بسم الله الرحمن الرحيم

MEGBI Vaccine Pilot Plant (MEGBI-VPP) - 3rd Project Report

(Jan 2014 – Sep 2015)

- Rough Business Plans and Commercializing Market Strategy
- Specification and System Design of Downstream Process of Hepatitis B DNA Vaccine Pilot Plant
- Detailed Mechanical Design of Devices
- Automation System (Graphical User Interface, Adaptation to Sensors/Actuators)

Authors:

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Jihad Samarji, Zaher Chendeb, Ibrahim Zaaroura, Fadi Yahya, Haitham Hindy

Initial Document: 10 Dec 2014

Last update/آخر تعديل: 24 September 2015

مركز أبحاث الشرق الأوسط للجينات والتقنية البيولوجية

رأسنحاش – قضاء البترون– لبنان

Middle East Genetics and Biotechnology Institute (MEGBI)

A Member Institute of AECENAR

Main Road, Ras-Nhache, Batroun, Lebanon

www.temo-ek.de

TEMO Biotechnology

TEMO e.K., Im Klingenbühl 2a, 69123 Heidelberg, Germany

المضمون

Project Status at beginning of this project phase

مركز ابحاث الشرق الاوسط
للجينات والتقنية البيولوجية
http://aecenar.com/institutes/megbi
ابحاث عن ألقحة

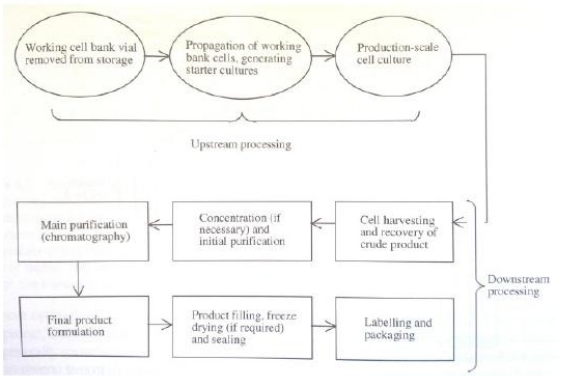
AECENAR
Association for Economical and Technological Cooperation in the Euro-Asian and North-African Region
www.aecenar.com

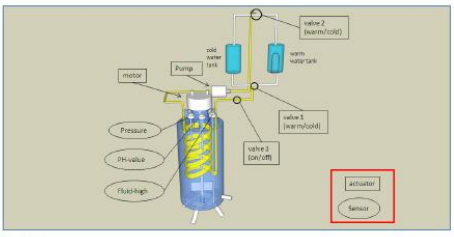
Recombinant Vaccine Technology / Biotechnological Upstream & Downstream Processing

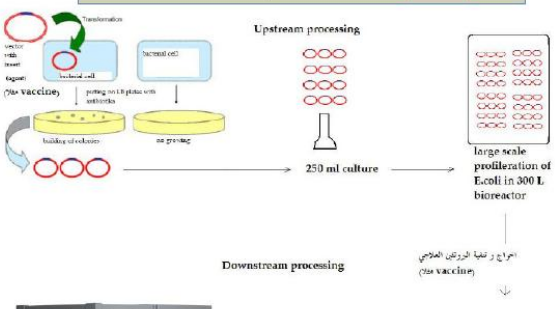
Hepatitis B DNA Vaccine Pilot Plant

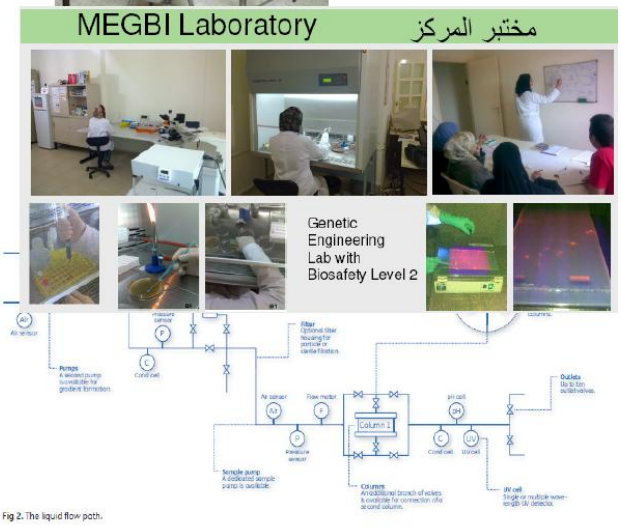
صناعة القحة عن طريق البيوتكنولوجيا

Last update: 03 December 2013









اعمال حالية:

- Prototype for a Chromatographic Purification Machine for Production Scale

الحاجيات لعام 2014:

- 2-1 اشخاص (مثلا طلاب ماستر في الهندسة الادواة الطبية او في الAutomation Engineering)
- $34.000 تكلفة مواد

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samir.mourad@aecenar.com

1. Project Management / إدارة المشروع

1.1 Project goal /هدف العمل

The goal is to install a DNA vaccine production plant.

1. HBSAg vaccine production based on S.cerevisae
2. later on migration to MAB production in E.coli

1.2 Budget Planning

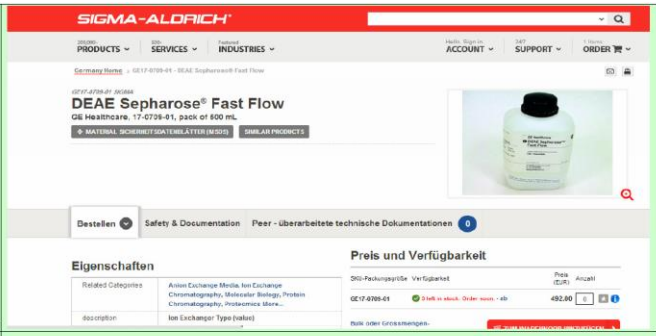
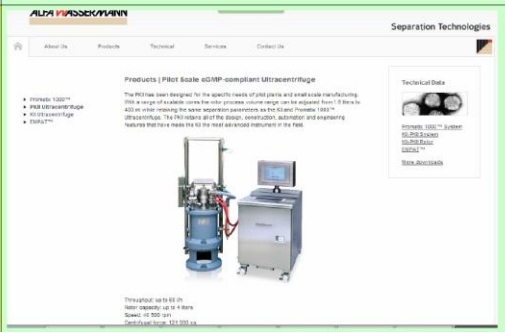
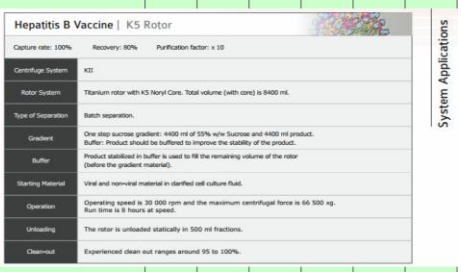
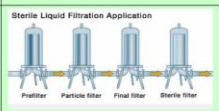
1.2.1 *Akta Based Plant*

Smaller Scale	Prices 2014	Prices 2015 including transport and douane in Lebanon
Bioreactor (offer from 2013)	130.000 €	175.760 €
Uniflux (Filtration) 10	172.000 €	232.544 €
Aktaready	140.000 €	189.280 €
Ultracentrifuge	50.000 €	67.600 €
DNA Laboratory		130.000 €
Total		795.184 €

Medium Scale	Prices 2014	Prices 2015 including transport and douane in Lebanon
Bioreactor Xcell	240.000 €	324.480 €
Uniflux (Filtration) 30	305.000 €	412.360 €
AktaProcess	170.000 €	229.840 €
Ultracentrifuge	50.000 €	67.600 €
DNA Laboratory		130.000 €
Total		1.164.280 €

1.2.2 *Patent Based semi-own manufactoring*

Last update: 24.12.2014

Device No.	Device Name	Price including transport & customs	Supplier/image
1	Disc Stack Centrifuge 1	$25.000	
2	Homogenizer	$15.000	 
3	Storage Tank/ PEG Precipitation mixing tank 100L	$2.000	
4	Disc Stack Centrifuge 2	$25.000	
5	Storage Tank/ CaCl2 Precipitation mixing tank 100L	$2.000	
6	Ultrafiltration Device	$5.000	
7	Storage Tank 100L for UF	$2.000	
8	20 L Chromatography column	$3.000	
9	20 L Sepharose 4B	$18.000	
10	Storage Tank 100L	$2.000	
11	20 L Chromatography column	$3.000	
12	10 L DEAE matrix DFF100 DEAE-Sepharose® Fast Flow (Sigma Aldrich)	$17.000	
13	Storage Tank 100L	$2.000	
14	Ultracentrifugation CsCl	$150.000	 
15	20 L Chromatography column	$3.000	
16	20 L Sepharose 4BCLFF	$18.000	
17	Storage Tank 100L	$2.000	
18	sterile filtration 0.22 Mikrometer	$2.500	
19	Chemicals	5000	
20	Engineering 6 man months	18000	
21	Project Management 6 man months	24000	
	Total	**$343.500**	

1.2.3 *Budget for MEGBI Vaccine Production Pilot Plant (MEGBI-VPP)*

MEGBI-VPP
DNA Vaccine Pilot Plant for production of Hepatitis B virus
2015

Last Update: 04. Feb 15

DNA Transfer Laboratory

device	price
Safety Cabinet B2	8.000 €
centrifuge	5.000 €
Shake incubator	2.500 €
Thermocycler	2.500 €
blood freezer	3.500 €
Gel rack with voltage	1.500 €
Precision Balance	800 €
Minus 80 °C freezer	10.000 €
UV Machine	1.100 €
vortex	300 €
precision pipettes	500 €
molecular biological reagences	4.000 €
chemicals	2.000 €
laboratory materials	1.500 €
laboratory tables	1.500 €
IT	1.000 €
fluorescence microscope	3.000 €

Transportation and customs	6.000 €
Building Lab 2009-11	25.000 €
Total Laboratory	**79.700 €**

Upstream Processing (Bioreactor)

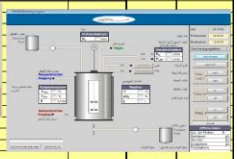
Material Costs

System	Piece	#	Piece price	Total
Behälter (1301 Stainless)		1	600 €	600 €
Temperiersystem	Behälter	2	50 €	100 €
	Abdeckwolle	1	100 €	100 €
	Blech	1	120 €	120 €
	Pumpe	1	80 €	80 €
	Beheizungsrad	1	60 €	60 €
Aut. Valve		2	200 €	400 €
Temp.Sensor		1	20 €	20 €
PH Sensor		1	100 €	100 €
PO_2 Sensor		1	1.200 €	1.200 €
Ausfluß	Aut.Valve	1	200 €	200 €
Medium Einfluß	Aut.Valve	1	200 €	200 €
	Behälter	1	50 €	50 €
PH Regulierung	Behälter	2	50 €	100 €
	Aut.Valve	2	200 €	400 €
Beimpfungsreinigung	Behälter	1	50 €	50 €
	Aut.Valve	1	200 €	200 €
Control System	Siemens S7 PLC	1	1.500 €	1.500 €
	PC	1	500 €	500 €
			Sum Material	5.980 €

Personnel Costs

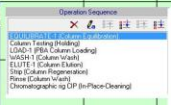
Task	duration (months)	Qualification	price / month	Total
Integration Mechanics	1	Eng.	2.000 €	2.000 €
Integration Automation System	1	Eng.	2.000 €	2.000 €
Programming Control AECENAR	1	Eng.	2.000 €	2.000 €
Project Managing	3	Eng.	2.000 €	6.000 €
			Sum Personnel Costs	**12.000 €**

Personal Specific Costs	cost per month
Student	0 €
Specialized Worker	1.000 €
Engineer	2.000 €



Total Upstream Processing Unit (Bioreactor) 17.980 €

Downstream Processing

		price per piece	pieces needed	Costs	Needed Staff
Homogenizer		1.000 €	1	1.000 €	
Prototype Chromatogr. Process Device (mech)		13.000 €	3	39.000 €	
Prototype Chromatogr. Process Device(autom)	 			0 €	
Ultrafiltration Unit		1.500 €	1	1.500 €	
Ultracentrifuge (used)				0 €	
Disc Stack Centrifuge	 	10.500 €	1	10.500 €	
Mixing/Storing tanks		1.000 €	4	4.000 €	
Pump		700 €	6	4.200 €	
Automation (PLC system, GUI)				10.000 €	
		Total Devices	73.200 €	Integration (piping, ...) 3.000 €	Total Downstream Processing Unit 76.200 €

Project Management

Program Managing over 9 months	#months 9	monthly rate for manage-ment 2.000,00 €	18.000 €
Documentation			4.000 €
			Total Project Management 22.000 €

Total MEGBI-VPP 195.880 €

planned project duration: Dez. 2014 - August 2015

MEGBI-VPP
DNA Vaccine Pilot Plant for production of Hepatitis B virus
2015

Last Update: 04. Feb 15

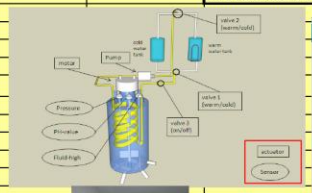
DNA Transfer Laboratory

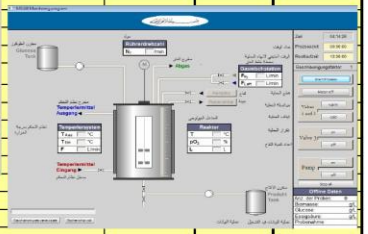
device	price
Safety Cabinet B2	8.000 €
centrifuge	5.000 €
Shake incubator	2.500 €
Thermocycler	2.500 €
blood freezer	3.500 €
Gel rack with voltage	1.500 €
Precision Balance	800 €
Minus 80 °C freezer	10.000 €
UV Machine	1.100 €
vortex	300 €
precision pipettes	500 €
molecular biological reagences	4.000 €
chemicals	2.000 €
laboratory materials	1.500 €
laboratory tables	1.500 €
IT	1.000 €
fluorescence microscope	3.000 €

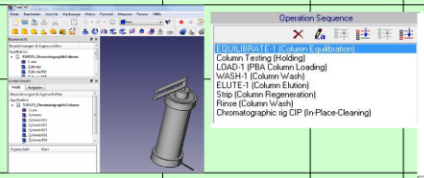
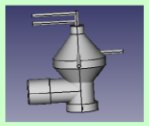
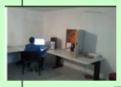
Transportation and customs	6.000 €
Building Lab 2009-11	25.000 €
Total Laboratory	**79.700 €**

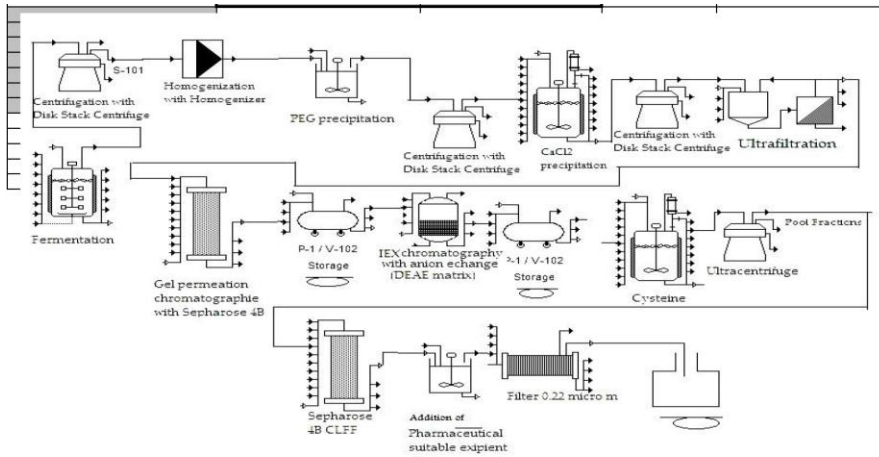
Upstream Processing (Bioreactor)

Material Costs					Personnel Costs					Personal Specific Costs	cost per month
System	Piece	#	Piece price	Total	Task	duration (months)	Qualification	price / month	Total	Student	0 €
Behälter (130l Stainless)		1	600 €	600 €	Integration Mechanics	1	Eng.	2.000 €	2.000 €	Specialized Worker	1.000 €
Temperiersystem	Behälter	2	50 €	100 €	Integration Automation System	1	Eng.	2.000 €	2.000 €	Engineer	2.000 €
	Abdeckwolle	1	100 €	100 €	Programming Control	1	Eng.	2.000 €	2.000 €		
	Blech	1	120 €	120 €	AECENAR Project Managing	3	Eng.	2.000 €	6.000 €		
	Pumpe	1	80 €	80 €				**Sum Personnel Costs**	**12.000 €**		
	Beheizungsrad	1	60 €	60 €							
Aut. Valve		2	200 €	400 €							
Temp.Sensor		1	20 €	20 €							
PH Sensor		1	100 €	100 €							
PO_2 Sensor		1	1.200 €	1.200 €							
Ausfluß	Aut.Valve	1	200 €	200 €							
Medium Einfluß	Aut.Valve	1	200 €	200 €							
	Behälter	1	50 €	50 €							
PH Regulierung	Behälter	2	50 €	100 €							
	Aut.Valve	2	200 €	400 €							
Beimpfungsreinigung	Behälter	1	50 €	50 €							
	Aut.Valve	1	200 €	200 €							
Control System	Siemens S7 PLC	1	1.500 €	1.500 €							
	PC	1	500 €	500 €							
			Sum Material	**5.980 €**				**Total Upstream Processing Unit (Bioreactor)**	**17.980 €**		





Downstream Processing					price per piece	pieces needed	Costs	Needed Staff		
Homogenizer					1.000 €	1	1.000 €			
Prototype Chromatogr. Process Device (mech)					13.000 €	3	39.000 €			
Prototype Chromatogr. Process Device(autom)							0 €			
Ultrafiltration Unit					1.500 €	1	1.500 €			
Ultracentrifuge (used)							0 €			
Disc Stack Centrifuge	 				10.500 €	1	10.500 €			
Mixing/Storing tanks					1.000 €	4	4.000 €			
Pump					700 €	6	4.200 €			
Automation (PLC system, GUI)	  						10.000 €			
				Total Devices	73.200 €	Integration (piping, ...)	3.000 €	Total Downstream Processing Unit		76.200 €
Project Management			#months	monthly rate for manage-ment						
	Program Managing over 9 months		9	2.000,00 €		18.000 €				
	Documentation					4.000 €		Total Project Management		22.000 €
	Total MEGBI-VPP	**195.880 €**		planned project duration: Dez. 2014 - August 2015						



Clean-in-place (CIP)&Steam-in-place (SIP) and intermediate storage	Upstream Processing (USP) – Downstream Processing (DSP)	Automation
 		   

Project Status Feb 2015

so far completed	67%	
still open	33%	**64.640 €**

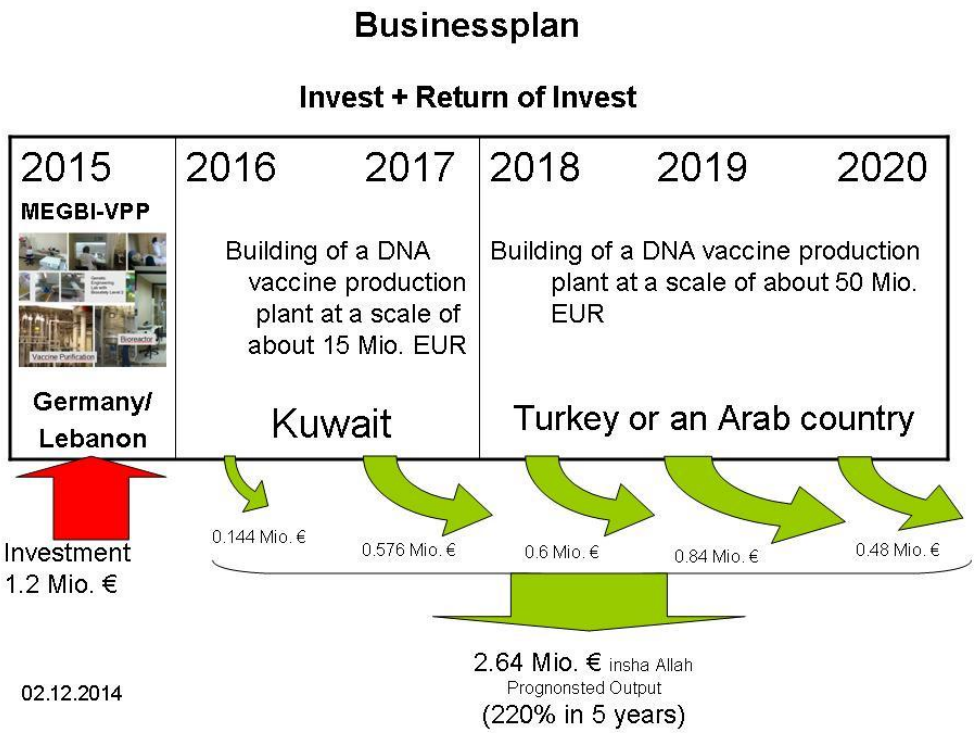
required financing to complete the pilot plant

Strategy for Market and Industrial Infrastructure Building in North Lebanon

2015	2016	2017	2018	2019	2020	2021
MEGBI VPP		MAB financed	market access	supplier startup companies		
DNA vaccine pilot plant			producing MAB (monoclonal antibodies)			

This calculation was presented to LASeR (Prof Moustafa Jazar, Dr Ammar Assoum, Dr Bachar al-Hasan) and discussed at LU Doctoral School in Tripoli (Dr Ammar Assoum, Dr Bachar al-Hasan, Dr Mohammad Khalil). Result: LASeR waits for the opinion of Prof Monzer Hamze (Status 17 March 2015).

1.3 Business Plan 1 (presented Dec 2014)



1.3.1 *Investors for Business Plan 1*

Übersicht der Investoren am MEGBI-VPP

Stand: **31.12.2014**

Investoren

Gesamtentwicklungswert **1.200.000 €**

Investor	Höhe des Investitionswertes	Anteile am Gewinn (Entwicklung) bis April 2011	Bemerkung/Datum der Investition
Amine Bouafif	100,25 €	0,0083542%	Investion bezahlt (Überweisung ca. 11.12.14)
Nasser Al Araïmi	1.200 €	0,1000000%	Investition bezahlt (Überweisung 27.12.14)
David Yildiz	600 €	0,0500000%	Investion bezahlt (bar ca. 8.12.14)
AECENAR	133.000€	11,0833333%	DNA Labor 130TEUR, Miete Jan-Jun 15 3TEUR
Summe:	134.900€	11,2416875%	
Restentwicklungsanteile TEMO	1.065.100 €	88,76%	

derzeit ist der größte Teil der Projektdokumente öffentlich zugänglich und hier einsehbar:

http://temo-ek.de/8.html

1.3.2 *Location Concept of Business Plan 1*

Cooperation between Europe and the Middle East

By the cooperation with Turkish and Arab partners the MEGBI-VPP project creates working possibilities for the young educated people in the Middle East region and helps to stabilize the region.

And with the help of God, the Almighty, this will be a big effort for a better and peaceful future for the two neighbour regions Europe and North-Africa/Middle East.

The genetic engineering laboratory of the pilot plant (for step 1) is based in North Lebanon at the nice village Ras Nhache. Threre were also undergone the prestudies of the project.

Bioreactor (step 2) and purification (step 3) will be implemented at TEMO Biotechnology site at Heidelberg, Germany

The administration of the project will be done by TEMO Biotechnology in Germany.

TEMO Biotechnology
TEMO e.K.
Im Klingenbühl 2a
69123 Heidelberg, Germany

Email: info@temo-ek.de
Website: www.temo-ek.de

1.4 Business Plan 2 (Presented Feb 2015 to Azm Association)

Bismillah

MEGBI-VPP Businessplan and Feasibility Study

18.2.2015

1. The Goal: a Facility able to produce vaccines and antibodies based on fermentation and purification (biotechnological upstream and downstream process)

The Goal is a company like this

Facility History

- **1988 -** Cetus Corporation, Emeryville, CA, US establishes its European headquarters, EuroCetus B.V. in Amsterdam, the Netherlands.
- **1990-1991 -** EuroCetus facility licensed for production and release of ProLeukin (IL-2) for Europe and rest of world, except US and Japan.
- **1992 –** Chiron Corporation acquires Cetus Corporation. Eurocetus B.V. becomes Chiron B.V.
- **1996 –** Facility licensed for commercial production of Pertussis vaccine.
- **1997 –** Facility licensed by WHO for commercial production of Meningitis A polysaccharides.
- **1997-1999 –** Facility licensed by UK, MCA and Italian Health Branch for Meningitis C polysaccharide and CRM197 carrier protein for conjugated vaccine Menjugate®.
- **2000 –** Facility acquired by Dr William J. Rutter. SynCo Bio Partners B.V. established.
- **2001 –** Facility licensed by EMA and Health Canada for Meningitis C polysaccharides and CRM197 carrier protein for Menjugate® production.
- **2004 –** Installation of new air handling systems completed for all of its facilities.
- **2005 –** Installation of new aseptic filling machine in its Class A zone, allowing larger batches sizes and expansion of filling ranges.
- **2005 –** Expansion of GMP facilities to allow production of a wider range of protein, vaccine and live biotherapeutic products.
- **2006 –** Facility licensed by Korean FDA for commercial production.

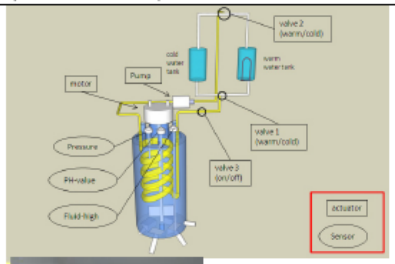
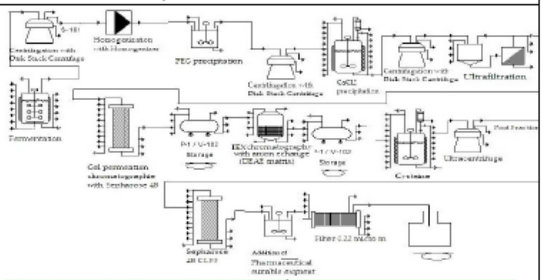
- **2008 –** Expansion of process development capabilities to enable a greater range of projects to be completed.
- **2009 –** Facility licensed by ANVISA for commercial production.
- **2011 –** Expansion of the Class A zone of its aseptic filling facility successfully completed, allowing SynCo to support new product launches in the US and the rest of the world.

2. Business Plan for our plant

Year	2009-2015	2016	2017	2018
	MEGBI Vaccine Production Pilot Plant (MEGBI-VPP)		Licenced for production of diagnostic antibodies (diagnosis of blood groups) in Lebanon/Jordan/Turkey and other Arab Countries	Licenced for production of Hepatitis B antigen vaccine (similar to Engerix) in Lebanon and other Arab Countries
Investment Costs	**120,000 €** **(still open to complete: 65.000 EUR)**	**4 Mio. EUR**		
Return of Invest			About 20 x 250 blood laboratories&blood banks: 2017-2019 5000x40x12 EUR = 2.4 Mio. EUR income per year **7.2 Mio. EUR**	

2.1. 2009-2015

MEGBI-VPP pilot plant shall convince an investor to invest in the next steps

Genetic Engineering Laboratory	Upstream Processing (Bioreactor)	Downstream Processing Unit (Protein Purification)
	 	  

2.2. 2016

A plant like this:

Azm Association (Faisal Maulawi, Dr Dani Saaduddin, Dr Kifah Tout) visited AECENAR Center at Ras Nhache on 6th March 2015 and Business Plan 2 was discussed. Result (Status 17th March 2015): Azm wants a more detailed business plan with detailed market strategy.

1.5 Time Schedule / الجدول الزمني

Nov/Dec 14: Financement and Concept Phase

Jan – June 15: Finishing of Development of MEGBI Vaccine Production Pilot Plant (MEGBI-VPP)

	Planned	Staff
System Design with SuperPro	15.12.-24.12.2014	
Design of mechanical parts with FreeCAD (Vessels, Storage Tanks, columns)		
Materials List (sensors, actuators, chemicals to be purchased)		
Automation System	16.3.-30.7.15	Haitham Hindi (Master Student)

2. Basics

2.1 Recovery of recombinant S.cerevisae cells

From The Elements of Immunology , https://books.google.com.lb/books?isbn=8131711587, Fahim Halim Khan:

use is hepatitis B vaccine. A single gene for the major surface antigen of *hepatitis B virus* (HbsAg) is cloned in yeast cells. The recombinant yeast cells are grown in fermenters. HbsAg, the surface antigen, is expressed and accumulates inside the yeast cells. The yeast cells are then harvested and then burst open by high pressure releasing the recombinant HbsAg (among other proteins). HbsAg is then purified by a standard biochemical technique such as affinity chromatography. The purified antigen has been shown to induce humoral immunity. This approach has been used to make several

From Catherine Charcosset, Membrane Processes in Biotechnology and Pharmaceutics:

3.3.2 Concentration and clarification of cells

Another common application of MF is the concentration and washing of cultures of single-cell organisms where the product is intracellular or cell associated [7]. Common cases include recombinant yeast cultures producing proteins and antigen particles, and recombinant *E. coli* producing proteins in the form of solid inclusion bodies. The next step is usually cell lysis and recovery of the product from cell debris. Separation of product from cell debris can often be performed by MF, as detailed in the next section.

Russotti et al. [107] have used cross-flow MF for the harvest of recombinant yeast in a short period of time to minimize the risk for product degradation. MF studies with flat sheet membranes showed high throughput with initial fluxes on the order of water fluxes (>1000 l m^{-2} h^{-1}, regime I, <2 min), followed by a rapid decay towards a low pseudo-steady state flux (20 l m^{-2} h^{-1}, regime II, >2 min). Large pore membranes (0.65 μm) were found to be more suitable for harvesting yeast (10 μm size) without cell leakage than smaller pore ones (0.22 μm and 0.45 μm). Among operating parameters, feed flow rate (i.e. shear rate) had a significant impact on average flux, whereas change in *TMP* afforded little improvement. In another recent example, Lee [108] concentrated recombinant yeast cells using a cross-flow MF unit containing a 0.2 μm membrane device. The concentrated cells were homogenized by several passes through a high-pressure homogenizer. The homogenate was then clarified using cross-flow MF. The clarified material was concentrated by UF and buffer-exchanged before delivering the material to down-stream for further purification.

3.3.3 Separation of products from fermentation broth

Microorganisms are sources of valuable enzymes, proteins and other bio-products. They produce two basic types of biological molecules: extracellular, which are excreted into a growth medium, and intracellular, which are retained inside the cytoplasm of the cells [9]. A variety of host microorganisms have been studied. The most often used organisms are *E. coli*, *S. cerevisiae* and *Bacillus subtilis*. Several other microbial strains have been used for production of microbial enzymes, such as *Aspergillus niger* and *Kluyveromyces fragilis* (for production of catalase), *Saccharomyces lactis* and *Kluyveromyces lactis* (β-galactosidase), *Bacillus coagulans* and

From Process Scale Bioseparations for the Biopharmaceutical Industry, edited by Abhinav A. Shukla, Mark R. Etzel, Shishir Gadam:

fouling due to media components, and influence of osmotic pressure. Patel et al. [21] have compared the different filter formats: pleated-sheet microfilter, tubular microfilter, and hollow fiber ultrafiltration (UF), in terms of flux and cell yields obtained with CFF of yeast cell suspensions. They found that the UF module had much lower fouling rate than with the pleated-sheet microfilter that had rapid plugging and significant cleaning issues. Bailey and Meagher [27] performed a similar comparison between the hollow fiber and plate and frame formats for microfiltration of recombinant *Escherichia coli* lysate and found both options to be comparable in performance under optimized conditions. Sheehan et al. [22] performed a comparison of the centrifugation vs. membrane-based separations of extracellular bacterial protease and found the membrane process to be twice as cost effective as the centrifuge and equivalent to a precoated filter, on the basis of unit cost of enzyme product recovered. Industrial studies demonstrating robust operation of tangential flow filtration (TFF) for harvest of mammalian cell culture [23] and CFF for harvest of recombinant yeast cell product [26] have also been reported. More fundamental studies investigating the various aspects of filtration processes such as membrane fouling, mathematical modeling, and critical flux determination have also been published [24,28,29].

Pilot-scale harvest of recombinant yeast employing microfiltration: a case study, Gregory Russotti[a], A.Edward Osawa[a], Robert D. Sitrin[a], Barry C. Buckland[a], William R. Adams[b], Steven S. Lee

Abstract

In order to develop a cost-effective recovery process for an intracellular product, crossflow microfiltration was studied for the harvest of a recombinant yeast under severe time constraint. It was required to process yeast broth in a short period of time to minimize the risk for product degradation. Preliminary microfiltration studies employing flat sheet membranes showed high throughout with initial fluxes on the order of water fluxes (> 1000 LMH, regime I, $2 min), followed by a rapid decay towards a low pseudo-steady state flux (20 LMH, regime II, > 2 min). Exploitation of these high fluxes and control of their eventual decline were crucial in establishing a rapid crossflow filtration process. The effect of several parameters, such as initial cell concentration, shear rate, transmembrane pressure, membrane pore size and medium composition on filtration performance were investigated to better understand the flux decline mechanisms. We found that the major contributor to flux decay was reversible fouling by the cake formation on the membrane surface. Within the operating boundaries of our microfiltration system, large-pore membrane (0.65 μm) was much more desirable for harvesting our yeast (10 μ size) without cell leakage than smaller pore ones (0.22 μm and 0.45 μm). Among adjustable operating parameters, feed flow rate (i.e., shear rate) exerted significant impact on average flux, whereas manipulation of transmembrane pressure afforded little improvement. Although initial cell concentration affected adversely the permeation rates, growth medium components, especially soy-peptone, was deemed pivotal in determining the characteristics of cell cake, thus controlling yeast microfiltration.

Keywords: Crossflow filtration; Microfiltration; *Saccharomyces cerevisiae*; Yeast; Recombinant

Biotechnology Techniques Vol 4 No 5 329-334 (1990)
Received 12th June

DISRUPTION OF *Saccharomyces cerevisiae* USING ENZYMATIC LYSIS COMBINED WITH HIGH-PRESSURE HOMOGENIZATION

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Waterloo, Ontario, Canada N2L 3G1

ABSTRACT

The disruption of commercially-available pressed Bakers' yeast (*Saccharomyces cerevisiae*) was studied using a relatively new high-pressure homogenizer (the Microfluidizer). Initial experiments using only mechanical disruption generally gave low disruption yields (i.e., less than 40 % disruption in 5 passes). Consequently combinations of two disruption methods, namely enzymatic lysis and subsequent homogenization, were tested to identify achievable levels of disruption. The enzyme preparation employed was Zymolyase, which has been shown to effectively lyse the walls of viable yeast. Yeast cell suspensions ranging in concentration from 0.6 to 15 gDW/L were disrupted with and without enzymatic pre-treatment. Final total disruption obtained using the combined protocol approached 100 % with 4 passes at a pressure of 95 MPa, as compared to only 32 % disruption with 4 passes at 95 MPa using only homogenization. A model is presented to predict the fraction disrupted while employing this novel enzymatic pre-treatment.

INTRODUCTION

An abundance of valuable biochemicals can be produced by microorganisms. Some of these products can be made to be secreted by the microorganism; however in some cases this is not possible to achieve and the cells must be disrupted to release their contents. There are many different methods for cell disintegration. Some of these methods can be used in association with one another, in order to take advantage of their combined benefits [1].

The Microfluidizer, a high-pressure homogenizer, has recently been tested with native and recombinant strains of *E. coli* [2]. The cell suspension is forced through two parallel channels (2x100 µm) and the separate high-velocity streams are directed at each other in front of a wall.

Preliminary disruption experiments with *Saccharomyces cerevisiae* using Microfluidization alone indicated the need for an improved disruption methodology. A combination of methods was suggested and an enzymatic pre-treatment using Zymolyase to "soften up" the cells was employed.

Zymolyase-20T is commercially available from Seikagaku Kogyo Co., Ltd. It is produced by a submerged culture of *Oerskovia xanthineolytica* (previously classified as *Arthrobacter luteus*). An essential enzyme responsible for lysis of viable yeast cells in this preparation is β-1,3-glucan laminaripentaohydrolase [3-6].

Using Zymolyase as a pre-treatment to Microfluidization, a study of various homogenization operating conditions (i.e., disruption pressure and number of passes) has been undertaken in order to determine the acceptability of using the Microfluidizer as a method for mechanical disruption of yeast. An empirical model is presented to account for the introduction of this novel enzymatic pre-treatment regime.

MATERIALS AND METHODS

ENZYME EMPLOYED FOR PRE-TREATMENT

Zymolyase-20T, produced by a submerged culture of *Oerskovia xanthineolytica*, is a relatively new enzyme preparation which effectively lyses the walls of viable yeast cells [7,8]. Zymolyase-20T has 20,000 units/g of lytic activity, defined below, toward Brewers' yeast (*Saccharomyces uvarum*, resting stage).

One unit of lytic activity is defined as that amount which results in a 30 % decrease in absorbance at 800 nm (A 800) of the reaction mixture under the following conditions:

Reaction Mixture	
Enzyme.....0.1 mg/mL solution	1 mL
Substrate.....Yeast Cell Suspension (2 mgDW/mL)	3 mL
Buffer.....M/15 Phosphate Buffer pH 7.5	5 mL
Deionized water	1 mL
Total volume	10 mL

After incubation for 2 h at 25 °C with gentle shaking, A 800 of the mixture is determined. As a reference, 1 mL of deionized water is used instead of the enzyme solution.

$$\% \text{ decrease in A 800} = \frac{(\text{A 800 ref.} - \text{A 800 react. mixture})}{\text{initial A 800 ref.}} \times 100\%$$

When an A 800 decrease of 60 %, equivalent to 2 units of activity, is observed in the reaction system, the yeast cells are completely lysed, namely 1 unit of Zymolyase-20T lyses 3 mg dry weight of Brewers' yeast [9].

DISRUPTION EQUIPMENT

The disruption device used in this work was a Microfluidizer high-pressure homogenizer (model M110T with extra heavy duty pump; Microfluidics Corp., Newton, MA., U.S.). The disruptor consisted of an air-driven, high-pressure pump (ratio 1:250; required air pressure 0.6-1 MPa) and a special disruption chamber with an additional back pressure unit. A minimum sample size of 20 mL is required for processing. Further details are given in [2].

STRAINS AND ANALYTICAL

Commercially-available pressed Bakers' yeast (*Saccharomyces cerevisiae*) was resuspended in deionized water to a cell concentration of 2 mgDW/mL. The resulting reaction mixture thus has a yeast cell concentration of 0.6 gDW/L. The above enzymatic pre-treatment (prior to homogenization) was carried out for 2 h, with the percent decrease in A 800 found at the end of the 2 h incubation (the course of the enzymatic lysis was followed by sampling the reaction and reference mixtures every 15 min.).

The buffered and partially lysed yeast suspension after enzymatic pre-treatment was cooled to 4 °C and was subsequently homogenized at various operating pressures (30-95 MPa) and at up to five passes through the homogenizer. The resulting homogenized yeast suspensions were cooled using ice packs, as was the disruption chamber. The Microfluidizer

2.1.1 ***How to Use Avestin Emulsiflex C3 Homogenizer to Disrupt Cells***

Cell Biology > Cell viability > Cell lysis

Fungi > Saccharomyces > Saccharomyces cerevisiae > Other compound

Author: Zongtian Tong
1/5/2011, 7257 views, 1 Q&A

[Abstract] The EmulsiFlex-C3 homogenizer is powered by an electric motor. The pump does not require a compressor for it to run. This equipment can be used to disrupt cells at a large scale. The EmulsiFlex-C3 has a fixed flow-through capacity of 3L/h. It has the ability to process samples as small as 10 ml. The homogenizing pressure is adjustable between 500 and 30,000 psi. In this protocol, we describe the use of the Avestin Emulsiflex C3 Homogenizer to disrupt *S. pombe* and *S. cerevisiae* cells.

Equipment

Figure 1. Avestin Emulsiflex C3 homogenizer

Procedure

1. Switch on the homogenizer at the back.
2. Turn on nitrogen. Pressure reads 80 psi.
3. Unscrew the funnel cap. Check if the funnel cap is on to make sure ethanol does not evaporate.
4. Turn red stop knob close wise and push green knob to start.
5. Pump residual ethanol out of the tubing.
6. Pour DI water into the funnel to wash ethanol out. Keep air pressure on occasionally to make sure no cell debris is left from the last user.
7. Before load your samples, take the funnel off and roll it on ice to keep it cool. Install the funnel back to the top. Put the steel coil heat exchanger into ice to cool down the samples.
8. Load your samples into the funnel. Turn on the homogenizer. Let the samples run through the tubing back to the funnel before air pressure is on.
9. Turn on air pressure. Air pressure at 40 psi, gauge pressure ≥ 20,000 psi and < 25,000 psi. The maximum pressure is 30,000 psi. Leave the tubing in a sample collection tube chilled on ice.
10. *S. Pombe* samples need to be passed through 5~6 times to reach 80~90% efficiency. *S. cerevisiae* samples need to be passed through 8~9 times to reach 80~90% lysis efficiency. Check samples under a microscope.
11. If the homogenizer is clogged by the samples, cap the funnel and blow with nitrogen tube.
12. After samples are done, take off the funnel and rinse it with DI water.
13. Run more water to flush out cell debris. Keep the air pressure on occasionally.

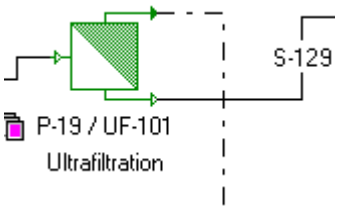
14. Run ethanol and leave 1/3 of a funnel volume of ethanol in the funnel.

References

1. http://www.avestin.com/English/c3page.html

How to cite this protocol: (2011). How to Use Avestin Emulsiflex C3 Homogenizer to Disrupt Cells. *Bio-protocol* Bio101: e11. http://www.bio-protocol.org/e11

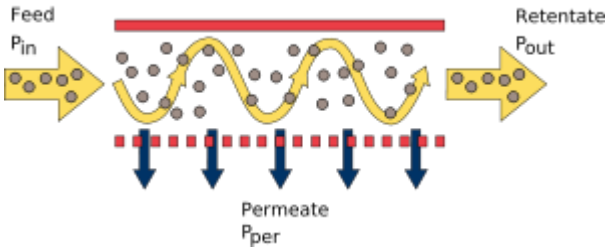
2.2 Crossflow filtration



The whey proteins are concentrated using crossflow ultrafilters (UF-101).

From Wikipedia, the free encyclopedia

Jump to: navigation, search



In chemical engineering, biochemical engineering and protein purification, crossflow filtration[1] (also known as tangential flow filtration[2]) is a type of filtration (a particular unit operation). Crossflow filtration is different from dead-end filtration in which the feed is passed through a membrane or bed, the solids being trapped in the filter and the filtrate being released at the other end. Cross-flow filtration gets its name because the majority of the feed flow travels tangentially across the surface of the filter, rather than into the filter.[1] The principal advantage of this is that the filter cake (which can blind the filter) is substantially washed away during the filtration process, increasing the length of time that a filter unit can be operational. It can be a continuous process, unlike batch-wise dead-end filtration.

Besonders dafür geeignet sind Hohlfasern (Kapillarmembran oder auch Hohlfäden genannt), deren Leistungsfähigkeit noch durch den sogenannten Pinch-Effekt verstärkt wird. Eine übliche Hohlfaser hat einen Innendurchmesser von circa 1,5 mm (3,0 mm bis 0,1 µm möglich) und eine Porengröße von 200 bis 5 nm (2 µm bis 1,0 nm möglich). Je nach Anwendung werden hunderte bis tausende Kapillaren in Modulen zusammengefasst und vergossen (Hohlfasermodule). Mit Hilfe einer Zirkulationspumpe wird das unfiltrierte Produkt solange durch die Kapillaren zirkuliert, bis die Trubstoffe im Retentat so konzentriert sind, dass eine Entleerung und Reinigung erforderlich wird.

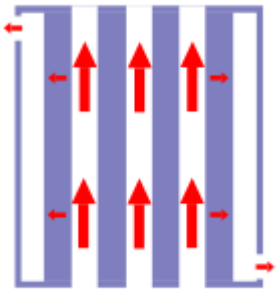


Diagram of cross-flow filtration

This type of filtration is typically selected for feeds containing a high proportion of small particle size solids (where the permeate is of most value) because solid material can quickly block (blind) the filter surface with dead-end filtration. Industrial examples of this include the extraction of soluble antibiotics from fermentation liquors.

Filtration unit for industrial cross-flow filtration

Charakteristische Merkmale der Cross-Flow-Filtrationstechnologie sind die weitgehende Eliminierung von Filterhilfsmitteln, d. h. deren Beschaffung, Lagerung, Handhabung und Entsorgung, die rasche, arbeitsextensive sowie die qualitätsschonende Verarbeitung.

Die Cross-Flow-Filtration ist sehr energieaufwändig. Ein großer Teil der in die Förderung des Feeds investierten Energie geht durch das Retentat verloren. Deshalb wird an den Stellen, wo darauf verzichtet werden kann, immer stärker auf die Dead-End-Filtration zurückgegriffen.

2.3 Dead-End Filtration

To be described

2.4 Anion exchange chromatography[1]

Anion-exchange chromatography is a process that separates substances based on their charges using an ion-exchange resin containing positively charged groups, such as diethyl-aminoethyl groups (DEAE).[2] In solution, the resin is coated with positively charged counter-ions (cations). Anion exchange resins will bind to negatively charged molecules, displacing the counter-ion. Anion exchange chromatography is commonly used to purify proteins, amino acids, sugars/carbohydrates and other acidic substances [3] with a negative charge at higher pH levels. The tightness of the binding between the substance and the resin is based on the strength of the negative charge of the substance.

General technique for protein purification

A slurry of resin, such as DEAE-Sephadex is poured into the column. After settling, the column is pre-equilibrated in buffer before the protein mixture is applied. Unbound proteins are collected in the flow-through and/or in subsequent buffer washes. Proteins that bind to the resin are retained and can be eluted one of two ways. First, the salt concentration in the elution buffer is gradually increased. The negative ions in the salt solution (e.g. Cl-) compete with protein in binding to the resin. Second, the pH of the solution can be gradually decreased which results in a more positive charge on the protein, releasing it from the resin. As buffer elutes from the column, the samples are collected using a fraction collector.

2.5 Final product formulation[2]

High-resolution chromatography normally yields a protein that is 98-99 per cent pure.

Final product formulation bings the product in the final format. This envolves:

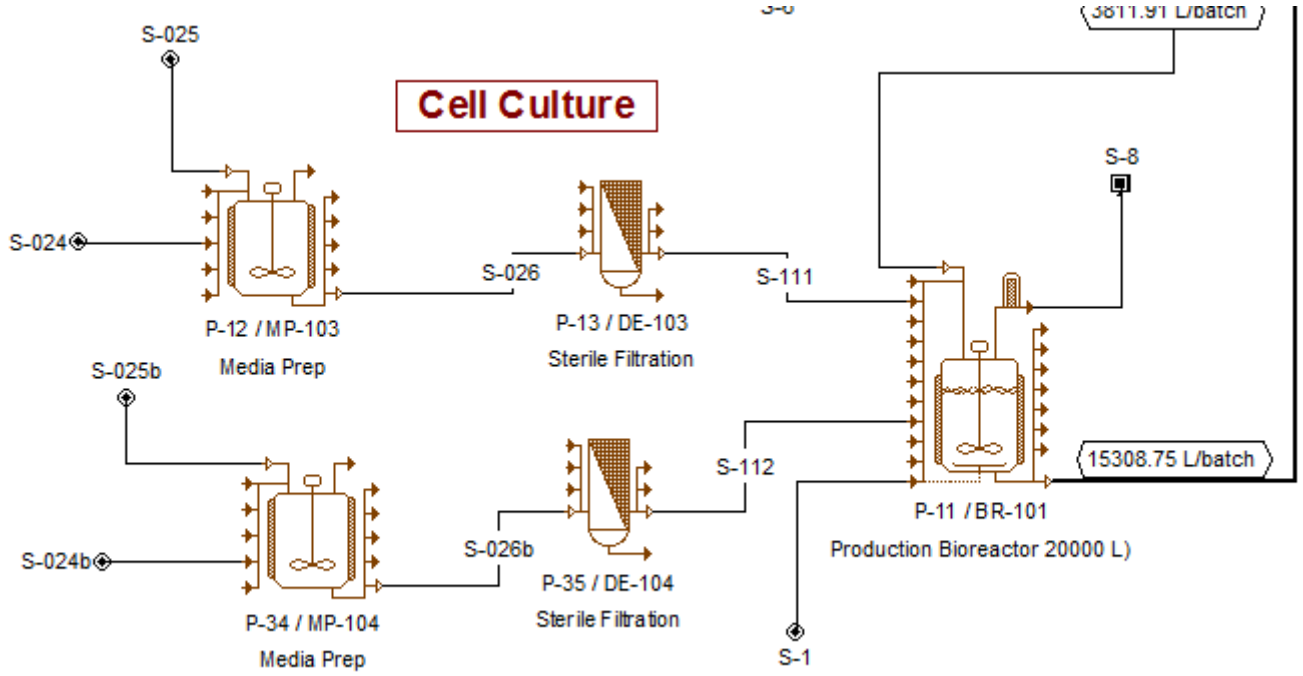
- Addition of various excipients
- Filtration of the final product through a 0.22 μm absoulute filter, then aseptic filling into final containers.
- Freeze-drying if the product is to be marketed in a powedered format.

Fig.: 0.22 μm filter

[1] http://en.wikipedia.org/wiki/Anion-exchange_chromatography

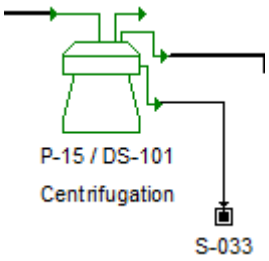
[2] [Walsh 2007], ch.6.9

2.6 **Bioreactor/Tank**

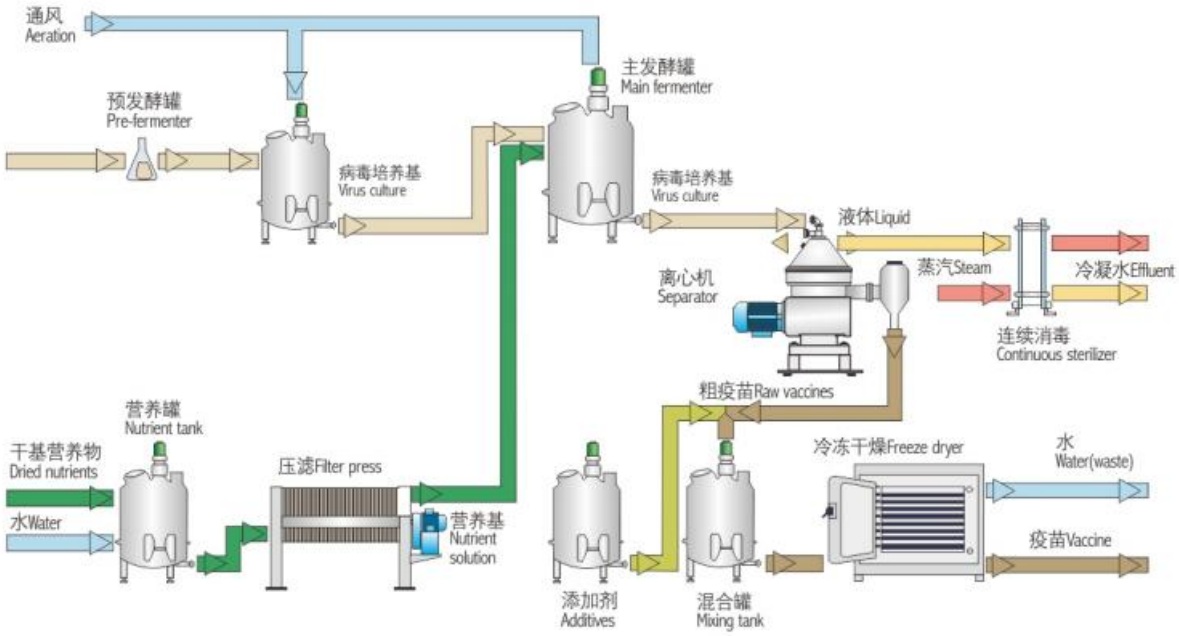


S.cerevisiae needs as nutrition only sugar.

2.7 **Disc-Stack Centrifuge**



2.7.1 *Example for Supplier: Huanding China*



型号 Model	处理量 Capacity(L/h)	重量 Weight(Kg)	外型尺寸(长*宽*高) Dimension(L*W*H)
BTSX15	400~1000	1100	950×950×1250
BTSX35	1000~3000	1500	1400×1400×1450
BTSX85	3000~70000	1800	1750×1450×1850
BTSX150	14000-30000	3700	1850×1500×1850
BTSX200	20000-40000	4800	2100×1700×2200

2014-12-26 13:57 GMT+02:00 Chouse Hu <chousehu@huading-separator.com>:
Dear Samir Mourad

do you think $250,000 dollar in your budget ?

Best Regards

Chouse Hu

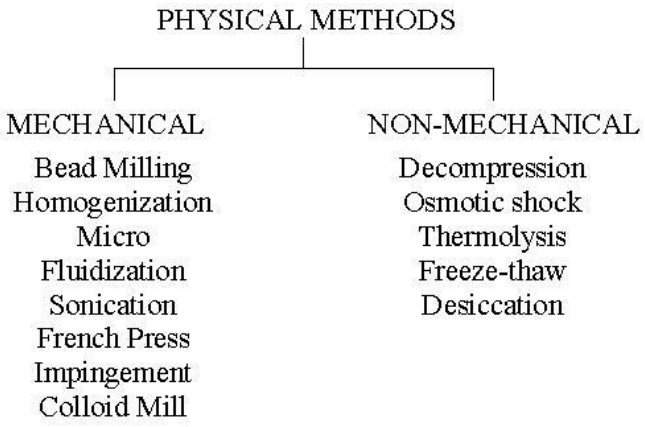
On Dec 27, 2014 8:50 AM, "Chouse Hu" <chousehu@huading-separator.com> wrote:
our cheapest centrifuge can be used in this case at all, that means nothing for you.

please refer to Westalia Separator, and learn what a particular centrifuge can meet your demand.

then, you could try to find out an alternative from China, to reduce your cost.

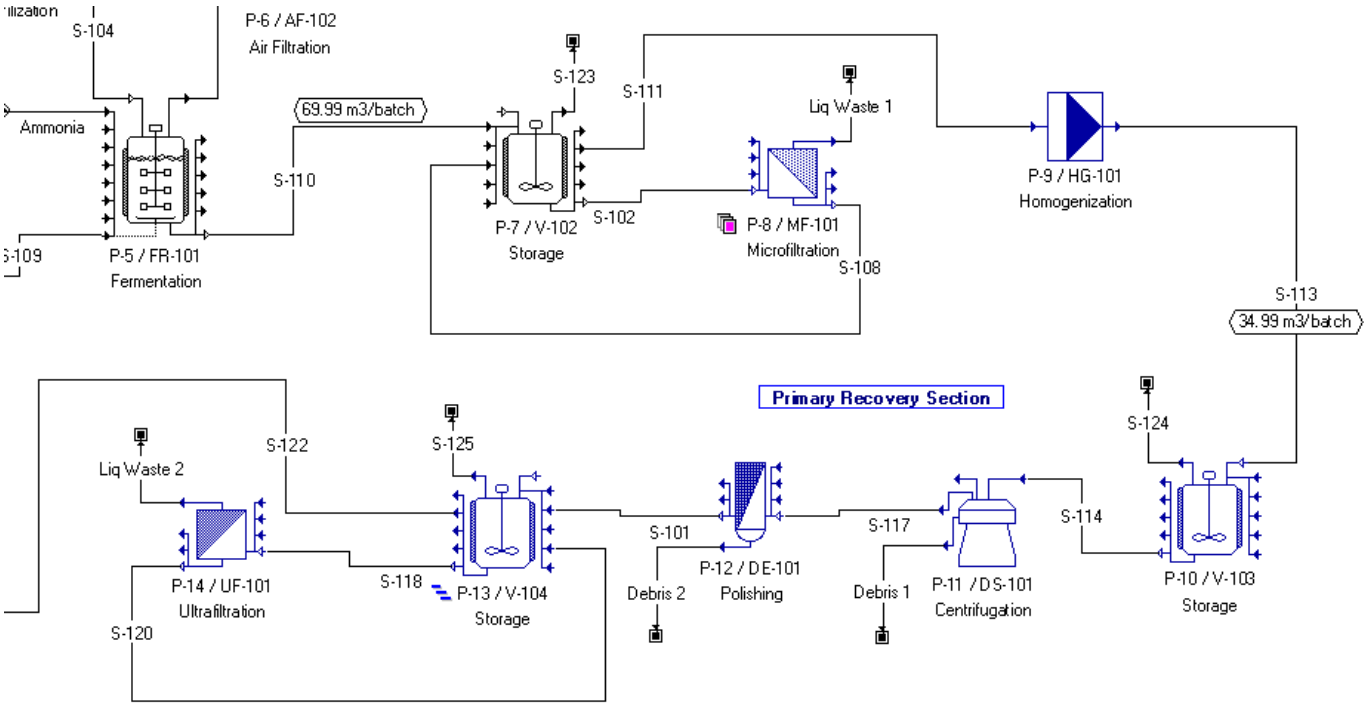
http://www.westfalia-separator.com/no_cache/contact/locations-worldwide/view.html

2.8 Homogenization



Method of cell lysis	disadvantages
freeze-thaw	very slow
chemical lysis	can cause changes in protein structure, difficulties in purification, expensive detergents
enzymatic lysis	often not reproducible, enzyme stability, long incubation time, necessity of removing the lysis enzymes, expensive scale-up, often combination with other method necessary
cell bomb	only applicable to specific cell types
high pressure homogenizer (e.g. french press)	expensive equipment, high maintenance due prone of orifices' clogging
centrifugation	only for very weak cell walls
ball mill / bead mill	uneven processing = incomplete lysis, protein denaturation, low efficiency whilst relatively high energy consumption, complex separation of milling medium and product, time-consuming cleaning

2.8.1 *Principle*



2.8.2 ***Suppliers***

2.8.2.1 Ningbo Scientz company

High Pressure Homogenizer JG-IA Series

Acting on high-pressure principle, the instrument is used for extruding cells, especially suitable for smashing thick-wall cells, germs and denser solution samples. Having no noise, less temperature rise and no metal ion contamination, it has wide application of such research fields as protein study, nucleic acid extraction, cell disruption in genetic engineering labs of colleges, scientific research institutes and pharmaceutical factories.

● Specifications:

Model	JG－IA	Capacity	50ml/times Continuous increase samples for scientific research
Voltage	380 VAC	Maximum operating pressure	256 Mpa (37120 psi)
Pressure device	Hydraulic System	Max Pressure stroke	170 mm
Sample tube	Φ25mm Stainless	Pressure plate speed	6.8 mm/s
Dimensions	555×600×1170 mm	Largest volume of sample	50 ml/times

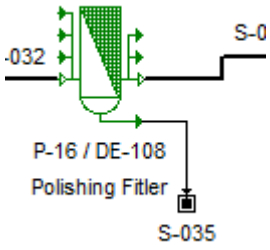
2.8.2.2 Zhangjiagang Beyond Machinery Co., Ltd.

Model Number: GJB, Shipping Terms: FOB Port: Shanghai

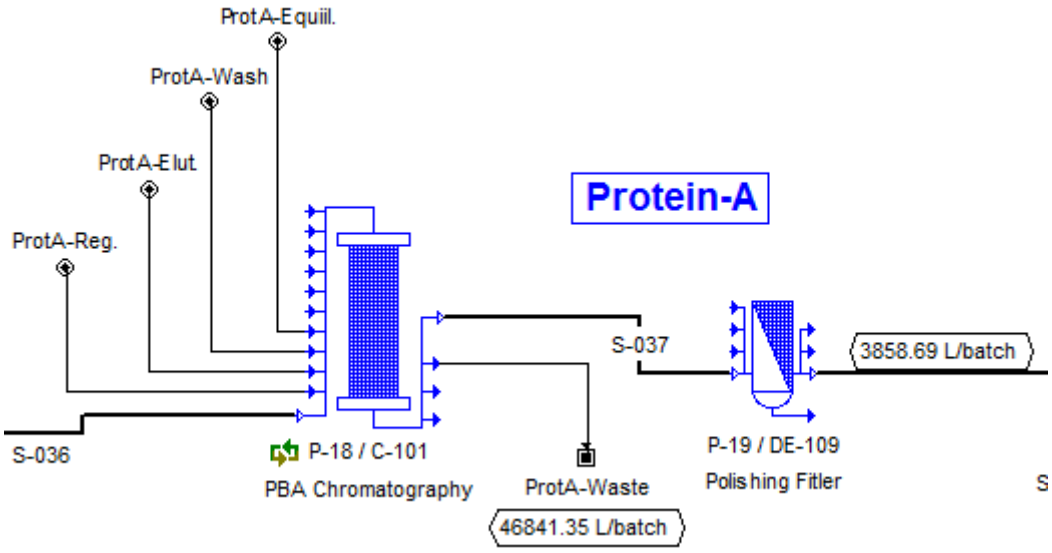
Unit Price: USD 10,000/Sets quantity: 1 Sets

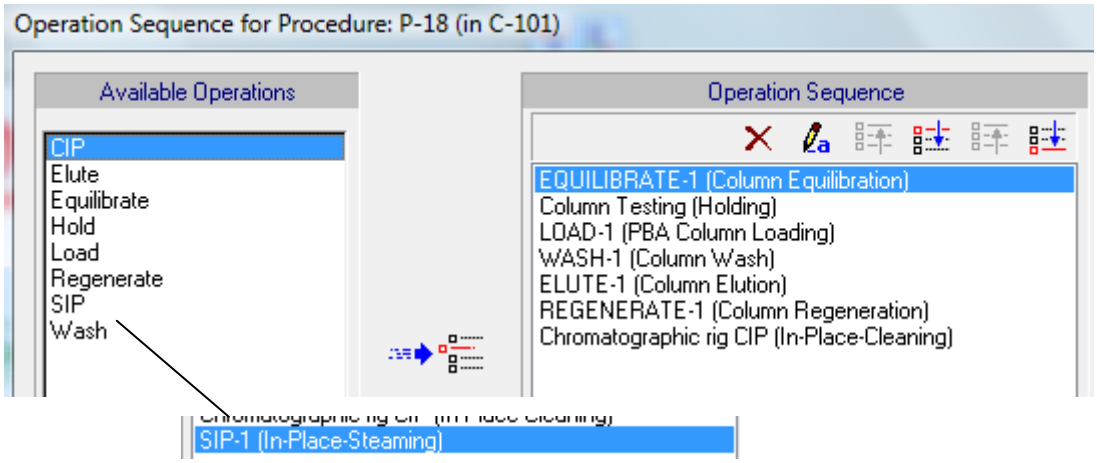
Lead Time: 30days Payment Terms: T/T Quotation Valid Till: 2015-1-28

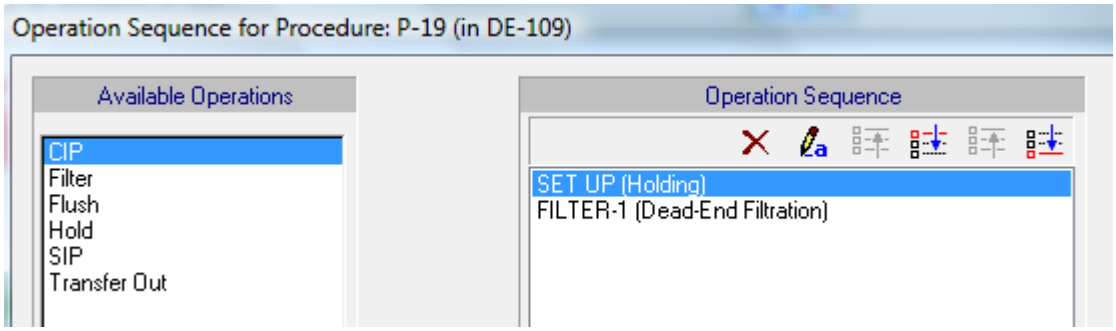
2.9 Dead-End Filter



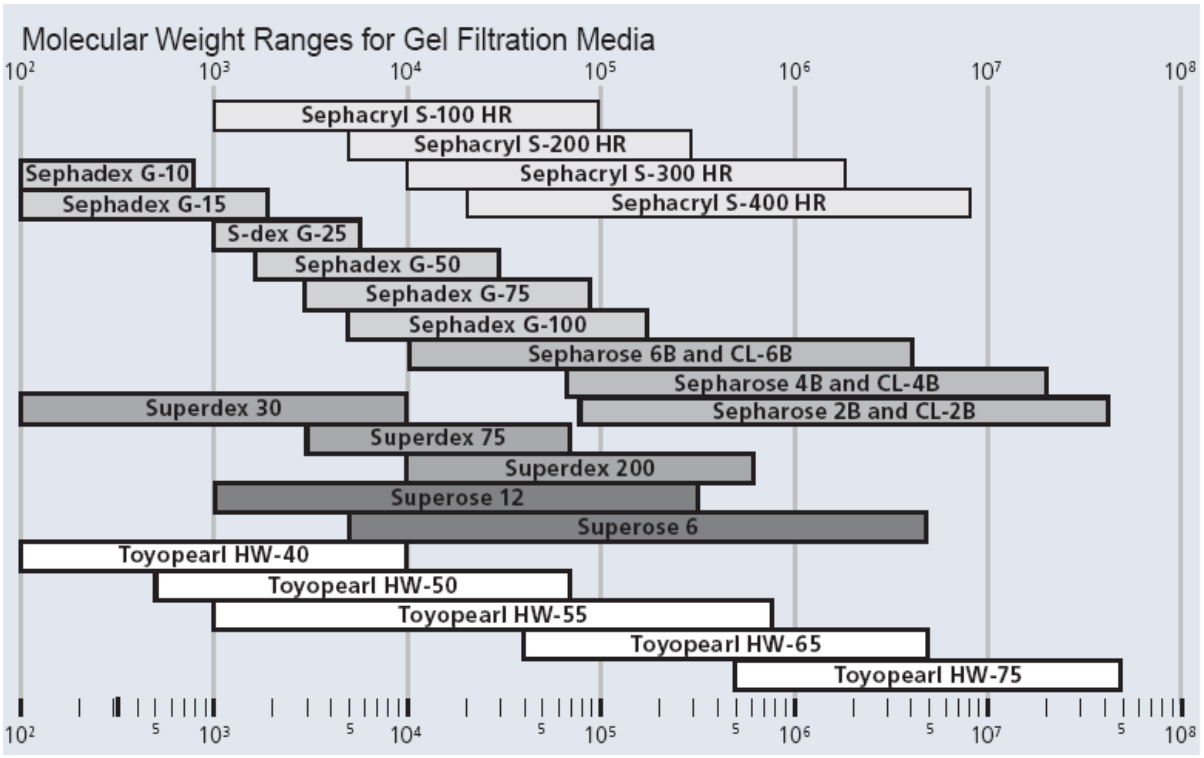
2.10**Protein-A Chromatography**



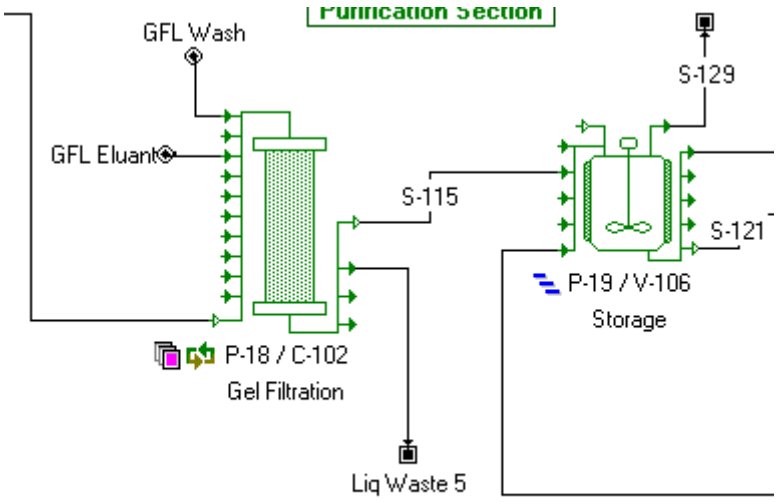




2.11 Gel filtration chromatography



4B Sepharose



2.11.1 *sensor/actuator list*

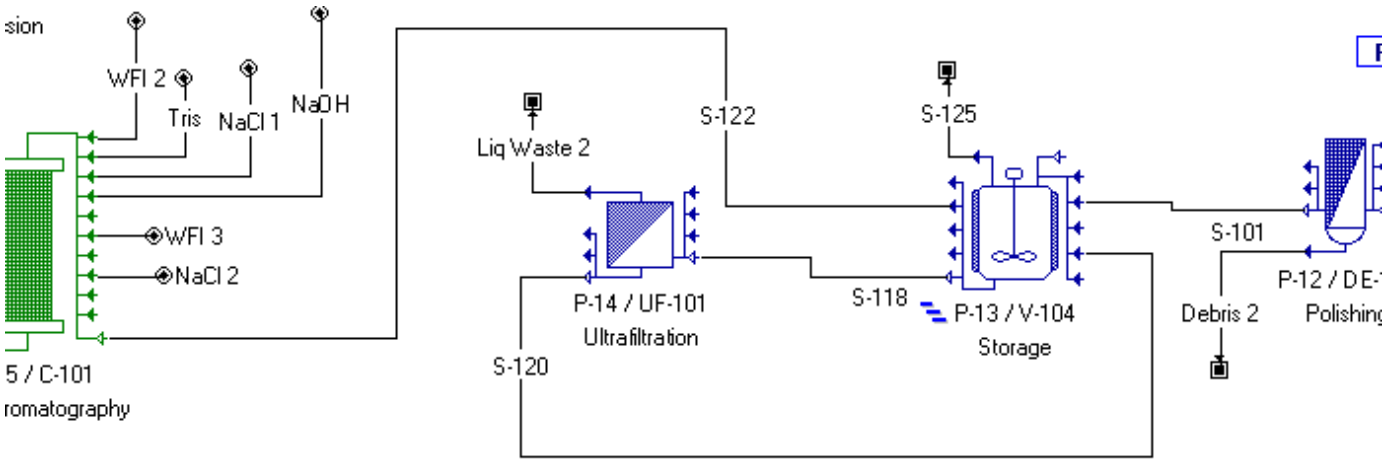
similiar to AKTA process

Akta process Sensors and actuators 13.12.13

Teil	**Anzahl**	Item Price
Air trap	1	
Filter	1	
Filter vent valve	1	
Capsule filter bottom manual valve	1	
Capsule filter top manual valve	1	
System pump	2	
Sample pump	1	

Pressure control valve	2
Buffer A inlet valves	10
Buffer B inlet valves	6
Sample connection valve	1
Sample inlets valves	2
Air trap inlet valve	1
Air trap bypass valve	1
Air trap vent valve	1
Air trap outlet valve	1
Filter inlet valve	1
Filter bypass valve	1
Filter outlet valve	1
System connection valve	1
Column 1 top inlet valve	1
Column 1 bottom inlet valve	1
Column 1 top valve	1
Column 1 bottom valve	1
Column 1 top outlet valve	1
Column 1 bottom outlet valve	1
Column 2 top inlet valve	1
Column 2 bottom inlet valve	1
Column 2 top valve	1
Column 2 bottom valve	1
Column 2 top outlet valve	1
Column 2 bottom outlet valve	1
Outlet valves	9
Air trap drain valve	1
Filter drain valve	1
CIP / AxiChrom manifold	1
Buffer inlet air sensor	1
Pre-column air sensor	1
Post-column pH-meter	1
Post-column UV-meter	1
Pre-column conductivity meter	1
Post-column conductivity	1
System flow meter	1
Air trap high level meter	1
Air trap low level meter	1
Pre-filter pressure meter	1
Pre-column pressure meter	1
Sample pump pressure meter	1
PCV pressure meter, A inlets	1
PCV pressure meter, B inlets	1

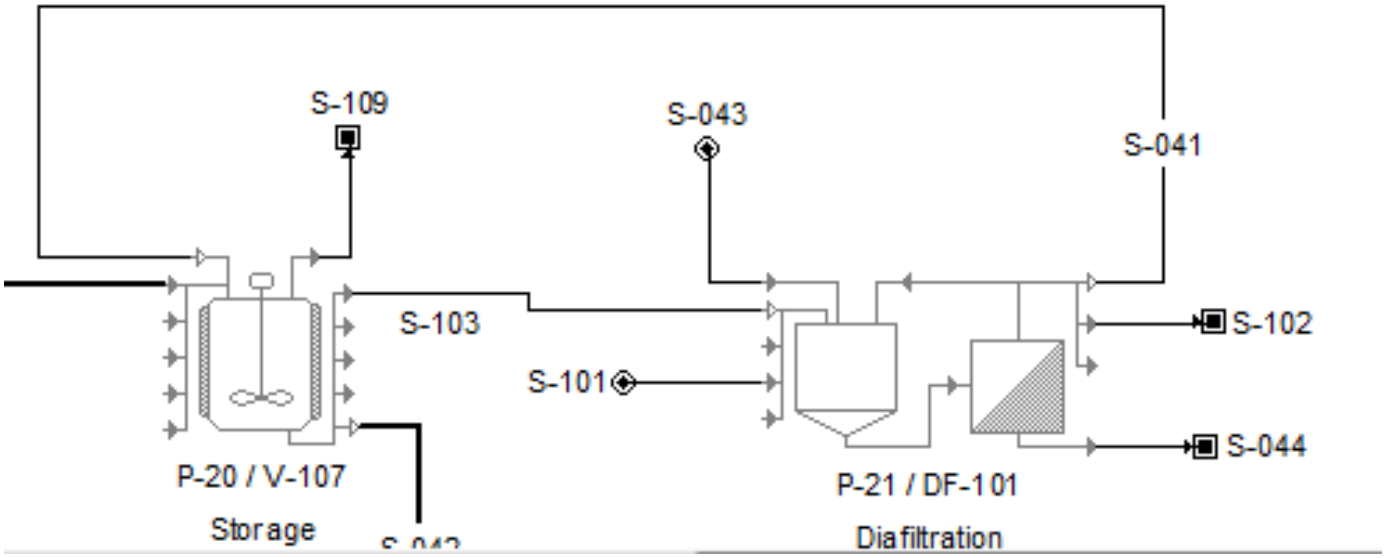
2.12**Ultrafiltration**

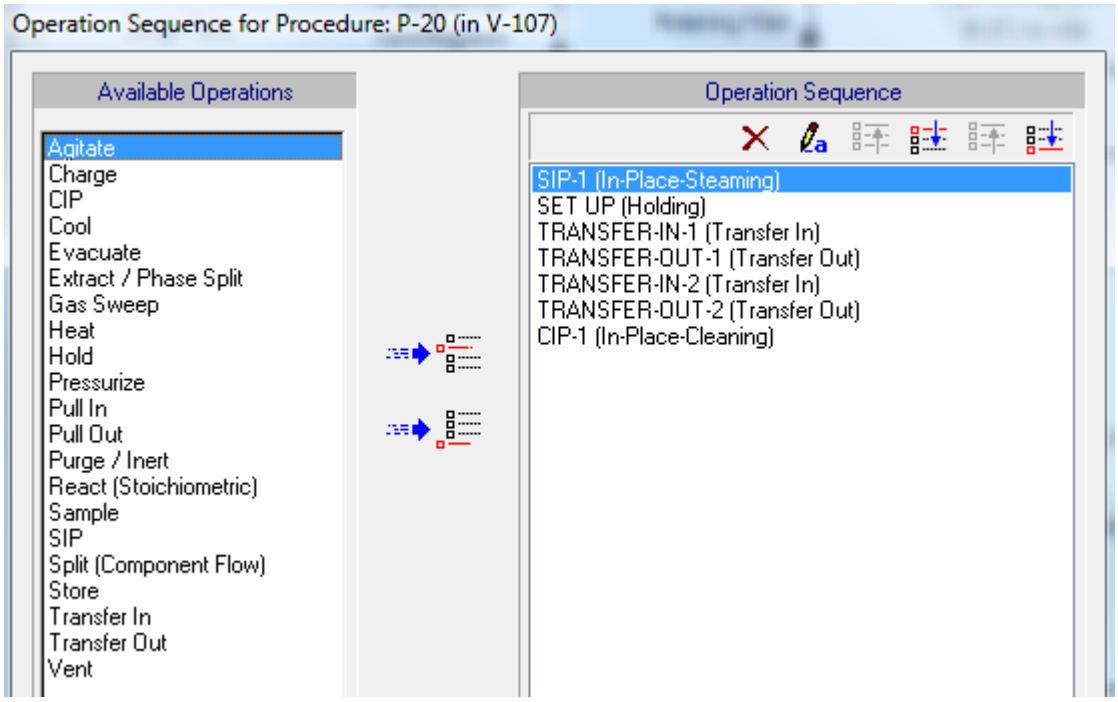


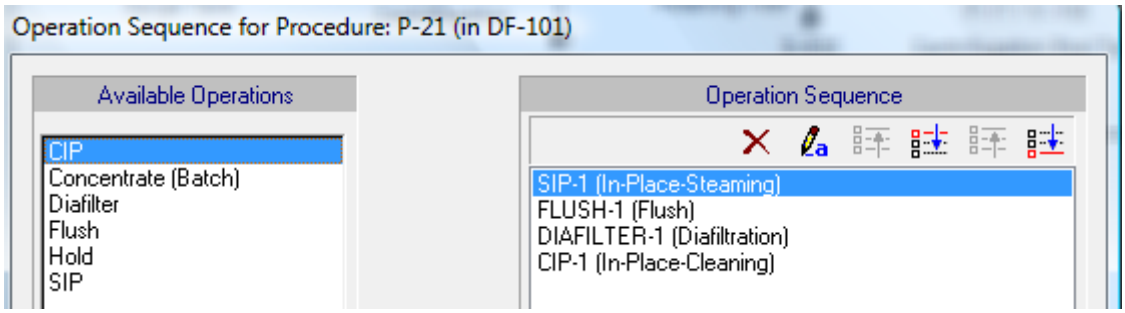
From SuperPro Example Bgal

2.13**Diafiltration**

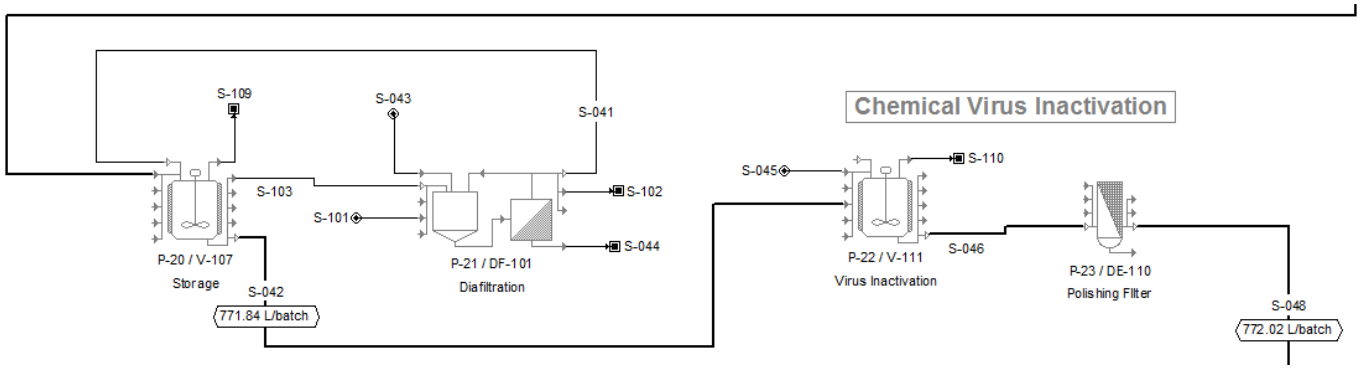
Same equipment as ultrafiltration

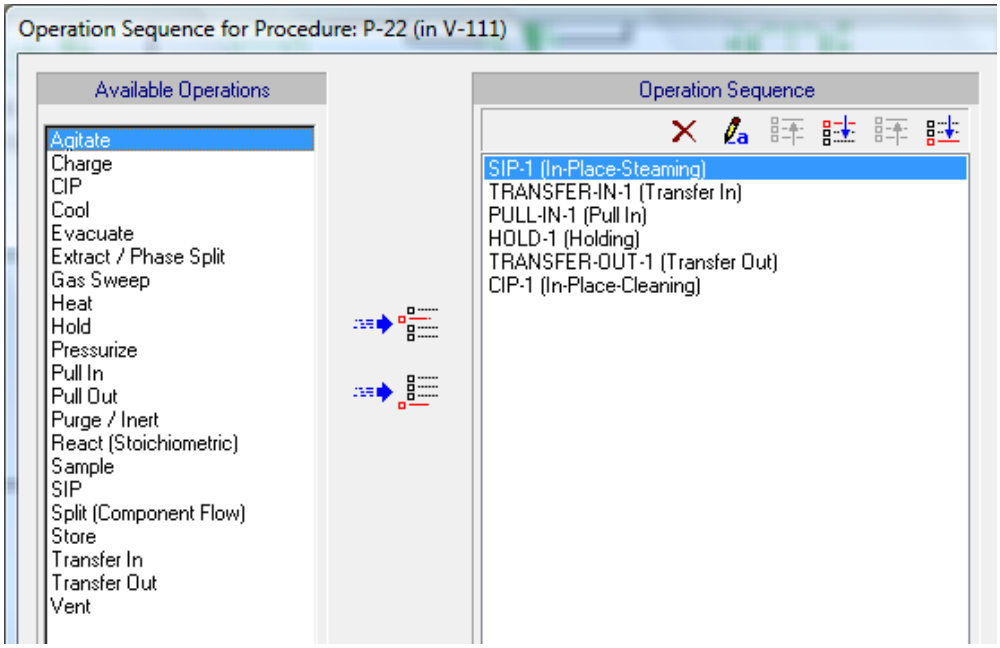






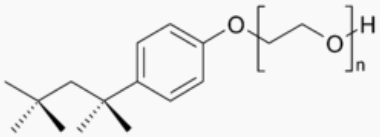
2.14 Chemical Virus Inactivation





2.14.1 ***Solvent/detergent (S/D) inactivation***[3]

This process, developed by the New York Blood Center,[5] is the most widely used viral inactivation method to date. It is predominantly used in the blood plasma industry, by over 50 organizations worldwide and by the American Red Cross [1]. This process is only effective for viruses enveloped in a lipid coat, however. The detergents used in this method interrupt the interactions between the molecules in the virus's lipid coating. Most enveloped viruses cannot exist without their lipid coating so are destroyed when exposed to these detergents. Other viruses may not be destroyed but they are unable to reproduce rendering them non-infective. The solvent creates an environment in which the aggregation reaction between the lipid coat and the detergent happen more rapidly. The detergent typically used is Triton-X 100.



Chemical Structure of Triton X-100 (n = 9-10).

This process has many of the advantages of the "traditional" removal techniques. This process does not denature proteins, because the detergents only affect lipids and lipid derivatives. There is a 100% viral death achieved by this process and the equipment is relatively simple and easy to use. Equipment designed to purify post-virus inactivated material would be necessary to guard against contamination of subsequent process streams.

S/D treatment utilizes readily available and relatively inexpensive reagents, but these reagents must be removed from the product prior to distribution which would require extra process steps. Because this process removes/inactivates the lipid coating of a virus, viruses without any sort of lipid envelope will be unaffected. There is also no inactivation effect by the buffers used in this process.

---

3 http://en.wikipedia.org/wiki/Virus_processing#Viral_inactivation

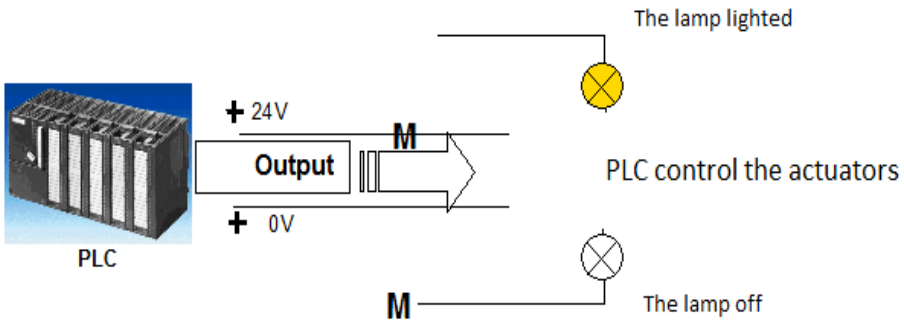
2.15Introduction to Siemens S7 PLC system

2.15.1 *Definition of PLC:*

PLC is an acronym for Programmable Logic Control (logic controllers programmable). This description of the system that controls the process (exp machine to print newspapers, facility for packing cement, piston to cut plastic ...). This process is carried out in accordance with the instructions of the program in the device memory.

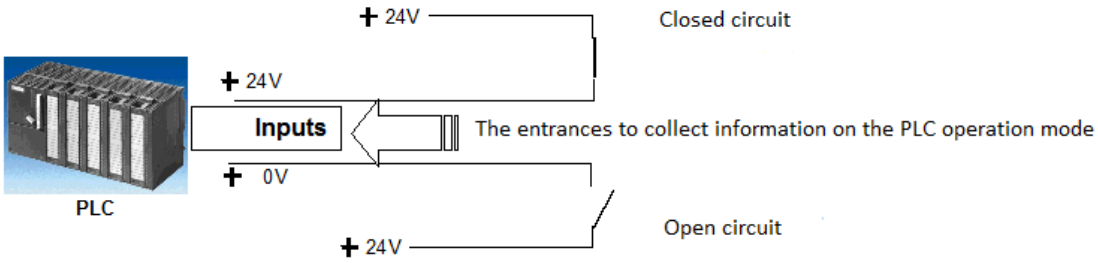
2.15.2 *Function of PLC:*

PLC controls the process in which the actuators are connecting to links feed (for example, 24 volts) specific to the PLC exits Outputs. Through these links we can run and turn off the engine, open and close the valves, or turn on and turn off the lights.

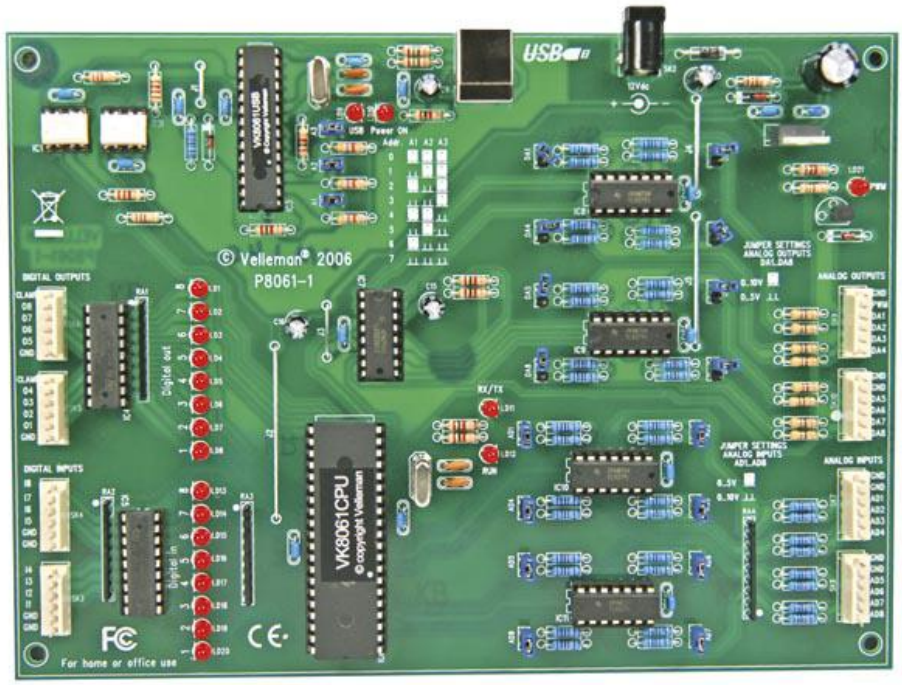


2.15.3 *Where to get the information about the PLC operation mode:*

PLC receives the information about the operation from the signal - generator connected to the entrances of the PLC. These signal generators can be, for example, sensors recognize the status of the working parts, keys or buttons. This specific situation can be open or closed. The difference between Note normally open NC: Normally Closed is ineffective when they are closed and petitions NO: Normally Open Normally open that are not effective when they are open:



2.16 Introduction to the DLL for the USB Interface Board K8061:

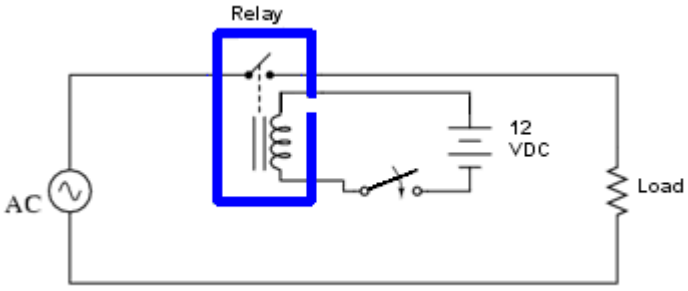


The K8061 interface board has 8 digital input channels and 8 digital output channels. In addition, there are 8 analogue inputs, 8 analogue outputs and one PWM output. The number of inputs/outputs can be further expanded by connecting more (up to a maximum of eight) cards to the PC's USB connectors. Each card is given its own identification number by means of three jumpers, A1, A2 and A3 (see table 1 below for card numbering).

Connection to the computer is optically isolated, so that damage to the computer from the card is not possible.

2.17**Definition of a relay**

A relay is usually an electromechanical device that is actuated by an electrical current. The current flowing in one circuit causes the opening or closing of another circuit. Relays are like remote control switcher and are used in many applications because of their relative simplicity, long life, and proven high reliability. Relays are used in a wide variety of applications throughout industry, such as in telephone exchanges, digital computers and automation systems. Highly sophisticated relays are utilized to protect electric power systems against trouble and power blackouts as well as to regulate and control the generation and distribution of power.



How do relays work?

All relays contain a sensing unit, the electric coil, which is powered by AC or DC current. When the applied current or voltage exceeds a threshold value, the coil activates the armature, which operates either to close the open contacts or to open the closed contacts. When a power is supplied to the coil, it generates a magnetic force that actuates the switch mechanism. The magnetic force is, in effect, relaying the action from one circuit to another. The first circuit is called the control circuit; the second is called the load circuit.
There are three basic functions of a relay: On/Off Control, Limit Control and Logic Operation.

3. Concept

3.1 Mechanical structure

The concept is to install a simplified DNA vaccine production line based on devices of GE Health and other suppliers.

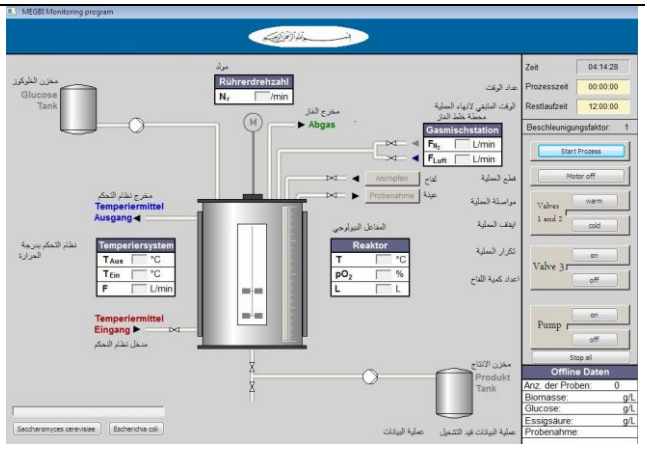
(As increment: simplified monoclonal antibodies production line using the same equipment)

The stainless structure shall be built by TEMO. The instrumentation and special pipes etc. shall be buyed for GE Health or others.

Fig.: Solaris downstream line. The MEGBI-VPP downstream line shall be similar to this.

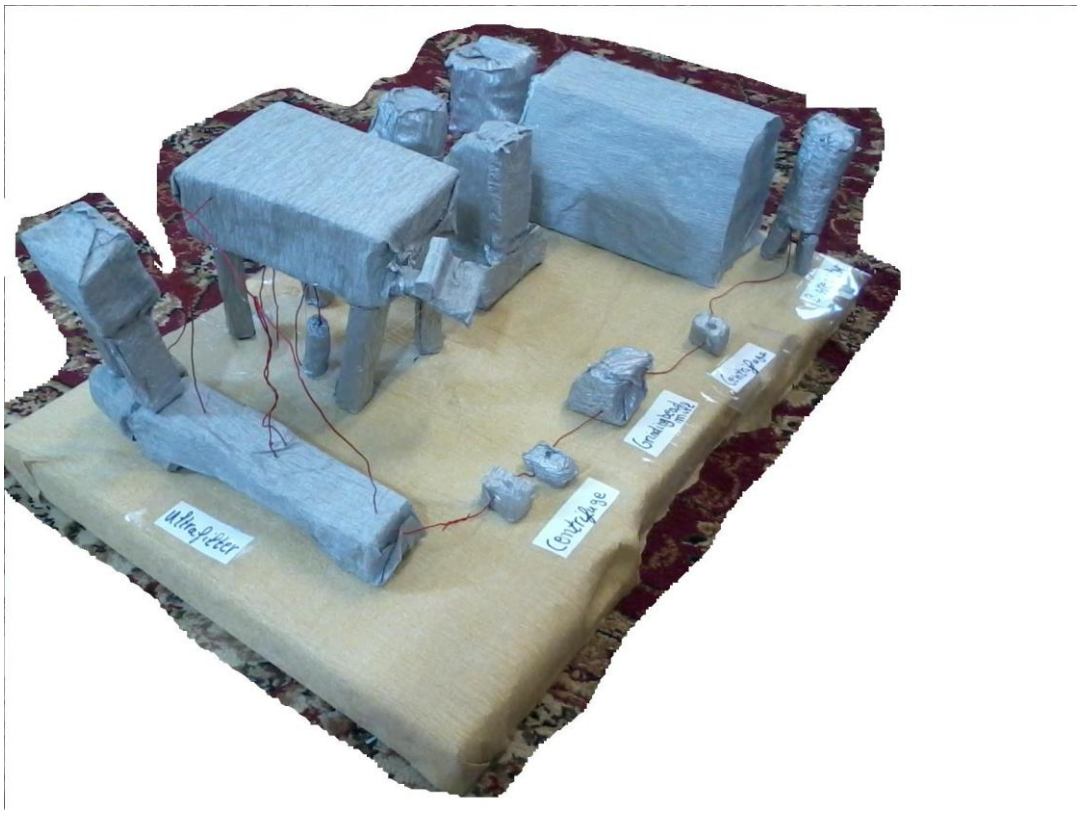
3.2 Automation System

The automation system shall have a C++/phython user interface and a Simatic S7 interface to the sensors/actuators.



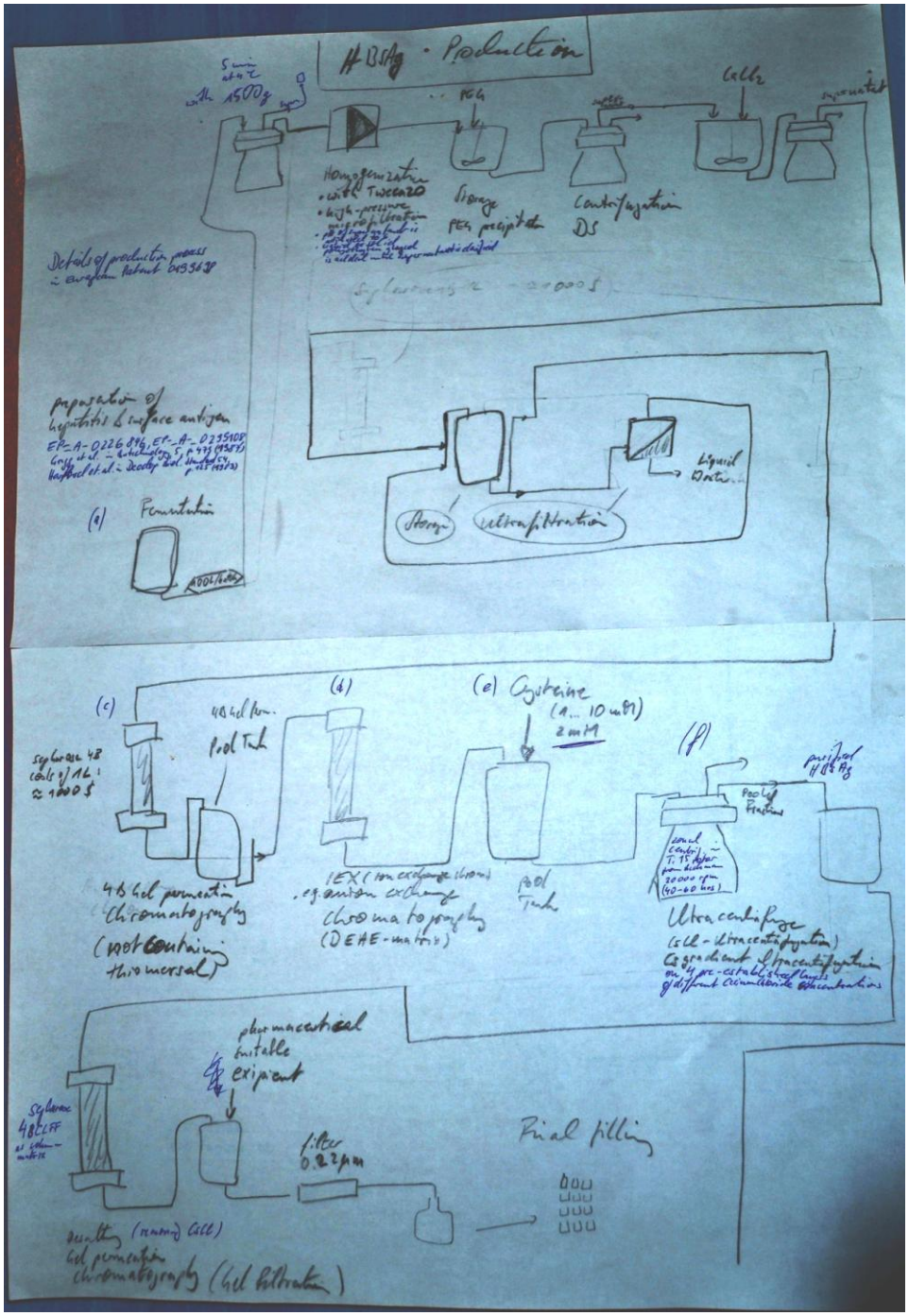
4. System Design

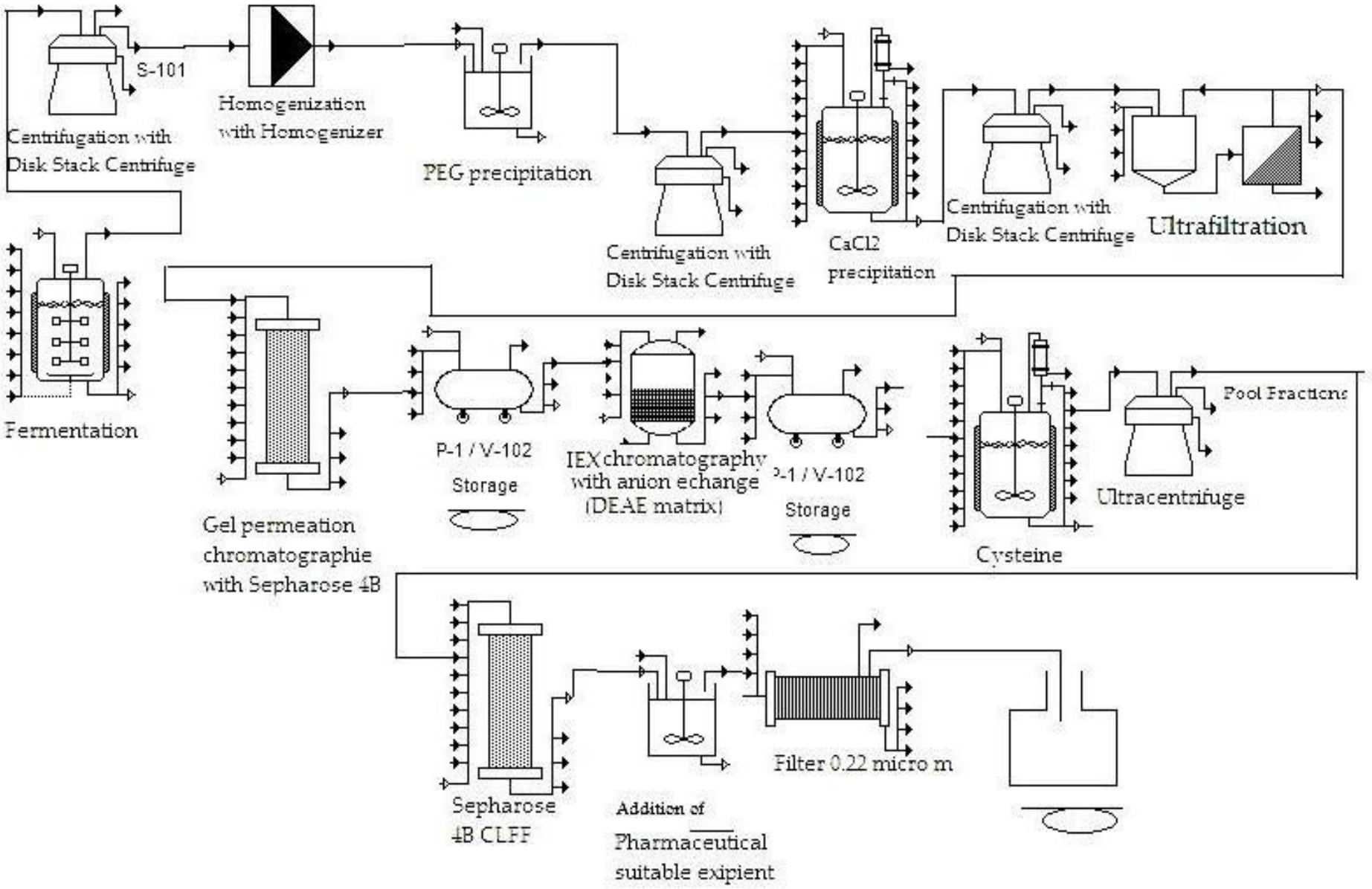
4.1 Mock-up model for the MEBGI vaccine pilot plant



4.2 Integration Overview

Based on the EngerixB patent and the films Bioprocessing Part 2_ Separation _ Recovery and Bioprocessing Part 3_Purification which describe in details the process of Fluorecence Protein Production in E.coli there shall be designed a simplified system for HBSAg vaccine. Using SuperPro SW for describing the design.

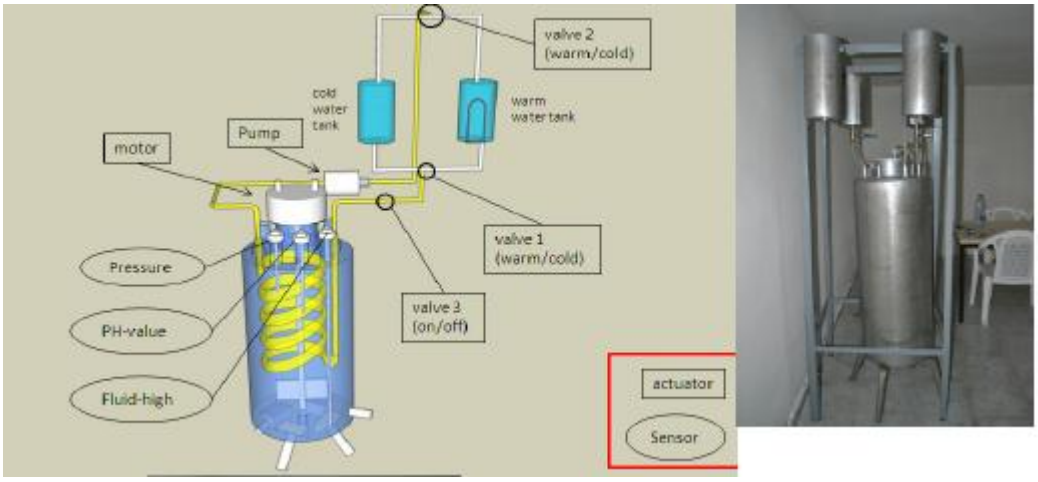



S-101
Centrifugation with
Disk Stack Centrifuge
Homogenization
with Homogenizer
PEG precipitation
Centrifugation with
Disk Stack Centrifuge
CaCl2
precipitation
Centrifugation with
Disk Stack Centrifuge
Ultrafiltration
Fermentation
Gel permeation
chromatographie
with Sepharose 4B
P-1 / V-102
Storage
IEX chromatography
with anion echange
(DEAE matrix)
P-1 / V-102
Storage
Ultracentrifuge
Pool Fractions
Cysteine
Sepharose
4B CLFF
Addition of
Pharmaceutical
suitable exipient
Filter 0.22 micro m

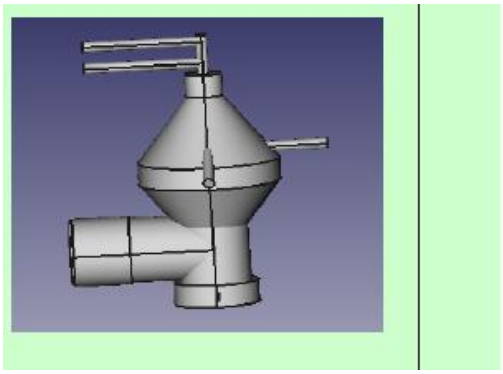
Clean-in-place (CIP)&Steam-in-place (SIP) and intermediate storage	Upstream Processing (USP) – Downstream Processing (DSP)	Automation
 		 

4.3 **Fermentation**

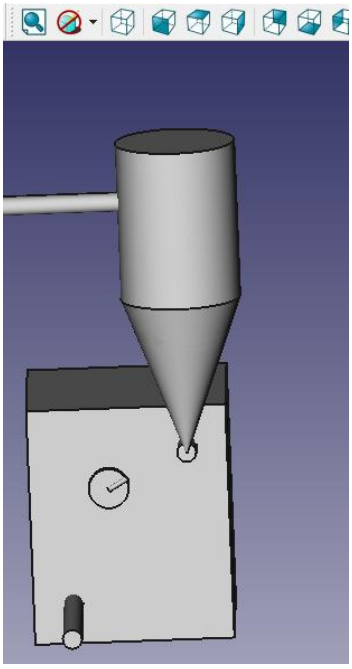
75 L/batch



4.4 Centrifugation with Disc Stack Centrifuge (1)

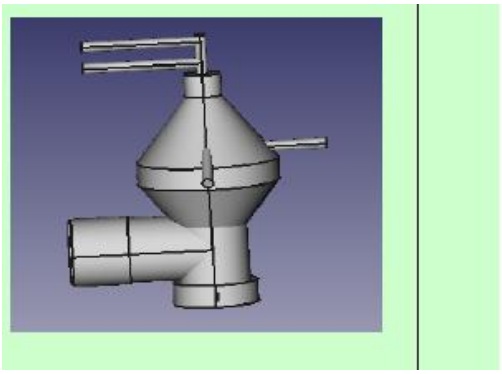


4.5 Homogenization with Homogenizer



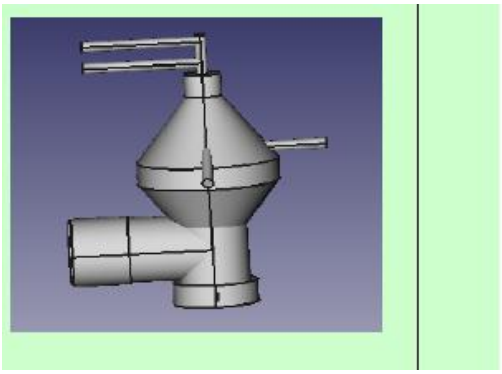
4.6 PEG precipitation

4.7 Centrifugation with Disc Stack Centrifuge (2)



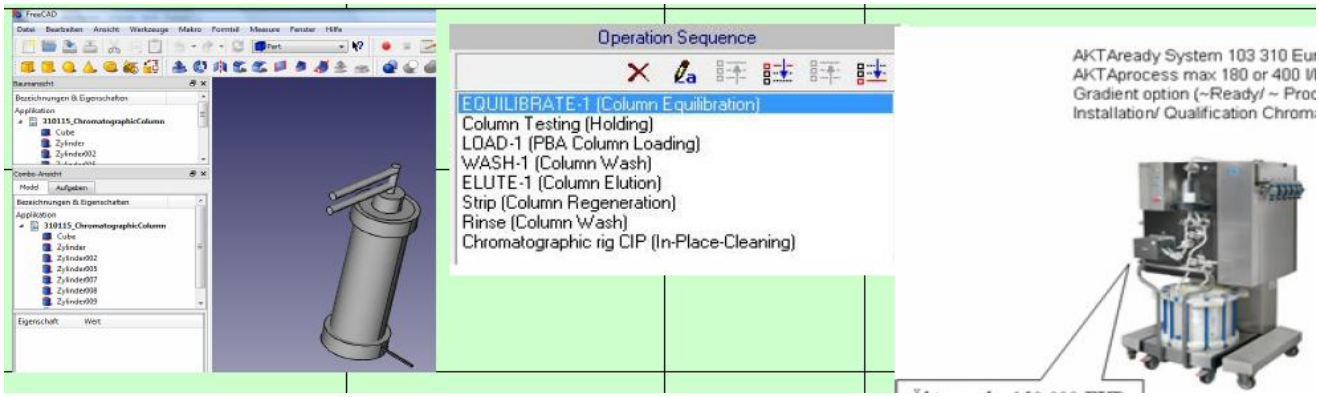
4.8 CaCl2 precipitation

4.9 Centrifugation with Disc Stack Centrifuge (3)

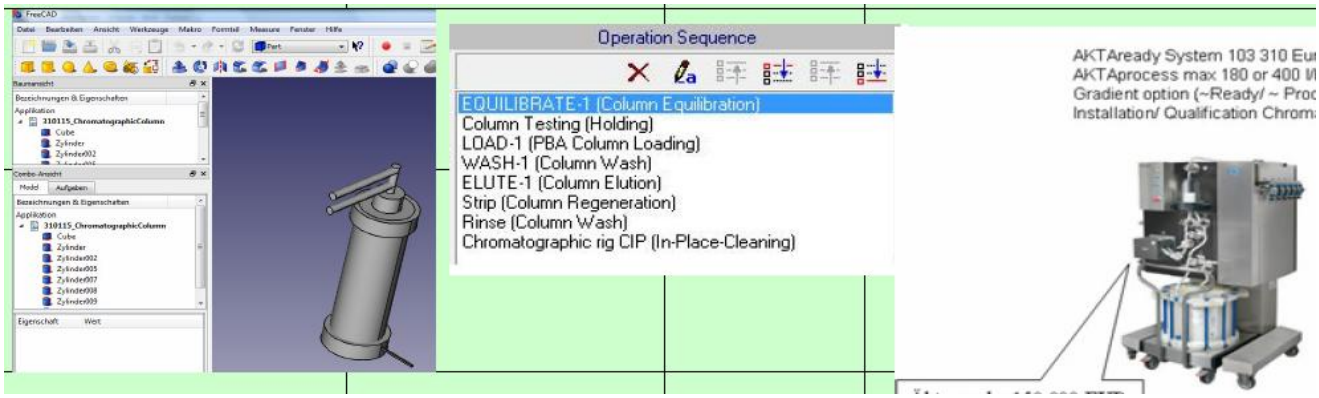


4.10Ultrafiltration

4.11Gel permeation chromatography with Sepharose 4B



4.12IEX chromatography with anion echange (DEAE matrix)



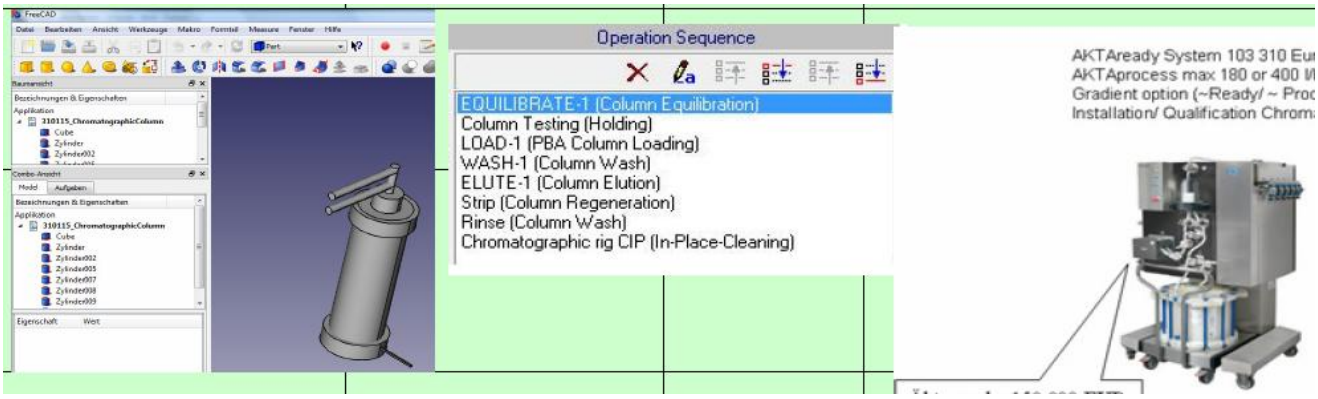
4.13Cystein addition

4.14Ultracentrifugation

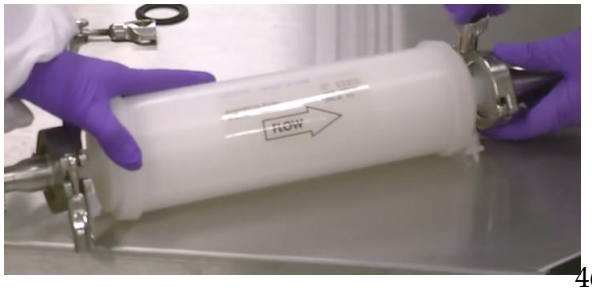
Pooled fractions contain HBSAg

Figure: Alfa Wassermann Ultracentrifuges

4.15 Desalting Gel permeation chromatography with Sepharose 4BCLFF



4.16 Filtering with 0.22 µm filter



5. Detailed Devices Mechanical Design

Done as 6 weeks practical work for 4 students (former planned as master thesis)

بسم الله الرحمن الرحيم

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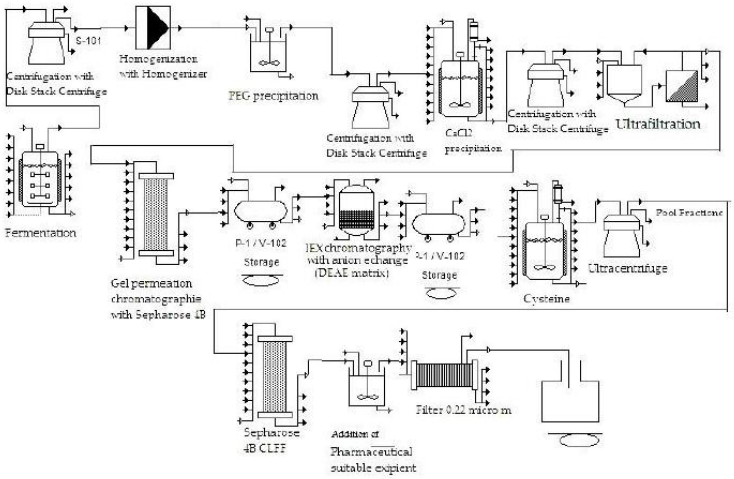
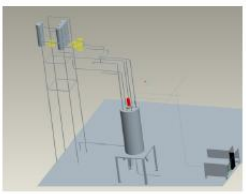
الجمعية اللبنانية لدعم البحث العلمي

TEMO
Hardware & Consulting e.K. - Software

Ras Nhache/Batroun - Tripoli, 12th Jan 2015

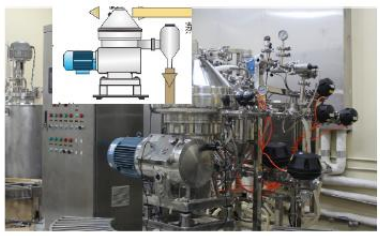
MEGBI Bioreactor





MEGBI-VPP system design:

Downstream System (Purification) from Solaris Group (in this way MEGBI-VPP downstream processing shall be developed)



Disc stack centrifuge from Huading/China

Master Thesis

Modeling and Integration of devices for the vaccine production plant MEGBI-VPP

- Modeling of Homogenizer (3 weeks)
- Modeling of Disc Stack Centrifuge in including CIP/SIP functional elements (mechanics) (3 weeks)
- Modeling Process Scale Gel Permeation and ion exchange chromatographic devices (3 weeks)
- Modeling of Process Scale Ultrafiltration Device (3 weeks)
- Integration of Devices to MEGBI-VPP downstream processing (DSP) unit (2 weeks)
- Documentation (3 weeks)

Keywords: CAD, Tool ProEngineer, Process Scale Homogenizer, Process Scale Centrifugation, Process Scale Protein Chromatography, Biotechnology

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بسم الله الرحمن الرحيم

Project: MEGBI-VPP (Detailed Devices Mechanical Design for 3rd MEGBI-VPP Report)

Report of Practical Work Jun/Jul 2015 (6 Weeks), Final version: 19 Aug 2015

Authors: Jihad Samarji, ZaherChendeb, Ibrahim Zaaroura, FadiYahya

OBJECTIVE AND PURPOSE OF THE ASSOCIATION:

The association is committed to the promotion of international cooperation in the economic and scientific fields in order to achieve the idea of international understanding and a closer relationship between institutions the Middle East, in Europe and its neighbors. To Download the AECENAR flyer as pdf please click here: AECENAR Brochure 2014

INSTITUTE:

MEGBI - Middle East Genetics and Biotechnology Institute

PROJECT:

MEGBI-VPP / DNA Vaccine Pilot Plant, Recombinant Vaccine Technology / Biotechnological Upstream & Downstream Processing Hepatitis B DNA Vaccine Pilot Plant

PROJET CURRENT TASKS:

Modelling of homogenizer

Modelling of Disc Stack Centrifuge in including CIP/SIP functional elements (mechanics)

Modelling Process Scale Gel Permeation and Ion Exchange Chromatography Devices

Modelling of Process Scale Ultrafiltration Device

Integration of Devices to MEGBI-VPP Downstream processing (DSP) unit

Documentation

SKILLS NEEDED:

CAD, Process Scale Homogenizer, Process Scale Centrifugation, Process Scale Protein Chromatography

SOFTWARE USED:

FREECAD (+ Python MACROS), Microsoft OFFICE, Adobe Suite, Windows OS / Linux OS





5.1 Demonstration and Modelling of the Ultracentrifuge.

"To Separate the High Density from the Low Density Molecules, We Use The Ultracentrifuge." –Jihad Samarji

Figure 1.1 PKII Ultra Centrifuge

5.1.1 *DEVICE DETAILS & SPECIFICATIONS*

The **ultracentrifuge** is a centrifuge optimized for spinning a rotor at very high speeds, capable of generating acceleration as high as 2 000 000 g (approx. 19 600 km/s²). There are two kinds of ultracentrifuges, the preparative and the analytical ultracentrifuge. Both classes of instruments find important uses in molecular biology, biochemistry, and polymer science.

In our Case we're using the Preparative Ultracentrifuge

Preparative ultracentrifuges are available with a wide variety of rotors suitable for a great range of experiments. Most rotors are designed to hold tubes that contain the samples. *Swinging bucket rotors* allow the tubes to hang on hinges so the tubes reorient to the horizontal as the rotor initially accelerates. *Fixed angle rotors* are made of a single block of material and hold the tubes in cavities bored at a predetermined angle.

In our Case we're using the Zonal Rotor

Zonal rotors are designed to contain a large volume of sample in a single central cavity rather than in tubes. Some zonal rotors are capable of dynamic loading and unloading of samples while the rotor is spinning at high speed.

Preparative rotors are used in biology for pelleting of fine particulate fractions, such as cellular organelles (mitochondria, microsomes, and ribosomes) and viruses. They can also be used for gradient separations, in which the tubes are filled from top to bottom with an increasing concentration of a dense substance in solution. Sucrose gradients are typically used for separation of cellular organelles. Gradients of caesium salts are used for separation of nucleic acids. After the sample has spun at high speed for sufficient time to produce the separation, the rotor is allowed to come to a

smooth stop and the gradient is gently pumped out of each tube to isolate the separated components. In our Case we're using it to produce anti-bodies

We Need To Design a Density Gradient Ultracentrifugation System for large scale and pilot scale downstream processing of viral vaccines and virus like particles.

	Air Drive
Models Available	KII and PKII
Process Air	2.83 m^3/min
Process Cooling	4.5 °C, 4 l/min
Electrical Supply	15A, 1ph 230V
Environmental Conditions	0-40°C, RH 85%
Clean Room Classifications	Class B,C,D
Bio-containment	Up to BL3
System Footprint	2140 x 1330 (W x D)
Height Requirements	295 cm (PKII 220 cm)
System Weight	1270 kg (PKII 1204 kg)

Table 1.1 Ultracentrifuge KII&PKII specifications

So we're going to design something close to the ALFA WASSERMAN KII & PKII Continuous Flow Ultracentrifuge model. See figure 1.1 and 1.2

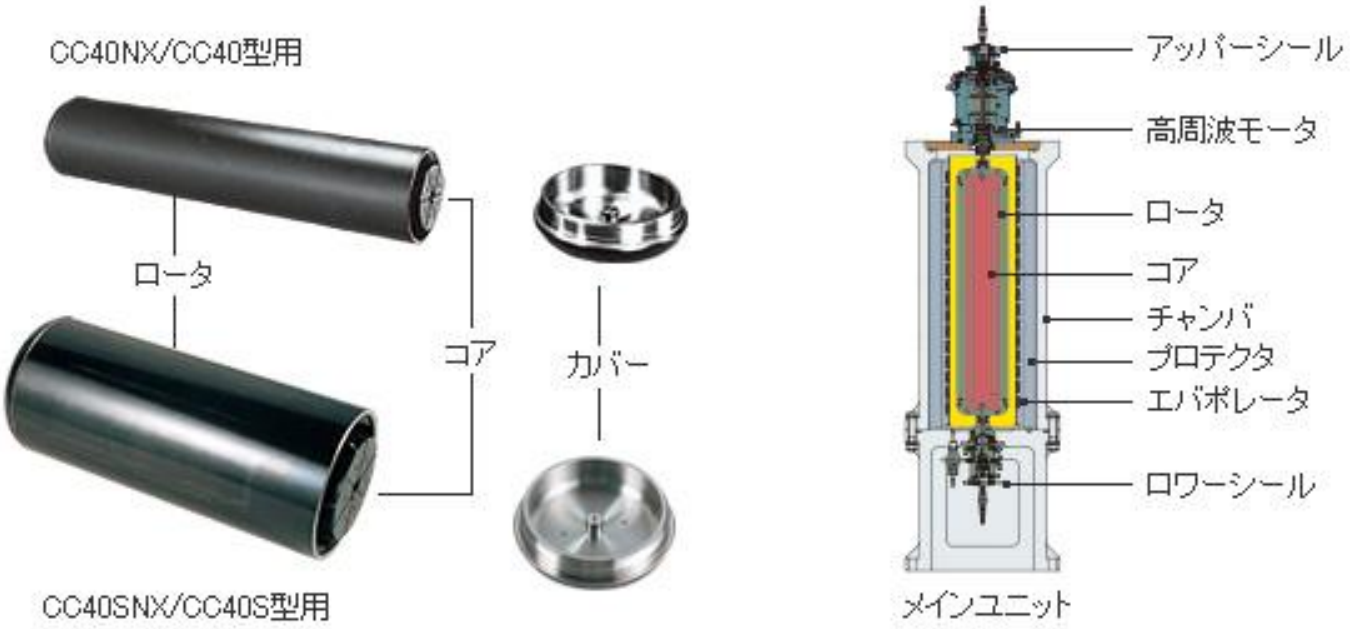
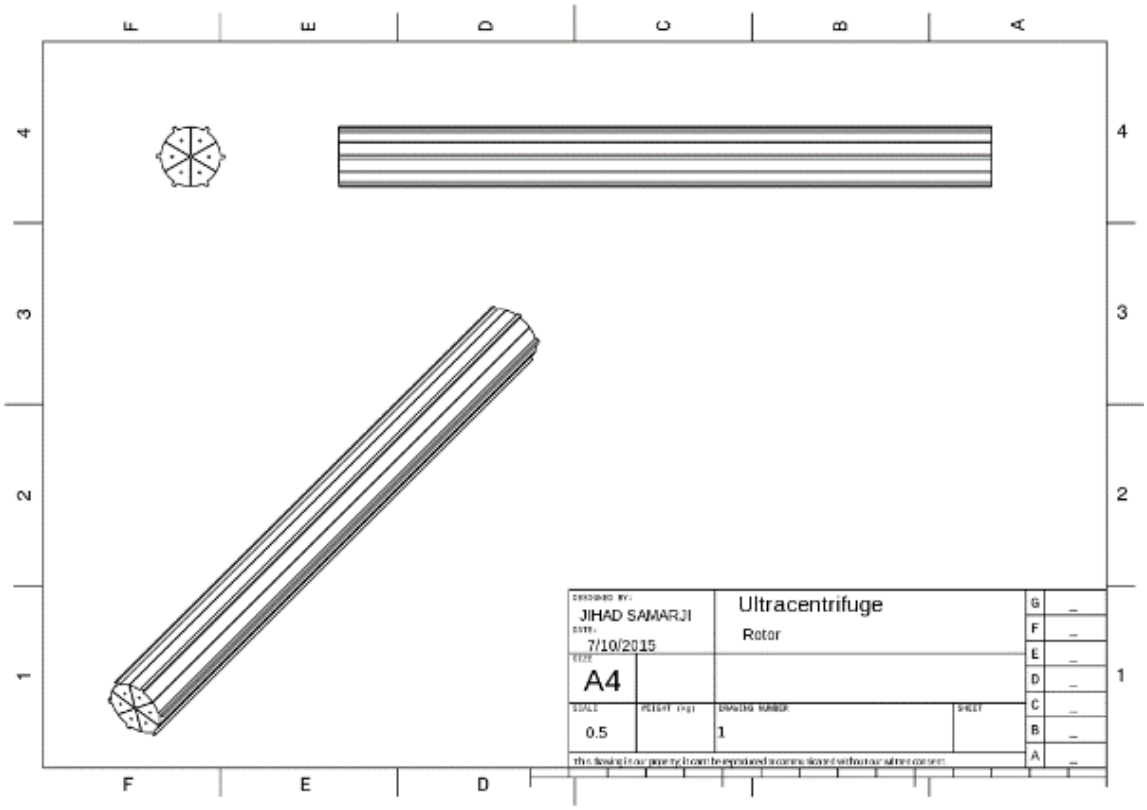


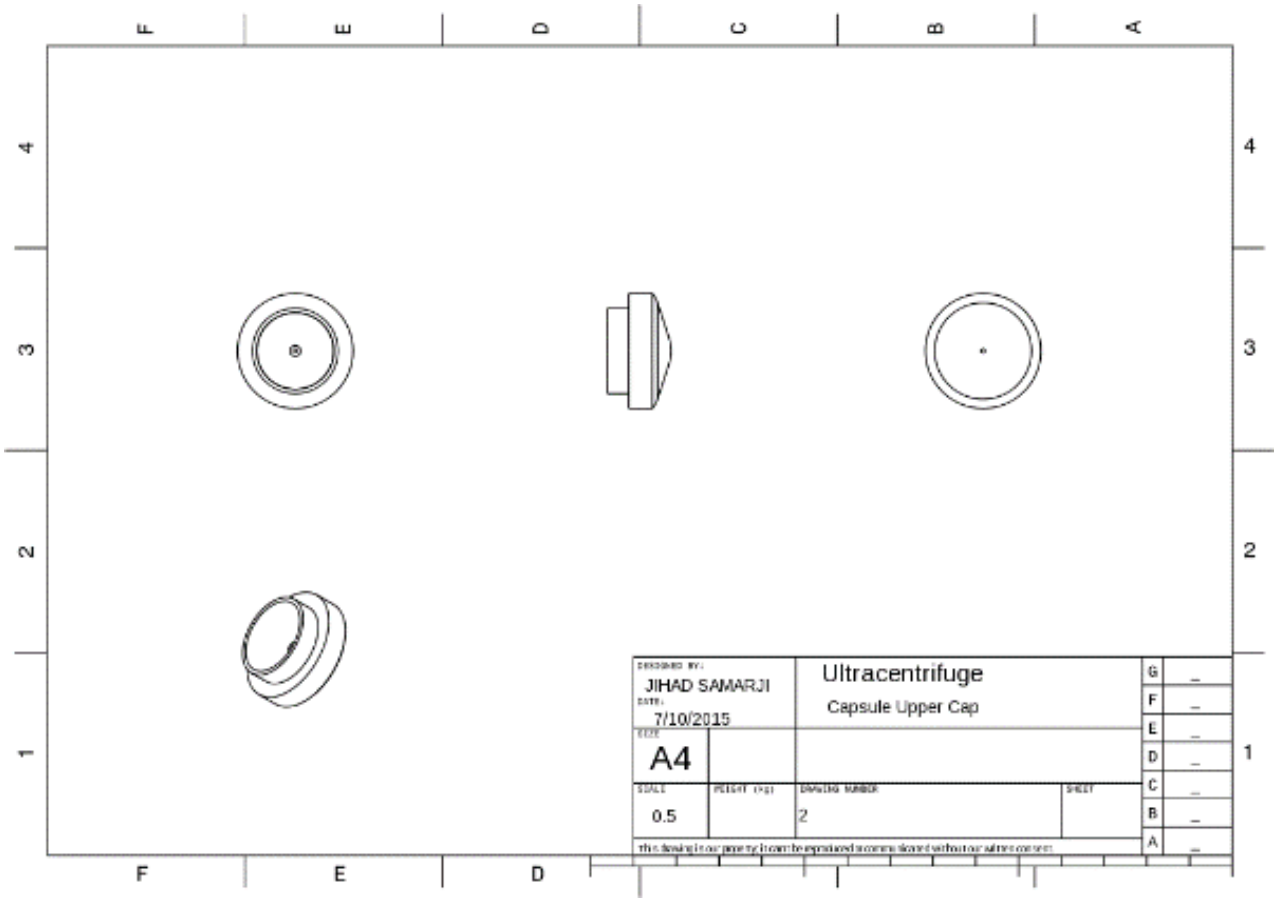
Figure 1.2 Ultracentrifuge annotation in Chinese

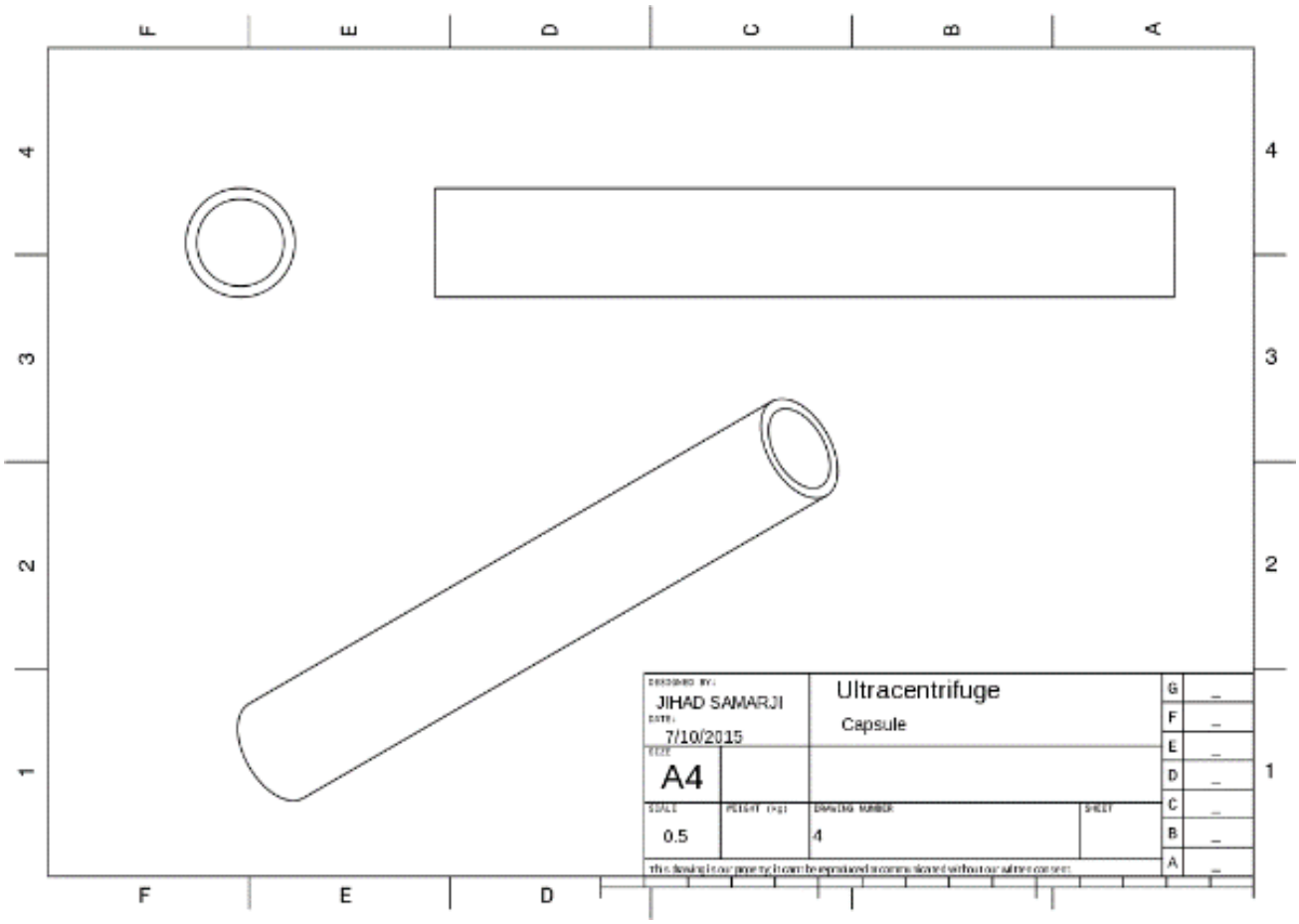
The Ultracentrifuge can be used For Uranium Enrichment which makes it very dangerous and Complicated, Therefore gaining information about this particular machine is very limited. This is by far the clearest picture describing the Ultracentrifuge on the internet.

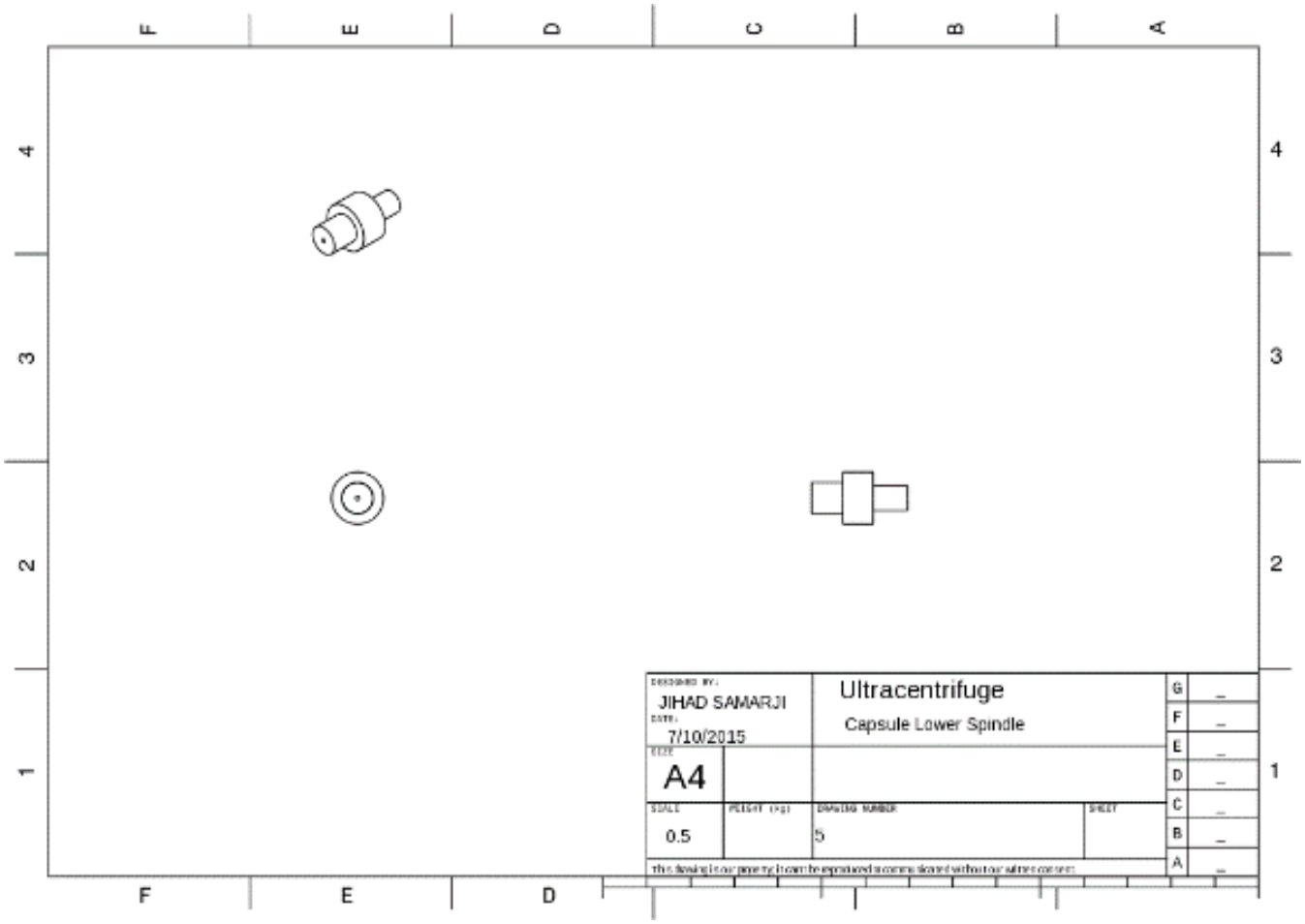
In fact, searching for the Keyword “Ultracentrifuge” on Google makes you suspicious for Global Terrorism. BE CAUTIOUS

5.1.2 *PART DIMENSIONS & DESIGN*






DESIGNED BY:
JIHAD SAMARJI
DATE:
7/10/2015
SIZE
A4
SCALE
0.5
WEIGHT (kg)
DRAWING NUMBER
4
SHEET
Ultracentrifuge
Capsule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JIHAD SAMARJI
DATE:
7/10/2015
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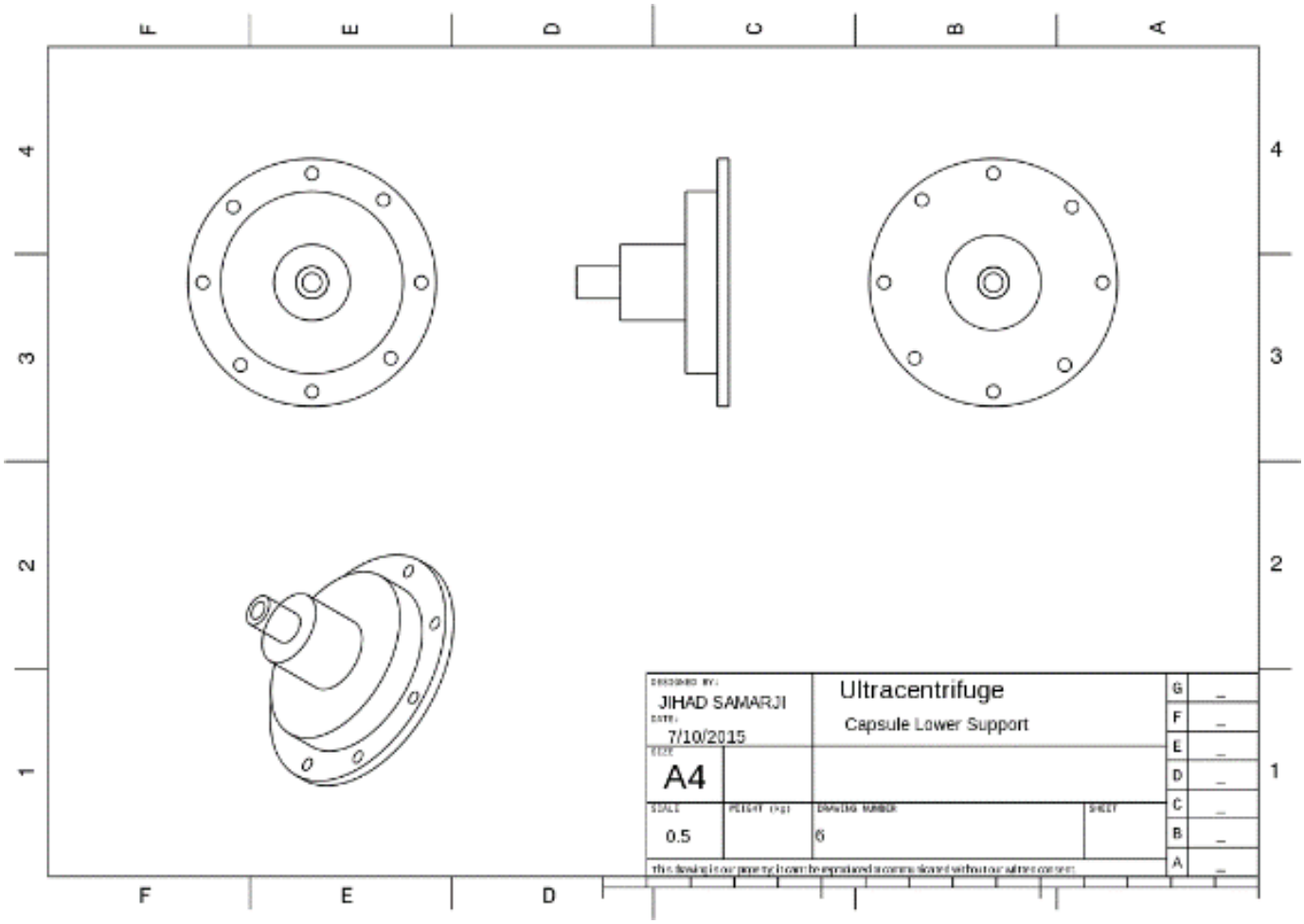
A4
SCALE
0.5
WEIGHT (kg)
DRAWING NUMBER
5
SHEET
Ultracentrifuge
Capsule Lower Spindle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JIHAD SAMARJI
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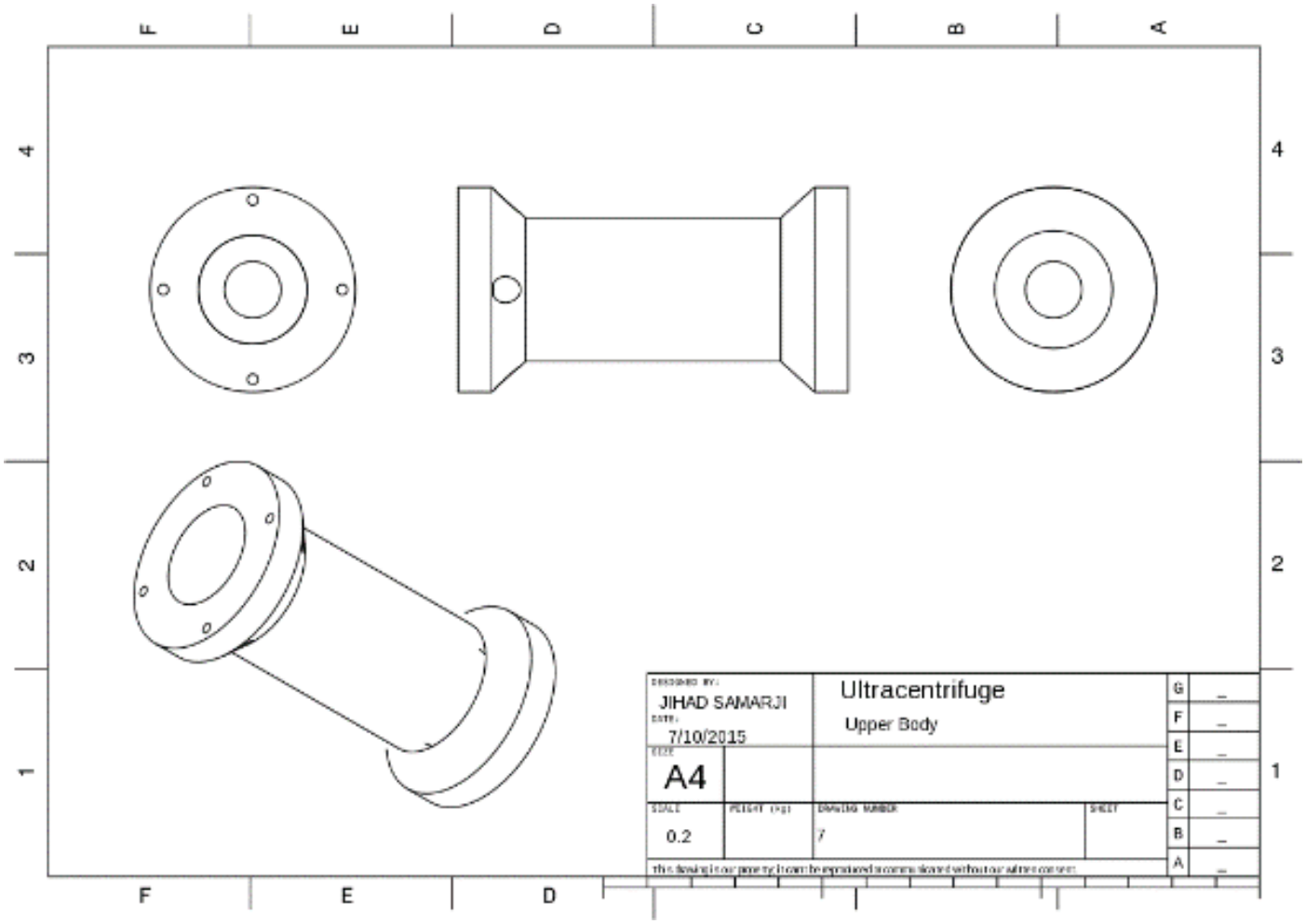
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WEIGHT (kg)
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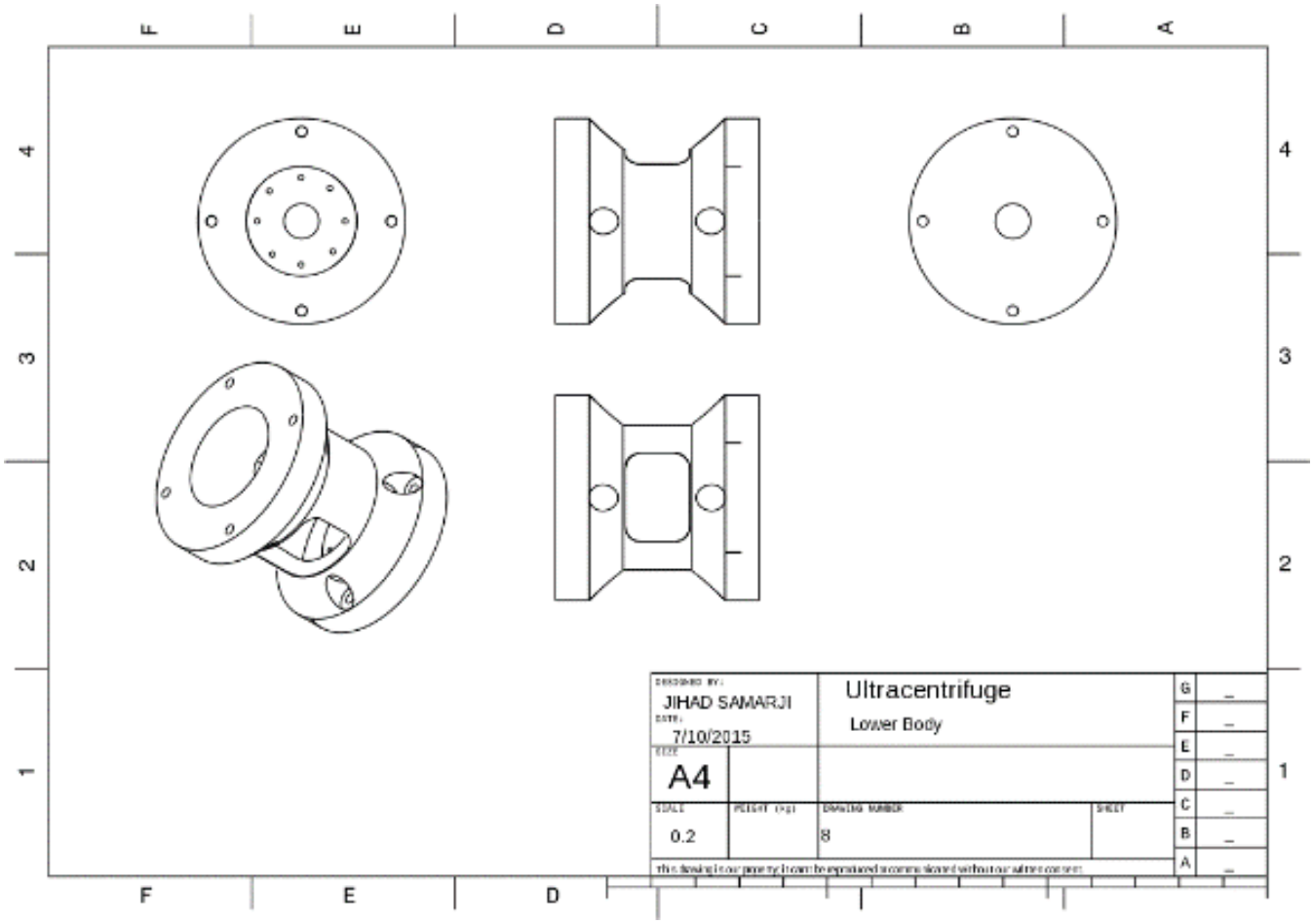
Ultracentrifuge
Upper Body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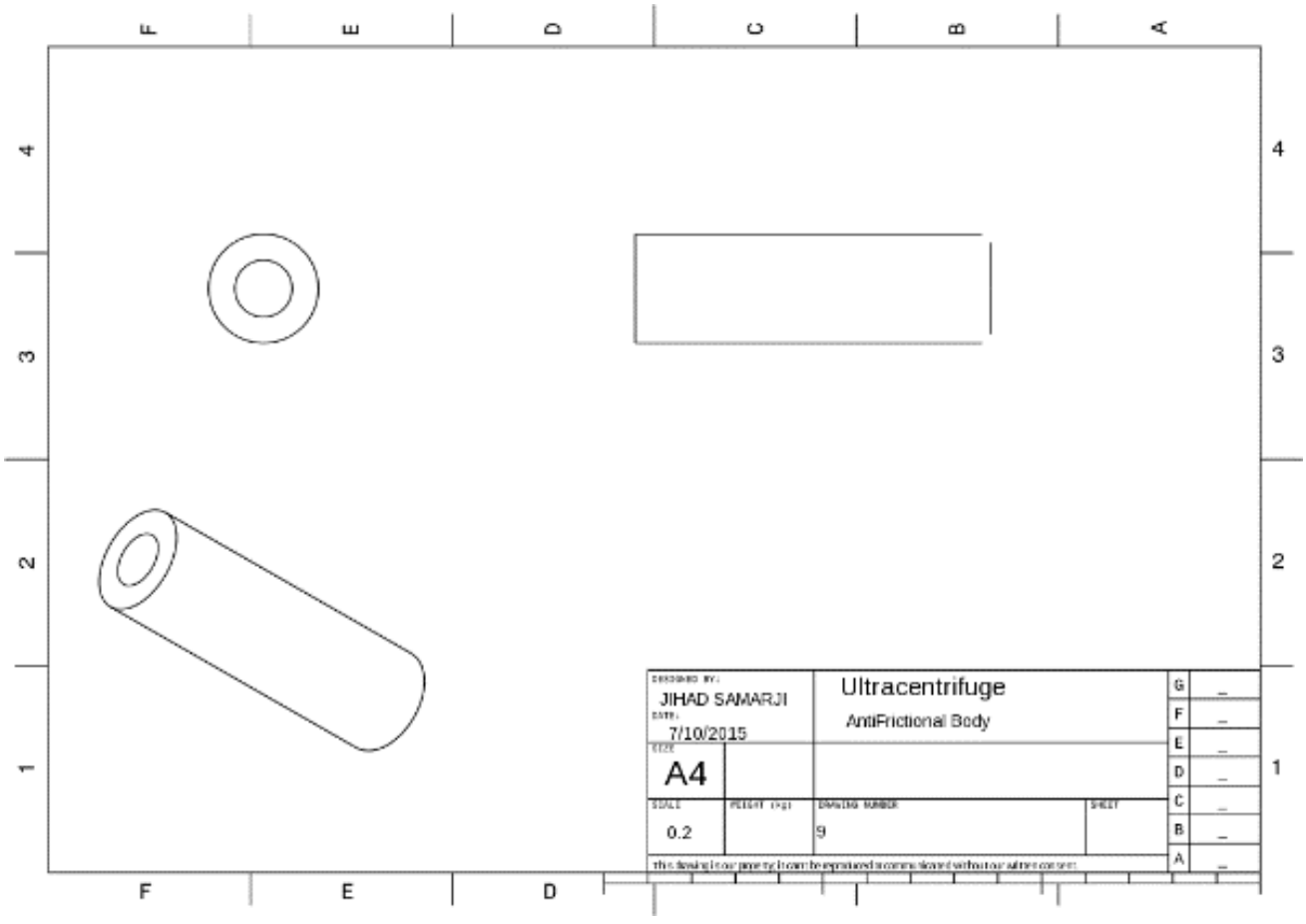
Lower Body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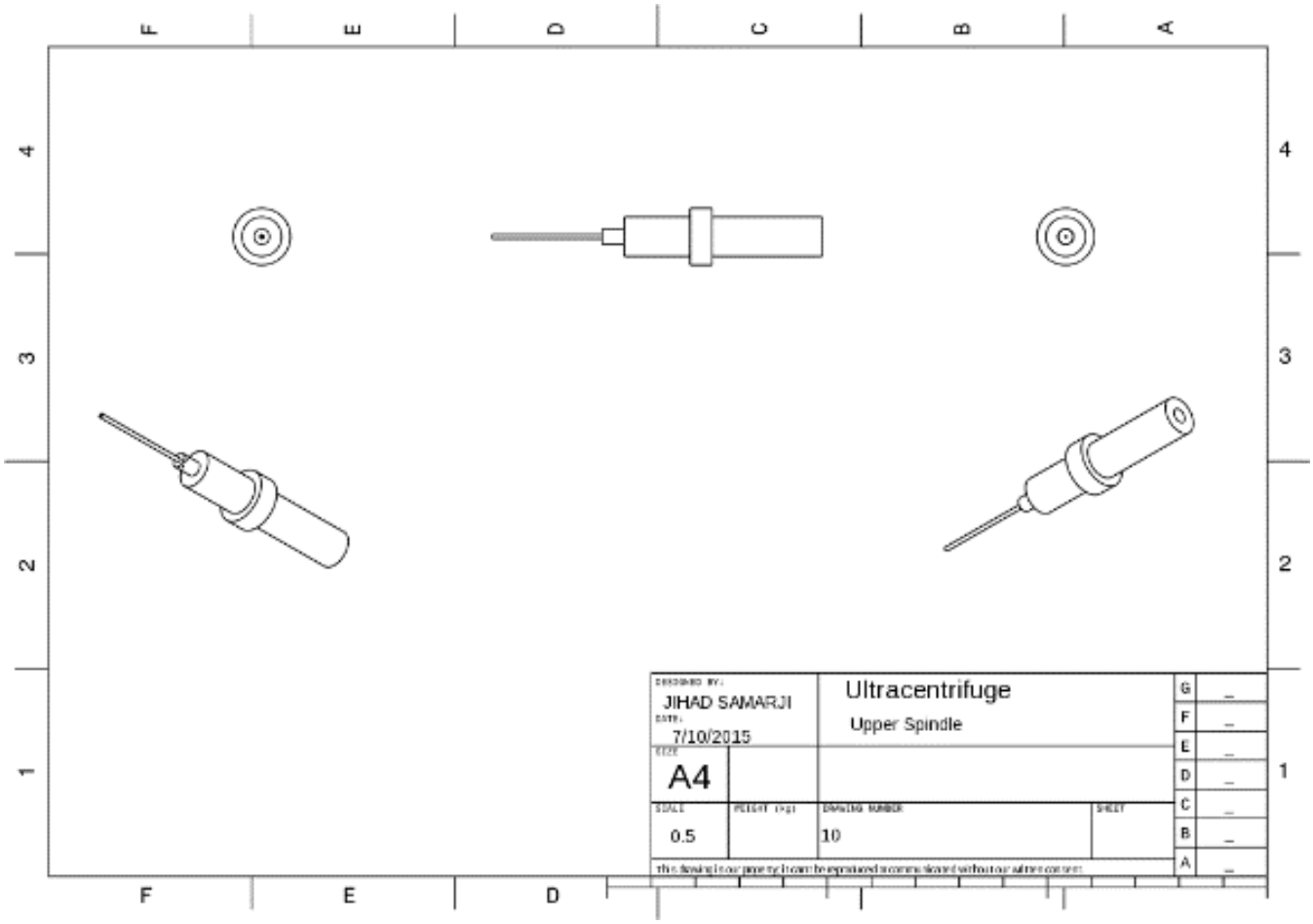
AntiFrictional Body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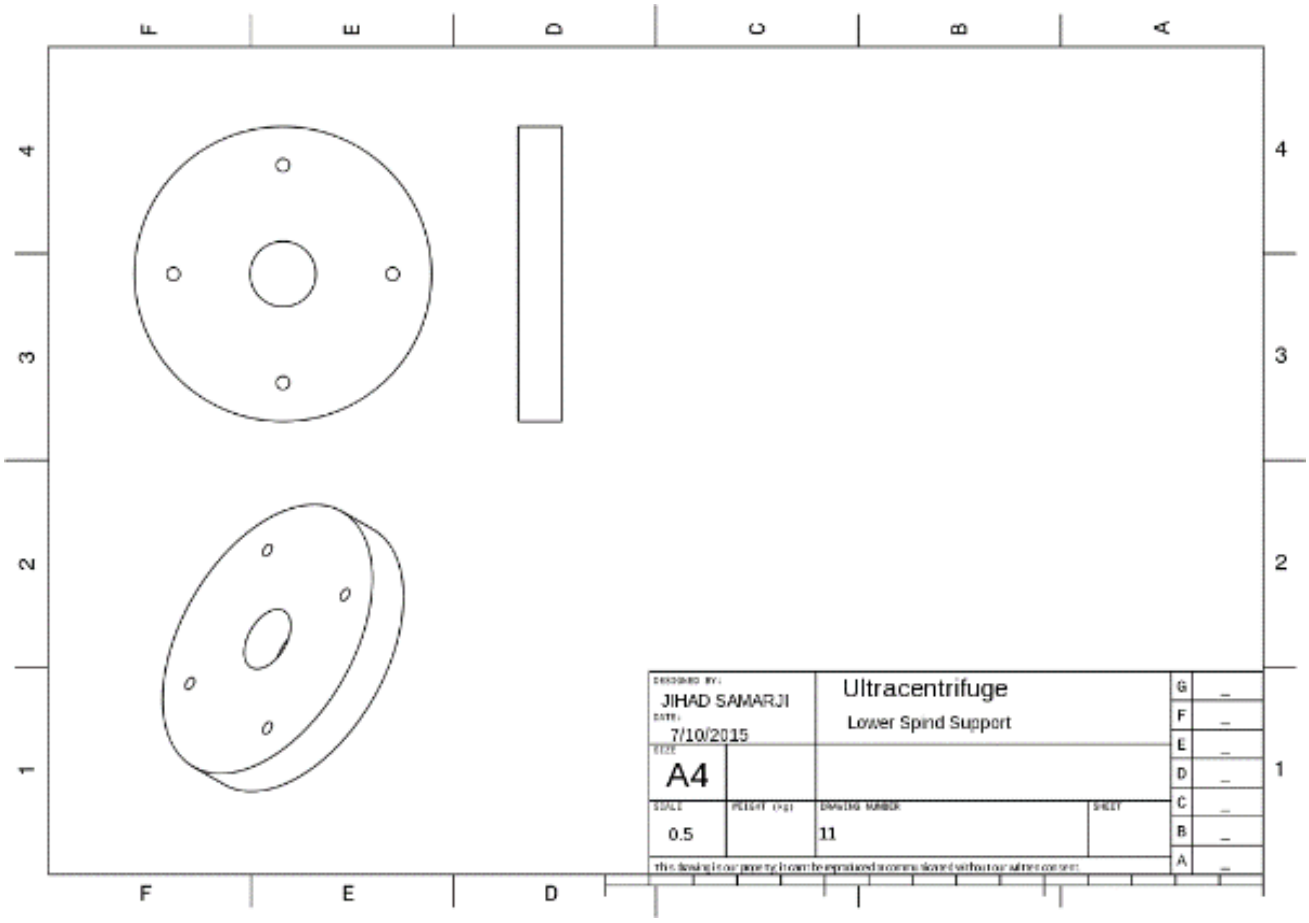
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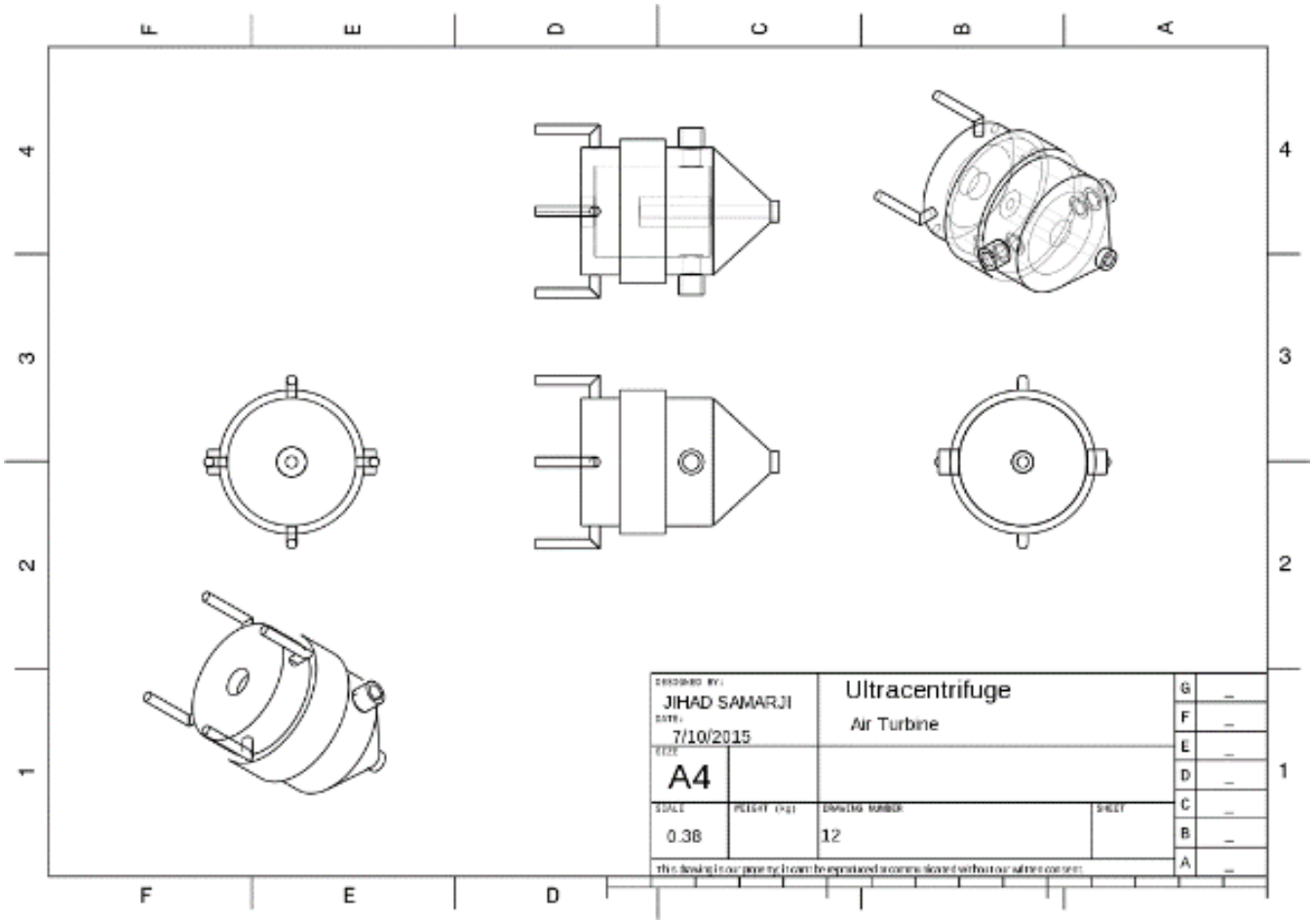
Lower Spind Support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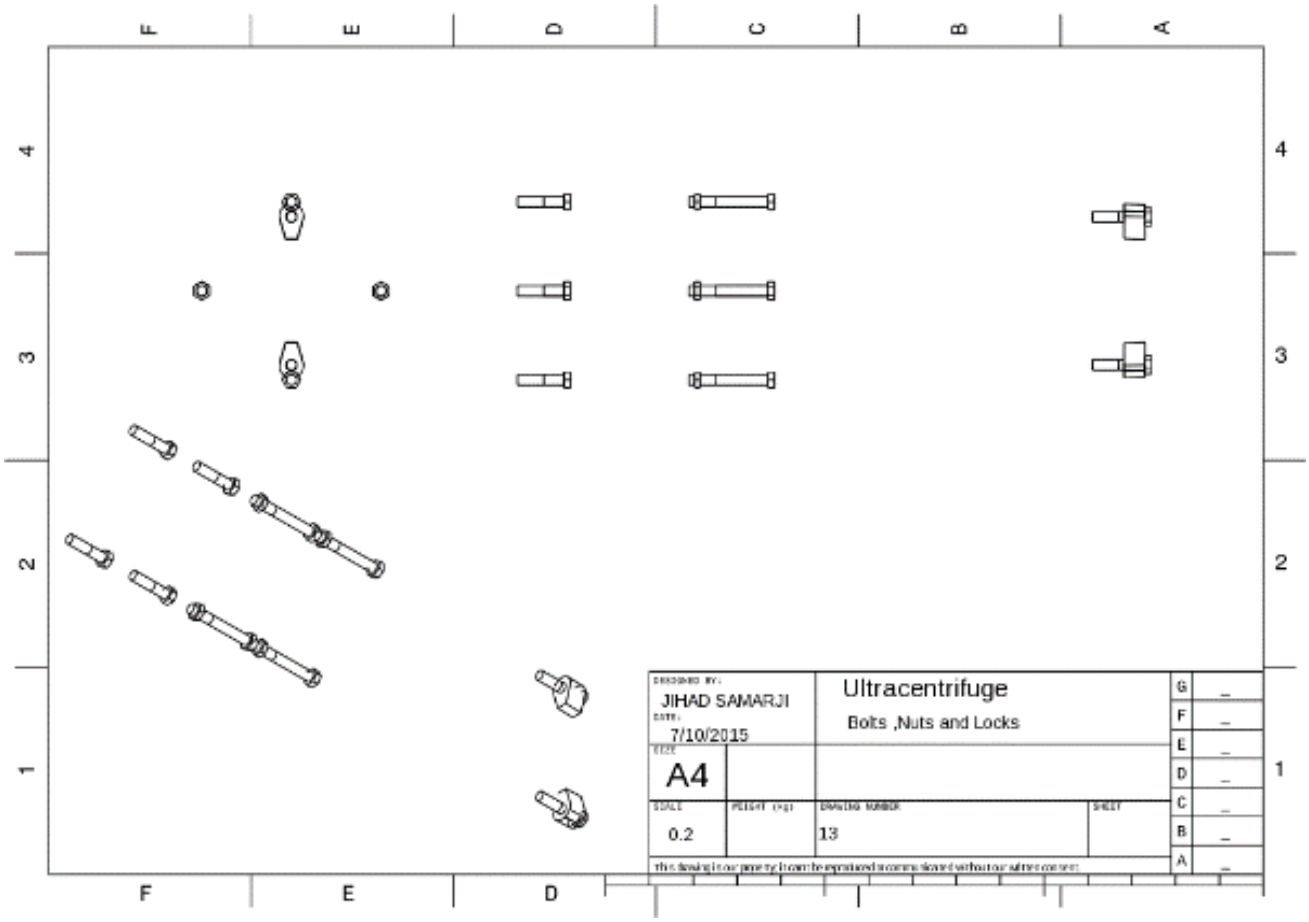
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DATE:
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WEIGHT (kg)
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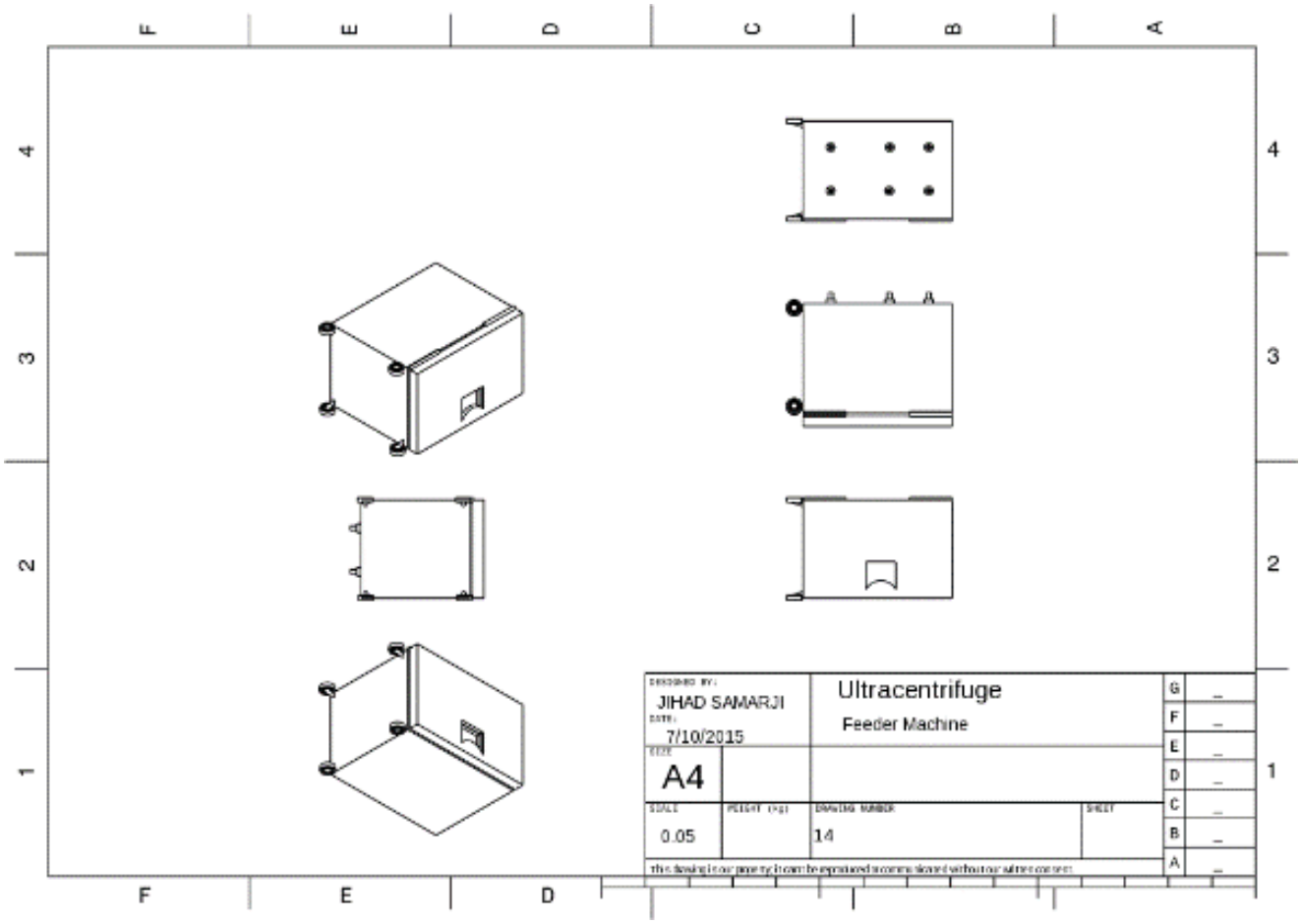
SHEET
Ultracentrifuge
Bolts ,Nuts and Locks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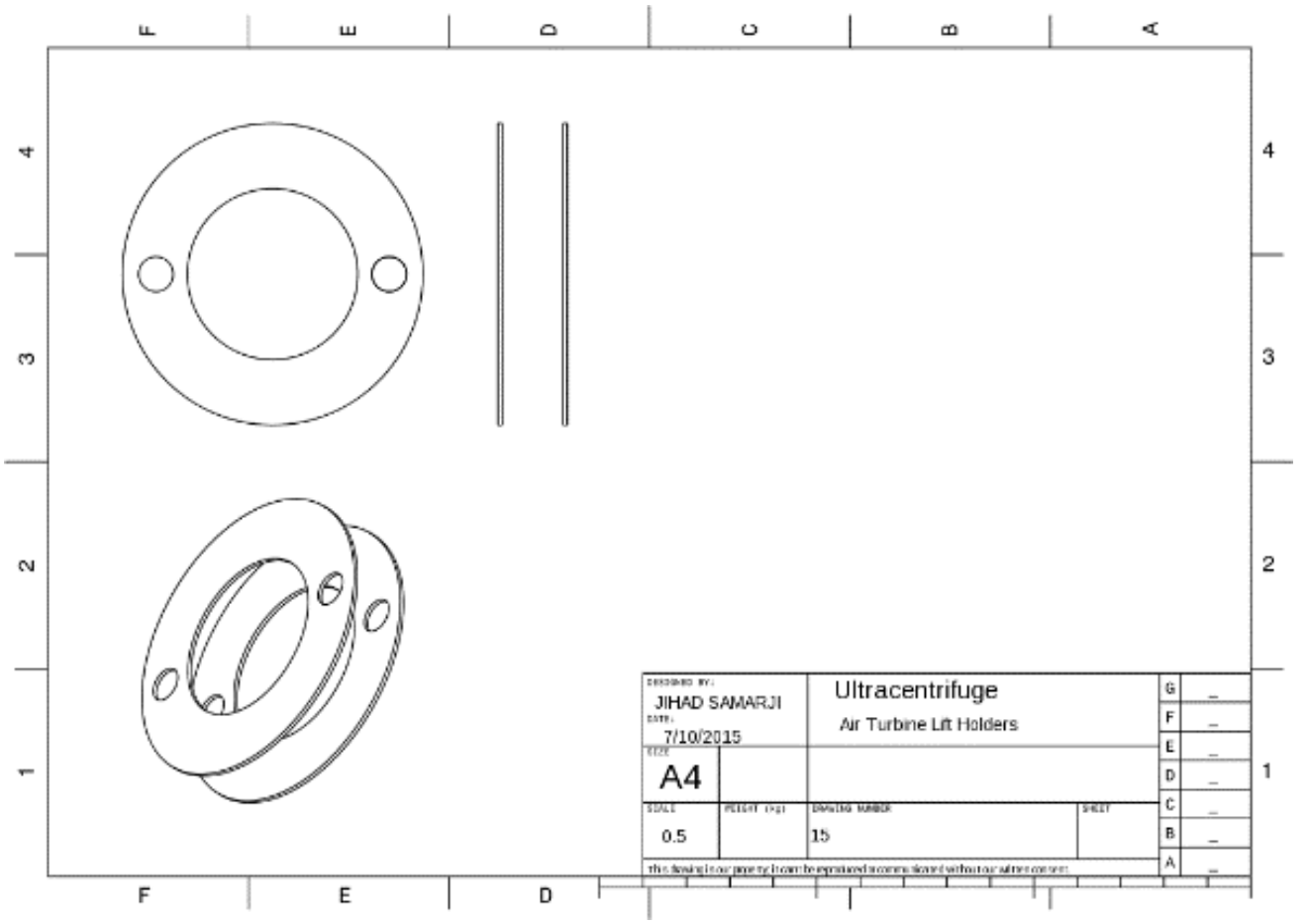
Feeder Machine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7/10/2015
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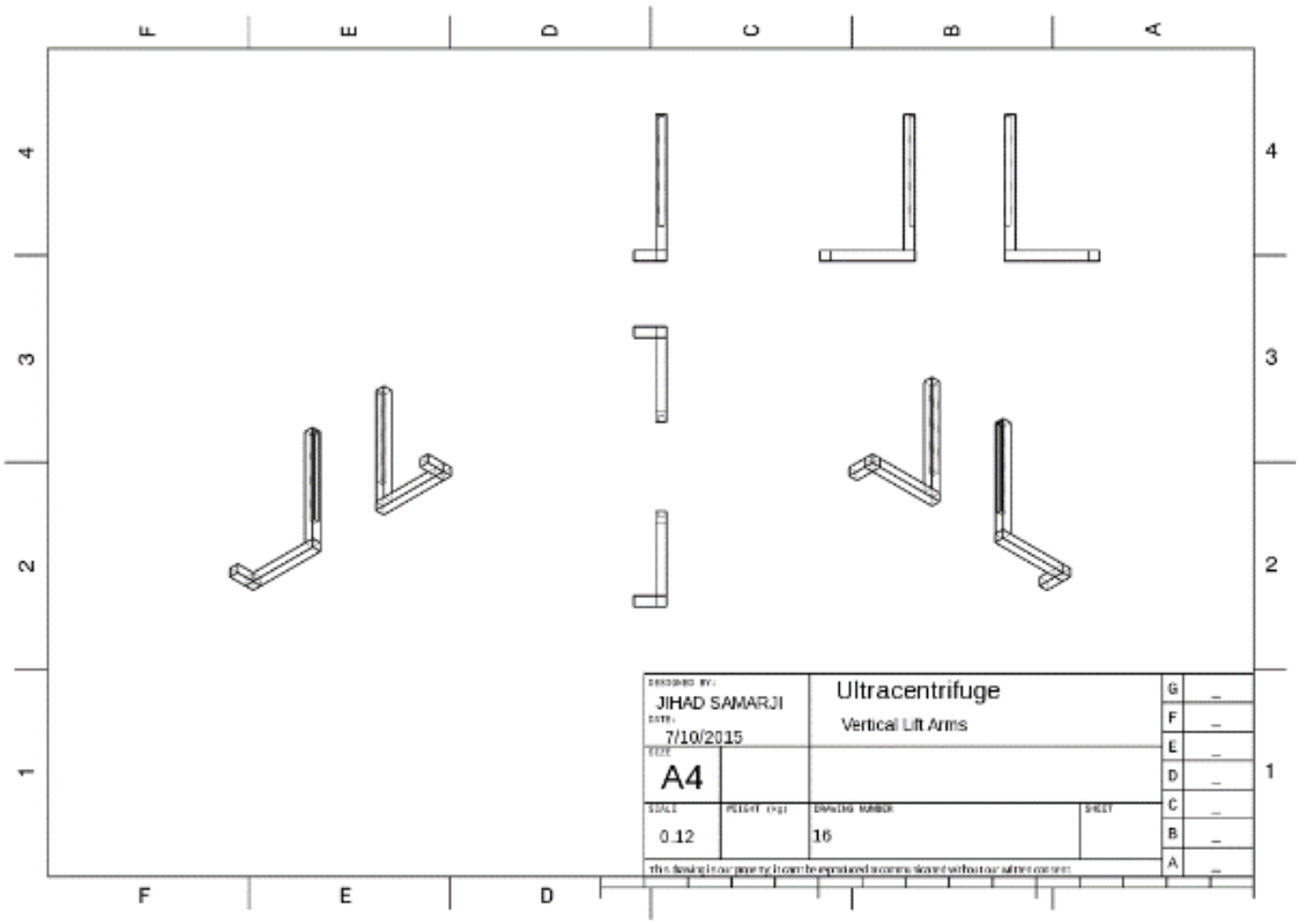
SHEET
Ultracentrifuge
Air Turbine Lift Holders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JIHAD SAMARJI
DATE:
7/10/2015
SIZE
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DRAWING NUMBER
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Ultracentrifuge
Vertical Lift Arms
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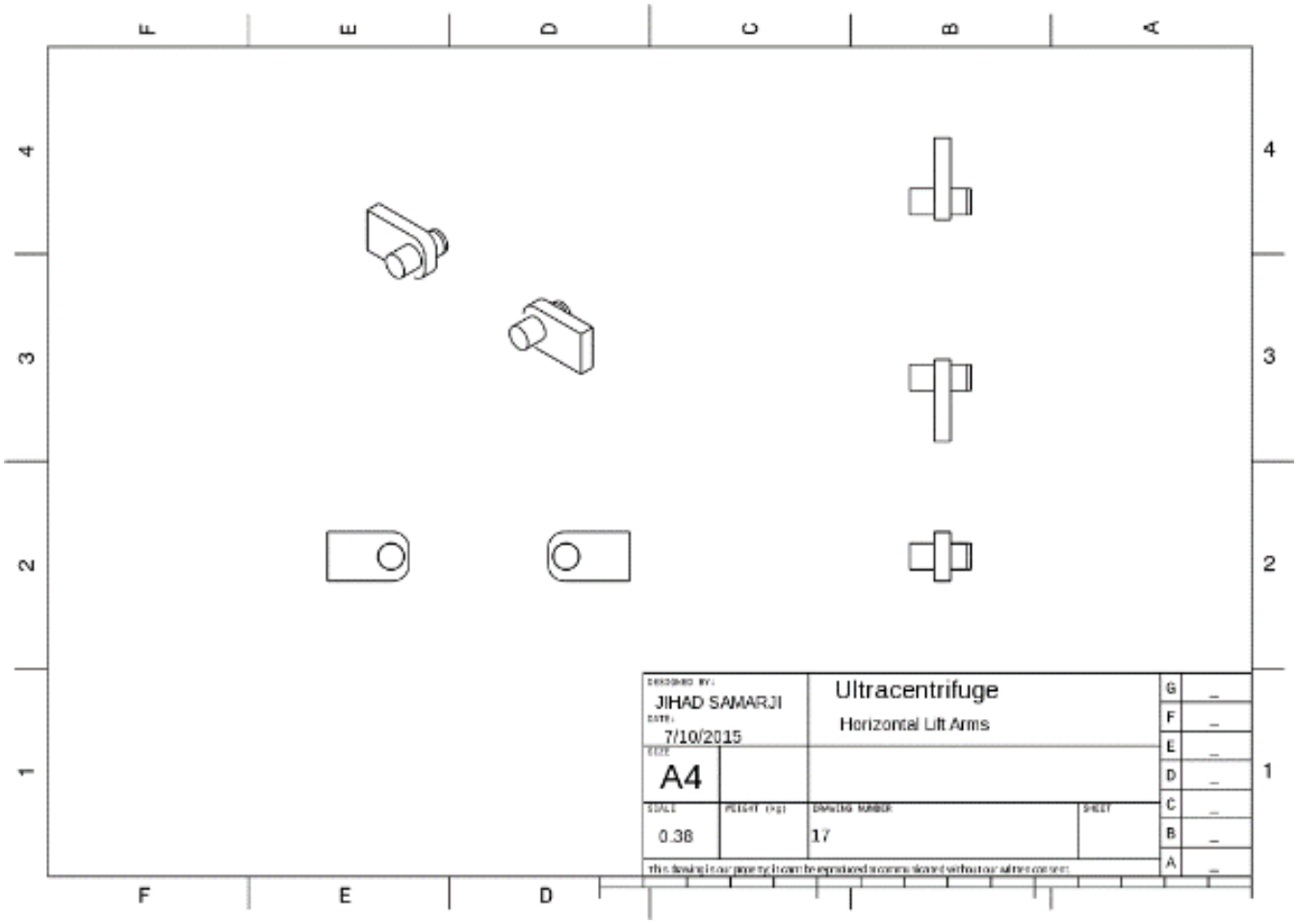
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DATE:
7/10/2015
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DRAWING NUMBER
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SHEET
Ultracentrifuge
Horizontal Lift Arms
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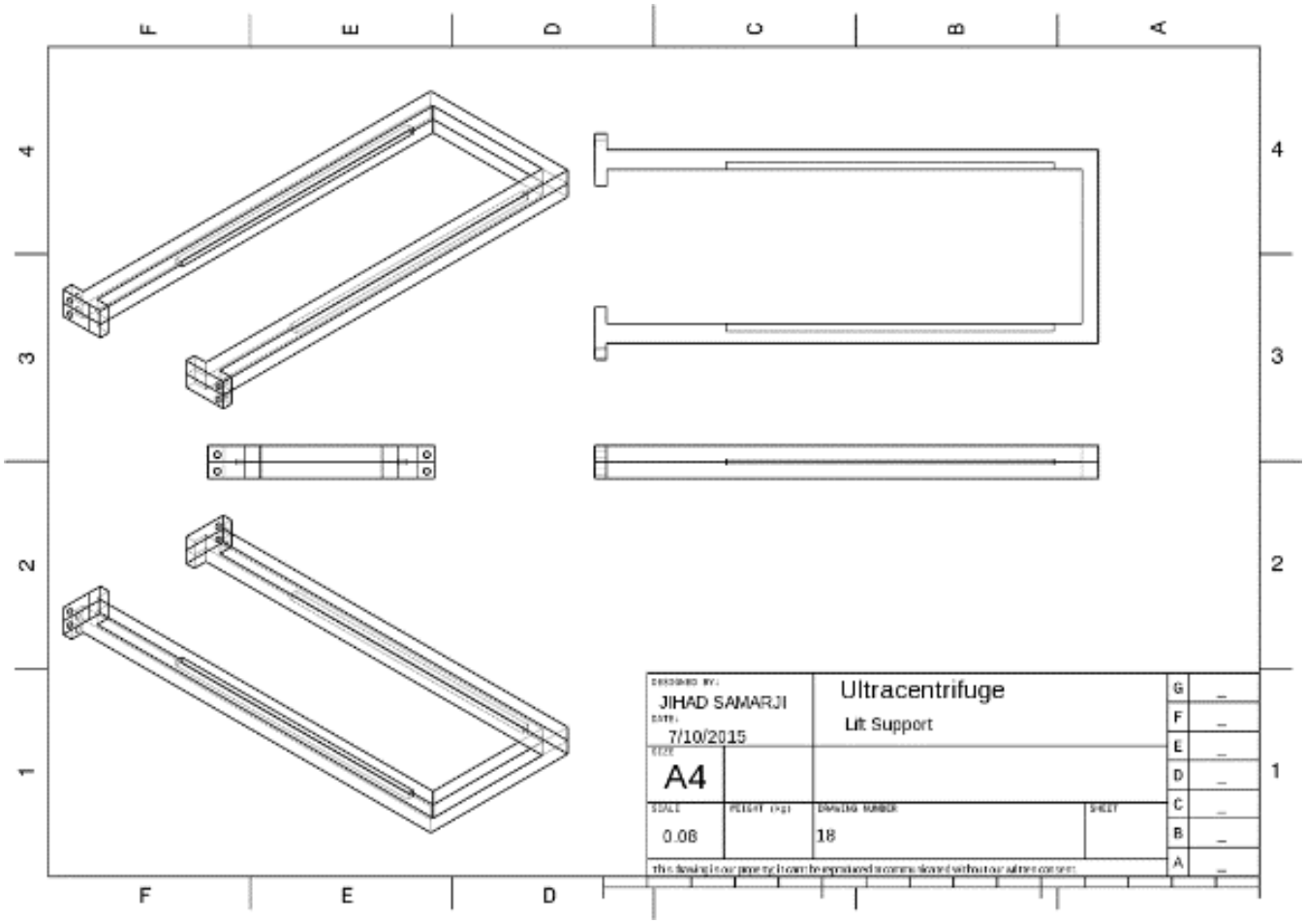
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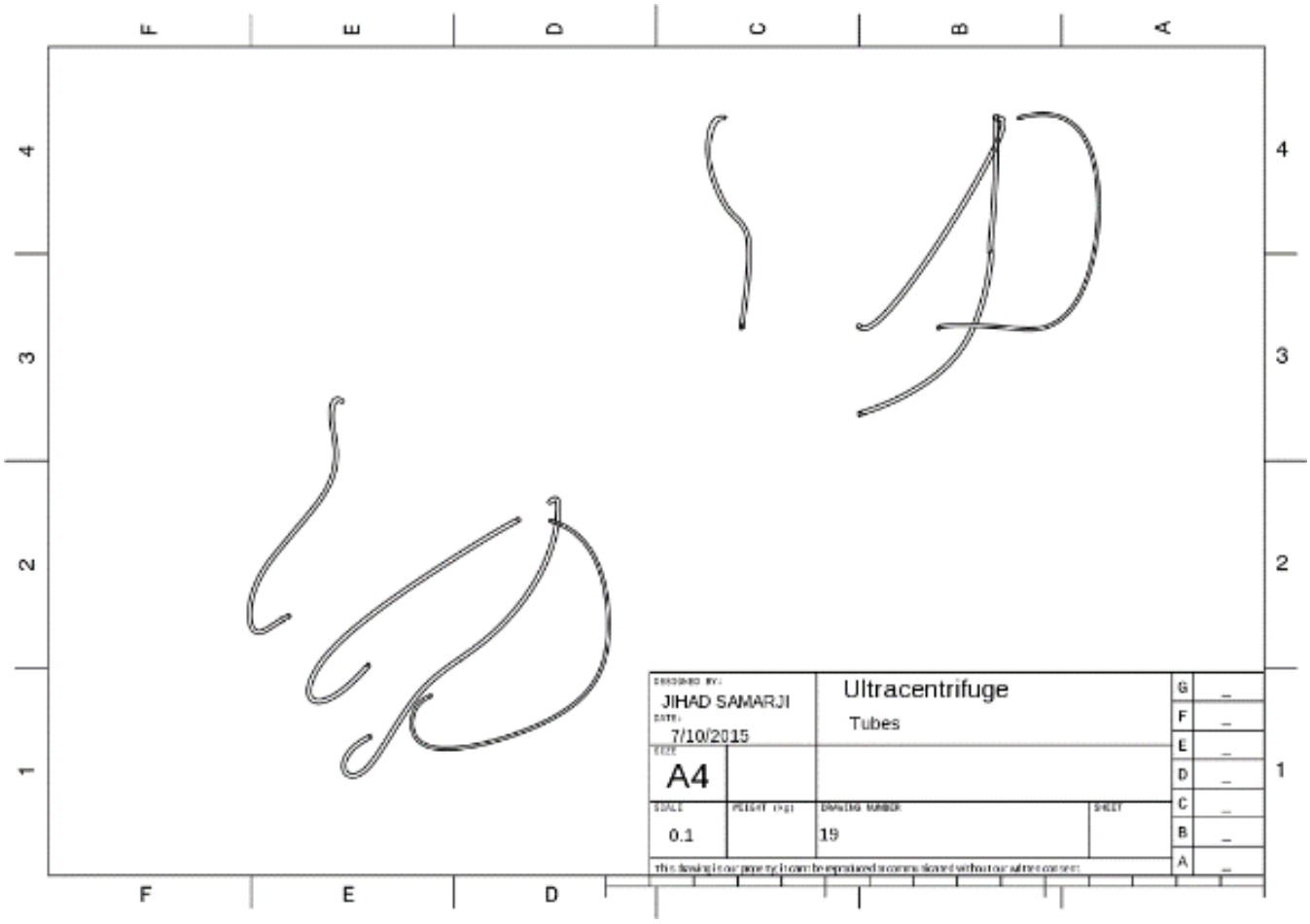
Ultracentrifuge
Lift Support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DATE:
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WEIGHT (kg)
DRAWING NUMBER
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SHEET
Ultracentrifuge
Tubes

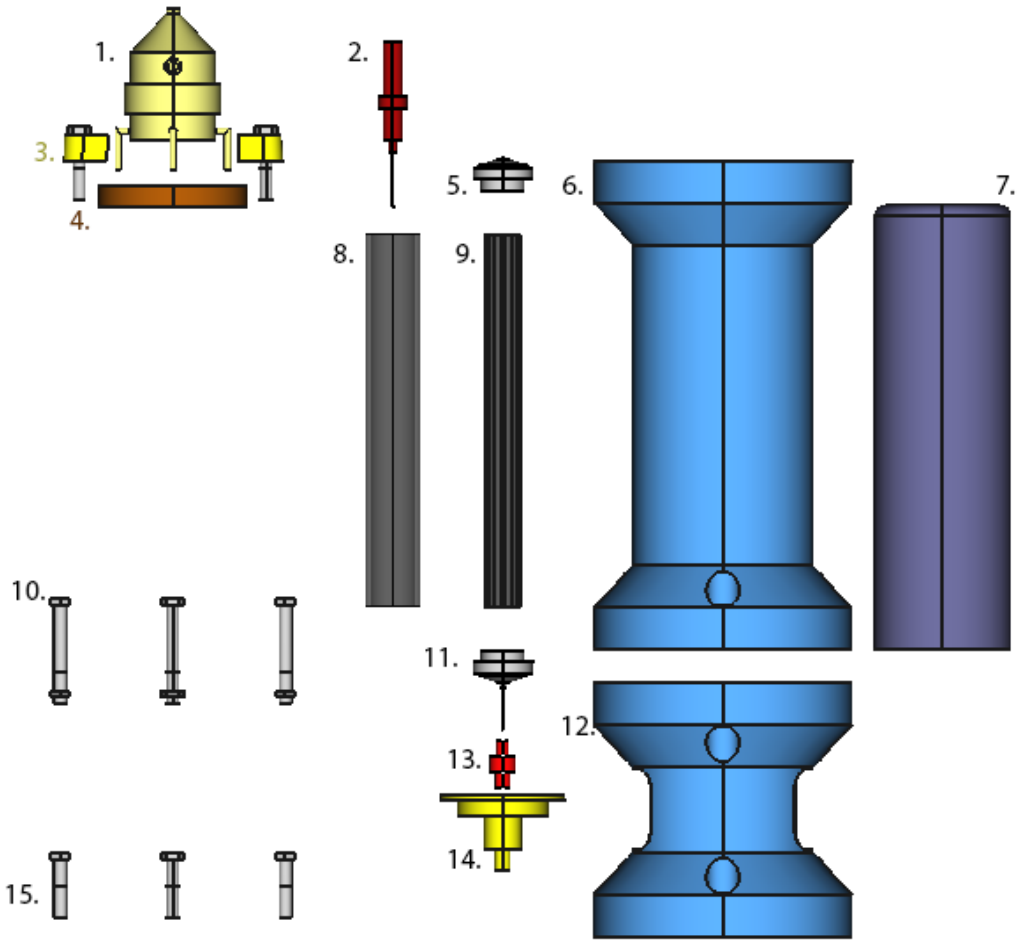


Figure 1.3 Ultracentrifuge First Group Parts Annotations

1. Air Turbine 2.Upper spindle 3.Locks 4.Air Turbine Support 5.Upper Capsule Cap 6.Upper Body 7. Anti-frictional body 8.Capsule 9.Rotor 10.Bolts & Nuts 11.Lower Capsule Cap 12.Lower Body 13. Lower Spindle 14.Lower Spindle Support 15.Bolts

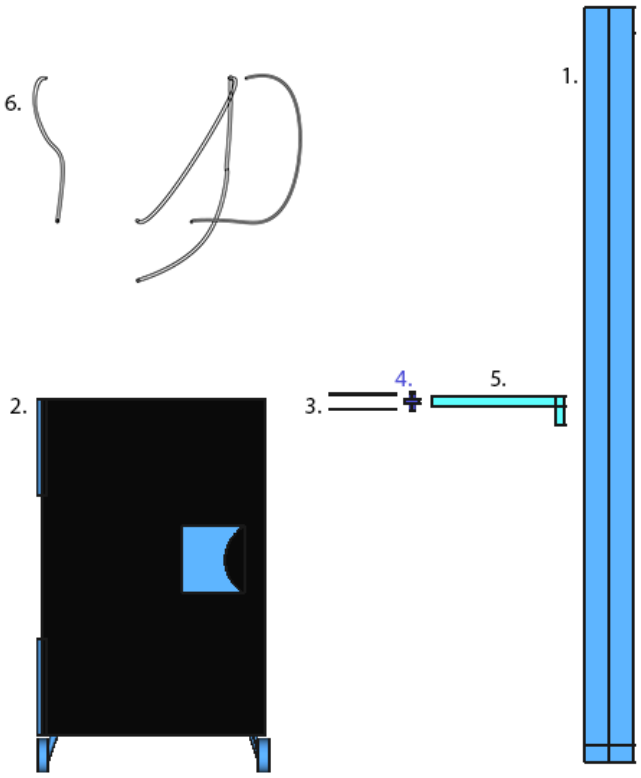
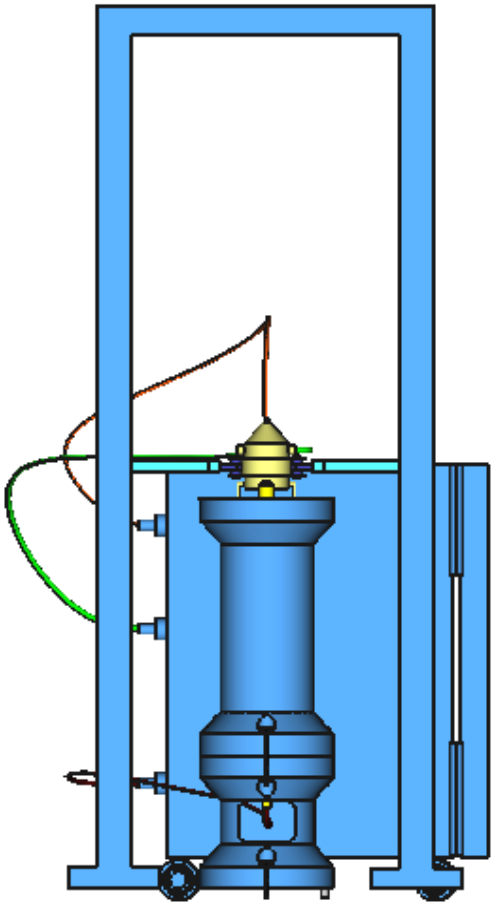
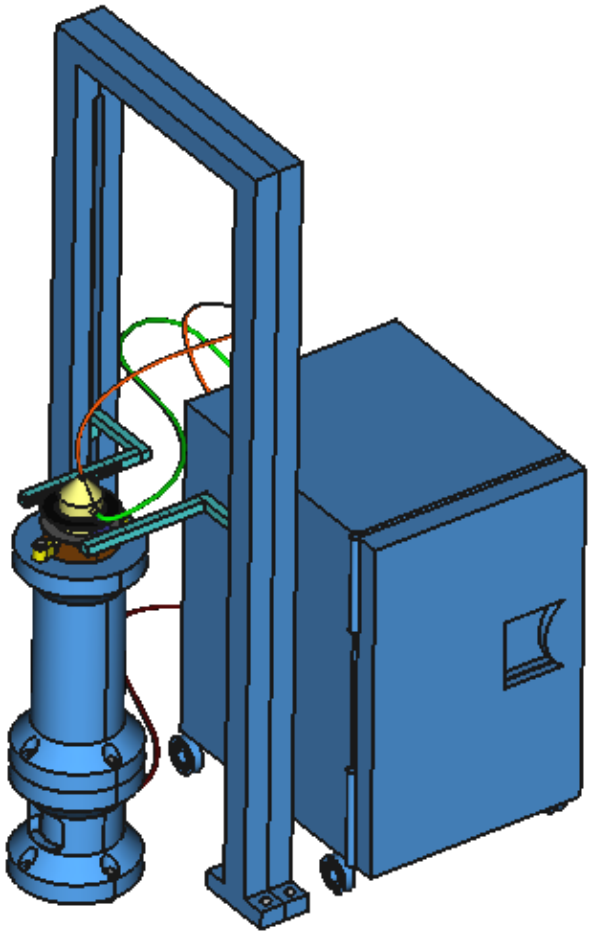


Figure 1.4 ULTRACENTRIFUGE SECOND GROUP PART ANNOTATIONS

1. Lift Support 2.Feeding Machine 3.Air Turbine Lift Holders 4.Horizontal Lift Arm 5.Vertical Lift Arm 6. Tubes

Remark: This Design Is for Educational Purposes. This is just a Prototype Concept Which will Not Work Because Some Parts Are Missing.





5.2 Demonstration and Modelling of the Disc Stack Centrifuge

"To Separate a Liquid from Solid or Liquid from Liquid, We Use the Decanter or the Disc Stack or a Filter Depending On the Amount ofSolids"-Jihad Samarji

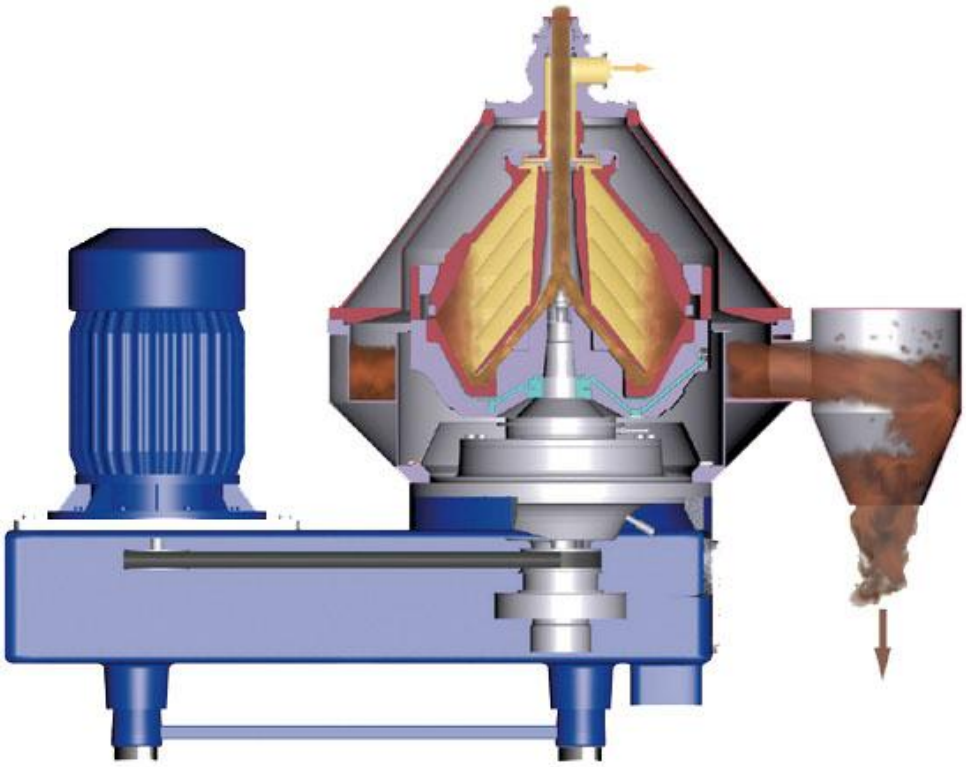


Figure 2.1 Disc Stack Centrifuge

5.2.1 *DEVICE DETAILS & SPECIFICATIONS*

Conical plate centrifuge (also known as disc bowl centrifuge or disc stack separator) is a type of centrifuge that has a series of conical discs which provides a parallel configuration of centrifugation spaces.

The conical plate centrifuge is used to remove solids (usually impurities) from liquids or to separate two liquid phases from each other by means of an enormously high centrifugal force. The denser solids or liquids which are subjected to these forces move outwards towards the rotating bowl wall while the less dense fluids moves towards the center. The special plates (known as disc stacks) increase the surface settling area which speeds up the separation process. Different stack designs, arrangements and shapes are used for different processes depending on the type of feed present. The concentrated denser solid or liquid is then removed continuously, manually or intermittently, depending on the design of the conical plate centrifuge. This centrifuge is very suitable for clarifying liquids that have small proportion of suspended solids

There is 3 Designs for the disc Stack Centrifuge

In our case, we need to make mass production of substance in big scale and without stopping to clean the disc stack centrifuge from solids so we're going to use the Self-Cleaning Centrifuge.

The Self Cleaning Centrifuge has a Movable plate that operates by the pressure of water underneath it,

When the water is sank, the movable plate goes down to open small nozzles for the solids to pass outside. When all the solids are ejected, water is re-flooded beneath the movable plate to make it goes up and block the nozzles.

We should come up with something similar to this:

Utilities consumption	
Electric power	max. 46 kW
Operating liquid during discharge	10 l/h
Cooling water, jacket	300 l/h
Cooling water, oil	80 l/h
Sealing liquid	100 l/h
Flushing liquid, per discharge	25–30 l
ATEX design codes	
BD 95X: EX II 2 G T4 X for zone 1 & 2	Inert gas design
BD 95Y: EX II 3 G T4 X for zone 2	Electrically protected
Material data	
Bowl body, hood and lock ring	s.s. 1.4418
Frame top part and hood	s.s. 1.4401 UNS 31600
Frame bottom part	Cast iron
Gaskets and O-rings	Fluorocarbon rubber

Technical specifications	
Throughput capacity	max. 52 m³/h
Bowl speed	4,300 rpm
Bowl volume	66 l
Sludge space	17 l
Motor speed synchron. 60.7 Hz	1,821 rpm
Motor power installed	52 kW
Starting time	6–8 min
Stopping time without brake	80 min
Inlet pressure at 46 m³/h	300 kPa
Outlet pressure, methyl ester phase	min. 200 kPa
Outlet pressure, heavy phase	800 kPa
Sound pressure	78 dB(A)
Overhead hoist lifting capacity	min. 1,200 kg

Table2.1 Disc Stack Centrifuge Specifications. See also figure 2.1 and 2.2

1. Feed valve
2. Centrate out
3. Pneumatic butterfly valve
4. Cooling jacket
5. Bowl
6. Cooling water
7. Axial seal
8. Check valve
9. Spindle cartridge
10. Oil filler and gauge
11. Flat driving belt
12. Cyclone with recirculation tube
13. Solids pockets
14. Pneumatically operated slide
15. Motor
16. Unibase frame
17. Foundation

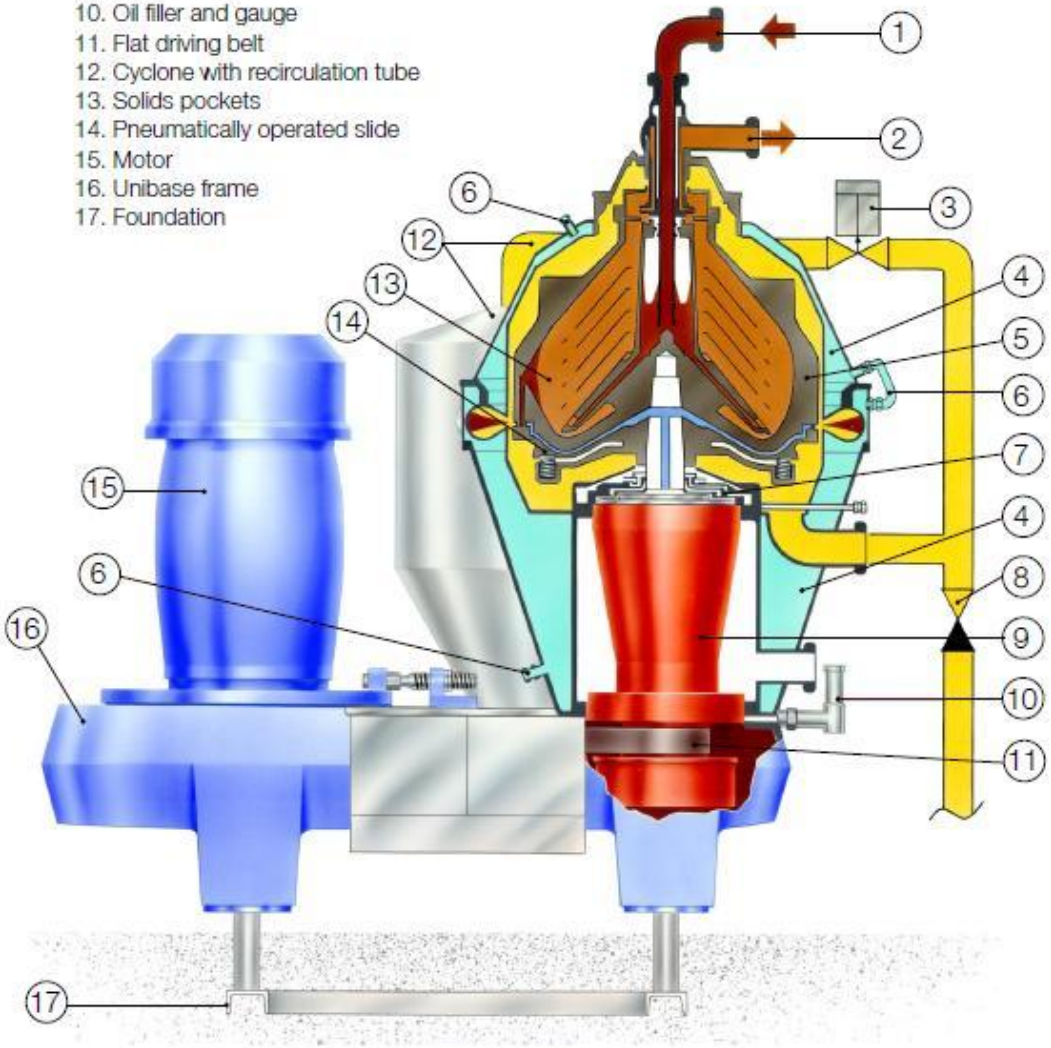
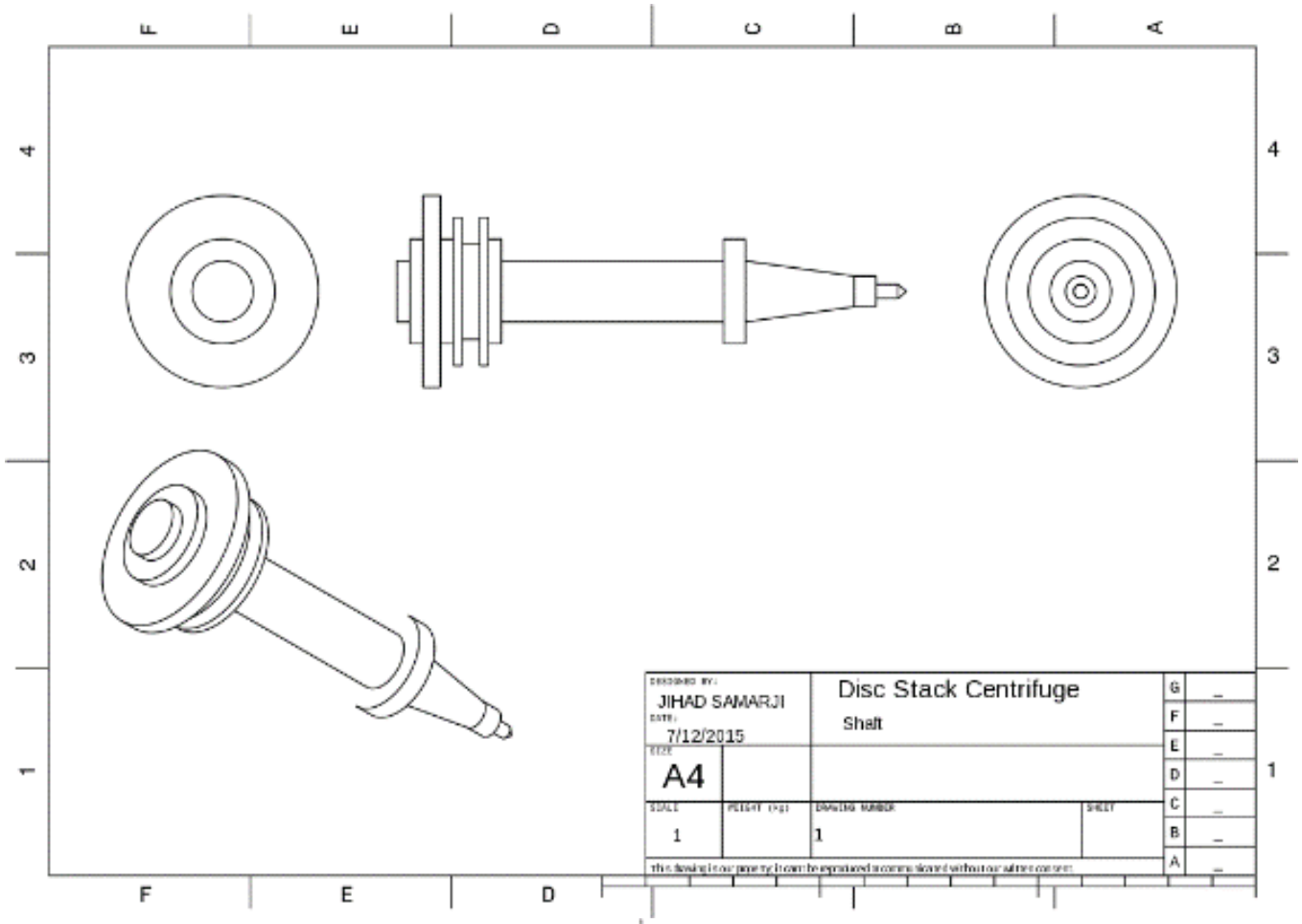
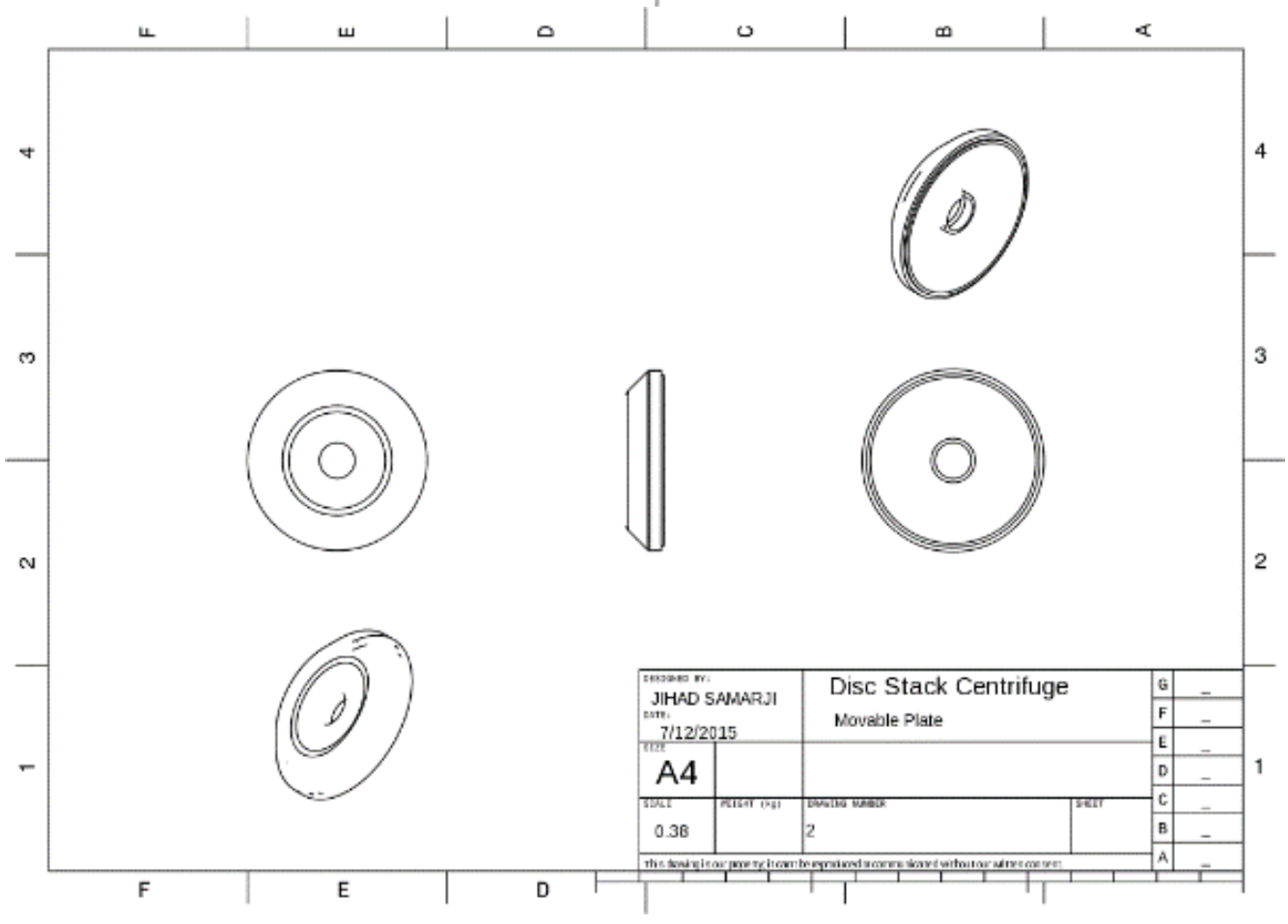
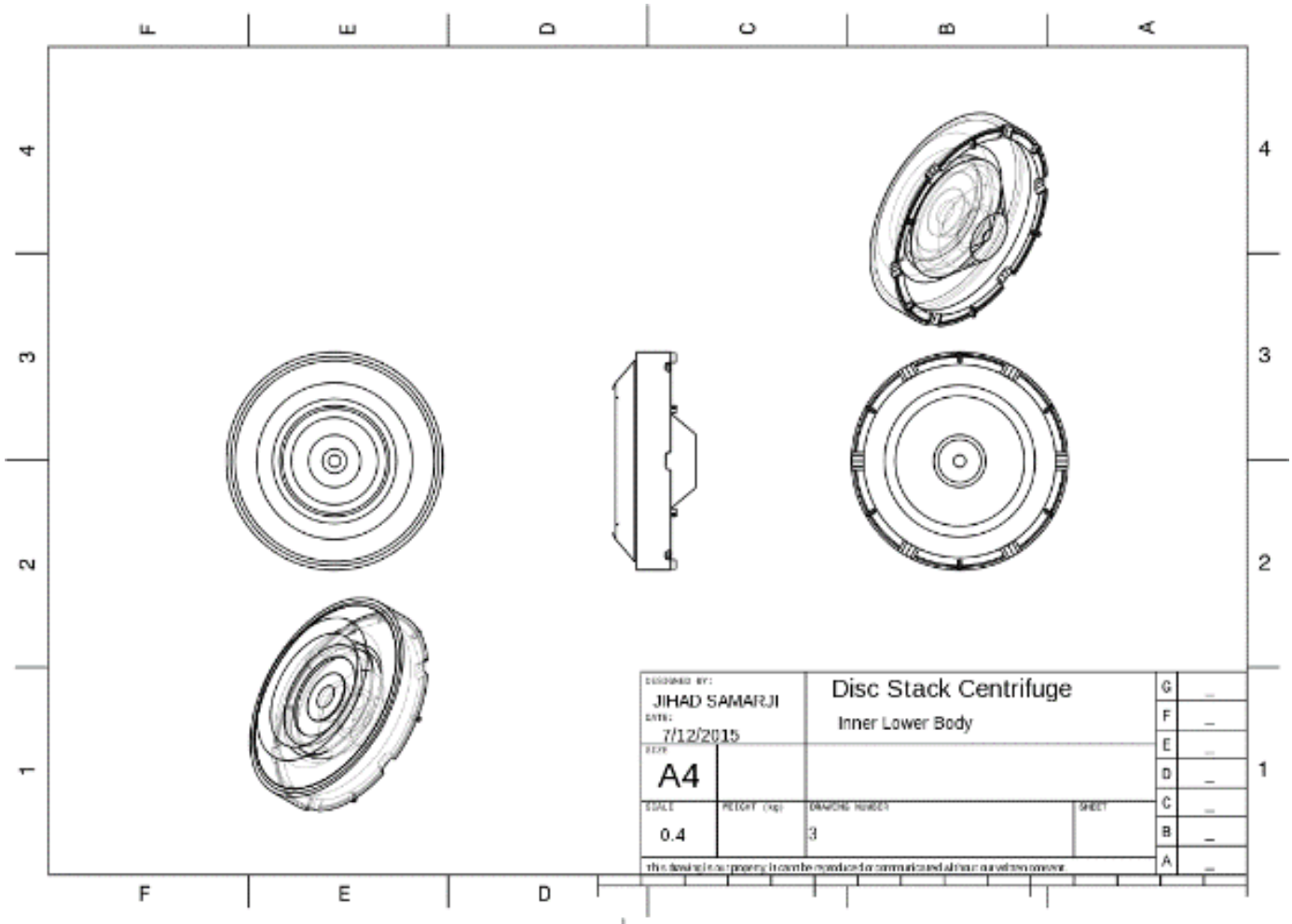


Figure 2.2 Disc Stack Centrifuge Annotation

5.2.2 *PART DIMENSIONS & DESIGN*







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SCALE: 0.4	WEIGHT (kg):	DRAWING NUMBER: 3	SHEET:

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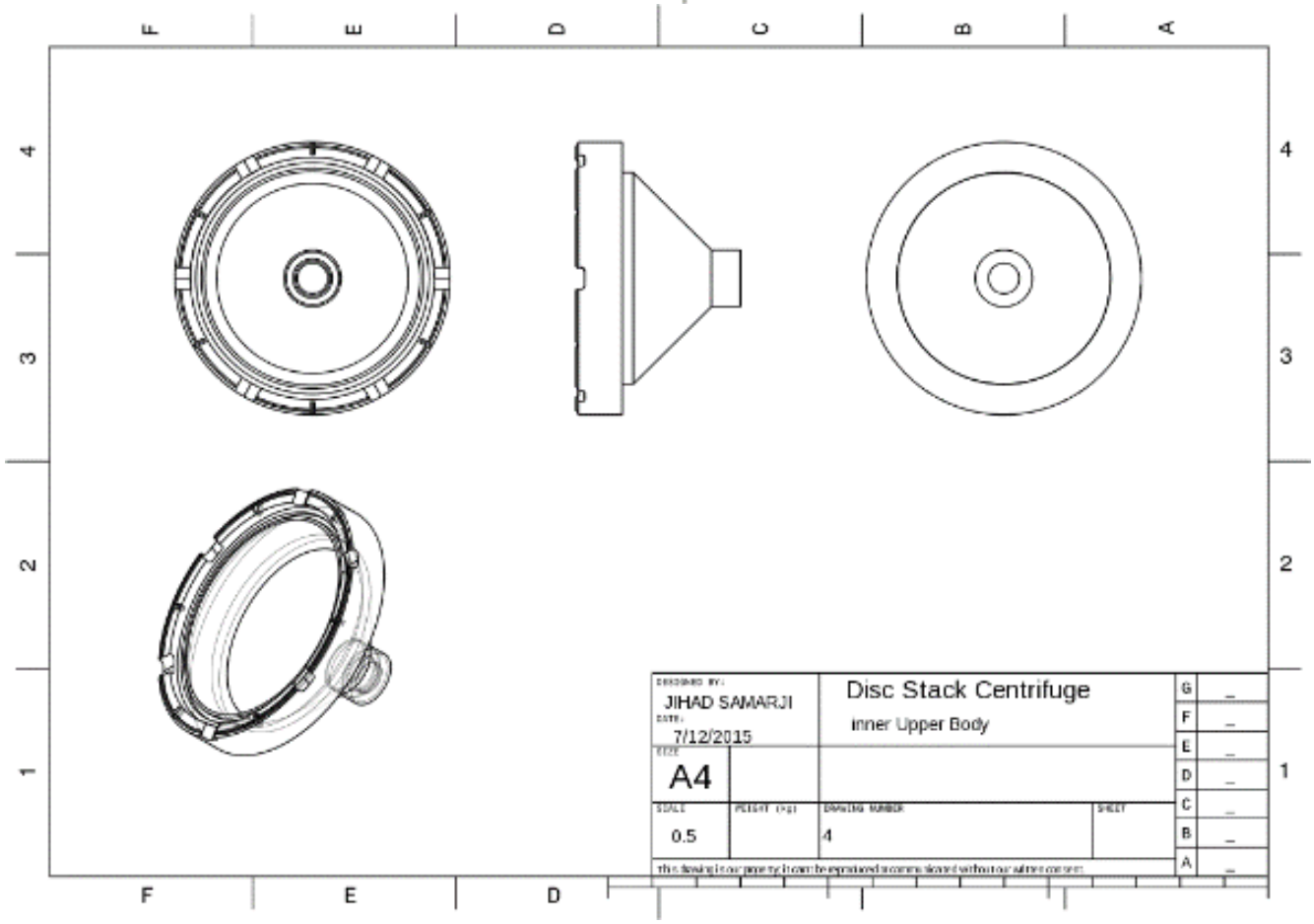

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DATE: 7/12/2015	inner Upper Body		
SIZE: A4			
SCALE: 0.5	WEIGHT (kg):	DRAWING NUMBER: 4	SHEET:

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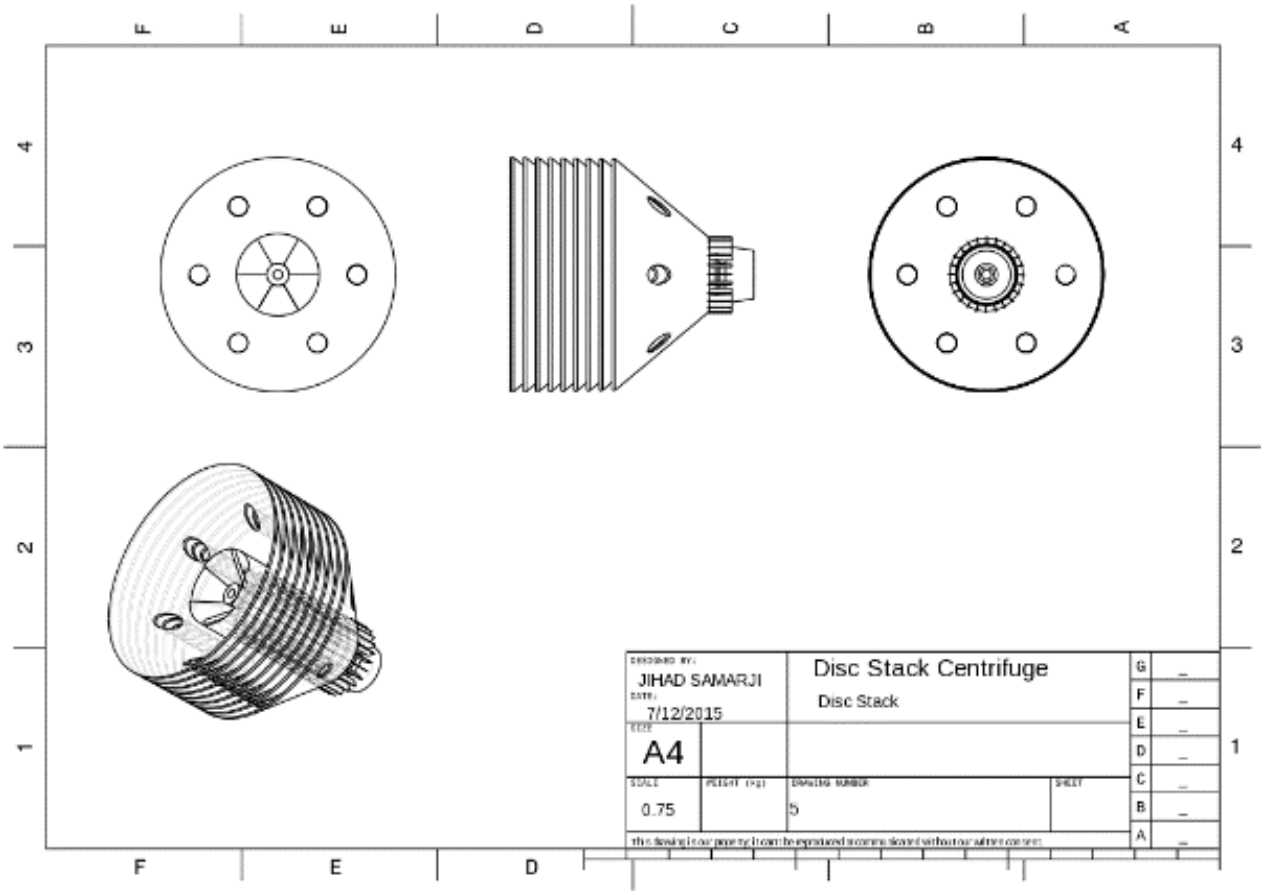
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JIHAD SAMARJI
DATE:
7/12/2015
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WEIGHT (kg)
DRAWING NUMBER
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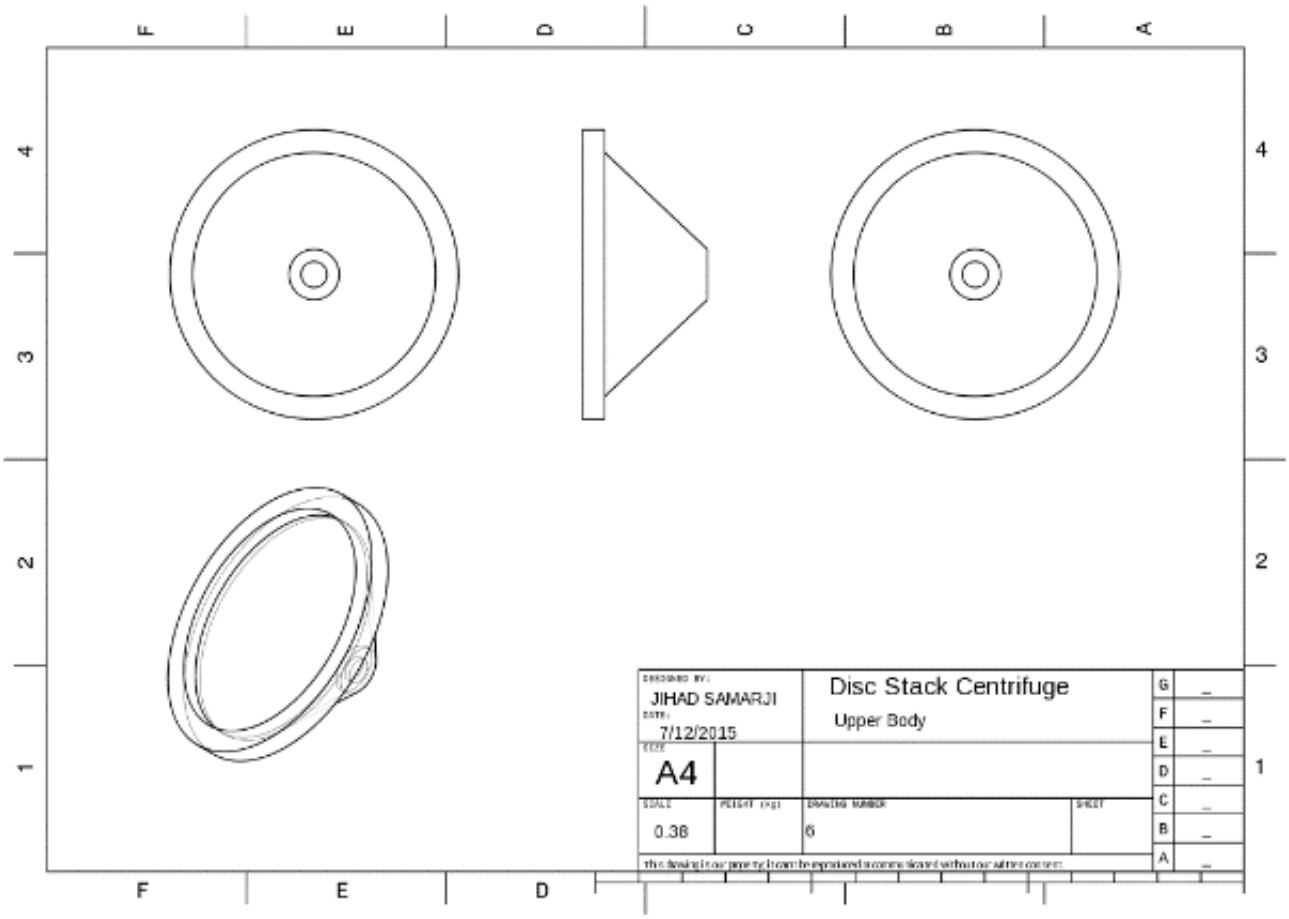
Disc Stack Centrifuge
Disc Stack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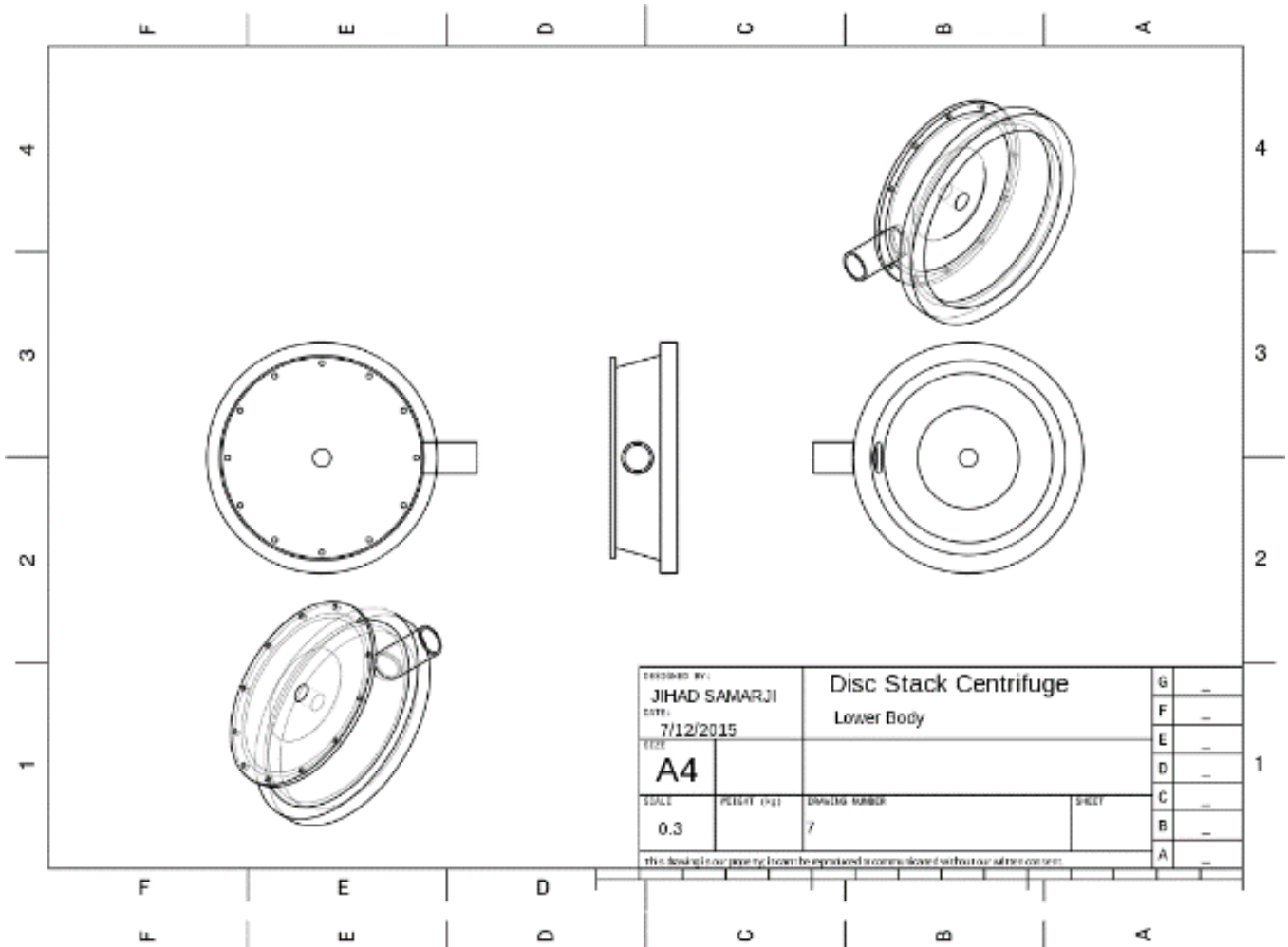
Upper Body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Disc Stack Centrifuge
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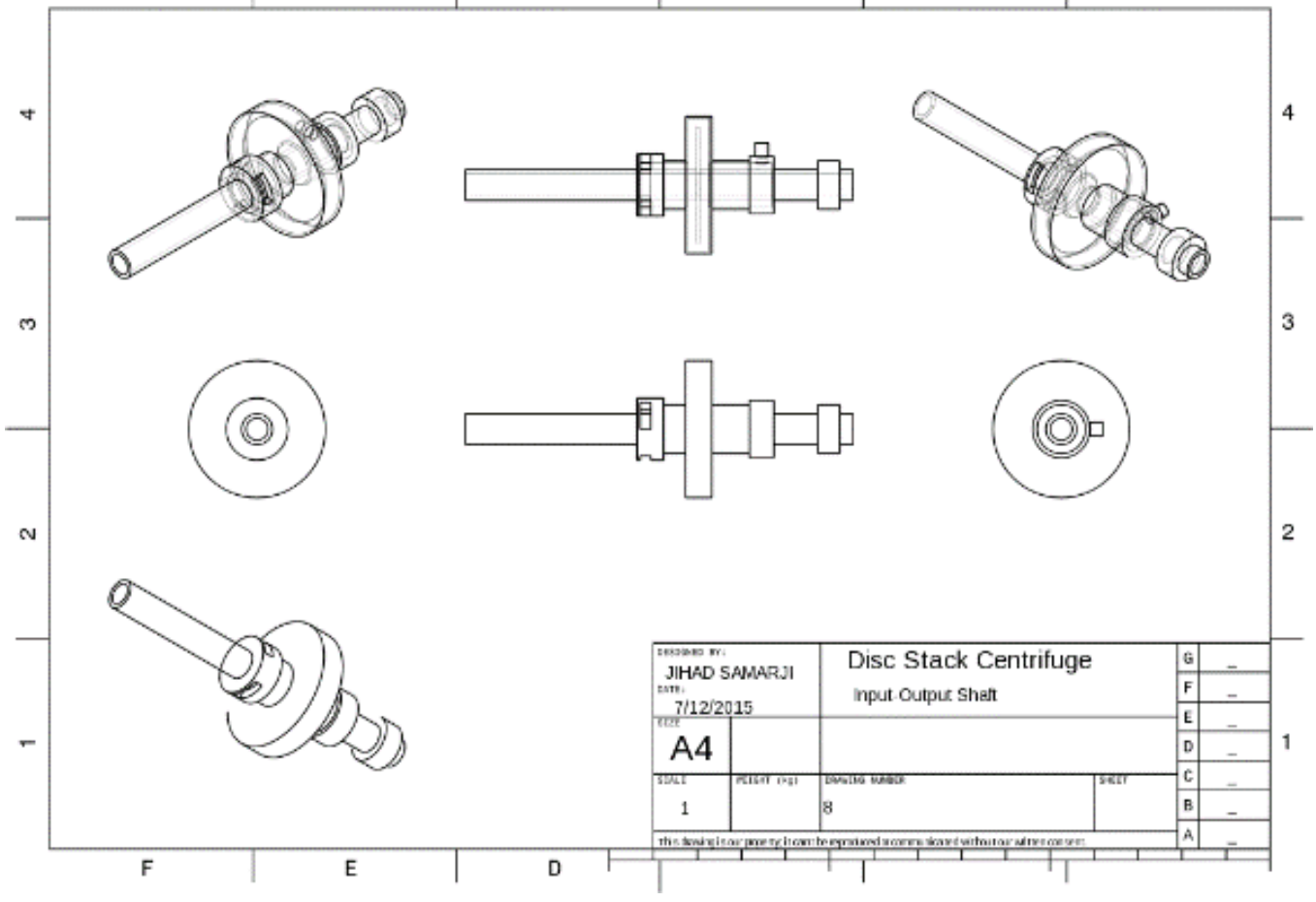
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DATE:
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WEIGHT (kg)
DRAWING NUMBER
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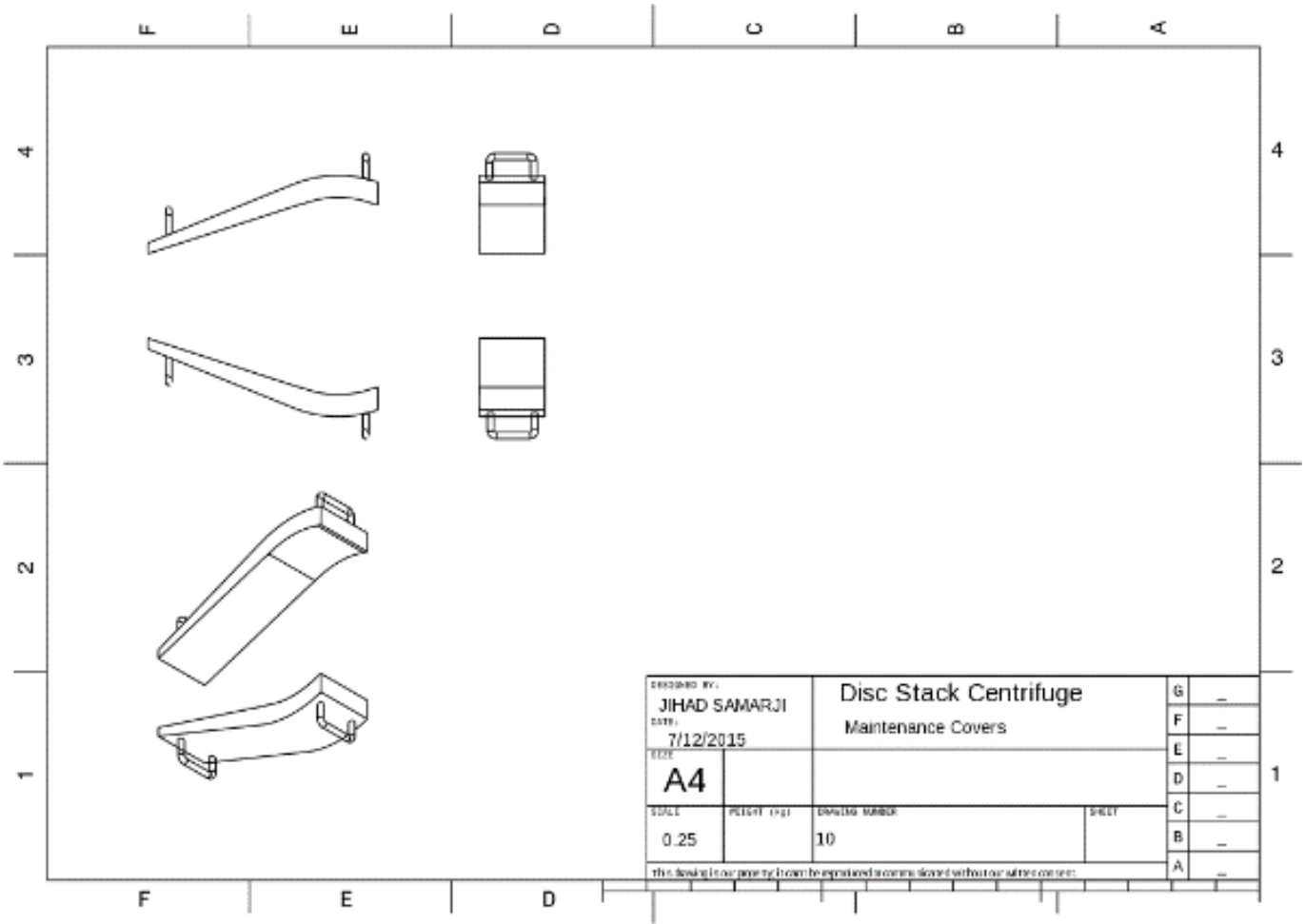
Disc Stack Centrifuge
Input Output Shaft



DESIGNED BY: JIHAD SAMARJI	Disc Stack Centrifuge		
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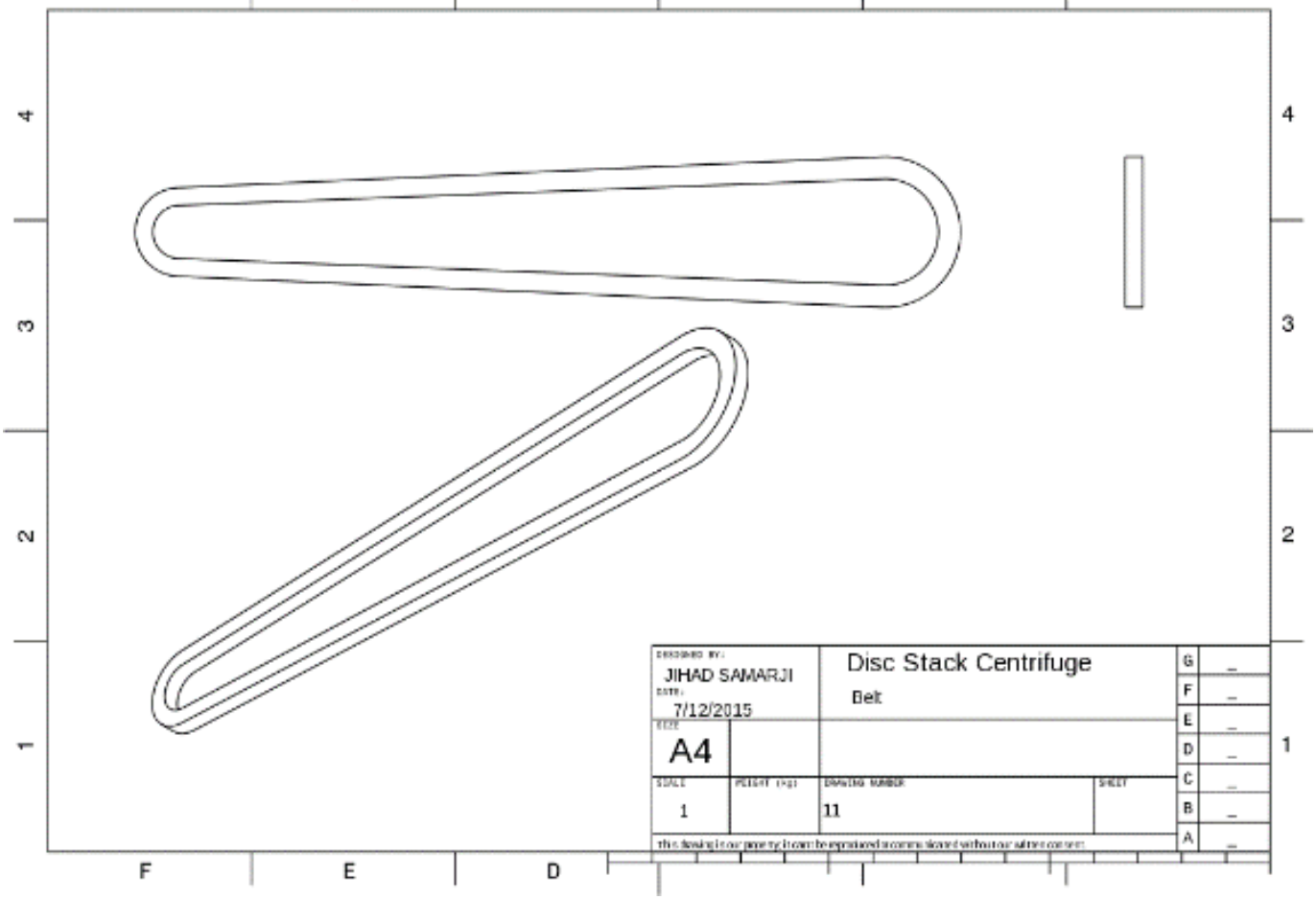

DESIGNED BY: JIHAD SAMARJI	Disc Stack Centrifuge		
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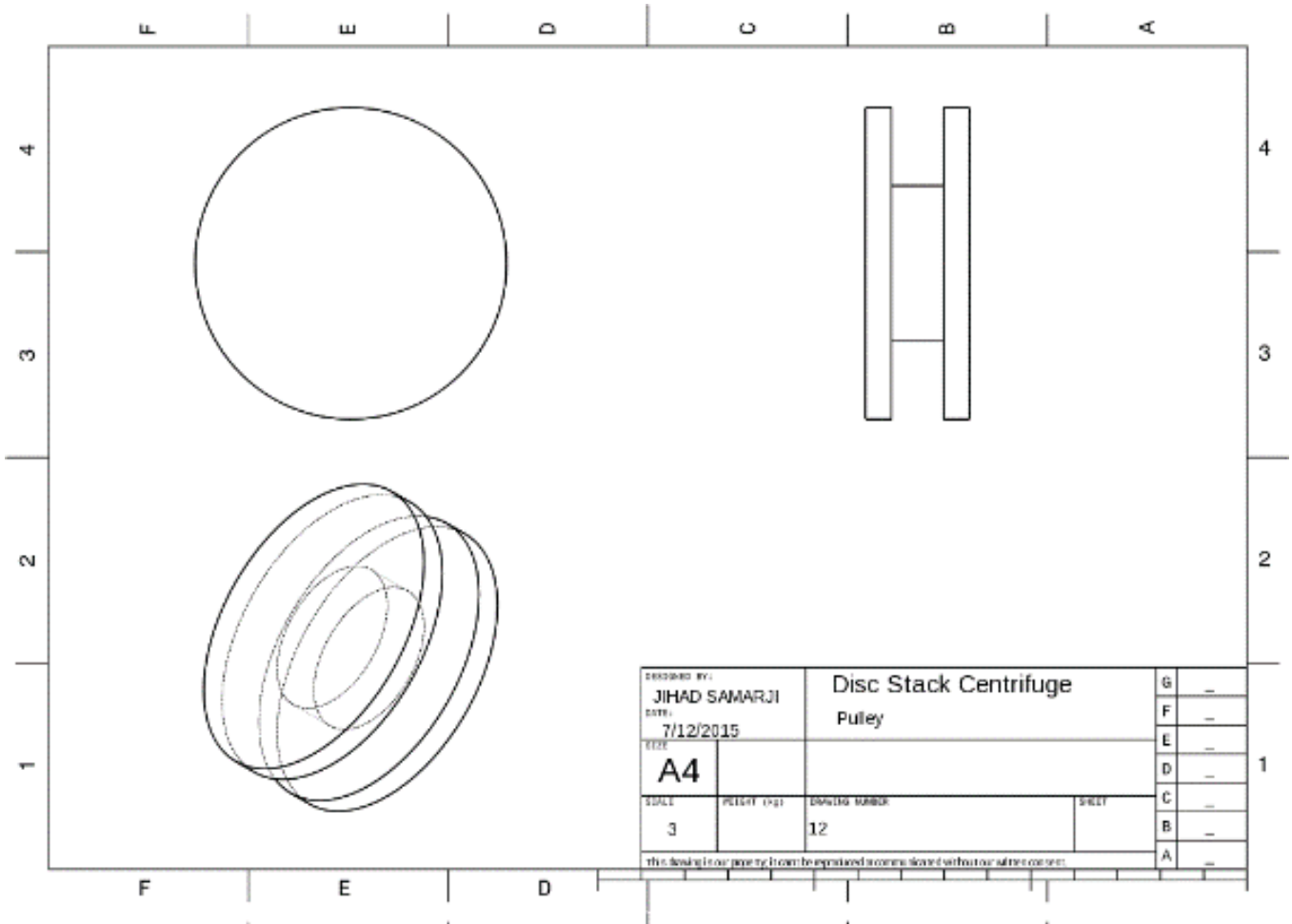
SIZE: A4			
SCALE: 1	WEIGHT (kg):	DRAWING NUMBER: 11	SHEET:



DESIGNED BY: JIHAD SAMARJI	Disc Stack Centrifuge		
DATE: 7/12/2015	Pulley		
SIZE A4			
SCALE 3	WEIGHT (kg)	DRAWING NUMBER 12	SHEET

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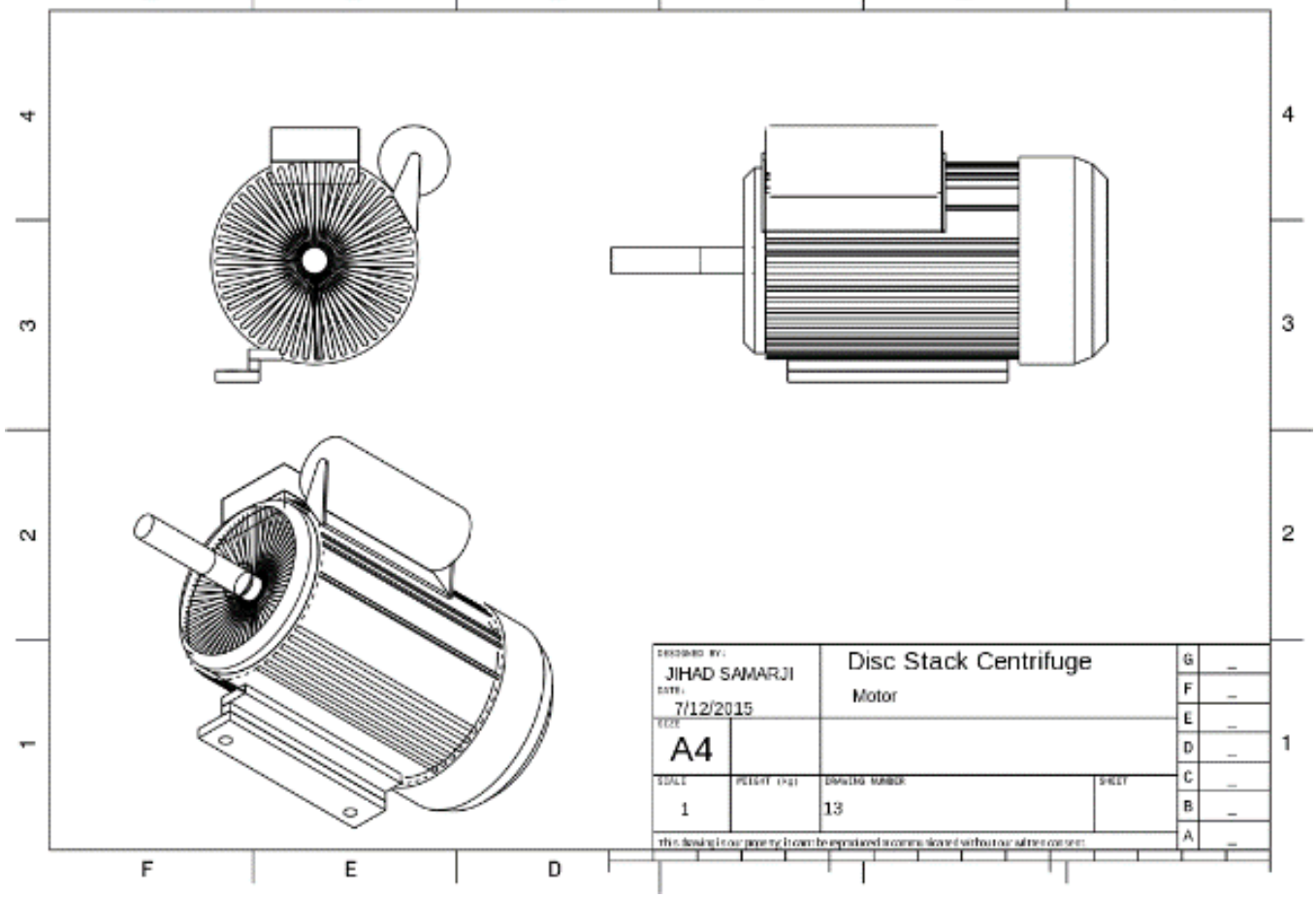

DESIGNED BY: JIHAD SAMARJI	Disc Stack Centrifuge		
DATE: 7/12/2015	Motor		
SIZE A4			
SCALE 1	WEIGHT (kg)	DRAWING NUMBER 13	SHEET

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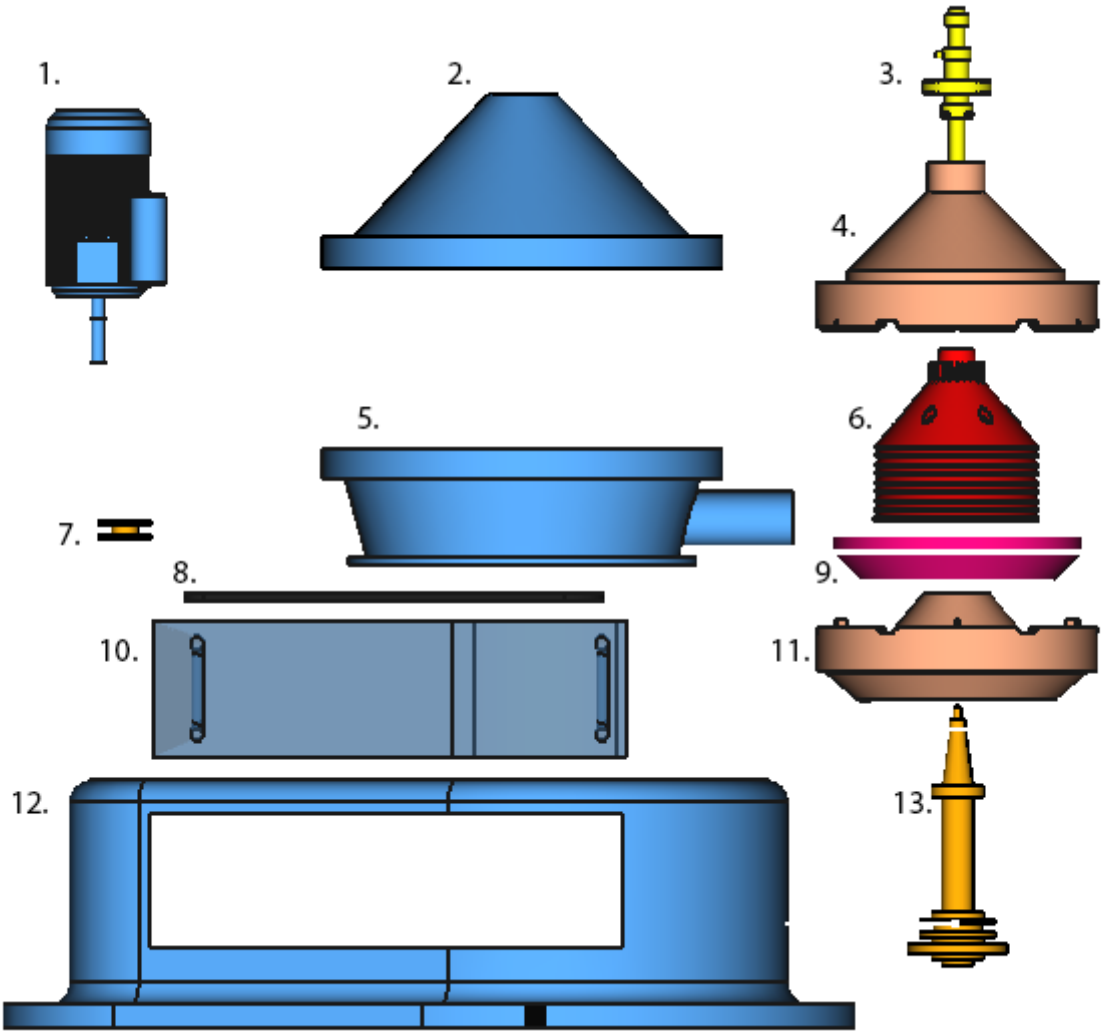
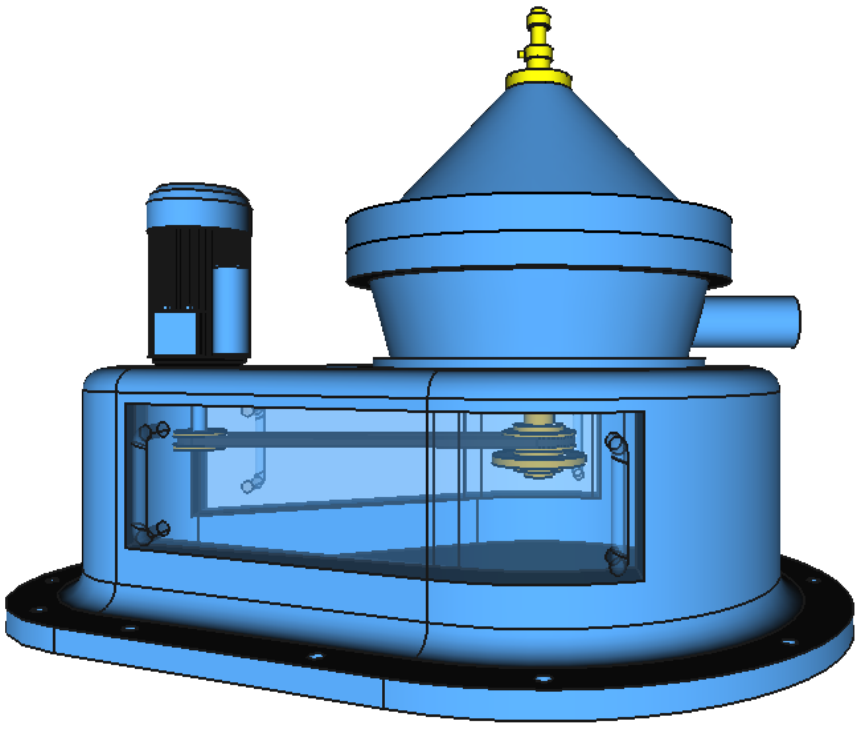


Figure 2.3 Disc Stack Centrifuge Annotations

1. Motor 2.Upper Body 3.Inlet-Outlet Shaft 4.Inner Upper Body 5.Lower Body 6.Disc Stack 7. Pulley 8.Belt 9.Movable Plate 10.Maintenance Cover 11.Inner Lower Body 12.Support 13. Shaft



5.3 Chapter 3: Demonstration and Modelling of the Ultrafiltration.

"To Filtrate a Huge Supply of Substance or Water we need an Ultrafiltration Device"-Jihad Samarji

Figure 3.1 Ultrafiltration Machine

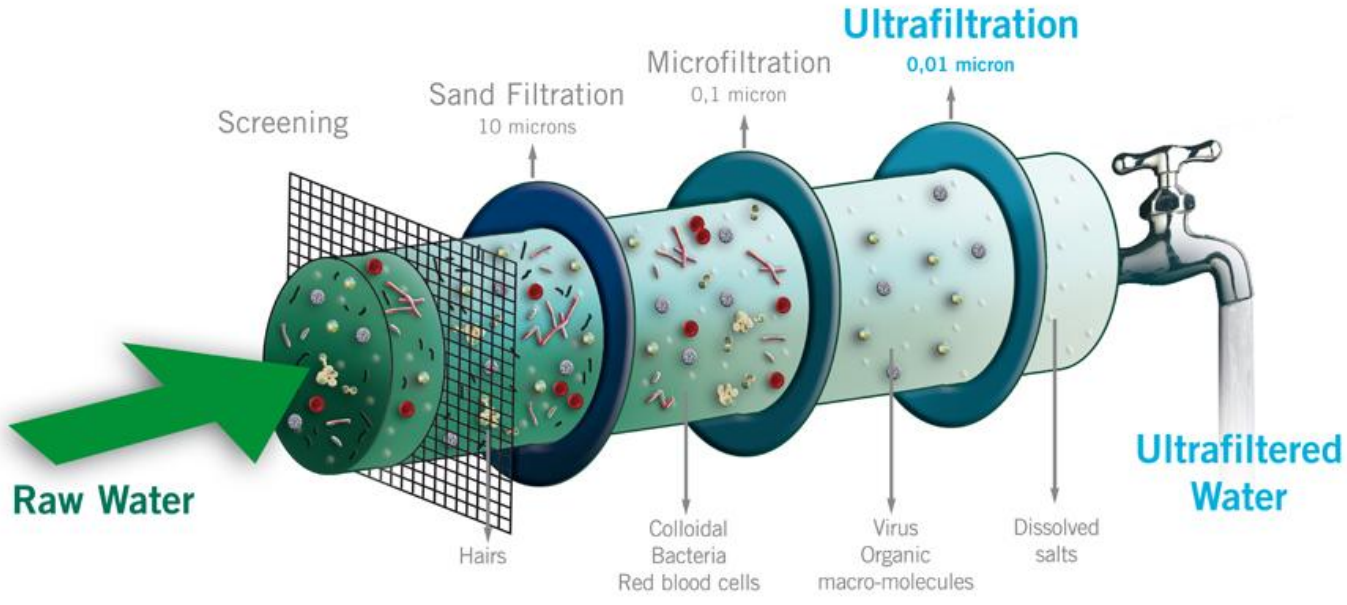


Figure 3.2 a small demonstration about the Ultrafiltration Process

5.3.1 *DEVICE DETAILS & SPECIFICATIONS*

Ultrafiltration (UF) is a variety of membrane filtration in which forces like pressure or concentration gradients lead to a separation through a membrane. Suspended and solutes of high molecular weight are retained in the so-called retentive, while water and low molecular weight solutes pass through the membrane in thepermeate. This separation process is used in industry and research for purifying and concentrating macromolecular (103 - 106 Da) solutions, especially protein solutions. Ultrafiltration is not fundamentally different from microfiltration. Both of these separate based on size exclusion or particle capture. It is fundamentally different from membrane gas separation, which separate based on different amounts of absorption and different rates of diffusion. Ultrafiltration membranes are defined by the molecular weight cut-off(MWCO) of the membrane used. Ultrafiltration is applied in cross-flow or dead-end mode.

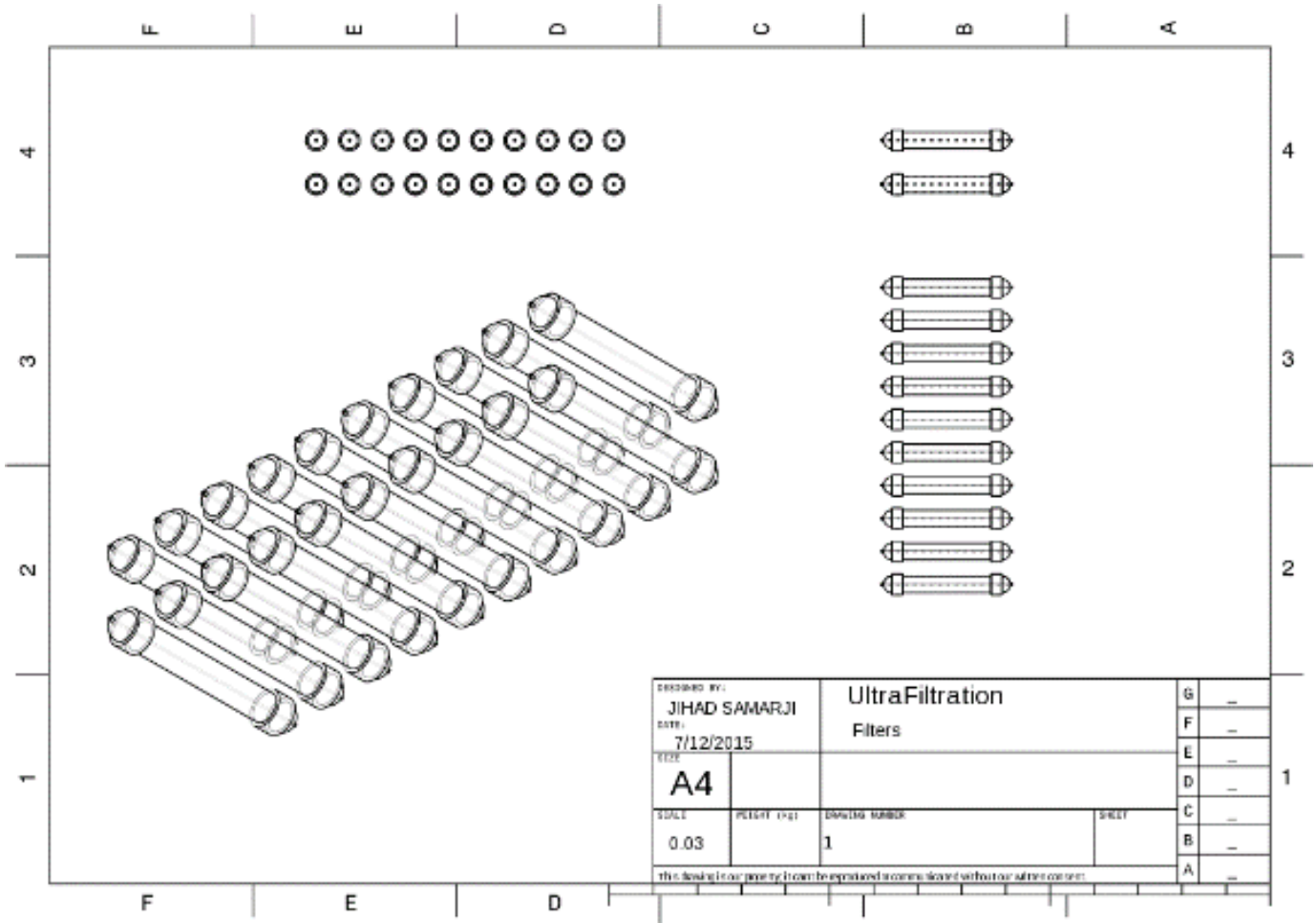
Ultrafiltration is used in Industries such as chemical and pharmaceutical manufacturing, food and beverage processing, and waste water treatment, employ ultrafiltration in order to recycle flow or add value to later products. Blood dialysis also utilizes ultrafiltration.

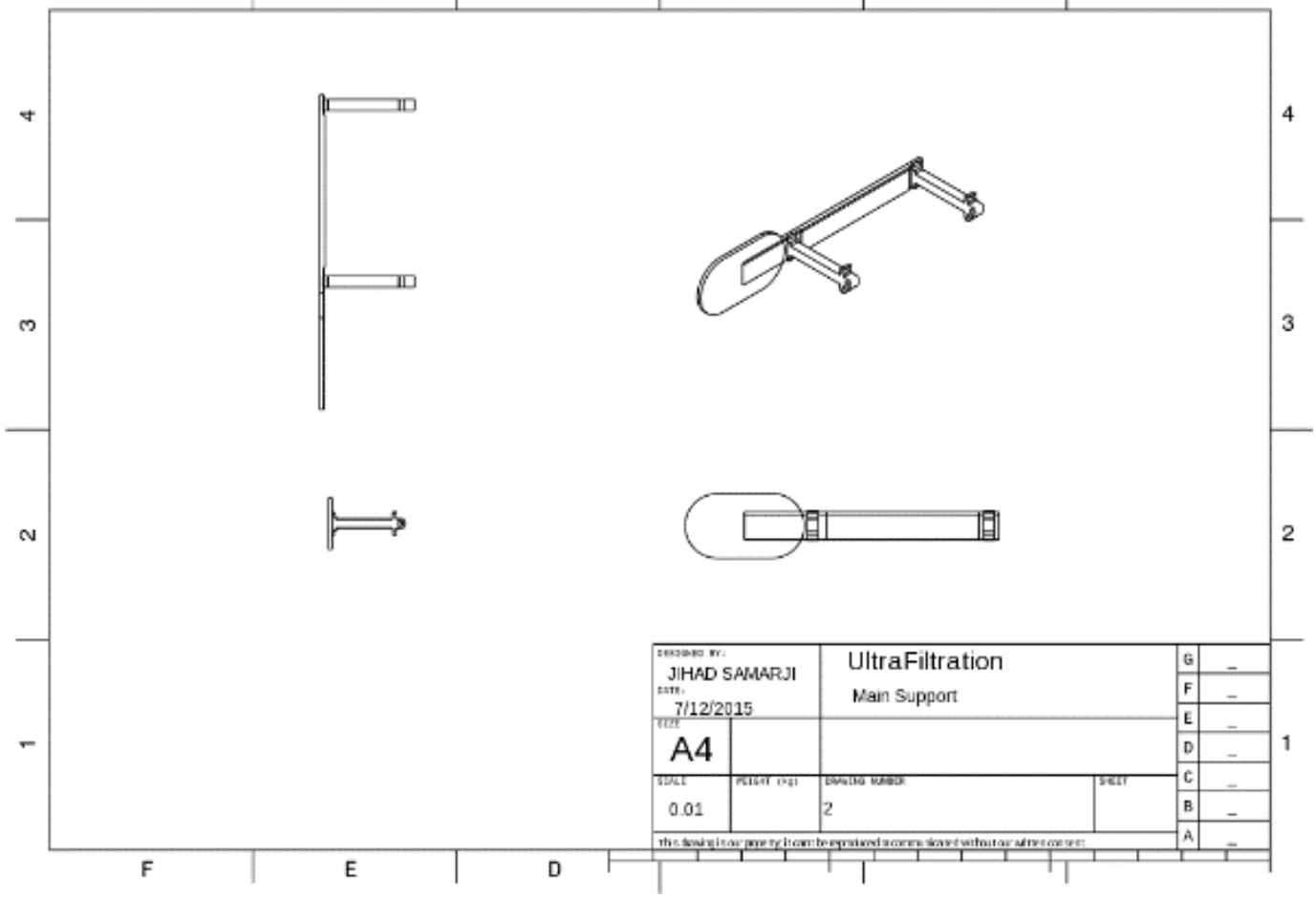
In our case we're going to use the ultrafiltration device for Protein concentration. See Figure.3.1 and 3.2

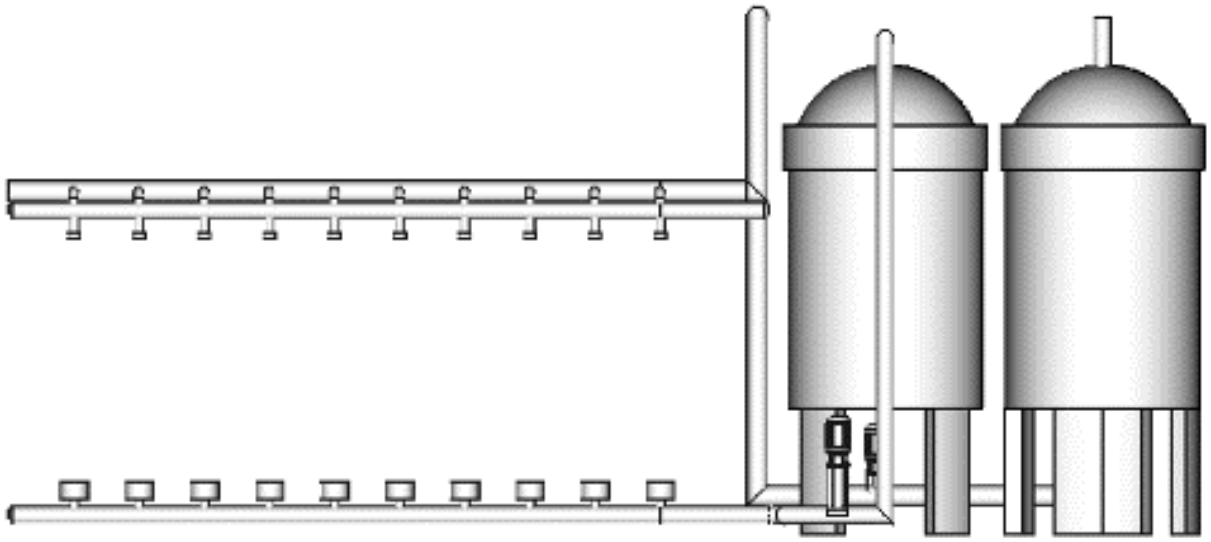
Foulant	Reagent	Time and Temperature	Mode of Action
Fats and oils, proteins, polysaccharides, bacteria	0.5M NaOH with 200 ppm Cl2	30-60 min 25-55 °C	Hydrolysis and oxidation
DNA, mineral salts	0.1M – 0.5M acid (acetic, citric, nitric)	30-60 min 25-35 °C	Solubilization
Fats, oils, biopolymers, proteins	0.1% SDS, 0.1% Triton X-100	30 min – overnight 25-55 °C	Wetting, emulsifying, suspending, dispersing
Cell fragments, fats, oils, proteins	Enzyme detergents	30 min – overnight 30 – 40 °C	Catalytic breakdown
DNA	0.5% DNAase	30 min – overnight 20 – 40 °C	Enzyme hydrolysis

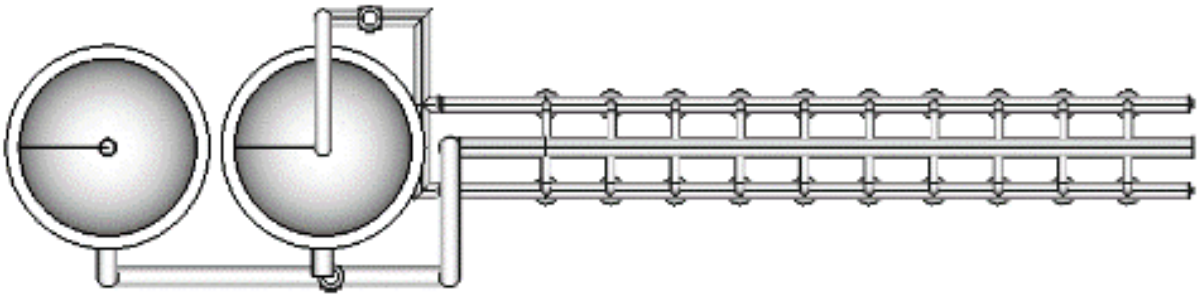
Table 3.2 SUMMARY OF COMMON TYPES OF FOULING AND THEIR RESPECTIVE CHEMICAL TREATMENTS

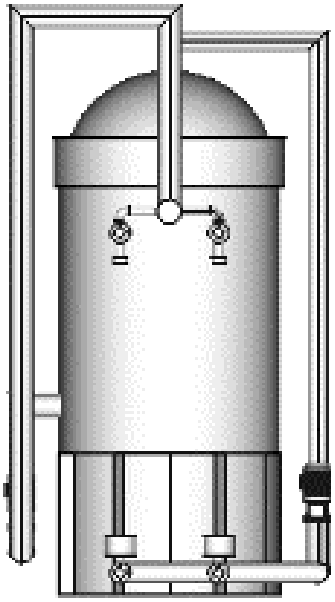
5.3.2 *PART DIMENSIONS & DESIGN*

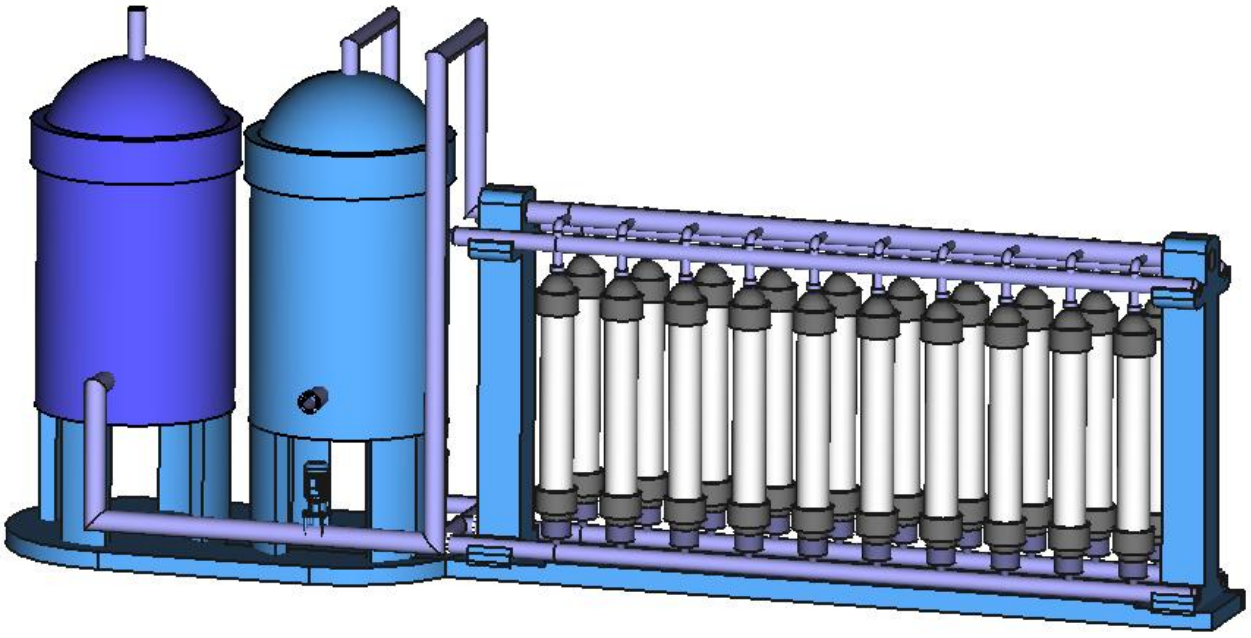












The Ultrafiltration is Simple, no need for further dimensions nor annotations. It contains 2 Tanks, one for filtrated substance and the other for non-filtrated substance which is connected to a set of filters. The device also contains 2 pumps to boost the substance into the pipes. For more information about the ultrafiltration process, see figure.5

5.4 **Demonstration and Modelling of the Chromatography Device**

-ZaherChendeb-

5.4.1 ***DEVICE DETAILS & SPECIFICATIONS***

AxiChrom<Chromatography>

The AxiChrom column platform is a revolutionary concept in column chromatography that simplifies column handling at all scales from process development to full-scale production. AxiChrom columns introduce three key features – Intelligent Packing, Intuitive handling, and Predictable scale-up – that together make process chromatography easier, safer, and more efficient.

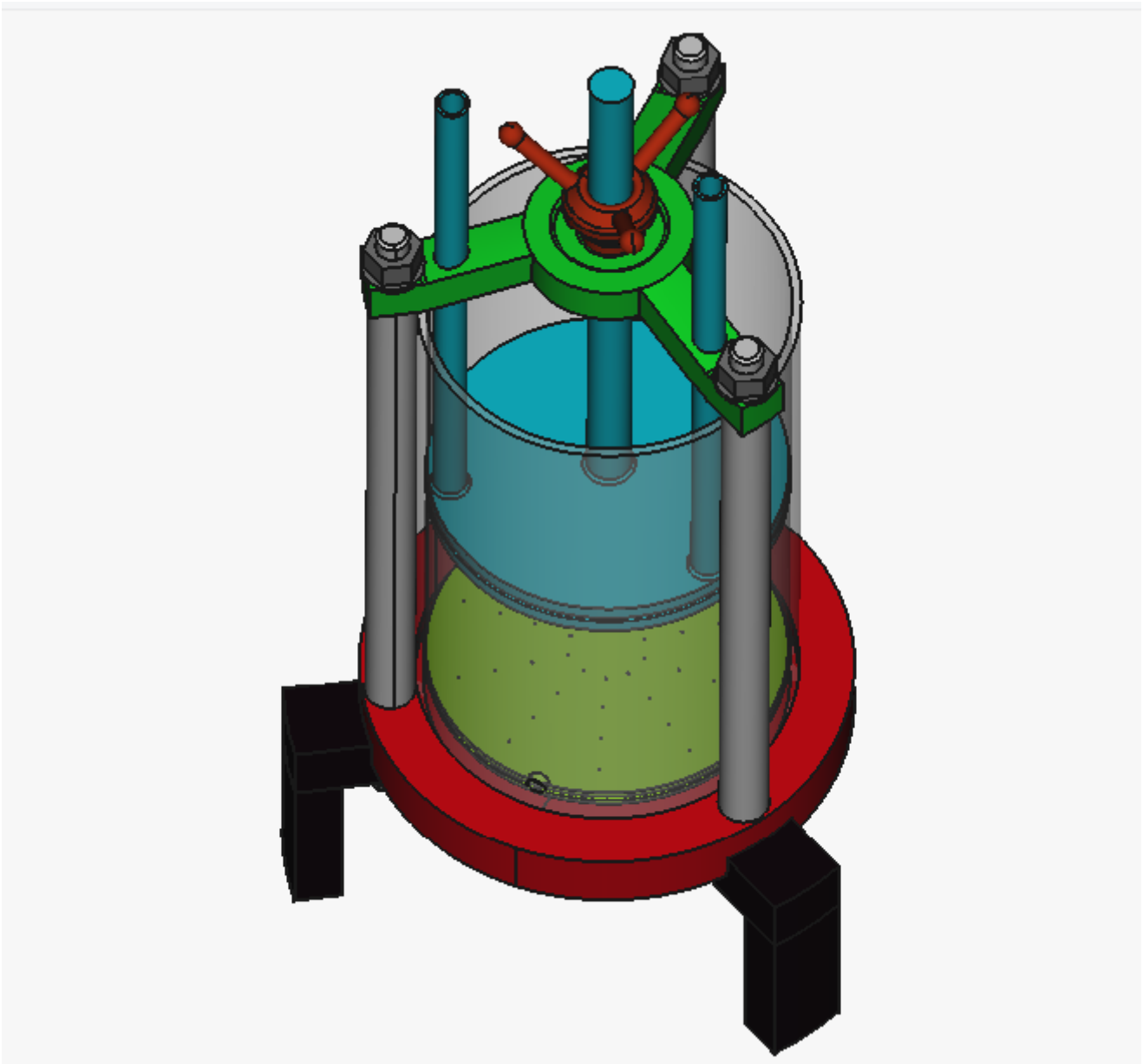
The AxiChrom process column family has been designed to deliver reproducible results from process development to production scales. This is facilitated by the innovative Intelligent Packing where UNICORN™ software, ÄKTA™ systems and AxiChrom columns work together to facilitate a convenient operation for packing of the bed via axial compression.

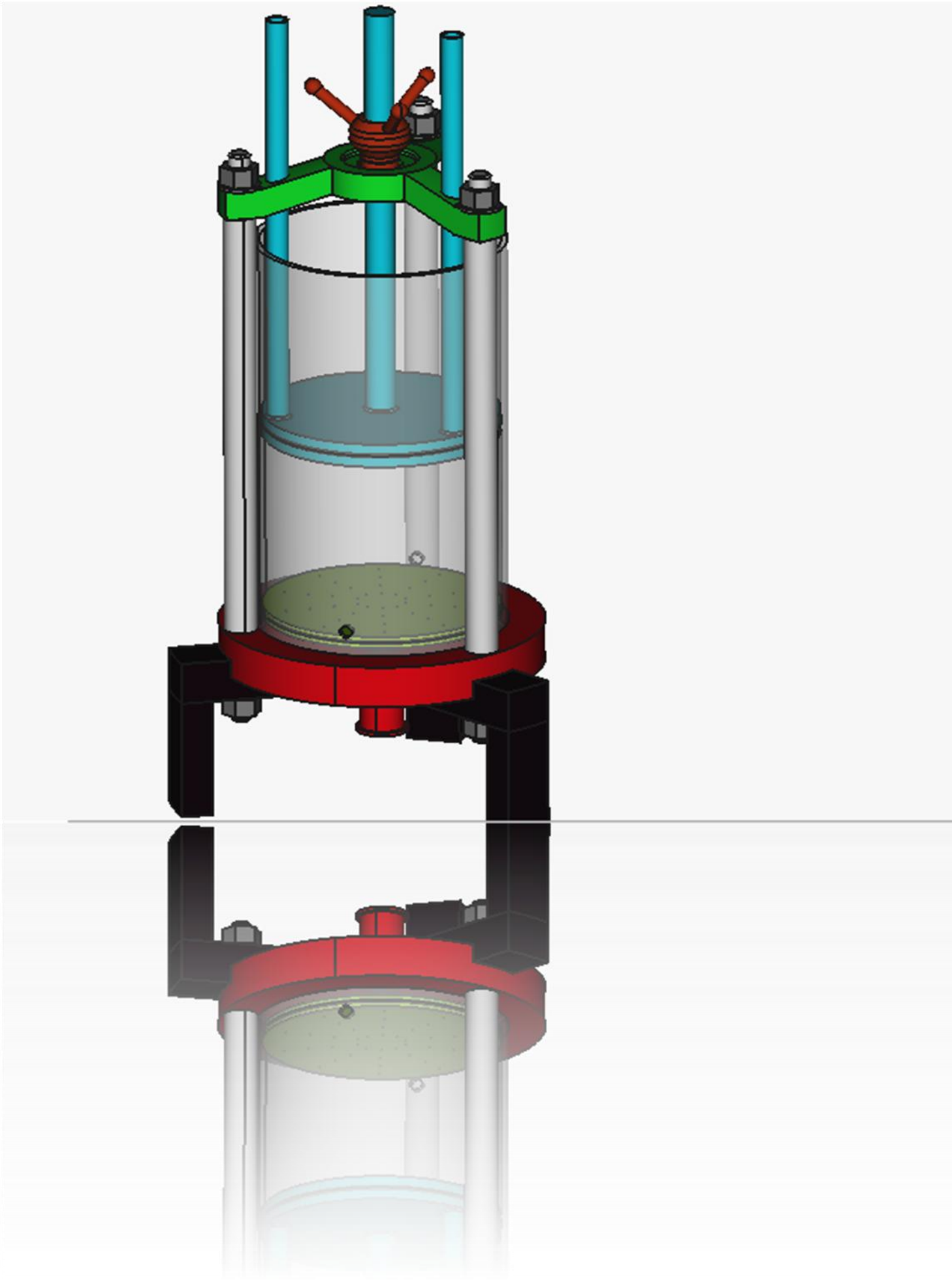
The Intended use of The AxiChrom family of process columns has been designed for low pressure chromatographic separation of biomolecules such as proteins, peptides and oligonucleotides in GMP-regulated environments.

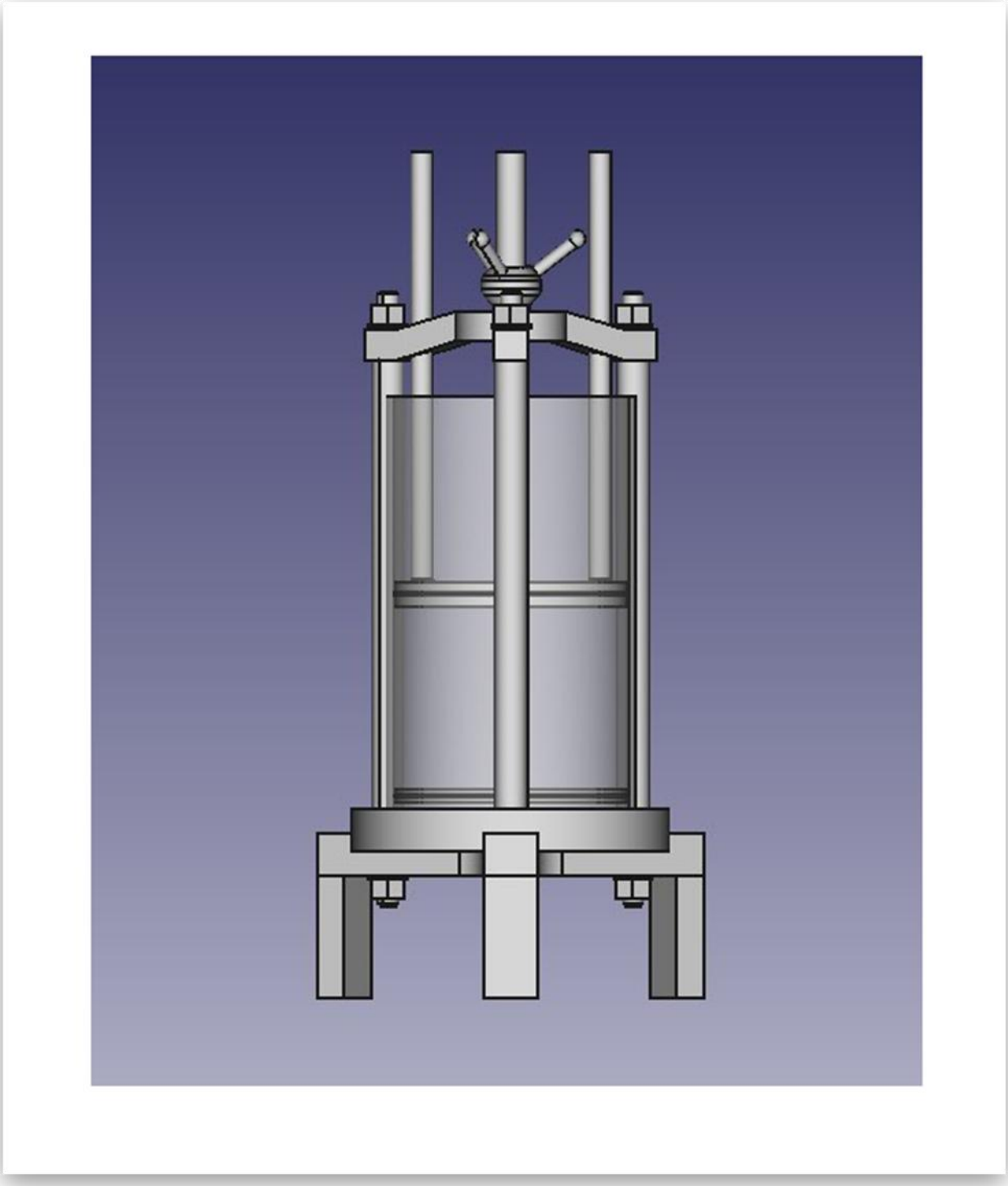
The AxiChrom columns are intended for production use only and should not be used for diagnostic purposes in any clinical or in vitro procedures. The columns are not suitable for operation in a

potentially explosive atmosphere or for handling flammable liquids. If the columns are used for purposes other than those specified in the user documentation, safe operation and the protection provided by the system may be impaired.

5.4.2 *PART DIMENSIONS & DESIGN*



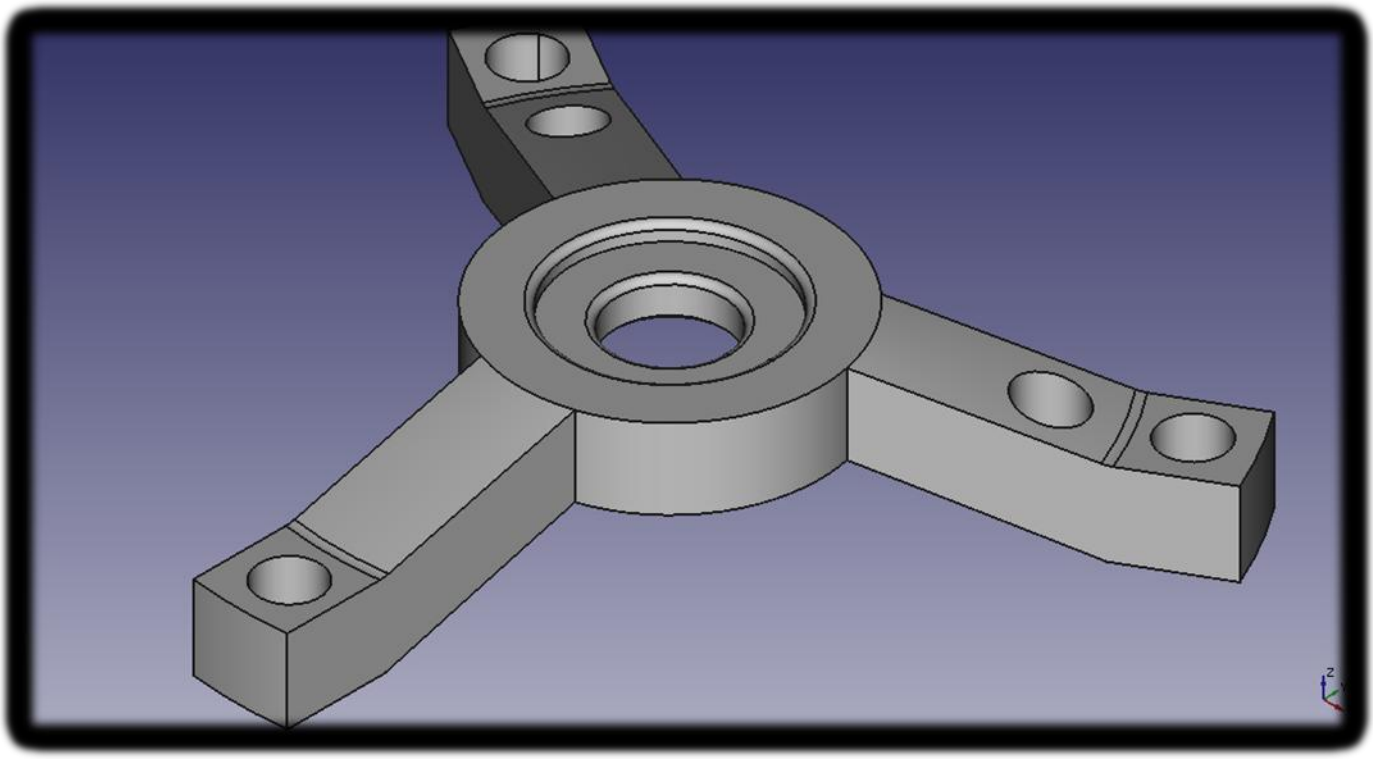


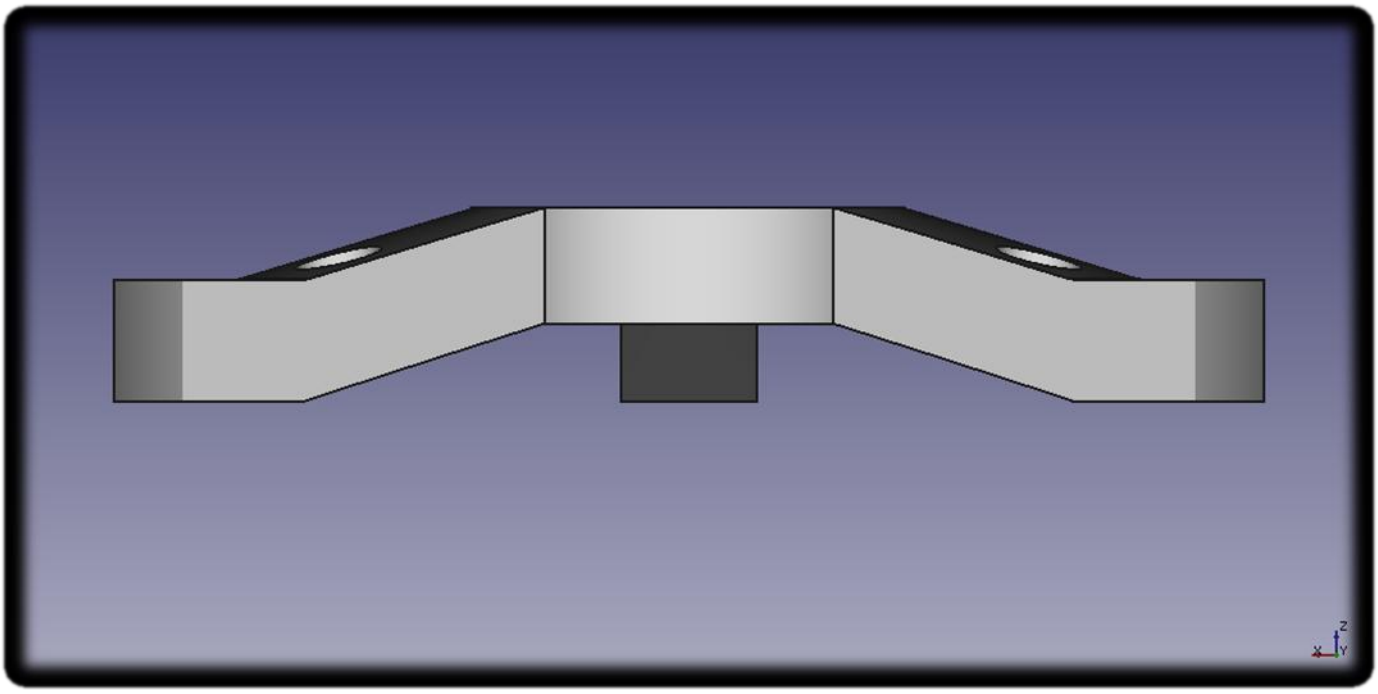


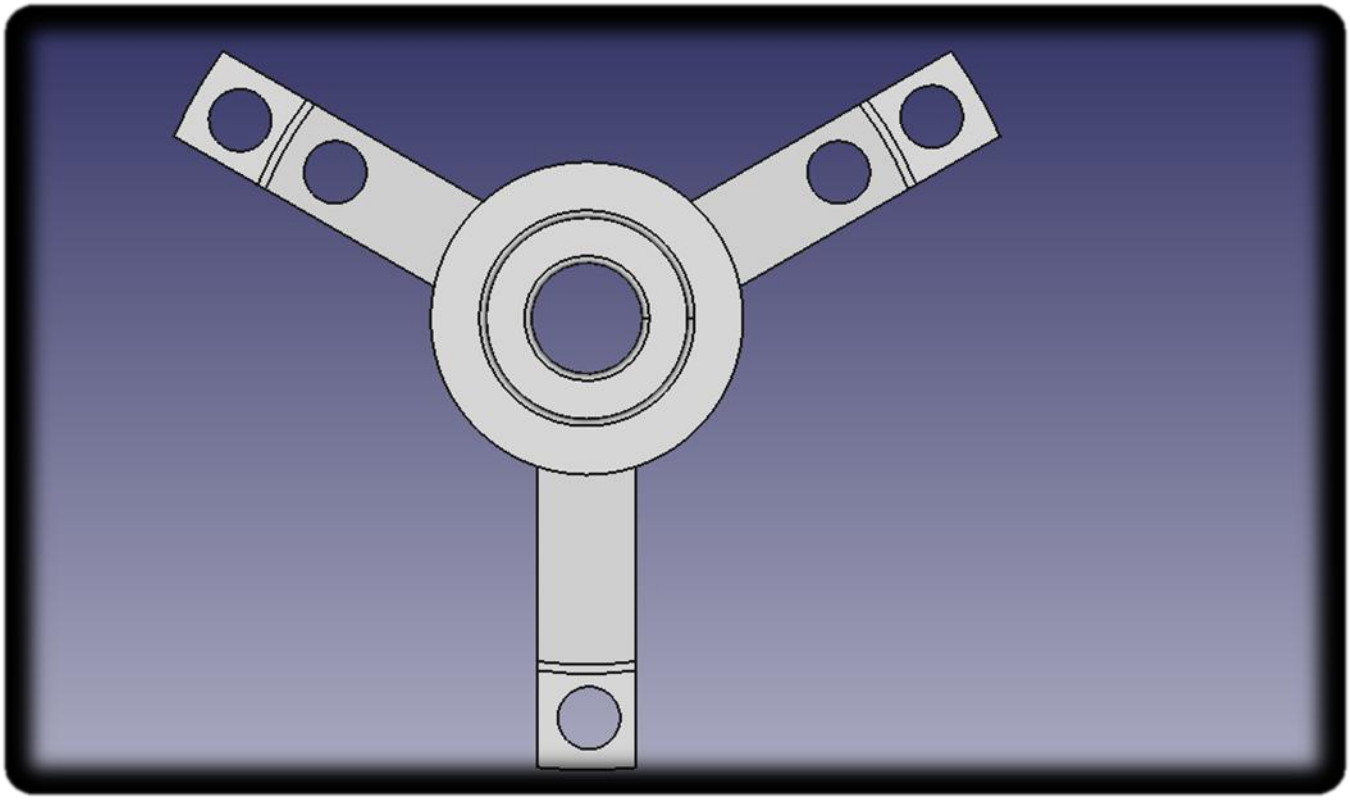
The Chromatography column contains eight main parts: top, handle, cylinder, piston, disc, bottom, base, and column.

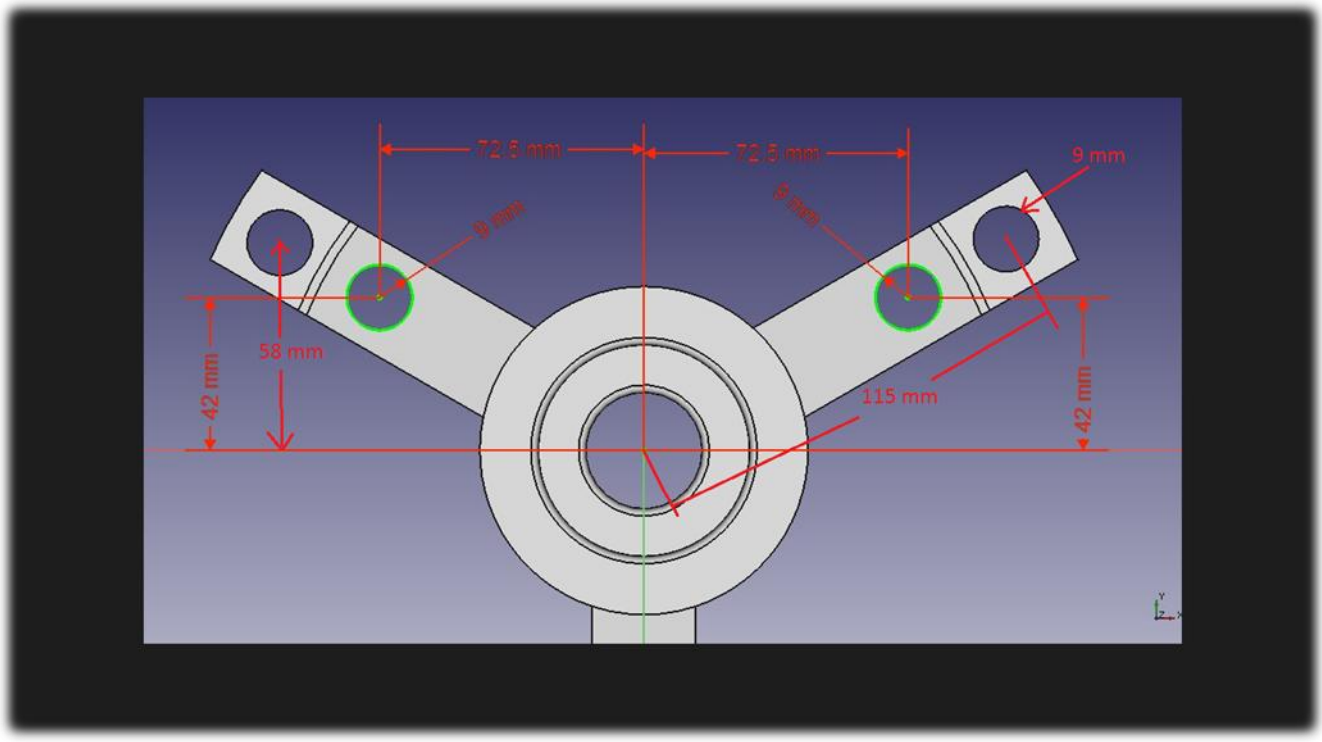
5.4.2.1 Top

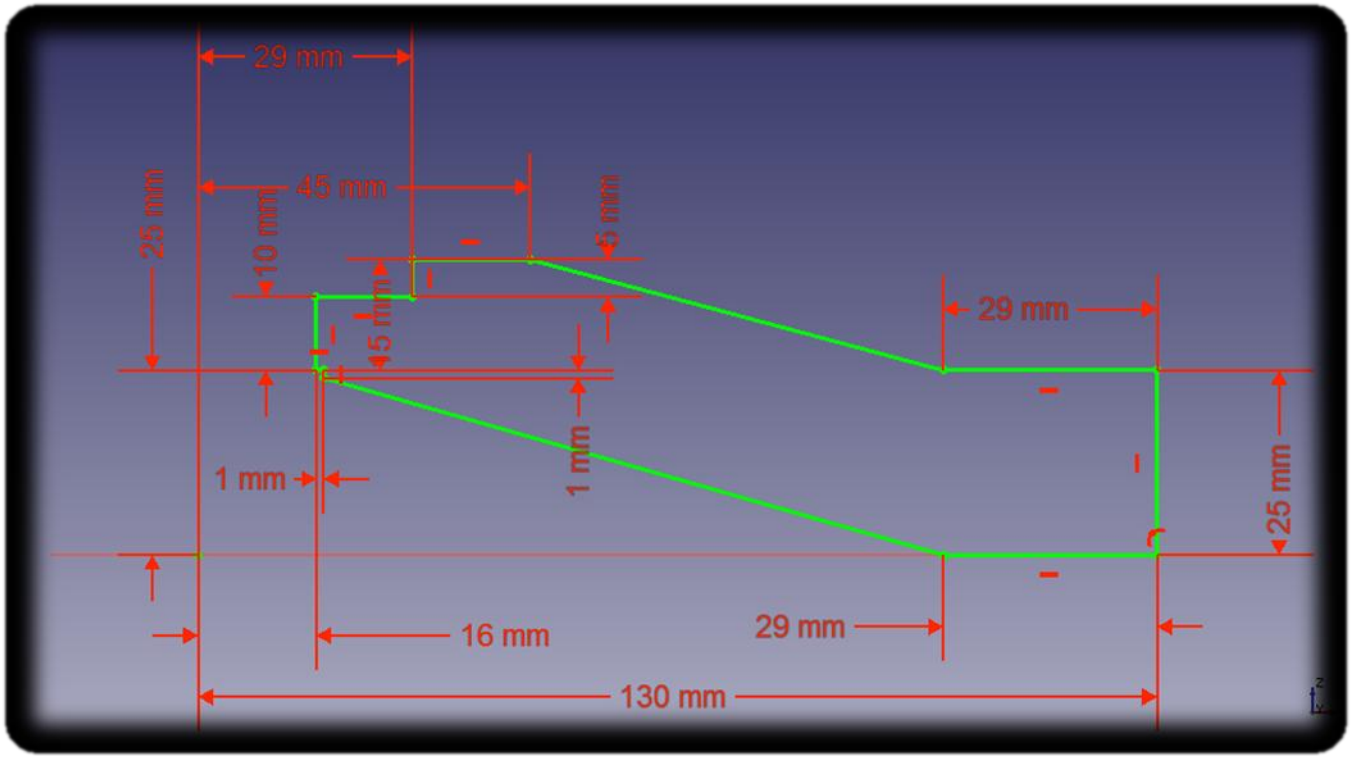
The top is fixed by nuts with three columns that pass through it, and it contains two other holes to let the tube of the piston pass through it







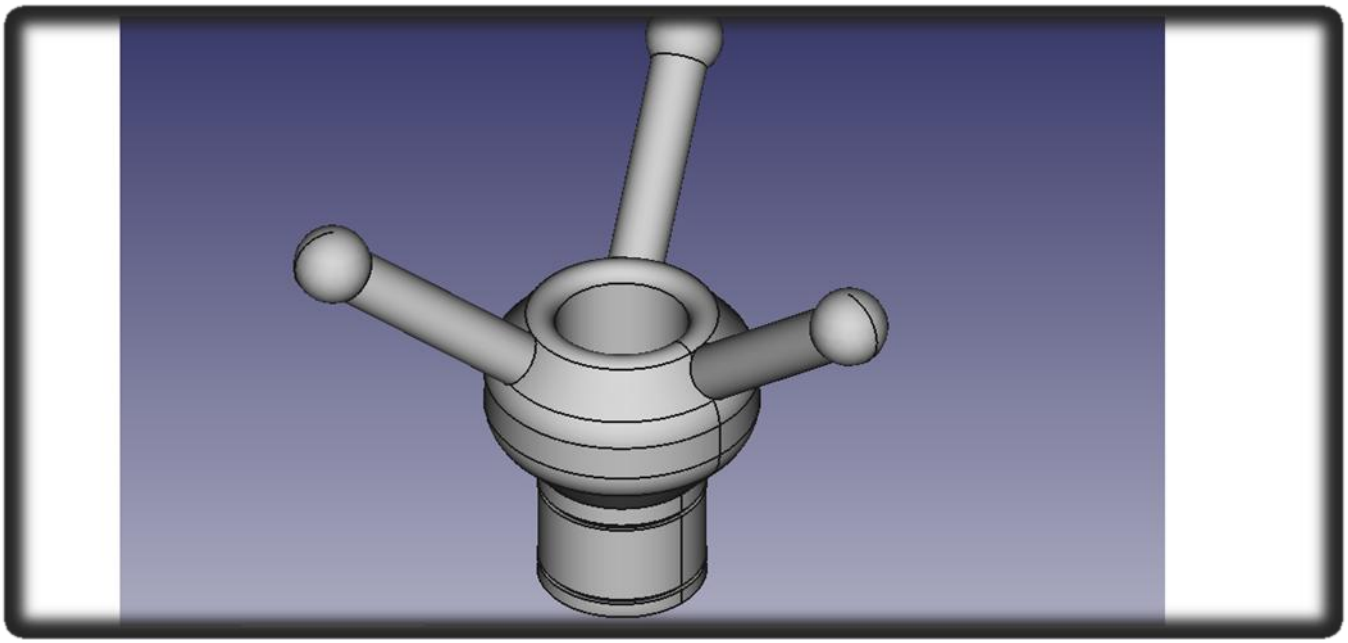

72.5 mm
72.5 mm
9 mm
9 mm
9 mm
58 mm
42 mm
115 mm
42 mm

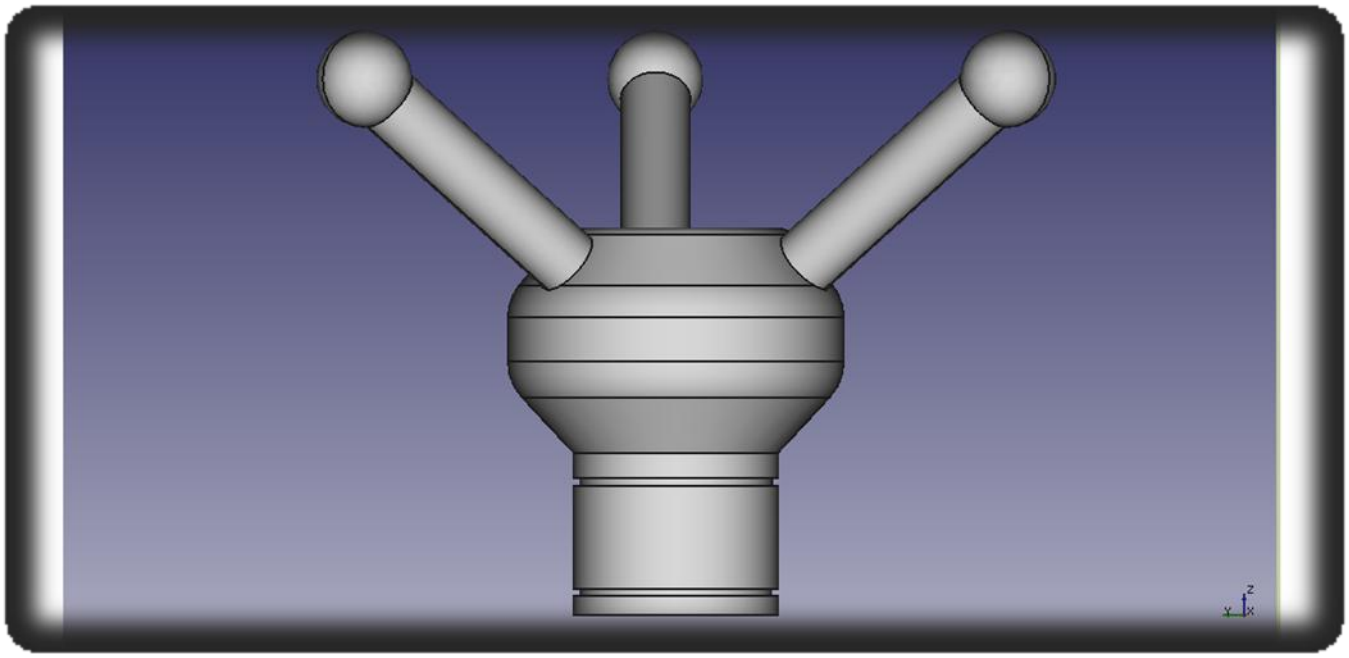


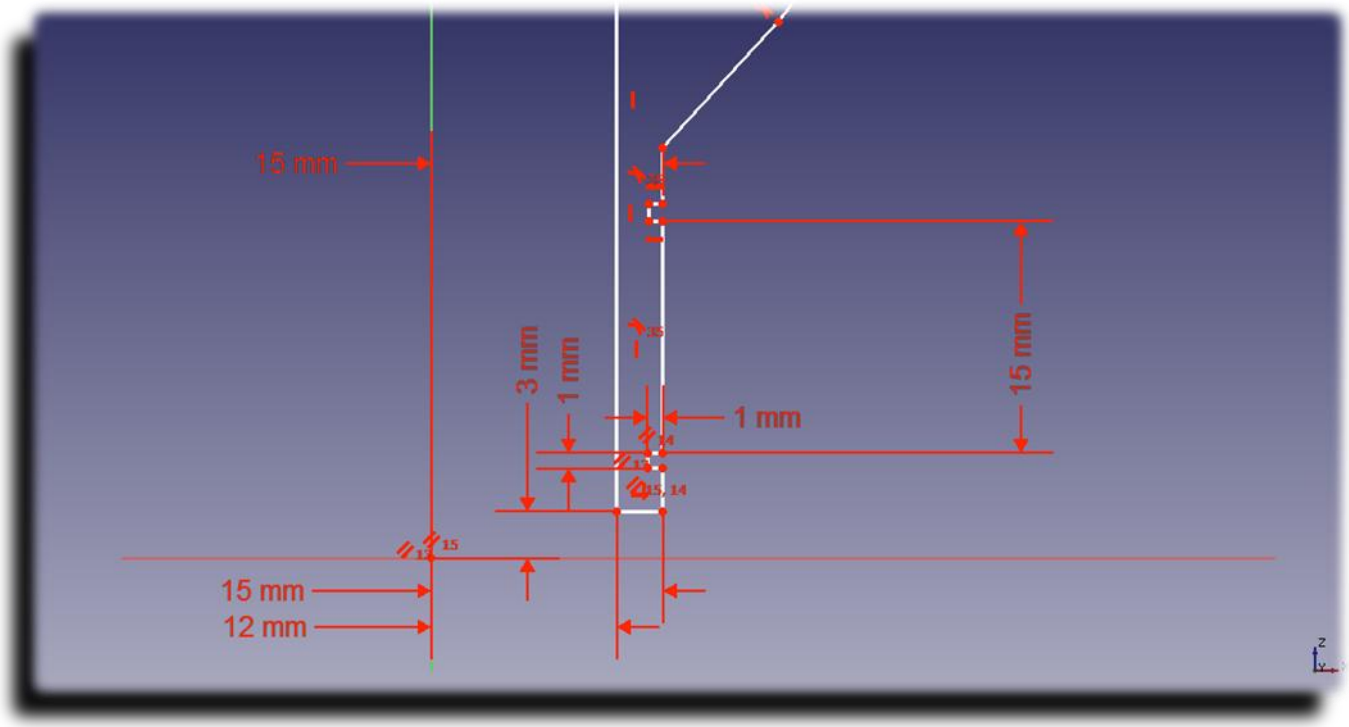
5.4.2.2 Handle

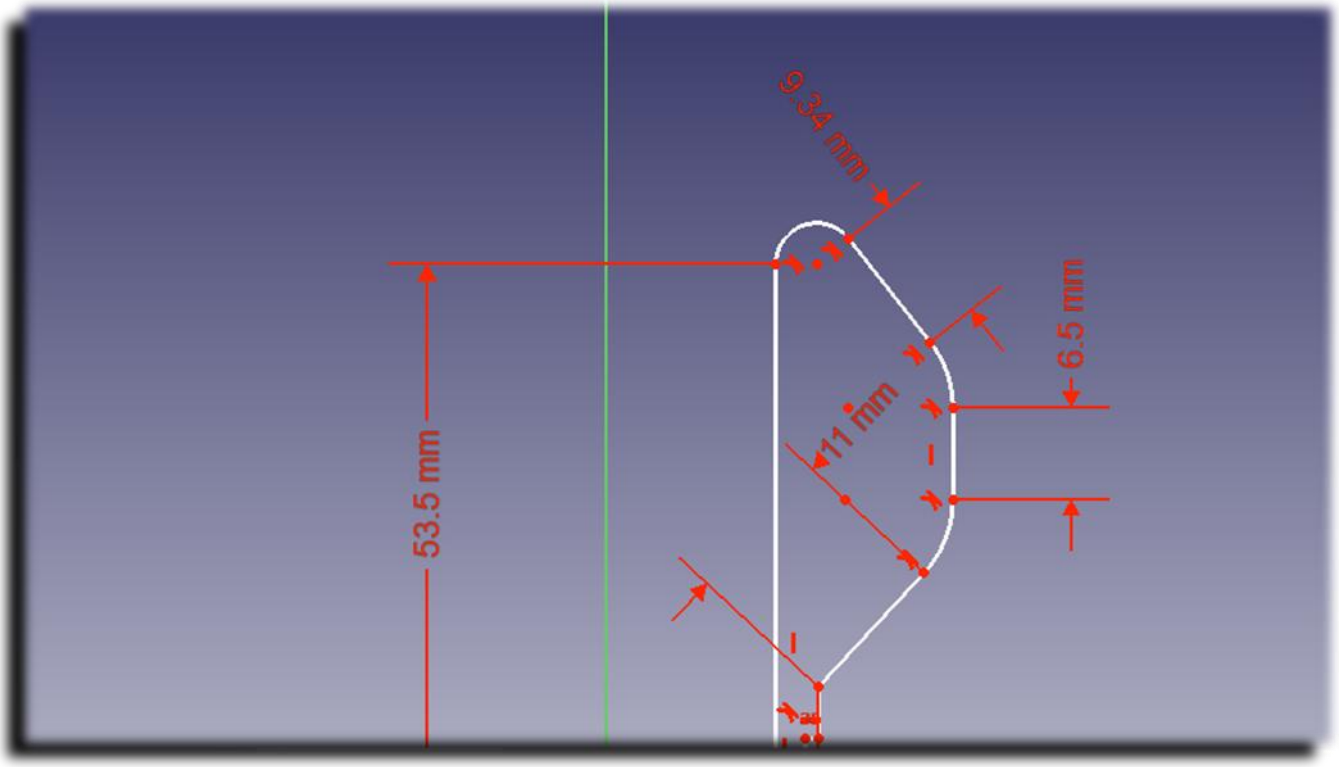
I used a handle instead of a motor, because it doesn't need a high power to rotate it and it is cheaper

Bearing is placed on the bottom between two rings



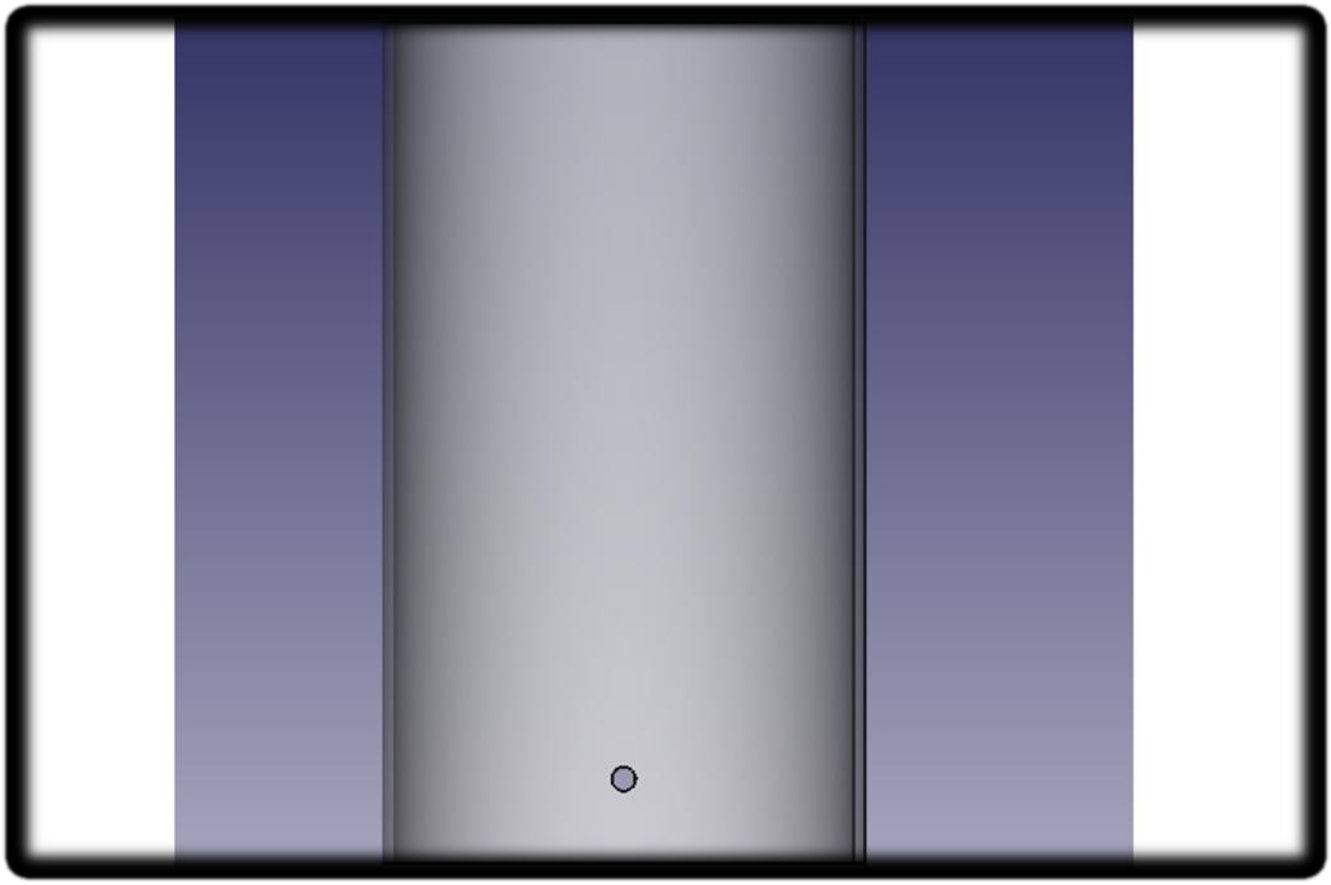


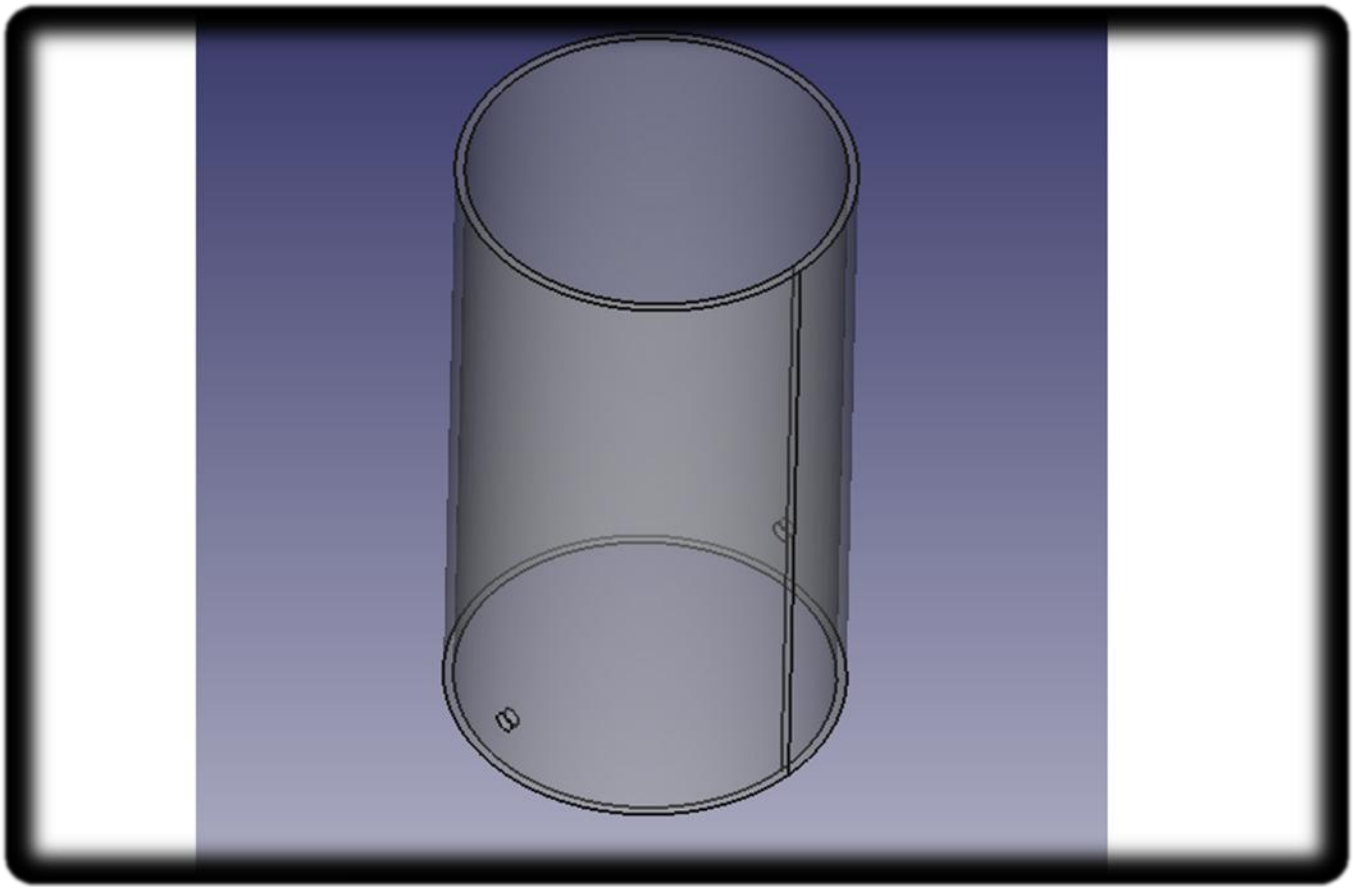

15 mm
3 mm
1 mm
1 mm
15 mm
15 mm
12 mm

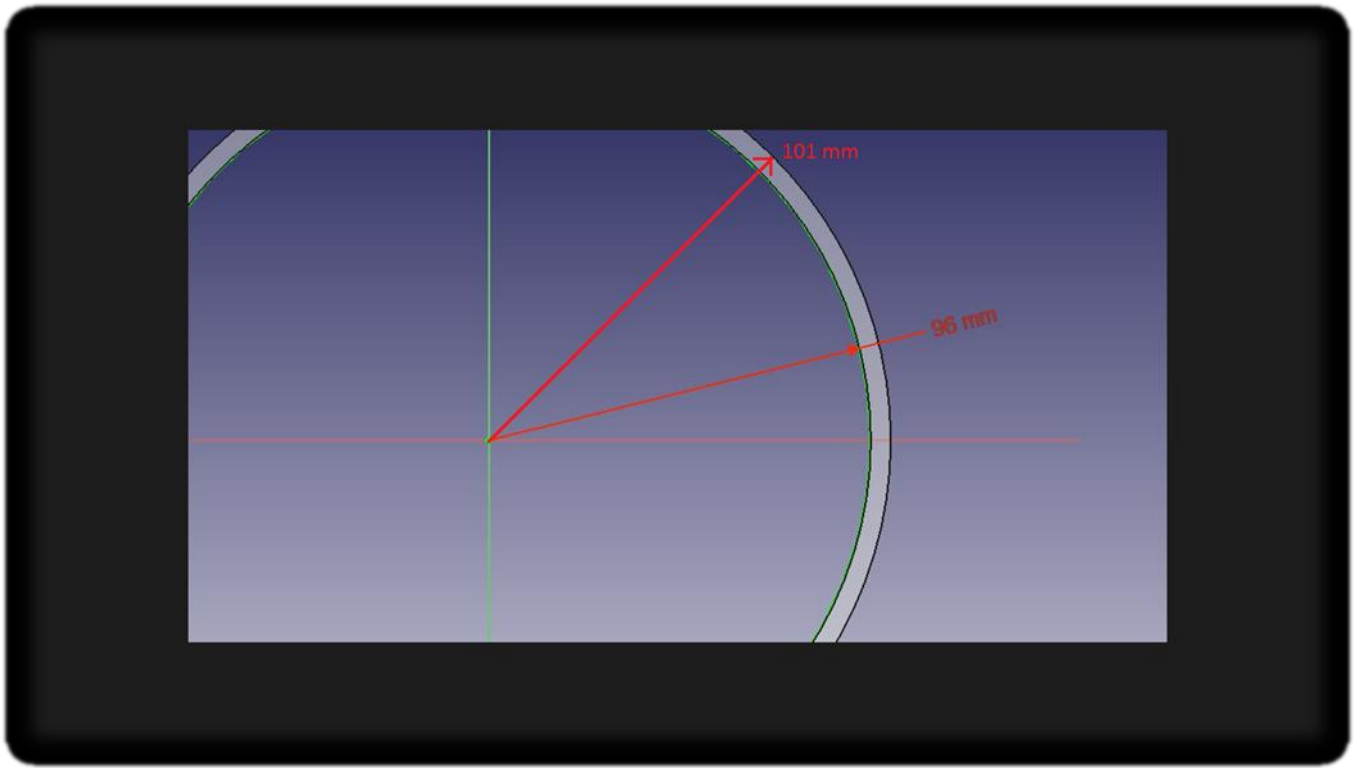


5.4.2.3 *Cylinder*

Is made of Plexiglas, it's where we put the solid particles

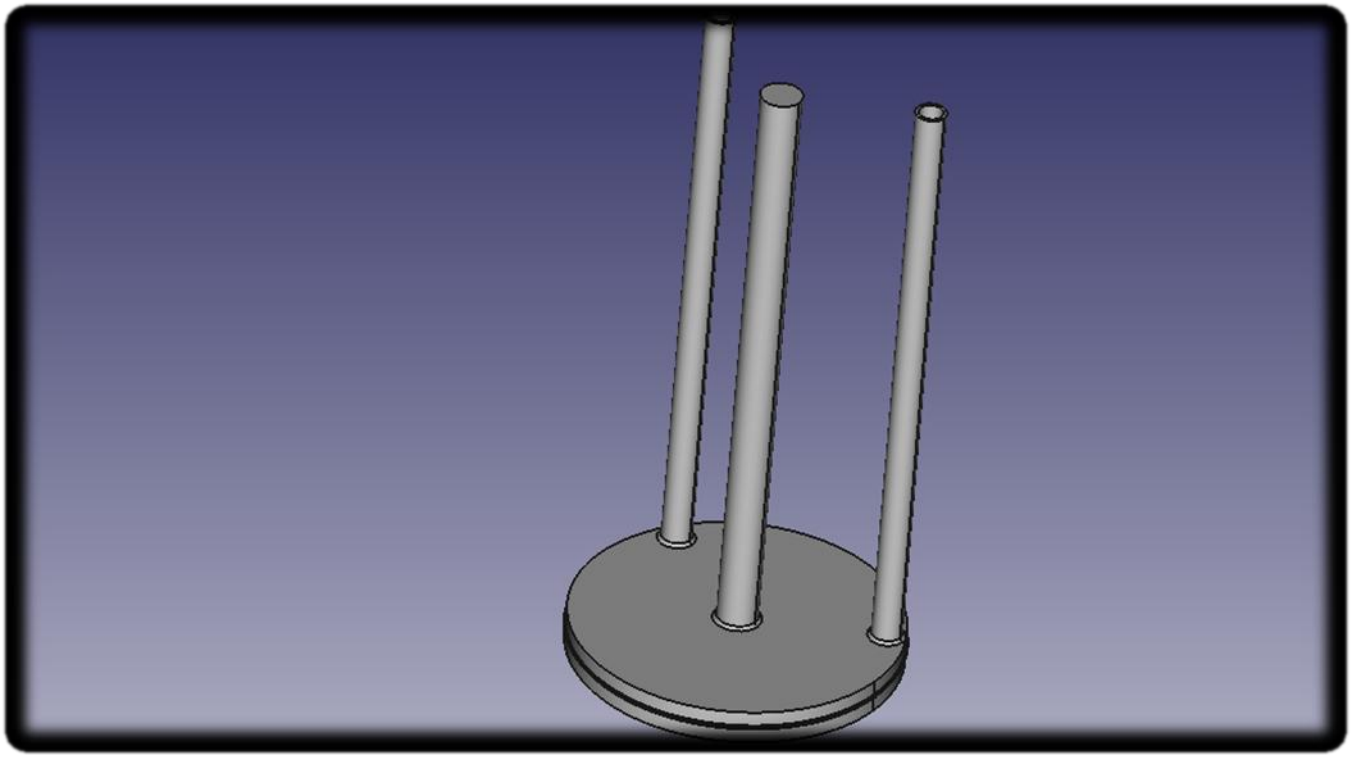


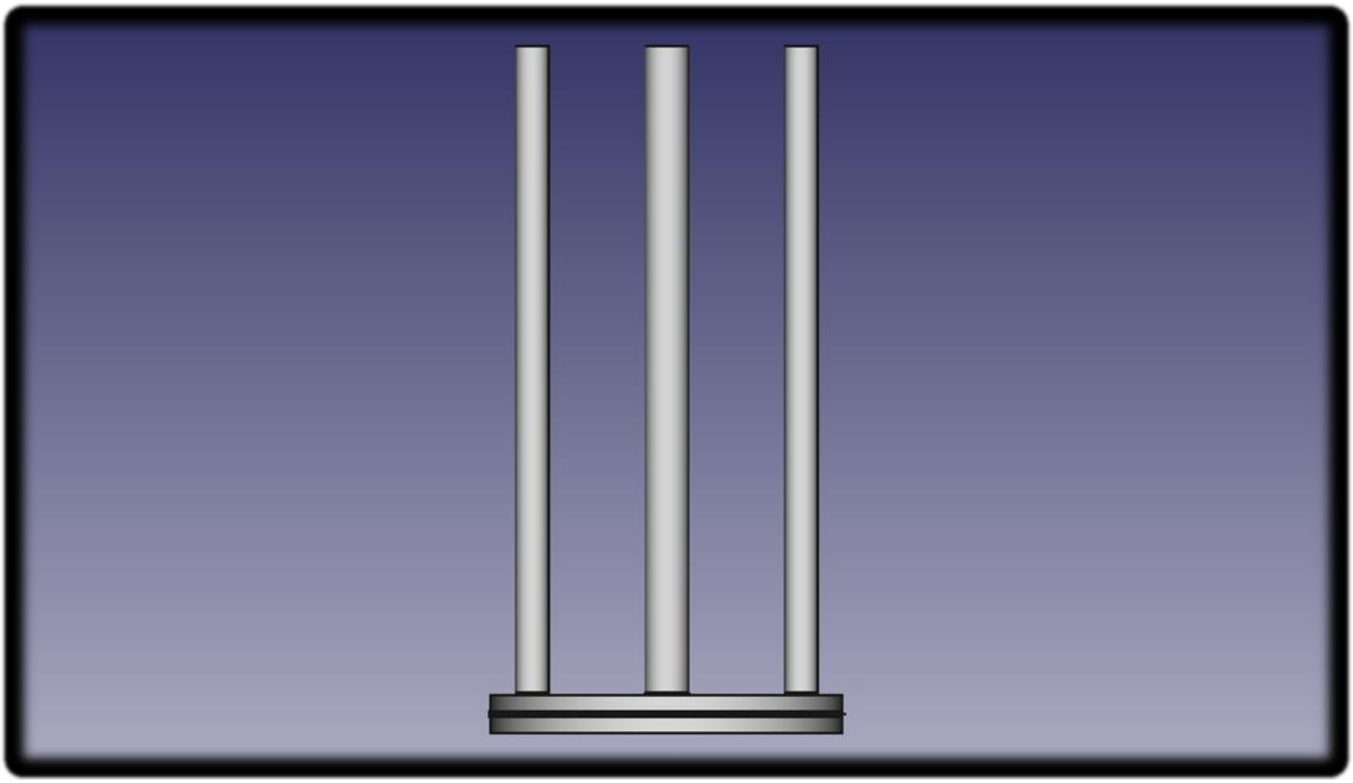


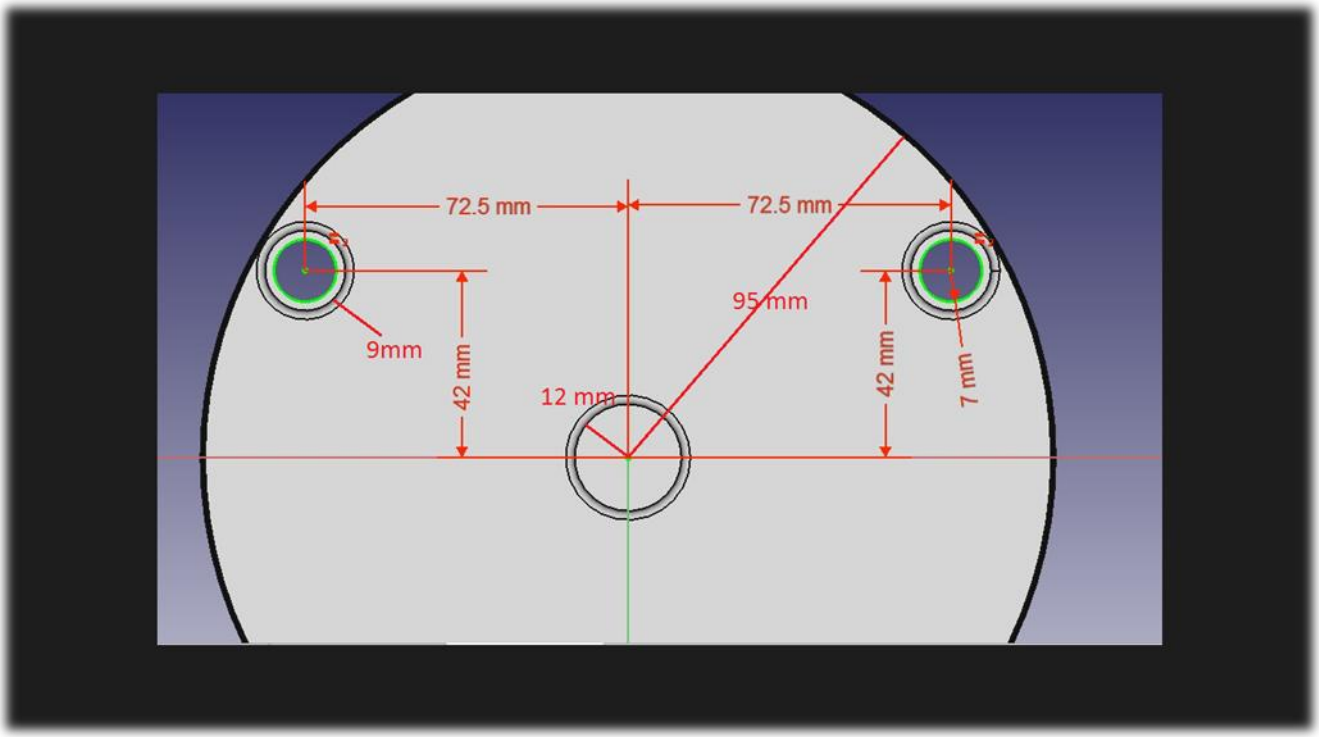


5.4.2.4 *Piston*

Contains a ring to avoid fluid leaks and to let the piston move easily and two tubes where fluid passes through into the piston

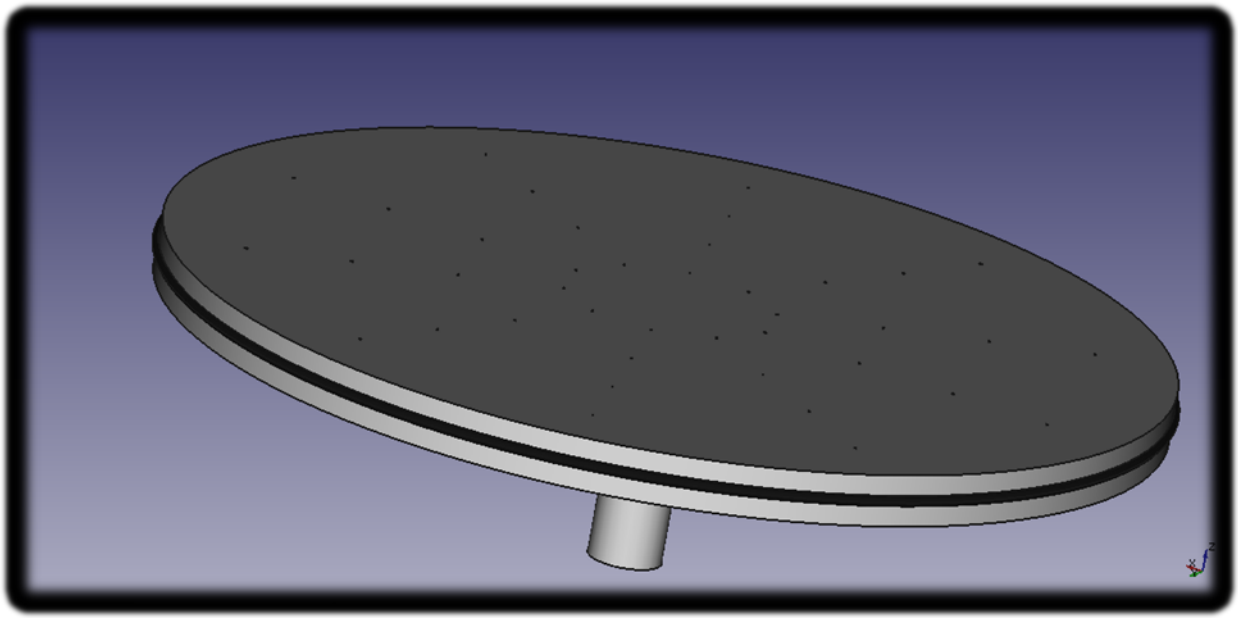


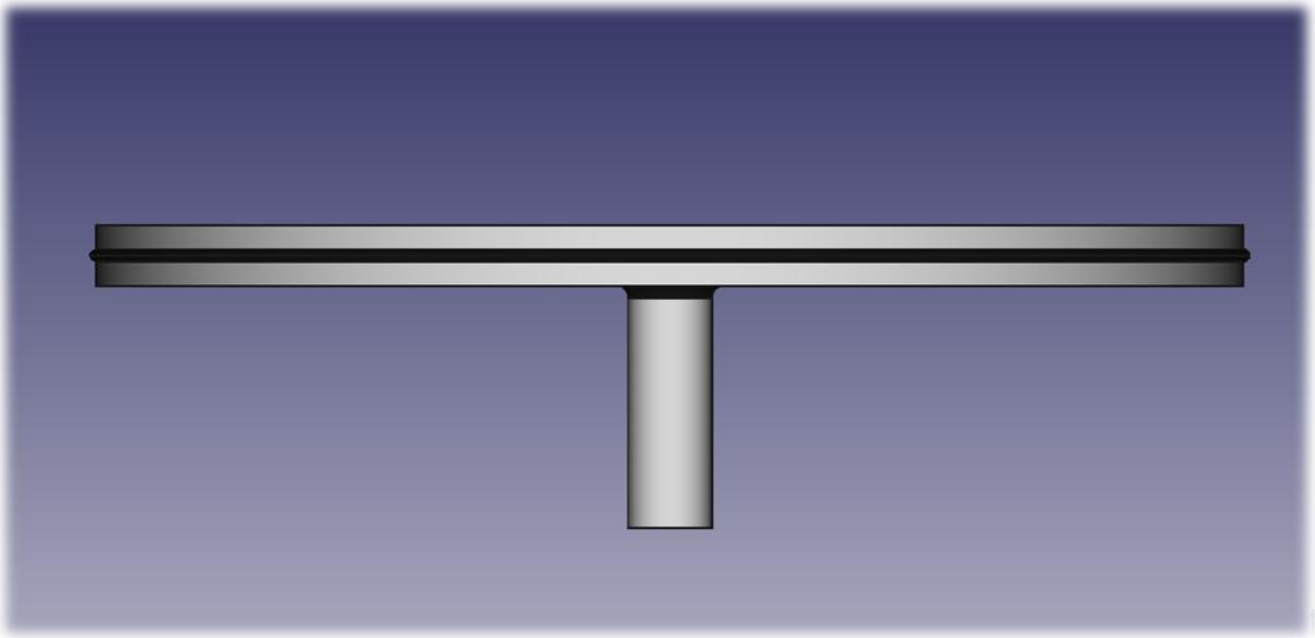


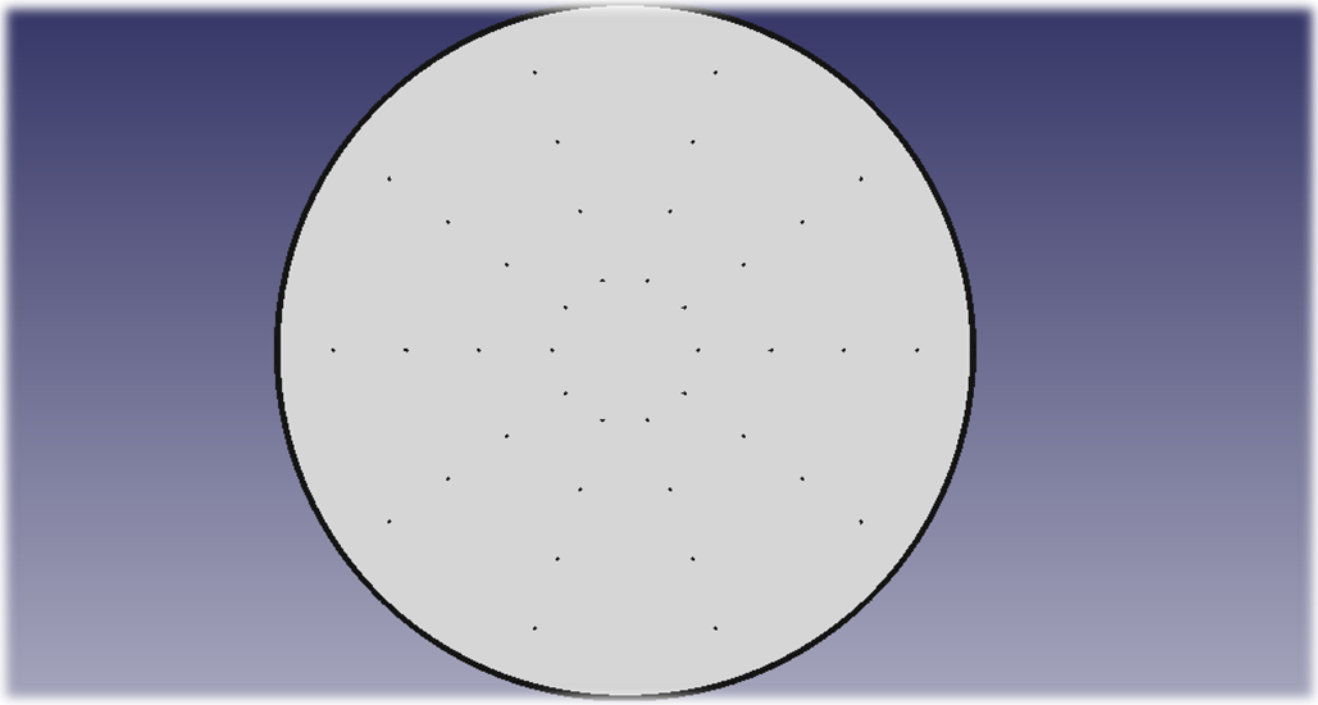


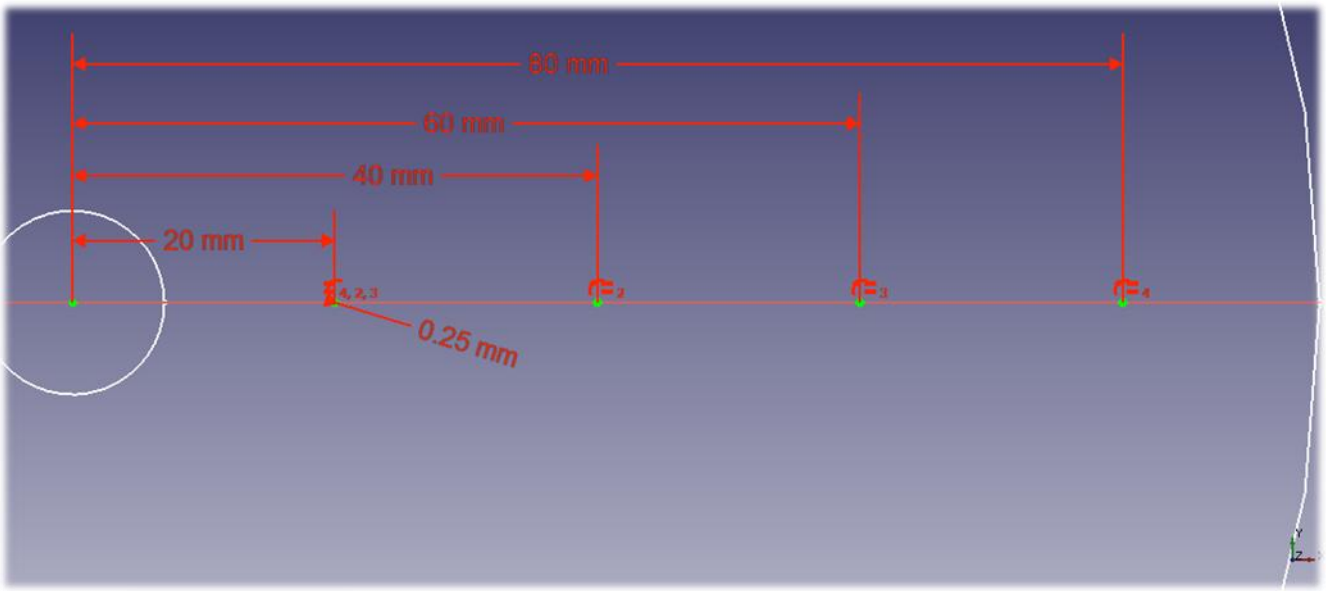
5.4.2.5 *Disc*

Is placed on the bottom of the cylinder. It contains holes of diameter 0.25 mm to separate big molecules from small ones and it contains a ring to avoid leakage



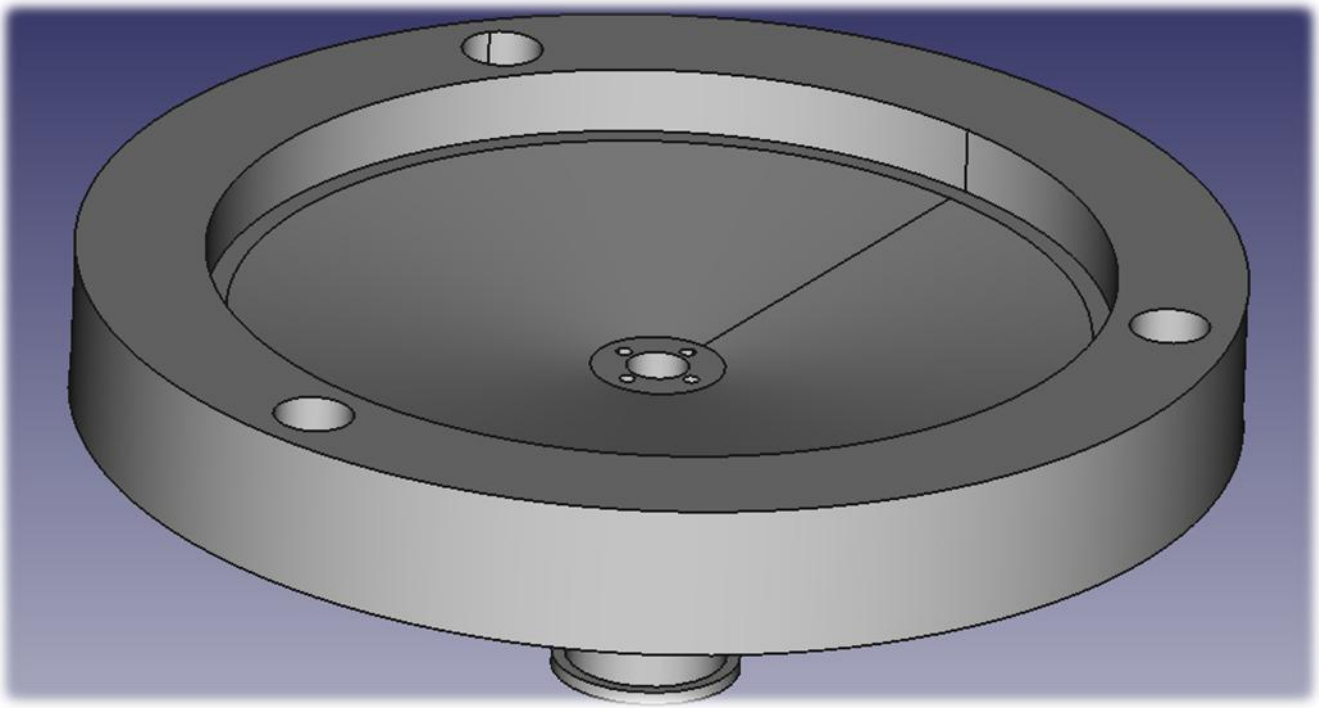


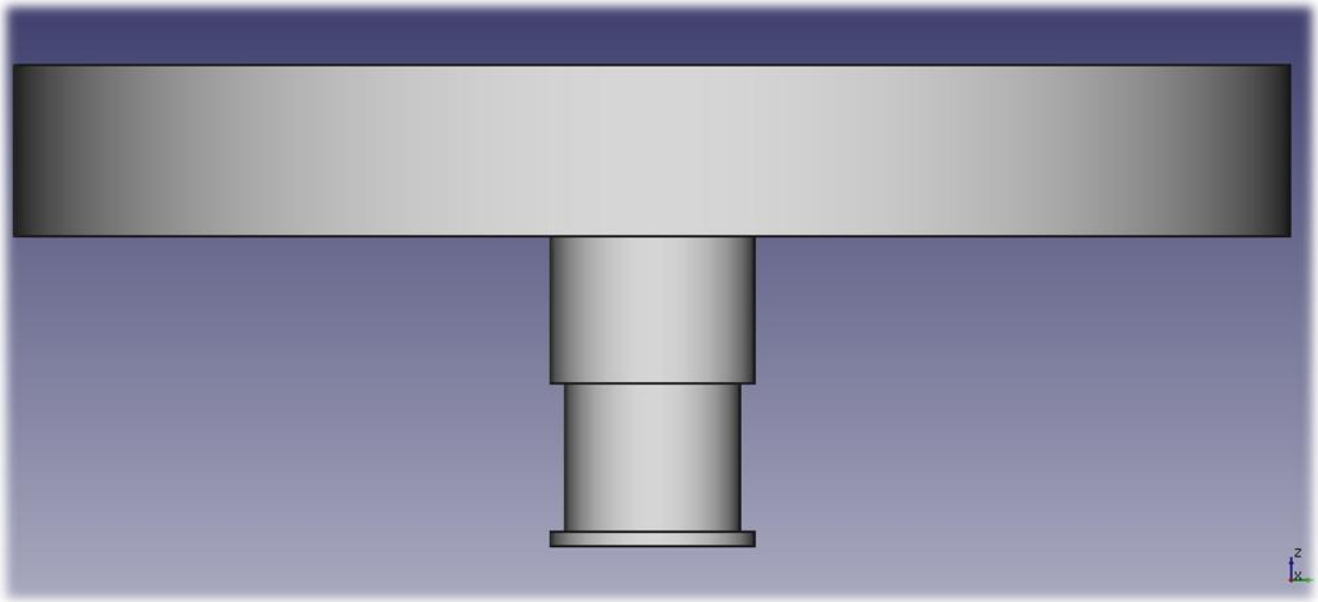


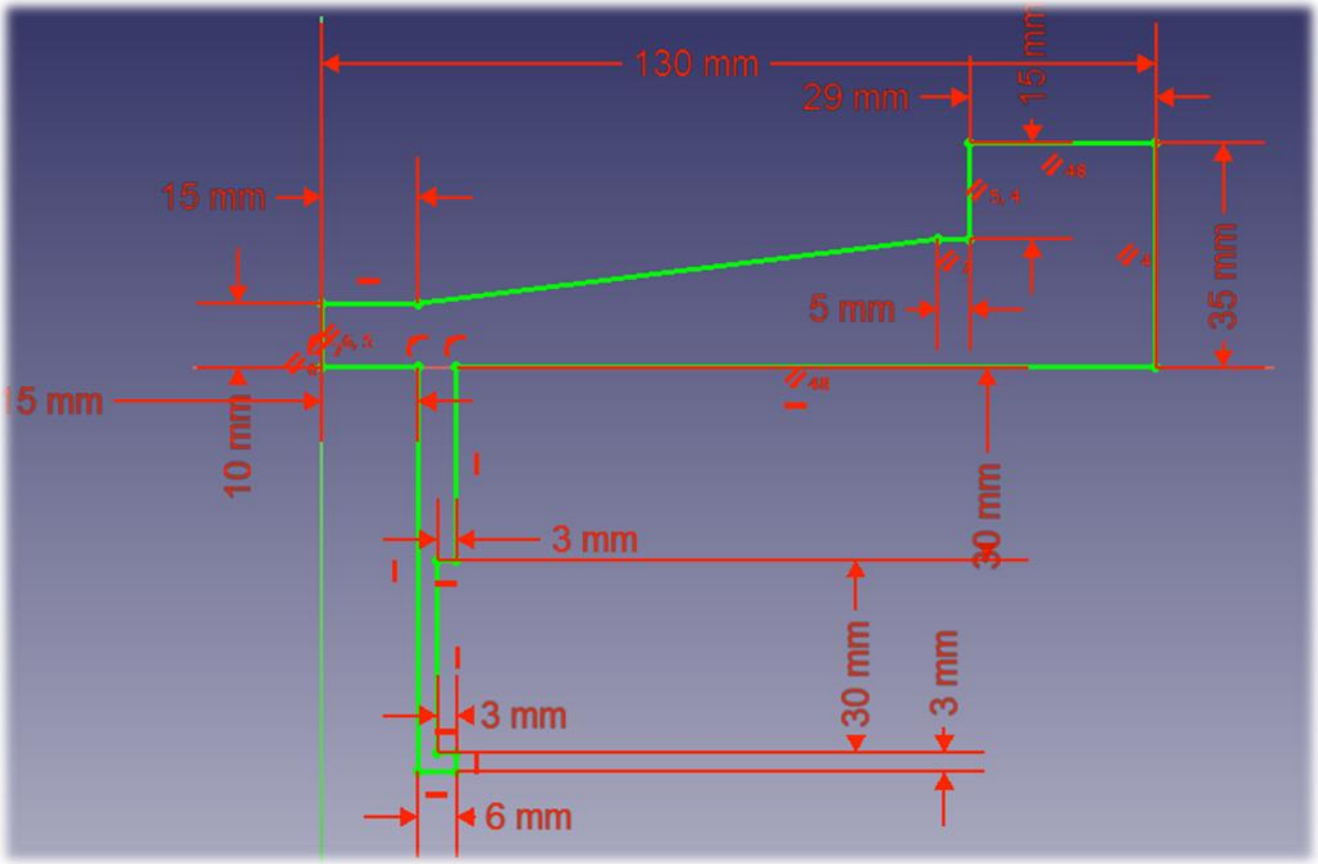


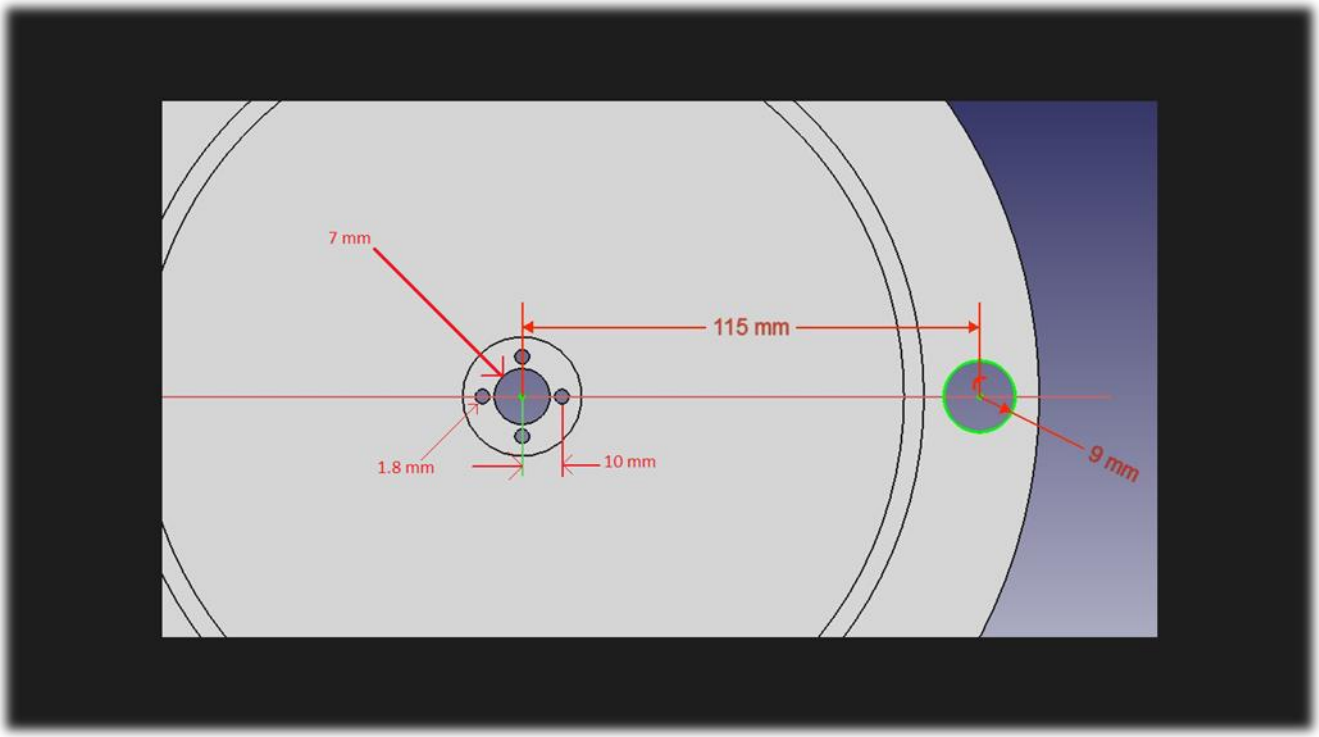
5.4.2.6 *Bottom*

Contains three external holes fixed to the three columns by bolts.it has a conical shape to let the fluid reach to the center



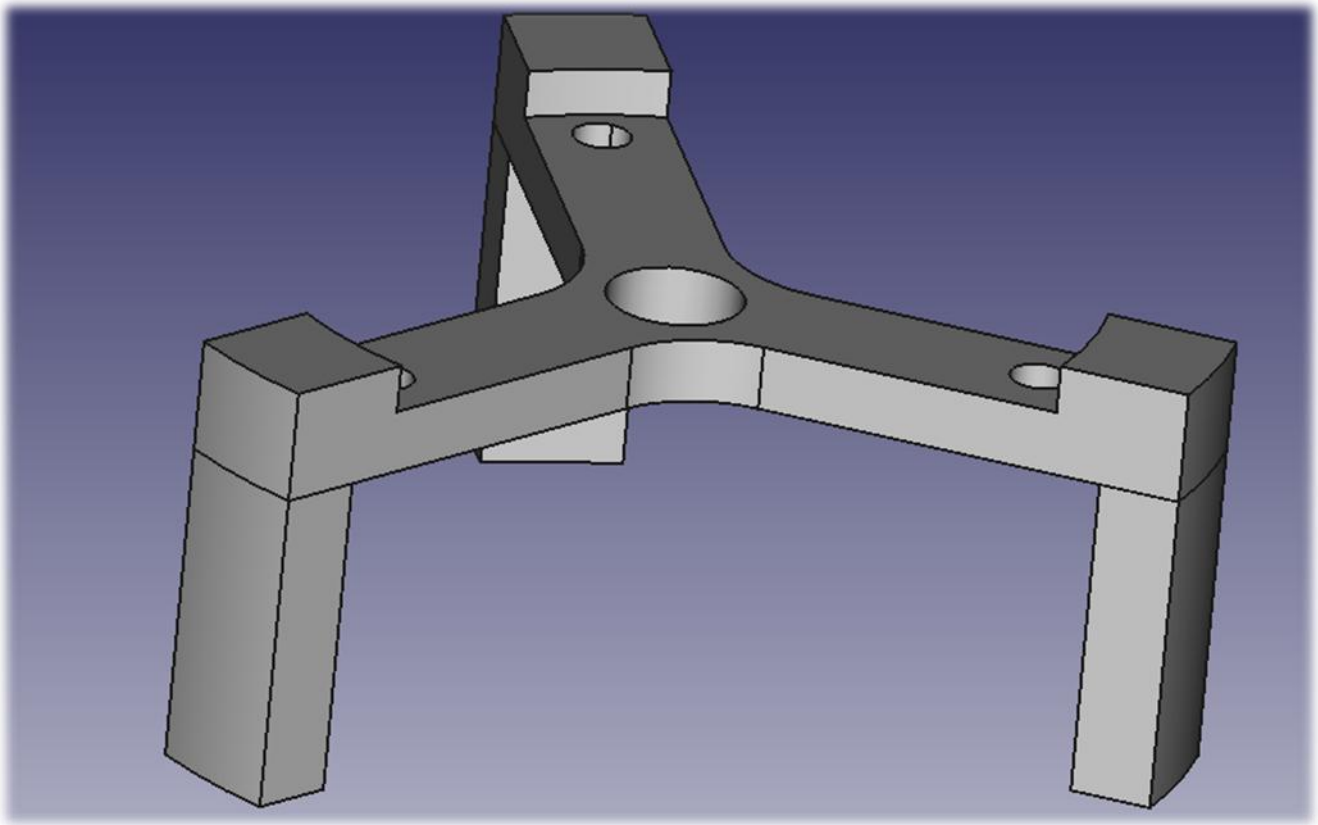


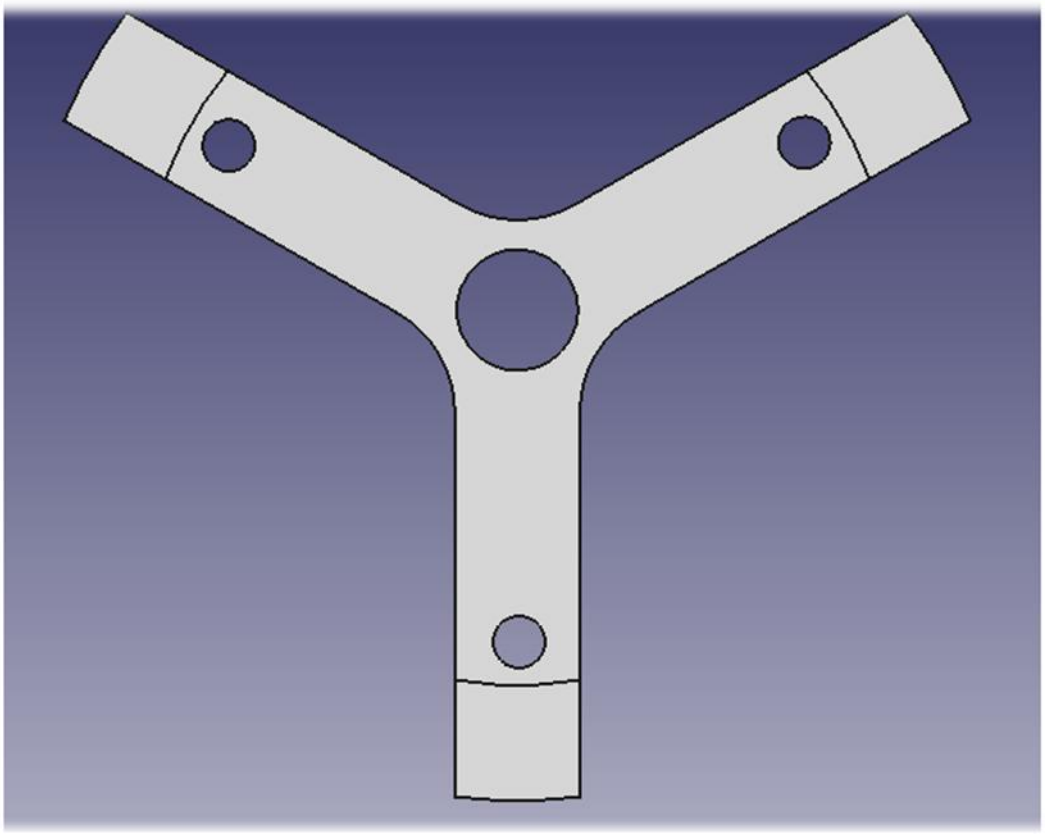

130 mm
29 mm
15 mm
15 mm
35 mm
5 mm
15 mm
10 mm
30 mm
3 mm
30 mm
3 mm
3 mm
6 mm

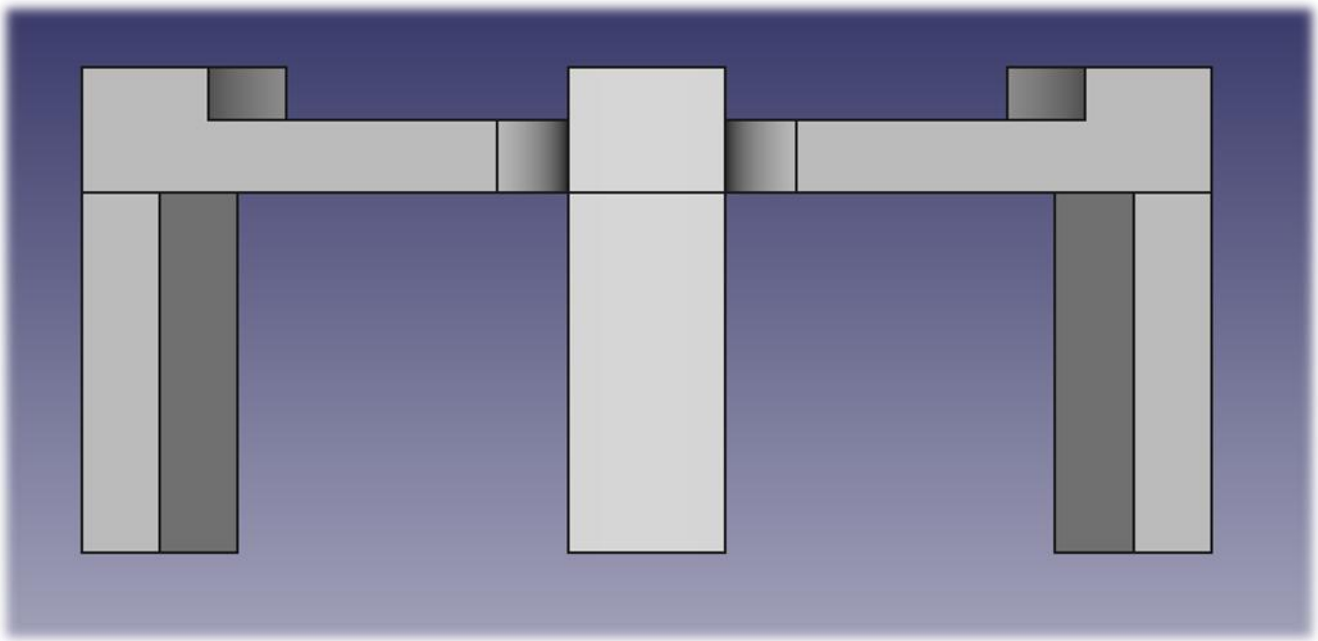


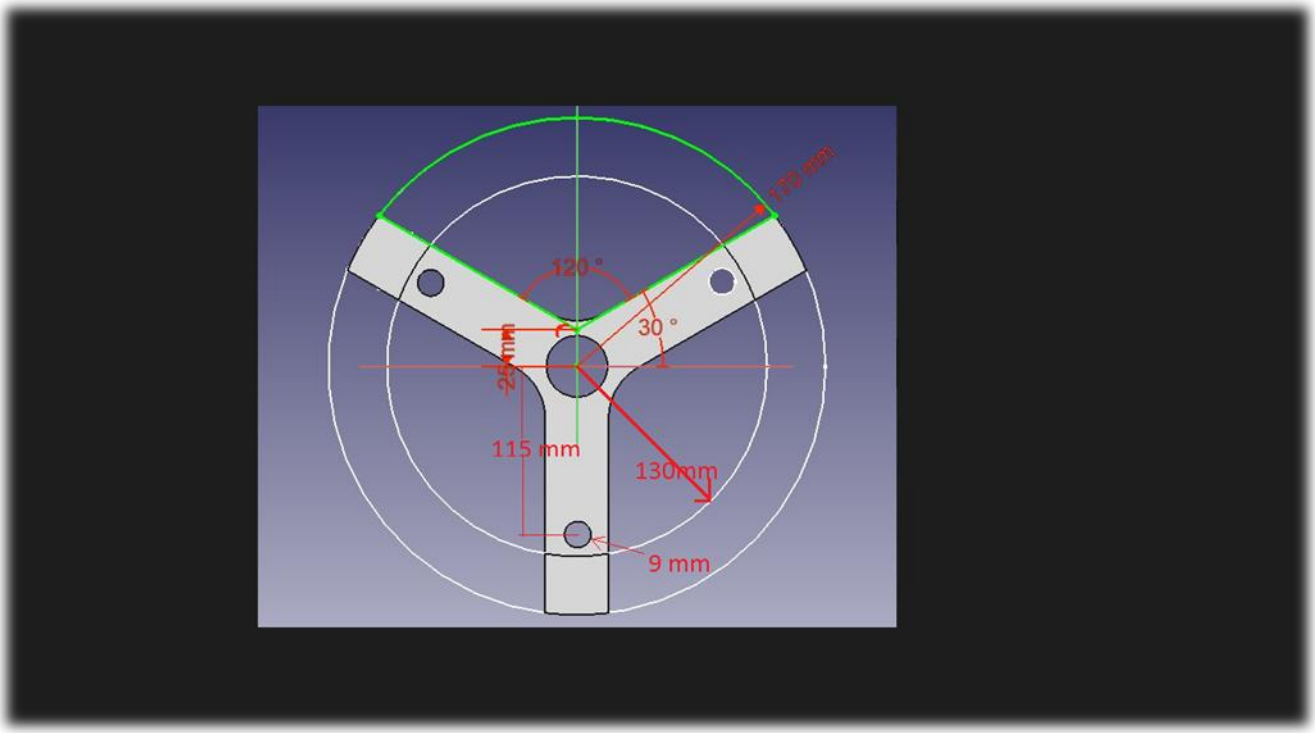
5.4.2.7 Base

Contains three legs and three external holes to fix the columns by bolts



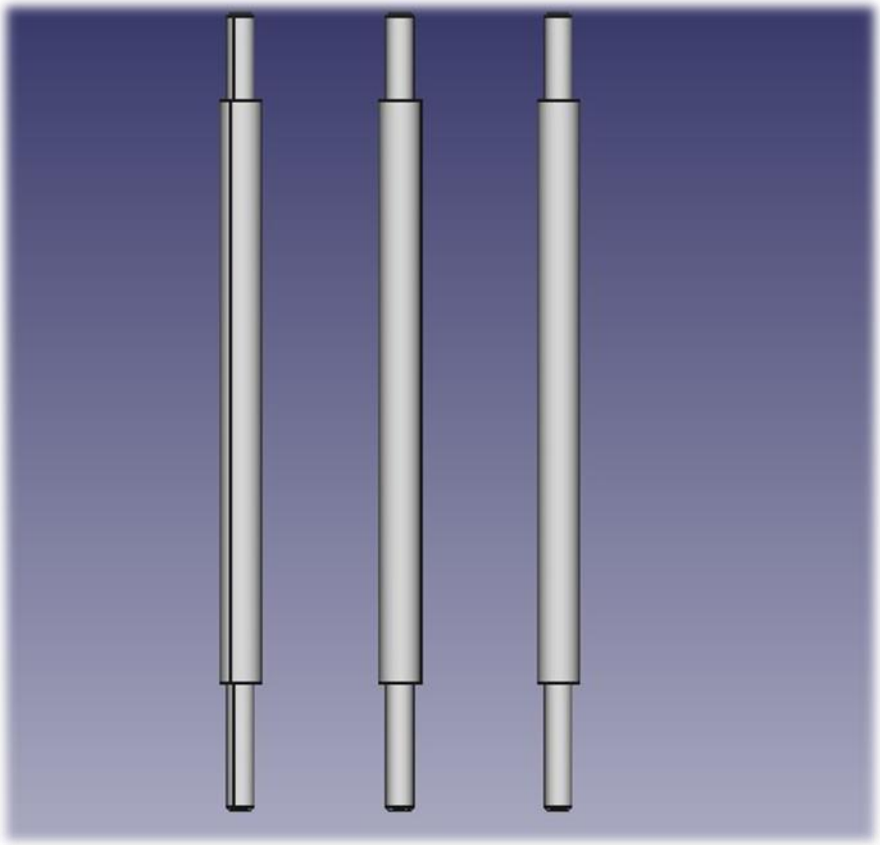


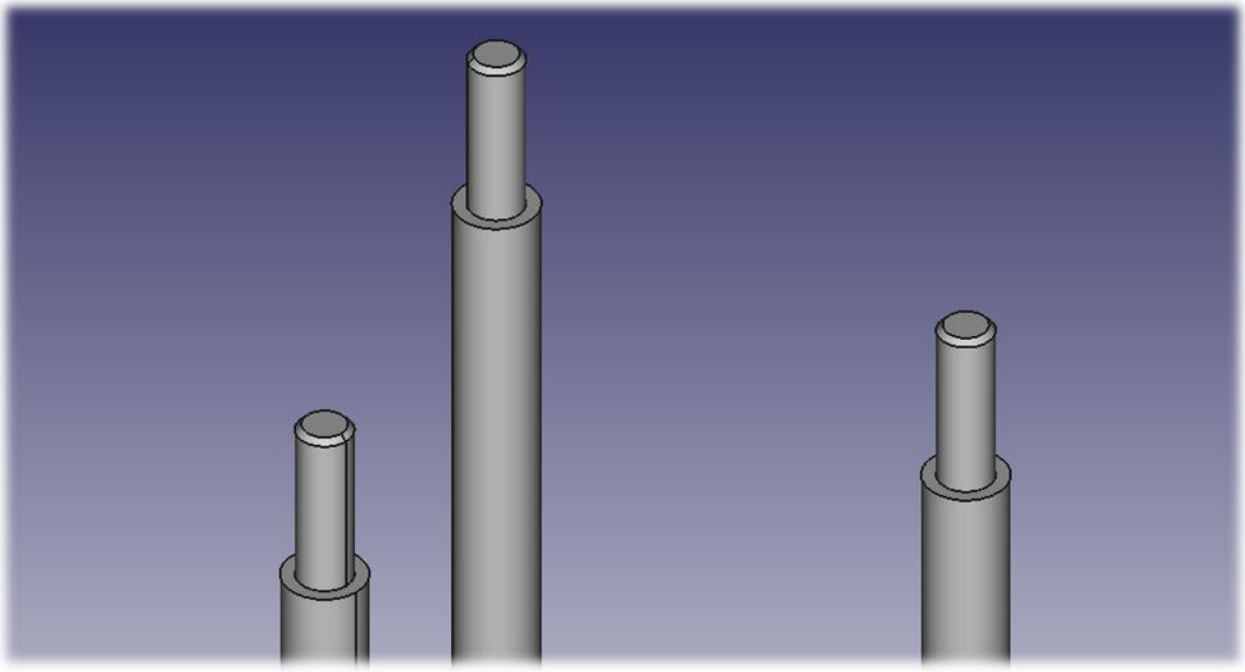


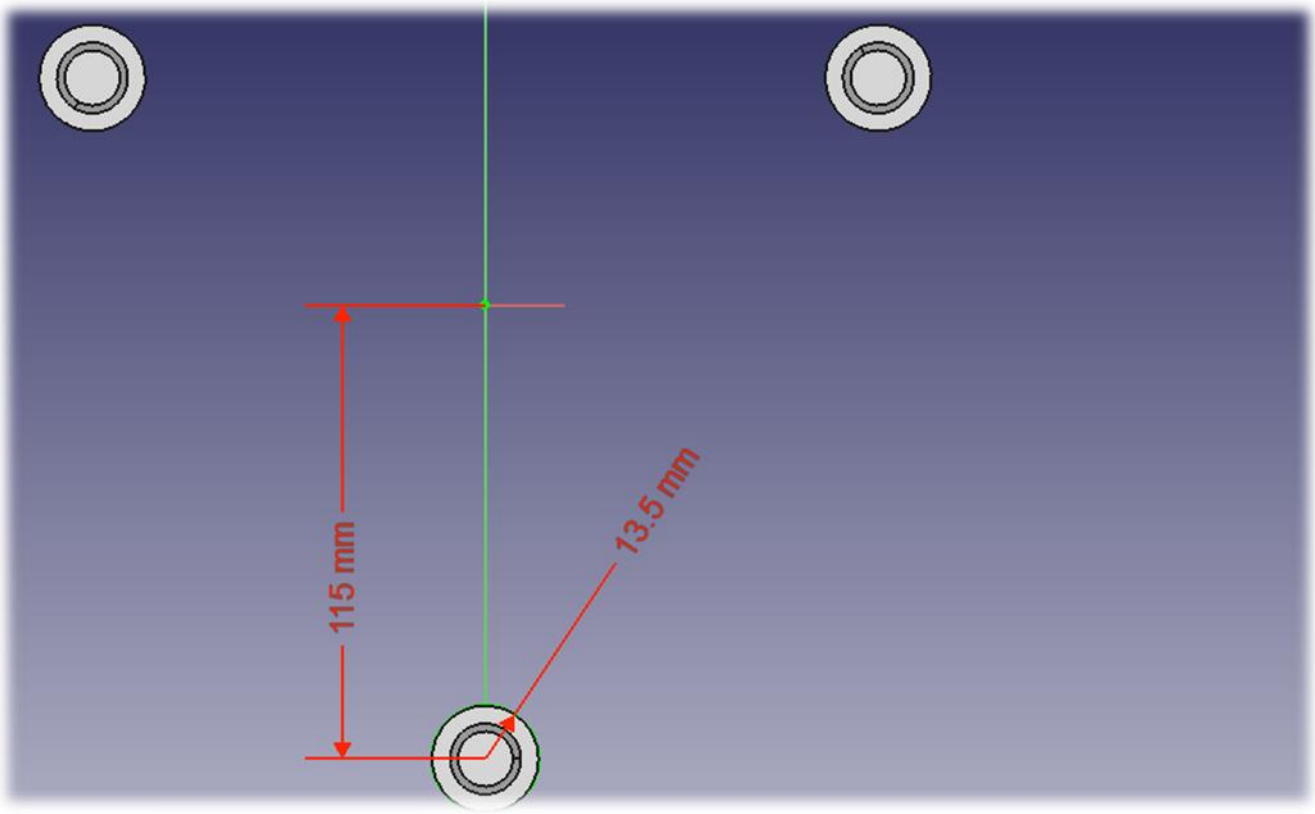


5.4.2.8 Column

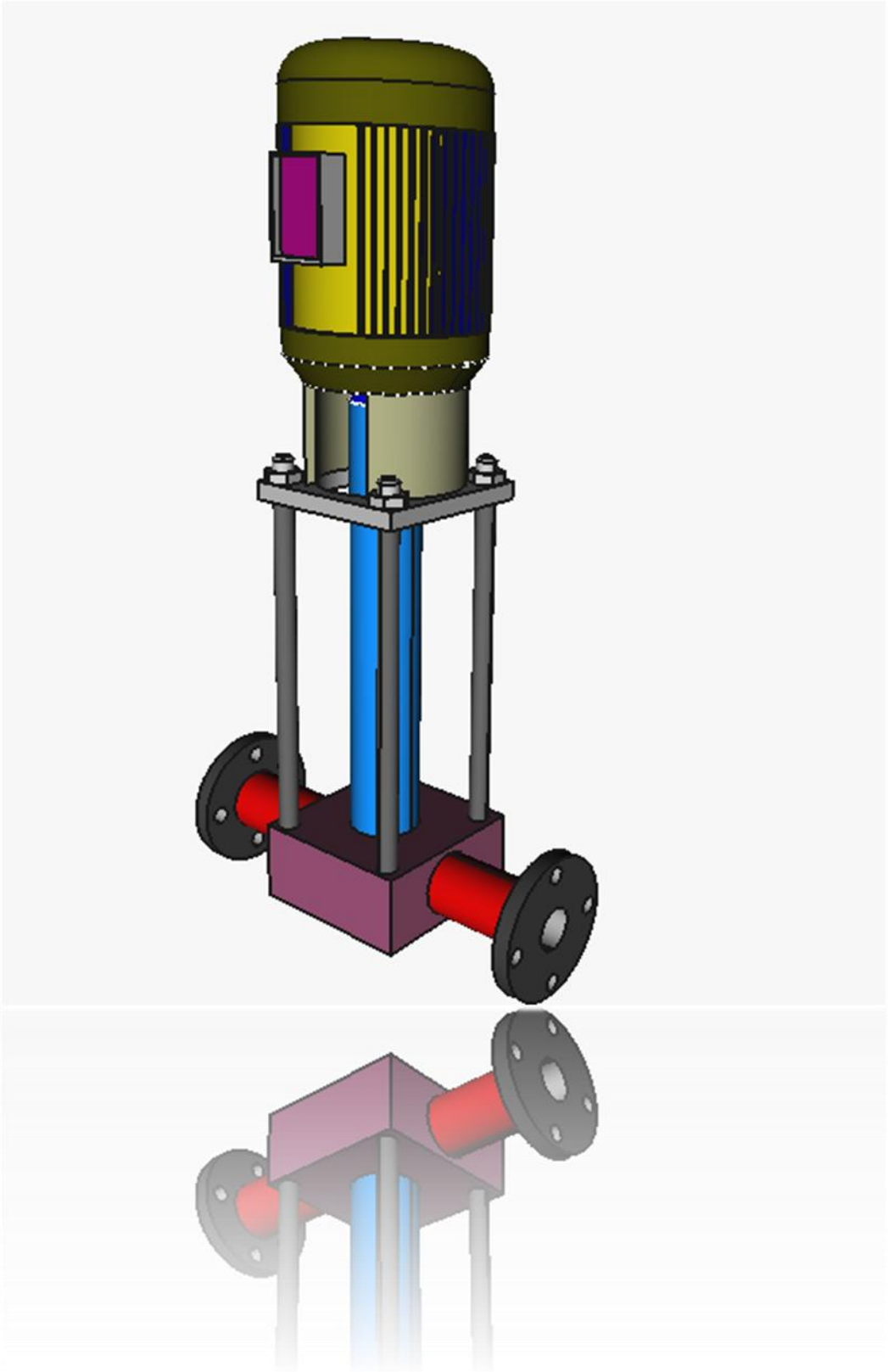
It is the part that connects the top, bottom and base parts together.

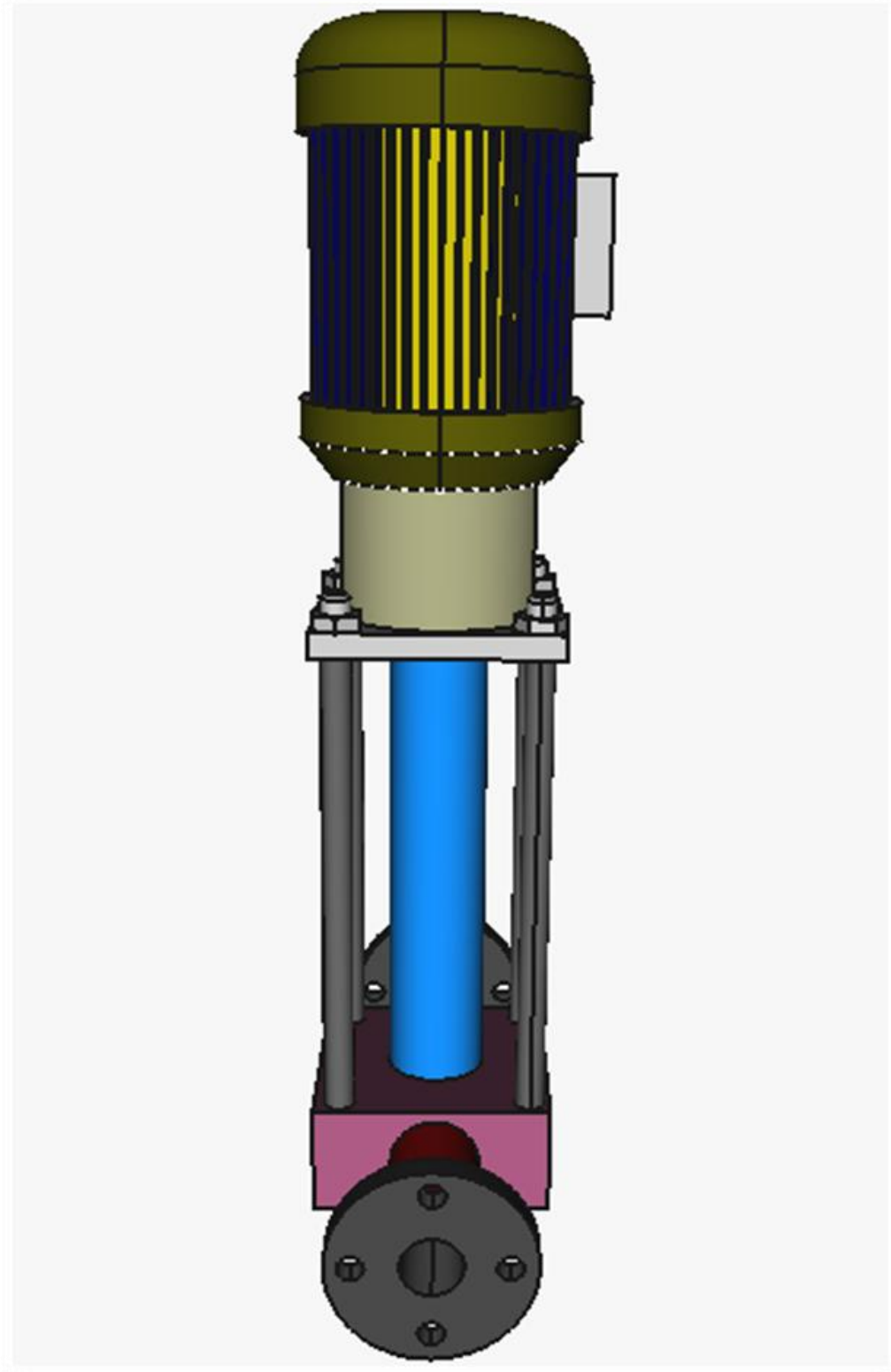


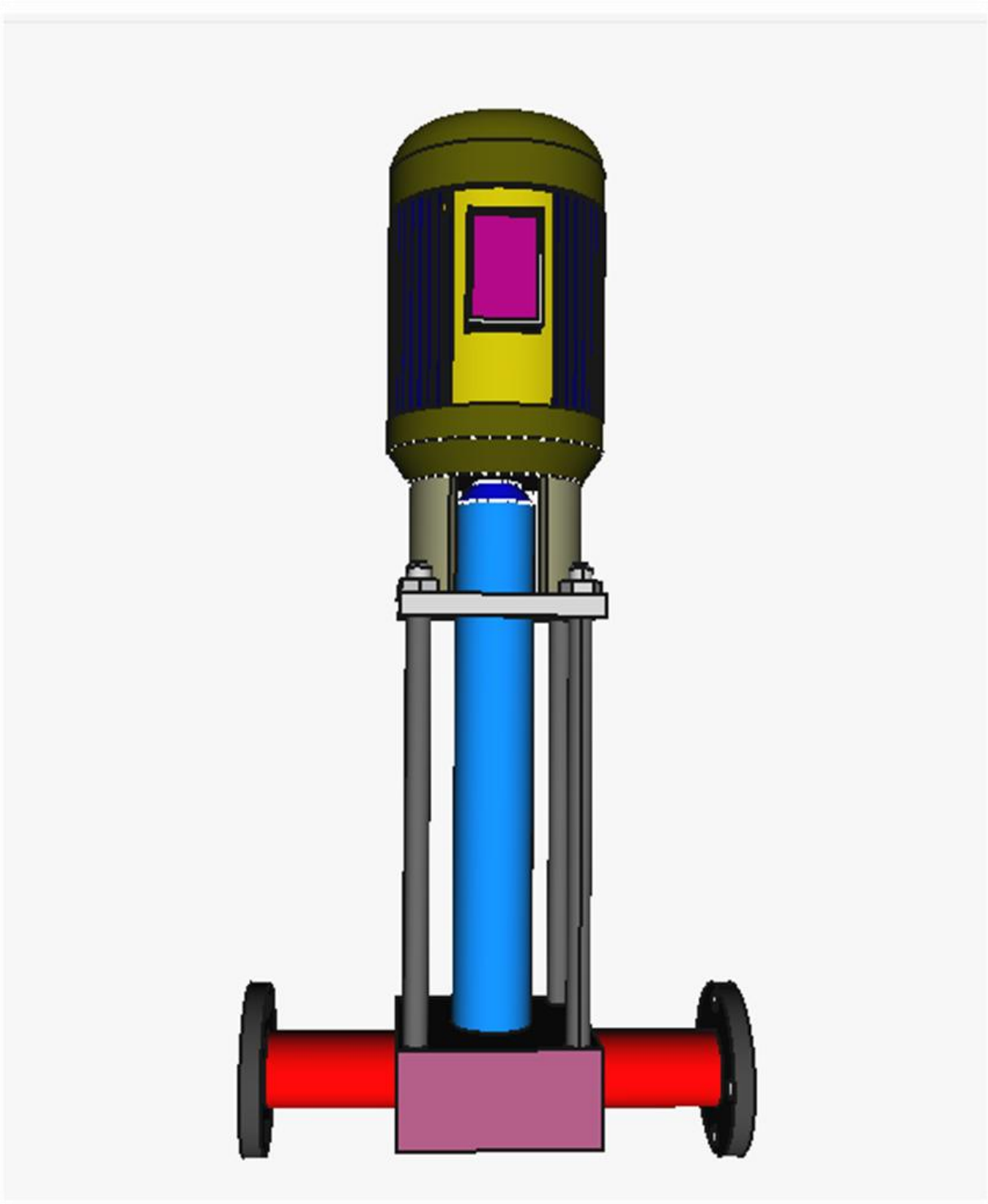



115 mm
13.5 mm

5.5 Demonstration and Modelling of the Pump







5.6 Demonstration and Modelling of the Homogenizer

-Ibrahim Zaaroura-

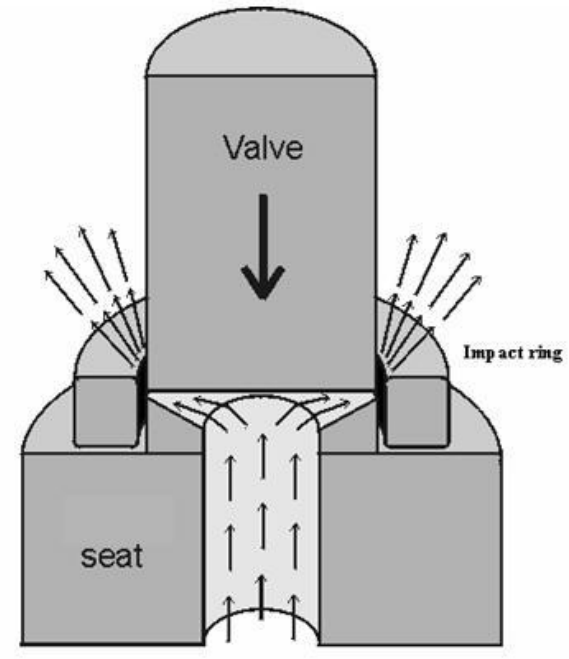
5.6.1 *DEVICE DETAILS & SPECIFICATIONS*

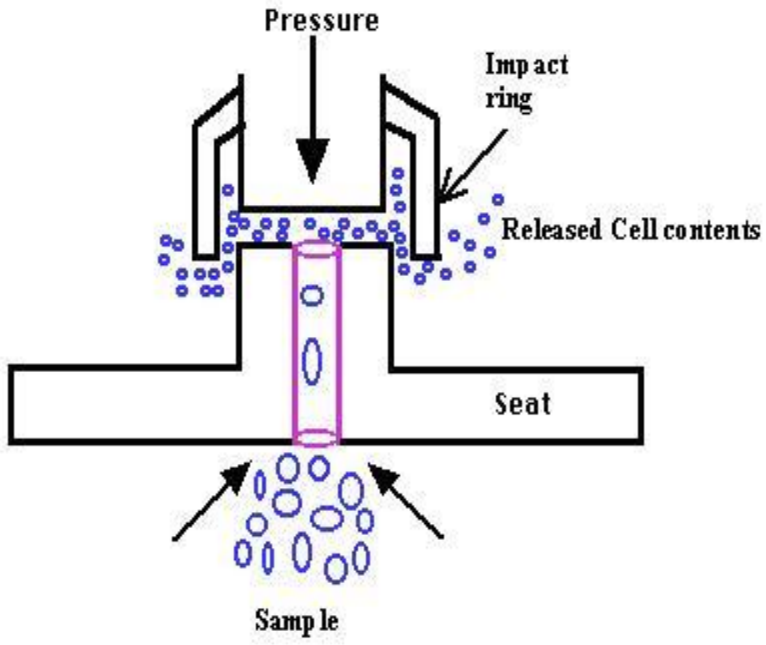
A homogenizer is a piece of laboratory or industrial equipment used for the homogenization of various types of material, such as tissue, plant, food, soil, and many others. Many different models have been developed using various physical technologies for disruption.

Homogenization is a very common sample preparation step prior to the analysis of nucleic acids, proteins, cells, metabolism, pathogens, and many other targets.

5.6.1.1 Working principle

High Pressure Homogenization is a process of increasing the consistency of a product by means of dispersions. The product is displaced under the generation of high pressure and is forced through homogenizing valve gap. Cavitation's turbulence and sheer force break the product into particles of size less than 1 microns.





Acting on high-pressure principle, the instrument is used for extruding cells, especially suitable for smashing thick-wall cells, germs and denser solution samples. Having no noise, less temperature rise and no 28 metal ion contamination, it has wide application of such research fields as protein study, nucleic acid extraction, cell disruption in genetic engineering labs of colleges, scientific research institutes and pharmaceutical factories.

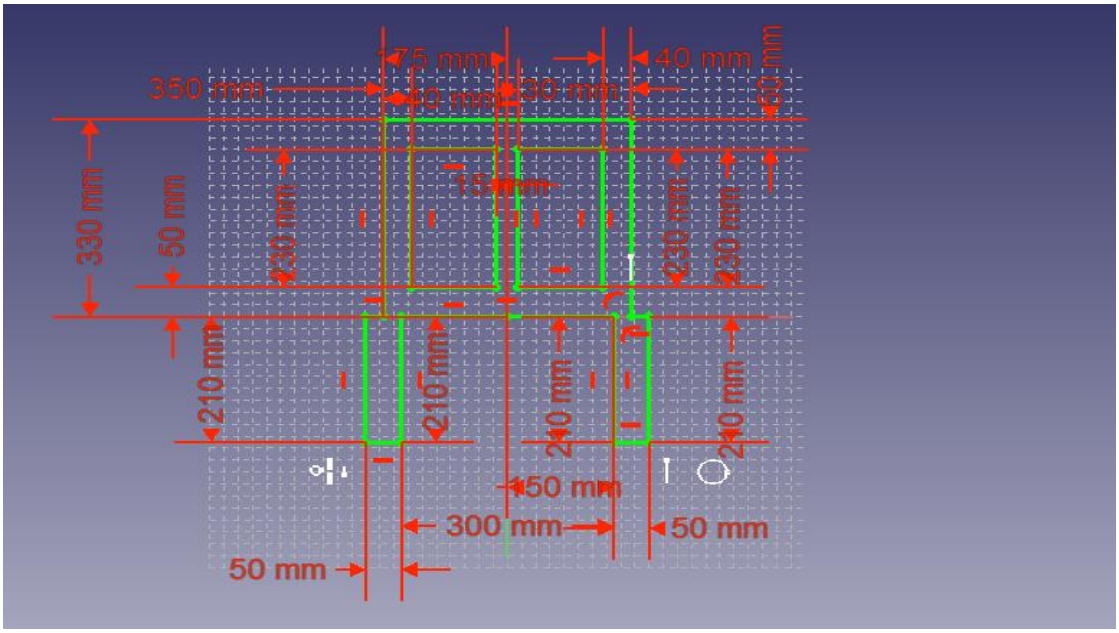
5.6.1.2 Specifications

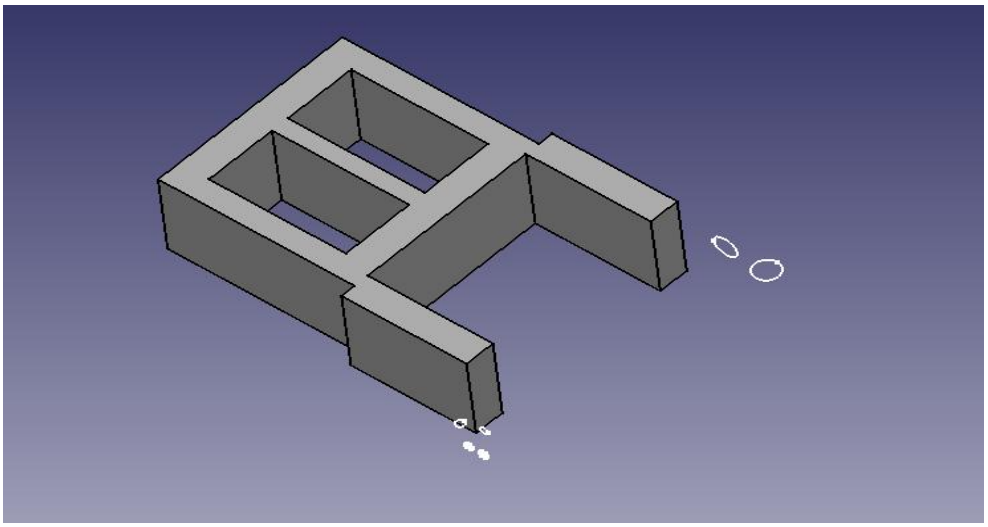
MODEL	JG-IA	CAPACITY	50/ML SCIENTIFIC RESEARCH
VOLTAGE	380 VAC	MAX OPERATING PRESSURE	256 MPA
PRESSURE DEVICE	HYDRAULIC SYSYTEM	MAX PRESSURE STROKE	170 MM
SAMPLE TUBE	Ø25 MM STAINLESS	PRESSURE PLATE SPEED	6.8 MM/S
DIMENSIONS	800×910×810	LARGEST VOLUME SAMPLE	50ML/TIMES

5.6.2 *PART DIMENSIONS & DESIGN*

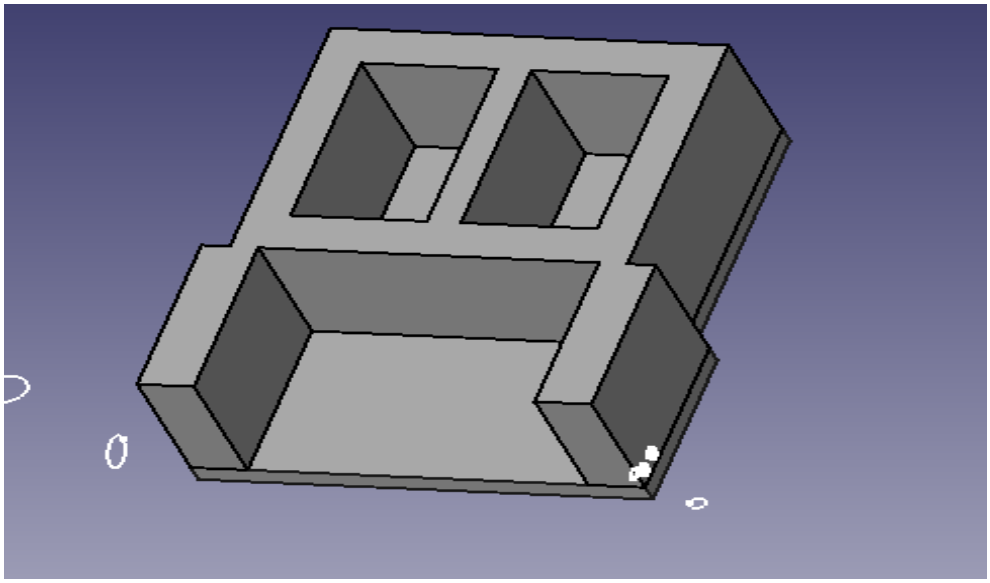
5.6.2.1 BASE PART

-box (sketch) with thickness =175 mm that contains the crankshaft and pistons to operate the process (breaking cells).



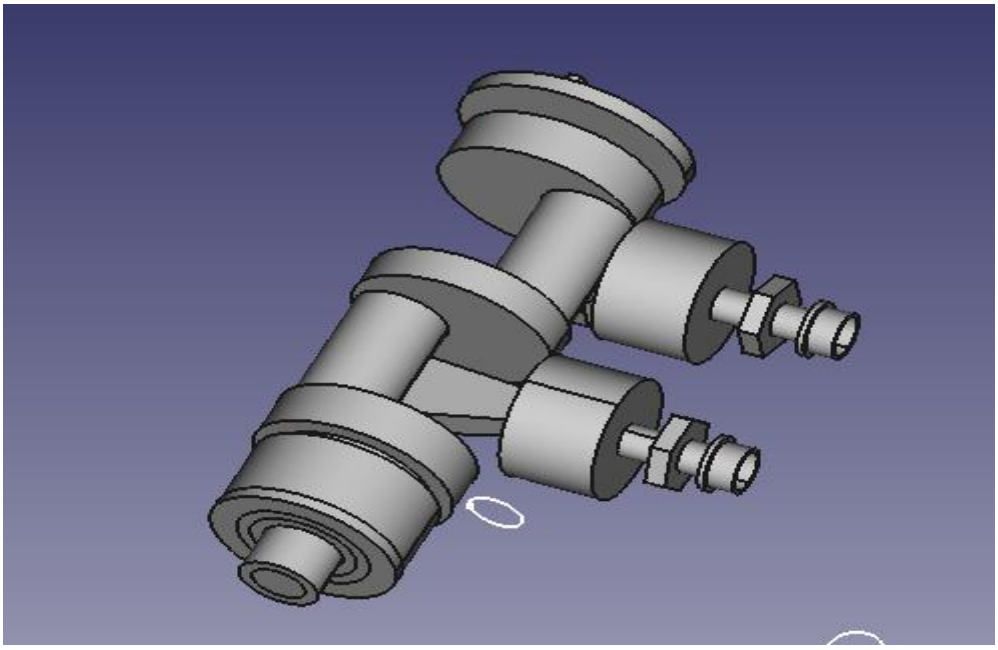


-Cover for Base with thickness =25mm

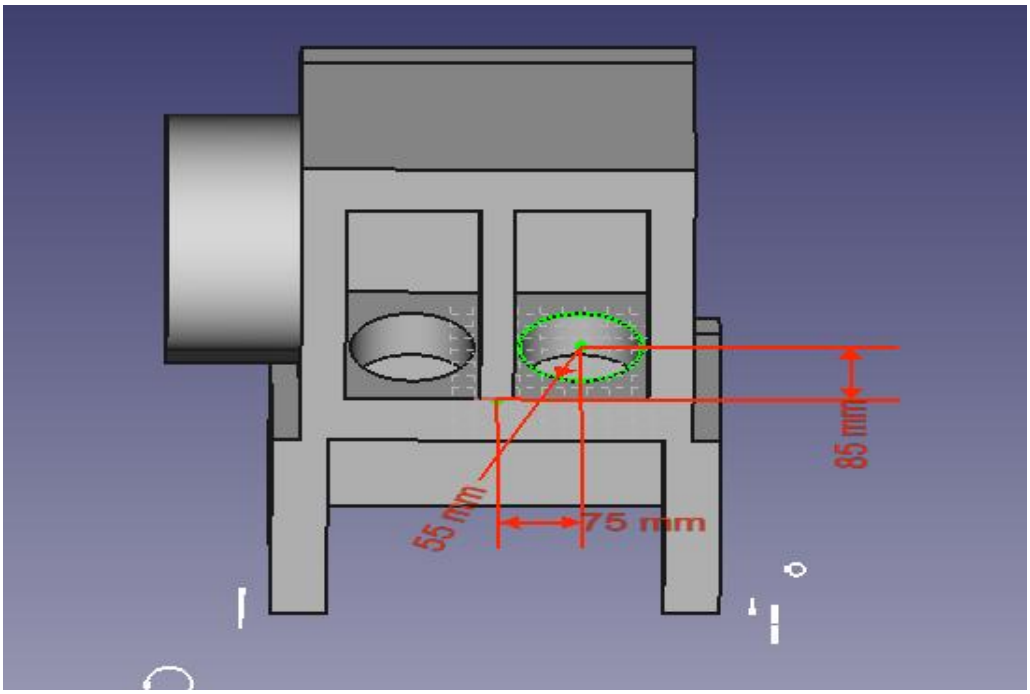


5.6.2.2 CRANK SHAFT AND PISTONS

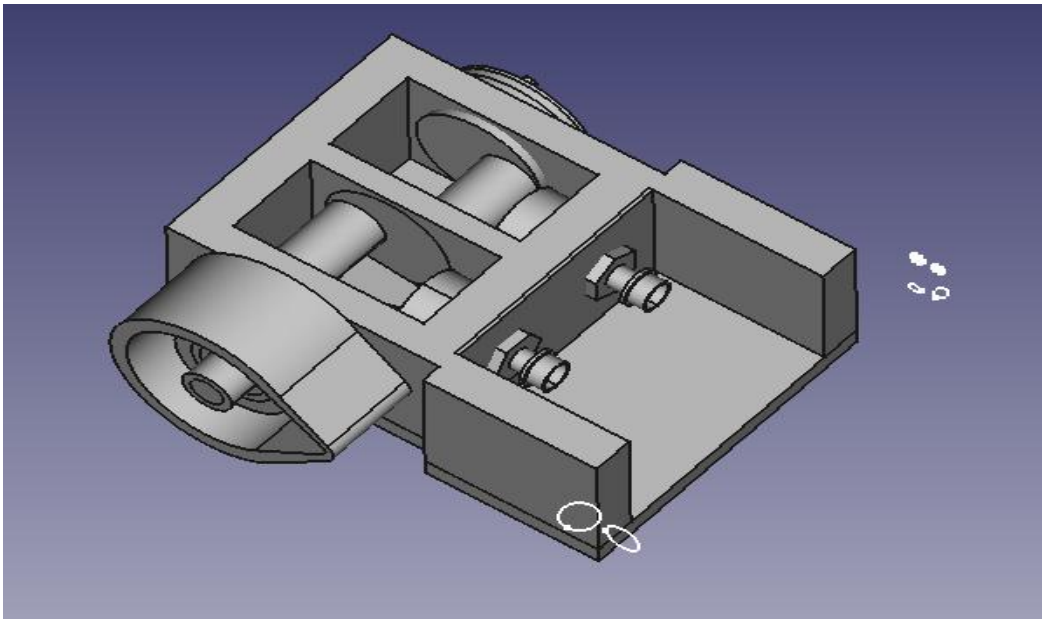
-Taking into account the dimensions of the shaft and pistons according to the box dimensions.



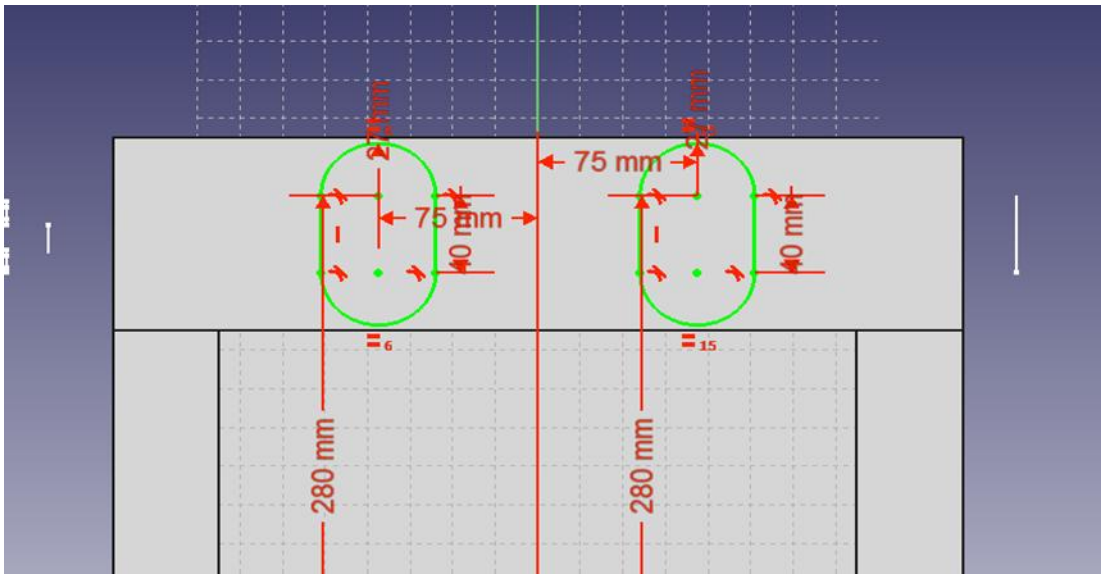
5.6.2.3 Pocket circle with R= 55mm to pass the cylinder of the pistons

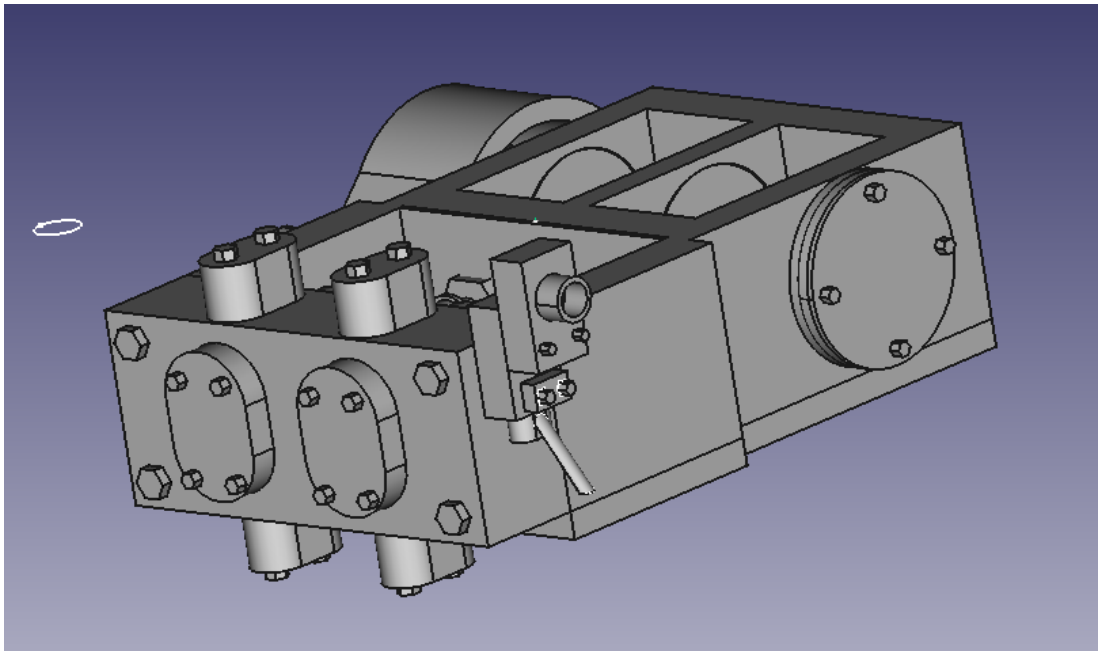


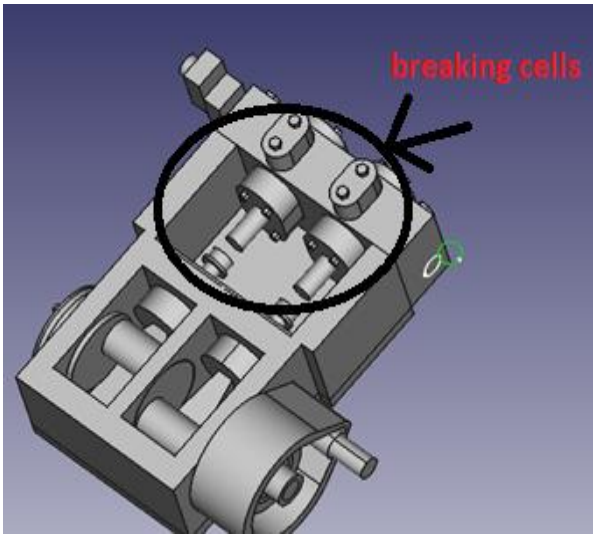
5.6.2.4 With same sketch for pistons and box bind the two parts together.

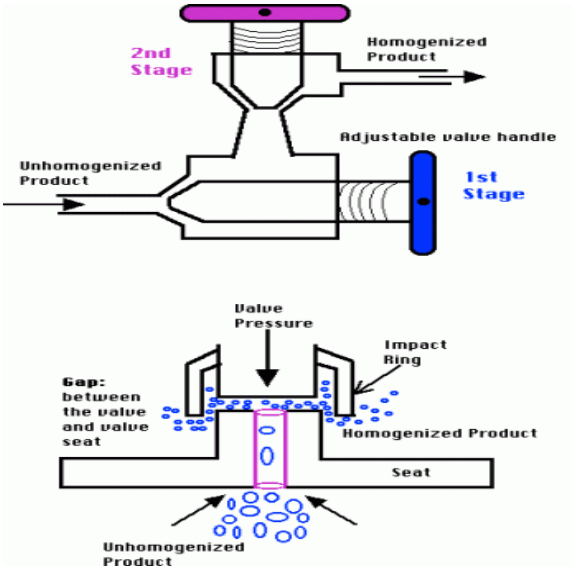


5.6.2.5 Draw the head of the machine where the cell will break due to the pistons and valve.

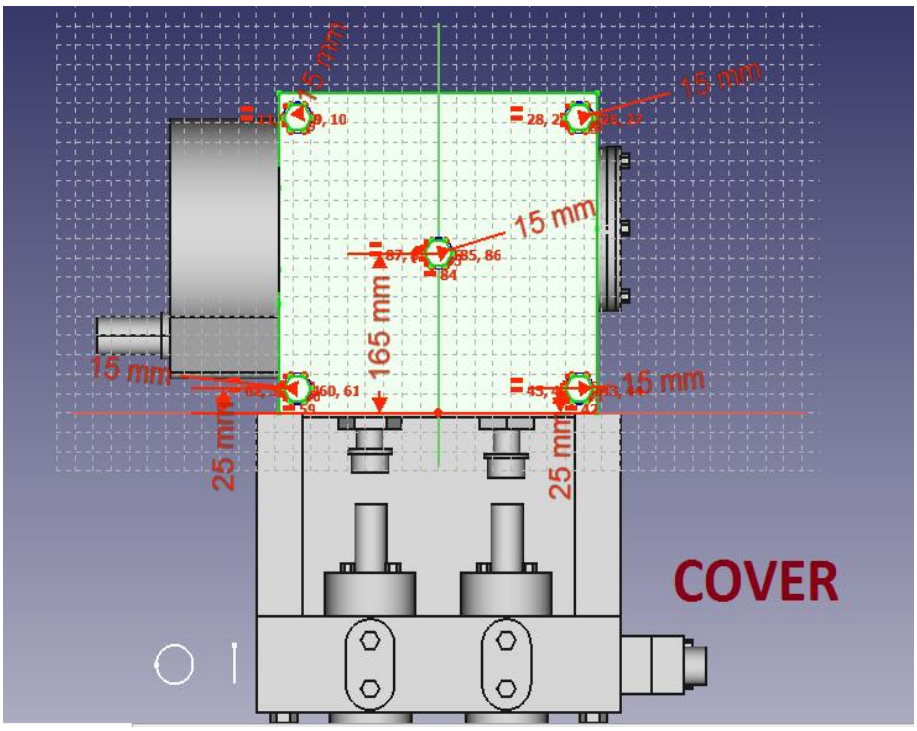


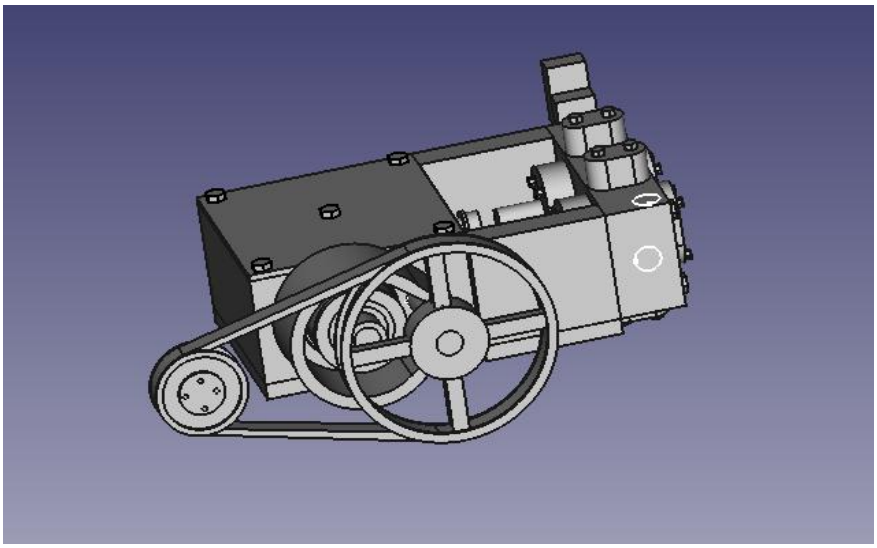






5.6.2.6 Putting the cover over the box and fixed it with bolts and added the shaft comes out from crank shaft and connecting to pulley, this pulley also connected to other one with belt.

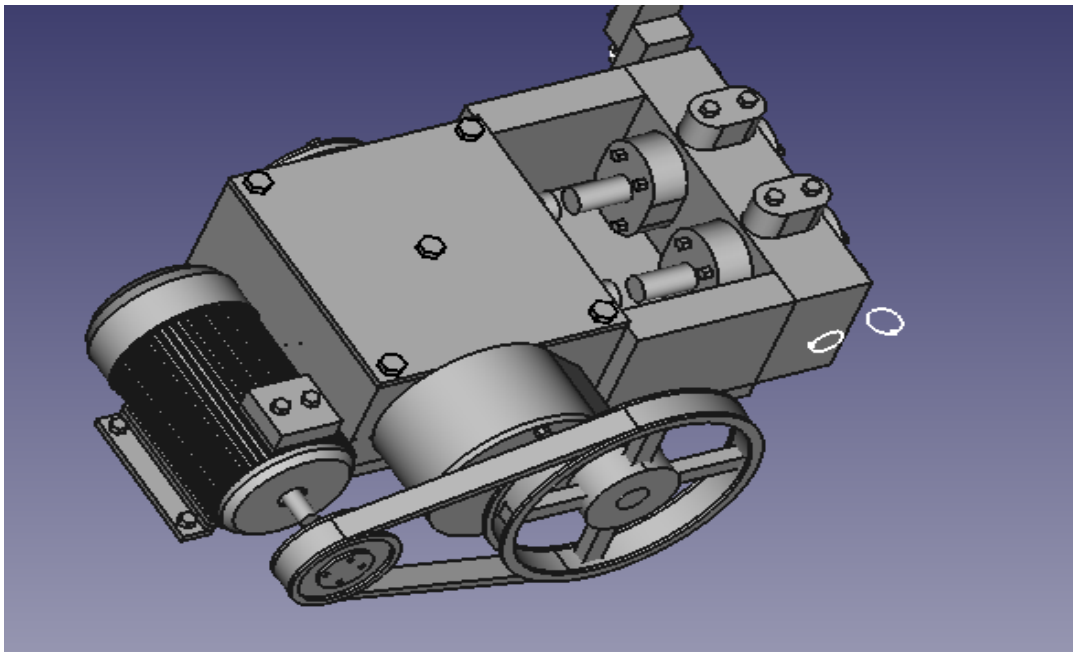




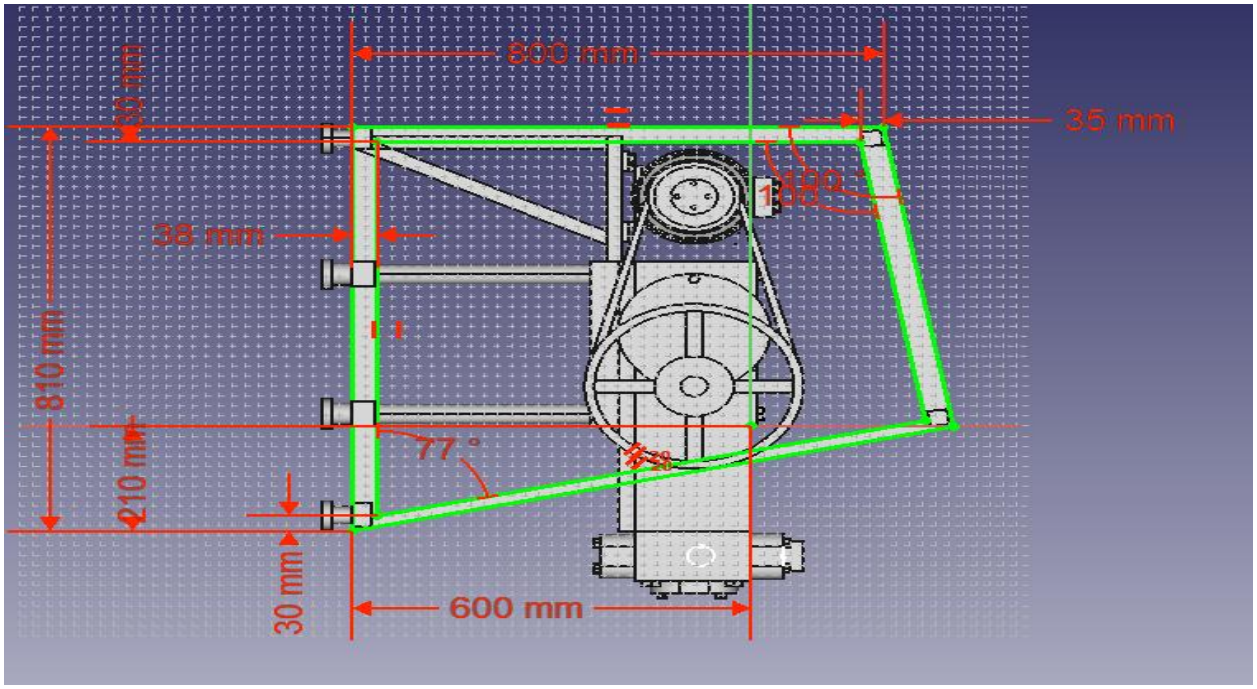
-belt thickness =10 mm

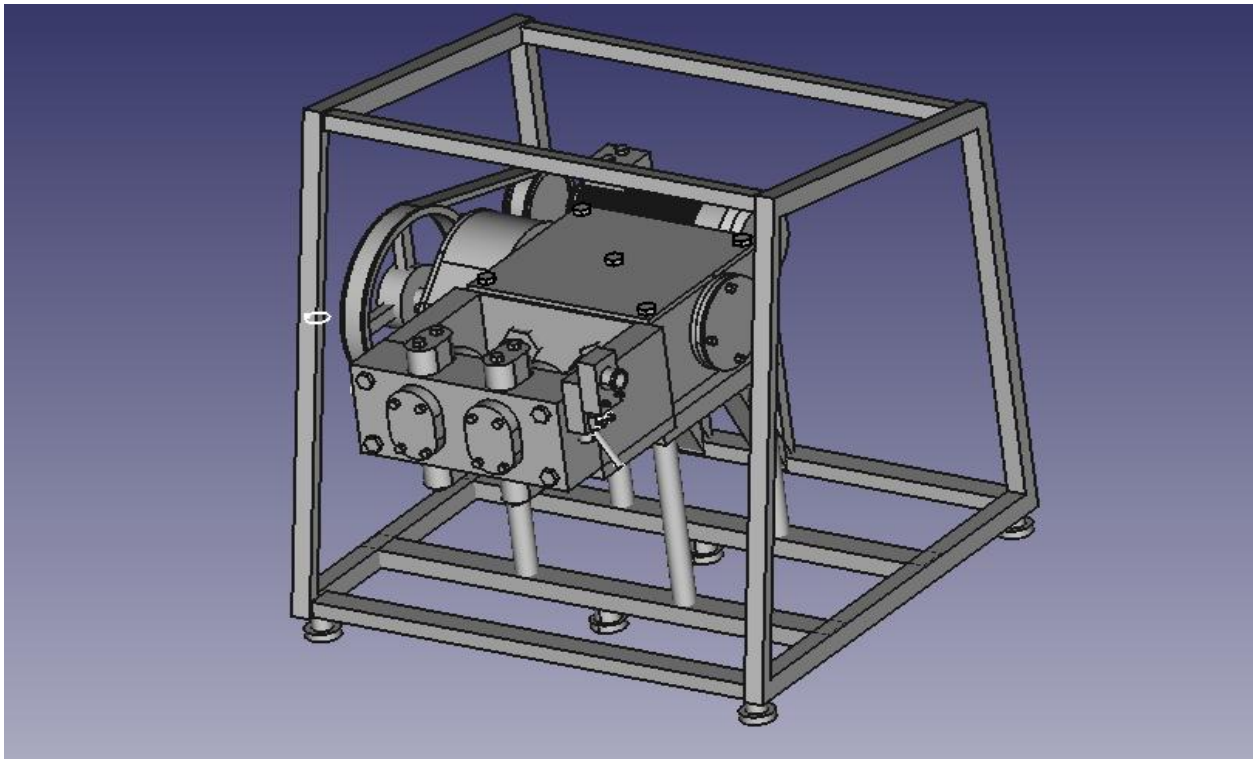
-Large circle with R=165 mm and small R=70 mm

5.6.2.7 Select a motor drive and connected to the small pulley to drive the second and the pistons start work

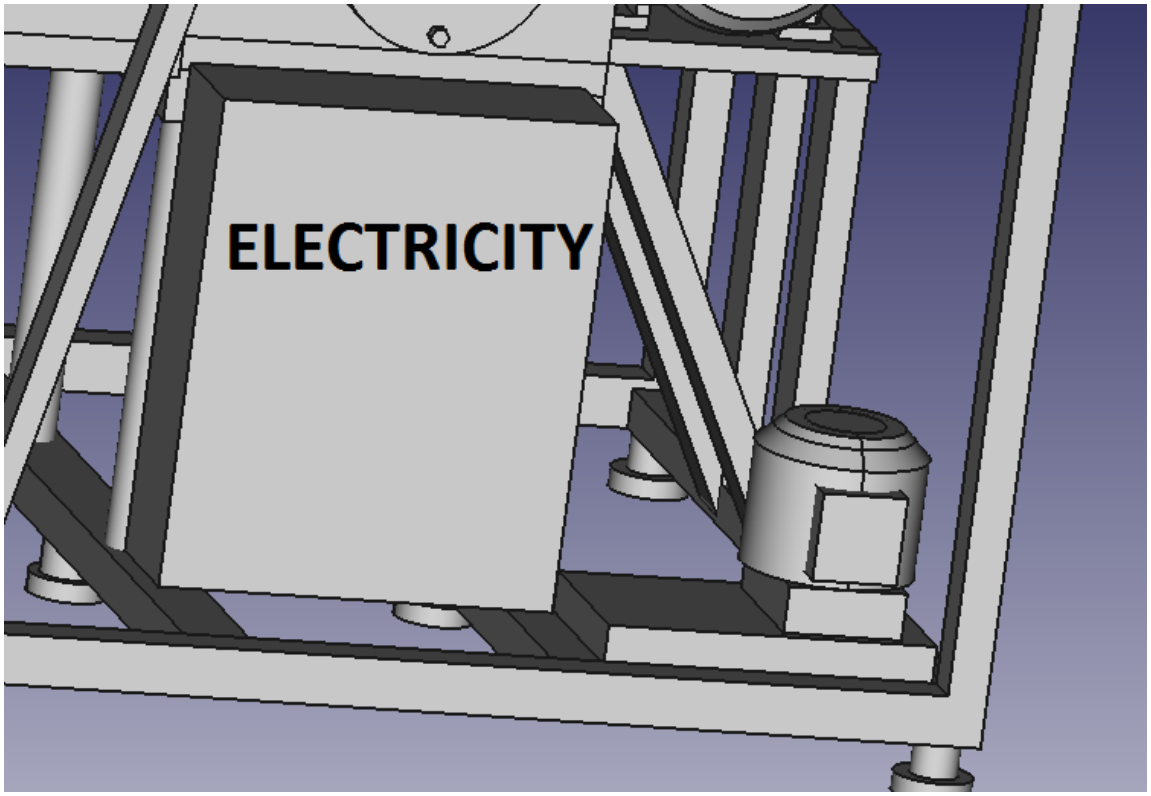


5.6.2.8 Drawing the boundaries of the machine and the basement for the box

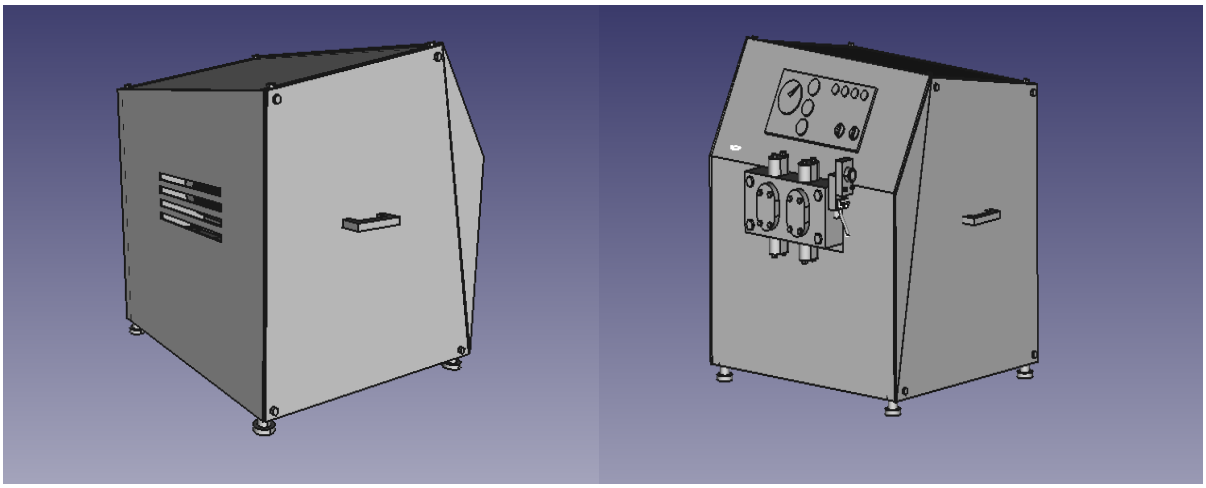




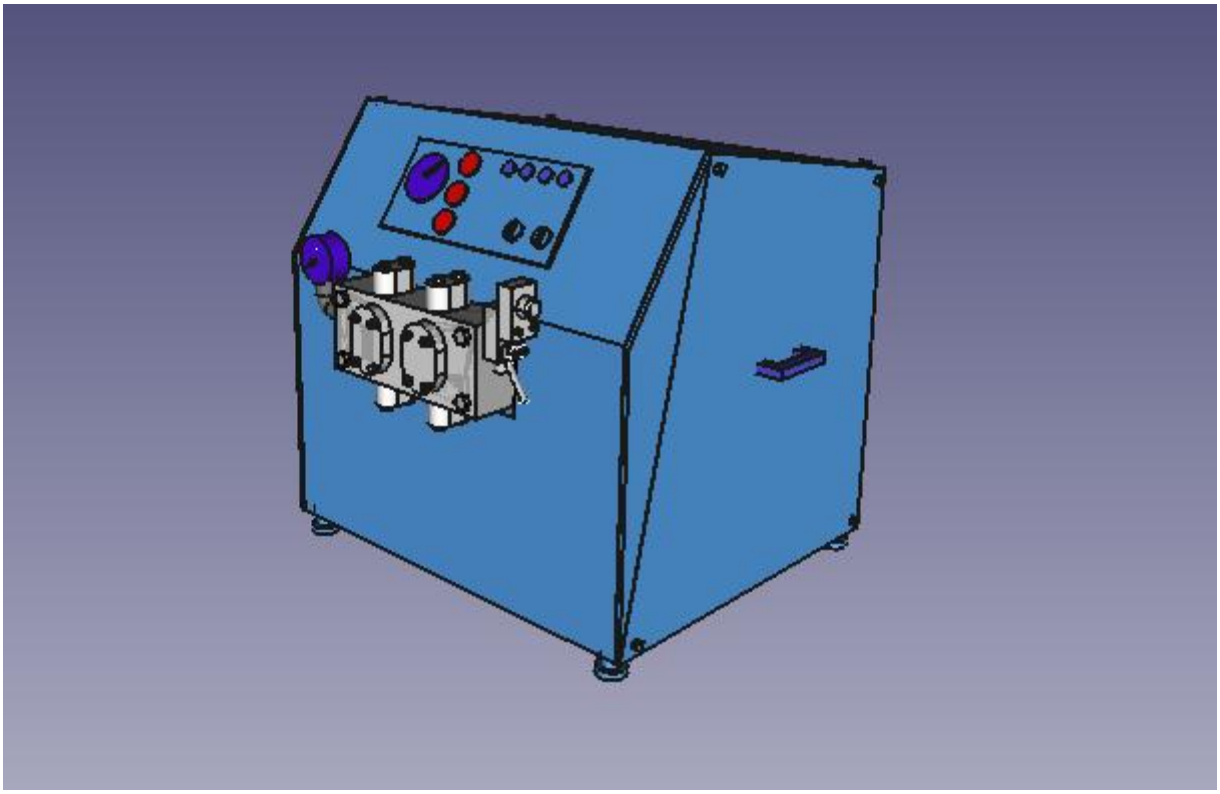
-Small motor and electricity box



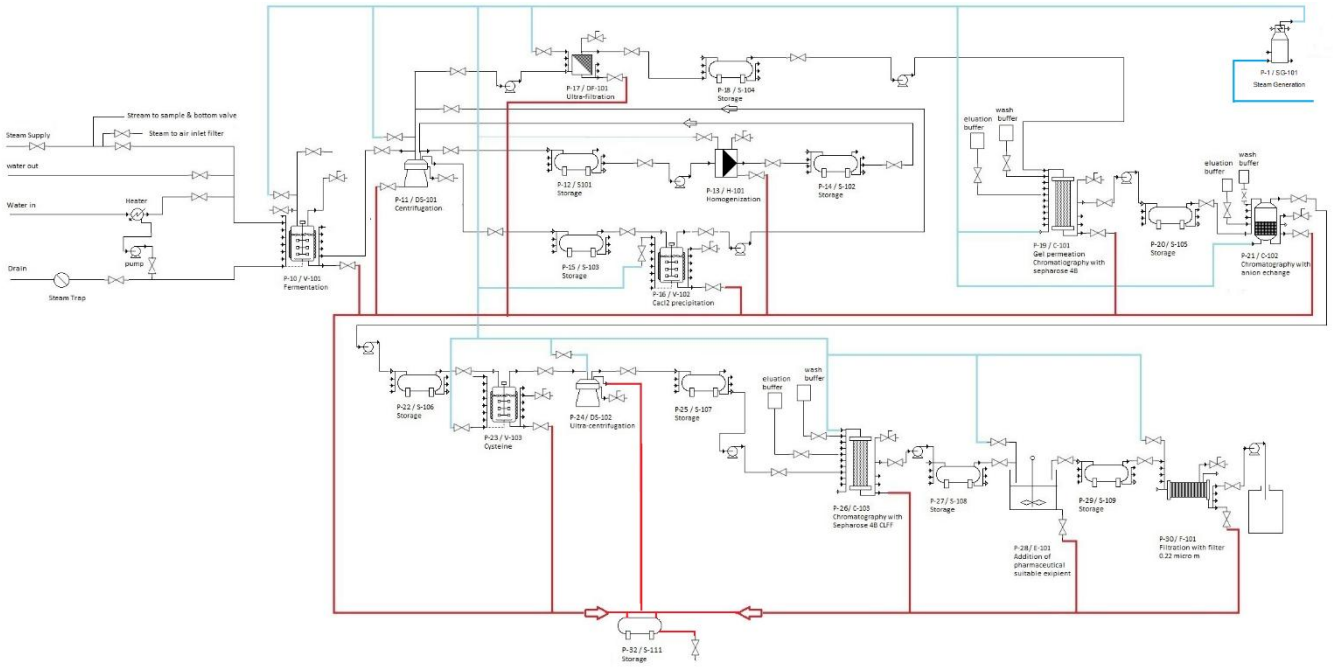
5.6.2.9 Finally close the machine with stainless wall



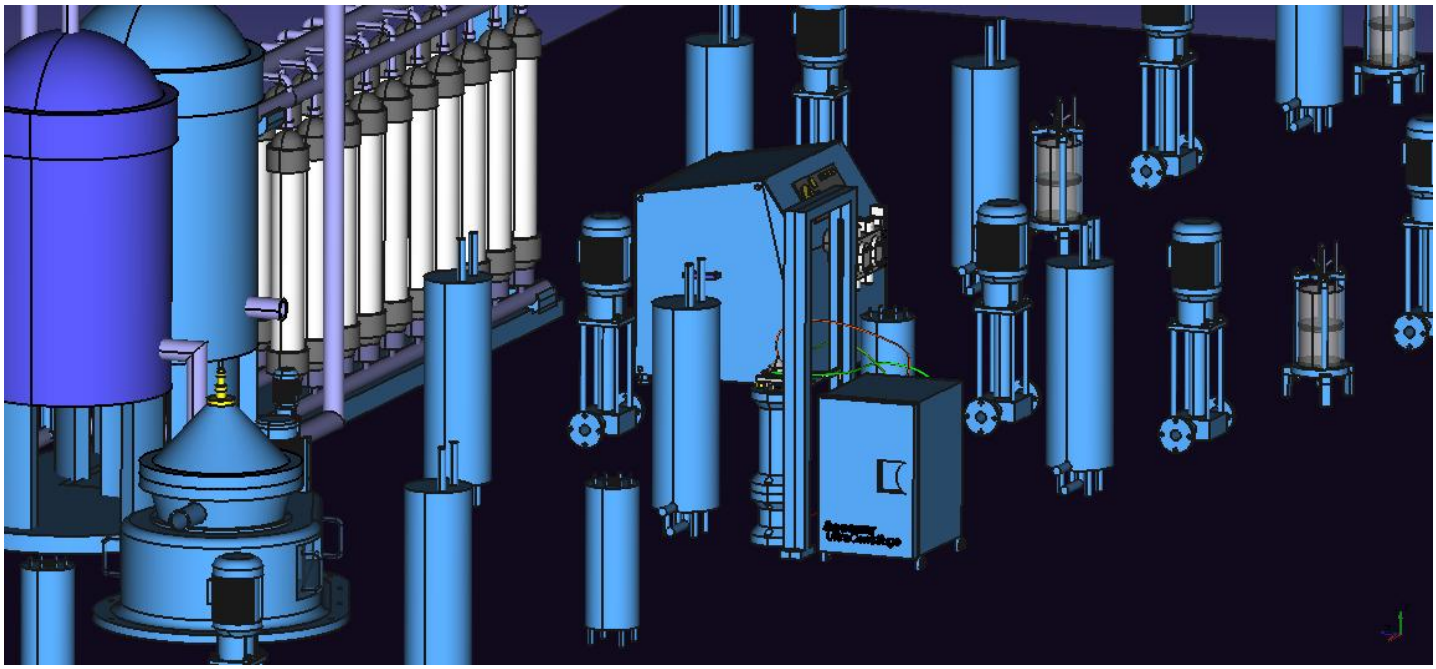
FINAL IMAGE: HOMOGENIZER

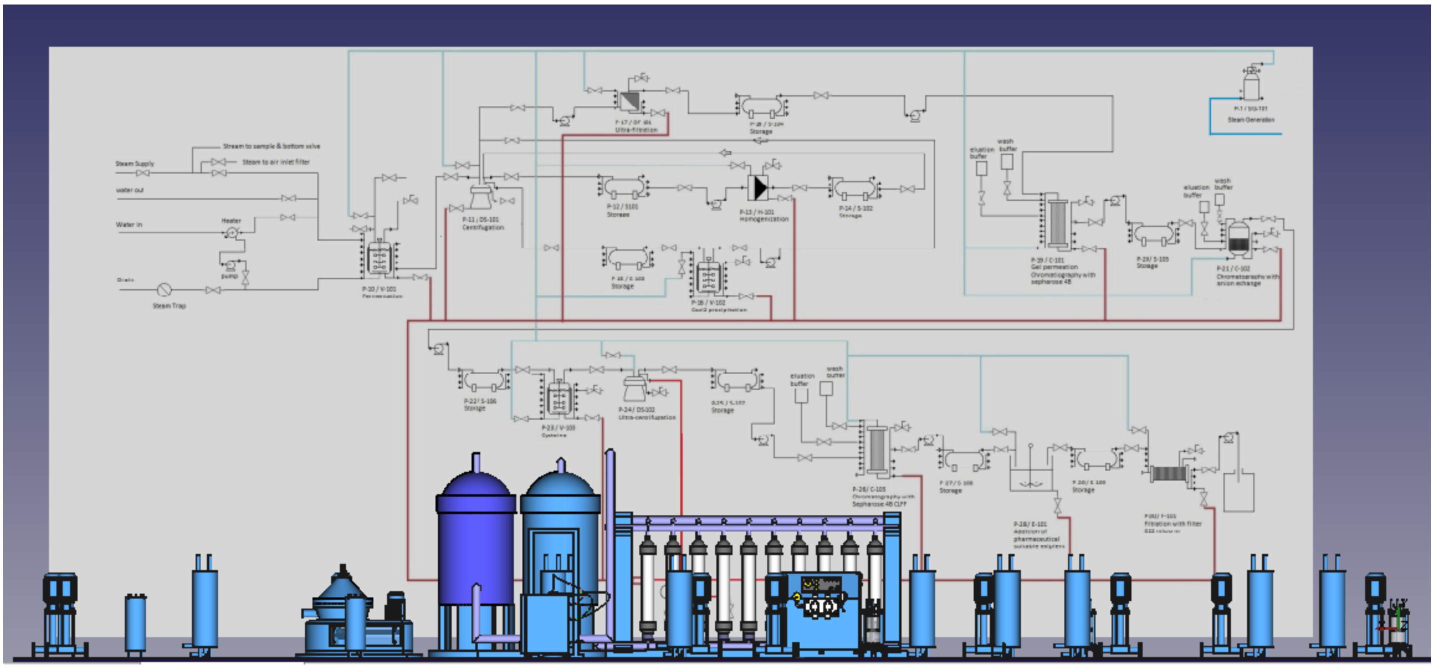


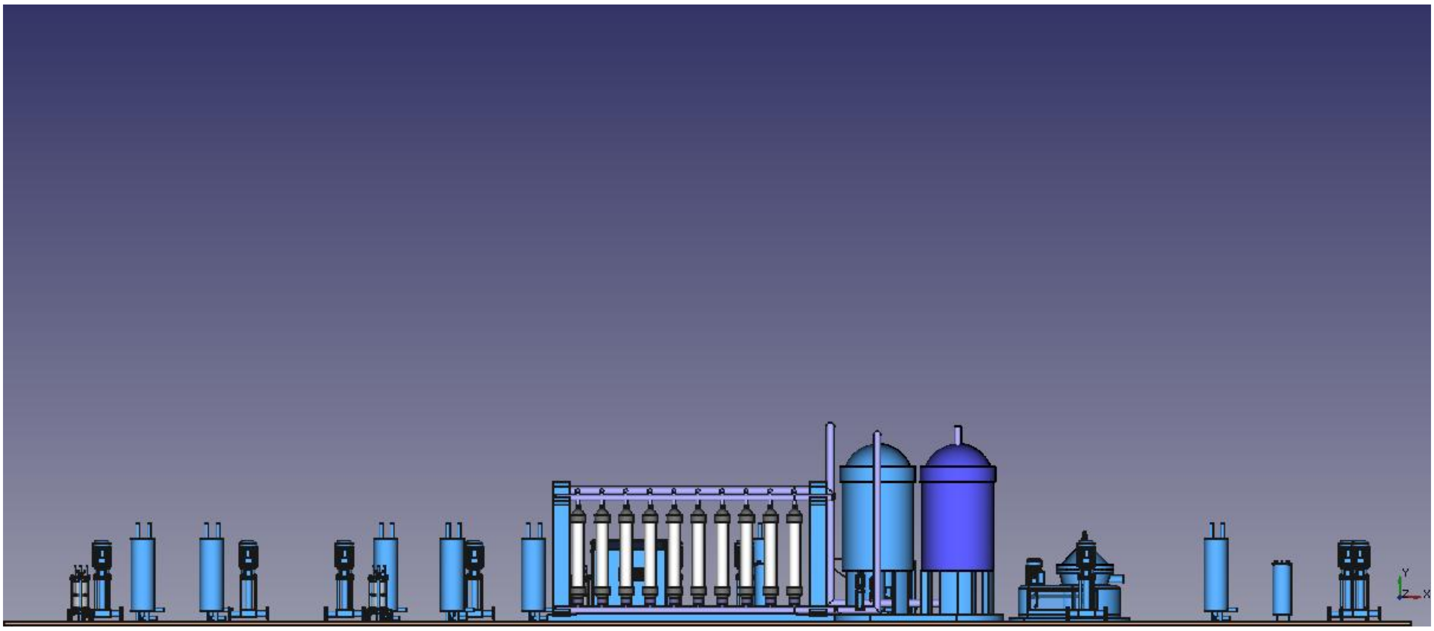
5.7 **Final Assembly of the MEGBI Plant**

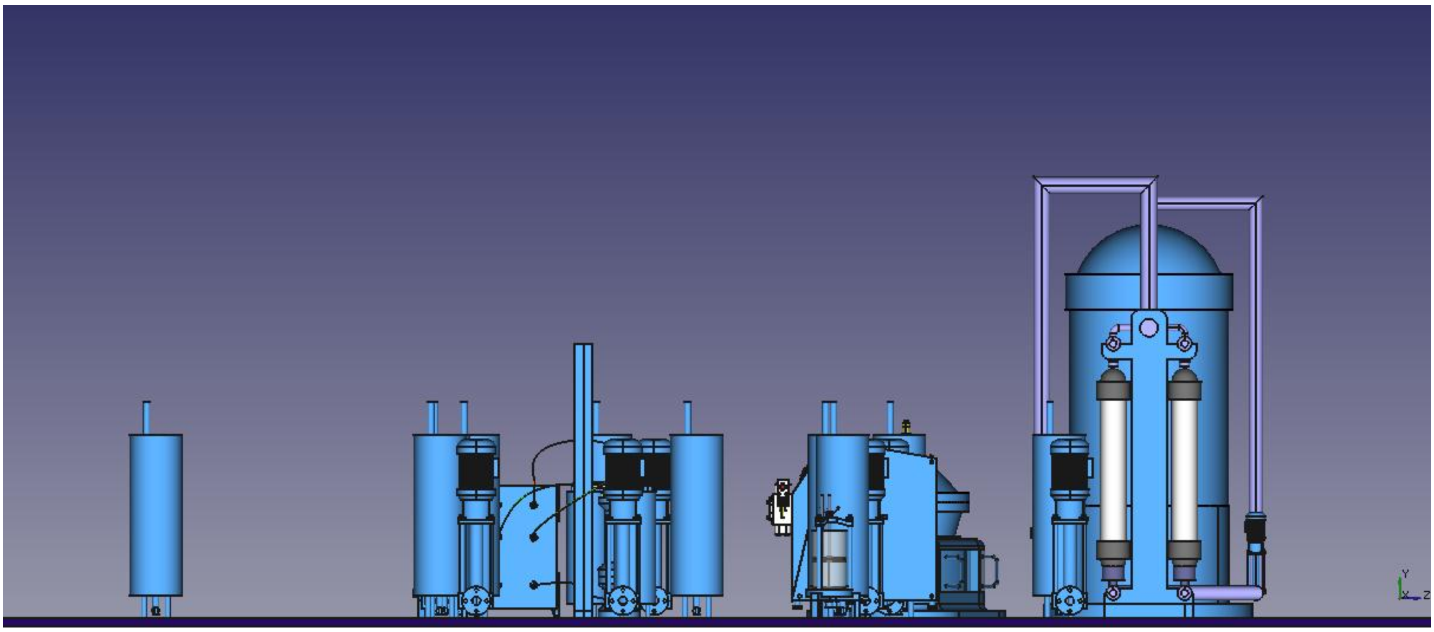


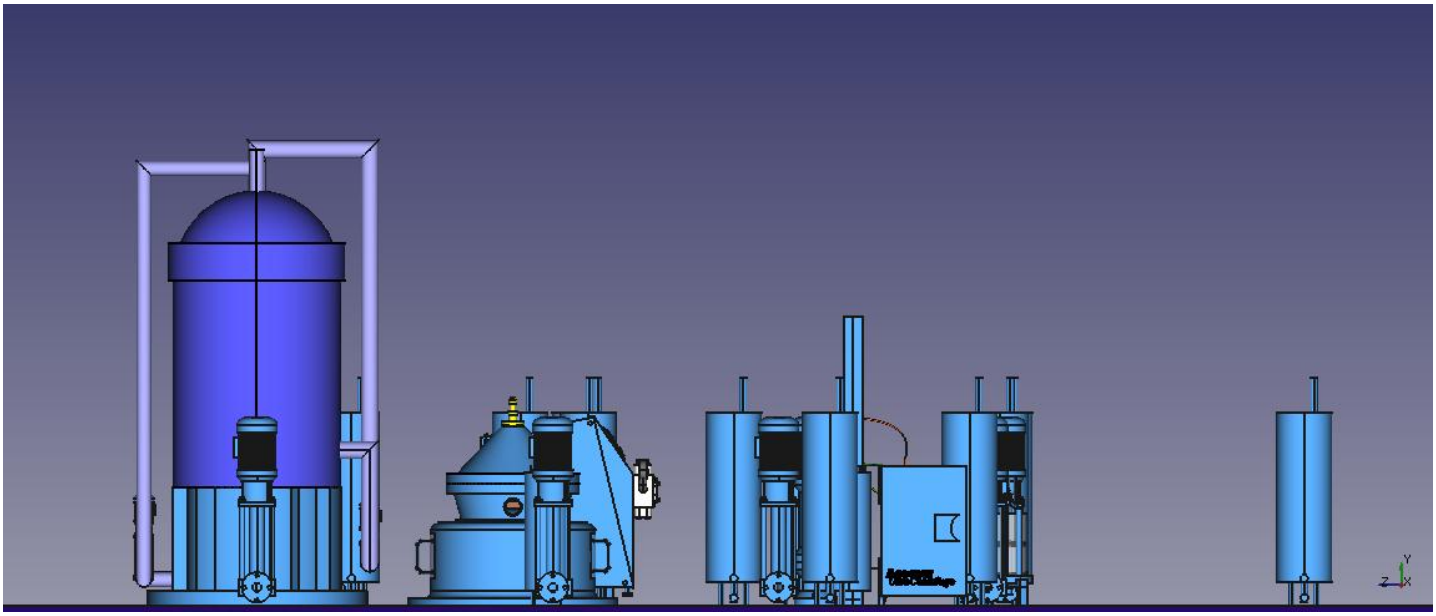
"Due to the lack of Assembly Features in FREECAD, Assembling These Parts Together. Was harder than Modelling Itself "– Jihad Samarji

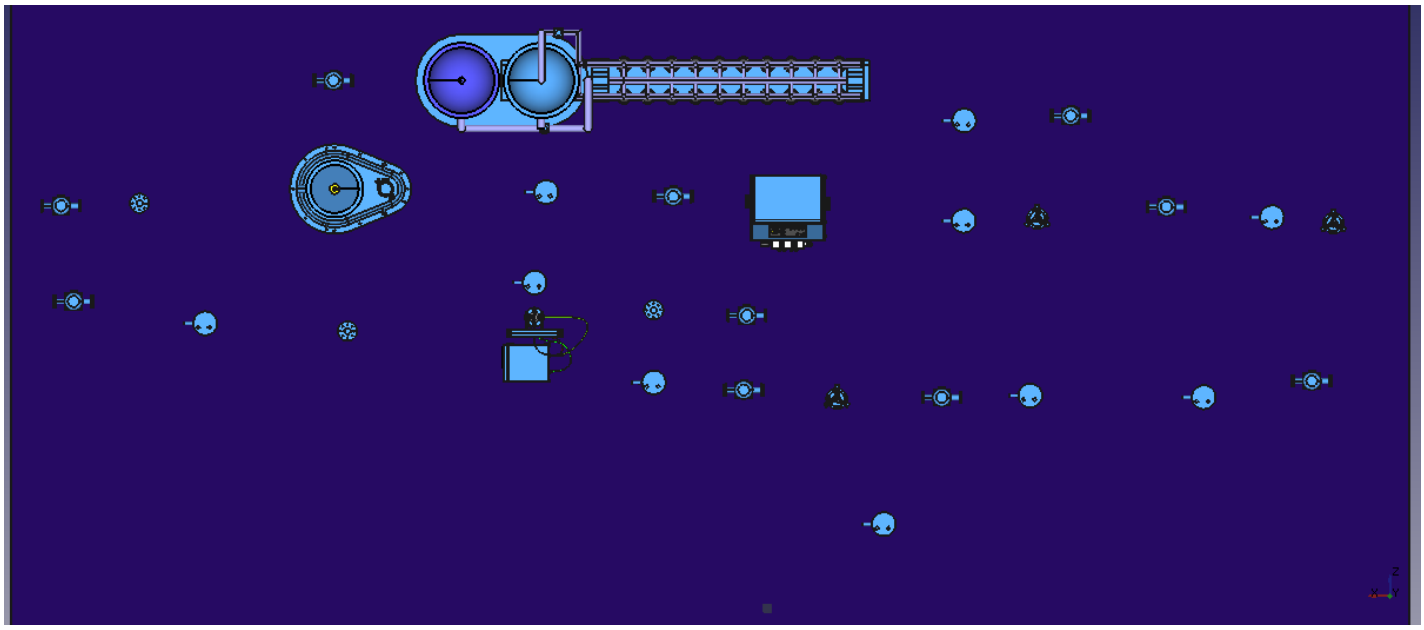


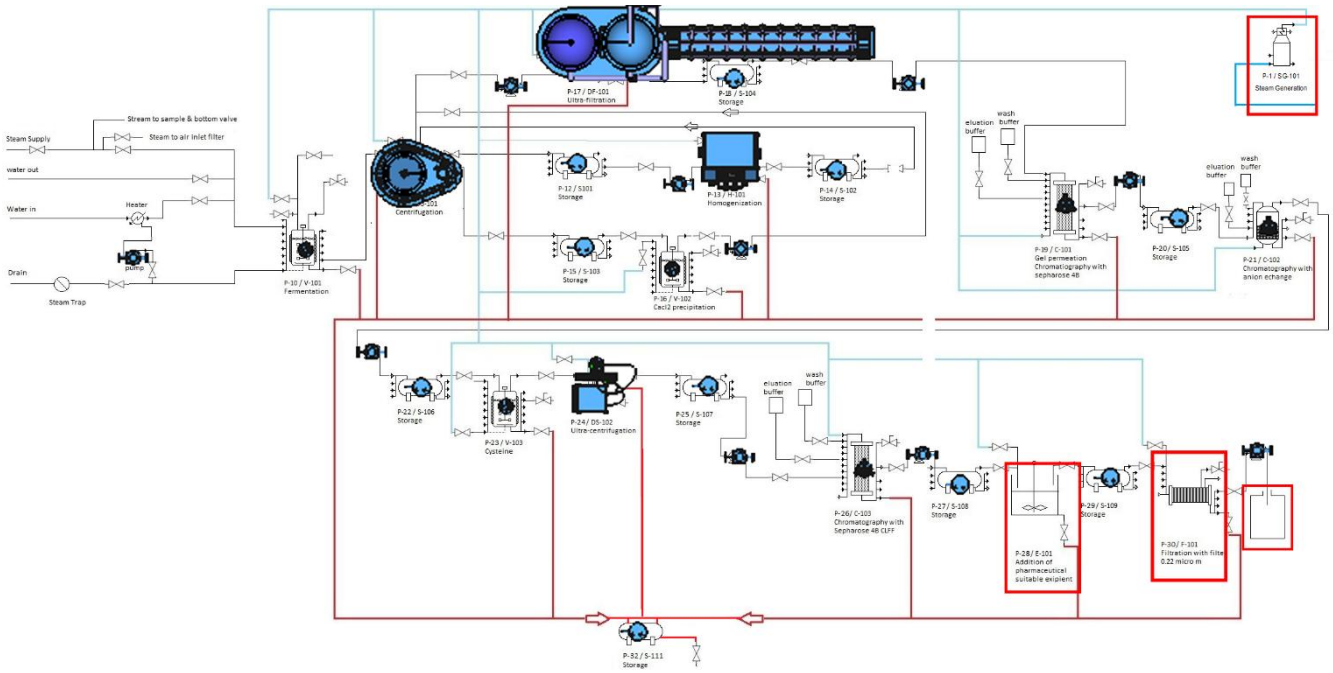

Stream to sample & bottom valve
Steam Supply
Steam to air inlet filter
water out
Water in
Heater
pump
Drain
Steam Trap

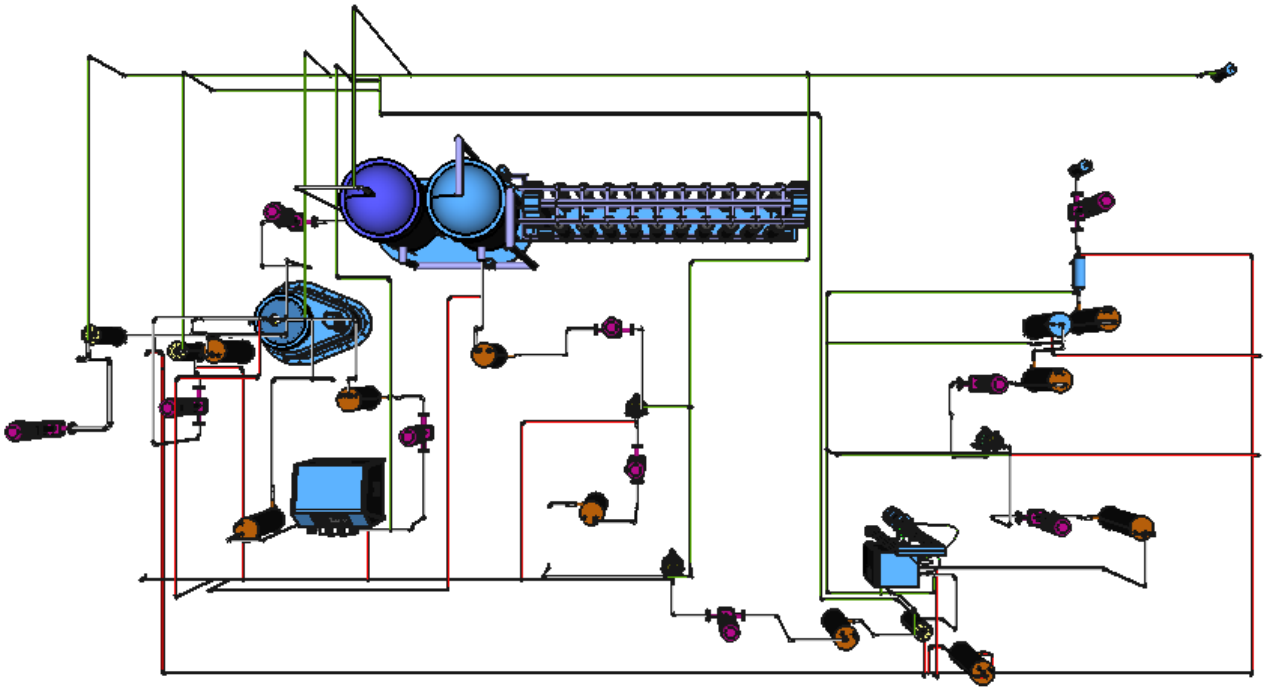


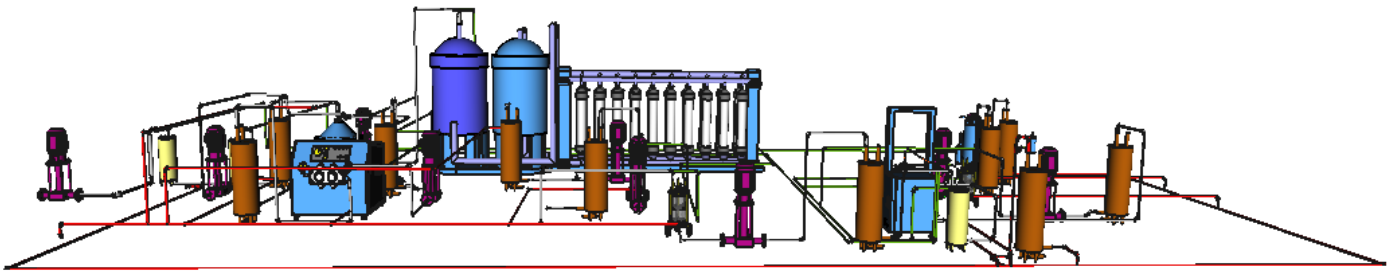







Stream to sample & bottom valve
Steam Supply
Steam to air inlet filter
water out
Water in
Heater
pump
Drain
Steam Trap
P-10 / V-101
Fermentation
P-17 / DF-101
Ultra-filtration
P-18 / S-104
Storage
P-1 / SG-101
Steam Generation
P-12 / S101
Storage
P-13 / H-101
Homogenization
P-14 / S-102
Storage
P-15 / S-103
Storage
P-35 / V-102
CaCl2 precipitation
eluation buffer
wash buffer
P-19 / C-101
Gel permeation Chromatography with sepharose 4B
P-20 / S-105
Storage
P-21 / C-102
Chromatography with anion exchange
P-22 / S-106
Storage
P-23 / V-103
Cysteine
P-24 / DS-102
Ultra-centrifugation
P-25 / S-107
Storage
P-26/ C-103
Chromatography with Sepharose 4B CLFF
P-27 / S-108
Storage
P-28 / E-101
Addition of pharmaceutical suitable exipient
P-29 / S-109
Storage
P-30/ F-101
Filtration with filte 0.22 micro m
P-32 / S-111
Storage





MODELLING AND ASSEMBLING MEGBI-VPP IS DONE

NEXT STEP: IS TO MAKE AN AUTOMATION SYSTEM TO SIMULATE THE WORK OF THIS PLANT

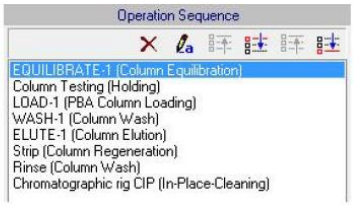
THIS CHAPTER CONTAINS INFORMATION GATHERED FROM PERSONAL EXPERIENCE AND THE INTERNET.

6. Automation System Design and GUI

This task was done as master thesis from Haitham Hindy, Lebanese University, Tripoli

Downstream System (Purification) from Solaris Group (in this way MEGBI-VPP downstream processing shall be developed)

Operation sequence of ion exchange chromatography device (to be automated)



PLC System for MEGBI-VPP downstream processing system: Siemens S7-300 with PROFIBUS

Master Thesis

Automation of measurement of temperature, pressure and pH data and automation of fluid flow of a biotechnological production plant

- Design of Software (State machines) for Homogenizer, Disc Stack Centrifuge in including CIP/SIP functional elements, Process Scale Gel Permeation and ion exchange chromatographic devices, Process Scale Ultrafiltration Device (6 weeks)
- Graphical User Interface for the automation of MEGBI-VPP downstream processing (DSP) unit (4 weeks)
- Adaptation of a Graphical User Interface to a Siemens S7 PLC system (6 weeks)
- Documentation (3 weeks)

Keywords: measurement of temperature, pressure and pH data, Automation of fluid flow, PLC, Siemens S7, Programming, User Interface, C++/Java, Biotechnology

For automation system basics please refer to chapter 2, sections 15-17

6.1 Automation System Specification

First this thesis is about automation of system for The DNA Vaccine, for that we should create a design for the Control screen and adaption with Velleman board and PLC Siemens.

We start with search about the subject to know what we will do in this thesis, we get some words keys like Downstream Processing, Graphical user interface (GUI), adaption to Velleman board or S7.

We had read about Downstream Processing, it's the finale process to create the vaccine of hepatitis B, it contains many steps begin by Bioreactor to ends by Storage.

And we had continued to know every step on this processing and we had written a list in which the step or process name and how many valve and sensor needed for it.

The list of the instruments:

1-Bioreactor, 2-Centrifuge, 3-Homogenizer, 4-Storage, 5-ultra0-filtration, 6-precipitation,

7-chromatography with gel permission,8-Filter,9-Cysteine.

With this 9 process i have developed a table to the downstream processing.

After that we had used the SuperPro to get the form of each instrument, for example bioreactor:

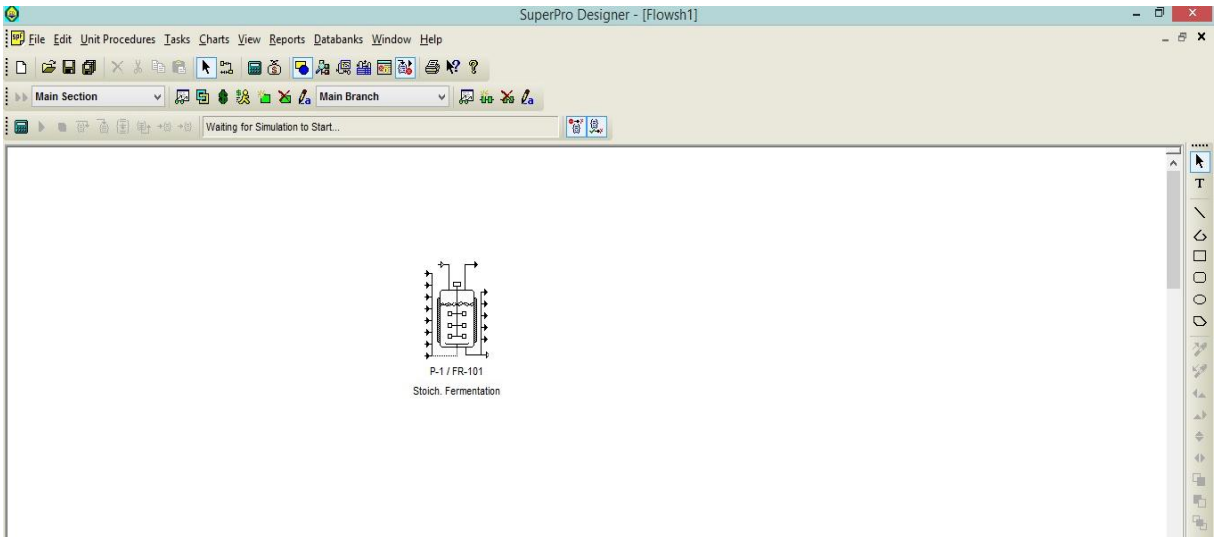
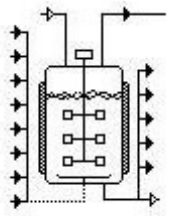
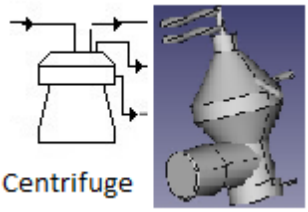
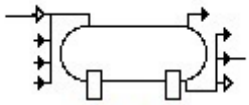
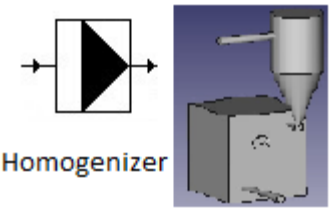
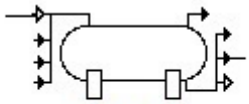
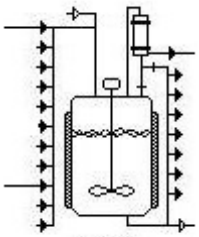
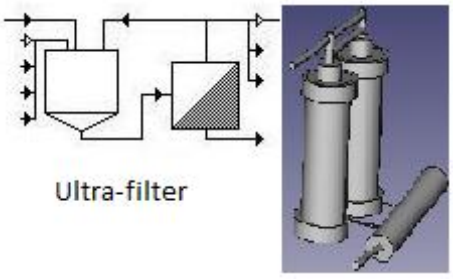
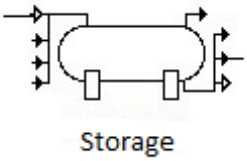
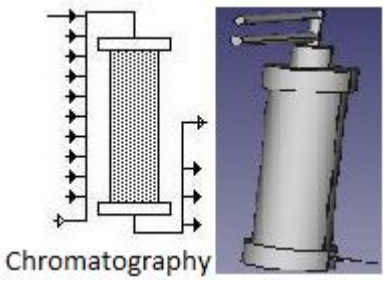
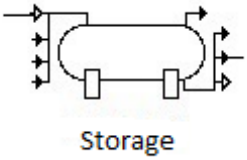
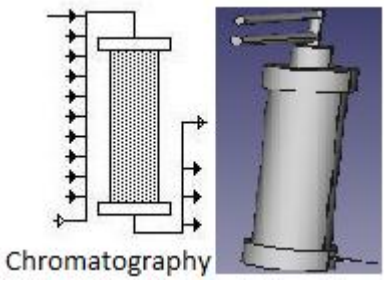
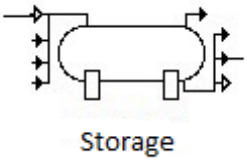
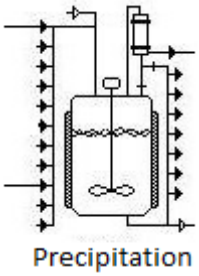


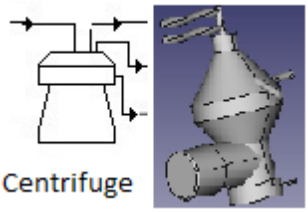
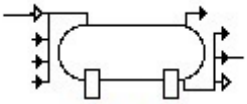
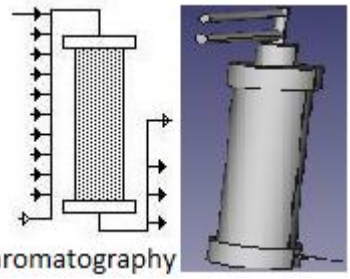
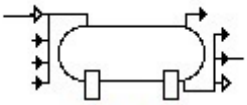
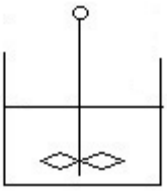
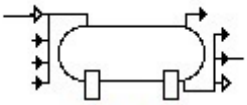
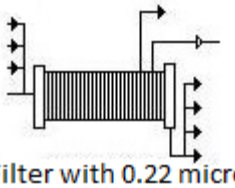
***Figure 3**: Shot of the bioreactor from Super-Pro.*

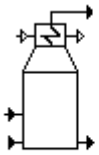
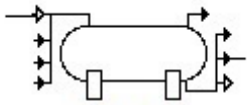
To Facility the work, we have put a table to insert in it all process with number, PI diagram symbol with CAD mode of some instruments, number of valves, sensors needed.

Table 3: List of Process with PI diagram and instruments linked to valve and pump.

PROCESS NUMBERS	DEVICES NUMBERS	PI DIAGRAM SYMBOL/CAD MODEL	VALVES / PUMPS	SENSORS
P-10	V-101	 Bioreactor	V1 V2 V3 V4 V5	T1 PH1 MF1 PR1 PO1
P-11	DS-101	 Centrifuge	V1 V2 V3 V4 V5 V6	T2 MF2 PR2
P-12	S-103	 Storage	V1	---
P-13	H-101	 Homogenizer	V1 V2 V3 PU1	T3 MF3 PR3
P-14	S-102	 Storage	V1	---
P-16	V-102	 Precipitation	V1 V2 V3 V4 V5	T4 MF4 PR4

P-16	DF-101		V1 V2 V3 V4 V5 PU2	T5 MF5 PR5
P-17	S-103		V1	
P-18	C-101		V1 V2 V3 V4 V5 V6 PU3	T6 MF6 PR6
P-19	S-104		V1 PU4	
P-20	C-102		V1 V2 V3 V4 V5 V6	T7 MF7 PR7
P-21	S-105		V1 PU4	
P-22	V-103		V1 V2 V3 V4 V5	T8 MF8 PR8

P-23	DS-102	 Centrifuge	V1 V2 V3 V4	T9 MF9 PR9
P-24	S-106	 Storage	V1	
P-25	C-103	 Chromatography	V1 V2 V3 V4 V5 V6 PU5	T10 MF10 PR10
P-26	S-107	 Storage	V1	
P-27	E-101	 Blender	V1 V2 V3 V4	
P-28	S-108	 Storage	V1	
P-29	F-101	 Filter with 0.22 micro m	V1 V2 V3 V4 V5	T11 PR11 MF11

P-30	S-110	 Steam generater	V1	P1
P-31	S-109	 Storage	V1	---

6.2 **Automation System Design**

With this list can make the screen, we have painted a miniature in a paper A4 link the instruments together in order to process the next process and we have introduced the valves to get this:

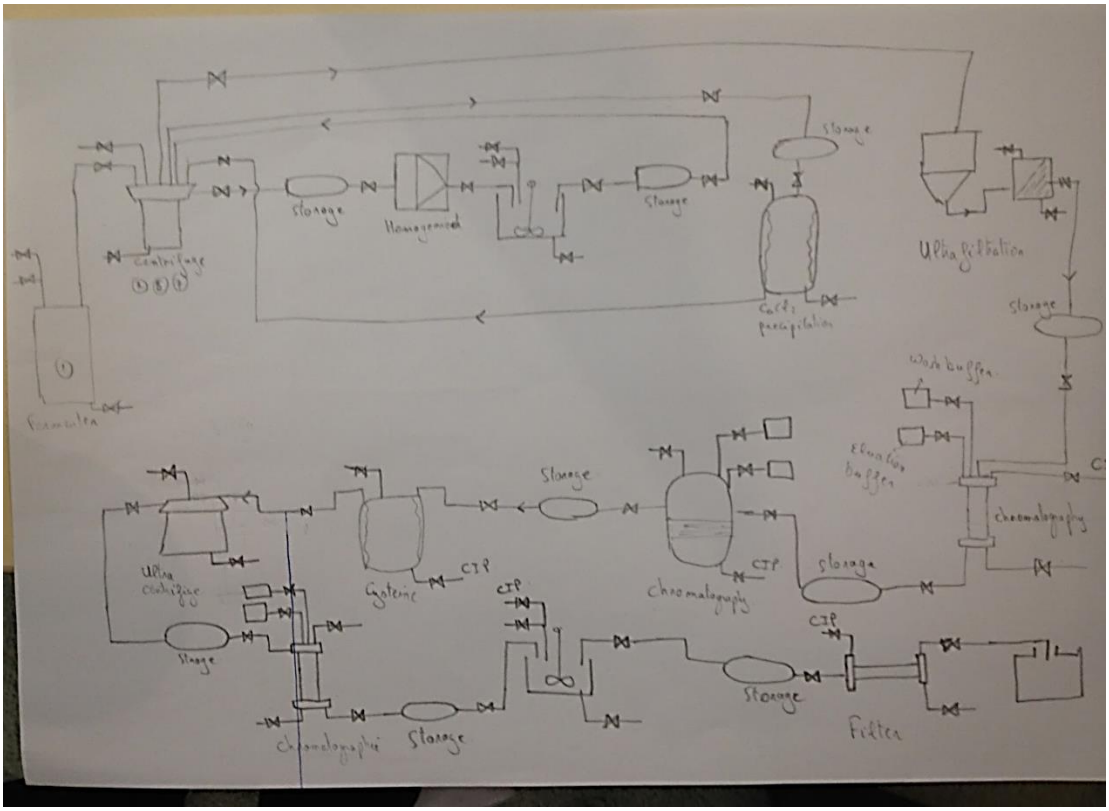
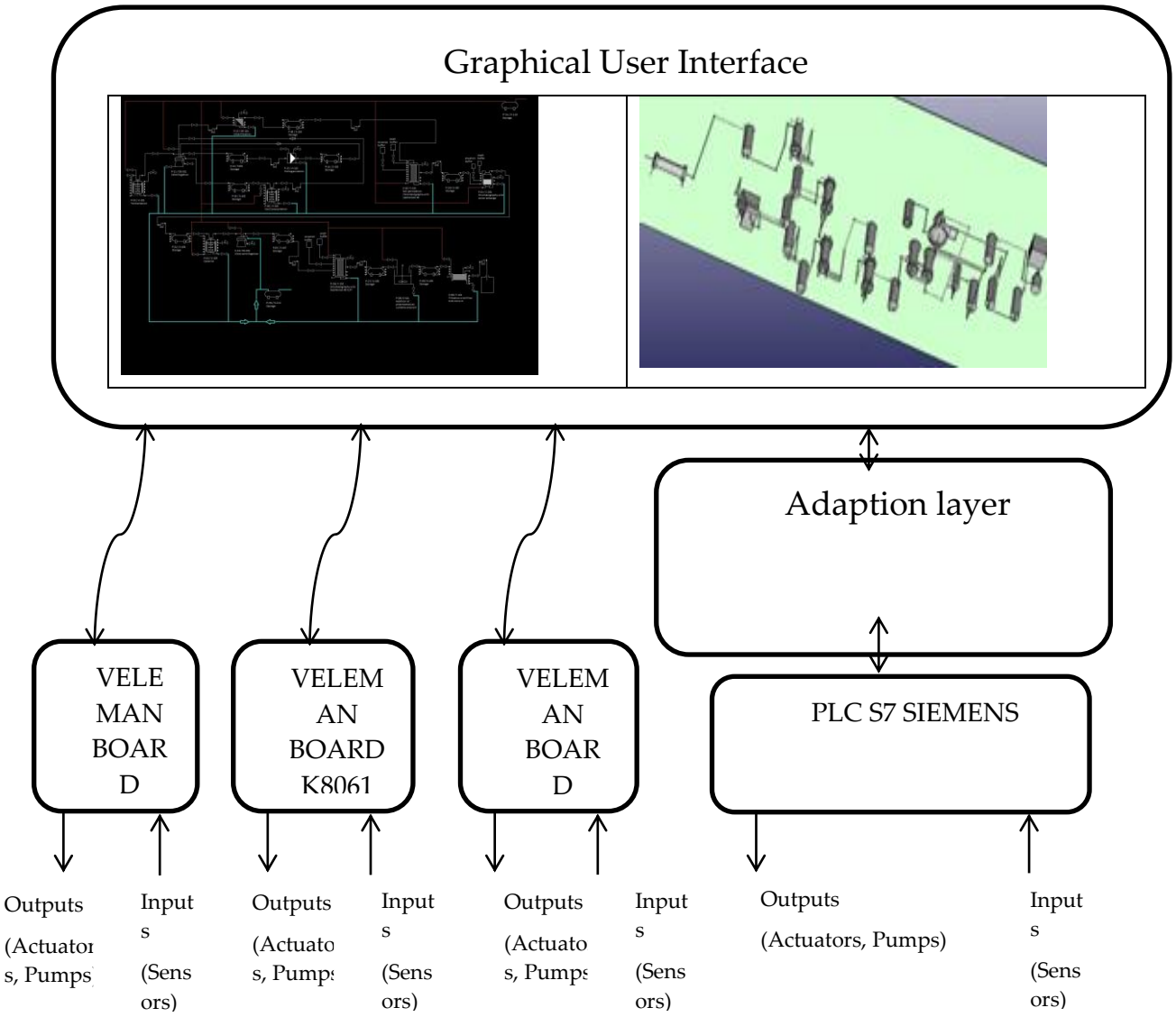


Figure 4: *Painting the Downstream Processing.*

Next step we have used this image to create the graphical user interface, we have collected the SuperPro with paint to make all instruments and process together and get the image:


Graphical User Interface
Adaption layer
VELE MAN BOAR D
VELEM AN BOARD K8061
VELEM AN BOAR D
PLC S7 SIEMENS
Outputs (Actuator s, Pumps
Input s (Sens ors)
Outputs (Actuato s, Pumps
Input s (Sens ors)
Outputs (Actuato s, Pumps
Input s (Sens ors)
Outputs (Actuators, Pumps)
Input s (Sens ors)

6.3 **Graphical User Interface**

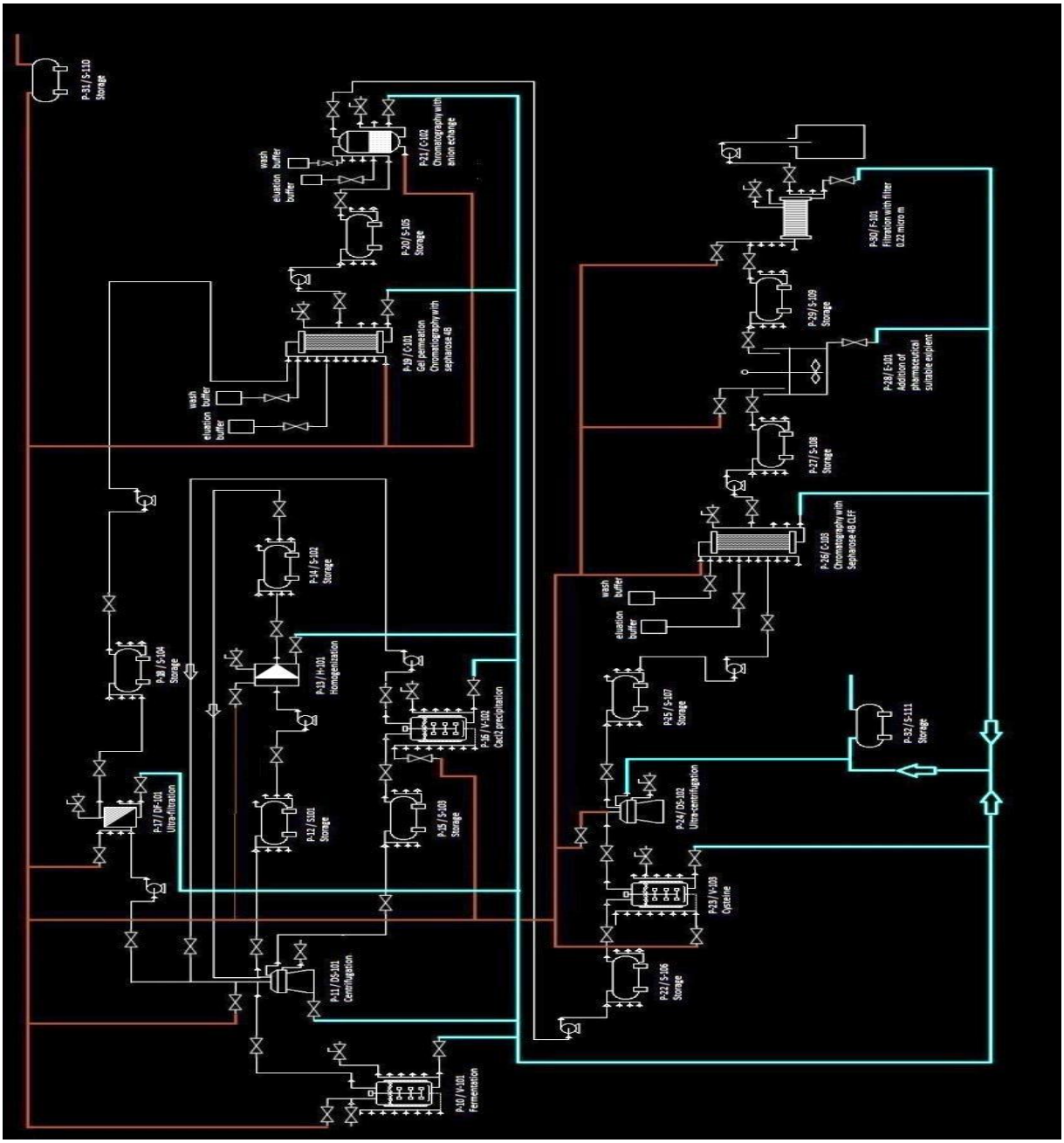


Figure 5: *First result Of the Controller Screen.*

This was the first result, after that we have conducted some adjustments and increases to get this finale result of this screen:

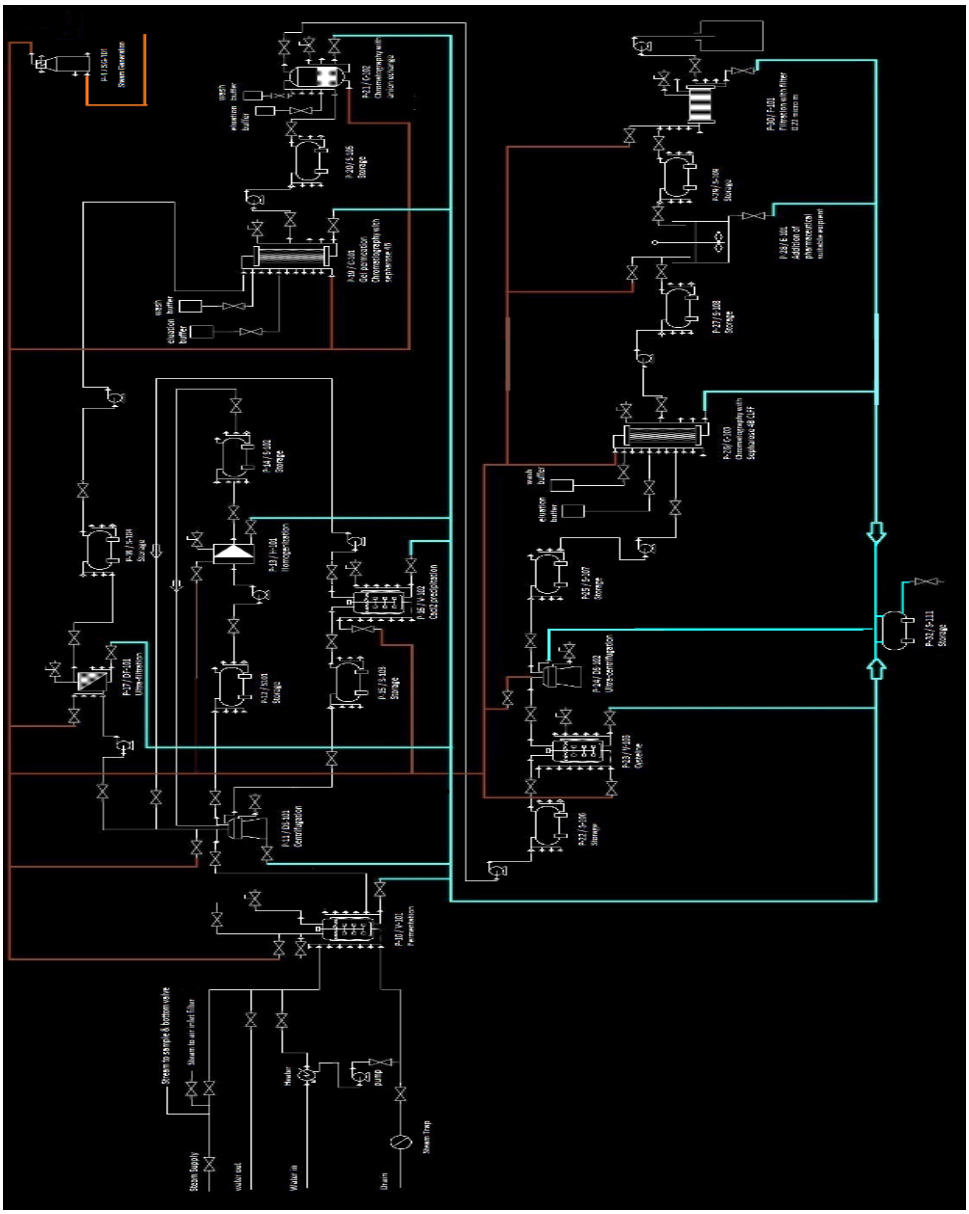


Figure 6 : *final result Of the Controller Screen.*

Previously, we had some problems with the process and how we will join these instruments together and what the next, for that, we working on it to know how many process needed, step with step and what we want to joint with the instrument and finally we get it full for the Downstream processing.

Next move, we have installed the Python 3.4, it's a programming language of which we can create the Control screen.

We had used the Python book and internet to learn and training about that language and to know how we can put the code and witch the code will used and with some problems, we create it.

But we have passed a problem with how to download the python ,run it and use it ,cause the form of program need much of package like wx package and more just for use the code, for that we have worked hard to search for the package need and how we will install it , how to run a command and more ,this take much time from me to continue the work , but finally we learn how to install it, what package needed and how to run it, for example this error:

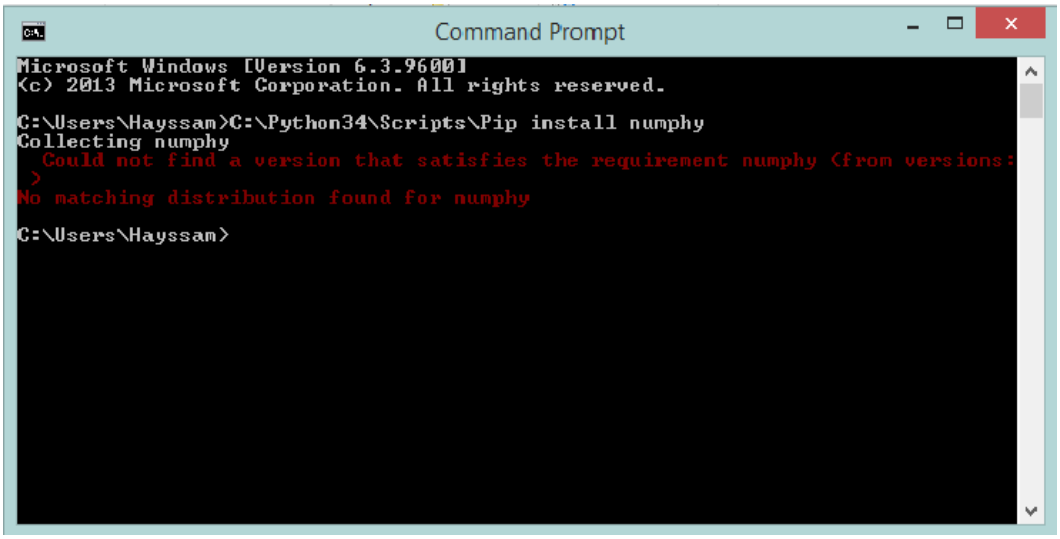


Figure 7 : *Error Command 1.*

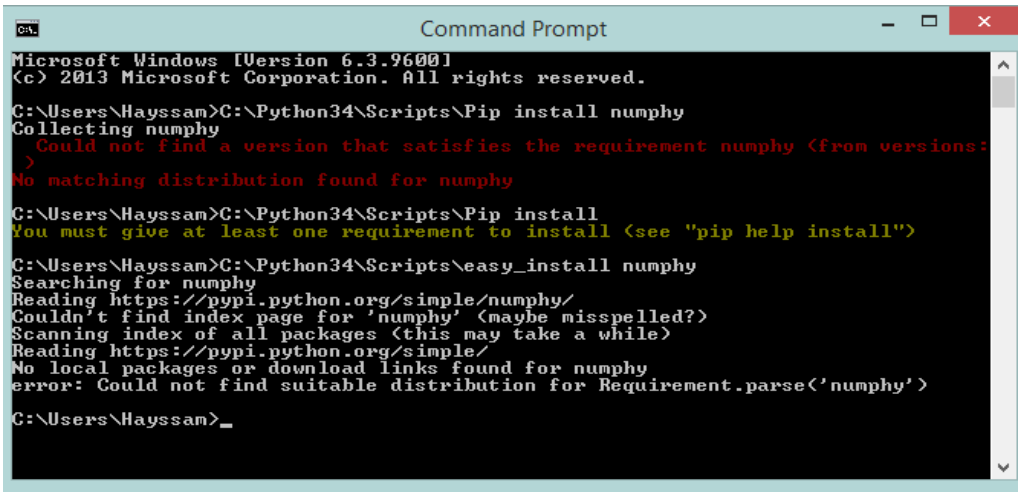


Figure 8 : ***Error Command 2***

To repair this error, must upgrade the pip package:

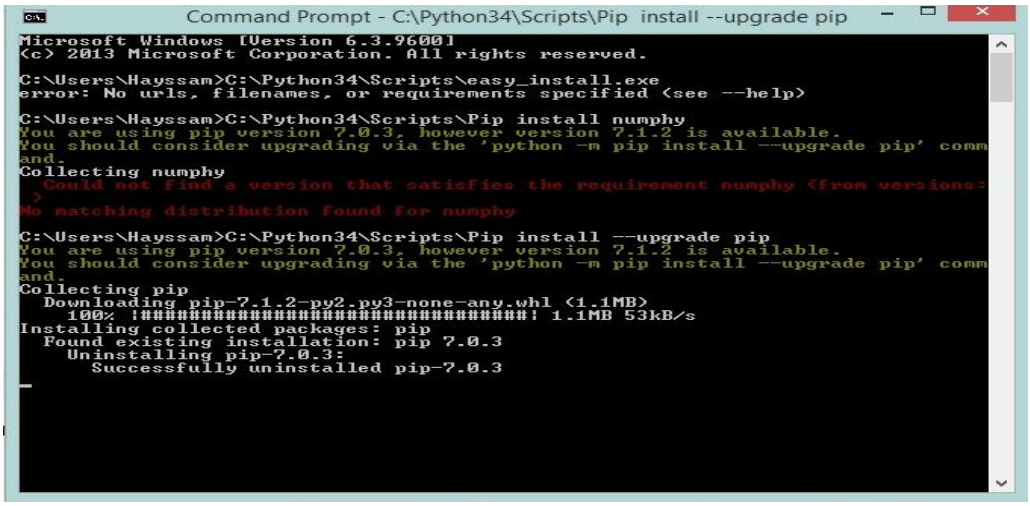


Figure 9: ***First step of upgrading.***

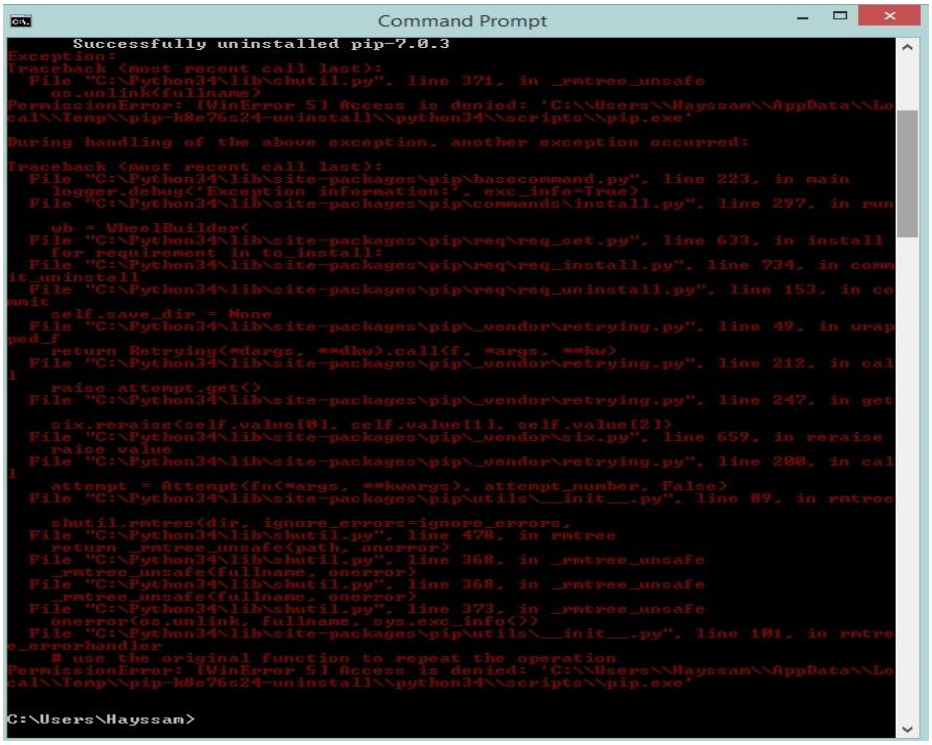


Figure 10 : *Done step of upgrading.*

For complete the installing must define the entire package like that example and run it:

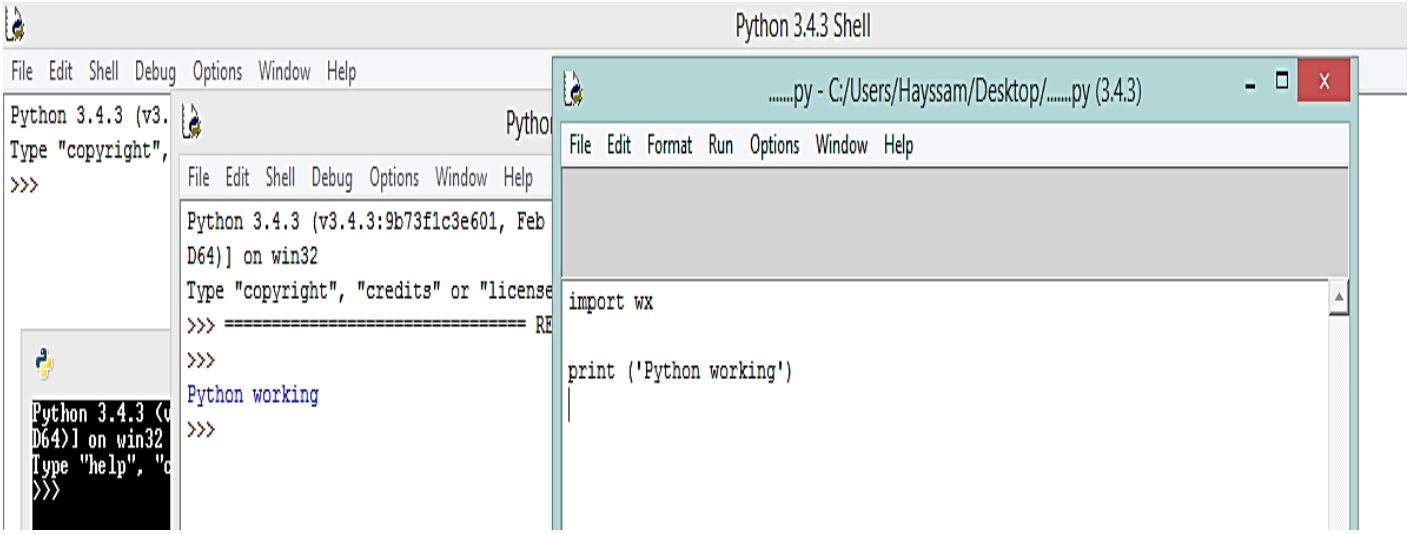


Figure 11 : *Python working.*

After installing and running the Python, must continue to written:

First how to insert a background, that background should be the screen, the previous image was painted must be that background, the Python had a code to use an image like a background we used the code to my image.

This is the code:

```
#**********************                    panel frame                    **********************
#*************************************************************************************************

BACKGROUND_IMAGENAME =\"C:\Users\Hayssam\Desktop\GUI 220415MEGPI-VPP_PI_DIAGRAM.PNG"

class MyBackgroundPanel(wx.Panel):

    def __init__(self, parent):
        wx.Panel.__init__(self, parent)
        self.bmp = wx.Bitmap(BACKGROUND_IMAGENAME)
        self.SetSize(self.bmp.GetSize())
        self.Bind(wx.EVT_PAINT, self.on_paint)


    def on_paint(self, event = None):
        dc = wx.BufferedPaintDC(self, self.bmp)
```

Figure 12 : *Python 1*

After this we had created a window from a code across written a new class, use the wx.Frame function, put name for this window and some developed.This is the code:

```
class MyFrame(wx.Frame):

    def __init__(self, parent = None, title = "MEGBI Vaccine Pilot Plant (MEGBI-VPP) Overview Upstream & Downstream Process"):


        wx.Frame.__init__(self, parent, -1, title)

        panel = MyBackgroundPanel(self)

        LABELSTYLE = wx.BORDER_SUNKEN | wx.ST_NO_AUTORESIZE | wx.ALIGN_CENTER_HORIZONTAL


        menuFile = wx.Menu()
        menuFile.Append(1, "&About...")
        menuFile.AppendSeparator()
        menuFile.Append(2, "Exit")
        menuBar = wx.MenuBar()
        menuBar.Append(menuFile, "File")
        self.SetMenuBar(menuBar)
        self.CreateStatusBar()
        self.SetStatusText("Welcome to MEGPI Project!")
        self.Bind(wx.EVT_MENU, self.OnAbout, id=1)
        self.Bind(wx.EVT_MENU, self.OnQuit, id=2)


    def OnAbout(self, event):
        wx.MessageBox("This is a Screen controller of MEGPI Project","Welcome to my python", wx.OK | wx.ICON_INFORMATION, self)
    def OnQuit(self, event):
        self.Close()
```

Figure 13 : *Python 2*

After the frame class, must put the finale class for the main loop, this is for show the background and the frame without it nothing done.

This is the code:

```
#**********************                main definition and loop              ********************
#*****************************************************************************************************

def main():
    """Testing"""
    MyBackgroundPanel = wx.PySimpleApp(wx.Panel)
    f = MyFrame()
    d=ValveListBox()
    d.Show()
    f.Center()
    f.Show()
    MyBackgroundPanel.MainLoop()

if __name__ == "__main__":

    main()
```

Figure 14 : *Python 3*

Next increased buttons for valves and pumps cause needed to click on it to open and close the valve, turn on or to turn off the pump, for that we used the code in python to create the buttons but we must specify the position of the valve or pump to put the button near of it, to know that button to that valve or pump.

The code in python given much of details like we can name the button and position for it in screen so that easy to create in my screen . I have 67 valves and 10 pumps.

This is the code:

```
            # Create Valves

               #V1

wx.StaticText(panel,-1," V1 ",(18,300))


self.button_V_1 = wx.Button(panel, -1, "V1", pos=(95,100),size=(20,20))
self.Bind(wx.EVT_BUTTON, self.V1, self.button_V_1)


def V1(self, event):
        wx.MessageBox("V1 is Open ","Open", wx.OK|wx.ICON_INFORMATION)
```

Figure 15 : *Python 4*

Getting this screen in first try, if we click on V1 will be given “V1 is Open”:

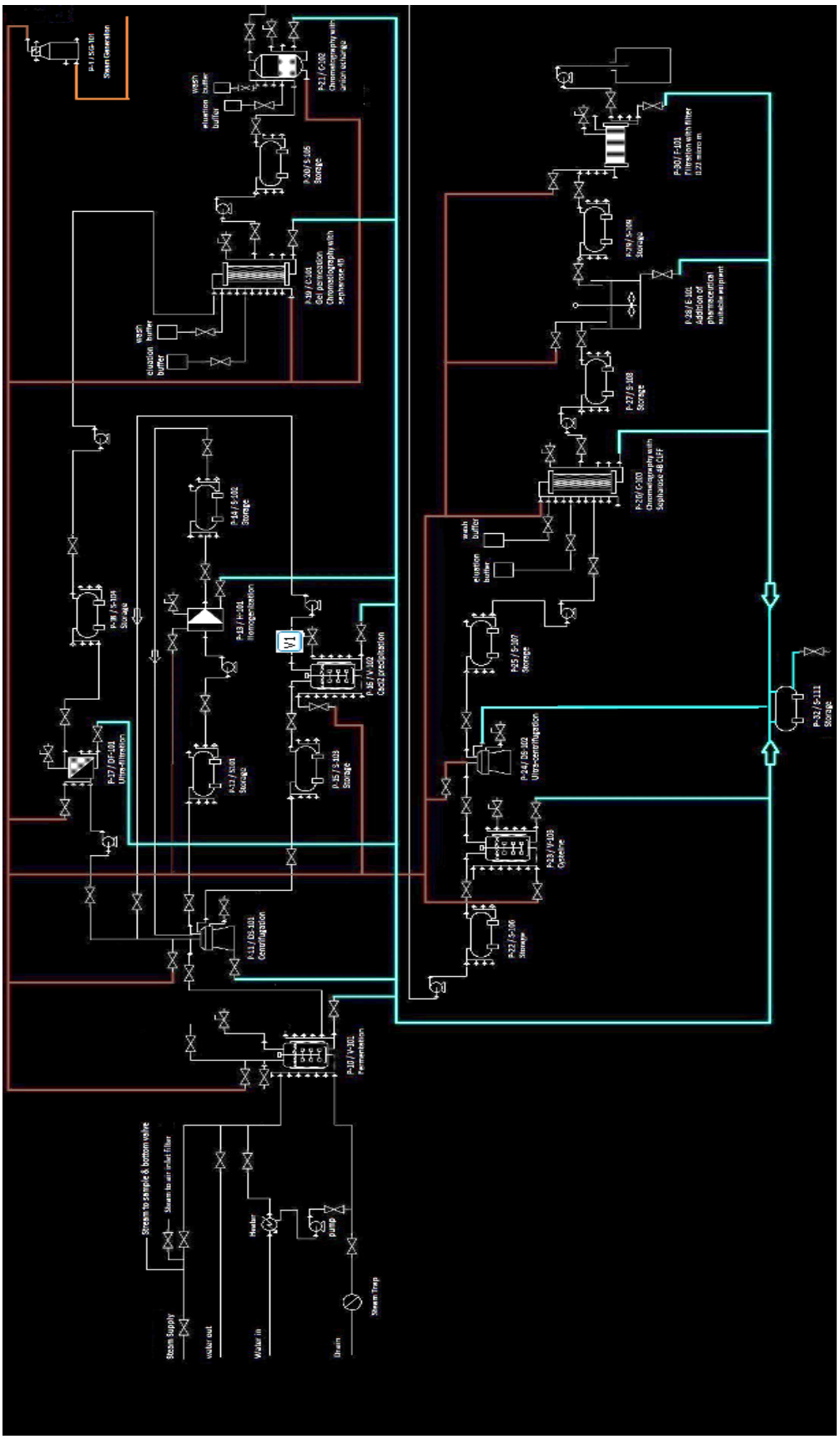


Figure 16 : *Button V1 Created*

Next step is creating label and collected it with buttons to show if we click the valve button will give “ON” or “OFF”

We need a code through which we can enter a condition and we can access to open-close the valve plus give in the label “ON”-“OFF”, and turn off-on the pump plus give “ON”-“OFF”.

This is the code:

```
          # Create Valves

             #V1
wx.StaticText(panel,-1," V1 ",(18,300))

self.button_V_1 = wx.Button(panel, -1, "V1", pos=(95,100),size=(20,20))
self.Bind(wx.EVT_BUTTON, self.V1, self.button_V_1)


def V1(self, event):
    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'

    if  self.Valve1.GetLabel()==valve_status_ON:
        self.Valve1.SetLabel(valve_status_OFF)
        wx.MessageBox("V1 is Open ","Open", wx.OK|wx.ICON_INFORMATION)
    else:
        self.Valve1.SetLabel(valve_status_ON)
        wx.MessageBox("V1 is Close","Closed", wx.OK|wx.ICON_INFORMATION)
```

Figure 17 : *Python 5*

With this code we have continued the Screen Controller to get it, this all buttons and labels represent the actuators, for the sensors we have developed some labels because the data of the sensors are taked from Velleman board.

We passed by some problems with function, how we will join the button with label and how to connect the label with condition ‘if …else’ to given “ON” or “OFF”, and the order of steps. With the help from www.Python-forum.org, there we asked about the problem, and how to fix it.

They have given us help and repaired the code just in exception case.

This was the wrong code:

CODE: SELECT ALL

```
              #V1
       self.button_V_1 = wx.Button(panel, -1, "V1", pos=(95,100),size=(20,20))
       self.Bind(wx.EVT_BUTTON, self.V1, self.button_V_1)

def V1(self, event):

            valve_status_ON = 'ON'
            valve_status_OFF = 'OFF'
            if self.V1.Bind("<Button-1>", callback):
            #self.V1 == True:
            #valve_status = valve_status_ON
             self.Valve1.SetLabel(valve_status_ON)
             wx.MessageBox("V1 is Open ","Open", wx.OK|wx.ICON_INFORMATION)
```

Figure 18: *Shot of the wrong code in forum.*

And this was the right code after the help from the forum python site:

CODE: SELECT ALL

```
def V1(self, event):
            valve_status_ON = 'ON'
            valve_status_OFF = 'OFF'

            if self.Valve1.GetLabel()==valve_status_ON:
                self.Valve1.SetLabel(valve_status_OFF)
                wx.MessageBox("V1 is Open ","Open", wx.OK|wx.ICON_INFORMATION)
            else: :
                self.Valve1.SetLabel(valve_status_ON)
                wx.MessageBox("V1 is Close","Closed", wx.OK|wx.ICON_INFORMATION)
```

Figure 19 : *Shot of the right code in forum.*

After writing the code for all the buttons, labels, text, we go the Controller Screen:

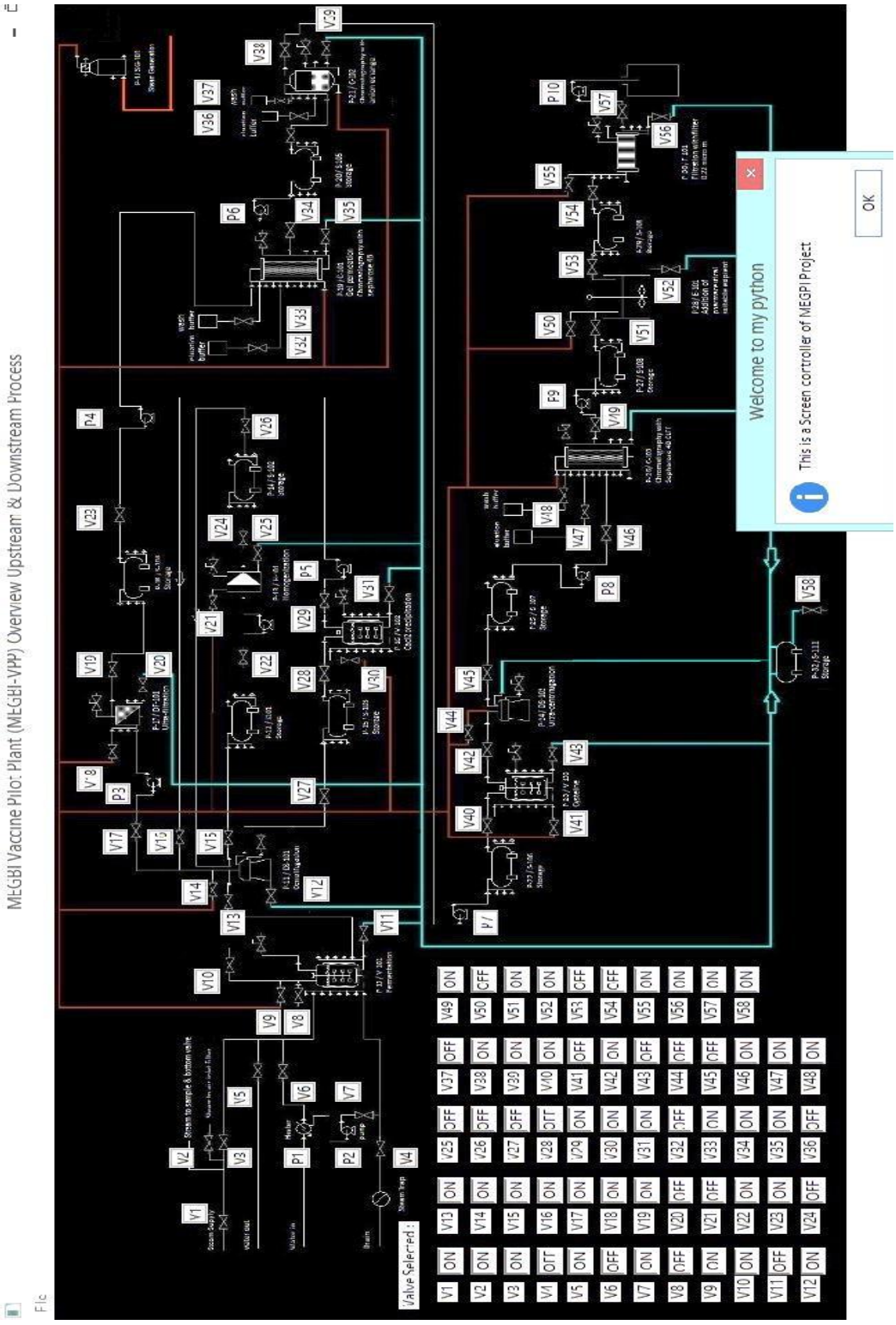


Figure 20 : *The Screen Controller it's done.*

Next step is to connect this controller screen to the Velleman board, and run it, for that too we needed a code to make the connection between the controller screen and the Velleman board.

This is the code:

```
#**********************                Run USB System                      **********************
#*****************************************************************************************************
    def OpenUSBBoardThread(self):
        self.dll = windll.K8061
        i = self.counterUSBBoards
        for doit in range(0,i+1):
            try:
                self.dll.OpenDevice()
                self.USBOpened = True
# debug info
                print ('USB Board is now connected!')
#end debug info
            except:
                txt = ('Please Check USB Board connection')
                print ('txt')
                return
```

Figure 21 : *Python 6*

Next of this code, must know how to connect the screen to the Velleman board, for that we must read about Velleman board, how to connect it ,how many inputs outputs ,the channels it has, and the actuators sensors which represents the outputs inputs .

And to connect the temperature sensors and get value, we must use a function to make the Velleman board read the value of the sensors and represents it in the label, to read the value should we use self.dll.ReadAnalogChannel(Adress,Channel) function ,the address of the board used is 0 and channel was 1. , next is the code:

```
            # temperature sensors value

        wx.StaticText(panel,-1," Temperature Value ",(100,12))
        self.temp_Vaporizer_out = wx.StaticText(panel, size = (26, -1), pos = (200, 10), style = LABELSTYLE )



        new_value = str(self.dll.ReadAnalogChannel(0,1))
        self.temp_Vaporizer_out.SetLabel(new_value)
        self.temp_Vaporizer_out.Refresh()
```

Figure 22 : *Python 7*

After knowing how to run, we must collect the circuit of the temperature sensor:

6.4 Adaptation of Daveices Sensors and Actuators

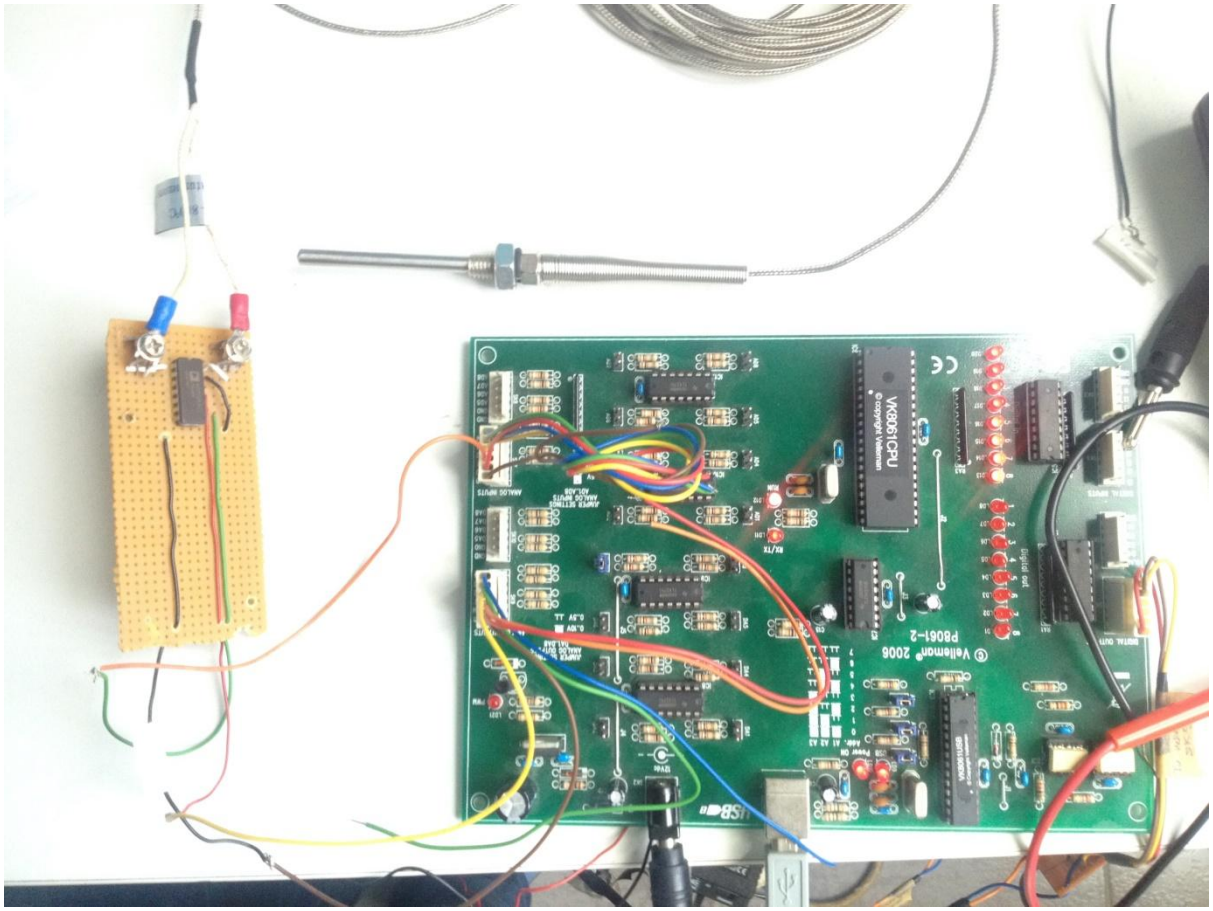


Figure 23 : *The circuit of Temperature sensor with Velleman board.*

This is the circuit, in which we have connected the temperature sensor to Velleman board across a connection, we have put it on inputs channel one but we have to connect it to output at same time, for even take electricity from that output it just the only way to filled.

So we need 2 connections, one on inputs channel 1 position 1, and other in outputs channel 2 position 1 plus ground and it's ready to work.

After the scan and test shows that there is the temperature sensor malfunction and it should be removed from the project for a reason of time constraints.

We take 3 shots to show how the sensor didn't give a constant value this shows that there malfunction, plus all the connection was right.

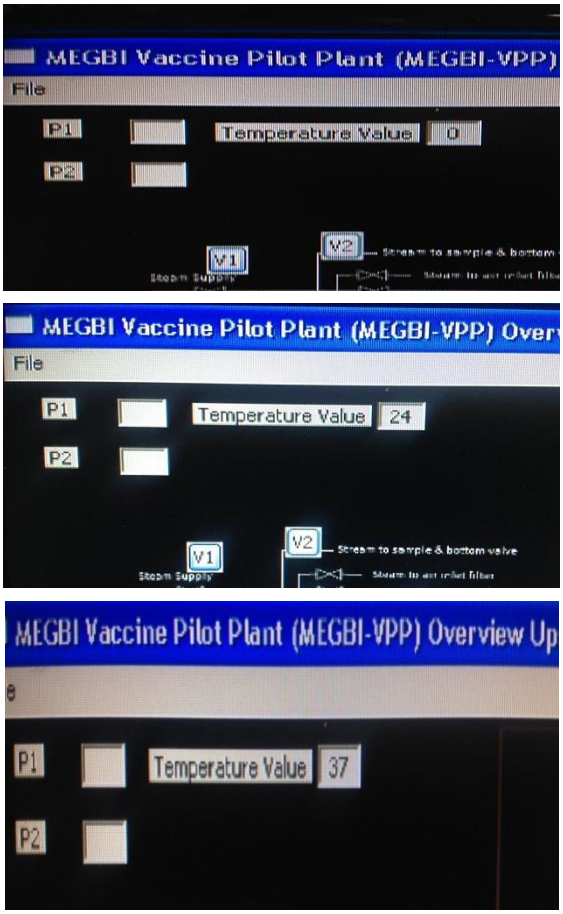


Figure 24 : *3 Shots show the malfunction.*

Now, how to connect the outputs represents across valves and pumps.

For example we worked on valve, must click on V1, that button give order to Velleman board to open or close the valve. So we have used a code to connect the python code valve to the board.

This is the code:

```
    def V1(self, event):
            valve_status_ON = 'ON'
            valve_status_OFF = 'OFF'

# open the USB board

        if  self.Valve1.GetLabel()==valve_status_OFF:
            self.Valve1.SetLabel(valve_status_ON)


            self.OpenUSBBoardThread()
            self.dll.SetDigitalChannel(0,1)
            wx.MessageBox("V1 is open ", "Open", wx.OK|wx.ICON_INFORMATION)
            self.timer = wx.Timer()
            self.timer.Bind(wx.EVT_TIMER, self.on_timer)
            self.timer.Start(0)
            wx.MessageBox("V1 is Open ","Open", wx.OK|wx.ICON_INFORMATION)
        else:
            self.Valve1.SetLabel(valve_status_OFF)
            time.sleep(2)
            self.dll.ClearDigitalChannel(0,1)
            print 'Digital Channel Cleared, V1 turn off'
            wx.MessageBox("V1 is Close","Closed", wx.OK|wx.ICON_INFORMATION)
```

Figure 25 : Python 8

We have used timer to get order fast if we click it opens directly and same to close it.

Now if I click on valve V1 they give V1 is open and the board take the order to open it, same to close it.

How to connect the valve to the Velleman board:

There should be a relay, like we see:

Must make a relay, we start with collect the contents

Needed:

- relay
- Small board
- Tapes
- Welding

the relay play the role of the brood , but with order.

For example , if a request from the board to open the actuators ,this brood will hatched and give the power required to actuators.

So when we click on button at screen,the board get order to open or close the actuators,and the relay is helping to do this order,across a signal from the board represent by 0.5V.

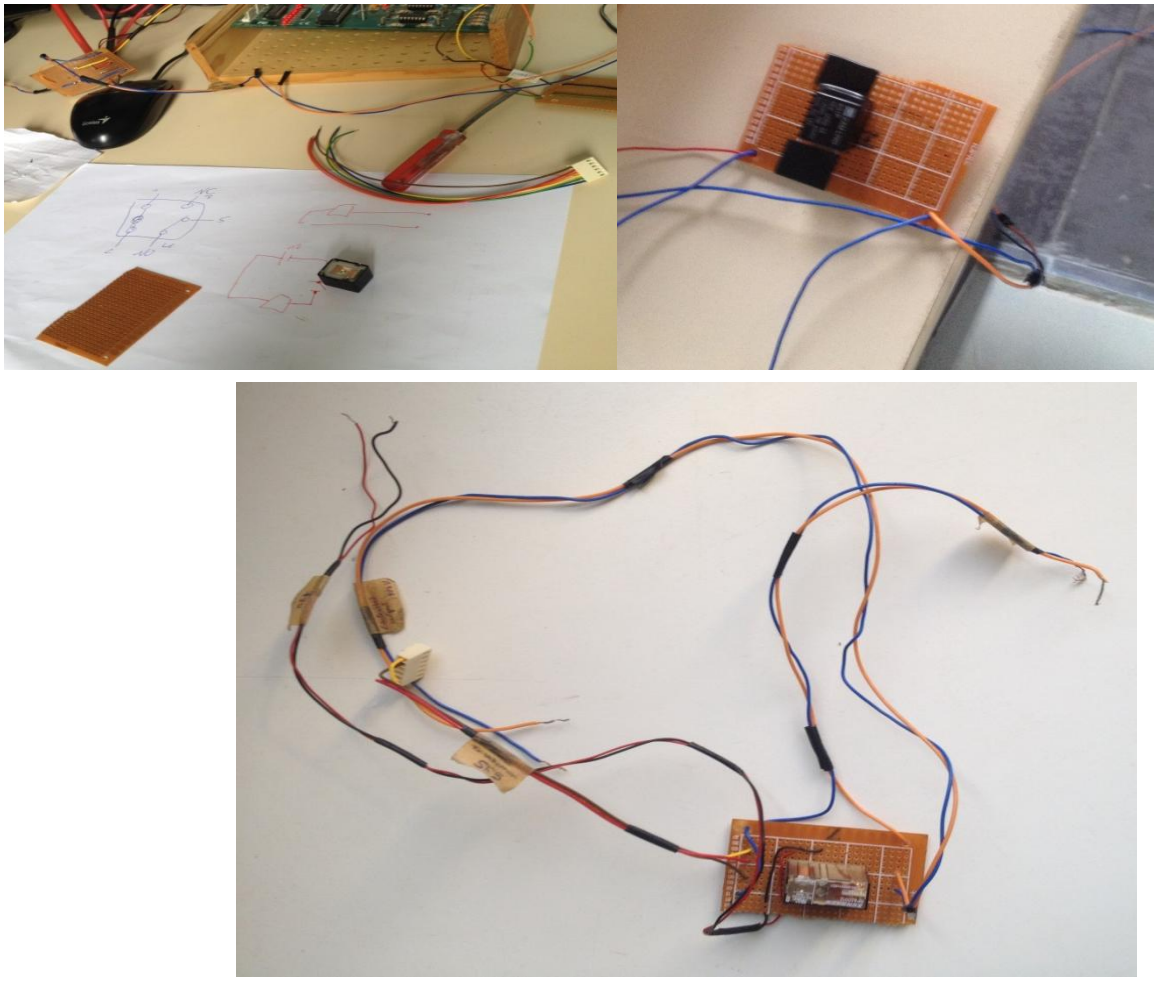


Figure 26: *Building a relay.*

This work like an electric transformer:

4 tapes:

- First one is getting 220V from source.
- the other is 220V linked to Valve(because the valve working on 220V)
- the other is connected to the source of Velleman board that mean take 12V
- The last one is connected to channel Analog outputs.

And we take a picture of the circuit:

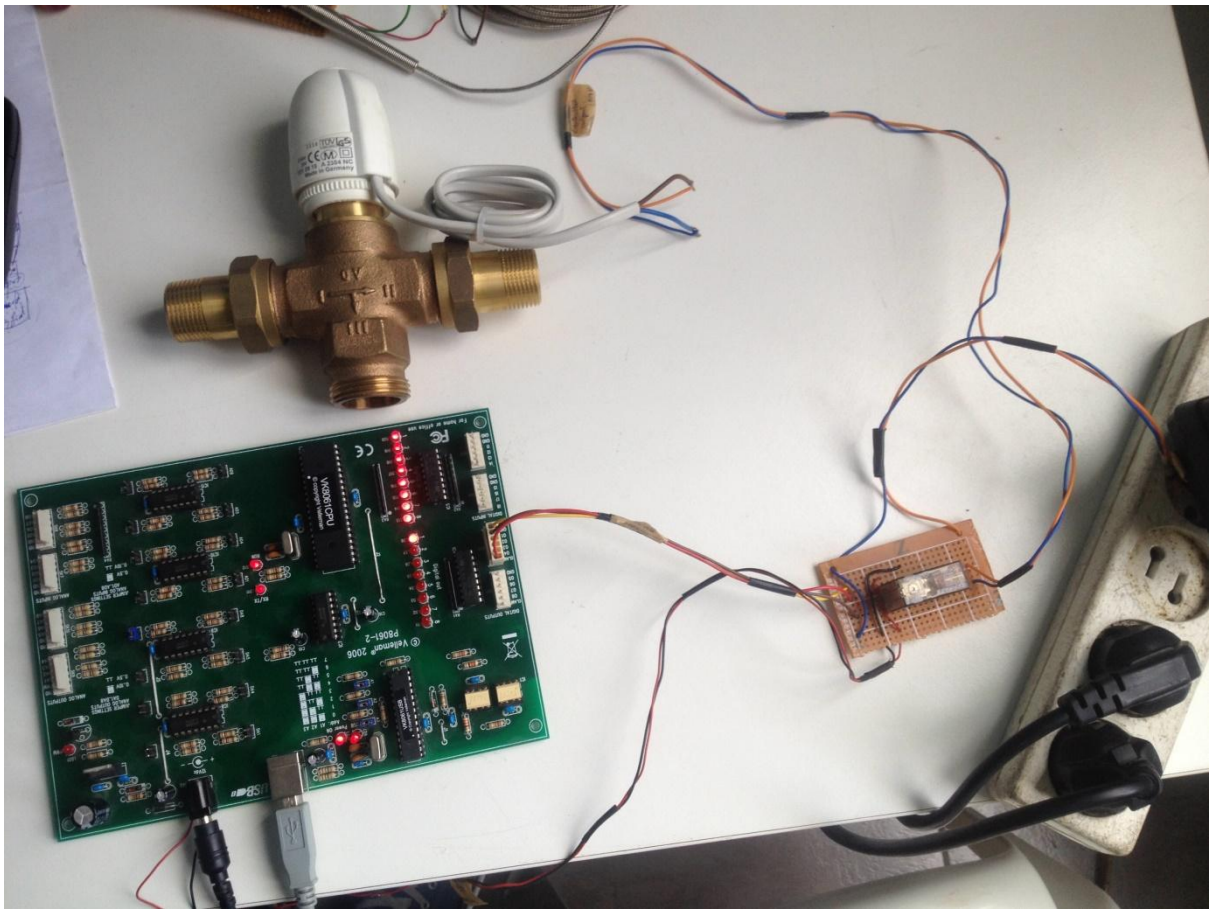


Figure 27 : *The circuit of Valve with Velleman board*

Now must test it:

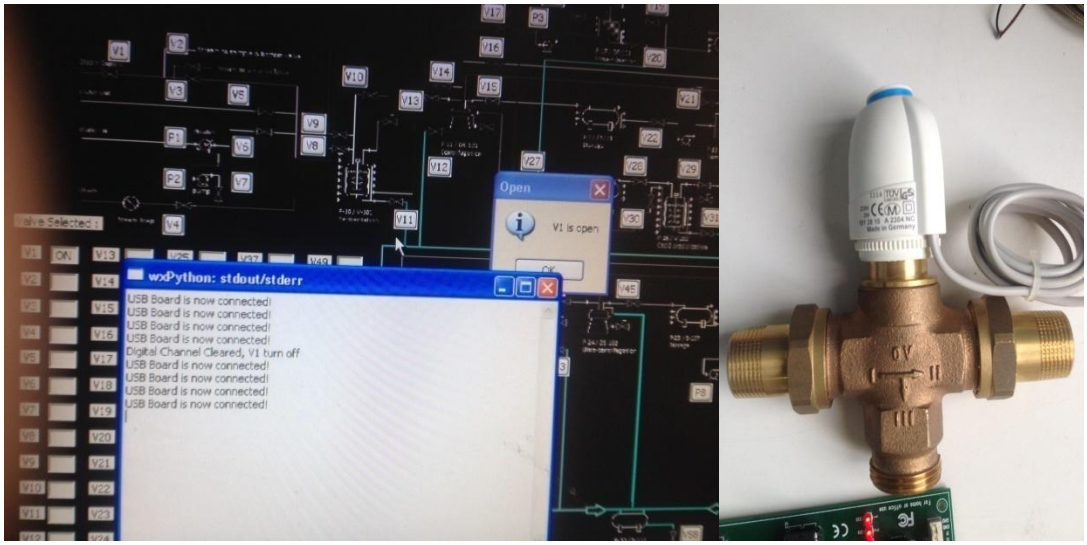


Figure 28 : *Testing the process.*

The valve is open, that blue color on valve means that the valve was open.

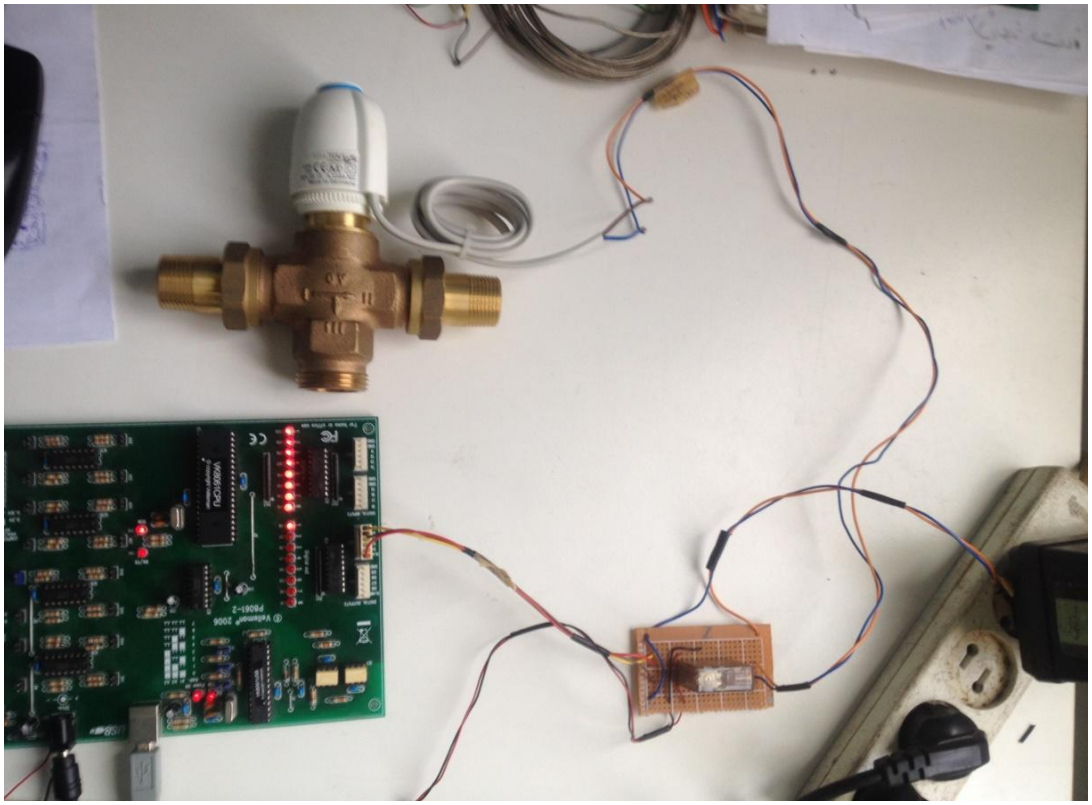


Figure 29 : *The circuit when the valve gets opened.*

They is red light on near the series of light, that light meaning the valve is getting order and open that is working when The button was clicked, so the valve was open.

Next click to close it, and we should showing it too:

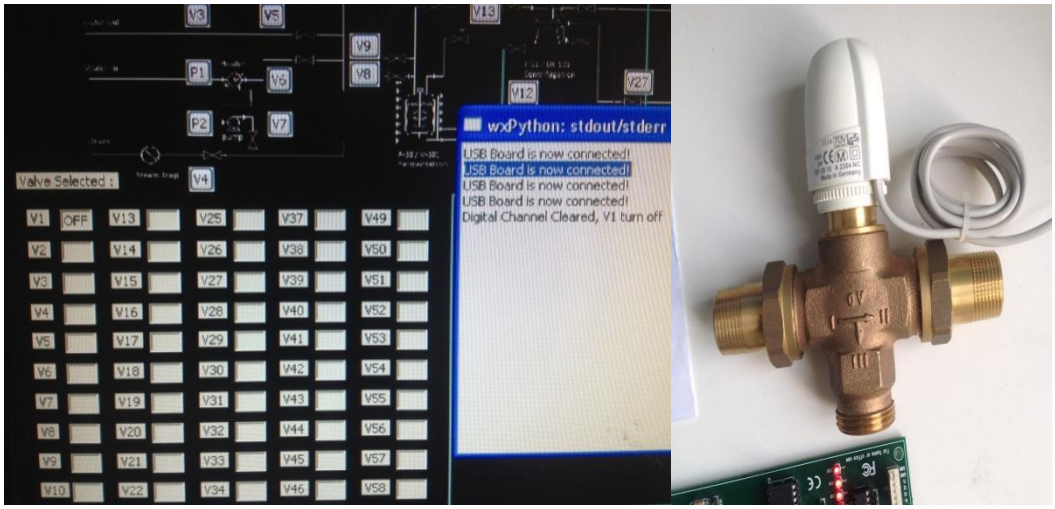


Figure 30 : *Testing Process.*

The valve is closed; we didn't saw the blue color.

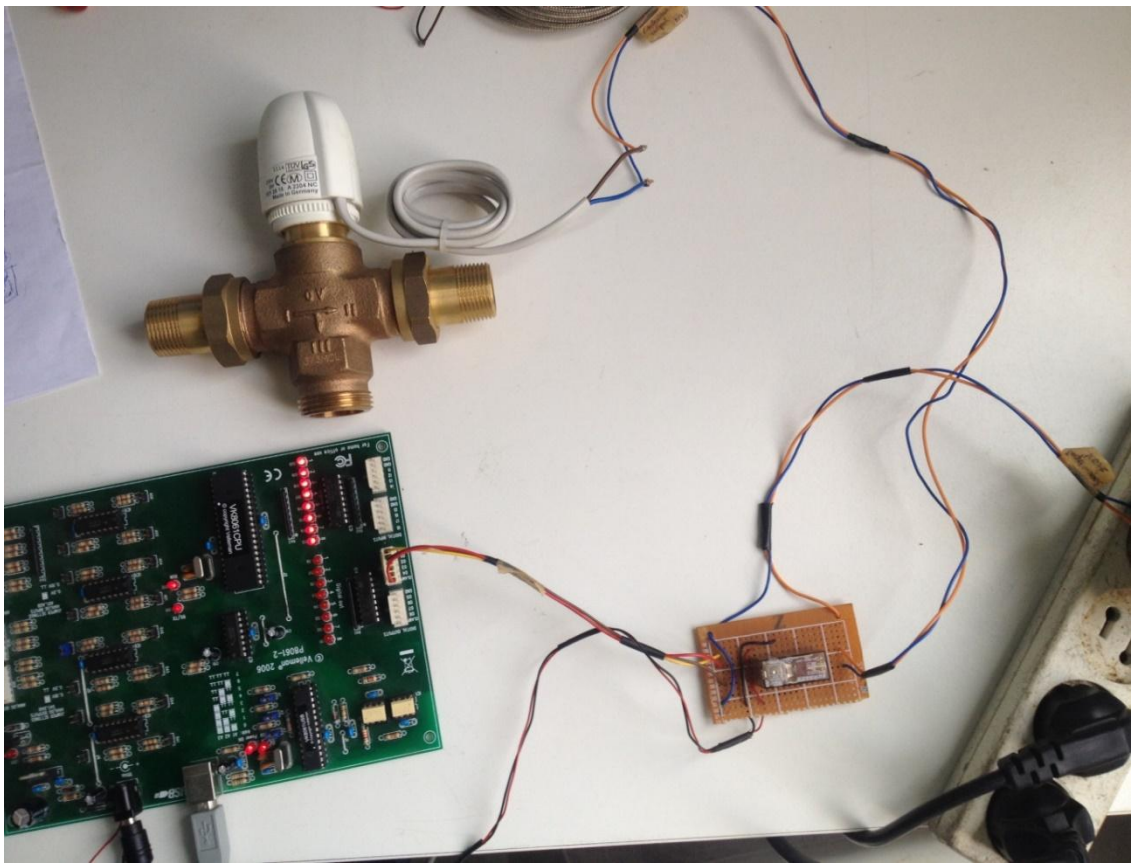


Figure 31 : *The circuit when the valve get closed.*

We saw in this picture the red light is off, that means the board gives order to valve to turn off, and is sawing in screen V1 "OFF".

Next, we have moved to the test stand, we have painted the circuit needed, across a website [2].

We have taken part of The Downstream processing, and we will test it across the board, valve, pump, and the control screen.

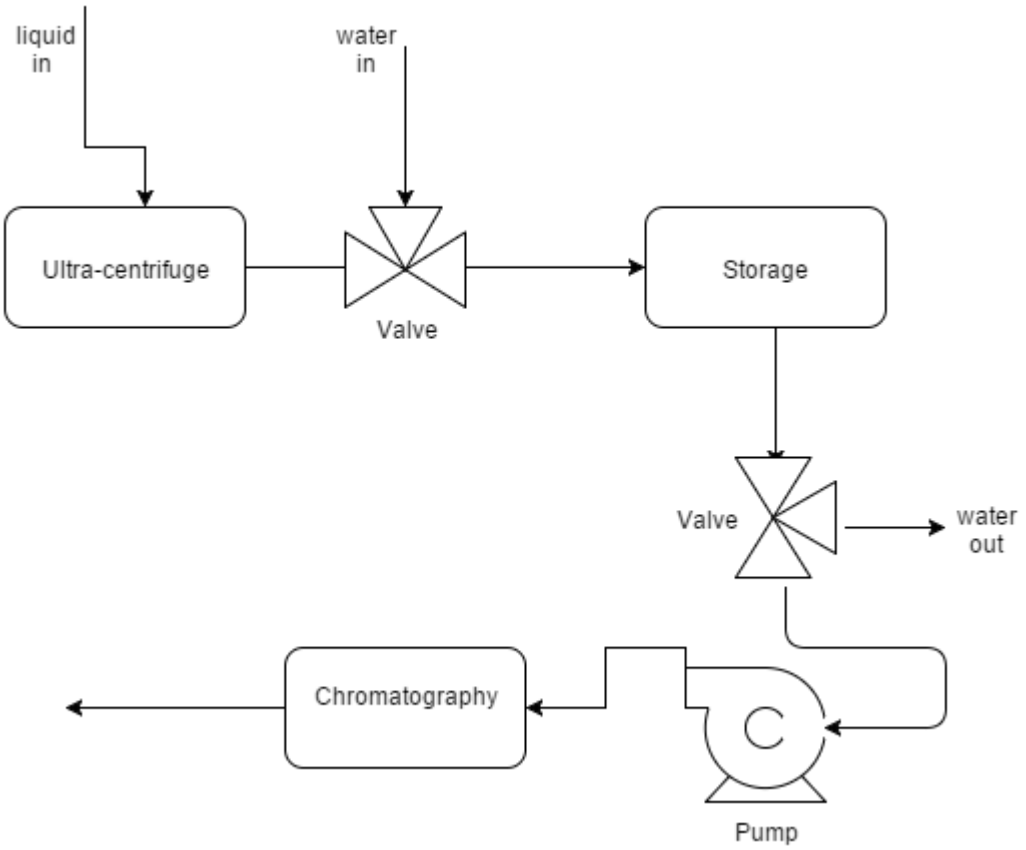


Figure 32 : *Diagram for the part of downstream processing used.*

We saw liquid enter to the ultra-centrifuge and continue across the valve to Storage.

After the storage, the liquid continue to Chromatography across valve and pump.

We saw in the way a line to water-in, that line is used to clean the storage, and more the line of water-out is used to get rid of the dirty water.

Across the diagram we will create a schema to the process that will test it.

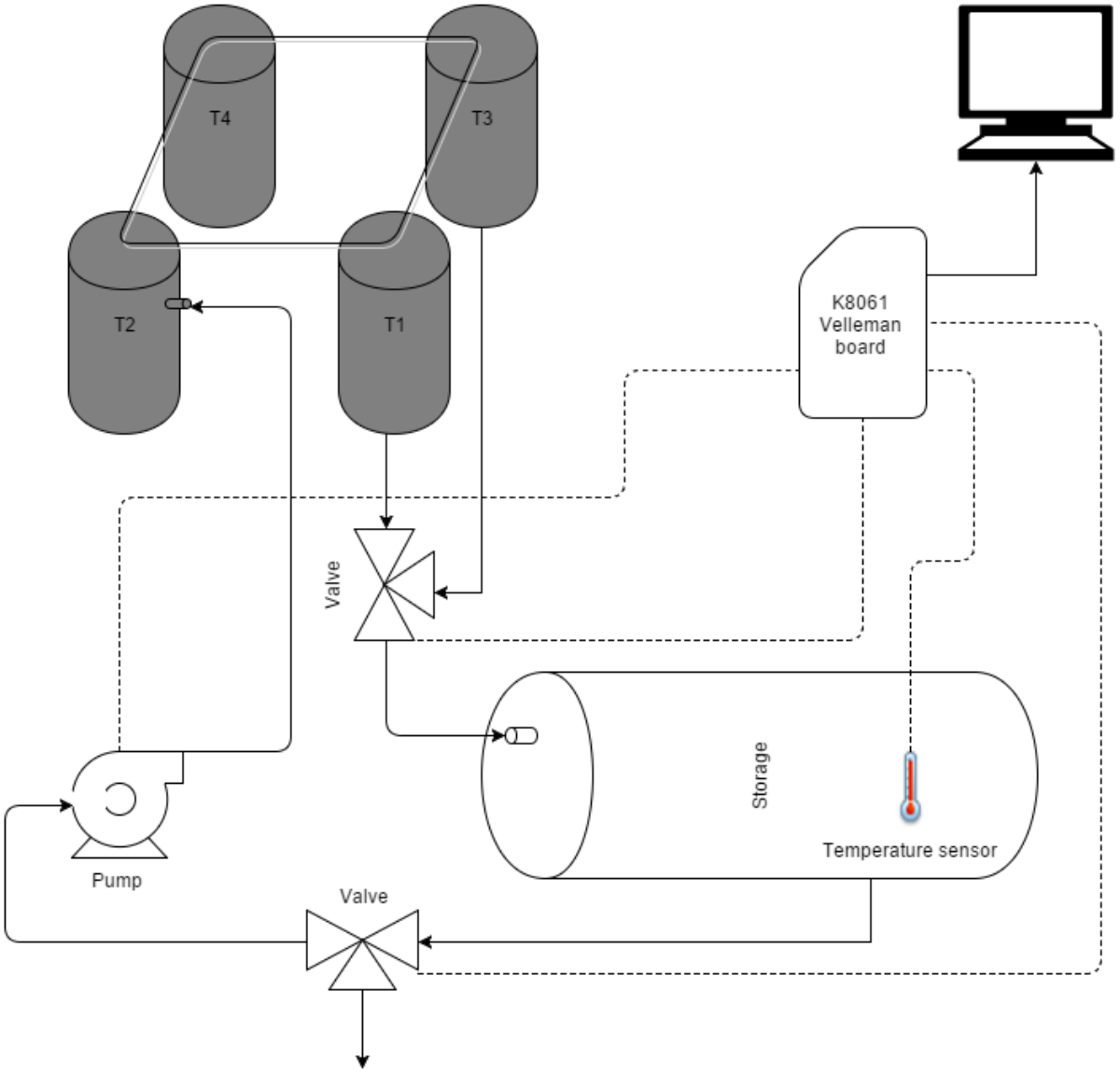


Figure 33 : *Schema of the part of downstream processing used.*

The contents of this schema:

- T1 represent the ultra-centrifuge.
- T2 represent the Chromatography.
- T3 represent the Storage of water used for cleaning.
- USB Velleman K8061 board connected to laptop.

This schema is a miniature of the really circuit.

Figure 34 : *Test stand.*

Like we saw in schema we have built the test stand:

The board is connected to pc, and the control screen was working and ready to test,

With using of plastic pipes, we have linked T1 to valve and the valve to the storage, plus T3 to the valve too.

On the other we have linked the storage to valve, the valve to pump and pump to T2.

We have worked hard to build it, like we saw, we used plastic pipes, the mechanics tools, cash cables …

Finally getting the test stand:

Figure 35 : *The test stand ready for work.*

6.5 Testing the automation System

We will examine all the actuators in the test stand to see if there is any problem or to see if everything is alright, it has been working on three tests:

1. *Test 1*

On this test , we will examine the valve by sending water from the ultra-centrifuge to the storage and we will exercise control over them and see if we can introduce water into the storage when we open the valve across pressing the button of valve on controller screen and the USB board will give the order to open.

In short,we will click the button of valve on screen to open it and the other click to close it.

Figure 36 : *Test 1.*

PRECONDITION	TEST ACTIVITY	POST CONDITION	TEST SUCCEED/FAILED
PIPES WITHOUT WATER	OPENING AND CLOSING THE VALVE.	FIRST CLICK VALVE OPEN OTHER CLICK VALVE CLOSE	SUCCEED

2. *Test 2:*

On this test ,will examine the valve with the pump by sending water from Storage to the Chromatography and will exercise control over them and see if we can introduce water into the Chromatography when we open the valve and turn on the pump,used pump here because the slot was from under, so the liquid have a low pressure, plus the Chromatography exist at high, so we must use pump to increase pressure.

Across pressing the button of valve with pump on controller screen and the USB board will give the order to open and turn on the pump.

In short,will click the button of valve with pump on screen to open it ,turn on the pump and other click to close it and turn off the pump.

Figure 37 : *Test 2.*

PRECONDITION	TEST ACTIVITY	POST CONDITION	TEST SUCCEED/FAILED
PIPES WITHOUT WATER	OPENING AND CLOSING THE VALVE WITH TURNING ON AND OFF THE PUMP.	FIRST CLICK VALVE OPEN AND PUMP TURN ON OTHER CLICK VALVE CLOSE AND PUMP TURN OFF	SUCCEED

3. *Test 3:*

In this test ,will examine the two previous two tests together.

Figure 38 : *Test 3.*

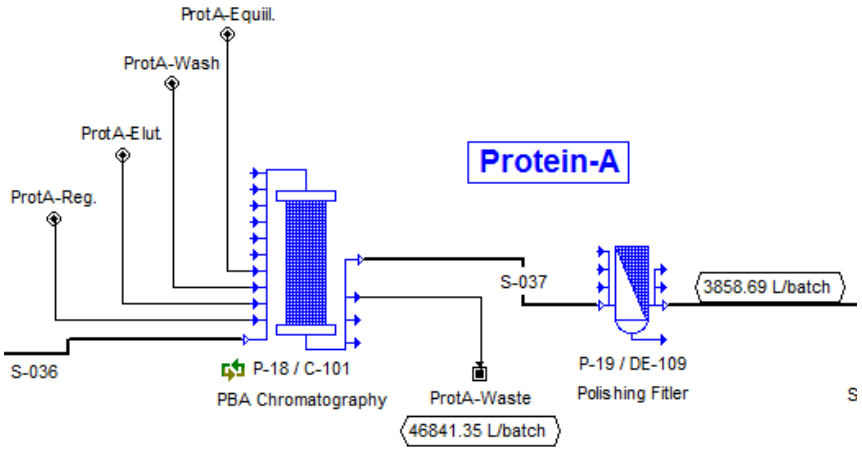
PRECONDITION	TEST ACTIVITY	POST CONDITION	TEST SUCCEED/FAILED
PIPES WITHOUT WATER	OPENING AND CLOSING THE VALVE(V1) WITH TURNING ON AND OFF THE PUMP.	VALVE1 OPEN PUMP TURN ON VALVE 2 OPEN	SUCCEED
	OPENING AND CLOSING THE VALVE (V2) AT SAME TIME.	VALVE 1 CLOSE PUMP TURN OFF VALVE 2 CLOSE	SUCCEED

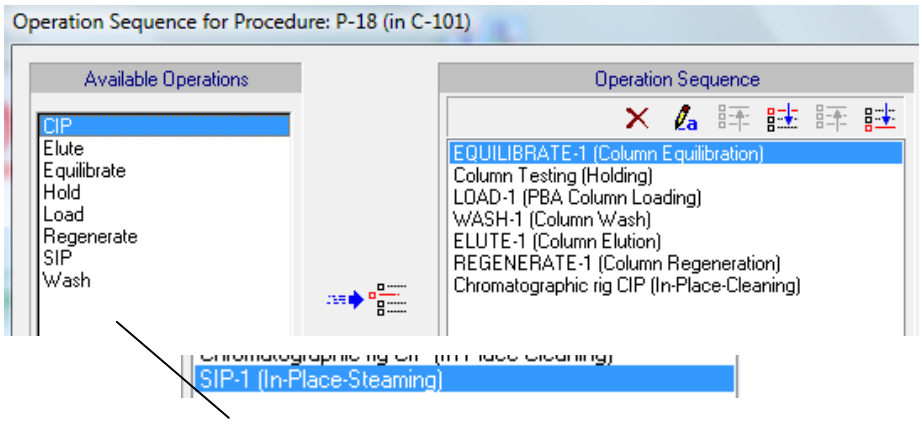
7. Functional Operation Algorithm of Automation System

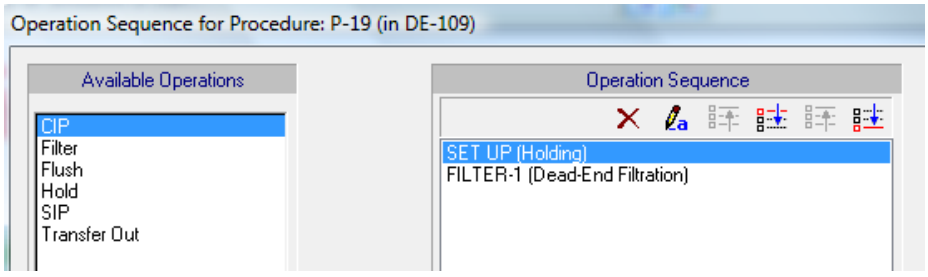
Shall be done in a pratical work (algorithm in python program): about 3 man months

State machine, Algorithm

For the whole plant devices such algorithms shall be implemented and then integrated. Please refer to the Automation System for TEMO-STPP (master thesis of Said Elmsaadi) in the 1stTEMO-STPP project report (2006-2008)







8. Integration of MEGBI–VPP

- Manufactoring of Devices and Integration. **Budget: 20.000 USD** (Material Costs: 10.000 USD, Personnel Cost: 4 months welder: 4000 USD, 4 months Eng.: 6000 USD)

Planned for Nov 15 - Feb 2016

- Integration of Automation System to the whole plant (**Material Budget: 10 000 USD**),

Planned for Mar-Aug 16 (as Master Thesis)

9. References / مراجع

Most Devices manufactor contacts from www.alibaba.com

SOFTWARES AND VIDEO DOCUMENTATION ARE FOUND ON THE DISC SUPPLIED WITH THIS DOCUMENT IN THE LAST PAGE