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SEC-MALS analysis of a pHPMA-based drug delivery polymer

Your Challenge

- You struggle to measure polymer sizes below 10 nm with conventional light scattering.
- You want to analyze complex conformation changes from architecture and drug conjugation.

Our Solution

EcoSEC Elite™ GPC system and LenS™$_3$ MALS detector

- An optimized SEC-MALS solution for MW & R_g determination.

What was done?

- SEC-MALS analysis of pHPMA based homo/copolymers, drug conjugates, and a star-shaped copolymer.

What was the result?

- High-MW pHPMA copolymers are compact, but drug conjugation expands them into random coils.

The LenS$_3$ MALS detector accurately distinguishes pHPMA drug delivery conformations—rod, random coil, and sphere—by providing precise MW and R_g measurements with exceptional sensitivity.

Analysis of drug delivery vehicles

Your Benefit

An easy and trusted solution to elucidate the structure of polymeric drug delivery systems

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Application Note

Characterization of poly(N-(2-hydroxypropyl)methacrylamide) as biocompatible and non-immunogenic polymer for drug delivery

Drug delivery systems involve a broad range of technologies used to transport the active payload to the site of action with the aim of improving therapeutic while minimizing side effects and ensuring controlled release and enhanced bioavailability. One of the strategies to achieve the targeted delivery is to conjugate a pharmacologically active small molecule to a polymer via a stimuli-responsive linker that is stable in the bloodstream but cleavable at the site of action under a specific stimulus (e.g. low pH, reductive environment, presence of cathepsin B, etc.).[1]

Due to their high water-solubility, biocompatibility, and non-immunogenic properties, poly(N-(2-hydroxypropyl) methacrylamide) (pHPMA) copolymers are one of the most studied water-soluble synthetic polymer carriers.[1] pHPMA copolymers with high molecular weight prolong the blood circulation time of conjugated small molecules and increase their accumulation in solid tumors via the enhanced permeability and retention effect.[2] However, the tumor accumulation and the renal elimination of pHPMA-drug conjugates depend strongly on the size, molecular weight, architecture, and rigidity of polymer chains.[3] Therefore, an adequate physicochemical characterization of the designed pHPMA-drug conjugates is crucial for better understanding of their behavior under physiological conditions that determine their in *vivo fate*.[4]

Size exclusion chromatography (SEC) coupled with refractive index (RI) and multi-angle light scattering (MALS) detection is widely used as the technique of choice to better understand structure-property relationships of pHPMA polymers.[5–7] The ability to monitor and correlate size as a function of molecular weight (MW) can help elucidate structural conformation; however, since the single polymer chains for drug delivery can often have a radius of gyration (R_g) below 10 nm, this requires the ability to accurately measure such a low size.[8] Conventional MALS technologies are limited to a detection threshold of approximately 10-12 nm, highlighting the need for a more sensitive MALS detector.

Experimental Conditions:

System:	EcoSEC Elite™ GPC system
Columns:	TSKgel® Alpha-4000, 10 µm, 7.8 mm × 30 cm + TSKgel Alpha-3000, 7 µm, 7.8 mm × 30 cm
Mobile phase:	Methanol/0.3 mol/L Sodium Acetate buffer (80/20), pH 6.5
Flow rate:	0.7 mL/min
Detectors:	RI and LenS™ 3 MALS detector
Software:	SECView™

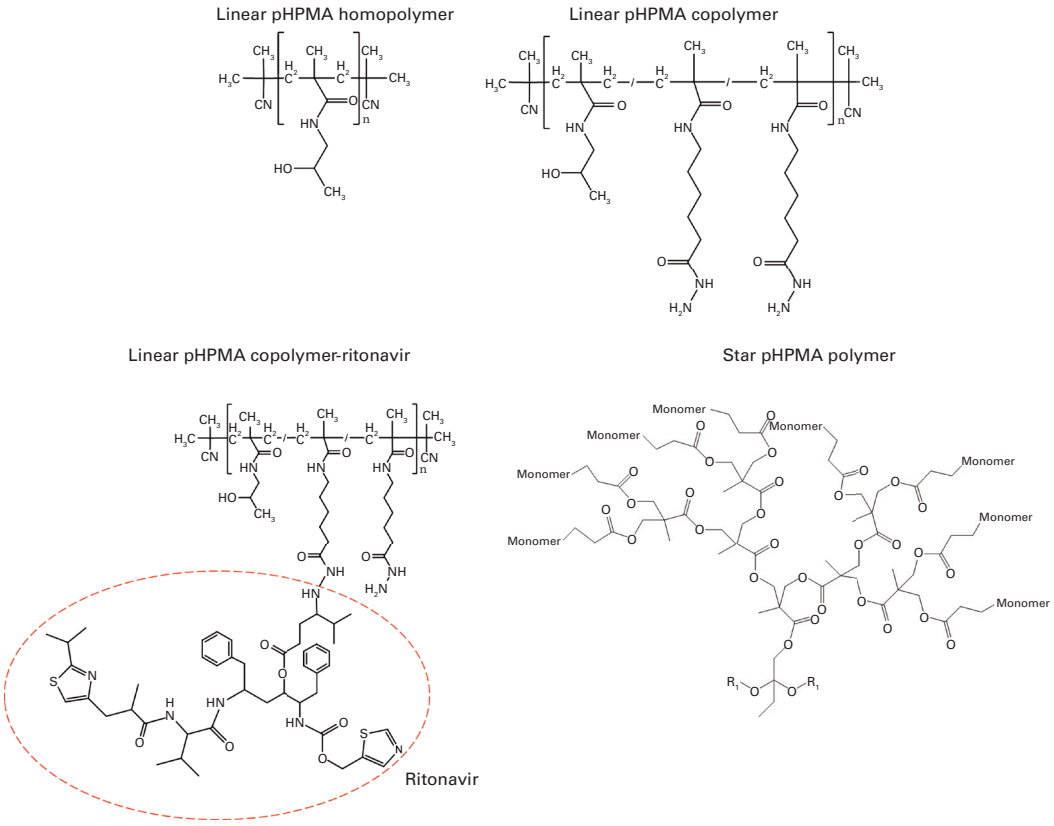
The MALS detector was calibrated using a low polydispersity polyethylene oxide (PEO) standard with a MW of 27 kDa dissolved in mobile phase at a concentration of 1.73 mg/mL with a specific refractive index increment (dn/dc) of 0.145 mL/g and injection volume of 80 µL. To determine the MW, signals from the low-angle light scattering detector at 10° (LALS) were used, whereas the Rg determination was carried out using the signals from all three angles of the LenS3 MALS detector (10°, 90°, and 170°).[9]

The stock solutions of pHPMA samples were prepared by dissolving the polymers in the mobile phase overnight on a shaking platform. The working solutions were then prepared by filtering the stock solutions through a 0.45 µm nylon syringe filter and diluting them with mobile phase to the following concentrations: linear homopolymers pHPMA 1 (2.47 mg/mL), pHPMA 2 (2 mg/mL), pHPMA 3 (1.6 mg/mL), linear pHPMA copolymer (1.93 mg/mL), linear pHPMA copolymer-drug conjugate (2 mg/mL), and star-shaped pHPMA (1.33 mg/mL). A dn/dc value of 0.167 mL/g was used for all pHPMA samples, and hydrodynamic radius (R_h) values were measured by Dynamic Light Scattering (DLS).

Results and Discussion

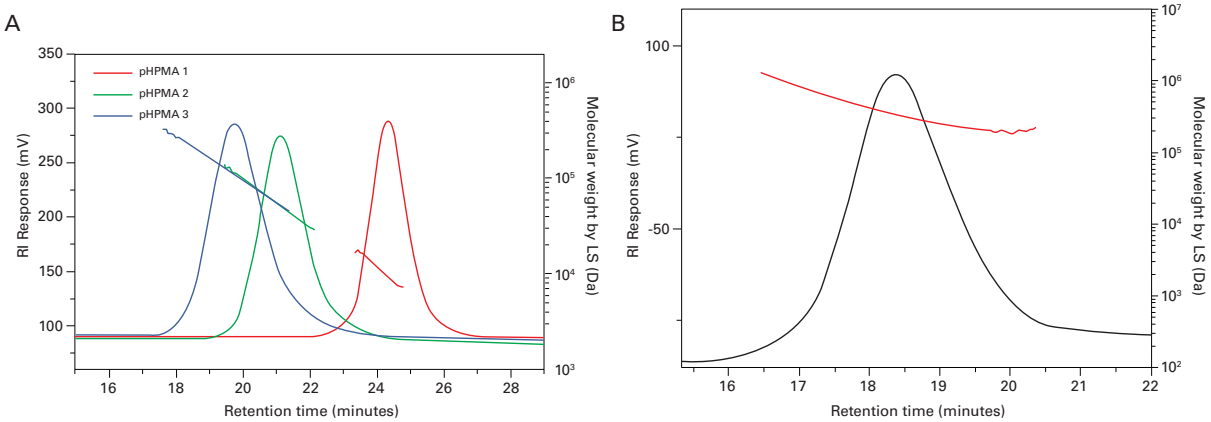
We performed SEC-RI-MALS analyses to characterize the MW and size distributions of six pHPMA-based polymers with distinct architectures and compositions *(Figure 1)*. These included three linear pHPMA homopolymers with different MW ranges (pHPMA 1, pHPMA 2, pHPMA 3), a linear pHPMA copolymer, a linear pHPMA copolymer with drug conjugated through a pH sensitive linker (ritonavir - potent anti-tumor activity *in vivo* via inhibition of proteasome and STAT3 signaling reference), and a star-shaped pHPMA polymer.

Figure 1. Structure of linear pHPMA homopolymer, pHPMA copolymer, copolymer-drug conjugate (pHPMA-ritonavir), and star pHPMA polymer



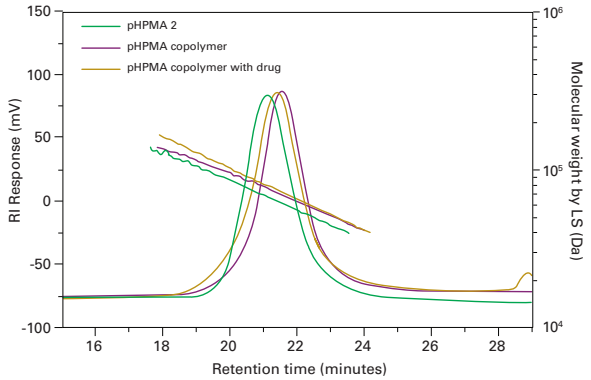
The SEC–RI–MALS data revealed clear differences in weight average molecular weight (M_w) and R_g among the samples, reflecting the influence of molecular architecture and drug conjugation on polymer conformation. The continuous decrease in M_w with increasing retention time for three pHPMA homopolymers and star-shaped pHPMA copolymer confirms a good SEC separation of the macromolecules without any secondary interactions *(Figure 2)*.

Figure 2. A) RI chromatograms and absolute M_w distributions of linear pHPMA homopolymers B) RI chromatogram and the absolute MW distribution of pHPMA star copolymer



We observed earlier elution of the linear homopolymer pHPMA 2 compared to the linear pHPMA copolymer, even though the latter has a higher M_w than the homopolymer *(Figure 3)*. This elution behavior can be attributed to the R_h values of pHPMA 2 and pHPMA copolymer (5.25 and 5.03 nm, respectively) *(Table 1)*. The linear homopolymer (pHPMA 2) consists of only HPMA monomers (N-(2hydroxypropyl)-methacrilamide), whereas the copolymer additionally contains N-(6-hydrazinyl-6-oxohexyl)-2-methylprop-2-enamid as a second monomer *(Figure 1)*. The hydrazone can react intramolecularly, allowing the structure of the linear copolymer to get more compact. As a result, its size is smaller than the homopolymer, despite having a higher M_w, and thus the linear copolymer elutes later than the linear homopolymer (pHPMA 2). We also observed that the R_h value of the copolymer-drug conjugate is 5.15 nm (*Figure 3*, yellow), leading to its elution between the linear homopolymer pHPMA 2 (5.25 nm, *Figure 3*, green) and the linear pHPMA copolymer (5.03 nm, *Figure 3*, purple).

Figure 3. RI chromatogram and M_w distribution of pHPMA 2, pHPMA copolymer, and pHPMA copolymer-drug conjugate



To elucidate the conformation of the analyzed polymers, we calculated the ratio of R_g to R_h *(Table 1)*. The R_g/R_h values of the two homopolymers pHPMA 2 and pHPMA 3 (1.048 and 1.089, respectively) are lower than that of pHPMA 1 (1.25), indicating that an increase in M_w of pHPMA homopolymer leads to a change of solution conformation from random coil to more compact structures.[10,11]

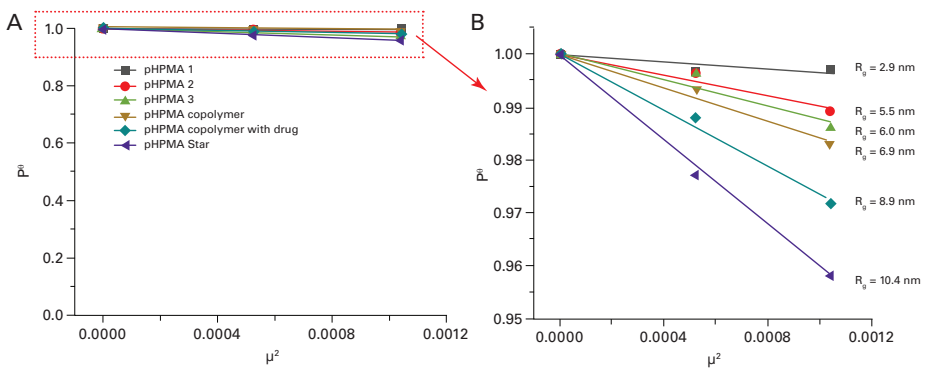
Moreover, conjugation of drug to a pHPMA homopolymer increases the R_g/R_h from 1.194 to 1.34, which is indicative of a random coil structure. This R_g/R_h increase could be attributed to the large size of the drug molecule, which expands the compact copolymer structure and impedes the non-covalent intramolecular interactions. Moreover, we obtained an R_g/R_h ratio of 0.87 for the star-shaped pHPMA copolymer, which agrees with a compact spherical structure.

Table 1. Molecular weight and size characteristics of the analyzed polymers

	Molecular weight by LS		Size			
Sample	**M_w (kDa)**	**CV (%)**	**R_g (nm)**	**CV (%)**	**R_h (nm)**	**R_g/R_h**
Homopolymer pHPMA 1	9.8	1.3	2.9	2.3	2.33	1.247
Homopolymer pHPMA 2	52.1	0.5	5.5	0.7	5.25	1.048
Homopolymer pHPMA 3	109.8	0.3	8.9	2.8	8.17	1.089
Copolymer pHPMA	51.4	0.5	6.0	0.6	5.03	1.194
Copolymer pHPMA-drug conjugate	61.4	2.1	6.9	1.2	5.15	1.340
Star pHPMA copolymer	361.9	0.5	10.4	1.8	11.93	0.872

Understanding the structural differences of pHPMA polymers via SEC-MALS characterization is challenging primarily due to their small size in solution. The difference in scattered light intensity at the different angles of observation for polymers with R_g below 10 nm in solution is small and can only be detected by a highly sensitive MALS detector with very low baseline noise at extremely low (<10°) and extremely high (>170°) angles. We demonstrated the sensitivity of the LenS3 MALS detector by providing the angular dissymmetry plot[9] obtained for all the samples *(Figure 4)*.

Figure 4. Angular dissymmetry plot for R_g determination of pHPMA polymers and its drug-conjugate.



The particle scattering function (Pθ) value describes the ratio of the light intensity at a given angle (10°, 90°, and 170°) to that at the reference angle (10°), illustrating the change in Pθ used to calculate R_g. For example, in the case of pHPMA 1, which has the lowest M_w and R_g, the difference in Pθ between the 10° and the 170° detector is only 0.3%, a value that is not detectable with a conventional MALS design. Using the LenS3 MALS detector, we were able to observe this difference and determine a size of 2.9 nm with good confidence (CV = 2.3%). Moreover, we clearly observed that – as expected from the theory - the Pθ values calculated from the three detector angles decreased significantly with increasing polymer size, a difference that was readily detectable with the LenS3 MALS detector *(Figure 4)*.

Conclusions

In conclusion, the SEC-RI-MALS analysis revealed important insights about the structural characteristics of various pHPMA polymers, including homopolymers, copolymers, copolymer-drug conjugates, and a star-shaped pHPMA copolymer. The results demonstrated effective SEC separation based on molecular weight, with distinct elution behaviors influenced by the physical properties and conformational changes of the polymers. Furthermore, the high sensitivity of the LenS3 MALS detector enabled the successful differentiation of polymers with R_g values below 10 nm. Overall, this study highlights the importance of implementing effective advanced detection methods to determine molecular weight, size and polymer structure, for better understanding of the behavior of pHPMA polymers and their drug-conjugates in solution, which ultimately influence their *in vivo* fate.

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