T0930-11-61

# Elevating Liposomal Formulation Analysis: An Advanced CE-C4D Method for Quantification of Ionic Excipients in Liposomal Doxorubicin


**Savithra Jayaraj[1], Wenlei Jiang[2], Thilak Mudalige[1]**

[1] Arkansas Laboratory, Office of Regulatory Affairs, U.S. Food and Drug Administration, Jefferson, Arkansas, 72079

[2] Office of Research and Standards, Office of Generic Drugs, Center for Drug Evaluation and Research, U.S. Food and Drug Administration, Silver Spring, Maryland, 20993

**CONTACT INFORMATION:** E-mail: Wenlei.Jiang@fda.hhs.gov, Tel.: +1-240-402-7963; Thilak.Mudalige@fda.hhs.gov, Tel.: +1-870-543-4665


aaps PharmSci 360

## PURPOSE

Liposomal drug delivery systems offer a transformative platform for delivering diverse active pharmaceutical ingredients (APIs). **Liposomal Doxorubicin Hydrochloride** (**DOX**) is widely used for treating ovarian cancer, AIDS-related Kaposi's Sarcoma, and multiple myeloma. Ammonium sulfate **$(NH_4)_2SO_4$** aids in loading and crystallizing DOX within the liposomes. Maintaining intraliposomal $(NH_4)_2SO_4$ concentrations over 200 mM is crucial for forming DOX sulfate nanorod crystals and ensuring sustained DOX release following injection in vivo. Traditional inorganic ion analysis of liposomal formulations, like ion chromatography, poses challenges, including potential liposome rupture and ion adsorption. We have demonstrated that capillary electrophoresis coupled with inductively coupled plasma mass spectrometry (CE-ICPMS) method selectively quantifies sulfate; however, this approach has some limitations and is restricted to sulfate due to ICPMS constraints.

## OBJECTIVE

Our GOAL was to demonstrate the use of **Capillary Electrophoresis** combined with **Capacitively Coupled Contactless Conductivity Detection** (**CE-C4D**) for high-resolution separation and detection of inorganic ions, enabling direct injection of liposomal formulations without the need for preparatory or separation steps.

## METHODS

The background electrolyte (BGE) solution consists of 2-(N-morpholino)ethanesulfonic acid (MES), L-histidine, 18-Crown-6 (18-C-6), and sucrose at pH 6. The samples were spiked with lithium fluoride (LiF) as an internal standard and hydrodynamically injected while maintaining the capillary temperature at 15°C. For CE separation, 30 kV positive polarity voltage was applied for 5 mins for cations and -30 kV negative polarity was applied for 5 mins for anion analysis. The conductivity was measured using eDAQ C4D. For external liposomal inorganic ions, the samples were directly injected after adding LiF. For total inorganic ions, the DOX formulation was lysed with methanol prior to injection. Measurements of 5 DOX formulations were performed in triplicate, and are reported as mean ± SD.

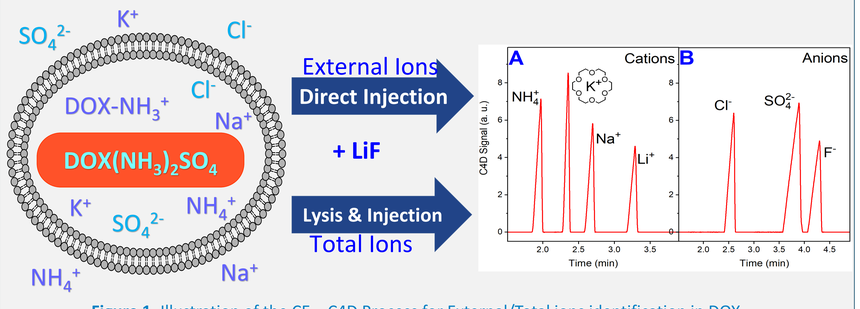


**Figure 1.** Illustration of the CE – C4D Process for External/Total ions identification in DOX

## RESULTS

**Table 1. Total ions, external ions, and intra-liposomal ions in doxorubicin HCl liposome formulations**

| Formulation | *Total Ion Concentration (mM) | | | | | *External Ion Concentration (mM) | | | | | *Intra-Liposomal Ion Concentration (mM) | | | | | #Intra-Liposomal Con (mM) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $NH_4^+$ | $Na^+$ | $K^+$ | $Cl^-$ | $SO_4^{2-}$ | $NH_4^+$ | $Na^+$ | $K^+$ | $Cl^-$ | $SO_4^{2-}$ | $NH_4^+$ | $Na^+$ | $K^+$ | $Cl^-$ | $SO_4^{2-}$ | $NH_4^+$ | $SO_4^{2-}$ |
| 1 | 12.36 | 0.42 | | 6.33 | 5.67 | 4.80 | 0.40 | | 6.13 | 1.25 | 7.56 | 0.01 | | 0.20 | 4.42 | 444.9 | 260.2 |
| 2 | 9.42 | 0.21 | 0.39 | 5.86 | 4.44 | 3.31 | 0.19 | 0.27 | 5.68 | 0.21 | 6.11 | 0.02 | 0.11 | 0.18 | 4.23 | 359.6 | 248.9 |
| 3 | 11.00 | 0.22 | | 6.03 | 4.44 | 3.69 | 0.20 | | 5.97 | 0.53 | 7.31 | 0.03 | | 0.06 | 3.92 | 430.2 | 230.5 |
| 4 | 10.15 | 0.34 | | 5.81 | 4.38 | 3.47 | 0.28 | | 5.80 | 0.34 | 6.68 | 0.05 | | 0.02 | 4.04 | 392.7 | 237.7 |
| 5 | 9.34 | 0.51 | TA | 6.06 | 4.14 | 3.63 | 0.32 | TA | 6.02 | 0.44 | 5.71 | 0.20 | TA | 0.05 | 3.70 | 336.0 | 217.7 |
| CE-ICPMS Siyam Ansar et al [1] | | | | | 4.22 | | | | | 0.16 - 1.28 | | | | | | | 238.8 - 313.5 |
| HPLC Jiewei Wu et at. [2] | 8.9 | | | | 4.2 | 3.5 | | | | 0.8 | | | | | | | |

*The concentrations were calculated based on the total sample volume #The concentration was calculated based on the total liposome internal volume that is 0.017 mL per mL of DOXIL[3] TA= Trace amounts

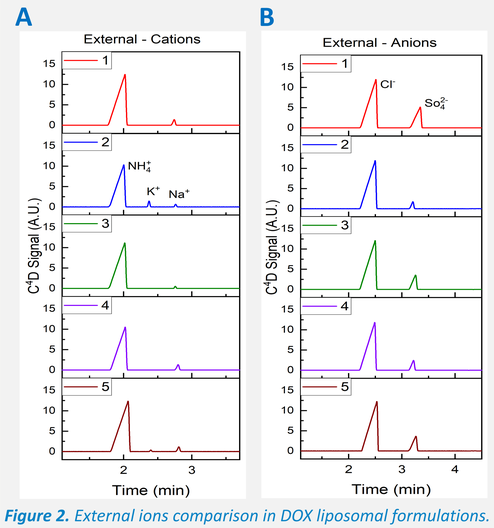


***Figure 2.*** *External ions comparison in DOX liposomal formulations. A) Cation analysis; and B) Anion analysis. (No internal standard added)*

## CONCLUSIONS

- It was determined that the **external $NH_4^+$** concentration ranged from 3.5-5.2 mM and **external $SO_4^{2-}$** concentration ranged from 0.3-1.4 mM based on total liposomal sample volume.
- Additionally, 270 µM **external $K^+$** ions were identified in the 2nd DOX formulation.
- **Total $NH_4^+$** ion concentration ranged from 9.3-12.4 mM, and **total $SO_4^{2-}$** ion concentration ranged from 5.8-6.3 mM based on total liposomal formulation.
- Though there are some variation in the levels of total and external ions when comparing **DOXIL®** and its generic formulations, all formulations contain an excess of **$(NH_4)_2SO_4$ (> 200 mM)**, sufficient for generating rod-like DOX nanocrystals and maintaining the stability of the liposomal formulation.
- Data obtained using CE-C4D method are comparable to other published method data for $NH_4^+$ and $SO_4^{2-}$ ions.
- This method offers unique advantage over other methods, allowing simultaneous detection of diverse ions, providing a more **efficient and comprehensive analytical solution** for ion.
- This method can be utilized for determination of inorganic ions in other drug products, supporting formulation development, quality test, and post-approval monitoring.


## ACKNOWLEDGEMENT AND DISCLAIMER

These studies were conducted using the Nanotechnology Core Facility (NanoCore) located on the U.S. Food and Drug Administration's Jefferson Laboratories campus (Jefferson, AR) with collaborations among ORA/ORS, ORA/ORS/ORCET, and CDER/OGD/ORS. We thank CDER/OGD/ORS and ORA/ORS for financial support, and ORA/ORS/ORCET for technical support. The opinions expressed in this poster are those of the authors. The opinions should not be interpreted as current or future policy of the U.S. Food & Drug Administration or any other agency of the U.S. government. The mention of manufacturers or trade names are for experimental clarity and does not constitute product endorsement.


## REFERENCES


1. S.M. Ansar, T. Mudalige, Direct and simultaneous determination of intra-liposomal and external sulfate in liposomal doxorubicin formulations by capillary electrophoresis/inductively coupled plasma-tandem mass spectrometry (CE/ICP-MS/MS), Int J Pharm 561 (2019) 283-288.
2. J. Wu, R.M. Crist, S.E. McNiel, J.D. Clogston, Ion quantification in liposomal drug products using high performance liquid chromatography, J. Pharm. Biomed. Anal. 165 (2019)41-46.
3. X. Wei, D. Shamrakov, S. Nudelman, S. Peretz-Damari, E. Nativ-Roth, O. Regev and Y. Barenholz, Cardinal Role of Intraliposome Doxorubicin-Sulfate Nanorod Crystalin Doxil Properties and Performance, ACS Omega, 3 (2018) 2508-2517.


U.S. FOOD & DRUG ADMINISTRATION