Defining Non-Animal Derived Supplements For the Optimisation of Mammalian Cell Culture Media Using DoE Methodologies

Chemically defined and animal-derived free components media are required for bioprocess development and operation using mammalian cell as platform for the production of biopharmaceuticals

The supplementation of commercial chemically defined media with specific compounds is studied for the optimisation of Chinese hamster ovary (CHO) and HEK 293 cell culture.

The design of experiments (DoE) approach is used to first screen the most efficient compounds and further determine their optimal conditions. This methodology allows to test simultaneously various compounds and also to determine potential interaction among them, additionally to their individual effects.

Using this methodology, the culture of CHO S cell growth could be improved by using a supplementation of selenium (1 µg/L), r-transferrin (20.2 mg/L) and r-insulin (26.1 mg/L). Further, HEK 293 cells could also be improved both in cell growth and also transfection efficiency using FreeStyle medium supplemented with CHO S cells, the optimal conditions were found for FreeStyle medium supplemented with r-insulin (19.8 mg/L), r-transferrin (1.6 mg/L) and lipid mix (0.9X).

Editorial

Mammalian cells are one of the most widely used platforms for the production of biopharmaceuticals. Among them, CHO and HEK 293 cells are extensively used for the production of recombinant proteins, vaccines and viral vectors.

The use of serum-free medium has grown significantly in industrial applications where the use of bovine serum represents a safety hazard as well as a source of unwanted contamination for the production of biopharmaceuticals. Furthermore, it has become more generally adopted the use of animal derived free and chemically defined culture media. Indeed, the culture media used for animal cell culture are very complex.

Historically serum has been a crucial component of their composition, as a provider of complex biological molecules such as hormones, growth factors as well as numerous low molecular weight nutrients [1] . The emergence of industrial scale mammalian cell culture for the production of protein pharmaceuticals presented a new challenge for cell culture medium design, and quality control aspects arose from the use of foetal bovine serum (FBS). The issues of reliability of supply, variability in performance and the risk for biological contaminants (mycoplasmas and viruses), created serious safety concerns in the regulatory agencies, further increased by the emergence of prion related diseases, specifically bovine spongiform encephalomyelitis.

This turned in an increasing use of chemically defined non-animal sourced medium components to replace both serum and medium supplements purified from animal sources, such as insulin, transferrin and albumin [2] [3] [4]. Insulin serves as a growth and maintenance factor and is considered to be important for serum-free cultures [5]. Insulin

stimulates uridine and glucose uptake and synthesis of RNA, proteins and lipids; it also increases fatty acid and glycogen synthesis [6].

Transferrin is one of the most essential growth promoting supplements in serum-free medium, and its omission causes severe inhibition of cell growth [7]. Transferrin is an iron binding glycoprotein that interacts with surface receptors. It is closely related to the transport of iron across the plasma membrane [8]. Transferrin has additional in-vitro functions, e.g., chelation of deleterious trace materials that are unlikely replaced by other components.

Selenium is a trace element essential for mammalian cell cultures [9]; its mechanism is poorly understood although there is evidence that selenium enhances growth rate in serum free-cultures [10]. Lipids are required for proliferation, differentiation, and antibody secretion. They play a major role in the cell membrane which is composed of a phospholipid bilayer, and help in the transmission of nutrients into the cell and excretion of proteins out the cell [11]. Albumin, most commonly known as human serum albumin (HSA), prevents toxic effects of free fatty acids on cells in culture, acts as a metal ion binding protein and it also has antioxidant effects [12].

The optimisation of the media composition in terms of selecting the most appropriate components as well as their optimal concentration can be performed using Design of experiments (DoE). DoE is a useful tool that enables to determine simultaneously the individual and interactive effects of many factors that could affect the response function [13]. The DoE can be divided in two steps; one for screening the most effective compounds and one to optimise their concentration, for example to support cell growth.

Plackett-Burman design is a two-level multifactorial design based on the rationale known as balanced incomplete blocks. With it, up to N-1 components can be studied in N experiments, where N must be a multiple of 4 [14]. To be mentioned here than the two levels tested are usually defined performing a series of toxicity tests for each individual compound. In them, the dose-response effect of a given compound on cell viability is studied, and the upper limit for the screening tests is set at the concentration showing a clear decline in the viability curve.

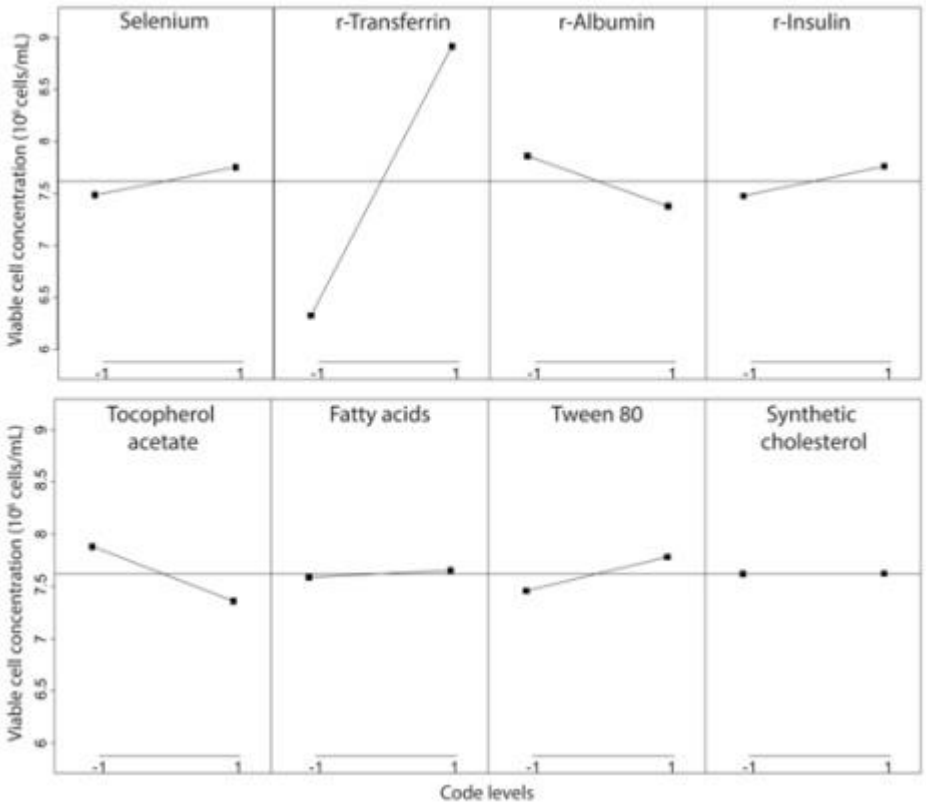
After finding the most relevant factors that influence cell growth, the next step is to optimise the concentrations of these components in the growth medium. Response surface methodology (RSM), a powerful experimental methodology for seeking the optimum conditions for a multivariable system, is the chosen technique for optimisation [15]. RSM comprises mathematical and statistical procedures that can be used to study relationships between one or more responses and a number of independent variables, and it also generates a mathematical model that describes the overall process [16]. A Box-Behnken design has been chosen as the RSM. Combining 2k factorials with incomplete block designs forms this design. The resulting designs are usually very efficient since they require few number experiments and allow determining combined effects of different factors.

CHO S cell growth optimisations were carried out using the chemically defined medium FreeStyleCHO, and up to eight non-animal derived compounds were tested as supplements (r-insulin, r-transferrin, r-albumin, selenium, tocopherol acetate, synthetic cholesterol, tween 80 and fatty acids) in order to increase maximum cell density. It is generally recognised that the expression of a recombinant protein in CHO cell cultures is directly proportional to the integer of viable cells (IVC) of the growth curve. Plackett-Burman initial screening

experiments indicated that only three of the supplements (r-insulin, r-transferrin and selenium) had positive effects on cell growth as presented in Figure 1A. To be noted here that in spite of Twin 80 having a positive slope in Figure 1A, therefore, and individual positive effect, it was discarded for the next round of optimisation due to its negative interaction with some of the other supplements tested. The concentration of the three selected components was further optimised by means of a Box-Behnken, and surface responses are presented in Figure 2A. A maximum cell density of 10 x 106 cells/mL in batch mode was achieved for FreeStyleCHO medium supplemented with selenium (1 µg/L), r-transferrin (20.2 mg/L) and r-insulin (26.1 mg/L), while only 8 x 106 cells/mL could be achieved with unsupplemented medium.

For HEK 293 cells, two aspects were tested, cell growth as well as efficiency of transient transfection, since this is one of the specific uses of this cell line. In general, transient transfection is used and the process development level, when relatively small quantities of a given molecule need to be produced rapidly for structural and functional testing, including pre-clinical trials. Transient transfection in this particular case was performed to produce HIV-1 Gag-GFP virus like particles. The medium selected was FreeStyle, and supplementation with non-animal derived components including recombinant insulin, transferrin, albumin and a mixture of lipids.

Plackett-Burman initial screening experiments indicated that only three of the supplements (r-insulin, r-transferrin and lipid mix) had positive effects as presented in Figure 1B. The concentration of these components was further optimised by means of a Box-Behnken, and surface responses are presented in Figure 2B. Such optimisation resulted in improved HEK 293 cell growth and VLP production for optimal concentrations of r-insulin (19.8 mg/L), r-transfeerin (1.6 mg/L) and lipid mix (0.9X). The maximum cell density attained using the optimised culture medium was 5.4×106 cells/mL in batch mode, almost double of that observed using the unsupplemented medium (2.9×106 cells/mL). Best production performance was attained when cells were transfected at mid-log phase (2-3×106 cells/mL) with medium exchange at the time of transfection using standard amounts of plasmid DNA and polyethylenimine as previously reported (17). By using optimised production protocol, VLP titers were increased 2.4-fold obtaining 2.8 µg of Gag-GFP/mL corresponding to approximately 8.2×109 VLPs/mL.



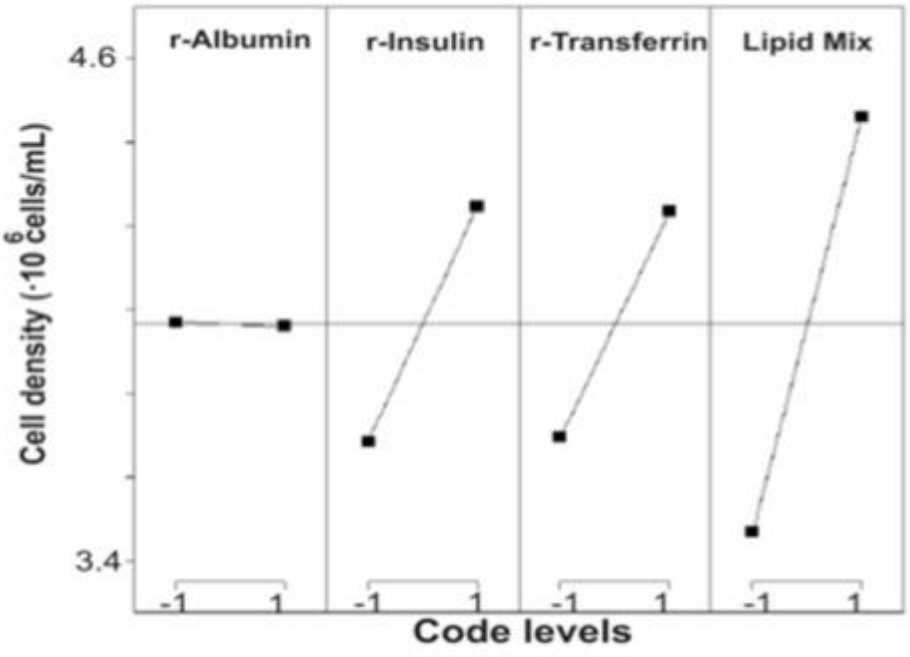


Figure 1. Results for the screening step in the selection of supplements using the Plackett-Burman methodology. Positive slopes indicate a positive effect of a particular supplement. A (top), results obtained for the CHO cell line with FreeStyleCHO medium. B (bottom), results obtained for HEK 293cells with FreeStyle medium.

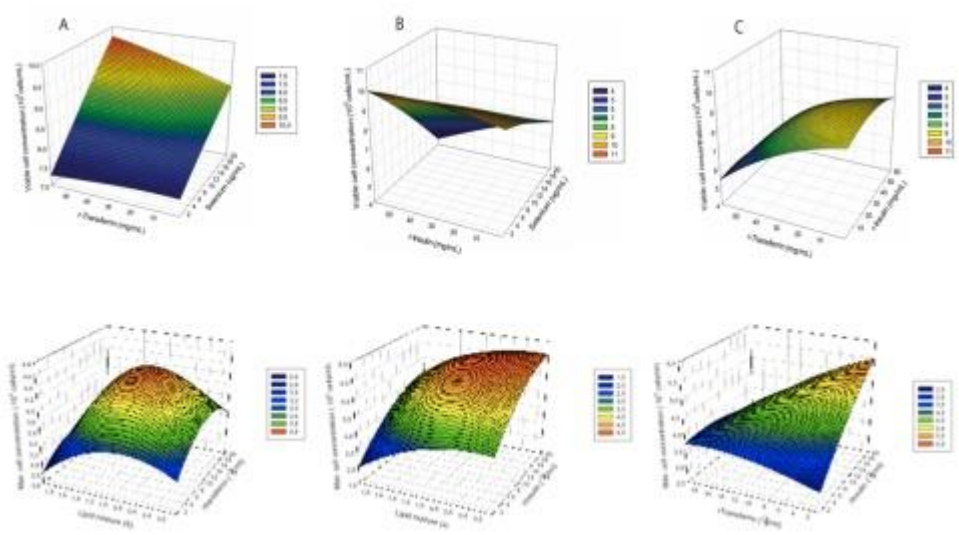


Figure 2. Results for the optimisation step using the Plackett-Burman methodology. Surface responses show the combined effect of components. A (top), results obtained for the CHO cell line with FreeStyleCHO medium. B (bottom), results obtained for HEK 293 cells with FreeStyle medium.

In conclusion two study cases have been performed, illustrating an approach to optimise a given chemically defined commercial cell culture medium by its supplementation with non-animal derived compounds. The use of Design of Experiment as a tool to accelerate both the screening of the most efficient compounds and the determination of the corresponding optimal conditions is very effective. For CHO S cells, the optimal conditions were found for FreeStyleCHO medium supplemented with Selenium (1 µg/L), r-transferrin (20.2 mg/L) and r-insulin (26.1 mg/L). For HEK 293 cells the optimal conditions were found for FreeStyle medium supplemented with r-insulin (19.8 mg/L), r-transfeerin (1.6 mg/L) and lipid mix (0.9X).

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