Simultaneous Comparison of Fluorescence Spectrometry With Multiphoton Ionization Spectrometry Using a Supersonic Jet Spectrometer

Cheng-Huang Lin, Masami Hozumi, Totaro Imasaka* and Nobuhiko Ishibashi
Faculty of Engineering, Kyushu University, Hakozaki, Fukuoka 812, Japan

The ratio of the signal intensities obtained in multiphoton ionization spectrometry and fluorescence spectrometry is compared semi-quantitatively for several aromatic compounds. The ratios, normalized against *N*-ethylaniline, are 0.2–1 for aniline derivatives, however, the value for anthracene is 7×10^{-6} in a one-colour ionization scheme and 7×10^{-4} in a two-colour ionization scheme. The small value obtained for the 'large' aromatic compound is ascribed to the high ionization potential relative to the two-photon energy for excitation and subsequent ionization; three photons are required in the one-colour scheme and an additional laser with sufficient energy must be aligned temporally and spatially for ionization in the two-colour scheme.

Keywords: *Supersonic jet spectrometry; fluorescence spectrometry; multiphoton ionization spectrometry; aromatic compound*

A very well-resolved spectrum is obtained by using supersonic jet (SSJ) spectrometry, which is useful for the selective analysis of samples with closely related chemical structures.[1-3] For the detection of chemical species, two major methods, *i.e.*, fluorescence spectrometry and multiphoton ionization spectrometry, are used in analytical studies, although other spectrometric methods such as phosphorescence spectrometry, absorption-and-Stokes-Raman spectroscopy, gas-phase thermal lens spectroscopy, infrared diode laser absorption spectroscopy, *etc.*, are currently used in spectroscopic studies.

Multiphoton ionization spectrometry is known to be a sensitive analytical tool. It is particularly useful when used in combination with mass spectrometry, as valuable information is concerned not only with the spectral data but also with the relative molecular mass (M_r) of the sample can be obtained. Alternatively, identification of the sample molecule is achieved from the features of the fluorescence spectrum. However, it gives no direct information regarding chemical structure. Multiphoton ionization spectrometry is also applicable to non-fluorescent samples, so that it is considered to have a wide potential for use in analytical spectroscopy.

It is, however, puzzling that only very few studies have used multiphoton ionization spectrometry for the determination of large aromatic hydrocarbons in SSJ spectrometry.[9,10] The word 'large' in this study indicates an extension of the π-electrons in the fused aromatic rings and does not refer to the physical size of the molecule. For example, anthracene and pyrene are classified as large molecules because they possess large fused aromatic ring structures. However, amino acids and polypeptides, whose π-electrons are localized in a benzene ring or a related analogue, are classified as small molecules even when the M_r is much greater than that of anthracene and pyrene. The reason why only a few multiphoton ionization spectra are reported for a large molecule is not clear so far, but is probably due to the difficulty in the quantitative analysis of the information that can be obtained from the signal intensities observed using the two different spectrometric methods.

In this study, an SSJ spectrometer is constructed, which allows alternative measurements of either the fluorescence or multiphoton ionization mass spectra. After careful calibration of the sample vapour pressure and the laser-power dependence, the ratio of the signal intensities obtained from

multiphoton ionization spectrometry and fluorescence spectrometry, is calculated. It is found that a large aromatic molecule such as anthracene gives a very small signal intensity in multiphoton ionization spectrometry. The advantages and limitations of the two methods are also discussed in this study.

Experimental

Apparatus

A schematic diagram of the experimental apparatus is shown in Fig. 1. The sample is placed in the reservoir, and the sample vapour pressure is controlled by adjusting the temperature using heating tape. The sample gas is diluted with the carrier gas argon, and the stagnation pressure is adjusted to 100 Torr, which is maintained throughout the experiment. The gas mixture is expanded into a vacuum from a laboratory-built pulsed nozzle, the pulse width being 1 ms.[11] The expansion chamber is evacuated by a 10.16 cm (4 in) oil ejector pump (Ulvac, PBL-04, 18 m^3 min^{-1}) followed by a mechanical booster pump (Ulvac, PMB-001B, 1800 l min^{-1}) backed by a rotary pump (Ulvac, D-330, 300 l min^{-1}). The pressure of the expansion chamber is maintained below 3×10^{-3} Torr during
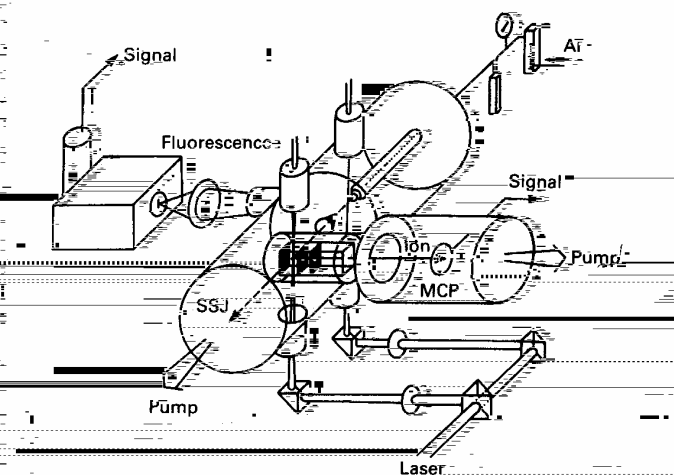
* To whom correspondence should be addressed.

Fig. 1 Supersonic-jet spectrometer allowing simultaneous fluorescence and multiphoton ionization spectrometry

the experiment. The pressure is monitored using a Pirani gauge (Ulvac, GP-2T). The ionization chamber is evacuated by use of an oil diffusion pump (Ulvac, ULK-06, 84 m³ min⁻¹) followed by a mechanical booster pump (Shimadzu, MB-30, 520 l min⁻¹) backed by a rotary pump (Ulvac, PVD-180K, 186 l min⁻¹), which is maintained at a pressure below 6 × 10⁻⁶ Torr. The pressure is monitored by an ionization gauge (Ulvac, GI-TL2). The time-of-flight tube is evacuated by a turbo-molecular pump (Osaka Vacuum, OV-TH520C, 31 m³ min⁻¹) followed by a rotary pump (Alcatel, T2012A, 310 l min⁻¹), which is maintained at a pressure below 1 × 10⁻⁶ Torr.

A Nd:YAG laser-pumped dye laser (Quantel, YG581C-20, TDL50, linewidth 0.08 cm⁻¹) is used for measurements of aniline derivatives and naphthalene. The laser wavelength is converted to the ultraviolet region by a KDP (KH_2PO_4) crystal using an autotracking system (Quantel, UVX-2). An excimer laser-pumped dye laser (Lambda Physik, LPX205, FL2002) is used for excitation of anthracene. A part of the excimer laser beam is split out by a quartz plate and is used for the ionization of anthracene. The laser beam can be operated in two directions, either for fluorescence excitation or for multiphoton ionization using a rotating prism. This configuration allows alternative excitation or multiphoton ionization, hence, allows the alternative measurement of either the fluorescence or the mass spectra with only a minor change in the experimental conditions.

For fluorescence measurements, the laser beam is focused by a quartz lens (focal length, 50 cm) into a molecular jet 7.5 mm away from the nozzle. Fluorescence is collected, by a quartz lens, onto the slit of a monochromator (Jasco, CT-25CP), which is equipped with a photomultiplier (Hamamatsu, R585). The slit-width is adjusted to 0.1 mm, corresponding to a spectral resolution of 0.21 nm. Capacitors are wired to all of the dynodes of the photomultiplier in order to avoid saturation of the output signal.[12] The voltage of the photomultiplier typically used is −900 V. It was confirmed experimentally that no signal saturation occurs under these conditions. For multiphoton ionization, the laser beam is focused by a quartz lens (focal length, 100 cm) into a molecular beam 105 mm away from the skimmer (117.5 mm away from the nozzle). Induced ions are accelerated by a repulsion potential (+1400 V) through grids (50 mesh, 0.02 mm wire, 92% optical transmission) and travel 48 cm prior to mass analysis. They are detected by an assembly of three microchannel plates (MCP, in Fig. 1, Hamamatsu, F1094-32S). The applied voltage is −3000 V. The time-of-flight signal is recorded by a digital memory (Iwatsu, DM901) and is converted to a signal that is 100-times slower. It is recorded again by an autodigitizer (Autonics, S-210) combined with a signal averager (Autonics, F610). The mass resolution achieved is 300. An excitation, fluorescence, or multiphoton ionization spectrum is measured by recording the signal with a boxcar integrator (NF Circuit Design Block, BX-530A) and is displayed by a strip-chart recorder.

Reagents

Aniline was obtained from Kishida and naphthalene from Kanto Kagaku. Other aromatic compounds such as N-ethyl-aniline, N-methylaniline, o-, m- and p-toluidine, and anthracene were supplied by Tokyo Kasei. Laser dyes such as Rhodamine 610 and Rhodamine 640 were purchased from Exciton and 2-(biphenyl-4-yl)-5-phenyl-1,3,4-oxadiazole (PBD) from Lambda Physik.

Results and Discussion

Sample Vapour Pressure

For comparison of fluorescence spectrometry and multiphoton ionization spectrometry, the signal intensities should

be calibrated in advance. The vapour pressure of the sample at a specified temperature is given in ref. 13. The vapour pressures of aniline derivatives at the different temperatures investigated are calculated from the Clausius–Clapeyron equation

$$Ln(P_2/P_1) = (- \Delta H_v/R)(1/T_2 - 1/T_1) \qquad (1)$$

where P_1 and P_2 are the pressures at temperatures T_1 and T_2, R is the gas constant and ΔH_v is the enthalpy of vaporization.[14] The vapour pressures of naphthalene and anthracene are calculated by following Antoine's equation:

$$Log_{10} P = A - B/(C + T) \qquad (2)$$

where P is the sample pressure, and A, B, and C are constants.[13] Calibration using these equations allows the comparison of the absolute signal intensities of the chemical species investigated. It is noted that the ratio of the signal intensities obtained by multiphoton ionization spectrometry and fluorescence spectrometry is unchanged even when there are substantial errors in the calibrations described above, as the measurements are carried out alternatively without changing the experimental conditions.

Dependences of the signal intensities in fluorescence and multiphoton ionization spectrometries on the partial sample vapour pressure in the nozzle were investigated. Linear relationships were observed from zero to 3 Torr in the two methods; this was ascertained by using anthracene in fluorescence spectrometry and p-toluidine in multiphoton ionization spectrometry, as representative compounds.

Laser Pulse Energy

Dependences of the fluorescence intensities on the laser pulse energy are shown in Fig. 2 for various aniline derivatives. The signal is almost saturated at 0.2 mJ, which is considered to be owing to a reduction of the population in the ground state by irradiation with a marked photon flux of the laser. For comparison of the signal intensity, the fluorescence measurements were performed or calibrated at a pulse energy of 1 mJ.

The dependence of the signal intensity, in multiphoton ionization spectrometry, on the laser pulse energy is shown in Fig. 3 for o-toluidine; the signal intensity changes sigmoidally. The signal increases in the second order below 0.2 mJ; two photons are required for excitation and ionization. The signal intensity is proportional to the square of the pulse energy, but it deviates from this relationship and saturates above 0.5 mJ; the molecule in the ground state is excited and is further ionized so that the population in the ground state decreases. For comparison of the signal intensity, the measurement was carried out at a pulse energy close to 1 mJ. The signal
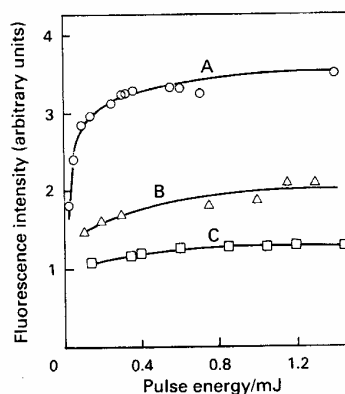

Fig. 2 Dependences of signal intensity on laser pulse energy in fluorescence spectrometry for: A, p-toluidine; B, N-methylaniline; and C; N-ethylaniline
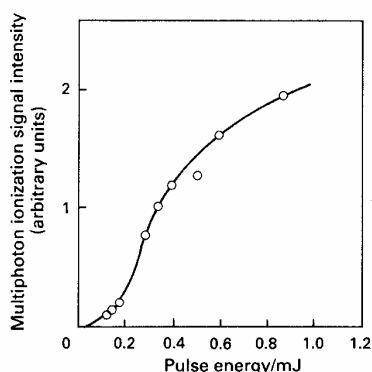
Fig. 3 Dependence of laser pulse energy on signal intensity in multiphoton ionization spectrometry for o-toluidine

Table 1 Relative signal intensities in multiphoton ionization and fluorescence spectrometries. The signal is normalized against N-ethylaniline. All the compounds are excited at 0–0 transition except for naphthalene specified by $\overline{8}_0^1$

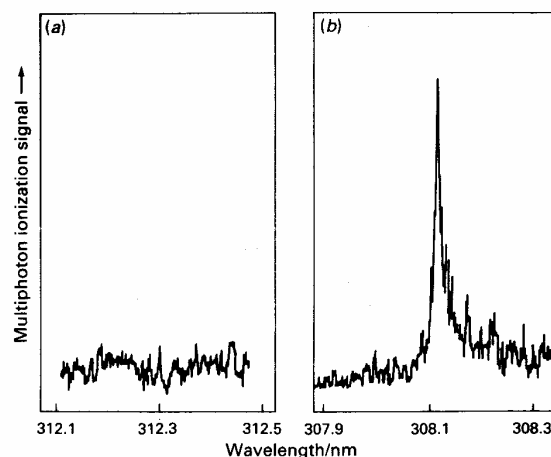| Compound	Fluorescence	Multiphoton ionization	Multiphoton ionization/ fluorescence
N-Ethylaniline	1.0	1	1
N-Methylaniline	1.6	2	1
o-Toluidine	2.6	0.4	0.2
m-Toluidine	1.4	0.4	0.2
p-Toluidine	2.0	0.8	0.4
Aniline	2.5	0.6	0.2
Naphthalene (0–0)	0.13	<0.0001	<0.001
Naphthalene ($\overline{8}_0^1$)	1.3	0.001	0.0008
Anthracene	1.5	0.00001	0.000007
Anthracene (+UV)	1.5	0.001	0.0007

Fig. 4 One-colour multiphoton ionization spectra for naphthalene excitation: (a) 0–0 transition; and (b) $\overline{8}_0^1$ transition

intensities measured at slightly different values (±15%) were properly corrected, according to the calibration graph obtained. The maximum pulse energy of the excimer laser-pumped dye laser applied to anthracene was 0.2–0.5 mJ. The signal intensity was measured at this energy level and was normalized by the signal intensity obtained for aniline measured at the same pulse energy.

It may be possible to measure and compare the signal intensities at lower pulse energies, e.g., ≈10 μJ per pulse, at which the first and second order dependences are observed for fluorescence and multiphoton ionization spectrometries, respectively. However, a laser with a large pulse energy was used to improve the sensitivity, which was necessary to detect multiphoton ionization signals for large aromatic hydrocarbons.

Standard Sample

The signal intensity is further calibrated against a standard molecule, i.e., N-ethylaniline, every day. This approach removes day-to-day variation in the signal measurements. However, variation of the absolute signal intensity was less than ±50%, even when the laser beam path was independently realigned and the measurement was performed on a different day.

Signal Intensity

Table 1 shows the signal intensities for several aromatic hydrocarbons measured by fluorescence spectrometry and multiphoton ionization spectrometry. The values are calculated by averaging 3–4 independent data values. In fluorescence spectrometry, the laser wavelength is adjusted to the strongest line in the excitation spectrum, and the strongest line in the fluorescence spectrum is monitored. The former corresponds to the 0–0 transition in all instances except for naphthalene, for which this electronic transition is forbidden and which has a larger signal peak at the $\overline{8}_0^1$ transition (λ_{ex} = 308.11 nm). In multiphoton ionization spectrometry, the mass spectrum was measured and accumulated over a period of about 1 min (20 Hz, 1024 shots). The parent ion signal, which is dominant in the mass spectrum, is used for discussion of the signal intensity.

At the start of the experiment, the partial vapour pressure of the sample was adjusted to 1 Torr in the nozzle. Under this condition, sharp multiphoton ionization spectra were observed for aniline derivatives. However, no such spectra were observed for naphthalene or anthracene. The partial vapour pressure was increased to 20 Torr, by heating the reservoir up to 102 and 195 °C for naphthalene and anthracene, respectively. Fig. 4 shows the multiphoton ionization spectrum

for naphthalene measured by the one-colour ionization scheme. When naphthalene is excited at the 0–0 transition (312.30 nm), no appreciable signal is observed. At a shorter wavelength (308.11 nm), a sharp signal peak appears at the $\overline{8}_0^1$ transition, the signal-to-noise ratio being 25. This is because the 0–0 transition is forbidden for naphthalene,[15,16] three-photons are required for excitation at 312.30 nm (3.97 eV), the two-photon energy (4.02 eV × 2 = 8.04 eV) at 308.11 is close to the ionization potential (8.13 eV) and naphthalene is further ionized from the Rydberg state by the electric potential applied for the extraction of ions to the drift tube. Fig. 5(a) shows the one-colour multiphoton ionization spectrum for anthracene excited at the 0–0 transition (361.08 nm). The 0–0 transition is strongly favoured for anthracene, and a sharp peak is observed even in the one-colour ionization scheme, although the two-photon energy (3.43 eV × 2 = 6.86 eV) is much less than the ionization potential (7.44 eV).[17] The signal intensity is strongly enhanced when an excimer laser (308 nm, 4.03 eV) is used for ionization, as shown in Fig. 5(b). The signal-to-noise ratio improved from 35 to 760. This is because the two-photon energy (3.43 eV + 4.03 eV = 7.46 eV) exceeds the ionization potential (7.44 eV).

As shown in Table 1, the aniline derivatives give similar signal intensities (0.2–1) in multiphoton ionization spectrometry. A large aromatic molecule has a similar fluorescence intensity relative to the aniline derivatives but has a very small signal intensity in multiphoton ionization spectrometry. The weak ionization signal for anthracene in the one-colour scheme is attributed to a high ionization potential
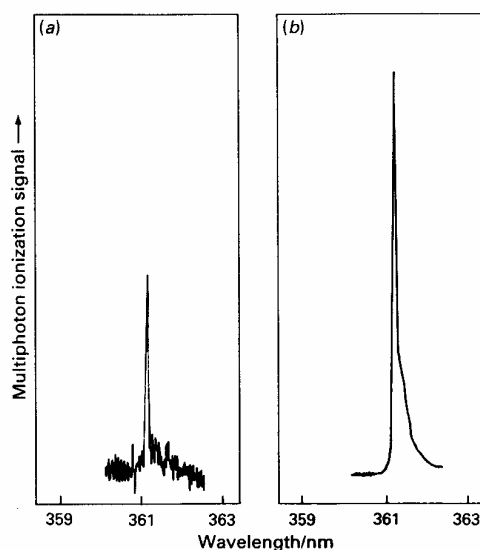
Fig. 5 (a) One-colour and (b) two-colour multiphoton ionization spectra for anthracene (0–0 transition). The pulse energy is increased from 0.2 to 0.5 mJ

relative to the two-photon energy at the 0–0 transition, as described. This is owing to delocalization of the π-electrons, resulting in a large stabilization energy in the first excited state. The two-colour, two-photon ionization scheme provides a better but still poor ionization yield. This is considered to be because the optimization, e.g., temporal and spatial overlaps of laser pulses, etc., is difficult to achieve practically in the two-colour scheme. The use of an intense ionization laser might increase the sensitivity, but it might also increase the non-resonant background signal.

In fact, very few multiphoton ionization spectra have been reported so far for a large molecule in SSJ spectrometry. Naphthalene has two aromatic rings and a sharp ionization spectrum is observed when this compound is excited to the high vibrational levels of the S_1 state in the 296.9–302.3 nm region (e.g. $\bar{8}_0^1 8_0^1$ or $\bar{8}_0^1 7_0^1$ transitions),[18] and a similar result is observed even at the longer wavelength of 308.11 nm (8_0^1 transition) in this study. Carbazole has two aromatic rings and a pyrrole ring, providing a sharp ionization spectrum at 324.56 nm (0–0 transition).[19] Phenanthrene has three aromatic rings, but it has a non-linear configuration in contrast to anthracene. A weak but well-resolved spectral feature is observed at 341.0 nm (0–0 transition) by the one-colour ionization scheme and is enhanced more than 10^4 times by the two-colour scheme.[17] A similar result is observed for anthracene even at 361.08 nm (0–0 transition) in this study; anthracene and pyrene (0–0 transition, 367.44 nm) have already been investigated but no sharp spectra are presented although the signals are reported to be enhanced 3×10^3 times.[17] More recently, tetracene derivatives[9] and perylene[10] have been measured by a two-colour scheme, providing sharp multiphoton ionization spectra.

The boundary compound might be anthracene, corresponding to an excitation wavelength of ≈ 360 nm. It is possible to ionize a large molecule by the one-colour, three-photon ionization scheme, but the signal is very weak at the power levels used in the present study. Even in the two-colour ionization scheme, the signal intensity is still ≈ 1000 times less than the aniline derivatives under the conditions used. It might be possible to ionize a large aromatic molecule by exciting it to the high vibrational levels of the S_1 or S_2 state, as demonstrated in non-jet multiphoton ionization spectrometry.[20] However, this ionization scheme might provide a congested and broadband spectrum in SSJ spectrometry, owing to a dense manifold of vibrational levels and an intramolecular radiationless transition.[21,22]

Comparison

Well-resolved spectral features are also observed in the fluorescence spectra of large aromatic compounds. This is useful for reliable identification of the sample. However, it is difficult to assign the molecule when no standard spectrum is available. A theoretical approach based on a molecular orbital calculation [complete neglect of differential overlap (CNDO/S) method][23] has been developed, but the error in the estimation of the wavelength for 0–0 transition (1–2%) is not sufficiently low for accurate assignment.[24] A discussion of spectral similarity using a cross-correlation factor gives additional information for assignment.[25] However, this is useful only for the determination of the basic outline of the molecular structure, at present. On the other hand, multiphoton ionization spectrometry gives direct information about the M_r, although the signal intensity is low especially for a large molecule. The signal-to-noise ratio in multiphoton ionization spectrometry is, unfortunately, poorer than that obtained in the excitation spectrum measured with a wide monochromator bandwidth, even for aniline derivatives, although it is much better than the value obtained with a narrow monochromator bandwidth. Of course, the sensitivity of the instrument depends on the individual design and the conditions used, but the sensitivity seems to be too low to apply it to trace analysis especially for large aromatic compounds.

In resonance-enhanced multiphoton ionization spectrometry, the ionization yield is low for a compound with a short fluorescence lifetime; the molecule rapidly relaxes from the excited state. It is possible to increase the sensitivity by increasing the laser power, but it might also increase the background signal occurring from non-resonant ionization of inert gases such as argon or a pump oil. It has been reported that the detection limit increases as the size of the peptide increases, which is considered to be due to less efficient ionization in the flexible molecule with a greater number of modes for energy disposal in the absorption process.[26] This implies that the resonant (sharp) multiphoton ionization spectrum is difficult to observe for non-fluorescent and flexible molecules. Thus, multiphoton ionization spectrometry provides a range of information that is of use in assignment, but it has several limitations especially for large aromatic hydrocarbons. On the other hand, fluorescence spectrometry gives a better signal-to-noise ratio, but further studies are necessary in order to construct a protocol to identify the sample molecule from the excitation and fluorescence spectrum. It is noted that a single instrument allowing both types of spectrometric analyses is particularly useful because of the availability of both sets of information in the identification of chemical species.

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