Biotech Diafiltration Process Optimization and Control Using Six Sigma Methods

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***Abstract* —** *Biologics parenteral downstream protein purification process often requires a buffer exchanges step. This process can be described also as a product washing step. In order to optimize a protein DF process is important to know what process initial conditions have a major effect on the process CTQ (critical to quality) attributes. For this case these attributes are; DF process pH, osmolality and conductivity. The main objective of this project was the optimization of Product X DF diafiltration process based on process factors Buffer and product initial conductivity, because these factors were found variable during several runs. A Six Sigma methodology was implemented in order to identify, how the factors initial conditions can be modify to optimize the DF operation. To identify which factor has the major effect on the DF process DMAIC, DOE, full Factorial Design 2^k experiment was used. Finally, experiments results, demonstrated that the DF buffer initial conductivity has the major effect on process final CTQ's. In addition, it was concluded that a formulation buffer initial conductivity of 13.5 mS/cm will result in a cost and cycle time reduction of 50 %, while DF process CTQ's are maintained under the specification limits.*

***Key Terms* —** *Biotech Process, Buffer Conductivity, Critical to Quality, Diafiltration*

PROBLEM STATEMENT

The buffer exchange step is performed using a DF or tangential flow filtration process. During this process the product is pumped through the filtration system with the purpose of remove the old buffer while the new buffer takes it position. Moreover, one of the main objectives of this process is to prepare the product in the final buffer (excipinets) to achieve the final formulation and CTQ (critical to quality) specifications. On the other hand, to maintain CTQ's under specifications, the control of specific parameters during the DF process is very essential. These parameters are; tangential filtration system inlet flow, inlet pressure, outlet pressure, formulation buffer total volume, product pH, product final conductivity, product final osmolality and product amount. The pH is one of the most important attributes, because an out of specification pH's affect patients during product use. Besides, osmolality assure drug effectiveness and process final conductivity will assure that both of the mentioned requirements stay within process specifications.

Today current process technologies assure optimal process control regarding variables as filtration system inlet flow, inlet pressure, outlet pressure and process temperature, these parameters can be effectively controlled using automatic recipes. However, variables as product final pH, osmolality, and conductivity really depend on the DF process initial conditions which can fluctuate within bathes. For this reason, the conditions evaluated during this project were the formulation buffer and the product conductivity initial conditions. Therefore, the characterization of the DF process was the main objective before implement this project. The method used for improvement and variability control was Six Sigma, DMAIC. The DMAIC implementation followed a DOE (design of experiment) full Factorial Design 2^k experiment based on product X CTQ specifications. Nonetheless, other aspect of the process as DF volume versus final pH, osmolality and conductivity were analyzed during the experiments with the intention of evaluates process cost and cycle time reduction scenarios.

PROJECT CONTRIBUTION

The project will contribute on the DF process optimization, since the implementation phase characterized the biotech process [1] final CTQ variables at the DF step against buffer and product initial conditions. Besides, with this information the operation can be re-designed to achieve a robust process that will reduce variability and defects while improve operational costs.

DIAFILTRATION PROCESS

Membrane-based Tangential Flow Filtration (TFF) unit operations are used for clarifying, concentrating, and purifying proteins [2]. This technical briefs a practical introduction to protein processing using tangential flow filtration.

Filtration is a pressure driven separation process that uses membranes to separate components in a liquid solution or suspension based on their size and charge differences. The process can be broken down into two different operational modes – Normal Flow Filtration and Tangential Flow Filtration. The difference in fluid flow between these two modes is illustrated in Figure 1.

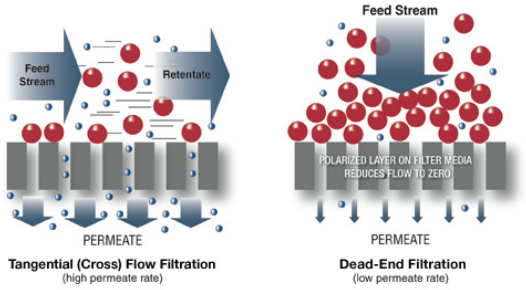

Figure 1
Filtration Modes

In Normal Flow Filtration (NFF), fluid is convicted directly toward the membrane under an applied pressure. Particulates that are too large to pass-through the pores of the membrane accumulate at the membrane surface or in the depth of the filtration media, while smaller molecules pass through to the downstream side. This type of process is often called dead-end filtration. However, the term “normal” indicates that the fluid flow occurs in the direction normal to the membrane surface, so NFF is a more descriptive and preferred name. NFF can be used for sterile filtration of clean streams, clarifying registration, and virus/protein separations. In Tangential Flow Filtration (TFF), the fluid is pumped tangentially along the surface of the membrane. An applied pressure serves to force a portion of the fluid through the membrane to the filtrate side. As in NFF, particulates and macromolecules that are too large to pass through the membrane pores are retained on the upstream side. However, in this case the retained components do not build up at the surface of the membrane. Instead, they are swept along by the tangential flow. This feature of TFF makes it an ideal process for finer sized-based separations. TFF is also commonly called cross-flow filtration. However, the term “tangential” is descriptive of the direction of fluid flow relative to the membrane, so it is the preferred name. In a TFF unit operation, a pump is used to generate flow of the feed stream through the channel between two membrane surfaces. A schematic of a simple TFF system is shown in Figure 2. During each pass of fluid over the surface of the membrane, the applied pressure forces a portion of the fluid through the membrane and into the filtrate stream. The result is a gradient in the feedstock concentration from the bulk conditions at the center of the channel to the more concentrated wall conditions at the membrane surface. There is also a concentration gradient along the length of the feed channel from the inlet to the outlet (retentate) as progressively more fluid passes to the filtrate side.

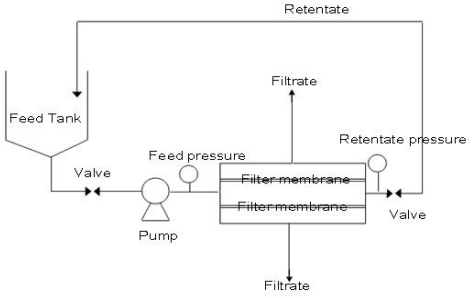

Figure 2
DF Process Diagram

METHODOLOGY

The project methodology followed fundamental concepts of Six Sigma, DMAIC, DOE Factorial Design Experiment [4]. The DMAIC define phase identified the characterization and improve needs for the DF process. The DMAIC measure phase used the DOE, factorial design experiment 2^k (k=2) with the factors of interest based on operational final CTQ control charts. Following the DMAIC methodology the factors affecting the DF process CTQ parameters were analyzed. In conclusion, DMAIC first three phases were adequate to identify all possible DF process defects and variability causes scenarios.

Define

During this phase the scope, objectives, problem statement and methodology were identified. Nevertheless, the define phase passed through a brainstorming step in where the opportunity to optimize the DF process was identified. The DF process was found with opportunities for improvement based on recent reworks and defects events. The process was monitored following a SIPOC [3] (Supply, Input, Process, Output and Customer) analysis and various operations visits. Afterwards, there was found that factors product initial conductivity (mS/cm) and formulation buffer Initial conductivity (mS/cm) were related to the rework and defect events. Control Chart, Figure 3 shows how process initial conductivity fluctuates within 10 manufacturing batches. Here, batches 1, 7 and 8 final conductivity reached the specification limits. These batches were reworked to meet up final conductivity specification range of 10-16 mS/cm. In addition, Control Chart Figure 4 shows how the formulation buffer initial conductivity runs closed to the upper specification limit. Besides, Figure 5 shows batch # 7 with an out of specification osmolality event. As well, batch 7 showed an out of specification event for the process final conductivity, but with an additional event in batch 1 on Figure 6.

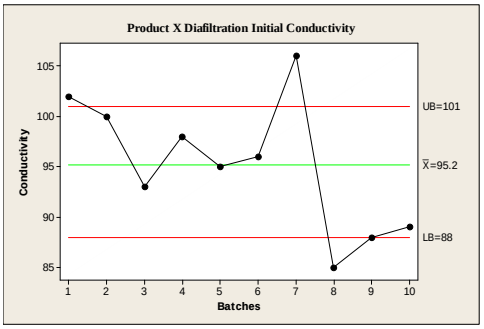

Figure 3
Product X DF Initial Conductivity 10 Batches during DF

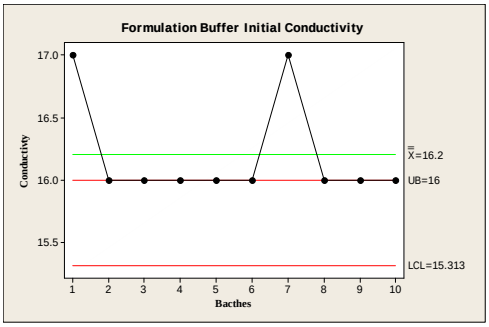

Figure 4
Formulation Buffer Initial Conductivity before the DF Process

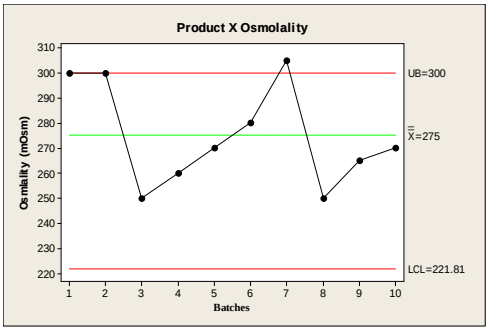

Figure 5
Final Product X Osmality within 10 Batches after the DF

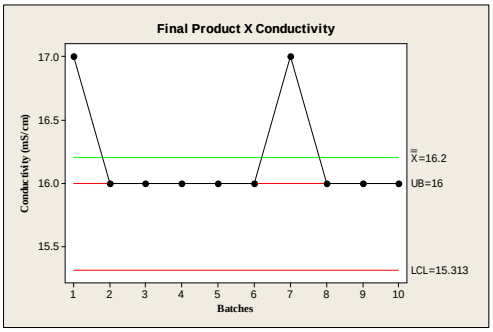

Figure 6
Final Product X Conductivity within 10 Batches

Measure

A DOE strategy was implemented based on factorial design experiment with factors Product Initial Conductivity (mS/cm) and Formulation Buffer Initial Conductivity (mS/cm) at two evaluation levels. The factors treatments were tested on an initial conductivity of 101 mS/cm or it high level and a conductivity of 89 mS/cm or it low level. The formulation buffer initial conductivity treatments were a high level of 16 mS/cm and a low level of 10 mS/cm. Table 1 show how the factorial experiment was run.

Table 1
Full Factorial Design Conditions

Factorial Design Experiment 2^k					
StdOrder	RunOrder	CenterPt	Blocks	Con	Buffer
1	1	1	1	89	10
2	2	1	1	101	10
3	3	1	1	89	16
4	4	1	1	101	16

Analyze

Using the gather information from the factorial design experiments the behavior of product initial conductivity and buffer initial conductivity during the DF process were analyzed.

Improve

A new process was designed to recommend improvements strategies to reduce DF process defects, variability, time and cost.

Control

After the project implementation procedures will be generated to monitor the DF process CTQ using control charts and to instruct manufacturing personnel on how identify any possible DF scenario that may generate defects as result of process variability. The control charts will be retrieved automatically from the process on each manufacturing batch. Afterwards, the expectation is that downstream personnel react to any diafiltration initial condition that may affect the DF process final CTQ's.

DESIGN OF EXPERIMENT

The project experiments were conducted a technical support pilot plant. The equipment used was a pilot plant scale Ultra Filtration and DF skid with 5 m^2 filter cassette membrane area. The process was run at the specified factorial design levels of 101 mS/cm-89 mS/cm for the product initial conductivity and 16 mS/cm-10 mS/cm for the buffer on a DF flow of 15 L/min at 25 C. The retentate (product) volume for the DF process (wash) was 30 L on each experiment. In addition each experiment was run under a protein concentration of 100 g/L. The target DV formulation buffer was 8X (8 x 30 L = 240 L) or 240 L. Process transmebrane (TMP) pressure was setup at 12 psig.

RESULTS AND ANALYSIS

Experiment I was conducted on a product conductivity of 101 mS/cm and a buffer conductivity of 16 mS/cm. Figure 7 shows the dialfiltration process factorial treatment conditions results for the DF process on factor A high level and factor B high level.

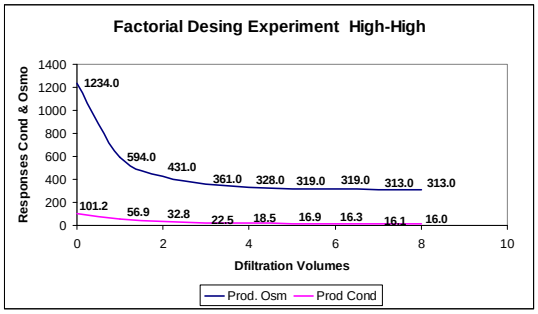

Figure 7
Factorial Design Experiment High-High

Figure 7 shows that a conductivity of 16 mS/cm (target range 16-10 mS/cm) was reached at 8 DV's. In addition, the DF process took 5 hours to reach a product osmolality of 313 mOms (target range 300-200 mOms) using a total buffer volume of 240 L or 8 DV. Process final pH result was 7.20 (target range 7.3 +/- 0.10 pH), which was within the lower specification limit.

Experiment II was conducted on a process conductivity of 101 mS/cm and a buffer conductivity of 10 mS/cm. Figure 8 shows the DF process factorial treatment conditions results for DF process factor A high level and factor B low level.

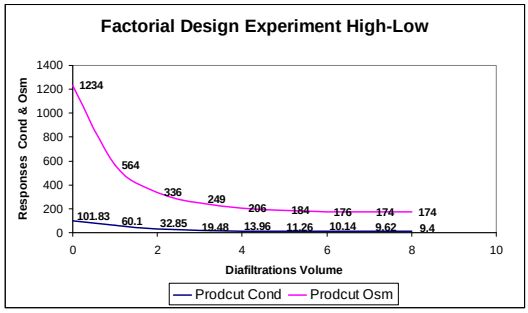

Figure 8
Factorial Design Experiment High-Low

Figure 8 showed that a conductivity of 16 mS/cm was reached approximately at 4 DV's or 1.5 hours after process started. At this point the process osmolality was approximately 200 mOms using a total buffer volume of 240 L. Process final pH result was 7.40, which is within the high specification CTQ.

Experiment III was conducted on a product conductivity of 90.2 mS/cm and a buffer conductivity of 16 mS/cm. Figure 9 shows the DF process factorial treatment conditions results.

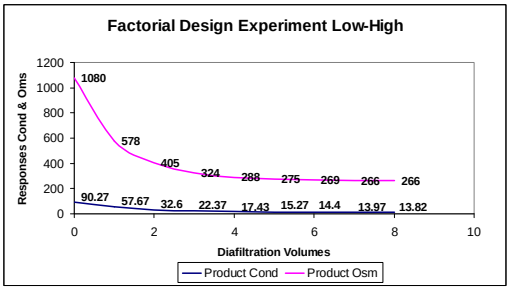

Figure 9
Factorial Design Experiment Low-High

Figure 9 conductivity of 16 mS/cm was reached approximately at 5 DV's or 1.6 hours. At this DV result, the product osmolality was approximately 280 mOms using a total buffer volume of 240 L. The process final pH result was 7.3, which is within the specification CTQ expected value.

Experiment IV was run on a product conductivity of 90.2 mS/cm and a buffer conductivity of 10 mS/cm. Below Figure 10 shows the results for the process on a product initial conductivity at a low level and a buffer initial conductivity at a low level.

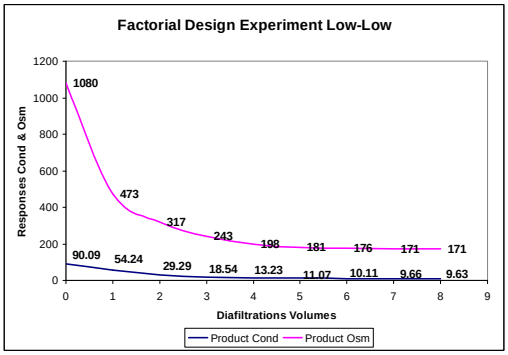

Figure 10
Factorial Design Experiment Low-Low

The process osmolality was approximately 240 mOms at 4 DV using a total buffer volume of 240 L. Product final pH result was 7.4, which is within the high specification CTQ limit.

Factorial Design Results

The information generated were analyzed following a two level factorial design using Minitab. Table 2 show the result obtained from the four experiments.

Table 2
Factorial Design Levels Treatment Results

Factorial Design Experiment Treatments & Results				
Product Initial Cond (mS/cm)	Buffer Initial Cond (mS/cm)	Product Final Cond (mS/cm)	Product Final Osm (mOms)	Product Final pH
101	16	16	313	7.2
90.2	10	9.63	171	7.41
90.2	16	13.82	266	7.32
101	10	9.4	174	7.4

Pareto chart (Figure 11) for the factors effects on process final conductivity showed that factor B or formulation buffer initial conductivity has a major effect. In addition, Figure 12 shows the same effect on the DF process final osmolality. Same effect is observed at Figure 13 in where the buffer initial conductivity was found with major effect on the final pH. Therefore there is a tendency of factor B or DF buffer initial conductivity to have an effect on the all CTQ's.

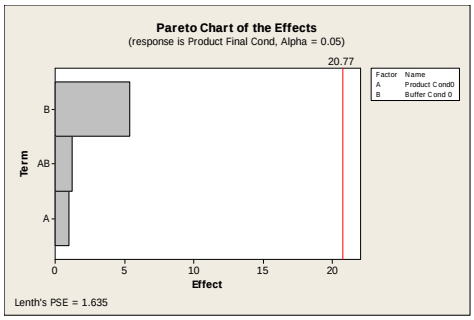

Figure 11
Pareto Effect for Product X Final Conductivity

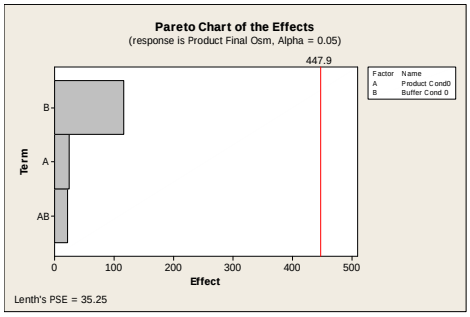

Figure 12
Pareto Effect for Product X Final Osmolality

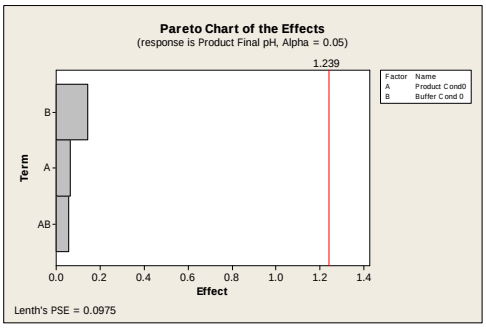

Figure 13,
Pareto Effect for Product X Final Ph

On Figure 14 the product initial conductivity showed a positive slope as well, buffer initial conductivity showed same behavior for the main effects for process final conductivity. Therefore, as these factors increase from their lower limit to their maximum limits, the process final conductivity increases. However, buffer initial conductivity has the greater slope or effect. The same effect was observed on the process final osmolality, which confirmed factor B with a major effect on the process CTQ's. Refer to Figure 15 for the osmolality main effect plot. On the other hand, the main effect plot for final pH showed that both factors have an effect. Figure 16 shows the effects of factors A and B on the DF process final pH. Here, as both factors move from their low levels to their high levels the pH tended to decrease.

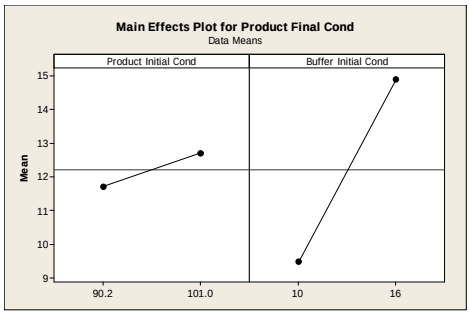

Figure 14
Main Effect for Product X Final Conductivity

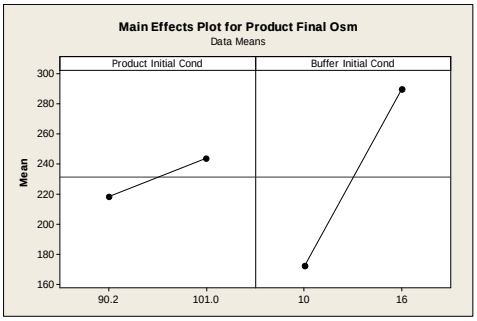

Figure 15
Main Effect for Product X Final Osmolality

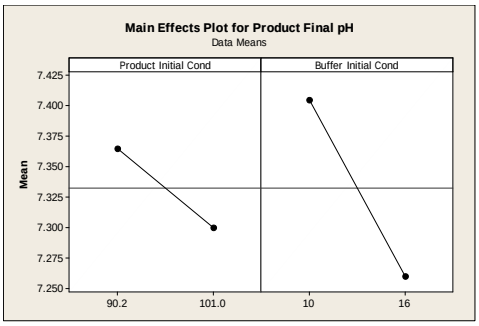

Figure 16
Main Effect for Product X Final pH

Factorial design cube plots for each one of the analyzed responses were created. Figure 17 shows the cube plot result for final conductivity. At this point the Figure demonstrated that in order to maintain a final conductivity under the specification range (10-16 mS/cm), the process conductivity must be maintained close to it lower specification limit. Additionally, process final osmolality can be maintained under the specification range (200-300 mOsm) following this

approach. On the other hand, if the buffer initial conductivity is move from it lower specification limit to it high one, the process final osmolality passed the lower specification limit. Refer to Figure 18 for cube plot of final osmolality. Figure 19 demonstrate that pH can be maintained at it specification limit following same approach as the described for the DF final osmolality and conductivity.

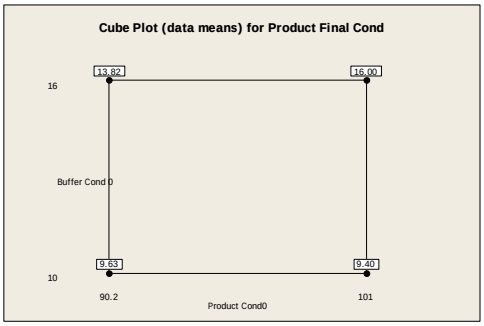

Figure 17
Cube Plot for Product X Final Conductivity

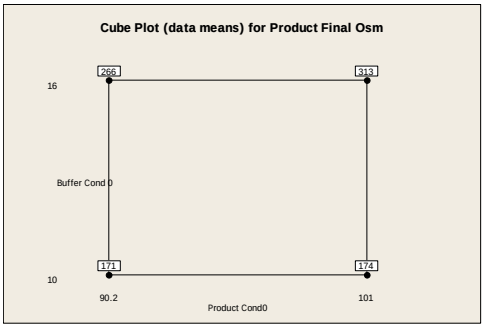

Figure 18
Cube Plot for Product X Final Osmolality

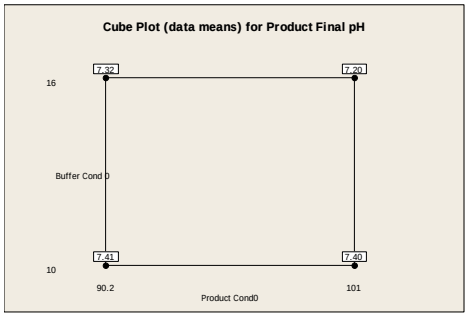

Figure 19
Cube Plot for Product X Final pH

3 axes surface plot using factor product initial conductivity buffer initial conductivity and product final pH as x, y and z variables respectively were generated. Figure 20 show the behavior of pH under DF process product initial conductivity and buffer initial conductivity changes. The Figure showed that even product initial conductivity varied from it lower specification limit to it high specification limit a buffer initial conductivity of 13.5 mS/cm maintained a pH of approximately 7.3. Therefore, as observed on pareto charts, DF process buffer initial conductivity control will satisfy pH requirements. As well, this behavior was observed on final osmolality, here the buffer initial conductivity of 13.5 mS/cm will result on an osmolality approximate value of 250 mOsm. Refer to Figure 21 for the surface plot of final osmolality. Furthermore, based on the surface Figure for the final conductivity, the expect condition is the same value set for the buffer initial conductivity (13.5 mS/cm). See Figure 22 for of the final conductivity.

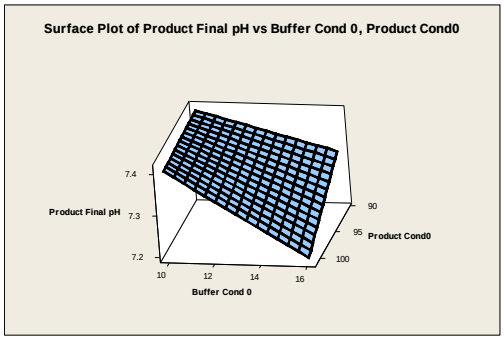

Figure 20
3 axes Surface Plot for Product X Final pH

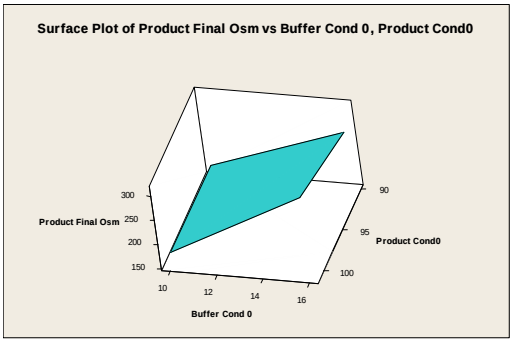

Figure 21
3 axes Surface Plot for Product X Final Osmolality.

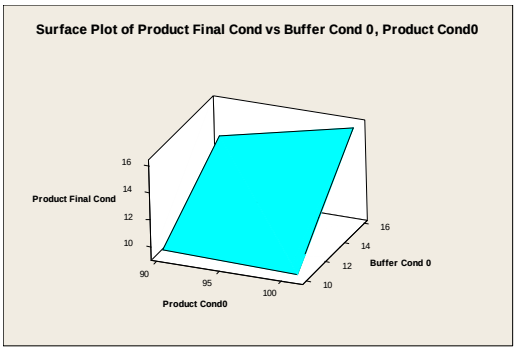

Figure 22
3 axes Surface Plot for Product X Final Conductivity.

CONCLUSION

After evaluated the gathered information from the experiments, there was found that a buffer initial conductivity of 13.5 mS/cm will result in a process optimization opportunity, also for cost and cycle time reduction. The new process design should use a buffer initial conductivity of 13.5 mS/cm, in order to achieve a DF final conductivity of 13.5 mS/cm at approximately 4 DV, which represent a process cycle time reduction of 50 % or 1.5 hours. Also the formulation buffer process real scale consumption will be reduced by approximately 1500 L/batch or 50 %. Finally, based in these facts, the potential economic impact was calculated as $ 72,420/year after the implementation of the recommended improvement strategy. The change will not have any adverse impact on the operation, since the recommended formulation buffer preparation conductivity range is under the process specification of 10-16 mS/cm. In addition, all the results obtained during factorial experiments demonstrated effective control on process final conductivity, osmolality and pH. Table 3 shows the economic potential analysis for the recommended strategy.

Table 3
New Process Cost Reduction Analysis

DMAIC Cost Reduction Analysis	
Actual Process Cost Analysis	
Manpower Utilization	8
Total Manpower hours	16
Cost per Manpower Hour ($)	15
Cost per Diafiltration Process ($)	1920
Annual Cost Based on 50 batches or full production year ($)	99840
Buffer Cost/L ($)	15
Actual Buffer Consumption/batch (L)	3000
Actual Buffer Consumption (L) Cost 50 batches or full production year	45000
Process total Cost Based on a full production year ($)	144840
New Process Cost Analysis	
Manpower Utilization	4
Total Manpower hours	16
Cost per Manpower Hour ($)	15
Cost per Diafiltration Process ($)	960
Annual Cost Based on 50 batches or full production year ($)	49920
Buffer Cost/L	15
Actual Buffer Consumption/batch (L)	1500
Actual Buffer Consumption (L) Cost 50 batches or full production year	22500
Process total Cost Based on a full production year ($)	72420
New Process Implementation Total Operational Cost Reduction ($)	72420

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