

Application Note

Polymer Analysis by MALDI-Tof MS

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Abstract

Molecular weight is an important parameter for synthetic polymers because it relates directly to their physical properties.

The most commonly used techniques to characterize synthetic polymers, such as osmometry, cryscopy, end-group titration and light scattering, only yield an average molecular weight and do not yield any information about chemical structure or chain branching, etc.

Other methods, such as gel permeation chromatography (GPC) and high performance liquid chromatography (HPLC), separate the oligomeric components of the polymer system with limited resolution.

Furthermore, the accuracy of molecular weight values is limited by the need to calibrate against reference compounds.

Thus these techniques are not suitable for the determination of absolute molecular weight distributions of the individual components of the polymer distribution.

MALDI MS has an important advantage in synthetic polymer analysis: absolute molecular weights of oligomers can be determined, as opposed to obtaining relative molecular weights by chromatographic techniques. MALDI polymer analysis permits accurate determination of molecular weights from narrowly distributed polymers (polydispersity <1.2).

This application note demonstrates the use of the Waters MALDI micro MX for polymer characterization. This includes molecular weight averages (both the number [Mn] and weight [Mw] averaged molecular weights), polydispersity, mass of repeat units and end-group mass structure.

Introduction

Polymer distributions are typically characterized by Mn and Mw, which are calculated from the formula:

$$M_n = \frac{\sum N_i M_i}{\sum N_i} \text{ and } M_w = \frac{\sum N_i M_i^2}{\sum N_i M_i}$$

Ni and Mi are the abundance and mass of the ith oligomer, respectively. For the polystyrene 2000 MALDI MX spectrum (Figure 1), the molecular weight averages can be calculated directly from the spectrum: Mn = 2079, Mw = 2232, and polydispersity (D = Mw/Mn) = 1.07, using programs such as Polymerix (Sierra Analytics, Modesto, CA, Figure 2 and 3)

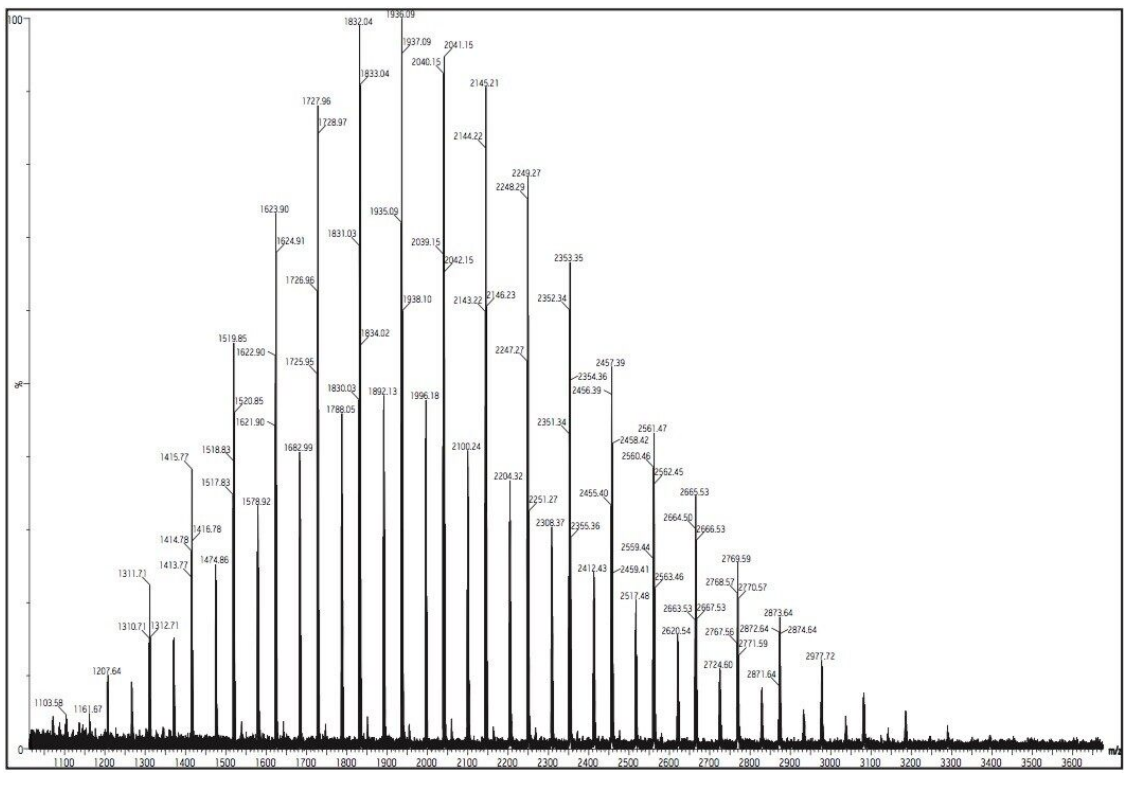
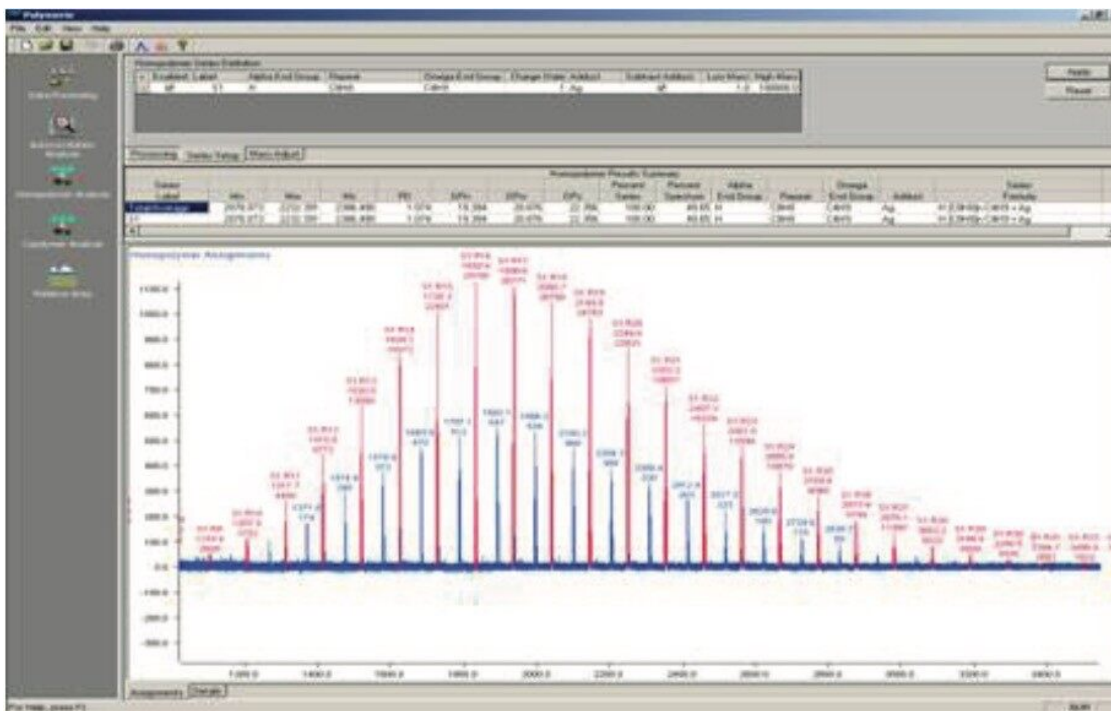


Figure 1. Polystyrene 2000 MALDI micro MX spectrum.



Found	Library	Found	Library	Found	Library	Found	Library
m/z	Relative Intensity	Elemental Analysis	Molecular Formula	m/z	Relative Intensity	Elemental Analysis	Molecular Formula
104.063	100	C ₈ H ₈	104.063	1934.083	100	C ₁₇ H ₁₄	1934.083
136.074	30	C ₉ H ₈	136.074	2252.139	10	C ₁₈ H ₁₄	2252.139
168.085	15	C ₁₀ H ₈	168.085	2570.195	5	C ₁₉ H ₁₄	2570.195
200.096	10	C ₁₁ H ₈	200.096	2888.251	3	C ₂₀ H ₁₄	2888.251
232.107	8	C ₁₂ H ₈	232.107	3206.307	2	C ₂₁ H ₁₄	3206.307
264.118	6	C ₁₃ H ₈	264.118	3524.363	1	C ₂₂ H ₁₄	3524.363
296.129	5	C ₁₄ H ₈	296.129	3842.419	1	C ₂₃ H ₁₄	3842.419
328.140	4	C ₁₅ H ₈	328.140	4160.475	1	C ₂₄ H ₁₄	4160.475
360.151	3	C ₁₆ H ₈	360.151	4478.531	1	C ₂₅ H ₁₄	4478.531
392.162	2	C ₁₇ H ₈	392.162	4796.587	1	C ₂₆ H ₁₄	4796.587
424.173	1	C ₁₈ H ₈	424.173	5114.643	1	C ₂₇ H ₁₄	5114.643
456.184	1	C ₁₉ H ₈	456.184	5432.699	1	C ₂₈ H ₁₄	5432.699
488.195	1	C ₂₀ H ₈	488.195	5750.755	1	C ₂₉ H ₁₄	5750.755
520.206	1	C ₂₁ H ₈	520.206	6068.811	1	C ₃₀ H ₁₄	6068.811
552.217	1	C ₂₂ H ₈	552.217	6386.867	1	C ₃₁ H ₁₄	6386.867
584.228	1	C ₂₃ H ₈	584.228	6704.923	1	C ₃₂ H ₁₄	6704.923
616.239	1	C ₂₄ H ₈	616.239	7022.979	1	C ₃₃ H ₁₄	7022.979
648.250	1	C ₂₅ H ₈	648.250	7341.035	1	C ₃₄ H ₁₄	7341.035
680.261	1	C ₂₆ H ₈	680.261	7659.091	1	C ₃₅ H ₁₄	7659.091
712.272	1	C ₂₇ H ₈	712.272	7977.147	1	C ₃₆ H ₁₄	7977.147
744.283	1	C ₂₈ H ₈	744.283	8295.203	1	C ₃₇ H ₁₄	8295.203
776.294	1	C ₂₉ H ₈	776.294	8613.259	1	C ₃₈ H ₁₄	8613.259
808.305	1	C ₃₀ H ₈	808.305	8931.315	1	C ₃₉ H ₁₄	8931.315
840.316	1	C ₃₁ H ₈	840.316	9249.371	1	C ₄₀ H ₁₄	9249.371
872.327	1	C ₃₂ H ₈	872.327	9567.427	1	C ₄₁ H ₁₄	9567.427
904.338	1	C ₃₃ H ₈	904.338	9885.483	1	C ₄₂ H ₁₄	9885.483
936.349	1	C ₃₄ H ₈	936.349	10203.539	1	C ₄₃ H ₁₄	10203.539
968.360	1	C ₃₅ H ₈	968.360	10521.595	1	C ₄₄ H ₁₄	10521.595
1000.371	1	C ₃₆ H ₈	1000.371	10839.651	1	C ₄₅ H ₁₄	10839.651
1032.382	1	C ₃₇ H ₈	1032.382	11157.707	1	C ₄₆ H ₁₄	11157.707
1064.393	1	C ₃₈ H ₈	1064.393	11475.763	1	C ₄₇ H ₁₄	11475.763
1096.404	1	C ₃₉ H ₈	1096.404	11793.819	1	C ₄₈ H ₁₄	11793.819
1128.415	1	C ₄₀ H ₈	1128.415	12111.875	1	C ₄₉ H ₁₄	12111.875
1160.426	1	C ₄₁ H ₈	1160.426	12429.931	1	C ₅₀ H ₁₄	12429.931
1192.437	1	C ₄₂ H ₈	1192.437	12747.987	1	C ₅₁ H ₁₄	12747.987
1224.448	1	C ₄₃ H ₈	1224.448	13066.043	1	C ₅₂ H ₁₄	13066.043
1256.459	1	C ₄₄ H ₈	1256.459	13384.099	1	C ₅₃ H ₁₄	13384.099
1288.470	1	C ₄₅ H ₈	1288.470	13702.155	1	C ₅₄ H ₁₄	13702.155
1320.481	1	C ₄₆ H ₈	1320.481	14020.211	1	C ₅₅ H ₁₄	14020.211
1352.492	1	C ₄₇ H ₈	1352.492	14338.267	1	C ₅₆ H ₁₄	14338.267
1384.503	1	C ₄₈ H ₈	1384.503	14656.323	1	C ₅₇ H ₁₄	14656.323
1416.514	1	C ₄₉ H ₈	1416.514	14974.379	1	C ₅₈ H ₁₄	14974.379
1448.525	1	C ₅₀ H ₈	1448.525	15292.435	1	C ₅₉ H ₁₄	15292.435
1480.536	1	C ₅₁ H ₈	1480.536	15610.491	1	C ₆₀ H ₁₄	15610.491
1512.547	1	C ₅₂ H ₈	1512.547	15928.547	1	C ₆₁ H ₁₄	15928.547
1544.558	1	C ₅₃ H ₈	1544.558	16246.603	1	C ₆₂ H ₁₄	16246.603
1576.569	1	C ₅₄ H ₈	1576.569	16564.659	1	C ₆₃ H ₁₄	16564.659
1608.580	1	C ₅₅ H ₈	1608.580	16882.715	1	C ₆₄ H ₁₄	16882.715
1640.591	1	C ₅₆ H ₈	1640.591	17200.771	1	C ₆₅ H ₁₄	17200.771
1672.602	1	C ₅₇ H ₈	1672.602	17518.827	1	C ₆₆ H ₁₄	17518.827
1704.613	1	C ₅₈ H ₈	1704.613	17836.883	1	C ₆₇ H ₁₄	17836.883
1736.624	1	C ₅₉ H ₈	1736.624	18154.939	1	C ₆₈ H ₁₄	18154.939
1768.635	1	C ₆₀ H ₈	1768.635	18472.995	1	C ₆₉ H ₁₄	18472.995
1800.646	1	C ₆₁ H ₈	1800.646	18791.051	1	C ₇₀ H ₁₄	18791.051
1832.657	1	C ₆₂ H ₈	1832.657	19109.107	1	C ₇₁ H ₁₄	19109.107
1864.668	1	C ₆₃ H ₈	1864.668	19427.163	1	C ₇₂ H ₁₄	19427.163
1896.679	1	C ₆₄ H ₈	1896.679	19745.219	1	C ₇₃ H ₁₄	19745.219
1928.690	1	C ₆₅ H ₈	1928.690	20063.275	1	C ₇₄ H ₁₄	20063.275
1960.701	1	C ₆₆ H ₈	1960.701	20381.331	1	C ₇₅ H ₁₄	20381.331
1992.712	1	C ₆₇ H ₈	1992.712	20699.387	1	C ₇₆ H ₁₄	20699.387
2024.723	1	C ₆₈ H ₈	2024.723	21017.443	1	C ₇₇ H ₁₄	21017.443
2056.734	1	C ₆₉ H ₈	2056.734	21335.499	1	C ₇₈ H ₁₄	21335.499
2088.745	1	C ₇₀ H ₈	2088.745	21653.555	1	C ₇₉ H ₁₄	21653.555
2120.756	1	C ₇₁ H ₈	2120.756	21971.611	1	C ₈₀ H ₁₄	21971.611
2152.767	1	C ₇₂ H ₈	2152.767	22289.667	1	C ₈₁ H ₁₄	22289.667
2184.778	1	C ₇₃ H ₈	2184.778	22607.723	1	C ₈₂ H ₁₄	22607.723
2216.789	1	C ₇₄ H ₈	2216.789	22925.779	1	C ₈₃ H ₁₄	22925.779
2248.800	1	C ₇₅ H ₈	2248.800	23243.835	1	C ₈₄ H ₁₄	23243.835
2280.811	1	C ₇₆ H ₈	2280.811	23561.891	1	C ₈₅ H ₁₄	23561.891
2312.822	1	C ₇₇ H ₈	2312.822	23879.947	1	C ₈₆ H ₁₄	23879.947
2344.833	1	C ₇₈ H ₈	2344.833	24197.003	1	C ₈₇ H ₁₄	24197.003
2376.844	1	C ₇₉ H ₈	2376.844	24515.059	1	C ₈₈ H ₁₄	24515.059
2408.855	1	C ₈₀ H ₈	2408.855	24833.115	1	C ₈₉ H ₁₄	24833.115
2440.866	1	C ₈₁ H ₈	2440.866	25151.171	1	C ₉₀ H ₁₄	25151.171
2472.877	1	C ₈₂ H ₈	2472.877	25469.227	1	C ₉₁ H ₁₄	25469.227
2504.888	1	C ₈₃ H ₈	2504.888	25787.283	1	C ₉₂ H ₁₄	25787.283
2536.899	1	C ₈₄ H ₈	2536.899	26105.339	1	C ₉₃ H ₁₄	26105.339
2568.910	1	C ₈₅ H ₈	2568.910	26423.395	1	C ₉₄ H ₁₄	26423.395
2600.921	1	C ₈₆ H ₈	2600.921	26741.451	1	C ₉₅ H ₁₄	26741.451
2632.932	1	C ₈₇ H ₈	2632.932	27059.507	1	C ₉₆ H ₁₄	27059.507
2664.943	1	C ₈₈ H ₈	2664.943	27377.563	1	C ₉₇ H ₁₄	27377.563
2696.954	1	C ₈₉ H ₈	2696.954	27695.619	1	C ₉₈ H ₁₄	27695.619
2728.965	1	C ₉₀ H ₈	2728.965	28013.675	1	C ₉₉ H ₁₄	28013.675
2760.976	1	C ₉₁ H ₈	2760.976	28331.731	1	C ₁₀₀ H ₁₄	28331.731

Figures 2 and 3. Polymerix (Sierra Analytics) is used for molecular weight distribution measurement for Polystyrene 2000.

The repeating mass unit of 104.063 confirms that the analyte is a styrene-based polymer. For the peak at m/z = 1934.083, it can easily calculate that the peak is a PS with 17 repeat styrene units (mass = 1769.071 Da).

The difference of 165.012 mass units (1934.083 – 1769.071) is made up by the addition of 107.913 and 57.099 with 107.913 is the average mass of the silver atom (from silver-cationized PS ions). Mass 57.099 is the butyl end group.

In this case, MALDI results provide good confirmation of the polymer structure. The technique can accurately determine the masses of oligomers, confirming that MALDI is a useful tool for end-group analysis as well as product confirmation.

Experimental

The major difference between MALDI analysis of proteins/peptides and synthetic polymers is in the ionization process. For protein/peptide MALDI analysis, most samples are ionized through protonation; for synthetic polymer MALDI experiments most samples are ionized by cationization.

Some polymers, such as polyethylene glycols and polymethylmethacrylate form ions with alkali metals (added to the sample or simply present as an impurity) in the form of MLi^+ , MNa^+ , and MK^+ , etc. Others, such as polystyrene, undergo a cationization process which preferentially involves transition metal ions, such as silver and copper.¹ The actual mechanism of the cationization process remains unclear, but it appears that as the result of a gas phase collision between cation and polymer chain, the cation will link itself in some fashion to an electron-rich site on the polymer chain.

Therefore, it is more challenging to analyze unknown polymer samples by MALDI MS than unknown peptides and proteins samples because of the sample preparation issues. Very often different MALDI MS sample preparation methods (matrices, solvents, adding/removing salts, etc.) are employed for different types of polymers. Here are some of the examples from MALDI polymer analysis experiments.

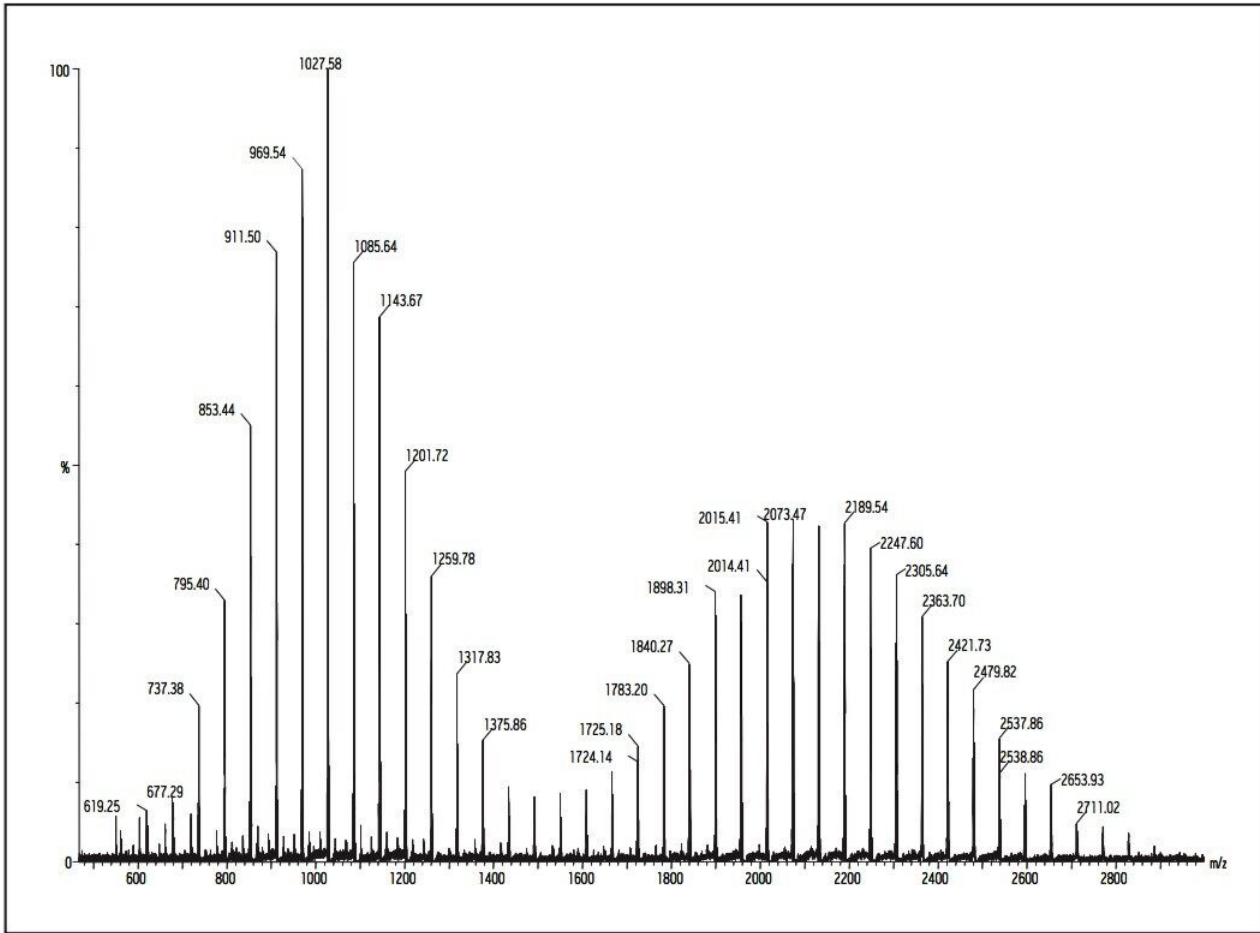


Figure 4. Polypropylene glycol 1000 and 2000 (1 mg/mL) mixture MALDI micro MX spectrum. DHB (10 mg/mL) was used as the matrix. Data was acquired in reflectron mode. All major peaks are sodiated peaks.

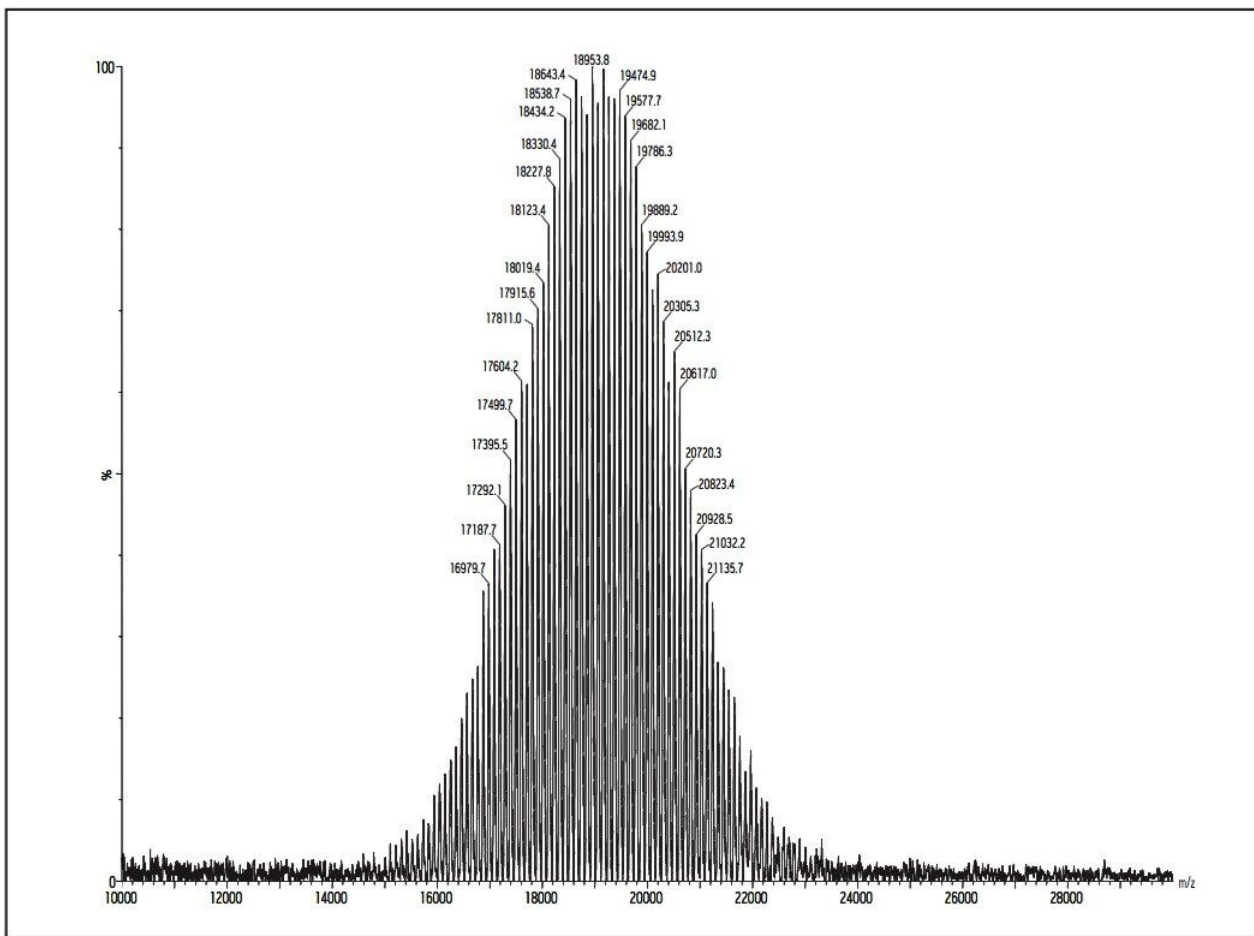


Figure 5. Polystyrene 19K (5 mg/mL in THF) MALDI micro MX spectrum. Dithranol (50 mg/mL) was used as the matrix. AgTFA (5 mg/mL) was added to the matrix and sample. Data was acquired in linear mode. All major peaks are silver cationized peaks.

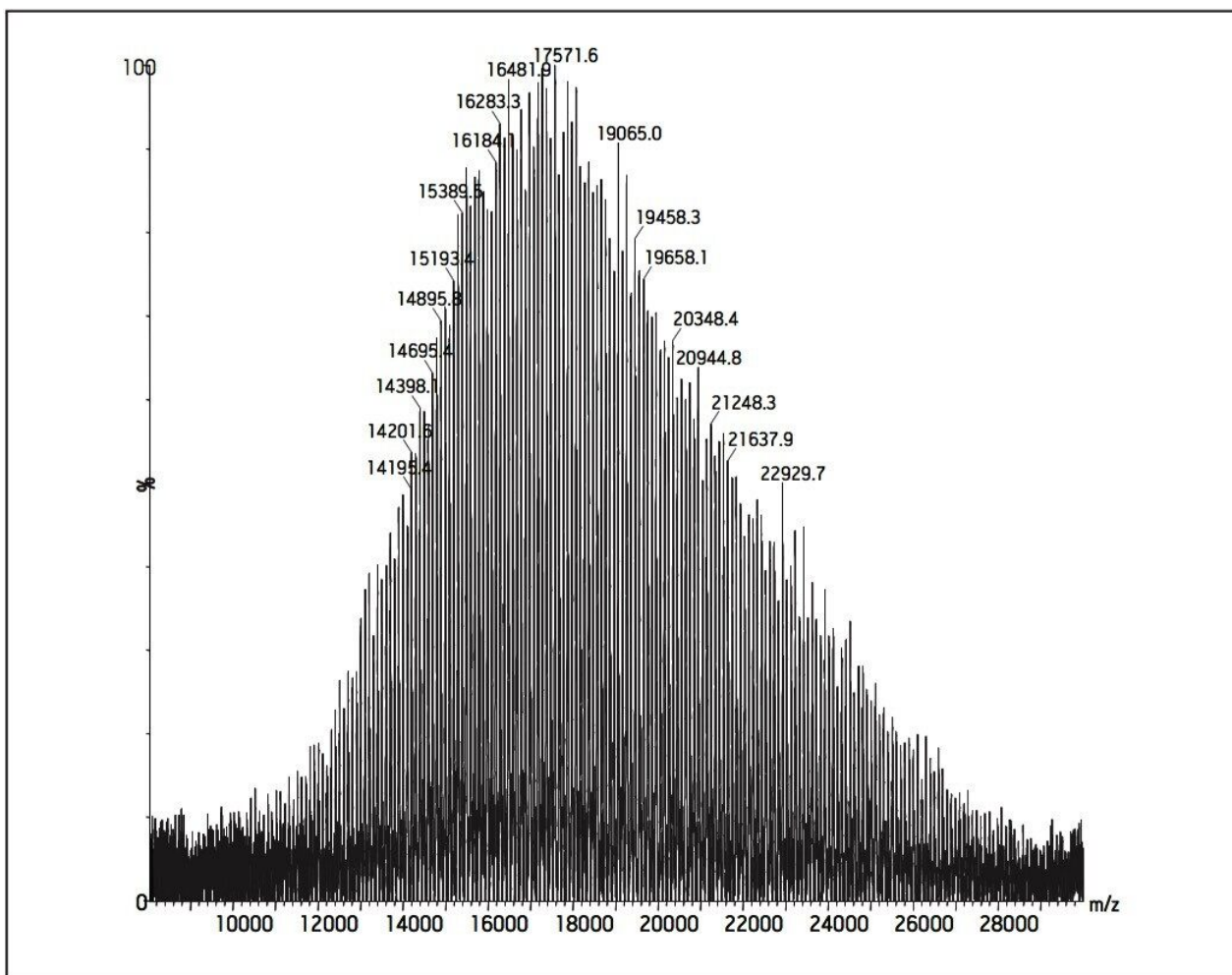


Figure 6. Polymethyl methacrylate 20K (1 mg/mL) MALDI micro MX spectrum. Dithranol (25 mg/mL) was used as the matrix. Data was acquired in linear mode. All major peaks are sodiated peaks.

Results and Discussion

GPC MALDI Polymer Analysis

Although MALDI MS has been used widely to provide molecular weight and structural and compositional information of synthetic polymers, one limitation of the technique is that it fails to provide correct molecular-weight values for polydisperse polymers (polydispersity, $M_w/M_n > 1.2$).

To overcome this limitation, the output of GPC can be coupled to the MALDI micro MX system by collecting

GPC fractions and performing MALDI analysis off-line. The average molecular weight of each fraction is then determined, allowing calibration of the GPC curve against absolute molecular weight. The calibrated GPC trace can then be used to compute average molecular weight and molecular-weight distribution of the unfractionated polymer samples.

Here is an example of GPC MALDI analysis for Poly-(DL-Lactide-co-glycolide) (PLG). OLG polymers are biocompatible and biodegradable polyesters used in drug delivery. Molecular weight data for these polymers is of importance as it relates to performance characteristics. PLG polymers are usually highly polydisperse ($P_d > 1.5$), thus present a challenge for MALDI analysis. Figures 7 and 8 show MALDI data obtained before and after GPC (Waters Styragel HR2 4.6 x 300 mm on a Waters 616 pump with 600S controller) separation.

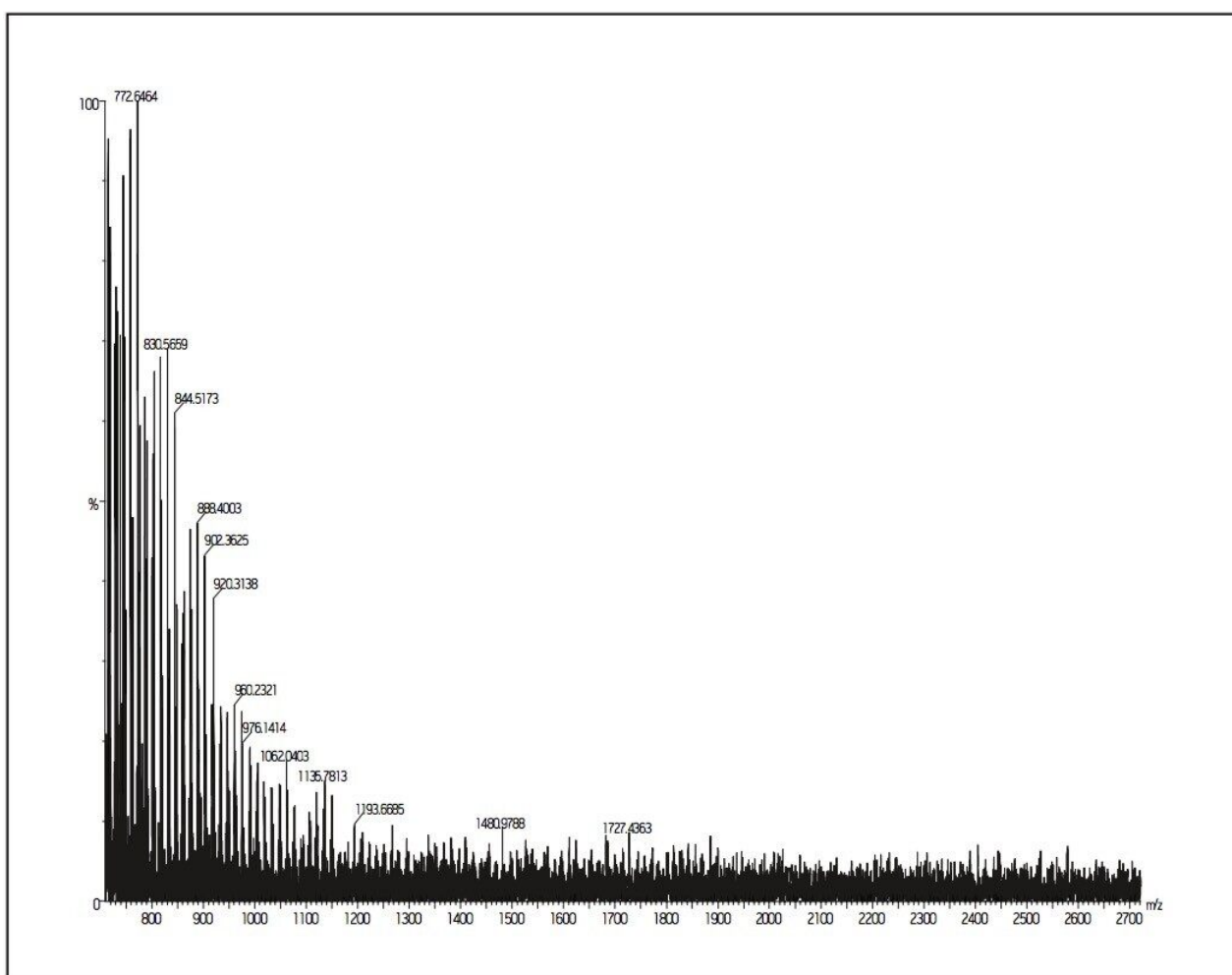


Figure 7. Poly-(DL-Lactide-co-glycolide) (1 mg/mL) MALDI micro MX spectrum before GPC separation. Dithranol (10 mg/mL) was used as the matrix. Data was acquired in reflectron mode. All major peaks are sodiated peaks. The spectrum does not reflect the real molecular distribution of the polymer sample.

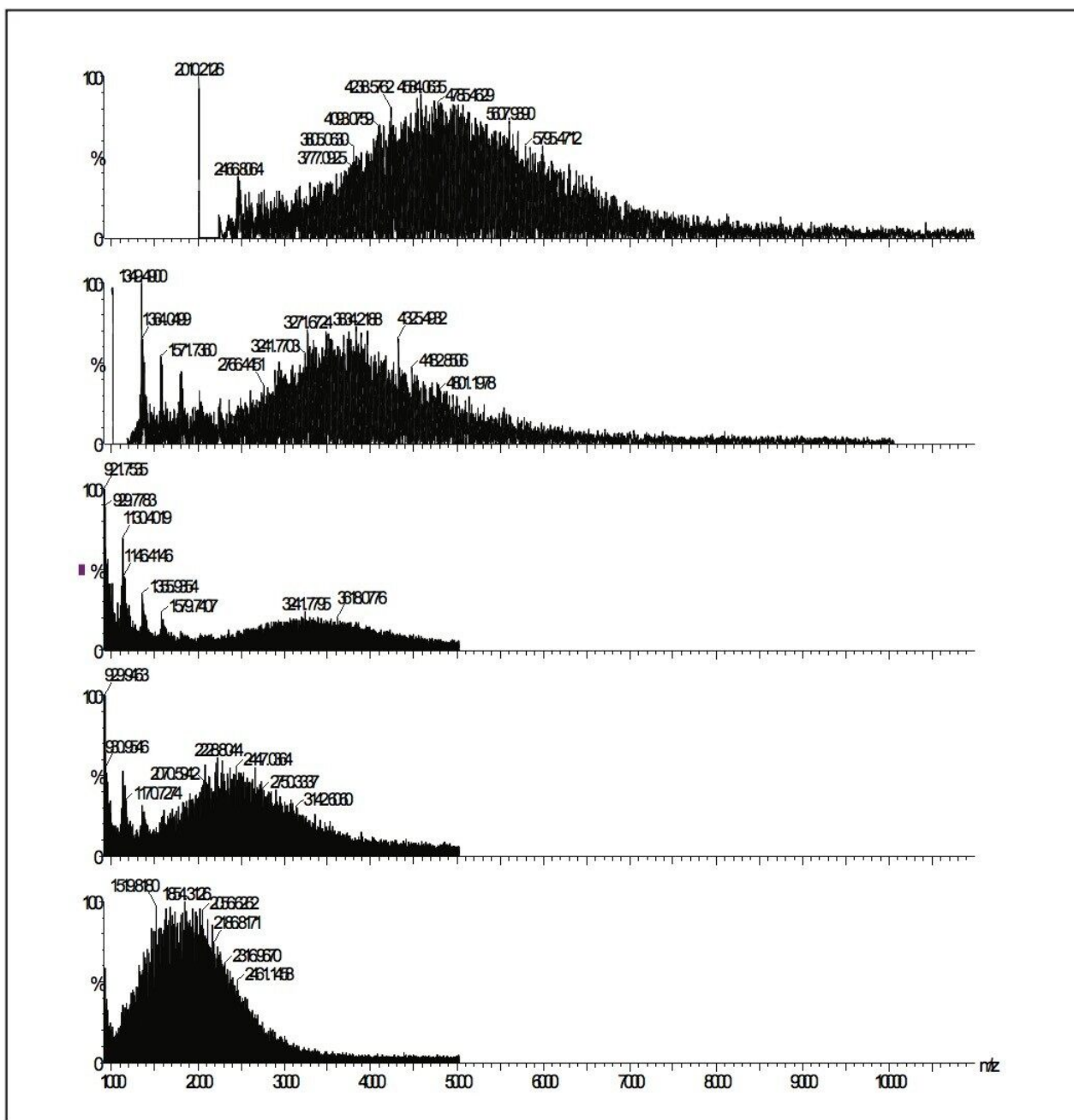


Figure 8. Poly-(DL-Lactide-co-glycolide) (1 mg/mL) MALDI micro MX spectrum after GPC separation.

Dithranol (10 mg/mL) was used as the matrix. Data was acquired in reflectron mode. All major peaks are sodiated peaks.

The molecular weight distribution of the polymer sample can be calculated by combining the spectra.

MALDI analysis of polyamidoamine (PAMAM) dendrimers PAMAM dendrimers represent an exciting new class of macromolecular architecture called "dense star" polymers. Unlike classical polymers, dendrimers

have a high degree of molecular uniformity, narrow molecular weight distribution, specific size and shape characteristics, and a highly-functionalized terminal surface. The manufacturing process is a series of repetitive steps starting with a central initiator core. Each subsequent growth step represents a new generation of polymer with a larger molecular diameter, twice the number of reactive surface sites, and approximately double the molecular weight of the preceding generation.

First discovered in the early 1980s by Dr. Donald A. Tomalia² at Dow Chemical, these polymers were called dendrimers to describe their tree-like branching structure. Figure 9 shows the polymerization process of dendrimers.

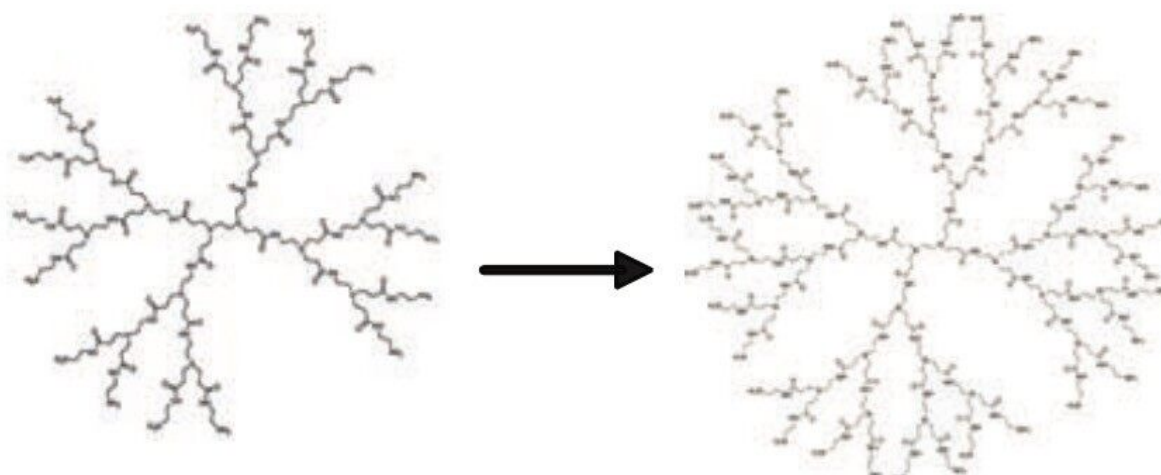


Figure 9. Polymerization of polyamidoamine (PAMAM) dendrimer.

Because of the structure, dendrimer samples tend to grasp as much salt as possible from the surroundings; thus better results are obtained by desalting the samples before mixing with the matrix. The common matrix used for dendrimer samples is DHB (2,5-dihydroxybenzoic acid).

Figure 10 is a MALDI spectrum for polyamidoamine 3 (PAMAM3, third generation). Fragmentations from the branches of the intact dendrimer molecule are often observed in MALDI spectrum.

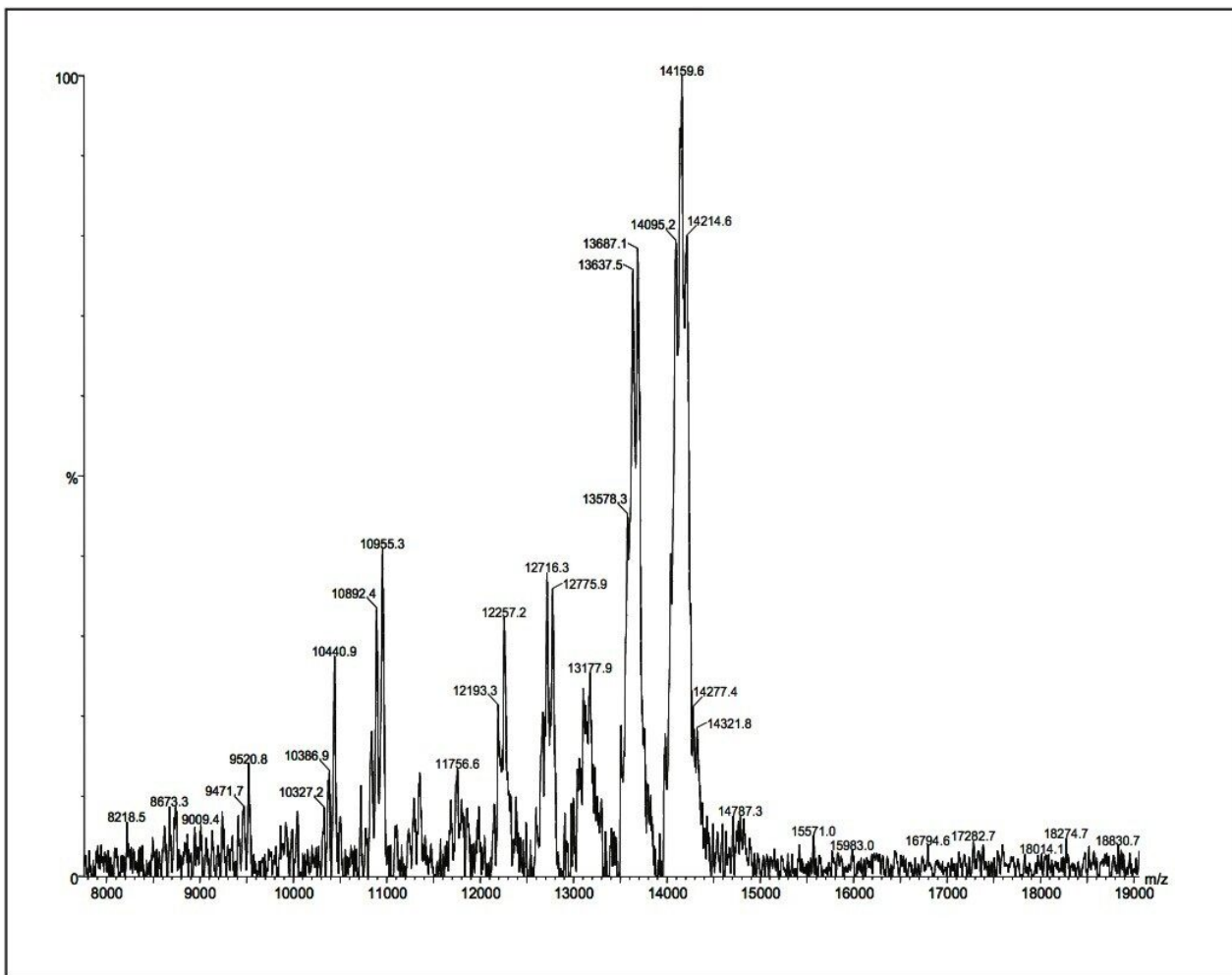


Figure 10. Polyamidoamine 3 (PAMAM3) (1 mg/mL) dendrimer MALDI micro MX spectrum. DHB (10 mg/mL) was used as the matrix. Data was acquired in linear mode. All major peaks are sodiated peaks.

Post acceleration detector (PAD) Higher molecular weight samples have less sensitivity in MALDI experiments. This is especially true when the molecular weights (distributions) of polymer samples are more than 50K Dalton. Therefore, a post acceleration detector (PAD) is equipped for all MALDI micro MX, to increase sensitivity for higher molecular weight analytes.

Figures 11 and 12 show two examples for polystyrene 190K and 410K, using PAD on the MALDI micro MX for the improvement of sensitivity.

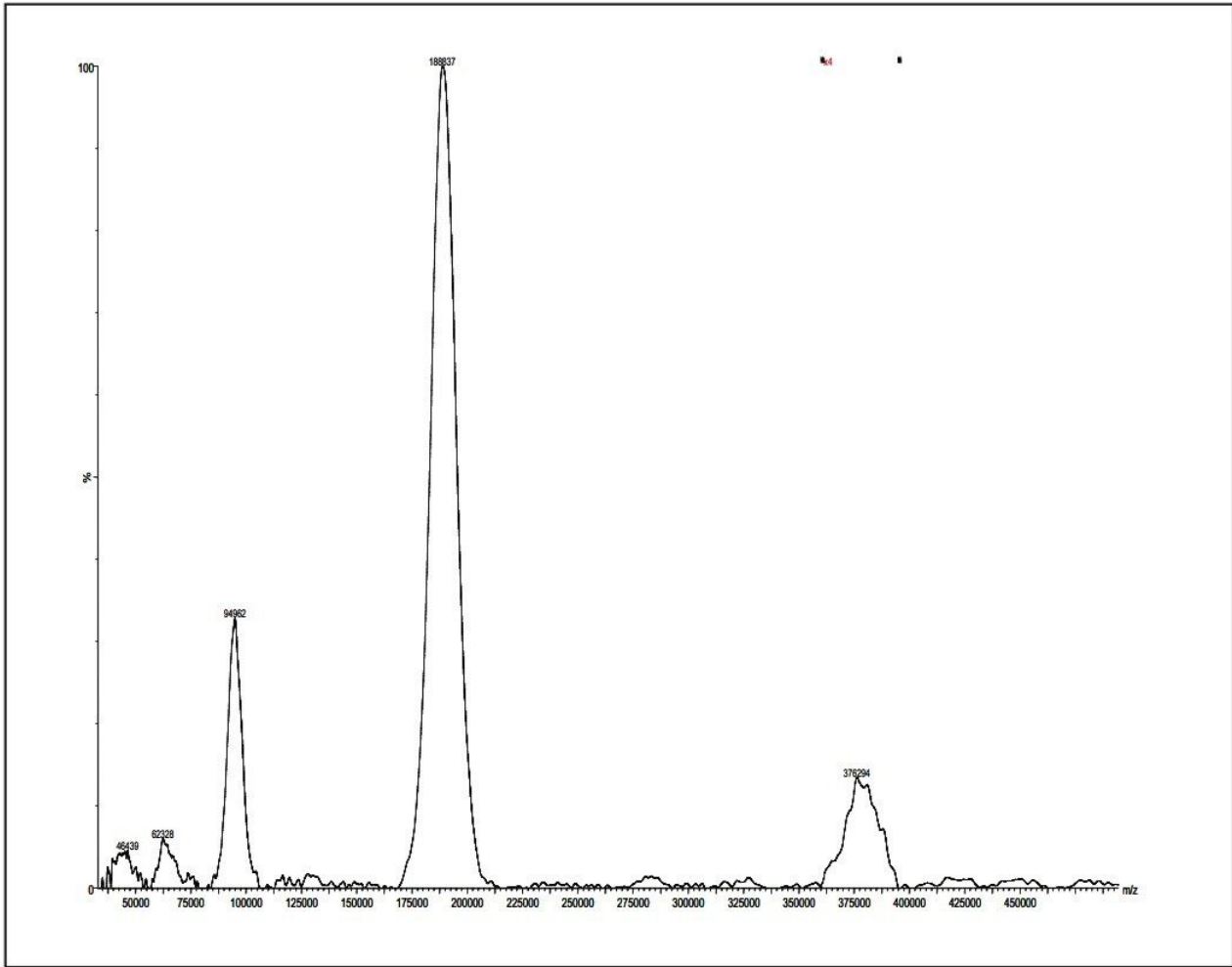


Figure 11. Polystyrene 190K (5 mg/mL) MALDI micro MX spectrum. All-trans retinoic acid (75 mg/mL) was used as the matrix. AgTFA (5 mg/mL) was added to the sample. Data was acquired in linear mode with PAD on. All major peaks (including dimer at m/z of 380K and doubly charged peak at m/z of 95K) are silver cationized peaks.

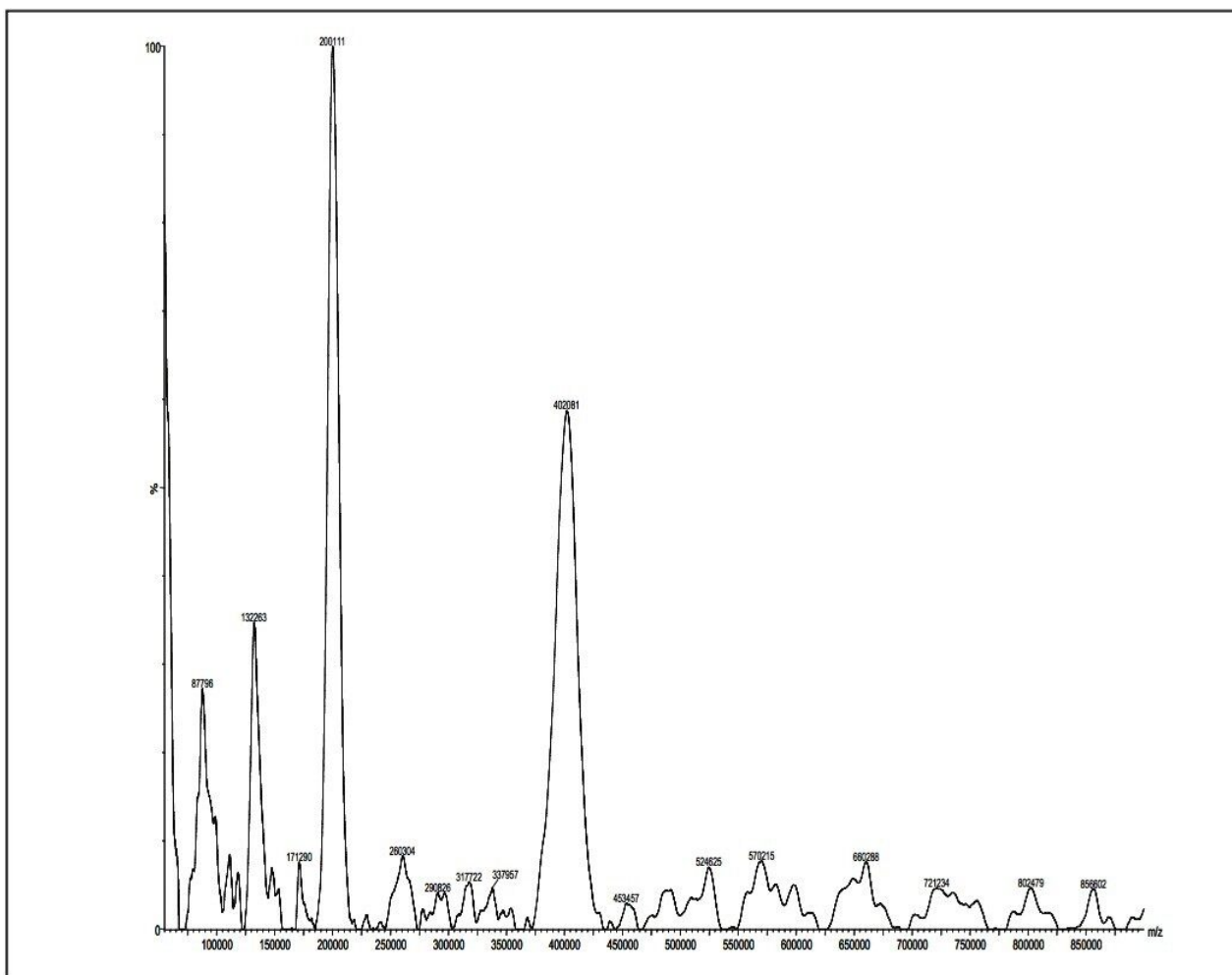


Figure 11. Polystyrene 410K (75 mg/mL) MALDI micro MX spectrum. All-trans retinoic acid (75 mg/mL) was used as the matrix. AgTFA (5 mg/mL) was added to the sample. Data was acquired in linear mode with PAD on. All major peaks (including doubly charged peak at m/z of 205K and triply charged peak at m/z of 137K) are silver cationized peaks.



MALDI micro-MDX[®]
BRUKER

2. Dow Patent 4,289,872 (published 1981, filed 1979).

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