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Characterization of implant device materials using size-exclusion chromatography with mass spectrometry and with triple detection

X. Michael Liu*, E. Peter Maziarz, David J. Heiler

Research, Development and Engineering, Bausch & Lomb Inc., 1400 N. Goodman Street, Rochester, NY 14609, USA

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Abstract

A more complete understanding of the raw materials used for making implant device materials becomes increasingly important in the medical device industry. Often such detailed information requires utilization of a combination of analytical techniques. In this work, we characterize a poly(dimethyl siloxane) (PDMS) material using on-line size exclusion chromatography (SEC) with electrospray ionization (ESI) and matrix assisted laser desorption ionization (MALDI) mass spectrometry (MS) techniques. Here, we obtain detailed molecular compositional information such as repeat units, end group chemistry, and identification of impurities in both the high and low mass range. SEC with light scattering, viscosity, and refractive index detection (triple detection) is used to obtain information on a small quantity of high mass impurity that was undetected by both SEC-ESI and MALDI MS techniques. SEC with triple detection measures absolute molecular weights and molecular weight distributions. We compare average molecular weight values of the implantable device polymer obtained by SEC with triple detection, SEC-ESI, and SEC-MALDI MS techniques.

Keywords: Implant device materials; Molecular mass distributions; Mass spectrometry; Triple detection; Polymers; Poly(dimethyl)siloxane

1. Introduction

Molecular weights and molecular weight distributions determine the performance properties of polymeric materials. The strict specifications on raw materials used to make implant devices require thorough knowledge of these molecular weight distributions. Conventional size exclusion chromatography (SEC) or gel permeation chromatography (GPC) has long been used successfully to measure relative molecular weights and molecular weight distributions with remarkable precision [1,2]. However conventional SEC is limited in that it only measures relative molecular weight values and does not provide any information on molecular composition, size, or conformation. The limitations of conventional SEC are most notable during the product development process where the polymer materials may be poorly understood.

One effective approach to overcome the limitations is to add more information rich detectors to the SEC system. These detectors may include light scattering detection (LS), differential viscometer (VISC), photodiode array detection (PDA), nuclear magnetic resonance (NMR), Fourier transform-infrared spectroscopy (FT-IR), and mass spectrometry [3–14]. In this study, we used SEC with a triple detection system that consists of a right angle light scattering detector, a differential refractometer (dRI), and a differential viscometer. A dRI and/or an UV spectrophotometer can accurately monitor the concentration change or selected compositional change (monomers containing UV chromophors compared those without the UV chromophors) over the molecular weight distribution. A differential viscometer combining a concentration detector can be used to determine the intrinsic viscosity of the polymer in solution and therefore indirectly calculate the conformation and sizes of the polymer [15–19]. By combining the universal calibration theory, the differential viscometer can also be used to calculate the absolute molecular weight values of the polymer. In addition, a light scattering detector can be used to determine absolute molecular weight values without a calibration curve [20,21]. Despite of all the great information that SEC with triple detection offers, it does not provide detailed information on the chemical composition of the sample.

* Corresponding author.
E-mail address: x.michael.liu@bausch.com (X.M. Liu).

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By contrast, mass spectrometry (MS) provides detailed chemical composition such as repeat unit, end-group chemistry, and presence of impurities of the polymers. With the rapid development of soft ionization techniques including especially electrospray ionization (ESI) and matrix-assisted laser desorption ionization (MALDI), it is practical to hyphenate (either on-line or off-line) SEC with ESI MS and MALDI MS. The hyphenation of SEC with MS combines many advantages from both techniques and overcomes most of limitations of individual techniques. Several studies have illustrated the power of combining SEC with MS for analysis of polymer materials [3–5,7,8,10,12,22–26]. Recently we published a paper comparing online SEC-ESI to automated SEC-MALDI MS analysis [3]. The data obtained suggested that SEC-ESI MS provided information within the low mass range where MALDI analysis may be difficult to obtain the same information due to matrix interference. On the other hand, SEC-MALDI MS analysis performed very well at the high mass range where no signal was obtained for the same analysis by SEC-ESI MS experiments. In this report, we extend the study with the use of triple detection SEC in combination with SEC-MS techniques to obtain a more complete description of a PDMS sample used as implantable device materials.

2. Experimental

2.1. Samples and materials

All PDMS samples used were synthesized in-house. A narrow molecular weight polystyrene standard with M_p of 13,100 Da (DP = 1.02) was purchased from Polymer Laboratories, MA, USA). Sodium nitrate, ammonium trifluoroacetate, and dithranol were obtained from Aldrich (Milwaukee, WI, USA). HPLC grade stabilized tetrahydrofuran (THF), toluene, and isopropanol (IPA) were all purchased from Fisher Scientific (Fairlawn, NJ, USA).

2.2. GPC-ESI MS

The solvent delivery system used an Agilent 1100 HPLC Series (Palo Alto, CA, USA). The separation was performed on two narrow-bore Plgel mixed-bed E (3 μm, 300 mm × 4.6 mm i.d.) columns (Polymer Laboratories, Amherst, MA, USA). THF was used as the mobile phase at a flow rate of 310 μl/min. Twenty microliters of sample solution at a concentration of 0.1% (w/v) were injected into the SEC system. The column temperature was set at 35 °C to ensure high resolution and reproducibility. The flow was split after the UV detector such that 50 μl/min of the SEC effluent was directed to the ESI MS instrument. Details of the SEC-ESI MS interface were described previously [3,27]. The internal diameter and the length of the waste line tubing controlled the flow–split ratio. The charge agent solution consisted of 1.0% (w/v) ammonium trifluoroacetate in isopropanol and was introduced into the SEC effluent downstream from the split at a flow rate of 5.0 μl/min. All ESI mass spectra were obtained from a Mariner mass spectrometer (Applied Biosystems, Framingham, MA, USA). The spray tip and nozzle potentials were set at 5000 and 100 V, respectively. Positive ion mode was used for all ESI MS measurements. Each mass spectrum was the result of 1 s accumulation of ions in the hexapole ion guide in order to maintain the high resolution from the SEC.

2.3. Automated SEC-MALDI-TOF MS

All experiments for Automated SEC-MALDI were performed using a modified Lab Connections LC-Transform Series 500 (Northborough, MA, USA) and an Applied Biosystems' Voyager DE-STR MALDI-TOF mass spectrometer (Framingham, MA, USA). The experimental conditions were the same as described in previous studies [3,26].

2.4. SEC with triple detection

The solvent delivery system used an Alliance 2695 Separation Module (Waters Corporation, Milford, MA, USA). The separation was performed on the two PLgel mixed-bed E columns (300 mm × 7.5 mm i.d.) that were purchased from Polymer Laboratories. The mobile phase was toluene that was flowed at 1.0 min/min. One hundred microliters of sample solution at a concentration of 3.0% (w/v) in toluene were injected into the SEC system. The triple detection consists of a right angle light scattering detector, a differential viscometer, and a differential refractometer that was purchased from Viscotek Corporation (Houston, TX, USA). The three detectors were connected in series that the light scattering detector was placed directly downstream of the SEC column set, followed by the refractometer, and then the viscometer.

One hundred microliters of a narrow polystyrene standard solution with M_p = 13,100 Da (Polymer Laboratories) at a concentration of 0.2% (w/v) in toluene were injected into the SEC with triple detection system. Three goals were achieved by the injection of such narrow polymer standard solution. First the inter-detector delay volumes among the three detectors were compared and corrected. Second, the calibration factors or constants for three detectors were obtained. Third, the band broadening effect due to serial connections of three detectors was effectively rectified. No flow rate marker was used in the analysis. All sample and standard solutions were filtered through 0.45 μm nylon Acrodisc syringe filters (Waters Corporation, Milford, MA, USA) prior to injection. The temperature for the SEC column set and the detector chamber was set at 45 °C ensuring high SEC efficiency, stables baseline, and consistent results. Data acquisition and calculation were performed using Viscotek's OmniSEC software version 2.0 (Viscotek Corporation, Houston, TX, USA).

The following simplified equations that reflect the correlation between the detector signals and molecular weights

of the polymer [28] are listed as follows:

$$\mathrm{dRI} = C \times \left(\frac{\mathrm{d}n}{\mathrm{d}c}\right) \quad (1)$$

$$R_{\mathrm{visc}} = K \times C \times M_{\mathrm{v}} \quad (2)$$

$$R(\theta)|_{\theta\to 0} = K \times C \times M_{\mathrm{w}} \quad (3)$$

$$[\eta] = [\mathrm{IV}] = K_{\mathrm{M}} \times M_{\mathrm{v}}^{a} \quad (4)$$

$$R_{\mathrm{g}}(i) = 0.328 \times (\mathrm{IV}_i \times Mi)^{1/3} \quad (5)$$

where dRI is the refractive index detector signal, dn/dc is the specific refractive index increment. R_{visc} is the viscosity detector signal. K is an optical constant that is approximately equal to $(\mathrm{d}n/\mathrm{d}c)^2$. C is the concentration of sample solution. $R(\theta)$ is the scattered light intensity. M_{v} and M_{w} are the viscosity and weight-average molecular weight, respectively. $[\eta]$ is the intrinsic viscosity [IV] of the polymer. K_{M} and exponent 'a' are Mark–Houwink's constants. The value of exponent a can also be used to predict the combined effect of polymeric chain conformations and the presence of branching. $R_{\mathrm{g}}(i)$ is the radius of gyration calculated at molecular weight 'i' for the sample.

3. Results and discussion

Fig. 1 illustrates a dRI trace of a methyl terminated PDMS (ME-PDMS) polymer. By using conventional polystyrene calibration method, the average mass values of M_{n} and M_{w} of the sample were calculated to be 5178 and 7504 Da, respectively. One interesting point about this sample is the inflexion that occurs at approximately 9.9 min elution time marked with an asterisk (∗) in the chromatogram. This unexpected inflexion was observed from repeated sample analysis and thus it is assumed that this indicates there is a high molecular weight impurity within the sample. In order to obtain quantitative information of this impurity, we assume that this impurity has *similar* specific refractive index increment (dn/dc) value to the main sample. The dRI trace represents the true concentration distribution of the polymer and thus the impurity was calculated to account for 0.17% of the main product by comparison of the areas. Although in many cases this small amount may be regarded as insignificant, it behooves us to examine such impurities further since it is unknown whether the performance and physical properties of the implant devices may be affected. This unexpected high mass impurity may also be indicative of a problem elsewhere in the polymer sample. Thus, it is advantageous to examine the entire molecular weight distribution of the polymer when such abnormalities are observed.

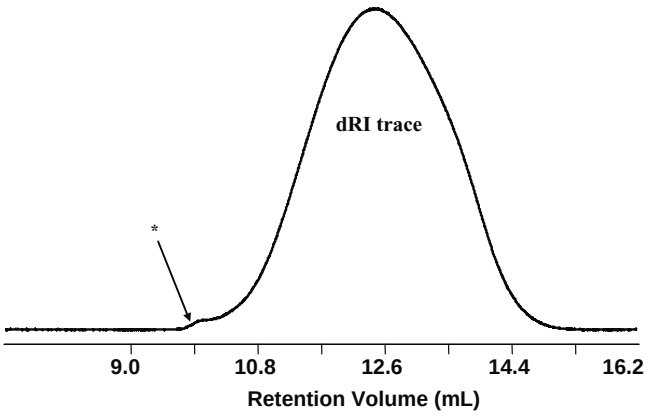

Fig. 1. dRI chromatogram of ME-PDMS that shows a small quantity of high molecular weight impurity marked with an asterisk (∗).

3.1. SEC mass spectrometry analysis

Although SEC with dRI detection seems to be the "industry standard" for calculating average mass values of polymer materials, it does not provide detailed information such as repeat unit sequence and end group chemistry of the oligomers. MALDI and ESI MS techniques provide this information and several studies for the application of these techniques for polymer analysis appears in the literature [4–6,29–32]. Specifically, the application of SEC prior to ESI and MALDI MS analysis can be used to overcome some mass discrimination effects imposed by direct analysis. The true on-line hyphenation of SEC with ESI MS analysis makes this combination very attractive with regards to the benefits gained from automation. The most notable benefit for us has been the time available for data analysis as opposed to instrument operation.

Fig. 2 illustrates the total ion chromatogram (TIC) (solid line), the dRI trace (dotted line) and selected mass spectra at different elution times of the ME-PDMS sample obtained from online SEC-ESI MS analysis. The mass spectrum at 14.8 min (Fig. 2b) contains a narrow distribution of singly charged ammoniated PDMS oligomers denoted as filled diamonds. The oligomers are separated by 74 Da, confirming the repeat unit as dimethyl siloxane (C_2H_6SiO). Additionally, the methyl end groups are confirmed from evaluation of the mono-isotopic mass peaks of selected oligomers in the exact mass measurements. A second singly charged ion distribution (minor) denoted with filled circles is also observed in Fig. 2b. From the exact mass data these oligomers were determined to be cyclic dimethyl siloxanes. It is worthwhile to mention that Aaserud et al. [24] also detected cyclic species present in their PMMA materials using SEC-ESI FTMS technique. Fig. 2a illustrates the mass spectrum at 13.0 min elution time that is the most concentrated spot according to the dRI chromatogram (the dotted trace in Fig. 2c). This spectrum contains both single and double charge states of the distribution. Interestingly the distribution, attributed to cyclic PDMS, is not observed in the higher mass fraction indicating that these impurities occur only in the low mass portion of the sample.

The TIC and dRI are superimposed in Fig. 2c. The poor overlap between the two traces indicates that, in this experiment, the TIC trace is a poor approximation of oligomeric

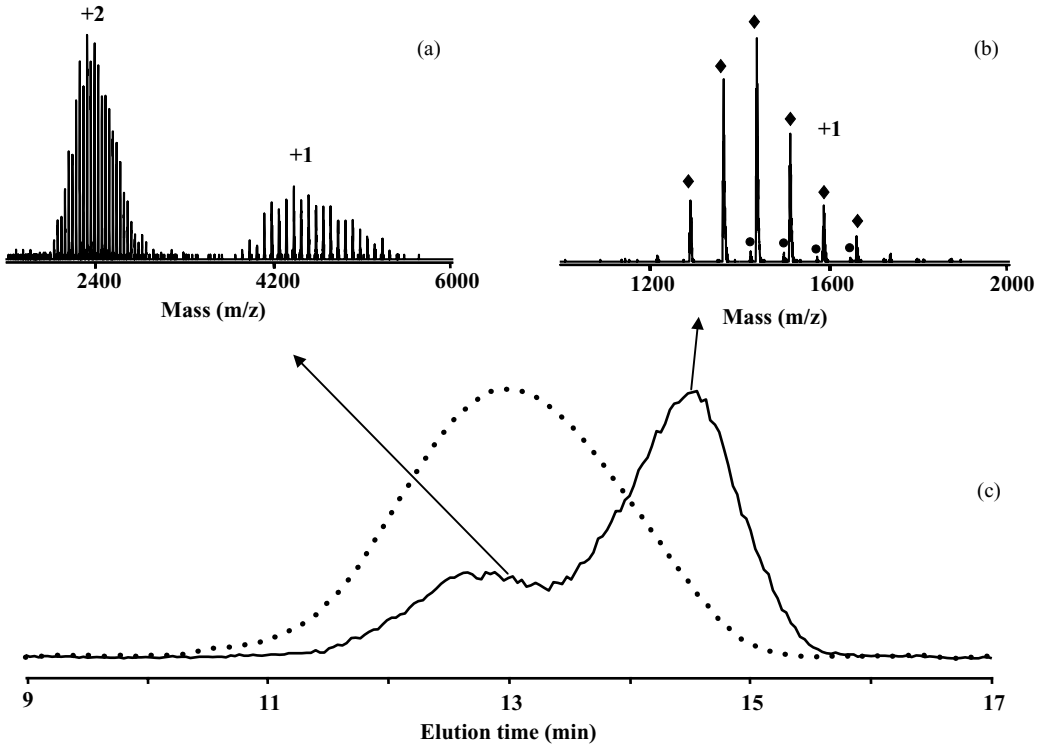

Fig. 2. Total ion chromatogram (TIC) and dRI chromatogram (c) of ME-PDMS and extracted MS spectra at elution times of (a) 13.0 min and (b) 14.8 min from SEC-ESI MS experiments.

concentration. The low mass and relatively low concentrated oligomers eluting at 14.8 min have the highest signal intensity (normalized to 100%) than the most concentrated oligomers eluting at 13.0 min (34% compared to the most intense peak at 14.8 min). In a previous study, we reported ion suppression within the TIC region where relatively high mass hydroxyl terminated PDMS (OH-PDMS) oligomers was observed [3]. We reported a modification to the GPC-ESI MS design that provided detection of the higher mass OH-PDMS compounds with higher S/N [27]. In the current study, higher mass ME-PDMS oligomers were indeed observed compared to the analysis of the same sample using the past SEC-ESI MS experimental design (data not illustrated). However, the data illustrated in Fig. 3 indicates that despite the more concentrated high mass oligomers present in solution, the lower mass oligomers are considerably more efficient in transferring into the gas phase for detection. Although this can be an advantage for trace analysis in the low mass portion of the mass distribution, it impedes the same efforts in the high mass region.

For comparative purposes, Fig. 3 illustrates mass spectra of the ME-PDMS sample obtained from SEC ESI and MALDI MS analysis. Fig. 3a and b illustrate the MALDI and ESI mass spectra, respectively, at 13.0 min elution time. This time corresponds to the most concentrated and most probable mass (M_p) along the dRI trace. As expected the MALDI spectrum shows a narrow distribution of singly charged ions while the ESI spectrum contains two narrow distributions of singly and doubly charged ions for this relatively high mass fraction. Comparatively the calculated M_n values for Fig. 3c and d were determined to be 4280 and 4330 Da, respectively.Fig. 3c and d illustrate the MALDI and ESI spectra of the highest mass fraction obtained while being mindful of acceptable S/N and oligomeric resolution. The calculated M_n for the MALDI mass fraction in Fig. 3c was calculated to be 12,000 Da while that of the ESI mass fraction in Fig. 3d was determined to be 7800 Da. Although we indeed observe improved results for obtaining high mass oligomers with online SEC-ESI MS, automated SEC-MALDI MS appears to be more effective for measuring the higher mass PDMS oligomers within a polymeric distribution. However, in this application neither of the SEC-MS techniques was able to obtain meaningful qualitative information of the inflexion part of the SEC trace at 9.9 min where the suspected mass distribution impurity for this sample elutes.

3.2. Triple detection analysis

This sample was analyzed by SEC with triple detection including dRI, LS, and VISC. Fig. 4 illustrates an overlay of the three chromatograms. While there is only a little indication in the traditional dRI trace (concentration response), the inflexion appears much more obvious in both LS and VISC traces. Eqs. (2) and (3) indicate that both LS and VISC signals are proportional to the molecular weight of the poly-

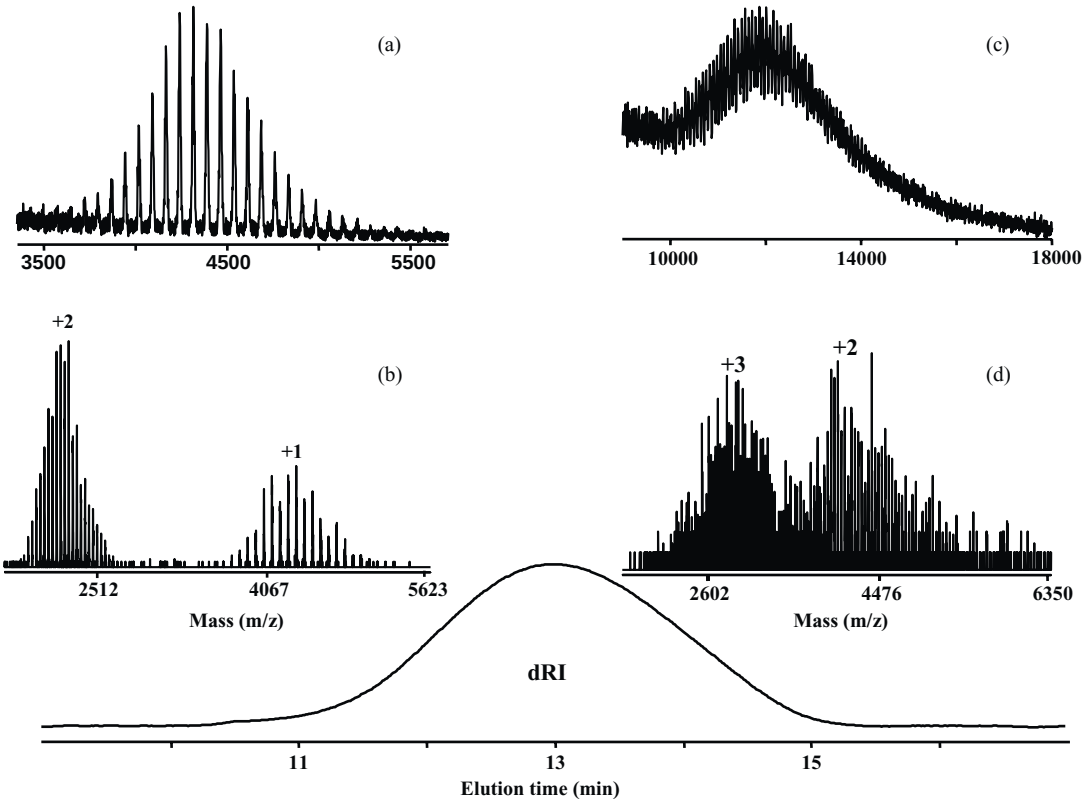

Fig. 3. SEC-MALDI (a) and SEC-ESI (b) mass spectra of ME-PDMS oligomers eluted at 13.0 min that are corresponding to the apex in the dRI trace (bottom). The highest molecular weight data obtainable by SEC-MALDI and SEC-ESI MS experiments are shown in (c) and (d), respectively.

mers. This explains the front shift in LS and VISC traces compared to the dRI trace. This front shift also demonstrates that the LS and VISC detectors are more sensitive to the high molecular weight oligomers within the polymeric mass distribution. Based on the correlation shown in Eq. (3), the absolute molecular weight of the fraction eluted at 9.9 min is calculated to be 75,000 Da. The distributions of absolute molecular weight [log(MW)] and intrinsic viscosity [log(IV)] are also obtained and illustrated in Fig. 4. The inflexion at 9.9 min in the log(MWD) and the dip in the log(IVD) suggests this small amount of impurity has a more compact conformation than the main sample. From this data, we propose that the configuration of the high mass impurity to be branched. From Eq. (4), the sample was determined to have a Mark–Houwink exponent '*a*' of 0.68. This indicates that the conformation of the main PDMS material is linear random coil [15–18].

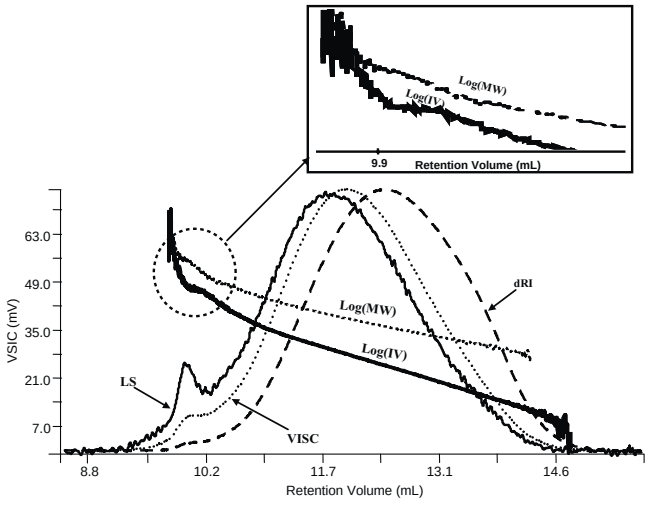

Fig. 4. dRI (- - -), VISC (···) and LS (—) chromatograms of ME-PDMS analyzed by SEC with triple detection. The absolute molecular weight [log(MW)] and intrinsic viscosity [log(IV)] are plotted as a function of elution time. The inset shows the expanded view at 9.9 min of the distributions of log(IV) and log(MW) where the high molecular weight impurity elutes.

3.3. Comparison of average mass determination

Selected ion chromatograms (SIC) of several mass fractions can be readily obtained from SEC-ESI MS experiments. Calibration curves can then be constructed by plotting the log(MW) obtained from the SIC as a function of elution time. The calibration curves from the conventional SEC technique and the SEC-ESI MS technique were created as illustrated in Fig. 5. The curve with conventional SEC calibration (unfilled squares) was constructed with a third order polynomial curve having a correlation coefficient (R^2) of 0.9998. The non-linear behavior can be explained by the deviation between hydrodynamic volume and molecular

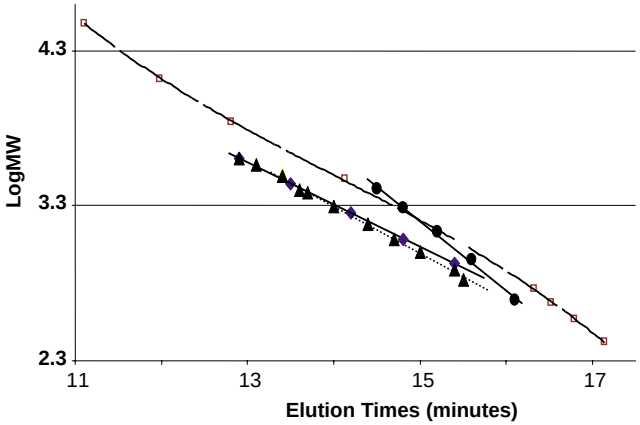

Fig. 5. Calibration curves generated from traditional SEC method, two linear PDMS samples, and one branched PDMS in the SEC-ESI MS experiments. The unfilled squares are generated from the conventional SEC method using narrow polystyrene standards. The filled cycles are the data obtained from the branched PDMS. The filled triangles and diamonds are data acquired from linear OH-PDMS and ME-PDMS, respectively.

weight over a relatively broad molecular weight range in the size exclusion process. In SEC-ESI MS calibration operations, the calibration curves were reconstructed for two linear (ME-PDMS and OH-PDMS) and a branched PDMS samples with R^2 from 0.9993 to 0.9998. The two linear PDMS samples include the highlighted ME-PDMS discussed previously in this paper (denoted as filled diamonds) and an OH-PDMS sample (denoted as filled triangles). A linear least square fit was used for the GPC-ESI MS data in Fig. 5. As expected the curves constructed from the SEC-ESI MS data are significantly different compared to the conventional polystyrene calibration curve. The difference in slope between the linear and branched PDMS (denoted as filled cycles) calibration curves indicates the branched sample has a more compact configuration than its linear counterparts. By comparing the OH and ME PDMS calibration curves in the low mass region (<2000 Da) it can be observed that at the same elution time the mass value of eluting OH-PDMS oligomers is smaller than that of ME-PDMS oligomers. A proposed explanation for this observation is that the hydrophilic OH end-groups repel from the hydrophobic siloxane backbone thus developing a more extended conformation. While the hydrophobic methyl end groups interact with the siloxane backbone to form conformations that are more compact. As the mass increases (>2000 Da), the calibration curves generated from both OH-PDMS and ME-PDMS merge as illustrated in Fig. 5. This suggests that the end groups have decreased impact on molecular size or hydrodynamic volume with increasing mass. Based on the calibration curves, the molecular weight and molecular weight distribution values of the polymer samples can be obtained.

Table 1 summarizes the average mass data for ME-PDMS obtained by direct ESI MS, conventional SEC, SEC with triple detection, and SEC-ESI MS experiments. SEC with triple detection is known to be an accurate method for determining average molecular weight and molecular weight distribution values of polymers [16–19,21,28]. In addition to the information of absolute molecular weights and molecular weight distribution, SEC with triple detection is the only technique that provides information of the weight-average IV and weight-average radius of gyration (R_g) of polymer molecules in the given solvent at the given temperature of the experiment. The average IV and R_g values of the ME-PDMS sample in toluene at 45 °C were determined to be 0.199 dl/g and 2.45 nm, respectively (Table 1).

In this study, we use the average molecular weight and molecular weight distribution values determined by SEC with triple detection as the benchmark values for clear comparison. The calculated M_n and M_w values by direct ESI MS are 61.7 and 69.7% less than the values determined by SEC with triple detection. This is due to the well-known mass discrimination effects associated with direct MS analysis of polymers [3,12]. The M_n and M_w values determined by conventional SEC are 57.4 and 39.4% higher than that by SEC with triple detection. This may be explained by the fact that polystyrene has a different hydrodynamic volume compared to PDMS at the same mass in toluene although they have similar linear conformations. It is amazing that the M_n value calculated by the on-line SEC-ESI MS is less than 1.0% deviated from that determined by SEC with triple detection. The mass difference is less than one dimethyl siloxane repeat unit! The M_w and M_z values obtained from SEC-ESI MS are both less than 5.0% from those determined by SEC with triple detection (Table 1). This study demonstrates that average molecular weights and molecular weight distributions determined by on-line SEC-ESI MS and SEC with triple detection agree remarkably well. On-line SEC-ESI MS

Table 1
Average mass values and other properties of ME-PDMS measured by different analytical techniques

Techniques	M_n (Da)	M_w (Da)	M_z (Da)	PD	IV (dl/g)	Weight-average R_g (nm)
Conventional GPC with PS calibration standards	5178	7504	11834	1.45	n.a.[a]	n.a.
ESI MS (direct infusion)	1260	1633	1877	1.30	n.a.	n.a.
GPC-ESI MS	3317	5547	15872	1.67	n.a.	n.a.
GPC with triple detection	3290	5384	15217	1.64	0.199[b]	2.45[b]

[a] n.a.: not applicable.
[b] The value was determined in toluene at 45 °C.

proves to be an excellent technique for determination of absolute molecular weights and molecular weight distributions of polymeric materials.

4. Conclusions

In this report, a PDMS sample was fully characterized using SEC with triple detection and SEC with mass spectrometry. A very small quantity of high mass impurity was detected by SEC with triple detection and was undetected by SEC MS. The absolute molecular weight of this impurity was determined to be 75000 Da and was believed to have branched conformation even though, the main sample was determined to have linear conformation. A small amount of cyclic PDMS species was detected only in the low molecular weight distribution of the ME-PDMS material by SEC-ESI MS. Based on the calibration curves generated from the on-line SEC-ESI MS experiments it was observed that the hydrophilicity of end groups (hydroxyl versus methyl) had an effect on the hydrodynamic volume of low mass (<2000 Da) linear PDMS oligomers. This study demonstrated that both on-line SEC-ESI MS and SEC with triple detection provided accurate average molecular weights and molecular weight distributions of this implant device material.

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