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

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PURPOSE

A considerable amount of research has been conducted over the past six decades on the use of liposomes as drug delivery systems. 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 vitro drug release test (IVRT) is a critical quality control method in both premarket and post-approval regulation of liposomal drug products. Most IVRTs for liposomes require a separation step such as dialysis or solid phase extraction to separate released active pharmaceutical ingredient (API) 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. Capillary electrophoresis (CE) is a high-resolution analytical technique that separates charged molecules based on their electrophoretic mobility under electric field.

OBJECTIVE(S)

Develop an automated IVRT method using CE to quantify released doxorubicin from liposomal encapsulated doxorubicin without additional sampling and separation steps.

METHOD(S)

CE method

The background electrolyte (BGE) solution consists of 5% sucrose, 2% PEG-600 and 40 mM potassium phosphate at pH 5.80. The sample was hydrodynamically injected, and for capillary electrophoresis separation of the sample, 30 kV positive polarity voltage was applied, and the absorbance monitored at 491 nm (595 nm reference). For in vitro drug release profiling, the data were collected for 24 hours in 45-min intervals continuously. CE requires only nanoliters of injection volume and can rapidly separate and quantitate liposomal doxorubicin from free doxorubicin *in situ* in less than 10 mins as an automated analysis.

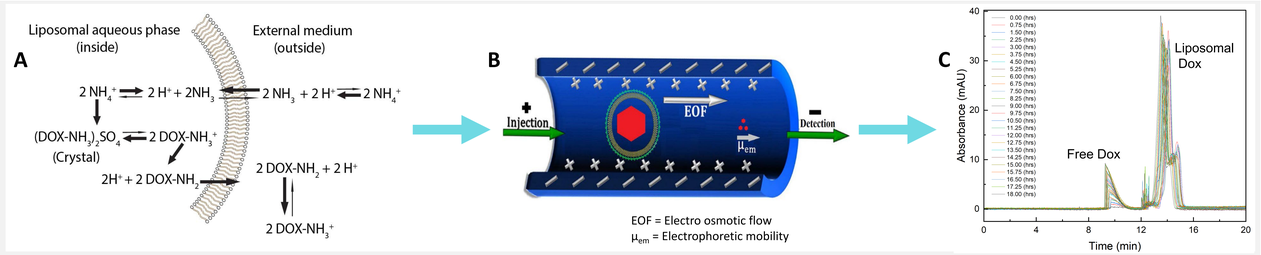
Doxorubicin Release

The optimized release buffer consists of 5% w/v sucrose, 20.0 mM L-histidine and 200.0 mM 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 with total sample volume of 0.50 mL. The release data were collected at three different temperatures (37ºC, 47ºC, and 52ºC). The in vitro liposomal doxorubicin drug release was repeated three times for each analytical condition.

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

RESULT(S)

Figure 1. *Illustration of the CE-based IVRT process.*
A) NH_4^+- assisted doxorubicin release from liposome[1]
B) Encapsulated and Free Doxorubicin separation by CE[2]
C) Electropherogram for drug release from liposomal formulation at 37ºC and pH 6.5



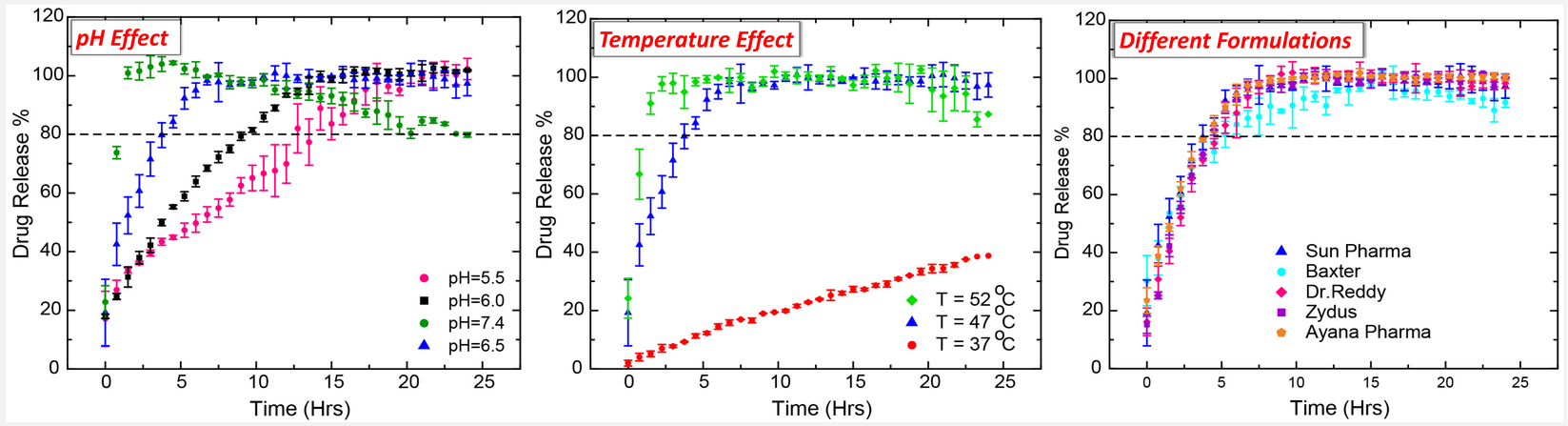


Figure 2. *Drug release profiles of the Sun Pharma formulation of the liposomal doxorubicin at 47ºC and different pH release mediums (pH 5.5, 6.0, 6.5 and 7.4). Greater than 80% drug release for pH 5.5, 6.0, 6.5 and 7.4 were achieved at 13 Hrs, 9 Hrs, 4 Hrs and 1 Hr respectively (mean ± SD, N=3).*

Figure 3. *Drug release profiles of the Sun Pharma formulation of the liposomal doxorubicin in pH 6.5 medium and different temperatures (37ºC, 47ºC and 52ºC). Greater than 80% drug release for temperatures 47ºC and 52ºC 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 at pH 6.5 and 47ºC (mean ± SD, N=3).*

CONCLUSION(S)

- 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.
- The drug release increased with increasing media pH and temperature. Complete doxorubicin release (100%) was obtained in 7 hours at pH 6.5 and 47ºC, and complete doxorubicin release (100%) was obtained in 3 hours at pH 6.5 and 52ºC.
- 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 47ºC.
- This method may be further applied in other liposomal formulations.

REFERENCES

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2. S.M. Ansar, W. Jiang, T. Mudalige, Direct quantification of unencapsulated doxorubicin in liposomal doxorubicin formulations using capillary electrophoresis, Int J Pharm 549(1-2) (2018) 109-114.

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