

MEGBI Vaccine Pilot Plant (MEGBI-VPP) - 3rd Project Report
(Jan 2014 – Dec 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, Functional operation algorithm for full automation)
- Manufacturing of a minimal system (without disc stack centrifuge, homogenizer, 2 columns)

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مركز أبحاث الشرق الأوسط للجينات والتقنية
البيولوجية

رأسنحاش – قضاء البترون – لبنان

**Middle East Genetics and
Biotechnology Institute (MEGBI)**

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TEMO Biotechnology

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Project Status at beginning of this project phase



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ابحاث عن الفحة



Recombinant Vaccine Technology / Biotechnological Upstream & Downstream Processing Hepatitis B DNA Vaccine Pilot Plant صناعة القحة عن طريق البيوتكنولوجيا Last update: 03 December 2013

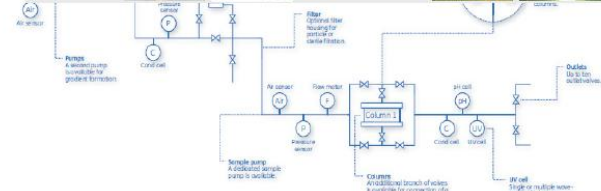
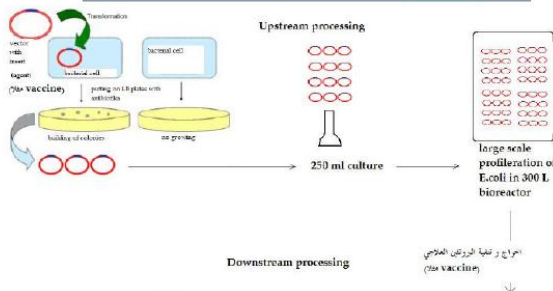
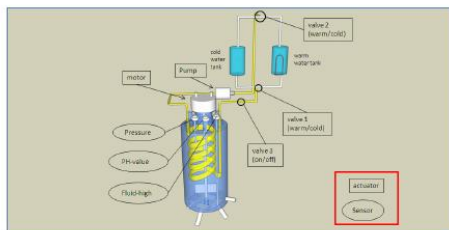
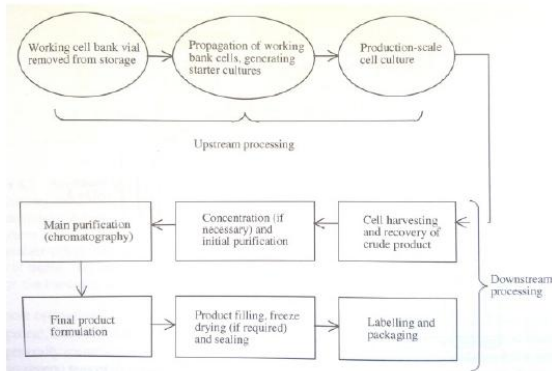


Fig 2. The liquid flow path.

اعمال حالية:

• Prototype for a Chromatographic Purification Machine for Production Scale

• الحاجيات لعام 2014:

- 1-2 اشخاص (مثلا طلاب ماستر في الهندسة الادوية الطبية او في الAutomation Engineering)
- \$34.000 تكلف مواد



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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) | | |
| Uniflux (Filtration) 10 | 130.000 € | 175.760 € |
| Akta ready | 140.000 € | 232.544 € |
| Ultracentrifuge | 50.000 € | 189.280 € |
| DNA Laboratory | | 67.600 € |
| 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 € |



Fig. 1. Uniflux 10 system configured for cassette filters and holder.



UNIFLUX 30: 161.200 Euro
 Filtration Tank (Basic: 250 L) 106.000 Euro
 UNIFLUX 10 (including 10 L tank) 135.800 Euro
 Installation/ Qualification Filtration 34.750 Euro

DF Uniflux: 305.000 EUR.



Akta ready: 150.000 EUR.



Xcellerex™ XDR cell culture bioreactor system: 240.000 EUR.

XDR-200 Bioreactor 190.360 Euro
 Fully configured: + 24.200 Euro
 Installation/ Qualification Bioreactor 34.750 Euro

MEGBI-VPP
 Akta based Draft Design
 (Prices 2014, +4% for 2015)


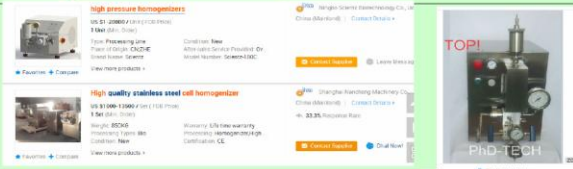

USP

AKTAreedy System 103.310 Euro
 AKTAProcess max.180 or 400 ltr: 131.200 Euro
 Gradient option (-Ready/-): Process: + 22.000 Euro
 Installation/ Qualification Chromatography : 20.300 Euro

DSF

1.2.2 Patent Based semi-own manufacturing

Last update: 24.12.2014

| Device No. | Device Name | Price including transport & customs | Supplier/Image |
|--------------|--|-------------------------------------|--|
| 1 | Disc Stack Centrifuge 1 | \$25,000 |  |
| 2 | Homogenizer Storage Tank PEG Precipitation mixing tank 3 100L | \$15,000 |  |
| 4 | Disc Stack Centrifuge 2 | \$25,000 |  |
| 5 | Storage Tank CaCl2 Precipitation mixing tank 5 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 10 100L | \$2,000 | |
| 11 | 20 L Chromatography column | \$3,000 | |
| 10 | 10 L DEAE matrix DEFF10 DEAE-Sepharose® Fast Flow | \$17,000 |  |
| 12 | Storage Tank 10 100L | \$2,000 | |
| 14 | Ultra-centrifugation CsCl | \$150,000 |  |
| 15 | 20 L Chromatography column | \$1,000 | |
| 16 | 20 L Sepharose 4BCLFF | \$18,000 | |
| 17 | Storage Tank 17 100L | \$2,000 | |
| 18 | sterile filtration 0.22 Micrometer | \$2,500 |  |
| 19 | Chemicals | 5000 | |
| 20 | Engineering 0 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

| | | |
|-------------------------------|----------|--|
| Service | | |
| Safety Cabinet B2 | 8,000 € | |
| centrifuge | 5,000 € | |
| Shake incubator | 7,500 € | |
| Thermocycler | 2,500 € | |
| Shed heater | 3,500 € | |
| Gel rack with voltage | 1,500 € | |
| Precision Balance | 800 € | |
| Mixing 80 °C freezer | 10,000 € | |
| UV Machine | 1,100 € | |
| water | 300 € | |
| precision pipettes | 500 € | |
| molecular biological reagents | 4,000 € | |
| chemicals | 2,000 € | |
| laboratory materials | 1,800 € | |
| laboratory tables | 1,500 € | |
| IT | 1,000 € | |
| Microscope | | |
| microscope | 3,000 € | |



Genetic Engineering Lab with Biosafety Level 2



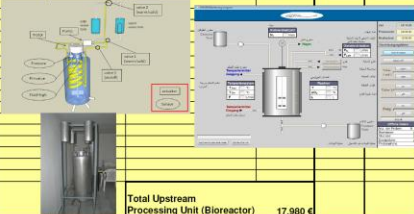
Transportation and customs
Building Lab 2009-11

6,000 €
25,000 €

Total Laboratory 79,700 €

Upstream Processing (Bioreactor)

| System | Piece | # | Price price | Total | Personnel Costs | price / month | Duration (months) | Qualification | price / month | Total | Personal Specific Costs | cost per month |
|---|------------------------|---|-------------|---------|------------------------|---------------|-------------------|---------------|---------------|----------|-------------------------|----------------|
| Behälter (150l Stainless) | | 1 | 600 € | 600 € | Integration Mechanics | 2,000 € | 1 Eng. | 2,000 € | 2,000 € | 2,000 € | Engineer | 2,000 € |
| Temperiersystem | Behälter Abdeckwolle | 1 | 50 € | 100 € | Integration Automation | 2,000 € | 1 Eng. | 2,000 € | 2,000 € | 2,000 € | Specialized Worker | 1,000 € |
| | Blech | 1 | 120 € | 120 € | System | 2,000 € | 1 Eng. | 2,000 € | 2,000 € | 2,000 € | | |
| | Pumpe | 1 | 80 € | 80 € | Programming Control | 2,000 € | 1 Eng. | 2,000 € | 2,000 € | 2,000 € | | |
| | Beheizungsrad | 1 | 60 € | 60 € | AECENAR | 2,000 € | 3 Eng. | 2,000 € | 2,000 € | 6,000 € | | |
| | Aut. Valve | 2 | 200 € | 400 € | Project Managing | 2,000 € | | | 2,000 € | 12,000 € | | |
| | Temp. Sensor | 1 | 20 € | 20 € | | | | | | | | |
| | PH Sensor | 1 | 100 € | 100 € | | | | | | | | |
| | PO ₂ Sensor | 1 | 1,200 € | 1,200 € | | | | | | | | |
| | Ausfluß | 1 | 200 € | 200 € | | | | | | | | |
| | Aut. Valve | 1 | 200 € | 200 € | | | | | | | | |
| | Medium Einfluß | 1 | 50 € | 50 € | | | | | | | | |
| | Behälter | 1 | 50 € | 50 € | | | | | | | | |
| | PH Regulierung | 1 | 50 € | 50 € | | | | | | | | |
| | Aut. Valve | 2 | 200 € | 400 € | | | | | | | | |
| | Aut. Valve | 1 | 200 € | 200 € | | | | | | | | |
| | Behälter | 1 | 50 € | 50 € | | | | | | | | |
| | Aut. Valve | 1 | 200 € | 200 € | | | | | | | | |
| | Siemens S7 PLC | 1 | 1,500 € | 1,500 € | | | | | | | | |
| | PC | 1 | 500 € | 500 € | | | | | | | | |
| Total Upstream Processing Unit (Bioreactor) 17,980 € | | | | | | | | | | | | |



Downstream Processing

| System | price per piece | pieces needed | Costs | Needed Staff |
|--|-----------------|------------------|------------|--|
| Hemogenizer | 1,000 € | 1 | 1,000 € | |
| Prototype Chromatogr. (Process Device (manu)) | 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 Downstream Processing Unit 76,200 € | | | | |
| Project Management | | | | |
| Program Managing over 9 months | | 9 months | 2,000,00 € | 18,000 € |
| Documentation | | | | 4,000 € |
| Total Project Management 22,000 € | | | | |
| Total MEGBI-VPP | | 195,880 € | | planned project duration: Dec 2014 - August 2015 |

DNA Transfer Laboratory

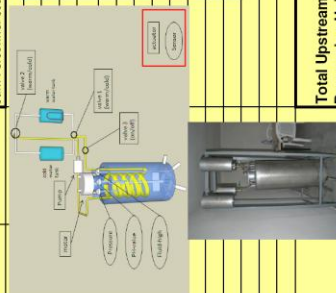
| device | price |
|-----------------------------|----------|
| Safety Cabinet B2 | 8.000 € |
| centrifuge | 5.000 € |
| Shake incubator | 2.500 € |
| Thermocycler | 2.500 € |
| Blood freezer | 3.500 € |
| Gel rack with voltage | 1.500 € |
| Precision Balance | 800 € |
| Minus 80 °C freezer | 10.000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



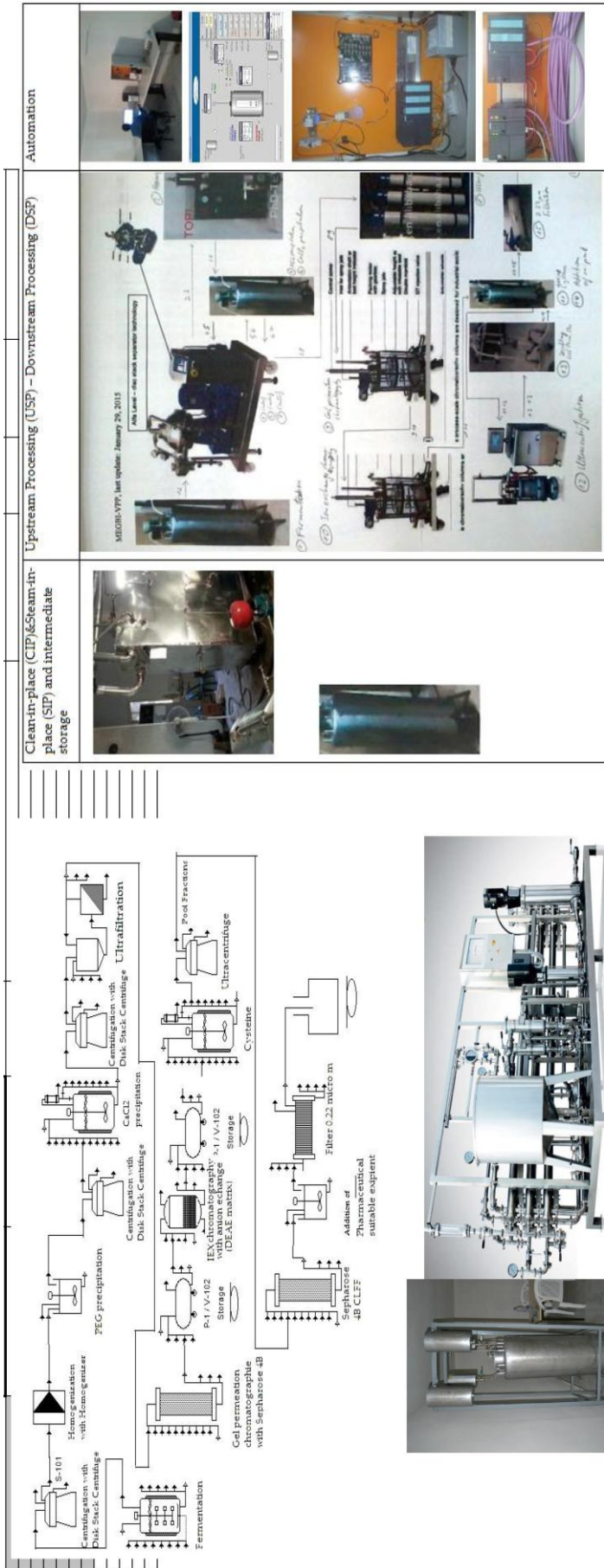
| Transportation and customs | 6.000 € |
|----------------------------|----------|
| Building Lab. 2009-11 | 25.000 € |

Total Laboratory 79.700 €

| Upstream Processing (Bioreactor) | | Material Costs | | Personnel Costs | | Personal Specific Costs | |
|----------------------------------|-------|---------------------|---------|-------------------------------|-------------------|---|---------------|
| System | Piece | # | price | Task | duration (months) | Qualification | price / month |
| Behälter (1300 Stainless) | 1 | 1 | 600 € | Integration Mechanics | 1 Eng. | 1 Eng. | 2.000 € |
| Temperiersystem | 2 | 2 | 50 € | Integration Automation System | 1 Eng. | 1 Eng. | 2.000 € |
| Abdeckwolle | 1 | 1 | 100 € | Programming Control | 1 Eng. | 1 Eng. | 2.000 € |
| Blech | 1 | 1 | 120 € | AECENAR | 3 Eng. | 3 Eng. | 2.000 € |
| Pumpe | 1 | 1 | 80 € | Project Managing | | | 6.000 € |
| Beheizungsrad | 1 | 1 | 60 € | | | | 2.000 € |
| | 2 | 2 | 200 € | | | | 2.000 € |
| Aut. Valve | 1 | 1 | 20 € | | | | 2.000 € |
| Temp.Sensor | 1 | 1 | 100 € | | | | 2.000 € |
| PH-Sensor | 1 | 1 | 100 € | | | | 2.000 € |
| PO ₂ Sensor | 1 | 1 | 1.200 € | | | | 6.000 € |
| Ausfluß | 1 | 1 | 200 € | | | | 2.000 € |
| Medium Einfluß | 1 | 1 | 200 € | | | | 2.000 € |
| Behälter | 1 | 1 | 50 € | | | | 50 € |
| Behälter | 2 | 2 | 50 € | | | | 100 € |
| Aut.Valve | 2 | 2 | 200 € | | | | 400 € |
| Behälter | 1 | 1 | 50 € | | | | 50 € |
| Aut.Valve | 1 | 1 | 200 € | | | | 200 € |
| Behälter | 1 | 1 | 1.500 € | | | | 1.500 € |
| Control System | 1 | 1 | 500 € | | | | 500 € |
| PC | 1 | 1 | 500 € | | | | 500 € |
| | | Sum Material | | | | 5.980 € | |
| | | | | | | Total Upstream Processing Unit (Bioreactor) 17.980 € | |
| | | | | | | 79.700 € | |
| | | | | | | 0 € | |
| | | | | | | 1.000 € | |
| | | | | | | 2.000 € | |
| | | | | | | 2.000 € | |
| | | | | | | 6.000 € | |
| | | | | | | 12.000 € | |
| | | | | | | 12.000 € | |



| 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 € | |
| Project Management | Total Devices 79,200 € | Integration (pipng,...) | 3,000 € | Total Downstream Processing Unit |
| Program Managing over 8 months Documentation | #months | 9 | 2,000,00 € | monthly rate for management |
| Total MEGBI-VPP | | | 195,880 € | Total Project Management |
| | | | | 76,200 € |
| | | | | 22,000 € |
| | | | | planned project duration: Dez. 2014 - August 2015 |



Automation

Upstream Processing (USP) – Downstream Processing (DSP)

Clean-in-place (CIP) & Steam-in-place (SIP) and intermediate storage

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

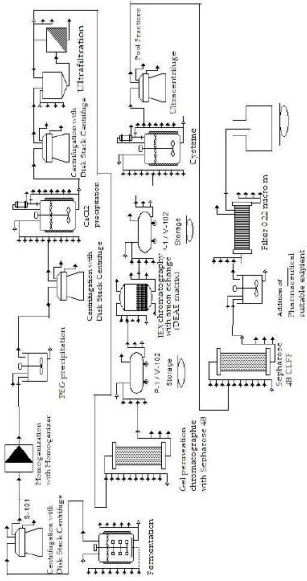
| | | | | | | |
|-----------------------------------|---------------|---|----------------------------|------|------|------|
| 2015 | 2016 | 2017 | 2018 | 2019 | 2020 | 2021 |
| MEGBI VPP DNA vaccine pilot plant | MAB (mabseed) | market access producing MAB (monoclonal antibodies) | supplier startup companies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

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), one phone call with Dr Monzer in March 15, then no further answers)

1.2.1 Budget Plan for completing a minimal Prototype Plan (Status November 15)

Missing Budget to complete a low cost MEGBI-VPP prototype

29.11.2015

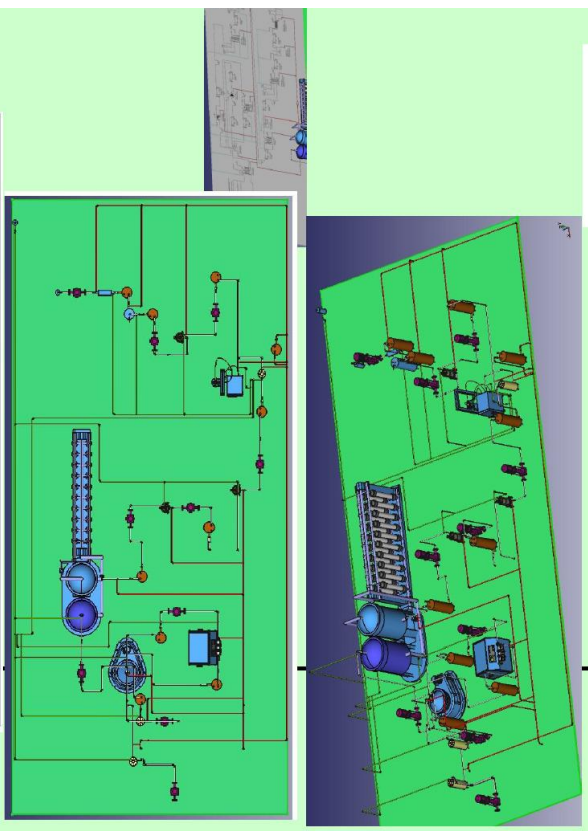


Clean-in-place (CIP) Steam-in-place (SIP) and intermediate storage

Upstream Processing (USP) – Downstream Processing (DSP)


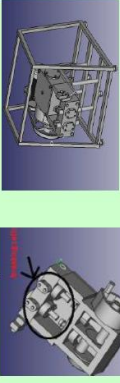
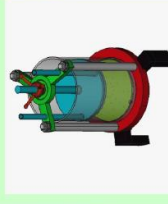
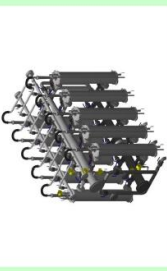

Automation


| System | Piece | # of open pieces | Piece price | Total |
|---------------------------|---------------|------------------|-------------|----------------|
| Behälter (130l Stainless) | 0 | 2 | \$500 | \$0 |
| 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 |
| PH ₂ -Sensoren | | 0 | \$1.200 | \$0 |
| Medium Einfluß | Aut. Valve | 1 | \$150 | \$150 |
| | Aut. Valve | 1 | \$50 | \$50 |
| PH Regulierung | Behälter | 1 | \$50 | \$50 |
| | Behälter | 2 | \$200 | \$400 |
| Beimpfungsreinigung | Behälter | 1 | \$50 | \$50 |
| | Aut. Valve | 1 | \$200 | \$200 |
| | | 0 | \$1.500 | \$0 |
| | PC | 0 | \$500 | \$0 |
| Sum Material | | | | \$2.080 |

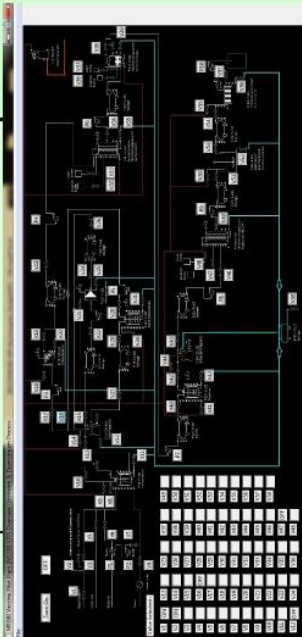


aus Deutschland zu holen ca. Dez. 2015

Open Issues

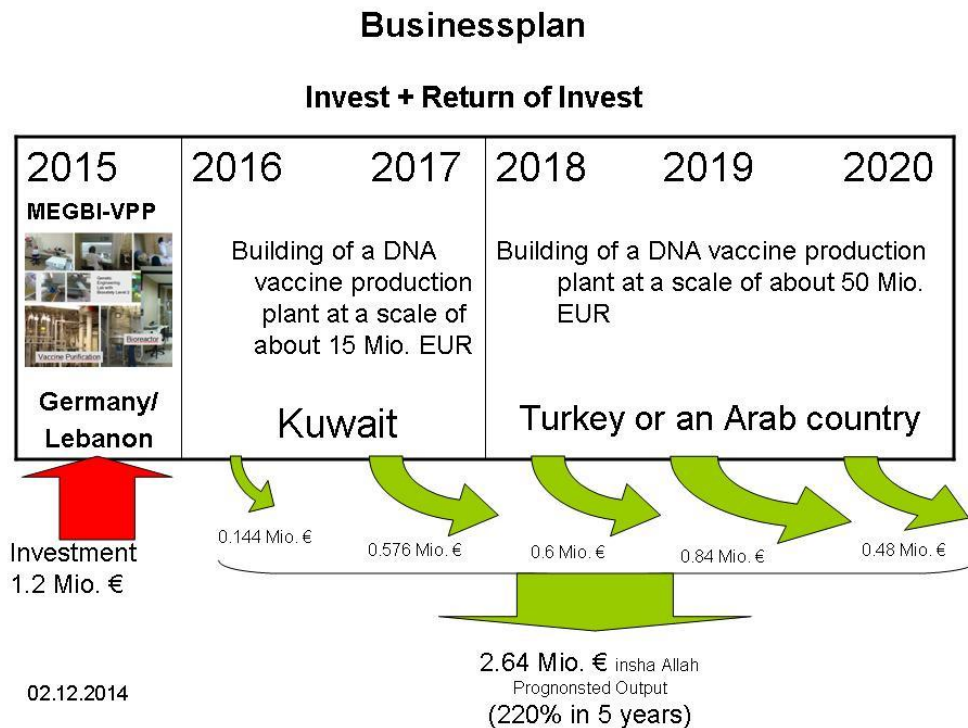
| Downstream Processing | # of mechanical pieces | average complexity/cost of part | price per piece | pieces needed | Costs | Needed Staff |
|---------------------------|------------------------|---------------------------------|--|---------------|---------|--------------|
| Disc Stack Centrifuge | 20 | \$70 |  \$1,400 | 1 | \$1,400 | |
| Homogenizer | 10 | \$120 |  \$1,200 | 1 | \$1,200 | |
| Chromatogr. Column (mech) | 8 | \$50 |  \$400 <div data-bbox="518 996 702 1198" style="border: 1px solid black; padding: 5px;"> <p>Operation Sequence</p> <ul style="list-style-type: none"> ELUTE-1 (Column Equilibration) Column Testing (Holding) LOAD-1 (PBA Column Loading) WASH-1 (Column Wash) ELUTE-1 (Column Elution) Strip (Column Regeneration) Pres (Column Wash) Chromatographic CP (In-Place-Cleaning) </div> | 3 | \$1,200 | |
| Ultrafiltration Unit | | |  \$200 <div data-bbox="710 1030 965 1288" style="border: 1px solid black; padding: 5px;"> <p>Operation Sequence</p> <ul style="list-style-type: none"> EQ (ULTRAFILTRATION) (Column Equilibration) Column Testing (Holding) LOAD-1 (PBA Column Loading) WASH-1 (Column Wash) ELUTE-1 (Column Elution) Strip (Column Regeneration) Pres (Column Wash) Chromatographic CP (In-Place-Cleaning) </div> | 1 | \$200 | |
| Ultracentrifuge (used) | | \$70 |  \$1,400 | 1 | \$1,400 | |

| | | | | | | | | | | | | | | | | | | | | | | | |
|----------------------|---|---|--|--|------|--|-------|---------|--|--|--|--|--|--|--|--|--|--|--|--|-----------|---------|----------|
| Mixing/Storing tanks |  | | | | | | | | | | | | | | | | | | | | | | |
| | | | | | \$60 | 14 | \$840 | | | | | | | | | | | | | | | | |
| | | | | | | Total Devices Integration (piping, ...) 1/2 inch | | \$6,240 | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | | | | | Total DSP | \$7,740 | |
| Automation System | | | | | | | | | | | | | | | | | | | | | | | |
| Item | | price per piece | | | | # of pieces | | | | | | | | | | | | | | | | | |
| pump | | \$60 | | | | 10 | | | | | | | | | | | | | | | | | |
| electronic valve | | \$50 | | | | 58 | | | | | | | | | | | | | | | | | |
| | | Total price | | | | | | | | | | | | | | | | | | | | | \$3,500 |
| | | | | | | | | | | | | | | | | | | | | | | | |
| | | Mechanical Parts USP | | | | | | | | | | | | | | | | | | | | | \$0 |
| | | Mechanical Parts DSP | | | | | | | | | | | | | | | | | | | | | \$7,740 |
| | | Automation System | | | | | | | | | | | | | | | | | | | | | \$3,500 |
| | | Total MEGBI-VPP Rest Budget Requirement | | | | | | | | | | | | | | | | | | | | | \$11,240 |



- This version includes:
- smaller vessels
 - simple disc stack centrifuge
 - cost reduction option: vessel with chemical opening of cells instead of homogenizer
 - disc stack centrifuge instead of ultracentrifuge
 - main new manufacturing: chromatographic columns (requires fine mechanical manufacturing facility)

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 Bouaffif | 100,25 € | 0,0083542% | Investition bezahlt (Überweisung ca. 11.12.14) |
| Nasser Al Araiimi | 1.200 € | 0,1000000% | Investition bezahlt (Überweisung 27.12.14) |
| David Yildiz | 600 € | 0,0500000% | Investition 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. There 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 Klingenhühl 2a
69123 Heidelberg, Germany

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

مركز البحوث المتكامل للبيولوجيا والتقنية الجينية والبيولوجية
رأسناش - قضاء النهرين - لبنان
Middle East Genetics and Biotechnology
Institute (MEGBI)
Main Road, Ras Nhache, Batroun, Lebanon,
www.aecenar.com/institutes/megbi
Email: info@aecenar.com

Genetic Engineering Lab with Biosafety Level 2

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



The screenshot shows the SynCo Bio Partners website. The header includes the company logo, the text "GMP Biopharmaceutical Manufacturing", and a search bar. A navigation menu contains links for Home, About Us, Services, How We Work, News & Events, Careers, and Contact Us. The "Services" section is expanded, listing various offerings such as Introduction, Process Development, Analytical, GMP Manufacturing (highlighted), Microbial Production, Process Validation, Aseptic Filling & Lyophilization, Quality Assurance, and Downloads. The "GMP Manufacturing" section features a photograph of a clean, industrial manufacturing facility with stainless steel equipment and a detailed text description of SynCo's multi-purpose GMP facilities, their capabilities for microbial-based biopharmaceuticals, and the benefits of outsourcing to SynCo.

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.


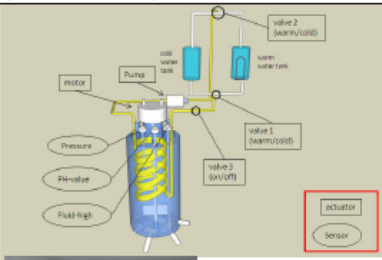

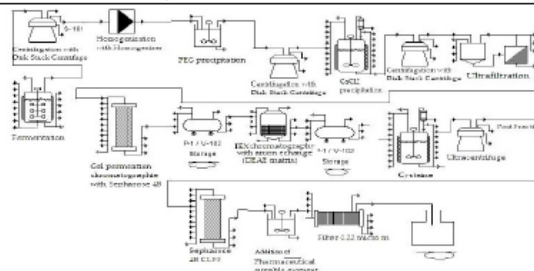
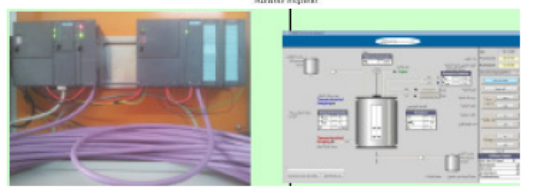
- **2008** – Expansion of process development capabilities to enable a greater range of projects to be completed.
- **2009** – Facility licensed by ANVISA for commercial production.
- **2011** – Expansion of the Class A zone of its aseptic filling facility successfully completed, allowing SynCo to support 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) | | Licensed for production of diagnostic antibodies (diagnosis of blood groups) in Lebanon/Jordan/Turkey and other Arab Countries | Licensed for production of Hepatitis B antigen vaccine (similar to Enderix) 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 Laboratory | Engineering | Upstream Processing (Bioreactor) | Processing | Downstream Processing Unit (Protein Purification) |
|---|---|---|---|---|
|  <p>Genetic Engineering Lab with Biosafety Level 2</p> |  |  |  |  |

2.2. 2016

A plant like this:

| 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 € |
| AltaReady | 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 € | 412.260 € |
| AltaProcess | 170.000 € | 223.840 € |
| Ultracentrifuge | 50.000 € | 67.600 € |
| DNA Laboratory | | 130.000 € |
| Total | | 1.164.280 € |



Fig. 1. Uniflux 30 system configured for smaller filtration volume.

Xcellerex™ XDR cell culture bioreactor system: 240 000 EUR

XDR-200 Bioreactor 180 380 Euro
Fully configured: + 24 200 Euro
Installation/ Qualification Bioreactor 34 750 Euro

UNIFLUX 30: 161 200 Euro
Filtration Tank (Basic 250 L) 108 080 Euro
UNIFLUX 10 (including 10 L tank) 135 800 Euro
Installation/ Qualification Filtration 24 750 Euro

DF Uniflux: 305 000 EUR

Alta ready: 150 000 EUR

MEGBI-VPP
Akta based Draft Design
(Prices 2014, +4% for 2015)

USP

AKTReady System 103 310 Euro
AKTProcess max. 180 or 400 l/h: 131 280 Euro
Gradient option [-Ready/ ~ Process: + 22 000 Euro
Installation/ Qualification Chromatography : 28 300 Euro

DSI

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 | |
| Automation System (GUI, veleman board) | 1.3.-15.10.2015 | Haitham Hindi (Master Student) |
| Design of mechanical parts with FreeCAD (Vessels, Storage Tanks, columns, Disc Stack Centrifuge, Ultracentrifuge, Homogenizer) | Mai - July 2015 | Practicants from BAU (Jihad, Zaher, Ibrahim, Fadi) |
| Materials List (sensors, actuators, chemicals to be purchased) | | |
| Manufacturing a low cost version of MEGBI-VPP (| 16.3.-30.7.15 | |

2. Basics

2.1 Recovery of recombinant *S.cerevisiae* 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 ($>1000 \text{ l m}^{-2} \text{ h}^{-1}$, regime I, $<2 \text{ min}$), followed by a rapid decay towards a low pseudo-steady state flux ($20 \text{ l m}^{-2} \text{ h}^{-1}$, regime II, $>2 \text{ min}$). Large pore membranes ($0.65 \text{ }\mu\text{m}$) were found to be more suitable for harvesting yeast ($10 \text{ }\mu\text{m}$ size) without cell leakage than smaller pore ones ($0.22 \text{ }\mu\text{m}$ and $0.45 \text{ }\mu\text{m}$). 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 $0.2 \text{ }\mu\text{m}$ 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^b](#) Copyright © 1995
Published by Elsevier B.V.:

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 throughput 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

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

Clark Baldwin and Campbell W. Robinson†

Department of Chemical Engineering, University of Waterloo,
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 | <u>10 mL</u> |

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 $\geq 20,000$ 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.

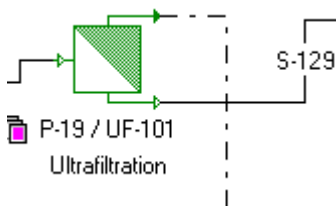
14. Run ethanol and leave 1/3 of a funnel volume of ethanol in the funnel.

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>

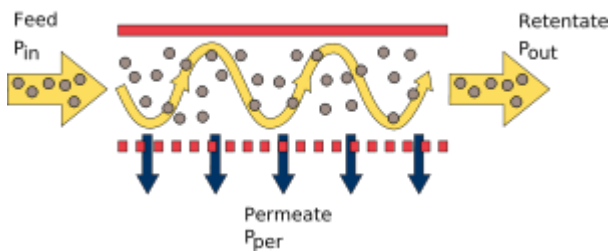
2.2 Crossflow filtration



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.

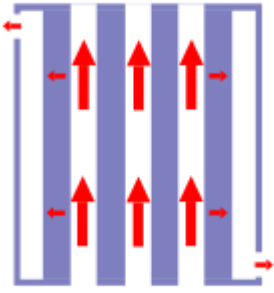


Diagram of cross-flow filtration

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, arbeitsexensive 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 chromatography¹

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 formulation²

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

Final product formulation brings the product in the final format. This involves:

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

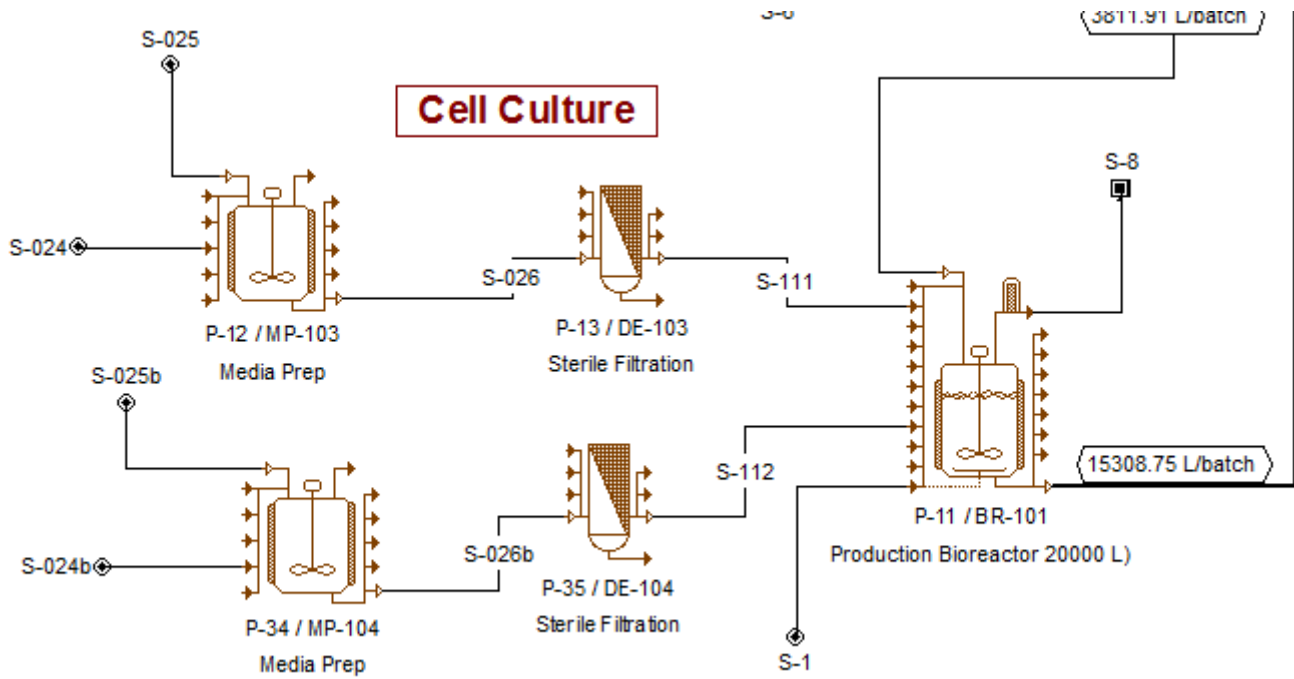


Fig.: 0.22 µm filter

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

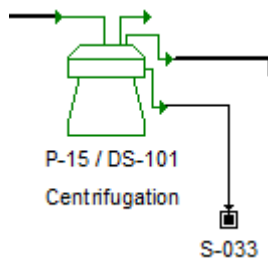
² [Walsh 2007], ch.6.9

2.6 Bioreactor/Tank

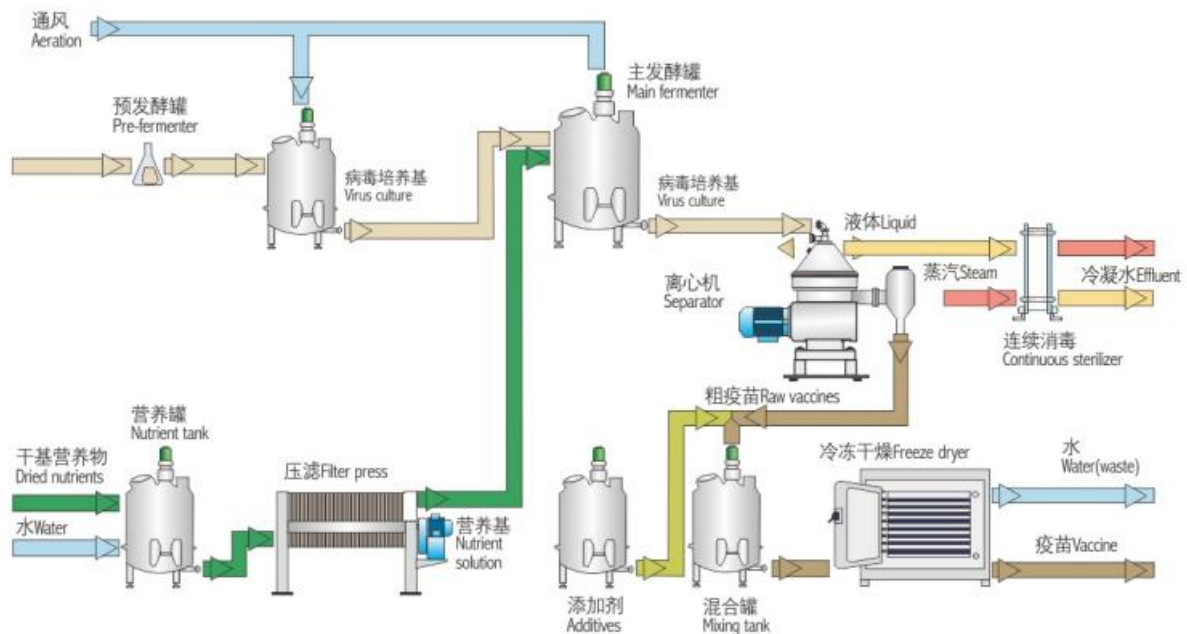


S.cerevisiae needs as nutrition only sugar.

2.7 Disc-Stack Centrifuge



2.7.1 Example for Supplier: Huading 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

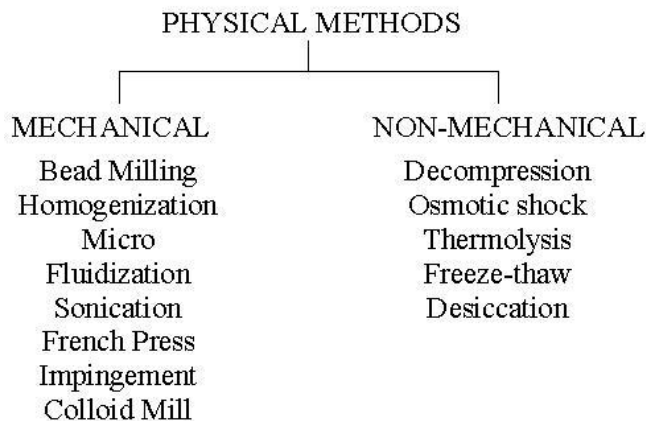
On Dec 27, 2014 8:50 AM, "Chouse Hu" <chousehu@huading-separator.com> wrote:
our cheapest centrifuge can be used in this case at all, that means nothing for you.

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.

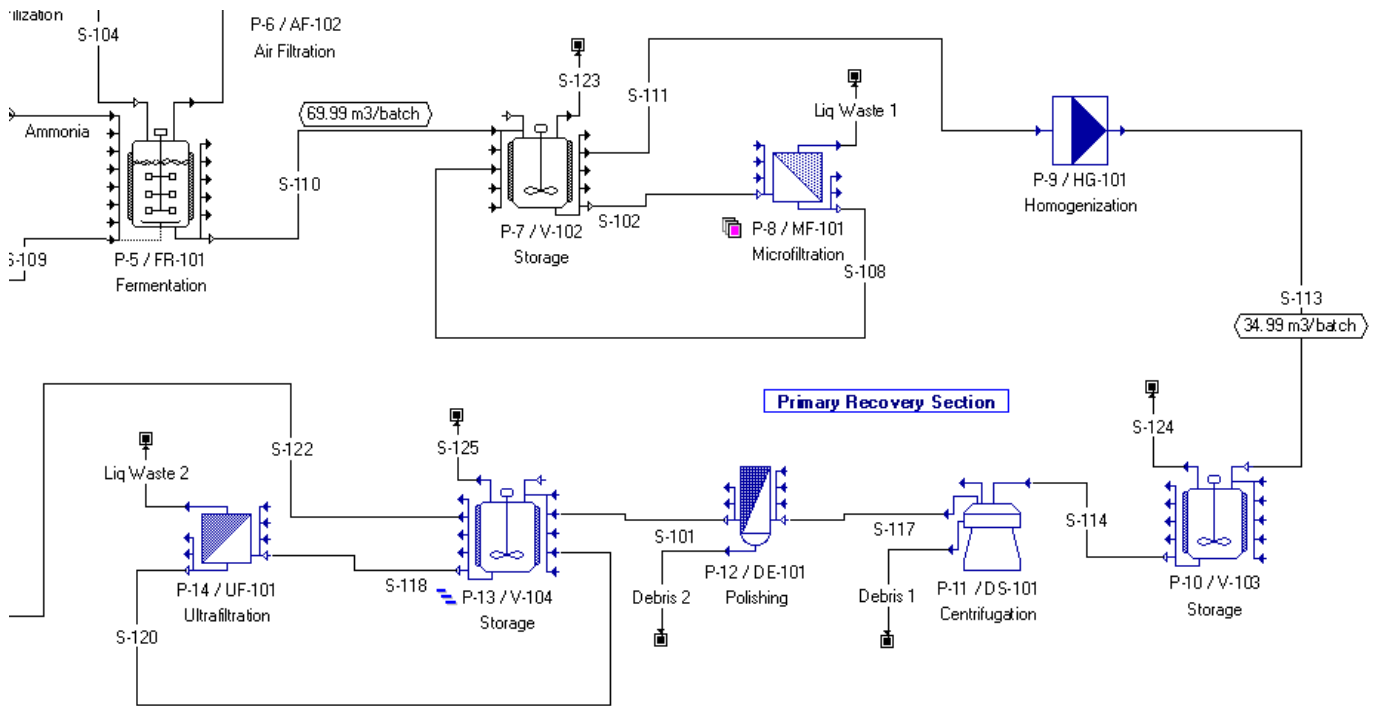
http://www.westfalia-separator.com/no_cache/contact/locations-worldwide/view.html

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.

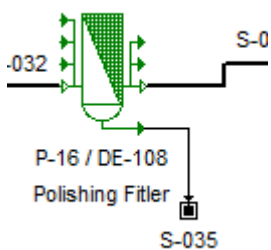
Model Number: GJB, Shipping Terms: FOB Port: Shanghai

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

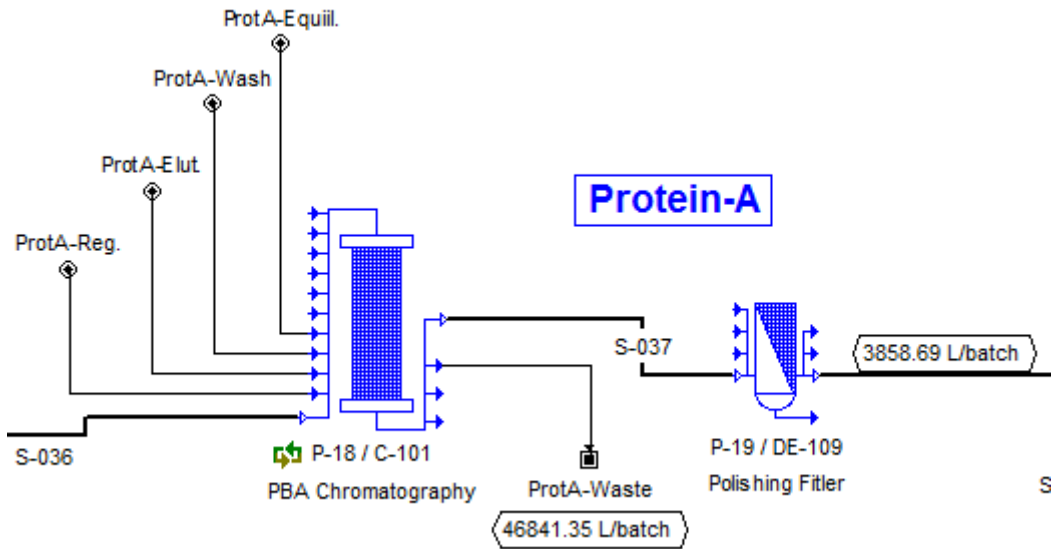
Lead Time: 30days Payment Terms: T/T Quotation Valid Till: 2015-1-28



2.9 Dead-End Filter



2.10 Protein-A Chromatography



Operation Sequence for Procedure: P-18 (in C-101)

Available Operations

- CIP
- Elute
- Equilibrate
- Hold
- Load
- Regenerate
- SIP
- Wash

Operation Sequence

- EQUILIBRATE-1 (Column Equilibration)
- Column Testing (Holding)
- LOAD-1 (PBA Column Loading)
- WASH-1 (Column Wash)
- ELUTE-1 (Column Elution)
- REGENERATE-1 (Column Regeneration)
- Chromatographic rig CIP (In-Place-Cleaning)

SIP-1 (In-Place-Steam)

Operation Sequence for Procedure: P-19 (in DE-109)

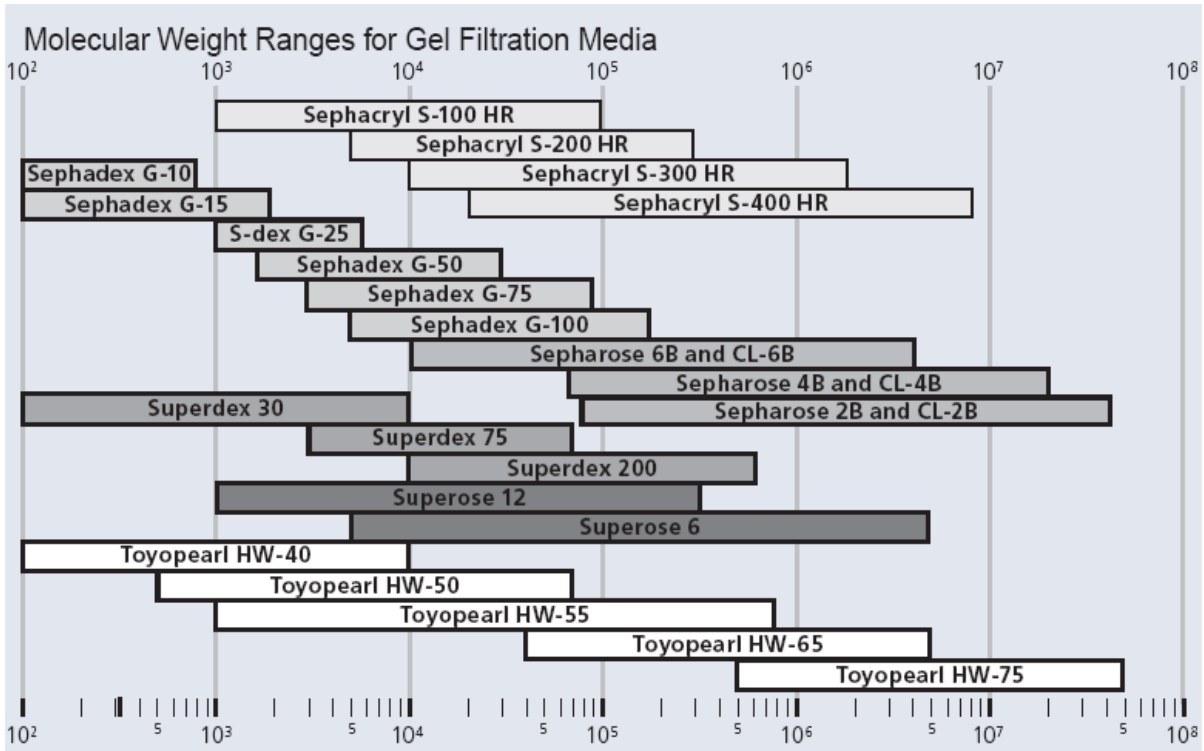
Available Operations

- CIP
- Filter
- Flush
- Hold
- SIP
- Transfer Out

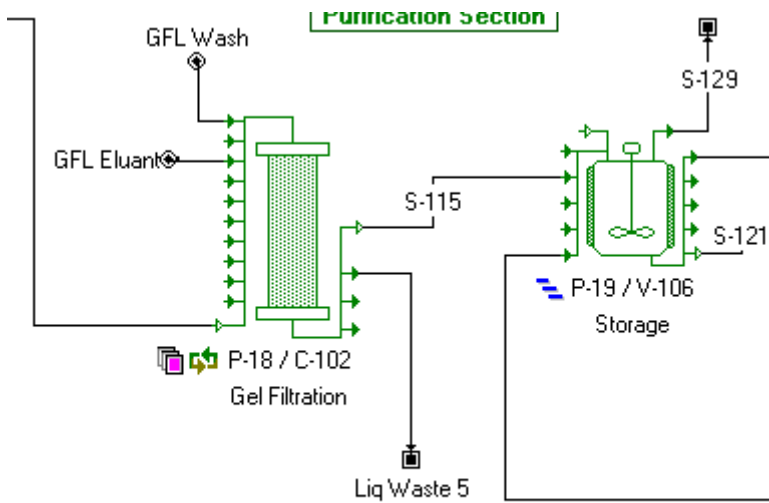
Operation Sequence

- SET UP (Holding)
- FILTER-1 (Dead-End Filtration)

2.11 Gel filtration chromatography



4B Sepharose



2.11.1 sensor/actuator list

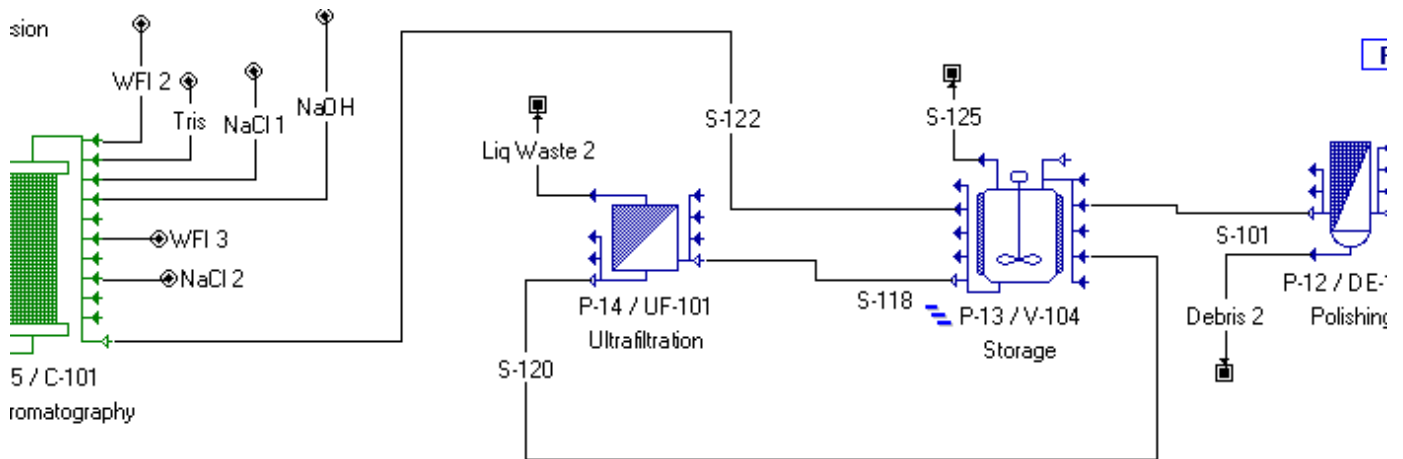
similar to AKTA process

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 |

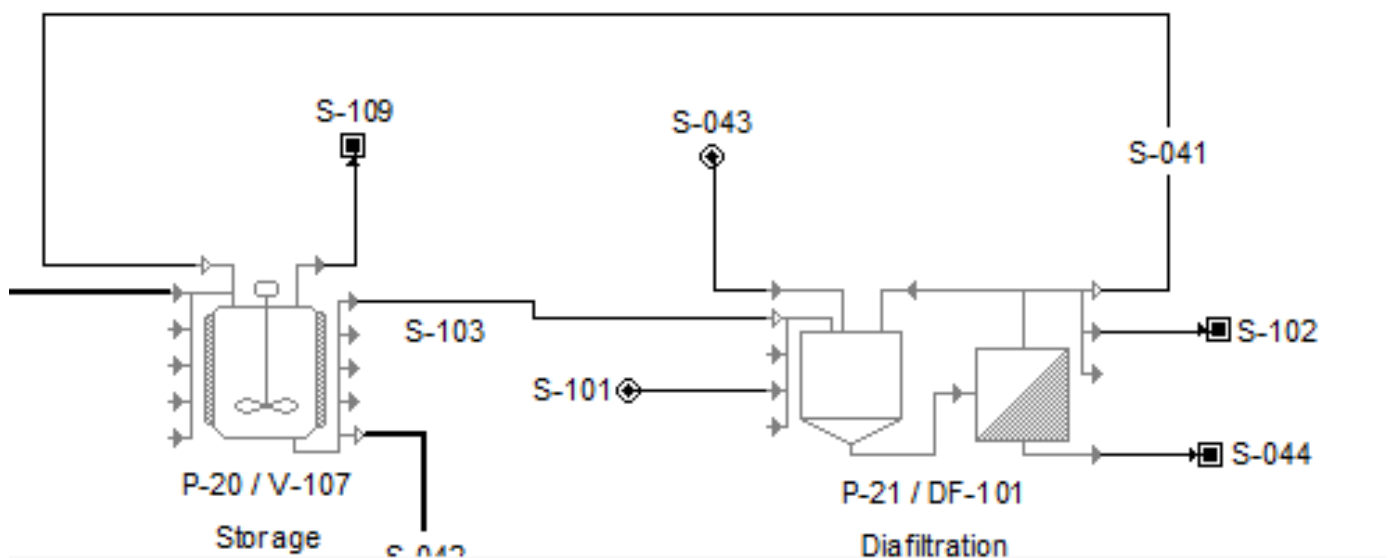
2.12 Ultrafiltration



From SuperPro Example Bgal

2.13 Diafiltration

Same equipment as ultrafiltration



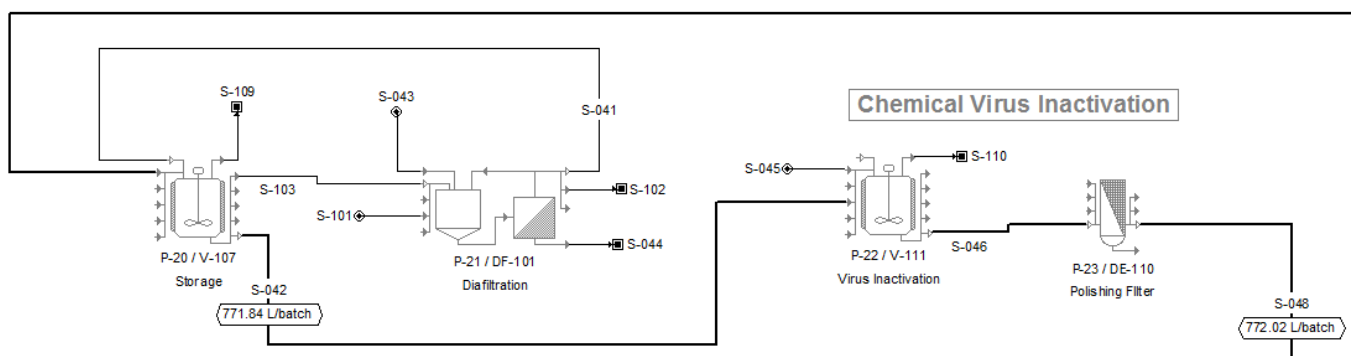
Operation Sequence for Procedure: P-20 (in V-107)

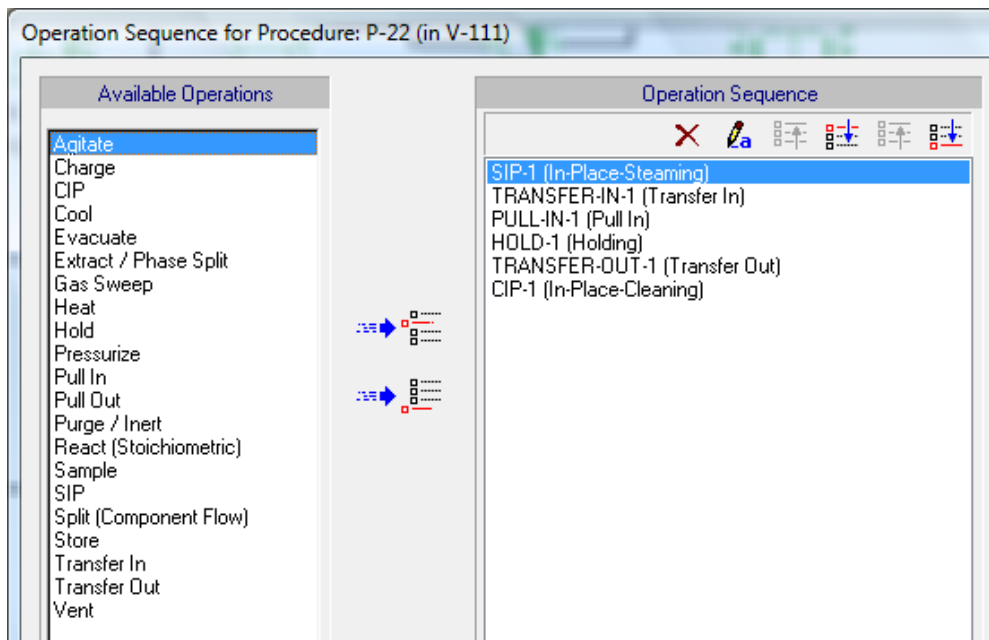
| Available Operations | Operation Sequence |
|------------------------|-------------------------------|
| Agitate | SIP-1 (In-Place-Steaming) |
| Charge | SET UP (Holding) |
| CIP | TRANSFER-IN-1 (Transfer In) |
| Cool | TRANSFER-OUT-1 (Transfer Out) |
| Evacuate | TRANSFER-IN-2 (Transfer In) |
| Extract / Phase Split | TRANSFER-OUT-2 (Transfer Out) |
| Gas Sweep | CIP-1 (In-Place-Cleaning) |
| Heat | |
| Hold | |
| Pressurize | |
| Pull In | |
| Pull Out | |
| Purge / Inert | |
| React (Stoichiometric) | |
| Sample | |
| SIP | |
| Split (Component Flow) | |
| Store | |
| Transfer In | |
| Transfer Out | |
| Vent | |

Operation Sequence for Procedure: P-21 (in DF-101)

| Available Operations | Operation Sequence |
|----------------------|-----------------------------|
| CIP | SIP-1 (In-Place-Steaming) |
| Concentrate (Batch) | FLUSH-1 (Flush) |
| Diafilter | DIAFILTER-1 (Diafiltration) |
| Flush | CIP-1 (In-Place-Cleaning) |
| Hold | |
| SIP | |

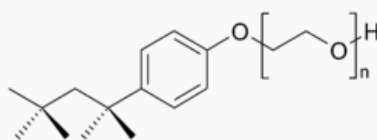
2.14 Chemical Virus Inactivation





2.14.1 Solvent/detergent (S/D) inactivation³

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

2.15 Introduction to Siemens S7 PLC system

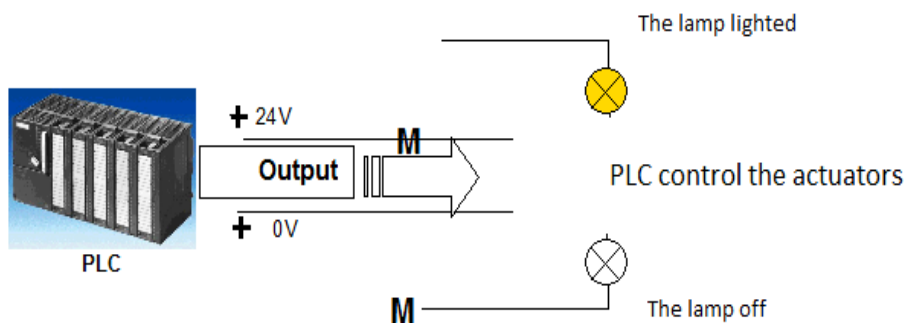
2.15.1 Definition of PLC:

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.



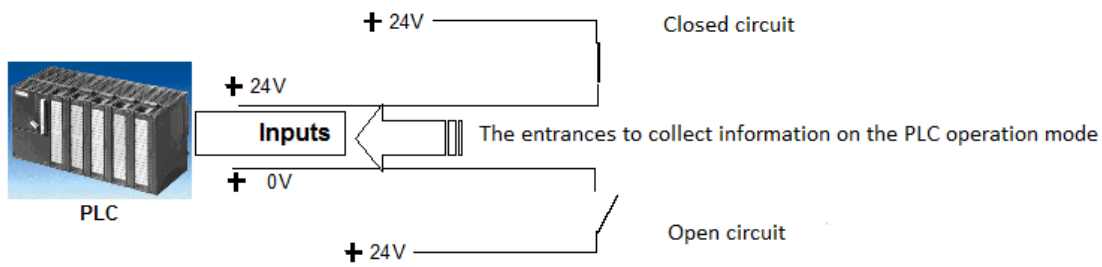
2.15.2 Function of PLC:

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.

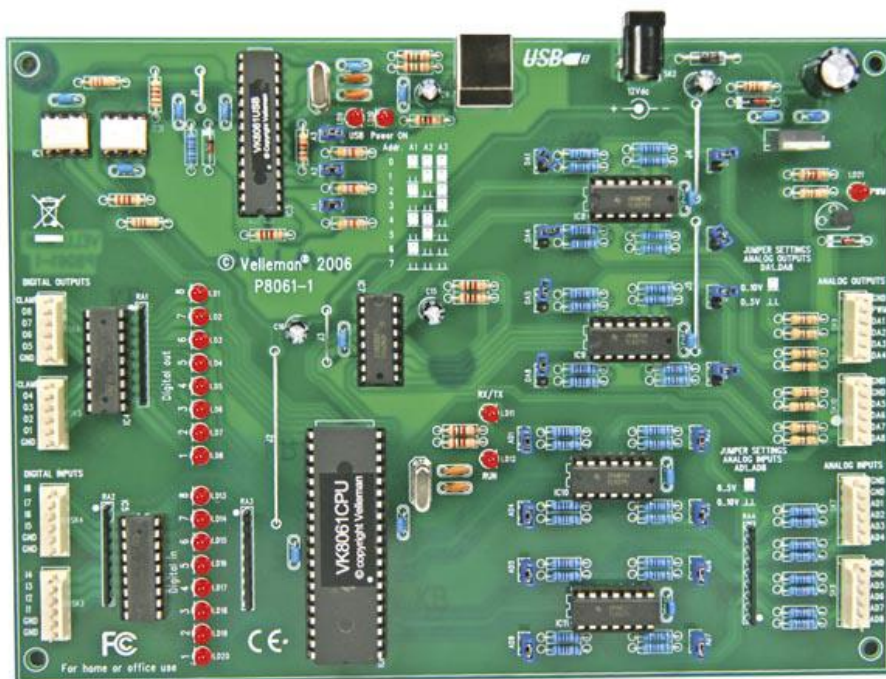


2.15.3 Where to get the information about the PLC operation mode:

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:

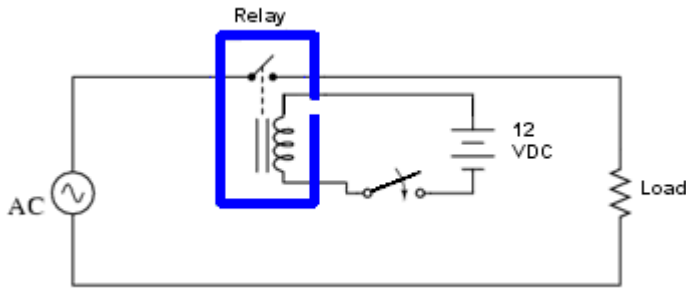


The K8061 interface board has 8 digital input channels and 8 digital output channels. In addition, there are 8 analogue inputs, 8 analogue outputs and one PWM output. The number of inputs/outputs can be further expanded by connecting more (up to a maximum of eight) cards to the PC's USB connectors. Each 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)

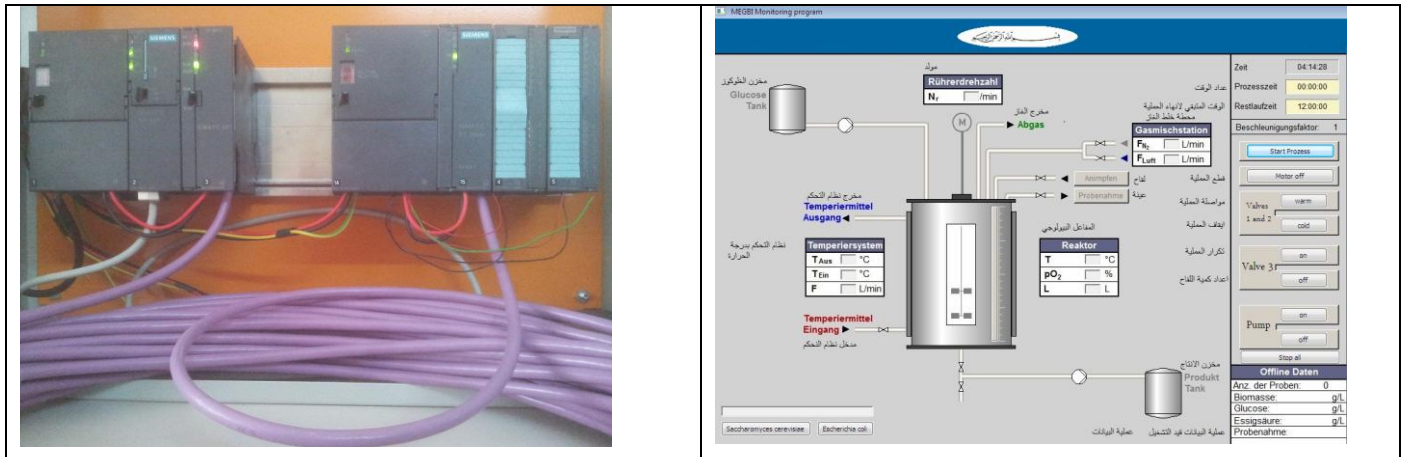
The stainless structure shall be built by TEMO. The instrumentation and special pipes etc. shall be bought for GE Health or others.



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

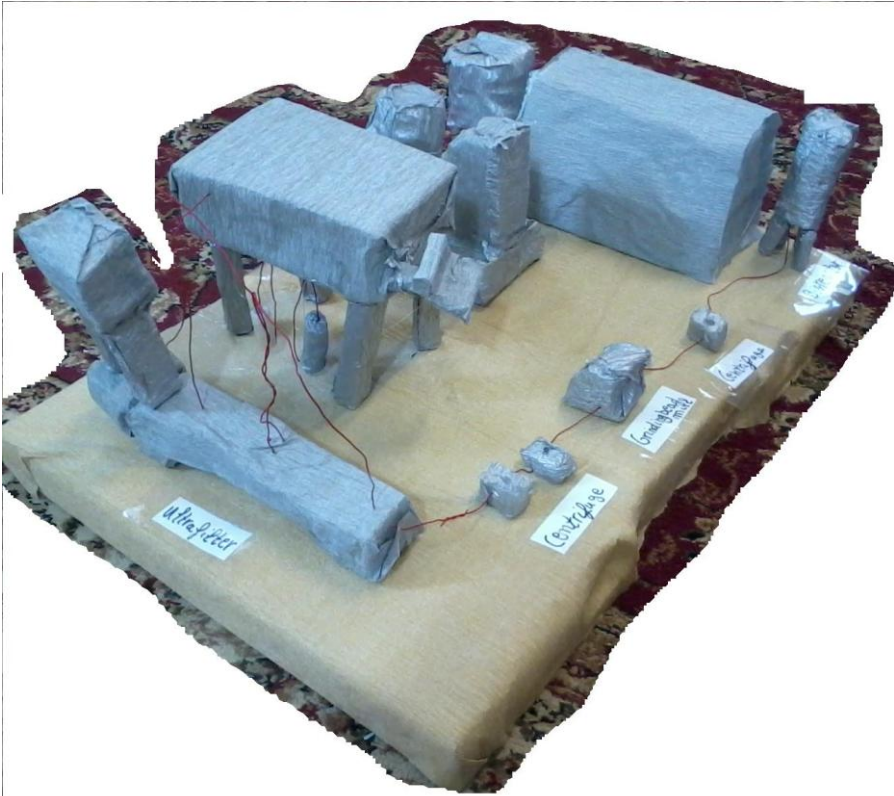
3.2 Automation System

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



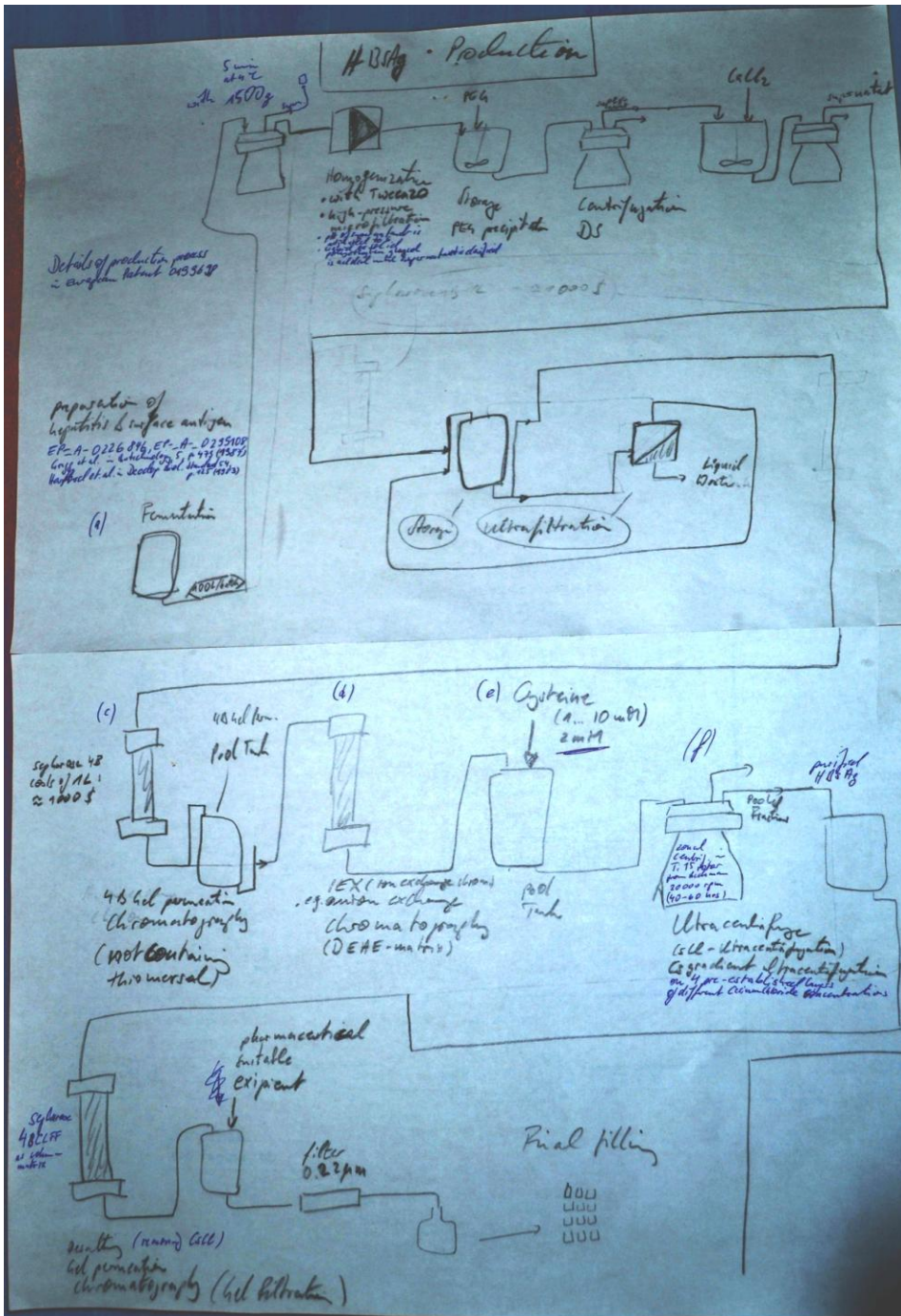
4. System Design

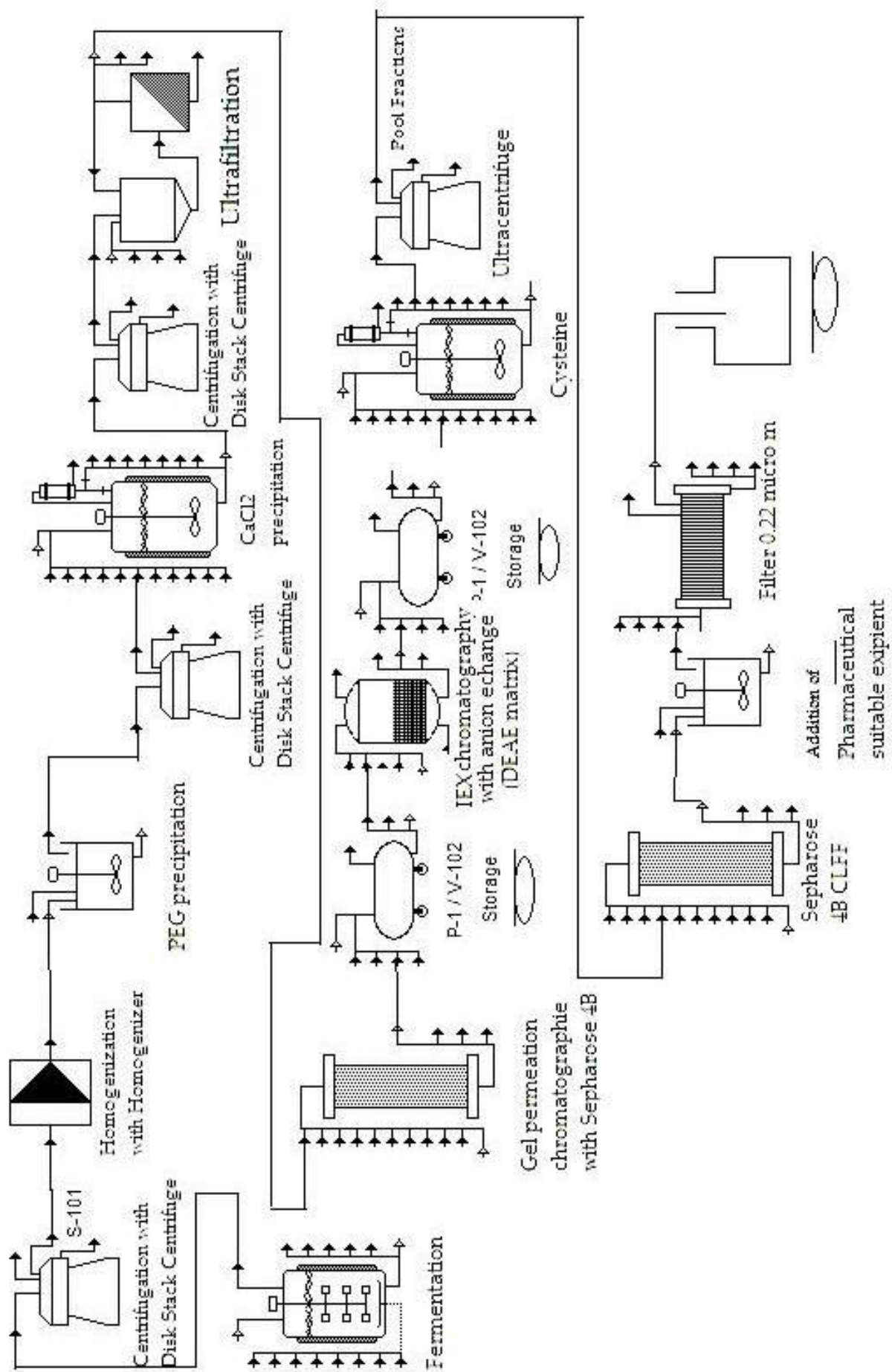
4.1 Mock-up model for the MEBGI vaccine pilot plant



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.





Clean-in-place (CIP)&Steam-in-place (SIP) and intermediate storage

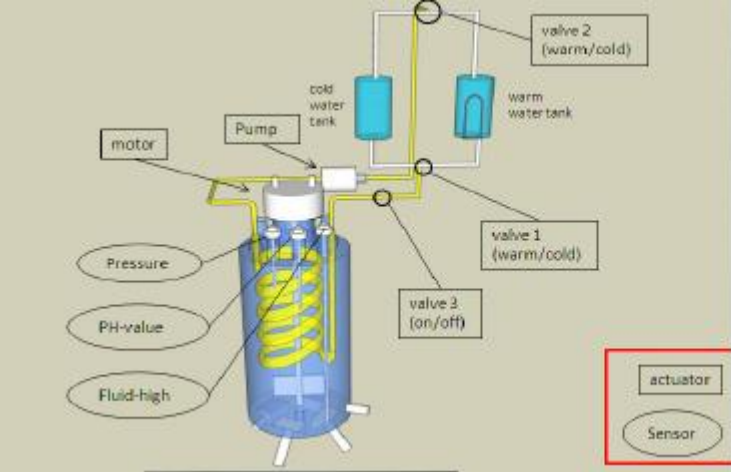
Upstream Processing (USP) – Downstream Processing (DSP)

Automation

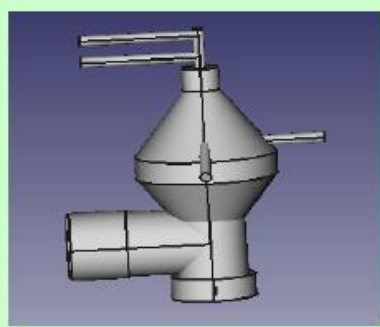


4.3 Fermentation

75 L/batch



4.4 Centrifugation with Disc Stack Centrifuge (1)



Disc Stack Centrifuge for Biotech & Pharmaceutical

FOB Price: US \$ 10,000 - 1,000,000 / Set | [Get Latest Price](#)

Min. Order Quantity: 1 Set/Sets

Supply Ability: 100 Set/Sets per Month

Port: SHANGHAI

[Contact Supplier](#)

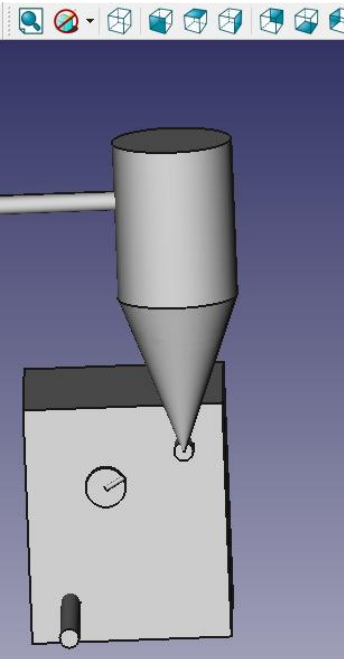
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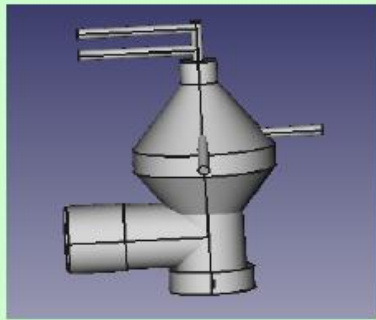
4.5 Homogenization with Homogenizer



4.6 PEG precipitation



4.7 Centrifugation with Disc Stack Centrifuge (2)



Disc Stack Centrifuge for Biotech & Pharmaceutical

FOB Price: US \$ 10,000 - 1,000,000 / Set | [Get Latest Price](#)
Min. Order Quantity: 1 Set/Sets
Supply Ability: 100 Set/Sets per Month
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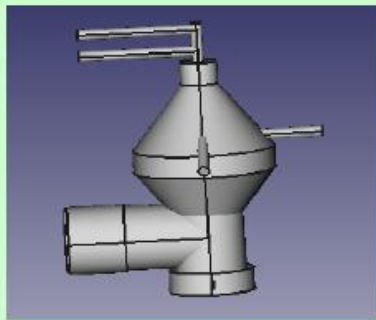
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4.8 CaCl₂ precipitation



4.9 Centrifugation with Disc Stack Centrifuge (3)



Disc Stack Centrifuge for Biotech & Pharmaceutical

FOB Price: US \$ 10,000 - 1,000,000 / Set | [Get Latest Price](#)
Min. Order Quantity: 1 Set/Sets
Supply Ability: 100 Set/Sets per Month
Port: SHANGHAI

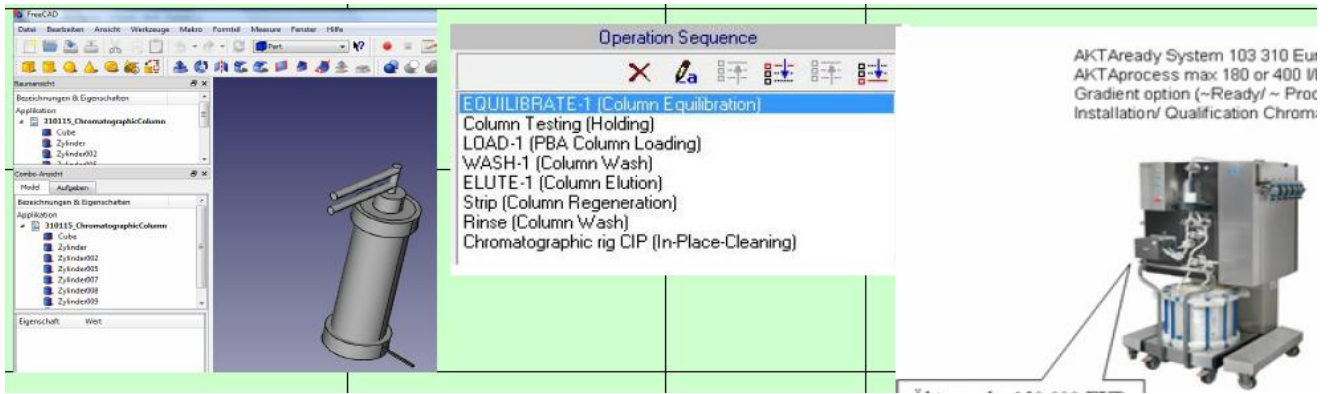
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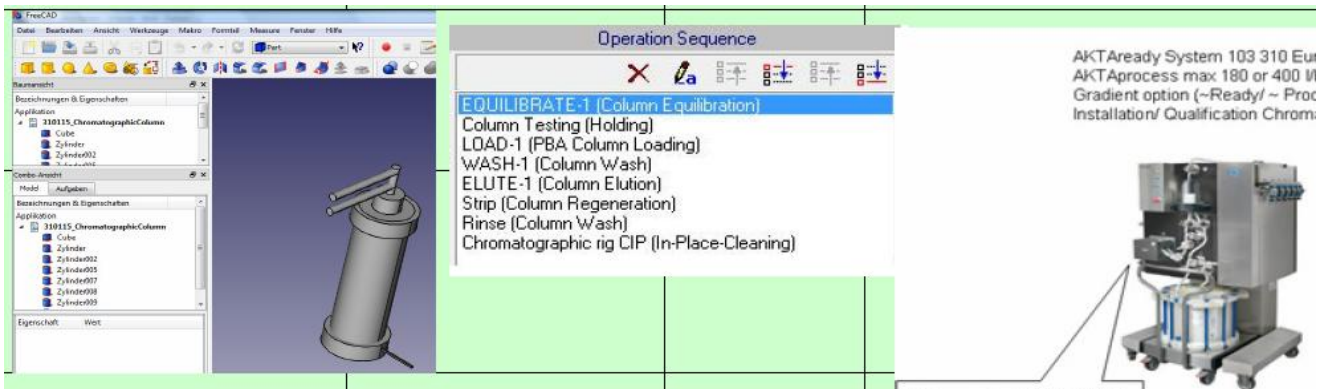
4.10 Ultrafiltration



4.11 Gel permeation chromatography with Sepharose 4B



4.12 IEX chromatography with anion exchange (DEAE matrix)



4.13 Cystein addition



4.14 Ultracentrifugation

Pooled fractions contain HBSAg



Figure: Alfa Wassermann Ultracentrifuges

4.15 Desalting Gel permeation chromatography with Sepharose 4BCLFF

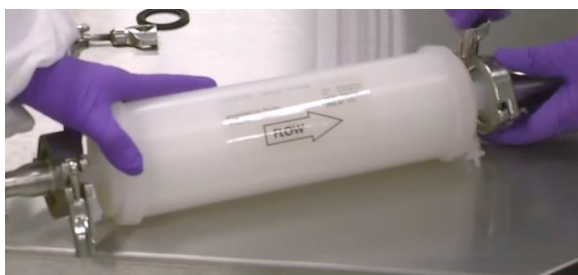
FreeCAD

Operation Sequence

- EQUILIBRATE-1 (Column Equilibration)
- Column Testing (Holding)
- LOAD-1 (PBA Column Loading)
- WASH-1 (Column Wash)
- ELUTE-1 (Column Elution)
- Strip (Column Regeneration)
- Rinse (Column Wash)
- Chromatographic rig CIP (In-Place-Cleaning)

AKTready System 103 310 Eur
 AKTprocess max 180 or 400 l
 Gradient option (~Ready/ ~ Proc
 Installation/ Qualification Chrom

4.16 Filtering with 0.22 µm filter

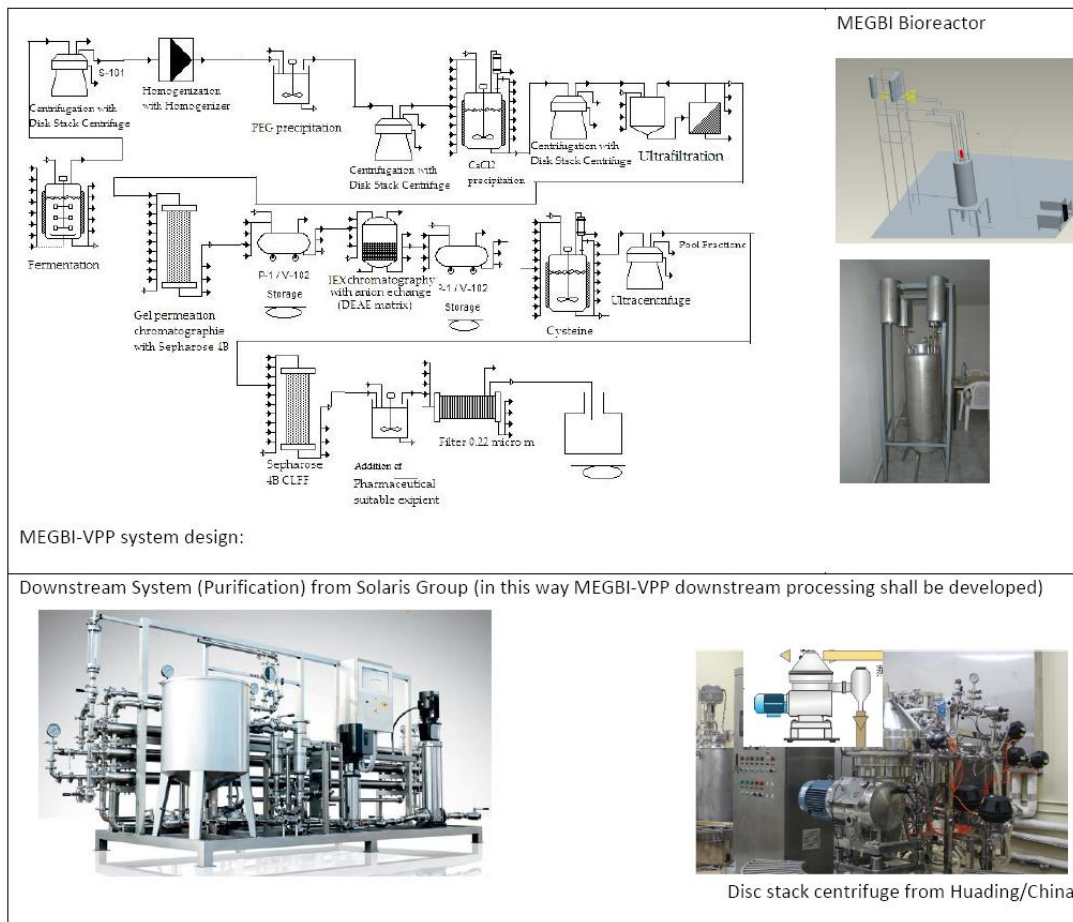


5. Detailed Devices Mechanical Design

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



Ras Nhache/Batroun - Tripoli, 12th Jan 2015



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



AECENAR

Association for Economical and Technological Cooperation
in the Euro-Asian and North-African Region

بِسْمِ اللّٰهِ الرَّحْمٰنِ الرَّحِیْمِ

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

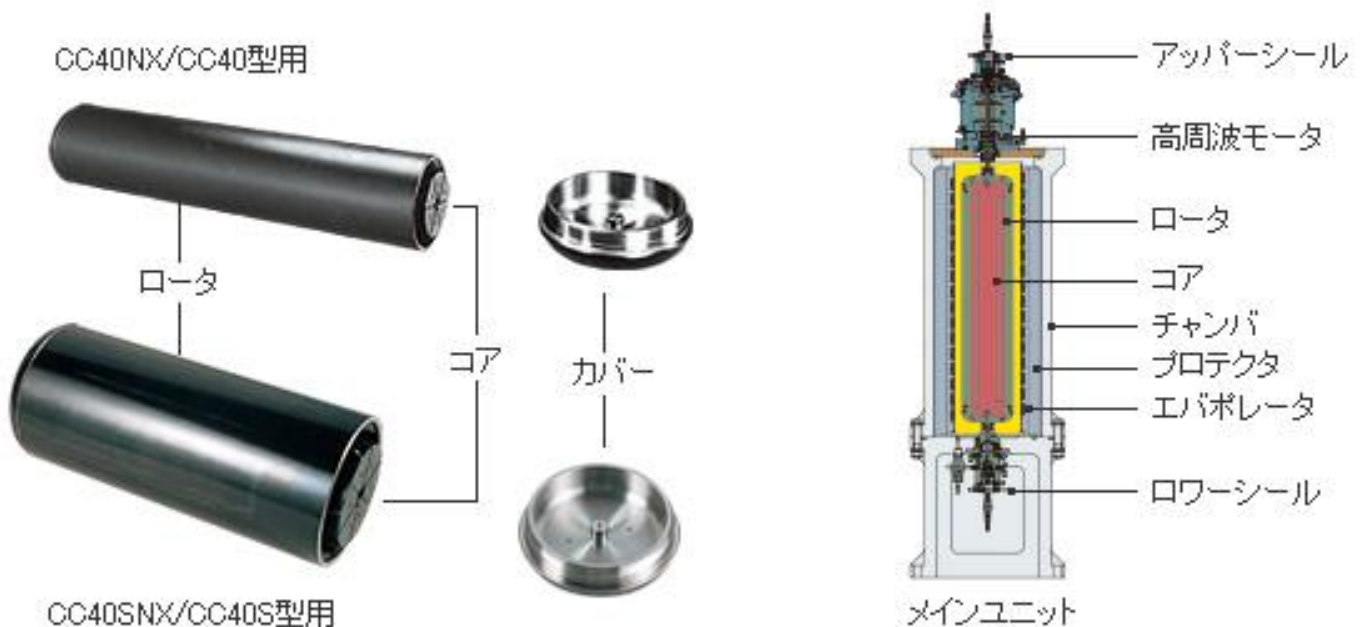
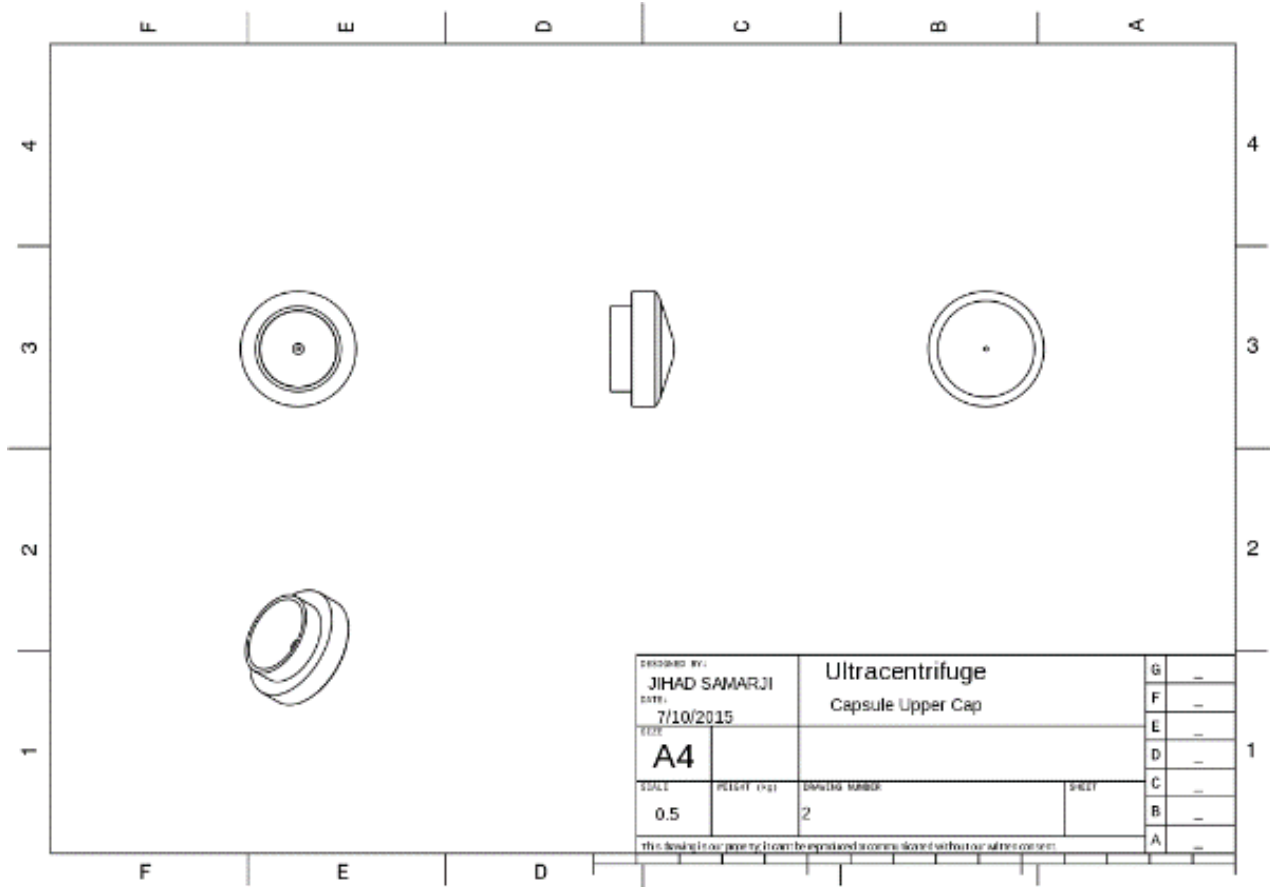
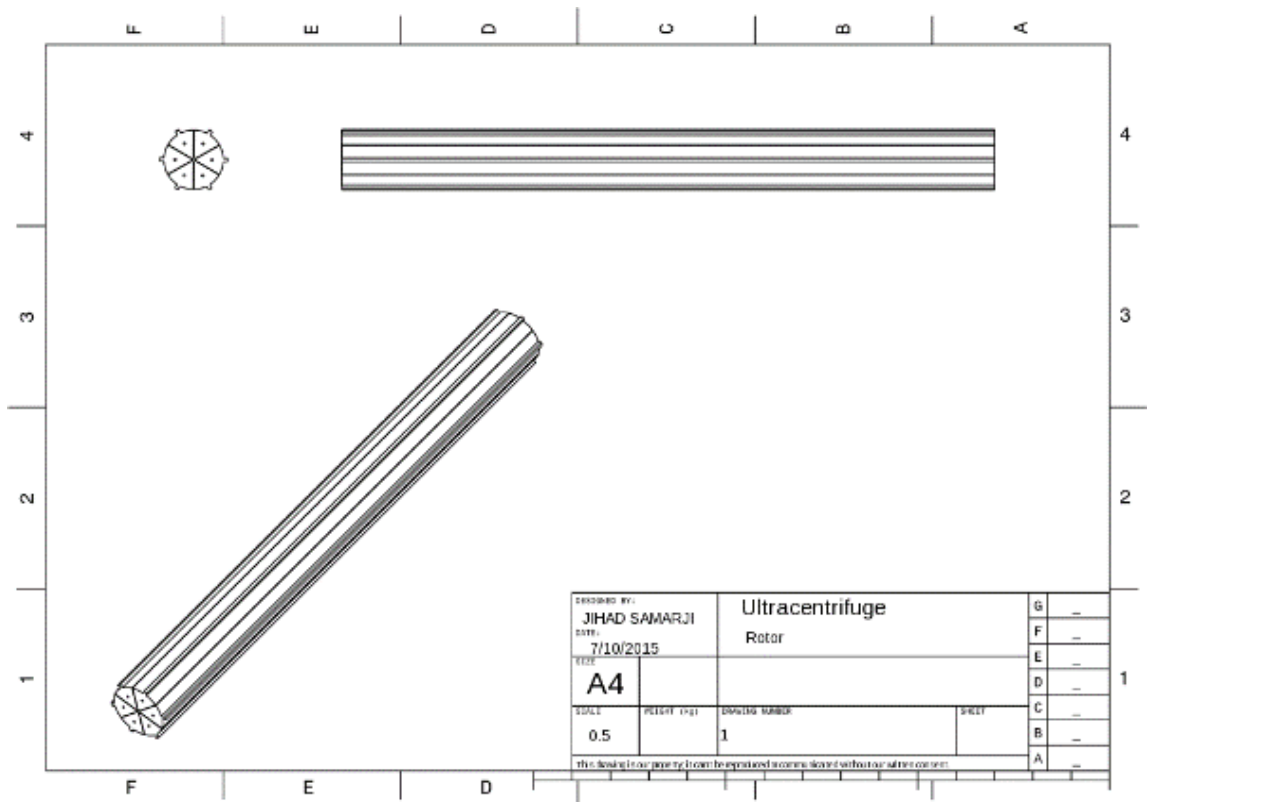


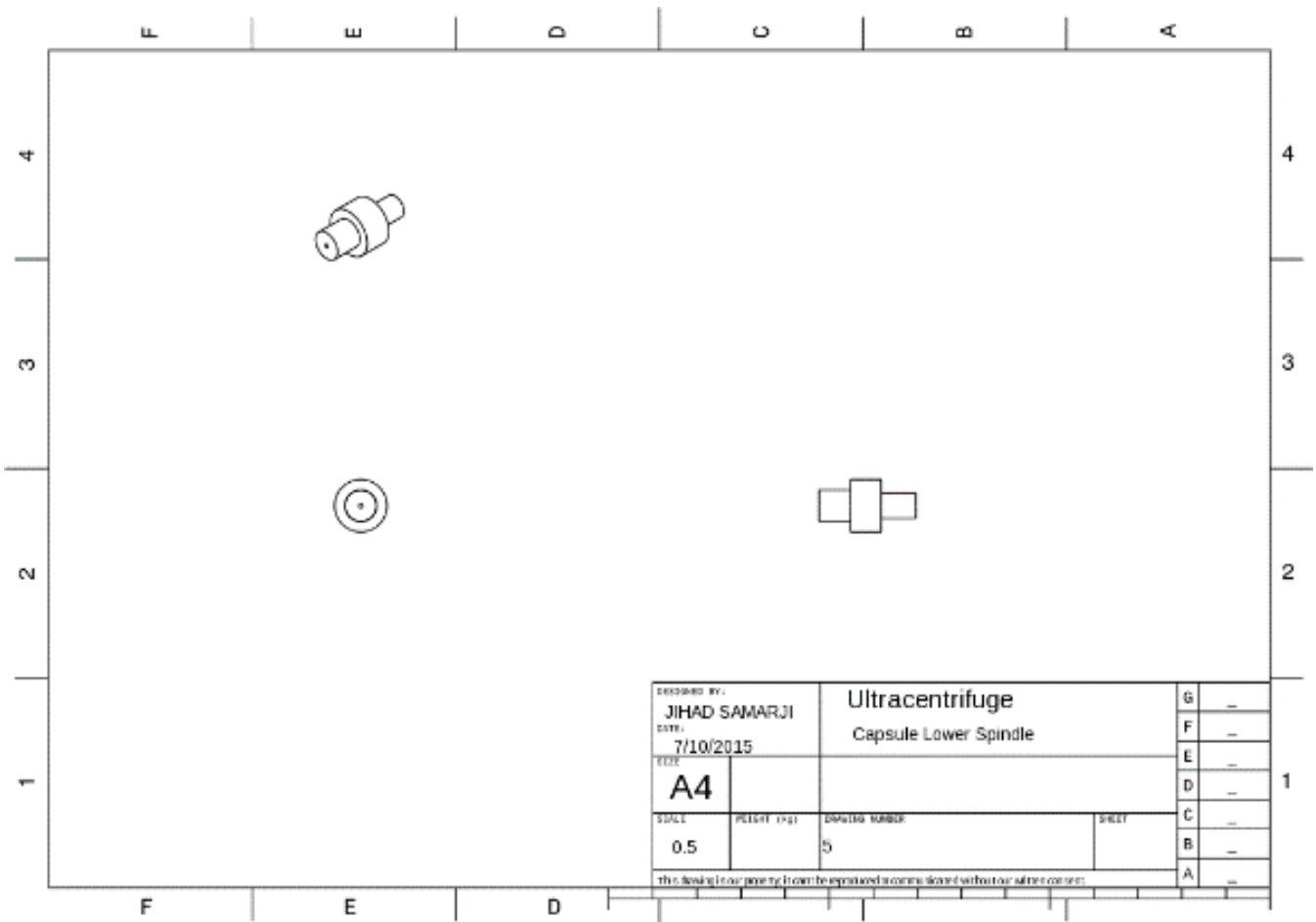
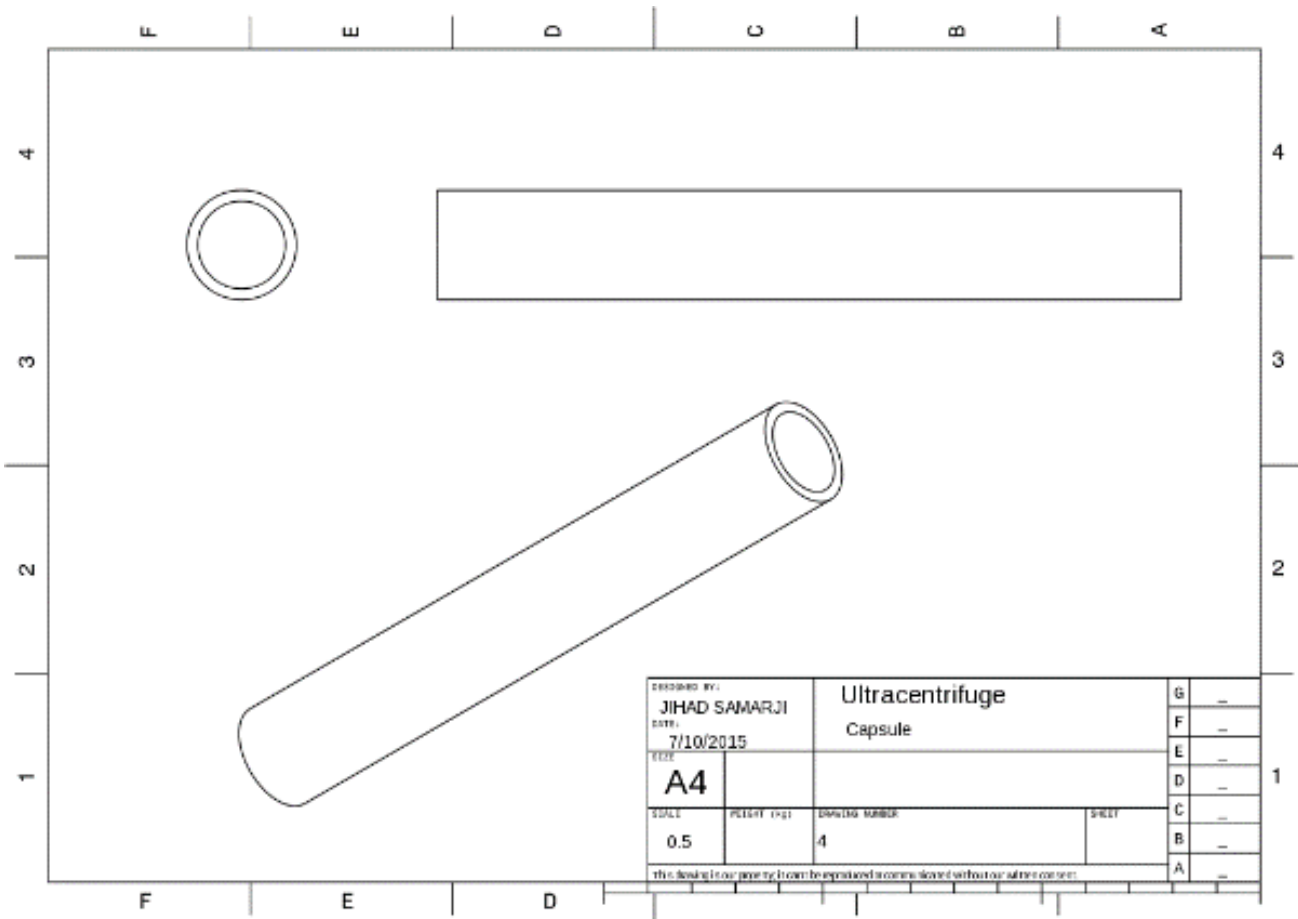
Figure 1.2 Ultracentrifuge annotation in Chinese

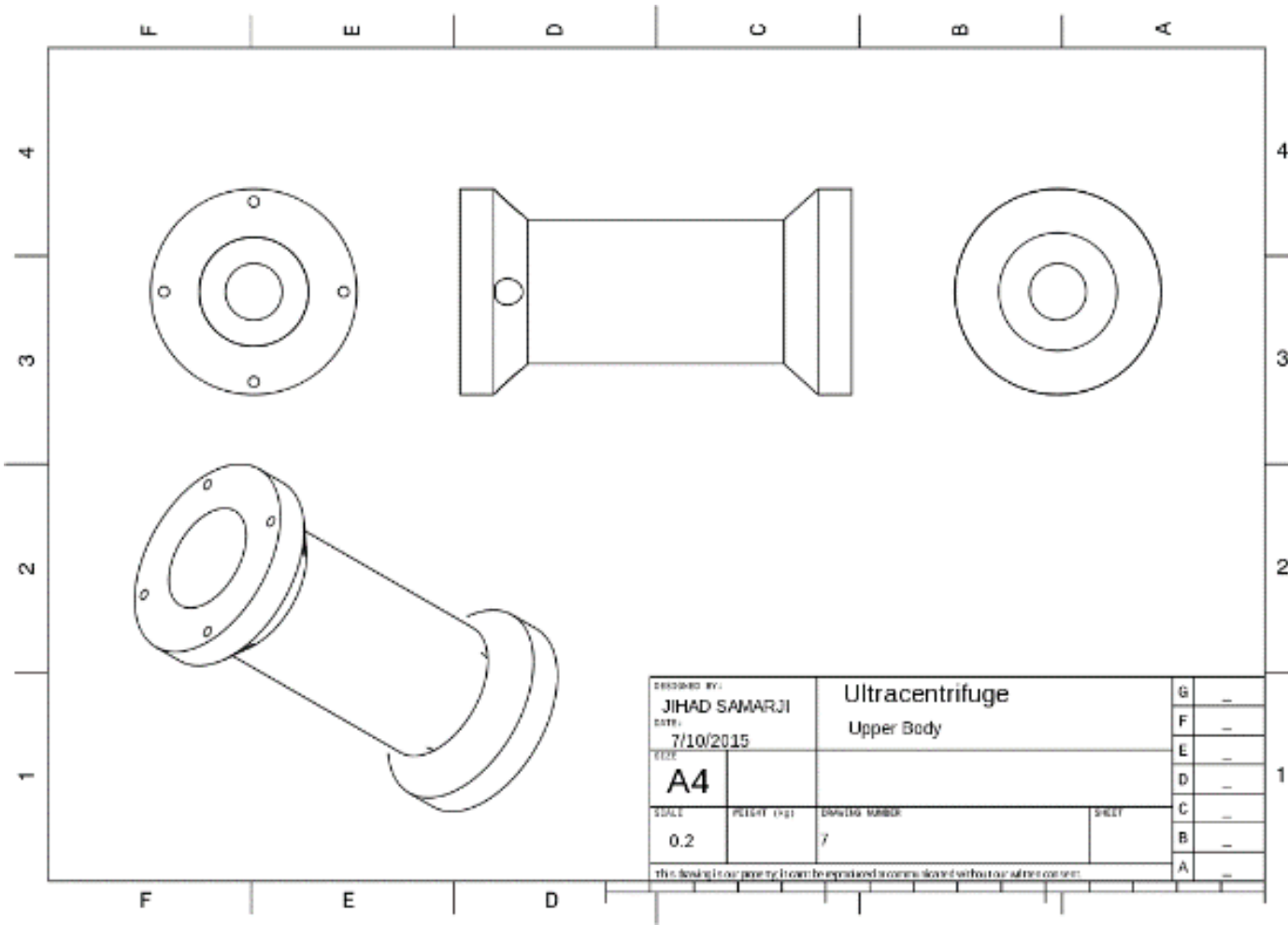
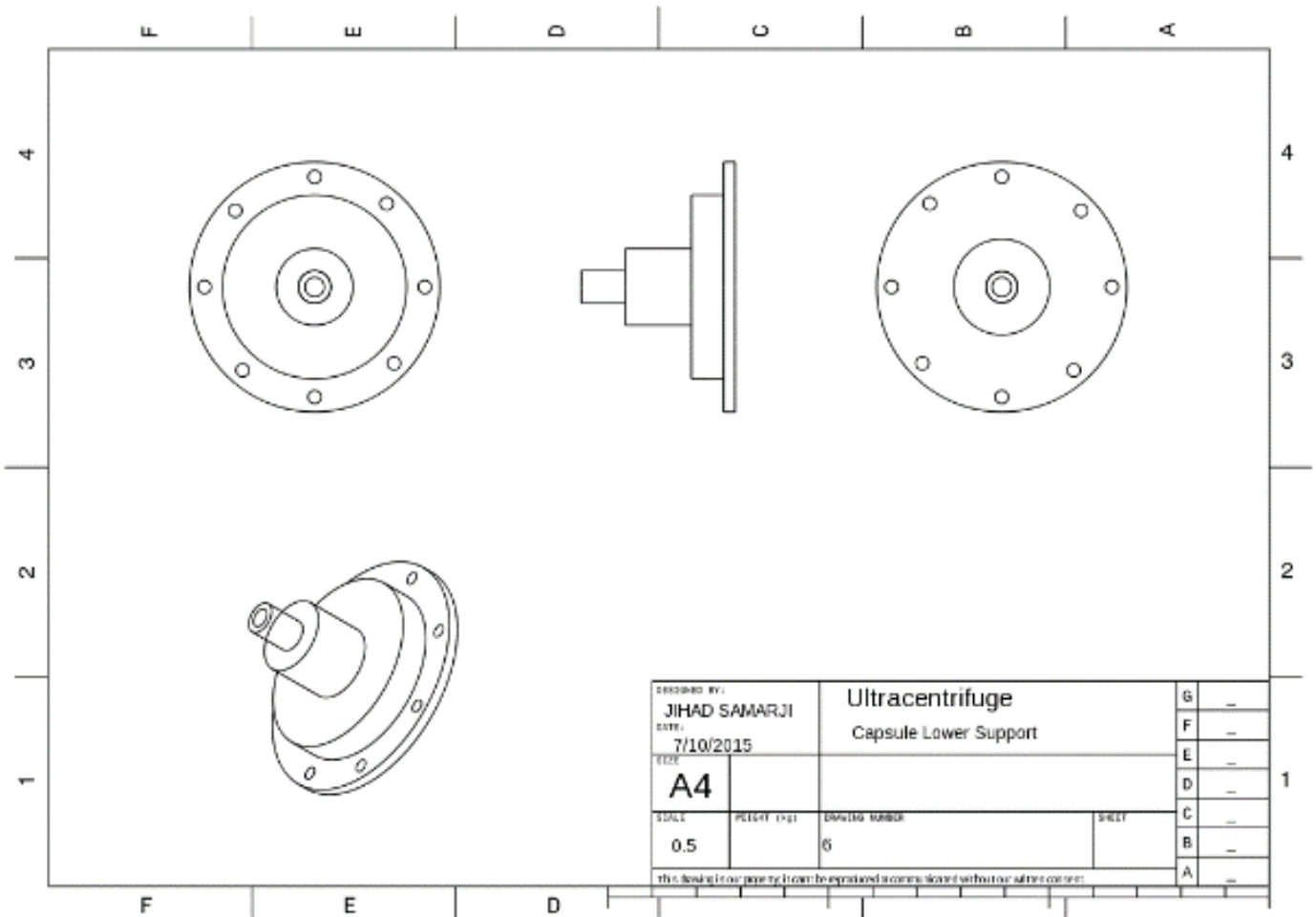
The Ultracentrifuge can be used For Uranium Enrichment which makes it very dangerous and Complicated, Therefore gaining information about this particular machine is very limited. This is by far the clearest picture describing the Ultracentrifuge on the internet.

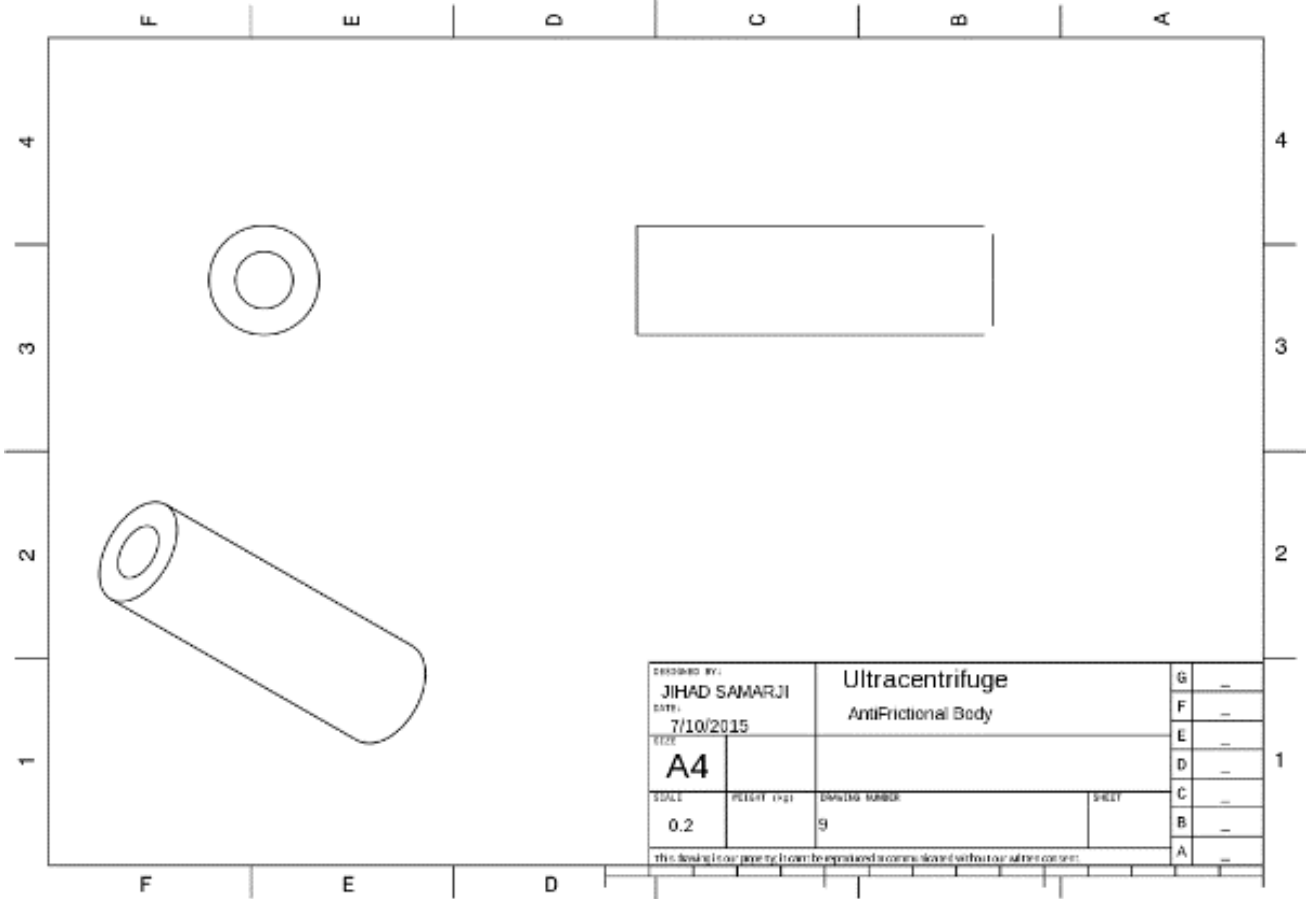
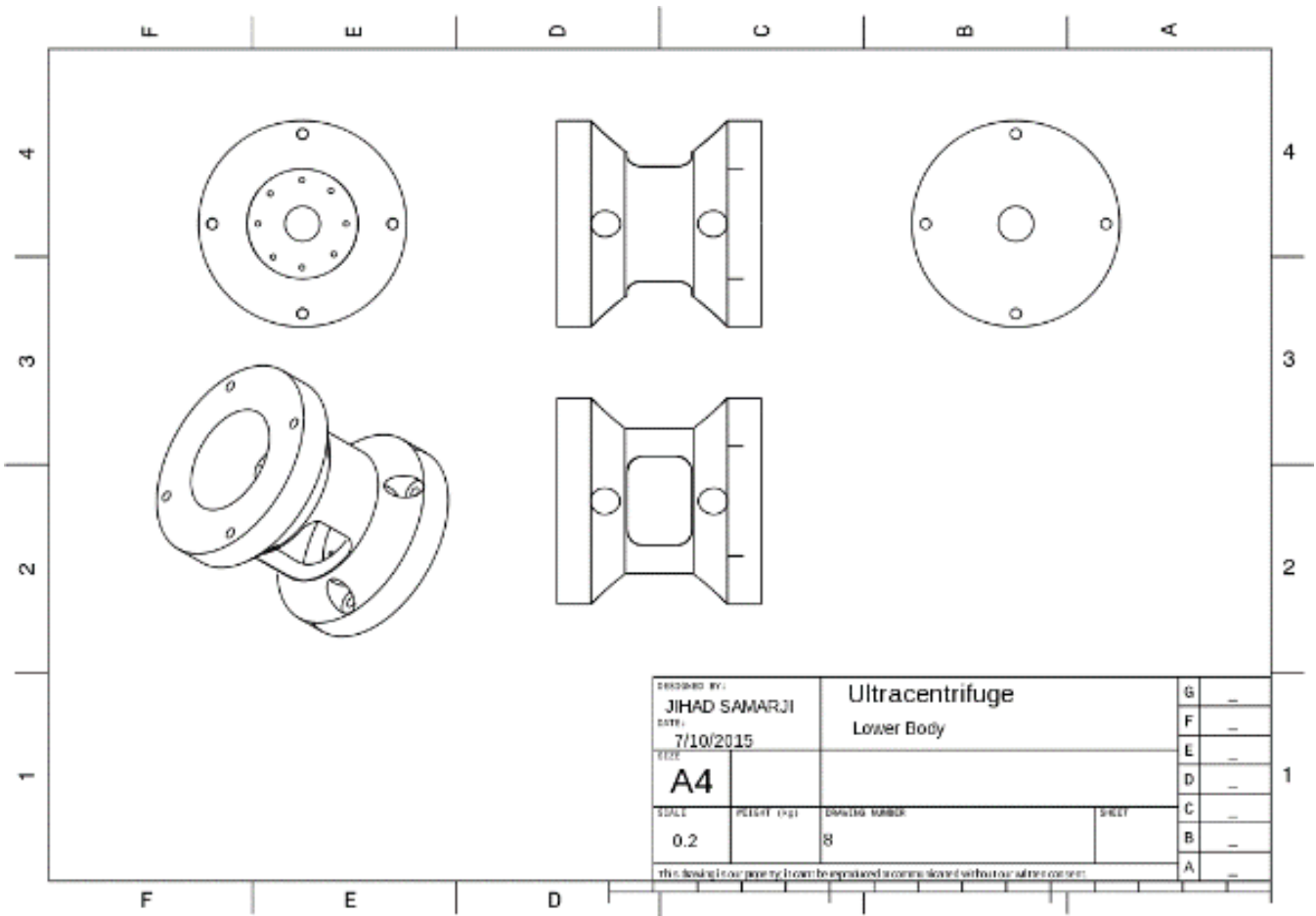
In fact, searching for the Keyword "Ultracentrifuge" on Google makes you suspicious for Global Terrorism. BE CAUTIOUS

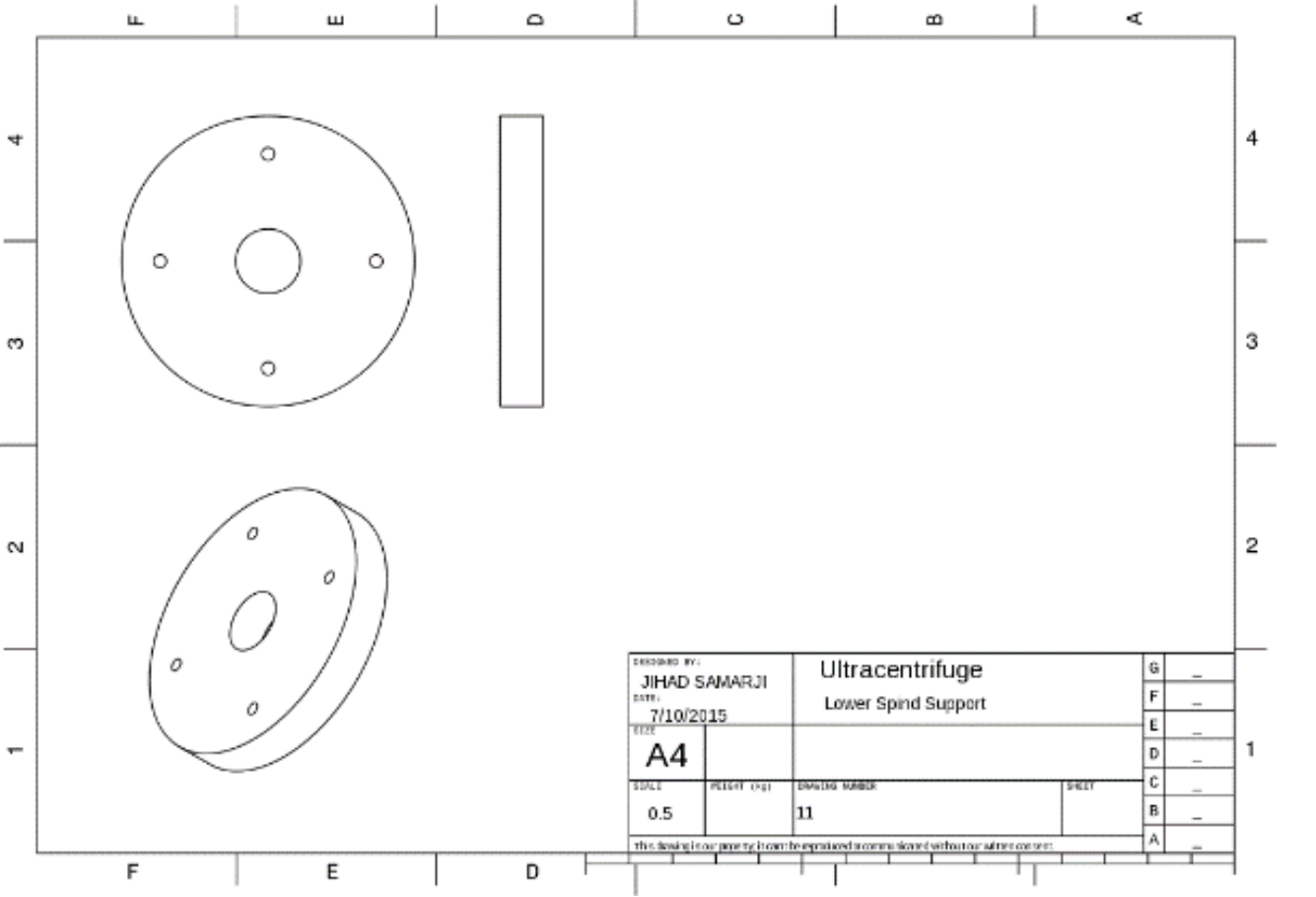
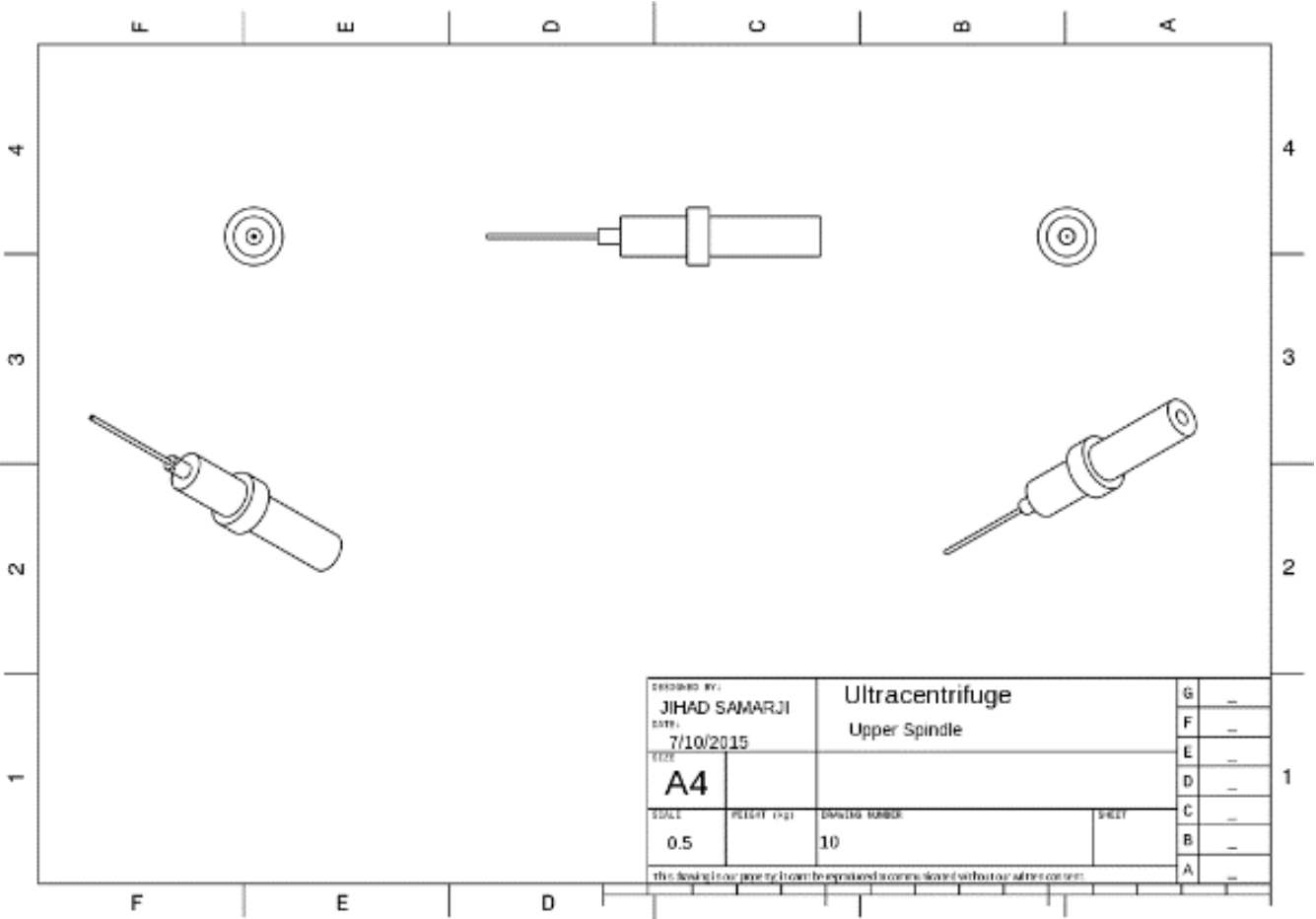
5.1.2 PART DIMENSIONS & DESIGN

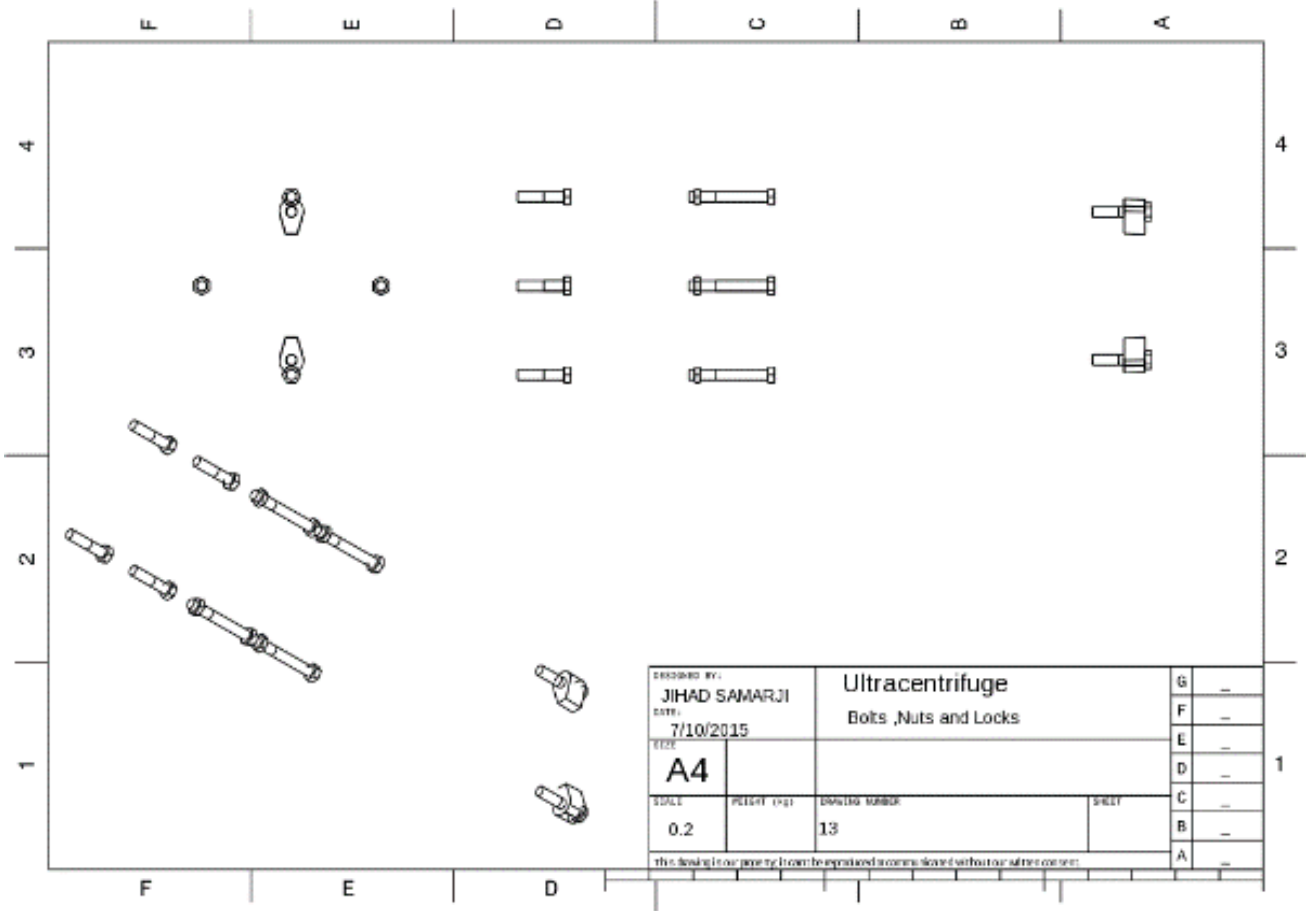
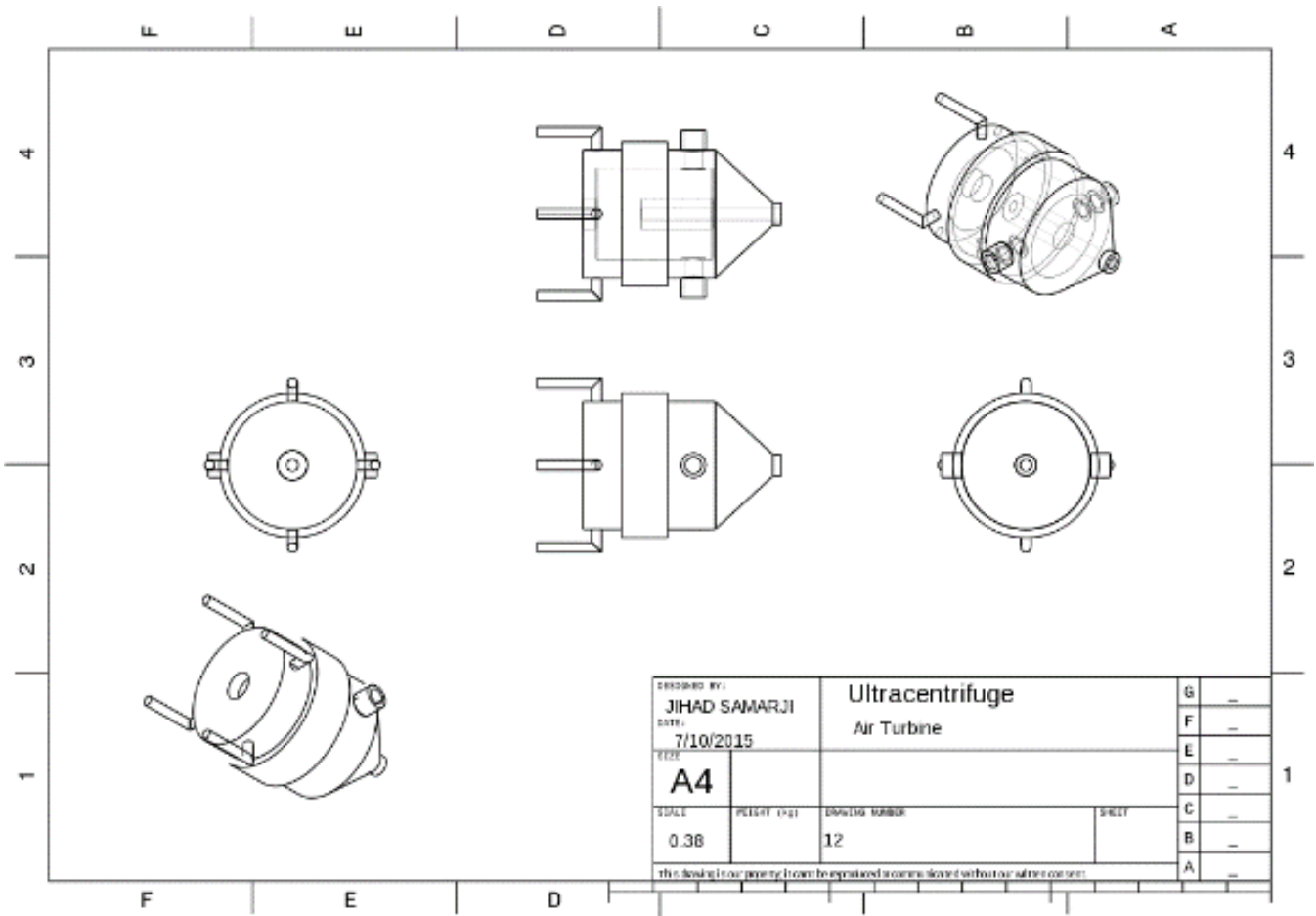


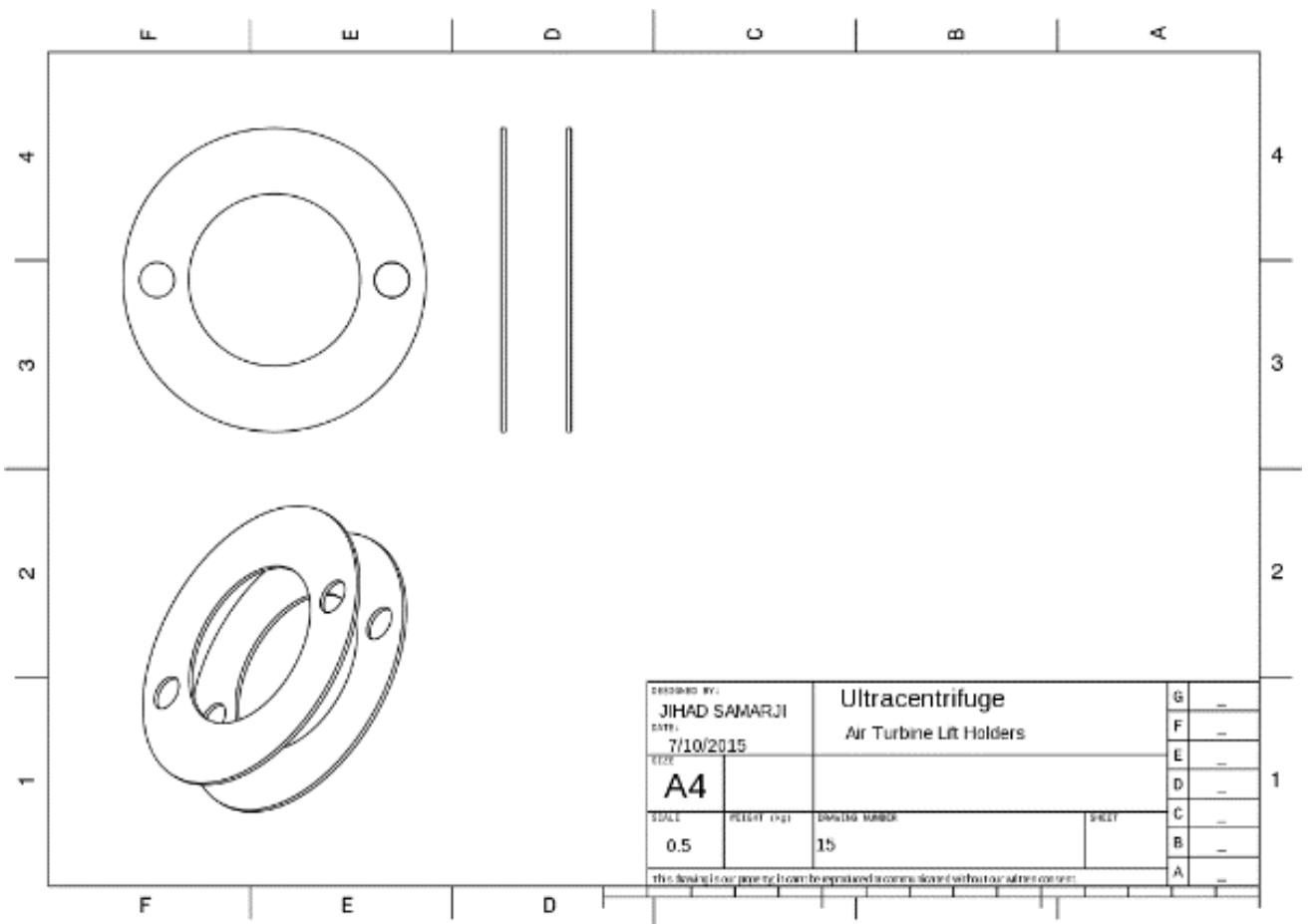
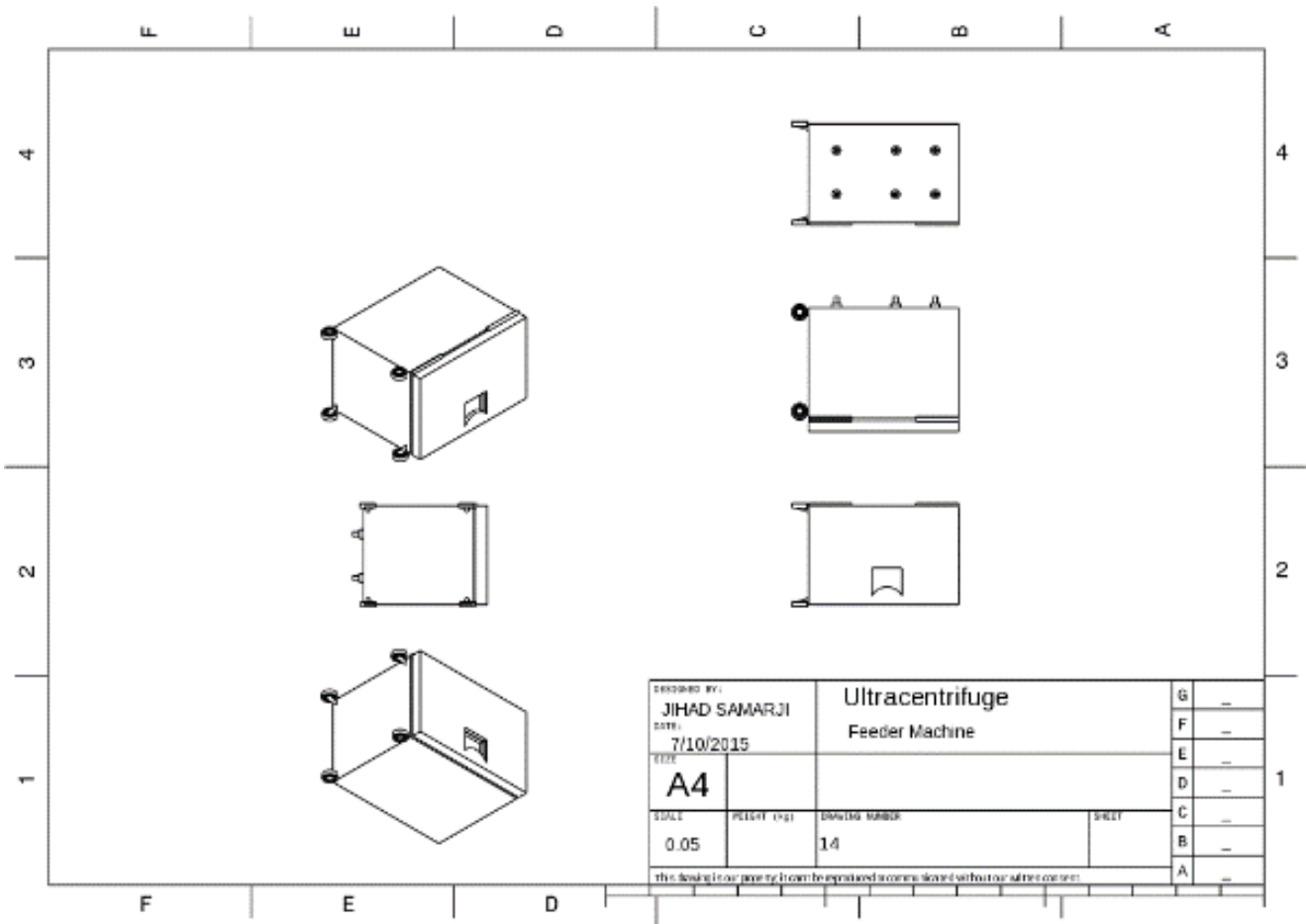


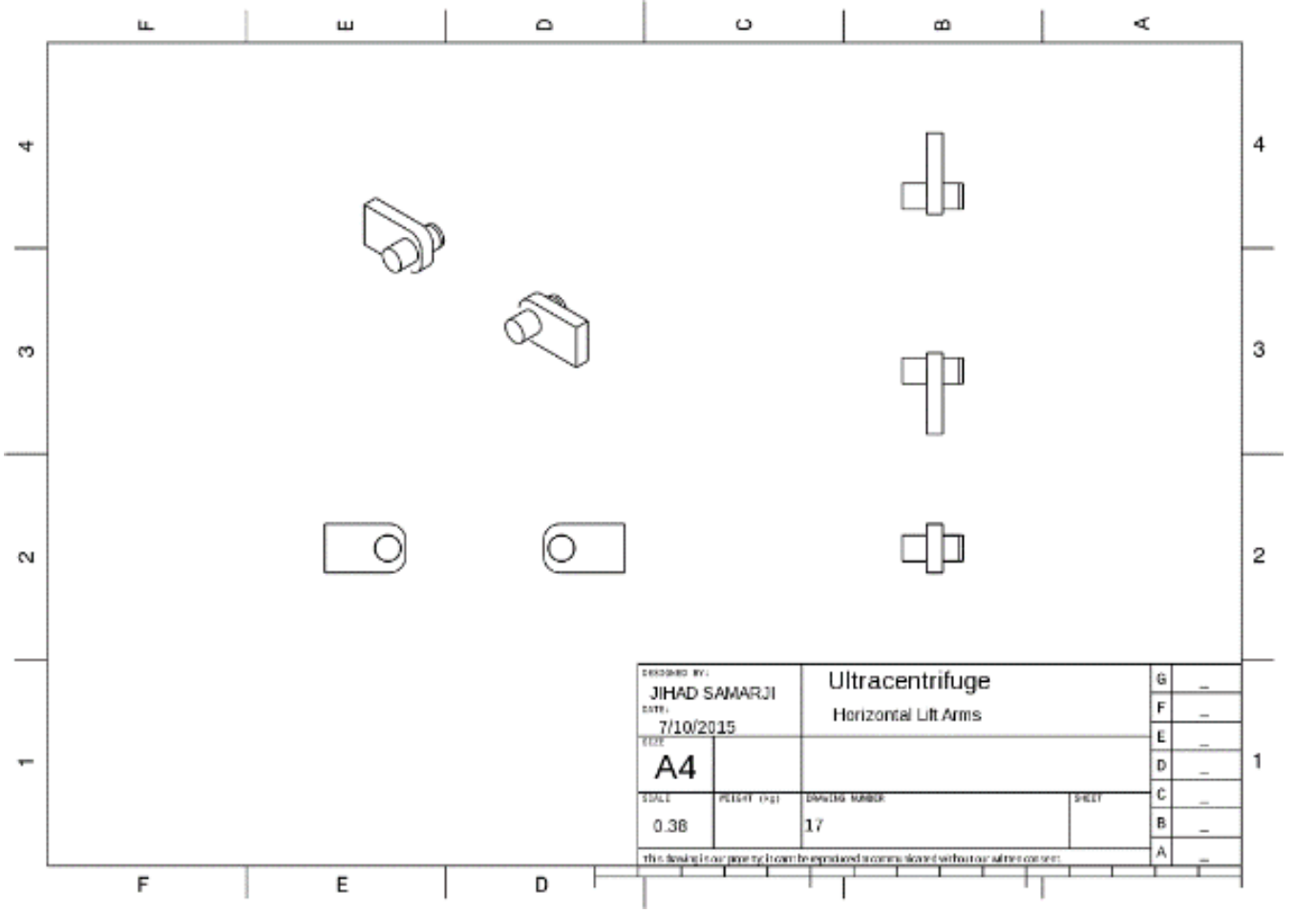
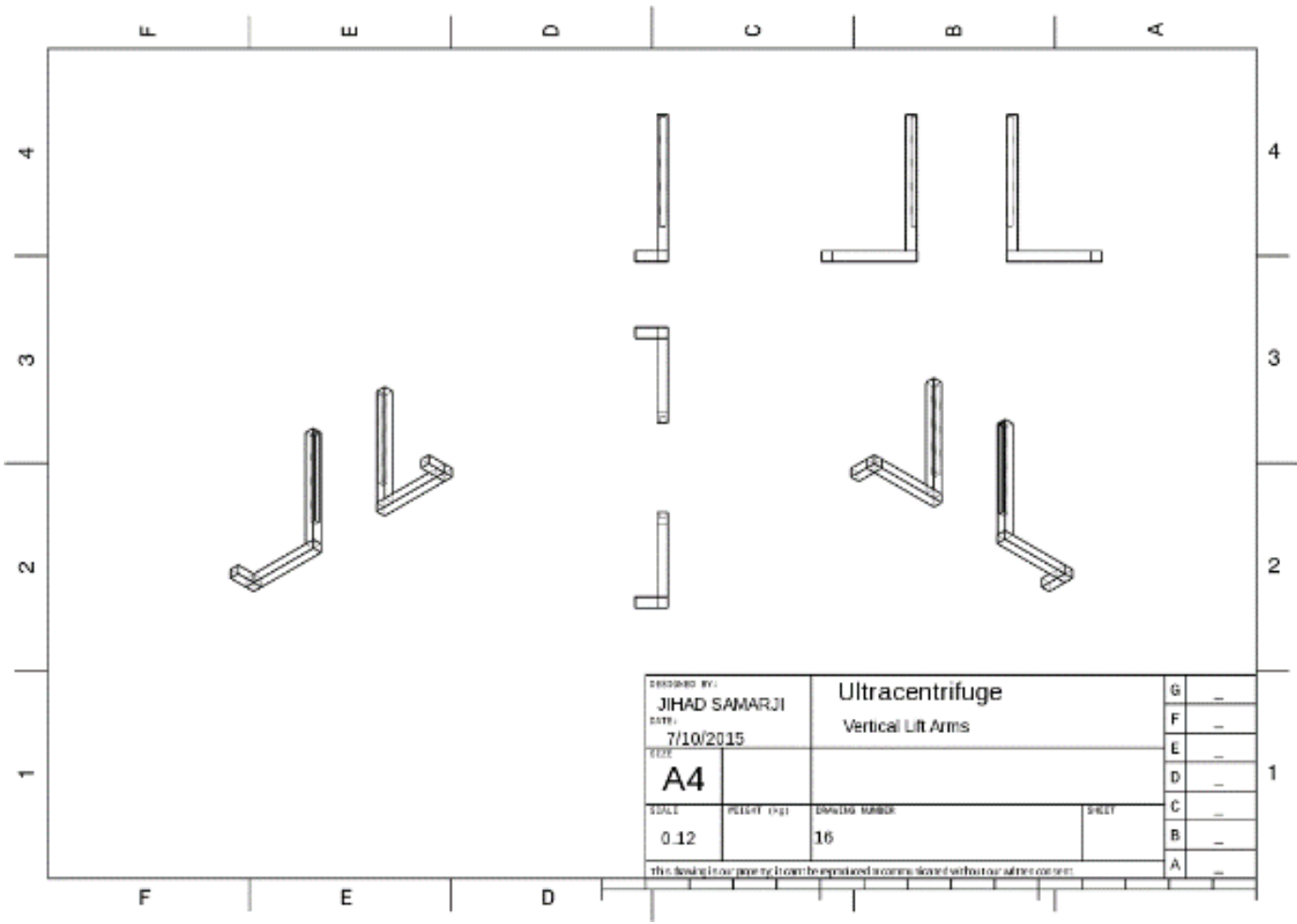


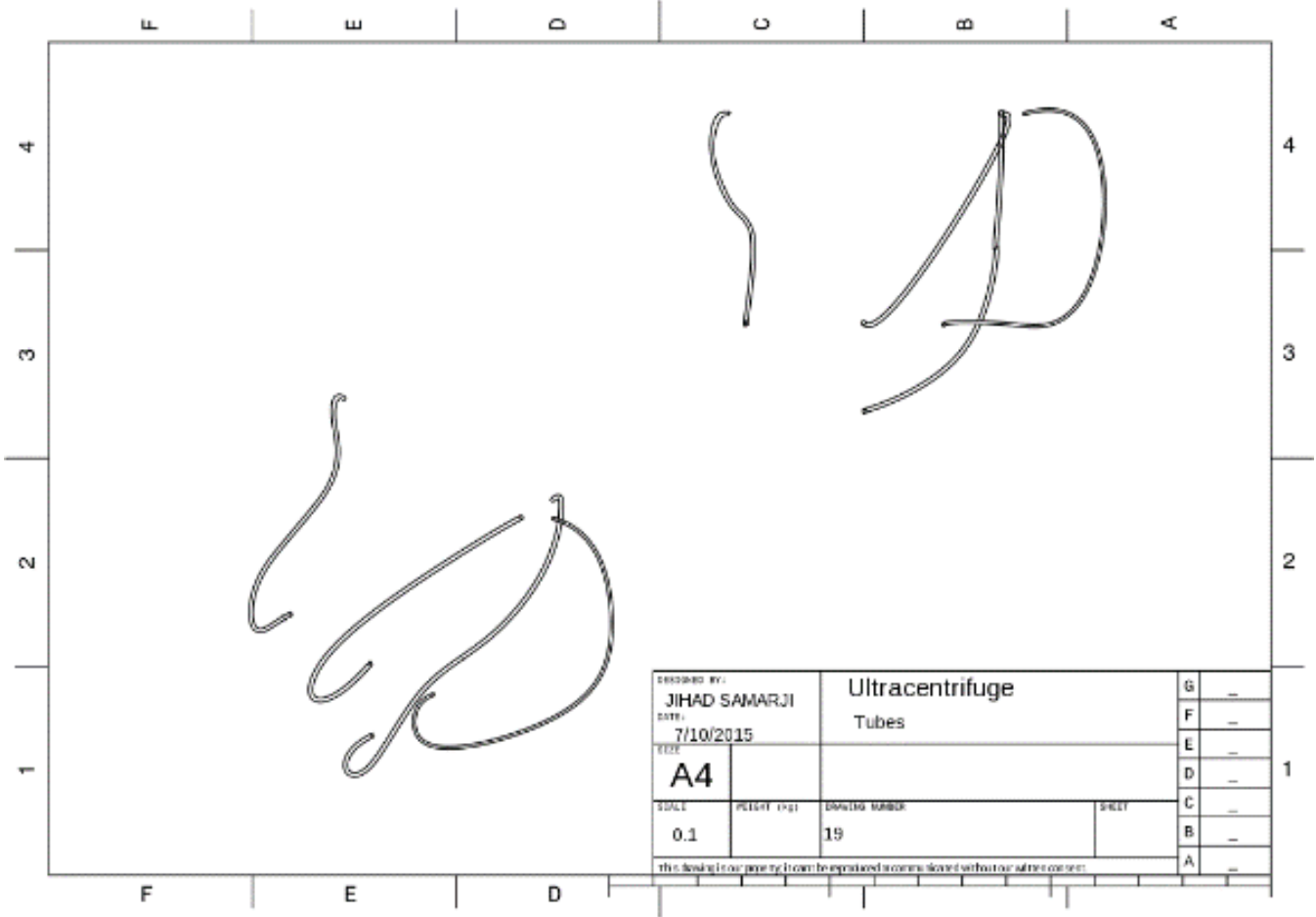
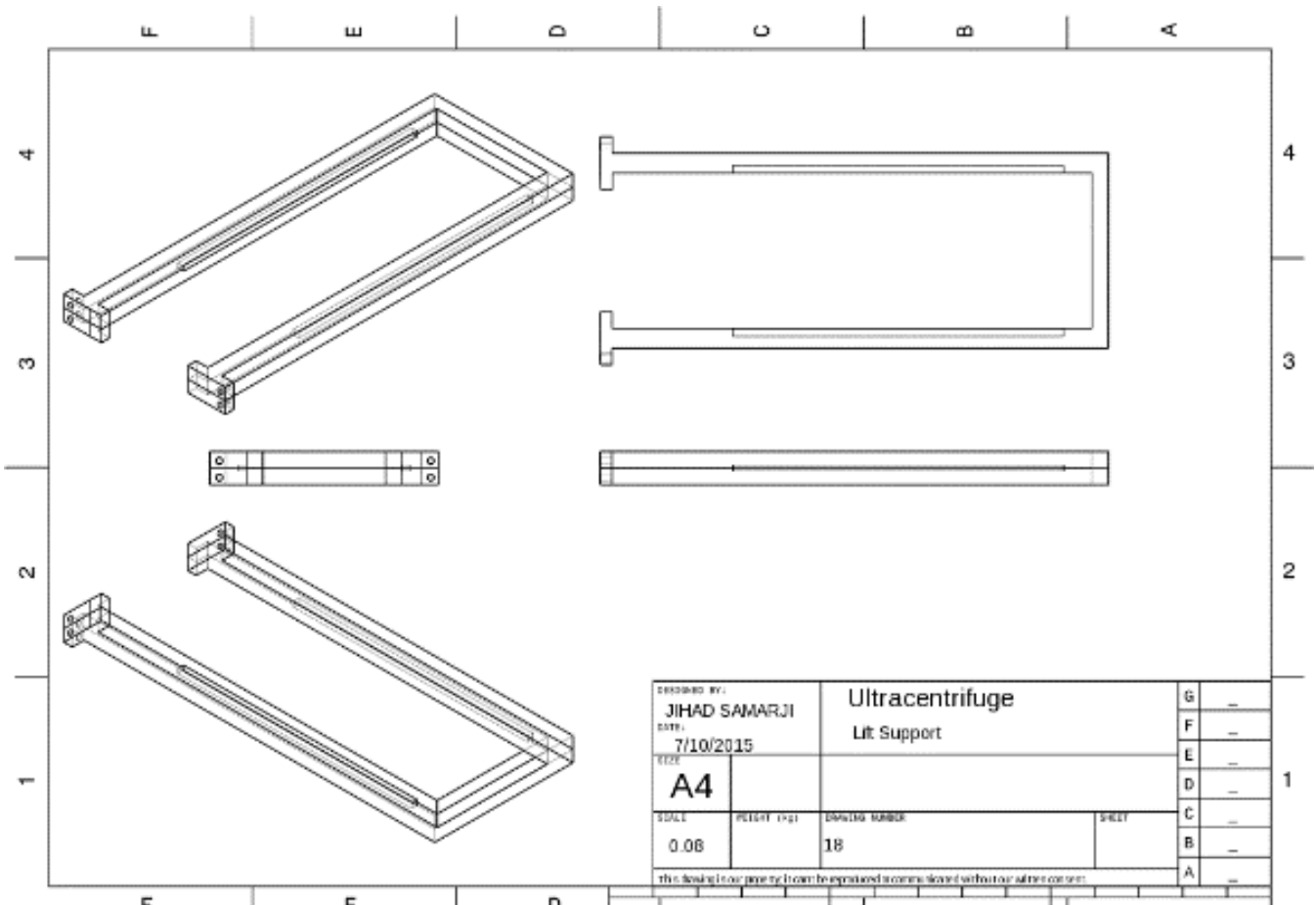












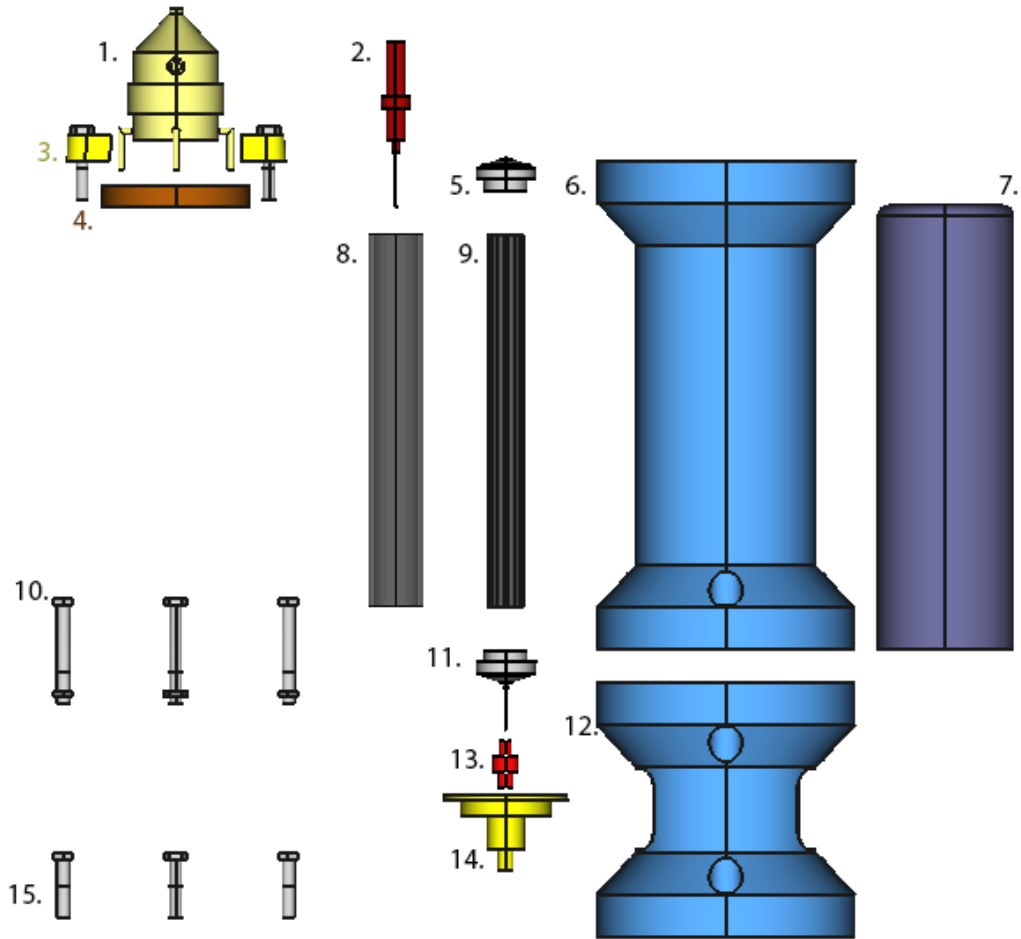


Figure 1.3 Ultracentrifuge First Group Parts Annotations

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

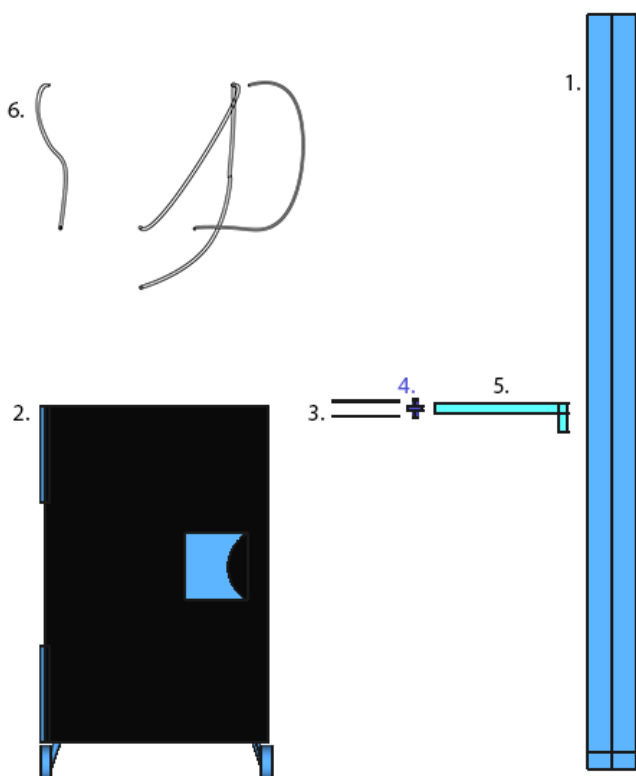
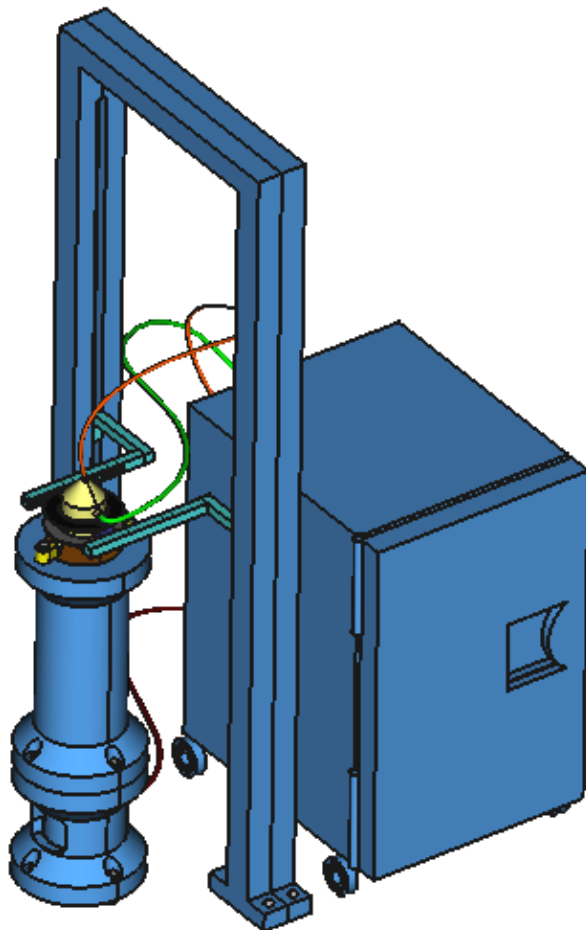
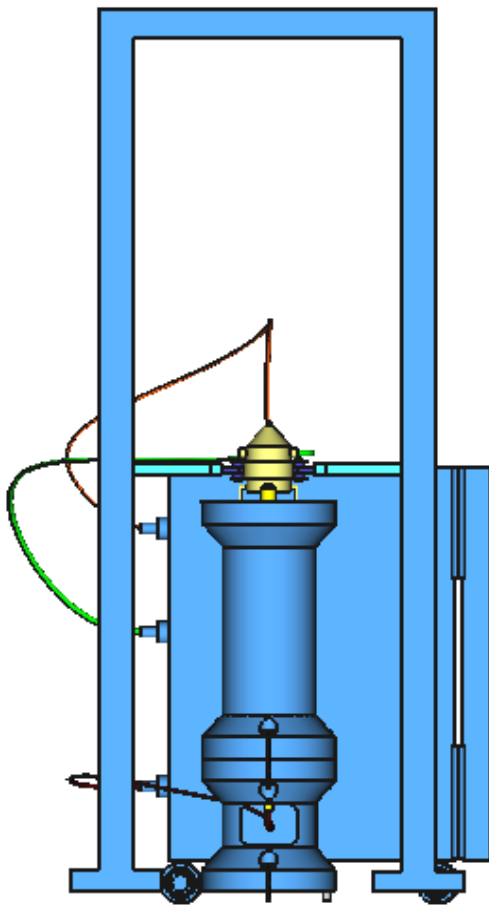


Figure 1.4 ULTRACENTRIFUGE SECOND GROUP PART ANNOTATIONS

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 of Solids”-Jihad Samarji

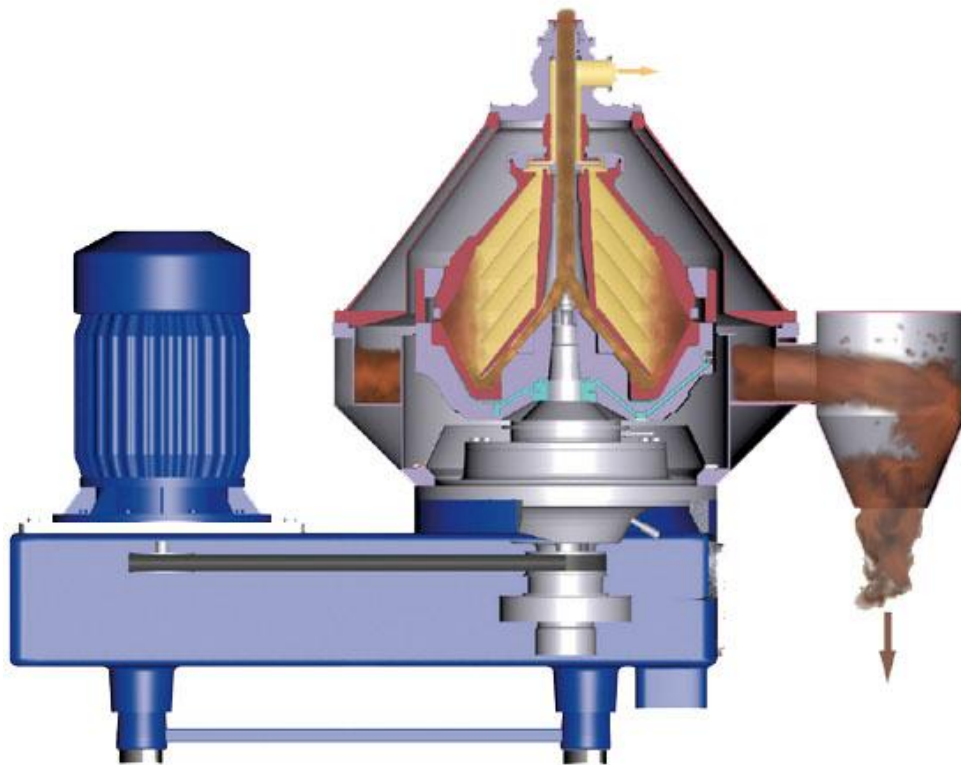


Figure 2.1 Disc Stack Centrifuge

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 m ³ /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 m ³ /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 |

Table 2.1 Disc Stack Centrifuge Specifications. See also figure 2.1 and 2.2

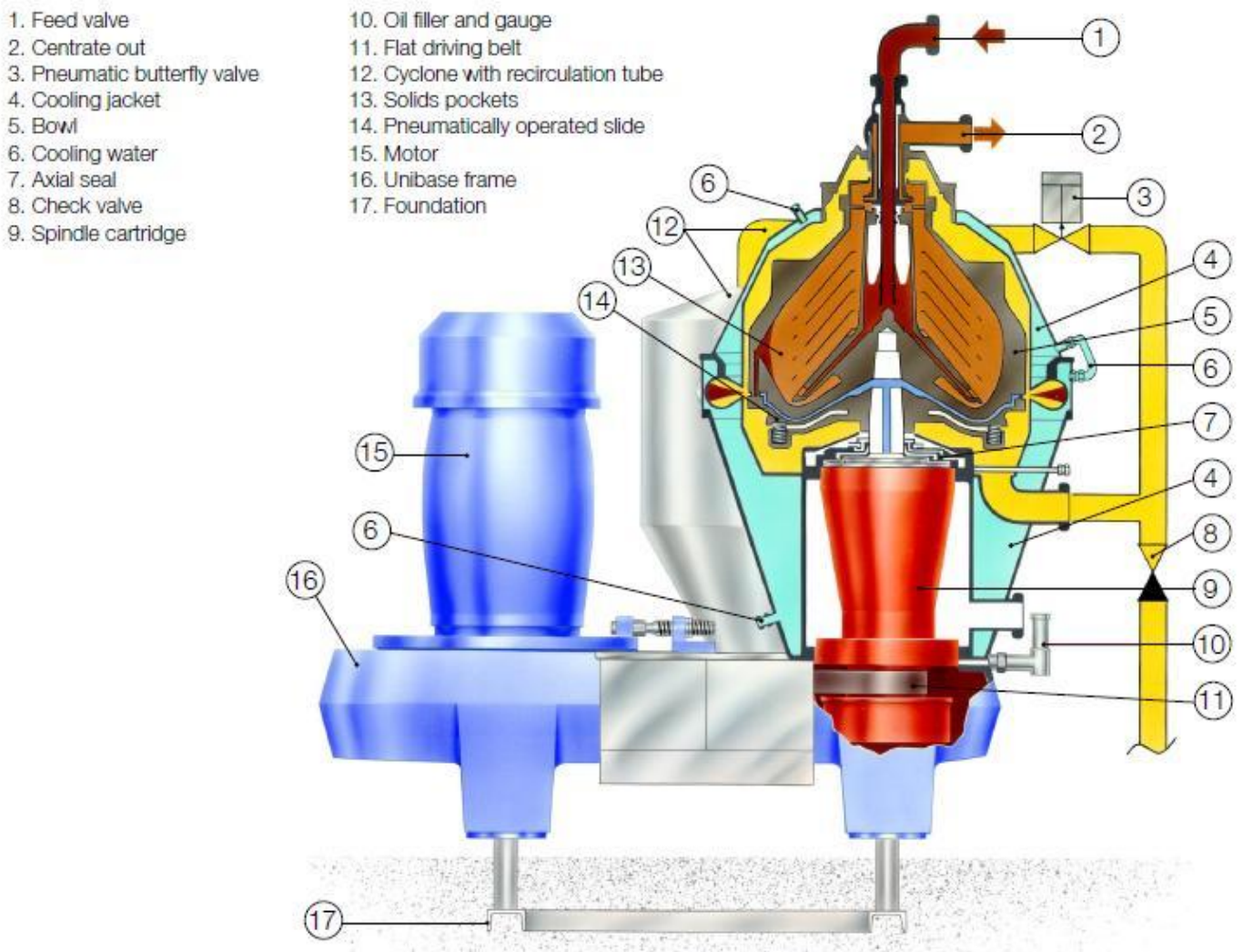
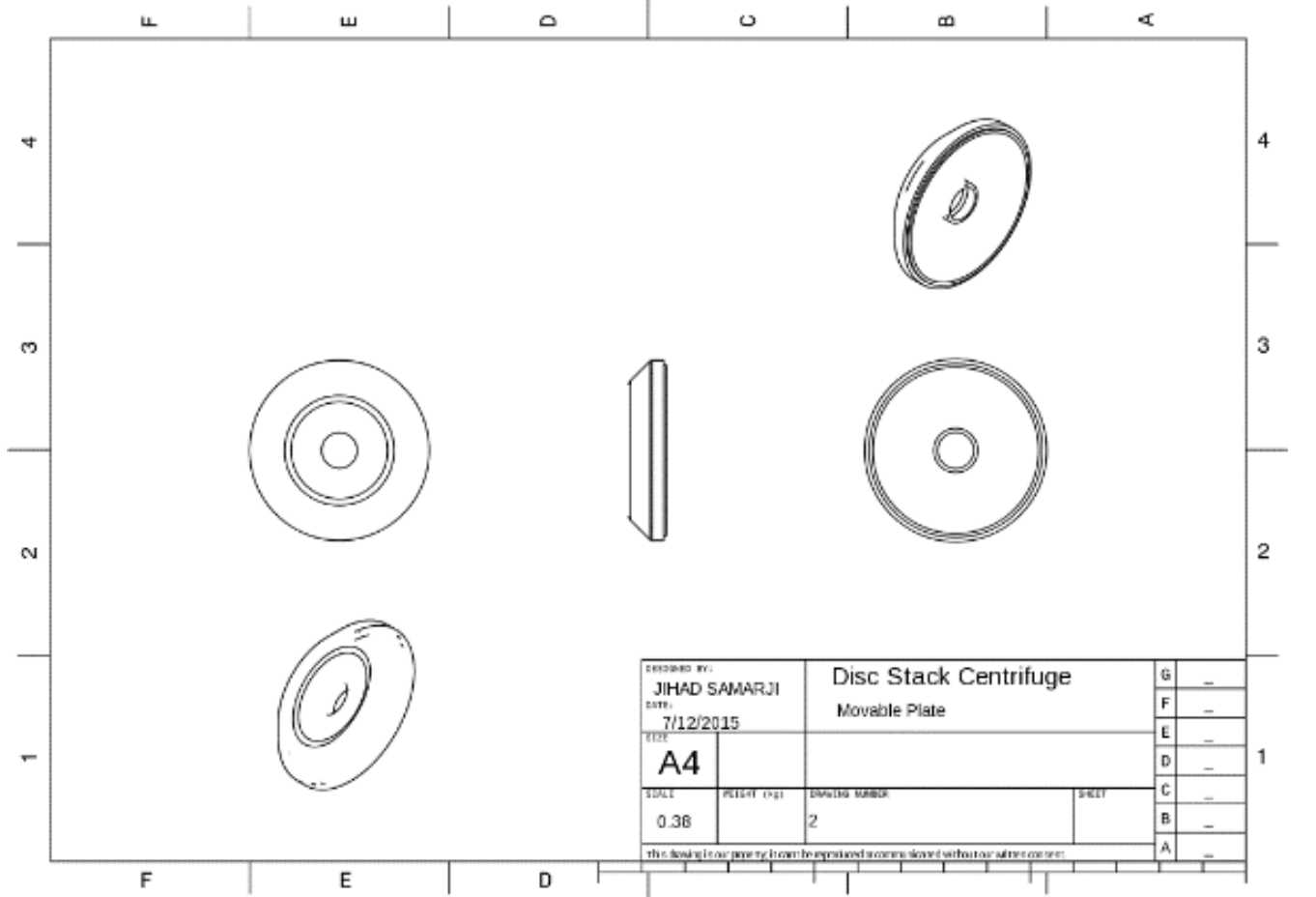
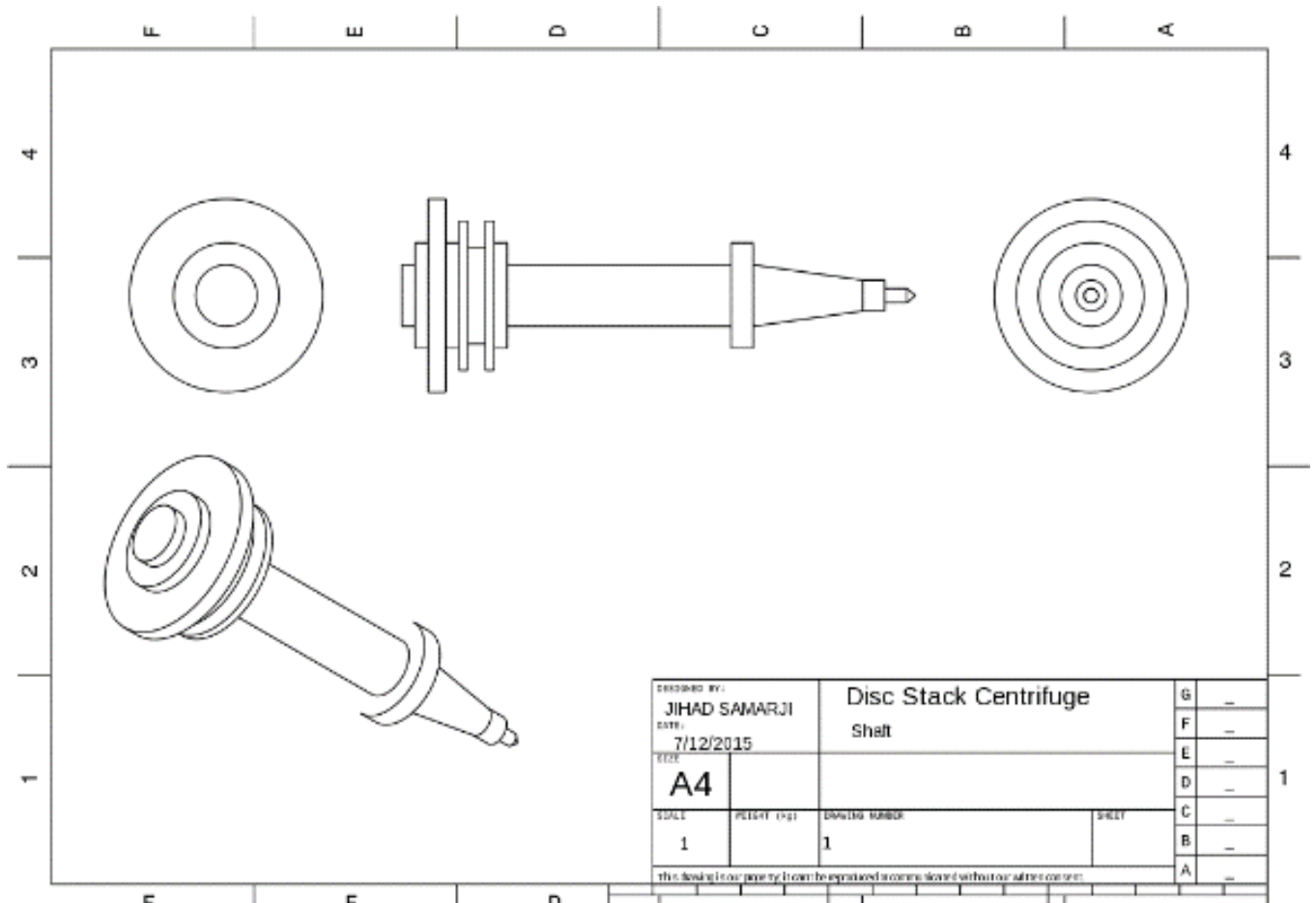
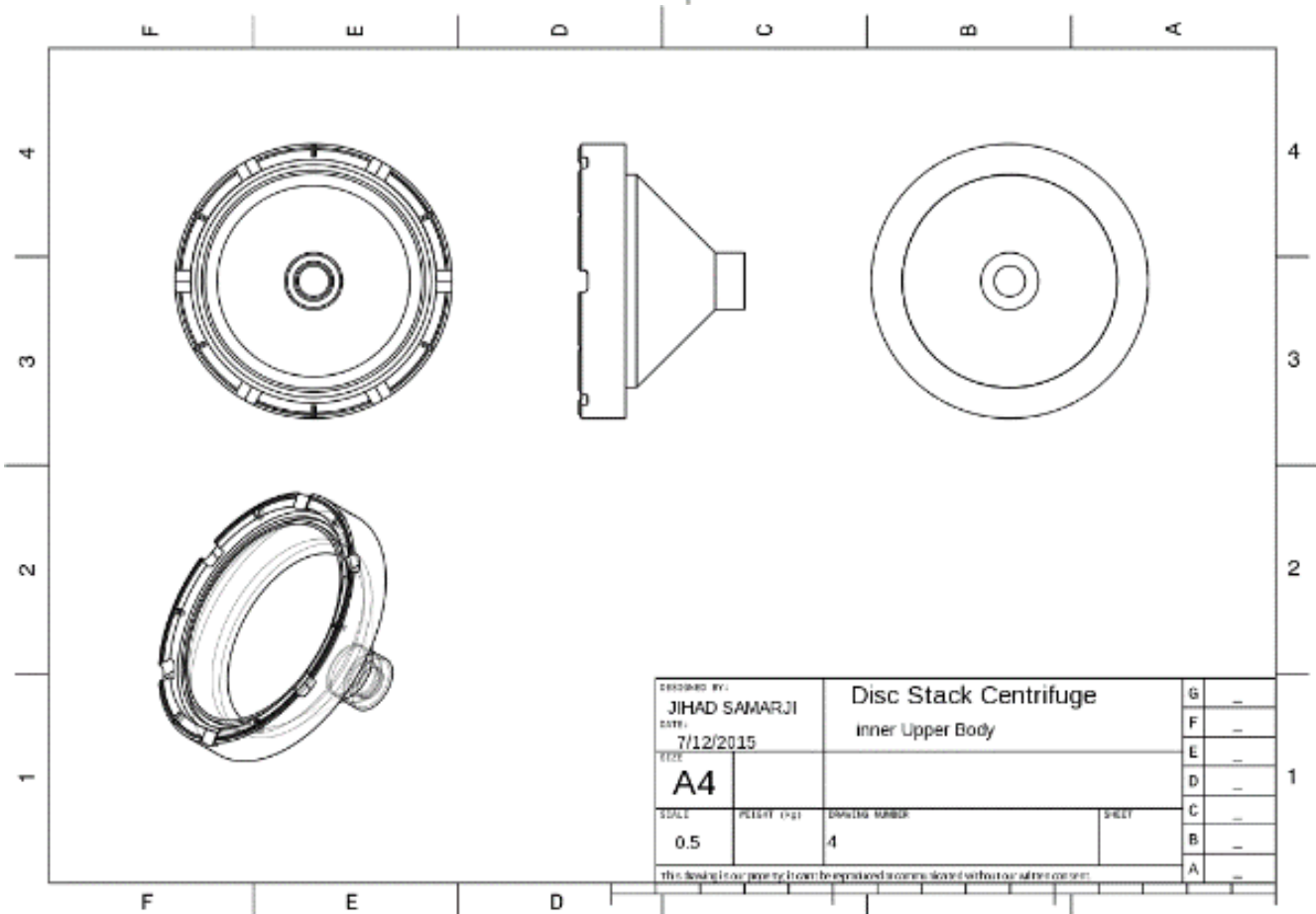
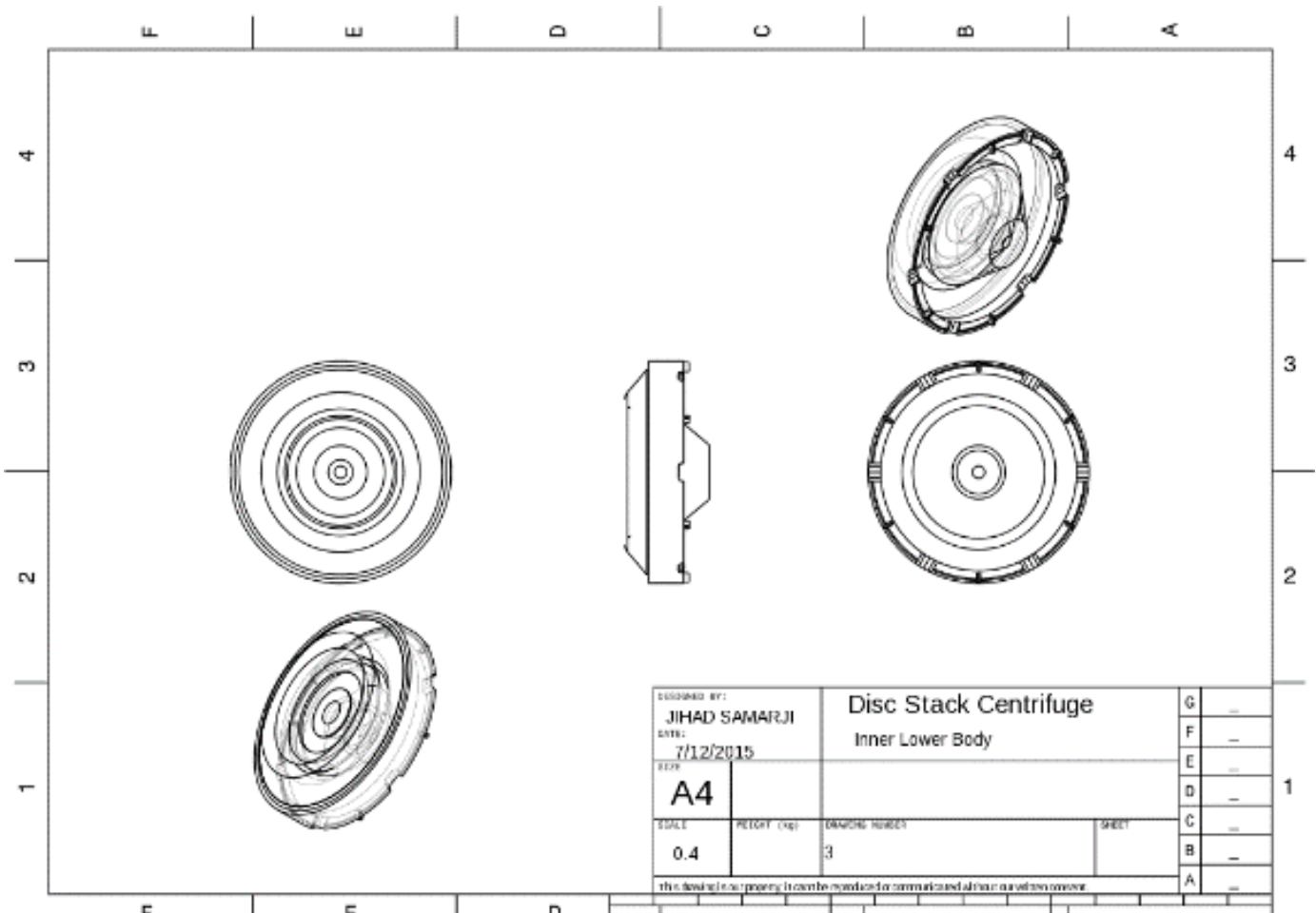
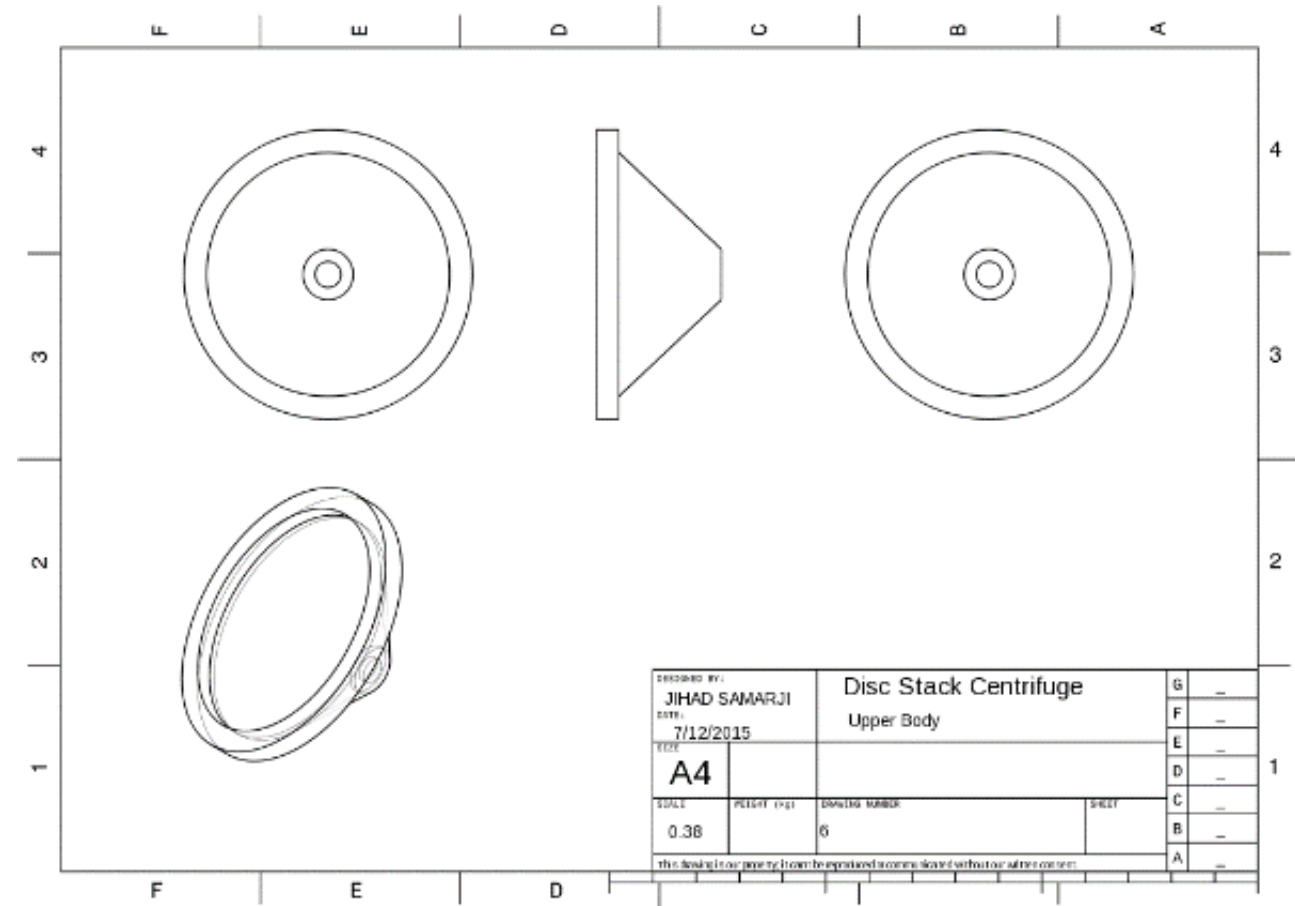
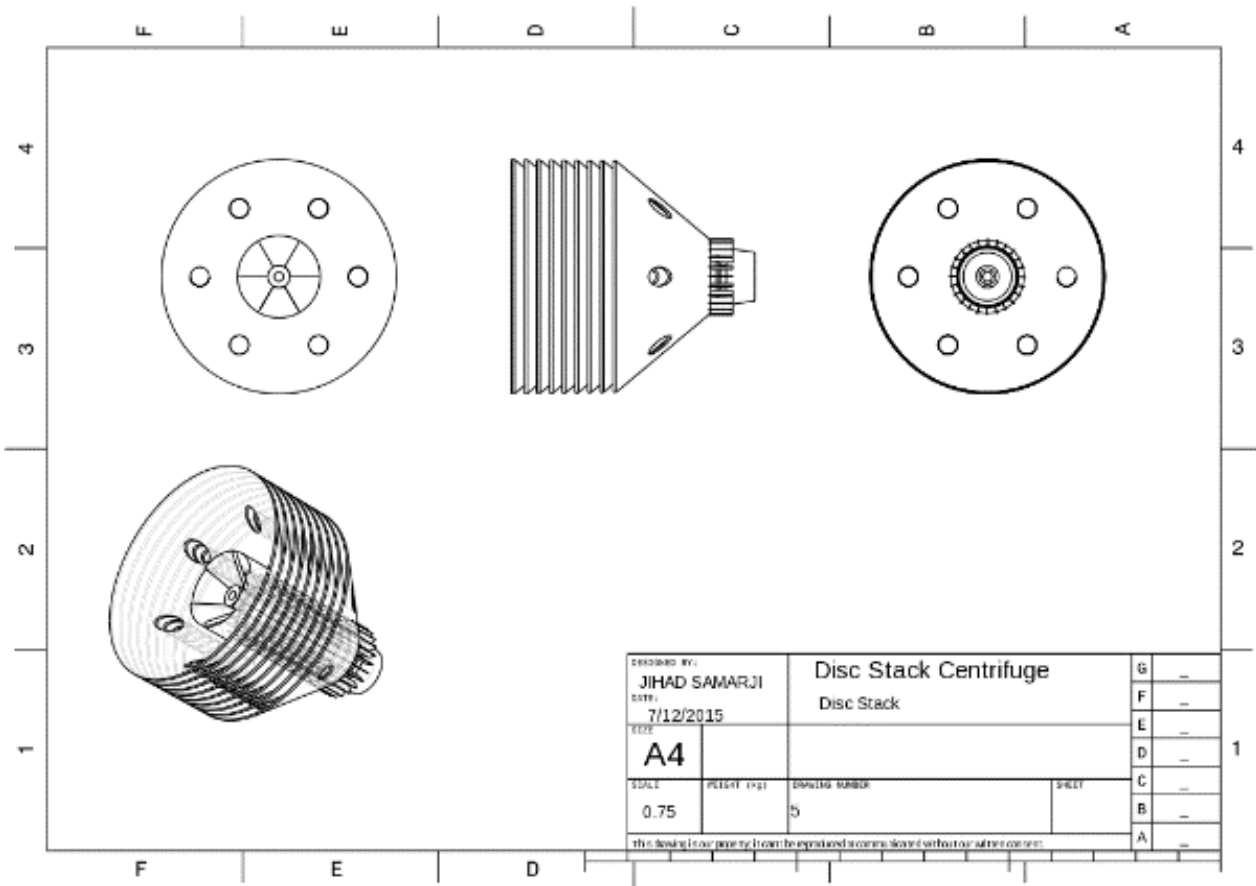


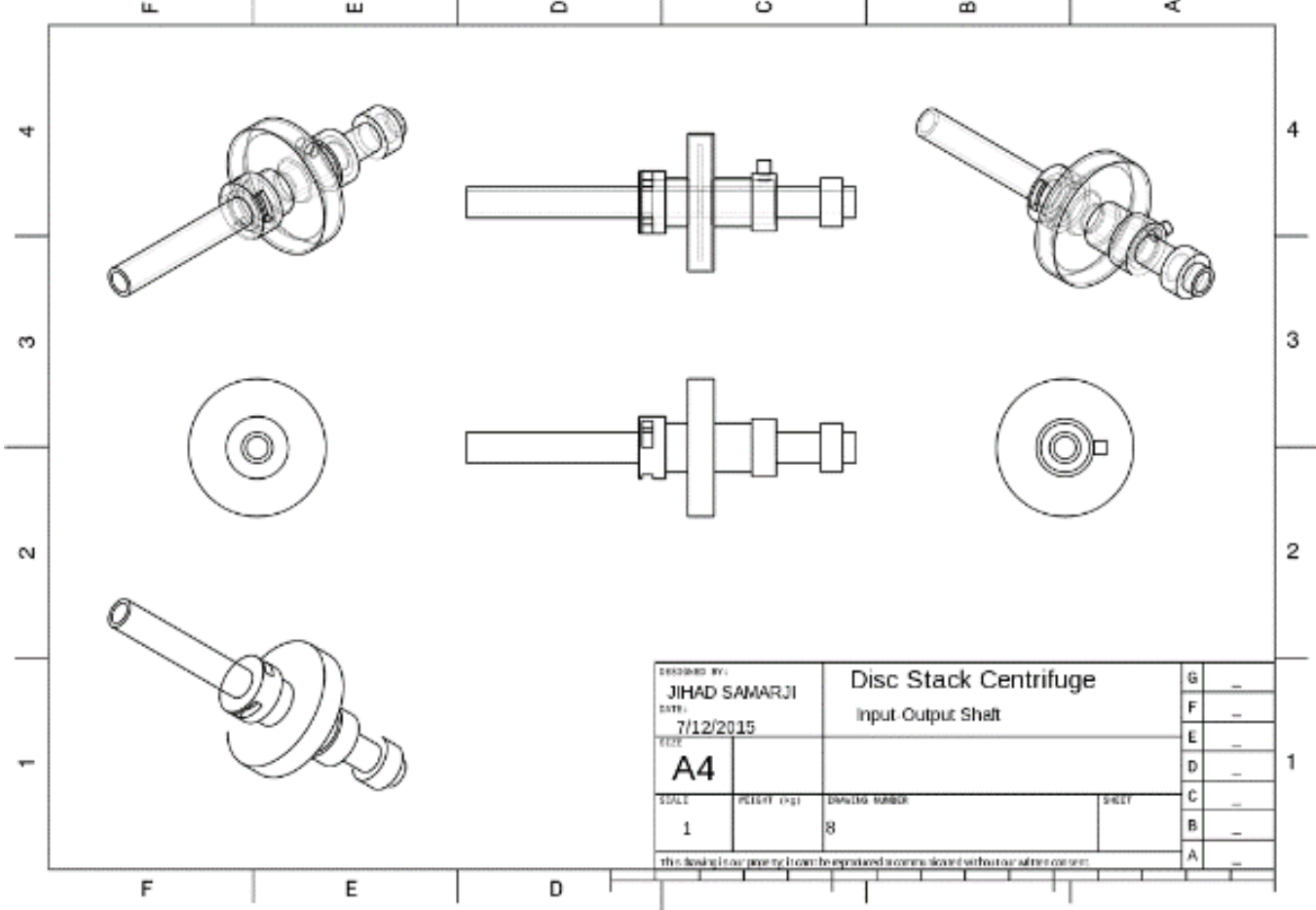
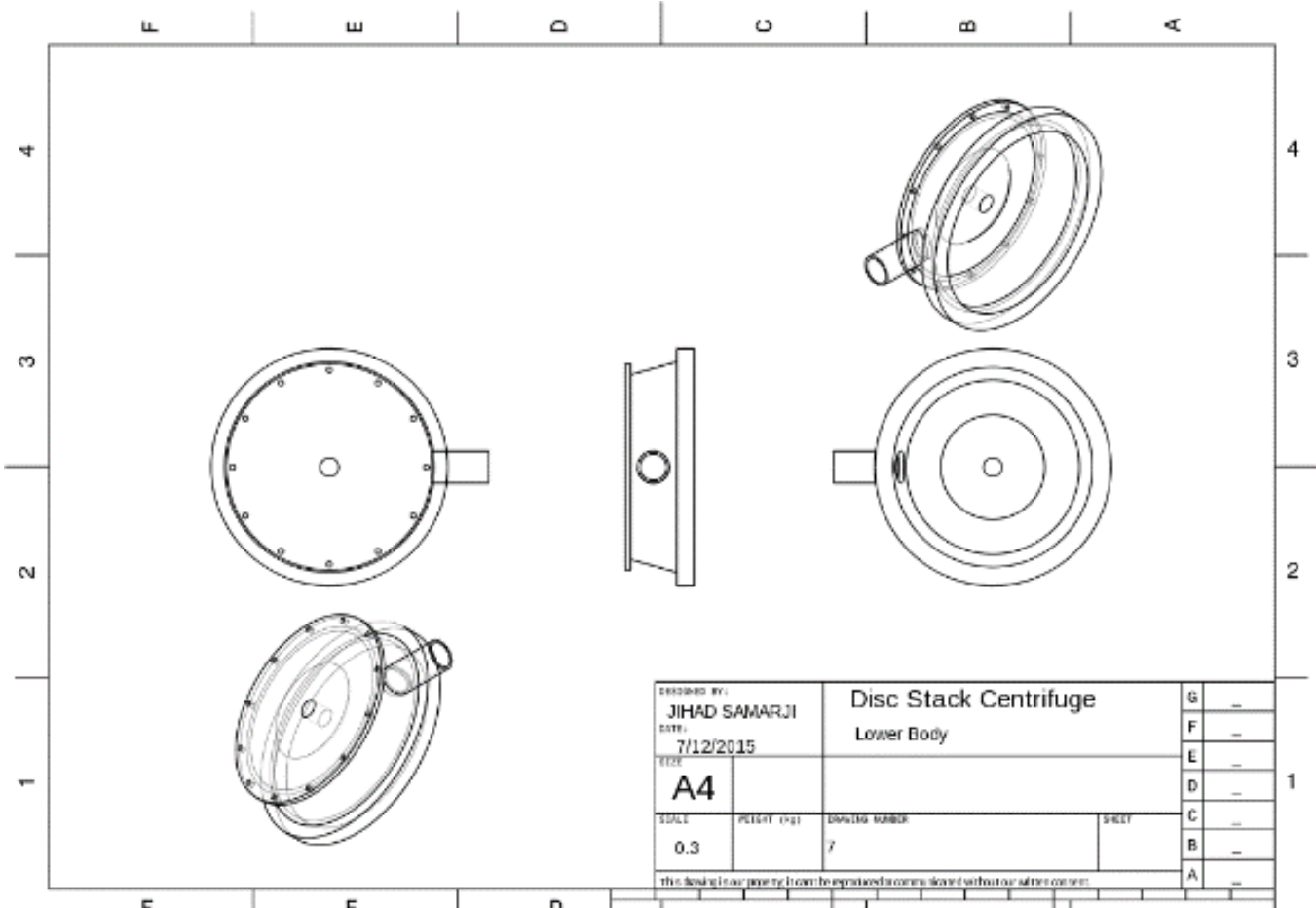
Figure 2.2 Disc Stack Centrifuge Annotation

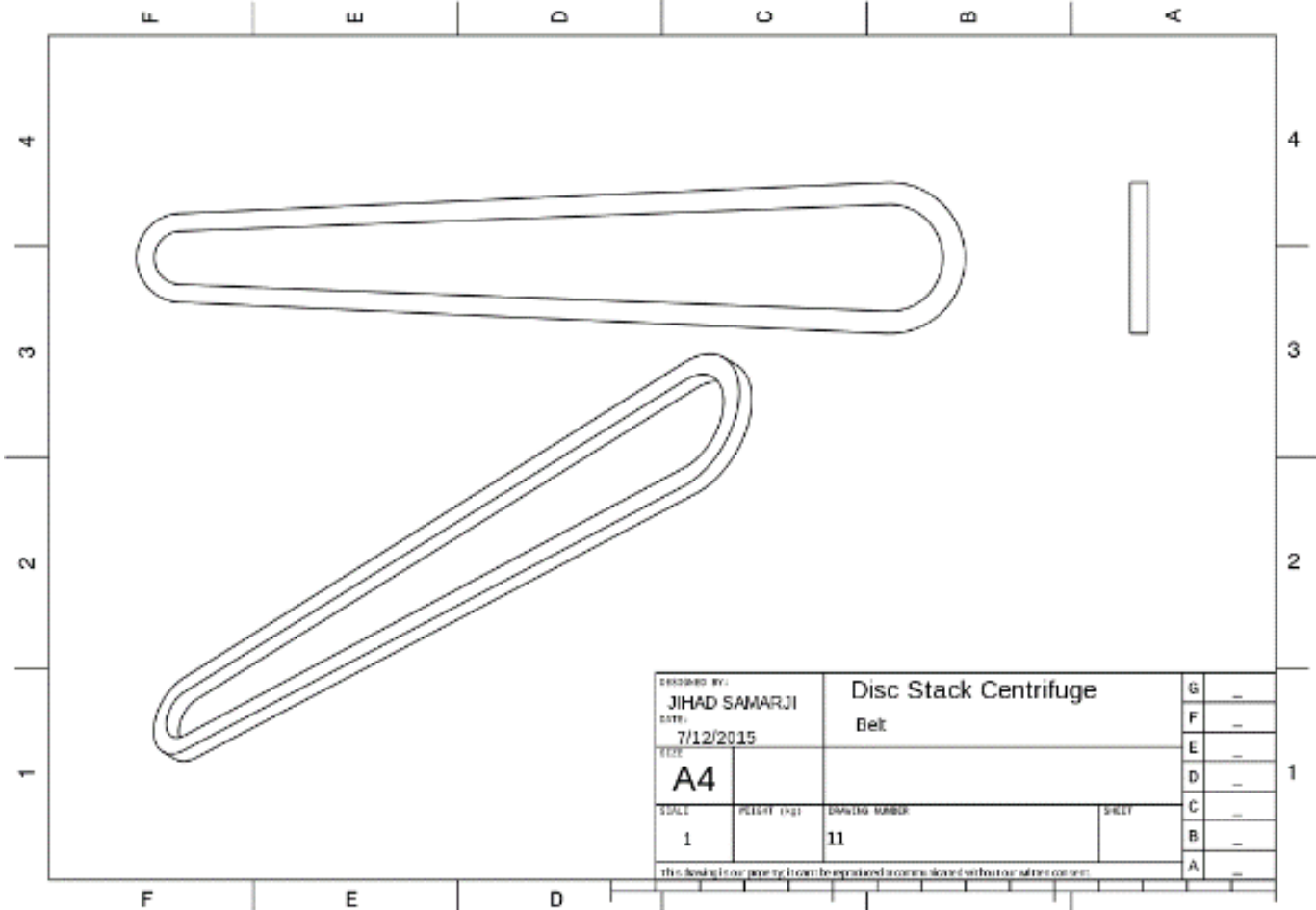
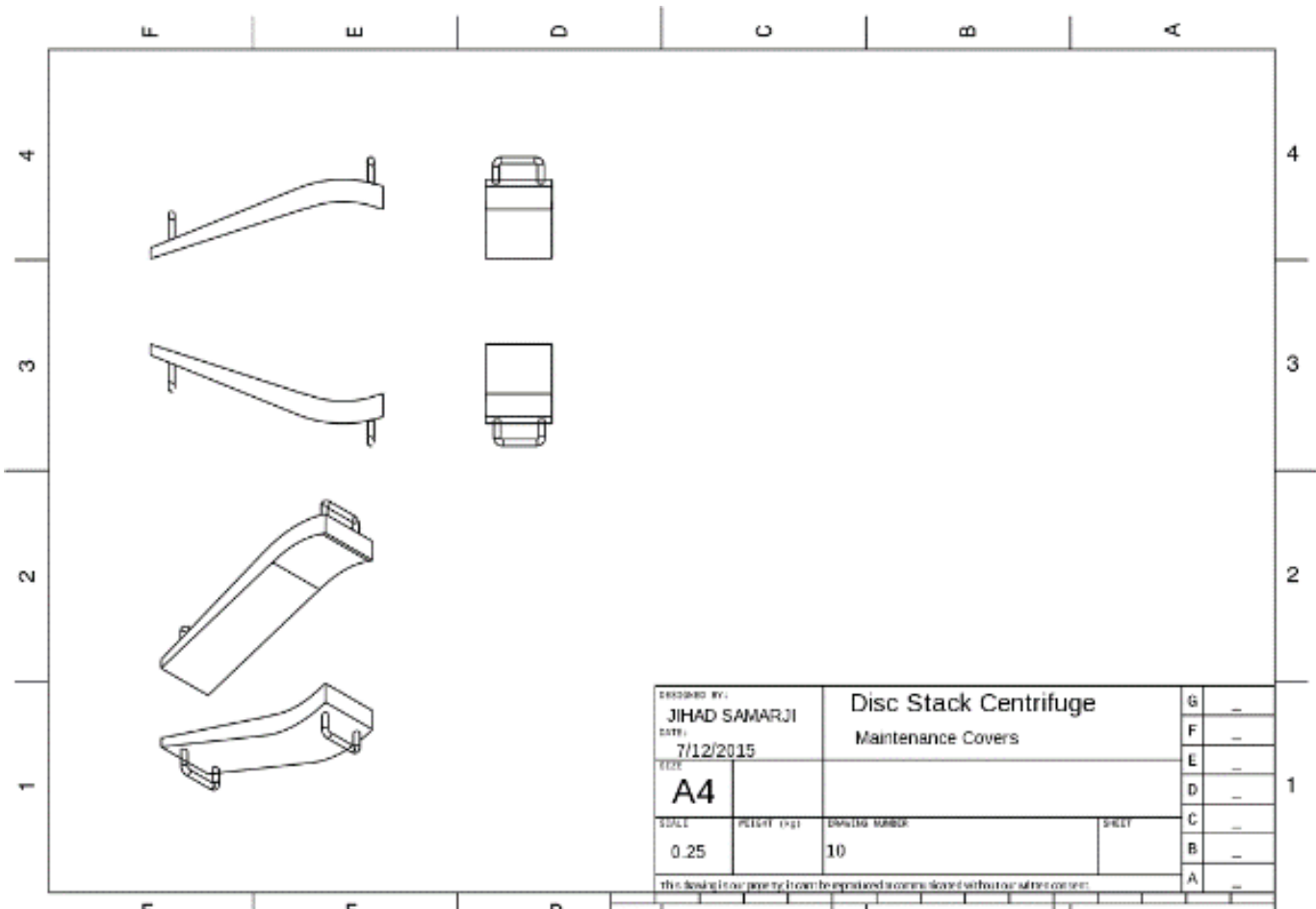
5.2.2 PART DIMENSIONS & DESIGN

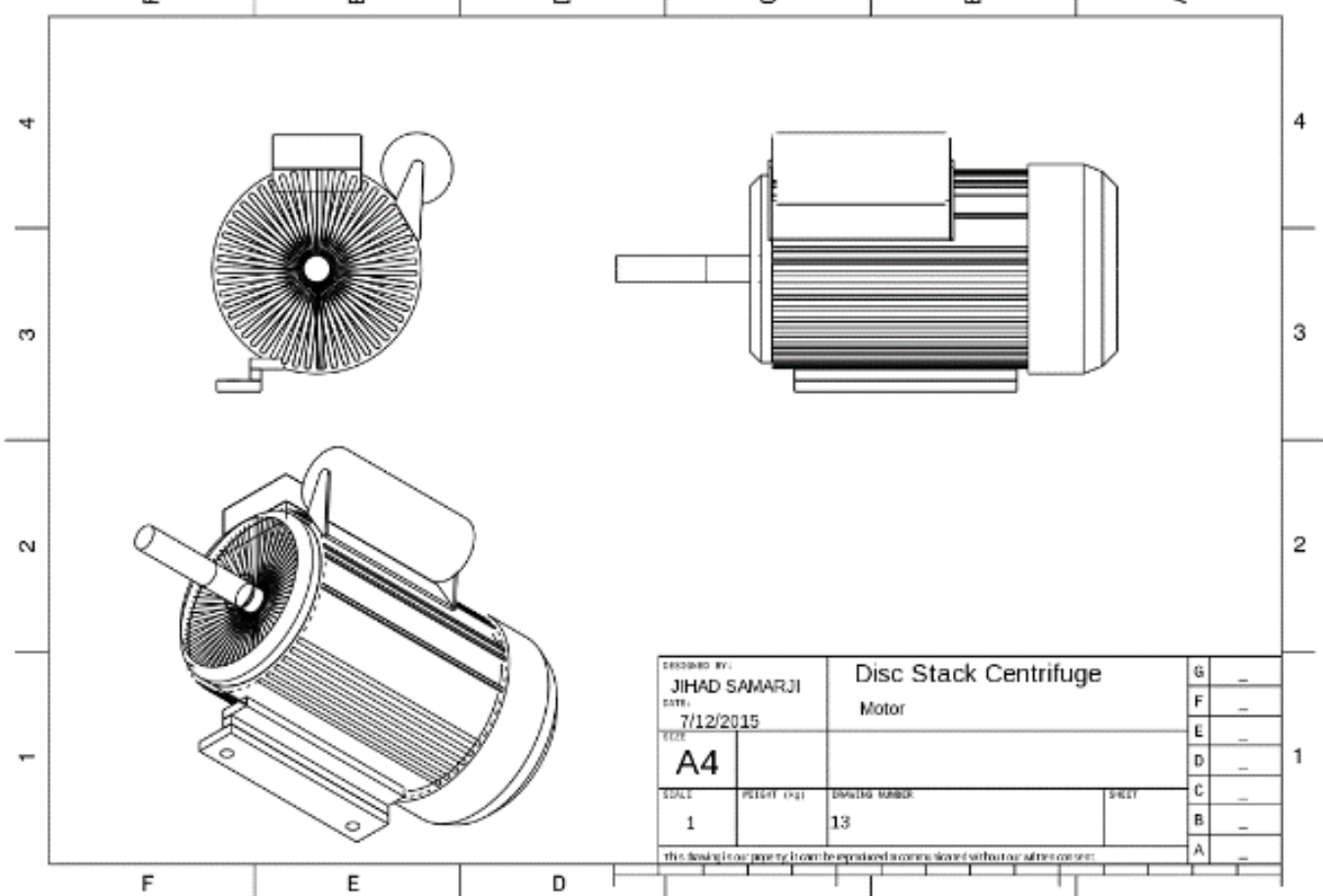
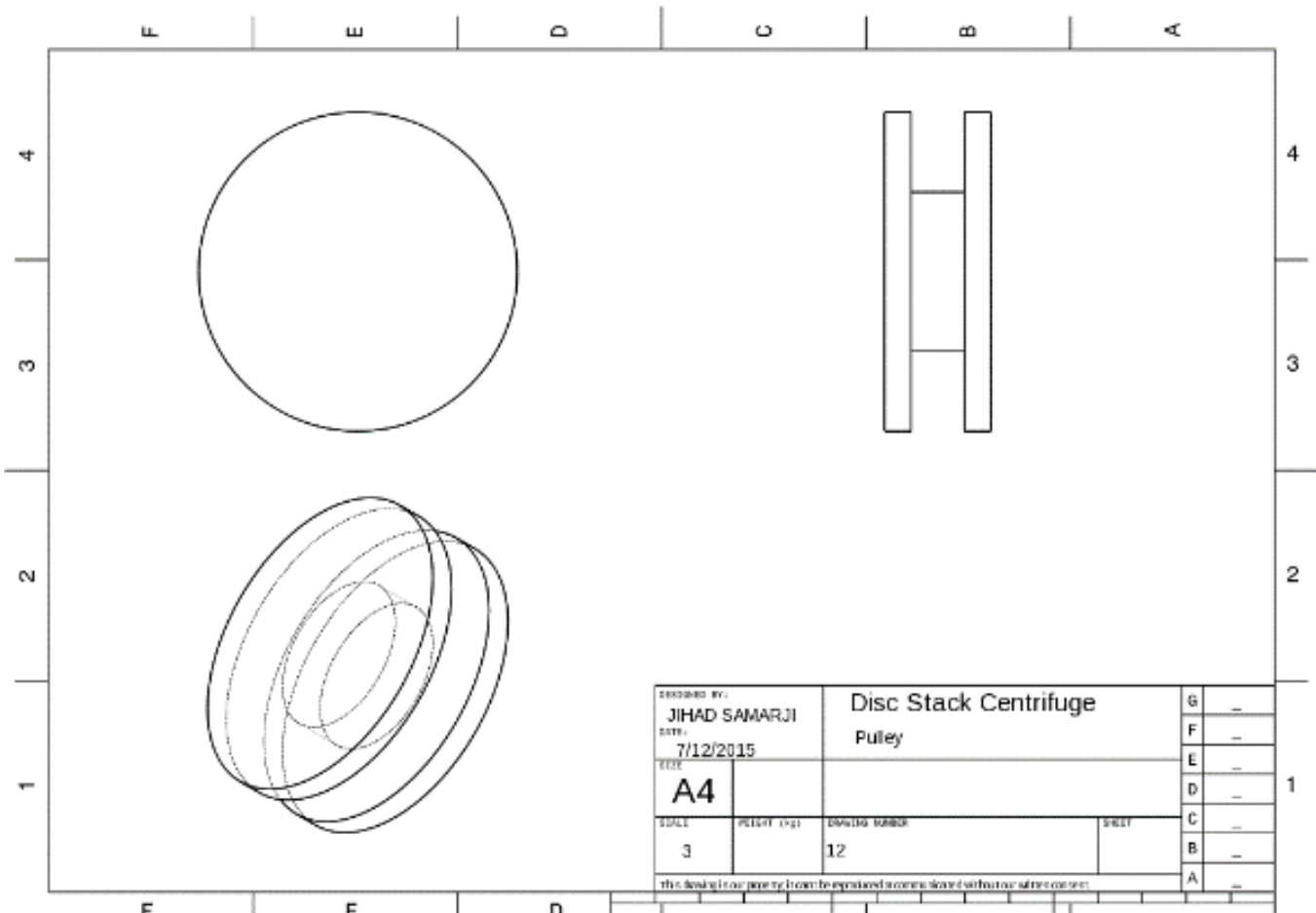












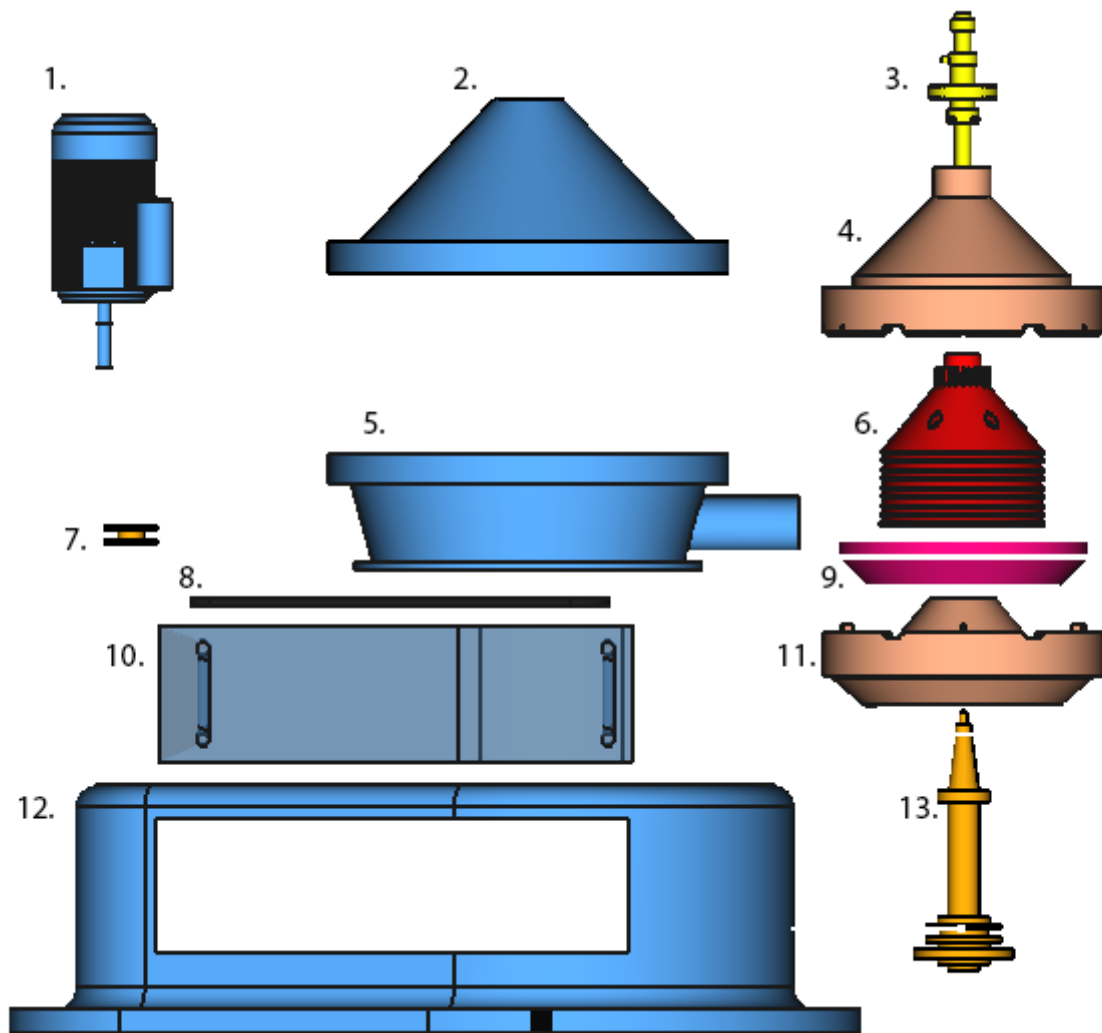
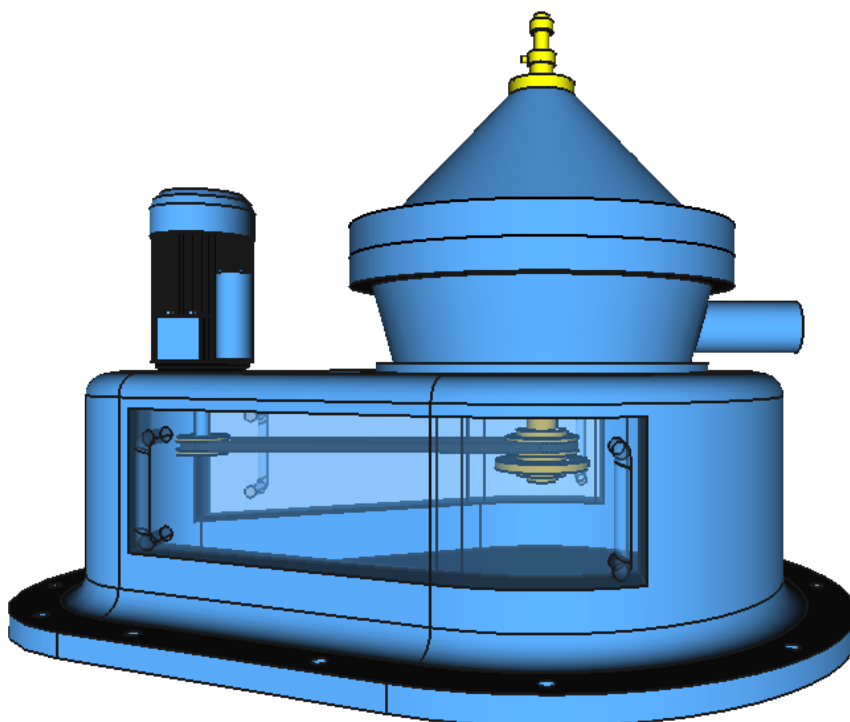


Figure 2.3 Disc Stack Centrifuge Annotations

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

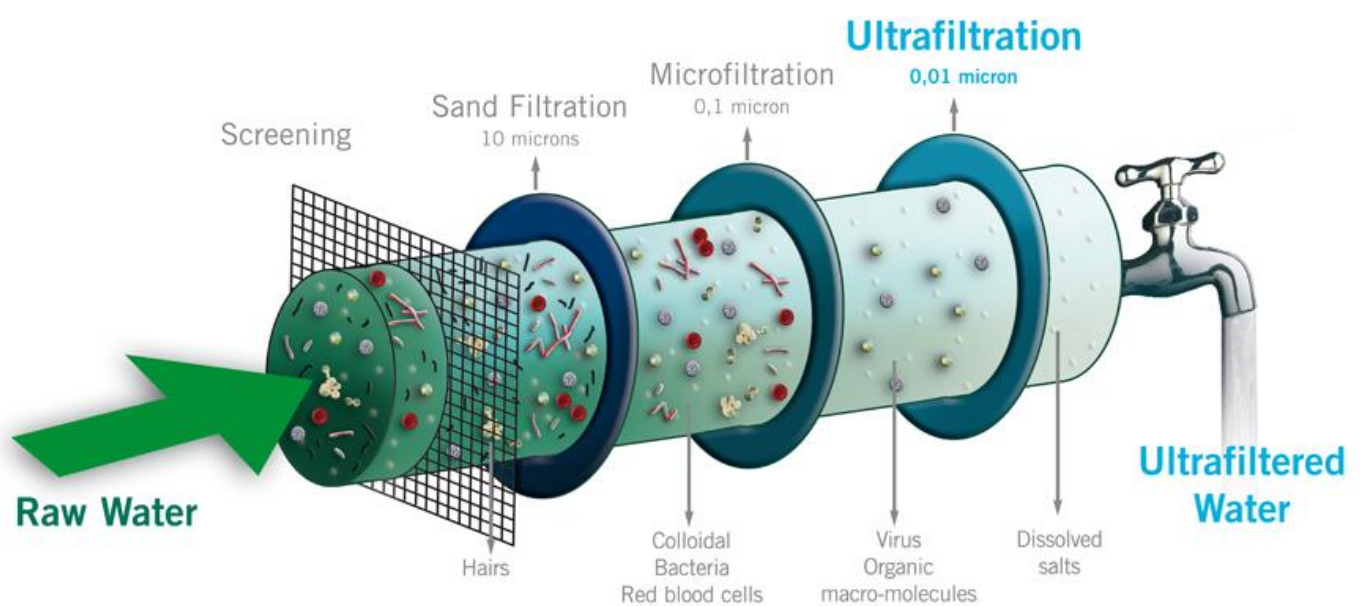


Figure 3.2 a small demonstration about the Ultrafiltration Process

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 the permeate. 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.

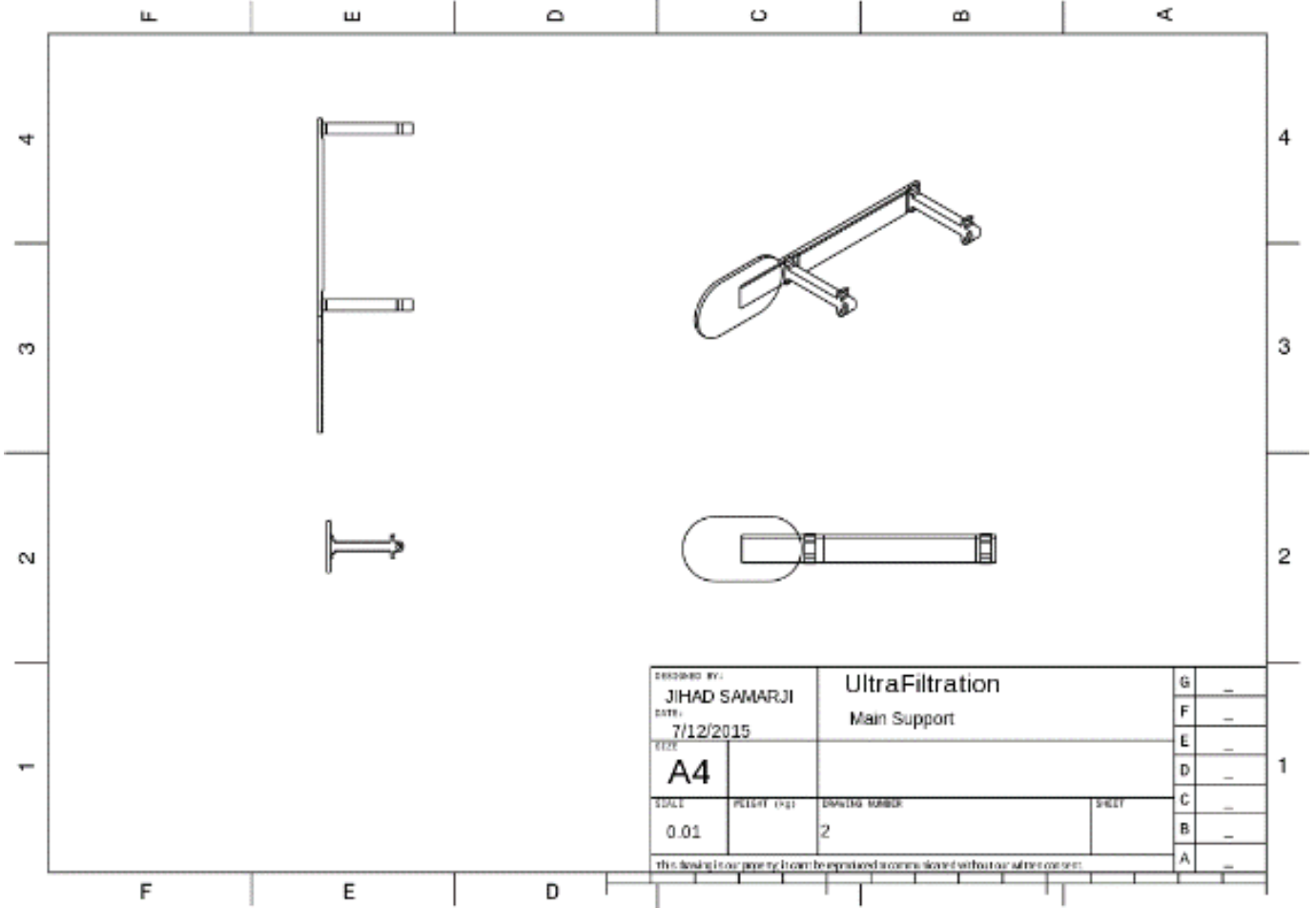
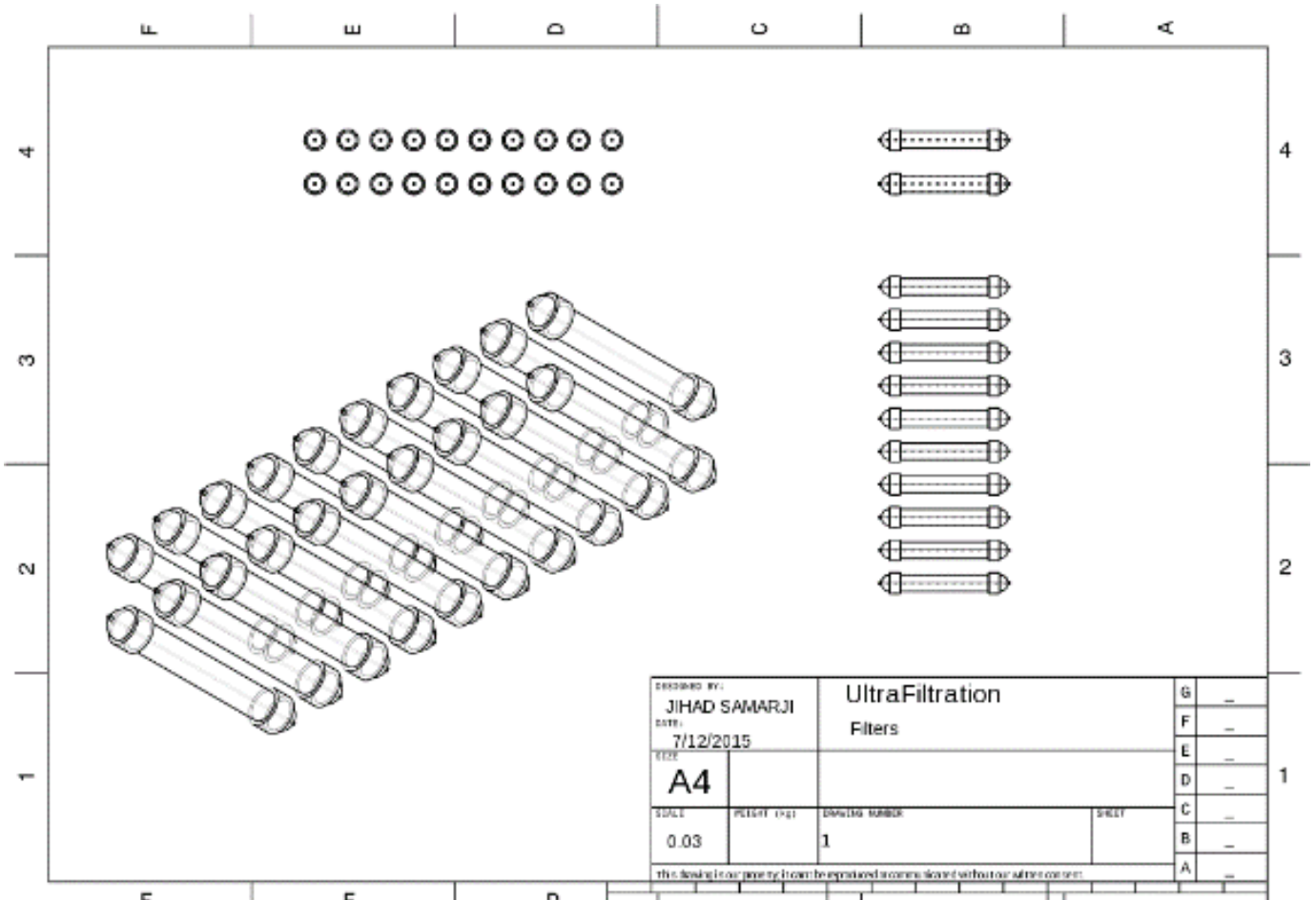
Ultrafiltration is used in Industries such as chemical and pharmaceutical manufacturing, food and beverage processing, and waste water treatment, employ ultrafiltration in order to recycle flow or add value to later products. Blood dialysis also utilizes ultrafiltration.

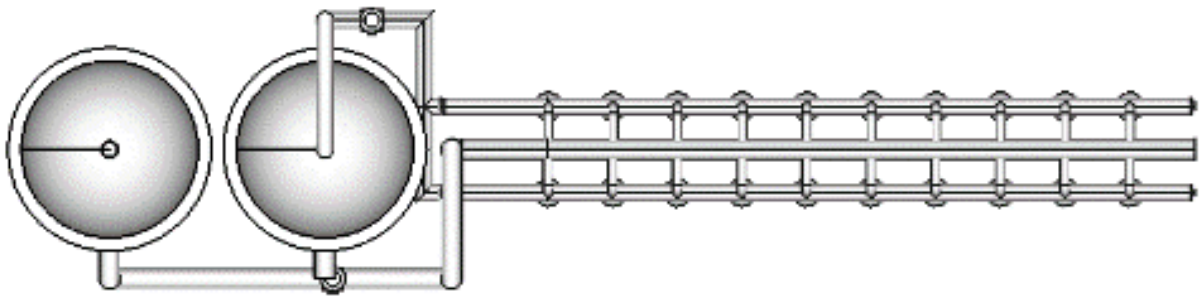
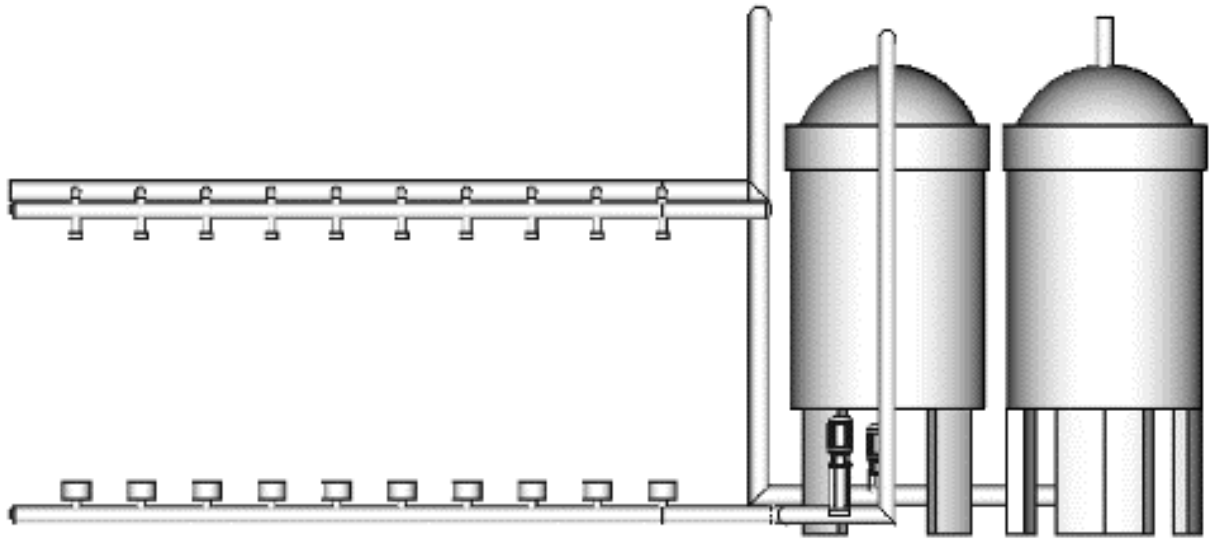
In our case we're going to use the ultrafiltration device for Protein concentration. See Figure.3.1 and 3.2

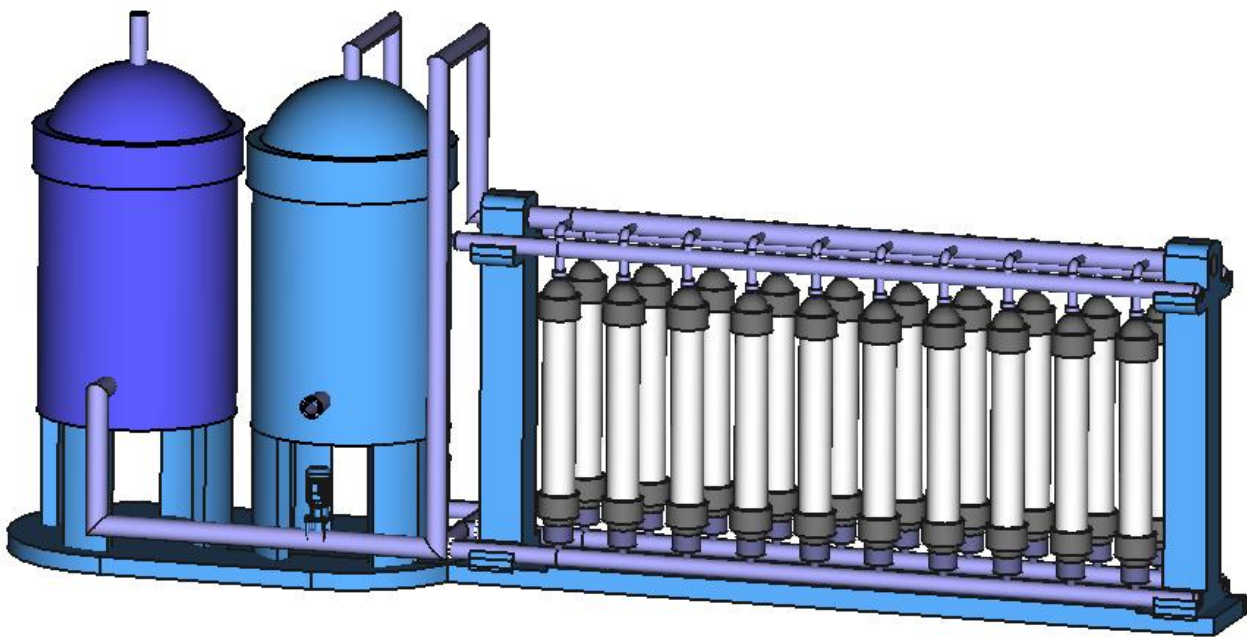
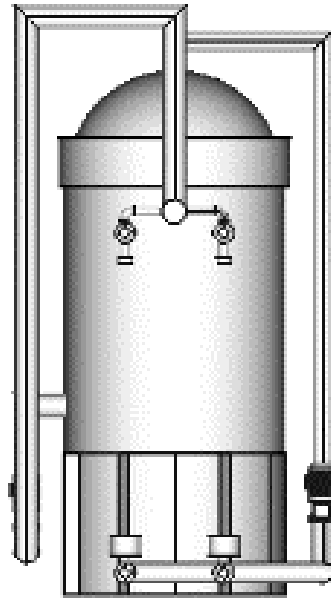
| Foulant | Reagent | Time and Temperature | Mode of Action |
|--|---|----------------------------------|--|
| Fats and oils, proteins, polysaccharides, bacteria | 0.5M NaOH with 200 ppm Cl ₂ | 30-60 min 25-55 °C | Hydrolysis and oxidation |
| DNA, mineral salts | 0.1M – 0.5M acid (acetic, citric, nitric) | 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.

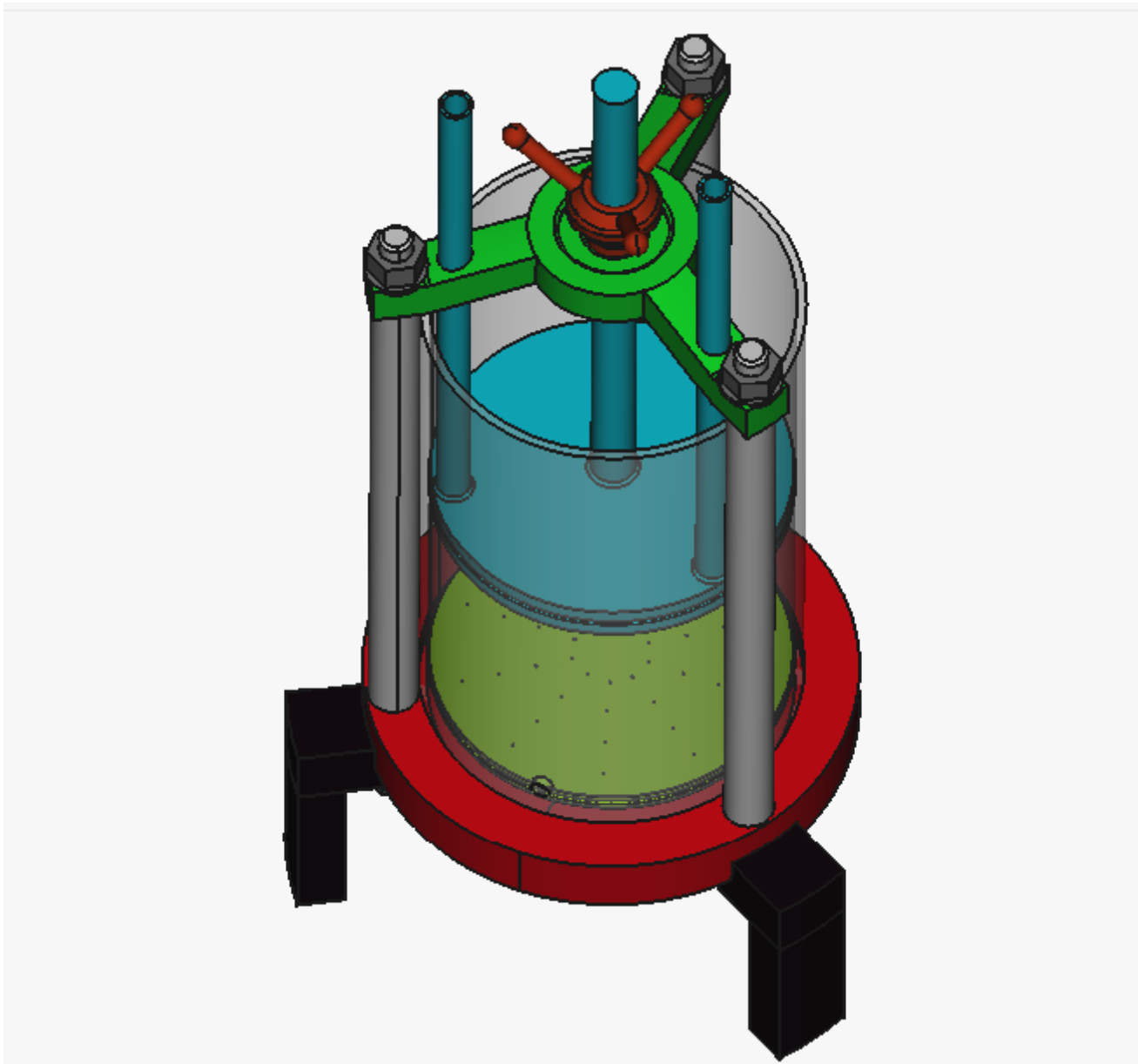
The AxiChrom process column family has been designed to deliver reproducible results from process development to production scales. This is facilitated by the innovative Intelligent Packing where UNICORN™ software, ÄKTA™ systems and AxiChrom columns work together to facilitate a convenient operation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

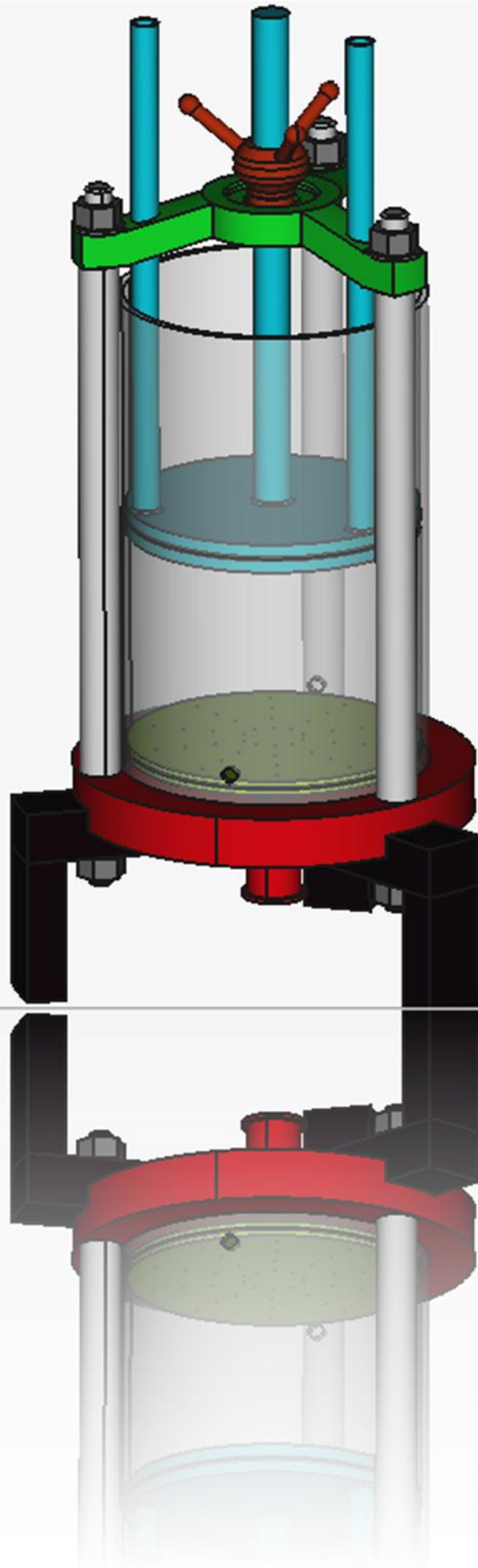
The Intended use of The AxiChrom family of process columns has been designed for low pressure chromatographic separation of biomolecules such as proteins, peptides and oligonucleotides in GMP-regulated environments.

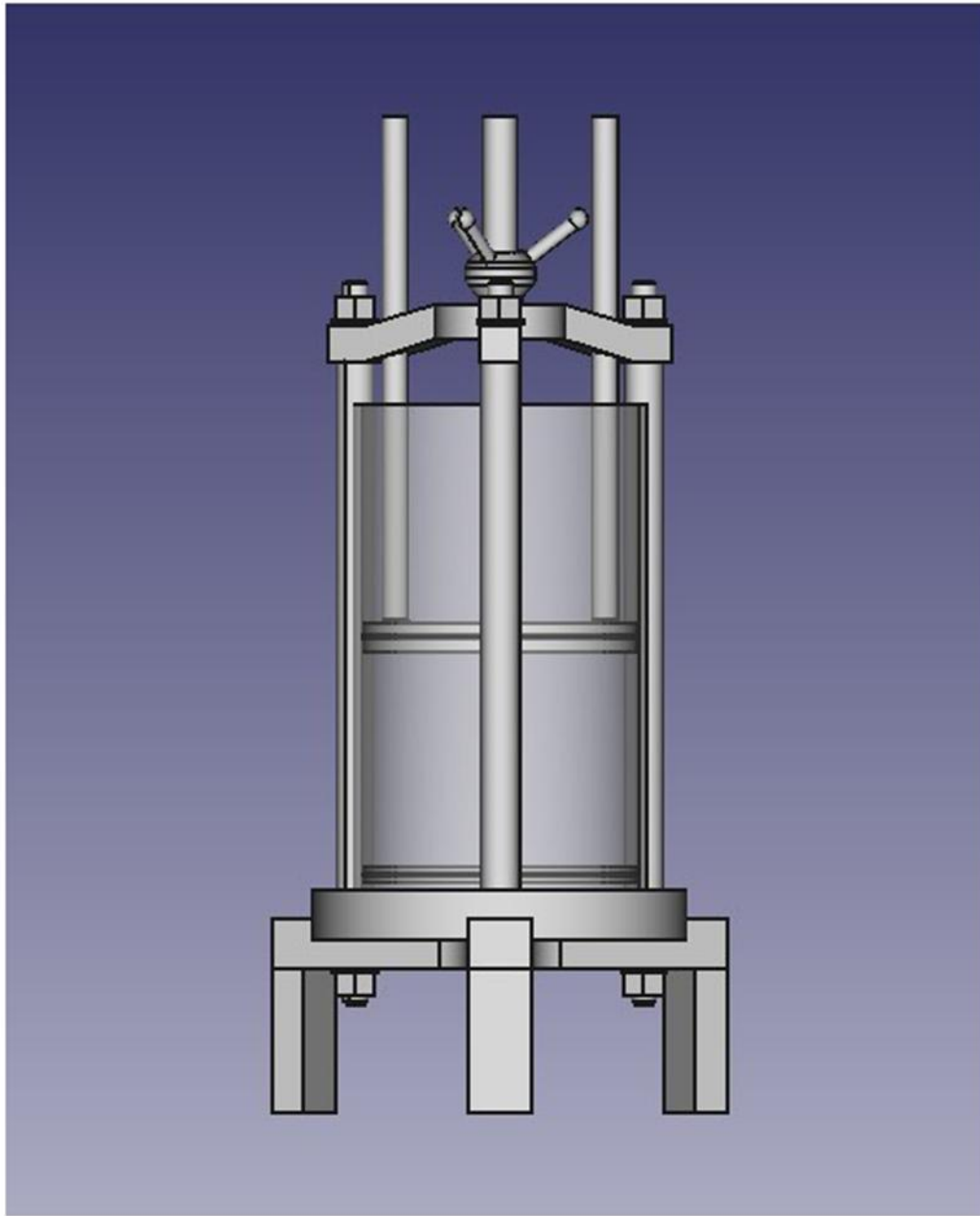
The AxiChrom columns are intended for production use only and should not be used for diagnostic purposes in any clinical or in vitro procedures. The columns are not suitable for operation in a

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



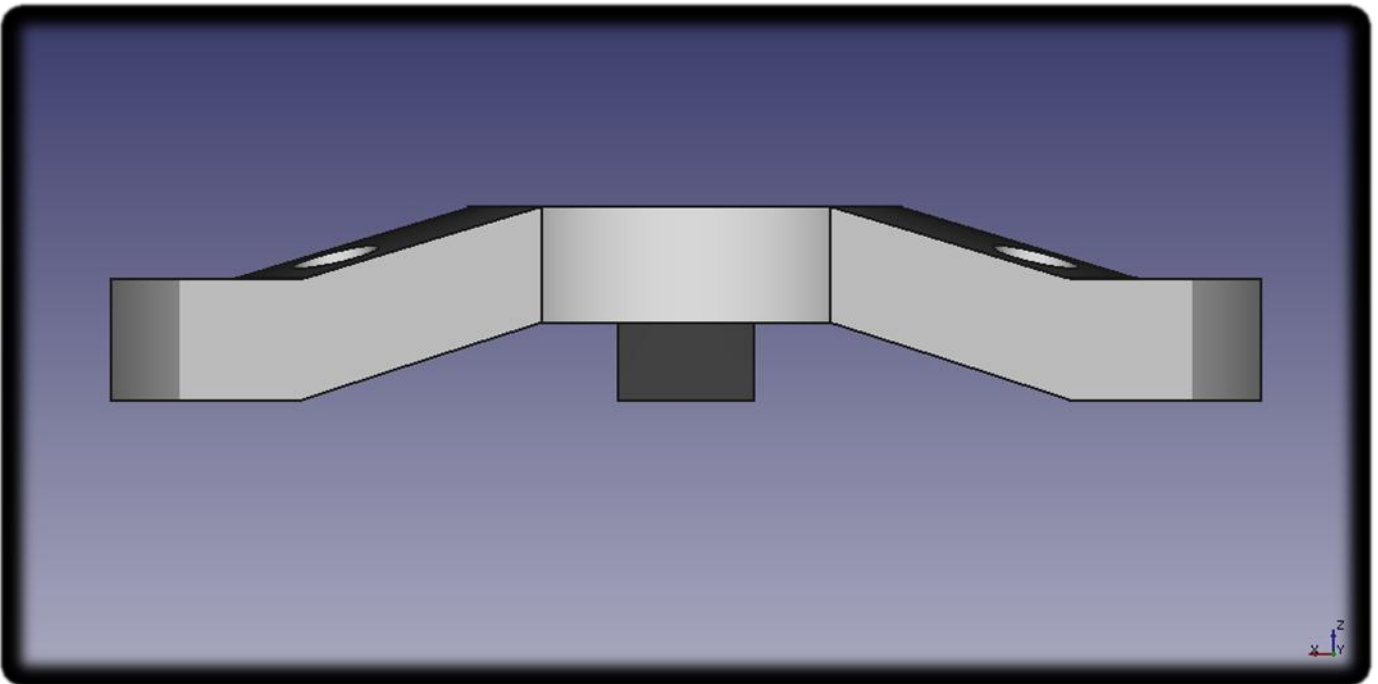
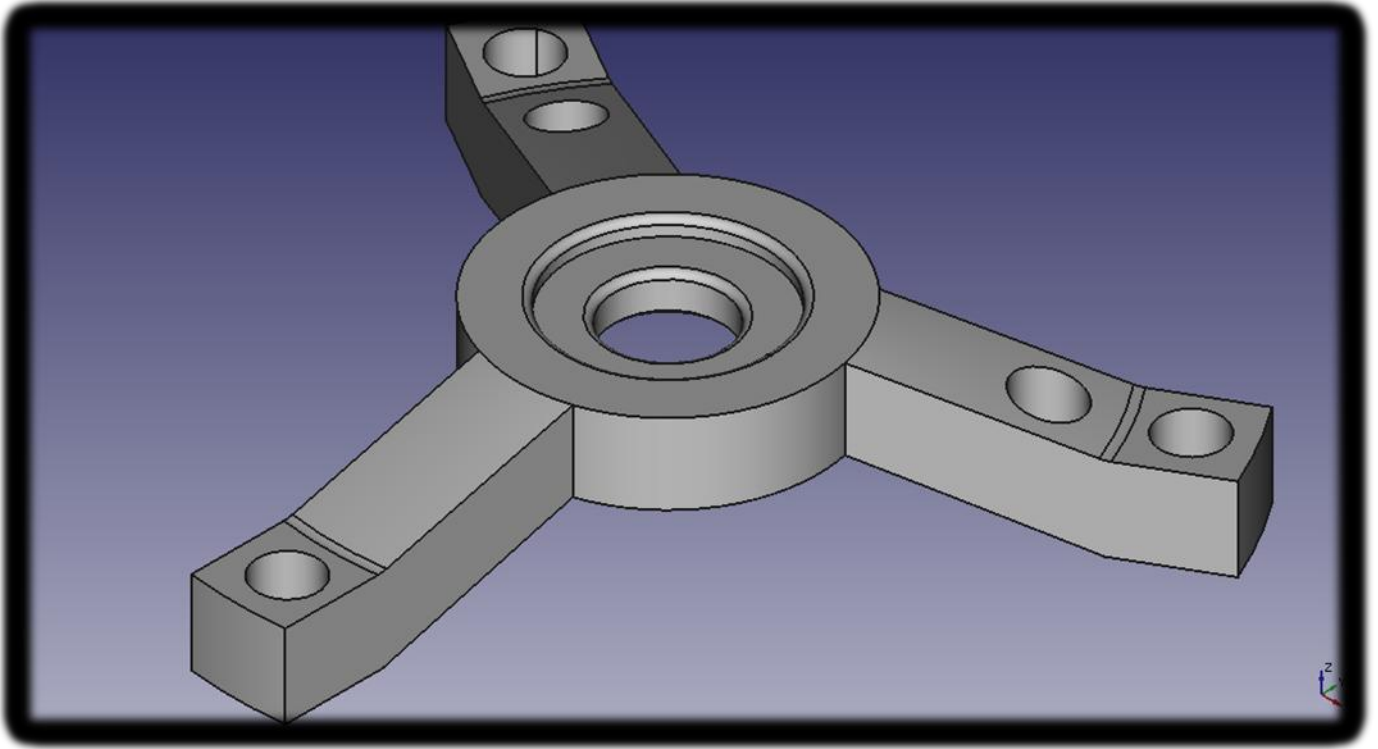


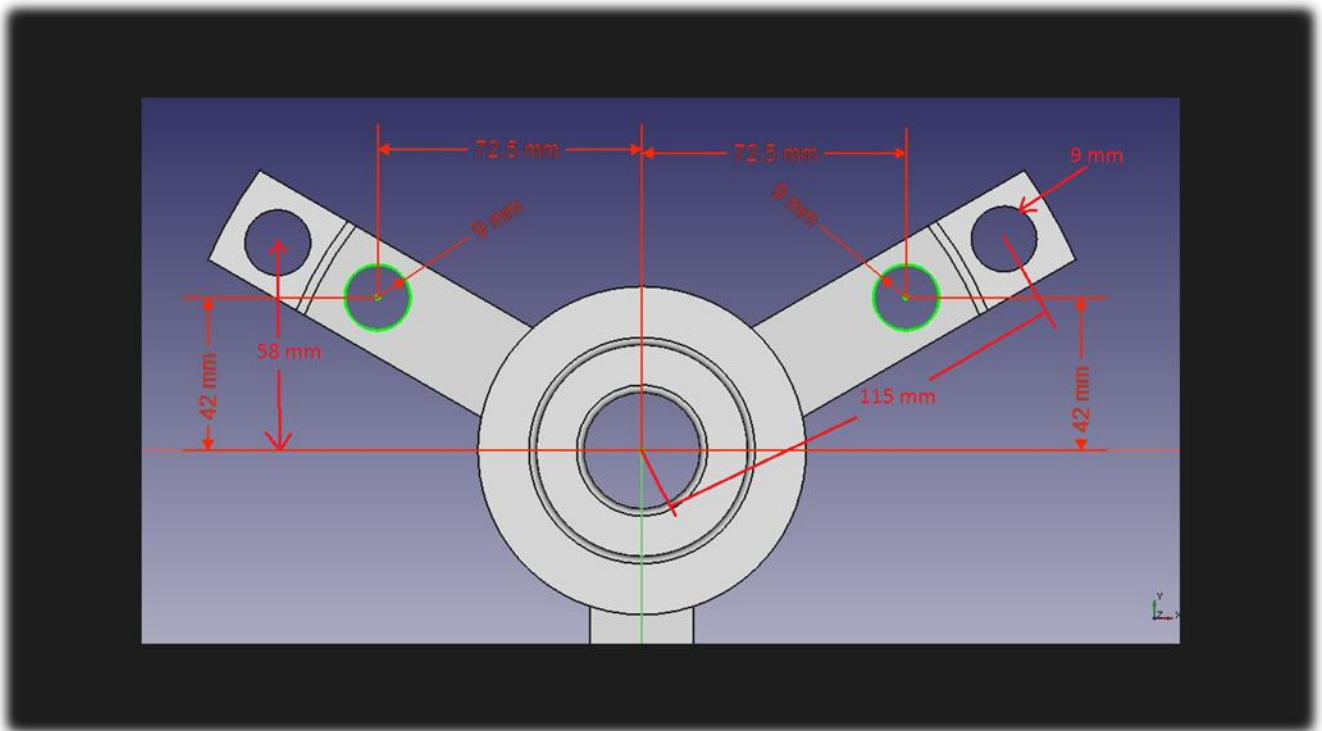
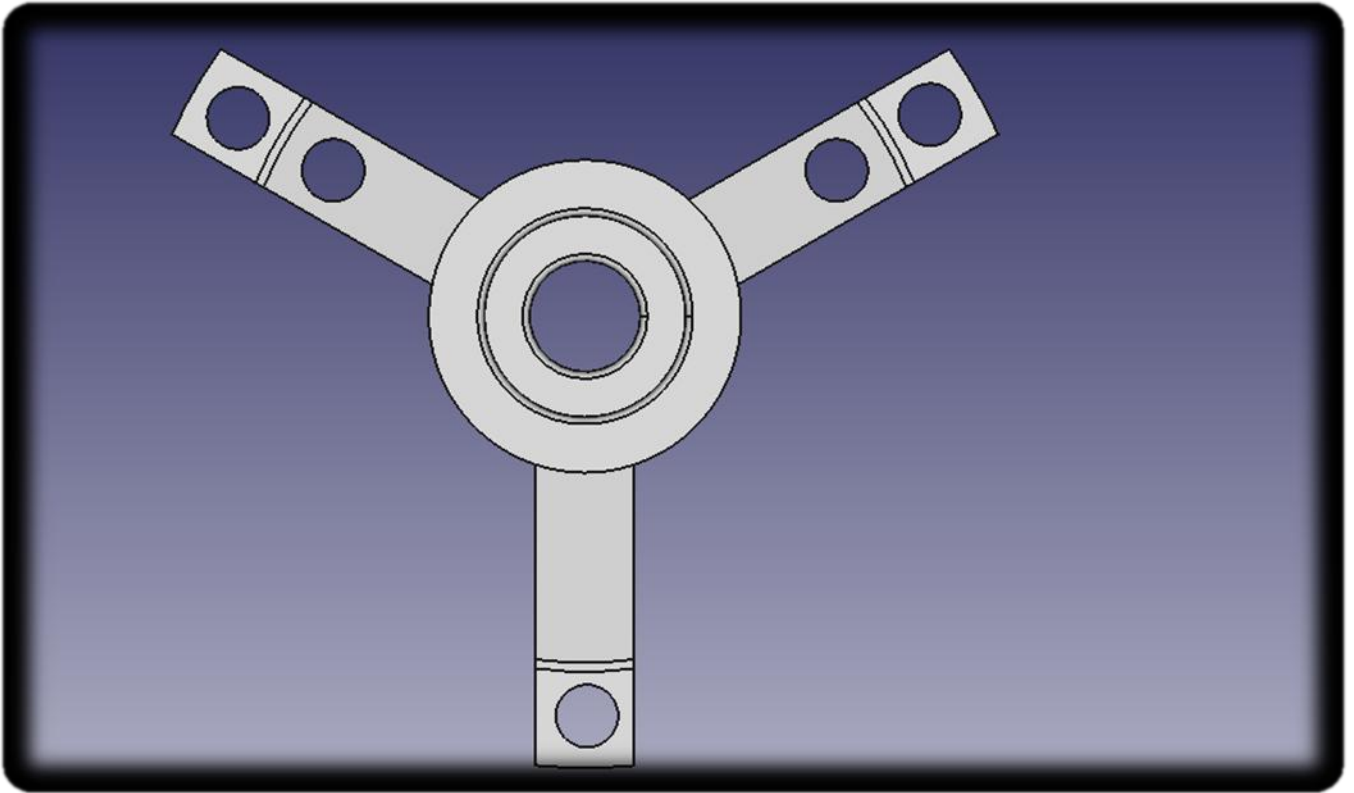


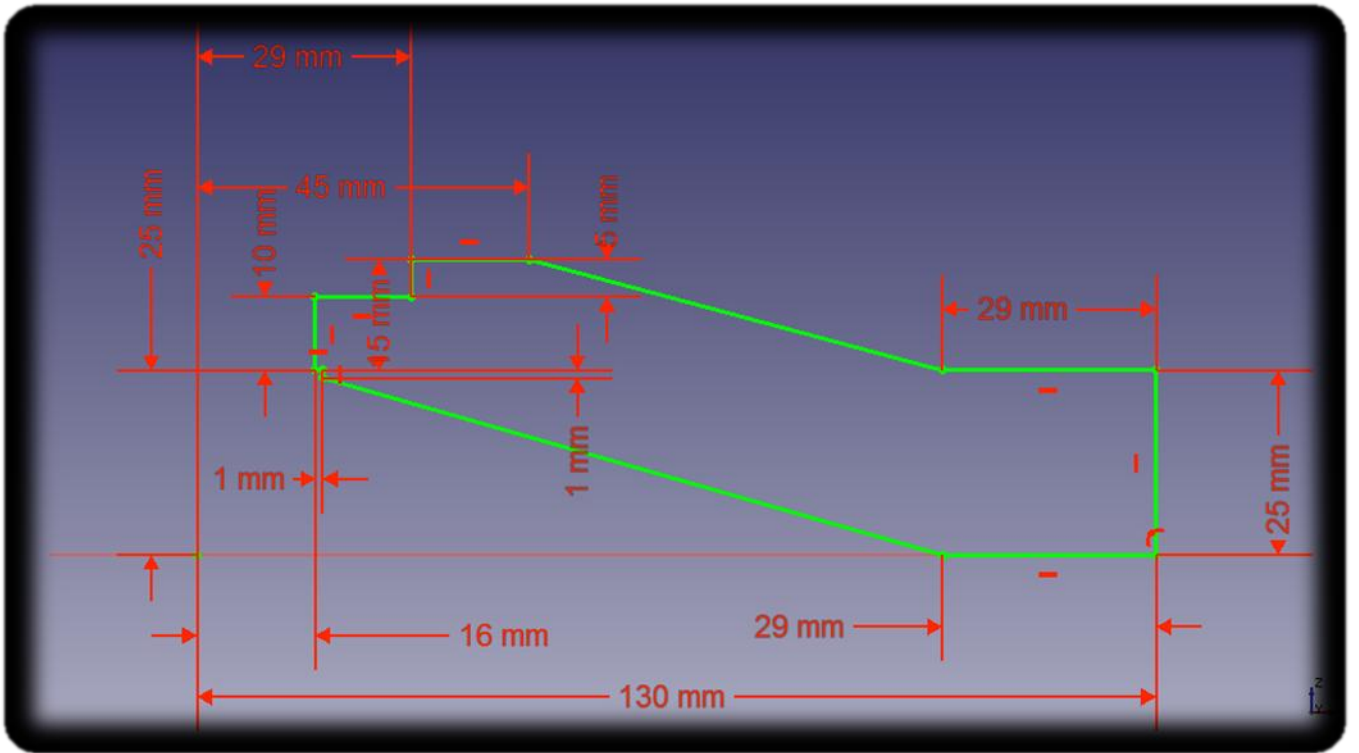
The Chromatography column contains eight main parts: top, handle, cylinder, piston, disc, bottom, base, and column.

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

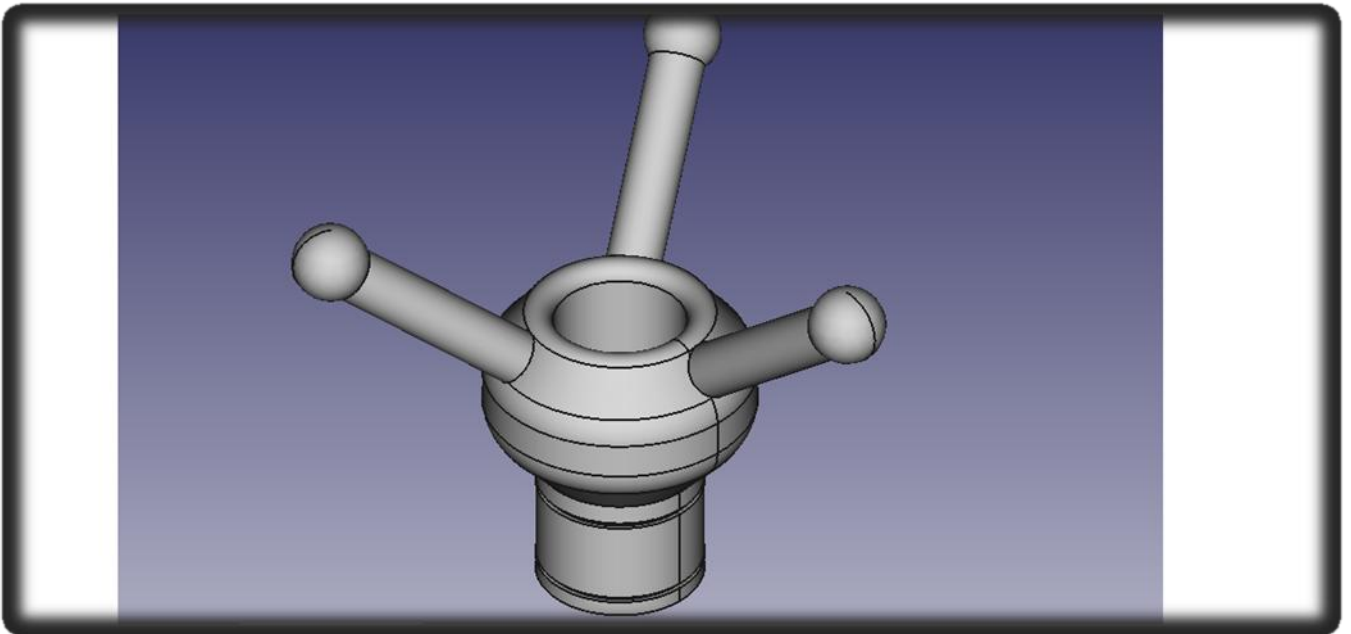


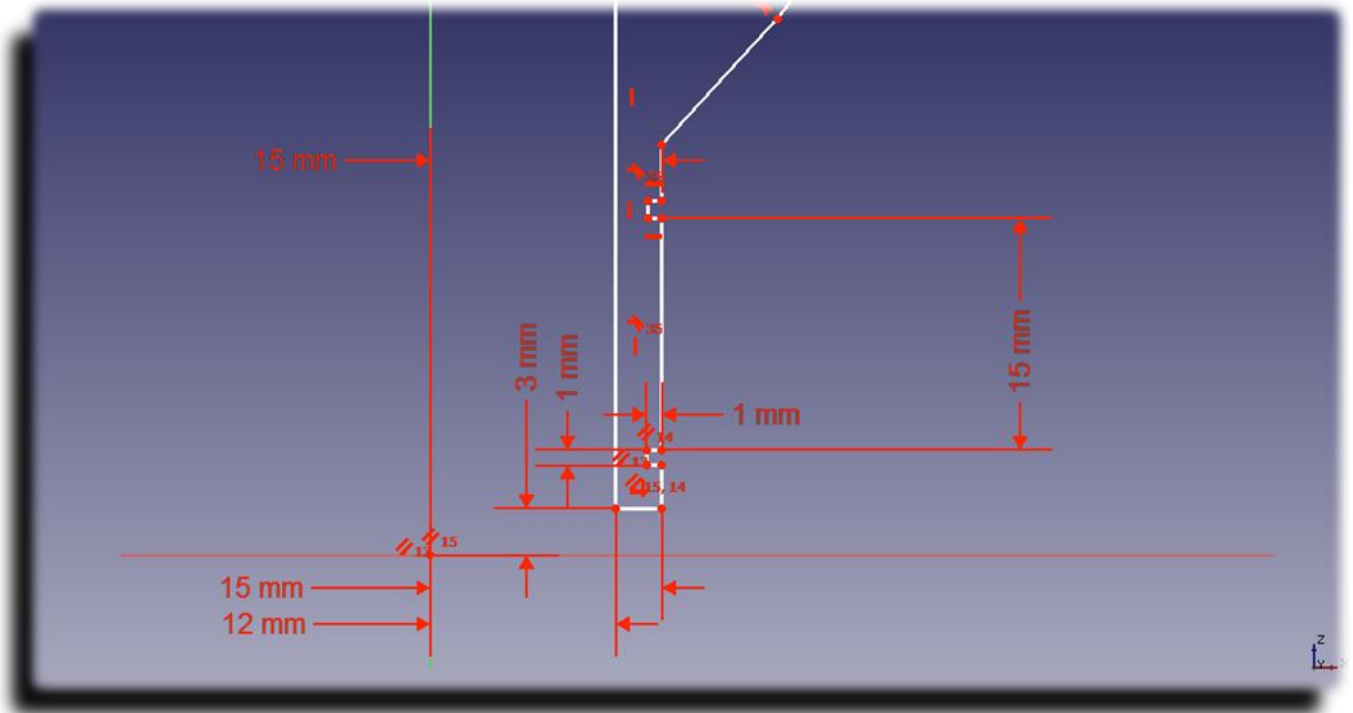
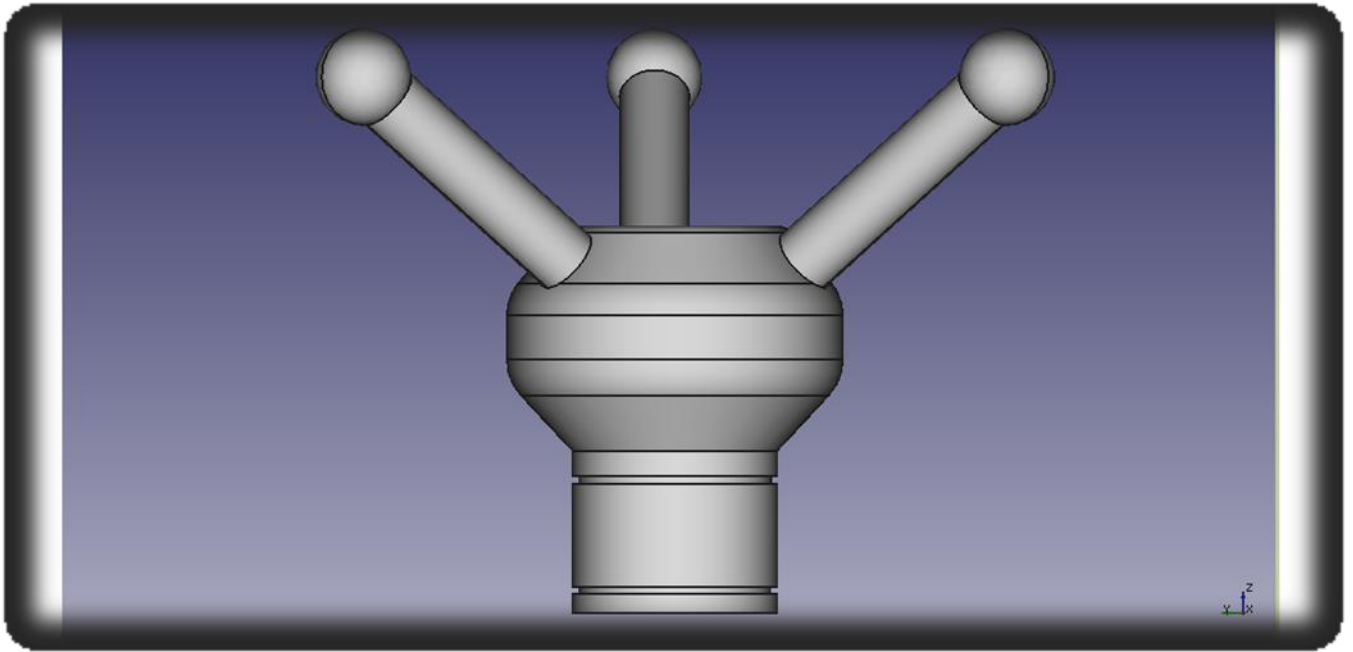


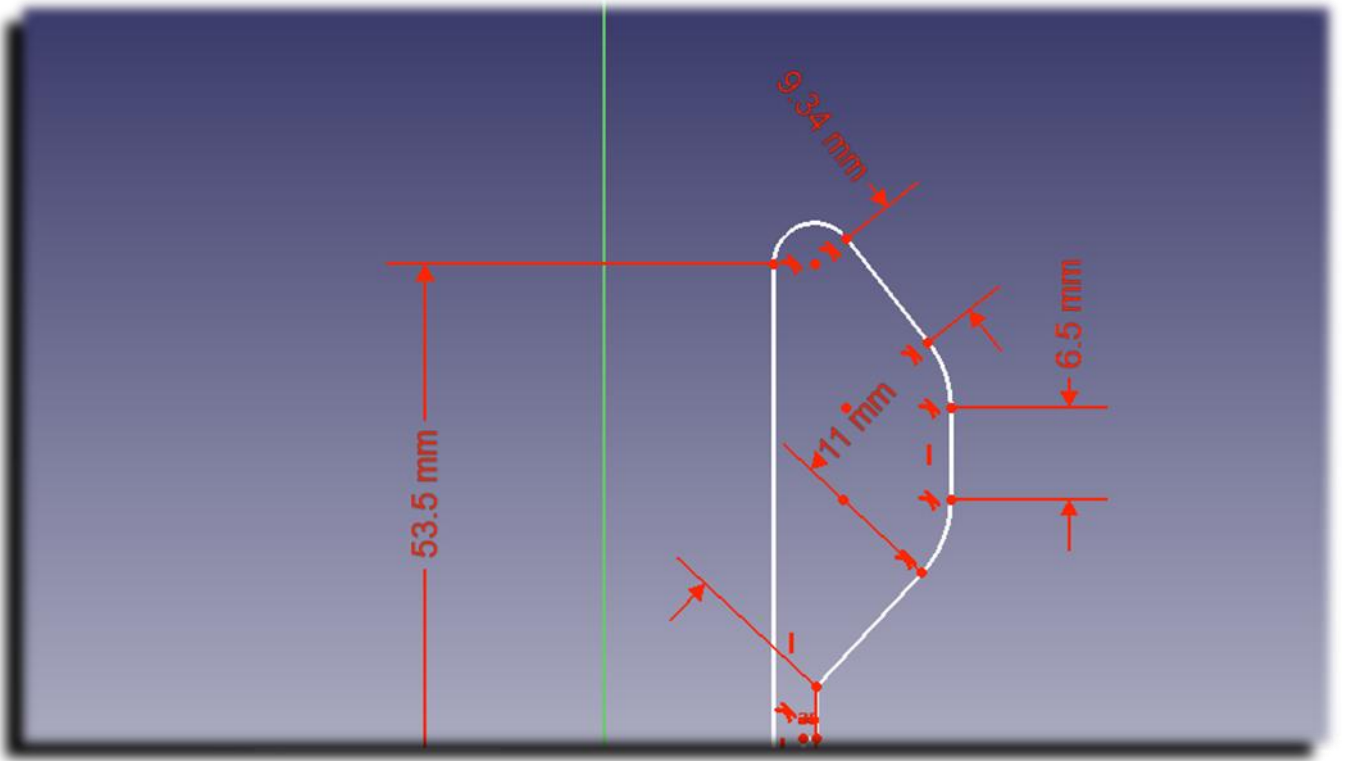


5.4.2.2 Handle

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

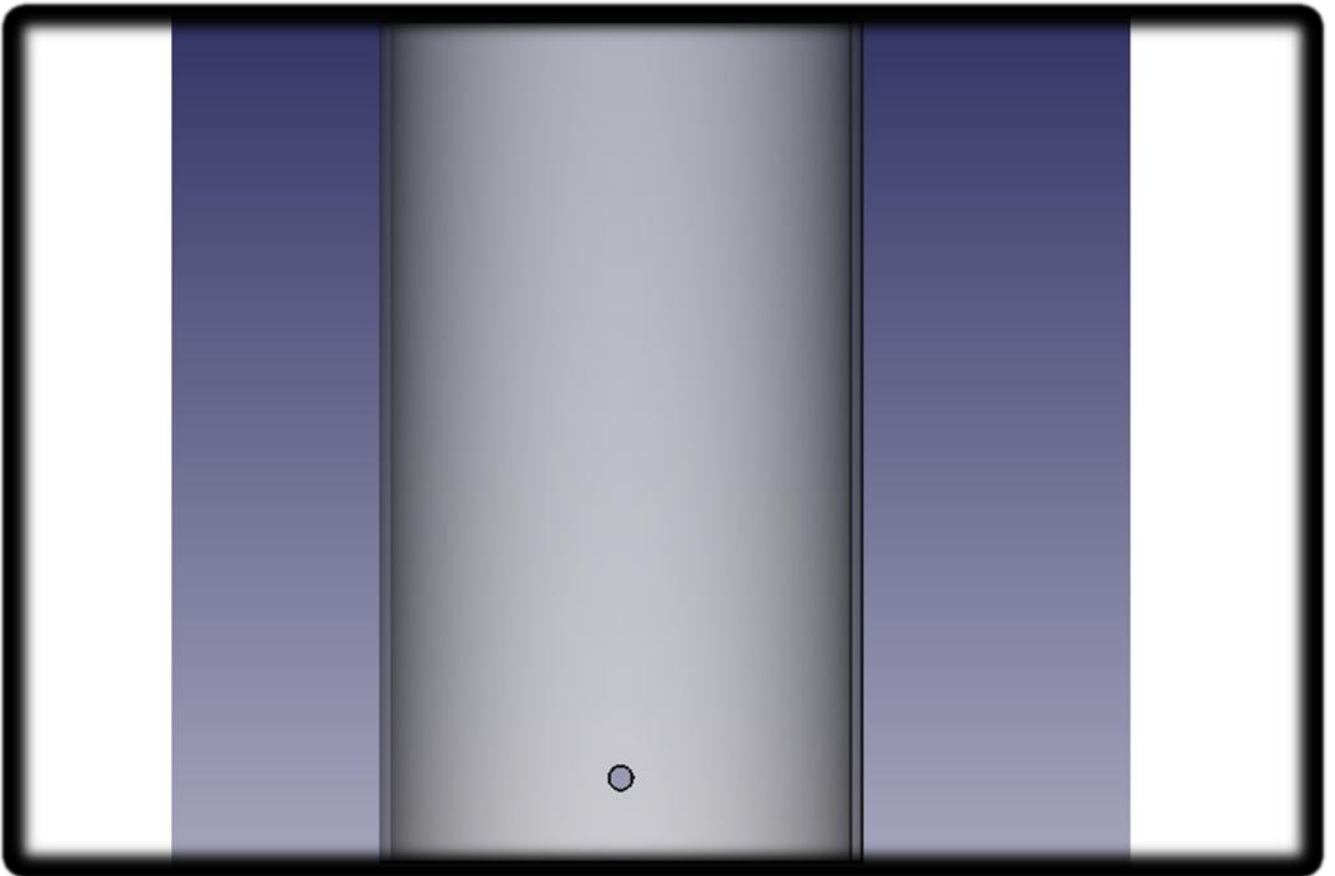


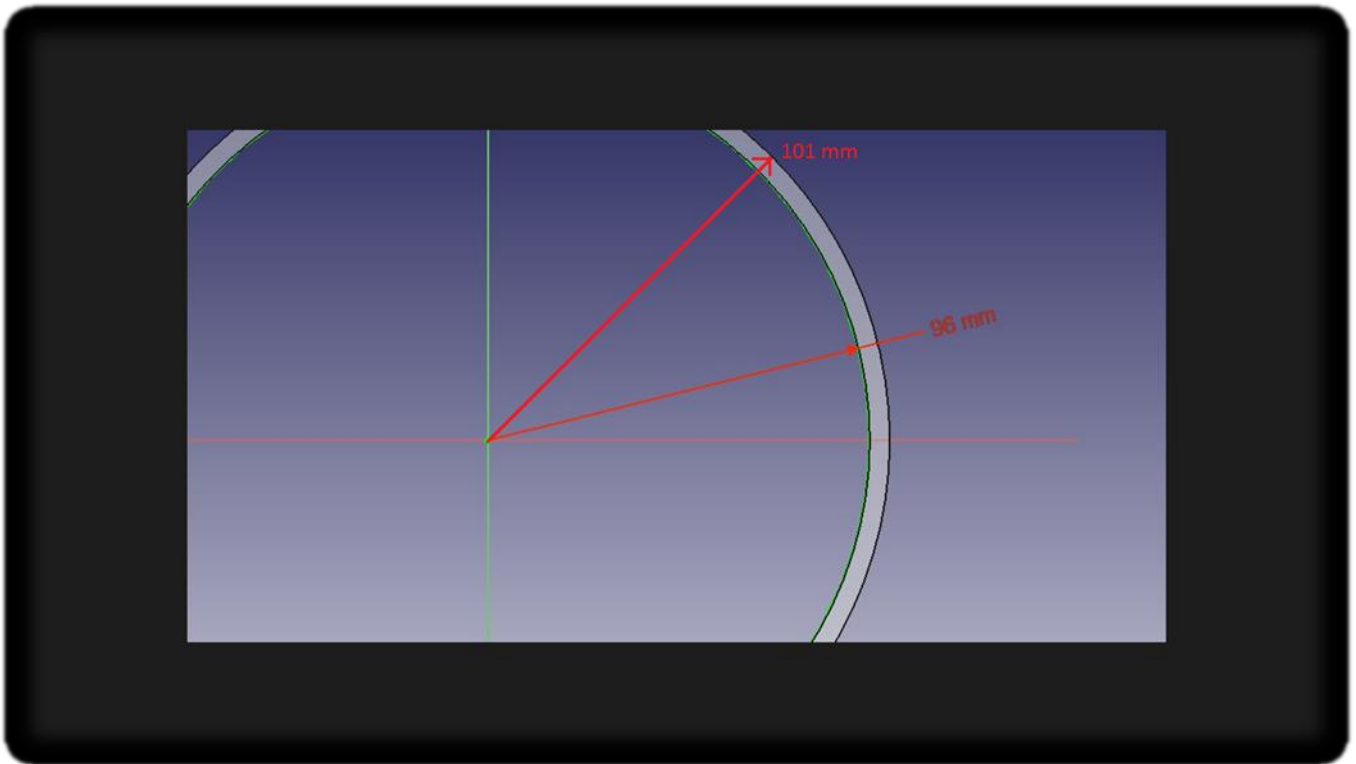
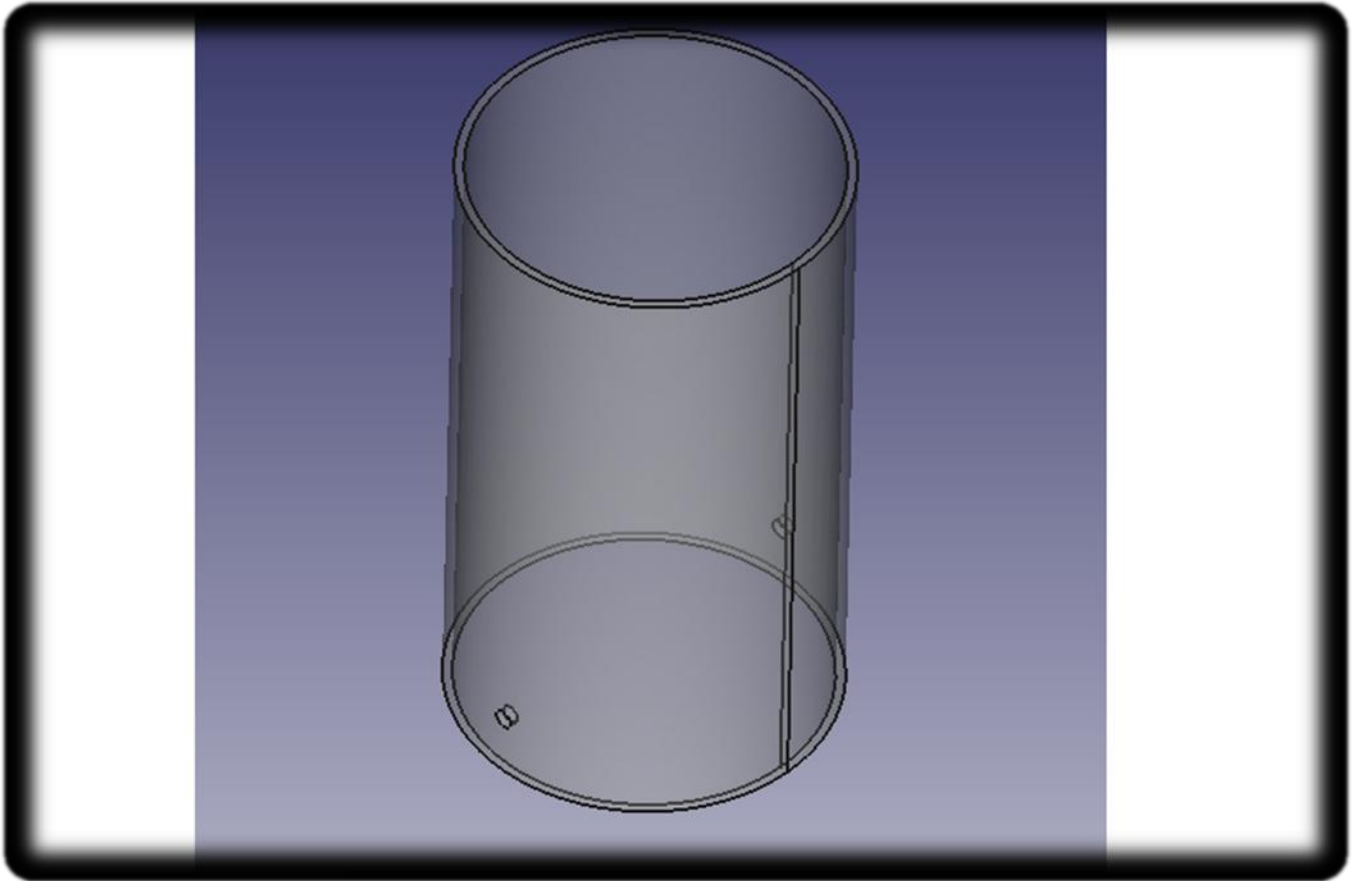




5.4.2.3 *Cylinder*

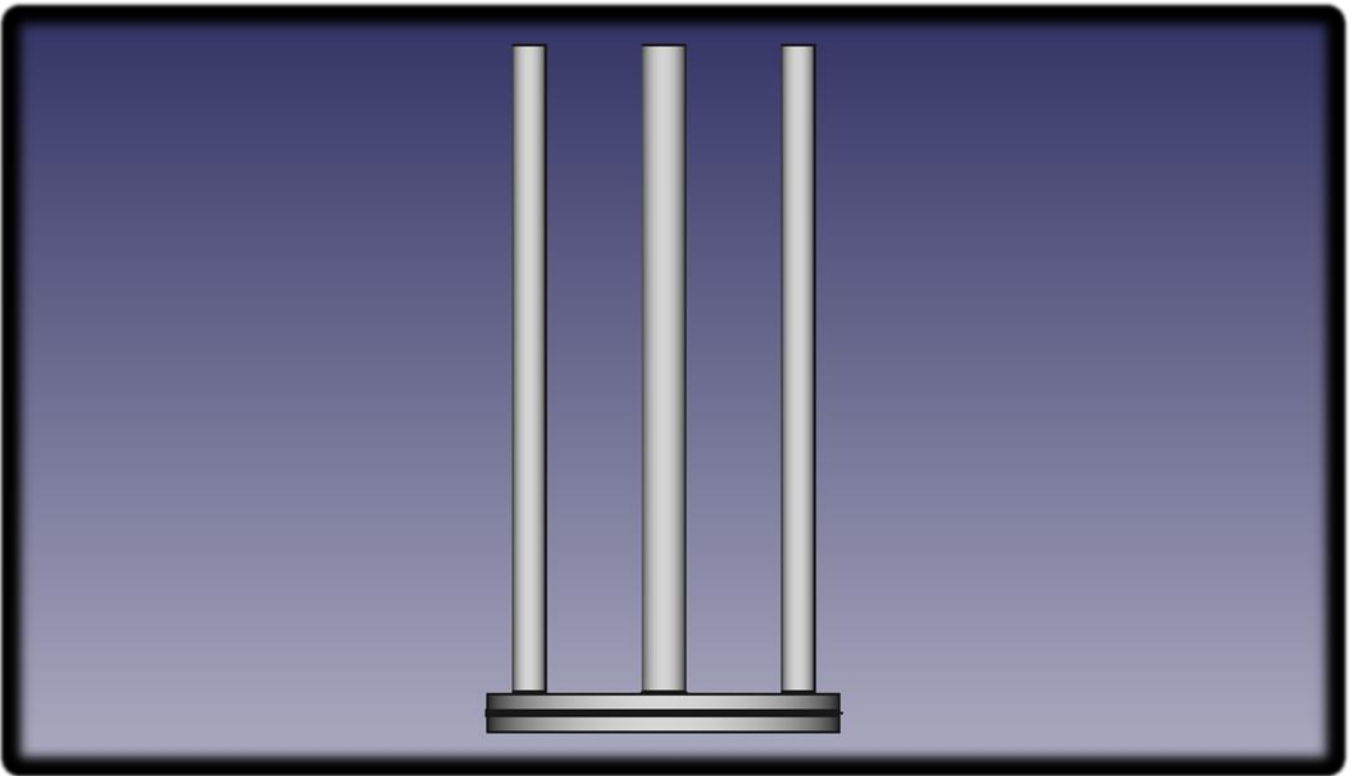
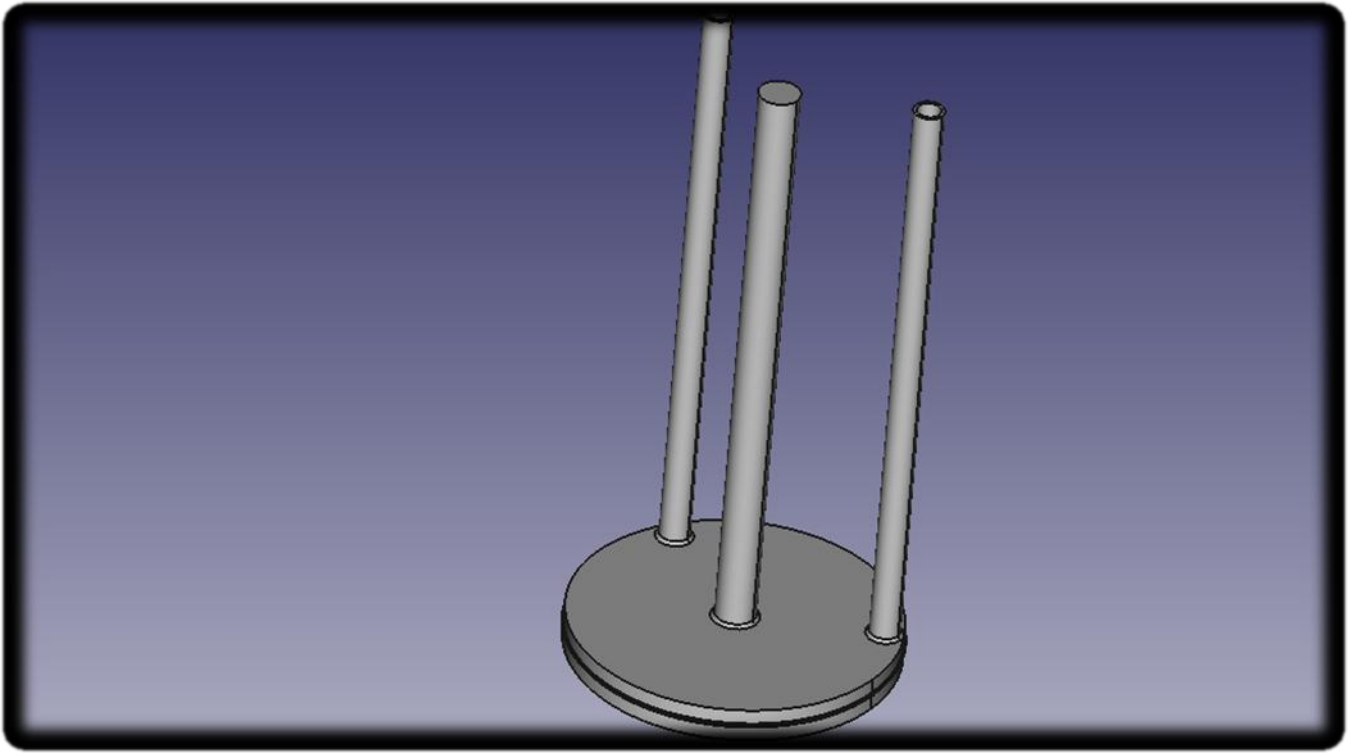
Is made of Plexiglas, it's where we put the solid particles

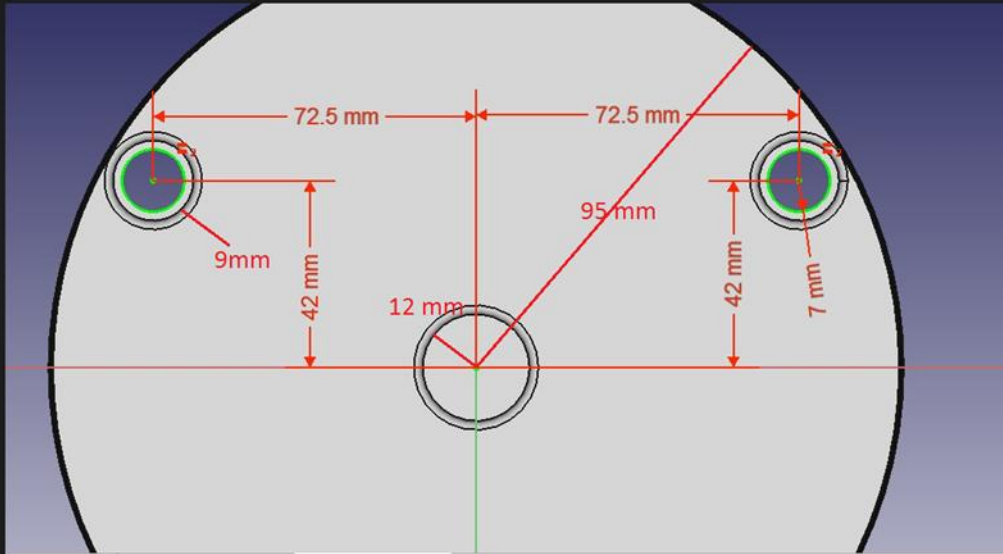




5.4.2.4 Piston

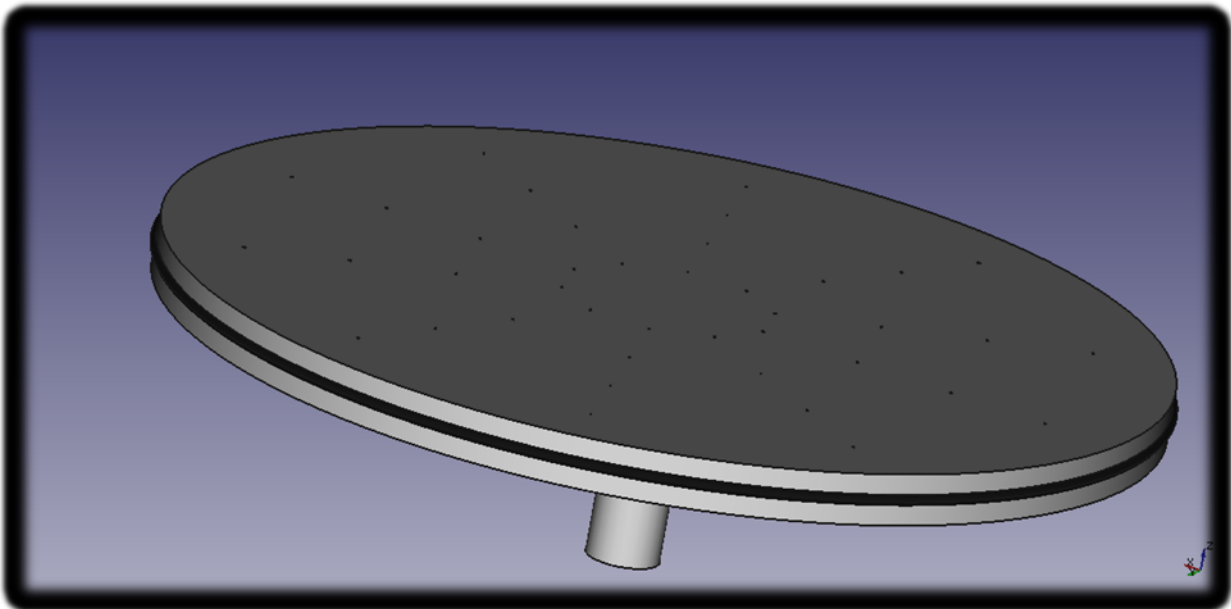
Contains a ring to avoid fluid leaks and to let the piston move easily and two tubes where fluid passes through into the piston

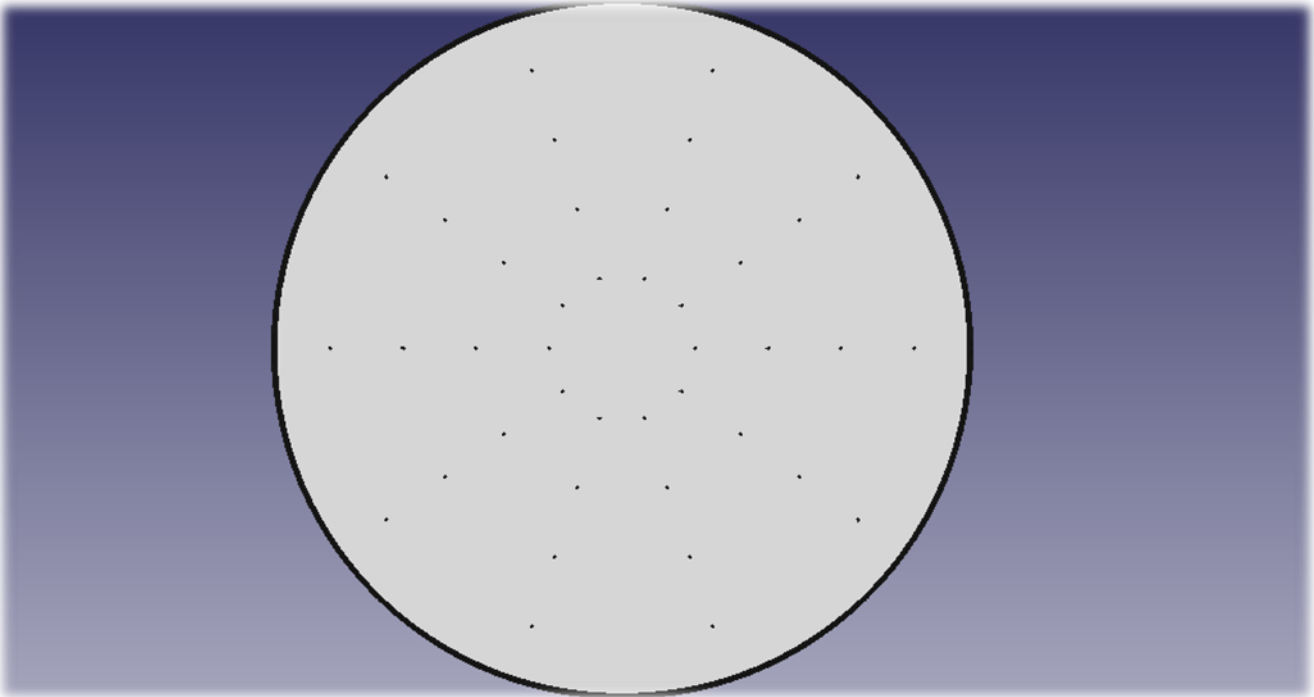
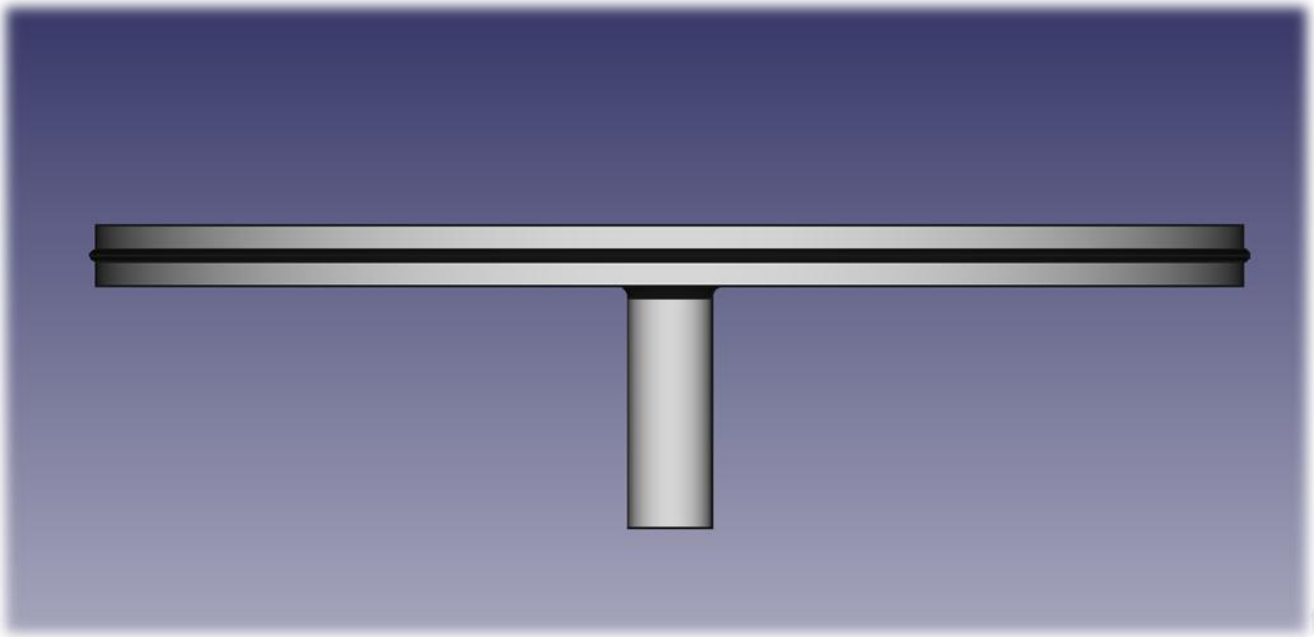


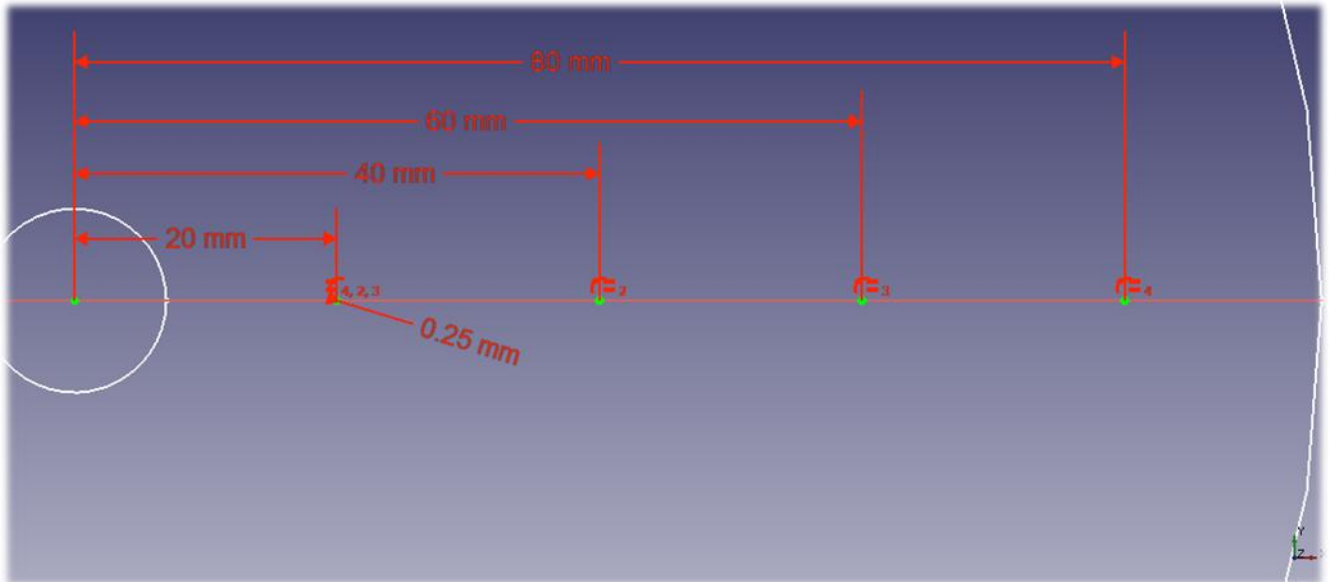


5.4.2.5 *Disc*

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

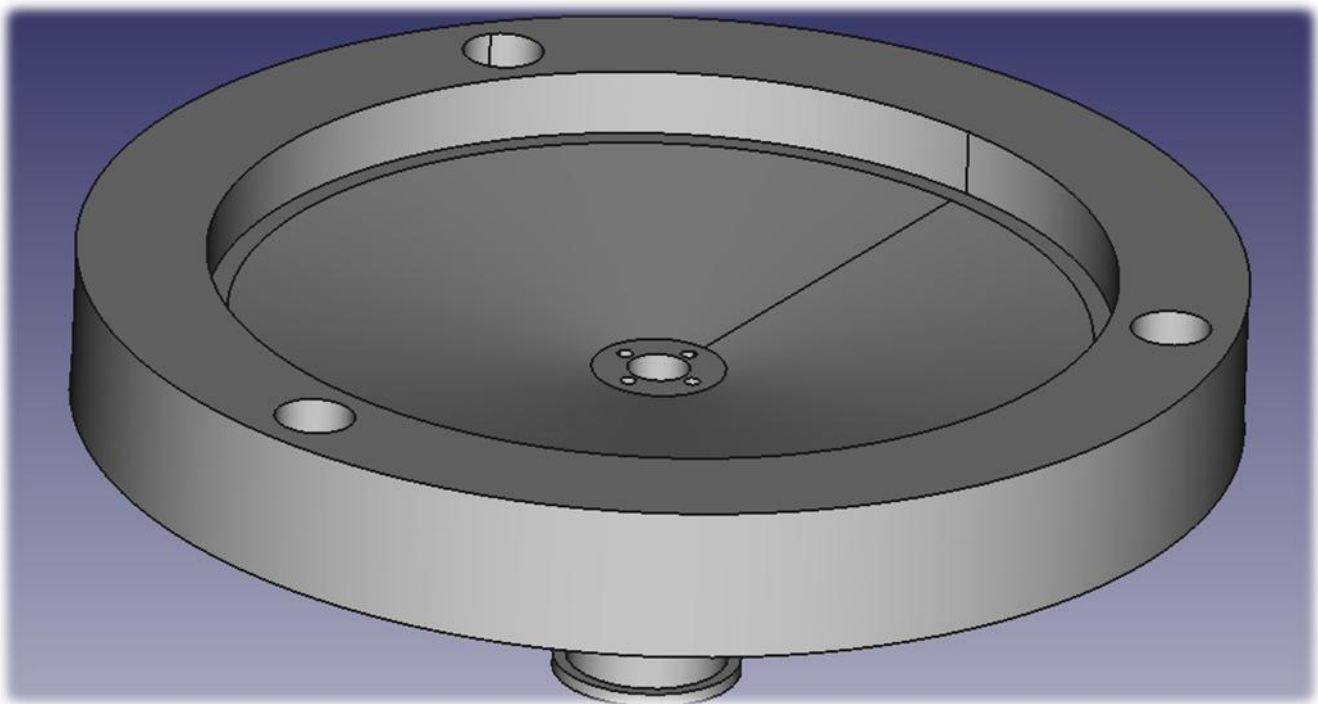


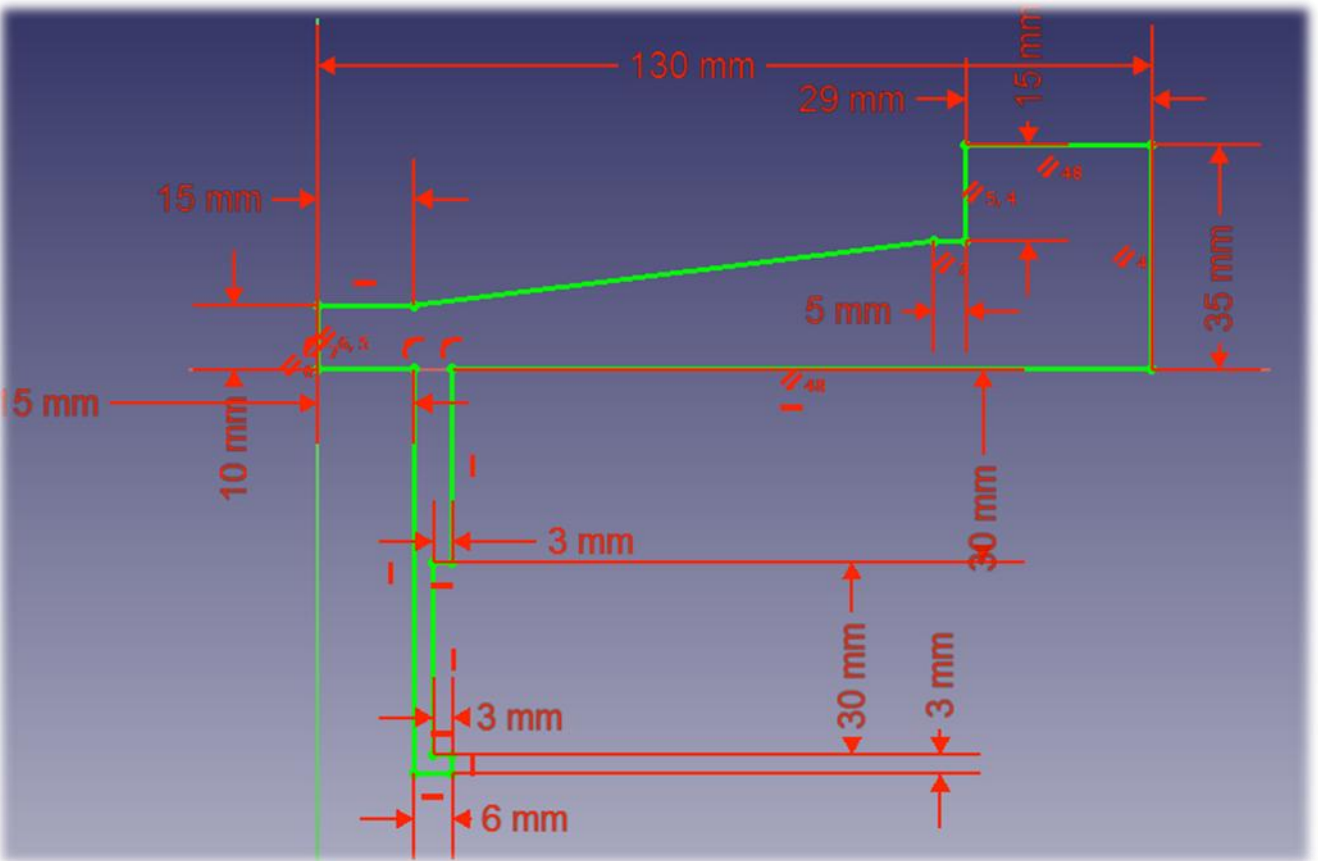
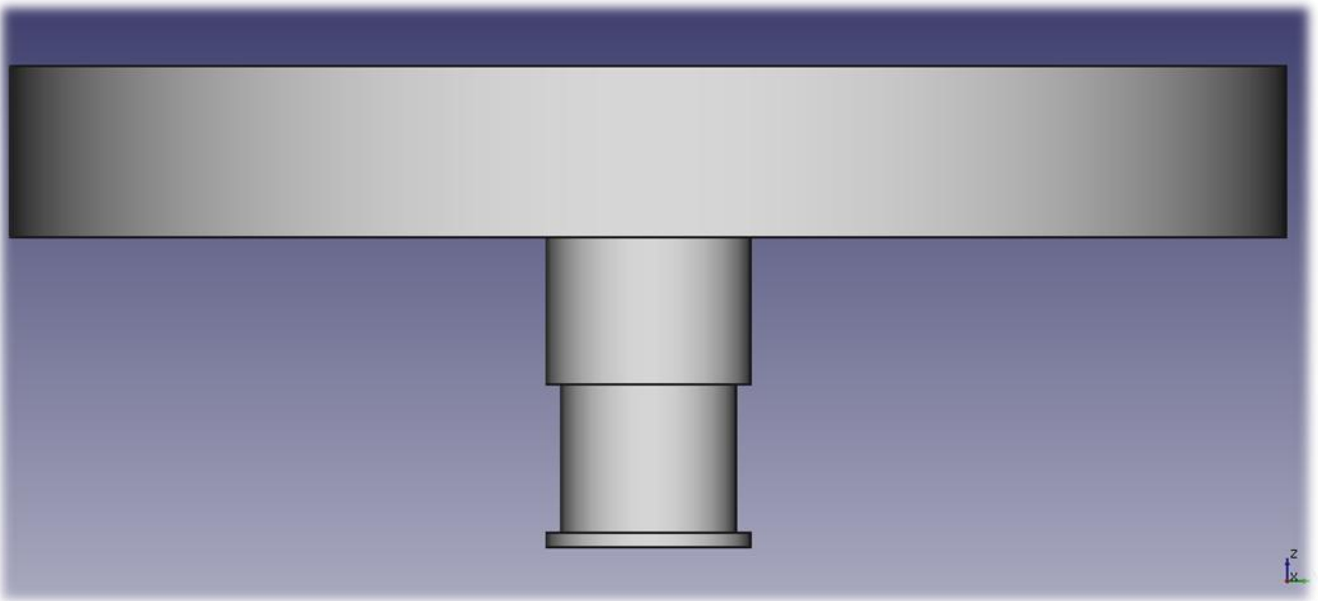


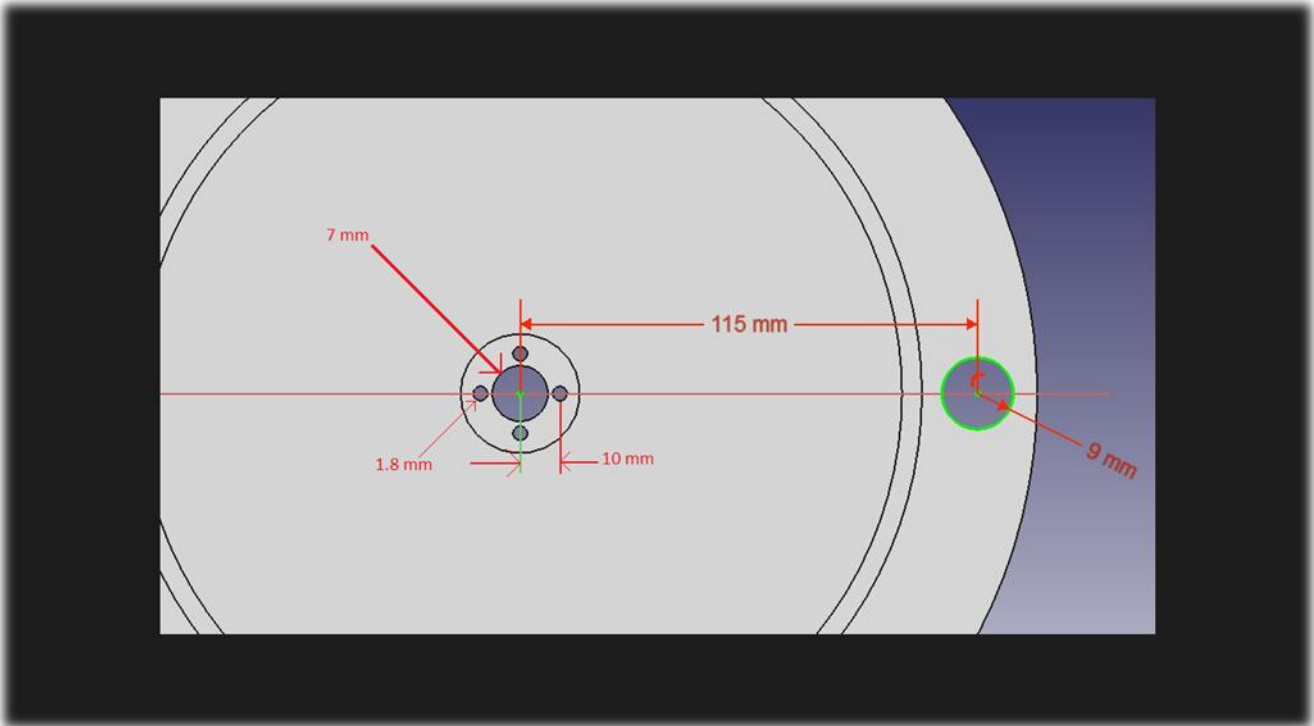


5.4.2.6 Bottom

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

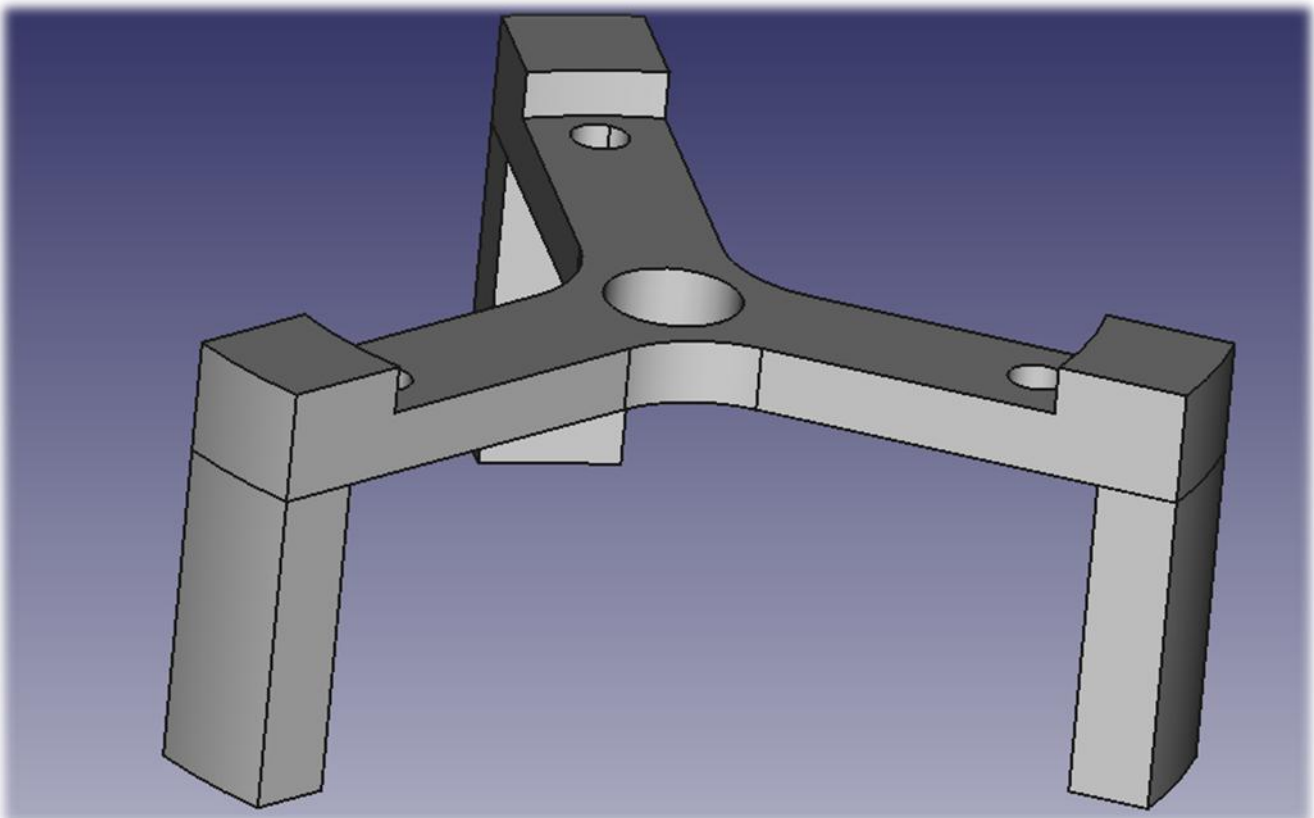


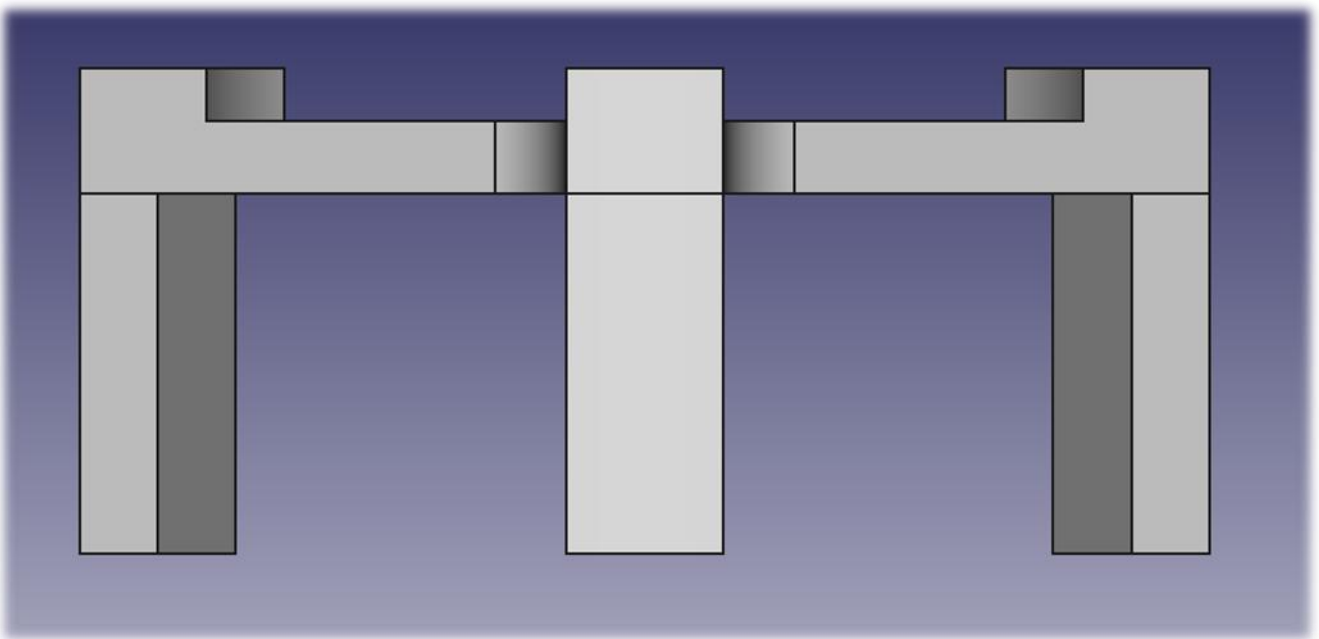
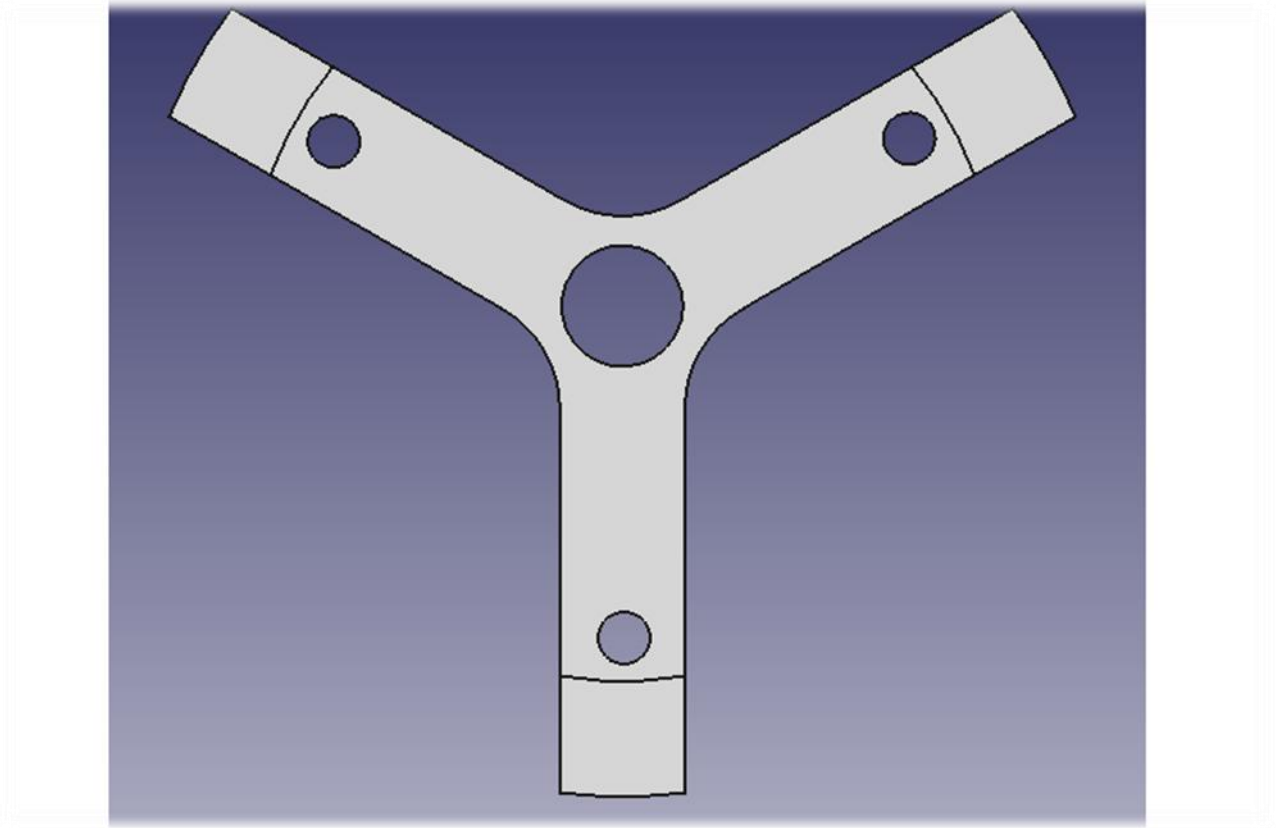


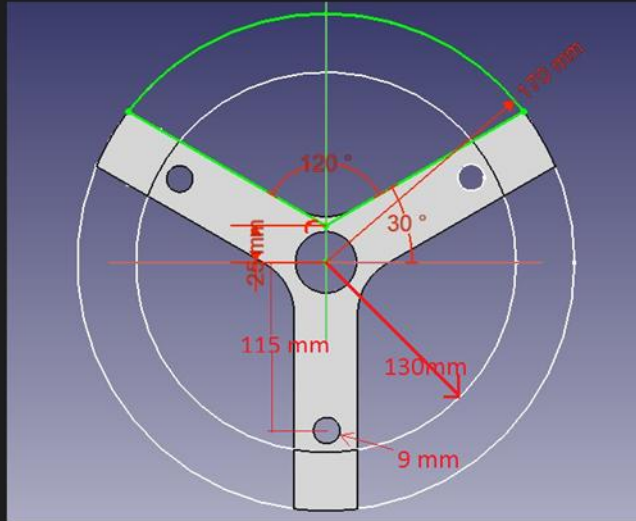


5.4.2.7 Base

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

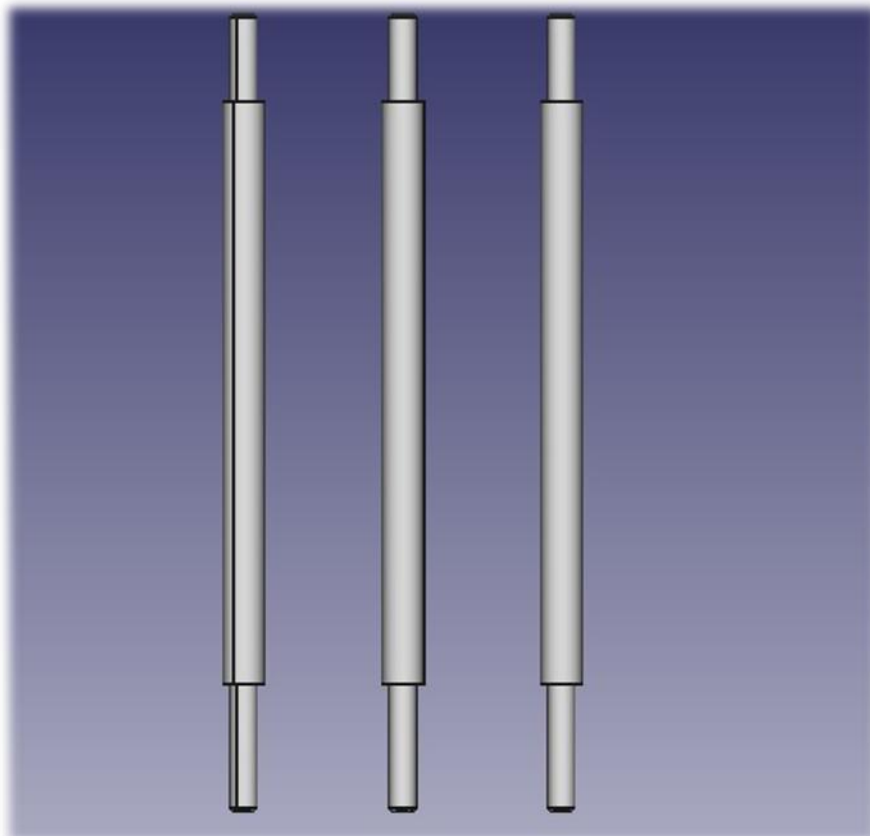


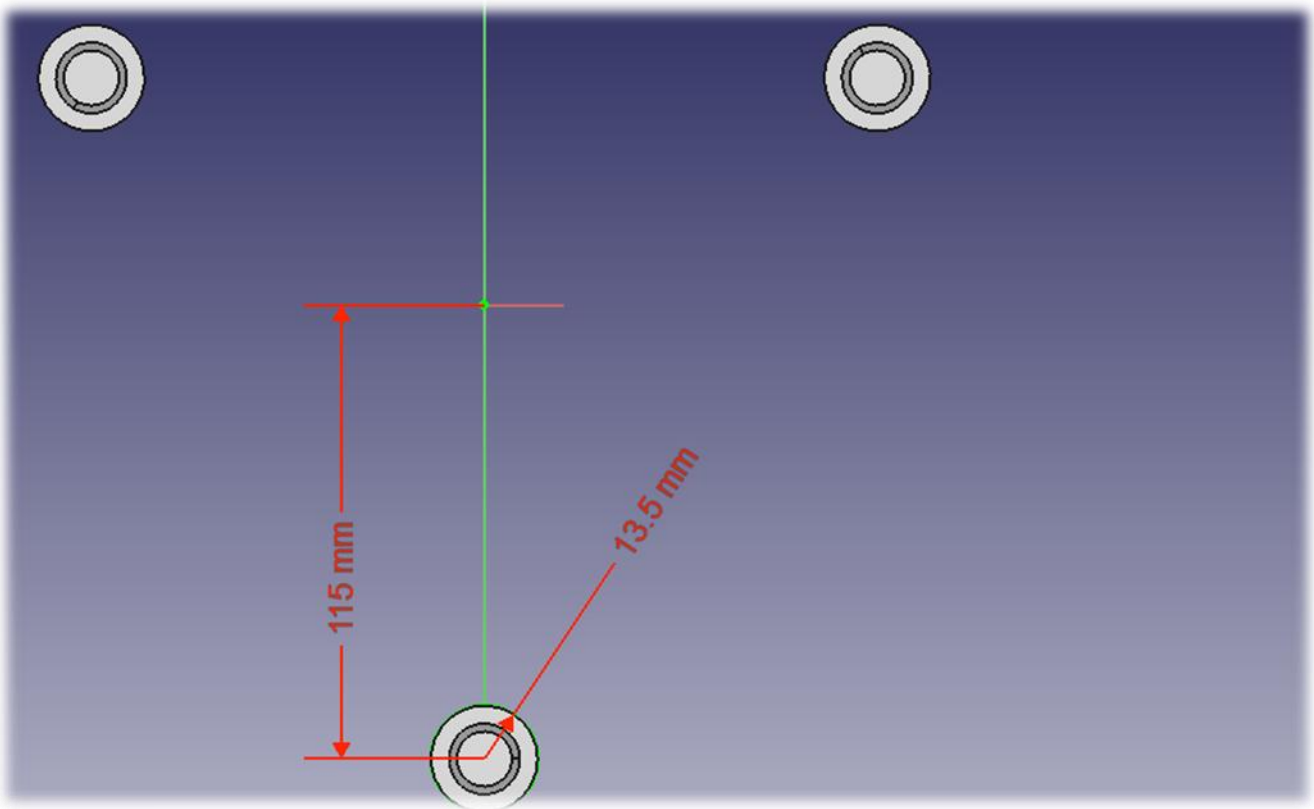
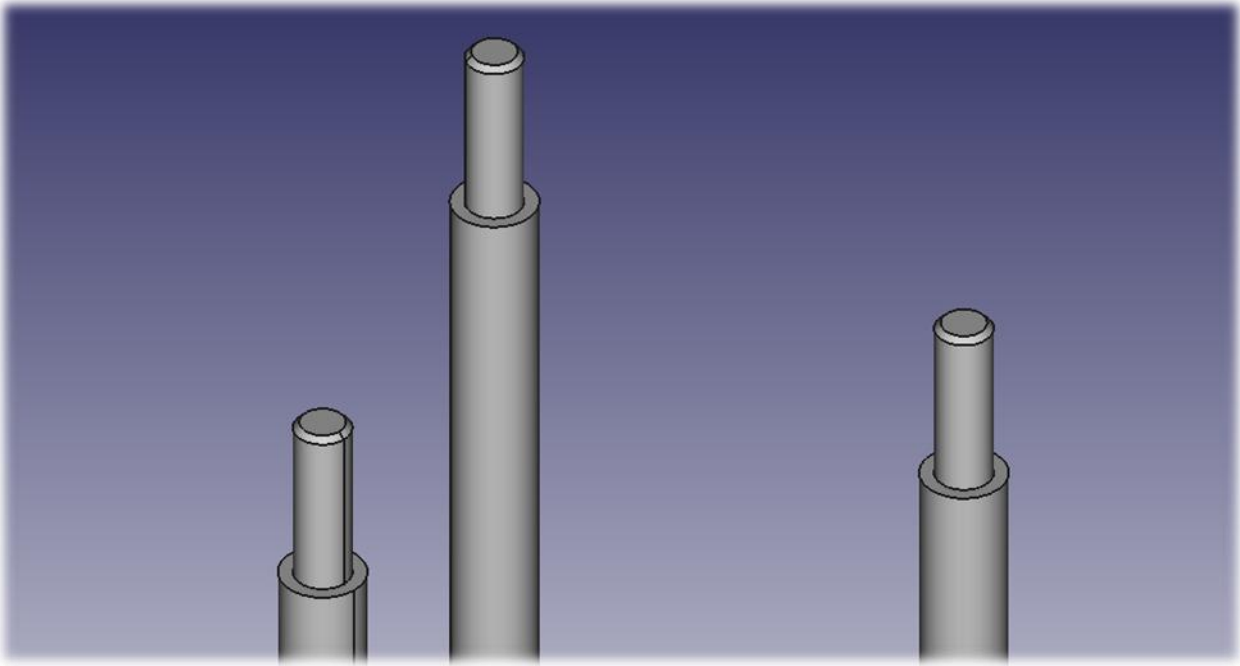




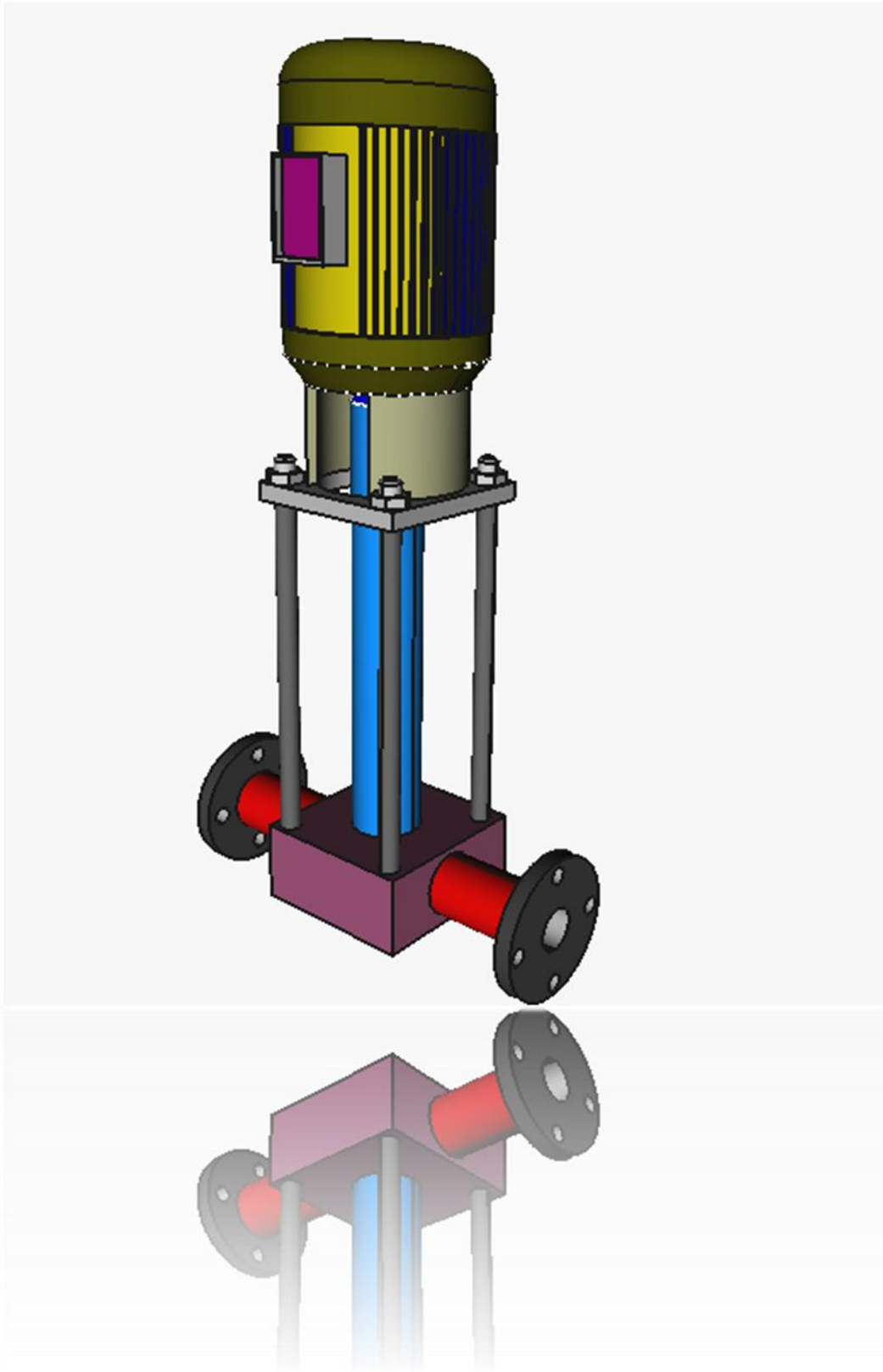
5.4.2.8 Column

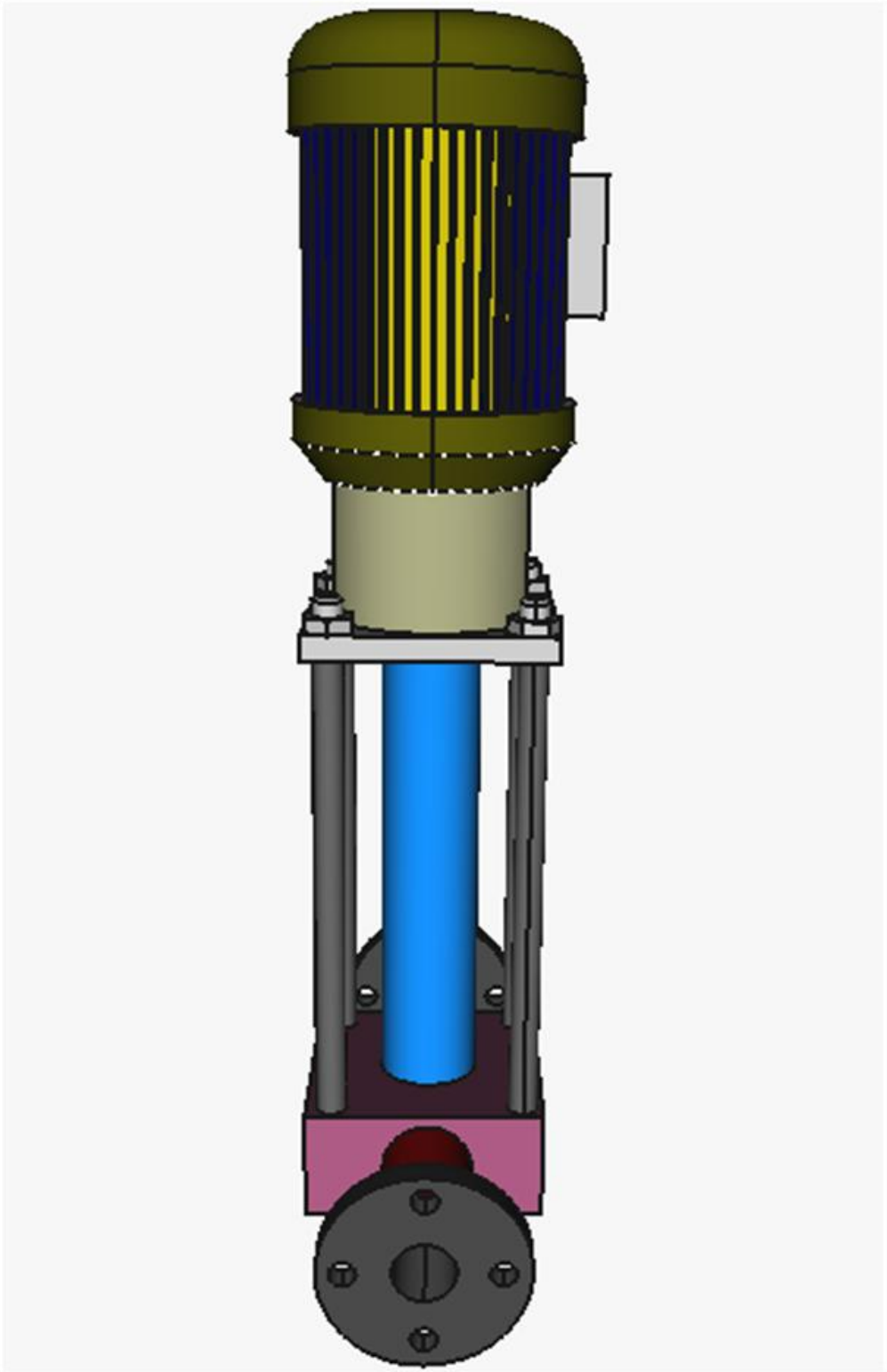
It is the part that connects the top, bottom and base parts together.

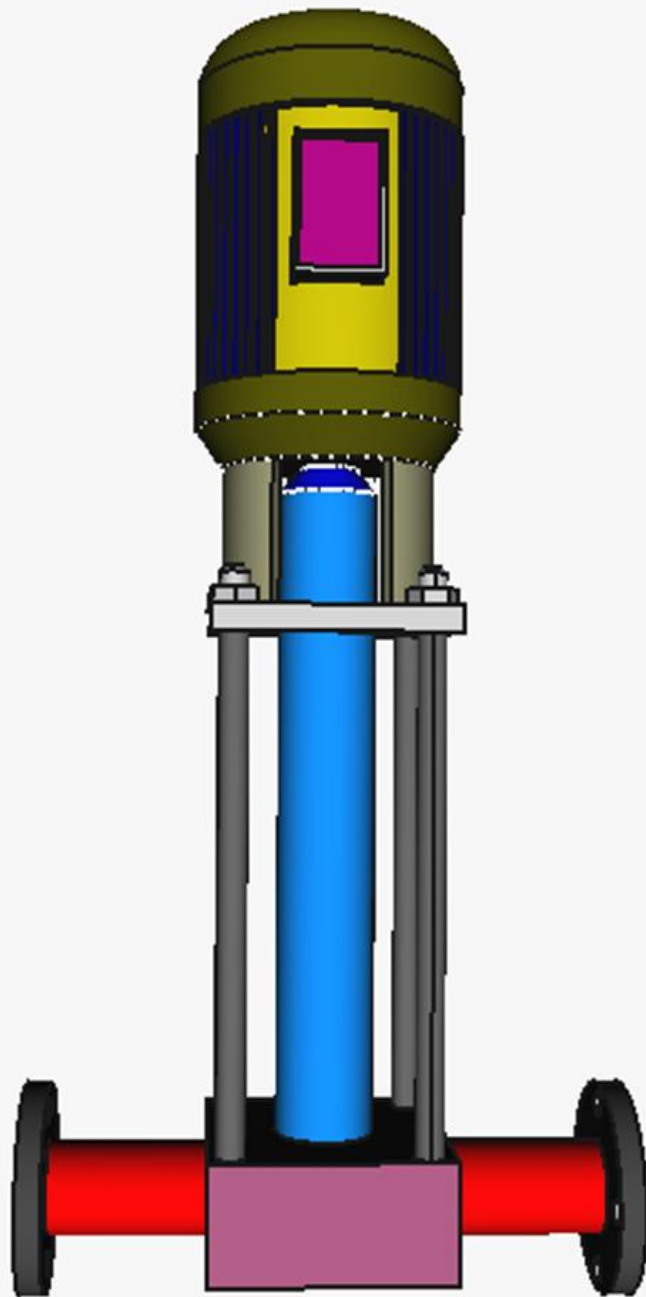




5.5 Demonstration and Modelling of the Pump







5.6 Demonstration and Modelling of the Homogenizer

-Ibrahim Zaaroura-



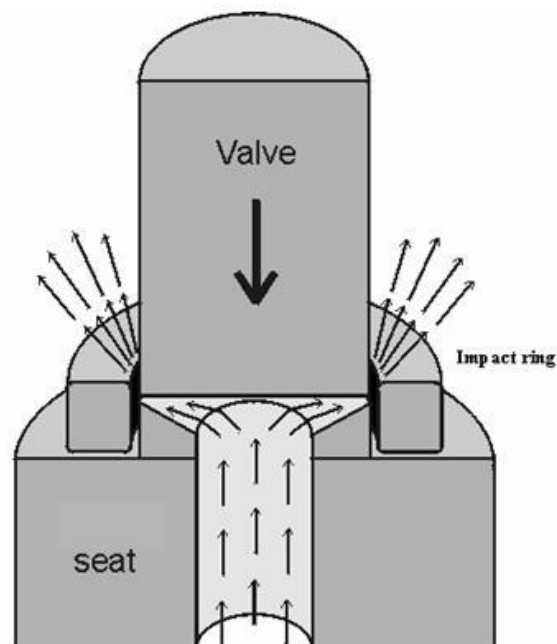
5.6.1 DEVICE DETAILS & SPECIFICATIONS

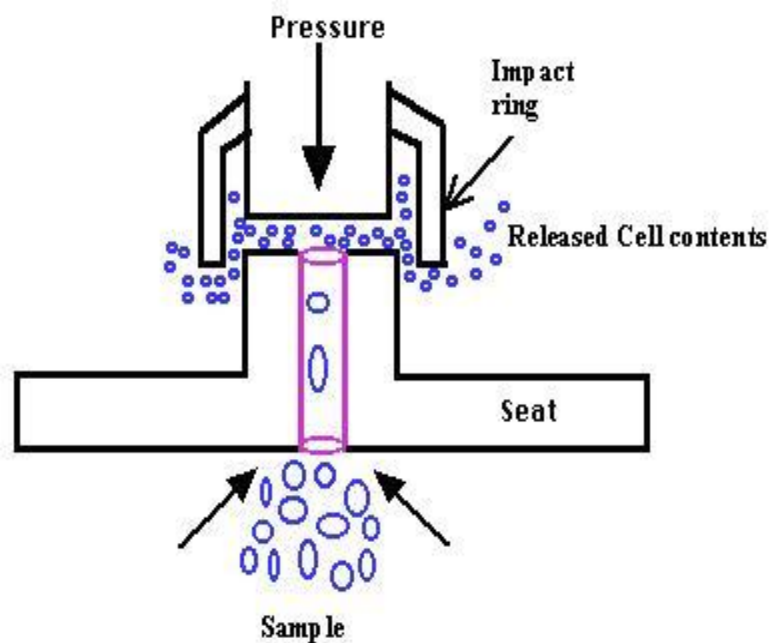
A homogenizer is a piece of laboratory or industrial equipment used for the homogenization of various types of material, such as tissue, plant, food, soil, and many others. Many different models have been developed using various physical technologies for disruption.

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 shear 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.

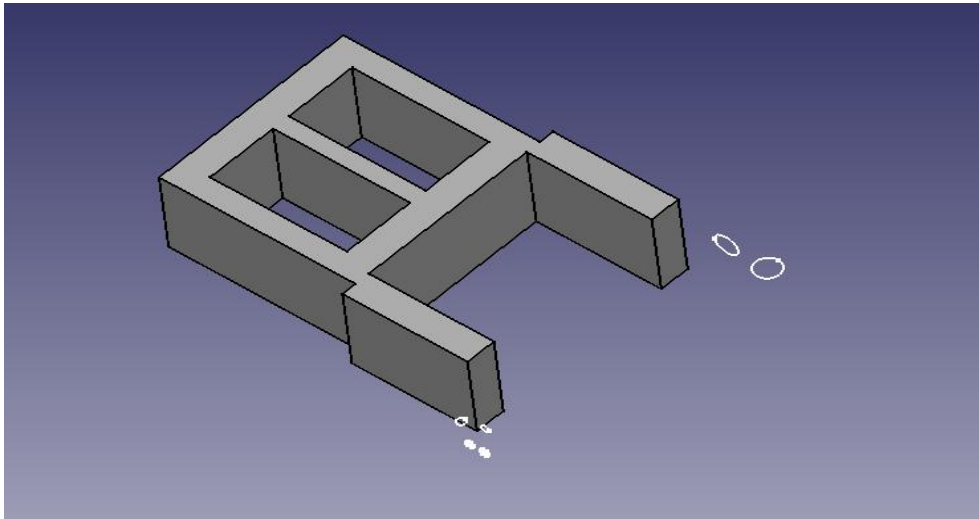
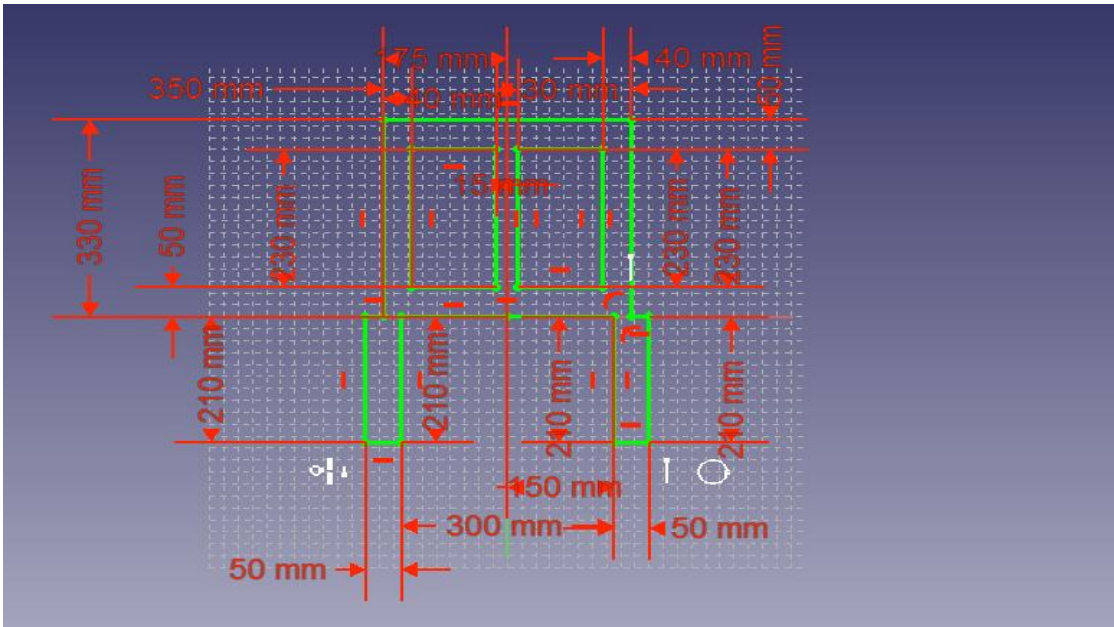
5.6.1.2 Specifications

| | | | |
|----------------------------|----------------------|---------------------------------------|---------------------------------|
| 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 |

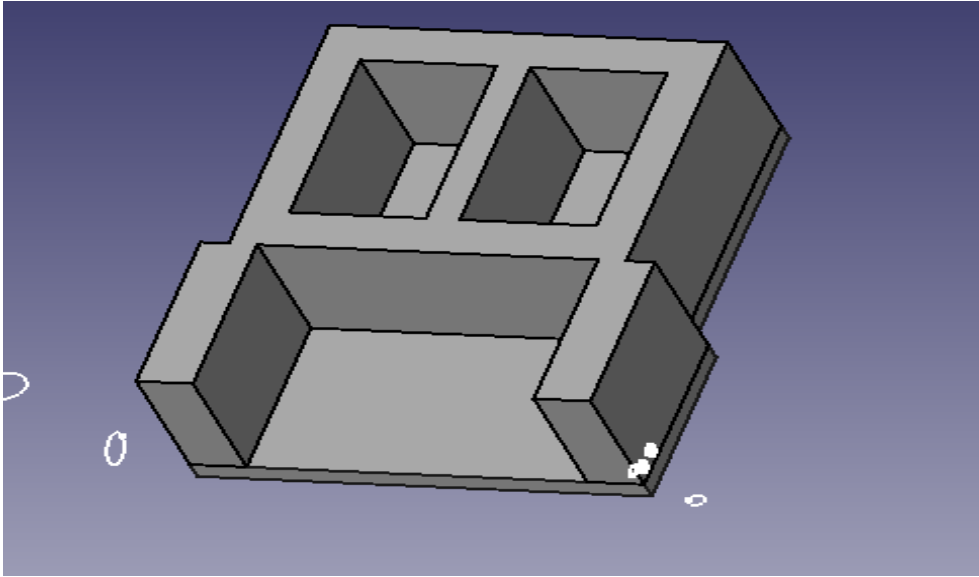
5.6.2 PART DIMENSIONS & DESIGN

5.6.2.1 BASE PART

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

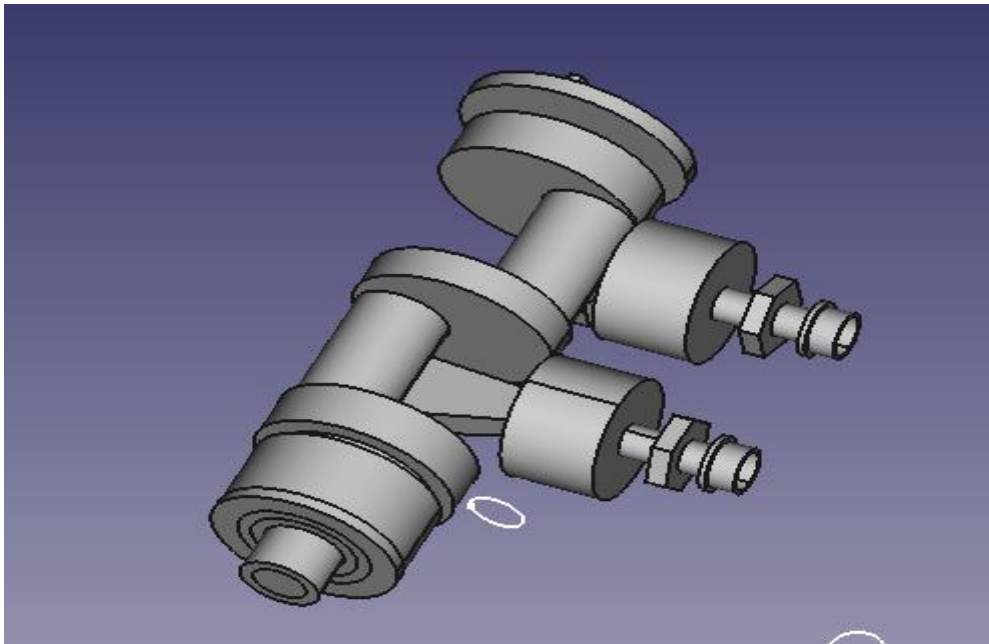


-Cover for Base with thickness =25mm

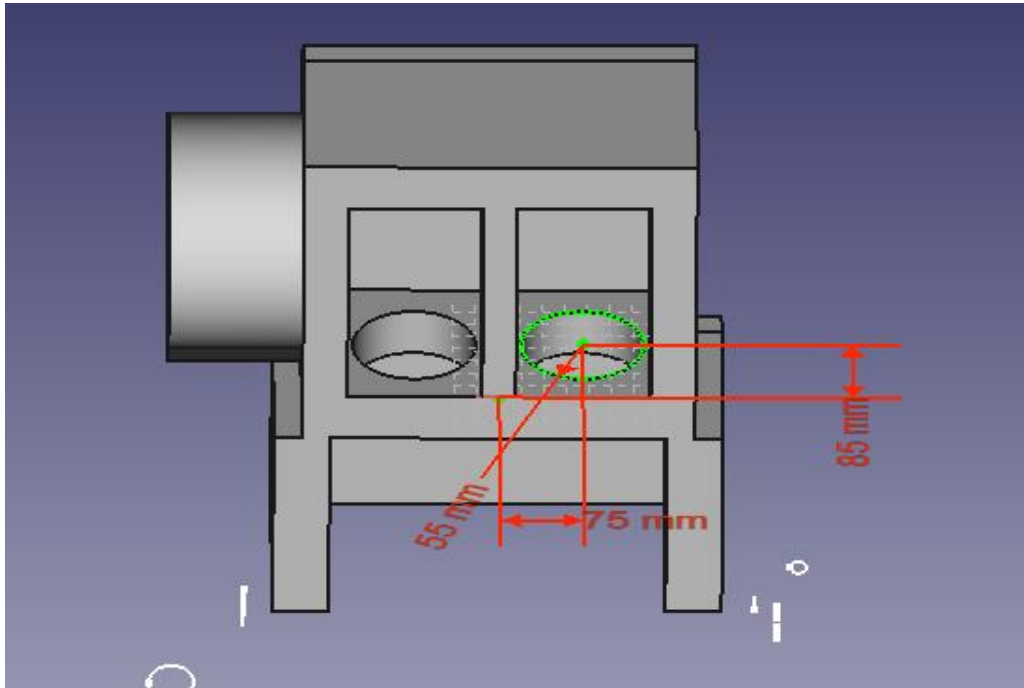


5.6.2.2 CRANK SHAFT AND PISTONS

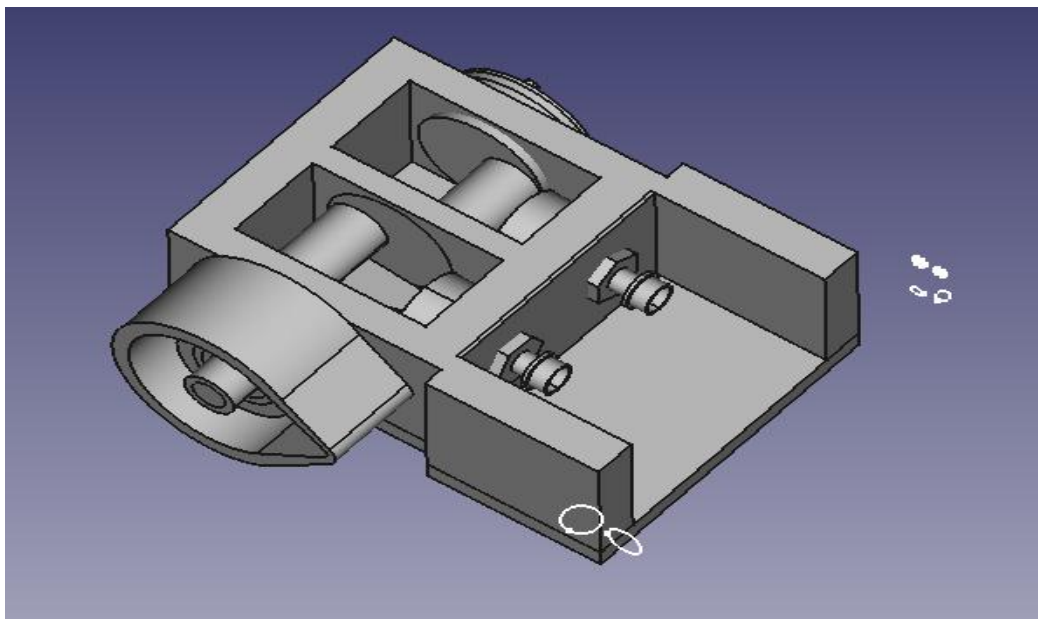
-Taking into account the dimensions of the shaft and pistons according to the box dimensions.



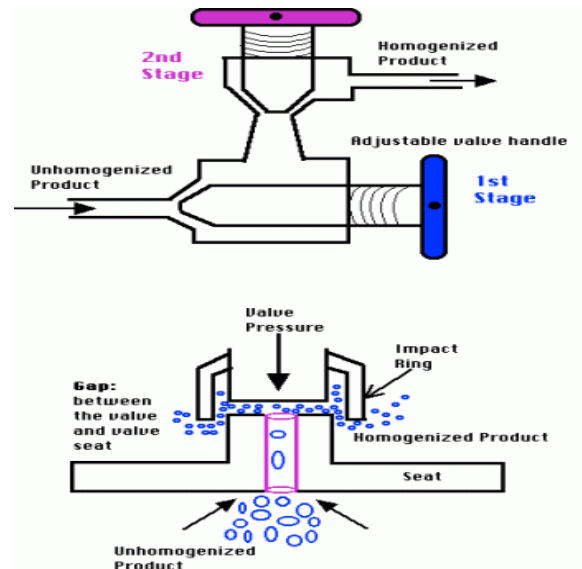
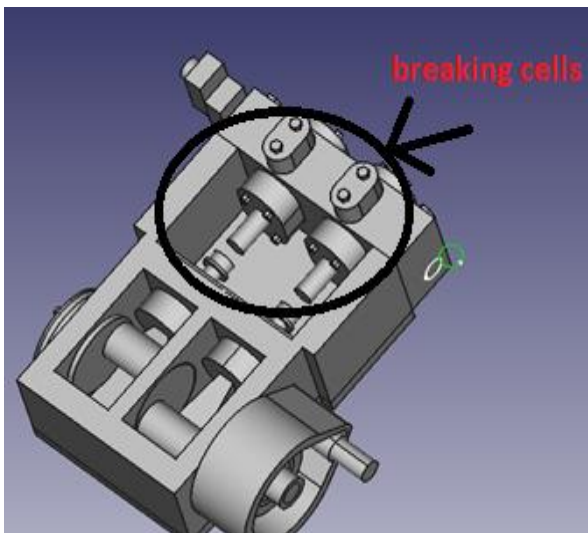
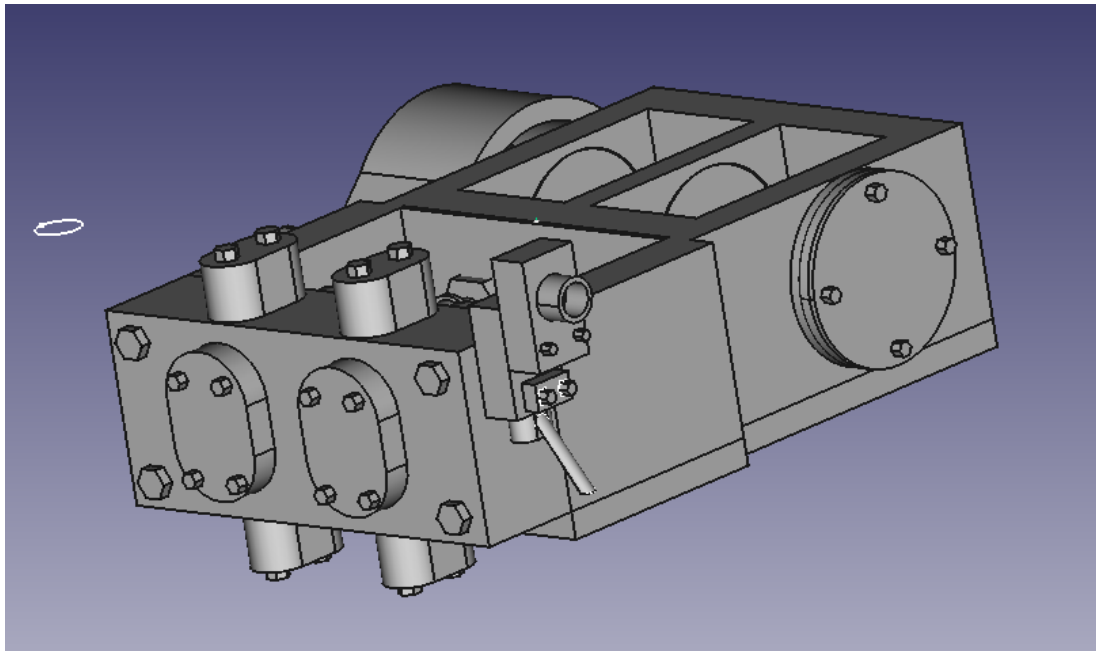
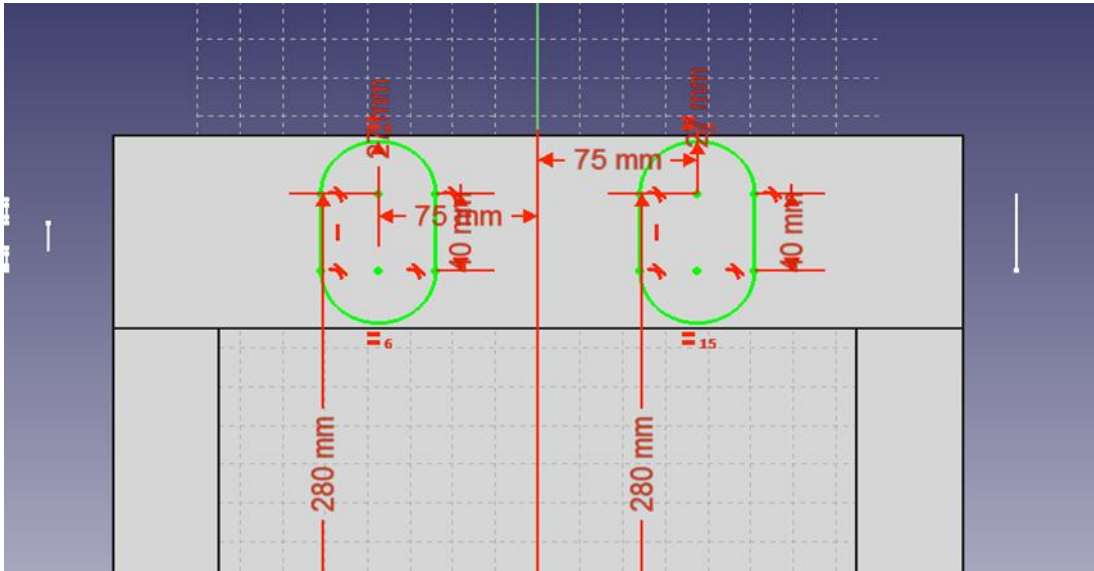
5.6.2.3 Pocket circle with $R=55\text{mm}$ to pass the cylinder of the pistons



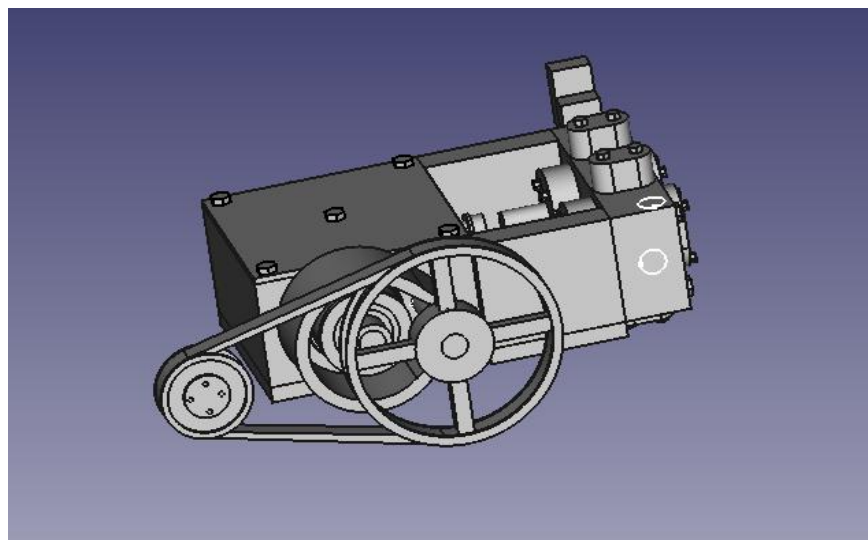
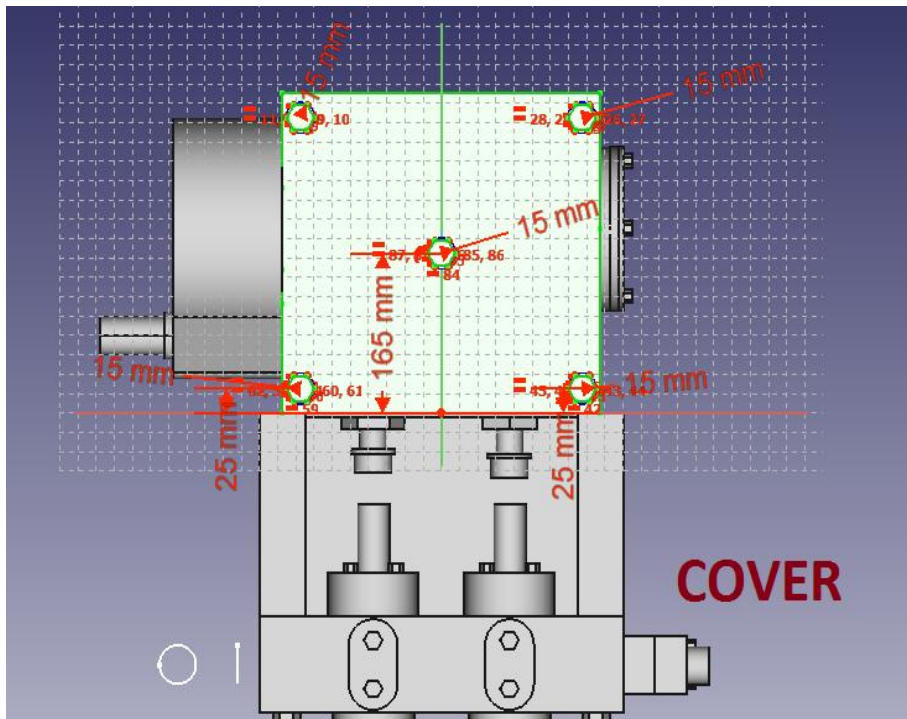
5.6.2.4 With same sketch for pistons and box bind the two parts together.



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



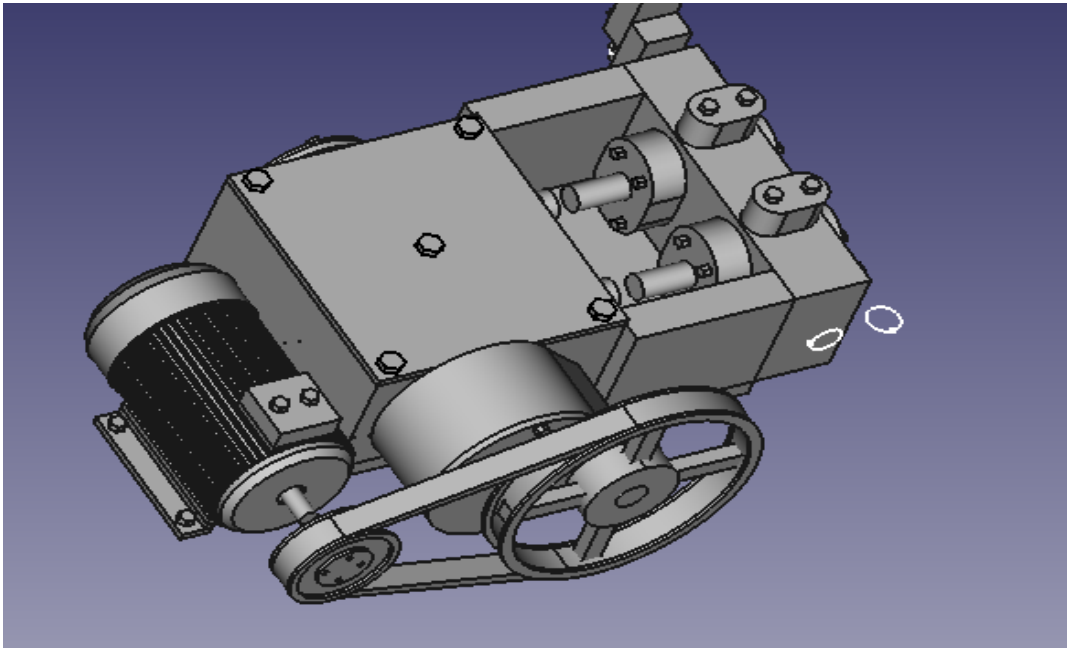
5.6.2.6 Putting the cover over the box and fixed it with bolts and added the shaft comes out from crank shaft and connecting to pulley, this pulley also connected to other one with belt.



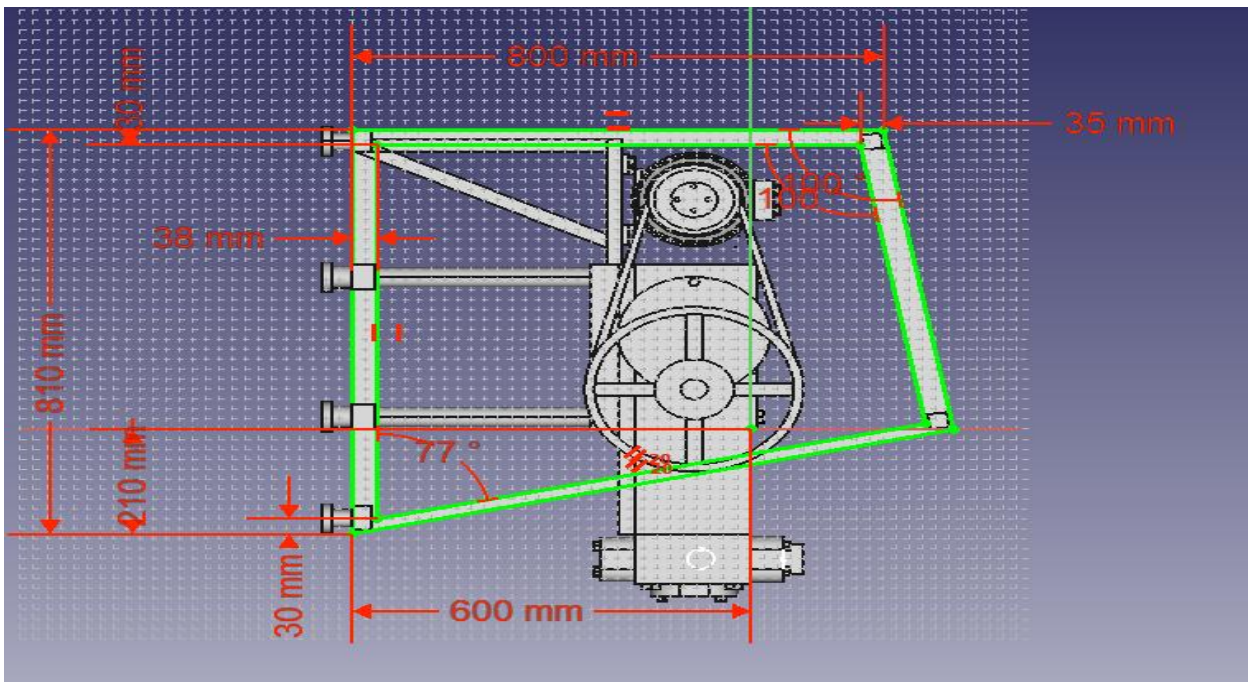
-belt thickness =10 mm

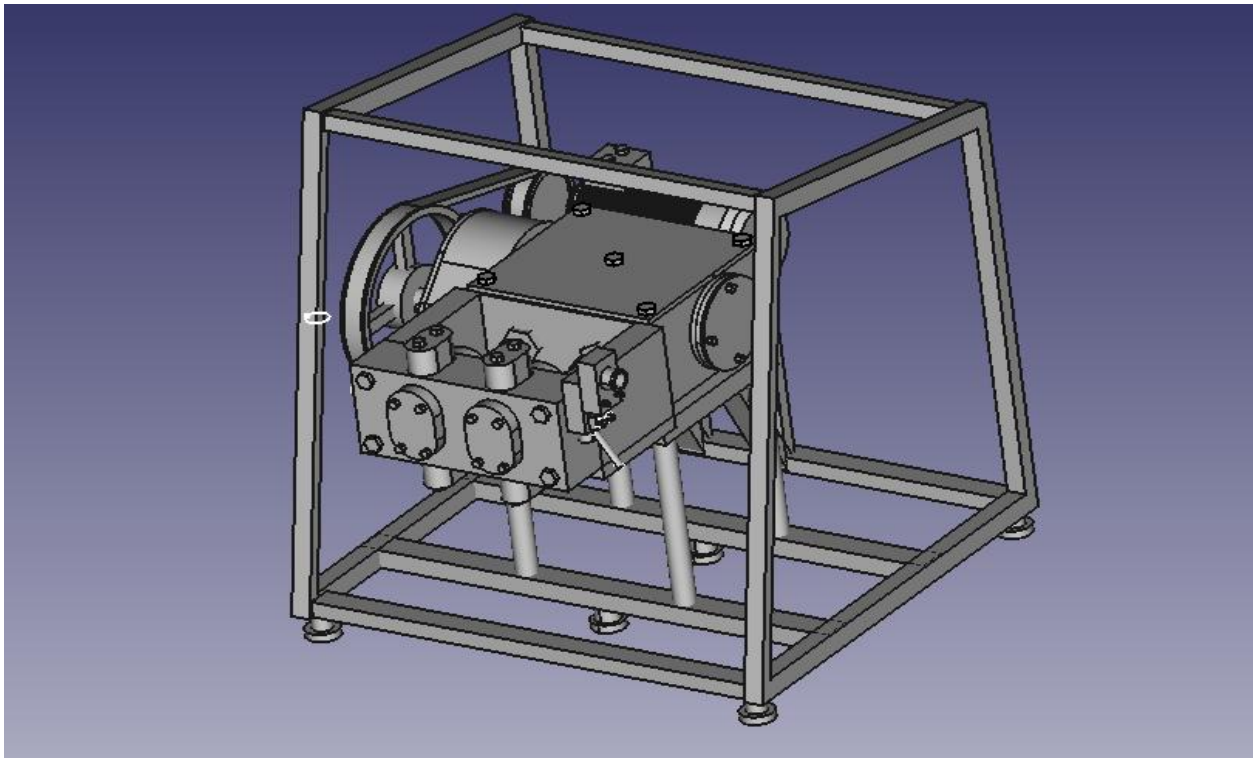
-Large circle with $R=165$ mm and small $R=70$ mm

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

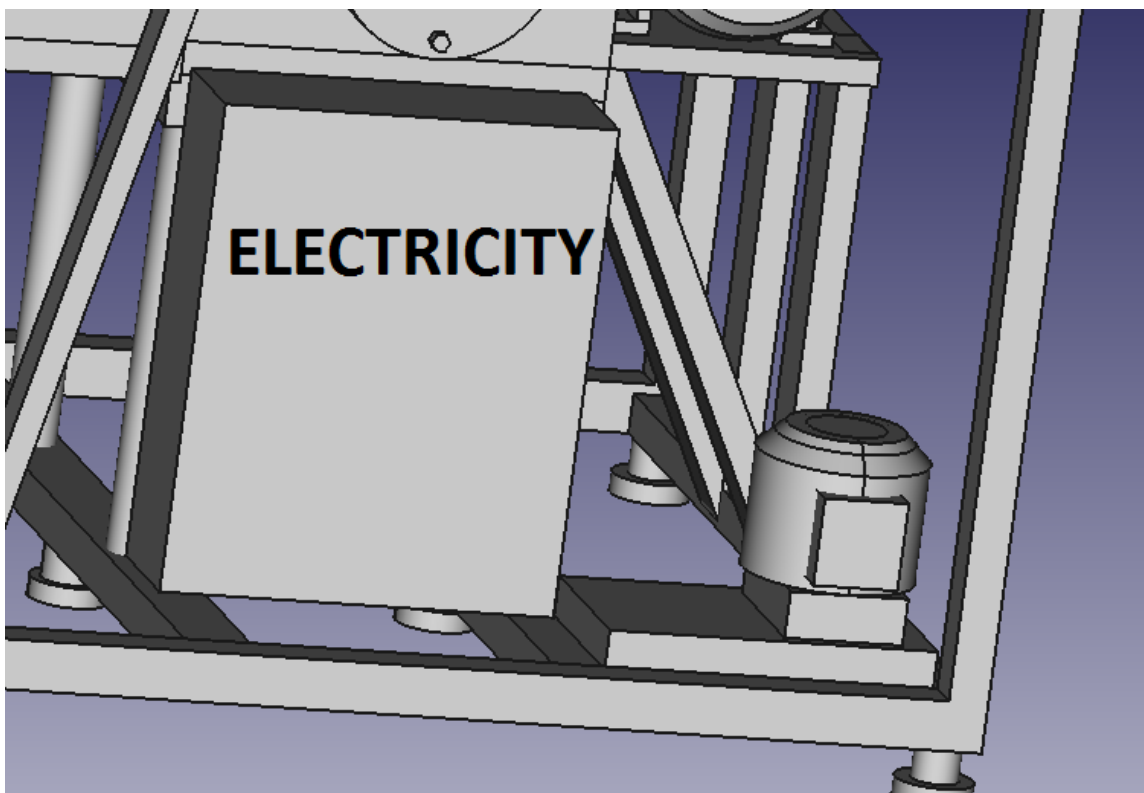


5.6.2.8 Drawing the boundaries of the machine and the basement for the box

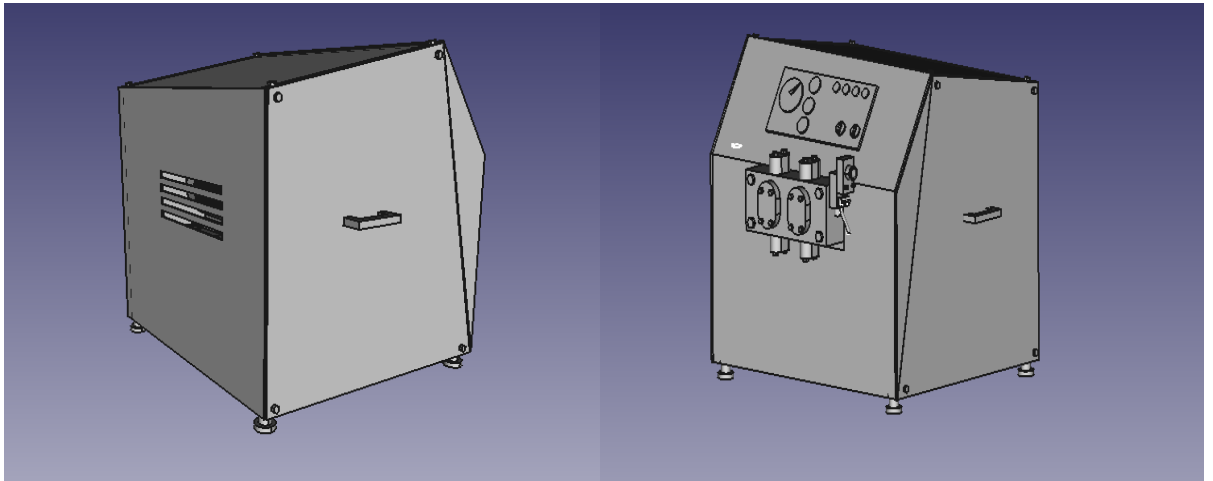




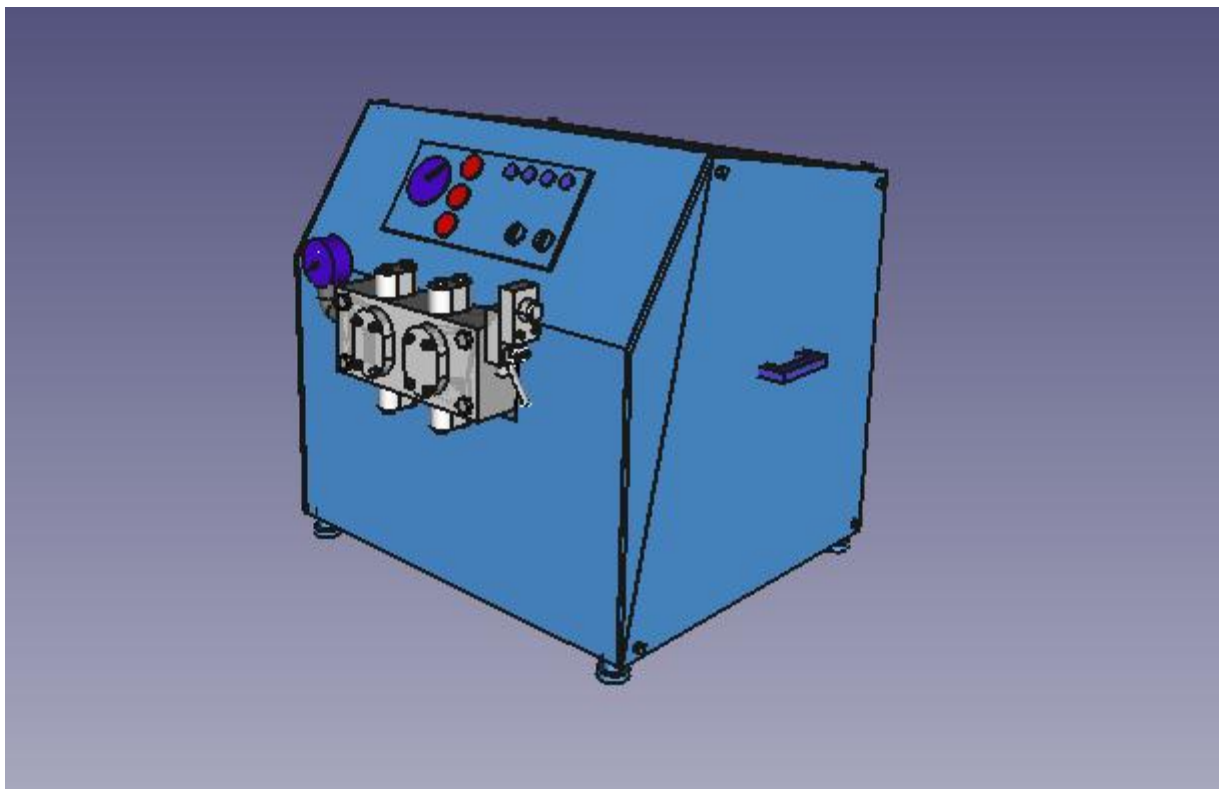
-Small motor and electricity box



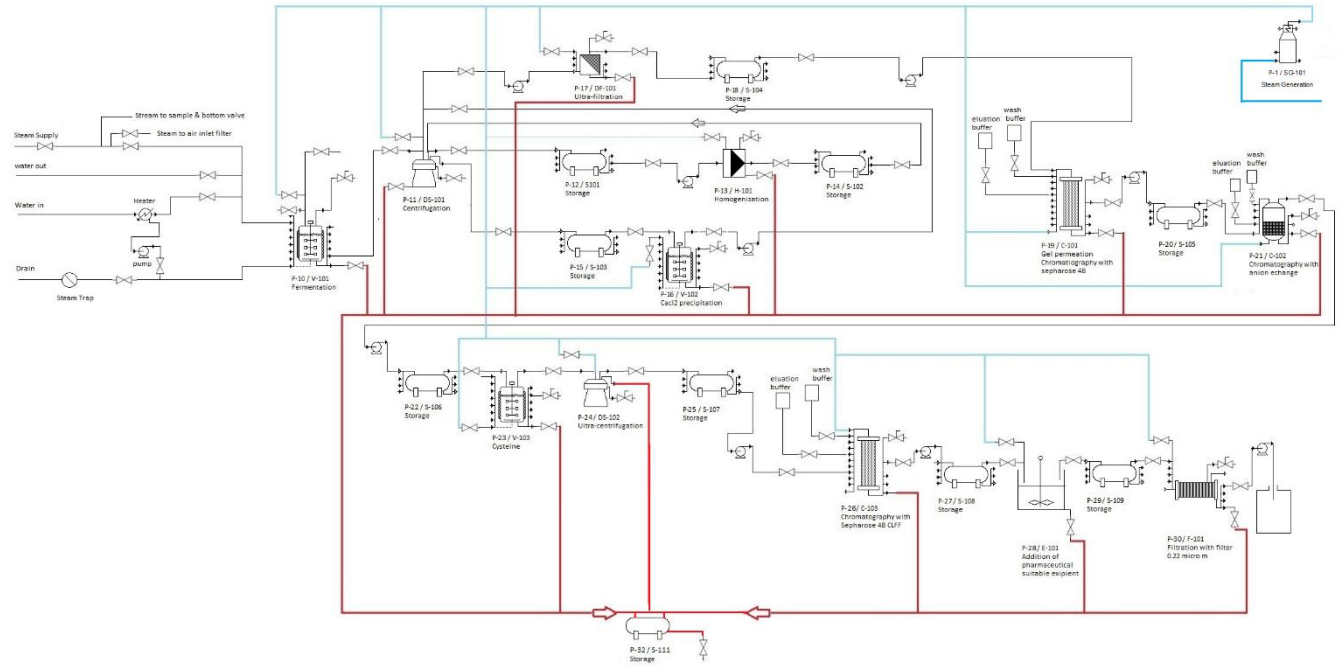
5.6.2.9 *Finally close the machine with stainless wall*



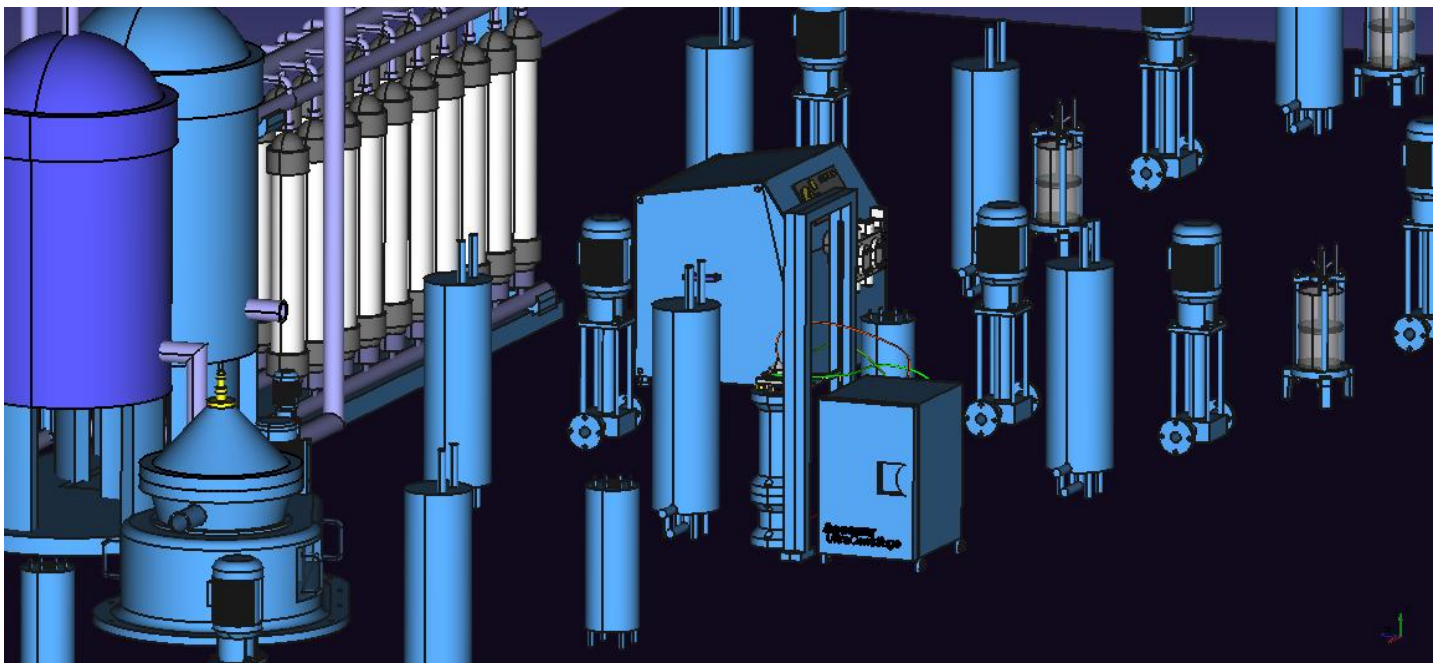
FINAL IMAGE: HOMOGENIZER

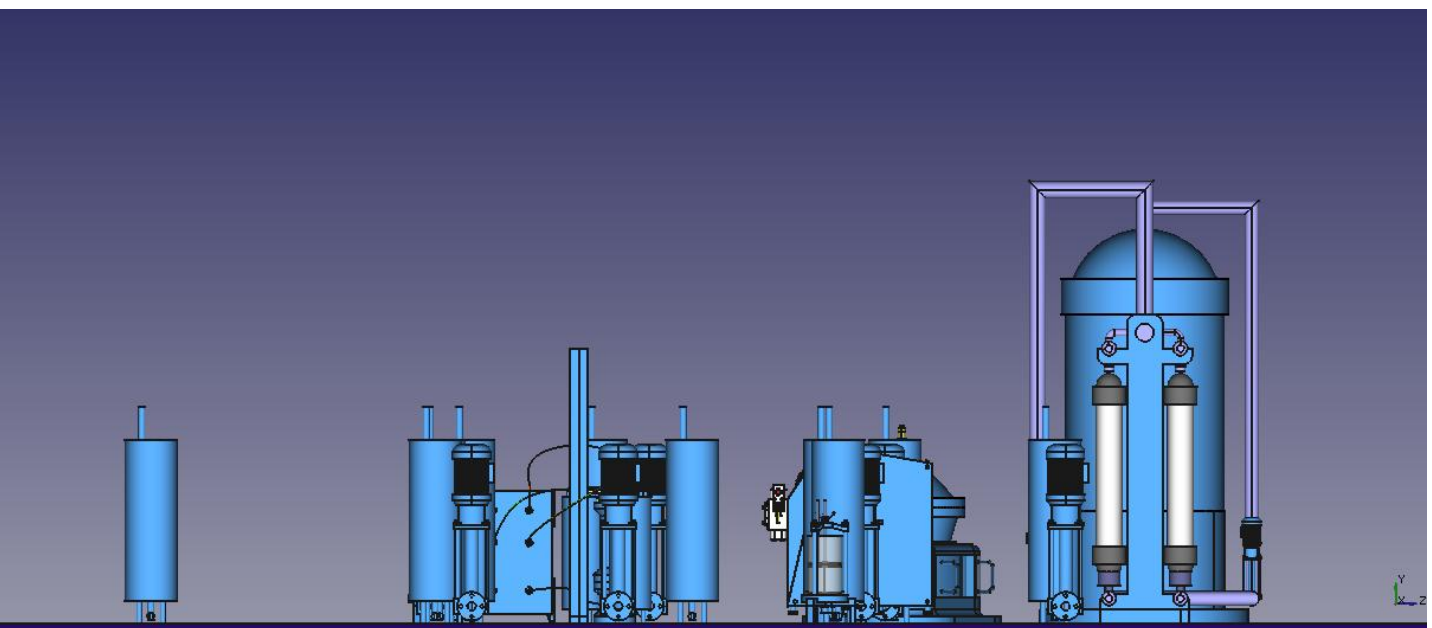
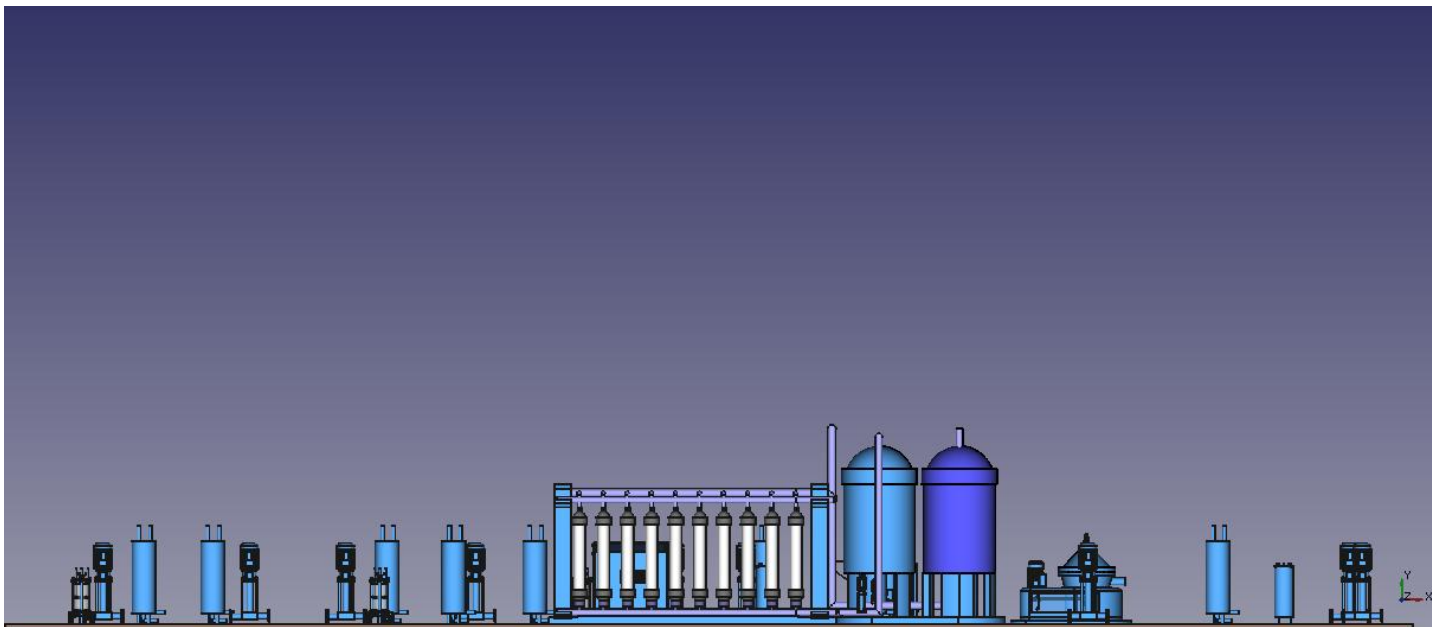
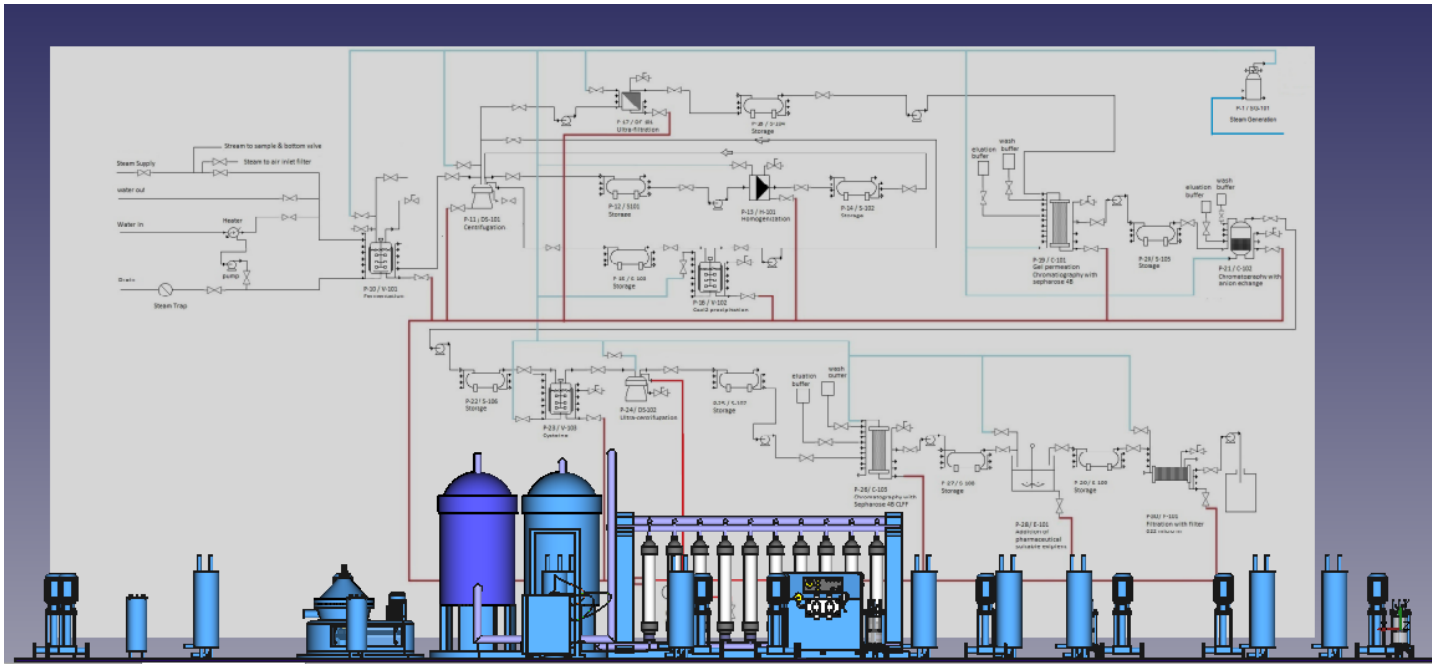


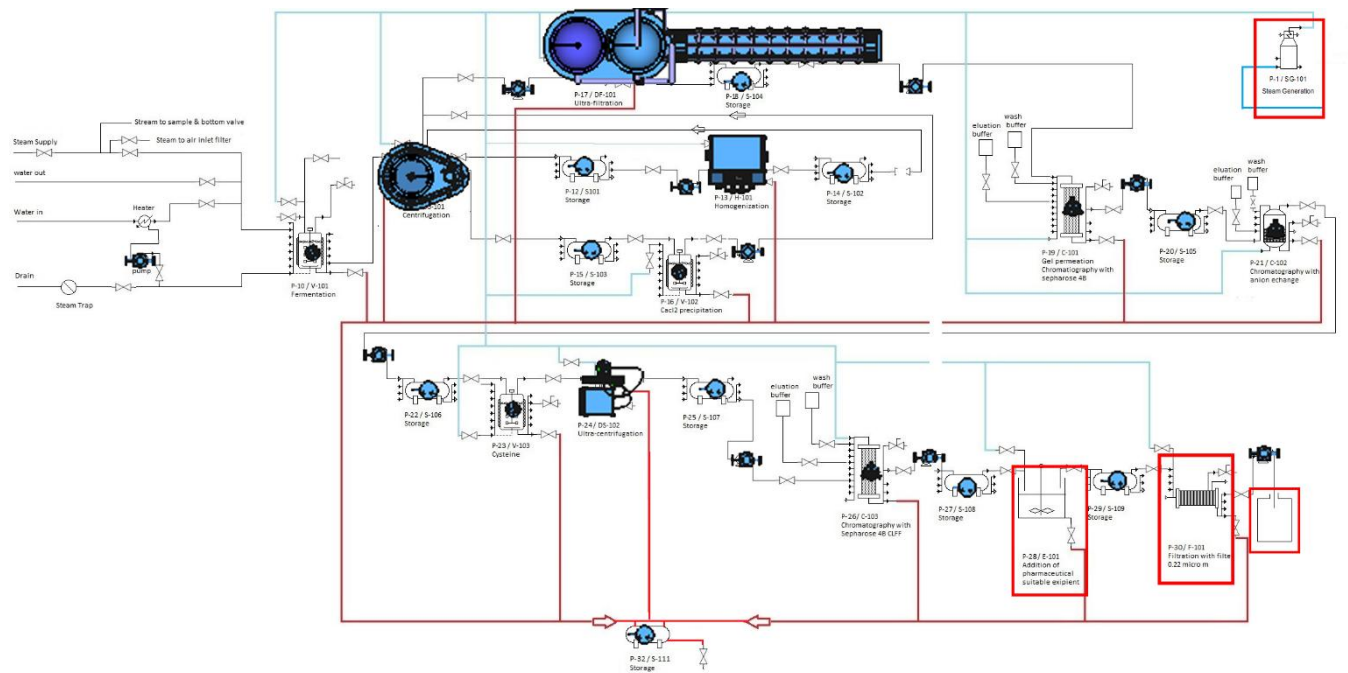
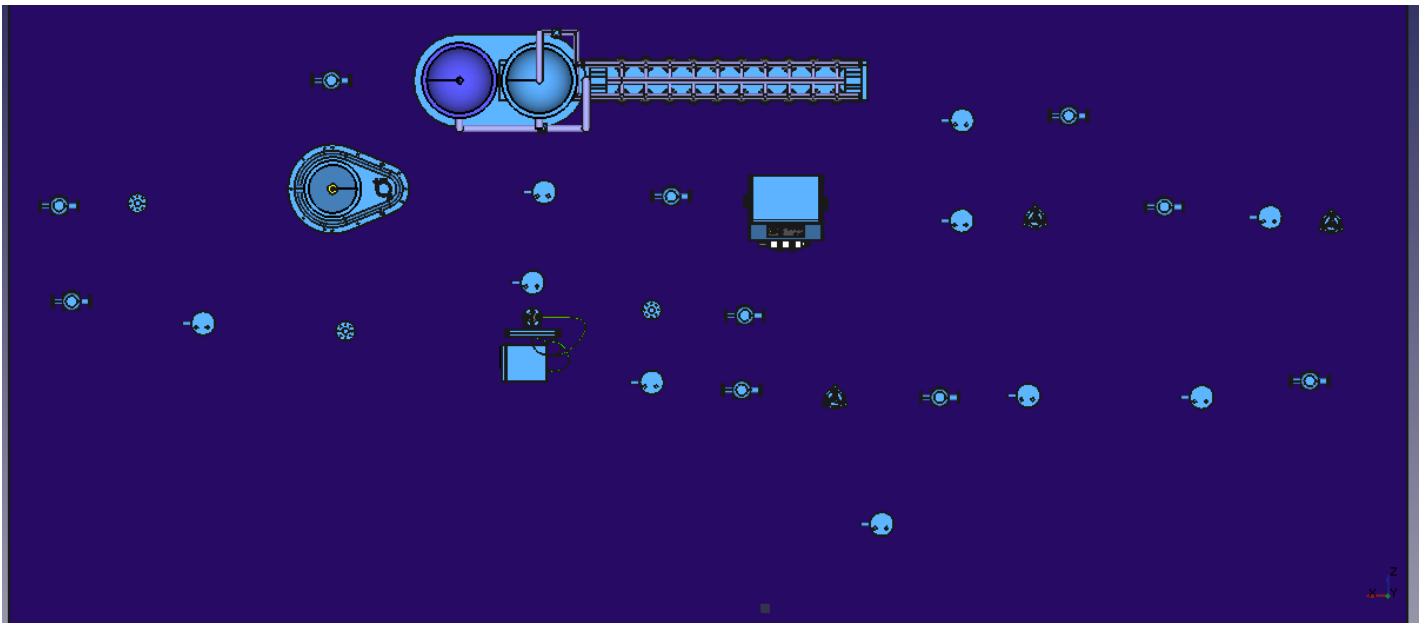
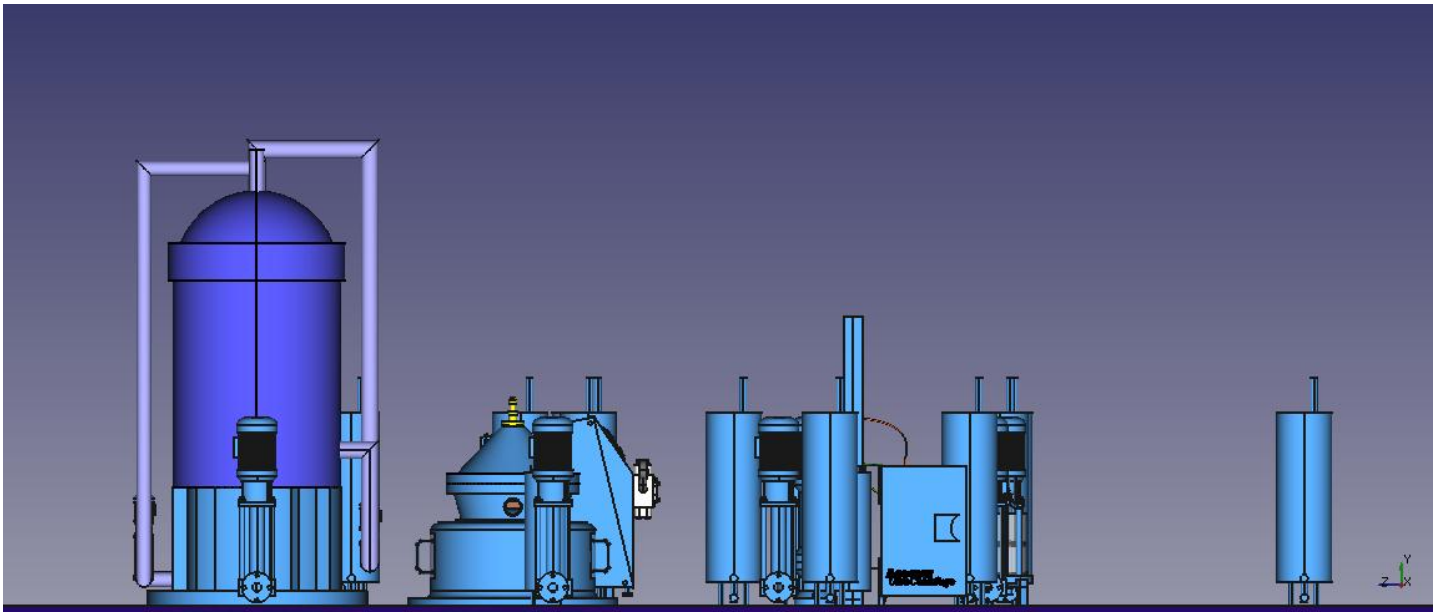
5.7 Final Assembly of the MEGBI Plant

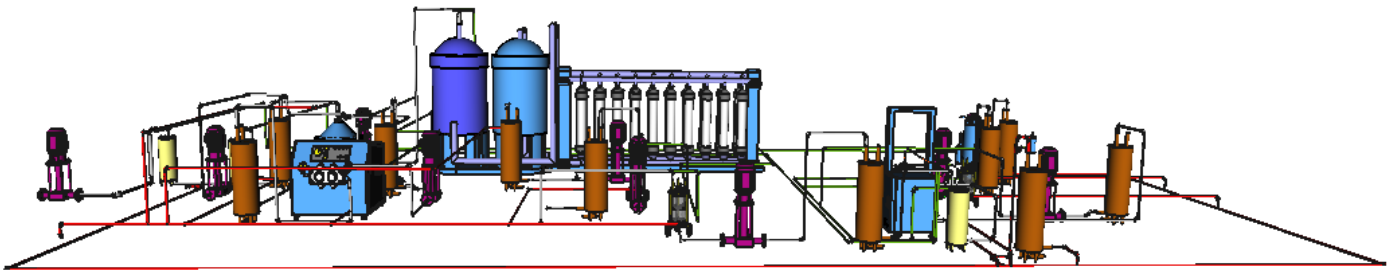
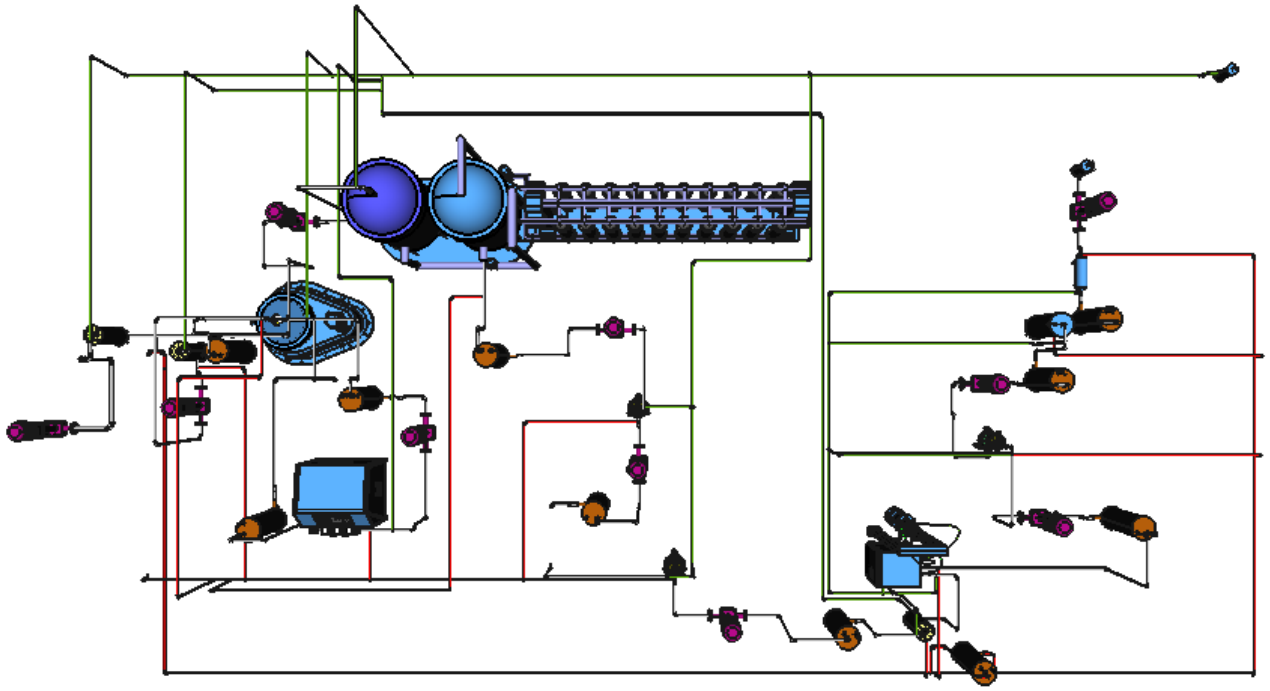


“Due to the lack of Assembly Features in FREECAD, Assembling These Parts Together. Was harder than Modelling Itself “– Jihad Samarji









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



الشركة اللبنانية للإنجاز التكنولوجي

رقم ٣٩٩ في سجل التجاري بيروت مسجل في تاريخ ٢٠٠٩/٥/٢٨

Ras Nhache/Batroun - Tripoli, 13th Jan 2015

MEGBI-VPP system design

البيوريكتور (Fermentation) و شاشة التحكم للبيوريكتور

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)

| Operation Sequence | |
|---|--|
| EQUILIBRATE-1 (Column Equilibration) | |
| Column Testing (Holding) | |
| LOAD-1 (PBA Column Loading) | |
| WASH-1 (Column Wash) | |
| ELUTE-1 (Column Elution) | |
| Strip (Column Regeneration) | |
| Rinse (Column Wash) | |
| Chromatographic rig CIP (In-Place-Cleaning) | |



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

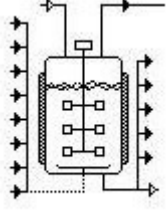
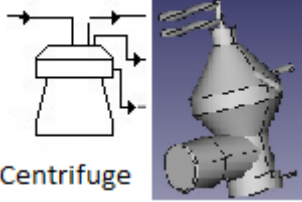
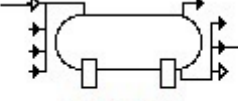
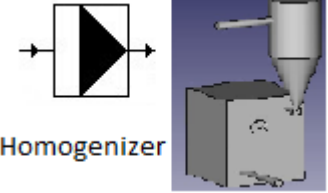
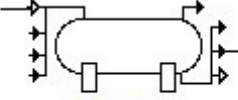
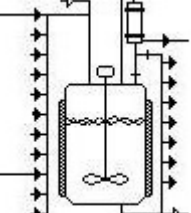
- Design of Software (State machines) for Homogenizer, Disc Stack Centrifuge in including CIP/SIP functional elements, Process Scale Gel Permeation and ion exchange chromatographic devices, Process Scale Ultrafiltration Device (6 weeks)
- Graphical User Interface for the automation of MEGBI-VPP downstream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

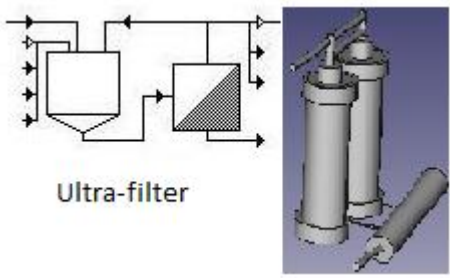
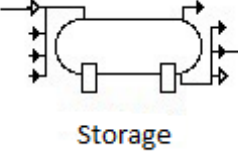
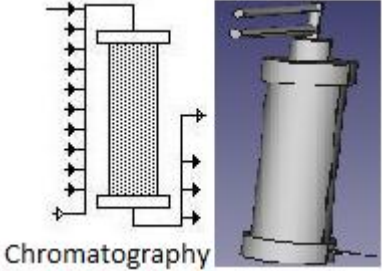
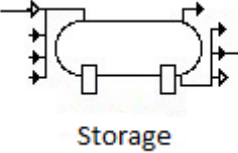
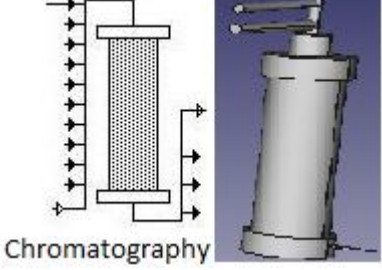
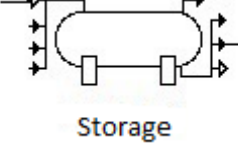
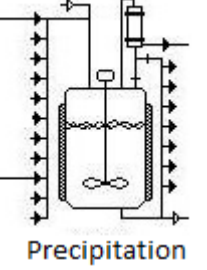
Keywords: measurement of temperature, pressure and pH data, Automation of fluid flow, PLC, Siemens S7, Programming, User Interface, C++/Java, Biotechnology

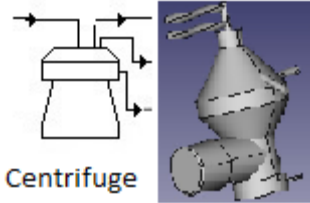
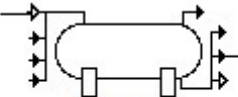
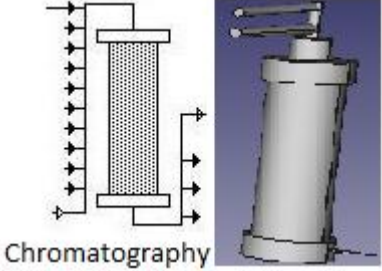
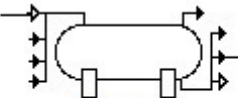
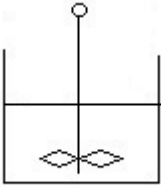
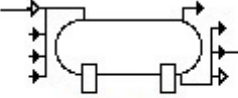
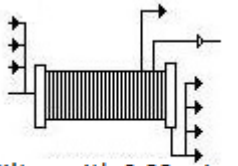
For automation system basics please refer to chapter 2, sections 15-17

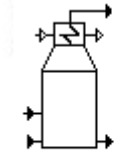
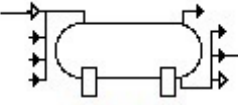
6.1 Automation System Specification

Table 3: List of Process *devices* with machine, PI diagram symbol and instruments needed.

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

| | | | | |
|------|--------|---|---|------------------|
| P-16 | DF-101 |  <p>Ultra-filter</p> | V1 V2 V3 V4 V5 PU2 | T5 MF5 PR5 |
| P-17 | S-103 |  <p>Storage</p> | V1 | |
| P-18 | C-101 |  <p>Chromatography</p> | V1 V2 V3 V4 V5 V6 PU3 | T6 MF6 PR6 |
| P-19 | S-104 |  <p>Storage</p> | V1 PU4 | |
| P-20 | C-102 |  <p>Chromatography</p> | V1 V2 V3 V4 V5 V6 | T7 MF7 PR7 |
| P-21 | S-105 |  <p>Storage</p> | V1 PU4 | |
| P-22 | V-103 |  <p>Precipitation</p> | V1 V2 V3 V4 V5 | T8 MF8 PR8 |

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

| | | | | |
|------|-------|--|----|-----|
| P-30 | S-110 |  <p>Steam generator</p> | V1 | P1 |
| P-31 | S-109 |  <p>Storage</p> | V1 | --- |

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:

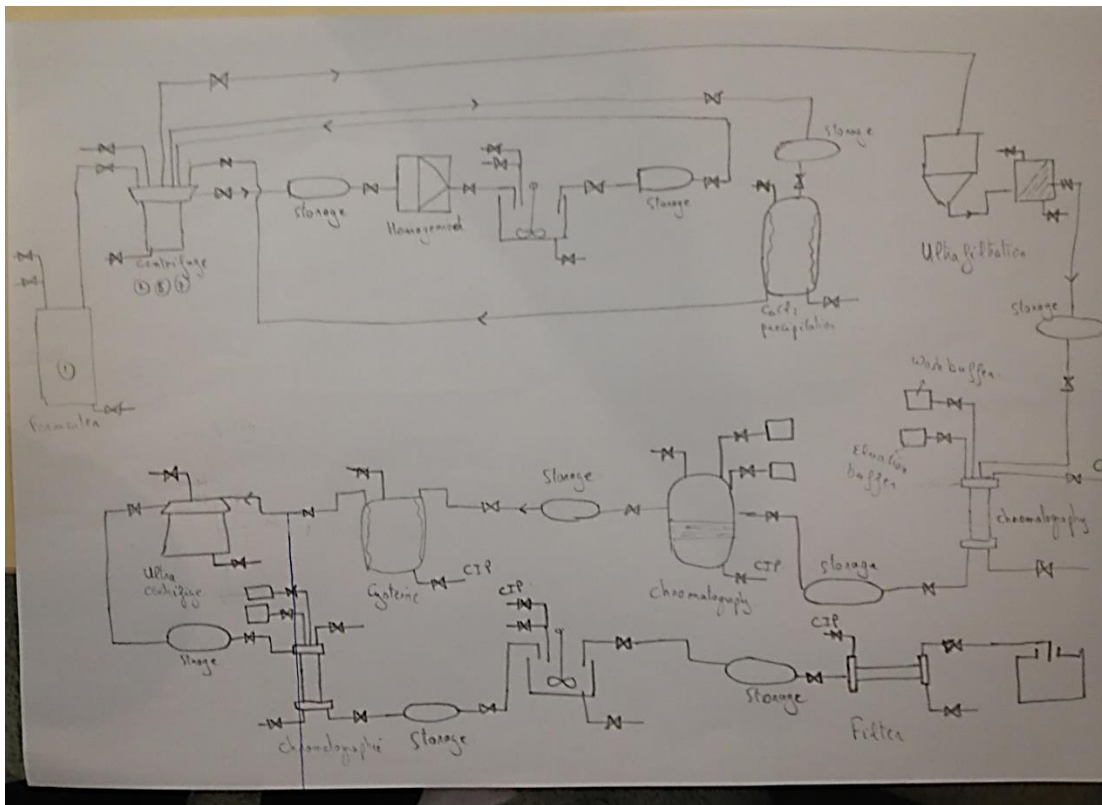
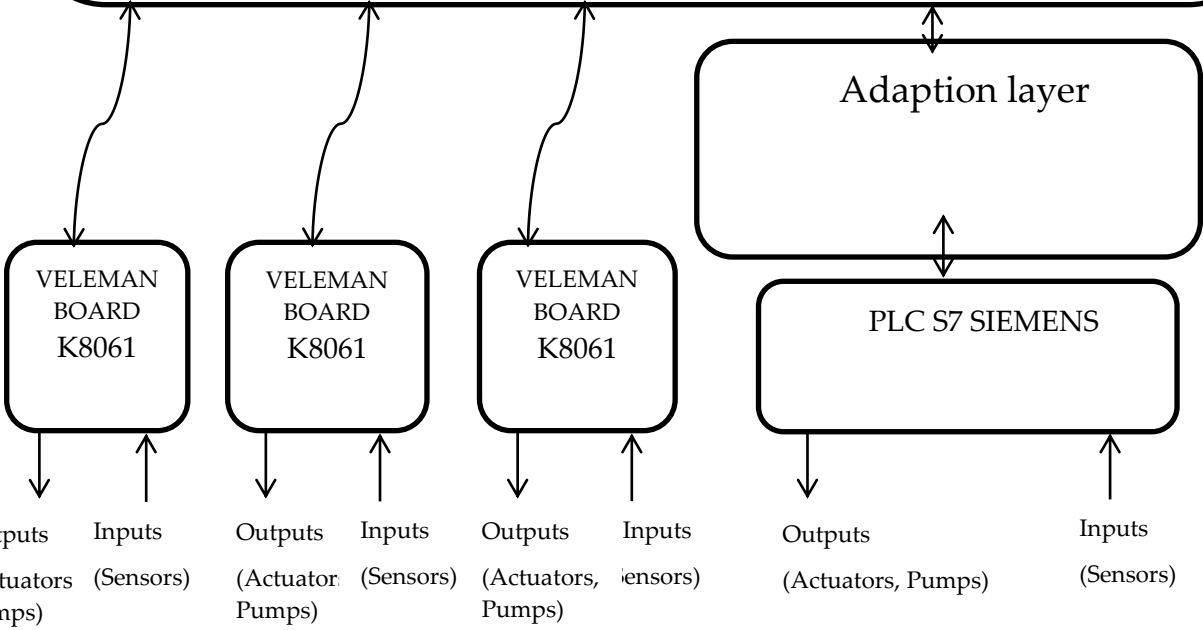
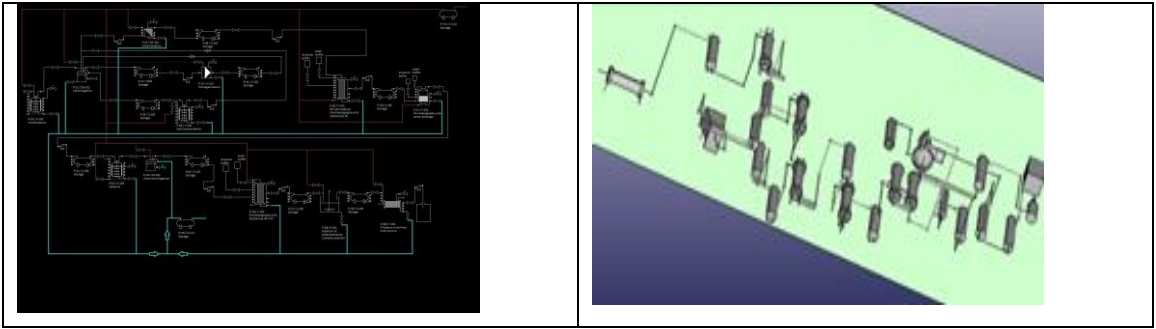


Figure 3: *Painting the Downstream Processing.*

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



6.2 Graphical User Interface

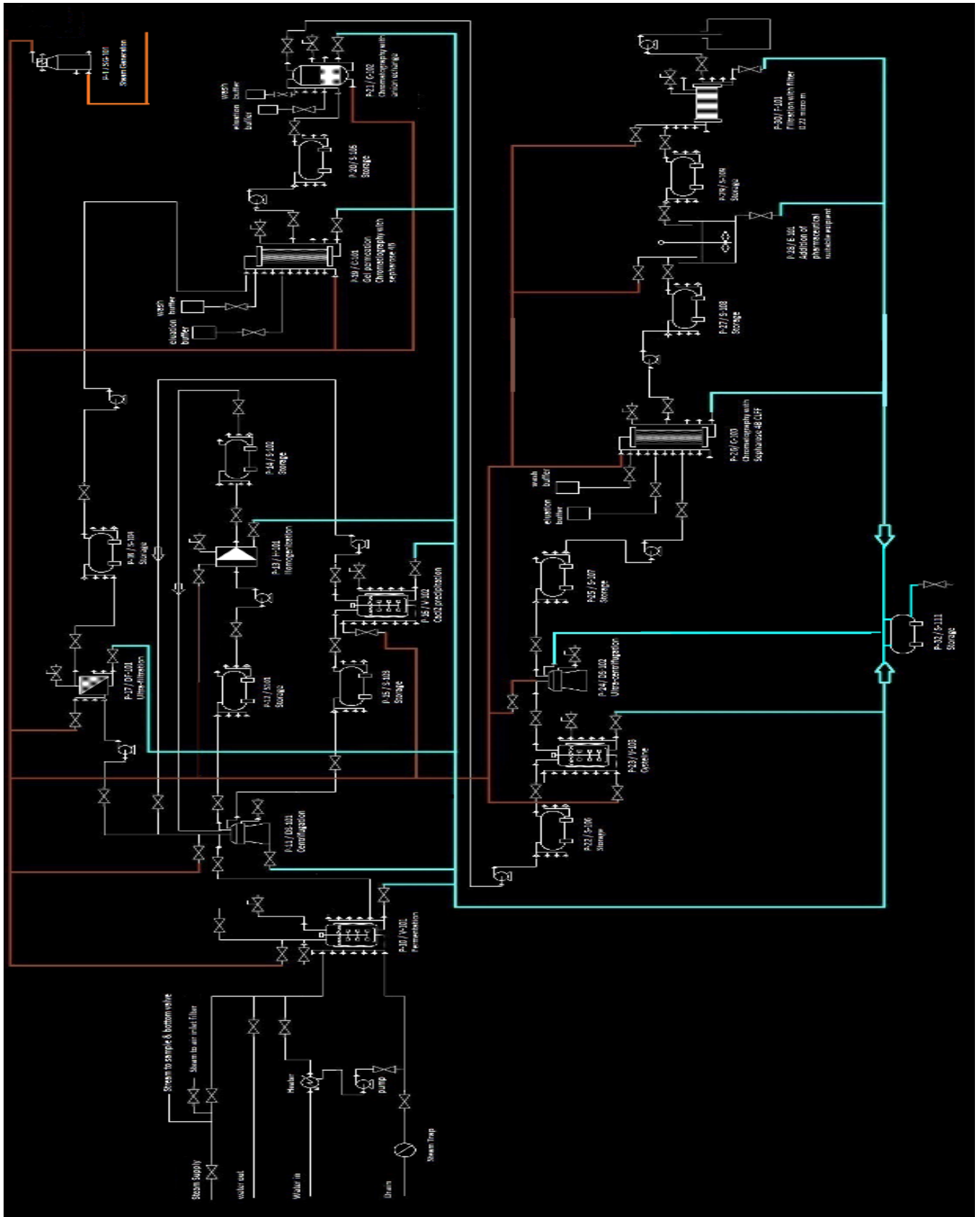


Figure 2.4: PI Diagram which represents the MEGBI-VPP Processing.

So by using the concepts and the specification of the production for the vaccine, we have organized the PI diagram for the MEGBI-VPP Processing and finally we get the PI diagram full needed to continue our project and to be the first step that has done and now this PI diagram (figure 2.1) represents the MEGBI-VPP Processing. On this PI diagram we have putted 3 pipelines white represents the road which will pass by the vaccine and some materials adding in many machines, we thought about cleaning the machines, everything need clean after use to get a perfect production, for that we have created the 2 next pipelines red and blue, the red one is the road of the steam water coming from a steam generator and the other one is the road of the dirty water ,it's the dirt coming from the production of the vaccine hepatitis b and beside the bioreactor we have added the temperature control system specific for it ,we have putted the valves necessary on the pipes to controls the in and out of fluid for the machine , and we have putted the pumps but we make look about the position that we have to use pump , so as we know if there is a elevation between the machine we need a pump so that is the condition that we have based on it.

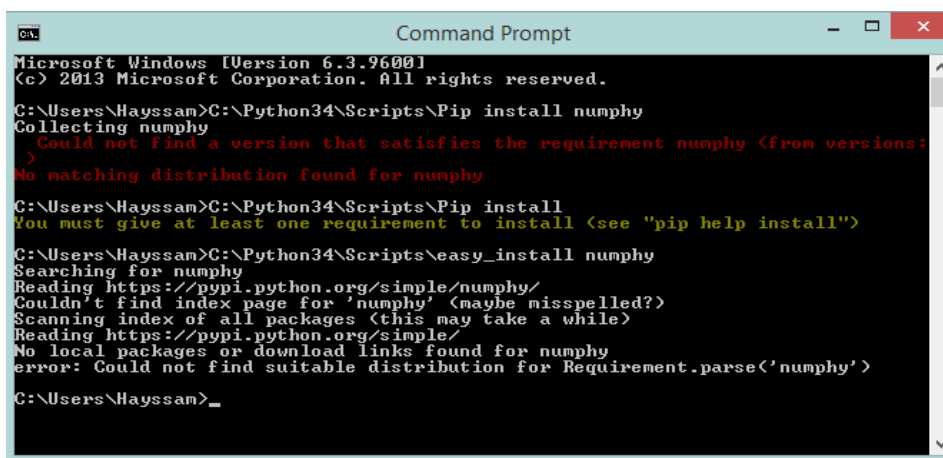
Next have to use the PI diagram on Python to create the control screen, we have passed by many problems on python start by how to install, how to run, and how to use, we have searched on internet sites to answer on this questions, and we get the answer needed by the Python book⁴ and internet sites[6] then we have used this reference for knowing the codes needed on Python to create this screen and how we should use it, first the idea of the control screen is controlling the fluid flow by open and close the valves, turning on and off the pumps, watching the status of each instruments on this screen for that we have needed buttons, labels texts list box background and window...

The hard job was on installing the Python, run it and how to choose the specific code for each thing we have to create on the screen so we have putted a plan about the things that we will show on the control screen, so as we talk before we need buttons by a specific code on Python we have created 68 buttons, 58 for valves and 10 for pumps , and we have organized it like each valve beside it the button specific for it , so we have identified the position of the valve to know where we will put this button and we did this for the 68 buttons. Before button we have used the PI diagram created with a specific code on Python to the background of the control screen

⁴Book name: Wx Python in Action

and with a specific code too we have created a window for this screen and we have created a bar status for this screen with 2 buttons 'exit' 'about', plus we have written on 'about' information about this screen and what it's represents. Next we have created two list box (one for valves, other for pumps) concerning the list name of the actuators (valve or pump) beside it we have putted a label for each actuators to shows on it the status of the actuators, this status we will use it to controls the actuators by open/close or turn on/off by a 'ON' or 'OFF' on label, so we have created on Python a condition between the label and button which we can use the click on button to get 'ON' for first click 'OFF' for other for a manual work, and for the full automation we have putted a "Turn ON" button that we will click on it to begin the production process. Now we have the window with the PI diagram background, buttons, and two list box and if we click on button we get the status of it on label in list box this for a manual work and we have the "Turn On" button, So the control screen is done and ready to use (figure2.2). About all specific codes used you can see the full program in Annex2 and see the codes.

Some difficulties during installation



```
Command Prompt
Microsoft Windows [Version 6.3.9600]
(c) 2013 Microsoft Corporation. All rights reserved.

C:\Users\Hayssam>C:\Python34\Scripts\Pip install numphy
Collecting numphy
  Could not find a version that satisfies the requirement numphy (from versions:
  )
No matching distribution found for numphy

C:\Users\Hayssam>C:\Python34\Scripts\Pip install
You must give at least one requirement to install (see "pip help install")

C:\Users\Hayssam>C:\Python34\Scripts\easy_install numphy
Searching for numphy
Reading https://pypi.python.org/simple/numphy/
Couldn't find index page for 'numphy' (maybe misspelled?)
Scanning index of all packages (this may take a while)
Reading https://pypi.python.org/simple/
No local packages or download links found for numphy
error: Could not find suitable distribution for Requirement.parse('numphy')

C:\Users\Hayssam>_
```

Figure 5 : Error Command 2

To repair this error, must upgrade the pip package:

```

Command Prompt - C:\Python34\Scripts\Pip install --upgrade pip
Microsoft Windows [Version 6.3.9600]
(c) 2013 Microsoft Corporation. All rights reserved.

C:\Users\Hayssam>C:\Python34\Scripts\easy_install.exe
error: No urls, filenames, or requirements specified (see --help)

C:\Users\Hayssam>C:\Python34\Scripts\Pip install numphy
You are using pip version 7.0.3, however version 7.1.2 is available.
You should consider upgrading via the 'python -m pip install --upgrade pip' command.
Collecting numphy
  Could not find a version that satisfies the requirement numphy (from versions:
)
No matching distribution found for numphy

C:\Users\Hayssam>C:\Python34\Scripts\Pip install --upgrade pip
You are using pip version 7.0.3, however version 7.1.2 is available.
You should consider upgrading via the 'python -m pip install --upgrade pip' command.
Collecting pip
  Downloading pip-7.1.2-py2.py3-none-any.whl (1.1MB)
    100% |#####| 1.1MB 53kB/s
Installing collected packages: pip
  Found existing installation: pip 7.0.3
  Uninstalling pip-7.0.3:
    Successfully uninstalled pip-7.0.3

```

Figure 6: First step of upgrading.

```

Command Prompt
Successfully uninstalled pip-7.0.3
Exception:
Traceback (most recent call last):
  File "C:\Python34\lib\shutil.py", line 371, in _mtree_unsafe
    os.unlink(fullname)
PermissionError: [WinError 5] Access is denied: 'C:\Users\Hayssam\AppData\Local\Temp\pip-k8e76e24-uninstall\python34\scripts\pip.exe'
During handling of the above exception, another exception occurred:
Traceback (most recent call last):
  File "C:\Python34\lib\site-packages\pip\basecommand.py", line 233, in main
    logger.debug("Exception information: ", exc_info=True)
  File "C:\Python34\lib\site-packages\pip\commands\install.py", line 297, in run
    wb = WheelBuilder<
  File "C:\Python34\lib\site-packages\pip\req\req_set.py", line 633, in install
    for requirement in rs.install:
  File "C:\Python34\lib\site-packages\pip\req\req_install.py", line 734, in commit_uninstall
  File "C:\Python34\lib\site-packages\pip\req\req_uninstall.py", line 153, in commit
    self.save_dir = None
  File "C:\Python34\lib\site-packages\pip\_vendor\retrying.py", line 49, in wrapped_f
    return Retrying(*args, **kwargs).call(f, *args, **kw)
  File "C:\Python34\lib\site-packages\pip\_vendor\retrying.py", line 212, in call
    raise attempt.get()
  File "C:\Python34\lib\site-packages\pip\_vendor\retrying.py", line 247, in get
    six.raise_from(self.value[0], self.value[1], self.value[2])
  File "C:\Python34\lib\site-packages\pip\_vendor\six.py", line 659, in raise_from
    raise value
  File "C:\Python34\lib\site-packages\pip\_vendor\retrying.py", line 208, in call
    attempt = attempt(f(*args, **kwargs), attempt_number, False)
  File "C:\Python34\lib\site-packages\pip\utils\__init__.py", line 89, in mtree_unsafe
    shutil.rmtree(dir, ignore_errors=ignore_errors,
  File "C:\Python34\lib\shutil.py", line 478, in rmtree
    return _mtree_unsafe(path, onerror)
  File "C:\Python34\lib\shutil.py", line 368, in _mtree_unsafe
    _mtree_unsafe(fullname, onerror)
  File "C:\Python34\lib\shutil.py", line 368, in _mtree_unsafe
    _mtree_unsafe(fullname, onerror)
  File "C:\Python34\lib\shutil.py", line 373, in _mtree_unsafe
    os.unlink(fullname, *args, **kwargs)
  File "C:\Python34\lib\site-packages\pip\utils\__init__.py", line 101, in mtree_errorhandler
    # use the original function to repeat the operation
PermissionError: [WinError 5] Access is denied: 'C:\Users\Hayssam\AppData\Local\Temp\pip-k8e76e24-uninstall\python34\scripts\pip.exe'

C:\Users\Hayssam>

```

Figure 7 : Done step of upgrading.

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

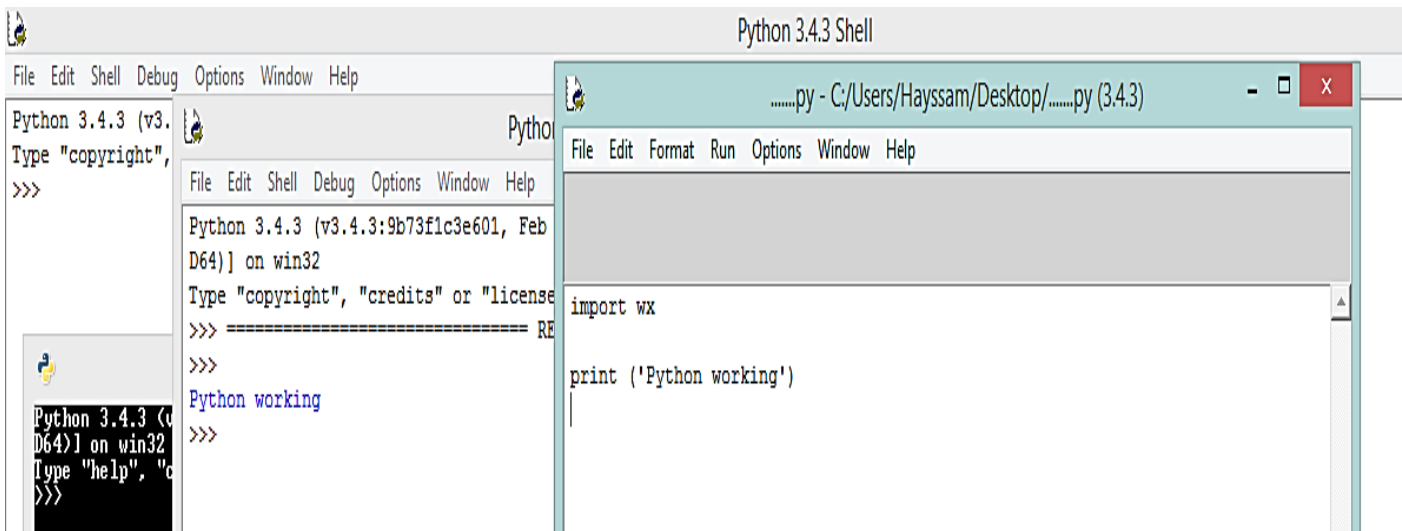


Figure 8 : Python working.

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_IMAGE_NAME = "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_IMAGE_NAME)
        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 9 : 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 = "MEGPI Vaccine Pilot Plant (MEGPI-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 10 : 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 11 : 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 12 : Python 4

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

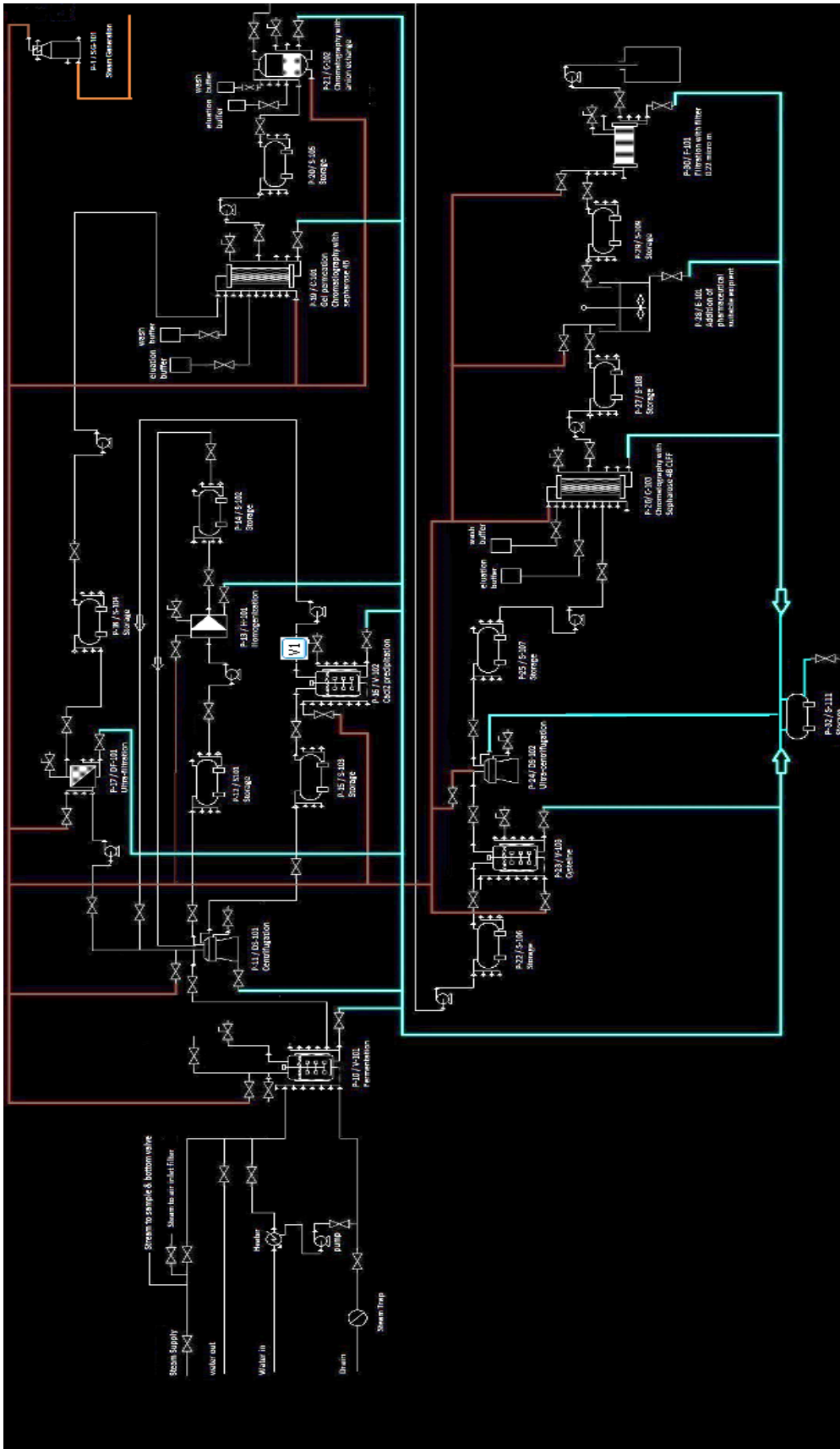


Figure 13 : Button VI Created

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 14 : 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 15: 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 16 : Shot of the right code in forum.

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

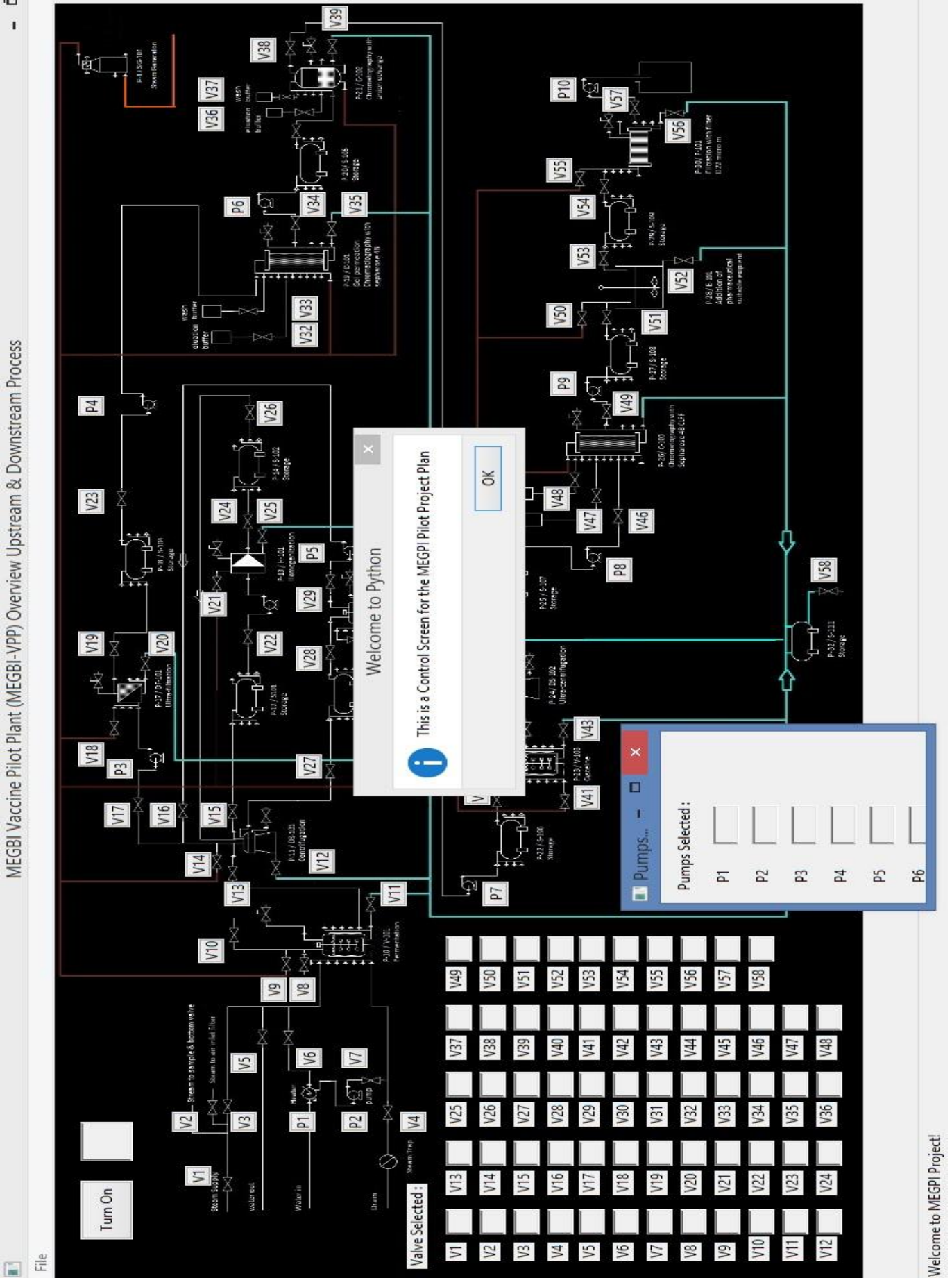


Figure 17 : The control screen full of buttons ,labels ,list box.

So as we talk before, the next step is connecting the control screen to USB Velleman K8061 board, we have used a specific code on Python to can the screen read the board and run it, before that we have defined the device of the USB board to pc by 2 dll files named "K8061.dll" "mpusbapi.dll" we downloading it from a site [7], when the board was connected to the screen we get a command shows the message between it (figure 2.3).

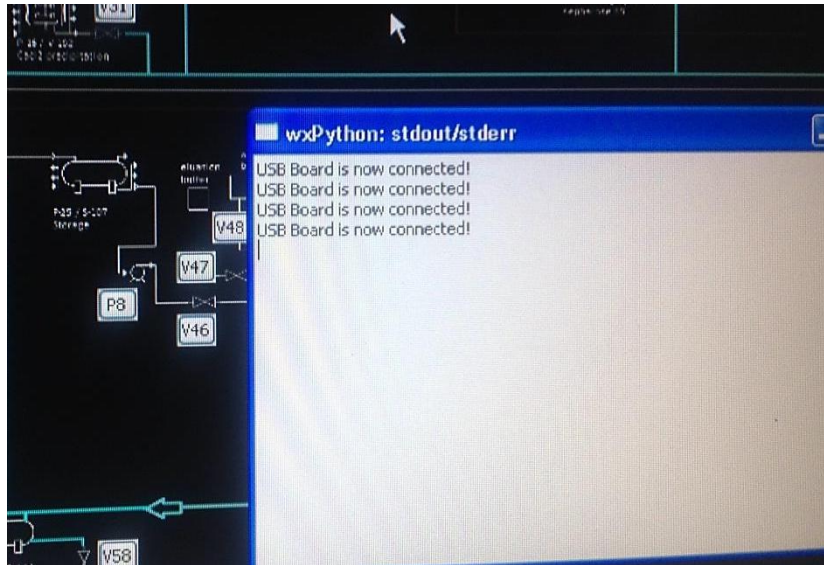


Figure 2.18 : Command between the USB board and the control screen .

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 19 : 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 20 : Python 7

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

6.3 Adaptation of Device Sensors and Actuators

6.3.1 Actuators (Valves)

Next we will talk about the actuators and sensors, and how we have connected it to the USB board to control them by the control screen. But when we makes the connection between the temperature sensors USB board we get wrong results that make a question about temperature so we have canceled it from the project and we continue about automation system by control the fluid flow from the actuators. To connect the actuators to USB board we need a relay, this relay will control the valve by open/close when they takes the order from the board same to pump by turn on/off, so we have built this relay by using a schema (figure 2.4).

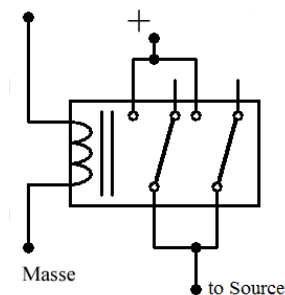


Figure 2.21: Schema represents the construction of relay.

So with this schema (figure 2.4), we build it by: relay-small board-tapes-welding

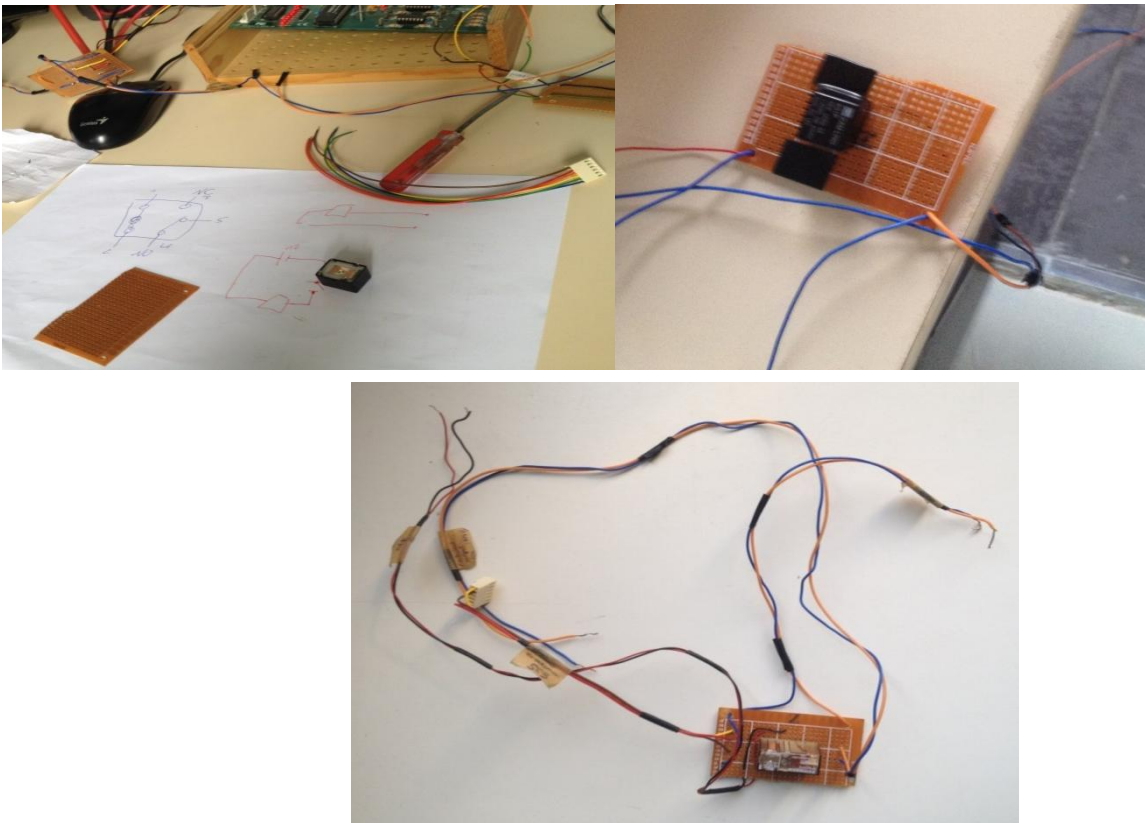
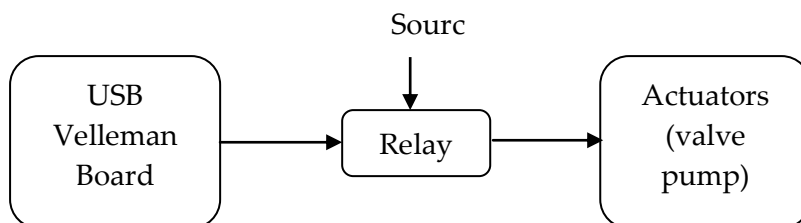


Figure 2.22: installation a relay.

And we use it to connect the actuators so as we can see on figure it's like a switch but with order this order come from the board, plus this actuators have connected to outputs (digital or analog).



4 steps to link the relay and make the connection between the board and the actuators:

- Connecting the relay to source (220V)
- Connecting the relay to actuators (220V)
- Connecting the relay to outputs channel(5V-12V)
- Connecting the relay to power of USB board(12V)

After knowing how to connect the actuators, we take a valve and we will try to connect it, so as we talk before, we have connected the valve to relay, then we have linked the relay and we get the circuit between the board, valve, relay, source (figure 2.6).

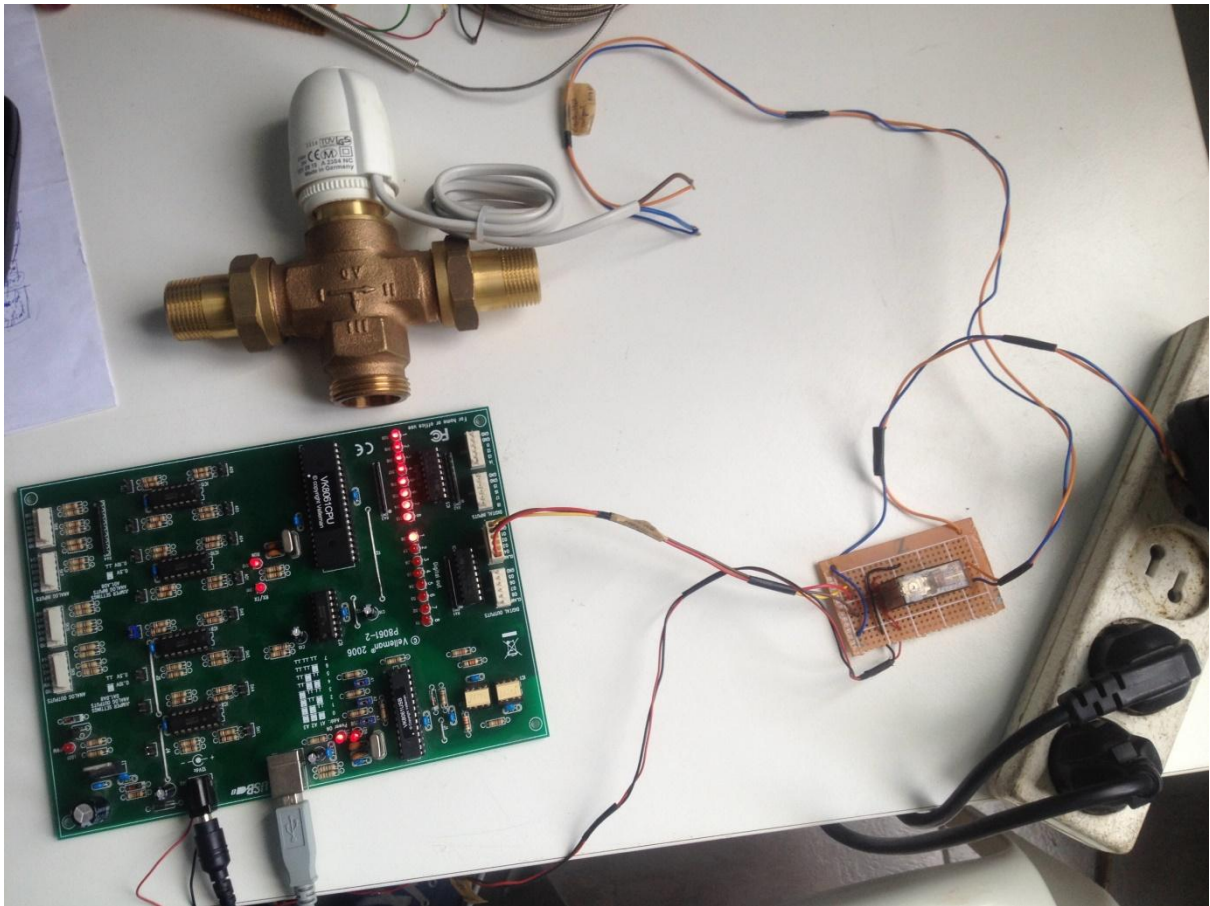


Figure 2.23 : The circuit between the board, valve, relay, source.

And we will test it to make sure the valve the circuit is working good and ready to use it in the test stand, so we will try to open and close it from the screen by clicking on button and we take many pictures showings the test, as we see the blue color mean the valve is getting open so the order from the board has been arrived to valve to open and the red light on the board in the outputs channel confirm the same thing (figure 2.7) ,and vice versa the red light disappeared when we close the valve so that confirm the same thing that the board gives the order to open or close(figure 2.8), so the control of the valve is working .

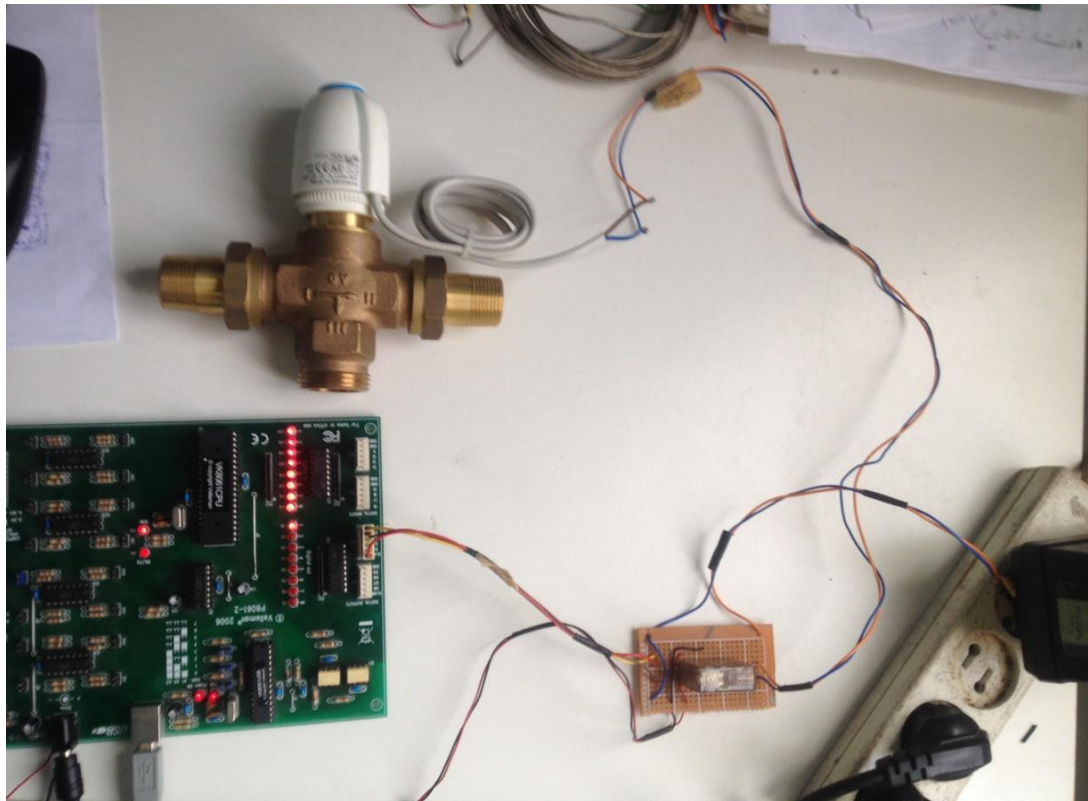


Figure 2.24 : The circuit when the valve gets opened.

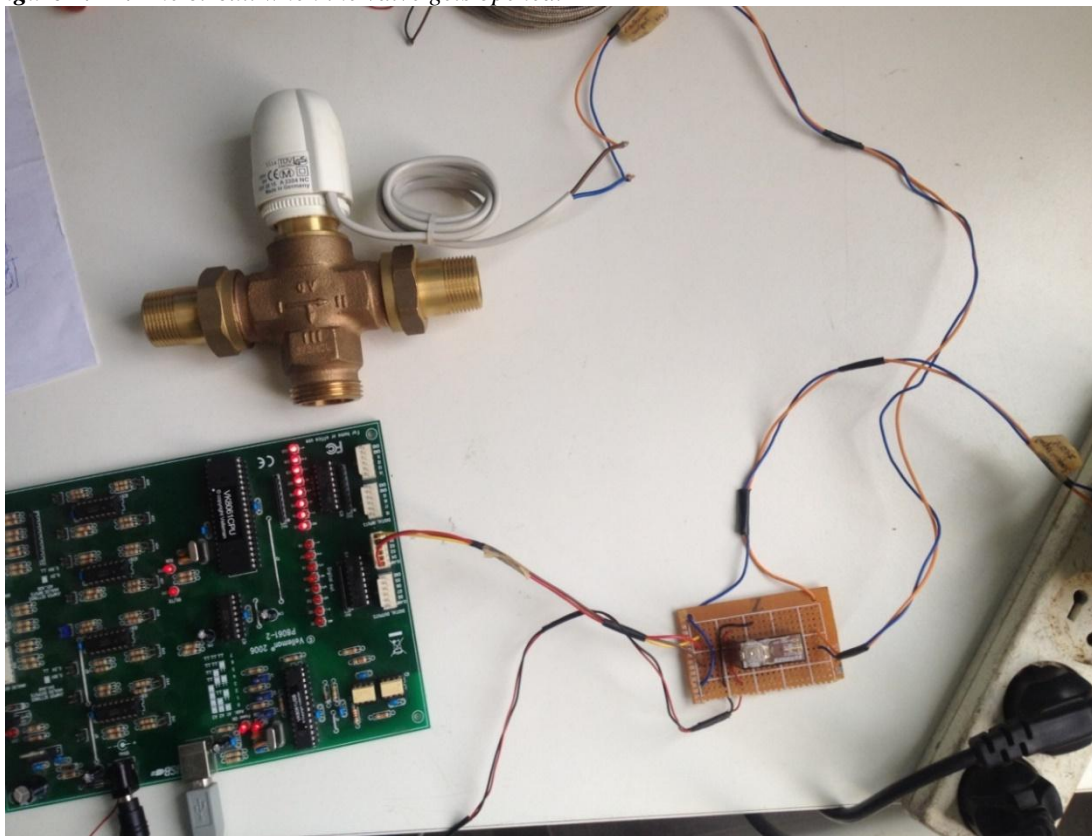


Figure 2.25 : The circuit when the valve get closed.

So we have 58 valves and 10 pumps concerning the production, and the valves, the pumps have used on temperature control system for bioreactor and the cleaning way, and we will make a connection on the PI diagram between the all actuators and the boards, PLC. so we have connected each valve to a output channel on board or PLC (figure 2.9), 16 outputs (digital + analoge) on board, 16 on PLC.

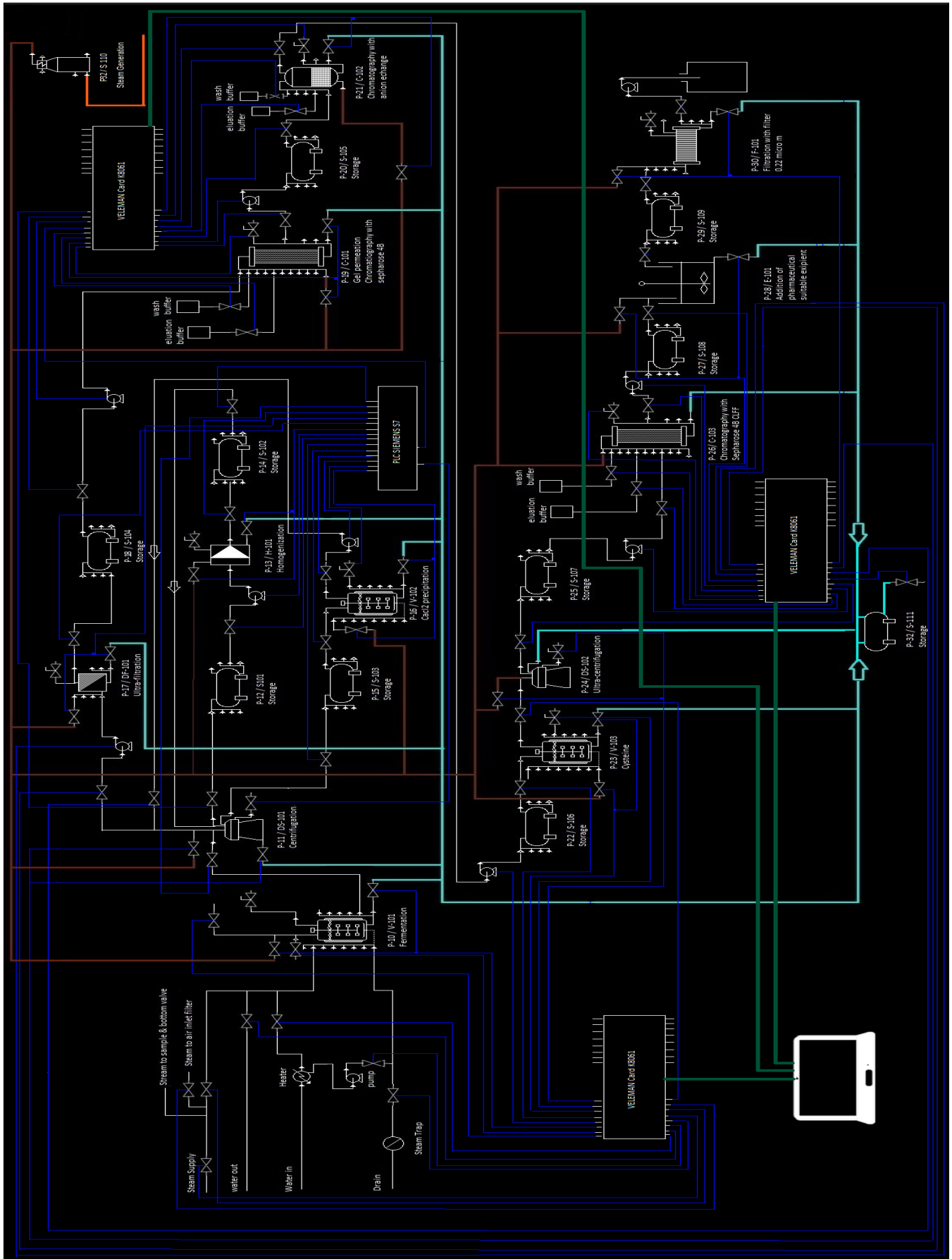


Figure 2.26 : Network between the actuators and the boards,PLC.

6.3.2 Sensors

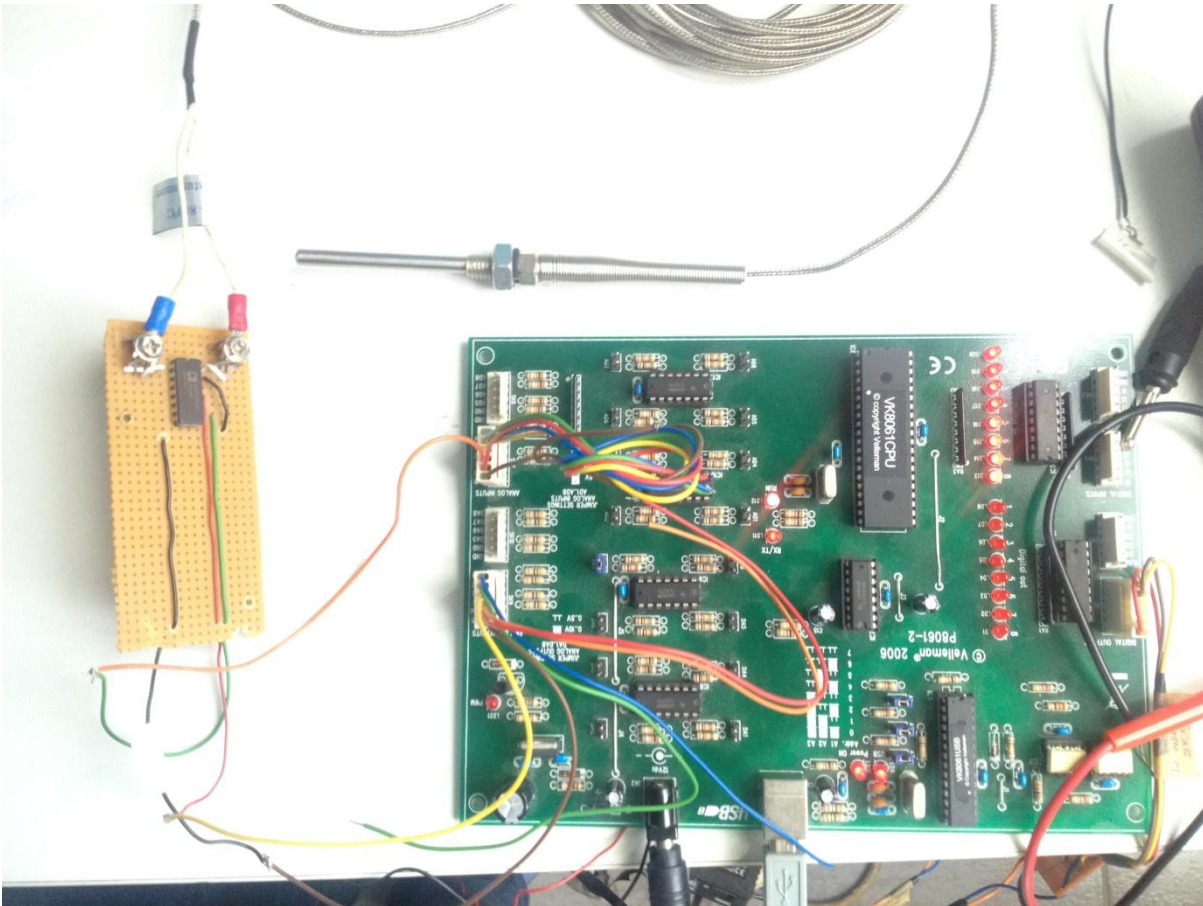


Figure 27 : The circuit of Temperature sensor with Velleman board.

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.

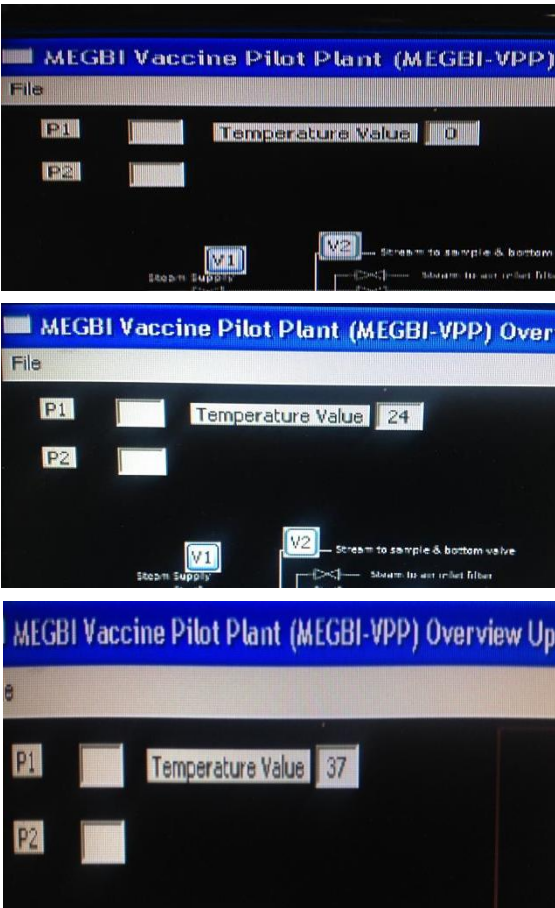


Figure 28 : 3 Shots show the malfunction.

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 29 : 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.

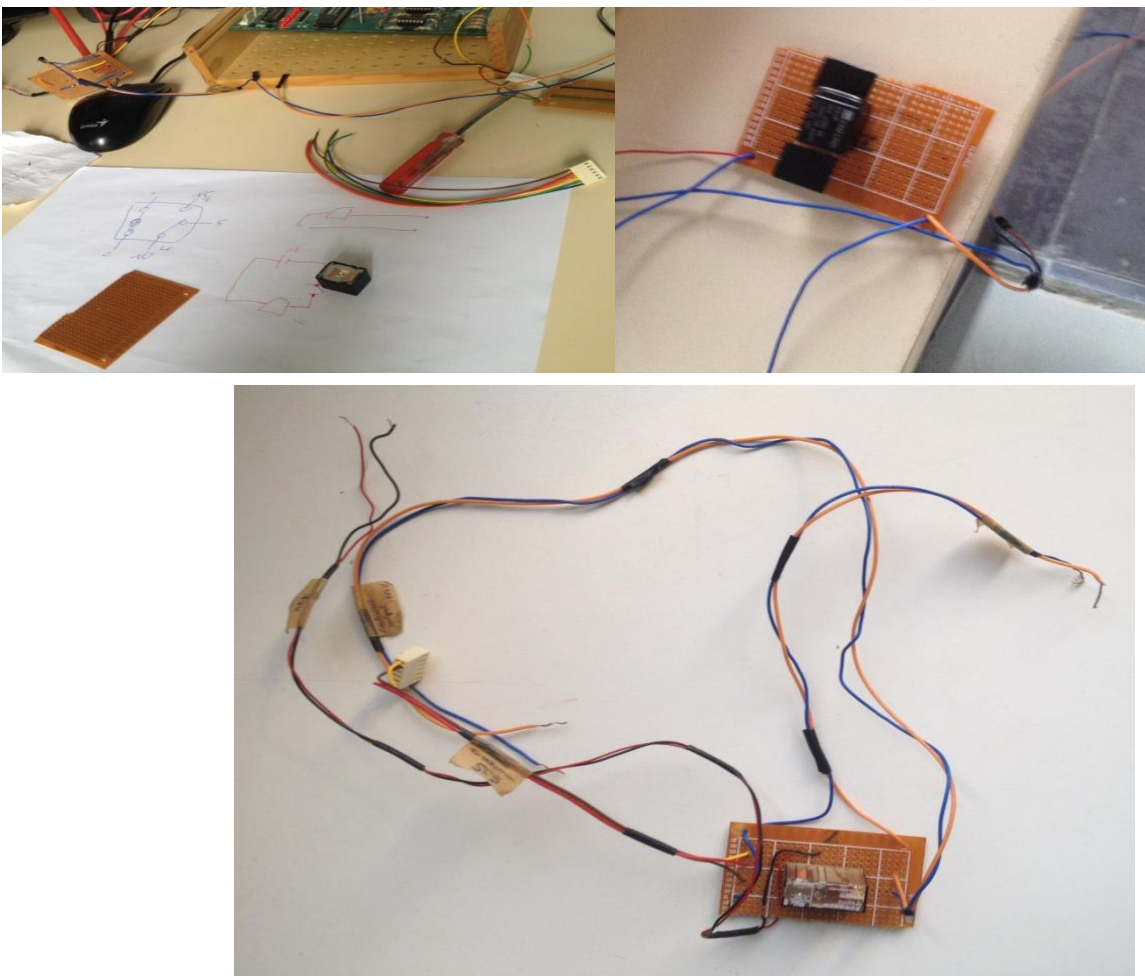


Figure 30: Building a relay.

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:

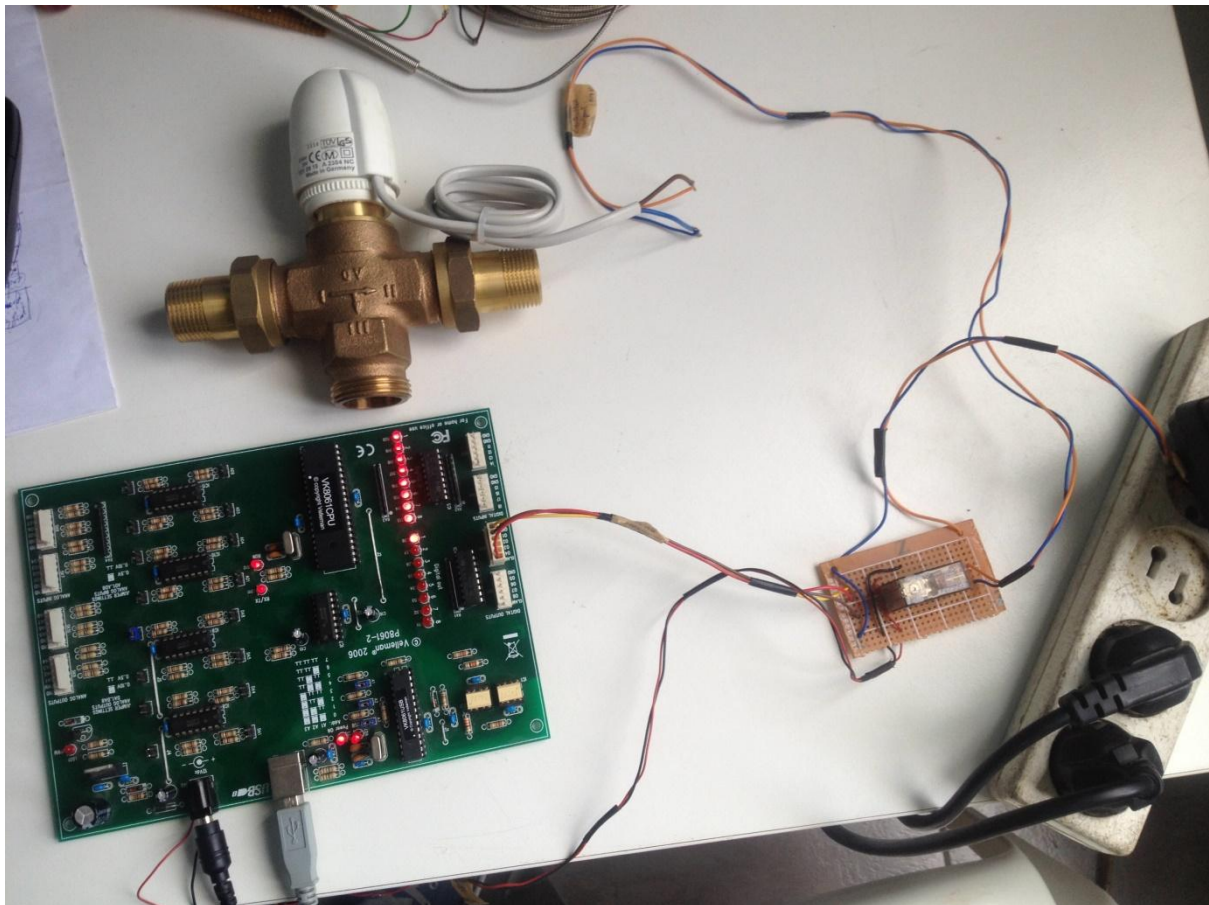


Figure 31 : The circuit of Valve with Velleman board

Now must test it:

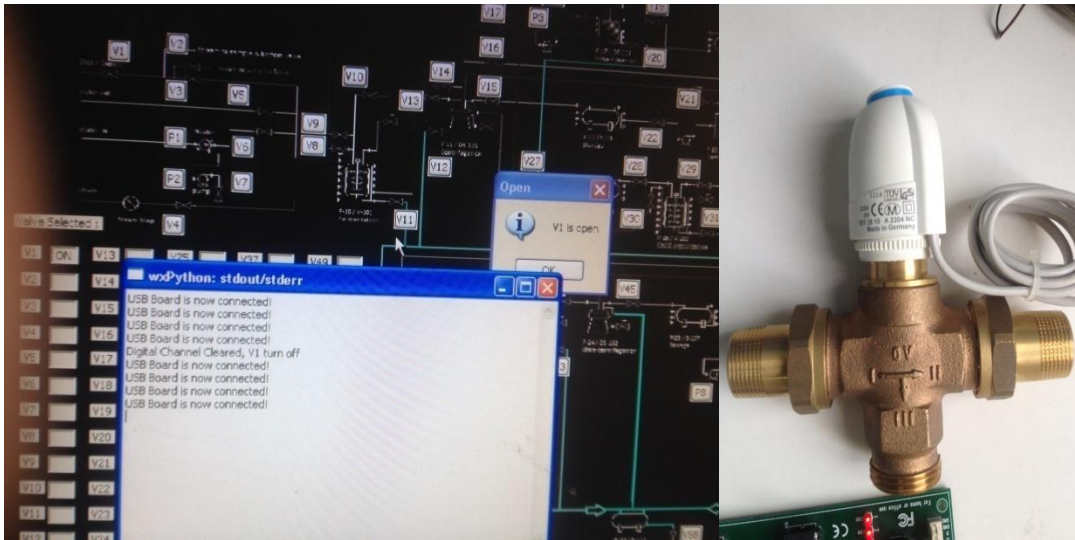


Figure 32 : Testing the process.

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

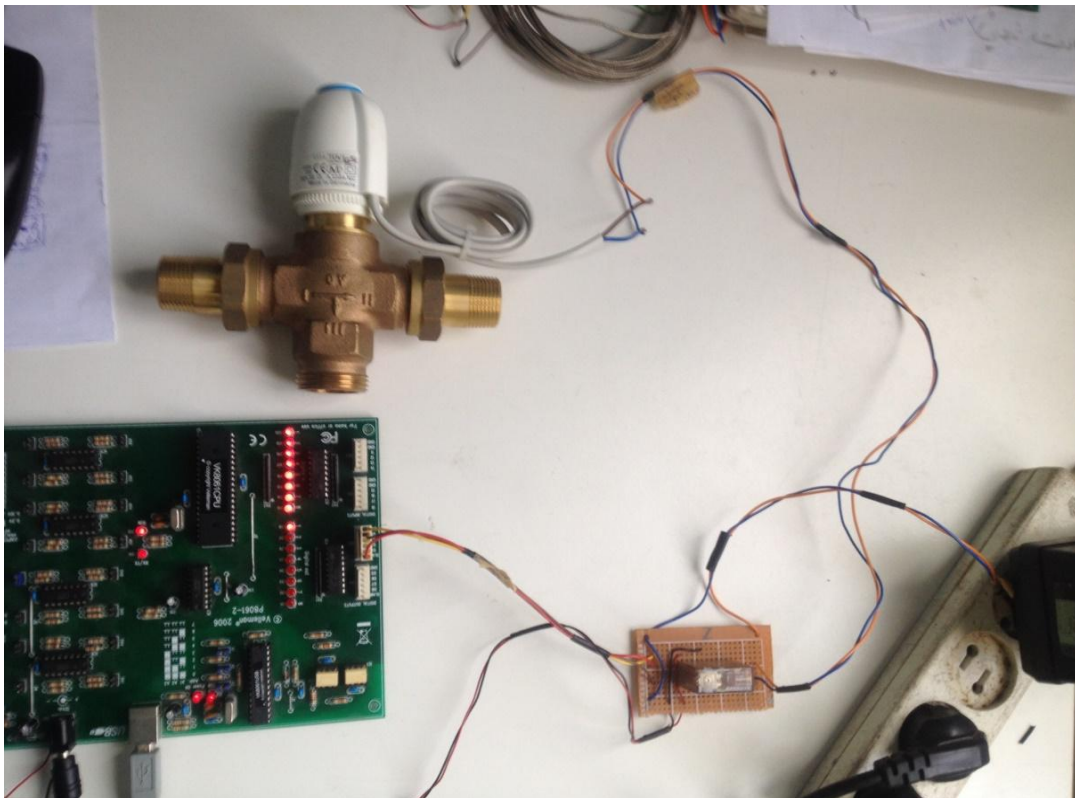


Figure 33 : The circuit when the valve gets opened.

There is a red light on near the series of light, that light means 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 show it too:

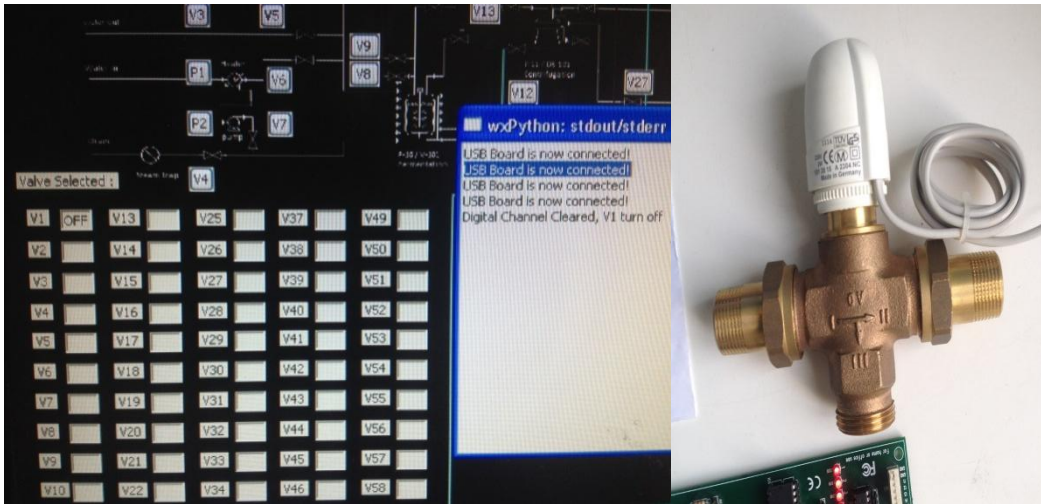


Figure 34 : Testing Process.

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

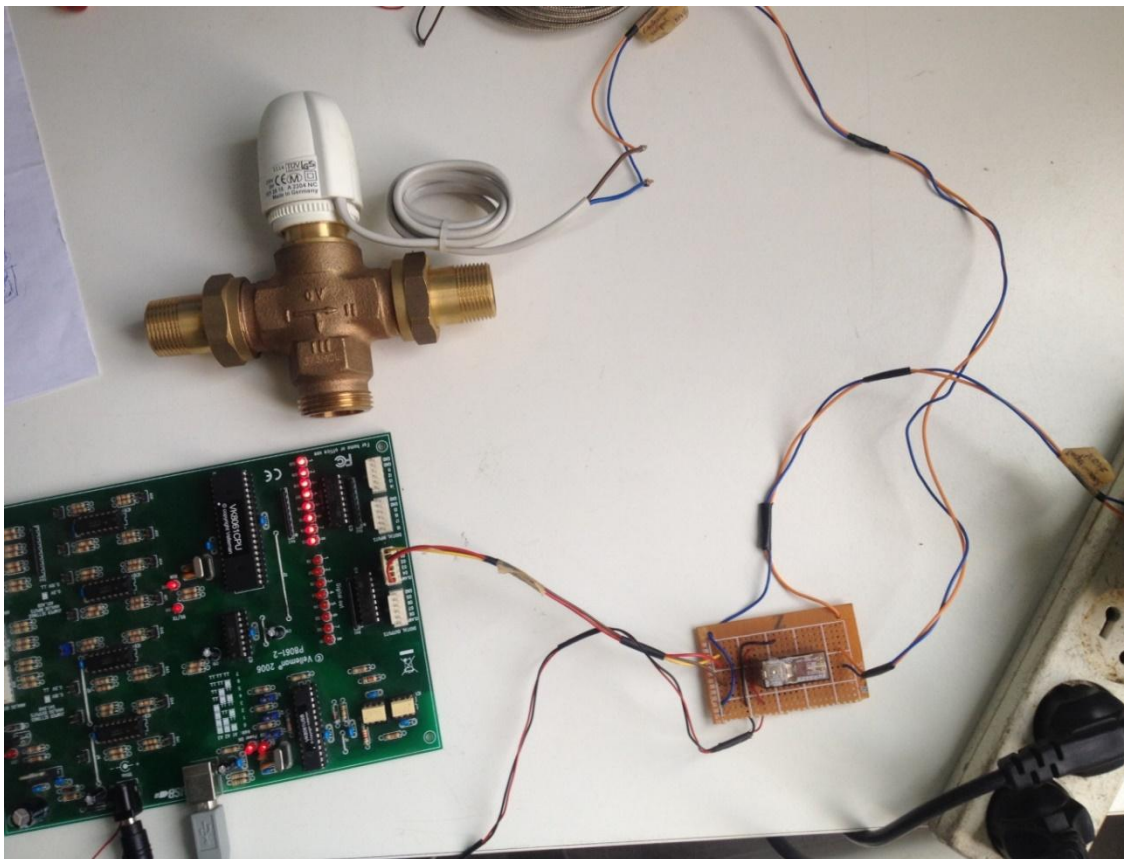


Figure 35 : The circuit when the valve get closed.

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.

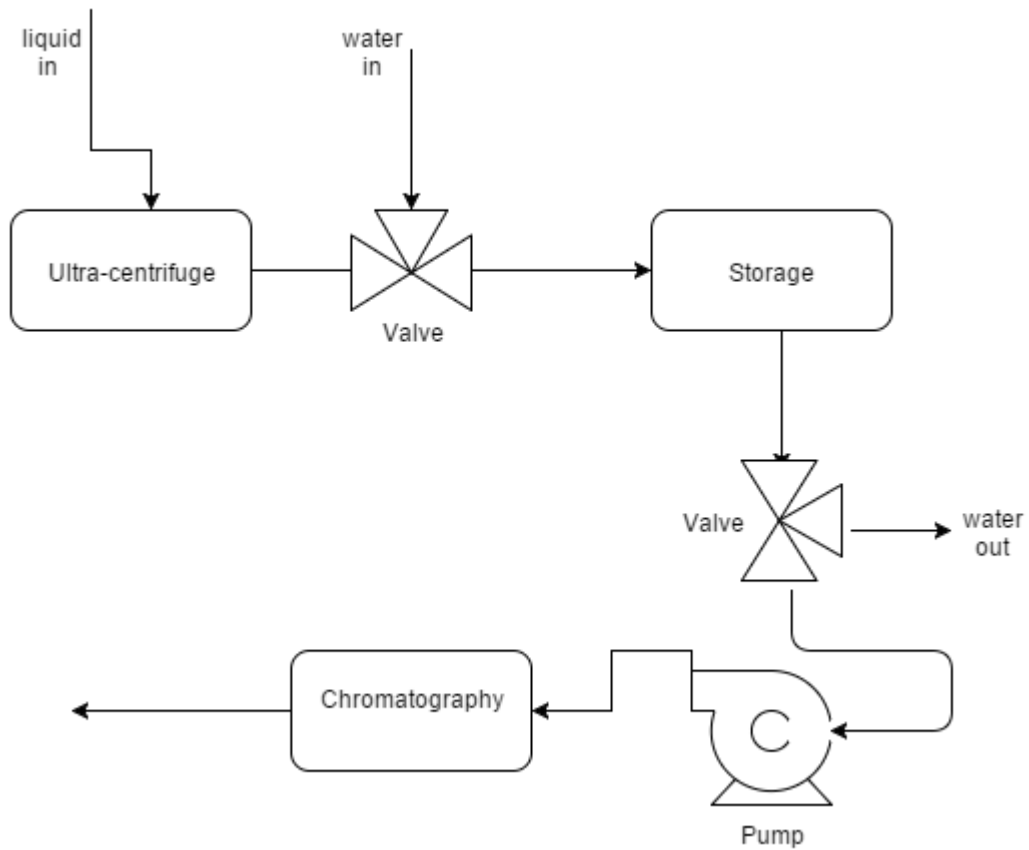


Figure 36 : Diagram for the part of downstream processing used.

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.

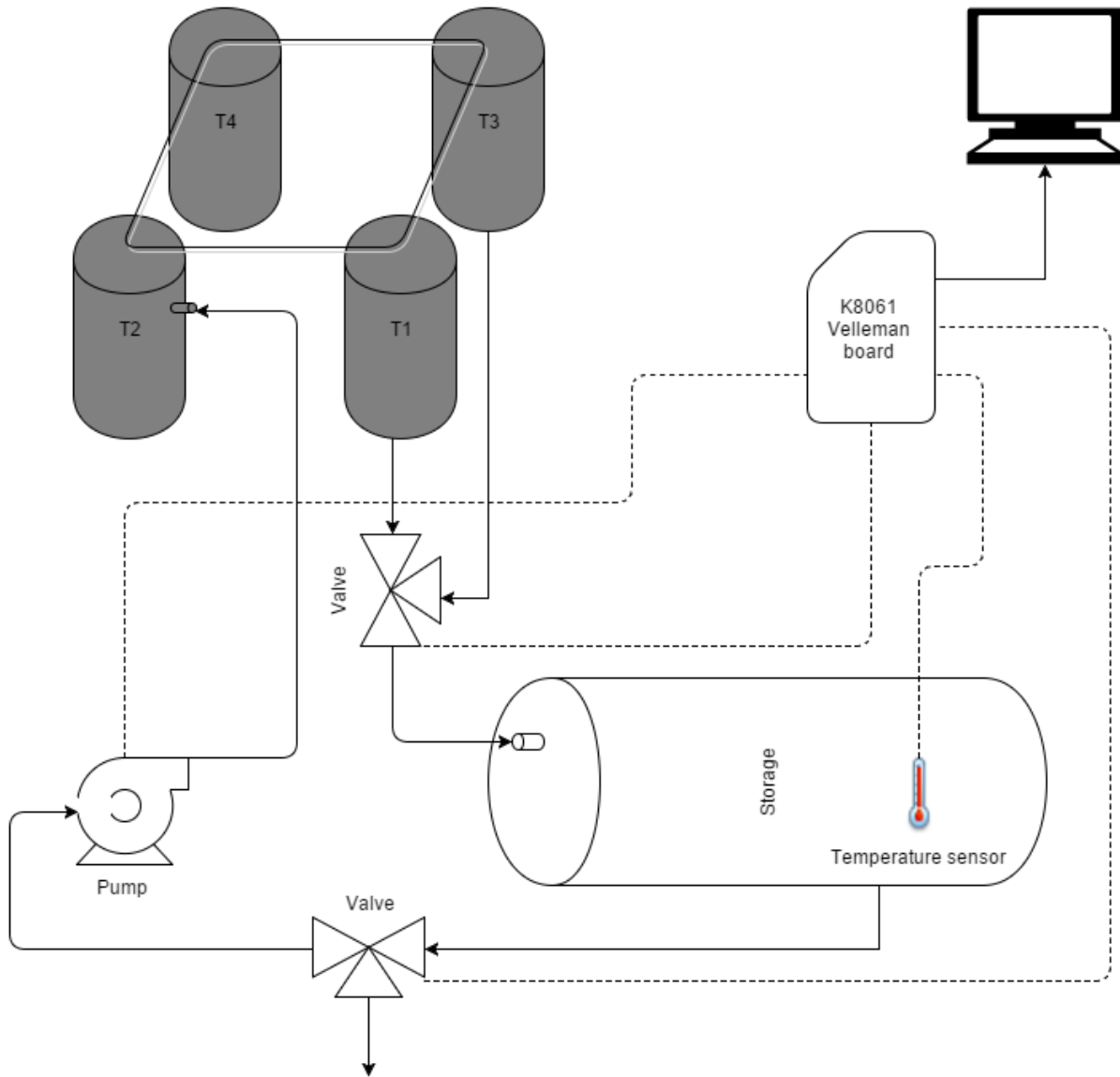


Figure 37 : Schema of the part of downstream processing used.

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 38 : 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.

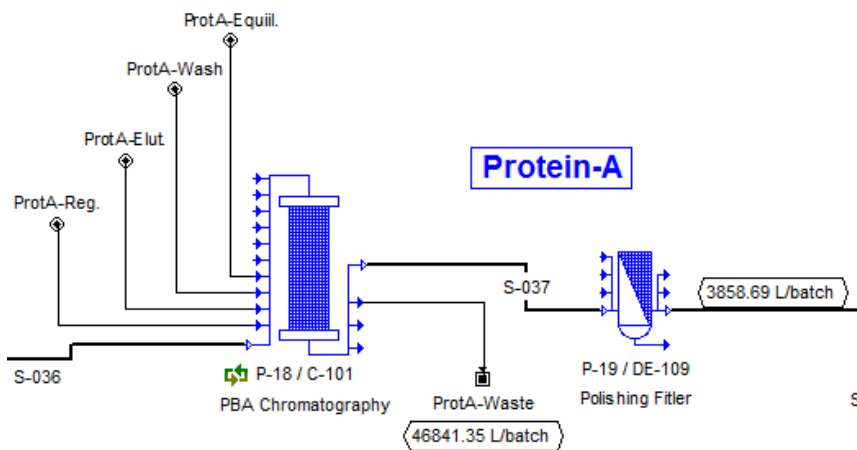
We have worked hard to build it, like we saw, we used plastic pipes, the mechanics tools, cash cables ...

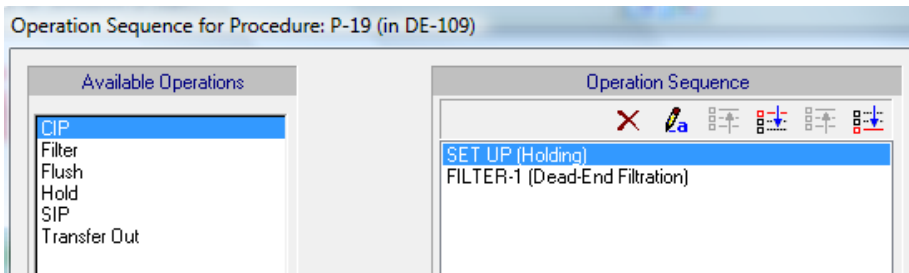
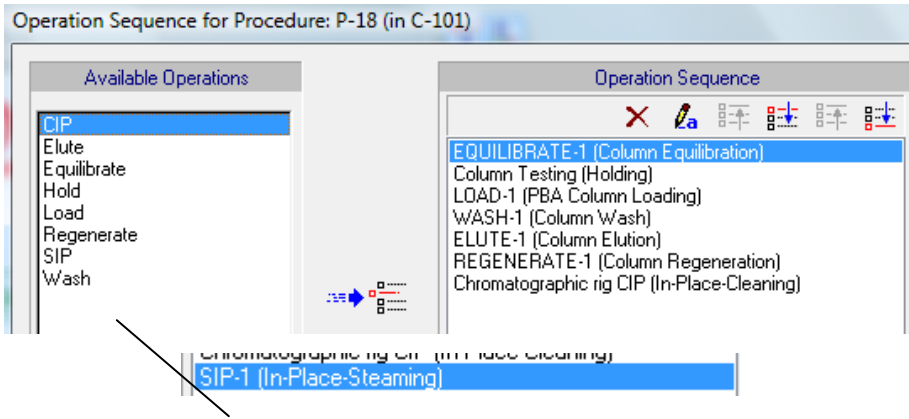
Finally getting the test stand:



Figure 39 : The test stand ready for work.

6.4 Functional Operation Algorithm of Automation System





Now we have created an algorithm that represents the production of the vaccine process by process plus the valve necessary and the order of open/close turn on/off for the process and we have based on this algorithm to make the full automation of the system of production of the vaccine by control the fluid flow, so we have based on this algorithm to collect on Python^[8] a code that with it "Turn On" the system to start the production, so with the algorithm we have created the Python code that we need for the automation process and it's done, ready to test, just click on "Turn On".

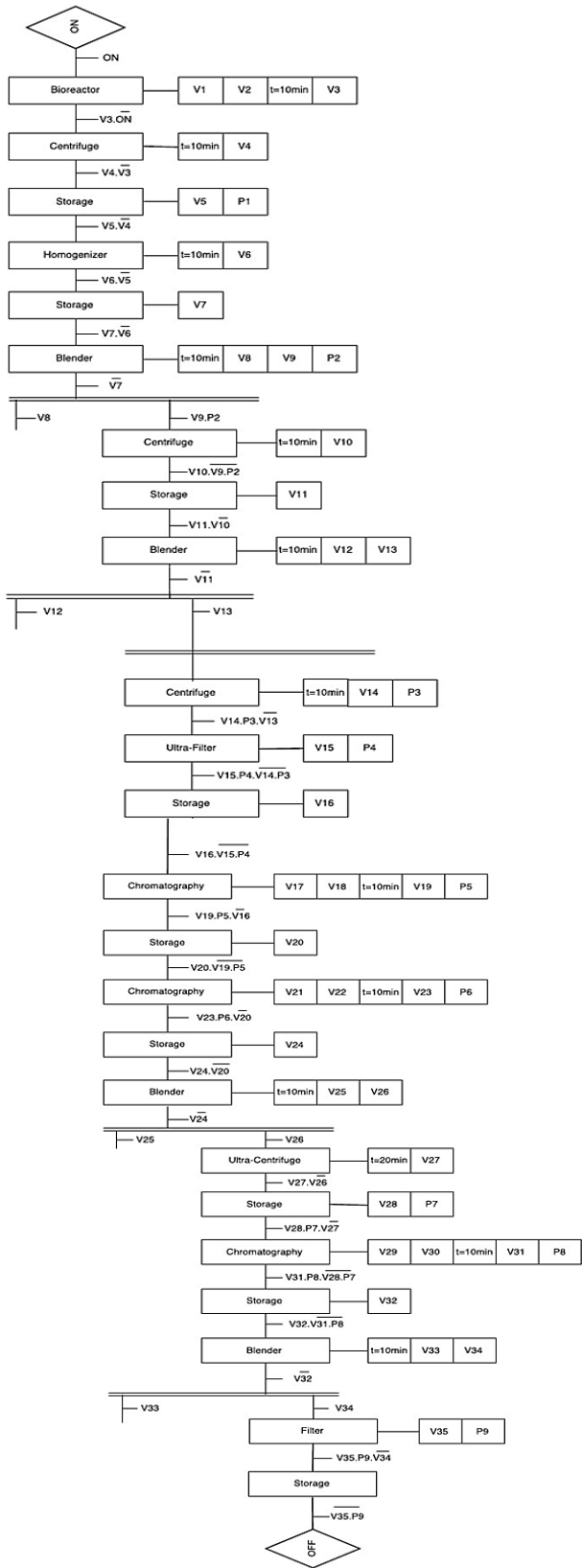
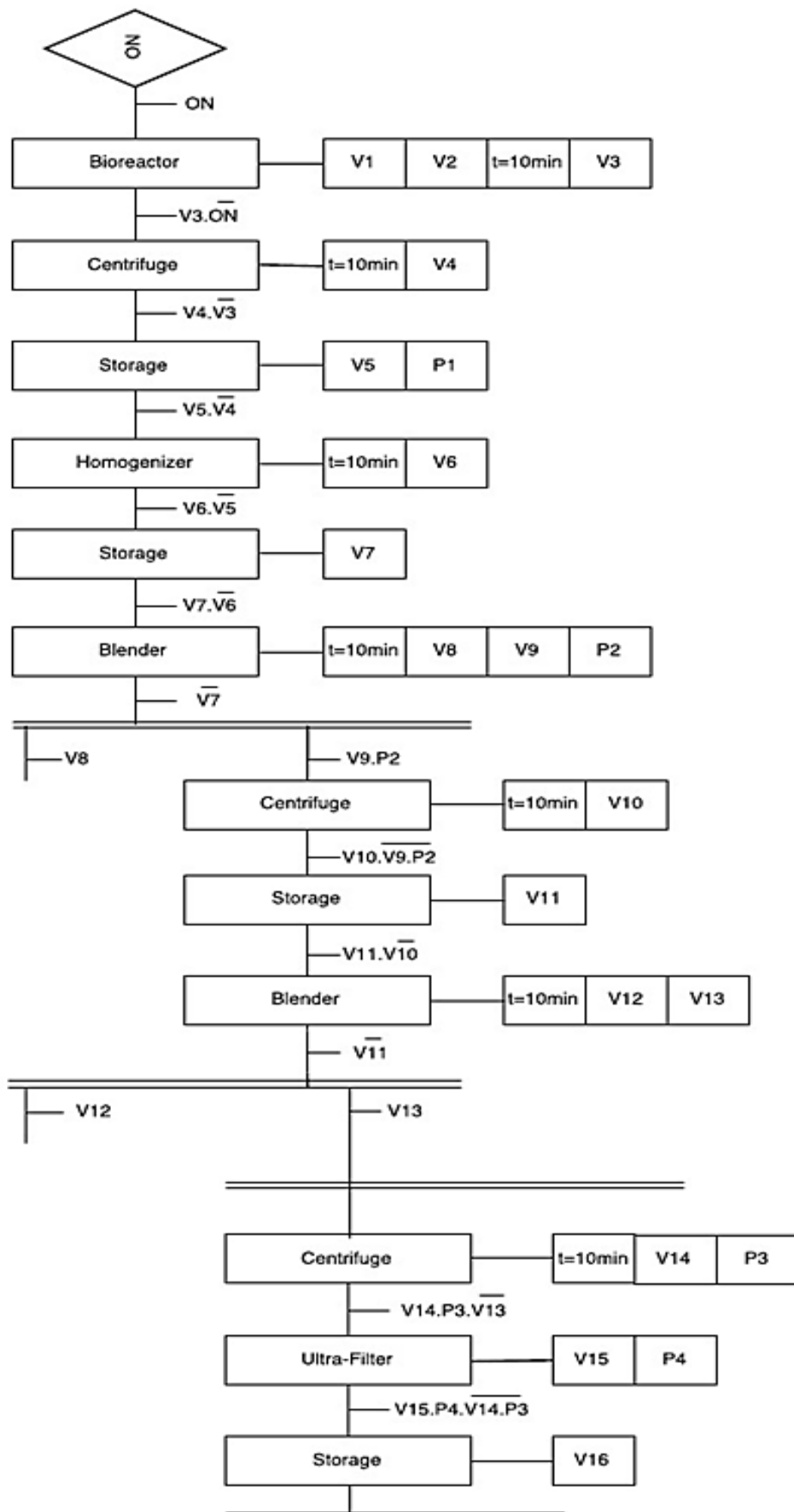


Figure 2.40a : Algorithm represents the automation of the production (Overview)



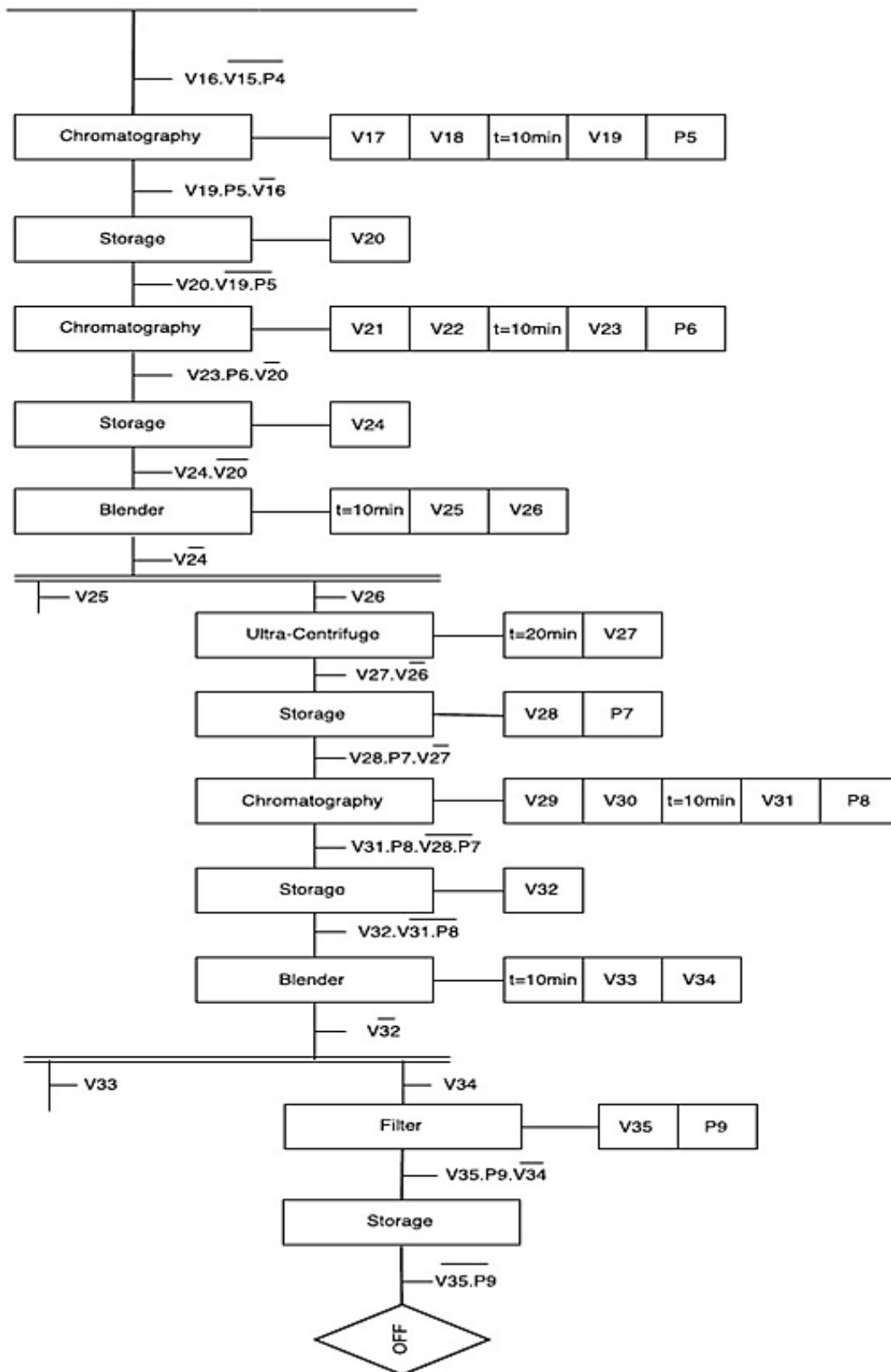


Figure 2.41b : Algorithm represents the automation of the production (as in overview, but two pictures)
 After creating that algorithm, we have moved to the test stand, the idea of this step is to build a part of the PI diagram of the MEGBI-VPP Processing and testing it ,so we have to choose the circuit that we will based on it build the test stand. By a website [9] we have created the diagram that represents this part. We will test it by the board, valve, pump, and the control screen.

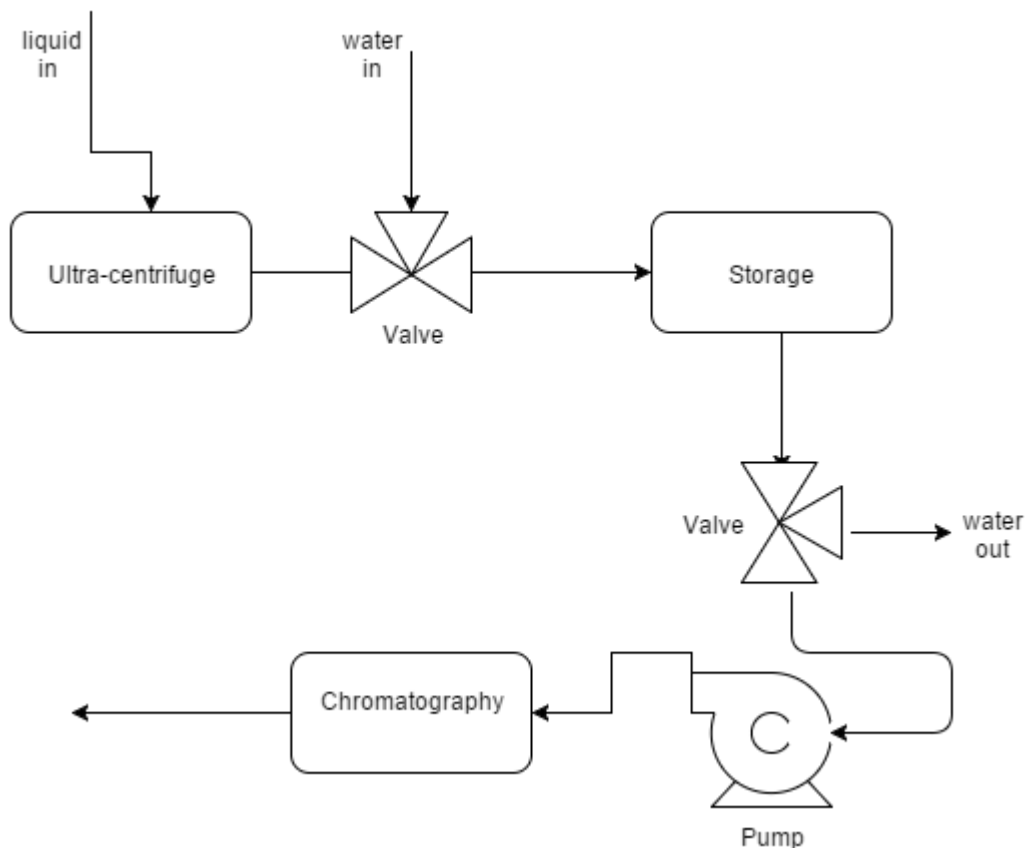


Figure 2.42 : *Diagram for the part of MEGBI-VPP Processing used.*

The liquid enter to ultra-centrifuge and continue by a valve to Storage, then to Chromatography by valve and pump (figure 2.11). This liquid represents the production and this is the part that we have chosen to base on it to build our test stand. We saw in the way a line to water-in that line is to clean the storage, and more the line of water-out is to get rid of the dirty water. With this Diagram we have created a Schema represents the test stand that we will build and each machine, instruments have to use (figure 2.12).

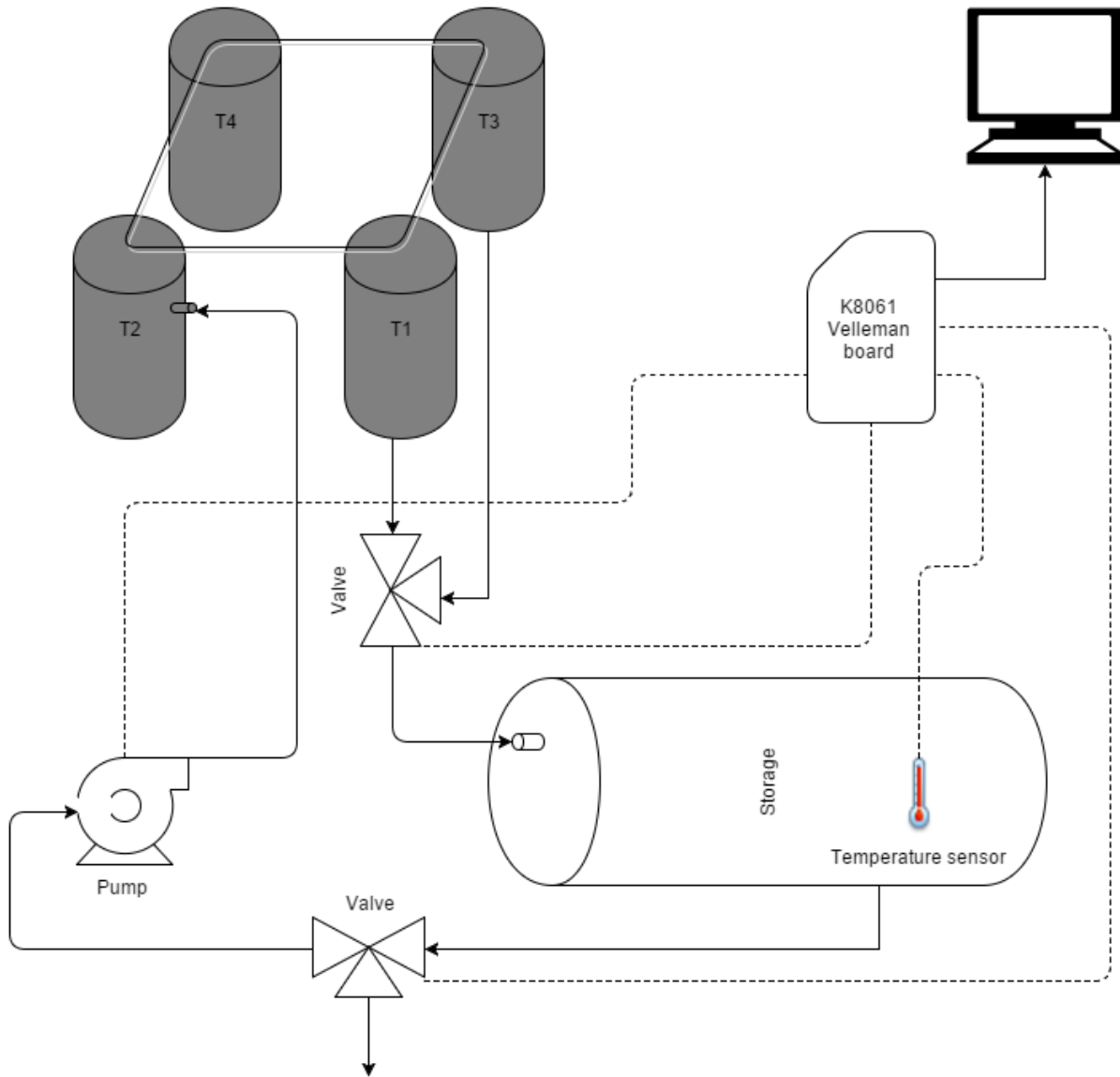


Figure 2.43 : Schema for the part of the MEGBI-VPP Processing used.

The contents of this schema:

- T1 represent the ultra-centrifuge.
- T2 represent the Chromatography.
- T3 represent the Storage of water used for cleaning.
- T4 is not used.
- USB Velleman K8061 board connected to laptop.

This schema is a miniature of the really circuit that we will build a really one like it.



Figure 2.44 : Test stand.

As we saw in schema we have built the test stand (figure 2.13). 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 valve too, T4 is not used. On the other we have linked the storage to valve, the valve to pump and pump to T2, here we have used a pump cause we want to send the production to T3 and T3 is located at a height of 2 meters We have worked hard to build it, like we saw we use plastic pipes, the mechanics tools, instruments, cash cable ...



Figure 6.14 a: The test stand after build it.



Figure 6.14b: Installed Electrical Control Cabinet

7. Testing the automation system

We have divided our work to two parts of results, the first one will examine all the actuators in the test stand to see if there is any problem or to see if everything is all right, it has been working on three tests by trying to control the actuators on the test stand from the control screen, then the other will examine the full automation system of the production of the vaccine by testing the Python code that based on algorithm to do this automation and control all the process of the production.

7.1 Testing the Test Stand

We start with the test of the test stand we made three tests:

7.1.1 Test 1

On this test, we will examine the valve by sending water from the ultra-centrifuge to the storage and will exercise control over them and see if we can introduce water into the storage when we open the valve by pressing the button of valve on control 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 other click to close it.



Figure 3.1 : The Test 1 concerning open and close a valve.

| 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 |

7.1.2 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 haved a low pressure,plus the Chromatography exist at high so must use pump to increase pressure.By pressing the button of valve with pump on the control 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 3.2: The test 2 concerning open/close valve turn on/off pump.

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

| | | | |
|--|------------------------------|-------------------------------|--|
| | TURNING ON AND OFF THE PUMP. | VALVE CLOSE AND PUMP TURN OFF | |
|--|------------------------------|-------------------------------|--|

7.1.3 Test 3:

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



Figure 3.3: The Test 3 concerning open/close 2 valves and turn on/off pump.

| 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 |

7.2 Testing the full automation of the MEGBI-VPP Processing:

On this part we will test the automation system that we have created before and get the results about it, this automation is for the production only so they have the valves and pumps

necessary for it , we not including the valves necessary to cleaning.so as we see on algorithm before we have 35 valves 9 pumps so we needed 42 outputs ,divided on 3 boards each one have 16 outputs(digital analogue),but we will test the automation on board and screen cause we haven't the 35 valves 9 pumps so test the automation system by the board and the screen is enough cause that mean we will test the outputs and it's enough. Now we will shows all the results on list of pictures started by turning on the system by click on "Turn on":

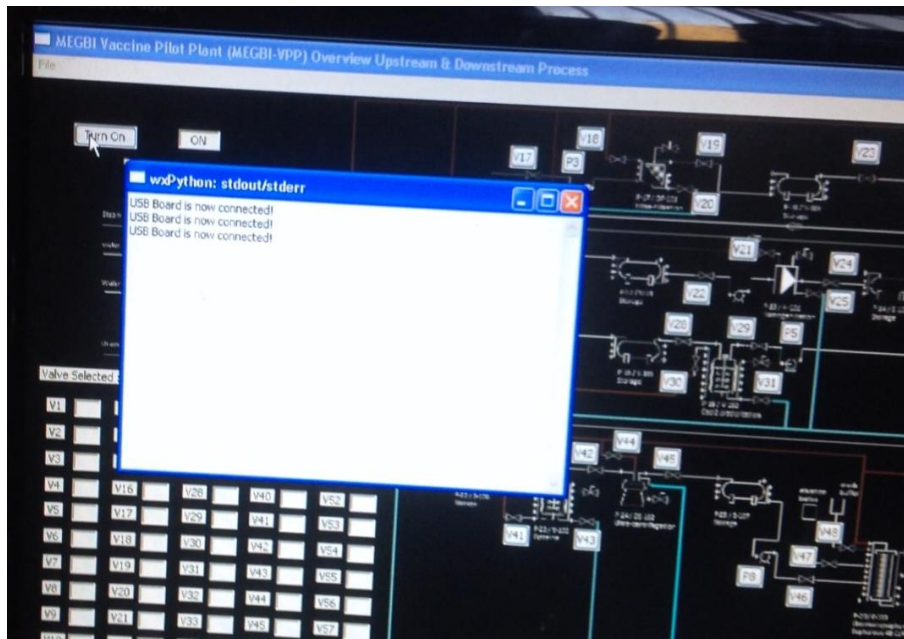


Figure 3.4: The test of the automation system by Turn on.

With clicking on "Turn On", the system is start (figure 3.4), and the full automation is started to test, this full automation can gives to us the controls of the fluid flow.

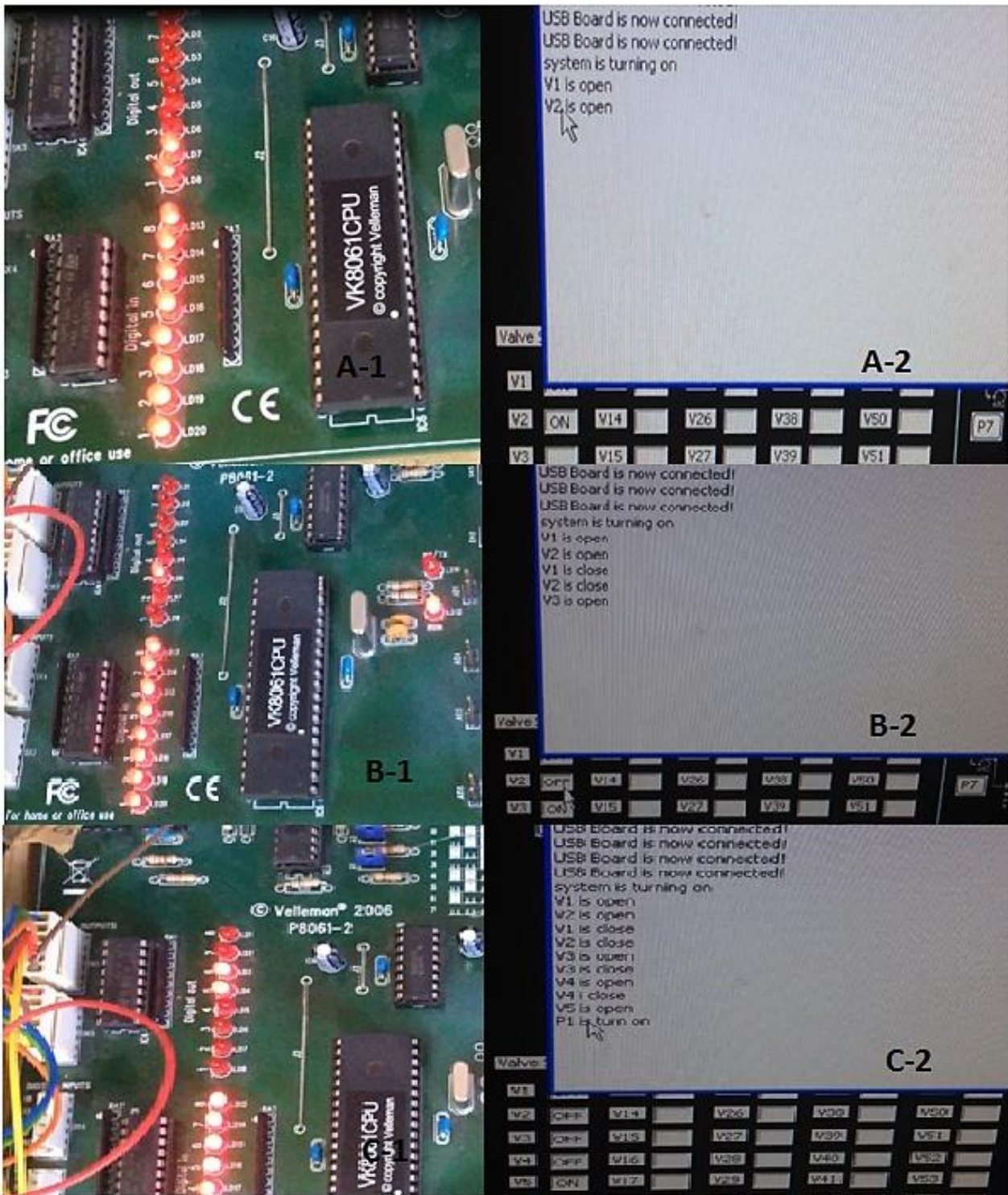


Figure 3.5: The test of the full automation system step A,B and C.

The automation is working good, as we see (figure 3.5) the valve V1 is open and the red light on board mean that , so when it close this red light will disappeared and that what we shows in this picture

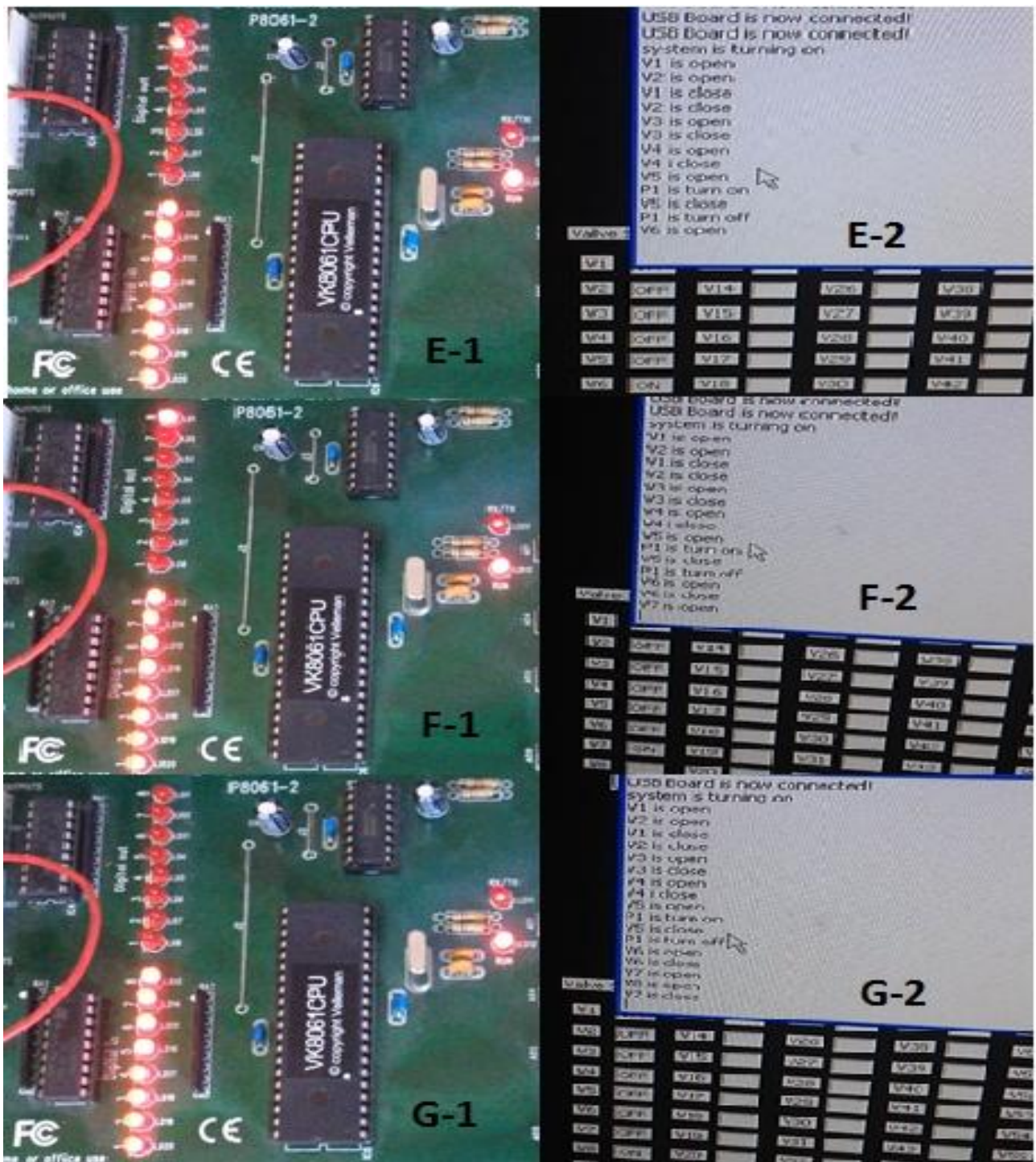


Figure 3.6: The test of the full automation system step E,F and G.

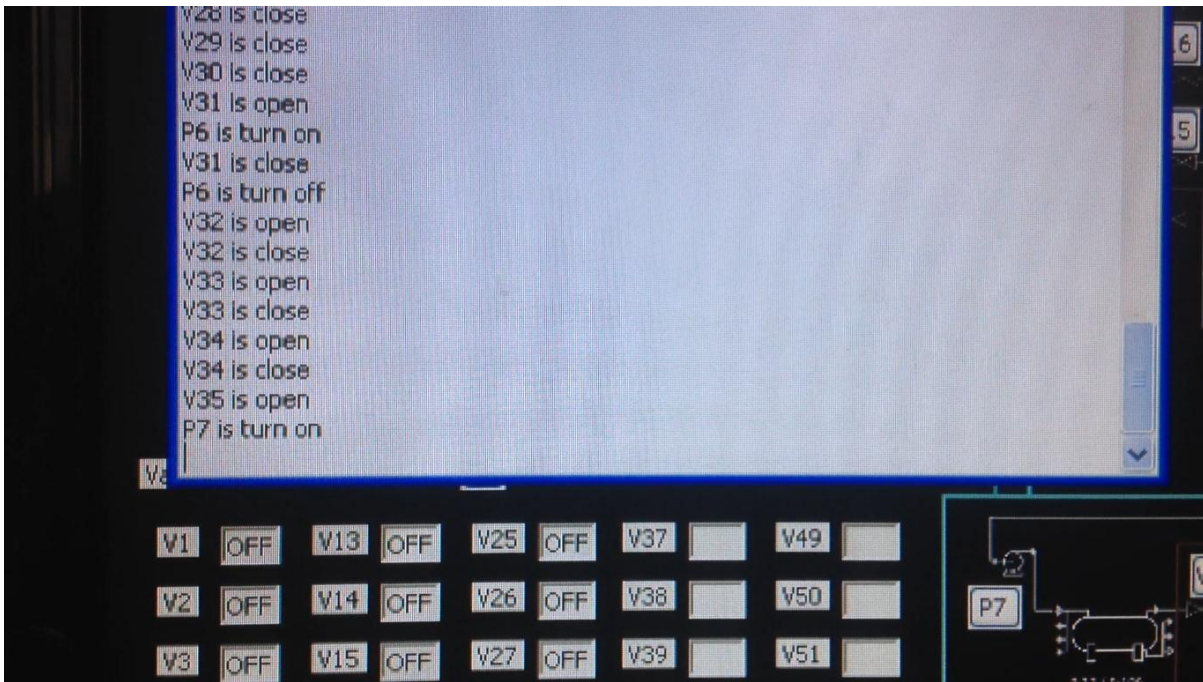
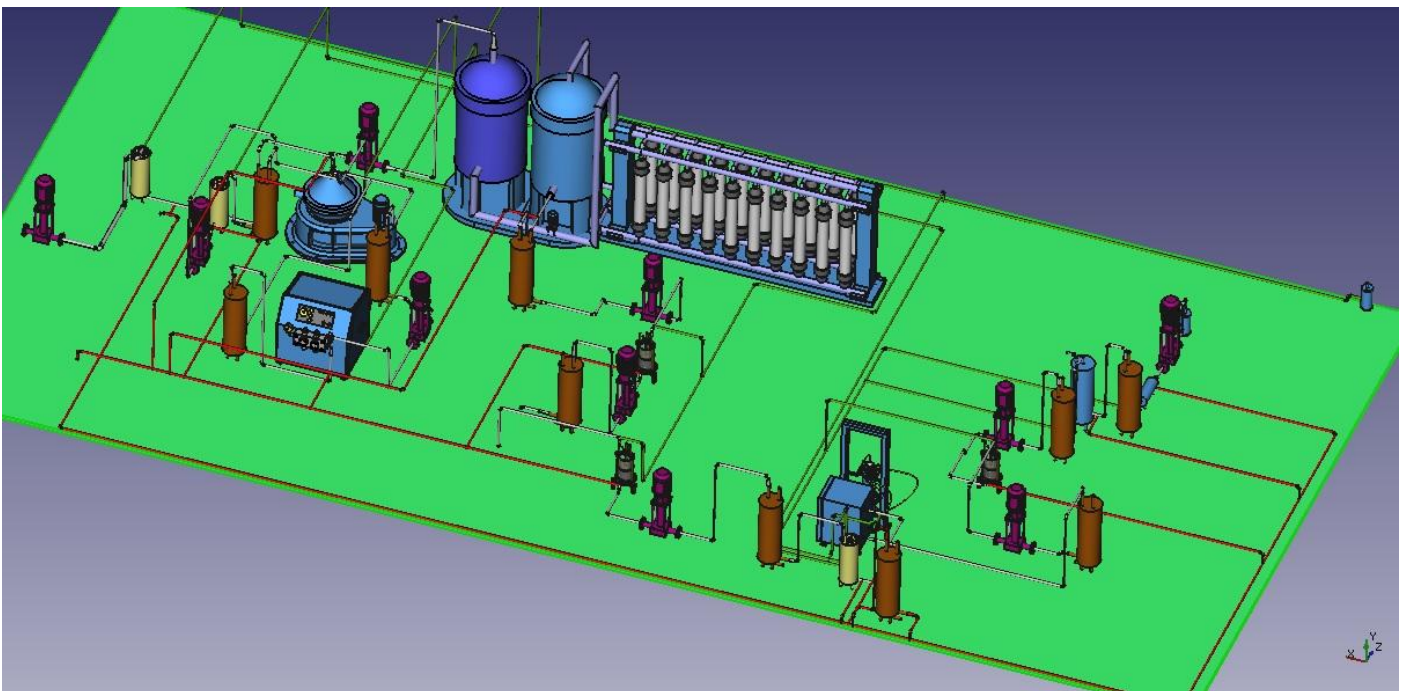
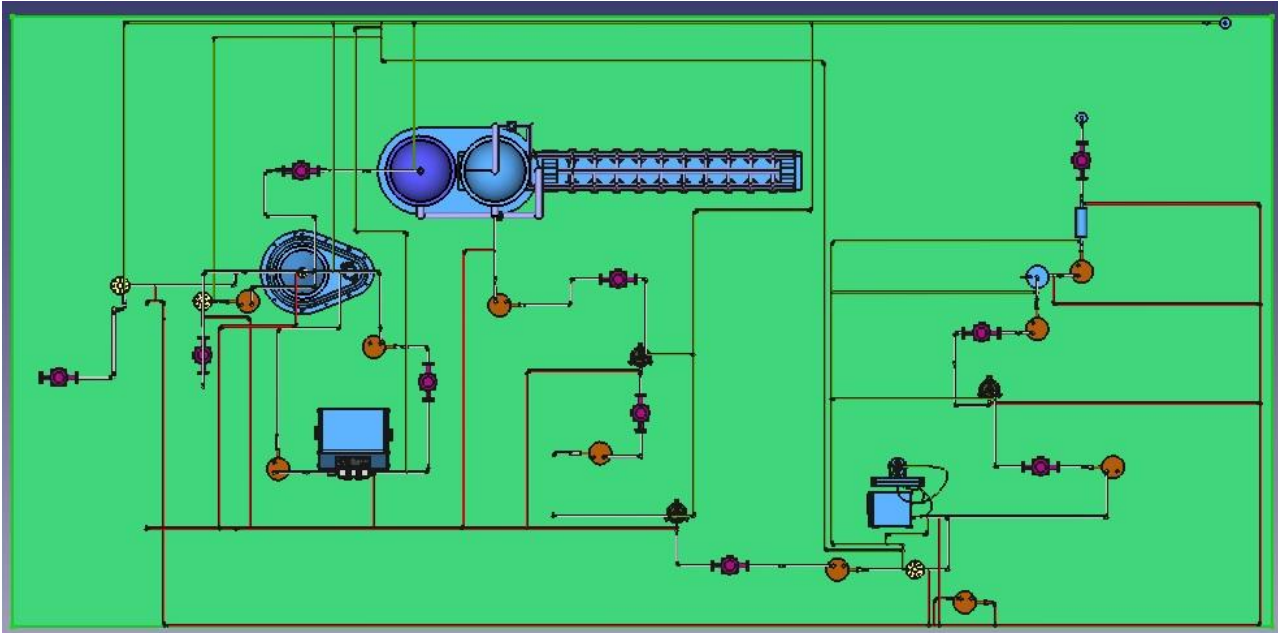


Figure 3.7: The test of the automation system by last step.

The system continues the step to finish at all the step of production and they automatically turned off, so the full automation is working and the test is succeed.

8. Integration of MEGBI-VPP based on Budget Plan for completing a minimal Prototype Plant

8.1 Overview



This version (minimal Prototype Plant) includes:

- smaller vessels











- simple disc stack centrifuge

- cost reduction option: vessel with chemical opening of cells instead of homogenizer

- disc stack centrifuge instead of ultracentrifuge

- main new manufacturing: chromatographic columns (requires fine mechanical manufacturing facility)

8.2 Cost Planning November 15

| Downstream Processing | # of mechanical pieces | average complexity/cost of part | | price per piece | pieces needed | Costs | |
|---------------------------|--|---------------------------------|--|--|---------------|------------------------------------|---------|
| Disc Stack Centrifuge | 20 | \$70 |  |  | \$1,400 | 1 \$1,400 | |
| Homogenizer | 10 | \$120 |  |  | \$1,200 | 1 \$1,200 | |
| Chromatogr. Column (mech) | 8 | \$50 |  |  Operation Sequence ELUTE-1 (Column Elution) Column Testing (Holding) LOAD-1 (PBA Column Loading) WASH-1 (Column Wash) ELUTE-1 (Column Elution) Stop (Column Regeneration) Rinse (Column Wash) Chromatographic iQ DP (In-Place Cleaning) | \$400 | 3 \$1,200 | |
| Ultrafiltration Unit | | |  | Operation Sequence EQUILIBRATE-1 (Column Equilibration) Column Testing (Holding) LOAD-1 (PBA Column Loading) WASH-1 (Column Wash) ELUTE-1 (Column Elution) Stop (Column Regeneration) Rinse (Column Wash) Chromatographic iQ DP (In-Place Cleaning) | \$200 | 1 \$200 | |
| Ultracentrifuge (used) | | \$70 |  | | \$1,400 | 1 \$1,400 | |
| Mixing/Storing tanks | | |  | Operation Sequence EQUILIBRATE-1 (Column Equilibration) Column Testing (Holding) LOAD-1 (PBA Column Loading) WASH-1 (Column Wash) ELUTE-1 (Column Elution) Stop (Column Regeneration) Rinse (Column Wash) Chromatographic iQ DP (In-Place Cleaning) | \$60 | 14 \$840 | |
| | | | | | | Total Devices | \$6,240 |
| | | | | | | Integration (piping, ...) 1/2 inch | \$1,500 |
| Automation System | | |  | | | | |
| Item | price per piece | # of pieces | | | | | |
| pump | \$60 | 10 | | | | | |
| electronic valve | \$50 | 58 | | | | | |
| | Total price | \$3,500 | | | | | |
| | Mechanical Parts USP | \$0 | | | | | |
| | Mechanical Parts DSP | \$7,740 | | | | | |
| | Automation System | \$3,500 | | | | | |
| | Total MEGBI-VPP Rest Budget Requirement | \$11,240 | | | | | |

Alternative was:

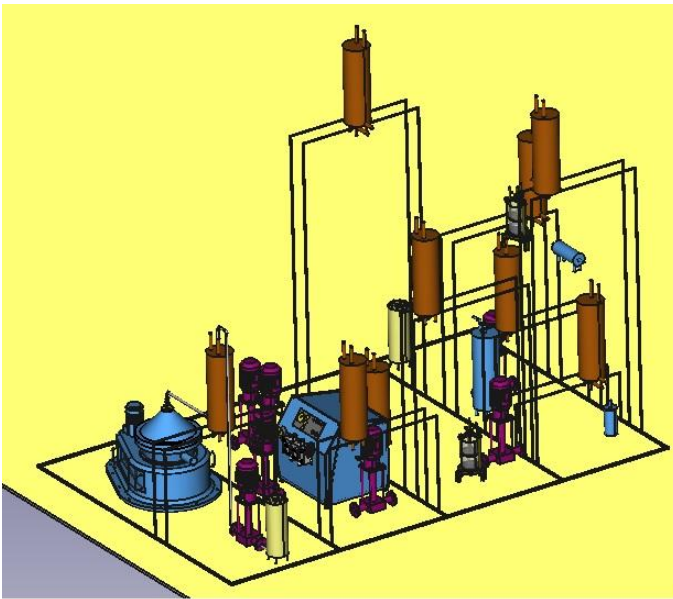
- Manufacturing of Devices and Integration. **Budget: 20.000 USD** (Material Costs: 10.000 USD, Personnel Cost: 4 months welder: 4000 USD, 4 months Eng.: 6000 USD)

Planned for Nov 15 - Feb 2016

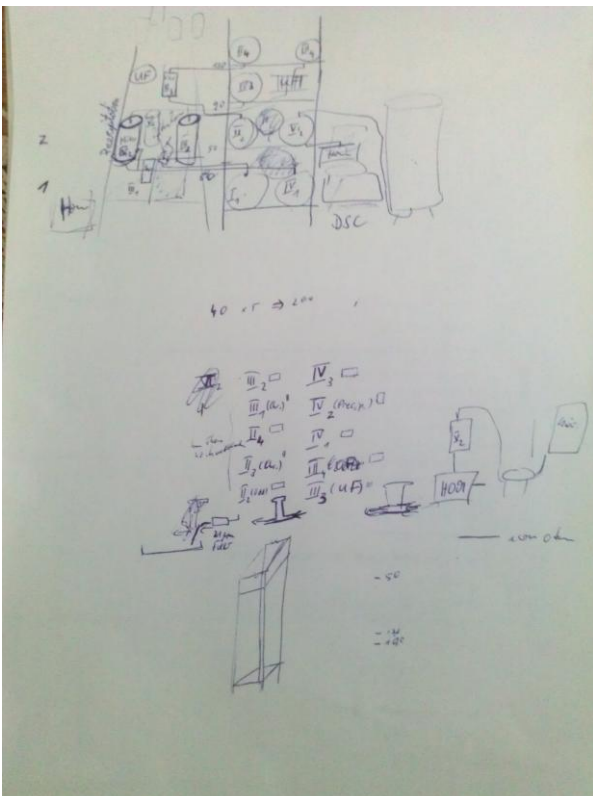
- Integration of Automation System to the whole plant (**Material Budget: 10 000 USD**),

Planned for Mar-Aug 16 (as Master Thesis)

8.3 Manufacturing Planning of minimal system



8.3.1 Package 1: vessels for storing and mixing



Placing of storages based on flowdiagram

Costs: 1500\$

8.3.2 Package 2: Chromatographic Columns, Disc Stack Centrifuge, Homogenizer

Costs: 1400\$

8.3.3 Package 3: Pumps & Valves

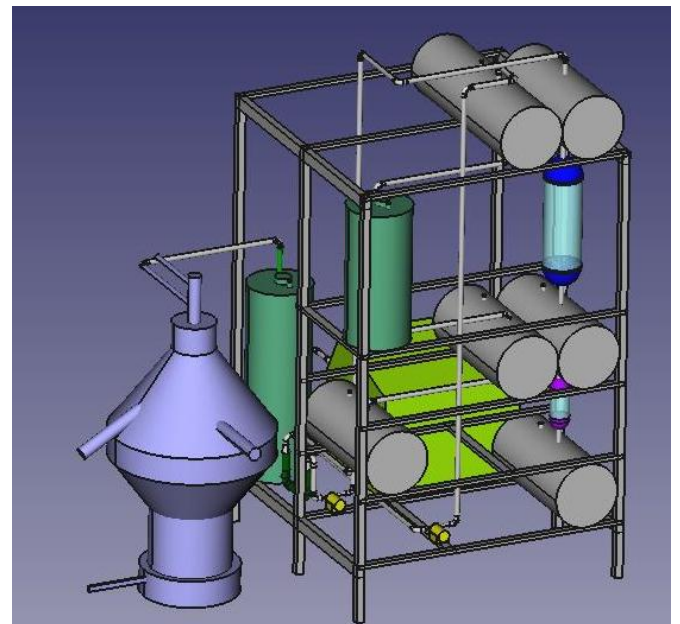
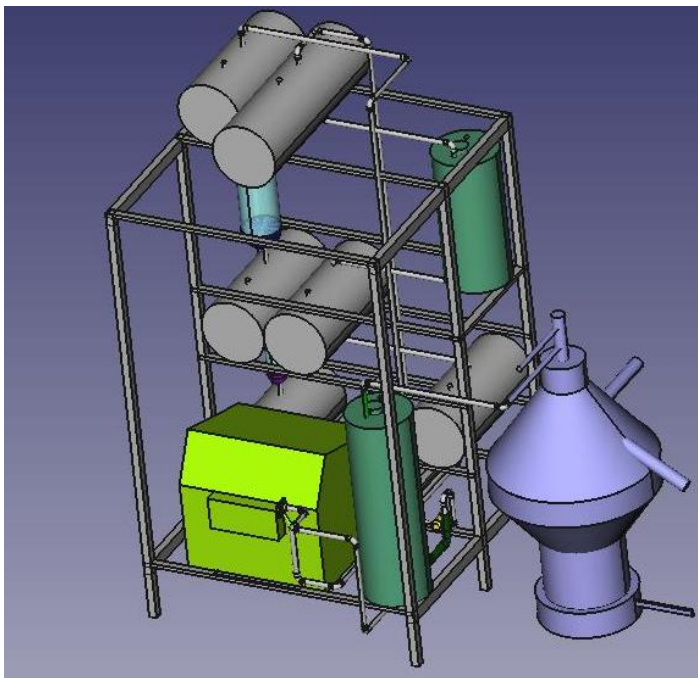
Costs: 3500\$

8.3.1 Package 4: Piping

Costs: 1500\$

8.4 Manufactured 24.12.-30.12.2015 (based on minimal system)

8.4.1 Design



8.4.2 Costs

1 sheet stainless steel 316: 1m x2m x 2mm -> about 150\$

1 sheet stainless steel 304: 1m x2m x 2mm -> about 100\$

stage material: 6m x 38 mm x 38 mm -> 35\$

| Material | Staff |
|---|---|
| storage & mixing tanks (stainless steel 316) and metal stage (stainless steel) -> about 850 \$ 4 rolls (15\$) 1 pump stainless steel 1,5 bar (55\$) | Welder (Mohammad Qammah) for 7 days (all in 315 \$) |

There were total costs of 1230 \$.













8.5 Still missing (to be manufactured/buyed in 2016 insha Allah)

pipng

disc stack centrifuge

homogenizer

2 columns

Adaption of automation system

For manufacturing disc stack centrifuge and homogenizer a CNC machine is needed.

9. Annex

9.1 Annex 1a: How to install Python

- download python from www.python.org , we downloading python 3.5 version for windows 8
- install Python by run the program and continue the steps.

Needed a series of packages:

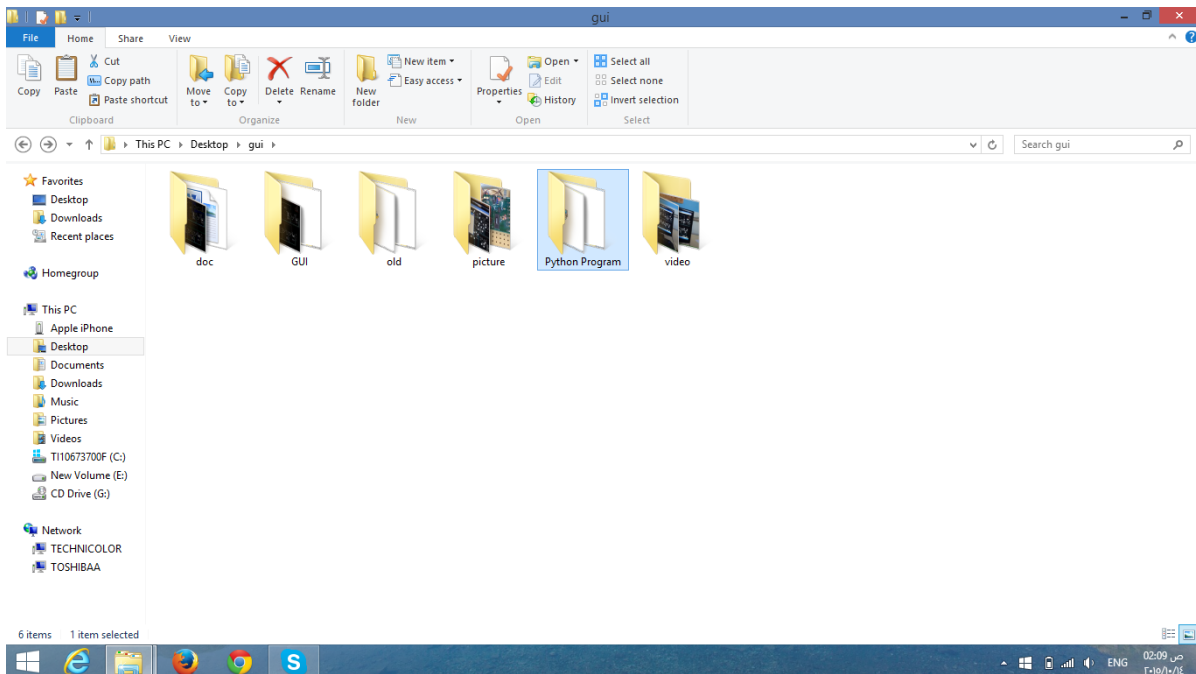
- Numpy-1.9.2
- Py2exe
- PyDDE
- wxPython-3.0.2.0

To install each package u should to open a command and write:

“C:\location of your python\Scripts\Pip install package name.”

We have putted a CD with this document

concerning:



9.2 Annex 1b: Connecting Veleman Board, Drivers

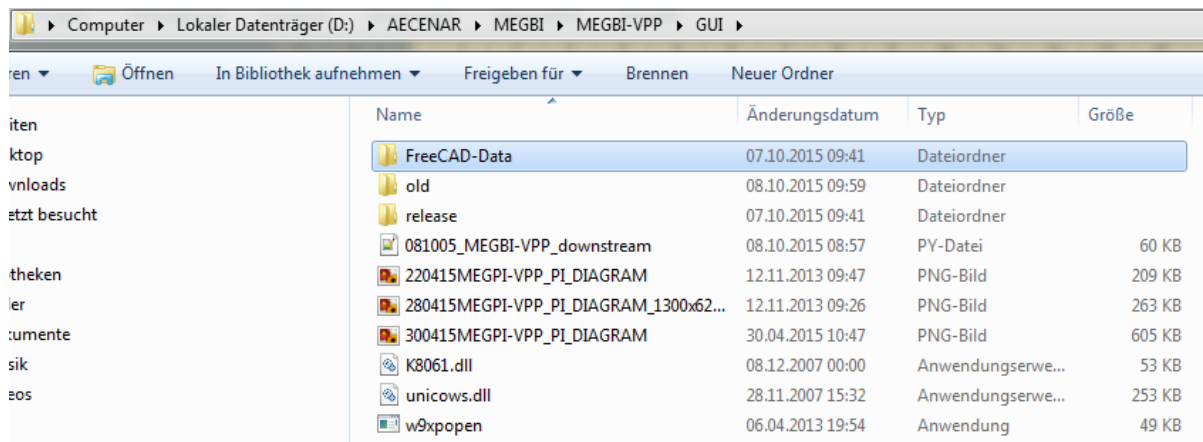
For the veleman board there are needed drivers. These drivers are only compatible to Windows XP, not to Windows 7. Following files are needed:

K8061.dll

unicows.dll

w9xpopen

The drivers must be in the same folder as the .py file.



The screenshot shows a Windows File Explorer window with the following path: Computer > Lokaler Datenträger (D:) > AECENAR > MEGBI > MEGBI-VPP > GUI. The window title is 'GUI'. The main pane displays a list of files and folders with columns for Name, Änderungsdatum, Typ, and Größe. The files listed are:

| Name | Änderungsdatum | Typ | Größe |
|---------------------------------------|------------------|-------------------|--------|
| FreeCAD-Data | 07.10.2015 09:41 | Dateiordner | |
| old | 08.10.2015 09:59 | Dateiordner | |
| release | 07.10.2015 09:41 | Dateiordner | |
| 081005_MEGBI-VPP_downstream | 08.10.2015 08:57 | PY-Datei | 60 KB |
| 220415MEGPI-VPP_PI_DIAGRAM | 12.11.2013 09:47 | PNG-Bild | 209 KB |
| 280415MEGPI-VPP_PI_DIAGRAM_1300x62... | 12.11.2013 09:26 | PNG-Bild | 263 KB |
| 300415MEGPI-VPP_PI_DIAGRAM | 30.04.2015 10:47 | PNG-Bild | 605 KB |
| K8061.dll | 08.12.2007 00:00 | Anwendungserwe... | 53 KB |
| unicows.dll | 28.11.2007 15:32 | Anwendungserwe... | 253 KB |
| w9xpopen | 06.04.2013 19:54 | Anwendung | 49 KB |

9.3 Annex 2a: python automation code only in maintance mode (without full automation)

```
#!/usr/bin/env python
## -*- coding: iso-8859-15 -*-
import sys
from tkinter import *
import random
import time
import wx
import thread

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

BACKGROUND_IMAGENAME = "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)

class MyFrame(wx.Frame):

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

        self.testUSB          = True
        self.dll               = None
        self.USBAdr0          = 0
        self.USBOpened        = False
        self.counterUSBBoards = 3

        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)
```

```

# 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()

#Valves

#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)

#V2
self.button_V_2 = wx.Button(panel, -1, "V2", pos=(150,90),size=(20,20))
self.Bind(wx.EVT_BUTTON, self.V2, self.button_V_2)

#V3
self.button_V_3 = wx.Button(panel, -1, "V3", pos=(150,135),size=(20,20))
self.Bind(wx.EVT_BUTTON, self.V3, self.button_V_3)

#V4
self.button_V_4 = wx.Button(panel, -1, "V4", pos=(150,265),size=(20,20))
self.Bind(wx.EVT_BUTTON, self.V4, self.button_V_4)

#V5
self.button_V_5 = wx.Button(panel, -1, "V5", pos=(210,135),size=(20,20))
self.Bind(wx.EVT_BUTTON, self.V5, self.button_V_5)

#V6
self.button_V_6 = wx.Button(panel, -1, "V6", pos=(215,185),size=(20,20))
self.Bind(wx.EVT_BUTTON, self.V6, self.button_V_6)

#V7
self.button_V_7 = wx.Button(panel, -1, "V7", pos=(215,220),size=(20,20))
self.Bind(wx.EVT_BUTTON, self.V7, self.button_V_7)

#V8
self.button_V_8 = wx.Button(panel, -1, "V8", pos=(282,180),size=(25,20))
self.Bind(wx.EVT_BUTTON, self.V8, self.button_V_8)

#V9
self.button_V_9 = wx.Button(panel, -1, "V9", pos=(282,158),size=(25,20))
self.Bind(wx.EVT_BUTTON, self.V9, self.button_V_9)

#V10
self.button_V_10 = wx.Button(panel, -1, "V10", pos=(322,110),size=(25,20))
self.Bind(wx.EVT_BUTTON, self.V10, self.button_V_10)

#V11
self.button_V_11 = wx.Button(panel, -1, "V11", pos=(380,250),size=(25,20))
self.Bind(wx.EVT_BUTTON, self.V11, self.button_V_11)

#V12
self.button_V_12 = wx.Button(panel, -1, "V12", pos=(413,195),size=(25,20))
self.Bind(wx.EVT_BUTTON, self.V12, self.button_V_12)

#V13
self.button_V_13 = wx.Button(panel, -1, "V13", pos=(380,130),size=(25,20))
self.Bind(wx.EVT_BUTTON, self.V13, self.button_V_13)

#V14
self.button_V_14 = wx.Button(panel, -1, "V14", pos=(410,100),size=(25,20))
self.Bind(wx.EVT_BUTTON, self.V14, self.button_V_14)

```

```

        #V15
self.button_V_15 = wx.Button(panel, -1, "V15", pos=(460,112),size=(25,20))
self.Bind(wx.EVT_BUTTON, self.V15, self.button_V_15)

        #V16
self.button_V_16 = wx.Button(panel, -1, "V16", pos=(460,73),size=(25,20))
self.Bind(wx.EVT_BUTTON, self.V16, self.button_V_16)

        #V17
self.button_V_17 = wx.Button(panel, -1, "V17", pos=(460,38),size=(25,20))
self.Bind(wx.EVT_BUTTON, self.V17, self.button_V_17)

        #V18
self.button_V_18 = wx.Button(panel, -1, "V18", pos=(522,18),size=(25,20))
self.Bind(wx.EVT_BUTTON, self.V18, self.button_V_18)

        #V19
self.button_V_19 = wx.Button(panel, -1, "V19", pos=(635,18),size=(25,20))
self.Bind(wx.EVT_BUTTON, self.V19, self.button_V_19)

        #V20
self.button_V_20 = wx.Button(panel, -1, "V20", pos=(635,72),size=(25,20))
self.Bind(wx.EVT_BUTTON, self.V20, self.button_V_20)

        #V21
self.button_V_21 = wx.Button(panel, -1, "V21", pos=(675,112),size=(25,20))
self.Bind(wx.EVT_BUTTON, self.V21, self.button_V_21)

        #V22
self.button_V_22 = wx.Button(panel, -1, "V22", pos=(635,155),size=(25,20))
self.Bind(wx.EVT_BUTTON, self.V22, self.button_V_22)

        #V23
self.button_V_23 = wx.Button(panel, -1, "V23", pos=(780,18),size=(25,20))
self.Bind(wx.EVT_BUTTON, self.V23, self.button_V_23)

        #V24
self.button_V_24 = wx.Button(panel, -1, "V24", pos=(770,120),size=(25,20))
self.Bind(wx.EVT_BUTTON, self.V24, self.button_V_24)

        #V25
self.button_V_25 = wx.Button(panel, -1, "V25", pos=(770,155),size=(25,20))
self.Bind(wx.EVT_BUTTON, self.V25, self.button_V_25)

        #V26
self.button_V_26 = wx.Button(panel, -1, "V26", pos=(870,155),size=(25,20))
self.Bind(wx.EVT_BUTTON, self.V26, self.button_V_26)

        #V27
self.button_V_27 = wx.Button(panel, -1, "V27", pos=(510,185),size=(25,20))
self.Bind(wx.EVT_BUTTON, self.V27, self.button_V_27)

        #V28
self.button_V_28 = wx.Button(panel, -1, "V28", pos=(620,185),size=(25,20))
self.Bind(wx.EVT_BUTTON, self.V28, self.button_V_28)

        #V29
self.button_V_29 = wx.Button(panel, -1, "V29", pos=(680,185),size=(25,20))
self.Bind(wx.EVT_BUTTON, self.V29, self.button_V_29)

        #V30
self.button_V_30 = wx.Button(panel, -1, "V30", pos=(620,240),size=(25,20))
self.Bind(wx.EVT_BUTTON, self.V30, self.button_V_30)

        #V31
self.button_V_31 = wx.Button(panel, -1, "V31", pos=(710,235),size=(25,20))
self.Bind(wx.EVT_BUTTON, self.V31, self.button_V_31)

```

```

#V32
self.button_V_32 = wx.Button(panel, -1, "V32", pos=(950,182),size=(25,20))
self.Bind(wx.EVT_BUTTON, self.V32, self.button_V_32)

#V33
self.button_V_33 = wx.Button(panel, -1, "V33", pos=(977,182),size=(25,20))
self.Bind(wx.EVT_BUTTON, self.V33, self.button_V_33)

#V34
self.button_V_34 = wx.Button(panel, -1, "V34", pos=(1083,188),size=(25,20))
self.Bind(wx.EVT_BUTTON, self.V34, self.button_V_34)

#V35
self.button_V_35 = wx.Button(panel, -1, "V35", pos=(1083,220),size=(25,20))
self.Bind(wx.EVT_BUTTON, self.V35, self.button_V_35)

#V36
self.button_V_36 = wx.Button(panel, -1, "V36", pos=(1170,110),size=(25,20))
self.Bind(wx.EVT_BUTTON, self.V36, self.button_V_36)

#V37
self.button_V_37 = wx.Button(panel, -1, "V37", pos=(1200,110),size=(25,20))
self.Bind(wx.EVT_BUTTON, self.V37, self.button_V_37)

#V38
self.button_V_38 = wx.Button(panel, -1, "V38", pos=(1240,150),size=(25,20))
self.Bind(wx.EVT_BUTTON, self.V38, self.button_V_38)

#V39
self.button_V_39 = wx.Button(panel, -1, "V39", pos=(1273,205),size=(25,20))
self.Bind(wx.EVT_BUTTON, self.V39, self.button_V_39)

#V40
self.button_V_40 = wx.Button(panel, -1, "V40", pos=(483,314),size=(25,20))
self.Bind(wx.EVT_BUTTON, self.V40, self.button_V_40)

#V41
self.button_V_41 = wx.Button(panel, -1, "V41", pos=(478,398),size=(25,20))
self.Bind(wx.EVT_BUTTON, self.V41, self.button_V_41)

#V42
self.button_V_42 = wx.Button(panel, -1, "V42", pos=(540,314),size=(25,20))
self.Bind(wx.EVT_BUTTON, self.V42, self.button_V_42)

#V43
self.button_V_43 = wx.Button(panel, -1, "V43", pos=(548,398),size=(25,20))
self.Bind(wx.EVT_BUTTON, self.V43, self.button_V_43)

#V44
self.button_V_44 = wx.Button(panel, -1, "V44", pos=(580,300),size=(25,20))
self.Bind(wx.EVT_BUTTON, self.V44, self.button_V_44)

#V45
self.button_V_45 = wx.Button(panel, -1, "V45", pos=(620,314),size=(25,20))
self.Bind(wx.EVT_BUTTON, self.V45, self.button_V_45)

#V46
self.button_V_46 = wx.Button(panel, -1, "V46", pos=(760,440),size=(25,20))
self.Bind(wx.EVT_BUTTON, self.V46, self.button_V_46)

#V47
self.button_V_47 = wx.Button(panel, -1, "V47", pos=(760,400),size=(25,20))
self.Bind(wx.EVT_BUTTON, self.V47, self.button_V_47)

#V48
self.button_V_48 = wx.Button(panel, -1, "V48", pos=(783,375),size=(25,20))
self.Bind(wx.EVT_BUTTON, self.V48, self.button_V_48)

#V49
self.button_V_49 = wx.Button(panel, -1, "V49", pos=(880,428),size=(25,20))
self.Bind(wx.EVT_BUTTON, self.V49, self.button_V_49)

#V50
self.button_V_50 = wx.Button(panel, -1, "V50", pos=(970,377),size=(25,20))
self.Bind(wx.EVT_BUTTON, self.V50, self.button_V_50)

#V51
self.button_V_51 = wx.Button(panel, -1, "V51", pos=(963,450),size=(25,20))
self.Bind(wx.EVT_BUTTON, self.V51, self.button_V_51)

#V52
self.button_V_52 = wx.Button(panel, -1, "V52", pos=(1005,470),size=(25,20))
self.Bind(wx.EVT_BUTTON, self.V52, self.button_V_52)

```



```

        #V52
self.button_V_52 = wx.Button(panel, -1, "V52", pos=(1005,470),size=(25,20))
self.Bind(wx.EVT_BUTTON, self.V52, self.button_V_52)
        #V53
self.button_V_53 = wx.Button(panel, -1, "V53", pos=(1030,395),size=(25,20))
self.Bind(wx.EVT_BUTTON, self.V53, self.button_V_53)
        #V54
self.button_V_54 = wx.Button(panel, -1, "V54", pos=(1080,395),size=(25,20))
self.Bind(wx.EVT_BUTTON, self.V54, self.button_V_54)
        #V55
self.button_V_55 = wx.Button(panel, -1, "V55", pos=(1118,377),size=(25,20))
self.Bind(wx.EVT_BUTTON, self.V55, self.button_V_55)
        #V56
self.button_V_56 = wx.Button(panel, -1, "V56", pos=(1158,468),size=(25,20))
self.Bind(wx.EVT_BUTTON, self.V56, self.button_V_56)
        #V57
self.button_V_57 = wx.Button(panel, -1, "V57", pos=(1190,420),size=(25,20))
self.Bind(wx.EVT_BUTTON, self.V57, self.button_V_57)
        #V58
self.button_V_58 = wx.Button(panel, -1, "V58", pos=(710,580),size=(25,20))
self.Bind(wx.EVT_BUTTON, self.V58, self.button_V_58)

        #Pumps

        #P1
self.button_P_1 = wx.Button(panel, -1, "P1", pos=(150,180),size=(20,20))
self.Bind(wx.EVT_BUTTON, self.P1, self.button_P_1)
        #P2
self.button_P_2 = wx.Button(panel, -1, "P2", pos=(150,220),size=(20,20))
self.Bind(wx.EVT_BUTTON, self.P2, self.button_P_2)
        #P3
self.button_P_3 = wx.Button(panel, -1, "P3", pos=(510,40),size=(20,20))
self.Bind(wx.EVT_BUTTON, self.P3, self.button_P_3)
        #P4
self.button_P_4 = wx.Button(panel, -1, "P4", pos=(880,18),size=(20,20))
self.Bind(wx.EVT_BUTTON, self.P4, self.button_P_4)|

        #P5
self.button_P_5 = wx.Button(panel, -1, "P5", pos=(730,187),size=(20,20))
self.Bind(wx.EVT_BUTTON, self.P5, self.button_P_5)

        #P6
self.button_P_6 = wx.Button(panel, -1, "P6", pos=(1083,130),size=(20,20))
self.Bind(wx.EVT_BUTTON, self.P6, self.button_P_6)

        #P7
self.button_P_7 = wx.Button(panel, -1, "P7", pos=(380,328),size=(25,20))
self.Bind(wx.EVT_BUTTON, self.P7, self.button_P_7)

        #P8
self.button_P_8 = wx.Button(panel, -1, "P8", pos=(710,423),size=(25,20))
self.Bind(wx.EVT_BUTTON, self.P8, self.button_P_8)

        #P9
self.button_P_9 = wx.Button(panel, -1, "P9", pos=(900,380),size=(25,20))
self.Bind(wx.EVT_BUTTON, self.P9, self.button_P_9)

        #P10
self.button_P_10 = wx.Button(panel, -1, "P10", pos=(1200,380),size=(25,20))
self.Bind(wx.EVT_BUTTON, self.P10, self.button_P_10)

```

: monitoring valve status

#Text column 1

```
wx.StaticText(panel,20," Valve Selected : ",(10,270))
wx.StaticText(panel,-1," V1 ",(18,300))
wx.StaticText(panel,-1," V2 ",(18,326))
wx.StaticText(panel,-1," V3 ",(18,352))
wx.StaticText(panel,-1," V4 ",(18,378))
wx.StaticText(panel,-1," V5 ",(18,402))
wx.StaticText(panel,-1," V6 ",(18,428))
wx.StaticText(panel,-1," V7 ",(18,454))
wx.StaticText(panel,-1," V8 ",(18,480))
wx.StaticText(panel,-1," V9 ",(18,506))
wx.StaticText(panel,-1," V10 ",(18,532))
wx.StaticText(panel,-1," V11 ",(18,558))
wx.StaticText(panel,-1," V12 ",(18,584))
```

#Text column 2

```
wx.StaticText(panel,-1," V13 ",(85,300))
wx.StaticText(panel,-1," V14 ",(85,326))
wx.StaticText(panel,-1," V15 ",(85,352))
wx.StaticText(panel,-1," V16 ",(85,378))
wx.StaticText(panel,-1," V17 ",(85,402))
wx.StaticText(panel,-1," V18 ",(85,428))
wx.StaticText(panel,-1," V19 ",(85,454))
wx.StaticText(panel,-1," V20 ",(85,480))
wx.StaticText(panel,-1," V21 ",(85,506))
wx.StaticText(panel,-1," V22 ",(85,532))
wx.StaticText(panel,-1," V23 ",(85,558))
wx.StaticText(panel,-1," V24 ",(85,584))
```

#Text column 3

```
wx.StaticText(panel,-1," V25 ",(155,300))
wx.StaticText(panel,-1," V26 ",(155,326))
wx.StaticText(panel,-1," V27 ",(155,352))
wx.StaticText(panel,-1," V28 ",(155,378))
wx.StaticText(panel,-1," V29 ",(155,402))
wx.StaticText(panel,-1," V30 ",(155,428))
wx.StaticText(panel,-1," V31 ",(155,454))
wx.StaticText(panel,-1," V32 ",(155,480))
wx.StaticText(panel,-1," V33 ",(155,506))
wx.StaticText(panel,-1," V34 ",(155,532))
wx.StaticText(panel,-1," V35 ",(155,558))
wx.StaticText(panel,-1," V36 ",(155,584))
```

#Text column 4

```
wx.StaticText(panel,-1," V37 ",(223,300))
wx.StaticText(panel,-1," V38 ",(223,326))
wx.StaticText(panel,-1," V39 ",(223,352))
wx.StaticText(panel,-1," V40 ",(223,378))
wx.StaticText(panel,-1," V41 ",(223,402))
wx.StaticText(panel,-1," V42 ",(223,428))
wx.StaticText(panel,-1," V43 ",(223,454))
wx.StaticText(panel,-1," V44 ",(223,480))
wx.StaticText(panel,-1," V45 ",(223,506))
wx.StaticText(panel,-1," V46 ",(223,532))
wx.StaticText(panel,-1," V47 ",(223,558))
wx.StaticText(panel,-1," V48 ",(223,584))
```

#Text column 5

```
wx.StaticText(panel,-1," V49 ",(293,300))
wx.StaticText(panel,-1," V50 ",(293,326))
wx.StaticText(panel,-1," V51 ",(293,352))
wx.StaticText(panel,-1," V52 ",(293,378))
wx.StaticText(panel,-1," V53 ",(293,402))
wx.StaticText(panel,-1," V54 ",(293,428))
wx.StaticText(panel,-1," V55 ",(293,454))
wx.StaticText(panel,-1," V56 ",(293,480))
wx.StaticText(panel,-1," V57 ",(293,506))
wx.StaticText(panel,-1," V58 ",(293,532))
```



```

##### 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

##### Menu Bar #####
#####
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()
def on_timer(self, event = None):
    new_value = str(windll.K8061.ReadAnalogChannel(0,1))

##### Define Valvue #####
#####
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)

    else:
        self.Valve1.SetLabel(valve_status_OFF)
        time.sleep(2)
        self.dll.ClearDigitalChannel(0,1)
        print 'Digital Channel Cleared, V1 turn off'

def V2(self, event):
    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'

    if self.Valve2.GetLabel()==valve_status_ON:
        self.Valve2.SetLabel(valve_status_OFF)

    else:
        self.Valve2.SetLabel(valve_status_ON)

```

```

def V3(self, event):
    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'
    if self.Valve3.GetLabel()==valve_status_ON:
        self.Valve3.SetLabel(valve_status_OFF)
    else:
        self.Valve3.SetLabel(valve_status_ON)

def V4(self, event):
    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'
    if self.Valve4.GetLabel()==valve_status_ON:
        self.Valve4.SetLabel(valve_status_OFF)
    else:
        self.Valve4.SetLabel(valve_status_ON)

def V5(self, event):
    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'
    if self.Valve5.GetLabel()==valve_status_ON:
        self.Valve5.SetLabel(valve_status_OFF)
    else:
        self.Valve5.SetLabel(valve_status_ON)

def V6(self, event):
    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'
    if self.Valve6.GetLabel()==valve_status_ON:
        self.Valve6.SetLabel(valve_status_OFF)
    else:
        self.Valve6.SetLabel(valve_status_ON)

def V7(self, event):
    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'
    if self.Valve7.GetLabel()==valve_status_ON:
        self.Valve7.SetLabel(valve_status_OFF)
    else:
        self.Valve7.SetLabel(valve_status_ON)

```

```

def V8(self, event):
    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'
    if self.Valve8.GetLabel()==valve_status_ON:
        self.Valve8.SetLabel(valve_status_OFF)
    else:
        self.Valve8.SetLabel(valve_status_ON)

def V9(self, event):
    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'
    if self.Valve9.GetLabel()==valve_status_ON:
        self.Valve9.SetLabel(valve_status_OFF)
    else:
        self.Valve9.SetLabel(valve_status_ON)

def V10(self, event):
    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'
    if self.Valve10.GetLabel()==valve_status_ON:
        self.Valve10.SetLabel(valve_status_OFF)
    else:
        self.Valve10.SetLabel(valve_status_ON)

def V11(self, event):
    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'
    if self.Valve11.GetLabel()==valve_status_ON:
        self.Valve11.SetLabel(valve_status_OFF)

    else:
        self.Valve11.SetLabel(valve_status_ON)

def V12(self, event):
    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'
    if self.Valve12.GetLabel()==valve_status_ON:
        self.Valve12.SetLabel(valve_status_OFF)
    else:
        self.Valve12.SetLabel(valve_status_ON)

def V13(self, event):
    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'
    if self.Valve13.GetLabel()==valve_status_ON:
        self.Valve13.SetLabel(valve_status_OFF)
    else:
        self.Valve13.SetLabel(valve_status_ON)

def V14(self, event):
    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'
    if self.Valve14.GetLabel()==valve_status_ON:
        self.Valve14.SetLabel(valve_status_OFF)
    else:
        self.Valve14.SetLabel(valve_status_ON)

def V15(self, event):
    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'
    if self.Valve15.GetLabel()==valve_status_ON:
        self.Valve15.SetLabel(valve_status_OFF)
    else:
        self.Valve15.SetLabel(valve_status_ON)

def V16(self, event):
    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'
    if self.Valve16.GetLabel()==valve_status_ON:
        self.Valve16.SetLabel(valve_status_OFF)
    else:
        self.Valve16.SetLabel(valve_status_ON)

```

```

def V17(self, event):
    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'
    if self.Valve17.GetLabel() == valve_status_ON:
        self.Valve17.SetLabel(valve_status_OFF)
    else:
        self.Valve17.SetLabel(valve_status_ON)

def V18(self, event):
    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'
    if self.Valve18.GetLabel() == valve_status_ON:
        self.Valve18.SetLabel(valve_status_OFF)
    else:
        self.Valve18.SetLabel(valve_status_ON)

def V19(self, event):
    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'
    if self.Valve19.GetLabel() == valve_status_ON:
        self.Valve19.SetLabel(valve_status_OFF)
    else:
        self.Valve19.SetLabel(valve_status_ON)

def V20(self, event):
    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'
    if self.Valve20.GetLabel() == valve_status_ON:
        self.Valve20.SetLabel(valve_status_OFF)
    else:
        self.Valve20.SetLabel(valve_status_ON)

def V21(self, event):
    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'
    if self.Valve21.GetLabel() == valve_status_ON:
        self.Valve21.SetLabel(valve_status_OFF)
    else:
        self.Valve21.SetLabel(valve_status_ON)

def V22(self, event):
    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'
    if self.Valve22.GetLabel() == valve_status_ON:
        self.Valve22.SetLabel(valve_status_OFF)
    else:
        self.Valve22.SetLabel(valve_status_ON)

def V23(self, event):
    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'
    if self.Valve23.GetLabel() == valve_status_ON:
        self.Valve23.SetLabel(valve_status_OFF)
    else:
        self.Valve23.SetLabel(valve_status_ON)

def V24(self, event):
    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'
    if self.Valve24.GetLabel() == valve_status_ON:
        self.Valve24.SetLabel(valve_status_OFF)
    else:
        self.Valve24.SetLabel(valve_status_ON)

def V25(self, event):
    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'
    if self.Valve25.GetLabel() == valve_status_ON:
        self.Valve25.SetLabel(valve_status_OFF)
    else:
        self.Valve25.SetLabel(valve_status_ON)

```

```

def V26(self, event):
    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'
    if self.Valve26.GetLabel() == valve_status_ON:
        self.Valve26.SetLabel(valve_status_OFF)
    else:
        self.Valve26.SetLabel(valve_status_ON)

def V27(self, event):
    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'
    if self.Valve27.GetLabel() == valve_status_ON:
        self.Valve27.SetLabel(valve_status_OFF)
    else:
        self.Valve27.SetLabel(valve_status_ON)

def V28(self, event):
    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'
    if self.Valve28.GetLabel() == valve_status_ON:
        self.Valve28.SetLabel(valve_status_OFF)
    else:
        self.Valve28.SetLabel(valve_status_ON)

def V29(self, event):
    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'

    if self.Valve29.GetLabel() == valve_status_ON:
        self.Valve29.SetLabel(valve_status_OFF)
    else:
        self.Valve29.SetLabel(valve_status_ON)

def V30(self, event):
    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'
    if self.Valve30.GetLabel() == valve_status_ON:
        self.Valve30.SetLabel(valve_status_OFF)
    else:
        self.Valve30.SetLabel(valve_status_ON)

def V31(self, event):
    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'
    if self.Valve31.GetLabel() == valve_status_ON:
        self.Valve31.SetLabel(valve_status_OFF)
    else:
        self.Valve31.SetLabel(valve_status_ON)

def V32(self, event):
    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'
    if self.Valve32.GetLabel() == valve_status_ON:
        self.Valve32.SetLabel(valve_status_OFF)
    else:
        self.Valve32.SetLabel(valve_status_ON)

def V33(self, event):
    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'
    if self.Valve33.GetLabel() == valve_status_ON:
        self.Valve33.SetLabel(valve_status_OFF)
    else:
        self.Valve33.SetLabel(valve_status_ON)

def V34(self, event):
    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'

    if self.Valve34.GetLabel() == valve_status_ON:
        self.Valve34.SetLabel(valve_status_OFF)

    else:
        self.Valve34.SetLabel(valve_status_ON)

```



```

def V35(self, event):
    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'
    if self.Valve35.GetLabel() == valve_status_ON:
        self.Valve35.SetLabel(valve_status_OFF)
    else:
        self.Valve35.SetLabel(valve_status_ON)

def V36(self, event):
    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'
    if self.Valve36.GetLabel() == valve_status_ON:
        self.Valve36.SetLabel(valve_status_OFF)
    else:
        self.Valve36.SetLabel(valve_status_ON)

def V37(self, event):
    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'
    if self.Valve37.GetLabel() == valve_status_ON:
        self.Valve37.SetLabel(valve_status_OFF)
    else:
        self.Valve37.SetLabel(valve_status_ON)

def V38(self, event):
    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'
    if self.Valve38.GetLabel() == valve_status_ON:
        self.Valve38.SetLabel(valve_status_OFF)
    else:
        self.Valve38.SetLabel(valve_status_ON)

def V39(self, event):
    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'
    if self.Valve39.GetLabel() == valve_status_ON:
        self.Valve39.SetLabel(valve_status_OFF)
    else:
        self.Valve39.SetLabel(valve_status_ON)

def V40(self, event):
    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'
    if self.Valve40.GetLabel() == valve_status_ON:
        self.Valve40.SetLabel(valve_status_OFF)
    else:
        self.Valve40.SetLabel(valve_status_ON)

def V41(self, event):
    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'
    if self.Valve41.GetLabel() == valve_status_ON:
        self.Valve41.SetLabel(valve_status_OFF)
    else:
        self.Valve41.SetLabel(valve_status_ON)

def V42(self, event):
    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'
    if self.Valve42.GetLabel() == valve_status_ON:
        self.Valve42.SetLabel(valve_status_OFF)
    else:
        self.Valve42.SetLabel(valve_status_ON)

def V43(self, event):
    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'

    if self.Valve43.GetLabel() == valve_status_ON:
        self.Valve43.SetLabel(valve_status_OFF)

    else:
        self.Valve43.SetLabel(valve_status_ON)

```

```

def V44(self, event):
    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'
    if self.Valve44.GetLabel() == valve_status_ON:
        self.Valve44.SetLabel(valve_status_OFF)
    else:
        self.Valve44.SetLabel(valve_status_ON)

def V45(self, event):
    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'
    if self.Valve45.GetLabel() == valve_status_ON:
        self.Valve45.SetLabel(valve_status_OFF)
    else:
        self.Valve45.SetLabel(valve_status_ON)

def V46(self, event):
    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'
    if self.Valve46.GetLabel() == valve_status_ON:
        self.Valve46.SetLabel(valve_status_OFF)
    else:
        self.Valve46.SetLabel(valve_status_ON)

def V47(self, event):
    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'
    if self.Valve47.GetLabel() == valve_status_ON:
        self.Valve47.SetLabel(valve_status_OFF)
    else:
        self.Valve47.SetLabel(valve_status_ON)

def V48(self, event):
    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'
    if self.Valve48.GetLabel() == valve_status_ON:
        self.Valve48.SetLabel(valve_status_OFF)
    else:
        self.Valve48.SetLabel(valve_status_ON)

def V49(self, event):
    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'
    if self.Valve49.GetLabel() == valve_status_ON:
        self.Valve49.SetLabel(valve_status_OFF)
    else:
        self.Valve49.SetLabel(valve_status_ON)

def V50(self, event):
    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'
    if self.Valve50.GetLabel() == valve_status_ON:
        self.Valve50.SetLabel(valve_status_OFF)
    else:
        self.Valve50.SetLabel(valve_status_ON)

def V51(self, event):
    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'
    if self.Valve51.GetLabel() == valve_status_ON:
        self.Valve51.SetLabel(valve_status_OFF)
    else:
        self.Valve51.SetLabel(valve_status_ON)

def V52(self, event):
    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'

    if self.Valve52.GetLabel() == valve_status_ON:
        self.Valve52.SetLabel(valve_status_OFF)

    else:
        self.Valve52.SetLabel(valve_status_ON)

```

```

def V53(self, event):
    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'
    if self.Valve53.GetLabel() == valve_status_ON:
        self.Valve53.SetLabel(valve_status_OFF)
    else:
        self.Valve53.SetLabel(valve_status_ON)

def V54(self, event):
    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'
    if self.Valve54.GetLabel() == valve_status_ON:
        self.Valve54.SetLabel(valve_status_OFF)
    else:
        self.Valve54.SetLabel(valve_status_ON)

def V55(self, event):
    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'
    if self.Valve55.GetLabel() == valve_status_ON:
        self.Valve55.SetLabel(valve_status_OFF)
    else:
        self.Valve55.SetLabel(valve_status_ON)

def V56(self, event):
    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'
    if self.Valve56.GetLabel() == valve_status_ON:
        self.Valve56.SetLabel(valve_status_OFF)
    else:
        self.Valve56.SetLabel(valve_status_ON)

def V57(self, event):
    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'
    if self.Valve57.GetLabel() == valve_status_ON:
        self.Valve57.SetLabel(valve_status_OFF)
    else:
        self.Valve57.SetLabel(valve_status_ON)

def V58(self, event):
    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'
    if self.Valve58.GetLabel() == valve_status_ON:
        self.Valve58.SetLabel(valve_status_OFF)
    else:
        self.Valve58.SetLabel(valve_status_ON)

***** Define Pumps *****
*****

def P1 (self,event):
    wx.MessageBox("P3 is Open ", "Pump", wx.OK|wx.ICON_INFORMATION)
def P2 (self,event):
    wx.MessageBox("P3 is Open ", "Pump", wx.OK|wx.ICON_INFORMATION)
def P3 (self,event):
    wx.MessageBox("P3 is Open ", "Pump", wx.OK|wx.ICON_INFORMATION)
def P4 (self,event):
    wx.MessageBox("P4 is Open ", "Pump", wx.OK|wx.ICON_INFORMATION)

def P5 (self,event):
    wx.MessageBox("P5 is Open ", "Pump", wx.OK|wx.ICON_INFORMATION)

def P6 (self,event):
    wx.MessageBox("P6 is Open ", "Pump", wx.OK|wx.ICON_INFORMATION)

def P7 (self,event):
    wx.MessageBox("P7 is Open ", "Pump", wx.OK|wx.ICON_INFORMATION)

def P8 (self,event):
    wx.MessageBox("P8 is Open ", "Pump", wx.OK|wx.ICON_INFORMATION)

def P9 (self,event):
    wx.MessageBox("P9 is Open ", "Pump", wx.OK|wx.ICON_INFORMATION)

def P10 (self,event):
    wx.MessageBox("P10 is Open ", "Pump", wx.OK|wx.ICON_INFORMATION)

```

```

#***** 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()

```

9.4 Annex 2b: python automation code including full automation

D:\AECENAR\MEGBI\MEGBI-VPP\AUT\CDMasterThesisHayssamHindy\GUI\101015_14MEGBI-VPP_automation.py

```

#-----
# Name:    MEGBI monitoring software
# Purpose:
#
# Author:  abd el rahman, Samir, hayssam
#
# Created: 09/05/2013
# Copyright: (c) aecenar 2013, 2015
# Licence: <AECENAR>
#-----

#!/usr/bin/env python
## -*- coding: iso-8859-15 -*-
import sys

#from tkinter import *
import threading
#from PIL import Image, ImageTk
import random

#import tkinter

```

```

import time
#from pymouse import PyMouse

#import ctypes
from ctypes import *

import wx
#import thread

#####                panel frame                #####
#####
#wx.SetDefaultPyEncoding("iso-8859-15")
BACKGROUND_IMAGENAME = "220415MEGPI-VPP_PI_DIAGRAM.PNG"
#BACKGROUND_IMAGENAME = "\"C:\Users\Hayssam\Desktop\GUI 220415MEGPI-
VPP_PI_DIAGRAM.PNG"
##"hintergrundbild.jpg"

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)

class MyFrame(wx.Frame):

```

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

```
self.testUSB          = True  
self.dll              = None  
self.USBAdr0         = 0  
self.USBAdr1         = 1  
self.USBAdr2         = 2  
self.USBOpened       = False  
self.counterUSBBoards = 3
```

```
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)
```

#Valves

#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)
```

#V2

```
self.button_V_2 = wx.Button(panel, -1, "V2", pos=(150,90),size=(20,20))
self.Bind(wx.EVT_BUTTON, self.V2, self.button_V_2)
```

#V3

```
self.button_V_3 = wx.Button(panel, -1, "V3", pos=(150,135),size=(20,20))
self.Bind(wx.EVT_BUTTON, self.V3, self.button_V_3)
```

#V4

```
self.button_V_4 = wx.Button(panel, -1, "V4", pos=(150,265),size=(20,20))
self.Bind(wx.EVT_BUTTON, self.V4, self.button_V_4)
```

#V5

```
self.button_V_5 = wx.Button(panel, -1, "V5", pos=(210,135),size=(20,20))
self.Bind(wx.EVT_BUTTON, self.V5, self.button_V_5)
```

#V6

```
self.button_V_6 = wx.Button(panel, -1, "V6", pos=(215,185),size=(20,20))
self.Bind(wx.EVT_BUTTON, self.V6, self.button_V_6)
```

#V7

```
self.button_V_7 = wx.Button(panel, -1, "V7", pos=(215,220),size=(20,20))
self.Bind(wx.EVT_BUTTON, self.V7, self.button_V_7)
```

#V8

```
self.button_V_8 = wx.Button(panel, -1, "V8", pos=(282,180),size=(25,20))
self.Bind(wx.EVT_BUTTON, self.V8, self.button_V_8)
```

```
#V9
self.button_V_9 = wx.Button(panel, -1, "V9", pos=(282,158),size=(25,20))
self.Bind(wx.EVT_BUTTON, self.V9, self.button_V_9)

#V10
self.button_V_10 = wx.Button(panel, -1, "V10", pos=(322,110),size=(25,20))
self.Bind(wx.EVT_BUTTON, self.V10, self.button_V_10)

#V11
self.button_V_11 = wx.Button(panel, -1, "V11", pos=(380,250),size=(25,20))
self.Bind(wx.EVT_BUTTON, self.V11, self.button_V_11)

#V12
self.button_V_12 = wx.Button(panel, -1, "V12", pos=(413,195),size=(25,20))
self.Bind(wx.EVT_BUTTON, self.V12, self.button_V_12)

#V13
self.button_V_13 = wx.Button(panel, -1, "V13", pos=(380,130),size=(25,20))
self.Bind(wx.EVT_BUTTON, self.V13, self.button_V_13)

#V14
self.button_V_14 = wx.Button(panel, -1, "V14", pos=(410,100),size=(25,20))
self.Bind(wx.EVT_BUTTON, self.V14, self.button_V_14)

#V15
self.button_V_15 = wx.Button(panel, -1, "V15", pos=(460,112),size=(25,20))
self.Bind(wx.EVT_BUTTON, self.V15, self.button_V_15)

#V16
self.button_V_16 = wx.Button(panel, -1, "V16", pos=(460,73),size=(25,20))
self.Bind(wx.EVT_BUTTON, self.V16, self.button_V_16)

#V17
self.button_V_17 = wx.Button(panel, -1, "V17", pos=(460,38),size=(25,20))
self.Bind(wx.EVT_BUTTON, self.V17, self.button_V_17)
```


#V18

```
self.button_V_18 = wx.Button(panel, -1, "V18", pos=(522,18),size=(25,20))  
self.Bind(wx.EVT_BUTTON, self.V18, self.button_V_18)
```

#V19

```
self.button_V_19 = wx.Button(panel, -1, "V19", pos=(635,18),size=(25,20))  
self.Bind(wx.EVT_BUTTON, self.V19, self.button_V_19)
```

#V20

```
self.button_V_20 = wx.Button(panel, -1, "V20", pos=(635,72),size=(25,20))  
self.Bind(wx.EVT_BUTTON, self.V20, self.button_V_20)
```

#V21

```
self.button_V_21 = wx.Button(panel, -1, "V21", pos=(675,112),size=(25,20))  
self.Bind(wx.EVT_BUTTON, self.V21, self.button_V_21)
```

#V22

```
self.button_V_22 = wx.Button(panel, -1, "V22", pos=(635,155),size=(25,20))  
self.Bind(wx.EVT_BUTTON, self.V22, self.button_V_22)
```

#V23

```
self.button_V_23 = wx.Button(panel, -1, "V23", pos=(780,18),size=(25,20))  
self.Bind(wx.EVT_BUTTON, self.V23, self.button_V_23)
```

#V24

```
self.button_V_24 = wx.Button(panel, -1, "V24", pos=(770,120),size=(25,20))  
self.Bind(wx.EVT_BUTTON, self.V24, self.button_V_24)
```

#V25

```
self.button_V_25 = wx.Button(panel, -1, "V25", pos=(770,155),size=(25,20))  
self.Bind(wx.EVT_BUTTON, self.V25, self.button_V_25)
```

#V26

```
self.button_V_26 = wx.Button(panel, -1, "V26", pos=(870,155),size=(25,20))  
self.Bind(wx.EVT_BUTTON, self.V26, self.button_V_26)
```

#V27

```
self.button_V_27 = wx.Button(panel, -1, "V27", pos=(510,185),size=(25,20))
self.Bind(wx.EVT_BUTTON, self.V27, self.button_V_27)

#V28
self.button_V_28 = wx.Button(panel, -1, "V28", pos=(620,185),size=(25,20))
self.Bind(wx.EVT_BUTTON, self.V28, self.button_V_28)

#V29
self.button_V_29 = wx.Button(panel, -1, "V29", pos=(680,185),size=(25,20))
self.Bind(wx.EVT_BUTTON, self.V29, self.button_V_29)

#V30
self.button_V_30 = wx.Button(panel, -1, "V30", pos=(620,240),size=(25,20))
self.Bind(wx.EVT_BUTTON, self.V30, self.button_V_30)

#V31
self.button_V_31 = wx.Button(panel, -1, "V31", pos=(710,235),size=(25,20))
self.Bind(wx.EVT_BUTTON, self.V31, self.button_V_31)

#V32
self.button_V_32 = wx.Button(panel, -1, "V32", pos=(950,182),size=(25,20))
self.Bind(wx.EVT_BUTTON, self.V32, self.button_V_32)

#V33
self.button_V_33 = wx.Button(panel, -1, "V33", pos=(977,182),size=(25,20))
self.Bind(wx.EVT_BUTTON, self.V33, self.button_V_33)

#V34
self.button_V_34 = wx.Button(panel, -1, "V34", pos=(1083,188),size=(25,20))
self.Bind(wx.EVT_BUTTON, self.V34, self.button_V_34)

#V35
self.button_V_35 = wx.Button(panel, -1, "V35", pos=(1083,220),size=(25,20))
self.Bind(wx.EVT_BUTTON, self.V35, self.button_V_35)
```

#V36

```
self.button_V_36 = wx.Button(panel, -1, "V36", pos=(1170,110),size=(25,20))  
self.Bind(wx.EVT_BUTTON, self.V36, self.button_V_36)
```

#V37

```
self.button_V_37 = wx.Button(panel, -1, "V37", pos=(1200,110),size=(25,20))  
self.Bind(wx.EVT_BUTTON, self.V37, self.button_V_37)
```

#V38

```
self.button_V_38 = wx.Button(panel, -1, "V38", pos=(1240,150),size=(25,20))  
self.Bind(wx.EVT_BUTTON, self.V38, self.button_V_38)
```

#V39

```
self.button_V_39 = wx.Button(panel, -1, "V39", pos=(1273,205),size=(25,20))  
self.Bind(wx.EVT_BUTTON, self.V39, self.button_V_39)
```

#V40

```
self.button_V_40 = wx.Button(panel, -1, "V40", pos=(483,314),size=(25,20))  
self.Bind(wx.EVT_BUTTON, self.V40, self.button_V_40)
```

#V41

```
self.button_V_41 = wx.Button(panel, -1, "V41", pos=(478,398),size=(25,20))  
self.Bind(wx.EVT_BUTTON, self.V41, self.button_V_41)
```

#V42

```
self.button_V_42 = wx.Button(panel, -1, "V42", pos=(540,314),size=(25,20))  
self.Bind(wx.EVT_BUTTON, self.V42, self.button_V_42)
```

#V43

```
self.button_V_43 = wx.Button(panel, -1, "V43", pos=(548,398),size=(25,20))  
self.Bind(wx.EVT_BUTTON, self.V43, self.button_V_43)
```

#V44

```
self.button_V_44 = wx.Button(panel, -1, "V44", pos=(580,300),size=(25,20))
```

```
self.Bind(wx.EVT_BUTTON, self.V44, self.button_V_44)
```

```
#V45
```

```
self.button_V_45 = wx.Button(panel, -1, "V45", pos=(620,314),size=(25,20))
```

```
self.Bind(wx.EVT_BUTTON, self.V45, self.button_V_45)
```

```
#V46
```

```
self.button_V_46 = wx.Button(panel, -1, "V46", pos=(760,440),size=(25,20))
```

```
self.Bind(wx.EVT_BUTTON, self.V46, self.button_V_46)
```

```
#V47
```

```
self.button_V_47 = wx.Button(panel, -1, "V47", pos=(760,400),size=(25,20))
```

```
self.Bind(wx.EVT_BUTTON, self.V47, self.button_V_47)
```

```
#V48
```

```
self.button_V_48 = wx.Button(panel, -1, "V48", pos=(783,375),size=(25,20))
```

```
self.Bind(wx.EVT_BUTTON, self.V48, self.button_V_48)
```

```
#V49
```

```
self.button_V_49 = wx.Button(panel, -1, "V49", pos=(880,428),size=(25,20))
```

```
self.Bind(wx.EVT_BUTTON, self.V49, self.button_V_49)
```

```
#V50
```

```
self.button_V_50 = wx.Button(panel, -1, "V50", pos=(970,377),size=(25,20))
```

```
self.Bind(wx.EVT_BUTTON, self.V50, self.button_V_50)
```

```
#V51
```

```
self.button_V_51 = wx.Button(panel, -1, "V51", pos=(963,450),size=(25,20))
```

```
self.Bind(wx.EVT_BUTTON, self.V51, self.button_V_51)
```

```
#V52
```

```
self.button_V_52 = wx.Button(panel, -1, "V52", pos=(1005,470),size=(25,20))
```

```
self.Bind(wx.EVT_BUTTON, self.V52, self.button_V_52)
```

```
#V53
```

```

self.button_V_53 = wx.Button(panel, -1, "V53", pos=(1030,395),size=(25,20))
self.Bind(wx.EVT_BUTTON, self.V53, self.button_V_53)

#V54
self.button_V_54 = wx.Button(panel, -1, "V54", pos=(1080,395),size=(25,20))
self.Bind(wx.EVT_BUTTON, self.V54, self.button_V_54)

#V55
self.button_V_55 = wx.Button(panel, -1, "V55", pos=(1118,377),size=(25,20))
self.Bind(wx.EVT_BUTTON, self.V55, self.button_V_55)

#V56
self.button_V_56 = wx.Button(panel, -1, "V56", pos=(1158,468),size=(25,20))
self.Bind(wx.EVT_BUTTON, self.V56, self.button_V_56)

#V57
self.button_V_57 = wx.Button(panel, -1, "V57", pos=(1190,420),size=(25,20))
self.Bind(wx.EVT_BUTTON, self.V57, self.button_V_57)

#V58
self.button_V_58 = wx.Button(panel, -1, "V58", pos=(710,580),size=(25,20))
self.Bind(wx.EVT_BUTTON, self.V58, self.button_V_58)

#Pumps

#P1
self.button_P_1 = wx.Button(panel, -1, "P1", pos=(150,180),size=(20,20))
self.Bind(wx.EVT_BUTTON, self.P1, self.button_P_1)

#P2
self.button_P_2 = wx.Button(panel, -1, "P2", pos=(150,220),size=(20,20))
self.Bind(wx.EVT_BUTTON, self.P2, self.button_P_2)

#P3
self.button_P_3 = wx.Button(panel, -1, "P3", pos=(510,40),size=(20,20))

```

```
self.Bind(wx.EVT_BUTTON, self.P3, self.button_P_3)
```

```
#P4
```

```
self.button_P_4 = wx.Button(panel, -1, "P4", pos=(880,18),size=(20,20))
```

```
self.Bind(wx.EVT_BUTTON, self.P4, self.button_P_4)
```

```
#P5
```

```
self.button_P_5 = wx.Button(panel, -1, "P5", pos=(730,187),size=(20,20))
```

```
self.Bind(wx.EVT_BUTTON, self.P5, self.button_P_5)
```

```
#P6
```

```
self.button_P_6 = wx.Button(panel, -1, "P6", pos=(1083,130),size=(20,20))
```

```
self.Bind(wx.EVT_BUTTON, self.P6, self.button_P_6)
```

```
#P7
```

```
self.button_P_7 = wx.Button(panel, -1, "P7", pos=(380,328),size=(25,20))
```

```
self.Bind(wx.EVT_BUTTON, self.P7, self.button_P_7)
```

```
#P8
```

```
self.button_P_8 = wx.Button(panel, -1, "P8", pos=(710,423),size=(25,20))
```

```
self.Bind(wx.EVT_BUTTON, self.P8, self.button_P_8)
```

```
#P9
```

```
self.button_P_9 = wx.Button(panel, -1, "P9", pos=(900,380),size=(25,20))
```

```
self.Bind(wx.EVT_BUTTON, self.P9, self.button_P_9)
```

```
#P10
```

```
self.button_P_10 = wx.Button(panel, -1, "P10", pos=(1200,380),size=(25,20))
```

```
self.Bind(wx.EVT_BUTTON, self.P10, self.button_P_10)
```

```
#control buttons
```

```
#Turn ON button
```

```
self.button_Turn_ON = wx.Button(panel, -1, "Turn On", pos=(40,40),size=(60,20))
```

```
self.Bind(wx.EVT_BUTTON, self.MEGBIVPPFullAutomationStart, self.button_Turn_ON)
```

```
self.ON = wx.StaticText(panel, size = (40, -1), pos = (140, 40), style = LABELSTYLE)
```

#Text column 1

```
wx.StaticText(panel,20," Valve Selected : ",(10,270))  
wx.StaticText(panel,-1," V1 ",(18,300))  
wx.StaticText(panel,-1," V2 ",(18,326))  
wx.StaticText(panel,-1," V3 ",(18,352))  
wx.StaticText(panel,-1," V4 ",(18,378))  
wx.StaticText(panel,-1," V5 ",(18,402))  
wx.StaticText(panel,-1," V6 ",(18,428))  
wx.StaticText(panel,-1," V7 ",(18,454))  
wx.StaticText(panel,-1," V8 ",(18,480))  
wx.StaticText(panel,-1," V9 ",(18,506))  
wx.StaticText(panel,-1," V10 ",(18,532))  
wx.StaticText(panel,-1," V11 ",(18,558))  
wx.StaticText(panel,-1," V12 ",(18,584))
```

#Text column 2

```
wx.StaticText(panel,-1," V13 ",(85,300))  
wx.StaticText(panel,-1," V14 ",(85,326))  
wx.StaticText(panel,-1," V15 ",(85,352))  
wx.StaticText(panel,-1," V16 ",(85,378))  
wx.StaticText(panel,-1," V17 ",(85,402))  
wx.StaticText(panel,-1," V18 ",(85,428))  
wx.StaticText(panel,-1," V19 ",(85,454))  
wx.StaticText(panel,-1," V20 ",(85,480))  
wx.StaticText(panel,-1," V21 ",(85,506))  
wx.StaticText(panel,-1," V22 ",(85,532))  
wx.StaticText(panel,-1," V23 ",(85,558))  
wx.StaticText(panel,-1," V24 ",(85,584))
```

#Text column 3

```
wx.StaticText(panel,-1," V25 ",(155,300))  
wx.StaticText(panel,-1," V26 ",(155,326))  
wx.StaticText(panel,-1," V27 ",(155,352))  
wx.StaticText(panel,-1," V28 ",(155,378))  
wx.StaticText(panel,-1," V29 ",(155,402))  
wx.StaticText(panel,-1," V30 ",(155,428))
```

```
wx.StaticText(panel,-1," V31 ",(155,454))
wx.StaticText(panel,-1," V32 ",(155,480))
wx.StaticText(panel,-1," V33 ",(155,506))
wx.StaticText(panel,-1," V34 ",(155,532))
wx.StaticText(panel,-1," V35 ",(155,558))
wx.StaticText(panel,-1," V36 ",(155,584))
```

#Text column 4

```
wx.StaticText(panel,-1," V37 ",(223,300))
wx.StaticText(panel,-1," V38 ",(223,326))
wx.StaticText(panel,-1," V39 ",(223,352))
wx.StaticText(panel,-1," V40 ",(223,378))
wx.StaticText(panel,-1," V41 ",(223,402))
wx.StaticText(panel,-1," V42 ",(223,428))
wx.StaticText(panel,-1," V43 ",(223,454))
wx.StaticText(panel,-1," V44 ",(223,480))
wx.StaticText(panel,-1," V45 ",(223,506))
wx.StaticText(panel,-1," V46 ",(223,532))
wx.StaticText(panel,-1," V47 ",(223,558))
wx.StaticText(panel,-1," V48 ",(223,584))
```

#Text column 5

```
wx.StaticText(panel,-1," V49 ",(293,300))
wx.StaticText(panel,-1," V50 ",(293,326))
wx.StaticText(panel,-1," V51 ",(293,352))
wx.StaticText(panel,-1," V52 ",(293,378))
wx.StaticText(panel,-1," V53 ",(293,402))
wx.StaticText(panel,-1," V54 ",(293,428))
wx.StaticText(panel,-1," V55 ",(293,454))
wx.StaticText(panel,-1," V56 ",(293,480))
wx.StaticText(panel,-1," V57 ",(293,506))
wx.StaticText(panel,-1," V58 ",(293,532))
#wx.StaticText(panel,-1," V59 ",(293,558))
# wx.StaticText(panel,-1," V60 ",(293,584))
```



```
#Label Column 1
```

```
self.Valve1 = wx.StaticText(panel, size = (26, -1), pos = (45, 300), style = LABELSTYLE)
self.Valve2 = wx.StaticText(panel, size = (26, -1), pos = (45, 326), style = LABELSTYLE)
self.Valve3 = wx.StaticText(panel, size = (26, -1), pos = (45, 352), style = LABELSTYLE)
self.Valve4 = wx.StaticText(panel, size = (26, -1), pos = (45, 378), style = LABELSTYLE)
self.Valve5 = wx.StaticText(panel, size = (26, -1), pos = (45, 402), style = LABELSTYLE)
self.Valve6 = wx.StaticText(panel, size = (26, -1), pos = (45, 428), style = LABELSTYLE)
self.Valve7 = wx.StaticText(panel, size = (26, -1), pos = (45, 454), style = LABELSTYLE)
self.Valve8 = wx.StaticText(panel, size = (26, -1), pos = (45, 480), style = LABELSTYLE)
self.Valve9 = wx.StaticText(panel, size = (26, -1), pos = (45, 506), style = LABELSTYLE)
self.Valve10 = wx.StaticText(panel, size = (26, -1), pos = (45, 532), style = LABELSTYLE)
self.Valve11 = wx.StaticText(panel, size = (26, -1), pos = (45, 558), style = LABELSTYLE)
self.Valve12 = wx.StaticText(panel, size = (26, -1), pos = (45, 584), style = LABELSTYLE)
```

```
#Label column 2
```

```
self.Valve13 = wx.StaticText(panel, size = (26, -1), pos = (115, 300), style = LABELSTYLE)
self.Valve14 = wx.StaticText(panel, size = (26, -1), pos = (115, 326), style = LABELSTYLE)
self.Valve15 = wx.StaticText(panel, size = (26, -1), pos = (115, 352), style = LABELSTYLE)
self.Valve16 = wx.StaticText(panel, size = (26, -1), pos = (115, 378), style = LABELSTYLE)
self.Valve17 = wx.StaticText(panel, size = (26, -1), pos = (115, 402), style = LABELSTYLE)
self.Valve18 = wx.StaticText(panel, size = (26, -1), pos = (115, 428), style = LABELSTYLE)
self.Valve19 = wx.StaticText(panel, size = (26, -1), pos = (115, 454), style = LABELSTYLE)
self.Valve20 = wx.StaticText(panel, size = (26, -1), pos = (115, 480), style = LABELSTYLE)
self.Valve21 = wx.StaticText(panel, size = (26, -1), pos = (115, 506), style = LABELSTYLE)
self.Valve22 = wx.StaticText(panel, size = (26, -1), pos = (115, 532), style = LABELSTYLE)
self.Valve23 = wx.StaticText(panel, size = (26, -1), pos = (115, 558), style = LABELSTYLE)
self.Valve24 = wx.StaticText(panel, size = (26, -1), pos = (115, 584), style = LABELSTYLE)
```

```
#Label column 3
```

```
self.Valve25 = wx.StaticText(panel, size = (26, -1), pos = (185, 300), style = LABELSTYLE)
self.Valve26 = wx.StaticText(panel, size = (26, -1), pos = (185, 326), style = LABELSTYLE)
self.Valve27 = wx.StaticText(panel, size = (26, -1), pos = (185, 352), style = LABELSTYLE)
self.Valve28 = wx.StaticText(panel, size = (26, -1), pos = (185, 378), style = LABELSTYLE)
self.Valve29 = wx.StaticText(panel, size = (26, -1), pos = (185, 402), style = LABELSTYLE)
self.Valve30 = wx.StaticText(panel, size = (26, -1), pos = (185, 428), style = LABELSTYLE)
self.Valve31 = wx.StaticText(panel, size = (26, -1), pos = (185, 454), style = LABELSTYLE)
self.Valve32 = wx.StaticText(panel, size = (26, -1), pos = (185, 480), style = LABELSTYLE)
self.Valve33 = wx.StaticText(panel, size = (26, -1), pos = (185, 506), style = LABELSTYLE)
self.Valve34 = wx.StaticText(panel, size = (26, -1), pos = (185, 532), style = LABELSTYLE)
self.Valve35 = wx.StaticText(panel, size = (26, -1), pos = (185, 558), style = LABELSTYLE)
self.Valve36 = wx.StaticText(panel, size = (26, -1), pos = (185, 584), style = LABELSTYLE)
```

#Label column 4

```
self.Valve37 = wx.StaticText(panel, size = (26, -1), pos = (253, 300), style = LABELSTYLE)
self.Valve38 = wx.StaticText(panel, size = (26, -1), pos = (253, 326), style = LABELSTYLE)
self.Valve39 = wx.StaticText(panel, size = (26, -1), pos = (253, 352), style = LABELSTYLE)
self.Valve40 = wx.StaticText(panel, size = (26, -1), pos = (253, 378), style = LABELSTYLE)
self.Valve41 = wx.StaticText(panel, size = (26, -1), pos = (253, 402), style = LABELSTYLE)
self.Valve42 = wx.StaticText(panel, size = (26, -1), pos = (253, 428), style = LABELSTYLE)
self.Valve43 = wx.StaticText(panel, size = (26, -1), pos = (253, 454), style = LABELSTYLE)
self.Valve44 = wx.StaticText(panel, size = (26, -1), pos = (253, 480), style = LABELSTYLE)
self.Valve45 = wx.StaticText(panel, size = (26, -1), pos = (253, 506), style = LABELSTYLE)
self.Valve46 = wx.StaticText(panel, size = (26, -1), pos = (253, 532), style = LABELSTYLE)
self.Valve47 = wx.StaticText(panel, size = (26, -1), pos = (253, 558), style = LABELSTYLE)
self.Valve48 = wx.StaticText(panel, size = (26, -1), pos = (253, 584), style = LABELSTYLE)
```

#Label column 5

```
self.Valve49 = wx.StaticText(panel, size = (26, -1), pos = (323, 300), style = LABELSTYLE)
self.Valve50 = wx.StaticText(panel, size = (26, -1), pos = (323, 326), style = LABELSTYLE)
self.Valve51 = wx.StaticText(panel, size = (26, -1), pos = (323, 352), style = LABELSTYLE)
self.Valve52 = wx.StaticText(panel, size = (26, -1), pos = (323, 378), style = LABELSTYLE)
self.Valve53 = wx.StaticText(panel, size = (26, -1), pos = (323, 402), style = LABELSTYLE)
self.Valve54 = wx.StaticText(panel, size = (26, -1), pos = (323, 428), style = LABELSTYLE)
```

```

self.Valve55 = wx.StaticText(panel, size = (26, -1), pos = (323, 454), style = LABELSTYLE)
self.Valve56 = wx.StaticText(panel, size = (26, -1), pos = (323, 480), style = LABELSTYLE)
self.Valve57 = wx.StaticText(panel, size = (26, -1), pos = (323, 506), style = LABELSTYLE)
self.Valve58 = wx.StaticText(panel, size = (26, -1), pos = (323, 532), style = LABELSTYLE)
#self.Valve59 = wx.StaticText(panel, size = (26, -1), pos = (323, 558), style = LABELSTYLE)

```

```
#####temperature sensors
```

```

wx.StaticText(panel,-1," Temperature : ",(1100,592))
self.temp = wx.StaticText(panel, size = (40, -1), pos = (1200, 590), style = LABELSTYLE)

```

```

##### 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

```

```
#####
```

```
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()
def on_timer(self, event = None):
    self.Close()

def Sensors(self,event):
    answer = self.dll.ReadAnalogChannel(0,1)
    new_value = str(answer)
    self.temp.SetLabel(answer)
    #power for the temperature sensor (255 >>>> 5V power)
    self.dll.OutputAnalogChannel(0,6,255)
```

```
##### Define Valve #####
#####
```

```
def MEGBIVPPFullAutomationStart (self, event):
```

```
    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'
```

```
if self.ON.GetLabel()==valve_status_OFF:
    self.ON.SetLabel(valve_status_ON)
    self.OpenUSBBoardThread()
    self.timer = wx.Timer()
    self.timer.Bind(wx.EVT_TIMER, self.on_timer)
    self.timer.Start(100)
    print'system is turning on'
    self.dll.SetDigitalChannel(0,1)
    print'V1 is open'
    self.Valve1.SetLabel(valve_status_ON)
    time.sleep(5)
    self.dll.SetDigitalChannel(0,2)
```

```

print'V2 is open'
self.Valve2.SetLabel(valve_status_ON)
self.timer.Start(40)
time.sleep(20)
self.dll.ClearDigitalChannel(0,1)
self.dll.ClearDigitalChannel(0,2)
print'V1 is close'
self.Valve1.SetLabel(valve_status_OFF)
print'V2 is close'
self.Valve2.SetLabel(valve_status_OFF)
self.dll.SetDigitalChannel(0,3)
print'V3 is open'
self.Valve3.SetLabel(valve_status_ON)
self.timer.Start(40)
time.sleep(20)
self.dll.SetDigitalChannel(0,4)
self.dll.ClearDigitalChannel(0,3)
self.Valve3.SetLabel(valve_status_OFF)
print'V3 is close'
print'V4 is open'
self.Valve4.SetLabel(valve_status_ON)
#self.timer.Start(30)
#time.sleep(20)
self.dll.SetDigitalChannel(0,5)
self.dll.ClearDigitalChannel(0,4)
print'V4 i close'

self.Valve4.SetLabel(valve_status_OFF)
print'V5 is open'
self.Valve5.SetLabel(valve_status_ON)
self.dll.SetDigitalChannel(0,6)
print'P1 is turn on'
#self.timer.Start(400)
time.sleep(20)
self.dll.ClearDigitalChannel(0,5)
self.dll.ClearDigitalChannel(0,6)
print'V5 is close'
self.Valve5.SetLabel(valve_status_OFF)

```

```

print'P1 is turn off'
self.dll.SetDigitalChannel(0,7)
print'V6 is open'
self.Valve6.SetLabel(valve_status_ON)
#self.timer.Start(40)
time.sleep(5)
self.dll.ClearDigitalChannel(0,7)
print'V6 is close'
self.Valve6.SetLabel(valve_status_OFF)
self.dll.SetDigitalChannel(0,8)
print'V7 is open'
self.Valve7.SetLabel(valve_status_ON)
#self.timer.Start(400)
time.sleep(20)
self.dll.OutputAnalogChannel(0,2,255)
print'V8 is open'
self.Valve8.SetLabel(valve_status_ON)
self.dll.ClearDigitalChannel(0,8)
print'V7 is close'
self.Valve7.SetLabel(valve_status_OFF)
#self.timer.Start(40)
time.sleep(5)
self.dll.OutputAnalogChannel(0,2,0)
print'V8 is close'
self.Valve8.SetLabel(valve_status_OFF)
self.dll.OutputAnalogChannel(0,3,255)
print'V9 is open'
self.Valve9.SetLabel(valve_status_ON)
self.dll.OutputAnalogChannel(0,4,255)
print'P2 is turn on'
#self.timer.Start(400)
time.sleep(20)
self.dll.OutputAnalogChannel(0,3,0)
self.dll.OutputAnalogChannel(0,4,0)
self.dll.OutputAnalogChannel(0,5,255)
print'V9 is close'
self.Valve9.SetLabel(valve_status_OFF)
print'P2 is turn off'

```

```

print'V10 is open'
self.Valve10.SetLabel(valve_status_ON)
#self.timer.Start(40)
time.sleep(5)
self.dll.OutputAnalogChannel(0,5,0)
print'V10 is close'
self.Valve10.SetLabel(valve_status_OFF)
self.dll.OutputAnalogChannel(0,1,255)
print'V11 is open'
self.Valve11.SetLabel(valve_status_ON)
self.timer.Start(400)
time.sleep(20)
self.dll.OutputAnalogChannel(0,1,0)
self.dll.OutputAnalogChannel(0,7,255)
print'V11 is close'
self.Valve11.SetLabel(valve_status_OFF)
print'V12 is open'
self.Valve12.SetLabel(valve_status_ON)
#self.timer.Start(40)
time.sleep(5)
self.dll.OutputAnalogChannel(0,7,0)
print'V12 is close'
self.Valve12.SetLabel(valve_status_OFF)
self.dll.OutputAnalogChannel(0,8,255)
print'V13 is open'
self.Valve13.SetLabel(valve_status_ON)
#self.timer.Start(400)
time.sleep(20)
self.dll.OutputAnalogChannel(0,8,0)
self.dll.SetDigitalChannel(1,1)
print'V13 is close'
self.Valve13.SetLabel(valve_status_OFF)
print'V14 is open'
self.Valve14.SetLabel(valve_status_ON)
self.dll.SetDigitalChannel(1,2)
print'P3 is turn on'
#self.timer.Start(40)
time.sleep(5)

```

```
self.dll.ClearDigitalChannel(1,1)
self.dll.ClearDigitalChannel(1,2)
print'V14 is close'
self.Valve14.SetLabel(valve_status_OFF)
print'P3 is turn on'
self.dll.SetDigitalChannel(1,3)
print'V15 is open'
self.Valve15.SetLabel(valve_status_ON)
#self.timer.Start(50)
time.sleep(5)
self.dll.ClearDigitalChannel(1,3)
print'V15 is close'
self.Valve15.SetLabel(valve_status_OFF)
self.timer.Start(100)
self.dll.SetDigitalChannel(1,4)
print'V16 is open'
self.Valve16.SetLabel(valve_status_ON)
#self.timer.Start(20)
time.sleep(10)
self.dll.ClearDigitalChannel(1,4)
self.dll.SetDigitalChannel(1,5)
self.dll.SetDigitalChannel(1,6)
print'V16 is close'
self.Valve16.SetLabel(valve_status_OFF)
print'V17 is open'
self.Valve17.SetLabel(valve_status_ON)
print'V18 is open'
self.Valve18.SetLabel(valve_status_ON)
#self.timer.Start(500)
time.sleep(20)
self.dll.ClearDigitalChannel(1,5)
self.dll.ClearDigitalChannel(1,6)
print'V17 is close'
self.Valve17.SetLabel(valve_status_OFF)
print'V18 is close'
self.Valve18.SetLabel(valve_status_OFF)
self.dll.SetDigitalChannel(1,7)
print'V19 is open'
```



```

self.Valve19.SetLabel(valve_status_ON)
self.dll.SetDigitalChannel(1,8)
print'P4 is turn on'
#self.timer.Start(50)
time.sleep(10)
self.dll.ClearDigitalChannel(1,7)
print'V19 is close'
self.Valve19.SetLabel(valve_status_OFF)
self.dll.ClearDigitalChannel(1,8)
print'P4 is turn off'
self.dll.OutputAnalogChannel(1,1,255)
print'V20 is open'
self.Valve20.SetLabel(valve_status_ON)
#self.timer.Start(50)
time.sleep(5)
self.dll.OutputAnalogChannel(1,1,0)
print'V20 is close'
self.Valve20.SetLabel(valve_status_OFF)
self.dll.OutputAnalogChannel(1,2,255)
self.dll.OutputAnalogChannel(1,3,255)
print'V21 is open'
self.Valve21.SetLabel(valve_status_ON)
print'V22 is open'
self.Valve22.SetLabel(valve_status_ON)
#self.timer.Start(500)
time.sleep(20)
self.dll.OutputAnalogChannel(1,2,0)
self.dll.OutputAnalogChannel(1,3,0)
print'V21 is close'
self.Valve21.SetLabel(valve_status_OFF)
print'V22 is close'
self.Valve22.SetLabel(valve_status_OFF)
self.dll.OutputAnalogChannel(1,4,255)
self.dll.OutputAnalogChannel(1,5,255)
print'V23 is open'
self.Valve23.SetLabel(valve_status_ON)
print'P5 is turn On'
#self.timer.Start(40)

```

```

time.sleep(5)
self.dll.OutputAnalogChannel(1,4,0)
self.dll.OutputAnalogChannel(1,5,0)
print'V23 is close'
self.Valve23.SetLabel(valve_status_OFF)
print'P5 is turn off'
self.dll.OutputAnalogChannel(1,6,255)
print'V24 is open'
self.Valve24.SetLabel(valve_status_ON)
#self.timer.Start(500)
time.sleep(20)
self.dll.OutputAnalogChannel(1,6,0)
print'V24 is close'
self.Valve24.SetLabel(valve_status_OFF)
self.dll.OutputAnalogChannel(1,7,255)
print'V25 is open'
self.Valve25.SetLabel(valve_status_ON)
#self.timer.Start(50)
time.sleep(5)
self.dll.OutputAnalogChannel(1,7,0)
print'V25 is close'
self.Valve25.SetLabel(valve_status_OFF)
self.dll.OutputAnalogChannel(1,8,255)
print'V26 is open'
self.Valve26.SetLabel(valve_status_ON)
#self.timer.Start(600)
time.sleep(20)
self.dll.OutputAnalogChannel(1,8,0)
print'V26 is close'
self.Valve26.SetLabel(valve_status_OFF)
self.dll.SetDigitalChannel(2,1)
print'V27 is open'
self.Valve27.SetLabel(valve_status_ON)
#self.timer.Start(40)
time.sleep(5)
self.dll.ClearDigitalChannel(2,1)
print'V27 is close'
self.Valve27.SetLabel(valve_status_OFF)

```

```

self.dll.SetDigitalChannel(2,2)
print'V28 is open'
self.Valve28.SetLabel(valve_status_ON)
#self.timer.Start(60)
time.sleep(10)
self.dll.SetDigitalChannel(2,3)
self.dll.SetDigitalChannel(2,4)
print'V29 is open'
self.Valve29.SetLabel(valve_status_ON)
print'V30 is open'
self.Valve30.SetLabel(valve_status_ON)
self.dll.ClearDigitalChannel(2,2)
print'V28 is close'
self.Valve28.SetLabel(valve_status_OFF)
#self.timer.Start(40)
time.sleep(5)
self.dll.ClearDigitalChannel(2,3)
self.dll.ClearDigitalChannel(2,4)
print'V29 is close'
self.Valve29.SetLabel(valve_status_OFF)
print'V30 is close'
self.Valve30.SetLabel(valve_status_OFF)
#self.timer.Start(600)
time.sleep(20)
self.dll.SetDigitalChannel(2,5)
print'V31 is open'
self.Valve31.SetLabel(valve_status_ON)
self.dll.SetDigitalChannel(2,6)
print'P6 is turn on'
#self.timer.Start(50)
time.sleep(5)
self.dll.ClearDigitalChannel(2,5)
self.dll.ClearDigitalChannel(2,6)
print'V31 is close'
self.Valve31.SetLabel(valve_status_OFF)
print'P6 is turn off'
#self.timer.Start(40)
time.sleep(5)

```

```

self.dll.SetDigitalChannel(2,7)
print'V32 is open'
self.Valve32.SetLabel(valve_status_ON)
#self.timer.Start(600)
time.sleep(20)
print'V32 is close'
self.Valve32.SetLabel(valve_status_OFF)
self.dll.ClearDigitalChannel(2,7)
self.dll.SetDigitalChannel(2,8)
print'V33 is open'
self.Valve33.SetLabel(valve_status_ON)
#self.timer.Start(50)
time.sleep(5)
self.dll.ClearDigitalChannel(2,8)
print'V33 is close'
self.Valve33.SetLabel(valve_status_OFF)
self.dll.OutputAnalogChannel(2,1,255)
print'V34 is open'
self.Valve34.SetLabel(valve_status_ON)
#self.timer.Start(50)
time.sleep(5)
self.dll.OutputAnalogChannel(2,1,0)
print'V34 is close'
self.Valve34.SetLabel(valve_status_OFF)
self.dll.OutputAnalogChannel(2,2,255)

self.dll.OutputAnalogChannel(2,3,255)
print'V35 is open'
self.Valve35.SetLabel(valve_status_ON)
print'P7 is turn on'
#self.timer.Start(50)
time.sleep(5)
self.dll.OutputAnalogChannel(2,2,0)
self.dll.OutputAnalogChannel(2,3,0)
print'V35 is close'
print'P7 is turn off'
print'System is turning off'

```

else:

```
self.ON.SetLabel(valve_status_OFF)
wx.MessageBox("Turning OFF ", "System", wx.OK | wx.ICON_INFORMATION)
```

```
#self.dll.OutputAnalogChannel(3,8,255)
#
#def V1(self,event):
# valve_status_ON = 'ON'
# valve_status_OFF = 'OFF'

# self.OpenUSBBoardThread()
#time.sleep(0.5)
#self.dll.SetDigitalChannel(0,1)
#self.timer = wx.Timer()
#print'V1 is open'
#self.V1.Open()
#self.timer.Bind(wx.EVT_TIMER, self.on_timer)
#self.timer.Start(120)
#def V2 (self,event):
# self.OpenUSBBoardThread()
# self.dll.SetDigitalChannel(0,2)
# self.Valve1.SetLabel(valve_status_ON)
```

#else:

```
#self.ON.SetLabel(valve_status_ON)
#wx.MessageBox("Turning ON ", "System", wx.OK | wx.ICON_INFORMATION)
```

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)

```

```

def V2(self, event):

```

```

    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'

```

```

if self.Valve2.GetLabel()==valve_status_ON:
    self.Valve2.SetLabel(valve_status_OFF)
    # wx.MessageBox("V1 is Open ","Open", wx.OK|wx.ICON_INFORMATION)
else:
    self.Valve2.SetLabel(valve_status_ON)
    #wx.MessageBox("V1 is Close","Closed", wx.OK|wx.ICON_INFORMATION)

```

```

def V3(self, event):

```

```

    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'

```

```

if self.Valve3.GetLabel()==valve_status_ON:
    self.Valve3.SetLabel(valve_status_OFF)
    # wx.MessageBox("V1 is Open ","Open", wx.OK|wx.ICON_INFORMATION)
else:
    self.Valve3.SetLabel(valve_status_ON)
    #wx.MessageBox("V1 is Close","Closed", wx.OK|wx.ICON_INFORMATION)

```

```

def V4(self, event):

```

```

    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'

```

```

if self.Valve4.GetLabel()==valve_status_ON:
    self.Valve4.SetLabel(valve_status_OFF)
    # wx.MessageBox("V1 is Open ","Open", wx.OK|wx.ICON_INFORMATION)
else:
    self.Valve4.SetLabel(valve_status_ON)
    #wx.MessageBox("V1 is Close","Closed", wx.OK|wx.ICON_INFORMATION)

```

```
def V5(self, event):
```

```

    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'

```

```

if self.Valve5.GetLabel()==valve_status_ON:
    self.Valve5.SetLabel(valve_status_OFF)
    # wx.MessageBox("V1 is Open ","Open", wx.OK|wx.ICON_INFORMATION)
else:
    self.Valve5.SetLabel(valve_status_ON)
    #wx.MessageBox("V1 is Close","Closed", wx.OK|wx.ICON_INFORMATION)

```

```
def V6(self, event):
```

```

    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'

```

```

if self.Valve6.GetLabel()==valve_status_ON:
    self.Valve6.SetLabel(valve_status_OFF)
    # wx.MessageBox("V1 is Open ","Open", wx.OK|wx.ICON_INFORMATION)
else:
    self.Valve6.SetLabel(valve_status_ON)
    #wx.MessageBox("V1 is Close","Closed", wx.OK|wx.ICON_INFORMATION)

```

```
def V7(self, event):
```

```

    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'

```

```

if self.Valve7.GetLabel()==valve_status_ON:
    self.Valve7.SetLabel(valve_status_OFF)
    # wx.MessageBox("V1 is Open ","Open", wx.OK|wx.ICON_INFORMATION)
else:

```

```
self.Valve7.SetLabel(valve_status_ON)
#wx.MessageBox("V1 is Close","Closed", wx.OK | wx.ICON_INFORMATION)
```

```
def V8(self, event):
```

```
    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'
```

```
    if self.Valve8.GetLabel()==valve_status_ON:
```

```
        self.Valve8.SetLabel(valve_status_OFF)
        # wx.MessageBox("V1 is Open ","Open", wx.OK | wx.ICON_INFORMATION)
```

```
    else:
```

```
        self.Valve8.SetLabel(valve_status_ON)
        #wx.MessageBox("V1 is Close","Closed", wx.OK | wx.ICON_INFORMATION)
```

```
def V9(self, event):
```

```
    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'
```

```
    if self.Valve9.GetLabel()==valve_status_ON:
```

```
        self.Valve9.SetLabel(valve_status_OFF)
        # wx.MessageBox("V1 is Open ","Open", wx.OK | wx.ICON_INFORMATION)
```

```
    else:
```

```
        self.Valve9.SetLabel(valve_status_ON)
        #wx.MessageBox("V1 is Close","Closed", wx.OK | wx.ICON_INFORMATION)
```

```
def V10(self, event):
```

```
    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'
```

```
    if self.Valve10.GetLabel()==valve_status_ON:
```

```
        self.Valve10.SetLabel(valve_status_OFF)
```

```
    else:
```

```
        self.Valve10.SetLabel(valve_status_ON)
```

```
def V11(self, event):
```

```
    valve_status_ON = 'ON'
```



```
valve_status_OFF = 'OFF'
```

```
if self.Valve11.GetLabel()==valve_status_ON:
```

```
    self.Valve11.SetLabel(valve_status_OFF)
```

```
else:
```

```
    self.Valve11.SetLabel(valve_status_ON)
```

```
def V12(self, event):
```

```
    valve_status_ON = 'ON'
```

```
    valve_status_OFF = 'OFF'
```

```
if self.Valve12.GetLabel()==valve_status_ON:
```

```
    self.Valve12.SetLabel(valve_status_OFF)
```

```
else:
```

```
    self.Valve12.SetLabel(valve_status_ON)
```

```
def V13(self, event):
```

```
    valve_status_ON = 'ON'
```

```
    valve_status_OFF = 'OFF'
```

```
if self.Valve13.GetLabel()==valve_status_ON:
```

```
    self.Valve13.SetLabel(valve_status_OFF)
```

```
else:
```

```
    self.Valve13.SetLabel(valve_status_ON)
```

```
def V14(self, event):
```

```
    valve_status_ON = 'ON'
```

```
    valve_status_OFF = 'OFF'
```

```
if self.Valve14.GetLabel()==valve_status_ON:
```

```
    self.Valve14.SetLabel(valve_status_OFF)
```

```
else:
```

```
    self.Valve14.SetLabel(valve_status_ON)
```

```

def V15(self, event):
    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'

    if self.Valve15.GetLabel()==valve_status_ON:
        self.Valve15.SetLabel(valve_status_OFF)

    else:
        self.Valve15.SetLabel(valve_status_ON)

def V16(self, event):
    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'

    if self.Valve16.GetLabel()==valve_status_ON:
        self.Valve16.SetLabel(valve_status_OFF)

    else:
        self.Valve16.SetLabel(valve_status_ON)

def V17(self, event):
    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'

    if self.Valve17.GetLabel()==valve_status_ON:
        self.Valve17.SetLabel(valve_status_OFF)

    else:
        self.Valve17.SetLabel(valve_status_ON)

def V18(self, event):
    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'

    if self.Valve18.GetLabel()==valve_status_ON:
        self.Valve18.SetLabel(valve_status_OFF)

    else:
        self.Valve18.SetLabel(valve_status_ON)

```

```

def V19(self, event):
    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'

    if self.Valve19.GetLabel()==valve_status_ON:
        self.Valve19.SetLabel(valve_status_OFF)

    else:
        self.Valve19.SetLabel(valve_status_ON)

def V20(self, event):
    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'

    if self.Valve20.GetLabel()==valve_status_ON:
        self.Valve20.SetLabel(valve_status_OFF)

    else:
        self.Valve20.SetLabel(valve_status_ON)

def V21(self, event):
    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'

    if self.Valve21.GetLabel()==valve_status_ON:
        self.Valve21.SetLabel(valve_status_OFF)

    else:
        self.Valve21.SetLabel(valve_status_ON)

def V22(self, event):
    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'

    if self.Valve22.GetLabel()==valve_status_ON:
        self.Valve22.SetLabel(valve_status_OFF)

```

```
else:  
    self.Valve22.SetLabel(valve_status_ON)
```

```
def V23(self, event):
```

```
    valve_status_ON = 'ON'  
    valve_status_OFF = 'OFF'
```

```
    if self.Valve23.GetLabel()==valve_status_ON:  
        self.Valve23.SetLabel(valve_status_OFF)
```

```
    else:  
        self.Valve23.SetLabel(valve_status_ON)
```

```
def V24(self, event):
```

```
    valve_status_ON = 'ON'  
    valve_status_OFF = 'OFF'
```

```
    if self.Valve24.GetLabel()==valve_status_ON:  
        self.Valve24.SetLabel(valve_status_OFF)
```

```
    else:  
        self.Valve24.SetLabel(valve_status_ON)
```

```
def V25(self, event):
```

```
    valve_status_ON = 'ON'  
    valve_status_OFF = 'OFF'
```

```
    if self.Valve25.GetLabel()==valve_status_ON:  
        self.Valve25.SetLabel(valve_status_OFF)
```

```
    else:  
        self.Valve25.SetLabel(valve_status_ON)
```

```
def V26(self, event):
```

```
    valve_status_ON = 'ON'  
    valve_status_OFF = 'OFF'
```

```
    if self.Valve26.GetLabel()==valve_status_ON:
```

```

        self.Valve26.SetLabel(valve_status_OFF)

    else:
        self.Valve26.SetLabel(valve_status_ON)

def V27(self, event):
    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'

    if self.Valve27.GetLabel()==valve_status_ON:
        self.Valve27.SetLabel(valve_status_OFF)

    else:
        self.Valve27.SetLabel(valve_status_ON)

def V28(self, event):
    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'

    if self.Valve28.GetLabel()==valve_status_ON:
        self.Valve28.SetLabel(valve_status_OFF)

    else:
        self.Valve28.SetLabel(valve_status_ON)

def V29(self, event):
    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'

    if self.Valve29.GetLabel()==valve_status_ON:
        self.Valve29.SetLabel(valve_status_OFF)

    else:
        self.Valve29.SetLabel(valve_status_ON)

def V30(self, event):
    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'

```

```

if self.Valve30.GetLabel()==valve_status_ON:
    self.Valve30.SetLabel(valve_status_OFF)

else:
    self.Valve30.SetLabel(valve_status_ON)

def V31(self, event):
    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'

    if self.Valve31.GetLabel()==valve_status_ON:
        self.Valve31.SetLabel(valve_status_OFF)

    else:
        self.Valve31.SetLabel(valve_status_ON)

def V32(self, event):
    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'

    if self.Valve32.GetLabel()==valve_status_ON:
        self.Valve32.SetLabel(valve_status_OFF)

    else:
        self.Valve32.SetLabel(valve_status_ON)

def V33(self, event):
    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'

    if self.Valve33.GetLabel()==valve_status_ON:
        self.Valve33.SetLabel(valve_status_OFF)

    else:
        self.Valve33.SetLabel(valve_status_ON)

```

```
def V34(self, event):
    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'

    if self.Valve34.GetLabel()==valve_status_ON:
        self.Valve34.SetLabel(valve_status_OFF)

    else:
        self.Valve34.SetLabel(valve_status_ON)
```

```
def V35(self, event):
    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'

    if self.Valve35.GetLabel()==valve_status_ON:
        self.Valve35.SetLabel(valve_status_OFF)

    else:
        self.Valve35.SetLabel(valve_status_ON)
```

```
def V36(self, event):
    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'

    if self.Valve36.GetLabel()==valve_status_ON:
        self.Valve36.SetLabel(valve_status_OFF)

    else:
        self.Valve36.SetLabel(valve_status_ON)
```

```
def V37(self, event):
    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'

    if self.Valve37.GetLabel()==valve_status_ON:
        self.Valve37.SetLabel(valve_status_OFF)

    else:
```

```
self.Valve37.SetLabel(valve_status_ON)
```

```
def V38(self, event):
```

```
    valve_status_ON = 'ON'
```

```
    valve_status_OFF = 'OFF'
```

```
    if self.Valve38.GetLabel()==valve_status_ON:
```

```
        self.Valve38.SetLabel(valve_status_OFF)
```

```
    else:
```

```
        self.Valve38.SetLabel(valve_status_ON)
```

```
def V39(self, event):
```

```
    valve_status_ON = 'ON'
```

```
    valve_status_OFF = 'OFF'
```

```
    if self.Valve39.GetLabel()==valve_status_ON:
```

```
        self.Valve39.SetLabel(valve_status_OFF)
```

```
    else:
```

```
        self.Valve39.SetLabel(valve_status_ON)
```

```
def V40(self, event):
```

```
    valve_status_ON = 'ON'
```

```
    valve_status_OFF = 'OFF'
```

```
    if self.Valve40.GetLabel()==valve_status_ON:
```

```
        self.Valve40.SetLabel(valve_status_OFF)
```

```
    else:
```

```
        self.Valve40.SetLabel(valve_status_ON)
```

```
def V41(self, event):
```

```
    valve_status_ON = 'ON'
```

```
    valve_status_OFF = 'OFF'
```

```
    if self.Valve41.GetLabel()==valve_status_ON:
```

```
        self.Valve41.SetLabel(valve_status_OFF)
```



```

else:
    self.Valve41.SetLabel(valve_status_ON)

def V42(self, event):
    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'

    if self.Valve42.GetLabel()==valve_status_ON:
        self.Valve42.SetLabel(valve_status_OFF)

    else:
        self.Valve42.SetLabel(valve_status_ON)

def V43(self, event):
    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'

    if self.Valve43.GetLabel()==valve_status_ON:
        self.Valve43.SetLabel(valve_status_OFF)

    else:
        self.Valve43.SetLabel(valve_status_ON)

def V44(self, event):
    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'

    if self.Valve44.GetLabel()==valve_status_ON:
        self.Valve44.SetLabel(valve_status_OFF)

    else:
        self.Valve44.SetLabel(valve_status_ON)

def V45(self, event):
    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'

```

```
if self.Valve45.GetLabel()==valve_status_ON:  
    self.Valve45.SetLabel(valve_status_OFF)
```

```
else:
```

```
    self.Valve45.SetLabel(valve_status_ON)
```

```
def V46(self, event):
```

```
    valve_status_ON = 'ON'
```

```
    valve_status_OFF = 'OFF'
```

```
if self.Valve46.GetLabel()==valve_status_ON:
```

```
    self.Valve46.SetLabel(valve_status_OFF)
```

```
else:
```

```
    self.Valve46.SetLabel(valve_status_ON)
```

```
def V47(self, event):
```

```
    valve_status_ON = 'ON'
```

```
    valve_status_OFF = 'OFF'
```

```
if self.Valve47.GetLabel()==valve_status_ON:
```

```
    self.Valve47.SetLabel(valve_status_OFF)
```

```
else:
```

```
    self.Valve47.SetLabel(valve_status_ON)
```

```
def V48(self, event):
```

```
    valve_status_ON = 'ON'
```

```
    valve_status_OFF = 'OFF'
```

```
if self.Valve48.GetLabel()==valve_status_ON:
```

```
    self.Valve48.SetLabel(valve_status_OFF)
```

```
else:
```

```
    self.Valve48.SetLabel(valve_status_ON)
```

```
def V49(self, event):
```

```
    valve_status_ON = 'ON'
```

```
valve_status_OFF = 'OFF'
```

```
if self.Valve49.GetLabel()==valve_status_ON:
```

```
    self.Valve49.SetLabel(valve_status_OFF)
```

```
else:
```

```
    self.Valve49.SetLabel(valve_status_ON)
```

```
def V50(self, event):
```

```
    valve_status_ON = 'ON'
```

```
    valve_status_OFF = 'OFF'
```

```
if self.Valve50.GetLabel()==valve_status_ON:
```

```
    self.Valve50.SetLabel(valve_status_OFF)
```

```
else:
```

```
    self.Valve50.SetLabel(valve_status_ON)
```

```
def V51(self, event):
```

```
    valve_status_ON = 'ON'
```

```
    valve_status_OFF = 'OFF'
```

```
if self.Valve51.GetLabel()==valve_status_ON:
```

```
    self.Valve51.SetLabel(valve_status_OFF)
```

```
else:
```

```
    self.Valve51.SetLabel(valve_status_ON)
```

```
def V52(self, event):
```

```
    valve_status_ON = 'ON'
```

```
    valve_status_OFF = 'OFF'
```

```
if self.Valve52.GetLabel()==valve_status_ON:
```

```
    self.Valve52.SetLabel(valve_status_OFF)
```

```
else:
```

```
    self.Valve52.SetLabel(valve_status_ON)
```

```
def V53(self, event):
    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'

    if self.Valve53.GetLabel()==valve_status_ON:
        self.Valve53.SetLabel(valve_status_OFF)

    else:
        self.Valve53.SetLabel(valve_status_ON)
```

```
def V54(self, event):
    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'

    if self.Valve54.GetLabel()==valve_status_ON:
        self.Valve54.SetLabel(valve_status_OFF)

    else:
        self.Valve54.SetLabel(valve_status_ON)
```

```
def V55(self, event):
    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'

    if self.Valve55.GetLabel()==valve_status_ON:
        self.Valve55.SetLabel(valve_status_OFF)

    else:
        self.Valve55.SetLabel(valve_status_ON)
```

```
def V56(self, event):
    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'

    if self.Valve56.GetLabel()==valve_status_ON:
        self.Valve56.SetLabel(valve_status_OFF)

    else:
```

```
self.Valve56.SetLabel(valve_status_ON)
```

```
def V57(self, event):
```

```
    valve_status_ON = 'ON'
```

```
    valve_status_OFF = 'OFF'
```

```
    if self.Valve57.GetLabel()==valve_status_ON:
```

```
        self.Valve57.SetLabel(valve_status_OFF)
```

```
    else:
```

```
        self.Valve57.SetLabel(valve_status_ON)
```

```
def V58(self, event):
```

```
    valve_status_ON = 'ON'
```

```
    valve_status_OFF = 'OFF'
```

```
    if self.Valve58.GetLabel()==valve_status_ON:
```

```
        self.Valve58.SetLabel(valve_status_OFF)
```

```
    else:
```

```
        self.Valve58.SetLabel(valve_status_ON)
```

```
def V59(self, event):
```

```
    valve_status_ON = 'ON'
```

```
    valve_status_OFF = 'OFF'
```

```
    if self.Valve59.GetLabel()==valve_status_ON:
```

```
        self.Valve59.SetLabel(valve_status_OFF)
```

```
    else:
```

```
        self.Valve59.SetLabel(valve_status_ON)
```

```
def V60(self, event):
```

```
    valve_status_ON = 'ON'
```

```
    valve_status_OFF = 'OFF'
```

```
    if self.Valve60.GetLabel()==valve_status_ON:
```

```
        self.Valve60.SetLabel(valve_status_OFF)
```

```
else:  
    self.Valve60.SetLabel(valve_status_ON)
```

```
def V61(self, event):
```

```
    valve_status_ON = 'ON'  
    valve_status_OFF = 'OFF'
```

```
    if self.Valve60.GetLabel()==valve_status_ON:  
        self.Valve60.SetLabel(valve_status_OFF)
```

```
    else:  
        self.Valve60.SetLabel(valve_status_ON)
```

```
def V62(self, event):
```

```
    valve_status_ON = 'ON'  
    valve_status_OFF = 'OFF'
```

```
    if self.Valve60.GetLabel()==valve_status_ON:  
        self.Valve60.SetLabel(valve_status_OFF)
```

```
    else:  
        self.Valve60.SetLabel(valve_status_ON)
```

```
def V63(self, event):
```

```
    valve_status_ON = 'ON'  
    valve_status_OFF = 'OFF'
```

```
    if self.Valve60.GetLabel()==valve_status_ON:  
        self.Valve60.SetLabel(valve_status_OFF)
```

```
    else:  
        self.Valve60.SetLabel(valve_status_ON)
```

```
def V64(self, event):
```

```
    valve_status_ON = 'ON'
```

```

valve_status_OFF = 'OFF'

if self.Valve60.GetLabel()==valve_status_ON:
    self.Valve60.SetLabel(valve_status_OFF)

else:
    self.Valve60.SetLabel(valve_status_ON)

```

```

def V65(self, event):
    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'

    if self.Valve60.GetLabel()==valve_status_ON:
        self.Valve60.SetLabel(valve_status_OFF)

    else:
        self.Valve60.SetLabel(valve_status_ON)

```

```

def V66(self, event):
    valve_status_ON = 'ON'
    valve_status_OFF = 'OFF'

    if self.Valve60.GetLabel()==valve_status_ON:
        self.Valve60.SetLabel(valve_status_OFF)

    else:
        self.Valve60.SetLabel(valve_status_ON)

```

```

##### Define Pumps #####
#####

```

```

def P1 (self,event):

    wx.MessageBox("P1 is Open ", "Pump", wx.OK | wx.ICON_INFORMATION)

def P2 (self,event):

```

```
wx.MessageBox("P2 is Open ", "Pump", wx.OK | wx.ICON_INFORMATION)
```

```
def P3 (self,event):
```

```
wx.MessageBox("P3 is Open ", "Pump", wx.OK | wx.ICON_INFORMATION)
```

```
def P4 (self,event):
```

```
wx.MessageBox("P4 is Open ", "Pump", wx.OK | wx.ICON_INFORMATION)
```

```
def P5 (self,event):
```

```
wx.MessageBox("P5 is Open ", "Pump", wx.OK | wx.ICON_INFORMATION)
```

```
def P6 (self,event):
```

```
wx.MessageBox("P6 is Open ", "Pump", wx.OK | wx.ICON_INFORMATION)
```

```
def P7 (self,event):
```

```
wx.MessageBox("P7 is Open ", "Pump", wx.OK | wx.ICON_INFORMATION)
```

```
def P8 (self,event):
```

```
wx.MessageBox("P8 is Open ", "Pump", wx.OK | wx.ICON_INFORMATION)
```

```
def P9 (self,event):
```

```
wx.MessageBox("P9 is Open ", "Pump", wx.OK | wx.ICON_INFORMATION)
```

```
def P10 (self,event):
```

```
wx.MessageBox("P10 is Open ", "Pump", wx.OK | wx.ICON_INFORMATION)
```

```
#####
```



```
##### listbox #####  
#####
```

```
class ValveListBox(wx.Frame):
```

```

def __init__(self):

    wx.Frame.__init__(self, None, -1, 'Pumps list', size=(250,300))

    panel = wx.Panel(self,-1)

    #List1=Listbox()
    #List1.insert(1,'P1')
    #List1.insert(2,'P2')
    #List1.insert(3,'P3')
    #List1.insert(4,'P4')
    #List1.insert(5,'P5')
    #List1.insert(6,'P6')
    #List1.insert(7,'P7')
    #List1.insert(8,'P8')
    #List1.insert(9,'P9')
    # List1.insert(10,'P10')

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

    wx.StaticText(panel,20," Pumps Selected : ",(10,10))
    wx.StaticText(panel,-1," P1 ",(18,40))
    wx.StaticText(panel,-1," P2 ",(18,70))
    wx.StaticText(panel,-1," P3 ",(18,100))
    wx.StaticText(panel,-1," P4 ",(18,130))
    wx.StaticText(panel,-1," P5 ",(18,160))
    wx.StaticText(panel,-1," P6 ",(18,190))
    wx.StaticText(panel,-1," P7 ",(18,220))
    wx.StaticText(panel,-1," P8 ",(18,250))

    wx.StaticText(panel,-1," P9 ",(18,280))
    wx.StaticText(panel,-1," P10 ",(18,310))

    self.Pump1 = wx.StaticText(panel, size = (40, -1), pos = (60, 40), style = LABELSTYLE)
    self.Pump2 = wx.StaticText(panel, size = (40, -1), pos = (60, 70), style = LABELSTYLE)
    self.Pump3 = wx.StaticText(panel, size = (40, -1), pos = (60, 100), style = LABELSTYLE)

```

```

self.Pump4 = wx.StaticText(panel, size = (40, -1), pos = (60, 130), style = LABELSTYLE)
self.Pump5 = wx.StaticText(panel, size = (40, -1), pos = (60, 160), style = LABELSTYLE)
self.Pump6 = wx.StaticText(panel, size = (40, -1), pos = (60, 190), style = LABELSTYLE)
self.Pump7 = wx.StaticText(panel, size = (40, -1), pos = (60, 220), style = LABELSTYLE)
self.Pump8 = wx.StaticText(panel, size = (40, -1), pos = (60, 250), style = LABELSTYLE)
self.Pump9 = wx.StaticText(panel, size = (40, -1), pos = (60, 280), style = LABELSTYLE)
self.Pump10 = wx.StaticText(panel, size = (40, -1), pos = (60, 310), style = LABELSTYLE)

```

```

#sampleList= ['P1','P2','P3','P4','P5','P6','P7','P8','P9','P10']
#
'V21','V22','V23','V24','V25','V26','V27','V28','V29','V30','V31','V32','V33','V34','V35','V36','V37','V38','V39','V
40',
#      'V41','V42','V43','V44','V45','V46','V47','V48']
#wx.StaticText(panel,-1,"Pumps",(20,5))
#wx.ComboBox(panel,-1," valve ",(20,30),wx.DefaultSize,sampleList,wx.CB_DROPDOWN)
#wx.ComboBox(panel,-1," valve ",(150,30),wx.DefaultSize,sampleList,wx.CB_SIMPLE)

# listBox = wx.ListBox(panel, -1, (0, 0), (250, 300), sampleList,wx.LB_EXTENDED)
# listBox.SetSelection(3)

# listbox = wx.CheckListBox(panel,-1,(20,20),(100,300),sampleList, wx.LB_SINGLE)
def P1 (self,event):

    pump_status_ON = 'ON'
    pump_status_OFF = 'OFF'

    if self.Pump1.GetLabel()==pump_status_ON:
        self.Pump1.SetLabel(pump_status_OFF)

    else:
        self.Pump1.SetLabel(pump_status_ON)

```

```
wx.MessageBox("P1 is Open ", "Pump", wx.OK | wx.ICON_INFORMATION)
```

```
def P2 (self,event):
```

```
    wx.MessageBox("P2 is Open ", "Pump", wx.OK | wx.ICON_INFORMATION)
```

```
def P3 (self,event):
```

```
    wx.MessageBox("P3 is Open ", "Pump", wx.OK | wx.ICON_INFORMATION)
```

```
def P4 (self,event):
```

```
    wx.MessageBox("P4 is Open ", "Pump", wx.OK | wx.ICON_INFORMATION)
```

```
def P5 (self,event):
```

```
    wx.MessageBox("P5 is Open ", "Pump", wx.OK | wx.ICON_INFORMATION)
```

```
def P6 (self,event):
```

```
    wx.MessageBox("P6 is Open ", "Pump", wx.OK | wx.ICON_INFORMATION)
```

```
def P7 (self,event):
```

```
    wx.MessageBox("P7 is Open ", "Pump", wx.OK | wx.ICON_INFORMATION)
```

```
def P8 (self,event):
```

```
    wx.MessageBox("P8 is Open ", "Pump", wx.OK | wx.ICON_INFORMATION)
```

```
def P9 (self,event):
```

```
    wx.MessageBox("P9 is Open ", "Pump", wx.OK | wx.ICON_INFORMATION)
```

```
def P10 (self,event):
```

```
    wx.MessageBox("P10 is Open ", "Pump", wx.OK | wx.ICON_INFORMATION)
```

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

```
def main():
```

```
    """"Testing""""
```

```

MyBackgroundPanel = wx.PySimpleApp(wx.Panel)
f = MyFrame()

```

#in D:\AECENAR\MEGBI\MEGBI-VPP\AUT\CDMasterThesisHayssamHindy\GUI\101005_MEGBI-VPP_downstream.py the following two lines are not in comment

```

#d=ValveListBox()
#d.Show()
f.Center()
f.Show()
MyBackgroundPanel.MainLoop()

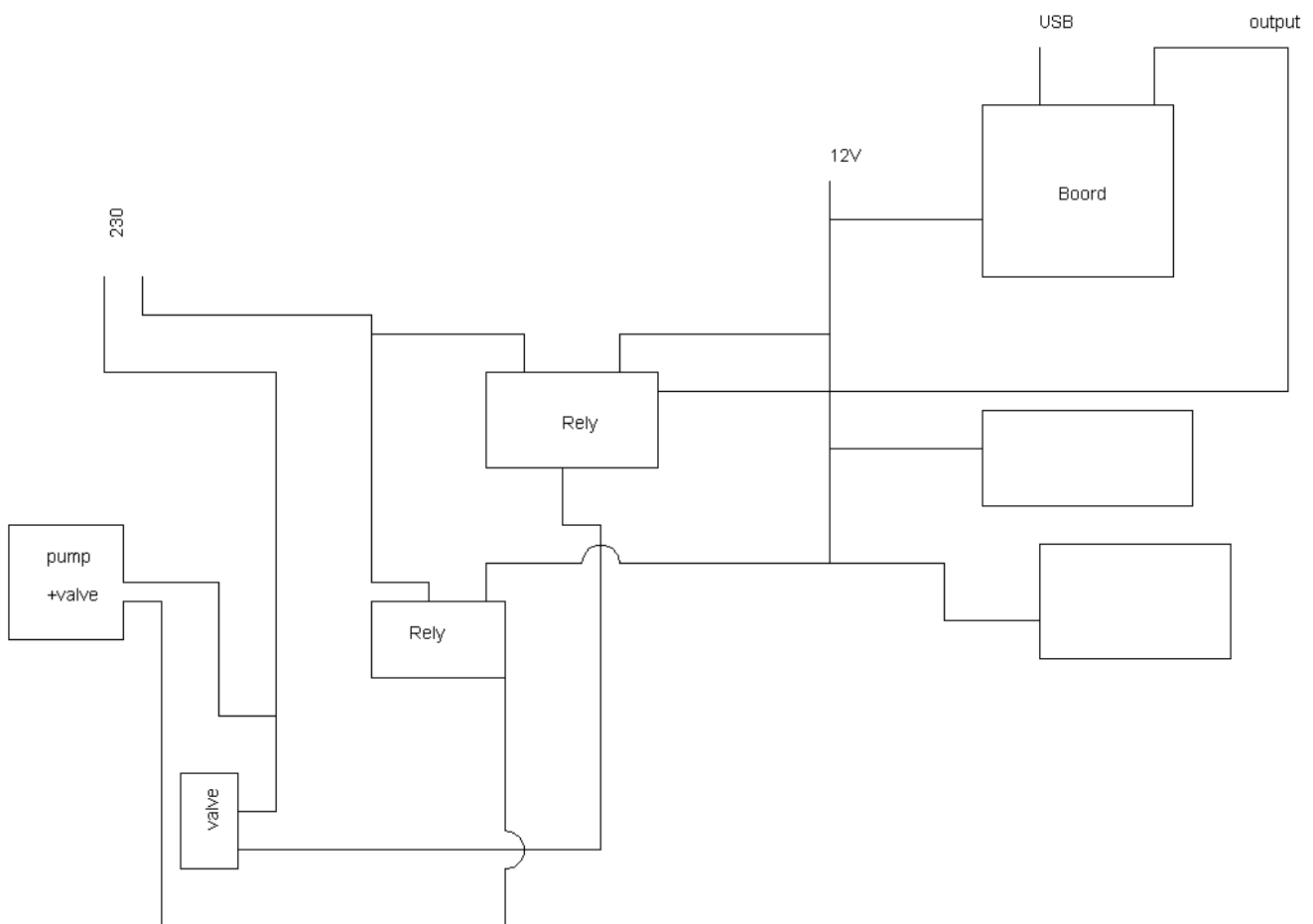
```

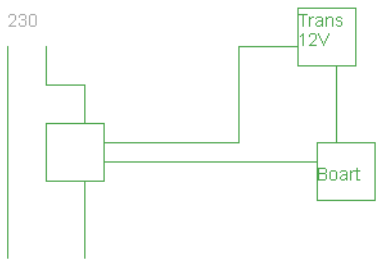
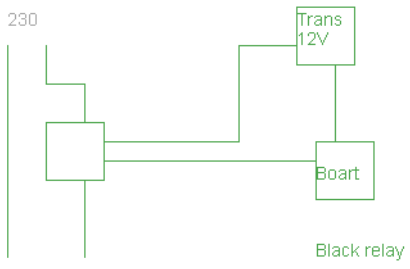
```

if __name__ == "__main__":
    main()

```

9.5 Annex 3: Electrical circuits for connection between veleman board, relais and actuators (pump. valve)





10. References / مراجع

- [1] <http://www.aecenar.com/publications>
- [2] http://www.aecenar.com/downloads/cat_view/7-megbi-institute
- [3] http://www.aecenar.com/downloads/cat_view/3-meae-institute?start=10
- [4] <http://www.eio.com/p-1603-velleman-k8061-extended-usb-interface-board.aspx>
- [5] http://www.apogeekits.com/images/usb_io_interface.jpg
- [6] www.pc-control.co.uk/relays.htm
- [7] <http://www.velleman.eu/products/view/?id=364910>
- [8] <http://www.tutorialspoint.com/python/>
- [9] <https://www.draw.io/>

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