Cell culture medium improvement by rigorous shuffling of components using media blending

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Abstract A novel high-throughput methodology for the simultaneous optimization of many cell culture media components is presented. The method is based on the media blending approach which has several advantages as it works with ready-to-use media. In particular it allows precise pH and osmolarity adjustments and eliminates the need of concentrated stock solutions, a frequent source of serious solubility issues. In addition, media blending easily generates a large number of new compositions providing a remarkable screening tool. However, media blending designs usually do not provide information on distinct factors or components that are causing the desired improvements. This paper addresses this last point by considering the concentration of individual medium components to fix the experimental design and for the interpretation of the results. The extended blending strategy was used to reshuffle the 20 amino acids in one round of experiments. A small set of 10 media was specifically designed to generate a large number of mixtures. 192 mixtures were then prepared by media blending and tested on a recombinant CHO cell line expressing a monoclonal antibody. A wide range of performances (titers and viable cell density) was achieved from the different mixtures with top titers

significantly above our previous results seen with this cell line. In addition, information about major effects of key amino acids on cell densities and titers could be extracted from the experimental results. This demonstrates that the extended blending approach is a powerful experimental tool which allows systematic and simultaneous reshuffling of multiple medium components.

Keywords Medium optimization · Screening · CHO cells · Mixture design · Media blending · Amino acids

Introduction

Mammalian cells have become the preferred host to produce recombinant proteins for the pharmaceutical industry. Dramatic improvements of titers of recombinant proteins (g/L) have been achieved during the last 20 years by process and medium optimization (Wurm 2004). Further improvements are still possible and there is a clear need to do so for industrial protein production at the tons/year scale.

Many medium components have been shown to be limiting factors for cell growth and/or productivity and their concentration has been considerably increased (Ozturk and Hu 2006). The optimization of cell culture medium is essential to improve bioprocess productivity. It can be performed after clone selection, before pivotal clinical trials or as a general approach to

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improve a generic medium. In addition to productivity, cell culture medium has been shown to have an impact on protein quality. Gawlitzek et al. (2009) has shown for example that medium components have an impact on recombinant protein glycoforms. Thus, the understanding of the relationships between medium composition and protein expression/quality is a key to improve process knowledge and enabling quality by design for manufacturing processes. Achieving medium optimization and understanding what components have an impact on the process is a challenging task, cell culture medium containing 50–100 components.

Different strategies to develop and improve media have been proposed (Kennedy and Krouse 1999). The best-known approach is the one factor at a time (OFAT) strategy, in which all factors except one component are kept constant. Although this is the simplest systematic approach, it is time consuming. Moreover, it suffers from severe limitations when it comes to identify an optimal combination of several components, because it cannot take into account interactions between components (Gheshlagi et al. 2005). Such interactions are ubiquitous within complex metabolic pathways and need special attention in medium optimization. With factorial designs, concentrations of several components are varied simultaneously and thus represent the method of choice to identify interactions and to find optimal combinations of components. Factorial designs have been used as medium development tools for bacterial systems (Wejse et al. 2003; Kiviharju et al. 2004; Zhao et al. 2005) and for mammalian cells such as CHO cells (Castro et al. 1992; Kim et al. 1998, 1999; Lee et al. 1999; Liu et al. 2001; Chun et al. 2003).

A drawback of factorial designs is the large number of experiments required to test a reasonable number of factors. To test six individual medium components in a full 2-level factorial design, 64 experiments are required. Considering the workload and the risk for human errors, 32–64 different conditions represent an upper limit if experiments are launched manually. This can be circumvented by using robotics such as the automated 96-deepwell cell culture platform, which allows to screen 96 (or a multiple of it) conditions per experiment (Kiss and Heath 2007).

However, increasing the number of components within a factorial experiment is problematic due to the fact that they are added as stock solutions. On one hand, the higher the number of added stock solutions, the higher the dilution of the original components, and

on the other hand, the smaller the added volume, the higher the stock solution concentrations. For current media enriched in amino acids and vitamins, this leads to severe solubility issues, sometimes excluding de facto large factorial experiments.

Various alternative approaches have been proposed for medium optimization, such as metabolic flux analysis, consumption profile study (Stoll et al. 1996; Rispoli and Shah 2009) and mixture designs (Yang and Jerums 2006). Mixture designs were shown to be appropriate for the identification of best media blends (Gifford et al. 2005). A mixture design was also used to identify the best mixture of three key components while keeping all the other required substances constant (Didier et al. 2007). Mixture designs are interesting because there is no issue of component solubility or medium dilution. Moreover, media blending executed on a high-throughput cell culture platform allows for a large panel of experiments to be performed.

Historically, media blending has been frequently used as a rapid approach to generate different mixtures from media that are available in the laboratory. These mixtures can be qualified as new media since they have unique compositions that differ from the original mother media. In many cases, a mixture will outperform any of the original mother media. A good example for such an approach using mixtures of two or more existing media with an improved performance is the DMEM/F12 ubiquitous medium that is frequently used as initial basal medium for further optimizations. However, up to this point the performance observed for such mixtures could not be linked to single medium components.

In this paper, the composition of the media to be blended has been specifically designed to obtain such additional information. The methodology of rational medium blending was applied to evaluate the effects of 20 amino acids at multiple concentrations on monoclonal antibody titers and cell growth. This article describes the new approach to generate a high quality data set that can be further exploited for medium optimization.

Materials and methods

Design and preparation of media formulations

A proprietary medium formulation based on DMEM/F12 components and supplemented with insulin,

pluronic F-68 and trace elements was further improved by media blending. Previously, the initial DMEM/F12 formulation went through several rounds of optimization by factorial designs resulting in a strongly fortified medium. Facing solubility issues with high concentrations of amino acids, the most recent fractional factorial designs testing more than 10 factors focused mainly on vitamins and trace elements. After several tests with these components it was assumed that they were no longer limiting factors of our enriched medium. Thus the next round focused on the 20 amino acids: all the amino acids were variable components, while the remaining components were kept constant.

The formal distribution of all the variable components is shown in Table 1. The concrete concentrations of the amino acids were chosen considering several points: (a) results and observations from previous factorial tests (b) the actual consumption rate (c) the chemical property of the amino acid. For most amino acids, the selected concentrations fitted the following criteria: level 0 is close to the concentration found in DMEM/F12, level 1 is the previously optimized concentration and level 2 is twice the concentration of level 1. For a formulation that would contain all components at level 2, the total concentration of all the amino acids would sum up to 143 mM. Since each formulation also contained several amino acids at the level 0, the actual sum was approximately 70 mM. Once the levels were set for all amino acids, the 10 different formulations were made according to Table 1.

These 10 different media were prepared from scratch by weighting the individual components. NaCl was added at the end in order to adjust the medium to 320 mOsm. The final composition of the 10 formulations corresponds to the previously enriched proprietary medium, except for the 20 amino acids. After sterilization by filtration, all the media were used immediately or stored for a maximum of 3 months at 2–8 °C.

Automated media blending

High throughput media blending was performed on a liquid handling workstation (Biomek FX, Beckman-Coulter, Inc., Fullerton, CA, USA). Table 2 shows some blending instructions and the resulting mixtures as examples. 192 mixtures were blended directly into

two 96-deepwell plates. The robot could complete this task within about 2 h. The presence of 15 mM of HEPES in the medium limited the pH drift during this period to an acceptable value. A few blends were mixed manually in order to run control experiments in shaken 50 mL tubes (De Jesus et al. 2004).

Cell cultures

For all experiments, a recombinant Chinese Hamster Ovary (CHO) cell line producing a monoclonal antibody (IgG) was used. Cells were grown in the in-house basal medium using ventilated 50 mL tubes (Filter Tube 50, TPP). Before launching experiments, the medium was exchanged by a brief centrifugation step of 10 min at 200 g. Cells were re-suspended in the basal medium with all the tested components at level 0 at a cell density of 10^7 viable cells/mL. This concentrated cell pool was diluted directly into the 192 medium formulations to a final density of 10^6 viable cells/mL. The 96-deepwell plates were incubated on an ISF-1 shaker (Kühner, Birsfelden, Switzerland) for a period of up to 2 weeks.

Analysis of samples

Samples were taken on day 6, 10 and 13. Cell densities were measured by cytometry (Guava PCA-96, Guava Technologies, Hayward CA, USA). The IgG titers were obtained by surface plasmon resonance (Biacore C, GE Healthcare, Chalfont St. Giles, UK).

Mixture design methodology for media blending

Media blending is an established approach to improve cell culture media. Multiple software supplying templates of mixture designs, including all the corresponding tools for statistical data analysis, are commercially available. Mixtures can contain 2, 3 or more mother media formulations. An example of mixtures proposed for 3 different media formulations (F1, F2 and F3) is shown in Fig. 1a. This classical approach is appropriate for the identification of a better formulation although it does not provide an explanation why a mixture performs better.

A more formal description of the example in Fig. 1a is represented in the first part of Fig. 1b as the “matrix of blends”. In order to correlate the results

Table 1 Matrix of formulations describing the composition of the 10 different medium formulations (F1-F10)

	c1	c2	c3	c4	c5	c6	c7	c8	c9	c10	c11	c12	c13	c14	c15	c16	c17	c18	c19	c20
F1	1	1	2	1	1	0	0	2	2	2	1	0	0	2	0	0	2	1	1	2
F2	2	1	1	1	2	0	2	2	1	0	0	2	0	1	2	1	0	2	0	1
F3	0	0	0	2	0	1	1	1	2	2	2	1	1	0	1	2	0	1	0	0
F4	2	0	0	2	2	0	1	1	1	1	1	2	2	0	0	1	2	0	2	1
F5	1	2	1	0	0	1	2	0	2	0	2	1	1	1	1	0	0	0	2	2
F6	2	1	2	1	1	2	1	2	0	1	1	0	2	0	0	1	1	1	1	2
F7	1	2	1	2	1	1	0	0	0	0	2	0	1	2	1	0	1	2	1	0
F8	0	1	0	0	2	2	1	0	1	1	1	1	0	1	1	2	1	2	2	1
F9	1	2	1	0	0	1	0	1	1	2	0	2	2	1	2	2	1	1	0	0
F10	0	0	2	1	1	2	2	1	0	1	0	1	1	2	2	1	2	0	1	1

This matrix considers only components that are variable within distinct formulations. While in this paper components c1-c20 corresponded to the 20 amino acids, c1-c20 could include any other component as well. The values of 0, 1 or 2 represent relative concentrations, absolute values can be different for each component

Table 2 Blending recipes (a: B1-B192) and the subsequent mixtures (b: M1-M192) created from the 10 formulations F1-F10 (see Table 1)

	F1			F2		F3		F4		F5		F6		F7		F8		F9		F10	
(a)																					
B1	–			4/9		–		1/9		–		–		4/9		–		–		–	
B2	–			–		2/9		–		–		–		4/9		–		3/9		–	
B3	–			–		4/9		–		–		–		3/9		–		2/9		–	
B4	1/9			4/9		–		–		4/9		–		–		–		–		–	
B189	–			4/9		3/9		–		2/9		–		–		–		–		–	
B190	–			–		–		–		1/9		4/9		–		–		–		4/9	
B191	–			3/9		–		–		–		4/9		2/9		–		–		–	
B192	–			–		–		–		–		2/9		–		4/9		–		3/9	
	c1	c2	c3	c4	c5	c6	c7	c8	c9	c10	c11	c12	c13	c14	c15	c16	c17	c18	c19	c20	
(b)																					
M1	1.6	1.3	0.9	1.6	1.6	0.4	1.0	1.0	0.6	0.1	1.0	1.1	0.7	1.3	1.3	0.6	0.7	1.8	0.7	0.6	
M2	0.8	1.6	0.8	1.3	0.4	1.0	0.2	0.6	0.8	1.1	1.3	0.9	1.3	1.2	1.3	1.1	0.8	1.4	0.4	0.0	
M3	0.6	1.1	0.6	1.6	0.3	1.0	0.4	0.7	1.1	1.3	1.6	0.9	1.2	0.9	1.2	1.3	0.6	1.3	0.3	0.0	
M4	1.4	1.4	1.1	0.6	1.0	0.4	1.8	1.1	1.6	0.2	1.0	1.3	0.4	1.1	1.3	0.4	0.2	1.0	1.0	1.6	
M189	1.1	0.9	0.7	1.1	0.9	0.6	1.7	1.2	1.6	0.7	1.1	1.4	0.6	0.7	1.4	1.1	0.0	1.2	0.4	0.9	
M190	1.0	0.7	1.9	0.9	0.9	1.9	1.6	1.3	0.2	0.9	0.7	0.6	1.4	1.0	1.0	0.9	1.3	0.4	1.1	1.6	
M191	1.8	1.2	1.4	1.2	1.3	1.1	1.1	1.6	0.3	0.4	0.9	0.7	1.1	0.8	0.9	0.8	0.7	1.6	0.7	1.2	
M192	0.4	0.7	1.1	0.6	1.4	2.0	1.3	0.8	0.4	1.0	0.7	0.8	0.8	1.1	1.1	1.4	1.3	1.1	1.4	1.2	

Row B1 describes the generation of the first mixture M1 containing the formulations F2, F4 and F7 at a ratio of 4:1:4. Row M1 lists the exact concentration of c1-c20 for this first mixture. The component c1 for example, is at the relative level of 1.6 in M1. From the 192 blending recipes and the subsequent mixtures, only 8 are shown as examples

with specific medium components and to develop data analysis and interpretation, this matrix had to be extended: first by considering the compositions of the mother media or formulations and second by taking

into account the new compositions of the resulting mixtures. Formally, as shown in Fig. 1b, a “matrix of formulations” and a “matrix of mixtures” are added to the “matrix of blends”.

Fig. 1 Basic mixture design and its extension for three different mother media formulations (F1, F2 and F3). **a** Classical mixture design representing pure formulations (1, 2 and 3), symmetric binary mixtures (4, 5 and 6), asymmetric ternary mixtures (7, 8 and 9) and symmetric ternary mixture (10). **b** Formal representation of the basic mixture design as a “matrix of blends” and extension of this design by two more matrices: a “matrix of formulations” describing the media formulations F1, F2 and F3 that contain components c1–c6 and a “matrix of mixtures” that describes the exact compositions of the mixtures M1–M10 for the components c1–c6

To our knowledge such an extension has not been reported for media blending experiments. A main reason might be that several conditions need to be fulfilled before such an extension is reasonable: the components of interest have to be variable within the different media, the media composition has to be known and the number of variable components should be realistic. By choosing media directly from the shelf, one has little control over the actual composition. In some cases, virtually all the components differ in their concentrations (e.g., DMEM and F12 medium), while other media differ only in few components. In contrast, this study uses a small set of specifically designed media in which components were either fixed at the desired concentration or were systematically changed for each medium formulation.

To demonstrate the feasibility of the extended media blending concept, the component variation was restricted to the different amino acids. Ideally, these 20 variable components should be studied in a total independency from each other. But in such a case, at least 21 different medium formulations should be designed and prepared from scratch—a number that exceeded our capacity for media preparation. As a compromise, the number of medium formulations to

be prepared from scratch was fixed to 10 at the beginning of this project. Even if this number is already at the upper level of what is used in typical blending experiments—it allows the creation of a large number of new mixtures—it is manifestly not sufficient to optimize 20 different components. Being aware of this limitation, it was taken into account that the new design would inevitably lead to some correlations among certain components which could cause confounding effects among the variables. Considering the negative impact of confounded variables on statistical analysis, minimizing correlations between variable components within the 10 media had the highest priority.

The design of these 10 media turned out to be more difficult than initially expected. Potentially the number of possibilities for how to fix the concentrations of 20 components for 10 media is endless, but only specific combinations represent a suitable “matrix of formulations”. It is an unsolved challenge to find the best matrix. Since no appropriate templates were found in the literature, a custom-made matrix was established. A normalized concentration level of 0, 1 or 2 was empirically assigned to every single amino acid for all the medium formulations F1–F10. The

levels 0 and 2 were enforced for each component to occur exactly 3 times within the 10 formulations. Moreover, correlations between the components of the “matrix of formulation” were used as the principal restrictive criterion (R^2 was limited to values of <0.3). The final design, obtained after several iterative rounds of improvements, is shown in Table 1. Within these 10 formulations, the amino acids are extensively shuffled and each formulation contains a unique combination of these components.

The next step was the definition of the blending recipes or the “matrix of blends”. This matrix had to fit to the “matrix of formulations”. A few examples of the final mixture design are given in Table 2. The mixture design exclusively focuses on ternary mixtures. For practical reasons, the mixture design and the blending ratios were adapted to the robotic platform that worked optimally when the mixtures for two 96-well plates were blended with 9 fractions. Furthermore it was desirable to test each component at several different levels. Applying asymmetric blends of 9 fractions to the 10 media formulations yielded in 19 different levels (concentrations) for each component within the resulting 192 mixtures. The exact number of mixtures containing component c1 at the different levels is given in Fig. 2a. All the other components had a similar frequency profile (data not shown).

All frequency profiles show a rather conservative distribution of the levels with a maximal frequency around level 1. Such a distribution should assure that only a few components are strongly modified at the same time, while the others are moderately shuffled close to level 1. Changing the concentration of a component certainly can improve the medium, but there is also a potential risk to get less performing compositions: lower concentrations (level 0) can cause limitations, while higher concentrations (level 2) can be inhibitory or toxic. When too many components simultaneously occur at such non-optimal concentrations, the chances for new mixtures to perform well would decrease considerably. Thus the proposed design avoids that any mixture contains more than one component at the lowest or highest level. As indicated in Table 2 b, M192 contains one component (c6) at level 2 while all the others are distributed between level 0.4 and 1.4.

A good mixing and formulation design minimizes the correlations between components within the final mixtures. Our final design was checked by calculating

Fig. 2 Statistical evaluation of the complete set of 192 mixtures. **a** Distribution profile of relative concentrations of components c1 within the different mixtures. While many additional levels between 0 and 2 are created by the asymmetric ternary mixtures, the most frequent levels are close to level 1 which occurs 18 times. **b** Correlations between c1 and the variable components of the final mixtures

all the correlations occurring in the “matrix of mixtures”. Correlations of c1 with the other components are shown as a representative example in Fig. 2b. Most correlations (R^2) with the other variable components are below values of 0.2. The number of confounding variables is acceptable and their impact is limited. In the case of c1, there is only c8 with a correlation above 0.2.

Results and discussions

Experimental results of medium blending

In this study, 10 mother media containing variable concentrations of 20 amino acids (c1-c20) were blended to obtain 192 different mixtures. Blending these 10 formulations in various combinations and ratios changed concentrations of the variable components (amino acids), but had no impact on the other medium components. This simultaneous shuffling of 20 amino acids at multiple concentrations was aimed

at improving an in-house medium (for more details see “Materials and methods”). Indeed, the approach was successful with nearly half of the mixtures outperforming the current medium. A selection of representative results is shown and discussed here.

The most relevant parameter, the final IgG titer at working day 13, ranged from 50 to 2,400 mg/L (Fig. 3a), indicating that protein expression is strongly linked to the amino acid composition.

Before analyzing the large data set in more details, the predictability of the 96-wells plate results was verified. For this purpose, two mixtures, the best performing M27 and the lower performing M86, were selected and manually reproduced in ventilated 50 mL tubes. The reproduction of the selected mixtures was straightforward (manual mixture of maximal 3 specific medium formulations according to original blending instructions). The titers obtained in the large blending experiment could be confirmed in this second test (Fig. 3b). The consistency between these results

indicates that the titer variations revealed in Fig. 2a are not due to experimental noise. As a consequence, the differences in titers are related to the composition and balance of the amino acids.

The viable cell count at day 6 was expected to be a suitable indicator for the evaluation of the impact of the medium composition on cell growth. At this point, the culture should have reached the maximal cell density. No further increase in cell number was typically observed for the initial medium, but cells maintained their good viability for a few more days (data not shown). On day 6, several mixtures delivered cell densities of 8–11 million cells per mL (Fig. 4a). Other mixtures yielded considerably lower cell densities, indicating either growth inhibition or a limitation due to an essential component depletion. Although high product titers are in general associated with a good cell growth, in certain cases cell growth has to be balanced with maximal titers, as observed for the M27 mixture generating the highest final IgG titer. The cell density for this mixture was 5×10^6 cells per mL at day 6, a value clearly below most of the other mixtures.

Fig. 3 Final titers measured after 13 days of batch culture. **a** Overview of IgG concentrations for all of the 192 mixtures. Full symbols assist the identification of selected mixtures in this and the following graphs. **b** Titer confirmation of the best performing mixture (M27) and a lower performing mixture (M86) in ventilated 50 mL tubes

Attribution of effects to single components

The matrices developed and tested for this project are essentially equivalent to those in Fig. 1b, except for the fact that they are larger. The “matrix of mixtures” described in this article is usually ignored in blending experiments. However, such a matrix is crucial for any further detailed data analysis, since it describes the exact composition of each mixture. Once the different compositions are known, the concentration of the variable components can be plotted against the viable cell counts or IgG titers. By systematically doing this for all amino acids, key components could be identified and main effects attributed to a small number of amino acids.

Asparagine (c3) was found to have a strong effect on cell growth (Fig. 4b). Whereas 10^7 cells per ml were obtained at relative levels of 1.3 and higher, such densities were not achieved at lower c3 concentrations (levels between 0 and 1). Less than 3×10^6 cells per mL were obtained for the two mixtures M72 and M188 containing the lowest c3 concentration. Since for these two particular mixtures all the other amino acids were present at intermediate concentrations, the poor cell growth was most likely caused by the low c3

Fig. 4 Viable cell densities obtained in the 192 mixtures at day 6. **a** Overview of viable cell numbers measured for all the mixtures without considering information about the concentration of components. Viable cell number plotted against encoded concentrations of c3 (**b**) and c1 (**c**)

concentration. c3 was suspected to be the main limiting factor in several other mixtures as well, particularly below a level of about 1. The potential limitation is emphasized by the gray line (Fig. 4b). The absence of data points above the gray line indicates that c3 is a dominant limiting factor for this cell line. Despite multiple indications for c3 limitations, the correlation between c3 concentrations and the viable cells numbers is rather weak (linear coefficient of determination $R^2 = 0.26$). Such a low coefficient seems to contrast with the strong effects of

c3 on cell growth. However, it has to be considered that many mixtures contain other amino acids at a limiting concentration. For these mixtures, c3 is not expected to be correlated with the cell number since c3 is no longer the principal limiting factor.

Several components had no apparent effect over the tested range. The profile of alanine (c1) illustrates such a case: c1 had little impact on cell growth (Fig. 4c). The profile of the plot for c1 is well distinguishable from the one obtained for c3 since the maximal cell concentration was reached whatever the concentration of c1 was. These two examples demonstrate that—despite the weak correlations between components—the non-orthogonal design is still useful for testing many components in parallel.

Another key component is tyrosine (c19). This component represents an interesting case since its effect strongly depends on the analyzed response. Regarding cell growth (Fig. 5a), c19 was potentially negative above the level 1 while it was only a weak limiting factor at low concentrations. At the relative level 0, the M151 mixture yielded 6×10^6 cells per mL, which still can be considered as a reasonable number. Regarding titers (Fig. 5b), the M151 mixture stayed far below the performance obtained with the other treatments, with less than 300 mg/L IgG. Up to the level 0.5, component c19 seemed to be a strong limiting factor for IgG production. The highest titer was obtained at the level 1.1 indicating that c19 was no longer a limiting factor at this concentration. It is not clear from these data, whether concentrations beyond this value cause a reduction of titers due to potential negative effects on cell growth. Additional experiments are needed to address this question.

Discussion and outlook on future designs

We present here an extended blending approach which has several practical advantages since its execution is simple and straightforward. While we formalized the approach and also demonstrated that the concept works nicely, we realized that no mathematical models and software is available that would be tailored to design and analyze this type of experiments. The simultaneous shuffling of the 20 amino acids at multiple concentrations by media blending created a large data set rich in information. While multivariate analysis (MVA) is a highly attractive method to analyze large data sets with many variables

Fig. 5 Distinct effects on cell growth and product titer related to the concentrations of c19. **a** Viable cells density at day 6. **b** IgG titers at day 13

and responses, this statistical tool did not provide a real added value for the analysis of our particular type of data. The main problem with the MVA is that for each variable several confounding factors exist. The weak correlations among variables dramatically reduce the application of MVA. Designs without any correlations would be feasible and examples have been described in the literature. For example, Plackett and Burman (1946) proposed a nice orthogonal design that would allow the user to distribute 19 different components into 20 mother media. Yet avoiding any correlation causes other constraints: this design doubles the number of mother media and leads to a reduced range of final concentrations, since the concentrations are set only at two levels. Alternatively, it would be possible to have an orthogonal design with components at three different levels, but this would further increase the number of mother media: an orthogonal design for 16 components would require 36 media (Muñoz and Brereton 1998).

Our systematic blending approach should be applicable to other desirable medium components and is not limited to chemically defined media. In fact any medium, including those containing undefined components such as serum or hydrolysates and those with an unknown composition, could be evaluated by this approach. In case that the component concentrations are not disclosed, the absolute value of the level 0 for the variables is not known. Nevertheless, as long as the desired components can be added to the medium to get level 1 and 2, testing and interpretation should be possible.

More sophisticated designs (e.g., larger sets of medium formulations, more components and reduced correlations) should be possible. Eventually, the template for variable components might be extended to all medium components. This would allow the user to shuffle and screen all components in one shot by a rationalized high throughput experiment. Such an approach could be applied to new recombinant cell clones in order to get early information about the real potential of individual clones and to identify components that are needed for specific clone optimization.

In conclusion, improved blending designs broaden the possibilities for medium screening/optimization. Media blending can be an efficient tool to screen and shuffle numerous components simultaneously, without the need for concentrated stock solutions. The data generated with such a methodology can be analyzed and used for further optimization. While in the current study cell density and titer were targeted, with a larger analytical panel other quality attributes such as glycosylation, deamidation, aggregation, methionine oxidation, fragmentation and loss of C-terminal lysine could be included to tailor the medium to specific molecules. Rational media blending is a promising approach that can lead to rapid identification of rebalanced high performing medium compositions and it has a potential to deliver valid information about the most important effects of individual key components.

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