Method Development Using a Multi-Angled Laser Light Scattering System for Characterization of Poly(ethylene)glycol Derivatives

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Method Development Using a Multi-angled Laser Light Scattering System For Characterization of Poly(ethylene)glycol Derivatives

Kerry J. Townsend

Advisors: Dr. Richard Congo, Shearwater Corp., and Dr. Carmen Scholz, UAH Chemistry Department
Abstract

Just within the last two decades, light scattering (LS) has tried to meet its applicable predictions and analytical capabilities for determining the weight-average molecular weight ($M_w$) for all molecules in solution. Progress includes LS differential systems, or multi-angled (2-18 angles) detectors. This project focused on developing an multi-angled (three detector) laser light-scattering (MALLS) system to determine the molecular weight of unknown impurities in poly(ethylene)glycol (PEG) species. This paper summarizes the instrument mechanics of the MALLS system as well as some of its advantages over Gel Filtration Chromatography (GFC) systems for determining absolute molecular weight of PEG species independent of column matrix interactions.
Theory

In nature, light scattering is evident as one looks up at the blue sky or sees an amazing red sunset. In fact, it was to answer this blue sky question that propelled Lord Raleigh, in 1871, to develop a theoretical description of light scattering.\(^1\) When light hits matter, most of the light continues in its original direction, whereas the rest of the light is scattered into new directions. Therefore, this scattering volume is related to properties of the matter.

In the modern laboratory, the conditions to retrieve information from light scattering are controlled by using lasers. In lasers, the wavelength, polarization, and intensity of incident light are manipulated. Furthermore, the size of the laser beam and the field of view of the detector can define a scattering volume.\(^1\) One can measure the scattered light \(I_s\) from this volume as the function of the angle and polarization (figure 1).

![Function of light scattering. Picture from Wyatt Technologies.\(^2\)](image)

There are many equations important in converting light scattering data for applicable investigations. The theory of light scattering was formulated and developed by Einstein, Raman, Debeye, Zimm, and many others. However, the most important equation for light scattering data is the Zimm Formalism (Figure 2). In the Zimm Formalism, the Raleigh, Gans, and Debeye equations are used to facilitate linearity between the excess scattered light or Raleigh ratio \(R(\theta)\), concentration \(c\), and weight-average molecular weight, \(M_w\).\(^2\) \(K*c\) is the index of refraction and \(A_2\) is the second virial coefficient which measures molecule-solvent interactions. The other elements to this equation include \(P(\theta)\), the form factor, which
describes the angular variation of the scattered intensity as function of particle size. The form factor is an integration over phase shifts from the extended particle, and thus, is a sine function based on the radius of gyration. The radius of gyration, or root mean square (rms), is the measure of the size of the particle, relating to the mass distribution of the particle about a point, not an axis (Figure 2). Therefore, by knowing only the concentration of the sample solution, its index of refraction, and the intensity of the scattered light, the molar mass of a molecule in solution is calculated.

Figure 2: (1) the Zimm Formalism. (2) the radius of gyration.

\[
\frac{K \cdot c}{R(\Theta)} = \frac{1}{MwP(\Theta)} + 2Ax
\]

\[
\langle r^2 \rangle_w = \sum \left( \frac{c_i \langle r^2 \rangle_i}{\sum c_i} \right)
\]

Experimental Procedures

This project consisted of investigating and developing methods to determine the molecular weight of unknown impurities in polyethylene glycol (PEG) species using an multi-angled laser light-scattering (MALLS) system. There are three goals of the project:

- Installing the miniDAWN MALLS detector to a Hewett Packard Model 1100 HPLC system equipped with a model 1100 Refractive Index Detector (RID),
- Developing analytical methods for determining molecular weight (Mw) and polydispersity of PEG-derivatives in accordance with the International Conference of Harmonization (ICH) and Federal Drug Administration (FDA) guidelines,
- Investigating PEG-derivative stability samples for the presence of degradants due to time, temperature, and environmental storage conditions. The degradants include chain cleavage of the PEG backbone, auto-oxidation, conversion to PEG acids, charge neutral compounds, and side reaction species.

The figure below shows the monomer of PEG. A polymer has at least five repeating monomers. Shearwater Corporation specializes in making PEG derivatives for coating of medicinal drugs (PEG is very site-specific).
1. **Installation of the miniDAWN**

Installation of the miniDAWN MALLS detector followed the instructions outlined in the *Light Scattering Conference Guide* written by the miniDAWN manufacturer, Wyatt Technologies Corporation. Toluene was used to determine the instrument constant. First, a 10mL Teflon syringe was filled with Drisolve toluene and using a syringe pump, volume was set to flow 0.5mL/min. Tubing was installed so that toluene flowed directly into the miniDAWN, and out into a waste beaker. The miniDAWN is controlled (the laser is turned on and off) by its parent software, “ASTRA.” Turning on the Astra software turns on the laser, but there is a 30-minute instrument warm up. One problem of the system was pressure build-up. The miniDAWN must be kept extremely clean; any dust particles can distort molecular weight readings with significant error. Therefore, on the end of the syringe a 0.02 µm filter was attached (different filters correspond to different solvents). This backflow pressure on the syringe pump was decreased by increasing the tubing width, and making sure all tubing within the system were the same width. As for determining the constant, the process was software controlled. The detector takes readings (based on the dn/dc value of toluene), returns the constant, and gives the error from the three detectors (angles). The instrument constant is very important because it converts the detector voltages into Raleigh ratios for the various angles, in this case 45°, 90°, and 135°. The Raleigh ratio is then used to compute the molecular weight (Mₜ) of the solute molecules. Therefore, the instrument constant should be within 5% of the constant reported by the manufacturer.

Manufacturer’s calibration constant: \[7.862 \times 10^{-6}\] @ 690 nm

Our calibration constant: \[7.205 \times 10^{-6}\] @ 690 nm

As evident, the calibration constant was not within the 5% guideline. However, Wyatt Technologies approved the constant.

II. **Refractive Index Calibration**

In addition to the MALLS calibration and nominalization, the Refractive Index Detector (RID) was calibrated to determine the inner delay volume. A set of concentrations of NaCl (AR022712) in water (SWP-12) flowed through the laser and the RI detector. The specific refractive index increment (dn/dc) of NaCl (0.1100) was used to compute the
calibration constant and to find the delay volume. Figure 3 shows the spectra from the miniDAWN, demonstrates the linearity of the concentrations, and gives the calibration constant in 1/volts. The delay volume was 0.250 ml.

![Calibration Curve](image)

**Known dn/dc (mL/g)**: 0.1100
**Calibration constant (1/volts)**: 3.5007e-04 ± 2.1e-06

III. **Implementation of Standards**

Once the miniDAWN was installed and operational, developmental methods for standards were established and qualified. This data was documented in an Analytical Method Report (AMR) and approved by both the Quality Control and Quality Assurance Departments for implementation as a Product Analytical Method (PAM) indicative to Shearwater Corporation, an Inhale Company. The standards used were PEG and PEO polymers ranging from 4,120 to 55,600 Dalton (Figures 6 and 7).

IV. **Investigation of Stability Products**

Information gathered from the stability program was the last goal of the project and the most challenging. Products in stability studies retain a strict storage schedule and
analytical studies are only allotted at specific times during the stability run time. The miniDAWN MALLS system has been an effective tool in investigations of stability products and identifications of impurities.

**Advantages of MALLS**

In Gel Filtration Chromatography (GFC) and Size Exclusion Chromatography (SEC) systems, compounds that disperse quickly through the columns are characterized as high molecular weight species. The theory is based on diffusion: larger molecules diffuse slower than small ones, thereby the larger molecules will not interact with the column and leave first. However, these elution readings depend upon more than just size of the compound; they depend on the shape and tendency of the species to interact with the column matrix (Figure 4). When a size exclusion chromatograph (SEC) is calibrated correctly, the molecular weight of a polymer is based on the time it takes to pass, or elute through to column. Henceforth, this is the major drawback of SEC/GFC systems. In such systems, the mass of what is in solution is not measured; instead the hydrodynamic volume of the polymer molecules is measured. The hydrodynamic volume is how much space a particular polymer molecule takes up when in solution. The molecular weight from SEC/GFC data is an approximation using the standards' (for example, polystyrene) hydrodynamic volumes and molecular weights to approximate the molecular weight for the sample polymer. Therefore, some compounds may not be fully characterized by an elution position, and to determine their molecular weight — independent of column matrix interactions — involves the use of a light scattering detector.³

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**Figure 4:** How SEC columns work. Picture from [http://www.chem.vt.edu/chem-ed/sep/le/size-exc.html](http://www.chem.vt.edu/chem-ed/sep/le/size-exc.html).
The miniDAWN light scattering data provides (particularly using Astra software) characterization of molecules in solution. The most important feature of the MALLS system is the determination of the molar mass moments. Number average ($M_n$) relates the brittleness, flow properties, and compression set of the molecule; weight average ($M_w$) gives molecular weight, strength properties, tensile, and impact resistance; z-average ($M_z$) relates the elongation, and flexibility properties of the molecule.\(^2\) In addition, the polydispersity hints at PEG degradation. Another feature is the Debeye plot, which manipulates the Zimm formalism to give the molecular weight and root mean square radius for a single slice on the curve (Figure 5). In this plot, $1/M_w$ is equal to the y-intercept, and the initial slope is equal to the root means square (rms) radius.

![Figure 5: Data from miniDAWN and Astra Software of 55600 Dalton PEO: (a) Strip chart of laser (red) readings and refractive index (blue). Laser detector measured voltages per volume of solution. (b) Debeye Plot.](image)
In addition, the Astra software returns 3-D plots (Figure 6), which, when not Gaussian, give indications of the presence of stray light, laser misalignment, problems with nominalization and baseline, and existence of a dirty flow cell.\textsuperscript{2}

![Figure 6](image)

Furthermore, molar masses versus elution volume plots are available to find molar mass distribution of samples. This plot (Figure 7) demonstrates the scattering data for PEG and PEO standards as a function of volume. As evident, the least amount of scatter was in the highest standard (55600 PEO). Finally, the cumulative molar mass is shown in molar mass versus weight fraction plots (Figure 8). This plot shows what percentage of the standard is at a specific molar mass.

![Figure 7](image)
Conclusion

Without the MALLS system, the elution order from SEC/GFC can lead to misleading assumptions about molecules in solution. For example, PEG acids typically elute rather quickly due to a charge effect on the matrix, and this early peak will give an apparent molecular weight several orders higher than the starting PEG derivative. MALLS helped to elucidate the true molecular weight without regards to elution order and thereby helped researchers identify and quantify these impurities.

This project’s three goals merged into an overall development of an important research tool for Shearwater Corporation. From qualitative and quantitative analytical method development, stability assessment, to potential usage in the manufacturing scale-up studies for optimization of reagent quantities and operational parameters, the miniDAWN MALLS
system provides abundant, necessary information for characterization of PEG derivatives and degradation.

Acknowledgements

I would thank Dr. Richard Congo, Dr. Carmen Scholz, and Shearwater Corporation (an Inhale Company) for their guidance and support of this project.

References


