A-TEEM Spectroscopic Characterization of Exosome Standards and Their Mixtures

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Abstract

Absorbance-Transmission Excitation Emission Matrix spectroscopy (A-TEEM) is:
1) sensitive to protein composition; 2) rapid (<60 sec data acquisition times); and 3) non-destructive.

As commercialization of exosome-based therapeutics will depend on robust and validatable analytical techniques that can move from R&D to QA/QC to PAT, we propose A-TEEM as a possible tool for the characterization of exosomes and ultimately exosome therapeutics.

The purpose of this study is to test the feasibility of using A-TEEM to quickly and sensitively differentiate exosomes in solution. With access limited to only commercially available samples, the study was limited in scope, but shows promise in rapid qualitative and quantitative characterization of exosomes.

Introduction

A-TEEM Method

A-TEEM is a spectroscopic technique that simultaneously acquires UV/Vis absorption and fluorescence Excitation Emission Matrix data. The Emission spectra are collected on a 2D-detector for < 1 min per-measurement acquisition times.

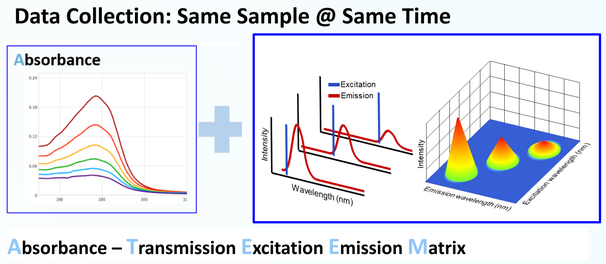
Absorbance/Transmittance (A-T)
The absorption spectrum is used for two purposes:
1) To correct for sample self-absorption (so-called IFE effect) of the fluorescence data (EEMs)
2) Provides two parameters that, together with fluorescence data, provide a molecular fingerprint of a sample: Extinction coefficient (ε) and Color (CIE)

Excitation Emission Matrix (EEM)
The fluorescence EEMs are used for in-depth characterization of samples:
1) EEMs provide a highly specific molecular fingerprint for characterization
2) Provides three parameters that, that along with the absorbance data provide a unique molecular fingerprint of a samples: Quantum Efficiency (ΦF), Fluorescence Excitation, and Fluorescence Emission spectra

A-TEEM for Exosome Characterization:

A-TEEM is a cuvette-based (potential for auto-sampling, at-line, and on-line deployment) technique for the characterization of protein-based samples. Potential to differentiate exosome types, detect contaminants, detect batch-to-batch variation, provide quantify payload.

Data Collection: Same Sample @ Same Time



Absorbance – **T**ransmission **E**xcitation **E**mission **M**atrix

A-TEM Eliminates "blur" of EEMs
- Clear molecular fingerprint

Attributes
- Low limit of detection (PPB/PPT)
- Sensitive and specific
- Rapid (< 60s)
- Low per-measurement cost

Methodology

Samples:

Lyophilized Exosome Standards PC-3 (ab239689) and MCF7 (ab239691) were purchased (Abcam, Cambridge, MA).

A-TEEM Measurements:

All sample measurements were made on the Aqualog UV-800 (HORIBA Scientific, Piscataway, NJ), with excitation from 220-450 nm. A blank measurement was collected with PBS solvent. PC-3 and MCF7 were reconstituted to 1 ug/ul with PBS, and then diluted an additional 200X and placed in a standard UV/Vis 1 cm path length cuvette for measurement. The PC-3/PBS solution was measured, and mixtures with MCF7/PBS solution in the following proportions were measured: 75%, 50%, 25%. Pure MCF7/PBS solution was also measured.

Exosome	Sample Composition - %				
PC-3	100	75	50	25	0
MCF7	0	25	50	75	100

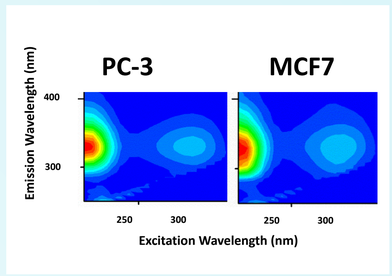
Data Analysis

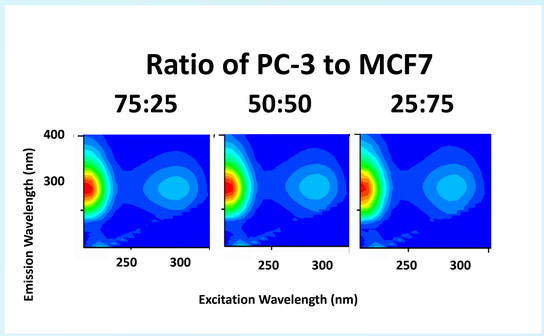
Data analysis employed multivariate methods. PARAFAC (Parallel Factor Analysis) was used for qualitative purposes, differentiate between the two exosome types. A PLS (Partial Least Squares) approach was used to predict the amount of the two in a mixture.

Results

A-TEEM Matrices of Pure Samples & A-TEEM Matrices of Mixtures

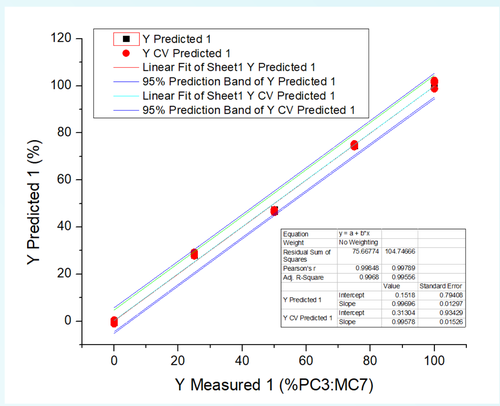
Changes are subtle, but apparent by eye. Multivariate methods are needed for more characterization.





PLS model - Predict Composition of Mixtures

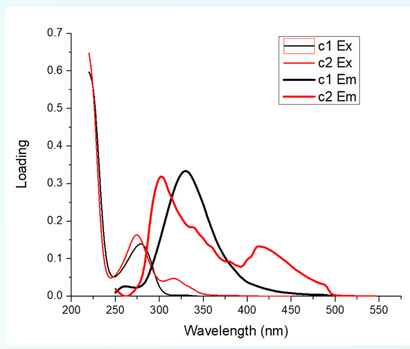
The PLS model of the A-TEEM data predicts composition of mixtures of PC-3 and MCF7 with an r-squared value of >0.99, and an SD of ~2%

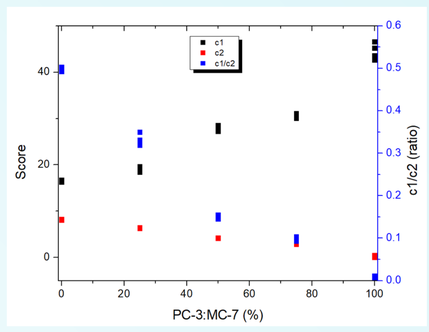


Literature Values			
Amino Acid	**λex (nm)**	**λem (nm)**	**Comments**
Tyr (Y)	275	305	
Trp (W)	275	330-332	Non-Polar
		340-342	Limited-polar
		350-353	Polar-exposed

PARAFAC Model to Interpret Variance

Component 1 (C1), with Ex 280/Em 328 is reflects a Tyrosine-like profile, in a non-polar environment.
Component 2 (C2), with Ex 275/Em 301 is reflects a Tryptophan-like profile.
PC-3 has a higher C1 score than MCF7.





Conclusion

The fluorescence EEMs profiles of these samples indicate that there are measurable differences in protein signatures between the samples. The PLS model was predictive of concentration.

The PARAFAC model gives some insight into the differences between the samples and is clearly able to distinguish between the exosome types, as well as their mixture. The ratio of C1/C2 reflects varying PC-3 to MCF7 concentrations.

This feasibility study was undertaken with a limited scope to test the potential for this approach for the characterization of exosomes in solution. A-TEEM was readily able to distinguish between the two exosomes, as well as their mixtures, and to predict the concentrations of the two components in the mixtures.