Exploring an Electroanalytical Method to Determine Drug Release from Liposomal Doxorubicin HCl

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Purpose

Develop an automated electroanalytical technique to measure in vitro drug release of doxorubicin from liposomal formulation.

Introduction

Liposomes are spherical colloidal vesicles containing one or more phospholipid bilayers, which are some of the most promising drug carriers in drug delivery applications for more than 50 years.

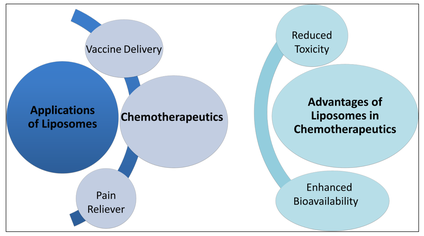

Figure 1. Applications and advantages of liposomal drug delivery

Liposomal doxorubicin hydrochloride (DOXIL) is the first anticancer liposomal formulation approved by U.S. FDA. It is a coffee bean shaped unilamellar and pegylated vesicle, remotely loaded with the doxorubicin, and having size range of 80-90 nm. The lipid membrane consists of hydrogenated soy phosphatidylcholine (HSPC), cholesterol, and polyethylene glycol linked distearoyl-phosphatidylethanolamine (mPEG2000-DSPE). Doxorubicin-HCl is loaded using an amomum sulfate gradient and the doxorubicin is contained inside the liposome as doxorubicin sulfate crystals.

Drug release profiling of liposomal formulations often requires a separation step such as dialysis or solid phase extraction to quantitate released active pharmaceutical ingredient (API) without interference from the liposome bound API. However, these separation methods are lengthy and may cause an artificial drug concentration gradient or liposome rupture, resulting in inaccurate quantitation of released drug. We developed an electroanalytical method for the continuous and direct quantitation of drug released from liposomes without additional separation steps.

Advantages of Electroanalytical Method

- Does not require additional sample preparation and separation step including dialysis, filtering and solid phase extraction
- Continuous monitoring at selected time intervals
- Quick response time (instantaneous quantitation)
- Requires buffer solution without organic solvent (green method)

Disadvantage of Electroanalytical Method

- API must be redox active.

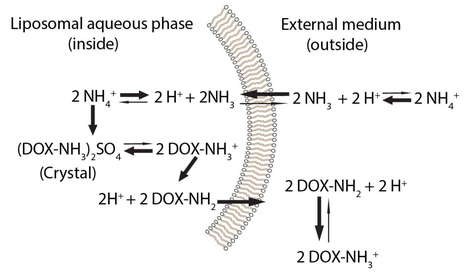

Figure 2. Illustration of ammonia induced drug release.

Materials and Methods

DOXIL (Liposomal Doxorubicin, Baxter Healthcare Corp.) and two generic formulations (Manufacturers: Dr. Reddy's Laboratories Inc. and Sun Pharmaceuticals.) were used as model liposome products in this project.

All measurements were carried out using CHI 630D bipotentiostat with three electrode system comprising of a glassy carbon disc electrode (Pine Instrument Inc., PA), Ag/AgCl electrode (BASi Corporate, IN), and a Pt wire (Pine Instrument Inc., PA) were used as a working electrode, reference electrode, and as a counter electrode, respectively. The sample compartment is a 10 mL water jacketed electrochemical cell (Pine Instrument Inc., PA) attached to the water bath circulator for temperature control. Water saturated nitrogen was used for the removal of oxygen from release buffer and headspace prior to introduction of liposomal suspension.

The redox reaction takes place in the quinone group of the released doxorubicin, which can be monitored using square wave voltammetry. All measurements were carried out using three electrode system.
200 mM ammonia formate was used to induce drug release.

The free drug concentrations were measured in every thirty minutes. Total encapsulated drug is measured by disrupting liposomes using Triton X 100.

The percent released is calculated by free drug concentration divided by total encapsulated drug concentration.

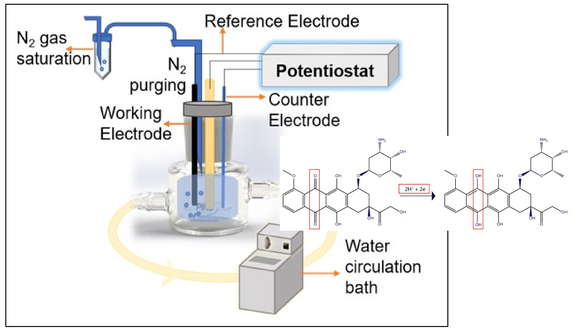

Figure 3. Illustration of the in-vitro drug release/dissolution set up and Illustration of quinone group redox reaction.

Results and Discussion

- In vitro drug leakage test conditions was chosen according to FDA Draft Product-specific Guidance for doxorubicin HCl liposomes.
- Drug release is induced by addition of ammonium formate and only uncharged molecules (Dox- NH_2 and NH_3) can translocate membrane.
- Uncharged ammonia (NH_3) concentration depends on pH conditions (pKa of NH_4^+ = 9.3) and increases with pH.
- At 37°C, the drug release increased from 7% to 40% at 24 hours when the pH increased from 5.5 to 7.4, indicating higher pH enhances the drug release.
- Over 80% drug release was obtained at pH 6.5 and 52°C in about 3 hours as shown in Figure 5.
- The brand name and the two generic formulations showed similar drug release profile in all experimental conditions.

Table 1. Temperature and pH-dependent drug release of reference formulation (mean ± SD, N=3)

Temperature	37°C	47°C	52°C
pH	24h	24h	15h
5.5	11.76 ± 4.13 %		
6.5	33.57 ± 4.56 %	82.54 ± 5.47 %	94.74 ± 10.44 %
7.4	52.79 ± 6.99 %		

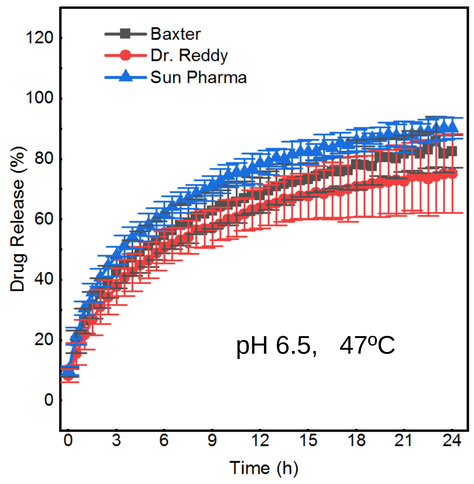

Figure 4. Drug release profiles of three different formulations of the liposomal doxorubicin HCl at pH 6.5 and 47°C (mean ± SD, N=3)

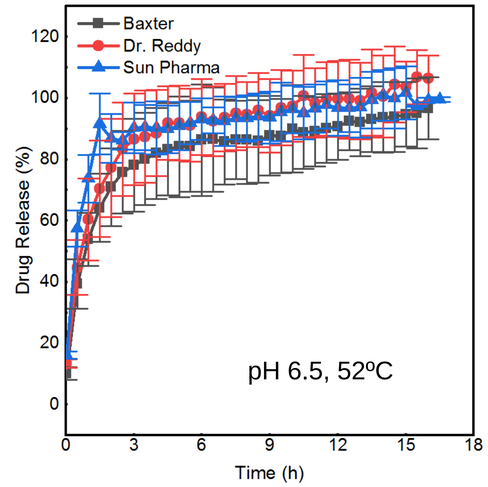

Figure 5. Drug release profiles of the three different formulation of the liposomal doxorubicin HCl at pH 6.5 and temperature of 52°C. The total dug release for refence, Dr. Reddy and Sunpharma formulations were 94.74 ± 10.44 %, 103.66 ± 8.25 % and 101.79 ± 8.67 respectively at 15 hours. (mean ± SD, N=3)

Conclusion

- In vitro drug release of liposomal doxorubicin was continuously monitored by using a square wave voltammetry electroanalytical method.
- As expected, total drug release was increased as the temperature increased.
- Complete drug release was achieved at pH 6.5 and 52°C .
- This method may be further applied in other liposomal formulations containing redox-active drug substances.

Acknowledgement and Disclaimer

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