Distinguish Coexistence of Nanoemulsion and Liposome in Propofol by Cryogenic Transmission Electron Microscopy

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INTRODUCTION

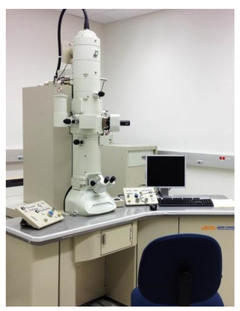
Propofol (2,6-diisopropylphenol) is an intravenously administered drug for the induction and maintenance of general anesthesia or sedation. It is formulated as an oil-in-water emulsion stabilized by egg lecithin phospholipids. Addition of an excessive amount of phospholipids in the formulation will, in practice, give rise to the formation of liposome structures (i.e., vesicular structures formed of a bilayer of phospholipid). If emulsion and liposome particles coexist in injectable propofol emulsion formulations, the two distinct contents would experience different metabolic processes upon injection and it would impact on the understanding of drug delivery mechanism of propofol. Currently, the presence, amount, morphology and potential impact of these structures are relatively unknown.

Transmission electron microscopy provides high resolution (~0.2 nm) and a broad range of operational magnifications. However, routine sample preparation procedure such as chemical fixation, rinsing and dehydration has long been recognized to introduce significant artifacts on sample morphology. In this study, we aim to investigate the use of high-resolution cryogenic transmission electron microscopy (cryo-TEM) to evaluate the size distribution and complex compositions of drug carriers in four propofol injectable emulsions. By rapid freezing, cryo-TEM can preserve the propofol colloidal suspensions in a frozen hydrated state and enable the direct visualization of the fine structures of propofol products.

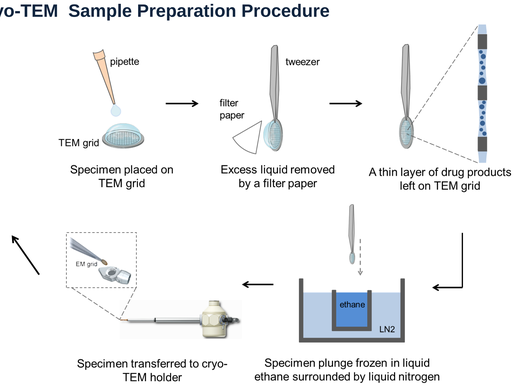
EXPERIMENTAL

- **Cryo transmission electron microscopy (cryo-TEM) was performed using a Jeol 1400 TEM/STEM equipped with a Leica EM GP grid plunge freezer.**
- **Dynamic light scattering (DLS) and zeta potential were performed using a Malvern Zetasizer. Three separate dilutions were run with at least 5 measurements per sample. Samples were diluted 1000X with DI water and borate buffer for DLS and zeta potential, respectively.**
- **Capillary electrophoresis (CE) was performed on an Agilent 7100 Capillary Electrophoresis system. For injection samples, a volume of 1 µL propofol sample is mixed with 100 µL sodium tetraborate buffer (Agilent; 20 mM, pH 9.3) and 5 µL dimethyl sulfoxide (DMSO) solution (1%, v/v in water; electroosmotic flow marker).**

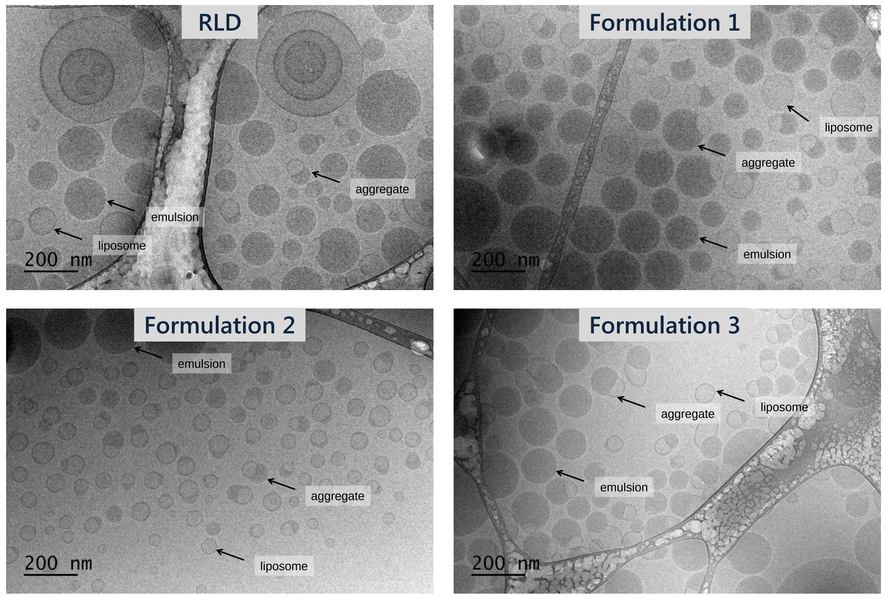
Cryo-TEM Sample Preparation Procedure



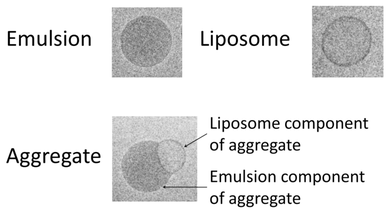
Jeol JEM 1400 TEM



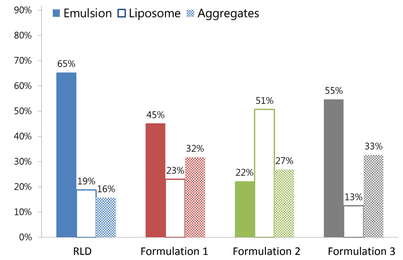
RESULTS



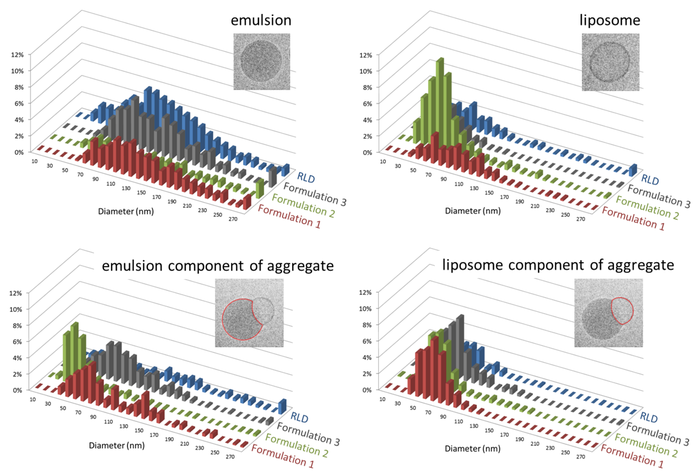
Cryo-TEM enables discrimination and measurement of nano and micro-structures within complex formulations such as the presence of both emulsion droplets and liposomes in propofol emulsion products from different manufacturers.



Proportion of Emulsion, Liposome and Aggregate



Particle Size Distribution of Emulsion, Liposome and Aggregate

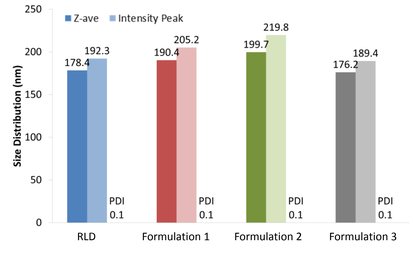


The population and size distribution of by-product structures, liposome and emulsion/liposome aggregate, varied among products.

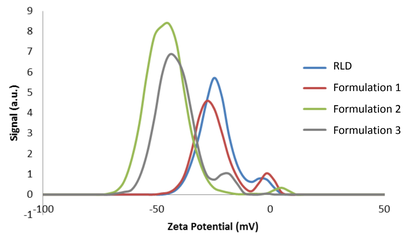
Summary Table of Proportion and Size Analysis of Emulsion, Liposome, Emulsion/Liposome Aggregates by Cryo-TEM

Particle Size (nm) /Proportion (%)	Emulsion (main carrier)	Liposome				Aggregates	
		SUV/LUV	Multilamellar	Multivesicular	Total	Emulsion component	Liposome component
RLD	124.0 ± 57.6	83.1 ± 62.9	209.7 ± 102.4	311.1 ± 89.4	98.5 ± 83.1	145.3 ± 89.8	65.0 ± 32.3
	65.4%	17.1%	1.1%	0.7%	18.9%	15.8%	
Formulation 1	147.0 ± 58.7	94.9 ± 35.1	190.3 ± 5.6	NA	96.5 ± 37.4	102.2 ± 51.2	64.8 ± 17.5
	45.2%	22.3%	0.8%	0.0%	23.1%	31.8%	
Formulation 2	168.7 ± 137.2	62.5 ± 32.2	84.8 ± 50.4	129.6	65.3 ± 35.7	52.5 ± 46.5	62.3 ± 26.9
	22.3%	44.3%	6.4%	0.1%	50.8%	27.0%	
Formulation 3	137.3 ± 79.1	68.3 ± 40.3	65.6	NA	68.2 ± 40.0	96.2 ± 59.0	65.1 ± 29.8
	54.7%	12.4%	0.2%	0.0%	12.6%	32.7%	

Hydrodynamic Size of Propofol by DLS

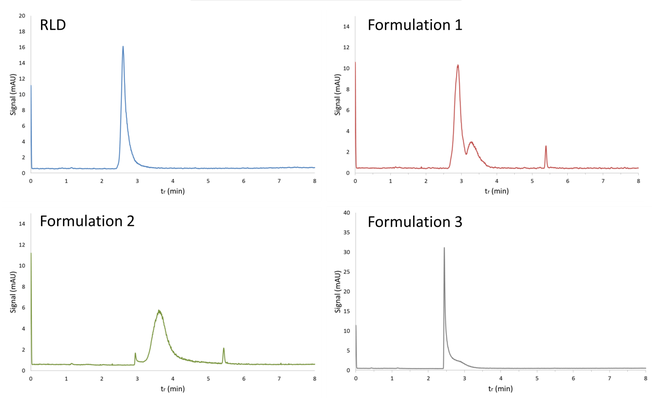


Zeta Potential Measurement



The multi-component property of propofol was not discerned by dynamic light scattering or zeta potential measurements.

Capillary Electrophoresis (CE)



- The capillary electrophoresis separation shows different retention time, indicating potential differences in particle size and/or surface charge.
- Broadened peak indicates heterogeneous particle size distribution.

CONCLUSIONS

- **Cryo-TEM results demonstrate that all propofol samples contain a mixture of oil-in-water emulsions, liposomes and emulsion/liposome aggregates.**
- **All products contain similar proportion and particle size distribution of the emulsions (the main carrier of the drug substance) whereas slight differences of the by-product structures (liposomes and aggregates) are observed.**
- **Cryo-TEM is a powerful characterization technique for analyzing complex drug products comprising coexisting morphologies.**

ACKNOWLEDGEMENTS

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