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Comparison of drug release profiles of doxorubicin liposomes prepared by different processes

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Introduction

The goal of this study is to discriminate liposome manufacturing and product compositional differences by measuring doxorubicin (DOX) release from liposomal formulations (L-DOX) using a USP-4 apparatus release assay.

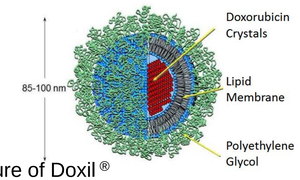

Figure1. Schematic picture of Doxil ®

	L-DOX lot #	Average size (nm)	Formulation description
MLV preparation technique effect	L-DOX-1	95.3 ± 0.6	Lipids pipetted into stirring ammonium sulfate over 10s
	L-DOX-2	82.9 ± 1.0	Lipids are high pressure injected into stirring ammonium sulfate
	L-DOX-3	83.6 ± 0.6	Lipids are poured into stirring ammonium sulfate
Extruded liposomes particle size effect	L-DOX-4	82.0 ± 0.7	Small size
	L-DOX-5	113.4 ± 0.5	Medium size
	L-DOX-6	125.5 ± 1.0	Large size
Homogenized liposome size effect	L-DOX-7	77.3 ± 0.2	Homogenized, 1 pass
	L-DOX-8	66.5 ± 0.8	homogenized, 2 passes
	L-DOX-9	65.8 ± 0.5	homogenized, 3 passes
Drug to lipid ratio effect	L-DOX-10	84.4 ± 0.7	drug to lipid ratio = 123 (g DOX per mol PL)
	L-DOX-11	85.9 ± 1.5	drug to lipid ratio = 143 (g DOX per mol PL)
	L-DOX-12	84.5 ± 0.1	drug to lipid ratio = 166 (g DOX per mol PL)
	L-DOX-13	84.2 ± 0.2	drug to lipid ratio = 186 (g DOX per mol PL)
Cooling rate following drug loading effect	L-DOX-14	86.1 ± 1.5	cooled in iced bath after drug loading
	L-DOX-15	86.2 ± 1.8	cooled from 60 ℃ to RT in water after drug loading
	L-DOX-16	85.0 ± 0.7	cooled to RT in air after drug loading
	L-DOX-17	84.9 ± 0.6	cooled to RT in water after drug loading
	L-DOX-18	84.3 ± 0.8	cooled 4 ℃ in water after drug loading
POPC-L-DOX	POPC-L-DOX	84.2	HSPC is replaced with POPC
Doxil®	Doxil®	87.4 ± 0.9	
Lipodox	Lipodox	76.2 ± 0.2	Liposomal Doxorubicin by Sun Pharma

Table1. Different DOX liposome (L-DOX) formulations used in the release study

Methods

The effect of different manufacturing processes such as extrusion vs. homogenization, liposome multi-lamellar vehicles (MLV) preparation techniques and the number of extrusion/homogenization on DOX release profiles were examined in USP-4 flow-through release assay. Doxorubicin liposomes (L-DOX) were placed in the dialysis tubes and inserted in flow-through cells of USP-4 apparatus CE7-smart (Sotax®). The release kinetics was examined at 45° C for 24h in the media containing 100 mM NH_4HCO_3, 5% w/w of hydroxypropyl-cyclodextrin (HP-CD), 75 mM MES, 5% sucrose and 0.02% NaN_3 (pH 6).

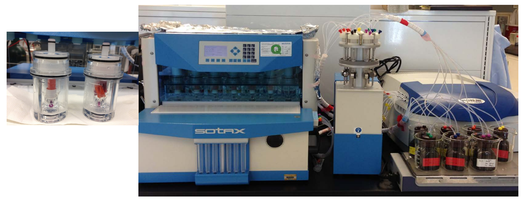

Figure 2. Picture of USP 4 apparatus CE7-smart (SOTAX ®) dissolution system

Results

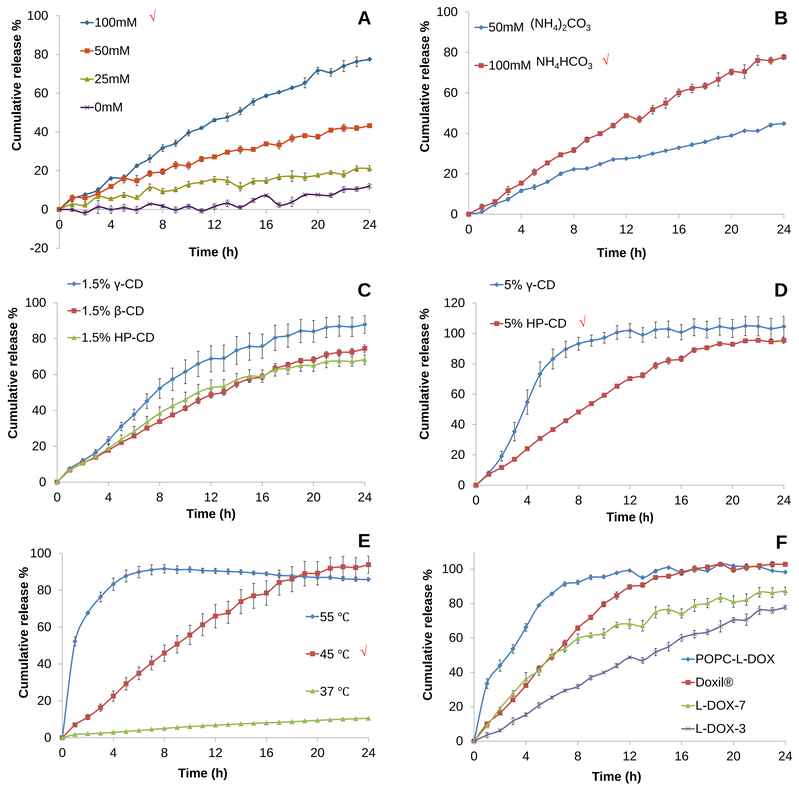

Figure 3. Optimization of condition for doxorubicin release from liposome formulations on Sotax®. **A-B.** Effect of NH_4HCO_3 concentration (**A**) and NH_4^+ salt types (**B**) on L-DOX-3 release profile; **C-D.** Effect of cyclodextrin (CD) types (**C** - 1.5%CD, **D** - 5%CD) on L-DOX-2 release profile; **E.** Effect of temperature on L-DOX-2 release profile; **F.** Release profiles of different DOX liposome formulations using the optimized release condition.

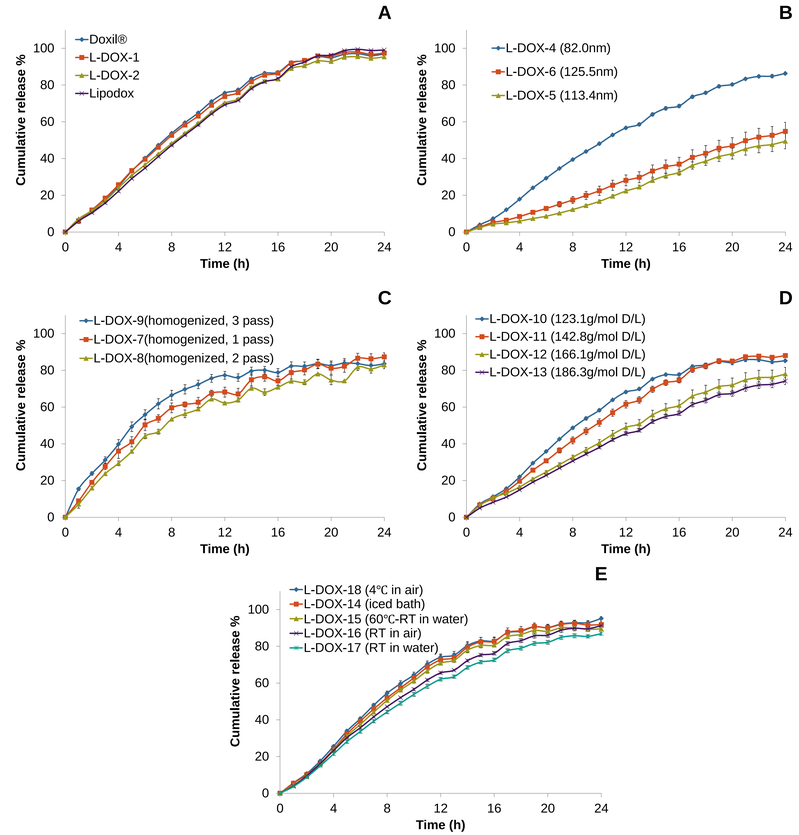

Figure 4. Comparing the release profile of DOX liposomes prepared by different manufacturing processes. **A.** Effect of MLV preparation technique; **B.** Effect of Extruded liposomes particle size; **C.** Effect of homogenized liposome size; **D.** Effect of drug to lipid ratio; **E.** Effect of cooling rate. The result revealed that, extruded liposome size and drug/lipid ratio significantly affected DOX release profiles as determined by the optimized USP-4 release assay.

Conclusion

The USP-4 release assay was used to discriminate differences in drug release from doxorubicin liposomes that were prepared by different processes. It was revealed that liposome size and drug/lipid ratio significantly affected the in vitro drug release profile using the USP-4 release assay. The established USP-4 release method can be used to distinguish possible differences between generic and innovator L-DOX and guide the design of generic products.

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