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DETECTION OF HIGH MASS MOLECULES BY LASER DESORPTION TIME-OF-FLIGHT MASS SPECTROMETRY

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[Introduction]

The laser desorption time-of-flight mass spectrometer has been developed in order to analyze non-volatile, thermally labile and high mass organic molecules. In this spectrometer we have made improvements on all stages of mass spectrometer (ion source, mass separation, detector, electronics).

[Equipment]

The construction of the laser desorption time-of-flight mass analyzer is shown in Fig.1. Figure 2 shows the block diagram of TOF spectrum measurement system.

-- Ion source --

N_2 laser (Wavelength:337nm, Pulse width:about 15nsec, Pulse energy:4mJ Max.) was used for ionization. "Rapid heating" [1] is achieved by irradiating pulsed laser on sample surface. As for sample preparation, "Ultra fine metal powder (UFP) and glycerol matrix method " was found to be very effective for increasing the yield of high mass molecular ions, and decreasing the yield of fragment ions[2].

In comparison with Bulk, UFP has the following features

- High photo-absorption
- Low heat capacity
- Extremely large surface area per unit volume

This UFP matrix method seemed to enhance the speed of heating even further.

-- Mass separation --

Generally, TOF-MS has the following characteristics

- Very high transmission
- Measurement time of less than a few hundreds μsec

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- Unlimited mass range
- Low mass resolution

A new gradient-electric field ion reflector for a time-of-flight mass spectrometer has been developed in order to improve mass spectral resolution by energy focusing [3]. In the TOF mass spectrometer consisting of a free ion drift region and a new ion reflector, the motion of the same m/z ions is quasi-single oscillation of the same period. Therefore, the flight-times of the same m/z ions are focused to a constant even if the initial kinetic energy of the emitted ions are scattered.

The TOF mass separation system was designed to permit easy switching between "Reflector type" ($V_R > V_0$) and "Linear type" ($V_R = 0$).

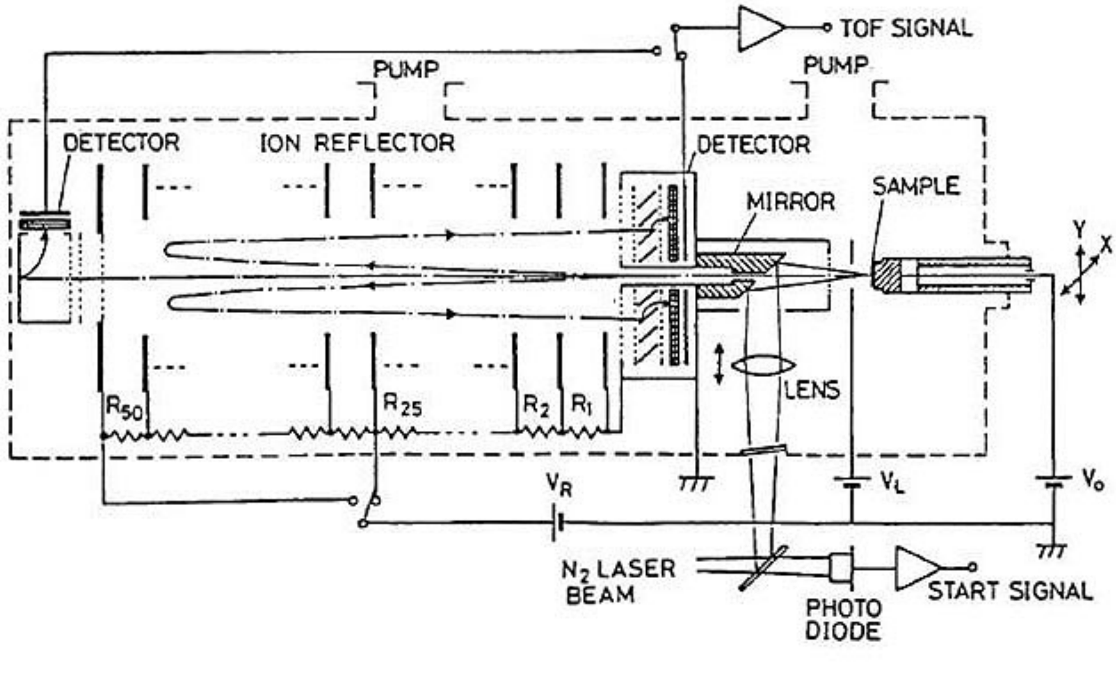


Fig. 1 Construction of the Laser Desorption TOF Mass Analyzer.

-- Detector --

Micro channel plate (MCP) or secondary electron multiplier (SEM) is usually used to detect ions, electrons or photons. Ions of larger m/z generally have low velocities in TOF-MS. So the detection sensitivity of MCP has a tendency to decrease in higher mass regions.

Higher detection sensitivity for high mass ions was

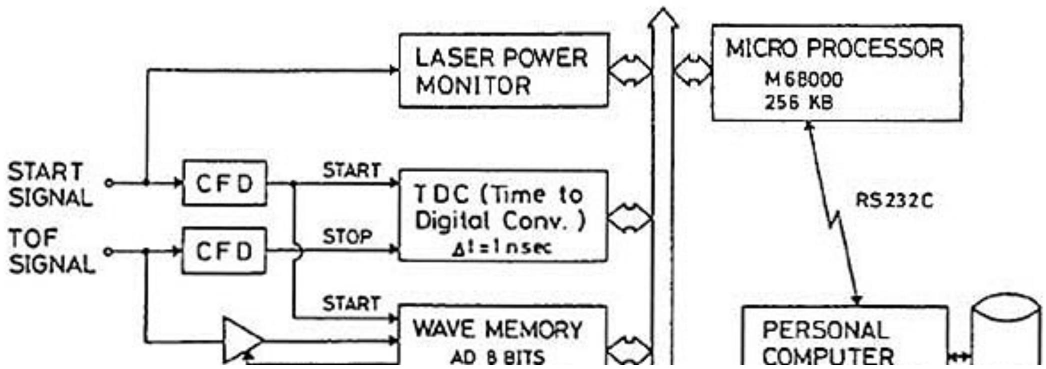
achieved by adding a blind type ion-electron converter to the MCP ion detector. And this converter was found to provide a longer life of the detector.

-- Electronics --

Two TOF spectrum measurement systems were constructed, one using the "AD method" to ensure high sensitivity, and the other using the "TDC method" to ensure high accuracy, were selectively used.

The system for "AD method" mainly consists of a digital wave memory (Analog to digital converter; 100MHz,8bits) and accumulation circuits (24bits,8kwords)[Fig.2]. This system can accumulate the spectrum of 8k words within 1 msec.

The system for "TDC method" mainly consists of a constant fraction discriminator (CFD) and a multi-stop time to digital converter (TDC). The time intervals between "start" and "stop" pulses are measured with a time resolution of 1nsec. And "stop" pulses are detected up to 255 at each "start" pulse. Dead time is 43nsec.



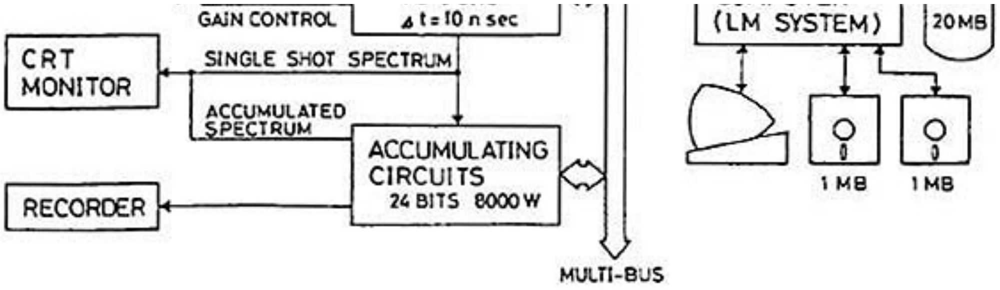


Fig. 2 Block Diagram of the TOF Spectrum Measurement System.

[Results and Discussion]

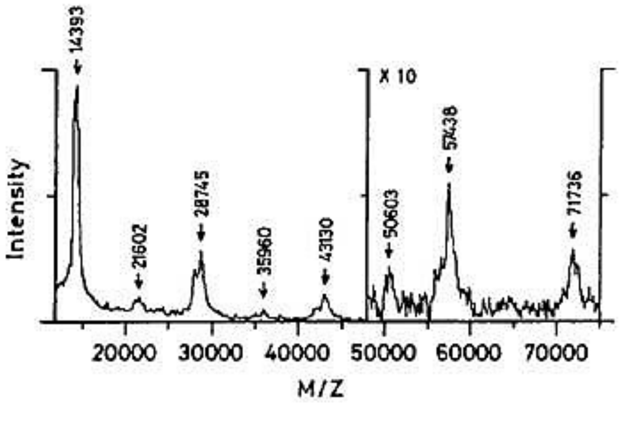
This mass spectrometer was successfully applied to the

detection of high mass molecular ions, for example, lysozyme (M.W. ~ 14,300)[Fig.3] and carboxypeptidase-A (M.W. 34,472) [Fig.4].

Quasi-molecular ion※ of lysozyme were found clearly, and the singly or doubly charged cluster ions were also found in the spectrum. Thus fairly significant information on molecular weights was easily obtained.

※ This ion may be mostly in the form of $[M+Cation]^+$

These spectra show that the laser desorption TOF mass spectrometer is capable of detecting organic ions of m/z up to 71,000, and that quasi-molecular ions are readily formed from proteins of molecular weight up to 34,000 u.



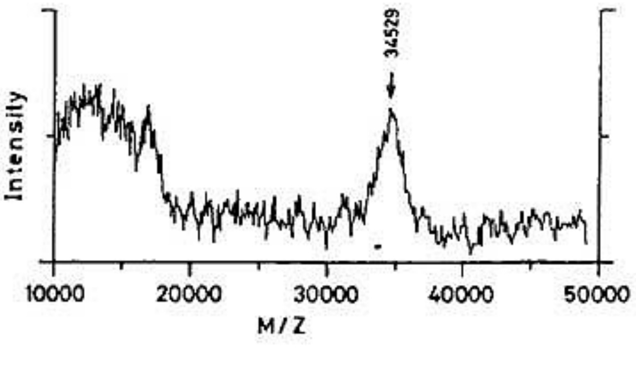


Fig. 3 Laser Desorption Mass Spectrum of Lysozyme

Fig. 4 Laser Desorption Mass Spectrum of Carboxypeptidase-A

The protein sample used in the present study was about 10 μg in quantity, and measurement time was less than several minutes. Sample preparation was simple and preparation time was less than a few minutes.

At present, the precision of the molecular weight(M.W.) determination is better than 1 percent in the region from M.W. 10,000 to M.W.34,000. This precision is approximately one order of magnitude higher than that in SDS-PAGE or gel permeation HPLC.

1. R.J.Cotter, Amal.Chem., 1589A, Vol.52, No.14, (1980)
2. T.Yoshida, et al., Mass Spectroscopy, submitted
3. Y.Yoshida, et al., Mass Spectroscopy, submitted

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