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ANALYSIS OF DISPERSANTS IN GASOLINE BY MASS SPECTROMETRY

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งานวิจัยนี้เกี่ยวข้องกับการพัฒนาเทคนิคสำหรับการวิเคราะห์สารช่วยกระจายตัวโดยใช้
เทคนิคแมสสเปกโทรเมทรีแบบเมทริกซ์แอสซิสเทดเลเซอร์ดีซอร์พชันไอออนไนเซชัน (MALDI-
MS) การวิเคราะห์ทำได้โดยการผสมสารช่วยกระจายตัวกับเมทริกซ์ชนิดต่าง ๆ เช่น ไดทรานอล
2,5-ไดไฮดรอกซิเบนโซอิกแอซิด อัลฟาไฮดรอกซิไซยาโนซินนามิกแอซิด 2-(4-ไฮดรอกซิเฟนิลอะ
โซ)เบนโซอิกแอซิด และ ออลทรานสเรติโนอิกแอซิด เมื่อนำของผสมของสารช่วยกระจายตัวและ
เมทริกซ์ใส่ลงในเป้าหมายแล้วทำการยิงของผสมนั้นด้วยเลเซอร์เพื่อให้สารตัวอย่างกลายเป็น
ไอออน พบว่าสภาวะต่อไปนี้ให้แมสสเปกตรัมที่ดีซึ่งแสดงถึงการกระจายน้ำหนักโมเลกุลของสาร
ช่วยกระจายตัว: ใช้ 2,5-ไดไฮดรอกซิเบนโซอิกแอซิด โดยมีเททระไฮโดรฟูแรนเป็นตัวทำละลาย
อัตราส่วนของสารละลายสารช่วยกระจายตัวต่อสารละลายเมทริกซ์เท่ากับ 1:50 โดยปริมาตร
และใช้พลังงานเลเซอร์ที่ 190 ไมโครจูล แมสสเปกตรัมที่ได้ให้ข้อมูลการกระจายน้ำหนักโมเลกุล
ของสารช่วยกระจายตัว 4 ช่วงมวลคือ ช่วงมวล 200-600, 390-1000 และ 520-1260 ซึ่งสารช่วย
กระจายตัวใน 3 ช่วงมวลนี้เป็นสารช่วยกระจายตัวชนิดโพลีไอโซบิวทีนิลเอมีน และช่วงมวล
1200-2300 ซึ่งเป็นสารช่วยกระจายตัวชนิดโพลีอีเทอร์เอมีน จากนั้นได้ใช้วิธีที่พัฒนาขึ้นนี้ในการ
วิเคราะห์สารช่วยกระจายตัวในตัวอย่างน้ำมันแกโซลีนจาก 8 บริษัทที่มีจำหน่ายในท้องตลาด ผล
การวิเคราะห์ที่ได้แสดงให้เห็นว่าน้ำมันแกโซลีนจากบริษัทต่าง ๆ มีชนิดและการกระจายน้ำหนัก
โมเลกุลของสารช่วยกระจายตัวที่แตกต่างกัน โดยชนิดของสารช่วยกระจายตัวที่พบในน้ำมันแกโซ
ลีนคือโพลีไอโซบิวทีนิลเอมีนและโพลีอีเทอร์เอมีน

สาขาวิชา ปิโตรเคมีและวิทยาศาสตร์พอลิเมอร์ ลายมือชื่อนิสิต ....
ปีการศึกษา 2547   ลายมือชื่ออาจารย์ที่ปรึกษา ....
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SUTHASINEE RAUNGWINYOUWETCH: ANALYSIS OF DISPERSANTS IN GASOLINE BY MASS SPECTROMETRY. THESIS ADVISOR: ASSOC. PROF. AMORN PETSOM, Ph.D., THESIS COADVISOR: ASSIST. PROF. POLKIT SANGVANICH, Ph.D., 103 pp. ISBN: 974-17-6702-1.

This research involves the development of a method for analysis of dispersants, using matrix assisted laser desorption ionization-mass spectrometry (MALDI MS). The MALDI-MS analysis was carried out by mixing the dispersants with various types of matrix such as dithranol, 2-5-dihydroxybenzoic acid, 2-(4-hydroxyphenylazo)-benzoic acid and *all-trans* retinoic acid. The dispersants-matrix mixture was applied to the target and bombarded with laser to generate ionic analyte. The following MALDI-MS condition were found to provide the good MS spectrum of molecular weight distribution of the dispersants: DHB as a matrix with THF as a solvent, the dispersants solution: matrix solution of 1:50 by volume, and the laser power of 190 µJ. The MALDI MS spectrum gave the information of dispersants molecular weight distribution in 4 range of m/z. Dispersants in m/z range of 200-600, 390-1000 and 520-1260 were polyisobutenylamine and dispersants in m/z range of 1200-2300 were polyether amine. The developed MALDI-MS was used for characterization of dispersants in the commercial gasoline from 8 companies. The results showed that dispersants which added in commercial gasoline from each companies were different in type and molecular weight distribution. The types of dispersants which found in commercial gasoline were polyisobutenylamine and polyether amine.

Field of study: Petrochemistry and Polymer Science   Student's signature ....

Academic year: 2004   Advisor's signature ....

Co-advisor's signature ....

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LIST OF ABBREVIATIONS

CI	=	Chemical Ionization
cm	=	centimeter
Da	=	Dalton
EI	=	Electron Impact
ESI	=	Electrospray Ionization
FAB	=	Fast Ion or Atom Bombardment Ionization
FTIR	=	Fourier Transform Infrared spectroscopy
FTICR	=	Fourier Transform Ion Cyclotron Resonance
GPC	=	Gel Permeation Chromatography
HPLC	=	High Performance Liquid Chromatography
IR	=	Infrared
kV	=	kilovolt
LS	=	Light Scattering
MALDI	=	Matrix Assisted Laser Desorption Ionization
mL	=	millilitre
mmHg	=	millimeter of Hg
MS	=	Mass spectrometry
m/z	=	mass to charge ratio
nm	=	nanometer
NMR	=	Nuclear Magnetic Resonance spectroscopy
PEG	=	Polyethylene glycol
PIB	=	Polyisobutylene

PMMA	=	Polymethyl methacrylate
PPG	=	Polypropylene glycol
PS	=	Polystyrene
SEC	=	Size Exclusion Chromatography
TOF	=	Time of Flight
TSP	=	Thermospray Ionization
UV	=	Ultraviolet
VPO	=	Vapor pressure Osmometry
(V/V)	=	volume by volume
µJ	=	microjoule
µL	=	microlitre

CHAPTER I

INTRODUCTION

The purpose of investigation

Gasoline additives have grown in importance in recent times for a number of reasons. Modern engines need to maintain their tune more closely and for longer durations in order to meet the stringent emissions and economy requirements. Gasoline have become more of the barrel is cracked into gasoline fuel product, and this has led to the presence of more olefins, which are oxidative reactive species. Fuels may also contain oxygenate blending components and these can bring added problems of corrosion and deposits. Finally, it is realized that additives can continue to provide marketers with a basis for product differentiation and, in a world of competitive marketing coupled with base fuel agreements, such uniqueness is greatly valued.

Dispersants is one of the important additives in fuel oil. It plays a major role in fuel oil to provide fuel injector, carburetor and valve cleanliness. It has a long chain hydrocarbon with polar ends containing oxygen and/or nitrogen. In engine oil, the harmful products of combustion and other contaminate are renders harmless by the polar ends and the long chain hydrocarbon of this additive helps to solubilizer suspend the debris in oil [1].

Previous techniques for determination of dispersants in gasoline include HPLC and GPC [2], electrospray ionization (ESI) [3]. Because of the ions which occur from ESI are multiplet charge species consequently the interpretation from spectra is complicated. In this research, matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS) was used for analysis of dispersants. MALDI-MS produces only singlet charges therefore it was much easier in interpretation. MALDI-MS was shown to be very useful tool for polymer characterization. It was used to determine average molecular weight distributions of polymers, and to provide structural information on end group, repeating unit and chemical modification of polymeric system. However, the success of this technique

for polymer analysis depends on the availability of a suitable sample/matrix preparation.

Objectives

The objective of this research is to develop mass spectrometric method for analysis of dispersants in gasoline.

Scope of research

In initial step, dispersants will be separated from gasoline additive package. The MALDI-MS conditions were studied for determination of molecular weight distribution of dispersants. Various factors effecting the analysis of dispersants would be determined. After that, a developed method will be used to determine polymeric dispersants in commercial gasoline.

CHAPTER II

THEORETICAL AND LITERATURE REVIEWS

2.1   Gasoline Additive [4]

Gasoline is a complex liquid mixture of hydrocarbon of four basic types: paraffins, olefins, naphthalenes, and aromatics, differing in their properties according to the number and agreement of carbon and hydrogen atoms in the molecule. Gasoline, having a boiling range of 38-200 °C is derived from crude petroleum by refining and contains 5-12 carbon atoms in each molecule.

There are a large number of additives that can confer benefits before the fuel reaches the combustion chamber of an engine. These may be added to ensure that the gasoline does not deteriorate on storage that it is free from problems during distribution and that deposit formation and corrosion do not occur in the fuel system of an engine. They may also be required for marketing or legal reasons.

The need for these additives has been growing for many years because of changes in gasoline composition arising from three main causes:

1. *Crude oil price changes*. Although crude oil prices have been rather volatile in recent years it is generally accepted that, in the longer term, because of the limited nature of crude oil reserves, they must increase very significantly. After the first oil crisis in 1973, prices rose rapidly for several years, and this had the effect of considerably reducing the use of fuel oil in power stations, steelworks, shipping, etc. in favor of other energy sources such as coal, hydroelectric and nuclear. The resulting excess of fuel oil left the oil companies with a severe imbalance in their product requirement, and there has been a massive move towards converting this heavy material to lighter products such as gasoline and middle distillates. This trend is expected to continue for several years to come.

   The conversion of heavier to lighter streams involves cracking the large hydrocarbon molecules and, unless accompanies by hydrogenation, it

inevitably means that olefinic compounds are formed. Olefins are much more reactive than other hydrocarbons present in gasoline and oxidize polymerize readily to form gummy deposits. These deposits upset the metering of fuel and air into the combustion chamber and, because of this, can cause vehicle driveability malfunction. They can also result in sticking of valves and some other moving parts.

2. *Measures to improve exhaust gas emissions*. The need to reduce exhaust emissions of hydrocarbons, carbon monoxide and nitrogen oxides has been recognized for many years, and legislation to control these emissions has become increasingly restrictive and severe. At present, in many countries it is necessary to use exhaust catalyst systems in new car models to achieve the required levels, and this has meant that unleaded fuel is required. Concerns about the toxicity of lead itself have also played a large part in forcing the introduction of unleaded fuels.

Compared with leaded, unleaded gasoline needs higher octane quality components in order to achieve the specified octane levels and this has given rise to major changes in composition. These, in turn, have increased the need for additives in many cases. The use of oxygenated components, for example, has meant that gasolines are more likely to cause corrosion problems and therefore have a greater need for corrosion inhibitors. Some oxygenates also increase the dissolved metals present in gasoline because they are corrosive to such metals as aluminium, magnesium, etc. Since these metals catalyze oxidation reactions there is a greater need for metal deactivators and antioxidants to counteract this problem. More severe catalytic reforming can give rise to heavy aromatic molecules which, together with any gums present, can lay down as deposits in the fuel intake system, needing detergents/dispersants to prevent excessive build-up.

The use of exhaust gas recirculation to reduce nitrogen oxide emissions can also worsen intake system deposits, since the exhaust gases contain particulates such as partially oxidized hydrocarbons which can deposit on surfaces in the intake system. PCV (positive crankcase ventilation) valves also

operate in an environment that makes them susceptible to sticking unless suitable additives are present in gasoline.

Port fuel injectors and more sophisticated carburetors, to provide better control of air/fuel ratios under all driving conditions; rely on absence of deposits to maintain their performance over the life of the vehicle. Again, detergent/dispersants additive in fuel can play a large part in helping to achieve this end.

In the USA, vehicles must be capable of meeting the stipulated exhaust emission requirements without adjustment over a distance of 50,000 miles. Under these conditions it is extremely important that gasolines do not lay down deposits which will degrade the quality of the exhaust emissions. The use of appropriate gasoline additives helps to ensure that this need is met.

3. ***Requirement to improve fuel consumption***. Fuel economy is an extremely important marketing aspect for motor vehicles and has been so ever since the price of fuel started to rise in 1973. The amount of fuel consumption control required by governments varies throughout the world. In European there is no legislation for mandatory reductions in fuel consumption, although voluntary agreements have been made by motor manufactures in several countries and there is a requirement that fuel consumption data be published. In the USA and Japan there is enforcement of staged improvements in fuel consumption

The motor industry continues to make considerable progress in improving the fuel economy of vehicles. Two important steps that they have taken and which are influenced by additives are to maximize compression ratios as far as possible, consistent with fuel octane quality, and to use leaner mixture strengths.

High compression ratios improve the thermal efficiency of an engine but at the same time increase its octane requirement. Changes in gasoline composition are necessary to achieve an acceptable octane level in the absence of lead, as has already been discussed, and this can require a greater use of additives. Leaner mixture strengths to improve fuel economy tend to make a vehicle more prone to driveability malfunctions and hence more critical to anything that leans the mixture strength still further, such as oxygenates.

Again, additives have been uses to reduce mixture maldistribution between cylinders, which is particularly important under lean mixture conditions. They are also uses to ensure that deposits in critical parts of the system are not allowed to build up.

Thus it can be seen that precombustion gasoline additives can play an extremely important part in ensuring that vehicle performance is maintained as mileage is accumulated, in spite of pronounced changed in gasoline composition and vehicle design.

2.2 Dispersants Gasoline Additive [1,4-7]

Dispersants are non-metallic or ashless cleansing agents. The structure of ashless dispersants is similar to the structure of a detergent in that the dispersants has a hydrocarbon tail or oleophilic group which enables the dispersants to be fully soluble in the base fuel used. The dispersants also has a polar head. The polarity of a dispersants is derived from the inclusion of oxygen, phosphorous, or nitrogen atoms into molecule. Figure 2.1 shows the stylized structure of an ashless dispersants.

Polar Head	Hydrocarbon Tail
Oxygen and/or	Oleophilic Group
Nitrogen Atom	Solubilizer
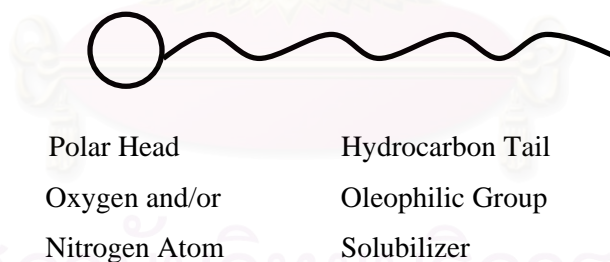
Figure 2.1 Stylized dispersants

The dispersants are used in gasoline fuel to provide fuel inject, carburetor, and valve cleanliness. Engine life is prolonged and undesirable engine exhaust emissions are reduced. The terms 'detergent' and 'dispersants' are often used interchangeably because both additive types keep insoluble combustion debris and oxidation products dispersed with the fuel. Detergents are normally utilized to minimize high-temperature engine varnish and lacquer deposits while dispersants are used to control low-temperature engine sludge deposits. In addition to the cleansing action, the dispersants have the ability to disperse particulate mater in an extremely fine and harmless state

and hold it in suspension. In this way it will pass through the fuel system into the combustion and other contaminants are rendered harmless by the polar ends of dispersants. The hydrocarbon chain of dispersants helps to solubilize or suspend the debris.

The first dispersants additive, used at very low levels, were long chain amides. They were introduced in the mid 1950s. Alkenyl succinimides were then introduced as dispersants in crankcase lubricants toward the end of the 1950s, a short time after they were tried as dispersants additive in gasoline. In 1970's increased conversion of crude to transportation fuels resulted higher amounts of high olefin cracked stocks being used in gasoline blending. The quality of the gasoline, and in particular olefin content where higher levels can lead to a reduction in fuel stability, plays a big part in the treatment required for particular batches of fuel. This also corresponds to a time of increased susceptibility to performance problems due to fuel-derived deposits in engines tuned to meet tighter emissions requirements. Polyolefin amines were introduced during this time as being more effective deposit control agents. More recently, further developments have lead to the introduction of polyether amines (mainly in North America, Korea and Japan) and combinations of alkenyl succinimide or polyolefin amines with polyether based carrier fluids for improved cost-effectiveness.

Deposits are laid down in the pre-combustion are of the fuel inlet system of all vehicles. The nature of the deposit can be sticky, soft or hard, and its position in the inlet system is a function of a large number of a large number of factors. These include the degree of oxidation/polymerization, storage temperature, and contamination by blow and exhaust gas recirculation. Deposits can also be derived from the crankcase lubricant. All of these deposits can affect the regulated and unregulated emissions of the vehicle, and its fuel economy, other impacts are to startabilty, idling and power.

The mechanism of the dispersants interaction with sludge was shown in Figure 2.2. The polar groups in the dispersants preferentially attach themselves to metal surface and to any particulates present since these also have a polar nature. The non-polar part of the molecule sticks out into the fuel hydrocarbons so that a monomolecular film is formed on the surfaces, preventing deposition and particle aggregation. Particles are thus carried on into combustion chamber and burnt. Deposits that already been laid down can gradually be removed by this mechanism, which, in effect, solubilized them.
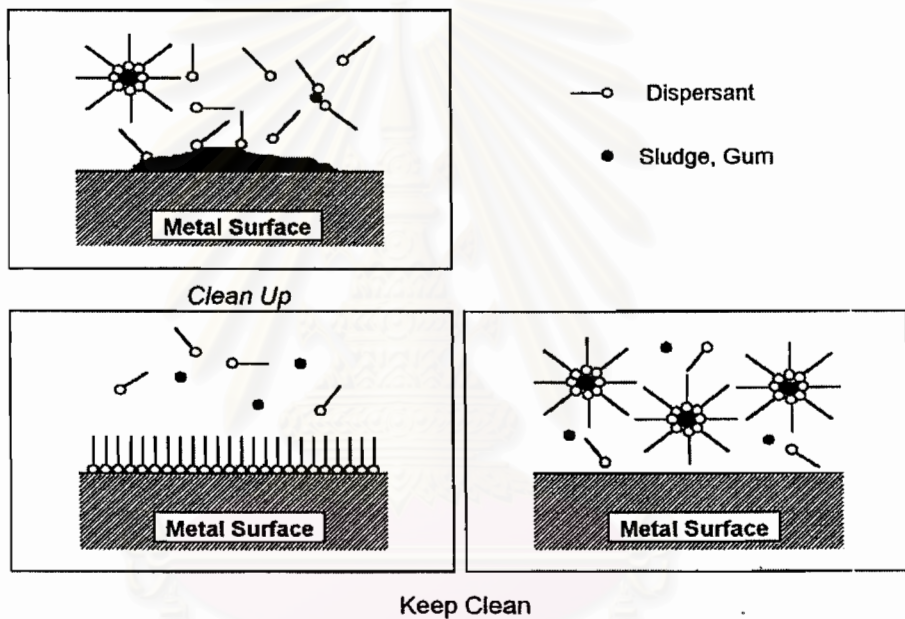
Figure 2.2 Sludge dispersion

Chemically, dispersants are relatively high molecular weight materials and can be divided into three chemical groups, polybutene succinimides, polybutene amines and polyether amines (Figure 2.3). The concentration of dispersants added to fuel is 20-600 ppm.

polyisobutenylsuccinimide

polyisobutenyl amine

polyether amine
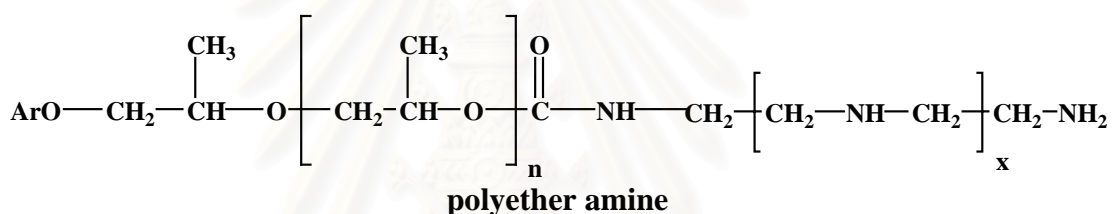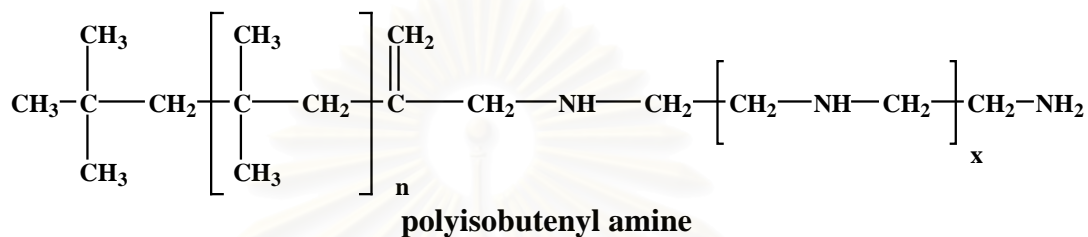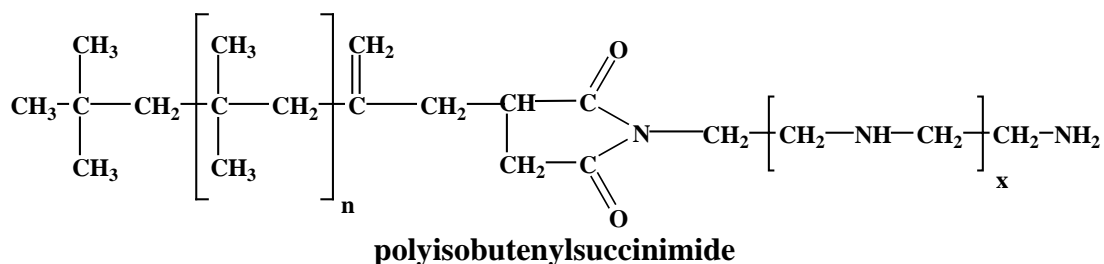
Figure 2.3 Three types of gasoline dispersants

2.3 Mass Spectrometry (MS) [8-12]

Mass spectrometry probably is the most versatile and comprehensive analytical technique currently at the disposal of chemists and biochemists. Since the early 1900s, it has enjoyed prominence in several areas of physics, chemistry, geology, cosmochemistry, nuclear science, material science, archeology, petroleum industry, forensic science, and environmental science. The ultrahigh detection sensitivity and high molecular specificity are the hallmarks of this technique. Molecular mass determination, structure elucidation, quantification at trace levels, and mixture analysis are some of the major applications of mass spectrometry. In addition, the technique has been used to study ion chemistry and ion-molecule reaction dynamics; to provide data on physical properties such as ionizing energy, appearance energy, enthalpy of a reaction, and proton affinities; and to verify theoretical predictions that are based on molecular orbital calculations.

2.3.1 Basic Concept of Mass Spectrometry

Mass spectrometry is an analytical technique that measures the masses of individual molecules and atoms. As conceptualized in Figure 2.4, the first essential step in mass spectrometry analysis is to convert the analyte molecules into gas-phase ionic species because one can experimentally manipulate the motion of ions, and to direct them (which are not possible with neutral species). The excess energy transferred to the molecule during the ionization event leads to fragmentation. Next, a mass analyzer separates these molecular ions and their charged fragments according to their m/z (mass/charge) ratio. The ion current due to these mass-separated ions is detected by a suitable detector and displayed in the form of a mass spectrum. To enable the ions to move freely in space without colliding or interacting with other species, each of these steps is carried out under high vacuum (10^{-4}-10^{-8} torr).
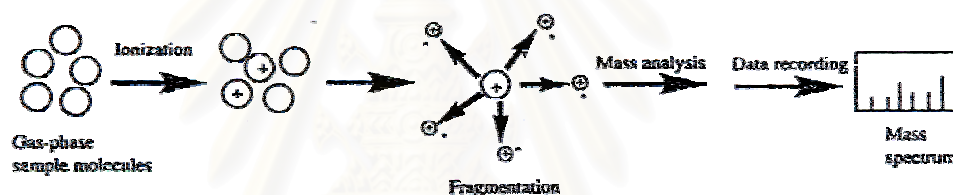
Figure 2.4 Basic concept of mass spectrometry analysis

Thus, a mass spectrometer consists of several essential functional units; they are depicted in Scheme 2.1 in the form of a block diagram. These units are:

- An inlet system to transfer a sample to the ion source
- A vacuum system to maintain a very low pressure in the mass spectrometer
- An ion source to convert the neutral sample molecule into gas-phase ions
- A mass analyzer to separate and mass-analyze ionic species
- A detector to measure the relative abundance of the mass-resolved ions
- Electronics to control the operation of various units
- A data system to record, process, store, and display the data

The overall analytic capability of a mass spectrometry system depends on the combined performance of these individual units. Several ionization techniques and mass analyzer have emerged, each with special purpose.
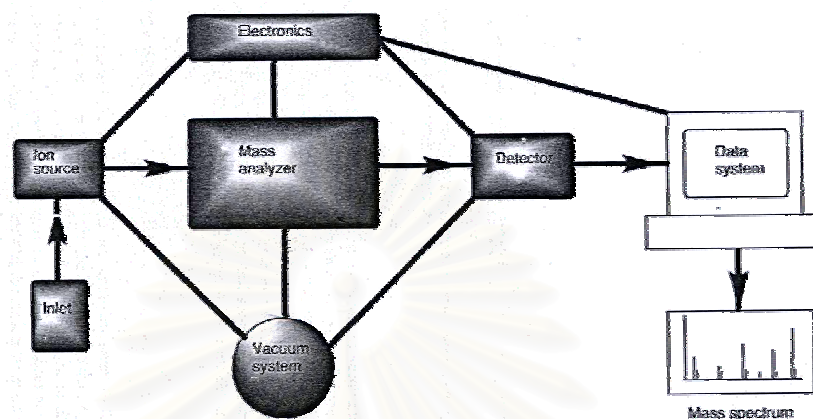
Scheme 2.1   Basic component of a mass spectrometer

Inlet systems

There are several means of introducing samples into the mass spectrometer and the inlet system used will normally depend on the volatility and nature of the sample, the task in hand, and the method of ionization (in particular, the gas pressure in the ion source). The introduction of samples into a mass spectrometer is not a trivial task because, in the most common instruments, the whole system must be maintained at very low pressure (high vacuum) to allow unrestricted movement of ions. The examples of inlet system are

- Batch gas and vapor inlets
- Direct probe inlets
- Membrane interfaces
- Gas or liquid chromatographic inlets

Ionization source

An ion source may be defined simply as the region in which ionization occurs. The region is usually enclosed in a small ion chamber in which the sample is ionized. The ions produced are propelled out of the chamber towards an exit slit by a low positive potential applied to a 'repeller' plate. On leaving the ion chamber of magnetic sector mass spectrometer, the ions are accelerated through a high potential of 2-8 kV

and passed into the analyzer for separation according to mass-to-charge ratio. The most common ionization sources uses in analysis today are below

- Electron Impact (EI)
- Chemical Ionization (CI)
- Fast atom/ion bombardment Ionization (FAB)
- Thermospray Ionization (TSP)
- Electrospray Ionization (ESI)
- Matrix-Assisted Laser Desorption/Ionization (MALDI) etc.

Mass analyzer

Mass analyzers disperse ion space or time according to their mass-to-charge ratios (m/z). Certain analyzers separate the ions simultaneously, while others are scanned to transmit to the detector a narrow m/z range at a given time. The examples of mass analyzer are below

- Quadrupolar Analyzers
- The Quadrupole Ion Trap or Quistor
- Time-of-Flight Analyzers
- Magnetic and electromagnetic Analyzers

Detectors

The detector allows a mass spectrometer to generate a signal (current) from incident ions, by generating secondary electrons, which are further amplified, or by inducing a current generated by a moving charge. The most common types of detector are below

- Photographic plates and faraday cylinders
- Electron Multipliers
- Array Detectors
- Photon Multipliers

For this research, the ionization technique which was chosen for analysis of dispersants is Matrix-Assisted Laser Desorption/Ionization and the mass analyzer is Time-of-flight. The principles of these techniques were explained, respectively.

2.3.2 Matrix-Assisted Laser Desorption Ionization (MALDI)

The irradiation by an intense laser beam is another suitable mode of depositing a large amount of energy into sample molecules for their desorption into gas phase. In early mass spectrometry applications of lasers, the sample was irradiated directly by a laser beam. Infrared (IR) laser, the pulsed CO_2 laser, and UV lasers were used. In this direct mode, termed laser desorption/ionization (LDI), the extent of energy transfer is difficult to control and often leads to excessive thermal degradation. Another pitfall of the direct mode is that not all compounds absorb radiation at the laser wavelength. As a consequence, the direct LDI mode is applicable to a limited number of compounds, usually those with molecular mass < 1000 Da.

Currently, LDI is practiced in the MALDI mode. Two research group, Karas and Hillenkamp and Tanaka and co-workers, nearly revolutionized the approached to the study of large biopolymers. This development has provided a unique opportunity to apply mass spectrometry to the analysis of proteins and other biomolecules with masses in excess of 200 kDa. The key to the pioneer work, Karas and Hillenkamp used nicotinic acid as the matrix, whereas Tanaka and co-workers embedded the sample in the slurry of finely divided platinum (10 nm size) in glycerol.

In practice, the sample (guest) is admixed with an excess of the host matrix material, and is irradiated with a laser beam of short pulses of 10-20 ns duration and ~10^{-6} W/cm^2 irradiance power. The main criterion of choosing a host matrix is that it absorbs energy at the wavelength of the laser radiation. This matrix-wavelength combination permits a large amount of energy to be absorbed efficiently by the matrix, and subsequently transferred to the sample in a controlled manner. Absorption of energy from the laser beam causes evaporation of the matrix. The analyte molecules are entrained in the resultant gas-phase plume and become ionized via gas-phase proton-transfer reactions. An astonishing feature of this process is that very large molecules are desorbed into gas phase without undergoing thermal degradation as shown for a monoclonal antibody in Figure 2.5.

Figure 2.5 MALDI mass spectrum of monoclonal antibody

Positive- and negative-ion analyses can both be performed with MALDI. The MALDI mass spectra of proteins and peptides typically contain signals due to singly protonated target molecules and their oligomeric ions (e.g. $[M + H]^+$, $[2M + H]^+$). In some cases, doubly and triply charged protonated ions of low abundance are also formed. With increasing mass of the analytes, multiply charged ions increase in abundance. In addition, the Na^+ and K^+ adducts are also a common feature of MALDI spectra of biological extracts.

Mechanism of Desorption and Ion Formation

The process by which large molecules are desorbed and ionized by absorption of photons is not clearly understood. Three different models have been proposed to explain desorption of the matrix-sample material from the crystal surface: (1) quasithermal evaporation as a result of increased molecular motion, (2) expulsion of upper lattice layers, and (3) an increase in the hydrodynamic pressure due to rapidly expanding molecules in the crystal lattice. The analyte species are entrained in a dense plume of the desorbed matrix molecules. The initial velocity is considered an important value in characterizing the desorption process. However, there is no consensus yet as to how the sample molecules are ionized. The widely accepted view is that, following their desorption as neutrals, the sample molecules are ionized by acid-base proton transfer reactions with the protonated matrix ions in a dense phase just above the surface of matrix. This view has been supported by the fact that the

spectra of proteins obtained with UV- or IRMALDI are similar. The protonated matrix molecules are generated by a series of photochemical reactions. An alternate view is that the singly excited matrix molecules and not the frequently invoked photoionized matrix molecules are common precursors for all subsequent ionization events. According to this model, two excited matrix molecule are require for the generation of free gaseous ions from the sample molecules. A 1998 study by Beavis and collaged has advocated that the ionization of the sample molecules occurs in a warm polar fluid that is formed from the matrix-sample crystals by absorption of laser energy. The ionization of acidic matrices in this warm fluid creates a population of free protons. The acceptance or loss of a proton in this fluid results in the formation of the sample ion. This proposal, however, does, not overrule the possibility of gas-phase ionization. The unified model presented by Karas et al. assumes the formation of initial cluster that comprise of matrix, protonated or deprotonated sample molecules, and counter ions and desolvation of the desorbed clusters to free ionic species via proton transfer and evaporation of neutrals. Highly charged clusters are neutralized to produce singly charged ions by capture of the electrons that are generated during photoionization of the matrix. The electron capture process is also assumed to be the cause fragmentation reactions.
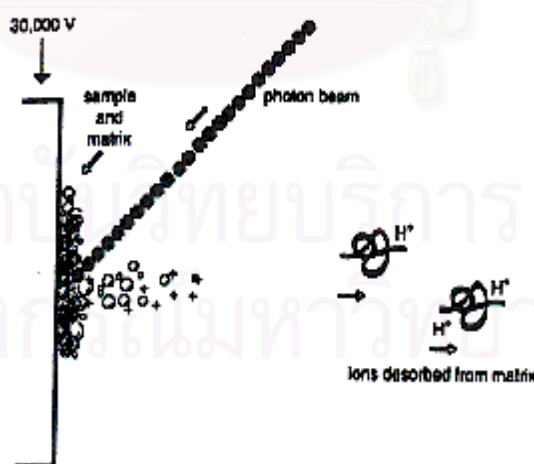
Figure 2.6 Diagram MALDI source

Instrumentation

Current applications of MALDI overwhelmingly use TOF instruments because the pulsed nature of a laser beam matches well with the pulsed scanning mode of TOFMS. In addition, the unlimited mass range, short duty cycle, high ion transmission, and multichannel detection features of TOFMS are also highly desirable for MALDIMS experiments. A schematic diagram of MALDI-TOFMS is presented in Figure 2.7. Linear and reflectron TOF instruments have both been used for MALDI-TOFMS. The combinations of MALDI with magnetic sector, quadrupole ion trap, and FTICR instruments have also emerged. Especially FTIRC has made a significant contribution to the accurate mass measurements of proteins.

Various laser systems have been used to rapidly deposit energy into the matrix-sample combination. Most applications have used UV lasers, such as the N_2 laser (337 nm), the frequency-tripled (335 nm) and frequency-quardrupled (226 nm) Nd:YAG laser and the ArF excimer laser (193 nm). IR lasers have also been used to produce the MALDI effect. The TEA CO_2 laser (10.6 μm), the Q-switched Er:YAG laser (2.94 μm), and the Cr : LiSAF or Nd : YAG pumped optical parametric oscillators (OPO) laser (3.28) are the most common IR lasers .UV and IR lasers both yield similar spectra for proteins, although a better resolution has been obtained for some proteins with an IR laser.
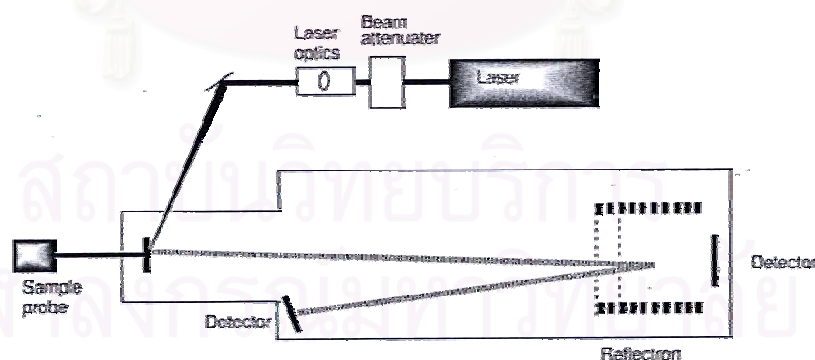
Figure 2.7 A schematic of MALDI-TOF MS

The Role of a Matrix

The matrix performs two important functions; (1) it absorbs photon energy from the laser beam and transfers it into excitation energy of the solid system; and (2) it serves as a solvent for the analyte, so that the intermolecular forces are reduced and aggregation of the analyte molecules is held to a minimum. Some desirable characteristics of a typical MALDI matrix are

- A strong light absorption property at the wavelength of the laser flux.
- The ability to form microcrystals with the sample.
- A low sublimation temperature, which facilitates the formation of an instantaneous high-pressure plume of matrix-sample material during the laser pulse duration.
- The participation in some kind of a photochemical reaction so that the sample molecules can be ionized with high yields.

Several matrix-laser combinations have been tested successfully. Some commonly used matrices, the solvents in which they can be dissolved, and fields of their applications, are listed in Table 2.1. For peptides and small-molecular-mass proteins (<10,000 Da), good results are obtained with α-cyano-4-hydroxycinnamic acid, whereas high-mass proteins are analyzed with sinapic acid. The use of 3-amino-4-hydroxybenzoic acid and 2,5-dihydroxybenzoic acid has been recommended for the analysis of oligosaccharides. Ice has been used as a matrix for IRMALDI of proteins. 3-Hydroxypicolinic acid (HPA) has gained a wide acceptance for the analysis of oligonucleotides. The most commonly used matrices for synthetic polymer is shown in Table 2.1.

Table 2.1   The common matrices used in MALDI MS

Matrix	Mass (Da)	Solvents	Applications
3-Amino-4-hydroxybenzoic acid	153	ACN, water	oligosacharides
2,5-Dihydroxybenzoic acid (DHB)	154	ACN, water, methanol, acetone, chloroform	oligosacharides, peptide, nucleotides, oligosacharides
2[4-hydroxyphenylazo]benzoic acid (HABA)	242	ACN, water, methanol	proteins, lipid
Cinnamic acid	148	ACN, water	general
α-Cyano-4-hydroxycinnamic acid (CCA)	189	ACN, water, ethanol, acetone	peptides, lipids, nucleotides
Sinapinic acid (3,5-dimethoxy-4-hydroxycinnamic acid)	224	ACN, water, acetone, chloroform	lipids, peptides proteins
Dithranol	226	THF	polymers
all-trans retinoic acid (RTA)	300	THF	polymers

Sample preparation

As with the other desorption ionization methods, the preparation of the sample for MALDI analysis requires utmost care. The homogeneity of the sample-matrix mixture is a critical factor to obtain good sample ion yields. Fortunately, MALDIMS is somewhat more tolerant of impurities, buffers, salts and mixtures. Several techniques have emerged for the sample preparation. These techniques include the

- Dried-droplet technique
- Fast evaporation technique
- Sandwich matrix technique
- Spin-dry technique
- Seed-layer technique

A saturated solution (or at least a mmol/mL concentration) of matrix is first preparation in the deionized water. Other solvents, such as acetonitrile or methanol, may be added to increase its solubility. The solvent used to dissolve the matrix must also be compatible with the sample to be analyzed. For example, for protein analysis, a solvent mixture of water-acetonitrile that contains 0.1 % TFA is recommended. A few microliters of a μmol/liter sample solution in 0.1 % TFA is mixed with equal volume of the matrix solution to give a matrix:sample molar ratio of 1000-10,000:1. In the dried-droplet technique, a 1 μL portion of this mixture is applied to a stainless-steel or gold-coated sample well. In order to obtain a fine-grained morphology of the crystal formation, the sample spot is evaporated slowly in the ambient air or by a gentle stream of cold air. Other researchers have advocated a fast evaporation of the matrix-sample mixture. In this procedure, a 0.5-μL drop of the matrix solution in acetone that contains 1-2 % water is rapidly deposited on the probe. The acetone rapidly evaporates to leave behind a homogeneous surface of small crystals. This procedure yields enhanced resolution, sensitivity, and mass accuracy. In the sandwich matrix technique, a thin layer of the matrix is applied first, followed by 0.1 % aqueous TFA, the sample solution, and an additional layer of the matrix. This mixture is allowed to dry. In the spin-dry technique, a solution that contains equal volumes of a nitrocellulose (NC) membrane and a matrix is applied to the rotating target. The solution is immediately spin-dried and yields a uniform NC-matrix layer. In the seed-layer technique, a seed layer of the matrix is prepared by deposition a droplet (0.5 μL) of the matrix solution on a sample target. The drop is allowed to spread and dry in ambient air. A 0.5- μL drop of 1:1 (v,v) analyte-matrix mixture is deposited on the seed layer, and allowed to dry in ambient air. This technique allows rapid crystallization with a high degree of sample homogeneity.

2.3.3   Time-of-Flight Mass Spectrometer

A time-of-flight (TOF) mass spectrometer is one of the simplest mass-analyzing devices. Since the 1990s, it has reestablished itself as a mainstream technique and is becoming increasingly useful in meeting the demands of contemporary research in biomedical sciences. The recent successes of TOFMS can be attributed to the development of MALDI, high-speed data processing devices, and focal plane detectors. A reviews and books can be referred to as additional useful reading material on this subject.

Principle of Operation

A TOF mass spectrometer behaves as a velocity spectrometer, in which ions are separated on the basis of their velocity differences. A short pulse of ions, after exiting the source, is dispersed in time by allowing it to drift in an FFR of a long flight tube. The principle behind the mass analysis is that after acceleration to a constant kinetic energy (equal to zV, where z is the charge on the ion and V the accelerating potential), ions travel at velocities, v, that are an inverse function of the square root of their m/z values:

$$v = \left(\frac{2zV}{m} \right)^{1/2} \tag{2.1}$$

The lighter ions travel faster and reach the detector placed at the end of the flight tube (of length L) earlier than do the heavier ones. Thus, a short pulse of ions is dispersed into packets of isomass ions (Figure 2.8). Therefore, mass analysis of ions that enter the flight tube can be accomplished by determining their time of arrival given by

$$t = \frac{L}{V} = L \left(\frac{m}{2zV} \right)^{1/2} \tag{2.2}$$

In order to convert the time spectrum into a mass spectrum, the instrument is mass calibrated by measuring the flight times of two different known mass ions.

A primary requirement in the operation of a TOFMS is that all ions enter the flight tube precisely at the same time. Generating ions in short bursts fulfills this condition. In this respect, TOF instruments are well matched to ^{252}Cf-plasma desorption (PD) and MALDI ion sources. The continuous ion beam sources (e.g., electron ionization and ESI), however, can be coupled with a TOF mass spectrometer,

but only after conversion of the generated ions into discrete packets. Pulsing of accelerating potential converts a continuous ion beam into discrete ion packets.
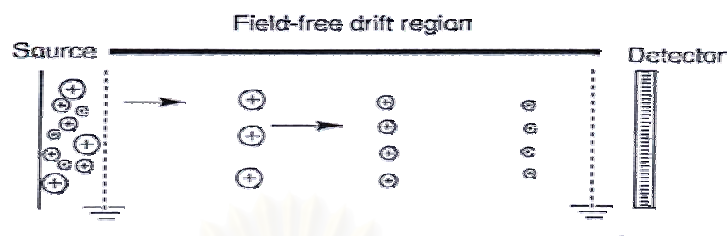

Figure 2.8 Principle of the mass separation by a time-of-flight mass analyzer. Ions are separated on the basic of their size; high-ions (big circles) travel more slowly than the lighter ions

Mass Resolution

In the past, poor mass resolution was one of the major limitations of TOFMS. The mass resolution of simple linear TOF mass spectrometers is usually less than 500. In TOFMS, this term is given by Eq. 2.3, where Δt is commonly measured in terms of FWHM. The spatial, temporal, and velocity dispersions of ions are the limiting factors in achieving higher resolution in TOF instruments. A higher resolution is obtained only when all ions are formed in the source at the same time and the same location (i.e., their temporal and spatial distributions are minimum), and all have the same kinetic energy. The temporal distribution, which is a combined effect of uncertainties in the time of ion formation as well as limitations of ion detection and time-recording devices, can be minimized with the use of very short ionization pulsed and also by increasing the difference in arrival times of two adjacent ions. Increasing the flight path of ions or reducing their velocities (i.e., with lower accelerating potentials) has the effect of increasing the arrival times of different mass ions. The use of lower accelerating potentials is counterproductive because of the concomitant loss of transmission, and of the greater impact of the energy spread on resolution at lower accelerating potentials. The pulsed ion extraction with very fast rise time can also be used to correct the temporal distribution. The spatial distribution degrades resolution because ions formed in different regions of the ion source are accelerated to different kinetic energies. Ions that are formed to the left of the central linear accelerated to a higher velocity than are ion formed to the right of the central line, resulting in loss of

mass resolution. The use of ionization methods that produce ions from a surface, such as [252]Cf-PD and MALDI, eliminates the spatial width to some extent because in these ionization techniques the plane of ion formation is well defined. Another way to correct for the spatial distribution is to use dual-stage ion extraction optics:

$$R = \frac{m}{\Delta m} = \frac{t}{\Delta t} \qquad (2.3)$$

The dominating factor that restricts the resolution in TOF instruments, especially with desorption ionization methods, is the kinetic energy in homogeneity within the ion beam. The higher-initial-energy ions arrive at the detector sooner than do the same-mass lower initial energy ions. The acceleration region energy spread can be eliminated by the prompt ejection of ions from the source (i.e., by using high accelerating potentials, usually > 10 kV). A further reduction in the energy spread is achieved through an energy-correcting device, known as the *reflectron.* Another factor that limits resolution is the *turnaround time* taken by the ions that are traveling initially away from the exit slit. These ions take extra time in exciting the source than ions that have the same initial velocity, but are facing the exit slit. Longer flight tubes and longer flight times can reduce the effect of turnaround time.

In TOF instruments, the time difference in the arrival of various ions at the detector is very short. As an example, *m/z* 2500 after acceleration through a potential of 6000 V will reach the end of the one-meter-long flight path 5.01 μs after the arrival of *m/z* 2000. The difference in the arrival times of ions differing by one dalton (say, 2000 and 2001) is even shorter (in nanoseconds). Therefore, the mass selectivity of TOF instruments is also limited by the accuracy with which short intervals of time can be measured.

Time-of-flight mass spectrometers have a number of attractive features, such as theoretically unlimited mass range, high ion transmission, very high spectrum acquisition rate, multiplex detection capability, simplicity, in instruments design and operation, reasonable mass resolution, and low cost. The detection sensitivity of TOF instruments is much higher than in scanning instruments because they can record all the ions that reach the detector after each ionization event, and they have a high ion transmission efficiency. A major asset of TOF mass spectrometers is their ability to

record a complete mass spectrum in time intervals as short as 25 μs. These attributes make TOFMS an attractive research instrument as well as a valuable analytical tool.

Reflectron

A reflectron is a new development that corrects for any initial position and energy dispersions in the accelerator region of a TOF instrument. This elegant device is in fact an electrostatic mirror that consists of a series of electrical lenses, each with progressively increasing repelling potential (Figure 2.9).
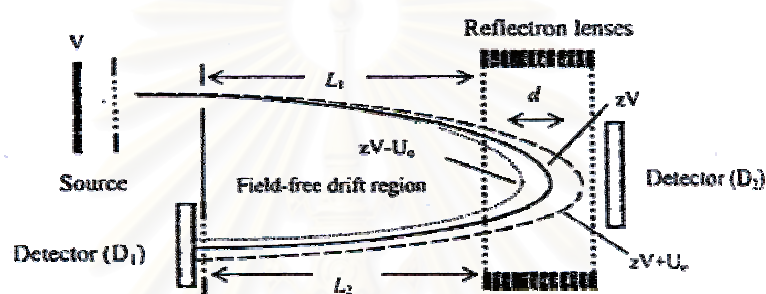

Figure 2.9 A sketch of a reflectron time-of-flight mass analyzer. All ions of the same mass, but that differ in kinetic energy, are made to arrive at the same time at a detector (D_1) that is located at the end of second field region (L_2)

The initial spatial spread is translated into a velocity spread, which can be readily corrected by the mirror. A reflectron works on a principle that the ions that enter this device after traversing the first FFR (L_1) are slowed down until they come to rest, and then their direction of motion is reversed, and finally, they are reaccelerated into a second FFR (L_2). Qualitatively, the faster-moving ions (i.e., ions with excess energy $zV + U_0$) spend less time in the drift regions, but penetrate to a greater depth (d) into the reflecting field and consequently, spend more time there. This extra time in the mirror compensates for the shorter flight times of faster ions in the drift regions, with the result that all isomass ions arrive simultaneously at the detector that is located at the end of the flight path L_2. Mass resolution is, thus, improved. An additional contributing factor in improving the mass resolution is the longer pathlength of the reflectron (i.e., $L_1 + L_2$). A mass resolution of > 20,000 (FWHM) has been achieved with a grid-free reflectron. A considerable improvement in performance is realized

over linear TOFMS instruments for < 10-kDa molecular mass compounds. The total flight time of an ion in the reflectron is given by

$$t = \left(\frac{m}{2zV}\right)^{1/2} \left(L_1 + L_2 + 4d\right) \qquad (2.4)$$

The single-state and dual-stage reflectrons are both in common use. A *single-stage reflectron* is a simple ion mirror in which a single retarding-reflecting field is used. It consists of an entrance grid electron and a series of ring electrodes for the Figure 2.9. The single-stage reflectron provides a first-order correction for the kinetic energy distribution. The *dual-stage reflectron* contains two linear retarding voltage regions that are separated by an additional grid. The first stage is smaller than the second state, but with considerably higher field strength capable of reducing ion kinetic energies to about one-third of their initial values. The dual-stage reflectrons provide higher mass resolution than do the single-stage devices. The gain in resolution, however, is at the expense of sensitivity because transmission losses occur when ions pass through the additional grid lenses. The reflecting TOF instruments are usually outfitted with an additional detector behind the reflectron. A conventional linear TOF mass spectrum is recorded with this detector when the reflectron voltage is turned off.

Usually, the detection region of a reflectron is separate from the ionization region by reflecting the ions at a small angle with respect to the incoming ions. In an alternative design, called axial *symmetry reflectron*, the reflected ions travel back along the flight axis of the incoming ions, but with a slight angular divergence. The multichannel plate (MCP) detector used in this design is of annular shape, and is located just outside the ion source.

2.4   Application of MALDI-TOF-MS to Synthetic Polymers

MALDI is the newest and most promising desorption method for synthetic macromolecules. The polymer dissolved in appropriate solvent and mixed with a solution matrix to achieve a molar ratio of analyte to matrix of 1:100-1:50,000. A solution of auxiliary ionization agent (e.g., a metal ion salt) may be added and a small droplet ($\leq$ 1 μL) of the resulting mixture are loaded onto target surface. As the solvent evaporates, a solid solution of sample (an auxiliary agent) in the matrix is obtained, which is bombarded by lasers light. MALDI is extremely sensitive, with the total amount of sample deposited onto the target being in the pico- to femtomole range. Polymer up to approximately 10^6 Da can be ionization by this method. Up to approximately 50,000 Da, singly charged ions are formed exclusively or predominantly, while at higher molecular weights multiply charged ions are usually coproduced in considerable abundance. The high dilution of the analyte in the matrix prohibits analyte-analyte interactions, which could to the formation of analyte cluster, thereby complicating molecular-weight assignments. MALDI-TOF allows desorption and ionization of very large molecules, even in complex mixtures. It gives information on the mass of individual oligomers from which repeat units, end-groups, the presence of rings, molar mass distributions and other information can be derived.

In contrast to biopolymers, the ionization of synthetic polymers usually occurs by cationization rather than protonation. Most of synthetic polymers having heteroatoms (e.g., polyesters, polyamides, polyethers, polyacrylates, and the like) are cationized by addition of metal salts (e.g., Na^+X^-, Ag^+X^-). MALDI has the advantage that the singly charged ion, either a proton or metal ion adduct, is typically the most intense high-mass ion. Maltiple charging is not usually observed, except for high $\overline{Mw}$ polymers.

The most commonly used matrices for synthetic polymers are 2,5-dihydroxybenzoic acid (DHB), 2[4-hydroxyphenylazo]benzoic acid (HABA), 3-β-indoleacrylc acid (IAA), dithranol, all trans-retinoic acid, 3,5-dimethoxy-4-hydroxy cinnamic acid (sinnapic acid) and 5-chlorosalicylic acid. The selection of a good matrix is still a trial and error process in MALDI research.

In principle, in the desorption/ionization process, the amount of pulsed laser energy transferred to the analyte via matrix will depend on the laser power, on the nature of the matrix and sample, and on the dispersion of analyte molecules within the matrix. There exists a threshold irradiance that is matrix-dependent, below which ionization is not observed. Above this level ion production increases nonlinearly.

This research is to study for good MALDI-MS condition, such as types of matrix, analyte to matrix ratio laser power and the presence of cationizing agent, for analysis dispersants.

Determination of Average Molecular Weights of Polydispersity Polymer

1. Mass-average molecular weight; $\overline{M}w$

$$\overline{M}w = \frac{\sum\left(N_i M_i{}^2\right)}{\sum\left(N_i M_i\right)} \tag{2.5}$$

2. Number-average molecular weight; $\overline{M}n$

$$\overline{M}n = \frac{\sum\left(N_i M_i\right)}{\sum N_i} \tag{2.6}$$

Where N_i, M_i represent signal intensity in peak area and mass for the oligomer containing i monomer.

2.5   Literature Reviews

Dispersants play a major role in gasoline especially to prevent agglomeration of particles produced by the oil degradation and metallic parts wear and maintain them in suspension the oil. Six research groups have been published works on analysis of dispersants with different technique. The first group, Malone *et al.* [13] studied the method of analyzing high molecular weight dispersants in motor oils by GPC and VPO determination. Hui *et al.* [14-15], published two works on isolation of polybutenylsuccinimide-type dispersants. In 1995 they isolated polybutenyl succinimide-type dispersants from monograde and multigrade lubricating oil by classical liquid-adsorption chromatography on florisil column and characterize through FT-IR and size exclusion chromatography (SEC). In 1997 they isolated polybutenylsuccinimide-type dispersants from multigrade lubricating oils by classical cation exchange chromatography. Ampha [2] determined dispersants in gasoline and diesel oil by separating dispersants from fuel oil with alumina and monitored the results with FT-IR, GPC, and NMR. Van der hage *et al.* [16] characterized polyoxyalkenylamines structural by MALDI on an external ion source FT-ICR-MS. They confirmed and quantified structural assignments by NMR. Alemán *et al.* [17] synthesized polyisobutenylsuccimimides as dispersants in gasoline by reaction of polyisobutenylsuccinic anhydride and primary amine and identified by FT-IR. The alkenylsuccinimide showed bands 1700 to 1707 cm^{-1} of C=O stretching, in addition to a C-N band at 1390 cm^{-1}. The absorption of N-H stretching in case of polyamines in the 3000-3500 cm^{-1} range was also observed. Sinjaroen [18] determined of molecular weight distribution of polyisobutenyl succinimide-type dispersant by MALDI-TOF MS. She found that the best condition to provide the good MS spectrum and molecular weight distribution of polymer dispersants are using dried droplet method as sample preparation, α-hydroxycyanocinnamic acid as matrix with tetrahydrofuran as a solvent, the dispersants solution:matrix solution of 1:5 by volume and the laser power of 170-180 μJ. In 2004 Carraze *et al.* [3] utilized ESI MS to study of fingerprints mass spectral of detergents in Austrian, Hungarian, Uruganyan, and Chilean gasolines. They observed polymers or copolymers based on ether motifs (inter-peak spacing of 44, 58, and 72 u). They found that polymers based on isobutene-amine were contained in Austrian gasoline.

MALDI is a useful technique for biological and polymer analysis. In field of polymers, many studies were reported on measurements of molecular weight distributions (polydisperse) for a variety of polymers including polystyrene (PS), polyethylene glycol (PEG), polypropylene glycol (PPG), polymethyl methacrylate (PMMA), polybutylene and polyisobutylene etc. In these works, the following optimum factors and parameters were studies: types of matrix, solvent effect, cationization reagent effect for obtaining good spectra. Belu *et al.* [19] studied MALDI MS for polymer characterization with different polymers, different matrices, different laser power and different cationizing agent. The results showed that optimum quantities of polymer, matrix and cationizing agent were established specifically for PS. Dithranol is the best matrix for PS, and addition of Ag^+ for cationization of molecules was necessary. Polymers up to 49 Ku were analyzed. Effect of laser power found to be the most significant for lower molecular weight polymers and skew the distribution of oligomers. In 1998 Zhu *et al.* [20] determined the average molecular weight of narrow polydispersity polymers by MALDI-TOF MS. Polymer in this experiment are PS 5050, PS 7000 and PS 1160 which used *all-trans* retinoic acid as a matrix, $AgNO_3$ and LiOH were used as a cationizing agent. The results showed that under the experimental conditions used in this work, asymmetric distortion of molecular weight function did not occur during the MALDI analysis of the PS 5050 and PS 7000. It was concluded that MALDI mass spectrometry can provide accurate molecular weight distribution information for narrow polydispersity polymers.

Dispersants in this research has PIB as hydrocarbon tail. Ji *et al.* [21] studied four types of PIB by MALDI. The sulfonation samples were employed before analysis by MALDI. All MALDI-TOF MS spectra reported in this paper were generated using *all-trans* retinoic acid matrix. Tetrahydrofuran was used as solvent for both polymers and matrix. Because the difficulty in attaching ions for such materials, the ionization for MALDI-TOF MS analysis is realized by sulfonating the olefin end groups to introduce a readily ionized moiety into the polymer. The number average molecular weight and weight average molecular weights of these materials were also measured by LS and VPO.

CHAPTER III

EXPERIMENTAL

3.1   Materials

1. LZ 8218C; as gasoline additive package

  Lubrizol

2. Aluminum oxide 90 (70-230 mesh)

  For column chromatography; Merck

3. Hexane

  Commercial grade; J.T. Baker

4. Tetrahydrofuran

  HPLC grade; Lab scan

5. Acetone

  Commercial grade; J.T. Baker

6. Dithranol

  Aldrich

7. 2,5-Dihydroxybenzoic acid

  Aldrich

8. 2-(4-Hydroxyphenylazo)-benzoic acid

  Aldrich

9. *all*-trans Retinoic acid

  Sigma

10. α-cyano-4-hydroxycinnamic acid

  Sigma

11. Angiotensin II

  Sigma

17. Neurotensin acetate

  Sigma

18. Adenocorticotropic hormone

    Sigma

19. Silver nitrate

    Sigma

3.2 Apparatus and Instruments

1. Matrix-Assisted Laser Desorption Ionization Mass spectrometer (MALDI-MS)

    BIFLEX, BRUKER

2. Vortex mixer

    Vortex-genie No.2, Scientific Industries

3. Multichannel probe

    BRUKER

4. Autopipett

    Pipetman, Gilson

5. Electronic Balance

    Fx-180, A&D

6. Pipette tip

7. Eppendorf

3.3 PROCEDURE

3.3.1 Separation of dispersants from base oil

Aluminium oxide (50 g) was packed in glass column. The gasoline additive package (50 mL of LZ 8218C) containing base oil and dispersants 50 mL was loaded into the column. Hexane (100 mL) was added into the column to elute base oil and 50 mL tetrahydrofuran was added to elute dispersants from aluminnium oxide. The solvent in this fraction was evaporated using a rotary evaporator.

Prior to analysis using MALDI-TOF mass spectrometry, the dispersants was dissolved in THF to give concentration at 100 mg/mL.

3.3.2 Condition for MALDI Analysis

The overall process of MALDI is shown in Scheme 3.1

```
┌──────────────┐     ┌──────────────┐     ┌──────────────┐
│    Sample    │ ⇒  │ Drop 0.5 µL to│ ⇒  │   Take into  │
│ preparation  │     │ multiprobe, dry│     │   MALDI MS   │
└──────────────┘     └──────────────┘     └──────────────┘
                                                   ⇓
┌──────────────┐     ┌──────────────┐     ┌──────────────┐
│    Obtain    │ ⇐  │ Mass analyzer│ ⇐  │ Bombard with │
│   spectrum   │     │    (TOF)     │     │    laser     │
└──────────────┘     └──────────────┘     └──────────────┘
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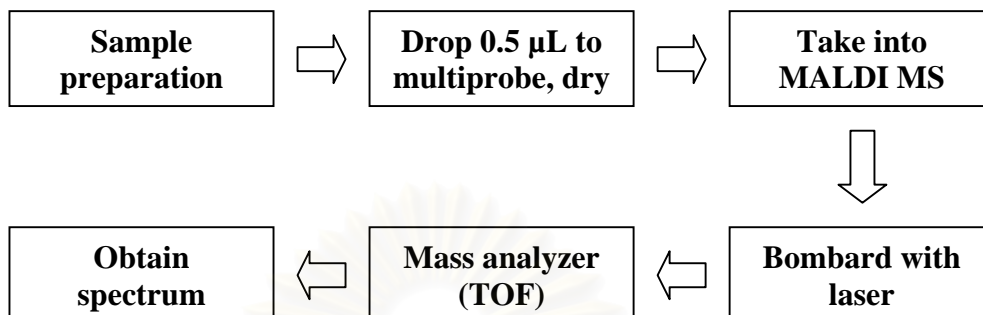
Scheme 3.1   The overall process of MALDI analysis

Sample and target preparation for MALDI

Polymer (dispersants) for MALDI analysis was prepared by combining the dispersant solution and various matrix solutions with various analyte to matrix ratios. Dispersants solution and matrix were mixed and vortexed, then a mixture of 0.5 µL was deposited on multiprobe and allowed to dry.

For MALDI-TOF MS analysis, the following conditions and parameter were studies:

- Type of matrix
- Analyte to matrix ratio
- Laser power
- Cationizing agent

Type of matrix

The following types of matrices in this experiment were separately used: dithranol, 2,5-dihydroxybenzoic acid (DHB), α-cyano-4-hydroxycinnamic acid (CCA), 2-(4-hydroxyphenylazo)-benzoic acid (HABA), *all-trans* retinoic acid (RTA). Dispersants sample (100 mg/mL) and matrices were mixed (1:50) and vortexed.

- 10 mg/mL dithranol was prepared in THF
- 10 mg/mL DHB was prepared in THF
- 10 mg/mL CCA was prepared in THF
- 10 mg/mL HABA was prepared in THF
- 10 mg/mL RTA was prepared in THF

Analyte to matrix ratio(A/M)

The ratio of analyte to matrix was varied as the following:

- 1:25
- 1:50
- 1:75
- 1:100

Laser power

Different degree of laser power has influence on MS Spectrum. Some sample may require high degree of laser power, whereas in some cases, spectrum can be shown by using low degree of laser power. Thus various values of laser power were used:

- 180 μJ
- 190 μJ
- 200 μJ

Cationizing agent

The metal solution, 0.15 M AgNO$_3$ dissolved in ethanol was used.

CHAPTER IV

RESULTS AND DISCUSSION

This research was related to development of mass spectrometry for determination of dispersants additive in gasoline the results will be shown and discussed in each part, respectively.

4.1 The optimized condition and parameter for determination of molecular weight distribution of dispersants

The optimized conditions and parameters for determination of molecular weight distribution of dispersants were performed to obtain good spectra. The results are shown as follow;

4.1.1 Types of matrices

Types of matrices are the most significant factors for MALDI analysis because the matrix has selectivity for particular sample. Moreover, the matrices yield the difference in range of molecular weight distribution, laser power and noise. The matrices: dithranol, 2,5-dihydroxybenzoic acid (DHB), α-cyano-4-hydroxycinnamic acid (CCA), 2-(4-hydroxyphenylazo)-benzoic acid (HABA) and *all*-trans retinoic acid (RTA) were used in the experiment.

Types of matrices yield a difference of m/z range of molecular weight distribution. The spectra are shown in Figures A1-A5 and summarized in Table 4.1.

Table 4.1 Mass to charge range of molecular weight distribution and laser power of matrices

Types of matrix	Laser power (μJ)	m/z range of molecular weight distribution	MW of repeating unit
dithranol	160	200-600	56
		390-1000	56
		1200-2000	58
DHB	190	200-600	56
		390-1000	56
		520-1260	56
		1200-2300	58
CCA	130	200-600	56
		390-1000	56
		520-1260	56
		1200-2300	58
HABA	160	1200-2300	58
RTA	160	400-1000	56
		1200-2300	58

From Table 4.1, it is concluded that four ranges of dispersants molecular weight distribution were found. The first range is m/z of 200-600 with repeating unit of 56 Da, by using dithranol, DHB, and CCA as matrix. The second range is m/z of 390-1000 with repeating unit of 56 Da, by using dithranol, DHB, CCA, and RTA as matrix. The third range of dispersants is the range of m/z of 520-1260 with repeating unit of 56 Da, by using DHB and CCA as matrix. The fourth range is m/z of 1200-2300 with repeating unit of 58 Da, by using dithranol, DHB, CCA, HABA and RTA as matrix. Therefore the complete molecular weight distribution information of dispersants was shown when using DHB and CCA as matrix. Because of the high intensity of noise, CCA is not the appropriate matrix for dispersants. Thus, the use of DHB as matrix was found to be the best condition for the analysis of molecular weight distribution of dispersants.

4.1.2 Analyte-to-matrix ratio

The samples, which have the amount ratio of analyte to matrix (DHB); 1:25, 1:50, 1:75 and 1:100 were prepared. The obtained MALDI-MS spectra are shown in Figure A6-A9. At the sample:matrix ratio of 1:25 (Figure A6), the obtained MALDI-MS spectrum was poor because of low intensity detection response in the third and the fourth range. The obtained spectrum of sample:matrix ratio of 1:50 (Figure A7) is very good. It gave a clearly molecular weight distribution of dispersants with low intensity of noise. At sample:matrix ratios 1:75 and 1:100 (Figure A8 and A9) the patterns of dispersants molecular weight distribution were found to be similar to that of sample:matrix ratio of 1:50, although at sample:matrix ratio of 1:75 and 1:100 have higher intensity of detection response than that of 1:50 but the high intensity of matrices peak at the first and the second range were also obtained. Thus, a suitable dispersants:matrix ratio was chosen to be 1:50.

4.1.3 Laser power

Laser power is the significant factors for MALDI analysis because laser power has selectivity for particular sample. The obtained MALDI-MS spectra of dispersants at laser power of 180 µJ, 190 µJ and 200 µJ are shown in Figure A10-A12. From Figure A10, no spectrum of dispersants was observed. This indicates that the laser power of 180 µJ has not enough energy for dispersants ionization. The obtained MALDI-MS spectrum of dispersants at laser power of 190 µJ and 200 µJ were found to be similar. In comparison of MALDI-MS spectra of dispersants at laser power of 190 µJ and 200 µJ (Figure A11 and A12), the MALDI-MS spectrum of dispersants at laser power of 200 µJ has a higher intensity of detection response but the intensity of noise is too high. Therefore, laser power of 190 µJ was suitable to be use for the analysis of dispersants.

4.1.4   Cationizing agent

The MALDI-MS spectra of dispersants using DHB as matrix and AgNO$_3$ as matrix are shown in Figures A13. MALDI-MS spectra of dispersants in the present and absent of cationizing agent (Figure A11) show the same pattern molecular weight distribution pattern. Thus, cationizing agent did not improve on ionization of dispersants.

4.2   Data processing from MALDI analysis

From the previous results and Figure 4.1-4.4, molecular weight distribution of dispersants in gasoline additive package (LZ8218C) may be divided into four ranges as shown in Table 4.2.

Table 4.2 Four range of dispersants molecular weight distribution from MALDI-MS

m/z Range of molecular weight distribution	MW of repeating unit (Da)
❶ 200 - 600	56
❷ 390-1000	56
❸ 520-1260	56
❹ 1200-2300	58
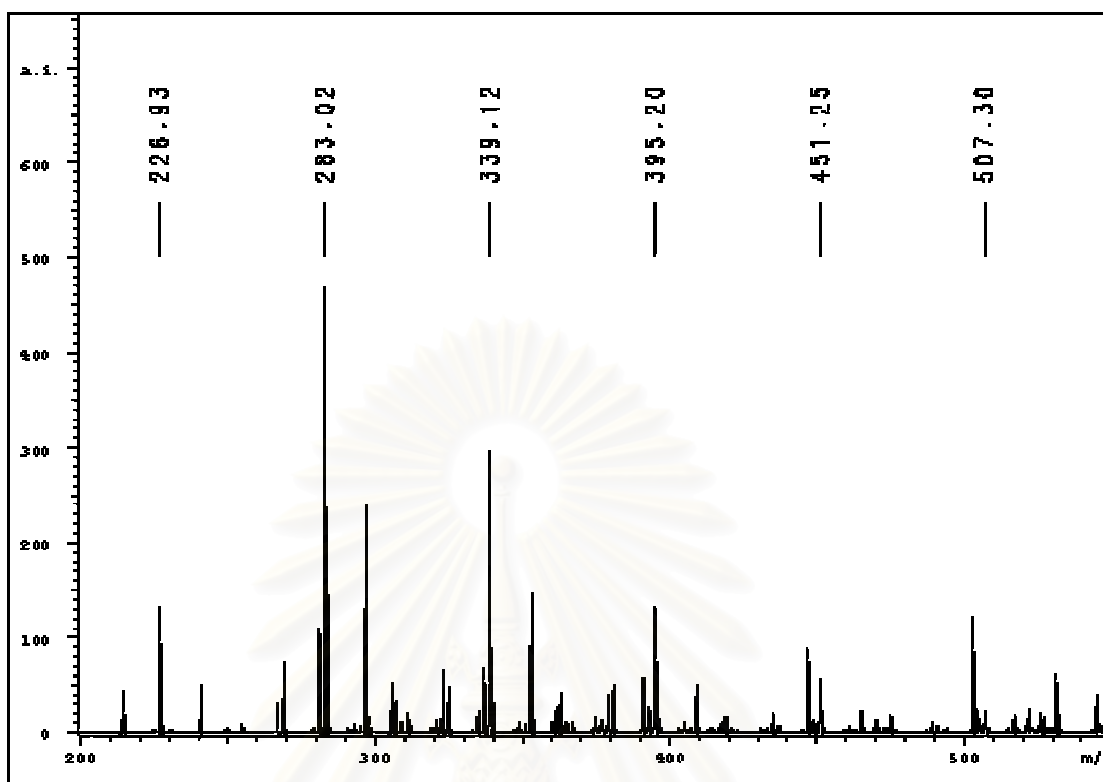
Figure 4.1 Molecular weight distribution of dispersants in range ❶ m/z of 200-600
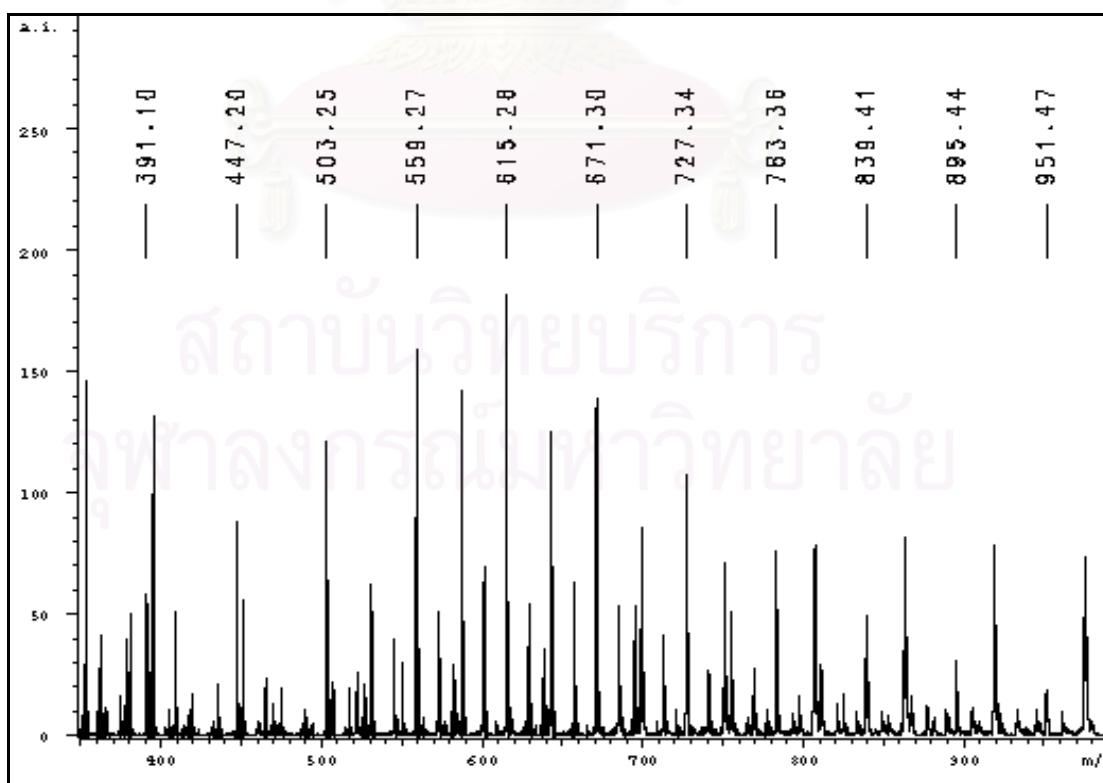
Figure 4.2 Molecular weight distribution of dispersants in range ❷ m/z of 390-1000
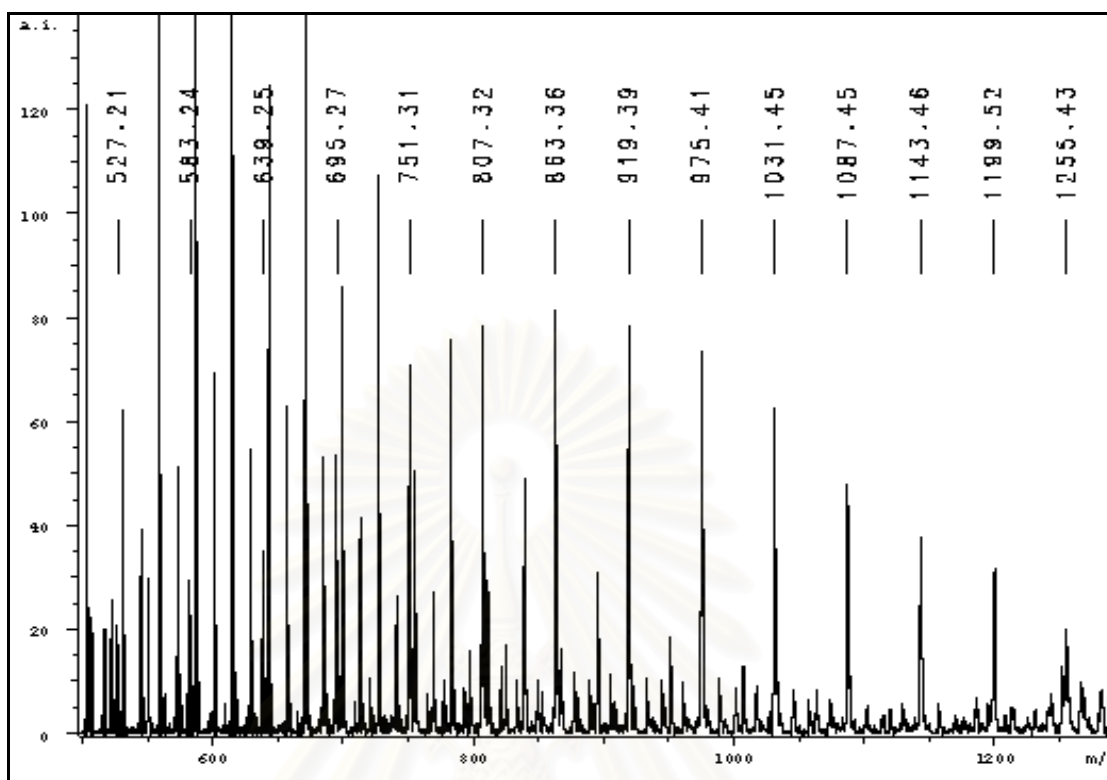
Figure 4.3 Molecular weight distribution of dispersants in range ❸ m/z of 520-1260
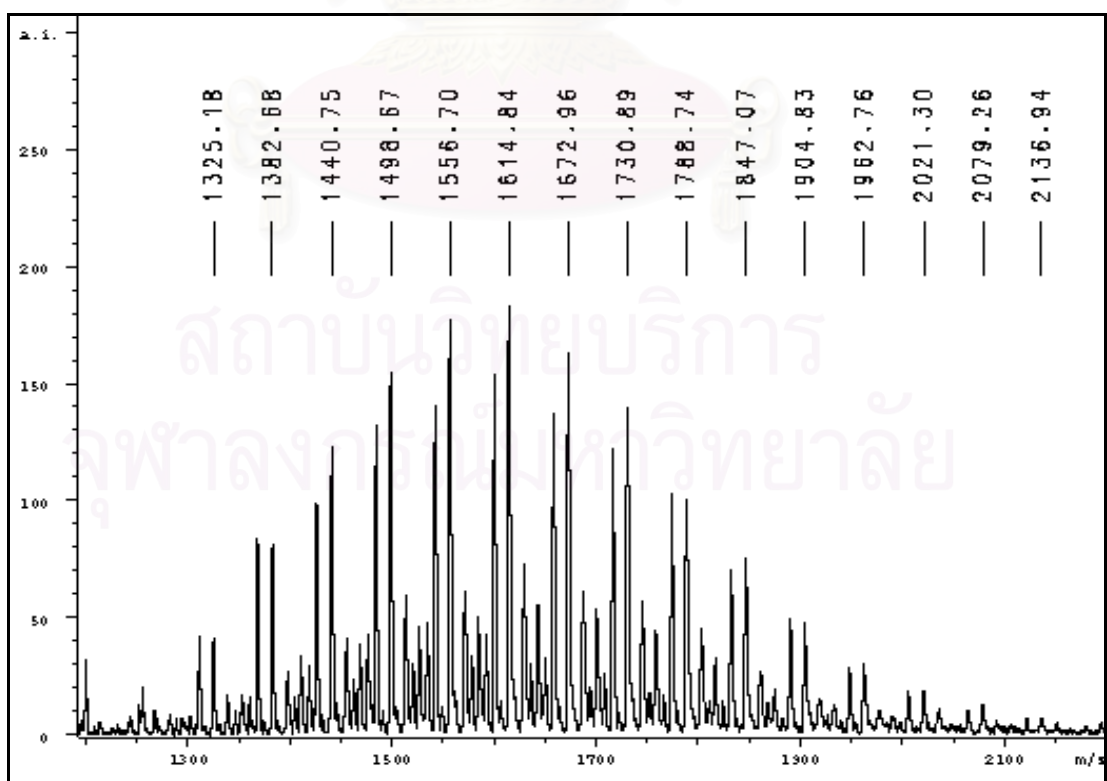
Figure 4.4 Molecular weight distribution of dispersants in range ❹ m/z of 1200- 2300

4.2.1   Calculation of average molecular weight of dispersants

Mass average molecular weight ($\overline{M}w$) and number-average molecular weight ($\overline{M}n$) are calculated using the following equation.

1)   Mass-average molecular weight; $\overline{M}w$

$$\overline{M}w = \frac{\sum\left(N_i M_i^{\,2}\right)}{\sum\left(N_i M_i\right)} \tag{4.1}$$

2)   Number-average molecular weight; $\overline{M}n$

$$\overline{M}n = \frac{\sum\left(N_i M_i\right)}{\sum N_i} \tag{4.2}$$

Where N_i and M_i represent signal intensity in peak area and mass for the oligomer containing *i* monomer.

Example 4.1

Dispersants in range ❹ 1200-2300
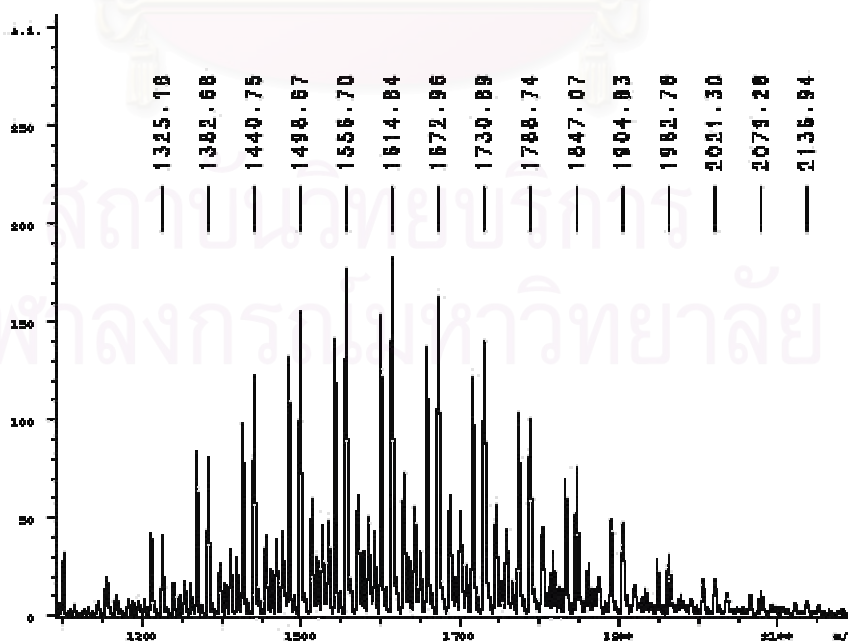
$$\overline{M}w = \frac{\sum\left(N_i M_i{}^2\right)}{\sum\left(N_i M_i\right)}$$

$\overline{M}w$ = [(0.0081×1325.18^2) + (0.0160×1382.68^2) + (0.0242×1440.75^2) +

(0.0305×1498.67^2) + (0.0349×1556.70^2) + (0.0361×1614.84^2) +

(0.0320×1672.96^2) + (0.0275×1730.89^2) + (0.0198×1788.74^2) +

(0.0149×1847.07^2) + (0.0094×1904.83^2) + (0.0060×1962.76^2) +

(0.0037×2021.30^2) + (0.0025×2079.26^2) + (0.0014×2136.94^2)] /

[(0.0081×1325.18) + (0.0160×1382.68) + (0.0242×1440.75) +

(0.0305×1498.67) + (0.0349×1556.70) + (0.0361×1614.84) +

(0.0320×1672.96) + (0.0275×1730.89) + (0.0198×1788.74) +

(0.0149×1847.07) + (0.0094×1904.83) + (0.0060×1962.76) +

(0.0037×2021.30) + (0.0025×2079.26) + (0.0014×2136.94)]

= 1648

$$\overline{M}n = \frac{\sum\left(N_i M_i\right)}{\sum N_i}$$

$\overline{M}n$ = [(0.0081×1325.18) + (0.0160×1382.68) + (0.0242×1440.75) +

(0.0305×1498.67) + (0.0349×1556.70) + (0.0361×1614.84) +

(0.0320×1672.96) + (0.0275×1730.89) + (0.0198×1788.74) +

(0.0149×1847.07) + (0.0094×1904.83) + (0.0060×1962.76) +

(0.0037×2021.30) + (0.0025×2079.26) + (0.0014×2136.94)] /

(0.0081 + 0.0160 + 0.0242 + 0.0305 + 0.0349 + 0.0361 + 0.0320 +

0.0275 + 0.0198 + 0.0149 + 0.0094 + 0.0060 + 0.0037 + 0.0025

+0.0014)

= 1630

$$D_p = \frac{\overline{M}w}{\overline{M}n} \ = \ 1648/1630 \ = \ 1.01$$

$\overline{M}w$, $\overline{M}n$ of dispersants are shown in Table 4.3.

Table 4.3 $\overline{M}w$ and $\overline{M}n$ of dispersants

Range	MW of repeating unit (Da)	$\overline{M}w$	$\overline{M}n$	D_p
❶ m/z of 200- 600	56	330	318	1.04
❷ m/z of 390-1000	56	839	839	1.00
❸ m/z of 520-1260	56	925	891	1.04
❹ m/z of 1200-2300	58	1648	1630	1.01

4.2.2 Characterization of dispersants

There are three types of gasoline dispersants that are polyisobutenyl-succinimide, polyisobutenyl amine and polyether amine. Gasoline additive package (LZ8218C) has four range of dispersants molecular weight distribution. The repeating unit of molecular weight range ❶, ❷ and ❸ are 56 Da, indicates that dispersants in these range have consisted of polyisobutylene (PIB). Thus, dispersants in these ranges might be polyisobutenylsuccinimide and/or polyisobutenyl amine. Dispersants in range ❹ had repeating unit of 58 Da, indicated that dispersants in this range might be polyether amine with polypropylene oxide repeating unit $-[-(CH_2)_3-O-]_n-$ and/ or $-[-CH_2-CH(CH_3)-O-]_n-$ polymers.

Molecular weight of polyisobutenylsuccinimide polyisobutenyl amine and polyether amine can be calculated from Equation 4.3, 4.4 and 4.5.

polyisobutenylsuccinimide
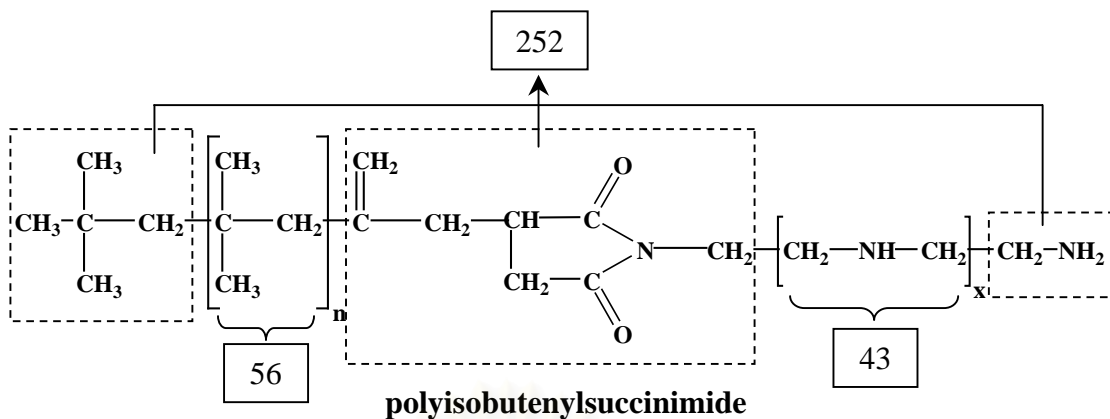
$$252 + 56n + 43x = M \qquad (4.3)$$

Where n is the number of repeating unit of PIB group and x is the number of repeating unit of polyethylene amine

polyisobutenyl amine
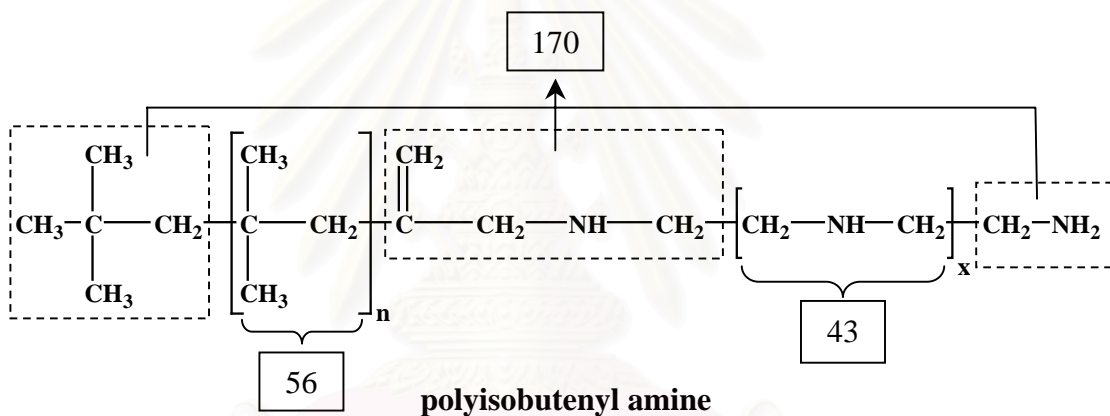
$$170 + 56n + 43x = M \qquad (4.4)$$

Where n is the number of repeating unit of PIB group and x is the number of repeating unit of polyethylene diamine.
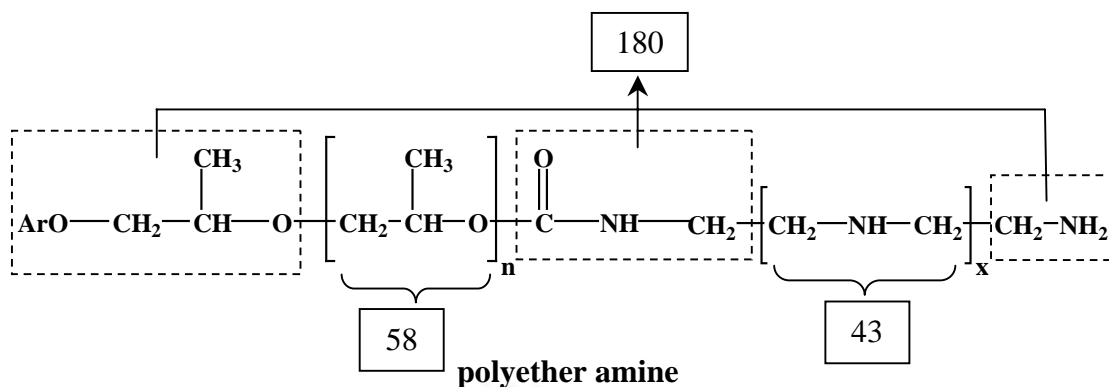
$$180 + 58n + 43x = M \qquad (4.5)$$

Where n is the number of repeating unit of propylene oxide and x is the number of repeating unit of polyethylene diamine.

From Equations 4.3, 4.4 and 4.5 there are three parameters M, n and x. A peak from MALDI-MS spectra in range ❶, ❷, ❸ and ❹ (Figure 4.1-4.4) was chosen to substitute M. Both n and x parameter are unknown.

Dispersants in range ❶

In Figure 4.1, peak of m/z of 283.03 were chosen for calculation of n value.
From Equation 4.3

If x=0 ⟶ n= 0.54

From Equation 4.4

If x=0 ⟶ n= 2.00

From calculation results as mentioned above, dispersants in range ❶ should be polyisobutenyl amine and not polyisobutenylsuccinimide. Moreover, other peaks in this range (see Figure 4.1) were also indicated that this range of molecular weight distribution should belong to polyisobutenyl amine of ethylenediamine.

Dispersants in range ❷

In Figure 4.2, peak of m/z of 391.10 were chosen for calculation of n value. From Equation 4.3

If x=2 ➡ n= 0.93

From Equation 4.4

If x=0 ➡ n= 3.93

From calculation results as mentioned above dispersants in range ❷ might be either polyisobutenylsuccinimide of triethylenetetramine or polyisobutenyl amine of ethylenediamine. Moreover, other peaks in this range (see Figure 4.2) were also agreed with this calculation results.

Dispersants in range ❸

In Figure 4.3, peak of m/z of 527.21 were chosen for calculation of n value. From Equation 4.3

If x=5 ➡ n= 1.06

From Equation 4.4

If x=3 ➡ n= 4.06
If x=7 ➡ n= 0.99

From calculation results as mentioned above dispersants in range ❸ might be polyisobutenylsuccinimide of hexaethyleneheptamine or polyisobutenyl amine of tetraethylenepentamine or oxtaethylenenonylamine. Moreover, other peaks in this range (see Figure4.3) were also agreed with this calculation results.

Dispersants in range ❹

In Figure 4.4, peak of m/z of 1325.18 were chosen for calculation of n value. From Equation 4.5

If x=1 ➤ n= 18.99

If x=5 ➤ n= 16.02

If x=9 ➤ n= 13.05

If x=13 ➤ n= 10.90

If x=24 ➤ n= 1.93

From calclation results as mentioned above indicates that dispersants in range ❹ should be polyether amine of polypropylene oxide repeating unit.

The calculation results of dispersants in range ❶ ,❷ and ❸ which have consisted of PIB from Equation 4.3 and 4.4 indicated that the dispersants in range ❶ should be polyisobutenylsuccinimide and dispersants in range ❷ and ❸ might be either polyisobutenylsuccinimide or polyisobutenyl amine. The calculation results of dispersants in range ❹ from Equation 4.5 indicated that dispersants in this range should be polyether amine.

4.2.3 Characterization of dispersants by infared spectroscopy

Dispersants obtained from removal of base oil in gasoline additive package (LZ 8218C) was identified by FTIR. The obtained FTIR spectrum is shown in Figure B1. From Figure B1, it was concluded that the dispersants in LZ 8218C package is not contained polyisobutenylsuccinimide because there is no C=O peak at 1700-1707 cm^{-1}. Thus, dispersants in range ❶, ❷ and ❸ should be polyisobutenyl amine.

Therefore, four ranges of dispersants found in gasoline additive package (LZ8218C) are polyisobutenyl amine and polyether amine of propylene oxide repeating unit.

This research focus on dispersants in gasoline, which found with this condition; using DHB as matrix with analyte: matrix ratio 1:50 at laser power of 190 μJ. This condition was used for analysis of dispersants molecular weight distribution in commercial gasoline oil.

4.3 Application of MALDI-MS for analysis of dispersants in commercial gasoline

Commercial gasoline are always added dispersants to improve property of gasoline on regulation. Therefore this method can be used to analyze dispersants which are added in each commercial gasoline.

The commercial gasoline from eight companies, B (BANGCHAK), C (CALTEX), E (ESSO), J (JET), P (PTT), S (SHELL), T (TPI), and Q (Q8), were taken for analysis. The obtained MALDI-MS spectrums of dispersants in each commercial gasoline are shown in Figure A14-A29.

From MALDI-MS spectrum, of each commercial gasoline, dispersants were found in commercial gasoline from B, E, J, S, C and P companies (Figure A14-A25). In comparison with the MALDI-MS spectrum of dispersants in commercial gasoline from these companies and gasoline additive package (LZ 8218C) (Figure A11), the pattern of dispersants molecular weight distribution of commercial gasoline from these companies were found to be different with gasoline additive package (LZ 8218C). Therefore commercial gasoline from these companies did not use gasoline additive package (LZ 8218C). The information of dispersants in each commercial gasoline which obtained from MALDI-MS spectra are shown in Table 4.4.

Table 4.4 Mass to charge range of molecular weight distribution and MW of repeating unit of commercial gasoline

Commercial gasoline	m/z range of molecular weight distribution	MW of repeating unit (Da)
B 91	❶ 600-1300	56
	❷ 1200-2100	58
B 95	❶ 430-1050	56
	❷ 730-1230	56
	❸ 1280-2050	58
E 91	❶ 330-1005	56
	❷ 1090-1970	58
E 95	❶ 690-1200	56
	❷ 1150-1970	58
J 91	❶ 515 - 850	56
	❷ 800-1620	58
J 95	❶ 290 – 850	56
	❷ 860-1440	58
S 91	❶ 400 - 800	56
	❷ 840-1440	58
S 95	❶ 400 - 850	56
	❷ 800-1440	58
C 91	❶ 1320-2020	58
C 95	❶ 1320-2020	58
P 91	❶ 570 - 920	58
P 95	❶ 570-1035	58
T 91	-	-
T 95	-	-
Q 91	-	-
Q 95	-	-

From Table 4.8, dispersants with repeating unit 56 Da which have consisted of PIB were found in the range ❶ of B 91, E 91, E 95, J 91, J 95, S 91, S 95 and range ❶, ❷ of B 95. The calculation results from Equation 4.3 and 4.4 indicates that these dispersants might be either polyisobutenylsuccinimide or polyisobutenyl amine. The IR spectra of dispersants of each commercial gasoline are shown in Figure B2-B17. From IR-spectra of B 91, B 95, E 91, E 95, J 91, J 95, S 91 and S 95 (Figure B2-B9), there is no peak of C=O peak at 1700-1707 cm^{-1}. Therefore dispersants with repeating unit 56 Da in these commercial gasoline are polyisobutenyl amine.

Similar to dispersants in LZ 8218C (gasoline additive), dispersants with repeating unit 58 Da in the range ❶ of C 91, C 95, P 91, P 95, range ❷ of B 91, E 91, E 95, J 91, J 95, S 91, S 95, and range ❸ of B 95 are polyether amine of polypropylene oxide repeating unit.

The type $\overline{M}w$ and $\overline{M}n$ of dispersants in commercial gasoline from B, E, J, S, P and C companies are shown in Table 4.5.

Table 4.5 Type, $\overline{M}w$ and $\overline{M}n$ of dispersants in commercial gasoline

m/z range of molecular weight distribution of commercial gasoline	$\overline{M}w$	$\overline{M}n$	D_p	Type of dispersants
B 91				
❶ 600-1300	850	850	1.00	polyisobutenyl amine
❷ 1200-2100	1588	1575	1.01	polyether amine
B 95				
❶ 430-1050	702	679	1.03	polyisobutenyl amine
❷ 730-1230	874	858	1.02	polyisobutenyl amine
❸ 1280-2050	1579	1561	1.01	polyether amine
E 91				
❶ 330-1005	620	560	1.12	polyisobutenyl amine
❷ 1090-1970	1526	1502	1.02	polyether amine
E 95				
❶ 690-1200	929	909	1.02	polyisobutenyl amine
❷ 1150-1970	1499	1478	1.01	polyether amine

Table 4.5 (continued)   Type, $\overline{M}w$ and $\overline{M}n$ of dispersants in commercial gasoline

m/z range of molecular weight distribution of commercial gasoline	$\overline{M}w$	$\overline{M}n$	D_p	Type of dispersants
J 91				
❶ 515 - 850	644	628	1.03	polyisobutenyl amine
❷ 800-1620	1126	1096	1.03	polyether amine
J 95				
❶ 290 – 850	548	505	1.09	polyisobutenyl amine
❷ 860-1440	1089	1071	1.02	polyether amine
S 91				
❶ 400 - 800	590	560	1.05	polyisobutenyl amine
❷ 840-1440	1092	1074	1.02	polyether amine
S 95				
❶ 400 - 850	538	526	1.02	polyisobutenyl amine
❷ 800-1440	1064	1044	1.02	polyether amine
C 91				
❶ 1320-2020	1599	1512	1.06	polyether amine
C 95				
❶ 1320-2020	1611	1611	1.00	polyether amine
P 91				
❶ 570 - 920	728	718	1.01	polyether amine
P 95				
❶ 570-1035	768	755	1.02	polyether amine

CHAPTER V

CONCLUSION

In this research, MALDI-MS was developed to determine the molecular weight distribution of dispersants. The dispersant was separated from gasoline additive package (LZ 8218C). Then, the dispersants were analyzed by MALDI-MS. The optimum conditions and parameters were studied for determination of molecular weight distribution of dispersants.

The results show that the best analyte-to-matrix ratio was found to be 1:50. The optimal condition for this analysis is: 2-5 dihydroxybenzoic acid (DHB) as a matrix and laser power 190 μJ which provided a good MALDI-MS spectrum for determination of molecular weight distribution of dispersants. Cationizing agent was found to have no improvement on ionization of dispersants.

From MALDI-MS and FTIR spectroscopy analysis of LZ 8218C gasoline additive package, two kinds of polymer were found, polyisobutenyl amine in the m/z range of 200-600, 390-1000 and 630-1150 and polyether amine in the m/z range of 1200-2300.

A developed method was used to analyze dispersants in commercial gasoline from eight companies, B, C, E, J, P, S, T, and Q. Results showed that dispersants which different pattern of molecular weight distribution with gasoline additive package (LZ8218C) were found in commercial gasoline from B, C, E, J, P, and S companies. Two types of dispersants, polyisobutenyl amine and polyether amine, were found in commercial gasoline from B, E, J and S companies. Commercial gasolines from C and P companies were found only polyether amine as dispersants.

It is concluded that MALDI-MS is an excellent method for determination of type and molecular weight distribution of dispersants. This method can be used for identification of dispersant in commercial gasoline oils.

It is suggested that a develop MALDI-MS method may be used for quality control of dispersant production and identify the source of unknown commercial gasoline by observation of the pattern of molecular weight distribution of dispersants. For further research, MALDI-MS method could be developed to quantitative analysis of dispersants in fuel oils if reference standard is available.

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APPENDICES

APPENDIX A
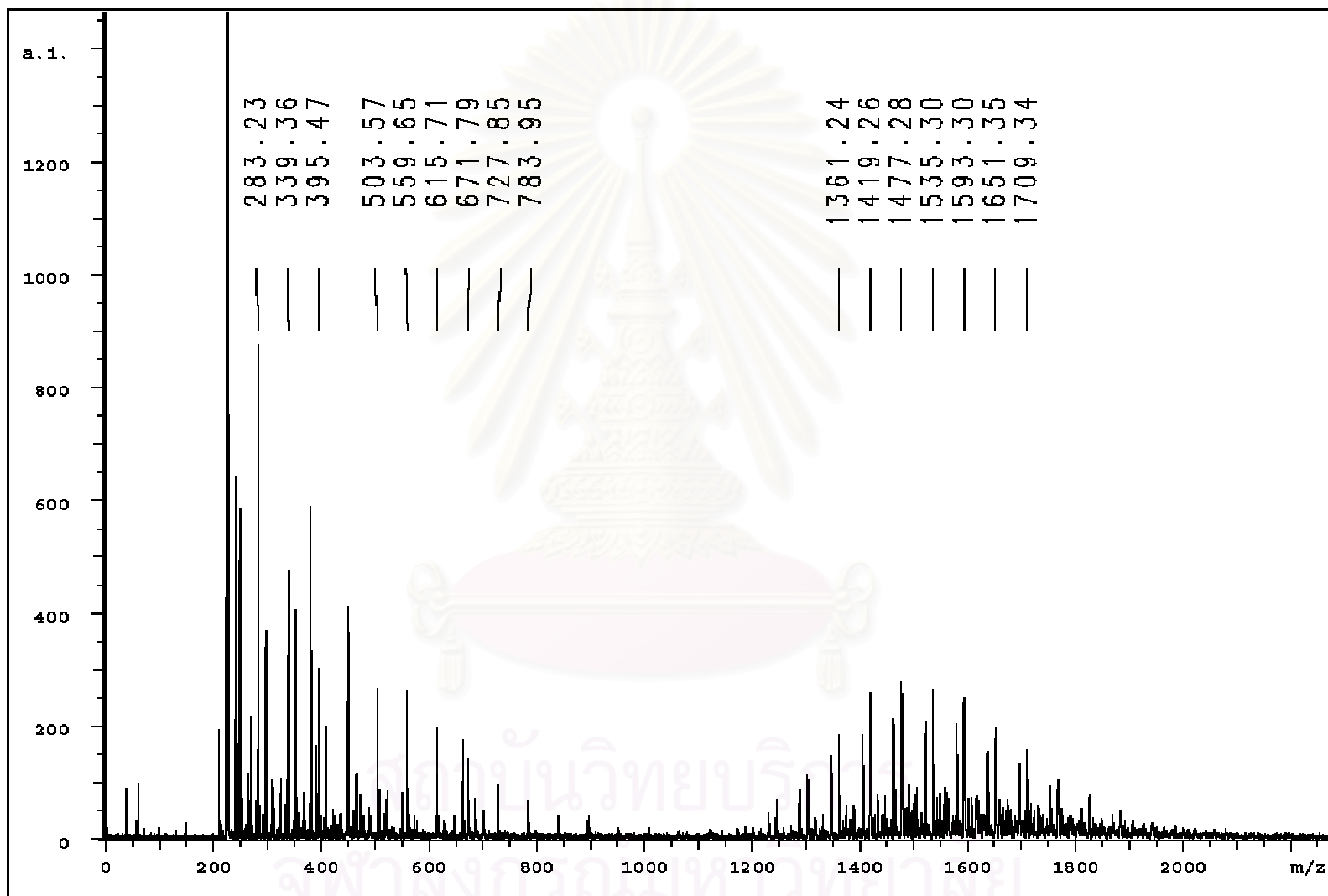
Figure A1   MALDI MS spectrum of dispersants using dithranol as matrix.
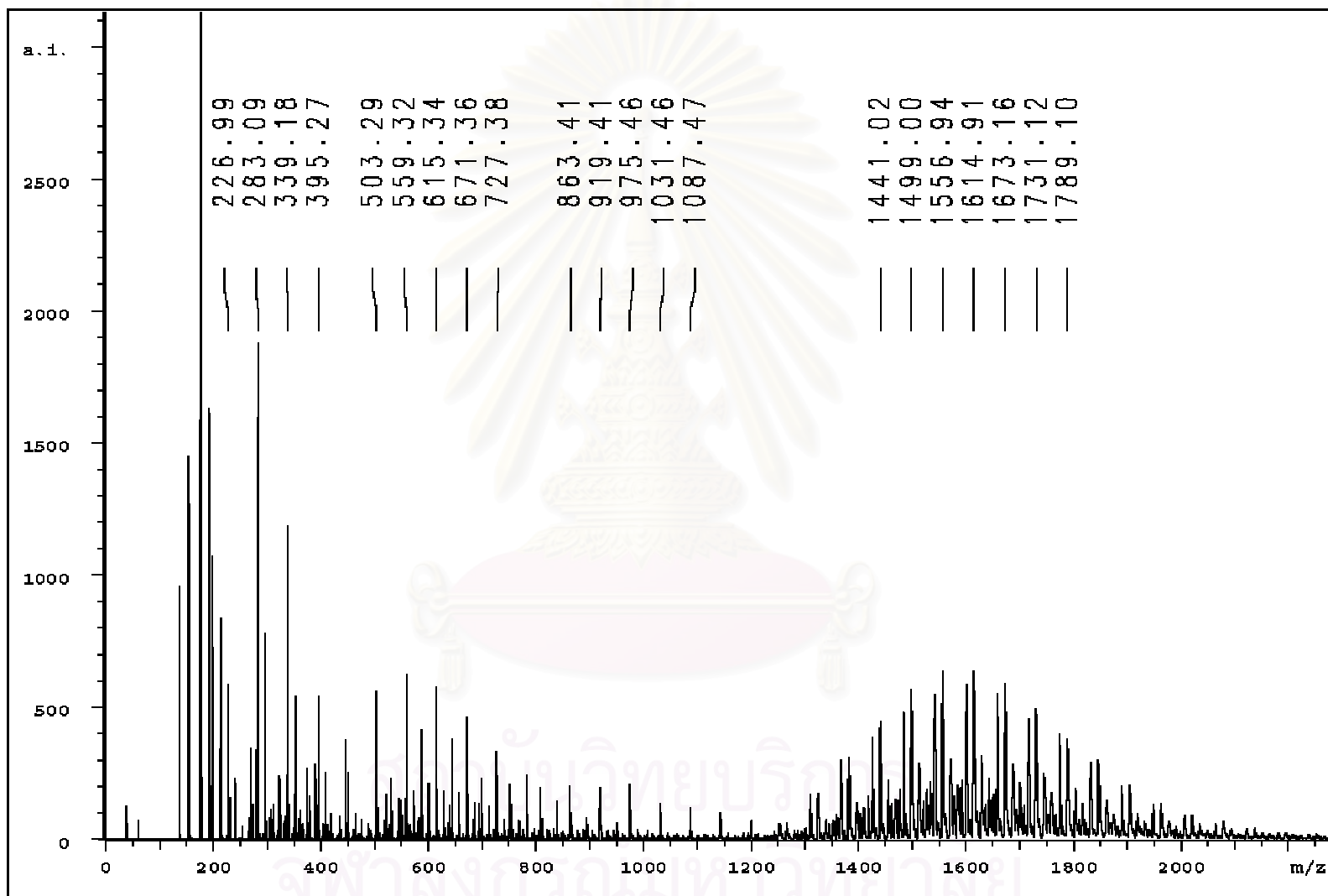
Figure A2 MALDI MS spectrum of dispersants using 2,5-dihydroxybenzoic acid (DHB) as matrix.

227.08
283.18
339.29
395.39
451.47
531.51
587.55
643.59
699.63
863.74
919.77
975.83
1031.85
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1790.02
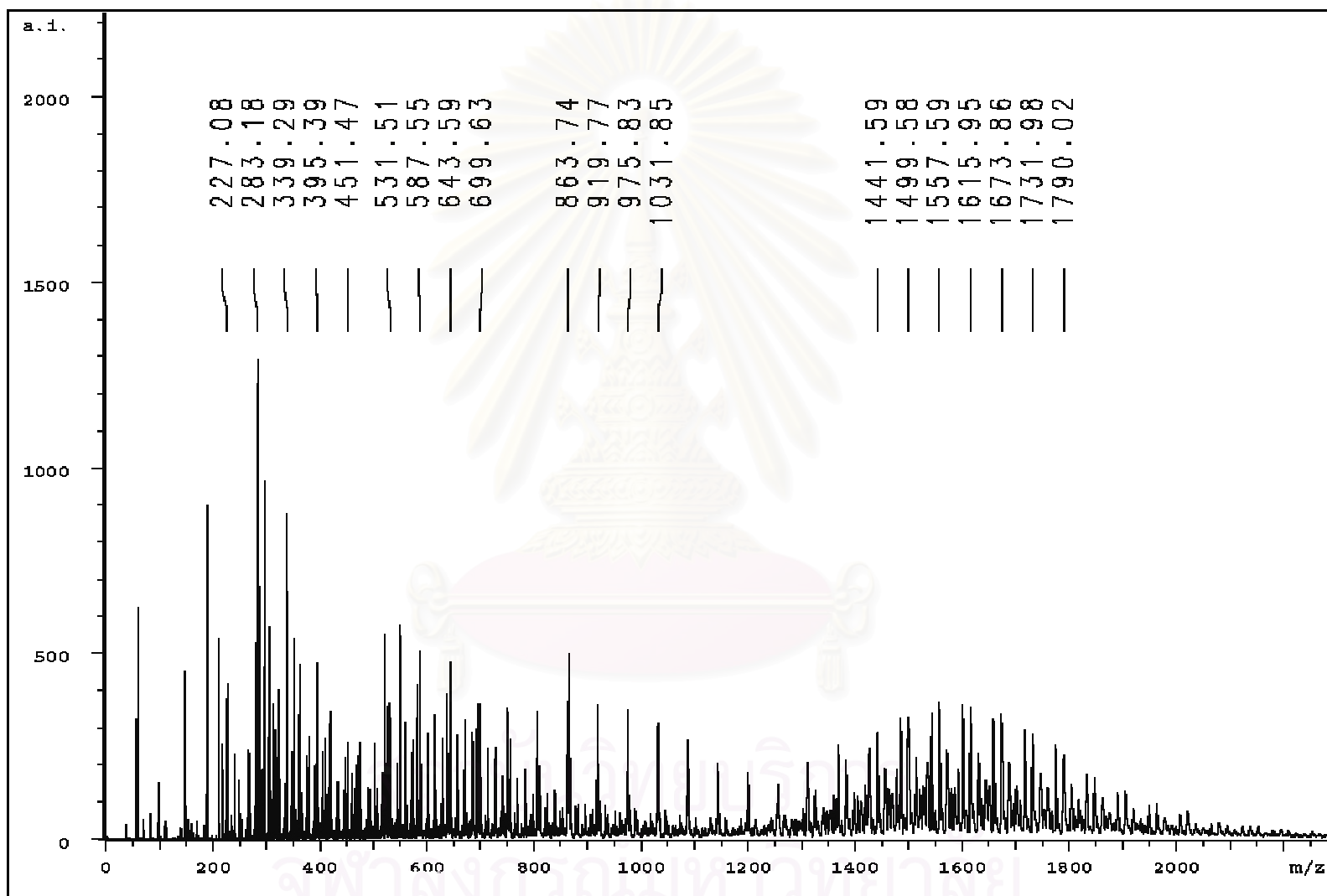
Figure A3 MALDI MS spectrum of dispersants using ∝-hydroxycyanocinamic acid as matrix.
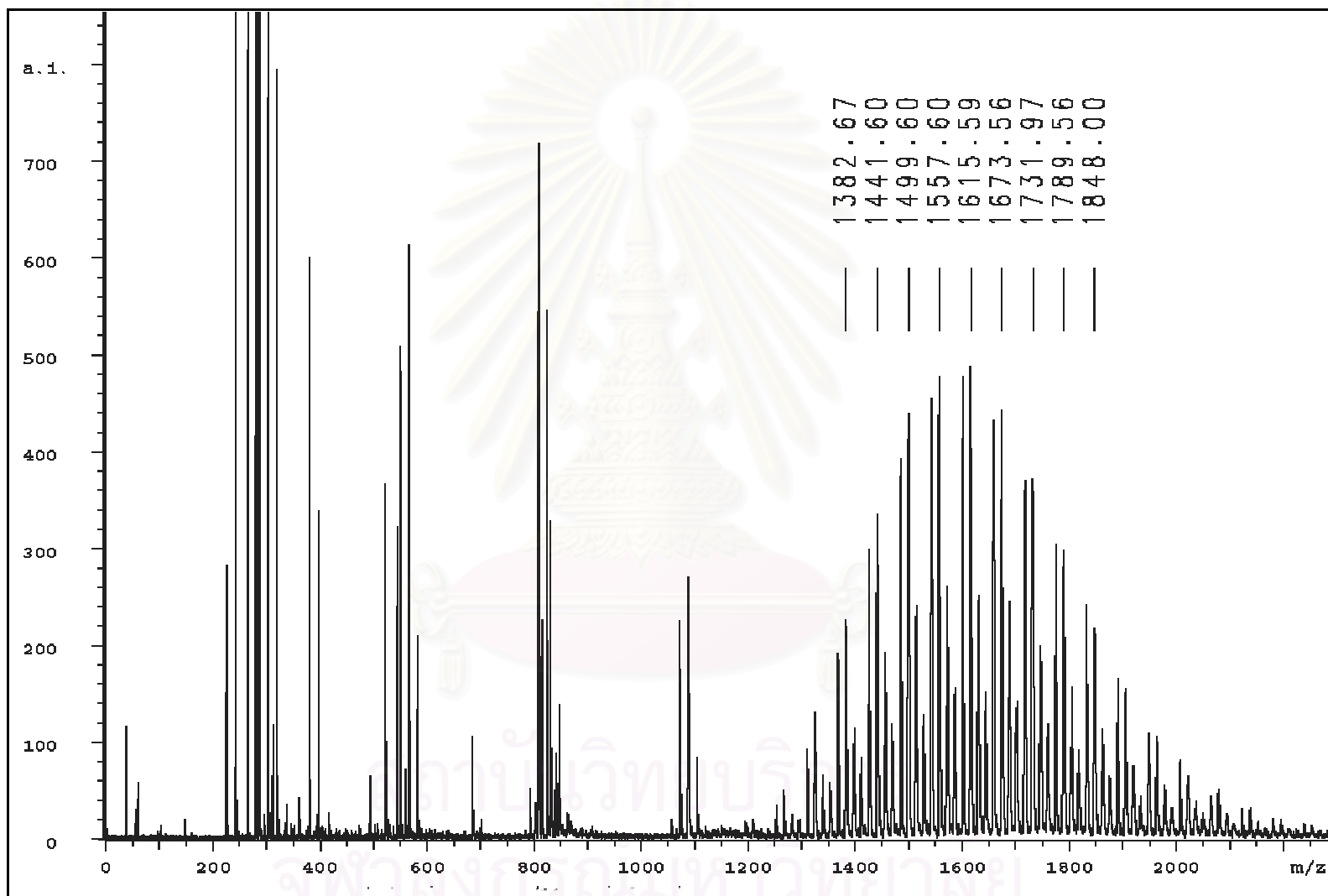
Figure A4 MALDI MS spectrum of dispersants using 2-(4-hydroxyphenylazo)-benzoic acid (HABA) as matrix.
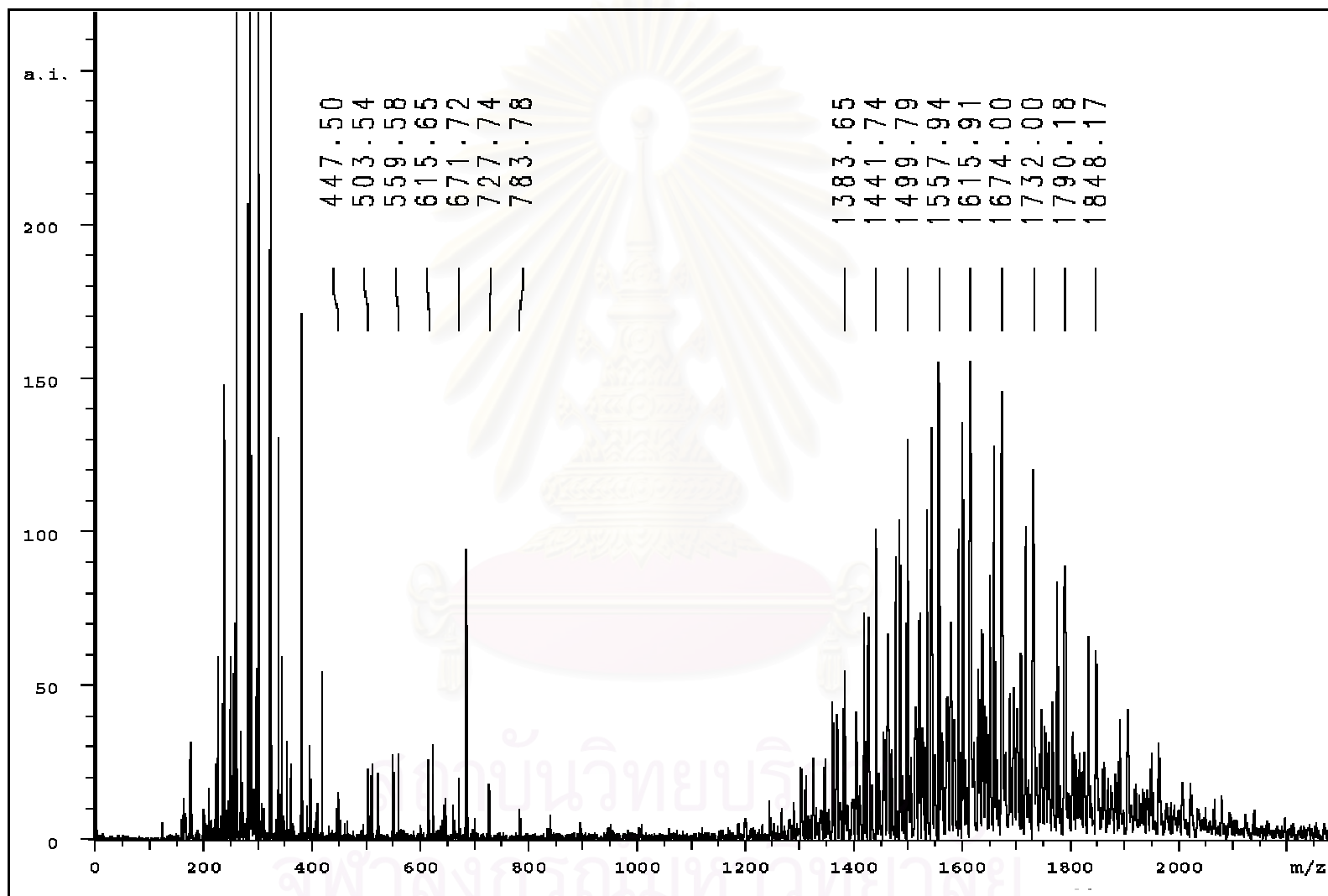
Figure A5 MALDI MS spectrum of dispersants using *all-trans* retinoic acid as matrix.
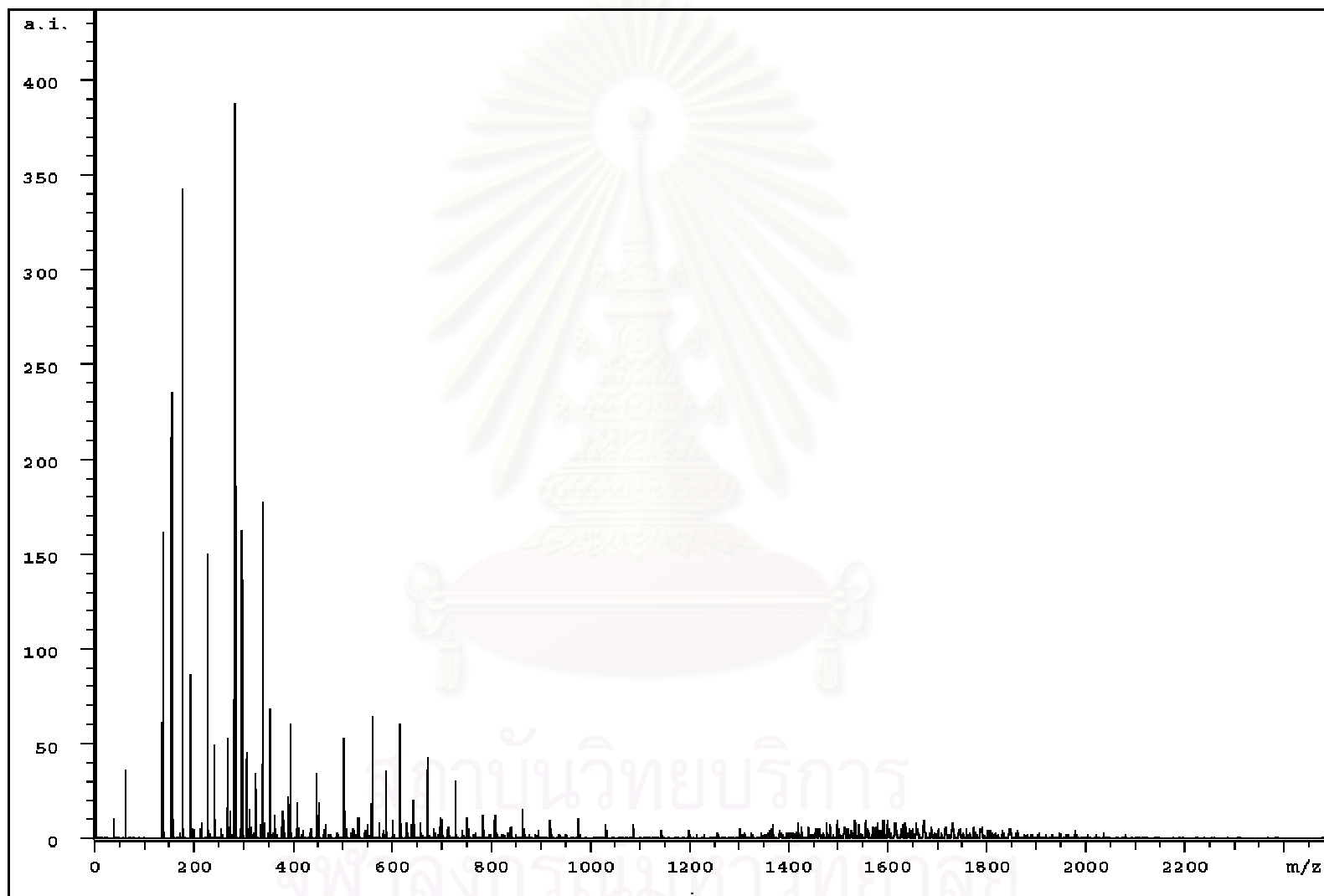
Figure A6 MALDI MS spectrum of dispersants using DHB as matrix, an analyte to matrix ratio of 1:25.
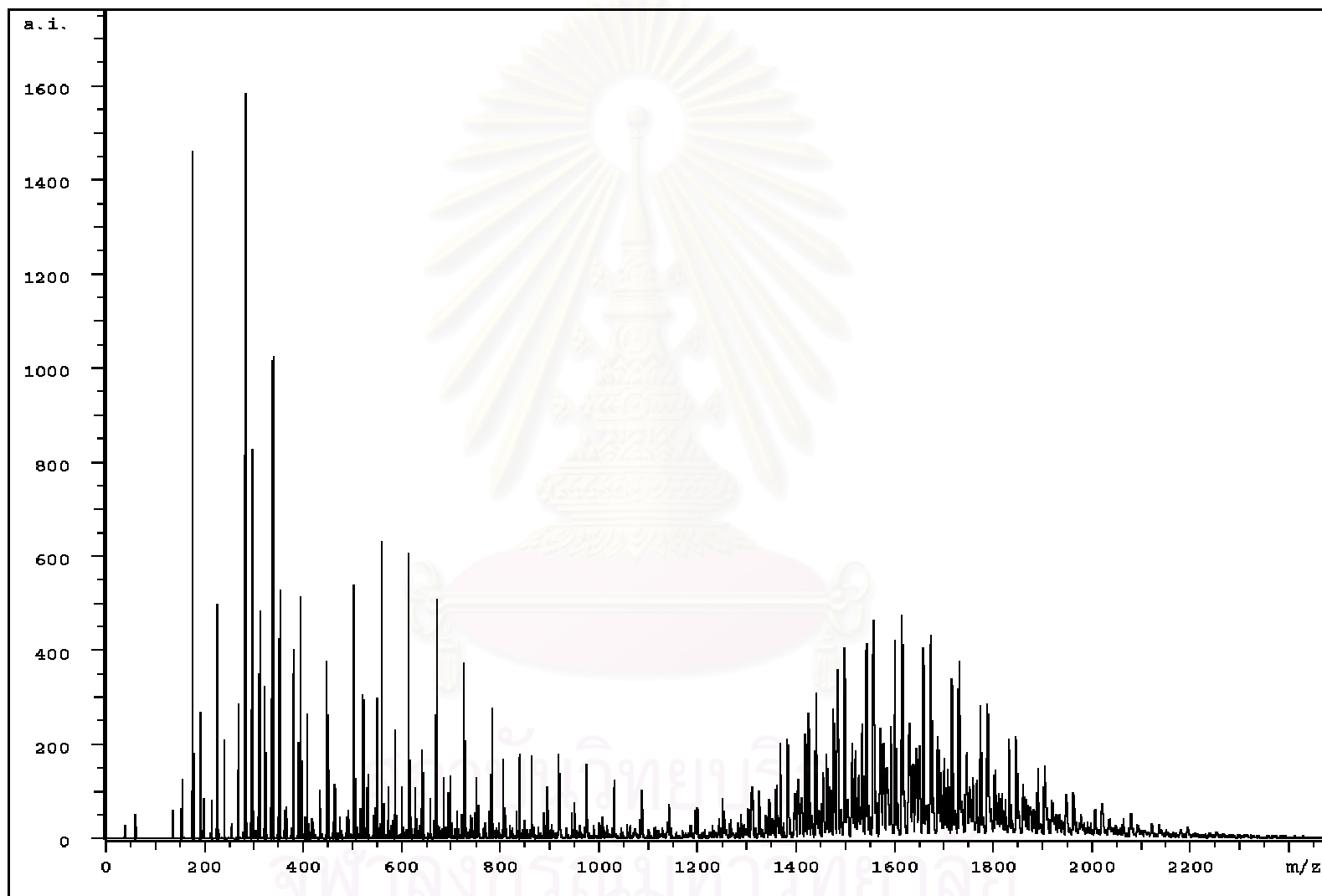
Figure A7   MALDI MS spectrum of dispersants using DHB as matrix, an analyte to matrix ratio of 1:50.
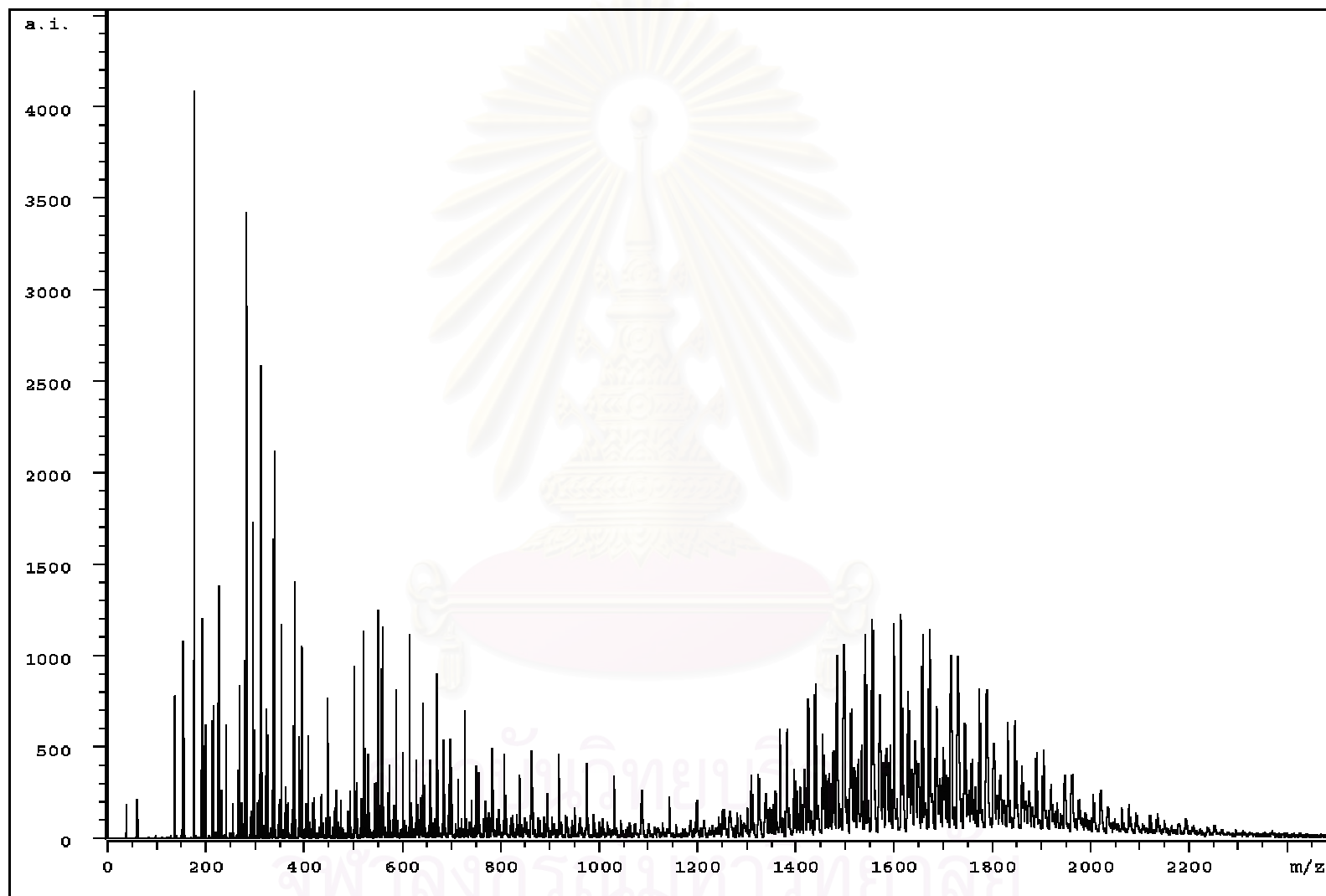
Figure A8 MALDI MS spectrum of dispersants using DHB as matrix, an analyte to matrix ratio of 1:75.
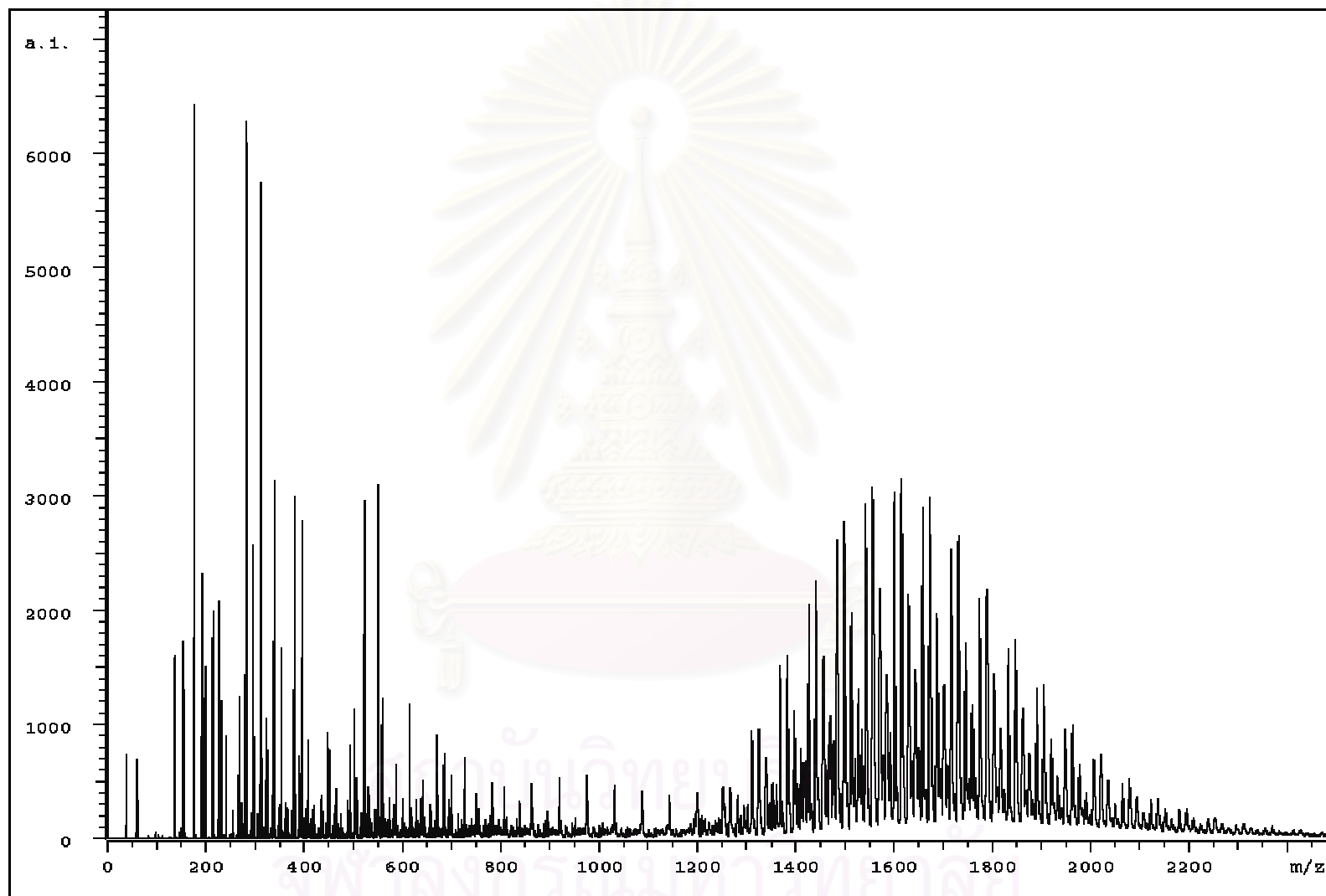
Figure A9   MALDI MS spectrum of dispersants using DHB as matrix, an analyte to matrix ratio of 1:100.
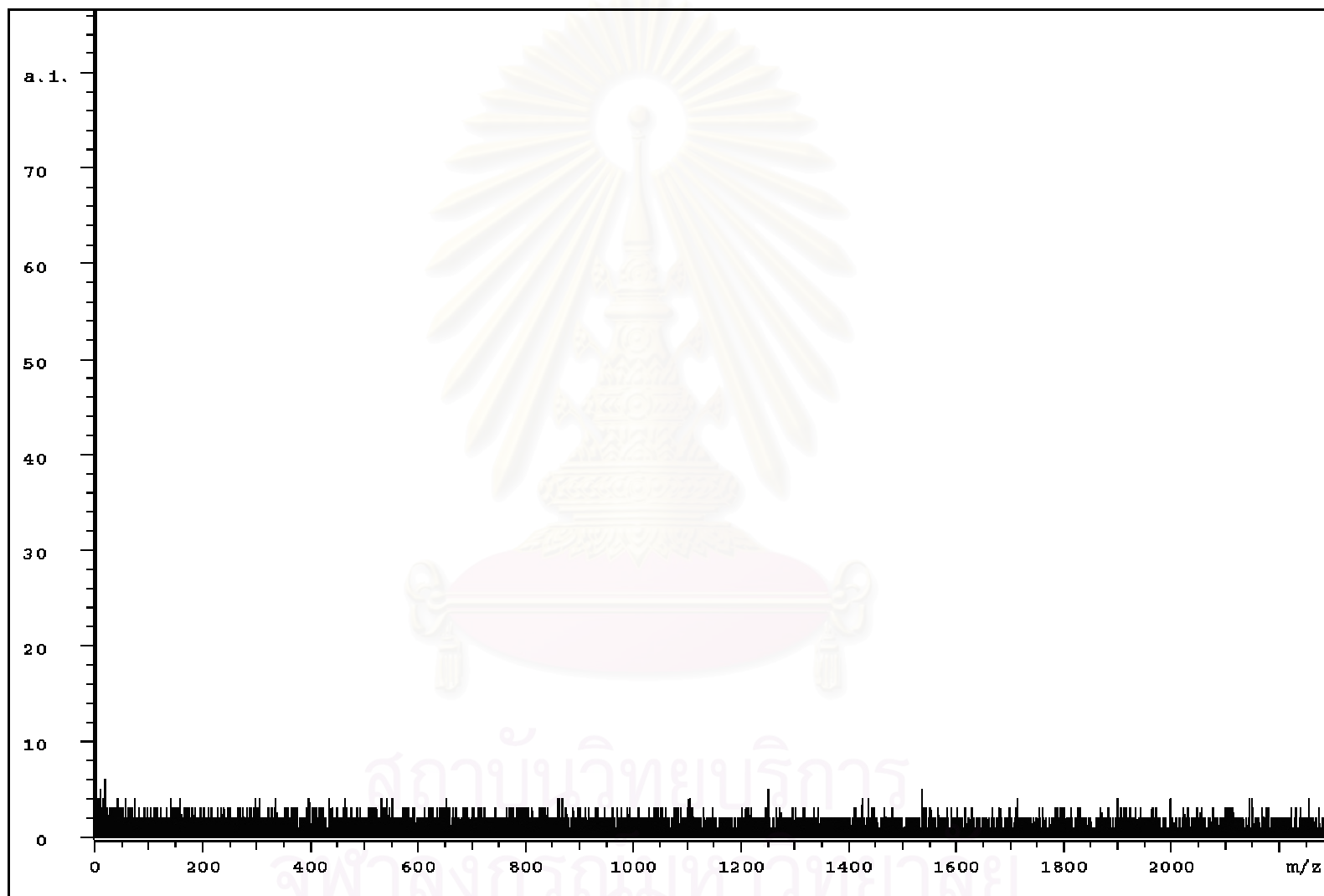
Figure A10   MALDI MS spectrum of dispersants using DHB as matrix, an analyte to matrix ratio of 1:50, laser power values 180 µJ.
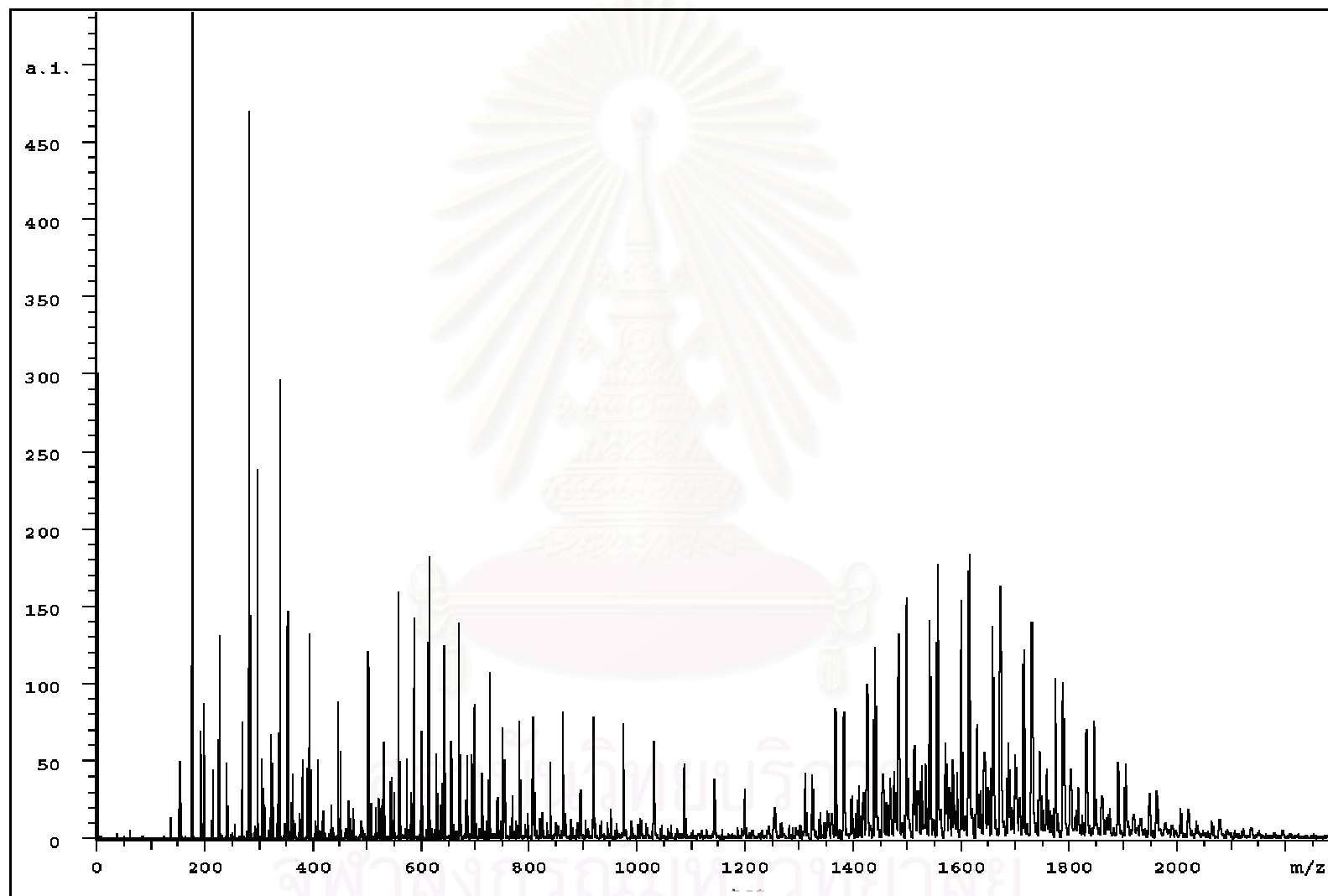
Figure A11 MALDI MS spectrum of dispersants using DHB as matrix, an analyte to matrix ratio of 1:50, laser power values 190 µJ.
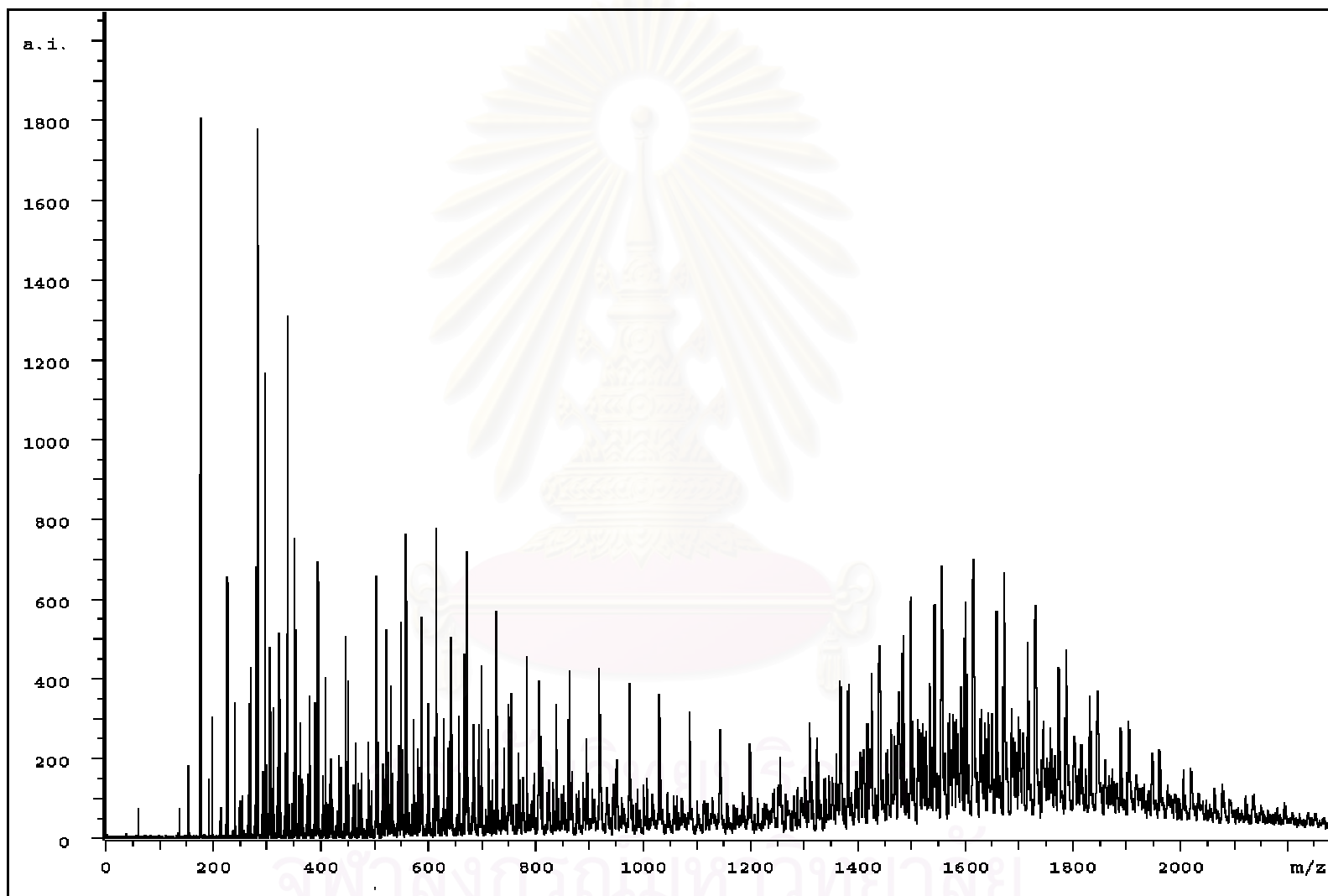
Figure A12 MALDI MS spectrum of dispersants using DHB as matrix, an analyte to matrix ratio of 1:50, laser power values 200 µJ.
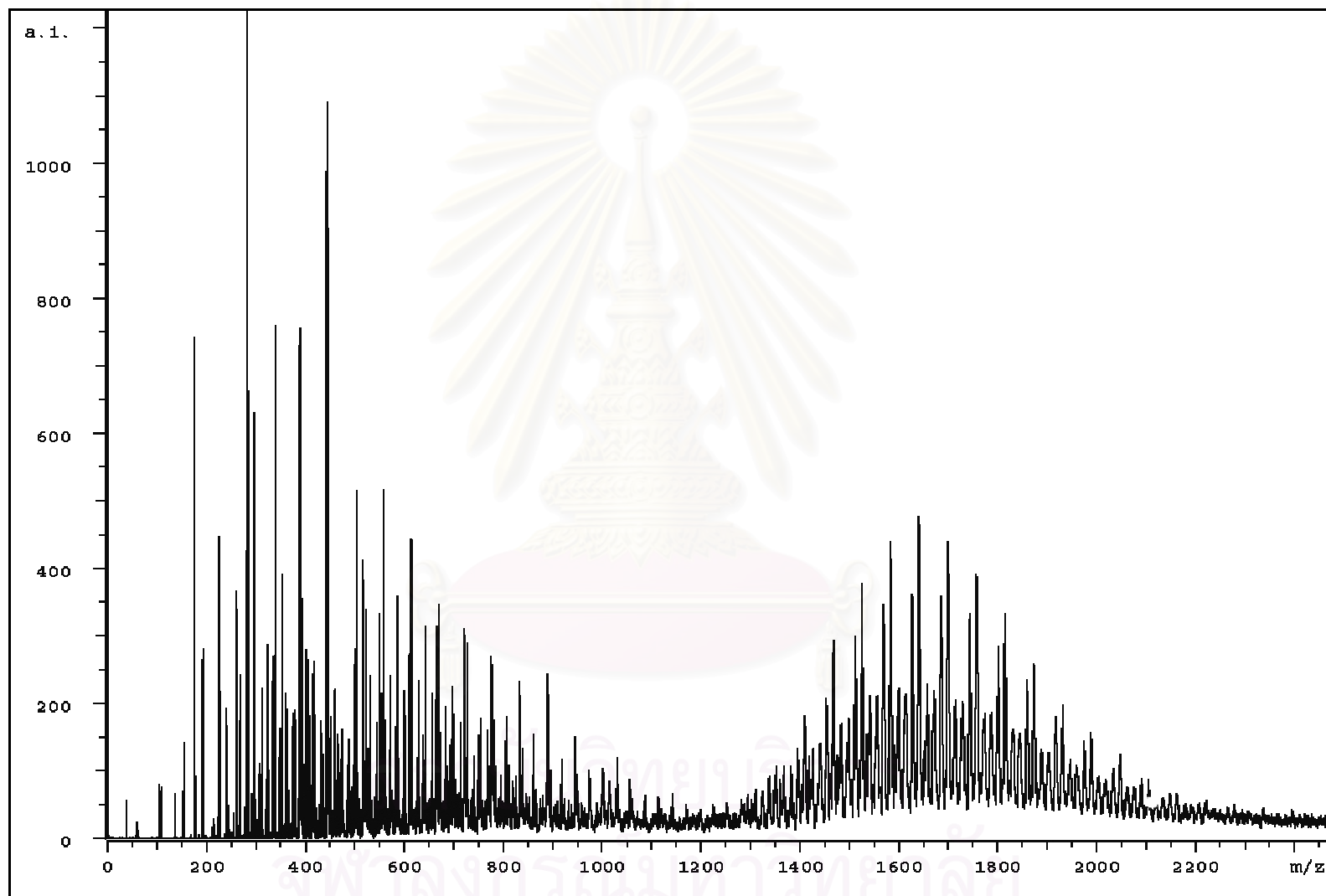
Figure A13   MALDI MS spectrum of dispersants using DHB as matrix, an analyte to matrix ratio of 1:50,
AgNO$_3$ as a cationizing agent, laser power values 190 µJ.
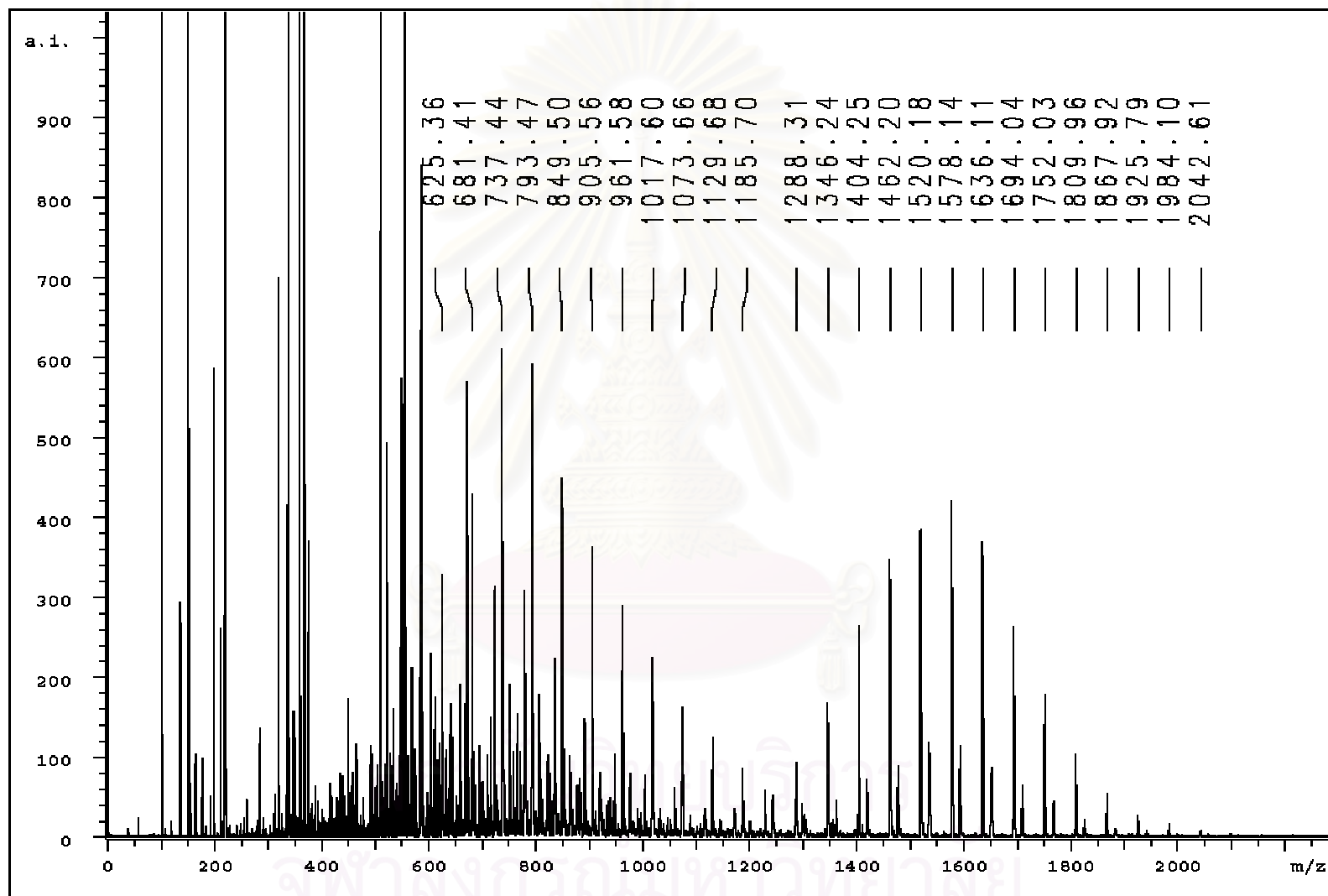
Figure A14 MALDI MS spectrum of dispersants separated from B 91 gasoline oil.

(MALDI-TOF MS condition as shown in Figure A11.)
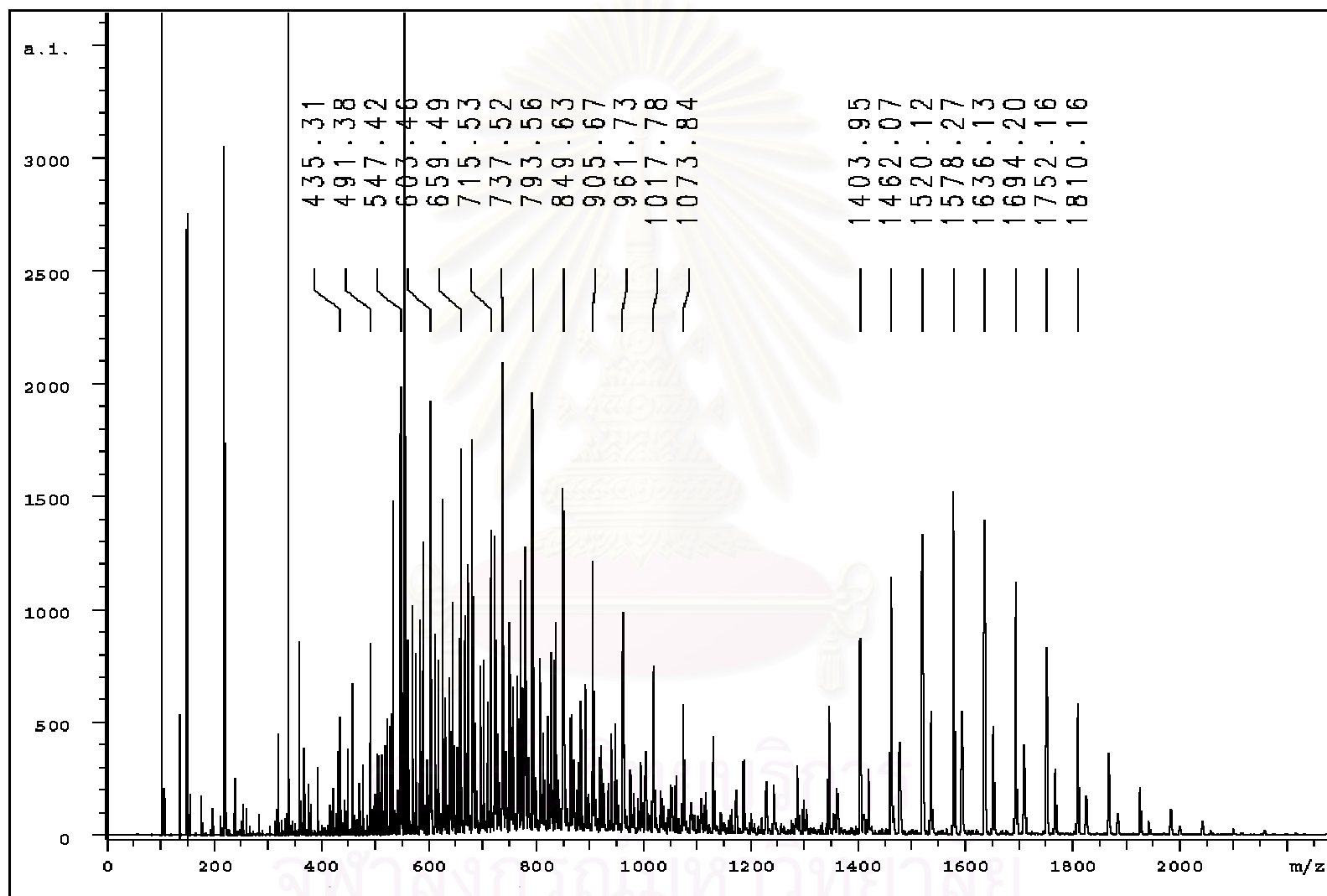
Figure A15 MALDI MS spectrum of dispersants separated from B 95 gasoline oil.
(MALDI-TOF MS condition as shown in Figure A11.)
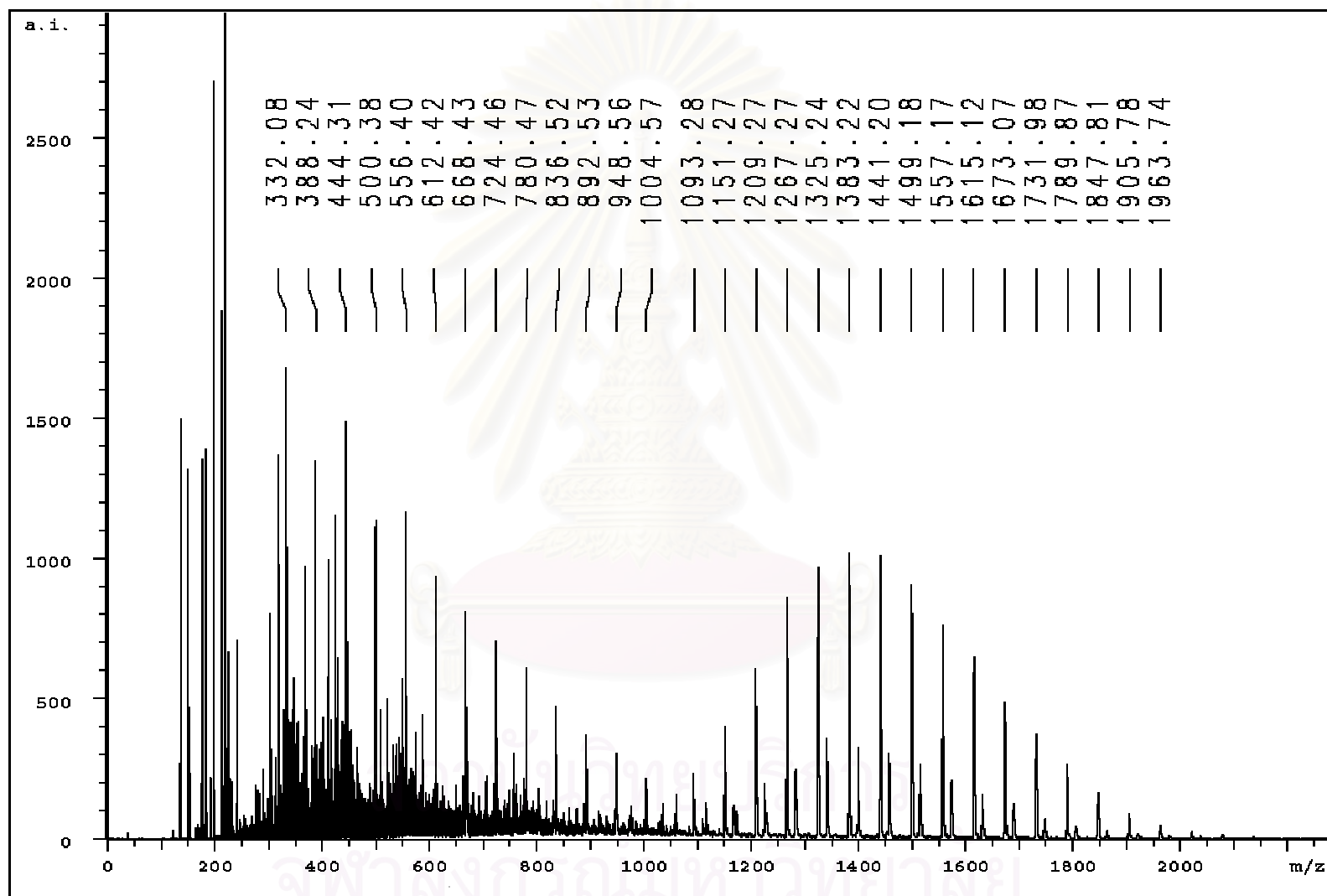
Figure A16 MALDI MS spectrum of dispersants separated from E 91 gasoline oil.

(MALDI-TOF MS condition as shown in Figure A11.)
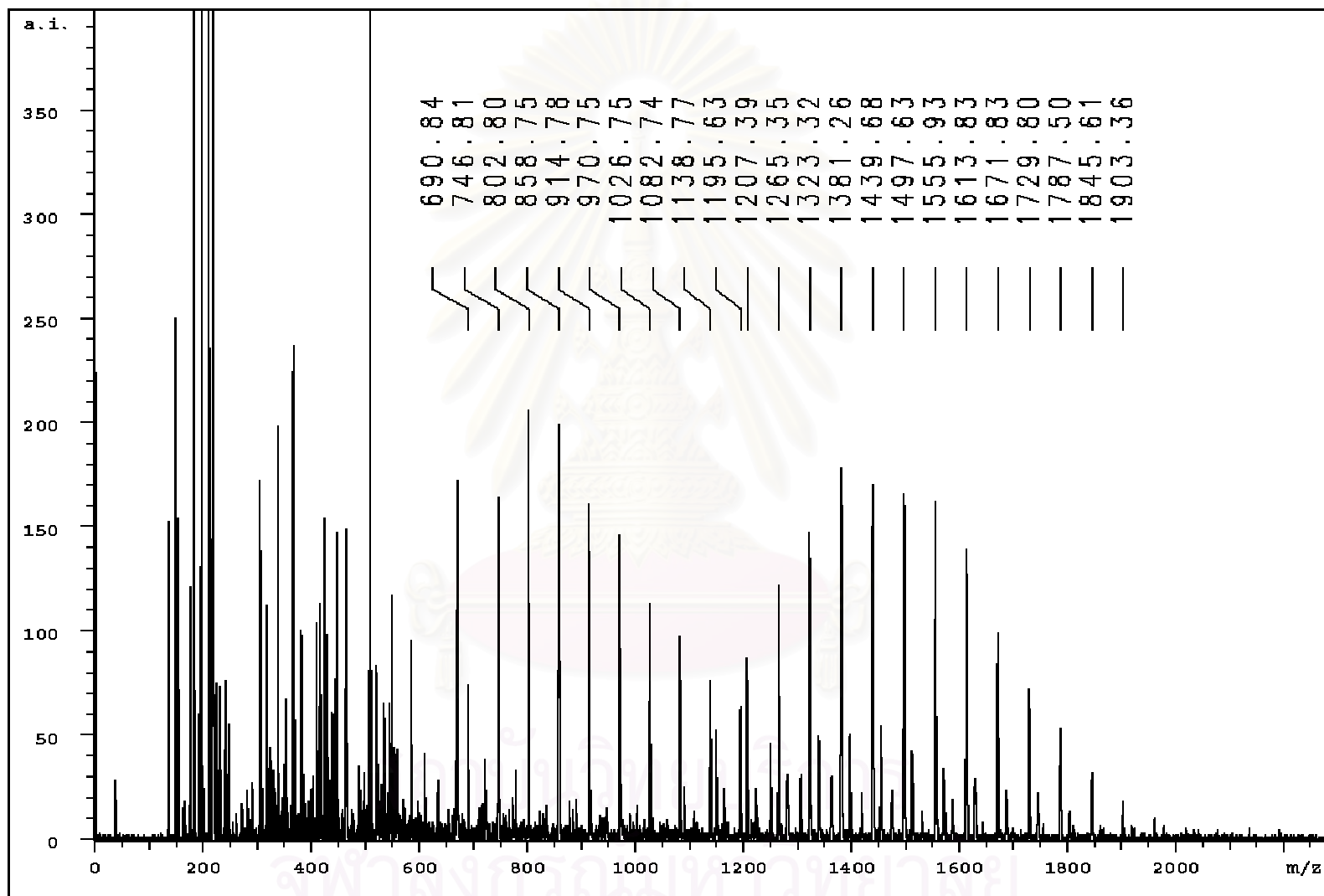
Figure A17 MALDI MS spectrum of dispersants separated from E 95 gasoline oil.
(MALDI-TOF MS condition as shown in Figure A11.)
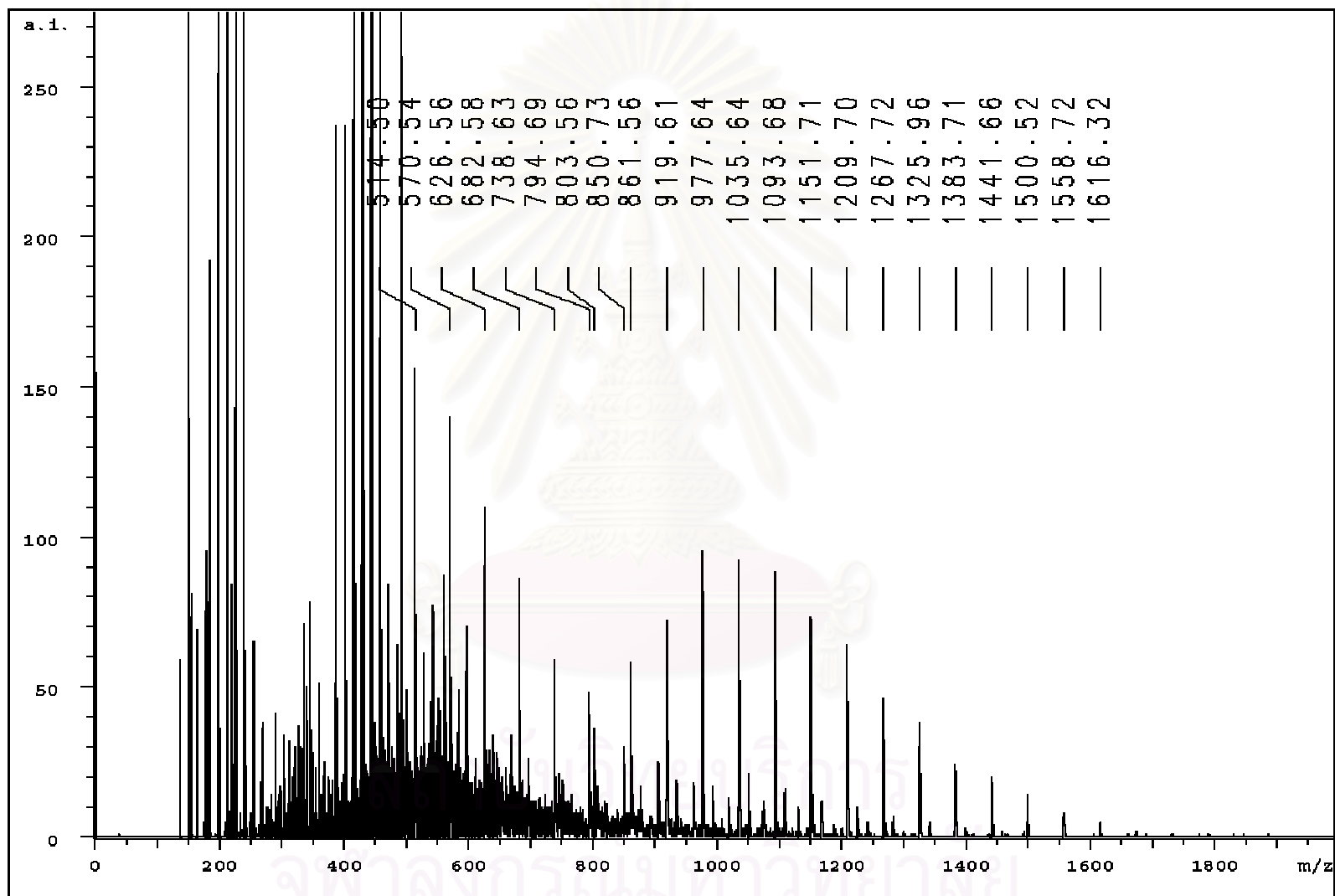
Figure A18 MALDI MS spectrum of dispersants separated from J 91 gasoline oil.
(MALDI-TOF MS condition as shown in Figure A11.)
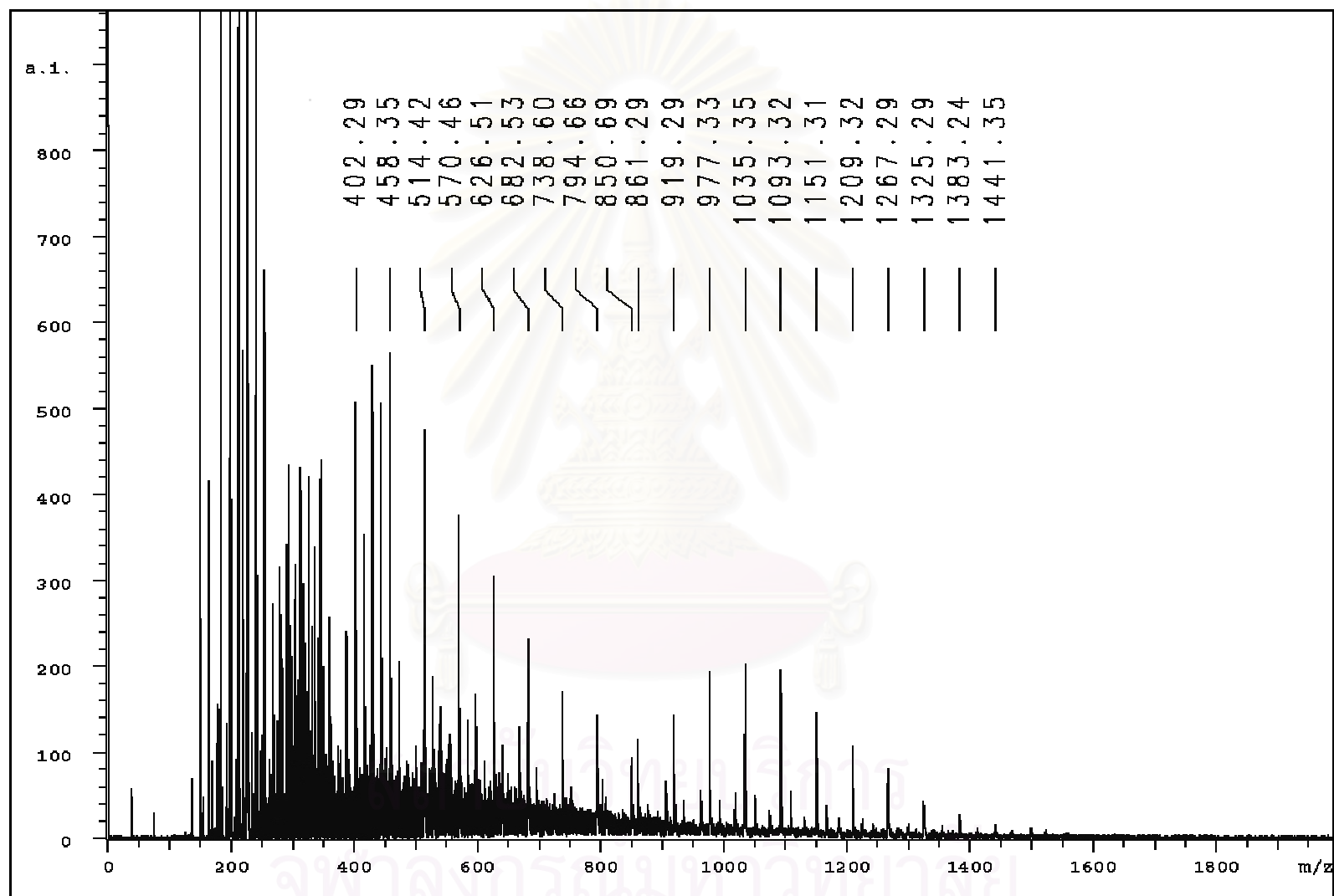
Figure A19 MALDI MS spectrum of dispersants separated from J 95 gasoline oil.

(MALDI-TOF MS condition as shown in Figure A11.)
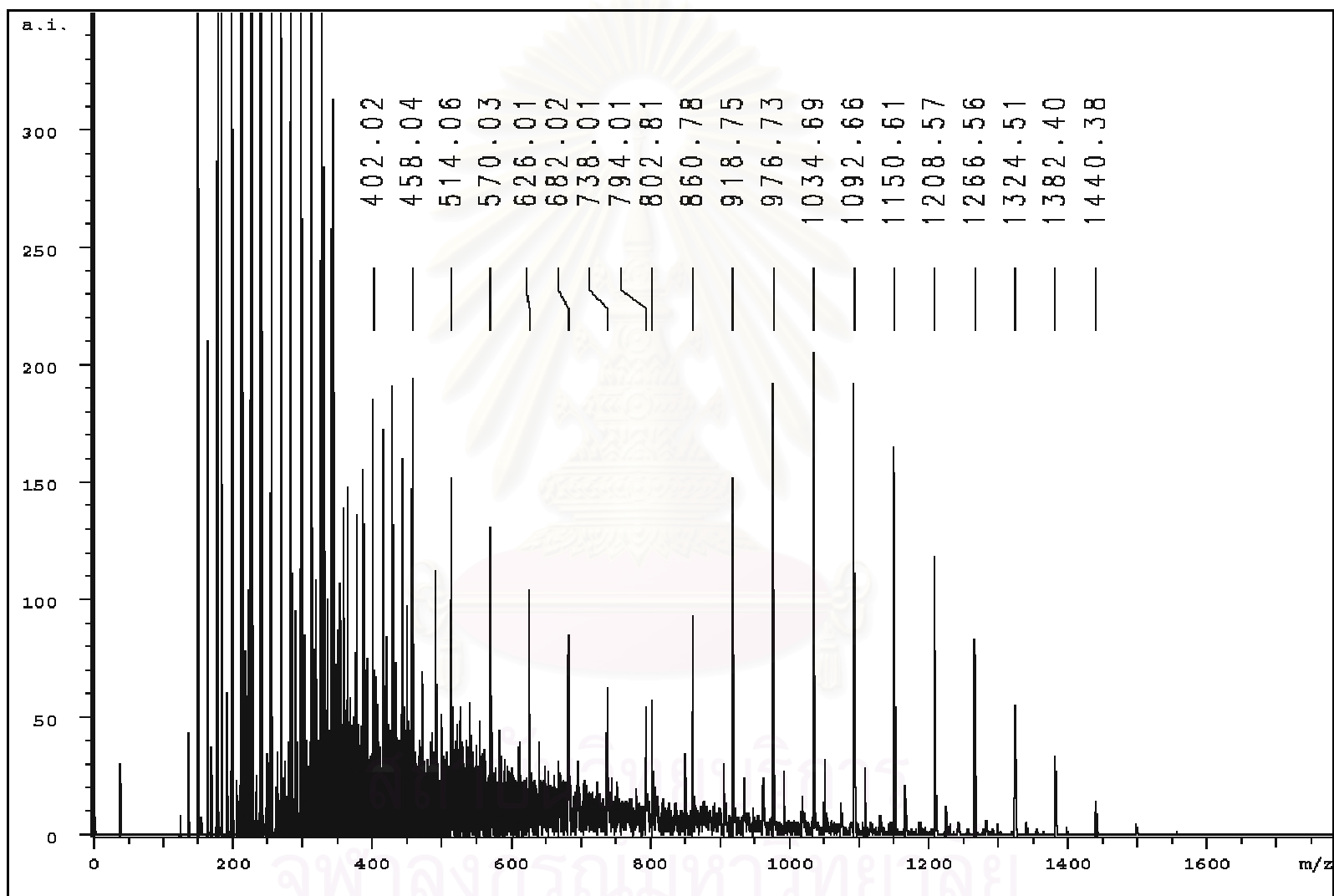
Figure A20   MALDI MS spectrum of dispersants separated from S 91 gasoline oil.
(MALDI-TOF MS condition as shown in Figure A11.)
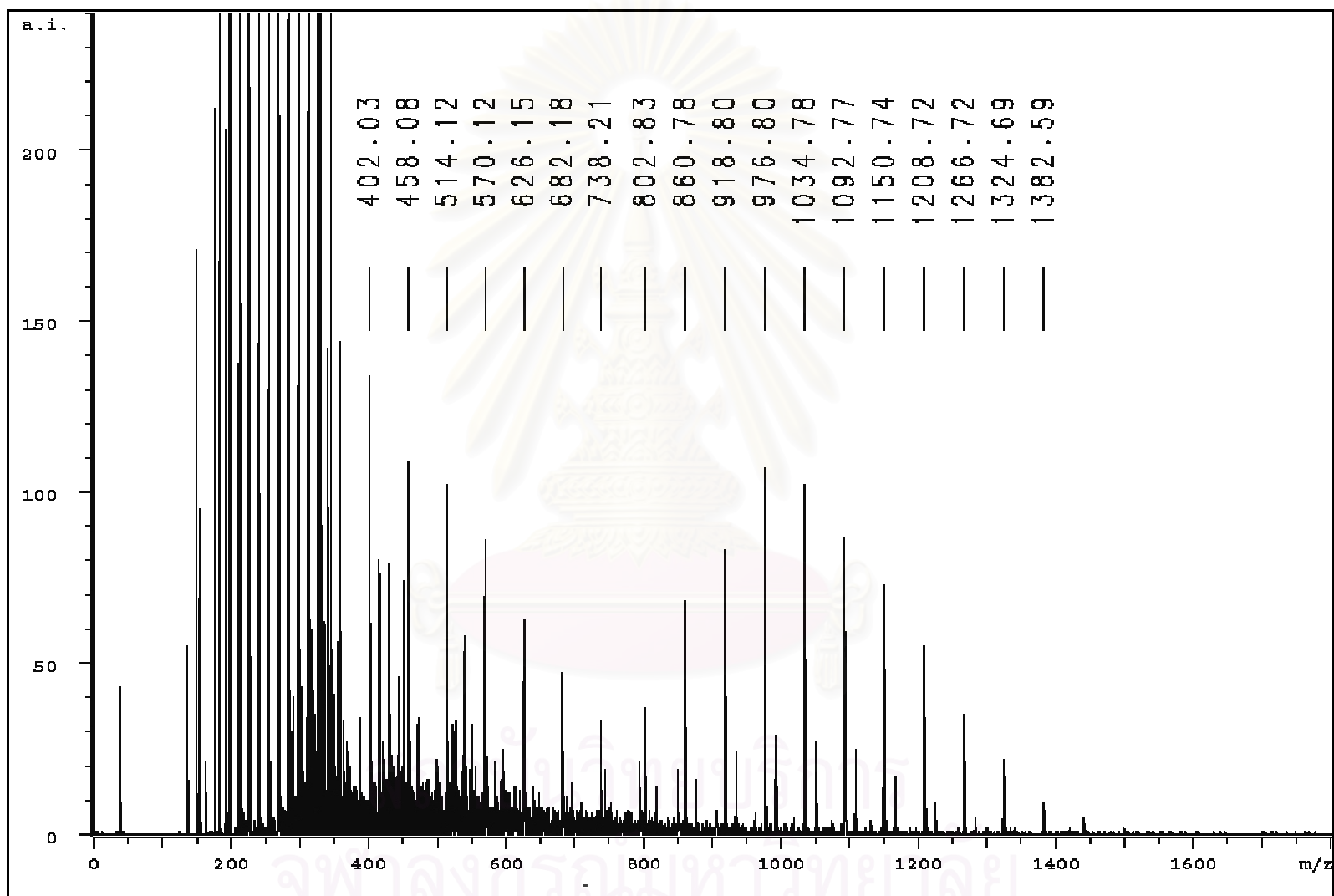
Figure A21 MALDI MS spectrum of dispersants separated from S 95 gasoline oil.
(MALDI-TOF MS condition as shown in Figure A11.)
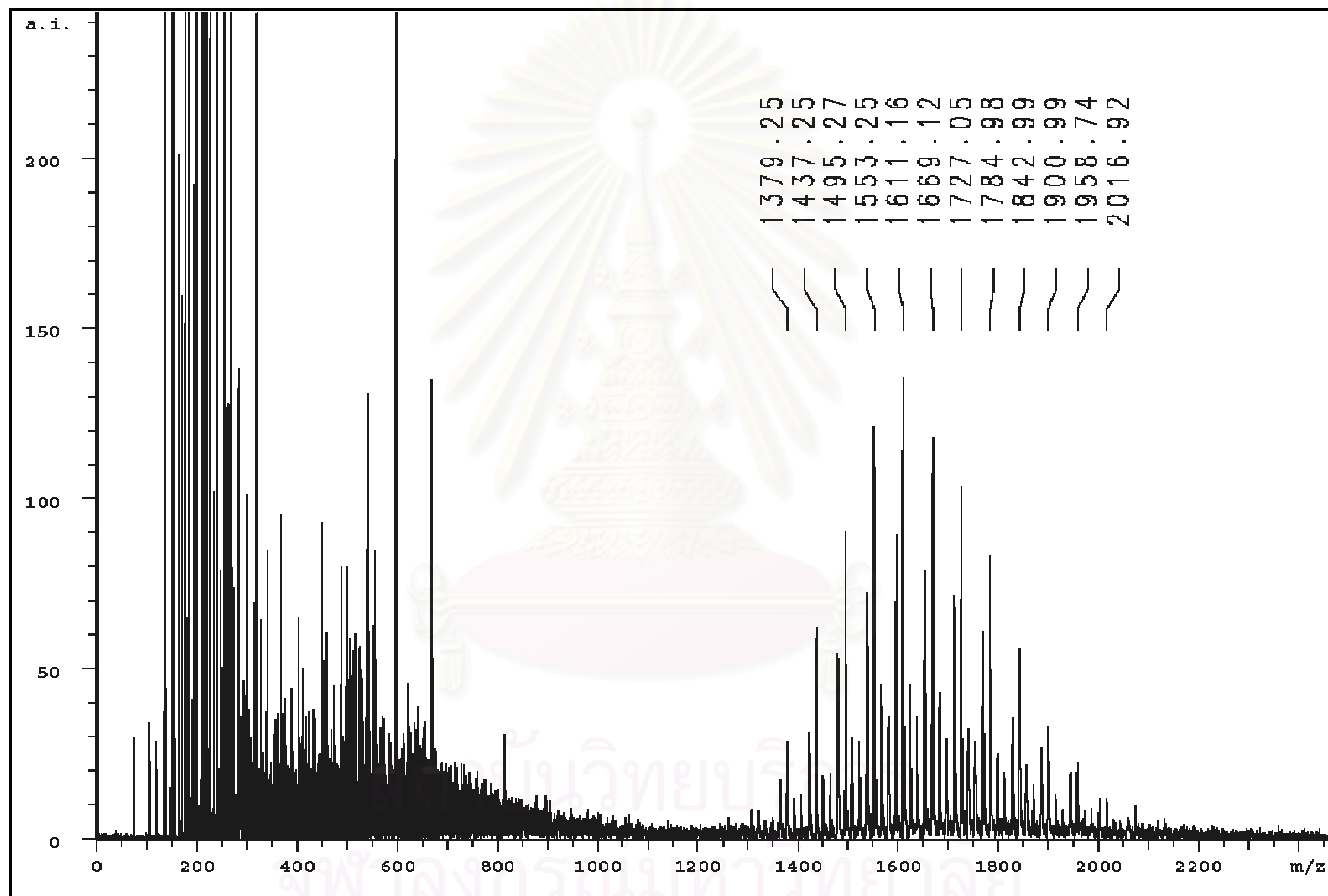
Figure A22 MALDI MS spectrum of dispersants separated from C 91 gasoline oil.

(MALDI-TOF MS condition as shown in Figure A11.)
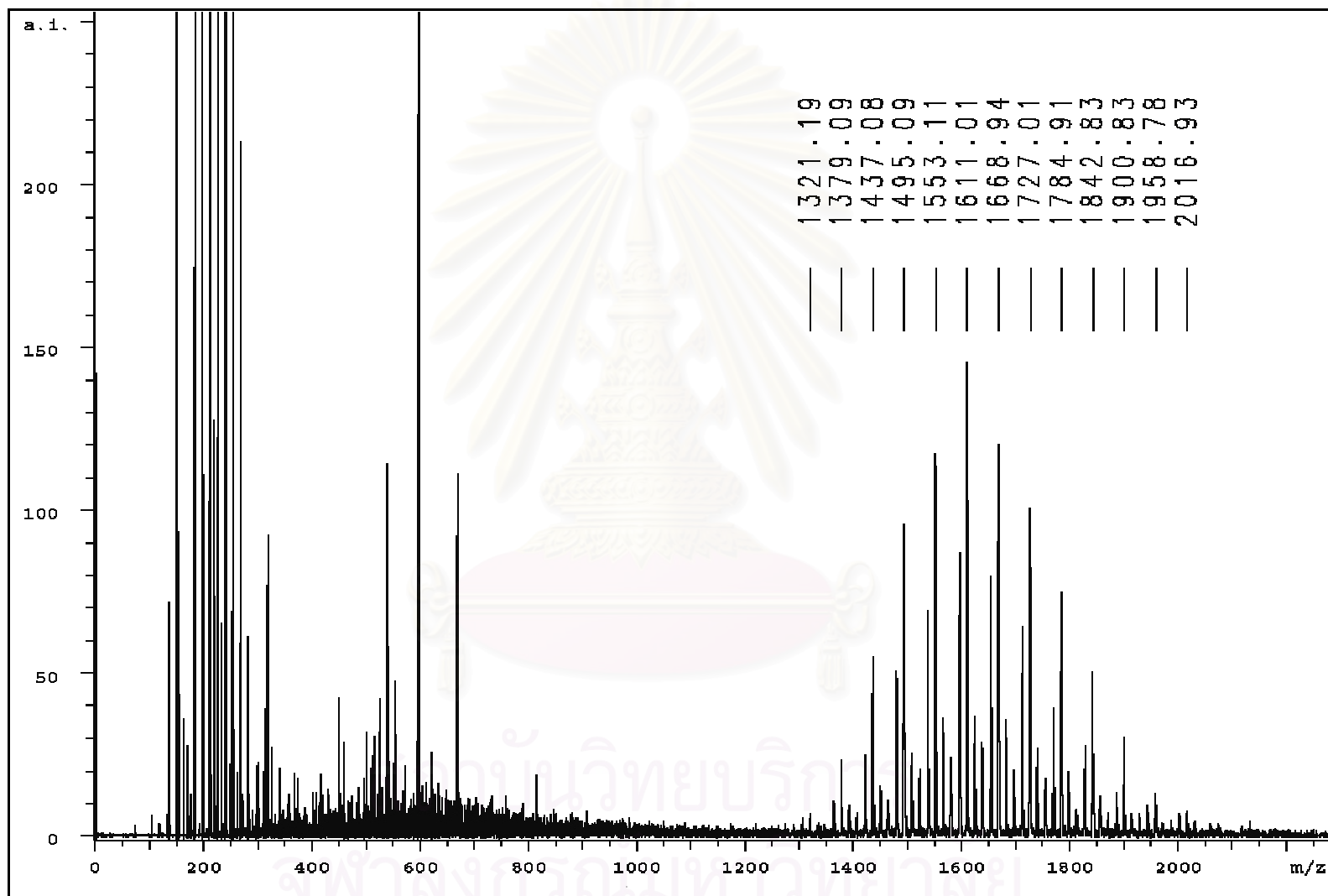
Figure A23 MALDI MS spectrum of dispersants separated from C 95 gasoline oil.
(MALDI-TOF MS condition as shown in Figure A11.)
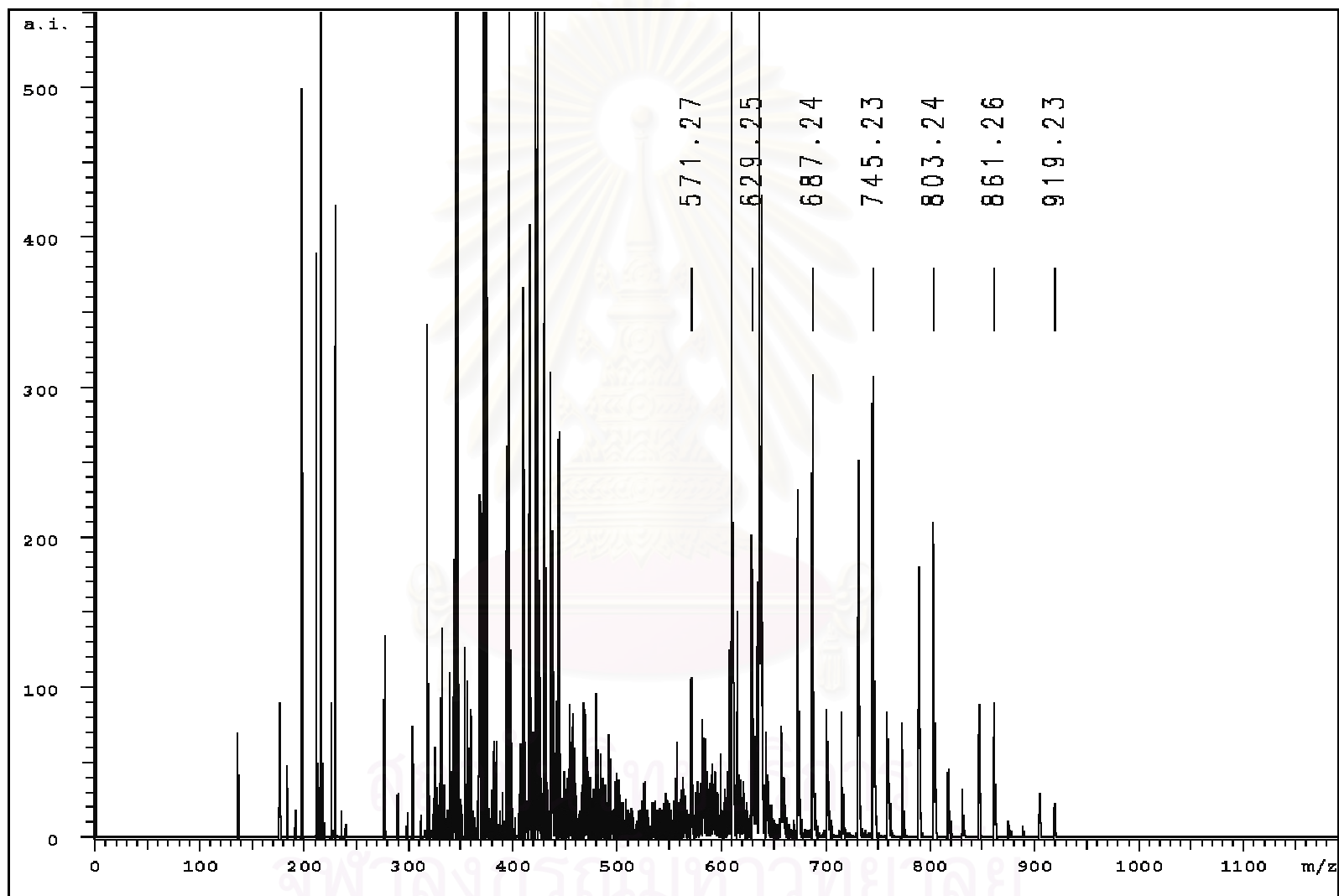
Figure A24 MALDI MS spectrum of dispersants separated from P 91 gassoline oil.

(MALDI-TOF MS condition as shown in Figure A11.)

Figure A25 MALDI MS spectrum of dispersants separated from P 95 gasoline oil.
(MALDI-TOF MS condition as shown in Figure A11.)
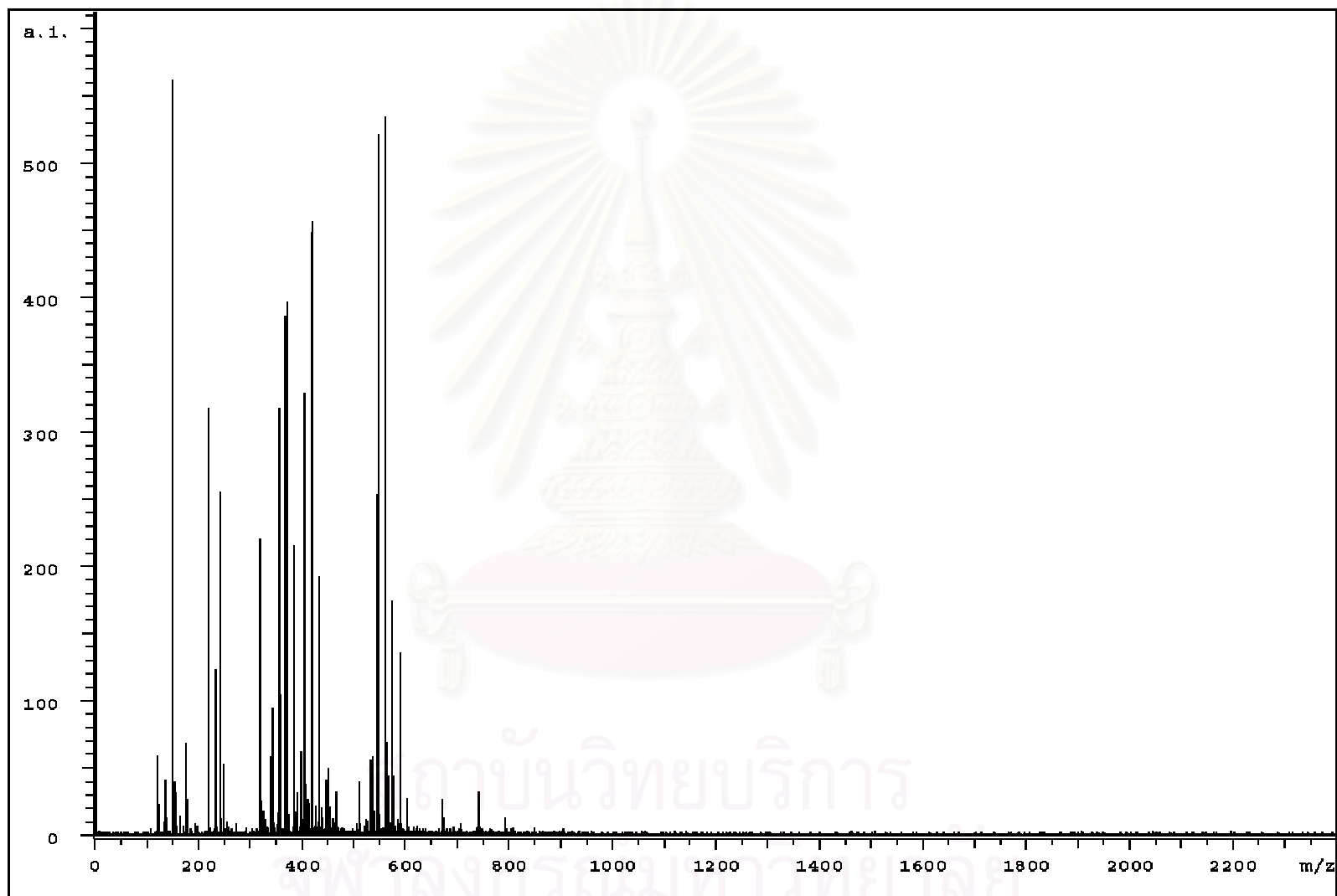
Figure A26 MALDI MS spectrum of dispersants separated from T 91 gasoline oil.

(MALDI-TOF MS condition as shown in Figure A11.)
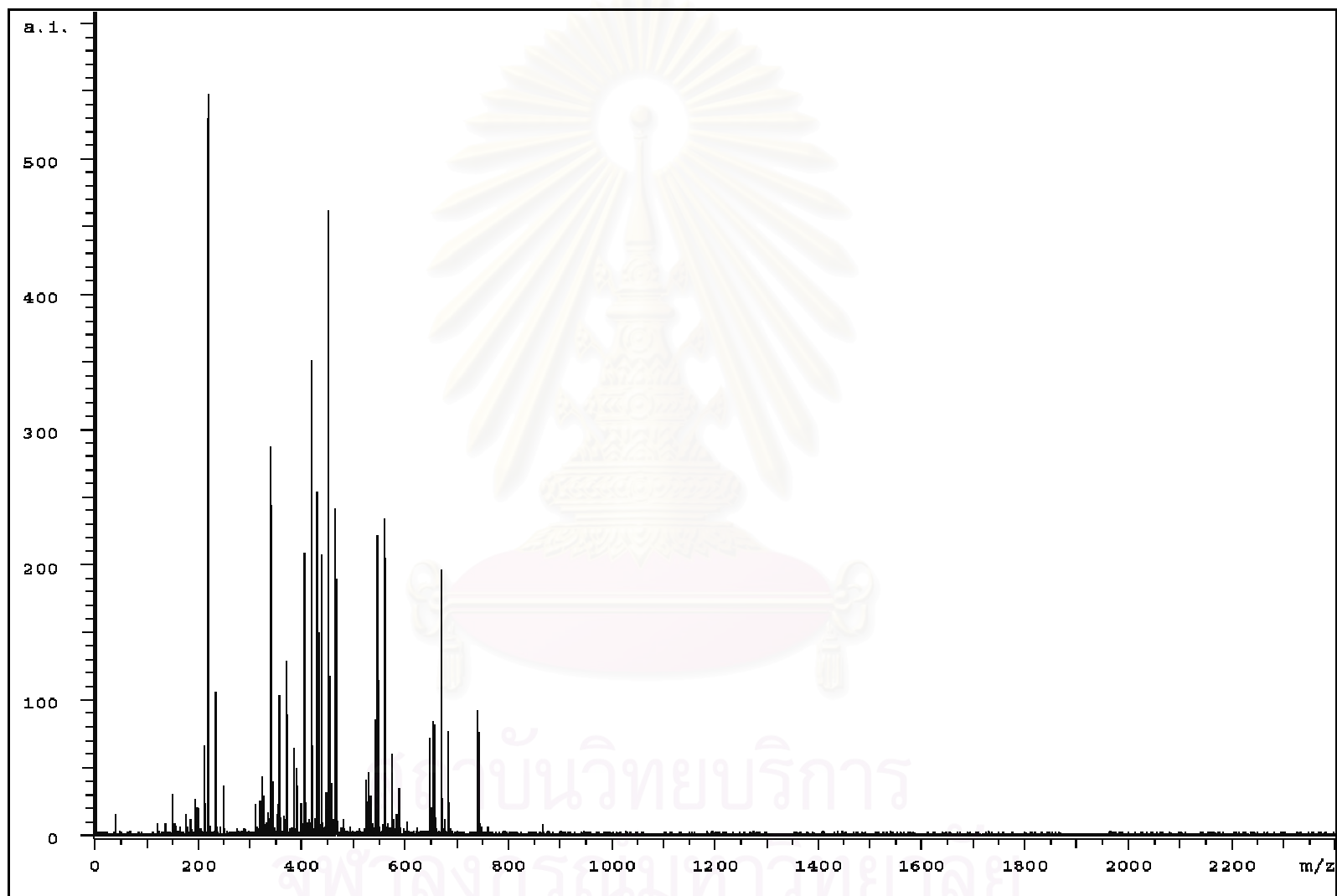
Figure A27 MALDI MS spectrum of dispersants separated from T 95 gasoline oil.
(MALDI-TOF MS condition as shown in Figure A11.)
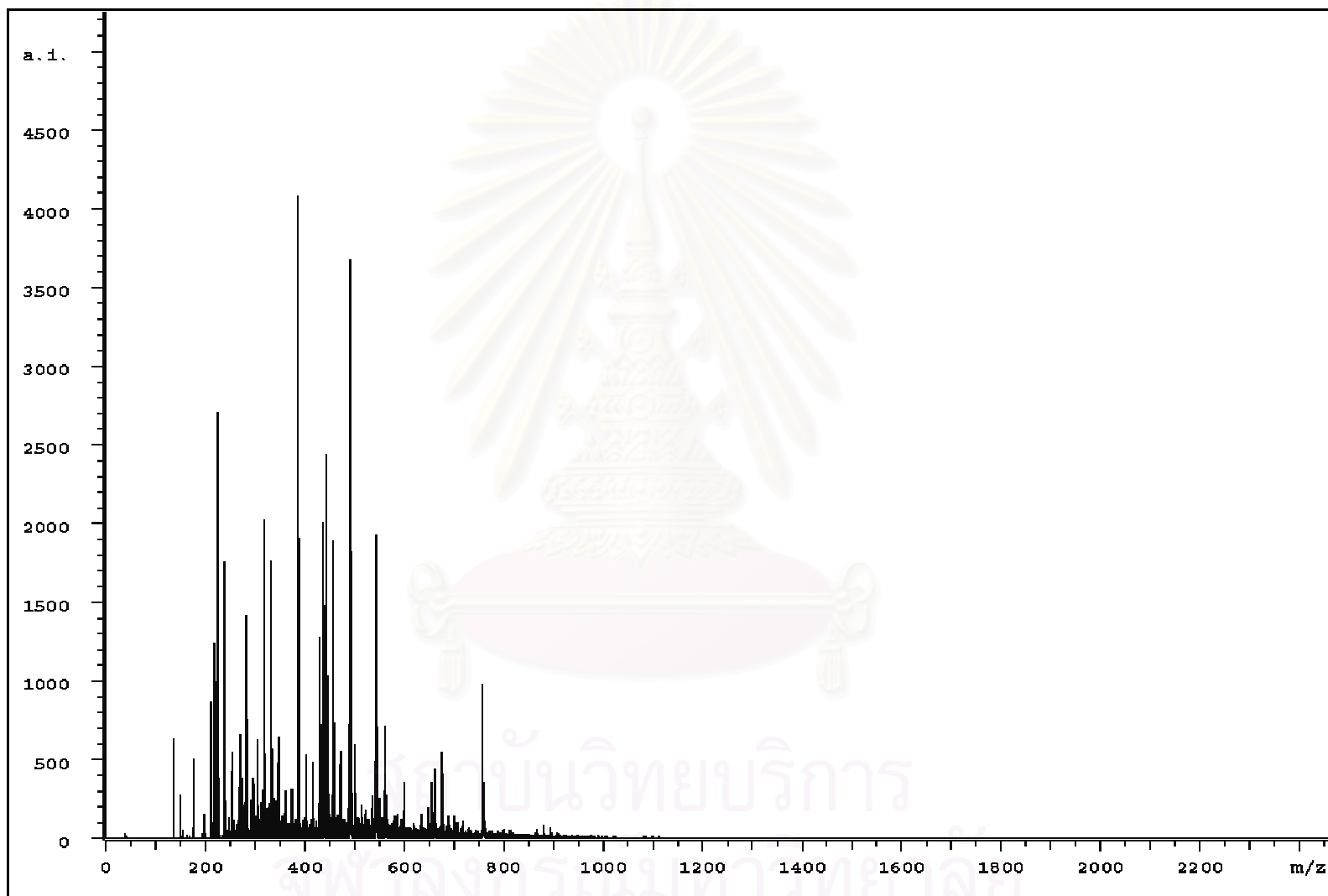
Figure A28 MALDI MS spectrum of dispersants separated from Q 91 gasoline oil.
(MALDI-TOF MS condition as shown in Figure A11.)
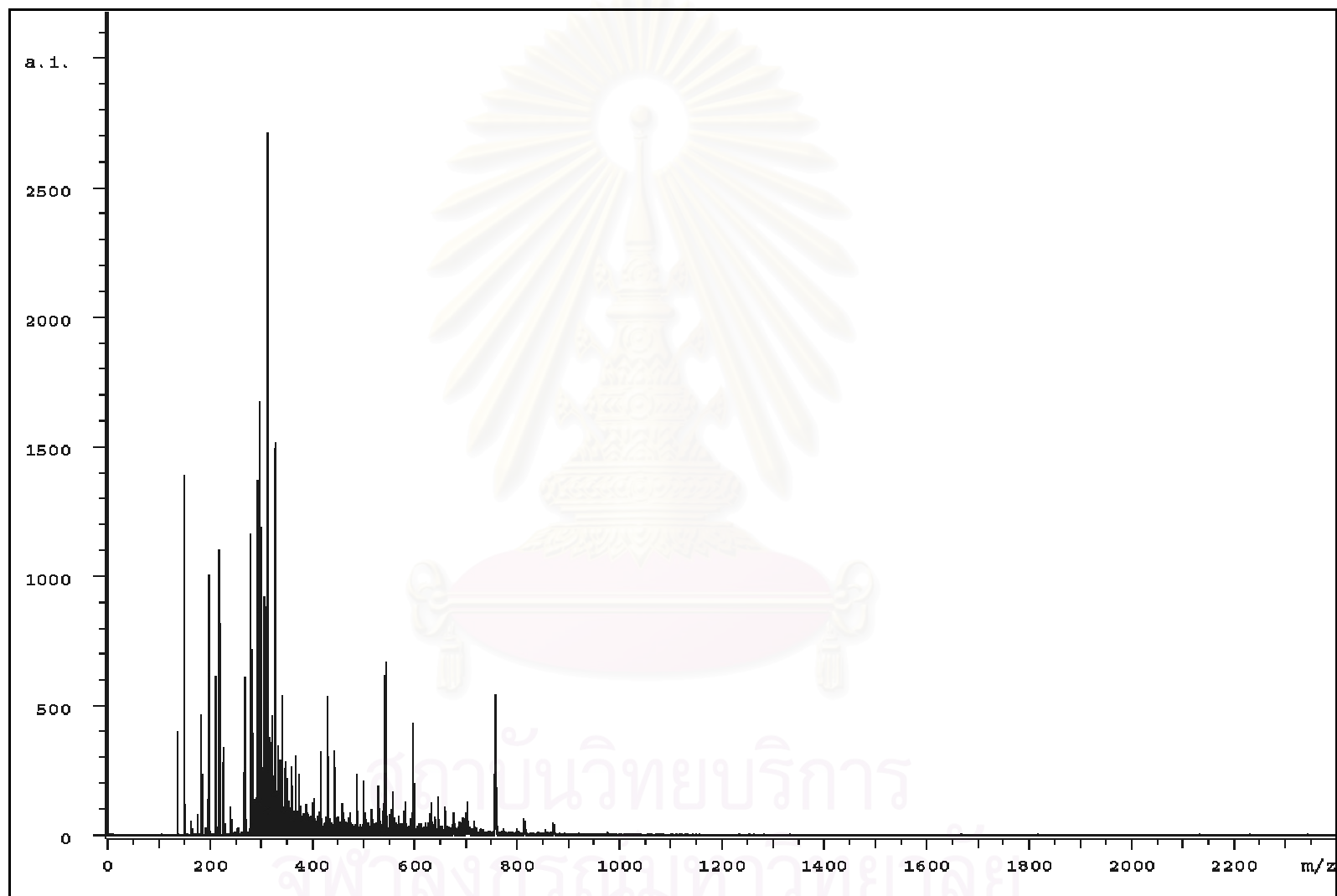
Figure A29 MALDI MS spectrum of dispersants separated from Q 95 gasoline oil.
(MALDI-TOF MS condition as shown in Figure A11.)
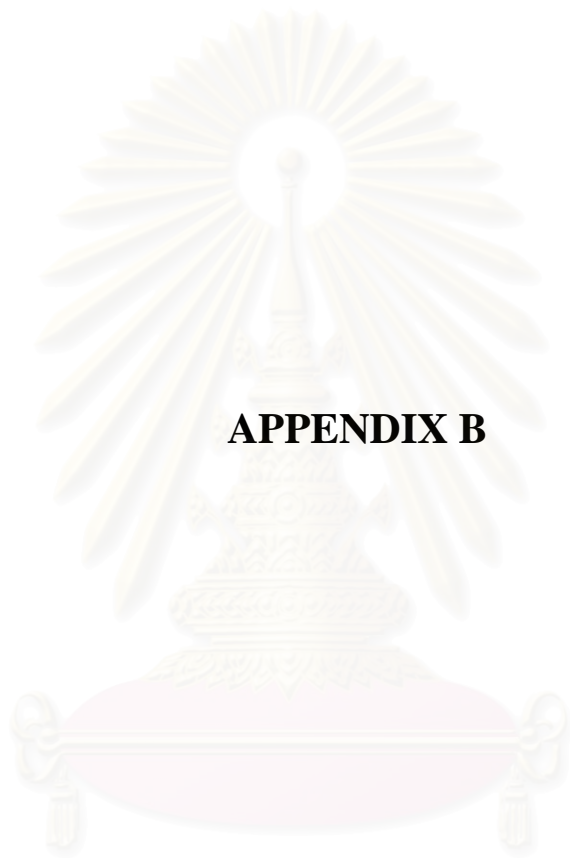
APPENDIX B
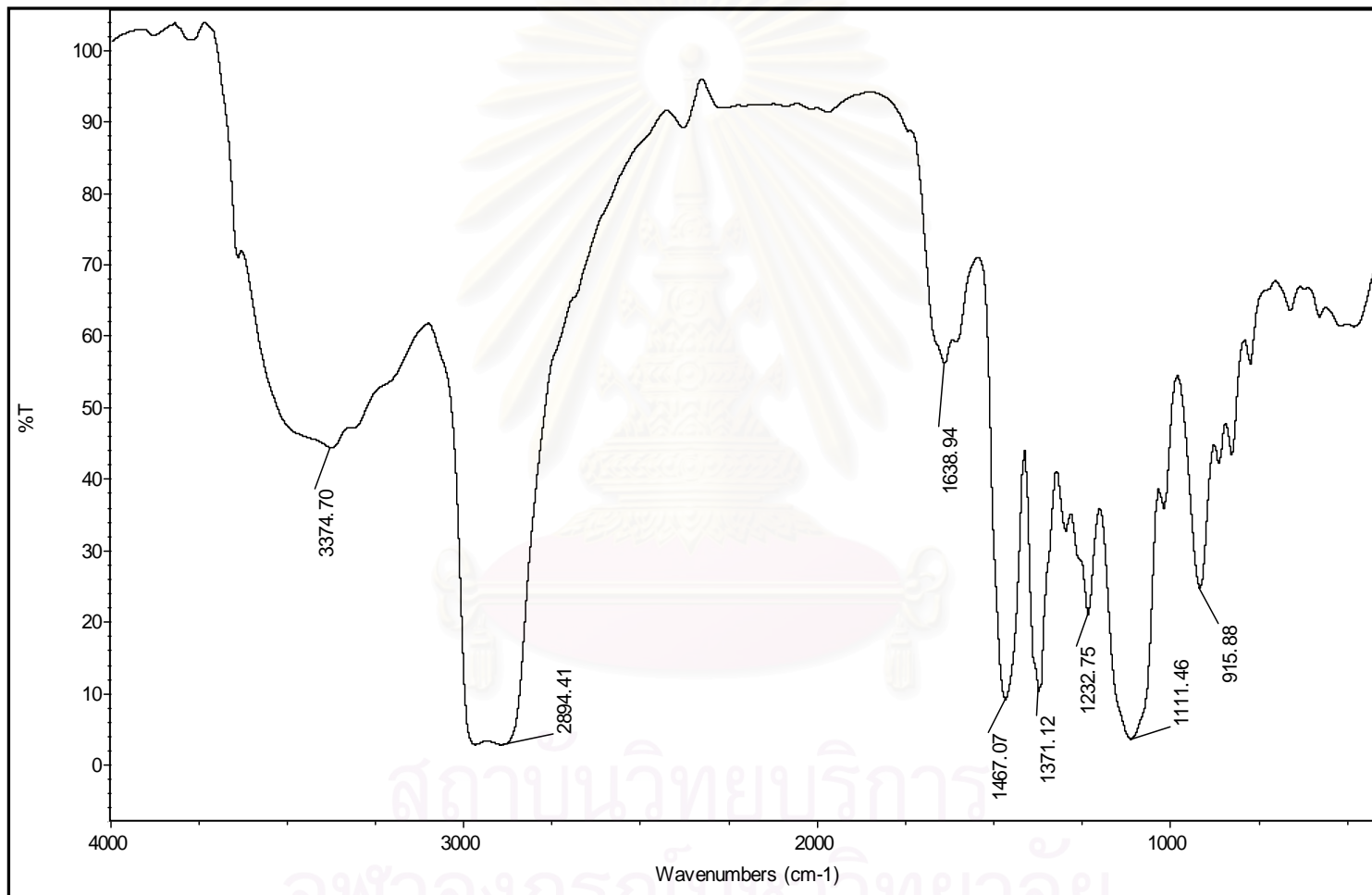
Figure B1 FT-IR spectrum of dispersants separated from LZ 8218C.
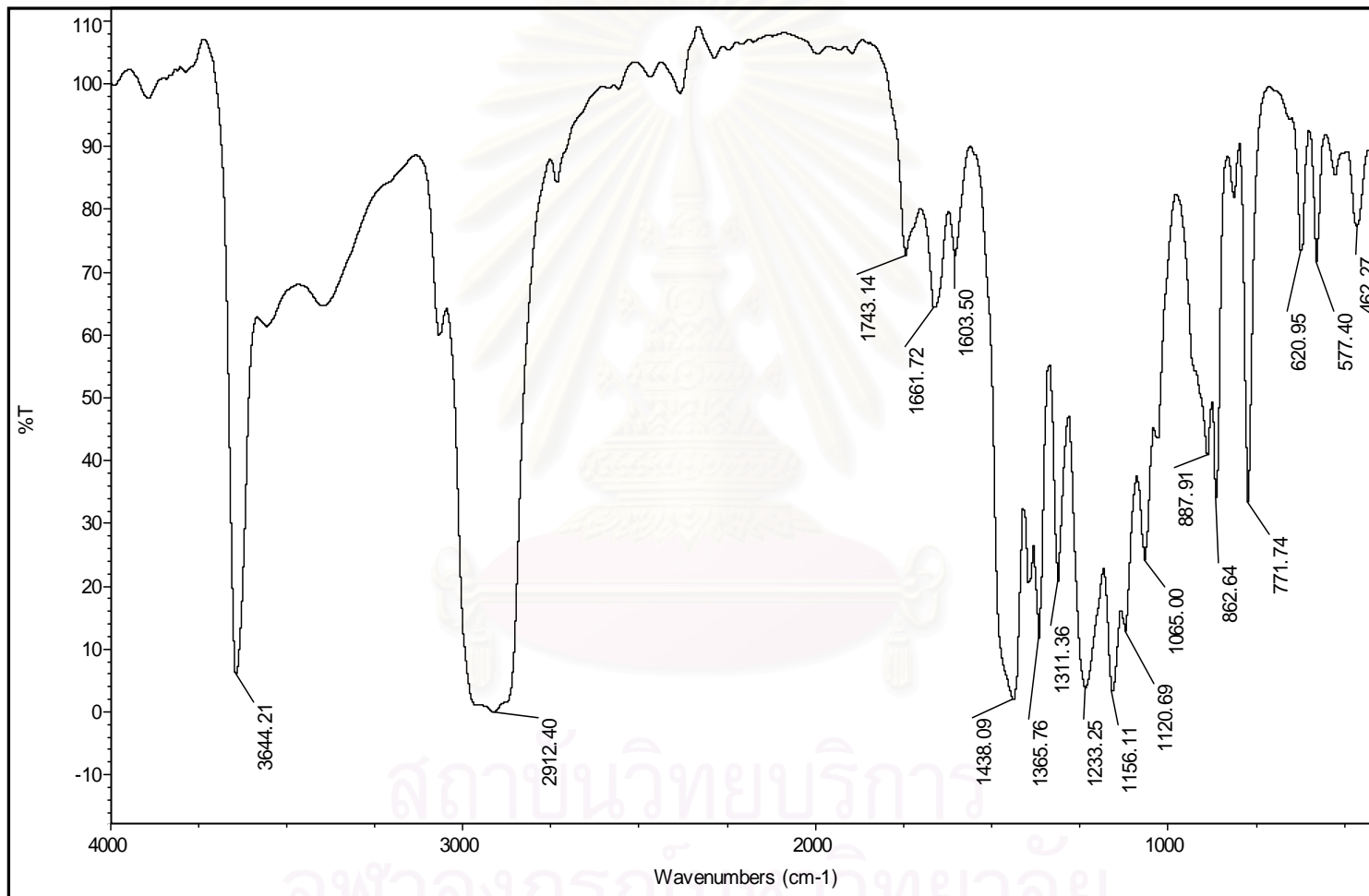
Figure B2 FT-IR spectrum of dispersants separated from B 16 gasoline oil.
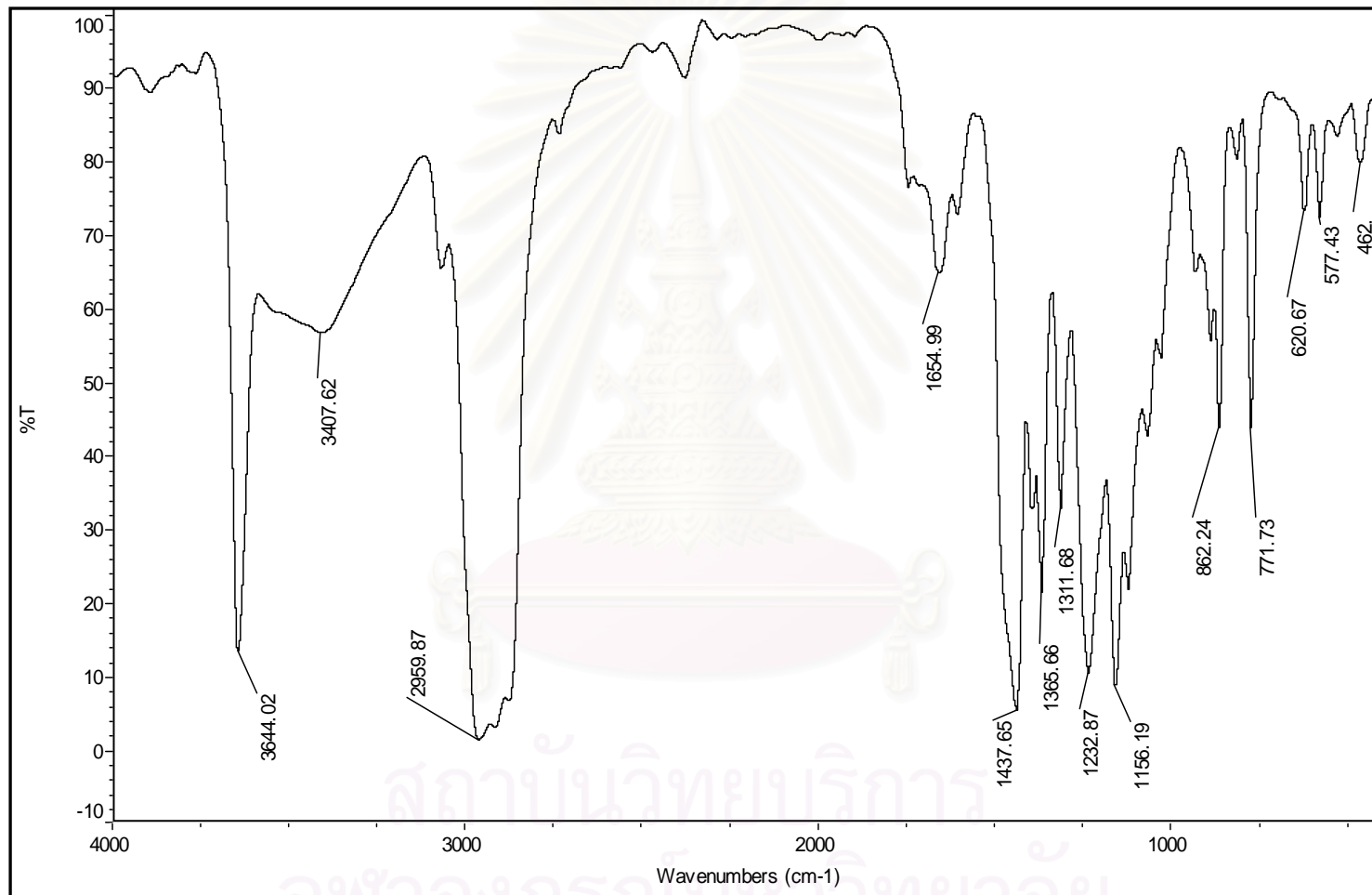
Figure B3 FT-IR spectrum of dispersants separated from B 95 gasline oil.
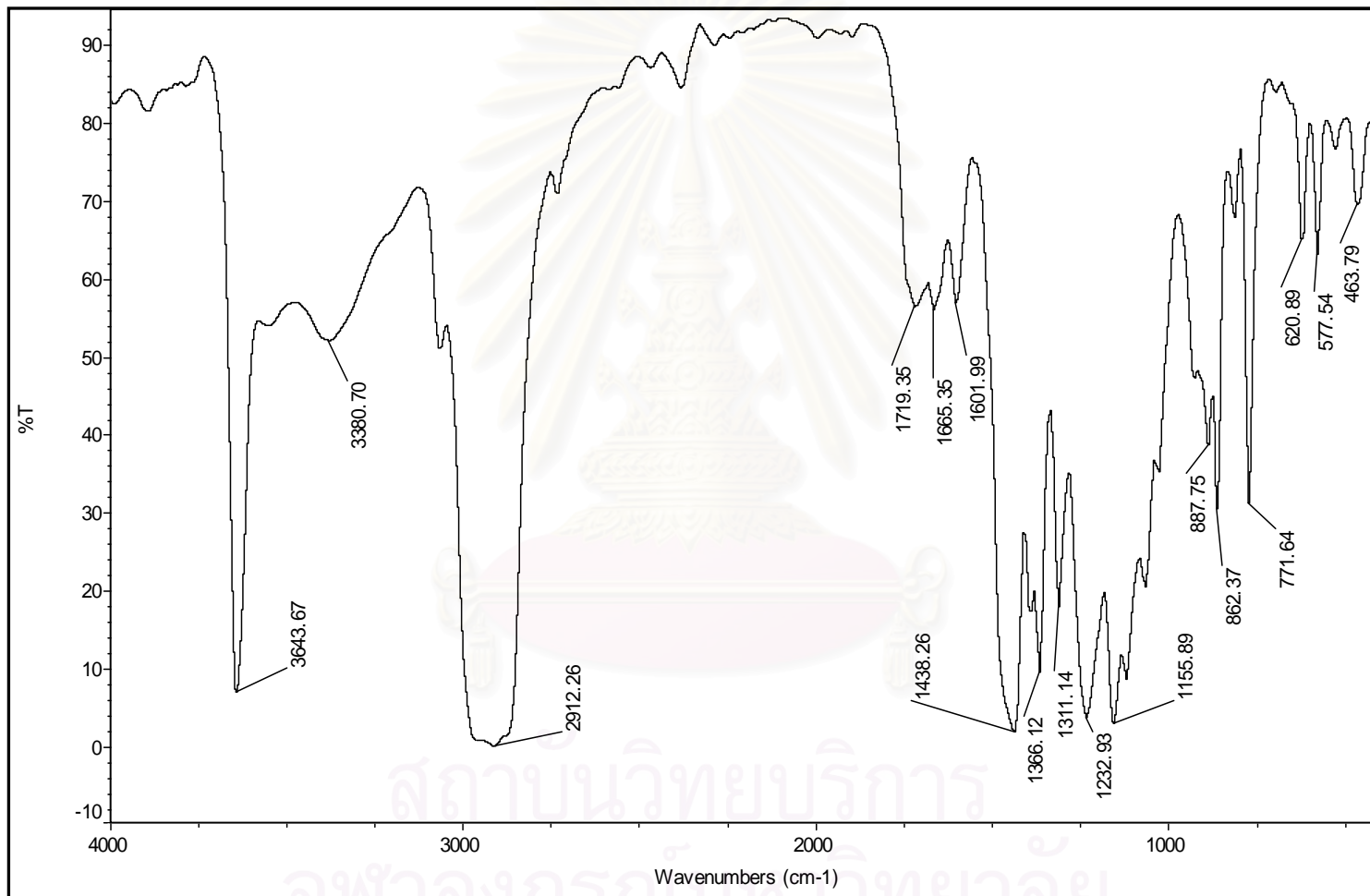
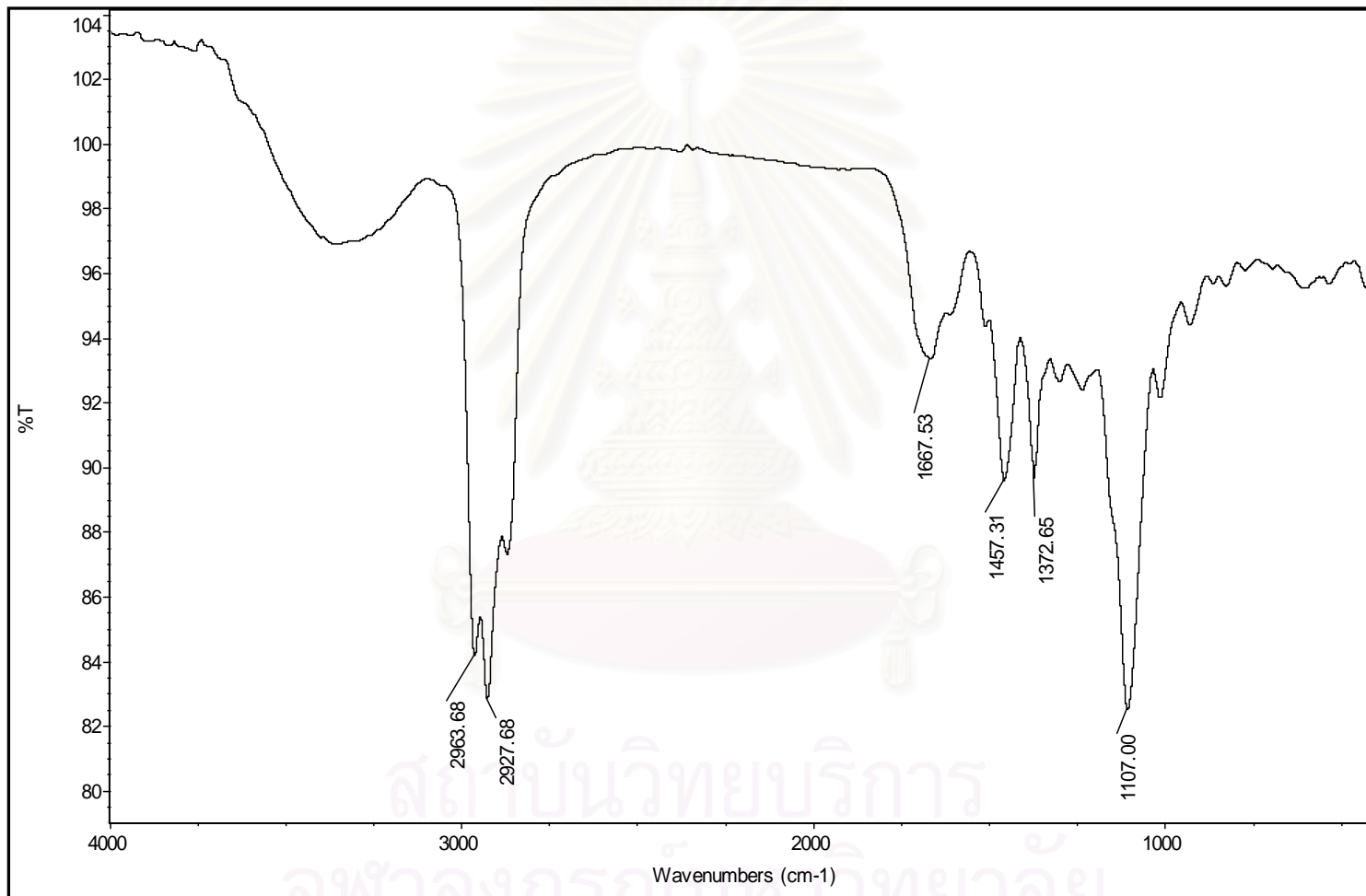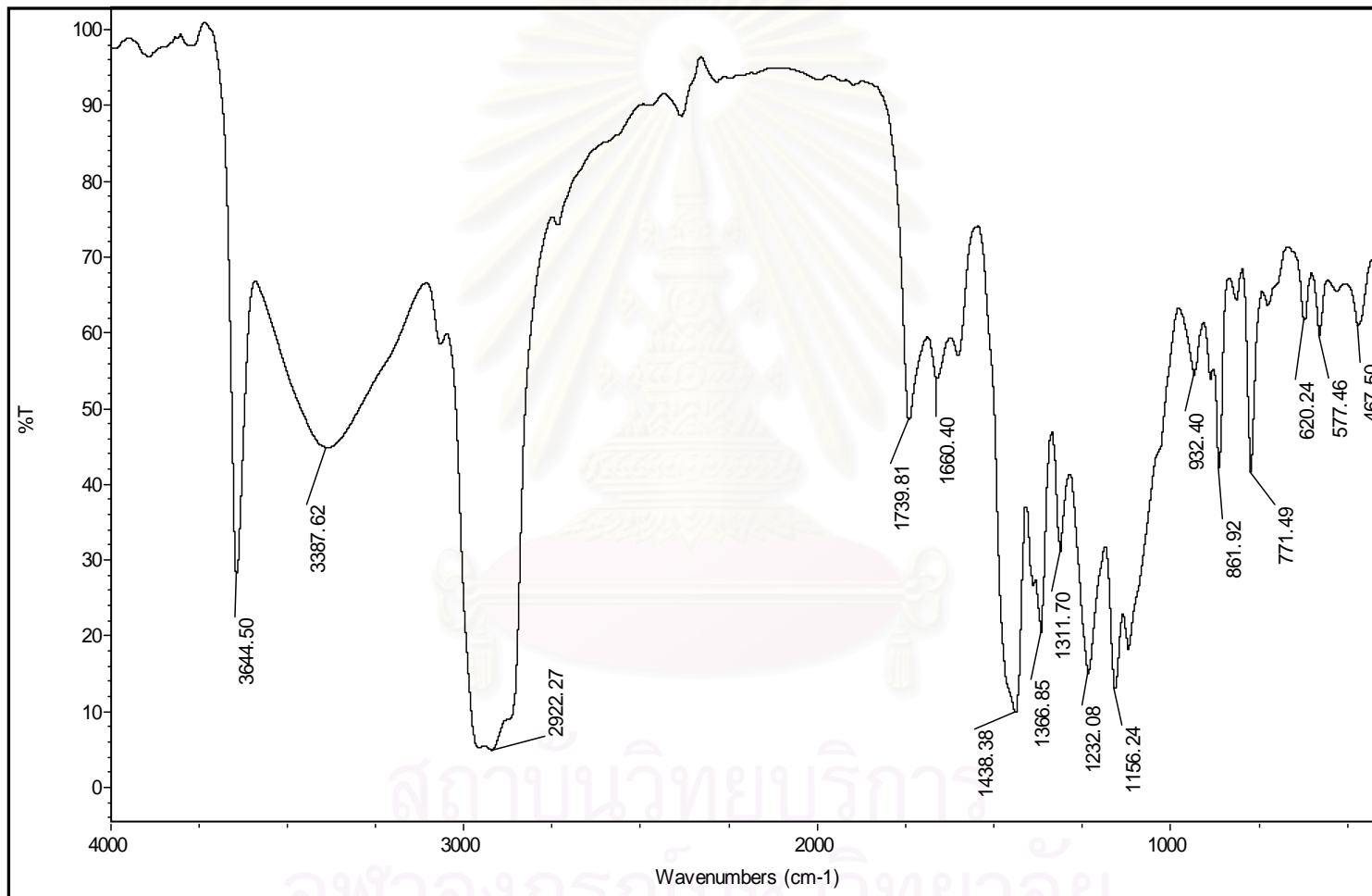**Figure B4** FT-IR spectrum of dispersants separated from E 91 gasoline oil.
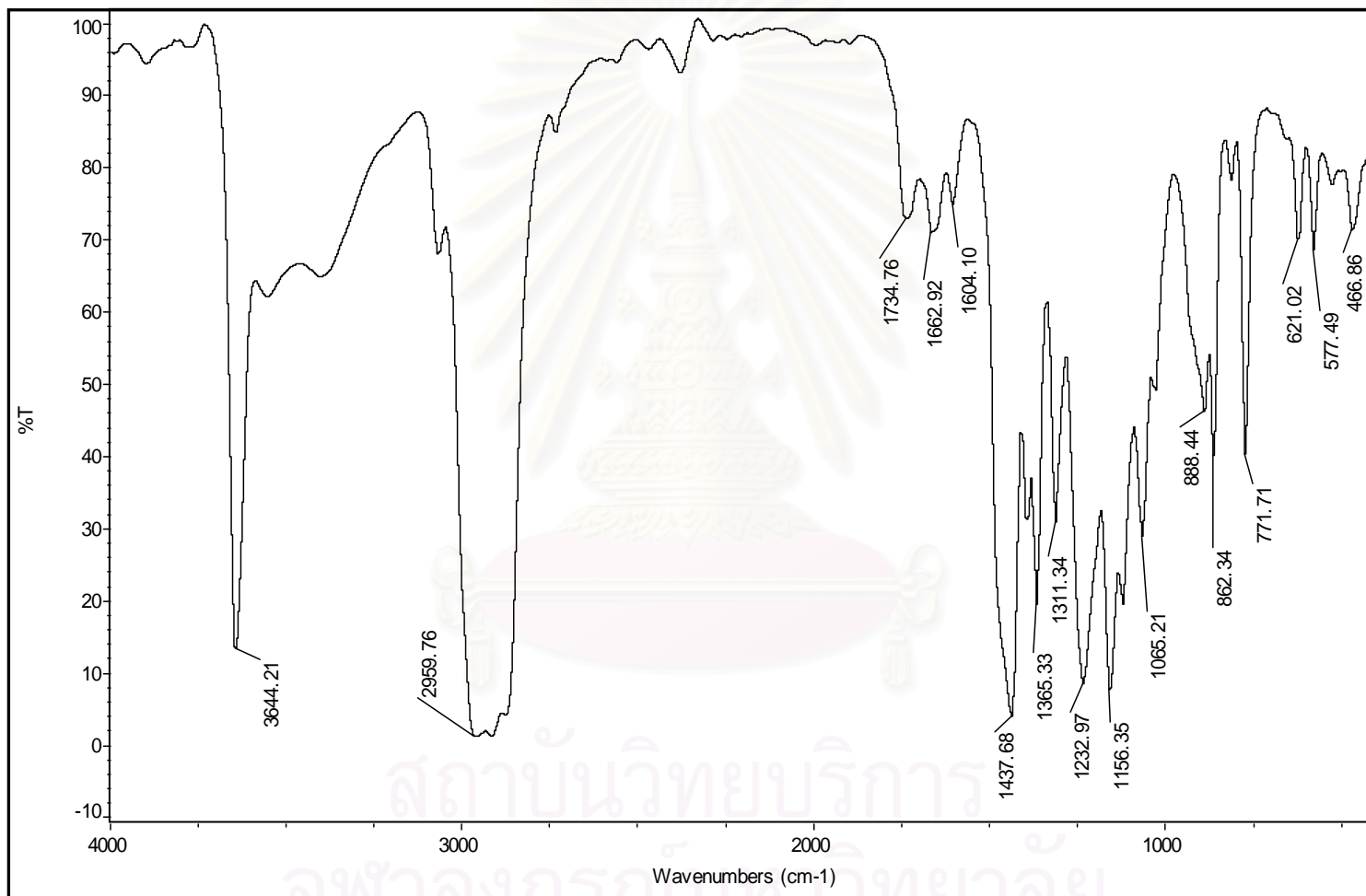
Figure B5   FT-IR spectrum of dispersants separated from E 95 gasoline oil.
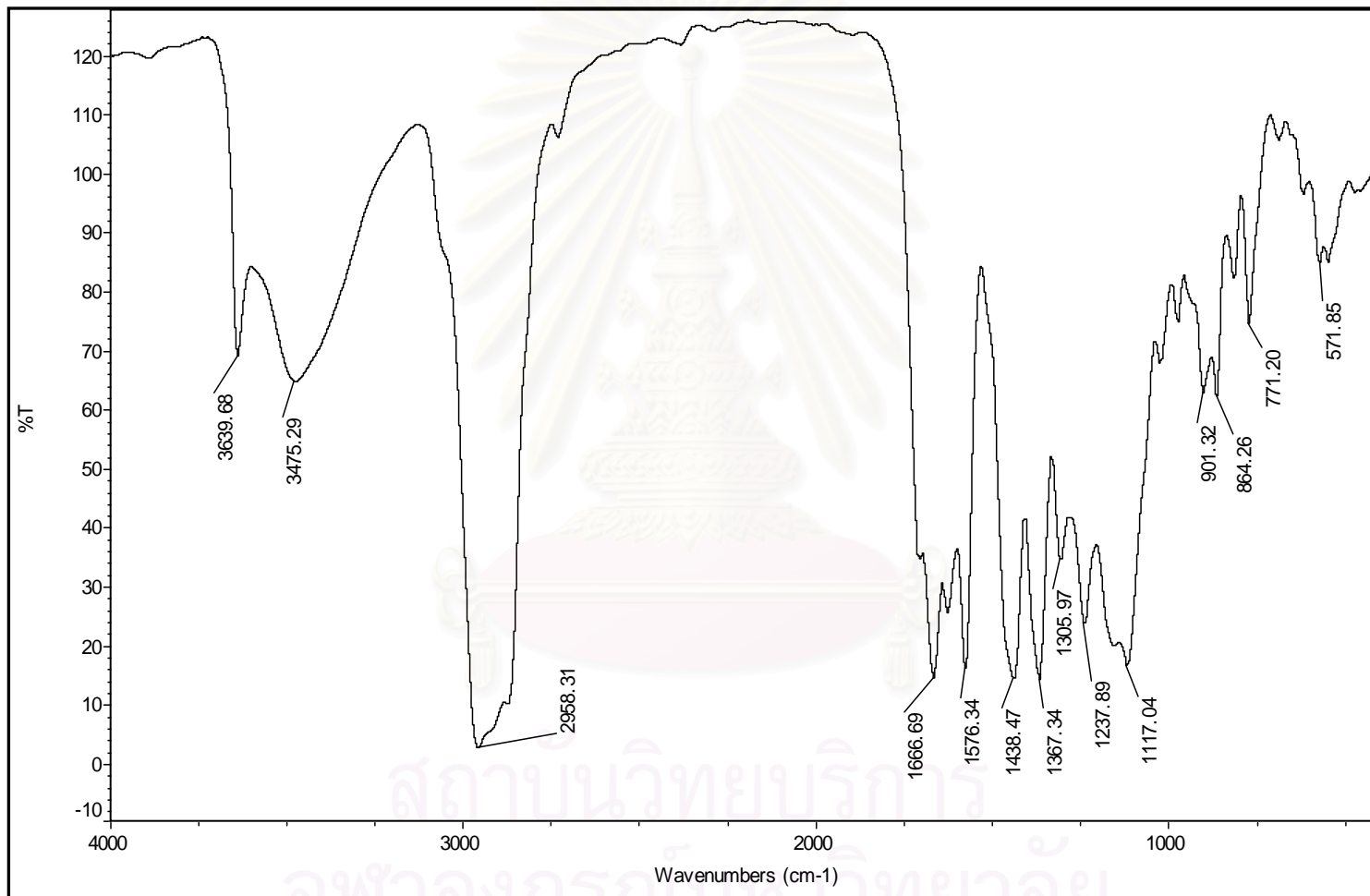
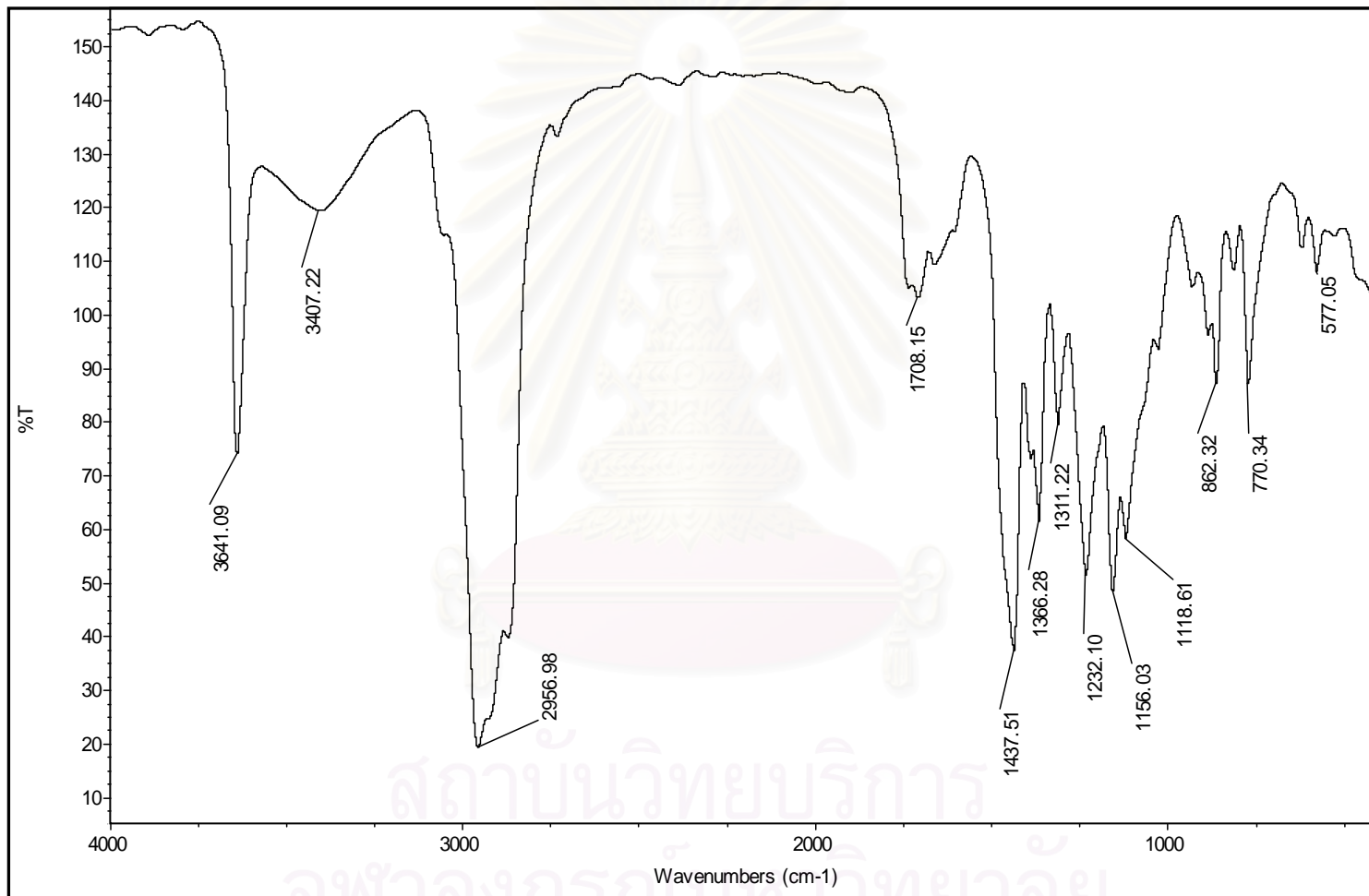**Figure B6** FT-IR spectrum of dispersants separated from J 91 gasoline oil.
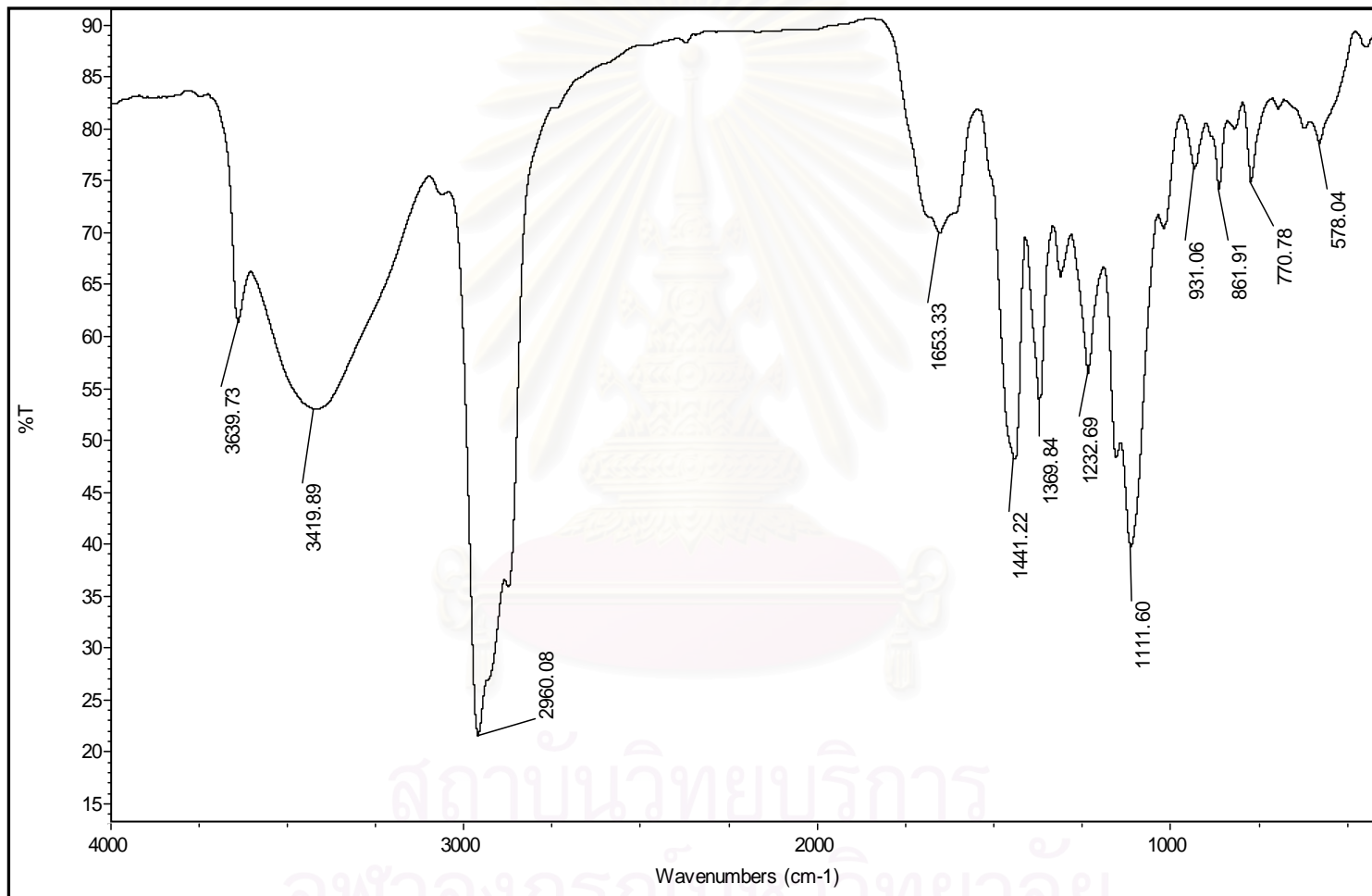
Figure B7 FT-IR spectrum of dispersants separated from J 95 gasoline oil.
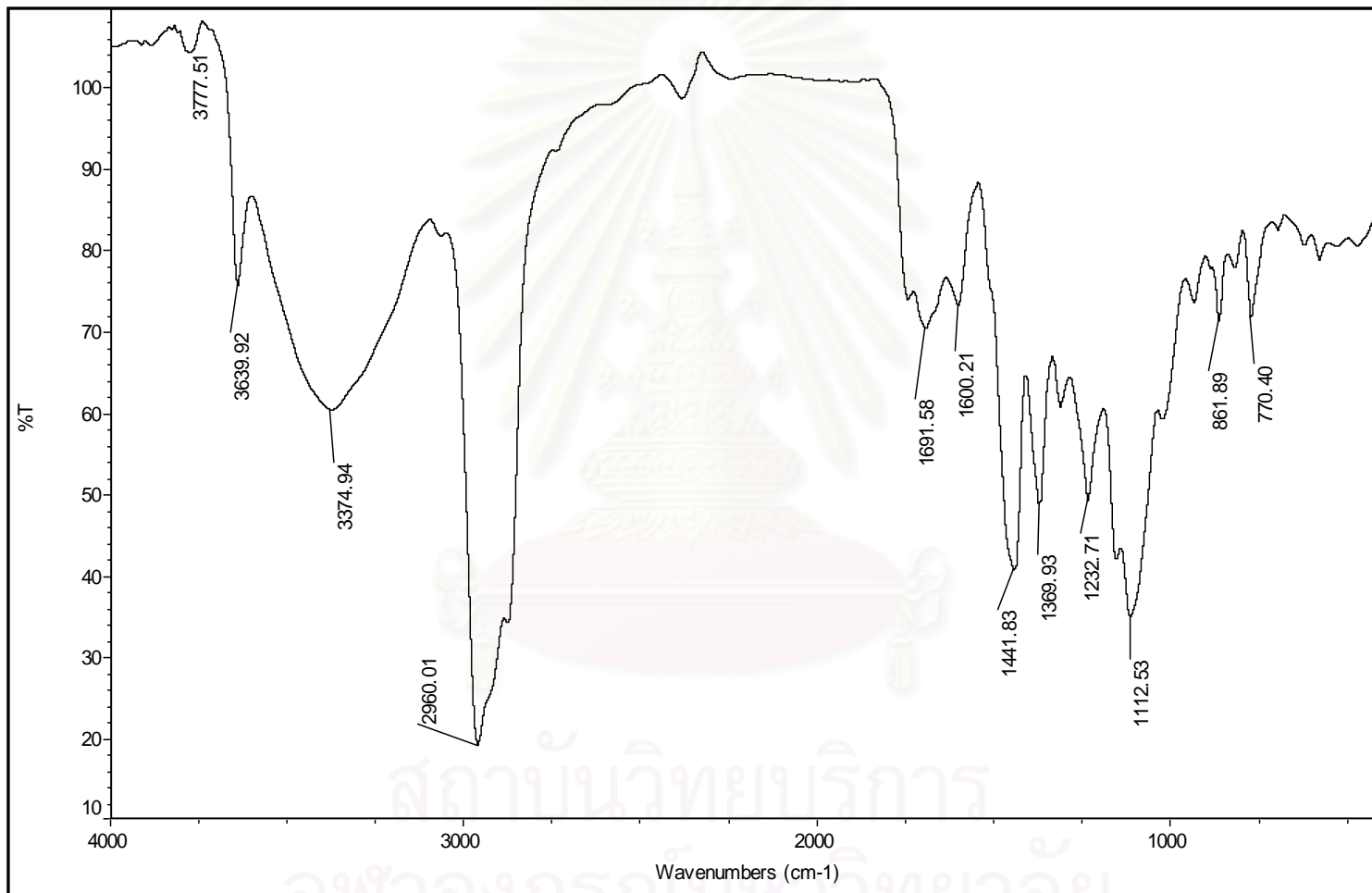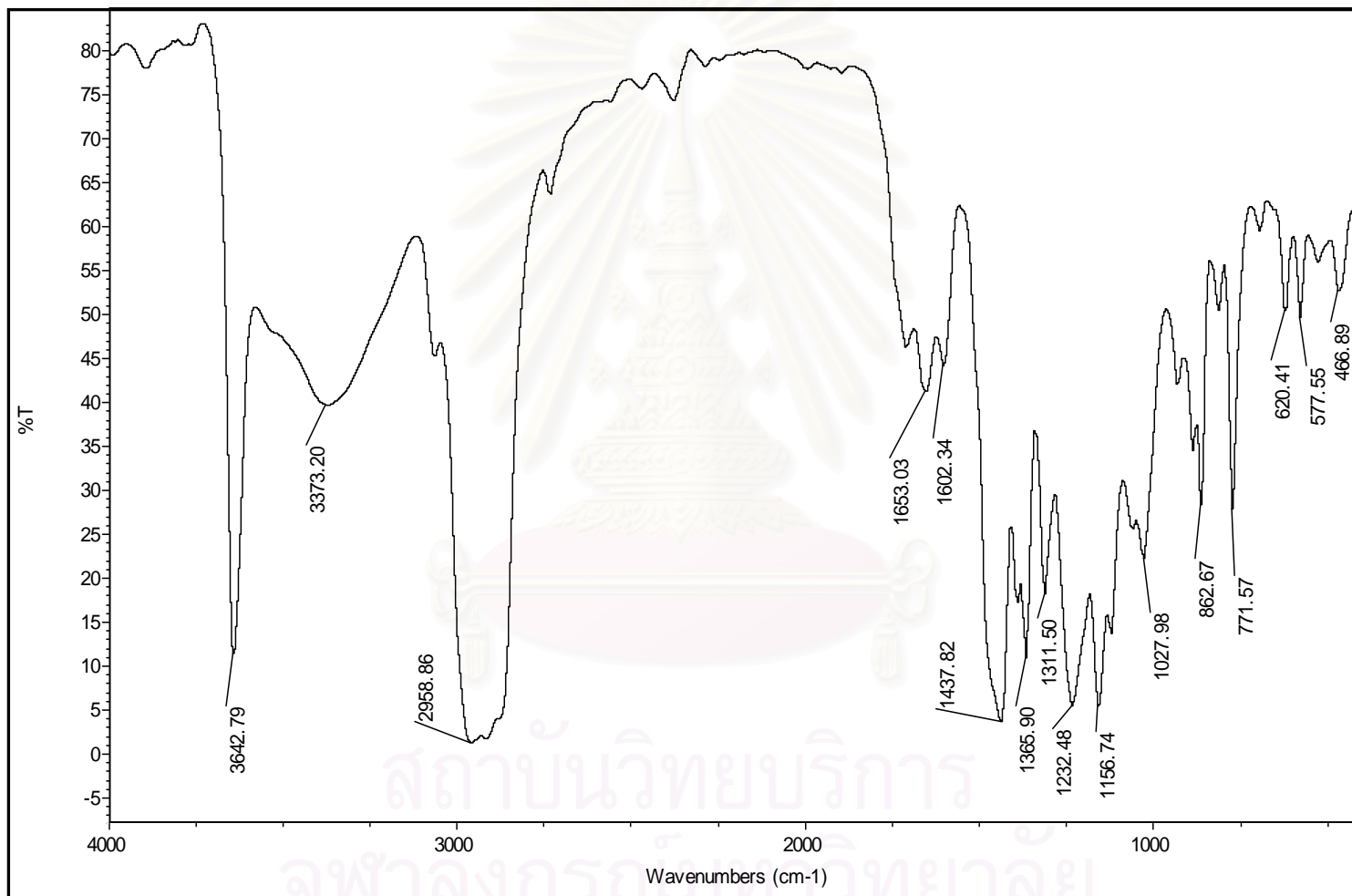
Figure B8   FT-IR spectrum of dispersants separated from S 91 gasoline oil.
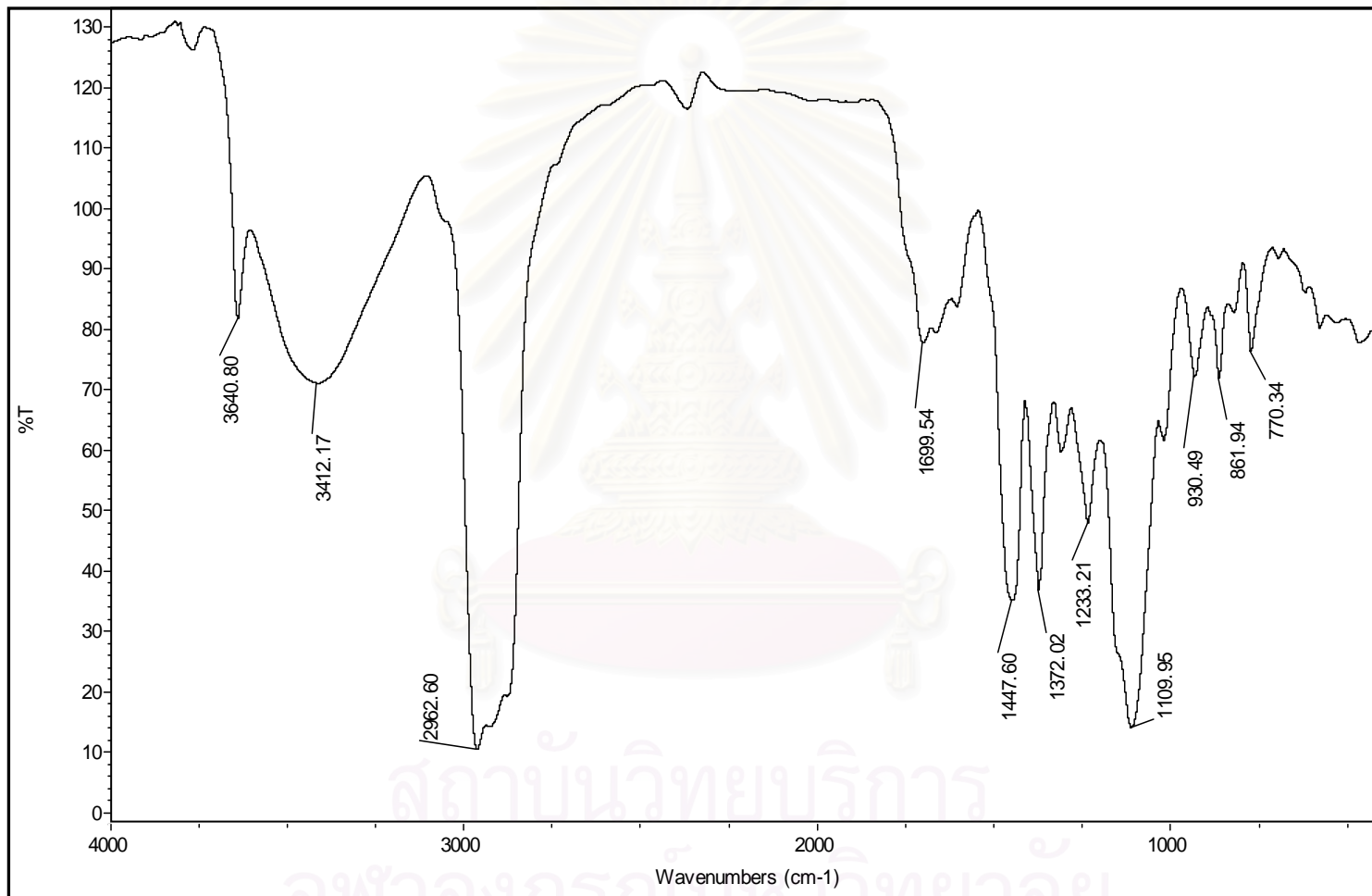
Figure B9 FT-IR spectrum of dispersants separated from S 95 gasoline oil.
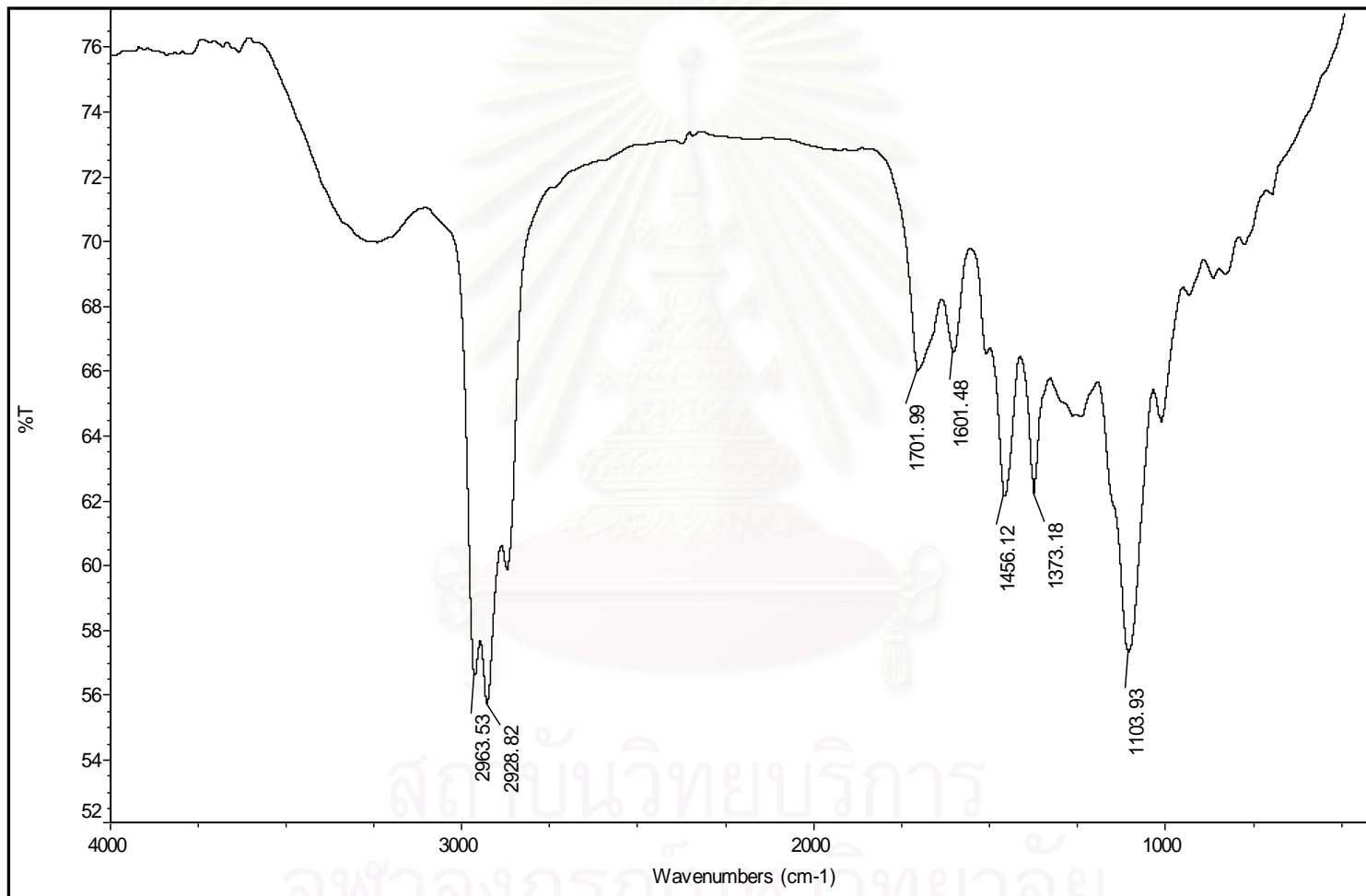
Figure B10 FT-IR spectrum of dispersants separated from C 91 gasoline oil.

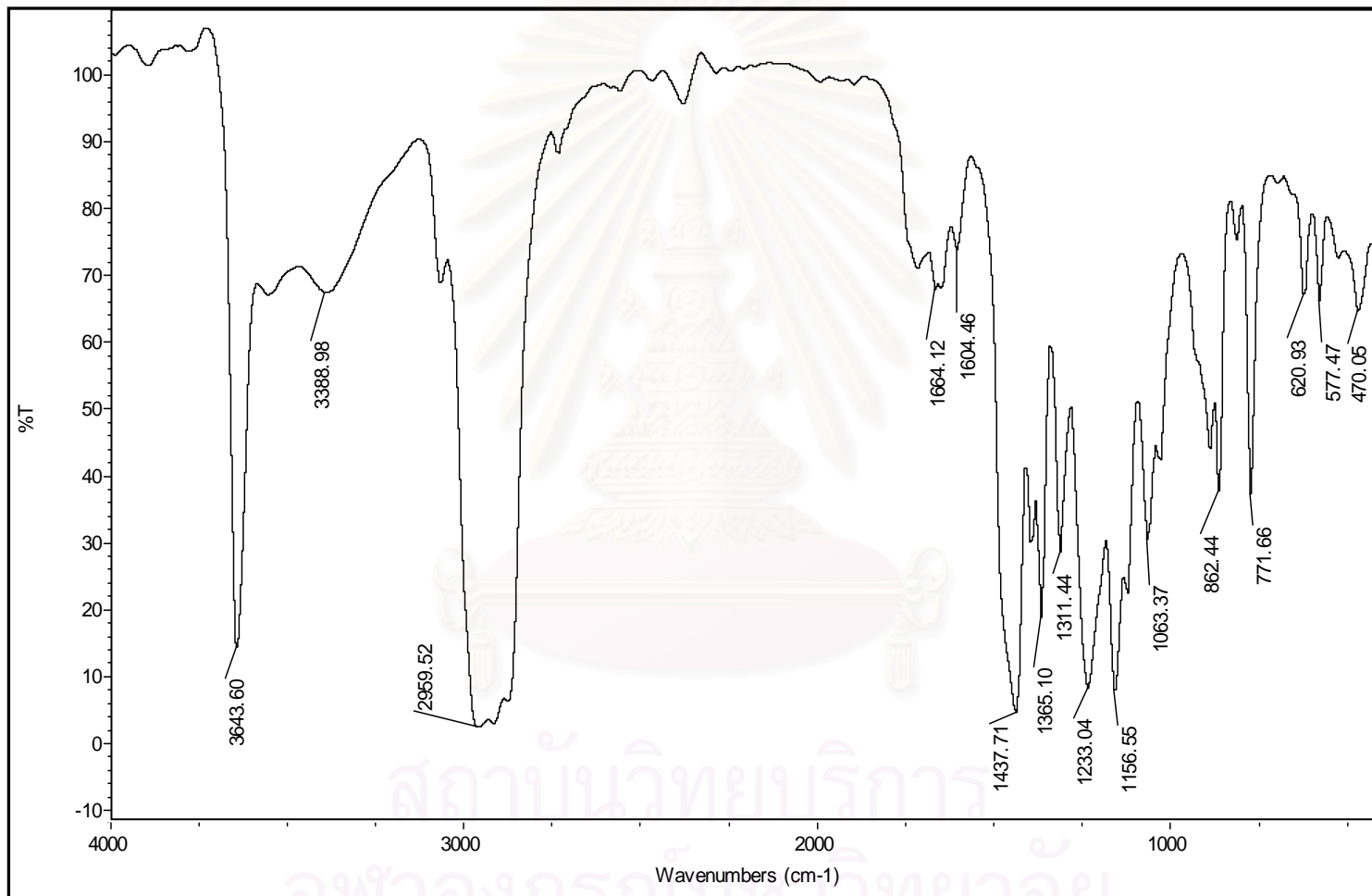**Figure B11** FT-IR spectrum of dispersants separated from C 95 gasoline oil.

Figure B12 FT-IR spectrum of dispersants separated from P 91 gasoline oil.

Figure B13 FT-IR spectrum of dispersants separated from P 95 gasoline oil.

Figure B14 FT-IR spectrum of dispersants separated from T 91 gasoline oil.

Figure B15 FT-IR spectrum of dispersants separated from T 95 gasoline oil.
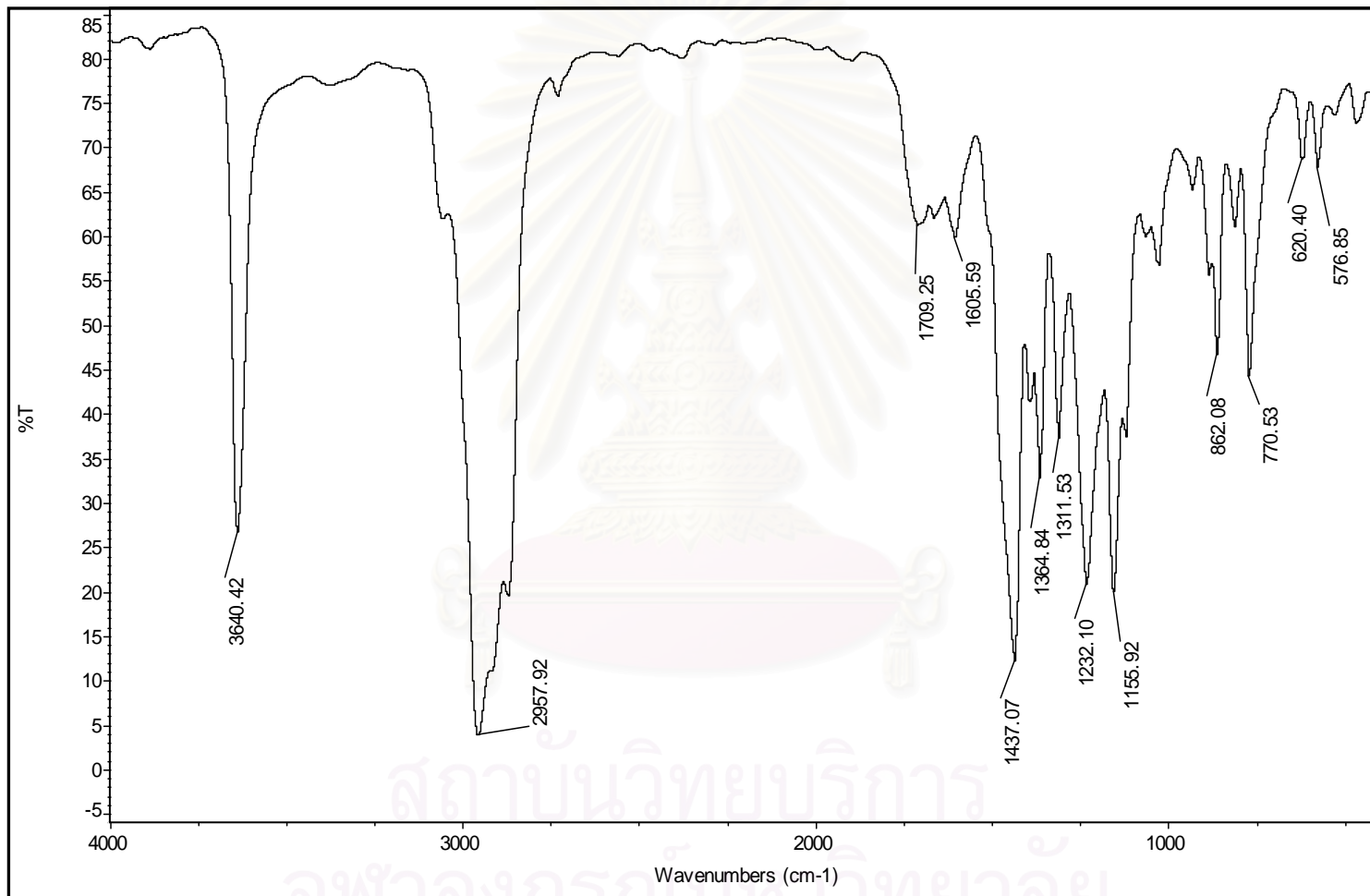
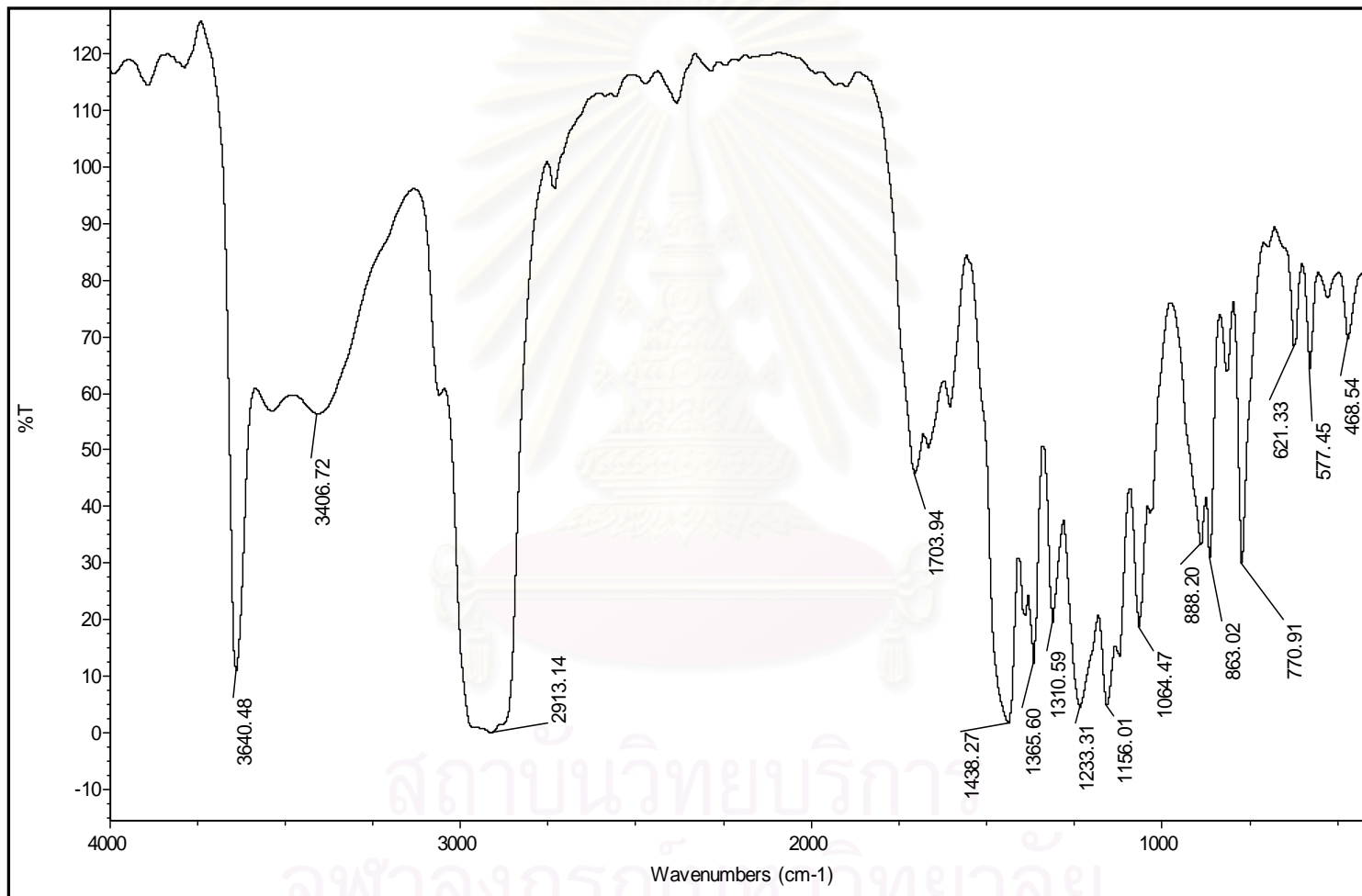**Figure B16** FT-IR spectrum of dispersants separated from Q 91gasoline oil.

Figure B17 FT-IR spectrum of dispersants separated from Q 95 gasoline oil.

VITA

Suthasinee Raungwinyouwetch was born on April 11, 1980, in Ratchaburi Province, Thailand. She received her Bachelor's degree of Science in Chemistry, Silpakorn University in 2002. She continued the Master Program of Multidisplinary of Petrochemistry and Polymer Science, Faculty of Science, Chulalongkorn University and completed the program in 2005.