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Media formulation optimization: current and future opportunities

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Chemically defined cell culture media is a complex mixture of molecules used for cellular metabolism and growth. Decisions surrounding which molecules to include in a formulation and their optimal concentration is a major endeavor in biomanufacturing. Cellular metabolic complexity and stringent quality requirements that vary across cell lines require time-consuming, iterative experimentation to optimize media formulation. Exacerbating this is the orders-of-magnitude concentration differences among media components making media characterization difficult. This review describes the challenges, advances and strategies currently used to optimize media formulations as well as future opportunities for improvement.

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Introduction

Cell culture media is an important factor within biomanufacturing as it impacts cell growth [1], productivity [2], and product quality [3]. Components within the media are incorporated into the drug molecule itself, affecting drug safety and efficacy. Chemically defined media (CDM) is extremely complex due to the uncertainty around the requirements of different cell lines, uncertainty around the effect of specific components on different cell lines, and the sheer number of components in the media (>50). This makes the process of formulating a CDM and then optimizing the concentration of components an incredibly difficult task.

For example, the depletion of an essential amino acid can lead to a different amino acid being used in its place, and therefore structural changes in the protein [4–6] resulting in regulatory impacts. Furthermore, biochemical cofactors, for example metals ions, are required for necessary cellular functions and the depletion or overabundance of cofactors can cause abnormalities. Another example is that media lean in copper ions can prevent the commonly observed metabolic lactate shift which hinders cell growth whereas a media rich in copper ions can lead to high levels of protein deamidation [7–12].

There are also process issues to consider in formulating CDM. Concentrating components to reduce liquid volumes and prevent large working volumes leads to high osmolality and solubility issues in feed media. Furthermore, the highly concentrated feed solutions cause pH perturbations requiring buffer additions which exacerbate osmolality issues. This contradicts the drivers for media formulation in perfusion cell culture where leaner media at a reduced cost is preferred.

The raw materials sourced for components in CDM can also impact the process, product yield and product quality, however, the exact impact has not been fully characterized. Compounding all of the previously discussed sources of uncertainty in media formulation, is the difficulty to even accurately measure all the components within CDM. This is primarily due to the difference in scales of concentration for different components with glucose in the order of 1–10 mmol/L and some metal ions in the parts per billion level.

Media formulation optimization should be undertaken for each individual process. Pan *et al.* [13] demonstrated that the optimal media for one clone is not optimal for another. The optimal media for clone A when applied to clone B exhibited a productivity that was approximately 70% of the optimal media for clone B [13]. Therefore, media formulation optimization is a critical part of process development. The decision on which strategy to use for media formulation optimization depends on many factors such as product type, cell type and process type [14].

Current industrial practices

There are several heuristics or industry practices, but no universal approach for media formulation and optimization, and a combination of approaches should be used to develop and optimize a formulation. Kennedy

and Krouse [15] broadly classify these approaches as open and closed strategies (Figure 1). Closed strategies involve a fixed number of known medium components and finding their optimum concentrations. The major disadvantage with closed strategies is components which could be beneficial will not be found, however, the constraints mean the optimization process is much quicker.

Open strategies do not constrain the number or identity of media components. However, the number of variables to be investigated in a truly open approach makes the task of media optimization unworkable. Therefore, researchers generally begin with a fixed initial media and published studies that closely match their cell line and process. Decisions about additional components and potential ranges are based on previous work, making scope of the task manageable.

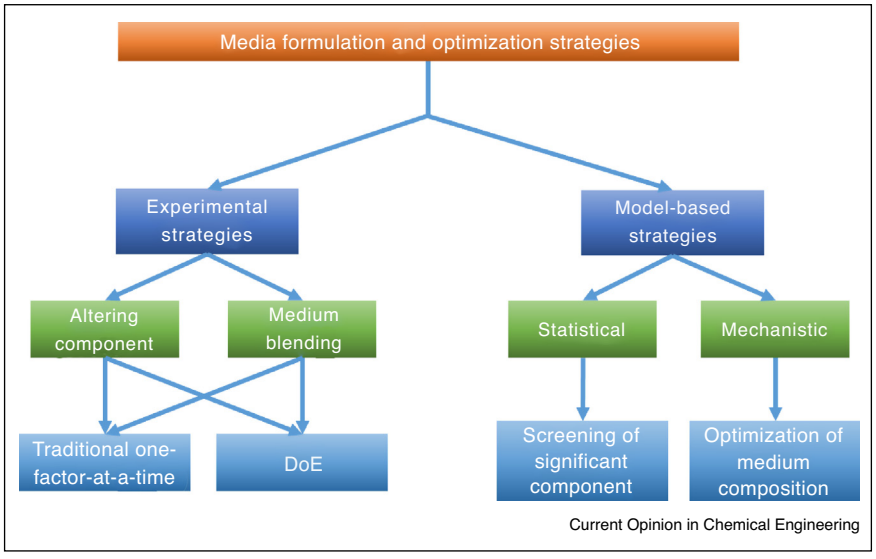
The **traditional approach** for media formulation involves changing one component at a time. It takes a number of iterations to optimize the concentration of all components. Many groups have used this traditional approach due to its ease of implementation. However, the experimental load can be burdensome as the number of components investigated increases, for example 10 components at 5 levels of concentration would require 50 experiments. It should be noted also, that since only one component is varied at a time, the interaction between the components is not considered, for example the optimal concentration of component A may be different once component B is varied. Therefore, even though the formulation will be improved it may not be truly optimized.

Design of Experiment (DoE) approaches allow the interaction effects of components to be explored. The response surface methodology (RSM) developed by Box and Wilson [16] has been used to formulate media for bacteria, fungi, and mammalian cells [17–19]. Several researchers have used the Central Composite design (CCC or CCF) with RSM optimization technique for media optimization [20–22].

Media Blending is a common technique used to optimize many media ingredients simultaneously [23,24••]. In this approach, mixtures are ranked on the basis of the highest performing blends, by mixing with already available media. When blending media, their composition should be known and significantly different so that the resulting blends are unique. However, since all components are changed simultaneously, drawing conclusions about causal relationships is impossible. Ultimately, media blending reduces the time and cost of optimization compared to the traditional and DoE approaches. As an example, Roullier *et al.* [24••] used media blending to increase the process titer by 40% using a high throughput methodology that took six weeks. 16 base CDMs containing the same 47 components were blended to create 376 unique media formulations. The collected data was used to build statistical models that could identify key components in the media. It was found that ferric ammonium citrate, panthothenic acid, valine, methionine, arginine, biotin, and serine were the components with the greatest impact on titer.

Effective media formulation requires accurate **analytical methods** to measure metabolites over the culture duration. Generally, metabolic analyzers (using combinations

Figure 1



Media formulation and optimization approaches.