MaDDOSY (Mass Determination Diffusion Ordered Spectroscopy) using an 80 MHz Bench Top NMR for the Rapid Determination of Polymer and Macromolecular Molecular Weight

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Measurement of molecular weight is an integral part of macromolecular and polymer characterization which usually has limitations. Herein, this article presents the use of a bench-top 80 MHz Nuclear Magnetic Resonance (NMR) spectrometer for diffusion-ordered spectroscopy as a practical and rapid approach for the determination of molecular weight/size using a novel solvent and polymer-independent universal calibration.

1. Introduction

A cornerstone of polymer analysis is the determination of molecular weight, with a significant amount of analysis being performed through gel permeation chromatography (GPC) with an increasing use of mass spectrometry.[1] GPC offers benefits using conventional High Performance Liquid Chromatogrpahy (HPLC) hardware with well-established and community-accepted columns and there are facilities available at most institutions. However, this usually relies on narrow molecular weight standards being available which have very limited availability unless light scattering or viscometry are used which although being termed absolute methods also suffer from errors arising from sample preparation and hardware limitations. Although mass spectrometry can give accurate masses for individual chains, polymers by definition are always dispersed and no mass spectrometry technique is able to be reliable over the wide mass ranges most polymers exhibit and are essentially incapable of providing dispersity information.[2]

GPC, meanwhile, requires large volumes of solvents and regular calibration with limited availability of molecular weight standards. GPC also struggles to characterize polymers that cannot be readily dissolved in common laboratory solvents, including Polyethylene terephthalate (PET) and nylon, often requiring harsh conditions, high temperatures, and highly hazardous solvents.[3] In addition, polar and water-soluble polymers are complicated by interactions between the analyte and the columns which causes adsorption separation pathways as opposed to the required separation on molecular size alone. As polymers are complex mixtures, new and complementary techniques for determining polymer molecular weight are desirable. An attractive example is the use of DOSY which is routinely being used with relatively expensive and difficult-to-access high-field NMR which requires cryogenic magnets.[4,5]

The DOSY experiment is well understood, and literature resources for the NMR theory are plentiful.[6,7] In an idealized, monodisperse case, the diffusion constant, *D*, the output of the DOSY experiment, is related to the hydrodynamic radius, r_h, of a substance measured by the Stokes–Einstein equation (Equation (1))[8]

$$D = \frac{k_B T}{6\pi\eta r_h} \tag{1}$$

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here, k_B is the Boltzmann constant, T is the temperature, and η is the bulk viscosity of the solvent. The hydrodynamic radius can itself be related to the molecular weight by the Rouse–Zimm model (Equation (2)) in the case of an ideal, monodisperse, polymer.[9,10]

$$r_h \approx bM^\nu \qquad (2)$$

However, polymers are not monodisperse, and thus in the polydisperse case, the DOSY experiment will provide an average diffusion constant, and therefore an average molecular weight.

Here, ν is a parameter describing the relationship between the polymer and its solvent. With a perfectly linear polymer in a theta-solvent, it would be expected to be 0.5, but it may vary, potentially being lower for highly branched polymers, and higher for linear polymers in a good solvent. The key takeaway from this relationship, however, is that the diffusion coefficient can be directly related to the average molecular weight through a series of constants and measurable parameters. It is also important to note that ν is unlikely to be exactly the same for all polymers in solution, however, variances are likely to be small if "good" solvents are chosen for measurements, and thus a calibration can be made, similarly to those used in GPC.

To determine the molecular weight of an unknown polymer, a calibration must first be constructed, and molecular weight correlated with the parameter measured. In GPC elution volume (or time as usually isocratic pump speeds are set at 1 mL min^{-1}) is measured, and calibration is generated for each polymer type in each required solvent system, as each polymer occupies a different hydrodynamic volume depending on molecular characteristics, solvent compatibility, temperature, chain length, etc., in order to optimize accuracy.[11] Indeed, many calibrations of this nature have been provided for a wide range of polymer and solvent systems by DOSY, examples of which are well outlined in the work by Voorter et al.[9,12]

In 2012, Grubbs and Johnson introduced the polymer community to DOSY as an alternative to GPC for measuring molecular weights of a number of narrow molecular weight distribution polymers.[11,13] DOSY was heralded as a replacement for conventional polymer molecular weight determination by GPC and was shown to be very accurate. However, although it is routinely used it has probably not widely been seen as an alternative to GPC. In their work concentrations were between 0.5 and 0.02 mg mL^{-1} in deuterated benzene run at 300 MHz with a non-spinning sample and in our own experience collecting a DOSY spectrum and a value for the diffusion coefficient with a high field NMR service is achievable but is not a replacement for a modern GPC instrument with an autosampler.

However, this approach is limited by the requirement for external calibration and calibration standards. Thus, the preparation required for the experiment is arguably cumbersome and limits routine use especially where many samples are being analyzed. This has been significantly improved with the promise of solvent-independent DOSY calibration, which has recently had its efficacy shown on high-field instruments, where diffusion coefficients can be correlated to average molecular weights (Equation (3))[9,14,15]

$$\log(D) + \log(\eta) = \log(c) - \nu \log(M) \qquad (3)$$

Equation (3) describes a viscosity-corrected situation where concentrations of up to 15 mg mL^{-1} can be used which then leads to the ability to use non-deuterated solvents with samples directly taken from a reaction mixture. Here, c is a proportionality parameter and, as in Equation (2), ν is an arbitrary parameter. Both parameters can be determined experimentally with a calibration curve of $\log(D) + \log(\eta)$ versus $\log(M)$, where the gradient will be $-\nu$ and the intercept $\log(c)$.

There are some drawbacks to this elegant approach. Most important of which is that these experiments have previously only been performed on relatively expensive high-field instruments. These require regular cryogen fills, use large amounts of electricity, are usually set up to spin samples at high speed, require deuterated solvents, and can be much more expensive than a conventional GPC. This can limit accessibility and provide little potential for instrument mobility. Benchtop instruments require no cryogens, do not spin the samples, and are much more sustainable, mobile, and versatile in their operation. While calibrations from one polymer type have been used, Grubbs used a polystyrene calibration, to analyze different polymer types, a fully universal approach has not yet been explored, and yet, may be constructed using similar assumptions as made in "universal GPC." That is, in solution the polymer forms essentially ideal coils, and thus, Equation (2) applies.[16] This of course is only the case in a theta-like solvent to exclude specific solvent-polymer interactions, and so solvent choice is crucial in the building and use of such a calibration.[17]

The promise of being able to perform similar experiments on new modern and developing benchtop instruments has recently become possible, thanks to the development of gradient-based solvent suppression NMR pulse sequences, which make use of the same z-axis gradient coil required for DOSY experiments.[18] This presents the use of benchtop NMR as an attractive solution for molecular weight determination when compared to high-field measurements using the principles set out by Grubbs in a way that could well displace GPC as the go-to method, especially for polymers that are difficult to solubilize apart from inexpensive eluents. The instruments are generally less expensive, approximately the same price as a full GPC system, and allow for fast measurements in close proximity to the synthetic experiment if required. An external lock means the instruments require no deuterated solvents, which means that reaction mixtures can be directly sampled, and the molecular weight measured with no additional workup or sample preparation.

2. Main

A key factor to consider is the effect of polymer concentration. It is well understood that DOSY should be conducted at the lowest concentration possible, to minimize any analyte-analyte interactions, and thus allow free diffusion within the solvent. The concentration at which the diffusion constant is affected by analyte-analyte interactions is known as C^*. This has been widely explored in the literature, with a range of values of C^* being reported for various polymer systems.[13,19]

In order to create a "universal calibration" we initially investigated trends in C^* for multiple polymer systems. To do this, we measured diffusion coefficients at varying concentrations for a variety of polymer systems, **Figure 1**. Generally, it was observed
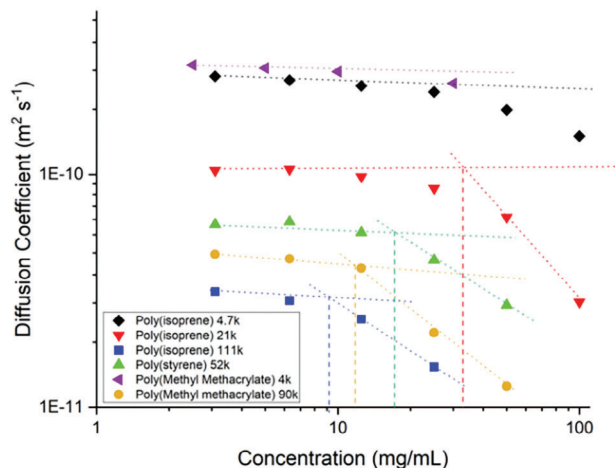
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Figure 1. Determination of $C*$ for various polymer systems.

Figure 2. Solvent-independent universal calibration.

that as polymer size increases $C*$ decreases. This is not particularly surprising, as larger polymers not only increase the viscosity of the system, greatly changing D, (Equation (1)), but also, polymer-polymer interactions are more likely in larger polymers inhibiting free diffusion. Both factors in combination explain why the diffusion constant decreases at higher concentrations.

The results of this study indicate that polymers with molecular weights <100 kDa can be measured at concentrations up to 10 mg mL^{-1}, which agrees with the previously cited literature.[19] This notwithstanding, the lowest practical concentration, that is, the lowest concentration whilst still maintaining a good signal in the NMR experiment, should always be used, in order to minimize the possibility of analyte-analyte interactions in unknown systems.

Using this concentration limit, a calibration curve was developed using a broad range of different polymer types, particularly with respect to solubility in different solvents and polymer branching. The solvents chosen in each case were those known to be good solvents for the respective polymer, allowing the assumptions previously discussed to apply. Detailed procedures regarding the sample preparation and spectroscopic parameters are available in the Supporting Information. The bulk viscosities used were those reported by Evans et al. for deuterated solvents.[20] The polymer concentrations chosen were sufficiently below $C*$ that the effect on the viscosity of the system is negligible. A linear calibration was achieved after accounting for solvent viscosity, which is described by Equation (4), **Figure 2**.

$$M_w = 10^{\frac{(\log D + \log \eta) + 7.74 \pm 0.086}{-0.597 \pm 0.021}} \qquad (4)$$

It should be noted that this calibration only holds true at a temperature of 26.5 °C, the measured internal temperature of the magnet, variations from this temperature will change molecular diffusion, Equation (1), and thus the calibration needs to be duly accounted for. It is also worth noting that while most of the polymers are randomly distributed around the calibration fit, the samples for poly(styrene sulfonate) are consistently higher than the fit, this is likely due to the charged nature of the polymer in deionized water. This polymer has, nevertheless, been included
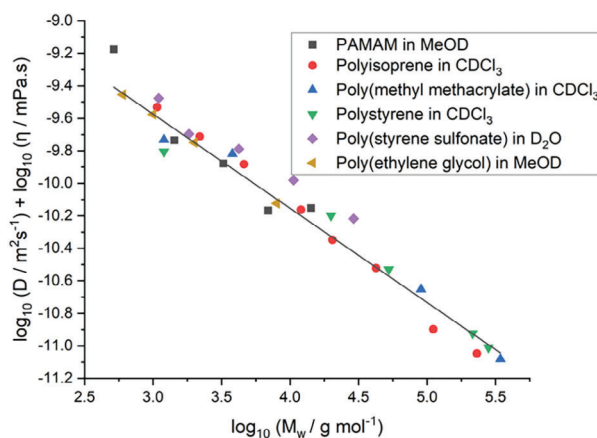
in the calibration to consider the effect charging may have on D, and therefore molecular weight.

To confirm the solvent independence of this calibration, which was already highlighted in the work by Voorter et al., a selection of the polystyrene and PMMA calibrants were also run in deuterated THF, and the corrected diffusion constants were compared, **Figure 3**.

In these data, the solvent correction can be seen to be very effective, with excellent agreement between the corrected diffusion coefficients for all polymers with the exception of the high, 343 kDa, Poly(methyl methacrylate) (PMMA). This is likely due to the fact this sample is at a concentration very close to $C*$, which means that analyte-analyte interactions may be affecting the diffusion. With this in mind, we suggest that the calibration should only be used on polymers with molecular weights below ≈200 kDa, to allow for samples with a low enough concentration to be sufficiently below $C*$ but high enough to generate an adequate NMR signal.

Using this calibration, the molecular weight of test polymers was determined. To facilitate easier calculation of the molecular
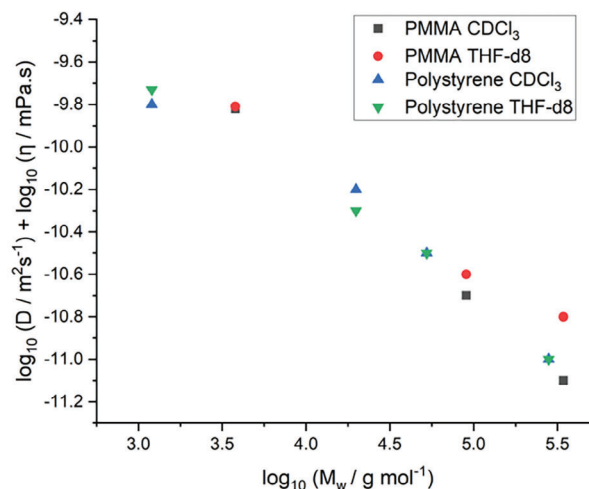

Figure 3. Solvent corrected diffusion constants for PMMA and polystyrene in both CDCl$_3$ and THF-d8.

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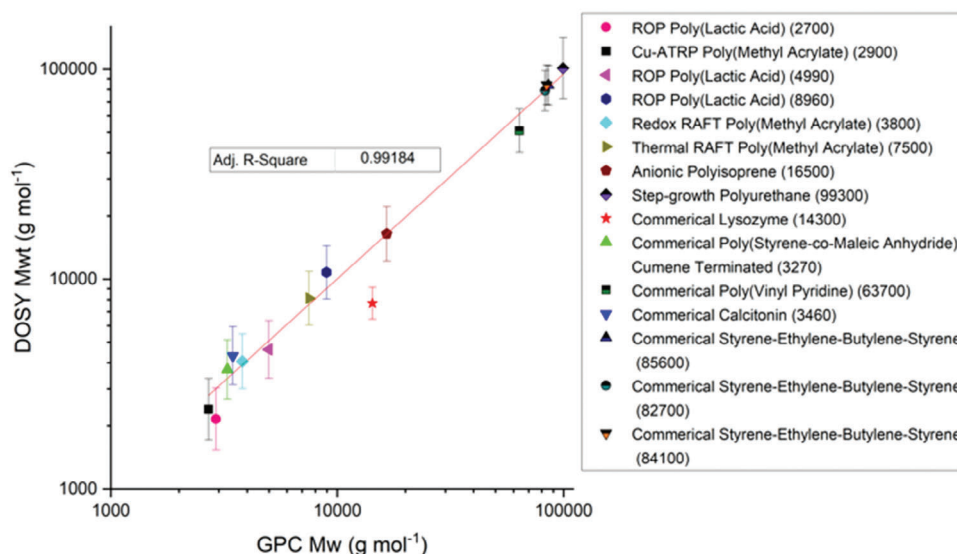olecular
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Figure 4. Comparison of molecular weights calculated by MaDDOSY and GPC for a selection of test polymers.

weight, we developed an interactive web-based tool that is available to freely use.[21] As the diffusion coefficient is, in principle, an intrinsic value of the polymer measured, it is independent of instrumentation, and therefore this tool can be used in any lab with any NMR spectrometer capable of DOSY. Alongside molecular weight, this tool also provides 95% confidence intervals about the molecular weight. Two approaches to the calculation of confidence intervals were explored, the methodology behind which is detailed in the Supporting Information.[22] The use of calibration to calculate molecular weight has been given the moniker MaD-DOSY (Mass Determination Diffusion Ordered Spectroscopy).

To test the calibration, several polymers were synthesized (Supporting Information), or purchased. These polymers were chosen as their syntheses make use of several different synthetic methods, and so allowed us to show applicability in most standard polymer syntheses. The final polymers were tested through both MaDDOSY and GPC analysis, and the results from each technique were compared (**Figure 4**).

We see excellent agreement between the results from GPC analysis, which was obtained using narrow polystyrene and PMMA standards and conventional GPC analysis using the refractive index detector for all samples other than Poly(lactic acid) (PLA), in which universal calibration was used, and those obtained through MaDDOSY. Of course, there are error margins within the GPC results, often up to 10–20%,[9,23] and so this agreement suggests the molecular weight results are comparable to those obtained via GPC.

In this data, we see that the use of MaDDOSY is applicable to a wide range of polymers, including those which are hydrogen bonding and block co-polymers. We also see in the case of a short peptide, calcitonin, the technique performs well. However, for a larger protein, lysozyme, the technique does not perform as well. This is likely due to the large number of proton environments present making it difficult to accurately integrate a region of interest, and thus calculate a diffusion constant. The spectra for the anionic, ring opening, and reversible-addition-fragmentation chain-transfer (RAFT) polymerizations, as well as

the commercial poly(vinyl pyridine) (PVP), were obtained using non-deuterated solvents, which is a great advantage of benchtop NMR instrumentation allowing for polymerization reactions to be monitored in real-time, while the other spectra used the more conventional deuterated NMR solvents. Data in both cases provide good agreement with the GPC results, suggesting that both the diffusion coefficient and calibration result are unaffected by the use of non-deuterated solvents. This notwithstanding, the data obtained for the step-growth polyurethane was collected in <1 min, with the speed being possible through the use of deuterated solvent, and resultant intensity reduction in the solvent peak. This shows that the usefulness of the MaDDOSY analysis system is twofold, first, rapid data acquisition is possible, with analysis times significantly faster than other mass determination techniques, but also that analysis is possible in conventional laboratory systems, with minimal sample preparation, only dilution to the appropriate concentration, required.

It should be noted that in all cases here, solvent choice was important, as with GPC. All polymers were fully dissolved in appropriate solvents; it was found that using solvents in which the polymers are poorly soluble resulted in different diffusion coefficients compared to those in a good solvent. This is not surprising, given the previously discussed assumptions whereby the polymer should be acting as an ideal chain. However, this is not necessarily a drawback of the method and is indeed the case in other molecular weight analysis techniques, but it should be considered when used in everyday analysis.

3. Conclusion

In conclusion, we have extended the use of DOSY for molecular weight measurement and demonstrated the use of a non-cryogenic 80 MHz benchtop NMR capable of DOSY as a versatile and simple tool for the determination of polymer molecular weight. We also present a new calibration approach, using a universal solvent and polymer-independent calibration for the calculation of molecular weight, which results in the calibration

being transferrable across institutions/laboratories studying different polymer systems. It is noted that at present this is limited to one number only with no measure of dispersity. Acquisition times are short, with run times in standard solvents of ≈8 min, and in deuterated solvents of <1 min, and a requirement for minimal amounts of solvent especially when compared to GPC. This is a sustainable (low solvent and low power usage) and cost-effective alternative for routine analysis in conventional laboratories and we are even using it in our undergraduate laboratories. Future work from the group is being directed toward the implementation of this system into furthermore complex analysis, including online/inline reaction monitoring. Further work should also aim to develop algorithms to extract dispersity data and aim to build a temperature-independent calibration. Current methods for the calculation of dispersity from DOSY spectra may be implemented, and calculation could be automated, however, currently, these methods require more extensive work prior to analysis.[24] Work is also focused on larger polymer systems especially water-soluble gums and natural polymers, in these cases, more in-depth calculations may be required to account for the polymer geometry in solution.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

Acknowledgements

Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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