Employing design of experiments in custom medium development to increase protein quantity and quality

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Employing design of experiments in custom medium development to increase protein quantity and quality

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Introduction

This work describes the development of a custom cell culture medium for the production of a highly glycosylated therapeutic protein. During the initial medium screening campaign, several commercial media were evaluated. The control medium used in this study provided valuable results during the initial screening.

As a first step, we reviewed information specific to the CHO H cell clone and selected seven prototypes from a formulation library. Four of the seven prototypes were selected for DoE simplex lattice mixture design studies based on growth and viability performance. Prototype mixtures were evaluated for peak viable cell density (PVCD), integral viable cell area (IVCA), product levels, and product quality.

Results for the top two prototypes showed dramatic increases in PVCD, IVCA, and product levels as compared with the control. Additionally, noticeable improvements in quality were seen when compared with the control. Specifically, increases in tri-sialylated and tetra-sialylated glycans as well as tri-antennary and tetra-antennary glycan structures.

Materials and methods

Cell culture growth parameters and analysis
Cells were grown in 37°C, 5% CO_2 incubators in 125 mL flasks (50 mL culture volume) on shaker platforms with 19 mm orbits set at ~ 120 rpm. Beginning at ~ 48 h post-inoculation and continuing daily, cultures were sampled and cell denisties and viabilities were monitored using Vi-CELL™ cell viability analyzer (Beckman Coulter) utilizing trypan blue exclusion technique.

Preliminary medium screening and analysis
Basal media from prototype families known to perform well with CHO clones were selected. Media were supplemented with 4 mM HyClone™ L-glutamine (GE Healthcare) and cells were grown in terminal batch cultures to < 90% viability. Growth curve and viability profiles were used to select media that supported high cell densities for the CHO H clone.

DoE mixture design
A DoE simplex lattice mixture design study was created using the top four prototypes selected from the preliminary medium screening. JMP™ statistical software (SAS Institute Inc.) was used to generate mixture conditions. The resulting study consisted of 27 conditions plus control medium.

Batch cell growth studies and analysis
All prototype mixtures were supplemented wiht 4 mM L-glutamine and growth in terminal batch cultures to < 80% viability. Growth curves and viability profiles were generated for the 27 prototypes and control. PVCD and IVCA were generated for each condition and compared with product quantity.

Protein quantification and quality analysis
ELISA was used to determine protein quantity. ELISA conditions were analyzed in triplicate at a 1:40 000 dilution factor. Due to high sample volume and large dilution factor in this study, dilutions were accomplished using a HTS robotics system (Biotech Precision). This use of HTS equipment allowed consistent fluid manipulation on the 25 96-well plates used for the ELISA analysis.

Protein quality was assessed using HPLC. Sialylated structures were analyzed using weak anion exchange HPLC (WAXHPLC). Antennary structures were analyzed using normal phase HPLC (NPHPLC).

Conclusions

- Prototypes 6 and 11 were chosen based on PVCD, IVCA, product yield, and quality improvements over the control. Note that even though prototype 8 exhibited slightly higher IVCA and product yield that prototype 11, 8 was excluded based on formulation similarity to 6.
- In addition to quality improvements in sialylation and antennary structures, prototype 11 exhibited a PVCD at 230%, an IVCA at 256%, and peak product levels at 162% of the control medium.
- In addition to quality improvements in sialylation and antennary structures, prototype 6 exhibited a PVCD at 219%, an IVCA at 276%, and peak product levels at 192% of the control medium, Ultimately, Prototype 6 was chosen for cell banking and further studies because of slightly higher product levels and quality.

Abstract

Nutrient requirements differ widely among mammalian cell clones producing recombinant proteins. This variation is also evident among various clones derived from the same parental cell line. In this case, a proprietary Chinese hamster ovary (CHO) cell clone (CHO H) is producing a glycosylated protein in which the glycosylation patterns dictate the bioreactivity of the compound. To approximate optimized formulations meeting specific clonal requirements, we utilized design of experiments (DoE) methods. After initial screening, DoE mixture designs were evaluated to determine optimized mixtures supporting higher protein quantity and quality. High-throughput screening (HTS) equipment aided evaluations. During this study, protein quantities were notably improved over the control medium. More importantly, the medium development campaign results indicated glycosylation quality improvements over the control medium.

Results

By the use of DoE mixture design, growth characteristics (PVCD and IVCA) were greatly improved as compared with the control medium (Fig 1 to 4). Additional IVCA offered increased opportunity for cells to produce and secrete valuable therapeutic proteins. Using DoE mixture design, protein yields could be dramatically improved as compared with the control medium (Fig 5 to 7). Analysis of PVCD, IVCA, and peak product level shows the superior performance of prototypes 6, 8, and 11 compared with other tested prototypes (Fig 8). The ternary plots highlight the hot spots in the mixtures with respect to the optimal medium composition of the four prototypes taken from our CHO prototype library (Fig 9 to 11). These plots indicate that mixtures higher in medium prototype 3 tend to yield higher PVCD, IVCA, and peak product levels. Figures 12 to 14 show the improvements in ratios of tri-sialylated and tetra-sialylated glycans of proteins produced in prototypes 6 and 11 as well as in the control medium. Figure 15 shows the profiles of tri-antennary and tetra-antennary glucan structures of proteins produced in prototypes 6 and 11 as well as in the control medium.

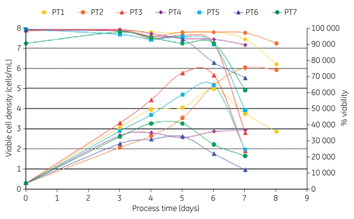

Fig 1. Growth curves and viability profiles during initial screening. Selected prototypes from our CHO formulation library were used. Note that prototypes 1, 2, 3, and 5 were used in DoE mixture design.

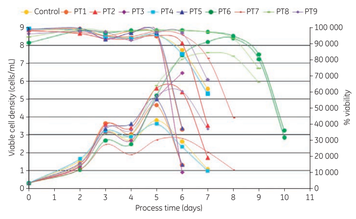

Fig 2. Growth curves and viability profiles for the control medium and DoE mixture design prototypes 1 to 9. Prototypes 6 and 8 yielded the highest PVCDs and IVCAs.

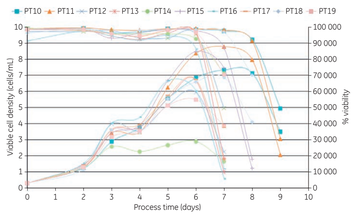

Fig 3. Growth curves and viability profiles for DoE mixture design prototypes 10 to 19. Prototype 11 yielded the highest PVCD and IVCA.

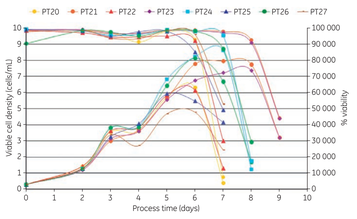

Fig 4. Growth curves and viability profiles for DoE mixture design prototypes 20 to 27. Prototype 21 yielded the highest IVCA.

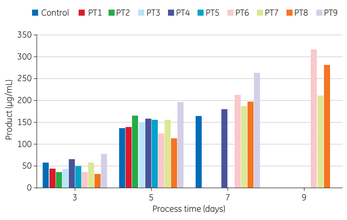

Fig 5. Productivity profiles for the control medium and DoE mixture design prototypes 1 to 9. Prototypes 6 and 8 yielded the highest protein levels.

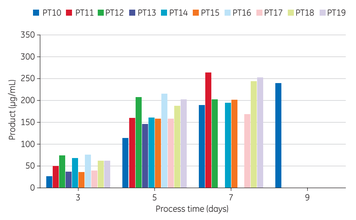

Fig 6. Productivity profiles for the control medium and DoE mixture design prototypes 10 to 19. Prototypes 11 yielded the highest protein level.

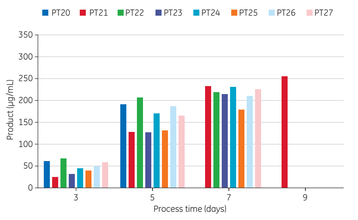

Fig 7. Productivity profiles for the control medium and DoE mixture design prototypes 20 to 27. Prototypes from this set produced less than prototypes 6, 8, and 11.

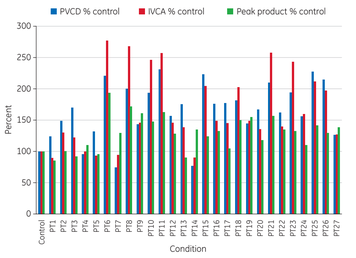

Fig 8. PVCD, IVCA, and peak product levels are expressed as a percentage of the control medium. Prototypes 6, 8, and 11 were most desirable based on high peak product levels (most important), IVCA, and PVCD.

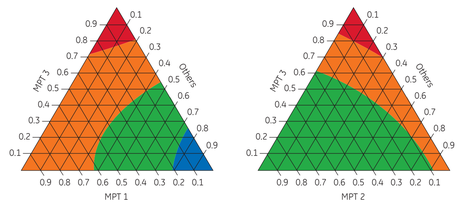

Fig 9. Ternary plots for peak product of DoE mixture design prototype conditions. "Hot spots" (red) on these plots show that mixtures higher in medium prototype 3 (MPT3) from the initial screening yielded higher product levels.

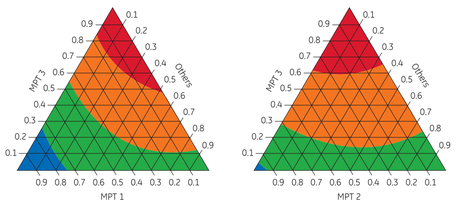

Fig 10. Ternary plots for peak product of DoE mixture design prototype conditions. "Hot Spots" (red) on these plots show that mixtures higher in medium prototype 3 (MPT3) from the initial screening yielded higher IVCA.

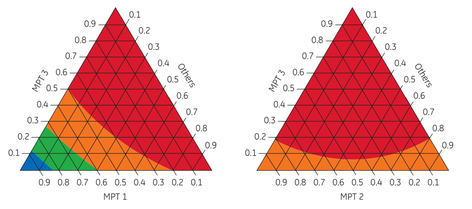

Fig 11. Ternary plots for peak product of DoE mixture design prototype conditions. "Hot Spots" (red) on these plots show that mixtures higher in medium prototype 3 (MPT3) from the initial screening yielded higher product PVCD.

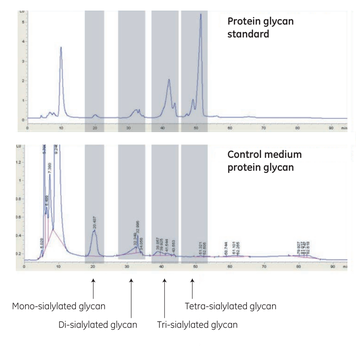

Fig 12. WAXHPLC analysis of protein glycan standard and control medium protein glycan. Note the lack of tri-sialylation and tetra-sialylation.

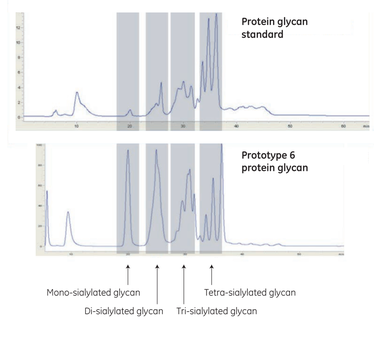

Fig 13. WAXHPLC analysis of protein glycan standard and prototype 6 protein glycan. Note the increase in tri-sialylation and tetra-sialylation compared with the control medium protein glycan.

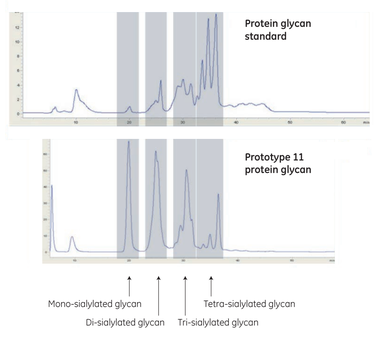

Fig 14. WAXHPLC analysis of protein glycan standard and prototype 11 protein glycan. Note the increase in tri-sialylation and tetra-sialylation compared with the control medium protein glycan.

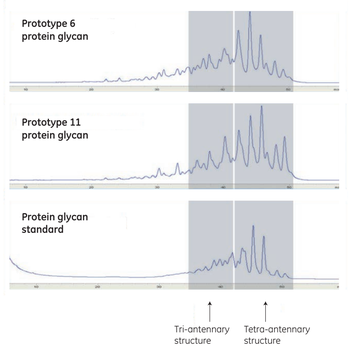

Fig 15. NPHPLC analysis of protein glycan standard prototype 6 protein glycan, and prototype 11 protein glycan. Note similarity of the tri-antennary and tetra-antennary profiles of the prototype media compared with the protein glycan standard.

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