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Oligomeric Cationic Polymethacrylates: A Comparison of Methods for Determining Molecular Weight

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S *Supporting Information*

ABSTRACT: This study compares three common laboratory methods, size-exclusion chromatography (SEC), ^{1}H nuclear magnetic resonance (NMR), and matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF), to determine the molecular weight of oligomeric cationic copolymers. The potential bias for each method was examined across a series of polymers that varied in molecular weight and cationic character (both choice of cation (amine versus guanidine) and relative proportion present). SEC was found to be the least accurate, overestimating M_n by an average of 140%, owing to the lack of appropriate cationic standards available, and the complexity involved in estimating the hydrodynamic volume of copolymers. MALDI-TOF approximated M_n well for the highly monodisperse (Đ < 1.1), low molecular weight (degree of polymerization (DP) <50) species but appeared unsuitable for the largest polymers in the series due to the mass bias associated with the technique. ^{1}H NMR was found to most accurately estimate M_n in this study, differing to theoretical values by only 5.2%. ^{1}H NMR end-group analysis is therefore an inexpensive and facile, primary quantitative method to estimate the molecular weight of oliogomeric cationic polymethacrylates if suitably distinct end-groups signals are present in the spectrum.
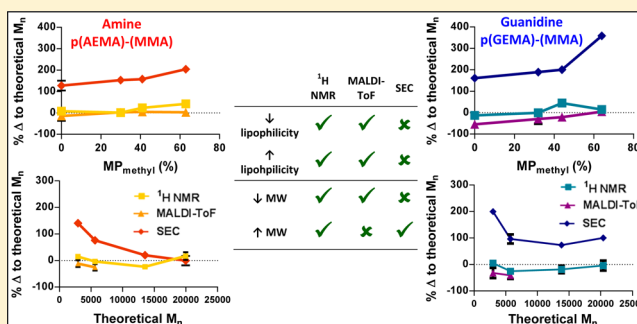
Water-soluble, low molecular weight cationic polymers have found applications in a variety of fields including the pharmaceutical industry as drug delivery systems[1−3] or antimicrobial agents,[4−7] the cosmetic field as conditioning agents,[8,9] and in industrial processes as coagulants or detergents.[10−13] The behavior of polymers in such applications is controlled by both their chemical composition and molecular weight, thus illustrating the need for accurate methods to characterize these materials. The determination of the molecular weight of oligomeric cationic species can be difficult using conventional methods such as size exclusion chromatography (SEC) coupled with multiple angle, static light scattering detection, which in principal can give absolute molecular weight distribution(s). However, owing to the inverse relationship between the molecular weight of species and the signal obtained, chemists are often forced to turn to more sensitive methods of detection for low-molecular weight species such as UV or refractive index (RI). These methods, however, require comparison to structurally related polymer standards in order to calibrate the instruments for a comparative measure of molecular weight. While there are a wide variety of standards commercially available for neutral species such as polymethyl-methacrylate, poly(ethylene oxide) or polystyrene, and anionic species such as poly(acrylic acid), the availability of suitable cationic polymer standards is very limited.

An alternative for gaining access to absolute molecular weight is that of SEC analysis using intrinsic viscosity (η) detection to give "universal calibration" measures. This theory assumes that polymers are separated only according to their hydrodynamic volume (V_h) and that this is the same for all polymers that elute at a particular elution volume. Following from this, a plot of $\log([\eta]M)$ versus elution volume gives a universal curve for polymers irrespective of architecture or composition.[14] This method avoids the necessity of structurally identical, low dispersity standards for accurate calculation of polymer molecular weight. This relationship is, however, only valid if separation occurs purely via size exclusion mechanisms. There are numerous studies that suggest that highly polar or ionic polymers do not confer with this relationship as they experience secondary separation effects from interactions with the typically negatively charged column surface.[15−20] It has also been shown that the use of universal calibration is invalid within the oligomeric size range where, for example, negative intrinsic viscosities have been observed.[21−25] Both of these limitations are highly relevant to the applicability of this method to the polymer class under investigation. There is, hence, little agreement within the community as to the best method to determine the molecular weight of oligomeric cationic polymers.

A recent study[26] completed in our laboratory involved the synthesis of oligomeric cationic polymethacrylates as potential antimicrobial agents using a reversible addition−fragmentation

chain transfer (RAFT) protocol to control the molecular structure of the polymer chain.[27,28] This work investigated the relationship between antimicrobial potency and selectivity to cationic character, composition, and polymer molecular weight. Two cationic series were examined, one encompassing amino pendant groups (PA1-8, $pK_a \sim 8–10$[29]), the other highly basic guanidine groups (PG1-8, $pK_a \sim 12–14$[30]). In addition, the relative proportion of cationic monomers (2-aminoethylmethacrylate (2-AEMA) or 2-guanidinoethyl methacrylate (2-GEMA)) to that of the neutral methylmethacrylate monomer (MMA) was varied within a range of molecular weights. For valid conclusions to be drawn regarding the relationship between structure and measured antimicrobial activity or structure–activity relationships (SAR), it was essential that we had an accurate method for determining molecular weights.

A survey of a variety of common laboratories techniques was undertaken to identify the most suitable method for this purpose including size-exclusion chromatography (SEC), end-group analysis by ^{1}H nuclear magnetic resonance (NMR), and matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) spectroscopy. There was the potential that the accuracy of each method may not only be affected by the presence of cationic groups but also by the type of cationic group and the relative number found within each polymer chain. Additionally, results obtained using each method may contain a molecular weight bias. The cohort of polymers described above would hence allow a unique investigation into the relative accuracy of each characterization method across a range of polymers that differ in cationic character and molecular weight. The use of the RAFT method also allowed for the calculation of theoretical molecular weight values based on the relative concentrations and molecular weights of each of the reagents (monomer, initiator, RAFT agent).

One of the most common methods utilized to determine molecular weights of polymers is SEC.[31] This method separates molecules based on their hydrodynamic volume. A porous stationary phase column packing is used such that with decreasing hydrodynamic volume, polymer chains experience higher accessibility to pore volume. This slows the progress of smaller chains through the column, allowing larger sized molecules to exit first. Chromatograms obtained hence correlate hydrodynamic volume to retention time. Following calibration with low dispersity standards, estimates of the number average molecular weight (M_n), weighted average molecular weight (M_w), and dispersity ($Đ$) can be obtained for a given polymer sample. The accuracy of this method is in part determined by the utilization of an appropriate detection method. Owing to the unsuitability of light scattering detection to such low molecular weight species, refractive-index (RI), a universal detection method, was chosen for this study. However, this method of detection being relative required the comparison of elution profiles to structurally related poly-methylmethacrylate (PMMA) standards.

The utilization of RAFT synthetic methods in combination with the low molecular weight of the polymers also allowed an estimation of molecular weight by ^{1}H NMR end-group analysis.[32] This method requires signals for the RAFT end-groups to be distinct from those of the polymer backbone in the NMR spectra. This method is typically more accurate in the low-molecular weight range, where the end-group signals are large enough to be unaffected by integration errors associated with low signal-to-noise ratios.[33] If these criteria are met,

however, this method can be powerful for determining polymer molecular weight without the use of external standards.[34]

One method that is gaining more attention as a means for determining absolute molecular weight is that of MALDI-TOF spectrometry.[35−40] This is a soft ionization technique that allows the analysis of much larger organic molecules than conventional mass spectrometry methods. Here, a polymer sample is cocrystallized with a "matrix", typically an aromatic cation. The matrix is selected to have suitable characteristics to provide a homogeneous polymer–matrix mixture, absorb sufficient energy from the UV ionizing source, desorb the analyte, as well as provide a pathway for ionization.[41,42] If any of these criteria are not fully met, accurate spectra will not be obtained; hence, two of the major hurdles for this technique are identification of an appropriate matrix and sample preparation methods. MALDI-TOF is also known to suffer from a mass bias phenomena, meaning that accurate molecular weight distributions can be difficult to obtain for disperse samples ($Đ >$ 1.1).[42−46] This method has, however, been identified as being particularly successful for a number of oligomeric polymer samples with low dispersities.[39]

The purpose of this work was to systematically examine and compare common characterization methods that may be used to determine the molecular weight of oligomeric cationic polymers. The techniques examined encompass analytical SEC, end-group analysis using ^{1}H NMR, and mass distribution analysis using MALDI-TOF. Such results will not only provide information regarding the ability of common laboratory methods to accurately estimate the molecular weights of oligomeric cationic polymethacrylates but will also present in-depth correlations between molecular and structural compositions and the analytical method of choice.

■ EXPERIMENTAL SECTION

Materials and General Methods. 2-Aminoethylmethacrylate hydrochloride (2-AEMA) was purchased from Polysciences (Warrington, PA). The RAFT agent, 4-cyano-4-[(dodecylsulfanylthiocarbonyl)sulfanyl]pentanoic acid, was synthesized according to literature methods.[32] Methyl methacrylate (MMA), N,N'-azobisisobutyronitrile (AIBN), dimethyl sulfoxide (DMSO), ethanol (EtOH), 1H-pyrazole-1-carboxamidine hydrochloride, N,N-diisopropylethylamine (DIEA), 2,5-dihydrobenzoic acid (DHB), a 9:1 mixture of DHB and 2-hydroxy-5-methoxybenzoic acid (super-DHB), dianthrol, α-cyano-4-hydroxycinnamic acid, sinapic acid, trans-3-indoleacrylic acid, and a 1:1 mixture of DHB and α-cyano-4-hydroxycinnamic acid (Universal) and all other reagents were purchased from Sigma-Aldrich (St. Louis, MO) and used as received.

Polymer Synthesis. Polymers PA1-8 and PG1-8 were synthesized as detailed in Locock et al.[26]

Amine Polymers PA1−8. In short, 2-AEMA and MMA were polymerized under RAFT conditions, using 4-cyano-4-[(dodecylsulfanylthiocarbonyl)sulfanyl]pentanoic acid as a chain transfer agent, AIBN as an initiator (1:5 ratio with CTA), and with DMSO as a solvent for 18 h at 70 °C. The resultant polymer was isolated by precipitation from methanol–acetone three times and stored under vacuum to remove residual solvent.

Guanidine Polymers PG1-8. Postpolymerization guanylation conditions were used to directly convert amine polymers PA1-8 into the corresponding guanidines PG1-8. The amine polymer of interest was dissolved in ethanol to give a 3% w/v solution. A

Table 1. Theoretical and Experimentally Derived M_n Values for Cationic Polymers

polymer	MP$_{methyl}$[a] (%)	theoretical M_n[b]	^{1}H NMR M_n[c]	Δ (%)	MALDI-TOF M_n[d]	Δ (%)	Đ[d]	SEC M_n[e]	Δ (%)	Đ[e]
PA1	35	2 940	3 370	14.7	2 590	−11.7	1.05	7 060	140.4	1.07
PA2	41	3 060	3 770	23.3	3 200	4.7	1.04	7 890	158.0	1.04
PA3	47	5 640	5 460	−3.3	4 180	−25.8	1.03	9 950	76.3	1.02
PA4	30	3 310	3 360	1.4	3 390	2.4	1.03	8 390	153.3	1.04
PA5	63	2 790	3 970	42.3	2 870	2.8	1.00	8 490	203.9	1.07
PA6	0	3 710	4 000	7.7	3 180	−14.4	1.11	8 450	127.6	1.15
PA7	49	13 540	10 380	−23.3	n.m.[f]			16 290	20.3	1.13
PA8	51	19 980	23 420	17.2	n.m.[f]			19 880	−0.5	1.12
average difference (%):				10.0		−7.0			109.9	
PG1	39	2 990	3 140	4.9	2 040	−31.8	1.08	8 970	199.9	1.15
PG2	44	3 110	4 510	45.0	2 450	−21.2	1.04	9 360	200.8	1.24
PG3	50	5 750	4 300	−25.3	3 390	−41.1	1.03	11 300	96.4	1.12
PG4	32	3 390	3 370	−0.6	2 370	−29.9	1.00	9 800	189.3	1.18
PG5	64	2 830	3 240	14.5	2 950	4.5	1.03	12 970	359.0	1.19
PG6	0	3 820	3 320	−13.2	1 740	−54.6	1.05	9 970	160.8	1.16
PG7	47	13 810	11 270	−18.4	n.m.[f]			23 940	73.3	1.25
PG8	49	20 390	19 610	−3.8	n.m.[f]			40 780	100.0	n/a[f]
average difference (%):				0.4		−29.0			172.4	
overall difference (%):				5.2		−18.0			141.2	

[a]MP$_{methyl}$ (mole percentage of methyl side chains). [b]Theoretical M_n was calculated using the following formula where DP refers to degrees of polymerization and MW to molecular weight: $(MW_{RAFT} + DP_{cationic} \times MW_{cationic} + DP_{methyl} \times MW_{methyl}) \times$ % conversion. [c]^{1}H NMR M_n was calculated by first comparing the integration of RAFT end-groups to that of pendant methylenes to give DP for each monomer. The final estimate of M_n was given by $MW_{RAFT} + DP_{cationic} \times MW_{cationic} + DP_{methyl} \times MW_{methyl}$. [d]MALDI-TOF M_n and dispersity (Đ) values were calculated from spectra obtain on a Bruker Autoflex III MALDI TOF/TOF mass spectrometer in positive ion mode using super-DHB as a matrix in a ratio of 100:1 to analyte. Final values were calculated using the Bruker PolyTools Synthetic Polymer Analysis software package v1.2. [e]SEC M_n and Đ was determined by gel permeation chromatography (GPC) analysis in DMAC against PMMA standards and calculated using the Shimadzu software package LC Solution v1.25. [f]n.m., not measured. n/a, not applicable.

total of 1.5 equiv of 1*H*-pyrazole-1-carboxamidine hydrochloride and 3 equiv of *N,N*-diisopropylethylamine relative to the number of pendant amine groups per chain was added to the mixture before heating at 55 °C overnight. Solvent was removed and the resultant polymer isolated by precipitation from methanol—acetone three times and stored under vacuum to remove residual solvent.

SEC Characterization. Size exclusion chromatography was performed on a Shimadzu system comprising a CMB-20A controller system, a SIL-20A HT autosampler, a LC-20AT tandem pump system, a DGU-20A degasser unit, a CTO-20AC column oven, a RDI-10A refractive index detector, and four Waters Styragel columns (HT2, HT3, HT4, HT5). Each column was 300 mm × 7.8 mm, providing an effective molar mass range of 100 to 4 × 10^6. *N,N*-Dimethylacetamide (DMAc) (with 2.1 g L^{-1} of lithium chloride (LiCl)) was used as an eluent with a flow rate of 1 mL min^{-1} at 80 °C. Calibration curves were obtained using low dispersity poly-(methylmethacrylate) standards purchased from Polymer Laboratories. Determined polymer molar mass are relative to PMMA standard molar masses. Dispersity (Đ) values were calculated using the Shimadzu software package LC Solution v1.25.

^{1}H NMR Characterization. ^{1}H and two-dimensional nuclear Overhauser effect spectroscopy (NOESY) NMR spectra were recorded at 400 MHz using a Bruker BioSpin 400-MHz NMR spectrometer (Billerica, MA) using a 10 s delay between acquisitions. This was found to be a sufficient relaxation time to allow accurate peak analysis across the polymer cohort. Chemical shifts (δ_H) were reported in parts per million (ppm). NMR solvents ($(CD_3)_2SO$ for NOESY to imitate the polar aprotic conditions used for SEC and CD_3OD for ^{1}H) were purchased from Cambridge Isotope Laboratories

and used as received. For details of end-group analysis, please refer to the Supporting Information.

MALDI-TOF Characterization. Spectra were run on a Bruker Autoflex III MALDI TOF/TOF mass spectrometer using a positive ion mode. Laser power was optimized to provide sufficient ionization of the analyte but to minimize fragmentation of lower molecular weight fractions and ranged from 30 to 70%. The matrix was super-DHB (9:1 mixture of 2,5-dihydroxybenzoic acid (DHB) and 2-hydroxy-5-methoxybenzoic acid) at a concentration of 10 mg/mL in methanol. A volume of 10 μL of this solution was coapplied with 1 μL of the analyte (1 mg/mL in methanol) on the target plate for analysis. M_n, M_w, and Đ values were calculated using the Bruker PolyTools Synthetic Polymer Analysis software package v1.2. For representative mass distribution spectra, please refer to the Supporting Information.

■ RESULTS AND DISCUSSION

Table 1 depicts the results obtained for the two series of cationic polymers across three different molecular weight characterization methods: ^{1}H NMR, MALDI-TOF, and SEC. Results have been compared to theoretical values for molecular weight, based on monomer feed ratios and percent conversion. It can be seen that the results obtained via each method varied from the theoretical values both positively and negatively with different magnitudes. The biggest variance was seen for SEC; here reported molecular weights appear inflated, between 2 and 3-fold higher on average. It should be noted that, owing to the lack of commercially available cationic standards, the neutral PMMA was utilized to construct a standard calibration curve for this method. While PMMA retains the same backbone to that of the polymers of interest, it will not accurately represent

effects on the hydrodynamic volume that may be present as a result of the cationic functional groups. The presence of such groups would, however, be expected to have a profound effect on the secondary structures adopted by the polymer chains in the SEC mobile phase and hence is the most likely explanation for the apparent errors associated with these measurements.

To allow analysis of molecular weight distributions via MALDI-TOF, an extensive investigation of the most appropriate matrix and method of sample preparation technique was undertaken. These included common agents such as 2,5-dihydrobenzoic acid (DHB), a 9:1 mixture of DHB and 2-hydroxy-5-methoxybenzoic acid (super-DHB), dianthrol, α-cyano-4-hydroxycinnamic acid, sinapic acid, trans-3-indolea-crylic acid, and a 1:1 mixture of DHB and α-cyano-4-hydroxycinnamic acid (Universal) in various solvents and concentrations.[39,41,42] High-resolution ionization spectra were obtained for PA1-4 and PG1-4 using super-DHB in a 100:1 ratio with analyte, cocrystallizing from methanol. Spectra could not, however, be generated for the two highest molecular weight polymers in each series, PA5-6 and PG5-6. This was presumably due to the difficulty associated with ionizing larger MW polymer samples with higher Đ values.[42−46] On average, results from MALDI-TOF analysis were shown to approximate the expected molecular weights for the amino series, PA1−PA6 fairly well, with only on average a 7% underestimation. The technique does, however, appear less accurate for the corresponding guanidine series, underestimating the molecular weight of those polymer samples by 20% on average and indicating that the accuracy of the techniques could be dependent also on the functional group present. This may be explained by the reduced solubility of the more polar guanidine series in methanol, when compared to that of the amine functionalized polymers. This could result in a less homogeneous cocrystallization of matrix and analyte during sample preparation and hence the accuracy of mass distributions obtained.[39,41,42,47]

The most accurate method across both series was that of end-group analysis by ^{1}H NMR. Here, an estimate of molecular weight was obtained through the relative ratio of peak areas resulting from signals from polymer pendant groups compared to signals from RAFT end-groups.[33,34,48] While this method does depend on the peaks for each signal being distinct, in all it provided a discrepancy of only 5% compared with theoretical molecular weights when averaged across both series (calculated from 10% for the PA series and 0.4% for PG).

The two series of polymers were prepared such that the overall lipophilicity of the final species was controlled through the feed ratios of the two monomers. This meant that the composition of the series varied from the highly lipophilic PA5 and PG5, with 63% and 64% methyl content, respectively, through to the least lipophilic with the cationic homopolymers PA6 and PG6. This in turn, allowed an examination of whether the global lipophilicity of the polymers also affected the accuracy of each molecular weight measurement technique.

Analysis of the data presented in Figures 1 and 2 show little relationship between lipophilicity of cationic polymers and either the evaluations resulting from ^{1}H NMR or MALDI-TOF analysis. These techniques are not known to be significantly affected by lipophilicity as long as suitable sample preparation procedures are followed.[34,39,41,42] There is, however, an apparent enhancement in the overestimation of molecular weight with SEC with the most lipophilic of the series. Here, analysis of PA5 gives a result which was double that of the
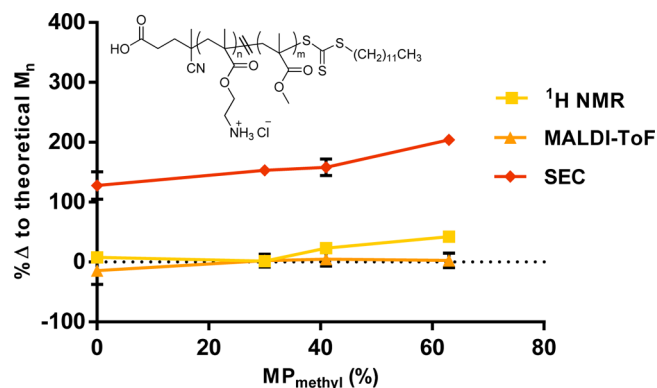

Figure 1. Percent difference between measured and theoretical M_n values for amine polymers as a function of mole-percent methyl groups (MP_{methyl}).
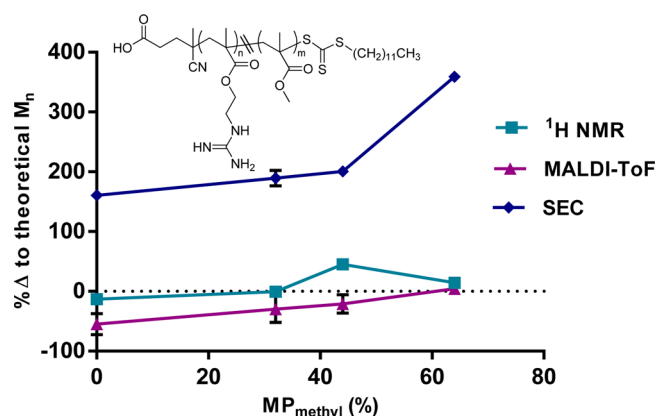

Figure 2. Percent difference between measured and theoretical M_n values for guanidine polymers as a function of mole-percent methyl groups (MP_{methyl}).

theoretical M_n, and for PG5, the M_n value was overestimated by over 350%. The explanation for this is not immediately apparent, the higher the methyl side chain composition of these polymers compared to others in the series means that they more closely resembled the monomer composition of the PMMA standards utilized for this technique. It would hence follow that the derived M_n would be more accurate. It appears therefore that there is something additional driving the hydrodynamic volume of these polymers beyond the relative contribution of the monomers. Previous studies have shown copolymers having the same molecular weight but different architectures (block versus random) have different retention rates in SEC.[49,50] Studies have also shown changes to hydrodynamic volume according to the number of cationic groups present within a polymer chain.[11,51,52] Such discrepancies between theoretical and SEC-derived M_n values can relate not only to the interactivity of cationic groups with the stationary phase (secondary separation mechanisms) but may also relate to the effect that the charged groups have on the adopted polymer conformation in solution. Evidence for this with the current series was obtained using NOESY. This is a through-space correlation method and allowed the investigation of the three-dimensional structure of polymers in solution. PA5, the most lipophilic amine polymer studied (63% methyl content), showed a strong association between pendant methyl groups from MMA and the polymer backbone, while there was little to no such correlation with pendant methylenes stemming

from AEMA (see the Supporting Information for relevant spectra). This could indicate an organized secondary structure for the amphiphilic PA5 that segregates cationic and lipophilic groups. The existence of such an extended conformation and its effect on polymer hydrodynamic volume may explain the derived 204% overestimation of M_n via SEC. While NOESY investigations for PG5 were not possible due to the presence of overlapping peaks in the NMR spectrum, it is thought that this explanation extends to the guanidines as very similar trends can be observed across the series.

This clearly demonstrates the difficulties that can be experienced when using SEC to determine the molecular weight of copolymers. Even if appropriate homopolymers are commercially available for the constituent monomers, the resulting hydrodynamic volume of copolymers cannot simply be determined via a proportionate summation of standard curves but requires careful consideration of other influences on hydrodynamic volume.

One potential method to circumvent such issues is to combine the powers of SEC with that of mass spectrometry (MS). A number of studies[53−61] have shown the power of online coupling of SEC with a soft ionization MS technique such as electrospray ionization (ESI) to derive accurate polymer molecular weight distributions. While MS has the ability to derive both molecular weight and chemical composition information from a sample, it can suffer from significant mass bias, particularly in the case of complex, polydisperse polymers. SEC on the other hand, allows the user to separate polymers based on hydrodynamic volume and then gain concentration information from universal detectors such as RI. The lack of suitable SEC calibration standards, however, limits the ability of this technique to derive accurate molecular weight information. If, on the other hand, SEC is used to fractionate a complex polymer sample which is then online-coupled to both a conventional concentration detector such as RI and a MS method, this allows both techniques to work to their strengths. Here, the accurate molecular weight information gathered from MS on each monodisperse polymer fraction serves as calibrant information for RI measures. The applicability of such methods to the area of oligomeric cationic polymers may be the focus of future work.

In addition to the effect that cationic character may have on the accuracy of the measured M_n value, it is furthermore assumed that the results obtained using each method may also be dependent in some way on the molecular weight of each sample itself. In this study, we controlled the effect of lipophilicity by selecting polymers that contained approximately the same ratio of the two monomers, but with increasing chain length, and examined the relative accuracy across the series as depicted in Figures 3 and 4.

While MALDI-TOF can be seen to provide good approximations of molecular weights within the range of 3000 to 6000 Da, suitably high-resolution spectra with appropriate Gaussian distributions could not be obtained for polymers above this threshold. It is well established that MALDI-TOF can suffer from significant mass discrimination, particularly for disperse or high molecular weight samples. In this case a decrease in detector response was observed with increasing molecular weight.[42−46] This occurs because higher laser intensities are often required to gain sufficient ionization of higher weight polymer chains. Such intensities can result in fragmentation of the lower weight fractions, saturating the detector and giving an inaccurate depiction of the sample
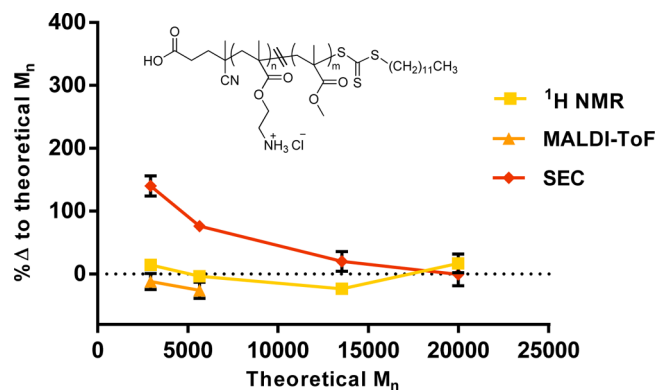

Figure 3. Percent difference between measured and theoretical M_n values for amine polymers as a function of polymer chain length (DP).
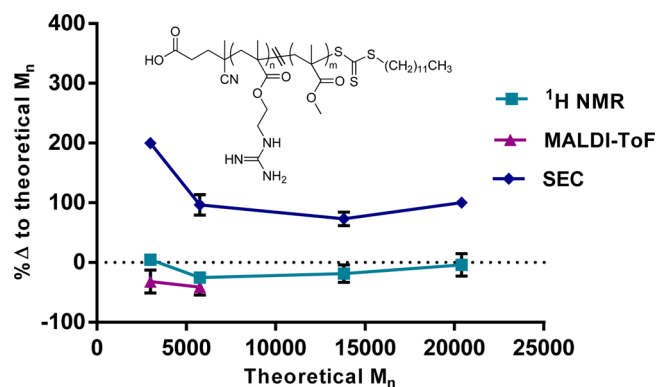

Figure 4. Percent difference between measured and theoretical M_n values for guanidine polymers as a function of polymer chain length (DP).

molecular weight distribution.[45] Evidence for this was observed with PA6 and PG4 which displayed bimodal distributions, corresponding to the detection of low-molecular weight species in addition to the parent polymer chains (for relevant spectra see Supporting Information). This accounts for the underestimation of M_n by 14.4% and 29.9%, respectively, using this method. Additionally, higher mass ions typically suffer from a lower detection rate as they arrive at the detector with too low an impact velocity to be detected over the relevant noise during data collection. These phenomena were also likely to be responsible for the difficulties associated with obtaining appropriately Gaussian molecular weight distributions for the two highest molecular weight samples in each series, PA7-PA8 and PG7-PG8. Alternatively, the size of these samples ($M_n >$ 6000) may give sufficient sensitivity with light scattering to allow detection via this means.

It is also interesting to note that differences in Đ measurements derived from SEC and MALDI-TOF in Table 1. While a number of values were similar for both methods (e.g., PA1-PA4), analysis of MALDI-TOF data appeared to give impossibly low Đ values in some cases, for example, values of 1.00 for PA5 and PG4. A number of studies[39,43,46,62−64] have identified significant differences between the two techniques, often deeming MALDI-TOF as inappropriate for measures of dispersity due to issues of mass bias. Take for instance a high weight fraction of a polymer population. MALDI-TOF ideally relies on each polymer chain obtaining a single charge irrespective of molecular weight. This would mean there would be only a few high molecular weight molecules available

to generate the ions for detection. These are hence often occluded by noise in the spectrum and result in a measured distribution that is truncated, giving significantly lower average molecular weight values.

The use of SEC, on the other hand, appeared to enable better approximation of M_n as the molecular weight increased, in particular for the amine polymer with the highest molecular weight, PA8, where experimentally derived and theoretical molecular weight differed by only 0.5%. One contributing factor to this result is that the relative influence of the RAFT end-groups on the hydrodynamic volume of species would be expected to be minimal for higher molecular weight samples. Additionally, while refractive index is often assumed to be a universal method of detection, various investigators have observed a molecular weight dependence with RI detection that is generally linear with $1/M_n$.[22,65,66] A combination of such effects could explain the decreased discrepancy observed for higher molecular weight species observed between M_n values obtained using SEC measurements and the theoretical M_n values.

^{1}H NMR end-group analysis appears to approximate fairly well across the series that stretches from a M_n of 3 000 through to over 20 000 Da. This illustrates the power that ^{1}H NMR analysis has for estimation of molecular weight within the oligomeric region, where signals from RAFT end-groups are strong enough to minimize error associated with signal-to-noise interference.

◾ CONCLUSIONS

This work compares three commonly used laboratory methods, SEC, ^{1}H NMR and MALDI-TOF, to determine the molecular weight of two series of oligomeric cationic polymers, PA1-8 and PG1-8. Because of the variation in composition and molecular weight across these two series, we were able to examine each method for any bias relating to cationic functional group chemistry, lipophilicity, and molecular weight.

The results obtained show SEC to be the least accurate for both series, appearing to overestimate molecular weight on average by 140%. While SEC is a widely used and accepted characterization method, it is generally performed with the assumption that the hydrodynamic volume of samples directly relates to that of the standards used for calibration. This translates to two issues for the current cohort. First, there was a lack of commercially available well-defined cationic polymethacrylate standards. This forced the use of neutral polymethylmethacrylate standards for the construction of standard calibration curves, which were used to correlate hydrodynamic volume, retention time and polymer molecular weight. The use of such standards would be unable to account for the affect that the presence of cationic groups may have on the hydrodynamic volume of the polymers of interest, leading to inaccurate estimates of M_n. Second, this work has also clearly illustrated that the observed hydrodynamic volume of a given copolymer does not relate to the simple summation of homopolymer values according to the relative contribution of each monomer. Caution should therefore be applied when attempting to apply common SEC protocols to the more complex cationic copolymer samples.

MALDI-TOF was shown to produce results which gave reasonably accurate M_n values for the 12 lowest molecular weight polymers (PA1-6 and PG1-6) but could not readily be applied to the four largest in the two series (PA7-8 and PG7-8). This was thought to be due to difficulties associated with

ionizing larger species, leading to saturation of the detector with smaller fragments and an overall mass bias. The data presented here do provide strong evidence that provided the correct matrix and samples preparation methods can be identified, MALDI-TOF can be a powerful method to determine M_n for low dispersity ($Đ < 1.1$), low molecular weight (DP <50) cationic polymers.

^{1}H NMR end-group analysis was found to, on average, most accurately estimate the molecular weight of the oligomeric cationic polymethacrylates in this study. ^{1}H NMR is a primary quantitative method that requires no external calibration to allow the user to estimate M_n values from the relative ratio of polymer end-group to pendants. It does, however, require the end-group signals to be suitably distinct from that of the polymer backbone if accurate ratios are to be calculated. Our results showed that this method was only minorly affected by the type of cation present in the polymers, the relative proportion of the cations compared to hydrophobic residues, or the length of the polymer chains. ^{1}H NMR hence represents an efficient and easy-to-use method to estimate the molecular weight of oligomeric cationic polymethacrylates.

◾ ASSOCIATED CONTENT

ⓢ Supporting Information
Details of ^{1}H, NOESY NMR and MALDI-TOF analyses. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions
The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes
The authors declare no competing financial interest.

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