Doxorubicin HCl Release from Liposomal Doxorubicin Formulations Autonomous Capillary Electrophoretic (CE) In Vitro Release Test (IVRT) Method

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Purpose

The objective of the current work was to develop an automated IVRT method based on real time quantitation of released doxorubicin from liposomal encapsulated doxorubicin without additional sampling and separation steps.

Introduction

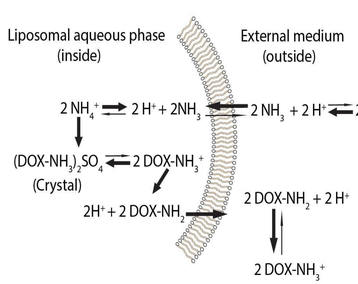
Liposomes are one of the common, widely investigated drug delivery systems, having reduced toxicity and enhanced circulation time through evading the immune system in some cases.

Liposomal Doxorubicin Hydrochloride is one of the extensively studied pegylated liposomal chemotherapy medications to treat Ovarian cancer, AIDS-related Kaposi's Sarcoma and Multiple myeloma. In 1995, U. S. Food and Drug Administration (US-FDA) approved the first liposomal doxorubicin HCl formulation, DOXIL®, by Baxter Healthcare Corporation and currently five generic formulations are available in the U.S. market.

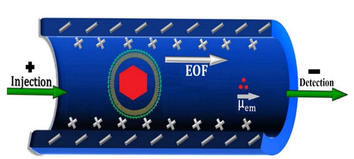
In vitro drug release test (IVRT) is a critical quality control method in both premarket and post-approval evaluation of liposomal drug products. Most IVRTs for liposomes require a separation step such as filtering, dialysis and solid phase extraction for selective quantitation of released active pharmaceutical 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 in addition to being labor intensive.

We employed a high-resolution analytical technique, capillary electrophoresis (CE) that separates ions based on their electrophoretic mobility with the use of an applied voltage.

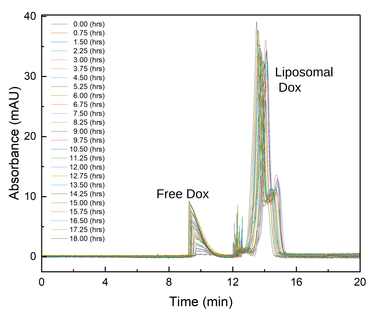
- ❖ CE requires only nanoliters of injection volume and can rapidly separate and detect liposomal doxorubicin from free doxorubicin in-situ with the use of UV-Vis detector in one step.
- ❖ IVRT coupled with CE can be easily automated.
- ❖ Compared to other techniques used for Doxorubicin HCl release measurement, CE separation and quantification is very reliable, selective, sensitive with minimum to no interferences, making it an efficient technique.



NH_4^+ Assisted Doxorubicin Release from Liposome



Encapsulated and Free Doxorubicin separation



Electropherogram for drug release from liposomal formulation (Sun Pharma) at 37°C and pH 6.5

Figure 1. Illustration of the CE based IVRT process

Materials and Instrumentation

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

All the Capillary Electrophoresis experiments were performed using Agilent CE 7100 (Agilent technologies, Santa Clara, CA, USA) which was equipped with diode array optical absorbance detector. A high sensitivity detection cell (Agilent Technologies, Germany) was used for the UV-Vis detection. Experiment samples were carried out on fused silica capillary (Agilent Technologies, Santa Clara, CA, USA) with an internal diameter of 75 µm and with a total length of 71.5 cm (63 cm to the detector window).

Methods

The background electrolyte (BGE) solution consists of sucrose, PEG and phosphate at pH 6. The background electrolyte solution was heated to 40 °C and vacuum degassed. The fused silica capillary was cleaned using a home-made cleaning solution. The capillary was conditioned with background electrolyte solution for 30 min prior to the analysis.

The sample was hydrodynamically injected and the capillary temperature was maintained at 15°C. For capillary electrophoresis separation of the sample, 30 kV positive polarity voltage was applied for 20 mins and the absorbance monitored at 491 nm. The in-vitro drug release was automated, and the data were collected for 24 hours in 45 mins intervals continuously.

Doxorubicin HCl Release

The optimized release buffer consists of sucrose, L-histidine and ammonium formate at various pH (5.5, 6.5 and 7.4). For each in-vitro release, 200 µM liposomal Doxorubicin HCl was prepared in release buffer while maintaining a total sample volume of 0.50 mL.

The release data were collected at three different temperatures (37 °C, 47 °C and 52 °C) by maintaining the vial temperature at the specified values. The in-vitro liposomal Doxorubicin HCl drug release was repeated (3x) for each analytical condition.

Results

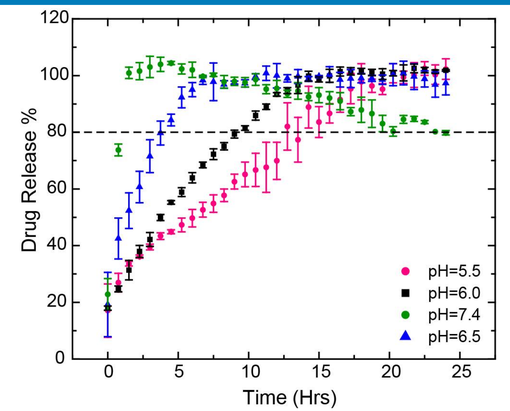


Figure 2. Drug release profiles of the Sun Pharma formulation of the liposomal doxorubicin HCl at 47°C and different pH release mediums (pH 5.5, pH 6.0, pH 6.5 and pH 7.4). Greater than 80% drug release for pH 5.5, 6.0, 6.5 and 7.4 were achieved at 13 Hrs, 9 Hrs, 4 Hrs and 1 Hr respectively (mean ± SD, N=3).

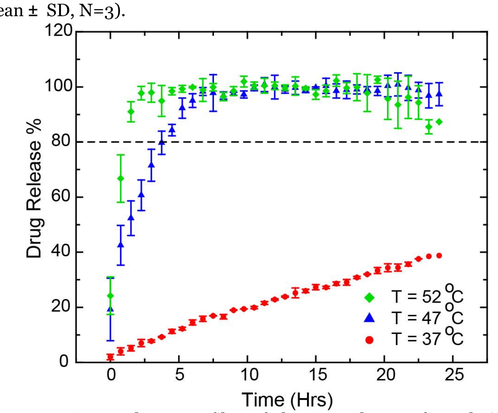


Figure 3. Drug release profiles of the Sun Pharma formulation of the liposomal doxorubicin HCl in pH 6.5 medium and different temperatures (37°C, 47°C and 52°C). Greater than 80% drug release for temperatures 47°C and 52°C were achieved at 4 Hrs and 1 Hr respectively (mean ± SD, N=3).

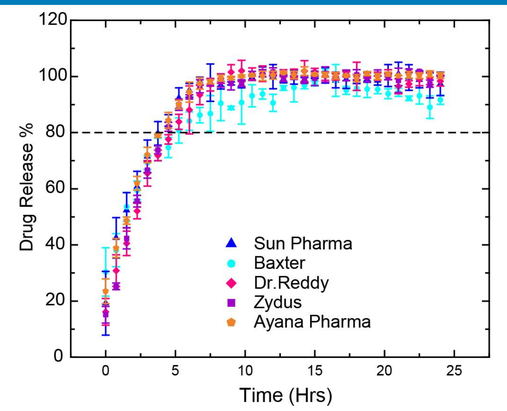


Figure 4. Drug release profiles of five different formulations of the liposomal doxorubicin HCl at pH 6.5 and 47°C (mean ± SD, N=3).

Summary & Conclusions

- ❖ The automated CE-based IVRT method can separate liposomal doxorubicin and released free doxorubicin at different CE elution times and quantitate them without additional sample preparation step.
- ❖ The drug release increased with increasing media pH. Drug release is induced by ammonia introduced in the release medium and uncharged ammonia (NH_3) concentration increase with increase of pH of the medium. The drug release increased with the increase of media temperature. Complete doxorubicin release (100%) was obtained in 7 hours at pH 6.5 and 47°C, and complete doxorubicin release (100%) was obtained in 3 hours at pH 6.5 and 52°C.
- ❖ The release profiles obtained for the brand name formulation (DOXIL®, Baxter Health Corp.) and four generic formulations (Manufacturers: Sun Pharmaceuticals, Dr. Reddy's Laboratories Inc., Ayana Pharma, and Zydus) were similar at pH 6.5 and 47°C.
- ❖ This method may be further applied in other liposomal formulations.

Acknowledgement and Disclaimer

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