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Size exclusion chromatography with superficially porous particles

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Abstract

A comparison is made using size-exclusion chromatography (SEC) of synthetic polymers between fully porous particles (FPPs) and superficially porous particles (SPPs) with similar particle diameters, pore sizes and equal flow rates. Polystyrene molecular weight standards with a mobile phase of tetrahydrofuran are utilized for all measurements conducted with standard HPLC equipment.

Although it is traditionally thought that larger pore volume is thermodynamically advantageous in SEC for better separations, SPPs have kinetic advantages and these will be shown to compensate for the loss in pore volume compared to FPPs. The comparison metrics include the elution range (smaller with SPPs), the plate count (larger for SPPs), the rate production of theoretical plates (larger for SPPs) and the specific resolution (larger with FPPs). Advantages to using SPPs for SEC are discussed such that similar separations can be conducted faster using SPPs.

SEC using SPPs offers similar peak capacities to that using FPPs but with faster operation. This also suggests that SEC conducted in the second dimension of a two-dimensional liquid chromatograph may benefit with reduced run time and with equivalently reduced peak width making SPPs advantageous for sampling the first dimension by the second dimension separator. Additional advantages are discussed for biomolecules along with a discussion of optimization criteria for size-based separations.

Keywords

size-exclusion chromatography; core-shell particles; superficially porous particles; efficiency; pore diffusion; peak capacity

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1. Introduction

Size exclusion chromatography (SEC) is a form of chromatography that separates molecules by size. It is also known as gel-filtration chromatography (GFC) and gel permeation chromatography (GPC) which often refers to SEC with organic solvents. The primary applications of this technique are polymer and medium to large biomolecule separations. The performance characteristics of SEC are not on the scale of the efficiencies typical of columns used for reversed-phase or normal-phase separations. However, SEC continues to be highly utilized for separations where fractionation can be accomplished based on molecular size.

The first SEC columns were made of starch (1,2), however, dextran gels were soon developed for biochemical applications (3). After this initial introduction, cross-linked polystyrene was introduced (4) as a material useful for fractionating industrial polymers. Silica is also often utilized as a porous medium and complements other media, especially when small particles are used to minimize zone broadening and provide a stable mechanical particle for use at higher pressures (5,6). In most cases, particles are used for SEC although polymer monoliths have been reported that can function in the size-exclusion mode (7).

In all cases where particles are used for SEC, the particle is made of organic or inorganic materials and is a fully porous particle (FPP) morphology. Superficially porous particles (SPPs) (8–13), also known as core-shell particles, offer the performance of a smaller diameter particle, for example a sub 2- μm diameter particle, with the smaller pressure drop of a standard-sized particle, for example a 2.7 μm particle. The SPP morphology has become a serious choice for high performance chromatographic media. In the early development of SPP materials, specifically from 1978, it was written by one of the developers of the SPP technology (14): “However, it is anticipated that, while wide-linear calibrations should result, resolution of polymers with these particular particles would be relatively poor because of their low specific porosity.”

The assumption inherent in this quote is that it is necessary to have as much pore volume as possible to get maximum performance from the SEC technique. This assumption is partly driven by the low peak capacity inherent in the SEC technique where getting a 10 peak separation would be considered a large number of peaks (15–17). This is in contrast to getting peak counts greater than 60 which is not uncommon (18) using the partition and adsorptive retention mechanisms inherent in reversed-phase liquid chromatography (19).

Recent theoretical work (20,21) has concluded that reducing the pore volume by using a SPP for SEC would not be especially deleterious for large-sized solutes with slow diffusional characteristics. This work highlighted that the loss of pore volume can be compensated by shortening the diffusion length of the solutes inside the pore and theoretical elution curves which showed this effect were given in detail (20,21). These results suggest that SPPs could be used successfully for SEC.

From the thermodynamic point of view, one will spread the SEC chromatogram across a greater range of retention volume V_R when the pore volume is increased. This can easily be shown by Eq 1

$$V_R = V_0 + KV_i \quad \{1\}$$

where V_0 is the interstitial pore volume, V_i is the particle pore volume and K is the distribution constant such that K varies from an excluded zone ($=0$) when the solute is larger than the pore to a fully included zone ($=1$) when the solute is very much smaller than the pore. As the volume of internal pores V_i increases, the retention volume, V_R , will also increase. This shows that the range of separation will be increased by a larger pore volume. SPPs have less pore volume than FPPs so SPPs are at a thermodynamic disadvantage for SEC. This was thought to be a limitation of SPPs for use as SEC materials, as mentioned previously.

The reduced pore length, inherent in the SPP shell, has specific advantages in reducing the resistance to mass transport in the particle and faster transport kinetics provide for higher efficiency. This is also important because slow diffusional properties of large molecules that are typically separated with SEC, are compounded by the additional slowing when the solute molecular size is on the order of the pore size (22, 23). Hence, the experimental determination of whether faster diffusional kinetics can overcome thermodynamic limitations is long overdue. Some of these issues were recently discussed (24) in the experimental demonstration of SEC with SPPs. In one case, SEC has been reported (25) to occur without pores.

In this paper we explore the use of SPP technology for SEC in the context of the interplay between particle pore volume and the efficiency of separation. It will be shown that for small and wide-pore materials that SEC with SPPs can deliver a faster separation while retaining most of the resolution of a FPP. This compromise will be suggested to be extremely important in two-dimensional separations where speed constraints often apply in the second dimension separation system. We also discuss some aspects of bioseparations pertinent to SEC using SPPs where adsorptive and partitioning mechanisms of retention exist alongside with the size-exclusion mechanism.

2. Experimental and data processing conditions

Particles and columns

FPPs were obtained from the Osaka Soda Co, (Osaka, Japan). The two silica-based FPPs used in this study are SP-200-3-P and SP-1000-3 which are both of diameter 3.2 μm and had pore sizes nominally of 200 $\text{\AA}$ and 1000 $\text{\AA}$ respectively with pore volumes of 1.06 cc/g and 0.80 cc/g respectively, as provided by the manufacturer. SPPs, also made of silica, are from Advanced Materials Technology (Wilmington, Delaware, USA) and have nominal pore sizes of 160 $\text{\AA}$ and 1000 $\text{\AA}$. These particles have outer diameters of 2.7 μm and 4.18 μm respectively and have solid core diameters of 1.7 μm and 3.3 μm respectively. These particles had pore volumes of 0.29 cc/g and 0.20 cc/g for the 160 $\text{\AA}$ and 1000 $\text{\AA}$ particles, as measured in-house using nitrogen adsorption methods. The pore size distribution of the 160 $\text{\AA}$ SPPs was narrow and measured in-house. The 1000 $\text{\AA}$ SPPs have a wider distribution and this is shown in a recent paper (26). The pore size distributions of the 200 $\text{\AA}$

and 1000 Å FPPs were not supplied by the manufacturer nor determined in-house. All particles of both morphologies and both pore sizes were packed into columns of dimension 4.6 mm i.d. and length 50 mm using an in-house developed proprietary packing process.

HPLC conditions

Individual solutes were run at 0.25 mL/min and 0.50 mL/min flow rates at a temperature of 25 °C using a Shimadzu Nexera™ X2 liquid chromatograph (Shimadzu, Columbus, Maryland). The UV detector wavelength is 254 nm and the solutes were polystyrene standards of molecular weight 2.5 kDa, 5.0 kDa, 9.0 kDa, 17.5 kDa, 30 kDa, 50 kDa, 110 kDa, 220 kDa, 400 kDa, 600 kDa, 900 kDa and 1.8 mDa. The standards are from a low and high molecular weight polystyrene standards kit (Supelco, Bellefonte, PA, part numbers 4-8937 and 4-8938). The polydispersity index of these standards was not supplied by the manufacturer. The molecular weights and log molecular weights are given in Table 1 along with the radius of gyration and the diameter of gyration calculated from the formula $R_g = 0.137 M^{0.589}$ (14) for polystyrene in tetrahydrofuran (THF). THF was used for all mobile phase solvents in the unstabilized, HPLC grade form, and was from J. T. Baker (Center Valley, PA). Typically, polystyrene standards were made up as solutions in THF at 1 mg/mL concentration with 1 µL injections.

Data analysis

The data, stored in Excel© spreadsheets (Microsoft, Redmond, WA), was processed for plotting using MATLAB© (MathWorks, Natick, MA) version R2016a. Individual zone broadening estimates of plates and plates per unit time were obtained using the plates calculator in the Shimadzu HPLC instrument software utilizing the width at half-height method (full width at half maximum or FWHM). When interpolation is employed in the plots, the interpolation method is the cubic spline method native to MATLAB. Results were checked for spline artifacts with visual graph inspection.

It is well known that using the width at half height method can overestimate the number of plates (27–29) for peaks that are non-Gaussian, typically with tailing that can be modelled with a peak model formed by the convolution of a Gaussian with an exponential tail (30). Obtaining high accuracy plate count measurements is difficult because the peak shape is not Gaussian nor Gaussian with exponential convolution across the full molecular weight range.

The specific resolution was described by Yau *et al* (31, 32) and originally defined as:

$$R_{sp} = \frac{2(V_{R2} - V_{R1})}{(W_1 + W_2)} \cdot \frac{1}{\log_{10}(M_{W1}/M_{W2})} \quad \{2\}$$

where W_1 and W_2 are the volume-based peak widths of adjacent peaks obtained by the tangent drop method. In addition, V_{R1} and V_{R2} are the retention volumes of adjacent peaks and M_{W1} and M_{W2} are the molecular weights of adjacent solute peaks.

In this paper we use a very similar approach. However, we use a continuous measure of the specific resolution which is constructed using interpolated time t , standard deviation σ and molecular weight M_w so that:

$$R_{sp_i} = \frac{(t_{i+1} - t_i)}{2(\sigma_{i+1} + \sigma_i)} \cdot \frac{1}{\log_{10}(M_{w_{i+1}}/M_{w_i})} \quad \{3\}$$

where the 1 and 2 subscripts are replaced by the i^{th} and $i^{\text{th}}+1$ numbers in the interpolation vector of t , M_w and of σ . The standard deviation σ is obtained from the FWHM measurement assuming a Gaussian zone so that $\sigma = FWHM/2\sqrt{2\ln 2}$. The specific resolution here is not normalized for comparison of columns of different lengths (31) because all of the compared columns are of the same 50 mm length.

The specific resolution R_{sp} is similar to other measures of separation for polymers and colloids, for example Giddings' mass-based fractionating power (33,34) which is nearly identical to Eqs 2 and 3 and is expressed in compact form as:

$$F_m = \frac{R}{\delta(M_w)/M_w} \quad \{4\}$$

where δ is the differential operator.

3. Results

3.1 Chromatograms

The individual chromatograms of the solutes run with SPPs and FPPs are shown in Figure 1 for all molecular weight solutes run at the flow rate of 0.25 mL/min. These elution curves are overlaid for specific molecular weight analytes run on 160 Å SPP to 200 Å FPP pore size materials (Figure 1a and Figure 1c respectively). The elution curves are also shown for the larger pore (1000 Å) SPP and FPP materials (Figure 1b and Figure 1d respectively). The results for both SPPs and FPPs run at 0.50 ml/min flow rate are not shown but look similar to that shown in Figure 1.

One of the first things that is seen when comparing superficially porous and fully porous SEC results is that the range between the exclusion limit, where the retention volume is due to the interstitial volume only and the full pore volume, which includes the particle pore volume, is reduced for SPPs. Hence, the elution range, which is typically small in FPP SEC, is even smaller when using SPPs. This should not be surprising because the pore volume in an SPP is lower than in a FPP due to the presence of the solid core. In both particle morphologies the small pore (160 Å and 200 Å pore) particles show resolution below ≈ 50 kDa and the larger pore materials (mean pore size of 1000 Å) show resolution of the larger solutes above ≈ 50 kDa. As shown in Table 1, 50 kDa corresponds to a diameter of gyration of 160 Å, which agrees well with the pore size of the solute fractionation range. It is well

known that a single pore size cannot fractionate solutes over the complete range used in these experiments (14,17).

3.2 Retention volume range

Figure 2 shows the comparison of the retention volumes of the SPP and FPP elution results across the logarithm of the molecular weight range. It is seen that the retention volume range of the SPPs is less than the range of retention volumes for the FPPs. For both particle types, there is little difference in the retention volumes when comparing the two flow rate results. This suggests that for the flow rates used in this study, there is little effect of flow velocity on the distribution constant K which is thermodynamic in origin. The SPP results cross at lower retention volume because the two SPP particles pack differently and the interstitial pore volume, V_0 , is smaller for 1000 Å particle than the 160 Å particle. For the lowest molecular weight solute, the difference in V_0 also contributes to the crossing at highest retention volume, although this is hard to ascertain because of differences in the particle pore volume, V_i .

As expected for the largest pore size particles, the retention volumes for both particle morphologies are similar for the largest molecules because these retention volumes should approach the exclusion limit. These retention volumes differ in the void (interstitial) volumes of the columns. For both particle morphologies in the largest pore particles, the shape of these curves is consistent with selectivity diminishing as smaller solutes are fractionated. The selectivity reduction here is equated to the larger slope of log MW versus retention volume shown in this figure in the vicinity of the lower molecular weight solutes.

As given in Table 1 for the largest solute of 1.8 mDa, the diameter of gyration is larger than the mean pore size of the large-pore particles and should be excluded from the pore. However, the pore size distribution of these materials is wide and therefore there is no hard cut-off into solute exclusion, besides the possibility that some of the solute can be included in the pore as the solutes are flexible random coil polymers in THF. Another possibility to explain the lack of constant retention volume as the exclusion limit is approached (i.e. as the molecular size is increased beyond the pore diameter), is the presence of hydrodynamic chromatography (HDC) effects (17, 35). For larger solutes, relative to the pore diameter, the center-of-mass velocity near the outer hull of the particle surface causes faster elution as molecular size increases, which is the basis for HDC. This effect could contribute to the smaller retention volumes seen in Figure 2 as compared to a near-vertical (constant) retention volume characteristic of pure SEC.

Figure 2 also shows that the smaller solutes are separated at higher selectivity than larger solutes with the smaller pore materials for both particle morphologies, as judged by the smaller slopes in the log M_w versus retention volume curves at smaller molecular weights.

3.3 Efficiency

The number of plates N is shown in Figures 3a and 3b for the two flow rates used in this study. Immediately one can see that the SPPs exhibit higher plate counts than the FPP results for both flow rates. The plate counts are generally higher at lower velocities for both particle morphologies than at higher velocities.

One of the general trends seen in Figure 3 is that the number of plates for excluded solutes, where larger solutes are excluded by the two smaller-pore materials independent of particle type, increases after the exclusion limit is reached. This increase is due to a decrease in zone broadening when solutes are excluded from the pore; i.e. mass transport in and out of the particle phase no longer occurs. The radius and diameter of gyration of polystyrene in THF, given in Table 1, shows that molecular weights in the vicinity of 50 kDa to 100 kDa have diameters of gyration between 160 Å and 200 Å and this is the range where increased plate counts occur as shown in Figure 3.

For the 1000 Å pore size particles of both morphologies, the plate numbers drop with an increase in solute molecular weight (and size). This is most likely due to the decrease in diffusion coefficient with increase in molecular weight (and size). Most of the solutes used here, except for perhaps the highest molecular weight solute, access the pore structure. The pore size distribution of most particles is rather broad for wide-pore materials and this may also explain why there appears to be access to pores with the largest solute. In the case of the 1000 Å SPP at the higher flow rate it is noted that σ is ≈ 0.015 min (≈ 0.9 sec). Given 6σ as a baseline width this gives peaks which are 5.4 seconds (at baseline) wide. This is very short for SEC and shows very good performance. The contrast in efficiency with the FPP, as measured by the number of plates, suggests the potential superior use of SPPs for conducting fast separations in the SEC mode of operation.

3.4 Rate of plate generation

While efficiency, as expressed in the number of plates is important, the speed of a separation is often identified with the number of plates generated per unit time (36). This key parameter has been discussed in the context of column optimization (36, 37) and is most important for SPPs as these have long been recognized as a technique which promotes faster speed operation.

Figures 4a and 4b show the rate of plate generation given by N/t_R as a function of the log of the solute molecular weight for the small and large pore size particles. These figures show the same pattern as in Figure 3; SPPs for the most part generate plates at a higher rate than FPPs given approximately the same pore size, flow rate, and particle diameter. However, in this case, faster flow rates give faster rate of plate generation. The same hump of higher plate number per minute for smaller pore material (Figure 4a) due to solute occlusion are shown here as in Figure 3. For the 1000 Å materials at both flow rates there is seen to be a relatively constant rate of plate generation across the molecular weight range as shown in Figure 4b.

3.5 Specific resolution

The specific resolution is shown in Figure 5 for the four series of experiments each at the two flow rates. As can be seen in this figure, there are two regions of maxima. For small pore materials (160 Å and 200 Å) this region is at approximately $\log M_w \approx 4.25$ and for large-pore materials (1000 Å) these maxima are between $\log M_w \approx 5-5.5$. For both small and large pore materials, the FPPs (as annotated by dotted-lines) show higher maxima in both regions. Clearly as shown in Figure 1, the resolution between zones in the respective

molecular weight regions show higher resolution for FPPs than the results for SPPs. There appears to be little difference with flow rate with constant particle morphology and pore size, although the lowest flow rate gives highest specific resolution in all cases.

Note that specific resolution should not be interpreted as having complete resolution above the value of 1.5, as is noted with traditional resolution. Rather these values here are biased by a molecular weight term, as shown in Eq 2 and Eq 3. However, the specific resolution can be compared between the curves shown in Figure 5. While FPPs clearly dominate in specific resolution, in specific regions, the wide-pore SPP 1000 Å material shows higher specific resolution up to log M_w of 5 than the FPP material. Furthermore, the performance of the SPP material, from the viewpoint of specific resolution, shows a rather fast decline as molecular weight is increased further. It is suspected that increasing pore volume of the SPP may promote further specific resolution increases and overtake the 1000 Å FPP material's performance using this metric. The optimization of shell thickness is discussed below.

3.6 Peak capacity

The concept of peak capacity in SEC was developed by Giddings (15) for a model that assumed a constant ratio of peak width to retention time (or volume). Horváth and Lipsky contributed to this theory with a constant peak width model (38) and this was generalized by Grushka (39). Later work by Hagel (16) assumed a variable peak width model more suitable for SEC and we will use that model here.

The Giddings model is approximated as

$$n_c = 1 + 0.2 \sqrt{N} \quad \{5\}$$

where n_c is the peak capacity. Hagel's model for SEC (16) with a peak resolution of unity is given as:

$$n_c = 1 + \frac{(V_p/V_t)}{4} \sqrt{N} \quad \{6\}$$

where V_p is the particle pore retention volume and V_t is the total pore retention volume (which includes the interstitial and particle pore retention volume). The plate number utilized in Eq 6 is that of a totally permeating (low molecular weight) component (16).

The square root of the number of plates $\sqrt{N}$ can be expressed as the ratio of retention time to standard deviation of a Gaussian zone t_r / σ . In Eq 6, the peak capacity is determined by the amount of particle porosity and by the ratio of a thermodynamic parameter t_r and a kinetic parameter σ . While the retention time is less for SPPs because of the loss of some pore volume, the extent of this loss can be compensated for smaller values in σ .

The values of t_r / σ for FPPs and SPPs are compared in Table 2 for the totally permeating peak where it is shown that the SPPs at approximately equal pore size have larger t_r / σ values. Also included in Table 2 is the ratio of V_p / V_t and the calculation of n_c . The values

of V_t are obtained from the toluene void retention volume and V_p is obtained by the difference in retention volumes between toluene and the 900 kDa solute which should be near the exclusion limit. The exclusion limit is not particularly distinct for both SPP and FPP experiments, as viewed by the results in Figure 2. This may be due to solute adsorption, partial exclusion, HDC effects or a combination of these effects.

The comparison of peak capacity values of the smaller pore size particles favors the FPP morphology. However, it is shown that the wide pore particles have very similar peak capacities. Clearly the ratio V_p/V_t favors FPPs as the core-shell morphology has less volume in the shell than a FPP. The diffusion length is much shorter in the superficially porous material and the peaks are narrower which would tend to preserve the peak capacity of the separation system and make the limited elution range faster for SPPs. With a somewhat larger shell thickness, the peak capacity could probably be made to favor the SPP for a smaller pore particle.

4. Discussion

Due to sampling considerations (40,41) it is often necessary to run a fast second dimension separator in two-dimensional liquid chromatography (2DLC) so that the first dimension peak can be adequately sampled by the second dimension separator (42). Proper sampling aids quantitation, peak detection algorithms, and helps prevent ionization suppression when mass spectrometry is used as a detector (43). In many applications of 2DLC (40,44–46) SEC has been utilized as the second dimension separation column. In these cases, SEC with SPPs could enhance the application by allowing for faster operation and/or more sampling. SEC in the second dimension is also useful as a desalting step prior to mass spectrometry.

The choice of pore size is an often critical choice for SEC. Column coupling, in which two columns of different pore sizes are used in series to expand the separation range (5,14) is well known. The results shown in this paper used two different pore sizes for the whole range of molecular weight solute standards and these pore sizes are not optimized for performance. The optimization of the pore size and choice of shell thickness for SEC with SPPs is not a trivial matter (47). This is because increasing the shell thickness gives more pore volume at a reduction in column efficiency. However, that balance is very dependent on the solute size and the pore size. This may result in the situation where maximizing speed and/or resolution of separation will depend both on the pore size and shell thickness across the molecular weight range.

A further aspect of this optimization is that for many biomolecules of substantial molecular weight (and size), the traditional retention mechanism and subsequent elution is controlled by the solvent gradient. This is exemplified in reversed phase, normal phase and ion exchange chromatography where enthalpy-driven retention results in almost infinite retention until a critical concentration of the gradient is reached. As retention is reduced at or near the critical gradient composition, SPPs have been shown to offer substantial performance advantages in speed and efficiency for larger biomolecules (48–50). This is attributed to the mass transport advantages of having thin shells and shorter diffusion distances.

It is possible that selectivity is then enhanced with size-exclusion processes aiding the separation as enthalpic retention approaches near zero during gradient elution and the SEC mechanism becomes more important when the solute size is some fraction of the average pore size.

This mechanism appears even more complex as mass transport studies of model wide-pore materials has shown that wide-pore SPPs have perfusive (diffusion aided by convection) flow in a substantial region of the porous shell with 10% of the mass flux in the particle bed being in the shell region (51). This can facilitate faster mass transport for even more efficiency (52–54).

With SPPs on the order of $2\text{ }\mu\text{m}$ particle diameters, the pressure drop is not excessive and this aids in using longer columns (and columns in series), a common practice in SEC (5,14). Both SPP and FPP particles can be used in longer columns to increase resolution. When time is not critical, the FPPs are expected to outperform SPPs with respect to specific resolution. If speed is important, SPPs can perform faster separations in the allotted time.

It has been established that chromatographic elution of small molecules in SPPs is affected by both the B term and C terms of the van Deemter equation (55–57). Both terms, for small molecule separations, appear smaller for SPPs as compared with FPPs (55, 56). For larger molecules, as are typically fractionated with SEC, both terms will be affected by the inherently smaller diffusion coefficient of larger molecules. Further details of the theoretical development of SEC with SPPs is forthcoming (47) along with plate height data for this application.

As we have shown in this paper, SPPs may have some very useful properties in the SEC mode. Future optimization of particle morphology and applications which clearly show the speed of SEC with SPPs will further demonstrate potential advantages of this technology.

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Highlights

- Size exclusion chromatography is shown with superficially porous particles (SPP's)
- SPP's demonstrate faster separations
- Fully porous particles show higher resolution than SPP's
- SPP's have higher efficiency and rate of plate production

Figure 1.

Chromatographic elution data of polystyrene solutes and toluene superimposed on the time axis. a) SPPs with pore size of 160 Å, b) SPPs with pore size of 1000 Å, c) FPPs with pore size 200 Å d) FPPs with pore size of 1000 Å. All data shown here is with the flow rate of 0.25 mL/min.

Figure 2.

Retention volumes of peaks across the chromatograms for the two flow rates, two pore sizes and the two different particle morphologies. The data with circles and asterisks and solid lines are for SPPs and the data with diamonds and triangles and dashed lines are for FPPs.

Figure 3.

The number of theoretical plates as a function of log molecular weight for a) 0.25 mL/min flow rate b) 0.50 mL/min flow rate. The data with circles and asterisks and solid lines are for SPPs and the data with diamonds and triangles and dashed lines are for FPPs.

Figure 4.

Plates per unit time as a function of log molecular weight for a) the smaller pore sizes, 160 Å and 200 Å b) the wide pore size, 1000 Å. The data with circles and asterisks and solid lines are for SPPs and the data with diamonds and triangles and dashed lines are for FPPs.

Figure 5. Specific resolution as a function of log molecular weight for the eight datasets of 0.25 mL/min and 0.50 mL/min flow rates, two pore sizes and the two different particle morphologies. The data with circles and asterisks and solid lines are for SPPs and the data with diamonds and triangles and dashed lines are for FPPs.

Table 1

Radius of gyration of Polystyrene in THF

Molecular weight (Da)	Log molecular weight	Radius of gyration (Å)	Diameter of gyration (Å)
1800000	6.26	662	1325
900000	5.95	440	881
600000	5.78	347	694
400000	5.60	273	546
220000	5.34	192	384
110000	5.04	128	255
50000	4.70	80	160
30000	4.48	59	119
17500	4.24	43	86
9000	3.95	29	58
5000	3.70	21	41
2500	3.40	14	27

Table 2

The values used in calculating peak capacity for the SPP and FPP results. The units of the pore volumes V_p and V_t is mL.

Morphology	Pore size (Å)	flow rate (mL/min)	V_p	V_t	V_p/V_t	N	$\sqrt{N=t/\sigma}$	n_c
SPP	160	0.25	0.164	0.544	0.301	4907	70.0	6.3
SPP	160	0.5	0.164	0.542	0.303	7056	84.0	7.4
SPP	1000	0.25	0.176	0.497	0.354	3410	58.4	6.2
SPP	1000	0.5	0.176	0.497	0.354	5399	73.5	7.5
FPP	200	0.25	0.379	0.666	0.569	2658	51.6	8.3
FPP	200	0.5	0.376	0.663	0.567	3731	61.1	9.7
FPP	1000	0.25	0.303	0.675	0.448	2717	52.1	6.8
FPP	1000	0.5	0.299	0.675	0.443	3926	62.7	7.9