# Molar Mass Determination of Water-Soluble Light-Emitting Conjugated Polymers by Fluorescence-Based Analytical Ultracentrifugation

**Xiao-Tao Hao,[†,§] Tim Ryan,[‡,§] Michael F. Bailey,[‡] and Trevor A. Smith*[,†]**

*School of Chemistry, The University of Melbourne, Victoria 3010, Australia, and Department of Biochemistry and Molecular Biology and The Bio21 Molecular Science and Biotechnology Institute, The University of Melbourne, Victoria 3010, Australia*

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ABSTRACT: Determining the molar mass of conjugated polymers has proven difficult in many situations using conventional means, and this is often exacerbated in the case of polyelectrolytes. In this paper we report on a new method of determining the molar mass of soluble, light-emitting conjugated polymers by coupling emission-based detection with analytical ultracentrifugation. This method is shown to be capable of recovering accurate molar masses with high sensitivity, without the need for standards, and can also identify aggregation behavior.

## 1. Introduction

$\pi$-Conjugated polymers are a most promising class of materials able to serve as primary active components for the development of plastic electronics because of their excellent electrical and optical properties, reasonable chemical stability, and easy processability.[1−3] The optoelectronic properties of one-dimensional conjugated $\pi$-electron systems are highly dependent on their chain length, i.e., the molar mass.[4] However, the determination of the molar mass of conjugated polymers, especially water-soluble polymers, is challenging due to limitations of the various conventional techniques including gel permeation chromatography (GPC),[5] nuclear magnetic resonance (NMR) spectroscopy,[5] matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry,[6,7] and static[8,9] or dynamic light scattering. Briefly, GPC is the most commonly used technique for determining the molar mass distribution and thereby calculation of both the number-average molar mass ($M_n$) and the weight-average molar mass ($M_w$). However, the lack of suitable calibration standards greatly handicaps utilization of this technique for some new types of polymeric species. This is especially troublesome for water-soluble polymers which require different columns and calibration standards to the more conventional organic-soluble polymers for which standards such as poly(styrene) can be used as the calibrant. Determination of polymer molar mass by GPC is further hampered for polymers that undergo interactions with the column, for example, for charged or strongly adsorbing polymers, such as that used in this work. NMR spectroscopy is limited to lower molar masses although it can be used to determine $M_n$ for polymers with defined end groups. MALDI-TOF mass spectrometry, which in principle can determine molar masses accurately, is also limited to rather low molar mass polymers, and weakly linked assemblies can be easily broken during the measurement. A suitable matrix for the given polymer must also be predetermined. Light scattering methods can be used to determine polydispersity of macromolecules, but the strong light absorption in these assemblies can complicate the analysis of the light scattering data for determination of the $M_n$.

Despite of the power and proven performance of these conventional techniques applied to a wide range of polymers, their disadvantages can be overwhelming for conjugated polyelectrolyte systems. Alternatively, analytical ultracentrifugation,[10−15] which has been developing in the past few years, can provide the absolute molar mass of species over a large range from hundreds up to several million g/mol. It also provides the quantitative information on molecular shape, polydispersity, reversible macromolecular interactions, and thermodynamic nonideality. Analytical ultracentrifugation is widely used for the analysis of biological macromolecules, and since the study of weakly linked assemblies and interactions of the species with matrices or surfaces are not limiting factors, this technique is expected to be an effective technique to determine the molar mass of water-soluble conjugated polymers. However, it is currently still underutilized in the analysis of synthetic polymers[7,10] partly due to its lack of sensitivity.

Analytical ultracentrifuges can be equipped with a variety of optical systems with which to measure the sedimentation profiles. Many $\pi$-conjugated polymers, including that under discussion in this paper, have the added feature of being photoluminescent. This enables the exploitation of the exquisite sensitivity and spectral selectivity of fluorescence-based detection to be coupled with analytical ultracentrifugation, which overcomes the traditional limitations in terms of sensitivity of this technique in the determination of molar masses of photoluminescent conjugated polymer materials. In this paper, we report the first application of fluorescence-based analytical ultracentrifugation for the determination of the molar mass of a water-soluble light-emitting conjugated polymer, namely [poly(2,5-dipropoxysulfonato-*p*-phenylene)vinylene] (DPS-PPV). We show that this novel coupling of techniques provides an ideal alternative method for the elucidation of the molar mass of photoluminescent materials for which more conventional methods have proved difficult or ineffective.

## 2. Experimental Details

The DPS-PPV was synthesized in our laboratory.[16] Solutions of DPS-PPV in $H_2O$ or $D_2O$ with various concentrations were prepared for measurements. The absorption and fluorescence spectra were measured using Cary Bio50 UV−vis and Eclipse fluorescence spectrometers, respectively.

Sedimentation experiments were conducted using an Optima XL-A analytical ultracentrifuge (Beckman Coulter Instruments, Inc., Fullarton, CA), an An-Ti60 rotor, and double-sector 12 mm path

* Corresponding author: E-mail: trevoras@unimelb.edu.au.
[†] School of Chemistry.
[‡] Department of Biochemistry and Molecular Biology and The Bio21 Molecular Science and Biotechnology Institute.
[§] X.T.H. and T.R. contributed equally to this paper.

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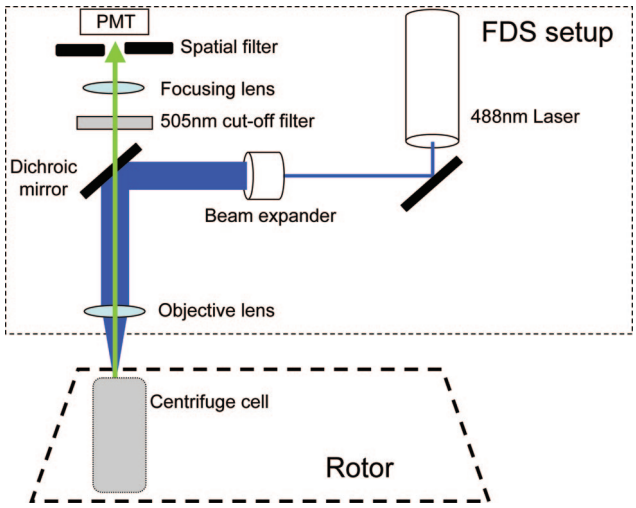


**Figure 1.** Schematic diagram of Beckman Optima XL-A analytical ultracentrifuge.

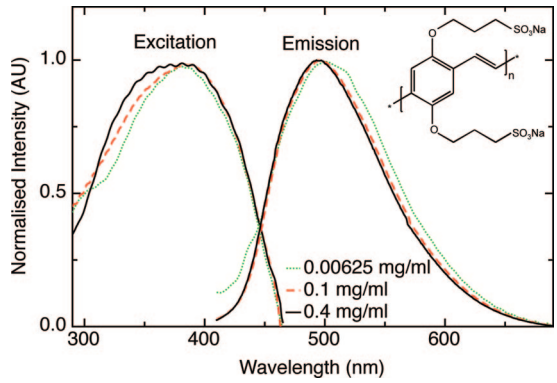


**Figure 2.** Normalized excitation ($\lambda_{em}$ = 495 nm) and fluorescence emission ($\lambda_{exc}$ = 380 nm) spectra of DPS-PPV in aqueous solution with different concentration of 0.4, 0.1, and 0.006 25 mg/mL. The inset shows the chemical structure of DPS-PPV.

length cells containing sapphire windows and charcoal-filled Epon centerpieces. A fluorescence detection system (FDS, Aviv Biomedical Inc.) was adopted to monitor the fluorescence of the DPS-PPV solutions, using a 488 nm, 13 mW solid-state laser and confocal optics to excite a small spot in the solution column. The emission is directed through a 520 nm long-pass filter before detection by a photomultiplier tube, the output of which was digitized[11] to measure the sedimentation profiles. The evolution of the sample concentration versus the axis of rotation profile can be observed as a result of the applied centrifugal field. These observations are then stored for further mathematical analysis. Sedimentation equilibrium and velocity measurements were carried out to determine the partial specific volume of the species in the DPS-PPV aqueous solutions and subsequently provide the continuous distribution of molar masses. A set of intermediate profiles is recorded during the sedimentation process in the sedimentation velocity measurement, while the sedimentation−diffusion equilibrium profile is analyzed in the sedimentation equilibrium measurement. A schematic diagram of the apparatus is shown in Figure 1.

## 3. Results and Discussion

DPS-PPV (inset of Figure 2) is a water-soluble PPV derivative with sulfonate-containing side chains that has a relatively high emission quantum yield compared to the other water-soluble PPV derivatives such as MPS-PPV [poly[2-methoxy-5-sulfopropoxy]-1,4-phenylenevinylene][16] and comparable with the more commonly studied organic-soluble poly[2-methoxy-5-(2′-ethylhexyloxy)-1,4-phenylenevinylene] (MEH-PPV). Figure 2 illustrates the fluorescence excitation and emission spectra of DPS-PPV in aqueous solution as a function of concentration.

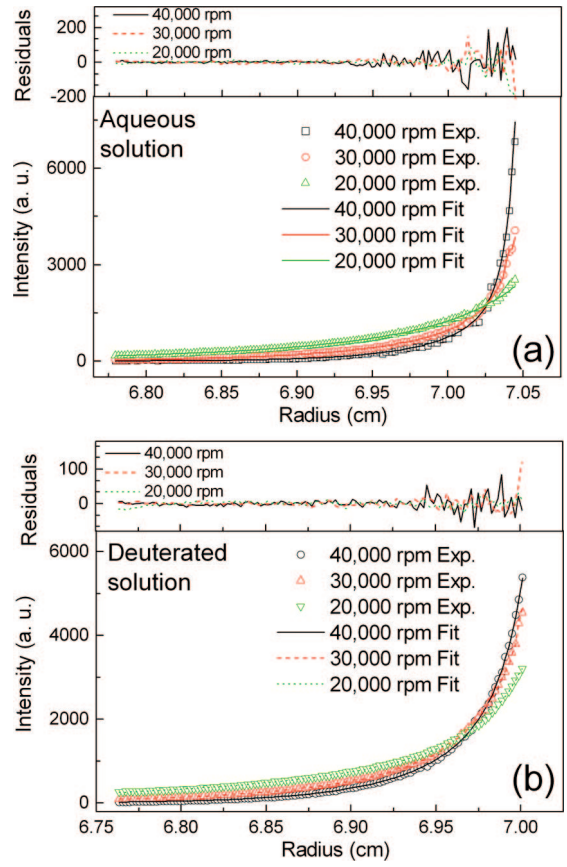


**Figure 3.** Sedimentation equilibrium profile with global fit for DPS-PPV at 20 000, 30 000, and 40 000 rpm in (a) aqueous and (b) deuterated solutions. The global fit residuals are also shown in the upper panel of each figure.

The maxima in the fluorescence emission spectra lie at 495, 497, and 502 nm for concentrations of 0.4, 0.1, and 0.00625 mg/mL, respectively. However, the maxima of the excitation spectra remain at the same wavelength of 380 nm for different concentrations. The slight wavelength red shift in the emission spectra may be due to aggregation or concentration-induced conformational changes that modify the effective conjugation lengths of the emitting chromophore,[17,18] i.e., the existence of the more extended chain structure with lower energy in the diluted solutions than that in the concentrated ones. Previous attempts to confirm this interpretation have been inconclusive.

Sedimentation equilibrium profiles were collected using the fluorescence detection attachment with rotor speeds of 20 000, 30 000, and 40 000 rpm for the DPS-PPV solution with a concentration of 0.4 mg/mL. The profiles for the polymer in aqueous and deuterated solutions are shown in parts a and b of Figure 3, respectively. It should be noted that 150 mM NaCl conditions were used in all the solutions to avoid the charging effect due to the charged polymer chains. Sedimentation equilibrium data were analyzed globally[13] assuming a noninteracting species model using the SEDPHAT program,[12] i.e., for jointly fitting data sets obtained at different experimental parameters.

The curves with symbols in Figure 3 show the experimental results, while the solid lines give the global fits. The buoyant molar mass ($M_b$) can be obtained, and correspondingly the partial specific volumes were determined using the "buoyant density method".[7] It is deduced from the analysis that the DPS-PPV in both solutions exhibits three dominant components with $M_b$ of

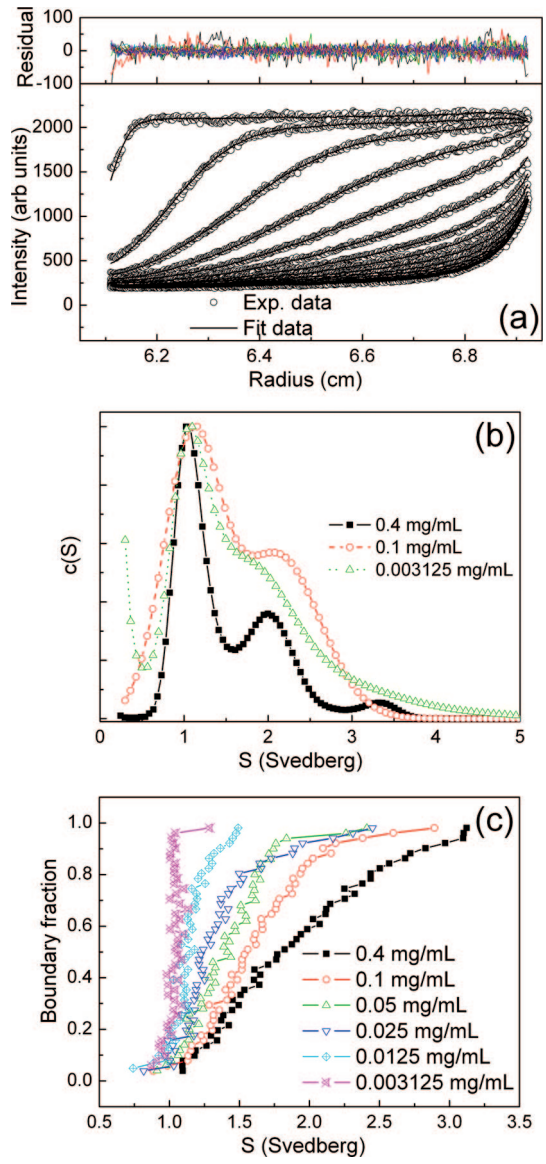


**Figure 4.** Sedimentation velocity analysis of 0.4 mg/mL DPS-PPV: (a) sedimentation velocity data acquired at 50 000 rpm, 20 °C (every fourth scan shown); (b) the sedimentation coefficient distribution $c(S)$; (c) corresponding Van Holde−Weischet analysis.

2531, 8414, and 18 877 g/mol and 2402, 8047, and 18 053 g/mol for aqueous and deuterated solution, respectively. Since the density of the buffer solution, $\rho$, and the buoyant molecular weight in different density solvents ($M_b(H_2O)$ and $M_b(D_2O)$) are known, if the molecular weight is assumed to be identical regardless of the buffer density, the partial specific volumes ($\bar{v}$) can be determined through the correlation of the molecular weight, buffer density, buoyant molecular weight, and $\bar{v}$ (eq 1).[13] Values for $\rho(H_2O)$ and $\rho(D_2O)$ were measured to be 0.991 and 1.106, respectively. $\bar{v}$ is independent of density, and the solution can be determined by solving simultaneously the expressions for the different solvents. Subsequently, the value for $\bar{v}$ was calculated to be around 0.89 mL/g, which can then be used to determine the molar mass of the polymer when coupled with sedimentation velocity measurements below.

$$M_w = M_b(1 - \bar{v}\rho) \quad (1)$$

Figure 4a depicts the sedimentation velocity profiles for DPS-PPV at a concentration of 0.4 mg/mL in aqueous solution. The measurements were carried out at 20 °C using a rotor speed of

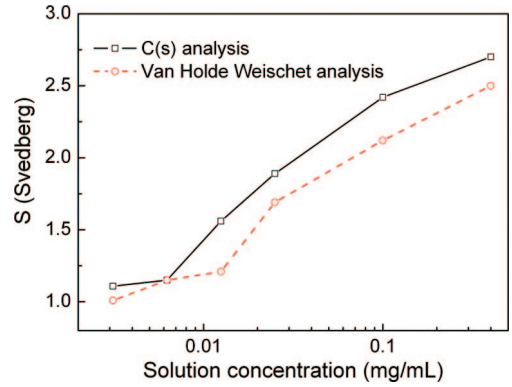


**Figure 5.** Weight-average sedimentation coefficient $S$ as a function of the solution concentration of DPS-PPV from integrating the $c(S)$ distributions and Van Holde−Weischet analysis.

50 000 rpm. Fluorescence data were collected at 1 min intervals from regions 6−7.25 cm from the rotor axis with the excitation laser focused to a spot ∼20 $\mu$m in diameter, 31 $\mu$m below the surface of the sapphire window. The sedimentation velocity distribution $c(S)$ was converted from the experimental data using the program SEDFIT 9.4[14] and maximum entropy regularization. The $c(S)$ analysis in Figure 4b revealed three peaks for the concentration of 0.4 mg/mL, which provides further evidence for the existence of three components of different molar mass in the solution. When combined with the $M_b$ value obtained above, molar masses of 2.48 × $10^4$, 8.25 × $10^4$, and 1.85 × $10^5$ g/mol are determined for these components within the assumptions inherent in the analysis program concerning reversible equilibrium processes. Rather than assigning these components as three distinct molecular weight species, it is more likely that the DPS-PPV in aqueous solution is aggregated as different macromolecular weight entities, which may include soluble monomers and aggregated clusters.

Sedimentation velocity measurements were also conducted for the solutions at a range of concentrations from 0.4 to 0.003125 mg/mL. The sedimentation coefficient distribution $c(S)$ for the concentration of 0.1 and 0.003125 mg/mL is also shown in Figure 4b. The frictional ratio ranged from 1.43 (for the lowest concentration) to 1.64 (for the highest concentration). Further analysis was then conducted using the Van Holde−Weischet analysis in the SEDFIT program, as shown in Figure 4c, suggesting that the sample with higher solution concentration is heterogeneous and the population of sedimentation coefficients shifts toward homogeneity at ∼1 Sv when reducing the solution concentration.

The weight-average sedimentation coefficient $S$ was deduced from integrating the $c(S)$ distributions and Van Holde−Weischet analysis, which is depicted in Figure 5. Both curves show a clear similar trend reflecting a decreasing sedimentation coefficient as the solution concentration is decreased. To fit the data, a minimum of three sedimenting components was required—any fewer and the fit was poor, while any more and the quality of the fit, in terms of the root mean square deviation (rmsd) and the Runs $Z$ test, did not improve. The three bands dominant in the $c(S)$ profiles (Figure 4b) can be attributed to concentration-dependent reversible polymer association.[15] While aggregation of PPV polymers is well-known,[19,20] the charged nature of this polymer might have been expected to help overcome or reduce this phenomenon in DPS-PPV, but it has clearly not been eradicated completely. This is of importance in understanding the properties of this polymer in film formation studies and for comparisons of thin films with single-chain spectroscopic studies.

## 4. Conclusions

In summary, fluorescence-based analytical ultracentrifugation by equilibrium sedimentation is shown to be a very practical and effective method to determine the average molar mass of water-soluble, photoluminescent conjugated polymers. Thus, this provides an accurate, sensitive, and convenient method for the determination of the molar mass of new photoluminescence materials, which can be difficult by other more common methods. A combined sedimentation velocity and equilibrium analysis was used to determine the molar mass distributions of DPS-PPV that have eluded determination by our attempts using alternative techniques. In the aqueous solution, the DPS-PPV contains at least three sedimenting components with average weight molar masses of the order of 24 800, 82 500, and 185 000 g/mol. The proportion of these species is highly dependent on the solution concentration, suggesting reversible association of the polymer in solution to form the higher molar mass components observed. The fluorescence-based analytical ultracentrifuge is unsurpassed for the direct measurement of molar masses of luminescent macromolecular solutes in solution, and it is not necessary to rely on calibration standards or to make assumptions concerning the macromolecular shape.

While we have illustrated this technique for the water-soluble polymer DPS-PPV, the technique is also applicable to organic-soluble polymers such as MEH-PPV if solubility issues relating to the sample cell in the present instrument are overcome. We point out that at this stage the emission quantum yield of each species has been assumed to be equal, which may affect the fractional quantities of the various components. This will be addressed as more detail concerning these polymers is acquired.

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