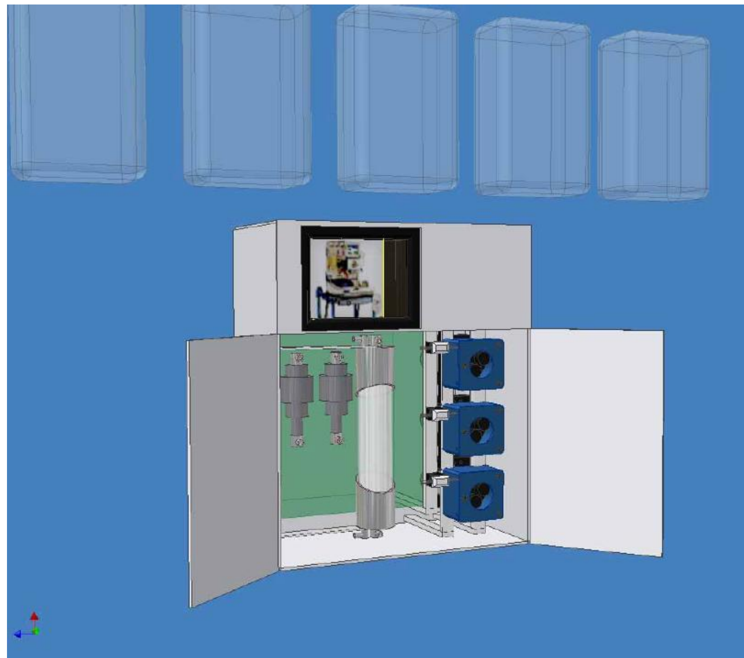
Development of a Portable Method for Preparing Previously Frozen Red Blood Cells for Transfusion

Final Project Report

IPRO 304C

Spring 2005



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ABSTRACT

When blood is collected from a donor, it is typically separated into its various components (red blood cells, white blood cells, plasma, and platelets). Red blood cells may be stored for up to 42 days after donation. If frozen, however, they may be stored for ten years or more. In order to freeze and thaw red blood cells without damaging them, it is necessary to use a cryoprotective agent such as glycerol. After the cells have been thawed, the cryoprotective agent must be removed before the cells can be transfused to a patient. Since the need for red blood cells often arises under adverse conditions, such as warfare or major disasters, there is a need for a portable method of preparing previously frozen red blood cells for transfusion. The objective of this project was to develop a device for washing multiple units of frozen red blood cells in emergency situations. The key parameters emphasized in this project were: selection of the optimal technology for the washing process; making the device portable by minimizing its weight, dimensions, and power requirements while maximizing its speed and degree of automation; minimization of wash-solution usage by considering techniques such as recycling; minimization and efficient disposal of biological waste; and compliance of the washed blood with the relevant standards for transfusion. In this project, our group successfully constructed computer models for the operation of both centrifugal and membrane-based cell washing devices. We have designed a membrane-based device, using off-the shelf components, that will produce the same quality of red blood cells for transfusion as the centrifugal device currently used by the U.S. military, at approximately one quarter of the cost and in approximately one third of the time.

TABLE OF CONTENTS

BACKGROUND 2

OBJECTIVE 5

METHODOLOGY 5

ASSIGNMENTS 6

OBSTACLES 7

RESULTS 8

CONCLUSIONS AND RECOMMENDATIONS 28

REFERENCES 31

ACKNOWLEDGEMENTS 34

BACKGROUND

Human blood is a suspension of three types of blood cells (red blood cells, white blood cells, and platelets) in an aqueous solution called plasma. Each type of cell plays a specific role in maintaining life. The current trend in medical blood transfusion is to deliver to the patient only the specific blood component or components that he or she needs. This allows efficient use of the existing blood supply. Therefore, when whole blood is collected from a donor, it is typically separated into its various cellular and plasma components.

If refrigerated at 1-6 °C, red blood cells may be stored for up to 42 days after donation, with the use of appropriate additive solutions. If frozen, however, they may be stored safely for ten years or more. The United States military currently maintains stockpiles of frozen red blood cells in bases around the world. Non-governmental organizations such as the American Red Cross also store frozen red blood cells for use in emergency situations.

In order to freeze and thaw red blood cells without inducing cell rupture (hemolysis), it is necessary to use a cryoprotective agent such as glycerol. After the cells have been thawed, the cryoprotective agent must be removed before the cells can be transfused to a patient.

Since the 1960s, deglycerolization of frozen red blood cells has typically been accomplished using various centrifugal cell washing devices. In general, these devices are not portable. For example, one such device, the COBE 2991 Cell Processor, distributed by Gambro Blood Component Technologies, is over five feet tall, and weighs 435 pounds. The size, weight, and power requirements of these devices mean that their use is restricted to a laboratory environment.

The need for deglycerolized red blood cells often arises under adverse conditions, such as warfare or major disasters. Under such conditions, a well-equipped, modern blood component laboratory may not be available. Therefore, there is a need for a portable method of preparing previously frozen red blood cells for transfusion.

In order to be portable, a device for washing multiple units of frozen red blood cells in emergency situations must be both lightweight and compact. Its power requirements must be minimized. Because it may be used in remote locations with minimal logistical support, utilization of consumables such as wash solutions must also be minimized. Because a dedicated, trained operator may not be available, the device should have the highest obtainable degree of automation. Because the device will be used in situations in which there is an immediate need for red blood cells for transfusion, it should accomplish the washing process in the shortest possible time. Finally, the deglycerolized red blood cells must comply with the relevant standards for blood transfusion.

The vast majority of deglycerolization processes that have been used or proposed in the past make use of one of two separation technologies: centrifugation or membrane filtration.

In centrifugation, solid particles suspended in a liquid medium (in this case, red blood cells suspended in a glycerol-saline solution) are separated on the basis of their density by means of rotation at a high angular velocity. A centrifugal force is exerted on the particles, causing them to sediment in the direction outward from the center of rotation. The supernatant (i.e. the glycerol-saline solution) may then be drawn off and disposed of. Additional wash solution may then be added, and this process may be repeated until the cells have been washed to a sufficient extent. The application of this technique to the deglycerolization of red blood cells was first demonstrated by Tullis et al in 1960. During the following decade, a number of workers, including Tullis, Latham, Haynes, Valeri, and others refined the technique, and, in 1969, the American Red Cross adopted this method for use in its blood banks (Neva, 1974; Valeri, 1976). Currently available commercial devices based on this technique include the COBE 2991 Cell Processor, mentioned above, and the Haemonetics ACP 215, which is currently used by the U.S. military for the deglycerolization of frozen red blood cells.

In membrane filtration, materials are separated by selective exchange across the walls of a membrane. Typically, for filtration applications, the walls of the membrane are porous, and the separation is by size. Liquids and particles smaller than the pore size may pass freely through the pores, while larger particles are excluded. The material passing through the walls of the

membrane is known as the permeate, while the excluded material is called the retentate. Membranes of numerous types and geometries are available. Hollow fiber membranes consist of hollow cylinders with porous walls. The permeate passes through the walls of the cylinder, while the retentate flows through the cylinder's interior, called the lumen. Typically, the outer diameter of the cylinder is less than 1 mm. Tens of thousands of individual hollow fibers are often grouped together in a bundle in order to achieve a large surface area (Moch, 1997).

Membrane technologies are widely used for numerous blood cell processing applications, including hemodialysis, hemofiltration, autotransfusion, and plasmapheresis. A membrane filtration device for deglycerolization of thawed red blood cells is not currently available commercially; however, such devices have been described in the medical literature. In 1980, Zelman et al reported on the use of a novel blood bag constructed of a semipermeable membrane material for glycerolization and deglycerolization of red blood cells. In the late 1980s and early 1990s, workers including Radovich and Van Reis investigated deglycerolization procedures using hollow fiber and flat sheet membranes, respectively. In 1996, Castino and Wickramasinghe described parameters for the optimization of a hollow fiber microfiltration system for deglycerolization of red blood cells.

Over the course of this project, our group has evaluated the suitability of each of these two technologies (centrifugation and membrane filtration) for application in a portable deglycerolization device for red blood cells. The two technologies were evaluated based on the criteria of weight, size, power requirements, consumables utilization, automation, speed, and standards compliance, as described above.

As mentioned above, the United States military currently uses the Haemonetics ACP 215, a benchtop centrifugal cell washing device supplied by Haemonetics Corporation, for deglycerolization of frozen red blood cells. Our group has successfully modeled the performance of this device, and determined that it is the optimal configuration for a centrifugal device, based on the criteria listed above.

Our group has also constructed a computer model of a membrane separation process. On the basis of this model, we have designed a hollow fiber membrane-based system for deglycerolizing packed red blood cells, using off-the shelf components. This device will produce the same quality of red blood cells for transfusion as the Haemonetics ACP 215, at approximately one quarter of the cost per unit, and in approximately one third of the time. This objective, and the methods we have used to accomplish our goal, are outlined below.

OBJECTIVE

The objective of this project was to develop a system for washing multiple units of frozen red blood cells in emergency situations. The key parameters considered in the project were:

- Selection of the optimal technology for the washing process.
- Portability of the system, in terms of weight, dimensions, and power requirements.
- Speed and automation of the system.
- Consumption of disposables, such as wash solution.
- Disposal of biological waste generated in the process.
- Cost of the device and related disposables.
- Compliance of the washed blood with the relevant standards for blood transfusion.

METHODOLOGY

To accomplish the goals set forth in the project plan, the team divided into two groups: one to research centrifugation, the other to research membrane filtration. A third group, consisting of members of the two previous groups, researched the relevant standards for blood transfusion. During this research phase, the team consulted with Dr. Radovich on a regular basis in order to refine its goals.

The two groups constructed computer models of the centrifugal and membrane processes. On the basis of these models, the team selected the membrane-based process as the optimal process

for our application. The team then divided into new groups in order to design a membrane-based system for deglycerolizing packed red blood cells, using off-the-shelf components.

The tasks divided among the new groups included: refining the computer model for the membrane process; designing process control systems; determining procedures for disposal of biowaste; locating and obtaining prices for off-the-shelf components (including membranes, pumps, and sensors); drafting the device using Autodesk Inventor; and performing a detailed cost analysis.

ASSIGNMENTS

	Eric	**Derek**	**Karen**	**Jennifer**	**Suman**	**Brogan**	**Ahlam**	**Oscar**	**Venkata**	**Clara**	**David**	**Joseph**
Project plan			■	■	■	■						
Meeting minutes					■	■					■	
Centrifugation research group		■	■			■	■		■			■
Membrane filtration group	■			■	■			■		■	■	
Standards research group								■	■	■		■
Mid-term progress report	■	■	■	■	■	■		■	■	■	■	
Optimization of membrane parameters			■	■		■	■	■				
Process control for membrane system		■				■				■	■	■
Cost analysis and selection of membranes		■	■		■		■	■				

	Eric	Derek	Karen	Jennifer	Suman	Brogan	Ahlam	Oscar	Venkata	Clara	David	Joseph
Cost analysis and selection of pumps												■
Cost analysis and selection of sensors											■	
Cost analysis of ACP-215					■		■					■
AutoCAD drawing												■
Detailed cost analysis		■										
PowerPoint presentation	■	■	■	■		■					■	
IPRO Day poster			■	■								
Abstract		■	■	■							■	
Final report		■	■	■	■			■			■	■
CD-ROM						■						

OBSTACLES

Since this is the first semester of this IPRO, our group has had to start from scratch. It was therefore a challenge to learn all of the material required for this project in a short period of time. There were many new concepts to learn, and a great deal of new terminology to master. However, as a team, we managed to accomplish a great deal.

A second obstacle involved was presented by the hesitancy of many suppliers to share pricing information with us. While some suppliers were willing to divulge pricing information over the phone, others were less cooperative. In particular, obtaining price information regarding the Haemonetics ACP-215 took over a week and required numerous e-mails. Manufacturers of hollow fiber membranes were also reluctant to divulge prices.

A third obstacle involved the project goal of developing a method for recovery or recycling of wash solution. While the amount of wash solution used was minimized, no satisfactory method of recycling or recovery of the wash solution was found. A number of methods for separating glycerol from saline wash solution were proposed, including distillation and chromatography; however, they were considered to be impractical, and were not pursued further. It is hoped that a future IPRO team may find a more practical means of achieving this goal.

The above were the main problems encountered during the semester. Most of the problems were tackled as they arose, and as a team, we were able to find solutions to them.

RESULTS

At present, the deglycerolization of red blood cells for transfusion is typically accomplished through the process of centrifugation. In this process, solid particles are separated from a fluid in which they are suspended by means of centrifugal forces acting on the particles as they are rotated at a high angular velocity. In this case, the particles are red blood cells, and the fluid is a glycerol-saline solution. Figure 1, on the following page, is a schematic representation of the operation of the Haemonetics ACP 215, a centrifugal cell washing device currently used by the U.S. military for the deglycerolization of frozen red blood cells. The process parameters used in Figure 1 are taken from the standard operating procedure issued by the U.S. Naval Blood Research Laboratory. The Haemonetics ACP 215 is described in U.S. Patents No. 6,267,925 and 6,440,372. Product specifications are available on the Haemonetics Corporation website.

According to the product specification sheet, the Haemonetics ACP 215 unit has a volume of 2.55 cubic feet and weighs 55 pounds. Using this device, and following the Naval Blood Research Laboratory standard operating procedure, the deglycerolization process for a single unit of red blood cells takes between 40 and 60 minutes. The current market price for this device, as provided by Haemonetics Corporation, is $46,995.

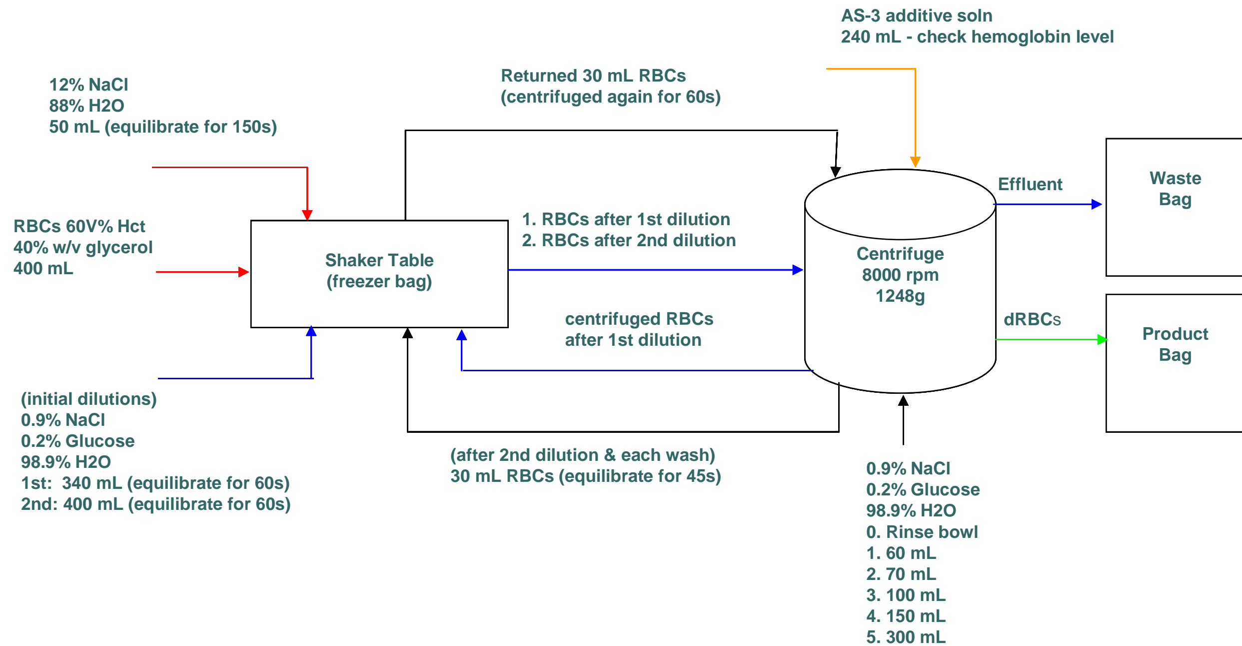

Figure 1. Deglycerolization of red blood cells using centrifugation in the Haemonetics ACP 215 system.

The process begins with 400 mL of thawed, glycerolized red blood cells, at a hematocrit (volume fraction of red blood cells) of 60%. The red blood cells are contained in a 800 mL polyvinyl chloride (PVC) freezer bag. A 50 mL portion of 12% sodium chloride saline solution is added to the bag, and the mixture is agitated on a shaker table. The saline causes the glycerol to be expelled from the red blood cells due to the osmotic pressure difference between the saline and glycerol. After shaking, the thawed red blood cells are allowed to equilibrate with the hypertonic saline solution for 150 seconds. The contents of the bag are then diluted with 340 mL of a wash solution containing 0.9% sodium chloride and 0.2% glucose, and allowed to equilibrate for an additional 60 seconds. The contents of the bag are then transferred by means of a pump into the centrifuge bowl, where they are spun at 8000 rpm. As the centrifuge spins, the supernatant is continuously is drawn off to a waste bag. The red blood cells are then returned from the centrifuge bowl to the freezer bag, and a second dilution is performed, using 400 mL of wash solution. Following a 60 second equilibration delay, the contents of the bag are transferred back into the centrifuge bowl. At this point, the blood cells are ready to be washed five times, with each succeeding wash increasing in volume, starting with 60 mL and finishing with 300 mL. After the five wash cycles have been completed, the washed red blood cells are resuspended in AS-3 additive solution. The AS-3 additive solution allows the deglycerolized red blood cells to

be stored for up to two weeks at 4°C. After the AS-3 additive solution has been added, the operator of the device must manually assess the degree of hemolysis of the red blood cells. Hemolysis (rupture) of the red blood cells may occur during the process for either two reasons. First, when the hypertonic saline solution is added at the beginning of the process, the rapid entry of saline and rapid exit of glycerol from the cells may cause the cell membrane to rupture, particularly if the cells have been glycerolized incorrectly. Second, the cells may rupture due to shear stress during centrifugation, if the centrifuge speed is too high. In either case, the rupture of the red blood cells releases hemoglobin, which will affect the color of the solution. The operator therefore manually examines the color of the solution and compares it to a set of printed cards provided with the device. If the operator finds the color of the solution to be acceptable, the final product is transferred to the product bag.

The operation of the Haemonetics ACP 215 was modeled in Microsoft Excel, using Equations 1-6, below. These equations were obtained from Geankoplis, 2003.

$$v_t = \frac{\varpi^2 r D_p^2(\rho_p - \rho)}{18\mu} = \frac{a_e D_p^2(\rho_p - \rho)}{18\mu}$$

Where:
v_t = settling velocity, m/sec
ω = angular velocity, rad/sec
r = centrifuge bowl radius, m
D_p = radius of solid particles, m
ρ_p = density of solid, kg/m^3
ρ = density of liquid, kg/m^3
μ = viscosity of liquid, Pa·s
a_e = angular acceleration, rad/s^2

Equation 1. Settling velocity for centrifugal process.

$$t_{cycle} = \frac{r}{v_t}$$

Where:
t_{cycle} = time per cycle, sec
r = centrifuge bowl radius, m
v_t = settling velocity, m/sec

Equation 2. Cycle time for centrifugal process.

$$V_{cycle} = \left(\frac{5}{3}\frac{mL}{s}\right) * t_{cycle}$$

Where: V_{cycle} = volume per cycle, mL
t_{cycle} = time per cycle, sec

Equation 3. Volume per cycle for centrifugal process.

$$N = \frac{V}{V_{cycle}}$$

Where: N = number of cycles
V = total volume, mL
V_{cycle} = volume per cycle, mL

Equation 4. Number of cycles for centrifugal process.

$$t = N \cdot t_{cycle}$$

Where: t = total time, sec
N = number of cycles
t_{cycle} = time per cycle, sec

Equation 5. Total time for centrifugal process.

$$V_{bowl} = \pi b(r_2^2 - r_1^2)$$

Where: V_{bowl} = effective centrifuge bowl volume, m^3
b = height of bowl, m
r_2 = outer radius, m
r_1 = inner radius, m

Equation 6. Volume of bowl used in centrifugal process.

The total process time for the ACP 215, determined by substituting the relevant parameters for the ACP 215 into the equations above, was approximately 40 minutes, which is consistent with the time given in the standard operating procedure. In order to decrease the cycle time, two main parameters can be changed: bowl dimensions or speed of rotation. An increase in the bowl dimensions was ruled out, as this would require an overall increase in the dimensions of the

device, which would have and adverse affect on portability. Increasing the rotational velocity is also not practical; the maximum acceleration for red blood cells is 1248g, where g is the gravitational acceleration (9.81 m/s^2). At greater accelerations, hemolysis may occur. In addition, increasing the centrifuge speed would require a larger motor, which would require an increase in weight and power consumption. Again, such an increase would have a detrimental affect on the portability of the system. As a result, it was concluded that a portable device for preparing previously frozen red blood cells for transfusion could not be obtained by making modifications to the ACP 215.

Since there is not currently a commercially-available membrane filtration device for the deglycerolization of red blood cells that can be directly compared to the Haemonetics ACP 215, it was necessary to design a model membrane process, in order to establish a basis for comparison. This model could be used to determine the process time and volume of wash solution required for the membrane process. These values of these parameters could then be compared to those calculated for the centrifugal process.

The first decision in the design of the membrane filtration process revolved around whether to employ a continuous process or a discontinuous process. In a discontinuous process, a set volume of wash solution is added. The solution is run through the membrane continuously for a set period of time, while permeate is removed. Another volume of wash solution is then added, and this process is repeated until the glycerol concentration is reduced to an acceptable level. The volume of wash solution required for a discontinuous process is given by Equation 7, below.

$$V_w = \frac{V_0}{\left(\frac{C_f}{C_0}\right)^{\frac{1}{n}}} - V_0$$

Where:
V_w = total volume of wash solution required, mL
V_0 = initial volume of glycerolized red blood cells, mL
C_f = final concentration of glycerol
C_0 = initial concentration of glycerol
n = number of process stages

Equation 7. Volume of wash solution required for discontinuous membrane filtration.

Using Equation 7, and setting the initial concentration of glycerol C_0 to 0.24, the final glycerol concentration C_f to 0.0079, and the initial volume of glycerolized red blood cells V_0 to 333 mL, if the total volume of wash solution V_w is plotted against the number of process steps n, the graph shown in Figure 2, below, is obtained. The initial glycerol concentration of 24% represents the glycerol concentration after the thawed red blood cells have been treated with the hypertonic 12% saline solution, as described above. It is assumed that the cells have been equilibrated with the hypertonic saline solution prior to the start of membrane filtration. The final glycerol concentration was set to 0.79% because the maximum permissible level of residual glycerol in red blood cells for transfusion, as established by the American Association of Blood Banks (AABB), is 1%. The value of 0.79% was chosen in order to provide a margin of safety.

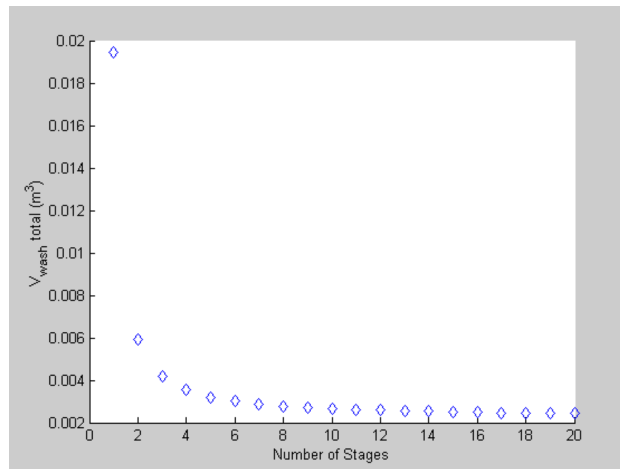

Figure 2. Number of stages vs. wash solution for a discontinuous membrane filtration process.

Figure 2, above, demonstrates that in order to minimize the volume of wash solution required, the number of wash stages should be maximized. This is realized in a continuous process, which is equivalent to an infinite number of wash stages.

In a continuous membrane filtration process, wash solution is added in a continuous fashion, at the same rate at which permeate is removed. The retentate is continuously recirculated through the membrane until the concentration of glycerol is reduced to an acceptable level. Equations 8-10 express, respectively, the volume of wash solution required, the volumetric flow rate, and the total process time, for a continuous membrane filtration process.

$$V_w = V_0 \ln(\frac{C_0}{C_f})$$

Where: V_w = volume of wash solution, mL
V_0 = initial volume of glycerolized red blood cells, mL
C_0 = initial concentration of glycerol
C_f = final concentration of glycerol

Equation 8. Volume of wash solution required for continuous membrane filtration.

$$Q = \mathrm{UF} \cdot \Delta P \cdot A$$

Where: Q = volumetric flow rate, mL/sec
UF = ultrafiltration coefficient, N·mL/sec
ΔP = transmembrane pressure, Pa
A = total effective surface area of membrane, m^2

Equation 9. Volumetric flow rate for continuous membrane filtration process.

$$t = \frac{V_0 + V_w}{Q}$$

Where: t = total time required for filtration, seconds
V_0 = initial volume of glycerolized red blood cells, mL
V_w = initial volume of glycerolized red blood cells, mL

Equation 10. Total time required for continuous membrane filtration process.

Using Equations 8, above, the total volume of wash solution required was calculated to be 1.1 L. Using equations 9 and 10, the graphs shown in Figures 3 and 4, on the following page, may be obtained. The upper graph in Figure 3 shows the required volumetric flow rate as a function of the desired process time for a constant membrane surface area; the lower graph in Figure 3 shows the required membrane surface area as a function of the desired process time for a constant volumetric flow rate. The upper graph in Figure 4 shows the required ultrafiltration coefficient as a function of the membrane surface area for a constant transmembrane pressure, and the lower graph in Figure 4 shows the required transmembrane pressure as a function of the

membrane surface area for a constant ultrafiltration coefficient. These graphs were generated using MatLab, version 6.1.

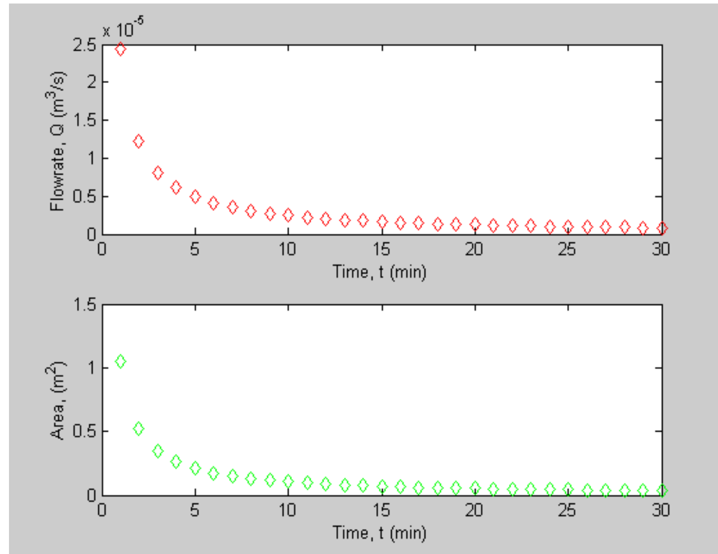

Figure 3. Volumetric flow rate and membrane surface area vs. process time for continuous membrane filtration process.

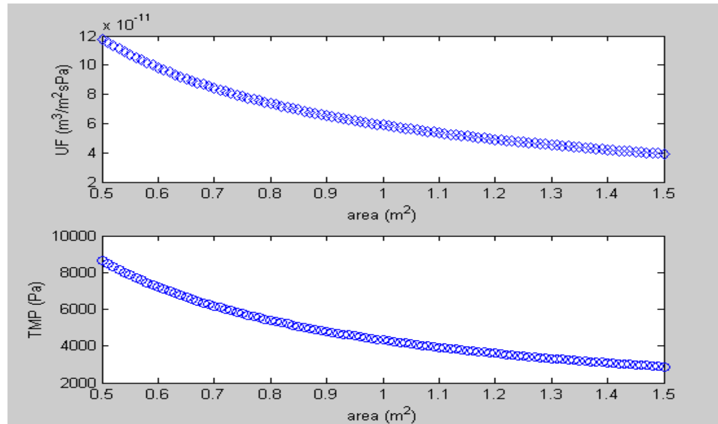

Figure 4. Ultrafiltration coefficient and transmembrane pressure vs. membrane surface area for continuous membrane filtration process.

In order to avoid stress-induced hemolysis, it is necessary to consider the shear stresses developed in the red blood cells as they flow through the membrane device. The relationship between shear stress, exposure time, and hemolysis is quite complex (Paul et al, 2003). The chemical and phyisical nature of the surface to which the red blood cells are exposed also affects their susceptibility to hemolysis (Sowemimo-Coker, 2002). However, numerous investigators

agree on the existence of a critical shear stress, τ_{crit}, below which hemolysis will not occur; the various models that have been proposed diverge on the value of τ_{crit} and on the exact nature of the mathematical relationship between shear stress, exposure time, and hemolysis at stresses above τ_{crit}. The lowest value of τ_{crit} reported in the literature is 150 Pa (Leverett et al, 1972). Other studies have found values of τ_{crit} that are several times higher; most recently, Paul et al reported a value 350 Pa. For purposes of our design, we adopted the low value of τ_{crit} reported by Leverett.

Shear stress is defined as the shear rate multiplied times the viscosity. Treating each individual fiber of the hollow fiber bundle as a cylindrical pipe, the shear rate is expressed by Equation 11, below.

$$\dot{\gamma} = \frac{4Q_{fiber}}{\pi R^3}$$

Where: $\dot{\gamma}$ = shear rate, sec^{-1}
Q_{fiber} = volumetric flow rate through individual fiber of hollow fiber bundle, mL/sec
R = inner radius of hollow fiber, cm

Equation 11. Shear rate for cylindrical pipe flow.

The volumetric flow rate through each individual fiber of the hollow fiber bundle was obtained by dividing the total volumetric flow rate, Q, by the number of fibers in the bundle.

The viscosity of blood is a function of its hematocrit (Wells and Merrill, 1962). A number of theoretical expressions relating viscosity and hematocrit have been proposed in the medical literature. For the purposes of our design, a simplified expression was used. This expression was obtained from a Microsoft Excel spreadsheet distributed by the Medical Algorithms Project, an online service of the Institute for Algorithmic Medicine. The expression is given in Equation 12, on the following page.

$$\log \mu = 0.01138(\mathrm{Hct}) + 0.03699$$

Where: μ = viscosity, cP
Hct = hematocrit, percent

Equation 12. Viscosity as a function of hematocrit.

The hematocrit of the solution remains essentially constant throughout the continuous membrane filtration process; since wash solution is added at the same rate that permeate is removed, the total volume remains constant at all times, and since the red blood cells cannot pass through the walls of the membrane, the volume of red blood cells also remains constant. Therefore, the volume fraction occupied by red blood cells remains constant. This permits a simpler analysis than would be required for a discontinuous process, in which the hematocrit varies as a function of time as permeate is removed during each wash cycle.

The shear stress experienced by the red blood cells during the washing process was calculated according to Equation 13, below.

$$\tau = \mu(\mathrm{Hct})\dot{\gamma}$$

Where: τ = shear stress, Pa
μ = viscosity, Pa·sec
$\dot{\gamma}$ = shear rate, sec^{-1}

Equation 13. Shear stress developed in fluid flow.

Due to the number of parameters that can be independently varied, there exist an infinite number of solution sets for A, UF, ΔP, and Q that will minimize the total process time t, volume of wash solution V_w, and shear stress τ. Many hollow fiber membrane modules for blood applications are available commercially. Each of these membrane modules has a well-characterized surface area, ultrafiltration coefficient, number of fibers, and fiber diameter. The values of these parameters are available from the membrane manufacturers. Therefore, rather than designing a custom membrane for our application, our group evaluated a number of off-the-shelf hollow fiber membranes.

The values obtained for the Hemocor HPH series of hemoconcentrators, manufactured and distributed by Minntech Filtration Technologies, are summarized in Table 1, below. Each module contains a bundle of polysulfone fibers, each having an inner diameter of 200 μm. The dimensions and operating pressures for each module were obtained from the manufacturer's specifications. The shear stress and process time for each membrane were then calculated, using the equations above.

	HPH 400	**HPH 700**	**HPH 1000**	**HPH 1400**
Pressure, kPa	8.1	19	11	10
Transmembrane pressure, kPa	105.0	110.8	107.0	106.5
Unit inner diameter, cm	3	3.2	3.2	3.6
Length, cm	13.8	25.3	25.3	25.3
Total membrane surface area, m^2	0.3	0.71	1.1	1.3
Number of hollow fibers	3,500	4,500	6,900	8,200
Shear stress, Pa	2.95	3.74	2.24	2.06
Process time, min	19.2	11.7	12.6	11.6

Table 1. Comparison of Minntech Hemocor HPH hemoconcentration membranes for use in a portable device for deglycerolization of red blood cells.

On the basis of the parameters considered in Table 1, above, the HPH 1400 membrane module was selected. The use of this module will allow a rapid process time (under 12 minutes) at a low shear stress (less than 1.5% of τ_{crit}). The module is shown in Figure 5, on the following page.

After determining the process parameters and selecting a membrane, it was necessary to design a set of process controls. Again, the decision was made to evaluate off-the-shelf components rather than designing custom components.

The key process control parameter was determined to be the concentration of glycerol. When the glycerol concentration falls below the target value of 0.79%, the washing process is complete,

Figure 5. Hemocor HPH 1400 hollow fiber membrane module (Minntech Filtration Technologies).

and the washed red blood cells can be returned to the storage bag. In order to monitor the glycerol concentration, it was decided to use an inline refractometer on the permeate line. This device measures the concentration of glycerol by its effect on the refractive index of the solution. It was decided to locate the refractometer on the permeate line for three reasons. First, due to the presence of the membrane, there will be no red blood cells on the permeate side; the presence of solid particles such as red blood cells would interfere with the accurate measurement of the refractive index. Furthermore, since the concentration of glycerol in the retentate should be lower than the concentration of glycerol in the permeate, basing the process control on the concentration of glycerol in the permeate provides a margin of safety. Finally, locating the refractometer on the permeate side eliminates issues regarding blood contact and sterility; the presence of the membrane should prevent any blood cells from reaching the refractometer, and, at any rate, all of the solution flowing past the refractometer is directed into the waste bag.

A number of inline process refractometers are commercially available; however, most are far too large for use in a portable device. For instance, the UR-20 In-Line Process Refractometer, manufactured and distributed by Maselli Measurements, is approximately seven inches in diameter, five inches long, and weighs seven pounds. While this may be suitable for industrial applications, it is clearly not appropriate for a portable medical device. Furthermore, its cost is prohibitive ($8200). However, the PR-111 Refractometer, manufactured and distributed by AFAB Industries, was found to be a good fit for our application. The sensor head is 1.485 inches

in diameter and 2.215 inches long, and weighs less than 1 pound. The device contains a built-in thermistor in order to compensate for the variation of refractive index with temperature. While the device is sold with an electronics console for autonomous operation, the sensor itself provides a 4-20 mA analog output which could be fed into the process control circuitry for our device. The PR-111 Refractometer costs $995; an adapter for 1/8-inch tubing costs an additional $135, and there is an optional calibration fee of $225. The PR-111 Refractometer is shown in Figure 6, below.

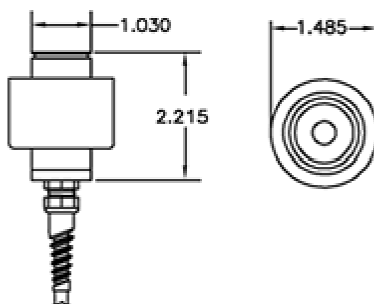

Figure 6. PR-111 Refractometer (AFAB Industries).

The next important process parameter is the volumetric flow rate. In order for the process to be continuous, it is necessary for wash solution to be added at the same rate that permeate is removed. Therefore, it is necessary to continuously monitor the flow rates at these two points and adjust pump speeds so that the two rates are equal. It is also necessary to monitor the flow rate of thawed red blood cells from the storage bag. Furthermore, in order to maintain sterile conditions, it is desirable that the flow sensors do not have direct blood contact. (Otherwise, it would be necessary to sterilize the flow sensors after deglycerolizing each unit of blood). Initially, Ultrasonic Flow-25 flow sensors, distributed by Crydom Magnetics, were considered. These sensors automatically compensate for viscosity and temperature, and have high accuracy; however, they can not be attached over the outside of sterile tubing. Therefore, it was decided to use ME-2PXL Sterile Tubing Flowsensors, manufactured and distributed by Transonic Systems, Inc. These ultrasonic flow meters are specifically designed to be clamped to the outside of medical tubing, and can read blood flow from 15 to 600 mL/min, which goes well beyond the operational limits of our device.

As an additional process control, it was decided to install a UV-visible spectrophotometer on the permeate line. The purpose of the spectrophotometer is to measure the concentration of free hemoglobin. As mentioned above, hemolysis will cause hemoglobin to be released into the solution. We do not anticipate the occurrence of hemolysis during the normal operation of the device, so the presence of free hemoglobin in the permeate would indicate a serious problem requiring operator Hemoglobin has an absorbance maximum at 576 nm. A standard spectrophotometric method for the determination of hemoglobin, known as the Cripps method, measures absorbance at 560, 576, and 592 nm. The absorbance values at 560 nm and 592 nm are used to construct a linear baseline (Sowemimo-Coker, 2002). In order to carry out this measurement, our group selected the S2000 Miniature Fiber Optic Spectrophotometer, from Ocean Optics. This is a low-cost, compact, high-performance spectrophotometer. Its weight is under 1 pound, and its cost is under $2000. Since it will be installed on the permeate line, blood contact is not an issue.

After designing and selecting components for the process control systems, it was necessary to select pumps and motors. A total of three pumps, with associated motors, are needed: one for the addition of wash solution, another for the removal of permeate, and a recirculating pump for the retentate. Three Randolph-Austin 250-PHO small volume pump heads were chosen. This peristaltic pump head readily fits standard medical tubing, and has quick release pins to expedite the tubing replacement process. The pump head is shown in Figure 7, below. For the motors, three LEXEL Corporation 2-pole shaded pole motors were chosen. These are 1/100 fractional horsepower, 3000 rpm maximum motors for pump use. They are relatively inexpensive and light, and fit well with the pump heads chosen.

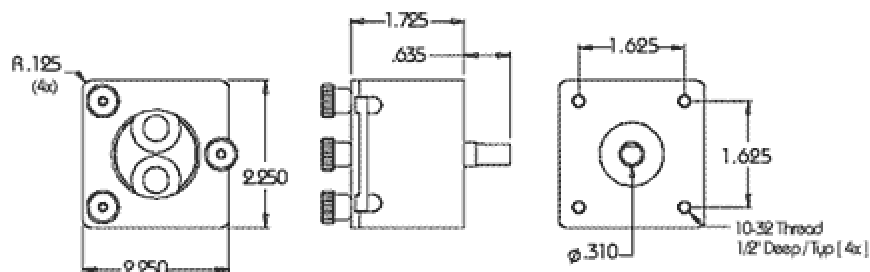

Figure 7. 250-PHO small-volume pump head (Randolph-Austin Co.).

Finally, a visual display for the user interface was selected. One model considered was the MMI 5.7" DSTN 256 Color LCD Display. This color display is specially designed to replace mechanical pushbuttons in harsh manufacturing and commercial conditions. However, this barebones unit does not have the printing features or the color quality that would best fit with this machines use. Also, alarm data is not automatically displayed on the screen with the software provided. Therefore, an IDEC SmartTouch 5.7" Color LCD Touchscreen (HG2F-SS22VCF) was selected. This LCD touchscreen has a small size and is relatively cheap. It is designed for commercial and industrial use, and would be quite durable. Alarm data can be displayed on the monitor. The device also has a serial printer output to facilitate the printing of records directly from a screen capture of the monitor. Accurate records can therefore be kept in both electronic and hard copy format.

Based on the components selected, and making estimates regarding the weight of the enclosure, power supply, and miscellaneous electronic components, the total weight of the membrane filtration device was estimated. This is shown in Table 2, below.

Component	Unit weight, lbs.	Number required	Total weight, lbs.
Pump head	2	3	6
Motor	3	3	9
Flow meters	0.1	3	0.3
Refractometer	1	1	1
UV-visible spectrophotometer	1	1	1
LCD touchscreen	2	1	2
Tubing	0.5	1	0.5
Miscellaneous electronics (estimated)	3	1	3
Enclosure (estimated)	6	1	6
Power supply (estimated)	5	1	5
Total Weight:			33.8

Table 2. Weight of continuous membrane filtration device for deglycerolization of red blood cells.

This is represents a low-end estimate for the weight of the device. Without additional electrical design work, and a final design for the enclosure, this is the closest estimate available. To be safe, an additional ten pounds were added to the estimated weight as the upper limit (35-45 lbs).

Figure 8, below, is a schematic representation of the membrane filtration process our team designed.

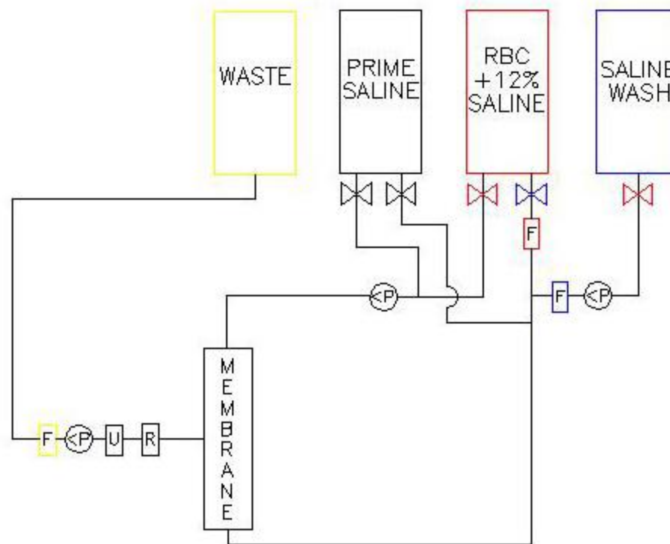

Figure 8. Deglycerolization of red blood cells using continuous membrane filtration process.

In Figure 8, above, the small rectangles marked "F" represent flow meters; the small circles marked "P" represent pumps; the small rectangle marked "R" represents the refractometer; and the small rectangle marked "U" represents the UV-visible spectrophotometer.

Prior to the start of the procedure, the two valves connected to the bag marked "Prime Saline" are opened, and the recirculating pump (located in the center of the diagram) is switched on. Saline solution flows through the system in a clockwise direction to prime the system. The valves are then closed and the pump is shut off. The valves beneath the bag marked "RBC + 12% Saline" are then opened, and the recirculating pump is switched on. This bag contains the thawed, glycerolized red blood cells, pre-equilibrated with the hypertonic 12% saline solution. At the same time, the valve beneath the bag marked "Saline Wash" is opened. This bag contains a solution of 0.9% sodium chloride and 0.2% glucose. The wash solution pump (on the right)

and the permeate pump (on the left) are switched on. The red blood cells flow through the system in a clockwise fashion, while permeate is removed to the waste bag. The concentration of glycerol in the permeate is continuously monitored with the refractometer. When the concentration of glycerol in the permeate falls below 0.79%, the valve beneath the wash solution bag is closed; the left valve beneath the blood cell bag is closed; and the wash solution and permeate pumps are shut off. The washed red blood cells are returned to the blood cell bag by the action of the recirculating pump. The right valve beneath the blood cell bag is closed, and the recirculating pump is shut off.

A mechanical draft of the membrane filtration device designed by our team was executed in Autodesk Inventor. This is shown in Figure 9, below.

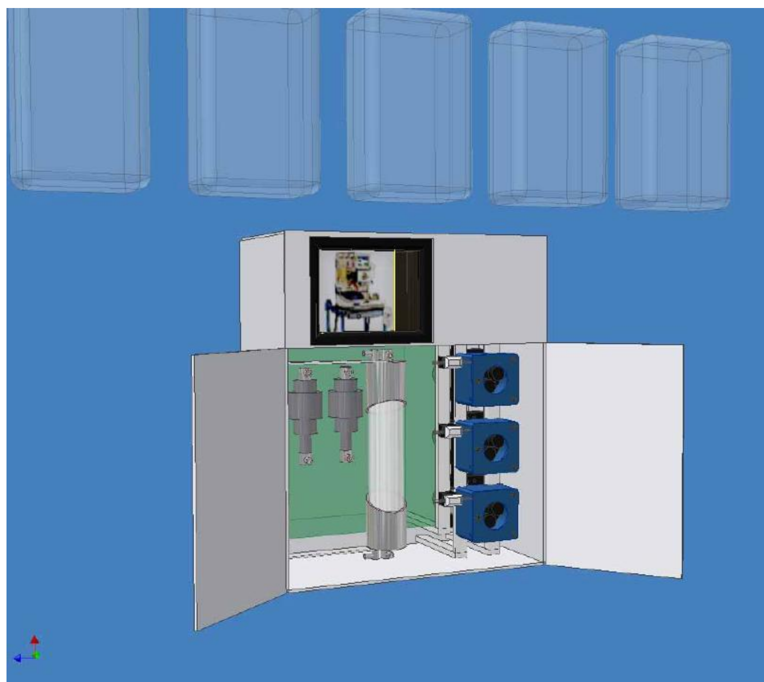

Figure 9. Mechanical draft of membrane filtration device for deglycerolization of red blood cells.

Our group has chosen to name this device PUR 304C. The name is an acronym for "portable ultrafiltration of red blood cells." The numerical designation refers to the IPRO team which designed it.

After designing and selecting components for the PUR 304C membrane filtration device, the capital and operating costs were calculated and compared for the centrifuge and membrane devices.

The capital cost of the Haemonetics ACP 215 is $46,995. The capital cost for the PUR 304C was determined by adding the total equipment cost, based upon the off-the-shelf prices for each of the selected components, to the estimated labor cost for assembly of the device. The estimated cost for each component in the membrane filtration device is listed in Table 3, below.

Component	Model	Supplier	Unit cost	Number required	Total cost
Membrane module	HPH 1400	Minntech Filtration Technologies	$128	1	$128
Shaker table	Genesis Blood Collection Mixer	National Hospital Specialties	$3000	1	$3000
Peristaltic pump head	250-PHO	Randolph-Austin	$290	3	$870
Motor	2-pole shaded pole motor	LEXEL Corporation	$150	3	$450
Flow meter	ME-2PXL	Transonic Systems	$200	3	$600
Refractometer	PR-111	AFAB	$1355	1	$1355
LCD touchscreen	HG2F-SS22VCF	IDEC	$995	1	$995
UV-visible spectrophotometer	S2000	Ocean Optics	$2000	1	$2000
Total equipment cost					$9398

Table 3. Equipment cost for PUR 304C membrane filtration device.

The estimated labor cost to assemble and perform quality tests on the membrane filtration device is $480 (based upon 16 labor hours at $30 per hour). Therefore, the total capital cost for the PUR 304C is $9878.

The two devices were then compared on the basis of their power requirements and utility cost. The power requirement for the Haemonetics ACP 215 was provided by Haemonetics as 600 watts. The annual power requirement was calculated for a period of 1872 hours (52 weeks per year, 5 days per week, 8 hours per day, 90% utilization). The electricity cost was determined based upon a market price of $0.05 per kilowatt-hour. Using these values, the total annual energy cost for the Haemonetics ACP 215 was calculated to be $56. This is summarized in Table 4, below.

Unit	Power requirement (W)	Annual power requirement (kWh)	Electricity cost (dollars per kWh)	Total annual electricity cost
Haemonetics ACP 215	600	1123	$0.05	$56

Table 4. Total annual electricity cost for Haemonetics ACP 215.

The power requirements for each of the components of the membrane device were provided by their respective manufacturers. The annual power requirement was calculated for a period of 1872 hours. The electricity cost was determined based upon a market price of $0.05 per kilowatt-hour. Using these values, the total annual energy cost for the PUR 304C was calculated to be $3. This is summarized in Table 5, below.

Unit	Power requirement (W)	Annual power requirement (kWh)	Electricity cost (dollars per kWh)	Total annual electricity cost
Mixer	2.00	4	$ 0.05	$ 0.19
Pumps (3)	3 x 7.46	3 x 14	$ 0.05	$ 2.10
Refractometer	2.72	5	$ 0.05	$ 0.25
LCD	1.00	2	$ 0.05	$ 0.09
Spectrometer	0.55	1	$ 0.05	$ 0.05
			Total	$ 3

Table 5. Total annual electricity cost for Haemonetics ACP 215.

The two devices were then compared on the basis of the cost of disposables and solutions. For each unit of deglycerolized blood produced with the Haemonetics ACP 215, three disposable

items are required: a Glyc Kit, a waste bag, and a final product bag. The cost for the Haemonetics Glyc Kit is $55.68. The cost of the waste bag (2000 milliliter capacity) is $5.25. The final product bag holds 500 milliliters and costs $2.25. The Haemonetics ACP 215 produces 1.25 bags of deglycerolized blood per hour. Using this rate and 1872 annual operating hours, the total annual cost for disposables is $147,841.

To deglycerolize one unit of blood using the Haemonetics ACP 215, two wash solutions and AS-3 solution are required. A solution of 0.9% sodium chloride and 0.2% glucose costs $6.00 per 2000 milliliter bag. A solution of 12% sodium chloride has an estimated cost of $3.00 per 500 milliliter bag. The AS-3 solution provided by Haemonetics has a cost of $6.10 per 240 milliliter bag. The Haemonetics ACP 215 produces 1.25 units of deglycerolized blood per hour. Using this rate and 1,872 annual operating hours, the total annual cost for solutions is $35,334.

These results are summarized in Table 6, below.

	Cost per unit	**Units per hour**	**Cost per hour**	**Operating hours per year**	**Total annual cost**
Disposables					
Glyc Kit	$ 55.68	1.25	$ 69.60	1872	$ 130,291
Waste bag	$ 5.25	1.25	$ 6.56	1872	$ 12,285
Final product bag	$ 2.25	1.25	$ 2.81	1872	$ 5,265
					$ 147,841
Solutions					
Wash solution	$ 9.00	1.25	$ 11.25	1872	$ 21,060
AS-3 solution	$ 6.10	1.25	$ 7.63	1872	$ 14,274
					$ 35,334

Table 6. Cost of disposables and solutions for Haemonetics ACP 215.

With the PUR 304C, one membrane module with tubing is used to produce 32 units of deglycerolized blood. Each unit of deglycerolized blood requires a waste bag and a final product bag. The membrane module with tubing costs $128 per unit. The cost for the waste bag (2000 milliliters) is $5.25. The final product bag holds 500 milliliters and costs $2.25. The PUR 304C produces four units of deglycerolized blood per hour. Using this rate and 1872 annual operating hours, the total annual cost for disposables is $86,112.

To deglycerolize one unit of blood using the PUR 304C, two wash solutions are required. A solution of 0.9% sodium chloride and 0.2% glucose costs $6.00 per 2000 milliliter bag. A solution of 12% sodium chloride has an estimated cost of $3.00 per 500 milliliter bag. The PUR 304C produces four units of deglycerolized blood per hour. Using this rate and 1872 annual operating hours, the total annual cost for solutions is $67,392. These results are summarized in Table 7, below.

	Cost per unit	Units per hour	Cost per hour	Operating hours per year	Total annual cost
Disposables					
Membrane with tubing	$ 128.00	0.13	$ 16.00	1872	$ 29,952
Waste bag	$ 5.25	4.00	$ 21.00	1872	$ 39,312
Final product bag	$ 2.25	4.00	$ 9.00	1872	$ 16,848
					$ 86,112
Solutions					
Wash solution	$ 9.00	4.00	$ 36.00	1872	$ 67,392
					$ 67,392

Table 7. Cost of disposables and solutions for PUR 304C.

With regard to labor costs, each device requires one operator. The estimated annual wages and benefits for one operator is $40,000. This may be a conservative estimate; the actual wages and benefits may be greater.

CONCLUSIONS AND RECOMMENDATIONS

Over the course of this semester, our group has evaluated the suitability of centrifugation and membrane filtration for application in a portable deglycerolization device for red blood cells. The Haemonetics ACP 215, currently used by the U.S. military, was taken as the model centrifugal separation device. A model membrane filtration device, called the PUR 304C, was designed by our team. The two devices are compared on the basis of cycle time, weight, size, power requirements, consumables utilization, and cost in Table 8, on the following page.

	Haemonetics ACP 215	PUR 304C
Process time	~40 min	~15 min
Weight	55 lbs	~35-45 lbs
Size	2.55 ft^3	0.58 ft^3
Volume of wash solution	1.6 L	1.1 L
Annual power consumption	1123 kWh	51 kWh
Estimated capital cost	$46,995	$9,878
Estimated cost per unit of deglycerolized red blood cells	$93	$26

Table 8. Comparison of Haemonetics ACP 215 centrifugal device with PUR 304C membrane device for deglycerolization of thawed red blood cells.

As can be seen from Table 8, above, the PUR 304C accomplishes the deglycerolization process in approximately one third of the time required by the Haemonetics ACP 215. The weight of the PUR 304C is at least 20% less than that of the Haemonetics ACP 215. The volume of the PUR 304C is approximately one quarter of the volume of the Haemonetics ACP 215. The volume of wash solution required by the PUR 304C is approximately two thirds of the volume of wash solution required by the Haemonetics ACP 215. The annual power consumption of the PUR 304C is less than 5% of the annual power consumption of the Haemonetics ACP 215. The estimated capital cost of the PUR 304C is approximately one fifth of the capital cost of the Haemonetics ACP 215. The estimated cost per unit of deglycerolized red blood cells produced using the PUR 304C is approximately one fourth of the cost per unit of deglycerolized red blood cells produced using the Haemonetics ACP 215. The actual difference in cost per unit of deglycerolized red blood cells may be even greater, as a low estimate of labor costs was used.

With regard to process automation, both systems are substantially automated; however, the PUR 304C eliminates the need for manual examination of the deglycerolized red blood cells for hemolysis by using a spectrophotometer to measure free hemoglobin. With regard to compliance of the washed red blood cells with the standards for transfusion, both systems comply with AABB standards; however, the PUR 304C provides additional quality assurance by measuring the concentration of glycerol. The Haemonetics ACP 215 has no means of measuring the concentration of glycerol.

It is strongly hoped that this project will be continued in future semesters. Starting from scratch, our group has accomplished a great deal in a short period of time. It is hoped that future IPRO teams will be able to build upon our successes.

A future IPRO team should include at least one student of electrical engineering. It is believed that further reductions in cost and weight could be obtained by replacing the refractometer and the spectrophotometer with more compact arrangements of LEDs, CCDs, and other optical devices. An electrical engineering student is also needed to design the power supply and process control logic.

We believe that we have advanced this project to the point at which a future IPRO should be able to build a prototype device and begin testing. We believe that this device has strong market potential and fulfils a serious medical need. We are confident in the advantages of our design over the currently available devices. We hope that the work we have begun will be continued in future semesters.

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