

Quantitative Risk Assessment of Product Disulfide Bond Reduction in A Recombinant Protein Manufacturing

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Abstract

Disulfide bond reduction occurred now and then during a recombinant protein manufacturing. Their reduction often led in the loss of batches. Various prevention measures were developed. Their implementation in a manufacturing inevitably increases its operation complexity and even might impact its product qualities and process performances. It is highly desirable to apply a reduction measure only if necessary and to apply no strong measure if a moderate one works. The reduction occurrence is determined both by the reduction sensitivity of a recombinant molecule and by the reduction power of harvested cell culture fluids. The reduction power of harvested cell culture fluids varies largely and depends on cells, lysis level, growth stage, and culture conditions. The method of the quantitative risk assessment of disulfide bond reduction was reported here. The quantitative assessment is realized by comparing the reduction sensitivity of a recombinant molecule with the reduction power of its harvested cell culture fluids.

Keywords: Disulfide Bond Reduction, Reduction Prevention, Antibody, Recombinant Protein, Reduction Risk Assessment

Introduction

Recombinant proteins for human therapeutic use have increased significantly in the last 25 years. Disulfide bonds play an important role in maintaining and stabilizing the three-dimensional structure of those proteins [1, 2]. Their reduction was observed now and then during a recombinant protein manufacturing [3-5]. Such a reduction event was catastrophic as the product failed to meet the drug substance specifications and the bulk product was lost. The reduction has a substantial impact on the functional effects of a mAb, including variable effects on antigen binding and Fc function, with the potential to significantly impact mAb efficacy in vivo [6]. The reduction mainly took place in the harvested cell culture fluids (HCCF). The release of intracellular reducing components was widely considered to be the cause of the observed reductions [3, 4]. The release took place both in cell culture bioreactors and in the harvest process of cell culture fluids (CCF). However, the harvest process was widely considered to be major [3, 4]. At the end of a production phase, the cell culture fluid was usually harvested either by disc stack centrifugation followed by depth filtration or by depth filtration only. The mechanical stresses from centrifugation and depth filtration were reported to cause cell lysis.

The disulfide bond reduction of monoclonal antibodies has been studied intensively. The thioredoxin reduction pathway, including

thioredoxin reductase (TrxR) and NADPH, has been proposed as a primary contributor [4, 7]. The monoclonal antibodies contain both intra chain disulfide bonds and inter chain disulfide bonds. The inter chain disulfide bonds are more susceptible to reduction than intra ones [8]. For the two studied IgG1, the disulfide bonds between the light chain and heavy chain were more susceptible for reduction than those between two heavy chains. The upper disulfide bond of the two inter heavy chain disulfide bonds was more susceptible than the lower one [9]. Reduction-susceptible disulfide bonds are frequently located at the surface of a protein [2]. There were large differences in reduction sensitivities between different monoclonal antibodies [10, 11]. Moreover, the reduction power in CCF and HCCF could vary largely for different molecules and different processes as well [10]. The reduction occurrence depends on molecule, cell culture process, and harvest process.

The various measures to prevent the disulfide reduction occurrence had been developed. Those measures included air sparging, air overlay, pH adjustment, cooling down, TrxR inhibitors and chemical additions. The added chemicals could be H₂O₂, EDTA, Cu²⁺, Hg²⁺, Zn²⁺, Co²⁺, Mn²⁺, and so on [12-15]. Among them, air sparging or air overlay is most often used due to its simplicity and minimal potential impact on product qualities [13]. The implementation of the above measures in a recombinant protein

manufacturing will inevitably increase its operation complexity somewhat and might impact its product qualities as well sometimes. A decrease in main peak and an increase in acidic peak were reported when air sparging was applied during a HCCF hold for a given monoclonal antibody [13]. Such a decrease in main peak and an increase in acidic peak had been seen in our labs as well for some molecules (data not shown here). Besides that, the decrease in the filterability of HCCF was observed in our labs and at-scale manufacturing (data not shown here) when air sparging or air overlay was applied.

If air sparging only doesn't work, the cooling HCCF down plus air sparging might be worth to be considered next. In the case that the cooling down plus air sparging was still not sufficient, the addition of a weak oxidant, such as Cu_2^+ , might be a choice. Such an addition certainly would introduce additional complexity to a process. It is highly desirable to apply a reduction measure only if necessary and to apply no strong measure if a moderate one works. How to assess the level of the risk of disulfide bond reduction for a given process quantitatively? How to determine what measure is

appropriate for a given process? This study reported a quantitative method to assess the risk of disulfide bond reduction for a given process. The outcome of the assessment could tell whether a reduction measure should be taken and what measure is appropriate if yes. Answer what measure (mild, moderate, or strong) shall be appropriate for a given process.

Materials and Methods

CCF, HCCF, Recombinant Proteins, and Other Materials

CHO CCF and recombinant proteins were produced at WuXi Biologics using standard cell culture procedures. The bioreactors used for cell cultures varied from a lab scale to at-scale manufacturing. The cell cultures were harvested either using centrifugation followed by depth filtration or depth filtration only. The molecules studied in this article are produced by CHO cell. The information of the molecule types is listed in the table 1. Nicotinamide adenine dinucleotide phosphate (NADPH) was from EDM Chemicals; 2,6-Dichlorophenolindophenol (DCPIP) from Sigma-Aldrich; Thioredoxin reductase (TrxR) from Sigma-Aldrich. 0.22 μm filter from Millipore and D0HC depth filter from Millipore.

Table 1: Information of The Molecule Type

Product	Molecule Type
Molecule 1	IgG4
Molecule 2	IgG1
Molecule 3	IgG4
Molecule 4	Fc-Fusion Protein
Molecule 5	IgG4
Molecule 6	Fc-Fusion Protein
Molecule 7	IgG1
Molecule 8	IgG1
Molecule 9	Fc-Fusion Protein
Molecule 10	IgG1

Low-speed centrifugation supernatant and cell lysate: CCF was centrifuged in a Sorvall ST 16R rotor centrifuge (Thermo) at 800 x g for 10min at room temperature. The 800 x g for 10min is considered as a moderate centrifugation condition and would not cause cell lysis. The obtained up-liquid is the low-speed centrifugation supernatant; i.e., the supernatant of CCF. The cell pellets were re-suspended into original volume using 50 mM HAc-NaAc buffer. The cell lysis of the suspension was realized either by centrifugation in a Sorvall ST 16R rotor centrifuge at 10000 x g for 30min or by freeze-thaw at -70°C/room temperature followed by a centrifugation at 10000 x g for 30 min. The up-liquid is the cell lysate.

Reduction simulation study: 1-2 mL of samples were transferred into 10ml glass bottles filled with nitrogen and sealed with a rubber plug. The bottles with the samples were incubated at intended temperature for up to 12 hours. The samples were taken at pre-determined time-points with syringe and immediately kept at <-70°C until analysis.

Harvest depth filtration processing: CCF was clarified with lab-scale depth filters (23cm² D0HC). The filters were rinsed with purified water at a flow rate of 600LMH (L/m²/h), equilibrated with the equilibration buffer (50mM Tris, HAc 50mM NaCl, pH7.4) at a flow rate of 300LMH for more than 20L/m² before use. The speed of the peristaltic pump was set at 50-60LMH for CCF filtrations. The delta pressure of depth filter was monitored with pressure sensors (PENDOTECH). After the CCF filtration, the filters were flushed with the equilibration buffer.

Assays

Reduction of antibody was tested by non-reduced SDS-PAGE using the NuPAGE 4-12%Bis-Tris Gel (Thermo Fisher). Sample preparation was carried out as described in the Thermo manual. TrxR activity and NADPH were determined by Thioredoxin Reductase Activity Assay Kit (BioVision) and Amplitude Colorimetric NADPH Assay Kit (AAT Bioquest), respectively. The tests were carried out as described in the kit manuals. LDH activity was de-

terminated by Cedex (Roch). The viable cell density and the viability during cell lysis were determined by Vi-cell (Backman Coulter). For HCCF, a HPLC-based protein A method was used to measure the titers of antibodies and Fc-Fusion proteins. For the purified antibodies and Fc-Fusion proteins, the concentration was measured using UV spectrometry (Thermofisher) at 280nm.

Results and Discussion

TrxR Activity and NADPH Concentration at Different Harvest Conditions

TrxR and NADPH were reported to be the primary contributor for the disulfide bond reduction of a recombinant protein manufacturing [4, 7]. To study them, TrxR activity and NADPH concentration were measured at different harvest conditions. Figure 1 shows the TrxR activities in the supernatant of the low-speed

centrifugation (800 x g for 10min at room temperature), the filtrate of the low-pressure depth filtration (<0.8 bar), the filtrate of the high-pressure depth filtration (> 0.8 bar), and the post-filtration flush of CHO cell culture of molecule 1, respectively. As shown, the TrxR activities presented in all of them except the post-filtration flush. The TrxR activity was the lowest in the low-speed centrifugation supernatant, the middle in the filtrate of low-pressure depth filtration, and the highest in the filtrate of the high-pressure depth filtration. The differences between them were limited. The TrxR activity in the flush was below the quantification limit of the used test method. Since the low-speed centrifugation would not cause cell lysis, the TrxR in the low-speed centrifugation supernatant would attribute to the accumulation of TrxR released during the cell culture process instead of its harvest. The post-filtration flush didn't cause a practical release of TrxR into the flush sample.

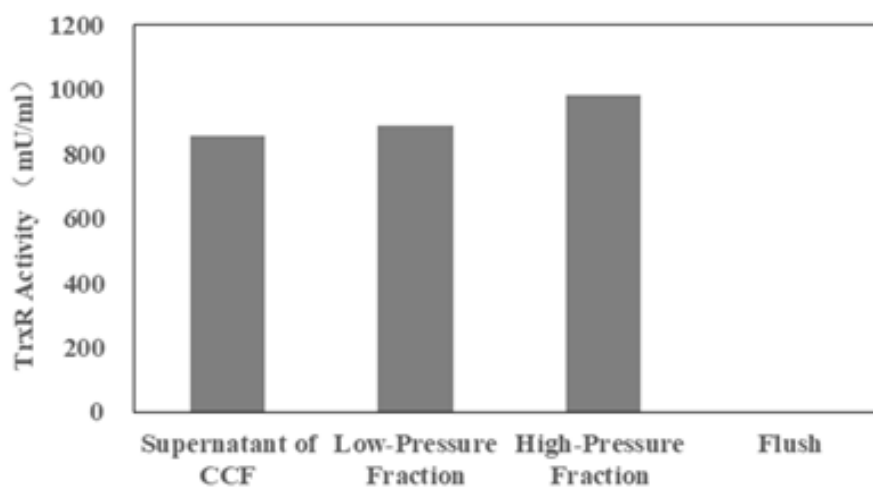


Figure 1: TrxR activities in the supernatant of CCF, the low-pressure fraction (< 0.8 bar), the high-pressure fraction (> 0.8 bar) and the flush of the depth filtration of the molecule 1 cell culture

For the CCF of molecule 2, the depth filtration was conducted using Millipore's D0HC at the feed flux of 50-60 LMH. The filtration pressure was monitored. The three fractions, 0.4-0.6 bar, >0.8bar, and flush, were collected. Additionally, the cell lysate was made by following the preparation method of cell lysate described above. Figure 2 and 3 show the TrxR activities and NADPH concentration in the different fractions of its depth filtration harvest process, respectively. As shown in Figure 2, the TrxR activities were 1100 mU/ml for the >0.8 bar fraction, 1050 mU/ml for the 0.4-0.6 bar fraction, 300 mU/ml for the flush, and 680 mU/ml for the

cell lysate. The >0.8 bar fraction had the highest TrxR activities, followed by the 0.4-0.6bar fraction, the cell lysate, and the flush. The NADPH concentration shown in Figure 3 was 0.4 μ M for the 0.4-0.6 bar fraction, 1.05 μ M for the >0.8 bar fraction, 0.5 μ M for the flush, and 2.0 μ M for the cell lysate. The observation suggested that the NADPH accumulated during the cell culture process was limited, the high-pressure filtration could increase the release of NADPH largely, the flush did contain NADPH at a comparable level as the 0.4-0.6 bar fraction, but the intact cells contained NADPH at the highest level among them.

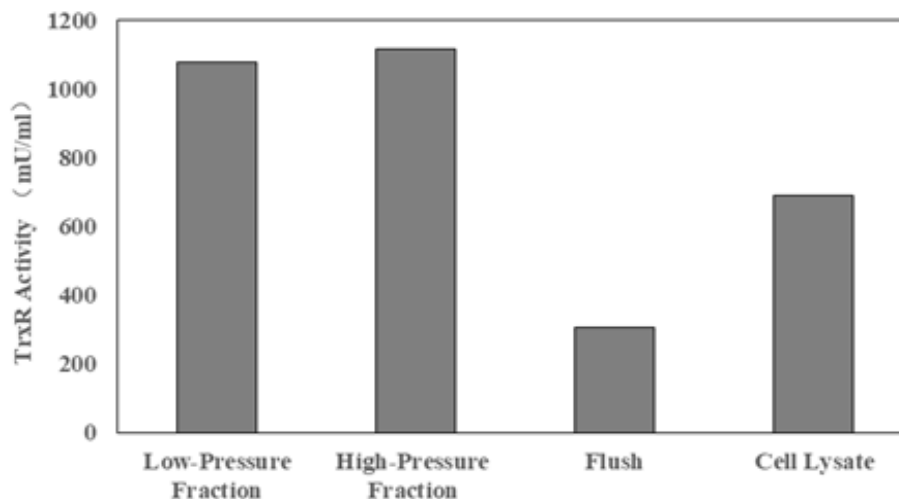


Figure 2: TrxR activities in the low-pressure fraction (< 0.8 bar), the high-pressure fraction (> 0.8 bar) the flush of the depth filtration, and the cell lysate of the molecule 2 cell culture

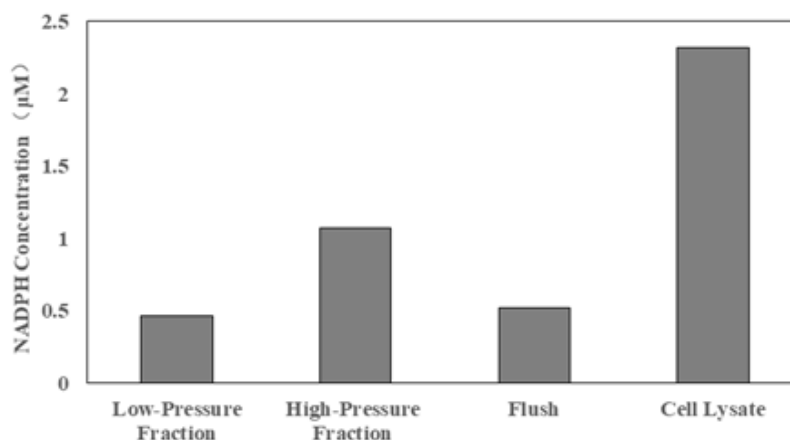


Figure 3: NADPH concentration in the low-pressure fraction (< 0.8 bar), the high-pressure fraction (> 0.8 bar), the flush of the depth filtration, and the cell lysate of the molecule 2 cell culture

Effect of TrxR Activity and NADPH Concentration on Disulfide Bond Reduction

To study the effect of TrxR activity and NADPH concentration on disulfide bond reduction, the four combinations with two TrxR activities and two NADPH concentrations were constituted using the cell lysate of the CHO cell culture of molecule 2 and TrxR from Sigma. Table 2 showed the TrxR activity and the NADPH concentration of the four constitutes. The purified molecule 2 was spiked to the final concentration of 1 g/L in each of them. The spiked constitutes were incubated at room temperature under nitrogen overlay for 12h. The obtained samples were analyzed on reduction occurrence by non-reduced SDS-PAGE. The results were given in Table 2. As seen, the reduction did not occur when the NADPH concentration was at 0.25 µM no matter the TrxR activity was either 300 mU/ml or 1200 mU/ml. However, the reduction did occur when the NADPH concentration was 1 µM even at the

TrxR activity of 300 mU/ml. The results suggested the NADPH concentration was better correlated with the reduction occurrence than the TrxR activity. To further evaluate the effect of NADPH concentration on reduction, the cell lysate was diluted with 50 mM HAc-NaAc buffer into three concentrations, >1.5 µM, 1 µM, and 0.5 µM. After spiked with the purified molecule 2 to a final concentration of 1 g/L, the samples were incubated at room temperature for 12 h under nitrogen overlay. The reduction by DTT, the positive control, was prepared by incubating molecule 2 with 1 % (W/V) of DTT in the non-reduced SDS-PAGE loading buffer at 75°C for 10 min. Figure 4 showed the non-reduced SDS-PAGE of the incubated samples. As seen, the intensity of the band at ~70 kD, ~50 kD and ~25 kD increased with the NADPH concentration. The level of the reduction at 0.5 µM of NADPH was much less than those at 1 µM and >1.5 µM.

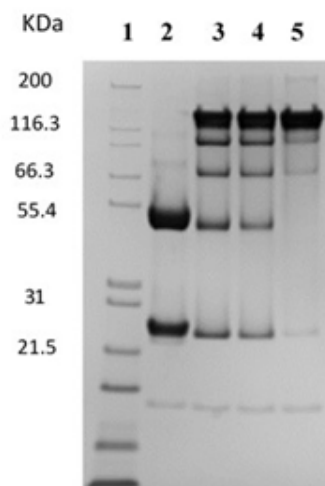


Figure 4: The non-reduced SDS-PAGE of molecule2 incubated in cell lysates with different NADPH concentrations. Lane: 1. Marker; 2. Reduction by DTT; 3. NADPH >1.5 μM cell lysate; 4. NADPH 1.0 μM cell lysate; 5. NADPH 0.5 μM cell lysate. Reduction by DTT was used as a positive control.

Table 2: Effect of TrxR Activities and NADPH Concentration on Disulfide Bond Reduction

NADPH concentration	TrxR activities	Reduction occurred (Y/N)
0.25 μM	300mU/ml	N
0.25 μM	1200mU/ml	N
1 μM	300mU/ml	Y
1 μM	1200mU/ml	Y

NADPH Concentration in Cell Culture Fluids and Cell Lysate

The CHO cell cultures from five different recombinant molecules were centrifuged at 800 x g for 10min at room temperature. The supernatants were sampled and removed. The cell pellets were re-suspended to their original volumes in 50 mM HAc-NaAc buffer and followed by 10000 x g for 30min at 4 $^{\circ}\text{C}$. In total, the five supernatants of the CFFs and the five cell lysates were obtained. Their NADPH concentrations were measured and the results were depicted in Figure 5. Among the five supernatants of the CFFs, the highest NADPH concentrations was 0.1 μM and the two of

them were below the quantification limit (0.01 μM). The observation suggested that the NADPH concentrations were low in the CFFs. The NADPH concentrations in the five cell lysates varied from 0.1 μM to 2.4 μM . The variability of NADPH concentration was large both in the CFFs and in the cell lysates. The NADPH concentration in the cell lysates was much higher than that in the CFFs. This partially explained why the bisulfite bond reductions only occurred in some manufacturing processes and why the harvest process could impact their occurrence for a given CCF.

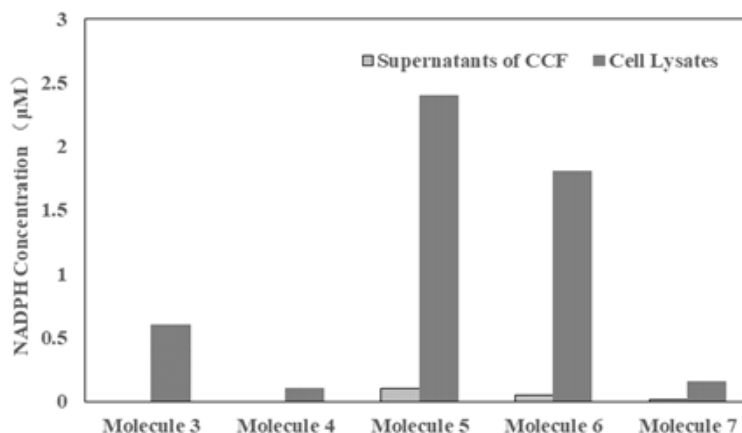


Figure 5: NADPH Concentration in The Cell Lysates and The Supernatants of CCF of five Recombinant Molecules

NADPH Concentration in Different Stage of Cell Culture

The CHO cell fed-batch cultures of three molecules, molecule 8, molecule 9, molecule 10, were sampled on Day 9, Day 11 and Day 14. The cell pellets of the low-speed centrifugation (800 x g for 10 min at room temperature) were collected and re-suspended to their initial cell density. Similar to the above, the cell lysates were generated. Their NADPH concentrations were measured and the results were shown in Figure 6. The NADPH concentration in all the three different cells varied with cell culture stage largely. The concentration was the highest on Day 11. The sharp increase in the intracellular ratio GSH/GSSG from day 7 onwards of CHO cultures was reported [16]. The both observations suggested the

intracellular redox potential was not constant but changed with cell culture stages. Figure 7 showed the NADPH concentration in the cell lysate of the seed, the cell lysate at harvest, and the supernatant of the CCF for molecules 1. The NADPH concentration in the cell lysate of the seed was about five times of the cell lysate at harvest. The NADPH concentration in the supernatant of the CCF was below the quantification limit of the test method. Both Figure 6 and Figure 7 suggested that the NADPH concentration in the cells varied largely with cell culture stages. The minimization of cell lysis during its harvest process could minimize the release of NADPH into its HCCF so that the risk of disulfide bond reduction could be reduced at least if it can't be prevented completely.

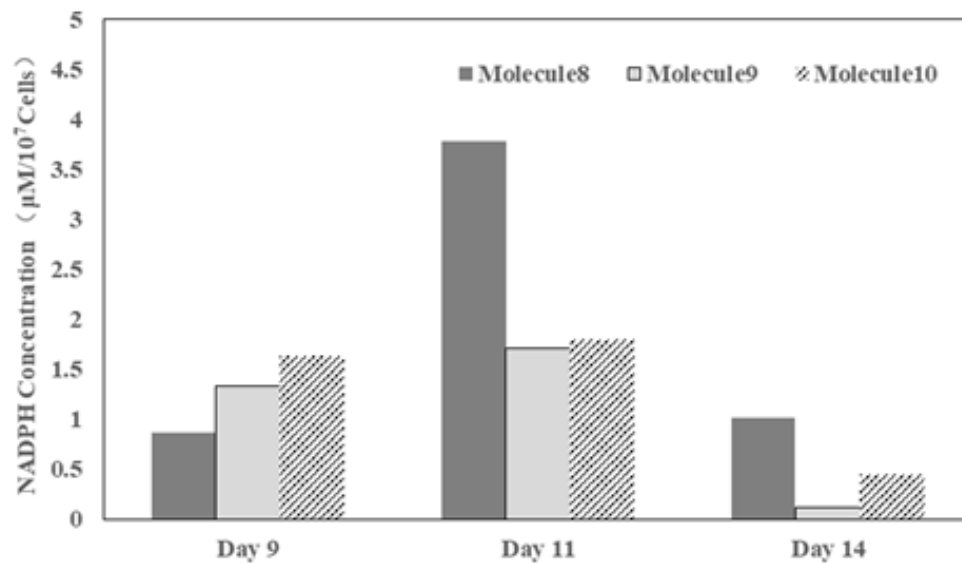


Figure 6: NADPH Concentration in Cell Lysates of Three Recombinant Molecules on Different Culture Days.

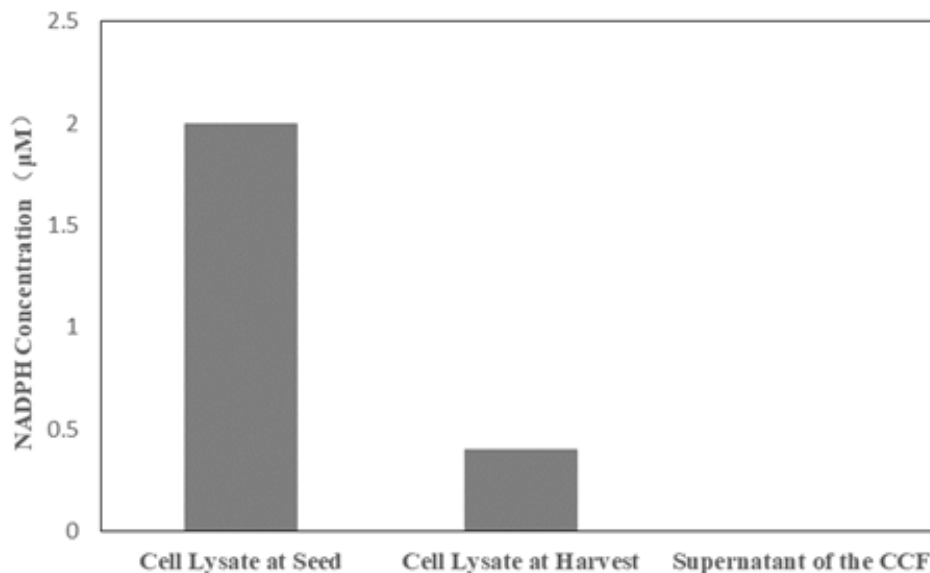


Figure 7: NADPH Concentration in The Cell Lysate of The Seed, The Cell Lysate at Harvest, and the Supernatant of the CCF.

The summarization of the above results leads to the following conclusions. The reducing agents such as NADPH varied largely not only with cell clones but also with growth stages. NADPH presented both in intact cells and in the cell culture supernatants. But the quantities of NADPH in the intact cells were far more than in the cell culture supernatants. Besides cell clones, the growth stage and harvest process could affect the NADPH concentration in HCCF largely. The reduction power of HCCF is correlated with its NADPH concentration better than its TrxR activity. Another key factor for a reduction occurrence is recombinant molecule itself. It has been reported in the literature [10] and observed in this study (data not shown here) that the reduction sensitivities of recombinant molecules varied largely. The reduction sensitivity of a given molecule shall not vary with cell culture conditions, growth stages, and harvest processes and can be quantified prior to its manufacturing run. However, the level of the reducing agents such as NADPH in HCCF varies largely not only with cell clones but also might vary with cell culture conditions, growth stages, and harvest processes. For each given HCCF, its reduction power needs to be measured individually.

Reduction Risk Assessment for a Given Manufacturing

The reduction power of HCCF and the reduction sensitivity of a recombinant protein are the two determining factors for a reduction occurrence. If the power and the sensitivity can be quantified using the same indicator, their comparison will be straightforward and the difference between them will tell the likelihood of a reduction occurrence for a given manufacturing. The following reduction risk assessment approach is formulated.

The quantification of the reduction power of HCCF: The reduction power of HCCF is correlated with the levels of its NADPH, reducing agents, and cell lysis. The NADPH can be quantified by using its specific kits. The level of the reducing agents can be measured by using DCPIP or other appropriate redox sensitive dyes and indicators. DCPIP is a weak oxidant and its aqueous solution has a blue color, but decolors if being reduced. The higher the reducing agent level is, the faster the decoloring rate will be. The NADPH concentration and the DCPIP decolor rate are correlated with the cell lysis level. The reduction power of a HCCF can be quantified either by the concentration of NADPH or the OD of decolored DCPIP.

The quantification of the reduction sensitivity of a recombinant protein: Dilute the cell lysate of a given molecule in a series for example 100%, 80%, 60%, 40%, and 20%, determine their NA-

DPH concentration and their OD of decolored DCPIP, spike the diluted samples with the purified target molecule to a final concentration of 1 mg/mL, overlay the spiked samples with nitrogen and incubate them for 12h at room temperature, and analyze the obtained samples with non-reduced SDS PAGE. The results will tell whether the reduction has occurred and if occurred, what is the minimum concentration of NADPH and the maximum OD of decolored DCPIP for the reduction occurrence of the tested molecule. The reduction sensitivity of the molecule can be quantified either by the minimum concentration of NADPH or by the maximum OD of decolored DCPIP for its reduction occurrence.

For a given manufacturing, the risk level of reduction occurrence is correlated with the difference between the reduction power of its HCCF and the reduction sensitivity of its recombinant protein. The difference can be quantified by using anyone of those two indicators (the concentration of NADPH and the OD of decolored DCPIP) as long as the same indicator is used for the quantifications. If the reduction power of the HCCF was lower than the reduction sensitivity of the recombinant protein, the reduction unlikely will occur. If the reduction power of the HCCF was higher than the reduction sensitivity of the recombinant protein, the reduction likely will occur without the implementation of a reduction prevention measure. The larger the difference is, the stronger the reduction measure would be needed for its reduction prevention.

NADPH Indicator Application Case

Generated the cell lysate for molecule 2 prior to its manufacturing, determined the NADPH concentration in the cell lysate. Diluted the cell lysate in a series based on NADPH concentration. Spiked the diluted cell lysates with purified molecule 2 to a final concentration of 1 mg/mL. Split each diluted and spiked cell lysate into two portions. One series was overlaid with nitrogen and the another was sparged with air. Incubated the two series of the cell lysates at room temperature for 12h. The incubated samples were tested on reduction occurrence with non-reduced SDS PAGE. The results were given in Table 3 and Table 4. The reduction under nitrogen overlay occurred when the NADPH concentration was $\geq 0.5 \mu\text{M}$ while the reduction under air sparging only occurred when the NADPH concentration was $\geq 1.25 \mu\text{M}$. $0.5 \mu\text{M}$ and $1.25 \mu\text{M}$ of NADPH are corresponding to 25% and 100% of cell lysis, respectively. In the GMP manufacturing of molecule 2, the HCCF was sampled and tested. Its NADPH concentration was $0.4 \mu\text{M}$, which was close to $0.5 \mu\text{M}$. The reduction risk was considered to be moderate. The air sparging was applied as a prevention measure. The manufacturing went successfully without reduction.

Table 3: NADPH Concentration and Molecule 2 Reduction in The Diluted Cell Lysates with Nitrogen Overlay

NADPH concentration (μM)	2	1.25	1	0.5	0.25
Cell lysate [%]	100	62.5	50	25	12.5
Reduction occurrence (Y/N)	Y	Y	Y	Y	N

Table 4: NADPH Concentration and Molecule 2 Reduction in The Diluted Cell Lysates with Air Sparging

NADPH concentration (μM)	2	1.25	1	0.5	0.25
Reduction occurrence (Y/N)	Y	N	N	N	N

DCPIP Indicator Application Case

Generated the cell lysate for molecule 6 prior to its manufacturing. Diluted the cell lysate in a series of 100%, 75%, 50%, 30%, 25%, 20%. Putted 199.2 μL of each diluted cell lysate on 96-well plate. Spiked 0.8 μL of DCPIP stock solution of 10 mg/mL into each

diluted cell lysate to a final concentration of 0.04 mg/mL. After incubated at room temperature for 10 min, the OD at 600 nm of both the series and the control were measured. The results were given in Table 5. As seen, the OD at 600nm increased with the dilution factor. The less the dilution was, the lower the OD at 600 nm was.

Table 5: The OD at 600 nm in the Diluted Cell Lysates Spiked With DCPIP

Cell lysate [%]	100	75	50	30	25	20	0
OD at 600nm	0.2994	0.3808	0.4076	0.4196	0.4825	1.0619	1.7869

Putted each diluted cell lysate into a vial. Spiked the purified molecule 6 into each diluted cell lysate to a final concentration of 1 mg/mL. Displaced air in the head space of each vial with nitrogen. After incubated at room temperature for 12h, the samples were tested on reduction occurrence with non-reduced SDS PAGE. The results were given in Table 6. For molecule 6, the reduction occurred when the cell lysis level reached 75% and above. According to

Table 5, the OD of 0.381 was correlated with 75% cell lysis. In the GMP manufacturing of molecule 6, the HCCF was sampled and tested using DCPIP. Its OD at 600 nm was 1.14 which was much higher than 0.381, the reduction sensitivity of molecule 6. The reduction risk was considered to be low. No prevention measure was taken. The manufacturing went successfully without reduction.

Table 6: Molecule 6 Reduction Occurrence in The Diluted Cell Lysates

Cell lysate [%]	100%	75%	50%	30%	25%	20%
Reduction occurrence (Y/N)	Y	Y	N	N	N	N

In the above two cases, the concentration of the purified recombinant molecules used was > 10 mg/mL. The target concentration of recombinant molecules in a spiked series of cell lysates was 1 mg/mL. The spiking of recombinant molecules should dilute the corresponding cell lysates somewhat. For simplicity, such a dilution effect was neglected as long as the dilution factor was less than 10% or the purified recombinant molecules was more than 10 times concentrated than the target one.

Conclusions

The reduction power of HCCF varied largely with cell clones, cell culture conditions, growth stages, and harvest processes. The harvest processes and cell clones showed strong effects on the NADPH concentrations but not on the TrxR activities in HCCF. The NADPH concentration is correlated with the reduction power of HCCF instead of the TrxR activity. NADPH was found both in intact cells and in the supernatant of CCF. The amount of NADPH in intact cells was much more than that in the supernatant of CCF. The method and operation condition of a clarification affected the level of cell lysis in harvest processes and impacted the release of NADPH and reducing agents in HCCF. The amount of NADPH per cell varied largely with the stage of a cell culture. Due to multiple influencing factors and their complexities, it is hard to predict the reduction power of HCCF quantitatively. Moreover, large differences in the reduction sensitivities of recombinant molecules existed as well.

The method for the quantitative risk assessment of disulfide bond reduction was developed. The cell lysate at different dilutions was used to evaluate the reduction sensitivity of recombinant proteins. NADPH concentration and DCPIP decolor rate were used to quantify the reduction power of HCCF and the reduction sensitivity of recombinant proteins. The difference between the reduction power of HCCF and the reduction sensitivity of recombinant protein determined whether the reduction might occur.

Abbreviations

CHO, Chinese hamster ovary; CCF, cell culture fluids; HCCF, harvested cell culture fluids; TrxR, thioredoxin reductase; NADPH, nicotinamide adenine dinucleotide phosphate; LDH, lactate dehydrogenase; DCPIP, 2,6-Dichlorophenolindophenol; DTT, dithiothreitol; LMH, liters per square meter per hour; GMP, good manufacturing practice; OD, optical density.

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