Manufacture of Verteporfin Loaded Liposomes using a Scalable Microfluidic Platform

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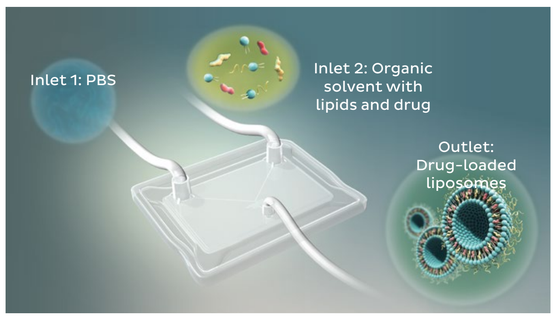
Introduction

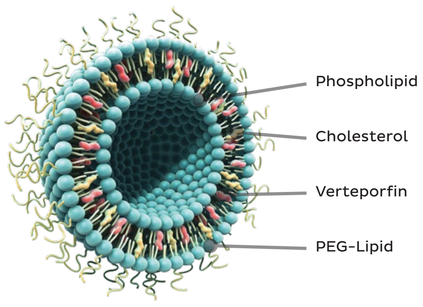
- Over 75% of new chemical entities from drug discovery programs are low solubility biopharmaceuticals
- Suitable drug delivery systems necessary to advance low solubility candidates into clinic
- Verteporfin is a highly hydrophobic sensitizer for photodynamic therapy for wet age-related macular degeneration (AMD) (trade name Visudyne)
- Free verteporfin dimerizes in water, losing pharmacological activity - Liposomal encapsulation overcomes this limitation
- Scaling up the production of liposomes is challenging: changing batch size affects liposome properties
- The NanoAssemblr™ platform allows scale up of liposomes without changing process parameters
- We developed *in situ* loading for liposomal verteporfin with natural and synthetic lipids using the NanoAssemblr™ Benchtop and scaled 10 times via the Blaze™ with the same size, PDI, and encapsulation efficiency

Microfluidic Nanoprecipitation



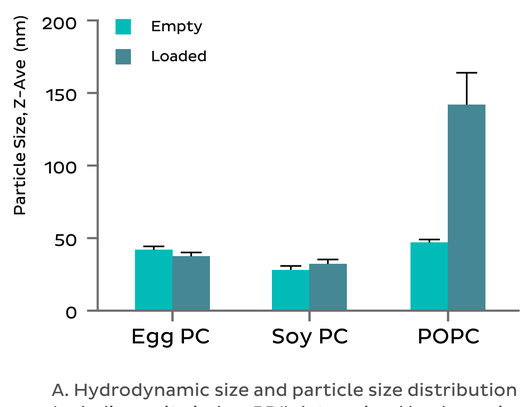
Aqueous buffer and organic solvent with dissolved lipids & verteporfin are mixed in the NanoAssemblr™ microfluidic cartridge. Liposomes formed by controlled nanoprecipitation

Verteporfin Loaded Liposomes

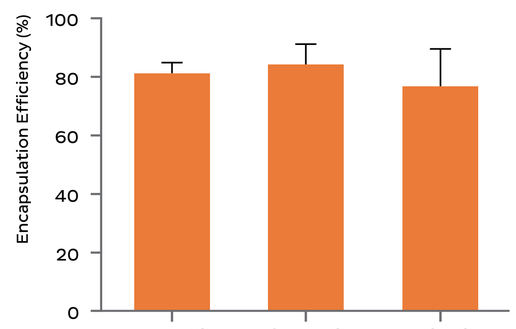


Drug Loading & Formulation Optimization Using Benchtop

A Sizes of soy- and egg-PC liposomes not affected by drug loading



B Encapsulation efficiencies > 80%



A. Hydrodynamic size and particle size distribution (polydispersity index, PDI) determined by dynamic light scattering. Synthetic lipid formulations aggregated in the presence of verteporfin, resulting in larger liposomes upon loading.

B. Encapsulation efficiency (final drug-to-lipid ratio as a percentage of the initial drug-to-lipid ratio). Lower encapsulation and loss of 70% of material observed in POPC-based liposomes due to aggregation upon loading.

Samples prepared in triplicate; values represent mean and error bars represent standard deviation of the mean.

Formulation Conditions

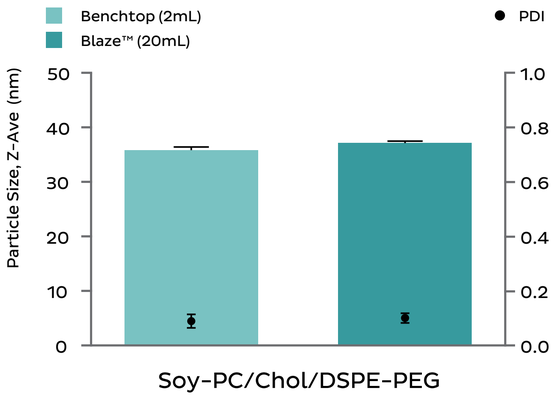
Lipid Composition	PC*:Chol:DSPE-PEG$_{2000}$ (52:45:3 mol%)
Total Lipid Concentration	10 mg/mL
Verteporfin Concentration	1 mg/mL
Drug/Lipid Ratio	0.1 (w/w), 0.09 (mol/mol)
Organic Solvent	Ethanol/DMF (97.5:2.5 v/v)
Aqueous Solvent	PBS pH 7.4
Total Flow Rate	12 mL/min
Flow Rate Ratio (Aq: Or)	2:1
Formulation Volume	2mL
Solvent Removal	Dialysis

* PC as labeled on x-axis

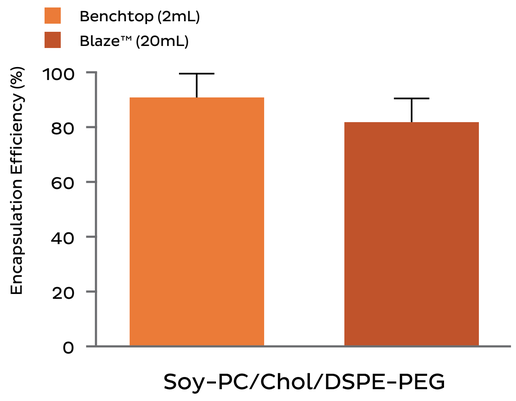
Scaling Up Optimized Formulations Using Blaze™

Soy-PC Based Liposomal Verteporfin

A Particles produced on the Benchtop and Blaze™ have identical size, PDI below 0.2



B Particles produced on Benchtop and Blaze™ have encapsulation efficiencies > 80%



A. Hydrodynamic size and particle size distribution (polydispersity index, PDI) determined by dynamic light scattering.

B. Encapsulation efficiency (final drug-to-lipid ratio as a percentage of the initial drug-to-lipid ratio).

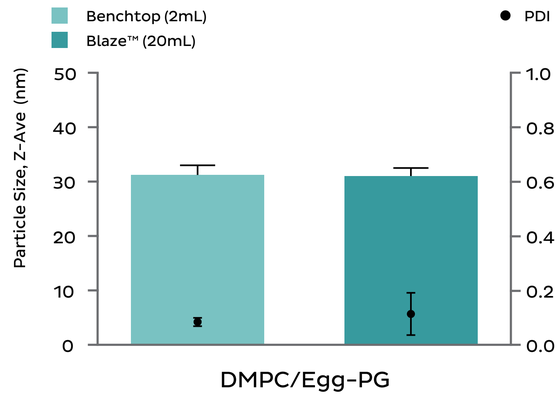
Formulation volumes: 2 mL and 20 mL for the NanoAssemblr™, Benchtop and Blaze™, respectively. Samples prepared in triplicate; values represent mean & error bars represent standard deviation of the mean.

Formulation Conditions

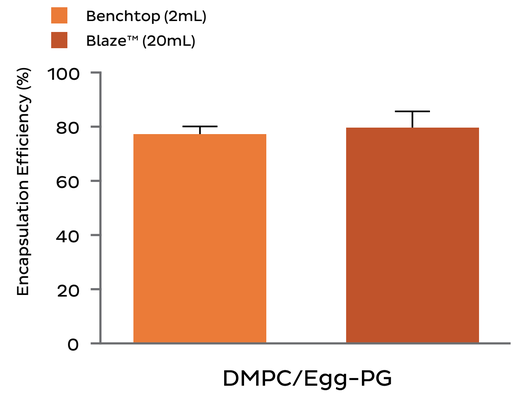
Lipid Composition	Soy-PC:Chol:DSPE-PEG$_{2000}$ (52:45:3 mol%)
Total Lipid Concentration	10 mg/mL
Verteporfin Concentration	1 mg/mL
Drug/Lipid Ratio	0.1 (w/w), 0.09 (mol/mol)
Organic Solvent	Ethanol/DMF (97.5:2.5 v/v)
Aqueous Solvent	PBS pH 7.4
Total Flow Rate	12 mL/min
Flow Rate Ratio (Aq: Or)	2:1
Solvent Removal	Benchtop: Dialysis Blaze™: Ultrafiltration

DMPC:Egg-PG Based Liposomal Verteporfin

A Particles produced on the Benchtop and Blaze™ have identical size, PDI below 0.2



B Particles produced on Benchtop and Blaze™ have encapsulation efficiencies of ~80%



A. Hydrodynamic size and size distribution (polydispersity index, PDI) determined by dynamic light scattering.

B. Encapsulation efficiency (final drug-to-lipid ratio as a percentage of the initial drug-to-lipid ratio).

Formulation volumes : 2 mL and 20 mL for the NanoAssemblr™, Benchtop and Blaze™, respectively. Samples prepared in triplicate; values represent mean & error bars represent standard deviation of the mean.

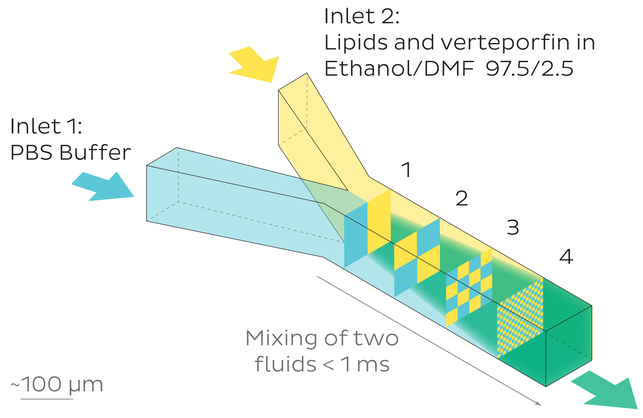
Formulation Conditions

Lipid Composition	DMPC:Egg-PG (67:33 mol%)
Total Lipid Concentration	10 mg/mL
Verteporfin Concentration	1 mg/mL
Drug/Lipid Ratio	0.1 (w/w), 0.09 (mol/mol)
Organic Solvent	Ethanol/DMF (97.5:2.5 v/v)
Aqueous Solvent	PBS pH 7.4
Total Flow Rate	12 mL/min
Flow Rate Ratio (Aq: Or)	2:1
Solvent Removal	Benchtop: Dialysis Blaze™: Ultrafiltration

Materials and Methods

Experimental Design

- Drug loaded liposomes made by controlled nanoprecipitation in microfuidic mixer
- Hydrophobic verteporfin loaded *in situ* by including in organic phase before nanoprecipitation
- NanoAssemblr Benchtop (1mL to 15mL) used to optimize formulations at 2mL
- Conserved microfluidic geometry allows optimized Benchtop parameters to be used on the Blaze (10mL to 1L) without modification
- Results expected to be very similar between Benchtop and Blaze
- Different lipid compositions can also be compared



1. Liquids are injected into each inlet of the NanoAssemblr™ microfludic mixer under laminar flow and hence, do not mix.
2. Microscopic features in the channel are engineered to cause the fluid streams to mingle in a controlled, reproducible, and non-turbulent way.
3. Intermingling of the fluids increases as they continue through the mixer.
4. Fluids emerge from the mixer completely mixed. The process occurs in less than 1 ms and triggers molecules to homogeneously coalesce into nanoparticles.

Materials
Liposomes containing 3 components were composed of one of either Soy-PC (Lipoid, Germany), Egg-PC (Lipoid, Germany), or POPC (1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine), cholesterol and DSPE-PEG(1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[amino(polyethylene glycol)-2000]). Liposomes containing 2 components were composed of DMPC (1,2 dimyristoyl-sn-glycero-3-phosphocholine) and egg-PG (L-α-phosphatidylglycerol).

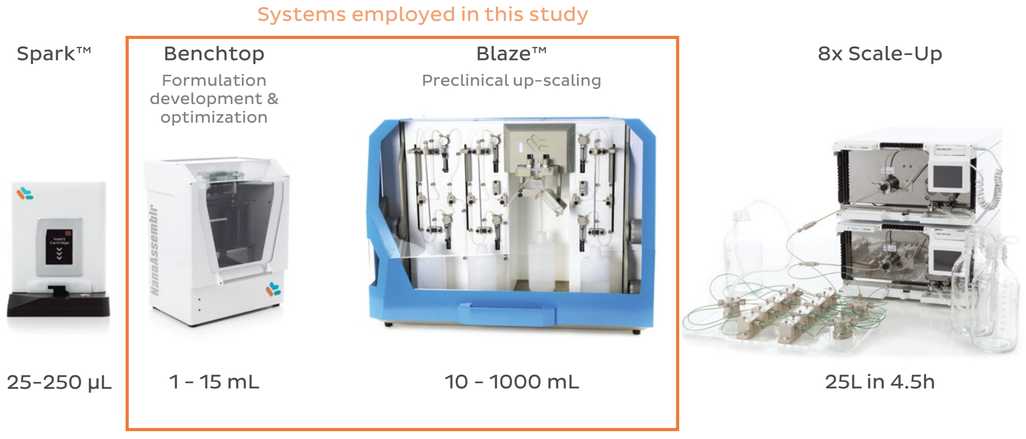
Liposome Preparation
Phospholipids, and either cholesterol and pegylated lipid or egg-PG were dissolved in absolute ethanol at a 52:45:3 molar ratio. Verteporfin was loaded by dissolving it in the lipid solution at a concentration of 1 mg/mL (0.09 molar ratio to total lipids. Here, 2.5% by volume DMF was added to ethanol as a co-solvent. Calcium- (Ca^{2+}) and magnesium- (Mg^{2+}) free PBS buffer at neutral pH was used as the aqueous phase. The organic and aqueous phases were rapidly mixed using the NanoAssemblr™ Benchtop (2mL) or Blaze (20mL) microfluidic instrument at a 2:1 flow rate and 12 mL/min total flow rate to form unilamellar liposomes.

Solvent Removal
Formulations produced on the Benchtop were dialyzed against PBS for ethanol removal. DMPC:Egg-PG formulations were diluted to 10% ethanol immediately following the mixing process and before dialysis, since high amounts of ethanol can destabilize liposomes. An aliquot of Blaze™ formulations were diluted 4X with PBS, then concentrated using Amicon® ultra-15 centrifugal filters. Ultrafiltered formulations underwent 3 cycles of dilution and subsequent concentration.

Particle Analysis
Particle size and integrity were measured before and after dialysis using Dynamic Light Scattering (Zetasizer, Malvern Instruments, UK). Unless otherwise indicated, formulations were prepared in triplicate. Size and polydispersity index (PDI) were represented as the mean of 3 samples, and error bars represent standard deviation of the mean. Verteporfin content was quantified by fluorescence spectroscopy.

NanoAssemblr™ Systems

Systems employed in this study



Conclusions

Liposomes containing natural lipids, cholesterol and a DSPE-PEG$_{2000}$ were formulated and *in situ*-loaded with verteporfin using the NanoAssemblr™ platform.

Substituting egg-PC with a synthetic analogue, POPC, resulted in aggregation and loss of 70% of the material. This POPC formulation assembles into stable 49 nm liposomes in the absence of verteporfin.

Verteporfin encapsulation efficiencies of >90% for soy-PC and >80% with egg-PC were obtained.

Naturally derived phospholipid formulations can be produced with the NanoAssemblr™ platform and natural phospholipids appear advantageous for encapsulating hydrophobic small molecules.

The Blaze™ enables seamless scale-up of the formulation volume while maintaining particle size, PDI and achieving high encapsulation efficiencies.

The *in situ* loading method enabled by the NanoAssemblr™ platform is applicable to clinically relevant DMPC/egg-PG/verteporfin liposomal formulations such as Visudyne®.

The NanoAssemblr™ platform provides versatile technology for liposomal drug formulations, and addresses an unmet industry need for robust formulation and scale-up of highly hydrophobic drugs.