Development of a novel, long-lifetime supersonic jet source for laser spectroscopy of biological molecules

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<th>Description</th>
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<tbody>
<tr>
<td>A</td>
<td>Adenine</td>
</tr>
<tr>
<td>Ala</td>
<td>Alanine</td>
</tr>
<tr>
<td>AIE</td>
<td>Adiabatic ionisation energy</td>
</tr>
<tr>
<td>C</td>
<td>Cytosine</td>
</tr>
<tr>
<td>CBS</td>
<td>Complete-basis-set</td>
</tr>
<tr>
<td>cc</td>
<td>Correlation consistent</td>
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<tr>
<td>CC</td>
<td>Coupled cluster</td>
</tr>
<tr>
<td>CI</td>
<td>Configuration interaction</td>
</tr>
<tr>
<td>DFT</td>
<td>Density functional theory</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>FC</td>
<td>Franck-Condon</td>
</tr>
<tr>
<td>FSSFI</td>
<td>Fractional Stark-state selective field ionisation</td>
</tr>
<tr>
<td>FWHM</td>
<td>Full width at half maximum</td>
</tr>
<tr>
<td>G</td>
<td>Guanine or Gaussians</td>
</tr>
<tr>
<td>GGA</td>
<td>Generalised gradient approximations</td>
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<tr>
<td>Gly</td>
<td>Glycine</td>
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<tr>
<td>GTO</td>
<td>Gaussian-type orbitals</td>
</tr>
<tr>
<td>HF</td>
<td>Hartree-Fock</td>
</tr>
<tr>
<td>HF- RH</td>
<td>Hartree-Fock-Roothan-Hall</td>
</tr>
<tr>
<td>HOMO</td>
<td>Highest occupied molecular orbital</td>
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<tr>
<td>IE</td>
<td>Ionisation energy</td>
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<tr>
<td>IP</td>
<td>Ionisation potential</td>
</tr>
<tr>
<td>IR</td>
<td>Infrared</td>
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<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>KDP</td>
<td>Potassium dihydrogen phosphate</td>
</tr>
<tr>
<td>LCAO</td>
<td>Linear combination of atomic orbitals</td>
</tr>
<tr>
<td>LD</td>
<td>Laser desorption</td>
</tr>
<tr>
<td>LDA</td>
<td>Local density approximations</td>
</tr>
<tr>
<td>LIF</td>
<td>Laser induced fluorescence</td>
</tr>
<tr>
<td>MATI</td>
<td>Mass-analysed threshold ionisation</td>
</tr>
<tr>
<td>MALDI</td>
<td>Matrix-assisted laser desorption/ionisation</td>
</tr>
<tr>
<td>MCP</td>
<td>Multichannel plate</td>
</tr>
<tr>
<td>MCSCF</td>
<td>Multi-configurational self-consistent field</td>
</tr>
<tr>
<td>MPI</td>
<td>Multi photon ionisation</td>
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<tr>
<td>MPn</td>
<td>Möller-Plesset perturbation theory (to $n^{th}$ order)</td>
</tr>
<tr>
<td>PES</td>
<td>Photoelectron spectroscopy</td>
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<td>PFI</td>
<td>Pulsed field ionisation</td>
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<td>Phenylalanine</td>
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<td>Photoionisation efficiency</td>
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<td>R2PI</td>
<td>Resonant Two-photon ionisation</td>
</tr>
<tr>
<td>REMPI</td>
<td>Resonance-enhanced multi photon ionisation</td>
</tr>
<tr>
<td>RETOF</td>
<td>Reflectron time of flight</td>
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<tr>
<td>RI</td>
<td>Resolution of the identity</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribonucleic acid</td>
</tr>
<tr>
<td>SCF</td>
<td>Self-consistent field</td>
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<tr>
<td>SHG</td>
<td>Second harmonic generation</td>
</tr>
<tr>
<td>S/N</td>
<td>Signal to noise ratio</td>
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<tr>
<td>SPI</td>
<td>Single photon ionisation</td>
</tr>
<tr>
<td>STO</td>
<td>Slater-type orbitals</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<td>--------------</td>
<td>-----------------------------------------------------</td>
</tr>
<tr>
<td>T</td>
<td>Thymine</td>
</tr>
<tr>
<td>THG</td>
<td>Third harmonic generation</td>
</tr>
<tr>
<td>TOF</td>
<td>Time of flight</td>
</tr>
<tr>
<td>TOFMS</td>
<td>Time of flight mass spectrometry</td>
</tr>
<tr>
<td>Trp</td>
<td>Tryptophan</td>
</tr>
<tr>
<td>TTL</td>
<td>Transistor–transistor logic</td>
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<tr>
<td>Tyr</td>
<td>Tyrosine</td>
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<tr>
<td>U</td>
<td>Uracil</td>
</tr>
<tr>
<td>UV</td>
<td>Ultraviolet</td>
</tr>
<tr>
<td>VIE</td>
<td>Vertical ionisation energy</td>
</tr>
<tr>
<td>VUV</td>
<td>Vacuum ultraviolet</td>
</tr>
<tr>
<td>ZEKE</td>
<td>Zero electron kinetic energy</td>
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Abstract

A novel laser desorption system, with improved signal stability and extraordinary long lifetime, is presented for the study of jet cooled biomolecules in the gas phase using vibrationally resolved photoionisation spectroscopy. Tryptophan (Trp) is used as the test substance to characterize this desorption source. Here, the surface of a moving and rotating rod (graphite/Trp, 3mm diameter and 6 mm length) is exposed to a pulsed desorption beam from a Nd:YAG (1064 nm) laser running at 20Hz (Continuum Minilite).

The characteristics of the source developed here and its properties with respect to cooling and stability have been investigated. Good control over the rod movement and the delivery of the IR beam result in a highly stabilized source with no noticeable fragmentation products.

The combination of premixing within the source and using a pellet has made it possible to produce a stable jet-cooled beam of Trp, which lasts for several weeks without changing the sample.

Additionally, the stability and signal to noise ratio has been improved by averaging the signal over the entire sample pellet by synchronizing the data acquisition with the rotation of the sample rod. The results demonstrate how a combination of the above helps to produce stable time of flight (TOF) signal and good quality one- and two-colour resonant two-photon ionisation (R2PI), photoionisation efficiency (PIE) and mass analyzed threshold ionization (MATI) spectra of Trp. The existence of six low-lying conformers of Trp in the gas phase has been confirmed. The first MATI spectrum of an isolated biomolecule (Trp) via R2PI for the determination of IE with high accuracy as ± 3 cm⁻¹ has been recorded.
Declaration

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This work is dedicated to my father.

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Dr. Jiri Cerny, for introducing me to the ab initio methodologies.

The mechanical workshop at the University of Manchester.
Chapter 1 Introduction

The study of large biomolecules such as DNA and proteins can be supported by an in-depth investigation of their smaller building blocks (amino acids and nucleic acid bases) by both experimental and theoretical methods. Knowledge of the properties of “elementary blocks” can improve the prediction of structure and function of larger biomacromolecules. From the theoretical point of view, the spectra obtained by performing spectroscopy of small species in the gas phase can be directly compared to computational results, an approach that presently is not practical for biomacromolecules. Gas-phase spectroscopic studies of medium-sized biological systems have become increasingly common over recent years\textsuperscript{1-6}, although the associated computational studies of even modestly sized biological systems are still quite challenging. This is particularly true because of the propensity of such systems to exhibit conformational and tautermeric isomerisation. The conformations of a molecule play a crucial role in molecular recognition processes, which are the basis for all selective biochemical reactions. Even small biomolecules often show several conformers with comparable energies. Nevertheless, under physiological conditions, generally only a few conformers are relevant. This is a surprising and important observation, because the reactivity and functionality of biologically active substances are governed by their structure. Understanding the conformation of isolated biological molecules requires also a detailed characterisation of the inter- and intra-molecular interactions\textsuperscript{7} that govern their inherent preferences.

Hydrogen bonds are ubiquitous and play critical roles in biological systems. Intermolecular interactions between $\pi$-conjugated molecules are of great importance in
many biological systems (e.g., DNA, RNA, proteins). The presence of these aromatic side chains tends to favour the formation of $\pi$- and H-bonds, which can hinder the manifestation of the intrinsic backbone preferences.\textsuperscript{8-10} The DNA bases have been the subject of many theoretical and experimental investigations. The purines guanine (G) and adenine (A), and the pyrimidines cytosine (C) and thymine (T), are the complementary bases forming the hydrogen-bonded G-C and A-T Watson-Crick base pairs in DNA\textsuperscript{11} whose sequence stores the genetic code.

Gas phase optical spectroscopic techniques, such as IR/UV double resonance spectroscopy, provide an accurate insight into the intrinsically stable structures of biomolecules. For guanine, at least four different conformers have been observed in the R2PI (resonant two photon ionisation) spectrum, while only one species gives rise to the R2PI spectrum of the guanine-cytosine base pair.\textsuperscript{12} Similar results have been found for the guanine\textsuperscript{13} and cytosine dimers\textsuperscript{14} as well as guanosine.\textsuperscript{15,16}

The spectroscopy of proteins and polypeptides is dominated by three amino acids: phenylalanine (Phe), tyrosine (Tyr) and tryptophan (Trp). From a spectroscopic point of view, they contain a suitable chromophore (aromatic system) since their excited states $S_1\leftarrow S_0$ and $D_0\leftarrow S_1$ ($S_0 =$ Ground state, $S_1 =$ Excited state, $D_0 =$ Cation state) transitions are normally situated in the near-UV region. Protein shape is governed by several stabilizing interactions. It is widely agreed that protein stability is mainly due to the hydrophobic effect and van der Waals interactions. Amino acids and their derivates are of considerable interest in relation to their conformational behaviour and the structure of their aggregates. Even the absence or replacement of a single amino acid in a peptide chain may cause changes in the tertiary and quarternary structure.
From the experimental point of view, the spectroscopic investigation of these systems is a very big challenge indeed. Since biomolecules typically have extremely low vapour pressures and decompose easily upon heating, carefully designed ovens or laser desorption sources are required to bring these molecules into the gas phase cleanly and with sufficient number density to allow their spectroscopic study under molecular beam conditions.

The present work focuses on laser desorption methods, because these offer the potential for high density and very good rotational and vibrational cooling in jet expansions. The major experimental difficulties in laser desorption (LD) methods are in coupling the source to the supersonic jet expansion to obtain efficient cooling, and in obtaining a stable density of the desorbed sample molecules in the molecular beam for further spectroscopic studies. In surface desorption experiments, reproducibility and shot-to-shot stability are always an issue. In experimental systems, which have good shot-to-shot stability, the methods employed often result in a short lifetime of the desorption source, requiring very frequent replenishment of the sample on the matrix (graphite).

The aim of the present project is to develop a new experimental apparatus and a new laser desorption long-life source for the spectroscopic study of biological molecules such as tryptophan (Trp) as reported in this thesis. The new source is designed to desorb the biomolecule from a cylindrical pellet and mix it with the carrier gas in a volume within the source, followed by a supersonic expansion. The characteristics of the source developed here and its properties with respect to efficient cooling and stability are investigated. The combination of premixing within the source and using a pellet has made it possible to produce a stable jet-cooled beam of tryptophan (Trp), which lasts for
several weeks without changing the sample. The novel source has been applied to the spectroscopy of Trp. To investigate the conformational structure of Trp in the gas-phase, the experimental R2PI spectra have been combined with predictions of *ab initio* calculations. Threshold ionisation spectroscopy has been carried out for the first time for a biomolecular system to determine the ionisation energy of Trp with high accuracy (± 3cm⁻¹).

In Chapter 2 of the thesis, the most common experimental techniques used for analyzing the bimolecular samples are described. Chapter 3 gives an overview of the *ab initio* methodologies used to perform theoretical calculations. The description of the experimental apparatus and a general overview of the hardware and software techniques used to record the spectra reported in this thesis are given in Chapter 4. Chapter 5 describes the general performance of the system using the test molecule perdeutero benzene (benzene-d₆). In Chapter 6, the development of the novel laser desorption source and its characterisation in respect to desorption parameters are discussed. The first spectroscopic results for Trp are presented in Chapter 7. The determination of the geometries and the relative energies of tryptophan using the *ab initio* methods are reported in Chapter 8. A brief summary of the achieved results and perspectives is presented in the final chapter.
1.1 References


Chapter 2 Experimental methods

This chapter will give a general description of the experimental techniques used in this thesis. These methods are presented in the following order:

- Supersonic jet expansion and cooling.
- Time of flight (TOF) mass spectrometric detection methods.
- High resolution spectroscopic methods for study of van der Waals and other weakly bound molecular clusters in their exited cationic states.
- Laser desorption (LD) technique.

2.1 Supersonic gas expansion

Studies of collisional processes in expanding gases show that random thermal energy is transferred into directed translational energy, and internal energy distributions are cooled. The total energy $U$ of a molecule with mass, $m$, is the sum of the internal energy $U = U_{\text{trans}} + U_{\text{rot}} + U_{\text{vib}}$. With potential energy $pV$ and its kinetic energy $1/2 mv^2$ of expanding gas, the energy conversation for the total energy before and after the expansion can be written:

$$U_0 + p_0 V_0 + 1/2 mv_0^2 = U + pV + 1/2 mv^2$$  \hspace{1cm} (2.1)

The pressure after expansion is small ($p \ll p_0$) and we can assume thermal equilibrium inside the reservoir, which means $v_0 = 0$. Therefore we can approximate equation (2.1) by setting $v_0 = 0$ and $p = 0$, which results in:

$$U_0 + p_0 V_0 = U + 1/2 mv^2$$  \hspace{1cm} (2.2)
Equation (2.1) illustrates that a cold beam with small internal energy $U$ is obtained, if most of the initial energy $U_0 + pV_0$ is converted into kinetic energy $\frac{1}{2}mv^2$. The flow velocity $v$ may exceed the local sound velocity. In this case, a supersonic beam is produced.

In the expansion of an atomic gas through a nozzle, while the most probable velocity in the distribution increases with the distance from the nozzle, the translational energy spread decreases, or cools. In an expansion involving a molecule, similar translational cooling effects are observed as well as cooling of the rotational and vibrational degrees of freedom. When a heavy molecule is mixed in low concentration, or seeded, in a light drive gas, the heavier component attains almost the same velocity distribution as the lighter one (buffer gas). However, it is the nozzle geometry that determines supersonic jet properties, particularly the Mach number $M$. The relationship between supersonic velocity, $v$, and the Mach number, is given:

$$v = Mc = M \sqrt{\frac{\gamma kT}{m}}$$

(2.3)

where $c = \text{local speed of sound}$, and $\gamma = \frac{C_p}{C_v}$ is the specific heat ratio of the buffer gas,

$T = \text{final temperature of the gas}$ and $k = \text{Boltzmann constant}$.

The Mach number is inversely proportional to the final temperature $T$ and increases with the product of stagnation pressure and nozzle diameter,

$$\frac{T_0}{T} = 1 + 0.5(\gamma - 1)M^2$$

(2.4)
where $T_0 =$ initial temperature of the gas.

Combining the equations (2.3) and (2.4) gives the maximum speed (if $T_0 \gg T$) of the supersonic jet:

$$v_{\text{max}} = \sqrt{\frac{2\gamma kT_0}{(\gamma - 1) m}} \tag{2.5}$$

Therefore the lowest temperatures are obtained with the largest nozzle diameters and pressures, and thus with the largest gas load. Thus, pumping speed constitutes a limitation, necessitating either differential pumping or reduction of the duty cycle with a pulsed nozzle. The latter solution is quite compatible with laser desorption. As described later in 2.4.1.4, collisions occurring in the gas phase immediately after the desorption event result in the alteration of the translational and internal energy distributions measured after laser desorption. The advantages of jet cooling are threefold:

1. Cooling improves spectroscopic resolution.$^{1,2}$
2. Cooling reduces fragmentation of volatile biomolecules.
3. Cooling provides a pathway for cluster formation of biomolecules.$^3$

### 2.2 Time of flight mass spectrometry (TOFMS)

Time of flight mass spectrometry (TOFMS) is the technique used to separate gas phase ions according to their ratio of mass/charge ($m/z$). Time of flight (TOF) mass
spectrometers operate on the simple principle that a packet of ions of different ratio of mass/charge ($m/z$) can be separated by applying a constant (or static) electric field, $F$. In Fig. 2.1 the basic principle of linear TOF spectrometer based on single stage and two stage acceleration (Wiley-MacLaren design) is shown.

![Diagram of TOF mass spectrometer](image)

**Fig. 2.1:** Single stage ($d = 0; F_d = 0$) and two stage (Wiley-MacLaren design) collinear TOF mass spectrometer.

In a linear TOF mass spectrometer in its simple design (collinear, single acceleration region, when $d$ and $F_d$ are zero), ions are formed in a short source region(s) and extracted by two electrodes. The first electrode is the source backing plate (Ion repeller), on which a positive voltage, $V$, is applied and the second plate the acceleration plate (Ion accelerator, $V = 0$). The positive voltage imposes an electric field ($F_s = V/s$) across the source region and accelerates all ions to the same kinetic energy (if, $\Delta s = 0$), given by the equation (2.3):

$$\frac{mv^2}{2} = zeFs$$

(2.6)
where $m =$ mass of ions, $v =$ the velocity of ions, $e =$ the charge on an electron, $z =$ number of charges.

The ions travel through the field-free flight tube ($D$), where ions are directed towards the detector. However, in a non collinear design, used in the present work, extraction of ions occurs at 90 degrees to the axis of the molecular beam. In this case a set of ion optics needs to be placed at the front of the flight tube that compensates any residual horizontal velocity component in the direction of travel and collimates the ion beam to achieve a high intensity of ions on the detector. The x- and y-directions of the ion beam are controlled by a set of deflection plates, known as a capacitor. These are four small (several square centimetres) metal plates, two for the x-direction and two for the y-direction, that have relatively small (< 10 V) potentials applied to them. The potentials on each set of plates are adjusted until the maximum ion signal is detected. The collimation device is known as a lens, consisting of a set of plates, which uses a low voltage elliptical field to control ion beam diffusion. Flight tubes longer than 2 m require a guide wire, because the lens does not collimate ions sufficiently over this length.$^{5,6}$

According to equation (2.6), the velocity of ions after passing the acceleration region is given by:

$$v = \left( \frac{2zeF_s s}{m} \right)^{\frac{1}{2}}$$  \hspace{1cm} (2.7)

After passing through the acceleration region, the ions then arrive at the much longer field-free drift region ($D$). The drift time $t_\mu$ across the field free region is:
The ions spend most of their time in drift region. Therefore their time of flight ($t$) is given approximately by:

$$t = \left( \frac{m}{2zeF_s s} \right)^{\frac{1}{2}} D$$

(2.9)

and the mass dependence on their time of flight is given by:

$$\frac{m}{z} = 2eF_s s \left( \frac{t}{D} \right)^2$$

(2.10)

The accuracy of mass determination depends on how accurately the distance $D$, the time of flight $t$, and the acceleration voltage can be measured. In practice, however, one measures the TOF of one known mass for calibrating and then calculates the time of flight of different masses by using:

$$\left( \frac{m_1}{m_2} \right)^{\frac{1}{2}} = \left( \frac{t_1}{t_2} \right)$$

(2.11)

The mass resolution $\Delta m/m$ depends on the shortest time interval $\Delta t$ that can still be resolved and can be obtained from equation (2.10):

$$\left( \frac{\Delta m}{m} \right) = 2 \left( \frac{\Delta t}{t} \right)$$

(2.12)

In a single stage linear TOF mass spectrometer the mass resolution is generally one part in 300 to 400.$^7$ Different uncertainties in the time of ion formation, location in the
extraction field and the initial kinetic energy of ions are responsible for the low mass resolution. Using short ionisation pulses from nanosecond and picosecond lasers and desorption of a sample from a smooth surface (e.g. MALDI: matrix-assisted desorption/ionisation)\textsuperscript{8} minimizes the temporal and spatial distribution.

In the Wiley and MacLaren design, time of flight focussing method, the ion source contains two acceleration regions as shown in Fig. 2.1. Based on applying a delayed draw pulse of length up to 10 $\mu$s after ionisation to the ion repeller to produce the electric field $E_s$, ions are accelerated out of the source towards the detector. This pulse lasts until all the ions have left the first field region. At all times, the second acceleration region ($d$) has an electric field ($F_d$) by applying a positive voltage to the ion accelerator, the third plate is grounded ($V = 0$) and the region ($D$) is field free. The double-field source introduces two new parameters, $d$ and $F_d/E_s$, which are not found in the single source. This increased flexibility makes the double field source easier to adjust, and gives it higher resolution. In this system any ion, with an initial energy $U_0$, will have a total energy ($U$) and a flight time ($t$) through the three regions:

\begin{align}
U &= U_0 + qsF_s + qdF_d \\
t &= t_s + t_D + t_0
\end{align}

(2.13) \hspace{1cm} (2.14)

where $t_s$ = time in the source, $t_D$ = time in the drift region and $t_0$ = time of ion formation.

A more practical method, found in modern instruments, of compensating for the initial kinetic energy distribution is by use a reflectron. The reflectron time of flight (RETOF) mass spectrometer was invented in 1970 by Mamyrin \textit{et al.}\textsuperscript{9} The reflectron works as an
energy focusing device, which consists of series of retarding plates as shown in Fig. 2.2. Each plate in the reflectron has an electrical potential applied to it. The electrical field thus produced retards the ions and turns the direction of flight of the ions through nearly 180°, with only a slight angle to the original direction of travel. Ions of different kinetic energy, but the same \((m/z)\) penetrate to different depths in the retarding field. The more energetic ions penetrate the retarding field of the reflectron to a greater depth and travel a longer path to the detector than ions with less kinetic energy, so they arrive at the detector at the same time as the less energetic ions. The small difference in flight path introduced by the reflectron provides a re-focusing of ions with the same \((m/z)\) at the detector. This type of energy focusing is independent of the \((m/z)\) of an ion, so it is not necessary to adjust the extraction fields. The mass selectivity can be achieved by optimisation of the ratio of the braking \((V_B)\) and reflecting voltages \((V_R)\). RETOF mass spectrometers have a mass resolution of over one part in 10000.\(^7\)

Fig. 2.2: The Dual-stage Reflectron of Mamyrin.\(^9\)
2.3 Laser spectroscopic methods

2.3.1 Resonance-enhanced multi photon ionisation (REMPI)

REMPI is a highly sensitive, highly species-selective gas-phase analysis technique that has been applied to molecular spectroscopy. REMPI is known as a special method of multi photon ionisation (MPI) by the fact that the photons are resonant with an excited state (ro)vibronic energy level. This resonant ionisation is abbreviated to \((m + n)\) for one-colour and \((m + n')\) for two-colour, where \((m)\) gives the number of photons that are resonant with an intermediate state and \((n)\) the number of photons required for ionisation of the molecule. Fig. 2.3 shows a one-colour, two-photon \((1+1)\) REMPI excitation/ionisation scheme on the left side and two-colour, two-photon \((1+1')\) REMPI scheme on the right side. This special case of two photon ionisation is also known as R2PI.

![Diagram of REMPI](image)

**Fig. 2.3:** One-colour, two-photon \((1+1)\) R2PI (on the left side), two-colour, two-photon \((1+1')\) R2PI (on the right side) excitation/ionisation.
The (1+1) scheme can be used when the first excited state \((S_1)\) lies at least half way between the neutral and ionic state. Resonant absorption of a single photon excites the molecule from the ground state \((S_0)\) to \(S_1\), where absorption of a second photon of the same energy results in ionisation. On the right side of Fig. 2.3 the two-colour, two-photon R2PI \((1+1')\) scheme is shown, where the first photon is used to excite the molecule to the first excited state \((S_1)\) resonantly, and a second photon of different energy is used to achieve ionisation. Two colour R2PI is preferred for studies of van der Waals complexes since the energy of the ionising photon can be lowered (or otherwise controlled) to reduce the probability of cluster fragmentation. The most important advantage of the R2PI technique is its mass selectivity and access to exited states, which are forbidden in a one photon process, but can be reached in a multi photon process by the more relaxed selection rules, for instance, by two or more photon absorption. R2PI provides information about an intermediate excited electronic state.

### 2.3.2 Photoionisation efficiency (PIE)

The experimental procedure used in PIE spectroscopy\(^{11-13}\) is similar to R2PI spectroscopy with the difference being that the excitation laser is tuned on to an intermediate resonance, whilst the ionisation laser is scanned over the ionisation threshold. The ion yield is determined as a function of the total photon energy. For every (ro)vibronic ionisation threshold, the cross-section for photoionisation rises in the form of a step-like function with the energy. From a PIE spectrum the position of cationic eigenstates can be extrapolated. However, this technique is not very accurate, since for many systems, especially molecular complexes, these steps are not pronounced.
2.3.3 Zero electron kinetic energy (ZEKE)

ZEKE spectroscopy\textsuperscript{14-16} is a modification to the well-known method of photoelectron spectroscopy (PES). In PES, the target is bombarded with photons and the photoelectrons emerge from the chamber to the electron energy analyzer, which separates the electrons according to their kinetic energy. The electrons pass from the analyzer to an electron detector and the spectrum recorded is the number of electrons per unit time as a function of electron energy. A multichannel photoelectron multiplier (MCP) can be used as the detector to collect electrons with a certain kinetic energy. The relationship between the energy of the photons and the ejected photoelectrons is given in equation (2.15):

\begin{equation}
\hbar \nu = \frac{1}{2} m_e v^2 + I_p
\end{equation}

In equation (2.15), $I_p$ is the Ionisation potential of the atom or molecule and $m_e$ and $v$ the mass and velocity of ejected electrons, respectively.

PES provides quantitative information about the energy of the molecular orbitals and the electronic and vibronic states of the molecular ion from the kinetic energy distribution that is detected. However, the resolution of PES is limited by its very nature to ca. 10 meV (80 cm\textsuperscript{-1})\textsuperscript{17}. The most important factor limiting the resolution in PES is the inability to measure the kinetic energy of electrons with sufficient accuracy, because the resolution of commercial time of flight (TOF) photoelectron analyzers generally limited to this value (80 cm\textsuperscript{-1}). This deficiency means that it is not possible to resolve fine structure in molecular spectra, except for molecules with large rotational constants,
like $H_2$. In theory, then, producing photoelectrons with zero kinetic energy would remove this limitation; this is basis of ZEKE spectroscopy. ZEKE spectroscopy differs from PES by the fact that the ionisation is achieved in two steps. First, the system is exited to a high n-Rydberg state using (multiple) photon(s), and then after a time delay of several microseconds, ionisation is induced by a pulsed electric field. The success of ZEKE depends on the properties of high n-Rydberg states ($n > 150$). Such states exist in a very narrow band below the ionisation threshold and have a lifetime of up to 10s of microseconds. In the original ZEKE\textsuperscript{18} experimental technique, the laser(s) excite the molecule into these states; a pulsed voltage is applied, rising slowly over about 0.4 $\mu$s, with a magnitude varying from 0 to 1 Vcm\textsuperscript{-1}. This slowly increasing pulse removes photoelectrons from a very narrow range of Rydberg states. However, this technique remained very susceptible to stray fields, since even small stray fields (20 mV cm\textsuperscript{-1}) can divert the ZEKE electrons from their original position during relatively long delay times.\textsuperscript{19}

A pulse field ionisation variant of the original ZEKE technique is known as ZEKE-PFI spectroscopy. Compared to the original ZEKE technique, this method is less susceptible to stray fields. In the classical picture, the electric field $F$ generates an energy shift (Stark shift) on the Coulomb potential between the ionic core and the high n-Rydberg electron. Fig. 2.4 shows the field-free Coulomb potential situation compared to the Coulomb Stark potential in presence of an external electric field, $F_z$:

$$V(z) = -\frac{e^2}{z} - eF_z z$$

(2.16)

$z$ = Distance to the nucleus, $V$= Coulomb potential, $F_z$ = Electric field
The local maximum energy in the Coulomb Stark potential is defined by a saddle. The energy shift (in cm\(^{-1}\)) for states lying between the saddle point and ionisation threshold in an adiabatic case (static field) is given:\(^\text{20}\)

\[
\Delta E = -6.1\sqrt{F_z} \text{ V cm}^{-1}
\]  

(2.17)

The quantum mechanical treatment leads to a fundamentally different picture of pulsed field ionisation. States located energetically below the saddle point can ionise by tunnelling of the electron through the potential barrier in the z direction.

At energies above the saddle point the rates vary rapidly from one Stark state to the next, which strongly depends on the parabolic principal quantum numbers of the

\[ V(z) = -\frac{e^2}{4\pi\varepsilon_0 z} \]
Rydberg electron density in the saddle point region. Stark states that are shifted to higher energies by the electric field (the so-called blue Stark states) have electronic wavefunctions that are located far away from the saddle point. The Stark states that are shifted to lower energies by the field (the red Stark states) on the other hand have wavefunctions located near the saddle point. The red limit of a given Stark manifold has the lowest energy but highest ionisation rate, since its wavefunction has a greater electron density close to the saddle point, leading to a minimum field ionisation at \[-4.6\sqrt{F_z} \text{ V cm}^{-1}\]. In contrast the higher energy blue states show a lower ionisation rate leading to a maximum in the ionisation signal at \[-3.1\sqrt{F_z} \text{ V cm}^{-1}\]) below the ionisation threshold.\textsuperscript{20,21} For the work presented in this thesis, the average field correction term:

$$\Delta E = -4\sqrt{F_z} \text{ V cm}^{-1}$$ \hspace{1cm} (2.18)

is used for the correction of ionisation energy (IE).

To reduce peak broadening from field ionisation, the fractional Stark state selective field ionisation (FSSFI)\textsuperscript{22} scheme was introduced with the objective of improving the resolution of ZEKE spectroscopy. The pulse sequence used in FSSFI spectroscopy is shown in Fig. 2.5. Two successive pulses of opposite polarity are applied. The first pulse (called the \textit{pre pulse}) removes the angular momentum degeneracy of the high-n Rydberg states and the Rydberg molecule are subdivided into the two regions of higher energy “blue” Stark states (orbital dipole antiparallel to the electric field) and lower energy “red” Stark states (parallel alignment). The first pulse leads to ionisation of the more fragile red states, but the more resilient blue states survive. After applying of a second pulse (called the \textit{extraction pulse}) of inverted polarity the surviving blue states
are transferred into less resilient red ones (Stark state inversion) with respect to the second field and can be ionised. Thus in the FSSFI scheme the large slice of Rydberg are divided into two smaller slices, resulting in two bunches of electrons that are extracted by different time of flights. In the time-of-flight spectrum of the extracted electrons three peaks can be observed corresponding to prompt electrons, low and high resolution ZEKE electrons.

Fig. 2.5: Pulsing scheme in the field ionisation of long-lived Rydberg states in the ZEKE (FSSFI) spectroscopy.

The best resolution available in ZEKE spectroscopy used to be about 0.2 cm\(^{-1}\), allowing, at least, partial resolution of rotational fine structure. However, by using modern pulse generators, which produce rise times up to 10-20 ns with pulse widths of 50-100 ns, it is now possible to obtain a resolution of up to 0.1 cm\(^{-1}\). ZEKE provides information about the ionic state of the molecular system and, by careful selection in its multi photon variant, can provide this information for a variety of unique rotational, vibrational and electronic intermediate states.
2.3.4 Mass-analyzed threshold ionisation (MATI)

Mass-analyzed threshold ionisation (MATI)\(^{25}\) was developed with the idea of collecting the ions produced in a ZEKE experiment. Like to ZEKE, MATI spectroscopy takes advantage of the field ionisation of long-lived high Rydberg states. After excitation of the molecules to high Rydberg states, the weakly bound Rydberg electrons are detached from the ionic cores by applying a pulsed electric field. The threshold ions, produced in this way, are detected as a function of the ionising laser wavelength in a TOF mass spectrometer to record the MATI. The mass-selective detection scheme is one of the advantages of MATI compared to ZEKE as it allows monitoring of the fragmentation process of the studied ionic system and, of course, the spectrum produced is indicative of a species of a given, unique mass (which is obviously not possible for electrons). However, MATI spectroscopy is a more challenging experiment, since the kinetic energy differences between spontaneous ions and Rydberg molecules are very small. This means that the separation of the Rydberg molecules from the spontaneous ions needs a longer delay time between photoionisation and pulsed-field ionisation. Therefore the ionisation area (defined by the intersection of ionisation laser beam and the molecular beam) needs to be moved away from the centre of the analyzer to a point upstream in the molecular beam. About 0.5 \(\mu\)s after laser excitation, an offset field of 1~3 V cm\(^{-1}\) is typically applied to the ion accelerator. After a delay time of 12-20 \(\mu\)s, separation is achieved and the Rydberg states have drifted with the molecular beam velocity to the centre of the analyser. A high voltage pulse (900 V/cm) is applied to the ion repeller which ionises the Rydberg states and accelerates the ions through the ion optics towards the MCP. The spontaneous ions will reach the detector before the MATI ions, since they are closer to the ion repeller plate at the time of the pulsed field ionisation and therefore experience a higher acceleration field. However, in RETOF
mode the arrival time of MATI and spontaneous ions can be influenced by adjusting the braking voltages $V_B$ and reflecting voltage $V_R$. Fig. 2.6 illustrates the schematic principle of the pulsing scheme and the spatial separation in MATI spectroscopy. The light blue circles represent the Rydberg states; the red circles are prompt cations, and the dark blue circles are MATI ions. The high voltage extraction pulse used to field ionise the neutral Rydberg molecules, causes a very large Stark shift in the ionisation energy, thus field ionising a very large slice of high-$n$ Rydberg states and consequently generating considerable band broadening. In this low resolution MATI, an overall resolution of $\sim 10$ cm$^{-1}$ is achieved.$^{26}$

Fig. 2.6: Schematic illustration of spatial separation in the low resolution MATI Spectroscopy. The details are explained in the text.

Similar to ZEKE spectroscopy, a pulse sequence based on the FSSFI technique can be applied in MATI to achieve high resolution. In high resolution MATI (Fig. 2.7), a low
voltage pulse separates prompt ions from neutral Rydberg molecules. Then a short low voltage pulse of inverted polarity (0.5-2 V) is applied. This pulse ionises some of the higher Rydberg molecules and the ions thus generated give rise to the high resolution MATI signal. A third pulse, which is applied for a further 8 to 12 µs separates the high resolution MATI ions from the remaining neutral Rydberg molecules, which will be ionised by a subsequent high voltage field. The high voltage pulse in the ion repeller extracts prompt ions, high resolution MATI ions and low resolution MATI ions each to a different time-of-flight, hence three peaks are seen on the mass spectrum.

![Fig. 2.7: Schematic illustration of spatial separation in the high resolution MATI Spectroscopy. The details are explained in the text.](image)

The high resolution MATI signal is directly equivalent to the ZEKE signal, and it is often of better quality as there is usually minimal background noise. In addition, the ion signal is more stable than the electron signal as it is less susceptible to stray fields.
2.4 Laser desorption (LD)

There exist generally two methods to vaporise condensed molecules. The first method uses thermal heating to evaporate a condensed sample. However, thermal heating causes fragmentation of larger molecules which is a particularly serious issue when considering biomolecules. The second, softer, method uses lasers to transfer fragile biomolecules, with molecular weight of hundreds to hundreds of thousands of atomic mass units, from the condensed phase into the gas phase with low accompanying decomposition by careful selection of factors such as the desorption wavelength and focus of the desorption laser on the sample.  

Fig. 2.8: (a) laser desorption of biomolecules and (b) laser ejection of biomolecules out of a matrix.

Fig. 2.8 shows the two most common techniques for producing a gas of biomolecules using lasers: (a) laser desorption (LD) of biomolecules and (b) laser ejection of
biomolecules out of a matrix. The difference between these approaches lies in the fact that laser desorption is based on thermal models. In contrast, laser ejection from a matrix is primarily a non-thermal process.

A number of reviews of various aspects of the laser desorption field have been published. Ready et al.\textsuperscript{31} discussed the interaction of laser radiation with solid-phase systems in detail in 1971. In 1980, Conzemius & Capellen\textsuperscript{32} prepared a comprehensive review of laser-solid interactions for mass spectrometry. The review by Chuang et al.\textsuperscript{33} of the field of laser surface interactions is of considerable importance for understanding the physical principles of laser-induced thermal desorption. The literature pertaining to laser desorption of biomolecules was reviewed by Shibanov et al. in 1985\textsuperscript{34} and Cotter et al. in 1987\textsuperscript{35}. Zare & Zenobi\textsuperscript{36} used laser desorption technique in two-step laser mass spectroscopy in 1991. Several other reviews regarding laser-induced thermal desorption have also been published.\textsuperscript{37-45} The field of laser ejection of biological molecules from a matrix (specifically matrix-assisted laser desorption/ionisation, MALDI) can also be widely found in literature.\textsuperscript{46-48} One experimental scheme employs pulsed-jet entrainment for cooling the internal energy distribution and pulsed-dye lasers to perform resonance-enhanced multiphoton ionisation (REMPI).\textsuperscript{49-65} Using such methods, researchers were able to detect biomolecules of molecular weight less than 2000 amu, including catecholamines, indoleamines, vitamins, acids, neuroleptic drugs, nucleotides and nucleosides, small peptides, protected dinucleotides, and polyenes such as flacarotene.
2.4.1 Theoretical background of Laser desorption (LD)

Three mechanisms are primarily responsible for the ability to transfer a biomolecule from the condensed phase into the gas phase by using laser induced thermal desorption. The three mechanisms result from the high heating rates inherent in the laser-desorption process. Typical heating rates range from $10^8$ to $10^{13}$ K/s and depend on the laser pulse energy density and absorption cross sections.66-75

2.4.1.1 Laser induced heating of solids

The classic model used to calculate the temperature jump caused by absorption of pulsed laser radiation was investigated by Ready et al.31 The model has been further refined by several researchers.76-78 Desorption may be stimulated by excitation of electronic or vibrational states of the adsorbate or by excitation of the valence or conduction electrons of the substrate itself, primarily through surface valence electrons. By way of introduction, the case of laser heating of a metal will be considered. For laser-induced thermal heating of a metal or semiconductor system, the radiation in the laser pulse interacts with the valence band electrons exciting them. Since the optical absorption coefficients are large ($10^5$ to $10^6$ cm$^{-1}$), the optical penetration depth is much shorter than the thermal diffusion length. The accelerated electrons then have approximately 100 femtoseconds (fs) mean-free time between collisions with lattice phonons. The oscillating electrons undergo collisions with lattice phonons or with themselves. Through these collisions, the adsorbed energy is converted to thermal energy. The time scale for this conversion is comparable to the electron-phonon collision time, which is of order of several picoseconds (ps). Thus, for laser pulse widths on the order of nanoseconds (ns) it is safe to assume that the laser energy is converted into heat at the point at which the photon was adsorbed. Ultrafast
measurements of electron relaxation\textsuperscript{73,74,79} and adsorbate vibrational excitation\textsuperscript{75} rates suggest that within several picoseconds electron-phonon collisions thermalize the electron kinetic energy.

The heating profile at the surface can be solved using the differential equation for heat flow:

\[
\nabla^2 T(x, y, z, t) - \frac{1}{D} \frac{dT(x, y, z, t)}{dt} = -\frac{A(x, y, z, t)}{\lambda} \quad (2.19)
\]

where \( T \) is the temperature as a function both of position \( x, y, z \) and time \( t \), \( D \) is the thermal diffusivity of the bulk. The thermal diffusivity is related to the other material parameters by \( D = \lambda \rho c_p \) where \( \rho \) (g cm\(^{-3}\)) is the sample density, \( c_p \) (J g\(^{-1}\)K\(^{-1}\)) is the sample heat capacity and \( \lambda \) (W cm\(^{-1}\) K\(^{-1}\)) is the thermal conductivity. \( A \) is the heat produced by the laser as a function of both position and time (the energy source term).

In the case that the optical penetration depth is much smaller than the lateral dimensions of the laser beam in the desorption experiment, the three-dimensional case can be approximated by the one dimensional case with the energy source term \( A(z, t) \) approximated by equation (2.16).\textsuperscript{80}

\[
A(z, t) = I_m(1-R)\alpha \exp(-\alpha z)f(x, y)q(t) \quad (2.20)
\]

and the solution to this differential equation is:

\[
T(z = 0, t) = \frac{I_m(1-R)(\pi D c)}{2} \int_{0}^{\frac{t}{\tau}} q(t - \tau) \tau^{-\frac{1}{2}} d\tau \quad (2.21)
\]
where \( I_m \) is the maximum laser intensity, \( R \) is the reflectance, \( \alpha \) is the absorption coefficient, \( f(x, y) \) is the spatial profile, \( q(t) \) is the temporal profile of the laser pulse, \( c \) is the heat capacity, and \( \tau \) is the time after the laser pulse \( t = 0 \).

A number of solutions have been proposed by Van der Peyl and coworkers\(^{81, 82}\) as well as Wedler\(^{83}\) and Hall\(^{84}\) using a measured temporal profile of the laser for \( q(t) \). Bechtel\(^{85}\) and Brand & George\(^{86}\) solved the heat flow equation using a Gaussian profile temporal probe. Cowin and coworkers,\(^{87}\) Burgess and coworkers,\(^{88}\) and Dai and coworkers\(^{89}\) employed a triangular pulse shape for \( q(t) \). The analytical solution to the heat flow equation fails for the important case of laser-induced desorption of thick films of biomolecules when the laser penetration depth approaches the thermal diffusion length.

### 2.4.1.2 Kinetics in LD process

The first mechanism involves the kinetics of desorption and decomposition at extremely high heating rates.\(^{90-96}\) The physical principle behind the kinetic mechanism for molecular desorption in laser-induced thermal desorption can be understood using the Polanyi-Wigner model\(^ {96}\) for the desorption rate. The rate expressions for an adsorbate given a desorption and reaction channel can be written as:

\[
\frac{d[\theta]_{des}}{dt} = [\theta]_{ads}^n v_{des} \exp \left[ -\frac{E_{des}}{RT} \right]
\]

(2.22)

where equation 2.22 describes the rate for desorption given the energy barrier for desorption \( E_{des} \), the frequency factor \( v_{des} \) and the adsorbate coverage \( [\theta]_{ads} \). \( R \) represents the universal gas constant and the temperature \( T \).
2.4.1.3 Energy transfer in LD process

The second mechanism involves the efficiency of energy transfer during laser desorption.\textsuperscript{97-101} As seen previously, the temperature of the surface can approach thousands of K, but fragile molecules can still be desorbed intact.

A theoretical model\textsuperscript{102} based on vibrational energy transfer suggests that the efficiency of energy transfer is low during the desorption step for weakly coupled adsorbate surface systems. In a simulation of laser desorption of isolated Nitrogen Oxide (NO) from LiF(100), Lucchese & Tully\textsuperscript{103} found that all degrees of freedom of the gas-phase NO were considerably less populated than expected from the surface temperature. The reason for this is the incomplete equilibration of the thermal energy due to differing relaxation times. This nonequilibrium distribution was attributed to the rapid, temperature jump. Based on this model, the vibrational temperature was the coolest (209 K), followed by the rotational temperature (302 K). The normal translational temperature was the warmest (638 K), but was still three times cooler than the mean surface temperature (1900 K). Thus, because vibrational energy transfer requires the most time, excited vibrational levels are least populated in the laser desorption experiment. To determine the amount of energy transferred through the adsorbate bond, the kinetic equation for energy transfer is written:

$$\frac{dE}{dt} = \frac{dE_0}{dt} - kE(t) \tag{2.23}$$

where $E_0$ is the initial energy found in the surface adsorbate bond, $kE(t)$ is the quantity of energy flowing into the adsorbate’s internal vibrational mode and $dE/dt$ is the rate of energy transferred through the surface adsorbate bond. In an attempt to quantify this model, Zare & Levine\textsuperscript{102} developed an expression based on the adiabaticity parameter $\xi$. 
For the case of energy transfer through vibrational modes \((\nu \rightarrow \nu')\) of the surface adsorbate bond, the adiabaticity parameter is defined as:

\[
\xi = 2\pi \sqrt{\frac{\nu'}{\nu}} \tag{2.24}
\]

The rate constant for energy flow into the adsorbate’s vibrational mode is then given by:

\[
k = \nu \exp(-\xi) \tag{2.25}
\]

When integrated, equation (2.23) provides the amount of energy lost at time \(\tau\)

\[
E(\tau) = D_0 \left( 1 - e^{k\tau} \right) \tag{2.26}
\]

where \(E_0\) has been set to the bond dissociation energy \(D_0\).

In the case that \(k\tau\) is small \((e^{k\tau} << 1)\), no energy transfers into the internal modes of the adsorbate. Thus, the criteria for the desorption of internally cold molecules can be fulfilled, if \(\nu \tau < \exp(\xi)\). The theory predicts that if the product of the surface adsorbate bond frequency and the interaction time is less than the quantity \(\exp(\xi)\), then little energy will flow from the surface adsorbate bond into the internal modes of the adsorbate. A key issue for this model, then, concerns the residence time of an adsorbate on the surface during the laser-induced thermal desorption. Thus if the measured residence time is less than the time scale for vibrational energy transfer, molecules can desorb intact even at very high heating rates after laser-induced thermal desorption.
2.4.1.4 Postdesorption collisional cooling of internal energy in LD process

The last mechanism, perhaps of less importance, is the effect of postdesorption collisions on the internal energy distributions of the desorbing species.\textsuperscript{104-111} Collisions occurring in the gas phase immediately after the desorption event serve to markedly alter the translational and internal energy distributions measured after laser desorption. Studies of collisional processes in expanding gases reveal that random thermal energy is transferred into directed translational energy, and internal energy distributions are cooled. Measurement of the kinetic energy distribution of laser desorbed ions reveals kinetic energy distributions of the range of tens of eV.\textsuperscript{112-114}

The velocity distribution of the gas-phase biomolecules produced in the laser-desorption process is perhaps the most convincing evidence for the thermal nature of the desorption mechanism. Velocity distributions for thermal desorption processes from surfaces often fit the following functional form:\textsuperscript{115}

\begin{equation}
    g(v)dv \sim \left(\frac{m}{kT}\right)^2 v^3 \exp\left(-\frac{mv^2}{kT}\right)dv
\end{equation}

In equation (2.27), \(v\) is the velocity of the gas, \(m\) is the molecular mass of the gas, \(T\) is the thermodynamic temperature and \(k\) is the Boltzmann constant.

Temperatures distributions described by thermal desorption are in the range of 250-450 K. Distributions resulting from a desorption process that involves collisional cooling or nonthermal mechanisms have been observed to be non-Maxwellian. The reasons for these excursions from ideal behaviour result from the nonequilibrium nature of the desorption process at these high heating rates and from postdesorption collisions. In a
number of experimental measurements, the velocity distributions for laser-desorbed molecules can be shown to be anisotropic with respect to angle of desorption\textsuperscript{116} or may require a stream velocity $v_0$, to fit the distribution.\textsuperscript{117}

\begin{equation}
    g(v)dv \sim \left( \frac{m}{kT} \right)^2 v^3 \exp\left(-\frac{m(v-v_0)^2}{kT}\right) dv
\end{equation}

Essentially two temperatures are required for the fit. One temperature is required to fit the stream velocity, which can be quite high, and one for the random translational energy (related to the kinetic energy distribution), which can be much lower.

In a study of UV laser desorption of tryptophan molecules using postionisation, Spengler and co-workers\textsuperscript{118} determined the most probable velocity of the gas phase neutral tryptophan products to be approximately 200 m/s, corresponding to a kinetic energy of 40 meV.
2.5 References


Chapter 3 Theoretical methods

Ab initio electronic structure techniques

This chapter provides an overview of the ab initio methodologies that have been used to calculate the structures and conformational energies of molecules. This section on the theoretical background is based on references.1-4

3.1 Schrodinger equation

The starting point of the following overview is the Schrodinger equation in its time independent form given by (3.1):

\[ \hat{H}\psi = E\psi \]  

where the wave function \( \psi \) is a function of all coordinates of the relevant system. In our case, a molecular Hamiltonian \( \hat{H} \) is given by:

\[ \hat{H} = -\frac{1}{2} \sum_i \nabla_i^2 - \sum_i \frac{1}{2M_i} \nabla_i^2 + \sum_{i>j} \frac{Z_iZ_j}{R_{ij}} - \sum_i \frac{Z_i}{r_i} + \sum_{i>j} \frac{1}{r_{ij}} \]  

(3.2)

where I and J refer to the nuclei and i and j refer to electrons or, in more compact notation:

\[ \hat{H} = \hat{T}_e + \hat{T}_N + \hat{V}_{NN} + \hat{V}_{Ne} + \hat{V}_{ee} \]  

(3.3)

The first term in (3.2) is the operator of the kinetic energy of the electrons. The second term is the operator of the kinetic energy of the nuclei. The third term is the potential energy arising from Coulomb repulsions between the nuclei, \( R_{ij} \) being the distance between the nuclei I and J with atomic numbers \( Z_I \) and \( Z_J \). The fourth term represents the potential energy arising from Coulomb attractions between the electrons and the
nuclei, \( r_{hi} \) being the distance between nucleus \( i \) and electron \( i \). The last term represents the potential energy due to Coulomb repulsions between the electrons, \( r_{ij} \) being the distance between electrons \( i \) and \( j \).

The Born-Oppenheimer approximation simplifies the general molecular problem by separating the nuclear and electronic motions. This approximation is reasonable since the mass of a typical nucleus is thousands of times greater than that of an electron. The nuclei move very slowly with respect to the electrons. Thus, the electronic motion can be described as occurring in a field of fixed nuclei. We can use the Born-Oppenheimer approximation to construct an electronic Hamiltonian, which neglects the kinetic energy term of the nuclei. Within this approximation, the electronic Hamiltonian is written as:

\[
\hat{H}_e = -\frac{1}{2} \sum_i \nabla_i^2 + \sum_{i>j} \frac{Z_i Z_j}{R_{ij}} - \sum_i \frac{Z_i}{r_i} + \sum_{i>j} \frac{1}{r_{ij}} \tag{3.4}
\]

or

\[
\hat{H}_e \Psi_e = E_{\text{eff}}(R) \Psi_e \tag{3.5}
\]

Solving the equation (3.4) for the electronic wave function gives the effective nuclear potential function \( E_{\text{eff}} \) that depends on the nuclear coordinates and describes the potential energy surface of the system.

### 3.2 The Hartree-Fock (HF) method

\( ab\ initio \) methods are the most accurate of all of the techniques currently in use in the field of molecular modeling. A significant reason for this is that, unlike other methods, the \( ab\ initio \) method really does start “from scratch” with just the molecular structure and a few constants. \( ab\ initio \) methods do not use any experimental data or other parameters in order to solve the Schrodinger equation for a molecular system. However,
this approach overestimates the predictive power of *ab initio* methods since all *ab initio* calculations must be evaluated via comparison with experimental data. The most common type of *ab initio* calculation is called the Hartree-Fock calculation (abbreviated to HF), in which the primary approximation is called the mean field approximation. Under this approximation, the Coulombic electron-electron repulsion is not explicitly taken into account. However, its average effect is included in the calculation. This is a variational calculation (Variational Principle), which implies that the approximate energies calculated are all equal to or greater than the exact energy. The Variational Principle provides the starting point for almost all methods which aim to find an approximate solution to the Schrodinger equation.

The accuracy of the calculation depends on the size of the basis set (set of functions used to create the molecular orbitals) used. However, because of the mean field approximation, the energies from HF calculations are always greater than the exact energy and tend, with increasing basis size, to a limiting value called the Hartree-Fock limit. Many types of calculations begin with a HF calculation and subsequently correct for electron-electron repulsion, referred to also as electronic correlation. Møller–Plesset perturbation theory (MP\textit{n}), configuration interaction (CI) and coupled cluster theory (CC) are examples of these post-Hartree–Fock (post-HF) methods. \textsuperscript{5,6}

In some cases, particularly for bond breaking processes, the HF method is inadequate and this single-determinant reference function (see equation 3.6) is not a good basis for post-HF methods. It is then necessary to start with a wave function that includes more than one determinant such as multi-configurational self-consistent field (MCSCF) \textsuperscript{5,6} and methods have been developed that use these multi-determinant references for improvements of orbital energies.
Method Description

The HF method seeks to approximately solve the electronic Schrödinger equation, and it assumes that the wavefunction can be approximated by a single Slater (see equation 3.6) determinant made up of one spin orbital per electron. Since the energy expression is symmetric with respect to exchange of any two particles, the variational principle holds, and so we know that the Slater determinant with the lowest energy is as close as we can get to the true wavefunction for the assumed functional form of a single Slater determinant. The HF method determines the set of spin orbitals, which minimize the energy and give us this best single determinant.

The Hartree–Fock method makes five major simplifications in order to deal with this task:

- The Born–Oppenheimer approximation is inherently assumed. The full molecular wave function is actually a function of the coordinates of each of the nuclei, in addition to those of the electrons.
- Typically, relativistic effects are completely neglected. The momentum operator is assumed to be completely non-relativistic.
- The variational solution is assumed to be a linear combination of a finite number of basis functions, which are usually (but not always) chosen to be orthogonal. The finite basis set is assumed to be approximately complete.
- Each energy eigenfunction is assumed to be describable by a single Slater determinant, an antisymmetrized product of one-electron wave functions (i.e., orbitals).
- The mean field approximation is implied. Effects arising from deviations from this assumption, known as electron correlation, are completely neglected.
Electron correlation should not be confused with electron exchange, which is fully accounted for in the HF method. In the HF approximation, the electron-electron repulsion is treated in average way. Thus each electron has its own wave equation, and the other electrons are treated as an average field. The antisymmetric product of n-electron wavefunctions used in the HF method can be expressed in the form of a Slater determinant shown in equation (3.6):

\[
\Psi(1,2,...,n) = \frac{1}{\sqrt{n!}} \text{det}[\varphi_i] = \frac{1}{\sqrt{n!}} \begin{bmatrix}
\varphi_1(1) & \varphi_2(1) & \cdots & \varphi_n(1) \\
\varphi_1(2) & \varphi_2(2) & \cdots & \varphi_n(2) \\
\vdots & \vdots & \ddots & \vdots \\
\varphi_1(n) & \varphi_2(n) & \cdots & \varphi_n(n)
\end{bmatrix}
\]

The electronic Hamiltonian shown in equation (3.4) can be simplified as a sum of one and two-electron operator, as shown in equation (3.7):

\[
\hat{H} = \hat{H}_e + \hat{V}_{eN} + \hat{V}_{ee} = \sum_i \hat{h}(i) + \sum_{i>j} \frac{1}{r_{ij}}
\]

\[
\sum_{i} \sum_{j \neq i} \frac{1}{r_{ij}} = \sum_{i,j} \frac{1}{r_{ij}}
\]

\[
\hat{H}_e = \sum_i \hat{h}_i + \frac{1}{2} \sum_{i,j} \frac{1}{r_{ij}}
\]

The two electron repulsion \( \hat{V}_{ee} \) is a function of the coordinates of two electrons operator and prevents the separation of variables. In equation (3.8) the electron repulsion \( \hat{V}_{ee} \) is replaced by the one electron potential, which represents an averaged field generated by all the electrons in the system felt by electron \( i \). In this way the Hamiltonian can be written as one electron operator (see equation 3.9). The variational optimisation of the
wavefunction in equation (3.6) can be carried out as the separation of variables has been achieved.

For an n-electron Slater determinant the energy term is given by Equations (3.10). Here, $J_{ij}$ is called the Coulomb integral which presents a classical Coulomb repulsion force between two electrons and $K_{ij}$ is the exchange integral, which has no simple physical interpretation but arises from the requirement that the wave function must be antisymmetric with respect to electron exchange.

\[
E[\Psi] = \frac{\langle \Psi | \hat{H} | \Psi \rangle}{\langle \Psi | \Psi \rangle} = \sum_i h_i + \frac{1}{2} \sum_{i<j} (J_{ij} - K_{ij}) \tag{3.10}
\]

\[
h_i = \langle \phi_i | \hat{h} | \phi_i \rangle \tag{3.11}
\]

\[
J_{ij} = \left\langle \phi_i | \phi_j | \frac{1}{r_{ij}} | \phi_i \phi_j \right\rangle \tag{3.12}
\]

\[
K_{ij} = \left\langle \phi_i | \phi_j | \frac{1}{r_{ij}} | \phi_i \phi_j \right\rangle \tag{3.13}
\]

The optimisation of $\Psi$ with respect to variations of the molecular orbitals $\delta \phi_i$, can be carried out by defining two new operators, called the Coulomb and exchange operators (shown in equations (3.14) and (3.15), respectively). Furthermore we can introduce a new operator, the Fock operator $\hat{F}_i$, as a new one-electron Hamiltonian (see equation (3.16)).
In equation (3.16) the term \( \sum_j (\hat{J}_j - \hat{K}_j) \) is called the Fock potential, which represents the Coulomb repulsion felt by an electron in the averaged field of all the other electrons in the system. The variational optimisation with the newly defined operators lead to the HF equations shown in (3.17), where \( \epsilon_i \) are the molecular orbital (MO) energies, the eigenvalues of the Fock operator with the MOs as eigenfunctions.

\[
\hat{F}_i = \hat{h}_i + \sum_j (\hat{J}_j - \hat{K}_j)
\]  

(3.16)

The total energy (including the constant nuclear repulsion) in term of molecular orbital energies can be written as

\[
E = \sum_i \epsilon_i - \frac{1}{2} \sum_{ij} (J_{ij} - K_{ij}) + V_{NN}
\]  

(3.18)
The HF equations can be solved by applying a linear combination of atomic orbitals (LCAO) to each of the molecular orbitals comprising the n-electron Slater determinant. For molecules, basis set expansions are always used to describe the molecule orbitals usually with $M$ atom-centred Gaussian basis functions, $\chi_j$

$$\varphi_i(r) = \sum_{j=1}^{M} c_{ji} \chi_j(r) \quad (3.20)$$

Variational optimisation of the orbital coefficients $c_{ji}$ is achieved through the Hartree-Fock-Roothan-Hall (HF-RH) equation (3.21) shown below ($F$ is the Fock matrix, $C$ the matrix of the orbital coefficients, $S$ the overlap matrix between different atomic orbitals, and $e$ the diagonal matrix of the orbital energies)

$$FC = SCe \quad (3.21)$$

Since the Fock matrix itself depends on its own solutions (i.e. a specific Fock orbital can only be determined if all the other occupied orbitals are known), the HF-RH equation needs to be solved iterively using the Hartree-Fock self-consistent field (SCF) procedure:

1. evaluate all one- and two-electron integrals
2. guess starting molecular orbital coefficient
3. form Fock matrix
4. diagonalise the Fock matrix to determine energies $\varepsilon_i$ and new molecular orbital; coefficients $c_{ij}$

(converged? no $\rightarrow$ (3), yes $\rightarrow$ (done))
3.3 Electron correlation

Hartree-Fock (HF) theory is a single-particle approximation, and therefore cannot adequately treat the correlated motion of electrons that occurs due to electron-electron interactions. Neglect of electron correlation has been blamed for systematic HF errors such as underestimated bond lengths and overestimated vibrational frequencies. Modifications added to HF-SCF theory to remedy these errors are termed ‘electron correlation’ or ‘post-HF’ methods. There are four general types of electron correlation treatments: configuration-interaction (CI) methods, Møller-Plesset (MP) perturbation theory, coupled cluster theory (CC) and density functional theory (DFT). These are examples of post-Hartree–Fock methods.

3.3.1 Møller-Plesset (MP) perturbation

For typical molecules, most of the ground-state energy originates from one-electron HF contributions. Møller-Plesset perturbation theory assumes that the effects of electron correlation are minor, and can be described by small corrections (perturbations) to the HF solution. Essentially, MP methods assume the true molecular Hamiltonian (3.1) can be divided into two parts:

\[ \hat{H}_{\text{mol}} = \hat{H}_{\text{one-e}} + \lambda \hat{P}_{\text{many-e}} \]  \hspace{1cm} (3.22)

where \( \hat{H}_{\text{one-e}} \) denotes single-electron energy contributions that can be solved exactly by HF-SCF, and \( \hat{P}_{\text{many-e}} \) represents the operator for contributions due to electron correlation. The coefficient \( \lambda \) is used to generate power series expansions of the energy and the molecular wavefunction:

\[ E_{\text{mol}} = E_0 + \lambda E_1 + \lambda^2 E_2 + \lambda^3 E_3 + ... \]  \hspace{1cm} (3.23)
These series are then substituted back into the molecular Schrödinger equation with the modified Hamiltonian \((3.21)\), and each term is evaluated in turn. The sum of the 0\textsuperscript{th} and 1\textsuperscript{st} order terms

\[
E_0 = \sum_{i=1}^{n} \varepsilon_i \quad \text{and} \quad E_1 = -\frac{1}{2} \sum_{i,j} (J_{ij} - K_{ij}) \]

constitute the HF energy,

\[
E_{HF} = E_0 + E_1
\]

and are used to evaluate \(\Psi_1\), which is composed of a linear combination of substituted determinants

\[
\Psi_1 = \sum b_i \Psi_{S,D,R,...}
\]

with coefficients \(b_i\) that are inversely proportional to the difference in energy between the ground state and the associated excited states. The substituted wavefunctions close in energy to the ground state make larger contributions to the perturbation expansion. The first-order correction to the wavefunction, \(\Psi_1\), is then used to calculate the second-order correction to the total energy \(E_2\):

\[
E_2 = \sum_{i<j} \sum_{a<b} \left[ \frac{\langle \varphi_i \varphi_j | \varphi_a \varphi_b \rangle - \langle \varphi_i \varphi_j | \varphi_a \varphi_a \rangle}{\varepsilon_i + \varepsilon_j - \varepsilon_a - \varepsilon_b} \right]^2
\]

In this approach electron correlation is accounted for by promoting two electrons from the occupied spin orbitals \(\varphi_i\) and \(\varphi_j\) to the virtual orbitals \(\varphi_a\) and \(\varphi_b\).
Møller-Plesset (MP) theory is designated by the order of the perturbative corrections. Since the second-order energy term \( E_2 \) is the first-order correction to the HF energy, the designation begins at MP2, and continues with MP3, MP4, MP5, and so forth.

### 3.3.2 Density functional theory (DFT)

DFT theory models electron correlation as a function of the electron density, \( \rho \). The functional employed by current DFT methods partitions the electronic energy via the Kohn-Sham equations\(^7\) into several terms (see equation 3.28),

\[
E = ET + EV + EJ + EXC
\]  

(3.28)

where \( ET \) is the kinetic energy term (arising from the motion of the electrons), \( EV \) is the potential energy term that includes nuclear-electron and nuclear-nuclear interactions, \( EJ \) is the electron-electron repulsion term and \( EXC \) is the electron correlation term. All terms except nuclear-nuclear repulsions are functions of the electron density. The terms \( ET + EV + EJ \) represent the classical energy of the electron distribution, while \( EXC \) represents both the quantum mechanical exchange energy, which accounts for electron spin, and the dynamic correlation energy due to the concerted motion of individual electrons. Pure DFT methods calculate \( EXC \) by pairing an exchange functional with a correlation functional and so are designated by the choice of combination. For example, the most famous of these functionals, denoted BLYP combines Becke’s gradient-corrected exchange functional with the gradient-corrected correlation functional of Lee, Yang and Parr\(^9\). DFT calculations fall into three general categories: local density approximations (LDA), generalised gradient approximations (GGA), and ‘hybrid’ combinations of DFT and Hartree-Fock terms. LDA exchange and correlation functionals only contain terms related to electron density - an approach that works for
some bulk materials, but fails to accurately predict properties in isolated molecules. GGA (‘nonlocal’) functionals like TPSS\(^1^0\) (from the authors’ initials) contain terms that depend upon both the electron density and the density gradients.

### 3.4 Resolution of the identity (RI)

The RI is a well-known mathematical construct from vector algebra\(^1^1\). Boys and Shavitt\(^1^2\) first introduced the idea of reducing the computational cost of four-index integrals using a RI technique, while Löwdin\(^1^3\) discussed the use of RI in terms of describing quantum phenomena. Since then, the RI approximation has been employed in density functional theory RI-DFT\(^1^4\), self-consistent field RI-SCF\(^1^5\), RI-MP2\(^1^6\), and RI-CC\(^1^7\) theory. The basic approach to all these methods is the factorization of the four-center integral into two parts. The details vary in each application but the ultimate goal is qualitatively the same, e.g., the reduction in computational cost. In the RI approximation the reduction is formally introduced by inserting a resolution of the identity into the two-electron integrals:

\[
(ij|kl) \approx \sum_{\tau}^{N} (ij\tau)(\tau|kl)
\]

(3.29)

Unless the auxiliary basis \(|t\rangle\) spans the whole space of products \(|ij\rangle\); this expansion introduces an error that has to be minimized. Vahtras \textit{et al.}\(^1^5\) showed that by inserting the resolution of the identity more than once and minimizing different properties of the residual function

\[
R_0(r) = |ij\rangle - \sum_{\tau} c_{\tau}|t\rangle
\]

(3.30)
three different three-center approximations for the four-center two-electron integrals result:

\[
(ij \mid kl) = \sum_{nrw} (ijt) S_{n}^{-1} V_{av} S_{w}^{-1} (klw)
\]  
(3.31)

\[
(ij \mid kl) = \sum_{lu} (ijt) S_{l}^{-1} (kl \mid u)
\]  
(3.32)

\[
(ij \mid kl) = \sum_{lu} (ijt) V_{l}^{-1} (kl \mid u)
\]  
(3.33)

Here, the parenthetical terms are three-center overlap integrals, the \(S\) terms are overlap integrals between two auxiliary basis sets, and the \(V\) terms are two-center, two-electron Coulomb integrals. Equations (3.31) and (3.32) are called the “SVS” and “S” approximations, respectively, in which the individual charge distributions are projected onto an auxiliary basis set. Equation (3.33) is called the “V” approximation, in which the Coulomb operator is projected onto an auxiliary basis set. The V approximation more closely approximates the exact four-index integral in practice, and consequently, is the most commonly used RI approximation for four-index integrals in SCF, DFT, and correlated \textit{ab initio} methods.
3.5 Basis Sets

In modern computational chemistry, quantum chemical calculations are typically performed within a finite set of basis functions. In these cases, the wavefunctions under consideration are all represented as vectors, the components of which correspond to coefficients in a linear combination of the basis functions in the basis set used. The operators are then represented as matrices, (rank two tensors), in this finite basis. In this paragraph, basis function and atomic orbital are sometimes used interchangeably, although it should be noted that these basis functions are usually not actually the exact atomic orbitals, even for the corresponding hydrogen-like atoms, due to approximations and simplifications of their analytic formulas. If the finite basis is expanded towards an infinite complete set of functions, calculations using such a basis set are said to approach the basis set limit.

When molecular calculations are performed, it is common to use a basis composed of a finite number of atomic orbitals, centred at each atomic nucleus within the molecule (linear combination of atomic orbitals ansatz). Initially, these atomic orbitals were typically Slater-type orbitals (STOs), which corresponded to a set of functions, which decayed exponentially with distance from the nuclei. Later, it was realized by Frank Boys that these Slater-type orbitals could in turn be approximated as linear combinations of Gaussian-type orbitals (GTOs) instead. Because it is easier to calculate overlap and other integrals with Gaussian basis functions, this leads to huge computational savings.

Today, there are hundreds of basis sets composed of Gaussian-type orbitals (GTOs). The smallest of these are called minimal basis sets, and they are typically composed of the minimum number of basis functions required to represent all of the electrons in each
atom. The largest of these can contain literally dozens to hundreds of basis functions for each atom.

A minimum basis set is one in which, for each atom in the molecule, a single basis function is used for each orbital in a Hartree-Fock (HF) calculation for the free atom. However, for atoms such as lithium, basis functions of p type are added to the basis functions corresponding to the 1s and 2s orbitals of the free atom. For example, each atom in the second period of the periodic system (Li - Ne) would have a basis set of five functions (two s functions and three p functions).

The most common addition to minimal basis sets is probably the addition of polarization functions, denoted (in the names of basis sets developed by Pople)\(^{20-22}\) by an asterisk, *. Two asterisks, **, indicate that polarization functions are also added to light atoms (hydrogen and helium). These are auxiliary functions with one additional node. For example, the only basis function located on a hydrogen atom in a minimal basis set would be a function approximating the 1s atomic orbital. When polarization is added to this basis set, a p-function is also added to the basis set. This adds some additional needed flexibility within the basis set, effectively allowing molecular orbitals involving the hydrogen atoms to be more asymmetric about the hydrogen nucleus. This is an important result when considering accurate representations of bonding between atoms, because the very presence of the bonded atom makes the energetic environment of the electrons spherically asymmetric. Similarly, d-type functions can be added to a basis set with valence p orbitals, and f-functions to a basis set with d-type orbitals, and so on. Another, more precise notation indicates exactly which and how many functions are added to the basis set, such as (p, d). Dunning used Huzinaga primitive GTOs to derive various contraction schemes.\(^{23-25}\) Dunning-Huzinaga-type basis sets do not have
the restriction of the Pople-type basis sets of equal exponents for the s- and p-function, and they are therefore somewhat more flexible, but computationally also more expensive.

Another common addition to basis sets is the addition of diffuse functions, denoted in Pople-type sets by a plus sign, +, and in Dunning-type sets by "aug" (from "augmented"). Two plus signs indicate that diffuse functions are also added to light atoms (hydrogen and helium). These are very shallow Gaussian basis functions, which more accurately represent the "tail" portion of the atomic orbitals distant from the atomic nuclei. These additional basis functions can be important when considering anions and other large, "soft" molecular systems.

**Minimal basis sets**

The most common minimal basis set is STO-nG, where n is an integer. This n value represents the number of Gaussian primitive functions comprising a single basis function. In these basis sets, the same number of Gaussian primitives comprises core and valence orbitals. Minimal basis sets typically give rough results that are insufficient for research-quality publication, but are much less expensive computationally than their larger counterparts. Commonly used minimal basis sets of this type are:

- STO-3G
- STO-4G
- STO-6G
- STO-3G* - Polarized version of STO-3G
3.5.1 Split-valence basis sets

In most molecular bonding, it is the valence electrons which principally take part in the bonding. In recognition of this fact, it is common to represent valence orbitals by more than one basis function, (each of which can in turn be composed of a fixed linear combination of primitive Gaussian functions). Basis sets in which there are multiple basis functions corresponding to each valence atomic orbital, are called valence double, triple, quadruple-zeta, ..(DZ, TZ, QZ, ..) basis sets. Since the different orbitals of an n-valence base have different spatial extents, the combination allows the electron density to adjust its spatial extent appropriate to the particular molecular environment. Minimum basis sets are fixed and are unable to adjust to different molecular environments.

3.5.2 Pople basis sets

The notation for the split-valence basis sets arising from the group of Pople is typically $X$-$YZG^{20-22}$. In this case, $X$ represents the number of primitive Gaussians (abbreviated to G) comprising each core atomic orbital basis function. The $Y$ and $Z$ indicate that the valence orbitals are composed of two basis functions each, the first one composed of a linear combination of $Y$ primitive Gaussian functions, the other composed of a linear combination of $Z$ primitive Gaussian functions. In this case, the presence of two numbers after the hyphens implies that this basis set is a split-valence double-zeta basis set. Split-valence triple- and quadruple-zeta basis sets are also used, denoted as $X$-$YZWg$, $X$-$YZWVg$, etc. Here is a list of commonly used split-valence basis sets of this type:

- 3-21G
- 3-21G* - Polarized
• 3-21+G - Diffuse functions
• 3-21+G* - With polarization and diffuse functions
• 6-31G
• 6-31G*
• 6-31+G*
• 6-31G(3df, 3pd)
• 6-311G
• 6-311G*
• 6-311+G*

The 6-31G* basis set (defined for the atoms H through Zn) is a valence double-zeta polarized basis set that adds to the 6-31G set six \(d\)-type Cartesian-Gaussian polarization functions for each of the atoms Li through Ca and ten \(f\)-type Cartesian-Gaussian polarization functions for each of the atoms Sc through Zn.

### 3.5.3 Correlation-consistent basis sets

Some of the most widely used basis sets are those developed by Dunning and co-workers\(^{23-25}\) since they are designed to converge systematically to the complete-basis-set (CBS) limit using extrapolation techniques. For first- and second-row atoms, the basis sets are cc-pVNZ where \(N= D, T, Q, 5, 6, ...\) (D=double, T=triples, etc.). The 'cc-p' stands for 'correlation-consistent polarized' and the 'V' indicates that they are valence-only basis sets. They include successively larger shells of polarization (correlating) functions (\(d, f, g,\) etc.). More recently these 'correlation-consistent polarized' basis sets have become widely used and are the current state of the art for correlated or post-Hartree-Fock calculations. Examples of these are:

• cc-pVDZ - Double-zeta
• cc-pVTZ - Triple-zeta
• cc-pVQZ - Quadruple-zeta
• cc-pV5Z - Quintuple-zeta, etc.
• aug-cc-pVDZ, etc. - Augmented versions of the preceding basis sets with added diffuse functions

For third-row atoms, additional functions are necessary; these are the cc-pV(N+d)Z basis sets. Even larger atoms require the cc-pVNZ-PP and cc-pVNZ-DK families of basis sets, where PP and DK stand for pseudopotential\textsuperscript{26-28}, and Douglas-Kroll\textsuperscript{29,30} respectively.

These basis sets can be augmented with core functions for calculations of geometric and nuclear properties, and with diffuse functions for electronic excited-state calculations, calculations of electric field properties, and long-range interactions, such as Van der Waals forces. A recipe for constructing additional augmented functions exists. As many as five augmented functions have been used in second hyperpolarizability calculations in the literature. Because of the rigorous construction of these basis sets, extrapolation can be done for almost any property. To understand how to get the number of functions, take the cc-pVDZ basis set for H: there are two $s$ ($L = 0$) orbitals and one $p$ ($L = 1$) orbital that has 3 components along the $z$-axis ($m_L = -1, 0, 1$) corresponding to $p_x, p_y$ and $p_z$. Thus, there are five spatial orbitals in total. Note that each orbital can hold two electrons of opposite spin.
### 3.5.4 Other split-valence basis sets

Other split-valence basis sets often have rather generic names such as:

- SV(P)
- SVP
- DZV
- TZV
- TZVPP - Valence triple-zeta plus polarization
- QZVPP - Valence quadruple-zeta plus polarization

### 3.6 Computational details

All calculation were performed using Turbomole package version 5.10. For the determination of the energies of the neutral conformers structures of tryptophan (Trp) later reported in this thesis, ground state geometry optimization were performed using RI-DFT and second order Møller-Plesset perturbation theory (RI-MP2). The RI-MP2 method was combined with correlation-consistent cc-pVDZ and cc-pVTZ basis set. For, the RI-DFT method the TPSS functional with the triple-zeta valence plus polarization (TZVP) basis sets was used.
3.7 References


Chapter 4 Experimental Set-up

This chapter will give a general description of the experimental apparatus in the laboratory in the following:

- Laser system
- Chamber and Vacuum system
- Analyser, Reflectron and Detectors (MCPs)
- Timing, System control and Data acquisition

4.1 Overview

Fig. 4.1a shows a picture of the laser desorption (LD) apparatus in the laboratory and a schematic of the apparatus is presented in Fig. 4.1b.

The experimental set-up consists of a home-built vacuum system (built by D. Jang and I. Pugliesi) and a Nd:YAG (Continuum Minilite II) laser system for the desorption and two frequency doubled dye lasers (Radiant NarrowScan) synchronously pumped by a Nd:YAG laser (Continuum 8020 Powerlite) for the excitation and ionisation of the molecules. The vacuum system is divided into two parts separated by a skimmer and evacuated by two turbo pumps backed by a scroll pump. The first part (source chamber) contains the novel desorption source which is attached to a pulsed General Valve. The pellet pressed from a mixture of graphite and the analyte molecule, is placed in the desorption source at a distance of a few millimetres from the nozzle of a pulsed General Valve. The sample rod is rotated by a motorized actuator. Following expansion of the desorbed sample together with the carrier gas (argon) through the nozzle of the General Valve, the supersonic jet is skimmed and the internally cooled sample molecules enter the second part of the vacuum system (ionisation chamber).
Fig. 4.1: a) Experimental setup of LD system in the laboratory b) Schematic of experimental setup showing laser system, chambers and vacuum system, probe assembly, ion optics and detection system.
Here the molecular beam is crossed in a perpendicular fashion by two counter propagating laser beams produced by the dye lasers. The ions are extracted perpendicularly to the axis of the molecular beam by a pair of parallel electrodes forming a Wiley MacLaren type time of flight mass spectrometer. The extracted ions travel through the drift tube, where their horizontal velocity component is compensated by a pair of parallel plates and the beam is focused by an einzel lens. After passing through the ion lens, the ions are reflected by a reflectron assembly before being detected by two stage multi-channel plates, MCP (1). The reflectron can be grounded, which makes it possible to let the ions travel straight to a second MCP (2) behind the reflector. In this way, RETOF and linear TOF experiments can be carried out. The signal from the MCP is amplified and displayed on a digital oscilloscope. The data acquisition is carried out on a PC via lab-developed software that also controls the scan of the lasers.

A more detailed description of the experimental apparatus, the hardware, and software techniques used for recording the data presented in this thesis are reported in the following sections.

4.2 Laser system

4.2.1 Nd:YAG-pumped dye laser system

The laser system consists of two dye lasers, which are pumped by a pump laser. The pump laser is a Continuum Powerlite Precision\textsuperscript{2} II 8020 flashlamp-pumped, Q-switched Nd:YAG laser with internal second and third harmonic generators and harmonic separation optics as shown in Fig. 4.2. The laser head consists of two flash lamps to pump Nd\textsuperscript{3+} doped YAG rods. The optical system consists of an optical cavity,
consisting of a Pockels cell, quarter waveplates, turning mirror and focusing lenses and
dichroic mirrors for the separation and generation of the second harmonic (SHG) 532 nm and third harmonic (THG) 355 nm.

Fig. 4.2: Nd:YAG laser used as the pump laser.²

1. Mirror, rear (rep rate dependent) 9. Con. lens, +155mm
2. Pockels cell 10. Dielectric polarizer
3. λ/4 plate 11. Pinhole
4. Dielectric polarizer 12. Head, rod (9 mm), flashlamp
5. Head, rod (6 mm), flashlamp 13. Dichroics, 532 nm
7. Turning mirror 45° 15. Dichroics, 355 nm
The YAG laser is operated at a repetition rate of 20 Hz with 5-9 ns pulse width of fundamental output 1064 nm (beam diameter = 9 mm, divergence = 0.45 mrad, internally blocked). The fundamental output of 1064 nm has a maximum energy of 1.3 J/pulse. The Q-switch delay for the maximum output energy is 180 µs. The maximal energy of SHG is 585 mJ/pulse and that of the THG is 330 mJ/pulse. Post-mounted dichroic steering optics are used to route the pump laser beams (SHG and THG outputs) to the dye lasers. A schematic representation of the dye laser optics is shown in Fig. 4.3. One laser is usually operated for the excitation and ionisation in accordance to the one- colour REMPI scheme. When the two-colour REMPI scheme is applied, a second laser is set for the ionisation stage.

![Fig. 4.3: Schematic of the optical components for frequency generation and autotracker unit of Narrow Scan dye laser.](image)

Dye solutions of different organic components are used as the lasing medium to cover the range of 400-800 nm. The dye solutions circulate inside two cells: the first cell is
used for the oscillation and pre–amplifier stage, while the second cell is responsible for the amplifier stage. The dye lasers are equipped with a 2400 lines/mm double grating allowing a maximum resolution of 0.04 cm\(^{-1}\) and have a tuning range of 322-711 nm.

The ultraviolet (UV) light for the electronic excitation or ionisation of the molecule under study is generated by combining two visible photons (fundamental emission of the dye laser) using sum frequency mixing, as shown by equation (4.1).

\[
\begin{align*}
\omega_2 &= 2\omega_1 \\
\vec{k}_2 &= \vec{k}_1 + \vec{k}_1 = \frac{2\pi}{\lambda_2} n(\omega_2) = \frac{4\pi}{\lambda_1} n(\omega_1)
\end{align*}
\]

where \(\vec{k}_{1,2} = n(\omega_{1,2}) \frac{\omega_{1,2}}{c}\) = wave vectors of the photons, \(n(\omega_{1,2})\) = refractive index of the photons, \(\omega = c \frac{2\pi}{\lambda}\) = angular frequency and \(c\) = velocity of light.

Constructive interference, and therefore a high-intensity \(\omega_2\) field, will occur only if the so-called phase matching condition is fulfilled, namely

\[
\vec{k}_2(2\omega_1) = 2\vec{k}(\omega_1) \quad (4.2)
\]

Sum frequency mixing is achieved by using a non-linear crystal (second harmonic generator) such as KDP (potassium dihydrogen phosphate). To achieve optimum conversion efficiency, the phase velocities of the fundamental and second harmonic have to be equal, as required by phase matching (equation 4.2), which only occurs at a certain, wavelength-dependent angle to the optical axis of the crystal.\(^4\) There are two different approaches to controlling the orientation of the tuning angle of the non-linear crystal as a function of the wavelengths during the laser scans. The first method uses lookup tables, and is generally used for fast scans over wide wavelength regions.
Lookup tables are created before any scan by storing the positions of the stepper motor, which adjusts the crystal position, as a function of wavelengths. In the second approach, the crystal employs the use of an "autotracker". The principle is based on the fact that the beam shape is distorted when the non-linear process is no longer in the optimal phase matching condition. The distortion of the beam is simultaneously detected using a photodiode. The voltage difference between the two photodiode channels is used as error signal and transferred to an autotracker module that changes the crystal position. The fundamental is separated from the second harmonic by a set of four Pelin-Broka separation units. The power of the fundamental radiation depends on the efficiency of the dye and on the pumping energy of the YAG laser. With 100 mJ/pulse pumping energy of the Nd:YAG laser, a maximum UV output between 10-20 mJ/pulse can be produced by the dye laser.

4.2.2 Desorption laser (Nd:YAG)

A Continuum Minilite II® flashlamp-pumped, Q-switched Nd:YAG laser is used as the desorption laser. The laser is triggered externally at a rate of 20 Hz to generate pulsed IR radiation (1064 nm – maximum power 1.08 W at 20 Hz, ~6 ns pulse, <3 mm beam diameter, <3 mrad divergence). This laser has also internal second, third and fourth harmonic generators and harmonic separation optics. The Minilite laser is located on the chamber table and the IR beam is guided to the window of the source chamber using two mirrors and a telescope.

4.3 Chamber and Vacuum system

The vacuum system is divided into two parts separated by a skimmer with a diameter of 1.5 mm. The first stage (source chamber) is evacuated by one 950 l/s turbo molecular pump. The main chamber (ionisation chamber and TOF- tube) is evacuated by one 950
l/s and one 550 l/s turbo molecular pump. The prevacuum is obtained by the use of a 30 m$^3$/h multi-stage dry vacuum scroll pump. The vacuum pressure in the chambers is measured using cold cathode and pirani gauges. A pressure of $8 \times 10^{-8}$ mbar can be achieved in both compartments. When the General Valve is operated, the source and ionisation chamber are held at $4 \times 10^{-4}$ and $5 \times 10^{-7}$ mbar respectively. The ion TOF tubes remain unaffected by the valve operation. The first stage (source chamber) contains the novel developed desorption source which is attached to a pulsed General Valve with a nozzle diameter of 0.8 mm. The pellet, pressed from a mixture of graphite and the analyte molecule, is placed in the desorption source at a distance of a few millimetres from the nozzle. It is rotated by a motorized actuator. The actuator (Thorlabs Z612B) simultaneously rotates and translates the sample in a helical fashion. The actuator can travel a maximum distance of 12 mm with a pitch of 0.5 mm per turn and 12288 steps complete one turn. Thus, a 6 mm rod allows for 12 turns. The speed of the turning motion can be adjusted via dedicated software. The output beam of the Nd:YAG laser (Minilite II) forms an angle of 45° with the sample rod. The distance of the pulsed valve to the skimmer is adjustable over a range of a few centimetres. The supersonic molecular beam expands with the carrier gas (argon or helium) through the skimmer to the ionisation chamber, where it is crossed in a perpendicular fashion by the two counter propagating laser beams produced by the dye lasers. The ionisation chamber is attached to the time of flight tube, which contains the analyser, reflectron and detectors (MCPs).

4.4 Analyser, Reflectron and Detectors (MCPs)

The ion optics assembly shown in Fig. 4.4, is situated in the main chamber. The laser beams interact with the molecular beam in the ionisation region between the copper meshes (70 lines inch$^{-1}$, transmission 90%) of the ion repeller and ion accelerator, which
are separated by 21.5 mm. Electrical voltages can be applied independently to either of the meshes to extract electrons or ions using one of the experimental techniques described previously. Ions are repelled and accelerated perpendicularly to the molecular beam and laser beams by applying a positive voltage (~900 V) to the ion repeller. In pulsed extraction mode, the ion repeller is connected to a high voltage pulser, which can generate pulses of up to 3000 V. In the MATI experiments, a digital delay pulse generator DG535 is used to apply a pulse (~2-3 V) or pulse sequence to the ion accelerator to separate prompt ions from MATI ions.

Fig. 4.4: Schematic diagram of the electronic optics: Analyser, Reflectron and Detectors.
The extracted ions travel through a 116 cm drift tube where their horizontal velocity component is compensated by applying a constant voltage difference of 5 V (± 2.5 V) to the capacitor plates (distance between the capacitor plates, d = 8 mm), and the ion beam is focused by applying a constant voltage of (~ 400-600 V) to an einzel lens. After passing through the ion lens, the ions are reflected by a reflectron assembly, which is oriented at five degrees to the axis of the electrode assembly, and drift into a 30 cm tube before being detected at a dual stage MCP(1). The reflectron consists of a 2 cm long braking and 13 cm long reflecting region. By optimising the ratio of braking and reflecting voltages (typically \( V_B = 350 \text{ V} \), \( V_R = 750 \text{ V} \)), a TOF of full width at half maximum (FWHM) of 30 ns can be achieved. For perdeutero benzene (Benzene-d6, m = 84), with a time of flight of \( t = 45 \mu\text{s} \), and TOF peak width of \( \Delta t = 30 \text{ ns} \), a relative mass resolution of 750 can be achieved. Alternatively the reflectron can be grounded, allowing the ions to travel straight to a second MCP(2) located behind the reflectron. In this way, RETOF and TOF experiments can be carried out. Recently, a third MCP(3) has been added to the ion optics assembly to detect the electrons produced in the MATI and REMPI experiments.

4.5 Timing, System control and Data acquisition

To control the timing of the experiment, digital delay pulse generators DG535 are used to produce Transistor–transistor logic (TTL) pulses of variable delay and width, which are used to trigger individual devices. Experiments are usually run at repetition rates of 20 Hz using a TTL pulse as the master trigger. Relative to the master trigger, which is usually the pulse for ion extraction, delayed TTL pulses of variable width are produced in order to trigger the General Valve and lasers. The valve is triggered first to allow enough time for the molecular beam to travel to the ionisation region. The desorption
laser needs to be triggered within the opening time of the General Valve to allow the desorbed material to be injected into the argon stream for efficient collisional cooling. Both dye lasers are fired and after a short delay extraction pulses for ions are applied to the corresponding plates.

The signal from the multi channel plates (MCPs) is amplified 10 times by a home-built pre-amplifier and displayed on a digital oscilloscope (LeCroy LT 354M, 1GS/s, 500 MHz)\(^7\). The data acquisition is carried out on a PC via home-written software (MB scan)\(^8\) that controls the scan of the laser via an Ethernet connection. The MB scan software allows the control read out of the mass spectrum to be displayed by the oscilloscope and then integration windows are set over the mass intervals. Typically 30-40 mass spectra are averaged at a repetition of 20 Hz. After the acquisition of a data point the laser is moved to the next wavelength, the frequency doubling crystals are tracked (either via the autotracker or a lookup table) to the optimum position and the oscilloscope starts averaging the ion signal again. In this way, a set of mass spectra for each wavelength can be collected and averaged. These wavelength spectra within the preset interval are simultaneously displayed in a second window.
4.6 References


8. Software built by Dr. M. Benyezzar (unpublished description).
Chapter 5 Testing the performance of the Laser system and Ion optics

It is convenient to test the laser system and ion optics by using conventional gas phase photo ionisation. The following chapter describes the performance of the system using the test molecule perdeutero benzene (benzene-d$_6$, atomic mass unit (amu) = 84). This was achieved by recording linear TOF, RETOF, R2PI and MATI spectra of benzene-d$_6$.

5.1 Benzene

The fact that that the benzene ring is a strong chromophore, coupled with the high vapour pressure of benzene, means that it is relatively easy to obtain good signal intensities. Callomon, Dunn and Miles have extensively studied the S$_1$ state of benzene.$^1$ They were able to demonstrate the importance of vibrational angular momentum. The neutral benzene molecule has a hexagonal, planar structure with D$_{6h}$ symmetry.$^{2-4}$ In the electronic ground state the electron configuration is (a$_{2u}$)$^2$(e$_{1g}$)$^4$. Upon ionisation, one e$_{1g}$ electron is removed from the highest occupied molecular orbital (HOMO) thus leaving one e$_{1g}$ electron unpaired. This results in a doubly degenerate $^2$E$_{1g}$ electronic ground state of the cation. The pure S$_1$←S$_0$ electronic transition has not been observed for the benzene molecule nor its noble gas heterodimer clusters. For benzene, it is well established that this is due to the high symmetry of the molecule (D$_{6h}$) and the symmetry of the S$_1$ state (B$_{2u}$) that make the S$_1$←S$_0$ transition symmetry forbidden. Instead of the pure electronic transition, the Herzberg–Teller theorem allows excitation of benzene from the zero level of the ground state to one quantum in the v$_6$ vibration in the exited state, written as $\sigma^1_0$. Thus, the band $\sigma^1_0$ is found as a “false origin” in the one-photon absorption and excitation spectrum of benzene.$^1$
5.2 R2PI Spectroscopy of Benzene-d$_6$ (C$_6$D$_6$)

The experimental apparatus has been described in the previous section and here the experimental conditions will be discussed only briefly. Expansion of benzene-d$_6$ is performed by the General Valve operating at 20 Hz with the sample reservoir located behind the valve outside the chamber. The carrier gas (argon) with a backing pressure of 1.5 bar was passed through the same reservoir. The one colour R2PI of perdeuterio benzene (benzene-d$_6$) were recorded by pumping the dye laser (Coumarin 307) with 70 mJ pulses of the Nd:YAG third harmonic. In the linear TOF mode the ions were extracted and accelerated in a Wiley MacLaren fashion by applying a pulsed voltage of 1300 V to the ion repeller and a constant voltage of 857 V to the ion accelerator. The voltages of the capacitor, ion lens and MCP (2) were set to $\sim$10 V (+4 V/-6.2 V), 480 V and 1900 V respectively. In the RETOF mode, a pulsed voltage of 739 V was applied to the ion repeller, the ion accelerator voltage was set to 0 V and the reflectron voltage was set to 1127 V ($V_B = 360$ V, $V_R = 767$ V). In Fig. 5.1, the timing setup for triggering the General Valve, pump laser and ion repeller are displayed.
The linear TOF and the RETOF mass spectra of benzene-d$_6$ are presented in Fig. 5.2. The one-colour (1+1) R2PI mass spectra were obtained by setting the photoionisation laser to the $6^1_0$ transition at 38786 cm$^{-1}$. The corresponding mass spectra show two different mass peaks due to the presence of one $^{13}$C in the benzene ring. The “heavy” benzene-d$_6$ ($^{13}$C$^{12}$C$_5$D$_6$) molecules of amu 79 are present in a concentration of 6.3% within the natural mixture due to the natural isotopic abundance of $^{13}$C atoms.
The linear TOF spectrum shows partial overlapping of both peaks and the FWHM (full-width at half-maximum) is $\Delta t \approx 83$ ns for a time of flight $t \approx 24.1 \mu s$ corresponding to a mass resolution of 151. The FWHM in RETOF mass spectrum is $\Delta t \approx 40$ ns for a flight time of $t \approx 45.5 \mu s$ resulting in a mass resolution of 569, nearly four times higher than the linear TOF. The RETOF mass spectrum shows a clear time separation of 300 ns between the $^{12}\text{C}$ and $^{13}\text{C}$ isotopic molecule. The signal ratio between both isotopes $^{12}\text{C}/^{13}\text{C}$ in RETOF is at least a factor of 16 corresponding to a concentration of 6.25%, which is good agreement with the expected value of 6.3%.

The experimental results for the one-photon ionisation of cooled $^{12}\text{C}_6\text{D}_6$ and $^{13}\text{C}^{12}\text{C}_5\text{D}_6$ molecules in the excitation energy range of 38780-38794 cm$^{-1}$ for the $S_1 6^1 \leftarrow S_0$ transition are shown in Fig. 5.3.
Fig. 5.3: The $S_1 6^1 - S_0$ band in (1+1) R2PI spectrum of benzene-$d_6$ ($^{12}\text{C}_6\text{D}_6$) and its isotopically substituted form ($^{13}\text{C}^{12}\text{C}_5\text{D}_6$).

The rotational contour of the band is partially resolved in the P, Q and R branches with increasing photon energy. The separation between both maxima is 1.7 cm$^{-1}$ in the $^{12}\text{C}_6\text{D}_6$ spectrum and there is a blue shift in the origin of the $^{13}\text{C}^{12}\text{C}_5\text{D}_6$ spectrum in agreement with refs. The observed shift to higher energies is explained in terms of both an isotope effect of the vibration and an isotope effect in the zero-point energy. The separation between the bands is 3.6 cm$^{-1}$ when a $^{13}\text{C}$ is present in the benzene-ring, and the $6^1_0$ band is split into two similar but not identical bands. The reason for this is the loss of the degeneracy of the $6^1$ level of $^{12}\text{C}_6\text{D}_6$ of $e_2g$ symmetry of the $D_{6h}$ point group, which splits the $^{13}\text{C}^{12}\text{C}_5\text{D}_6$ molecule into two levels of $a_1$ and $b_1$ symmetries of the $C_{2v}$ point group.
5.3 PIE and MATI spectroscopy of Benzene-d$_6$

The PIE and MATI spectra of benzene-d$_6$ recorded by ionising via the $6_1^1$ (38876 cm$^{-1}$) transition are shown in Fig. 5.4. The spectra were measured by applying a delayed pulsed voltage of 687V to the ion repeller and static voltages of $\pm$ 6V, 500V and 2100V to the capacitor, ion lens and MCP. The reflectron voltage was set to 1117 V ($V_B = 350$ V, $V_R = 767$ V).

![Pie and MATI spectra](image)

**Fig. 5.4:** Two-colour (1+1') MATI and PIE spectra of $^{12}$C$_6$D$_6$ obtained via the $S_1^1 6_0^1 \leftarrow S_0$ intermediate state.

In the case of MATI the separation of MATI ions from the prompt ions was achieved by setting the voltage of the ion accelerator to 1.3 V. The PIE spectrum was obtained by scanning the ionisation laser near the ionisation limit in the range 35712-35962 cm$^{-1}$. The PIE spectrum shows a sharp increase at the threshold 74577 $\pm$ 10 cm$^{-1}$ corresponding to the ionisation energy (IE) of benzene-d$_6$. 
A more accurate value for the IE can be obtained from the MATI spectrum via the \(6^1_0\) transition. The spectrum shows a sharp peak at 74583.0 cm\(^{-1}\), which can be clearly identified as the vibration 0\(^0\) in the cationic ground state. This value also includes the correction due to the separation field, which can be estimated by the relation \(\Delta E = 4F^{1/2}\) (F is the field strength in units of V/cm). The total two-colour two-photon MATI spectrum via the intermediate state \(6^1_0\) in the range of 1400 cm\(^{-1}\) above the cationic ground state \(D^0_0\) is shown in Fig. 5.5. The spectrum shows the vibrational structure of the benzene-d\(_6\) cation. The assignments of the vibrational peaks are based on the rotational ZEKE measurements carried out by Lindner \textit{et al.}\(^7\) Removal of an \(e_{1g}\) electron from benzene results in distorted structures of the benzene cation, which corresponds to a reduction in the symmetry from \(D^6_{6h}\) to \(D^2_{2h}\). The benzene cation has a doubly degenerate \(^2E_{1g}\) electronic ground state. According to the Jahn-Teller theorem\(^8\), for any nonlinear polyatomic molecule in a degenerate electronic state there exists a distortion of the nuclei along at least one non totally symmetric normal coordinate. This results in a splitting of the potential energy function, so that the potential minimum is no longer at the symmetrical position.\(^8\) This explains the split of the \(D^0_0\) vibronic level by the dynamical Jahn-Teller effect to give \(j = \pm 1/2, \pm 3/2\) bands in the MATI spectrum.\(^9\)
Fig. 5.5: MATI spectrum of the cationic ground state of benzene-$d_6$ obtained via the $S_1 6^1_0 \leftarrow S_0$ intermediate state.

5.4 Conclusion

The benzene-$d_6$ molecule has been used to test the performance of the TOF spectrometers employing the REMPI, PIE and the MATI spectroscopic methods. Table 5.1 are the Vibrational frequencies (in cm$^{-1}$) and assignments for $C_6D_6$ obtained from the MATI spectrum via the $S_1 6^1_0 \leftarrow S_0$ intermediate state listed and compared with the existing literature.$^{12,13}$ The peaks in the spectra could be assigned extremely well by referring to the previous assignments. At higher excess energies (> 1000 cm$^{-1}$) a direct assignment of the observed peaks to vibrational modes is not reasonable due to strong mixing of the Jahn-Teller active Modes.
Table 5.1: Vibrational band positions and assignments for the MATI spectrum of the $\tilde{X} \ 2E_{1g}$ state of $\text{C}_6\text{D}_6$ via the $S_1 6^1 \leftarrow S_0$ intermediate state.

<table>
<thead>
<tr>
<th></th>
<th>Experiment/ $\nu \pm 1 \text{ cm}^{-1}$</th>
<th>Literature$^{12,13}$ $\nu$ (cm$^{-1}$)</th>
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<tr>
<td>$S_1 6^1 \leftarrow S_0$</td>
<td>388786</td>
<td>38785.94</td>
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<tr>
<td>IE, $0^u (e_{1g})$</td>
<td>74583</td>
<td>74583.51</td>
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<tr>
<td>$16^1$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$16^1(e_{1u})$</td>
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<td>245</td>
</tr>
<tr>
<td>$16^1$</td>
<td>263</td>
<td>262</td>
</tr>
<tr>
<td>$16^1$</td>
<td>286</td>
<td>285</td>
</tr>
<tr>
<td>$6^1(\pm3/2) (b_{1g})$</td>
<td>337</td>
<td>335</td>
</tr>
<tr>
<td>$6^1(\pm3/2) b_{2g}$</td>
<td>354</td>
<td>356</td>
</tr>
<tr>
<td>?</td>
<td>485</td>
<td>488</td>
</tr>
<tr>
<td>$16^1 6^1(\pm3/2)$</td>
<td>602</td>
<td>603</td>
</tr>
<tr>
<td></td>
<td>619</td>
<td>620</td>
</tr>
<tr>
<td>$6^1(1/2) (b_{1u})$</td>
<td>632</td>
<td>634</td>
</tr>
<tr>
<td>?</td>
<td>671</td>
<td>673</td>
</tr>
<tr>
<td>?</td>
<td>735</td>
<td>-</td>
</tr>
<tr>
<td>?</td>
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<td>-</td>
</tr>
<tr>
<td>?</td>
<td>867</td>
<td>-</td>
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<td>923</td>
<td>925</td>
</tr>
</tbody>
</table>
5.5 References


Chapter 6 Development of a novel source with high stability and lifetime

The major experimental difficulties in laser desorption (LD) methods are in coupling the source to the supersonic jet expansion to obtain efficient cooling and in obtaining a stable density of the desorbed sample molecules in the molecular beam for further spectroscopic studies. Therefore, combining laser desorption with jet-cooling requires careful optimization of the source geometry. The following chapter presents the development of a new source for laser desorption jet-cooled spectroscopy of thermally fragile molecules. The characteristics of the source developed here and its properties with respect to cooling and stability have been investigated. A cylindrical pellet made from a compressed mixture of graphite and the sample, in this case the amino acid tryptophan (Trp), was used as the sample for the experiments presented here.

6.1 Laser desorption (LD) sources described in the literature

Hitherto published methods to produce sources for LD mainly differ in the preparation of the sample. The analyte molecules can be directly desorbed\(^1\) or with the aid of a supporting matrix (mostly graphite).\(^2,3\) When using a matrix it is possible to coat the matrix with a small analyte molecular\(^2\) layer or to produce a mixture, which is pressed to a pellet.\(^3\) Experimental work has shown that high stability can be achieved when desorbing from a porous matrix (graphite) coated by a thin layer of the sample, directly in front of the nozzle of a pulsed valve. This method was pioneered by de Vries \textit{et al.}\(^2\) and is generally recognized as the most successful in the literature. This technique requires that the sample is moved as the analyte molecules are completely desorbed
after one or several laser shots. Due to limitations in the length of rod that can be employed, then even with movement of the sample rod the lifetime is limited to about an hour. A source with a longer lifetime without needing to move the sample was realized by Piuzzi et al.\textsuperscript{3} Here the analyte molecules are desorbed from a solid disc (graphite / analyte). Very recently a source with a lifetime of 5-6 hours, with movement of the sample, was presented by Zhang et al.\textsuperscript{4}

However, in all these sources the desorption is performed in the low pressure region directly in front of the nozzle of a pulsed valve, which does not provide the best possible cooling. A further problem is the formation of higher clusters, which tends to occur when desorbing in front of the valve in the low pressure region. The main idea to overcome these shortcomings is that the desorbed material must be entrained in the jet as a small cloud, such that lasers downstream can interact with as much of the desorbed material as possible. The simplest way to achieve this is to desorb from a solid sample that is placed in the path of the molecular jet in a similar way to the source introduced by Smalley et al.\textsuperscript{5} as shown in Fig. 6.1. It was found that the volume, which is formed through the intersection of the desorption/ejection “cone” resulting from the desorption process and the carrier gas injection pulse is extremely important for the efficient mixing of the desorbed material and the carrier gas, leading to more efficient cooling and higher stability of the quantity of desorbed material and hence the observed spectroscopic signal.
In a more advanced design, Smalley et al.\textsuperscript{6} describe a source that fulfils the requirements for premixing of the carrier gas and the molecules under study. In the described method called “direct injection” they mention the supersonic jet and its “waiting room”. The “waiting room” can be described as a small chamber (D = 0.3 cm L = 1 cm) in the nozzle block through which the injected gas passes before expanding through the orifice. The “waiting room” is also the place where the vaporization laser beam passes perpendicular to the jet direction and hits the sample, which takes the form of a rotating disc placed in the “waiting room”. The design described enables a cloud of desorbed material to be produced in the molecular beam before the supersonic expansion through the orifice takes place. This Smalley source already fulfils some of the requirements for a long lasting sample; high density of the desorbed substance under study in the jet, high cooling efficiency and beam stability. However, the method described by Smalley et al.\textsuperscript{6} was applied only to non biomolecular samples, in particular to inorganic and metallic clusters. Hitherto only one group has implemented this technique for the spectroscopic investigation of the laser desorbed Guanine dimer.\textsuperscript{1}
Here a matrix free sample was used and the desorption was performed without movement of the sample. A lifetime of a few hours was achieved. It was shown that the efficiency of desorption from a matrix free pellet depends substantially on the wavelength used for the desorption process. However, among all the possible sources there is not one which uses a pressed pellet (analyte/graphite) that is moved during the desorption process. This should give the longest source lifetime. Such sources tend not to be used because the movement of a pressed probe generally suffers from a poor signal stability compared to a moved coated probe, due to the fact that its surface is much more irregular.

The designs of Smalley\(^6\) and Piuzzi\(^3\) have shown the complexity of creating a desorption source that provides a long lasting sample with a good shot to shot stability. Combining the ideas of both techniques gave the inspiration for the construction of the new vapourisation source presented here.

### 6.2 Designs from the early stages of development

The development process, resulting in the new desorption source presented here, has been lengthy and was begun by previous researchers in the group (I. Pugliesi’s PhD thesis and D. Jang)\(^7\) at the University of York. They have built the present TOF spectrometer and tested several designs of laser desorption sources as described below.

However, these previous designs have failed to deliver a stable molecular beam with sufficient intensity and cooling temperature. In the early stages of development, desorption from a fixed sample rod (analyte/graphite), mounted uncovered directly close to the orifice, was attempted. The observed TOF signal showed an initial signal that only last several hundred shots at a desorption frequency of 10 Hz. The high
intensity of the initial signal was most probably due to the surface characteristics of the pellet. When freshly pressed, the surface of the pellet is smooth and glasslike. The laser beam needs to break this surface before it can efficiently desorb from the underlying layers. It was also observed that a considerable amount of graphite and thus biomolecules were sputtered onto the source chamber walls. This indicated that the desorbed material was not seeded efficiently into the molecular beam. With the pellet source described above sufficient signal intensity was only produced for a few minutes making it unsuitable for REMPI / MATI / ZEKE spectroscopy.

The second design contained an actuator connected to a holder, continuously supplying a fresh surface for the desorbing laser beam, and thus giving a much longer signal lifetime. The sample rod was covered to achieve seeding of the desorbed material into the jet and avoid desorbed material from expanding in all directions. The sample rod could thus be put in the correct position with respect to the nozzle orifice. A groove was cut into the General Valve flange in order to eliminate any dead volume between the flange surface and the rod. The brass tip, on which the rod is impinged, was connected to the actuator via a connector, called the actuator-tip connector (see Fig. 6.2). The obtained TOF signal showed a sinusoidal variation in intensity due to the eccentric motion of the screw shaft of the actuator, resulting in transfer of the same to the motion of the turning sample rod. It was found that only a slight eccentricity (<0.5 mm from the axis of revolution) caused a sinusoidal variation in the signal intensity. The second design succeeded in extending the lifetime of the signal. However, the stability of the signal obtained was insufficient and the recorded R2PI spectra of desorbed tryptophan (Trp) were not reproducible. In Fig. 6.2, all the designs from the early stages of development of the new desorption source are depicted.
Fig. 6.2: LD sources from the early stages of the development process (a) is a picture of the whole assembly originating from the first design stage, (b) shows the uncovered design with the groove drilled into the General Valve flange to eliminate any volume otherwise created between the flange face and rod, (c) shows the actuator-tip connector, (d) is a picture of the valve cap and in the covered design (e) the actuator holder is shown, which in the schematic is shown in simplified form for clarity. (Schematic Fig. based on design created and edited by Dalin Yang).

6.3 Desorption Source: Design

The designs discussed above were not able to fulfil all basic criteria that are essential to achieve the goals:

- Long lifetime of signal with sufficient intensity at low desorption energy.
- Acceptable signal stability that allows recording of reproducible spectra with sufficient resolution.

This paragraph describes the novel source, which combines the advantages of the Smalley 	extit{et al.} design as well as overcoming the shortcomings normally associated with
the movement of a pressed sample. The new source can be described as a valve cap made from stainless steel, which is attached onto the nozzle block. The schematic picture of the source is shown in Fig. 6.3. The sample rod was placed directly in the path of the noble gas expansion. Placing the rod directly in the path of the expansion should therefore allow efficient mixing of the desorbed material and carrier gas that leads to more effective cooling and higher signal stability. The sample rod is rotated through a shaft, which is now part of an extended valve cap. The laser beam is shone perpendicular to the molecular beam through the inlet tube (D = 2.8 mm, L = 5 mm) on the side of the valve cap and passes through a Quartz window thus preventing the material from escaping. With this arrangement, the desorbed material can be seeded efficiently into the molecular beam without formation of any debris on the window. After passing the window the laser beam enters the outlet tube (D = 2.8 mm, L = 2 mm) and hits the rotating sample at the end of the tube. The laser outlet tube fulfils a similar role to that of the “waiting room” in the design of Smalley et al.\textsuperscript{6} described previously and provides the necessary premixing step of the carrier gas and the desorbed material before actual expansion through the orifice extension of the valve cap.
Fig. 6.3: a-b) Images of the present desorption source taken from different angles (scale 1:1) and c) technical drawing (dimensions in mm) of the present desorption source.
Furthermore, to avoid any eccentric motion of the rod caused by the wobbling of the actuator screw shaft, the connection between the actuator screw shaft and the rod has been separated by using a set of decoupling devices. This ensures that the centre of the holder part and the internal shaft of the cap overlap and are parallel to each other further diminishing any possibilities for eccentricity in the turning motion. In Fig. 6.4, the image of the actuator (Thorlabs, Z612B), coupling set and the valve cap in their order of connection are shown.

![Assembly of laser desorption source in order of connection including actuator, connection coupling set to the sample rod and the valve cap.](image)

The stability of the signal is determined by the mixing of desorbed molecules with the carrier gas (Argon) as well as the distance between the desorbed surface and the orifice of the valve. A good mixture of carrier and sample and a constant distance during the whole scan are essential for stability; both factors are fulfilled by the present design.

The whole assembly of the desorption source fitted on to the valve is shown in Fig. 6.5.
The top schematic shows the whole assembly, the bottom schematic a slice through the newly designed valve cap. The assembly is fitted in a desorption chamber in such a way that the rod connected to the actuator is positioned at an angle (45º) to the vertical axis of the molecular beam.

Fig. 6.5: Present desorption source in the apparatus. The top schematic shows the whole assembly, the bottom schematic a slice through the newly designed valve cap.

6.3.1 Sample preparation

The sample rod is made from a mixture of the molecule under study (Trp) and graphite powder as the matrix. The choice of graphite as the matrix material used to assist the desorption of the compound studied fulfils the necessary requirements. Graphite is
unreactive, apolar, a good heat conductor, an excellent absorber of IR and easily pressed into a solid geometric shape. The finest available graphite powder (Sigma Aldrich) with minimal particle size diameter of 20 µm is added to the fine milled powder of the biomolecule under study. The relative abundance of the molecule in the mixture depends on the species. In the case of Trp, three different sets with the weight ratio (graphite/Trp) of 1:1, 5:1 and 1:5 were prepared. However, the best results have been obtained using a ratio of graphite/Trp of 1:1, which was used in all following samples.

There are two possible ways to mix the two powders. In the first method, Trp is dissolved in a 30% ammonia solution and Graphite, in equal weight to Trp, was added to this solution. The solution/mixture was placed in to a supersonic bath in order to generate a suspension. Finally, the water inside the supersonic bath was heated to 60°C in order to drive off slowly the ammonia solution, and leaving a mixture of Trp/Graphite powder. However, the mass signal observed by employing this method suffers from a poor stability. This may be explained by the fact that the crystallisation process does not produce crystals of equal size and hence the sample is non-homogeneous.

In the other method, the two powders are well mixed in a mortar to produce as fine a mixture as possible. Using a hydraulic press (300-400 kg), the resulting fine powder was pressed on a brass tip forming a cylindrical rod. In this way 6 mm long rods with a diameter of 3 mm on brass tips were produced. The brass tip contains a slit at the bottom, which provides the connection to the rotating device. The resulting rod is impinged on the brass tip and ready to be fixed onto the actuator-tip connector. A selection of rods pressed is shown in Fig. 6.6.
Fig. 6.6: Sample rods. From left to right: fresh sample and used sample rod on brass tip.

6.4 Characterisation of the desorption source

The objective of the previous section was to optimize the desorption source with respect to its geometry and the sample preparation. The following section describes the characterization of the novel desorption source, and how the internal cooling in the molecular beam and the intensity and stability of the desorption signal are determined by the conditions under which the laser desorption takes place.

From the literature, laser desorption processes have been found to be dependent on a number of experimental parameters:

1. Laser desorption wavelength$^{9-11}$
2. Laser desorption power$^{11-13}$
3. Focussing condition$^{14,15}$
4. Sample nature (analyte and matrix)$^{2,3,16,17}$
5. Sample positioning in the source$^{2,3,6,18}$
To test the performance of the source and find out the optimal conditions, Trp was chosen as the test molecule, because of its special importance due to its dominant role in the near-ultraviolet (UV) absorption and fluorescence of many proteins. In addition to this, Trp has a large absorption cross section in the UV region thus giving a large R2PI ion signal. The following section discusses the experimental setup of the desorption source including the most important optimization steps from the initial stage to the final completion.

6.4.1 Desorption setup (initial stage)

The fresh sample rod of Trp/graphite pellet (1:1 weight ratio) was positioned in the 3 mm shaft channel (see Fig. 6.3) of the valve cap. The second harmonic (532 nm) of a Continuum Minilite II laser with a pulse energy of 3 mJ (0.3 J/cm²) is used for the desorption wavelength. The unfocussed laser beam is directed into the laser inlet tube hitting the rod at an angle of 45º perpendicular to the axis of the molecular beam. The size of the desorption beam is reduced to a diameter of 1-2 mm by passing through a pinhole. The actual energy density per pulse at the rod surface is below the stated value due to the attenuation by passing through the quartz window and the non-perfect alignment of the beam through the inlet tube of the valve cap. The motion of the actuator is controlled using the actuator software. To find the optimal speed for turning the actuator, the signal intensity was monitored for different speeds (see section 6.4.2.4). The desorbed molecules were seeded into an argon expansion jet with a backing pressure of 1 bar. Rhodamine 6-G was used as the dye to cover the scan range for excitation of Trp. The pulse energy of the probe laser for photoionisation was set between 2-3 mJ, using a pumping energy of 60 mJ. The delays between the opening time of the General Valve, desorption laser Q-switch and pump laser Q-switch are crucial, and need to be optimised. Fig. 6.7 reflects the timing scheme used at this stage.
The delay between the pulsed valve opening time and the desorption laser pulse must be set to entrain the maximum amount of material into the supersonic jet expansion. A long valve opening time (518 µs) pulse had to be set to in order to be able to obtain a measurable signal, indicating a low density of the desorbed material in the molecular beam. The time delay between the closing of the valve and desorption laser firing (Q-switch) pulse is 110 µs. However, there is a certain delay in the actual mechanical opening and closing of the General Valve. The delay between the two laser firings is around 120-220 µs, which reflects a broad flight time from the desorption rod surface to the acceleration region of the TOF mass spectrometer. This broad flight time reflects a poor cooling of the molecular beam under the described desorption conditions.

![Diagram showing TTL pulse setup for time delays between valve opening, desorption laser, pump laser, and ion repeller.]

Fig. 6.7: Schematic TTL pulse setup for the time delays between the opening of the General Valve, desorption laser ($\lambda = 532$ nm), pump laser, and ion repeller.
The linear TOF mass-spectrum of Trp obtained at excitation energy 34952 cm\(^{-1}\) is shown in Fig. 6.8. The spectrum was measured by setting the voltage of the ion repeller and ion accelerator to 1400V and 780 V. The voltages of the capacitor, ion lens and MCP were ±4.7V, 537V and 2100V. The spectrum reflects a set of overlapping peaks with apparent mass jitter, and makes it difficult to distinguish mass peaks from background contamination. This contamination results from neutral products of thermal decomposition occurring during the desorption process, which are also photoionized by the probe laser since all these molecules possess the same indole chromophore and the respective S\(_1\) ←S\(_0\) transitions lie in the same spectral domain as for Trp (see Table 7.1). In the spectrum in Fig. 6.8, the signal for Trp (\(m/z = 204\)) appears very weak at 37.8 \(\mu s\).

![Relative Ion Signal(arb. unit)](image)

**Fig. 6.8:** Linear TOF mass spectrum of desorbed Trp and its fragment masses obtained with the desorption laser wavelength set to 532nm at 3 mJ (0.13 J/cm\(^{-2}\)) and the probe laser energy set to 34952 cm\(^{-1}\) at 2.5 mJ.
The masses present at \( m/z = 160 \) can be assigned to tryptamine, as a result of breaking the \( C_{\alpha} – COOH \) bond of Trp and losing the formic acid \( (m/z = 46) \). The strongest signal appears at 31.53 \( \mu s \), which is assigned to the 3-ethylindole \( (m/z =145) \) fragment resulting from losing the amino acid and the carboxyl group by breaking the \( C_{\alpha} – COOH \) and \( C_{\alpha}-NH_2 \) bond in Trp. The bond between \( C_{\alpha} \) and the amino acid group seems to be the major breaking point of the molecule. Additionally, other masses appear at \( m/z = 131 \) and \( m/z = 117 \), which correspond to 3-methylindole and indole fragments. These masses are produced by breaking the \( C_{\alpha} - C_{\beta} \) bond, losing the entire amino acids glycine or \( C_{\beta} - C_{\gamma} \) losing the amino acid alanine.

When scanning the probe laser in the spectral range of 34782-35650 cm\(^{-1}\) (as per the literature\(^3\) for electronic transition in Trp), no major changes were obtained in the TOF spectrum. The most intense signal for Trp was found to be at 34952 cm\(^{-1}\), which lies more than 74 cm\(^{-1}\) above the \( S_1 \leftarrow S_0 \) transition as reported by Piuzzi \textit{et al.}\(^3\). This can be explained by the high temperature of the molecular beam resulting in the occupation of higher vibrational states and also reflected by the intense fragmentation of the parent ion.

The fragmentation path of laser desorbed Trp using 532 nm as the desorption wavelength is shown in Fig. 6.9.
6.4.1.1 Effect of desorption laser pulse energy on the desorption process

The dependence of the pyrolysis process on the desorption laser power can be followed by monitoring the variation of the fragment population as a function of the desorption laser energy at a fixed argon pressure of 2 bar and probe laser pulse energy of 2.5 mJ (0.03 J/cm²). Fig. 6.10 shows the linear TOF spectra of Trp and its fragments recorded at different desorption laser power in the range between 1 mJ to 4 mJ (corresponding to energy densities of 0.04 to 0.18 J/cm² at a spot diameter size of 1.5 mm).

Fig. 6.9: Fragmentation pattern of laser desorbed Trp using desorption wavelength 532 nm at 3 mJ (0.13 J/cm²) desorption energy.
As can easily be deduced from Fig. 6.10, increasing the power of the laser produces an increase in the number of fragment species. The fragmentation process can be seen in the routes reported in Fig 6.9. In particular, for 1 mJ (0.04 J/cm$^2$) only two species can be detected: 3-ethylindole ($m/z = 145$) and formic acid ($m/z = 46$). This fragmentation process represents the most favoured fragment channel for Trp, which is displayed in route (1). When a laser pulse of energy 2 mJ (0.09 J/cm$^2$) is applied, additional fragments at ($m/z = 160, 131$) are produced, as can be seen from channels 2 and 3 of the fragmentation pattern. Another peak ($m/z = 117$) appears for a pulse energy of 3 mJ (0.13 J/cm$^2$) which can be explained by the fragmentation process described in channel 4. The signal corresponding to the parent ion Trp ($m/z = 204$) does not appear within the
range 1-3 mJ (0.04-0.13 J/cm²). This could be due to the poor signal to noise (S/N) ratio which means that a possible Trp signal may be swamped. The signal of the parent ion Trp can be clearly distinguished when the desorption pulse energy is set to 4 mJ (Fig. 6.10d). However, the most intense features in the spectrum remain those corresponding to 3-ethylindole and formic acid. This demonstrates that it is only possible to detect the Trp signal at somewhat higher laser pulse energies at improved signal to noise ratio (S/N). However, if the laser pulse energy is further increased, then although the Trp signal becomes more intense, so do the fragment peaks and the width of the peaks becomes broader as demonstrated in Fig. 6.11. This is due to the increase in uncertainty of the initial kinetic velocity with the temperature of the desorbed molecules.

![Diagram of TOF mass spectrum](image)

**Fig. 6.11:** Linear TOF mass spectrum of Trp and its fragments at very high desorption pulse energy of 15 mJ (0.7 J/cm²).
6.4.1.2 Effect of excitation laser pulse energy on the desorbed species

To understand how the excitation power affects the desorption process, linear TOF mass spectra of desorbed Trp were recorded at different excitation laser pulse energies of 1.5-3.5 mJ (0.02-0.04 J/cm$^2$, corresponding to energy densities of 0.04 to 0.18 J/cm$^2$ at a spot of diameter 3 mm) at a fixed argon pressure of 2 bar and a desorption pulse energy of 4 mJ (0.18 J/cm$^2$). The spectra presented in Fig. 6.12 show that increasing the excitation energy leads only to a steady increase in the relative intensity of nearly all of the species, the parent ion Trp ($m/z = 204$), and all its fragments. This can be explained by the fact that the excitation energy of 34952 cm$^{-1}$ lies within the electronic transition band of all species with the same indole chromophore.

![Fig. 6.12: Linear TOF mass spectra of Trp ($m/z = 204$) and its fragments at different probe laser powers: a) 1.5 mJ, b) 2.5 mJ, c) 3.5 mJ; using 532 nm as the desorption wavelength.](image)
To evaluate this effect, the ratio \((M/F)\) of the intensity of the parent molecular ion Trp (M: \(m/z = 204\)) relative to the intensity of the fragment ions (F: \(m/z = 160, 145, 131, 189\)) has been calculated and presented in Fig. 6.13. The fact that fragment ions are present even at low excitation energies shows that the fragmentation process is not affected by the excitation laser pulse energy over the range of 1.5-3.5 mJ used to record the spectra presented.

**Fig. 6.13:** Dependence of the ratio \((M/F)\) of the parent molecular ion Trp \((m/z = 204)\) intensity to the fragment ions \((m/z = 160, 145, 131, 189)\) intensity on the different excitation laser pulse energies of Fig. 6.12.

### 6.4.1.3 Effect of argon backing pressure on the desorption process

The importance of the argon backing pressure in the desorption process is deduced from the linear TOF mass spectra shown in Fig. 6.14, recorded under different pressures of argon at a fixed desorption pulse energy of 2 mJ \((0.2 \text{ J/cm}^2)\) and excitation laser pulse energy of 2 mJ. Fig. 6.14 shows that the relative intensities of the parent ion and its
fragments decrease gradually with higher argon loads. The relative intensity of the major peak \( m/z = 145 \) corresponding to 3-ethylindole falls dramatically with increasing argon pressure. One possible explanation is that due to the inefficient seeding at high argon pressures the amount of desorbed material in the molecular beam falls and thus the total signal intensity drops. However, this explanation does not explain the appearance of fragment by-product formic acid \( m/z = 46 \) in higher intensity in the spectrum recorded at the pressure of 5 bar, which confirms that fragmentation takes place even at high argon pressures. From these observations it can be concluded that increasing the argon pressure does not prevent the fragmentation of the parent ion Trp, which indicates a very efficient pyrolysis process during the desorption.

![Linear TOF mass spectra of Trp and its fragments at different argon backing pressures.](image)

**Fig. 6.14**: Linear TOF mass spectra of Trp and its fragments at different argon backing pressures: a) 2 bar, b) 3 bar, c) 4 bar d) 5 bar; using 532 nm as the desorption wavelength.
To support the pyrolysis mechanism prior to expansion in the jet, mass selected excitation spectra were recorded for all the fragment masses observed, in the spectral domain starting around the $S_1 \leftarrow S_0$ transition of Trp. The spectrum in Fig. 6.15b, which represents the fragment molecule indole, shows a clear increase in relative intensity near the $S_1 \leftarrow S_0$ transition of indole at 35224 cm$^{-1}$. The respective $S_1 \leftarrow S_0$ transitions for 3-methylindole (Fig. 6.15c), 3-ethylindole (Fig. 6.15d) and tryptamine (Fig. 6.15e) are red-shifted by 300-400 cm$^{-1}$ with respect to their chromophore indole (see Table 7.1). In fact, Fig. 6.15 shows that in the region between 34800-34900 cm$^{-1}$ the spectra in Fig. 6.15 (e-c) show an increase of their relative intensity.

Fig. 6.15: Mass resolved excitation spectra (34843 cm$^{-1}$ - 35606 cm$^{-1}$) of the fragments of laser desorbed Trp parent ion.: a) Formic acid, b) Indole c) 3-methylindole, d) 3-ethylindole, e) Tryptamine.
The low S/N ratio and instability of the Trp parent ion signal obtained at the desorption pulse energies between 1-4 mJ (see Fig. 6.10) make it very difficult to set a mass window over its peak and execute a R2PI scan. An attempt to run a scan over the major fragment mass, 3-ethylindole and the parent ion mass, Trp with improved S/N ratio at a high desorption pulse energy of 15 mJ (0.7 J/cm$^{-1}$), is shown in Fig. 6.16.

![Fig. 6.16: (1+1) R2PI scan (34782- 35211 cm$^{-1}$) over desorbed a) Trp (m/z = 204); and its main fragment b) 3-ethylindole (m/z = 160). Desorption pulse energy was set to 15 mJ (0.7 J/cm$^{-1}$) at desorption wavelength 532 nm.](image)

The resolution of both spectra in Fig. 6.16 is very poor. The low S/N ratio in both spectra hardly allows any possible peaks to be distinguished from noise. However, the top spectrum in Fig. 6.16a, which represents the parent ion Trp, shows a resonant trend in the scanned region. In fact, despite the poor resolution the spectrum contains features indicating the presence of Trp. Unlike the spectrum 6.16a, the signal level is fairly constant in spectrum 6.16b, indicating the non-resonant ionisation of the molecule tryptamine in the scanned region. The features in the spectrum 6.16a will become clear
by filtering the noise using a six data point averaging method in Origin. Fig. 6.17 shows the result of smoothing the spectrum Fig. 6.16a by averaging six data points.

Fig. 6.17: Smoothed (1+1) R2PI of Trp in Fig 7.16a by averaging six data points.

The spectrum in Fig. 6.17 reveals the contour of the (1+1) R2PI of Trp. The appearance of an intense peak at 34883 cm$^{-1}$ corresponding to the origin $S_1$→$S_0$ electronic transition of Trp confirms this argument. However, the extremely curved baseline, the broad peak width of 4 cm$^{-1}$ and the appearance of several low-lying vibrational levels (hot bands) in the spectrum point to a high temperature of the molecular beam.
6.4.1.4 Discussion

The observations in the previous sections (6.4.1.1-6.4.1.2) show that fragmentation of Trp is mainly caused by the interaction of the desorption laser beam with the sample itself and is less affected by the excitation laser, at least over the range (1-3 mJ) normally used for excitation. Generally, a higher load of the carrier gas helps to improve cooling of the molecular beam and can reduce fragmentation of the parent ion. However, at this stage of the desorption setup the fragmentation problem remains unaffected by the argon pressure.

We have observed in Fig. 6.10 that higher desorption energies lead to an increase in intensity of the fragment peaks. This is due to accumulation of heat at the carbon surface, which rapidly attains high temperatures leading to thermal decomposition of the Trp molecule. However, the fact that even at low power density (< 0.04 J/cm$^2$) the fragment ion tryptamine appears as the major peak means that further factors affect the fragmentation process.

It should be noted that the amount of fragmentation is linked to the thermal and photochemical stability of the sample desorbed. Important parameters, which determine this interaction, include the cohesive energy of the biomolecule film, the absorption cross section of the light absorbing substrate or molecule, the film thickness if a substrate absorbs the light rather than the biomolecule, and the photon energy rather than the flux.

The following section is dedicated to optimization of the desorption conditions related to the interaction of the desorption laser beam with the sample.
6.4.2 Optimization of desorption conditions

The observations in the previous paragraphs have shown that the desorption setup requires optimization of the conditions to improve the intensity of the parent ion, the signal to noise ratio (S/N) and the cooling efficiency of the source. These quantities depend on the interaction of the desorption laser beam with the sample, which can be influenced by adjusting the following parameters:

- Desorption wavelength
- Absorption cross section of the desorption beam on the sample (irradiance)
- Optimizing the source mixing chamber (see section 6.3)
- Adjusting the speed of the sample rod rotating actuator

6.4.2.1 Desorption Wavelength (1064 nm)

The first parameter to be changed is the desorption wavelength from 532 to 1064 nm in order to optimize the condition for an indirect laser desorption (LD) as discussed above. At this point, referring to Grotemeyer et al. \textsuperscript{9}, we need to distinguish between the direct LD where the interaction occurs mainly with the analyte (Trp) and the indirect LD determined by the interaction of the matrix (graphite) with the light. By measuring the multi photon ionisation (MPI) spectra of the simple dipeptide alanyl-Trp using various desorption laser wavelengths of the Nd:YAG-laser, Grotemeyer et al. \textsuperscript{9} found that the use of high energy photons (e.g. UV-photons) in LD leads to ions and to a less intense appearance of neutral molecules, while longer wavelengths (e.g. IR-photons) produce more neutrals than ions from a surface. This behaviour can be explained through the fact that first the sample is removed from the surface without producing intact molecules. After this induction time, the substrate is heated by the laser leading to the
measurable desorption of intact neutral molecules. This process can be described as a thermal desorption or, since here the laser radiation is not absorbed by the sample, as a non resonant desorption process. Unlike this, in direct LD, it is more efficient when the desorbing wavelength is resonant with sample absorption. At a resonant laser wavelength, where only intact molecules without addition of substrate atoms are desorbed, resonant absorption of photons by the molecules on the surface must have occurred. Extensive investigations into the influence of the wavelength on laser desorption for various amino acids and dipeptides were carried out by Karas et al.\textsuperscript{20} However, it has been suggested that the resonant enhancement observed for direct LD of ions is more a function of the ion formation processes than a requirement for efficient sample desorption. In our case, the interaction may take place by excitation of electronic or vibrational states of the substrate, graphite, or by excitation of the valence or conduction electrons of the adsorbate, Trp. The substrate graphite acts as a black body and adsorbs nearly all incoming radiation from the near-IR to the near-UV. This makes the choice of desorption wavelength solely dependent on Trp. The absorption maxima of Trp lie in the UV region of the electronic spectrum (280-260 nm). In fact, it is to be expected that the desorption wavelength of 532 nm used is not adsorbed by Trp. However, the absorption of two photons (2 x 532 nm) would fulfil the condition for direct LD and explains the low intensity of neutral intact Trp molecules during the desorption process. However, from the problems described above it is still unclear whether absorption by the sample at the desorbing wavelength is desirable or undesirable for LD of neutral intact molecules. After optimizing the desorption parameter “Wavelength” the source can be tested for performance.
In order to obtain a signal under optimized desorption conditions, described in the following sections, the timing for the General Valve, desorption laser and excitation laser needs to be adjusted. The optimized time delay setup for obtaining the signal is shown in Fig. 6.18. The timing in Fig. 6.18 shows major changes in valve opening time and laser pulse delays from the timing setup in Fig. 6.7. The opening time for the valve is reduced and is about 200 µs (60%) shorter, reflecting a higher density of the desorbed material in the molecular beam.

**Fig. 6.18:** Schematic TTL pulse setup for the time delays between the General Valve, pump laser and desorption laser ($\lambda = 1064$ nm).

An improvement of the molecular beam cooling is obtained employing a fixed delay time ($\approx 250$ µs) between the two laser firings. Considering a travel distance of 145 mm
from the orifice of the General Valve (see Fig. 4.4) to the ionisation region in the analyzer, the velocity of the molecular beam can be estimated to be nearly 0.6 mm/s. This value is in good accordance with the typical velocity in a supersonic jet expansion. To test the performance under optimized conditions, the TOF mass spectrum of the Trp/graphite pellet (1:1 weight ratio) was recorded, as presented in Fig. 6.19. The experimental setup was broadly the same as described in 6.4.1. The position of the sample rod was fixed to simplify the experiment and exclude any other factors affecting the signal stability or source performance. For this reason, high desorption power was required to obtain sufficient signal intensities. The fundamental beam of the Minilight YAG at 1064 nm with a pulse energy of 4 mJ (corresponding to energy densities of 0.18 J/cm² at a spot of diameter 3 mm) was used for desorption. The incident angle of the desorption laser beam to the normal to the surface of the rod was set unchanged to 45° as before. The energy of the excitation laser was set between 2-3 mJ. The spectrum was measured by applying a pulsed voltage of 1400 V to the ion repeller and static voltages of 716 V, ±6 V, 500 V and 2100 V to the ion accelerator, capacitor, ion lens and MCP.

Fig. 6.19: Linear TOF spectrum of desorbed Trp obtained with desorption laser wavelength set to 1064 nm at 4 mJ (0.18 J/cm²) and excitation wavenumber at 34952 cm⁻¹.
From the TOF spectrum in Fig. 6.19 the most prominent peaks are the Trp parent ion ($m/z = 204$) and the fragment 3-ethylindole ($m/z = 145$). One more fragment appears at $m/z = 160$ corresponding to tryptamine. The spectrum shows an improvement in the signal intensity of the parent ion and a decrease in fragmentation under these new conditions. This observation confirms that the desorption wavelength has a much greater influence on the fragmentation process than the laser power density as discussed in the previous chapter.

6.4.2.2 Source mixing chamber

Another crucial factor, which determines the cooling efficiency and stability of the source, is the mixing chamber in the source. Initially, the source was affected by poor cooling and low S/N ratio. Increasing the volume of the mixing chamber will lead to better mixing and improved cooling of the source. Apart from this, it is necessary to increase the diameter of the outlet tube in order to be able to illuminate a larger area of the rod as described in the next section. The diameter of the inlet and outlet tube was almost doubled from 1.5 to 2.8 mm to meet the new requirements. Fig. 6.20 shows a cross section of the source with the sample rod positioned inside the shaft.

![Cross section through the desorption source with the sample rod positioned inside the shaft.](image)
6.4.2.3 Irradiance

The third parameter that needs to be considered is the influence of laser spot size and angle of incidence. Direct UV laser desorption-ionisation experiments presented by Karas et al.\(^\text{14}\) have shown that the threshold irradiance for obtaining a measurable ion signal is a function of the laser spot size at the sample surface. It was reported that if a larger area of sample is illuminated with a homogeneous fluence, then the ion yield should increase linearly with the area. The rise of the threshold irradiance for ion detection with decreasing spot size was explained by geometric considerations taking into account the Gaussian beam profile on the target. Generally, the best results with respect to the signal quality, both in terms of resolution and intensity, are obtained in a small irradiance range above threshold irradiance. By making the desorption IR beam wider the energy is distributed over a larger area, so that the increasing of the laser energy does not cause fragmentation. Furthermore, the use of a larger desorption spot has the advantage that the signal is not so strongly dependent on surface inhomogeneities as when a small spot is used. Therefore, an effort must be made to carefully control the laser intensity and the homogeneity of the beam profile at the sample surface. The simplest arrangement to control the beam size and focussing condition is by mounting a pair of lenses (concave /convex) on a x-y translation stage, thus allowing variation in the divergence of the outgoing beam by simply moving one of the lenses longitudinally along the optical axis. Fig. 6.21 shows the arrangement of a telescope for optimizing the focussing condition and beam size of the desorption laser. The limited space on the bench and variation of the translation stage meant that careful consideration of the focal lengths \((f_1, f_2)\) of the lenses was necessary.
Fig. 6.21: Schematic of the optical set-up for focusing the laser beam using a telescope \( f_1 = 50, f_2 = -150 \) for increasing the laser spot size (3-4 mm) at the sample rod.

A further point, which is found to have an influence on threshold irradiance, is the angle of incidence. Usually different angles between 70° and 15° to the normal to the surface of the rod are used in MALDI. In the present setup an angle of 45° to the normal to the surface is used but source modifications have been built to change the angle to 90°.

### 6.4.2.4 S/N ratio dependence on the speed of the motor

Finally, it was observed that for very slow speeds (0.0003 mm/s) of the sample rod rotating actuator, no measurable signal could be monitored. By increasing the speed to 0.05 mm/s a sufficiently intense signal was observed for the execution of a scan. The signal intensity depends not only on the desorption laser fluencies, but also on the speed of the rotating sample rod. An increase in signal can be achieved by increasing the speed of rotation of the actuator. This increase can be explained by the fact that when the sample is rotated, a new fresh surface will be provided for the desorption laser, which results in a larger amount of substance desorbed compared to the used surface.
The used surface will recover shortly after each shot and can be reused again after this recovery time. In this way, it is possible to use the sample rod for a long period of time by moving the rod in a closed cycle. Fig. 6.22 shows linear TOF mass spectra for laser desorbed Trp obtained at the excitation wavelength of 286.7 nm (origin of the $S_1 \rightarrow S_0$ transition of Trp) for different sample rod speeds (0.00005 mm/s - 0.5 mm/s). The spectra were recorded at a fixed desorption energy of 3.5 mJ (0.04 J/cm$^2$) and argon backing pressure of 2.5 bar. The embedded black line in Fig. 6.22 (a-e) presents the respective smoothed graph by averaging of six data points.

Rotating the rod leads to an increase in signal but also unfortunately causes signal instabilities. The reason for this is the non-homogeneity in the surface of the sample rod, which causes fluctuations in the amount of the material desorbed. Fig.6.23 shows the increase in signal fluctuation with increasing motor speed for each graph shown in

**Fig. 6.22: Linear TOF mass spectra of desorbed Trp with a desorption energy of 3.5 mJ (0.04 J/cm2) and excitation wavelength of 286.7 nm for different translational speeds of the sample rod (a: 0.5 mm s$^{-1}$, b: 0.05 mm s$^{-1}$, c: 0.0005 mm s$^{-1}$, d: 0.00005 mm s$^{-1}$, and e: fixed sample).**
Fig. 6.22. The signal fluctuation was calculated by subtracting the embedded black curve from the respective graph shown in Fig. 6.22, and integrating over the resulting difference. One can see that signal fluctuations increase considerably, by ca. 30%, when the motor is moving compared to the fixed motor position.

The samples consist of randomly oriented small crystals of graphite and Trp. The crystals themselves are usually a mixture of several different crystal habits. The crystals are also strongly birefringent. Because of their birefringence and random orientation, one would expect the reflection coefficient of the crystal to vary with the alignment of its optical axes and crystal faces to the laser wavefront. Therefore, assuming that a certain absorbed fluence results in desorption, crystals oriented so as to have a minimum reflection coefficient would produce Trp at significantly lower illumination fluence than other crystals. The crystals themselves may not be equivalent: some crystals may contain more Trp than others, depending on how the crystal was formed.
during the mixing and pressing process. These considerations suggest that there must be crystals that emit Trp at a lower apparent fluence than others do. This value of the threshold fluence is only approximate since there is both a temporal and a spatial distribution of deposited energy, neither of which is accurately known; the estimated desorption density of 0.04 J cm\(^{-2}\) was made assuming uniform distributions. Separate measurements showed a variation in signal intensity at different points on the rod surface at fixed desorption energy. The graph in Fig. 6.24 displays the fluctuation of the signal intensity of the origin peak at 34878 cm\(^{-1}\) by desorbing from different spots on the sample rod, as a function of the actuator travel distance. The spectrum was recorded by displacing the sample rod rotating actuator in steps of 0.2 mm over a distance of 4 mm in both forward and backward directions to form a closed cycle, allowing each spot to be irradiated by 40 shots.

![Graph showing fluctuation of signal intensity](image)

**Fig. 6.24:** Fluctuation of the signal intensity of the origin peak at 34878 cm\(^{-1}\) by desorbing from different spots on the sample rod, as a function of the actuator travel distance.
The graph in Fig. 6.24 presents an areal plot to demonstrate the sample rod surface. The plot reflects the fluctuation of the signal intensity over the entire rod surface with a standard deviation of over 0.25 indicating a high spread out over a large range of values. As mentioned before the quality of spectra obtained depends strongly on the desorption setup and its conditions. Fig. 6.25 shows the (1+1) R2PI spectrum of Trp in the excitation range of 34873-35087 cm\(^{-1}\) under two different desorption conditions. The spectrum in Fig. 6.25b was recorded for a fixed sample using a high desorption energy of 30 mJ (corresponding to an energy density of 0.3 J/cm\(^2\) at a spot diameter of 3 mm) in comparison to spectrum 6.25a where the sample was moved at the speed of 1 mm/s using a low desorption energy of 3.5 mJ (0.04 J/cm\(^2\)). The stationary conditions were: excitation energy 2.5 mJ, argon pressure 2.5 bar and sampling rate 30 shots.

![Fig. 6.25: One colour R2PI of desorbed Trp between 34783-35088 cm\(^{-1}\) under two different desorption conditions: a) Sample moving at 1 mm/s, low desorption energy (0.04 J/cm\(^2\)), and b) Fixed sample rod, high desorption energy (0.33 J/cm\(^2\)).](image-url)
The peak located at 34878 cm\(^{-1}\) belongs to the origin \(S_1\leftrightarrow S_0\) electronic transition of one of the Trp conformers. The main peaks are resolved in both spectra, presented in Fig. 6.25, and their wavenumbers are in good accordance with the work of Piuzzi et al.\(^3\) The spectra show the same features and differ only in their spectral resolution. Fig. 6.25a has poor resolution; the reason is that the spectrum also includes the noise caused by the rotating actuator, which overlaps with the actual peaks. The curved base line of the spectrum reflects the instability of the TOF signal. On the other hand, Fig. 6.25b shows a spectrum with a flat base line and higher peak resolution. A peak width of 2.9 cm\(^{-1}\) implies fairly good cooling by the supersonic expansion through the source. The relative peak intensities in Fig. 6.25a-b are not equivalent. Fig. 6.25b shows a less intense origin transition. This can be explained by changes in the occupation of the population of different states at different desorption conditions. Despite the higher resolution in spectrum 6.25b, the desorption conditions in 6.25a are preferred; because a much lower desorption energy is employed (see below for the removal of the fluctuations from the rotation of the pellet).

In order to optimize the condition for a moving sample, the first step that needs to be taken is to adjust the translation speed of the sample rod rotating actuator. For this reason, a set of spectra were recorded under the low desorption energy conditions of 3.5 mJ (0.03 J/cm\(^2\)) at different translational speeds of the sample rod. The spectra are presented in Fig. 6.26 and show a different progress of the spectra as the speed of the actuator increases. The spectrum for the fixed sample reflects a very low S/N ratio and does not allow any ion peaks to be distinguished from noise. As the speed of the actuator increases, the spectrum begins to take on a certain shape. At the actuator speed of 0.005 mm/s, most of the peaks are resolved.
Fig. 6.26: One colour R2PI scans of desorbed Trp in the excitation range of 34783-35088 cm\(^{-1}\) for different translational speeds of the sample rod rotating actuator. a: fixed sample rod, b: 0.00005 mm/s, c: 0.0005 mm/s, d: 0.005 mm/s, e: 0.05 mm/s, f: 0.5 mm/s.

However, further increases in speed lead also to more fluctuation of the signal intensity and result in noisy spectra. The ideal speed for moving the sample rod lies between 0.005-0.05 mm/s.

6.4.2.5 Improving the signal stability

The following paragraph presents a methodology to improve the signal stability based on instrumentation, signal handling and analysis of collected data and the effect of these methods is assessed. To improve the signal stability, one needs to recall that the signal fluctuations are mainly caused by non-homogeneities in the sample rod, resulting in a variation of the concentration of desorbed material entrained into the supersonic expansion. Using an overall signal average of the irradiated area of the sample rod will
improve the stability in the obtained spectra. The first step in this direction was taken by trying to synchronize the data acquisition with the movement of the sample rod rotating actuator. In this way an averaging of the signal over the entire desorbed sample can be achieved. Fig. 6.27 shows the evolution with time of the signal intensity of the origin peak with and without synchronisation. An example of the synchronization of data acquisition with the movement of the sample rod is shown in the spectrum 6.27a, and was achieved in the following way: using control software for the actuator, a looped movement with a rod travel distance of 3 mm and a speed of 1 mm/s was set. Controlling the data acquisition via MB software, an averaging of 60 shots with the photo ionisation laser (2.5 mJ) running at 20 Hz was chosen. In this way the desorption signal over the illuminated area of sample rod can be collected and averaged to one data point.

Fig. 6.27: Evolution with time of the signal intensity of the origin peak at 286.7 nm ($S_1 \leftrightarrow S_0$ transition of one conformer of the Trp. a) with synchronisation of the movement of the sample rod with the data acquisition, b) without synchronisation

The spectrum in 6.27 b presents the unsynchronised case by setting the actuator speed to 0.33 mm/s. Comparing the two spectra in Fig 6.27 one can see a higher signal stability over time in the synchronised spectrum. The standard deviation from the mean for the signal obtained using synchronisation is 3%, which makes it twice as stable as the
signal in the unsynchronised case. The spectrum 6.27 b shows a periodic signal fluctuation similar to the R2PI spectrum in 6.25a. The method presented helps to improve the signal stability. However, as the precision actuator is cycled around to cover the same series of spots on the pellet the surface structure will change throughout the experiment and this results in a fluctuation of the overall averaged signal. The signal fluctuation in the desorption experiment remains a major challenge and further actions need to be taken to improve the stability of the signal over long periods of time.

Another point that affects the signal stability is the shot-to-shot fluctuation in the desorption laser power. A small fluctuation can cause a large fluctuation in the amount of desorbed material. Measurements made on the profile of the power stability of the desorption laser using a photodiode showed that laser intensity fluctuations can exceed 15% of the overall peak-to-peak intensity. By substantially reducing the effects caused by this instability in desorption laser intensity, a sizeable improvement in signal stability during measurement can be achieved. The desorption process is an early event in the experiment and this allows for ample time (several microseconds) to select data shots that fall within a preset power window of the monitored laser output. Two methods for alleviating the instability caused by fluctuations in the laser power are outlined. Both involve the measurement of laser power within a comparator window before a decision is made on whether to record the ion intensities. A power window discriminator is used in the first instance as a straight add-on instrument which enables triggered data recording when the power level falls within a preset threshold window. A software/firmware based acquisition is used in the second approach where a mathematical comparison is undertaken on the laser pulse intensities sampled by a photodiode. The presented approaches associated with shot to shot stability are in the
testing phase and need to be further developed to produce consistent correlation between the measured desorption by-product densities and the intensity of the desorbing laser in desorption systems employing homogenous surfaces.

The method employed above has helped with the provision of highly accurate R2PI data. The following chapter is dedicated to the interpretation of the spectroscopic results obtained for the essential amino acid tryptophan (Trp).

### 6.5 Conclusion

The development of a novel laser vaporisation source, coupled with a supersonic expansion, for laser spectroscopy of biomolecules has been described. The performance and characteristics of the source have been investigated by using a cylindrical pellet from mixture of Trp/graphite (weight ratio 1:1).

By enlarging the critical “waiting room” region and opening up the diameter of the entrance orifice to the expansion room, it has been achieved that most of the vaporised molecules entraining in the inert carrier gas will accelerate with the carrier gas to the terminal velocity of the supersonic beam.

The highest number-average of intact neutral Trp molecules was observed at a desorption wavelength of 1064 nm. Increasing the incident power density at this wavelength, probably creating shockwave type desorption conditions, resulted in extensive fragmentation of the Trp molecule. By optimization of the experimental parameters such as laser spot size, laser irradiance, laser wavelength and sample
movement, a stable cold molecular beam of Trp for spectroscopic investigation has been produced. The spectra presented in the next chapter were recorded under the following optimized desorption conditions:

1. Sample: Pellet from mixture of Trp/graphite (1:1 weight ratio)
2. Desorption beam size: 2-3 mm
3. Desorption energy: 3-4 mJ
4. Rotating speed of the sample rod, 0.005-0.05 mm/s
5. Desorption wavelength: 1064 nm
6. Synchronisation of the movement of the sample rod and the data acquisition
6.6 References


Chapter 7 Spectroscopy of Tryptophan (Trp)

The methods described in the preceding chapter allow highly accurate R2PI data to be obtained. The present chapter is dedicated to the interpretation of the spectroscopic result obtained for tryptophan (Trp).

7.1 One colour (1+1) R2PI spectroscopy of Trp

The purpose of this research is to contribute to the understanding of the complex photophysics of the amino acid Trp through gas-phase molecular spectroscopy. The approach taken is to first understand the spectroscopy and photophysics of Trp and to examine the properties of isolated Trp molecules to understand the effects of the solvent. In neutral aqueous solution, Trp exists as a zwitterion, since the interaction of the solvent dipoles with the charged ends of the molecule stabilizes the charge separation. However, in the gas phase, the lack of an equivalent stabilizing interaction makes it unlikely that Trp will form a zwitterion in its ground electronic state, and the molecular form should be the predominant form.

The spectroscopic investigation of isolated Trp is the first step towards understanding the effects of the bulk solvent by forming van der Waals complexes and the behaviour of Trp in proteins. Investigations into the near-ultraviolet spectroscopy of Trp often employ simpler indole derivates to find out the influence of the environment on the absorption and fluorescence spectroscopy.¹ The indole chromophore is responsible for the near-UV absorption seen in proteins and polypeptides and can be found in the amino acids phenylalanine and tyrosine as well as in Trp. In order to understand the effect of conformation on the electronic spectrum of Trp, Levy et al.¹ have measured the electronic spectra of four smaller more volatile analogues of Trp compounds, which can
be more easily vaporised by thermal methods. Since all these molecules possess the same indole conformer chromophore, the respective $S_1 \leftarrow S_0$ transition lies in the same spectral region as for Trp. The electronic transition of the lowest excited states of these indole analogues are listed in Table 7.1. The origin transition of these indole derivatives depends upon their particular substitution group and electron-releasing or -withdrawing abilities. Generally, the further away a group is from the indole centre, the smaller the expected shift of the origin transition. There are two key points common to all the results. Namely, that these compounds tend to strongly absorb through their $\pi-\pi^*$ transition between 280 and 290 nm, and that they can be ionised softly or with minimal fragmentation due to rearrangements in the gas phase. Fluorescence polarization spectroscopy of indole and its derivatives in the polar condensed phase has shown the existence of two overlapping $\pi, \pi^*$ electronic states, designated historically as $^1L_a$ and $^1L_b$. These investigations imply that the $^1L_a$ electronic state exhibits large stabilization energies in the solvent and is much more sensitive to solvent polarity than the $^1L_b$ electronic state, since the two electronic transitions are polarized in different directions. However, the existence of these two close-lying electronic transitions in the gas phase is not fully understood. The investigations suggest that $^1L_a$ state is dissociative for the isolated molecule and $^1L_b$ state is the lowest excited state for all of these molecules in Table 7.1, which can be assigned to the origin transition $S_1 \leftarrow S_0$. UV spectra have shown the existence of several conformers of the indole derivatives shown in Table 7.1, which lists the wavenumber of $S_1(^1L_b) \leftarrow S_0$ origin transition of the selected aromatic chromophore.
Important aspects related to Trp spectroscopy are the nature and location of the participating electronic states and how the lowest electronic transition(s) shifts when the environment changes. A further unresolved question is the non-exponential fluorescence decay of Trp in aqueous solution.\textsuperscript{6-9}

The one colour (1+1) R2PI spectrum of Trp in the range 34843-35087 cm\textsuperscript{-1} is shown in Fig. 7.1. The obtained spectrum is in consistent with the work of Levy et al.\textsuperscript{10,11} using different vaporisation methods and either Laser induced fluorescence (LIF) or REMPI techniques.

The spectrum shows sharp, clearly resolved features with a peak width of 1.9 cm\textsuperscript{-1} reflecting their rotational contour. The features arise primarily from transitions in the cold isolated Trp molecule and not from van der Waals molecules or hot band transitions. For a van der Waals complex to contribute to the mass resolved ionisation spectrum, it would have to fragment to form an ion of mass 204. Since no ion peaks of mass greater than 204 were observed for the experimental conditions used to acquire

Table 7.1: Electronic transition S\textsubscript{1}(^1L\textsubscript{b}) \leftrightarrow S\textsubscript{0} for the lowest excited states of indole derivates.\textsuperscript{1,5}

<table>
<thead>
<tr>
<th>Molecule</th>
<th>(\nu) (cm\textsuperscript{-1})</th>
<th>(\nu-\nu_{\text{indole}}) (cm\textsuperscript{-1})</th>
</tr>
</thead>
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<tr>
<td>Indole</td>
<td>35224</td>
<td>0</td>
</tr>
<tr>
<td>3-methylindole</td>
<td>34877</td>
<td>-347</td>
</tr>
<tr>
<td>3-ethylindole</td>
<td>34810</td>
<td>-414</td>
</tr>
<tr>
<td>3-indole acetic acid</td>
<td>35039</td>
<td>-185</td>
</tr>
<tr>
<td>3-indole propionic acid</td>
<td>34965</td>
<td>-259</td>
</tr>
<tr>
<td>Tryptamine</td>
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<td>-306</td>
</tr>
<tr>
<td>Trp</td>
<td>34878</td>
<td>-351</td>
</tr>
</tbody>
</table>
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spectrum 7.1, this possibility can be eliminated. The most prominent feature occurs at 34878 cm\(^{-1}\) and corresponds to the lowest electronic transition of neutral Trp. The wavenumber of the lowest electronic transition in Trp differs by only 1 cm\(^{-1}\) from the \(^1L_b\) state in 3-methylindole (34877 cm\(^{-1}\)). This suggests for this transition in Trp the electronic state involved is the \(^1L_b\) state.

![One colour (1+1) R2PI spectrum of laser desorbed jet cooled Trp.](image)

**Fig. 7.1:** One colour (1+1) R2PI spectrum of laser desorbed jet cooled Trp. The bands labelled \(A_{0_0}^0\) - \(F_{0_0}^0\) are assigned to different conformers according to Ref\(^{10}\).

Four intense bands with an almost harmonic vibrational progression of 26 cm\(^{-1}\) above the Trp origin transition, followed by a few more peaks, but this time irregularly spaced, are visible. The intensity pattern of the low frequency vibrational progression indicates that Trp undergoes a considerable change in the complex geometry of the coordinate of this vibration upon electronic excitation. Calculation of the Frank-Condon factors
Chapter 7

(reported in Ref.\textsuperscript{10}) using harmonic oscillators and calculating the overlap of the Hermite polynomials also results in a harmonic progression similar to that observed, which is in consistent with a change in geometry upon the first electronic excitation. These low frequency vibrational bands do not appear in the spectra of indole\textsuperscript{12,13} or 3-methylindole\textsuperscript{12,14} indicating that they are due to the amino acid group of Trp. The spectrum also includes features which are not due to the vibrational modes.

The early work by Levy \textit{et al.}\textsuperscript{10,11} gave an indirect method to find out the origin of these features. Using the power saturation technique Levy and coworkers\textsuperscript{10,11} measured the response of the spectral features to the power saturation of the excitation laser. They found out that the power saturation does not affect the intensity of the peaks labelled $A_0^0$ - $F_0^0$ in the spectrum 7.1, but that the intensity of the remaining unlabeled peaks increases until they have all the same intensity. Similar observations were made by us as we measured the one colour R2PI spectrum in 7.1 using higher excitation laser power densities. To explain this behaviour of the spectrum, the possible electronic transitions involved need to be considered. Generally there are two possible explanations for the difference in intensity of two peaks in the spectrum. The first is that the two peaks arise from transitions with the same initial state, which means that the difference in intensity must be due to the different Frank-Condon factor for the transition. In this case an increase in laser power density will lead first to the saturation of the peak with the higher intensity and then the relative intensity of the weaker peak will increase until the two peaks have the same intensity. The second possible reason is that the two peaks arise from transitions with different initial states. In this case, the difference in intensity could arise from the difference in population of the initial levels or from different
Frank-Condon factors. If the Frank-Condon factors are different for the two transitions, an increase in laser power density will still increase the intensity of the weaker peak. However, at the maximum saturation, the two peaks will still have unequal intensity because of the difference in population of their initial states. On the other hand, if the transitions have equal Frank-Condon factors originating from different initial levels, the relative intensity of the two peaks will not be affected by the laser power. The peaks labelled $A_0^0 - F_0^0$ in the spectrum 7.1 thus represent transitions starting from different initial states. Consequently, these peaks can be assigned to different conformers of the Trp.

The fact that only the conformer A shows an extensive vibrational progression in the low frequency region indicates that the conformers B - F have a similar geometry to that in the ground ($S_0$) state in the Franck-Condon region of the potential surface. The ultimate proof for the existence of six conformers was provided by Piuzzi et al.\textsuperscript{15} using double resonance techniques: both UV/UV hole-burning and IR/R2PI spectroscopy confirmed the existence of six Trp conformers. However, at this stage, we are not able to carry out these double resonance techniques, and our assumption of the existence of six conformers is based on Piuzzi’s et al.\textsuperscript{15} work.

A one colour (1+1) R2PI spectrum of desorbed Trp for a long scan region is displayed in Fig. 7.2. This long spectrum (768 cm\textsuperscript{-1}) shows the efficiency of the desorption source for a very long scan (83 minutes). Compared to the spectrum measured by Levy et al.\textsuperscript{10} for the nearly same spectral range using thermal vapourisation method, the spectrum in Fig. 7.2 shows a higher resolution.
Table 7.2 presents a list of the spectral transitions observed in the one colour R2PI spectrum of Fig 7.2. In Table 7.2, the new peaks, which are not resolved in the spectrum by Levy et al.\textsuperscript{10}, are marked with an asterix *.

![](image)

**Fig. 7.2:** One colour R2PI spectrum of laser desorbed jet-cooled Trp in the range 34843 cm\textsuperscript{-1} to 35611 cm\textsuperscript{-1}.

The assignment of peaks in Table 7.2 is based on the vibrational progression built on the origin transition of the conformer A of Trp. In addition, combination bands with the 26 cm\textsuperscript{-1} vibration separation are assigned, which show a distinctive vibrational progression upon electronic excitation of Trp.
Table 7.2: Transitions in the electronic spectrum of Trp.

<table>
<thead>
<tr>
<th>Peak Nr.</th>
<th>Relative intensity</th>
<th>$\Delta \nu^{(a)}$ (cm$^{-1}$)</th>
<th>Standard deviation$^{(b)}$ (cm$^{-1}$)</th>
<th>Assignment$^{(c)}$</th>
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<tr>
<td>1</td>
<td>0.99</td>
<td>0</td>
<td>0</td>
<td>$A0_0^0$</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>25.9</td>
<td>0.3</td>
<td>26</td>
</tr>
<tr>
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<td>35.9</td>
<td>1.3</td>
<td>$B0_0^0$</td>
</tr>
<tr>
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<td>2x26</td>
</tr>
<tr>
<td>6</td>
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<td>$C0_0^0$</td>
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<tr>
<td>7</td>
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<td>70.8</td>
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<td>45 + 26</td>
</tr>
<tr>
<td>8</td>
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<td>0.3</td>
<td>3 x 26</td>
</tr>
<tr>
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<td></td>
</tr>
<tr>
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<td>0.3</td>
<td>45 + (2x26)</td>
</tr>
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<td>1.3</td>
<td>$E0_0^0$</td>
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<td>0.9</td>
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<td>0.6</td>
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</tr>
<tr>
<td>15</td>
<td>0.11</td>
<td>109.7</td>
<td>0.6</td>
<td>$F0_0^0$</td>
</tr>
<tr>
<td>*16</td>
<td>0.11</td>
<td>125.9</td>
<td>0.3</td>
<td>45 + (3x26)</td>
</tr>
<tr>
<td>*17</td>
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<td>131.1</td>
<td>0.9</td>
<td>5x26</td>
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<tr>
<td>18</td>
<td>0.28</td>
<td>136.9</td>
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</tr>
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<td>20</td>
<td>0.45</td>
<td>162.7</td>
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</tr>
<tr>
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<td></td>
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<tr>
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<td>188.8</td>
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</tr>
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</tr>
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<td>1.4</td>
<td>163 + (2x26)</td>
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<td>26</td>
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</tr>
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<td>240.7</td>
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<td>163 + (3x26)</td>
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<td>*28</td>
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<td>1</td>
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<tr>
<td>29</td>
<td>0.35</td>
<td>259.8</td>
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<td></td>
</tr>
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<td>163 + (4x26)</td>
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<td>295.1</td>
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<td></td>
</tr>
<tr>
<td>34</td>
<td>0.28</td>
<td>303.4</td>
<td>0.6</td>
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</tr>
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<td>35</td>
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<td>319.5</td>
<td>1.8</td>
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</tr>
<tr>
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<td>338.2</td>
<td>1.9</td>
<td></td>
</tr>
<tr>
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<td>0.10</td>
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<tr>
<td>*39</td>
<td>0.08</td>
<td>365.5</td>
<td>1.5</td>
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7.2 Two colour (1+1′) R2PI spectroscopy of Trp

A two-colour two-photon (1+1′) R2PI spectrum was carried out to improve the quality of spectrum of the $S_1$ state previously obtained through one colour (1+1) R2PI spectroscopy. This was achieved by using a second photon wavenumber of 32000 cm$^{-1}$ to minimize fragmentation from higher mass species. The pulse energy of the excitation laser (scanning laser) was set to 0.3 mJ while the power of the ionisation laser was set to 4 mJ. Fig 7.3 shows the two-colour R2PI spectrum. It shows peak widths of 0.6 cm$^{-1}$ and a much better S/N ratio compared to the one-colour R2PI and confirms the excellent cooling properties of the novel desorption source under optimized conditions. The
electronic origin transitions $A^0_0 - F^0_0$ for the different conformers are clearly resolved and much better resolved than Ref. 10.

![Image](image.png)

**Fig. 7.3:** Two colour (1+1′) R2PI spectrum of desorbed Trp between 34843 - 35087 cm$^{-1}$. The bands labelled $A^0_0 - F^0_0$ represent the electronic origin transition for different conformers as according to Ref. 10.

### 7.3 PIE spectroscopy of Trp

The determination of ionisation energies and other properties of amino acids in the gas phase is quite a challenge. A number of photoelectron spectroscopic (PES) determinations of the ionisation energies of amino acids have been performed. 16-19 There has been also a large body of theoretical work characterizing the electronic properties of amino acids. 20,21 However, the ionisation energies of 7 of the 20 naturally occurring amino acids are still unknown. Campbell et al. 19 carried out extensive correlation studies of adiabatic ionisation energies with proton affinities to derive qualitative and mechanistic information for the amino acids and related compounds. In Table 7.3
estimations of the adiabatic ionisation energies (IEs) of amino acids obtained from PES data references are summarized. The values of IEs quoted in the Table 7.3 may vary by as much as ± 0.2 eV, especially in the case where the nitrogen lone pair is not the highest occupied molecular orbital (HOMO). As the structure becomes more complex, the accuracy with which the IE can be determined decreases. This is because as the geometry of the structure changes, the accompanying ionisation results in broad spectral bands and overlap of the bands for ionisation from the amine nitrogen lone pair orbital and the side-chain orbitals.

These experimental and theoretical studies\textsuperscript{20} have shown that the lowest ionisation energy is generally attributed to the nitrogen lone pair (n\textsubscript{N}) orbital, and is spectrally isolated from the carbonyl oxygen lone pair (n\textsubscript{o}), and oxygen π (π\textsubscript{oo}) orbitals. The oxygen lone pair (n\textsubscript{o}) ionisation potential is found to be approximately 1 eV higher in energy than the n\textsubscript{N} orbital. However, the adiabatic ionisation potential (IP) of the nitrogen lone pair (n\textsubscript{N}) orbital changes as the amino acid side-chain varies. As the electron donating ability of the side-chain increases, the n\textsubscript{N} IP shifts to lower energy since the lone pair orbital destabilizes. The highest occupied molecular orbital (lowest IP) of some amino acids is not the nitrogen lone pair (n\textsubscript{N}) orbital, but is an orbital associated with a functional group of the side-chain. For tyrosine and Trp, ionisation should occur by electron loss from the aromatic rings similar. The lowest unoccupied molecular orbitals for these molecules are localized on the aromatic rings in the π* orbital.\textsuperscript{21}
Table 7.3: Ionisation energies for the amino acids obtained from the PES References listed in the table for photoelectron data.

<table>
<thead>
<tr>
<th></th>
<th>1st IE(^{(a)}) (eV)</th>
<th>N IE(^{(b)}) (eV)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycine</td>
<td>8.8</td>
<td>8.8</td>
<td></td>
</tr>
<tr>
<td>Tryptophan</td>
<td>7.3</td>
<td>8.7</td>
<td>22,23</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>8.6</td>
<td>8.5</td>
<td>16, 23</td>
</tr>
<tr>
<td>Alanin</td>
<td>8.88</td>
<td>8.88</td>
<td>24</td>
</tr>
<tr>
<td>Cysteine</td>
<td>8.0</td>
<td>8.83</td>
<td>17</td>
</tr>
<tr>
<td>Serine</td>
<td>8.8</td>
<td>8.8</td>
<td>17</td>
</tr>
<tr>
<td>Valine</td>
<td>8.71</td>
<td>8.71</td>
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</tr>
<tr>
<td>Leucine</td>
<td>8.51</td>
<td>8.51</td>
<td>24</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>8.66</td>
<td>8.66</td>
<td>24</td>
</tr>
<tr>
<td>Methionine</td>
<td>8.0</td>
<td>8.8</td>
<td>17</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>8.0</td>
<td>8.6</td>
<td>17</td>
</tr>
<tr>
<td>Proline</td>
<td>8.2</td>
<td>8.2</td>
<td>17</td>
</tr>
<tr>
<td>Lysine</td>
<td>8.6</td>
<td>8.7</td>
<td>17</td>
</tr>
</tbody>
</table>

\(^{(a)}\)Lowest adiabatic IE in eV, \(^{(b)}\)Nitrogen lone pair IE in eV

The ionisation values in Table 7.3 originate from a thermal vaporisation techniques and the use of non-resonant single photons from a dispersed cw light source. The ions produced in this way will have the distribution of internal energy of the neutral state and the cationic state. To best of our knowledge, no precise determination of the ionisation energy of Trp via REMPI has been done. Fig. 7.4 displays the PIE spectrum of laser desorbed jet cooled Trp via the \(S_1 \leftarrow S_0\) intermediate state of conformer A.
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Fig. 7.4: PIE spectrum of laser desorbed jet cooled Trp recorded via the $S_1 \leftrightarrow S_0$ intermediate state of conformer A.

The spectrum was measured by applying a pulsed voltage of 660 V to the ion repeller, which was delayed by 18 µs relative to the excitation event. Static voltages of 12 V, 400 V and 2100 V were applied to the capacitor, ion lens and MCP. The reflectron voltage was set to 1080 V ($V_B = 350$ V, $V_R = 767$ V). The ionisation threshold was measured by scanning the ionisation laser over the range 31446-32252 cm$^{-1}$. The excitation laser was fixed at the $S_1 \leftrightarrow S_0$ origin band of the conformer A of Trp (34878 cm$^{-1}$). The PIE curve (Fig. 7.4) shows a well-defined baseline with a small step in the threshold region followed by a sharp rise. The first jump in the spectrum corresponds to the adiabatic IE, which can be estimated to be at 66694 ± 20 cm$^{-1}$ (8.269 ± 0.002 eV).

The value obtained is nearly 1 eV above the IE reported by non-resonant single photon ionisation (SPI) methods in the Table 7.3. To find an explanation for this significant
difference, we need to compare the methods used for the determination of the IE, which are related to the vaporisation and ionisation techniques.

Zhou et al.$^{25}$ have carried out thermal vaporization and laser desorption SPI experiments of guanine with tuneable vacuum ultraviolet (VUV) synchrotron radiation and found out a dramatic difference in IE for the two. The PIE curves obtained from the thermal vaporization experiments were very different in shape to those from the laser desorption experiments. The thermal vaporization PIE curves showed a very gentle curvature with an onset at 7.75 eV, while the laser desorption experiments showed a rise in intensity at 8.2 eV. Zhou et al.$^{25}$ realized that their experimental observations arose from different guanine tautomers being populated in the two different experimental conditions. The Franck-Condon (FC) overlap of the neutral and cation ground electronic states differs for the different tautomers. It was suggested that in thermal desorption experiments, only the first 5 conformers were populated, producing gentle PIE onsets due to the poor FC overlap associated with a large difference between adiabatic ionisation energy (AIE) and vertical ionisation energy (VIE). On the other hand, in laser desorption experiments, tautomers 6 and 7 were also present, and because of their better FC overlap, these tautomers dominated the PIE signal with a sharp onset.

The difference in IE for different conformers was reported by Lee et al.$^{26}$ in a study of the photoionisation of the aromatic amino acid phenylalanine (Phe) generated by supersonic expansion. They measured the IEs of all low-energy conformers by R2PI. This revealed strikingly different IE values that range from 8.80 to 9.15 eV. The wavelength selectivity is achieved in R2PI when the excitation frequency is resonant with an electronic state, whereas in non-resonance direct photoionisation no such
selectivity is possible since this process does not proceed through an intermediate state. This means that the difference in IE in a R2PI process may be due to the different electronic transitions which are not the same as in non resonance SPI process.

A clear example of how the ionisation technique affects the determination of the IE is depicted in Fig. 7.5. Fig 7.5 shows the PIE spectra of laser desorbed jet cooled Trp under identical conditions as in Fig. 7.4. The only difference is that the spectrum in Fig. 7.5b was measured under static field extraction mode and the spectrum in Fig. 7.5a under delayed pulsed field extraction. In the latter case the molecules were excited under near field free condition. The PIE spectrum in Fig. 7.5b shows a gradual slow increase in relative intensity. The AIE can be estimated from these profiles by a linear extrapolation of the steepest part of the profile back to the baseline signal level. The IE of the conformer A at the best-fit in this way was derived to be 66340 ± 50 cm⁻¹ (8.225 ± 0.006 eV). This value lies more than 354 ± 70 cm⁻¹ below the IE obtained in the delayed pulsed field extraction mode in Fig. 7.5a.
Fig. 7.5: PIE spectrum of laser desorbed jet cooled Trp recorded via the $S_1 \leftarrow S_0$ intermediate state of conformer A: a) under pulsed field extraction mode, which was delayed by 18 µs with respect to the excitation event, b) under static field extraction mode.

Theoretically, the energy shift caused by the static field can be calculated using the equation (2.17), $\Delta E \approx -6.12 \sqrt{F \text{ Vcm}^{-1}}$. Using a static voltage of 670 V and a distance of 2.1 cm between the ion repeller and ion accelerator plates, the energy shift is $\sim 110 \text{ cm}^{-1}$.

This value is less than a third of the obtained value, which means that it is still unclear why the large red shift of IE in Fig 7.5b occurs. This difference in the spectrum profiles can be explained qualitatively by making the following consideration. In the spectrum in Fig. 7.5 higher long lived n-Rydberg states within the magic region (5-10 cm$^{-1}$ below the IE) are affected by ionisation, whereas, in the spectrum in Fig. 7.5b, the Rydberg states participating in the ionisation process are originated from much broader region and for this reason the spectrum appears less pronounced.
The results in this paragraph provide a clear picture of the advantages of the laser desorbed REMPI technique over the non resonance photoionisation technique for the determination of IE.

### 7.4 MATI spectroscopy of Trp

A more accurate value for the IE of Trp can be obtained from an analysis of the MATI spectrum of Trp. The MATI spectrum was difficult to obtain due to the challenge of separating the prompt ions from the Rydberg molecules of interest. Fig. 7.6 presents the MATI spectrum of laser desorbed jet cooled Trp recorded via the $S_1 \leftarrow S_0$ origin band of the conformer A at 34878 cm$^{-1}$. This spectrum represents the first MATI spectrum ever recorded for a biomolecular system, using laser desorption method. The separation of MATI ions from the prompt ions was achieved by setting the voltage of the ion accelerator to 1.6 V. The ionisation threshold was measured by scanning the ionisation laser over the range 31574 - 32258 cm$^{-1}$.

The spectrum presents a broad structure suggesting that the prompt ions are not completely suppressed. A vibrational progression of almost equal spacing of 110 cm$^{-1}$ can be clearly observed. The feature at 66704 ± 3 cm$^{-1}$ is thought to correspond to the IE for Trp due to the fact that no further feature can be seen at lower energies in the spectrum. This value is in full agreement with the value obtained from the PIE spectroscopy (IE = 66694 ± 20 cm$^{-1}$). It has to be underlined that this peak is not the most intense in the spectrum due to the fact that the $\Delta v = 0$ propensity rule is violated as the vertical ionisation is not the strongest transition. The third overtone ($v = 3$) in the
progression at 66926 cm$^{-1}$ is the one of highest intensity. This observation indicates a dramatic change in the geometry of Trp upon ionisation.

![MATI spectrum of the cationic ground state of laser desorbed jet cooled Trp obtained by (1+1)$^\prime\prime$ R2PI recorded via the $S_1\leftarrow S_0$ intermediate state of the conformer A.]

### 7.5 Conclusion

The electronic spectrum of the amino acid Tryptophan (Trp) has been measured using the novel laser desorption source. The R2PI of Trp for a long scan has been performed showing the efficiency of the source. The R2PI spectrum is in full agreement with the previous work in the literature, and confirms the appearance of the features, which are attributed to six most stable conformers (A-F) of Trp in the molecular beam. Conformer A shows a vibrational progression of nearly 26 cm$^{-1}$ and the intensity pattern of this
progression indicates that this conformer undergoes a significant geometry change upon ionisation. To determine the IE of Trp, PIE and MATI spectra of Trp have been recorded. The IE value determined by these methods lies about 1eV above the value obtained from PES. This significant difference outlines the importance of the techniques used to determine the IE. In SPI technique used in PES the ionisation of the Trp molecule occurs by removing an electron directly from the aromatic ring. In contrast, in resonant MPI methods used in MATI/PIE the ionisation of the molecule takes place via an intermediate state, which affects the electronic states of the ion that can be reached in the ionisation process. In Table 7.4, the results obtained from the electronic spectra of Trp are summarized and compared to the values obtained from the literature.\textsuperscript{10,23}

**Table 7.4: Comparison of the experimental S\textsubscript{1}←S\textsubscript{0} transition frequencies of conformers A\textsubscript{0}\textsuperscript{0} - F\textsubscript{0}\textsuperscript{0} and IE (conformer A\textsubscript{0}\textsuperscript{0}) of Trp to the values found in the literature.**\textsuperscript{10,23}

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<td>F\textsubscript{0}\textsuperscript{0}</td>
<td>34986.6</td>
<td>34982</td>
</tr>
<tr>
<td>IE (PES)</td>
<td>-</td>
<td>58878 ± 1613 via SPI</td>
</tr>
<tr>
<td>IE (PIE)</td>
<td>66694 ± 20 cm\textsuperscript{-1}</td>
<td>58878 ± 1613 via SPI</td>
</tr>
<tr>
<td>IE (MATI)</td>
<td>66704 ± 3</td>
<td>-</td>
</tr>
</tbody>
</table>
7.6 References


Chapter 8 Computational results

The following chapter presents the computational results used to assign the conformational structures of tryptophan (Trp) in the gas phase.

8.1 Conformational structures of Trp in the gas phase

The planar structure of Trp is shown in Figure 8.1.

![Schematic structure of Trp molecule.](image)

**Fig. 8.1: Schematic structure of Trp molecule.**

The relative energies of the Trp conformers are determined by different interaction strengths of the flexible side chain, namely: the interaction between the amino group and the indole ring plane, the interaction between the carboxyl group and the indole ring plane, the steric interference and the repulsion between the lone pairs on the oxygen and the nitrogen atoms. Fig 8.2 demonstrates an overview of the relevant hydrogen-bonding types between the amino group and the carboxyl functional group of Trp.
The rigid bonds of the indole ring do not provide for any internal rotamer. In contrast, different conformers of the Trp molecule result from rotating the five internal axes of the substituent, that is, the Cα-N, Cα-C, C-O, Cα-Cβ and Cβ-Cγ bonds, and the conformational structure of the gaseous Trp molecule may be characterized by five internal torsion parameters.

Snoeck et al.\textsuperscript{1} have generated multiple conformers of Trp by systematic search of four dihedral angles in the alanyl side chain in Trp, whilst the fifth torsion, about the bond connecting to the aromatic ring, was set to 90° or 270°. The generated conformers were subjected to full geometry optimization using density functional theory and Møller-Plesset perturbation theory. In that article, the structure of the lowest energy conformers

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**Fig. 8.2: Hydrogen bonding types between the carboxyl group and the amino functional group of Trp.**
of Trp was fully optimized at the B3LYP/6-31+G(d) and also at the MP2/6-311+G(d,p), including the zero-point energies.

In our investigation to find the structure of the lowest energy of Trp we reoptimized the geometry of Trp at different levels for which Snoeck et al.\textsuperscript{1} published data. For the determination of the energies of the conformers, ground state geometry optimization and frequency calculations were performed with the RI-DFT method implemented in Turbomole (resolution-of-identity approximation with the TPSS functional) with the triple-zeta valence plus polarization (TZVP) basis sets. The Trp conformers were also submitted to full geometry optimisation at RI-MP2. In the MP2 calculation the cc-pVDZ and cc-pVTZ basis sets were used to obtain reasonable results. The structures of the ten most stable conformers of Trp in the ground state ($S_0$), calculated at MP2/cc-pVTZ level are shown in Fig. 8.3. In Table 8.1 their optimized lowest relative energies at higher level of theory are listed. Table 8.1 includes also Snoek’s et al.\textsuperscript{1} calculated relative energies at the higher level of theory, MP2/6-311+G (d,p), including zero-point energies. The italic number in brackets next to the energy gives the rank order of the particular conformer relative to the conformer of the lowest energy. Conformer 1 is found to be the global minimum for Trp at both the DFT and the MP2 level. Its total electronic energy is -684.3959 au at the RI-MP2/cc-pVDZ level, -685.0598 au at the RI-MP2/cc-pVTZ and -686.6512 au at the RI-DFT/TZVP.
Fig. 8.3: Structures of the ten lowest energy conformers of Trp optimized at the MP2/cc-pVTZ level of theory. The corresponding hydrogen-bonding types are shown in brackets.
Table 8.1: Relative energies for the ten most stable conformers of Trp.

<table>
<thead>
<tr>
<th>Conformer structure(^a)</th>
<th>RI-MP2/ cc-pVDZ(^b)</th>
<th>RI-MP2/ cc-pVTZ(^b)</th>
<th>RI-DFT/TZVP(^b)</th>
<th>MP2/6-311 G(d,p).(^{b,c})</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0 (1)</td>
<td>0 (1)</td>
<td>0 (1)</td>
<td>0 (1)</td>
</tr>
<tr>
<td>2</td>
<td>0.0701 (2)</td>
<td>0.0514 (8)</td>
<td>0.0519 (5)</td>
<td>0.0529 (5)</td>
</tr>
<tr>
<td>3</td>
<td>0.0724 (3)</td>
<td>0.0242 (5)</td>
<td>0.1622 (6)</td>
<td>0.0448 (2)</td>
</tr>
<tr>
<td>4</td>
<td>0.0787 (4)</td>
<td>0.0285 (6)</td>
<td>0.1671 (7)</td>
<td>0.0466 (3)</td>
</tr>
<tr>
<td>5</td>
<td>0.1005 (5, 6)</td>
<td>0.0028 (2, 3)</td>
<td>0.0209 (3)</td>
<td>0.0471 (4)</td>
</tr>
<tr>
<td>6</td>
<td>0.1005 (5, 6)</td>
<td>0.0028 (2, 3)</td>
<td>0.0207 (2)</td>
<td>0.0841 (8)</td>
</tr>
<tr>
<td>7</td>
<td>0.1027 (7)</td>
<td>0.0521 (9)</td>
<td>0.1817 (8)</td>
<td>0.0850 (9)</td>
</tr>
<tr>
<td>8</td>
<td>0.1132 (8)</td>
<td>0.0470 (7)</td>
<td>0.1871 (9)</td>
<td>0.0746 (6)</td>
</tr>
<tr>
<td>9</td>
<td>0.1135 (9)</td>
<td>0.1262 (10)</td>
<td>0.2457 (10)</td>
<td>0.0760 (7)</td>
</tr>
<tr>
<td>10</td>
<td>0.2035 (10)</td>
<td>0.0207 (4)</td>
<td>0.0468 (4)</td>
<td>0.0892 (10)</td>
</tr>
</tbody>
</table>

\(^{(a)}\) Conformational structure in Fig. 8.3.
\(^{(b)}\) The italic numbers in brackets indicate here the relative energy ordering at the respective level of theory.
\(^{(c)}\) Zero-point energies are included.

A comparison of the relative energies in Table 8.1 reveals some differences in rank order of the remaining conformers (2-10) depending on the method used. However, the energies calculated at higher level of theories at RI-MP2/cc-pVTZ and RI-DFT/TZVP show good agreement, allowing one to form an overall impression of the rank order of the conformers. Four of the six most stable conformers 1, 2, 5 and 6 form an anti conformation of the C=COH group in accordance with the bonding type A. These conformers are stabilized through an intramolecular H-bond (COOH…..NH\(_2\)) and an
additional H-bonding interaction between the amino group and π-electron system of the aromatic ring, which leads to an increased electron density around the naked nitrogen atom making the O-H…NH-bond stronger.

However, a comparison of the relative global minima of aliphatic amino acids glycine, alanine and valine\textsuperscript{2,4} shows a preference of the syn to the anti conformation of the C=COH group. The bifurcated interaction in the syn confirmation is compensated in the conformers 1, 2, 5, and 6 by creating the alternative OH…NH\textsubscript{2} hydrogen bond and the co-operative between NH\textsubscript{2} and the π-electron system.\textsuperscript{5}

The global minimum of conformer 1 also appears to profit from the additional benefit of the electrostatic interaction between the carbonyl oxygen and neighbouring C-H group in the pyrrole ring. In order to be able to assign the conformational structures in Fig. 8.3 to the experimentally observed conformers (A - F) in the R2PI spectrum of Trp, associated IR spectra for these conformers are necessary. This can be achieved by recording IR-UV hole-burn spectra for each of the conformers A – F, as Snoek et al. did. Snoek et al. compared the experimental R2PI peak intensities, infrared frequencies (and intensities) from IR-UV depletion spectra; to the MP2 calculated relative energies and the DFT calculated infrared frequencies.

However, as currently we are not able to carry out those double resonance techniques, our qualitative prediction of the structural assignment of the Trp conformers relies only on a comparison of the relative intensities (see Table 7.2) of the transitions A\textsubscript{0}\textsuperscript{0} - F\textsubscript{0}\textsuperscript{0} obtained from the R2PI spectrum with the energies calculated in Table 8.1. According to Table 7.2 the most intense signal of Trp in the gas phase corresponds to conformer A, which reflects the fact that conformer A has the highest population of all conformers in neutral Trp in the ground state. This observation, combined with the fact that structure 1

in the Fig 8.3 is the most stable conformer of Trp as per all three levels of theory, confirms the conformational assignment A(1). The same approach can be used to reveal the structure of conformers B-F, although with less certainty, as the calculated energy of the conformer depends on the level of theory used and the three levels of theory give different answers. In Table 8.2 are the experimental R2PI intensities from Table 7.2 ordered to the relative energies calculated in RI-DFT and RI-MP2/cc-pVDZ and compared to the more rigorous assignments of Snoek et al.¹

Table 8.2: Assignments of conformers (A₀₀⁰⁻ F₀₀⁰) in experimental R2PI to the theoretical optimized structures.

<table>
<thead>
<tr>
<th>Conformer</th>
<th>(I)ᵃ</th>
<th>RI-DFTᵇ</th>
<th>RI-MP2ᵇ</th>
<th>Snoek et al.¹ᵇ</th>
</tr>
</thead>
<tbody>
<tr>
<td>A₀₀⁰</td>
<td>0.99</td>
<td>(1)</td>
<td>(1)</td>
<td>(1)</td>
</tr>
<tr>
<td>E₀₀⁰</td>
<td>0.36</td>
<td>(6)</td>
<td>(2)</td>
<td>(3)</td>
</tr>
<tr>
<td>D₀₀⁰</td>
<td>0.20</td>
<td>(5)</td>
<td>(3)</td>
<td>(8)</td>
</tr>
<tr>
<td>C₀₀⁰</td>
<td>0.14</td>
<td>(10)</td>
<td>(4)</td>
<td>(4)</td>
</tr>
<tr>
<td>F₀₀⁰</td>
<td>0.11</td>
<td>(2)</td>
<td>(5)</td>
<td>(5)</td>
</tr>
<tr>
<td>B₀₀⁰</td>
<td>0.08</td>
<td>(3)</td>
<td>(6)</td>
<td>(7) ²</td>
</tr>
</tbody>
</table>

(a) Relative intensities from R2PI spectrum in Table 7.3. (b) Conformational structure in Fig. 8.3.

A comparison of the energies in Table 8.2, shows that the RI-MP2/ cc-pVDZ are more in consistent with the assignments of Snoek et al.¹, which are definitely more accurate.
8.2 Conclusion

The calculation of the relative energies of 10 most stable conformer structure of Trp (see Fig. 8.30) all indicate that conformer structure 1 is the dominating conformer. This combined with experimental R2PI allows us to assign A0\(^0\) (1) as the most populated conformer in the supersonic beam. Trp conformer 1 is stabilized by the anti configuration of the COOH group, which allows the formation of the formation of H-bonded interactions between the side chain and the indole ring. The assignments of the remaining conformers (B-F) can be done with less certainty as the relative energies calculated at three levels of theory (see Table 8.2) are not consistent. In order to be able to make accurate assignments IR-UV depletion spectra of conformers (A-F) need to be recorded.
8.3 References


Chapter 9 Concluding remarks and future work

In this thesis I have developed a laser desorption source coupled with a pulsed jet expansion to produce cold molecular beams of thermally fragile molecules and their complexes. This source has been further refined by a method that can significantly improve the signal/noise ratio under such circumstances by eliminating the signals associated with the non-homogeneity of the sample surface and shot to shot fluctuation of the desorption laser.

The main advantages of the novel desorption source are:

- Simple sample preparation by pressing a mixture of the substance to be laser-desorbed with graphite powder.
- Long lifetime of the above sample with no change after 1 month.
- Excellent supersonic jet expansion cooling and good signal stability.

The approaches presented here can be further developed to improve the desorption process and shot-to-shot stability. Several new sources with a larger desorption rod (larger surface), bigger volume of the mixing chamber and different angle of the sample rod/ desorption beam have been constructed and are ready to be tested for performance. Furthermore, an actuator with high precision speed and position control is ready to be used for patterning and synchronisation techniques.

The novel source has been so far applied to the spectroscopic study of the amino acid tryptophan (Trp). The methods employed above have helped with the provision of highly accurate mass resolved electronic spectra (REMPI, PIE and MATI) of Trp. The results achieved are:
• Confirmation of the existence of six low-lying conformers of Trp in the gas phase.

• First ever threshold ionisation MATI spectrum of a biomolecule (Trp) via R2PI for the determination of IE with high accuracy as ± 3 cm⁻¹.

The source and methodologies developed demonstrate the potential for the analysis of other biological molecules and their clusters. In Table 9.1 we report a list of different classes of biomolecules, with some examples of molecules appertaining to each classes which can appropriately be studied using the LD source shown in this thesis. A common point, which characterizes those molecules, is their fragility upon thermal heating. Therefore, our LD source is a very appropriate method for the spectroscopic investigation of the biomolecules listed as examples in Table 9.1.

The long lifetime of the source has the advantage that one can carry out experiments where scans in a large range are required to explore the region above excitation or ionisation of the molecular system. While the LD methods of de Vries et al.¹ can guarantee a good stability of the signal for a short scan, this novel source can offer a better stability equal stability for a much longer time.
Table 9.1: List of biomolecules, suitable for using the novel LD source for spectroscopic studies.

<table>
<thead>
<tr>
<th>Biomolecule</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neurotransmitters, enzymes and</td>
<td>Phenylethylamine, catecholamines,(^2)</td>
</tr>
<tr>
<td>hormones</td>
<td>dopamine, histamine, tryptamine,</td>
</tr>
<tr>
<td></td>
<td>serotonin, acetylcholine, ephedrine, adrenaline, 5-phenylimidazole</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Amino acids, amides and small</td>
<td>Phenylalanine,(^3) tyrosine,(^4,5) arginine, valine, N-phenylformamide,(^5,7) N-</td>
</tr>
<tr>
<td>peptides</td>
<td>benzylformamide,(^8) di- and tripeptides</td>
</tr>
<tr>
<td></td>
<td>(Gly-Trp, Phe-Gly, Gly-Tyr Ala-Tyr, Trp-Gly-Gly)(^9,10,11)</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Nucleic acid bases</td>
<td>Uracil(^12), thymine,(^13) cytosine,(^14) Guanine(^15)</td>
</tr>
<tr>
<td></td>
<td>adenine(^16), Guanosine(^17)</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Their clusters and molecular</td>
<td>Guanine-cytosine,(^18) Guanine-guanine(^19)</td>
</tr>
<tr>
<td>hydrates</td>
<td>Cytosine-cytosine(^19), Guanine-Aspartic Acid,(^20) and GG(H(_2)O)(^{21}), GG(H(_2)O)(_2)(^{21})</td>
</tr>
</tbody>
</table>

With increasing complexity of the molecular components the possibility for forming structural isomers grows and an important goal is to determine the infrared and ultraviolet spectral signatures of each conformational or structural isomer. To accomplish this goal, UV-UV\(^{22,23}\) and IR-UV\(^{24,25}\) double resonance methods can be
employed to identify different tautomers and structures for non-covalent bound complexes.

The experiments presented here have shown some fragmentation channels and pathways for Trp as an example of the dissociation dynamics of polyatomic biomolecules. The techniques presented here provide a powerful tool for investigating the photoinduced fragmentation behaviour of larger molecules, and this could be useful for determining the sequence of small peptide chains.
9.1 References

5. L. Li, and D.M. Lubman, Appl. Spectrosc, 1988, **42**, 418.


