

# Characterization of Crosslinked Polymer Nanoparticles by Size-Exclusion Chromatography Coupled to Differential Viscometry

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## ABSTRACT

Conventional size-exclusion chromatography (SEC) and size-exclusion chromatography coupled to differential viscometry (SEC/DV) methodologies have been developed and applied to the dilute suspension characterization of solvent-swollen crosslinked unimolecular polymeric nanoparticles (PNPs) in the 5 ~ 50 nm weight-average diameter ( $d_w$ ) range. SEC and SEC/DV characterization techniques yield, based on the principle of universal calibration, apparent and absolute molecular weight distributions (MWD), apparent and absolute molecular weight averages, peak and weight-average particle diameters, particle size distributions in both the solvent-swollen and solvent-free states, particle conformation (shape), and an estimate of the volumetric swell factor. The approach for extracting size and volumetric swelling parameters (VSF) from SEC/DV data will be discussed, as will solute conformation gleaned from Mark-Houwink plots of the nanoparticle samples will be illustrated.

**Keywords:** polymer nanoparticle, size exclusion chromatography, differential viscometry, Mark – Houwink

## 1 BACKGROUND

Historically, particle size distributions of PNPs have been measured by a variety of techniques including hydrodynamic chromatography (HDC),[1] dynamic light scattering (DLS),[2] field flow fractionation (FFF),[3] ultracentrifugation,[4] transmission electron microscopy (TEM), and static multi-angle light scattering (MALS).[5] HDC, FFF, ultracentrifugation, and MALS are not well suited for measurement of the particle radius of gyration,  $R_g$ , in the size regime ~  $\leq 10$  nm.[6] Static light scattering provides bulk property measurements. DLS methods can provide both size distributions as well as concentration.[7] The number of particles studied in TEM experiments is usually small, and therefore does not always represent the entire sample, and small organic particles can have low contrast and be hard to image.

Information regarding suspended particle shape, or conformation, can be gleaned from studying the

relationship between size and molecular weight, information readily obtainable in a multidetector SEC experiment.

SEC and SEC/DV methods were used to characterize a number of crosslinked (4 – 50 wt% crosslinking agent based on monomer) PNPs with respect to their size distribution and suspension conformation. Determination of the MWD of the PNP samples relative to linear PS standards provides a measure of the hydrodynamic volume distribution of the particles. Additionally, the use of an on-line differential viscometer allows for measurement of intrinsic viscosity,  $[\eta]$ , at each SEC elution volume increment. The measured  $[\eta]$  can be used, in combination with universal calibration,[8] to provide the absolute molecular weight of the PNPs at each SEC elution volume increment. Thus, the relationship between molecular weight and  $[\eta]$  can be gleaned for each sample; the Mark – Houwink relationship then provides insight into the shape or conformation of the particles. Because SEC is performed under thermodynamically good conditions (thermodynamically good solvent; THF),  $[\eta]$  provides a direct measure of the VSF for the particles. Finally, from the molecular weight and the polymer density, one can calculate the solvent-free volume, and thus diameter, of a solvent-free, spherical PNP.

## 2 EXPERIMENTAL PROCEDURES

**Particle Synthesis:** Polymerizations performed in batch or semi-batch mode in microemulsion media offer a convenient route to well-defined, narrow-polydispersity microlatex particles that are typically an order of magnitude smaller than the particles obtained from standard emulsion polymerization processes. Batch and semi-batch microemulsion polymerization methods were used at 1.5 ~ 30 wt% monomer solids to prepare dense, spherical particles with  $d_w$  in the range of 5 – 35 nm.[9]

**SEC:** SEC was performed on a modular system of components in THF as the eluent. The columns (various PLgel mixed bed from Agilent and TSKgel Alpha from TosoHaas; two or three in series) were operated at 35 °C. A Waters 2410 differential refractive index detector was used and was maintained at 35 °C. A Viscotek Model

H502 four capillary bridge-based differential viscometer, also operated at 35 °C, was used to detect the specific viscosity at each SEC elution volume increment.

**Static Light Scattering (SEC/SLS):** The light scattering values were obtained with either mini-Dawn or Dawn MALS detectors from Wyatt Technology (Santa Barbara, CA) attached to an SEC system with RI detection as well.

**DLS:** DLS measurements were performed on a custom-built DLS instrument of the rotating arm type.[10]

**Other:** HDC and TEM analyses were performed by standard methods.

### 3 PARTICLE SIZE

SEC/DV with universal calibration has been used to determine the molecular weight of polymers.[11] In SEC, polymer molecules are ideally separated by equivalent sphere volume (the product of intrinsic viscosity and molecular weight  $[\eta]M$ ).[12] Under ideal SEC conditions, a plot of  $\log [\eta]M$  versus SEC elution volume will result in a single, universal, calibration curve that is independent of polymer composition or topology.

Knowledge of the weight average molecular weight derived from the SEC/DV measurement ( $M_w$ ) and polymer concentration at each SEC elution volume increment allows one to calculate the absolute molecular weight averages of interest and to calculate molecular weight and diameter distributions. Particle diameter (solvent-free) is given by Equation (1), below. Particle sizes determined under such conditions are in excellent agreement with those obtained by DLS, TEM, HDC, and (SEC/SLS).

$$d = \left( \frac{6M}{\pi N_a \rho} \right)^{1/3} * 10^7 \quad (1)$$

in which  $N_a$  is Avogadro's number, density  $\rho$  is expressed in  $\text{g/cm}^3$  (1.0 for polystyrene), molecular weight ( $M$ ) in  $\text{g/mol}$  and diameter  $d$  in nm.

### 4 VOLUME SWELL FACTOR

Because intrinsic viscosity,  $[\eta]$ , is nearly independent of molecular weight for most PNPs, one can quickly obtain a rough estimate of the average, or bulk,  $[\eta]$  from the Mark – Houwink plot. The bulk  $[\eta]$  is directly proportional to the VSF and inversely dependent on the level of crosslinking monomer, as shown below. VSF is given simply (Equation (2)) by the bulk intrinsic viscosity as measured in the swollen state in THF, in units of  $\text{cm}^3/\text{g}$ , divided by  $2.5 \text{ cm}^3/\text{g}$ :

$$\text{VSF} = \frac{V(\text{swollen})}{V(\text{non-swollen})} = \frac{[\eta](\text{swollen})}{[\eta](\text{non-swollen})} = \frac{[\eta](\text{swollen})}{2.5 \text{ cm}^3/\text{g}} \quad (2)$$

Particle VSF was found to decrease and the density to increase with increasing molecular weight within the MWD of any given particle (Figure 1). VSFs of  $\sim 2.0$  have been observed, indicating that extremely compact PNPs can be made at very small particle diameters.

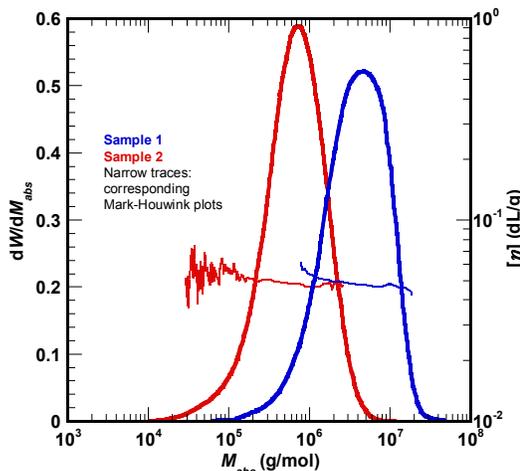


Figure 1. MWDs, based on SEC/DV and universal calibration, and Mark – Houwink plots for a large PNP (1; heavy and light blue traces) and small PNP (2; heavy and light red traces).

### 5 PARTICLE CONFORMATION

The relationship between molecular weight and intrinsic viscosity (the Mark – Houwink relationship) provides information regarding solute conformation. The Mark – Houwink power law relationship is given by,

$$[\eta] = KM^\alpha \quad (3)$$

where  $K$  and  $\alpha$  are empirical constants that depend on polymer composition, solvent, temperature and solute conformation. The product of intrinsic viscosity and molecular weight is proportional to hydrodynamic volume. The Mark – Houwink power-law exponent,  $\alpha$ , is an indicator of solute (or, more accurately in this case, of the solvent swollen suspension-) conformation; the expected value of  $\alpha$  for spherical solutes is zero.[13,14]

Molecular weight and intrinsic viscosity pairs for PNPs can be obtained from the SEC/DV experiment. Examination of the resulting Mark – Houwink plot provides insight regarding particle conformations present in PNP samples. Typical Mark – Houwink plots are shown in Figure 1. Particles containing adequate crosslinker and having a high parent chain molecular weight are very compact; over the higher signal/noise regions of the Mark –

Houwink plots, they are relatively flat (the power-law slope,  $\alpha$ ,  $d\log[\eta]/d\log M$ , is approximately zero), consistent with spherical architecture, even at very small particle diameters.

## 6 COMPARISON TO OTHER METHODS OF SIZE DETERMINATION

**HDC:** A series of relatively large PNPs was characterized by SEC/DV and by HDC. Volume median diameter (median  $d_v$ ) from HDC, and  $d_w$  (or, equivalently, volume average diameter) from SEC/DV are compared in Figure 2. There is good correlation between the SEC/DV-based diameters and the HDC-based diameters. HDC-based diameters of the microlatex tend to be larger than the SEC-DV derived diameters likely because the measurement is performed on the dilute microlatex itself in an aqueous surfactant solution. Thus, the adsorbed surfactants and the electrical double layer will contribute to the measured particle diameter.

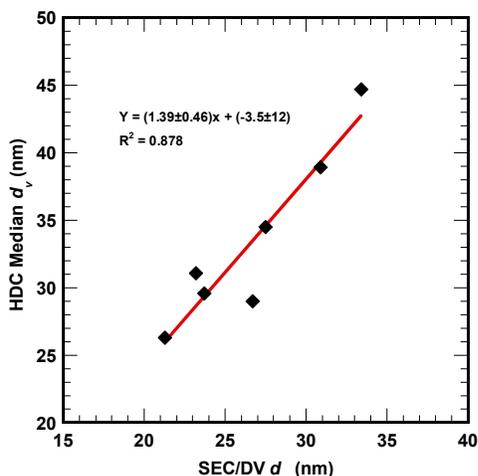


Figure 2. Correlation of SEC/DV-based diameters with diameters determined *via* HDC (median  $d_v$  by HDC *vs.*  $d_w$  by SEC/DV) for a series of PNPs.

**TEM:** The large and small PNPs (**1** and **2**) described above were analyzed by TEM. For sample **1**, a  $d_w$  of  $25.3 \pm 0.5$  nm (standard error of the mean at  $2\sigma$ ) was obtained by SEC/DV, in excellent agreement with the  $25.7 \pm 1.2$  nm volume (weight) average diameter obtained *via* TEM image analysis. Similar good agreement was observed for sample **2**, with SEC/DV providing a solvent-free weight-average diameter of  $12.9 \pm 0.3$  nm *vs.*  $14.6 \pm 0.5$  nm by TEM. (compare Figures 1 and 3).

**SEC/SLS:** Equation (1) was used to calculate  $d_w$  from weight-average molecular weights,  $M_w$ , determined by SEC/DV with universal calibration and SEC/SLS methods. A plot of SEC/SLS-based  $d_w$  versus SEC/DV based  $d_w$  is shown in Figure 4. A good correlation ( $R^2 = 0.997$ ) and

slope of 1.12 were observed between the two measurements.

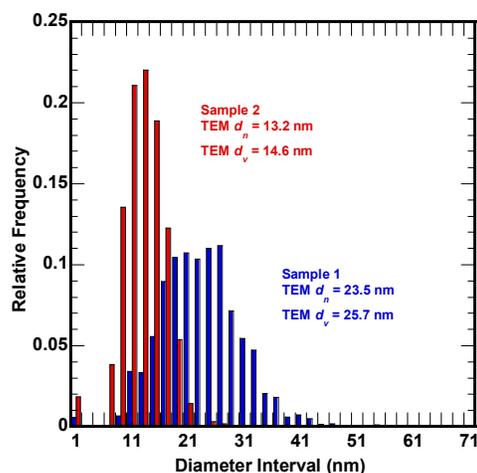


Figure 3. Particle size distribution histograms for particles **1** and **2** determined from TEM.

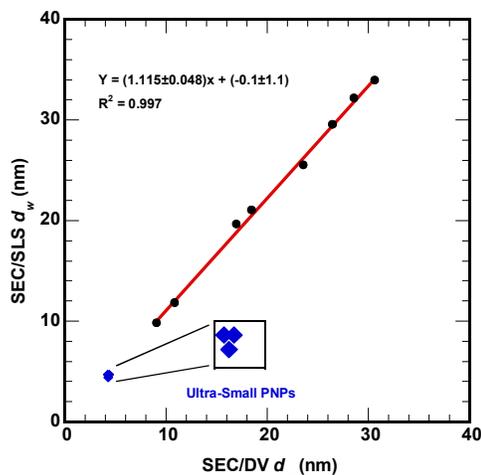


Figure 4.  $d_w$  derived from the  $M_w$  determined by SEC/SLS (SEC/SLS  $d_w$ ) *vs.* the  $d_w$  from SEC/DV (SEC/DV  $d_w$ ) for a series of PNPs.

**DLS:** DLS measurements of PNPs yield  $R_h$  values, and because the measurements were performed in a good solvent, the measurement provides solvent-swollen  $R_h$ . For comparison, SEC/DV  $d_w$  values were converted to solvent-swollen particle diameters by multiplying  $d_w$  by the cube root of the VSF. The resulting “solvent-swollen” diameters were then compared to the hydrodynamic diameters,  $2R_h$ , determined by DLS (Figure 5). Despite one extremely broad MWD outlier, the particle sizes determined by the two techniques correlate very well. Excluding the outlier, the correlation coefficient was 0.997 and the slope was 1.23. The fact that DLS-based sizes were  $\sim 25\%$  larger than the SEC/DV-based sizes is not too surprising because

DLS is sensitive to the  $z$ -average size of the distribution, while SEC/DV results were determined from the weight-average size of the distribution.

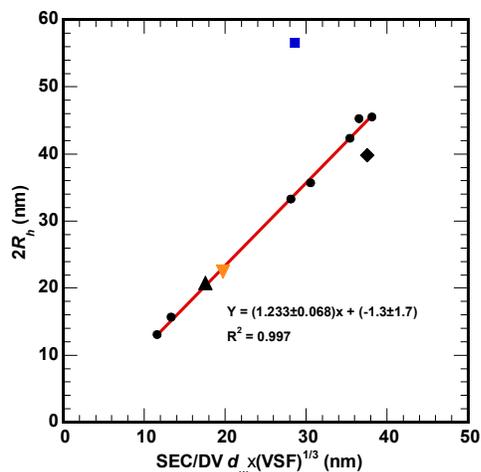


Figure 5. Volume-average solvent-swollen particle diameter from SEC/DV ( $d_w \times \text{VSF}^{1/3}$ ) vs. the hydrodynamic diameter,  $d_h$ , (equivalent to,  $2R_h$ ) determined by DLS. ●: (SEC/DV data collected on ALPHA-M column set; ■: broad MWD PNP; ◆:  $R_h$  determined in GBL; ▼: SEC data collected on a Mixed A column; ▲: SEC data collected on a Mixed B column.

## 7 CONCLUSION

PNPs can be studied in suspension by SEC with concentration and differential viscometric detectors to obtain valuable information about their structure (apparent and absolute molecular weight and size distributions, solvent-swollen suspension conformation and VSF) in a single rapid experiment. SEC/DV based sizes compared favorably to those obtained by DLS, SEC/SLS, HDC, and TEM methods. Both SEC/MALS and SEC/DV revealed volumetric swelling gradients with respect to molecular weight across the particle MWD. Because  $[\eta]$  is nearly independent of molecular weight for most PNPs, one can quickly obtain a rough estimate of the average, or bulk,  $[\eta]$  from the Mark – Houwink plot. The bulk  $[\eta]$  is directly proportional to the VSF and inversely dependent on the level of crosslinking monomer.

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