Neue Entwicklungen und Trends in der Feld-Fluss Fraktionierung (FFF): Elektrische Asymmetrische FFF zur Trennung von geladenen und polarisierbaren Nanopartikeln und (Bio)Molekülen und Hochtemperatur FFF zur Charakterisierung von Polyolefinen

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13. Tagung des Arbeitskreises Polymeranalytik, Fraunhofer LBF, Darmstadt, 08. Juni 2018

Content

- Principles of Flow Field-Flow Fractionation (FFF)
- FFF-Applications
- Electrical Asymmetrical Flow Field-Flow Fractionation
- High Temperature Field-Flow Fractionation

Company & Product History

2017	***NEW EAF2000 ELECTRICAL FLOW FFF***
2015	**SC2000 SEC Separator**
2010	**CF2000 Centri FFF Separator**
2008	**AF2000 Flow FFF Separator**
2007	**TF2000 Thermal FFF Separator**
2015	**Postnova UK, Malvern, UK**
2009	**Postnova Nordic, Norlab, Finland**
2006	**Postnova China, Jili Scientific, Shanghai**
2001	**Postnova USA, Merger FFFractionation**
1997	**Foundation Postnova, Munich, Germany**
1986	**Foundation of FFFractionation, SLC, Utah**
1966	**FFF Invention, C. Giddings, Univ. Utah, SLC**

Nanomaterials: EU Legislation

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European Commission

Nanomaterials

In cosmetic products, reference to "nanotechnology" usually means the use of insoluble nanoparticles as ingredients. EU legislation provides a high level of protection of human health where nanomaterials are used in cosmetic products.

Use of insoluble nanoparticles in cosmetics

According to the Regulation (EC) No 1223/2009 Art.16 (3), the manufacturer or importer of a cosmetic product has to assess and document the safety of the product prior to placing it on the market. This has to be done while taking into consideration 'the general toxicological profile of the ingredients, their

Definition of a nanomaterial

The EU adopted a definition of a nanomaterial in 2011 (Recommendation on the definition of a nanomaterial (2011/696/EU)). Its provisions include a requirement for review "*in the light of experience and of scientific and technological developments. The review should particularly focus on whether the number size distribution threshold of 50 % should be increased or decreased*". The Commission is expected to conclude the review in 2016, following the consultation of its draft findings with the stakeholders towards the end of 2015 and a public consultation planned to be published in Summer 2016 or shortly after.

According to the Recommendation a "Nanomaterial" means:

A natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50 % or more of the particles in the number size distribution, one or more external dimensions is in the size range 1 nm - 100 nm.

In specific cases and where warranted by concerns for the environment, health, safety or competitiveness the number size distribution threshold of 50 % may be replaced by a threshold between 1 and 50 %.

By derogation from the above, fullerenes, graphene flakes and single wall carbon nanotubes with one or more external dimensions below 1 nm should be considered as nanomaterials.

Nanomaterials in Polymer Research

ELSEVIER

Polymer

Volume 49, Issue 15, 7 July 2008, Pages 3187-3204

polymer

Feature Article

Polymer nanotechnology: Nanocomposites

D.R. Paul [a, 1], L.M. Robeson [b]

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https://doi.org/10.1016/j.polymer.2008.04.017

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Abstract

In the large field of nanotechnology, polymer matrix based nanocomposites have become a prominent area of current research and development. Exfoliated clay-based nanocomposites have dominated the polymer literature but there are a large number of other significant areas of current and emerging interest. This review will detail the technology involved with exfoliated clay-based nanocomposites and also include other important areas including barrier properties, flammability resistance, biomedical applicatio
electrical/electronic/optoelectronic applications and fuel cell inter
question of the "nano-effect" of nanoparticle or fiber inclusion rel
counterparts is addressed relative to crystallization and glass tra
other polymer (and composite)-based properties derive benefits
addition and these are addressed.

- **Drug Delivery**
- **Exosomes**
- **Viruses**
- **Polymer-NP-Conjugates**
- **PEG/HES-Protein Conjugates**
- **Sunscreens**
- **Tomato Soup**
- **...**

Anal Bioanal Chem. 2014 June; 406 (16): 3895-3907

Production of reference materials for the detection and size determination of Silica Nanoparticles in Tomato Soup

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Environ. Sci. Technol., 2014, *48* (10), pp 5415–5422

Release of TiO_2 Nanoparticles from Sunscreens into Surface Waters: A One-Year Survey at the Old Danube Recreational Lake

The New Postnova FFF-Platform

The New Postnova Platform

The New Postnova Platform

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PN3600 MALS Detector

Separation by electrophoretic mobility
Method fine tuning by reverse charge
Combine Size + Charge Separations

Malvern Zetasizer Nano

Particle Size R_g
Molar Mass Mw
Branching

Particle Size R_h
Zetapotential
Rel. Particle Density

Particle Size R_h
Elemental
Composition

Concentration
Intrinsic Viscosity
Branching

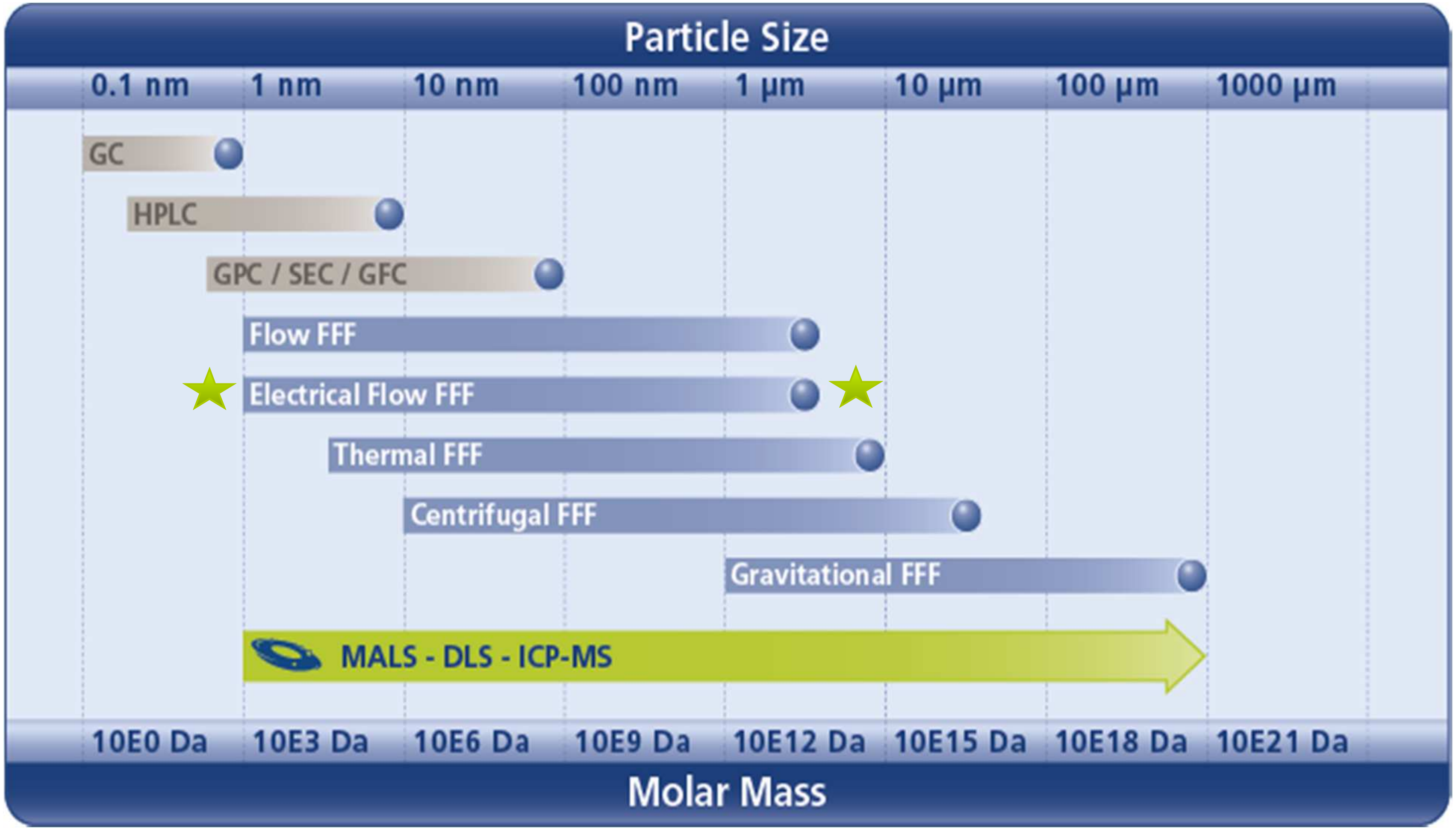
Agilent 7900 ICP-MS

PN3150 Refractive Index Detector

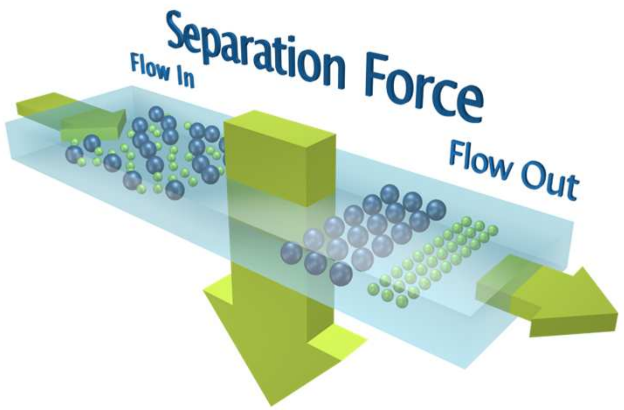
PN3310 Viscometric Detector

The New Postnova Platform

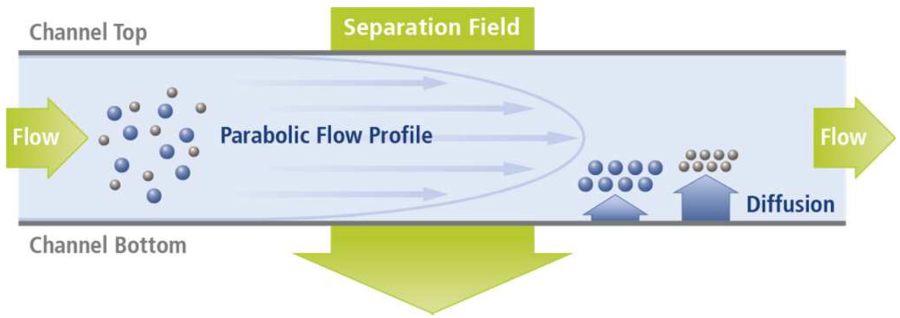
New Member EAF4



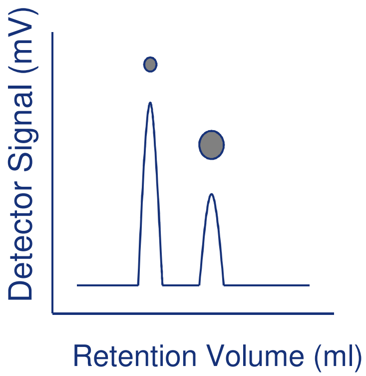
Separation Mechanism



- Separation in a narrow ribbon-like channel
- Laminar flow inside the channel
- External field perpendicular to the solvent flow

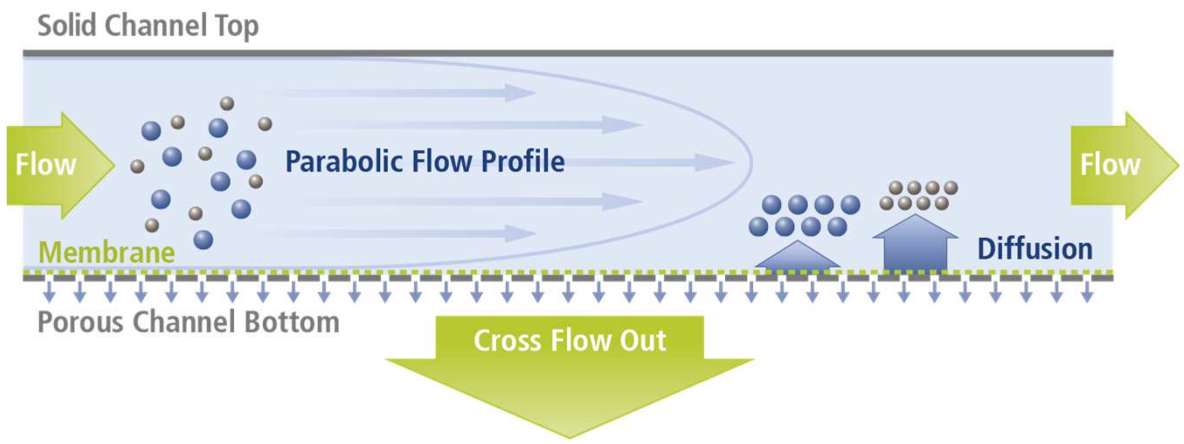


Different Separation Fields:
Flow
Centrifugal
Thermal
Gravitational



Flow FFF – Principle

Asymmetric Flow FFF Principle (AF4)



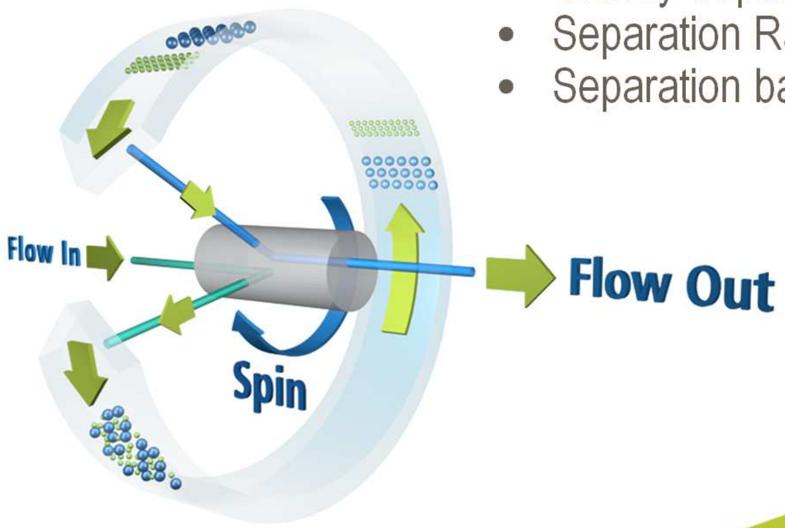
Applications

- Peptides
- Proteins
- Antibodies
- Virus
- Liposomes
- Latex Bead
- Nano Particles
- Synthetic / natural Polymers

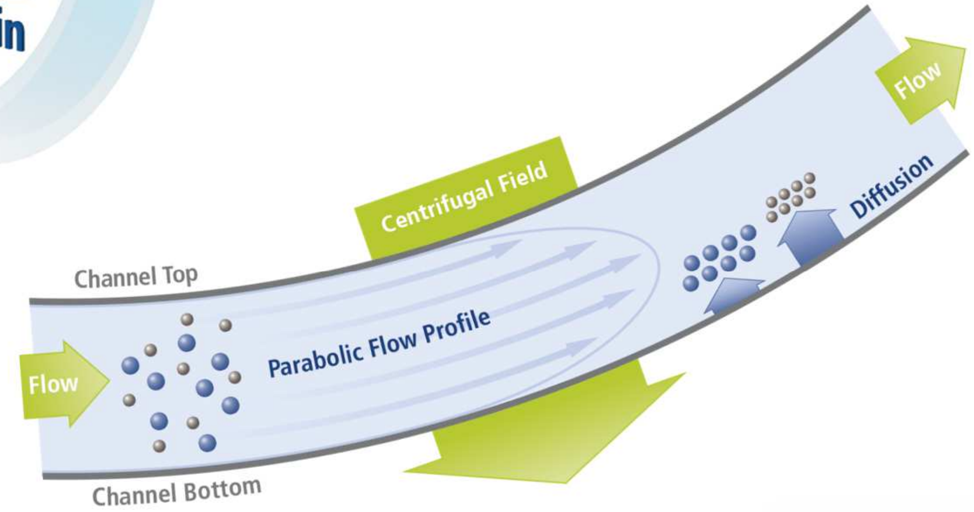
- Cross-flow (hydraulic pressure gradient field) controlling separation
- Separation based on **Size (Hydrodynamic Radius)**
- Channel at ambient, mid or high temperature

CF2000 - Centri FFF

Centrifugal FFF Channel



- Gravity Separation Field up to 2.500 g
- Separation Range: 7 nm – 50 µm
- Separation based on Size and Density



Applications

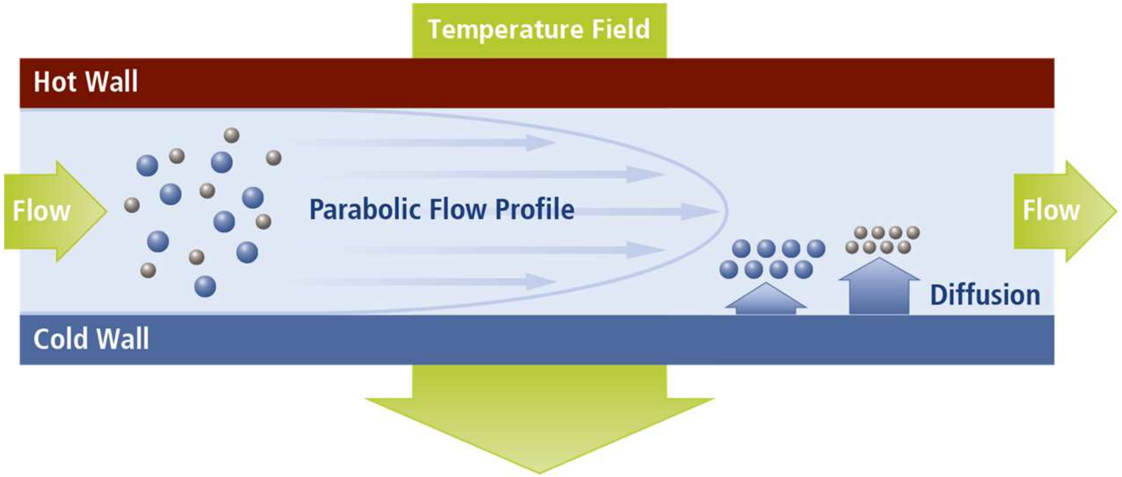
- Nanoparticle
- Microparticle
- Latices
- Gold / Silver Nano
- Liposomes
- Emulsions

Thermal FFF – Principle

TF2000: Separation

$V_E \sim D_T \Delta T / D$

Thermal Diffusion Coeffcient
-> Depends on chemical composition



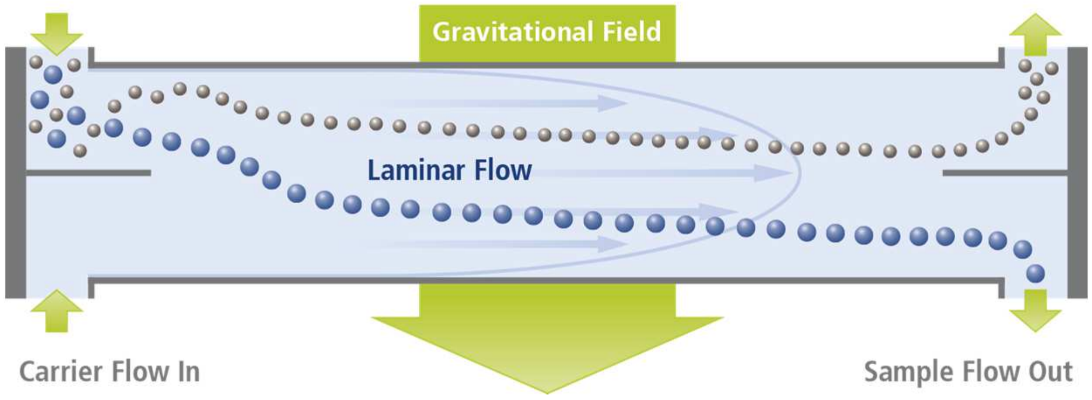
- Thermal gradient up to Δ120°C
- Separation kDa up to several MDa
- Separation depends on **Size** and **Chemical Composition**

Applications

- Rubbers / Polymers
- Gels / Latexes
- Cross-linked Polymers

Gravitational SPLITT FFF – Principle

SF2000: Separation



- Sedimentation by gravity
- Particles 1 – 100µm
- Continuous, semi prep (mg/day up to g/day)

Applications

- Microparticles
- Cells
- Algae
- Diatoms
- Pollen
- Sediments

FFF Applications

AF4-UV-MALS-DLS

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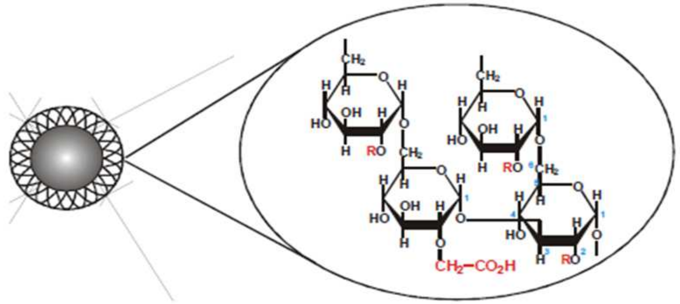
Polymer-Coated Nanoparticles

Core: Magnetite (Fe_3O_4)

Matrix: Sodium-Carboxymethyldextran

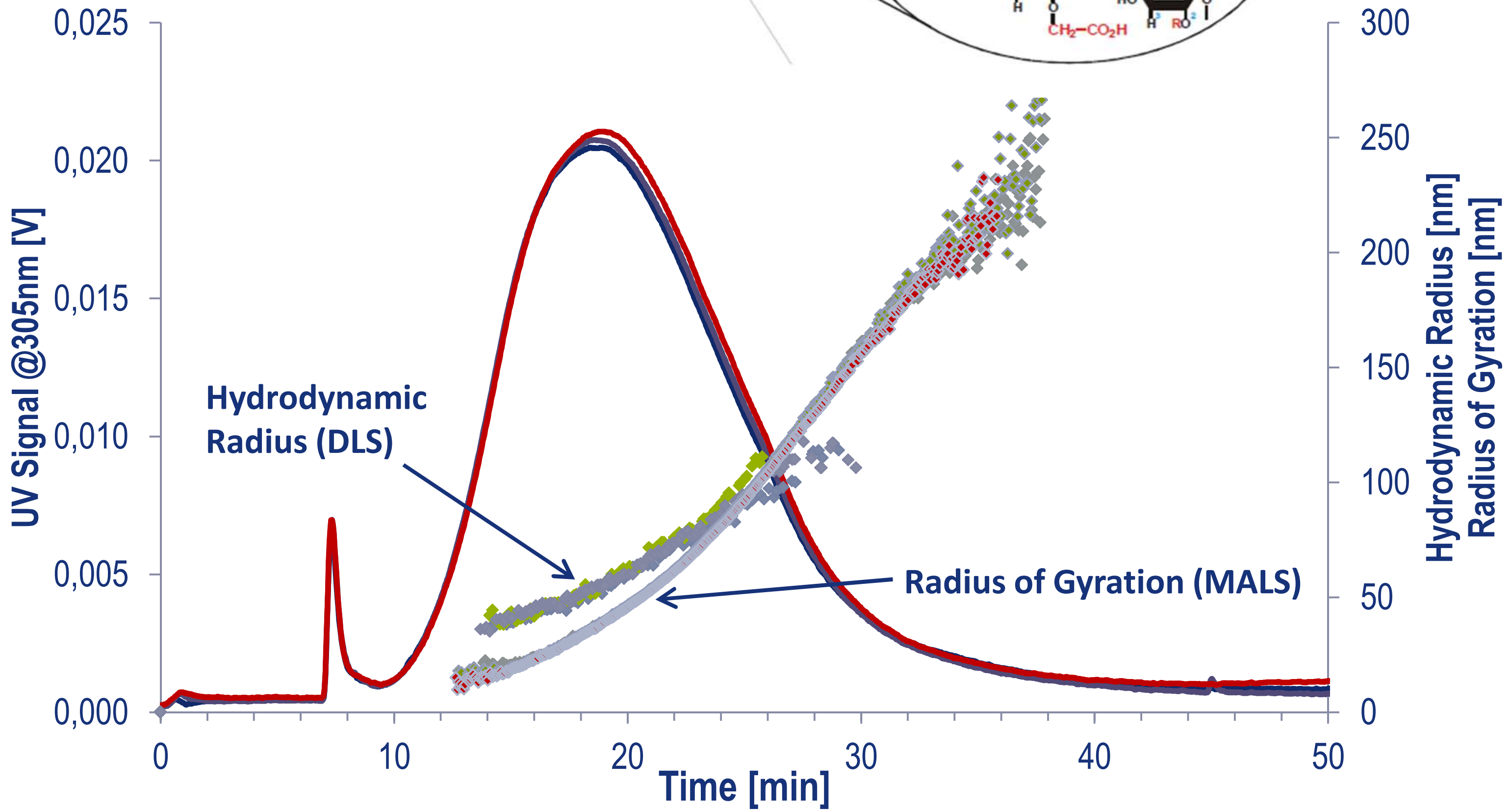


Shape Information:

Sphere: $R_g = \sqrt{3/5} \times R_h$

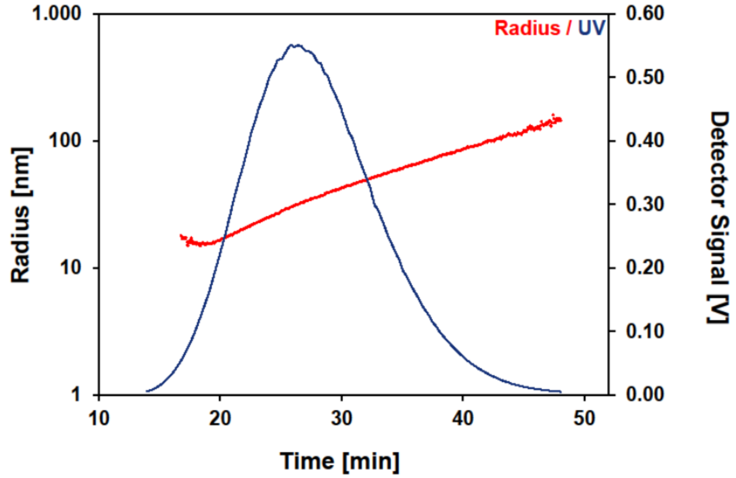
Spherical Shell: $R_g = R_h$

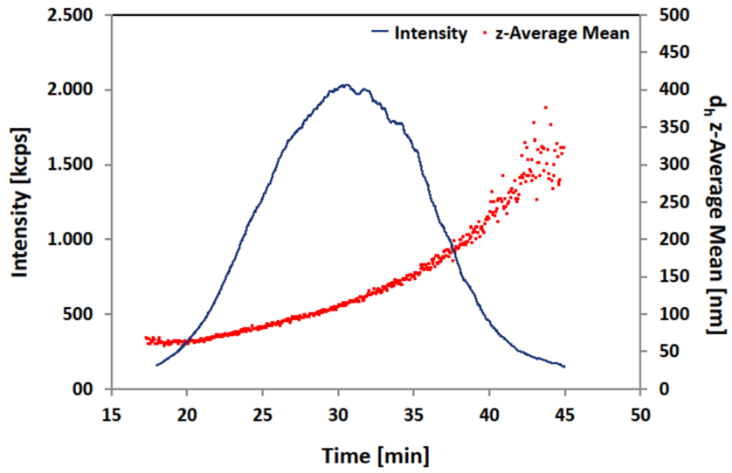
Infenitily thin Rod with length L: $R_g = L / \sqrt{12}$



AF2000-UV-MALS-DLS

Characterization of TiO_2 (AeroDisp® W740X, Evonik)





R_g Distrib. [nm]	R_g UV_{max} [nm]	D_h z-Ave. Flow [nm]	D_h z-Ave. Batch [nm]	PDI
16 - 80	30	114	115	0.127

➡ R_g from MALS
➡ D_h from DLS

- Application in routine monitoring of product quality of bulk (nano)-materials
- TiO_2 used in sunscreen lotions => cosmetics industry

AF4 Separation Channels

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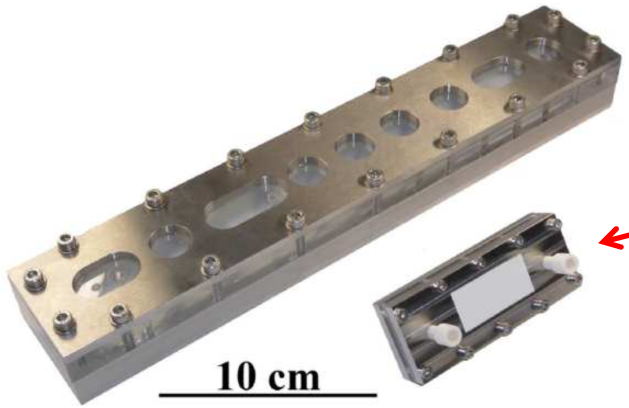
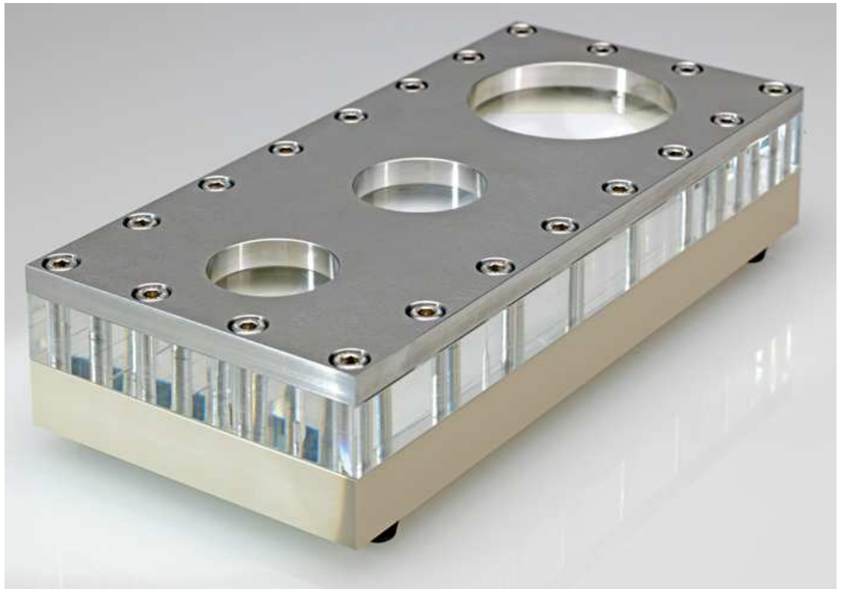


Analytical
Channel
and
Microchannel



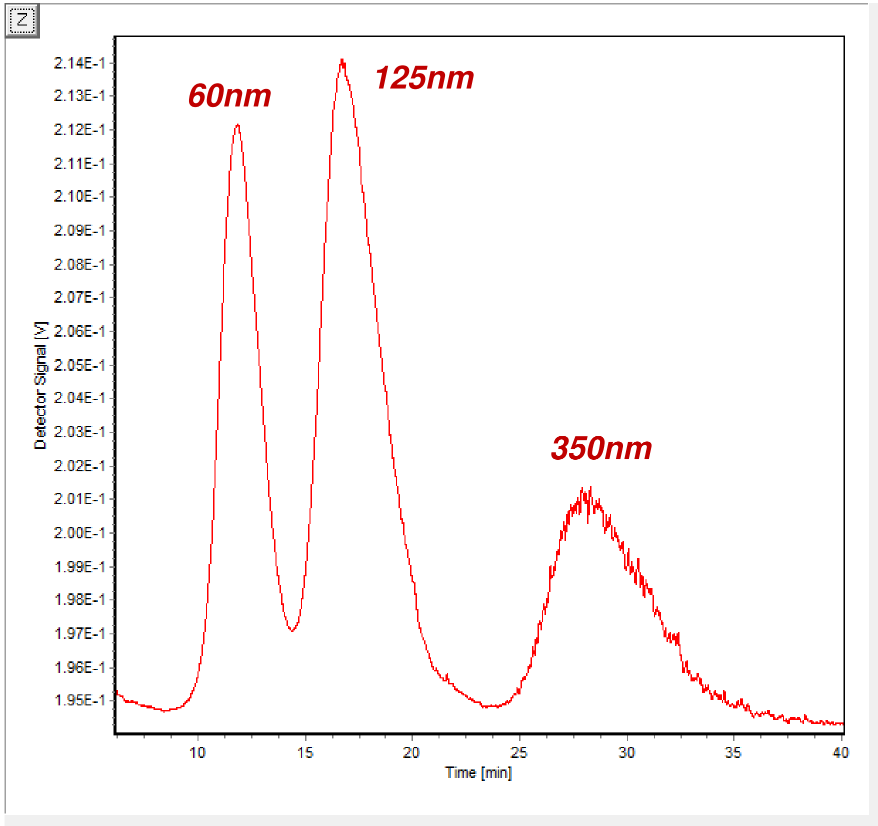
Preparative
Channel

Hollow Fiber

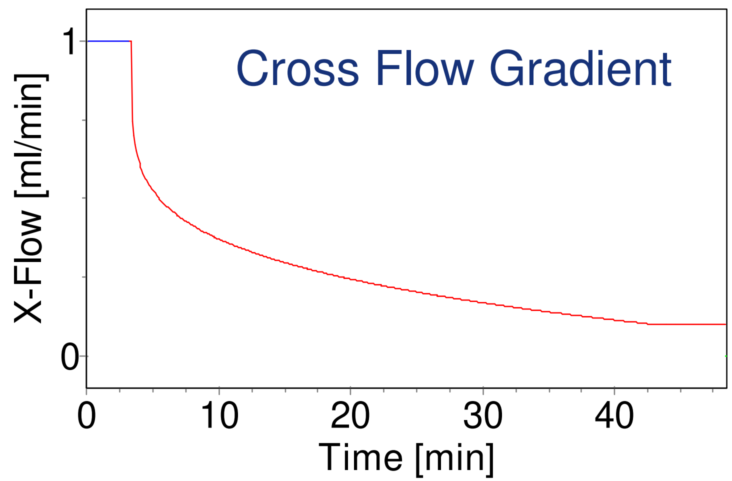
AF4–Microchannel: Latex Nanoparticles

Separation of latex nanoparticles with diameter of 60 nm, 125 nm and 350 nm with microchannel

MALS Signal (90° Angle)



- Separation of mixture of latex nanoparticle mixture with diameter of 60 nm, 125 nm and 350 nm
- Solvent: 0.05% Novachem solution with 0.05% NaCl



NEW: EAF2000 Electrical Flow FFF – Working Principle –

EAF2000 Electrical Flow FFF

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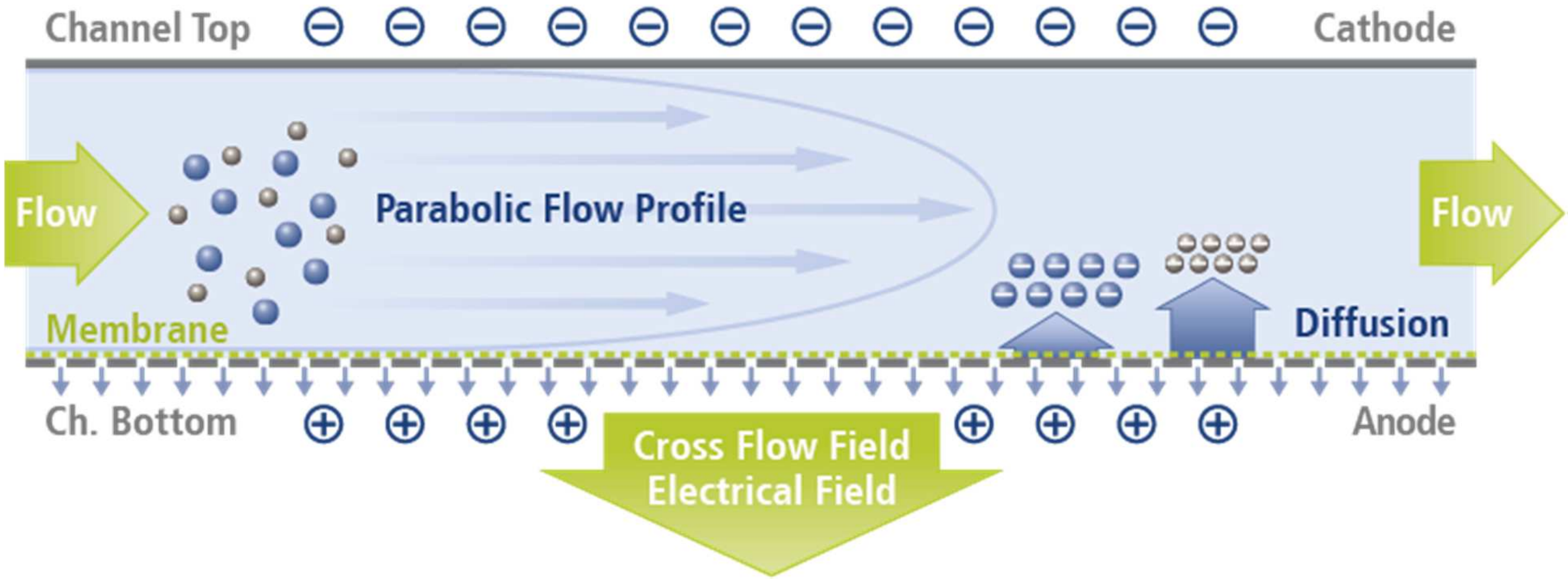
Principle of Electrical Asymmetrical Flow FFF – EAF4



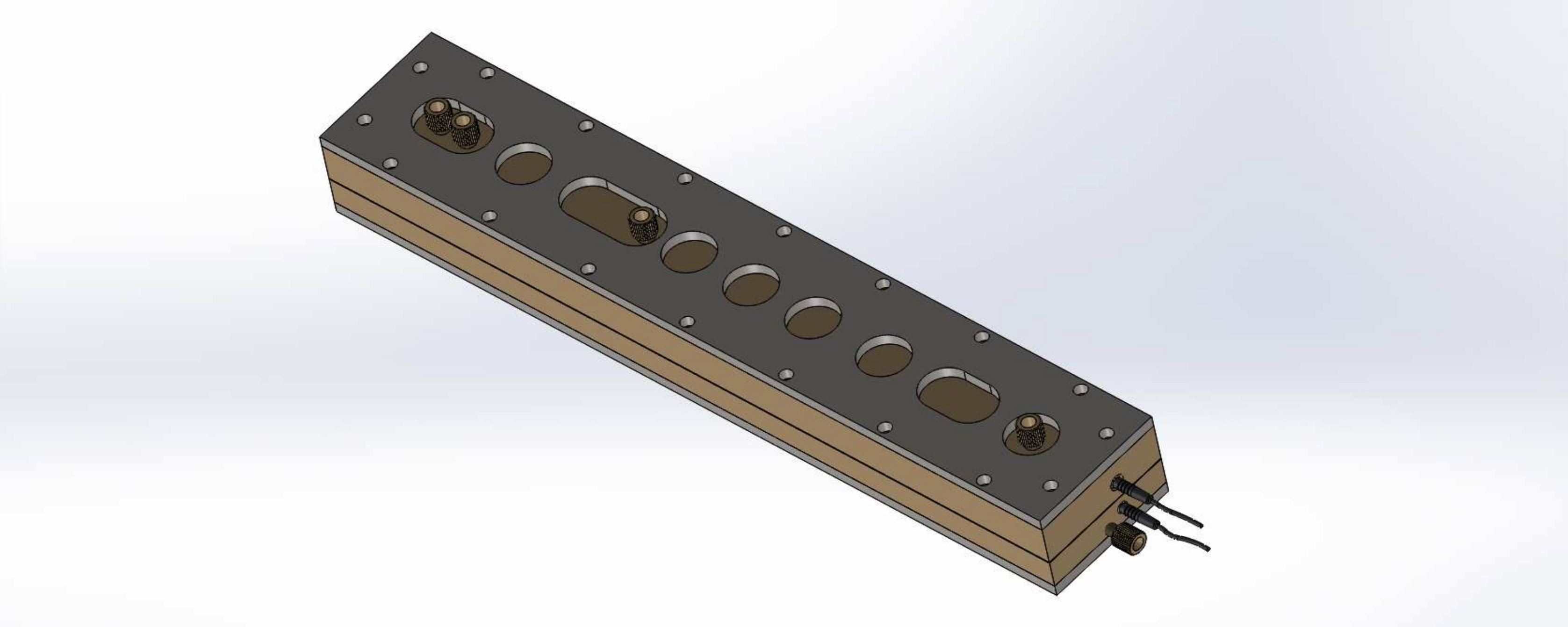
- EAF4 combines the principle of Electrical and Asymmetrical Flow FFF in one Channel
- Electric and Cross Flow Fields are applied simultaneously across the channel
- Separations by particle size and electrophoretic mobility (particle charge)

New

EAF2000 Electrical Flow FFF – Hardware Set-up –

EAF2000 Electrical Flow FFF

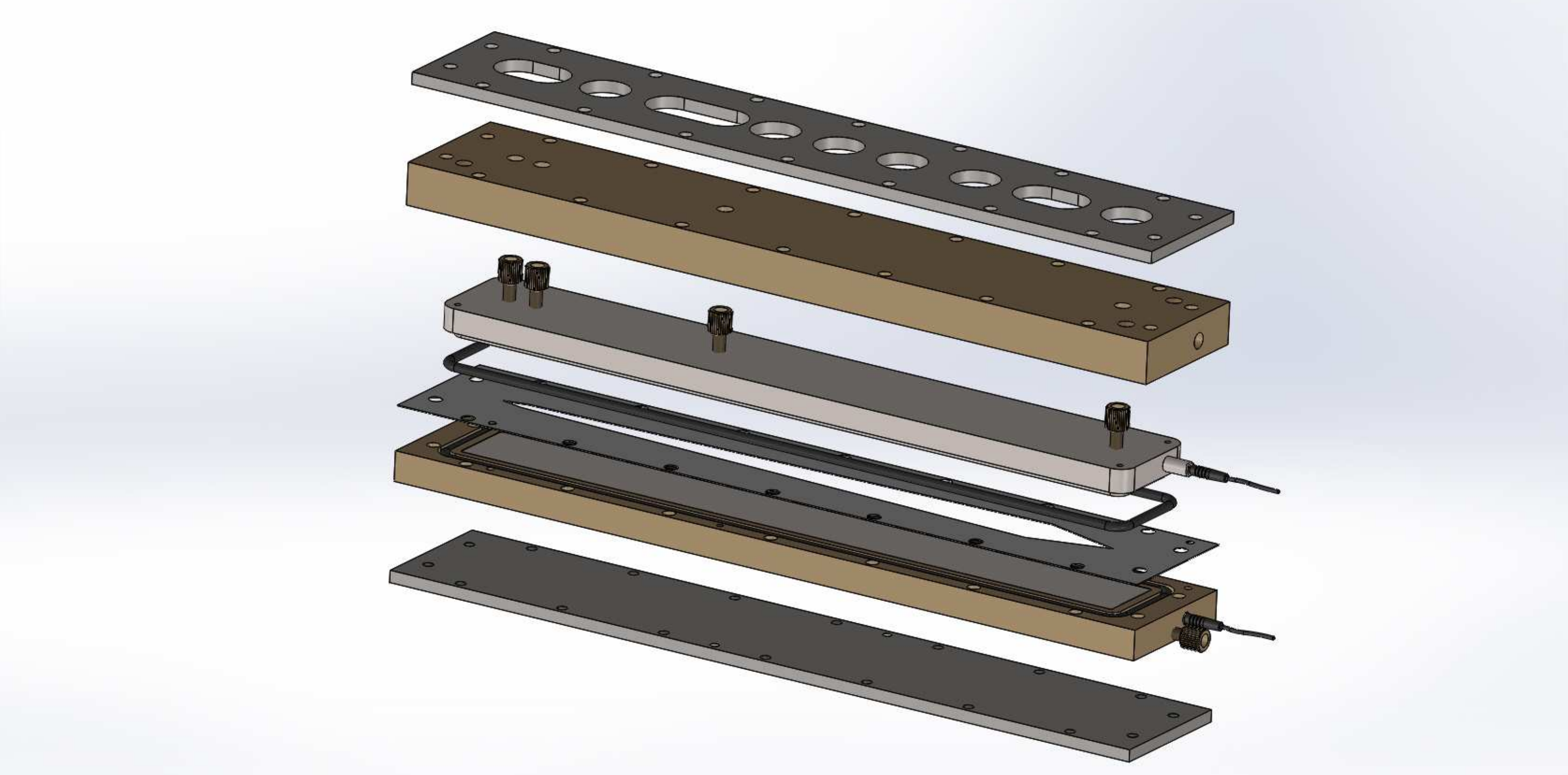
EAF4 – Electrical Flow FFF Channel – Outside View



- New designed EAF4 Channel for combined Electric Asymmetrical Flow FFF
- Inert PEEK Channel cartridge with stainless-steel metal holder plates on top and bottom
- Same outside and inside dimensions as normal standard AF4 Analytical channel
- Same range of existing AF4 analytical channel membranes can be used as well
- Focusing and Smart Stream Splitting possible but Frit-Inlet technology not implemented

EAF2000 Electrical Flow FFF

EAF4 – Electrical Flow FFF Channel – Explosion View



- TOP: Metal electrode with incorporated ports and fittings for inlet/outlet/focus/split
- BOTTOM: Metal electrode inside porous frit which support separation membrane (not shown)
- INSIDE: Same dimensions/materials for spacers/membranes as standard analytical channel

EAF2000 Electrical Flow FFF

EAF4 – Electrical FFF Module – Outside View

- The PN2410 Electrical FFF Module is needed to establish and control the Electrical Field
- The Module and the EAF4 channel are connected to an Standard AF2000 Flow FFF
- Additional NovaFFF – EAF4 Software module is needed for upgrading to an EAF2000

EAF2000 Electrical Flow FFF – Total System View

- **PN2410 Electrical FFF Module** for precise control of Electric Field allowing constant and reproducible separation conditions
- **EAF2000 Electrical Flow FFF Channel** with special build-in pole-reversible metal electrodes and membrane for cross flow
- **Software control** module for constant current with security features
- **Electrode commutation** for increased or reduced retention times
- **The Electric Flow FFF Technology** is compatible with any AF2000!
- **Specifications:** Aqueous Solvents; pH: 2–11; Constant Current Mode

EAF2000 Electrical Flow FFF

EAF2000 Electrical Flow FFF Channel

PN2410 Electrical FFF Module

New

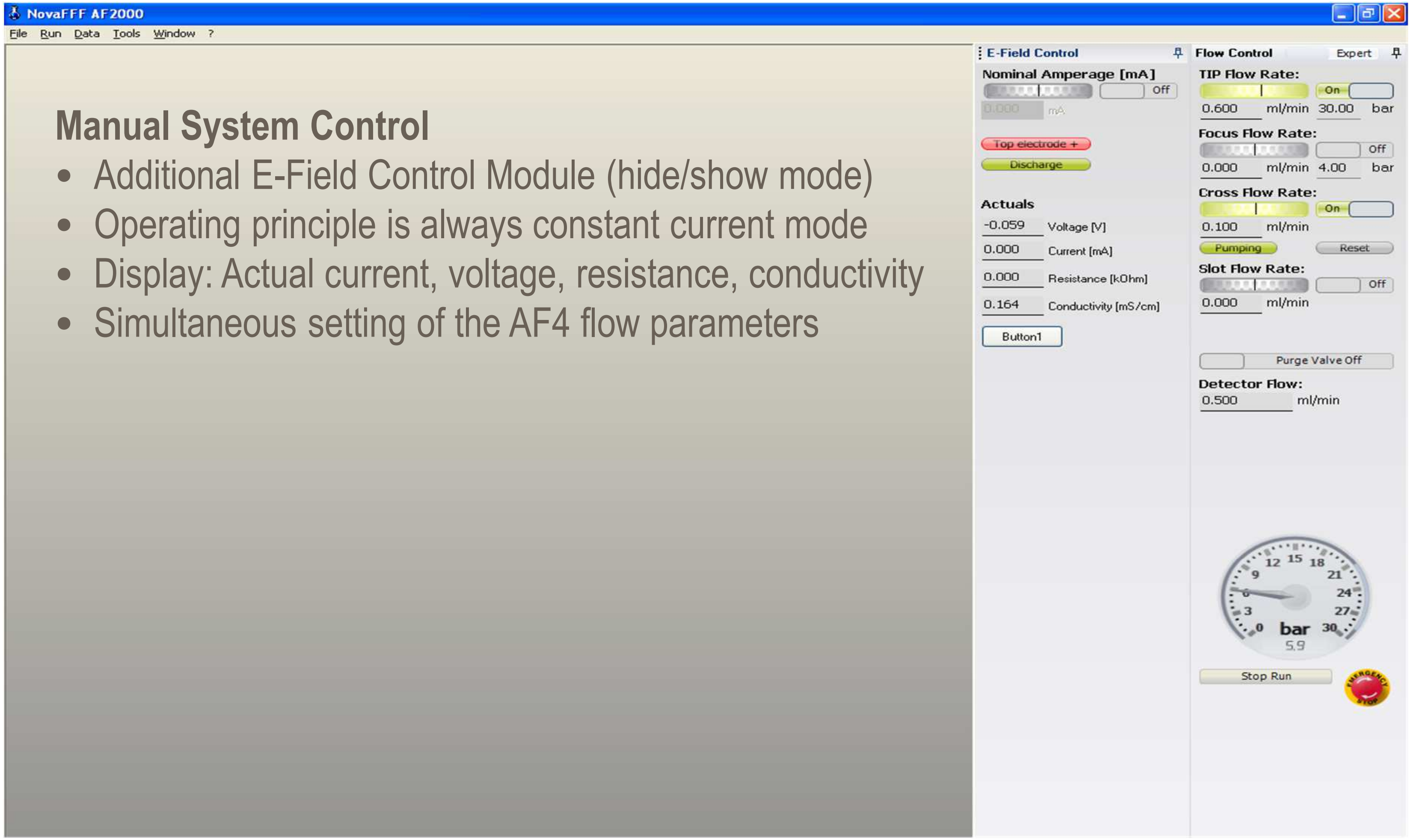
EAF2000 Electrical Flow FFF
– Software –

EAF2000 Electrical Flow FFF

NovaFFF – EAF4 Software Module

Manual System Control

- Additional E-Field Control Module (hide/show mode)
- Operating principle is always constant current mode
- Display: Actual current, voltage, resistance, conductivity
- Simultaneous setting of the AF4 flow parameters



EAF2000 Electrical Flow FFF

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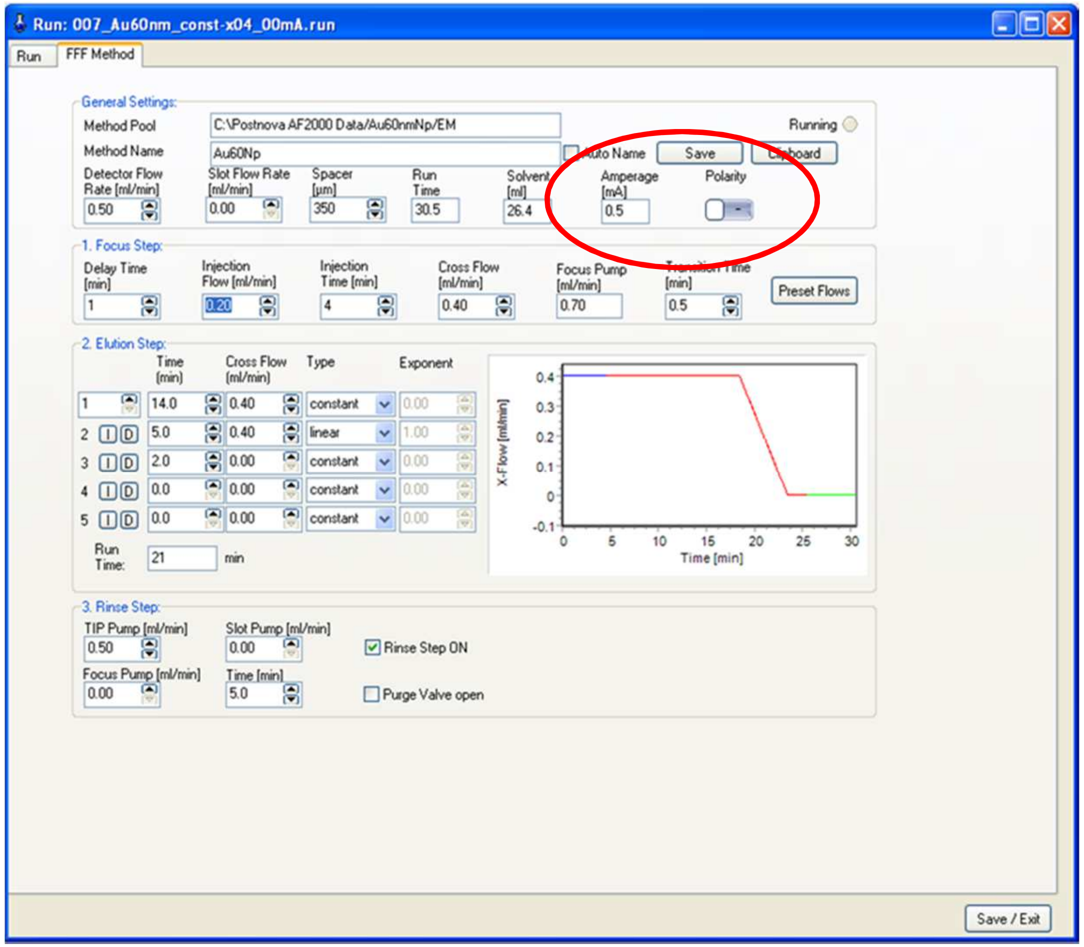
NovaFFF – EAF4 Software Module



FFF Method

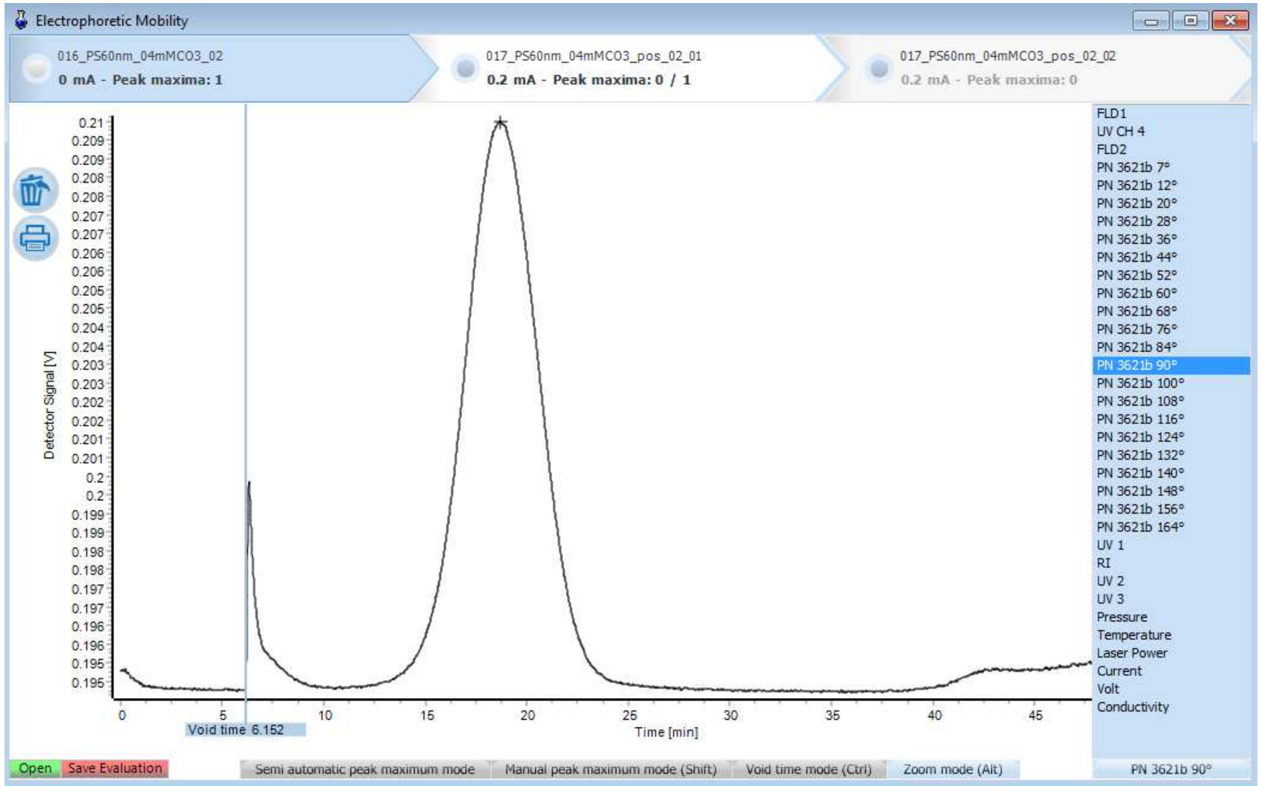
- Same as AF4 method
- ONLY additional Entries:

-> Define Amperage

-> Select Electrode Polarity

EAF2000 Electrical Flow FFF

NovaFFF – EAF4 Software Module



Independent Electrophoretic Mobility Determination

- Calculation of EM directly from retention time difference of at least two runs with different currents
- Need to know channel dimensions and conductivity to calculate effective field strength
- Conductivity is monitored online by sensor build into the PN2410 Electrical FFF Module

Theory

Evaluation of electrophoretic mobility

Calculations:

Definition: $\mu = \frac{\nu_{em}}{E}$ $\quad E = \frac{I}{A \cdot \kappa}$ $\quad \nu_c = \frac{F_c}{A}$ $\quad \nu = \nu_c + \nu_{em}$

μ	electrophoretic mobility
E	electrical field strength
I	applied current
A	channel area
κ	conductivity

ν_c	drift velocity induced by the cross flow
F_c	cross flow
A	channel area
ν	total drift velocity
ν_{em}	drift velocity induced by the electrical field

$$\nu_{em} = \left[e^{\frac{t_{r,i}}{t_r} \ln\left(1+\frac{fF_c}{F_{det}}\right)} - \left[1 + \frac{fF_c}{F_{det}}\right]\right] \cdot \frac{F_{det}}{A \cdot f}$$

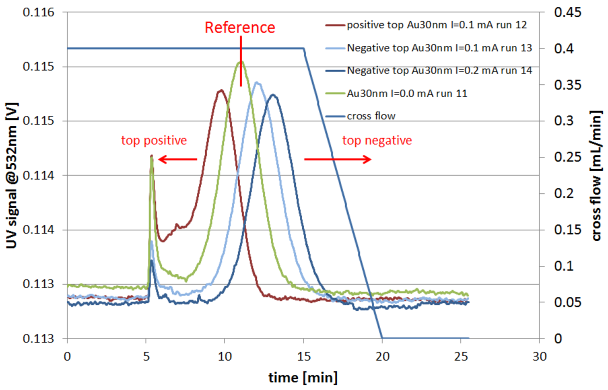
$t_{r,i}$	retention time with applied current (index i)
t_r	retention time of a measurement without current/voltage(**net retention time**)
f	focussing parameter
F_c /F_{det}	cross flow / detector flow rate
A	channel area

Evaluation of electrophoretic mobility

AF4 – measurements: 1. Reference measurement
2. Measurement with electrical field

Calculation of **ratio** of **net retention times** between measurements

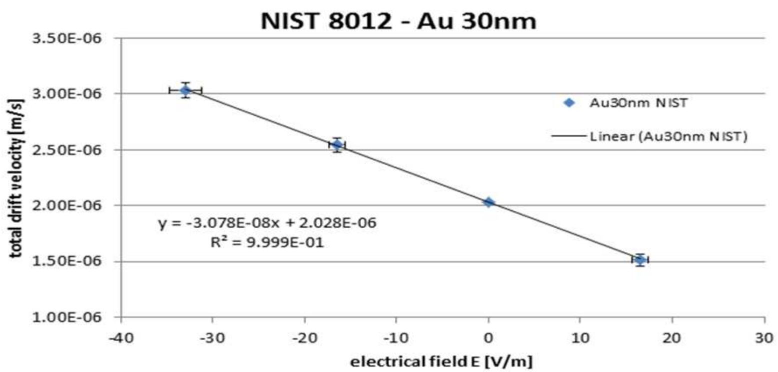
Calculation of drift velocity and **total drift velocity** for each measurement with ratio of cross flow and detector flow



Measuring conductivity during elution, current is set before measurement

Calculation of **electrical field strength E**

Plotting the total drift velocity against electrical field strength
→ the **slope** (linear least squared fit) represents the **electrophoretic mobility** μ_{em}
→ Calculation of a correlation coefficient to check the quality of the results



Evaluation of zeta potential

Start: electrophoretic mobility

Input parameter:

a) Smoluchowski Approximation
$f(\kappa a) = 1.5$
standard for nanoparticles

b) Hückel Approximation
$f(\kappa a) = 1.0$
small particles and proteins in non-polar media

c) Custom Approximation
$f(\kappa a)$ adjustable

↘ ↓ ↙

Temperature: 25°C (Default)

η	Viscosity of at 25 °Ccarrier liquid [Pa s]
ε_0	vacuum permittivity (physical constant) ≈ $8.854187 \cdot 10^{-12}$ [A s V^{-1} m^{-1}]
ε_r	relative permittivity of water at 25 °C ≈ 78.53114 [-]
μ_{em}	calculated electrophoretic mobility
$f(\kappa a)$	"Henry's Function" with $\kappa a = \kappa R_h$
κ	reciprocal of the Debye length κ^{-1} ("thickness" of the electrical double layer)
R_h	hydrodynamic radius of a particle in solution.
κa	estimates the ratio of the particle radius to the electrical double layer.

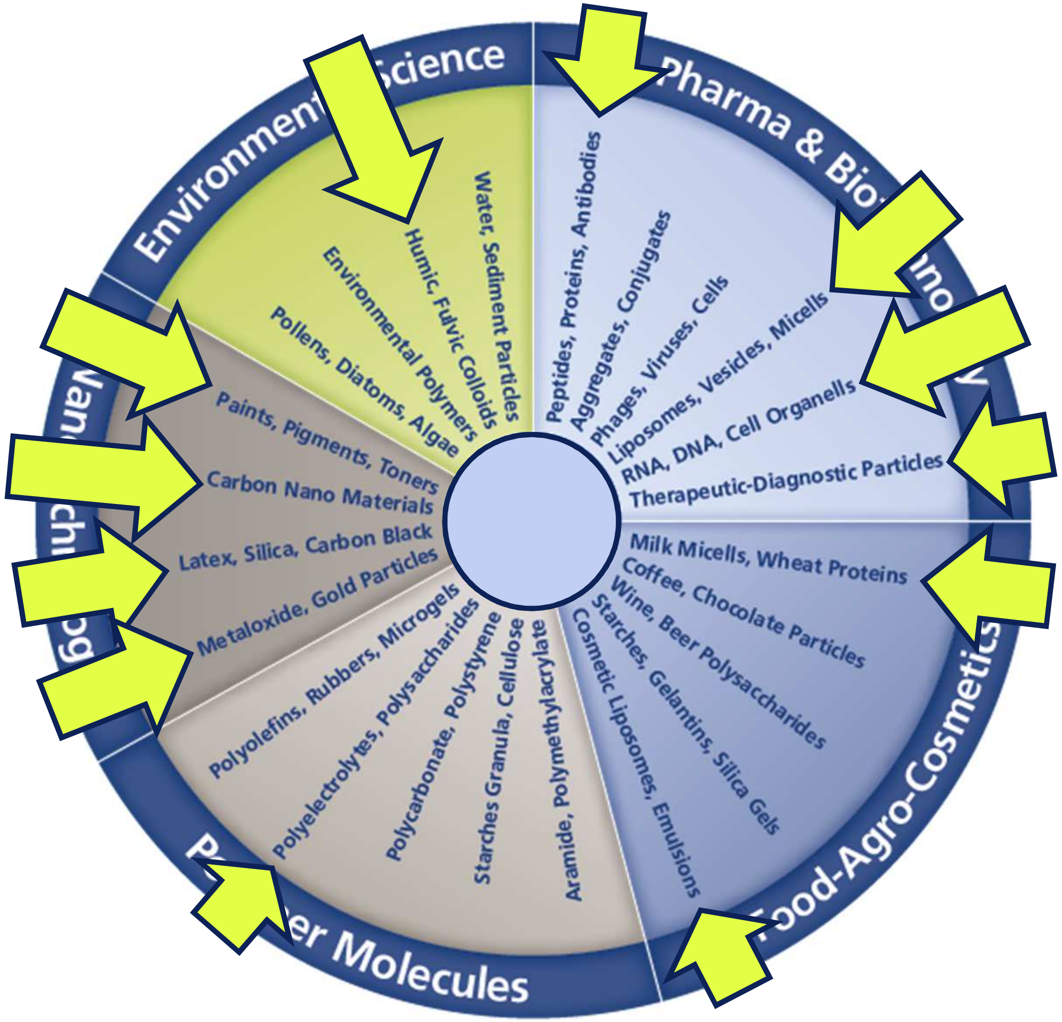
↓

$$\zeta = \frac{3}{2} \frac{\eta \cdot \mu_{em}}{\epsilon_0 \epsilon_r f(\kappa a)} \cdot 1000 \text{ [mV]}$$

New

EAF2000 Electrical Flow FFF – Applications –

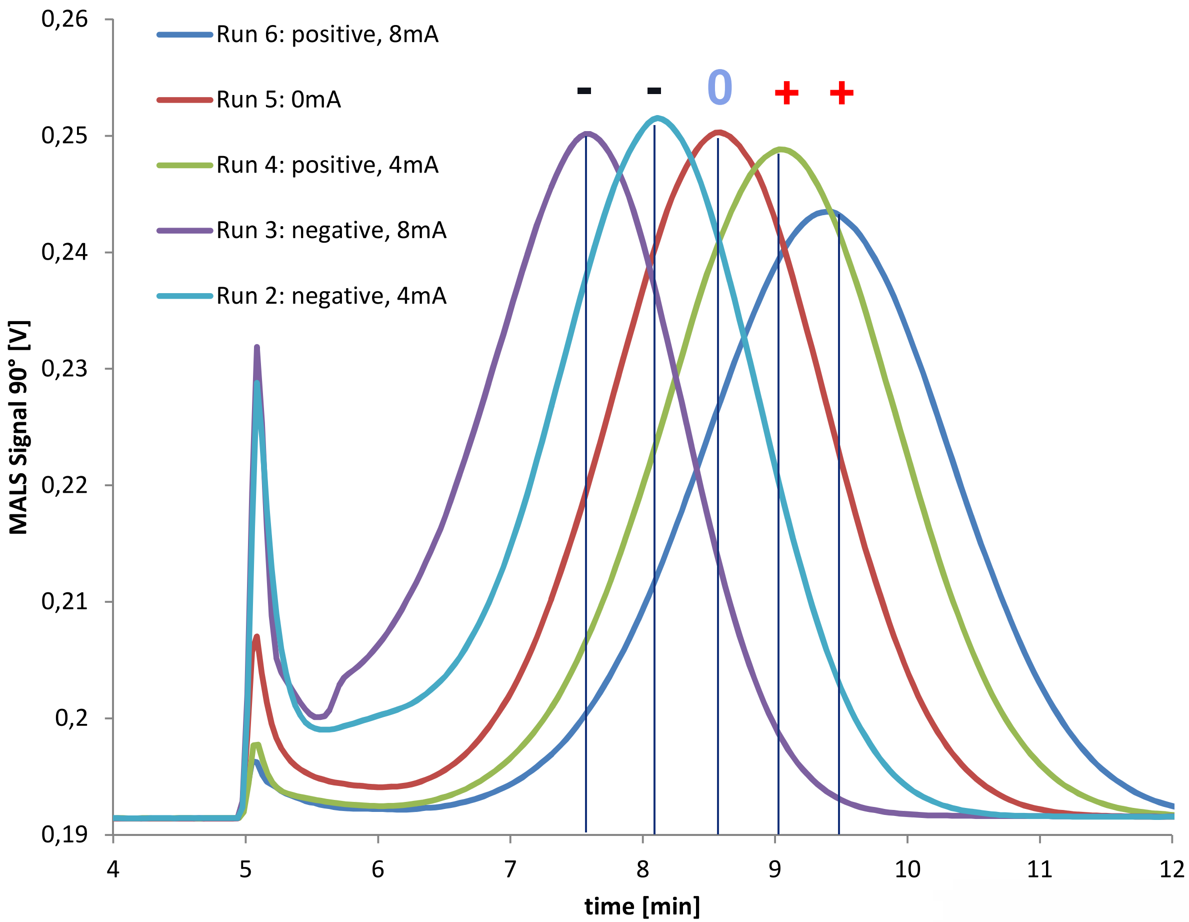
Platform Applications



PS Sulfate Latex Particles (negative Charge)

EAF2000 Electrical Flow FFF

EAF4 Separation of Polystyrene Latex 26 nm



Sulfate stabilized PS NP 26 nm

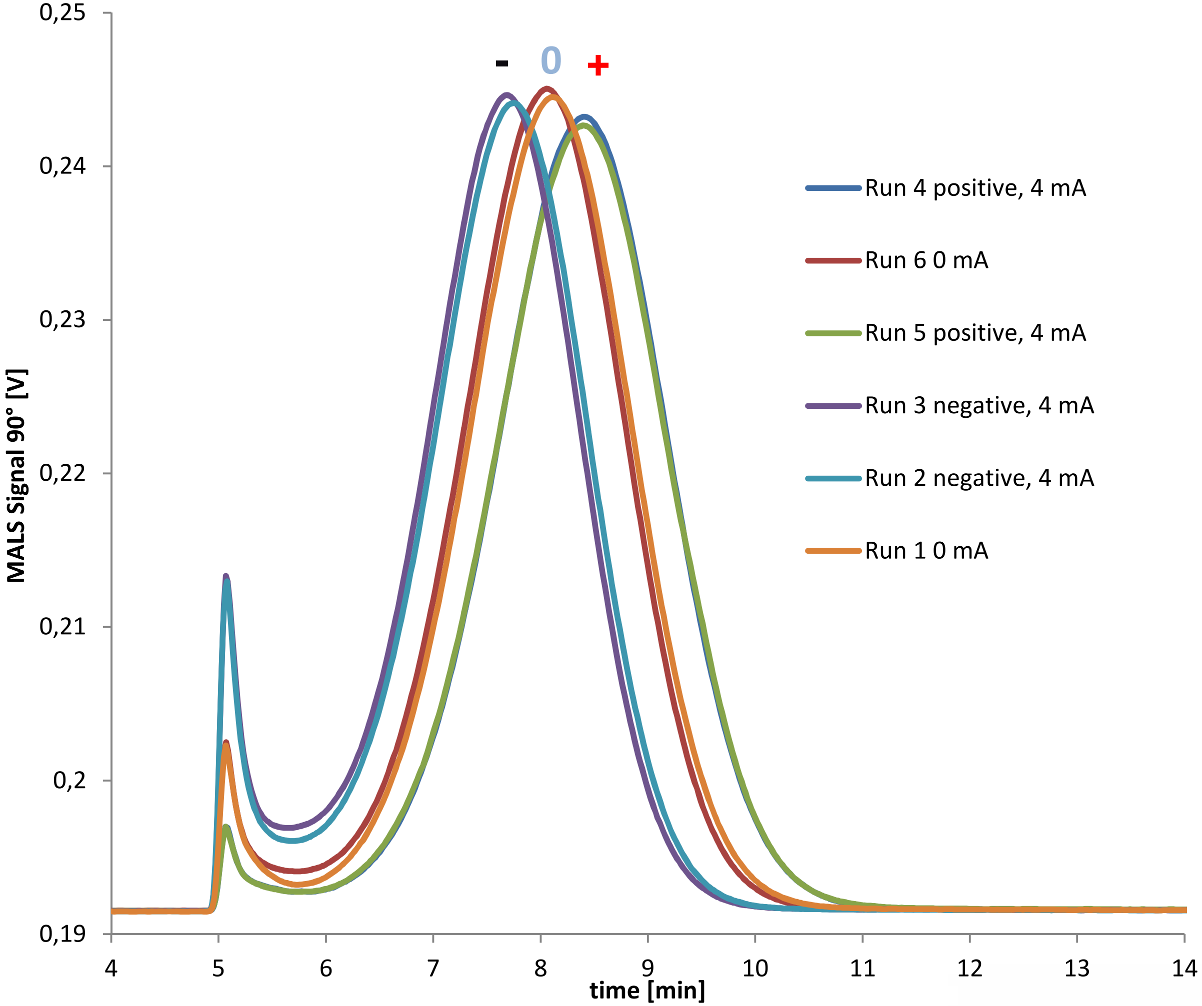
polarity of bottom electrode is named

injection time		4 min
injection flow		0.2 ml/min
detector flow		1 ml/min
cross flow		0.6 ml/min
elution time		
constant cross flow	linear decay	no cross flow
0.6 ml/min 10 min	0 ml/min 5 min	0ml/min 5 min

Run	R_t [min]	ΔR_t [min]
3	7.60	- 0.99
2	8.11	- 0.48
5	8.59	0.00
4	9.03	+ 0.44
6	9.42	+ 0.83

EAF2000 Electrical Flow FFF

1-Day Reproducibility PS Latex 26 nm



Sulfate stabilized PS NP 26 nm

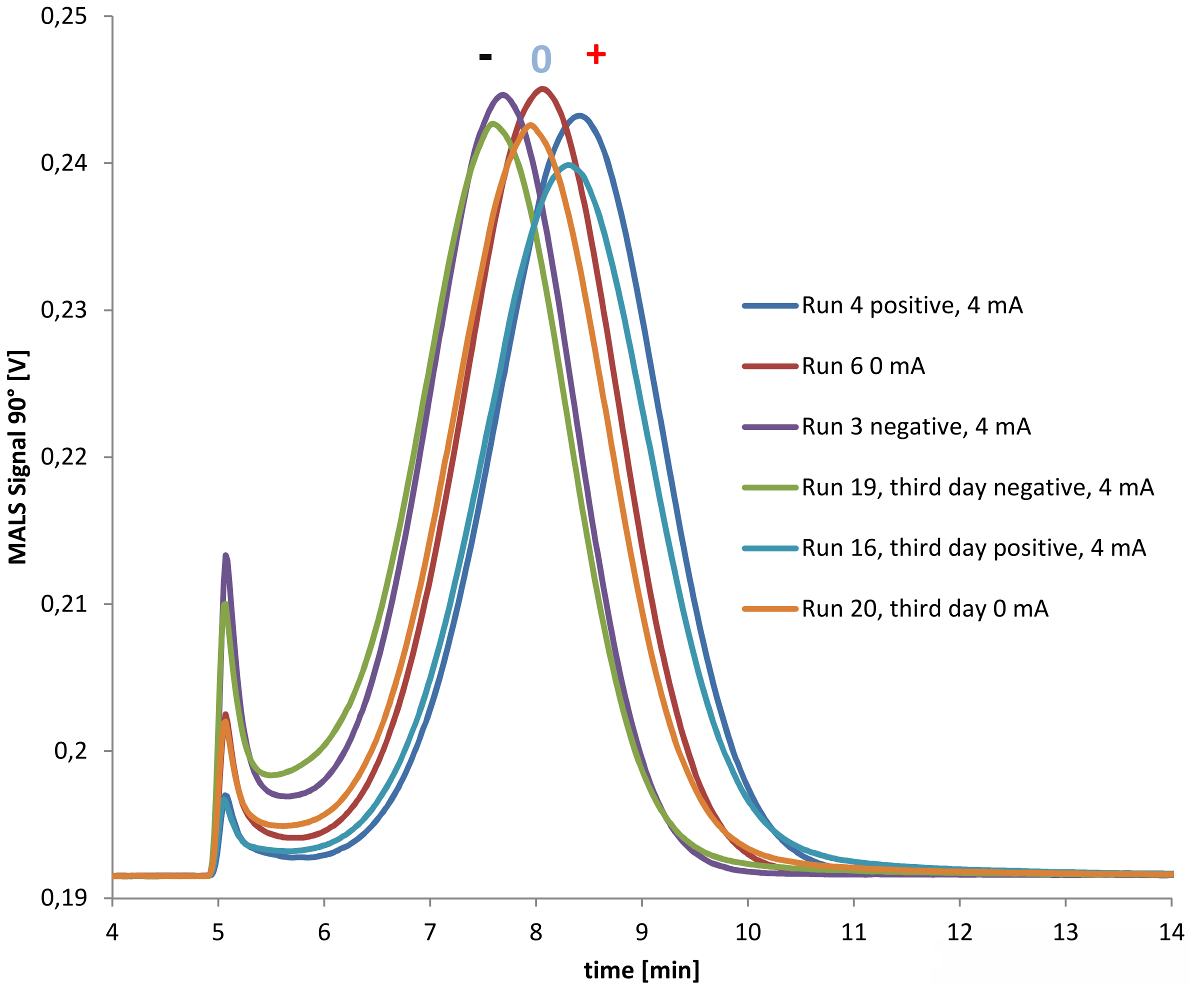
polarity of bottom electrode is named

injection time		4 min
injection flow		0.2 ml/min
detector flow		1 ml/min
cross flow		0.6 ml/min
elution time		
constant cross flow	linear decay	no cross flow
0.6 ml/min	0 ml/min	0ml/min
10 min	5 min	5 min

Run	R_t [min]	Δ [min]
3	7.69	- 0.06
2	7.75	0.00
1	8.11	0.00
6	8.07	- 0.04
5	8.40	+ 0.83
4	8.41	+ 0.01

EAF2000 Electrical Flow FFF

3-Day Reproducibility PS Latex 26 nm



Sulfate stabilized PS NP 26 nm

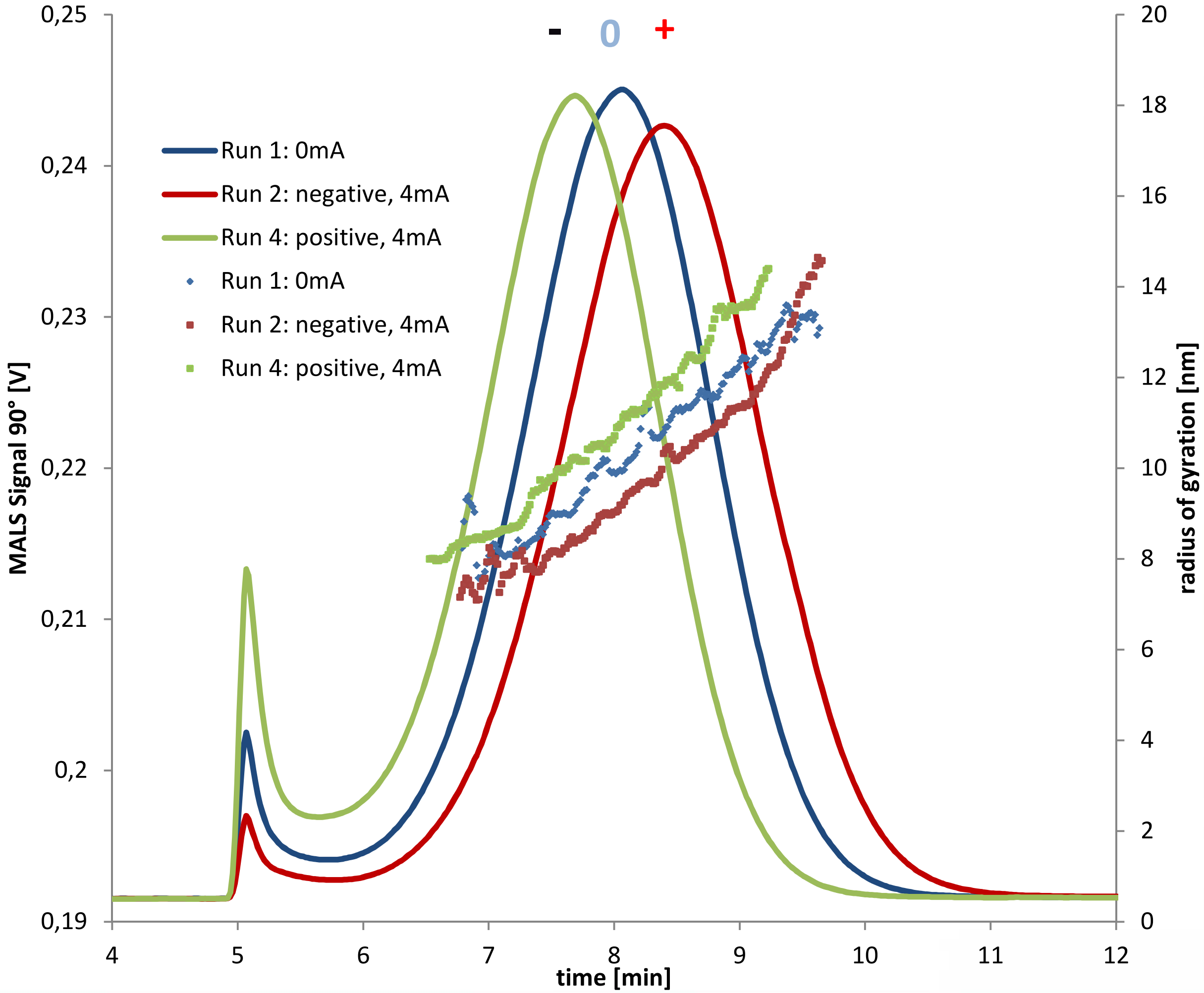
polarity of bottom electrode is named

injection time		4 min
injection flow		0.2 ml/min
detector flow		1 ml/min
cross flow		0.6 ml/min
elution time		
constant cross flow	linear decay	no cross flow
0.6 ml/min	0 ml/min	0ml/min
10 min	5 min	5 min

Run	R_t [min]	Δ [min]
3	7.69	0.00
19	7.60	- 0.09
6	8.07	0.00
20	7.94	- 0.13
4	8.41	0.00
16	8.31	- 0.10

EAF2000 Electrical Flow FFF

Gyration Radius R_g by MALS for PS Latex 26 nm



Sulfate stabilized PS NP 26 nm

polarity of bottom electrode is named

injection time	4 min
injection flow	0.2 ml/min
detector flow	1 ml/min
cross flow	0.6 ml/min

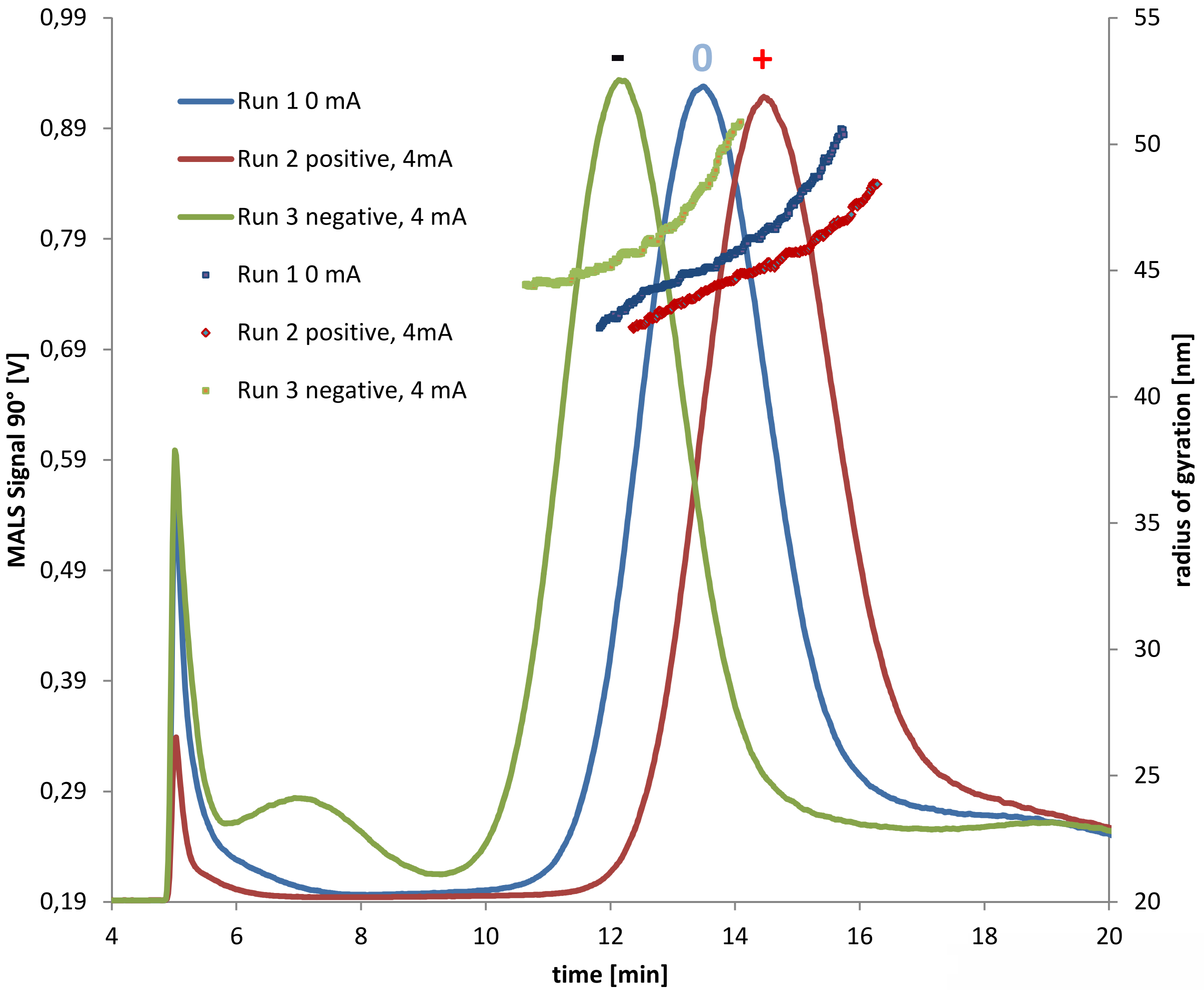
elution time

constant cross flow	linear decay	no cross flow
0.6 ml/min 10 min	0 ml/min 5 min	0ml/min 5 min

Run	R_t [min]	Rg [nm]
4	7.69	10.24
1	8.07	10.10
2	8.40	10.33

EAF2000 Electrical Flow FFF

Gyration Radius R_g by MALS for PS Latex 120 nm



Sulfate stabilized PS Np 120 nm

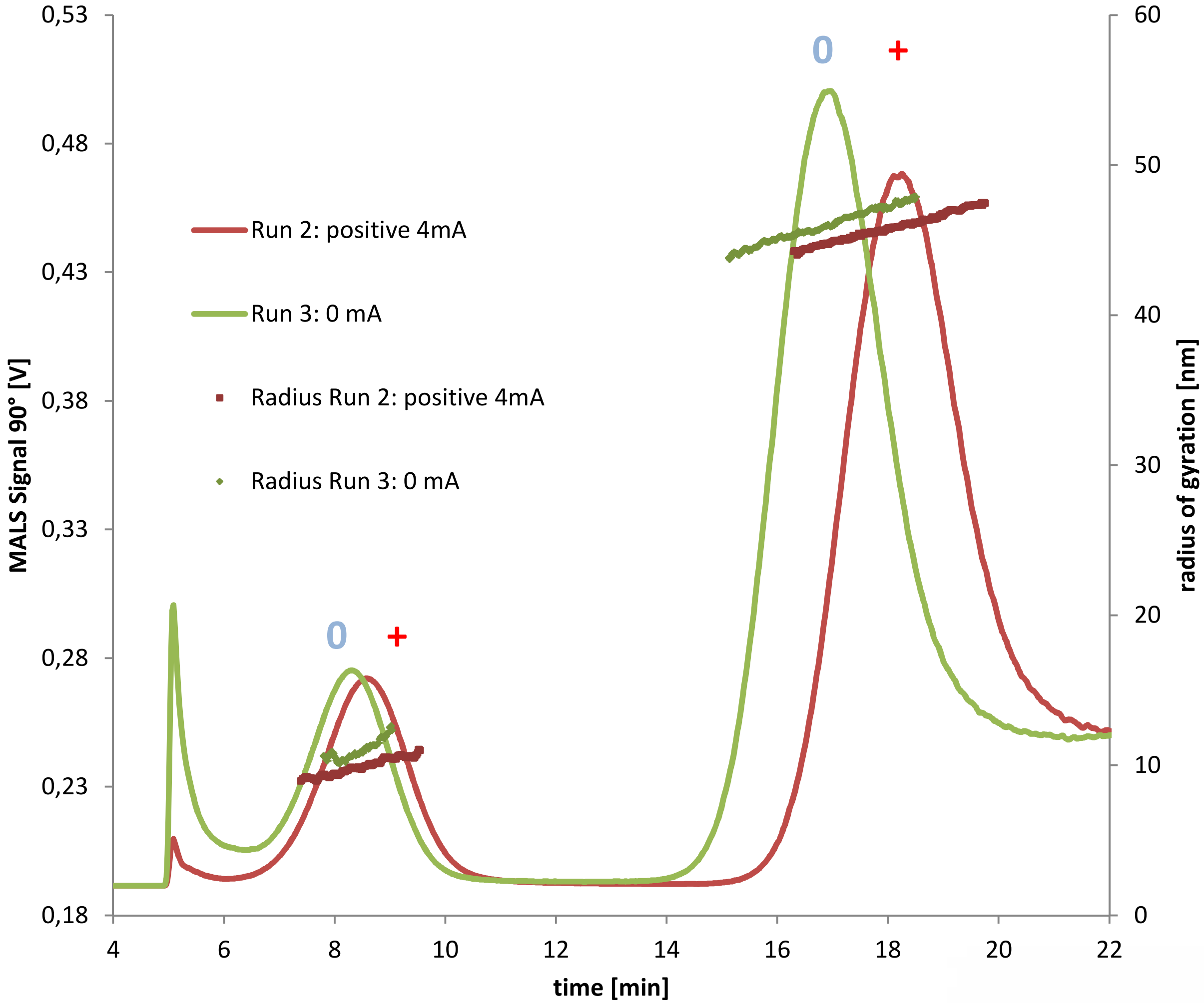
polarity of bottom electrode is named

injection time		4 min
injection flow		0.2 ml/min
detector flow		1 ml/min
cross flow		0.5 ml/min
elution time		
linear decay cross flow		no cross flow
0.5 ml/min		0ml/min
15 min		10 min

Run	R_t [min]	Rg [nm]
3	12.22	45.69
1	13.50	45.04
2	14.46	45.10

EAF2000 Electrical Flow FFF

Gyration Radius R_g by MALS for PS Latex 26 + 120 nm



Sulfate stabilized PS Np 120 nm

polarity of bottom electrode is named

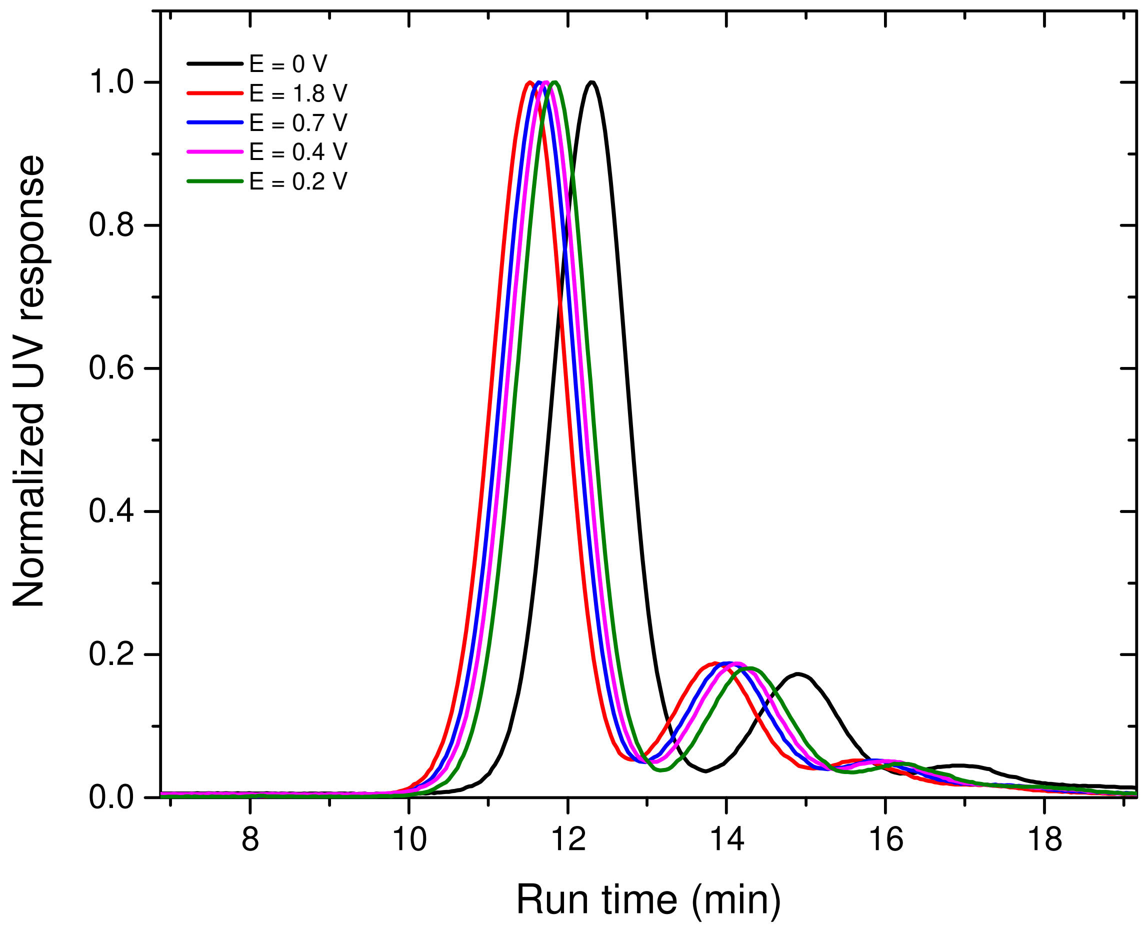
injection time	4 min
injection flow	0.2 ml/min
detector flow	1 ml/min
cross flow	0.5 ml/min
elution time	
linear decay cross flow	no cross flow
0.5 ml/min	0ml/min
15 min	10 min

Run	R_t [min]	Rg [nm]
3 [26]	8.31	10.68
2 [26]	8.58	10.00
3 [120]	16.96	45.90
2 [120]	18.25	45.97

BSA – Protein (negative Charge)

EAF2000 Electrical Flow FFF

EAF4 Separation of BSA with Monomer, Dimer, Trimer



BSA – Bovine Serum Albumin

Bottom Electrode negative (Cathode)

injection time	4 min
injection flow	0.2 ml/min
detector flow	1 ml/min
cross flow	3 ml/min
elution time	
Constant cross flow	3.0 ml/min
Membrane: RC 10 kDA	PBS buffer, pH 7.5

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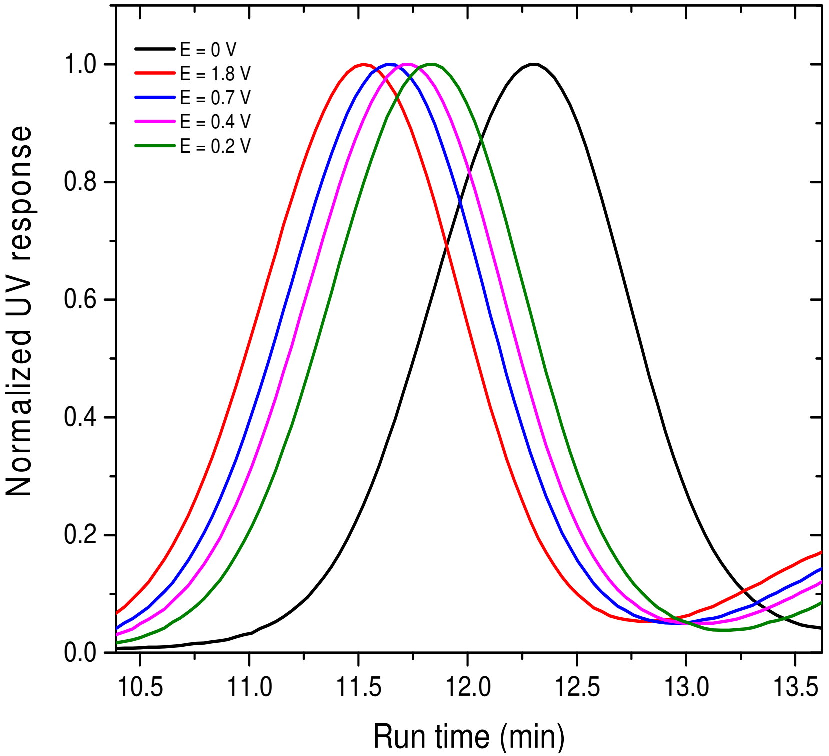
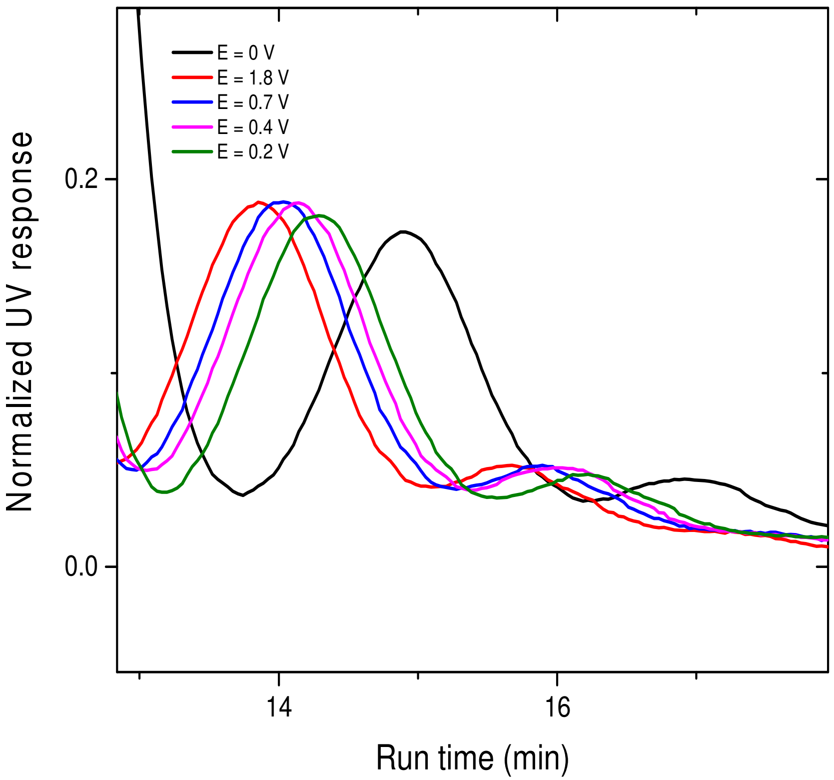
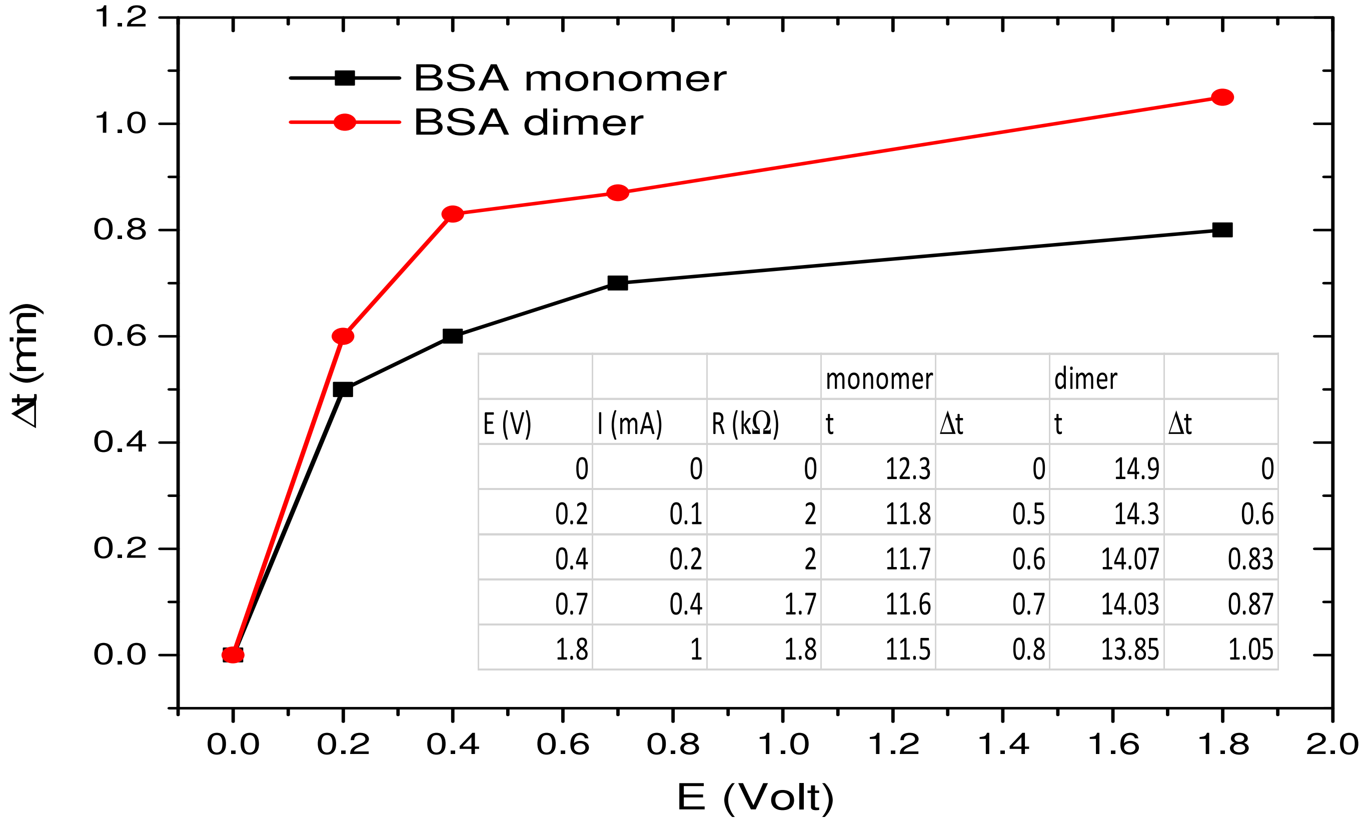
EAF4 Separation of BSA Monomer and Dimer





EAF2000 Electrical Flow FFF

EAF4 Separation of BSA Monomer and Dimer



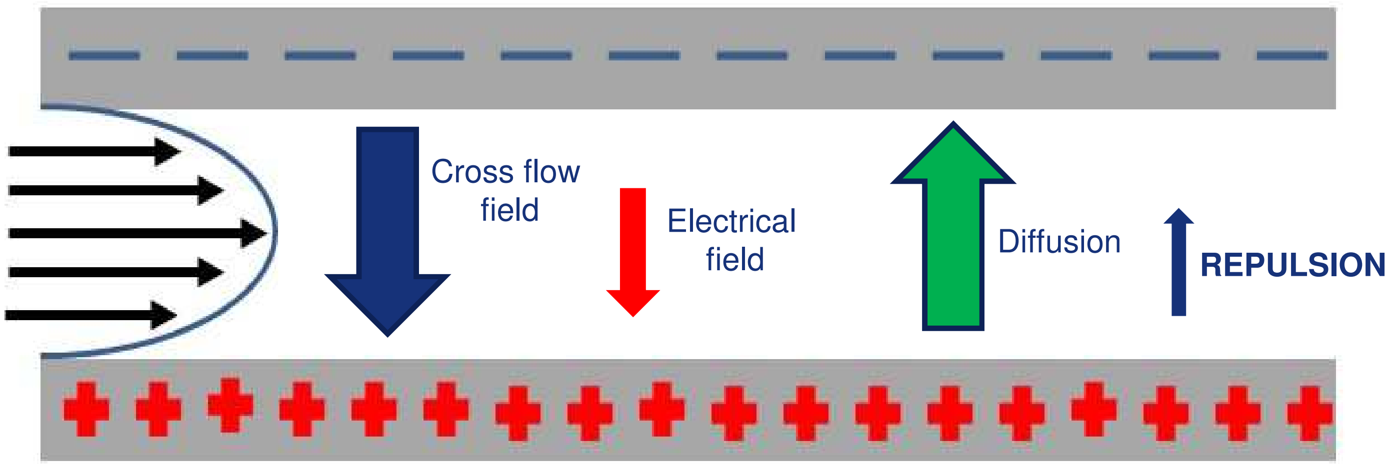
			monomer		dimer	
E (V)	I (mA)	R (kΩ)	t	Δt	t	Δt
0	0	0	12.3	0	14.9	0
0.2	0.1	2	11.8	0.5	14.3	0.6
0.4	0.2	2	11.7	0.6	14.07	0.83
0.7	0.4	1.7	11.6	0.7	14.03	0.87
1.8	1	1.8	11.5	0.8	13.85	1.05

- Additional increased separation can be achieved after applying additional E-Field
- Dimer Separation improves more compared to Monomer, then constant
- After E=0.4 V field is applied no more increase of resolution for monomer/dimer

Pharmaceutical Protein (positive Charge)

EAF2000 Electrical Flow FFF

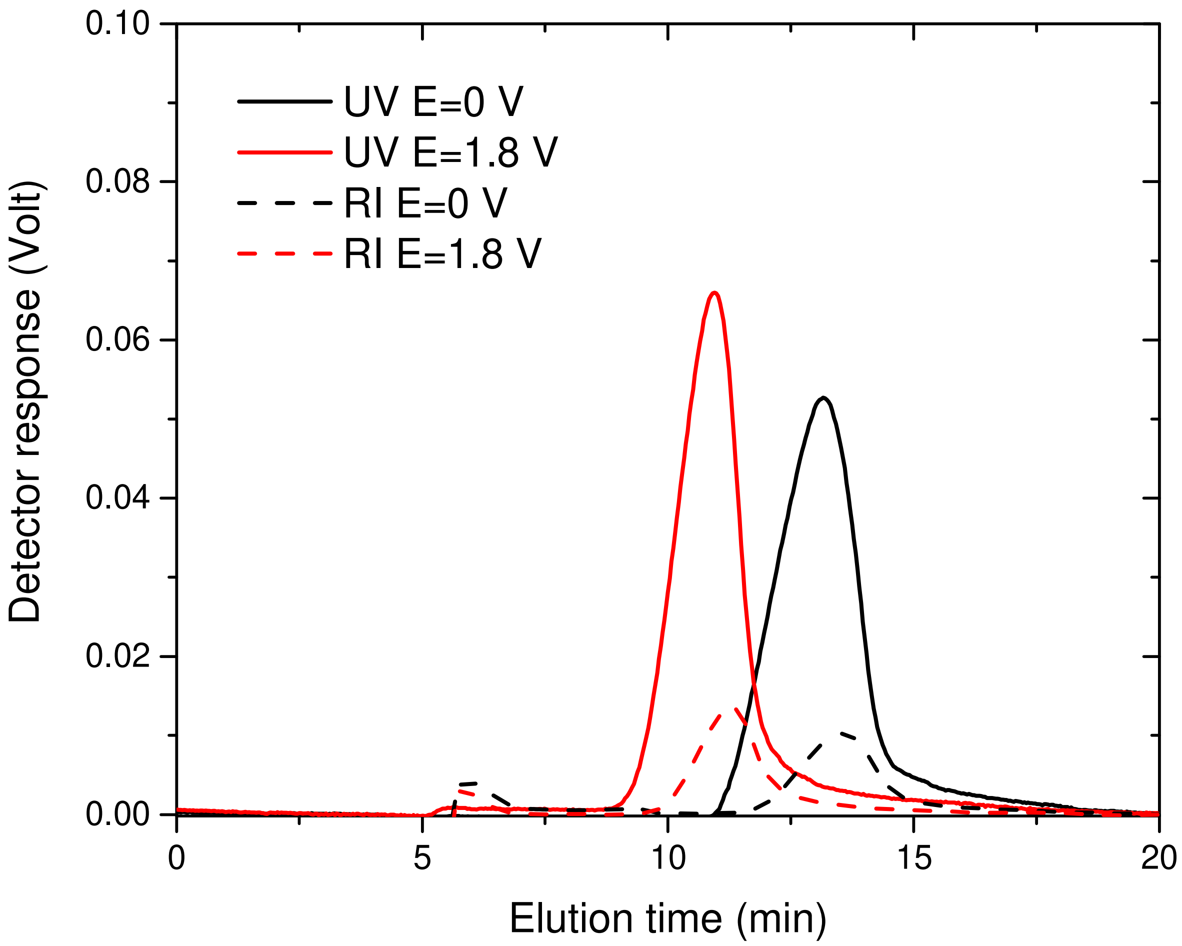
RCP – Reversed Charge Polarization technique creating Analyte Repulsion



Inducing a positive charge on the membrane by selecting the Anode to be at the bottom wall and by applying sufficient voltage will result in a repulsion of positively charged analytes from the membrane and thus cause earlier elution and reduced interaction and/or conformational changes of the protein!

EAF2000 Electrical Flow FFF

EAF4 Separation of Pharma Protein (Positive Charge)



Unknown Protein

Bottom Electrode positive (Anode)

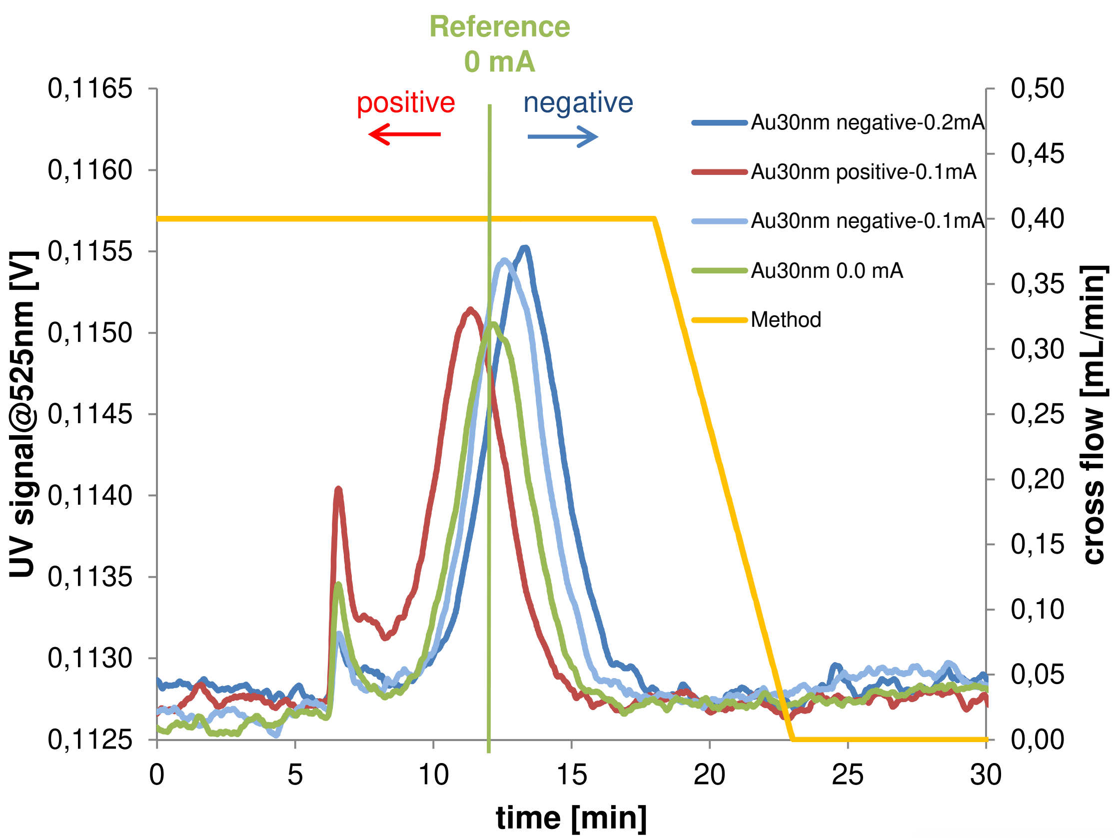
injection time	4 min
injection flow	0.2 ml/min
detector flow	1 ml/min
cross flow	3 ml/min
elution time	
Constant cross flow	3.0 ml/min
Membrane: RC 10 kDA	PBS buffer pH 7.5

- Positively charged protein shows broadened peak with shoulders, possibly caused by interaction with negative charged membrane surface.
- RCP technique allows better peak shape, less interaction and conformational changes

Gold 30 nm Nanoparticle (NIST)

EAF2000 Electrical Flow FFF

EAF4 Separation of Au 30nm Nanoparticle



Au 30 nm (NIST)

polarity of top electrode is named

injection time	5 min
injection flow	0.2 ml/min
detector flow	0.5 ml/min
cross flow	0.4 ml/min
elution time	

constant cross flow	linear decay	no cross flow
0.4 ml/min 12 min	0 ml/min 5 min	0ml/min 5 min

Membrane type:
10kDa RC

0.2 mM
Na_2CO_3

Concentration
5 mg/L

V(inj)
21 µL

EAF2000 Electrical Flow FFF

Zeta potential: Comparison EAF4 and Zetasizer

Au 30nm - Nanoparticles							
		Zeta Potential [mV]	**± SD [mV]**	**electrophoretic mobility [µm cm/Vs]**	**± SD [µm cm/Vs]**	**comments**
	Measurement 1	-25.58		-2.00		
	Measurement 2	-26.89		-2.10		
EAF4	**average**	**-26.24**	**1.0**	**-2.05**	**0.1**	25°C, 5 mg/L
Zetasizer		**-30.76**	**2.8**	**-2.41**	**0.3**	25°C, same solvent as for EAF4
NIST certificate		**-33.6**[1]	**6.9**	**-2.38**	**0.49***	20°C, stock solution 50 mg/L, preconditioning with 2mM NaCl

[1] Calcluation based on Smoluchowski approximation

- Zeta potential was calculated based on the Smoluchowski-Approximation
- Zetasizer results were determined from 10 sub-measurements
- Zetasizer results are in good agreement with the EAF4 results

Coated Iron Nanoparticle (FeMAG)

EAF2000 Electrical Flow FFF

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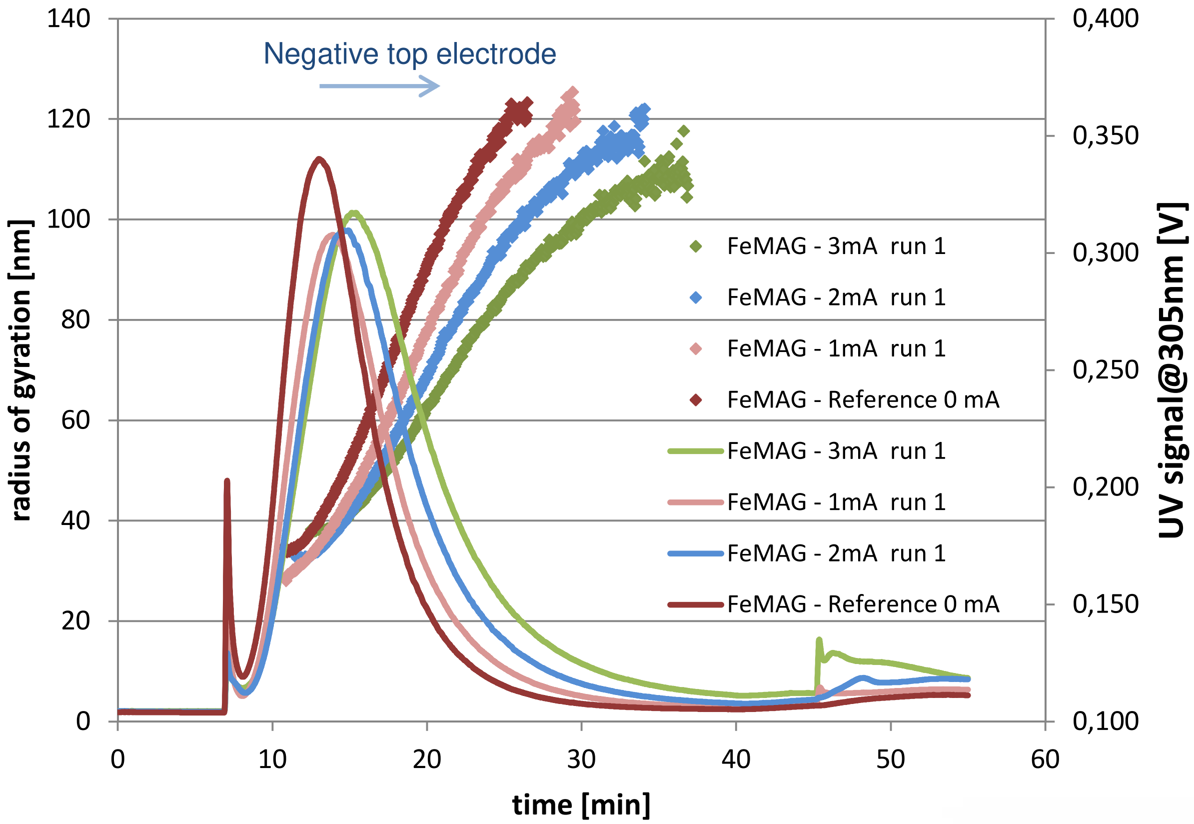
EAF4 Separation of coated magnetic Fe Nanoparticle



coated FeMAG nanoparticles

polarity of top electrode is named

injection time	7 min
injection flow	0.2 ml/min
detector flow	0.5 ml/min
cross flow	0.15 ml/min
elution time	

constant cross flow	linear decay	no cross flow
0.15 ml/min	0 ml/min	0ml/min
30 min	5 min	10 min

Membrane type: 10kDa RC

4 mM Phosphate buffer

Concentration 250 mg/L

V(inj) 21 µL

EAF2000 Electrical Flow FFF

Zeta potential: Comparison EAF4 and Zetasizer

FeMAG- Nanoparticles					
	Zeta Potential [mV]	± SD [mV]	electrophoretic mobility [µm cm/Vs]	± SD [µm cm/Vs]	comments
EAF4	-33.77	-	-2.64	-	25° C, 250 mg/L, result of 7 individual runs
Zetasizer	-36.53	1.5	-2.86	0.2	25°C, same solvent as for EAF4

- Zeta potential was calculated based on the Smoluchowski-Approximation
- Zetasizer results were determined from 6 sub-measurements
- Zetasizer results are in good agreement with the EAF4 results

High-Temperature FFF (HT-AF4): Advanced Characterization of Ultrahigh Molar Mass and Highly branched Polyolefins

Why FFF?

Limitations of SEC-Light Scattering:

Shear degradation in porous columns / frits

→ Detected mass too low

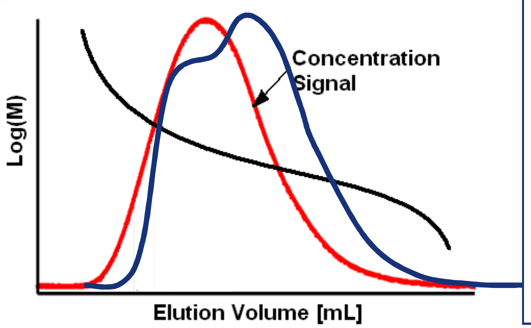
Filtration of gel, clogging of columns

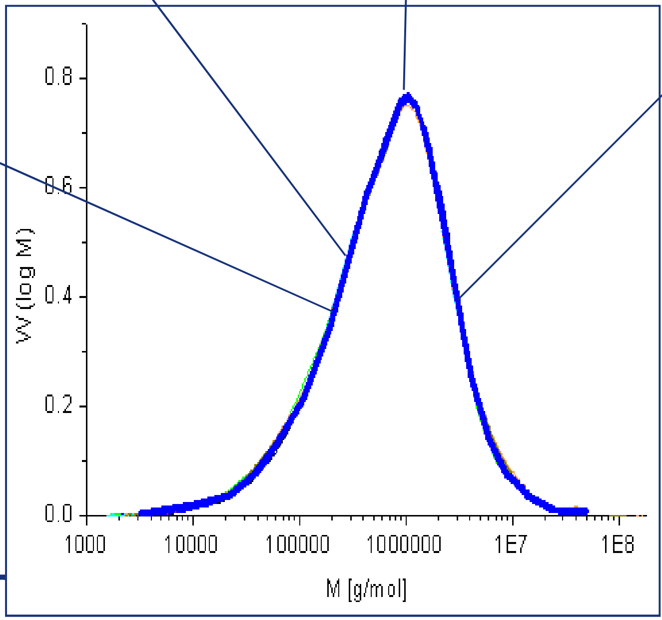
Unwanted interaction with stationary phase

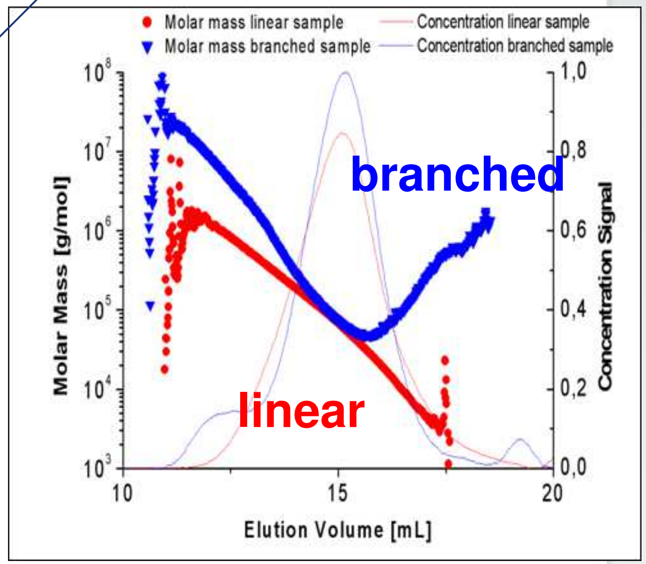
→ Wrong branching / MMD

Low separation for high M_w

´Exclusion Limit´

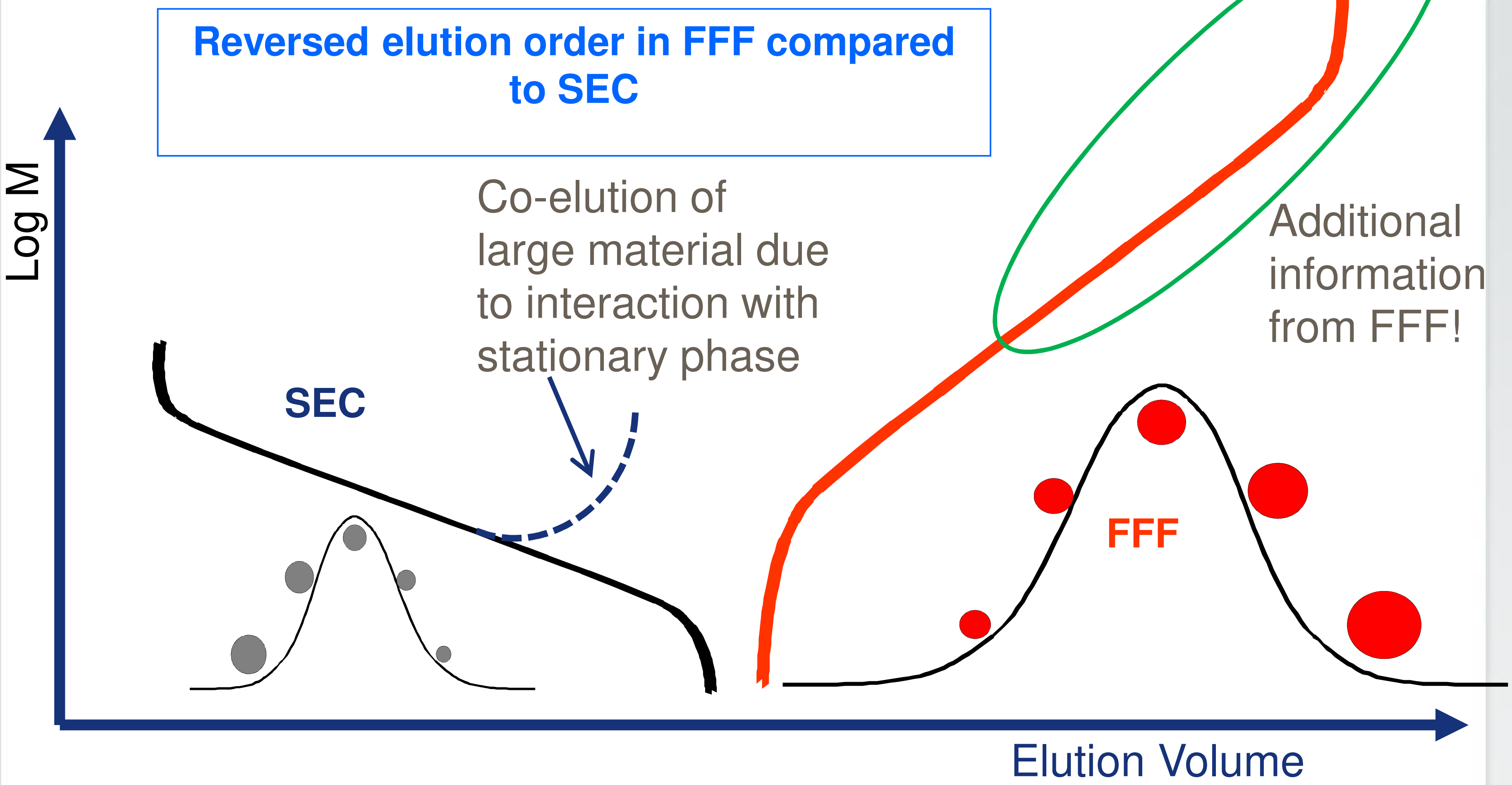






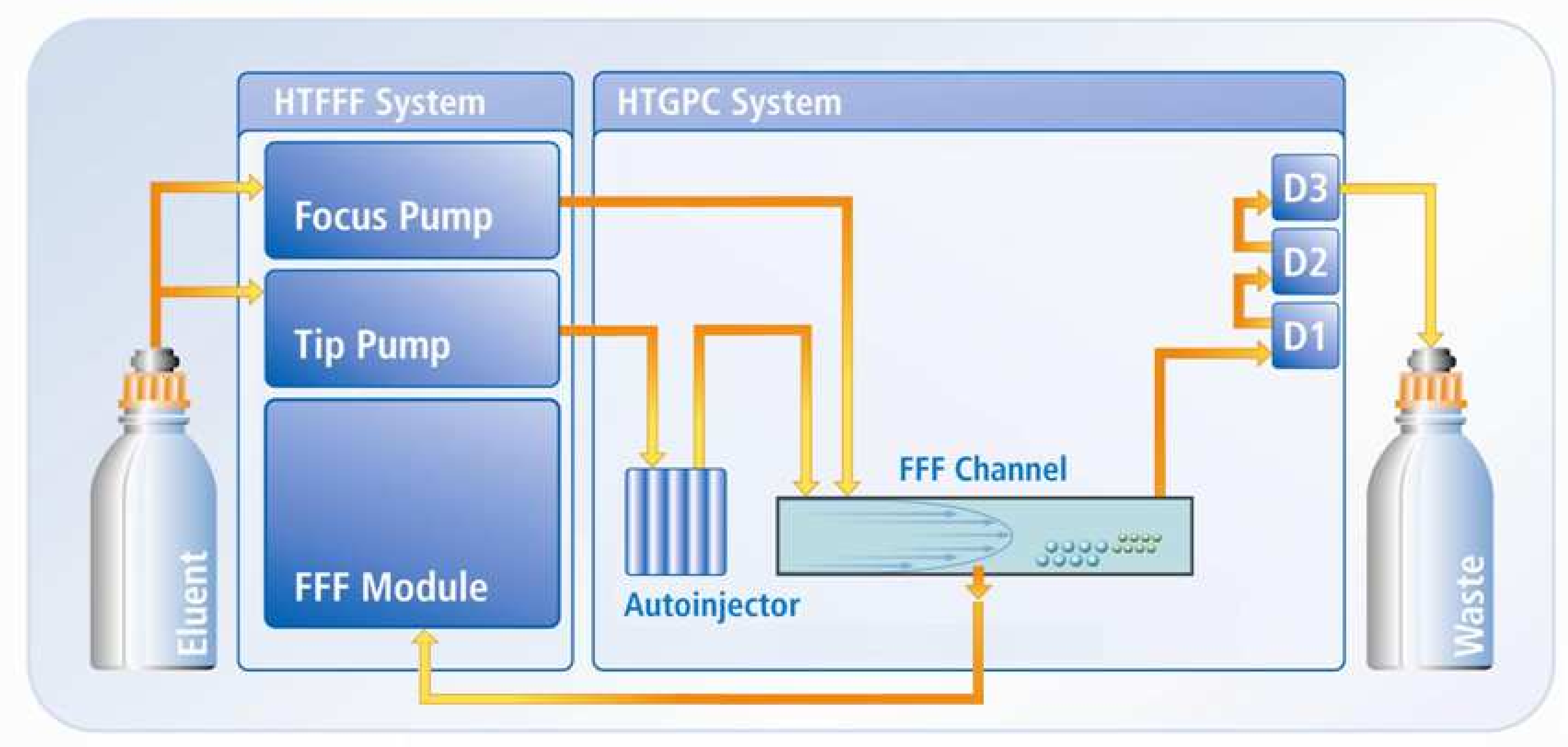
FFF vs. SEC

Elugram from SEC vs. Fractogram from FFF

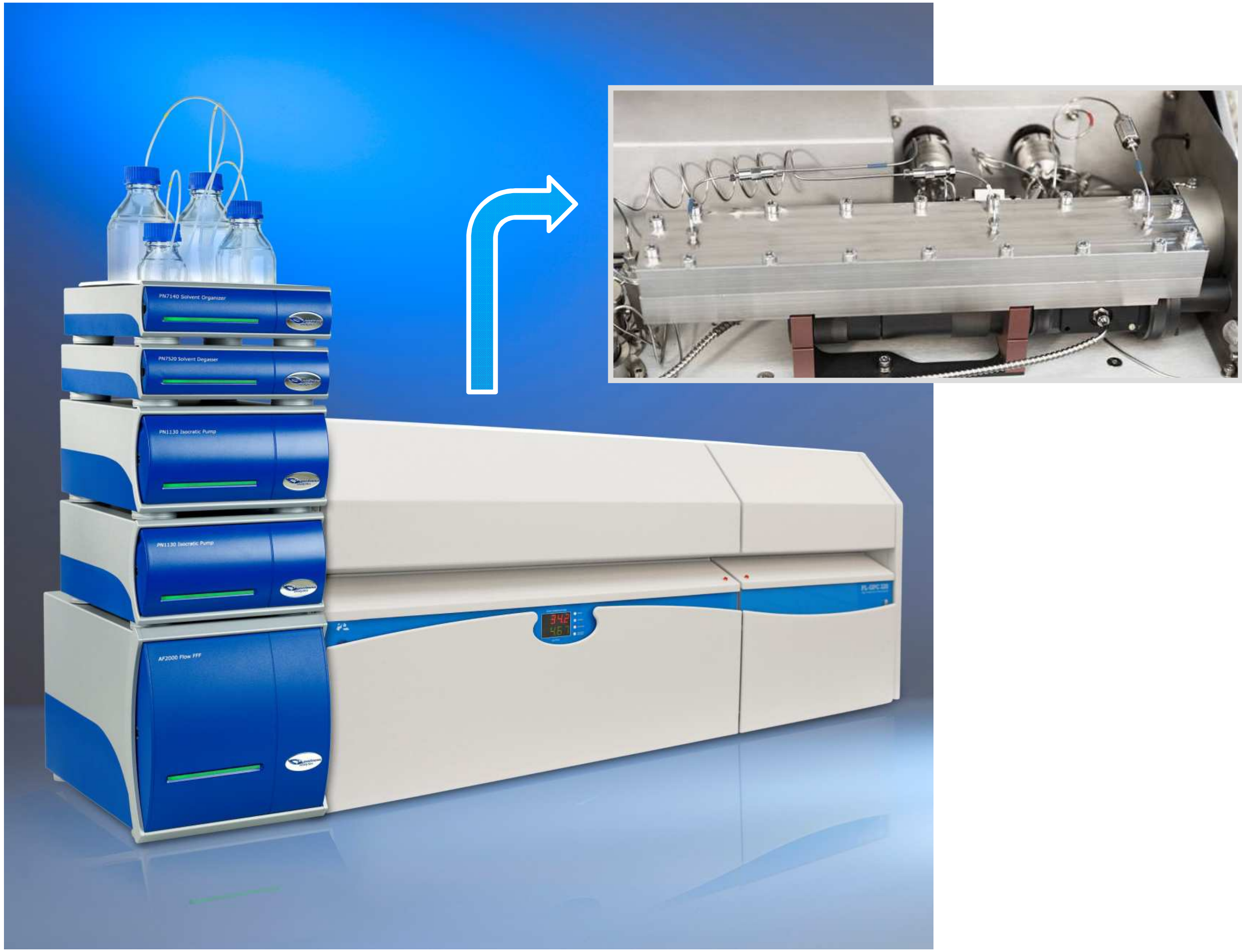


HT-AF4 Instrumentation

Flow Scheme of the System



HT-AF4 Instrumentation

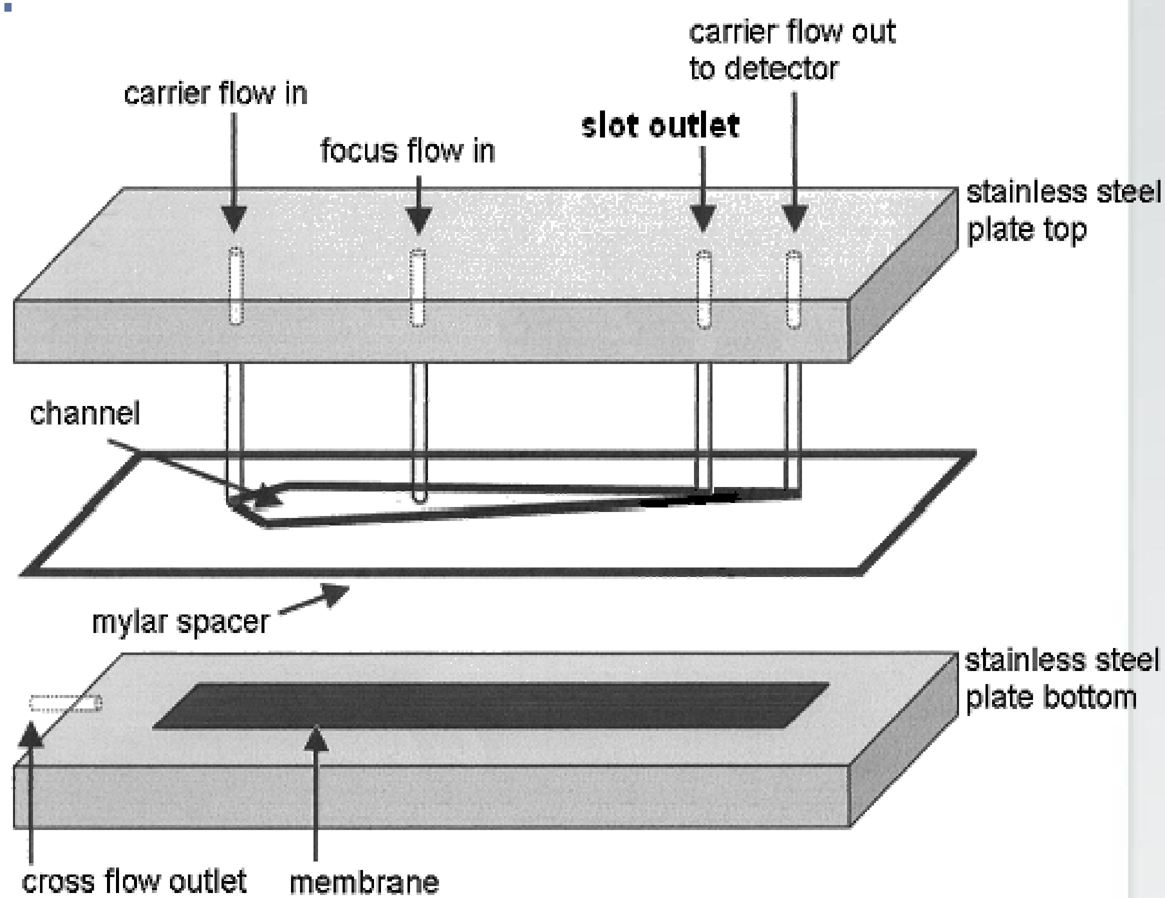


HT-Asymmetrical FFF (HT-AF4)

Setup of HT-AF4 Channel:

High temperature of above 130°C requires resistant materials!

- Steel channel / capillaries
- Ceramic frit / membrane



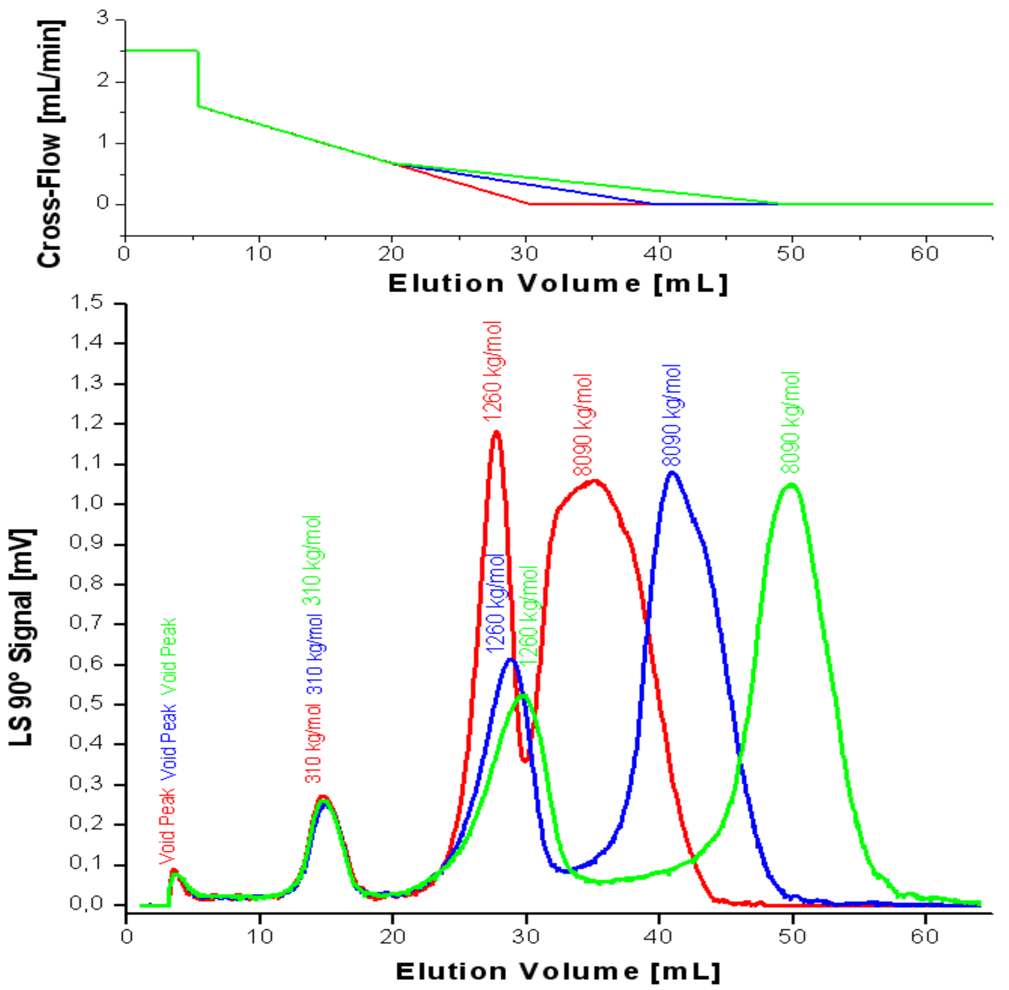
Flexibility of the Cross-Flow Gradient

"Tailor-made" separation
in AF4 realized by cross-flow-adjustment

- **Extension of Cross-Flow causes better separation**
- **Selective enhancement of separation**
- **<u>In SEC not possible!</u>**

→ *Column change is time consuming + expensive*

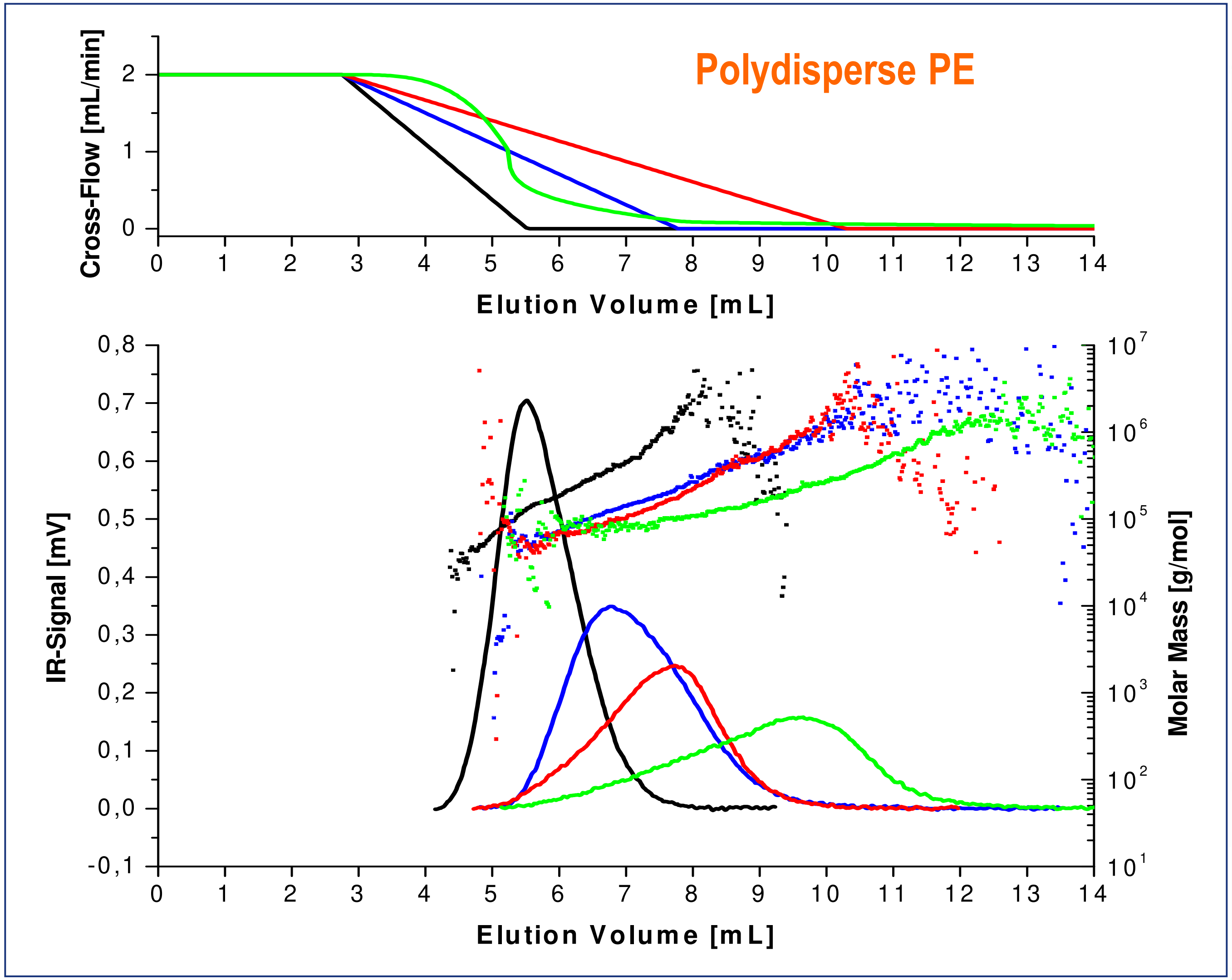
→ *Calibration of column determines separation*



Mixture of narrow PS: 300, 1200 & 8000 kg/mol

Flexibility of the Cross-Flow Gradient

- **Gradient of any shape can be used**
- **Adjustment of separation according to special requirements of the sample**



HT-AF4 vs. HT-SEC in Overlap Size Range

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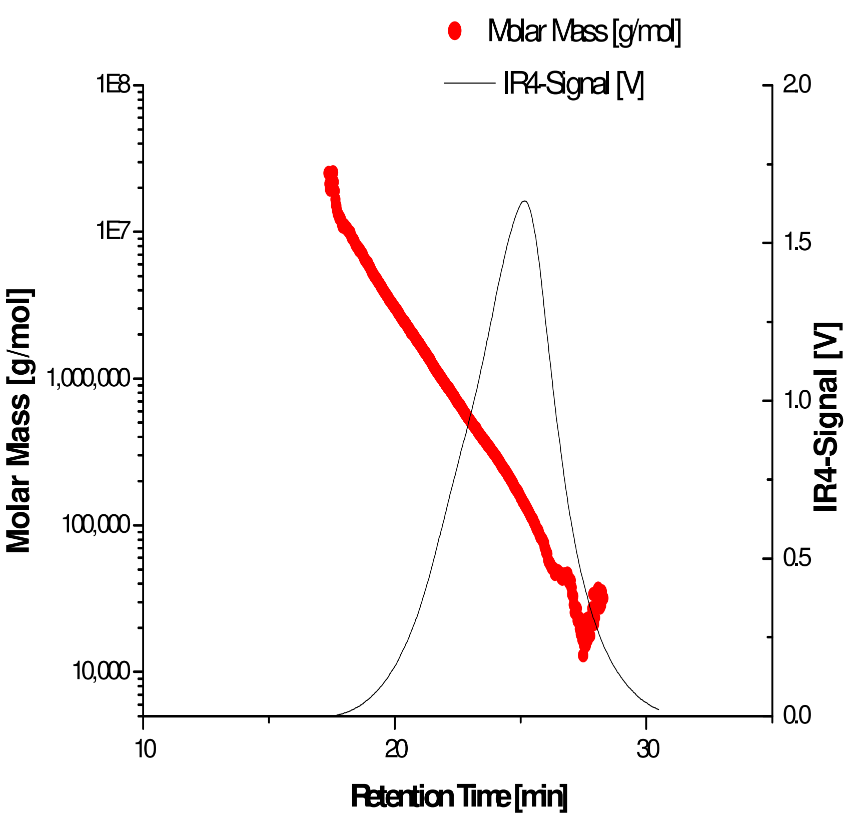
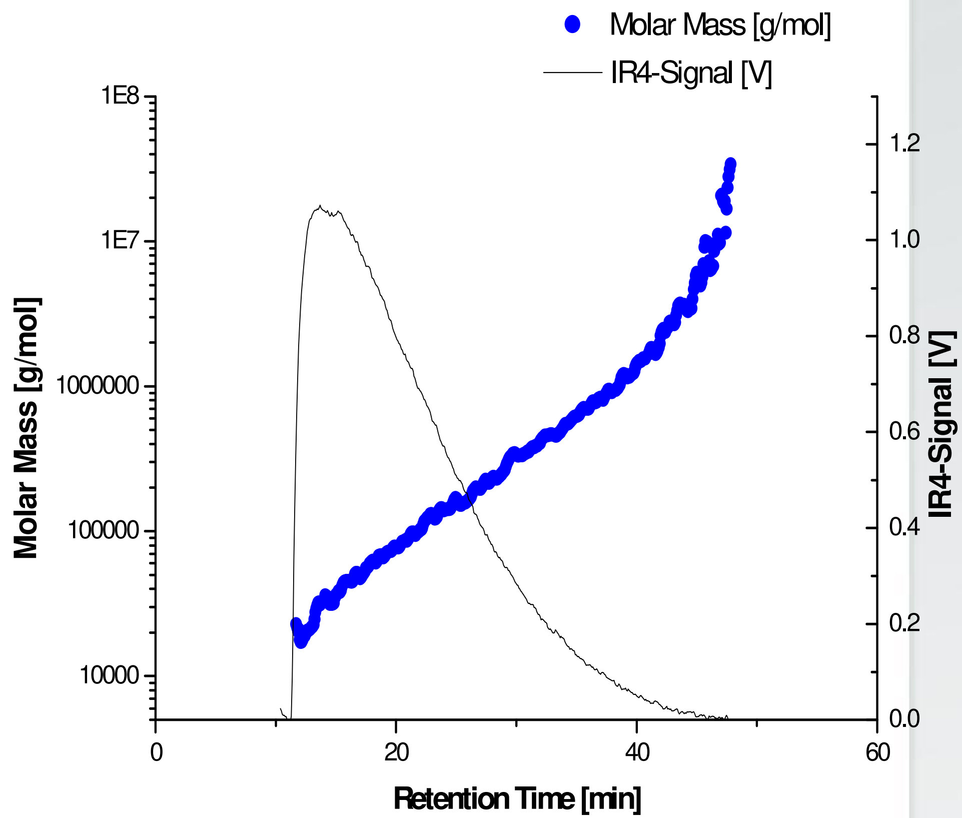
Linear HDPE Reference NIST 1496 was separated with both methods

HT-SEC



HT-AF4



IR4+Light Scattering Detection

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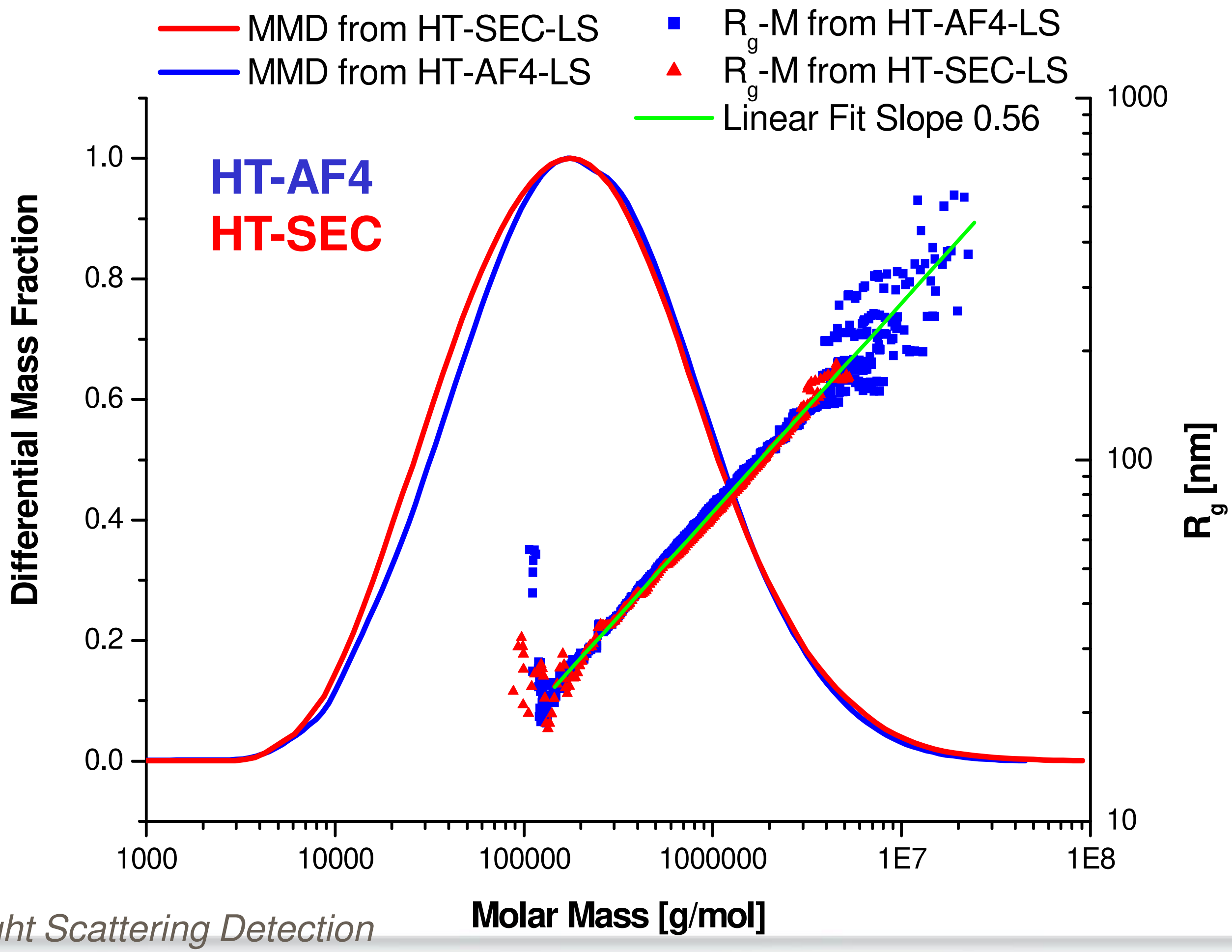
HT-AF4 vs. HT-SEC in Overlap Size Range

Both separation methods deliver the same molar mass distribution!



- **Slope of Rg-M is similar for AF4 and SEC**
- **Theoretical value for linear material of 0.56-0.58 indicates proper separation with both methods**

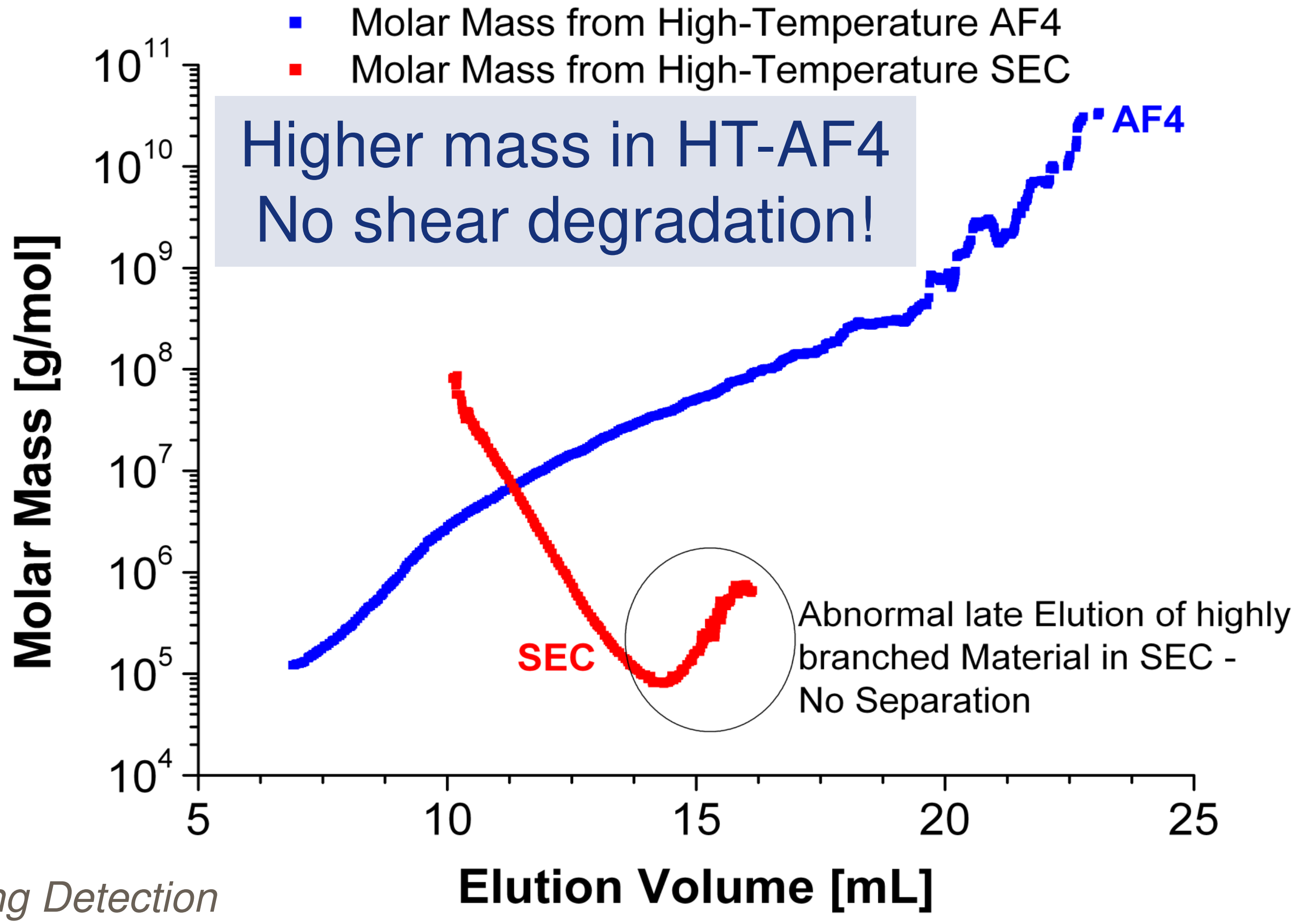
IR4+Light Scattering Detection

Problematic Material: LDPE

HT-AF4 vs. HT-SEC

AF4 vs. SEC separation of highly branched LDPE

HT-AF4
HT-SEC

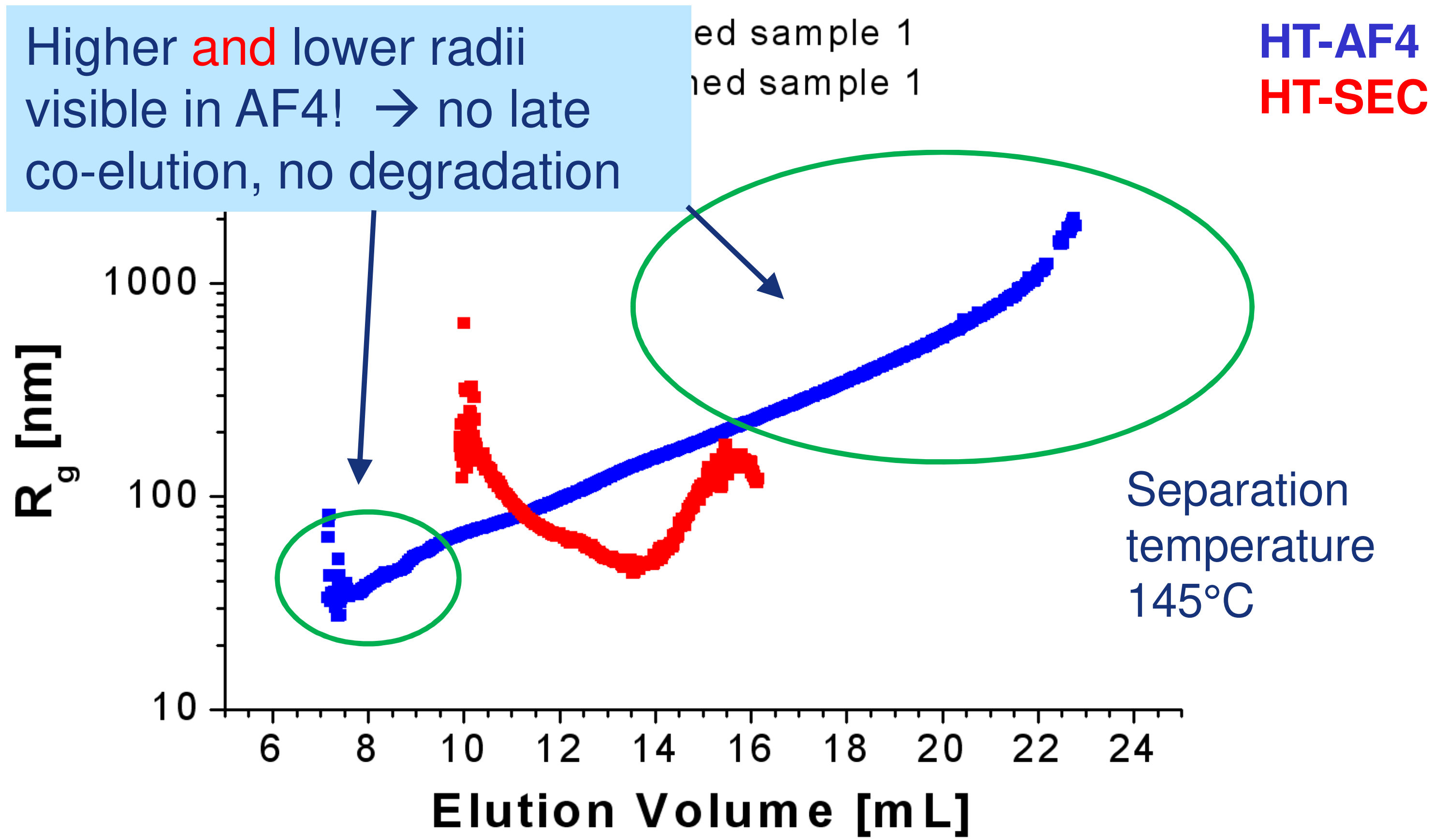


IR4+Light Scattering Detection

Problematic Material: LDPE

HT-AF4 vs. HT-SEC

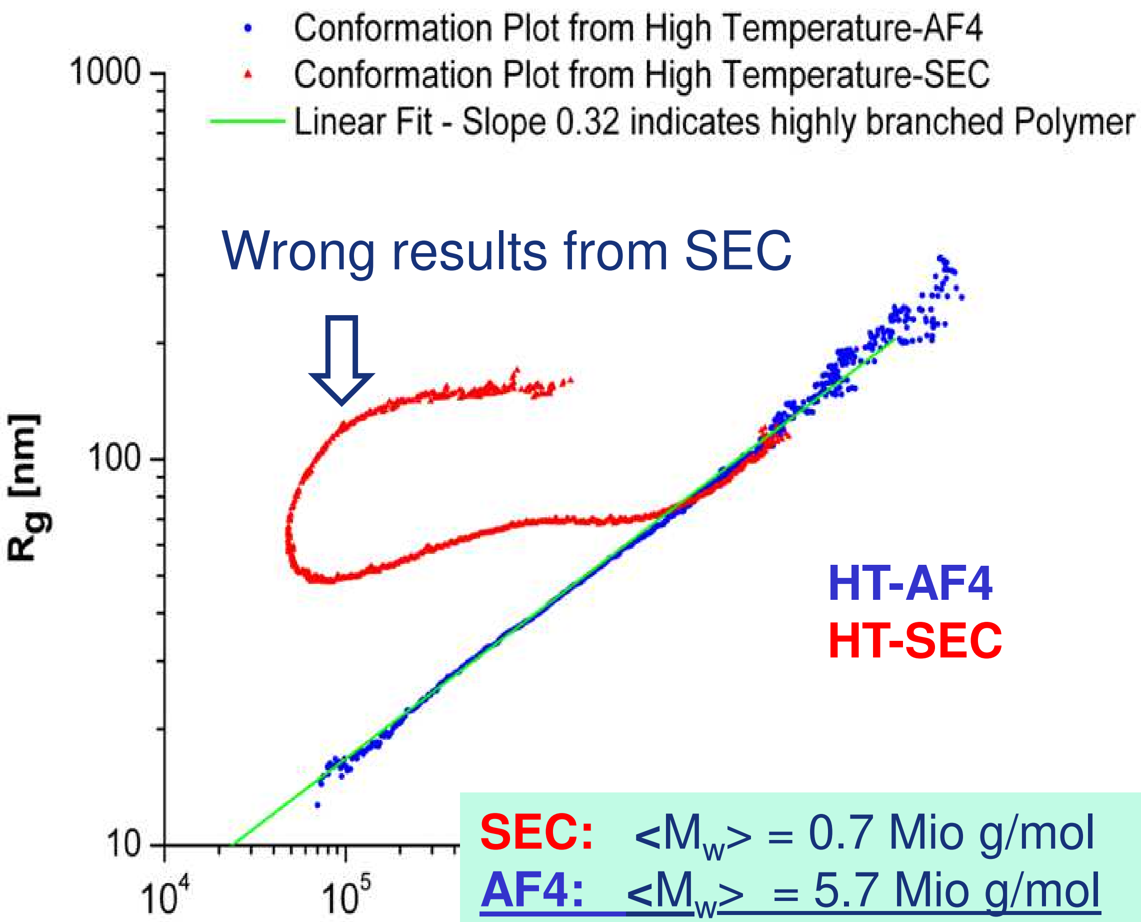
AF4 vs. SEC separation of <u>highly branched LDPE</u>



IR4+Light Scattering Detection

Problematic Material: LDPE

HT-AF4 vs. HT-SEC



SEC: $\langle M_w \rangle$ = 0.7 Mio g/mol
AF4: $\langle M_w \rangle$ = 5.7 Mio g/mol

- **HT-AF4 shows no abnormal co-elution**
- **Determination of the correct slope from AF4 possible**

FhG-LBF

GPC vs. AF4 – reicht GPC nicht aus?

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GPC zeigt zwei gravierende Nachteile:

- Scherdegradation / Filtereffekte → Sehr große Moleküle können nicht ausreichend gut analysiert werden

1

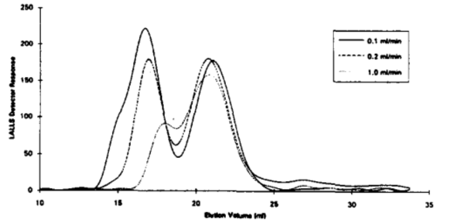


Fig. 4. LALLS detector response for NBS 1476. Polymer Labs.' 20-μm columns.

Labs.' 20-μm columns.

- Abnorm späte Coelution → Langkettenverzweigte Moleküle werden zusätzlich nach Anzahl der Verzweigungen getrennt → Verzerrung der realen Verteilungen

2

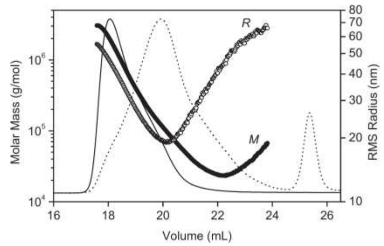
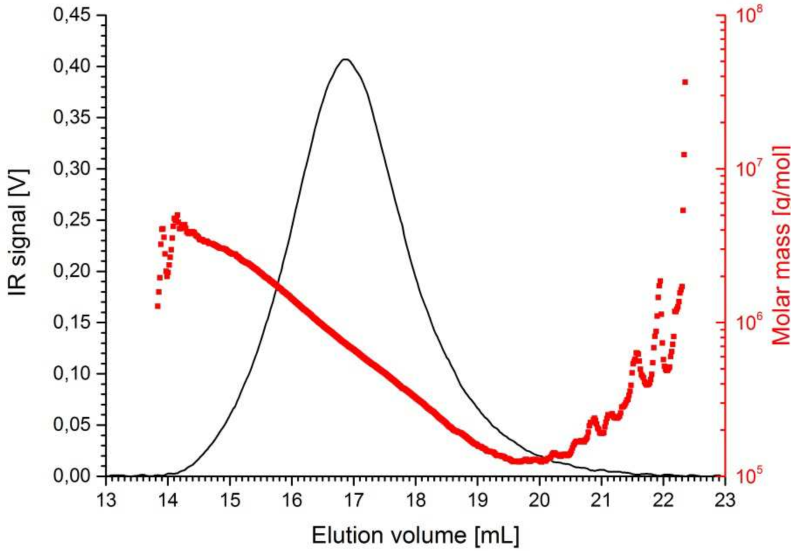


Figure 3.
Molar mass (•, *M*) and RMS Radius (○, *R*) versus elution volume plots for low density PE. MALS (solid line) and RI (dashed line) chromatograms are overlaid here.

[1] A. W. deGroot et al., *Journal of Chromatography*, 648 (**1993**) 33
[2] S. Podzimek, *Macromolecular Symposia*, 330 (**2013**) 81

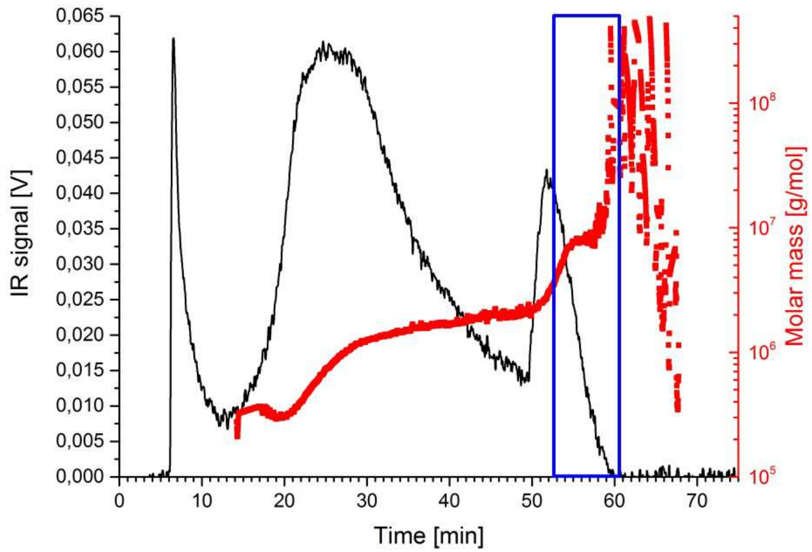
Scherdegradation/Filtereffekte am praktischen Beispiel: UHMWPE Braskem UTEC 3041

GPC



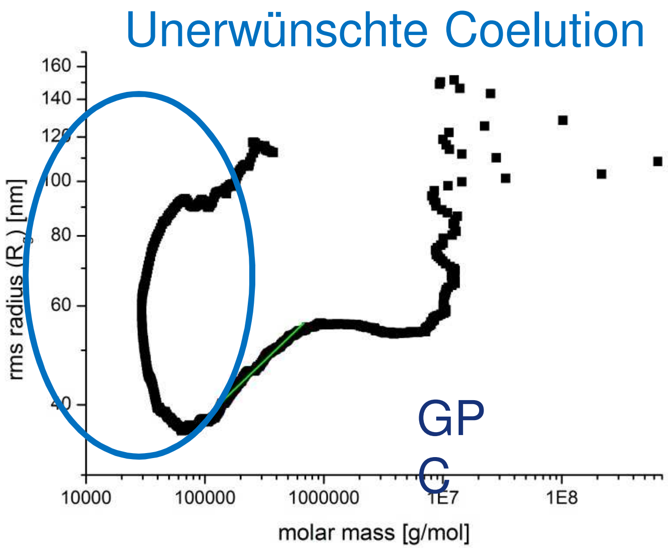
AF4

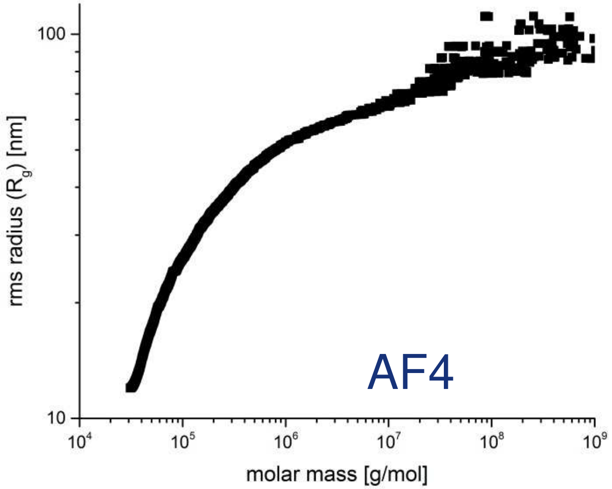
Mit GPC nicht nachweisbar



Die besonders hochmolekularen Anteile der Probe konnten bei der GPC-Trennung nicht nachgewiesen werden.
Erst die AF4 zeigt den hochmolekularen Anteil deutlich

Abnorm späte Coelution: LDPE





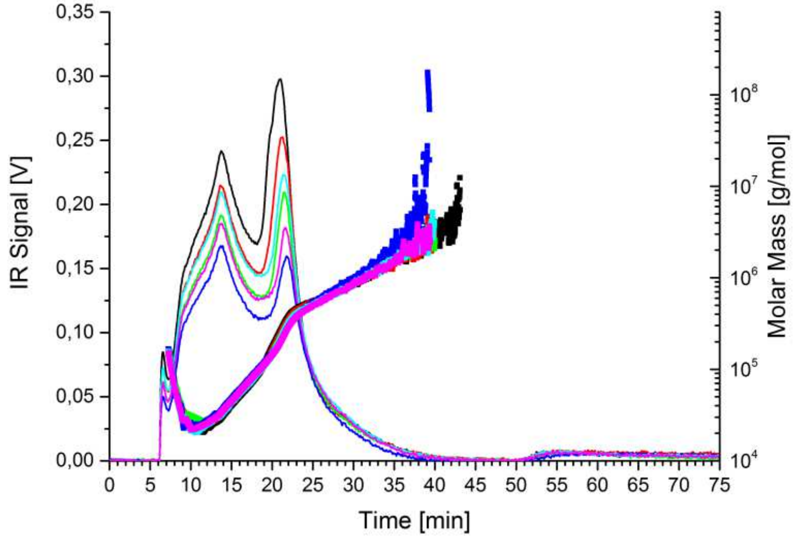
Coelution wird besonders deutlich beim Auftrag von Radien gegen Molekulargewichte

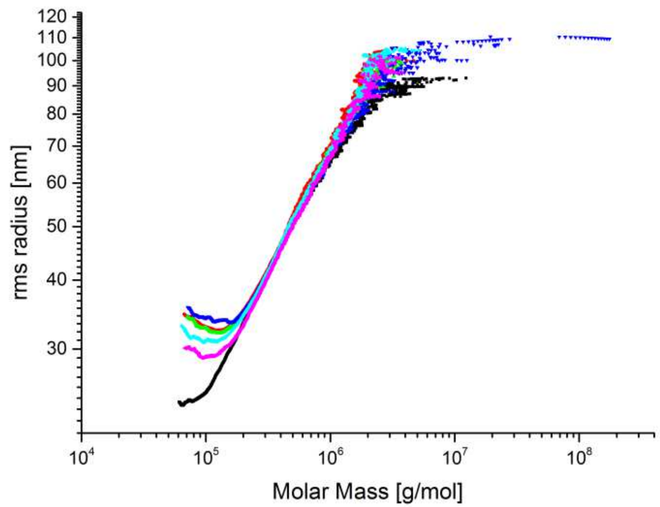
GPC: Typisches Aufkrümmen im niedermolekularen Bereich

AF4: Stetige Zunahme der Radien mit steigendem Molekulargewicht (erwünscht!)

AF4 vs. GPC – Vergleich der Reproduzierbarkeit

HDPE





Reproduzierbarkeit ist essentiell für jede analytische Methode
→ Verlässlichkeit der Ergebnisse
Ergebnis: Reproduzierbarkeit von AF4-Ergebnissen (Molekulargewichtsmittel) genauso hoch wie die von GPC Ergebnissen

AF4 – Anwendungsbeispiel 1:
Postreaktormodifiziertes Polypropylen

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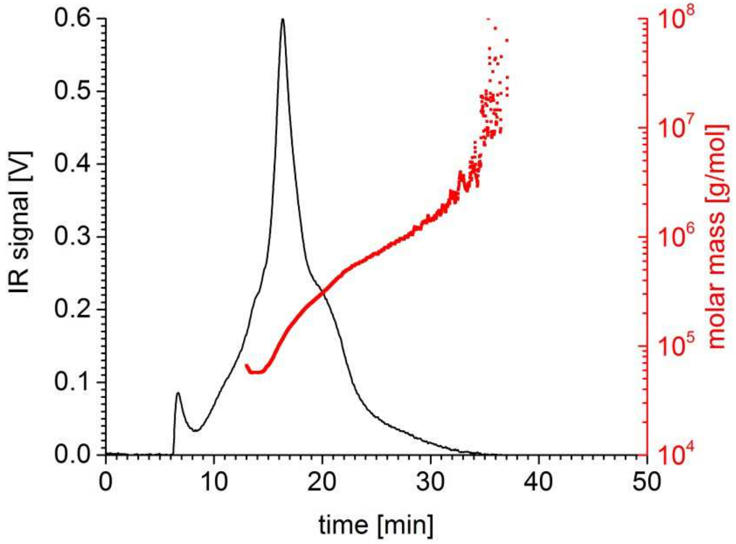
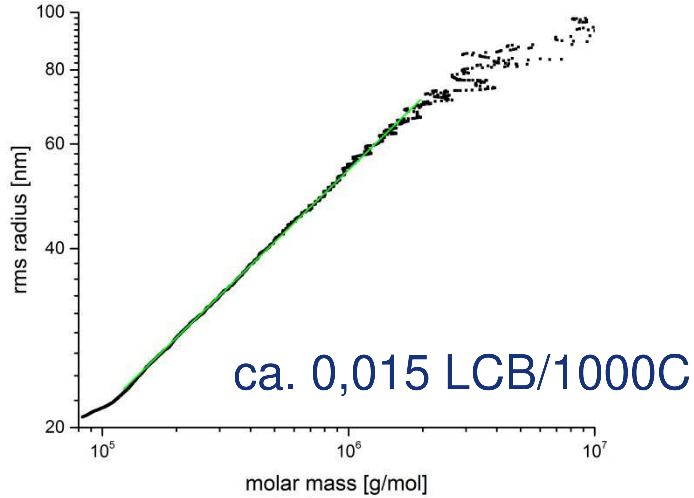
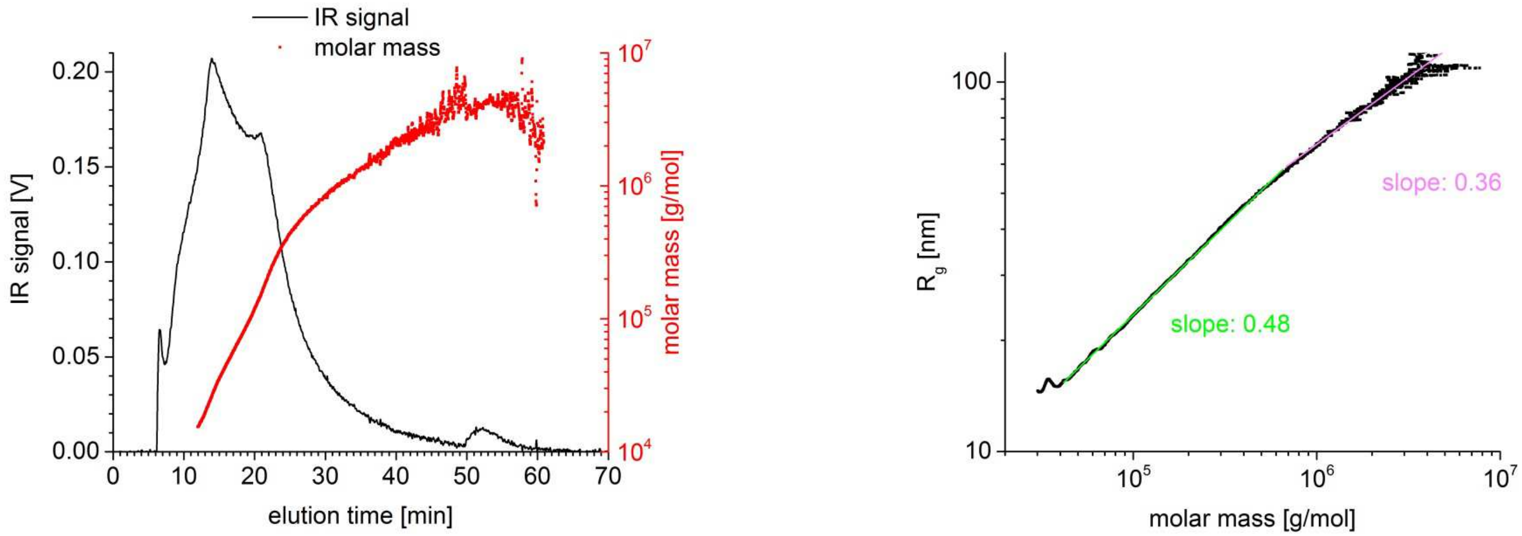




Langkettenverzweigungen in PP verbessern Verarbeitungseigenschaften
Häufig Postreaktormodifikation (reaktive Extrusion)
Veränderung von Molekulargewicht UND Einführung von LCB
→ AF4 ist ideal geeignet um die Veränderungen nachzuvollziehen
→ Prozessoptimierung

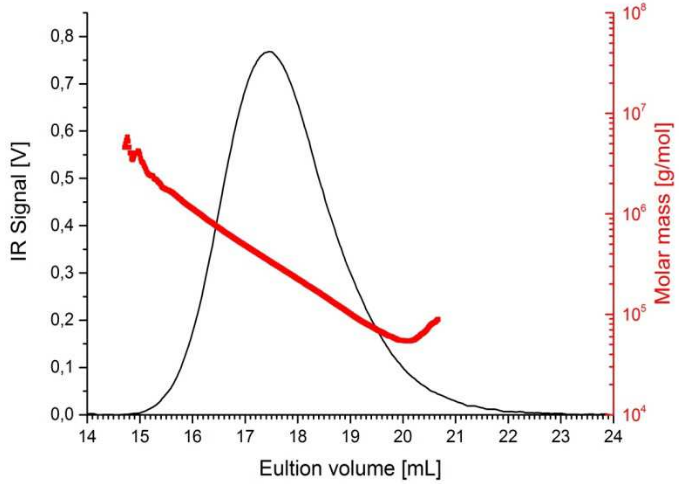
AF4 – Anwendungsbeispiel 2: Langkettenverzweigungen in Polyethylen



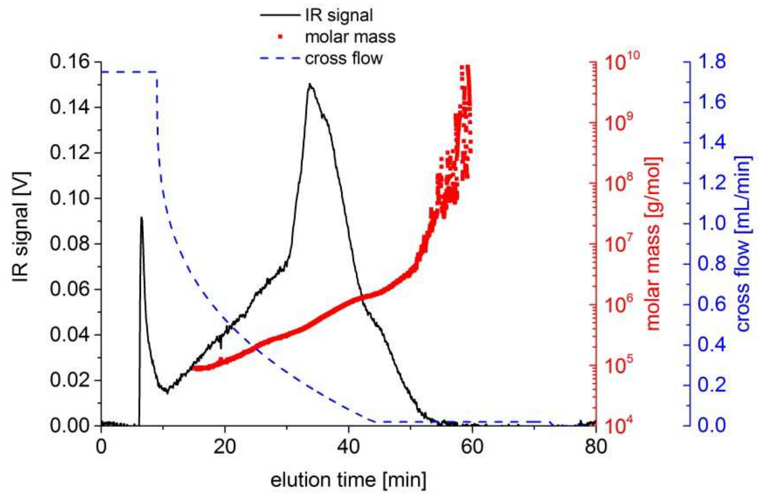
Langkettenverzweigungen auch bei PE häufig erwünscht
Berechnung des Anteils an Verzweigungen aus Abhängigkeit von Radius und Molmasse
Bei dieser Probe: Nachweis eines größeren Anteils an Langkettenverzweigungen im hochmolekularen Probenanteil

AF4 – Anwendungsbeispiel 3:
PE mit ultrahochmolekularen Anteilen

GPC



AF4



Ultrahochmolekulare Anteile erhöhen mechanische Belastbarkeit von PE deutlich!

Idealer Anwendungsbereich für AF4 → Keine Scherdegradation, keine Filtereffekte

Nachweis von Anteilen > 10^7 g/mol gelang nur mittels AF4!

AF4 – Besonderheit Querflussprogramme

Trennstärke der AF4 hängt von der Querflussrate ab

- Flussraten sind leicht einstellbar → Trennstärke kann angepasst werden

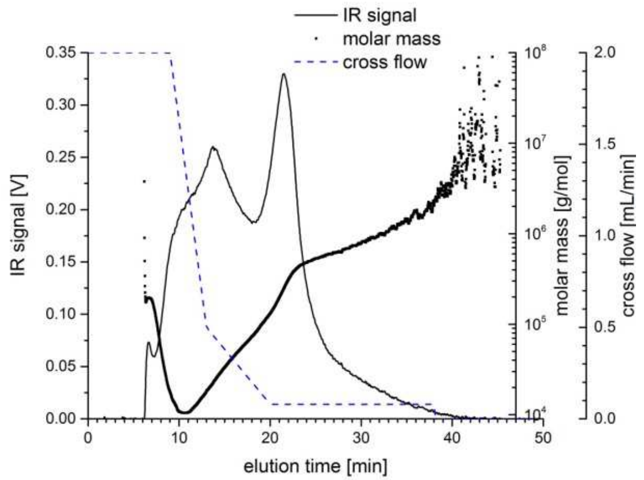
Konstante Querflüsse liefern meist unbefriedigende Ergebnisse für Polymere

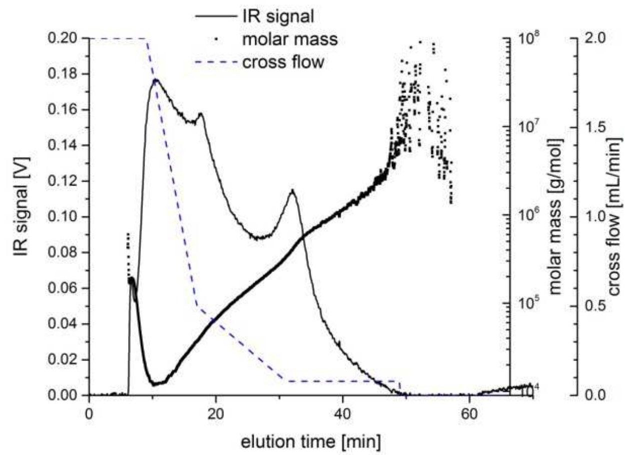
- Zu geringe Trennung der geringen Molmassen, zu starke Trennung der hohen Molmassen

Daher: Reduktion der Querflussrate während einer Analyse → Querflussgradient/Querflussprogramm

- Verschiedene Querflussprogramme = verschiedene Elutionszeiten
- Bestimmung absoluter Molmassen über MALS-Detektor
- Für optimale Ergebnisse: Gewisse Anpassung des Querflussprogramms an die Probencharakteristik
- Mittleres Molekulargewicht; Breite der Molekulargewichtsverteilung

AF4 – Optimierung der Querflussprogramme 1



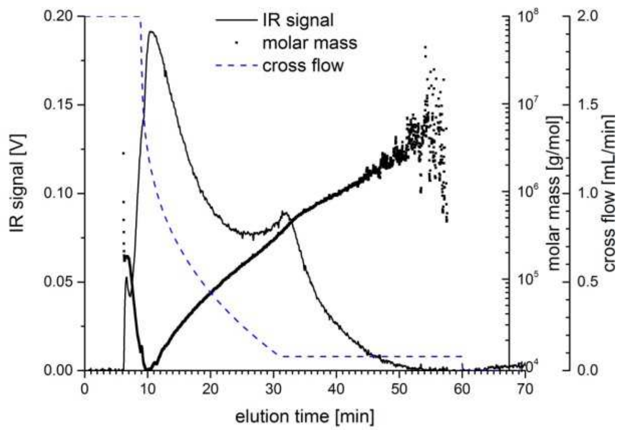


Lineares PE
M_w: 200 kg/mol
PD: 6,7 (MALS)

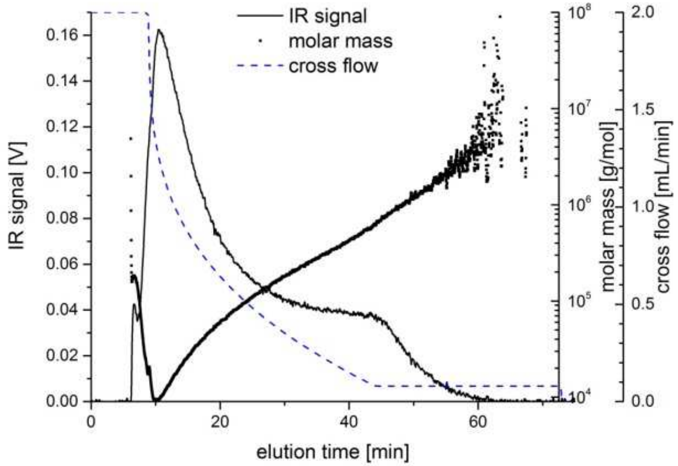
Intial verwendetes Programm   Längere lineare Schritte

- Ziel: Lineare Trennung (analog GPC) → Einfache Rückschlüsse auf MMD
- Intial: lineares Programm mit zwei Schritten
- Veränderungen der Steigung deutlich erkennbar im Elutionsprofil
- Längere lineare Schritte deutlich vorteilhafter, Potential für weitere Verbesserungen

AF4 – Optimierung der Querflussprogramme 2



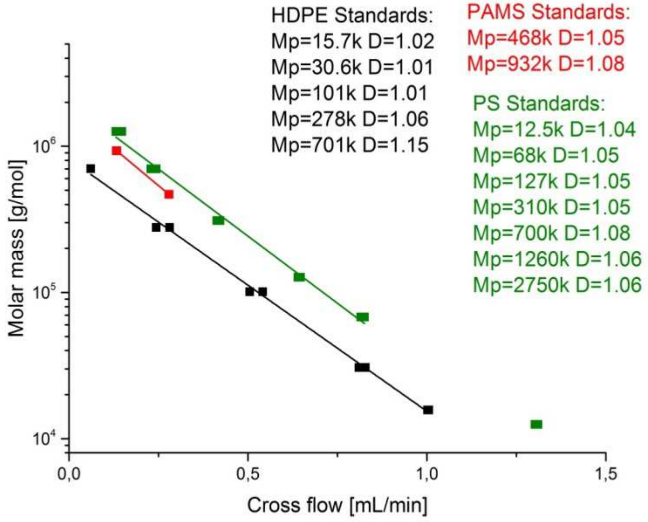
Exponentielles Flussprogramm

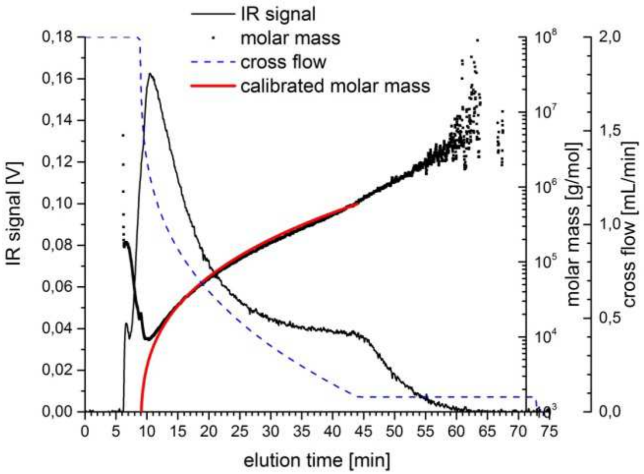


Längere Laufzeit

Plötzliche Veränderungen der Steigung erwiesen sich als ungünstig
→ Wechsel zu exponentiellem Flussprogramm
Weitere Optimierung der Trennung durch Verlängerung der Gradientendauer
Ergebnis: Deutlich linearere Trennung

AF4 - Kalibration





GPC: Kalibration der Elutionszeiten mit Standards
→ Bestimmung der Molmassen
AF4: Gleichsam möglich aber für Routine zu aufwendig
→ Hohe Flexibilität der Trennkraft über Querflussprogramm
→ Jedes Querflussprogramm erfordert eigene Kalibration
Daher: Verwendung eines Vielwinkellichtstreudetektor (MALS)
→ Direkte Bestimmung absoluter Molmassen

Summary

- **New EAF2000 Electrical Flow FFF**
  - Separation by Size and Charge (electrophoretic mobility)
  - One channel for both techniques in one design
  - Same dimensions, spacer, membranes as analytical AF4 channel
  - Tune separation conditions, RCP-Reversed Charge Polarization
  - Avoid membrane interaction and conformational changes
  - Extend and shorten separation times as needed
  - Ideally for charged proteins, nanoparticles and limposomes
  - **=> Absolute Determination of Electrophoretic Mobility and Zetapotential by EAF4 !**

- **The New Postnova Platform**
  - Unique Platform for macromolecule/nanoparticle Analysis
  - Techniques: FFF, **HT-FFF**, SEC, MALS, VISC, RI, DLS, ICP-MS
  - Parameters: Molecular Weight/Size, Particle Size/Density, Structure/Charge and Elemental Composition.

- **Potential Application Range**
  - Proteins, Antibodies, Viruses
  - Synthetic/Natural Polyelectrolytes
  - Charged Nanoparticles and Liposomes
  - Polyolefins

Thank you for your attention!