

**ECONOMIC FEASIBILITY OF THE RECOVERY OF THERAPEUTIC PROTEINS
FROM THE MILK OF TRANSGENIC LIVESTOCK**

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Abstract

The recovery of pharmaceuticals from blood plasma is often expensive, limited, and is subject to contamination. The objective of this feasibility study is to present recovery designs and the most significant parameters affecting manufacturing costs of human Serum Albumin (hSA), human Protein C (hPC), and Factor VIII (FVIII) from the milk of transgenic livestock. All the processes presented are based on chromatography columns to achieve separation. The sensitivity of the manufacturing cost of the protein to various parameters are illustrated graphically and discussed, along with the cost distribution percentages for each type of process. It was found that manufacturing costs decrease with superficial velocity, expression levels, column yields, column capacities, and scale of production increases with all other parameters remaining constant. However the estimated cost decreases tend to level out at higher values of the parameters. Finally a judicious choice of separation scheme, such as Fall-Through (FT) versus Direct Adsorption (DA) for hSA production, can decrease the protein cost by as much as 38%, due to savings from wash and elution buffers. The costs associated with the production of hSA (DA process), hSA (FT process), hPC, and FVIII are 3.56, 2.20, 129, and 11,780 \$/gr respectively. By means of the optimization of manufacturing parameters to reduce costs, key areas of emphasis for process development were identified.

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Introduction

The demand for therapeutic proteins is rising with population growth. Hence the need to produce them in larger quantities and cheaper. These proteins are typically extracted from donated blood where their concentrations are typically very minute which makes them too expensive for widespread treatments. With current technology, continuous replacement of Factor VIII necessary to hemophiliacs would cost over \$100,000 per year [Velander, 1997]. A cheaper, more productive, and safer alternative for the production of proteins such as Factor VIII, human Protein C, and human Serum Albumin reside in transgenic livestock as bioreactors. Expression of human proteins in the milk of cows (hSA), pigs (hPC, FVIII), sheep, or mice [Paleyanda, 1997, Velander, 1992] is now possible. These proteins help in the treatment of individuals suffering from hemophilia A and severe burns. The current expression levels in milk are 5, 0.5, and 0.05 gr/L for hSA, hPC, and FVIII respectively. Costs involved in obtaining blood plasma proteins can be prohibitive for sustained treatment [Velander, 1998] and the supplies are well below the demand [Paleyanda, 1991] since they depend on blood plasma availability. The production of these proteins in the mammary glands of transgenic livestock provide virtually unlimited supplies of drug therapies. It also reduces potential threats from contaminated blood [Ehmann, 1995, Lubon, 1996].

The next step after the expression of therapeutic proteins is to design and scale up a recovery process that will achieve specified levels of purity. The recovery represents most of the costs of the protein and we need optimize the manufacturing parameters to reduce them to a minimum. It is the intent of this paper to explore the economic feasibility of producing recombinant proteins with transgenic livestock. Costs sensitivity and cost distribution for the production of hSA, hPC, and FVIII are the main focus of this study. The backbone of the recovery process for each protein consist of chromatography columns in series. Also the potential for achieving process-scale using these chromatographic steps has been validated in laboratory experiments at approximately one liter scale [Degener, 1997]. A centrifuge step is present before the whey is fed to the columns to prevent clogging of the columns by lipids. Furthermore the first separation step is performed in an expanded bed (EB) mode to remove remaining solid particles in the whey. The EB thus circumvents the need for a counter flow filtration unit. This first step also acts to reduce the amounts of volume processed. Ultrafiltration units allows the feed to have the proper concentration of buffers and to prepare the proteins for separation in the columns. A viral inactivation step using specific solvents [Velander, 1990] is present after the first column in order to prevent contamination. In addition, each independent chromatographic step in the process represents a barrier to pathogen contamination. Other type of ancillary equipment such as heat exchangers, cooling units, pumps, vessels, and warehouse were also scaled up. Most of the manufacturing is done in a chilled environment (4 °C) to avoid contamination. The processes described above were all modeled using a spreadsheet (Excel™) and include over a 100 parameters, only a fraction of which are discussed in this study. Proteins costs can thus be determined instantaneously for any given set of these parameters.

Process description

Three separation schemes were design for the recovery of hSA, hPC, and FVIII. A Fall-Through and a direct adsorption process were investigated for the purification of hSA. An Immunoaffinity (IA) process was designed for the purification of hPC and FVIII. The flowsheet for each process is shown in figure 1. The applicable base production scales are 35,000 kg/yr, 90 kg/yr, and 0.12 kg/yr for hSA, hPC, and FVIII respectively. These figures are based on market demand and represent 20%, 100%, and 25% of total U.S. yearly requirements respectively [Paleyanda, 1991]. Further base values of the process parameters are shown in table 2. Table 1 indicates most significant unit operations present in each process.

Fall-Through Process (hSA)

The Fall-Through process utilizes a chromatography column which does not capture but allow the target protein to pass through unadsorbed. As part of this process a high superficial velocity (10 cm/min) is used. In addition, specialized conditions of adjusted buffer ionic strength and metal binding are used to reduce the adsorption potential of the target protein relative to other milk proteins in the feed [Degener, 1997]. The second step consists of an ion exchange column which provides medium resolution (50% purity) and the hydrophobic interaction column brings the hSA purity to 99% [Degener, 1998]. The last column removes bovine Serum Albumin (bSA) which is expected to be at a concentration of the order of micrograms per liter. Hence the size of the column necessary to capture bSA is small relative to hSA columns (283 L/column) and contribute little to the overall cost (less than 2 %.) The last ultrafiltration unit is used for formulation of hSA.

Direct Adsorption Process (hSA)

An alternative to the Fall-Through process is the direct adsorption process which, instead of *not* capturing the target protein in the first step, captures the target protein by adsorption onto three ion exchange columns in series. The purity goes from 20 % after the first column to 50% after the second column and, finally, reaches 99% after the third column. Again the anti-bSA column is present to remove bovine serum impurities.

Immunoaffinity Process (hPC, FVIII)

The Immunoaffinity process is also based on the capture of the target protein at each step. However an immunoaffinity column constitutes the high resolution step. The anti-bSA column is not necessary since the hPC and FVIII are currently being expressed, at a large scale, in pigs only. An ion exchange column is required as a last step to remove leached immunoglobulin. Whereas hSA and hPC are formulated in solution it is more common to lyophilize FVIII and still preserve the appropriate protein structure. Hence freeze drying kits are indicated in figure 1 and table 1.

Costs modeling

The manufacturing costs were estimated as suggested by Bailey [Bailey, 1986]. They include the installed equipment cost using the appropriate bare module factors, salaries and wages, raw materials and supplies, and utilities. From these items the total capital investment and the yearly manufacturing costs were estimated. Equipment was depreciated linearly over 10 years using an interest rate of 12%. A list of the critical equipment utilized in each process is given in table 1. Most of the salaries and wages were scaled up as suggested by Ulrich [Ulrich, 1984] and most depend on the number of unit operations involved in the process. It should be noted that this dependence was linearly scaled up for large production volumes of hPC and hSA. For FVIII the scale of production being relatively limited (0.12 kg/yr), allows a direct analysis of the process for the determination of the workforce necessary and, hence, of the salaries and wages. The cost of many unit operations were scaled up using given correlation [Bailey, 1986] when the cost could not be determined directly for the unit operation size. Costs of raw materials may vary depending on the supply source and the amounts involved.

Recovery process parameters

In this feasibility study six manufacturing parameters were varied to determine their effects on the recovery costs of milk proteins. Protein expression level, column yield, column superficial velocity, column capacity, production scale, and separation scheme alternatives are all parameters that need be adjusted to minimize overall costs. Some of these parameters are technology dependent while others depend on the capital available and strategic choices. The effects on costs that each of these parameter has should allow to prioritize process improvement investments and maximize returns. Figures 2-9 pertaining to the process parameters and cost distribution discussed below, were all obtained using base values shown in table 2 and the independent variables involved. Finally, purity levels after each separation were set as suggested by experimental data [Degener, 1998].

Protein expression level

Expression levels greater than about 1 to 5 gr/L in milk are difficult to accomplish due to rate limitations in post-translational modifications in mammary glands of transgenic livestock [Degener, 1998]. Without these modifications the protein will not be produced in an active form. However relatively important cost reduction in the Fall-Through process could be achieved if the expression of hSA in milk can be increased to 7 gr/L where the hSA cost is 1.79 \$/gr, from 5 gr/L currently achieved where the cost is 2.20 \$/gr. As expression level is increased the rate of cost decrease levels out beyond 7 gr/L as shown in Figure 2. It should be noted that the expression or the feed concentration becomes more important to reduce as the milk cost increases. This is demonstrated for hSA in Figure 2 where the hSA cost at every expression level increases with increasing milk costs. Milk from swine is more expensive at 10 \$/L, than the milk from cows at 0.53 \$/L [Van Cott, personal communication, 1997]. Thus for processes where the share of raw materials of

manufacturing costs is important one would favor using transgenic cows as opposed to pigs, sheep or others. However expression of certain proteins such as hPC is not yet possible in cows. Swine do offer the advantages of short gestation periods, short generational times, and larger litter sizes compared to other types of livestock [Velander, 1997]. Producing transgenic pigs is thus more rapid than for other dairy animals. The price of raw materials increase significantly the cost of the protein recovered in particular at larger production scales. Any parameter such as expression level that tend to increase the feed volume, and thus the raw material volume, need be optimize. The main reason for the cost decrease with increasing expression is a reduction in volume processed hence less equipment, labor, and buffer is necessary to recover a given amount of protein. However for values of expression levels ranging from 7 to 15 gr/L the reduction in costs are less important.

Column yield

The yield assumed in the model were 50 % for the affinity columns and 80% for the ion exchange columns. Typically the handling of the proteins with any unit operation leads to a loss of about 5 to 10 % of the target protein. These values were used to take into account losses in ultrafiltration units, centrifuge, etc. Hence the overall yield, which is significantly reduced as the number of unit operation in the process increases, varied from 20% for FVIII to 40% for hSA with a Fall-Through process. The largest contributor in the reduction of the overall yield are the column yields. The higher the yield one can achieve, the lower the costs as shown in Figure 3. The cost decreases from about 350 \$/gr hSA using a direct adsorption process at 10% yield to about 3 \$/gr near 90% column yield. However the cost starts to level out in the range of 70 to 90% yield and no major improvement in cost reduction can be expected thereafter. The yield impacts the cost by increasing the amount of raw materials, labor and equipment necessary to obtain a given amount of product and this becomes even more important at higher raw materials costs. One way to improve yield in a Fall-Through scheme, would be to add specific complexing agents of the eluted target protein for reasons explained below.

Superficial velocity

Superficial velocity is defined as the ratio of volumetric flow rate to the column cross sectional area. It is an indication of the column feed rate and is important as both a constraint and a separation parameter. First the superficial velocity generates a pressure drop across the column and thus limits the height of the column. In the design used the column height and diameter are limited to values of up to 25 cm and 120 cm respectively. These constraints, however, depend on the type of matrix used. A maximum of 200 cycles before replacement was used in the models. Second, superficial velocity influences the time permitted for adsorption of the target protein onto the column. Hence, in the direct adsorption process, the superficial velocity was set at 3 cm/min to maximize the bound ligand concentration. However for the Fall-Through column this velocity was increased to 10 cm/min since we want the target protein *not* to be adsorbed. Again, to enhance this effect, metallic agents were added to the buffer in order to complex the hSA molecules together thereby increasing

there size and reducing their residence time through the matrix of the column [Degener, 1998].

For a Fall-Through process we observe that the cost of hSA decreases from 2.44 to 2.20 \$/gr as the superficial velocity varies from 1 cm/min to 10 cm/min as shown in figure 4. Beyond 7 cm/min it can be seen the rate of cost decrease becomes insignificant. Superficial velocity reduces processing time, labor, and equipment to achieve a given product output. It should be noted that as the superficial velocity varies it is assumed that all other parameters remain constant, which is not necessarily true. However for the Fall-Through process it is not only appropriate to increase the superficial velocity, as it favors the separation, but it is also cost effective. This is not the case for a direct adsorption process where increasing superficial velocity could decrease yield and purity. For the Fall-Through process superficial velocity should be increased cautiously, as it may affect purity unless the subsequent high resolution step can achieve the desired purity. Indeed, the protein impurities may fall through with the target if not given time to adsorb. If a correlation between column feed rate and column yield and purity is available then one could optimize the superficial velocity to minimize costs.

Column capacity

For the hSA adsorption process, column capacity has a significant impact on hSA cost but this impact becomes less important at capacities of 6 gr/L or more, as shown in figure 5. As column capacity increases hSA cost decreases. Values for DA process are shown in figure 5 with only the *first* column's capacity being changed from a value of 1gr/L to a final value of 10 gr/L (the other two following columns have base values of 5 gr/L). However there is a limitation to the extend of column capacity, especially for immunoaffinity columns where the antibody density, as it increases, starts to exhibit steric effect preventing the binding of the target protein [Subramanian, 1994] It was calculated that a reasonable capacity for an affinity column would be 0.88 gram target protein per liter matrix as opposed to 3 to 7 gr/L for direct adsorption columns. The capacity of the volume reduction step is usually lower than that of subsequent steps since high concentrations of impurities competitively bind to immobilized sites.

The decrease of the cost shown in figure 5 occurs because of a reduction in the yearly quantities of resin necessary to adsorb the proteins. In fact over 36% of the raw material cost can be due to matrix costs in large scale processes as in the DA process for hSA recovery. With raw material costs representing over 85% of the total costs, this parameter can be very significant. As was discussed earlier, superficial velocity and column height both increase shear stresses experienced by the matrix. Developing a more resilient matrix, lowering column height to practical levels, or reducing column superficial velocity may increase the number of cycles the column matrix can be subjected to before replacement. It is thus a matter of obtaining the appropriate experimental data pertaining to matrix resiliency versus column height, and column feed rates. The second step is then to optimize these parameters based on economic considerations. Following this line of reasoning, adding a high yield low cost pretreatment step prior to the feed of the first chromatography column can improve that

column capacity. This step would remove specific species present in the milk that compete with the target protein for the binding sites. Such an alternative was not investigated in this study.

Production scale

This parameter has the most impact on cost considering figure 6. The costs decrease exponentially with increasing volume of production going from 11,780 \$/gr for FVIII at a scale of 0.12 kg/yr to 129 \$/gr for hPC at a scale of 90 kg/yr and, finally, to 2.20 \$/gr for hSA at a scale of 35,000 kg/yr with a FT process. The difference between the data point representing hPC cost on figure 6 and the curve for hSA is primarily due to a difference in milk costs. hPC is extracted from transgenic pig milk which has an estimated cost of 10 \$/L as opposed to hSA which is extracted from transgenic cow milk and costs only 0.53 \$/L. Figure 6 shows that, independently of the process type, hSA costs decrease rapidly and in a similar fashion with increasing production scale but tend to level out at higher values of production scales of about 4000 kg/yr. As discussed below, increase of production scale decreases unit costs of hSA by shifting the dominant share of cost from labor to raw materials. However, as the production scale increases beyond the 4000 kg/yr for a DA process, the share of raw materials cost varies only slightly from approximately 70 to 90%. Hence, the cost decrease also is limited varying from 4.26 \$/gr for hSA DA process at 4000 kg/yr to 3.56 \$/gr at 35,000 kg/yr, while the raw materials share of the cost varies from 76% to 87% respectively.

Separation scheme alternatives

The type of process utilized can make a large difference in protein costs as shown in table 2 where the costs per gram protein are given. hSA produced by a Fall-Trough process costs only 2.20 \$/gr as opposed to 3.56 \$/gr for the hSA produced using a direct adsorption process. While market forces change the value of hSA derived on a day to day basis, from blood plasma, the cost is greater than about 3 to 5 \$/gr [Velandar, W.H., Personal communications, 1998]. When evaluating the design of a process, simple ion exchange type columns instead of immunoaffinity columns should be favored. Especially since, in a three step process, the desired purity tolerances *can* be achieved. Non-immunoaffinity column capacities tend to be higher, their cost two order of magnitude lower, and their efficiency is usually superior. Indeed, immunoaffinity binding has, typically, lower binding probabilities than non-covalent type binding. Nevertheless, immunoaffinity separation achieve very high purity after only one step, while most other processes require additional steps to achieve specified purity levels. However, a one step process would not provide the number of independent steps that reduce contamination risks from pathogens as required by FDA guidelines.

A Fall-Through process is more competitive because of the savings induced by using less buffer and equipment. Over 85% of the total cost at the hSA scale is due to raw material costs. In the fall through column no eluent and wash steps are necessary, only the usual

regeneration steps are required. Also there is no need to reequilibrate the feed to the second column (table 1) since the original loading buffer is, without excessive addition of buffers, appropriate. It is only necessary to add or mix chelating agents such as EDTA in order to decomplex hSA as explained earlier, particularly since the cost of adding the chelating is insignificant compared to the savings generated. Observe also that the second ultrafiltration unit and its buffers are no longer required, reducing further hSA costs.

Recombinant protein costs and cost distribution

Table 2 provides the cost in dollar per gram of protein and the capital investment for the recovery of each protein with the corresponding base set of parameters. hSA cost produced by a direct adsorption process is 3.56 \$/gr while cost of hSA from a Fall-Trough process is only 2.20 \$/gr. hPC cost and FVIII are 129 \$/gr and 11,780 \$/gr respectively. The difference in the scale of production of these proteins (35,000 kg/yr, 90 kg/yr, and 0.12 kg/yr for hSA, hPC, and FVIII respectively) is at the origin of the bulk of the differences in costs. Clearly the capital investments for the recovery of each protein must differ. For hSA with a FT process, hSA with a DA process, hPC, and FVIII the capital investments are \$36,868,000, \$66,615,000, \$4,606,000, and \$540,150 respectively. To obtain the yearly manufacturing costs one needs only multiply the production scale for a given protein with the cost per unit weight.

Figures 7-11 represent the manufacturing cost distribution for hSA, hPC, and FVIII at various production scales. These costs distribute amongst raw materials, labor, equipment, and a grouping of taxes, building, overhead, and utilities (represented as G on figures 7-11.) Also the detailed raw material costs are shown on the right hand side of each pie chart. The raw material costs include column matrix, milk, buffer, contingency, and other items such as operating and lab supplies, maintenance, and viral inactivation solvent costs. Note that the equipment manufacturing cost share never exceeds 5% of the total manufacturing costs. Raw materials share varies between 20 and 87%, labor share varies between 58 and 4%, and the grouping share varies between 5 and 18%. These percentages represent the extreme of low and high production scale. At the lowest scale of production of 0.12 kg/yr for FVIII (figure 11), the dominant share is that of labor with 58% versus 20% for raw materials, and 4% for equipment. At the intermediate scale of production of 90 kg/yr for hSA with a FT process (figure 8), the shares of labor (55%) and raw materials (26%) are closest of all three scales, while equipment share remains low at 2%. At the largest scale of production of 35,000 kg/yr for hSA with a FT process (figure 7), the dominant share is that of raw materials with 86% versus 4% for labors, and 3% for equipment.

The major trends with respect to manufacturing cost distribution is that as the scale of production increases the labor share decreases, the raw materials share increases, the equipment share increases but remains small, and, finally, the grouping of taxes, building, overhead, and utilities share remains somewhat constant. Labor and equipment tend to be more capital intensive than raw materials, thus, augmenting the second relative to the first, will reduce significantly the unit cost of the products. Indeed, if we compare figures 7 and 8 we see that for two identical FT processes but at scales of production of 35,000 and 90 kg per

year, the labor costs contribution goes from 4 to 55% and the materials costs contribution goes from 86 to 26% respectively. The hSA cost per gram also changes significantly from \$2.20 to \$34 at the lower scale.

From the cost distributions we can also infer what specific cost item of a process tend to make it more or less costly. If we compare figures 7 and 10 for a FT and DA process respectively we see that higher relative buffer costs are associated with a DA process (42%), making it more costly than a FT process (29%) even when the scales of production are identical. Comparing figures 8 and 11 for the production of 90 kg per year of protein, we observe that much higher relative materials costs are associated with a IA process (69%) compared those of a FT process (26%). The IA process is more expensive because the volume of material processed per year is 478,250 L with a cost of 10 \$/L versus only 43,163 L with a cost of 0.53 \$/L for a FT process. Recall that the milk PC concentration is 0.5 gr/L while that of hSA is 5 gr/L. It is thus not surprising to see that 42% of the total manufacturing cost for PC is that of milk while being only 1 % of the total cost for hSA at the same production scales. In this case hPC cost is 129 \$/gr versus 34 \$/gr for hSA from a FT process.

Conclusions

The recovery of recombinant therapeutic proteins from the milk of transgenic livestock milk is likely to be economically competitive with traditional methods. It is also a more reliable source in terms of satisfying demand and health safety requirements. The economic feasibility study of the recovery of these proteins from milk focuses on the impact of six manufacturing parameters. Increasing column yields and protein expression levels in milk was shown to decrease costs by lowering the feed volumes to be processed. These two parameters become especially important to optimize with respect to the total manufacturing costs, as raw materials and volumes processed increase. This is generally true at large production scales. When the mass throughput is high, the more economical process may include a first step which does not absorb the target protein but absorbs the impurities from the milk. Improving post-translational processing of the proteins can augment active protein expression levels and reduce drastically protein costs two to three fold, depending on the specific level.

Increased superficial velocity reduces processing time, labor costs, and equipment costs. The appropriate velocity need be utilized such that column yields are not reduced excessively. For a Fall-Through type process increasing velocity leads higher yields but, possibly, lower purity. In the case of direct adsorption type processes, yields generally decrease with increasing superficial velocities. Experimental data are needed to determine the effects of superficial velocity on column yields. Increasing column capacity of just the first column in the process decreases costs significantly. Indeed, protein cost goes from 7 \$/gr hSA at 1 gr/L capacity to 3.56 \$/gr hSA at 3 gr/L capacity for a DA process. The lowering of the costs are due to the decrease in the amount of column matrix required. If column capacity can not be augmented then increasing the number of cycles tolerated by the matrix before replacement will reduce costs. Especially since matrix replacement costs may

represent as much as 31% of the total manufacturing costs. This could possibly be achieved by lowering column heights, superficial velocity, or by improving matrix resiliency. Again, experimental data need be obtained to establish the relationship among these parameters and allow for parameter optimization. One should consider adding a high yield low cost pretreatment step of the whey that reduces impurities concentration. A priori, this step would improve column capacity enough to more than offset the extra costs incurred.

The appropriate choice of a separation scheme can also reduce costs. Using a Fall-Through process as opposed to a direct adsorption process can lower the cost from 3.56 \$/gr to 2.20 \$/gr hSA. Mainly because the Fall-Through scheme does not require the use of wash and eluent buffers. Nevertheless, production scale remains the most important parameter in terms of reducing costs and affecting distribution of manufacturing costs. The decrease in protein costs as production scale increases is essentially exponential but is less significant beyond 4000 kg/yr for a DA process. This parameter is determined by market demand and availability of capital investment. Furthermore, increasing production scale has the effect of decreasing the labor share of manufacturing costs while increasing the raw materials share, a more desirable situation. For the base parameters as given in table 2, the costs of hSA (DA process), hSA (FT process), hPC, and FVIII (both IA process) are 3.56, 2.20, 129, and 11,780 \$/gr respectively. Finally, for the same base parameters, the capital costs of hSA (DA process), hSA (FT process), hPC, and FVIII (both IA process) are \$36,868,000, \$66,615,000, \$4,606,000, and \$540,150 respectively. In general the rates of cost decreases become less significant at higher values of the parameters. Ideally, a unique set of parameters could be obtained that would minimize manufacturing costs of the recovery of therapeutic proteins from milk. However correlations from experimental data and/or constitutive equations would be needed for this purpose.

Appendix: Figures and Tables

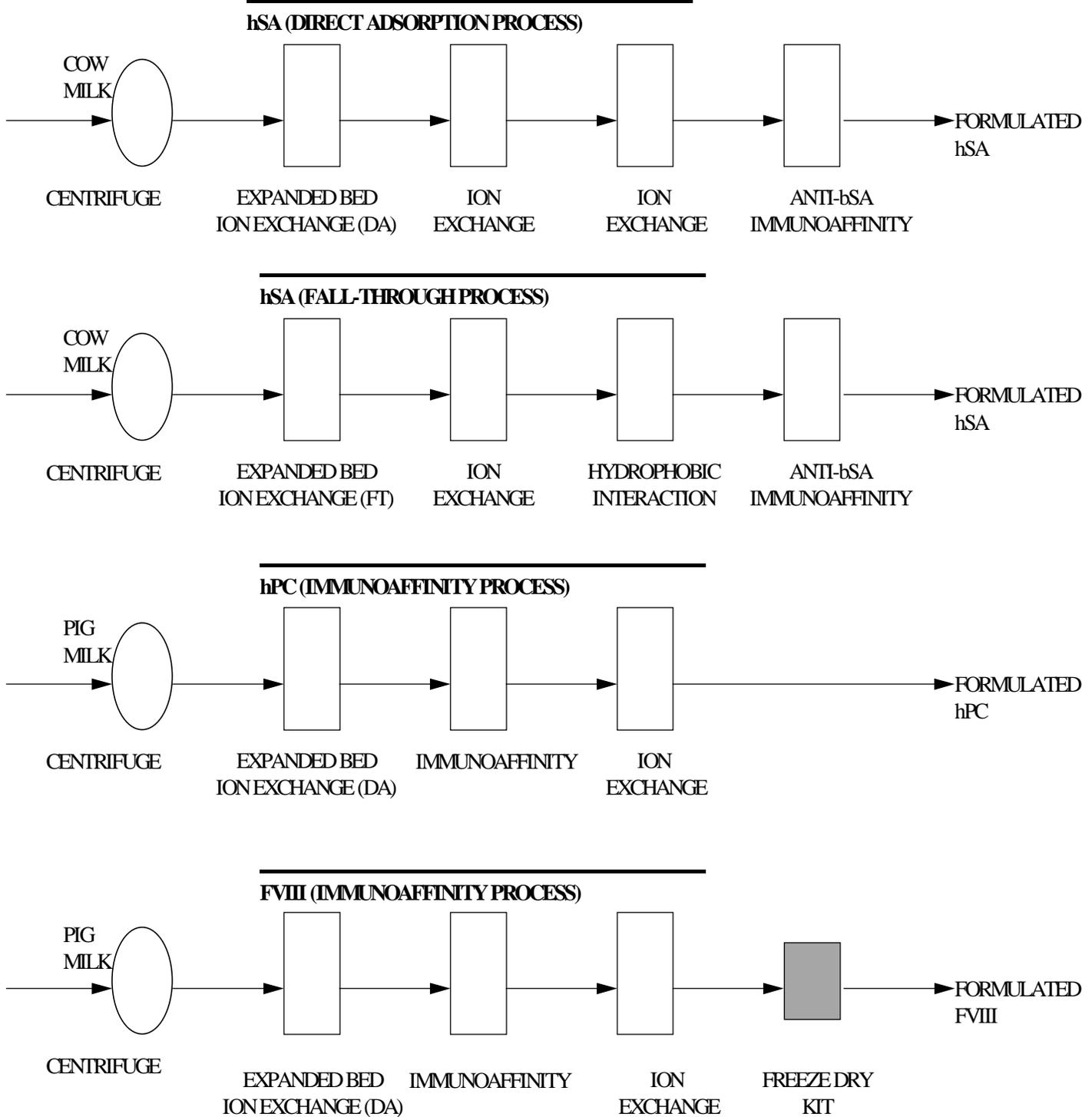


Figure 1. Flowsheets for hSA direct adsorption process, hSA Fall-Through process, hPC Immunoaffinity process, and FVIII Immunoaffinity process

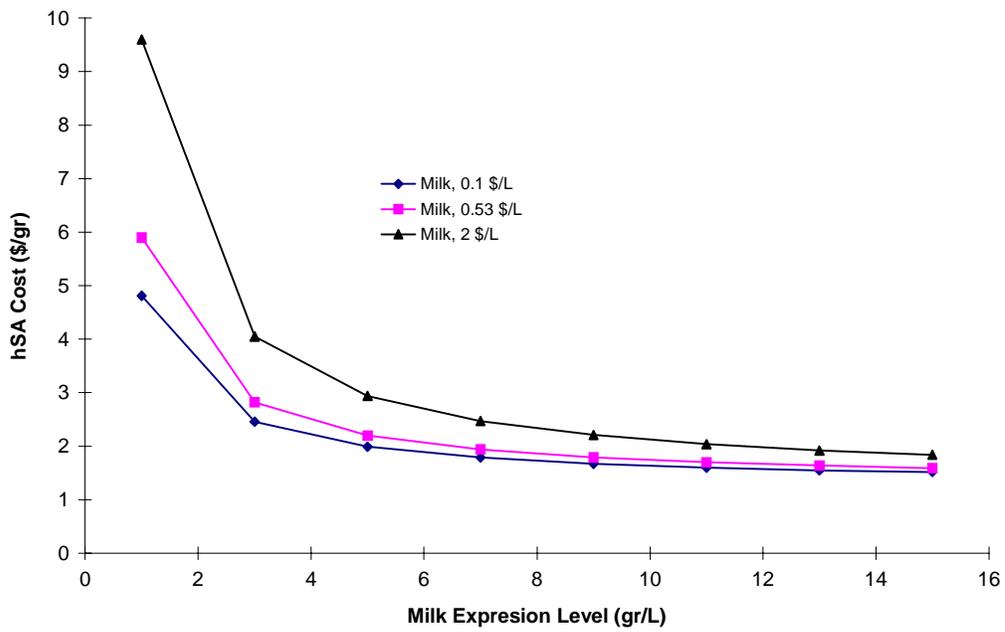


Figure 2. *hSA costs for FT process versus expression levels in transgenic cow milk and milk costs.*

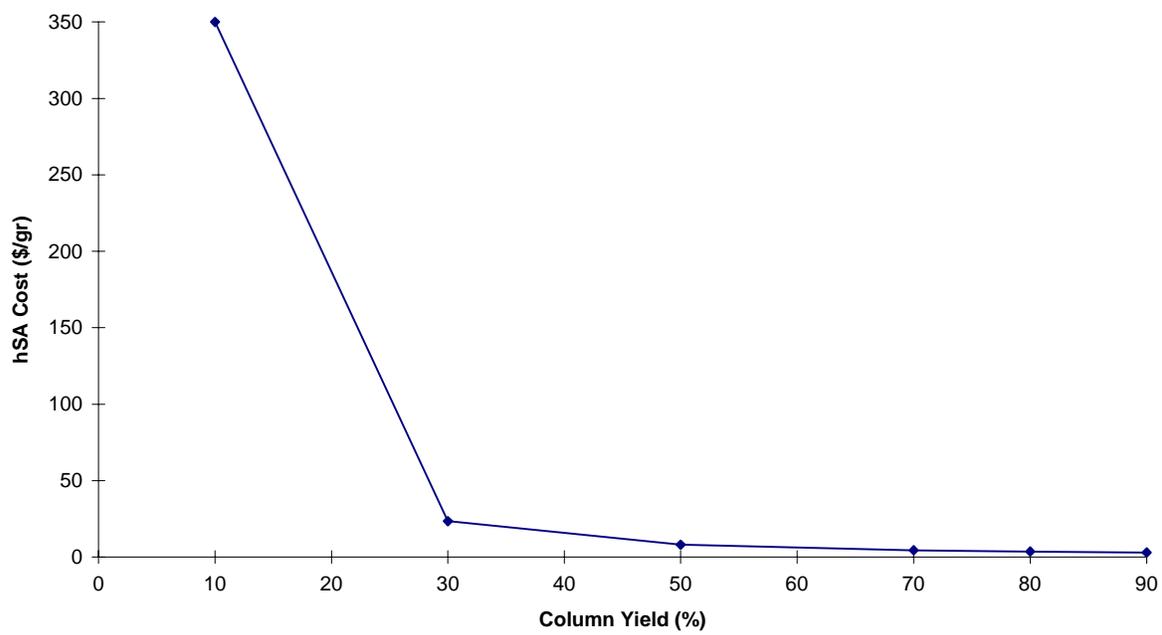


Figure 3. hSA costs for DA process versus column yield.

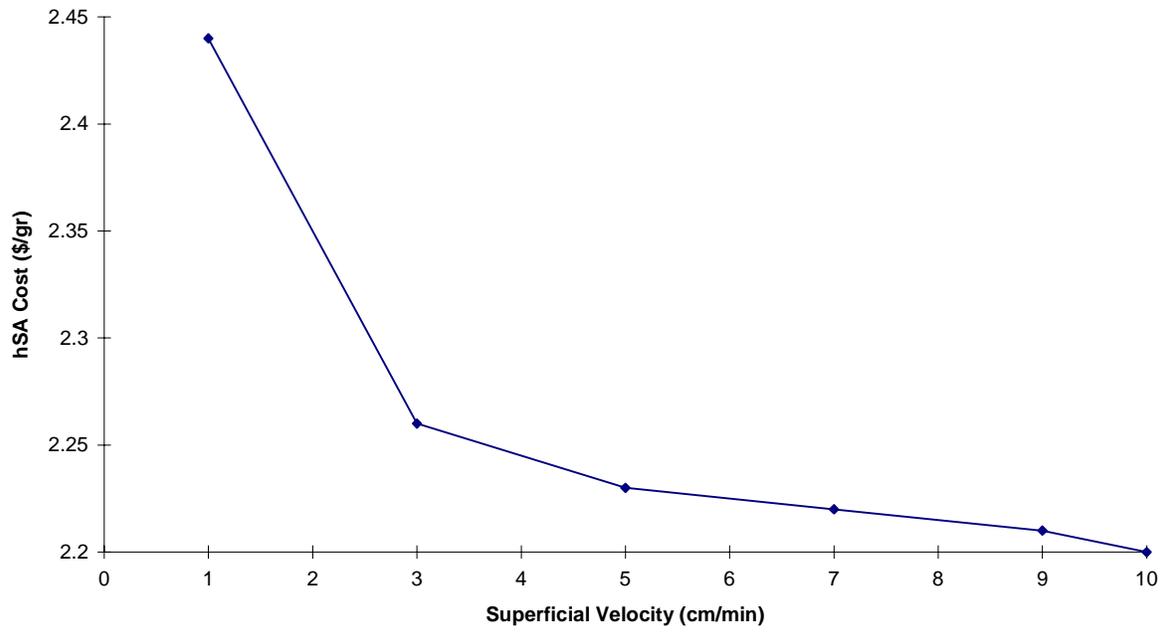


Figure 4. hSA costs for FT process versus column superficial velocity.

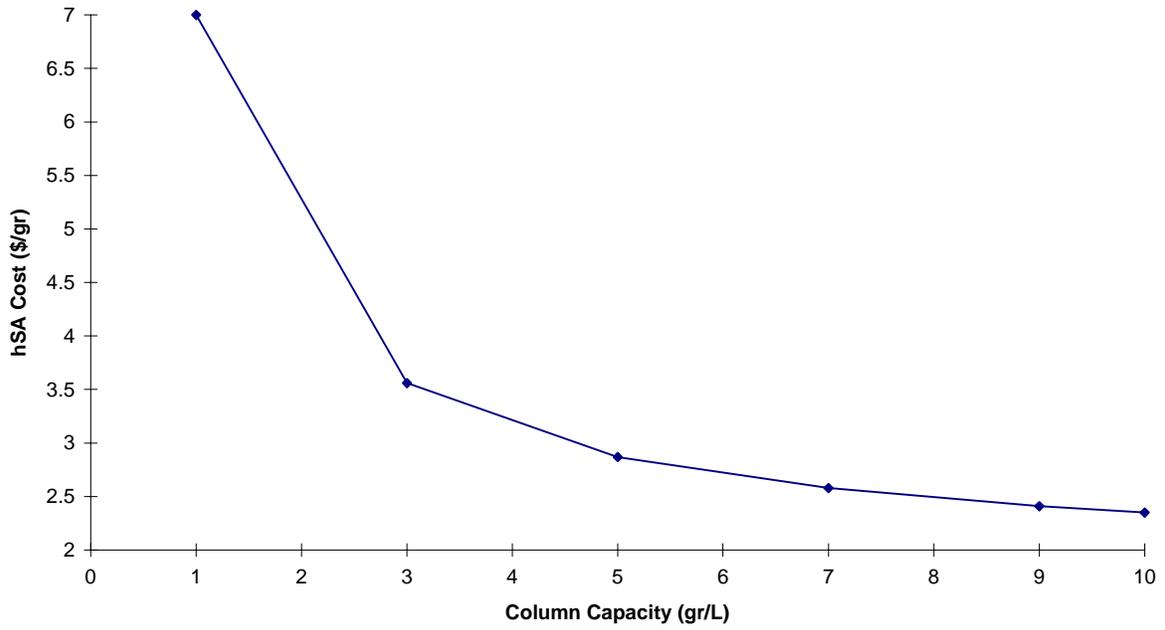


Figure 5. hSA costs for DA process versus first column capacity. Base value for following two columns is 5 gr/L.

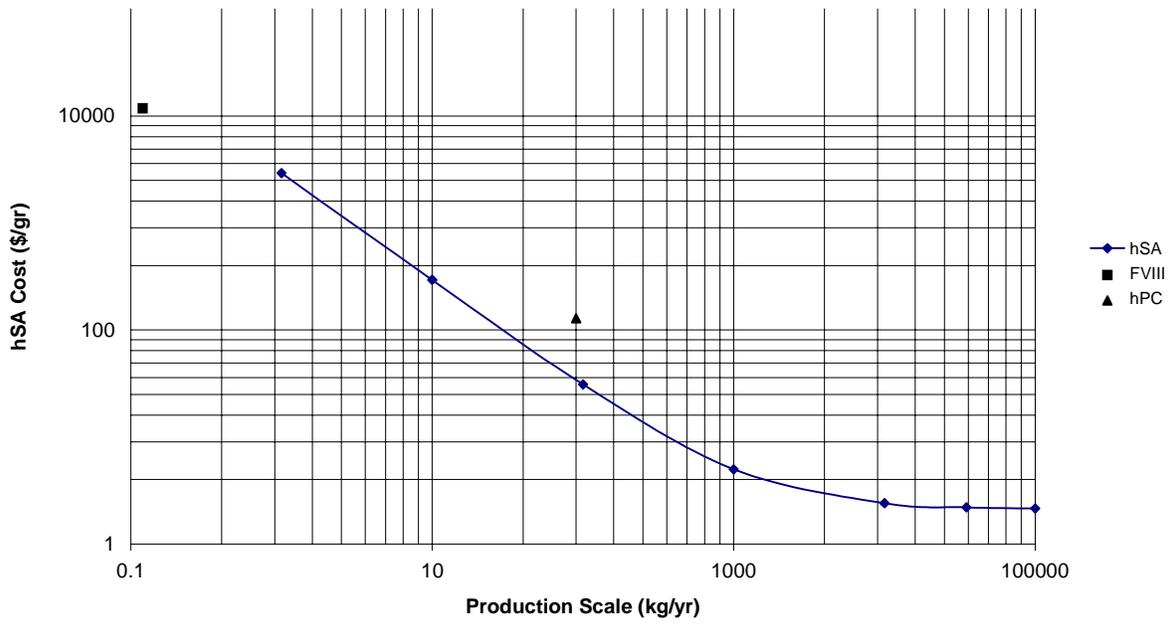


Figure 6. hSA costs for FT process versus production scale. Costs for hPC at 90 kg/yr and FVIII at 0.12 kg/yr are also included.

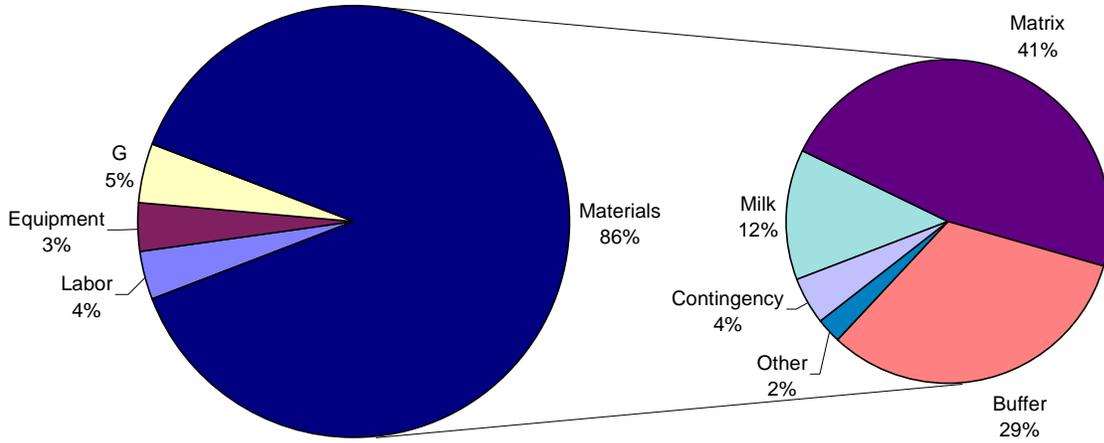


Figure 7. Manufacturing cost distribution for hSA FT process at a production scale of 35,000 kg/yr. The costs distribute among raw materials, labor, equipment, and a grouping of utilities, taxes, building, and overheads (G.) The pie chart on the right represents the contribution of matrix, milk, buffer, contingency, and other (operating and lab supplies, maintenance, and viral inactivation solvent) to raw material costs.

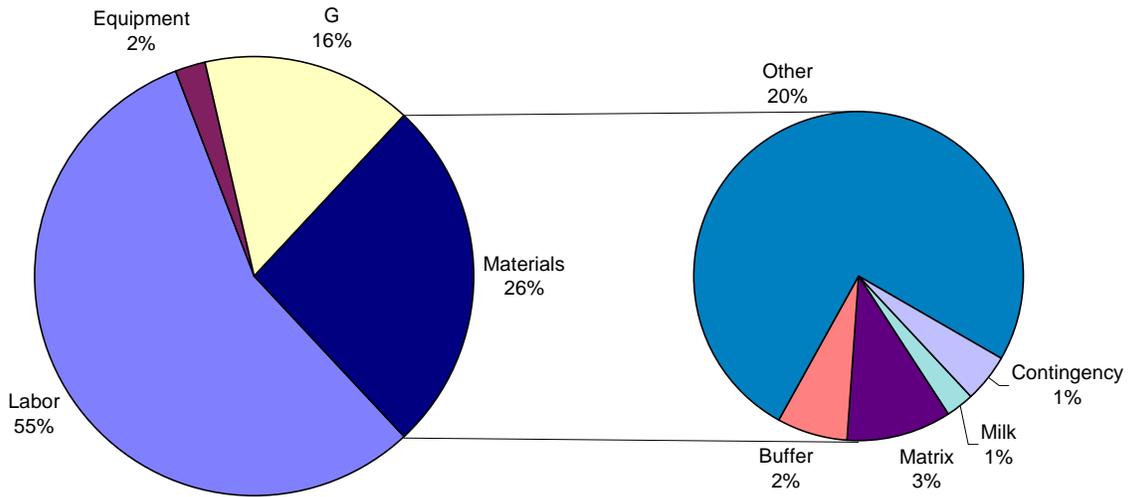


Figure 8. Manufacturing cost distribution for hSA FT process at a production scale of 90 kg/yr. The costs distribute among raw materials, labor, equipment, and a grouping of utilities, taxes, building, and overheads (G.) The pie chart on the right represents the contribution of matrix, milk, buffer, contingency, and other (operating and lab supplies, maintenance, and viral inactivation solvent) to raw material costs.

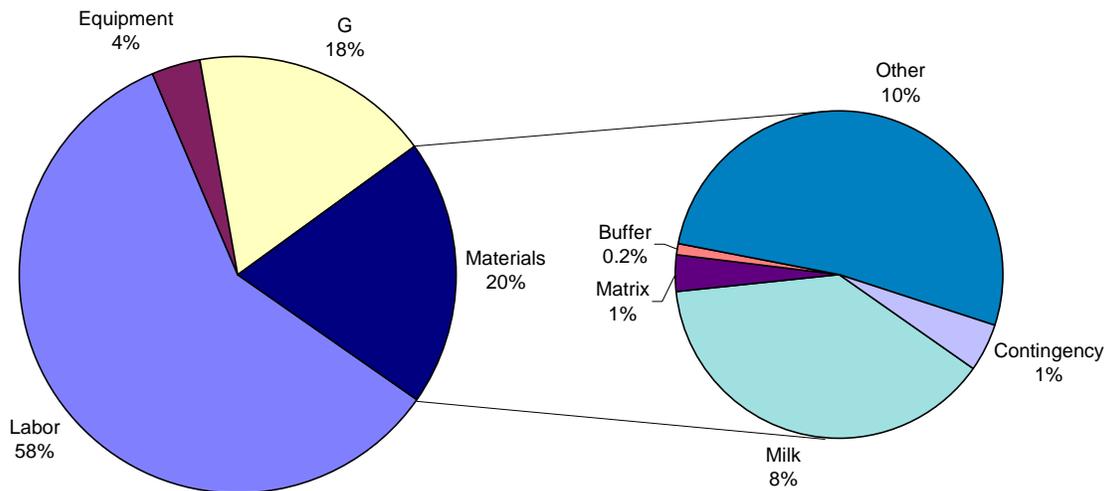


Figure 9. Manufacturing cost distribution for FVIII immunoaffinity process at a production scale of 0.12 kg/yr. The costs distribute among raw materials, labor, equipment, and a grouping of utilities, taxes, building, and overheads (G.) The pie chart on the right represents the contribution of matrix, milk, buffer, contingency, and other (operating and lab supplies, maintenance, and viral inactivation solvent) to raw material costs.

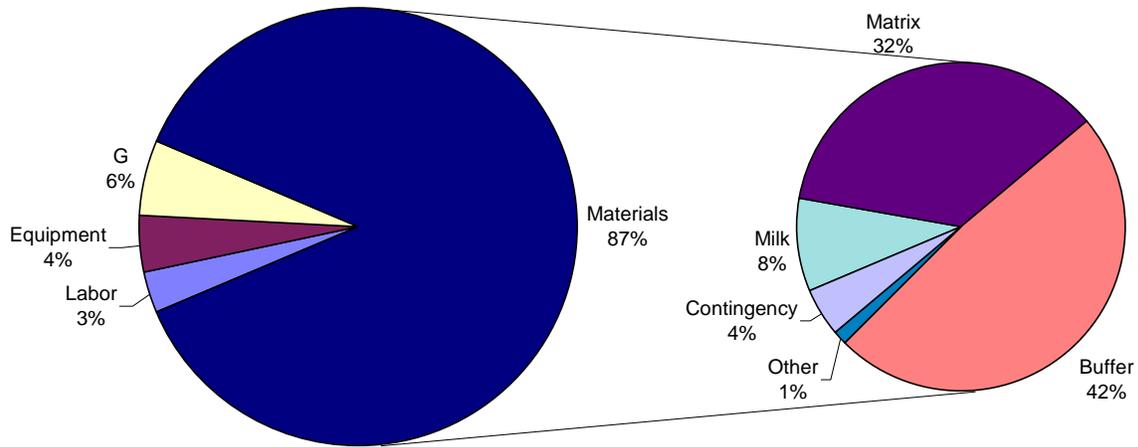


Figure 10. Manufacturing cost distribution for hSA DA process at a production scale of 35,000 kg/yr. The costs distribute among raw materials, labor, equipment, and a grouping of utilities, taxes, building, and overheads (G.) The pie chart on the right represents the contribution of matrix, milk, buffer, contingency, and other (operating and lab supplies, maintenance, and viral inactivation solvent) to raw material costs.

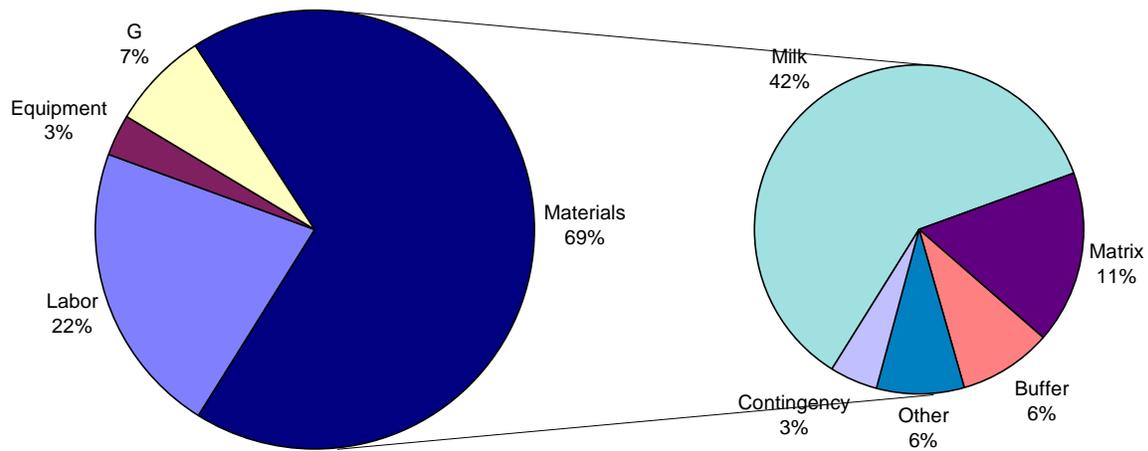


Figure 11. Manufacturing cost distribution for PC immunoaffinity process at a production scale of 90 kg/yr. The costs distribute among raw materials, labor, equipment, and a grouping of utilities, taxes, building, and overheads (G.) The pie chart on the right represents the contribution of matrix, milk, buffer, contingency, and other (operating and lab supplies, maintenance, and viral inactivation solvent) to raw material costs

Table 1. Process Specifications for Recovery of hSA, hPC, and FVIII.

	hSA Fall-Through Cow Milk	hSA Direct Adsorption Cow Milk	hPC Immunoaffinity Pig Milk	FVIII Immunoaffinity Pig Milk
Step 1	Ion Exchange*	Ion Exchange*	Ion Exchange*	Ion Exchange*
Step 2	Ion Exchange	Ion Exchange	Immunoaffinity	Immunoaffinity
Step 3	Hydrophobic	Ion Exchange	Ion Exchange	Ion Exchange
Step 4	Affinity (Anti-bSA)	Affinity (Anti-bSA)	Not Present	Not Present
UF1**	Present	Present	Present	Present
UF2**	Not Present	Present	Present	Present
UF3**	Present	Present	Present	Present
UF4**	Present	Present	Present	Present
Centrifuge	Present	Present	Present	Present
Viral Inactivation	Present	Present	Present	Present
Formulation	Solution	Solution	Solution	Freeze Dried

* Expanded Bed Mode, Fall Through or Direct Adsorption

** Ultrafiltration Units

Table 2. Formulated hSA, hPC, FVIII Costs for Given Base Sets of Parameters.

	hSA Direct Adsorption	hSA Fall- Through	hPC Immuno affinity	FVIII Immuno affinity
Expression level, gr/L	5	5	0.5	0.05
Ion Exchange				
Column Yield, %	80**	80	80	80
Immunoaffinity				
Column Yield, %	50	50	50	50
Hydrophobic				
Column Yield, %	---	80**	---	---
Superficial				
Velocity, cm/min	3	10	3	3
Ion Exchange				
Column Capacity, gr/L	5	20	5	5
Immunoaffinity				
Column Capacity, gr/L	0.88*	0.88*	0.88**	0.88**
Hydrophobic				
Column Capacity, gr/L	---	5	---	---
Production Scale, kg/yr	35,000	35,000	90	0.12
Cost, \$/gr	3.56	2.20	129	11,780
Capital Investment, \$	66,615,000	36,868,000	4,606,000	540,150

* For Anti-bSA column

** For high resolution step

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Vita

I, Selim Elhadj, was born in Algiers (Algeria) and came to the U.S. for graduate studies. I am currently holding B.S. and M.E. degrees in Chemical Engineering from Virginia Tech. My main field of interest is biotechnology and am pursuing PhD studies in this field. Drug therapies are of special interest to me since I believe much work remains to be done to achieve effective, safe, and affordable drugs.