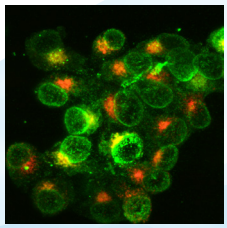
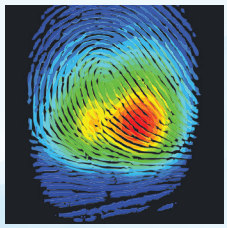
HORIBA Scientific

Fluorescence

Monitoring Cell Culture Media Variability using a Simple Optical Technique (A-TEEM Molecular Fingerprinting)





Application Note
Life Sciences
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Introduction

Cell culture media for bioreactors are usually prepared as an aqueous solution and provide everything a cell line needs for optimal growth as well as product yield and quality. Even subtle variations in composition could have a noticeable impact on the growth rate of the cell culture and its yield. Thus identifying and analyzing cell culture media is important. As a result, the pharmaceutical industry has begun to turn to spectroscopic methods such as fluorescence for cell culture media analysis due to the speed of testing, minimal sample handling requirements, and relatively lower cost when compared to mass spectrometry and chromatography. Of particular interest are Fluorescence Excitation-Emission Matrix molecular fingerprints complemented by simultaneous Absorbance and Transmission measurements (A-TEEM) (Fig. 1).

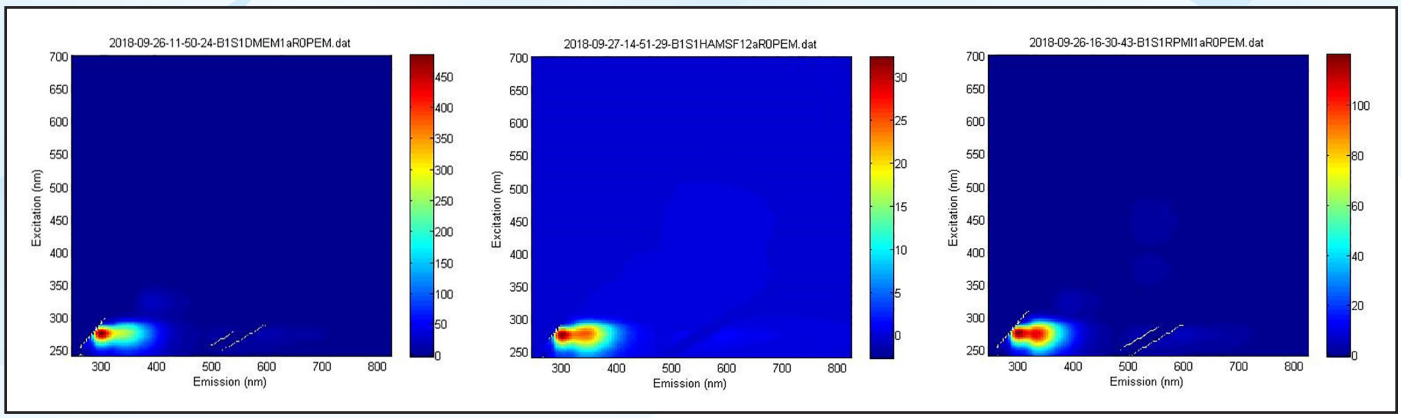

Figure 1: Examples of cell culture media molecular fingerprint A-TEEMs.
The media shown are from the samples DMEM1 (left), HAMSF12 (middle), and RPMI1 (right).

Recently, it was shown[1] that A-TEEM, along with the aid of certain chemometric methods (PARAFAC, PCA) provides a fast, effective, and inexpensive solution to identify and assess the quality of cell culture media. Aqualog's A-TEEM ability to acquire Absorbance, Transmittance and Excitation-Emission Matrices simultaneously along with the instrument's real-time ability to correct for inner filter effects (IFE) is an enhancement on traditional EEM techniques and provides a more precise molecular fingerprint for cell culture media samples and can identify and detect signs of change or degradation within cell culture media due to storage at ambient temperature over time.

Samples were stored at 4°C and were allowed to equilibrate to ambient laboratory temperature prior to analysis. A total of five sample aliquots were taken for each type of media and measured using 1 cm pathlength quartz cuvettes in triplicate to establish A-TEEM fingerprints.

Additional samples for each media type were stored at room temperature. These samples were then analyzed at various time points to observe any degradation effects due to ambient temperature and light exposure over time.

Materials & Methods

Media Samples measured for this project are listed below.
1) Dulbecco's Modified Eagle Medium (DMEM), no glucose, no glutamine, no phenol red (DMEM1)
2) DMEM, high glucose, HEPES, no phenol red (DMEM2)
3) DMEM, high glucose, no glutamine, no phenol red (DMEM3)
4) Roswell Park Memorial Institute (RPMI) 1640 Medium, no phenol red (RPMI1)
5) RPMI 1640 Medium (ATCC modification) (RPMI2)
6) Ham's F-10 Nutrient Mix (HAMSF10)
7) Ham's F-12 Nutrient Mix (HAMSF12)
8) Ex-Cell CD CHO Serum-Free Medium for CHO Cells, Chemically Defined (EXCELL)

Instrument and Data Collection

The A-TEEMs were obtained using excitation scans from 200 to 700 nm in 3 nm increments and emission scans from 250 to 800 nm using approximately 4.66 nm increments (8 pixel binning). The band path was set at 5 nm. NIST Traceable Excitation and Emission spectral correction factors, inner-filter correction, Rayleigh scatter masking and Raman scatter unit normalization were applied. Blank reference was recorded using Starna 3Q-10 water. For chemometric analysis, PCA modeling was performed with the aid of Eigenvector Inc.'s Solo software.

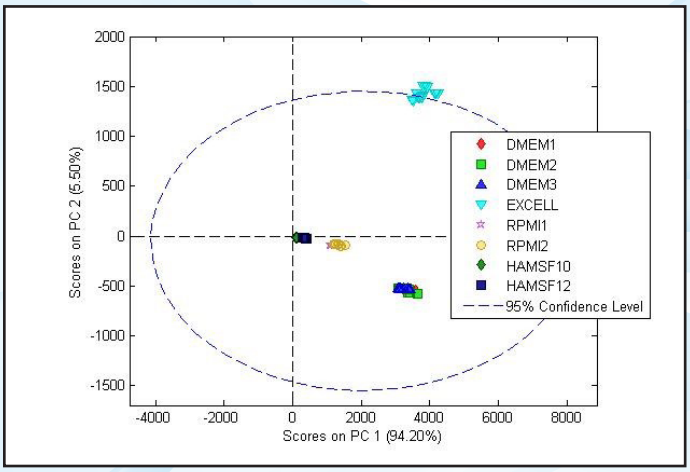

Figure 2: PCA model built from data of all eight media samples.

PCA chemometric analysis on the initial, time=0, samples provided easy classification (Figure 2.) Similar media types (for example, the three DMEM mixtures) were clustered together and visibly discernible from other media types, with no overlap (Figure 3). This result indicates that we can successfully classify different media types using the combination of A-TEEM spectroscopy and chemometrics. In Figure 3 only data from the three DMEM mixtures were included but similarly distinct results were obtained for the RPMI and Ham's mixtures.

The time-dependent course of PCA score changes at ambient temperature vs. the 4°C storage temperature is seen in Figure 4. The measurements on EXCELL media were conducted over five days with datapoints obtained at 0, 2 and 5 days for both sets of samples stored at different temperatures. The cold-stored samples clustered together regardless of elapsed time whereas the ambient conditions clearly affected the samples and led to a change in their molecular fingerprint attributes resulting in a noticeable trend of changes in the PC scores.

Conclusion

Based on the data collected and the PCA models utilized, it can be shown that the combination of A-TEEM spectroscopy and chemometrics provides a viable and rapid method of identifying and evaluating cell culture media. As shown in the figures above, the A-TEEM fingerprints serve as a way of visually observing and discriminating between sample types. In addition, the use of chemometrics allows the classification of the media types being analyzed. The combination of chemometrics and A-TEEM spectroscopy is a promising and viable method of cell culture media evaluation prior to their use in bioreactors and can also be employed at-line or in-line in the manufacturing process. This is especially important since the media composition will change as a function of cell growth and will need to be supplemented or replenished.

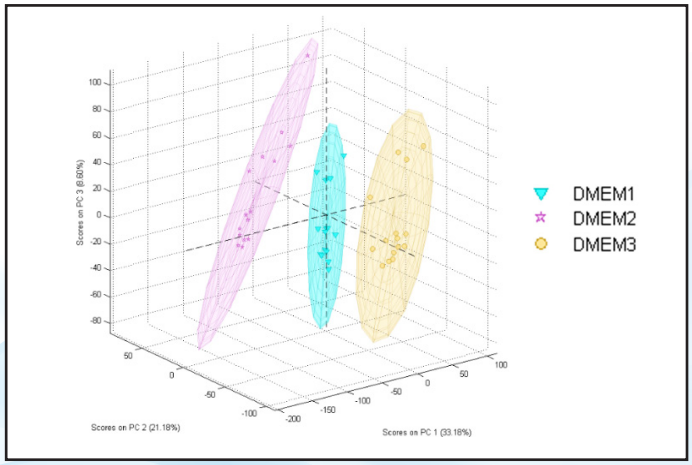

Figure 3: PCA model built using data of all three DMEM samples to discriminate between samples of the same media type but with different modifications.

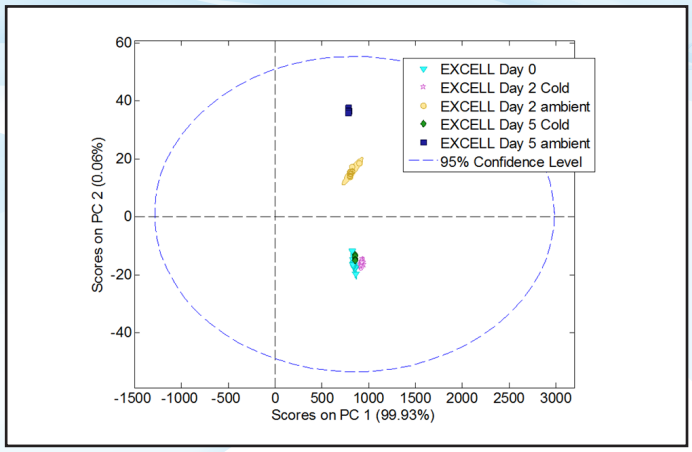

Figure 4: PCA model built for EXCELL media samples using data from the initial Day 0 sample and Day 2 and Day 5 day ambient storage samples to observe effects of potential degradation due to temperature and light exposure over time.

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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