HOW TO MAKE OPTIMISATION DEAL WITH TROUBLESHOOTING

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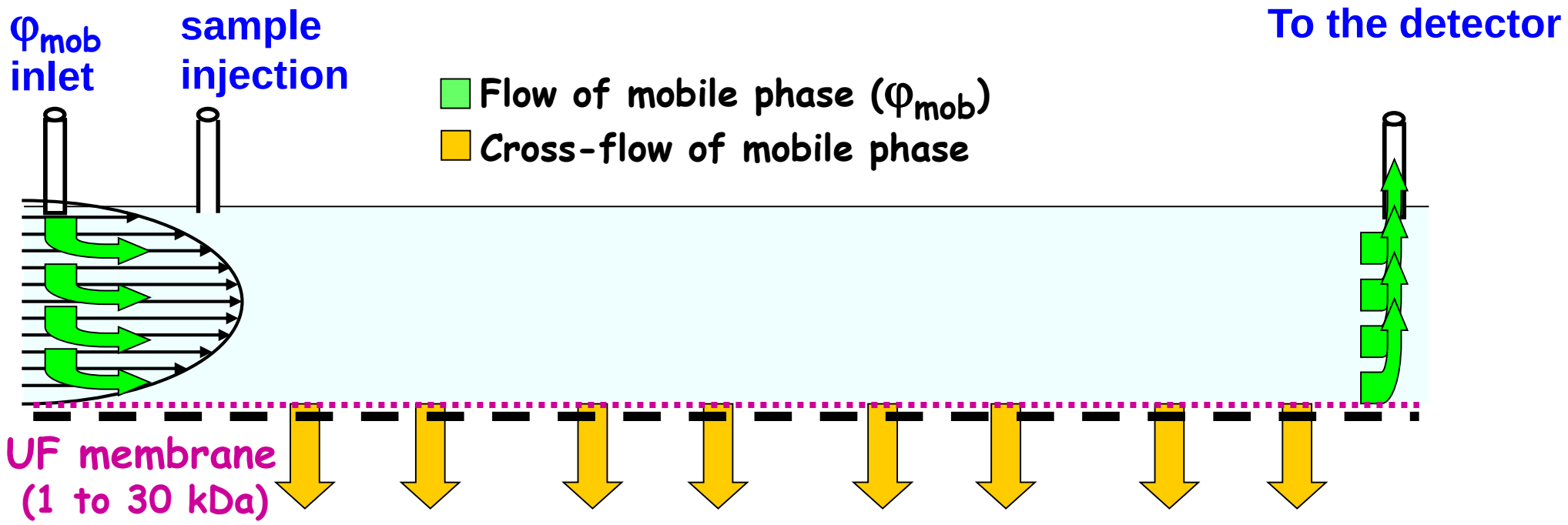
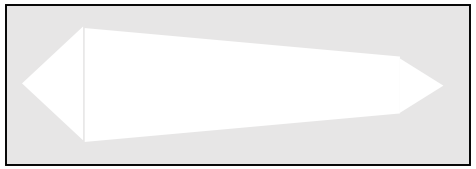
Asymmetric Flow-Field-Flow Fractionation (AF4)

The different analysis step



The cross flow is generate by aspiration, only one porous wall⇒ Asymmetrical Fl-FFF : { **As-Fl-FFF** or **AF4**

To have a constant flow-rate thorough the channel:



Asymmetric Flow-Field-Flow Fractionation (AF4)

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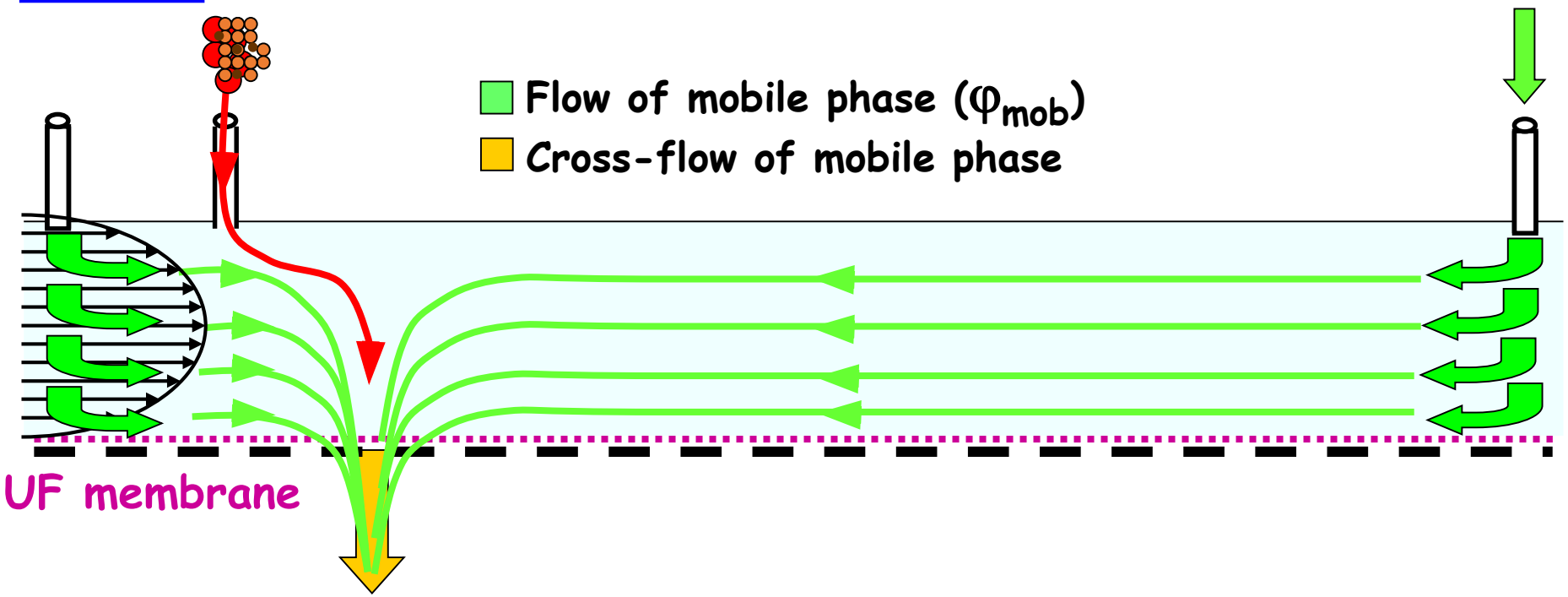
injection/focus/relaxation



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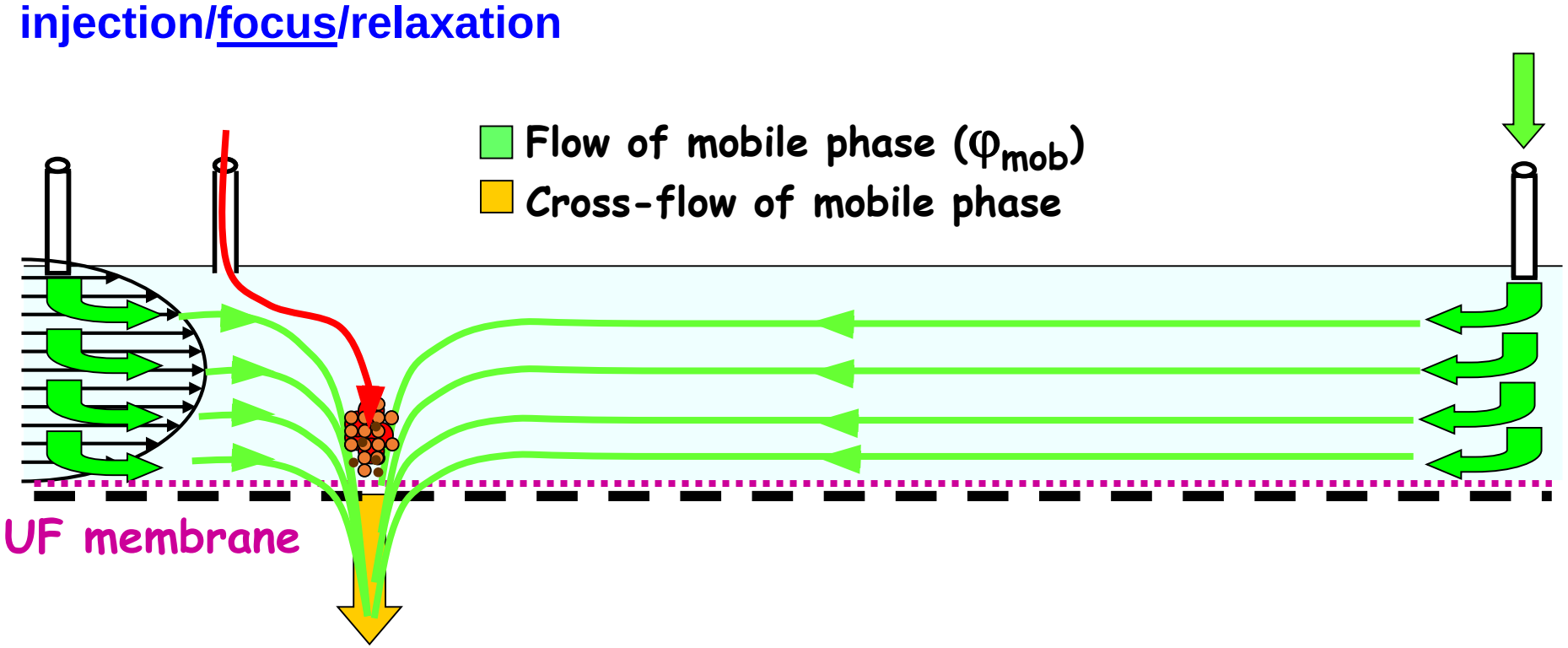

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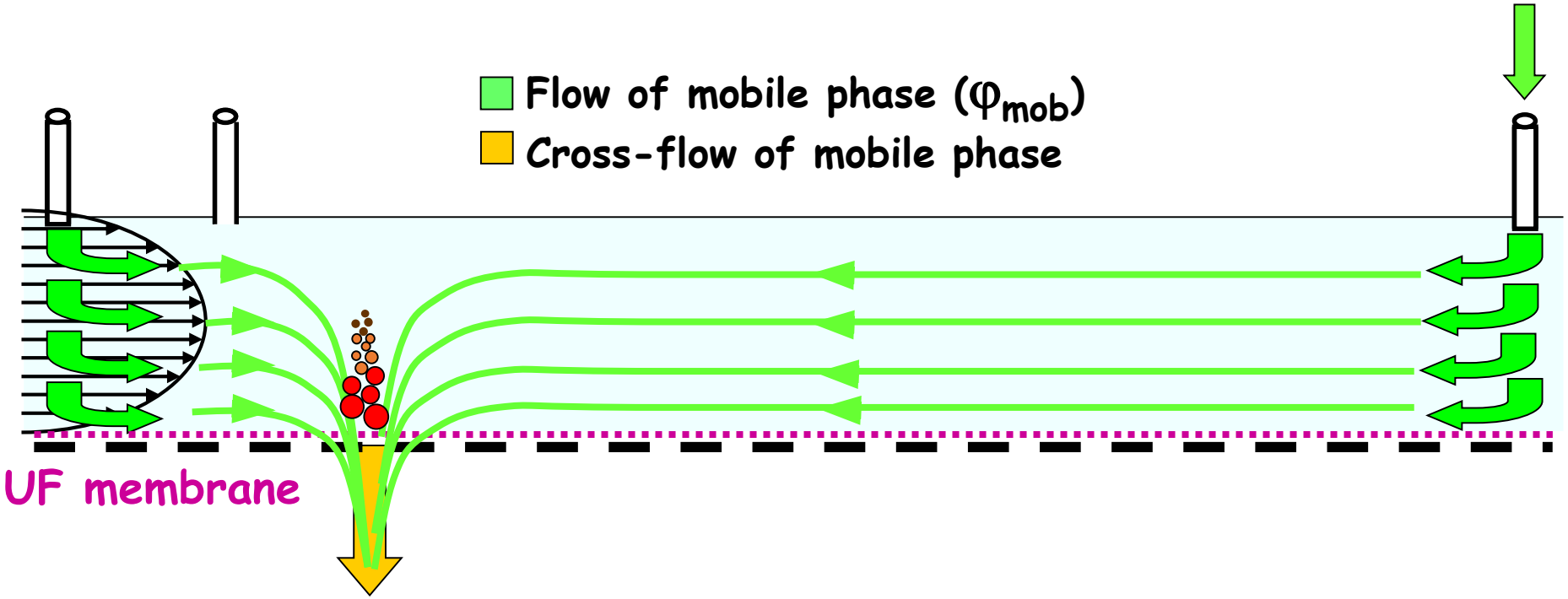

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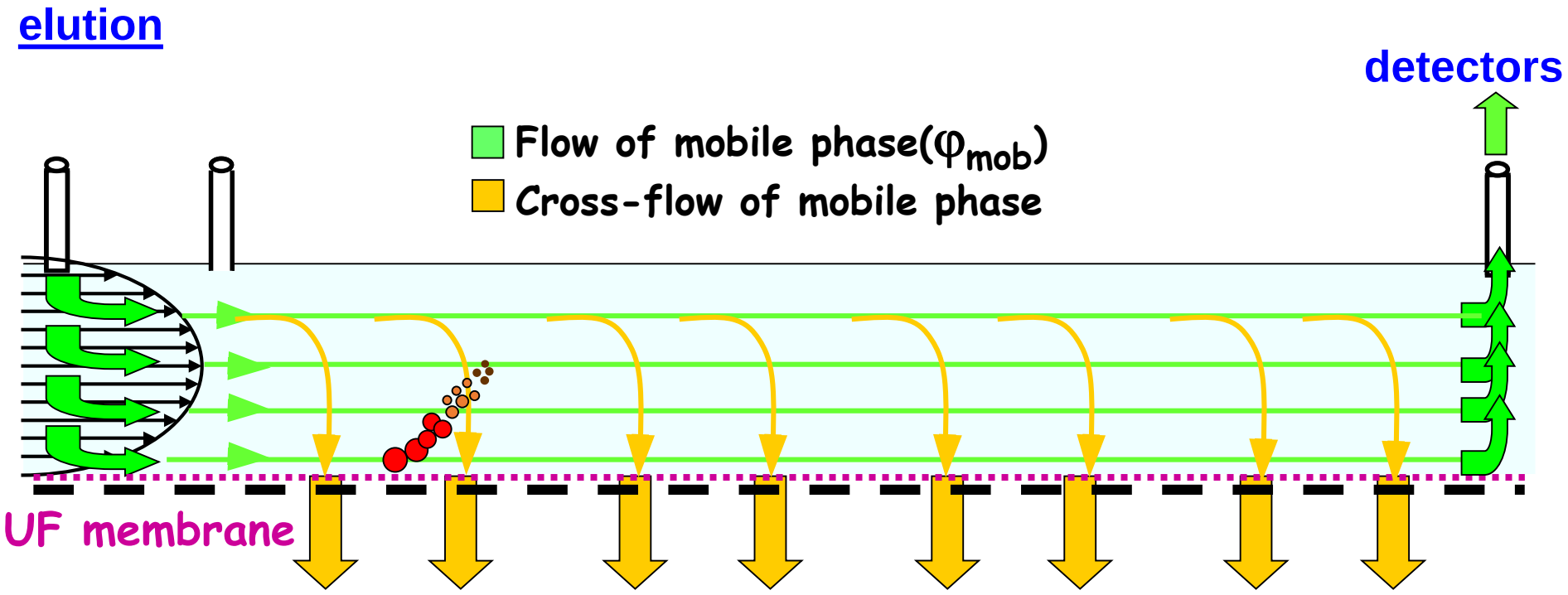

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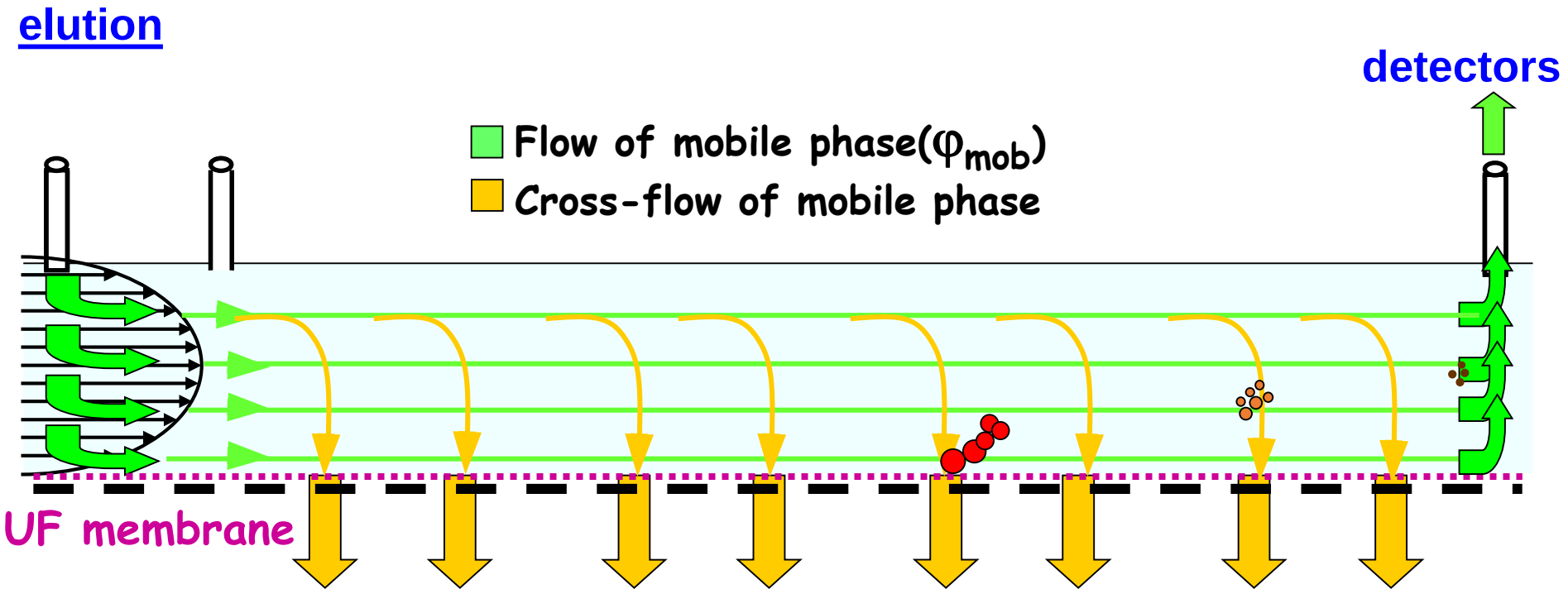

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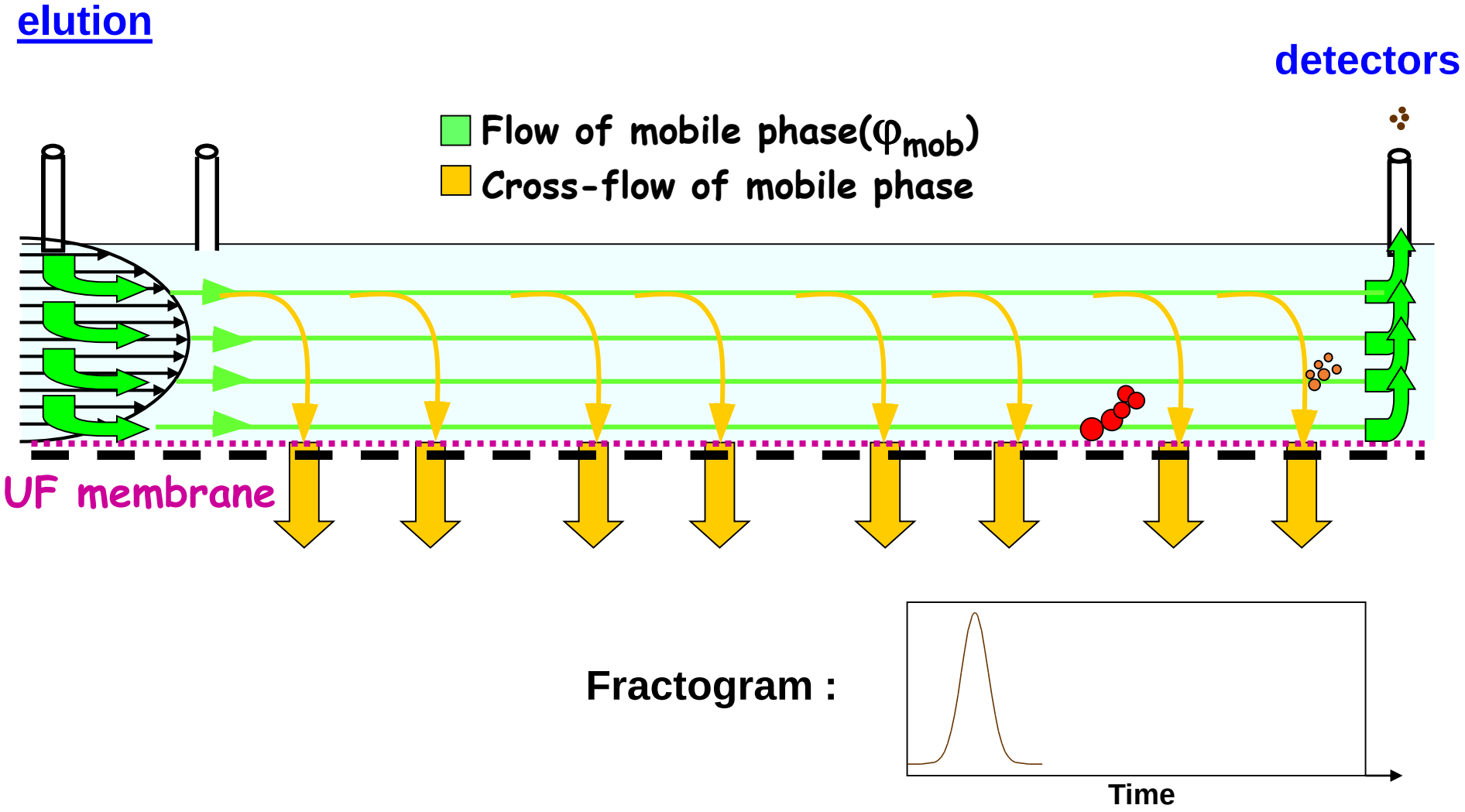

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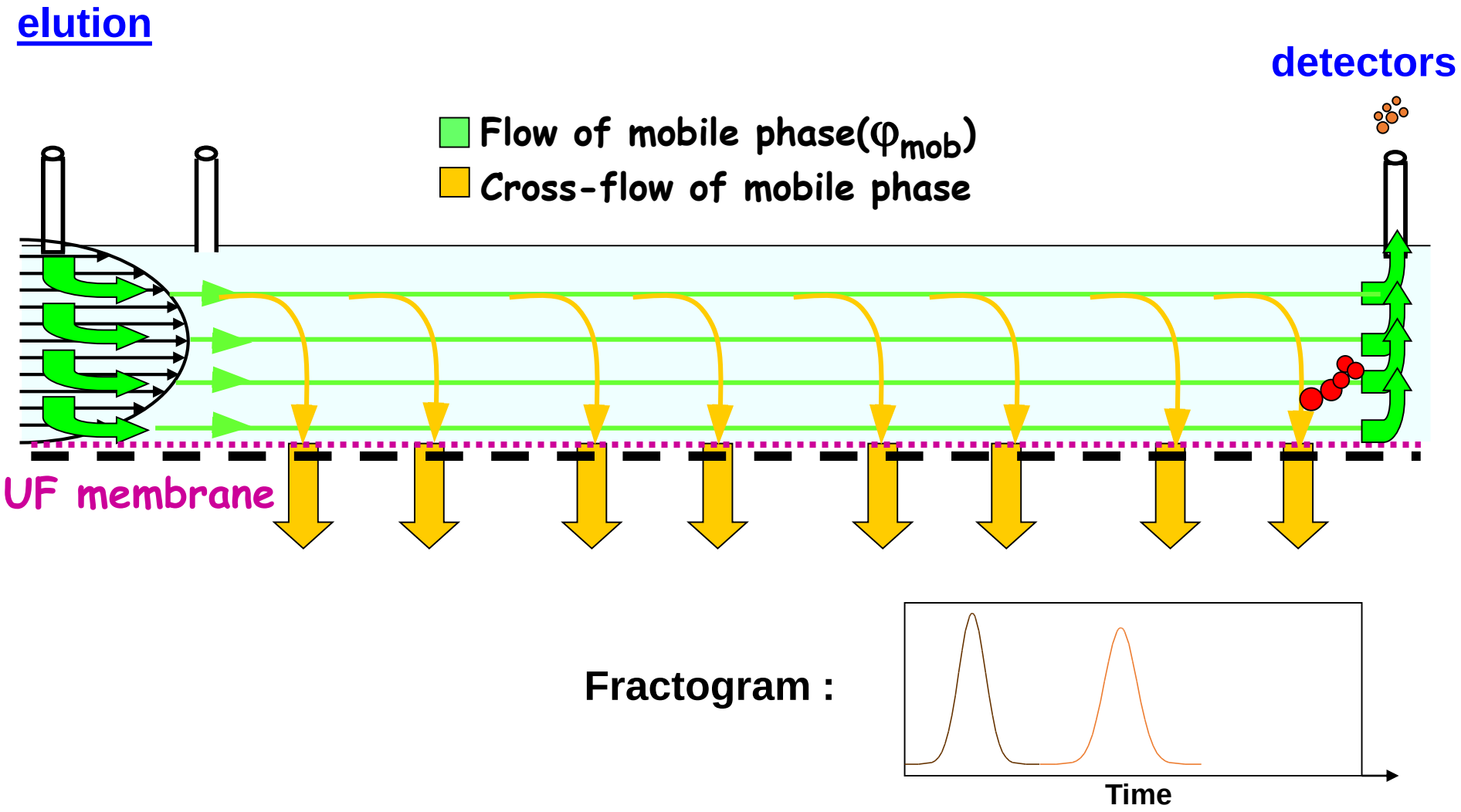

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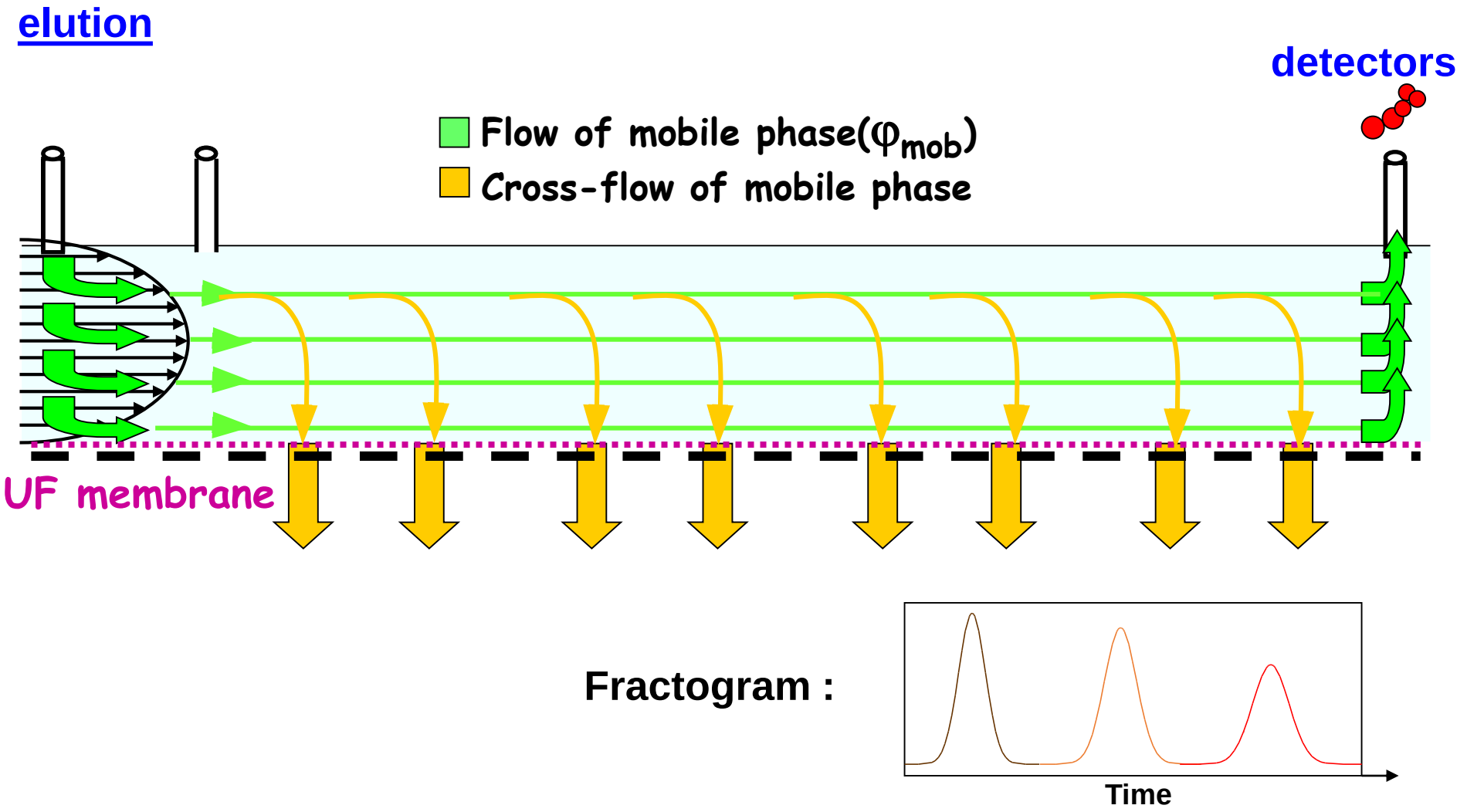


Why Optimising an FFF method ?

Why do we use FFF ?

To fractionate polydisperse sample in order to:

- **Characterize the sample** (size, concentration, composition, …)
- **Collect fractions** (offline measurements, compounds isolations, …)
- Quality control (stability, batch variation, …)

- **There is no universal method which works well for all kind of samples**
- **An optimisation is therefore necessary for each couple sample/matrix**

An ideal FFF method

An ideal FFF method should be able to correctly fractionate the sample according to its diffusion coefficient and its size, without sample loss during the analysis

Performance criteria

Recovery

$$R(\%) = \frac{A_F}{A_{inj}}$$

A_F: Area of the sample with the field force applied [detector signal*s]
A_{inj}: Area of the sample eluted without field force applied

Retention ratio

$$R = \frac{t_0}{t_r}$$

t_0: Void time [min]
t_r: Retention time [min]

Selectivity

$$S_d = \left| \frac{d \log(t_r)}{d \log(d)} \right|$$

d: particle diameter [m]

Resolution

$$R_s = 1.18 \frac{t_{r2} - t_{r1}}{\langle w_{FWHM} \rangle}$$

$\langle w_{FWHM} \rangle$: Average value of the peak width at half maximum [min]

An ideal FFF method

Satisfaction criteria

ISO/TS 21362:2018

Nanotechnologies — Analysis of nano-objects using asymmetrical-flow and centrifugal field-flow fractionation

$R(\%) \geq 70\%$

$S_{dmethod} \longrightarrow S_{dmax}$

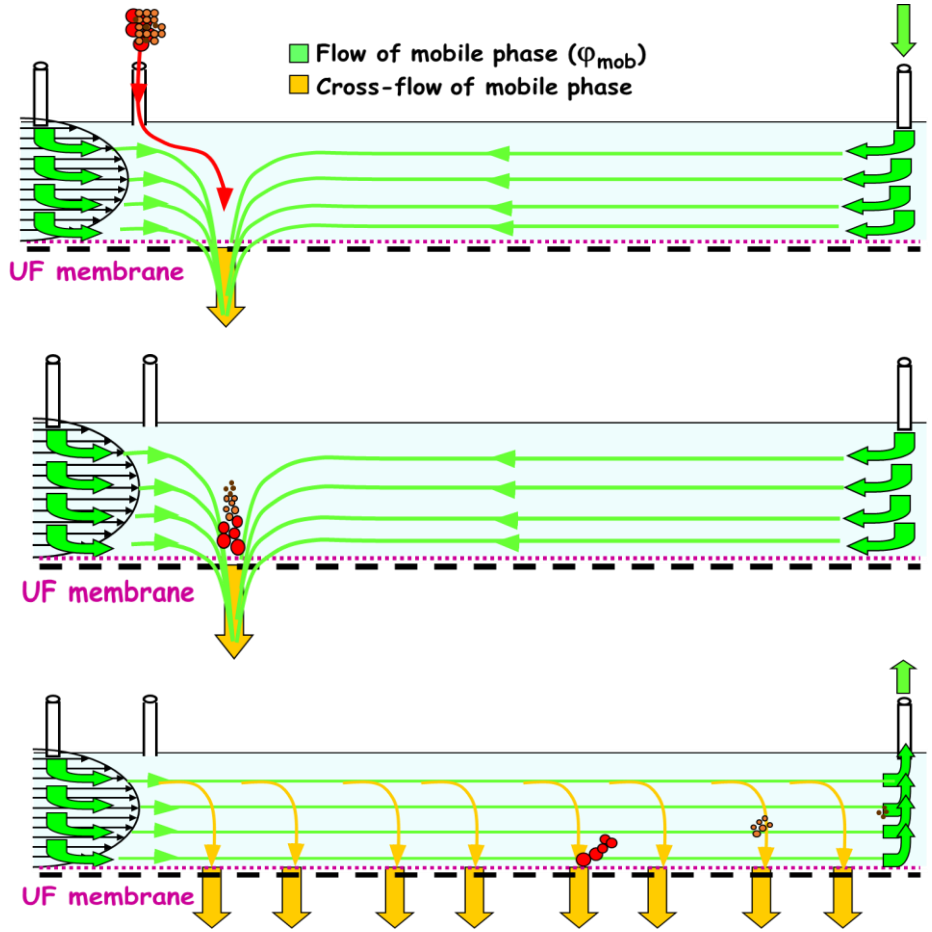
$R_S > 1.5$

Parameters available to optimisation: case of the flow-FFF

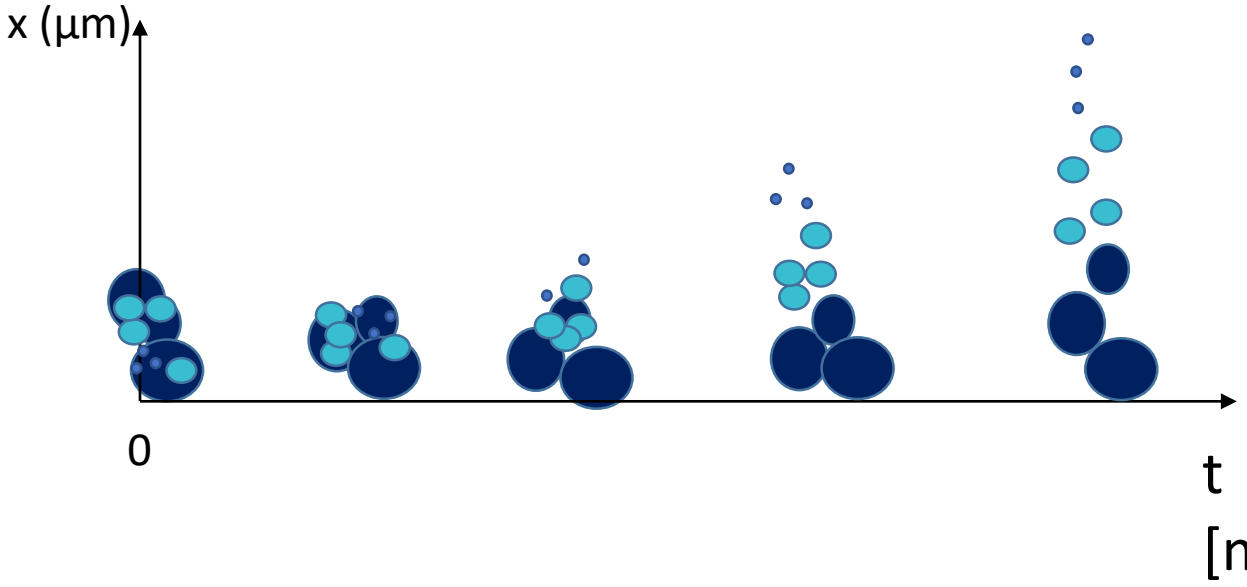
Injection

Focusing

Elution



Optimisation of the focusing step



$$\tau = 1.2 \frac{V_0}{V_c}$$

τ : minimal relaxation time [min]
V_0: Channel volume [mL]
V_c: cross flow rate [mL min^{-1}]
(Moon et al., 2000)

The focusing time should be long enough to allow the sample to attain a steady state

There is a risk of agglomeration or sample loss due to the interaction with the membrane

- Reduce the injected quantity
- Change the membrane or the carrier
- Reduce the cross flow rate applied

If the recovery is low, perform an analysis with just the focus step to see how much the focusing contribute to the sample loss

Optimisation of the elution step

$$t_r = \frac{\pi \eta w^2 d_h Q_c}{2kTV^0 t_0}$$

d_h: hydrodynamic diameter
k: Boltzmann constant
Q_c: cross flow rate
T: temperature
t_0: temps mort
t_r: retention time
V^0: channel volume
w: channel thickness
η: carrier viscosity

This equation assume that the interactions between the particles and the membrane are negligible

In theory

t_r= f(d_h, channel geometry, Q_c)

In practice

The composition of the carrier (ion concentration, pH) and the nature of the membrane will influence the retention time and the sample loss

Key parameters for method optimisation

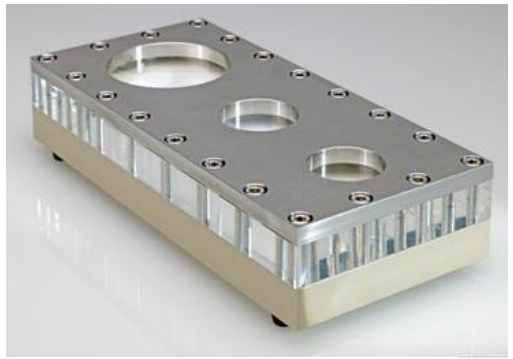
PHYSICAL PARAMETERS

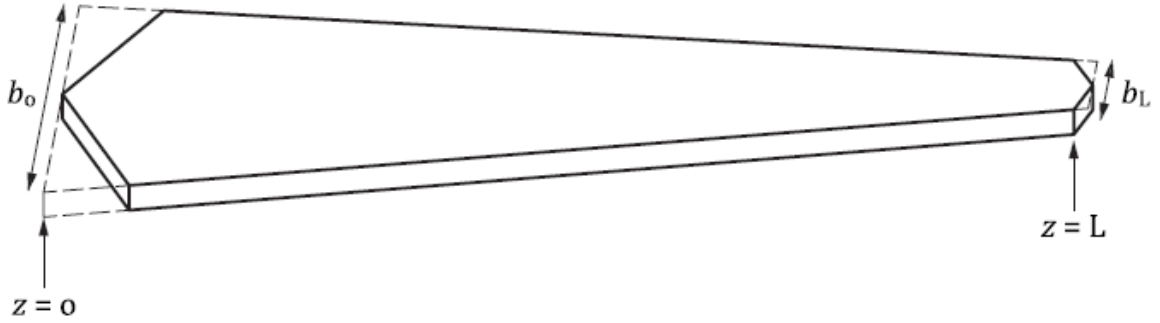
1. Channel length
2. Channel thickness
3. Injected sample mass
4. Cross flow rate
5. Detector flow rate

CHEMICAL PARAMETERS

1. Carrier composition (ionic strength, pH, surfactant)
2. Membrane nature and cut-off

Channel length





70 mm < Length < 300 mm

Short channel: shorter analysis, better LOD but less fractionated *(You et al. 2017)*

Long channel: better fractionation

Channel thickness

The channel thickness influence greatly on the particle retention time

Nano-objects nanometric scale → w = 350 µm

high channel thickness

- Sample dilution
- peak broadening
- better fractionation/resolution
- increase the time of analysis

small channel thickness

- less sample dilution
- thinner peak width
- reduce the selectivity of the fractionation
- reduce the time of the analysis

Injected sample mass

- Identify a concentration/ mass injected range that give the same recovery rate and retention time
- Compromise between operating in dilute condition but sufficient for the LOD/LOQ of the associated detectors

« When the sample mass injected is too high, the sample will not be able to relax correctly, which will induce a shift in the retention time »

Overloading effect

ISO recommendation

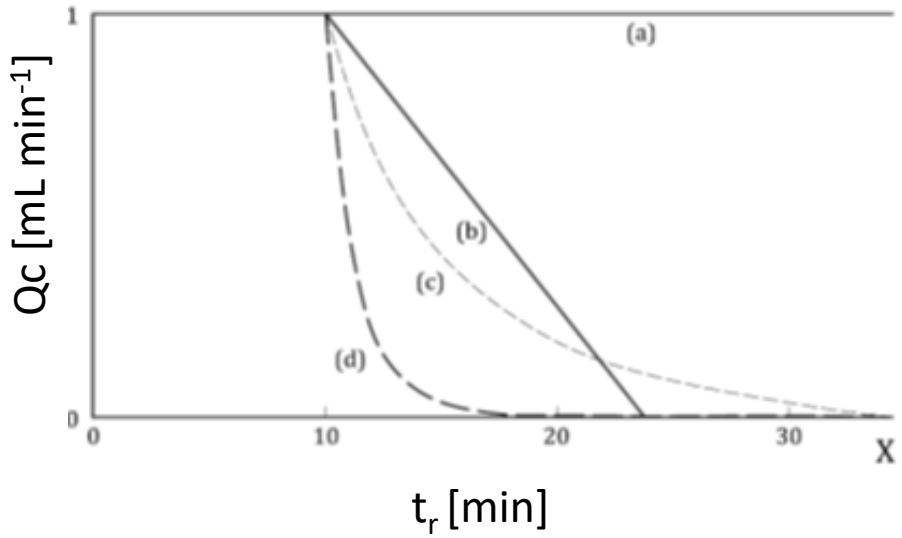
- ❑ Don't work with concentration higher than 1 g L^{-1}
- ❑ Spherical nano-objects : 10^7 – 10^{11} total injected particles (*Gigault et al. 2014)*

The polydispersity of the sample influence the limit where you begin to see overloading

Cross flow rate

The cross flow need to retain the particle inside the channel sufficiently to produce a separation. The cross flow value need to be:

- high enough to separate the small particles from the void peak
- not too high to let the big particles eluting from the channel



(a) Q_c constant
(b) linear decrease
(c) power decrease
(d) exponential decrease

Detector flow rate

The particle retention time depend on the ratio between the detector flow rate and cross flow: $\frac{Q_{out}}{Q_c}$

Recommended flow rate 0.5 < Q_{out}< 1 mL min^{-1}

Q_{out}< 0.5 mL min^{-1} → Long analysis, peak broadening

Q_{out}> 1 mL min^{-1} → High pressure, the cross flow rate need to be high enough to sufficiently retain the particle

Optimisation of the focusing step

- **Determine the focalisation position**

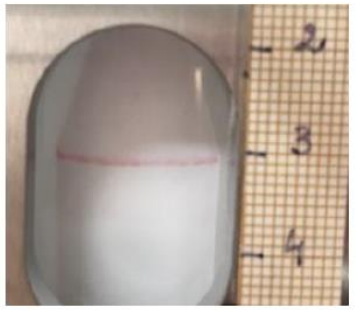
$$z_{foc} = z_1 + \frac{b_1 - \sqrt{b_1^2 - 2S\frac{Q_{in}}{Q_c}A_{tot} + b_1 z_1 S}}{S}$$

(*Wang, Jour. of Chrom. A, 2018*)

- **Check the focalisation position** using a dye (bleu dextran, ferritine, gold NPs)

- z_{foc} is linked to the channel geometry and to the ratio (Q_{in}/Q_c)
- z_{foc} est is indipendent from the others flow rates (Q_{out} et Q_{foc}) and from the other characteristics of the system

Au NPs



$z_{foc} = 3.5 \pm 0.1$ cm

Mobile phase

Compatibility with the sample ⇔ Optimisation of analytical performances

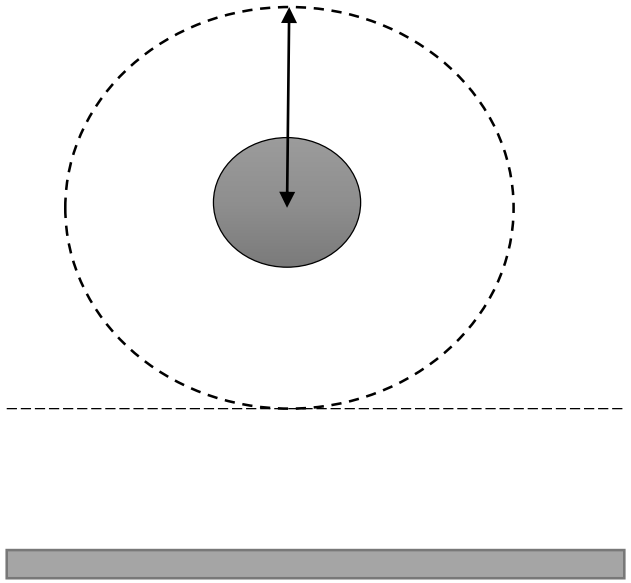
- ❑ Composition
- ❑ Ionic strength
- ❑ pH

Wide choice of carriers

- ❑ ultrapure water
- ❑ ammonium nitrate
- ❑ sodium chloride
- ❑ sodium azide

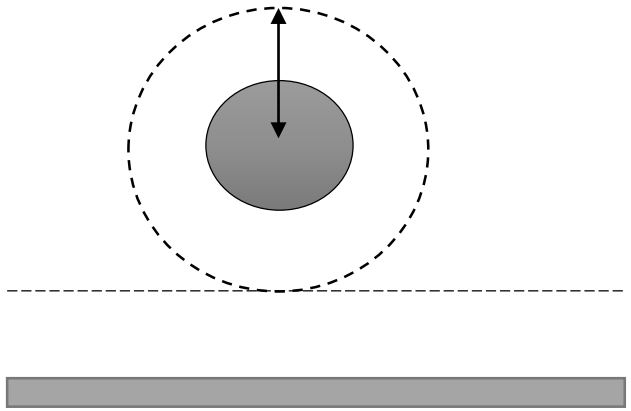
Ionic strength effect

The carrier ionic strength influences the electrical double layer thickness (Deby length κ^{-1}) around the particle and the membrane, hence the electrostatic repulsion between the particle and the membrane

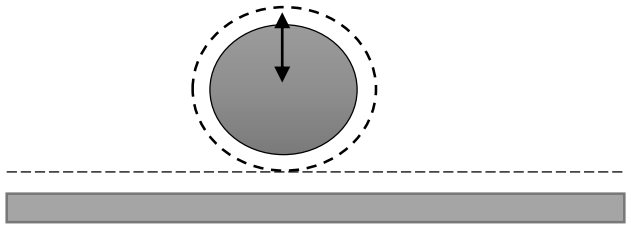


Low ionic strength

High Debye length
High recovery rate
Pre-elution



Optimal condition

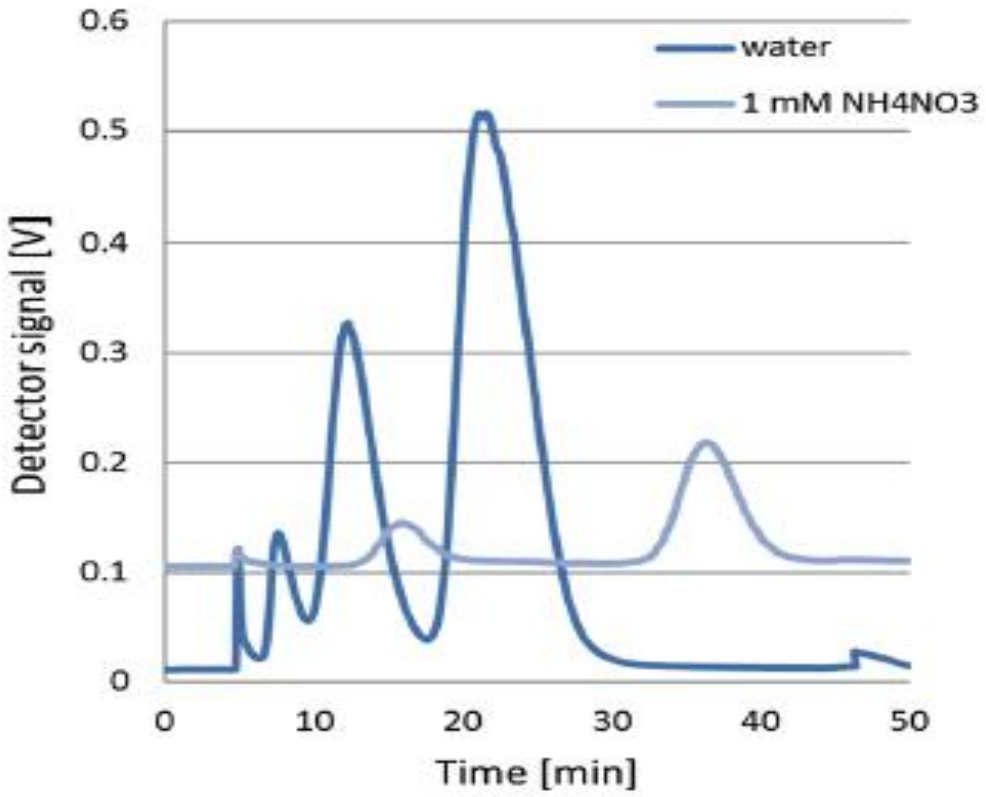


High ionic strength

Small Debye length
Low recovery rate
Risk of adsorption

Ionic strength effect

Example: fractionation of 3 size populations polystyrene latex standards (60-151-356 nm of diameter)



(Kowalski et al. 2018)

The three populations are more fractionated when the ionic strength is high but the recovery decreased.

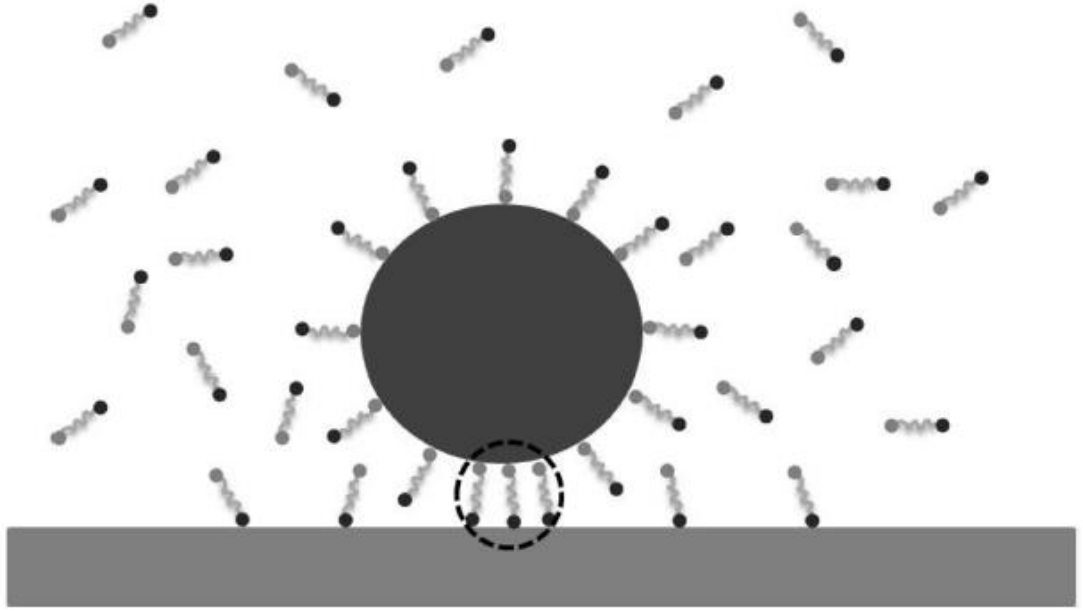
Surfactant in the carrier

Using surfactant allows to reduce the interaction particle-membrane and to increase the recovery rate.
However it can also increase detectors baseline (MALS, UV-Vis) and decrease the method LOD.

List of most used surfactants in literature:

- FL-70/Novachem
- Tween 20
- SDS
- Triton X-100
- CTAB

Surfactant concentration need to be << critical micellar concentration.



(Kim et al. 2012)

Membrane nature and cut-off

- Molecular weight cut off (MWCO) availables:
  1-30 kDa
  retention in the channel/ resistance against cross flow
- Surface charge

MWCO 10KDa

Typical available membranes:

- Regenerated cellulose (RC)
- Polyethersulfone (PES)
- Polyvinylidene fluoride (PVDF)
- Cellulose triacetate (CTA)

Negative surface charge

The couple membrane/ carrier influence the possibility of sample adsorption on the membrane

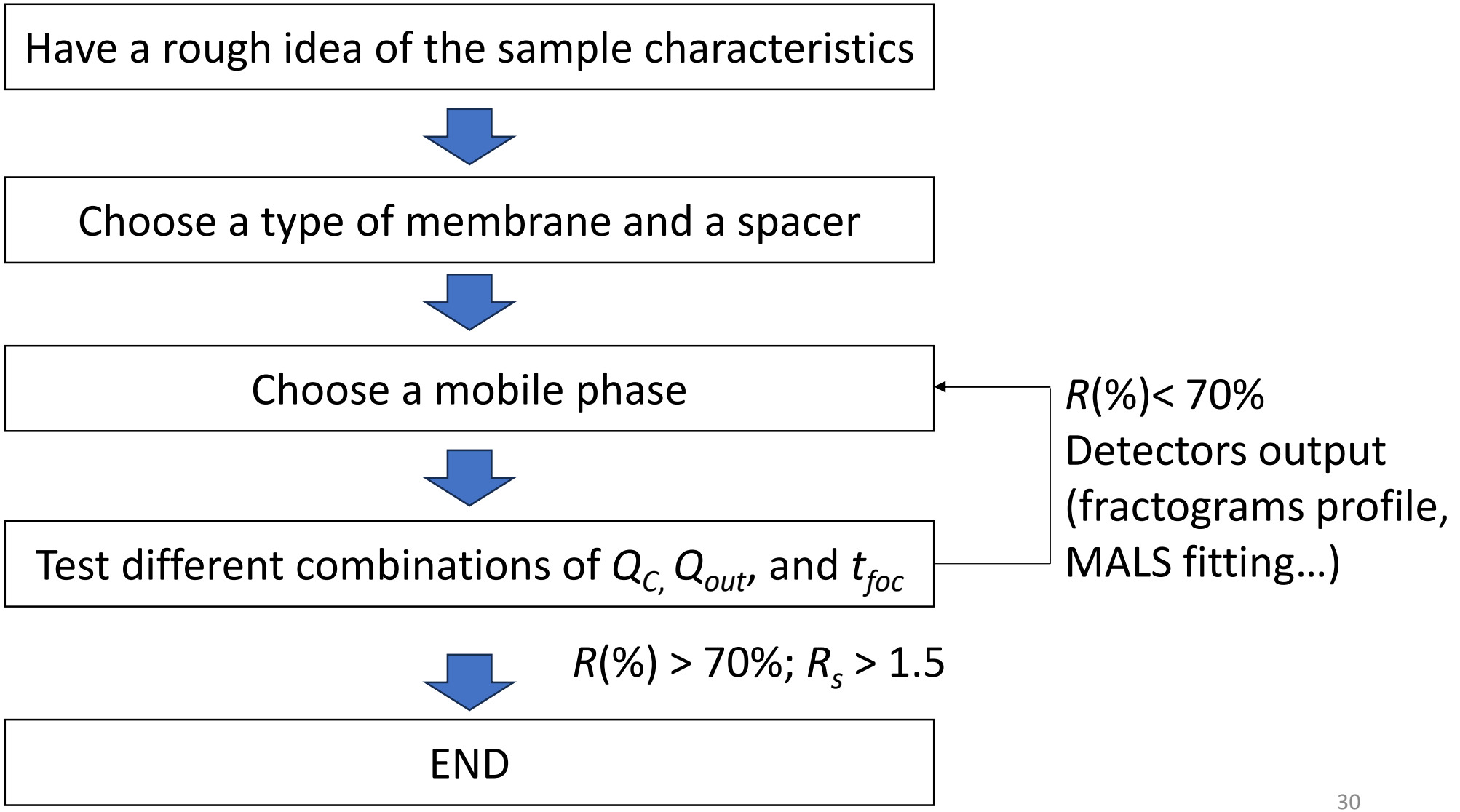
Membrane behaviour

The membrane can have two opposite behaviours depending on the sample.

In the first case the recovery rate is weak at the first injection and increases in the following analyses, this is called the membrane conditioning.

In the second case the recovery decreases with the membrane aging (number of injections) (cf. troubleshooting part)

Optimization Steps



ISO/TS 21362:2018 SCOPE

- This document identifies **parameters and conditions**, as part of an integrated measurement system, necessary to **develop and validate methods** for the application of **asymmetrical-flow** and **centrifugal field-flow fractionation** to the analysis of **nano-objects and their aggregates and agglomerates** dispersed in aqueous media. In addition to constituent **fractionation**, analysis can include **size, size distribution, concentration and material identification** using one or more suitable detectors.
- **General guidelines and procedures** are provided for application, and minimal reporting requirements necessary to reproduce a method and to convey critical aspects are specified.

ISO/TS 21362:2018

- **Terms and definitions**

- **Principles of operation and main equations**

- **Method development for AF4/CF3**
  - Sample specifications
  - Mobile phase specifications
  - Fractionation (channel/membrane selection, injection/relaxation, optimizing flow conditions, elution programme…)

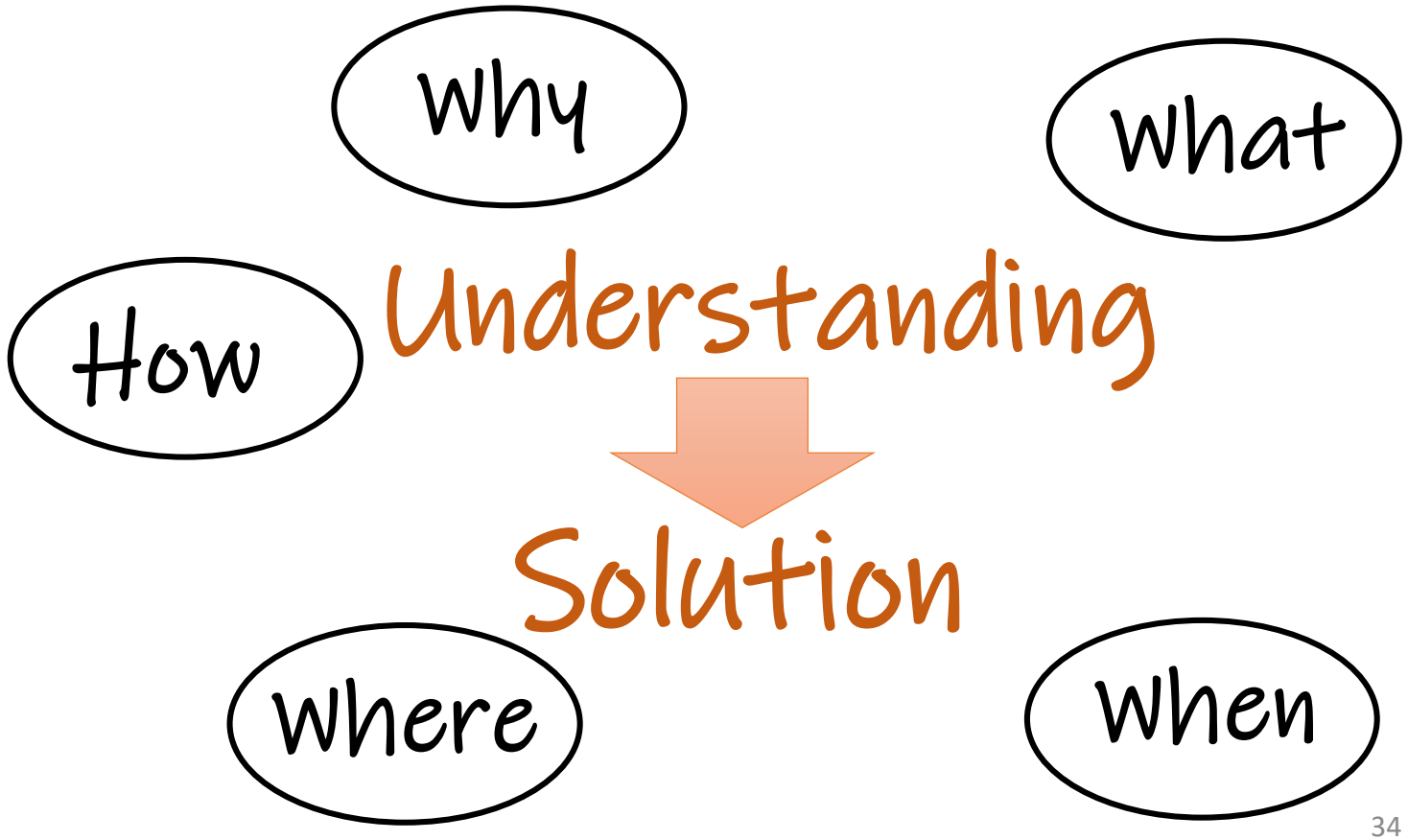
AF4

- **Analysis of nano-objects**
  - Online size analysis
  - Online concentration analysis (mass-based and number-based methods)
  - Online material identification or composition
  - Off-line analyses (fraction collection)

ISO/TS 21362:2018

- **Qualification, performance criteria et measurement uncertainty**
  - System qualification and quality control
  - Method performance criteria (recovery, selectivity, retention, resolution)
  - Method precision and uncertainty measurement
- **General procedure for measurement of samples**
  - Calibration of retention time for online size analysis
  - Calibration of AF4 retention time for online size measurements
  - General measurement procedure for AF4/CF3
- **Test report**

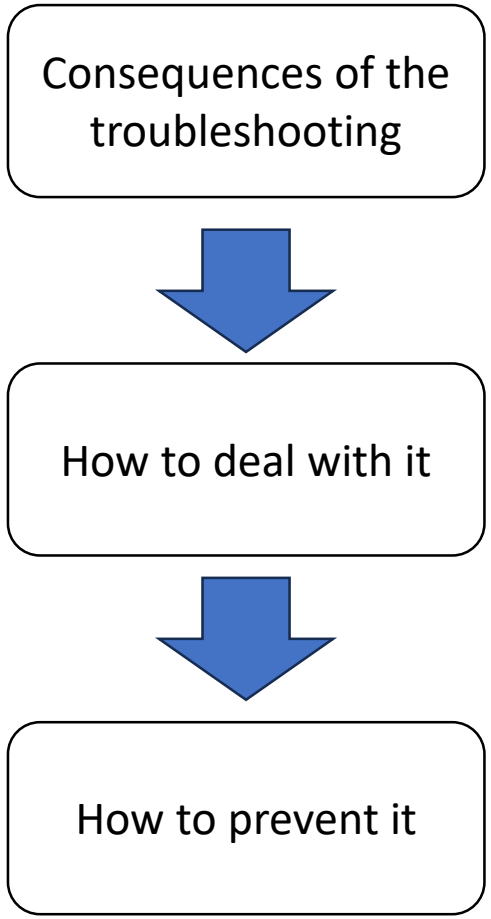
Troubleshooting



Deal with troubleshooting

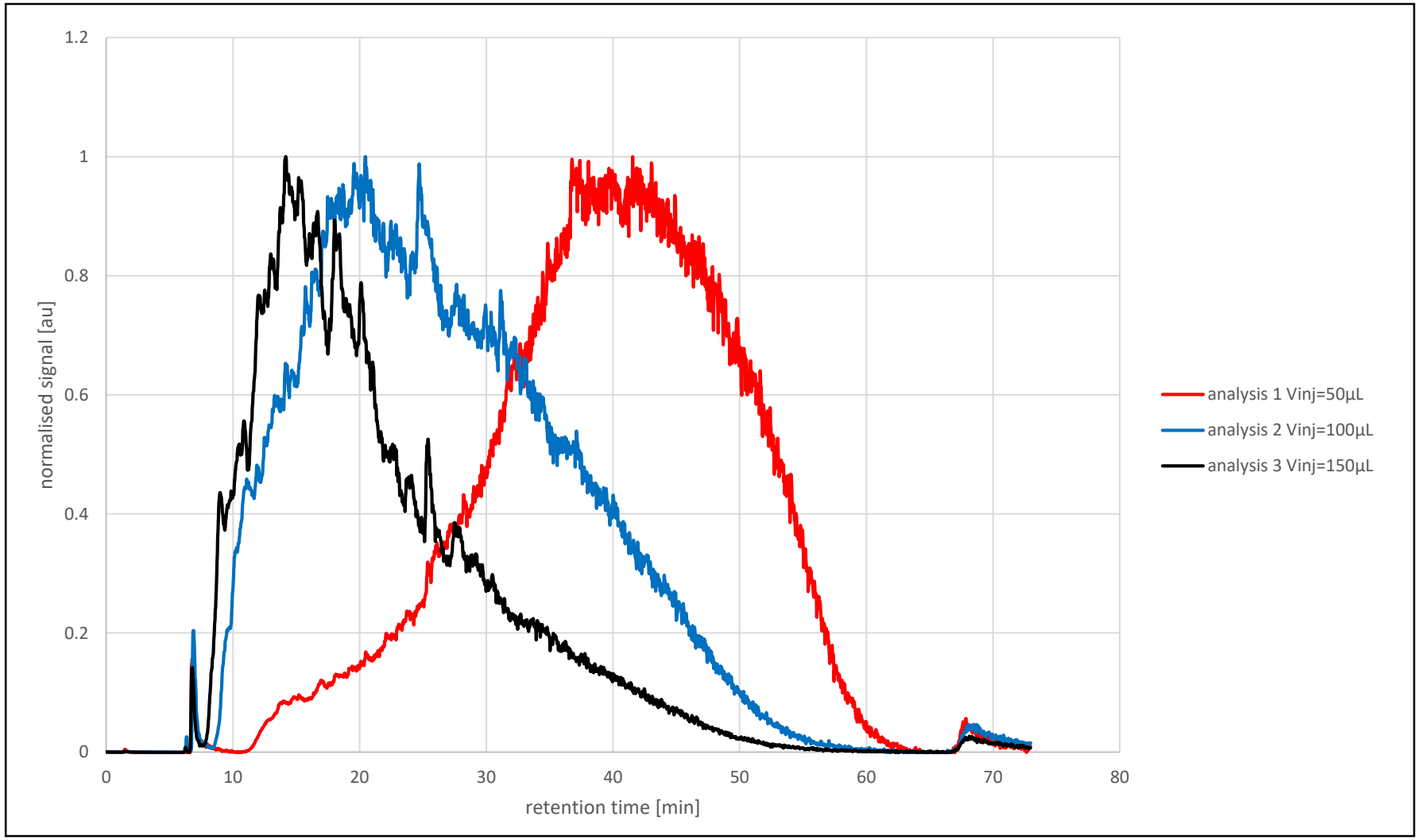
Examples of troubleshooting

1. Overloading effect
2. Overpressure
3. Membrane related problem



Overloading effect

Overloading phenomena happen when too much sample have been injected in the channel and result in a different retention behaviour (sample dependent)



Soil leachate suspension
solid/liquid ratio =1/10

Injected at different volumes

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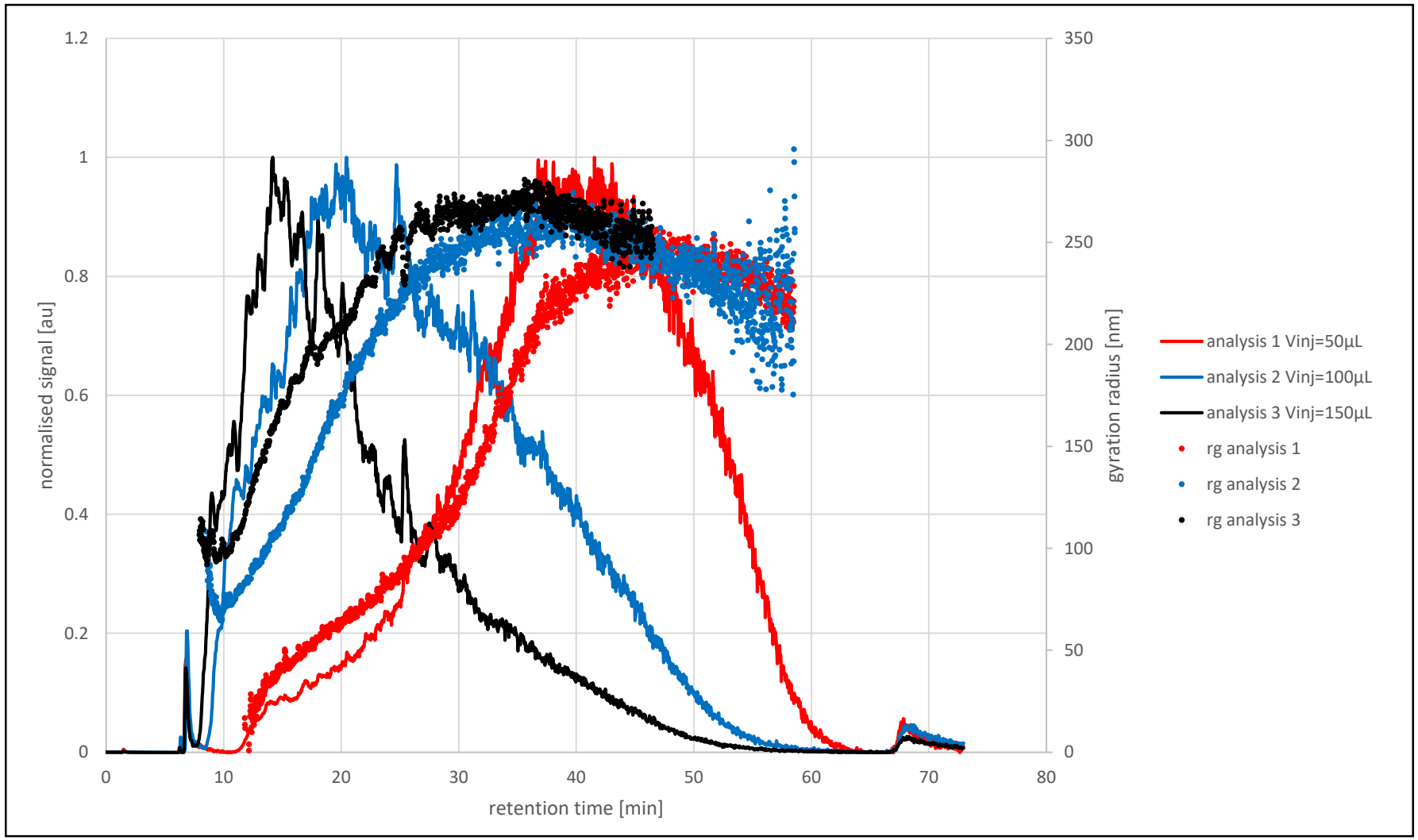


Soil leachate suspension solid/liquid ratio =1/10

Injected at different volumes

Overloading effect

Consequence of overloading

- Shift in the retention time (sample dependent)
- Poor fractionation

How to deal with overloading

- Re-optimize the method
- Increase the focusing time

How to prevent overloading

- Inject small quantity of sample in the channel
- The mass limit is sample dependent (nature and polydispersity)

Overpressure

Consequence of overpressure

- The analysis is stopped (loss of the sample, stop of overnight sequences)
- Leakage can appear

How to deal with overpressure

- Check if tubing are not obstructed
- Rinse the system (Hellmanex 0.1%, ultrasound bath, ultrapure water)
- Check if the change in the cross flow rate are not too fast for the system
- Change the membrane

How to prevent overpressure

- Decrease slowly the cross flow during the analysis
- Use, if possible, a low detector flow rate ≤ 1 mL min $^{-1}$
- Monitor pressure increasing in the time (days)

Membrane related problems

Consequence of membrane related problems

- Shift in retention time
- Decrease in recovery rate
- Peak disappearance

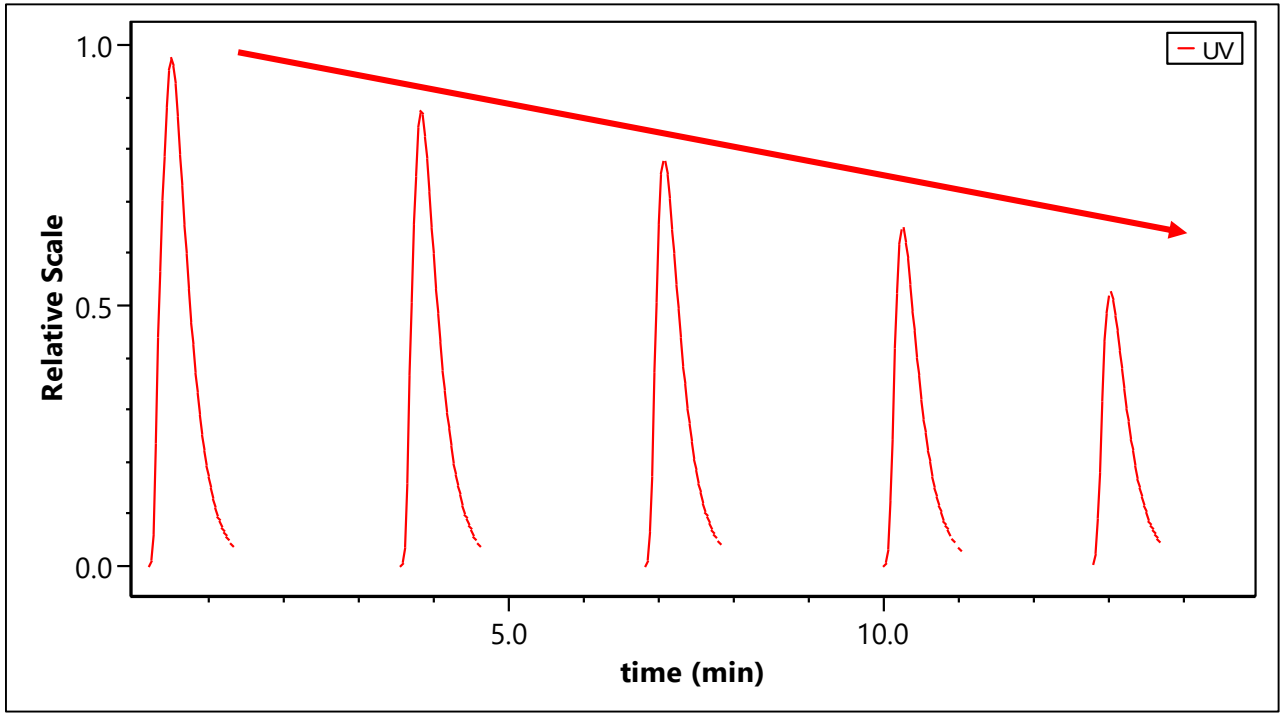
How to deal with it

- Rinse the system (Hellmanex 0.1%, ultrasound bath, ultrapure water)
- Change the membrane

How to prevent it

- Numerate the analysis to follow the “membrane age”
- If possible, use one membrane for a category of sample
- Rinse between injections and frequently inject blanks (carryover monitoring)

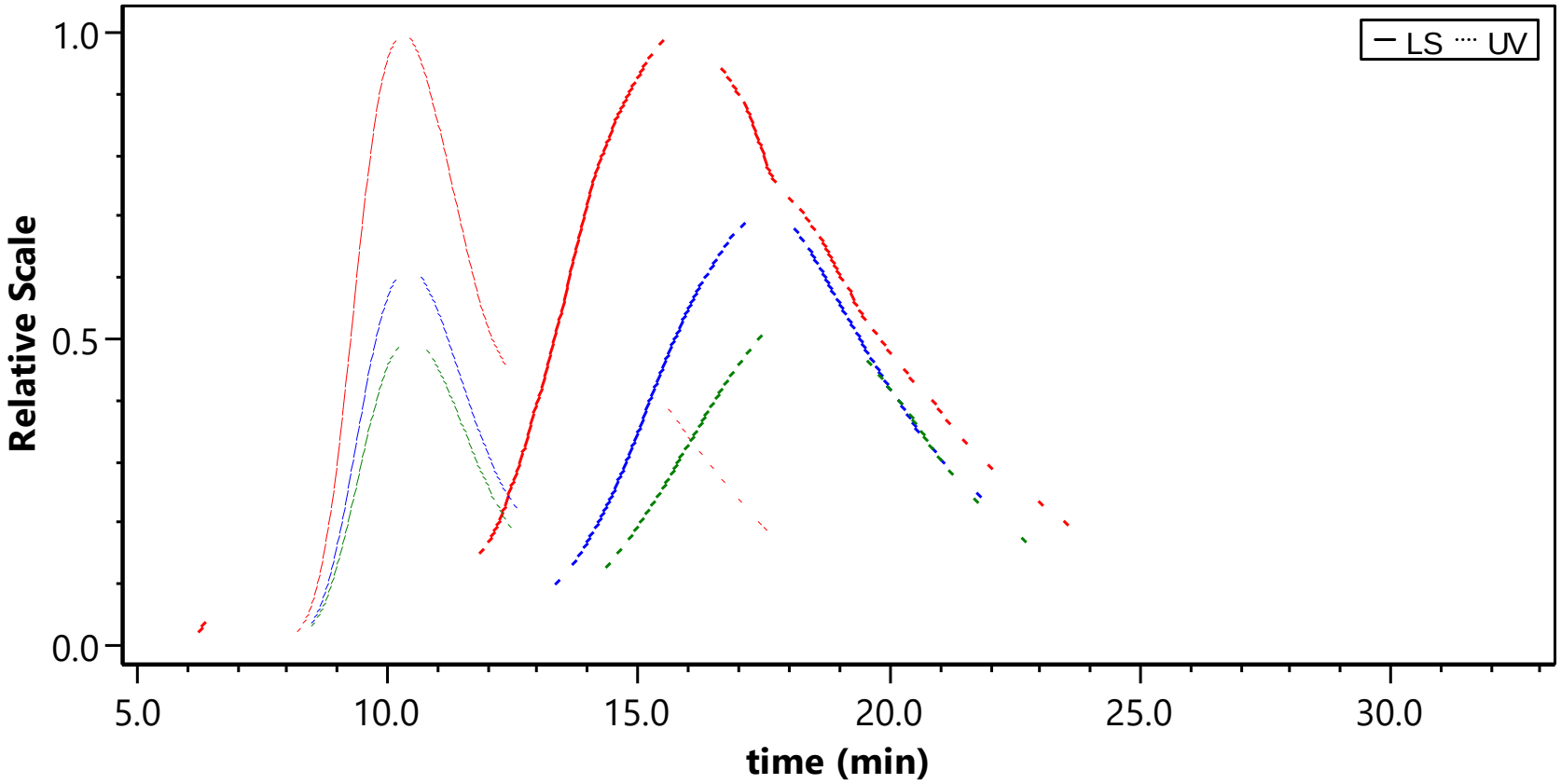
Other examples: Recovery issues



- Decrease of the peak recovery after several injections
- Absorption on injection system/tubing, on the membrane, unstable sample… ?
- Bias on effective recoveries values

- Sample/matrix depending (understanding)
- Stabilise the sample
- Change the membrane

Other examples: Repeatability issues



- Decrease or increase of the peak area after several injections
- Stability of the sample, absorption, conditioning ?
- May stabilise after some injections
- May affect or not the size distribution

- Sample/matrix depending (understanding)
- Stabilise the sample
- Change the membrane

Thank you for your participation

Enjoy the iSFFF Nantes 2024 symposium!