Micellar encapsulation of hydrophobic drugs to prevent their adsorption on extracorporeal membrane oxygenation (ECMO) circuits and to increase drug bioavailability

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Introduction

Extracorporeal membrane oxygenation (ECMO) is a life-saving cardiopulmonary bypass device used in patients with heart and lung failure (**Fig.1**).

- Patients on ECMO receive numerous drugs. [1] Dosing is often unknown because the ECMO circuit components can adsorb drugs [2]
- The adsorption is driven primarily by hydrophobic and electrostatic interactions [3]
- Mortality of ECMO-patients often exceeds 40% and is suspected to be due, in part, to significantly altered drug disposition by the ECMO circuit, resulting in suboptimal dosing [4]

Poloxamer 407 (P407) is a triblock copolymer consisting of a central hydrophobic block of Poly(propylenglycol) (PPG) flanked by two hydrophilic blocks of poly(ethylene glycol) (PEG).

- P407 spontaneously assembles to micelles in aqueous solutions, where PPG forms the hydrophobic core and PEG a hydrophilic shell
- Hydrophobic drugs or drugs with poor water solubility can be encapsulated in micelles, the hydrophilic shell will avoid the interactions of drugs with the hydrophobic or charged surfaces

Propofol was selected as a model drug because it is widely used in ECMO patients, highly adsorbed [5] and has favorable physicochemical properties for micellar encapsulation.

We aim to reduce drug adsorption by the ECMO-system by micellar encapsulation, increase overall drug-bioavailability and optimize dosing. (**Fig.2**).

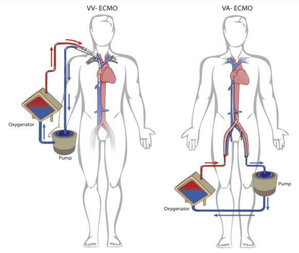

Figure 1: ECMO modalities [7]
VV: venovenous, for respiratory support,
VA: venoarterial, for circulatory and/or respiratory support

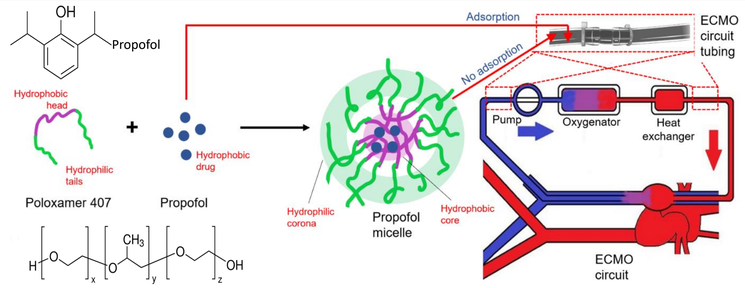

Figure 2: Drug adsorption by the ECMO circuit is avoided by micellar encapsulation.

Method

- The **Thin-Film Method** was utilized for micelle manufacturing (**Fig.3**)
- Physiochemical properties such as hydrodynamic radius, polydispersity (PDI), zeta potential (ZP) and micelle morphology were determined by Dynamic Light Scattering (DLS) & Scanning Electron Microscopy (SEM), respectively
- Propofol was quantified by reverse phase HPLC

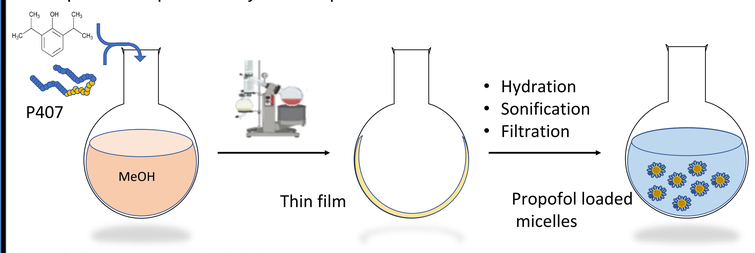

Figure 3: Thin-film method: P407 & Propofol are dissolved in MeOH. A rotary evaporator is used to form a homogenous thin film inside the round bottom flask. After rehydration with an aqueous medium micelles are formed.

Results

- The **hydrodynamic radius** and **PDI** for drug-free micelles were 20-23 nm and 0.200, respectively
- The size remained constant at different concentrations
- An increase in size was observed after incorporation of propofol (**Fig.4a**)
- The PDI dramatically decreased to as low as 0.04 (**Fig.4b**)
- 2% (w/v) P407 micelles were homogenously formed in the presence of 1 mg/ml propofol but showed a strong increase in size and PDI when 10 mg/ml propofol was used. (**Fig.4a/b**)
- With increasing the P407 concentration, 10 mg/ml propofol also resulted in lower PDI values

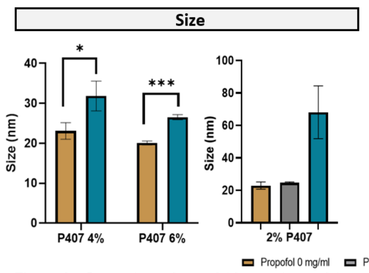

Figure 4a: Comparison of propofol free and propofol loaded micelles in size. Data represents mean ± SD.
p ≤ 0.05 *, p ≤ 0.01 **, p ≤ 0.001 ***

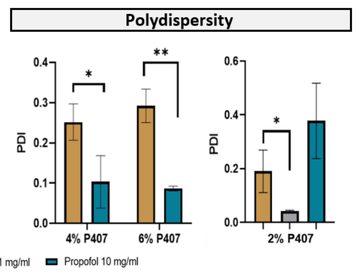

Figure 4b: Comparison of propofol free and propofol loaded micelles in polydispersity. Data represents mean ± SD.
p < 0.05 *, p ≤ 0.01 **, p ≤ 0.001 ***

- The **zeta potential** of propofol free micelles was -5.0 mV. After incorporation of propofol the ZP slightly increased. (**Fig. 5**)
- Micelles were stored for 30 days at 4°C. The PDI and size remained constant

P407	ZP
2%	-1.42 mV
4%	-0.33 mV
6%	-0.17 mV

Figure 5: ZP of Propofol loaded micelles (10 mg/ml).

- Stability upon lyophilization was evaluated:

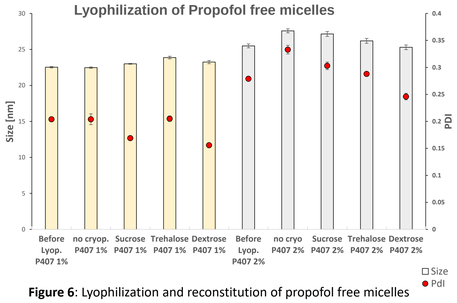

Figure 6: Lyophilization and reconstitution of propofol free micelles with different cryoprotective agents. Dextrose showed the best results after reconstitution. Data represent the mean ± SD.

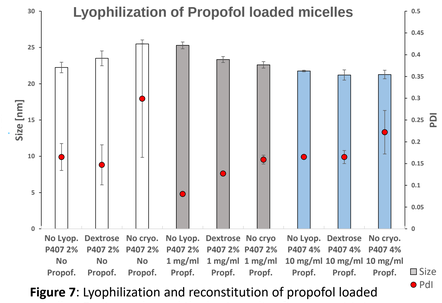

Figure 7: Lyophilization and reconstitution of propofol loaded micelles. Dextrose showed the best results after reconstitution. Data represent the mean ± SD.

- The PDI increased after reconstitution when no cryoprotectant was used. Cryoprotectants such as sucrose, dextrose and trehalose stabilized the micelle properties during the lyophilization process. (**Fig.6**)
- Dextrose performed best in maintaining size and PDI of propofol free and loaded micelles.(**Fig.6/7**)

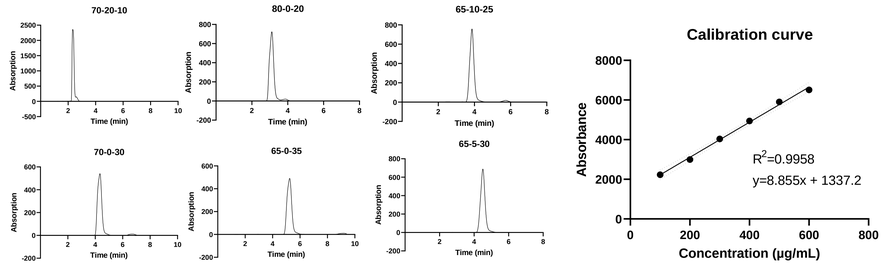

Figure 8: HPLC method implementation with variating the mobile phase composition. ACN-MeOH-H_2O.

- A reverse phase/UV-HPLC method was implemented for propofol quantification
- The mobile phase consisted of Acetonitrile (ACN) – Methanol (MeOH) – Water (H_2O)
- Different mobile phase ratios were tested to optimize the peak resolution
- A proportion of 65-5-30 % was selected and a calibration curve was created. (**Fig.8**)

SEM-Imagines: SEM images were taken from propofol free & propofol loaded micelles.

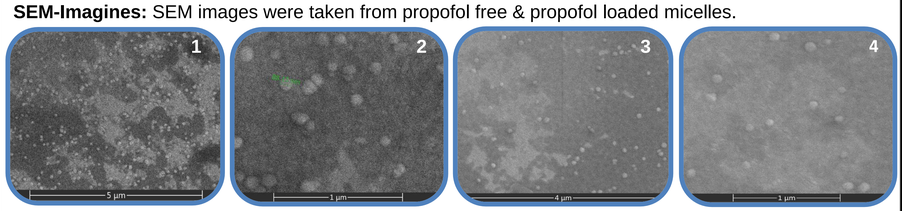

Figure 9: SEM images: Propofol loaded P407 micelles (1&2), Propofol free micelles (3&4), P407 = 4% (w/v) Propofol 10 mg/ml

Conclusion

- Micelles were successfully formulated and loaded with propofol
- Reproducible results for physicochemical micelle properties were demonstrated
- The incorporation of propofol dramatically reduced the PDI of the formulation indicating lower polydispersity of the propofol loaded micelles
- The size of the micelles increased significantly after the incorporation of propofol
- 10 mg/ml propofol was incorporated in 4 & 6% P407 micelles
- Morphology of propofol loaded and free P407 micelles was demonstrated by SEM
- By optimizing the mobile phase composition, were able to quantify propofol via HPLC to set up the analytical backbone for subsequent experiments

Future directions

To investigate the encapsulation efficiency, drug loading capacity and release kinetics of propofol, followed by ex-vivo studies with the ECMO device to compare adsorption between free and micellar propofol. (**Fig.10**)

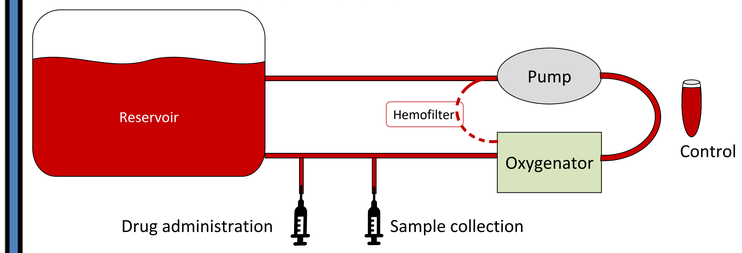

Figure 10: Ex-vivo studies, experimental design. ECMO parts like pump and oxygenator can be excluded from the circuit so drug adsorption by the individual ECMO-part can be determined.

Acknowledgements

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