University of Mississippi

eGrove

Annual Poster Session 2020 | Annual Poster Session

10-23-2020

R16. Formulation and Evaluation of Doxorubicin HCl Nanoliposomes by Ethanol Injection Method

Arun Kumar Kotha
University of Mississippi, akotha@olemiss.edu

Bhavani Prasad Vinjamuri
University of Mississippi

Mahavir B. Chougule
University of Mississippi

Follow this and additional works at: https://egrove.olemiss.edu/pharm_annual_posters

Recommended Citation

Kotha, Arun Kumar; Vinjamuri, Bhavani Prasad; and Chougule, Mahavir B., "R16. Formulation and Evaluation of Doxorubicin HCl Nanoliposomes by Ethanol Injection Method" (2020). *Annual Poster Session 2020*. 16.
https://egrove.olemiss.edu/pharm_annual_posters/16

This Book is brought to you for free and open access by the Annual Poster Session at eGrove. It has been accepted for inclusion in Annual Poster Session 2020 by an authorized administrator of eGrove. For more information, please contact egrove@olemiss.edu.

Formulation and Evaluation of Doxorubicin HCl Nanoliposomes by Ethanol Injection Method

Arun Kumar Kotha[a], Bhavani Prasad Vinjamuri[a], Mahavir B. Chougule[a,b]

[a]Department of Pharmaceutics and Drug Delivery, School of Pharmacy, The University of Mississippi. Oxford, MS. 38677, USA

[b]The Research Institute of Pharmaceutical Sciences, School of Pharmacy, The University of Mississippi. Oxford, MS. 38677, USA.

PURPOSE

Nanoliposomes (NLs) are complex systems, which have tremendous therapeutic applications for treating various diseases when compared to conventional drug delivery systems. Doxorubicin HCl (Dox) is anthracycline antibiotic which exerts antineoplastic activity against numerous human cancers. It acts by intercalating between base pairs in DNA which prevents DNA replication and results in inhibition of protein synthesis. Doxil® approved by FDA in 1995 and is used to treat ovarian, multiple myeloma and Kaposi sarcoma. Different methods are available to formulate NLs i.e., thin-film hydration method, coaxial turbulent jet in co-flow and continuous manufacturing microfluidic hydrodynamic flow focusing method, among which industrially applicable ethanol injection method offer several advantages such as it is easy to scale up, rapid, form small size NLs with narrow size distribution, and this method doesn't cause lipid degradation and oxidative alterations.

HYPOTHESIS AND OBJECTIVE

- The hypothesis of the project is the development of Doxorubicin HCl (Dox) loaded PEGylated (peg) nanoliposome (NL) formulation using endogenous lipids and scalable industrially applicable ethanol injection method will produce NLs in range of 50 to 150 nm.

- Analysis of Stability, entrapment efficiency and drug leakage.

- Study the thermal behavior of lipids and cholesterol by using Differential scanning calorimetry.

METHOD(S)

Formulation of Dox-pegNL: The 191.6 mg of DPPC, 63.8 mg of cholesterol (Chol) and 63.8 mg of mPEG-2000-DSPE were dissolved in ethanol (90%) with constant stirring (600 rpm) at 65°C. The lipid mixture was slowly injected at a rate of 1ml/min into the ammonium sulphate solution (250mM). The formed liposome suspension stirred for 30min at 600 rpm while maintaining the temperature at 65°C. The liposome suspension subjected to extrusion process through a mini-extruder at 65°C. In active drug loading step, Dox was dissolved in 10% sucrose solution at 60°C with continuous stirring. Sufficient amount of histidine (10mM) was added. After 25 min. incubation, liposomes cooled to a temperature of 2 to 8°C and incubated for an hour. Finally, pH was adjusted to 6.65 using NaOH/HCl.

Particle size and Zeta Potential of Dox-pegNLs was measured using Malvern Zetasizer Nano ZS.

Entrapment efficiency: Dox-pegNLs (2ml) was centrifuged using Amicon ultra filters (10kDa) at 13000 rpm for 15min. The eluent was collected and analyzed for Dox concentration by using Fluorescence spectrometry .

Stability and drug leakage: The Dox-pegNLs stability at 37°C in Phophate buffered Saline (PBS), pH 7.4 for 24 h was analyzed using size, zeta potential. Drug leakage was analyzed at 37°C in PBS buffer (200µl of PBS + 800µl of Dox-pegNL) at different pH of 5.5, 6.5, and 7.5 for 24 h. Dox leakage was estimated by entrapment efficiency method.

Calorimetry studies : Approximately 4 mg of DPPC or Chol samples, "as is" and dried, were weighed into an open aluminum pan or an aluminum pan that was subsequently nonhermetically crimped closed. DSC studies were performed by heating the samples at 8°C/min from -40°C to 250°C. Nitrogen was used as the purge gas with a flow rate of 40 mL/min.

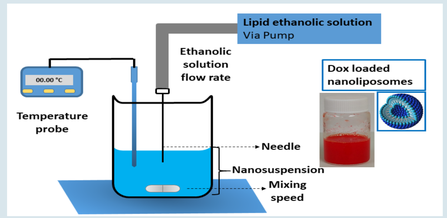

Figure 1: Preparation of Doxorubicin HCl loaded NLs by ethanol injection method

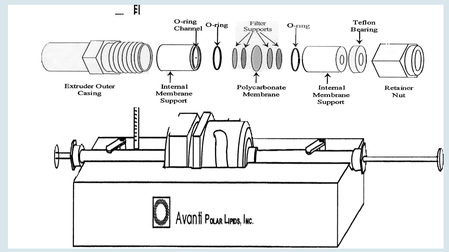

Figure 2: Mini-Extruder set up used for Nanoliposomes size reduction

RESULT(S)

- The developed NLs showed particle size in the range 100nm (102.10nm ± 0.44) and PDI of 0.06 ± 0.02. Zeta Potential of the NLs was 4.64 ± 11.48mV
After active drug loading, particle size increased to 387.43nm ± 50.11, with PDI of 1 and Zeta Potential 17.20mV

- Extrusion of NLs was optimized using 0.1µm Whatman filter followed by 0.05µm Whatman filter.

- Dox-pegNL showed high drug entrapment efficiency which is above 90% (91.45%)

- Dox-peg NLs are stable at pH 7.5 in PBS Buffer for 24hrs

- Drug leakage from liposomes was below 2.5% in all three pH (5.5, 6.5 and 7.5) PBS buffers conditions which shows that formed Dox-peg NL are stable

- Tm for DPPC was 61.25°C and Cholesterol : ~36.25°C.

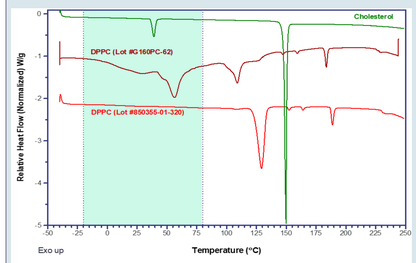

Fig 3: DSC overlays of two different lots of DPPC and Cholesterol differential scanning calorimetry thermograms

	Before extrusion	After extrusion	Final Dox-peg NL
Particle size	784.60 ± 4.40 nm	102.10 ± 0.44 nm	387.43 ± 50.11 nm
Zeta potential	1.49 ± 10.47 mV	4.64 ± 11.48 mV	17.20 ± 1.75 mV
Polydispersity index	0.48 ± 0.02	0.06 ± 0.02	1.0

Table 1: Particle size, zeta potential and PDI of Dox-pegNLs during the formulation development

	pH 5.5	pH 6.5	pH 7.5
Particle size	111.77 ± 1.33 nm	132.17 ± 2.42 nm	139.13 ± 1.50 nm
Zeta potential	11.35 ± 2.63 mV	7.63 ± 0.90 mV	5.62 ± 1.0 mV
Polydispersity index	0.14 ± 0.01	0.32 ± 0.05	0.34 ± 0.01
Drug leakage	2.06%	1.87%	2.4%

Table 2: Dox-peg NLs , Particle size, zeta potential, PDI and drug leakage at different pH (5.5, 6.5 and 7.5) of PBS buffer.

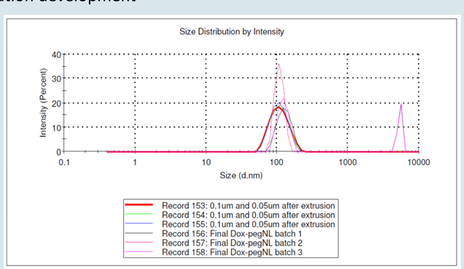

Figure 4: Particle size distribution after extrusion and after Dox loading

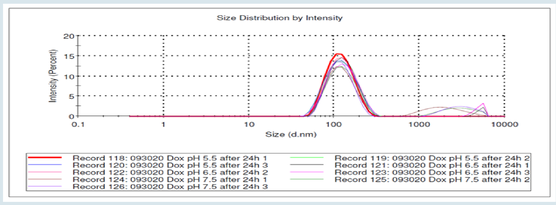

Figure 5: Particle size distribution of Dox-pegNL at different pH

CONCLUSION(S)

- Dox-pegNLs produced by ethanol injection method. Method needs to be optimized

- Entrapment efficiency of Dox-pegNLs is very high

- Dox-pegNLs Stable in PBS buffer at pH7.5 and integrity of NLs maintained at different pH PBS buffers

FUTURE WORK:

- In vitro and in vivo studies needs to be performed to evaluate Dox-pegNLs

- Stability in Human plasms at 37°C for 24hrs going to be analyzed using size, zeta potential and drug release rate

- DSC studies are going to be performed on liquid Dox-pegNL formulation.

REFERENCES:

Ming Xie, Yuxiang Chen, Lixiang Wu. Preparation of Doxorubicin-Hydrochloride Nanoliposomes by Ethanol injection-pH Gradient Method and Their Safety Evaluation. J Nanosci Nanotechnol. 2013;13(1):216-21.

Wagner, A, Platzgummer, M. Kreismayr, G. Quendler, H. Stiegler, G. Ferko, B. Vecera, G, Vorauer-Uhl, K, Katinger, H.GMP production of liposomes--a new industrial approach. *J Liposome Res* **2006,** *16* (3), 311-9.

ACKNOWLEDGEMENTS:

Author would like to thank Department of Pharmaceutics and Drug Delivery, School of Pharmacy, University of Mississippi, University, MS, USA.