DoE-Guided Liposomal Delivery of a Novel Pyrrolobenzodiazepine for TNBC Anticancer Therapy

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INTRODUCTION

Pyrrolobenzodiazepines (PBDs) are a class of potent anticancer agents with promising therapeutic potential. However, their clinical application is often limited by **poor solubility and systemic toxicity. Liposomal encapsulation** can **enhance drug bioavailability** and **reduce off-target effects**. MH10 is a **novel PBD** with **potent cytotoxicity against TNBC**, making it a promising targeted therapy candidate.

HYPOTHESIS

Liposomal encapsulation of MH10 **enhances intracellular delivery** and **sustained exposure** in TNBC cells, thereby **increasing uptake-dependent cytotoxicity** and **improving *in vivo* antitumour efficacy and survival** compared to free MH10.

1. PBD and CTX Cytotoxicity Study in 4T1 Cells

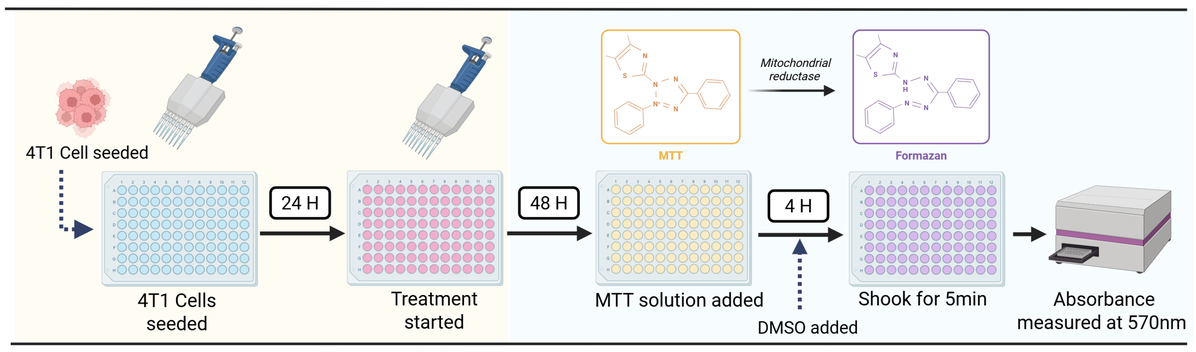
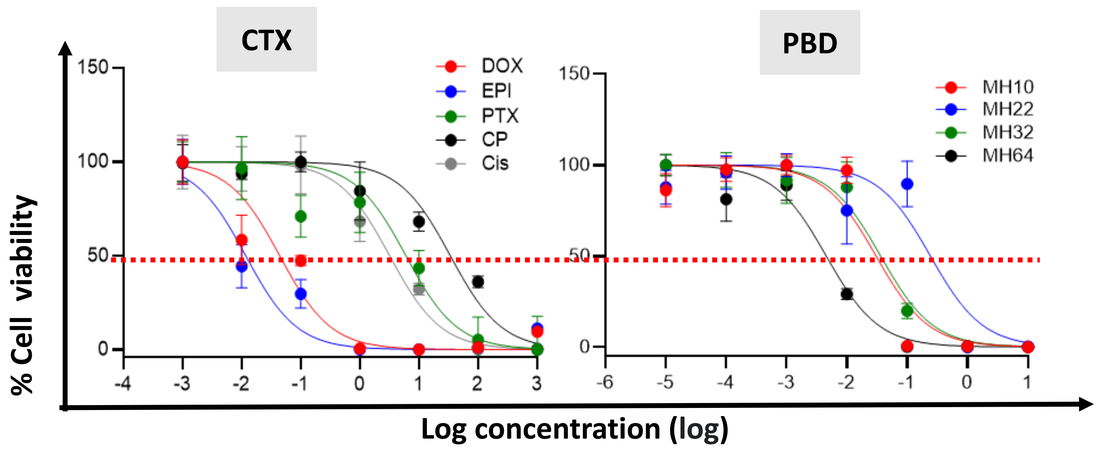


Figure 1: MTT assay for cytotoxicity study



Compounds	DOX	EPI	CP	Cis	PTX	MH10	MH22	MH32	MH64
µM	0.13	0.2	27.73	3.01	7.51	0.03	0.242	0.036	0.005

- **Most PBDs demonstrated superior potency compared to conventional CTX**

2. Liposomal MH10 Formulation and DoE

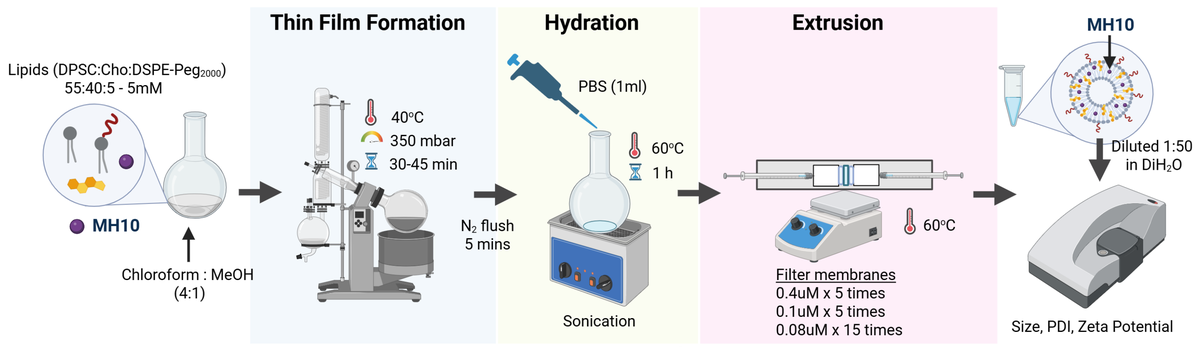


Figure 2: Liposomal MH10 formulation process

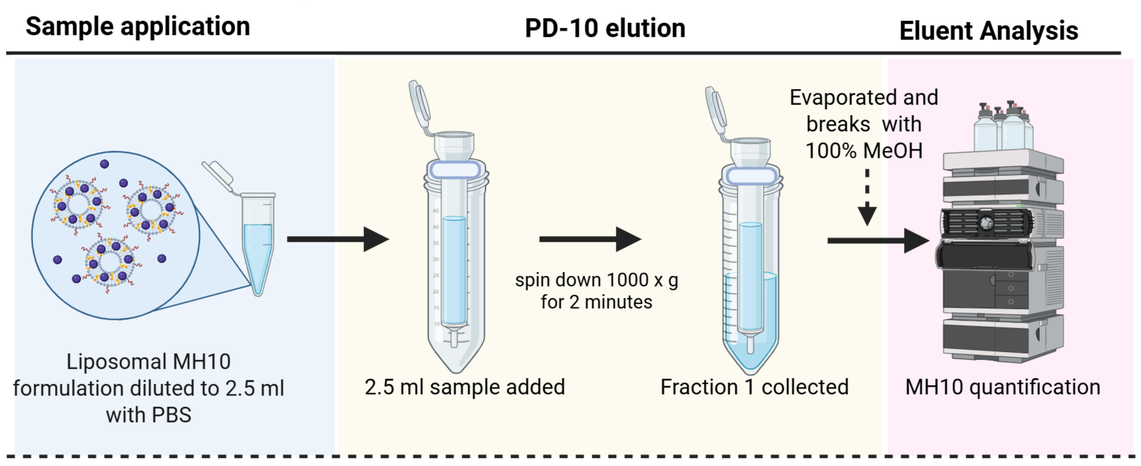
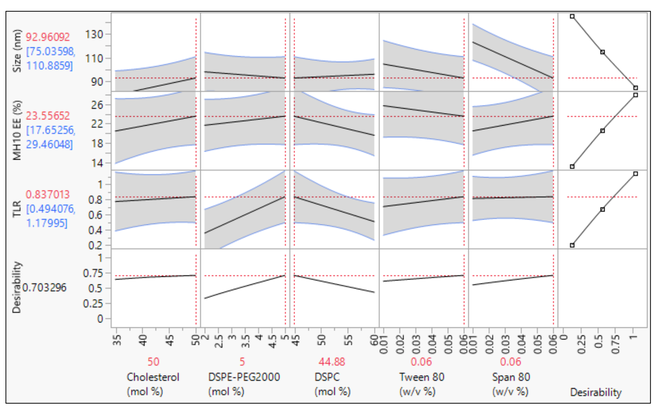


Figure 3: Purification of liposomal MH10

Formulation	Size ± SD	PDI ± SD	ζ-potential ± SD	EE (%) ± SD
Empty liposome	94.35±1.44	0.08±0.01	-25.85±1.12	
Lip-MH10	107.5±3.14	0.19±0.02	-20.94±0.49	20.40±9.47

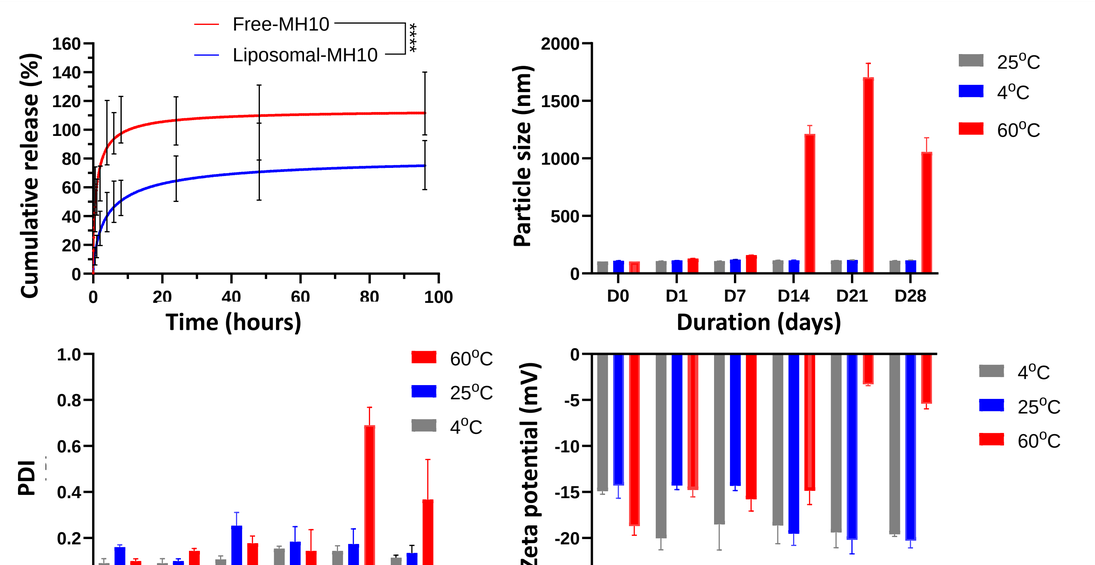


Optimized formulation				
DSPC (mol %)	DSPE-PEG2000 (mol %)	Chol (mol %)	Tween 80 (v/v %)	Span 80 (v/v %)
45	5	50	0.06	0.06

Figure 4 : The Prediction Profiler shows the effects of formulation factors using JMP software.

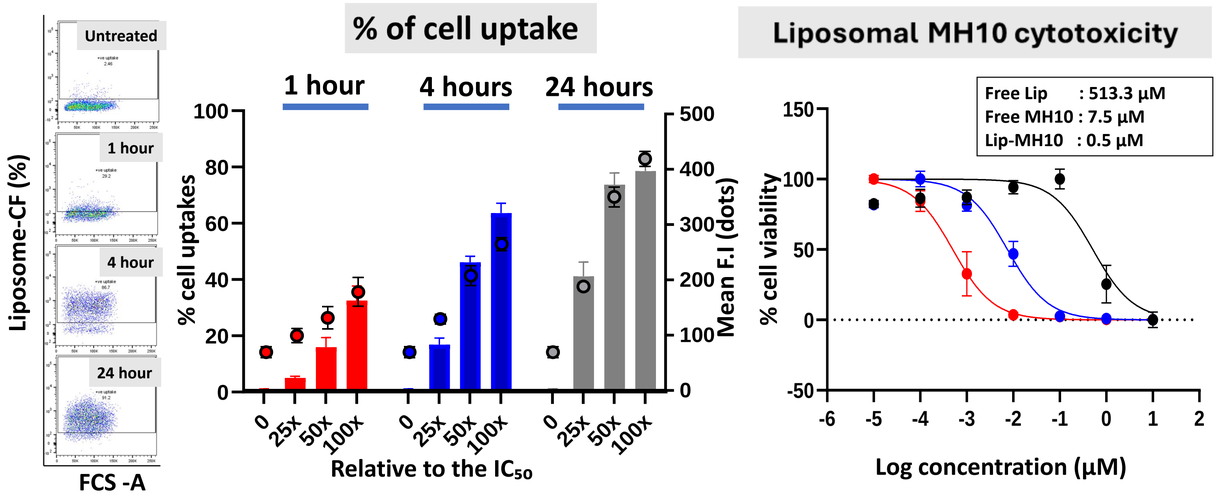
- **The optimized formulation achieved a particle size below 120 nm, narrow size distribution, an encapsulation efficiency of ~ 20%, and a drug loading of ~ 3%.**

3. Lip-MH10 Release and Stability Profile



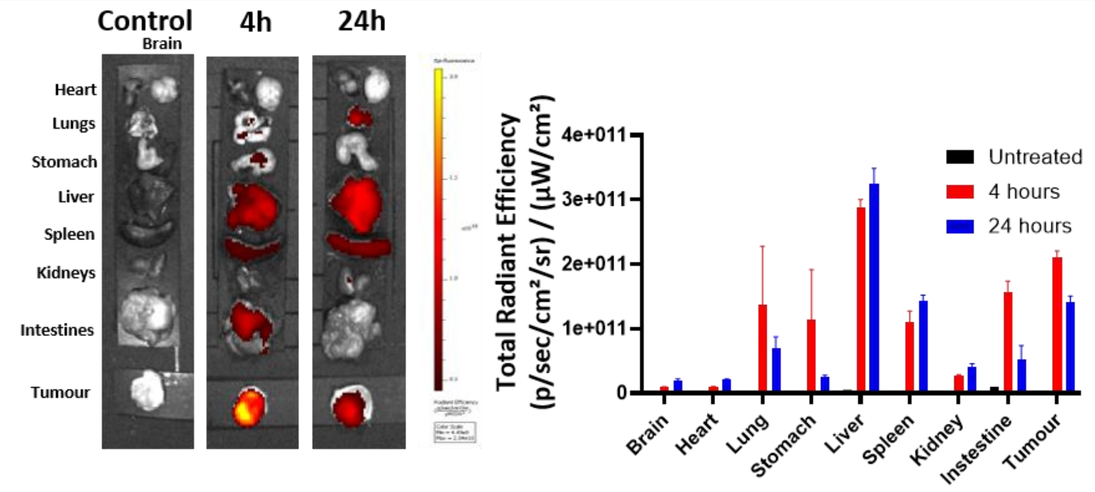
- **Lip-MH10 showed sustained release and good stability over 28 days.**

4. Lip-MH10 Cytotoxicity and Cells Uptake in 4T1 Cells



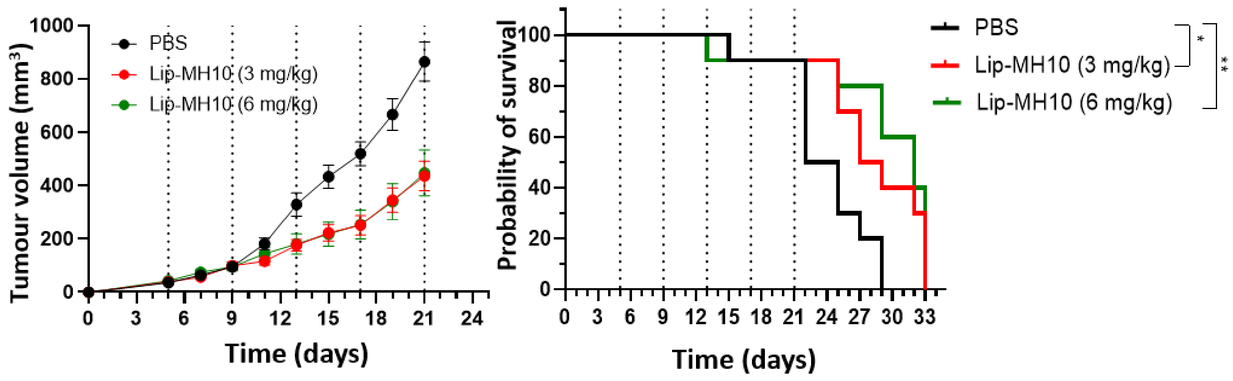
- **~ 80% of liposome were internalized at 4 and 24 hours and lip-MH10 retained anticancer activity after 48 h treatment.**

5. Liposomes Biodistribution in Major Organs at 4 and 24h



- **Preferential tumour accumulation with concurrent liver and spleen uptake.**

6. *In vivo* Antitumour Efficacy and Survival in 4T1 Model



- **Lip-MH10 delayed tumour progression and improved overall survival**

6. Conclusion

The DoE-guided liposomal MH10 formulation showed sustained release, efficient cellular uptake, and antitumour activity in the 4T1 TNBC model, supporting further preclinical studies.