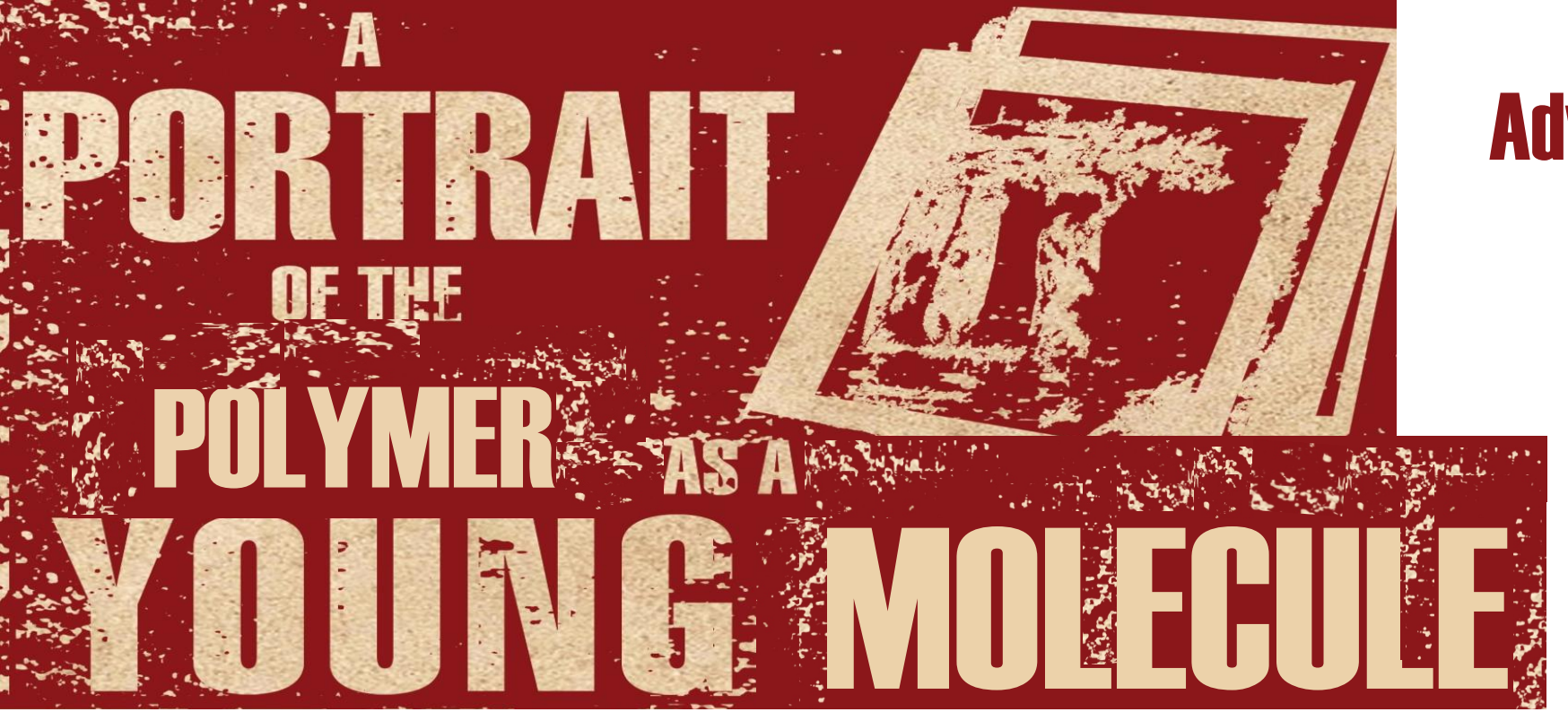


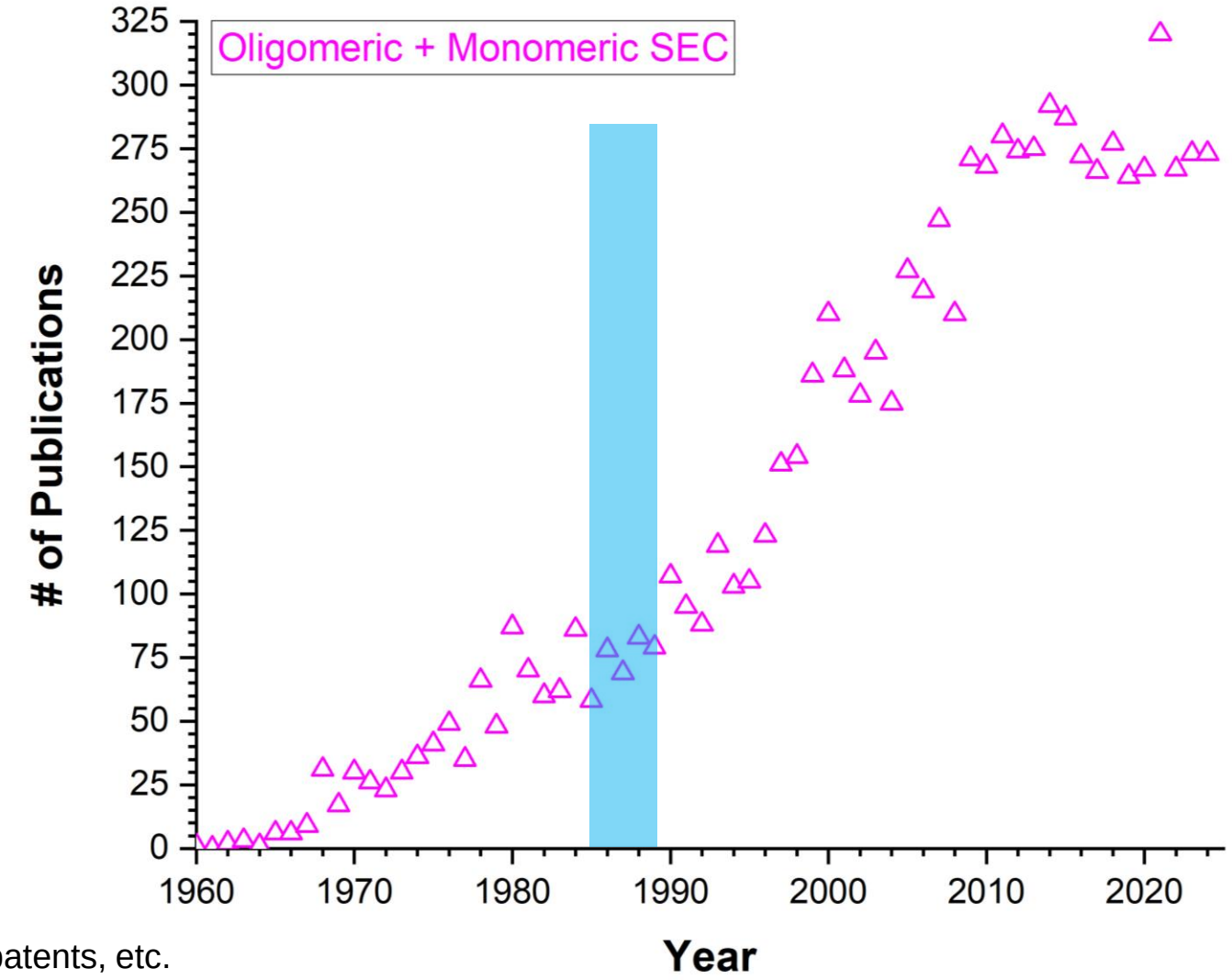
Adventures & Challenges in Oligomeric SEC

André M. Striegel*

National Institute of Standards & Technology

*with apologies to James Joyce

Interest in Characterizing Monomers & Oligomers & Oligomeric MMD



Results include papers, reviews, patents, etc.
Search terms: SEC, GPC, GFC

SciFinder search, January 2025

Interest in Characterizing Monomers & Oligomers & Oligomeric MMD

- ***Oligomeric portion of MMD governs flow properties, brittleness, etc. of macromolecules.***
- ***Monomers and oligomers oftentimes behave as individual chemical entities, even when composed of same repeat unit.***
- ***Even if polymer is considered "safe," monomer can be highly toxic or hazardous (e.g., styrene vs. PS, or vinyl chloride vs. PVC).***
- ***Synthetic oligomer use in, e.g., lubricants, plasticizers, coatings. Oligosaccharides used in foods, feeds, biomolecular recognition processes, etc.***
- ***Information on oligomeric content of polymers required (or being considered) by regulatory agencies for import/export purposes.***

CHALLENGES

Some Questions to Ponder

- **What is an *oligomer*?**
- **What is a *polymer*?**
- **What distinguishes an *oligomer* from a *polymer*?**
- **When does a molecule become a *polymer*? I.e., when does the transition from *oligomer* to *polymer* occur?**

Some Answers

- "*Polymers* are macromolecules built up by linking together larger numbers of smaller molecules." (Odian, *Principles of Polymerization, 3rd ed,* 1991).

- "If only a few monomer units are joined together, the resulting low-[molar mass] polymer is called an *oligomer*." (Stevens, *Polymer Chemistry, 2nd ed,* 1990).

- "A *polymer* may be defined as a large molecule comprised of repeating structural units joined by covalent bonds… In this context , a large molecule is commonly arbitrarily regarded either as having a [molar mass] of at least 1000 or as one containing 100 structural units or more." (Saunders, *Organic Polymer Chemistry,* 1973).

- "Polymers with only a few structural units (from 5 to 10) are usually called 'oligomers'." (Vollmert, *Polymer Chemistry,* 1973).

Some Answers

- “This word [polymer] means ‘many parts’ and designates a large molecule made up of smaller units… Polymers generally have [molar masses] greater than about 5000 but no firm lower limit need be defined since the meaning of the word is nearly always clear from its use… An oligomer is a low-[molar mass] polymer. It will contain at least two monomer units… The distinction between the sizes of oligomers and the corresponding polymers is left vague, however, because there is no sharp transition in most properties of interest.” (Rudin, *The Elements of Polymer Science & Engineering*, 1982).

- “**Polymer molecule –** a molecule that contains a sequence of at least 3 monomer units, which are covalently bound to at least one other monomer unit or other reactant” and “**Oligomer –** a low [molar mass] species derived from the polymerization reaction.” (*US EPA Polymer Exemption Guidance Manual,* 1997).

"When Does a Molecule Become a Polymer" Ding, Kisliuk, Sokolov, *Macromolecules* 2004

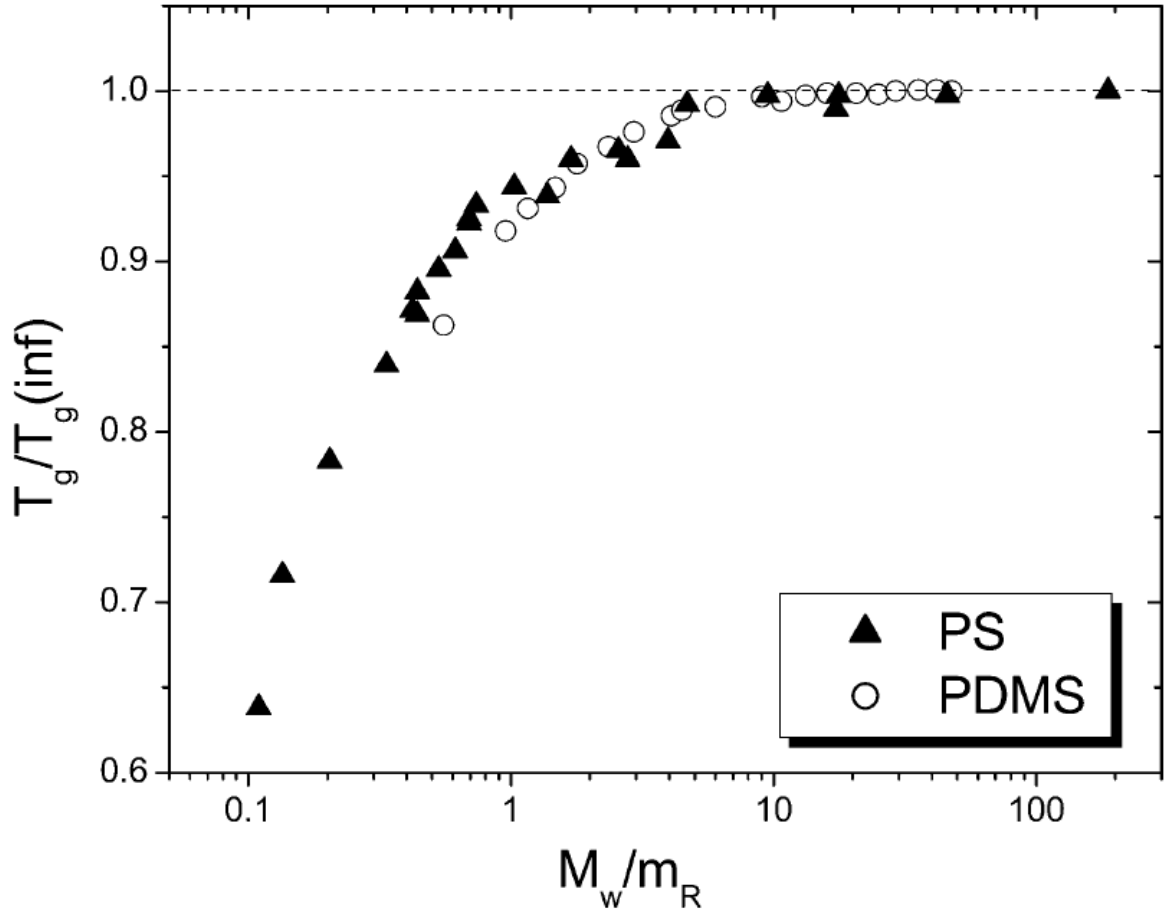


Figure 5. $T_g/T_g(\infty)$ as a function of molecular weight scaled by the mass of random step m_R. $m_R \sim 560$ for PDMS and $m_R \sim 5100$ for PS (see the text for definition of m_R).

Considering Oligomer/Oligomeric

- Here, we will consider the terms “oligomer/oligomeric” two different ways.
- First, we shall consider these from a *regulatory* viewpoint, as regards the importance of quantitating the oligomeric portion of a macromolecule.
- This is a US-based viewpoint. An EU-based definition is still in the works. (See talks by Ron Peters, Laszlo Mojoros, Timo Beskers, Matthias Pursch, Adriana Carvalho de Souza, etc., and *Industry Round Table* with Bastiaan Staal).
- Second, we shall consider the terms “oligomer/oligomeric” from a *“macromolecular separation science”* viewpoint.

US EPA Polymer Exemptions for Import/Export

- *The (e)(1) exemption*. This concerns polymers with 1000 g $mol^{-1} \leq M_n <$ 10,000 g mol^{-1}. Oligomer content must be less than 10 % by mass below 500 g mol^{-1} and less than 25 % by mass below 1000 g mol^{-1}. Polymers must also meet certain function group criteria (not discussed here).

- *The (e)(2) exemption*. This concerns polymers with $M_n \geq$ 10,000 g mol^{-1} and oligomer content less than 2 % below 500 g mol^{-1} and less than 5 % below 1000 g mol^{-1}.

- *The (e)(3) exemption*. This concerns certain polyesters composed solely of listed monomers and reactants.

- Other rules may apply in conjunction with the above criteria; *e.g.,* in the case of cationic or degradable polymers.

- *US EPA Polymer Exemption Guidance Manual*, EPA 744-B-97-001, June 1997.

Polymer REACH: Pray or be Prepared? A Polymer Chromatography Viewpoint on What to Expect

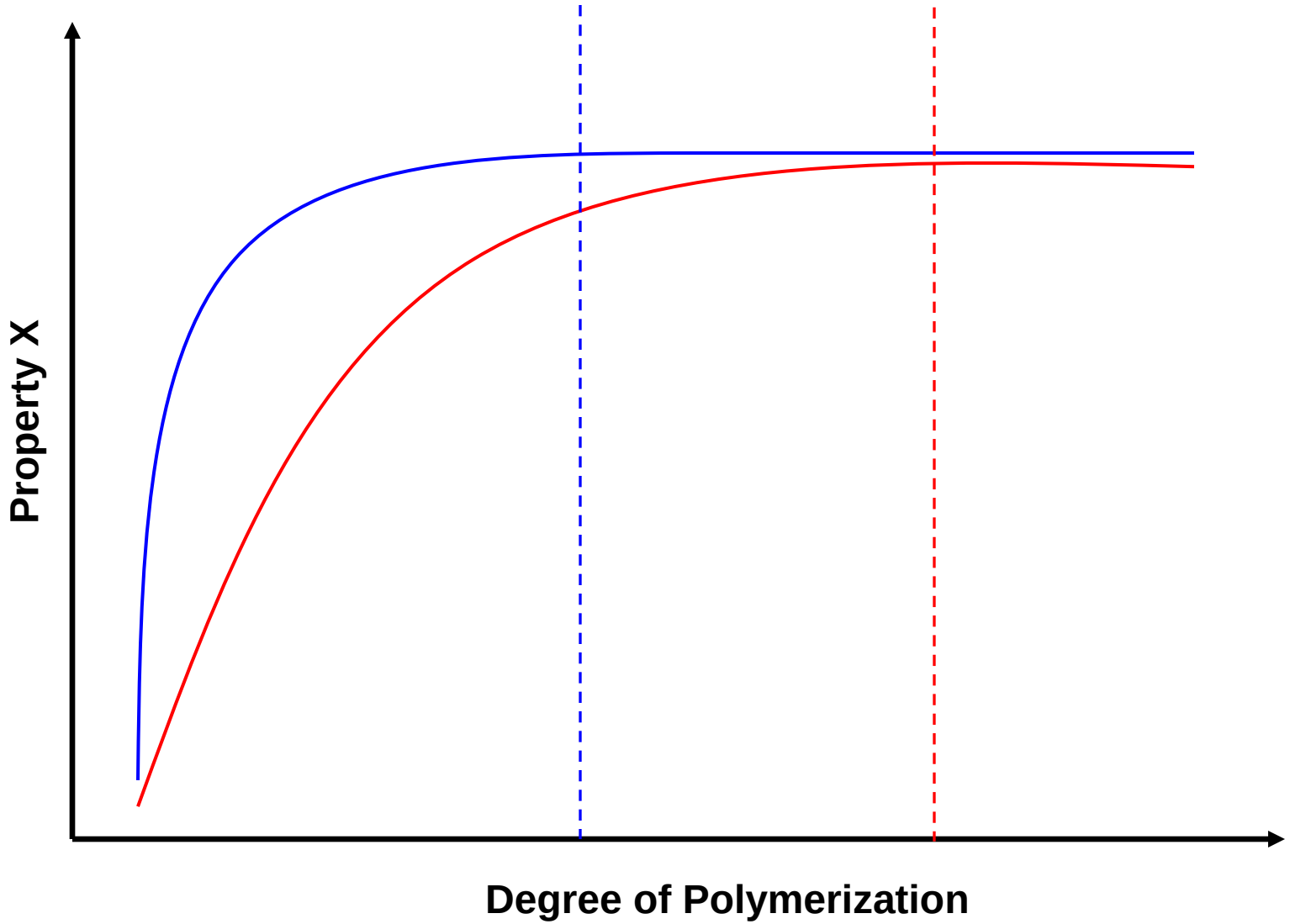
Timo F. Beskers, BASF SE, Ludwigshafen am Rhein, Germany

Timo F. Beskers previews his tutorial lecture at HPLC 2023 in Düsseldorf, where he will give an overview on polymer REACH, its expected regulatory requirements, challenges, and analytical needs, as well as strategies to deal with those from the perspective of polymer chromatography.

LCGC Europe **2023,** *35(s6),* 16-19

OK, fine. We'll return to that later.

In the meantime, how can we,
as *macromolecular separation scientists,*
distinguish a polymer from an oligomer?


Property X
Degree of Polymerization

“Our” Polymer/Oligomer Definition

- **We consider a molecule a polymer once the *property of interest* (∂*n*/∂*c*, ε, A_2, etc.) has achieved a constant value.**

- **As long as this *property of interest* changes appreciably from one DP to the next, we consider the molecule an oligomer.**

- **If using more than one detector, the we shall consider a molecule a polymer once all the *properties of interest* have achieved a constant value.**

Challenges to Characterizing Oligomers

- Non-constant $\partial n/\partial c$ and ε in oligomeric region.
- Non-constant A_2 in oligomeric region.
- Negative viscosities of dilute solutions; failure of universal calibration.
- Non-SEC behavior.
- Low response of LS and VISC detectors.
- Counterintuitive solubility behavior.
- Other electromagnetic and hydrodynamic effects.

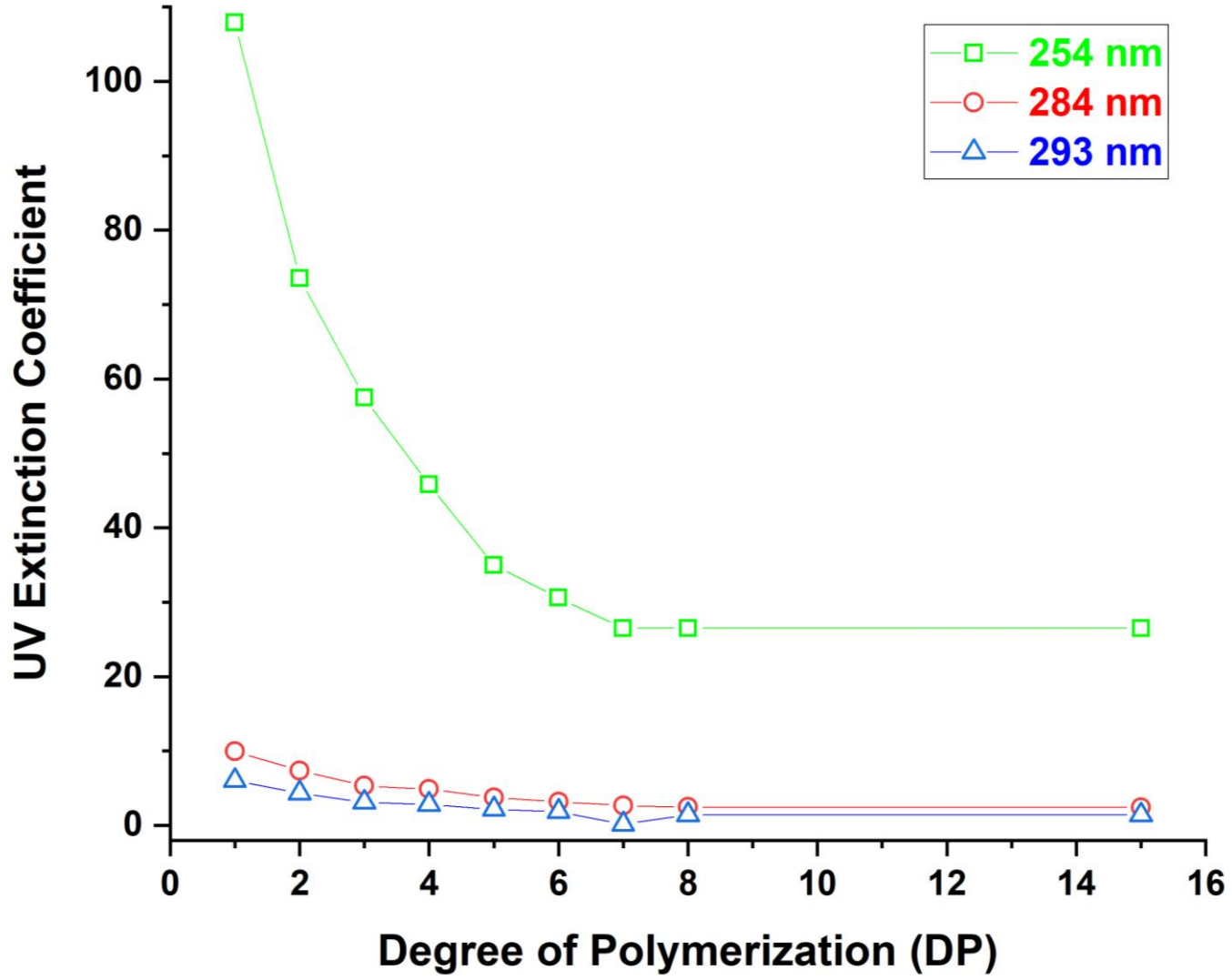
Anal Bioanal Chem (2013) 405:8959–8967
DOI 10.1007/s00216-013-7198-1

TRENDS

There's plenty of gloom at the bottom: the many challenges of accurate quantitation in size-based oligomeric separations

André M. Striegel

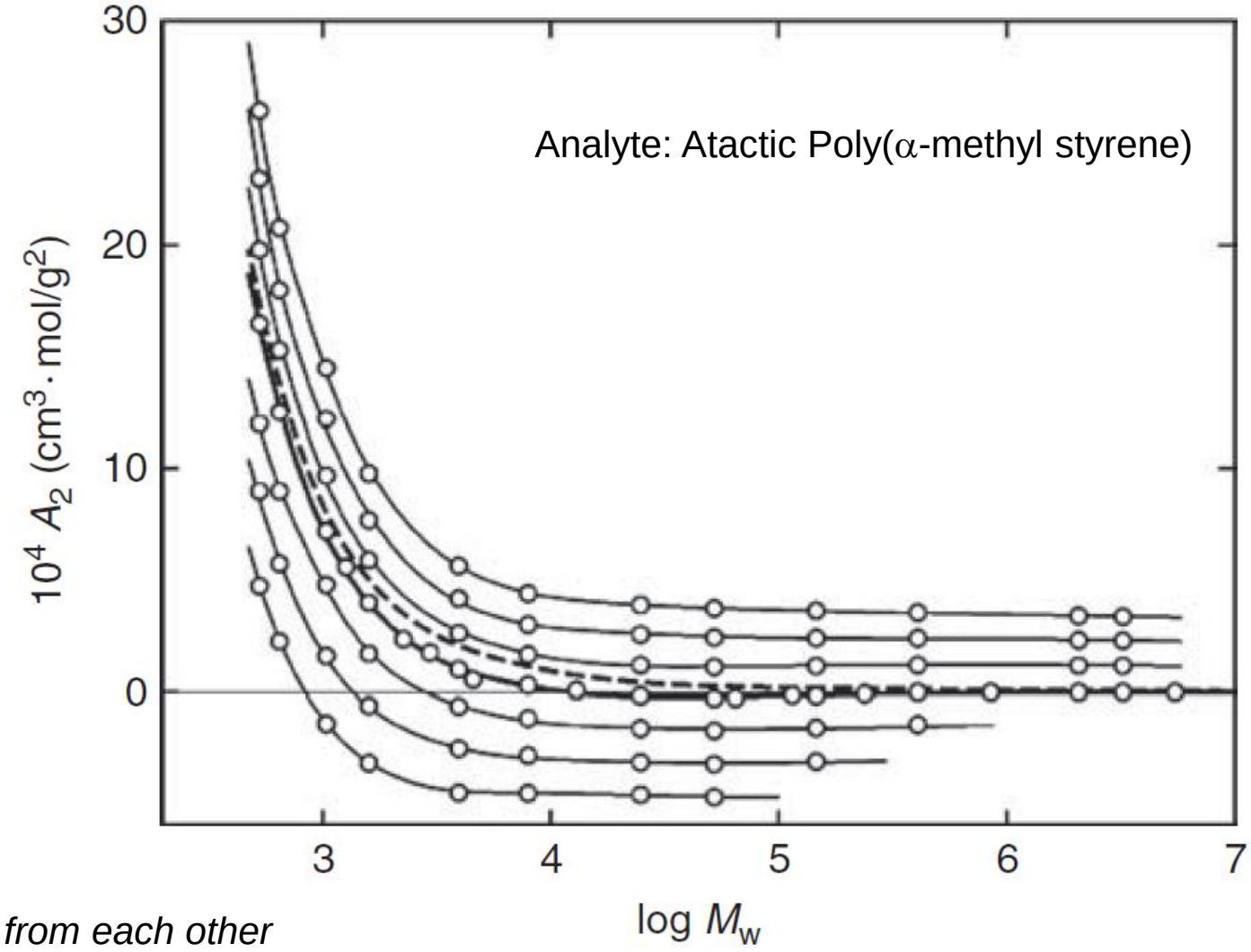
Non-Constant ε in Oligomeric Region



Vinyl-terminated styrene oligomers in THF at room temperature

From data in Gridnev *et al., J Polym Sci A* **2001,** *39,* 1099

Non-Constant A_2 in Oligomeric Region

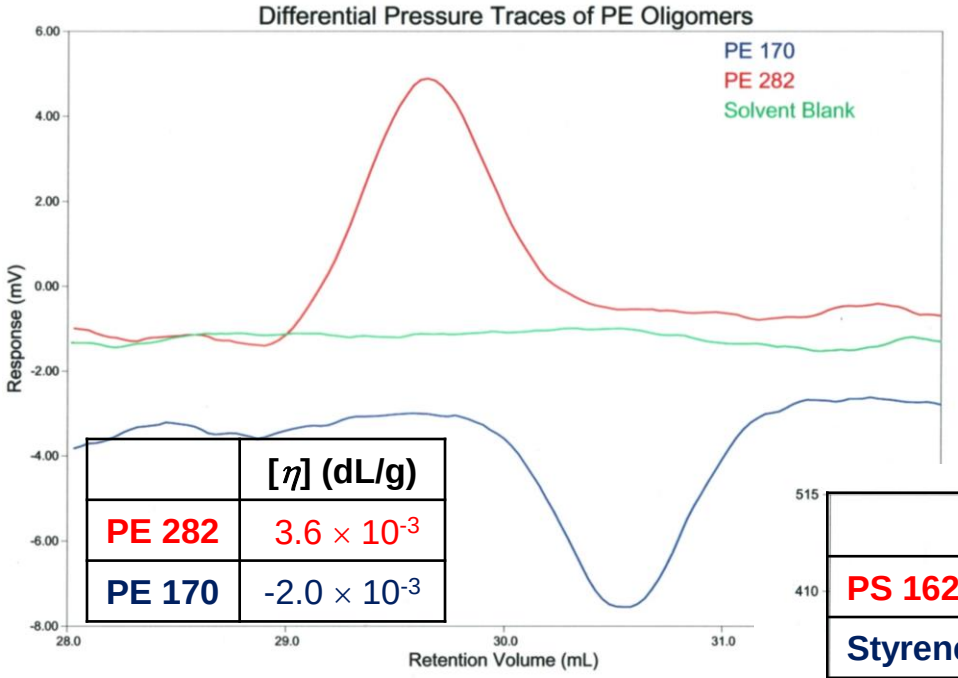


Temperature (°C)
from top to bottom:
45.0
40.0
35.0
30.5 (θ)
25.0
20.0
15.0

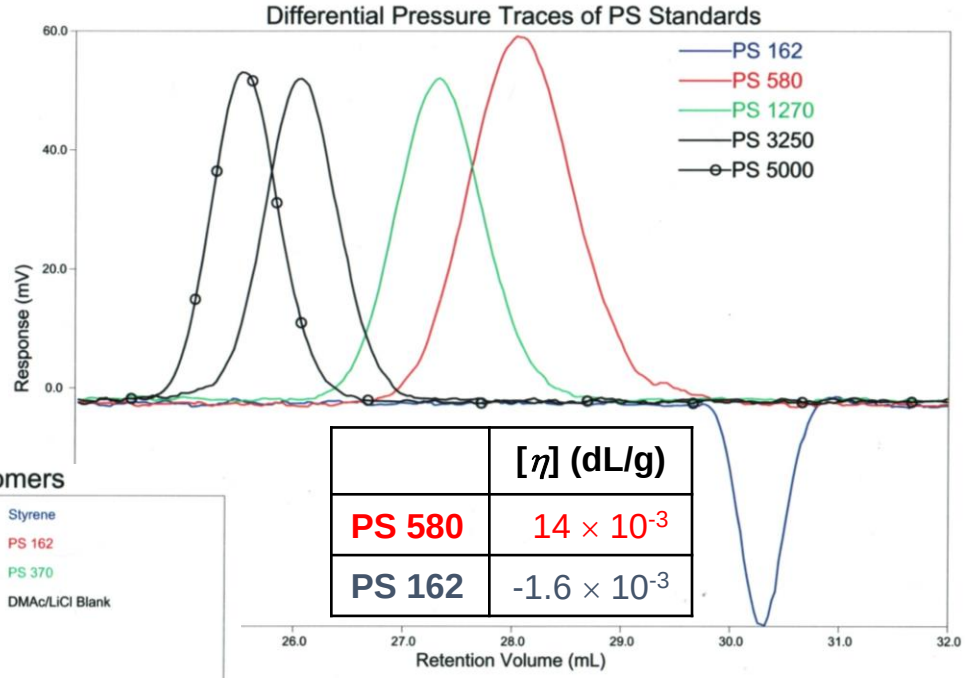
NB: Data sets are offset from each other vertically for viewing clarity

Kawaguchi *et al., Macromolecules* **2004,** *37,* 2240

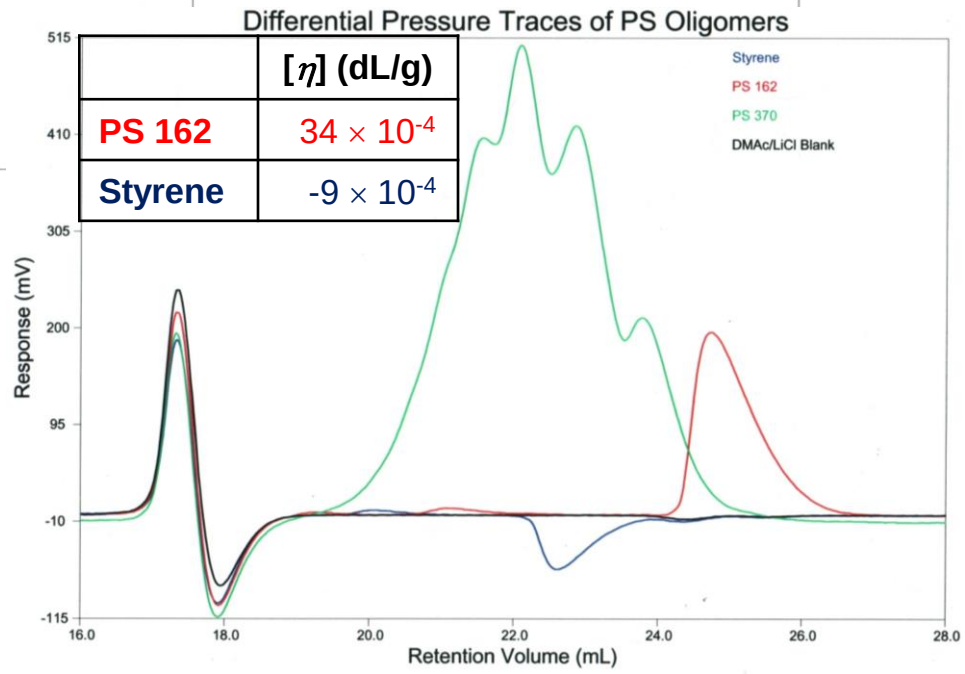
Negative Viscosities & Non-SEC Behavior



	[η] (dL/g)
PE 282	3.6×10^{-3}
PE 170	-2.0×10^{-3}



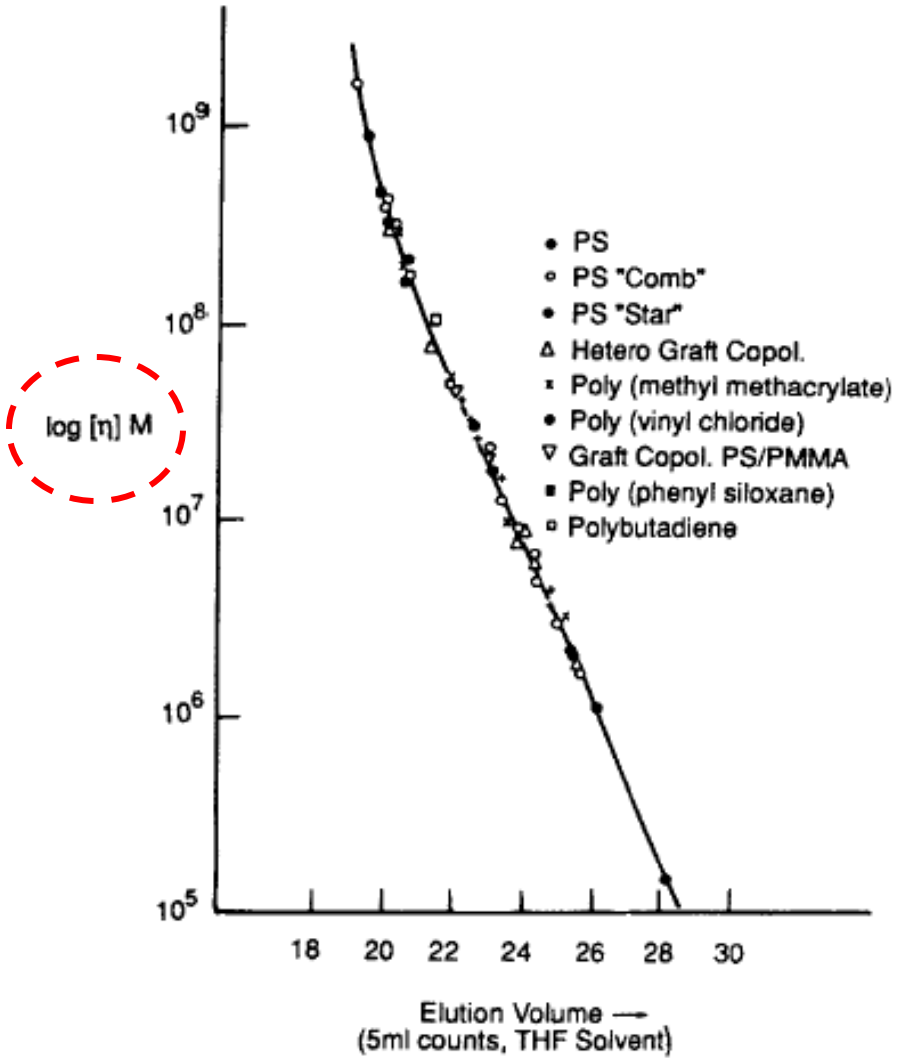
	[η] (dL/g)
PS 580	14×10^{-3}
PS 162	-1.6×10^{-3}



	[η] (dL/g)
PS 162	34×10^{-4}
Styrene	-9×10^{-4}

Alward & Striegel, *J. Liq. Chromatogr. Rel. Technol.* **2003,** 26, 157

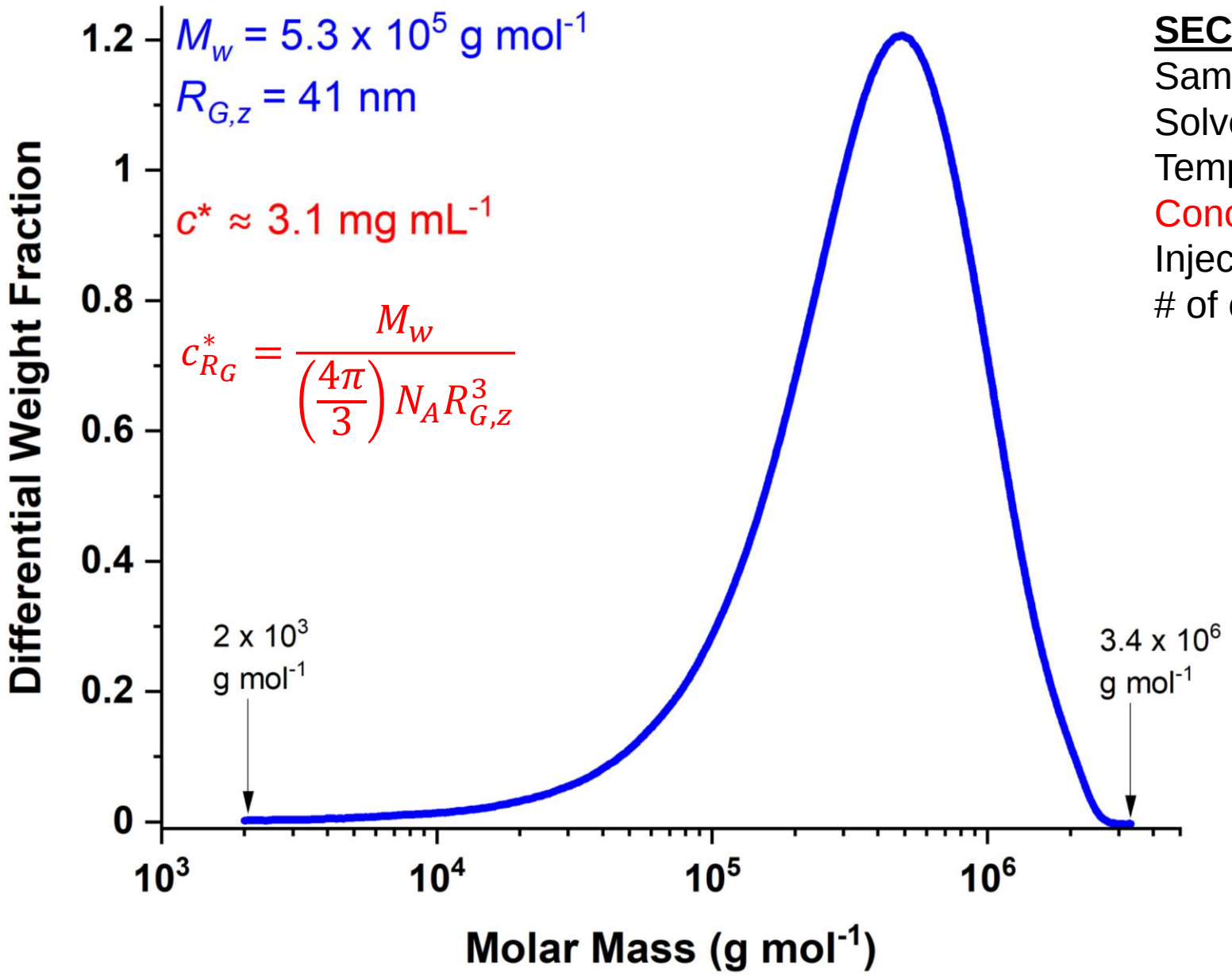
Failure of Universal Calibration



Z Grubisic, P Rempp, H Benoit, *J. Polym. Sci.: Polym. Lett.*, 5 (1967) 753

Low Response of LS & VISC Detectors

- Not as bad a problem as usually thought.
- For monomeric/oligomeric samples, we'll discuss later ("Adventures").
- For broad MMD polymers with oligomeric component, we have more latitude w/r to concentration and injection volume that typically believed.



SEC/MALS/DRI

Sample: PS

Solvent: THF

Temperature: 35 °C

Concentration: 1 mg mL^{-1}

Injection volume: 400 µL

# of columns in set: 4

Counterintuitive Solubility Behavior

Solubility (g/g solvent) of Cyclodextrins in H_2O and D_2O at 25 °C

Solute	H_2O	D_2O	Relative Difference (%)
α-Cyclodextrin	0.1295 ± 0.0007	0.0758 ± 0.0005	41
β-Cyclodextrin	0.0184 ± 0.0002	0.0108 ± 0.0001	41
γ-Cyclodextrin	0.2492 ± 0.0002	0.1988 ± 0.0006	20

Sabadini *et al., Carbohydr. Res.* **2006,** *341,* 270

ADVENTURES

Journal of Chromatography A 1719 (2024) 464750

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Online coupling of liquid chromatography and two-dimensional diffusion ordered spectroscopy for the analysis of oligostyrenes

Bastian Grabe, Wolf Hiller *

Chromatographia (2024) 87:95–104
https://doi.org/10.1007/s10337-023-04306-8

ORIGINAL

The Column, February 2024

Calibrating the Low Molar Mass Range in GPC/SEC Using Oligomer Peaks

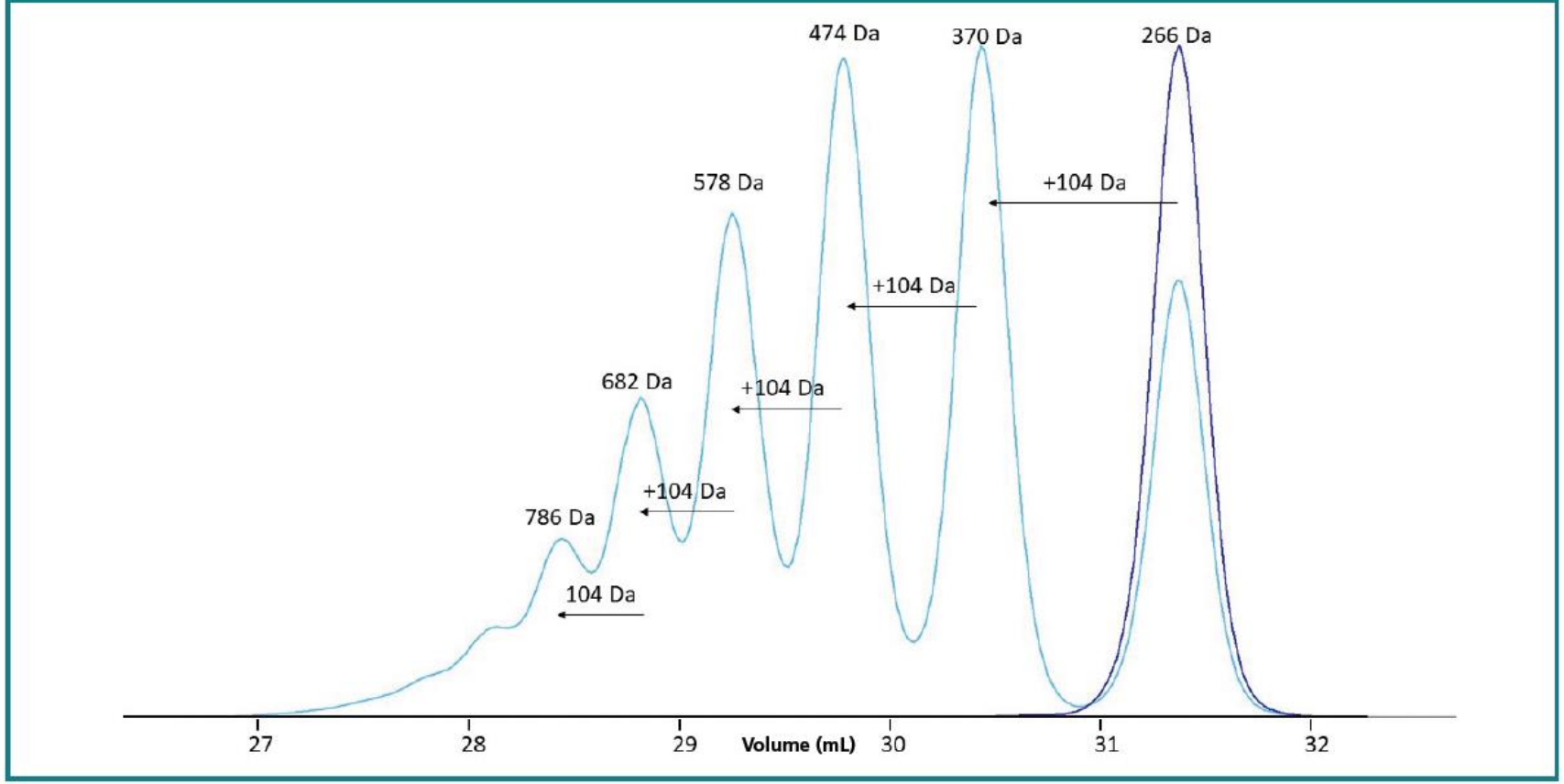
Dr. Wolfgang Radke, *PSS – Part of Agilent, Mainz, Germany*

Characterizing Styrene Monomer and Oligomers by SEC/MALS/VISC/DRI

André M. Striegel[1]

Calibrating Low-*M*-Range in SEC using Oligomers

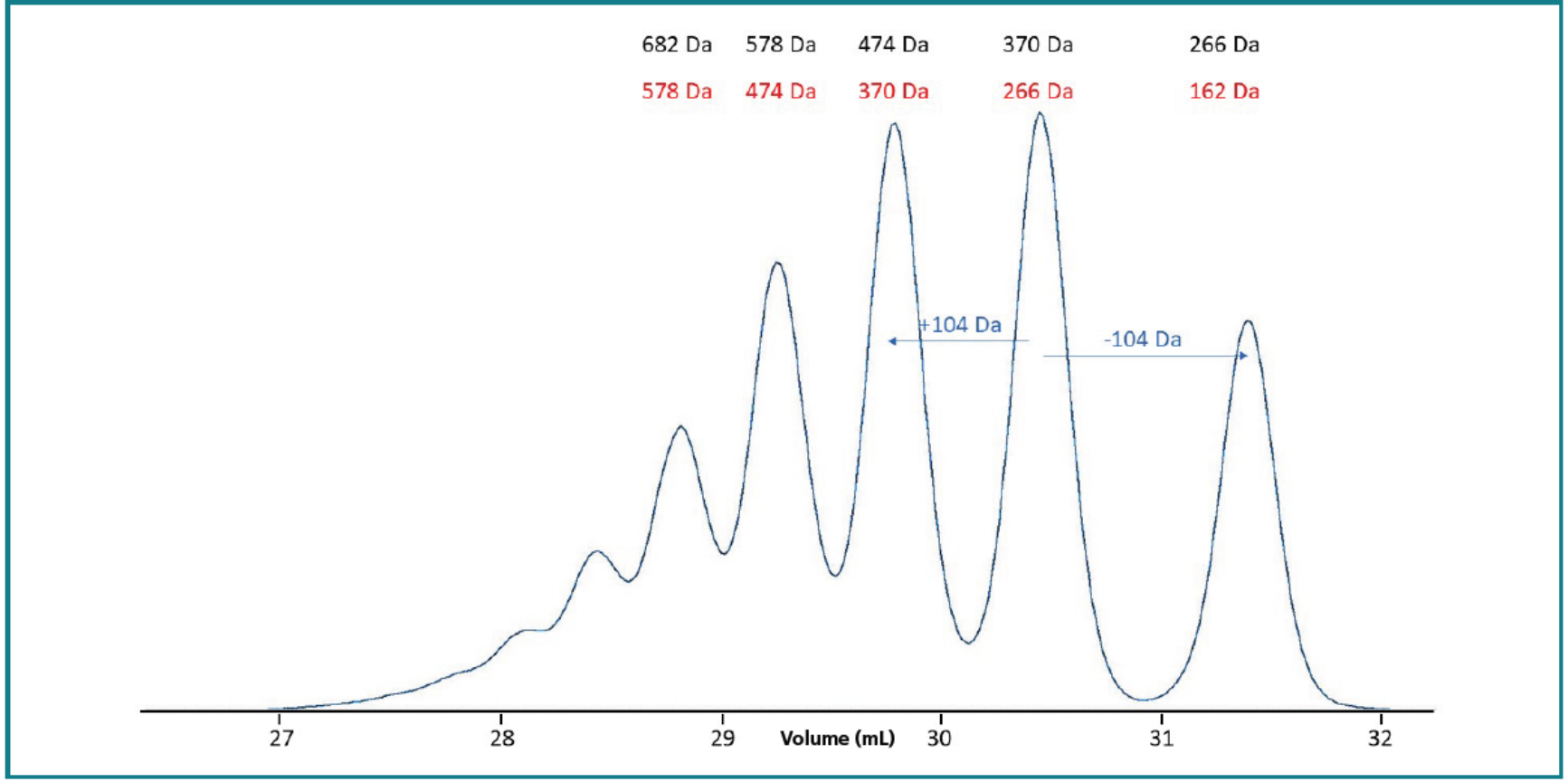
Figure 3: Chromatogram of a low molar mass polystyrene standard (light blue) overlaid with a uniform reference material of molar mass M_p = 266 Da (dark blue).



Radke, *The Column*, February 2024

Calibrating Low-*M*-Range in SEC using Oligomers

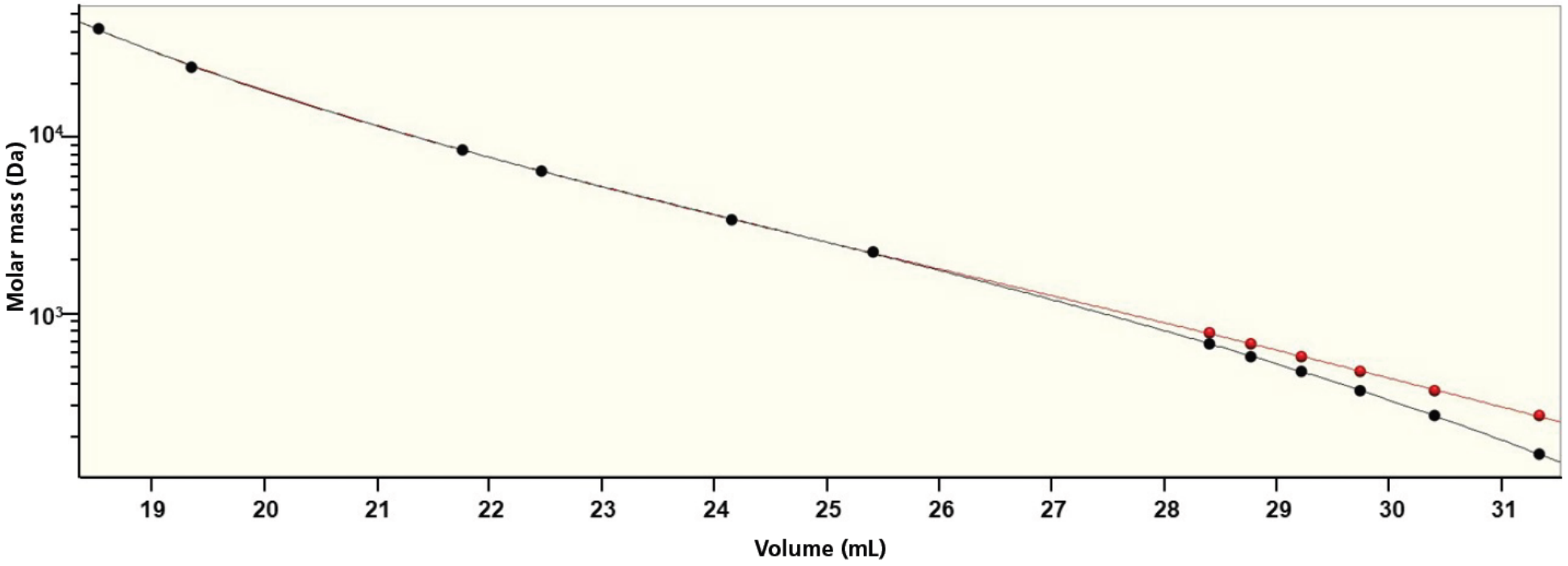
Figure 4: Chromatograms of a low molar mass polystyrene standard. Red numeration: Incorrect assignment of molar mass 370 Da to peak, resulting in M_w = 399 Da; M_n = 321 Da. Black numeration: Correct assignment of molar mass 370 Da to peak, resulting in M_w = 507 Da; M_n = 466 Da. Certified values M_w = 498 Da; M_n = 435 Da.



Radke, *The Column*, February 2024

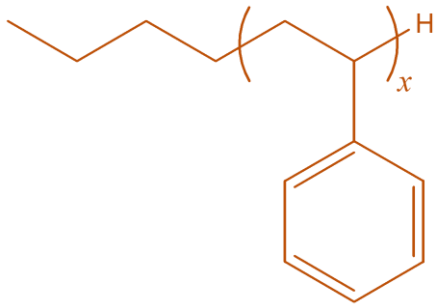
Calibrating Low-*M*-Range in SEC using Oligomers

Figure 5: Calibration curves resulting from peak assignments in Figure 4. Red calibration curve corresponds to red assignment in Figure 4, black curve corresponds to black peak molar mass assignment.



Radke, *The Column*, February 2024

SEC/MALS/VISC/DRI Characterization of Styrene Oligomers



Influence of $\partial n/\partial c$ on Calculations

$$\frac{\partial n}{\partial c} \equiv \lim_{c \to 0} \frac{n - n_0}{c}$$

DRI:

$$DRI_{resp} = k_{DRI} \times c \times \frac{\partial n}{\partial c}$$

MALS:

$$\frac{\Delta R(\theta)}{K^* c} = M_w P(\theta)[1 - 2A_2 c M_w P(\theta)]$$

$$K^* = \frac{4\pi^2 n_0^2 (\partial n/\partial c)^2}{\lambda_0^4 N_A}$$

UC:

$$[\eta] \equiv \lim_{c \to 0} \frac{\eta_{sp}}{c}$$

Specific Refractive Index Increment (*∂n/∂c*)

- ***Constant for a given macromolecule at a given set of experimental (solvent, temperature, wavelength) conditions.***
- ***Non-constant in the oligomeric region.***
- ***For oligomers, ∂n/∂c changes from mer to mer, as a result of chain end effects.***
- ***∂n/∂c of, e.g., tetramer will differ from those of trimer and pentamer. All will differ from ∂n/∂c of polymer.***
- ***Accurate determination of MMD and M averages, and of %MMD < 500 & 1000 g mol^{-1}, depends on accurate knowledge of oligomeric ∂n/∂c values.***

Characterizing PS by SEC/MALS/VISC/DRI

- ***Most SEC analyses of PS performed using THF at slightly above r.t. (e.g., 35 ^{o}C).***
- ***Most analyses conducted at 1 mL min^{-1} (except for ultra-high-M PS).***
- ***These conditions also lead to nearly optimal R_s when using S/DVB oligomeric columns.***
- ***For sake of time, will dispense with discussion of SEC optimization experiments.***
- ***Rather, let's discuss ∂n/∂c determination for n-butyl-terminated styrene monomer and oligomers.***

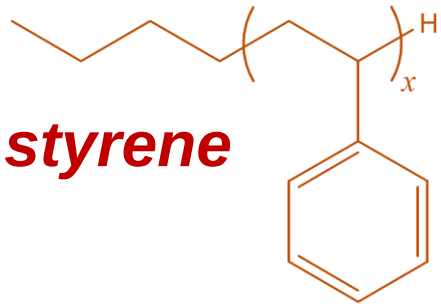


Table 3 Individual effects of flow rate and temperature on R_s: "PS 580"

Peak pair	1 mL min^{-1}		0.5 mL min^{-1}	
	35 °C	50 °C	35 °C	50 °C
266–370	1.56	1.23	1.26	1.33
370–474	1.13	0.92	0.93	0.98
474–578	1.09	0.83	0.83	0.93

With VISC detector. Numbers of peak pair components refer to molar masses of oligomers in g mol^{-1}, as per Fig. 1B. Injection volume: 100 µL. All results based on at least triplicate determinations; in all cases, standard deviations are less than ± 1 in the last significant figure

Today's Menu

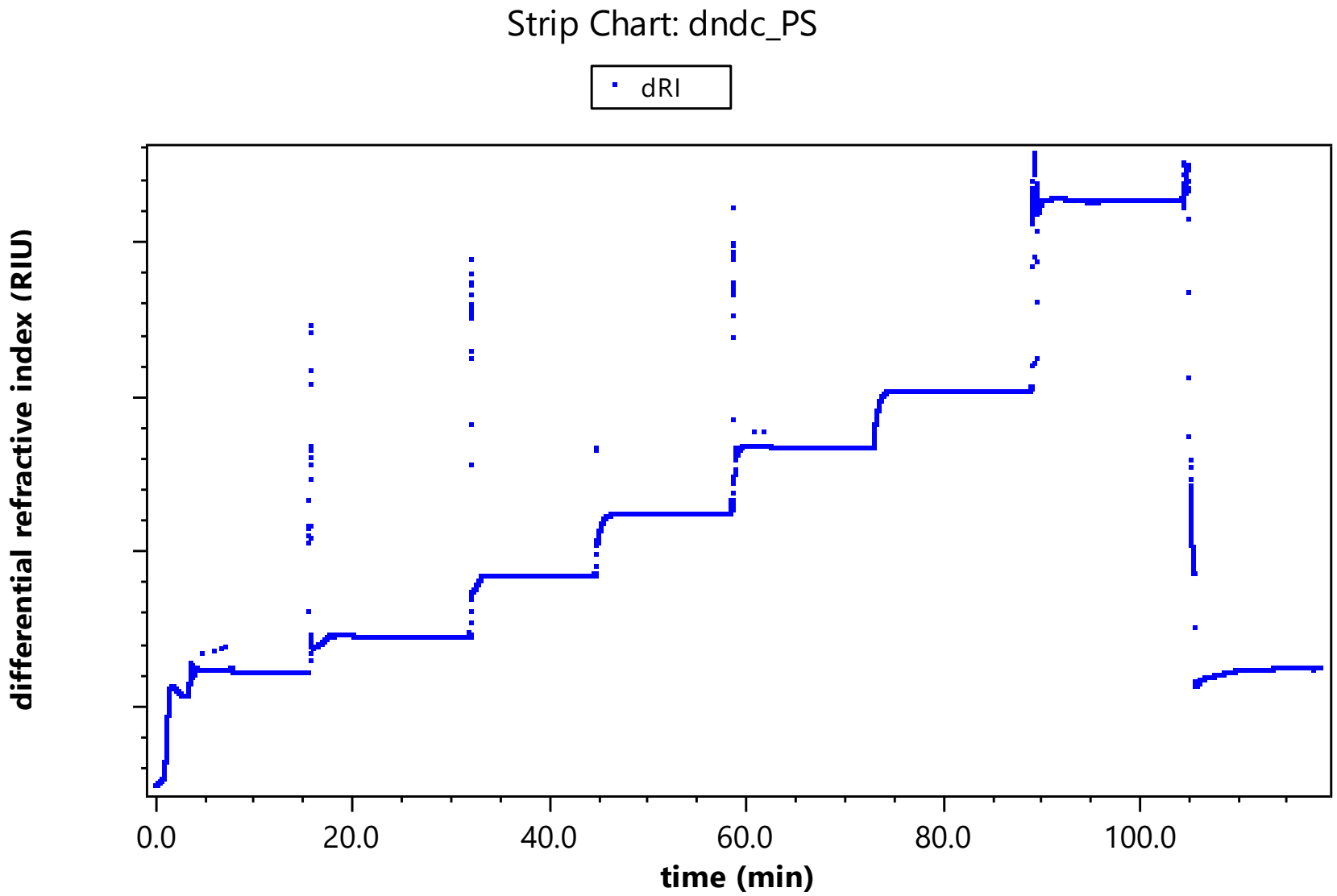
∂n/∂c Three Ways

- ***Method 1:*** ***Off-line, batch-mode DRI (only for n-butyl-terminated Styrene monomer).***

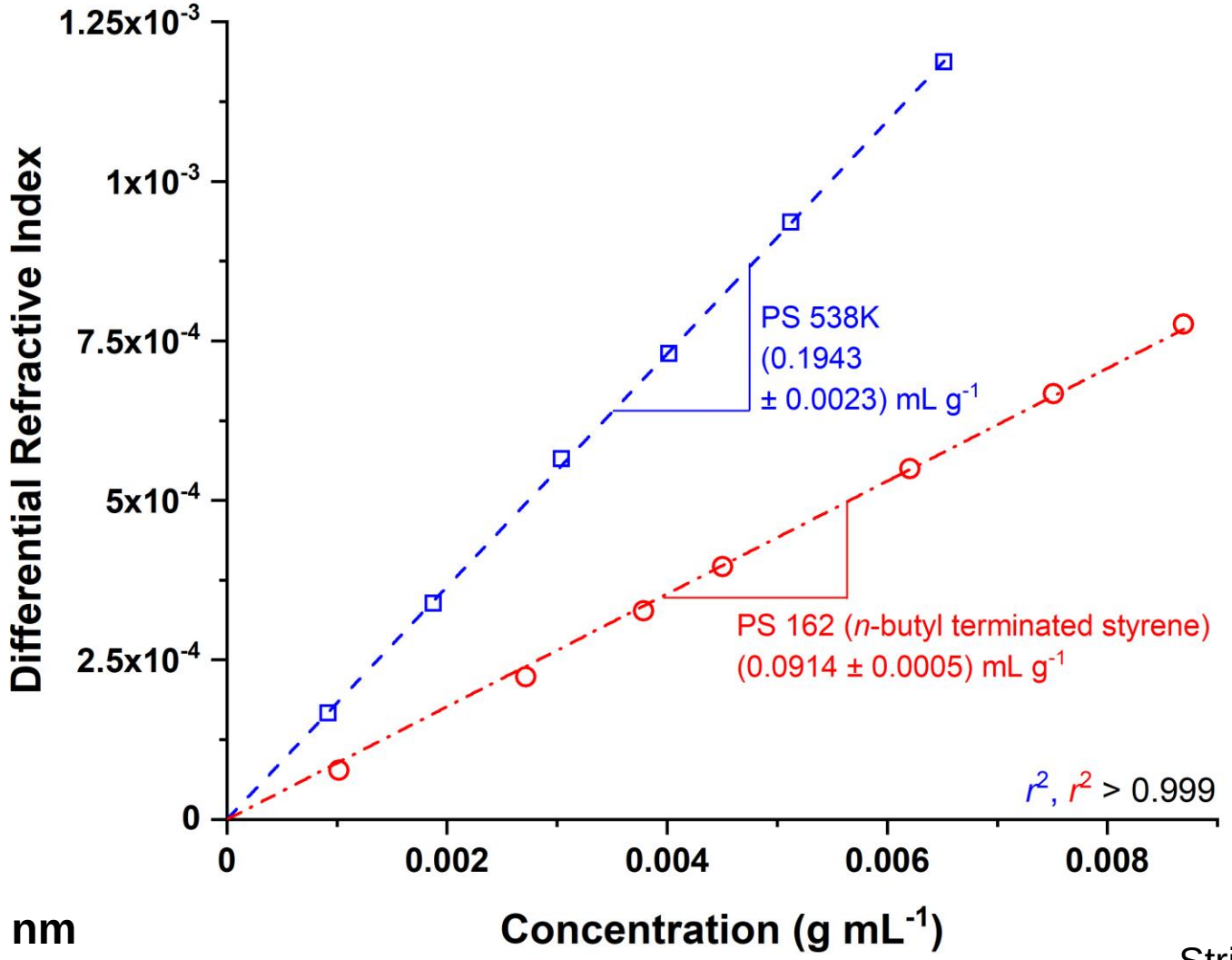
- ***Method 2:*** ***Assume 100% recovery. I.e., assume 100% of injected analyte eluted from SEC columns and is contained within integrated DRI peak.***

- ***Method 3:*** ***For each analyte (peak), vary ∂n/∂c value in software until correct (known) molar mass is obtained.***

Method 1
Determine $\partial n/\partial c$ by Off-Line, Batch-Mode DRI



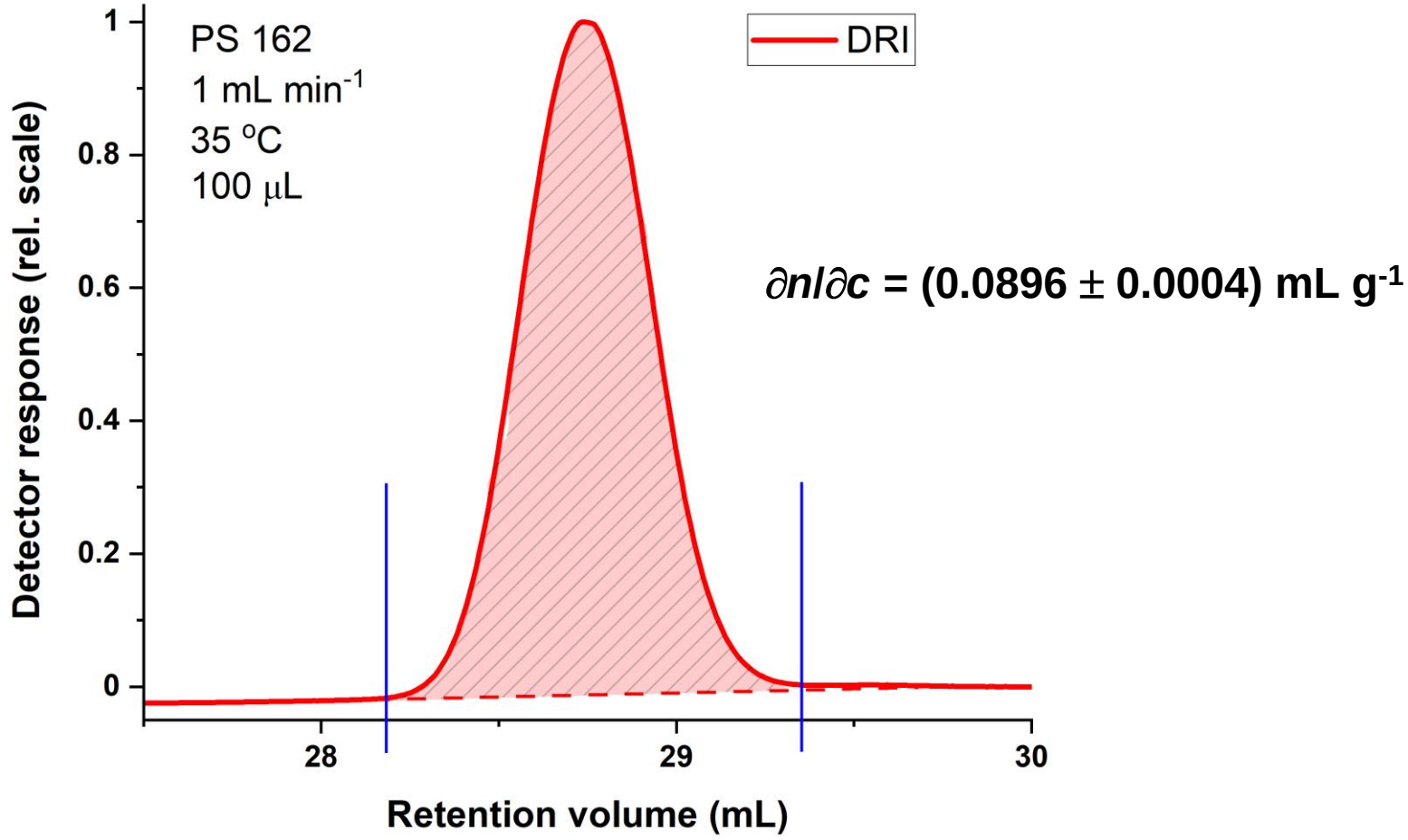
Method 1
Determine $\partial n/\partial c$ by Off-Line, Batch-Mode DRI



THF, 35 °C, λ_0 = 658 nm

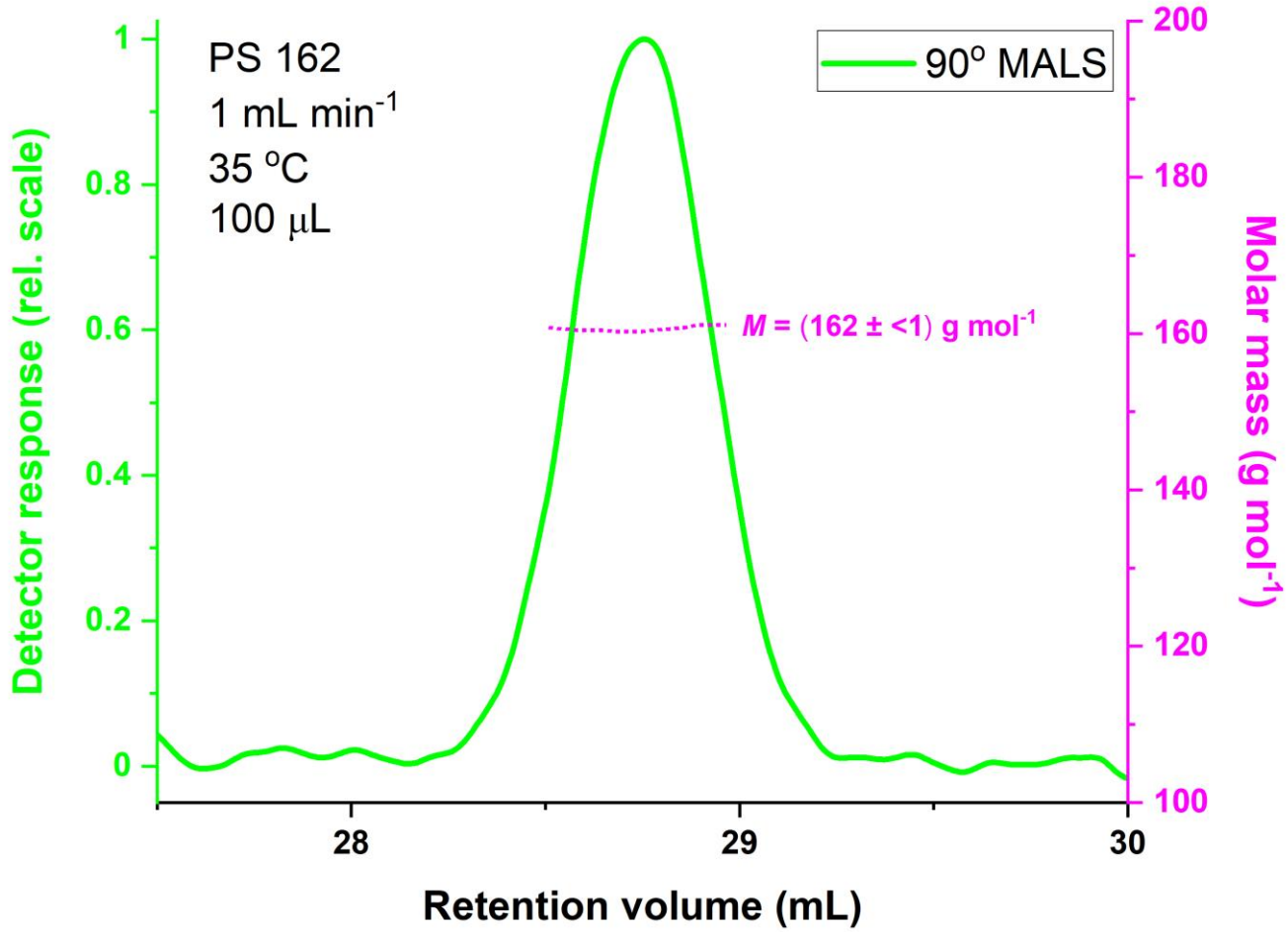
Striegel, *Chromatographia* **2024,** *87,* 95

Method 2
Determine $\partial n/\partial c$ by Assuming 100% Recovery from SEC Columns



$\partial n/\partial c$ = (0.0896 ± 0.0004) mL g^{-1}

Method 2
Determine $\partial n/\partial c$ by Changing Value in MALS Software until Correct M is Obtained



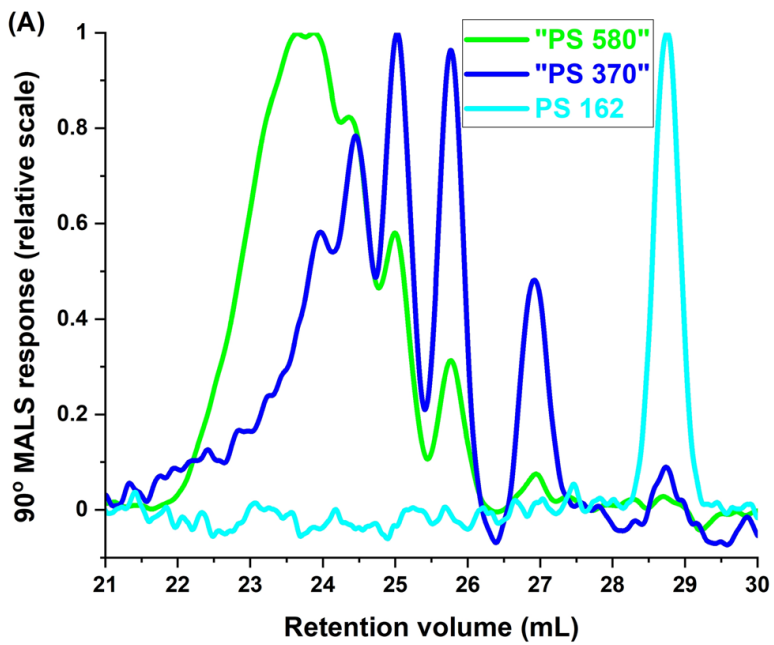
$\partial n/\partial c$ = (0.0916 ± 0.0012 mL g^{-1})

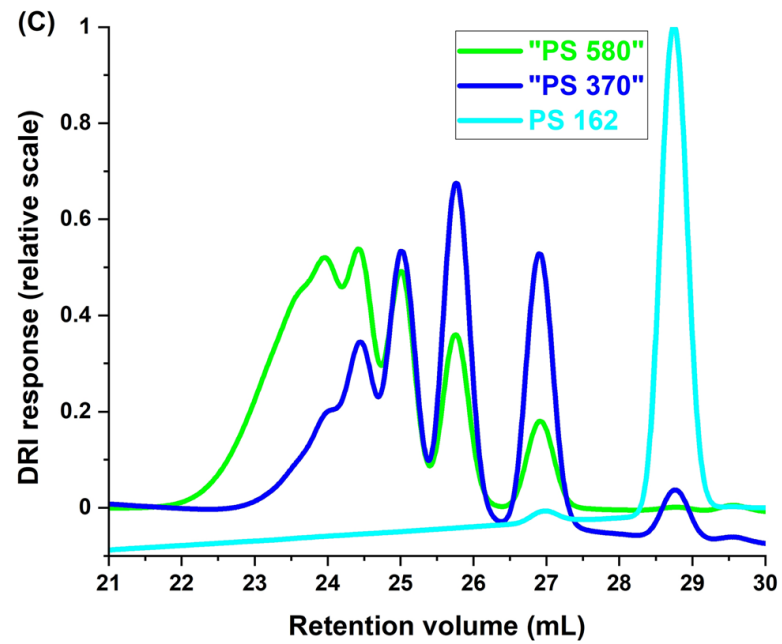
Comparison of $\partial n/\partial c$ Determination Methods

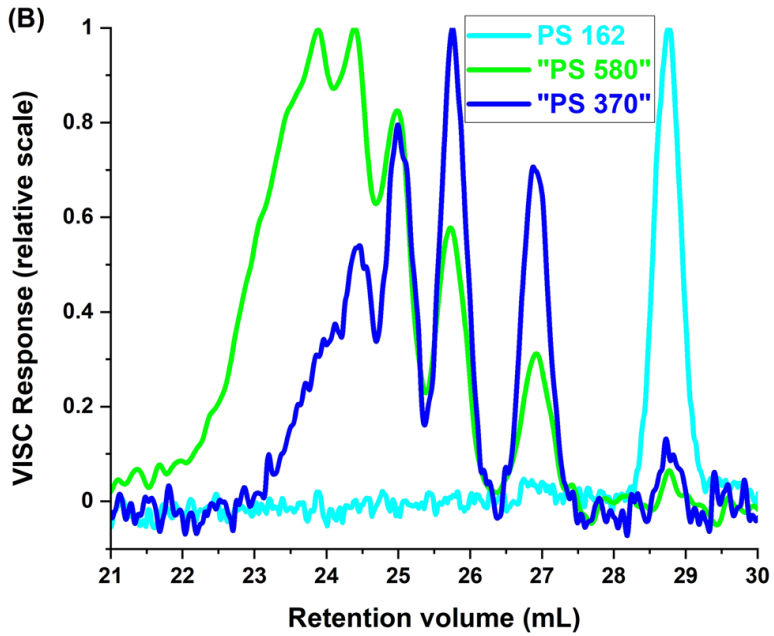
	$\partial n/\partial c$ (mL g^{-1})
Method 1 (off-line, batch-mode DRI)	0.0914 ± 0.0005
Method 2 (assume 100% recovery)	0.0896 ± 0.0004
Method 3 (adjust value to give accurate *M*)	0.0916 ± 0.0012

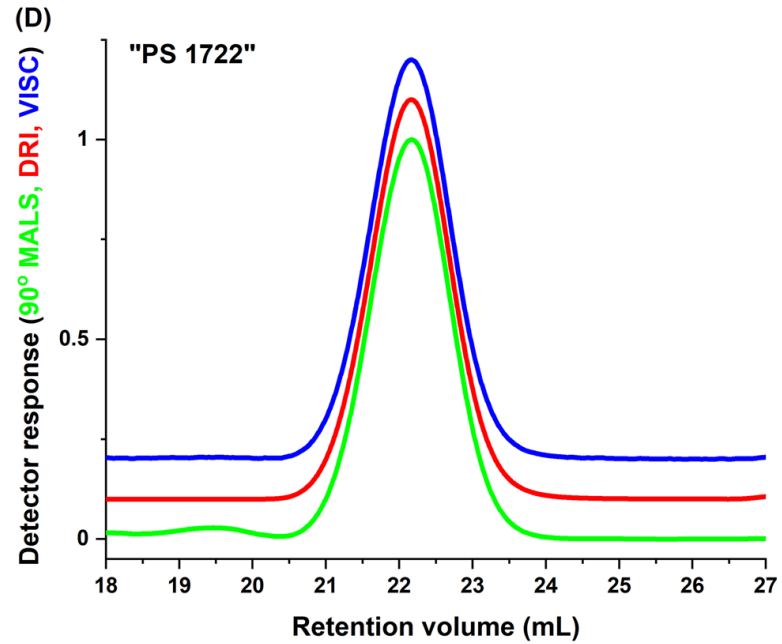
THF, 35 °C, λ_0 = 658 nm

Striegel, *Chromatographia* **2024,** *87,* 95

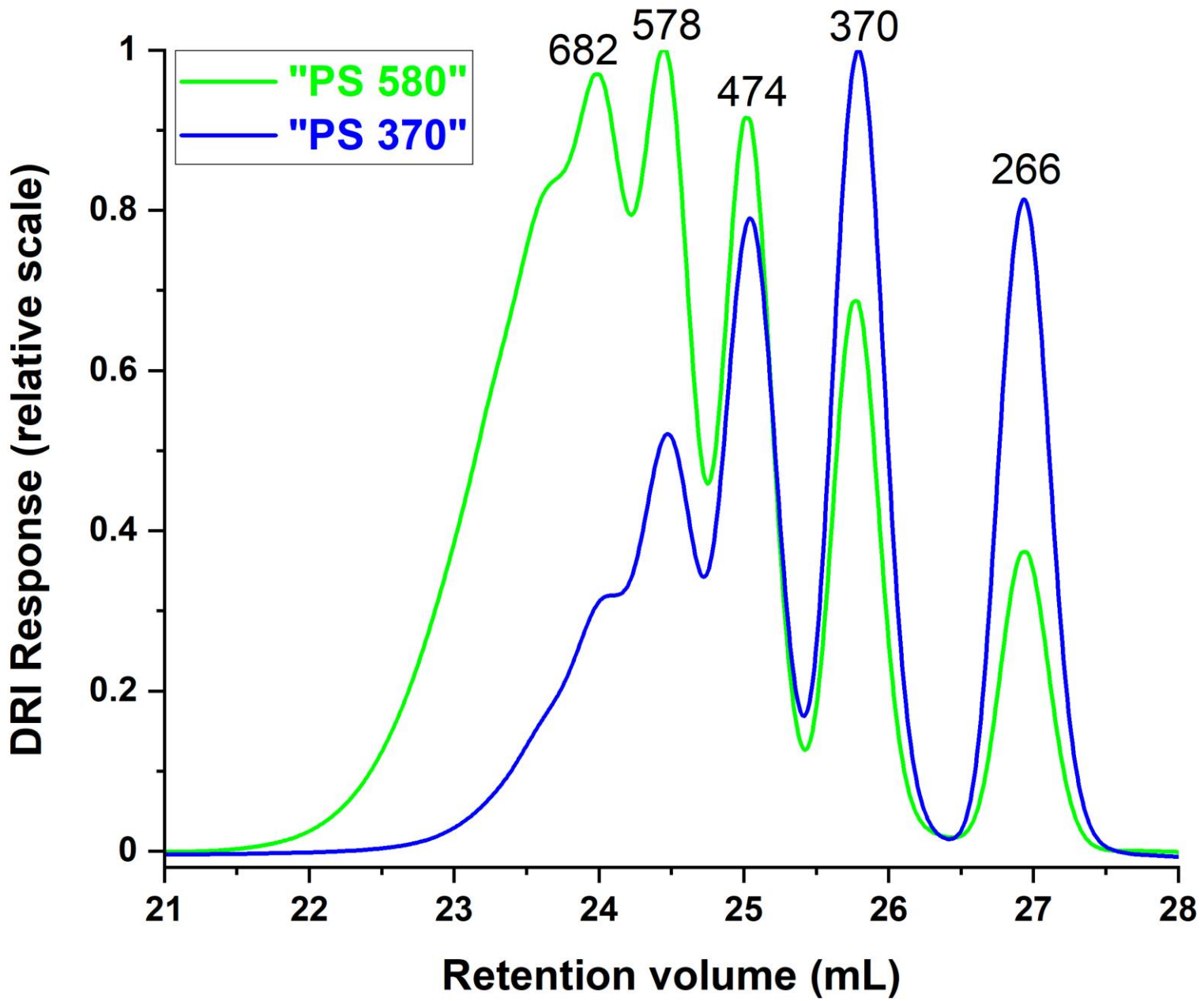




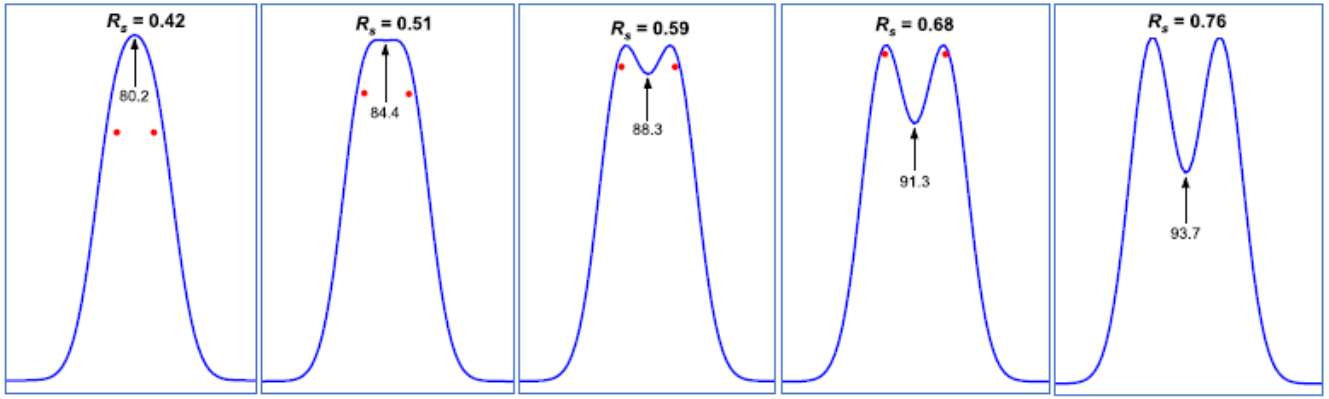


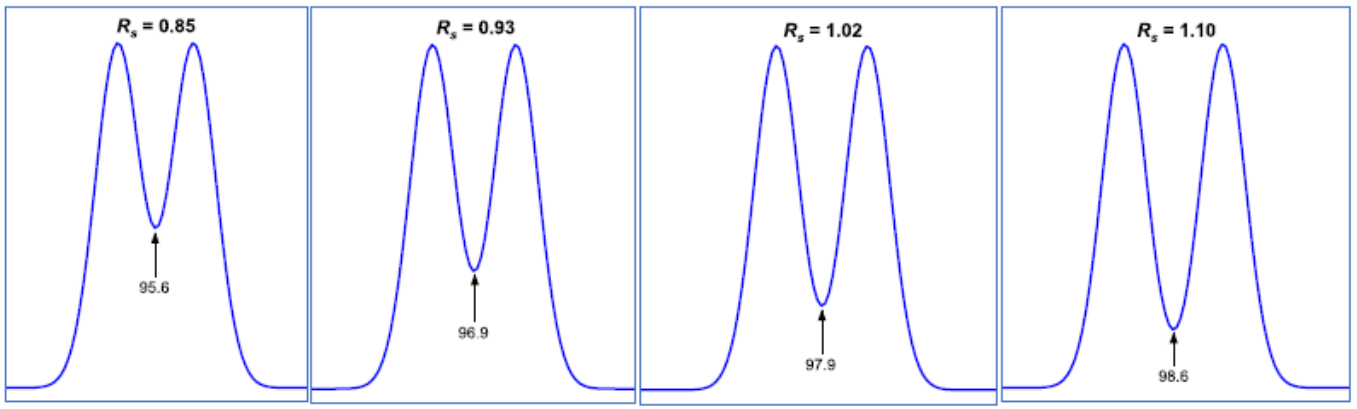


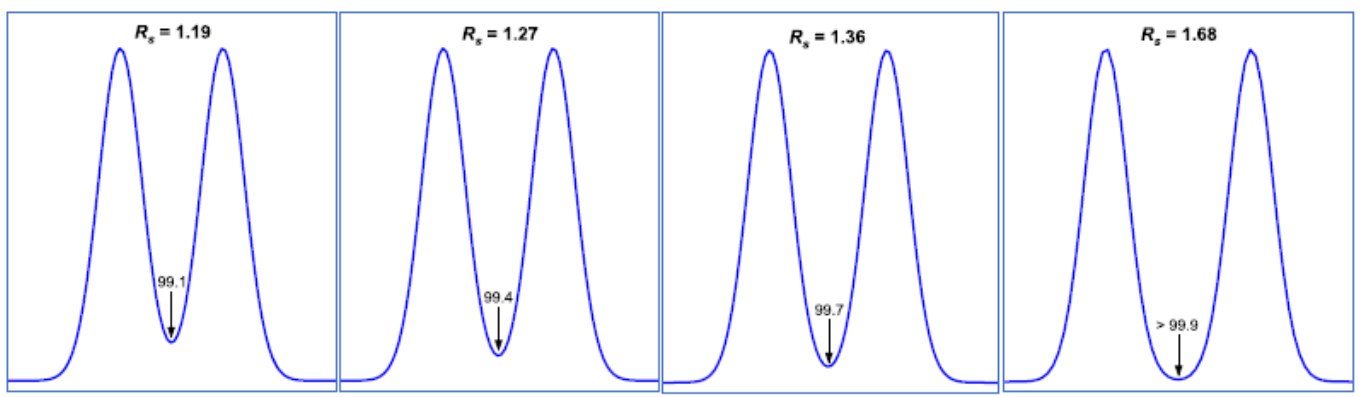
Striegel, *Chromatographia* **2024,** *87,* 95

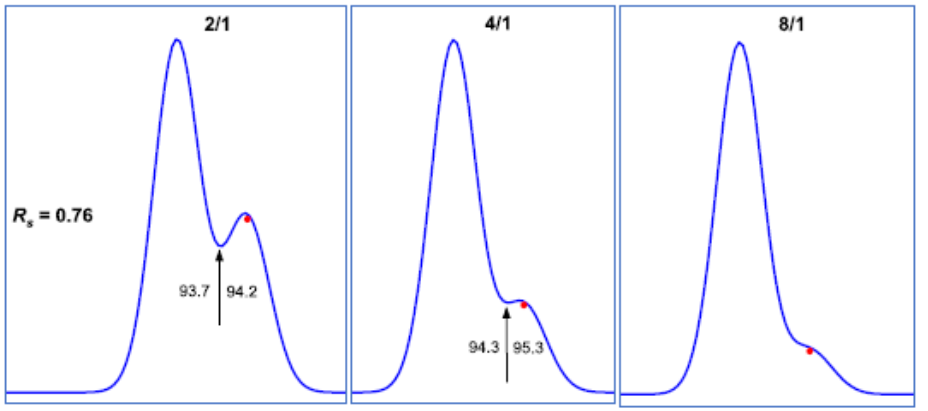


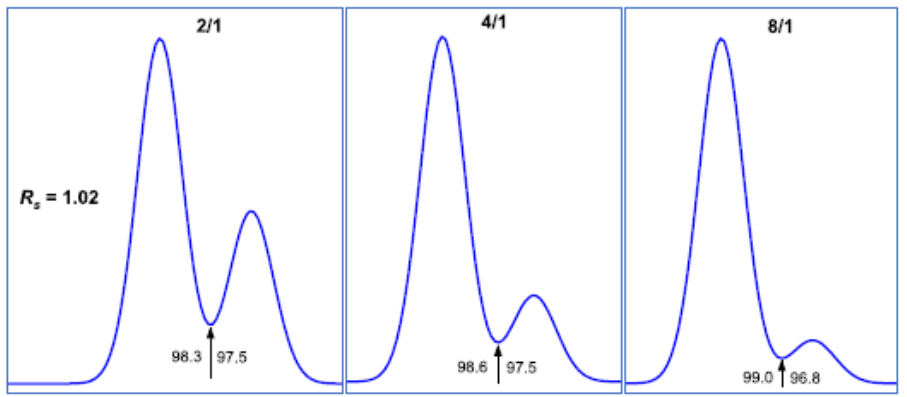
Striegel, *Chromatographia* **2024,** *87,* 95

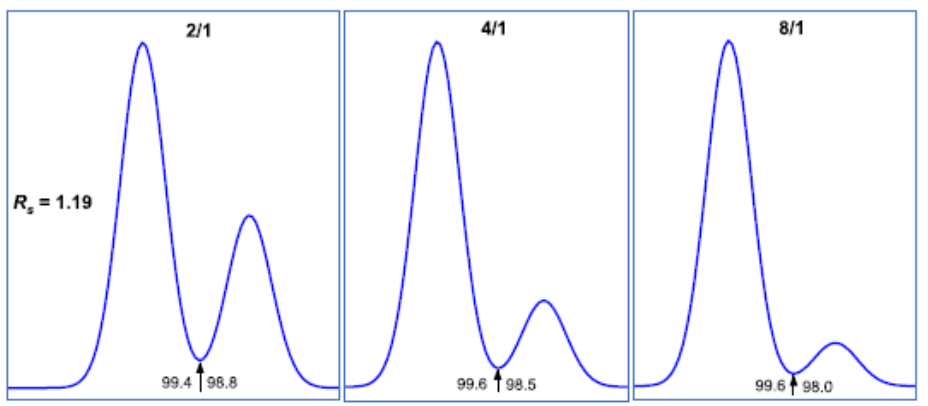




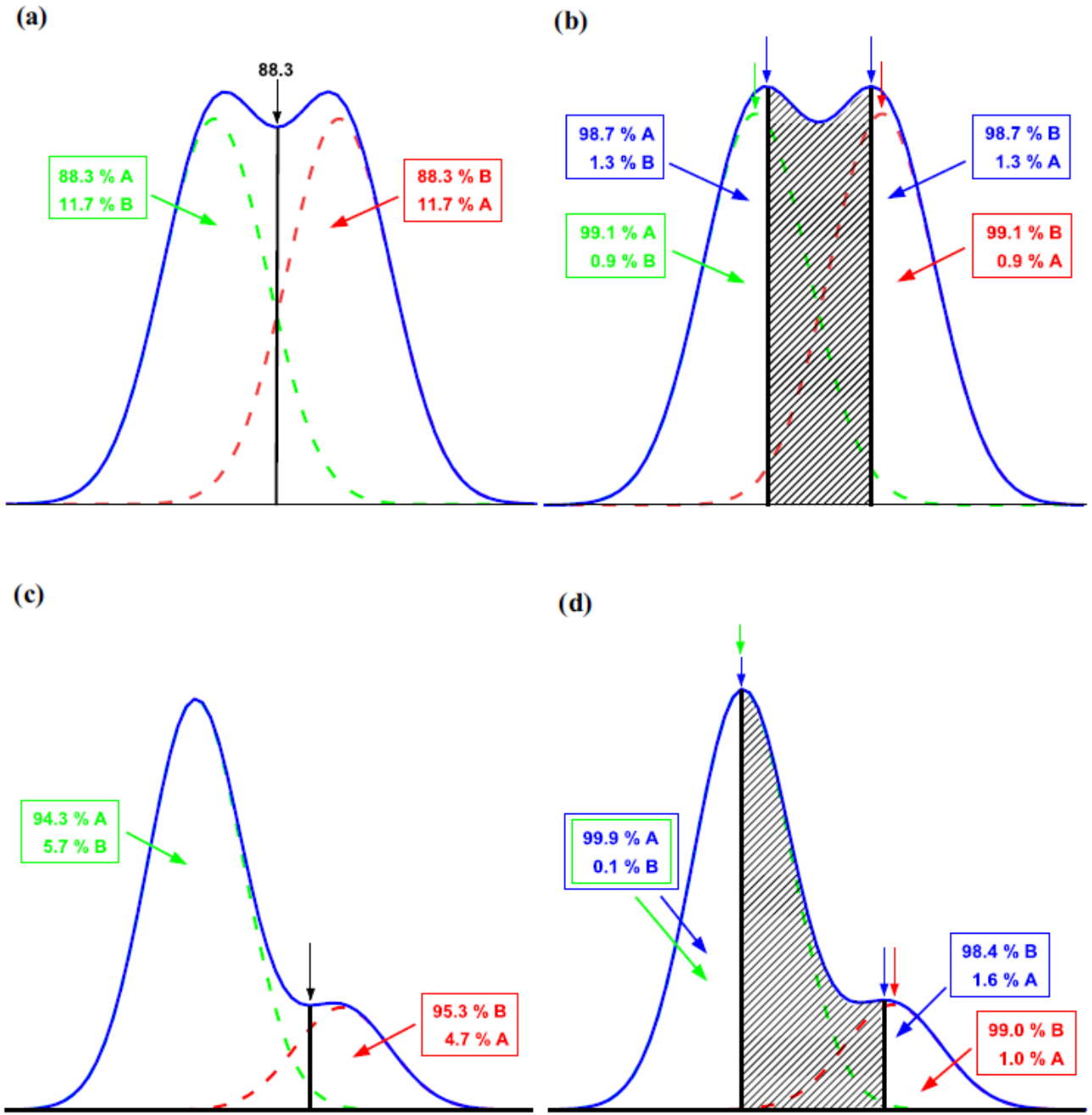




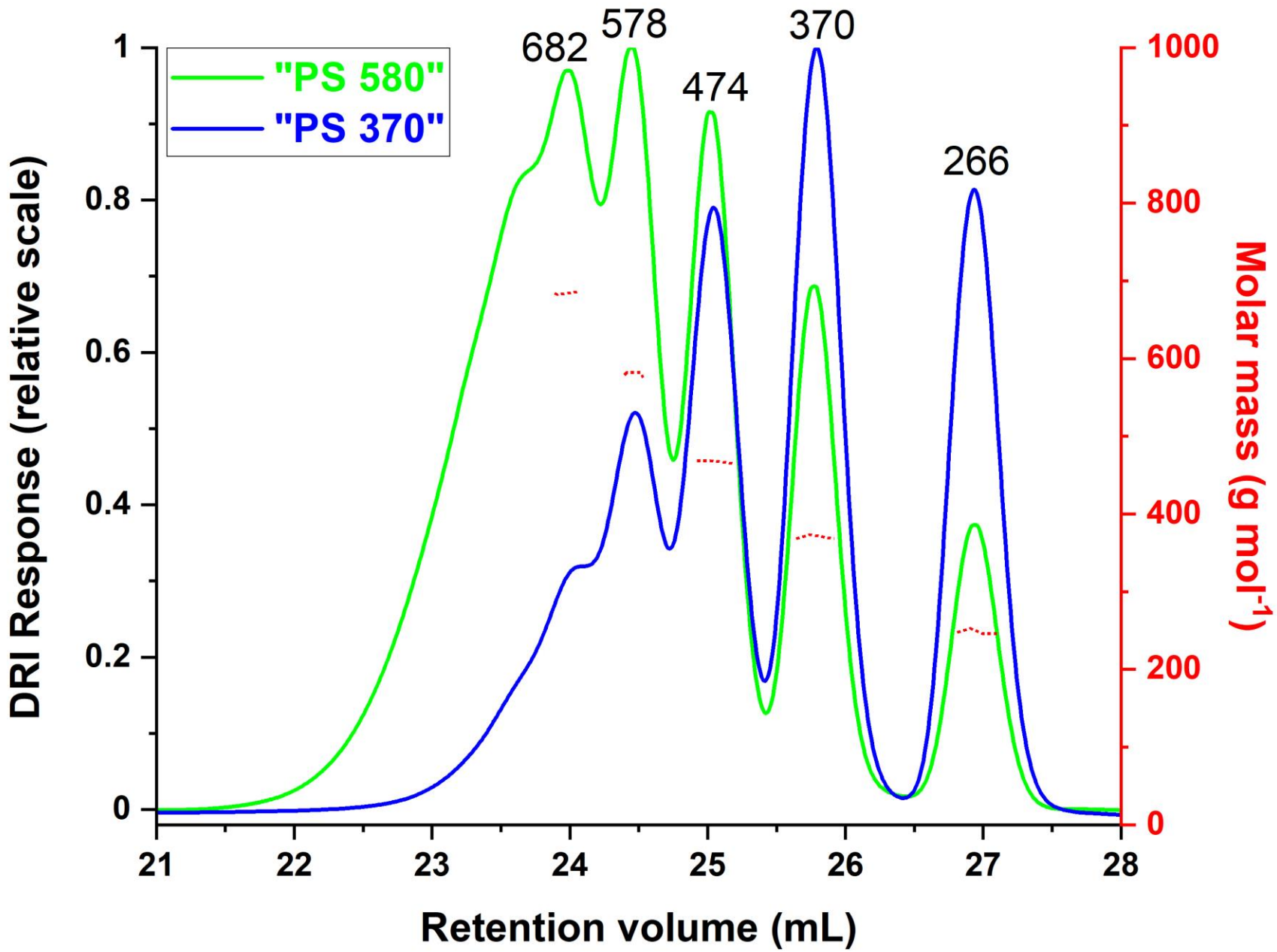




Striegel & Striegel, *Chromatographia* **2022,** *85,* 65



Striegel & Striegel, *Chromatographia* **2022,** *85,* 65


"PS 580"
"PS 370"
682
578
474
370
266
DRI Response (relative scale)
Molar mass (g mol-1)
Retention volume (mL)
0
0.2
0.4
0.6
0.8
1
0
200
400
600
800
1000
21
22
23
24
25
26
27
28

$\partial n/\partial c$ of Styrene Oligomers (THF, 35 °C, λ_0 = 658 nm)

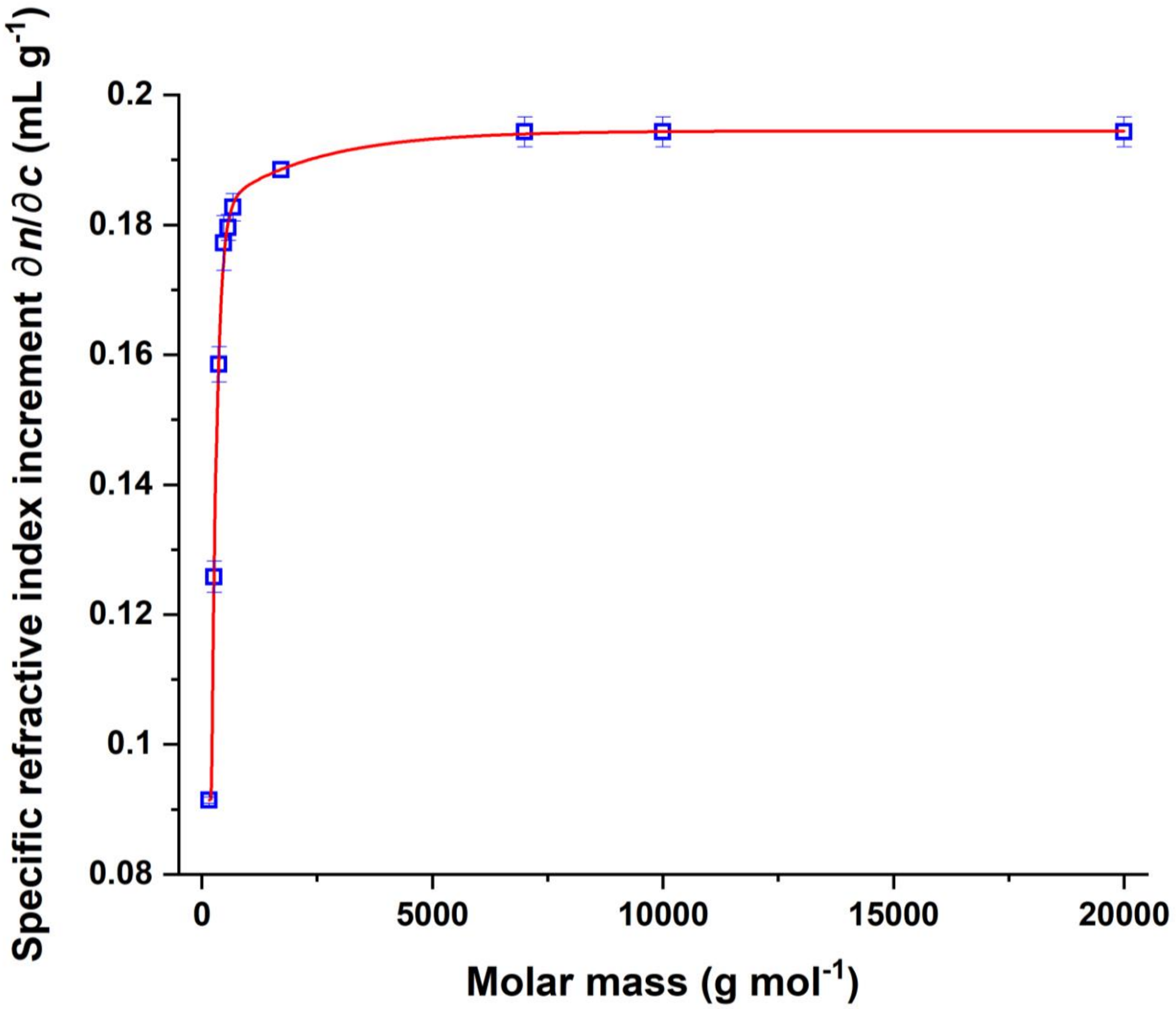


Table 4 Specific refractive index increment, intrinsic viscosity, and viscometric radius of styrene monomer and oligomers

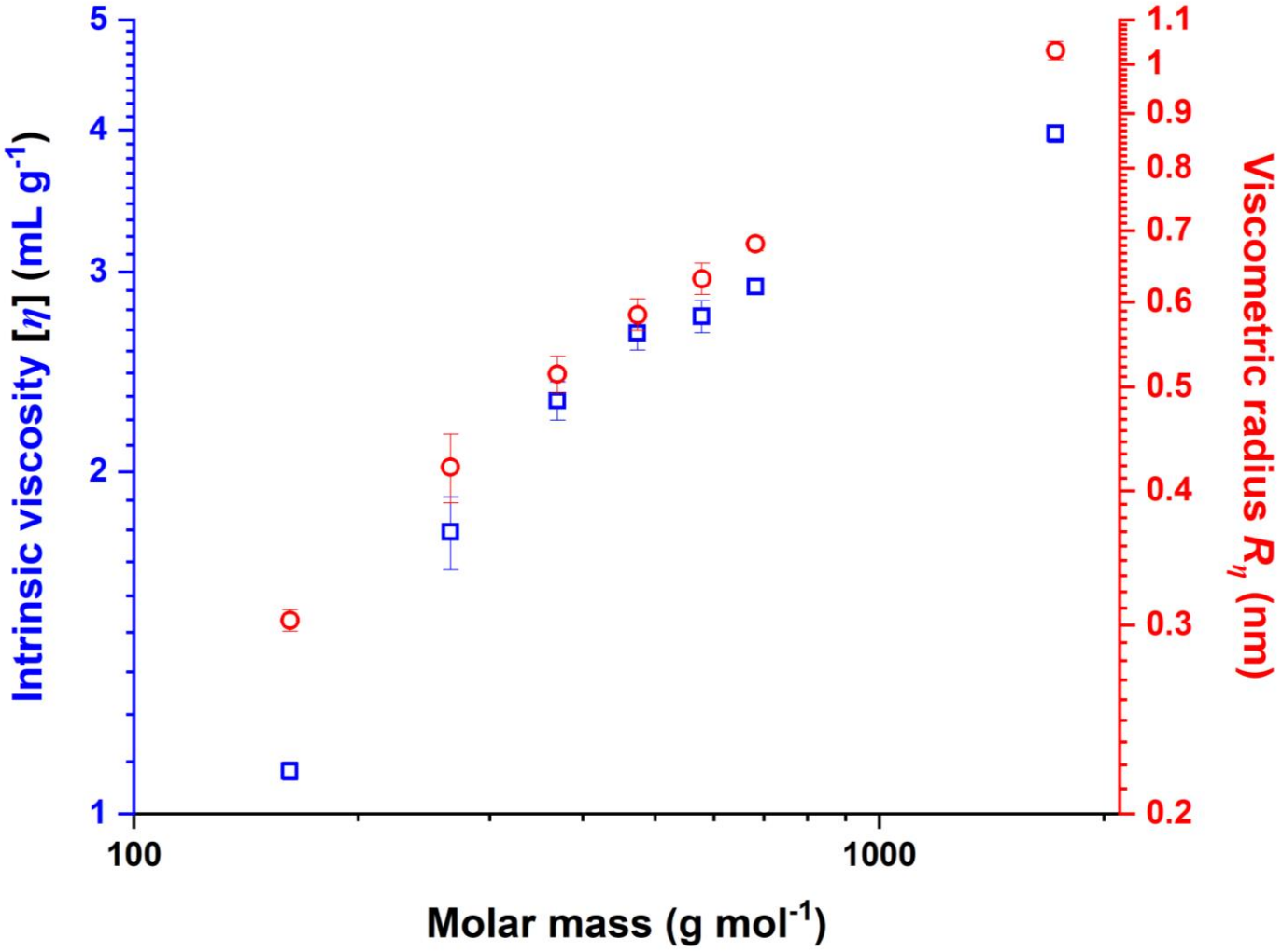
x	Molar mass (g mol^{-1})	$\partial n/\partial c$ (mL g^{-1})	$[\eta]$ (mL g^{-1})	R_η (nm)
1	162	0.0914 ± 0.0005	1.09 ± 0.02	0.303 ± 0.007
2	266	0.1258 ± 0.0024	1.77 ± 0.13	0.421 ± 0.031
3	370	0.1585 ± 0.0027	2.31 ± 0.09	0.514 ± 0.020
4	474	0.1772 ± 0.0042	2.65 ± 0.09	0.584 ± 0.020
5	578	0.1796 ± 0.0020	2.74 ± 0.09	0.631 ± 0.021
6	682	0.1827 ± 0.0021	2.91 ± 0.04	0.680 ± 0.010
16[a]	1722	0.1885 ± 0.0009	3.97 ± 0.07	1.03 ± 0.02

x corresponds to degree of polymerization, as per Scheme 1. Solvent: THF; temperature: 35 °C; λ_0 = 658 nm

[a]Likely contains some chains with $x > 16$ and $x < 16$

Striegel, *Chromatographia* **2024,** *87,* 95

Incorporating VISC
Mark-Houwink and R_η Conformation Plots



$$R_\eta \equiv \left(\frac{3[\eta]M}{10\pi N_A}\right)^{1/3}$$

Striegel, *Chromatographia* **2024,** *87,* 95

Influence of Low-*M* Fraction on SEC of Cellulose

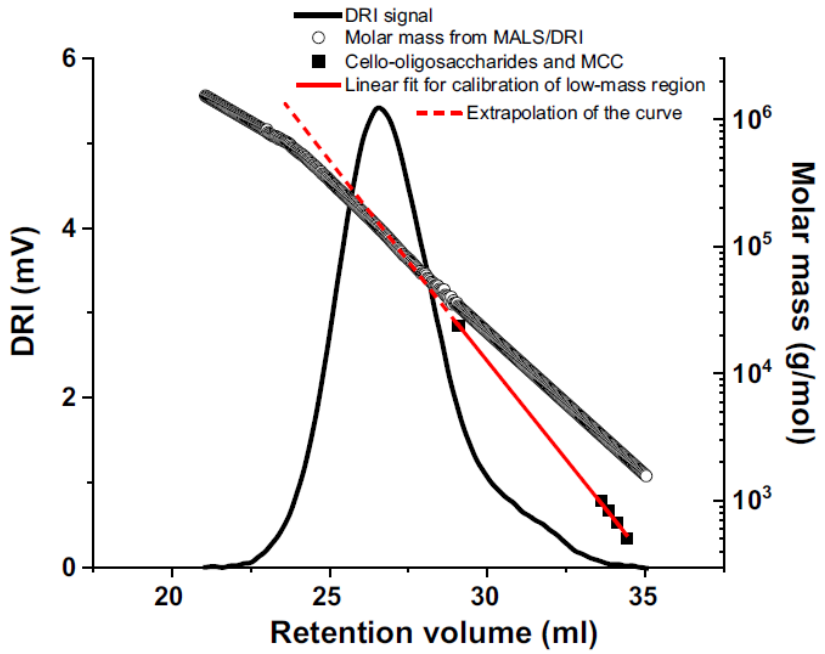


Fig. 1 DRI chromatogram and molar mass for a birch pulp sample. Open circles represent the calibration curve based on the MALS/DRI detection and red line calibration curve based on the cello-oligomers and MCC

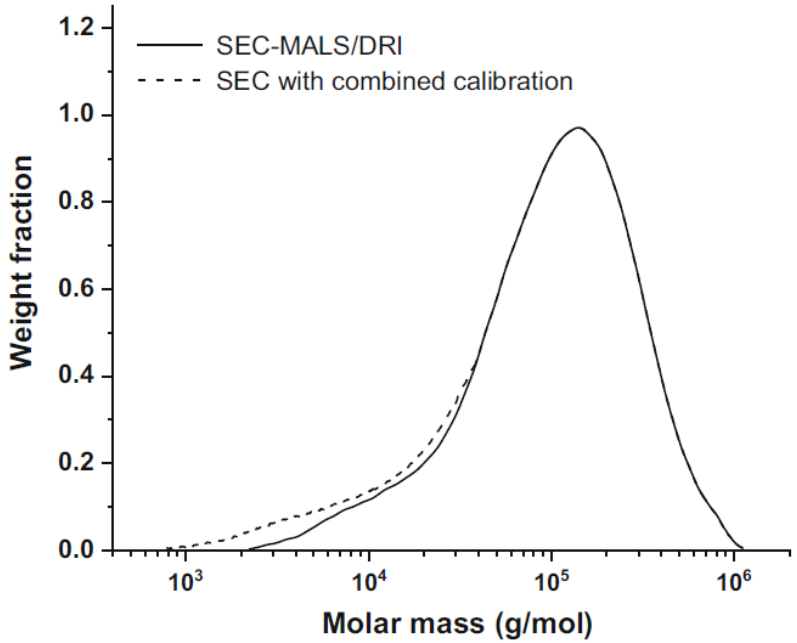
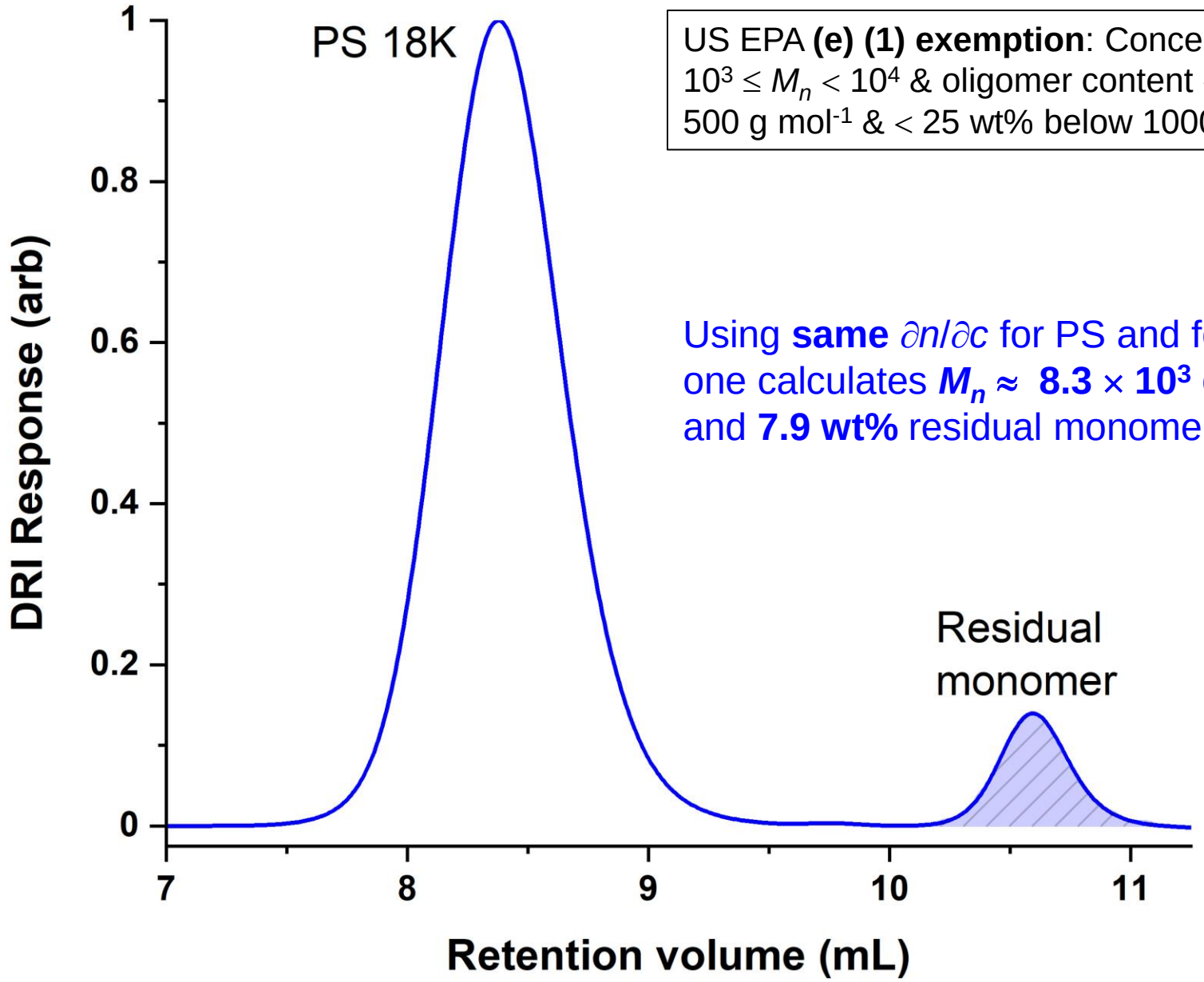


Fig. 2 Molar mass distribution of a birch pulp sample determined using MALS/DRI method and calibration where cello-oligosaccharides have been used for low-molar mass region (M $\leq \sim$ 39,000 g/mol; the point in which the two calibration lines intersect in Fig. 1)

Table 1 Molar mass averages for standard pulp sample calculated using different approaches

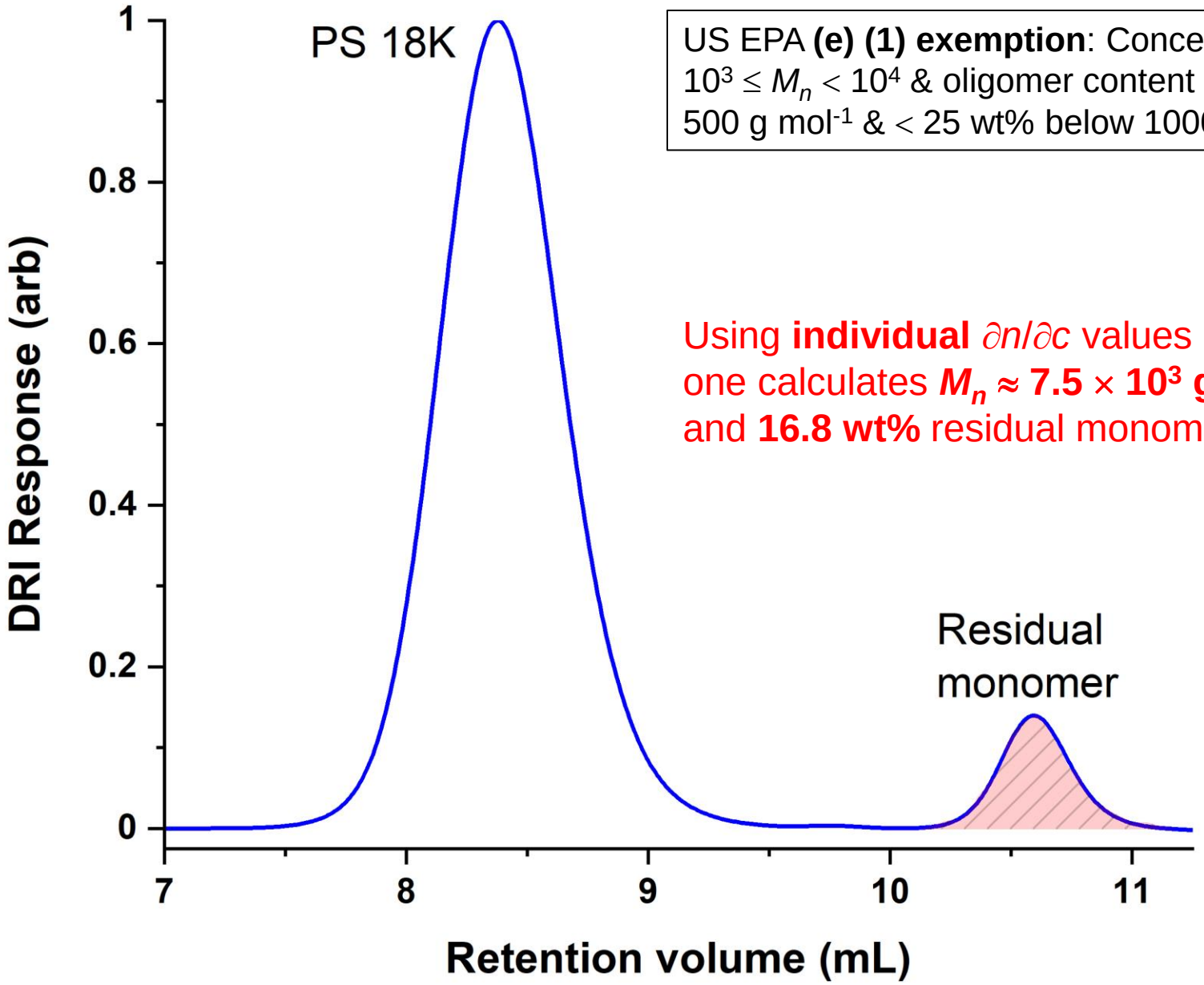
	M_w (g/mol)	M_n (g/mol)	$Đ$	DP < 100 (%)	DP > 2000 (%)
SEC-MALS/DRI	156,400	50,300	3.11	6.6	11
SEC with combined calibration	156,800	38,800	4.04	7.4	11

Pitkänen & Sixta,
Cellulose **2020,** *27,* 9217



US EPA **(e) (1) exemption**: Concerns polymers with $10^3 \leq M_n < 10^4$ & oligomer content < 10 wt% below 500 g mol^{-1} & < 25 wt% below 1000 g mol^{-1}.

Using **same** $\partial n/\partial c$ for PS and for Sty monomer, one calculates $\boldsymbol{M_n \approx 8.3 \times 10^3}$ **g mol^{-1}** and **7.9 wt%** residual monomer in sample.



US EPA **(e) (1) exemption**: Concerns polymers with $10^3 \leq M_n < 10^4$ & oligomer content < 10 wt% below 500 g mol^{-1} & < 25 wt% below 1000 g mol^{-1}.

Using **individual** $\partial n/\partial c$ values of PS and Sty monomer, one calculates $\boldsymbol{M_n \approx 7.5 \times 10^3}$ **g mol^{-1}** and **16.8 wt%** residual monomer in sample.

CONCLUSIONS

- **Analysis of oligomers, and accurate quantitation of oligomeric portion of macromolecules, is fraught with challenges.**
- **These include, but are not limited to, non-constancy of key parameters (ε, *∂nl∂c*, etc.) in oligomeric region.**
- **For *macromolecular separation science* purposes, suggest "polymer" designation for species with *M* (or DP) greater/equal to value at which property/properties of interest become constant.**
- **Specific refractive index increment *∂nl∂c* shows exponential dependence of *M* in oligomeric region. Same applies to absorptivity, density, etc.**

CONCLUSIONS

- **Accurate measurement of *∂n/∂c* of monomer and oligomers is needed for accurate determination of polymer MMDs, *M* averages, and mass percentages below 500 g mol^{-1} and 1000 g mol^{-1}.**
- **Essential for US and EU regulatory purposes, *inter alia*.**
- **Ignoring *∂n/∂c* differences between polymer and oligomers leads to large errors in calculated quantities (*M*, mass percentages, etc.).**
- **Decisions regarding oligomeric calculations should be based on *required scientific accuracy* and/or *potential regulatory liability*.**

In Memoriam

Walther Burchard
1930 – 2024

Charles Guttman
1939 – 2023