Monitoring Single-chain Nanoparticle Formation and Characterization of Nanoparticles using the EcoSEC® GPC System

EcoSEC GPC System
APPLICATION NOTE

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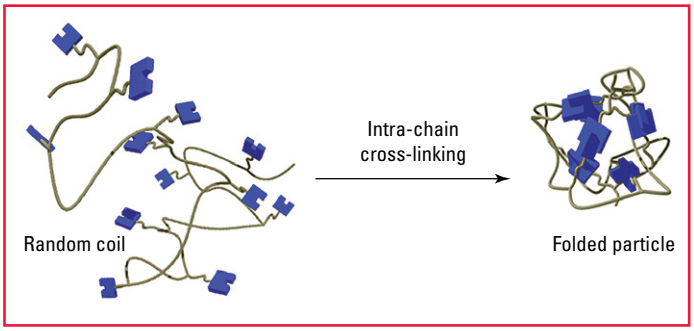
Introduction

Single-chain polymer nanoparticles (SCNPs) are well-defined linear polymers folded into nanostructures of defined architecture which can be used for tasks such as catalysis, sensors and nanomedicine. The general scheme to forming SCNPs is to take a linear polymer as a random coil with functionalities that allow cross-linking, and induce intra-chain, cross-linking to form a folded particle, as shown in the cartoon in *Figure 1*. The polymer can either require an external cross-linker or the cross-linking agent can be included in the polymer backbone as pendant functional groups. In order to ensure intramolecular cross-linking over intermolecular cross-linking, the cross-linking reaction is done under ultra-dilute conditions.

Figure 1. The transition from random coil to a folded nanoparticle



The folding and unfolding of the SCNPs from the random coil to globule structure can either be reversible or irreversible depending on the chemistry behind the cross-linking reaction. SCNPs can be formed using a variety of methods utilizing many different types of chemistry. For example, poly(norbornene-exo-anhydride) (P1) can be synthesized, *via* ROMP using third generation Grubbs catalyst as an initiator and the degree of collapse that occurs during nanoparticle (N1) formation can be controlled by varying the amount of difunctional cross-linker added.

The formation of SCNPs from the polymer coils can be characterized using gel permeation chromatography (GPC), based on polymer size and molar mass. GPC, a separation method which separates molecules based on their size in solution, is ideal for analyzing the nanoparticle formation as the size in solution of the random polymer varies from that of the collapsed nanoparticle, thus resulting in a variation of GPC retention times and elution profiles. GPC can also be used to monitor the unfolding of the SCNPs as the SCNPs can reversibly undergo a coil to particle transition through the formation and cleavage of intramolecular disulfide cross-links. Here we report the use of single and multi-detector GPC analysis, using the EcoSEC GPC System coupled to various detection methods for the monitoring of the folding and unfolding of SCNPs.

Experimental

Sample analysis was performed on an EcoSEC GPC System (HLC-8320) equipped with a dual flow refractive index (RI) detector coupled in series to a multi-angle light scattering detector, MALS (Wyatt miniDAWN™ TREOS®). Unfiltered 25 µL injections of 1 mg/mL samples occurred over a column bank consisting of two 4.6 mm ID x 15 cm, 4 µm particle size TSKgel® SuperMultiporeHZ-M columns (Tosoh Bioscience LLC). The solvent and mobile phase were tetrahydrofuran (THF) (Fisher Chemical) at a flow rate of 0.35 mL/min. Detector, pump oven, and column oven were maintained at 35 °C.

The miniDAWN detector was normalized in-house using a polystyrene standard. Calculation of interdetector delays and interdetector band broadening correction were performed using polystyrene. Calibration of the MALS unit was performed using toluene. The differential refractive index increment values (dn/dc) were calculated online assuming 100% mass recovery (RI as the concentration detector) using the Astra 6.0 software package (Wyatt Technologies). Absolute molar mass and molar mass distributions were calculated using the Astra 6 software package. Relative molar masses were obtained against polystyrene standards (PStQuick MP-M, Tosoh Bioscience) and calculated using the EcoSEC Workstation software.

Results and Discussion

As mentioned in the "Introduction," SCNPs can reversibly undergo a coil to particle transition via formation and cleavage of intramolecular disulfide cross-links.[1] The coil to particle transition of poly(norbornene-*exo*-anhydride) (P1), synthesized *via* ROMP using third generation Grubbs catalyst as an initiator was collapsed into nanoparticles (N1) of different degrees by varying the amount of difunctional cross-linker added. The coil and particles were then characterized using the EcoSEC GPC System with dual flow RI via polystyrene relative molar mass averages. *Figure 2* shows a series of GPC traces for P1 and its corresponding N1 after various extents of intramolecular cross-linking. As expected, an increase in GPC retention time is observed as the intramolecular cross-linking reaction progresses. The increase is GPC retention time is due to a decrease in hydrodynamic volume that occurs as the coil collapses, as the elution order in GPC is that of an "inverse-sieving" technique, smaller analytes sample a larger pore volume than larger analytes resulting in the smaller analytes eluting from the GPC column later than the larger analytes.

After confirmation of the nanoparticles formed by disulfide bridges was confirmed via the GPC retention times, redox chemistry was employed to reduce the disulfide linkages to corresponding thiols, thus unfolding the nanoparticle, N1, back to the polymer in a coil conformation, uN1. Using catalytic amounts of ferric chloride, the thiols were oxidized to re-fold the polymer to a globule-like conformation. The transition from particle to coil was confirmed via a decrease in GPC retention time, signifying an increase in hydrodynamic volume, as shown in *Figure 3*. The unfolded nanoparticle returns to the original size and conformation based on the identical trace of the GPC elution profile.

Figure 2. GPC elution profile as monitored by the RI for the polymer and nanoparticles formed with varying amounts of cross-linker added

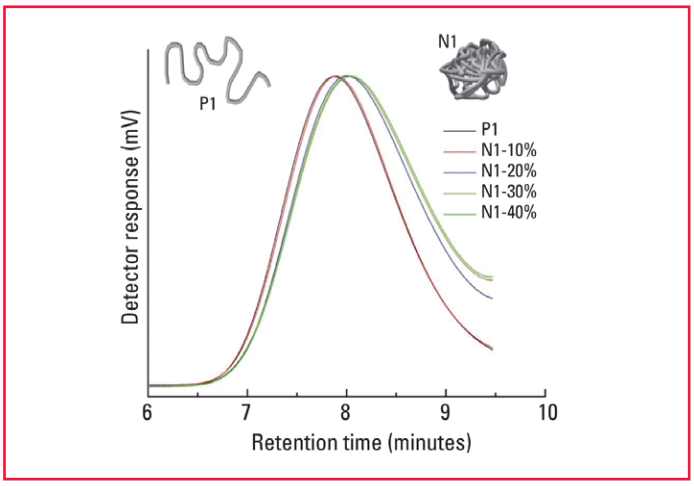


Figure 3. GPC elution profile as monitored by the RI of the original polymer with unfolded nanoparticles

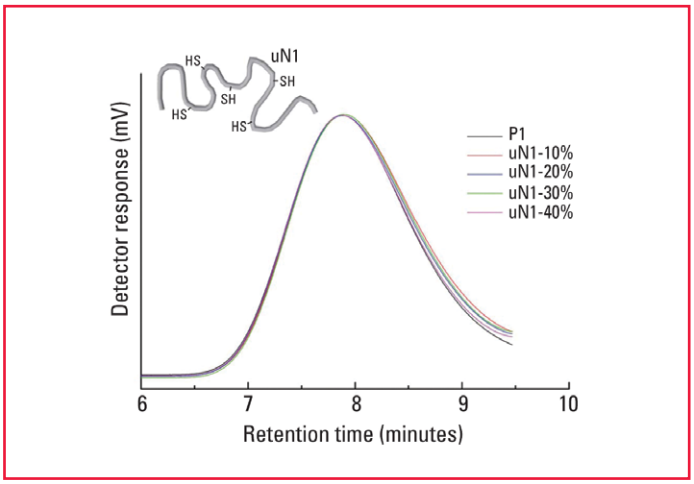
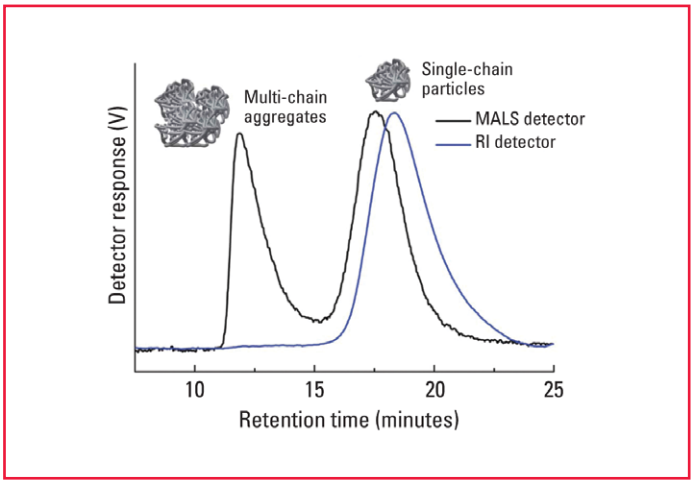


Figure 4. GPC elution profile as monitored by the MALS (black) and RI (blue) detectors for the SCNP



The next part of the SCNPs study was to differentiate between single-chain particles and aggregates of a few chains and to determine the absolute molar mass via dual-detector GPC (GPC/RI/MALS). Copolymerizing the anhydride monomer previously used with cyclooctadiene (COD) allowed control and a way to predict the amount of mass that should be added upon application of the folding mechanism. The folding reaction was repeated with the same cross-linking agent in excess and for an extended reaction time to encourage a small amount of intermolecular coupling. *Figure 4* shows an overlay of the MALS and RI traces when the intramolecular cross-linking reaction was extended with a slight excess of the cross-linker to encourage intermolecular coupling. The RI detector shows a single peak that can be attributed to single-chain particles, while the MALS detector shows two peaks of nearly equal intensity. The single peak shifted to longer retention time in the RI trace can be attributed to the single-chain particles that were formed. The first peak in the MALS trace, with the shorter retention time, represents the small amount of multi-chain aggregates that formed. Light scattering is very sensitive to larger objects and they scatter light to a greater extent and therefore will give a stronger signal than something smaller in size. RI, on the other hand, is concentration sensitive. Therefore, the lack of the earlier eluting peak in the RI trace portrays the low concentration of multi-chain aggregates that formed compared to the much higher concentration of the single-chain particles. For this particular sample analysis, single-detector GPC would not have revealed the presence of the larger aggregates.

Conclusions

The transition of a random coil polymer to and from a folded single chain nanoparticle was monitored using the EcoSEC GPC System with a refractive index detector coupled to a multi-angle light scattering detector. The GPC elution profile was used to monitor the reaction progress and limitations of disulfide linkages formed. The formation or folding of the SCNPs results in an increase in GPC retention time as a direct result of a decrease in polymeric size. On the other hand the unfolding of the SCNPs back to their original random coil formation results in an increase in GPC retention time as the polymeric size is increasing. The dual detector set up was also used to monitor changes in the GPC elution profile when an intramolecular cross-linking reaction was extended with a slight excess of the cross-linker to encourage intermolecular coupling. The intermolecular coupling resulted in a single RI peak, while the MALS detector shows two peaks. The peak present in the MALS detector but not the RI detector is a result of a very low concentration of multi-chain aggregates and thus only revealed through a dual-detector GPC set up (GPC/RI/MALS). The SCNP experiment showed the versatility of the EcoSEC GPC System when coupled to MALS to determine polymer chain folding and unfolding, and interchain versus intrachain coupling.

References

[1]Tuten, B.T.; Chao, D.; Lyon. C.K.; Berda, E.B. *Polym. Chem.*, ***2012***, *3, 3068-3071*

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