Interactive Session:

Designing a virus filtration process - assumption and points to consider, How to design a process, Calculating production costs

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Designing a Virus Filtration Process

In the following slides we will discuss filtration process:

- related to **Filterability** (Flux, Volume)
- but NOT related to virus removal (LRV)

The presentation will give guidelines and strategical approaches, but cannot guarantee the performances for the manufacturers particular process and protein- solutions

Designing a Virus Filtration Process

Vocabulary:

TransMembrane Pressure (TMP): $P_{Feed} - P_{Filtrate}$ = kPa

Flow rate: Filtration Volume / min or h = L/h or L/min

Flux: « Flow rate / Filtration surface area» = $L/h/m^2$ or LMH

Filtration Capacity: « Volume / Filtration surface area» = L/m^2

Mass Throughput: « g of target protein / Filtration surface area» = g or kg / m^2

Before the filter: Feed solution **After the filter:** Permeate or Filtrate solution

Designing a Virus Filtration Process

- Main equations:

  S (filtration surface area) = Volume of protein solution / (**Flux** x Time)

  or

  S (filtration surface area) = Volume of protein solution / (**Filtration Capacity**)

- Why **filtration flux & capacity** important ?
  - To reduce the filtration surface area (S)
  - To reduce filtration time
  - To reduce cost !

Designing a Virus Filtration Process

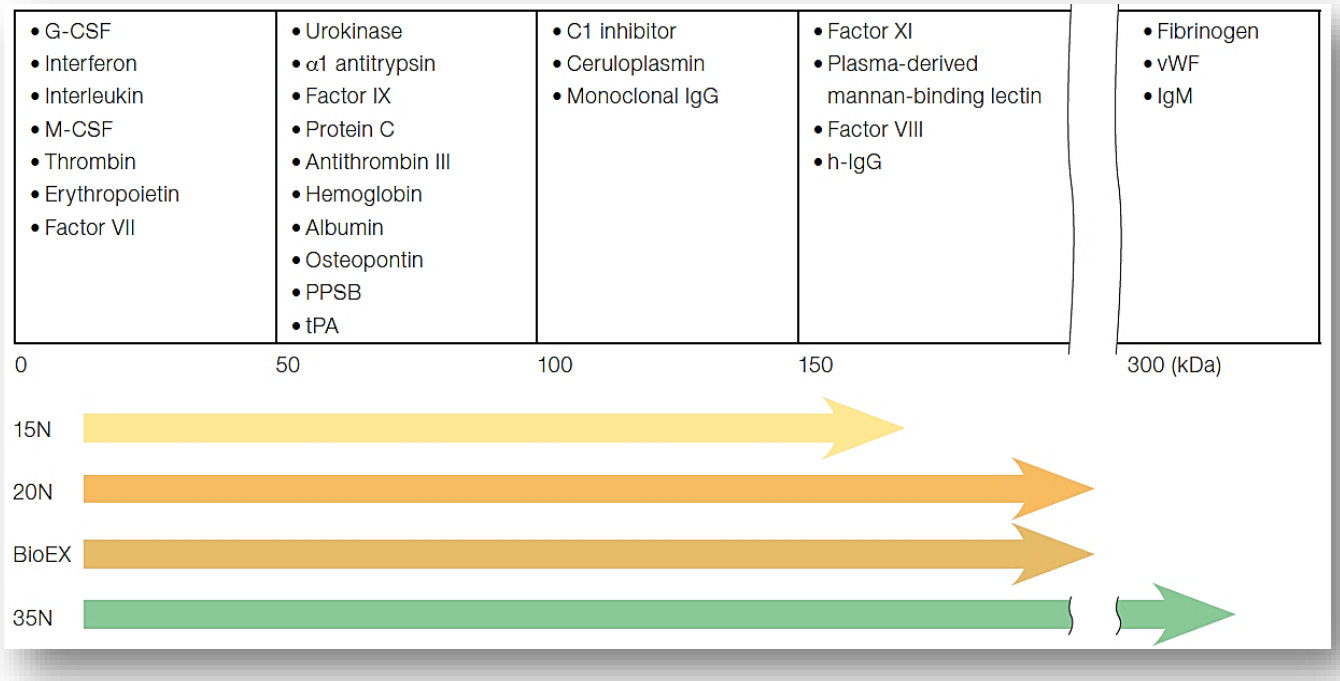
- **Preliminary information required (if known):**

  - Plasma derived product or product from cell culture?
  - Protein type (mAb, Fab, clotting factor, etc..), sensitivity to temperature & other stress
  - Molecular weight
  - Buffer composition, concentrations, pH and conductivity
  - Concentration of protein prior to nanofiltration step
  - Commercial batch volume to be nanofiltered at once
  - Maximum acceptable time for nanofiltration
  - Purification steps prior to nanofiltration
  - Target virus to remove, expected removal (LRV)
  - Other removal/inactivation steps
  - Availability of fresh solution for lab scale trials
  - Single use or SS equipment

Designing a Virus Filtration Process

- **Preselection of the nanofilter (pore size, parvovirus/retrovirus removal):**
  - ✓ What is the size of my target protein ?
  - ✓ Can I use a parvovirus removal filter grade ?

Example: easier to nanofilter Fibrinogen with Planova 35N vs. 20N

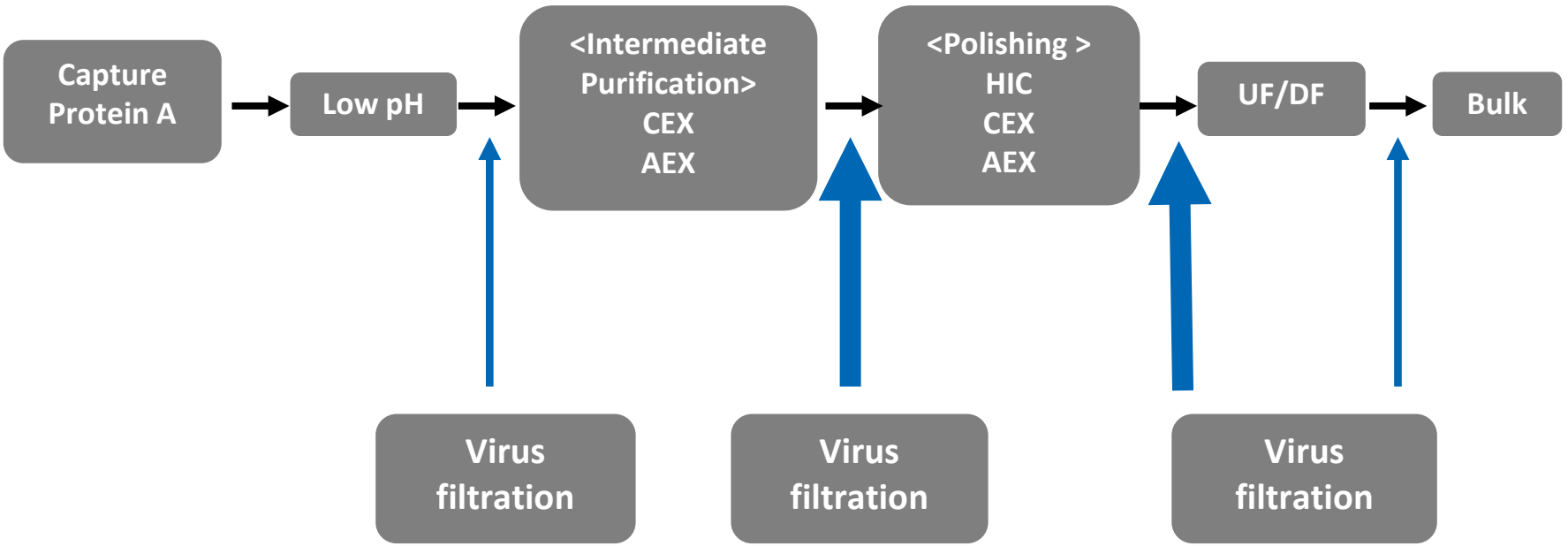


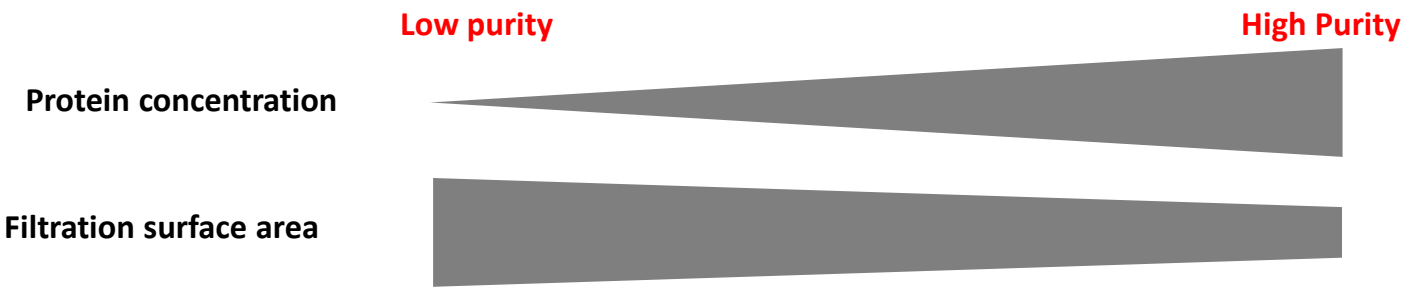
Designing a Virus Filtration Process

- **What protein concentration ?**
  - ✓ Always an optimal concentration to get the maximum mass throughput (g or kg /m^2)
  - ✓ The higher the concentration, the higher the mass throughput until a certain point, then decline
  - ✓ Main reason: **Polarization Layer** (= “traffic jam")

Designing a Virus Filtration Process

- **What protein concentration ?**

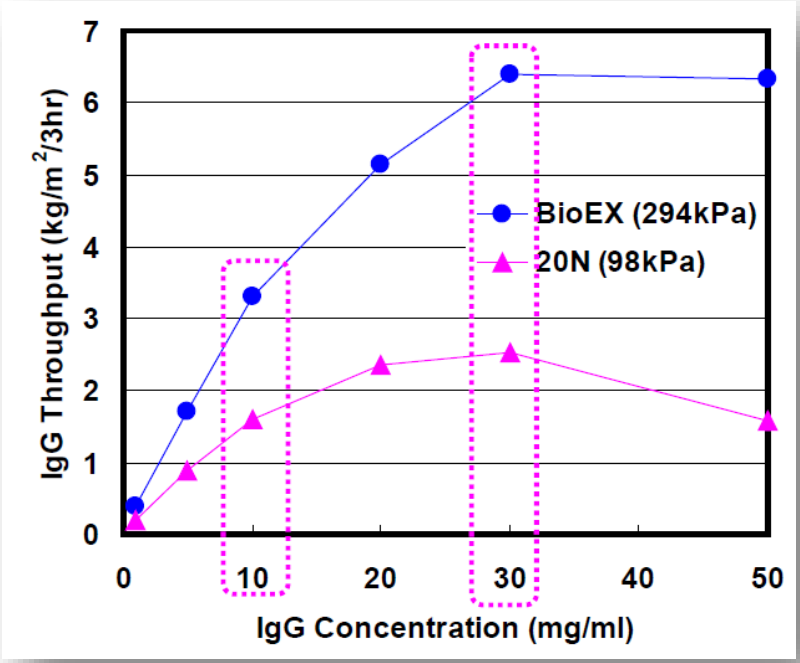




Designing a Virus Filtration Process

- **Which protein concentration ?**

Solution : **h-IgG** (polyclonal) in 100 mM NaCl, pH 4.5
Filtration pressure : 79 kPa (20N), 294 kPa (BioEX) **Filtration time : 3 h**

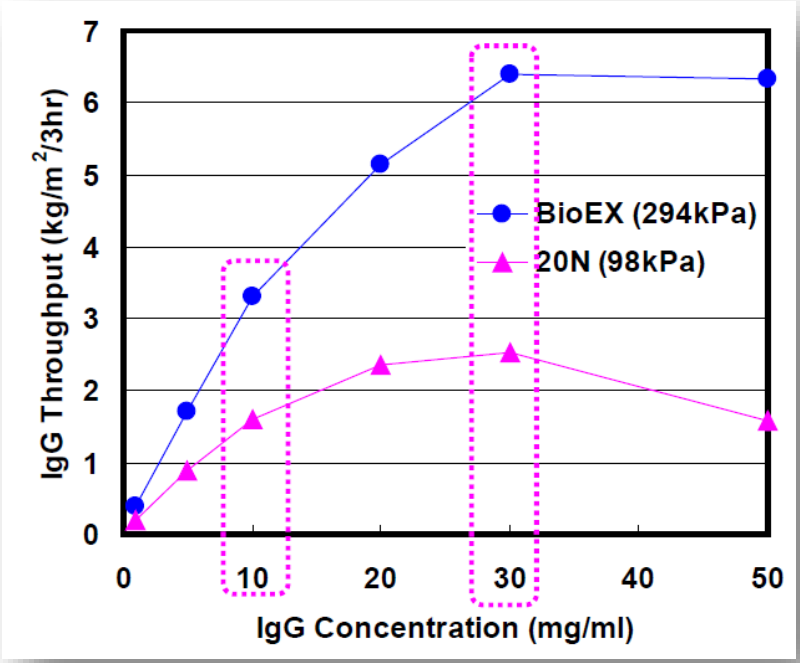


- ✓ Could be different with another product, buffer or operating conditions
- ✓ Optimal concentration **~30 g/L**

Designing a Virus Filtration Process

- **Which protein concentration ?**

Solution : **h-IgG** (polyclonal) in 100 mM NaCl, pH 4.5
Filtration pressure : 79 kPa (20N), 294 kPa (BioEX) **Filtration time : 3 h**

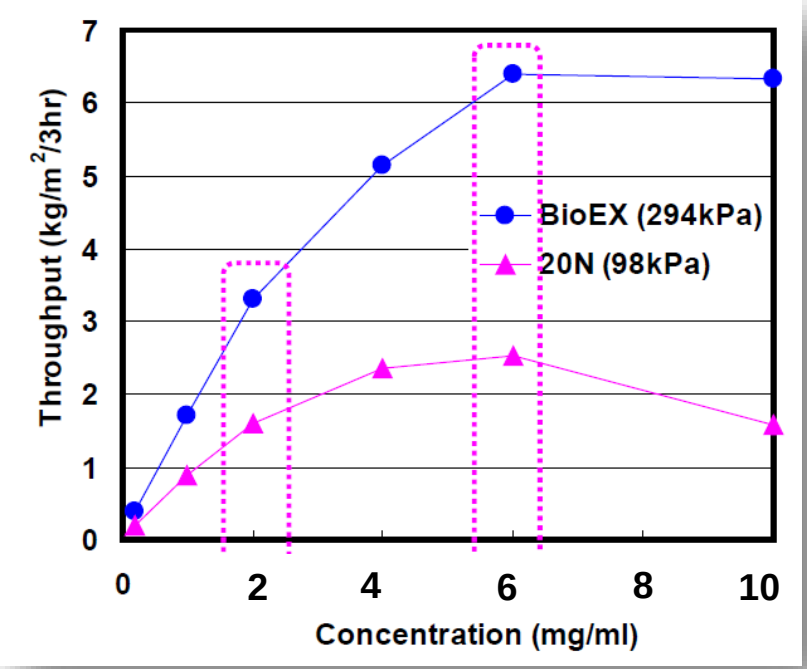


10 kg IgG to nanofilter:

- ✓ **at 10 mg/mL:**
  - 20N: 1.5 kg/m² ➔ 6.7 m²
  - BioEX: 3.5 kg/m² ➔ 2.9 m²
- ✓ **at 30 mg/mL:**
  - 20N: 2.5 kg/m² ➔ 4 m²
  - BioEX: 6.5 kg/m² ➔ 1.5 m²

Designing a Virus Filtration Process

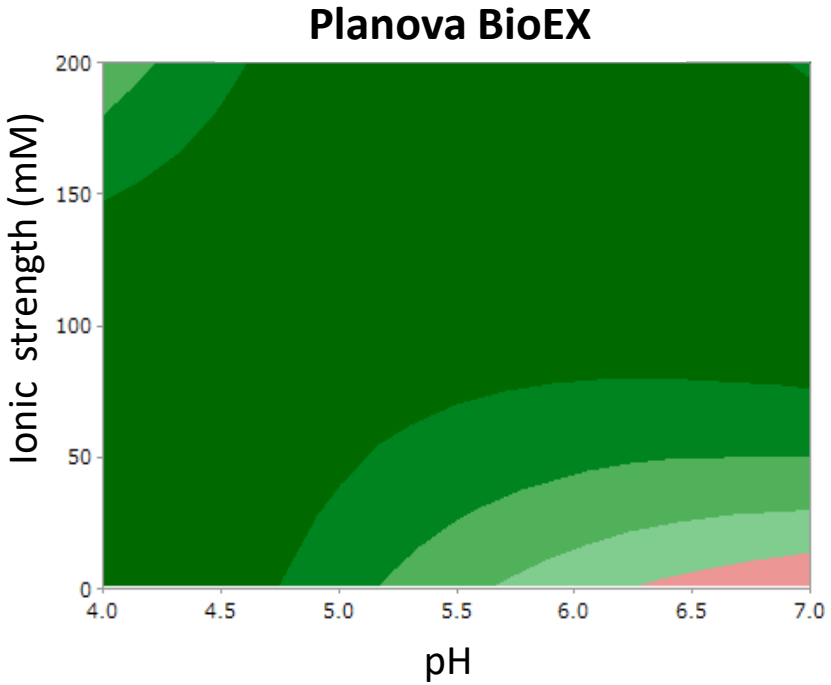
- **What protein concentration ?**
  - ✓ Could be different with another product, buffer or operating conditions
  - ✓ Sometime it is needed to dilute to lower the protein concentration !



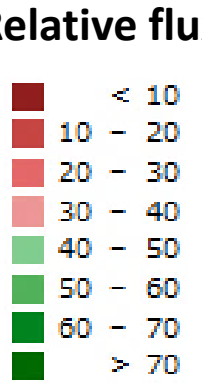
Designing a Virus Filtration Process

- **What pH and Conductivity?**

  Product solution: 10 mg/mL h-IgG
  Filtration time: 3 h



Relative flux (%) = (3 h flux / initial flux) x 100



Hongo-Hirasaki, Asahi Kasei Medical,
Planova Workshop, Prague, 2017 (adapted)

Designing a Virus Filtration Process

- **What prefiltration?**

	High Flux Nanofilter	High Capacity Nanofilter
Brand	VPro (Merck) Virosart CPV & HF (Sartorius), Pegasus Prime (Pall)	Virosart HC (Sartorius), Pegasus SV4 & DV20 (Pall), Planova 20N & BioEX (Asahi)
Prefiltration	Special or adsorptive prefilters	None or simple 0.2 or 0.1µm

- ✓ Main parameter impacting flux or filtration capacity ➔ aggregate content
- ✓ Other process-specific contaminants possible
- ✓ **In case of very difficult filterability ➔ Prefilter**

Designing a Virus Filtration Process

- **What Transmembrane Pressure (TMP)?**

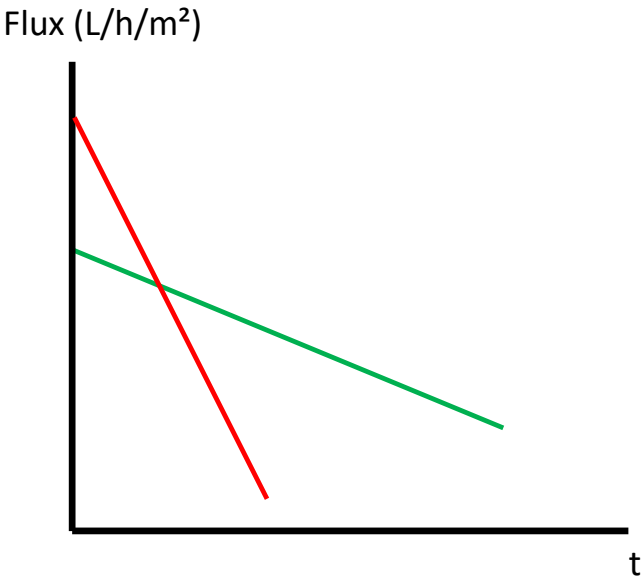
  - ✓ To follow the recommendations of each nanofilter supplier
  - ✓ To anticipate Manufacturing constraints (max pressure, equipment)
  - ✓ Increase of TMP can improve flux (= lower filtration area) if “reasonable” flux decay. From 80 kPa to 98 kPa ➔ + 22.5% water flux
  - ✓ With a too prominent flux decay, or too high impurity-levels, or high Molecular Weight protein ➔ Decrease TMP !

Designing a Virus Filtration Process

- **What filtration time?**

  - ✓ To anticipate Manufacturing constraints (working time shift, etc…)

  - ✓ The shorter not always the better in terms of cost effectiveness! Calculate the gain of longer filtration time on filtration surface area



For the green curve, extending filtration time can be very beneficial

Designing a Virus Filtration Process

- **What DoE for preliminary trials?**
  - ✓ Use the experience of the nanofilter supplier!
  - ✓ As shown until now, many parameters can impact on the nanofiltration performances
  - ✓ **Strategy:**
    1) **Understand your product:** what protein, MW, pI, pressure or temp sensitive, etc…
    2) **Understand your process conditions & constraints:** buffer, max desired filtration time, max possible pressure, place of the nanofiltration in the process strain, etc…
    3) **Preliminary DOE** with a few variables and using current buffer
    4) **Results** of the preliminary experiments ➔ "**optimized" DOE**

Designing a Virus Filtration Process

- **What DoE for preliminary trials?**

  Example:

  ✓ **Product:** ~ 100 kD, no temp or pressure sensitive, tendency to aggregation

  ✓ **Process:** no specific process constraints, 6 h filtration time max

  ✓ **Preliminary DOE:**

  1) **Nanofilters:** 20N, BioEX
  2) **Prefilter:** standard 0.1 µm (off-line is ok)
  3) **TMP:** 50 & 98 kPa for 20N; 150 & 300 kPa for BioEX
  4) **Conc.:** before & after last chrom., before & after last UF/DF
  5) **Product:** fresh (if possible)
  6) **Buffers:** as currently in the different process steps
  7) **Filtration time:** 6 h, at least 3 h

Designing a Virus Filtration Process

- **What DoE for preliminary trials?**

  Example:

  ✓ **Product:** ~ 100 kD, no temp or pressure sensitive, tendency to aggregation

  ✓ **Process:** no specific process constraints, 6 h filtration time max

  ✓ **Preliminary DOE:** **limited volume available or no time…**

  1) **Nanofilters:** 20N, BioEX
  2) **Prefilter:** standard 0.1 µm (off-line is ok)
  3) **TMP:** ~~50 &~~ **98 kPa for 20N**; ~~150 &~~ **300 kPa for BioEX**
  4) **Conc.:** ~~before & after last chrom.~~, **before & after last UF/DF**
  5) **Product:** fresh (if possible)
  6) **Buffers:** as currently in the different process steps
  7) **Filtration time:** ~~6 h,~~ **at least 3 h**

Designing a Virus Filtration Process

- **Reality on the field can be different… "evolving" DoE**

Solution : **monoclonal IgG 1,** 24 mg/mL, 10 mM NaCl, pH 5.5
Filtration pressure : 98 kPa (20N-0.001m^2); 294 kPa (BioEX-0.001m^2)
0.1 µm prefiltration

Objectif: use of BioEX to filter high concentration solution…

Test 1:

Filter	BioEx
Product concentration (mg/mL)	24
Buffer	pH 5.5
Total filtration time (min) :	15
Total filtration volume (mL) :	0,6
Average flux (L/h/m2) :	2,4
Pressure (bar) :	2,0
Vmax (L/m2) :	1,5

✓ It seems it will not be so easy ☺

Designing a Virus Filtration Process

- **Reality on the field can be different… "evolving" DoE**

Test 2:

Filter	BioEx
Product concentration (mg/mL)	12
Buffer	pH 5.5
Total filtration time (min) :	38
Total filtration volume (mL) :	7,8
Average flux (L/h/m2) :	12,3
Pressure (bar) :	2,0
Vmax (L/m2) :	25

Test 3:

Filter	P20N
Product concentration (mg/mL)	12
Buffer	pH 5.5
Total filtration time (min) :	65
Total filtration volume (mL) :	4,0
Average flux (L/h/m2) :	3,7
Pressure (bar) :	0,8
Vmax (L/m2) :	9

✓ First action: decrease protein concentration.

… but no significant positive impact

✓ Even 20N cannot nanofilter the solution

Designing a Virus Filtration Process

- **Reality on the field can be different… "evolving" DoE**

Test 4:

Filter	P20N
Product concentration (mg/mL)	12
Buffer	pH 6.6 - 60 mM NaCl
Total filtration time (min) :	35
Total filtration volume (mL) :	22,8
Average flux (L/h/m2) :	39,0
Pressure (bar) :	0,8
Vmax (L/m2) :	> 500

Test 5:

Filter	P20N
Product concentration (mg/mL)	12
Buffer	pH 5.5 - 100 mM NaCl
Total filtration time (min) :	30
Total filtration volume (mL) :	20,1
Average flux (L/h/m2) :	40,2
Pressure (bar) :	0,8
Vmax (L/m2) :	> 800

- ✓ Second action: change pH and conductivity
- ✓ Higher conductivity has a significant positive impact (High Vmax)

Designing a Virus Filtration Process

- **Reality on the field can be different… "evolving" DoE**

Test 6:

Filter	P20N
Product concentration (mg/mL)	24
Buffer	pH 5.5 - 100 mM NaCl
Total filtration time (min) :	10
Total filtration volume (mL) :	1,3
Average flux (L/h/m2) :	7,5
Pressure (bar) :	0,8
Vmax (L/m2) :	3,15

Test 7:

Filter	BioEx
Product concentration (mg/mL)	24
Buffer	pH 5.5 - 100 mM NaCl
Total filtration time (min) :	35
Total filtration volume (mL) :	30,6
Average flux (L/h/m2) :	52,4
Pressure (bar) :	2 - 3
Vmax (L/m2) :	> 300

- ✓ Third action: back to the initial high concentration
- ✓ Concentration too high for 20N but BioEX works well !

Designing a Virus Filtration Process

- **Data Analysis (Filtration Study Report, Estimation L/m^2, Vmax)**

AsahiKASEI BIOPROCESS

Planova Filtration Report - Study n° XX/XXX/XX
Confidential

1. Objective

The following filtration study took place on the 26th and 27th of January 2016 at X. It was carried out using Planova™ 20N and Planova™ BioEX filters.

The 3 main objectives were 1) to compare the performances of Planova™ 20N and Planova™ BioEX filters at lab scale, 2) to evaluate the impact of a dilution on the filterability of the plasma derived IVIG solution at lab scale, and 3) to perform nanofiltration runs at pilot scale.

3. Manufacturing conditions

Protein:	IVIG (160 kD)
Concentration (total proteins):	~ 33 g/L; possibility to dilute down to ~25 g/L
Buffer/Washing solution:	10 mM NaCl, 275 mM Glycine, pH 4.136, Conductivity: 1.6 mS/cm
Volume to nanofilter:	810 L or 1070 L at respectively 33 g/L and 25 g/L (total proteins)
Acceptable nanofiltration time:	6 h maximum

Designing a Virus Filtration Process

- **Data Analysis (Filtration Study Report, Estimation L/m^2, Vmax)**

4. Experimental materials

Planova™ filters:	20N (0.001 m^2 and 0.12 m^2) and BioEX (0.001 m^2)
Pre-filter(s):	0.2/0.1 µm (Pall Fluorodyne II Mini Kleenpak)
Protein solution:	~ 3 L were prepared by X (each day) from their pilot scale production process
Total proteins concentration:	Day 1: 32.61 g/L; 25.22 g/L (after dilution) Day 2: 33.53 g/L; 26.39 g/L (after dilution)

Designing a Virus Filtration Process

- **Data Analysis (Filtration Study Report, Estimation L/m^2, Vmax)**

Photo 1 - Setups with 2 filters 20N-0.001m^2 and 2 filters BioEX-0.001m^2

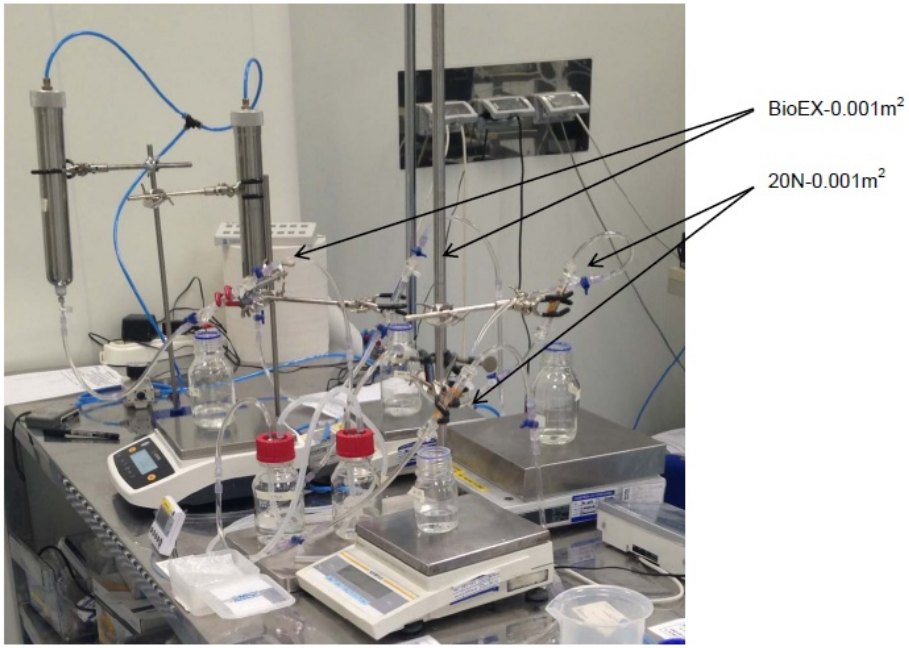


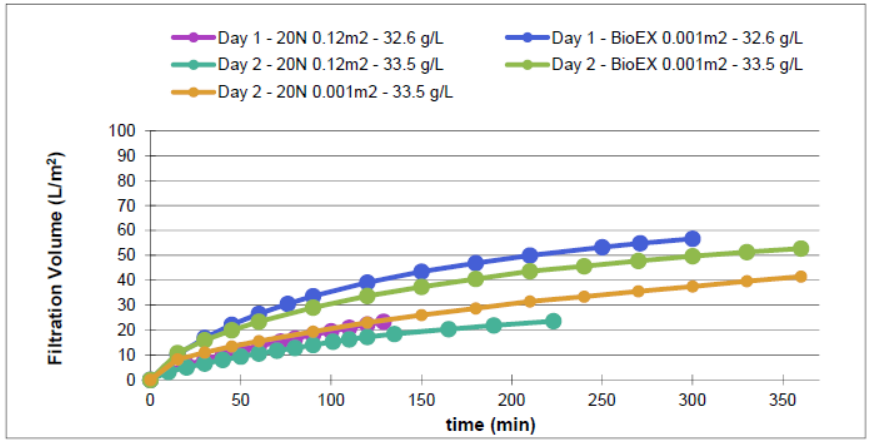
Photo 2 - Setup with 20N-0.12m^2

Designing a Virus Filtration Process

- **Data Analysis (Filtration Study Report, Estimation L/m², Vmax)**

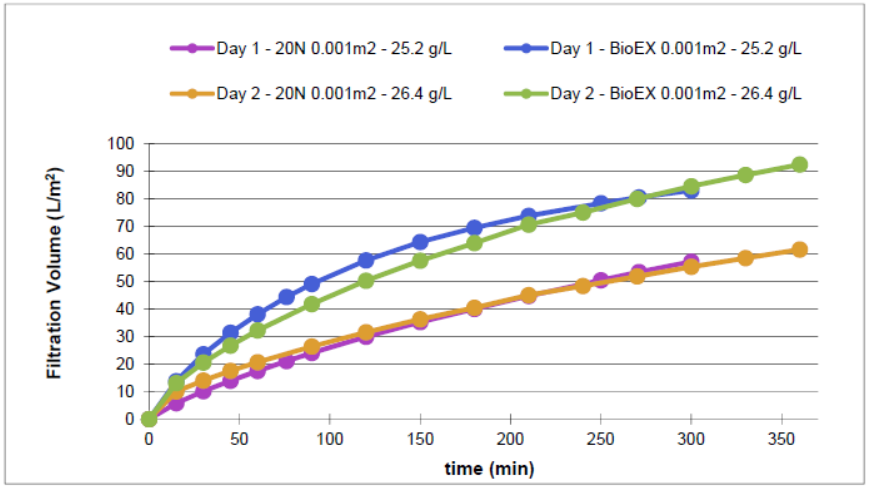
6.2.1 Total proteins concentration ~ 33 g/L

Graph 1 - Filtration Volume vs. Time



6.2.2 Total proteins concentration ~ 25 g/L

Graph 2 - Filtration Volume vs. Time

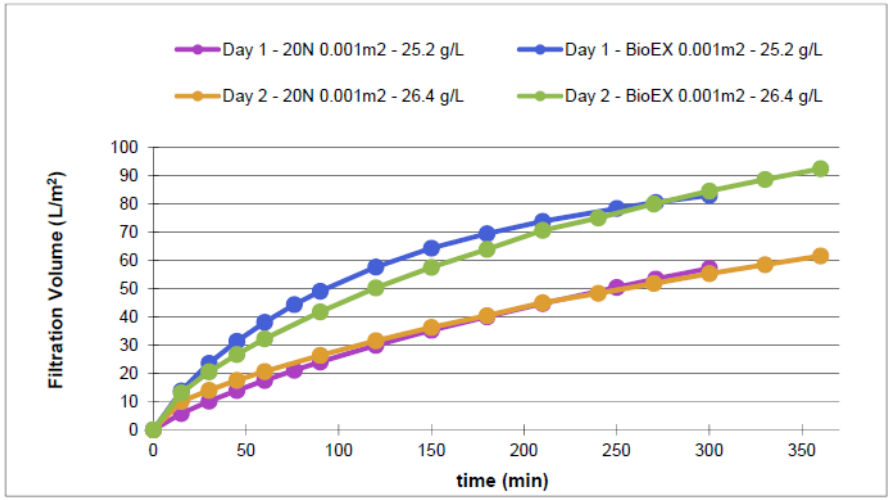


High consistency and scalability of the performances
Positive impact of the dilution on the Filtration Capacity (L/m²)

Designing a Virus Filtration Process

- **Data Analysis (Filtration Study Report, Estimation L/m², Vmax)**

Total proteins conc. (g/L)	Volume (L)	Filter type	TMP (kPa)	Filter Configuration	Filtration Area (m²)	Filtration Volume (L/m²)	Filtration Time (h)
25	1070						



Filter range:
20N-1m², 20N-4m2
BioEX-1m2, BioEX-4m2

Filter cost (example only):
20N: 3000 €/m2
BioEX: 6000 €/m2

Designing a Virus Filtration Process

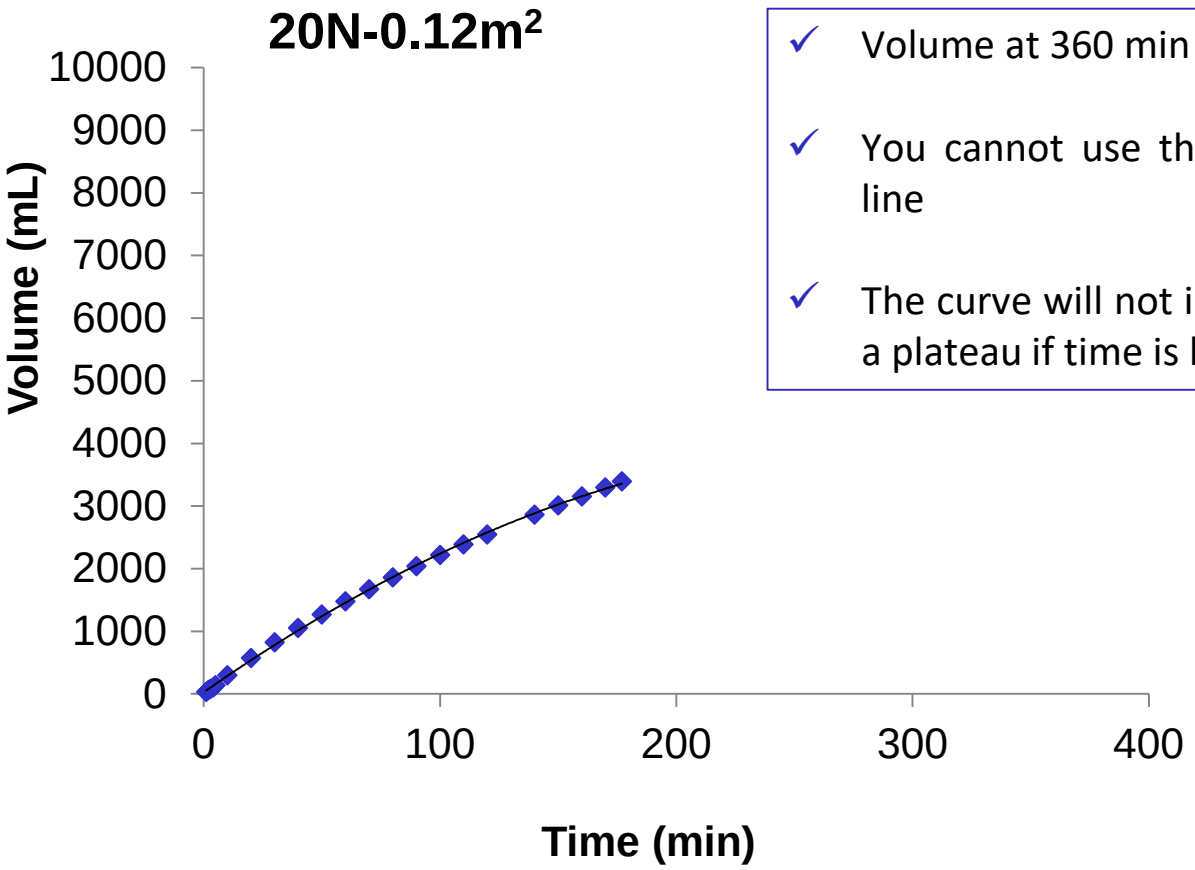
- **Data Analysis (Filtration Study Report, Estimation L/m², Vmax)**

Total proteins conc. (g/L)	Volume (L)	Filter type	TMP (kPa)	Filter Configuration	Filtration Area (m²)	Filtration Volume (L/m²)	Filtration Time (h)
25	1070	20N	98	5 filters 20N-4.0 m²	20	54	5
		BioEX	300	3 filters BioEX-4.0 m²	12	89	6
		BioEX	300	4 filters BioEX-4.0 m²	16	67	3,5

1. "Fix" the filter configuration based on the product ranges available ➔ Total filtration surface area (m^2)
2. Nanofiltration Volume / Total filtration surface area ➔ Filtration capacity (L/m^2)
3. 2 situations:
   - if the Filtration Capacity was already reached during the trial run you can directly pinpoint the corresponding filtration time.
   - if you stopped the trial run before achieving the needed Filtration Capacity, you will have to estimate the filtration time (*next slides*).

Designing a Virus Filtration Process

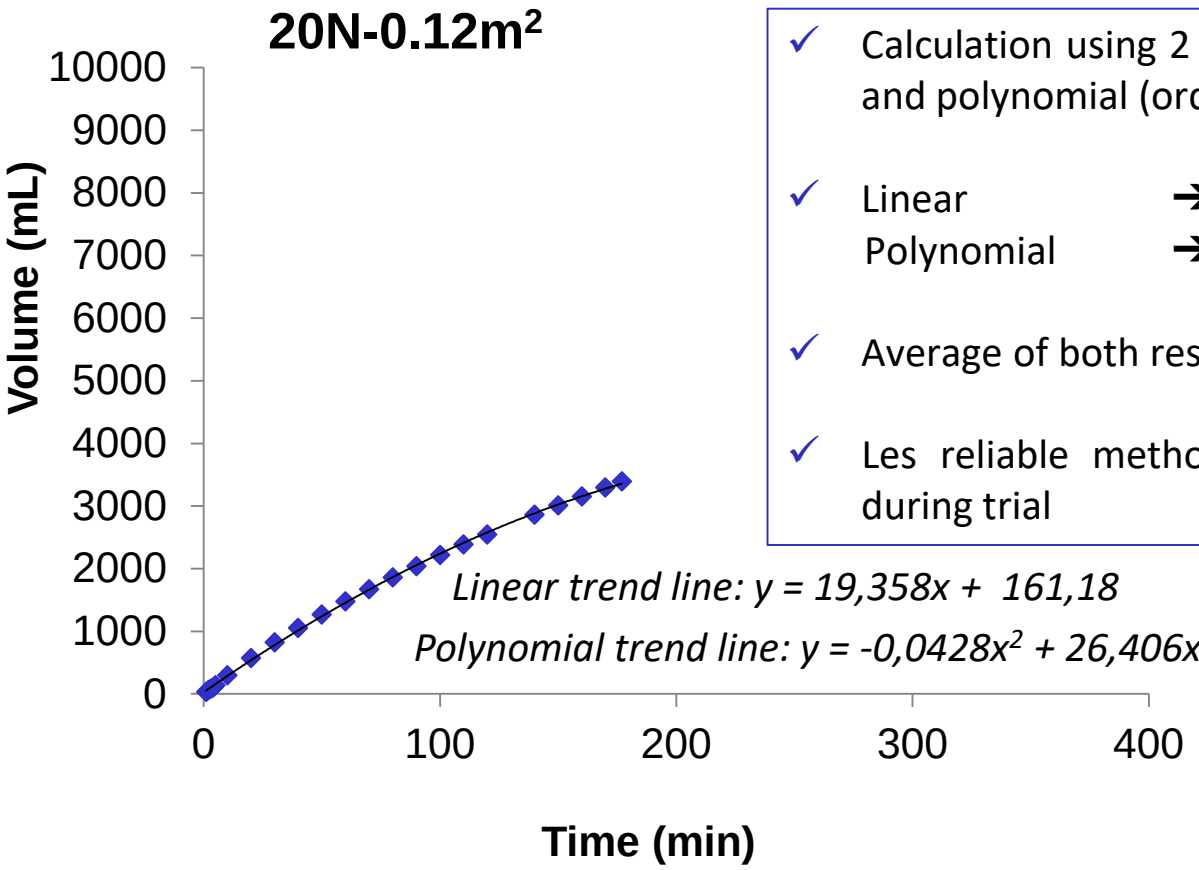
- **Data Analysis (Filtration Study Report, Estimation L/m², Vmax)**



- ✓ Volume at 360 min ?
- ✓ You cannot use the linear equation of the trend line
- ✓ The curve will not increase linearly but will tend to a plateau if time is long enough.

Designing a Virus Filtration Process

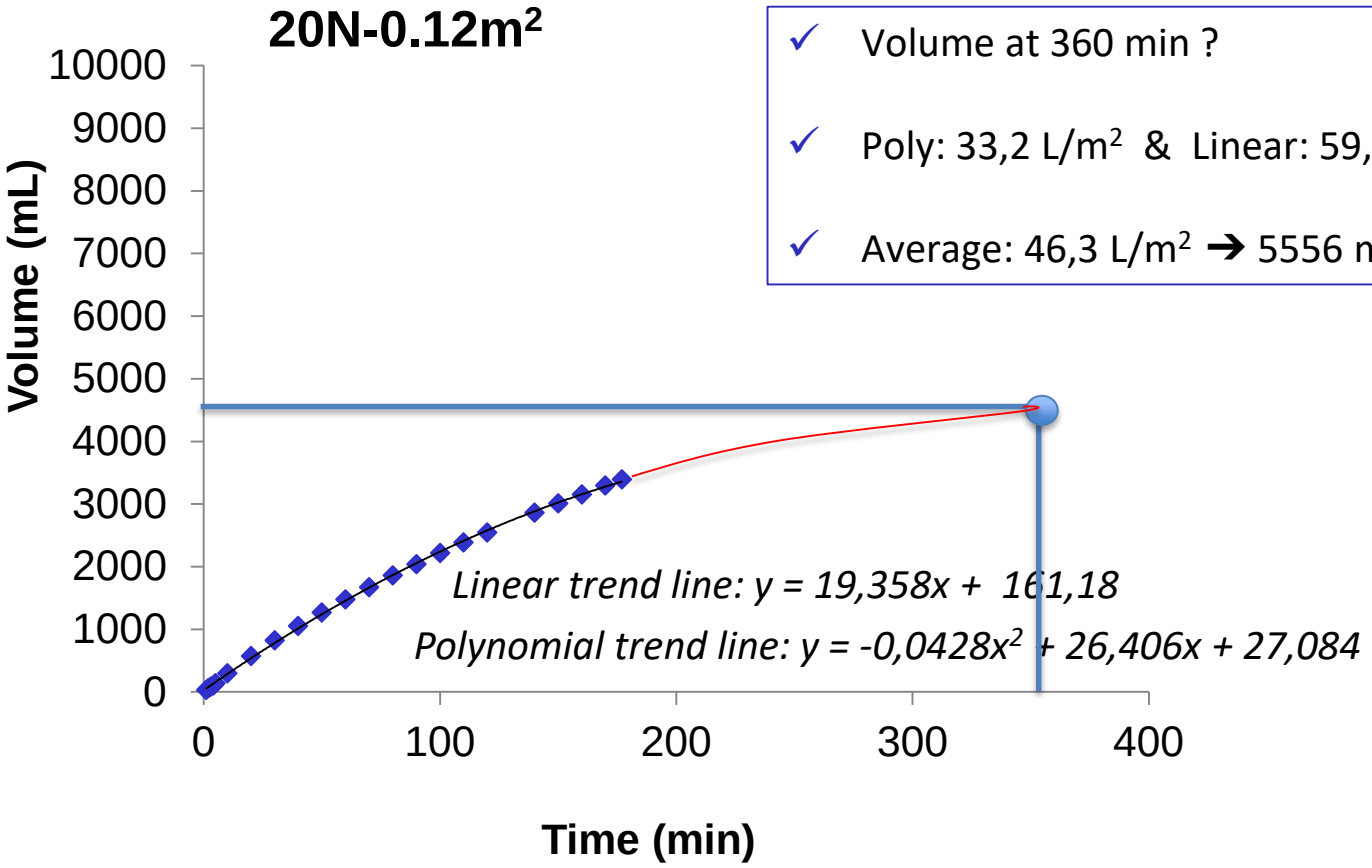
- **Data Analysis (Filtration Study Report, Estimation L/m², Vmax)**



- ✓ Calculation using 2 trend lines (2 equations) linear and polynomial (order 2)
- ✓ Linear → overestimated
  Polynomial → underestimated
- ✓ Average of both results → fair estimation
- ✓ Les reliable method if too short filtration time during trial

Designing a Virus Filtration Process

- **Data Analysis (Filtration Study Report, Estimation L/m², Vmax)**



- ✓ Volume at 360 min ?
- ✓ Poly: 33,2 L/m² & Linear: 59,4 L/m²
- ✓ Average: 46,3 L/m² ➔ 5556 mL with 0.12m²

Designing a Virus Filtration Process

- **Data Analysis (Filtration Study Report, Estimation L/m², Vmax)**

$$\frac{F_{min}}{V} = \frac{1}{V_{max}} + \frac{1}{R \times t}$$

$F = filtration\ area\ (mm^2)$ $V_{max} = maximum\ capacity\ (l/m^2)$

$V = process\ volume\ (l)$ $t = process\ time\ (min)$

$R = initial\ volumetric\ flow\ rate\ (l/m^2 min)$

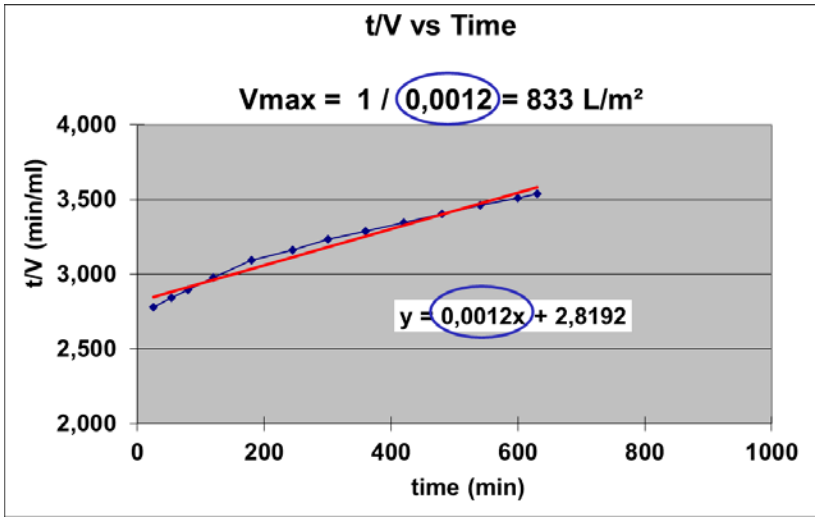
Translation…

"Vmax is the filtration capacity (L/m²) achieved if the time would be infinite"

Designing a Virus Filtration Process

- **Data Analysis (Filtration Study Report, Estimation L/m², Vmax)**

t (min)	Volume (mL)	dV/dt (mL/min)	t/V	Flux (LMH)
0,0	0,00	-	-	-
25	9,00	0,36	2,778	21,60
54	19,00	0,34	2,842	20,69
80	27,63	0,33	2,895	19,92
120	40,30	0,32	2,978	19,01
180	58,16	0,30	3,095	17,86
245	77,52	0,30	3,160	17,87
300	92,75	0,28	3,235	16,61
360	109,50	0,28	3,288	16,75
420	125,50	0,27	3,347	16,00
480	141,00	0,26	3,404	15,50
540	156,00	0,25	3,462	15,00
600	171,00	0,25	3,509	15,00
630	178,10	0,24	3,537	14,20



- ✓ The main interest of Vmax is to inform about the possibility to filter longer and then to achieve high filtration capacity
- ✓ The higher Vmax, the lower the flux decay

Designing a Virus Filtration Process

Exercise! ☺

→ Calculation of Production Costs

Questions ?