

A High Yielding, Chemically Defined, Animal Component-free and Protein-free Process for Biopharmaceutical Manufacturing from GS-NS0 Cell Lines

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Introduction

As the market for biopharmaceutical production using mammalian cell cultures matures, there is an increasing demand for higher yielding processes that are free of animal-derived components. Animal-derived components are potential sources of adventitious agents, batch-to-batch variability may cause inconsistent growth and productivity, and their presence complicates product purification.

The adaptation of NS0 cell lines from serum-free medium (contains animal-derived components e.g. BSA) to a chemically defined, animal component-free medium which is also free of protein (CDACF & PF), was:

- Traditionally a lengthy procedure, often taking up to 16 weeks;
- Often accompanied by transient poor growth and viability;
- Achieved by the serial reduction of animal-derived components over numerous successive subcultures.

A recurring problem encountered with NS0 cell lines after adaptation to CDACF & PF medium was poor viability and growth on revival of cryopreserved cell stocks.

- Prolonged processing time prior to the initiation of cryopreservation, a consequence of the length of time required to aliquot large cell banks, often exacerbated the problem;
- This often limited cell bank size.

The removal of serum or BSA (and any other animal-derived component) from the cryopreservation mixture is also highly desirable as they are potential sources of adventitious agents.

Potentially CDACF & PF processes are less productive than serum-free ones. The strategy used at Lonza Biologics to increase productivity is to perform process improvements using a model cell line for each cell line type (e.g. GS-CHO, GS-NS0, etc.). Updated platform processes for each cell type are generated from these and are employed for incoming cell lines used to supply material for early stage clinical trials:

- This provides the customer with clinical trial material more quickly (and cheaply) than undergoing specific optimisation process prior to first manufacturing batch.

These processes are designed to be operable within the constraints of a manufacturing environment, facilitating process transfer from development into cGMP manufacturing.

A new CDACF & PF medium for glutamine synthetase transfected NS0 (GS-NS0) cell lines was developed at Lonza Biologics in an attempt to eliminate these problems. This new medium allows for rapid adaptation with minimal loss of viability and also allows cryopreservation in the absence of serum. An example of an adaptation of a GS-NS0 cell line to the new CDACF & PF medium is shown here.

An evaluation of this GS-NS0 cell line adapted to the new CDACF & PF medium was undertaken in laboratory-scale fermenters. Lonza Biologic's current CDACF & PF platform process (developed using a different GS-NS0 cell line) was used. A comparison was made with serum-free adapted cells of this cell line in the serum-free platform process.

Methods

A GS-NS0 cell line was created by transfection of NS0 myeloma cells with a GS expression vector (Lonza Biologics) encoding the heavy and light chain genes for a humanised antibody.

After successful adaptation to Lonza Biologics' proprietary serum-free medium (containing animal-derived components) for GS-NS0 cell lines, the cells were inoculated directly into Lonza Biologics' newly developed proprietary CDACF & PF medium. For comparison a lineage was maintained in the serum-free medium during the period of the adaptation. Once acceptable and reproducible growth at high viability were seen during serial subculture in CDACF & PF conditions a cryopreservation experiment was performed.

Growth and productivity kinetics of serum-free and CDACF & PF adapted cells were evaluated in 10 litre airlift fermenters (ALF) operated in fed-batch mode. The current serum-free and CDACF & PF fed-batch platform processes for GS-NS0 cell lines were used respectively.

Results

The data from the lineage maintained in serum-free medium are presented in Figure 1. The data from the adaptation to the new CDACF & PF medium are presented in Figure 2. The following observations were made:

- Growth in the CDACF & PF medium was quickly established and growth at subculture was similar to that seen in serum-free medium.
- Viability throughout the CDACF & PF adaptation period was high, never dropping below 89%. In general the viability was slightly higher in CDACF & PF conditions than that observed in serum-free culture.
- The CDACF & PF adaptation was rapid, taking less than 35 days. Previously this had taken up to 16 weeks.

Figure 1. Serial Subculture of the Model GS-NS0 Cell Line in Serum-free Medium (contains animal-derived components).

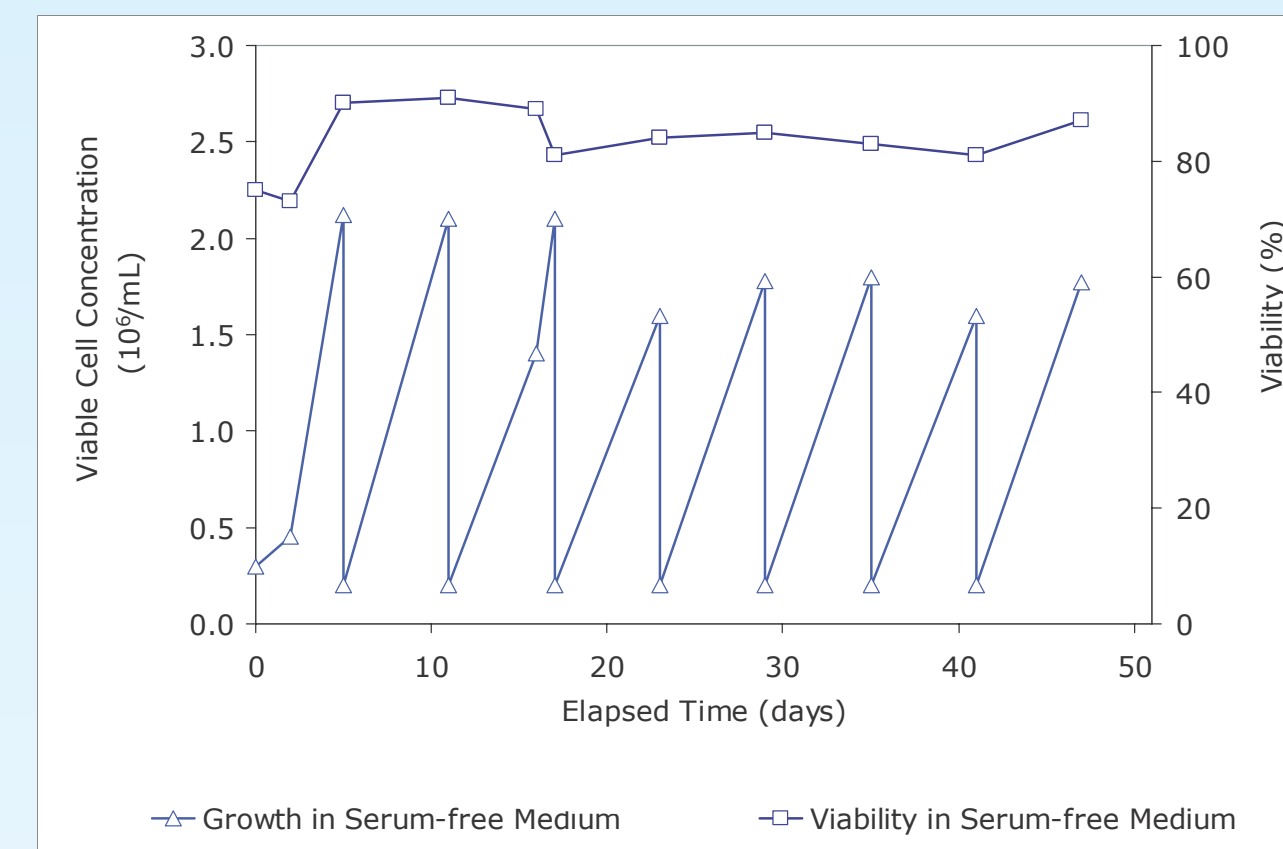


Figure 2. Serial Subculture of the Model GS-NS0 Cell Line During Adaptation to CDACF & PF Medium from Serum-free Medium.

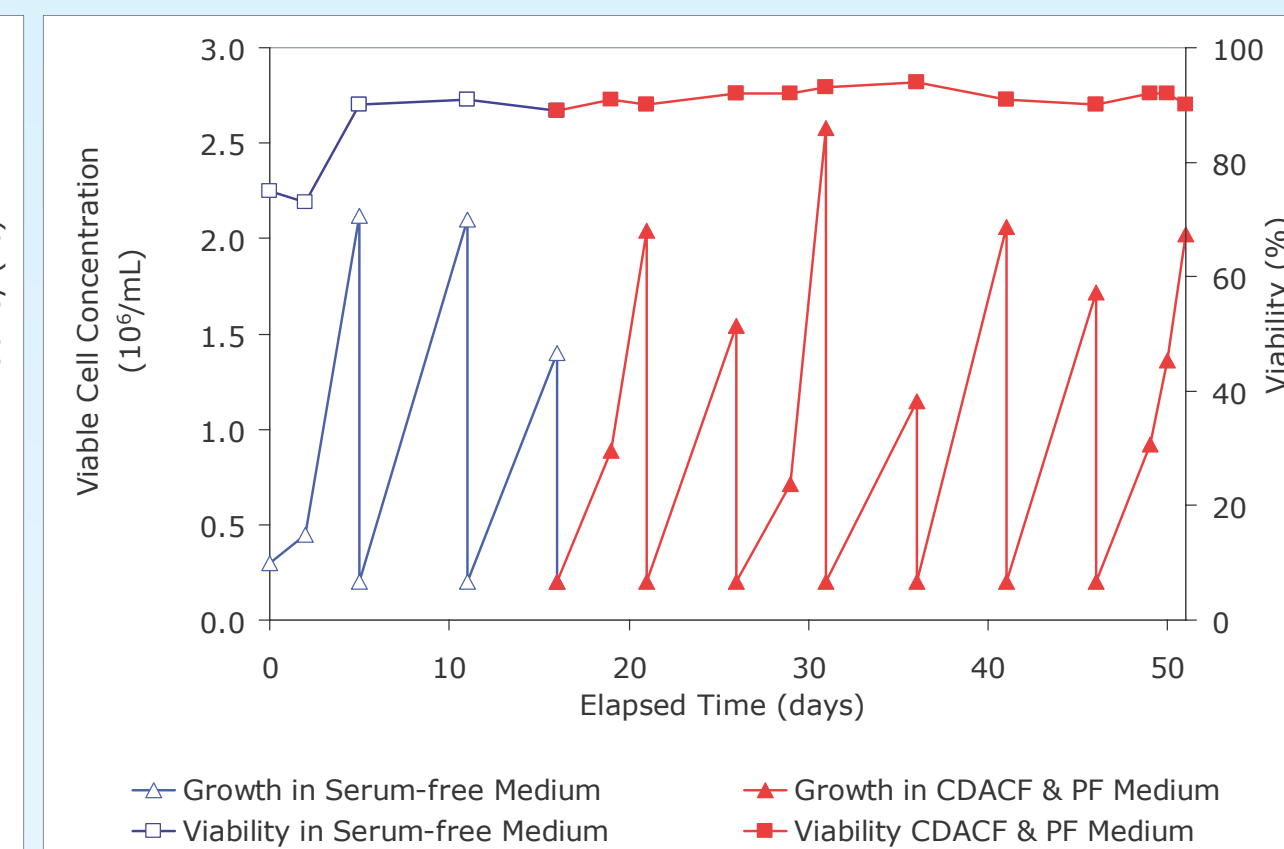


Figure 3. Cell Growth Profiles for the Laboratory-scale Fermentations of the Model GS-NS0 Cell Line.

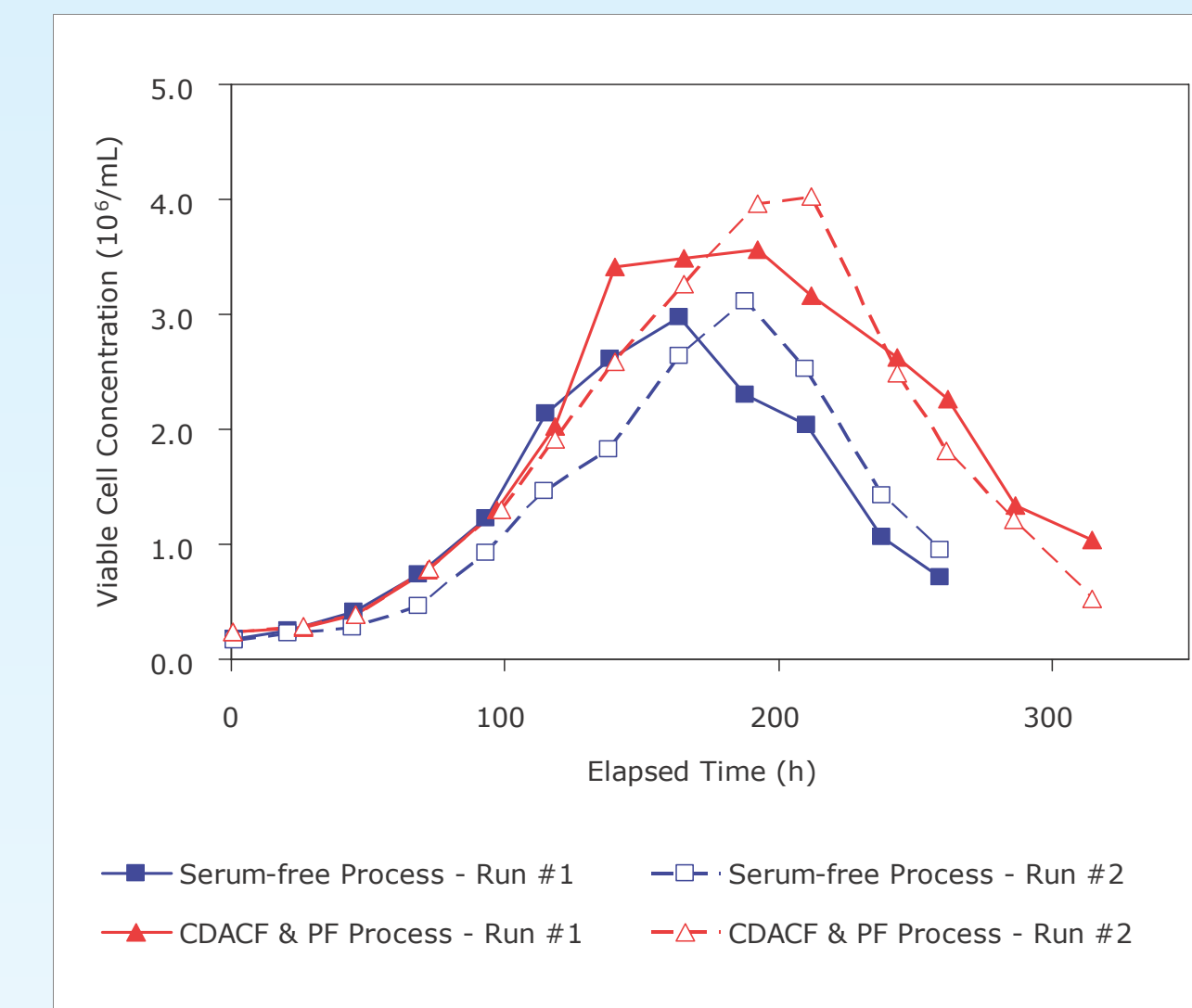
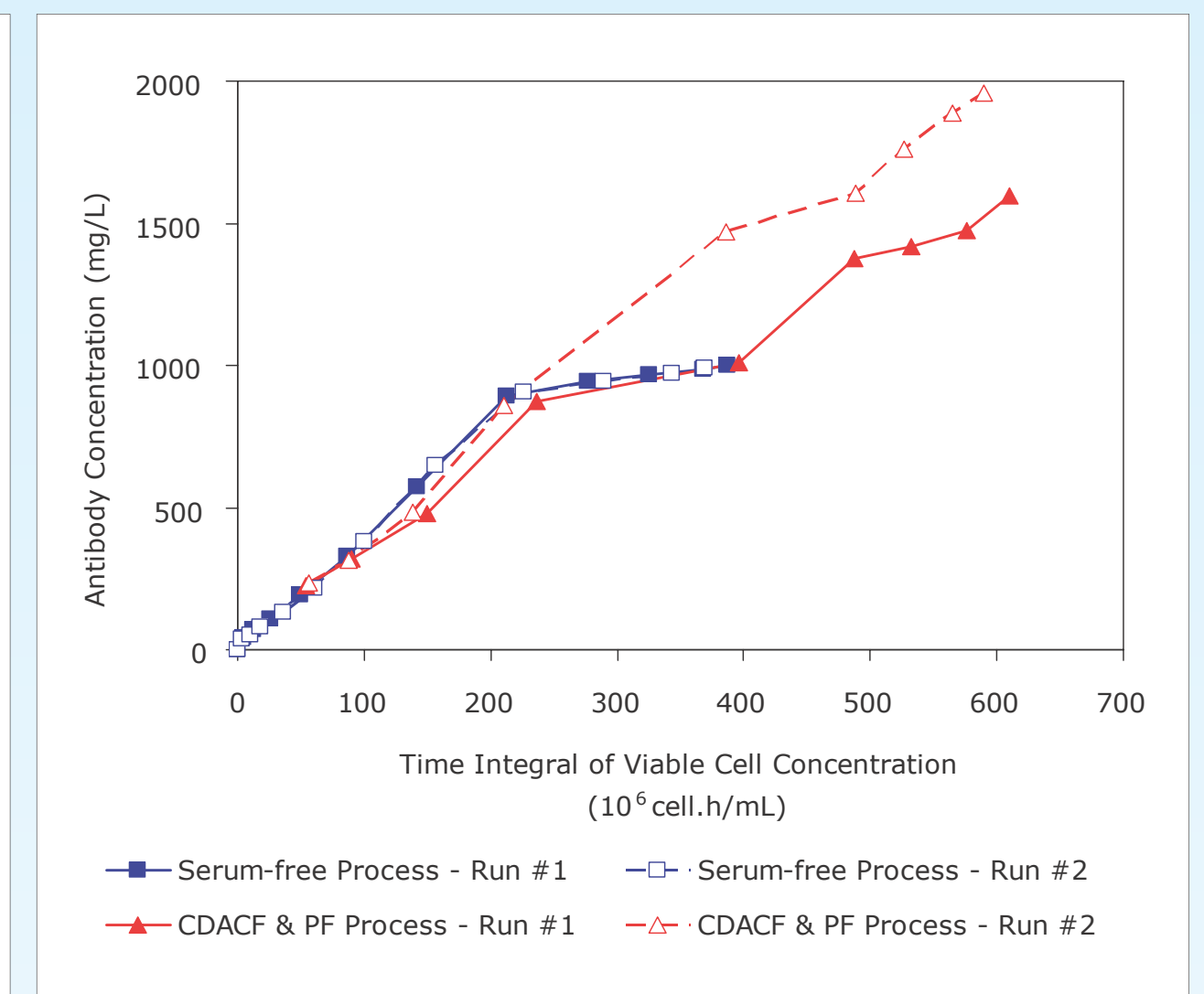


Figure 4. Productivity for the Laboratory-scale Fermentations of the Model GS-NS0 Cell Line.



The results from the cryopreservation experiment using the CDACF & PF adapted GS-NS0 cell line are presented in Table 1. It can be seen that:

- All the revivals of cryopreserved cell stocks yielded high viability ($\geq 83\%$) and good growth upon recovery. All revivals generated sustainable cultures.
- Exposing the CDACF & PF adapted cells to the cryopreservation mixture for 3 hours prior to 'freezing' had no detrimental effect on revival of the cell stocks. This was true both with and without serum (or any other protein) present in the cryopreservation mixture.

Table 1. Summary of Cryopreservation Conditions Tested and the Effect on Revival of the Model GS-NS0 Cell Line Adapted to Growth in CDACF & PF Medium.

Viability Prior to Cryopreservation (%)	Time Cells in the Cryopreservation Mixture Prior to Cryopreservation (hrs)	Concn. of Serum in the Cryopreservation Mixture (%)	Viability upon Revival (%)	Viability of Revival Culture at Time of 1 st Subculture (%)	Viable cell Concn. at Time of 1 st Subculture (10 ⁶ cells/mL)
92	0.5	0	89 & 84	91 & 92	2.19 & 1.69
		10	83 & 89	93 & 93	2.50 & 1.96
	3	0	90 & 85	92 & 91	2.09 & 2.03
		10	87 & 86	89 & 92	2.17 & 2.24

The data from the fermentations are presented in Table 2 and Figures 3 and 4. The following observations can be made:

- The initial specific rate of antibody accumulation in the platform CDACF & PF process was similar to that seen in the serum-free process of this cell line.
- The CDACF & PF platform process generated 1594 and 1957 mg/L antibody concentrations at harvest. These harvest antibody concentrations were approximately double those seen with the serum-free process.
- This improvement in harvest antibody concentration using the current CDACF & PF platform process was due to a combination of factors:
 - Increased time integral of viable cell concentration and
 - The high initial specific rate of antibody accumulation (seen in both processes) was maintained over the entire course of the fermentation in the CDACF & PF medium. In contrast the fermentations using the serum-free process, demonstrated a marked decrease in the rate of antibody accumulation in the latter half of the culture.

Table 2. Summary of Growth and Productivity of the Model GS-NS0 Cell Line in 10 Litre Airlift Fermentations.

Process	Maximum Viable Cell Concn. (10 ⁶ /mL)	Time Integral of Viable Cell Concn. (10 ⁶ cell.h/mL)	Harvest Antibody Concn. (mg/L)	Specific Rate of Antibody Accumulation at Harvest ⁽¹⁾ (µg/10 ⁶ cells/h)	Process Duration (Days)
Serum-free Process	3.0 & 3.1	369 & 387	989 & 1002	2.59 & 2.68	11 & 11
CDACF & PF Process	3.6 & 4.0	590 & 610	1594 & 1957	2.61 & 3.32	13 & 13

⁽¹⁾ The specific rate of antibody accumulation at harvest is a single point estimation. It was calculated by dividing the antibody concentration at harvest by the value for time integral of viable cell concentration at harvest.

Summary

The new CDACF & PF medium for GS-NS0 cell lines has eradicated many of the problems previously experienced with CDACF & PF GS-NS0 processes. With the model GS-NS0 cell line this medium:

- Allowed the rapid and simple, single step adaptation with no negative impact on growth or viability compared to serum-free culture in a medium containing animal-derived components.
- Permitted successful cryopreservation and revival without serum or protein in the cryopreservation medium.
- Facilitated a reduction in the sensitivity to the cryopreservation mixture, allowing the production of larger cell banks.

Since the implementation of this medium as the platform CDACF & PF medium for GS-NS0 cell lines:

- Over 130 (100% success) cell lines (both transfectants and clones) have successfully undergone CDACF & PF adaptation.
- The duration required for adaptation to CDACF & PF medium has been shortened from 16 weeks to 4 - 7 weeks.

For the model GS-NS0 cell line, the new CDACF & PF medium in combination with the current platform CDACF & PF process:

- Generated harvest antibody concentrations of 1594 and 1957 mg/L which were approximately double those seen with the serum-free process.
- No loss in the specific rate of antibody accumulation compared to the serum-free process.
- The data support the strategy employed of using model cell lines to improve our CDACF & PF platform processes. The current CDACF & PF platform process was developed using another GS-NS0 cell line.

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