Designing Culture Media for Recombinant Protein Production

A Rational Approach

Tom Fletcher

Developing an industrial manufacturing process for production of a therapeutic protein in cell culture can seem like an overwhelming responsibility. A company's research and development group may provide its responsible process development team with little more than a cell line that produces a certain therapeutic protein. Developing an efficient, commercially viable process to produce that therapeutic protein involves solving a wide range of highly technical problems. Among the most significant is finding the cell culture medium that is best suited to achieve the goals of the process.

Finding a good medium is important, first of all, because it affects process performance. Investing effort into media development for every individual process is easily justified — not only because the nutritional requirements are different for every process, but also because the impact of satisfying those requirements on process performance can be significant. After the cell line, there is perhaps no other factor that more greatly influences process performance.

The chosen medium must also meet applicable quality standards and regulatory requirements. Consider what a critical role a culture medium plays in providing the primary source of raw materials that actually end up in the drug itself. It is believed that most amino acids making up the primary structure of a therapeutic protein produced in cell culture are derived directly from the culture medium.

So finding a culture medium that meets all process requirements will certainly be a good return on the effort and investment typically required to discover it. The importance of media development to overall process development is indicated by the fact that, once a successful process has been developed, the culture medium formula and details of how it was developed are often two of the most closely guarded secrets of the manufacturer.

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THE CHALLENGE OF MEDIA DEVELOPMENT

Cell culture media technology has advanced tremendously during the past few decades. In some ways, it still seems amazing that we can grow such a wide range of cell lines outside the animals from which they were derived. But the practice of in vitro cell culture for research — and even for large-scale manufacturing processes — is now considered quite routine.

It is interesting to reflect on how much culture media have advanced since the days when most classical media were developed in the 1950s and 1960s. The "Media Examples" box lists some of those early media, which were all developed for use with blood serum supplements that supply the highly complex mixture of once-unknown nutrients necessary for in vitro culture (1). As our understanding of cellular nutritional requirements improved, many serum-free media were developed in the 1970s and 1980s to provide a more defined culture

PRODUCT FOCUS: PRODUCTS OF ANIMAL CELL CULTURE

PROCESS FOCUS: PRODUCTION AND PROCESS DEVELOPMENT/OPTIMIZATION

WHO SHOULD READ: CELL CULTURE ENGINEERS, PROCESS DEVELOPERS, ANALYTICAL LABORATORIES

KEYWORDS: CULTURE MEDIA, SERUM-FREE, CHEMICALLY DEFINED, ANIMAL CELLS, DESIGN-OF-EXPERIMENTS

LEVEL: BASIC

environment. The "Media Examples" box lists a few.

When the risk of previously unknown pathogens became a concern after the discovery of prions in the 1980s (**2**), efforts were initiated to completely eliminate all animal-derived components from the culture media used to produce human therapeutics. The latest step in this advancing technology has been to develop culture media that are not only free of animal-derived components, but are also chemically defined (**3**). Many first-generation animal-component–free media relied on vegetable-derived protein hydrolysates or other undefined components to achieve successful performance.

Understanding the challenge of developing media that meet today's stringent requirements begins by recognizing how far removed these artificial growth conditions we create actually are from the native environment of animal cells. To meet today's increasingly stringent standards, media must grow cell cultures without the use of serum, animal-derived components, or indeed any undefined components. But this is in great contrast to the cells' native environment, where they are nourished by an extremely complex, high-protein nutrient mixture composed exclusively of animal-derived components that are derived primarily from the bloodstream.

Today's challenge, then, is to develop sophisticated media that can be individually optimized for a range of processes within a short amount of time. The difficulty derives from several aspects including the diversity of cell lines and production processes in use, the large number of media components involved, and the fact that many of those components are interdependent on others because of the complexity of cellular metabolic pathways.

Each process has its own set of requirements, and the choice of medium so greatly affects how well a process will meet those requirements that media development is generally a standard component of any process development effort. The media requirements for two different processes may differ greatly even when all other aspects of those processes are very similar. Even cell lines derived from a common parent often exhibit different nutritional requirements. For media manufacturers, the diversity of requirements is increased even more by the number of different cell line varieties and processes in common use today. Thus, for example, we wouldn't expect a medium designed for the batch culture of Chinese hamster ovary (CHO) cells to be the best medium for a fed-batch culture of mouse myeloma (NS0) cells. Both the nutritional requirements of the different cell lines (CHO and NS0) and the requirements of those particular processes (batch and fed-batch) are dissimilar.

The complex problem of determining the best blend of media components for a given cell line used in a particular process deserves a carefully devised strategy. This becomes immediately apparent to anyone familiar with the complexity of cellular metabolism and the large number of components in media. And not only will a typical cell line require 50–75 different components to perform best in culture, but the optimum concentrations of many components are interrelated. By varying the concentration of one component, you often change the requirement for another.

In addition to the restrictions against using certain types of components, the diversity of process requirements, and complexity of the associated problem to be solved, the next greatest factor affecting most culture media development projects is time. The entire task of process development seems to be carried out most often on an accelerated time schedule, and the media development component of this work obviously needs to comply. Experienced process engineers often practice the art of finding the best balance of tradeoffs to meet a particular deadline. Choosing an effective media development strategy often comes down to knowing how to design the right experiments that will quickly deliver the best information about how to gain the most improvement in culture

Media Examples

Early Media

Ham's F-10 nutrient mixture was formulated for serum-free growth of Chinese hamster ovary (CHO) cells and growth of various mammalian cell lines with serum supplementation.

Ham's F-12 nutrient mixture was formulated for single-cell plating of near-diploid Chinese hamster ovary (CHO) cells.

Minimum Essential Media (MEM) are are well suited for the growth of a broad spectrum of mammalian cells.

Dulbecco's Modified Eagle Media (D-MEM) are well suited for supporting the growth of a broad spectrum of mammalian cell lines.

RPMI Media are enriched formulations with extensive applications for mammalian cells. They were formulated for suspension cultures or monolayer culture of human leukemic cells.

Later Media

Iscove's Modified Dulbecco's Media (IMDM) are highly enriched synthetic media well suited for rapidly proliferating, high-density cell cultures.

CMRL Medium is especially useful for cloning monkey kidney cell cultures and for growth of other mammalian cell lines when enriched with horse or calf serum.

MCDB 131 Medium was developed in 1987 as a reduced serum-supplemented medium for the culture of human microvascular endothelial cells.

NCTC-109 is a synthetic medium suitable for general use in generating and maintaining hybridoma cells. Like MCDB, this medium typically requires the addition of a growth factor cocktail or serum.

performance. That's where rational culture media design comes in.

What It Isn't . . .

Rational culture media design can best be understood if it is first explained in terms of what it is not. Using a rational design approach means being careful not to overcommit to any single method of media development. The problems associated with depending too much on any single method might best be illustrated if we conduct a critical survey of the common individual methods currently used. Each has its merits, but each suffers from particular weaknesses as well. None of the methods alone can meet the challenge as we have defined it above.

Component titration (Figure 1) is the classic approach to media development. It involves performing a series of experiments to determine the "dose response" of a cell line to various media components by adding each one in varying amounts to individual cultures.

Media blending (Figure 2) is a method that rapidly generates many new media by simply blending existing formulations. By evaluating these combinations and then iteratively performing additional blending and testing of those that perform well, process developers can rapidly zero in on the best blend.

Spent media analysis (Figure 3) can provide important information using chemical analysis to measure how a medium changes during the culture process. By comparing spent medium with a fresh sample, process developers can make calculations describing both nutrient depletion and metabolite accumulation.

Automated screening is focused primarily on throughput. The use of robotic fluids handling and multiwell plates can rapidly create and analyze large numbers of candidate media. So this method generates a large dataset of performance results obtained in parallel conditions.

Comparing the Methods: How useful are those methods? When should each be used, and when should it be avoided? What are some of their particular strengths and weaknesses? A brief evaluation might consider the following important characteristics:

- Throughput — how many samples can be evaluated in a given time?
- Modeling — how well does the method represent the real application?
- Instructive — does the method effectively reveal useful information about the cell culture process requirements (e.g., nutritional requirements)? Does it contribute to a knowledge base?
- Scope — how well does the method consider all possible solutions to the problem?

With those working definitions in mind, we might summarize our survey results as in Table 1. Notice that none of the methods is best in every way, and each has its own particular weakness. Depending on any one method alone would not be the most effective way to develop a new medium rapidly.

Component Titration: For example, if component titration were the only method used, then the sheer number of samples to be created and analyzed would be overwhelming. Consider that testing even just three concentrations each, of the more than 50 components in a typical chemically defined medium, would require at least 150 samples. And even after testing all of those samples individually, you still would not have obtained important information about how the components interact. More experiments would be necessary. Using component titration alone would make the project exceedingly labor intensive because of the inherently low throughput. Statistically savvy scientists sometimes refer to this as the "OFAT" (one factor at a time) approach.

Media Blending: If media blending were the chosen method, throughput could be improved, but the approach would be weak in two other aspects: it is poorly instructive and limited in scope. Practically speaking, this is a "blind" method to rapidly generate new component combinations. Although it is effective for creating many new combinations, "untangling" the results and determining which components are providing benefits or detriments is difficult when performance testing those combinations. You may by chance discover that a certain media blend works better than the others you

Figure 1: Component titration is the classic approach to media development.

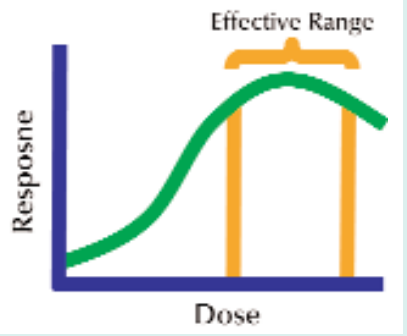


Figure 2: Media blending — the shaded area represents the range of concentrations that can be derived by blending three media (A, B, and C).

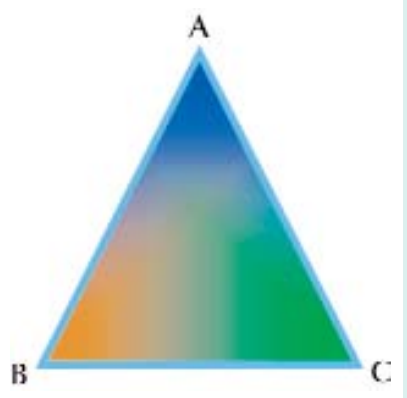
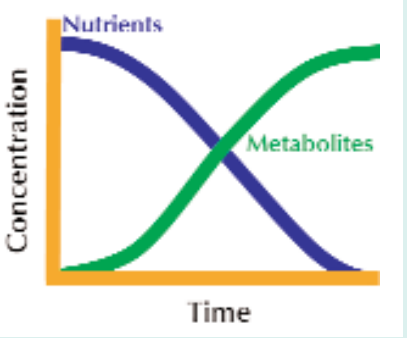


Figure 3: Spent media analysis measures how the medium changes throughout the culture process.



happened to test. However, you probably can't determine what makes it better, nor can you further adjust critical components without first identifying what they are.

So the scope of this method is bound by the diversity of the media used. Consider that any medium created by blending cannot contain component concentrations exceeding those of the media from which it is derived. Graphically mapping the range of possible combinations illustrates clearly where the boundaries of this method lie (Figure 2).

Spent Media Analysis: A media development effort based on spent media analysis could provide valuable insight into the specific requirements for any given cell culture process. Perhaps the most instructive of the four methods considered here, this approach can provide important information about how culture chemistry changes over the course of a process.

The risk of using this method exclusively, however, is that it generally cannot provide a complete picture of process requirements. Based on the fact that spent media analysis generally focuses on a limited number of analytes (usually glucose, lactate, ammonia, and amino acids), it provides a simple chemical view into what is actually a much more complex biological problem.

Automated Screening: The obvious tradeoff in any method that seeks to increase throughput through miniaturization is the loss of accuracy in modeling a large-scale process. The automated screening approach to media development is generally tied to the use of multiwell plates that work well with robotic instrumentation.

The risk of reducing the cell culture model to such a small scale is that those culture conditions will not represent the actual process. A proper understanding of its limitations and use will make this approach a valuable tool within a comprehensive media development program. In fact, all four of the methods described here can be effective if they are used appropriately and within their respective limits.

Table 1: Summary table comparing four methods of media development

	Component Titration	Media Blending	Spent Media Analysis	Automated Screening
Throughput	Low	High	Medium	High
Modeling	Good	Good	Good	Poor
Instructive	Good	Poor	Good	Fair
Scope	Unlimited	Limited	Limited	Unlimited

Rational Culture Media Design in Practice

Rational culture media design makes use of several complementary methods to meet the aggressive requirements of process development projects that aim to achieve the best culture medium in the shortest amount of time. Although each project may be quite different, below a typical example is outlined showing how the four methods described may be effectively combined to meet specific process requirements in a given amount of time. The four methods are abbreviated as follows: component titration = CT, media blending = MB, spent media analysis = SMA, automated screening = AS. The total time for the project described here would be 18–32 weeks.

Phase 1: Screen
Using 96-well plates, with the assay endpoint being growth, the best starting medium is determined in about 2–6 weeks. AS uses metabolic dyes, and MB uses 1:1 blends in addition to initial candidates to increase the scope from $n = x$ to $n = x^2$.

Phase 2: Optimize
For the Basal Medium: Shaker flasks are used, with the assay endpoint being growth and production, to optimize a chemically defined basal medium in about 4–12 weeks. MB and/or AS uses component groups, SMA identifies limiting nutrients and metabolites, and CT involves only critical components.

For the Supplements: Shaker flasks are used, with growth and production being the assay endpoints, to optimize non-animal sourced media in 4–12 weeks. CT features hydrolysates.

For the Feeds: A bioreactor is used, with the assay endpoint being growth and production, to optimize a basal + feed combination in 4–10 weeks. SMA identifies limiting nutrients and metabolites.

Phase 3: Verify
A bioreactor is used, with the assay endpoint being growth and production, to find an effective process-specific medium in 4–8 weeks. SMA is used for monitoring batch performance.

What It Is . . .

So what is rational culture media design? It can be described as a multidimensional approach because instead of relying on a single technique, rational culture media design makes use of several complementary methods. A media development project is generally divided into phases that guide its progression from screening through optimization to verification.

All the methods described above are used in rational design, but they are chosen for use with careful regard to their strengths and weaknesses. The combination is carefully chosen for each project to fit the goals of each particular phase and provide results that will be relevant to those goals. Project management tools (such as Gantt charts) are used to facilitate efficient planning and coordination of project tasks. That becomes especially important when operating under accelerated timelines that require performing many experiments concurrently.

As would seem fitting for its use in solving industrial problems, rational culture media design can be termed an applied science because it

is concerned with effectively solving "real-world" problems by properly combining tools that fit each specific problem. In fact, before any experiments begin, the development goals must be carefully defined in terms of not only process performance, but also regulatory and any other requirements. The same care is applied to choosing appropriate models for the actual experiments — in regards to both scaled-down cell culture methods and using appropriate end-points.

Finally, the rational culture media design approach does not allow you to avoid performing cell culture work during media development. But it is both rapid and unbound by the limitations inherent in any single-method approach. Appropriate software tools help design an efficient discovery process. For example, by using a statistical Design of Experiments (DoE) program — such as ECHIP (ECHIP, Inc., www.echip.com), Design-Ease and Design-Expert (Stat-Ease, Inc., www.statease.com) — you can perform experiments that capture the complex interactions of multiple components without testing the full factorial number of combinations. Statistical tools help you choose the lowest possible number of conditions for your experiments without sacrificing statistical confidence in your results.

By using several carefully chosen methods that are effective in the rapid development of culture media optimized for specific processes, rational culture media design can help you meet the stringent challenges presented by today's cell culture processes.

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