A-TEEM Multidimensional Fluorescence Spectroscopy as a Powerful Tool for Unveiling Drug Release Kinetics in Liposomal Delivery Systems

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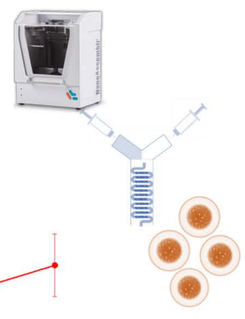
Introduction

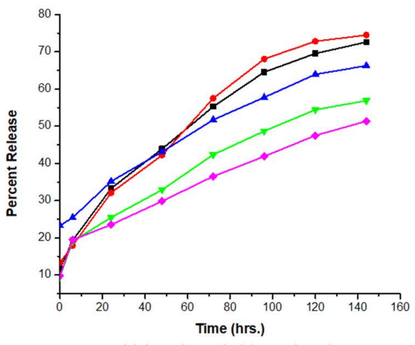
Recent advancements in microfluidic technology have revolutionized drug delivery by enabling precise fabrication and manipulation of liposomes for controlled drug release. Liposomes, versatile carriers for therapeutic agents, have shown substantial efficacy in enhancing drug delivery efficiency. This study pioneers using Absorbance-Transmission Excitation Emission Matrix (A-TEEM) spectroscopy to investigate doxorubicin (DOX) release from liposomes. A-TEEM combines absorbance and fluorescence spectroscopy, providing a contour plot that utilizes the UV/Vis absorbance spectrum and full fluorescence excitation and emission spectra to perform an automated correction of the Inner Filter Effect (IFE). Unlike traditional fluorescence EEMs, A-TEEM provides a true molecular fingerprint, enabling quantitative analysis of responsive components

This study highlights A-TEEM spectroscopy as a novel, powerful method for analysing drug release dynamics from liposomal formulations. A-TEEM offers a sensitive, rapid, non-destructive, and cost-effective way to quantify drug release from liposomal nanoparticles, avoiding shear stress and potential damage associated with established centrifugation methods. Initially, DOX release was detected and quantified in various buffered media at different temperatures in DSPC/cholesterol/DSPE-PEG2000 liposomes but has now expanded to include cellular analyses in MDA-MB-231 breast cancer cell lines

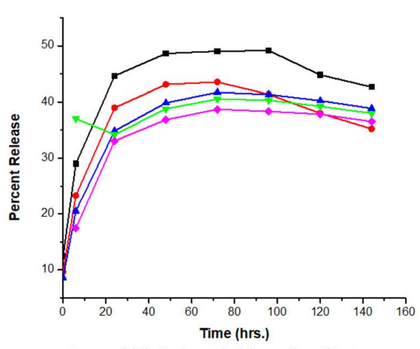
Material and method

Liposomes were synthesized using the NanoAssemblr™ Benchtop and a staggered herringbone micromixer. The formulations consisted of Distearoylphosphatidylcholine (DSPC), cholesterol, and N-(carbonyl-methoxy (polyethylene glycol)-2000)-1,2-distearoyl-sn-glycero-3-phosphoethanolamine (DSPE-PEG2000-SH), in a w/w ratio of 3:1:1 labeled with 1% w/w DiR'; DilC18(7) (1,1'-Dioctadecyl-3,3,3',3'-Tetramethylindotricarbocyanine Iodide)Additionally. Purification was achieved via Tangential Flow Filtration, and loading with 12.5% w/w doxorubicin using the pH gradient method. Following this, the liposomes underwent characterization to assess their size, polydispersity index (PDI), and zeta potential. Additionally, UV-Vis from, FTIR analyses were carried out to further evaluate their properties, and the drug release was monitored over 144 hours at varying temperatures and different media using Absorbance-Transmission Excitation Emission Matrix (A-TEEM) spectroscopy from HORIBA.

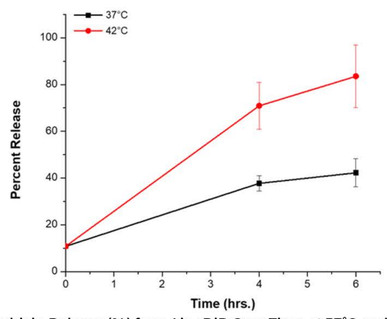




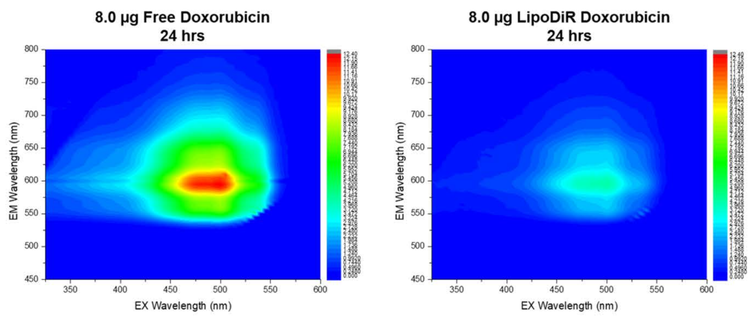
Doxorubicin Release (%) from LipoDiR Over Time at pH 4 for Different Concentrations



Doxorubicin Release (%) from LipoDiR Over Time at pH 7.4 for Different Concentrations

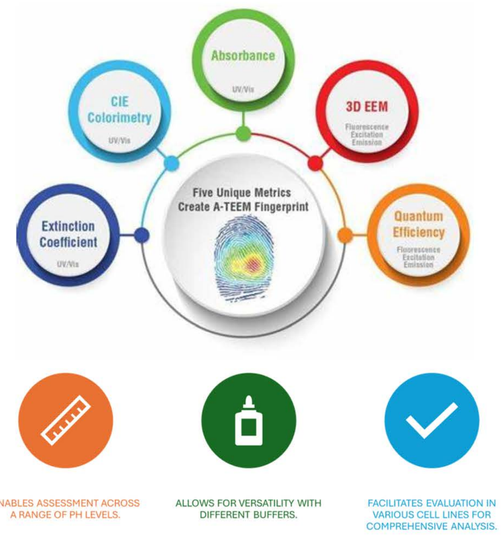


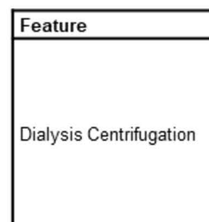
Doxorubicin Release (%) from LipoDiR Over Time at 37°C and 42°C

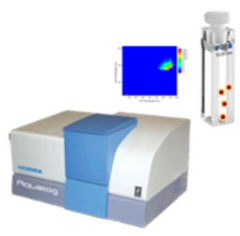


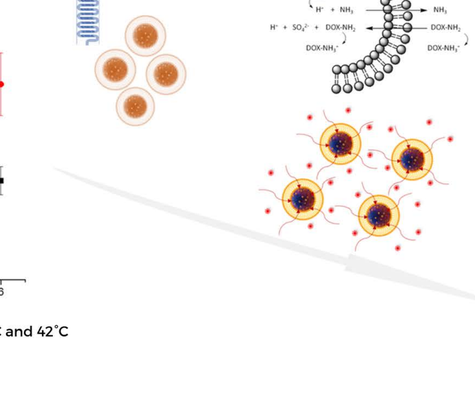
LipoDiR signal ~34% in comparison to Free doxo

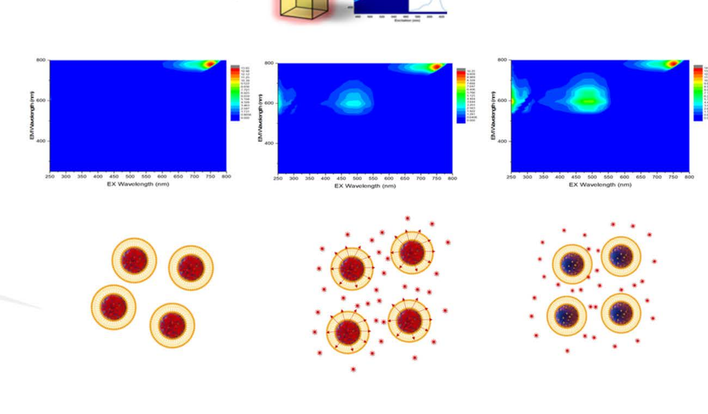
Doxorubicin Release from Liposomes and Free Doxorubicin in MDA-MB-231 Cancer Cells After 24 Hours of Treatment

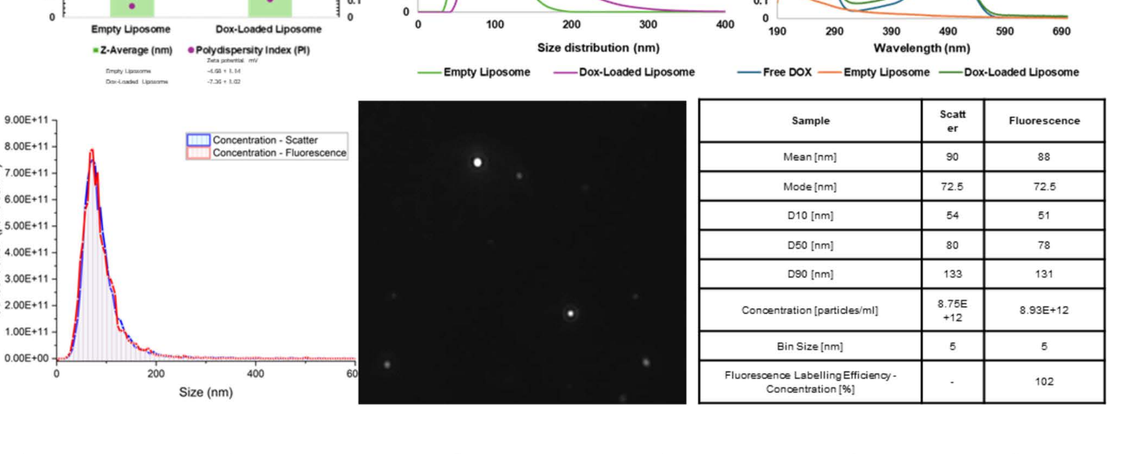


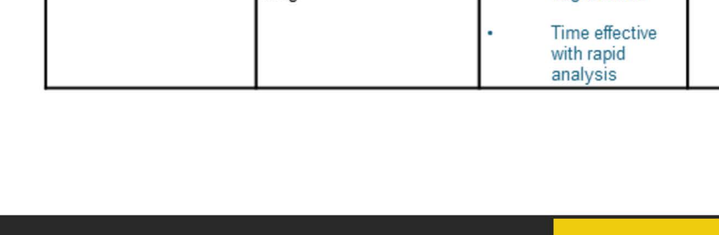












Sample	Scatter	Fluorescence
Mean [nm]	90	88
Mode [nm]	72.5	72.5
D10 [nm]	54	51
D50 [nm]	80	78
D90 [nm]	133	131
Concentration [particles/ml]	8.75E+12	8.93E+12
Bin Size [nm]	5	5
Fluorescence Labelling Efficiency - Concentration [%]	-	102

Feature	Principle	Strengths	Weaknesses	Data Output	Suitability for
Dialysis Centrifugation	Separates drug release media from dosage form using a semi-permeable membrane	• Simple setup. • Suitable for small sample volumes	• Time-consuming process (40 min per sample) • Costly • Yields varying outcomes	Cumulative amount of drug released over time	In vitro studies for initial screening
Dissolution Apparatus	Flow-through system continuously pumps dissolution media past the dosage form	• Mimics in vivo conditions with continuous flow. • Suitable for a wide range of dosage forms	• Potential for shear stress affecting release kinetics, • limited to sink conditions	Dissolution profile (concentration vs. time)	In vitro studies for formulation development, USP compliance testing
A-TEEM (Spectroscopy)	Measures drug release by monitoring changes in absorbance or fluorescence of released drug	• Non-destructive analysis • provides information on both drug release and degradation • Time effective with rapid analysis	• May not be suitable for all drugs	Dissolution profile (concentration vs. time)	In vitro studies for detailed analysis of release mechanisms, compatibility studies, and stability studies

Conclusion

•A-TEEM has effectively measured drug release from liposomes and from within cells, proving its capability to detect drugs in buffer solutions and within cells.

• This versatility and precision make A-TEEM an invaluable tool for studying drug release mechanisms, ensuring accurate monitoring and understanding of therapeutic agents' behaviour within biological systems

Acknowledgments

We would like to express our gratitude to the Centre for Process Analytics and Control Technology (CPACT) at the University of Strathclyde for their invaluable support and collaboration.

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