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Application of Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry and Gel Permeation Chromatography to Polymer Analysis

by

Honghui Zhu

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of *Master of Science*

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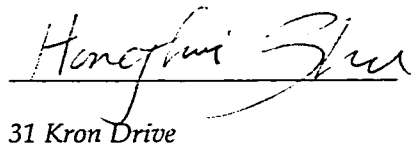

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ABSTRACT

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI TOF-MS) has been demonstrated as a powerful technique to analyze synthetic polymers. However, the accuracy of this method is difficult to assess due to the lack of suitable polymer standards of accurately known molecular weights and molecular weight distributions. Furthermore, the comparison of MALDI TOF-MS and gel permeation chromatography (GPC) methods has been found varied from one lab to another. As a result, this study investigated the accuracy of MALDI results using a particular polymeric system; examined several important factors affecting the accurate determination of polymer molecular weights; and explored possible causes for the variations between the MALDI and GPC results.

A series of narrowly distributed polystyrene standards were selected in this study. It was found that when using a very sensitive sample preparation method for MALDI TOF-MS analysis, neither symmetric nor asymmetric mass spectra truncations were observed. Second, the determination of polymer molecular weights was found to strongly depend on instrumental configuration, laser power, time-lag focusing point and spectra interpretation method. It was believed that different instrumental configurations and laser power would produce the different sensitivity and resolution spectra, thereby, causing variation in polymer molecular weights. Third, a comparison of MALDI and GPC results showed that significant difference exists in terms of resultant average molecular weights and polydispersity. It is believed that this discrepancy is caused by the different mass separation mechanisms between the two techniques, the band broadening effect and the accuracy of instrument calibration in GPC analysis. Finally, a new approach was proposed to analyze the GPC band broadening function by using the MALDI result as true polymer molecular weight distribution.

University of Alberta

Faculty of Graduate Studies and Research

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled **Application of Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry and Gel Permeation Chromatography to Polymer Analysis** submitted by Honghui Zhu in partial fulfillment of the requirements for the degree of Master of Science in Analytical Chemistry.

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Sept. 22, 2000

Dedicated to my parents, my husband, my son and my daughter

GUOCIONG ZHU

HUILANG LEI

HONGDE ZHOU

KEVIN ZHOU

CATHY ZHOU

For their encouragement, understanding and support.

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LIST OF ABBREVIATIONS

a	pore radius
A_i	area of the *i*th slice
E	electronic field of source
e	fundamental unit of charges
f(v)	experimental chromatogram function
FWHM	full width at half maximum of the ion peak
g(v-y)	band broadening function
GPC	gel permeation chromatography
K	distribution coefficient
L	length of drift region
m	mass of ions
m/z	mass-to-charge ratio
MALDI	matrix-assisted laser desorption/ionization
MCP	microchannel plate
M_i	mass for the oligomer containing *i* monomers
M_n	number average molecular weights
M_p	most probable molecular weight
M_w	weight average molecular weights
Nd:YAG	neodymium: yttrium aluminum garnet
N_i	signal intensity in peak area respectively
PD	polydispersity, PD = Mw/Mn
PMMA	poly(methyl methacrylate)
PS	polystyrene
R	shell of thickness
RSD	relative standard deviation
v	ionic velocity
s	distance between extraction grid and backing plate

t	total flight time
t_d	flight time of drift region
THF	tetrahydrofuran
TOF MS	time-of-flight mass spectrometry
t_s	flight time in source region
UV	ultraviolet
v/v	volume-to-volume ratio
V_o	void volume
Vp	porous volume
w(y)	true polymer oligomer distribution
y	mean elution volume of individual species
z	number of charges
DHB	2,5-dihydroxybenzoic acid
SEC	size exclusion chromatograph
VPO	vapor pressure osmometry
IV	intrinsic viscosity
LLS	laser light scattering
Mv	viscosity average molecular weight

CHAPTER 1 INTRODUCTION

Matrix-assisted laser desorption/ionization (MALDI) time-of-flight mass spectrometry has been proved to be a powerful technique for the analysis of synthetic polymers. The greatest advantage of this method is that with the assistance of a suitable matrix, large polymer molecules can be analyzed without fragmentation. Compared to some conventional polymer analysis techniques, it can provide polymer molecular weight and molecular weight distribution information with high analysis speed, minimum sample preparation and high accuracy. It also has the potential to provide oligomer structural information [1-18]. This makes it feasible to analyze much larger polymers that could not be examined with other mass spectrometric methods such as field desorption (FD), fast atom bombardment (FAB) and plasma desorption [1].

In this chapter, the principles of matrix-assisted laser desorption/ionization (MALDI) time-of-flight mass spectrometry will be reviewed. In addition, the principles of gel permeation chromatography (GPC) will also be reviewed in view of its wide application in polymer molecular weight determination.

1.1 MALDI Time-of-Flight Mass Spectrometry

1.1.1 Mechanism of a Linear Time-of-Flight Mass Spectrometer

Time-of-flight mass spectrometry is a type of mass measurement instrument in which the mass to charge ratio (m/z) can be determined by measuring its flight time. In practice, it is done by accelerating an ion electrostatically to a defined kinetic energy and measuring its time-of-flight through a field free or drift region. For a linear time-of-flight mass spectrometer, the flight time is generally measured by positioning a detector at the opposite end of the drift region.

The development of time-of-flight mass spectrometer could be dated back a long time ago. During the 1960s it became prominent in the mass spectrometric field, but was soon displaced by magnetic and quadrupole instruments. One reason for its failure to mature was that magnetic and quadrupole instruments could give higher sensitivity and mass resolving power. Another problem was the lack of technologies to record ions and process the mass spectrum in a microsecond flight time scale. With recent advances in technology, recording the microsecond time scale has become possible. Accompanying it is the emergence of ionization techniques for high molecular weight biological compounds [1]. There was also an increasing need to perform faster mixture separation. Time-of-flight mass spectrometers reappeared as a major mass analyzer in the mass spectrometry field in the 1990s [19].

Compared to other types of mass spectrometers, the time-of-flight mass spectrometer offers several distinct advantages. First of all, it has a theoretically unlimited mass range. Second, mass determination is very fast since mass spectra can be obtained in every single cycle without the need to scan any voltages or currents. Finally, ion transmission efficiency is very high because only few ion optical elements are needed [20, 21]. As a result, time-of-flight mass spectrometers have been rapidly developed and extensively applied in many different fields. Figure 1.1 shows a schematic diagram of a simple linear time-of-flight mass spectrometer.

The operational mechanism of a time-of-flight mass spectrometer can be explained by the following equations

$$Esze = 1/2\ mv^2 \tag{1.1}$$

where E is the electronic field of the source, s is the distance between the extraction grid and backing plate, z is the number of the charge, e is the fundamental unit of the charge, m is the mass of the ion and v is the ion velocity. Since the velocity of the ion is

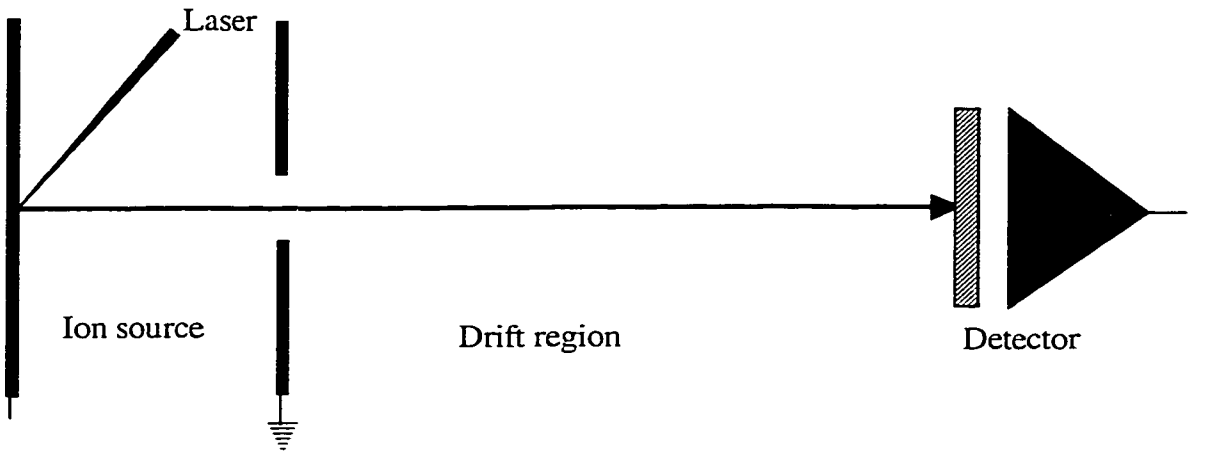

Figure 1.1. The schematic diagram of a simple linear time-of-flight mass spectrometer

determined by the flight time of drift region (t_d) and the length of the drift region (L), therefore

$$t_d = L(m/2ezEs)^{1/2} \tag{1.2}$$

In addition to the flight time (t_d) that an ion will experience in the drift region, there is also another flight time (t_s), which is the time that the ion spends in the source region. Thus, the total flight time (t) of an ion is:

$$t_s = 2s(m/2eEsz)^{1/2} \tag{1.3}$$

$$t = t_s + t_d = (m/2eEsz)^{1/2} (2s + L) \tag{1.4}$$

Equation 1.4 shows that m/z is dependent on the total flight time of an ion in both of the source region and drift region. With knowledge of the instrumental design and the ion arriving time, the m/z value can be determined.

1.1.2 Factors Affecting the Mass Resolution in a Time-of-Fight Mass Spectrometer

Because of these advantages, time-of-flight mass spectrometers have great potentials to be used in different areas of analysis. However, the low mass resolution inherited in initial instrumental designs was a major obstacle in their wide applications. The mass resolution in a time-of-flight mass spectrometer can be calculated as:

$$\text{Resolution} = m/\Delta m = t/2\Delta t \tag{1.5}$$

where m is the ion mass and t is the flight time of ion, Δm and Δt are measured as the full width at half maximum (FWHM) of the ion peak. The mass resolution depends on ions' temporal distribution, spatial distribution and initial kinetic energy distribution. Mass

spectral recording in TOF usually involves three major steps, namely ionization, ion acceleration and ion flight-time measurement. The three distributions are related to the ionization process.

Temporal distribution results from the time difference during ion formation for the ions that have the same mass and initial kinetic energy. Different ion formation times result in different times at which the ions of same mass and initial kinetic energy could reach the detector. This variation will result in a boarder peak, hence a reduction in mass resolution. If the time difference between ion formations remains constant, mass resolution can be improved by several different ways. For example, it can be done by reducing the accelerating voltage (i.e., reducing Δt) or increasing the length of flight tube (i.e., increasing flight time t) [2].

Spatial distribution is a result of ion formation at different locations along the direction of the electric field. The ions that have the same mass and initial kinetic energy, but are formed at different locations will gain different energy from the electric field in the ionization source region. The ions formed toward the rear of the ionization source get higher kinetic energy than the ions formed near the front of the source. This kind of mass resolution reduction problem is generally solved in the following two ways. The first approach is the famous two-stage extraction system developed by Wiley and McLaren in 1955 [22]. The two-stage extraction system is based on a mass independent space-focus plane existing the drift region. At the space-focus plane, faster ions formed toward the rear of the source will catch up with the slower ions formed near the front of the source. The two-stage extraction system can push the space-focus plane to the entrance of the detector. This approach has resulted in a substantial improvement in mass resolution. Another approach is to consider the space-focus plane as an ion source in which ions having different energy are focused by using reflectron mode operation [2].

Initial kinetic energy distribution deals with the variation in the initial kinetic energy of

the formed ions. Ions with higher initial kinetic energy will have larger velocities and reach the detector sooner than those with lower initial kinetic energy. In addition, the direction of the ion velocity is also important. If the ions have a velocity opposite the direction of source exit, there is a time delay for these ions to turn around their directions and catch up with other ions. This time delay, known as the turn-around time, will also reduce the mass resolution.

1.1.3 Combination of MALDI and Time-of-Flight Mass Spectrometry

Matrix-assisted laser desorption/ionization (MALDI) is a relative new laser desorption technique in which analyte molecules are premixed with a matrix to form crystals after solvent evaporation, and then these crystals are irradiated by a pulsed laser beam to produce analyte ions. The greatest advantage of this technique is that with the assist of a matrix, large thermally labile molecules can be ionized without fragmentation and the molecular weight information can be directly obtained, even for mixtures containing many species. Because of the pulsed nature of this ionization technique, time-of-flight mass spectrometry has been considered as an ideal mass analyzer due to its theoretically unlimited mass range and high ion transmission efficiency. However, the mass resolution obtained from MALDI time-of-flight mass spectrometry is often poor and the severity of this problem appears to increase rapidly with the increase of ion mass [20].

In the MALDI process, a sample is deposited onto the probe tip as a very thin crystal film and the analyte ions will be produced by irradiating this film with a pulsed laser beam with 1-10 ns pulse width. Therefore, the effect of temporal and spatial distribution on mass resolution reduction should be low. Metastable decay or post-source-decay may be another resolution reduction factor in MALDI process because ions will pick up internal energy from the desorption process. Under usual MALDI conditions, fragmentation on the time scale across the drift region is insignificant as compared to that of the ion generation.

It is generally accepted that in the MALDI process, a large amount of ions with a broad initial kinetic energy distribution will be produced after laser irradiation. The initial velocity of desorbed analyte ions is nearly independent of mass, thus the initial kinetic energy is proportional to the mass of analyte only. In addition, when the desorption occurs in a strong electrical field, energy will be lost presumably by collisions with the neutral plume, resulting in further mass dependent energy dispersion. It is this broad initial kinetic energy distribution, particularly in the axial direction, is the main cause of poor mass resolution. To minimize this kind of problem, a variety of techniques have been suggested. These include using higher acceleration voltage [2], non-linear (reflectron) time-of-flight instruments [23, 24], time-lag focusing [22], impulse-field focusing [25], dynamic-field focusing [26] or post-source pulse focusing [27]. Among them, the reflectron and time-lag focusing techniques are most commonly used. The major difference between these two methods is that the reflectron technique is a post-source initial energy correction method, while the time-lag focusing technique is an in-source energy correction method.

1.1.4 Mechanism of Reflectron and Time-Lag Focusing TOF Mass Spectrometers

Reflectron time-of-flight mass spectrometry was first suggested by Alikanov in 1957 [28]. He suggested that the lower resolution produced by the different initial kinetic energy could be compensated by stopping them in an electric deceleration field and reversing their flight direction before detecting them. This electric deceleration field is called as reflector. The reflector is suggested to locate at the end of the flight tube and consists of a series of rings and/or grids with voltages that increase up to a value slightly greater than the voltage at the ion source. The ions penetrate the reflector until they reach zero energy, turn around, and are reaccelerated back through the reflector, exiting with energies identical to their in-coming energy but with velocities in the opposite direction. High-energy ions penetrate deeper into the reflection field and, therefore, spend more time there than low-energy ions. If the retarding field of the reflector is adjusted properly, then this effect compensates for the different flight times of the ions in the drift regions

and mass resolution is greatly improved [23, 24].

There are two devices of reflectors: single-stage and two-stage. The single-stage reflector can only reach first-order time focusing of the energy spread, however the two-stage reflector can obtain second order focusing which is necessary for high resolution at large ion energy spread. The most commonly used reflector is the two-stage device. Ions penetrate the first grid whose potential is the same as that of the flight tube. The second grid, located at about 10% of the depth of the reflector, is placed at about two-thirds of their kinetic energy. The voltage on the third grid is adjusted slightly above the accelerating voltage to provide different penetration depths over the longer stage. The reflectron time-of-flight mass spectrometer can also be used for the analysis of the fragment ions formed in the drift region due to metastable decay.

Time-lag focusing is an excellent technique to improve mass resolution in MALDI time-of-flight mass spectrometry. Willy and McLaren first suggested the time-lag focusing in 1955 [22]. In this method, a two-stage extraction system was used in order to push the space-focus plane to the detector. The laser-generated ions are first allowed to expand into a field-free region between the repeller and the first extraction grid. After a certain time lag or delay (hundreds of nanoseconds to several microseconds), a voltage pulse is applied to the repeller to extract the ions into the flight tube. The initial velocities of the ions depend on their initial kinetic energy. The ions with higher initial kinetic energy will move farther from the repeller, and the ions with lower initial kinetic energy will move closer to the repeller. This will result in the ions farther from the repeller will gain less energy than the ions closer to the repeller after a voltage pulse is added. Finally, a compromise is made between the best space and initial kinetic energy focusing [20].

This kind of energy focusing can also be achieved by adjusting the delay time. Longer delay time will allow the ions of the same m/z to move further from the repeller. As a result, the effect of amplitude pulse potential will be reduced. For optimal operation,

both the pulse voltage and delay time can be adjusted individually. The optimal pulse voltage or delay time is mass dependent; higher pulse voltages or longer delay times are required to focus ions with higher mass.

1.2 Matrix-Assisted Laser Desorption/Ionization (MALDI)

1.2.1 Lasers in MALDI Experiment

In the MALDI process, the laser plays a predominant role in generating intact gas phase molecular ions from compounds that are usually solids and degrade or decompose when being exposed to thermal heating. Using lasers to generate ions for analysis in mass spectrometers started as early as 1960 [29]. Initially, a variety of lasers with vastly different wavelengths and pulse widths were used to combine with every available type of mass spectrometer. Because of the large variation in basic experimental parameters, different results were obtained. The first systematic attempts to generate ions of organic molecules with lasers dated back to the early 1970s [30, 31]. Up to then, two general principles had been developed for choosing an appropriate laser for MALDI time-of-flight mass spectrometers. First, an efficient and controllable energy transfer to the sample requires the resonant absorption of molecule at the wavelength. Consequently, lasers emitting in the far-UV, which can couple to electronic states give the best results. Second, to avoid thermal decomposition of thermally labile molecules, the energy must be transferred within a very short time. The short-duration laser beam can easily be focused to spot sizes that are small compared with the dimensions of other ion sources. This feature makes them ideally suitable for combining with time-of-flight mass spectrometers [29].

In the design of a MALDI time-of-flight mass spectrometer, the laser beam is generally focused to a spot size of 100-1000 μm by either a single or a multi-element optical system. In this range of spot size, the threshold at which laser can generate ions is almost

independent of spot size. The angle of incidence of the laser beam on the sample surface can be from 30 to 70 degree without significantly altering the ion signals produced [29]. The position of the laser focusing on the sample surface can be changed either by shifting the optical axis of the focusing system or by moving the sample probe. A critical parameter in a MALDI experiment is the laser power. It has been suggested that in order to avoid fragmentation for thermally very labile species, the laser irradiance should be controlled just above the threshold [32].

1.2.2 Matrix Selection

The choice of a proper matrix is the most decisive factor for a successful MALDI analysis. There are at least several basic properties needed for a potential matrix. First, the matrix should have a strong absorbance at the wavelength at which the sample(s) only weakly absorbs. Secondly, for one layer method the matrix should be dissolved in the comparable solvent as the analyte. Third, the matrix should induce efficient desorption and ionization. Finally, the matrix should have good vacuum stability. From a practical point of view, the matrix should not form aducts with the analyte molecules and must retain its properties in the presence of contamination [33]. Although the above properties of a matrix are generally agreed upon, the identification of new matrices has been slow. Even if almost all of the criteria stated above are met, a compound may not be a good matrix. Therefore, searching for a proper matrix is usually based on trial-and-error tests. The main criterion in evaluating a matrix is the ability to provide maximum ion intensity and signal reproducibility while minimizing fragmentation. To date, hundreds of different substances have been tested for their performance as matrices, but only a handful of them were found to be really useful. It was proven that solid matrices could produce better signals with wider applicability than liquid matrix [34-37]. Due to the limited number of matrices, a number of research activities such as development of multicomponent matrices, co-matrices and using different procedures to prepare the matrix/analyte mixture have been carried out [21].

1.2.3 Sample Preparation Methods

The sample preparation method in MALDI experiment is also very important. It affects detection sensitivity, selectivity and mass resolution. Up to now, the development of sample preparation technique has mainly focusing on biomolecules. But a growing number of studies have been focused on synthetic polymers. For biomolecules, five methods have been well developed: dried-droplet, vacuum drying, crushed crystal, fast evaporation and two-layer methods.

Dried-droplet is a method introduced by Karas and Hillenkamp in 1988 [38]. In this method, the matrix and analyte are dissolved in an appropriate mixture of organic-water solvent, and either applied sequentially onto a solid substrate or mixed together followed by deposition on the probe tip. Solvent evaporation occurs under ambient conditions or is accelerated by drying with compressed air or N_2. The most intense ion signals are usually associated with the sample that appears crystalline [21]. The advantage of this preparation method is its ease and speed, taking usually only a few minutes. The disadvantage is that the samples prepared in this way are heterogeneous. X-ray studies showed that the matrix incorporated protein molecules into its crystal structure [39]. To improve the homogeneity of the formed crystals, many studies have been conducted such as scratching the sample until the crystals start to form or use of multi-component matrices [40-42].

The vacuum drying method is an improvement of the dried-droplet method. In 1993, Weinberger *et al.* [43] found that if the sample was dried under vacuum (10^{-2} torr), the analyte-matrix cocrystallization would be more uniform and the relative standard deviations of the intensity ratio (analyte/internal standard) could be improved from 20-50% to 10-15%.

The crushed crystal method is a method introduced by Xiang and Beavis [44] to increase

the tolerance of the matrix to contamination. In this method, the saturated matrix solution was applied to the probe tip, and after crystallization, the crystals were crushed. A second solution, which contains both matrix and analyte, was later applied on top of the crushed polycrystalline film. The first polycrystalline film provides seed sites for the subsequent matrix/analyte deposition and induces rapid crystallization in a more uniform manner. In addition, they found that matrix-analyte crystallization process acts as purification step in which only analyte is included in the matrix structure but not contaminants. If the formed crystals are rinsed by pure water, the surface contaminants and some nonvolatile solvents can be removed. The method provides a means to analyze extremely contaminated samples.

Fast evaporation is a method reported by Vorm *et al.* in 1994 [45, 46]. In this method, matrix was dissolved in a highly volatile solution such as acetone, and then apply this solution to sample probe tip. A dense, flat and thin film of small crystals of matrix was formed by fast evaporation of the solution. A small volume of aqueous analyte solution is then placed on top of the matrix surface and allows evaporating slowly, forming a very homogeneous sample layer. Sample impurities such as salts and buffers will be washed off without noticeable loss of analyte. The results of this preparation method are an increased sensitivity and mass resolution. This method is useful for peptides, but not for proteins.

Two-layer method is a technique introduced by Dai *et al.* in 1996 [47]. In this method, the first layer of crystals was prepared by dissolving the matrix in a mixture of solvents and then applied to the sample probe tip to allow air dry. Depositing a mixture of analyte and matrix solution on the top of the first layer formed the second layer. Confocal fluorescence microscopic imaging study found that the analyte is uniformly distributed and the crystals are small, uniform and densely packed. It was noted that this method could provide high detection sensitivity and excellent spot to spot reproducibility for both peptides and proteins.

The analysis of synthetic polymers could require different sample preparation methods as compared to those used for biomolecules. No standard protocol has been developed yet because of the diversity of polymeric materials, the different instrumental conditions under which the measurements are made, and the ambiguous role the matrix plays in desorption and ionization of the analyte [48]. Nevertheless, some rules-of-thumb have been generalized for synthetic polymers. Based on the solution properties of the polymer to be studied, synthetic polymers can be divided into four categories, which include water-soluble polymers, polar organic-soluble polymers, non-polar organic-soluble polymers, and polymers soluble only in 'difficult' solvents such as hot 1, 2-dichlorobenzene or sulfuric acid [49]. For water-soluble polymers, such as poly(acrylic acid), poly(ethylene glycol) and poly(styrene sulfonate), matrix conditions similar to those used for peptides and proteins could be used [1, 4, 50, 51]. For polar organic-soluble polymers, such as acrylics, poly(hydroxybutanoate) and poly(vinyl acetate), the use of the common MALDI matrices with a solvent, such as acetone, tetrahydrofuran or methanol, yields good spectra [1, 5-7, 11, 52]. For polymers requiring difficult solvents, proper matrix preparation is still to be developed [53]. For non-polar organic-soluble polymers, finding an appropriate matrix and preparation method is especially difficult because of their nonaqueous and nonpolar nature. It was suggested that hydrocarbon polymers lack an effective site for ionization, and laser desorption thereby yields neutral gas-phase molecules. Polystyrene may be the easiest hydrocarbon to be ionized because its phenyl functionality allows for a higher ionization probability. Ion yields may be increased by the presence of alkali metals, particularly for polar polymers where the heteroatoms (O, N) are the sites of alkali metal attachment. With nonaromatic hydrocarbon polymers, however, difficulty in their ionization remains due to their lack of energetically favorable sites for alkali metal attachment [54]. All of the above rules only give a general direction about the analysis of synthetic polymers. As mentioned early, because different instrumental conditions are used, even the same sample preparation method may generate different results.

1.2.4 Experimental Factors Affecting Mass Resolution in MALDI Experiments

In addition to the temporal, spatial and initial kinetic energy distributions that affect the mass resolution in a time-of-flight mass spectrometer, there are some other experimental factors which can also influence mass resolution in MALDI experiments. They can be grouped into sample preparation method, instrumental factors and data collection technique.

Sample preparation method is a step to produce *good* or *bad* crystals for laser desorption/ionization. Besides mass resolution, spot to spot reproducibility, detection sensitivity and selectivity are also dependent on this procedure. Many experiments have proven that small and flat matrix/analyte cocrystals with a uniform analyte distribution produce the best mass spectra [47, 55]. Therefore choosing a suitable sample preparation protocol is very important. Chemical contamination is the second factor that affects the mass resolution. Chemical contamination can yield unwanted adduct ions. If the instrumental resolution is not good enough to separate them from the principal ions, peak broadening will be resulted. Thus, purified matrix and HPLC grade solvents are recommended in the sample preparation procedure. Furthermore, matrix to analyte ratio is an important factor in a sample preparation procedure. A large amount of matrix and its fragment ions will lead ions repelled to each other, resulting in poor detection sensitivity. The use of a higher laser power will increase this interference. As a result, poor resolution spectra could be observed. Finally, sample thickness can also affect mass resolution. A thicker sample film will increase the degree of spatial distribution and result in poor mass resolution.

Instrumental factors include the instrumental configuration, data acquisition speed, length of flight tube, flight tube pressure, etc. The predominant factor is the instrumental configuration. Mass resolution is greatly affected by choosing linear, reflectron or time-lag focusing instrument. A longer flight tube can in theory increase mass resolution, but

the instrumental sensitivity may suffer. Higher vacuum condition can minimize multiple collisions between analytes and residual gas molecules in the flight tube, resulting in better resolution [56].

The data collection technique is also an important issue for mass resolution. Because of the inhomogeneities in the matrix and analyte crystals and laser power profile, the signal to noise ratio will vary from spot to spot. Extremely high signal to noise ratio may occur during data collection. High analyte signals usually yield broader spectral peaks due to coulombic repulsion, shielding and initial kinetic energy effects in the source [56]. Therefore controlling the laser power to its threshold and selecting individual spectra are recommended in data collection procedure.

1.3 Gel Permeation Chromatography (GPC)

1.3.1 Mechanism of Gel Permeation Chromatography

Gel permeation chromatography (GPC), also known as size exclusion chromatograph (SEC) or gel filtration chromatography, is based on the separation of molecules according to their sizes. It is a liquid chromatographic technique in which a sample solution is introduced onto a column filled with rigid porous gel with defined pore size. Size separation is achieved by differential pore permeation. Below a certain size, a molecule can freely enter the holes in the stationary phase. Above a certain size, the molecule cannot enter the pores at all and is completely excluded. Molecules between these two size limits will be able to penetrate the pores more or less deeply and thus will be separated based on their sizes. As a result, the larger molecules passing from the column are eluted earlier than the smaller ones. The reason that GPC can give molecular weight information according their sizes is based on the relationship between linear dimension and molecular weight in a freely jointed polymeric chain (random coil): either the root-mean-square end-to-end distance or the radius of gyration is proportional to the square

root of the molecular weight. It follows that the log of either distance is proportional to (one-half) the log of the molecular weight [57].

1.3.2 Solute Retention in GPC

The mechanism of solute retention is based on partitioning of solute between mobile phase and stationary phase. The retention volume (V) of a particular solute can be calculated as:

$$V = V_o + KV_p \tag{1.6}$$

where K is the distribution coefficient, Vo is the void volume and V_p is the porous volume containing stationary solvent. For very small solutes which can freely access the entire porous and void volume of the column, K = 1. For very large solutes which cannot fit in any of the pores, K = 0. For intermediate solutes which can partially access the pores, $0 < K < 1$. Many researchers have attempted to model K in terms of the size and shape of both solute and pore [57-63]. The simplest model is treating a solute molecule as a sphere with radius R which excludes in a cylindrical pore with a pore of length L and radius a. An excluded volume effect prevents the center of the solute molecule from approaching the wall any closer than a distance R. This reduces the volume accessible to the solute to a smaller cylinder of radius (a - R). Because of the shell of thickness R containing no solute, the average solute concentration in the pore as a whole is less than that outside. Therefore, the fraction of the external concentration in the pore is given by the ratio of two volumes, that is

$$K = \pi(a-R)^2L/\pi a^2L = 1-2R/a + R^2/a^2 \tag{1.7}$$

If the solute dimensions are much smaller than the pore,

$$K = 1 - 2R/a \tag{1.8}$$

For a polymer molecule, if it is treated as a random coil by visualizing the coil domain as a sphere with the radius of gyration r_g

$$K = \exp(-cr_g) = \exp(-kM) \tag{1.9}$$

where c and k are constants. Therefore

$$V = V_o + V_p\exp(-kM) = a - b\log M \tag{1.10}$$

where a and b are constants depending on instrumental parameters. Based on this equation, the GPC calibration curve is normally plotted as logM vs V or elution time (t). Due to the mechanism of GPC separation, the calibration curve for GPC is only linear in a certain region. For very small molecules, they will be totally included and elute without size separation. For very large molecules, they will be completely excluded. Therefore, a linear region is only exist in the molecular sizes between the two extremely cases.

1.3 Study Objectives

The overall objective of this study is to explore several important fundamental issues associated with the application of MALDI time-of-flight mass spectrometry for determining polymer molecular weights. Specifically, the objectives include:

1. to investigate any symmetric and asymmetrical spectra distortion that may occur in MALDI analysis of polystyrene samples,

2. to reveal the possible causes of error in polymer molecular weight determination

due to the limited sensitivity or dynamic range of the MALDI technique,

3. to examine the effect of instrumental configuration, laser power and time-lag focusing point on spectra sensitivity, oligomer resolution, spectra truncation and polymer molecular weight determination,

4. to study the effect of spectra analysis methods on the accuracy determination of polymer molecular weights,

5. to compare the polymer molecular weights and polydispersity obtained from MALDI and GPC techniques, and

6. to determine the possible causes that would generate the differences between the MALDI and GPC techniques in terms of average polymer molecular weights and polydispersity.

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CHAPTER 2. AN ANALYSIS OF THE ACCURACY OF DETERMINING AVERAGE MOLECULAR WEIGHTS OF NARROW POLYDISPERSITY POLYMERS BY MALDI TIME-OF-FLIGHT MASS SPECTROMETRY

2.1 Introduction

Matrix-assisted laser desorption ionization (MALDI) time-of-flight mass spectrometry (TOF MS) has a demonstrated utility in the analysis of polymeric systems [1-12]. In particular, it has been shown that this method can be used to deduce information on molecular weight and molecular weight distribution of narrow polydispersity polymers with high speed and precision [1, 2, 13-20]. However, the accuracy of this method is difficult to assess due to the lack of suitable polymer standards of accurately known molecular weights and molecular weight distributions. There are several experimental and instrumental factors that may affect the average polymer molecular weight measurement by MALDI time-of-flight mass spectrometry [21, 22]. A number of studies have shown that there is a dependence of the measured average molecular weight of narrow disperse polymers on the adduct-forming cation type [23-25], sample preparation method [26, 27], laser irradiance [28], and even the type of mass analyzer [17, 29]. However, in many of these cases, the variability imparted to the average molecular weight is minor and has an identifiable source. For example, average molecular weight differences arising from changing the nature of the adduct-forming cation can be attributed to a minimum size requirement in the stabilization of the cation in the gas phase [30].

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In order to measure the average molecular weight accurately, the MALDI method must be able to generate a mass spectrum that reflects the actual oligomer distribution as well as the relative amounts of all oligomers within the distribution. Sensitivity limitations, background interference, and/or mass discrimination can cause a change in the polymer distribution function, broadening or narrowing of the overall distribution, and/or truncation of detected oligomer peaks within a distribution (*i.e.*, missing low- or high-mass tails). Any one of these variations can result in errors in average weight determination. Thus, the question of how accurate the MALDI results are for a particular polymeric system can be addressed by examining the magnitude of the errors that might arise from these variations. We have designed several experiments to assess the extent of these variations and their effects on average molecular weight determined by MALDI time-of-flight mass spectrometry. Using polystyrenes as a model system, the experimental methods for evaluating the accuracy of the MALDI method are described.

2.2 Experimental

2.2.1 Instrumentation

Mass spectral data were collected on a linear time-lag focusing MALDI-TOF mass spectrometer. The ionization and ion extraction regions consist of four stainless steel plates seperated by 5-mm ruby balls. Both of the repeller side of the first extraction plate and the ground plate are covered with stainless steel grids. In order to insert the sample probe, the ion repeller plate was designed with a 4.2 mm diameter of opening at the center. The diameters of the center holes at the first extraction plate, second extraction plate and the ground extraction plate are 12 mm, 12 mm and 1 cm, respectively. A pair of ion deflectors was arranged after the ground plate. The flight tube was a 1-meter stainless steel tube. A dual multi-channel plate (MCP) was used for ion detection. The ionization and ion extraction regions are housed in a 22 x 22 x 22-cm cubic stainless steel vacuum chamber pumped by a 15-cm diffusion pump (Varian). A 10-cm diffusion pump

(Edwards) is connected to the middle of the flight tube to provide additional pumping. A DC power supply up to 30 kV was used to apply the potentials on the repeller and the first extraction plate and the potential on the second extraction plate through a voltage divider. A high voltage pulser built in-house was used to generate the delayed extraction pulse. A Hewlett-Packard pulser (Palo Alto, CA) was used to deflect the low mass ions by applying a positive pulse to the ion gate. The extraction pulse and time lag were chosen to focus the center of the analyzed mass range.

During operation, the dried droplet method was used for sample preparation. A sample was first placed onto a probe to form a very thin layer of crystals after solvent evaporation. The sample probe was then inserted into the instrument. A nitrogen laser (model VSL 337ND, Laser Sciences Inc., Newton MA) with a 3-ns pulse width was used for desorption at 67.5° normal to the probe tip surface. Laser fluence was maintained slightly above ion detection threshold in all analyses. An ion deflector was used to deflect matrix ions in order to avoid saturating the detector. A Hewlett Packard (HP) MALDI data system was used for mass spectral recording and data processing. This data system is a modified version of the software used for the HP Model G2025A MALDI time-of-flight mass spectrometer, in which the instrument control features have been disabled. All data were further processed using the Igor Pro software package (WaveMetrics, Lake Oswego, OR). No correction of 1/(dm/dt) was applied to the mass spectra during the conversion of the time domain to the mass domain [19, 21, 33].

Number average molecular weight (M_n) and weight average molecular weight (M_w) were determined directly from the time domain according to the following equations [21, 22]:

$$M_n = \Sigma(N_i M_i)/ \Sigma N_i \tag{2.1}$$

$$M_w = \Sigma(N_i M_i^2)/ \Sigma N_i M_i \tag{2.2}$$

where N_i and M_i represent signal intensity in peak area and mass for the oligomer containing i monomers, respectively. The polydispersity, PD, was determined from the ratio of M_w to M_n. Average molecular weights were corrected for the contribution of the cation (Ag^+). In general, mass spectra from 100 laser shots were summed to produce a final spectrum. The percent relative standard deviations (% RSD) for the measured M_n and M_w values are determined from five separate sample loadings. The precision of the method was determined to be generally better than 0.5% RSD. All mass spectra shown in the figures are the smoothed spectra using 30-point Savitzky-Golay smoothing. No baseline correction was performed.

2.2.2 Samples and Reagents.

Bovine insulin b-chain, bovine ubiquitin, and equine cytochrome c used in the calibration were obtained from Sigma (Milwaukee, WI). The matrix used in their analyses (sinapinic acid) was purchased from Aldrich (St. Louis, MO). Polystyrene standards with the following nominal molecular weights were used in this study: 5050 (Showa Denko, Tokyo, Japan), 7000 (Polymer Laboratories, Amherst, MA), and 11600 (Showa Denko). The molecular weight data from the suppliers along with the MALDI results obtained from this work are listed in Table 1. MALDI analyses of these polymers utilized *all-trans* retinoic acid as the organic matrix (Aldrich) [19]. The cationizing species ($AgNO_3$) was reagent grade and used without further purification. In order to eliminate the presence of water, tetrahydrofuran (THF) (VWR, Toronto, Canada) used in the dissolution of the polystyrene and retinoic acid was pretreated with potassium hydroxide, filtered, and then distilled over sodium metal, in the presence of benzophenone as an indicator of dryness.

2.2.3 Sample Preparation.

Polymer samples for MALDI analysis were prepared by combining the analyte, matrix and cationizing agent in THF [19, 21]. *All-trans* retinoic acid was prepared to a concentration of 0.15 M. In a typical experiment, polymer stock solutions (1 mM,

calculated from the nominal molecular weights) were diluted ten-fold with the matrix solution, and 1% (v/v) of a 0.15 M $AgNO_3$ ethanolic solution was added (for the less sensitive sample preparation method, 0.5% (v/v) of a 0.30 M LiOH solution was also added). In the analysis, 1 μL of the appropriate mixture was added to the MALDI probe tip and allowed to air-dry.

2.3 Results and Discussion

Polymers do not have exact molecular weight and they display a distribution of molecular weights. The molecular weight distribution of a particular polymeric system depends on the polymerization kinetics and mechanism [34]. Various mathematical functions can be used to describe the distribution [34, 35]. For a polymer synthesized by an anionic living polymerization process, the molecular weight distribution can be described by a Poisson distribution function from the statistical consideration of the polymerization kinetics [35, 36]. Such a distribution can be approximated by a normal or Gaussian distribution for a very narrow polydispersity polymer [36]. Many linear homopolymers with very narrow polydispersity, such as polystyrenes used for calibration of GPC, belong to this category of polymers. We focus our efforts on using low mass, narrow polydispersity polystyrenes as the model system to evaluate the accuracy of the MALDI method. For polystyrenes, a very sensitive sample preparation method can be used [19, 21, 22] and this method does not cause polymer dissociation during the MALDI analysis. In addition, for polystyrenes with masses less than ~12,000 u, only the singly charged principal distribution is detected [21, 22].

At present, there are a number of analytical techniques based on separations, osmometry, light scattering, and spectroscopy that can be used to determine the molecular weights of polymers [35, 37]. Among them, GPC has been most widely used to determine the average molecular weights as well as molecular weight distributions [35, 37, 38]. However, there are many factors, including axial dispersion in the column, adsorption and

partition effects, and concentration effects that can significantly influence the results [35, 37, 38]. Thus, the polymer molecular weight distribution function determined by GPC may not reflect the true distribution of the polymer. The M_n and M_w values derived from GPC are also prone to errors, particularly in light of the fact that GPC relies on the use of polymer standards for molecular weight calibration [38]. Other traditional molecular weight determination methods also have limitations in providing accurate M_n and M_w data [35, 37, 38]. Nevertheless, these methods are still widely used in polymer characterization, particularly in the application areas where only a general agreement of the molecular weight data obtained from different methods is required.

MALDI-TOF MS can potentially provide more accurate molecular weight results than those obtained from the traditional methods. In light of the above discussion, at present, the accuracy of the MALDI method for polymer analysis can only be assessed from the mass spectrometric detection point of view. In evaluating the accuracy of the MALDI method for a particular application, three possible variations, namely a change in the type of distribution function, broadening or narrowing of the overall distribution, and truncation of selected oligomer peaks within a distribution, need to be examined.

The change of the distribution function can be caused by any asymmetrical distortion that may occur during the process of obtaining a polymer mass spectrum by MALDI. Such a distortion may be due to mass dependence of desorption, ionization, and/or detection of individual oligomer. For example, detector saturation and/or mass dependence of ion-to-electron conversion efficiency in an MCP would result in the loss of detection sensitivity for the higher mass ions. On the other hand, background interference from the matrix and impurities is often more pronounced at the low mass region, causing elevation of the spectral baseline and/or signal overlap with the background ions [12]. This could reduce the detectability of the low-mass polymer signals, resulting in the skewing of the distribution to the high mass region. For a very narrow polydispersity polymer with its molecular weight distribution represented by a Gaussian distribution, it is expected that

Table 2.1. Molecular weight data for polystyrene standards.

Polymer Standard	Molecular Weight and Polydispersity	
	By Classical Methods[a]	By MALDI[b]
Polystyrene 5050	M_n = 4755 (GPC)	M_n = 5189 (0.5% RSD)
	M_w = 4992 (GPC)	M_w = 5329 (0.5% RSD)
	M_n = 4720 (VPO)	PD = 1.027 ± 0.001
	M_v = 4950 (IV)	
	PD = 1.05 (GPC)	
Polystyrene 7000	M_n = 6770 (GPC)	M_n = 6998 (0.4% RSD)
	M_w = 6962 (GPC)	M_w = 7132 (0.4% RSD)
	M_w = 7170 (LLS)	PD = 1.019 ± 0.001
	M_v = 6943 (IV)	
	PD = 1.03 (GPC)	
Polystyrene 11600	M_n = 11356 (GPC)	M_n = 11074 (0.3% RSD)
	M_w = 11687 (GPC)	M_w = 11187 (0.3% RSD)
	M_w = 11000 (LLS)	PD = 1.010 ± 0.001
	M_v = 10720 (IV)	
	PD = 1.03 (GPC)	

[a] These results are provided by the suppliers; IV, GPC, gel permeation chromatography; VPO, vapor pressure osmometry; IV, intrinsic viscosity; LLS, laser light scattering. [b] From five trials.

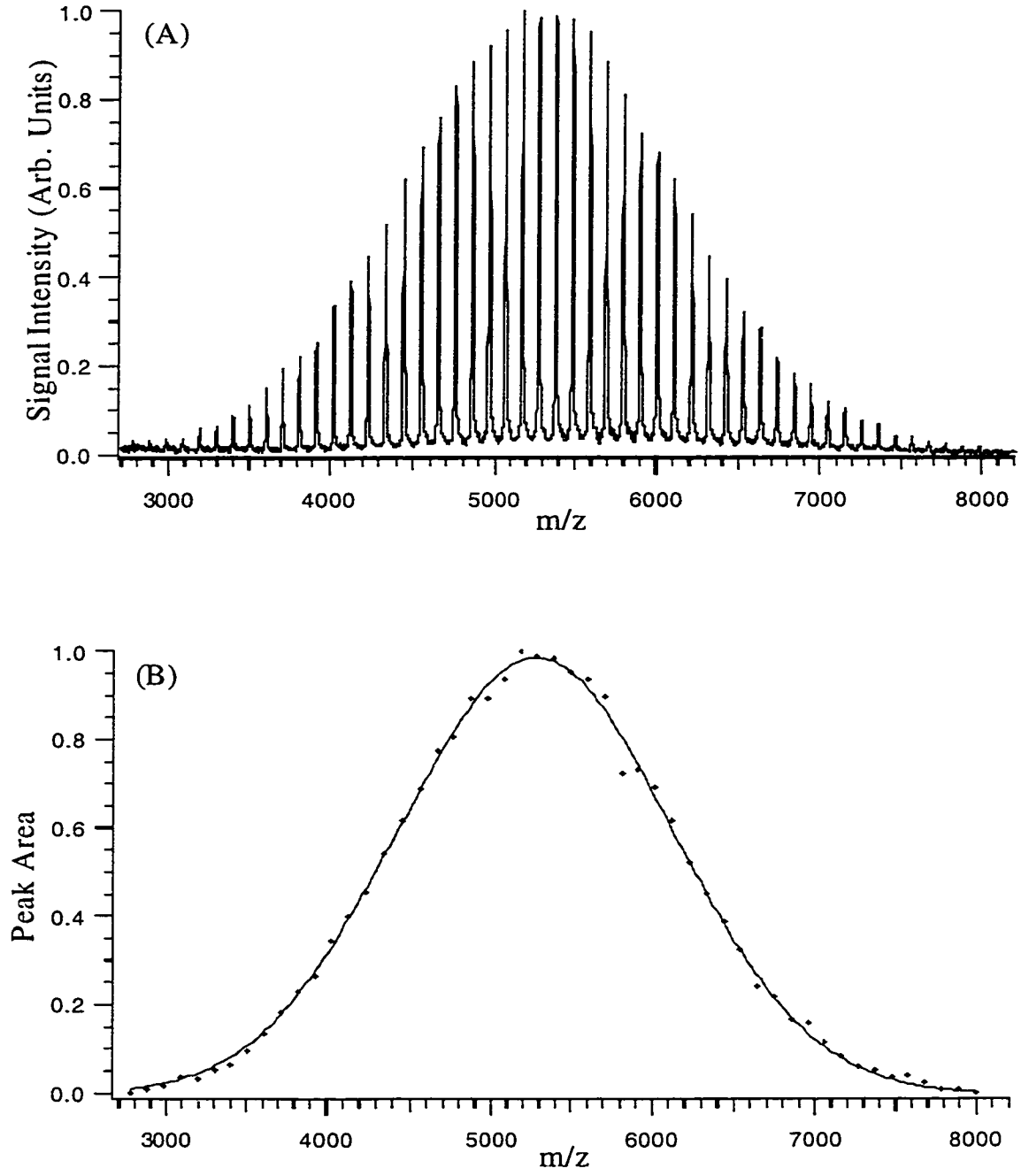

Figure 2.1. (A) MALDI mass spectrum of polystyrene 5050. (B) Individual peak areas of oligomers from the MALDI spectrum are plotted as the function of m/z for (B) polystyrene. The result of Gaussian curve fitting is shown as solid lines in (B).

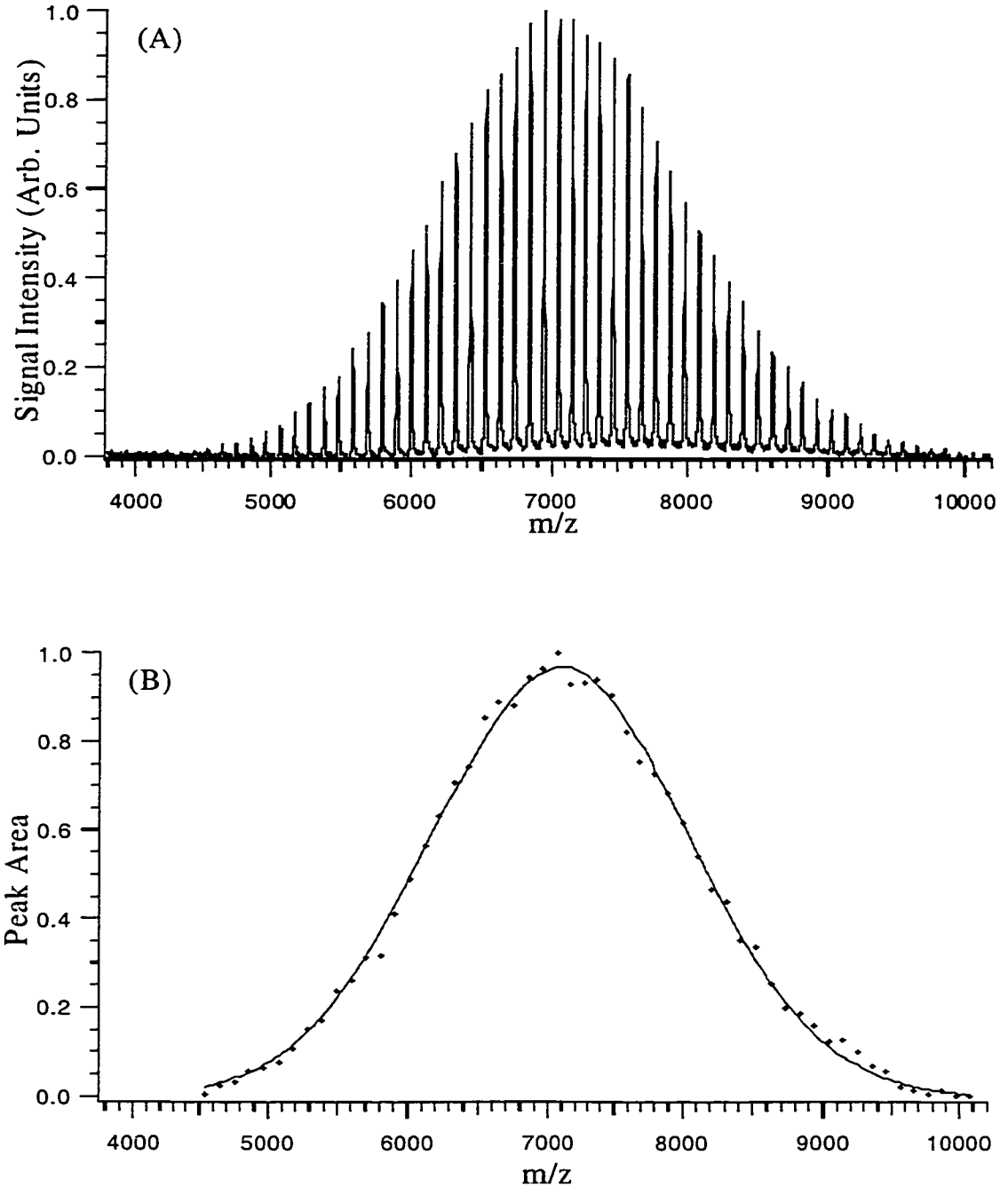

Figure 2.2. (A) MALDI mass spectrum of polystyrene 7000. (B) Individual peak areas of oligomers from the MALDI spectrum are plotted as the function of m/z for (B) polystyrene. The result of Gaussian curve fitting is shown as solid lines in (B).

the M_n value will be increased if the distribution skews to the high mass region or reduced in the case that the distribution skews to the low mass region.

We have examined two narrow polydispersity polystyrenes with expected Gaussian distributions (see Table 2.1). Figures 2.1A and 2.2A show the mass spectra of polystyrene 5050 and polystyrene 7000, respectively. These spectra were re-plotted as peak area of individual oligomers vs. m/z and are shown in Figures 2.1B and 2.2B, respectively. The spectra of Figures 2.1B and 2.2B are quite symmetric and can be well fitted to the Gaussian distributions (see the solid lines; the goodness of fit is indicated by $\chi^2 = 0.0156$ for polystyrene 5050 and $\chi^2 = 0.0201$ for polystyrene 7000; $\chi^2 = 0$ for a perfect fit). These results demonstrate that, under the experimental conditions used in this work, asymmetric distortion of the molecular weight distribution function did not occur during the MALDI analysis of polystyrene 5050 and polystyrene 7000. However, the results shown in Figure 2.1 and 2.2 could still be in error due to the broadening or narrowing of the overall Gaussian distribution, i.e., symmetric distortion of the distribution. The exact cause of a symmetric distortion is difficult to ascertain from the current understanding of the MALDI process and of the TOFMS ion detection. It could, however, come from the desorption and ionization process where mass discrimination has been known to occur [21, 22]. Fortunately, the extent of this mass discrimination can be readily probed by experiments involving the use of polymer blends [21, 22].

To examine any symmetric distortion that may occur in the MALDI analysis of polystyrene 7000, two blends of polystyrene 5050/polystyrene 7000 in different proportion: (65:35 and 50:50, mole ratios) were prepared and their spectra were recorded. Figure 2.3 shows the plots of peak area vs. m/z for polystyrene 7000 and two blends. Since the high-mass tail of the polystyrene 5050 distribution does not extend beyond m/z ~8000 (see Figure 2.1A), the amounts of the oligomers with m/z > 8000 in the blends should be the same as those in polystyrene 7000. However, there is a mass dependence in the analysis of these oligomers, the addition of the second component at the low mass

region of the polystyrene 7000 distribution may affect the analysis of the oligomers with m/z > 8000 [21]. For example, if mass discrimination occurs in the analysis of polystyrene 7000 and is caused by detector saturation, the addition of the low mass oligomers in the blends, will exacerbate detector saturation, resulting in the reduction of signals for oligomers with m/z >8000 in the spectra of the blends.

Figure 2.3 illustrates that very small change in peak areas of the oligomers with m/z >8000 are observed for polystyrene 7000 and the blends. To put into perspective the magnitude of the changes, Figure 2.3 also shows two theoretical curves reflecting the increase and decrease of the polydispersity by 1% from the polystyrene 7000 distribution (PD 1.019). Note that a small change of polydispersity can translate into a significant variation in the distribution, because the width of the Gaussian distribution is related to $M_n(PD-1)^{0.5}$ [21, 35]. For instance, oligomer peaks are expected to be present at m/z from ~3000 to 11000 for the distribution with PD 1.029 and from ~5200 to 9000 for the distribution with PD 1.009, while the m/z range covered by the distribution with PD 1.019 is from ~4000 to 10300. The differences in relative areas for any given oligomer peaks in the three Gaussian distributions are also quite large. For example, the relative intensities for the oligomer at m/z ~8000 are expected to be 76.7%, 65.3%, and 33.8% from the three distributions with PD 1.029, 1.019, and 1.009, respectively. It is clear that for symmetric distortion of a distribution, mass discrimination has to be very severe to cause any appreciable errors in MALDI analysis. In Figure 2.3, the variations of the relative peak areas of individual oligomers with m/z > 8000 for the two blends are certainly less than ±1% variations indicated by the two theoretical distributions. From this analysis, we can conclude that no symmetric distortion of the distribution is detectable in the MALDI analysis of polystyrene 7000.

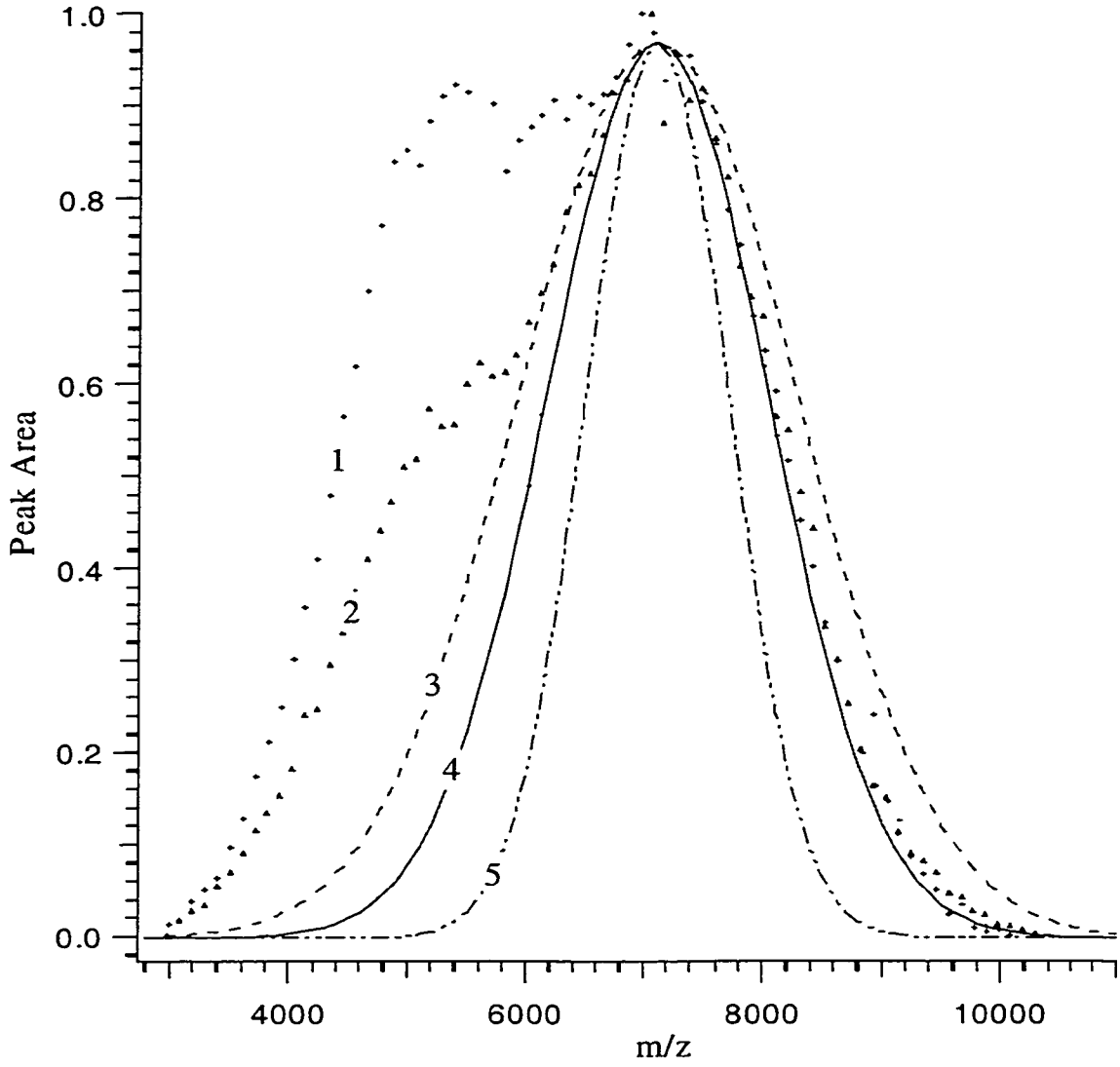

Figure 2.3. Line 1 is a blend containing 50% polystyrene 7000 and 50% polystyrene 5050, line 2 is a blend containing 65% polystyrene 7000 and 35% polystyrene 5050 (all in moles), line 4 is the theoretical Gaussian distributions derived from the Gaussian fit of the polystyrene 7000 distribution, line 3 is the increase of 1% of polydispersity and line 5 is the decrease of 1% of polydispersity.

The final consideration of the possible causes of error in molecular weight measurement is related to the truncation of some oligomer signals in the mass spectrum due to the limited sensitivity or dynamic range of the MALDI technique. The relative amounts of the oligomers in a polymer sample can span a wide range. In this context, oligomers with low abundance such as those at the tails of the distribution may not be detected in a MALDI analysis. A poor sample preparation protocol can cause reduction of the dynamic range (see below). This is particularly true if the sample preparation can cause severe background interference, in the form of baseline elevation and/or analyte signal overlap with the background ions. In addition, a MALDI instrument that is unable to provide highly sensitive detection of the MALDI ions or is operated under less than optimal conditions can also reduce the dynamic range of the MALDI analysis. The dynamic range of detection can be inferred from examining the polymer spectrum directly. In the case of polystyrene 7000, the most intense peak (100% relative intensity) is the peak at m/z 7055. The peak at m/z 4555 is the least intense peak detectable at the low mass tail of the distribution with S/N 3.9. It has a relative intensity of 1.8%. The least intense peak (S/N 3.1) from the high mass tail of the distribution is at m/z 10075 with a relative intensity of 1.4%. All data were the average values from five trials.

The dynamic range of the MALDI method and the extent of the oligomer peak truncation can be further examined by using polymer blends. In this case, the experiment involved the use of polymer blends prepared from polystyrene 7000 with the addition of small amounts of polystyrene 5050 or polystyrene 11600. Polystyrene 5050 contains oligomers with the same masses as those of oligomers at the low mass tail of the polystyrene 7000 distribution, while polystyrene 11600 has peak overlap in the high mass tail of polystyrene 7000. A sensitive method should be able to detect the mass spectral change after a small amount of a second component is added.

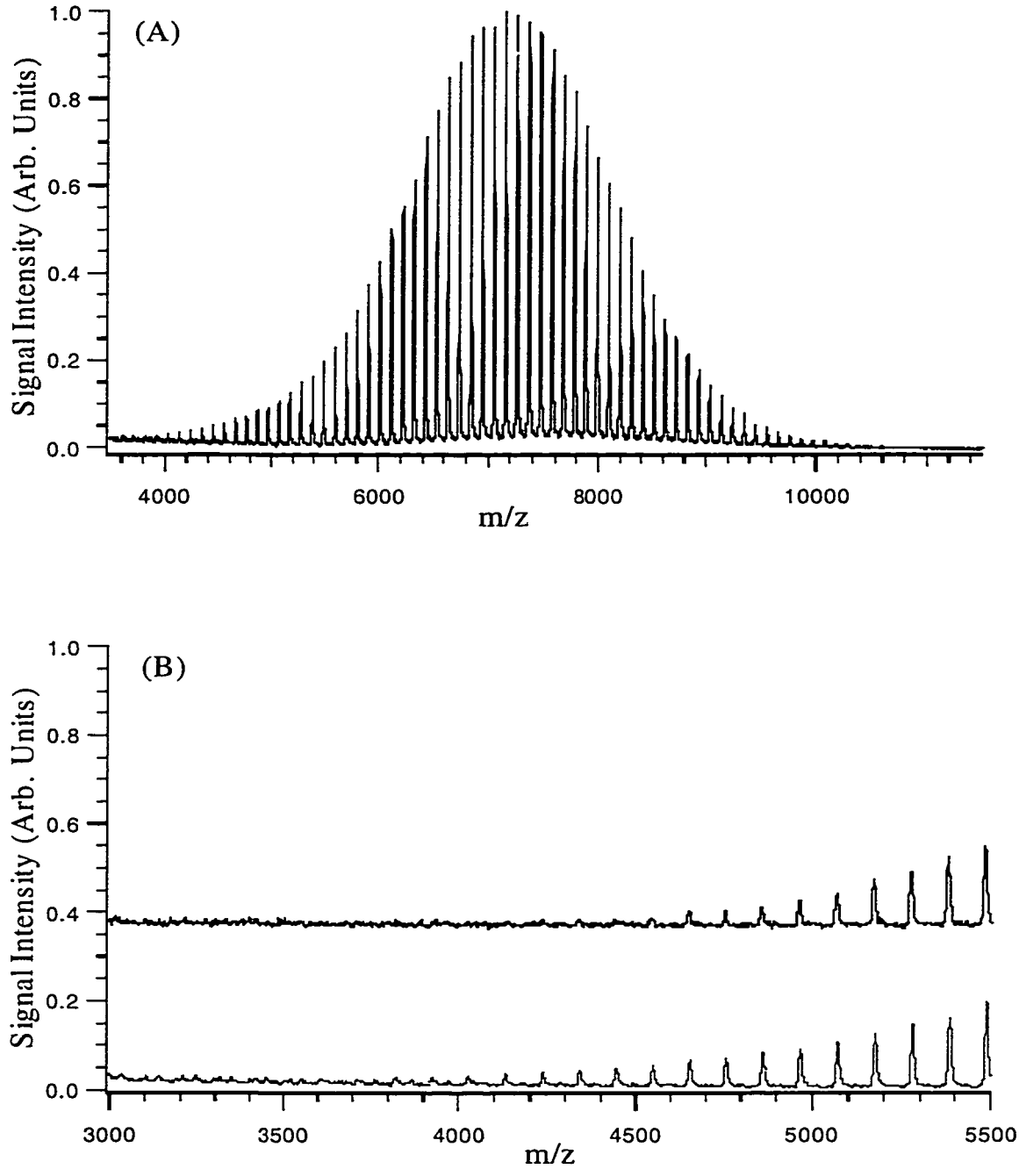

Figure 2.4. MALDI mass spectra of (A) a blend containing 95% polystyrene 7000 and 5% polystyrene 5050 (in moles) and (B) the expanded low mass region of polystyrene 7000 (upper spectrum) and the blend (lower spectrum).

Figure 2.4A shows the mass spectrum of a blend of 95% polystyrene 7000 and 5% polystyrene 5050 and Figure 2.4B shows the expanded spectra from the blend and polystyrene 7000 at the low mass region. From Figure 2.4B, it can be seen that the low-mass tail of the polystyrene 7000 distribution extends to m/z ~4400. For the blend, this low-mass tail has been further extended to m/z ~3600. Judging from the relative intensities of oligomer peaks in the spectrum of polystyrene 5050 shown in Figure 2.1A, it can be estimated that the amount of the various oligomers with masses < ~4400 is less than half of the most abundant oligomer in polystyrene 5050. In other words, these low mass oligomers are present in the blend with less than 2.5% of the most abundant at m/z 7055. Yet, these minor oligomer components are detected in the blend. Note that the peak at m/z ~4238, which is mainly from the oligomer present in polystyrene 5050, has a S/N of 9, about 3-fold above the detection limit of this peak. For this blend, M_n and M_w are determined by MALDI to be 7000 (0.2% RSD) and 7155 (0.1% RSD), respectively, from five trials. The polydispersity is found to be 1.022 ± 0.001. The theoretical molecular weight data for the blend can be obtained by considering the relative intensities of the individual oligomer peaks in the two polymer components and the weight ratio of the two components in the blend. They are calculated to be M_n 6977, M_w 7128, and PD 1.022. They are in agreement with the experimental data.

In the case of bi-component polymer blend containing polystyrene 7000 and a small amount of polystyrene 11600, it was found that the minor component could be detected in the mixture of polystyrene 7000 and polystyrene 11600 even at a mole ratio of 100 to 1. This result suggests that any oligomers with relative contents greater than 1% of the most abundant oligomer in the high mass tail of the polystyrene 7000 distribution should be detected in MALDI. For polystyrene 7000, the addition of any peaks with intensities less than 1% of the most abundant peak at both tails of the distribution does not affect the calculated M_n, M_w, and PD values, within the experimental precisions.

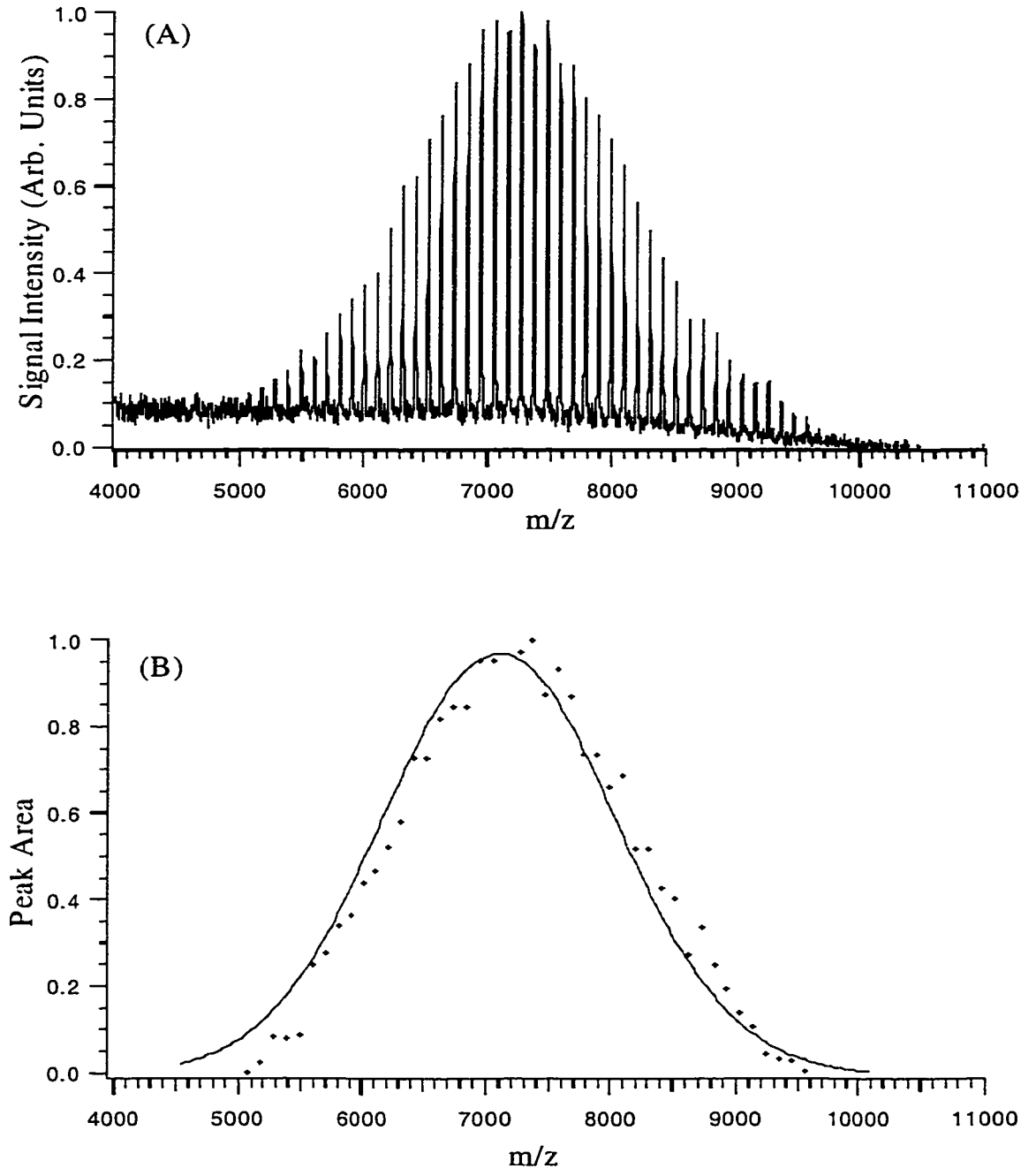

Figure 2.5. (A) MALDI mass spectra of polystyrene 7000 obtained under the same conditions as those used in Figure 2.2A except that LiOH was added to the sample preparation. (B) Plot of individual peak areas of oligomers as a function of m/z and the Gaussian fit from Figure 2.2B.

From the above discussion, it can be concluded that there are no detectable systematic errors associated with the average molecular weight determination by MALDI for polystyrene 7000. The actual error associated with the M_n and M_w measurement cannot be determined; but it should be governed by the random error. Considering that the precision of the technique is better than 0.5% RSD, we expect that the error of M_n and M_w measurement by the MALDI method described in the work is very small.

The utility of the aforementioned method of accuracy evaluation can be further demonstrated when it is applied to evaluate a less ideal MALDI method. In this case, polystyrene 7000 was analyzed using a MALDI preparation where lithium hydroxide was purposely added to the retinoic acid/silver nitrate formulation. The addition of LiOH reduces the detection sensitivity and increases the background level at the low mass region [39]. Figure 2.5A shows the MALDI spectrum of polystyrene 7000 obtained by using this less sensitive method. It can be clearly seen that the signal-to-noise ratio of this spectrum is relatively poor, compared to that shown in Figure 2.2A. The average molecular weight values are found to be M_n 7166 (0.8% RSD) and M_w 7292 (0.7% RSD) from five trials. Thus the precision of the method using this preparation is also degraded. Note that these M_n and M_w values are significantly different from those obtained by the more sensitive method (see Table 2.1 MALDI results) (99% confidence limit from t-test).

In the absence of any information from the more sensitive preparation, the less sensitive method can be evaluated by first considering the shape of the polymer distribution for polystyrene 7000. Figure 2.5B plots the peak area of individual oligomers as a function of m/z. This spectrum does not fit to a Gaussian distribution well, particularly for the low abundance oligomers (the overall fit has $\chi^2 = 0.0522$). It appears to be skewed to the high mass distribution. For visual comparison, the curve fit of polystyrene 7000 as shown in Figure 1D is included in Figure 2.5B. Since a Gaussian distribution is expected for this polymer, the departure from Gaussian distribution suggests that there could be an error associated with the MALDI measurement.

To further evaluate the accuracy of the less sensitive method, the dynamic range of detection is examined. In the mass spectra of polystyrene 7000 obtained by the less sensitive method, the m/z 7368 peak is the most intense peak. The least intense peak in the low mass region of the distribution is at m/z 5076 and has a relative intensity of 5.2% and a S/N of 4.0. The peak at m/z 9555 has a relative intensity of 4.7% and a S/N of 4.4. These data suggest the possible truncation of the peaks from low abundance oligomers in the distribution. To confirm this notion, two bi-component blends containing polystyrene 7000 and a second polymer component were prepared as before. The expanded mass spectra in the region of interest (not shown) indicate that, with the addition of 5% polystyrene 5050 to polystyrene 7000, the MALDI method is still not sensitive enough to extend the detection of oligomers to lower masses beyond those from polystyrene 7000. Similarly, very little signals from polystyrene 11600 is detected in the blend containing polystyrene 7000 and polystyrene 11600 in a mole ratio of 100 to 2. This reduced dynamic range can be attributed to both a decrease in detection sensitivity for the oligomers and an increase in background noise level. Thus, from the combined results, namely asymmetric distortion of the polymer distribution and truncation of the oligomer distribution, we can conclude that the M_n and M_w values determined by the MALDI method using this less sensitive sample preparation are not accurate.

The method of assessing accuracy from the mass spectrometric detection point of view, as described herein, should be applicable to other polymers. Polymers of narrow polydispersity can be prepared using GPC fractionation of a polydisperse polymer. In making the blends, it is preferable to choose a minor polymer component having some oligomer overlap with the major component. This closeness in masses would avoid the potential problem of mass discrimination that has been observed for polydisperse polymers [21, 22]. Another important consideration in applying this method of accuracy analysis to other polymers is related to the reproducibility of the technique. The sample and matrix preparation as well as the instrumental measurement should be optimized to achieve high precision. This is important for comparison of the results from different

experiments with statistical significance. Furthermore, the accuracy is ultimately limited by the precision of the method.

Determination of the accuracy of a MALDI method has other implications. It is well known that there are many variables in MALDI-TOFMS that can affect molecular weight determination [21-29]. Because of differences in instrumental design, method of sample preparation, and skill of the operator, interlaboratory comparison of molecular weight data obtained under diverse experimental conditions must be attempted with great care. To facilitate such a comparison, it is our view that each method to be included in such an exercise should be first qualified with a set of performance indicators such as resolution, precision and accuracy. Confirmation of the MALDI method providing accurate molecular weight data for polymers also has a significant implication in the understanding and further development of other molecular weight characterization methods such as GPC. Up to now, theoretical treatment of band broadening and other chromatographic behaviors for polymers cannot be rigorously tested [35, 38]. The use of a polymer standard with known distribution should open new opportunities to address these fundamentally important issues. A comparative study of determining M_n, M_w, and PD of narrow polydispersity polymers by MALDI and GPC will be discussed in Chapter 4.

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CHAPTER 3. INVESTIGATION OF THE EFFECTS OF INSTRUMENTAL CONFIGURATION, MASS RESOLUTION AND DATA ANALYSIS METHOD ON POLYMER MOLECULAR WEIGHT DETERMINATION BY MALDI-TOF MS

3.1 Introduction

The property of a polymer material can be significantly affected by a small variation in average molecular weights. Therefore, it is critical to determine polymer molecular weights accurately. At present, there are a number of analytical techniques based on separations, osmometry, light scattering and spectroscopy that can be used to determine polymer molecular weights [1, 2]. Among them, the most widely used is gel permeation chromatography (GPC) [1-3]. However, the accuracy of polymer molecular weight determination can be significantly affected by several factors, including chromatographic band broadening, solvent interaction, limited types of standards, adsorption and partition effect [1-3]. In contrast, matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry has the potential to provide more accurate molecular weight results at least for narrow polydispersity polymers. In addition, this technique offers several other advantages over GPC including direct mass measurement [6, 7, 10, 12], end-group confirmation [4, 7], oligomer structure analysis [6, 7], repeated unit identification [8, 9] and polymerization kinetic study [10]. However, the use of MALDI-TOF mass spectrometry to determine the molecular weight could also be affected by a

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number of experimental parameters. A systematic identification of these parameters as well as fundamentally understanding their effects on molecular weight analysis can be of significance in practical applications of MALDI-TOF. This work was to examine the effects of four important factors affecting MALDI-TOF molecular weight determination, including the type of instrument, mass resolution, time-lag focusing point and method of data analysis.

In MALDI-TOF, the number average M_n and weight average M_w of a polymer are defined by the following equations:

$$M_n = \Sigma N_i M_i / \Sigma N_i \tag{3.1}$$

$$M_w = \Sigma N_i M_i^2 / \Sigma N_i M_i \tag{3.2}$$

where N_i and M_i represent signal intensity in peak area and mass for the oligomer containing i monomers, respectively. Therefore, an accurate determination of polymer molecular weights needs to meet two important criteria. First, an obtained polymer mass spectrum must reflect the real oligomer distribution. In other words, all oligomers should be detected with constant response or detection efficiency. Second, an accurate spectral analysis must be performed. The change in spectral appearance brought about by the variations in baseline level and mass resolution can affect the peak area measurement, hence the M_n and M_w results

An analysis of polymers with MALDI-TOF MS generally consists of three steps. First, the sample must be prepared properly. This is usually done by premixing the analyte with selected matrix at a certain ratio, leading to the formation of a solid sample film in which analytes are incorporated into the matrix crystals. In many cases, a small amount of metal salt is added for cationization. The second step is the process of converting analytes into gas-phase ions through laser desorption/ionization. The third step is that the ions are

analyzed using a TOF mass spectrometer. Conducting any of these steps may cause the truncation of polymer distribution. Mass discrimination may occur in sample preparation step by producing unflavored crystals for laser desorption/ionization. Spectrum truncation may occur in the ionization step by fragmentation. Spectrum distortion may result from the extraction of ions by accelerating voltage, the low ion transmission and the incorrect response of the detector.

A number of studies have showed that sample preparation method [11-13], accelerating voltage [13], laser power [15] and type of cation addition [16-18] may play a substantial role in spectrum truncation. For example, Chen and Guo [13] noted that when a small amount of water was added to a poly(methyl methacrylates) (PMMA) solution (methanol), mass discrimination would occur, most likely, resulting in larger oligomers. If the amount of water is increased, more severe systemic mass discrimination was observed. Over 50% of weight average molecular weight (M_w) variation had been reported for the analysis of PMMA 4000 because of water addition. This observation can be explained by considering the solvent mixture properties [19]. Because PMMA cannot readily be dissolved in water, the use of water in a solvent mixture can lead to polymer precipitation prior to matrix/polymer cocrystalization [19]. Dogruel *et al.* [14] found that not only the mixture of solvent but also the solvent acidity can influence polymer molecular weight determination. They noticed that the mass spectrum of PMMA was strongly impacted by the pH of the sample solution when using 2,5-dihydroxybenzoic acid (DHB) as a matrix. It was observed that at acidic condition ($pH \leq 4$), similar mass spectrum was obtained. However, when the pH was increased from 5 to 9, there was no signal observed. Further increase in pH resulted in different shape of mass spectra. This phenomenon contributed to the effects of pH the structure of the matrix-analyte crystal and the cationization ability of cations.

Tang *et al.* reported that the mass spectrum of poly(methylmethacrylate) (PMMA 6000) was truncated when the accelerating voltage of TOF is decreased from 30 kV to 18 kV

[20]. This truncation results in lower molecular weight values at 18 kV as compared to that of 30 kV. They also noted that at 18 kV, there were a great reduction in spectrum intensity and mass resolution. It was speculated that at lower acceleration voltage, the ions spent more time in the relatively high-density environment of the acceleration region, thereby, experiencing a larger number of collisions. These collisions took place not only with small matrix-related particles but also with other oligomers similar in size. The collision-induced kinetic energy spread was responsible for the truncation of the polymer spectrum.

A comparison of previous polymer mass spectrometry researches shows that different molecular weight results are sometimes obtained from one lab to another even for the same type of polymers from the same supplies. While there are a number of possible variables in MALDI analysis from lab to lab that may affect the M_n and M_w results, several researchers have suggested that one important variable is sample preparation method. In this work, we focus on other four commonly encountered variables, i.e., the type of instrument or instrumental configuration, laser power, time-lag focusing point and data analysis method, and show how they can affect the molecular weight analysis.

3.2 Experimental

3.2.1 Instrumentation

Two home-built, one Hewlett Packard (HP) and one BRUKER Proflex^{Tm} linear MALDI-TOF mass spectrometers were used in this study. Two home-built instruments have similar configurations except that their resolving powers are slightly different. The basic constructions of the instruments have been described in Chapter 2. Both instruments have since been modified to operate up to 30 kV for ion extraction [21]. Briefly, they feature a four-plate source design with both of the repeller side of the first extraction plate and the ground plate covered with stainless steel grids, pulsed ion extraction for time-lag

focusing, and 1 m linear flight tube. Ions are generated using a 337 nm laser beam from a nitrogen laser, having a pulse width of 3 ns (model VSL, 337ND, Laser Sciences Inc., Newton, MA). The laser desorption was designed at 67.5° to the probe tip surface. Except in the laser effluence study, laser power was maintained slightly above ion detection threshold in all analyses. Under normal operation, the extraction voltage was set to 20 kV DC. A home built high voltage pulser was used to generate the delayed extraction pulse. The delay time was generally chosen to be 1 μs. In order to achieve better resolution, different pulse voltages were selected for different polymer analyses. To reduce detector saturation for the polymers due to the high abundance of ionized low mass matrix molecules, most of the matrix ions were deflected away by a strong-pulsed electric field applied to the ion gate of the time-of-flight mass spectrometer. A dual multi-channel plate (MCP) detector was used in both instruments for ion detection.

The HP MALDI time-of-flight system (Model G2025A, Palo Alto, CA) was also equipped with a pulsed nitrogen laser with 337 nm and 3 ns pulse width. It also has a 1 m long flight tube. Under normal operation, the extraction voltage was set to 28 kV. A dual MCP was used for ion detection. The BRUKER Proflex MALDI time-of-flight mass spectrometry was built with a 1.25 m long flight tube. Ion desorption was also performed by a pulsed nitrogen laser with 337 nm radiation and 3 ns pulse width. Under normal operation, the extraction voltage was set to 20 kV and one of the three time-lag focusing arrangements was used according to the polymer's mass range. In order to increase the sensitivity of the detector, a 2 kV of deflector voltage was used to deflect matrix and other irrelevant ions at the lower mass range. A MCP detector was used for ion detection.

3.2.2 Data Collection and Processing

The HP (Model G2025A) data collection system was used both in the HP and the home-built MALDI time-of-flight instruments. For the home-built instruments, the data system was modified in which the instrument control features have been disabled and a LeCrey

9350A digital oscilloscope at a sampling rate of 500 MHz to 1 GHz was used. The BRUKER Proflex MALDI system uses its own data collection system for data acquisition.

In general, mass spectra from 100 laser shots were summed to produce a final spectrum. All data were further processed using the Igor Pro software package (WaveMetrics, Lake Oswego, OR). No correction of 1/(dm/dt) was applied to the mass spectra during the conversion of the time domain to the mass domain [22-24]. Average molecular weights were corrected for the contribution of the cation (Ag^+). Polydispersity (PD) was determined from the ratio of M_w to M_n. The percent relative standard deviation (%RSD) for the measured M_w and M_n values are determined from five separate sample loadings. All mass spectra shown below are the smoothed spectra using 30-point Savitzky-Golay smoothing approach in Igor Pro software. No baseline correction was performed.

3.2.3 Samples and Reagents.

Bradykinin, bovine insulin b-chain, bovine ubiquitin, equine cytochrome c and bovin carbonic anhydrase II were obtained from Sigma (Milwaukee, WI). Water used as solvent for calibration was from a NANOpure water system (Barnstead/Thermolyne). Sinapinic acid was purchased from Aldrich (St. Louis, MO). Polystyrene standards with the following nominal molecular weights were used in this study: 1700 (Showa Denko, Tokyo, Japan), 7000 (Polymer Laboratories, Amherst, MA), and 11600 and 28500 (Showa Denko, Tokyo, Japan). *All-trans* retinoic acid was obtained from Aldrich (St. Louis, MO). $AgNO_3$ was reagent grade and used without further purification. Tetrahydrofuran (THF) was HPLC grade and obtained from VWR (Toronto, Canada).

3.2.4 Calibration

Mass calibrations were performed externally. Bradykinin, bovine insulin b-chain, bovine

ubiquitin, equine cytochrome c and bovin carbonic anhydrase II were used in the calibrations. Different proteins were selected for calibration of different mass range. The proteins were first dissolved in water and then stored in a freezer at -20° C. A solution containing a mixture of proteins was prepared fresh. The dried droplet method was used for mass calibration. In this method, sinapinic acid was used as the matrix. It was first prepared to 0.1 M in a solvent containing 60% CH_3CN, 36% MeOH and 4% H_2O and then mixed with an equal volume of protein solution. The concentrations of proteins and ratios among the test proteins had been adjusted before each calibration to reach their best sensitivities and resolutions.

3.2.5 Sample Preparation.

One layer method was used for polymer sample preparation for MALDI analysis. This was done by combining the analyte, matrix and cationization reagent [16, 18]. All polymer samples were prepared in glass vials. The polymer standards were prepared by dissolving in THF to a concentration of 1 mM (calculated from the nominal molecular weights). *All-trans* retinoic acid was used as the matrix and was prepared to 0.15 M in THF. The saturated $AgNO_3$ ethanolic solution was used as the cationization reagent. In a typical experiment, polymer stock solutions were diluted ten-fold with the matrix solution, and then 1% (v/v) of the $AgNO_3$ ethanolic solution was added. This will give a final polymer concentration of 100 μM and a matrix to analyte ratio of 1350. In the analysis, 1 μL of the appropriate mixture was added to the MALDI probe tip and allowed to air-dry.

3.3 Results and Discussion

3.3.1 Instrumental Configuration Effect

In MALDI-TOF analysis, the instrumental configuration of a mass analyzer largely

dictates its sensitivity and mass resolution, thereby affecting the mass detection range and accuracy. Different instrumental designs have been found in both home-built and commercial instruments by varying flight tube length, acceleration energy, type of laser, with or without time-lag focusing, ion source design and type of detector. In this experiment, four narrow distributed polystyrene standards, PS 1700, PS 7000, PS 11600 and PS 28500, were selected to evaluate the performance of one home-built, one HP and one BRUKER Proflex MALDI linear time-of-flight mass spectrometers. In the following discussion, the identity of these instruments is designated as TOFx (x = 3, 2, or 1), respectively.

Fig. 3.1 shows the PS 1700 mass spectra obtained from TOF1 (Fig. 3.1A), TOF2 (Fig. 3.1B) and TOF3 (Fig. 3.1C), respectively. Only the mass range showing the polymer distribution for Ag^+-attached oligomers is presented. As shown, the signal-to-noise ratio of the spectrum obtained from the TOF1 system is the lowest in all of the three spectra. Because of this lowest sensitivity, the interference of matrix molecules on polymer distribution would be the greatest. As a result, the baseline level of the spectrum was the highest at the low mass end and partial truncation of the polymer distribution was observed. Furthermore, different mass resolutions were evident from these three linear time-of-flight mass spectrometers.

The effects of instrumental configuration on polymer molecular weights for PS1700 and the other polymers are summarized in Table 3.1. The relative standard deviations of M_n and M_w were calculated based on 5 runs. It can be seen that the M_n and M_w of PS 1700 are significantly different. The difference in M_n between TOF1 and TOF2 is 100 Da while the difference between TOF3 and TOF2 is only 39 Da. These results clearly show that at low mass region, different instrument design gives different polymer molecular weight results.

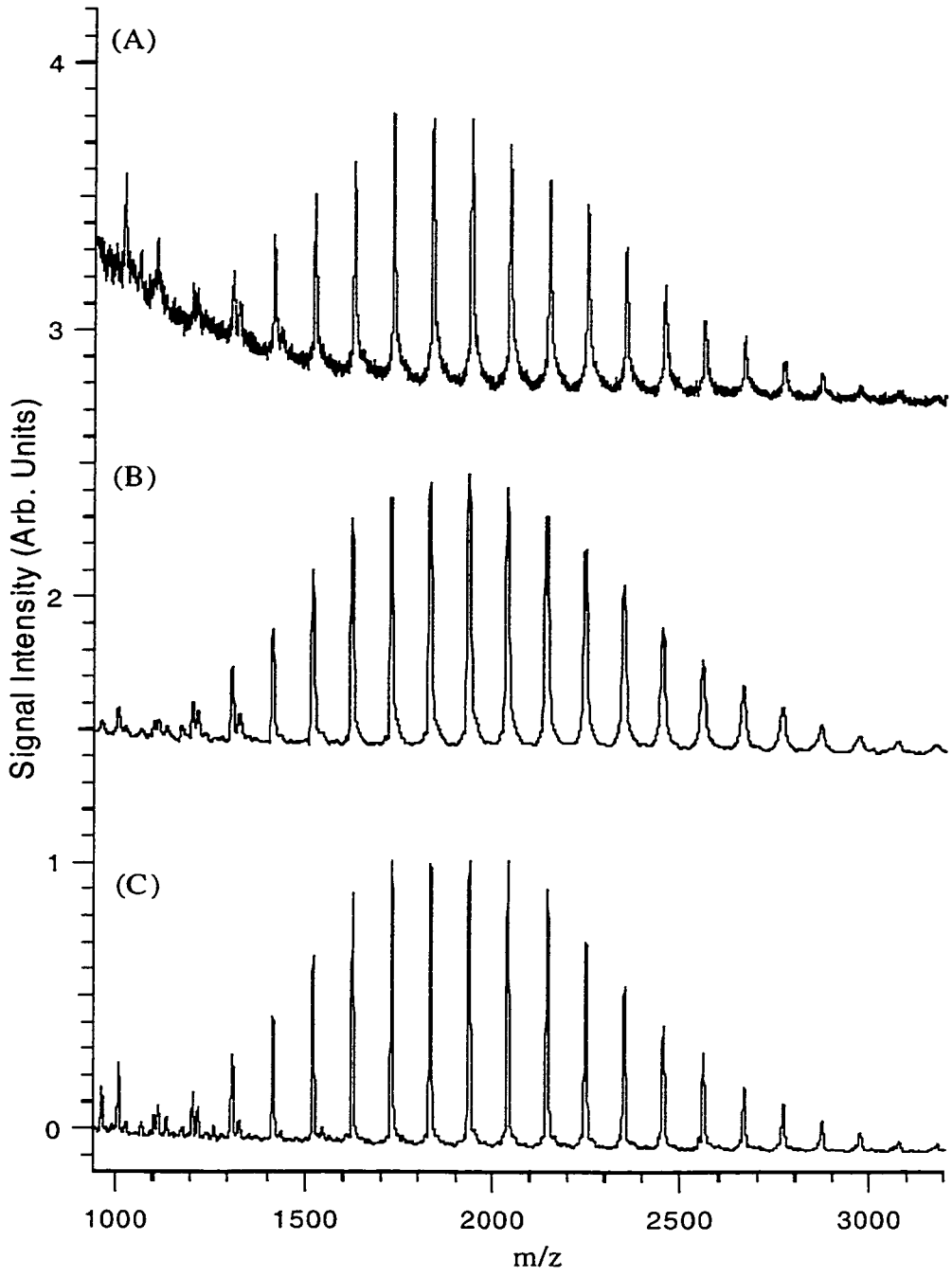

Figure 3.1. MALDI mass spectra of (A) TOF1 (B) TOF2 and (C) TOF3 instruments for polystyrene 1700

Table 3.1. Experimental Results From Three Different Instruments

Polymer	INSTRUMENTS														
	TOF3					TOF2					TOF1				
	Mn	RSD	Mw	RSD	PD	Mn	RSD	Mw	RSD	PD	Mn	RSD	Mw	RSD	PD
PS 1700	1878	0.70%	1963	0.90%	1.043	1917	1.10%	2060	0.90%	1.04	1817	1.00%	1892	0.90%	1.034
PS 7000	7050	0.03%	7166	0.03%	1.016	7152	0.20%	7256	0.17%	1.015	7051	0.80%	7145	0.80%	1.013
PS 11600	11080	0.09%	11182	0.10%	1.009	10925	0.40%	11019	0.40%	1.009	N/A	N/A	N/A	N/A	N/A
PS 28500	26569	0.04%	26672	0.04%	1.004	27025	0.15%	27231	0.15%	1.005	N/A	N/A	N/A	N/A	N/A

Note: N/A = no data available

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To further demonstrate the effect of instrumental configuration on polymer molecular weight, PS 7000 was selected to exam the effect on the higher mass range. Fig. 3.2 shows the mass spectra acquired from TOF1 (Fig. 3.2A), TOF2 (Fig. 3.2B) and TOF3 (Fig. 3.2C) instruments, respectively. Due to the low sensitivity of TOF1 system, the background interference becomes stronger, resulting in some of oligomer peaks lost at the both low and high mass tails of the polymer distribution. As a result, the polydispersity of PS 7000 determined by this instrument is smaller than those of other two instruments (Table 3.1).

Table 3.1 also shows that there are significant average molecular weight (M_n and M_w) differences existing in the three instruments for this sample. In addition, the relative standard deviations of M_n and M_w for PS 7000 are greatly reduced as compared to that of PS 1700 for both TOF2 and TOF3. At this mass range, these two instruments provided their very good instrumental sensitivity and shot to shot reproducibility.

Fig. 3.3 shows the spectra obtained by TOF2 (Fig. 3.3A) and TOF3 (Fig. 3.3B) for PS 11600, respectively. Because of the lower detection sensitivity of TOF1 instrument under the same sample preparation conditions, no signal could be recorded for this polymer. In contrast, TOF3 can still give a very nice polymer oligomer distribution with completely resolved oligomer peaks and very high resolution power at such high mass range. TOF2 system failed to give an accurate oligomer distribution spectrum. The serious spectrum truncation occurred due to the low-resolution ability. No adjacent oligomer peaks were completely resolved and the truncation at the right side of the polymer distribution is much stranger than that at the left side of the polymer distribution. Consequently, over 150 Da difference in M_n between TOF2 and TOF3 was observed (see Table 3.1).

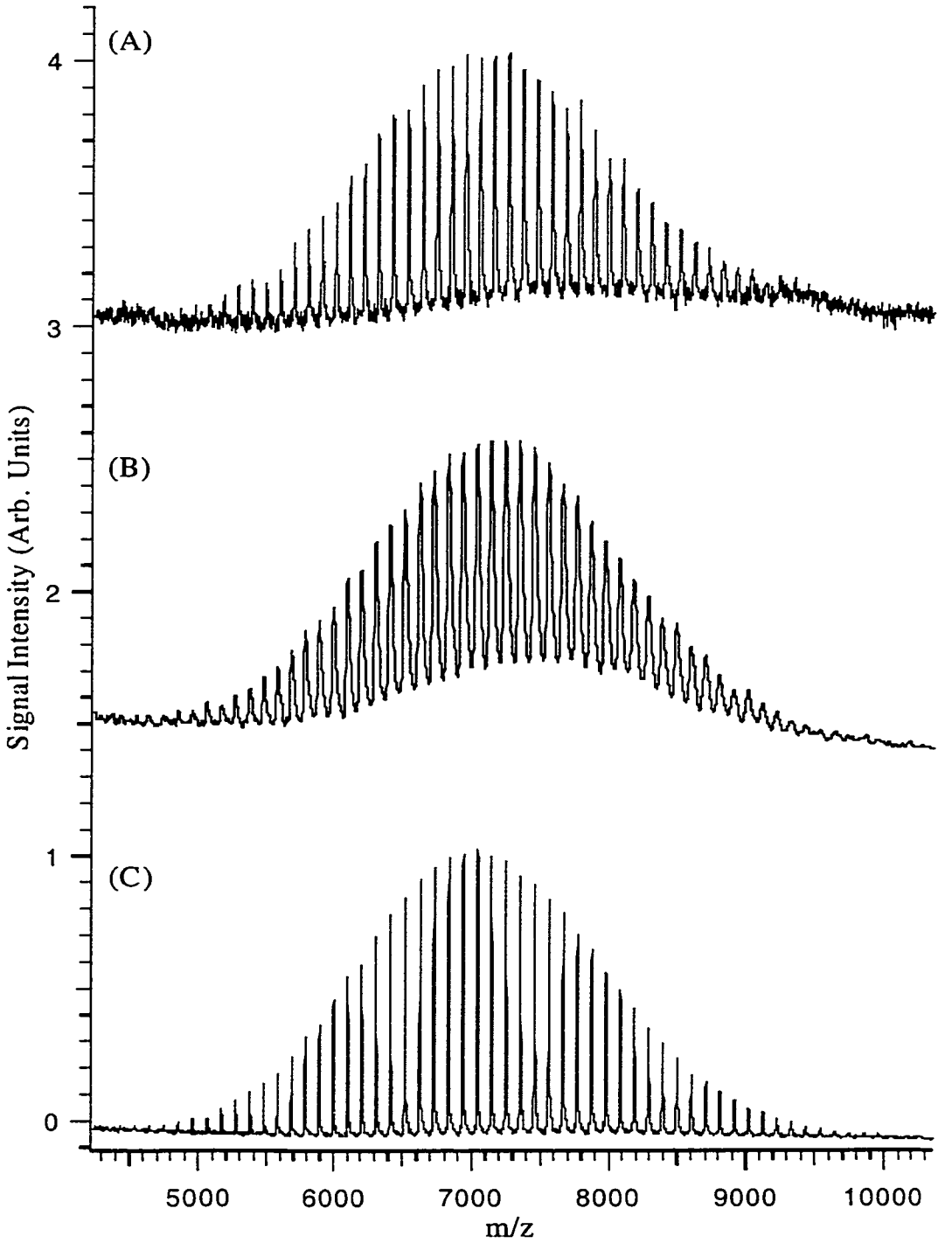

Figure 3.2. MALDI mass spectra of (A) TOF1 (B) TOF2 and (C) TOF3 instruments for polystyrene 7000.

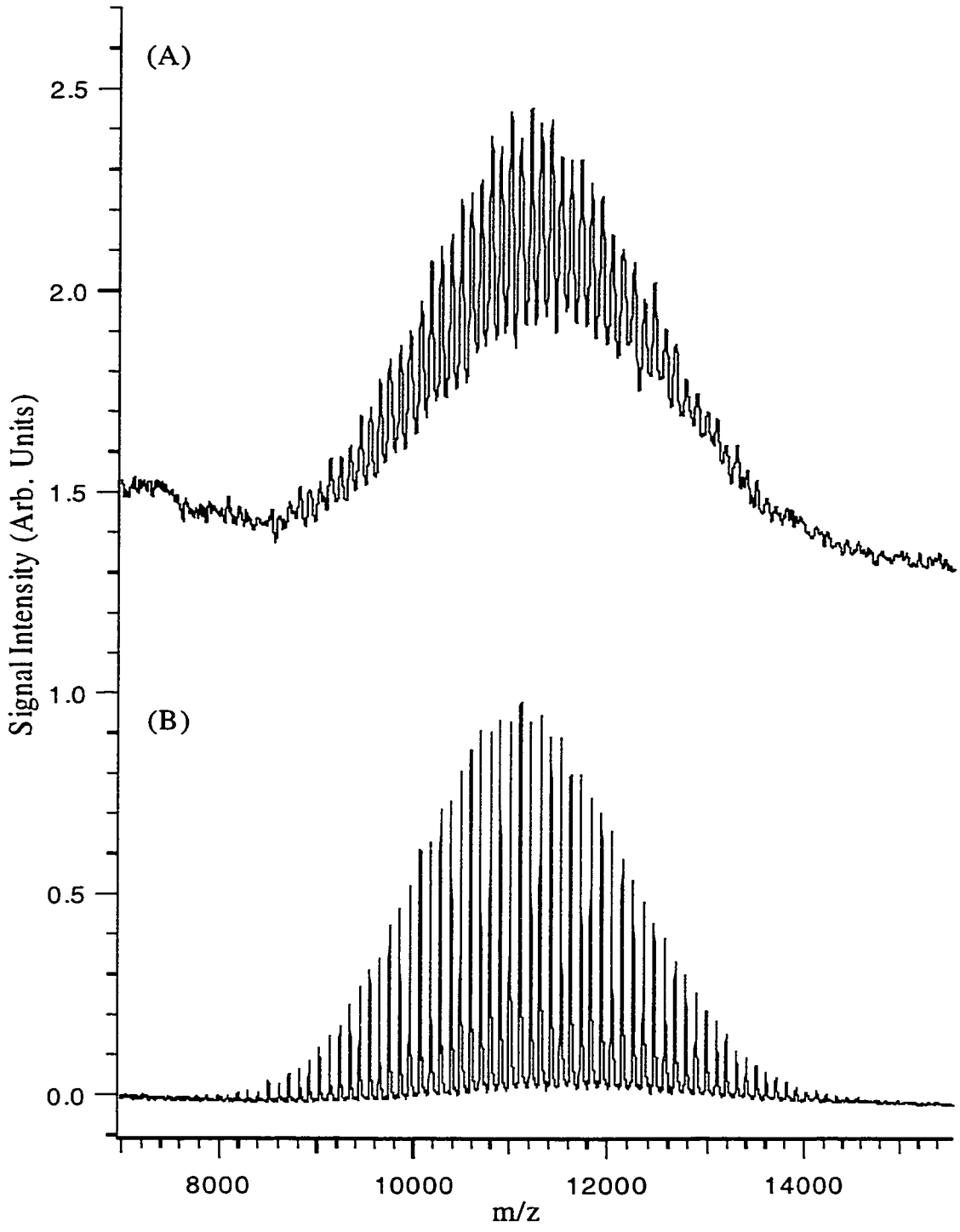

Figure 3.3. MALDI mass spectra of (A) TOF2 and (B) TOF3 instruments for polystyrene 11600.

Fig. 3.4 shows the spectra obtained using TOF2 (Fig. 3.4A) and TOF3 systems (Fig. 3.4B) for PS 28500, respectively. Even at such high mass range, TOF3 can still give a very good oligomer resolved polymer distribution. On the other hand, the resolution of TOF2 becomes so low that no adjacent oligomers are resolved in its spectrum. All of the peaks appeared in Fig. 3.4B belonged to PS 28500. The multi-peak appearance suggests the multimer formation for polystyrene at this mass range. The term "multimer" is referred to the aggregation of two or more polymeric distributions. The first peak is the major polymeric distribution, the second peak is the aggregation of two polymeric distributions, and the third one is the aggregation of three polymeric distributions and so on. As we can see that as the number of the aggregation of polymer chains increases, the intensity of the each peak decreases. The monomer peak gives the highest intensity. It is also interesting to note that after the dimer peak, as the number of the aggregation of polymer chains increases by one, the intensity of the peak decreases almost by half.

Multimer formation is normally found in the analysis of high molecular weight polymers. It is believed that the agglomeration of polymer chain happened in MALDI sample preparation stage [26]. At this stage, after solvent evaporation the concentration of polymer is quite high. As high molecular weight polymer has longer chain than that of low molecular weight one, chain entanglement becomes possible. Sampling at this condensed phase, multimer formation will be produced. Gas phase collisions in the clustering process may be another reason for polymer chain entanglement [27]. Furthermore, it is found that matrix plays an important role in the number of multimer formation [26]. In this experiment, the monomer peak was used for polymer molecular weight calculation. The calculated results (Table 3.1) show that the mass difference is 456 Da for M_n and 559 Da for M_w between two instruments.

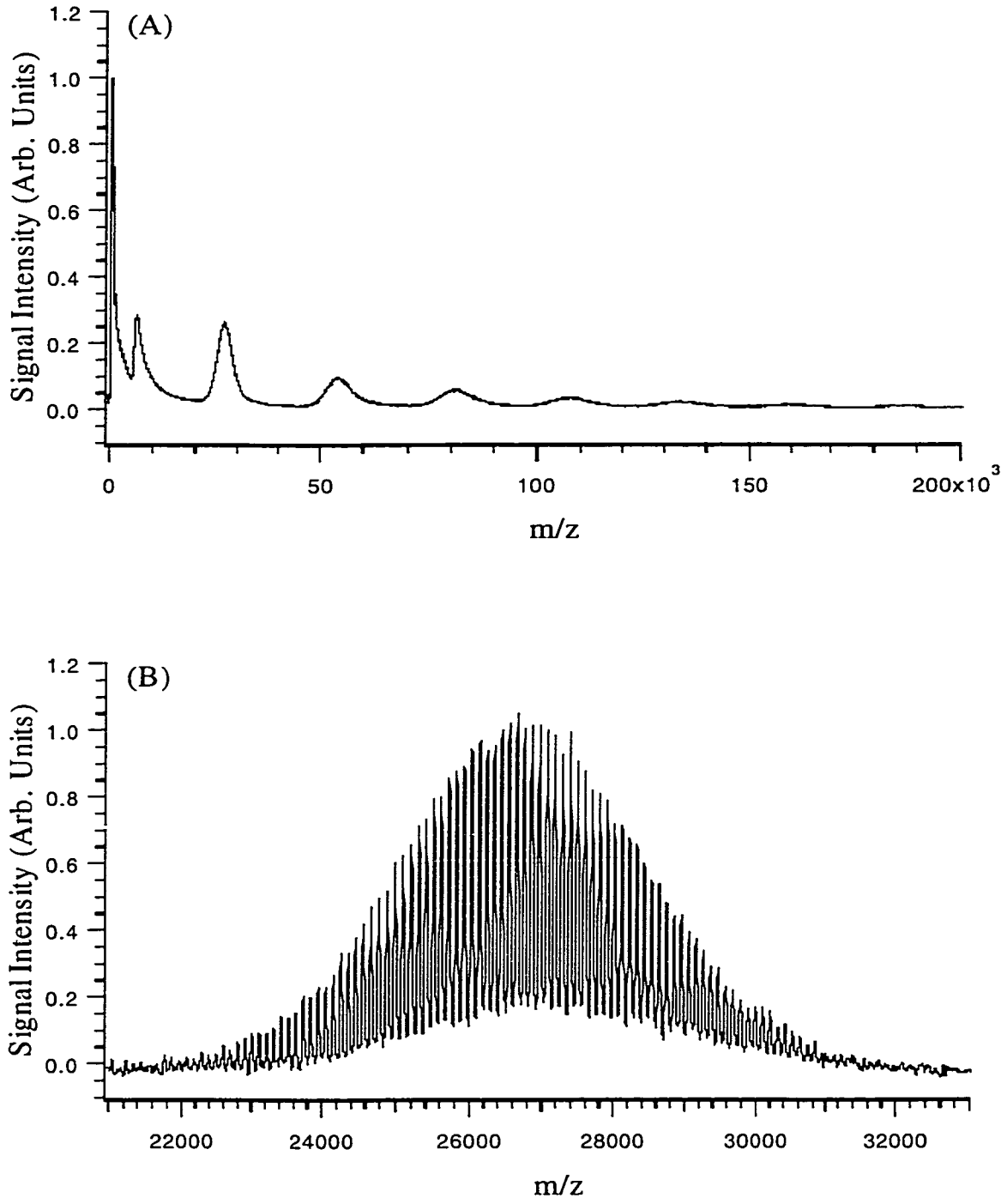

Figure 3.4. MALDI mass spectra of (A) TOF2 and (B) TOF3 instruments for polystyrene 28500.

The above experimental results demonstrate that instrument configuration affects the determination of polymer molecular weights. Different instrument design shows different spectrum appearance and polymer molecular weights. The different in mass resolution may affect the molecular weight results (see below). Instrumental sensitivity is another critical factor that would affect polymer molecular weight determination. Lower signal-to-noise ratio may cause spectrum truncation. Furthermore, accurate determination of polymer molecular weights also dependes on the analyte mass range. Finally, multimer formation becomes more important at high molecular weight mass spectrum.

3.3.2 Laser Power

In MALDI TOF MS measurements, the laser power must be adjusted slightly above the threshold for the desorption/ionization process. The term "threshold " is defined as the lowest laser energy required for obtaining a measurable signal. It has been shown that laser power plays an important role in polymer molecular weight determination. Lehrle and Sarson [28] found that high laser radiation could cause degradation of the poly(methyl methacrylate) (PMMA) structure, resulting in polymer molecular weight distribution skewed towards lower molecular weights. Martin *et al.* [29] suggested that the determination of polydisperse polymer molecular weights depends on the laser power used. Higher laser power produced higher average polymer molecular weights. They also noticed that the higher mass components would require a higher laser power for effective desorption/ionization. However, the lower mass components were fragmented by a very high laser power. For most polymers, slightly increasing laser power does not induce any fragmentation, rather the mass resolution of the polymer spectrum may be changed.

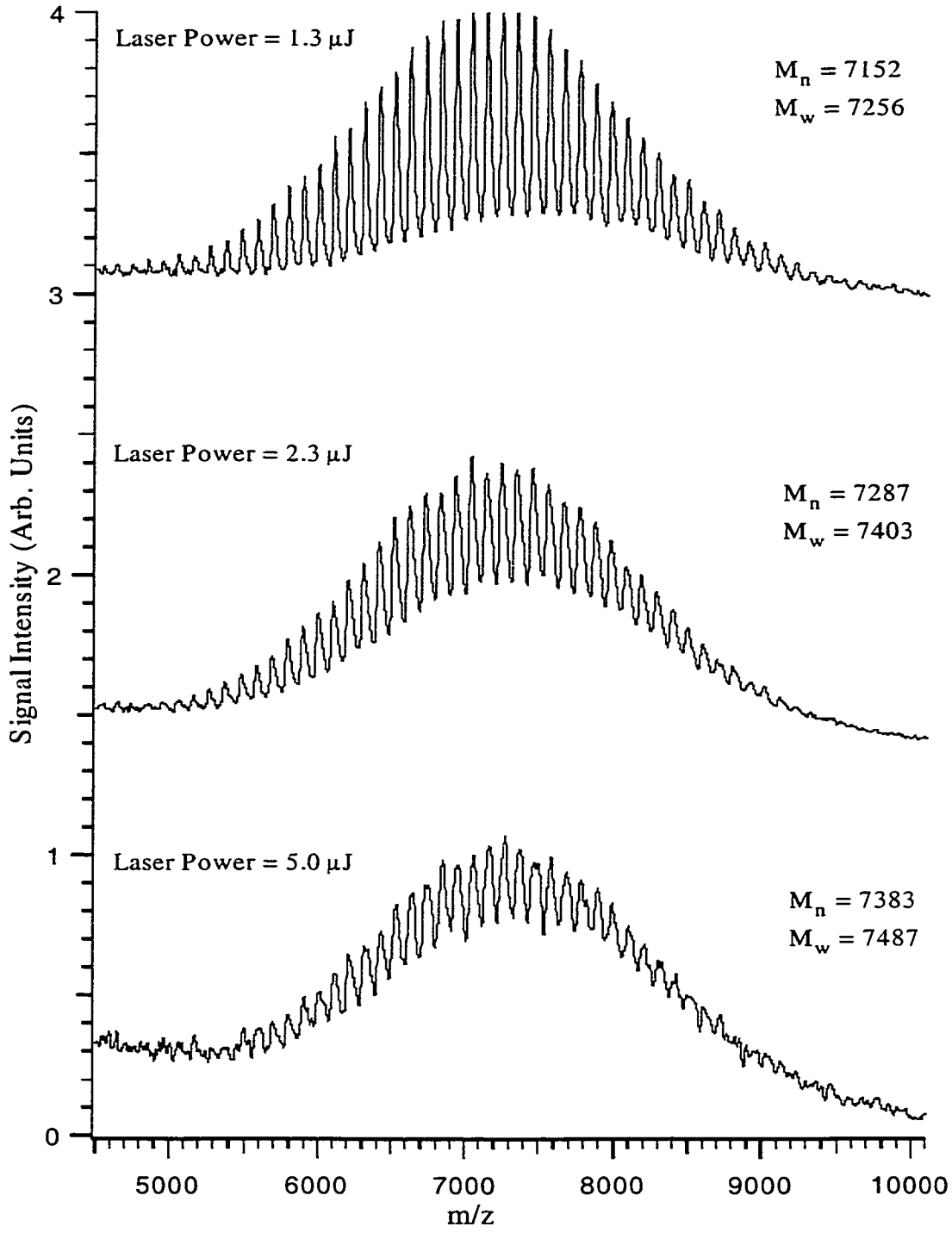

Figure 3.5. DC extraction MALDI spectra of polystyrene 7000 obtained by using different laser powers.

In order to illustrate the effect of laser power on polymer molecular weights, PS 7000 was selected as a model while *all-trans* retinoic acid was used as matrix. Previous results showed that the *all-trans* retinoic acid can provide a very good "shield" for the polystyrene molecules from UV irradiation [22]. Consequently, a very high laser power can be used without destroying the polystyrene molecules. TOF2 was used in this study. Laser power was controlled within a range from 1.3 μJ to 5.0 μJ. No molecular fragmentation was observed during data collection. The experimental results are shown in Fig. 3.5. As shown, the resolution decreases while the M_n value increases as laser power increases. The largest difference between the lowest and highest resolution spectra is 500 Da. This difference can be contributed to overestimating oligomer peak areas at low oligomer resolution and varying resolutions across the polymer distribution.

3.3.3 Time-Lag Focusing Point and Data Analysis Method

In the analysis of nonpolar narrow polydispersity polymers, the principal polymer distribution usually results from the adduct ions formed by the attachment of the cations from the cationization reagent to the polymer. In some cases, minor distributions from other cation attachments are also observed. These cations may come from the impurities of solvent, matrix or polymer itself. In the analysis of PS 7000 with the use of TOF3 and $AgNO_3$ as a cationization reagent, it was found that, in addition to the Ag^+-attached polymer distribution, there is another low intensity distribution from Na^+-attached oligomers. Similar Na^+ attachment has been observed by other investigators [8], although Na^+ cationization is not as efficient as Ag^+ cationization.

In the analysis of PS 7000 mass spectrum, the smallest mass difference between the two adjacent peaks from Ag^+-attached oligomer and Na^+-attached oligomer is 19 Da. To completely resolve these two peaks, high resolving power instruments need to be used. If the instrumental resolving power is not high enough, the two peaks may completely or partially overlap with each other. As a result, it will become difficult to accurately

Table 3.1. Molecular Weight Results of PS 7000 Obtained by TLF MALDI-TOFMS (from five trials)

TLF focusing:	Low Mass		Middle Mass		High Mass	
Calculation Method:	Method 1	Method 2	Method 1	Method 2	Method 1	Method 2
TOF3						
M_n	7086±10	7058±13	7010±12	7041±14	7022±11	7066±17
M_w	7195±11	7171±15	7126±11	7157±15	7140±10	7181±17
TOF4						
M_n	7078±10	7070±12	7050±2	7087±15	7043±11	7066±20
M_w	7192±11	7185±10	7166±2	7202±15	7157±10	7181±21

calculate the polymer average molecular weights, since peak areas of the individual oligomer need to be correctly determined. Thus the method of defining the oligomer peak in order to calculate its area becomes critical under these partial peak-overlap conditions.

Table 3.2 shows the molecular weight results of PS 7000 obtained by using TOF3 at different time-lag focusing pulse voltages. Two methods are used to calculate the oligomer peak area. Method 1 accounts for the peak areas mainly from the Ag^+-attached oligomers. The area of the Ag^+-attached oligomer peak was calculated by integrating the area from the peak to the baseline. The baseline of the peak is defined as a tangle line drawn from the base of the rising peak to the valley point between the Ag^+- and Na^+-attached peaks. Method 2 uses the total peak area from both the Ag^+- and Na^+-attached oligomer peaks. As shown, Method 2 gives consistent M_n and M_w results, independent of the focusing conditions used. In addition, except the data obtained from the middle mass focusing, both instruments yielded similar M_n and M_w results calculated from Method 2. In contrast, M_n and M_w values calculated by Method 1 are statistically different under different focusing conditions.

Fig. 3.6 shows the MALDI spectra obtained at three different focusing points from TOF3. Even within this narrow mass range, mass resolution was affected by the time-lag focusing pulse voltage used, resulting in different degrees of peak overlap between the Ag^+-attached and Na^+-attached oligomers. Focusing at the high (or low) mass of the distribution provides better resolution at the high (or low) mass end of the distribution. When the low mass ions are focused, the M_n and M_w values calculated by Method 1 are higher as compared to those obtained by focusing the high mass ions. In other words, the peak areas of the unfocused ion peaks with relatively lower resolution are overestimated, compared to those well-focused ion peaks. The same trend was observed for another home-built MALDI instrument (TOF4). It is interesting to note that both M_n and M_w values obtained by TOF3 focused at the high or middle mass ions are different from those obtained by TOF4 operated under the similar conditions. The mass resolution of the

focused ions from TOF4 is typically about 900 vs. 1100 from TOF3. Thus, it can be concluded that Method 1 is more sensitive to the change of mass resolution. In the cases where there are peak overlaps, Method 2 is recommended.

3.4 Conclusions

Instrumental configuration can significantly affect the determination of polymer molecular weights. This phenomenon can be explained by different instrumental sensitivity, resolution and mass detection range. For the same instrument, even a small change in laser power can affect mass resolution. The resolution was also affected by adjusting the time-lag focusing point. Finally, the resolution will be affected by the methods to define the baselines of response peaks. This is particularly true in the case of examining polymers with multiple molecular weight distributions from different cationization processes.

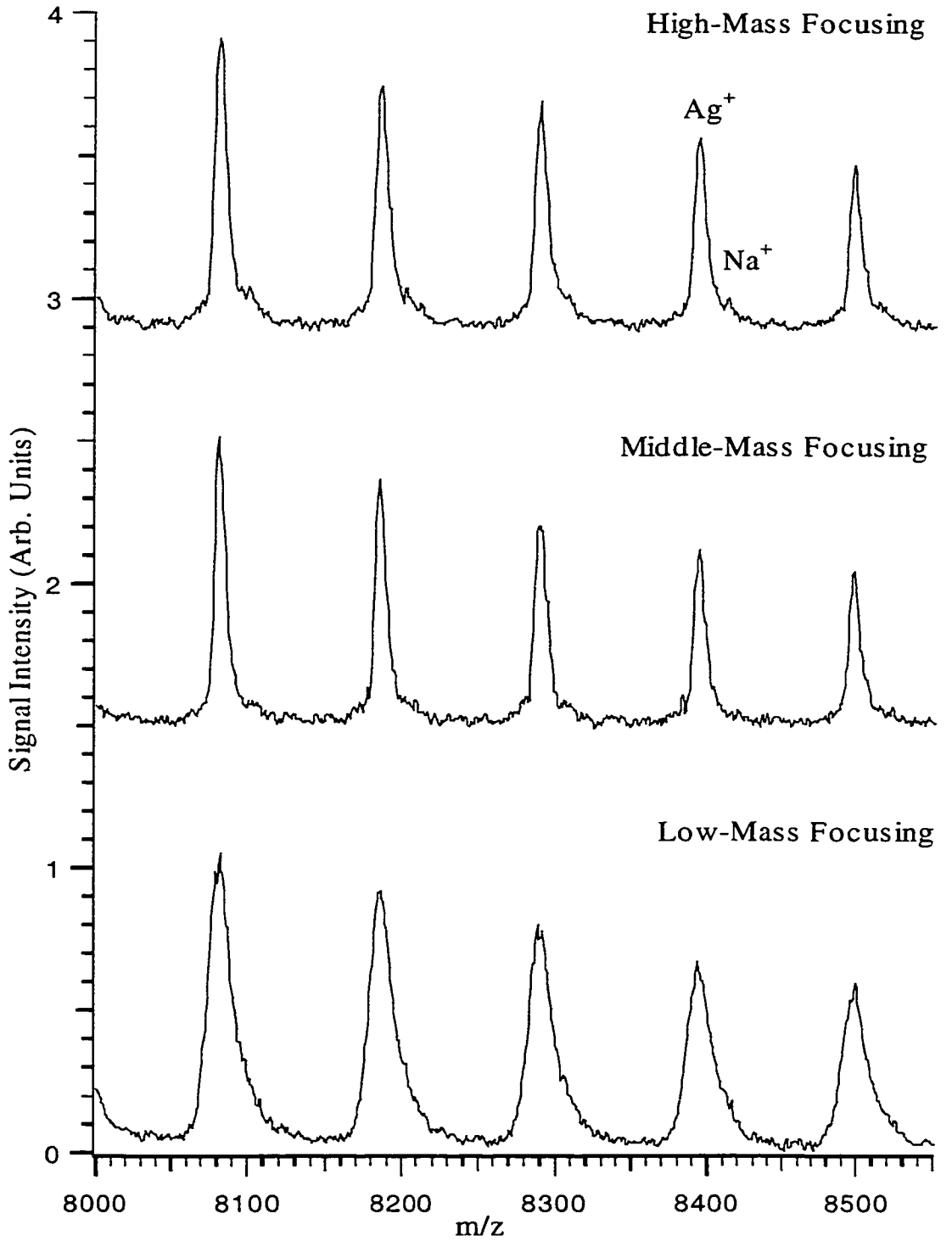

Figure 3.6. Expanded spectra of polystyrene 7000 obtained at different pulse voltages

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CHAPTER 4. COMPARISON OF MATRIX-ASSISTED LASER DESORPTION/IONIZATION TIME-OF-FLIGHT MASS SPECTROMETRY AND GEL PERMEATION CHROMATOGRAPHY

4.1 Introduction

Matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS) is a soft ionization technique that allows desorption and ionization of very large molecules without fragmentation. Since its introduction in 1988, this method has gained considerable interest as a new method for characterizing polymers [1-3]. A promising feature of this technique for polymer characterization is to perform direct identification of mass-resolved polymer chains [1, 4-6]. Consequently it provides a great potential for more accurately determination of polymer molecular weights than traditional methods such as gel permeation chromatography, light scattering or nuclear magnetic resonance spectroscopy [7]. In addition, the absence of substantial fragmentation, the extremely high sensitivity (pmol to fmol range), easy sample handing, and short analysis time make MALDI a nearly ideal technique for the analysis of complex polymer mixtures. MALDI mass spectra have been used to study repeat unit [1, 8], end-group, oligomer structures as well as tracking of polymerization kinetics [1, 4-6, 8-9].

Traditionally, gel permeation chromatography (GPC) has been widely used for polymer molecular weight determination. GPC is a liquid chromatographic technique in which a sample solution is introduced onto a column filled with rigid porous gel with defined pore size. Molecular separation is based on their sizes [12]. Size separation is achieved by differential pore permeation. The resulting chromatogram is a weight distribution of the polymer as a function of retention time or volume. The reason GPC can give molecular weight information according to their sizes is based on the relationship between linear dimension and molecular weight in a freely jointed polymeric chain (random coil): either the root-mean-square end-to-end distance or the radius of gyration is proportional to the

square root of the molecular weight. It follows that the log of either distance is proportional to (one-half) the log of the molecular weight [12].

As discussed, molecular separation mechanisms of MALDI TOF MS and GPC methods are different. In MALDI-TOF MS, molecules are separated by molecular mass, whereas GPC separates molecules according to their hydrodynamic size and is sensitive to polymer architecture (e.g., branching). GPC analysis has an inherent instrumental band-broadening problem that can greatly affect the experimental results. Instrumental band broadening is caused by many factors including solute longitudinal diffusion, eddy diffusion, resistance to mass transfer in stationary, characteristics of mobile and stagnant mobile phases, and the use of injector, detector, connecting tubing and end fittings of the column. When a very small amount of sample is injected at the top of the column as a narrow band, the bandwidth is increased after the sample elutes from the column. Band broadening can change the original polymer distribution, resulting in inaccurate polymer molecular weight determination. Thus, it is important to better understanding the band distribution function for GPC analysis as well as for comparing MALDI data with those of GPC. However, mainly because of the lack of information on the actual distribution of oligomers in a polymer standard, the determination of band broadening function is difficult in practice.

In this study, we present a method of combining the accurate molecular weight distribution data obtained from MALDI with those measured from GPC to quantify the band broadening function.

4.2 Experimental

4.2.1 Instrumentation

The GPC system used in this work consists of Waters 400 dual piston high performance liquid chromatography pumps, a Waters 715 ultra wisp sample processor with 96

injection valves, a Waters 600E system controller and a Waters 991 photodiode array detector (Waters Associates, Milford, MA). In order to get a large molecular detection range, three 7.8 mm x 300 mm Ultrastyragel columns (Waters Associates, Milford, MA) were used in series. Their pore sizes are 10^3 Å, 10^5 Å and 10^6 Å, respectively. The column temperature was controlled at 25.0 ± 0.1°C. Tetrahydrofuran (THF) was used as mobile phase at a flow rate of 1 mL/min. During the experiment, it was done by setting the pump at 0.0 mL/min first and then gradually increasing the flow rate in 0.1 mL/min increments. The injection loop value was set at 20 μL. Before operation, THF was degassed by helium for 15 min. During operation, the helium flow rate was controlled to continue degassing the solvent. A Waters 991 photodiode array detector software was used to collect and process the GPC chromatograms. The wavelength used was 260 μm.

The MALDI-TOF mass spectrometer used in this study was a home-built linear time-lag focusing system. The detail instrumental configuration has been presented in Chapter 2. Briefly, the ionization and ion extraction regions consist of four stainless steel plates and both of the repeller side of the first extraction plate and the ground plate is covered with stainless steel grids. A DC power supply was used to apply the potentials on the repeller and the first extraction plate and the potential on the second extraction plate through a voltage divider. A high voltage pulser built in-house and a Hewlett-Packard pulser (Palo Alto, CA) was used to generate the delayed extraction pulse. The length of the flight tube is 1 m. During operation, the extraction voltage was controlled at 20 kV DC and the extraction pulse and time lag were chosen to focus on the center of the analyte mass range. A nitrogen laser (model VSL 337ND, Laser Sciences Inc., Newton MA) with a 3-ns pulse width was used for desorption at 67.5° to the probe tip surface. Laser effluence was maintained slightly above ion detection threshold in all analyses. To reduce detector saturation for the polymers due to the high abundance of ionized low mass matrix molecules, most of the matrix ions were deflected away by a strong-pulsed electric field. A dual multi-channel plate (MCP) detector was used in the instrument for ion detection. MALDI mass spectra were recorded using a Hewlett-Packard MALDI data system. In general, 100 laser shots were summed to produce a final mass spectrum.

4.2.2 Data Analysis

The calculation of average molecular weights and polydispersity (M_n, M_w and PD) from the acquired GPC chromatography was performed using the Waters Maxima 820 software. In order to get accurate average polymer molecular weights, the polymer peak was divided into 150 slices. The average molecular weights (M_n and M_w) were calculated as followings:

$$M_n = \Sigma A_i / \Sigma (A_i / M_i) \tag{4.1}$$

$$M_w = \Sigma (A_i M_i) / \Sigma A_i \tag{4.2}$$

where A_i is the area of the ith slice and M_i is the molecular weight of the ith slice. The polydispersity, PD, was determined from the ratio of M_w to M_n.

The calculation of average molecular weights from the mass spectra data was processed using the Igor Pro software package (WaveMetrics, Lake Oswego, OR). Macros were written for this calculation. The average molecular weights are determined based on the following equations [10, 11]:

$$M_n = \Sigma (N_i M_i) / \Sigma N_i \tag{4.3}$$

$$M_w = \Sigma (N_i M_i^2) / \Sigma N_i M_i \tag{4.4}$$

where N_i and M_i represent signal intensity in peak area and mass for the oligomer containing i monomers, respectively. The polydispersity was also calculated from the ratio of M_w to M_n. Average molecular weights were corrected for the contribution of the cation (Ag^+).

4.2.3 Calibration

Narrow polydispersity polystyrene standards with nominal molecular weights: 2450, 3250, 5050, 9240, 11600, 22000 and 66000 were used for GPC calibration. Their concentrations and injection values were the same as the test polymer samples. Linear least square method was used for curving fitting. The calibration curve is shown in Fig. 4.1 and its coefficient of determination is 0.9995.

The MALDI-TOF instrument was calibrated using external calibrants. Bradykinin, bovine insulin b-chain and bovine ubiquitin were used for the calibration. The proteins were first dissolved in water and then stored in a freezer at -20° C. A solution containing a mixture of calibrants was prepared fresh. Sinapinic acid was used as the matrix. It was first prepared to 0.1 M in a solvent containing 60% CH_3CN, 36% MeOH and 4% H_2O and then mixed with an equal volume of the protein solution.

4.2.4 Samples and Reagents

Polystyrene standards with the following nominal molecular weights were used in this study: 2450, 3250, 5050, 7000, 9240, 11600, 22000, 28500 and 11600 (Showa Denko, Tokyo, Japan).). *All-trans* retinoic acid, used as the matrix for the analysis of polymers, was from Aldrich (St. Louis, MO). $AgNO_3$ was reagent grade and used without further purification. Tetrahydrofuran (THF) was HPLC grade and obtained from VWR (Toronto, Canada). Bradykinin, bovine insulin b-chain and bovine ubiquitin were obtained from Sigma (Milwaukee, WI). Water used as the solvent for calibration was from a NANOpure water system (Barnstead/Thermolyne). Sinapinic acid was purchased from Aldrich (St. Louis, MO).

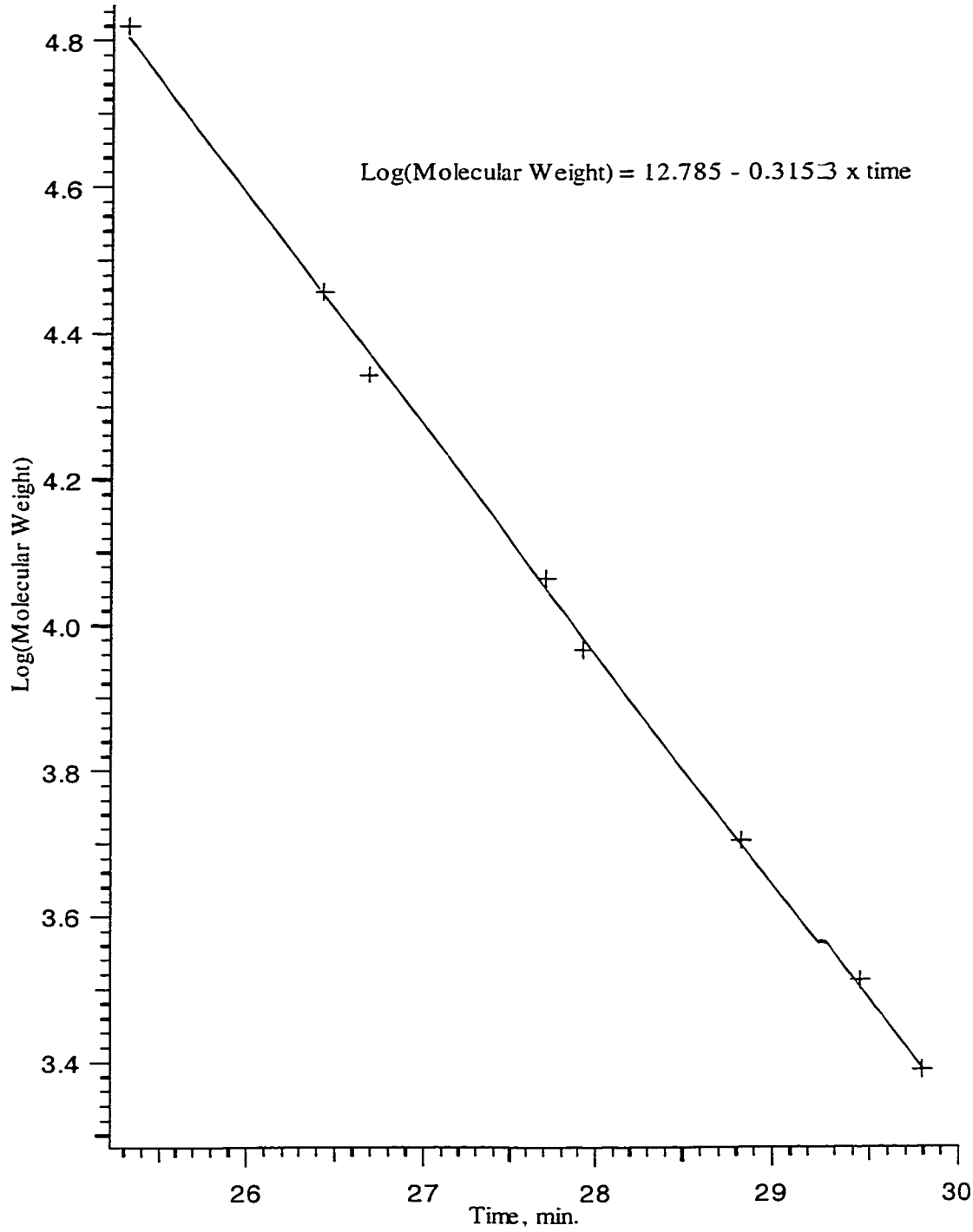

Figure 4.1 Calibration curve for gel permeation chromatography instrument.

4.2.5. Sample Preparation.

Polymer samples for GPC analysis were prepared by dissolving polymers in HPLC grade THF solvent. The concentrations of the polymers were controlled to be 0.05%. For mixture polymer analysis, all of the samples were prepared at the same time and then stocked in a fridge at 4°C. Before analysis, all of the samples were warmed up to room temperature.

Polymer samples for MALDI analysis were prepared by combining the analyte, matrix and cationization reagent in THF [10]. In a typical experiment, polymer stock solutions (1 mM, calculated from the nominal molecular weights) were diluted ten-fold with the *all-trans* retinoic acid matrix solution (0.15 M) plus 1% (v/v) of a 0.15 M $AgNO_3$ ethanolic solution. In the analysis, about 1 μL of the mixture was added to the MALDI probe tip and allowed to air-dry.

4.3 Results and Discussion

4.3.1 Comparison of MALDI-TOF MS and GPC Results

Two narrowly distributed polystyrene standards, PS 5050 and PS 7000 along with their mixtures with different proportions were used in this study. Table 4.1 summarizes the experimental results from MALDI-TOF MS and GPC. To quantify the uncertainty in the analysis, each sample was run in triplicate. The relative standard deviations were then calculated. As shown, there are systematic differences between the results of MALDI-TOF and those of GPC. MALDI always yielded higher average molecular weights (M_n and M_w) than GPC method does. The difference between M_n and M_w values can be as large as 720 and 427 Da, respectively. In addition, the polydispersity values from MALDI are much smaller than the GPC values.

These results are consistent with those reported in the literature. For instance, in their analysis of low molecular weight polystyrene and poly(methyl methacrylate) samples, Lloyd and coauthors also observed some difference in average molecule weights determined by MALDI and GPC [15], although the difference they found was not as large as our experimental results. This possible reason is the different samples used in these studies.

The variance between MALDI TOF MS and GPC can be mainly attributed to the different mass separation mechanisms underlying the two techniques, the band broadening effect and the accuracy of instrument calibration in GPC analysis. The principal separation mechanism of ions in MALDI TOF MS is based on their different mass to charge ratios with a MALDI detector to count the number of received ions. Therefore, the obtained spectrum reflects a number molecular weight distribution of a polymer. In contrast, in GPC analysis, molecular weight separation is according to their sizes. In general, the size separation is achieved by differential pore permeation. All molecules would experience a solute-to-wall exclusion effect inside the pore. Because of greater static interference, larger molecules are kept away from the wall of the pore and smaller molecules can approach the pore wall more closely. Under the influence of the solvent stream passing down the column, larger molecules are eluted from the column earlier than smaller ones. A GPC detector at the end of the columns determines the molecule concentrations. Therefore, the resulting chromatogram is a weight distribution of the polymer molecules as a function of retention time or volume. Since the calibration curve of the GPC instrument presents a linear relationship between the log molecular weight and the elution time, the obtained chromatogram is actually a weight distribution of the polymer as a function of log molecular weight. Consequently, GPC gives a distorted polymer molecular weight distribution as compared to the number based molecular weight distribution obtained from the mass spectra of MALDI-TOF MS. The average polymer molecular weights calculated from this chromatography are, of course, different from those obtained from MALDI-TOF MS spectra. Jackson *et al.* have used a

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Table 4.1. Calculated MALDI and GPC Results

Volume Percent of	MALDI					GPC					Difference		
PS 5000 in PS 7000	Mn	RSD	Mw	RSD	PD	Mn	RSD	Mw	RSD	PD	Mn(M)-Mn(G)	Mw(M)-Mw(G)	PD(G)-PD(M)
0%	7261	0.40%	7398	0.40%	1.019	6750	1.40%	7112	1.50%	1.054	511	286	0.0349
5%	7197	1.00%	7382	1.20%	1.025	6625	0.60%	7027	0.60%	1.061	572	355	0.0355
10%	7061	1.00%	7270	1.00%	1.029	6527	0.30%	6961	0.50%	1.066	534	303	0.0372
15%	7104	0.70%	7311	0.90%	1.029	6545	0.70%	6907	0.60%	1.063	559	404	0.0397
20%	7001	0.70%	7222	0.60%	1.034	6326	0.70%	6795	0.60%	1.074	675	427	0.0405
25%	6778	0.80%	7060	0.60%	1.041	6187	0.50%	6682	0.30%	1.080	591	378	0.0392
30%	6744	1.30%	7016	1.50%	1.057	6156	0.30%	6673	0.40%	1.084	588	343	0.0275
35%	6679	0.60%	6971	0.60%	1.043	6038	0.70%	6549	0.60%	1.085	641	422	0.0416
40%	6528	0.40%	6837	0.40%	1.047	5918	0.20%	6432	0.20%	1.087	610	405	0.0400
45%	6566	0.70%	6768	0.70%	1.046	5846	0.90%	6358	1.00%	1.088	720	410	0.0417
50%	6332	1.20%	6658	0.10%	1.051	5745	0.40%	6263	0.30%	1.090	587	395	0.0398

Note: Mn(M) = Mn (MALDI), Mw(M) = Mw(MALDI), Mn(G) = Mn(GPC), Mw(G) = Mw(GPC)

mathematical simulation to prove that the distorted polymer molecular weight distribution from GPC always provides about two more repeat unit masses (M_p) than the most probable peak value obtained by using MALDI TOF MS for narrow polydispersity polymers [16]. For wider polydispersity polymers, the difference between the two techniques will be much larger but also depend on the type of polymerization. M_p value can vary from the monomer mass in condensation polymerization to 1/2 M_n for addition polymerization [16].

Another important issue in comparing the results of MALDI-TOF MS and GPC is GPC band broadening. Band broadening is a phenomenon that is affected by many factors including the type of column, injector, detector, connecting tubes, solvent, flow rates and injection volumes. For example, longer columns always generate larger band broadening than short ones. So do the lower flow rates even though yielding better separation efficiency. Band broadening can cause the much broader chromatogram observed experimentally than the true polymer molecular weight distribution. As the chromatogram is on the log molecular weight scale, therefore it can cause large errors in polymer average molecular weight determination, especially enlarging the polydispersity of polymer. This is consistent with the experimental observations that the GPC method always has larger polydispersity than MALDI technique as shown in Table 4.1. Schriemer and Li and Jackson *et al* also noticed this phenomenon [15, 17]. In addition, the band broadening function in GPC analysis is nonuniform and time dependent. In the analysis of polymer molecular weight distribution, the smaller molecules come out of the column later than larger ones. Therefore, they will suffer larger band broadening effect than the larger ones, resulting in another large error in polymer molecular weight determination. When the polydispersity of a polymer becomes larger, much bigger errors will be produced because of the uneven band broadening distribution. Furthermore, the effects of individual factors on band broadening function are different. Some factors can produce Gaussian distribution function, while others may cause exponential band

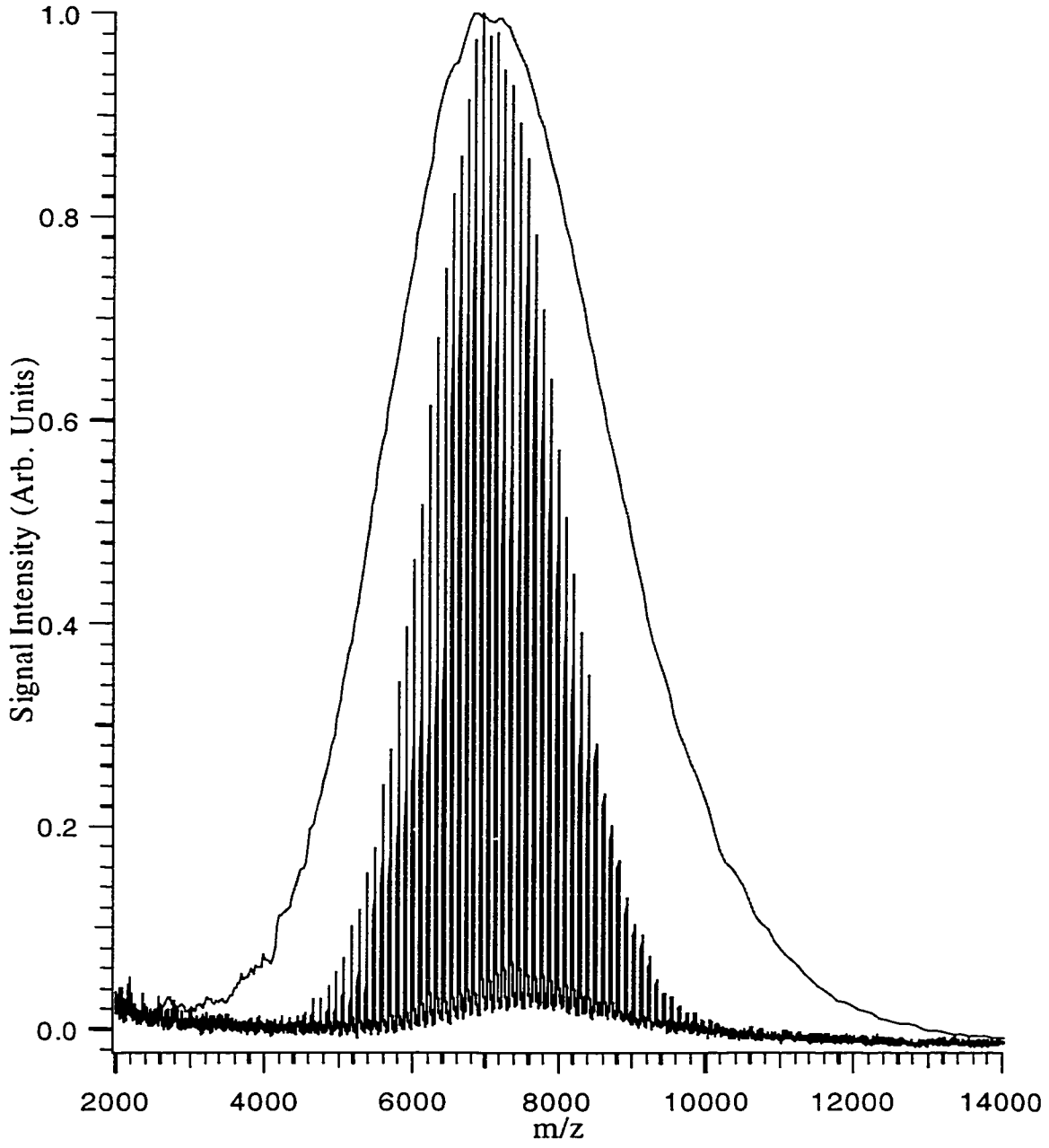

Figure 4.2. The comparison of GPC data and MALDI result for PS 7000

The calibration accuracy is another important issue in comparing the MALDI and GPC broadening function. This will further aggravate the complexity in the analysis of the results from GPC technique and cause more unidentified errors in molecular weight determination. Figure 4.1 shows a comparison of GPC data and MALDI result for PS 7000 results. As mentioned before, the separation of polymer molecules in GPC is based ontheir sizes and the resulting chromatogram is a weight distribution of the polymer as a function of retention volume or time. The shape of the chromatogram will be determined by the pore size distribution within the porous packing and the sizes of the polymer molecules in solution. If the theory of the separation mechanism were sufficiently precise and comprehensive, and if the nature of the pore structure and the distribution of pore sizes could be determined accurately experimentally, a molecular size distribution could be calculated from an observed chromatogram for a polymer. Unfortunately, this procedure is not always practical at present. Thus, GPC instrument must be calibrated with known molecular weight standards. However, obtaining polymer standards with known molecular weights are not always possible for many polymers. In some cases, the molecular weights of standards provided by manufacturers might be incorrect. For example, polymer characterization techniques such as light scattering and membrane osmometry will give an average molecular weight with an error of about 5% in most cases. Depending on the technique and molecular weight, this error could be even higher [18]. In some extreme cases, the calibration standards provided by manufacturers can have an error of as high as 20% [19, 20].

In summary, direct comparison of the average molecular weights determined by MALDI-TOF MS and GPC methods must be exercised with great care. Therefore, it is not recommended to use GPC results to evaluate the experimental values from MALDI-TOF MS although it was suggested and used by several researchers [15,17, 21-23].

4.3.2 Band Broadening Function

The uncertainness of the band broadening effect has raised a major concern about the accuracy of the GPC results for polymer analysis. Since the invention of the GPC

technique, a great deal of research on how to correct the obtained chromatogram have been conducted [25-30]. In order to better understand the applicability of these approaches, it is important to know the various sources and influence of band broadening in GPC analysis. It is generally recognized that band broadenings consists of column band broadening and extracolumn band broadening. Column band broadening occurs in column and is composed of interstitial band broadening and pore band broadening. Mixing (diffusion and convective) in the mobile phase within the packed column can produce interstitial band broadening. The transfer of solute between the mobile and stationary phases in the columns can cause pore band broadening. These two types of band broadening are independent and additive. For large molecules, the principal contribution to band broadening is interstitial band broadening because the molecules are too large to permeate the pores. For small molecules, on the other hand, pore band broadening would become predominant because a large percentage of the pores are penetrated. For medium molecules, interstitial and pore band broadening will both contribute significantly because of their partially penetration characteristics. Extracolumn band broadening is associated with injection valve, connecting tubes and detector cell, etc. When the analyte goes through them, mixing in the mobile phase can produce extracolumn band broadening. Since GPC instruments generally involve several columns, the extracolumn band broadening is relative much smaller than the column band broadening.

There are many processes which will cause band broadening in GPC analysis. Among them the most important include longitudinal diffusion, eddy diffusion, flow velocity variations caused by nonuniform packing, slow radical diffusion and the mass transfer resistances in the stationary phase, the mobile phase and the stagnant mobile phase. Some of these processes can cause symmetrical peak band broadening such as longitudinal diffusion and eddy diffusion, whereas others can produce unsymmetrical band broadening such as flow variations or slow solute exchange between the phases.

Mathematically, the instrumental band broadening can be determined by the following Tung's integral equation:

$$f(v) = \int_{-\infty}^{+\infty} g(v - y)w(y)dy \qquad (4.5)$$

where f(v) is the obtained experimental chromatogram, w(y) is the true polymer oligomer distribution, g(v-y) is the band broadening function, y is the mean elution volume of individual species and v is the elution volume variable [24]. The difference between y and v is that due to instrumental band broadening, some fraction of polymer that would elute at y in its absence actually elutes over a range of v.

In order to eliminate the effect of band broadening, a number of theoretical approaches have been developed to correct GPC data with analytical solution of the Tung's equation [25-30]. All of these approaches require to assume an appropriate band broadening function with determination of numerical values for its parameters and then to solve the equation with different mathematical manipulations. For example, Tung assumed that the band broadening function is a Guassian distribution function and then applied polynomial method to solve the equation [25]. Obviously, his assumption is inadequate because not the all the band broadening processes would result in symmetrical dispersion. Smith proposed a log-normal spreading function to take unsymmetrical band broadening effect into account [26]. Although this band broadening function is better than the Gaussian distribution, it is still not sufficient to represent the real band broadening function. Pickett and coworkers attempted to account for the unsymmetrical band broadening function using a function obtained from the chromatogram shapes of narrow polydispersity polymer standards [28]. Unfortunately, this technique overestimated the band broadening function because the narrow polydispersity polymer standards used are not truly monodisperse. Other band broadening functions were also tested, for example, Hess and Kratz's plug flow dispersion model [29] and Provder and Rosen's [30] general statistical band broadening model. All of these models could achieve limited satisfaction.

Nevertheless, the predictions based on these assumed band broadening functions always deviate from those obtained from GPC measurement. Consequently, the efforts on how to find a better band broadening function are still continuing.

In this study, we will introduce a new approach to find the band broadening function for GPC analysis. As demonstrated in Chapter 2, the home built MALDI TOF instrument can provide molecular weight and molecular weight distribution information with high precision and accuracy for the analysis of narrow polydispersity polystyrene standards when an appropriate sample preparation protocol was followed. The experiment was designed to use the MALDI TOF to determine the true molecular weight distribution of a polymer within the error of MALDI (<1%). With the known molecular weight distribution obtained from MALDI analysis, the band broadening function was determined by using function convolution to fit to the obtained GPC chromatogram. In the calculation, the GPC chromatogram data had already been transformed to a number molecular weight.

As discussed in the early part of this chapter, the mechanism of MALDI-TOF MS separation is based on separation of ions according to their mass to charge ratios, therefore the acquired spectra is a number molecular weight distributed mass spectra. This number molecular weight distributed mass spectra presents the real molecular weight distribution in the process of polymerization. For a very narrow distributed polystyrene sample, this distribution can be approximated by a Guassian distribution due to its polymerization kinetics and separation mechanism [31]. The symmetric molecular weight distribution can be well fitted to the Guassian distribution and the resultant Gaussian distribution has the following function:

$$w(y) = \exp[-((y - 7145.45)/1296.03))^2] \qquad (4.6)$$

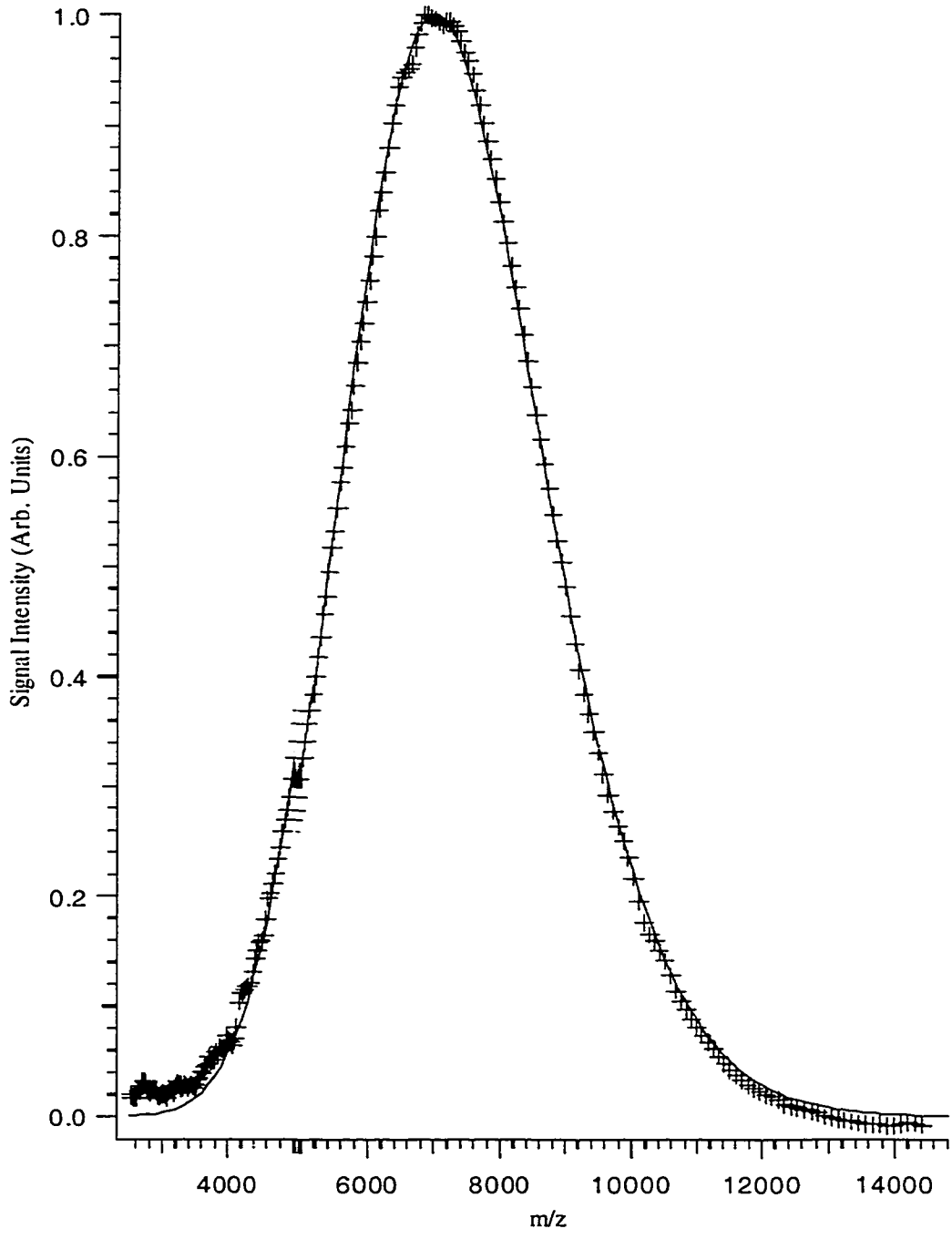

Figure 4.3. The result (solid line) of modeling of GPC data (plus) by convolution of band broadening and MALDI molecular weight distribution functions for polystyrene 7000.

The transformed GPC data was considered to follow the exponentially modified Guassian function because of its unsymmetrical and tailing caused by the intracolumn and extracolumn band broadening processes. This function has been generally accepted as an accurate model for evaluating the chromatographic peaks [32] and was obtained through the convolution of the Guassian function and an exponential decay function. After mathematical derivation, the band broadening function in terms of the number based molecular distribution for polystyrene 7000 was found to be:

$$g(v-y) = \mathrm{erf}((v-y)/7070) \times \exp(-((v-y)/2100)^{1.47}) \qquad (4.7)$$

As shown in Figure 4.3, the predicted intensity by convolving this assumed band broadening function together with the MALDI-TOF molecular weight distribution function fitted well to the number distributed GPC chromatograph for polystyrene 7000.

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CHAPTER 5. CONCLUSION AND FUTURE WORK

Based on the above experimental results, the following conclusions and the recommendations for future work can be drawn:

1. For the analysis of narrowly distributed polystyrene polymers (PS 5050 and PS 7000), when using *all-trans* retinoic acid as matrix with a matrix to analyte ratio of 1350 no asymmetric spectra distortions were found. Symmetric distortion was also not found in the analysis of PS7000 polymer. However, when a small amount of LiOH was added to the analyte solution, the mass spectra were truncated. Spectra sensitivity and reproducibility were poorer. Significant molecular weight difference was found using two different sample preparation methods. An evaluation of the effect of LiOH addition on crystal formation should be conducted in future with the use of confocal microscopy technique. It might provide more valuable information to better understand the spectra truncation. The methodology used in this study (Chapter 2) could be applicable to the study of other polymer systems.

2. Accurate determination of polymer molecular weight was affected by instrumental configuration. It was found that instrument design affect spectrum sensitivity, resolution and appearance. Low sensitivity instrument can introduce spectra truncation, especially at the lower mass range of the study because of the interference of matrix peaks. Due to the difference in spectrum sensitivity and resolution, the variation in polymer molecular weight was found among three different designed instruments. Large molecular weight differences had been found between the low- and high-resolution spectra. At high mass range, multimer formation was detected.

3. The determination of polymer molecular weights can also be affected by laser power and time-lag focusing point. It was observed that the resolution of spectrum decreased as the laser power increased. The largest difference between the lowest and

highest resolution spectra was found to be 500 Da for PS 7000 analysis. This difference partially results from an overestimation of oligomer peak areas over the mass range of low oligomer resolution. As well, different polymer molecular weights were obtained at different time-lag focusing points because of the changed oligomer resolution.

4. Polymer molecular weight determination also depends on the use of proper data analysis method. It was found that the peak area calculated only from the Ag^+-attached oligomer peaks resulted in different molecular weights as compared to the peak areas calculated from both the Ag^+-and Na^+-attached oligomer peaks. Other polymer molecular weight determination methods, such as those suggested by Kratos and BRUKER Proflex instruments, should be investigated in future in order to better understand their impact on molecular weight determination.

5. The comparison of MALDI and GPC results shows that the polymer molecular weights obtained from MALDI are always higher than those from GPC method, however, MALDI will exhibit smaller polydispersity than GPC method. It was concluded that these discrepancies are mainly attributed to different mass separation mechanisms, the band broadening effect and the accuracy of instrument calibration in GPC analysis.

6. A new approach was used to analyze the band broadening function associated with GPC analysis by using the MALDI-TOF molecular weight distribution function as true molecular weight distribution. The applicability of this method should be further verified for other narrowly distributed polymer systems in the future.