Utilizing Drug-Lipid Membrane Partitioning

Novel, highly efficient and scalable loading of poorly water soluble drugs into the lipid membranes of preformed liposomes

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Introduction

➢ Rational design of liposomal drug delivery systems (Ali, M.H. *et al.*, 2013) requires, among other aspects, a comprehensive understanding of the biophysics governing the interactions between lipid membranes and lipophilic drugs (small molecules such as curcumin).

➢ Currently, there are only few techniques available to address the question of optimal drug to lipid ratio and favourable lipid composition (e.g. addition of cholesterol, α-tocopherol) in a comprehensive fashion. In this study we introduce a promising high throughput approach to study this very important aspect.

➢ Microfluidics technology and online mixing modules as those used for the production of liposomes using cross flow solvent injection can be useful in utilizing drug-lipid membrane partitioning for highly efficient and scalable loading of small lipophilic drugs such as curcumin into preformed liposomes.

Materials & Methods

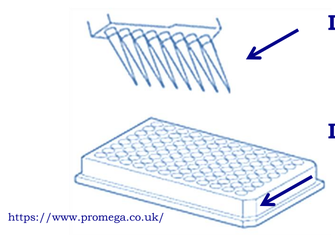
➢ Liposome preparation: Thin-film hydration method and extrusion or probe sonication.
➢ Size, PdI and zetapotential were determined using a Zetasizer Nano ZS.
➢ Drug content was determined using a validated spectrophotometric method.

Partitioning Assay

High Throughput Screening / Liposome Loading

➢ Submerse injection of an ethanolic curcumin solution to a serial dilution of liposomes under well-defined and monitored conditions (Temperature, mixing etc.).

➢ The effects of temperature, liposome size, mixing, incubation time, solvent concentration on the partitioning of curcumin were determined in a comprehensive study. Loading conditions were optimised to prevent ethanol induced interdigitation fusion of the liposomes (Smith, E. & Dea, P.K., 2013). Conditions were finally optimised to allow a maximum of drug partitioning / loading.



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Drug solution (organic solvent e.g. ethanol)
- ➢ volume = const.
- ➢ drug conc. = const.

Liposomes of desired size in appropriate buffer
- ➢ Serial dilution; Lipid conc.: 0.15 – 10 mM)
- ➢ volume = const.

➢ The liposome suspensions were then filtered (0.22 or 0.45 µm 96-well filter plates) to remove precipitated curcumin and subsequently analysed for loaded curcumin using a microplate reader.

Microfluidics Assisted Liposome Loading

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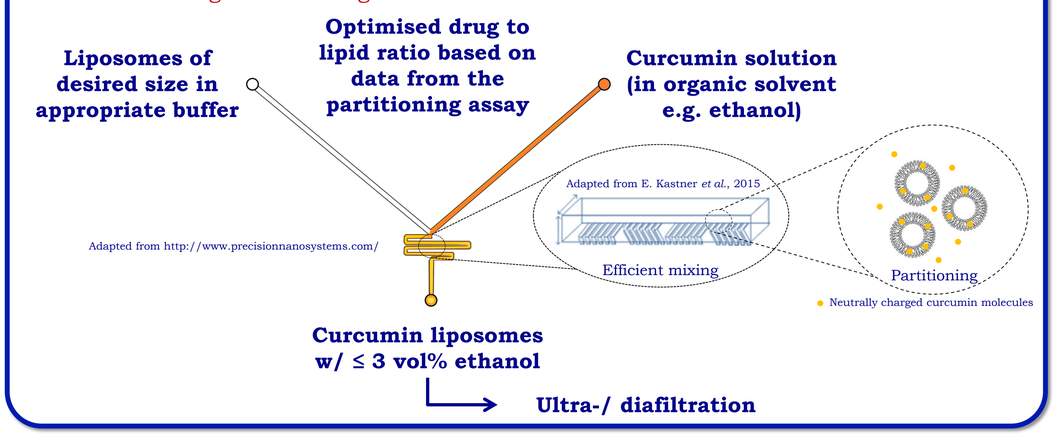
➢ Microfluidics assisted liposome loading for academic lab scale (3 mL loaded liposome suspension) was performed on the NanoAssemblr ™ Platform (Precision Nanosystems). Translation to large scale mixing modules is conceivable.



Conclusions

➢ The introduced method is a promising high throughput screening/manufacturing (loading) approach for liposomal drug delivery systems.

➢ Drug-lipid membrane partitioning as a method for liposome loading can be assisted by microfluidics systems. Translation to large scale mixing modules is conceivable.

➢ The drug-lipid membrane partitioning based approach is highly efficient and scalable.

➢ The proposed method was shown to be capable of identifying and characterising drug partitioning enhancing / increasing effects of a variety of membrane compounds such as cholesterol, α-tocopherol and DMPG.

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To learn more about the collaborating company and universities visit:
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References & Acknowledgements

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Read more about SignPath Pharma's liposomal curcumin formulation Lipocurc™ at signpathpharma.com. Lipocurc™ for the treatment of cancer is currently tested in clinical trials. (Storka, A. *et al.*, 2015).

Results

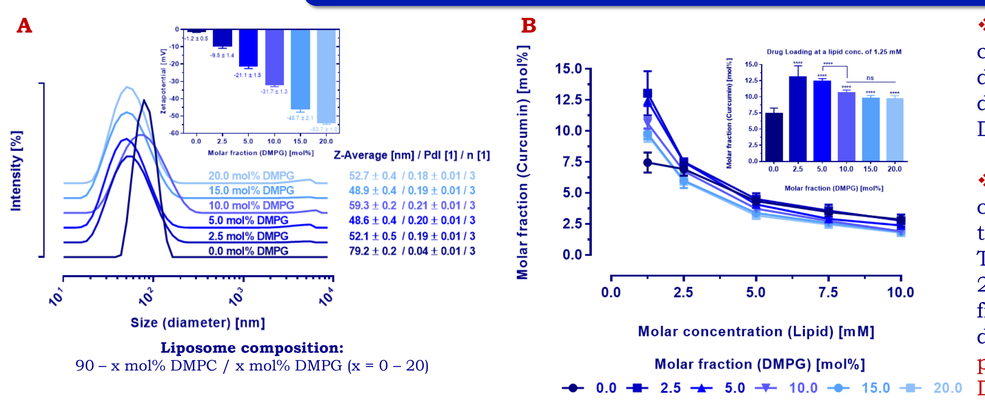


Figure 1. Effect of DMPG (Surface Charge) on membrane partitioning / loading. **A.** Physicochemical characterisation of the negatively charged liposomes (prior loading). **B.** Partitioning assay: Drug loading expressed as molar fraction of curcumin in the lipid membrane.

❖ Increasing fractions of the negatively charged lipid DMPG in the liposome decrease the zetapotential (**1A**). The data suggests a linear relation between DMPG content and the zetapotential.

❖ Addition of DMPG enhances curcumin membrane partitioning and therefore increases drug loading (**1B**) . The highest drug loading is seen at 2.5 mol% DMPG. Higher molar fractions of DMPG (≥ 10 mol%) decrease the drug loading. Note the plateau between 10 and 20 mol% DMPG (**1B**).

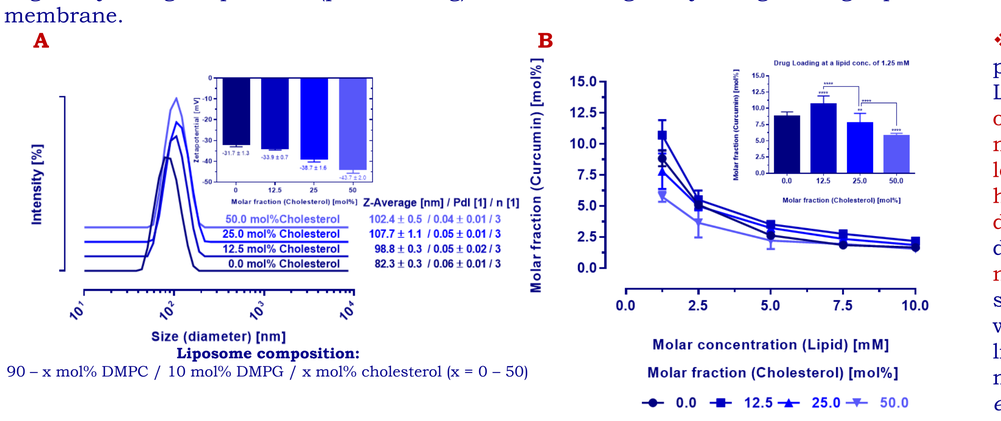
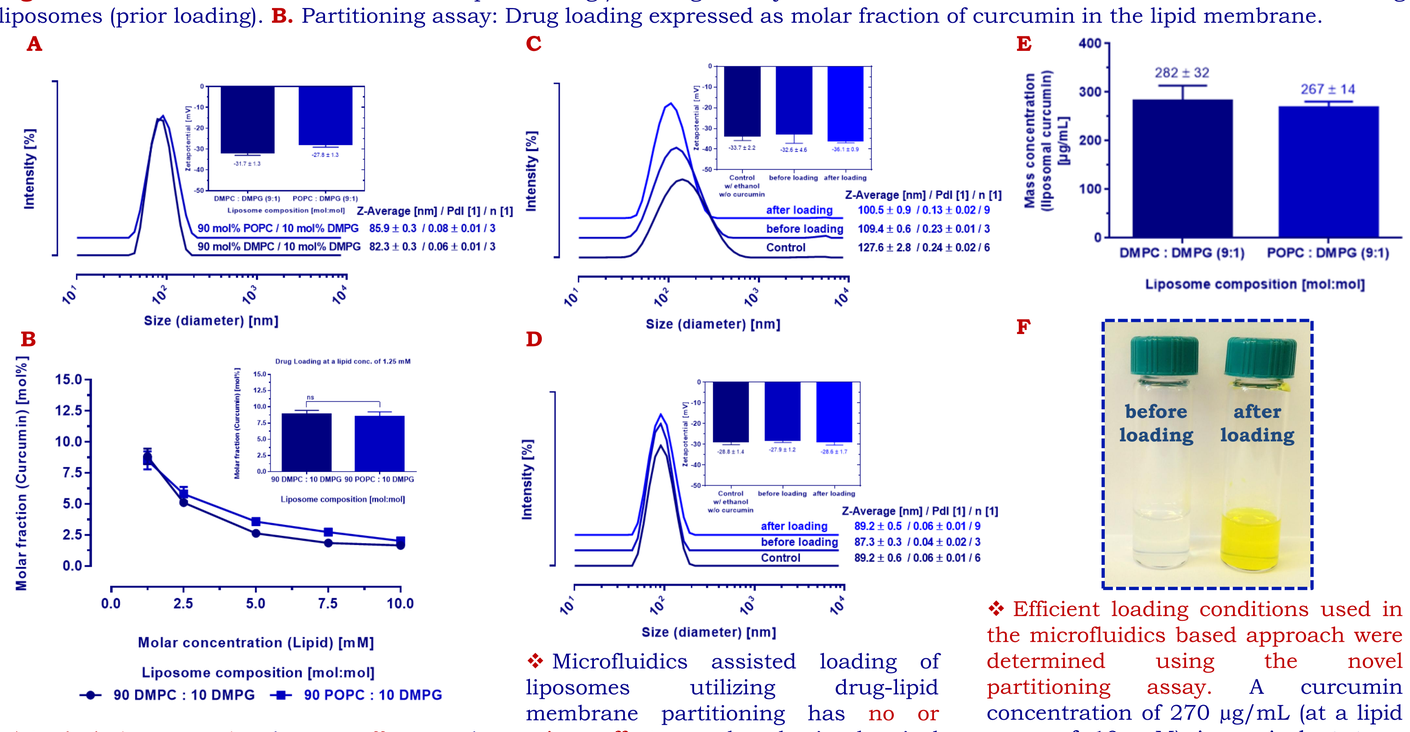


Figure 2. Effect of cholesterol on membrane partitioning / loading. **A.** Physicochemical characterisation of the cholesterol containing liposomes (prior loading). **B.** Partitioning assay: Drug loading expressed as molar fraction of curcumin in the lipid membrane.

❖ In accordance to previous data published by researchers of the Perrie Lab (Aston University) small fractions of cholesterol (≤ 12.5 mol%) in the lipid membrane of liposomes improve drug loading. However it can be seen that higher fractions (> 12.5 mol%) decrease the loading of small lipophilic drug molecules such as curcumin. The novel partitioning assay used in this study confirms this phenomena which was already observed with drug loaded liposomes produced with conventional methods such as film method (Liu, X.Y. *et al.*, 2001) and solvent injection (**2B**).

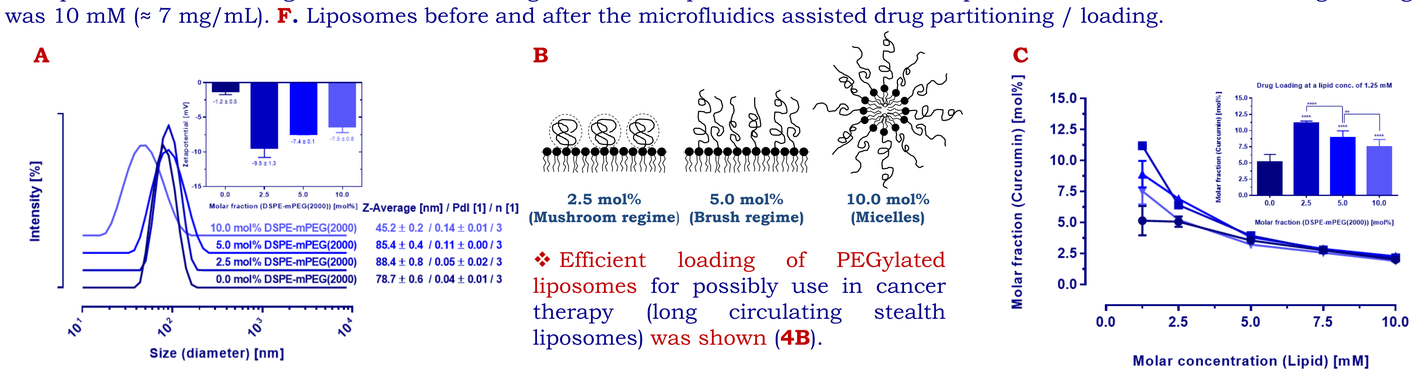


❖ Acyl chain saturation has no effect on the lipid membrane partitioning of curcumin (**3B**).

❖ Microfluidics assisted loading of liposomes utilizing drug-lipid membrane partitioning has no or minor effects on the physicochemical properties of liposomes (**3C and 3D**).

❖ Efficient loading conditions used in the microfluidics based approach were determined using the novel partitioning assay. A curcumin concentration of 270 µg/mL (at a lipid conc. of 10 mM) is equivalent to a molar fraction of ~7.5 mol% (**3E**).

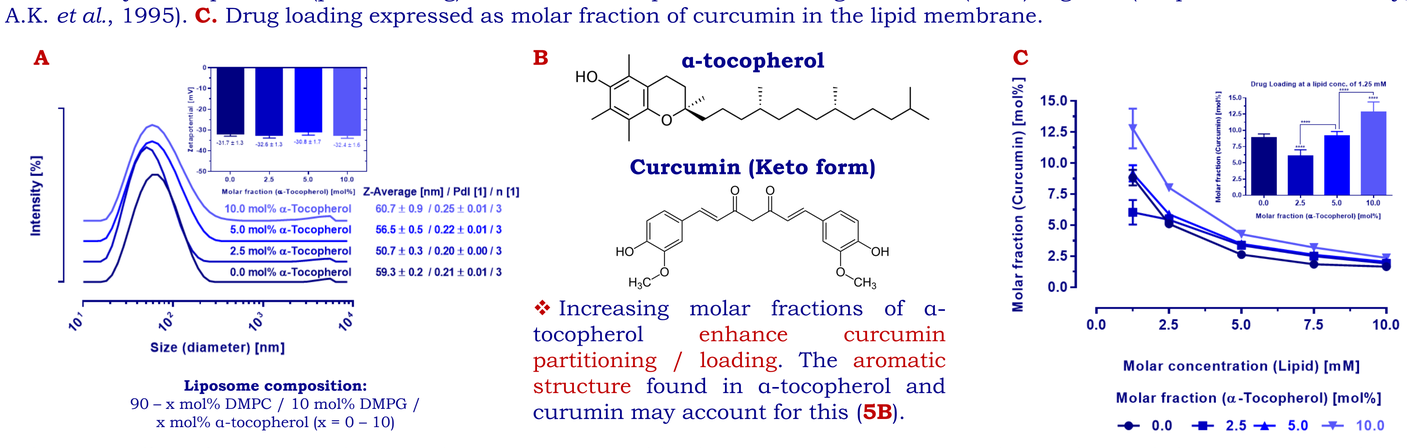
Figure 3. Effect of acyl chain saturation on membrane partitioning / loading. **A.** Physicochemical characterisation of the liposomes (prior loading). **B.** Partitioning assay (submerse injection based approach): Drug loading expressed as molar fraction of curcumin in the lipid membrane. **C-D**. Microfluidics assisted approach: Effect of the drug partitioning / loading on the physicochemical properties of the liposomes (DMPC : DMPG (9:1; mol:mol) (**C**) and POPC / DMPG (9:1; mol:mol) (**D**)). **E.** Curcumin concentration of the liposomes loaded using a microfluidics mixing device. The lipid concentration of the liposome formulation used for drug loading was 10 mM (≈ 7 mg/mL). **F.** Liposomes before and after the microfluidics assisted drug partitioning / loading.



❖ Efficient loading of PEGylated liposomes for possibly use in cancer therapy (long circulating stealth liposomes) was shown (**4B**).

❖ Conformation of grafted PEG(2000) effects partitioning of curcumin (**4A-C**).

Figure 4. Effect of grafted PEG(2000) on membrane partitioning / loading. **A.** Partitioning assay: Physicochemical characterisation of the PEGylated liposomes (prior loading). **B.** Schematic representations of grafted PEG(2000) regimes (adapted from Kenworthy, A.K. *et al.*, 1995). **C.** Drug loading expressed as molar fraction of curcumin in the lipid membrane.



❖ Increasing molar fractions of α-tocopherol enhance curcumin partitioning / loading. The aromatic structure found in α-tocopherol and curumin may account for this (**5B**).

Figure 5. Effect of α-tocopherol on membrane partitioning / loading. **A.** Partitioning assay: Physicochemical characterisation of the α-tocopherol containing liposomes (prior loading). **B.** Chemical structure of α-tocopherol and curcumin. **C.** Drug loading expressed as molar fraction of curcumin in the lipid membrane.