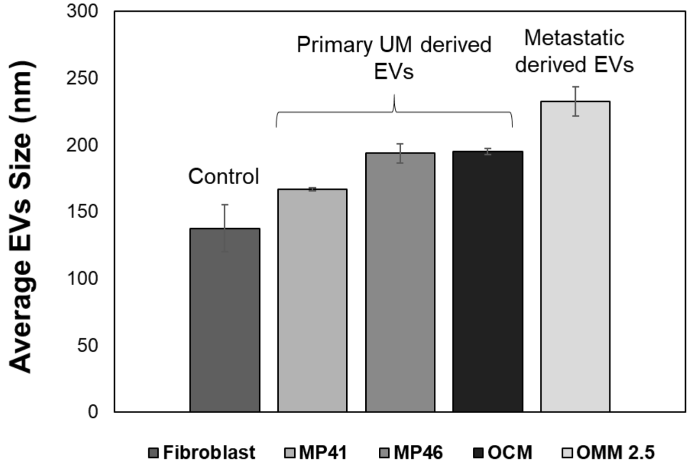
Challenges and future work

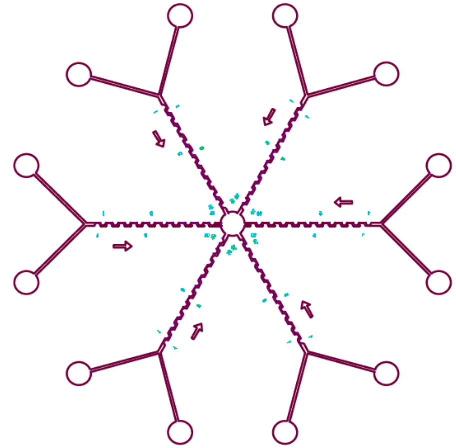
- Continuous solvent removal
- Production yield (PNI). Toroidal micromixer (TrM)
- Production parallelization
- Synthesis and size characterization integration

Cell to cell communication and cancer research EVs liposomes



(López et al. 2020) *ARVO 2020*

Parallelization



Unpublished

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Supplementary Materials and References

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Acknowledgments

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Université Concordia University
Khalifa University
Tecnológico de Monterrey
UQÀM
ÉTS
McGill University
Institut de recherche Centre universitaire de santé McGill
Research Institute McGill University Health Centre

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CONACYT
GATTEFOSSÉ
CMC Microsystems
NanoQAM

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