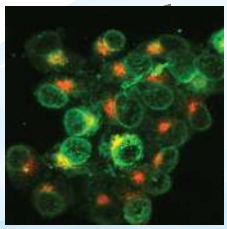
HORIBA

Fluorescence

Monitoring Cell Culture Media Variability Using the Veloci BioPharma Analyzer and A-TEEM™ Molecular FIngerprinting



Application Note

FL-2025-1-28

Introduction

Cell culture media for bioreactors are commonly prepared as aqueous solutions that provide the essential nutrients and conditions required for optimal cell growth, product yield, and quality. Even subtle variations in media composition can significantly impact cell growth rates and yield. Consequently, the identification and analysis of cell culture media are critical for maintaining consistency and optimizing performance.

A-TEEM technology enhances traditional Excitation-Emission Matrix (EEM) techniques by simultaneously acquiring Absorbance, Transmittance, and EEM data, while also applying real-time inner filter effect (IFE) corrections. This results in the ability to detect qualitative and quantitative changes in the molecular fingerprint for cell culture media samples, enabling the detection of degradation caused by storage conditions over time.

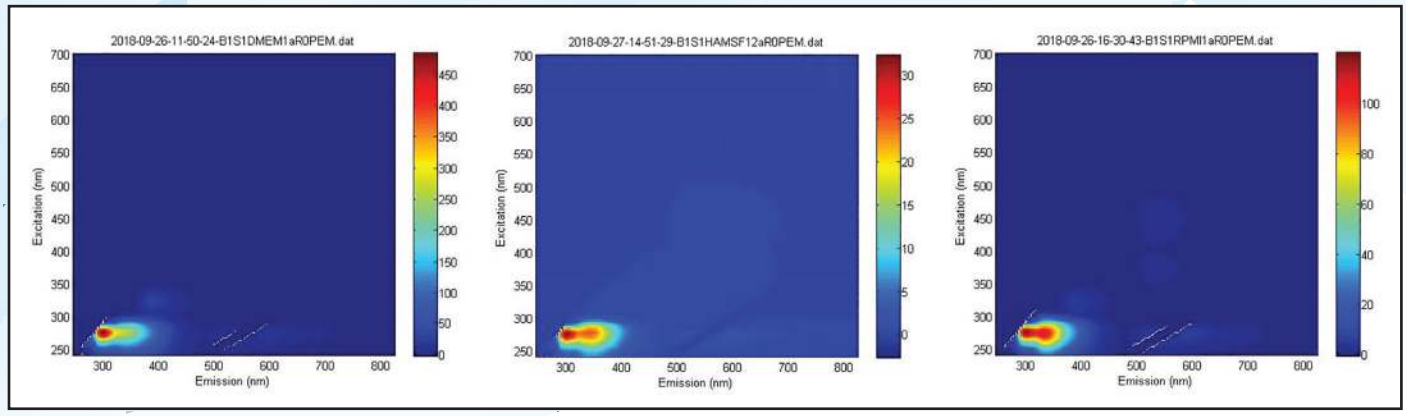


Figure 1: Examples of cell culture media molecular fingerprint A-TEEMs.
The media shown are from the samples DMEM1 (left), HAMSF12 (middle), and RPMI1 (right).

In recent years, the pharmaceutical industry has increasingly turned to spectroscopic techniques such as fluorescence for cell culture media analysis. These methods offer advantages including rapid testing, minimal sample handling, and relatively lower costs compared to traditional approaches like mass spectrometry and chromatography. Notably, Fluorescence Excitation-Emission Matrix molecular fingerprinting, combined with simultaneous Absorbance and Transmission measurements (A-TEEM), has emerged as a powerful tool for analyzing media composition (Fig. 1).

Recent studies have demonstrated that A-TEEM, when combined with chemometric methods such as Parallel Factor Analysis (PARAFAC) and Principal Component Analysis (PCA), provides a fast, effective, and economical solution for identifying and assessing the quality of cell culture media. The Veloci BioPharma Analyzer system's

In a recent evaluation, samples were stored at 4°C and equilibrated to ambient laboratory temperature before analysis. Five aliquots of each media type were measured using 1 cm pathlength quartz cuvettes in triplicate to establish A-TEEM fingerprints. Additional samples were stored at room temperature and analyzed at various time points to observe degradation effects caused by ambient temperature and light exposure.

Materials and Methods

The cell culture media samples analyzed include:

- Dulbecco's Modified Eagle Medium (DMEM), no glucose, no glutamine, no phenol red (DMEM1)
- DMEM, high glucose, HEPES, no phenol red (DMEM2)
- DMEM, high glucose, no glutamine, no phenol red (DMEM3)
- Roswell Park Memorial Institute (RPMI) 1640 Medium, no phenol red (RPMI1)

- RPMI 1640 Medium (ATCC modification) (RPMI2)
- Ham's F-10 Nutrient Mix (HAMSF10)
- Ham's F-12 Nutrient Mix (HAMSF12)
- Ex-Cell CD CHO Serum-Free Medium for CHO Cells, Chemically Defined (EXCELL)

Instrumentation and Data Collection

A-TEEM spectra were acquired with excitation scans from 200 to 700 nm in 3 nm increments and emission scans from 250 to 800 nm in approximately 4.66 nm increments (8-pixel binning). The bandpass was set to 5 nm. NIST-traceable spectral correction factors for excitation and emission, inner filter effect corrections, Rayleigh scatter masking, and Raman scatter unit normalization were applied. Blank references were recorded using Starna 3Q-10 water. PCA modeling was performed using Eigenvector Inc.'s Solo software.

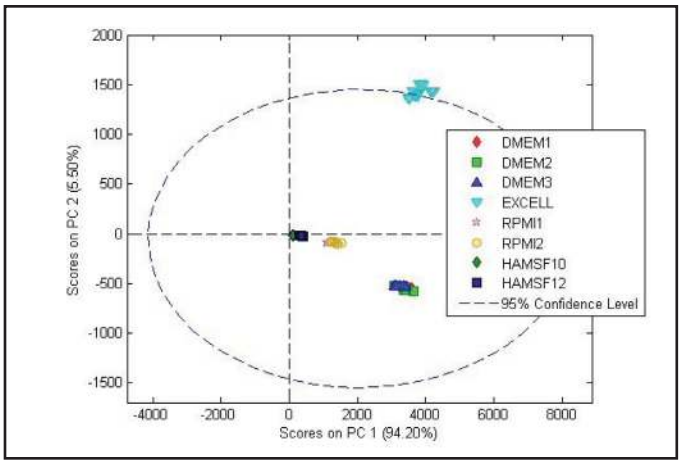


Figure 2: PCA model built from data of all eight media samples.

Results and Discussion

Initial Media Classification: PCA models effectively classified the cell culture media types. Similar media types, such as the three DMEM mixtures, clustered together and were distinctly separated from other media types with no overlap (Fig. 2). This demonstrates the ability of A-TEEM spectroscopy combined with chemometrics to differentiate media types.

Time-Dependent Degradation Analysis:
EXCELL media samples stored at 4°C remained stable over time, clustering closely in PCA space regardless of storage duration. In contrast, samples stored at ambient temperature displayed noticeable molecular fingerprint changes, reflecting degradation effects (Fig. 3). These changes were evident in PCA score trends over the five-day evaluation period, highlighting the sensitivity of A-TEEM to detect fluctuations in media quality.

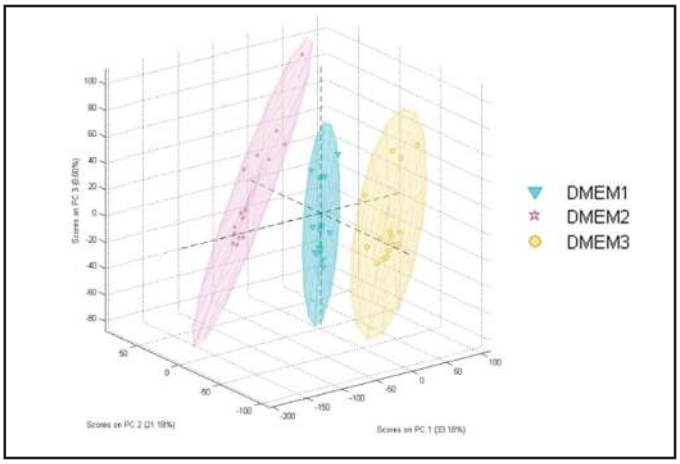


Figure 3: PCA model built using data of all three DMEM samples to discriminate between samples of the same media type but with different modifications.

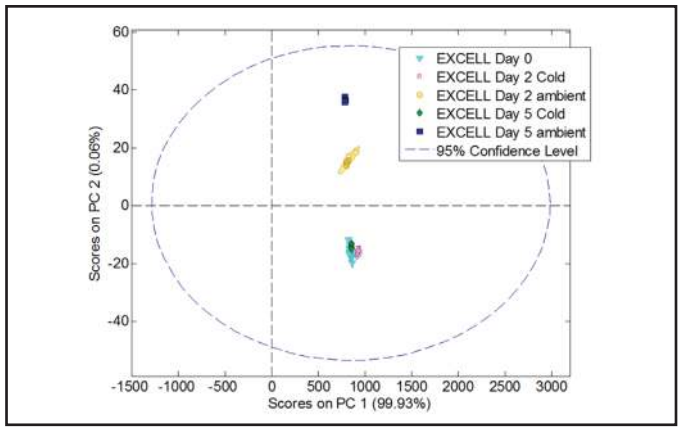


Figure 4: PCA model built for EXCELL media samples using data from the initial Day 0 sample and Day 2 and Day 5 day ambient storage samples to observe effects of potential degradation due to temperature and light exposure over time.

Conclusion

The combination of A-TEEM spectroscopy and chemometric analysis offers a robust, rapid method for identifying and evaluating cell culture media. As demonstrated, A-TEEM molecular fingerprints enable visual discrimination between sample types, while chemometric analysis facilitates media classification and monitoring of quality changes. This approach is particularly valuable for biomanufacturing, where media composition evolves during cell growth and requires supplementation or replenishment. Moreover, the method's potential for at-line or in-line implementation further supports its application in bioprocess monitoring and quality assurance.

References

1. Li, B., Ryan, P. W., Shanahan, M., Leister, K. J., & Ryder, A. G. (2011). Fluorescence Excitation-Emission Matrix Spectroscopy for Rapid Identification and Quality Evaluation of Cell Culture Media Components. Applied Spectroscopy, 20.

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