

The Challenge of Using Small Particle Packing Materials in SEC/GPC

Risks and Possibilities

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About 5 years ago, [1] the particle size of packing media in HPLC columns was reduced to 2 μm or smaller. This new column technology named U-HPLC or UPLC offers much better resolution and column efficiency compared to standard 5 μm packing materials. For SEC/GPC, the 2 μm range can also be quite interesting but very challenging as well. In contrast to HPLC, SEC mostly deals with very large molecules. The diameter of these large molecules can easily be in the range of the interstitial volume of the 2 μm particles.

Today a wide variety of porous column materials in different particle sizes are available for the separation and characterisation of macromolecules. The pore volume and the pore size distribution determine the molar mass range which can be separated, whereas the particle size determines the diffusion processes between the macromolecules on their way through the column. Smaller particles lead to narrower peaks resulting in a better column efficiency, separation and finally resolution. Column efficiency, as determined by the theoretical plate height, depends on the particle size (a power law $\sim d_p^2$) [2a] and the diffusion coefficient (linear reciprocal function $1/D_m$). Because of this, the particle size is very important as polymers diffuse slowly through a column [3]. A totally porous material with a small particle size seems to be the most favourable column material for SEC.

However reducing the particle size into the 2 μm range increases the risk of shear degradation [11].

Shear degradation is defined as a breakage or degradation of a polymer chain based on an external force or friction. If this external force is applied to a polymer chain in solution, the polymer chain can break into smaller pieces. Shear degradation lowers the molar mass of a polymer chain significantly. The molar mass average values such as M_w and M_n will be smaller and the polydispersity index [PDI] will increase. Shear degradation is a function of the solvent viscosity, the flow rate, the particle size and the temperature. In

this article we will discuss the shear degradation as a function of the flow rate, the particle size and the solvent viscosity.

The risk of shear degradation

Reducing the particle size to the sub-micron range, as in UPLC, causes some difficulties for the SEC separation mechanism. In SEC, polymers can easily have molar masses between 1000 Da and 10 Million Da. The size of a polymer, as determined by the radius of gyration depends on the molar mass, the polymer architecture, the polymer structure in solution and the solubility in the solvent. This can range from a few nm up to 300 nm and even larger.

The interstitial volume for a spherical particle is roughly 1/6 of the particle diameter [2c]. This means that a 3 μm particle has an average interstitial volume of 500 nm. According to Striegel et.al. [2c] the interstitial volume should be at least 3 times larger than the hydrodynamic volume of a polymer [2c]. If not, shear degradation, as a function of the particle size and polymer size, can take place. The applied shear force on the polymer also depends on the solvent viscosity and the flow rate. The larger the applied shear force, the greater is the risk that a polymer chain will break and be degraded.

Particle size

Reducing the particle size minimises the peak broadening and optimises the diffusion

processes for a sample molecule ([2b]). Smaller particles have a higher packing density resulting in more particles per unit volume, a smaller interstitial volume and therefore a smaller distance for a sample molecule to diffuse between the mobile and the stationary phase. Both, stagnant mobile phase and non-stagnant mobile phase mass transfer are much faster in much smaller particles [2a].

Polymer size scales with the molar mass as does the hydrodynamic volume. Due to the increase of hydrodynamic volume as a function of the molar mass, a dimensional comparison of the pores, the interstitial volume and the size of the polymer is necessary. The diffusion coefficient is also a function of the size of the molecule and thus - so is the equilibrium condition for the diffusion processes. The diffusion coefficient under equilibrium conditions has to be considered as a function of the flow rate and the particle size [2c].

Transport phenomena, sometimes called slalom chromatography, and mass transportation for polymers as a function of molar mass, also influence the elution profile of the polymers. Due to these phenomena, apparent shear degradation is often discussed in the literature [2,4-6]. This is hard to distinguish from real shear degradation.

What are the risks and potentials for SEC, if the particle size is in the 2 μm range and the polymer size in solution, the hydrodynamic volume, is therefore no longer negligible compared to the interstitial volume? How are the diffusion processes for large

macromolecules influenced and when does shear degradation [2a] take place?

In this paper the general advantage of the use of small particles is shown by comparison of 5 μm columns and 10 μm columns in aqueous solutions. More importantly, proof is given that large molecules show no shear degradation behaviour even for column particles in the low-micron range. In order to prove this, SEC-MALS (Size-Exclusion Chromatography - Multi angle Light scattering) studies for large polymers (polystyrene in THF) as a function of particle size and flow rate are presented. The authors present molar mass data for high molar mass polystyrenes as a function of particle size and flow rate. MALS studies allow the determination of the absolute molar mass of a polymer, independent of any flow rate or diffusion processes. The molar mass data for three different polymers are measured. The results are discussed with respect to calculations of the size of the interstitial volume for porous particles and the estimation of the diffusion coefficient as a function of the polymer molar mass.

Experimental

Pore size distribution, particle size and molar mass

Dimensional considerations

In order to discuss the phenomenon of shear degradation as a function of particle size and the porosity of the particle, some general considerations should be made. In Table 1 calculations about molar masses and corresponding dimensions for polymers, porosities, particle sizes and interstitial volume are summarised. The calculations are

Table 1. Dimensional information about polymers, particles and porosities; Diameter based on the calculation of Ref [7].

Particle size [μm] and back pressure [bar] [1 mL/min, Column: 8mm x 150 mm]	Max diameter [B] of the interstitial volume of a porous particle based on a spherical calculation [nm]	Diameter [C] of the interstitial volume of a non-porous particle based on a spherical calculation [nm]	molar mass ²⁾ of a polymer corresponding to the non-porous particle diameter [C] [g/mol]
1 μm 100 bar	167 ¹⁾	154	3,000,000
3 μm 33 bar	500 ¹⁾	460	22,000,000
5 μm 20 bar	833 ¹⁾	780	50,000,000
10 μm 10 bar	1667 ¹⁾	1540	180,000,000

1) Diameter for the spherical interstitial volume for particles with a large porosity and $d = 2 * dp / 3 * (\epsilon / (1 - \epsilon))$
 d = diameter of the channels in the interstitial volume, dp = diameter of the particle, $\epsilon = 0.2$ [9,11]

2) Calculation for Polystyrene in THF at RT based on the calculation $V_h = [\eta] * M$, $[\eta] = K * M^\alpha$ [intrinsic viscosity, K, α = Mark-Houwink parameter; $K = 1,363 \times 10^{-2}$ [ml/g], $\alpha = 0,714$]

based on the trigonometric concept of the equilateral triangle [7]. Based on this geometrical approximation (see Figure 1) the dimensions of the interstitial volume for the particles are calculated.

The hydrodynamic volume and corresponding diameter is a function of the molar mass, assuming that the polymer remains as a statistical coil (no coil deformation) in solution, in a good solvent. The molar mass of a polymer, corresponding to the diameter of the spherical non-porous interstitial volume is shown in the last column of Table 1. This is the largest possible molar mass that can pass through the column based on a non-porous particle (diameter C in Table 1). If the particle is porous, the interstitial volume diameter increases due to the porosity and the fractal dimension of the particle (diameter B in Table 1).

Figure 1) illustrates how the interstitial volume can be calculated. Based on the

given equation to describe the center of a triangle, and the radius of sphere, r_{iv} , of the inner blue circle can be calculated as

$$r_{iv} = r_u - r_p$$

(Eq. 1)

The radius, r_p , for a particle of a diameter of 1 μm is 0.5 μm . The distance, r_u , describes the distance from the corner of an isosceles triangle to the center of the triangle and is given as:

$$r_u = \left(\frac{a}{3}\right) * \sqrt{3}$$

(Eq. 2)

For a 1 μm particle based with $a = 1\mu\text{m}$ (see Figure 1) and Equation 1 and 2, the calculated value for the interstitial radius r_{iv} is

$$r_{iv} = r_u - r_p = 0,5773\mu\text{m} - 0,5\mu\text{m} = 0,0773\mu\text{m}$$

This corresponds to an interstitial diameter of 154nm as shown in Table 1.

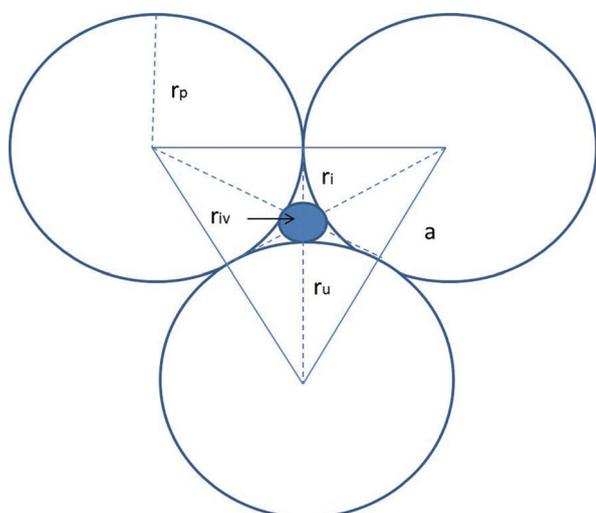


Figure 1a) interstitial volume (blue circle) for spherical particles, calculated based on the shown trigonometric ratios.

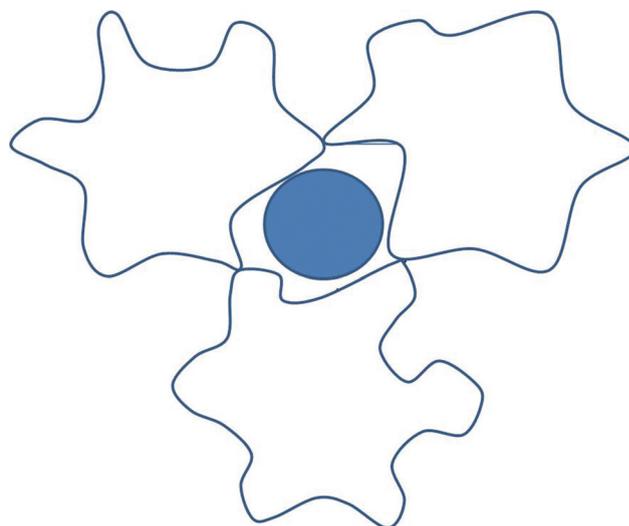


Figure 1b) Illustrated increased interstitial volume (blue circle) for porous particles of the same size as in Fig. 1a); Illustration based on the assumption, that the average pore size is 1/4 of the particle radius.

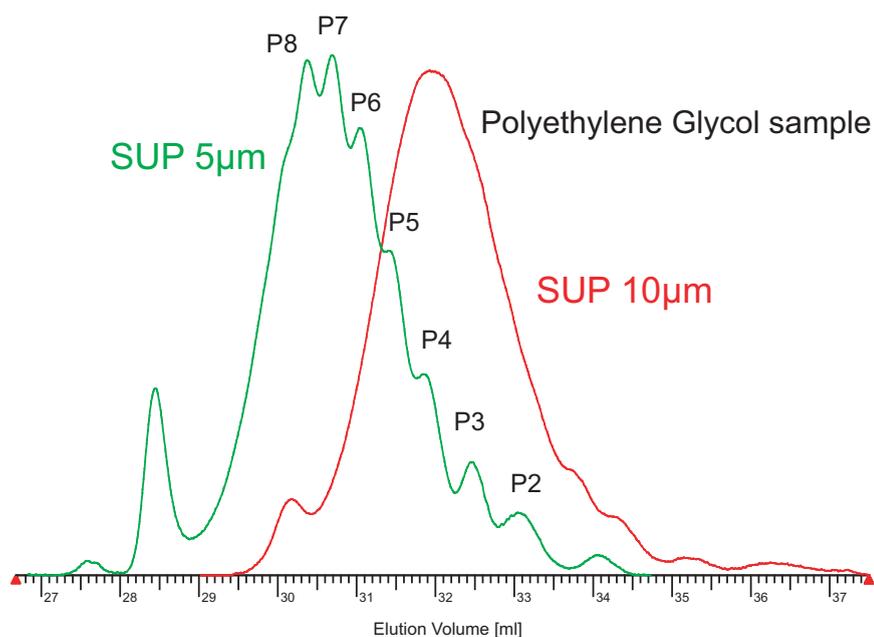


Figure 2. Improved resolution of 5 µm column (green line) compared to a 10 µm column (red line) both measured on a SUPREMA 30 Å, 1000 Å and 1000 Å column combination; a polyethylene glycol sample was measured under aqueous conditions with 1 ml/min at RT.

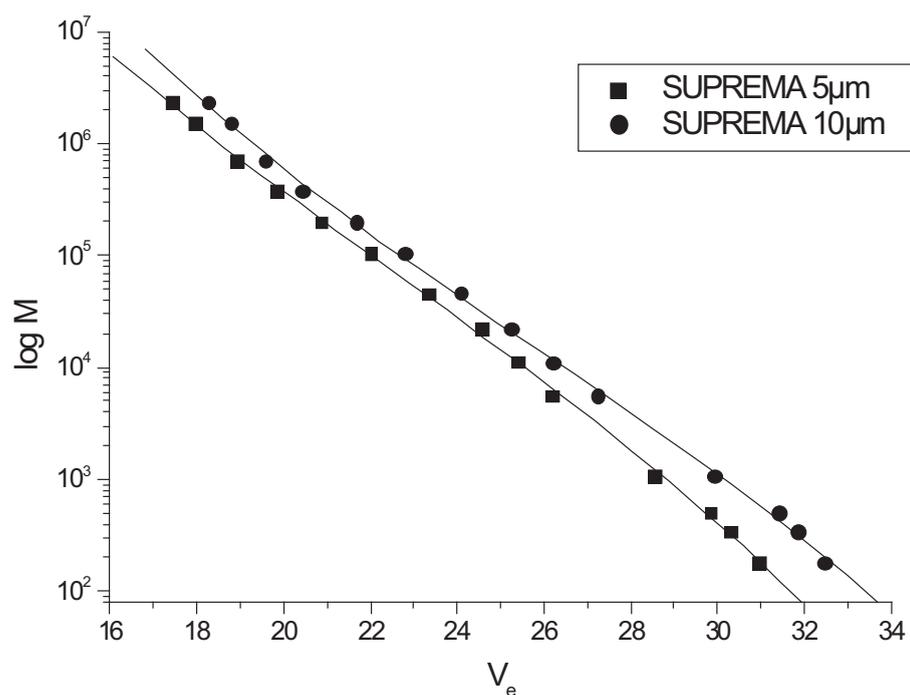


Figure 3. Comparison of the SUPREMA calibration curves for narrow distribution pullulan (500 – 2,340,000 Da) standards for 5 µm (■) and 10 µm (●) particles.

Table 2. Molar Mass information of the polymeric samples. (PS is polystyrene and PEG is Polyethylene Glycol. PDI is the polydispersity index $PDI = M_w/M_n$)

Sample	Mw [g/mol]	PDI
PSS pskit1h-04	474 - 2520000	< 1.1
PSS ps6097	2340000	1.15
PSS ps7025	4000000	1.18
PSS ps70203a	6010000	1.17
PEG sample	350	<1.25
Pullulan	1000 – 2600000	<1.25

Taking into account that the interstitial volume for a porous particle is even larger [9], the diameter for the interstitial volume for a 1 µm particle can increase up to 167 nm. This corresponds to a molar mass of 5,000,000 Da. Even if the maximum diameter of the interstitial volume for a 1 µm porous particle is 167 nm, it has to be considered that the porosity is statistical and not perfectly distributed among the particles in the column. For this reason the exclusion limit will have a minimum value of 3 Million Da and a maximum value of 5 Million Da.

Still, the effects of friction on the polymer have to be considered because the size of the interstitial volume and the hydrodynamic volume of the polymer are quite similar. In other words the interstitial volume for the non-porous particle is the limiting factor for the pore size for separation of the polymers. According to the calculations above, the interstitial volume for porous particles is much larger than for non-porous particle. The accessible molar mass increases by a factor of 6 based on the porosity and means that even for small 1 µm particles the elution of a polymer up to 5,000,000 Da is possible.

Transport and diffusion processes

GPC/SEC can be defined as pore size limited, diffusion controlled distribution chromatography. In addition to the requirement that the separation occurs without any kind of interactions, other kinetic equilibrium parameters, also have to be considered.

The diffusion coefficient describes the speed of the movement of a molecule or an ensemble of molecules in solution. Consequently, this coefficient also describes the diffusion process of a polymer into and out of the pores of a porous particle. The diffusion coefficient is strongly dependent on the size and therefore on the molar mass of a polymer. The larger the polymer is, the smaller the diffusion coefficient.

For a polymer of molar mass 100,000 Da, the diffusion coefficient is in the range of $4.5 \times 10^{-7} \text{ cm}^2/\text{s}$, whereas a polymer of molar mass of 3,000,000 Da has a value of only $0.5 \times 10^{-7} \text{ cm}^2/\text{s}$ [12]. If a polymer with 100,000 Da is eluting close to equilibrium conditions, then the flow rate is just low enough for all the molecules to be able to reach and diffuse into all of the available pores. These flow conditions do not hold for a larger molecule. Based on the given values for a molecule of molar mass of 3,000,000 Da, the flow rate has to be further reduced by at least a factor of 9 in order to measure under equilibrium conditions.

If we assume that the cross-sectional area of the interstitial volume for a 4 μm particle is in the range of 1 μm^2 , then a flow rate of 1ml/min has a linear velocity of 333 $\mu\text{m/s}$. The diffusion coefficient of a polymer of the molar mass of 3.000.000 million Da is about 5 $\mu\text{m}^2/\text{s}$. This diffusion coefficient is high enough, in relation to the interstitial area of 1 μm^2 , to allow the polymer to diffuse in and out of the pores under equilibrium conditions. Based on a linear velocity of 333 $\mu\text{m/s}$, the flow passes by approximately 80 particles of 4 μm size per second.

This indicates that not all pores of the particle are available under these flow conditions for the large polymer.

The adjustment of the flow rate with respect to the diffusion coefficient is one important requirement for the equilibrium conditions but not the only one. The flow resistance of a molecule in a mobile phase also has to be considered. Each real object creates a resistance in the mobile phase. A certain force has to be applied to a molecule to overcome this resistance for it to follow the flow profile in the flow direction. If the flow rate is too high, the polymer molecule can't adjust to the speed and the mobile phase moves faster than the polymer molecule. Due to this non- equilibrium state, large polymer molecules elute later [13]. In the GPC calibration curve an apparent exclusion limit due to the non-equilibrated flow will be observed. The increase in the slope of the calibration curve is not because of a limited pore size distribution but because of non-equilibrated polymer diffusion process. These phenomena should also be considered when GPC on different sized polymers is performed.

Smaller particles reduce the distance to a pore for a diffusing polymer molecule. Therefore even the mass transport for diffusion limited large molecules is enhanced and leads to much better resolution.

However if the size of the polymer molecule approaches the size of the interstitial volume, the polymer experiences shear force, which could start to degrade the large molecule. On the other hand, polymers have a statistical and flexible conformation in solution. The applied force or resistance could also force the polymer to change its conformation in solution to minimise the dimensional restrictions of the interstitial volume. The randomly coiled polymer could transform its coiled structure to a better adapted, more stretched structure, to fit into the limited interstitial volume. This effect is reported in the SEC literature as slalom

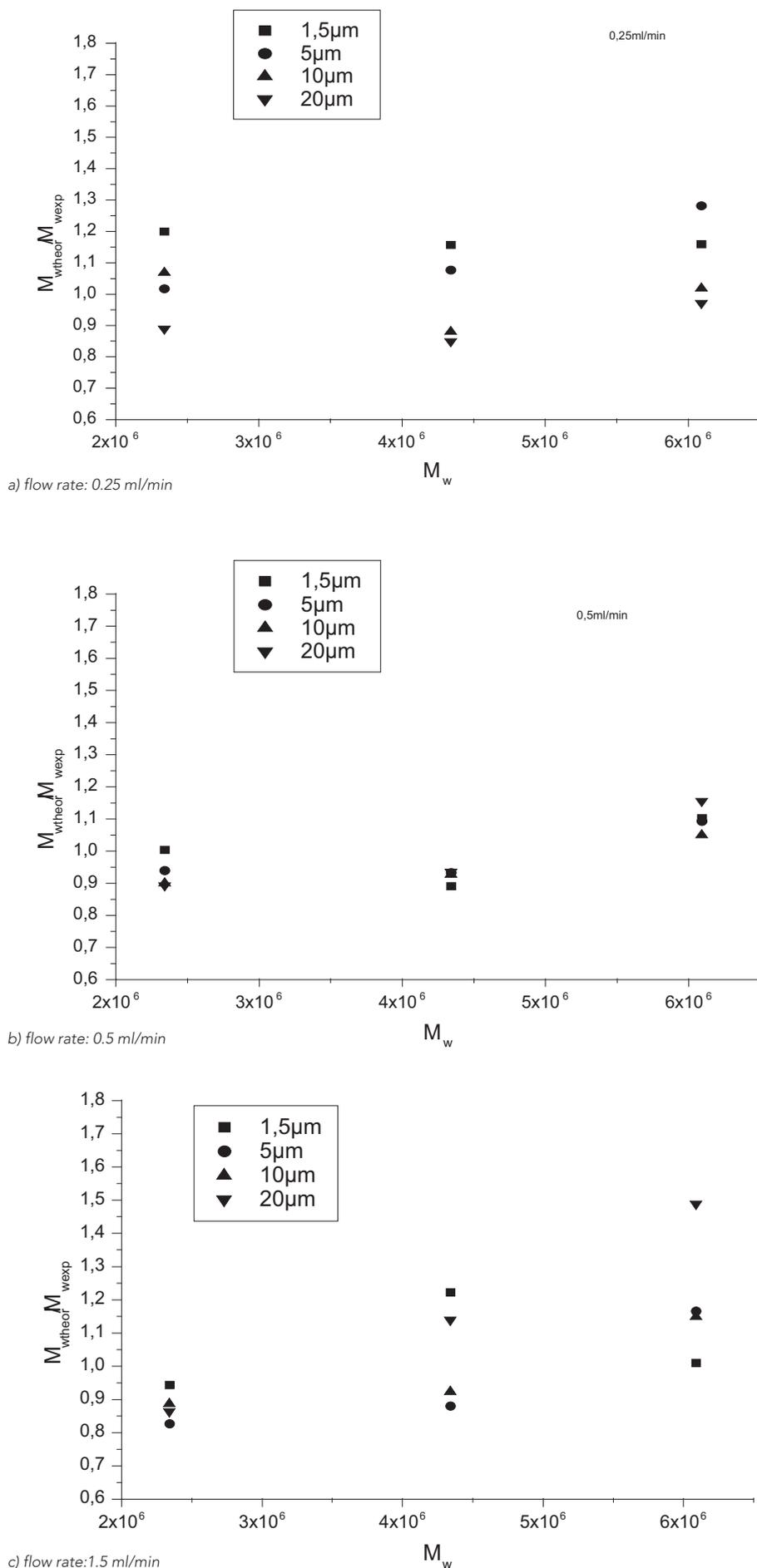


Figure 4: $M_{w,theor}/M_{w,exp}$ Ratio for various high molar mass polystyrene samples as a function of flow rate and particle size; molar mass values based on MALS results.

chromatography, which leads to larger elution volumes [11].

If non-equilibrium conditions are applied, or the interstitial volume is too small causing the polymer conformation to alter, then this change can be described and quantified by the Deborah number. This constant relates the relaxation time of a polymer to the observation times [10, 11].

Experimental conditions

All experiments were carried out with a PSS SECcurity SEC instrument fitted with RI and UV detectors and a PSS SLD7000 MALS (Multi Angle Light-Scattering) (7 angles between 35° and 145°) detector at 30°C. For the aqueous measurements PSS SUPREMA (100 Å, 3000 Å, 3000 Å) columns with 5 µm and 10 µm particle sizes and a flow rate of 1 ml/min were used.

The flow rate variation experiments for organic solvent were performed on an 8.0mm x 150mm column to reduce the back pressure, using flow rates between 0.25 ml/min and 1.5 ml/min. The solvent of choice was THF. For the organic SEC experiments, PSS SDV 10⁷ Å porosity columns in various particle sizes, 1.5 µm, 5 µm, 10 µm and 20 µm were used.

Data acquisition and data processing were done with the PSS WINGPC Unity. All polymers are reference materials from PSS GmbH

Results and discussion

Resolution as a function of the particle size

A low molar mass polyethylene glycol (PEG) polymer sample was measured on PSS SUPREMA 5 µm and also on PSS SUPREMA 10 µm column combinations. The results are shown in Figures 2 and 3.

As predicted and expected, with the smaller particle size, the resolution for small molar mass samples increases significantly. An oligomeric PEG polymer elutes on 10 µm columns as a single, almost symmetrical, Gaussian peak but on a 5 µm columns, the oligomeric nature is revealed showing rider peaks on the main peak, each corresponding to a different degree of polymerisation (Figure 2). The specific resolution increases from 0.7 to 2.1. The plate count increases from 25,000 [1/m] up to 45,000 [1/m].

The calibration curves for pullulan (a polysaccharide) reference standards on the SUPREMA 5 µm and SUPREMA 10 µm do not indicate any shear degradation as a function of the molar mass. The profile of the calibration curve (flatter slope in the high molar mass range) indicates better mass transport for the high molar mass samples on the 5 µm columns compared to the 10 µm columns (see Figure 3). As discussed before, on the 10 µm column, the larger polymer molecules can't reach all the accessible pores. Therefore the slope of the calibration increases due to a

diffusion process and not due to a pore size limitation process.

A combined GPC-MALS experiment shows no evidence that larger macromolecules break under the given conditions. The molar masses measured for the PSS Pullulan standards by GPC - MALS do not show any deviation from the true or given molar mass.

Shear degradation as a function of particle size and flow rate

In order to prove if and when shear degradation takes place in SEC, an experiment with various molar masses and various flow rates was designed. The aim was to increase the shear force on different sized polymers using step by step variation of the particle size and the flow rate. Three different high molar mass polystyrene polymers between 2 million and 6 million Da were selected and measured on a 10⁷ Å column. All three polymers had the same polydispersity. GPC-MALS coupling was used to detect the polymers and measure the change in the molar mass. The results are shown in Figure 3.

The molar mass ratios shown in Figure 4 do not indicate a strong dependency of the flow rate and the particle size with respect to shear degradation. The experimentally determined ratio, $M_{w,theor.}/M_{w,exp}$ varies between 0.8 and 1.2. The variations are statistical. No shear degradation could be detected with respect to the molar mass, neither at 0.25 ml/min on a 20 µm column nor at 1.5 ml/min on a 1.5 µm column. This holds for the 2 million Da polystyrene as well as for the 6 million Da polystyrene samples. The MALS results for the high molar mass polymers at high flow rates show some variation probably due to some flow phenomena. Nevertheless, neither for small particles, nor for large flow rates can any shear degradation be observed.

The calibration curve indicates an increasing resolution for larger molecules at smaller flow rates as predicted before. The calibration curves at various flow rates for the SDV 5 µm particles are shifted towards earlier elution times for lower flow rates see (Figure 5).

The MALS data show, that the molar mass is still the same regardless of the flow rate and the particle size. Due to the much lower diffusion coefficient, larger molecules will move much slower than smaller solvent particles and therefore be retarded during the journey through the porous column material.

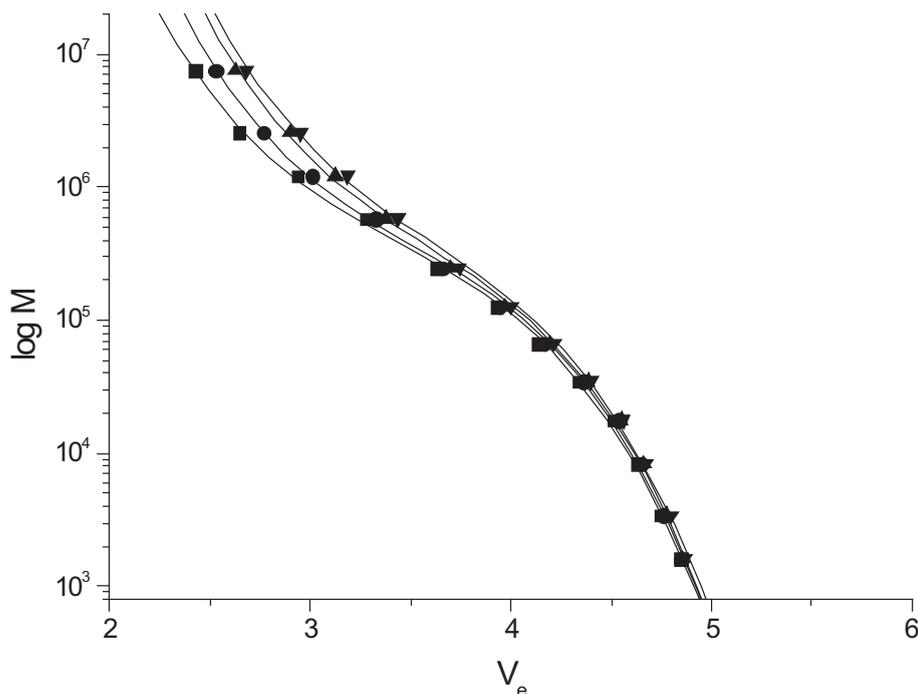


Figure 5. Calibration curves as a function of the flow rate for a SDV 5µm material, for PSS polystyrene polymers between 474 – 7,250,000 Da. Flow rates: 0.25 ml/min [■], 0.5 ml/min [●], 1.0 ml/min [▲], 1.5 ml/min [▼]

Summary:

As predicted from the calculations of the interstitial volume for a 1 μm porous particle no shear degradation could be observed. The minimum interstitial volume for a non porous particle corresponds to a polymer molar mass of 3 million Da and for a porous particle to a polymer molar mass of 5 million Da. From the GPC - MALS experiments, shear degradation, even for large polymers (6 million Da) on 1.5 μm particles could not be observed for polystyrene in THF. Also in a more viscous aqueous solvent no shear degradation was detectable on 5 μm particles. Due to non-equilibrium and diffusion effects the elution behaviour for larger molecules is influenced by the particle size of the column material and the applied flow rate.

For larger column particles, much lower flow rates have to be applied in order to have good separation for high molar mass polymers. Due to the much lower diffusion coefficients for large particles, the equilibrium conditions for the mass transportation in and outside the pores is only obtained at low flow rates.

The increasing slope and the shift of the exclusion limit to larger elution volumes for large molecules in the SEC/GPC calibration curves is not due to any shear degradation or exclusion effects. The reason for the shift are the non equilibrium conditions for larger molecules at high flow rates. The resolution for polymers on small column particles is much better even at higher flow rates. The calibration curves indicate better resolution due to optimised flow conditions. In conclusion, no detectable shear degradation for large molar mass polymers (up to several million Da), enables the analysis of macromolecules with improved separation efficiency using GPC/SEC columns packed with 3 or 5 μm particles.

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