Manufacture of fully synthetic liposomes using microfluidics

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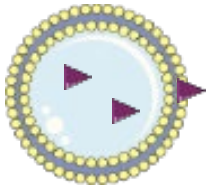
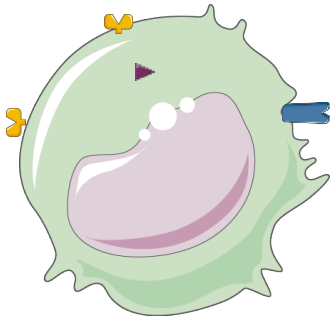
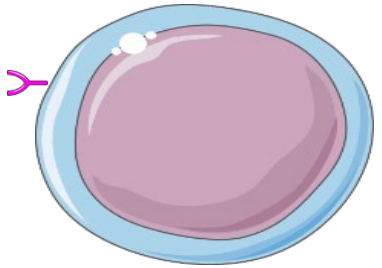
Liposomal delivery of antigens to APC and processing in vaccines

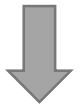




Liposome endocytosed by APC in the lymphatic system

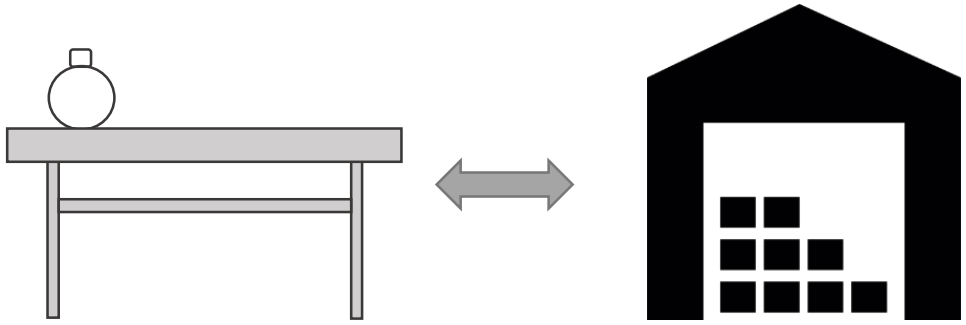


APC presents antigen to CD4 T cell



T cell initiates the appropriate immune response dependent on antigen characteristics

Challenges of manufacturing liposomal vaccines



Translation from bench to clinic is challenging:

- ✖ Batch processes
- ✖ Costly
- ✖ Time-consuming

Recent supply issues:

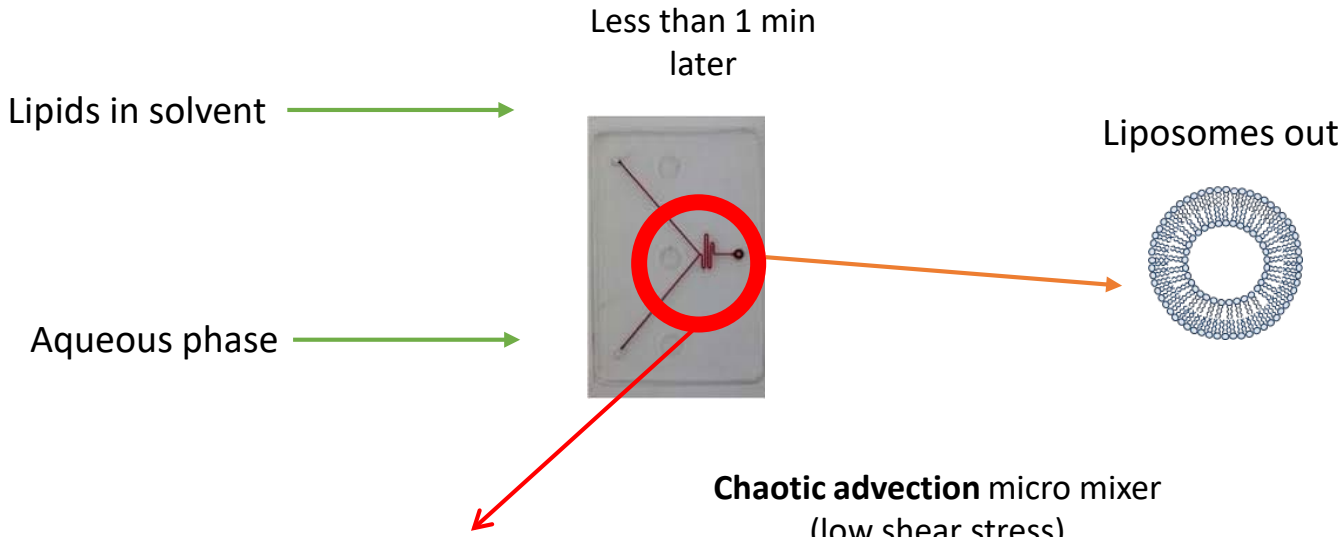
09 September 2011
EMA/718827/2011
EMEA/H/C/000089

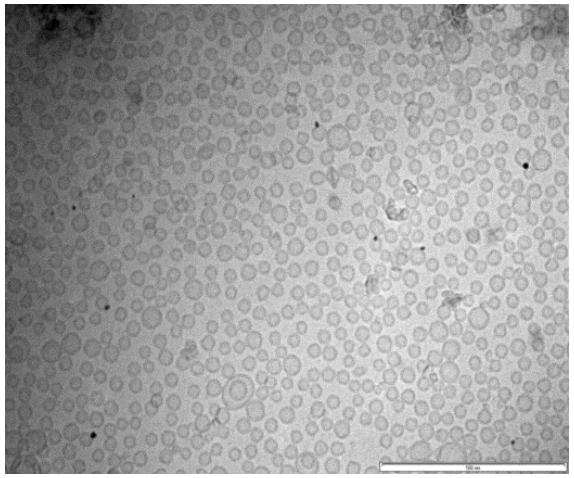
The recommendations in this document were valid during the supply shortage of Caelyx which was resolved in April 2013. For the updated recommendations see here.

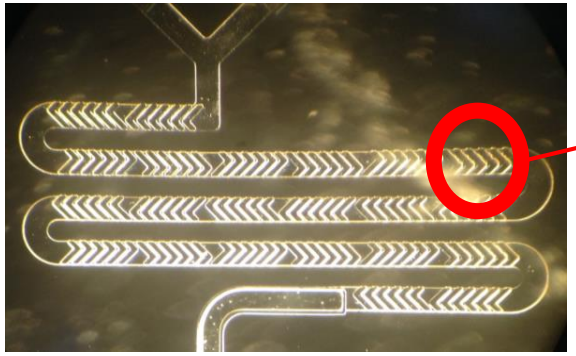
Shortage of Caelyx (doxorubicin hydrochloride)

The European Medicines Agency is aware of a shortage of the anticancer medicine Caelyx in several EU Member States. The Agency's Committee for Medicinal Products for Use (CHMP) is recommending that patients already receiving treatment with Caelyx be given priority and that alternative treatments be considered for new patients.

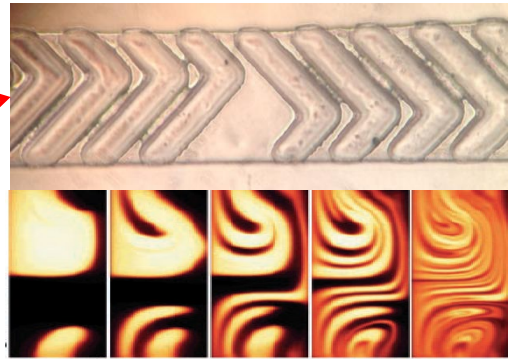
Concept of microfluidics to manufacture liposomes







Chaotic advection micro mixer (low shear stress)

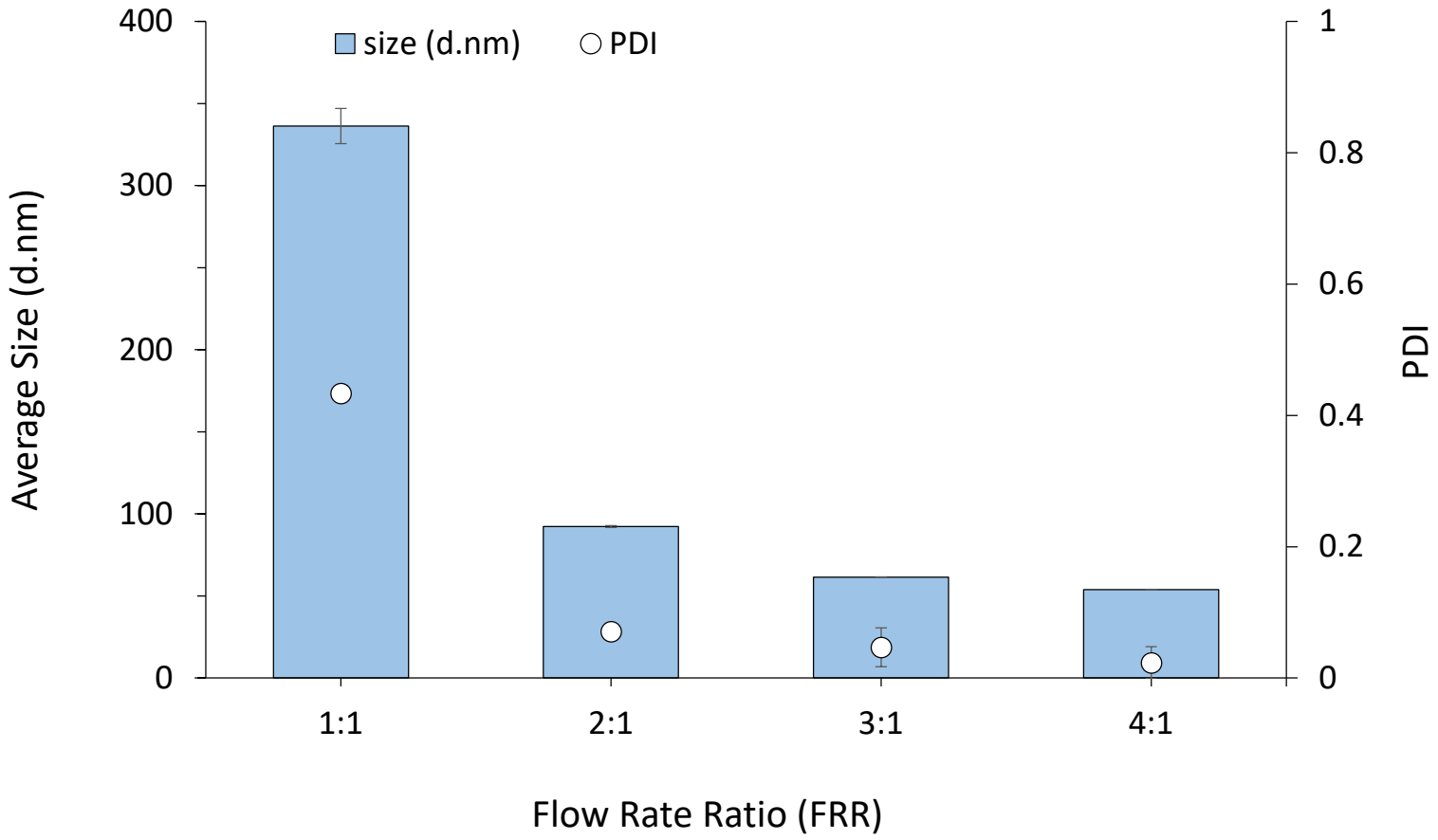


(Stroock et al., 2002)

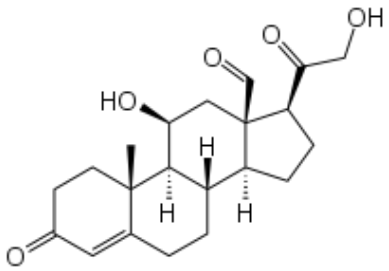
Increased surface area between streams

Nanoprecipitation reaction

Controllable manufacturing parameters available using microfluidics: Flow Rate Ratio

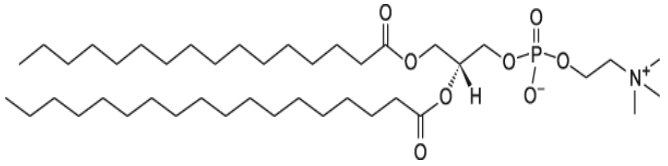


SyntheChol®



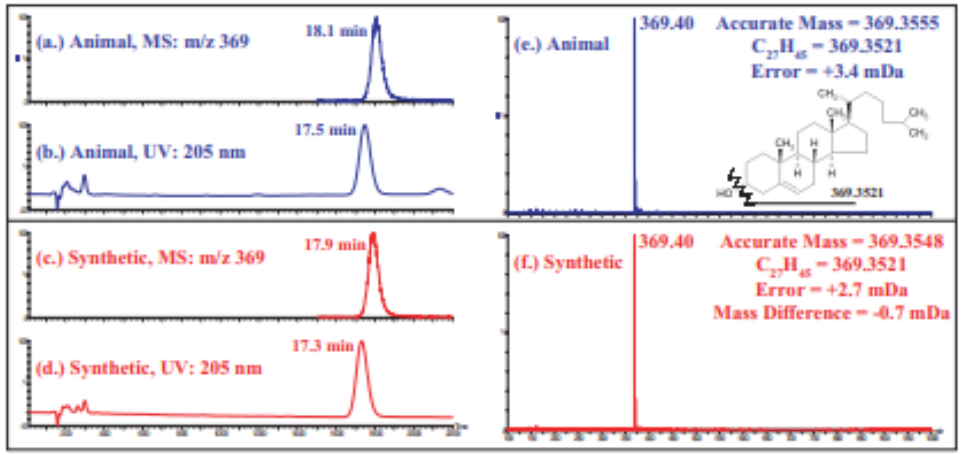
Cholesterol

HSPC



SyntheChol® what it is and importance

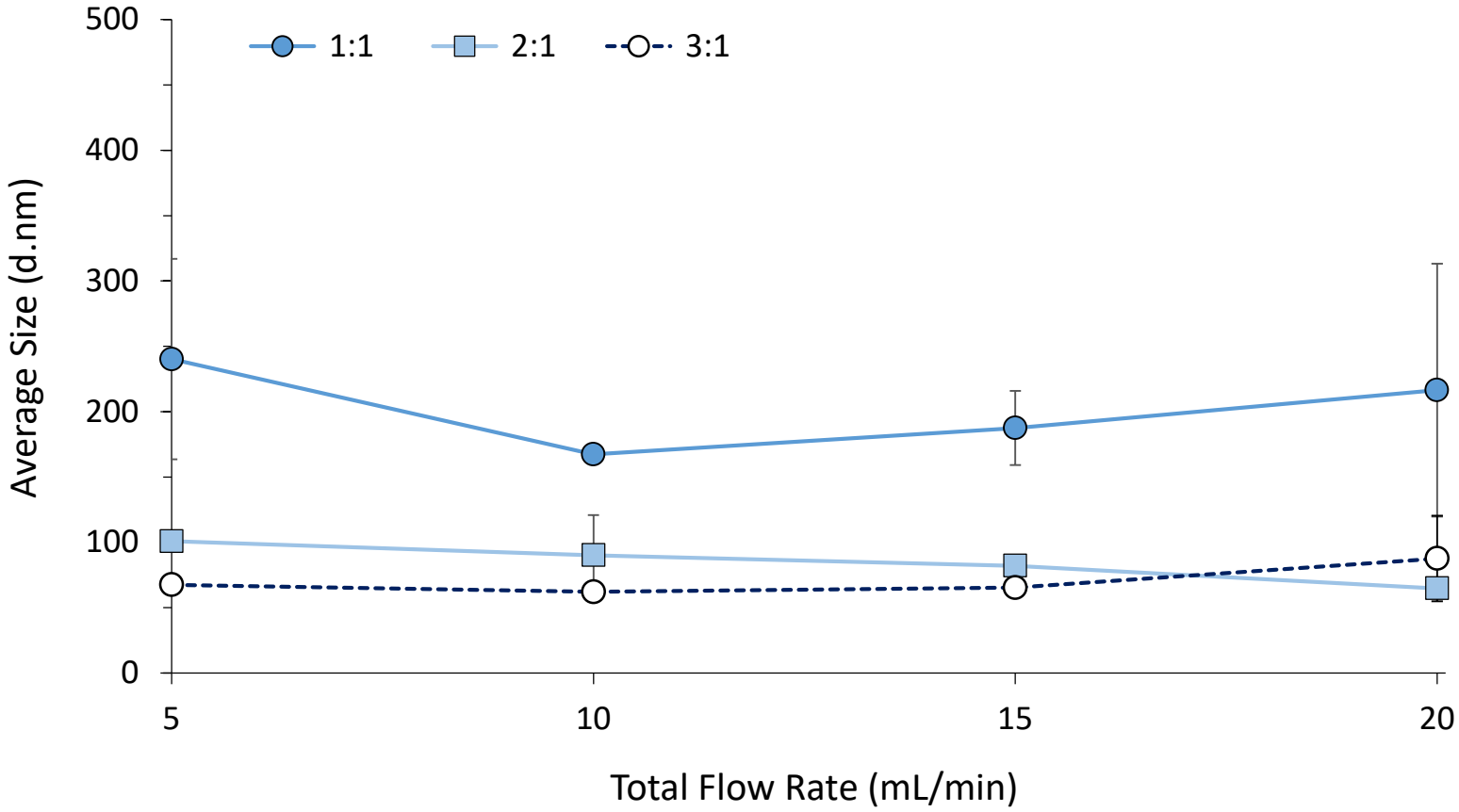
- First synthetic, non – animal derived cholesterol produced by Sigma- Aldrich
- Proven to have the same physio-chemical behaviour as animal derived cholesterol
- NS0 myeloma cell line are rising in popularity for biopharmaceutical production, due to their high cell growth potential and their subsequent high production yields
- Used in NS0 derived cell lines for large-scale manufacturing as cholesterol alternative due to cholesterol auxotrophic nature.



Tally *et al.* SyntheChol™ Synthetic Cholesterol for Cholesterol Dependent Cell Culture — Development of Non-Animal Derived Chemically Defined NS0 Medium, Sigma-Aldrich

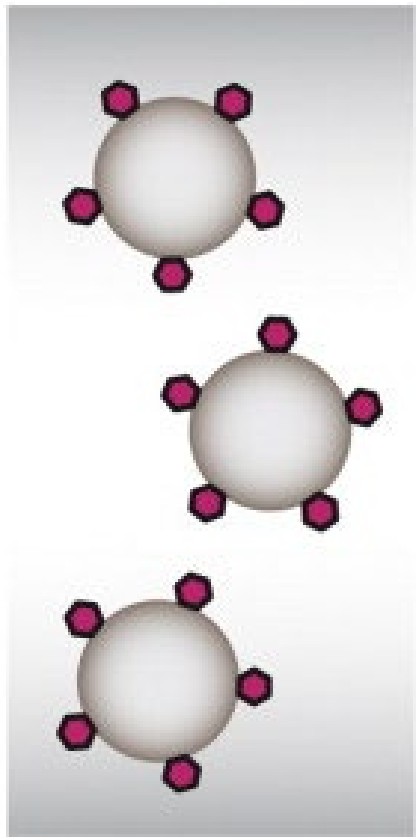
Controllable manufacturing parameters available using microfluidics: Total Flow Rate

- Flow rate ratio is a critical process parameter
- Total flow rate is not



Mechanisms of antigen loading in liposomes

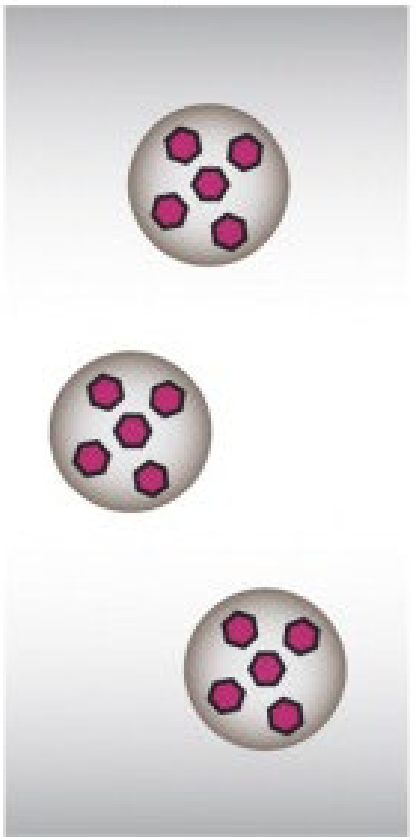
Adsorption



Often electrostatic interactions (+/-)

High load

Encapsulation

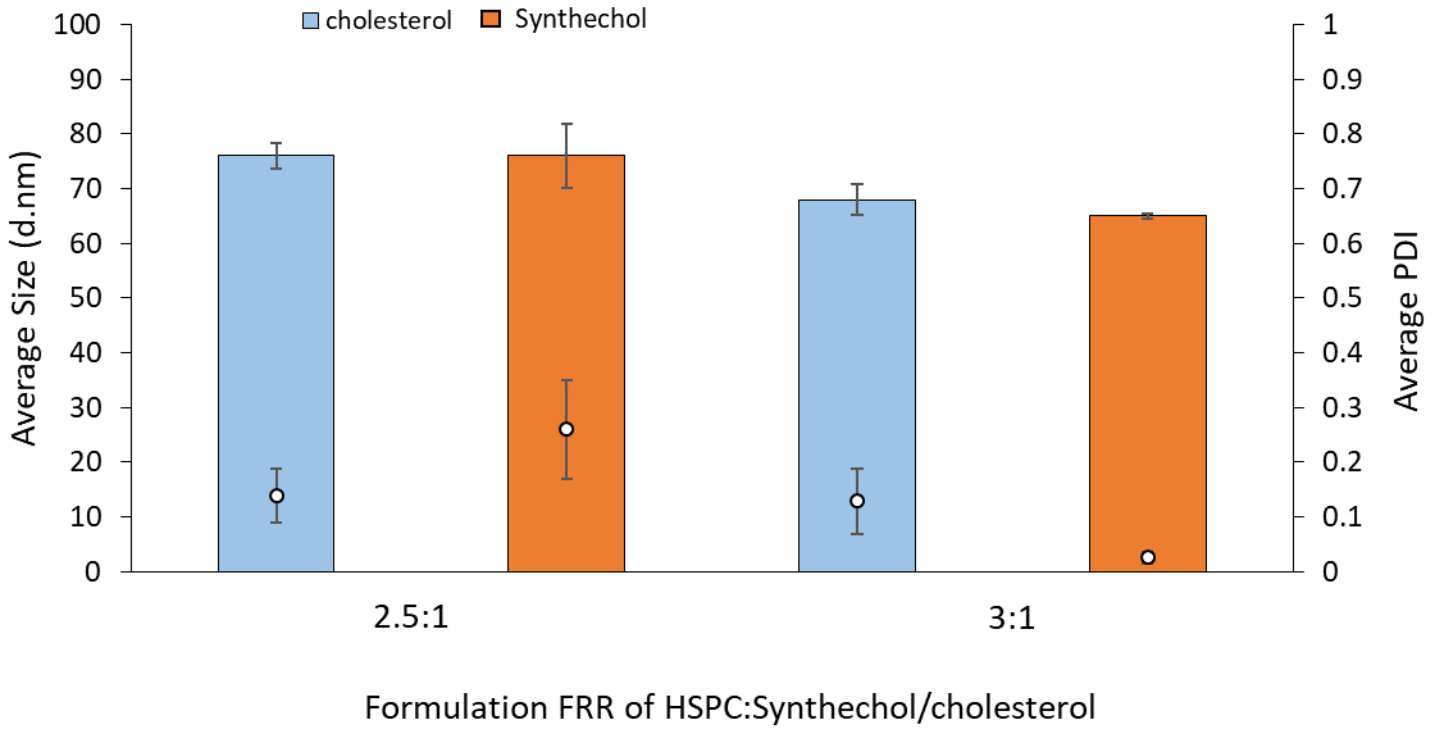


No electrostatics needed (can be any charge)

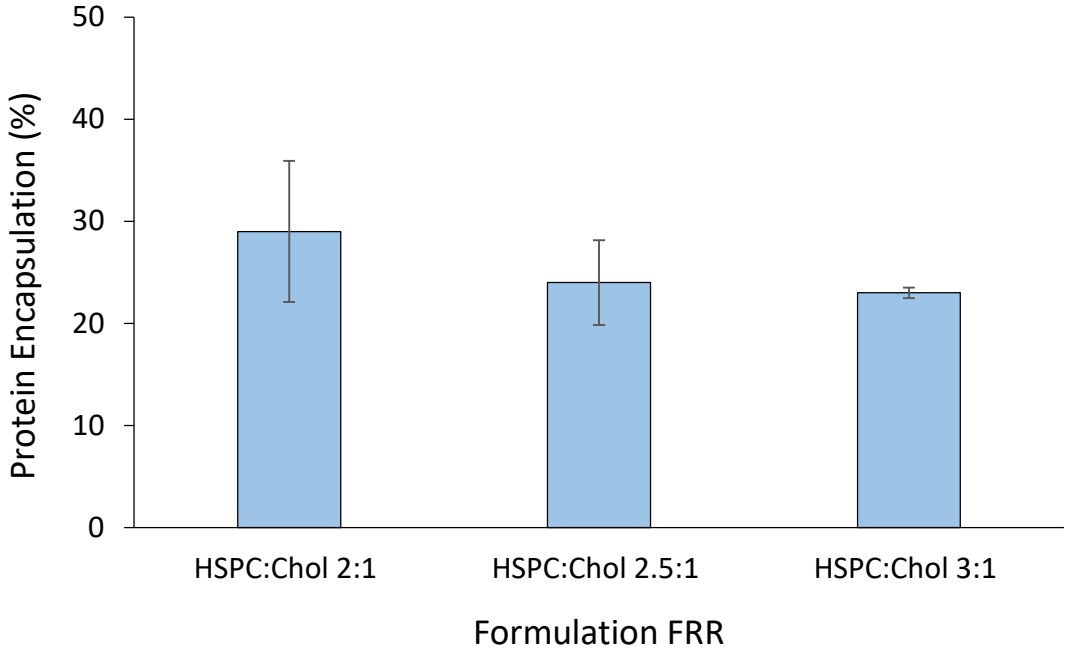
Lower loading

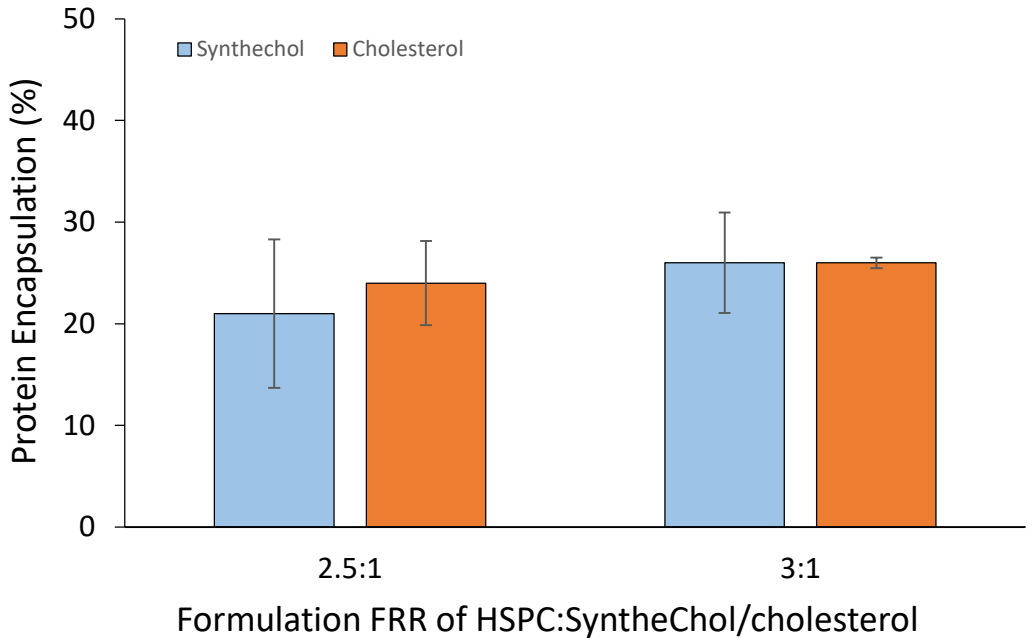
Comparison of Cholesterol substitution for SyntheChol® on formulation size and PDI values

- No difference between liposomes formulated using cholesterol vs SyntheChol®



Comparison of Cholesterol substitution for SyntheChol® on protein encapsulation efficiency

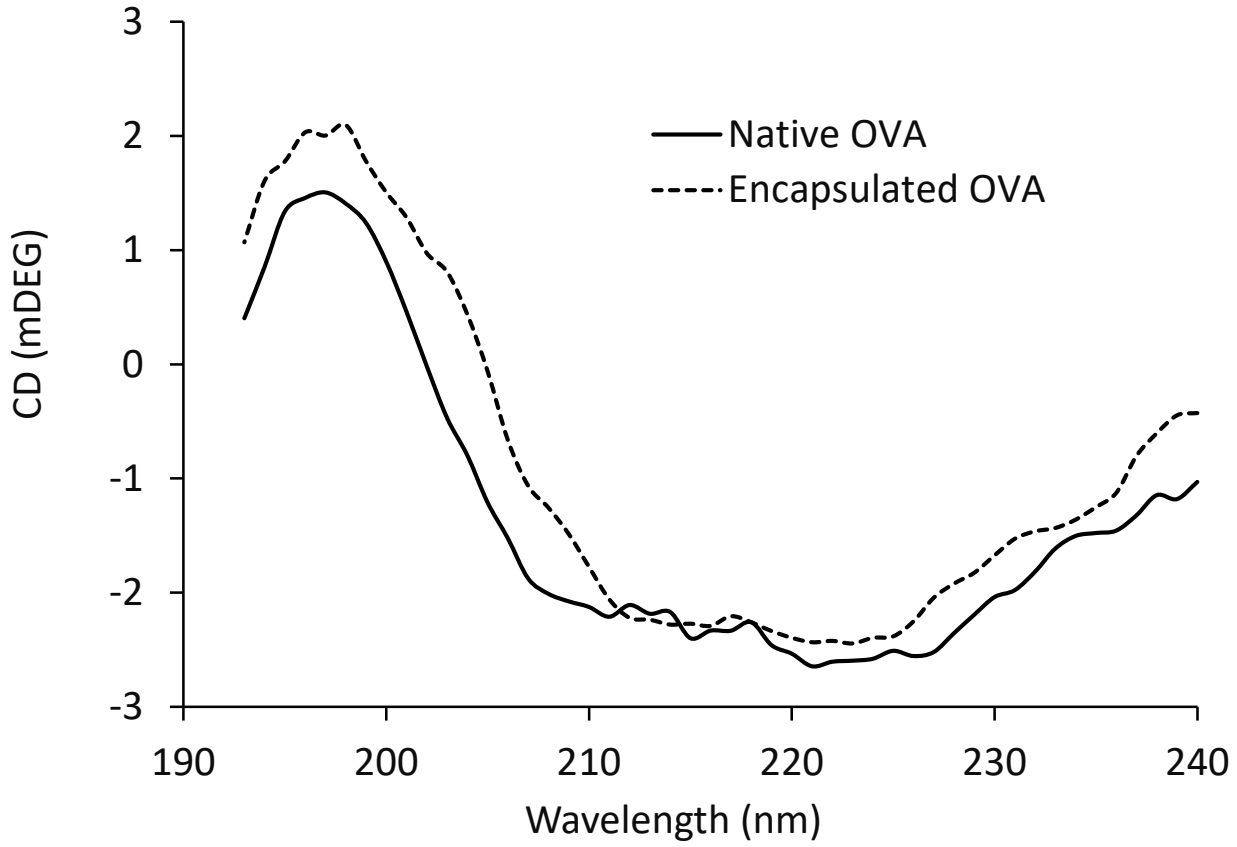




- From the results we were able to achieve high protein loading ranging between 20-30%
- The use of synthetic liposomes had little effect on entrapment efficiency

Circular Dichroism analysis of model antigen properties

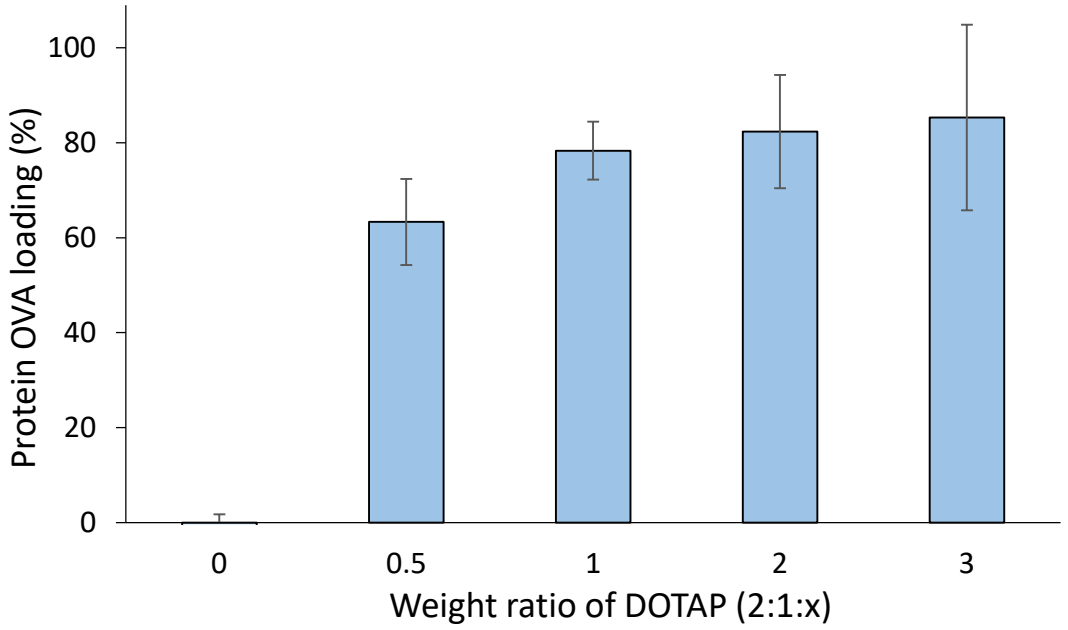
- Results show that there is no damage in OVA structure after encapsulation within liposomes

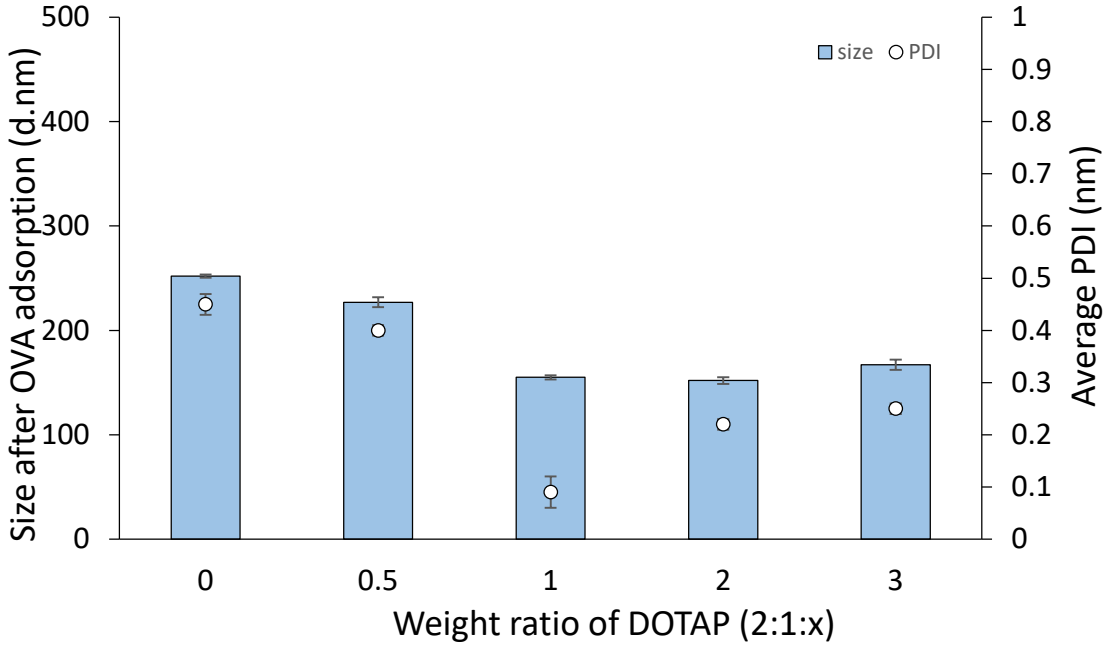


Forbes *et al.* (2019) Rapid and scale-independent microfluidic manufacture of liposomes entrapping protein incorporating in-line purification and at-line monitoring

The effect of cationic liposome formulation and surface adsorption of protein

- From the results we were able to achieve high protein loading ranging between 50-100%





Conclusion

- The substitution of SyntheChol® for cholesterol made no difference in liposome formation or protein loading
- Flow rate ratio is an important factor to consider for manufacturing of liposome size
- The amount of cationic lipid controls protein loading efficiency

Acknowledgements

- University of Strathclyde team
  - Prof Yvonne Perrie (PI)
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- UCL- EPSRC
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