Thermal Field-Flow Fractionation: A Polymer Separation Technique for MALDI-TOF MS

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Outline

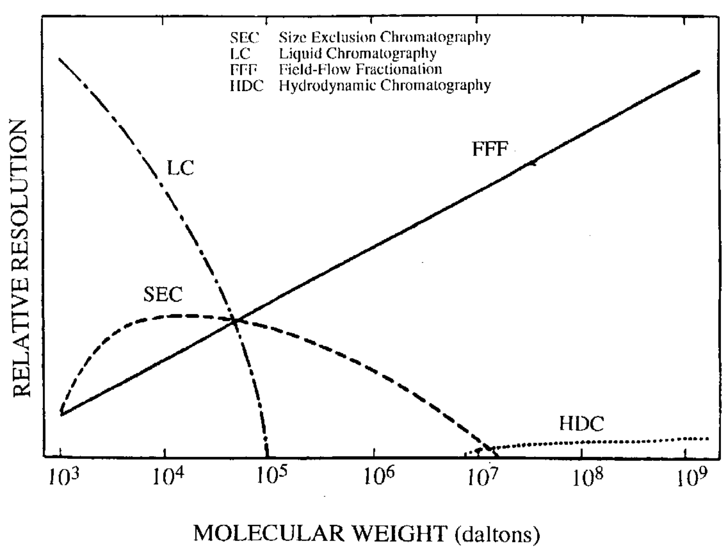
- FFF Basics
- Thermal FFF
  - Instrumentation and Practice
  - Example applications
- ThFFF-MALDI TOF MS
  - Compatibility and optimization
  - Example applications

Polymer separation techniques

Packed columns
- Size exclusion
- LC at critical point of adsorption
- Gradient polymer elution chromatography

Open channels
- Thermal FFF
- Flow FFF
- Sedimentation FFF
- Hydrodynamic chromatography
- Capillary electrophoresis

RELATIVE RESOLUTION OF ANALYTICAL SEPARATION TECHNIQUES



J. J. Kirkland, W. W. Yau, and W. A. Doemer, *Anal. Chem.*, **52**, 1944 (1980).

Benefits of Thermal FFF

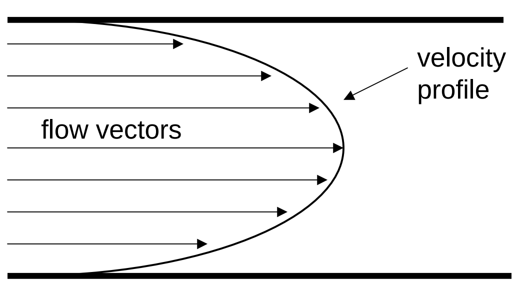
1. Wide MW range in a single channel.
2. Tunable retention.
3. Higher MW selectivity than single column SEC.
4. Well suited for:

    ultrahigh MW polymers analysis (low shear)

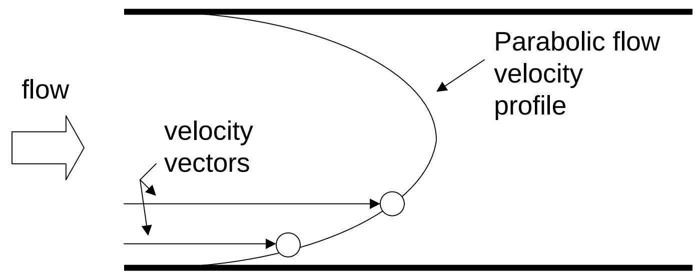
    studies of fragile aggregates

    microgels and particles analyses.

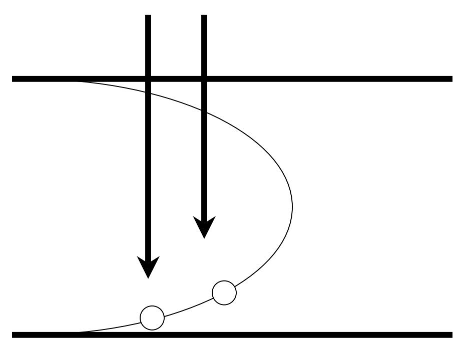
Laminar flow



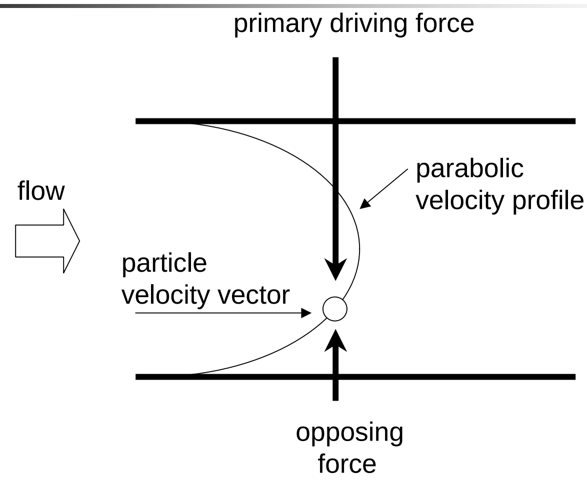
Separation



Parabolic flow
velocity
profile
flow
velocity
vectors



PRIMARY DRIVING FORCES
(externally generated)



Normal mode

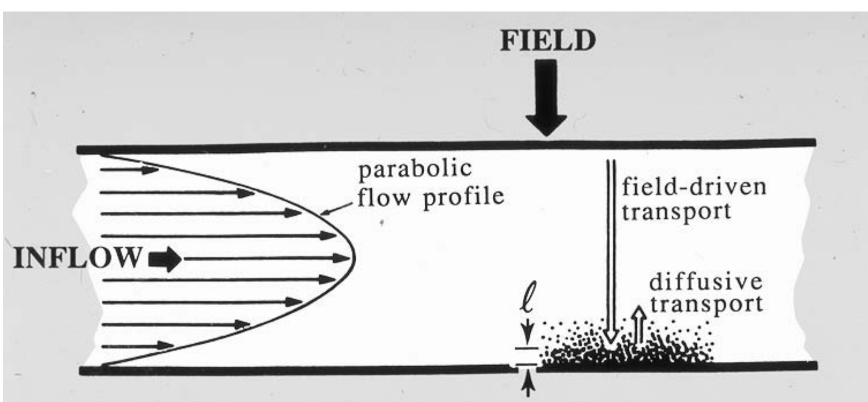
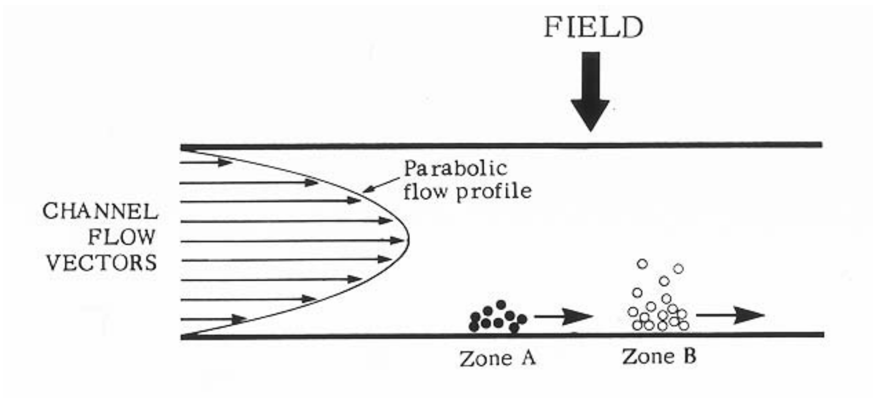


Figure 5. Different opposing forces or transport processes give rise to different operating modes of FFF. In so-called "normal FFF", used for most polymers and submicron particles, the opposing transport is caused by diffusion or Brownian motion.

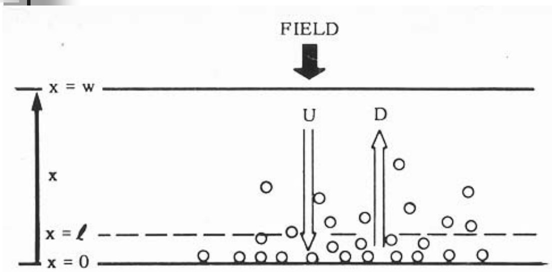


Fast diffusion or

small sizes

elute first.

Basic FFF Theory



$$J = Uc - D\frac{dc}{dx} = 0$$

$$\frac{dc}{c} = \frac{U}{D}dx$$

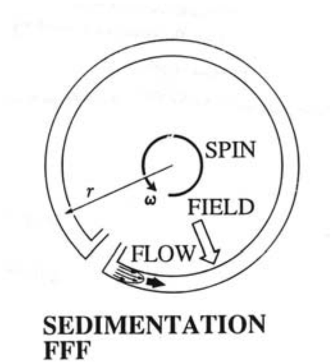
$$\ln\frac{c}{c_0} = \frac{U}{D}x = \frac{-|U|}{D}x$$

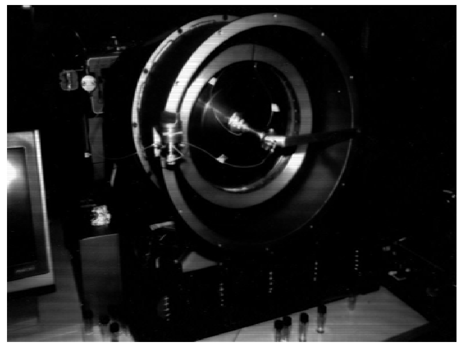
$$\frac{c}{c_0} = e^{-|U|x/D} = e^{-x/\ell}$$

$$\ell = D/|U|$$

$$\lambda = \frac{\ell}{w} = \frac{D}{|U|w} = \frac{kT}{|F|w}$$

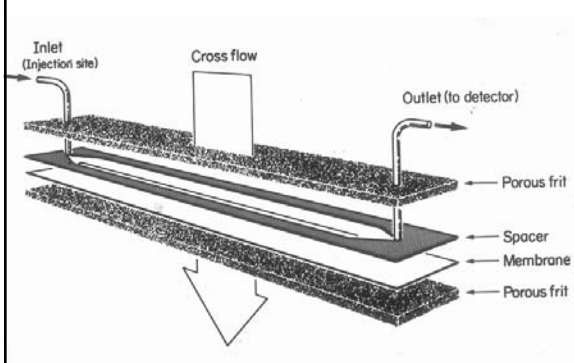
Sedimentation FFF

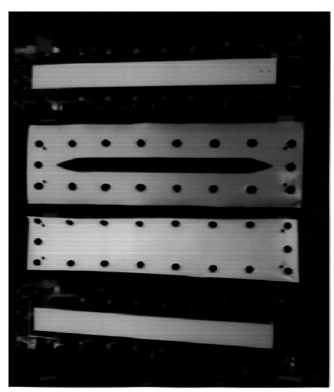


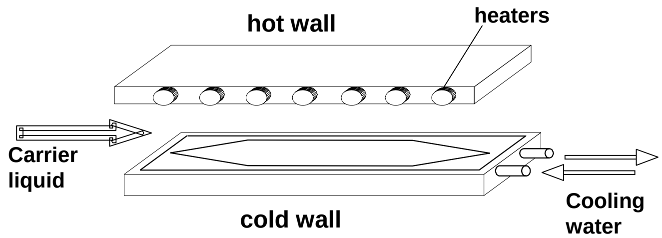


$$\frac{t_r}{t^0} = \frac{\pi w d^3 \Delta\rho G}{36kT}$$

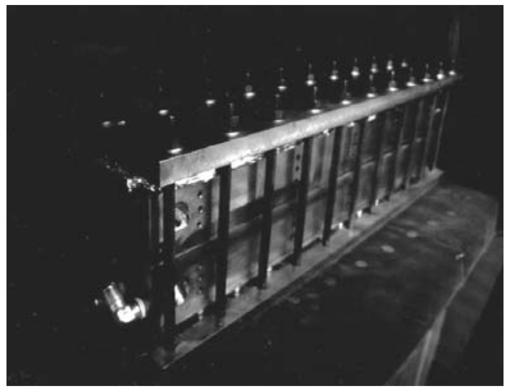
Flow FFF



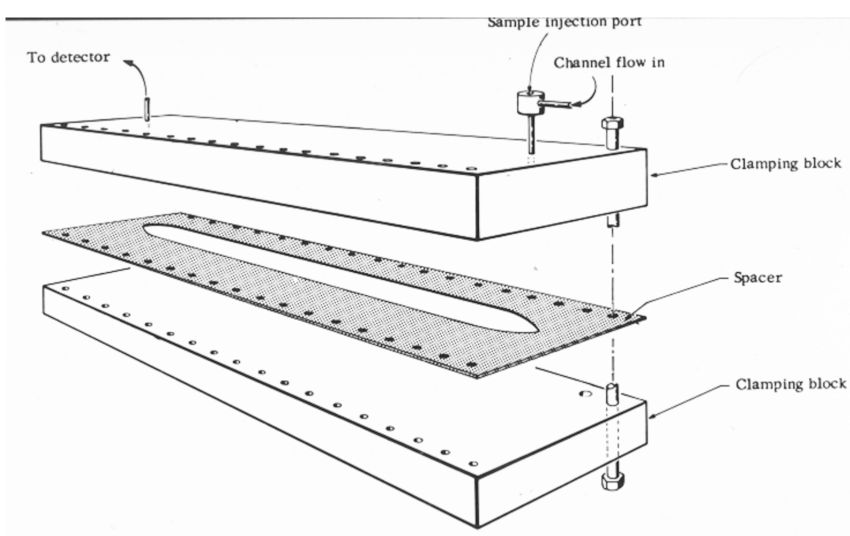




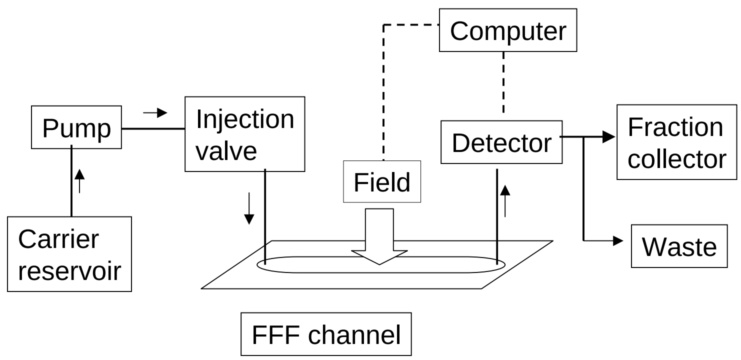
Thermal FFF



Basic Elements of an FFF Channel



Typical FFF System



Outline

- Thermal FFF
  - Theory
  - Instrumentation and Practice
  - Example applications

ThFFF Theory

For a parabolic velocity profile, the retention parameter λ and the retention time t_r are related by:

$$R = \frac{t^0}{t_r} = 6\lambda\left[\coth(1/2\lambda) - 2\lambda\right]$$

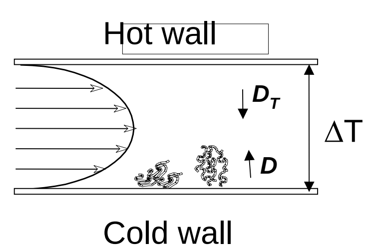
However...... temperature gradient → viscosity variations → deformed parabola

$$R = \frac{t^0}{t_r} = 6\lambda\left\{\nu + (1 - 6\lambda\nu)\left[\coth(1/2\lambda - 2\lambda)\right]\right\}$$

ThFFF Theory

Relationship between t_r, field strength, sample properties.

$$\frac{t_r}{t^0} = \frac{D_T \Delta T}{D}$$



t^0 void time
D_T thermal diffusion coefficient
ΔT temperature difference between hot and cold walls
D diffusion coefficient

Typical ThFFF Experiment

▪ MW range	$10^4 – 10^7$
▪ Sample amount	ng – μg
▪ Sample volume	5 – 40 μL
▪ Flow rate	0.2 mL/min
▪ Run time	5 – 60 min

Typical ThFFF equipment

- Pumps: high pressure not necessary
- Pulse dampener: needed if using RI detector
- Injection valve
- ThFFF channel (75 μm x 2 cm x tens of cms)
- Detector(s): UV, RI, viscosity, ELSD, MALS-DRI, QELS, MALDI-TOF MS
- Back pressure regulator (50 or 100 psi)
- Computer for equipment control and data acq.

Typical ThFFF solvents

- THF
- Acetonitrile
- Commonly used LC solvents.

Other considerations:

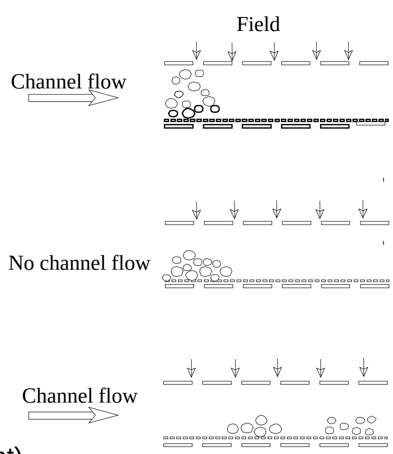
- Minimize use of chlorinated solvents.
- H-bonding solvents tend to show poor retention.
- Small amounts (25 mM) of salt can be added to improve retention in H-bonding solvents or in particle analyses.

Experimental Procedure

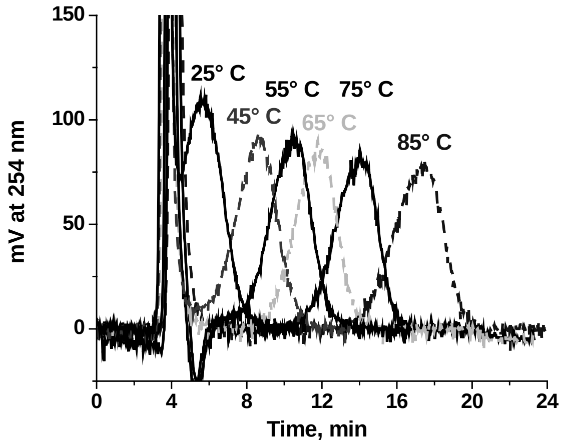
Sample Injection

Relaxation
(Apply driving force)

Separation
(Sample displacement)

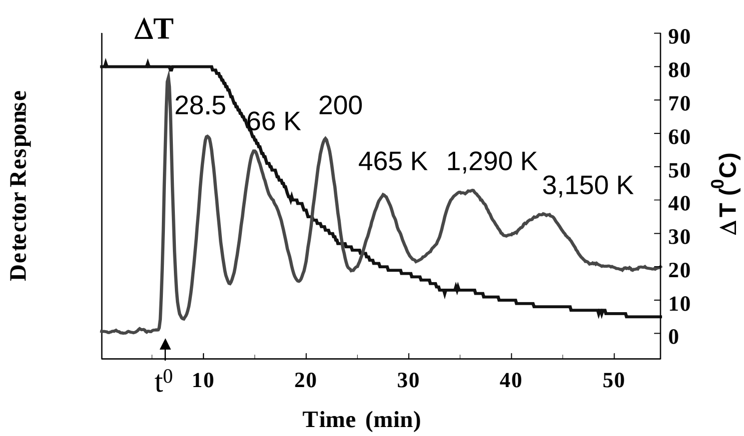


Effect of ΔT on Retention of PS 200K



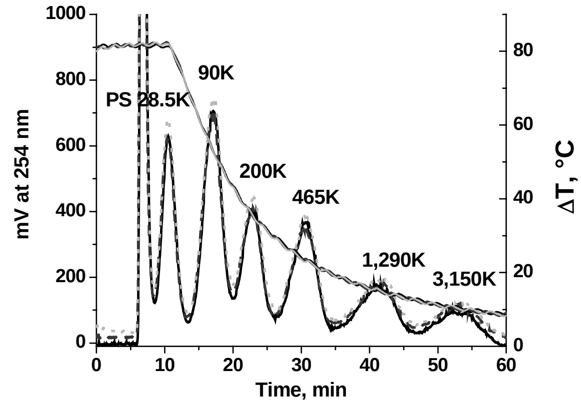
Flow rate: 0.2 mL/min; Inj. volume: 20 μL; Conc. 1 mg/mL

Temperature Programmed ThFFF Separation of PS Standards



Conditions: $\Delta T_0 = 80^0C$; $T_c = 26^0C$; $\Delta T_{final} = 5^0C$; V = 0.1 mL/min

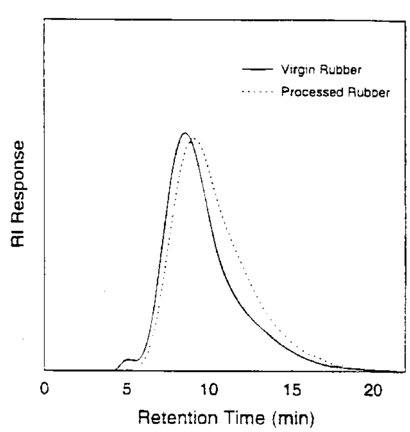
Run-to-Run Reproducibility



Flow rate: 0.1 mL/min; Inital ΔT: 80 °C for 10 min; Final ΔT: 5 °C

SEC CHROMATOGRAMS OF 0.2 μm FILTERED VIRGIN AND PROCESSED RUBBER

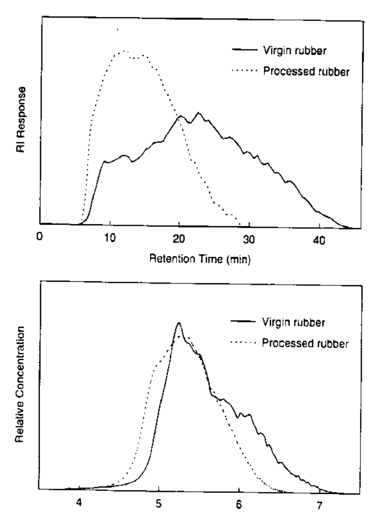
Permagel 6 column set (500, 10^3, 10^4, 10^5, 10^6, 100Å)
$\dot{V}$ = 1.0 mL/min, THF, RI detector, cis-polyisoprene standards



S. Lee and A. Molnár, Macromolecules, 28, 6354 (1995).

ThFFF FRACTOGRAMS AND MW DISTRIBUTIONS OF UNFILTERED VIRGIN AND PROCESSED RUBBER

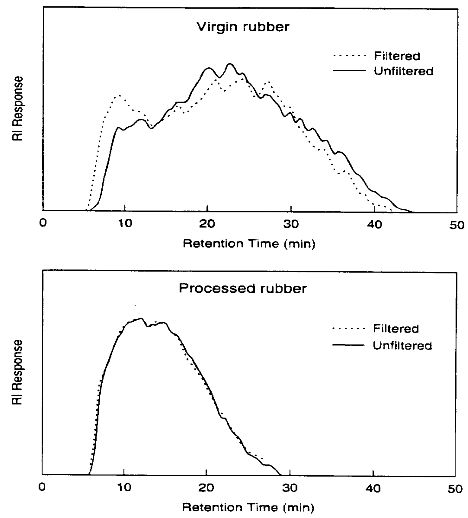
ΔT = 50 K→3 K, t_1 = 6 min, t_a = -12 min, $\dot{V}$ = 0.2 mL/min, THF, RI detector



S. Lee and A. Molnár, Macromolecules, 28, 6354 (1995).

ThFFF FRACTOGRAMS OF FILTERED AND UNFILTERED VIRGIN AND PROCESSED RUBBER

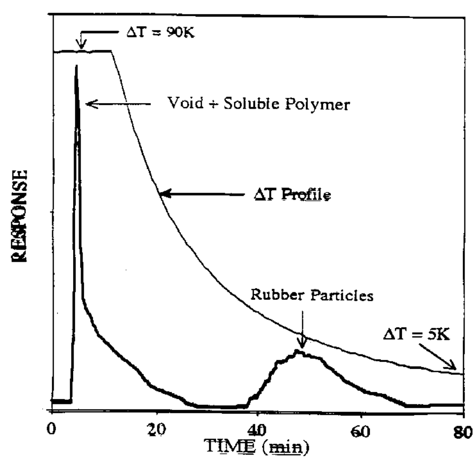
5 µm filter, $\Delta T = 50\ K \rightarrow 3\ K$, $t_l = 6$ min, $t_a = -12$ min, $\dot{V} = 0.2$ mL/min, THF, RI detector



S. Lee and A. Molnár, Macromolecules, 28, 6354 (1995).

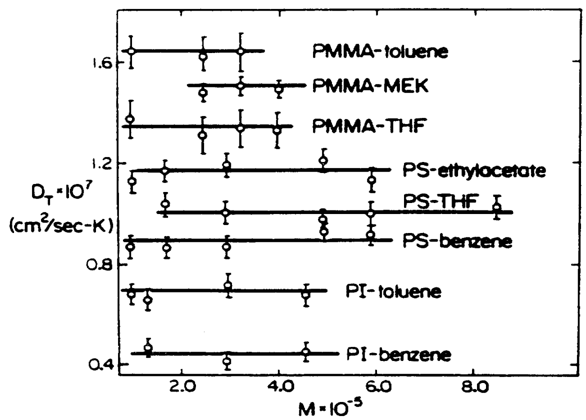
Thermal FFF Separation of ABS Sample

THF, [TBAP]=0.10 mM, ΔT=90 K$\rightarrow$5 K, t_l=10 min, $\dot{V}$=0.185 mL/min



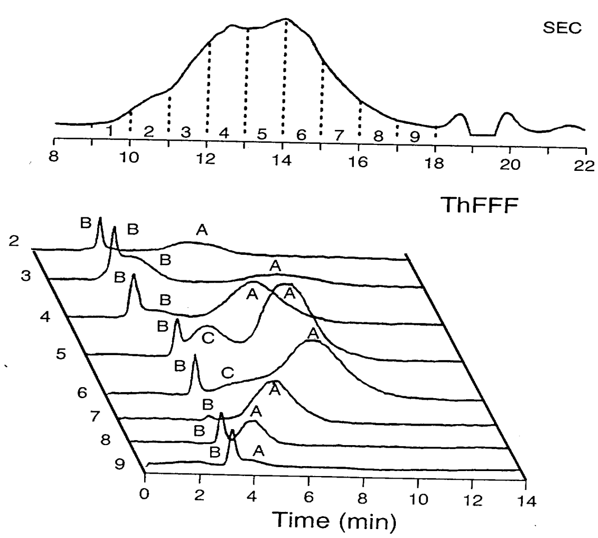
P. M. Shiundu, E. E. Remsen, and J. C. Giddings, J. Appl. Polym. Sci., 60, 1695 (1996).

THERMAL DIFFUSION COEFFICIENTS



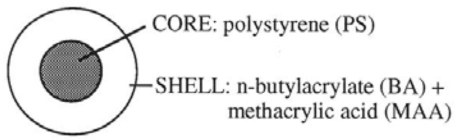
M. E. Schimpf and J. C. Giddings, J. Polym. Sci., Part B:Polymer Physics, 27, 1317 (1989).

SEC-ThFFF Cross-Fractionation of PS-PB- PTHF Blend



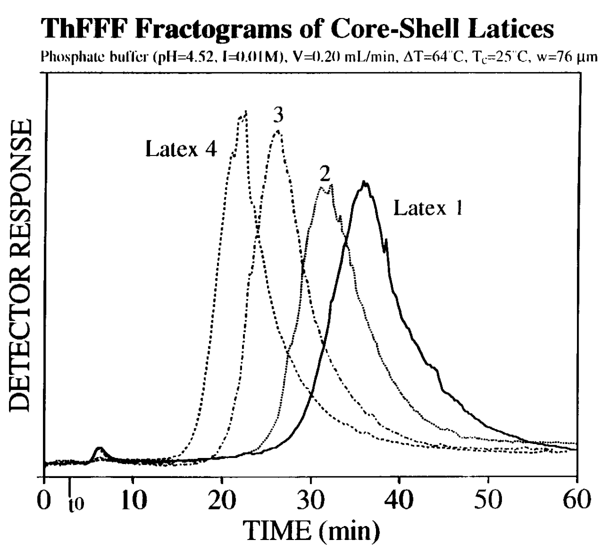
A. PS; B. Polybutadiene (333 kD); C. Polytetrahydrofuran (9.9 kD)

Core-Shell Particles



Designation	BA/MAA	%MAA
Latex 1	0	0
Latex 2	10/1	9
Latex 3	9/2	18
Latex 4	7/4	36

polymer	ρ (g/mL)
PS	1.05
BA	0.8898
MAA	1.1053



S. K. Ratanathanawongs, P. M. Shiundu, and J. C. Giddings, *Colloids and Surfaces A: Physicochem. Eng. Aspects*, **105**, 243-50 (1995).

Outline

- ThFFF-MALDI TOF MS
  - Compatibility and optimization
  - Example applications

Why ThFFF and MALDI-TOF MS?

ThFFF

- *separates polymers according to MW and chemical composition*
- *wide MW range*
- *high MW selectivity*
- *low shear rates*
- *requires calibration with standards*

MALDI-TOF MS

- measures MW for narrow polydispersity polymers
- provides data on repeat units and end groups for low MWs
- requires fractionation of polydisperse polymers and mixtures

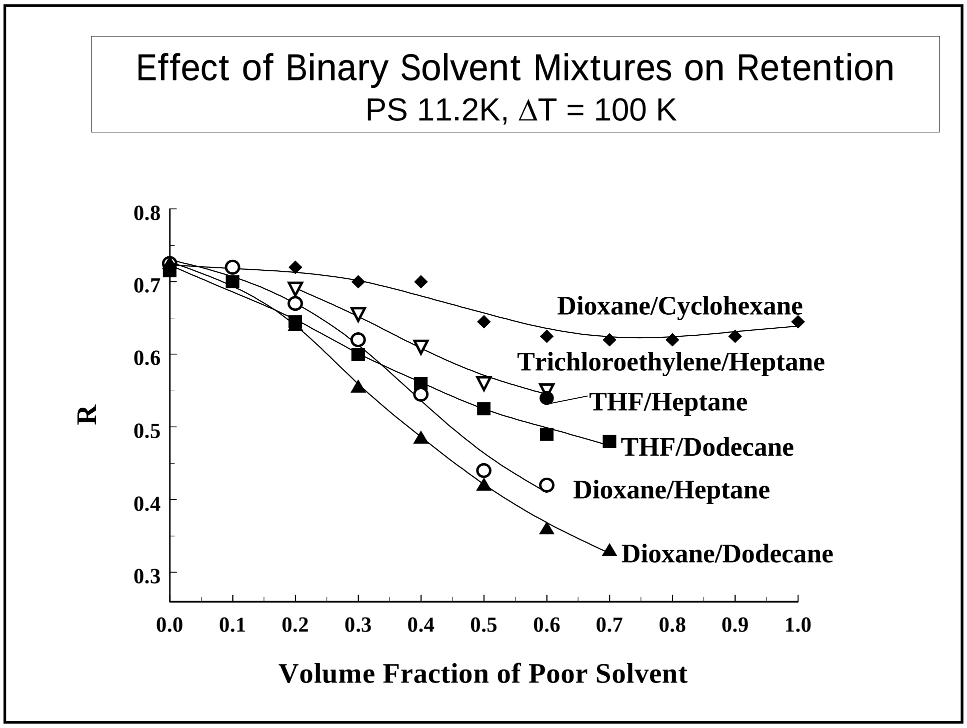
ThFFF/MALDI-TOF MS

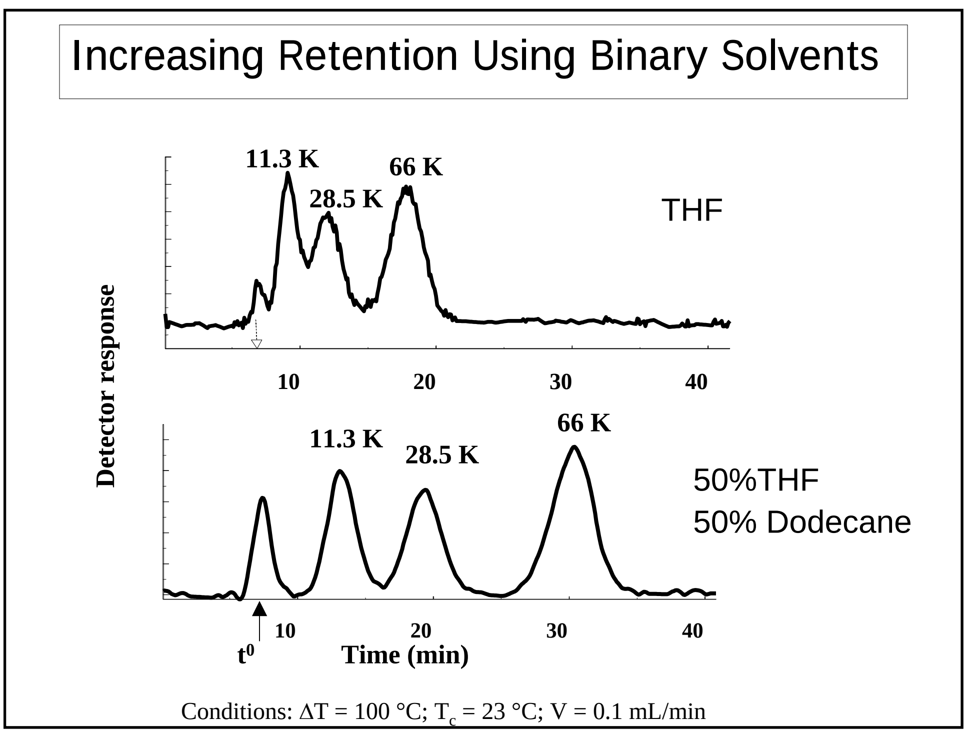
⇩

MW and composition analysis of polydisperse polymers and polymer mixtures over a wide MW range

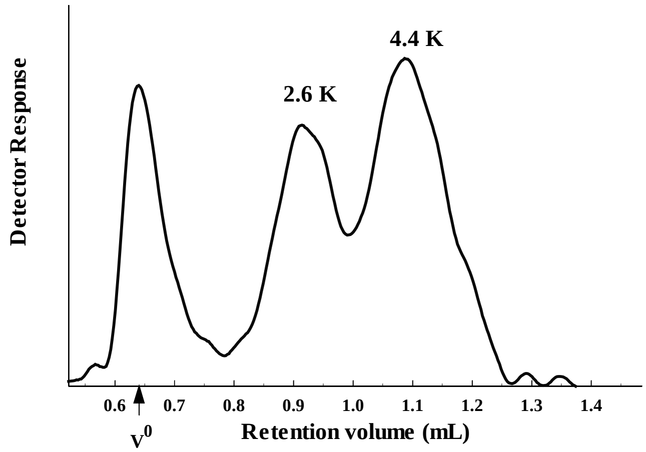
Compatibility and Optimization

- Sample amount
  100 μL fractions or 1-10 μg
- Solvent compatibility
- Molecular weight range


Effect of Binary Solvent Mixtures on Retention
PS 11.2K, ΔT = 100 K
0.8
0.7
0.6
0.5
0.4
0.3
R
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
Volume Fraction of Poor Solvent
Dioxane/Cyclohexane
Trichloroethylene/Heptane
THF/Heptane
THF/Dodecane
Dioxane/Heptane
Dioxane/Dodecane

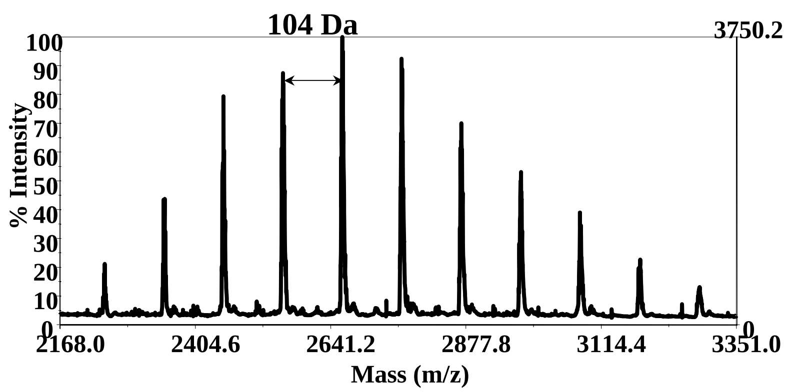

Increasing Retention Using Binary Solvents
11.3 K
66 K
28.5 K
THF
10
20
30
40
Detector response
66 K
11.3 K
28.5 K
50%THF
50% Dodecane
10
20
30
40
t0
Time (min)
Conditions: ΔT = 100 °C; Tc = 23 °C; V = 0.1 mL/min

Separation of Low MW Polystyrenes in THF/Dodecane (15:85)



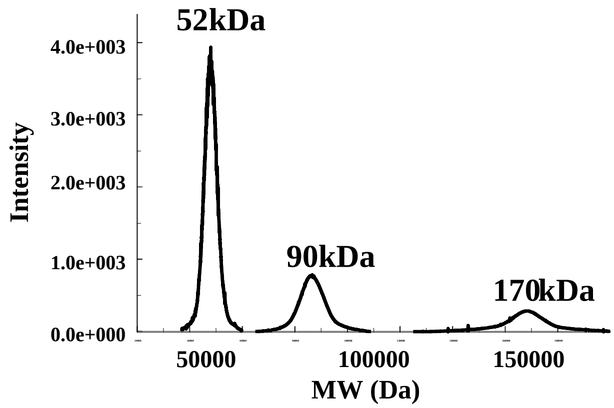
ΔT = 110 K; T_c = 299 K; V= 0.03 mL/min

MALDI-TOF MS: Polystyrene 2 kDa



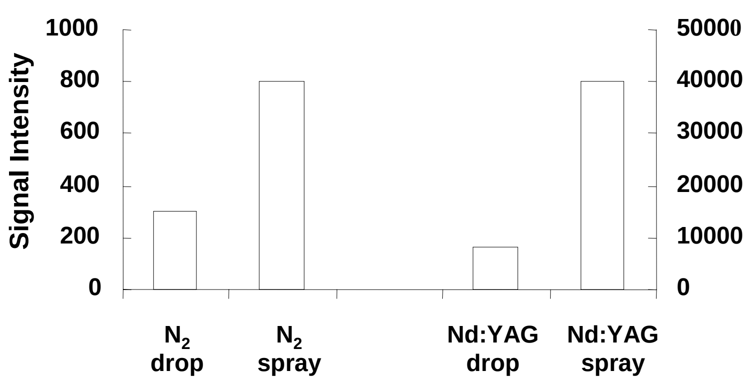
M_n=2.60 kDa; M_w=2.63 kDa; PD = 1.01;M_p = 2.6 kDa

MALDI Sensitivity for Higher MW Polymers

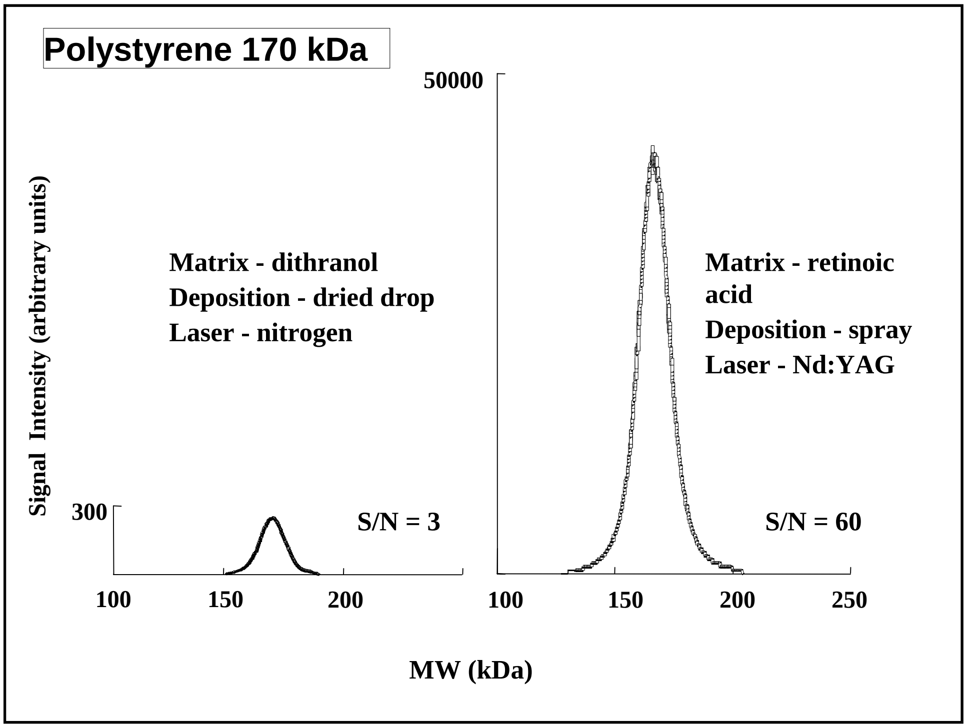


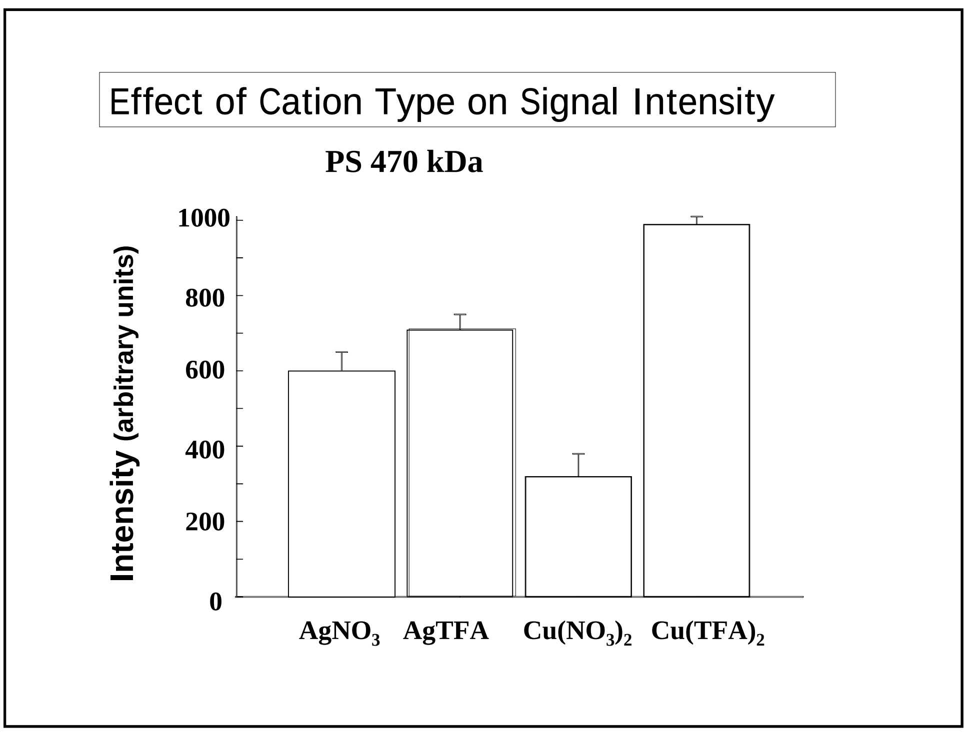
Conditions: matrix-dithranol; drop-wise deposition, N_2 laser

Effect of Sample Deposition and Laser on Signal Intensity of PS 170K

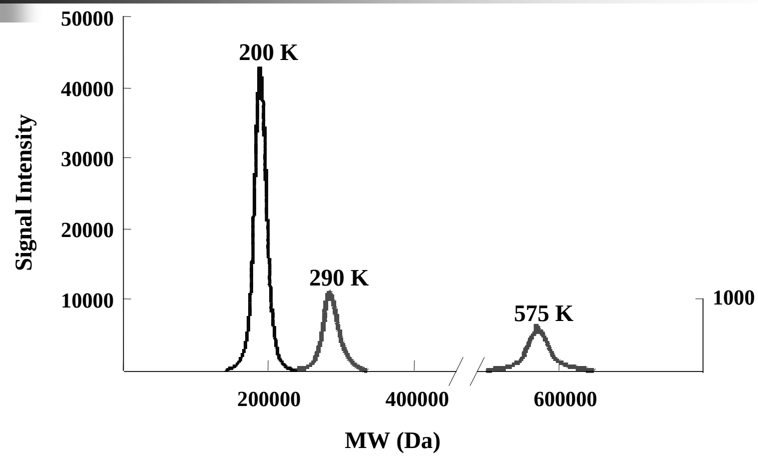


Matrix - 0.05M retinoic acid in THF, PS 170K -5 mg/mL in THF; salt - $AgNO_3$ saturated in methanol, mix - 125:7:1


Polystyrene 170 kDa
50000
300
Signal Intensity (arbitrary units)
Matrix - dithranol
Deposition - dried drop
Laser - nitrogen
S/N = 3
Matrix - retinoic acid
Deposition - spray
Laser - Nd:YAG
S/N = 60
100
150
200
100
150
200
250
MW (kDa)


Effect of Cation Type on Signal Intensity
PS 470 kDa
Intensity (arbitrary units)
1000
800
600
400
200
0
$AgNO_3$
AgTFA
$Cu(NO_3)_2$
$Cu(TFA)_2$

MALDI-TOF MS of High MW Polystyrenes



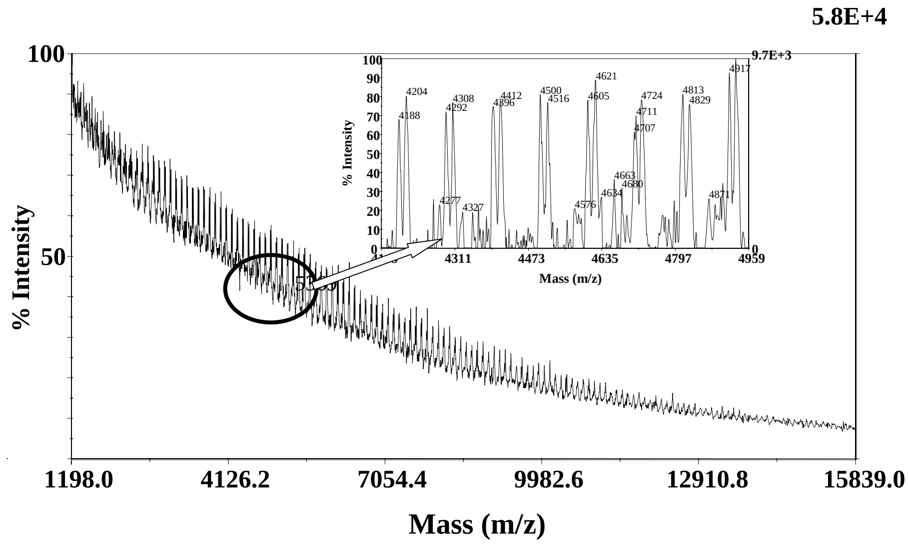
Conditions: matrix – retinoic acid; spray deposition; Nd:YAG laser

MALDI Conditions for Polystyrene

	I	II
Matrix	**Dithranol**	**All-trans-retinoic acid**
Cation	**Silver (AgTFA)**	**Silver ($AgNO_3$)**
Solvent	**Tetrahydrofuran**	**Tetrahydrofuran**
Deposition	**Drop-wise**	**Spray**
Laser	**N_2 (337 nm)**	**Nd:YAG (355 nm)**

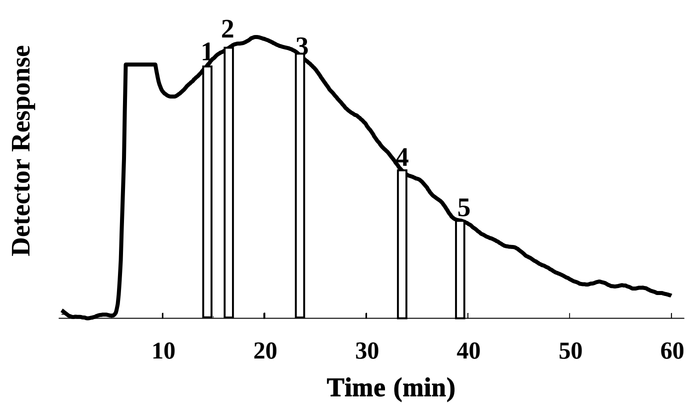
MALDI-TOF MS of Broad MWD Polystyrene

(M_n = 14.5 kDa; M_w = 33.0 kDa; PD = 2.28)

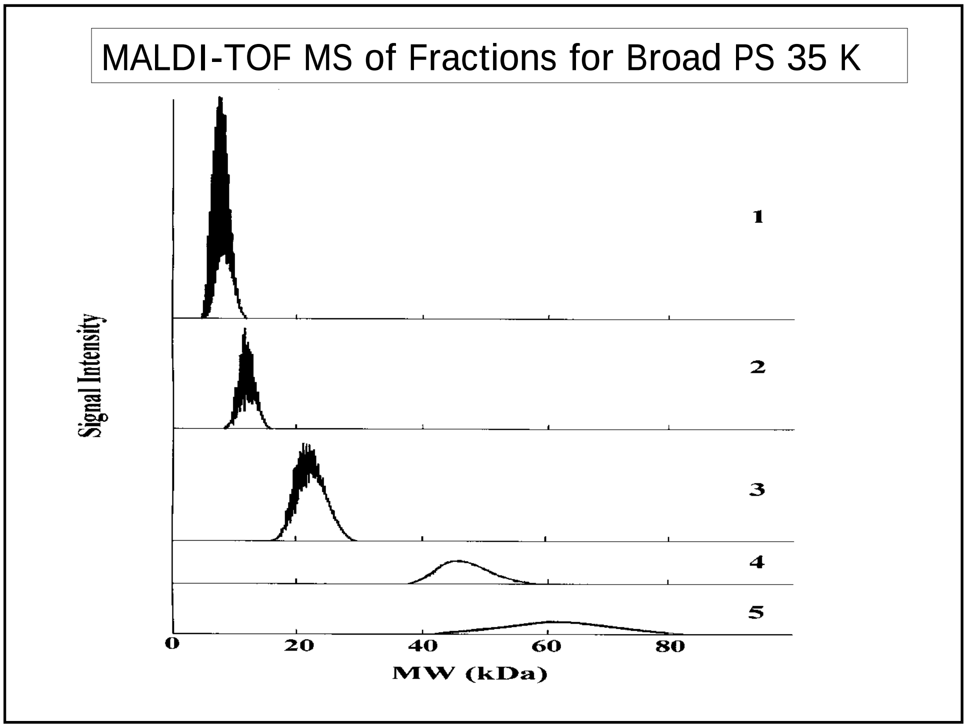


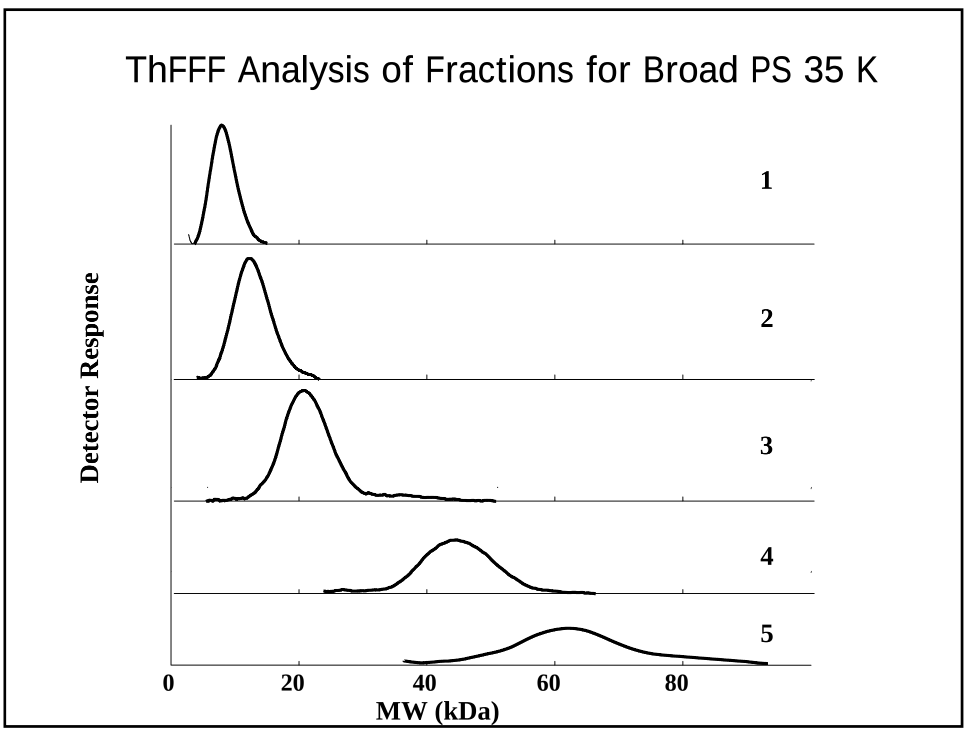
Fractionation of Broad MWD Polystyrene

(M_n = 14.5 kDa; M_w = 33.0 kDa; PD = 2.28)



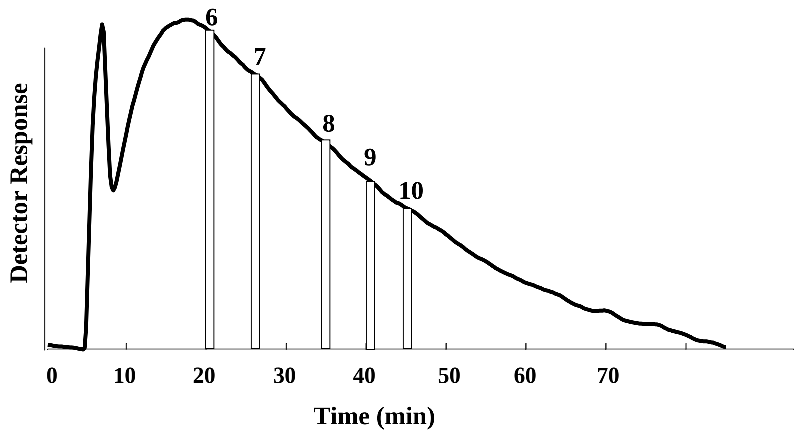
ThFFF conditions: solvent - 40% dioxane/ 60% heptane;
ΔT = 100 ^{0}C; T_c = 23 ^{0}C; V = 0.1 mL/min.


MALDI-TOF MS of Fractions for Broad PS 35 K
Signal Intensity
1
2
3
4
5
0
20
40
60
80
MW (kDa)


ThFFF Analysis of Fractions for Broad PS 35 K
Detector Response
1
2
3
4
5
0
20
40
60
80
MW (kDa)

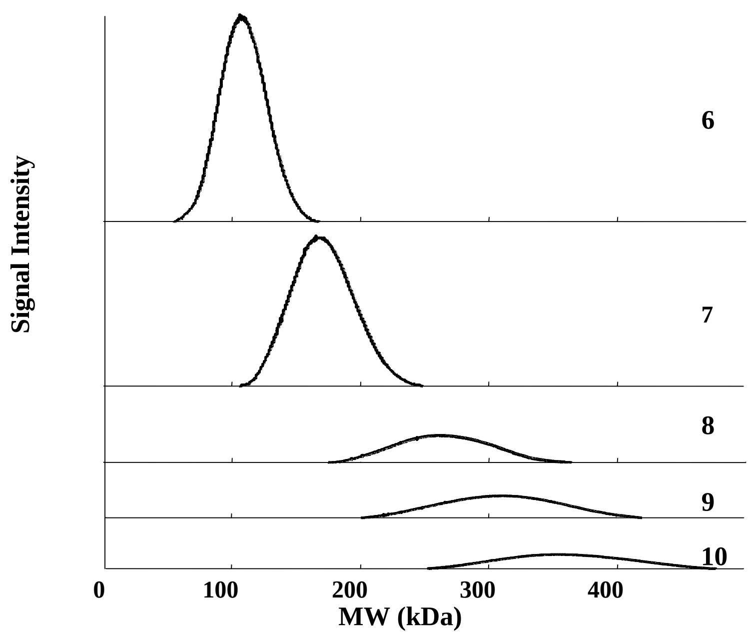
Fractionation of Broad MWD Polystyrene

(M_n = 100 kDa; M_w = 250 kDa; PD = 2.5)



Conditions: solvent - THF, $\Delta T = 80\ {}^0C$; $T_c = 22\ {}^0C$; V = 0.1 mL/min.

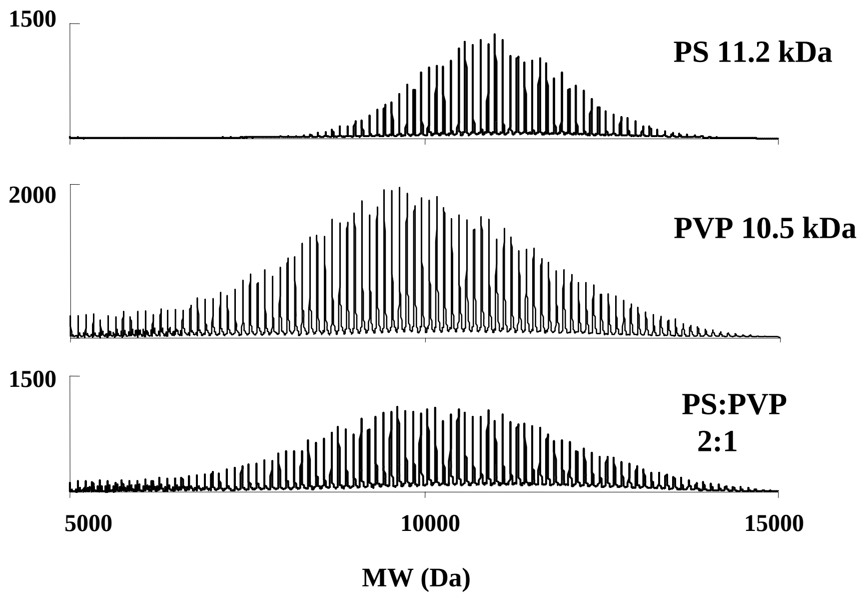
MALDI-TOF of Fractions for Broad PS250 K

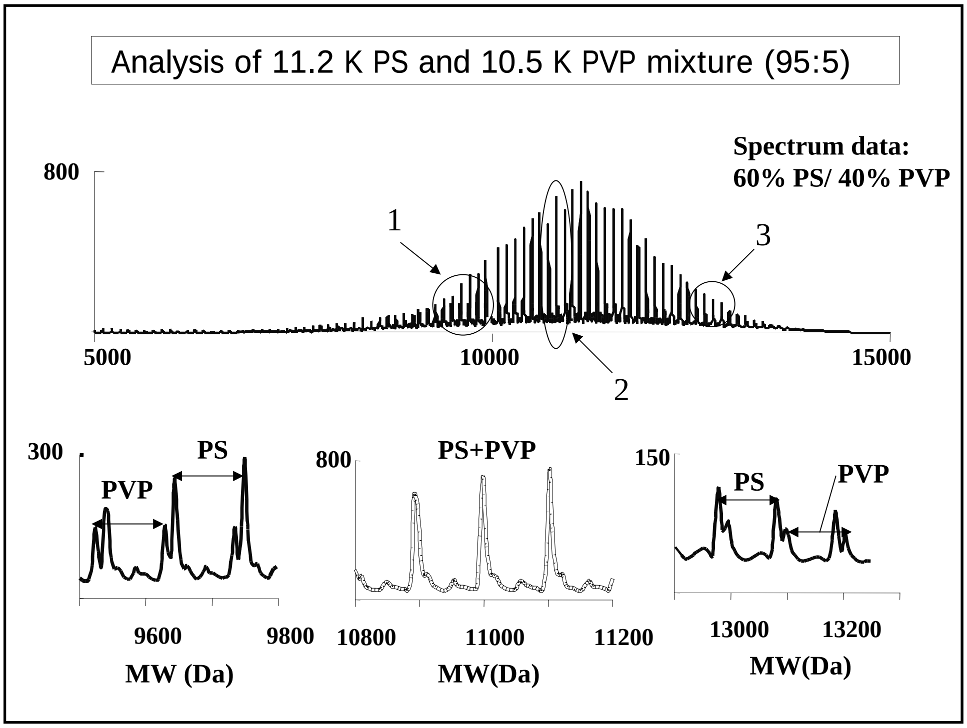


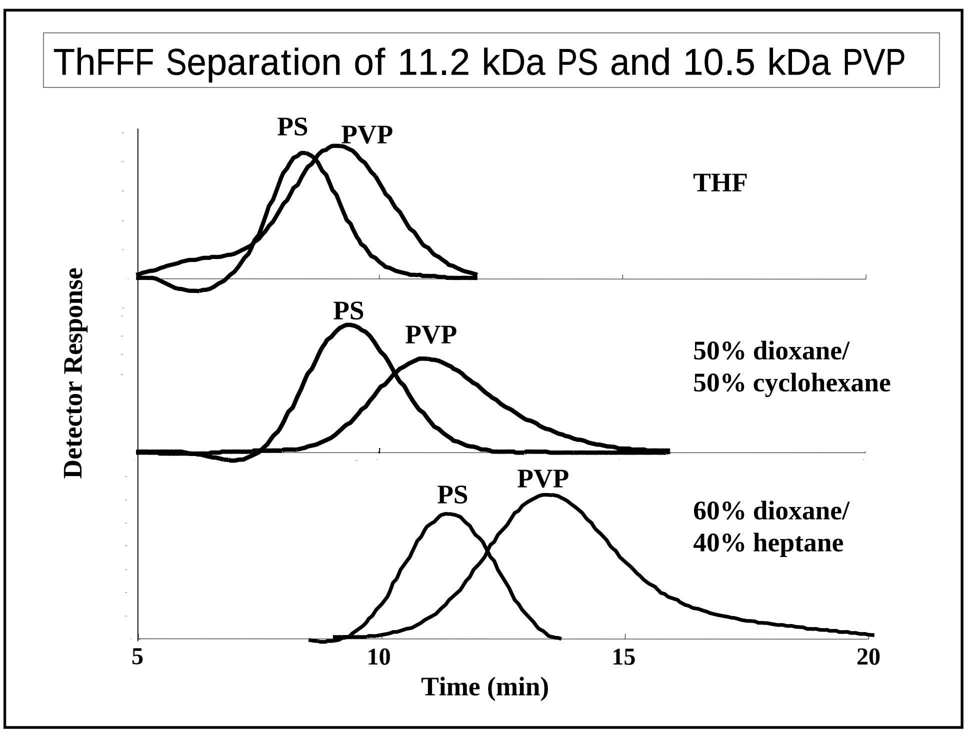
Comparison of MWD for Fractions

Fraction	MALDI-TOF MS (kDa)	ThFFF (kDa)
1	M_n = 7.8; M_w = 8.0 PD = 1.03	-
2	M_n = 12.0; M_w = 12.2 PD = 1.02	M_n = 13.8; M_w = 14.4 PD = 1.03
3	M_n = 21.4; M_w = 21.5 PD = 1.01	M_n = 22.1; M_w = 23.4 PD = 1.03
4	M_n = 46.3; M_w = 46.4 PD = 1.01	M_n = 44.1; M_w = 45.3 PD = 1.01
5	M_n = 61.5; M_w = 61.8 PD = 1.01	M_n = 61.4; M_w = 62.3 PD = 1.01
6	M_n = 109; M_w = 112 PD = 1.02	M_n = 114; M_w = 117 PD = 1.03
7	M_n = 166; M_w = 170 PD = 1.02	M_n = 167; M_w = 172 PD = 1.03
8	M_n = 262; M_w = 266 PD = 1.02	M_n = 260; M_w = 269 PD = 1.02
9	M_n = 309; M_w = 314 PD = 1.02	M_n = 299; M_w = 318 PD = 1.02
10	M_n = 359; M_w = 365 PD = 1.02	M_n = 346; M_w = 386 PD = 1.05

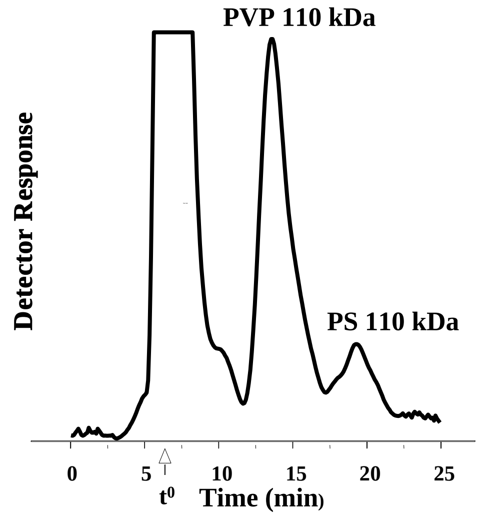
Analysis of Polystyrene / Polyvinylpyridine Mixture



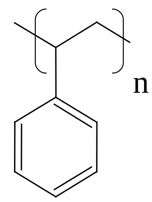

Analysis of 11.2 K PS and 10.5 K PVP mixture (95:5)
Spectrum data:
60% PS/ 40% PVP
800
1
3
2
5000
10000
15000
300
PS
PVP
9600
9800
MW (Da)
800
PS+PVP
10800
11000
11200
MW(Da)
150
PS
PVP
13000
13200
MW(Da)


ThFFF Separation of 11.2 kDa PS and 10.5 kDa PVP
PS
PVP
THF
PS
PVP
50% dioxane/
50% cyclohexane
PS
PVP
60% dioxane/
40% heptane
Detector Response
5
10
15
20
Time (min)

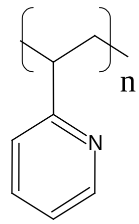
Separation by Chemical Composition



Polystyrene

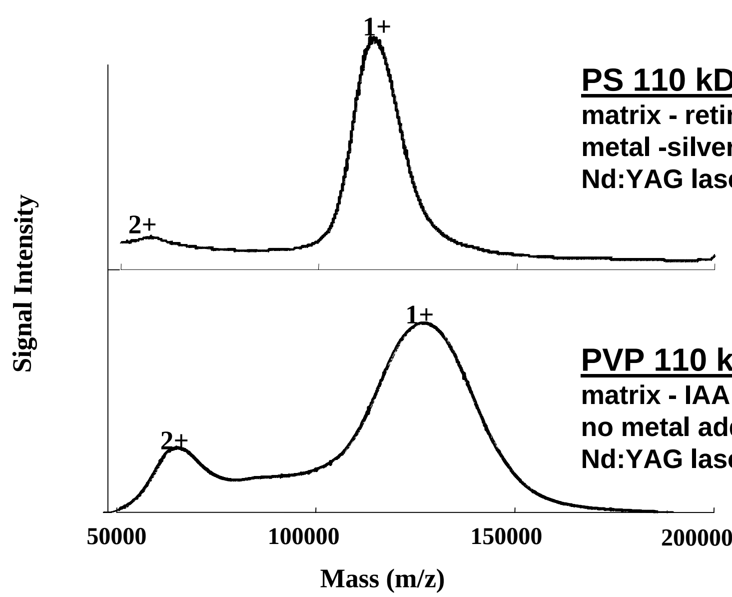


Poly(vinylpyridine)



Conditions: ΔT = 70 °C; T_c = 26 °C;
solvent - THF; V = 0.1 mL/min

Optimization of MALDI-TOF Analysis



PS 110 kDa:
matrix - retinoic acid;
metal -silver;
Nd:YAG laser

PVP 110 kDa:
matrix - IAA;
no metal added;
Nd:YAG laser

Nominal MW and Composition of Standards

Polymer	MW	PD	% (w) PS
Polystyrene	M_p= 110 K	<1.06	100
Random PS/PB	M_n =100.6 K	1.05	28
Diblock PS-PB	Mn = 96.8 K	1.06	28
Triblock PS-PB-PS	Mn = 102 K	1.05	28
Polybutadiene	Mn = 99.5 K	1.09	-

Random copolymer - S-B-S-B-B-S-B

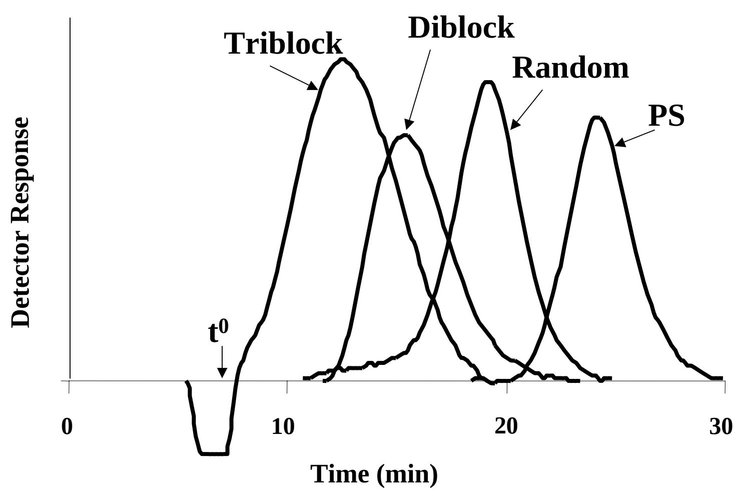
Diblock - S-S-S-B-B-B-B-B-B-B

27 K 70 K

Triblock - S-S-B-B-B-B-B-B-B-S-S

14 K 70 K 15 K

Analysis of Copolymers in Methyl Ethyl Ketone



Conditions: ΔT = 100 ^{0}C; T_c = 26 ^{0}C; V = 0.1 mL/min

Summary

- *ThFFF/MALDI-TOF MS allows analyses of:*
  - *wide polydispersity polymers*
  - *polymer mixtures of different compositions*
  - *polymer mixtures of different microstructures*
- ThFFF can be coupled with other separation methods for multidimensional separation.

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