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Fluorescence

Monitoring Cell Culture Media Variability using A-TEEM™

Application Note
Life Sciences

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Introduction: With the rise of protein production using mammalian cell culture, it has become increasingly important to control the quality of the cell culture media for use in production processes. Cell culture media are usually prepared as aqueous solutions and should provide everything a cell line needs for optimal cell growth as well as product yield and quality. This could include amino acids, glucose, vitamins and various other nutrients. The exact composition and concentrations of the components found in a given type of cell culture medium tend to vary depending on the unique needs of a given cell line and are usually considered proprietary information by the manufacturers of said media. In any given bioreactor process it is important to identify the proper type of cell culture medium and its quality because even subtle variations in composition could have a noticeable impact on the growth rate of the cell culture and its yield. Therefore, the composition and quality of cell culture media in bioreactors must be tightly controlled in order to maintain an optimal bioreactor process. As a result, methods of identifying and analyzing the quality of cell culture media have become an important focus in this field.

Existing solutions include methods such as chromatographic separations and mass spectrometry. However, these methods are generally considered to be too expensive and time consuming to be considered for routine analysis of cell culture media samples. As a result, the 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, and relatively lower cost when compared to mass spectrometry and chromatography. One technique of particular interest is Absorption and Transmission fluorescence Excitation Emission Matrix (A-TEEM) technology. Unlike other EEM based systems, ATEEM's ability to acquire absorbance, transmittance and excitation-emission matrices simultaneously along with the instrument's ability to correct for inner filter effects provides a more precise molecular fingerprint for cell culture media samples.

In this study, we demonstrate that the A-TEEM system is capable of producing precise molecular fingerprints to differentiate between cell culture media samples in a fast and cost-efficient manner. In addition, we demonstrate that this system can detect signs of change or degradation of cell culture media samples due to storage conditions.

Materials & Methods: The following cell culture media samples were provided in solution (Sample designations in parenthesis at the end of each description):

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)

All samples were stored at 4°C before testing and were allowed to equilibrate to ambient laboratory temperature prior to analysis. A total of five sample aliquots were taken for each type of medium and measured in triplicate to establish their A-TEEM fingerprints. Additional samples for each medium were prepared to be stored at room temperature. These samples were then analyzed at various time points over five days to observe any degradation effects due to light exposure and ambient temperature.

Instrument and Data Collection: A-TEEMs measurements were carried out at ambient laboratory temperature using a Horiba Scientific Aqualog system. The A-TEEMs were obtained using excitation scans from 200 to 700 nm in 3nm 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 and the integration time used differed as follows: EXCELL CHO – 0.25 sec, HAMS F10 & HAMS F12 – 0.15 sec, all other samples – 0.05 sec. NIST Traceable Excitation and Emission spectral correction factors, inner-filter correction, Rayleigh scatter masking and Raman scatter unit normalization were applied. Samples were analyzed in 1cm path quartz cuvettes at room temperature with Starna 3Q-10 sealed water sample used as blank.

*Chemometric Analysis*PCA modelling was performed with the aid of Eigenvector's Chemometric software named Solo.

Results and Discussion: Five samples were aliquoted from each of the eight types of media being analyzed. Each sample was then measured in triplicate resulting in 120 measurements for each time point. All cell culture media were stored at 4 °C before testing and were allowed to equilibrate to ambient laboratory temperature prior to analysis. A-TEEM data were analyzed using the chemometric method known as Principle Component Analysis (PCA) in order to classify each media type.

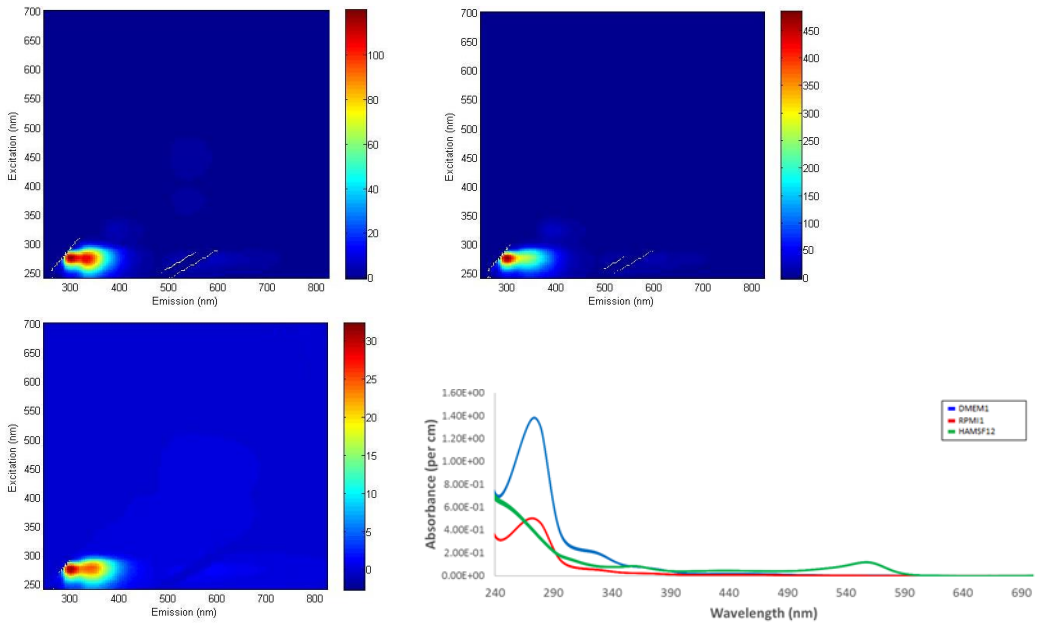


Figure 1: Examples of cell culture media fingerprint ATEEMs. The media shown are from the samples DMEM1 (top left), HAMSF12 (top right), RPMI1 (bottom left) and absorption spectra of all 3 Media (bottom right). Excitation wavelengths less than 240nm were excluded.

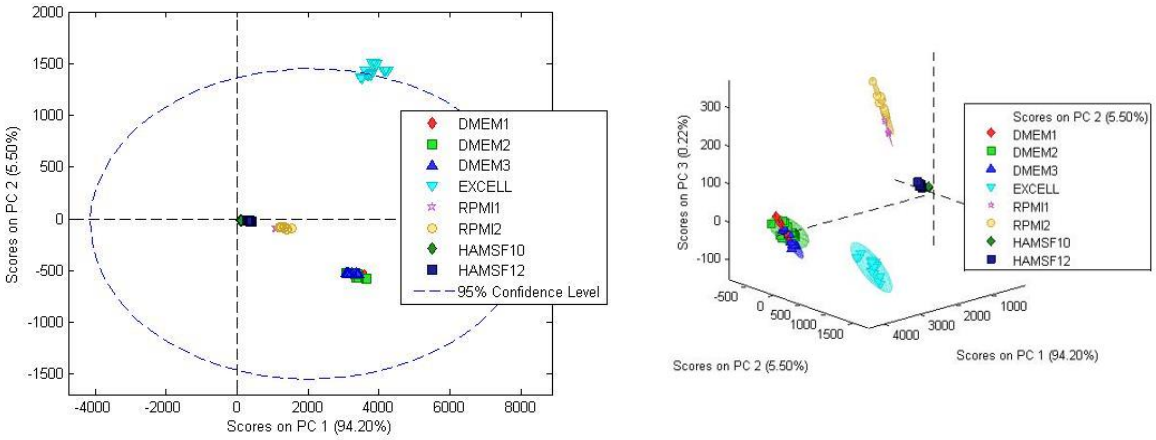
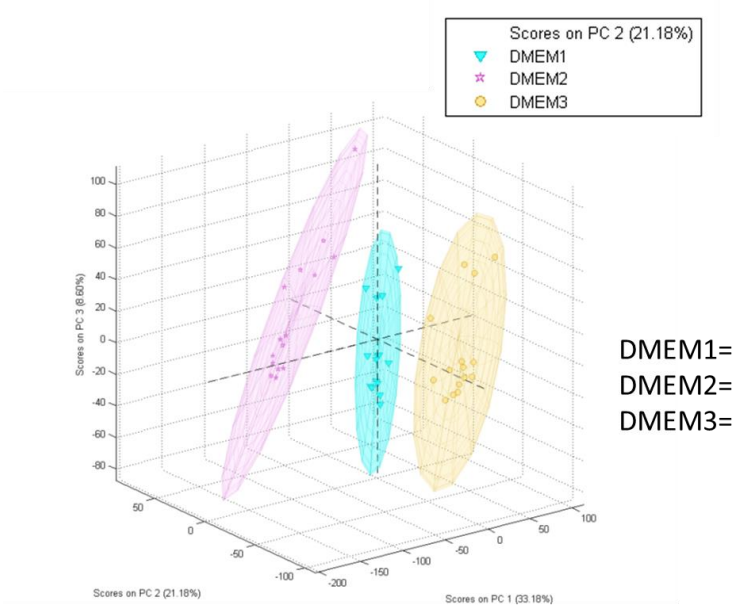


Figure 2: PCA model built from data from all eight media samples. The plot on the left shows the two dimensional score plot across the first two principal components (PCs). The plot on the right is the three dimensional score plot of the same model across three PCs.

Cell Culture Media Identification: Principal Component Analysis (PCA) provided classification of all eight media measured (Fig.2.). Similar media types (for example, the three different DMEM mixtures) were clustered together and discernible from the other media types with no overlap. This result indicates that we can successfully classify different media types using the combination of A-TEEM spectroscopy and chemometrics. In order to determine whether or not it would be possible to differentiate between samples of similar types, individual PCA models were created for each media type. For example, only data from the three DMEM mixtures were included in the DMEM model (Figure 3) and the same was done for the RPMI and Ham's mixtures (Figure 4).



DMEM1= no glucose, no glutamine, no phenol red
DMEM2= high glucose, HEPES, no phenol red
DMEM3= high glucose, no glutamine, no phenol red

Figure 3: PCA model built using data from all three DMEM samples to discriminate between different modifications of the same media type, three-dimensional scores plot shown on the left.

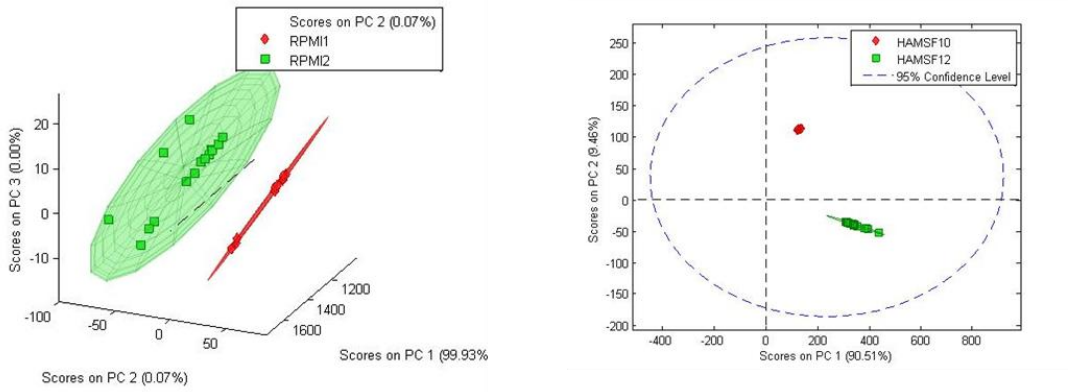


Figure 4: Scores plots shown for both RPMI and HAMS samples. 3D plot shown on left and 2D plot shown on right.

The PCA model is shown to be able to classify different modifications of the same type (Figures 3-4). More importantly to highlight that the influence of the absence and presence of glucose was clearly detected as we observed a clear difference between DMEM 1 and DMEM 3 (Figure 3).

Variability in Storage Conditions: Chemically-defined cell culture media are known to degrade if not stored properly or handled with care. In order to determine if A-TEEM is sensitive to such changes, we investigated the effect of storage temperature and exposure to light and how that influences the A-TEEM fingerprint. For EXCELL media samples that have been stored at room temperature and exposed to light for only 2 days, we observed changes a noticeable modification to the fingerprint (Figure 5, bottom). After 5 days, samples stored at ambient temperature could also be easily discerned from both the day 0 samples and the day 2 samples (Figure 5, bottom). The tryptophan region (exc: 275 nm and em: 350 nm) in the A-TEEM plot underwent most drastic change after 5 days of storage at room temperature. From this model, we can also conclude that both media types appear to be very stable when stored at the recommended temperature of 4°C. This is apparent from the clustering of the samples from both day 2 and day 5 cold samples with the initial day 0 samples (Figure 5, top).

Conclusion: Based on the data collected and the PCA models utilized, it can be shown that the combination of A-TEEM spectroscopy and chemometrics does provide a viable and rapid method of identifying and evaluating the quality of cell culture media. The A-TEEMs fingerprints collected by this method is one way of visually observing and discriminating between sample types. In addition, the use of chemometrics allows us to dive deeper into the data and classify each of the media types being analyzed, as shown in the PCA scores plots. Further research that could be of interest would include additional cell culture media types as there are many different kinds of media that could be used in bioprocesses. For the purposes of this study however, the results show that the combination of chemometrics and A-TEEM spectroscopy is a promising and viable method of cell culture media evaluation.

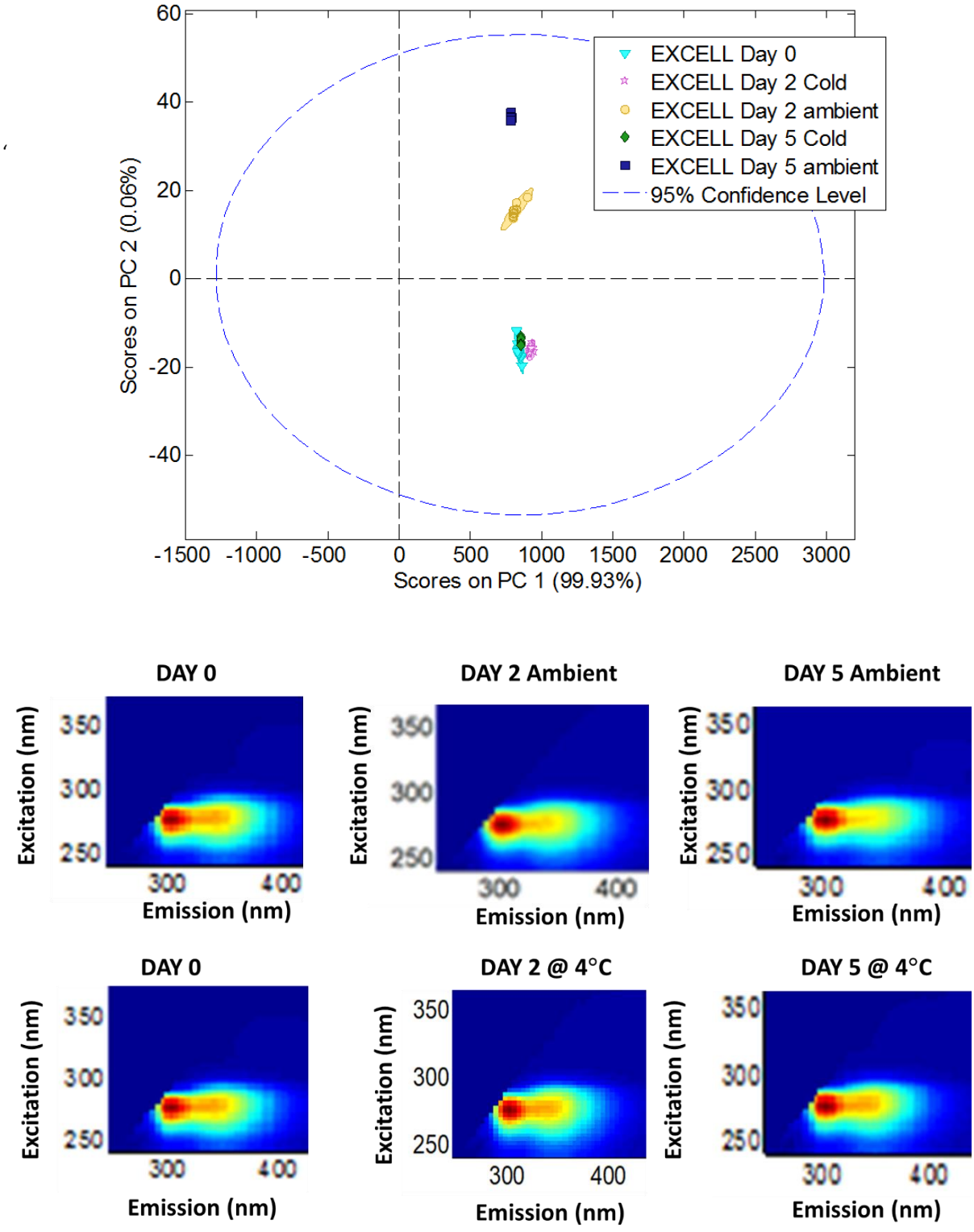


Figure 5: Scores plots (top) and A-TEEMs (bottom) of EXCELL (10x diluted) media samples under different conditions.