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Cell culture media analysis using rapid spectroscopic methods

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Cell culture media (CCM) used in industrial biotechnology are complex mixtures of molecules and elements that are inherently difficult to analyze comprehensively. CCM quality analysis is of utmost importance for efficient production of protein-based therapeutics with the correct Critical Quality Attributes (CQAs). Here we discuss the use of rapid spectroscopic methods for routine screening of CCM molecular variance which include electronic (UV–vis absorption and fluorescence) and vibrational (Raman, FT-IR, and Near-Infra-red) spectroscopies. CCM analysis needs to provide: identity testing, compositional variance analysis (i.e. lot-to-lot variation), validation of media preparation protocols, and correlations with Critical Process Parameters (CPPs) or product CQAs. Rapid spectroscopic methods can fulfil some of these requirements but only with correct sample handling and preparation. Accurate analysis requires the use of chemometrics combined with a detailed knowledge of sample behavior such as water absorption and chemical stability.

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Introduction

Cell culture media (CCM) are a critical element in biopharmaceutical manufacturing which directly affect process yield and product quality. Therefore, the analysis of media variance and prediction of performance is of high importance. The essential purpose of CCM is to produce and maintain an optimum physiological environment to allow large-scale culturing of cells where they remain healthy for an appropriate amount of time and express the right product with the correct Critical Quality Attributes (CQAs). For protein-based therapeutics CQAs include factors such as the glycosylation pattern, stability, aggregation, impurity profiles, and so on. Many CQAs are directly affected by CCM [1] and unknown changes in composition can have very serious adverse consequences for the process. CCM are carefully designed and optimized for each process (and often for different stages in the process) and cell line, which results in a very wide range of different CCM formulations. The resulting media are highly complex, molecular and elemental mixtures, containing amino acids, carbohydrates, vitamins, growth factors, trace and bulk minerals. The most complex of the raw material components used in media manufacture are the various hydrolysates of protein sources (like yeast or soy). These hydrolysates are a complex mixture of carbohydrates, amino acids, peptides, and a host of other often unknown molecules and minerals [2–5]. These contain many more components than chemically defined media and often display much greater molecular and elemental variance but are often critical for antibody manufacturing processes [6]. This variance can arise from changes in source material, manufacturing issues, or improper handling/storage. For these materials, both molecular [7] and elemental variance are important [8] in the context of their effect on cell culture. This molecular and elemental variance impacts on CCM quality in terms of its intended purpose, namely sustaining a specific bioprocess. Therefore, when we use the term media quality it refers to a specific media and a specific process. It should be noted that the same media could be used in two different processes and for each process, the effect of media variance may be different.

Another major issue is the fact that components are present in widely varying concentrations, from trace (ppm) levels for some elements to >5% w/w for carbohydrates. In liquid media water is the major component (>90% w/w) diluting components (millimolar, micromolar, and nanomolar concentrations) and making analyte detection more difficult for spectroscopic methods. Because of these compositional and concentration factors, media analysis using traditional analytical methods, is challenging, very time consuming, requiring multiple methods and techniques. This makes comprehensive analysis too expensive for routine use. As the industrial cell culture production of therapeutic proteins continues to grow so too does the need for rapid, inexpensive, reliable, robust, and non-destructive analytical methods that can be integrated into process control to improve end

product yield and quality. A recent review [9••] provides a more in-depth assessment of these raw materials issues. Whereas here we focus on the use of spectroscopic measurements on the media and take a critical look into the quality of the data being generated. The quality of the spectral data that these rapid methods can provide is very sensitive to sample handling and failure to recognize this can lead to the generation of erroneous data and ultimately misleading conclusions.

Fast spectroscopic methods

There are many potential spectroscopic methods that can be applied to media analysis, however, here we limit ourselves to commercially available, tried and tested methods. Relatively recent reviews describe the needs for Process Analytical Technologies (PAT) in biopharmaceutical manufacturing [10–13] and provide more background detail about how rapid spectroscopic techniques could be incorporated into manufacturing processes and are directly relevant to CCM analysis. The most important methods are the electronic spectroscopies (UV–vis absorption and fluorescence) and vibrational spectroscopies (MIR and NIR absorption and Raman scattering). The correct application of all methods requires an in-depth appreciation of the practical difficulties of dealing with chemically complex mixtures. Sample handling therefore plays a critical role in the collection of accurate, correct, and reproducible data which is required for the development of robust analytical methods.

Sample handling

A critical issue with CCM analysis relates to sample handling and stability both in powdered and solution forms (Table 1). CCM have complex physicochemical properties which mean that the samples are neither chemically nor physically stable for varying time periods of minutes to hours depending on the type. It is very important to recognize here that stability in the context of measurements is different from stability associated with CCM performance in a bioprocess. For example, CCM powders are usually hygroscopic, and will quickly absorb atmospheric water rapidly (within a couple of minutes) and are thus unsuitable for vibrational spectroscopy analysis of molecular variance (as opposed to just measuring the effects of water absorption) unless properly dried. Whereas small amounts of absorbed water are unlikely to have any impact on cell culture performance. Water adsorption from the atmosphere induces variations in baselines and erratic signals which are not always clearly evident to the analyst. One answer is to use specialist powder cells [14] to prevent water adsorption. Another approach is to use Spatially Offset Raman Spectroscopy (SORS) to analyse CCM powder in sealed packages and thus avoid the water adsorption problem [15], while this works well with simple media components it has not been proven with CCM powders, where fluorescence and the large number of components with varying concentrations are also major issues. MIR and EEM fluorescence have been used to measure media aging effects [16] and the authors here concluded that

Table 1

Some general sample handling and storage requirements for the spectroscopic methods used for cell culture media analysis

	Powders		Solutions	
	Hydrolysates	Chemically Defined	Hydrolysates	Chemically Defined
Hygroscopic	Yes, very [42]	Yes, very [16]	n/a, unless very concentrated	n/a, unless very concentrated
Drying required before NIR/IR/Raman measurements	Yes	Yes	n/a	n/a
Light sensitivity	Some	Some	Yes	Yes [23]
Protection measures	Avoid intense or prolonged light exposure	Avoid intense or prolonged light exposure	Store in dark containers	Store in dark containers
Temperature sensitivity	Some	Some	Yes	Yes [17]
Long-term storage conditions	−20 °C, dry Need to minimize potential microbial growth as these materials are usually not sterilized	−20 °C, dry Need to minimize potential microbial growth as these materials are usually not sterilized	−70 °C [20]	−70 °C
Microbial growth	Yes, if sufficient water absorbed	Yes, if sufficient water absorbed	Yes, very sensitive [22].	Yes, very sensitive
Protection measures	Store dry	Store dry	Aseptic sample preparation and 0.2 μm filtering recommended [16]	Aseptic sample preparation and 0.2 μm filtering recommended [16,23]