Summary

Human chorionic gonadotropin (hCG) is a glycoprotein hormone, which is used for infertility treatment. hCG appears in abundance in the urine of pregnant women during the first trimester of pregnancy. Some companies, like Diosynth, purify the hCG from urine and sell it for infertility drug. The current purification process in Diosynth does not incorporate a specific virus removal step, but according to the pathogenic virus tests, virus is never found in the final product. Although there is always a negative result in the tests, Diosynth needs to integrate a virus removal step in their current process. Another dangerous substance that is present in urine is endotoxin. Currently Diosynth uses a calcium precipitation method followed by ultra filtration.

The aim of this project is to design a plant to purify hCG, which incorporates virus removal step(s). The required specification of the final product is defined, as well as the composition of the incoming product stream, which is the outgoing product stream from the first ultra filtration step in the current process in Diosynth. The key technologies to be used are simulated moving bed chromatography-gel filtration (SMB-GF) and surfactant aided SMB-GF (SA-SMB-GF). Surfactant (Brij® 35) gradient in five sections SMB is introduced for the SA-SMB-GF system in order to improve the purification performance of this step. A process with cation exchange chromatography is used as a base case for comparison.

This report presents a complete design of the hCG purification processes mentioned above. The base case is designed so that all the requirements are just met. The detail design of base case is not shown in this report but an overview is given here. Economic aspects are also discussed in detail to be able to compare the economic performance of the processes.

In the process with SMB-GF or SA-SMB-GF, endotoxins are aggregated and then removed using ultrafiltration while virus is removed with the simulated moving bed process. During those processes, other protein contaminants are also removed. hCG is then precipitated, leaving the water, ethanol and salts in the liquid phase. Finally this purified hCG is sent to the drying system. In case of base case, endotoxin, virus and protein contaminants are removed together with cation exchange chromatography while the other steps are the same as the designed plants.

The purity of hCG produced by process with SMB-GF, SA-SMB-GF and base case is 52%, 39% and 50% respectively. Process with SA-SMB-GF does not meet the requirement for hCG purity while the other two do. The overall recovery of process with SMB-GF, SA-SMB-GF and base case is 92% and 93%, 77% respectively while virus and endotoxin are sufficiently removed from hCG in all of those processes. The Total Capital Investment (TCI) of the processes are 9,4 millions, 9,2 millions and 6,8 millions for process with SMB-GF, SA-SMB-GF and base case respectively. With 15 years as the economical plant life, both processes with SMB-GF and SA-SMB-GF have very good economic potential; Net Present Value (NPV) is 128.5 and 131.8 million Euros respectively, while the NPV from the base case process is only 87 million Euros. Other economic criteria such as rate of return and discount cash-flow rate of return also show that the processes with SMB are better than the base case. The base case is only better in term of payout time due to its low investment costs.

The best process for hCG purification is the process with SMB-GF. All the requirements are met, and the process has a good economic potential. Furthermore the final product of the process with SMB can be considered safer than the product of the process with SA-SMB, because it will not contain surfactants and it is not sure yet what impact Brij® 35 will have on humans when it is injected. Since using surfactant gives advantage in term of economic potential, it is recommended to improve the purity of SA-SMB-GF process by dedicate the SMB only for virus removal and add another equipment for protein separation like ion exchange chromatography. In this case, the SMB costs will also be less. Besides that, the SA-SMB-GF system could also be optimized for example by changing the surfactant concentration, column dimensions, number of columns per section etcetera. Furthermore, it will be useful to get some experimental data for Tween 20, since this surfactant is commonly used for injected pharmaceutical. It will be useful as well to check the performance of process with SMB-GF when SMB is only used for virus removal and not for proteins removal.

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1 Introduction

1.1 Human Chorionic Gonadotropin

Human Chorionic Gonadotropin (hCG) is a glycoprotein hormone, which is normally produced by the developing placenta, and aberrantly produced by some germ cell neoplasms (Ross, 1977). hCG is secreted by the trophoblast in increasing amount from the time of implantation of the fertilized ovum (blastocyst) in the uterine endometrium (Vankrieken et al, 2000). The hormone serves to maintain the steroid secretions of the corpus luteum. The resultant steroids maintain the lining of the uterus in a state suitable for development of the embryo after its implantation. (Birken et al, 2000).

In women who do not ovulate on their own, hCG is used for infertility treatment that brings on ovulation, or release of an egg from the ovary. In men, it is used to stimulate the production of testosterone. Some doctors also use hCG in men with erection problems or lack of sexual desire (health.yahoo.com).

hCG appears in abundance in the urine of women during the first trimester of pregnancy and reaches a peak concentration of 2-8 mg/L in both blood and urine during week 10-12 of pregnancy (Birken et al., 2000).

1.2 Project design background

Diosynth is one of the companies that produces hCG from urine of pregnant women. There are other companies that produce hCG, for example Serono. Recently more and more women and men have problems with fertility, thus there is an increasing need for infertility treatment medicines.

hCG is found in urine of pregnant women together with other substances, such as amino acids, vitamins, minerals, salt, hormones and proteins. Since hCG is administered to humans, the product needs to have a high degree of purity. One of these constraints regards the presence of viruses, which vary in dimension between 20 to 100 nm. Separating viruses from biopharmaceutical products becomes troublesome in the smaller ranges as they approach the dimensions of proteins. Although virus is hardly present in urine, a pharmaceutical company should show that during their production process, there is at least a step that removes virus to a certain level.

Another constraint is the presence of endotoxin in urine. Endotoxins's potent biological activities causes pyrogenic and shock reactions in mammals (Hirayama and Sakata, 2002). Endotoxins show strong biological effects at very low concentration in human beings when entering the blood streams. This requires removing even minute amounts of endotoxin in administered preparations. The threshold level of endotoxin for intravenous application is set to 5 endotoxin units (EU) per kg body weight and hour. It is taken as a rule of thumb that 1 EU corresponds to 100 pg of endotoxin (Petsch and Anspach, 2000).

Endotoxins are very stable molecules, their biologically active part surviving extremes of temperature and pH in comparison to proteins. Thus, it is a challenge to remove endotoxin from sensitive substances, such as proteins (Petsch and Anspach, 2000).

The present hCG purification process in Diosynth does not include a specific virus removal process, but according to the pathogenic virus tests, virus is never found in the final product. Although there is always a negative result in the tests, Diosynth needs to integrate a virus removal step in their current process, in line with the more and more strict pharmaceutical rules in the world. For endotoxin removal, Diosynth currently applies calcium precipitation method followed by ultrafiltration. There might be more efficient endotoxin removal processes or they can be removed at the same time with the viruses.

1.3 Objective of the project

The objective of this project is to design a plant that purifies human Chorionic Gonadotropin (hCG). The hCG obtained from the purification plant should have at least 50-wt% purity and should be sufficiently free from virus and endotoxin. The other 50-wt% will be other proteins normally present in urine. The level of virus in the product should be 10^3 particles/g, while the final endotoxin concentration should be 10^{-3} wt%. Basically there should be no virus in the final product. The value presented here is based on a starting value of 10^7 particle/ml raw material and the final product should contain virus below detection limit, for example < 10 particles/ml. The calculation of the final endotoxin concentration is presented in chapter 3.3.4.2. In this project, parvovirus will be taken as model virus to be separated because of its small dimension (about 20 nm) and endotoxin from *Escherichia coli* will be used as reference standard for endotoxin.

The type of hCG produced is intact hCG, which is α and β-subunits with glycosylation. The final product will be in lyophilized powder (dried) form. Detailed final product specifications are given in Table 3.5. Some other requirements, such as operational mode and capacity, which were defined in deliberation with the client, are mentioned in Table 3.1. The process will be run in a batch mode with a capacity of 100 litres per batch.

The process to be designed will be part of an existing plant in Diosynth. The team will design purification processes as the continuity of the previous existing concentration steps in Diosynth. Thus, the team will not deal with urine as the raw material but the team will deal with output stream of the last concentration process, which is ultrafiltration. The overall block diagram that shows the battery limit of the design is presented in Figure 3.1.

Delft Design Matrix (DDM) is used as guidance in doing the process design structurally. DDM describes the structure of the design framework and help to deal with the growth of information and complexity during the design process. The framework consists of 8 design spaces. These form a sequence of intermediate designs of increasing detail. The team uses Plant design Improvement by QUAlity Review (PIQUAR) as a tool for evaluationg the options. This tool helps the team to make a decision based on the important criteria that are formed from the opinions of the team, client and supervisor. Advance Activity Assistant (AAA) table is used to control the activity of the team members. AAA table is useful for keeping tract of the tasks of each team member.

1.4 Constraints on key technologies

The meaning of key technology is a technology that is used for virus removal. There are several demands for the technology used in the purification process. One process should be designed including Surfactant Aided Simulated Moving Bed technology; another process

should include Simulated Moving Bed technology (SMB) without surfactants. The designed plant with these two key technologies should be compared with the base case, which is the current process in Diosynth with ion exchange chromatography as an alternative step for virus and endotoxin removal.

2 Process options and selection

2.1 Base case

Because of confidentiality constraints, not much information on the current hCG-purification process that is performed at Diosynth is available. The base case that will be compared with the designs will therefore be a hypothetical one. In agreement with the client and all other participants, the team will also design the base case with the criteria given below.

The composition of the entering product stream is defined in Table 3.2. The main purification step in the base case is a Cation Exchange Chromatography step. This step will not run in SMB-mode. The constraints considering virus and endotoxin removal are met. The whole process of base case is run in a bacth mode with 5 batches per year. Since there is 500 litres of input stream per year, 100 litres of this will be processed in one bacth.

The binding capacity of the column material is assumed to be 20 mg of protein per ml of gel (van Dedem, 2003). The concentration of hCG in the in going stream is 2 wt%, or 20 mg/ml. Therefore in 100 litres, there is 2 kg of hCG present. If all hCG needs to be bound to the gel, 100 litre of gel material is needed. In Appendix 1, several gel materials are discussed, and SP Sepharose high performance is chosen to be the best option for the base case.

After the Cation Exchange step, the hCG is precipitated with ethanol and ammonium acetate. Now a filtration step is performed, after which the hCG is recovered and dried. All constraints and targets considering the product (purity, recovery, virus and endotoxin removal) are met. The out going product stream for the base case is defined in Table 3.5.

2.2 Key technologies in the designed plant

2.2.1 Options for key technologies

There are several options of the key technologies to be used for virus and endotoxin removal in the hCG purification process.

- Gel-SMB-chromatography
  Gel chromatography separates molecules based on their size. Since there is a difference in the size of hCG (6.4 nm) compared to virus (18-24 nm) and endotoxin (10-100 nm, in aggregate form), it is possible to use this process for hCG purification. The estimation of hCG's size is mentioned in pure components properties table in Appendix 2. In industrial scale, normal gel chromatography is less interesting because of its batch mode of operation. Simulated moving bed (SMB) technology can be applied to operate gel chromatography continuously. It can also improve eluent and resin inventory by an order of magnitude compared to traditional batch chromatography.

  A block diagram of the hCG purification process with Gel-SMB-Chromatography as the key technology is shown in Figure 2.1. The streams compositions are discussed further in chapter 4.4. The undefined stream is added because the other unit operations in the whole purification process besides SMB are still unknown. The chemicals needed in chromatography process are also included in this undefined stream. More information on

the principle of gel chromatography and SMB is presented in Appendix 3 and Appendix 4 respectively.

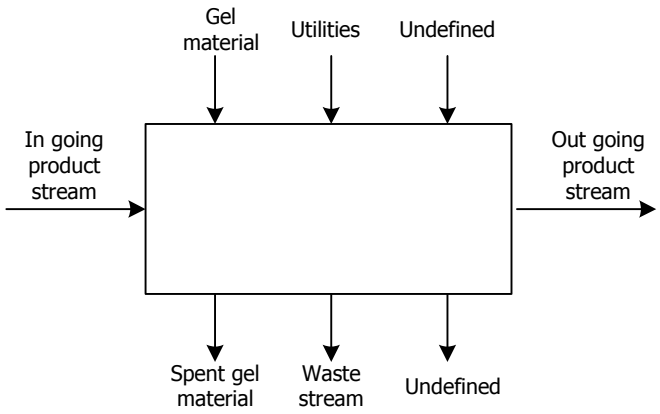


Figure 2.1 Block diagram of hCG purification process with Gel-SMB-Chromatography as the key technology

- Surfactant Aided Gel-SMB-chromatography (SA-SMB)
  The performance of SMB-gel filtration system could further be improved by adding surfactant. In an aqueous solution, with a certain concentration of surfactants, a two-phase system can be derived. One phase is the surfactant-rich phase, while the other phase only contains few micelles. This two-phase aqueous micellar system can be used to separate biomolecules. It was found that the biomolecules would have more affinity with the surfactant-poor phase (Liu et al., 1998). Surfactant changes the partition coefficient of proteins and virus in an order of magnitude depending upon the type and concentration of the surfactant, and operating condition used (Liu et al., 1998 and Kamei et al., 2002). A block diagram of the purification process with this key technology is presented in Figure 2.2.

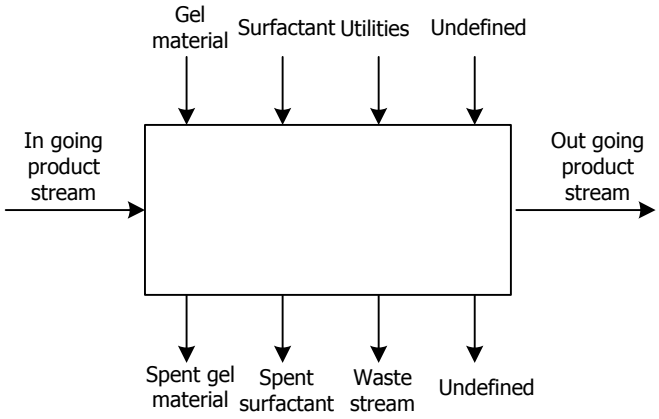


Figure 2.2 Block diagram of hCG purification process with Surfactant Aided-Gel-SMB-Chromatography as the key technology

- Immunoaffinity chromatography
  This is a separation technique in which the highly specific antibody-antigen interaction is incorporated into the separation process. Since the binding between the antibody and antigen is very specific and strong, the recovery in immunoaffinity chromatography can

be very high. Antibodies have been developed that are directed to specific epitopes on hCG (Liu and Bowers, 1996). Block diagram presented in Figure 2.1 also represent a purification process with immunoaffinity chromatography as the key technology.

- Membrane processes/filtration
  According to van Reis and Zydney there is much potential in membrane processes for virus removal. Several techniques can be used, like Ultra Filtration, Nano Filtration, Tangential flow systems, etcetera. (van Reis and Zydney, 2001). If Ca^{2+} is added, endotoxin will aggregate, and can then be removed with a filtration step (Petsch and Anspach, 2000). A block diagram of the process with membrane separation/filtration is shown in Figure 2.3.

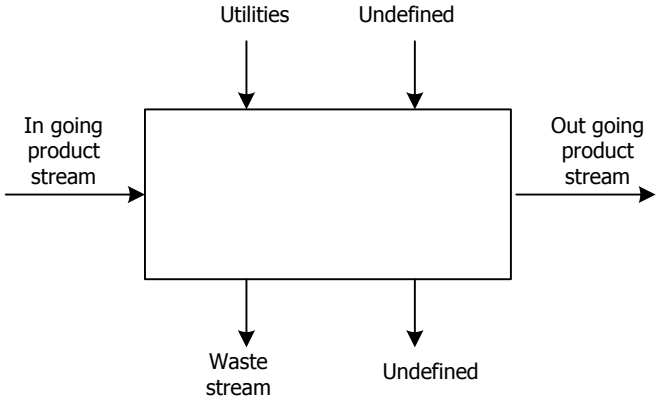


Figure 2.3 Block diagram of hCG purification process with membrane separation / filtration as the key technology

- Chemical inactivation of virus and/or endotoxin
  It is possible to add certain chemicals, which will inactivate virus or endotoxin. Examples of chemicals are β-propiolactone, formaldehyde and glutaraldehyde (Dichtelmüller *et al.*, 1993). A block diagram for this key technology is presented in Figure 2.4.

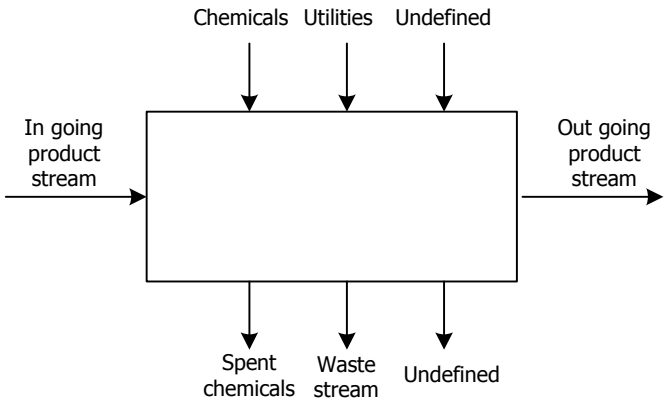


Figure 2.4 Block diagram of hCG purification process with chemical inactivation as the key technology

2.2.2 Analysis and evaluation of key technologies

The principle of separating virus from hCG could be based on charge, size, bioaffinity etcetera. The charge and affinity of virus are not known since many different types of viruses might be present in the urine. Therefore, the aim of the process is to separate virus based on their size difference with hCG. In this project, the parvo-virus (20 nm) will be taken as the model virus to be separated because of its small dimension. If the parvo-virus is sufficiently removed, other viruses, which are larger than the parvo-virus, will also be sufficiently removed.

Piquar is used as a tool in the analysis of key technologies. More information about this tool, like the Piquar factors chosen and their relative importance is available in Appendix 33. Table 2.1 to Table 2.5 show the detail results of Piquar Analysis while a summary of the total marks is given in Table 2.6. The reasons behind the marks given to the different technologies for the Piquar factors are explained below.

Immunoaffinity chromatography is highly specific but this method is relatively more expensive due to the use of antibody. Therefore this method gets the lowest score for criteria plant makes money. The filtration step and the addition of chemicals are given the highest scores for this factor, because these steps are considered to be relatively cheap. The SMB and SA-SMB step both need a chromatography column system, so both technologies are given a lower score for this factor.

For criteria specification is met, the following scores are given. Immunoaffinity chromatography enhances an interaction between hCG and antibody, which is highly specific. This will give a high purity of the product; therefore this step obtains the highest score. The addition of chemicals gets a low score, because separating the chemicals from the product might be a problem. SMB is considered to give a very good purification of the hCG, and surfactant aided SMB even better. Therefore SA-SMB obtains a high score, and SMB gets a slightly lower score.

For filtration no additional raw materials, other than a membrane, is needed, so filtration gets the highest score for criteria efficient use of raw materials. For SA-SMB, besides the gel material, also a raw material stream of surfactants is needed. Therefore, SA-SMB gets the lowest score. The other technologies score in the middle.

For safety criteria, addition of chemicals gets the lowest score, because the chemicals used might be not so safe for people and/or environment. SA-SMB also has a lower score, because surfactant also may cause safety problems. All other technologies get the same score.

For all other Piquar factors it is not sure which technologies will perform better or worse than the others. Therefore all technologies have got the same score for these factors. This will not affect the results of the Piquar analysis.

The closer the total weighted mark to 5, the better the technology. It is found that there are not so much differences between the total mark of SMB, SA-SMB and Filtration. Basically those three key technologies use a similar separation principle, which is based on the size of the compounds to be separated.

Table 2.1 Piquar analysis on SMB

Factor	Weighting factor	Marks	Weighted marks
Plant makes money	0.187	4	0.7
Specification is met	0.187	3	0.6
Safety	0.167	4	0.7
Quality of basis of design	0.146	4	0.6
Reliability	0.104	3	0.3
Efficient use of raw materials	0.083	4	0.3
Comply with construction regulations	0.042	4	0.2
Good documentation of project	0.042	4	0.2
Controllability	0.021	4	0.1
Meet functional, budget and time requirement concurrently	0.021	4	0.1
Final mark			3.7

Table 2.2 Piquar analysis on SA-SMB

Factor	Weighting factor	Marks	Weighted marks
Plant makes money	0.187	4	0.7
Specification is met	0.187	4	0.7
Safety	0.167	4	0.7
Quality of basis of design	0.146	4	0.6
Reliability	0.104	2	0.2
Efficient use of raw materials	0.083	3	0.2
Comply with construction regulations	0.042	4	0.2
Good documentation of project	0.042	4	0.2
Controllability	0.021	4	0.1
Meet functional, budget and time requirement concurrently	0.021	4	0.1
Final mark			3.7

Table 2.3 Piquar analysis on Immunoaffinity

Factor	Weighting factor	Marks	Weighted marks
Plant makes money	0.187	1	0.2
Specification is met	0.187	5	0.9
Safety	0.167	4	0.7
Quality of basis of design	0.146	4	0.6
Reliability	0.104	3	0.3
Efficient use of raw materials	0.083	4	0.3
Comply with construction regulations	0.042	4	0.2
Good documentation of project	0.042	4	0.2
Controllability	0.021	4	0.1
Meet functional, budget and time requirement concurrently	0.021	4	0.1
Final mark			3.5

Table 2.4 Piquar analysis on Filtration

Factor	Weighting factor	Marks	Weighted marks
Plant makes money	0.187	5	0.9
Specification is met	0.187	2	0.4
Safety	0.167	4	0.7
Quality of basis of design	0.146	4	0.6
Reliability	0.104	4	0.4
Efficient use of raw materials	0.083	4	0.3
Comply with construction regulations	0.042	4	0.2
Good documentation of project	0.042	4	0.2
Controllability	0.021	4	0.1
Meet functional, budget and time requirement concurrently	0.021	4	0.1
Final mark			3.8

Table 2.5 Piquar analysis on chemical inactivation

Factor	Weighting factor	Marks	Weighted marks
Plant makes money	0.187	5	0.9
Specification is met	0.187	1	0.2
Safety	0.167	4	0.7
Quality of basis of design	0.146	4	0.6
Reliability	0.104	3	0.3
Efficient use of raw materials	0.083	1	0.1
Comply with construction regulations	0.042	4	0.2
Good documentation of project	0.042	4	0.2
Controllability	0.021	4	0.1
Meet functional, budget and time requirement concurrently	0.021	4	0.1
Final mark			3.3

Table 2.6 Summary of Piquar analysis on key technologies

Key technology	Total weighted mark
Gel-SMB-Chromatography	3.7
Surfactant-Aided-Gel-SMB-Chromatography	3.7
Immunoaffinity chromatography	2.8
Membrane separation/filtration	3.8
Chemical inactivation	3.3

2.2.3 Key technologies chosen

Based on the analysis and constraints on the key technology to be used, the team decided to use Gel-SMB-Chromatography and Surfactant-Aided-Gel-SMB-Chromatography as the key technologies in hCG purification process. The filtration technology also scored very high in the Piquar Analysis, but because of the constraints on the key technologies, the team will focus on Gel-SMB-Chromatography and Surfactant-Aided-Gel-SMB-Chromatography. Some of the other technologies mentioned above will still be used in the process, but not as the key technology. As mentioned before, a base case with Ion-Exchange Chromatography as key technology also has to be designed.

2.3 Raw materials

It is mentioned earlier that the team will design purification processes after several existing concentration steps in Diosynth. Thus the main raw material of the designed plant will be the concentrated hCG in 40% ethanol. The estimated composition of this main raw material is defined in Table 3.2.

2.4 Auxiliary materials

Since one of the key technologies to be used in the design is SA-SMB-GF, the team will deal with surfactant and gel as the main auxiliary materials.

2.4.1 Surfactants

2.4.1.1 Options for surfactants

Using surfactant aided SMB is one of the process options. Thus it is important to select a proper surfactant to be used in the purification of hCG. When selecting a detergent, the first consideration is usually the ionic form of the hydrophilic group, which is anionic, cationic, zwitterionic, or non-ionic. Ease of removal of detergent from the main product is often a factor in the selection of a detergent. Some of the more common removal methods include dialysis, gel filtration chromatography, hydrophobic adsorption chromatography, and protein precipitation. The CMC value associated with the detergent is a useful guide to hydrophobic binding strengths – the higher the CMC, the weaker the binding and the easier the removal. Another useful parameter is the micelle molecular weight, which indicates relative micelle size. In most cases, the smaller the micelle, the easier the removal. If protein-detergent complexes are to be separated based on the molecular size of the protein, a small micelle size is usually preferred (www.sigmaaldrich.com).

In this project, surfactant is used to purify hCG, thus preferably the surfactant does not denaturize proteins. In this case, the non-ionic surfactant is the most suitable type of surfactants to be used. Several different companies produce many non-ionic surfactants. It is decided to choose 5 non-ionic surfactants as our options. They are Triton® X-100, Triton® X-114, Brij® 35, Brij® 58 and Tween® 20. The reasons of choosing those surfactants are described below and the main properties of these surfactants are listed in Table A 6.1 in Appendix 6.

Triton® X-100, Triton® X-114

Triton® X series are commonly used in virus inactivation followed by removal of the surfactants (Karlsson et al., 2002). Triton® X-100 and Triton® X-114 have been used in many protein purification procedures (Karlsson et al., 2002; Collen et al., 2002). Triton® X-114 is also used for selective removal of endotoxin from protein solution (Petsch and Anspach, 2000; Wilson et al., 2001).

Triton® X-100 and Triton® X-114 are non-ionic detergents, 100% active ingredient, which are often used in biochemical applications to solubilize proteins. They do absorb in the ultraviolet region of the spectrum, so it can interfere with protein quantification by absorption at A_{280nm}. A number of polymeric resins have been used to remove X-100 from solution, including Amberlite hydrophobic XAD resins and Rezorian A161 cartridges (www.sigmaaldrich.com). More information about these surfactants is described in Appendix 5.

Brij® 35 and Brij® 58

Brij® 35 and Brij® 58 were used in a research to purify Bovine Serum Albumin (BSA) and myoglobine (Myo) using surfactant aided size exclusion chromatography. These non-ionic surfactants are used to minimize interactions between the surfactant's micelles and the product, other than size exclusion interactions (Horneman et al., 2003). Some relevant data's needed in the calculation of surfactant aided size exclusion SMB are presented in Horneman et al. (2003) and Tanford et al. (1977). These two surfactants are also mentioned as pharmaceutical ingredients, but only for topical use (www.uniqema.com).

Tween® 20

Besides Brij® 35 and Brij® 58, Uniqema Health Care also mentioned Tween® 20 as pharmaceutical ingredients (www.uniqema.com). Tween® 20 is often used as pharmaceutical excipient (Lo, 2003; Kim et al., 2001; info.bio.cmu.edu.html) and it has been used as solubilizing agents in the dosage form for the intramuscular injection of poorly water-soluble drugs (Kim et al., 2001). Additionally, Food and Drug Administration (FDA) mentions the maximum use of Tween® 20 for intravenous injection is 0.4% (www.accessdata.fda.gov).

2.4.1.2 Analysis and evaluation of surfactants

Piquar is used as a tool in evaluating the surfactants. Complete Piquar analysis results are shown in Table 2.7 to Table 2.11. The reasons behind the marks given in Piquar analysis are mentioned below.

Since there is no experimental data, the amount of surfactant needed for hCG purification is not known. It may be that a different surfactant will need larger quantities or will be easier to separate from the product. Thus, the team will not look at the different prices when analyzing these surfactants. Based on these reasons, the team gives equal marks for category plant makes money in Piquar analysis for all surfactants, which are 3.

Triton® X-series have been used a lot for protein purification. The CMC values of the Triton® X-series are also higher than the other two surfactants, which might help in the detergent removal from the product. Unfortunately the Triton® X-series are not easily biodegradable. They leave relatively stable metabolite octylphenol compound that is toxic to both marine and fresh species (Li and Chen, 2002). The use of nonylphenol and octylphenol based surfactants is declining in Europe due to this environmental constraints (pubs.acs.org). Additionally, Brij® 35, Brij® 58 and Tween® 20 are in the list of pharmaceutical ingredients, while Tritons are not. Based on the above reviews, the team gives a very low point in the safety criteria for Tritons. Brijs are used as pharmaceutical excipients, but so far it is only used in topical used. Thus the team gives 2 as the mark for these surfactants. Tween® 20 is more frequently used in pharmaceutical formulation compared to Brijs. Moreover, Tween is used in injected pharmaceutical. That is why it gets the highest marks in this criterion.

More information is available on the micelle sizes of Brij® 35 and Brij® 58 than on the micelle sizes of Tritons and Tween® 20. Since micelle size is important information for the design, the team gives low values for Tritons and Tween in quality of basis of design criteria, which are 2. The same reasoning applies for reliability criteria.

For other criteria, the team gives the same marks for each surfactant, since there is no difference in those criteria for all surfactants analyzed. For all those criteria the team gives marks of 3 except for controllability and good documentation of project. The team gives 2 for controllability since using surfactant will decrease the controllability of the whole system. The summary of the final marks for each surfactant is mentioned in Table 2.12.

Table 2.7 Piquar analysis of Triton® X-100

Factor	Weighting factor	Marks	Weighted marks
Plant makes money	0.187	3	0.6
Specification is met	0.187	3	0.6
Safety	0.167	1	0.2
Quality of basis of design	0.146	2	0.3
Reliability	0.104	2	0.2
Efficient use of raw materials	0.083	3	0.2
Comply with construction regulations	0.042	3	0.1
Good documentation of project	0.042	4	0.2
Controllability	0.021	2	0.0
Meet functional, budget and time requirement concurrently	0.021	3	0.1
Final mark			2.4

Table 2.8 Piquar analysis of Triton® X-114

Factor	Weighting factor	Marks	Weighted marks
Plant makes money	0.187	3	0.6
Specification is met	0.187	3	0.6
Safety	0.167	1	0.2
Quality of basis of design	0.146	2	0.3
Reliability	0.104	2	0.2
Efficient use of raw materials	0.083	3	0.2
Comply with construction regulations	0.042	3	0.1
Good documentation of project	0.042	4	0.2
Controllability	0.021	2	0.0
Meet functional, budget and time requirement concurrently	0.021	3	0.1
Final mark			2.4

Table 2.9 Piquar analysis of Brij® 35

Factor	Weighting factor	Marks	Weighted marks
Plant makes money	0.187	3	0.6
Specification is met	0.187	3	0.6
Safety	0.167	2	0.3
Quality of basis of design	0.146	4	0.6
Reliability	0.104	4	0.4
Efficient use of raw materials	0.083	3	0.2
Comply with construction regulations	0.042	3	0.1
Good documentation of project	0.042	4	0.2
Controllability	0.021	2	0.0
Meet functional, budget and time requirement concurrently	0.021	3	0.1
Final mark			3.1

Table 2.10 Piquar analysis of Brij® 58

Factor	Weighting factor	Marks	Weighted marks
Plant makes money	0.187	3	0.6
Specification is met	0.187	3	0.6
Safety	0.167	2	0.3
Quality of basis of design	0.146	4	0.6
Reliability	0.104	4	0.4
Efficient use of raw materials	0.083	3	0.2
Comply with construction regulations	0.042	3	0.1
Good documentation of project	0.042	4	0.2
Controllability	0.021	2	0.0
Meet functional, budget and time requirement concurrently	0.021	3	0.1
Final mark			3.1

Table 2.11 Piquar analysis of Tween® 20

Factor	Weighting factor	Marks	Weighted marks
Plant makes money	0.187	3	0.6
Specification is met	0.187	3	0.6
Safety	0.167	4	0.7
Quality of basis of design	0.146	2	0.3
Reliability	0.104	2	0.2
Efficient use of raw materials	0.083	3	0.2
Comply with construction regulations	0.042	3	0.1
Good documentation of project	0.042	4	0.2
Controllability	0.021	2	0.0
Meet functional, budget and time requirement concurrently	0.021	3	0.1
Final mark			2.9

Table 2.12 Summary of Piquar analysis results on surfactants

Surfactant	Total weighted mark
Triton® X-100	2.4
Triton® X-114	2.4
Brij® 35	3.1
Brij® 58	3.1
Tween® 20	2.9

The maximum score for Piquar analysis is 5 and in this analysis the team only obtains 3 as the maximum. The reason for this is that the lack of some proven data. Although Brij® 35 and Brij® 58 have been used for purification of Bovine Serum Albumin (BSA) and Myoglobin (Myo) in normal gel-filtration (Horneman et. al, 2003), at this moment it is not known how exactly these surfactants will perform in hCG purification process. Moreover, so far those two surfactants are only found in topical pharmaceuticals. On the other hand, there is no enough data for Tween® 20, which is used in intravenous, inject able drugs.

Based on the above analysis, it is decided not to use Tween® 20 in this project design since there is not enough data to compute the simulation in SA-SMB-GF process. Brij® 35 and Brij® 58 are then analysed further to see how partition coefficients of hCG and virus changes with surfactants concentrations. For this, information on the gel to be used is important, thus the analysis of Brij® 35 and Brij® 58 are discussed in chapter 2.4.3.2, after the possible gels to be used are defined.
Although those three surfactants are used in pharmaceutical formulations, they still have to be removed from the final product since they also have negative effect on human health (Table A 6.1). This surfactant can be recycled or discharged to a wastewater treatment facility, which will be discussed further in chapter 2.5.2.

2.4.2 Gel

2.4.2.1 Options for gel

Choice of an appropriate gel depends on the sizes of the molecules to be separated (Gel filtration, Principles and Methods). In this project design, the target protein is hCG which has molecular weight of 36000 Da. Different types of gel suitable for separation regarding to the size of hCG are shown in Appendix 7.

2.4.2.2 Analysis and evaluation of gel

In the input stream, other compounds that need to be removed might be present: parvovirus and endotoxin. The molecular weight range of endotoxin is 10000-100000 and the parvovirus size is in the range of 18-24 nm. It can be seen that almost all types of gel listed above can be applied. However, this will lead to too many options for this project.

It was reported that Sephacryl HR would generally be the most suitable gel for the separation with molecular weights in a wide range (Gel filtration, Principles and Methods). In addition, Sephacryl HR is also preferable for fractionating components of very different in shape like in this project. Therefore, it is decided to use Sephacryl S-300 HR, which has been used in some studies in Kluyver Laboratory. One more gel that is chosen as an alternative is Sephacryl S-200 HR, which may have a better resolution for the target molecular weight range.

2.4.3 Surfactant and gel chosen

This chapter will discuss about the chosen gel that will be used in the SMB process with or without surfactant. The final decision on which surfactant will be used in SA-SMB process will also be described.

2.4.3.1 SMB process without surfactants

Sephacryl S-200 HR

In Appendix 8, the calculation of the partition coefficients of hCG and virus for Sephacryl S-200 HR without surfactants, is shown. The result can be seen in Table 2.13. It is obvious that the partition coefficient of hCG is much larger than the partition coefficient of virus, so hCG will be much more present in the gel than virus.

If the partition coefficient of hCG is divided by the coefficient of virus, a measure of the separation between hCG and virus is obtained. This separation factor (S) can be used to compare performance of gels types. S is defined as the highest partition coefficient divided by the smallest partition coefficient, as can be seen in Equation 2.1. Therefore, the separation factor always has a number equal to one, or higher. If the partition coefficient of both components is equal, the separation factor will be 1. In this case, the partition coefficient of hCG is higher than that of virus, so the separation factor S is defined as the partition coefficient of hCG divided by the partition coefficient of virus. This gives a separation factor of $6.2 \cdot 10^3$.

$$S = \frac{K_{highest}}{K_{smallest}}$$ Equation 2.1

Table 2.13 Partition coefficients of hCG and virus, with Sephacryl S-200 HR as gel, and no surfactants

K_{HCG}	K_{virus}	S
0.20	$3.2 \cdot 10^{-5}$	$6.2 \cdot 10^3$

Sephacryl S-300 HR without surfactants

In Appendix 8, the calculation of the partition coefficients of hCG and virus for Sephacryl S-300 HR without surfactants, is also shown. The result can be seen in Table 2.14. The partition coefficient of hCG is again much larger than the partition coefficient of virus. The separation factor S for Sephacryl S-300 HR is $1.7 \cdot 10^{2}$, which is much lower than S for Sephacryl S-200 HR.

Table 2.14 Partition coefficients of hCG and virus, with Sephacryl S-300 HR as gel, and no surfactants

K_{HCG}	K_{virus}	S
0.36	$2.0 \cdot 10^{-3}$	$1.7 \cdot 10^{2}$

The chosen gel for SMB process without surfactant

The system with the highest separation factor will be taken along for the final design. It is clear from the discussion above that a system with Sephacryl S-200 HR as a gel has the highest separation factor ($S = 6.2 \cdot 10^{3}$). Therefore, for the design of the process with an SMB-unit without surfactants, Sephacryl S-200 HR is the gel that will be used.

2.4.3.2 SMB process with surfactants

Sephacryl S-200 HR with Brij® 35

The partition coefficients of hCG and virus depend on the concentration of Brij® 35. In Appendix 8, the partition coefficients of hCG and virus as a function of the concentration of Brij® 35, for Sephacryl S-200 HR are calculated. The result, for a surfactant concentration range of zero to twenty-weight percentage, is shown in Figure 2.5.

The influence of the surfactant can be clearly seen in this figure. Under about 10 %, the partition coefficient of hCG is higher than that of virus. Above 10 %, the selectivity reverses, and virus will prefer to stay in the solid phase than hCG.

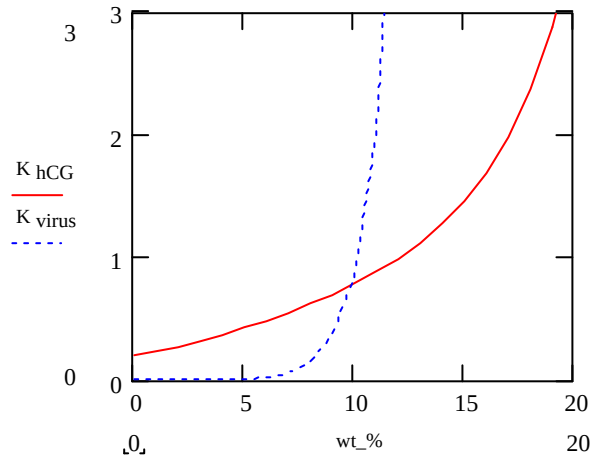


Figure 2.5 Partition coefficients of hCG and virus as a function of the concentration of Brij® 35 (weight percentage) with Sephacryl S-200 HR as gel

Sephacryl S-200 HR with Brij® 58

In Appendix 8 the partition coefficients of hCG and virus as a function of the concentration of Brij® 58, for Sephacryl S-200 HR are also calculated. The result, for a concentration range of zero to twenty-weight percentage, is shown in Figure 2.6. The lines follow the same course as the lines in Figure 2.5, only the point where the selectivity reverses lays at a higher concentration.

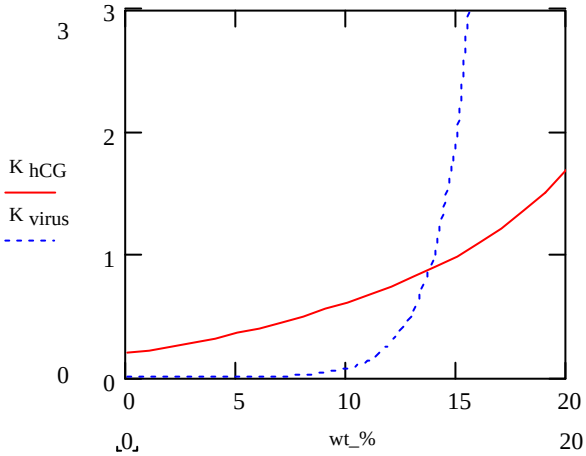


Figure 2.6 Partition coefficients of hCG and virus as a function of the concentration of Brij® 58 (weight percentage) with Sephacryl S-200 HR as gel

Sephacryl S-300 HR with Brij® 35

In Appendix 8 the partition coefficients of hCG and virus as a function of the concentration of Brij® 35, for Sephacryl S-300 HR are calculated. The result, for a concentration range of zero to twenty-weight percentage, is shown in Figure 2.7. The same kind of plot as in the previous paragraphs is obtained. Here, the selectivity reverses around 8%.

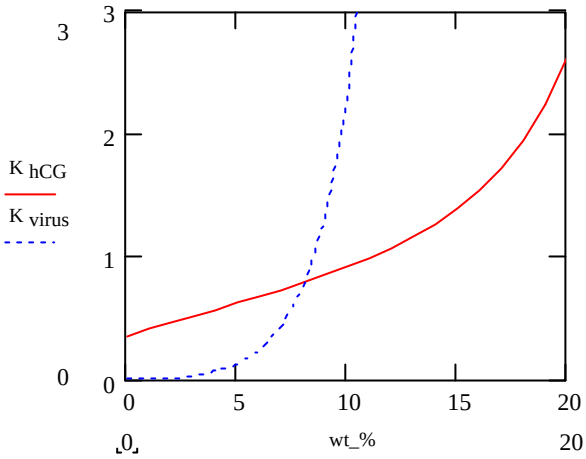


Figure 2.7 Partition coefficients of hCG and virus as a function of the concentration of Brij® 35 (weight percentage) with Sephacryl S-300 HR as gel

Sephacryl S-300 HR with Brij® 58

In Appendix 8 the partition coefficients of hCG and virus as a function of the concentration of Brij® 58, for Sephacryl S-300 HR are calculated. The result, for a concentration range of zero to twenty-weight percentage, is shown in Figure 2.8. Again, the plot is similar to the previous cases. The selectivity reverses this time around 11%.

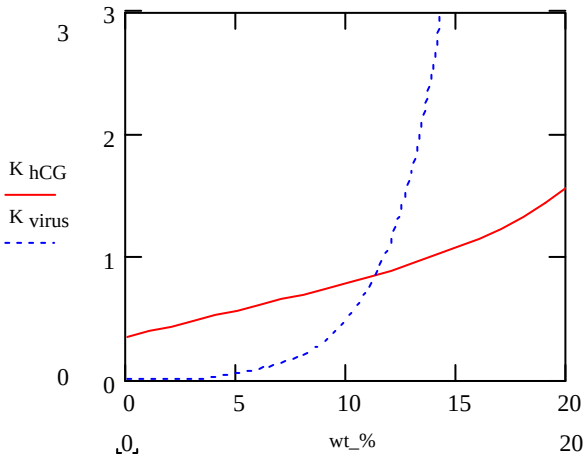


Figure 2.8 Partition coefficients of hCG and virus as a function of the concentration of Brij® 58 (weight percentage) with Sephacryl S-300 HR as gel

The surfactant chosen for SA-SMB process

According to Horneman (2003), the concentration of surfactant should not be higher than 10 wt-%. If the graphs with the same gel are compared (Figure 2.5 and Figure 2.6 or Figure 2.7 and Figure 2.8), it is only possible to work before or after the crossing point if Brij® 35 is used. In this case, the team has more freedom to choose the working area during the simulation for the SA-SMB-GF design. Thus, Brij® 35 is chosen as the surfactant used in this project design.

The gel chosen for SA-SMB process

Similarly, if Figure 2.5 and Figure 2.7 are compared, it is only possible to work before or after the crossing point if gel Sephacryl S-300 HR is used. Thus, with the same reasoning as above, this gel is chosen to be used in the project design.

2.5 Overall purification process

Before going into detail to the equipment's designs, it is important to define all kind of tasks needed to achieve the desired product specification. Although the key technologies to be used for virus removal have already been decided, there is still a need to look at the overall purification process. According to design objectives, targets and constraints, main tasks in the overall hCG purification process are:

- Removal of virus from input stream
- Removal of endotoxin
- Separation of salts and other soluble components
- Removal of ethanol
- Removal of water

The following assumptions are taken to perform the above tasks:

- As a design constraint, virus is removed only by SMB-Gel Filtration (SMB-GF) or surfactant-aided SMB-Gel Filtration (SA-SMB-GF) process. No other process option will be considered.
- Several options are available to separate endotoxin: precipitation, two phases extraction and adsorption techniques. These options are discussed in detail in Appendix 9, and it is decided that precipitation would be the best separation process for endotoxin among those three. In this chapter, a possibility of separating endotoxin with SMB-gel filtration and SMB-affinity will be discussed.

In order to have a good overview, the tasks are grouped into several categories:

- Design alternatives in the process containing SMB-GF
- Design alternatives in the process containing SA-SMB-GF
- Common design alternatives applicable for both process containing SMB-GF and SA-SMB-GF
- Generation of tasks in base case

2.5.1 Process with SMB-GF

2.5.1.1 Design alternatives

In this process option, virus is removed by SMB-GF chromatography, and endotoxin is removed either by SMB-GF chromatography or by other means. Different design alternatives in this category are presented in Figure 2.9 to Figure 2.11.

Design alternatives concerning virus and endotoxin removal

Design alternative 1 of the process containing SMB-GF (Figure 2.9) was generated based on the following assumptions:

- Virus is removed by SMB-GF technique.
- Endotoxin is removed by SMB (either gel filtration or affinity chromatography) system.

Virus can be removed first and then followed by endotoxin removal or the other way around. In Figure 2.9, it is shown that virus is removed first. Formulation of desorbent buffer is an additional task required in SMB system.

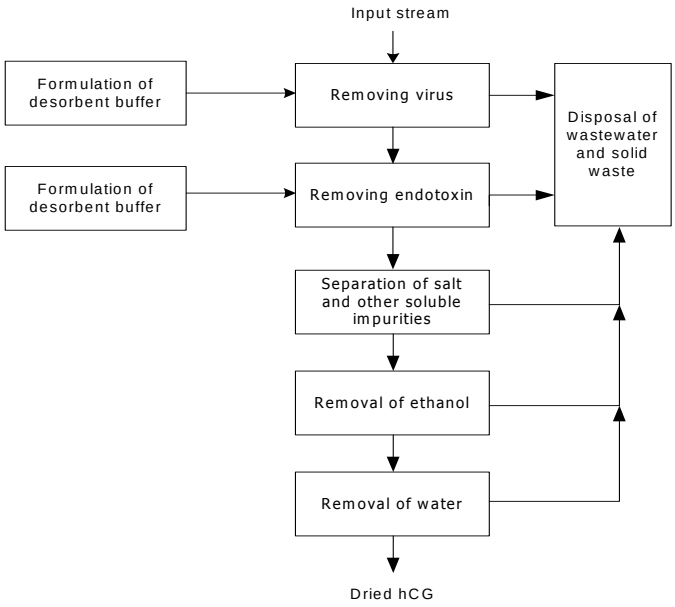


Figure 2.9 Design alternative 1 of the process containing SMB-GF

In design alternative 2, it is assumed that endotoxin exists as aggregates if Ca^{2+} are added into the medium (Petsch and Anspach, 2000). In this case, formulation of Ca^{2+} solution is required. Therefore, endotoxin can be separated by precipitation technique. As described before, the order of removing virus and endotoxin aggregates can be one or other way around.

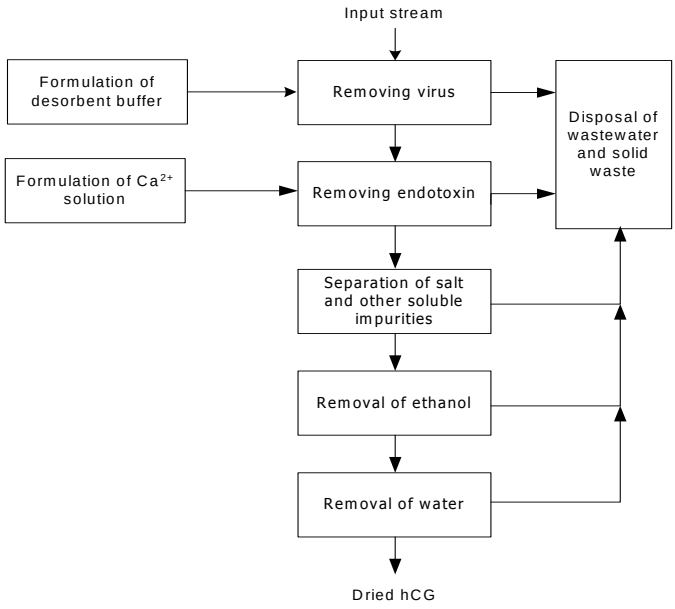


Figure 2.10 Design alternative 2 of the process containing SMB-GF

If endotoxin exists as aggregates, the particle size is bigger than hCG, and even bigger then virus (Petsch and Anspach, 2000). Consequently, virus and endotoxin could be separated together in one task by SMB-GF, which leads to the generation of design alternative 3 (Figure 2.11).

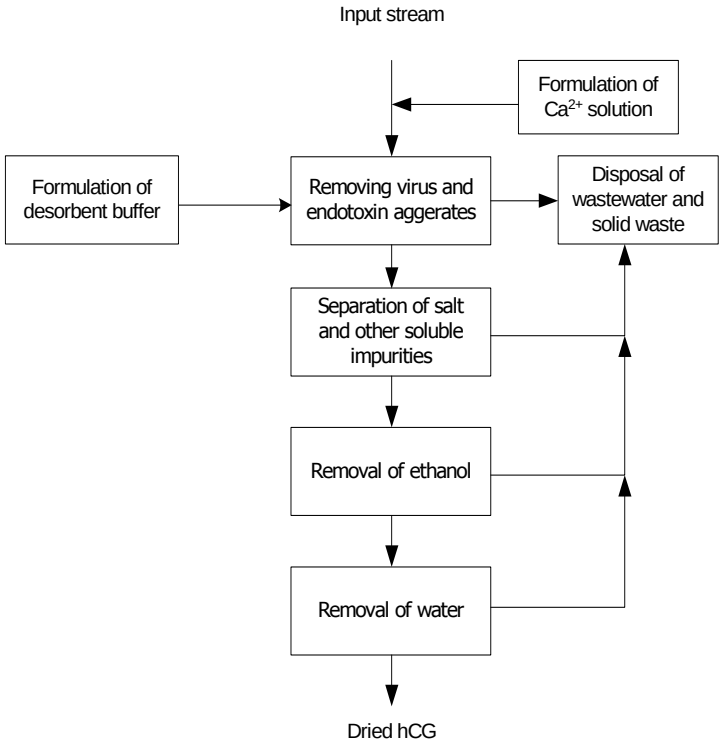


Figure 2.11 Design alternative 3 of the process containing SMB-GF

2.5.1.2 Analysis of the design alternatives

Analysis of the virus and endotoxin removal

It is possible to remove virus from hCG by SMB-GF chromatography (design alternative 1,Figure 2.9), since they are different from hCG in term of size. In Table 2.15 and Table 2.16, each task is described briefly and possible composition of streams is presented respectively (according to Figure 2.12).

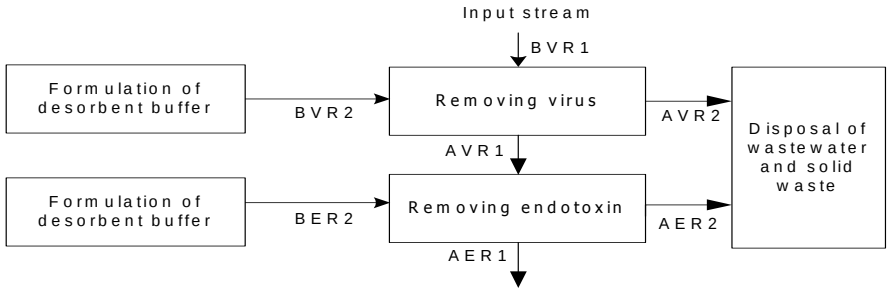


Figure 2.12 Major tasks and streams of design alternative 1

Table 2.15 Description of tasks according to Figure 2.12

Tasks	Activity
Removing virus	Remove virus from input stream (BVR1), thereby AVR1 is sufficiently free of virus
Removing endotoxin	Remove endotoxin from stream come after virus removal (AVR1), thereby AER1 is sufficiently free of endotoxin
Formulation of desorbent buffer	Prepare eluent to supply for the tasks require SMB chromatography process
Disposal of waste	Dispose all the waste of the process to the waste treatment area

Table 2.16 Description of the streams according to Figure 2.12

Stream No.	Description	Components
BVR1	Input stream	hCG, virus, endooxin, ethanol, other impurities and ethanol in water
BVR2/BER2	Desorbent solution	Phosphate buffer
AVR1	Virus free input stream	hCG, endooxin, ethanol, other impurities and ethanol in water
AVR2	Virus containing raffinate (waste) stream	Virus in water
AER1	Virus and endotoxin free input stream	hCG, ethanol, other impurities and ethanol in water
AER2	Endotoxin containing raffinate (waste) stream	Endotoxin in water

In design alternative 1, endotoxin can also be removed by SMB affinity chromatography. It was decided earlier that affinity chromatography would not be used for virus removal due to its high cost. Actually, this method is used in laboratory scale to separate endotoxin and it gives a good result, but in expense of high cost (Petsch and Anspach, 2000). Therefore, it is decided not to use affinity-SMB method for endotoxin removal.

As mentioned before, in design alternative 2 (Figure 2.10) endotoxin is removed by precipitation. Thus there is a need to provide appropriate concentration of Ca^{2+} to form endotoxin aggregates. Ca^{2+} is given in the form of $Ca_3(PO)_2$ in water.

Design alternative 3, where endotoxin aggregates and virus particles are removed at the same time, can also be a good option. Thus, design alternative 1 (with SMB-GF method for endotoxin removal), 2 and 3 will be further evaluated using Piquar method.

Table 2.17 Design alternatives under the process containing SMB-GF, which will be evaluated further

Design alternative no.	Assumptions
Design alternative 1	Endotoxin are removed by SMB gel filtration chromatography
Design alternative 2	Endotoxin is removed by precipitation
Design alternative 3	Endotoxin aggregate is removed at the same time with virus by SMB-GF

Analysis of the order of the tasks

In design alternative 1 and 2, order of the tasks (removing virus and endotoxin) is still flexible, but it is better to remove endotoxin before virus because relatively large amounts of endotoxin is present in the input stream than that of virus. If endotoxin is removed later, it could also cause problem in the virus removing gel column because endotoxin is a rather

sticky material (Petsch and Anspach, 2000). Thus, it is decided for design alternative 1 and 2, endotoxin is removed first before virus.

2.5.1.3 Evaluation of the design alternatives

Design alternatives 1, 2 and 3 are evaluated here. The detail Piquar analysis results are presented in Table 2.18 to Table 2.20 and the summary is presented in Table 2.21. The reasons of marking in the piquar factor are:

- Alternatives 1 and 2 contain two separate tasks for removing virus and endotoxin. Separating virus and endotoxin together is cheaper than separation in two units. Endotoxin removal by SMB unit is more expensive than removing by aggregation and filtration.
- It is hard to achieve 100% endotoxin aggregation by Ca^{2+} addition. If aggregation is less than 100%, the product may contain more endotoxin than what is required.
- All alternatives are considered to have the same level of safety.
- Not enough experimental data are available for removing virus and endotoxin aggregates together by SMB-GF. Also, experimental evidence is not sufficient for separating endotoxin from protein sample by gel filtration chromatography.
- Separation of endotoxin by aggregation and precipitation/filtration is more reliable than gel filtration.
- Achieving two tasks together causes less product loss during operation.
- Construction of two SMB processes is less handy than one SMB process.
- Alternatives would not affect the documentation of the project.
- All processes are controllable, but two SMB processes may cause difficulty in term of controllability.

Table 2.18 Piquar results of design alternative 1

Factor	Weighting factor	Marks	Weighted marks
Plant makes money	0.187	3	0.6
Specification is met	0.187	4	0.7
Safety	0.167	4	0.7
Quality of basis of design	0.146	4	0.6
Reliability	0.104	3	0.3
Efficient use of raw materials	0.083	3	0.2
Comply with construction regulations	0.042	3	0.1
Good documentation of project	0.042	4	0.2
Controllability	0.021	3	0.1
Meet functional, budget and time requirement concurrently	0.021	4	0.1
Final mark			3.6

Table 2.19 Piquar results of design alternative 2

Factor	Weighting factor	Marks	Weighted marks
Plant makes money	0.187	4	0.7
Specification is met	0.187	4	0.7
Safety	0.167	4	0.7
Quality of basis of design	0.146	5	0.7
Reliability	0.104	4	0.4
Efficient use of raw materials	0.083	3	0.2
Comply with construction regulations	0.042	4	0.2
Good documentation of project	0.042	4	0.2
Controllability	0.021	4	0.1
Meet functional, budget and time requirement concurrently	0.021	4	0.1
Final mark			4.1

Table 2.20 Piquar results of design alternative 3

Factor	Weighting factor	Marks	Weighted marks
Plant makes money	0.187	5	0.9
Specification is met	0.187	3	0.6
Safety	0.167	4	0.7
Quality of basis of design	0.146	3	0.4
Reliability	0.104	3	0.3
Efficient use of raw materials	0.083	4	0.3
Comply with construction regulations	0.042	4	0.2
Good documentation of project	0.042	4	0.2
Controllability	0.021	4	0.1
Meet functional, budget and time requirement concurrently	0.021	4	0.1
Final mark			3.8

Table 2.21 Summary of Piquar analysis results on design alternatives of process with SMB-GF

Design alternative	Total weighted marks
1	3.6
2	4.1
3	3.8

2.5.2 Process with SA-SMB-GF

2.5.2.1 Design alternatives

Under this process option, it is assumed that virus will be removed by SA-SMB-GF chromatography while endotoxin will be removed by either any SA-SMB process or precipitation technique.

Design alternatives concerning the virus and endotoxin removals

Design alternative 4 of process containing SA-SMB-GF (Figure 2.13) was generated based on the following assumptions:

- Virus and endotoxin are separated by SA-SMB-GF.
- Virus will be removed first, followed by endotoxin.
- Surfactant enters the SMB column with desorbent.
- Surfactant is recycled in order to reduce cost and environmental pollution.

It is also possible to remove endotoxin first before removing virus.

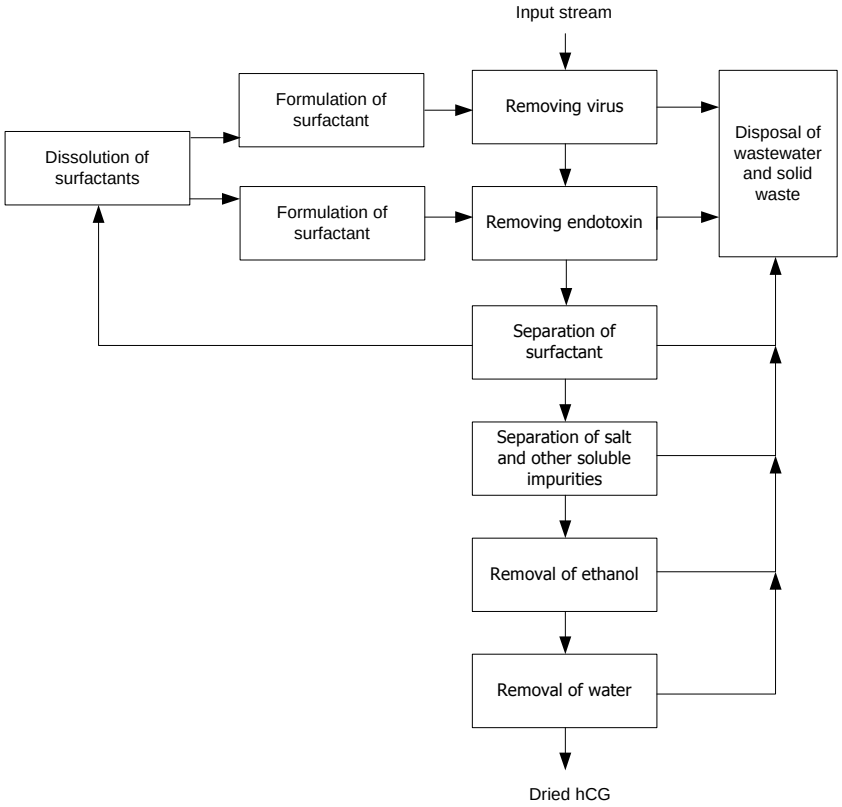


Figure 2.13 Design alternative 4 of the process containing SA-SMB-GF

In design alternative 4, endotoxin removal is done by SA-SMB-GF. According to the process option and constraints, endotoxin removal can be done by other SMB system or another process. Two more design alternatives are then generated: Design alternative 5, in which Endotoxin removal was achieved by other SMB system (Figure 2.14) and Design alternative 6, in which Endotoxin removal was achieved by another system rather than SMB, possibly by Ca^{2+} precipitation (Figure 2.15). In these design alternatives, the order of removing virus and endotoxin can be either way around.

In design alternative 7, it is assumed that endotoxin would exist as aggregates to make a bigger particle (Petsch and Anspach, 2000). Thus, virus and endotoxin can be separated together in one task with SA-SMB-GF (Figure 2.16).

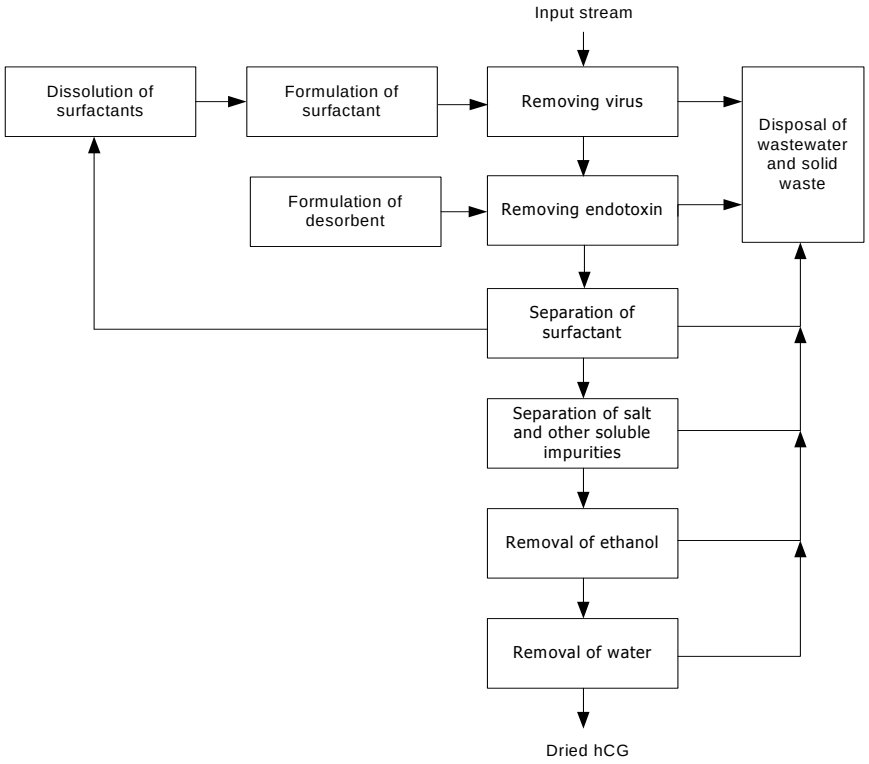


Figure 2.14 Design alternative 5 of the process containing SA-SMB-GF

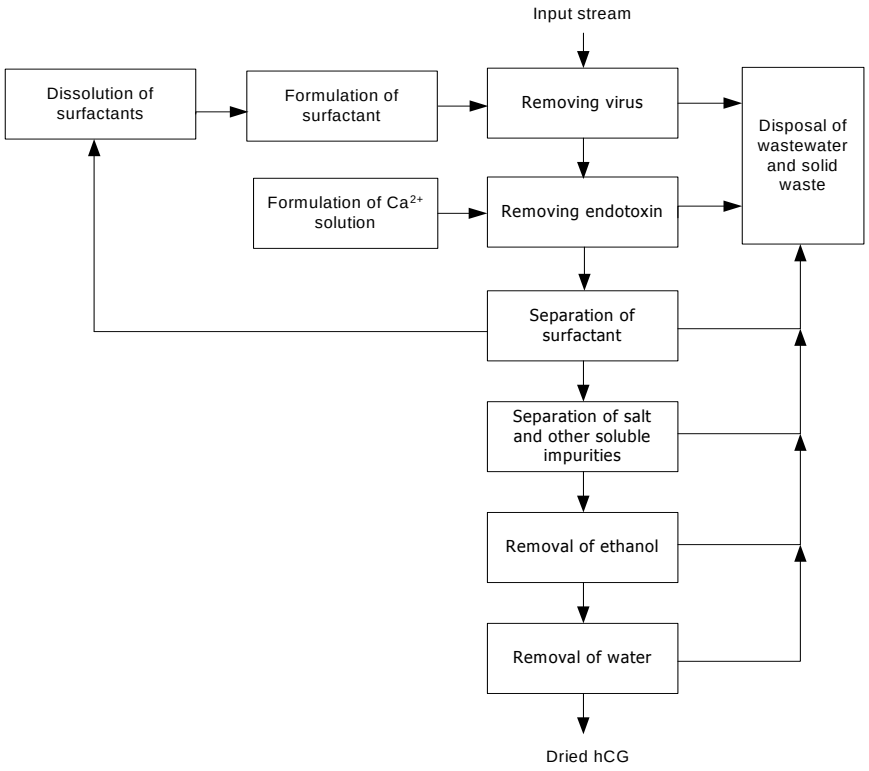


Figure 2.15 Design alternative 6 of the process containing SA-SMB-GF

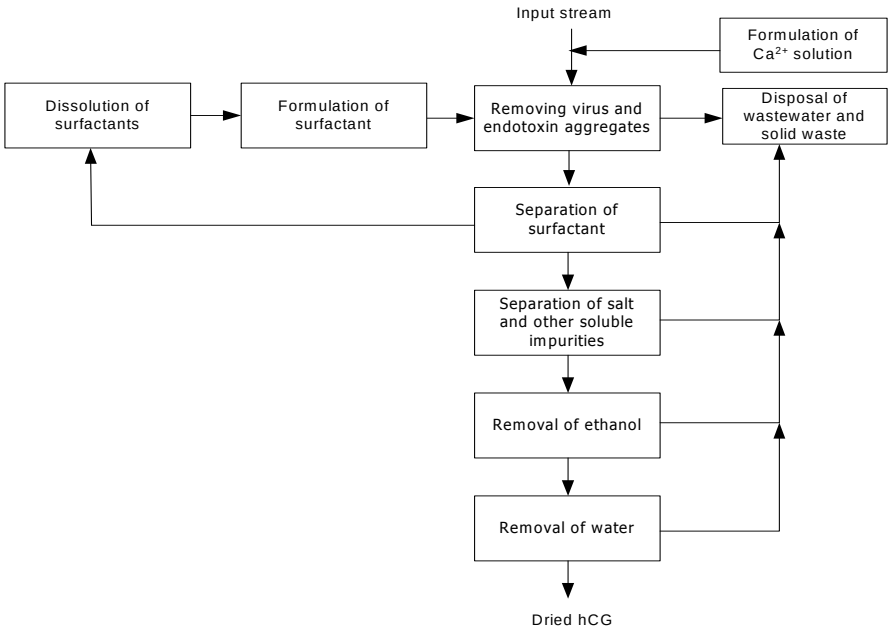


Figure 2.16 Design alternative 7 of the process containing SA-SMB-GF

Design alternatives concerning the entering stream of surfactant

In design alternatives 4 to 7 surfactant enters the SMB-column only with desorbent. But besides that, surfactant can also enter with feed or with both desorbent and feed. Thus, regarding the entering of surfactant to the SMB-column, two more design alternatives are generated. In design alternative 8, the surfactant enters the SMB column only with feed (Figure 2.17), while in design alternative 9 the surfactant enters the column through both desorbent and feed (Figure 2.18).

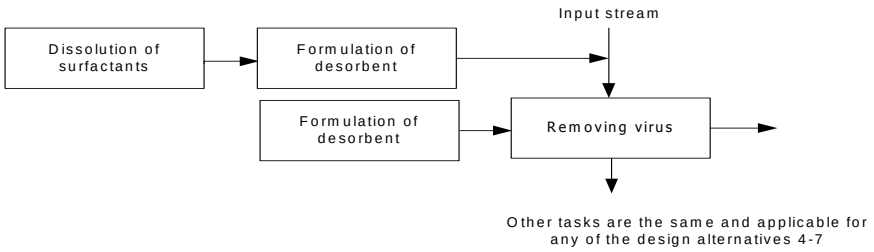


Figure 2.17 Design alternative 8 of process containing SA-SMB-GF

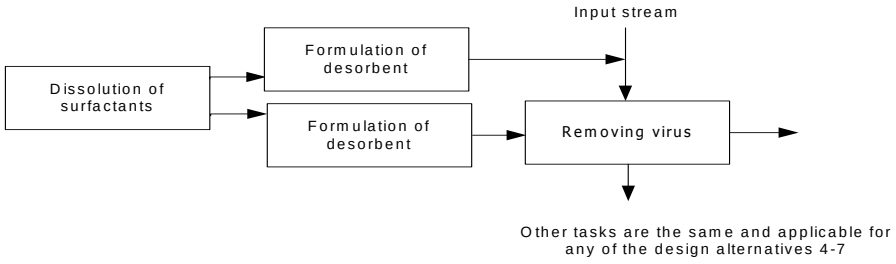


Figure 2.18 Design alternative 9 of process containing SA-SMB-GF

Design alternatives concerning the surfactant recycling

All the design alternatives under SA-SMB-GF (alternatives 4-9) are based on the assumption that surfactant is recycled. Design alternative 10 is generated with the assumption that surfactant is not recycled (Figure 2.19).

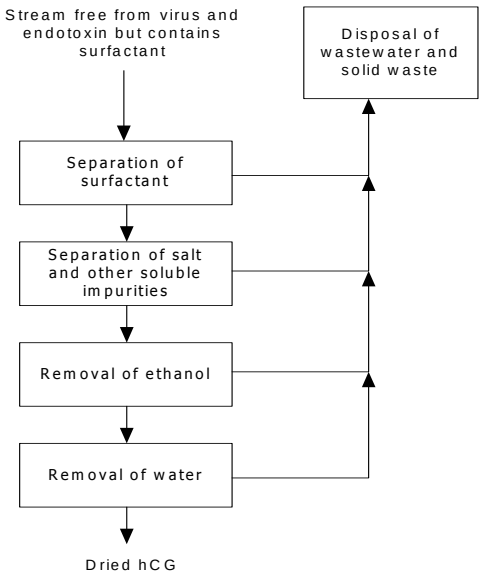


Figure 2.19 Design alternative without surfactant recycling in the process containing SA-SMB-GF

2.5.2.2 Analysis of the design alternatives

Analysis of the virus and endotoxin removal

It is possible to remove virus and endotoxin from hCG by SA-SMB-GF chromatography (design alternative 4, Figure 2.13). Additional tasks under this process option are dissolution and formulation of surfactant. Dissolution of surfactant has to be done to prepare required concentration of surfactant. Formulation of surfactant refers to mixing of surfactant with phosphate buffer hence the respective streams composed of surfactant and phosphate buffer. In Table 2.8 and Table 2.9, each task is described briefly and possible composition of streams is presented, respectively (according to Figure 2.20).

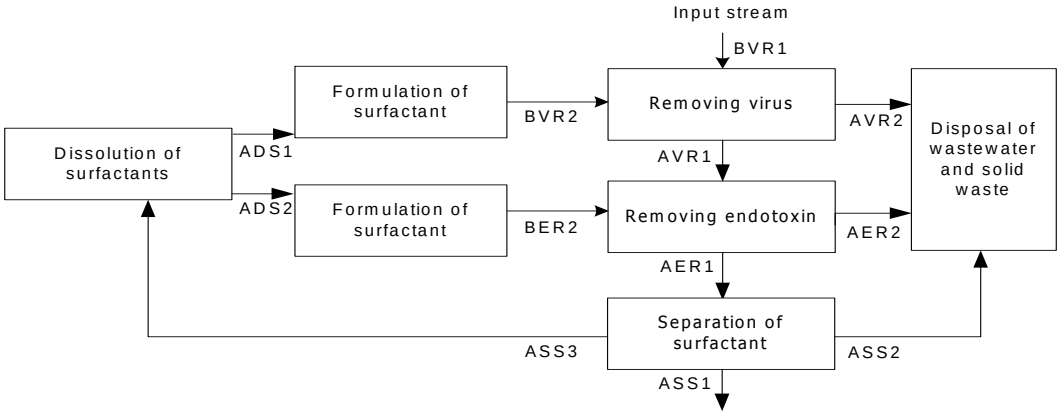


Figure 2.20 Major tasks and streams of design alternative 4

Table 2.22 Description of tasks according to Figure 2.20

Tasks	Activity
Removing virus	Remove virus from input stream (BVR1), thereby AVR1 is sufficiently free of virus
Removing endotoxin	Remove endotoxin from stream comes after virus removal (AVR1), thereby AER1 is sufficiently free of endotoxin
Dissolution of surfactant	Dissolve surfactant into water and make appropriate concentration (fix the concentration of ADS1 and ADS2)
Formulation of surfactant	Mix surfactant with phosphate buffer to supply as desorbent buffer for the tasks require SMB chromatography process
Separation of surfactant	Separate surfactant from the input stream after virus and endotoxin removal (AER1) so that ASS1 does not contain any surfactant
Disposal of waste	Dispose all the waste of the process to the waste treatment area

Table 2.23 Description of the streams according to Figure 2.20

Stream No.	Description	Components
BVR1	Input stream	hCG, virus, endotoxin, ethanol, other impurities and ethanol in water
ADS1/ADS2	Surfactant solution	Surfactant in water
BVR1/BVR2	Desorbent solution	Phosphate buffer and surfactant
AVR1	Virus free input stream	hCG, endotoxin, ethanol, other impurities and surfactant in water
AVR2	Virus containing raffinate (waste) stream	Virus and surfactant in water
AER1	Virus and endotoxin free input stream	hCG, ethanol, other impurities and surfactant in water
AER2	Endotoxin containing raffinate (waste) stream	Endotoxin and surfactant in water
ASS1	Virus, endotoxin and surfactant free input stream	hCG, ethanol and other impurities in water
ASS2	Surfactant disposal stream	Surfactant (and possible other impurities) in water
ASS3	Surfactant recycle stream	Surfactant in water

In design alternative 5, it is assumed that endotoxin removal would be achieved by SMB affinity chromatography. With the same reason as it is mentioned earlier, this design alternative will not be further evaluated.

All the assumptions in design alternative 6 are the same as alternatives 4 and 5, except endotoxin removal is done by precipitation rather than by the SMB process. Consequently, formulation of Ca^{2+} is the required task in place of formulation of desorbent buffer. This is also a potential alternative to be discussed further.

As it was mentioned earlier, the basis of design alternative 7 is the addition of Ca^{2+} in the input stream and the removal of virus and endotoxin aggregates together with SA-SMB-GF. This alternative will also be evaluated further.

Analysis of the surfactant entering stream

Design alternatives 8 and 9 focus on possible input of surfactant. The entering stream of surfactant will affect the separation process in SMB-GF. Performance of SA-SMB-GF depends on the surfactant entering port into SMB system. It determines the concentration of

surfactant in various parts of SMB columns. Decision has to be made based on the partition coefficient of each component and desired surfactant concentration in each part of SMB columns. In this process, surfactant entering point and concentration in each column is a vital design variable. Surfactant concentration affects the partition coefficient of each components and hence also the separation.

Analysis of the surfactant recycling system
Assumption of design alternative 10 is that there is no recycling of surfactants. In this case, the surfactant recycle stream does not exist and all separated surfactants are disposed as wastewater.

Analysis of the order of the tasks
As in design alternative 1 and 2, in design alternatives 4 and 6 endotoxin is removed before virus.

Table 2.24 Design alternatives under the process containing SA-SMB-GF, which will be evaluated further

Design alternative	Assumptions
Design alternative 4	Endotoxin are removed by SA-SMB gel filtration chromatography
Design alternative 6	Endotoxin is removed by precipitation
Design alternative 7	Endotoxin aggregates and virus are removed together by SA-SMB gel filtration chromatography
Design alternative 8	Surfactant enters into the SMB column with only feed
Design alternative 9	Surfactant enters into the SMB column with both feed and desorbent
Design alternative 10	No recycling of surfactant (applicable for design alternatives 4-7)

2.5.2.3 Evaluation of the design alternatives

Evaluation concerning the virus and endotoxin removal
Design alternatives 4, 6 and 7 are evaluated here. Piquar analysis of these alternatives is the same as piquar analysis for design alternatives 1, 2 and 3, only that surfactant is entered into the system. Here, alternatives 4, 6 and 7 replaced alternatives 1, 2 and 3, respectively.

Evaluation of optimum surfactant entering point in SA-SMB
It is less complicated if the same concentration of surfactant is used in all sections of SMB column to avoid the variation of partition coefficient. In this case, surfactant must enter with both feed and desorbent. Since more detail calculation will be performed later, the entering mode of surfactant into the system will not be decided in chapter.

Evaluation of surfactant recycling requirement in SA-SMB
Recycling of surfactant will save the surfactant cost. It is assumed that the stream entering the surfactant-recycling system contains surfactants, virus, endotoxin, proteins and salts. According to the physical properties of the components in that stream, the surfactant can be separated using ultra filtration. The size of the surfactant's micelles is much smaller than virus, endotoxin and other proteins, but it should be kept in mind that there are also salts, which are smaller in size compared to surfactants. Thus, besides using ultra filtration, an additional step is needed to remove the salts. This can be done by reversed osmosis.

Dialysis can also be used to separate surfactants from virus and endotoxin. Dialysis is a method of separating smaller compounds in a solution from the larger compounds, such as proteins, through a buffer exchange. The buffer has an osmolarity less than that of the sample solution, and therefore small solutes such as salts and surfactants diffuse through the dialysis membrane into the dialysis buffer (Asenjo, 1990). The same as recovering surfactant by ultra filtration, an additional step to remove the salts is also needed.

It is mentioned that Brij® and Tween® detergents are difficult to remove from solution by dialysis (www.piercenet.com). Thus, it is reasonable not to use dialysis to recover the surfactants in our particular process. Ultra filtration followed by reverse osmosis can be a good option for surfactant recovery. Ultra filtration is also used in the designed plant to remove endotoxin aggregates and the membrane can be changed for surfactant removal purpose. But the team needs to analyze the importance and the effect of surfactant recovery more thoroughly.

According to the preliminary economic analysis in chapter 3.4, surfactant cost is only a small part of the total production cost. The cost of urine is the most significant one. The amount of surfactant used is also relatively small. Thus, it is not worth installing reversed osmosis equipment only for this purpose. Besides that, there is a possibility that some salts are still present in the recovered surfactant solution or even endotoxin. Since the team is dealing with an injected pharmaceutical product, the purity of pure components including surfactants is very important.

Based on the analysis above, the team decided not to recycle the surfactant used in hCG purification process. Thus, the stream exiting the distillation column will be sent out to the wastewater treatment facility outside Diosynth.

2.5.3 General processes for the designed plant

2.5.3.1 Design alternatives

Some tasks in hCG purification process are common and applicable for all of the design alternatives 1 to 10. These tasks are:

- Temperature adjustment of input stream
- pH adjustment of input stream
- Separation of salt and other soluble impurities
- Removal of ethanol
- Removal of water

Design alternative concerning temperature and pH adjustment

In this process, pH and temperature are two important parameters that may affect the purification process. The input stream has pH and temperature of 7.0 and -20 °C, respectively. It needs to be heated up to 10 °C and its pH might need to be adjusted before entering the separation system. Design alternatives 1-10 are generated with the assumption that temperature and pH adjustment of the input stream is not required before entering the separation system. Now, design alternative 11 (Figure 2.21) is generated to take into account the requirement of temperature and pH adjustment.

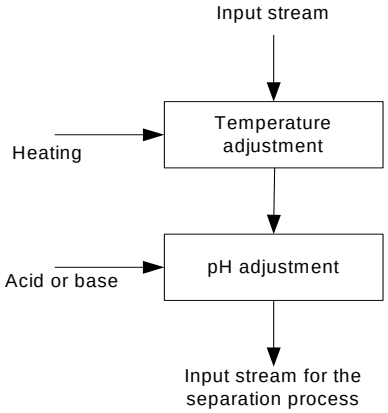


Figure 2.21 Design alternative 11 with temperature and pH adjustment of input stream

Design alternative concerning the removal of other impurities

When hCG containing stream is sufficiently free from virus, endotoxin and surfactant (if SA-SMB-GF is used), some more tasks are still required to achieve the final product specifications. All the design alternatives described above (alternatives 1-11) were based on the assumption that removing salt and other soluble components are done first before removing ethanol. One more design alternative is generated with the assumption that ethanol is removed first before removing salt and other soluble components (Figure 2.22).

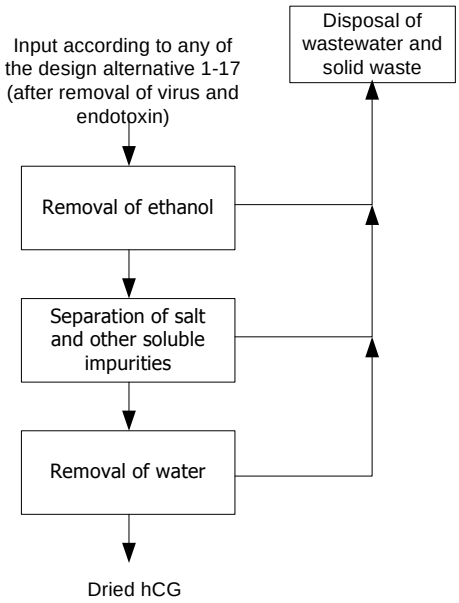


Figure 2.22 Design alternatives 12 with reversed order of ethanol and salt removal

2.5.3.2 Analysis of the design alternatives

The overall process or any task of the process may require pH or temperature adjustment or both adjustments. According to design alternative 11, two different tasks are required for temperature and pH adjustment. Heating of the input stream keeps the composition and phase the same, but results in a higher temperature. On the other hand, addition of acid or

base changes the composition of the input stream in term of only H^+ ion concentration. All other physical behaviours remain unchanged. Indeed, the order of these two tasks could also be reversed or could be achieved together. It is also possible that any one of the two tasks is not needed. Order of the tasks "ethanol removal" and "salt and other impurities removal" in design alternative 12 also can be one or the other way around.

2.5.3.3 Evaluation of the design alternatives

Evaluation of temperature and pH adjustment
It was already stated earlier that the working temperature should be kept below 10 ^oC as it is in the current hCG purification process in Diosynth. Since the ingoing stream has a temperature of –20 ^oC, there is a need to heat this stream to 10 ^oC. Thus, the temperature adjustment is needed. On the other hand, the pH of the ingoing stream is 7 and it will be constant during the purification process, thus pH adjustment of the ingoing stream is not needed.

Evaluation of removal of other impurities
Design alternative 12 was generated considering that removing ethanol is done before removing soluble components. From the point of view of performance, it is convenient to remove soluble components before removing ethanol and water. At the end, water has to be removed from the product. It might be possible to remove water and ethanol together.

2.5.4 Purification tasks chosen

Among the alternatives under the category of virus and endotoxin removal by SMB-GF, alternative 2 is selected. Similarly, design alternative 6 is selected for category virus and endotoxin removal by SA-SMB-GF. Some other decicions are:

- In case of process containing SA-SMB-GF, the entering port of surfactant will be decided later.
- Soluble impurities are removed first and followed by ethanol removal

Table 2.25 summarizes the selected design alternatives among all the design alternatives.

Table 2.25 Selected design alternatives

Design alternative	Assumptions
Alternative 2	Virus is removed by SMB-GF but endotoxin is removed by precipitation
Alternative 6	Virus is removed by SA-SMB-GF but endotoxin is removed by precipitation
Alternative 8	Surfactant enters into the SMB column with only feed (applicable for design alternative 6)
Alternative 9	Surfactant enters into the SMB column with both feed and desorbent (applicable for design alternative 6)
Alternative 10	No recycling of surfactant (applicable for design alternatives 6)
Alternative 11	Adjustment of temperature is required (applicable for design alternative 2 and 6)

2.5.5 Tasks in the base case

The base case is presented in Figure 2.23 for comparison with the generated design alternatives. It is stated earlier that the removal of virus and endotoxin is done with cation-exchange chromatography column. As it is in the process with SMB-GF and SA-SMB-GF, separation of salts, other impurities, ethanol and water will follow after the endotoxin and virus removal respectively.

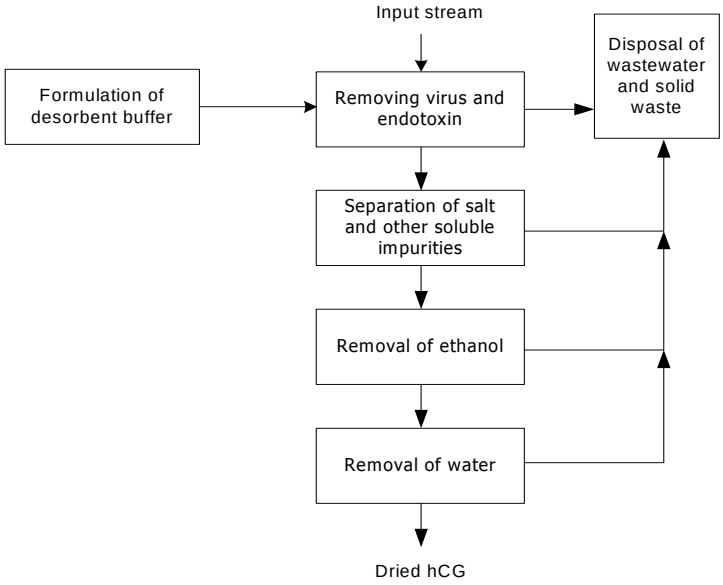


Figure 2.23 Tasks in the base case

2.5.6 Ethanol recycling process

In the whole hCG purification process, ethanol is used in two steps, which are hCG precipitation step and final washing step. All designed process options incorporate those two steps. Additionally, ethanol is already present in the first incoming product stream to the designed plant. It is used in the pre-concentration step, which is not included in our battery limits. The percentage amount of ethanol used in each of those steps is presented in Table 2.26.

Table 2.26 Percentage amount of ethanol used in the hCG purification process

Process	Amount (%)
Pre-concentration	40 wt-% of the stream going out of Ultrafiltration step.
Precipitation	77 wt-% of the stream coming in to the precipitation vessel
Final washing	96 wt-% of the wet precipitate

Diosynth recycles the ethanol by using a distillation column. It is assumed that the current ethanol recycling system in Diosynth has enough capacity to recycle the ethanol that is used from the designed plant. This assumption is reasonable since the three steps mentioned in Table 2.26 are also present in the existing hCG purification process. Since this distillation column for ethanol recycling is already exist in Diosynth, the team does not need to design it.

It should be kept in mind however, that in the process containing surfactant, there might be a problem during the distillation. Surfactant may cause a severe foaming in the distillation column. Thus, antifoam might be needed in case of purification process with SA-SMB-GF but the team will not incorporate this in the design. To reduce the amount of antifoam needed, surfactant can be removed before the stream enters the distillation column, for example by using ultrafiltration prior to ethanol recycling process, but the team will not deal with this. In this design project, it is assumed that there is no surfactant removal step prior to the distillation column.

2.6 Mode of operation

The current hCG production process in Diosynth is performed in batch. It is decided to use the batch mode as well in the designed plant. The overall operation depends very much on the supply on raw material, in this case urine, which varies throughout the year. In batch process, the treatment in each unit will be faster (Douglas, 1988), which means less possibility for deactivation of hCG. Also, batch process is more suitable for low production capacity, as it is in our case (15 kg hCG/year). Finally, a good trace-ability is needed. It must be possible to see from which urine-pool the product is coming, so if something is wrong only the batch in question will be rejected. After consultation with the client, it is decided that there will be 5 batches of 100 litres raw materials per year (van Dedem, 2003). It must be noted however, that in the designed plant, the SMB system will be operated in continuous mode, as it is explained in Appendix 4.

3 Basis of Design (BOD)

3.1 Description of the design

Human chorionic gonadotropin (hCG) is a steroid hormone that appears in abundance in the urine of women during the first trimester of pregnancy. This hormone is commonly used for infertility treatment in both men and women.

Diosynth is one of the companies that produce hCG from urine of pregnant women. It purifies hCG from other substances that are present in urine, such as amino acids, vitamins, minerals, salt, hormones and proteins. Since hCG is administered to humans, the product needs to have a high degree of purity. Two of these constraints regard the presence of virus and endotoxin in urine.

The team will design hCG purification processes as the continuity of the previous existing concentration steps in Diosynth. Thus, the input stream of the designed plant is the output stream of the last concentration step in the existing process. The composition of the input stream coming to the first step in the designed plant is described in Table 3.2. The hCG resulted from the purification plant should be sufficiently free from virus and endotoxin and should meet the desired purity. The specification of the hCG produced from the plant is presented in Table 3.5.

The team will design two purification plants, one with SMB-GF and the other one with surfactant-aided SMB-GF, as the key technology. The team will also design a base case that contains cation chromatography as the key technology, for comparison. The information on the base case was given by Diosynth (van Dedem, 2003). The overall designed plants will be run in batch mode and there will be 5 batches of 100 litres input per year.

Sephacryl S-200 HR will be used as the gel material in SMB-GF while Sephacryl S-300 HR will be used as the gel material in SA-SMB-GF. Brij® 35 will be used as the surfactant in SA-SMB-GF and SP Sepharose high performance resin will be used in the cation exchange chromatography.

Ethanol will be recycled using the existing recycling system available in Diosynth, thus the team will not include an ethanol-recycling process in the designed plant. Considering the safety of the product, surfactant will not be recycled but will be sent to the wastewater treatment plant. The team will not deal with the wastewater and solid waste treatment.

This BOD chapter presents the process concept chosen. The pure component properties and basic assumptions will be described. Margin that is calculated as the difference between income from sales minus costs for feedstock and waste streams will also be calculated and presented here.

3.2 Process definition

3.2.1 Process concept chosen

The detail options and analysis of the purification process are already explained in detail in chapter 2. In this part, the process chosen will be summarized.

3.2.1.1 Base case

The team will design a base case that contains cation exchange chromatography as the key technology to separate virus and endotoxin from hCG. The main purification steps in base case are cation exchange chromatography, precipitation of hCG with ethanol and ammonium acetate and recovery of hCG using filtration. The purified hCG will meet all the requirements.

This chromatography will be run in a normal mode and not in SMB mode. With this purification step, the requirements for virus and endotoxin removals are met. It is stated that the binding capacity of this chromatography is 2 wt% (van Dedem, 2003).

3.2.1.2 Key technology chosen

SMB-GF and SA-SMB-GF are chosen as the key technologies in the hCG purification plants that will be designed in detail by the team. For industrial scale, SMB-GF technology is more interested than the normal gel filtration chromatography since it is operated continuously. It can also improve eluent and resin inventory by an order of magnitude compared to traditional batch chromatography.

Surfactant micelles influence the selectivity of gel chromatography. Using non-ionic surfactants above their critical micelle concentration in the mobile phase increases the distribution coefficient of a protein in gel chromatography. The increase is different for proteins of different sizes. This means that changing the surfactant concentration in the mobile phase can change the selectivity in situ. It is expected to be the case for the mixture of proteins and viruses.

3.2.1.3 Raw materials and auxiliary materials

The raw materials entering the first designed equipment come from the first ultrafiltration step in the current hCG purification process in Diosynth. This stream contains hCG with 25% purity in 40%-wt ethanol. As mentioned before, the detail composition is presented in Table 3.2.

Non-ionic surfactants are chosen for this purification process to avoid hCG denaturation or inactivation. Triton® X-100, Triton® X-114, Brij® 35, Brij® 58 and Tween® 20 were considered as surfactant used in SA-SMB-GF system. The last three surfactants got the highest marks in piquar analysis. The main reasons are the fact that Tritons are not commonly used as pharmaceutical ingredients and not easily biodegradable. Brij® 35 and Brij® 58 get the highest marks since the experimental data for size exclusion chromatography is rather complete. These two surfactants are used as pharmaceutical ingredients but only for topical used. Although Tween® 20 is commonly used as pharmaceutical ingredient for inject able product, there is not enough data to do the calculation for SA-SMB-GF system. Considering the importance of experimental data for process simulation, Brij® 35 and Brij® 58 are chosen to be analyzed further.

There are many options for gel that can be used in SMB-GF and SA-SMB-GF system. Based on the analysis described in 2.4.2, Sephacryl S-200 HR and Sephacryl S-300 HR were chosen.

Since the main aim of using SMB-GF and SA-SMB-GF is to separate virus from hCG, the partition coefficients of the two components in the gels with and without Brij® 35 or Brij® 58 are analyzed. Based on the behavior of the partition coefficients, it was decided to use Sephacryl S-200 for SMB-GF system. In this gel and without any surfactant presents, the different in partition coefficient of virus and hCG are bigger than in the other gel. For process with SA-SMB-GF, Brij® 35 is chosen as surfactant and Sephacryl S-300 is chosen as the gel. The reason for this is that in this situation, it is possible to work before and after the crossing point since this point exists below 10% Brij® 35 concentration. In other situation, the crossing points happen above 10% surfactant concentration, which may give problem in viscosity.

3.2.1.4 Overall purification tasks chosen

Process with SMB-GF
The temperature of the entering stream to the first designed equipment is –20 ^{o}C while the process temperature should be in the range of 0 to 10 ^{o}C. Thus, there is a need for a temperature adjustment before the input stream enters the designed plant. pH adjustment is not needed since the pH of the input stream is 7 and the pH of the whole system will also be 7.

In this design criterion, virus is removed with SMB-GF. Several options are available to remove endotoxin, which are precipitation, two-phase extraction and adsorption techniques. The possibility of removing endotoxin during the virus removal with SMB-GF was also considered, but the precipitation technique is considered to be the best option. This technique is also applied to remove endotoxin in the current hCG purification plant in Diosynth. Precipitation is done by adding calcium ions to the hCG solution to form endotoxin aggregates so that they can be removed easily by ultrafiltration.

It is decided to remove endotoxin prior to virus removal since relatively large amount of endotoxin is present in the input stream than that of virus. More over endotoxin is a rather sticky material (Petch and Anspach, 2000) that may disturb the virus removal process in SMB-GF.

After endotoxin and virus removal steps, ethanol, water, salts and other soluble impurities will have to be removed to meet the product specifications. It was decided to remove salts and other impurities first prior to ethanol and water removal. Those last two components might be separated together.

Recycling of ethanol will save the costs of the materials used in the purification plant. It was agreed with the client that the team would not design the ethanol recycling system. In the current hCG purification process, Diosynth recycles the ethanol using distillation column. The ethanol used in the designed plant will be sent to this recycling system.

Process containing SA-SMB-GF

The whole purification process with SA-SMB-GF system is similar to the one with SMB-GF system. Temperature adjustment is also needed to heat-up the input stream from –20 ^{o}C to a certain temperature between 0 to 10 ^{o}C. As it is in the process with SMB-GF, pH adjustment is not needed.

In this design criterion, virus is removed with SA-SMB-GF. It is expected that surfactant will change the selectivity of hCG and other impurities in situ. The entering port of surfactant into the SMB-GF system is not yet decided. More insights are needed to make this decision.

As in process with SMB-GF system, endotoxin is removed by precipitation that is also applied in the current hCG purification plant in Diosynth. Adding calcium ions will precipitate endotoxin and the aggregates formed can be removed using ultrafiltration.

In this process, endotoxin will also be removed prior to virus removal for the same reasons as mentioned before. After endotoxin and virus removal steps, ethanol, water, salts and other soluble impurities will have to be removed to meet the product specifications. It was also decided to remove salts and other impurities prior to ethanol and water removal.

Ethanol recycling will also be done using the available recycling system in Diosynth. The team will not design it. On the other hand, considering the safety requirement for hCG, surfactant will not be recycled. Besides that, the amount of surfactant used and its price is much less compared to the other raw materials.

Process in base case

The whole purification process, in the base case is similar to the previous ones with SMB-GF and SA-SMB-GF system. Temperature adjustment is also needed and as it is in the designed processes, pH adjustment is not needed. The batch mode of operation is also applied in the base case with 5 batches per year.

In base case, virus and endotoxin are removed with cation exchange chromatography. The requirements for virus and endotoxin removal are fulfilled with this system. After this step, ethanol, water, salts and other soluble impurities will also be removed from the target protein and ethanol will also be recycled as it is mentioned above.

3.2.2 Pure component properties

Pure component properties are very important to know, since the purification or separation techniques will be based on the characteristics of each component. The pure components involved in the process are hCG, parvovirus, endotoxin, surfactant, ethanol and water. Their relevant properties are presented in Appendix 2.

3.2.2.1 Stability of hCG

The extent and nature of glycosylation is known to have a significant influence on stability of hCG in vitro and in vivo. Usually glycosylation reduces the rate of dissociation of hCG into it's subunits. The degradation process is more rapid at high temperature and slow in native medium like serum and defebrinated blood (Butler et al., 1998). At 37˚C, the rate of dissociation of intact hCG is about 15% and 32% per week in serum and urine, respectively. In addition, a relatively low pH of the sample also causes instability of hCG (Cole, 1997).

3.2.2.2 Stability of endotoxins

Endotoxins are very stable molecules, their biologically active part surviving extremes of temperature and pH in comparison to proteins. Routinely, temperatures of 180-250 °C and acids or alkalis of at least 0.1 M must be chosen to destroy endotoxin in laboratory equipments (Petch and Anspach, 2000).

3.2.2.3 Stability of parvovirus

Parvovirus is extremely physico-chemical resistant. Pasteurization for 10 hours at 60 °C results in a 50% loss of biological activity of parvovirus, while the reduction of parvovirus infectivity is negligible. Heat treatment at 80 °C (for 72 h), 90 °C (20 h) and 100 °C (8 h) results the reduction of infectivity by a factor of 4.2 log, 3.9 log, > 3.8 log, respectively (ter Hart et al., 1999).

From the stability information mentioned above, it is concluded that the temperature of the product stream should be kept relatively low and pH of it should be kept around neutral. Virus and endotoxin are much more stable than hCG, thus it is not possible to inactivate virus and endotoxin by increasing the temperature or using extreme pH. The current purification process temperature in Diosynth is 10 °C and the team will keep the same process temperature in the designed plant.

3.3 Basic assumptions

3.3.1 Plant capacity

Diosynth processes 5 millions litres of urine per year. This amount of urine is only collected from pregnant women in the Netherlands. The urine is collected during week 10 to 12 of pregnancy. The production process of hCG is mainly the purification process of hCG from other impurities that are present in urine. Thus, there is no conversion-taking place during hCG production. The waste resulting from this process will be the impurities from urine, such as virus, endotoxin and other proteins. Besides that, the waste also contains spent base chemicals that may be used during the purification process, for example surfactant and gel.

Based on the agreement with the client, some design requirements regarding the plant capacity and product recovery are set up. The production target is 15 kg of hCG per year. Overall recovery of the process is aimed to be at least 75% (5 unit operations with about 95% recovery in each). Total investment cost, including equipment, installation and piping, of the process should be less than half a million Euro. The lifetime of the equipment must be at least 15 years. The working time is 7200 hours per year, equivalent with 300 full working days. An overview of these constraints is given in Table 3.1.

Table 3.1 Requirements on the design (van Dedem, 2003)

	Specification
Production rate of hCG (1)	15 kg/year
Overall recovery (2)	≥ 75%
Investment costs	≤ 500 000 euro
Economic lifetime	15 years
Available working time (300 full working days)	7200 hours/year

(1) Explained in more detail in chapter 3.3.4.2
(2) This is the overall recovery of the new steps, excluding the existing previous processes in Diosynth

3.3.2 Location

As it is mentioned earlier, the designed plant will be part of the existing plant of Diosynth. This company is located in Oss, The Netherlands.

3.3.3 Battery limit

The designed processes will only consist of product purification steps. The team will only produce the wet-purified hCG. The drying system is not included in this prcess design. The wastewater treatment plant will not be designed and it is assumed that the waste can be directed to a wastewater treatment company nearby Diosynth (Mosmans, 2003). Utilities are assumed to be supplied from outside utility generating companies. Utility generating units will not be designed. The overall block diagram for this design is presented in Figure 3.1.

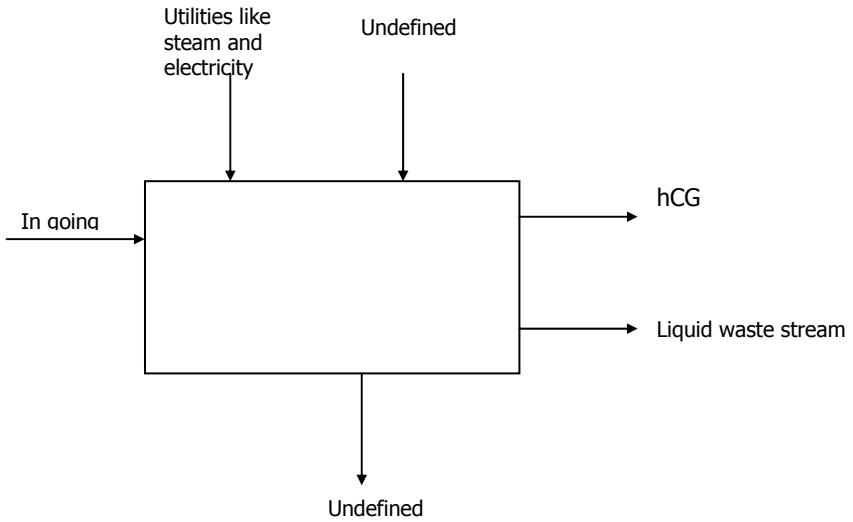


Figure 3.1 Overall block scheme

More detailed description of all streams is given in chapter 3.3.4. There is an undefined auxiliary stream, which will depend on the unit operations used in the process.

3.3.4 Streams passing the battery limit

3.3.4.1 Main input stream

The amount of urine processed per year is about 5 million litres with an hCG concentration of 2 mg/L. After several processes in Diosynth, the product stream is concentrated to reach an hCG concentration of 20 mg/ml. Thus the total outflow of those preliminary steps will be about 500 litres per year. This concentrated product stream is regarded as the main input stream for this design project, from which the hCG should be purified. The composition of this input stream is mentioned in Table 3.2.

The costs that are made to obtain this stream consist of the costs to get the urine at the site and the costs for the process steps performed. It costs approximately €2 per litre of urine to get the urine at the site (van Dedem, 2003). The costs for the process steps that are performed are difficult to estimate. An additional €1 per litre of urine processed is then considered to be sufficient (van Dedem, 2003). The total costs will therefore be 15 million Euros per year. Subsequently, the costs per litre of in going product stream are 30000 Euros. The process condition and price of the input stream are given in Table 3.2.

Table 3.2 Specifications, Process conditions and price of the main input stream

Stream name: main input stream		
Component	Specification (weight percentage)	
	Available	Design
hCG	1-3	2
Other proteins	3-9	6
Virus	0-1	$3 \cdot 10^7$ particles/ml
Endotoxin	0-0.05	0.01
Soluble components	0.5-2	1.0
Ethanol	35-45	40.0
Water	41-55	49.99
Total		100
Process Conditions and Price		
Flowrate	litre/year	500
Temperature	°C	-20
Density(1)	kg/L	0.91
Pressure	atm.	1
Phase		Liquid
Delivery mode		Direct piping from previous unit
Price	Euro/L	30000

(1) The density is calculated based on assumption that this stream contains 40 wt-% ethanol and 60 wt-% water

Other proteins in the input stream include urinary proteins as well as probably some microbial proteins. It is quite difficult to determine what are the other proteins that need to be separated and what are their sizes. Consequently, approximation is taken about the major 'other proteins' present in the input stream and their content.

Healthy human urine contains numerous peptides (www.mosaiques.de), albumin, cystatin C, microglobulin, retinol binding protein and growth hormone (www.aaltobioreagents.ie). In addition, urine contains a lot of different immunoglobulins. Probably, peptides and immunoglobulins constitute more than half of the other proteins. Albumin and growth hormone also present in abundant. Molecular weight of these proteins is given in Table 3.3.

Table 3.3 Major other proteins present in urine

Proteins	Molecular Weight (kDa)
Albumin	69
Cystatin C	13
Microglobulin- alpha 1	33
Microglobulin- beta 2	12
Immunoglobulins	~150
Growth hormone	22
Polypeptides	~10

Total amount of other proteins present in the input stream is 6 kg per batch of 100 litres. Separation of other proteins in this process is mainly based on their sizes. For design and calculation propose, 5 categories of other proteins are assumed in term of molecular weight.

Table 3.4 Category of other proteins and their amount

Proteins	Proportion	Amount (kg/batch)
150 kDa	30%	1.8
69 kDa	20%	1.2
22 kDa	10%	0.6
10 kDa	30%	1.8
Others	10%	0.6

3.3.4.2 Main output stream

Virus clearance is confirmed by 6-log removal of parvovirus. The product must contain less than 0.02 EU/IU of hCG. EU is Endotoxin Unit and IU is International Unit. Endotoxin from *Escherichia coli* will be used as reference standard, this gives 1 EU = 10^{-10} g. Therefore, maximum 0.002 ng of endotoxin can be present per IU of hCG. A potency of final product of 5000 IU/mg can be estimated (van Dedem, 2003). This leads to the maximum allowable limit of $1 \cdot 10^{-3}$ -wt% (w/w) for endotoxin in the final product.

The selling price for hCG was found to be around €14 per 10000 IU for crude product, which is 'ready to use' (www.affiland.com). As mentioned before, the potency of the final product is estimated to be 5000 IU/mg. This gives a selling price of €7 per mg, for the 'ready to use' product. Because the final product is not 'ready to use', but needs to be packaged, a selling price of €5 per mg is assumed. For 15 kg product per year, this gives a total selling price of 75 million Euros a year. The hCG exiting the designed plant will be in a wet solid form and will be sent to the drying equipment, which is not included in the design. In this case, the hCG will be transferred manually by the operator to the drying unit. An overview of the specifications of the out going product stream is given in Table 3.5.

Table 3.5 Specifications, Process conditions and price of the main output stream

Stream name: Out going product stream		
Component	Specification (weight percentage)	
	Available	Design
hCG	≥ 50	50
Other proteins	≤ 50	50
Virus	0	≤ 10^3 particles/g
endotoxin	0	≤ 10^{-3}
Total		100
Process Conditions and Price		
Flowrate	kg/year	15
Temperature	°C	10
Pressure	atm.	1
Phase		Solid
Delivery mode		Manual transfer to the dryer
Price	MEuro/kg	5

3.3.4.3 Waste stream

The wastewater streams will be directed to a wastewater treatment company in the neighborhood. This company is specialized in treating industrial wastewater. In principle all wastewater types can be handled in such a company, although there should be good communication before the wastewater is offered to the treatment company. The costs for the wastewater treatment depend on the specific composition of the wastewater, but the costs will not exceed €10 per m^3 (Mosmans, 2003). Thus the design will not include a wastewater treatment step. The precise composition of the wastewater is not yet known, but it will follow from the design. A first estimation of concentrations, conditions and costs can be given based on the main input stream and out going product stream. An overview is given in Table 3.6.

Table 3.6 Assumption on the liquid waste stream

Stream name: Liquid waste stream		
Component	Specification (weight percentage)	
hCG		0.5
Other proteins		4.5
Virus		$3 \cdot 10^7$ particles/ml
endotoxin		0.01
Soluble components		1.0
Ethanol		40.0
Water		53.99
Total		100
Process Conditions and Price		
Flowrate	litre/year	500
Temperature	°C	10
Pressure	atm.	1
Phase		Liquid
Delivery mode		Direct piping to waste container
Price	Euro/m^3	10

3.3.4.4 Utilities

As it is mentioned above, utilities are assumed to be supplied from outside utility generating companies. The utilities that may be used in this plant are Steam, Electricity and Cooling water or pressurized air. Depending on the properties of the product, impurities and base chemicals like surfactant and on the separation methods, which will be used in the process; and also the result from heat integration evaluation, different utilities will be used in the process. The following table gives the normal utilities cost taken from the Conceptual Process Design manual (Grievink et al, 2002).

Table 3.7 Utility costs

Utility	Unit	Costs (Euro per unit)
Steam	ton	20
Electricity	kWh	0.2
Cooling water	m^3	0.05
Pressurised Air	m^3	0.03

3.3.4.5 Undefined stream

For the unit operations used in the total process, auxiliary streams may be needed. For instance, if a column chromatography step is used, gel material is needed. For surfactant aided size-exclusion chromatography an additional stream containing surfactants will be needed. To account for these still to be defined auxiliary streams; the 'Undefined' in going and out going streams are added.

3.4 Economic margin

The margin calculation is estimated based on the difference in cost of raw materials and the selling price of the product. Utilities, equipments and other criteria are not yet included. Since the raw materials of the process containing SA-SMB is the most expensive (due to the use of surfactant), the margin calculation is performed for this process option. Later on, a detailed economic evaluation will be performed for all process options chosen.

As it is mentioned before, the first input stream to the designed plant is the output stream of the pre-concentration step using ultra filtration done in Diosynth. Thus, the calculates the raw material cost as the cost of urine, the cost of the previous step before the designed plant and the cost of surfactant. The cost of urine and the previous steps are already mentioned in Table 3.2. The amount of surfactant used is assumed to be 10% of the input stream. Since the cost of urine gives the biggest contribution in the cost of raw materials, the team adds other costs as much as 5 % of the cost of urine.

Table 3.8 Margin calculations

Components	Price M Eur/unit	Unit	Unit/year	Value M Eur/year
Raw materials				
Urine	0.002	m^3	5,000.00	10
Cost of previous concentration steps	0.001	m^3	5,000.00	5
Surfactant	0.031	ton	0.13	0
Other (15% of urine cost)				2
Total				17
Product				
hCG	5	ton	15	75
Margin (product - raw material costs)				**58**

In the calculation of maximum investment costs, it is assumed that the first year is used for construction. The maximum investment is defined as the maximum amount of money that one can spend in order to have a Net Present Value of zero at the end of the project at an annual interest rate of 10%. There are two methods for calculating the maximum investment costs. The first one is based on the lecture of Process System Design (PSD) given in Delft Technical University and the second one is based on the lecture of Economic Evaluation given by Professor Asselbergs. In the first method, tax and depreciation are not considered while the second method takes into account those two parameters. The team decided to use the second method.

Using the method of Asselbergs (Asselbergs, 2002), the maximum allowable investment is found to be EUR 323 M. Details on maximum investment calculation can be found in Appendix 10.

4 Process structure and description

In chapter two, two hCG purification processes have been selected and block schemes have been made available. In this chapter, the tasks in the purification processes will be translated into unit equipments. The Process Flow Schemes (PFS) will also be explained in this chapter.

4.1 Criteria and selection

The team will design two hCG purification plants, one with SMB-GF and the other one with SA-SMB-GF. Since most of the equipments used are the same, the criteria and selection for the designed plants are discussed in the same chapter. The numbering of the equipments is also the same for both processes and the overall PFS is presented in
Figure 4.2 and Figure 4.3 for process with SMB-GF and SA-SMB-GF respectively. The criteria and selection for the base case is discussed separately and the PFS for this process is available in Figure 4.4.

4.1.1 Criteria and selection for the designed process

4.1.1.1 Temperature adjustment of the input stream

The exiting flow from the ultrafiltration unit in Diosynth has a temperature of –20 °C. This stream needs to be heated-up to a temperature between 0 and 10 °C. To obtain this temperature, the team looks at the possibility to do heat integration at the first place.

Heat integration is applied if there are process streams that need to be cooled and/or heated, and these streams can exchange their heat. In the designed hCG purification process, there is no stream that needs to be cooled down and moreover the overall process is operated in a batch mode. Thus, heat integration cannot be applied in the designed plant. There are some hot utilities that can be used to heat up the entering product stream, such as steam, electricity and water. Each of those utilities is analysed below.

The first step is to calculate the heat duty to change the temperature from –20 °C to 5 °C. The calculation is shown in Appendix 11 and the result is presented in Table 4.1.

Table 4.1 Heat duty to increase the temperature of the ingoing product stream

Stream	T_{in} (°C)	T_{out} (°C)	Heat duty (kJ/batch)
Ingoing product stream	-20	5	7843.5

1 batch = 100 L

Steam as the hot utility

If low-pressure (LP) steam (3 bar) is used as the hot utility, 2.878 kg steam/batch will be needed. The calculation is shown below, where Q is the heat duty, m is the mass flow of steam and ΔH is the enthalpy for steam condensation.

$$Q = m \times \Delta H$$ Equation 4.1

$$m = \frac{Q}{\Delta H} = \frac{7843.5}{2724.88} = 2.878\ {}^{kg}/_{batch}$$

The heat between steam and the entering product stream will happen in a heat exchanger, which will be designed later.

Water as the hot utility

If water is used as the hot utility, 187.64 kg water/batch will be needed. The calculation is shown in Equation 4.2, where Q is the heat duty, m is the mass flow of water, Cp is the heat capacity of water (4.18 kJ/kg.°C), and ΔT is the temperature difference of water before and after heating up the ingoing stream. It is assumed that the temperature of water is 20 °C and it will be cooled down to 10 °C.

The water that will be used is a mixed between hot water available at Diosynth (80 °C) and the cold water (10 °C). Prof. van Dedem (2003) from Diosynth already confirmed the availability of the water. Equation 4.3 and Equation 4.4 shows the calculation of the hot and cold water needed per batch for that purpose. m_{10} and m_{80} is the mass of the water at 10 and 80 °C respectively. Based on those calculations, 160.83 kg/batch cold water and 26.81 kg/batch hot water are needed.

$$Q = m \times Cp \times \Delta T \qquad \text{Equation 4.2}$$

$$m = \frac{Q}{Cp \times \Delta T} = \frac{7843.5}{4.18 \times (20 - 10)} = 187.64\ {}^{kg}/_{batch}$$

$$(m_{10} \times 10) + (m_{80} \times 80) = 187.64 \times 20 \qquad \text{Equation 4.3}$$

$$m_{10} + m_{80} = 187.64 \qquad \text{Equation 4.4}$$

The heat between the heated water and the entering product stream will happen in a heat exchanger, which will be designed later.

Electricity as the hot utility

If electricity is used as the hot utility, 7843.5 kJ electricity/batch will need to be supplied. In this case, an electrical heater will be used in the form of plate or coil, to heat a vessel containing a batch (100 L) of the ingoing product stream. The total costs of those utilities are presented in Table 4.2.

Table 4.2 The cost of utilities needed per batch (1)

Utility		Unit	Price (EUR)/unit	Price/batch (EUR/batch)
Steam (LP)		ton	16	0.05
Water	Cold water	ton	0.023	0.004
	Process water	ton	1.14	0.03
Electricity		kJ	2.8×10^{-5}	0.22

(1) The prices are taken from Grievink et al. (2002)

Evaluation of the utilities option is done using Piquar method. From the above calculation, water is cheapest utility. Steam is also cheap, but it should be kept in mind that the constructions needed for steam is more expensive and complicated. Moreover the quantity of this purification process is quite small. It is not worth installing pipes and other equipments and controllers needed for steam installation for such a small scale. Thus, for criterion plant makes money, water gets a higher mark.

For personal safety, using electricity and water will be better than using the steam. Since the product is heat sensitive, for safety of the product, using water is better than using electricity directly, thus water gets the highest mark for safety in overall. For other criteria, the marks are the same. The Piquar results are shown in Table 4.3 to Table 4.5 and the summary is given in Table 4.6.

Table 4.3 Piquar results on steam

Factor	Weighting factor	Marks	Weighted marks
Plant makes money	0.187	2	0.4
Specification is met	0.187	4	0.7
Safety	0.167	2	0.3
Quality of basis of design	0.146	5	0.7
Reliability	0.104	4	0.4
Efficient use of raw materials	0.083	4	0.3
Comply with construction regulations	0.042	4	0.2
Good documentation of project	0.042	4	0.2
Controllability	0.021	4	0.1
Meet functional, budget and time requirement concurrently	0.021	4	0.1
Final mark			3.4

Table 4.4 Piquar results on water

Factor	Weighting factor	Marks	Weighted marks
Plant makes money	0.187	5	0.9
Specification is met	0.187	4	0.7
Safety	0.167	5	0.8
Quality of basis of design	0.146	5	0.7
Reliability	0.104	4	0.4
Efficient use of raw materials	0.083	4	0.3
Comply with construction regulations	0.042	4	0.2
Good documentation of project	0.042	4	0.2
Controllability	0.021	4	0.1
Meet functional, budget and time requirement concurrently	0.021	4	0.1
Final mark			4.5

Table 4.5 Piquar results on electricity

Factor	Weighting factor	Marks	Weighted marks
Plant makes money	0.187	3	0.6
Specification is met	0.187	4	0.7
Safety	0.167	3	0.5
Quality of basis of design	0.146	5	0.7
Reliability	0.104	4	0.4
Efficient use of raw materials	0.083	4	0.3
Comply with construction regulations	0.042	4	0.2
Good documentation of project	0.042	4	0.2
Controllability	0.021	4	0.1
Meet functional, budget and time requirement concurrently	0.021	4	0.1
Final mark			3.8

Table 4.6 Summary of piquar results on utilities

Utility	Total weighted marks
Steam	3.8
Electricity	3.4
Water	4.5

Based on the above evaluation, it is decided to use heat exchanger system with 20 ^{o}C of water as the hot stream. For this heating system, two equipments are needed:

- Water mixing vessel to mix the cold and hot water (this vessel is not included in process flowsheet).
- Heat exchanger (E101) to exchange the heat between the input stream as the cold stream and water as the hot stream.

  The shell and tube exchanger is by far the most commonly used type of heat–transfer equipment in the chemical and allied industries. The most simple and best-described shell and tube exchanger consists of 1 pass at the shell side and 2 passes at the tube side. Therefore, this type of heat exchanger is chosen. Furthermore a heat exchanger with an external floating head is chosen, because this type of heat exchanger can be cleaned very well, and will not have the risk of leaking (Coulson and Richardson, 2001).

  The product stream will flow through the tubes and the heating water will flow on the shell side. This choice is made by considering several factors as described in Coulson and Richardsons's Chemical Engineering (Coulson and Richardson, 2002). The most important factors are corrosion and fouling; it is best to allocate the most corrosive fluid to the tube-side. The same is true for the fluid that has the greatest tendency to foul the heat-transfer surfaces.

4.1.1.2 Endotoxin removal

The first purification step in the designed plant is to remove endotoxin. The method for endotoxin removal has been discussed earlier in chapter two. The options of endotoxin removal methods are described in Appendix 9. The precipitation method that is currently applied in the hCG purification process in Diosynth was chosen for endotoxin removal in the designed processes as well as in the base case.

Ultrafiltration using membranes with about 10-kDa Nominal Molecular Weight Cut-off (NMWCO) is employed to remove endotoxin from product solutions if the products have low molecular weights (Sweadner et al., 1977). The product in this project design, hCG, is much bigger than the subunit of endotoxin. Li and Luo (1998) improved the endotoxin separation process from protein solution by adding Ca^{2+}. According to their process, 45 mM of $CaCl_2$ was added to form large endotoxin aggregates (1000 kDa), which was retained by 300 kDa NMWCO membrane. The endotoxin removal system in the designed process is based on this principle, although there are some important differences as mentioned in Table 4.7.

Table 4.7 Differences between the designed process and process of Li and Luo (1998) for endotoxin removal

Parameter	Process of Li and Luo (1998)	The designed process
Target protein	Haemoglobin	hCG
Total protein concentration in the solution (mg/ml)	0.144	80
Endotoxin concentration in the solution (µg/ml)	5	0.1
Working temperature (ºC)	37	10
Endotoxin removal	6-log	2-log
Incubation time (min.)	30	60
NMWCO of membrane	300	100

All the specifications needed to design ultrafiltration unit in hCG purification process are based on either data of Li and Luo (1997) or assumptions. The reasons behind the assumptions are described below:

- Protein concentration in the designed process is around 550 times more and endotoxin concentration is 50 times less. Protein concentration has a significant effect on protein-LPS (Lipopolysacharides) binding and the amount of endotoxin disaggregated (Li and Luo, 1997). Endotoxin has remarkable capability to interact with proteins. Many of the interacting proteins are basic protein (Petsch and Anspach, 2000). Unfortunately, very little is known about interaction of hCG and endotoxin. Since hCG is highly basic protein, it is assumed that endotoxin has more interaction with hCG than haemoglobin (pI~7.1). Working temperature might also have effect on endotoxin aggregation. For these reasons, 2-log endotoxin removal is assumed in the designed process instead of 6-log.
- It is assumed that 2 gram of hCG is attached to 1 gram of endotoxin. Thus, during endotoxin removal, some hCG will be lost due to attachment with endotoxin and due to retentate volume, which is assumed to be 5% of total volume.
- The target protein in the designed process is hCG, whereas other proteins present in the input stream should be removed. Therefore, ultrafiltration membrane of 100 kDa NMWCO is selected so that bigger proteins are retained. In this condition, it is assumed that 5% of 10 kDa and 22 kDa proteins, 20% of 69 kDa proteins and 50% of 150 kDa proteins are removed from the product.
- Since there is no constraint about time, incubation time in the aggregation vessel is 60 minutes instead of 30 minutes. Double incubation time should confirm aggregation of all endotoxin.

Based on the above discussion, four units are needed for endotoxin removal system:

- Endotoxin aggregation stirred vessel (V101)
  This vessel contains the hCG solution exiting the heat exchanger (E101). $CaCl_2$ is added to this vessel to form endotoxin aggregates.
- Ultrafiltration with membrane of 100 kDa NMWCO (S101)
  This unit will separate endotoxin aggregates and some other proteins from the target protein: hCG.
- Filtrate collection stirred vessel (V102)
  The Filtrate resulted from ultrafiltration (S101) will be collected in this vessel. In case of process with SA-SMB-GF, surfactant will also be added into this vessel.
- Retentate collection vessel (V105)
  The retentate from ultrafiltration (S101) that mainly contains endotoxin will go to this vessel.

4.1.1.3 Virus removal

Virus removal methods include filtration, chromatographic separations, and partitioning into different fraction. As it is mentioned earlier, the constraint of technology for virus removal is using SMB-GF and SA-SMB-GF. Therefore only these two options are chosen as the unit operations for virus removal.

Process with SMB-GF

In simulated moving bed (SMB) chromatography the resin is kept stationary in place, the net movement of the resin relative to the in- and outlet positions is created by periodically switching the in- and outlet positions. The scheme of SMB and its description are shown in Appendix 4.

SMB chromatography has attracted more and more attention recently for the separation of fine chemicals and pharmaceuticals. The main advantages of SMB are high separating power, low solvent consumption and continuous operation. By applying gel chromatography in SMB operation, the virus removal can be done continuously.

The separation of viruses in SMB gel chromatography is based on the different in size of hCG and viruses. Parvovirus, taken as the model with the average size around 20 nm, is much bigger than hCG which is about 6 nm (Harris et al, 1989). As a result, in normal SBM gel chromatography, parvovirus has lower partition coefficient and goes out of the system in the raffinate stream. hCG, which has a higher affinity, is mainly present in the extract stream.

For this designed purification process, two equipments are needed:

- SMB-GF (S102)
  The filtrate from ultrafiltration that is collected in vessel V102 enters the SMB-GF system as feed stream. This SMB system is designed as an open system, where the desorbent stream is not recycled but sent out as a waste. This open process is chosen since by recycling the desorbent, the virus, which stays with the liquid flow will also be recycled and comes back to the system. At the end the virus requirement cannot be met with the close system.
- Desorbent buffer mixture vessel (M101)
  This vessel's function is to prepare the desorbent buffer needed in SMB-GF system.

Process with SA-SMB-GF

As it is mentioned earlier, using surfactants above their critical micelle concentration in the mobile phase increases the distribution coefficient of a protein in gel chromatography. The changes are different for proteins of different sizes, thus the addition of surfactant into SMB-GF system is expected to increase the SMB-GF performance in hCG purification process.

Based on Figure 4.1, the partition coefficient of other proteins become closer to that of hCG with increasing surfactant concentration. Thus, using surfactant at the same concentration in the all SMB sections will reduce its ability to separate other proteins. The calculations of this figure are shown in Appendix 12

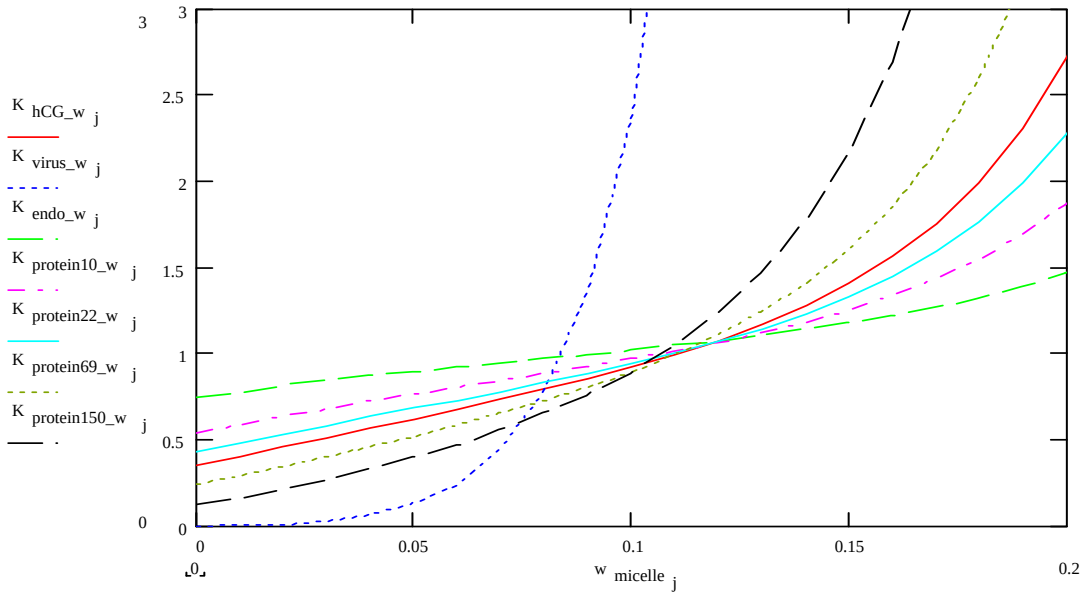


Figure 4.1 Partition coefficients of the components in SA-SMB-GF system as a function of surfactant concentrations

A novel development in SMB technology is the use of gradients in solvent strength. It introduces regions of high and low affinity of the solutes towards the resin in the SMB. This is highly beneficial for the throughput, the volume of feed that can be processed per resin volume. It also reduces solvent consumption in comparison to the isocratic situation (Houwing et al., 2002).

Thus, to overcome other proteins separation problem in this SA-SMB-GF process, surfactant is introduced to the system so that there is a surfactant concentration gradient in the SMB-GF unit. For this purpose, five-sections-SMB is needed instead of four-sections. Surfactant is introduced between the feed and raffinate stream so that there will be a lower surfactant concentration in the sections where components with higher partition coefficient (hCG and other proteins) are absorbed and desorbed.

For this virus removal system with SA-SMB-GF, three equipments are needed:

- SMB-GF (S102)
  The filtrate from ultrafiltration that is collected in vessel V102 enters the SMB-GF system as feed stream.
- Desorbent buffer mixture vessel (M101)
  This vessel's function is to prepare the desorbent buffer needed in SMB-GF system.
- Surfactant mixing vessel (M102)
  Surfactant solution will be prepared in this vessel before flowed to the SMB system.

4.1.1.4 Solvent and water removal

Solvent, in this case ethanol, and water can be separated using precipitation, filtration or evaporation. Each of those options is discussed below.

Precipitation

The addition of ethanol or other organic solvents decreases the polarity of the solution, which results in proteins precipitation. This normally needs to be done at low temperature to

reduce the possibility of proteins denaturation. The precipitation of hCG can be carried out in a mixture of 77% of ethanol, 6% of ammonium acetate and water (Van Dedem, G. 2003). After precipitation process, the target protein (hCG) is separated from the solution by filtration. This precipitation step also contributes to the removal of other proteins that will increase the purity.

Filtration

Ultrafiltration or nanofiltration can be used to separate solvents and water from the hCG solutions. Their common characteristic is the application of pressure differential as a driving force, besides the use of semi permeable membrane as a separation element. The differences between ultrafiltration and nanofiltration are in the pressure differential used, in the properties of membranes (particularly pore sizes), and in a dominant transport mechanism.

The principle of ultrafiltration process can be explained in a simple way as a separation of particles based on different sizes and shapes and also pores of the membrane. The membrane retains particles larger than the size of membrane's pores, while passes through the smaller particles.

The principle of nanofiltration is more complicated. Besides the pore size, the electric charge of the membrane surface and the diffusivity of the particles also play an important role. Small particles may have problem passing through the membrane if they have the same charge as the membrane and also there can be a preferential diffusion of some components through the membrane.

Evaporation

The separation of solvent and water can also be done by evaporation. Since hCG is a heat sensitive protein, the evaporation needs to be carried out at low temperature, which can be achieved by lowering the pressure.

Based on the above overview, the team decided to use the precipitation method to separate solvent and water from hCG. This method is already applied in the current hCG purification process in Diosynth and is also considered as the cheapest one. Additionally, the purified hCG will be dried and in this step, more ethanol and water will be removed, but the team will not deal with the drying process. The team only designs the microfiltration process. The wet hCG will be sent manually by the operator to the drying unit and this procedure is not discussed in this report.

For the solvent and water removal system, two equipments are needed:

- hCG precipitation vessel (V103)
  The extract exiting SMB-GF or SA-SMB-GF system is collected in this vessel and a mixture of ethanol, ammonium acetate and water is added to achieve the desired hCG precipitation.
- Microfiltration (S103)
  The precipitated hCG and other soluble components including the solvent and water are sent to the microfiltration unit. In this unit, the precipitated hCG will be separated from solvent, water, some other proteins and also salts. After this step, hCG will be sent to the drying system available at Diosynth to reduce the water and ethanol contents to the desired level.

4.1.1.5 Other proteins removal

The stream entering the process contains hCG with 25% purity while the product purity must be at least 50%. The recovery of the whole process is minimal 75% (Table 3.1). As a result, at least 75% of initial protein contaminants must be removed. In the separation of other components, proteins are also eliminated. An example of protein elimination is in the step of solvent removal by precipitation. Proteins that are still soluble when solvent is added will be eliminated from hCG. Therefore, it is assumed that other proteins are sufficiently removed during the separation steps of other components and the purity is met.

All proteins bigger than hCG will be removed by SMB-GF or SA-SMB-GF. Proteins bigger than 150 kDa are also removed during endotoxin separation in ultrafiltration unit. Some parts of smaller protein will also be removed during process in SMB-GF or SA-SMB-GF unit. Finally, the desired 50% purity will be achieved.

Thus there is no additional equipment is needed for other proteins removal. Some of the above mentioned equipments would also function as other proteins removal units.

4.1.1.6 Salts removal

It is assumed that the salts are removed during the precipitation of hCG. Thus, the task of removing salts from the purified hCG is also performed in vessel V103 and microfiltration unit (S103).

4.1.1.7 Surfactant removal (only applies for process with SA-SMB-GF)

In case of purification process using SA-SMB-GF, at least one step that removes surfactant is needed. Several options are mentioned below:

- Precipitation of surfactant
  It is mentioned that trichloroacetic acid (TCA) can be used to precipitate surfactant (Janson and Lars, 1998)
- Precipitation of protein
  Polyethylene glycol (PEG) can be used to precipitate proteins and leaving the surfactant in the supernatant (JRH Biosciences, 2001). In the present purification process, hCG is precipitated from the solution by the addition of ethanol (van Dedem, 2003).
- Chromatographic removal of surfactant
  SM-2 macroporous beads, batch or column chromatography (Bio-Rad) can be used to remove surfactant from protein solution (JRH Biosciences, 2001).
- Chromatographic removal of protein
  Hydroxyapatite coated ceramic beads in batch or column chromatography is used to remove proteins from surfactants (JRH Biosciences, 2001)
- Foam fractionation
  Foam fractionation is a process in which solute species adsorbed at the gas–liquid interface between a dispersed phase (gas bubble) and a continuous phase (bulk liquid). It has been shown to be an effective method of removing surfactant, especially anionic or cationic surfactants from water (Boonyasuwat et. al., 2003). However, foam fraction technique is mainly used in detergent industry for recycling and for removing surfactant from wastewater (Scamehorn et. al. 1996).
- Dialysis
  Dialysis is a method of separating smaller compounds in a solution from the larger compounds, such as proteins, through a buffer exchange. The buffer has an osmolarity

less than that of the sample solution, therefore small solutes such as salts and surfactants diffuse through the dialysis membrane into the dialysis buffer (Asenjo, 1990). As a general rule, dialysis membrane is chosen with a molecular weight cut-off that is at least one half of the size of the compound of interest (JRH Biosciences, 2000). By choosing a membrane with a pore size or molecular weight cut-off less than 20 kDa, the hCG will remain in the retentate, isolated from the buffer that now contains surfactant. However, Brij® detergents are difficult to remove from solution by dialysis (www.piercenet.com).

Based on the above analysis, it is decided to remove surfactant by precipitating the proteins. As it is in the current purification process, ethanol will be added to the hCG solution. In this case, hCG and other proteins will precipitate and surfactant will stay in the liquid phase together with ethanol and water.

4.1.1.8 Ethanol containing waste collection

The raffinate from SMB (S102) and the retentate from microfiltration (S103) are collected as liquid waste. Thus for this purpose, one more equipment unit is needed:

- Waste collection stirred vessel (V104)
  The waste collected in this vessel will be sent to the distillation system available at Diosynth for ethanol recovery.

4.1.2 Criteria and selection for base case

4.1.2.1 Temperature adjustment of the input stream

The exiting flow from the ultrafiltration unit in Diosynth has a temperature of –20 ^{o}C. As it is in the designed plant, this stream needs to be heated-up to a temperature between 0 and 10 ^{o}C.

Based on the same evaluation with the temperature adjustment system in the designed plants, it is decided to use heat exchanger system with 20 ^{o}C of water as the hot stream. For this heating system, two equipments are needed:

- Water mixing vessel to mix the cold and hot water (this vessel is not included in process flowsheet).
- Heat exchanger (E101) to exchange the heat between the input stream as the cold stream and water as the hot stream. The type of heat exchanger that will be used is also a shell and tube heat exchanger.

4.1.2.2 Endotoxin and virus removal

In the base case, both endotoxin and virus will be removed by the same system using cation exchange chromatography. Neutral pH is applied and the separation is principally based on the change of ionic strength. At this pH and low ionic strength of the loading solution, hCG is positively charged and is retained on the resin. Viruses and endotoxins that do not bind to resin are washed out with the washing solution. Then hCG is recovered with an eluting solution which has a higher ionic strength. The neutral pH is obtained by using phosphate buffer and the ionic strength is adjusted by the addition of sodium chloride.

For this process, six equipment units are needed:

- Washing buffer mixture stirred vessel (M101)
  In this vessel, washing buffer that is used in cation exchange chromatography is prepared. This solution will be sent to the chromatography system during the washing step.
- Eluent mixture stirred vessel (M102)
  In this vessel, eluent solution that will be used to recover hCG is prepared. This solution will be sent to the chromatography system during the elution step.
- Regeneration solution mixture stirred vessel (M103)
  In this vessel, regeneration buffer is prepared. This solution will be sent to the chromatography system during the regeneration step. The function of this step is to clean the column and its resin so that it can be used for the next purification process.
- Equilibration buffer mixture stirred vessel (M104)
  Equilibration buffer is prepared here and will be sent to the chromatography system during the equilibration step.
- Chromatography column (S101)
  This column will be packed with resin Sp Sepharose high performance that has been chosen earlier. The separation of hCG from endotoxin, virus and proteins contaminant happens in this column.
- Waste collection vessel (V101)
  This vessel collects some of the waste from cation exchange chromatography (S101) process. This waste does not contain a reasonable amount of ethanol to be sent to the distillation system. The liquid collected will then be sent to the wastewater treatment plant available at Diosynth.

4.1.2.3 Solvent, water and salts removal

As it is in the designed plants, the precipitation method will also be used to separate solvent, water and salts from hCG. By adding a mixture of 77% of ethanol, 6% of ammonium acetate and water, hCG and most of other protein contaminants will be precipitated.

For the solvent and water removal system, two equipments are needed:

- hCG precipitation vessel (V102)
  The eluted hCG from cation exchange chromatography (S101) is sent to this vessel and will be precipitated by the addition of a mixture of ethanol, ammonium acetate and water.
- Microfiltration (S102)
  The contents of hCG precipitation vessel (V102) are then sent to a microfiltration unit. In this unit, the precipitated hCG will be separated and then sent to the drying system available at Diosynth. The wet hCG will also be sent manually by the operator to the drying unit and this procedure is not discussed in this report.

4.1.2.4 Other proteins removal

As it is in the designed plants, at least 75% of initial contaminant proteins must be removed. In the base case, proteins are also removed during the separation steps of other components and the purity is met. Thus there is no additional equipment needed for other proteins removal. Some of the above mentioned units would also function as other proteins removal units.

4.1.2.5 Ethanol containing waste collection

Some parts of the waste from the cation exchange chromatography are collected for ethanol recovery treatment. Thus for this purpose, one more unit is needed:

- Waste collection stirred vessel for distillation (V103)
  The waste collected in this vessel will be sent to the distillation system available at Diosynth for ethanol recovery.

4.2 Process Flow Scheme (PFS)

4.2.1 Process Flow Scheme of process with SMB-GF

The overall PFS of hCG purification process with SMB-GF is presented in

Figure 4.2. While the step-by-step PFS is presented from Figure A 13.1 to Figure A 13.17 in Appendix 13 and the explanation of each figure is given below. In the step-by-step PFS, some of the pressure levels cannot be seen because the figures are smaller but those values are shown clearly in the overall PFS.

4.2.1.1 Step 1: heating the input stream and transferring it to vessel V101 (Figure A 13.1)

The first step of the designed plant is heating the product stream exiting the first ultrafiltration unit in the current process. This is done by pumping the product solution through a shell and tube heat exchanger where 20 ^{o}C water is used as the hot stream. The heated product stream is then directly transferred to vessel V101. The process is assumed to take place for five minutes.

4.2.1.2 Step 2: transfering $CaCl_2$ to vessel V101 (Figure A 13.2)

The second step is transferring $CaCl_2$ powder into vessel V101. This process takes place for 1 minute.

4.2.1.3 Step 3: stirring vessel V101 (Figure A 13.3)

The third step is stirring vessel V101, which contains impure hCG solution and CaCl2 solution. It is assumed that by mixing it for one hour, all endotoxin will be aggregated.

4.2.1.4 Step 4: ultrafiltration of endotoxin aggregate (Figure A 13.4)

The fourth step is the step to separate endotoxin aggregate from hCG solution. This is done by ultrafiltrating the stream exiting vessel V101. The total time of ultrafiltration is 72 minutes, but this fourth step is only 59 minutes.

The ultrafiltration process consists of transferring the exiting stream from vessel V101 into the ultrafiltration unit, the ultrafiltration itself, transferring the filtrate to vessel V102 and transferring the retentate to vessel V105.

4.2.1.5 Step 5: ultrafiltration of endotoxin aggregate and transferring water to vessel M101 (Figure A 13.5)

After 59 minutes of ultrafiltration, water is transferred to vessel M101. This vessel is used to prepare the desorbent solution for SMB-GF process. The transferring process is assumed to

take place for five minutes. During this process, ultrafiltration process to separate endotoxin is still going on.

4.2.1.6 Step 6: ultrafiltration of endotoxin aggregate and transferring chemicals to vessel M101 (Figure A 13.6)

After the transferring of water is finished, the solid chemicals (NaH_2PO_4, Na_2HPO_4 and NaCl) are transferred to vessel M101 for one minute. During this step, ultrafiltration step is still going on.

4.2.1.7 Step 7: ultrafiltration of endotoxin aggregate and transferring ethanol to vessel M101 (Figure A 13.7)

After the transferring of chemicals is finished, ethanol is then transferred to vessel M101. This process is assumed to take place in 5 minutes. At that moment, the endotoxin separation process is still happening in ultrafiltration unit.

4.2.1.8 Step 8: ultrafiltration of endotoxin aggregates and stirring vessel M101 (Figure A 13.8)

The next step is stirring vessel M101 to get a well-mixed desorbent solution. The mixing time is 2 minutes and during this time, ultrafiltration in S101 is also still taking place.

4.2.1.9 Step 9: re-ultrafiltration of endotoxin aggregates and stirring vessel M101 (Figure A 13.9)

After the ultrafiltration is complete, the retentate that is collected in vessel V105 is put into ultrafiltration process again. The aim of this step is to reduce the loss of hCG during the ultrafiltration step. The filtrate is transferred to vessel V102 and the raffinate is sent to the wastewater treatment facility available at Diosynth. While this process is taking place for three minutes, vessel M101 is still being stirred.

4.2.1.10 Step 10: SMB-GF process (Figure A 13.10)

The SMB-GF process takes place for 24 hours. The main function of this process is to separate virus from hCG, but it will also increase the purity of hCG by removing other proteins. This process includes the feed flow from vessel V102 to the SMB-GF unit, the desorbent flow from vessel M101 to the SMB-GF unit, the extract flow from SMB-GF unit to vessel V103, the raffinate flow from SMB-GF unit to vessel V104 and the waste flow from SMB-GF unit to vessel V104.

In this unit, the separation process is based on the difference in partition coefficient of all components present. In this case, the partition coefficient of hCG is bigger than that of virus, so that it will mainly be in the solid phase and flow to the extract stream. On the other hand, virus has a smaller partition coefficient thus it will mainly stay in liquid phase and flow to the raffinate stream.

4.2.1.11 Step 11: transferring NH_4-acetate to vessel V103 (Figure A 13.11)

After the SMB-GF process is finished and all of the extract is collected in vessel V103, NH_4-acetate is transferred into this vessel. This process takes place for one minute.

4.2.1.12 Step 12: transferring ethanol to vessel V103 (Figure A 13.12)

Once the transferring of NH_4-acetate to vessel V103 is finished, ethanol is transferred into the same vessel. This process is assumed to take place for 10 minutes.

4.2.1.13 Step 13: stirring vessel V103 (Figure A 13.13)

After all ethanol required is transferred to vessel V103, the vessel is stirred for two hours. During this process, hCG and other proteins will be precipitated, leaving the water, ethanol and salts in the liquid phase.

4.2.1.14 Step 14: microfiltration (Figure A 13.14)

Microfiltration is performed after the precipitation step in vessel V103 is finished. With this process the precipitated hCG will be separated from water, ethanol and salts. During the microfiltration process, hCG will be retained in the membrane while the filtrate is sent to vessel V104 where the waste containing a large amount of ethanol is collected. This process takes place for 10 minutes.

4.2.1.15 Step 15: transferring ethanol to S103 and microfiltration (Figure A 13.15)

After all the stream exiting vessel V103 is filtrated, ethanol is transferred to the microfiltration unit to wash the hCG left on the membrane. The filtrate is again sent to vessel V104 and the washed hCG stays in the membrane. This washing step takes place for 5 minutes.

4.2.1.16 Step 16: transferring hCG to the drying system (Figure A 13.16)

After the washing step is finished, the hCG is transferred to the drying system available at Diosynth. This process will take place for 5 minutes. The hCG is taken out manually from the microfiltration unit by the operator.

4.2.1.17 Step 17: transferring out the waste in V105 to the distillation plant (Figure A 13.17)

Now all the ethanol containing waste is collected in vessel V105 and ready to be transferred to the distillation plant for ethanol recovery. Thus, the last step transferring the liquid waste to the distillation plant, which takes place for 15 minutes.

Figure 4.2 Overall PFS of the process with SMB-GF

4.2.2 Process Flow Scheme of process with SA-SMB-GF

The overall PFS of hCG purification process with SMB-GF is presented in Figure 4.3. Step 1, 2, 3, 4 and step 5 of process with SA-SMB-GF are the same as those in process with SMB-GF. Thus the explanation can be referred to chapter 4.2.1.1 to chapter 4.2.1.5. The PFS of step by step process with SA-SMB-GF is presented from Figure A 14.1 to A 14.18 in Appendix 14.

4.2.2.1 Step 6: ultrafiltration of endotoxin aggregate, transferring chemicals to vessel M101 and transferring water to vessel M102 (Figure A 14.6)

After the transferring of water to vessel M101 is finish, the solid chemicals (NaH_2PO_4, Na_2HPO_4 and NaCl) are transferred to the same vessel. Besides that, at the same time water is also transferred to vessel M102. Those processes take place for one minute. During this process, ultrafiltration process is still going on.

4.2.2.2 Step 7: ultrafiltration of endotoxin aggregate and transferring water to vessel M102 (Figure A 14.7)

After the transferring of chemicals is finish, the endotoxin separation process is still happening in ultrafiltration unit and the water is still transferred to vessel M102. These processes take place for 4 minutes.

4.2.2.3 Step 8: ultrafiltration of endotoxin aggregates, transferring ethanol to vessel M101 and transferring surfactant to vessel M102 (Figure A 14.8)

In the 8^{th} step, ultrafiltration in S101 is also still taking place and besides that, ethanol is transferred to vessel M101 and surfactant is transferred to vessel M102. This step takes place for 1 minute.

4.2.2.4 Step 9: ultrafiltration of endotoxin aggregates, stirring vessel M101 and vessel M102 (Figure A 14.9)

In the 9^{th} step, ultrafiltration in S101 is still taking place and besides that, vessel M101 and M102 are mixed to obtain a desorbent solution and surfactant solution respectively. This step takes place for 2 minutes.

4.2.2.5 Step 10: re-ultrafiltration of endotoxin aggregates, stirring vessel M101 and vessel M102 (Figure A 14.10)

After the ultrafiltration is complete, the retentate that is collected in vessel V105 is put in ultrafiltration process again. The aim of this step is to reduce the loss of hCG during ultrafiltration step. The filtrate is transferred to vessel V102 and the raffinate is sent to the wastewater treatment facility available at Diosynth. While this process is taking place for three minutes, vessel M101 and M102 are still stirred.

4.2.2.6 Step 11: SA-SMB-GF process (Figure A 14.11)

The SA-SMB-GF process takes place for 24 hours. The main function of this process is to separate virus from hCG, but it will also increase the purity of hCG by removing other proteins. This process includes the feed flow from vessel V102 to the SA-SMB-GF unit, the

desorbent flow from vessel M101 to the SA-SMB-GF unit, the surfactant flow from M102 to the SA-SMB-GF unit, the extract flow from SMB-GF unit to vessel V103 and the raffinate flow from SMB-GF unit to vessel V104.

The separation principal of this unit is the same with SMB-GF unit, where the virus tends to stay in the liquid, thus will flow with the raffinate stream and hCG tends to stay in the solid phase, thus will flow with the extract stream. A gradient surfactant is introduced to obtain a better separation process as it is explained in chapter 4.1.1.3.

4.2.2.7 Step 12: transferring NH_4-acetate to vessel V103 (Figure A 14.12)

After the SMB-GF process is finish and all of the extract is collected in vessel V103, NH_4-acetate is transferred into this vessel. This process takes place for one minute.

4.2.2.8 Step 13: transferring ethanol to vessel V103 (Figure A 14.13)

Once the transferring of NH_4-acetate to vessel V103 is finish, ethanol is transferred into the same vessel. This process is assumed to take place for 10 minutes.

4.2.2.9 Step 14: stirring vessel V103 (Figure A 14.14)

After all ethanol required is transferred to vessel V103, the vessel is stirred for two hours. During this process, hCG and other proteins will be precipitated, leaving the water, ethanol and salts in the liquid phase.

4.2.2.10 Step 15: microfiltration (Figure A 14.15)

Microfiltration is performed after the precipitation step in vessel V103 is finish. With this process the precipitated hCG will be separated from water, ethanol and salts. During the microfiltration process, hCG will be retained in the membrane while the filtrate is sent to vessel V104 where the waste containing a large amount of ethanol is collected. This process takes place for 10 minutes.

4.2.2.11 Step 16: transferring ethanol to S103 and microfiltration (Figure A 14.16)

After all the stream exiting vessel V103 is filtrated, ethanol is transferred to the microfiltration unit to wash the hCG left on the membrane. The filtrate is again sent to vessel V104 and the washed hCG stays in the membrane. This washing step takes place for 5 minutes.

4.2.2.12 Step 17: transferring hCG to the drying system (Figure A 14.17)

After the washing step is finish, the hCG is transferred to the drying system available at Diosynth. This process will take place for 5 minutes.

4.2.2.13 Step 18: transferring out the waste in V105 to the distillation plant (Figure A 14.18)

Now all the ethanol containing waste is collected in vessel V105 and ready to be transferred to the distillation plant for ethanol recovery. Thus, the last step transferring the liquid waste to the distillation plant, which takes place for 15 minutes.

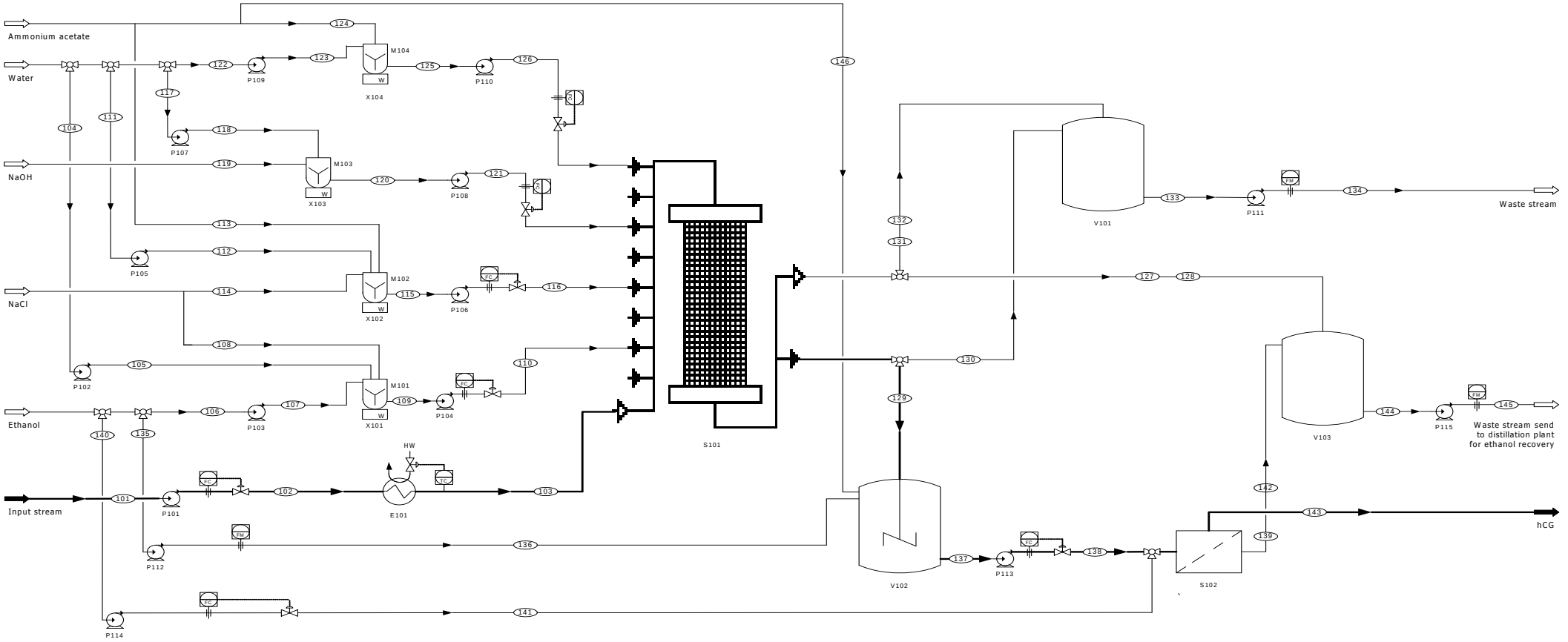
Figure 4.3 Overall PFS of the process with SA-SMB-GF

4.2.3 Process Flow Scheme of the base case

The overall PFS of the base case is presented in Figure 4.4. The step-by-step process for the base case will not be shown but an overview of the process is explained in this chapter. The main separation process is done with cation exchange chromatography. hCG is separated from virus, endotoxin and other proteins in this process.

Before entering the cation chromatography (S101) step, the exiting stream from the first ultrafiltration unit in the current purification process is heated in a heat exchanger (E101), as it is in the designed plants. The cation chromatography process itself consists of several steps, which are loading of the product stream, washing of the un-bound components, elution of the bound components, regeneration of the column and equilibration.

The waste stream from the regeneration and equilibration step is sent to vessel V101 and will be sent to the wastewater treatment plant available at Diosynth. The other waste stream is sent to vessel V103. The product stream exiting this chromatography step is sent to vessel V102 and mixed with ammonium acetate and ethanol to precipitate hCG and other proteins. The precipitated hCg is then sent to the microfiltration unit as it is in the designed plants. The separated hCG is sent to the drying system, while the filtrate is sent to vessel V103. The collected solution in this vessel will be sent to the distillation plant for ethanol recovery.



Process Equipment List		
E101: Heat up stream 102	P106: Elute hCG from column	S101: Ion exchange column
M101: Washing buffer mixture	P107: Feed water for regeneration solution	S102: Microfiltration
M102: Eluent mixture	P108: Regenerate column	V101: Waste collection vessel
M103: Regeneration solution mixture	P109: Feed water for equilibration mixture	V102: Protein precipitation vessel
M104: Equilibration buffer mixture	P110: Equilibrate column	V103: Waste collection vessel for distillation
P101: Heat up stream 101	P111: Discharge waste stream	X101: Weighing balance for M101
P102: Flow water to washing buffer mixture	P112: Feed ethanol to precipitation vessel	X102: Weighing balance for M102
P103: Flow ethanol to washing buffer mixture	P113: Microfiltrate stream 137	X103: Weighing balance for M103
P104: Wash column	P114: Wash retentate	X104: Weighing balance for M104
P105: Feed water for eluent mixture	P115: Discharge waste stream to distillate	

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Process Flow Scheme
Project: Conceptual design of purification process of human chrionic gonadotropin (hCG)
Project ID: CPD3292
Completion date: 22 August 2003
Stream number
Temperature of stream 101 and 102 is -20 ^{0}C. All other streams are between 5-10 ^{0}C.

Figure 4.4 Overall PFS of the base case

4.3 Batch Cycle Diagram (BCD)

Figure 4.5 Batch Cycle Diagram of process with SMB-GF

Figure 4.6 Batch Cycle Diagram of process with SA-SMB-GF

Figure 4.7 Batch Cycle Diagram of the base case

4.4 Process Stream Summary

In this chapter, three stream reports are presented in Table 4.10, Table 4.11 and Table 4.12 for process with SMB-GF, process with SA-SMB-GF and base case respectively. The calculations are based on the explanation given below.

4.4.1 Calculation of Stream Report in SMB Process

Some general assumptions are taken for the calculation of process stream summary in the process with SMB-GF:

- The working temperature of the overall process is between 5-10 °C. The densities of water and ethanol fluctuate very little in this range of temperature. Therefore, the density of water and ethanol is considered to be 1 and 0.8 kg/L, respectively, throughout the whole process.
- Volume of all streams is assumed as volume of ethanol plus water, since all other components exist mainly in dissolved form. Volume of endotoxin and virus is also neglected.
- Virus and endotoxin are important target components in this process. Their weight contribution to the total weight is negligible. Consequently, these are measured in term of particle and endotoxin unit (EU), respectively, rather than in weight.

Input stream/stream <101>

Total volume of input stream is 100 L/batch and the composition is presented in Table 4.8,based on following assumptions:

- It contains 20 mg/ml of hCG, which is 25% pure. Therefore, concentration of other proteins is 60 mg/ml.
- Input stream contains 40% (w/w) ethanol.
- Input stream contains 15 mM of salt. All of the salt is assumed to be NaCl, since it is the major urinary salt.
- Endotoxin concentration in the input stream is 10^6 EU/ml.
- Virus concentration in the input stream is assumed as 3×10^7 particles/ml, which indicates that after 6-log removal the product is allowed to contain 1 particle/mg.

Table 4.8 Detail composition of the input stream in process with SMB-GF

Total volume	100 L
hCG	2 kg
Proteins	6 kg
Virus	3×10^{12} particles
Endotoxin	10^{11} EU
NaCl	0.0878 kg
Ethanol	39.30 kg
Water	50.87 kg
Total weight	98.26 kg
Density	0.9826 kg/L
Temperature	-20 °C

Stream <102> and <103>

The composition of stream <102> and <103> is exactly the same as stream 101, but stream <103> is heated up to 5 °C.

Stream <104>
The aggregation vessel (V101) should contain 90 mM $CaCl_2$.

Stream <105> and <106>
Stream <105> or <106> is the sum of all components in stream <104> and <103>.

Stream <108> and <107>
The composition of stream <108> and <107> in ultrafiltration is determined based on the following assumptions in ultrafiltration (S101):

- Volume of the retentate is 5% of the total volume that passes through the membrane (www.millipore.com) and the other 95% is the permeate (stream <111>).
- 99% of endotoxin is retained by the membrane as aggregates.
- Because of the affinity with endotoxin, hCG is attached to the endotoxin aggregates. It is assumed that 2 gram of hCG molecule is attached to one gram of endotoxin.

  Total amount of endotoxin = 10^{11} EU
  = 0.1 x 10^{11} = 10^{10} ng = 10 g

  Total amount of hCG attached = 10 x 2 = 20 g = 0.02 kg

- The retentate stream <107> contains 5% of dissolved hCG. The rest of the hCG comes in the permeate. Thus, the amount of hCG goes with the permeate is 95% of dissolved hCG minus the amount that is attached to endotoxin. The retention of other 10 kDa and 22 kDa proteins are 5%, while the retention of 69 kDa protein is 20%. Since NMWCO of the membrane is 100 kDa, it was assumed that 50% of 150 kDa protein is retained (www.millipore.com).
- Virus, NaCl, $CaCl_2$ and ethanol concentration in permeate and retentate is equal (www.millipore.com).

Stream <109>-<112>
Retentate of ultrafiltration is recycled back to recover the lost hCG.

- Volume of retentate (stream <111>) is 5% of total volume passed through the membrane (www.millipore.com). The other 95% is permeate (stream <112>).
- All endotoxin is retained by the membrane as aggregates.
- Retentate stream <107> contains 10% dissolved hCG. The rest of the hCG comes in the permeate. Thus, the amount of hCG goes with the permeate is 95% of dissolved hCG minus the amount that is attached to endotoxin. The retention of other 10 kDa and 22 kDa proteins are 10%, while the retention of 69 kDa protein is 20%. Since NMWCO of the membrane is 100 kDa, it was assumed that 50% of 150 kDa protein is retained.
- Virus, NaCl, $CaCl_2$ and ethanol concentration in permeate and retentate is equal.

Stream <117> and <118>
The composition of stream <117> and <118> is exactly the sum of stream <108> and <112>. Stream <118> is the feed stream in SMB (S102).

Stream <119>-<127>
Stream <127> is the desorbent stream in SMB (S102). The required volume of desorbent stream is known from MathCad model (Appendix 21). The desorbent streams (<126> and <127>) contain 10 mM phosphate buffer at pH 7.0, and 0.1 M NaCl. Ethanol concentration

in desorbent stream is assumed to be the same (in volume percent) as that in thefeed stream <118>.

All streams required for desorbent preparation is mixed in mixing vessel (M102). The required ethanol is pumped through stream <124> and <125> to M102. The required Na_2HPO_4 and NaH_2PO_4 are calculated and added to M102 through stream <121> and <122>, respectively. The required NaCl is added to the same vessel through stream <123>. Finally, the amount of water needed is determined as total desorbent volume minus volume of ethanol in desorbent. Stream <119> and <120> represent the flow of water to the mixture.

Stream <128>
Stream <128> is the extract stream of SMB (S102). The concentration of hCG, proteins, virus and endotoxin in stream <114> depends on their partition coefficients and is calculated in MathCad model (Appendix 21). In addition, it is assumed that the concentration of salts and ethanol is the same as that in the desorbent stream <125>.

Stream <114>
Stream <114> is the raffinate stream of SMB (S102). Concentration of hCG, proteins, virus and endotoxin in stream <114> depends on their partition coefficients and is calculated in MathCad model (Appendix 21). In addition, it is assumed that the concentration of salts and ethanol in the raffinate is the same as that in the waste stream.

Stream <115>
Stream <115> is the waste stream of SMB (S102). Concentration of hCG, proteins, virus and endotoxin in stream <114> depends on their partition coefficients and is calculated in MathCad model (Appendix 21). In addition, it is assumed that the concentration of salts and ethanol is the same as in raffinate and waste stream.

Stream <129>
In the precipitation vessel, 6% ammonium acetate is required (van Dedem, 2003).

Stream <130> and <131>
Precipitation vessel (V103) should contain 85% (w/w) ethanol. Stream <127> already contained about 40% ethanol. The required additional ethanol is calculated and pumped through stream <130> and <131>.

Stream <132> and <133>
Stream <132> or <133> is the sum of all components in stream <127> and <131>.

Stream <134>
The composition of stream <134> is determined based on the following assumptions in microfiltration (S103):

- Volume of the retentate is 5% of the total volume passes through the membrane (www.millipore.com). The other 95% is the permeate (stream <134>).
- 5% of hCG and proteins is passed through the membrane. The other 95% is retained as precipitate (van Dedem, 2003).
- The retentate contains 5% of virus, endotoxin and salts (www.millipore.com). The rest of these components come in the permeate.
- Water and ethanol concentration in permeate and retentate is equal.

Stream <135> and <136>
Stream <136> is the washing solution of the retentate in microfiltration (S103) and it is 100% ethanol. The volume of stream <135> and <136> is calculated in MathCad to achieve 100 times hCG purity (Appendix 19).

Stream <137> and <138>
The composition of stream <137> and <138> is determined based on the following assumptions in washing in microfiltration (S103):

- Volume of stream <138> is the same as that of the retentate. Therefore, volume of stream <137> is equal to the volume of washing solution (stream <136>). Stream <138> is the product stream in the process.
- No hCG and proteins is passed through the membrane during washing, since these particles are large enough not to pass though the membrane.
- Stream <138> contains 1% of virus, endotoxin, salts, and water, since these components are very small (Appendix 19). The rest of these components come in stream <137>.
- The rest of the volume in stream <138> is ethanol.
- The composition of stream 137 can then be calculated.

Stream <139> and <140>
Stream <139> and <140> is the sum of streams 114, 115, 134 and 137. This stream is very rich in ethanol concentration and will be sent to distillation plant for ethanol recovery.

4.4.2 Calculation of Stream Report in SA-SMB Process

The calculations of the process stream in process with SA-SMB-GF are based on the same assumptions with the ones in process with SMB-GF. Thus, for this chapter one can refer to chapter 4.4.1.

4.4.3 Calculation of Stream Report in base case

Some general assumptions are taken for the calculation of process stream summary in the base case:

- The working temperature of the overall process is between 5-10 °C. The densities of water and ethanol fluctuate very little in this range of temperature. Therefore, the density of water and ethanol is considered to be 1 and 0.8 kg/L, respectively, throughout the whole process.
- The volume of all streams is assumed as the volume of ethanol plus water, since all other components exist mainly in dissolved form. Volume of endotoxin and virus is also neglected.
- Virus and endotoxin are important target components in this process. Their weight contribution to the total weight is negligible. Consequently, these are measured in term of particle and endotoxin unit (EU), respectively.

Input stream/stream <101>
The total volume of the input stream is 100 L/batch and the composition is presented in Table 4.9, based on following assumptions:

- It contains 20 mg/ml of hCG, which is 25% pure. Therefore, concentration of other proteins is 60 mg/ml.

- Input stream contains 40% (w/w) ethanol.
- Input stream contains 15 mM of salt. All of the salt is assumed to be NaCl, since it is the major urinary salt.
- Endotoxin concentration in the input stream is 10^6 EU/ml.
- Virus concentration in the input stream is assumed as 3×10^7 particles/ml, which indicates that after 6-log removal the product is allowed to contain 1 particle/mg.

Table 4.9 Detail composition of the input stream in base case

Total volume	100 L
hCG	2 kg
Proteins	6 kg
Virus	3×10^{12} particles
Endotoxin	10^{11} EU
NaCl	0.0878 kg
Ethanol	39.30 kg
Water	50.87 kg
Total weight	98.26 kg
Density	0.9826 kg/L
Temperature	-20 °C

Stream <102> and <103>
The composition of stream <102> and <103> is exactly the same as that of stream <101>, but stream <103> is heated up to 5 °C.

Stream <104>-<110>
The washing solution in M101 should contain 20 mM NaCl and 20% ethanol and the volume is 4 times bed volume.

Stream <111>-<116>
Elution buffer in M102 should contain 10 mM NaCl and 50 mM NH_4-acetate and the volume is 3 times bed volume.

Stream <117>-<121>
The regeneration solution volume is 2 times bed volume and contains 1 M NaOH

Stream <122>-<126>
The Equilibration solution volume is 8 times bed volume and contains 0.5 M of NH_4-acetate.

Stream <127>
When the feed is passed through the ion exchange column, some components are retained in the column and some will come out in stream <127>.

Stream <128>
When washing solution is passed through ion exchange column, some bound components are washed out with stream <128>.

Stream <129>-<130>
The components that are bound to the column are then eluted with elution buffer (stream <116>). It is assumed that hCG is eluted with the first 1/3 volume of elution buffer. Therefore, the first 1/3 of the volume of elution buffer coming out from the column is the extract (stream <129>). The remaining 2/3 of the elution buffer volume is the waste (stream <130>).

Stream <131>-<132>
The column is regenerated with NaOH and equilibrated with NH_4-acetate buffer. The composition of stream <131> and <132> are the same as that of stream <121> and <126>, respectively. However, pH is different in these streams.

Stream <146>
In the precipitation vessel, 6% ammonium acetate is required (van Dedem, 2003).

Stream <135> and <136>
The precipitation vessel (V103) should contain 85% (w/w) ethanol. Stream <127> already contained some ethanol. The required additional ethanol is calculated and pumped through stream <135> and <136>.

Stream <137> and <138>
Stream <137> or <138> is the sum of all components in stream <129>, <146> and <136>.

Stream <139>
The composition of stream <139> is determined based on the following assumptions in microfiltration (S102):

- Volume of retentate is 5% of total volume passes through the membrane (www.millipore.com). The other 95% is the permeate (stream <139>).
- 5% of hCG and proteins passes through the membrane and the other 95% is retained as precipitate (www.millipore.com).
- The retentate contains 5% of virus, endotoxin and salts (www.millipore.com). The rest of these components come in the permeate.
- Water and ethanol concentration in permeate and retentate is equal.

Stream <140> and <141>
Stream <140> is the washing solution of the retentate in microfiltration (S102) and it is 100% ethanol. The volume of stream <141> and <142> is calculated in MathCad file to achieve 100 times hCG purity (Appendix 19).

Stream <142> and <143>
The composition of stream <142> and <143> is determined based on the following assumptions in washing in microfiltration (S102):

- The volume of stream <143> is the same as retentate. Therefore, volume of stream <142> is equal to the volume of washing solution (stream <141>). Stream <143> is the product stream in the process.
- No hCG and proteins passes through the membrane during washing, since these particles are large enough not to pass though the membrane.
- Stream <143> contains 1% of virus, endotoxin, salts, and water present in retentate, since these components are very small and washed out by ethanol (Appendix 19). The rest of these components come in stream <142>.

- The rest of the volume in stream <143> is ethanol.
- Composition of stream <142> can then be calculated

Stream <144> and <145>

Stream <144> and <145> is the sum of streams <127>, <128>, <139> and <142>. This stream is very rich in ethanol concentration and will be sent to distillation plant for ethanol recovery.

Table 4.10 Process stream summary of the process with SMB-GF

Stream Nr.:		**101/102/103**			**104**			**105/106**			**107**			**108**		
Batch Cycle:		Feed to P101			Feed to V101			Feed to ultrafiltration			Retentate of ultrafiltration			Permeate of ultrafiltration		
Component	Mwt	Cycle kg	Cycle kg/h	Process kg/h avg.	Cycle kg	Cycle kg/h	Process kg/h avg.	Cycle kg	Cycle kg/h	Process kg/h avg.	Cycle kg	Cycle kg/h	Process kg/h avg.	Cycle kg	Cycle kg/h	Process kg/h avg.
hCG	37500,0	2,0000	24,0000	0,0687				2,0000	1,6667	0,0687	0,1200	0,1000	0,0041	1,8800	1,5667	0,0646
10 kDa protein	10000,0	1,8000	21,6000	0,0618				1,8000	1,5000	0,0618	0,0900	0,0750	0,0031	1,7100	1,4250	0,0587
22 kDa protein	22000,0	0,6000	7,2000	0,0206				0,6000	0,5000	0,0206	0,0300	0,0250	0,0010	0,5700	0,4750	0,0196
69 kDa protein	69000,0	1,5000	18,0000	0,0515				1,5000	1,2500	0,0515	0,3000	0,2500	0,0103	1,2000	1,0000	0,0412
150 kDa protein	150000,0	1,8000	21,6000	0,0618				1,8000	1,5000	0,0618	0,9000	0,7500	0,0309	0,9000	0,7500	0,0309
NS proteins	~50000,0	0,3000	3,6000	0,0103				0,3000	0,2500	0,0103	0,0225	0,0188	0,0008	0,2775	0,2313	0,0095
Virus (Part.)	~2000000,0	3,00E+12	3,60E+13	1,03E+11				3,00E+12	2,50E+12	1,03E+11	1,50E+11	1,25E+11	5,15E+09	2,85E+12	2,38E+12	9,79E+10
Endotoxin (EU)	10000,0	1,00E+11	1,20E+12	3,43E+09				1,00E+11	8,33E+10	3,43E+09	9,90E+10	8,25E+10	3,40E+09	1,00E+09	8,33E+08	3,43E+07
NaCl	58,5	0,0878	1,0530	0,0030				0,0878	0,0731	0,0030	0,0044	0,0037	0,0002	0,0834	0,0695	0,0029
$CaCl_2$	111,0				0,4995	29,9700	0,0172	0,4995	0,4163	0,0172	0,0250	0,0208	0,0009	0,4745	0,3954	0,0163
Ethanol	46,0	39,3047	471,6558	1,3499				39,3047	32,7539	1,3499	1,9652	1,6377	0,0675	37,34	31,1162	1,2824
Water	18,0	50,8692	610,4308	1,7471				50,8692	42,3910	1,7471	2,5435	2,1196	0,0874	48,33	40,2715	1,6597
Total	(kg)	98,262	1179,140	3,375	0,50	29,97	0,02	98,76	82,30	3,39	6,00	5,00	0,21	92,76	77,30	3,19
	(L)	100,000	1200,001	3,434				100,00	83,33	3,43	5,00	4,17	0,17	95,00	79,17	3,26
Density	(kg/L)	0,9826						0,9876			1,2001			0,9764		
Phase	(L/S)	L			S			S-L			S-L			L		
Temperature	(^{0}C)	20/-20/5			10			5			5			5		
Pressure	(bara)	1,0/4,5/4,0			1.0			4.5/7.5			3.5			3.5		
Cycle times	(h)															
Cycle & process			0,0833	29,1167		0,0167	29,1167		1,2000	29,1167		1,2000	29,1167		1,2000	29,1167

Stream Nr.:		**109/110**			**111**			**112**			**117/118**			**126/127**		
Batch Cycle:		Recycle of ultrafiltration			Retentate of recycle			Permeate of recycle			Feed to SMB			Desorbent of SMB		
Component	Mwt	Cycle kg	Cycle kg/h	Process kg/h avg.	Cycle kg	Cycle kg/h	Process kg/h avg.	Cycle kg	Cycle kg/h	Process kg/h avg.	Cycle kg	Cycle kg/h	Process kg/h avg.	Cycle kg	Cycle kg/h	Process kg/h avg.
hCG	37500,0	0,1200	2,4000	0,0041	0,0300	0,6000	0,0010	0,0900	1,8000	0,0031	1,9700	0,0821	0,0677			
10 kDa protein	10000,0	0,0900	1,8000	0,0031	0,0090	0,1800	0,0003	0,0810	1,6200	0,0028	1,7910	0,0746	0,0615			
22 kDa protein	22000,0	0,0300	0,6000	0,0010	0,0030	0,0600	0,0001	0,0270	0,5400	0,0009	0,5970	0,0249	0,0205			
69 kDa protein	69000,0	0,3000	6,0000	0,0103	0,0600	1,2000	0,0021	0,2400	4,8000	0,0082	1,4400	0,0600	0,0495			
150 kDa protein	150000,0	0,9000	18,0000	0,0309	0,4500	9,0000	0,0155	0,4500	9,0000	0,0155	1,3500	0,0563	0,0464			
NS proteins	~50000,0	0,0225	0,4500	0,0008	0,0023	0,0450	0,0001	0,0203	0,4050	0,0007	0,2978	0,0124	0,0102			
Virus (Part.)	~2000000,0	1,50E+11	3,00E+12	5,15E+09	1,50E+10	3,00E+11	5,15E+08	1,35E+11	2,70E+12	4,64E+09	2,99E+12	1,24E+11	1,03E+11			
Endotoxin (EU)	10000,0	9,90E+10	1,98E+12	3,40E+09	9,90E+10	1,98E+12	3,40E+09	0,00E+00	0,00E+00	0,00E+00	1,00E+09	4,17E+07	3,43E+07			
NaCl	58,5	0,0044	0,0878	0,0002	0,0004	0,0088	0,0000	0,0039	0,0790	0,0001	0,0873	0,0036	0,0030	1,9670	0,0820	0,0676
$CaCl_2$	111,0	0,0250	0,4995	0,0009	0,0025	0,0500	0,0001	0,0225	0,4496	0,0008	0,4970	0,0207	0,0171			
Na_2HPO_4	142,0													0,5207	0,0217	0,0179
NaH_2PO_4	120,0													0,1844	0,0077	0,0063
Ethanol	46,0	1,97	39,3047	0,0675	0,20	3,9305	0,0067	1,77	35,3742	0,0607	39,11	1,6295	1,3432	132,29	5,5122	4,5435
Water	18,0	2,54	50,8692	0,0874	0,25	5,0869	0,0087	2,29	45,7823	0,0786	50,61	2,1090	1,7383	171,22	7,1340	5,8804
Total (kg)		6,00	120,01	0,21	1,01	20,16	0,03	4,99	99,85	0,17	97,75	4,07	3,36	306,18	12,76	10,52
Total (L)		5,00	100,00	0,17	0,50	10,00	0,02	4,50	90,00	0,15	99,50	4,15	3,42	336,58	14,02	11,56
Density	(kg/L)	1,2001			2,0161			1,1094			0,9824			0,9097		
Phase	(L/S)	S-L			S-L			L			L			L		
Temperature	(^{0}C)	5			5			5			5			10		
Pressure	(bara)	3.5/7.5			3.5			3.5			4.0/11.0			1.5/11.0		
Cycle times	(h)															
Cycle & process			0,0500	29,1167		0,0500	29,1167		0,0500	29,1167		24,0000	29,1167		24,0000	29,1167

Stream Nr.:		**119/120**			**121**			**122**			**123**			**124/125**		
Batch Cycle:		Feed to M101			Feed to M101			Feed to M101			Feed to M101					
Component	Mwt	Cycle kg	Cycle kg/h	Process kg/h avg.	Cycle kg	Cycle kg/h	Process kg/h avg.	Cycle kg	Cycle kg/h	Process kg/h avg.	Cycle kg	Cycle kg/h	Process kg/h avg.	Cycle kg	Cycle kg/h	Process kg/h avg.
NaCl	58,5										1,9670	118,0189	0,0676			
$CaCl_2$	111,0															
Na_2HPO_4	142,0				0,5207	31,2415	0,0179									
NaH_2PO_4	120,0							0,1844	11,0668	0,0063						
Ethanol	46,0													132,29	1587,50	29,12
Water	18,0	171,22	2054,59	5,88												
Total	(kg)	171,22	2054,59	5,88	0,52	31,24	0,02	0,18	11,07	0,01	1,97	118,02	0,07	132,29	1587,50	29,12
	(L)	171,22	2054,59	5,88										165,37	1984,38	36,40
Density	(kg/L)	1,0000												0,8000		
Phase	(L/S)	L			S			S			S			L		
Temperature	(^{0}C)	10			10			10			10			10		
Pressure	(bara)	1.0/1.5			1.0			1.0			1.0			1.0/1.5		
Cycle times	(h)															
Cycle & process			0,0833	29,1167		0,0167	29,1167		0,0167	29,1167		0,0167	29,1167		0,0833	29,1167

Stream Nr.:		**114**			**115**			**128**			**129**			**130/131**		
Batch Cycle:		Raffinate of SMB			Waste of SMB			Extract of SMB			Feed to V103			Feed to V103		
Component	Mwt	Cycle kg	Cycle kg/h	Process kg/h avg.	Cycle kg	Cycle kg/h	Process kg/h avg.	Cycle kg	Cycle kg/h	Process kg/h avg.	Cycle kg	Cycle kg/h	Process kg/h avg.	Cycle kg	Cycle kg/h	Process kg/h avg.
hCG	37500,0	0,0180	0,0007	0,0006	0,0075	0,0003	0,0003	1,9446	0,0810	0,0668						
10 kDa protein	10000,0	0,3737	0,0156	0,0128	0,4229	0,0176	0,0145	0,9944	0,0414	0,0342						
22 kDa protein	22000,0	0,0740	0,0031	0,0025	0,0837	0,0035	0,0029	0,4393	0,0183	0,0151						
69 kDa protein	69000,0	1,4138	0,0589	0,0486	0,0106	0,0004	0,0004	0,0155	0,0006	0,0005						
150 kDa protein	150000,0	0,7809	0,0325	0,0268	0,5691	0,0237	0,0195									
NS proteins	~50000,0							0,2978	0,0124	0,0102						
Virus (Part.)	~2000000,0	1,54E+12	6,40E+10	5,27E+10	1,45E+12	6,06E+10	4,99E+10									
Endotoxin (EU)	10000,0	2,54E+08	1,06E+07	8,73E+06	2,87E+08	1,20E+07	9,87E+06	4,58E+08	1,91E+07	1,57E+07						
NaCl	58,5	0,53084	0,0221	0,0182	0,60060	0,0250	0,0206	0,9229	0,0385	0,0317						
$CaCl_2$	111,0	0,23318	0,0097	0,0080	0,26382	0,0110	0,0091									
Na_2HPO_4	142,0	0,12968	0,0054	0,0045	0,14672	0,0061	0,0050	0,2443	0,0102	0,0084						
NaH_2PO_4	120,0	0,04594	0,0019	0,0016	0,05197	0,0022	0,0018	0,0865	0,0036	0,0030						
NH_4-acetate	82,0										9,4749	568,4936	0,3254			
Ethanol	46,0	51,30	2,1373	1,7617	58,04	2,4182	1,9932	62,07	2,5862	2,1317				421,16	2526,95	14,46
Water	18,0	66,39	2,7662	2,2801	75,11	3,1297	2,5797	80,33	3,3471	2,7589						
Total (kg)		121,28	5,05	4,17	135,31	5,64	4,65	147,34	6,14	5,06	9,47	568,49	0,33	421,16	2526,95	14,46
Total (L)		130,51	5,44	4,48	147,66	6,15	5,07	157,91	6,58	5,42				526,45	3158,69	18,08
Density	(kg/L)	0,9293			0,9163			0,9331						0,8000		
Phase	(L/S)	L			L			L			S					
Temperature	(^{0}C)	8			8			10			10			10		
Pressure	(bara)	1.5			1.5			1.5			1.0			1.0/2.0		
Cycle times	(h)															
Cycle & process			24,0000	29,1167		24,0000	29,1167		24,0000	29,1167		0,0167	29,1167		0,1667	29,1167

Stream Nr.:		**132/133**			**134**			**135/136**			**137**			**138**		
Batch Cycle:		Feed to microfiltration			Permeate of microfiltration			Wash filter cake			Washout of filter cake			Product		
Component	Mwt	Cycle kg	Cycle kg/h	Process kg/h avg.	Cycle kg	Cycle kg/h	Process kg/h avg.	Cycle kg	Cycle kg/h	Process kg/h avg.	Cycle kg	Cycle kg/h	Process kg/h avg.	Cycle kg	Cycle kg/h	Process kg/h avg.
hCG	37500,0	1,9446	11,6673	0,0668	0,0972	0,5834	0,0033							1,8473	22,1679	0,0634
10 kDa protein	10000,0	0,9944	5,9664	0,0342	0,0497	0,2983	0,0017							0,9447	11,3362	0,0324
22 kDa protein	22000,0	0,4393	2,6358	0,0151	0,0220	0,1318	0,0008							0,4173	5,0080	0,0143
69 kDa protein	69000,0	0,0155	0,0930	0,0005	0,0008	0,0047	0,0000							0,0147	0,1767	0,0005
NS proteins	~50000,0	0,2978	1,7865	0,0102	0,0149	0,0893	0,0005							0,2829	3,3944	0,0097
Endotoxin (EU)	10000,0	4,58E+08	2,75E+09	1,57E+07	4,36E+08	2,61E+09	1,50E+07				2,27E+07	4,54E+08	1,56E+07	2,3E+05	2,75E+06	7,87E+03
NaCl	58,5	0,9229	5,5371	0,0317	0,8767	5,2603	0,0301				0,0457	0,9136	0,0314	0,0005	0,0055	0,0000
Na_2HPO_4	142,0	0,2443	1,4658	0,0084	0,2321	1,3925	0,0080				0,0121	0,2419	0,0083	0,0001	0,0015	0,0000
NaH_2PO_4	120,0	0,0865	0,5192	0,0030	0,0822	0,4933	0,0028				0,0043	0,0857	0,0029	0,0000	0,0005	0,0000
NH_4-acetate	82,0	9,4749	56,85	0,33	9,00	54,01	0,31				0,4690	9,3801	0,3222	0,0047	0,0568	0,0002
Ethanol	46,0	483,2264	2899,36	16,60	459,07	2754,39	15,77	126,07	2521,42	4,33	122,89	2457,80	84,4122	27,34	328,1087	0,9391
Water	18,0	80,3301	481,98	2,76	76,31	457,88	2,62				3,98	79,53	2,7313	0,04	0,4820	0,0014
Total (kg)		577,98	3467,86	19,85	545,76	3274,53	18,74	126,07	2521,42	4,33	127,40	2547,95	87,51	30,89	370,74	1,06
Total (L)		684,36	4106,18	23,50	650,14	3900,87	22,33	157,59	3151,78	5,41	157,59	3151,78	108,25	34,22	410,62	1,18
Density	(kg/L)		0,8445			0,8394			0,8000			0,8084			0,9029	
Phase	(L/S)		S-L			L			L			L			S-L	
Temperature	(^{0}C)		10			10			10			10			10	
Pressure	(bara)		1.5/2.5			1.5			1.0/2.5			1.5			1.0	
Cycle times	(h)															
Cycle & process			0,1667	29,1167		0,1667	29,1167		0,0500	29,1167		0,0500	29,1167		0,0833	29,1167

Stream Nr.:		**139/140**		
Batch Cycle:		Wastewater to distillate		
Component	Mwt	Cycle kg	Cycle kg/h	Process kg/h avg.
hCG	37500,0	0,1301	0,3902	0,0045
10 kDa protein	10000,0	0,8501	2,5503	0,0292
22 kDa protein	22000,0	0,1813	0,5440	0,0062
69 kDa protein	69000,0	1,4252	4,2757	0,0489
150 kDa protein	150000,0	1,3500	4,0500	0,0464
NS proteins	~50000,0	0,0160	0,0481	0,0006
Virus (Part.)	~2000000,0	2,99E+12	8,97E+12	1,03E+11
Endotoxin (EU)	10000,0	1,03E+09	3,10E+09	3,55E+07
NaCl	58,5	2,1239	6,3718	0,0729
$CaCl_2$	111,0	0,4970	1,4910	0,0171
Na_2HPO_4	142,0	0,5391	1,6174	0,0185
NaH_2PO_4	120,0	0,1910	0,5729	0,0066
NH_4-acetate	82,0	578,00	1734,01	19,85
Ethanol	46,0	695,53	2086,58	23,89
Water	18,0	227,89	683,68	7,83
Total (kg)		1508,73	4526,19	51,82
Total (L)		1097,30	3291,91	37,69
Density (kg/L)		1,3749		
Phase (L/S)		L		
Temperature (^{0}C)		10		
Pressure (bara)		1.0/2.0		
Cycle times (h)				
Cycle & process			0,3333	29,1167

Table 4.11 Process stream summary of the process with SA-SMB-GF

Stream Nr.:		**101/102/103**			**104**			**105/106**			**107**			**108**		
Batch Cycle:		Feed to P101			Feed to V101			Feed to ultrafiltration			Retentate of ultrafiltration			Permeate of ultrafiltration		
Component	Mwt	Cycle kg	Cycle kg/h	Process kg/h avg.	Cycle kg	Cycle kg/h	Process kg/h avg.	Cycle kg	Cycle kg/h	Process kg/h avg.	Cycle kg	Cycle kg/h	Process kg/h avg.	Cycle kg	Cycle kg/h	Process kg/h avg.
hCG	37500,0	2,0000	24,0000	0,0687				2,0000	1,6667	0,0687	0,1200	0,1000	0,0041	1,8800	1,5667	0,0646
10 kDa protein	10000,0	1,8000	21,6000	0,0618				1,8000	1,5000	0,0618	0,0900	0,0750	0,0031	1,7100	1,4250	0,0587
22 kDa protein	22000,0	0,6000	7,2000	0,0206				0,6000	0,5000	0,0206	0,0300	0,0250	0,0010	0,5700	0,4750	0,0196
69 kDa protein	69000,0	1,5000	18,0000	0,0515				1,5000	1,2500	0,0515	0,3000	0,2500	0,0103	1,2000	1,0000	0,0412
150 kDa protein	150000,0	1,8000	21,6000	0,0618				1,8000	1,5000	0,0618	0,9000	0,7500	0,0309	0,9000	0,7500	0,0309
NS proteins	~50000,0	0,3000	3,6000	0,0103				0,3000	0,2500	0,0103	0,0225	0,0188	0,0008	0,2775	0,2313	0,0095
Virus (Part.)	~2000000,0	3,00E+12	3,60E+13	1,03E+11				3,00E+12	2,50E+12	1,03E+11	1,50E+11	1,25E+11	5,15E+09	2,85E+12	2,38E+12	9,79E+10
Endotoxin (EU)	10000,0	1,00E+11	1,20E+12	3,43E+09				1,00E+11	8,33E+10	3,43E+09	9,90E+10	8,25E+10	3,40E+09	1,00E+09	8,33E+08	3,43E+07
NaCl	58,5	0,0878	1,0530	0,0030				0,0878	0,0731	0,0030	0,0044	0,0037	0,0002	0,0834	0,0695	0,0029
$CaCl_2$	111,0				0,4995	29,9700	0,0172	0,4995	0,4163	0,0172	0,0250	0,0208	0,0009	0,4745	0,3954	0,0163
Ethanol	46,0	39,3047	471,6558	1,3499				39,3047	32,7539	1,3499	1,9652	1,6377	0,0675	37,34	31,1162	1,2824
Water	18,0	50,8692	610,4308	1,7471				50,8692	42,3910	1,7471	2,5435	2,1196	0,0874	48,33	40,2715	1,6597
Total	(kg)	98,262	1179,140	3,375	0,50	29,97	0,02	98,76	82,30	3,39	6,00	5,00	0,21	92,76	77,30	3,19
	(L)	100,000	1200,001	3,434				100,00	83,33	3,43	5,00	4,17	0,17	95,00	79,17	3,26
Density	(kg/L)	0,9826						0,9876			1,2001			0,9764		
Phase	(L/S)	L			S			S-L			S-L			L		
Temperature	(^{0}C)	20/-20/5			10			5			5			5		
Pressure	(bara)	1,0/4,5/4,0			1.0			4.5/7.5			3.5			3.5		
Cycle times	(h)															
Cycle & process			0,0833	29,1167		0,0167	29,1167		1,2000	29,1167		1,2000	29,1167		1,2000	29,1167

Stream Nr.:		**109/110**			**111**			**112**			**117/118**			**141/142**		
Batch Cycle:		Recycle of ultrafiltration			Retentate of recycle			Permeate of recycle			Feed to SMB			Feed to M102		
Component	Mwt	Cycle kg	Cycle kg/h	Process kg/h avg.	Cycle kg	Cycle kg/h	Process kg/h avg.	Cycle kg	Cycle kg/h	Process kg/h avg.	Cycle kg	Cycle kg/h	Process kg/h avg.	Cycle kg	Cycle kg/h	Process kg/h avg.
hCG	37500,0	0,1200	2,4000	0,0041	0,0300	0,6000	0,0010	0,0900	1,8000	0,0031	1,9700	0,0821	0,0677			
10 kDa protein	10000,0	0,0900	1,8000	0,0031	0,0090	0,1800	0,0003	0,0810	1,6200	0,0028	1,7910	0,0746	0,0615			
22 kDa protein	22000,0	0,0300	0,6000	0,0010	0,0030	0,0600	0,0001	0,0270	0,5400	0,0009	0,5970	0,0249	0,0205			
69 kDa protein	69000,0	0,3000	6,0000	0,0103	0,0600	1,2000	0,0021	0,2400	4,8000	0,0082	1,4400	0,0600	0,0495			
150 kDa protein	150000,0	0,9000	18,0000	0,0309	0,4500	9,0000	0,0155	0,4500	9,0000	0,0155	1,3500	0,0563	0,0464			
NS proteins	~50000,0	0,0225	0,4500	0,0008	0,0023	0,0450	0,0001	0,0203	0,4050	0,0007	0,2978	0,0124	0,0102			
Virus (Part.)	~2000000,0	1,50E+11	3,00E+12	5,15E+09	1,50E+10	3,00E+11	5,15E+08	1,35E+11	2,70E+12	4,64E+09	2,99E+12	1,24E+11	1,03E+11			
Endotoxin (EU)	10000,0	9,90E+10	1,98E+12	3,40E+09	9,90E+10	1,98E+12	3,40E+09	0,00E+00	0,00E+00	0,00E+00	1,00E+09	4,17E+07	3,43E+07			
NaCl	58,5	0,0044	0,0878	0,0002	0,0004	0,0088	0,0000	0,0039	0,0790	0,0001	0,0873	0,0036	0,0030			
$CaCl_2$	111,0	0,0250	0,4995	0,0009	0,0025	0,0500	0,0001	0,0225	0,4496	0,0008	0,4970	0,0207	0,0171			
Ethanol	46,0	1,97	39,3047	0,0675	0,20	3,9305	0,0067	1,77	35,3742	0,0607	39,11	1,6295	1,3432			
Water	18,0	2,54	50,8692	0,0874	0,25	5,0869	0,0087	2,29	45,7823	0,0786	50,61	2,1090	1,7383	39,80	477,60	1,37
Total (kg)		6,00	120,01	0,21	1,01	20,16	0,03	4,99	99,85	0,17	97,75	4,07	3,36	39,80	477,60	1,37
Total (L)		5,00	100,00	0,17	0,50	10,00	0,02	4,50	90,00	0,15	99,50	4,15	3,42	39,80	477,60	1,37
Density	(kg/L)	1,2001			2,0161			1,1094			0,9824			1,0000		
Phase	(L/S)	S-L			S-L			L			L			L		
Temperature	(^{0}C)	5			5			5			5			10		
Pressure	(bara)	3.5/7.5			3.5			3.5			4.0/11.0			1.0/1.5		
Cycle times (h)																
Cycle & process			0,0500	29,1167		0,0500	29,1167		0,0500	29,1167		24,0000	29,1167		0,0833	29,1167

Stream Nr.:		**143**			**144/145**			**126/127**			**119/120**			**121**		
Batch Cycle:		Feed to M102			Feed 2 to SMB			Desorbent to SMB			Feed to M101			Feed to M101		
Component	Mwt	Cycle kg	Cycle kg/h	Process kg/h avg.	Cycle kg	Cycle kg/h	Process kg/h avg.	Cycle kg	Cycle kg/h	Process kg/h avg.	Cycle kg	Cycle kg/h	Process kg/h avg.	Cycle kg	Cycle kg/h	Process kg/h avg.
NaCl	58,5							1,1048	29,1167	0,0379						
Na_2HPO_4	142,0							0,2925	7,7076	0,0100				0,2925	52,6429	0,0100
NaH_2PO_4	120,0							0,1036	2,7303	0,0036						
Surfactant	1199.6	0,1035	6,2088	0,0036	0,1035	0,0043	0,0036									
Ethanol	46,0							74,31	1958,28	2,55						
Water	18,0				39,8000	1,6583	1,3669	96,17	2534,47	3,30	96,17	1154,02	3,30			
Total	(kg)	0,10	6,21	0,00	39,90	1,66	1,37	171,97	4532,30	5,91	96,17	1154,02	3,30	0,29	52,64	0,01
	(L)				39,80	1,66	1,37	189,05	4982,32	6,49	96,17	1154,02	3,30			
Density	(kg/L)				1,0026			0,9097			1,0000					
Phase	(L/S)	S			L			L			L			S		
Temperature	(^{0}C)	10			10			10			10			10		
Pressure	(bara)	1.0			1.0/11.0			5.0/11.0			1.0/1.5			1.0		
Cycle times	(h)															
Cycle & process			0,0167	29,1167		24,0000	29,1167		24,0000	29,1167		0,0833	29,1167		0,0056	29,1167

Stream Nr.:		**122**			**123**			**124/125**			**114**			**128**		
Batch Cycle:		Feed to M101			Feed to M101			Feed ethanol to M101			Raffinate of SMB			Extract of SMB		
Component	Mwt	Cycle kg	Cycle kg/h	Process kg/h avg.	Cycle kg	Cycle kg/h	Process kg/h avg.	Cycle kg	Cycle kg/h	Process kg/h avg.	Cycle kg	Cycle kg/h	Process kg/h avg.	Cycle kg	Cycle kg/h	Process kg/h avg.
hCG	37500,0										0,0034	0,0001	0,0001	1,9666	0,0819	0,0675
10 kDa protein	10000,0										0,0269	0,0011	0,0009	1,7642	0,0735	0,0606
22 kDa protein	22000,0													0,5970	0,0249	0,0205
69 kDa protein	69000,0										1,0314	0,0430	0,0354	0,4086	0,0170	0,0140
150 kDa protein	150000,0										1,3500	0,0563	0,0464			
NS proteins	~50000,0													0,2978	0,0124	0,0102
Virus (Part.)	~2000000,0										2,98E+12	1,24E+11	1,03E+11	2,60E+05	1,08E+04	8,93E+03
Endotoxin (EU)	10000,0										5,98E+07	2,49E+06	2,05E+06	9,42E+08	3,93E+07	3,24E+07
NaCl	58,5				1,1048	198,8655	0,0379				0,9983	0,0416	0,0343	0,1938	0,0081	0,0067
$CaCl_2$	111,0										0,4970	0,0207	0,0171			
Na_2HPO_4	142,0										0,2412	0,0100	0,0083	0,0513	0,0021	0,0018
NaH_2PO_4	120,0	0,1036	18,6479	0,0036							0,0854	0,0036	0,0029	0,0182	0,0008	0,0006
Surfactant	1199.6										0,10120	0,0042	0,0035	0,0023	0,0001	0,0001
Ethanol	46,0							74,31	891,66	2,55	100,38	4,1824	3,4474	13,04	0,54	0,45
Water	18,0										169,71	7,0713	5,8287	16,87	0,70	0,58
Total	(kg)	0,10	18,65	0,00	1,10	198,87	0,04	74,31	891,66	2,55	274,42	11,43	9,42	35,21	1,47	1,21
	(L)							92,88	1114,58	3,19	295,18	12,30	10,14	33,17	1,38	1,14
Density	(kg/L)							0,8000			0,9297			1,0615		
Phase	(L/S)	S			S			L			L			L		
Temperature	(^{0}C)	10			10			10			10			10		
Pressure	(bara)	1.0			1.0			1.0/1.5			1.5			1.5		
Cycle times	(h)															
Cycle & process			0,0056	29,1167		0,0056	29,1167		0,0833	29,1167		24,0000	29,1167		24,0000	29,1167

Stream Nr.:		**129**			**130/131**			**132/133**			**134**			**135/136**		
Batch Cycle:		Feed to V103			Feed to V103			Feed to microfiltration			Permeate of microfiltration					
Component	Mwt	Cycle kg	Cycle kg/h	Process kg/h avg.	Cycle kg	Cycle kg/h	Process kg/h avg.	Cycle kg	Cycle kg/h	Process kg/h avg.	Cycle kg	Cycle kg/h	Process kg/h avg.	Cycle kg	Cycle kg/h	Process kg/h avg.
hCG	37500,0							1,9666	29,1167	0,0675	0,0983	0,5900	0,0034			
10 kDa protein	10000,0							1,7642	26,1200	0,0606	0,0882	0,5293	0,0030			
22 kDa protein	22000,0							0,5970	8,8389	0,0205	0,0299	0,1791	0,0010			
69 kDa protein	69000,0							0,4086	6,0496	0,0140	0,0204	0,1226	0,0007			
NS proteins	~50000,0							0,2978	4,4084	0,0102	0,0149	0,0893	0,0005			
Virus (Part.)	~2000000,0							2,60E+05	3,85E+06	8,93E+03	2,47E+05	1,48E+06	8,48E+03			
Endotoxin (EU)	10000,0							9,42E+08	1,39E+10	3,24E+07	8,95E+08	5,37E+09	3,07E+07			
NaCl	58,5							0,1938	2,8697	0,0067	0,1841	1,1048	0,0063			
Na_2HPO_4	142,0							0,0513	0,7597	0,0018	0,0487	0,2925	0,0017			
NaH_2PO_4	120,0							0,0182	0,2691	0,0006	0,0173	0,1036	0,0006			
NH_4-acetate	82,0	1,9900	119,4001	0,0683				1,9900	29,4631	0,0683	1,8905	11,3430	0,0649			
Surfactant	1199.6							0,0023	0,0338	0,0001	0,0022	0,0130	0,0001			
Ethanol	46,0				112,60	675,61	3,87	125,64	1860,14	4,31	119,36	716,1355	4,0992	32,05	384,62	1,10
Water	18,0							16,87	249,79	0,58	16,03	96,1683	0,5505			
Total	(kg)	1,99	119,40	0,07	112,60	675,61	3,87	149,80	2217,86	5,14	137,78	826,67	4,73	32,05	384,62	1,10
	(L)				140,75	844,51	4,83	173,92	2574,97	5,97	165,22	991,34	5,67	40,07	480,78	1,38
Density	(kg/L)					0,8000			0,8613			0,8339			0,8000	
Phase	(L/S)		S			L			S-L			L			L	
Temperature	(^{0}C)		10			10			10			10			10	
Pressure	(bara)		1.0			1.0/2.0			1.5/2.5			1.5			1.0/2.5	
Cycle times	(h)															
Cycle & process			0,0167	29,1167		0,1667	29,1167		0,1667	29,1167		0,1667	29,1167		0,0833	29,1167

Stream Nr.:		**137**			**138**			**139/140**		
Batch Cycle:		Washout of microfiltration			Product			Wastewater to distillate		
Component	Mwt	Cycle kg	Cycle kg/h	Process kg/h avg.	Cycle kg	Cycle kg/h	Process kg/h avg.	Cycle kg	Cycle kg/h	Process kg/h avg.
hCG	37500,0				1,8683	22,4192	0,0642	0,1017	0,3052	0,0035
10 kDa protein	10000,0				1,6760	20,1119	0,0576	0,1151	0,3453	0,0040
22 kDa protein	22000,0				0,5672	6,8058	0,0195	0,0299	0,0896	0,0010
69 kDa protein	69000,0				0,3882	4,6580	0,0133	1,0518	3,1555	0,0361
150 kDa protein	150000,0				0,0000	0,0000	0,0000	1,3500	4,0500	0,0464
NS proteins	~50000,0				0,2829	3,3944	0,0097	0,0149	0,0447	0,0005
Virus (Part.)	~2000000,0	1,29E+04	1,54E+05	4,42E+02	1,3E+02	1,56E+03	4,46E+00	2,98E+12	8,95E+12	1,03E+11
Endotoxin (EU)	10000,0	4,66E+07	5,60E+08	1,60E+06	4,7E+05	5,65E+06	1,62E+04	1,00E+09	3,00E+09	3,44E+07
NaCl	58,5	0,0096	0,1151	0,0003	0,0001	0,0012	0,0000	1,1920	3,5761	0,0409
$CaCl_2$	111,0							0,4970	1,4910	0,0171
Na_2HPO_4	142,0	0,0025	0,0305	0,0001	0,0000	0,0003	0,0000	0,2924	0,8773	0,0100
NaH_2PO_4	120,0	0,0009	0,0108	0,0000	0,0000	0,0001	0,0000	0,1036	0,3108	0,0036
NH_4-acetate	82,0	0,0985	1,1821	0,0034	0,0010	0,0119	0,0000	1,9890	5,9670	0,0683
Surfactant	1199.6	0,0001	0,0014	0,0000	0,0000	0,0000	0,0000	0,1035	0,3104	0,0036
Ethanol	46,0	31,38	376,6066	1,0779	6,95	83,4001	0,2387	251,12	753,3519	8,6245
Water	18,0	0,84	10,0218	0,0287	0,01	0,1012	0,0003	186,57	559,7239	6,4078
Total	(kg)	32,33	387,97	1,11	11,74	140,90	0,40	444,53	1333,60	15,27
	(L)	40,07	480,78	1,38	8,70	104,35	0,30	500,47	1501,41	17,19
Density	(kg/L)		0,8070			1,3503			0,8882	
Phase	(L/S)		L			S-L			L	
Temperature	(^{0}C)		10			10			10	
Pressure	(bara)		1.5			1.0			1.0/2.0	
Cycle times	(h)									
Cycle & process			0,0833	29,1167		0,0833	29,1167		0,3333	29,1167

Table 4.12 Process stream summary of the base case

Stream Nr.:		Mwt	**101/102/103**			**127**			**109/110**		
Batch Cycle:			Column loading			Output of loading			Column washing		
Component			Cycle kg	Cycle kg/h	process kg/h avg.	Cycle kg	Cycle kg/h	process kg/h avg.	Cycle kg	Cycle kg/h	process kg/h avg.
hCG		37500.0	0.2000	1.3333	0.0713	0.0200	0.1333	0.0071			
Proteins		~50000.0	0.6000	4.0000	0.2139	0.4200	2.8000	0.1497			
Virus (Part.)		~2000000.0	3.00E+11	2.00E+12	1.07E+11	2.99E+11	1.99E+12	1.07E+11			
Endotoxin (EU)		10000.0	1.00E+10	6.67E+10	3.57E+09	9.00E+09	6.00E+10	3.21E+09			
NaCl		58.5	0.0088	0.0585	0.0031	0.0087	0.0579	0.0031	0.2338	0.3896	0.0833
Ethanol		46.0	3.93	26.2031	1.4012	3.9305	26.2031	1.4012	15.2826	25.4710	5.4483
Water		18.0	5.09	33.9128	1.8135	5.0869	33.9128	1.8135	60.8967	101.4945	21.7101
Total	(kg)		9.83	65.51	3.50	9.47	63.11	3.37	76.41	127.36	27.24
	(L)		10.00	66.67	3.57	10.00	66.67	3.57	80.00	133.33	28.52
Density	(kg/L)			0.9826			0.9466			0.9552	
Phase	(L/S)			L			L			L	
Temperature	(^{0}C)			-20/-20/5			5			10	
Pressure	(bara)			1/12/11			2			1/11	
Cycle times					10			10			10
Cycle & process	(h)			0.15	28.05		0.15	28.05		0.60	28.05

Stream Nr.:		Mwt	**108**			**106/107**			**104/105**		
Batch Cycle:			NaCl to M102			Ethanol to M102			Water to M102		
Component			Cycle kg	Cycle kg/h	process kg/h avg.	Cycle kg	Cycle kg/h	process kg/h avg.	Cycle kg	Cycle kg/h	process kg/h avg.
NaCl		58.5	0.2338	14.0256	0.0833						
Ethanol		46.0				15.2826	458.4786	5.4483			
Water		18.0							60.8967	1217.9345	21.7101
Total	(kg)		0.23	0.39	0.08	15.28	458.48	5.45	60.90	1217.93	21.71
	(L)					19.10	573.10	6.81	60.90	1217.93	21.71
Density	(kg/L)						0.8000			1.0000	
Phase	(L/S)			S			L			L	
Temperature	(^{0}C)			10			10			10	
Pressure	(bara)			1			1\2			1\2	
Cycle times					10			10			10
Cycle & process	(h)			0.02	28.05		0.03	28.05		0.05	28.05

Stream Nr.:		**128**			**115/116**			**114**		
Batch Cycle:		Output of washing			Elution			NaCl to M103		
Component	Mwt	Cycle kg	Cycle kg/h	process kg/h avg.	Cycle kg	Cycle kg/h	process kg/h avg.	Cycle kg	Cycle kg/h	process kg/h avg.
hCG	37500.0	0.0090	0.0150	0.0032						
Proteins	~50000.0	0.0090	0.0150	0.0032						
Virus (Part.)	~2000000.0	1.20E+09	2.00E+09	4.27E+08						
Endotoxin (EU)	10000.0	9.50E+08	1.58E+09	3.39E+08						
NaCl	58.5	0.2315	0.3858	0.0825	0.3506	0.7792	0.1250	0.3506	21.0384	0.1250
NH_4-acetate	82.0				0.0925	0.2055	0.0330			
Ethanol	46.0	15.28	25.4710	5.4483						
Water	18.0	60.90	101.4945	21.7101	60.00	133.3333	21.3904			
Total	(kg)	76.43			60.44	134.32	21.55	0.35	21.04	0.13
	(L)	80			60.00	133.33	21.39			
Density	(kg/L)	0.9554				1.0074				
Phase	(L/S)		L			L			S	
Temperature	(^{0}C)		10			10			10	
Pressure	(bara)		1			1/11			1	
Cycle times				10			10			10
Cycle & process	(h)		0.60	28.05		0.45	28.05		0.02	28.05

Stream Nr.:		**113**			**111/112**			**129**		
Batch Cycle:		NH_4-acetate to M103			Water to M103			Eluent		
Component	Mwt	Cycle kg	Cycle kg/h	process kg/h avg.	Cycle kg	Cycle kg/h	process kg/h avg.	Cycle kg	Cycle kg/h	process kg/h avg.
hCG	37500.0							0.1625	1.0830	0.0579
Proteins	~50000.0							0.1625	1.0830	0.0579
Virus (Part.)	~2000000.0							6.00E+05	4.00E+06	2.14E+05
Endotoxin (EU)	10000.0							5.00E+06	3.33E+07	1.78E+06
NaCl	58.5							0.1180	0.7870	0.0421
NH_4-acetate	82.0	0.0925	5.5498	0.0330				0.0308	0.2055	0.0110
Water	18.0				60.0000	720.0000	21.3904	20.0000	133.3333	7.1301
Total	(kg)	0.09	5.55	0.03	60.00	720.00	21.39	20.47	136.49	7.30
	(L)				60.00	720.00	21.39	20.00	133.33	7.13
Density	(kg/L)					1.0000			1.0237	
Phase	(L/S)		S			L			L	
Temperature	(^{0}C)		10			10			10	
Pressure	(bara)		1			1/2			2	
Cycle times				10			10			10
Cycle & process	(h)		0.02	28.05		0.08	28.05		0.15	28.05

Stream Nr.:		**130**			**120/121**			**119**		
Batch Cycle:		Waste after elution			Column regeneration			NaOH to M104		
Component	Mwt	Cycle kg	Cycle kg/h	process kg/h avg.	Cycle kg	Cycle kg/h	process kg/h avg.	Cycle kg	Cycle kg/h	process kg/h avg.
hCG	37500.0	0.0086	0.0285	0.0030						
Proteins	~50000.0	0.0086	0.0285	0.0030						
Virus (Part.)	~2000000.0	6.00E+05	2.00E+06	2.14E+05						
Endotoxin (EU)	10000.0	4.50E+07	1.50E+08	1.60E+07						
NaCl	58.5	0.2349	0.7831	0.0838						
NH_4-acetate	82.0	0.0617	0.2055	0.0220						
NaOH	40.0				1.6000	5.3333	0.5704	1.6000	96.0000	0.5704
Water	18.0	40.0000	133.3333	14.2602	40.0000	133.3333	14.2602			
Total	(kg)	40.31	134.38	14.37	41.60	138.67	14.83	1.60	96.00	0.57
	(L)	40.00	133.33	14.26	40.00	133.33	14.26			
Density	(kg/L)		1.0078			1.0400				
Phase	(L/S)		L			L			S	
Temperature	(^{0}C)		10			10			10	
Pressure	(bara)		2			1/11			1	
Cycle times				10			10			10
Cycle & process	(h)		0.30	28.05		0.30	28.05		0.02	28.05

Stream Nr.:		**117/118**			**131**			**125/126**		
Batch Cycle:		Water to M104			Output of regeneration			Equilibration of column		
Component	Mwt	Cycle kg	Cycle kg/h	process kg/h avg.	Cycle kg	Cycle kg/h	process kg/h avg.	Cycle kg	Cycle kg/h	process kg/h avg.
NH_4-acetate	82.0							6.1664	5.1387	2.1984
NaOH	40.0				1.5840	19.0080	0.5647			
Water	18.0	40.0000	480.0000	14.2602	40.0000	480.0000	14.2602	160.0000	133.3333	57.0410
Total	(kg)	40.00	480.00	14.26	41.58	499.01	14.82	166.17	138.47	59.24
	(L)	40.00	480.00	14.26	40.00	480.00	14.26	160.00	133.33	57.04
Density	(kg/L)		1.0000			1.0396			1.0385	
Phase	(L/S)		L			L			L	
Temperature	(^{0}C)		10			10			10	
Pressure	(bara)		1/2			2			1/11	
Cycle times				10			10			10
Cycle & process	(h)		0.08	28.05		0.08	28.05		1.20	28.05

Stream Nr.:		**124**			**122/123**			**132**		
Batch Cycle:		NH_4-acetate to M104			Water to M104			Output of equilibration		
Component	Mwt	Cycle kg	Cycle kg/h	process kg/h avg.	Cycle kg	Cycle kg/h	process kg/h avg.	Cycle kg	Cycle kg/h	process kg/h avg.
NH_4-acetate	82.0	6.1664	369.9840	2.1984				6.1664	5.1387	2.1984
NaOH	40.0							0.0160	0.0133	0.0057
Water	18.0				160.0000	1920.0000	57.0410	160.0000	133.3333	57.0410
Total	(kg)	6.17	369.98	2.20	160.00	1920.00	57.04	166.18	138.49	59.25
	(L)				160.00	1920.00	57.04	160.00	133.33	57.04
Density	(kg/L)					1.0000			1.0386	
Phase	(L/S)		S			L			L	
Temperature	(^{0}C)		10			10			10	
Pressure	(bara)		1			1/2			2	
Cycle times				10			10			10
Cycle & process	(h)		0.02	28.05		0.08	28.05		1.20	28.05

Stream Nr.:		**133/134**			**146**			**135/136**		
Batch Cycle:		Wastewater			NH_4-acetate to V102			Ethanol to V102		
Component	Mwt	Cycle kg	Cycle kg/h	process kg/h avg.	Cycle kg	Cycle kg/h	process kg/h avg.	Cycle kg	Cycle kg/h	process kg/h avg.
hCG	37500.0	0.0855	0.5130	0.0030						
Proteins	~50000.0	0.0855	0.5130	0.0030						
Virus (Part.)	~2000000.0	6.00E+06	3.60E+07	2.14E+05						
Endotoxin (EU)	10000.0	4.50E+08	2.70E+09	1.60E+07						
NaCl	58.5	2.3493	14.0958	0.0838						
NH_4-acetate	82.0	62.2806	373.6838	2.2203	11.6917	701.5008	0.4168			
NaOH	40.0	16.0000	96.0000	0.5704						
Ethanol	46.0							1160.1809	13922.1712	41.3612
Water	18.0	2400.0000	14400.0000	85.5615						
Total	(kg)	2480.80	14884.81	88.44	11.69	701.50	0.42	1160.18	13922.17	41.36
	(L)	2400.00	14400.00	85.56				1450.23	17402.71	51.70
Density	(kg/L)		1.0337						0.8000	
Phase	(L/S)		L			S			L	
Temperature	(^{0}C)		10			10			10	
Pressure	(bara)		1/2			1			1/2	
Cycle times				1			1			1
Cycle & process	(h)		0.17	28.05		0.02	28.05		0.08	28.05

Stream Nr.:		**137/138**			**139**			**140/141**		
Batch Cycle:		Feed to microfiltration			Permeate			Wash filtercake		
Component	Mwt	Cycle kg	Cycle kg/h	process kg/h avg.	Cycle kg	Cycle kg/h	process kg/h avg.	Cycle kg	Cycle kg/h	process kg/h avg.
hCG	37500.0	1.6245	9.7470	0.0579	0.0812	0.4874	0.0029			
Proteins	~50000.0	1.6245	9.7470	0.0579	0.0812	0.4874	0.0029			
Virus (Part.)	~2000000.0	6.00E+06	3.60E+07	2.14E+05	5.70E+06	3.42E+07	2.03E+05			
Endotoxin (EU)	10000.0	5.00E+07	3.00E+08	1.78E+06	4.75E+07	2.85E+08	1.69E+06			
NaCl	58.5	1.1805	7.0830	0.0421	1.1215	6.7288	0.0400			
NH_4-acetate	82.0	12.0000	72.0000	0.4278	11.4000	68.4000	0.4064			
Ethanol	46.0	1160.1809	6961.0856	41.3612	1102.1719	6613.0313	39.2931	303.9781	3647.7370	10.8370
Water	18.0	200.0000	1200.0000	7.1301	190.0000	1140.0000	6.7736			
Total	(kg)	1376.61	8259.66	49.08	1304.86	7829.13	46.52	303.98	3647.74	10.84
	(L)	1650.23	9901.36	58.83	1567.71	9406.29	55.89	379.97	4559.67	13.55
Density	(kg/L)		0.8342			0.8323			0.8000	
Phase	(L/S)		S-L			L			L	
Temperature	(^{0}C)		10			10			10	
Pressure	(bara)		1/3			2			1/3	
Cycle times				1			1			1
Cycle & process	(h)		0.17	28.05		0.17	28.05		0.08	28.05

Stream Nr.:		**142**			**143**			**144/145**		
Batch Cycle:		Permeate of washing			Product			Waste to distillate		
Component	Mwt	Cycle kg	Cycle kg/h	process kg/h avg.	Cycle kg	Cycle kg/h	process kg/h avg.	Cycle kg	Cycle kg/h	process kg/h avg.
hCG	37500.0				1.5433	18.5193	0.0550	0.3712	0.7425	0.0132
Proteins	~50000.0				1.5433	18.5193	0.0550	4.3712	8.7425	0.1558
Virus (Part.)	~2000000.0	2.97E+05	3.56E+06	1.06E+04	3.00E+03	3.60E+04	1.07E+02	3.00E+12	6.00E+12	1.07E+11
Endotoxin (EU)	10000.0	2.48E+06	2.97E+07	8.82E+04	2.50E+04	3.00E+05	8.91E+02	9.95E+10	1.99E+11	3.55E+09
NaCl	58.5	0.0584	0.7012	0.0021	0.0006	0.0071	0.0000	3.5819	7.1637	0.1277
NH_4-acetate	82.0	0.5940	7.1280	0.0212	0.0060	0.0720	0.0002	11.9940	23.9880	0.4276
Ethanol	46.0	296.06	3552.6970	10.5547	65.929	791.1486	2.3504	1590.3608	3180.7217	56.6974
Water	18.0	9.90	118.8000	0.3529	0.1000	1.2000	0.0036	859.7365	1719.4729	30.6501
Total	(kg)	306.61	3679.33	10.93	69.12	829.47	2.46	2470.42	4940.83	88.07
	(L)	379.97	4559.67	13.55	82.51	990.14	2.94	2847.69	5695.38	101.52
Density	(kg/L)		0.8069			0.8377			0.8675	
Phase	(L/S)		L			S-L			L	
Temperature	(^{0}C)		10			10			10	
Pressure	(bara)		2			1			1/2	
Cycle times				1			1			1
Cycle & process	(h)		0.08	28.05		0.08	28.05		0.50	28.05

4.5 Utilities

Utilities are needed for heating the input stream to the designed plants. The options and discussion about utilities used for this purpose are discussed in detail in chapter 4.1.1.1. Besides that, utilities are also needed for operating the stirrers, pumps and the equipments. For these purposes, electricity is used as the utility. Water is also used for cleaning the units. Two qualities of water are used for this purpose, which are process water and water for injection. It is important to rinse the units with water for injection before using it for hCG purification process since hCG will be injected to humans. The washing step is not included in the batch cycle diagram and nor in process stream summary. A summary of utilities is available in Appendix 15.

4.6 Process Yields

4.6.1 Process with SMB-GF

Table 4.13 Yields of process with SMB-GF

Process Streams					
Name	**Referance Stream**	**kg/batch**		**kg/kg dry product**	
		IN	**OUT**	**IN**	**OUT**
Input stream	101	98.26		56.15	
Sodium Chloride	123	1.97		1.13	
Calcium Chloride	104	0.50		0.29	
Sodium bi-phosphate- dibasic	121	0.52		0.30	
Sodium bi-phosphate- monobasic	122	0.18		0.10	
Ammonium acetate	129	9.47		5.41	
Ethanol	124, 130, 135	679.52		388.30	
Water	119, 141	171.22		97.84	
Product stream	138		30.89		17.65
Wastewater to distillate	140		929.74		531.28
Waste	111		1.01		0.58
Total		961.64	961.64	549.51	549.51

Table 4.14 Utilities of process with SMB-GF

Utilities					
Name	**Referance Stream**	**kg/ batch**	**Wh/ batch**	**kg/kg product**	**Wh/kg product**
Cleaning water		12,134		6,934	
Electricity			54.2		31.1

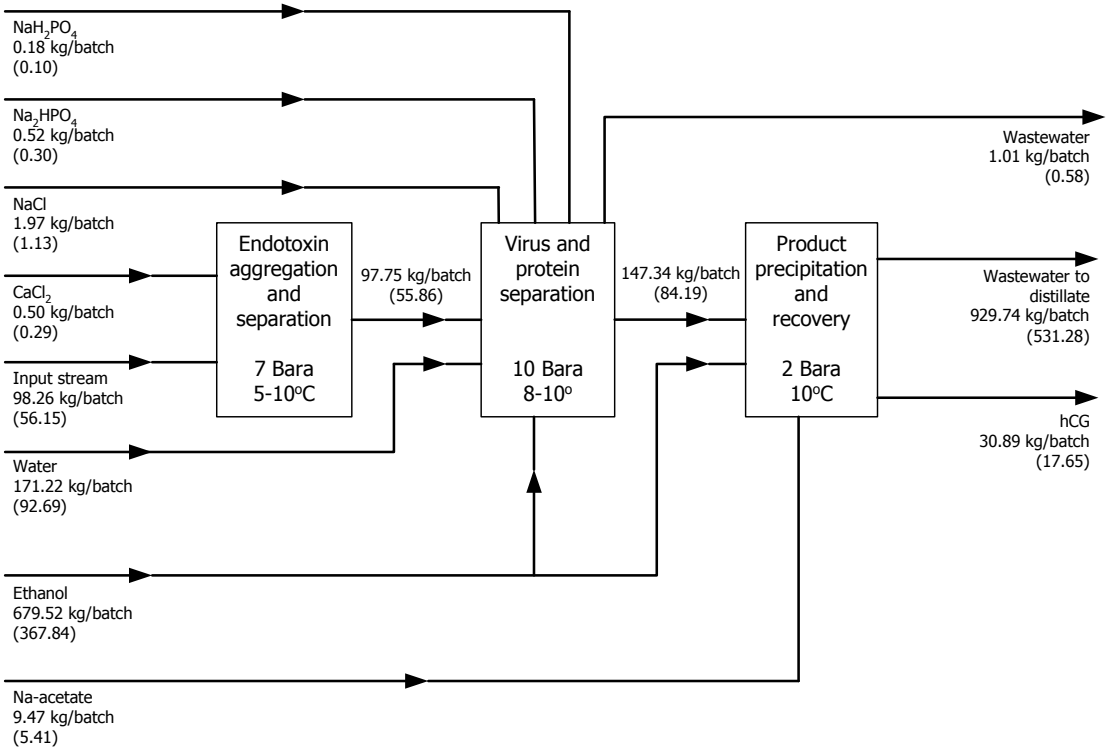

Figure 4.8 Process yields diagram of process with SMB-GF

Note: The number in the brackets is in kg/kg dry product

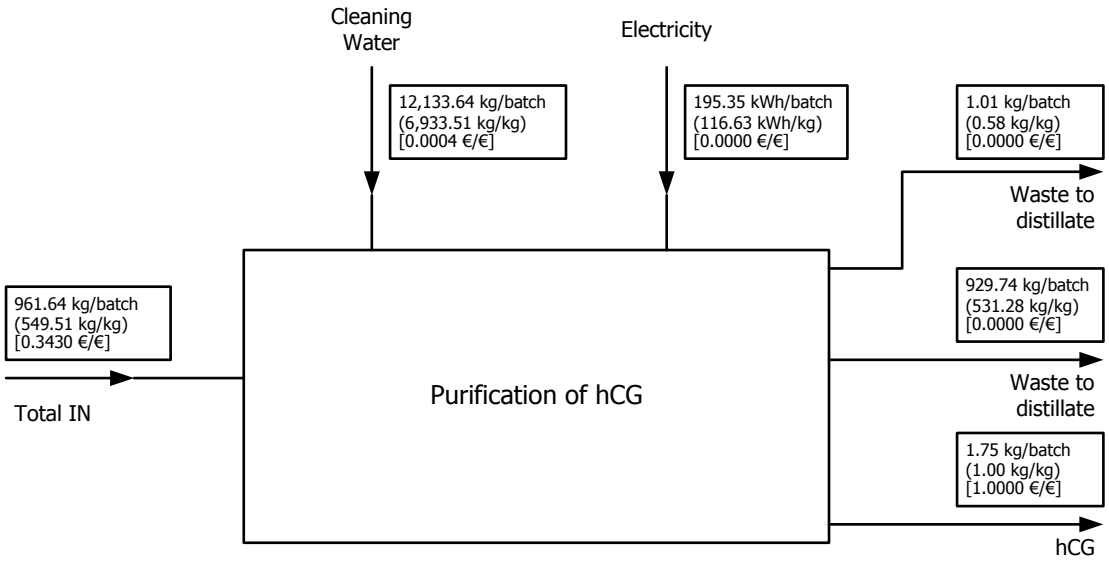

Figure 4.9 Overall yields of process with SMB-GF

4.6.2 Process with SA-SMB-GF

Table 4.15 Yields of process with SA-SMB-GF

Process Streams					
Name	Referance Stream	kg/batch		kg/kg dry product	
		IN	OUT	IN	OUT
Input stream	101	98.26		52.55	
Sodium Chloride	123	1.10		0.59	
Calcium Chloride	104	0.50		0.27	
Sodium bi-phosphate- dibasic	121	0.29		0.16	
Sodium bi-phosphate- monobasic	122	0.10		0.05	
Ammonium acetate	129	1.99		1.06	
Brij 35® surfactant	143	0.10		0.05	
Ethanol	124, 130, 135	218.96		117.09	
Water	119, 141	135.97		72.71	
Product stream	138		11.78		6.30
Wastewater to distillate	140		444.53		237.72
Waste	111		1.01		0.54
Total		457.27	457.32		

Table 4.16 Utilities of process with SMB-GF

Utilities					
Name	Referance Stream	kg/batch	Wh/batch	kg/kg product	Wh/kg product
Cleaning water		12,134		6,489	
Electricity			54.2		28.9

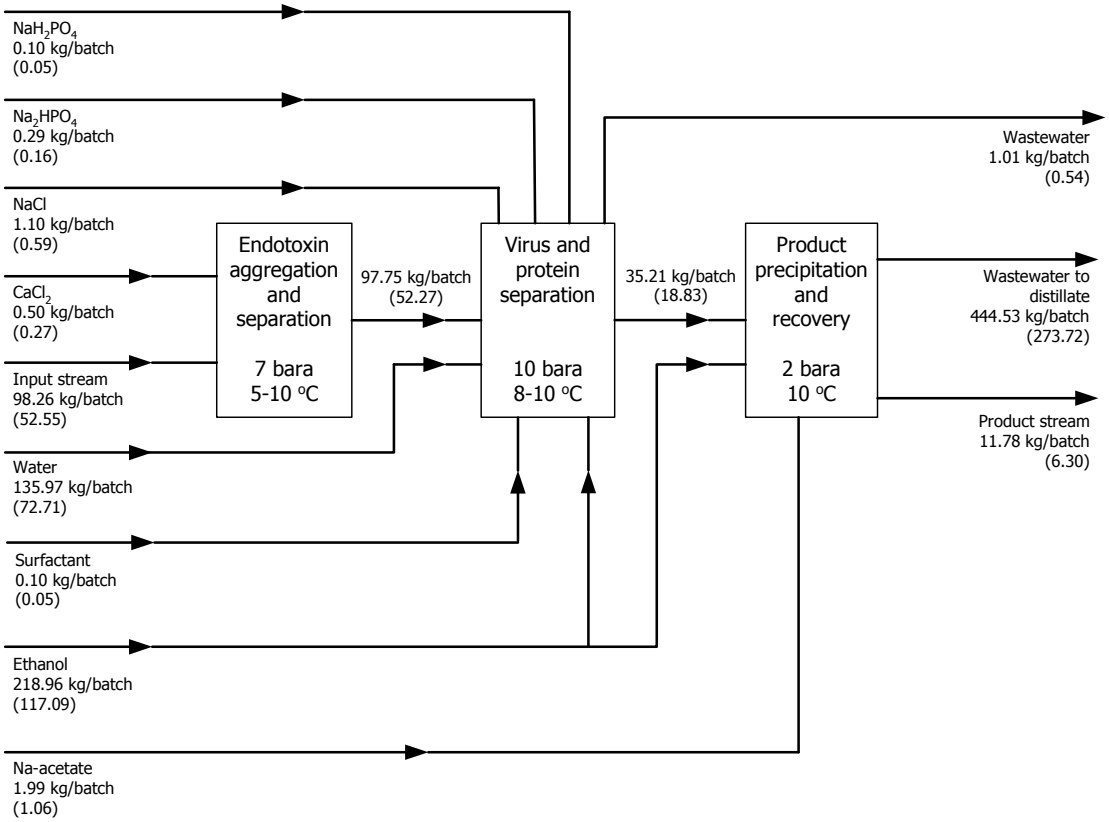

Figure 4.10 Process yields diagram of process with SA-SMB-GF

Note: The number in the brackets is in kg/kg dry product

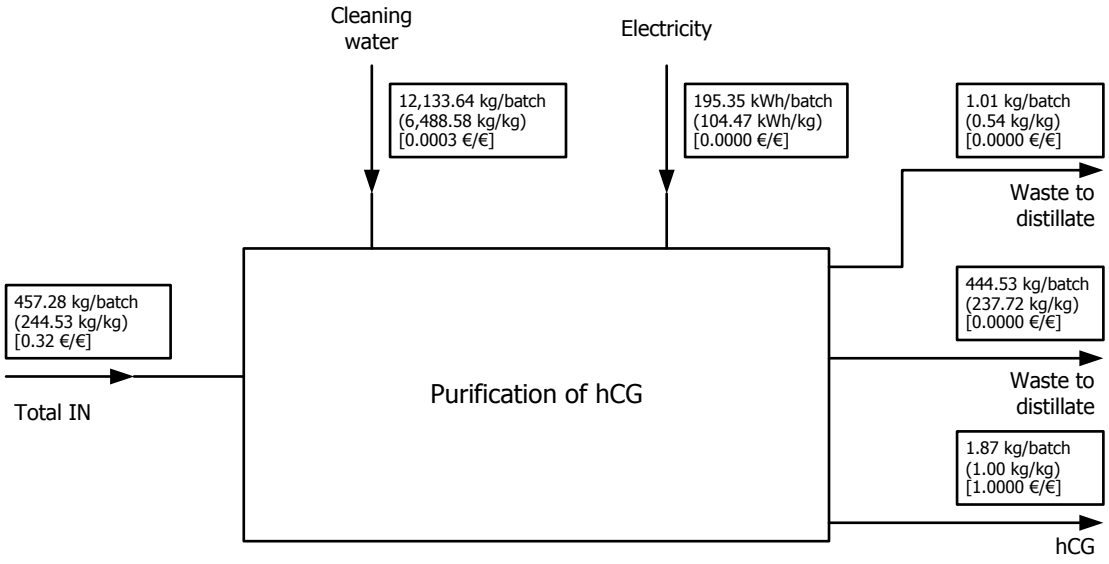

Figure 4.11 Overall yields of process with SA-SMB-GF

5 Process Control

5.1 Temperature control

The stream containing hCG from the storage vessel is at about –20 °C. It will be heated up to approximately 5 °C before entering the process. If the temperature of the stream entering the process is less than 5 °C, the valve of the hot water stream will be more open. As a result, the temperature of the input stream will be increased. The valve will be closed more in case the temperature of the input stream is higher than 5 °C. (Figure 5.1).

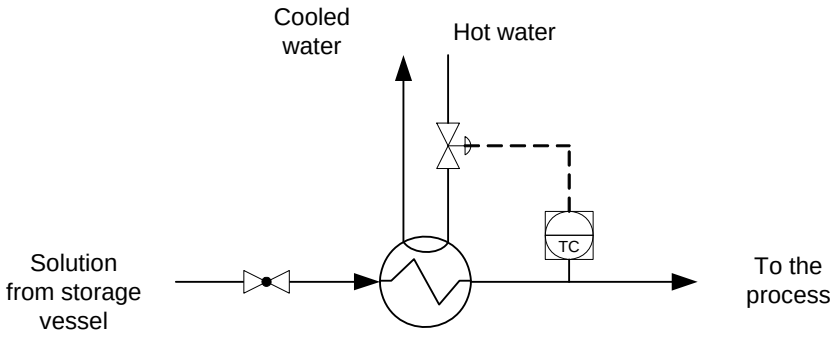

Figure 5.1 Temperature control of input stream

5.2 Flow control

Flow control is necessary for many streams of the processes:

- All streams entering the cation exchange column in the base case and to SMB units in the two other cases are supplied at accurate flow rates. Therefore flow controllers are needed.
- Streams entering the filtration units also require flow controllers, as they have to be accurately regulated.

Figure 5.2 shows a flow control diagram.

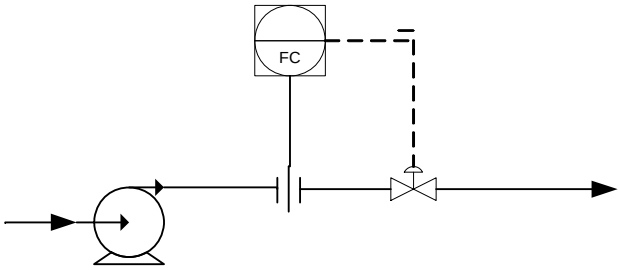

Figure 5.2 Flow control diagram for process with centrifugal pump

Some chemicals enter the processes in a solid form. They have to be transferred into the units when these units are already charged with liquid. Before entering the processes, those chemicals are weighted up to the amount needed corresponding to the amount of input stream.

5.3 Quality control

Quality control is really necessary in this process. For this issue, three types of detectors are used to monitor and control the progress of purification procedures: pH, conductivity and UV absorption detectors.

UV absorption, pH and conductivity detectors should be installed on the outlet stream of the cation exchange unit of the base case process. UV detector should be installed on the outlet stream of the SMB unit of the other processes.

Those detectors mentioned above cannot test the chemical composition of the stream but only provide qualitative values that can be used in operation. To know how effectively the processes and units perform, samples at certain points in the processes must be periodically taken and analysed to give exact results. For a good manufacturing practice (GMP), these activities must be clarified as guidance for operation.

5.4 Interlock and alarm

Alarm and interlock are needed for some units in the processes. All vessels are equipped with alarms and interlocks. An alarm of a vessel will sound when the liquid in that vessel exceeds a certain level. In this situation, it is assumed that the operator knows what corrective action to take, for instance to switch off the previous unit or pump. Any pump that transfer a liquid out from a vessel needs an interlock to switch off in case the vessel is empty. The static pressure on the liquid surface in the vessel can be used to actuate the interlock. Interlock system is also used for agitators. The system may prevent the agitators from starting when liquid is charging into the vessels or may turn off the agitators when they are running at a too high speed.

6 Mass Balances

6.1 Practical aspect

The input stream is heated up from –20 °C to 5 °C in heat exchanger (E101). No other heat transfer takes place in the overall process. All processes include only separator, no reactor or other heat transfer device. Therefore, heat balance is shown only in heat exchanger (E101). It is considered for other equipments that if the mass balance is correct, heat balance must be correct. Temperature of the equipment site is kept constant at 10 °C. Most of the streams in all processes contain complex biological components, in example proteins, virus, and endotoxin. Enthalphy or specific heat of these components is unavailable. For calculation of heat content of input stream, these components are neglected.

The mass balance is shown for both processes containing SMB and SA-SMB in the following sections. The mass balance is done firstly for every unit and then an overall component mass balance is done for all pure components. This provides the difference between overall mass of IN and OUT streams. Some minor imbalances have occurred in this process. The highest imbalance is 0.1%. Since most calculations are done in Microsoft Excel and MathCad, this inequality in mass balance might be due to round off error.

6.2 Mass and heat balance of process with SMB

Table 6.1 Equipment by equipment mass balance for process with SMB-GF

IN							IN			
Plant		EQUIPMENT			EQUIPM.		EQUIPMENT		Plant	
Mass kg/batch	Heat KJ/batch	Mass kg/batch	Heat KJ/batch	Stream Nr.	IDENTIF.	Stream Nr.	Mass kg/batch	Heat KJ/batch	Mass kg/batch	Heat kJbatch
		98,26		<102>	**E101**	<103>	98,28			
	8563		8563					95243		
		98,26	95242		Total		98,28	95243		
				<101>	**P101**	<102>				
		98,26		<103>	**V101**	<105>	98,76			
		0,50		<104>						
		98,76			Total		98,76			
		98,76		<106>	**S101**	<108>	92,76			
						<111>	1,01			
						<112>	4,99			
		98,76			Total		98,76			
		98,76		<105>	**P104**	<106>	98,76			
		6,00		<107>	**V105**	<109>	6,00			
		6,00			Total		6,00			
		6,00		<109>	**P103**	<110>	6,00			
		92,76		<108>	**V102**	<117>	97,75			
		4,99		<112>						
		97,75			Total		97,75			
		171,22		<120>	**M101**	<126>	306,18			

IN						IN			
Plant		EQUIPMENT			EQUIPM.		EQUIPMENT		Plant	
Mass kg/batch	Heat KJ/batch	Mass kg/batch	Heat KJ/batch	Stream Nr.	IDENTIF.	Stream Nr.	Mass kg/batch	Heat KJ/batch	Mass kg/batch	Heat kJbatch
		0,52		<121>						
		0,18		<122>						
		1,93		<123>						
		132,29		<125>						
		306,14			Total		306,18			
		171,22		<119>	**P105**	<120>	171,22			
		132,29		<124>	**P106**	<125>	132,29			
		97,75		<118>	**S102**	<114>	121,28			
		306,18		<127>		<115>	135,31			
						<128>	147,34			
		403,93			Total		403,93			
		97,75		<117>	**P107**	<118>	97,75			
		306,18		<126>	**P108**	<127>	306,18			
		147,34		<128>	**V103**	<132>	577,98			
		421,16		<131>						
		9,47		<129>						
		577,97			Total		577,98			
				<130>	**P111**	<131>				
		577,98		<133>	**S103**	<134>	545,76			
		126,07		<136>		<137>	127,40			
						<138>	30,89			
		704,05			Total		704,05			
		577,98		<132>	**P112**	<133>	577,98			
		126,07		<135>	**P113**	<136>	126,07			
		121,28		<114>	**V104**	<139>	929,74			
		135,31		<115>						
		545,76		<134>						
		127,40		<137>						
		929,75			Total		929,74			
		929,74		<139>	**P114**	<140>	929,74			

Table 6.2 Overall components mass balance for process with SMB-GF

Components	**Total IN** kg/batch	**Total OUT** kg/batch	**IN-OUT** kg/batch
Streams	<101>, <104>,<119>, <121>, <122>, <123>, <124>, <129>, <130>, <135>,	<111>, 137>, <140>	
hCG	2,00	2,00	
10 kDa protein	1,80	1,80	
22 kDa protein	0,60	0,60	
69 kDa protein	1,50	1,50	
150 kDa protein	1,80	1,80	
NS proteins	0,30	0,30	
Virus (particles)	3,00E+12	3,01E+12	-5,00E+09

Components	**Total IN** kg/batch	**Total OUT** kg/batch	**IN-OUT** kg/batch
Streams	<101>, <104>,<119>, <121>, <122>, <123>, <124>, <129>, <130>, <135>,	<111>, 137>, <140>	
Endotoxin (EU)	1,00E+11	1,00E+11	-2,00E+03
NaCl	2,05	2,05	
$CaCl_2$	0,50	0,50	
Na_2HPO_4	0,52	0,52	
NaH_2PO_4	0,18	0,18	
NH_4-acetate	9,47	9,47	
Ethanol	718,83	718,83	
Water	222,09	222,09	
Total	961,65	961,65	

6.3 Mass and heat balance process with SA-SMB-GF

Table 6.3 Equipment by equipment mass balance for process with SA-SMB-GF

IN							**IN**			
Plant		**EQUIPMENT**			**EQUIPM.**		**EQUIPMENT**		**Plant**	
Mass kg/batch	**Heat** kJ/batch	**Mass** kg/batch	**Heat** kJ/batch	**Stream** Nr.	**IDENTIF.**	**Stream** Nr.	**Mass** kg/batch	**Heat** kJ/batch	**Mass** kg/batch	**Heat** kJ/batch
		98,26	86679	<102>	**E101**	<103>	98,28	95243		
	7843,5		8563							
		98,26	95242		Total		98,28	95243		
				<101>	**P101**	<102>				
		98,26		<103>	**V101**	<105>	98,76			
		0,50		<104>						
		98,76			Total		98,76			
		98,76		<106>	**S101**	<108>	92,76			
						<111>	1,01			
						<112>	4,99			
		98,76			Total		98,76			
		98,76		<105>	**P104**	<106>	98,76			
		6,00		<107>	**V105**	<109>	6,00			
		6,00			Total		6,00			
		6,00		<109>	**P103**	<110>	6,00			
		92,76		<108>	**V102**	<117>	97,75			
		4,99		<112>						
		97,75			Total		97,75			
		96,17		<120>	**M101**	<126>	171,97			
		0,29		<121>						
		0,10		<122>						
		1,10		<123>						
		74,31		<125>						
		171,97			Total		171,97			

IN						IN			
Plant		EQUIPMENT			EQUIPM.		EQUIPMENT		Plant	
Mass	Heat	Mass	Heat	Stream	IDENTIF.	Stream	Mass	Heat	Mass	Heat
kg/batch	kJ/batch	kg/batch	kJ/batch	Nr.		Nr.	kg/batch	kJ/batch	kg/batch	kJ/batch
		96,17		<119>	**P105**	<120>	96,17			
		74,31		<124>	**P106**	<125>	74,31			
		39,80		<142>	**M102**	<144>	39,90			
		0,10		<143>						
		39,90			Total		39,90			
		39,80		<141>	**P102**	<142>	39,80			
		97,75		<118>	**S102**	<114>	274,42			
		171,97		<127>		<128>	35,21			
		39,90		<145>						
		309,62			Total		309,63			
		97,75		<117>	**P107**	<118>	97,75			
		39,90		<144>	**P109**	<145>	39,90			
		171,97		<126>	**P108**	<127>	171,97			
		35,21		<128>	**V103**	<132>	149,80			
		112,60		<131>						
		1,99		<129>						
		149,80			Total		149,80			
		112,60		<130>	**P111**	<131>	112,60			
		149,80		<133>	**S103**	<134>	137,78			
		32,05		<136>		<137>	32,33			
						<138>	11,74			
		181,85			Total		181,85			
		149,80		<132>	**P112**	<133>	149,80			
		32,05		<135>	**P113**	<136>	32,05			
		274,42		<114>	**V104**	<139>	444,53			
		137,78		<134>						
		32,33		<137>						
		444,53			Total		444,53			
		444,53		<139>	**P114**	<140>	444,53			

Table 6.4 Overall components mass balance for process with SA-SMB-GF

Components	**Total IN** kg/batch	**Total OUT** kg/batch	**IN-OUT** kg/batch
Streams	<101>, <104>, <119>, <121>, <122>, <123>, <124>, <129>, <130>, <135>, <141>, <143>	<111>, 137>, <140>	
hCG	2,00	2,00	
10 kDa protein	1,80	1,80	
22 kDa protein	0,60	0,60	
69 kDa protein	1,50	1,50	
150 kDa protein	1,80	1,80	
NS proteins	0,30	0,30	
Virus (particles)	3,00E+12	3,00E+12	0,00E+00

Components	**Total IN** kg/batch	**Total OUT** kg/batch	**IN-OUT** kg/batch
Streams	<101>, <104>, <119>, <121>, <122>, <123>, <124>, <129>, <130>, <135>, <141>, <143>	<111>, 137>, <140>	
Endotoxin (EU)	1,00E+11	1,00E+11	-1,80E+06
NaCl	1,19	1,19	
$CaCl_2$	0,50	0,50	
Na_2HPO_4	0,29	0,29	
NaH_2PO_4	0,10	0,10	
NH_4-acetate	1,99	1,99	
Surfactant	0,10348	0,10348	
Ethanol	258,26	258,26	
Water	186,84	186,84	
Total	457,28	457,28	

7 Process and Equipment Design

7.1 Integration by process simulation

Several computer tools were used for the design of the equipment. The pumps and vessels were designed with MS Excel. All filtration units were designed with Mathcad, as well as the heat exchangers and the SMB unit in the case where no surfactants are used. The SMB unit where surfactants were used was designed in Matlab. An overview of the tools used is given in Table 7.1.

Table 7.1 Computer tools used for designing equipment

Equipment	Computer tool
Pumps	Excel
Vessels	Excel
Filtration units	Mathcad
Heat exchangers	Mathcad
SMB unit without surfactants	Mathcad
SMB unit with surfactants	Matlab
Ion Exchange column	Mathcad

7.2 Equipment selection and design

For the process with SMB-GF and SA-SMB-GF, all the equipment is designed in detail. Normally, the base case is a real case so the equipment does not need to be designed. Because of time constraints, the team decided not to design all the equipment in the base case in full detail. The ion exchange column of the base case is fully designed, because it is needed to compare the three processes. Furthermore, the dimensions of the vessels and the flowrate of the pumps are calculated in order to obtain the costs for this equipment.

7.2.1 Pumps

For every pump the type is selected based on the flow rate, with the help of Table 10.17 of Coulson and Richardson's Chemical Engineering. The pumps are all designed as is described in Appendix 16. The construction materials are mild steel for the pump house and high tensile steel for the pump rotor and the shaft. These materials are commonly used for pumps. In Table 7.2 and Table 7.3 the most important design parameters of the pumps are summarised for the processes with SMB-GF and SA-SMB-GF. Table 7.4 gives the flowrate of the pumps used in the base case.

Table 7.2 Design parameters of pumps for process with SMB-GF

Pump	Type	Suction pressure (bara)	Discharge pressure (bara)	Flowrate (l/s)	Power (W)
P-101	Centrifugal	0.78	4.93	0.3300	691.0
P-103	Diaphragm	1.70	10.20	0.0280	118.0
P-104	Diaphragm	0.60	13.20	0.0230	146.0

Pump	Type	Suction pressure (bara)	Discharge pressure (bara)	Flowrate (l/s)	Power (W)
P-105	Centrifugal	0.86	1.30	0.5700	130.0
P-106	Centrifugal	0.52	1.75	0.5500	339.0
P-107	Diaphragm	0.73	15.30	0.0003	8.4
P-108	Diaphragm	0.61	11.80	0.0039	22.0
P-111	Centrifugal	0.79	1.44	0.8800	284.0
P-112	Centrifugal	0.94	2.31	1.1000	782.0
P-113	Centrifugal	0.57	2.73	0.5500	268.0
P-114	Centrifugal	0.67	2.52	0.9100	836.0

Table 7.3 Design parameters of pumps for process with SA-SMB-GF

Pump	Type	Suction pressure (bara)	Discharge pressure (bara)	Flowrate (l/s)	Power (W)
P-101	Centrifugal	0.78	4.93	0.3300	691.0
P-102	Centrifugal	0.58	2.70	0.1300	138.0
P-103	Diaphragm	1.70	10.20	0.0280	118.0
P-104	Diaphragm	0.60	13.20	0.0230	146.0
P-105	Centrifugal	1.00	5.20	0.3200	670.0
P-106	Centrifugal	0.30	6.00	0.3100	886.0
P-107	Diaphragm	0.73	15.30	0.0003	8.4
P-108	Diaphragm	0.91	17.30	0.0022	18.0
P-109	Diaphragm	1.13	12.55	0.0005	2.6
P-111	Centrifugal	0.34	1.98	0.0024	192.0
P-112	Centrifugal	1.03	2.18	0.0029	166.0
P-113	Centrifugal	0.57	2.77	0.0013	147.0
P-114	Centrifugal	0.92	2.23	0.0051	327.0

Table 7.4 Pump capacities for base case

Pump	Flowrate (l/s)
P-101	0.019
P-102	0.340
P-103	0.160
P-104	0.037
P-105	0.200
P-106	0.037
P-107	0.130
P-108	0.037
P-109	0.530
P-110	0.037
P-111	0.400
P-112	4.800
P-113	2.800
P-114	1.300
P-115	1.600

7.2.2 Vessels

The vessels are designed as it is explained in Appendix 17. The material of construction is stainless steel for both tank and stirrer. This is standard for pharmaceutical processes. Since operating pressure for all vessels is nearly 1 bar and processes do not involve any corrosive chemicals, stainless steel is sufficient for this process. The most important design parameters are summarised in Table 7.5, Table 7.6 and Table 7.7.

Table 7.5 Design parameters of vessels for process with SMB-GF

Vessel	Volume (m^3)	Diameter (m)	Height (m)
M-101	0.4400	0.57	1.71
V-101	0.1300	0.38	1.14
V-102	0.1300	0.38	1.14
V-103	0.8800	0.72	2.16
V-104	1.4000	0.84	2.52
V-105	0.0065	0.14	0.42

Table 7.6 Design parameters of vessels for process with SA-SMB-GF

Vessel	Volume (m^3)	Diameter (m)	Height (m)
M-101	0.2400	0.47	1.41
M-102	0.0500	0.28	0.84
V-101	0.1300	0.38	1.14
V-102	0.1300	0.38	1.14
V-103	0.2100	0.45	1.35
V-104	0.7500	0.68	2.04
V-105	0.0065	0.14	0.42

Table 7.7 Dimensions of vessels for base case

Vessel	Volume (m^3)	Diameter (m)	Height (m)
M-101	0.10	0.35	1.05
M-102	0.10	0.35	1.05
M-103	0.05	0.28	0.84
M-104	0.20	0.44	1.32
V-101	3.00	1.08	3.25
V-102	2.10	0.96	2.88
V-103	3.50	1.14	3.42

7.2.3 Filtration units

7.2.3.1 Ultra filtration

The ultra filtration unit for the process with SMB-GF will be exactly the same as the one for the process with SA-SMB-GF. The membrane for the ultra filtration unit is selected in terms of molecular weight cut-off (MWCO) and pore size. It is known that a 100 kDa MWCO membrane will let proteins of the size of hCG (38 kDa) through with very low solute retention (www.millipore.com; www.pall.com). In the process, low hCG retention is desired. A 100 kDa MWCO membrane has an average pore diameter of 10 nm, which will lead to a

high rejection for biomolecular components with a diameter of at least 30 nm. (www.pall.com/laboratory/genomics). The endotoxin aggregates will have an average diameter of 100 nm (Li and Luo, 1998). Therefore, the MWCO of the membrane will be 100 kDa.

The protein concentration in the feed of the ultra filtration will be quite high (8%), so there is a risk of foaming in the membrane. Therefore, a low protein binding membrane is preferable. In this case, modified polyethersulfone is the construction material for filter media (www.labfilters.com). Table 7.8 represents the membrane construction materials for different sections of the ultra filtration unit.

Table 7.8 Ultra filtration membrane construction materials

Section	Material
Filter Media	Modified polyethersulfone
Screen	Polyester
Backing	Polyolefin
Encapsulant	Polyurethane
Gasket	Silicone
Centramate systems	316L stainless steel
Centramate PE systems	Ultra-high molecular weight polyethylene
Tie Rods	Stainless steel
Nuts	Bronze

The ultra filtration unit is designed as is explained in Appendix 18. The most important design parameters are given in Table 7.9.

Table 7.9 Design parameters of ultra filtration unit

MWCO (kDa)	Average pore diameter (nm)	Membrane area (m^2)	Pressure difference (bar)
100	10	4	4

7.2.3.2 Micro filtration

For the micro filtration unit, almost the same materials are used. The only difference is the use of hydrophilic polyethersulfone as the filter media, instead of modified polyethersulfone (www.labfilters.com). The micro filtration units are designed as is explained in Appendix 19. The most important design parameters are given in Table 7.10 and Table 7.11.

Table 7.10 Design parameters of micro filtration unit for process with SMB-GF

Average pore diameter (μm)	Membrane area (m^2)	Pressure difference (bar)
0.8	0.4	1

Table 7.11 Design parameters of micro filtration unit for process with SA-SMB-GF

Average pore diameter (μm)	Membrane area (m^2)	Pressure difference (bar)
0.8	0.4	1

7.2.4 Heat exchanger

The heat exchanger for the process with SMB-GF is the same as the heat exchanger for the process with SA-SMB-GF, because the input stream is heated up and this stream is the same for both processes. The selection and calculation of the design can be found in Appendix 20. Stainless steel is again the material used. The most important design parameters are listed in Table 7.12.

Table 7.12 Design parameters of heat exchanger

Capacity (kW)	Heat exchange area (m^2)
26.2	1.8

7.2.5 SMB-GF

The selection of some design parameters has already been explained in paragraph 4.1.1.3. As is mentioned there, the SMB unit used in this process will have 4 sections, and it will be an open system. The model with which the SMB unit is designed is shown in Appendix 21. The most important design parameters are listed in Table 7.13.

Table 7.13 Design parameters of SMB-GF

Flowrate feed (m^3/s)	Operating time (h)	Number of columns	Diameter columns (m)	Length columns (m)
$1.2 \cdot 10^{-6}$	24	12 (4:2:4:2)	0.27	0.25

7.2.6 SA-SMB-GF

As is explained in paragraph 4.1.1.3., the SMB unit used in this process will have 5 sections, and it will be an open system. The design of this SMB unit is performed by Danielle Horneman in Matlab (see Appendix 22). The most important design parameters are listed in Table 7.14.

Table 7.14 Design parameters of SA-SMB-GF

Flowrate feed (m^3/s)	Operating time (h)	Number of columns	Diameter columns (m)	Length columns (m)
$1.2 \cdot 10^{-6}$	24	12 (2:2:2:2:2)	0.2	0.2

7.2.7 Ion Exchange column

The Ion Exchange column is designed as it is explained in Appendix 23. The most important design parameters are shown in Table 7.15.

Table 7.15 Design parameters of Ion Exchange column

Flowrate feed (m^3/s)	Operating time (h)	Amount of columns	Diameter columns (m)	Length columns (m)
$1.9 \cdot 10^{-5}$	24	1	0.41	0.15

7.3 Equipment data sheets

All Equipment Data Summary Sheets and Equipment Data Specification Sheets are given in Appendices 24 and 25.

8 Wastes

It was agreed with the client that no wastewater treatment unit would be designed in the process. Either the existing wastewater treatment facility at Diosynth will be used or the waste will be treated commercially. However, description of the waste must be given.

The total direct waste in these processes includes the waste streams from the process stream summary as well as the wastewater for cleaning. Each designed process produce two wastewater streams. The processes do not generate any gaseous waste. The mass and composition of these streams are given in Table 8.1 and Table 8.2.

Table 8.1 Composition of wastewater produced by process with SMB-GF

Components	Stream <111>		Stream <140>	
	Amount (kg/batch)	wt%	Amount (kg/batch)	wt%
hCG	0.12	0.01	0.03	2.97
Proteins	3.82	0.41	0.52	51.49
Virus (particles)	2.99×10^{12}		1.5×10^{10}	
Endotoxin (EU)	10^9		9.9×10^{10}	
Sodium chloride	2.05	0.22	0.00	0.04
Calcium chloride	0.50	0.05	0.00	0.25
Sodium bi-phosphate (monobasic)	0.18	0.02		
Sodium bi-phosphate (dibasic)	0.52	0.06		
Ammonium acetate	9.47	1.02		
Ethanol	691.29	74.35	0.20	19.80
Water	221.79	23.86	0.25	24.75
Total	929.74	100.00	1.01	100.00

Table 8.2 Composition of wastewater produced by process with SA-SMB-GF

Components	Stream <111>		Stream <140>	
	Amount (kg/batch)	wt%	Amount (kg/batch)	wt%
hCG	0.10	0.02	0.03	2.97
Proteins	2.56	0.58	0.52	51.49
Virus (particles)	2.98×10^{12}		1.5×10^{10}	
Endotoxin (EU)	10^9		9.9×10^{10}	
Sodium chloride	1.19	0.27	0.00	0.04
Calcium chloride	0.50	0.11	0.00	0.25
Sodium bi-phosphate (monobasic)	0.10	0.02		
Sodium bi-phosphate (dibasic)	0.29	0.07		
Ammonium acetate	1.99	0.45		
Surfactant	0.10	0.02		
Ethanol	251.12	56.49	0.20	19.80
Water	186.57	41.97	0.25	24.75
Total	444.53	100.00	1.01	100.00

It is clear from the above tables that stream <140> is very rich in recoverable ethanol in both cases. It was agreed with the client that the distillation column would not be designed in this process, since it is already exist in the company. Therefore, stream <140> is sent to distillation plant for ethanol recovery. The remaining waste stream is very small (stream <111>), but composed of biologically hazardous components. Although the waste will be sent to a commercial waste treatment plant, for extra safety, virus and endotoxin must be deactivated. Deactivation could be done chemically or physically. The best way of treating this waste stream is autoclaving, since the stream is quite small (only 1.01 kg/batch).

In addition to the above waste, a lot of wastewater is generated by cleaning of the equipment after each campaign. This wastewater will be treated either by the existing facility of Diosynth or by a commercial wastewater treatment company. This wastewater might contain some virus and endotoxin, thus it should be autoclaved as well. The action taken for all waste streams are represented in Table 8.3.

Table 8.3 Action taken for waste streams

Waste stream	Action
Stream <111>	Autoclaving and sent to treatment
Stream <140>	Sent to distillation plant
Cleaning water	Autoclaving and sent for treatment

9 Process safety

9.1 Fire and Explosion Index

To assess the potential hazard of the process, the Dow Fire and Explosion Index is first made as shown in Table 9.1. Notes on the decisions taken and the factors used are explained as follows:

Unit: consider the total plant, no separate areas, including the main storages

Material factor: for Ethyl alcohol MF = 16

Note: Ethyl alcohol is chosen to determine material factor, as it is the dominantly dangerous material in the plant.

General process hazards:

A. Exothermic chemical reaction: not applicable
B. Endothermic processes: not applicable
C. Material and handling and transfer: since N_f = 3, a penalty of 0.85 is applied
D. Enclosed or indoor process units: not applicable since the process temperature (10 ^oC) is less than the flash point of ethanol (12 ^oC)
E. Access of emergency equipment: adequate access will be provided, factor = 0.0
F. Drainage and spill control: adequate drainage will be provided,factor = 0.0

Special process hazards:

A. Toxic materials: not applicable since N_h is zero for ethanol
B. Sub-atmospheric pressure: not applicable since the absolute pressure is more than 500 mmHg (760 mmHg).
C. Operation in or near flammable range: not applicable
D. Dust explosion: not applicable
E. Pressure: using equation from Dow's Fire and Explosion Index Hazard Classification Guidebelow, the penalty factor is Y = 0.16

$$Y = 0.16109 + \frac{1.61503 \cdot X}{1000} - 1.42879 \cdot \left(\frac{X}{1000}\right)^2 + 0.5172 \cdot \left(\frac{X}{1000}\right)^3$$

where: Y is penalty factor
X is pressure in psig
In this case X = 0 psig since atmospheric pressure is used

F. Low temperature: not applicable as all materials are stainless steels
G. Quantity of flammable material:
The largest quantity of ethanol in the process will be in the liquid in the waste storage vessel. The amount is estimated to be about 900 litres.
Heat of combustion: Hc = 26.8 MJ/kg
Potential energy release = 900 * 0.8 * 26.8 = 19296 MJ = 0.018 * 10^9 Btu
Using equation from Dow's Fire and Explosion Index Hazard Classification Guide, the penalty factor is then Y = 0.23

$$\text{Log } Y = -0.403115 + 0.378703*\log X - 0.046402*(\log X)^2 - 0.015379*(\log X)^3$$

Where: Y is penalty factor
X is potential energy release in 10^9 Btu

H. Corrosion and erosion:
External corrosion is possible due to the presence of some corrosive ions: NH_4^+, Cl^-, OH^- . Thus factor = 0.1 is taken.
I. Leakage-joints and packing:
Use minimum factor 0.1
J. Use of fired heaters: not applicable
K. Hot oil heat exchange system: not applicable
L. Rotating equipment: not applicable

The index works out at 39: classified as "Light". Ethanol is considered as a dangerously flammable material but the process is running at temperatures lower than the flash point of ethanol. The danger of material handling and transfer, and internal explosion in the storage tank are the process hazards.

9.2 Hazard and operability study (HAZOP)

A limited Hazard and Operability study is carried out for main the equipments such as: endotoxin aggregation vessel and SMB.

9.2.1 HAZOP for endotoxin aggregation vessel

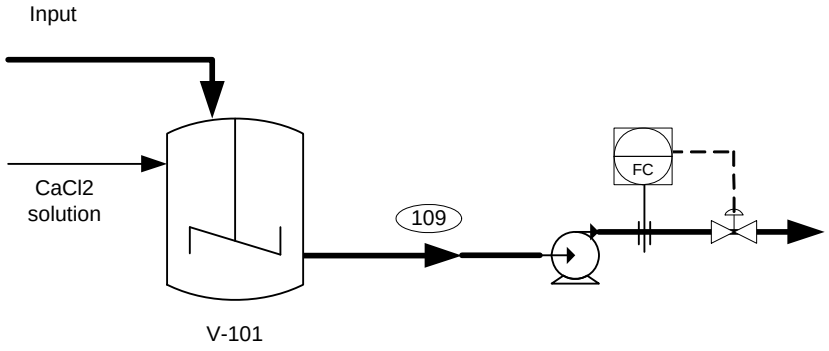

Figure 9.1 Endotoxin aggregation vessel section

V-101: Endotoxin aggregation vessel; to aggregate endotoxin with Ca^{2+}
Line <109>: aggregation mixture line; to transfer aggregation mixture to filter.

Table 9.1 Dow's fire and explosion index analysis

FIRE AND EXPLOSION INDEX			LOCATION The Netherlands		DATE
PLANT	PROCESS UNIT	EVALUATED BY Pham Minh Tuan	REVIEWED BY Tangir Ahamed		
MATERIALS AND PROCESS					
MATERIALS IN PROCESS UNIT					
STATE OF OPERATION [ ] START UP [ ] SHUT DOWN [ ] NORMAL OPERATION			Basic material for MF: ETHANOL		
MATERIAL FACTOR					16
1. GENARAL PROCESS HAZARD			Penalty factor range	Penalty factor used	
BASE FACTOR			1	1	
A. EXOTHERMIC CHEMICAL REACTIONS			0.3 -1.25		
B. ENDOTHERMIC PROCESSES			0.2-0.4		
C. MATERIAL HANDLING AND TRANSFER			0.25-1.05	0.85	
D. ENCLOSED OR INDOOR PROCESS UNITS			0.25-0.90		
E. ACCESS			0.20-0.35		
F. DRAINAGE AND SPILL CONTROL			0.25-0.5		
GENERAL PROCESS HAZARDS FACTOR (F_1)				1.85	
2. SPECIAL PROCESS HAZARDS BASE FACTOR			1	1	
A. TOXIC MATERIAL			0.20-0.80		
B. SUB-ATMOSPHERIC PRESSURE			0.50		
C. OPERATION IN OR NEAR FLAMMABLE RANGE [ ] INERTED [ ] NOT INERTED					
1. Tank farms storage flammable liquids			0.50		
2. Process upset or purge failure			0.30		
3. Always in flammable range			0.80		
D. DUST EXPLOSION			0.25-2.00		
E. PRESSURE: Operating pressure 0 psig					
F. LOW TEMPERATURE			0.20-0.30	0.23	
G. QUANTITY OF FLAMMABLE MATERIAL QUANTITY: 900 litres Hc = 26.8 MJ/kg					
1. Liquids, gases and reactive materials in process					
2. Liquids or gases in storage					
3. Combustible solids in storage dust in process					
H. CORROSION AND EROSION				0.1	
I. LEAKAGE-JOINTS AND PACKING					
J. USE OF FIRED HEATERS			0.10-0.75		
K. HOT OIL HEAT EXCHANGE SYSTEM			0.10-1.50		
L. ROTATING EQUIPMENT					
			0.15-1.15		
			0.5		
SPECIAL HAZARD FACTOR (F_2)				1.33	
UNIT HAZARD FACTOR (F_1 x F_2 = F_3)				2.46	
FIRE AND EXPLOSION INDEX (F_3 x MF = F&EI)					39

Table 9.2 HAZOP analysis for endotoxin aggregation vessel

Guide word	Deviation	Cause	Consequences and action
NO	No Flow	Pump/valve failure	Endotoxin aggregates stay in the vessel too long; their size may be changed. Action: replace pump/valve
LESS	Less Flow	Partial failure of pump/valve	As the case of no flow but may be less serious. Action: check the pump and valve periodically to ensure that they work correctly.
MORE	More Flow	Partial failure of pump/valve	Endotoxin aggregates might be damaged during the transfer. Action: as the case of less flow.

9.2.2 HAZOP for SMB-GF

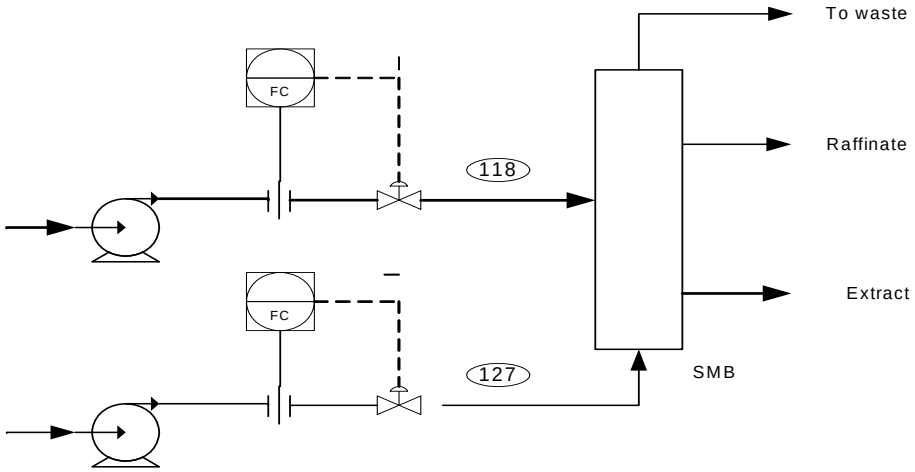

Figure 9.2 SMB section

SMB: Simulated moving bed chromatography; to separate hCG from other proteins and viruses
Line <118>: Feed stream for SMB; to transfer feeding solution to SMB

Table 9.3 HAZOP analysis for SMB feed stream

Guide word	Deviation	Cause	Consequences and action
LESS	Less Flow	Partial failure of pump/vale	Concentration of other components than hCG in the Extract stream will increase. As a consequence, requirements for purity of product or virus clearance may not be met. Action: check the pump/valve periodically to ensure that it works as expected.
MORE	More flow	Partial failure of pump/vale	Purity and recovery might be decreased. Action: as the case of less flow.

Line <127>: Desorbent stream for SMB; to transfer desorbent solution to SMB

Table 9.4 HAZOP analysis for SMB desorbent stream

Guide word	Deviation	Cause	Consequences and action
MORE	More Flow	Valve opens too wide	As the case less flow of feed stream

10 Economic

10.1 Cost estimation

Estimations of investment and manufacturing costs are made using values taken from the Techno Economic course (Asselbergs, 2002). The estimations are as follows.

10.1.1 Estimation of Total Capital Investment (Capex)

Typical breakdown of investment is presented in Table 10.1. Purchased Equipment Costs (PEC), which is calculated, based on the method in Chemical Engineering book by Coulson and Richardson's (2001), is only a fraction of the Fixed Capital Investment. The calculations of PEC are shown in Appendix 26.

Table 10.1 Typical breakdown of investment (Asselbergs, 2002)

<table>
<tr><th colspan="4">Total Capital Investment (Capex)</th></tr>
<tr><td colspan="3">Fixed Capital Investment</td><td>Working Capital</td><td>Start-up Costs</td></tr>
<tr><td colspan="2">Direct Costs</td><td>Indirect Costs</td><td>Working Capital</td><td>Start-up Costs</td></tr>
<tr><td>ISBL Costs (Onsite)</td><td>OSBL Costs (Offsite)</td><td>Indirect Costs</td><td>Working Capital</td><td>Start-up Costs</td></tr>
<tr><td>Puchase & Installation of:
• Process equipment
• Piping & appurtenances
• Instrumentation & controls
• Electrical equipment/materials
• Civil & structural
• Process Buildings</td><td>• Auxiliary buildings
• Yard Improvements
• Service facilities
• Storage/distribution
• Packaging plant
• Land</td><td>• Up-front R&D
• Up-front license
• Engineering
• Construction
• Contractor's fee
• Contingencies</td><td>• Inventories
• Salaries/wages due
• Receivable less payable
• Cash</td><td>• Modifications
• Start-up labour
• Loss in production</td></tr>
<tr><td colspan="3">73-83%</td><td>10-20%</td><td>6-8%</td></tr>
<tr><td colspan="3">100%</td><td>12-28%</td><td>8-10%</td></tr>
<tr><td colspan="2">70-85%</td><td>15-30%</td><td></td><td></td></tr>
</table>

There are several different methods that can be used to calculate the Fixed Capital Investment (FCI). The method that will be used in this design project is the one presented by Peters and Timmerhaus (1991), which is mentioned in the Techno Economic course (Asselbergs, 2002). Typical percentages of Fixed Capital Investment for elements of the costs are given in Table 10.2. First, the purchased process equipment (PEC) is estimated based on the detailed list of equipment for all processes. In the next step, the fixed capital investments (FCI) are calculated with the assumed percentage of PEC. The costs of buildings, yard improvement, service facilities and land are assumed to be the same for all processes and are calculated based on the base case. The working capital is assumed to be the costs of raw material for one month plus the production costs for 2 months. The indirect costs are calculated by following the percentage on the table. Validation costs are assumed to be twice the purchased equipment cost (van Dedem, 2003).

From the table, it is clear that the base case needs the lowest investment, while the process with SMB needs the highest investment. The reason that makes the total investment costs of SMB processes much higher than that of the base case is the costly investment in the SMB, which contributes the major part in the purchased equipment cost. By adding surfactant, the investment is slightly decreased, mainly due to the decrease in size of the SMB.

Table 10.2 Typical percentages of fixed capital investment and results of estimation

	Range (% of FCI)	Assumed percentage (%)	Costs (M€/a)		
			Base case	SMB	SA-SMB
Direct costs (ISBL+OSBL)					
Purchased process equipment (PEC)	15-40	25	0.314	0.723	0.658
Installation of purchased equipment	6-14	8	0.101	0.345	0.307
Instrumentation and controls	2-8	6	0.075	0.259	0.230
Piping	3-20	5	0.063	0.216	0.192
Electrical	2-10	4	0.050	0.173	0.153
Buildings and building services	3-18	5	0.063	0.063	0.063
Yard improvements	2-5	4	0.050	0.050	0.050
Service facilities	8-20	10	0.126	0.126	0.126
Land	1-2	1	0.013	0.013	0.013
Indirect costs					
Engineering	4-21	10	0.126	0.289	0.263
Construction expense	4-16	8	0.101	0.231	0.211
Contractor's fee	2-6	4	0.050	0.116	0.105
Contingency	5-15	10	0.126	0.289	0.263
Fixed Capital Investment (FCI)			**1.257**	**2.892**	**2.634**
Working capital (18% FCI)			4.774	4.768	4.767
Start-up expenses (10% FCI)			0.126	0.289	0.474
Validation cost (2 x PEC)			0.628	1.446	1.317
Total Capital Investment (TCI)			**6.784**	**9.395**	**9.192**

10.1.2 Estimation of Total Manufacturing Costs

Typical breakdown of Total Manufacturing Costs (TMC) is presented in Table 10.3. Common percentages of TMC for elements of the costs are given in Table 10.4.

Table 10.3 Typical breakdown of Total Manufacturing Costs (TMC) (Asselbergs, 2002)

Total Manufacturing Cost (Opex)			
Production Cost (fixed & variable)			General expences
Direct production costs	Fixed charges (direct/indirect cost)	Plant overhead (indirect cost)	General expences (indirect cost)

• Raw materials
• Utilities
• Maintenance & Repairs
• Operating Labour
• Supervision/Clerical
• Laboratory charges
• Operating Supplies/Packaging
• Patents & Royalties
	• Local taxes
	• Insurances
	• Rent (buildings/land)
	• Capital charges:
	  • Interest
	  • Depreciation
	  • Debt repayment
	  • Dividend
		• Purchasing/warehousing
		• Site overhead
		• Medical services
		• Safety & protection
		• Cafetaria & recreation
		• Laboratories
		• Logistic services
		• Administrative services (accounting)
			• Sales/Marketing/Advertising
			• Distribution
			• Quality management
			• Engineering services
			• Research & Development
			• Finance & Administration
			• Personnel & Organisation
			• Management Services

Table 10.4 Typical percentages of Total Manufacturing Costs (TMC) and results of estimation

	Range (%)	**Assumed percentage (%)**	**Costs (M€/a)**		
			Base case	**SMB**	**SA-SMB**
Direct Production costs					
Raw materials	20-60% TMC	75	15.022	15.004	15.001
Utilities	10-30% TMC	Calculated	0.002	0.002	0.002
Maintenance & repair	1-10% FCI	5	0.063	0.149	0.138
Operating supplies	0.5-2% FCI	5	0.063	0.149	0.138
Patents & royalties	0-6% FCI		2.549	1.991	2.065
Operating Labour (OL)	10-20% TMC	10	0.040	0.040	0.040
Supervision/Clerical	10-25% OL	15	0.006	0.006	0.006
Laboratory charges	10-20% OL	15	0.006	0.006	0.006
Fixed Charges					
Depreciation	10% FCI	10	0.126	0.299	0.275
Local taxes	1-4% FCI	4	0.050	0.120	0.110
Insurances	0.5-1% FCI	1	0.013	0.030	0.028
Interest	0-7% TCI	7	0.088	0.209	0.193
Plant overhead					
Plant overhead	5-15% TMC	10	2.003	2.001	2.000
Total Production Costs (TPC)			**20.029**	**20.006**	**20.002**
General expenses (10% TPC)			**2.003**	**2.001**	**2.000**
Total Manufacturing Costs (TMC)			**22.032**	**22.006**	**22.002**

The operating labour is estimated as follows: (5 batches/year) x (4 shifts/batch) x (5 persons/shift) x (8 hours/person) x (50 €/hour). From the table, it can be seen that the three processes have almost the same total manufacturing cost. This is due to the fact that the cost of raw material, the main cost in all three processes (75% of total production cost), is more or less the same.

10.2 Income

Based on the mass balance/stream report, it is known that the production rate are 9.235, 9.34 and 7.715 kg per year for process with SMB, surfactant-aided SMB and base case respectively. Based on equation 1.1, income of the plant with each purification process is calculated and the results are presented in Table 10.5.

$$\text{Income} = \text{Production\_rate} \times \text{hCG\_price} \qquad \text{Equation 10.1}$$

Table 10.5 Income of the plant for each different process

Process	Production rate (kg/a)	HCG price (M€/kg)	Income (M€/a)
Base case	7.715	5	38.58
SMB	9.235		46.18
Surfactant-aided SMB	9.340		46.70

It can be seen from Table 10.5 that the process with surfactant-aided SMB will gain the highest income. The income of SMB process is a little less than that of process with surfactant since the production rate is less. The lowest in production rate of the base case makes this process lest attractive in term of income.

10.3 Economic criteria

10.3.1 Net Cash Flow

Some means of comparing the economic performance of projects is needed since the purpose of investing money in a plant is to earn money. One of the means is by checking the value of the Net Cash Flow (NCF). The flow of cash is the lifeblood of any commercial organization. The cash flows in a manufacturing company can be likened to the material flows in a process plant. The inputs are the cash needed to pay for research and development; plant design and construction; and plant operation. The outputs are goods for sale; and cash returns, are recycled, to the organization from the profits earned (Coulson and Richardsons, 2000). The NCF, which is presented in Table 10.6 is calculated based on equation 10.2 to equation 10.6 with 35% as the tax rate.

$$\text{Gross\_profit} = \text{Income} - \text{TMC} \qquad \text{Equation 10.2}$$

$$\text{Taxable\_profit} = \text{Gross\_profit} - \text{Depreciation} \qquad \text{Equation 10.3}$$

$$\text{Tax} = \text{Tax\_rate} \times \text{Taxable\_profit} \qquad \text{Equation 10.4}$$

$$\text{Net\_Income} = \text{Taxable\_profit} - \text{Tax} \qquad \text{Equation 10.5}$$

$$\text{NCF} = \text{Net\_Income} + \text{Depreciation} \qquad \text{Equation 10.6}$$

Table 10.6: Net Cash Flow calculations

	Costs (M€/a)		
	Base case	**SMB**	**Surfactant-Aided SMB**
Gross profit	16.42	23.88	24.43
Depreciation	0.12	0.30	0.28
Taxable profit	16.30	23.58	24.15
Tax	5.70	8.25	8.45
Net Income	10.60	15.33	15.70
Net Cash Flow	10.72	15.63	15.98

10.3.2 Discounted Cash Flow (DCF)

The Net Cash Flow is shown as its value in the year in which it occurred. The money earned in any year can be put to work as soon as it is available and start to earn a return. So money earned in the early years of the project is more valuable than that earned in later years. The Net Cash Flow in each year is brought to its "present worth" at the start of the project by discounting it at some chosen compound interest rate (Coulson and Richardsons, 2001). The discount factor is usually taken as the interest rate in banks. Therefore, the discount factor may differ depending on the location of the investment. With this method, all debts have been taken into account.

The cumulative sum of the present values is termed the Net Present Value (NPV), which is the expected profit of the whole project. NPV is calculated based on equation 10.7 and 10.8, where i is the discount factor and j is the time in year. The whole calculations are done with Excell and presented in Appendix 26.

$$NPV = NCF \times (1+i)^{-j} \qquad \text{Equation 10.7}$$

$$Cumulative\_NPV = \sum_{j=-2}^{n} PV_j \qquad \text{Equation 10.8}$$

Some assumptions that are needed to calculate NPV are mentioned below:

- Economic plant life is 15 years.
- Straight-line depreciation method has been used for the first ten years of operation.
- Investment is assumed to take place at the end of year –1.
- The beginning of year zero is the start of the manufacturing operation.
- In the first year of operation (year 0), the plant will operate at 70% capacity. From the second year (year 1) until the end of the plant lifetime, the plant will operate at full capacity. In this case, the income in the first year is only 70% of the total income and the manufacturing costs for raw materials and utilities are also 70% of the calculated raw materials and utilities costs.
- Interest rate/discount factor is taken as 7%.
- The salvage value of the process equipment is assumed to be 0% of TCI

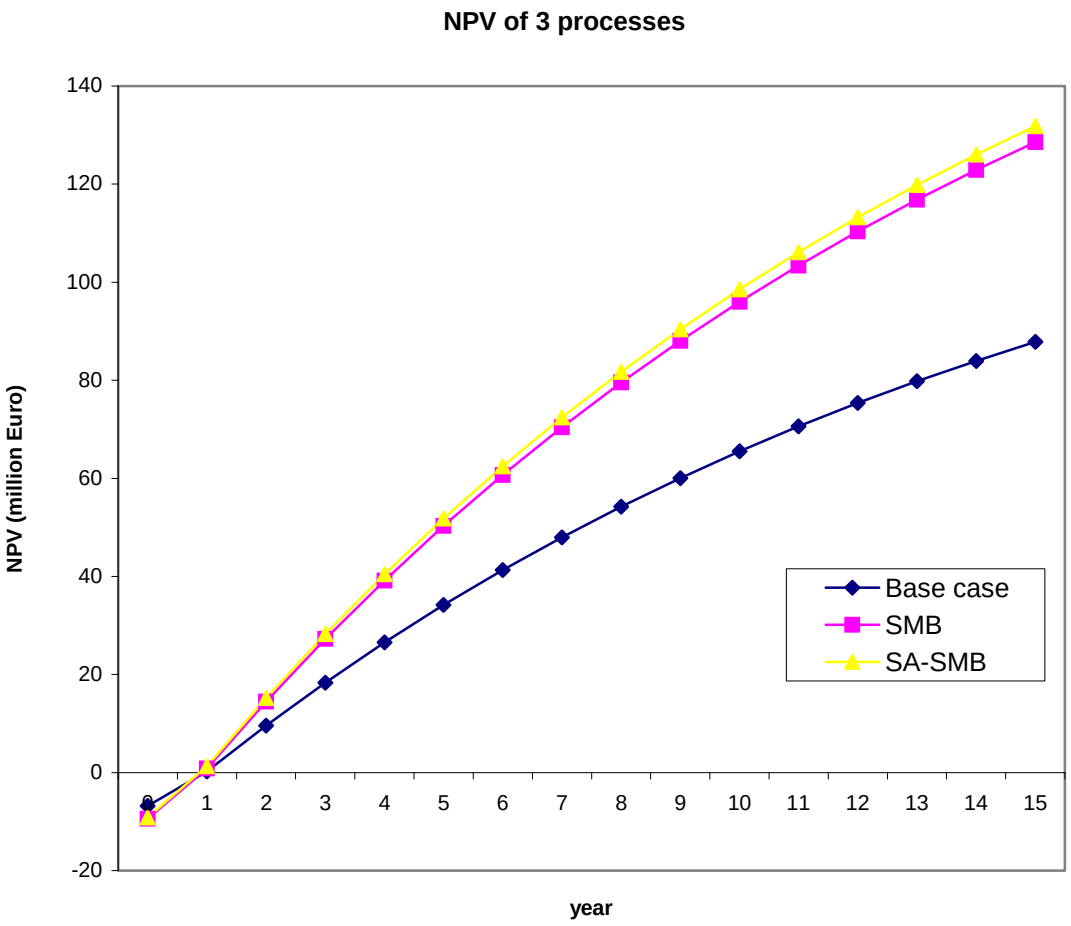

Figure 10.1 Cumulated NPV of 3 processes

10.3.3 Rate of Return (ROR)

Rate of Return (ROR), which is the ratio of annual profit to investment, is a simple index of performance of the money invested. ROR is calculated based on equation 10.9 and the results are presented in Table 10.7 for different purification processes.

$$ROR = \frac{CumulativeNCF}{Life\_of\_project \times TCI} \times 100\% \qquad \text{Equation 10.9}$$

Table 10.7 Rate of return for 3 processes

	Base Case	SMB	Surfactant-Aided SMB
ROR (%)	86	91	96

10.3.4 Discounted cash-flow rate of return (DCFRR)

DCF analysis, used to calculate the present worth of future earnings, is sensitive to the interest rate assumed. By calculating the NPV for various interest rates, it is possible to find an interest rate at which the cumulative NPV at the end of the project is zero. This particular rate is called the Discounted cash-flow rate of return (DCFRR) and is a measure of the maximum rate that the project could pay and still break even by the end of the project life (Coulson ad Richardson's, 2001). Table 10.8 shows DCFRR of 3 processes.

Table 10.8 Discounted cash-flow rate of return for 3 processes

	Base Case	SMB	Surfactant-Aided SMB
DCFRR (%)	131	137	143

10.3.5 Pay Out Time (POT)

Pay out time is the time in year it takes to recover the fund that is invested in the project. Pay out time is a useful criterion for judging projects that have a short life (Coulson ad Richardson's, 2001). Pay out time does not consider the performance of the project after the pay back time. POT is calculated based on equation 10.10 and the results are presented in Table 10.9 for different purification processes.

$$\text{Payout\_time} = \frac{\text{Fixed\_capital} + \text{Start} - \text{up}}{\text{Net\_cash\_flow}} \qquad \text{Equation 10.10}$$

Table 10.9 Pay out time of 3 processes

	Base Case	SMB	Surfactant-Aided SMB
POT (year)	0.19	0.30	0.42

10.4 Sensitivities

The results of economic analysis can be affected by any changes in operating costs as well as changes in the product volume and price. A sensitivity analysis is a way of examining the effects of uncertainties in the forecast on the viability of a project (Coulson ad Richardson's, 2001). In the economic analysis, it can be seen that this project is very profitable; its payback time is very short. In this report, the effects of the main raw material and product's price on some economic criteria are analysed.

Table 10.10 Sensitivity analysis for base case

	Change (%)	NPV (M€)	DCFRR (%)	ROR (%)	POT (year)
	0	88	131	86	0,19
Raw materials	-10	100 14%	147 12%	99 15%	0,17 -11%
	10	75 -14%	115 -12%	74 -14%	0,22 16%
Product's price	-10	66 -25%	103 -21%	65 -24%	0,25 32%
	10	110 25%	159 21%	108 26%	0,15 -21%

Table 10.11 Sensitivity analysis for the process with SMB

	Change (%)	NPV (M€)	DCFRR (%)	ROR (%)	POT (year)
	0	128	137	91	0,3
Raw materials	-10	141 10%	149 9%	100 10%	0,28 -7%
	10	116 -10%	126 -8%	82 -10%	0,33 10%
Product's price	-10	102 -21%	113 -18%	72 -21%	0,38 27%
	10	155 21%	161 18%	110 21%	0,25 -17%

Table 10.12 Sensitivity analysis for the process with SA-SMB

	Change (%)	NPV (M€)	DCFRR (%)	ROR (%)	POT (year)
	0	132	143	96	0,42
Raw materials	-10	144 10%	155 8%	105 9%	0,37 -12%
	10	119 -10%	131 -8%	86 -10%	0,48 14%
Product's price	-10	105 -20%	118 -17%	76 -21%	0,55 31%
	10	159 20%	168 17%	115 20%	0,34 -19%

Table 10.10 to Table 10.12 show that NPV and other economic criteria are very dependent on the changes of raw materials and product's price. If raw material cost increases by10%, NPV, DCFRR and ROR decrease about 10% while POT increases a little more than 10% in all processes. It is also clear that the economic items are more sensitive in changes of product's price than in those of raw materials. When product's price decreases 10%, NPV, DCFRR and ROR decrease almost 20%; and POT increases nearly 30%.

10.5 Discussion on economic results

The PEC fo base case is still below 500.000 Euros, which is the maximum PEC stated by the client, but this is not the case for the processes with SMB-GF and SA-SMB-GF. The high PEC in the last two processes is caused by the costs of SMB units. But as it is shown in the economic analysis, both processes with SMB-GF and SA-SMB-GF have very good economic potential; NPV is 128 and 132 million Euros respectively. These are much higher than the money that the company can get from the base case process, which is 87 million Euros. Other economic criteria such as rate of return and discount cash-flow rate of return also show that the processes with SMB are better than the base case. The base case is only superior in term of payout time due to its low investment costs.

The results of TCI for process with SA-SMB-GF is also compared to the maximum allowable investment calculated in chapter 3.4. The TCI: 9,2 M Euros, is much lower than the calculated maximum allowable investment, which is 355 M Euros. The reason for this is that in the previous maximum allowable investment calculation, only the revenues and raw materials costs were taken into account. The other costs like equipments, installation and some other criteria were not taken into consideration. Thus a very high investment costs were obtained.

Another point that should be emphasized here is the uncertainty of economic potential. This depends very much on the accuracy of the estimation of economic variables, especially raw material cost and product's price. The cost of input is the main cost of raw materials and it is rather accurately estimated since the cost information is given by the company. On the other hand, the product's price is more problematic as it has more effects on the economic potential. In addition, the product's price is estimated based mainly on the information found on the Internet. If hCG in the market is 10% cheaper than it is estimated in this report, NPV of SMB processes is 20% less than the numbers calculated. Basically, if the company cannot sell hCG at a price higher than 2.5 Euros/mg, none of the processes has positive economic potential.

11 Creativity and group process tools

This chapter describes the creativity and process tools used in the design. Besides that, the internal group functioning and the relationship with all partners outside of the design team are also discussed.

11.1 Team interaction

The design team interacts with:

- The project coordinator (Mr. Luuk van der Wielen).
- The principal, which is the person from the company (Mr. Van Dedem) who will function as the client commisioning the design.
- Mr. P. Swinkels, as the coach on conceptual process design, work process management and creativity & group p rocesses.
- Ms. Horneman and Mr. Ottens, as the technological coaches.
- Technical experts, who can help the team with technical problem.
- The design tools (DDM [Delft Design Matrix] (Grievink et al., 2001), design quality monitor (Piquar: Plant design Improvement by QUAlity Review (Herder and Weijnen, 1999))).
- Mr. A. Austin, who will assist in the use of the design tools.
- Work process tools (AAA, timetable Gantt chart, shared folders).
- The domain knowledge and information.

11.2 Exchange information with people outside the team

Table 11.1 describes the tools used for exchange information between the team and other people outside.

Table 11.1 Tools used for exchange information

Point of contact	Tools
Principal	- E-mails - Monthly meetings - Minutes of meetings - Design space report - Basis of design (written report and subsequent defense) - Final report
Coach on conceptual design methods and work processes	- E-mails - Weekly meetings - Minutes of meetings - Design space reports - Basis of design - Final report
Technical coach	- E-mails - Monthly meetings - Minutes of meetings - Design space reports - Basis of design - Final report

Point of contact	Tools
Austine Ajah	- Meetings as needed - Minutes of meetings
Luuk van der Wielen	- Progress meetings - Design space reports - Basis of design - Final report

11.3 Constraints

Some constraints on the whole design process are established and mentioned below:

- Dates set for Basis of Design (BoD) and Final report, as it is mentioned in chapter 5.
- Requirements on the contents and format of the memos, BoD and Final report (given in the CPD-manual, (Grievink et al., 2002)).
- Requirements on the quality of the design (Grievink et al., 2002).
- On the design method:
  - The Delft Design Matrix is to be used (Grievink et al., 2001).
  - For evaluation, the Piquar quality control tool is to be used (Herder and Weijnen, 1999).
  - For controlling the team's activities, AAA (Advanced Activity Asistant) table is to be used.

11.4 Variables in the team functioning

Some variables on the team functioning are defined below:

- Roles of team members
- Action distribution over team members
- Time/resource planning of actions
- Application of team capabilities through group profile
- Group rules
- Creative methods to be used
- Design tools (DDM [Delft Design Matrix (Grievink et al., 2001), design quality monitor (Piquar: Plant design Improvement by QUAlity Review (Herder and Weijnen, 1999)))
- Work process tools (AAA, timetable Gantt chart, shared folders)
- Number and type of Piquar quality factors

11.5 Building blocks

Some building blocks related to the team functioning are mentioned below:

- Creativity methods are designed to stimulate the generation and application of creativity during the design process. This will enable the creation of a more innovative (plant) design
- Time/resource planning of the project is performed to ensure that the project is finished in time, to make sure that all team members spend the required time on the project and to prevent loss of time
- Team capabilities and personal capabilities are examined, by group profiling, to use the capabilities of the group to maximum extent.
- The group structure is defined by establishing group rules to enable smooth operation of the group.

- The design method employed is the Delft Design Matrix. The evaluation of the design during the various design stages is done using the Piquar quality control tool; evaluation factors have to be chosen for this.

11.6 Available knowledge

The team has gathered necessary knowledge related to the building blocks mentioned in chapter 11.5. Brief explanations of the knowledge are listed below:

- Creativity methods: article on creative method have been supplied (Grunwald, 1997); each member of the design team has read this. Additional knowledge was obtained by performing a creativity assignment (Grievink et al., 2002) and browsing several websites.
- Time planning: Constraints put on the group by the principal (delivery date and requirements for reports/ presentations) and the Delft Design Matrix method (Grievink et al., 2002).
- Team capabilities: Members were asked to analyze their strengths and weaknesses in a predetermined format. These are combined to assess overall strengths and weaknesses of the group (appendix 27).
- Internal structure: Apart from some experience-based knowledge of the members some rules were established using the outcome of the creativity methods development.
- The Delft Design Matrix design method is specified in (Grieving et al., 2001).

11.7 Tools used for setting up the team functioning

The tools used in the synthesis, analysis, and evaluation phase to decide on the team function design are:

- Group meetings. During these meetings an open discussion is held to analyze and evaluate options. Group-wide consensus is the deciding factor.
- Individually assessing articles on creative methods and reporting the findings to the group
- Group profile analysis
- Brainstorming.

11.8 Tools and rules to be used in the design

The outcome of each building block specified before is given in a series of reports attached as appendices.

- Creative method
  Appendix 28 gives the methods to be used by the group. These methods were obtained partly from the individual creativity assignments and partly from information available in websites. They were analyzed and evaluated in group-discussions by looking at applicability and feasibility.
- Team capabilities are discussed in appendix 29.
- Group rules are given in appendix 30.
- A time planning of the group is given in Appendix 32.
- Piquar factors and weights are given in appendix 33

12 Conclusions and recommendations

The aim of this project is to design a plant that purifies human Chorionic Gonadotropin (hCG), with sufficient purity and virus and endotoxin removal. The purity should be at least 50-wt% and the level of virus in the product should be maximal 10^3 particles/g, while the final endotoxin concentration should be maximal 10^{-3} wt%.

Apart from the base case two complete processes are designed, which will be compared with each other. The first process makes use of a four-section simulated moving bed, the second process uses a five-section surfactant aided SMB and the base case operates with a cation exchange column. The operating times of the three different processes are kept the same (29 hours). A summary of the performance of the processes is given in Table 12.1.

Table 12.1 Performance of different processes

Process	Purity (%)	Virus concentration (part./g)	Endotoxin concentration (wt-%)	Overall recovery (%)
Base case	50	10^3	10^{-3}	77
Process with SMB-GF	52	0	$6.4 \cdot 10^{-4}$	92
Process with SA-SMB-GF	39	27	$9.8 \cdot 10^{-4}$	93

The overall recovery is the best for the process with SA-SMB-GF, and also the process with SMB-GF has a high recovery. The recovery in the base case is much lower. In the base case and the process with SMB-GF, all requirements are met. In the latter the purity is a bit higher than required, and the endotoxin and virus concentration are lower than required. All virus particles will be even removed. The process with SA-SMB-GF performs very well for virus and endotoxin removal; unfortunately the required purity is not met. This can be improved by optimisation of the system for example by changing the surfactant concentration, column dimensions, number of columns per section etcetera.

It can be seen in Table 12.2 that the processes with SMB-GF and SA-SMB-GF have a very good economic potential; the Net Present Value (NPV) after 15 years is 128.5 and 131.8 million Euro respectively. The NPV for the base case is only 87.8 million Euro. Also the Rate of Return is the lowest for the base case. The base case is only superior in terms of Pay Out Time (POT) due to its low investment costs.

Table 12.2 Economics of different processes

Process	TCI (million €)	POT (year)	ROR (%)	NPV (million €)
Base case	6.8	0.19	86	87.8
Process with SMB-GF	9.4	0.30	91	128.5
Process with SA-SMB-GF	9.2	0.42	96	131.8

The difference between the process with SMB-GF and the process with SA-SMB-GF is not very obvious. The process with SA-SMB-GF is, economically speaking, slightly better because the NPV and ROR are a bit higher. Furthermore the Total Capital Investment is lower for the process with SA-SMB-GF.

When all the above-mentioned is taken into account, the conclusion is that the best process for hCG purification is the process with SMB-GF. All the requirements are met, and the process has a very good economic potential. Furthermore the final product of the process with SMB can be considered safer than the product of the process with SA-SMB, because it will not contain surfactants and it is not sure yet what impact Brij® 35 will have on humans when it is injected.

To exploit the economic advantage of the SA-SMB-GF process, the purity should be increased. Because of the time limitations of this project, not all possibilities of SA-SMB-GF are fully investigated. If the SMB unit is used only for virus removal, another process step can be added for protein separation, like ion exchange chromatography. The SMB unit could then be smaller and the total economic potential should be investigated. Besides that, the SA-SMB-GF system could also be optimized, by changing some parameters, as mentioned above. If it turns out that the new process is viable, it is recommended to do research with Tween 20 as the surfactant, since it is commonly used for injected pharmaceuticals.

For the normal SMB process, it could also be beneficial to use the SMB unit only for virus removal and to add, for instance, an ion exchange chromatography step. This is not presented in the report, because the team focused first on virus and endotoxin removal and later there was not enough time to investigate all the possibilities with respect to protein removal.

List of symbols

Cp		heat capacity		[kJ/kg·°C]
ΔH		enthalpie		[J]
Hc		heat of combustion		[MJ/kg]
K		partition coefficient		[-]
	K_{hCG}		- of hCG	[-]
	K_{virus}		- of virus	[-]
m		mass flow		[kg/s]
m_{10}		mass of water at 10 °C		[kg]
m_{80}		mass of water at 80 °C		[kg]
Q		heat duty		[kJ]
S		separation factor		[-]
ΔT		temperature difference		[°C]
X		pressure		[psig](1)
X		potential energy release		[Btu](2)
Y		penalty factor		[-]

(1) 1 psig = $6.895 \cdot 10^3$ J
(2) 1 Btu = $1.054 \cdot 10^3$ Pa

List of abbreviations

a	annual
AAA	advanced activity asistant
atm.	atmosphere
avg.	average
BoD	basis of design
BSA	bovine serum albumin
Capex	total capital investment
CMC	critical micelle concentration
CPD	conceptual process design
Da	Dalton
DCF	discounted cash flow
DCFRR	discounted cash-flow rate of return
DDM	Delft design matrix
EU	endotoxin unit
F	hazard factor
FCA	fixed capital investment
FDA	food and drug administration
F&EI	fire and explosion index
GMP	good manufacturing practice
h	hour
HAZOP	hazard and operability study
hCG	human chorionic gonadotropin
ISBL	inside battery limits
IU	international unit
kDa	kilo Dalton
kg	kilogram
kJ	kilo Joule
kWh	kilo Watt hour
L	litre
L	liquid
LP	low pressure
LPS	lipopolysacharides
M	molarity
m	meter
MF	material factor
mg	milligram
min.	minute
ml	milliliter
MS	Microsoft
MWCO	molecular weight cut-off
Myo	myoglobine
N_f	flammability factor
N_h	health factor
NCF	net cash flow
nm	nanometer
NMWCO	nominal molecular weight cut-off
NPV	net present value

nr.	number
NS	not specified
OSBL	outside battery limits
part.	particles
PEC	purchased equipment costs
PEG	polyethylene glycol
PFS	process flow scheme
pg	picogram
Piquar	plant design improvement by quality review
POT	pay out time
PSD	process system design
ROR	rate of return
S	solid
SA-SMB	surfactant aided simulated moving bed
SA-SMB-GF	surfactant aided simulated moving bed gel filtration
SMB	simulated moving bed
SMB-GF	simulated moving bed gel filtration
TCA	trichloroacetic acid
TCI	total capital investment
TMC	total manufacturing costs
TPC	total production costs
UV	ultra violet
W	Watt
^{o}C	degree Celsius
€	Euro
µg	micro gram
wt%	weight percentage

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