# *Novel Methods of Monitoring the Synthesis of N-L-Leucyldoxorubicin: MEKC-LIF and HPLC-LIF-MS*

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## Introduction

•Normal methods of monitoring chemical reactions, such as Thin-Layer Chromatography (TLC), sometimes do not fully characterize products.

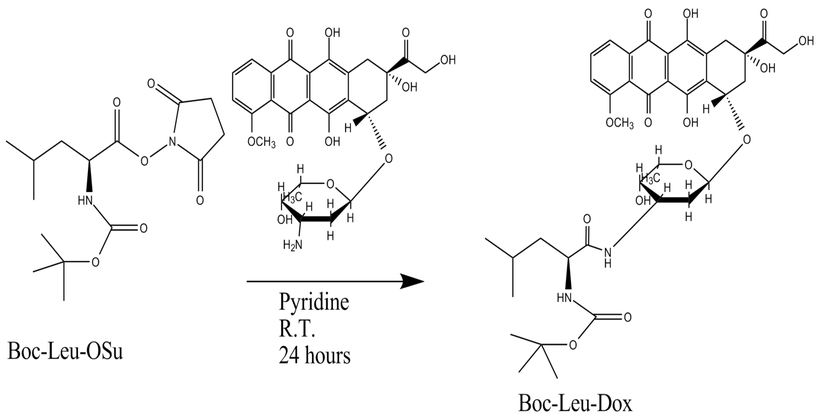
•Micellar Electrokinetic Chromatography coupled with Laser-Induced Fluorescence (MEKC-LIF) detection is a technique used to separate and detect fluorescent compounds.

•High-Performance Liquid Chromatography coupled with LIF and Mass Spectrometry (HPLC-LIF-MS) is a technique used to first separate compounds on an HPLC column, then to detect fluorescent compounds and determine m/z values of both fluorescent and non-fluorescent compounds.

•Doxorubicin (Dox) is an anti-cancer drug that is marred by many harsh side effects. The use of prodrugs, chemically altered drugs that are activated at the target site, could reduce these side effects. *N*-L-Leucyldoxorubicin (Leu-Dox) is a known prodrug of Dox.

## Purposes and Reaction Scheme

The addition of dox to *tert*-butoxycarbonyl (Boc)-L-leucine succinimide ester (step 1 of the synthesis) was monitored by TLC, MEKC-LIF, and HPLC-LIF-MS.



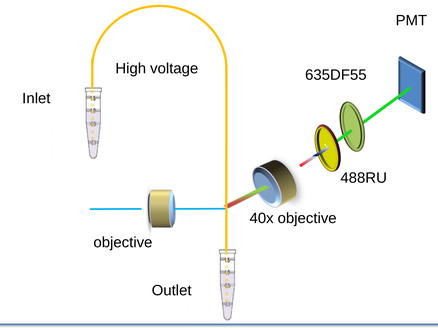

## TLC


**Advantages:**
•Fast
•Inexpensive

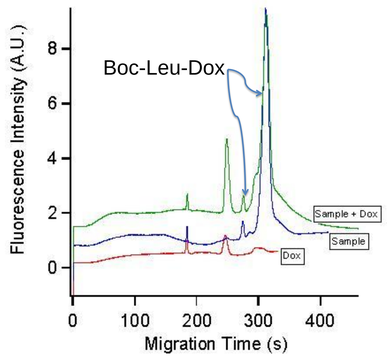
**Disadvantages:**
•Low resolution
•Large LOD

**Method:**
TLC analysis was performed on 0.3 mm silica plates with a solvent of 10:1 $CHCl_3$ : MeOH.

## Instrumentation for MEKC-LIF




## MEKC-LIF




**Method:**
MEKC-LIF was done in an uncoated silica capillary (i.d. = 50 µm, - 400V/cm, tricine/cetyl trimethyl ammonium bromide (surfactant)/NaOH, pH 8.5 buffer). Fluorescence excitation occurred at 488 nm and detection at 635 ±27.5 nm.

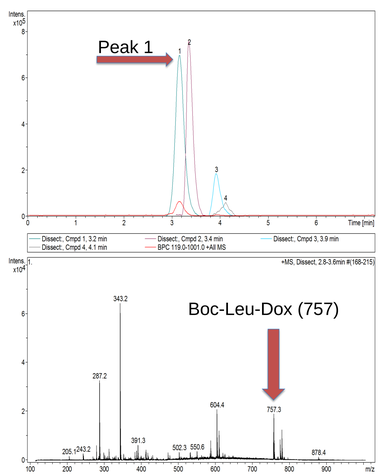
**Principles:**
• The buffer contains a surfactant that forms micelles.

•A high voltage applied at the ends of the capillary creates an electric field that causes differential movement of ionic species (i.e. micelles).

•During separation, the analytes partition differently into the micelles. Those with higher partition coefficients (e.g. more hydrophobic) spend more time in the micelles and exit the capillary later.

•Upon exit from the capillary, fluorescent compounds are excited and detected.

## HPLC-LIF-MS




**Method:**
HPLC-LIF-MS analysis used a reversed-phase HPLC column (isocratic $H_2O$ – $CH_3CN$, 2:1) followed by fluorescence detection (excitation at 488 nm, detection at 635 ±27.5 nm) and Time-Of-Flight MS (TOFMS) in a positive-ion electrospray mode.

**Results:**
Only peak 1 was fluorescent, and is thus the only peak of interest.

## Discussion and Conclusions

| Monitoring Method | Limit of Detection* (moles) |
|---|---|
| TLC | $1 \pm 0.5 \times 10^{-12}$ |
| MEKC-LIF | $1.58 \pm 0.03 \times 10^{-17}$ |
| HPLC-LIF-MS | $1.9 \pm 0.05 \times 10^{-12}$ |

• MEKC-LIF has the lowest LOD (by 5 orders of magnitude) of the three methods investigated here, which implies that less product can be used for monitoring.

• TLC showed only one red compound while MEKC-LIF showed two characteristic fluorescent peaks and HPLC-LIF-MS showed 5 MS peaks but only one that was fluorescent. This implies that TLC's resolution power is less than that of the other two methods.

•MEKC-LIF could be incorporated into an undergraduate teaching laboratory as a sensitive method for monitoring reactions involving fluorescent species.

* LODs are calculated for starting material, Dox, with n=3 for all three methods.

## Future Work

The Leu-Dox synthesized here will be used in the Arriaga lab for subcellular biotransformation studies.

## References

1. Wang, Yaohua; Arriaga, Edgar A. Monitoring incorporation, transformation and subcellular distribution of *N*-L-leucyl-doxorubicin in uterine sarcoma cells using capillary electrophoretic techniques, Cancer Letters 262 (2008) 123-132.

2. *Bioanalytical Chemistry*; Manz, Pamme et al. Imperial College Press, London, England, 2004.

## Acknowledgements

I would like to acknowledge Dan Mullen and Josh Ochocki for assistance, the Mark DiStefano laboratory for assisting in the purification of intermediates, and the Arriaga lab as a whole for assistance. This project was supported by the University of Minnesota's Undergraduate Research Opportunities Program.