Intrinsic Properties of Rhodamine B and Fluorescein Gas-phase Ions Studied using Laser-Induced Fluorescence and Photodissociation in a Quadrupole Ion Trap Mass Spectrometer.

by

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A thesis submitted in conformity with the requirements for the degree of Masters of Science

Department of Chemistry

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Abstract

Studying the intrinsic properties of molecules in the gas-phase is advantageous, since it reduces the complexity present in solution that arises from interactions between the molecule of interest and other species present in the local environment, including those with the solvent itself.

In this report, the photophysical properties of gaseous cationic rhodamine B (RBH$^+$) were determined and photodissociation reaction kinetics and power dependence of three prototropic forms of fluorescein; the cation ([F + H]$^+$), monoanion ([F - H]$^-$), and dianion ([F – 2H]$^{2-}$), each of which possesses their own distinct spectral properties, were measured. The analyte ions of interest were formed via electrospray ionization, mass-selected and stored in a quadrupole ion trap mass spectrometer which has been customized to enable gas-phase spectroscopic studies.

Knowledge of the intrinsic photophysical properties of such chromophores in the gas-phase will enable a better understanding of how the local environment of the molecule alters its properties.
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Chapter 1

Introduction

1.1 Mass Spectrometry

Mass spectrometry (MS) is a powerful, versatile and widely applicable analytical tool. It is an instrumental technique that is used to identify the chemical composition of compounds by forming gas-phase ions that are separated according to their mass and charge. MS is also very useful for elucidating the structures of various molecules as well as to provide qualitative and quantitative information on the composition of complex chemical mixtures.

Mass spectrometry was started by J.J. Thomson in 1897. By the 1940s, mass spectrometers were commercially available and MS was established as a useful and powerful technique among physicists and industrial chemists. Chemists in the petroleum industry were using the mass spectrometer to quantitatively analyze hydrocarbon mixtures. It was not until the 1950s, that commercial mass spectrometers began to be used by chemists for structure elucidation and identification of various compounds. The three main advantages of mass spectrometry are specificity, sensitivity, and speed. The sensitivity of MS-based methods (picomole to femtomole range) allows the study of minute quantities of material, which makes MS a useful method when only a small amount of a compound is available. Another advantage of MS is the ability to determine the number of ligands that form complexes via non-covalent interactions. As an analytical tool, mass spectrometry is also very useful, due to the ability to carry out tandem mass spectrometry experiments, thus providing the ability to identify unknown molecules.

1.1.1 What is Mass spectrometry?

A mass spectrometer is an instrument that produces ions from the analyte of interest, separates them according to their mass-to-charge ratio (m/z), and records the relative intensity of each ionic species. The three main components of a mass spectrometer are the ionization source, mass...
analyzer, and the detector as schematically shown in Figure 1.1. In this experiment, the molecules are ionized by an electrospray ionization (ESI) source, then the created ions are separated based on their mass to charge ratio ($m/z$) using a modified quadrupole ion trap, and finally the ion beam is detected by an electron multiplier.

Ionization is the process of transforming neutral sample components into charged species. This is done because only ions have the ability to change speed and direction which can be straightforwardly controlled by an electric field. Deprotonation leads to the formation of negatively charged ions ([M–nH]$^n$) and protonation leads to the formation of positively charged ions ([M+nH]$^+$). ESI is specifically used since it is an extremely soft ionization technique enabling a proton to be removed from (or added to) the analyte without breaking it apart.

The mass analyzer used in this experiment is the quadrupole ion trap (QIT) since this instrument is capable of trapping ions for an extended period of time as well as performing tandem mass spectrometry (MS/MS) experiments. The ions created by ESI enter the ion trap via the entrance end cap electrode and they get trapped through the action of a dynamic electric field generated by applying a radio frequency (RF) potential to the three hyperbolic electrodes (entrance end cap electrode, ring electrode, and exit end cap electrode). The ion trap is also filled with helium buffer gas which undergoes collisions with the trapped ions thus removing their kinetic energy, resulting in the ions being further focused to the centre of the trap which increases the ion cloud number density. This cooling process is also beneficial because it reduces the loss of ions out of the trap therefore increasing their maximum possible trapping time. The use of helium buffer gas is highly beneficial for our experimental set-up because we are collecting laser-induced fluorescence from the trapped ions and having a dense ion cloud that is well focused in the centre of the trap plus longer trapping times increase the fluorescence efficiency. When the kinetic energy of the trapped ions is high enough, they also undergo dissociation reactions through collisions with the buffer gas molecules (collision-induced dissociation – CID). Mass detection is achieved when the radio frequency voltage applied to the ring electrode is gradually increased, thus resulting in the destabilization of the ions oscillatory motion (secular frequency) which causes the ions to be ejected through the exit end cap electrode in order of increasing $m/z$ ratio. These ions are sent off to the electron multiplier to be detected and signal processing results in the formation of a mass spectrum (Figure 1.1).
Figure 1.1: A schematic representation of how the modified quadrupole ion trap mass spectrometer (coupled with laser-induced fluorescence spectroscopy) works.

1.1.2 Electrospray Ionization

The electrospray ionization (ESI) technique was invented by Malcolm Dole in the 1960’s. It was not until the 1980’s that work by John Fenn et al., showed that ESI could be used for the ionization of high-mass biologically significant compounds thus allowing such compounds to be analyzed by mass spectrometry. The development of ‘soft’ ionization methods, such as, matrix-assisted laser desorption/ionization (MALDI) and ESI has allowed the mass spectrometric analysis of large biomolecules such as proteins and DNAs. With these ionization techniques, fragile molecules can be successfully transferred into the gas phase without breaking apart.
The basic electrospray process is shown in Figure 1.2. As the sample solution passes through the capillary to which an electric field is applied, droplets are formed. These droplets undergo sequential evaporation and Coulombic explosions which causes them to get smaller and smaller\(^1\),\(^{20-21}\). Eventually, all the polar, volatile solvent evaporates from the droplets, leaving only gas-phase ions that are sent to the mass analyzer to be separated according to their \(m/z\) ratio.

![Figure 1.2: Schematic of the electrospray ionization (ESI) process.](image)

1.1.3 Theory of the Quadrupole Ion Trap

The quadrupole ion trap (QIT) mass analyzer (Figure 1.3) was developed at the same time as the quadrupole mass filter analyzer by Wolfgang Paul whose work in the 1950's lead to the development of the basic parameters of current QIT-mass spectrometers\(^{22-23}\). The QIT-MS is currently a versatile and simple to use analytical instrument due to technologically advances in design at Finnigan MAT by George Stafford and co-workers in the 1980’s\(^{24-25}\).

Since then, there have been various improvements made to the QIT-MS. The main advantage of ion trap techniques is the ability to perform tandem mass spectrometry and so far up to 12 stages of tandem mass spectrometry (MS\(^{12}\)) have been performed using a QIT\(^{26}\). This technique allows much more structural information to be obtainable for a given compound. This and other advances in QIT-MS have improved the performance of the ion trap and expanded its application to various molecules.
Three hyperbolic electrodes, consisting of a ring and two end caps, form the main components of this instrument (Figure 1.3). The ion trap is also filled with helium buffer gas which further focuses the ions to centre of the trap. Trapped ions are further focused toward the center of the trap through the use of an oscillating voltage, called the fundamental RF, applied to the ring electrode\textsuperscript{11}. An ion is trapped stably depending on the mass and charge of the ion, the radial size of the ion trap \((r_o)\), the separation of the two end-cap electrodes measured along the axis of the ion trap \((2z_o)\), the oscillating frequency of the fundamental rf \((\Omega)\), and the amplitude of the voltage on the ring electrode \((V)\). The dependence of ion motion on these parameters is described by the dimensionless parameter \(q_z\) (Equation 1.1):

\[
q_z = \frac{8eV}{m(r_o^2 + 2z_o^2)\Omega^2} \tag{1.1}
\]

An equivalent parameter \(a_z\) describes the effect on the motion of ions when a DC potential \((U)\) is applied to the ring electrode (Equation 1.2)\textsuperscript{11}.

\[
a_z = \frac{-16eU}{m(r_o^2 + 2z_o^2)\Omega^2} \tag{1.2}
\]
The parameters required for the stability (and instability) of the trajectory of an ion within the field provides the basis for the operation of a quadrupole ion trap\textsuperscript{11}. These experimental conditions determine whether an ion is stored within the trap or is ejected from the trap and either lost or externally detected. The `stability diagram´ (Figure 1.4) represents a theoretical region where radial ($r$) and axial ($z$) stabilities overlap. Depending on the amplitude of the voltage applied to the ring electrode, an ion of a given $m/z$ will have $a_z, q_z$ values that will fall within the boundaries of the stability diagram, and the ion will be trapped\textsuperscript{9,11}. If the $a_z, q_z$ values at a specific voltage fall outside of the boundaries of the stability diagram, the ion will hit the electrodes and be lost. For the QIT, when the $q_z$ is greater than 0.908, ions are no longer stable and are not trapped any more\textsuperscript{11,21}.

**Figure 1.3:** Schematic of the quadrupole ion trap (QIT).
1.2 Tandem Mass Spectrometry

Tandem mass spectrometry (MS/MS) relies on the selection of an investigation ion at a particular \( m/z \) ratio and subsequent activation of this ion via various processes, such as, collision-induced/activated dissociation (CID/CAD) and photodissociation (PD), to create fragment ions from the selected ion which provides structural information about the ion of interest\(^{27}\).

Tandem mass analysis occurs due to measurements being tandem in space, where two mass analyzers are assembled in tandem; or tandem in time, where only one analyzer is used for mass analysis and it isolates the specific analyte ion and fragments it, thus allowing analysis of the fragment ions\(^{28-29}\). Ion activation methods vary depending on how the energy is transferred to the ions and how this energy is distributed in the activated, isolated ions and not only do these
factors have a large effect on which fragment ions are produced from the precursor ion but they also affect the mass spectra produced in terms of efficiency, selectivity, and reproducibility.  

The ability to perform tandem in time mass analysis with high efficiency, is the main advantage of a quadrupole ion trap.

### 1.2.1 Collision-induced Dissociation (CID)

Collisional activation occurs when an isolated ion collides with a neutral atom/molecule (nitrogen, helium, or argon), and some of the ion's kinetic energy can be converted into internal energy (law of conservation of energy). CID occurs when this internal energy is high enough thus resulting in the breaking of chemical bonds which causes the isolated ions to fragment.

In a quadrupole ion trap, collision induced dissociation occurs by applying a small amplitude resonant excitation voltage, to the end-cap electrodes, which is at the same frequency as the secular frequency of the trapped precursor ion of interest. This causes the ions to move away from the centre of the trap, thus causing them to be accelerated which in turn increases the kinetic energy of the trapped ions. CID occurs when the excited ions undergo collisions with the helium buffer gas, causing the ions kinetic energy to be converted to internal energy.

In a QIT, a simple tandem mass spectrum of the precursor ion of interest is usually obtained because, the product ions, formed by fragmentation of the precursor ion via CID, do not usually have enough energy to fragment as well, since only the precursor ions are activated by collisions with the helium buffer gas. The main instrumental parameters that are optimized in the QIT in order to perform CID experiments are the mass isolation window, the $q_z$, the fragmentation amplitude and the time.

### 1.2.2 Photodissociation (PD)

An alternative method of fragmenting ions in a quadrupole ion trap is via photo-induced dissociation. Photodissociation (PD) is a mechanism in which isolated ions are activated by
absorption of electromagnetic radiation such as infrared, visible or ultraviolet light, causing them to then dissociate. In order for this to occur, the structure of the ions must contain a chromophore which allows them to absorb light at a specific wavelength and either multiple photons must be absorbed or the energy of the photons must be higher than the energy needed to break a chemical bond. This makes photodissociation a very selective process.

Initial photodissociation studies of gas-phase ions date back to the 1960's due to the work by Dehmelt and Dunn. Dehmelt was the first to study trapped ions (H$_2^+$) using photodissociation in a low-pressure QIT. From the early 1970's, photodissociation coupled with ion cyclotron resonance (ICR) mass spectroscopy became popular, with most of these experiments being done by Dunbar et al. Several other researchers including Brauman, Beauchamp and Freiser, Bowers and van der Hart, also used this technique.

1.3 Fluorescence Spectroscopy

Fluorescence spectroscopy is an important analytical method that is well recognized as a sensitive probe of the intrinsic electronic properties of various atoms, ions and organic dyes.

Although most fluorescence studies have been carried out in the condensed phases, studying the intrinsic properties of gas-phase ions using this technique has become a useful way of determining the photophysical properties of fluorophores in the absence of interfering solvent effects.

1.3.1 Theory of Fluorescence

Fluorescence spectroscopy is an analytical technique where analyte molecules of interest are excited by irradiation at a particular wavelength and emit radiation of (usually) a different wavelength. As shown in the Jablonski diagram (Figure 1.5), when a molecule absorbs light of a particular wavelength ($h\nu$), it gets excited from the electronic ground state ($S_0$) to one of many vibrational levels in one of the singlet excited electronic states ($S_n$). Once the molecule is in this excited state, relaxation can occur via several processes. The Jablonski diagram was created by.
Aleksander Jablonski in 1935 and it provides a suitable way to visualize vibronic transitions and other relevant processes involved in photoluminescence. The Jablonski diagram (Figure 1.5) illustrates the ground ($S_0$), first excited ($S_1$) and second excited ($S_2$) singlet electronic states of a fluorophore, arranged according to increasing energy (E). Fluorophores can exist in a number of vibrational energy levels (horizontal lines within each electronic state). The vertical lines represent transitions between states and these can be divided into two main categories, that is, radiative and non-radiative transitions. Radiative transitions, depicted with solid vertical arrows, involve the absorption and emission of a photon ($h\nu_A$ and $h\nu_F$ / $h\nu_P$, respectively) where as non-radiative transitions, shown by wavy arrows, include internal conversion, vibrational relaxation, and intersystem crossing. The usual time-frames of all these processes vary significantly, with absorption usually being very fast and occurring in $\sim 10^{-15} \text{s}$, fluorescence in $10^{-9} – 10^{-7} \text{s}$, vibrational relaxation and internal conversion in $10^{-14} – 10^{-11} \text{s}$, and non-radiative relaxation in $10^{-9} – 10^{-7} \text{s}$.

One of the main characteristics of fluorescence from a fluorophore is the Stokes shift ($E_{SS}$), which was first observed for quinine by Sir. G. G. Stokes in 1852. As seen from the Jablonski diagram (Figure 1.5), fluorescence usually occurs at longer wavelengths / lower energies, that is, the energy of emission is usually less than the energy of absorption. The magnitude of the fluorescence Stokes shift and the energies of the spectral bands provide information about the excited states ($S_n$) relative to the ground state ($S_0$), and the amount of molecular reorganization that occurs following absorption.
Two important characteristics of a fluorophore are its fluorescence lifetime ($\tau$) and the fluorescence quantum yield ($\phi_f$). The fluorescence lifetime is defined as the average time a molecule spends in the excited state before returning to the ground state and it is usually in the nanosecond range\(^{50}\). The fluorescence lifetime of a fluorophore can be expressed in terms of the
radiative ($k_r$) and/or non-radiative ($k_{nr}$) rate decay constants as shown in Equation 1.3. This equation shows that the lifetime of a fluorophore is the inverse of the total decay rate.

$$\tau = \frac{1}{k_r + \Sigma k_{nr}}$$  \hspace{1cm} (1.3)

The fluorescence quantum yield is the ratio of the number of photons emitted to the number of photons absorbed and it can also be expressed in terms of the radiative and non-radiative rate decay constants, as shown in Equation 1.4.

$$\phi_f = \frac{k_r}{k_r + \Sigma k_{nr}}$$  \hspace{1cm} (1.4)

A non-radiative transition that fluorophores in the excited state may undergo is intersystem crossing (ISC) from an excited singlet state to an excited triplet state ($T_1$). Intersystem crossing is formally spin forbidden since it involves a change in the spin multiplicity of the fluorophore from the singlet state (antisymmetric spin pairing of electrons) to a triplet state in which there are two electrons that have parallel spin to each other\textsuperscript{59}. Radiative transition from a triplet state is known as phosphorescence and is known to have a slow decay back to the ground state, typically having lifetimes in the range of milliseconds to seconds ($10^{-3}$ - $10^{2}$ s)\textsuperscript{50}.

The wavelength ($\lambda$) that a particular chromophore absorbs light at is inversely proportional to the energy ($\Delta E$) required to excite it from the ground state ($S_0$) to the excited state ($S_1$). This relationship can be expressed as shown in Equation 1.5, where $c$ is the speed of light and $h$ is Planck’s constant.

$$\Delta E = \frac{hc}{\lambda}$$  \hspace{1cm} (1.5)

A bathochromic (red) shift occurs when the maximum wavelength of absorption / emission shifts to a longer wavelength, which in turn corresponds to a decrease in the energy gap between $S_0$ and $S_1$ due to stabilisation of these states (Figure 1.6). A hypsochromic (blue) shift occurs due to an increase in the energy gap between ground and excited states (less stabilized) and this results in a shift of the maximum wavelength of absorption / emission to shorter wavelengths (Figure 1.6)\textsuperscript{59}.  

Figure 1.6: Schematic energy level diagram showing how the red and blue shifts of the maximum wavelength of absorption / emission are related to the energy level gap between the ground state ($S_0$) and the excited state ($S_1$).

These shifts usually occur due to a change in the environment conditions that the fluorophore is in. Any influence of the ‘medium’ on the electronic absorption and emission spectra of fluorophores is known as solvatochromism\textsuperscript{59}. For example, changes in the pH of the solvent, solvation / desolvation, or hydration all lead to solvatochromism.

1.3.2 Fluorescence from Trapped Ions

Studying the photophysical properties of molecular systems in the gas-phase is advantageous since it reduces the complexity often found in biomolecular systems due to a range of interfering interactions in solutions. The main advantage of combining ion spectroscopic measurements with mass spectrometry is the ability to mass-select and isolate a specific ionic species from a solution thus allowing a specific analyte ion of interest to be studied. Although fluorescence is an extremely sensitive technique, capable of producing high S/N data, practical limitations exist in the experimental set-up used in the work. The ion density in the mass spectrometer is usually very low (usually hundreds to thousands of ions are stored in a trapping MS) which greatly reduces the signal level\textsuperscript{51}. Other factors leading to low S/N are a low fluorescence collection solid angle and any potentially high background signals. To improve the S/N when spectroscopically studying gas-phase ions, many ions are stored for an extended period of time.
Laser-induced fluorescence (LIF) measurements of trapped ions have been used in spectroscopic studies of atomic structure\textsuperscript{61-64} and the first excitation spectrum for a gas-phase molecular ion (N\textsubscript{2}\textsuperscript{+}) was measured in 1975 by Engelking and Smith\textsuperscript{52}. Soon after, Grieman \textit{et al.} measured the first laser-induced fluorescence spectrum for trapped CD\textsuperscript{+} in a quadrupole ion trap\textsuperscript{53}. After about twenty years, Marshall \textit{et al.} measured the laser-induced fluorescence excitation spectrum of hexafluorobenzene cations\textsuperscript{54} and the emission spectrum of trifluorobenzene cations\textsuperscript{65} in an FT-ICR cell. In 2002, Joel Parks \textit{et al.} used a QIT to measure the gas-phase fluorescence with zero-background detection of two dyes, Alex Fluor 350 and Rhodamine 101\textsuperscript{55}. The Zenobi group have used FT-ICR mass spectrometry to measure the fluorescence emission from a number of commercial laser dyes\textsuperscript{56, 66-68}. Recent work by Kent Ervin’s group also involved the fluorescence and photodissociation of rhodamine 575 in a QIT\textsuperscript{57}. The Jockusch group have measured the fluorescence emission and excitation spectra, as well as the fluorescence lifetime of various laser dyes, by combining LIF with a QIT-MS\textsuperscript{51, 69-73}.

Although most fluorescence studies have been carried out in the condensed phase, studying the intrinsic properties of gas-phase ions using this technique has emerged as a useful way of determining the photophysical properties of fluorophores in the absence of interfering solvent effects\textsuperscript{51, 52-57, 66-75}.

1.4 Instrumental Set-up

The instrument used for laser-induced fluorescence and photodissociation of trapped ions is a modified commercial QIT mass spectrometer (Esquire 3000+, Bruker Daltonik GmbH, Bremen, Germany) equipped with an ESI source\textsuperscript{51} The basic components of the mass spectrometer are shown in Figure 1.7. Solutions containing the analyte ions (nM concentration) are sprayed through a syringe into the spray chamber with a flow of nitrogen (N\textsubscript{2}) nebulizing gas through the electrospray emitter. This produces droplets of the volatile solvent and analyte ions. Counter-current drying gas (also N\textsubscript{2}) at a temperature of 300 °C is introduced into the spray chamber to help evaporate the solvent from the droplets. In the Bruker ESI source, the ESI emitter is held at ground while the entrance of the capillary inlet to the mass spectrometer is adjusted to a suitable voltage (± 2 - 4 kV). The ESI spray chamber is at atmospheric pressure and the heated glass
capillary is shielded by an end plate / spray shield. Through the ESI process (Section 1.1.2) gas-phase ions are produced from the charged droplets containing analyte ions. These gas-phase analyte ions are directed through the skimmer, via a heated glass capillary, which removes most of the drying gas. The ions are further focused and transported to the quadrupole ion trap by the action of an octopole ion guide and the focusing/exit lenses.

The quadrupole ion trap consists of three electrodes (ring, entrance end-cap, and exit end-cap electrodes) that trap the ions due to the electrodynamic trapping field (Section 1.1.3). The vacuum in the main chamber is maintained at $\sim 10^{-5}$ mbar and the QIT is used with a significant pressure of helium bath gas ($0.6 - 2.5 \times 10^{-3}$ mbar) in order to efficiently trap the ions and to kinetically cool the ion cloud, thus focusing the ions to the centre of the trap. Destabilized, trapped ions are sent off to the electron multiplier to be detected and signal processing results in the formation of a mass spectrum. The QIT allows a large number of ions ($\sim 5 \times 10^4$) to be stored for extended periods of time (from milliseconds to several seconds), thus making it an ideal analyzer to carry out laser-induced fluorescence studies on trapped ions.

**Figure 1.7**: A schematic diagram of the Bruker Esquire 3000+ quadrupole ion trap mass spectrometer.
Ions generated by ESI are mass-selected and stored in the QIT where they are irradiated with the frequency-doubled output of an 80 MHz pulsed Ti:Sapphire laser (Tsunami, Spectra-Physics, Mountain View, CA). Upon second harmonic generation, visible light in the range of 345-540 nm can be generated from this laser source. The laser beam enters and exits through two holes in the ring electrode of the QIT assembly thus intersecting the cloud of trapped, mass-selected ions. Fluorescence is collected through a third hole on the ring electrode which is orthogonal to the path of the laser beam. The collected fluorescence light is directed through a high-pass filter (Chroma Technology Corp., Rockingham, VT) and focused on the slit of a spectrograph (Shamrock303i, Andor Technologies, Belfast, Ireland) which disperses the light onto an electron-multiplied charge-coupled device (Newton EM-CCD, Andor Technologies, Belfast, Ireland). The EM-CCD is cooled and operated with electron multiplying features activated. The entire experimental set-up is shown in Figure 1.8.
Figure 1.8: Scheme of the laser-induced fluorescence optical set-up coupled with the QIT mass spectrometer
1.5 Overview

With this experimental set-up, the intrinsic gas-phase properties of fluorophores, isolated and in well-defined micro-environments can be investigated. In Chapter 2, the intrinsic (gas-phase) properties of cationic rhodamine B, a xanthene dye, are determined using the quadrupole ion trap mass spectrometer coupled with laser-induced fluorescence spectroscopy. This information provides a better understanding of the effects of the solvent on the photophysical properties of the cationic rhodamine species. In this study, the excitation and emission maxima for RBH⁺ are found to lie at higher energy in the gas-phase than in the solution phase and the fluorescence lifetime for the cationic form of rhodamine B in the gas-phase is much longer than in solution phase. Preliminary work on the zwitterionic form of rhodamine B, complexed with metal cations, in the gas-phase is also shown.

Chapter 3 focuses on photodissociation studies in the QIT of fluorescein, also a xanthene dye. This chromophore can exist in a number of prototropic forms, each of which possesses their own distinct spectral properties. While many solution-phase studies of fluorescein exist, knowledge of its intrinsic (gas-phase) properties have until recently been limited to computational studies. In contrast to its solution-phase behavior, recent experimental results from our laboratory show that fluorescein does not fluoresce to a significant extent when isolated\(^7^4\). The monoanionic, dianionic and cationic forms of gas phase fluorescein are formed via electrospray ionization, mass-selected and stored in the modified QIT-MS. Photodissociation kinetics and power dependence studies on each of the ionic forms of fluorescein are discussed.
1.6 References

31. F.W. Mclaffery, *Sciences*, 1979, **293**(1400), 93.


Chapter 2

Gas-phase Fluorescence Properties of Rhodamine B in a Quadrupole Ion Trap Mass Spectrometer

Significant portions of this chapter will be submitted to the Journal of Photochemistry and Photobiology A - Chemistry. (S. Sagoo and R.A. Jockusch, in preparation.)

Abstract

Studying the photophysical properties of molecules in the gas-phase can be advantageous because it reduces the complexity present in solution that arises from interactions between the molecule of interest and other species present in the local environment, including those with the solvent itself. Here, we report on the intrinsic properties of gaseous protonated rhodamine B (RBH⁺), a well-known xanthene-based dye. Protonated rhodamine B was transferred into the gas phase using electrospray ionization (ESI) and isolated in a quadrupole ion trap (QIT) mass spectrometer which has been modified to enable laser-induced fluorescence spectroscopy of trapped ions. The gas-phase fluorescence emission and excitation spectra of RBH⁺ show maxima (λ_{ex (max)} = 531 nm and λ_{em (max)} = 542 nm, respectively) that lie at higher energy than those of RBH⁺ in solution. The fluorescence lifetime of gaseous RBH⁺ is found to be 5.97 ± 0.12 ns, which is significantly longer than that of solution-phase rhodamine B.

In order to study the zwitterionic form of rhodamine B (RB±) in the gas-phase, it was complexed to metal cations (Li⁺ and K⁺). A blue-shift in the fluorescence emission spectra was observed for the rhodamine B metal ion complexes, similar to that observed for the solution-phase emission spectra of zwitterionic rhodamine B compared to the protonated rhodamine B.

Knowledge of the intrinsic photophysical properties of chromophores, such as those presented here for rhodamine B, will enable a better understanding of how the local environment of the chromophore modulates its properties.
2.1 Introduction

Xanthene-based dyes are one of the most extensively studied families of luminescent dyes. This class of dyes includes fluorescein, eosins, and rhodamines. These dyes are used in a wide range of applications, for example, as biological stains, sensitizers, fluorescent probes, tracing agents, and laser dyes. Xanthene dyes exist in a variety of neutral and ionic forms in solution. Each form usually possesses unique spectral properties and these can be highly dependent on the local environment of the dye. Numerous studies have shown that the quantum yield, absorption and emission spectra, and fluorescence lifetimes for these dyes vary greatly depending on the solvent conditions. Despite extensive studies of many xanthene dyes in solution, their sensitivity to solvent, compounded with concentration and temperature dependencies, have resulted in difficulties in understanding their photophysical properties.

A useful way to better understand the effect of solvent on these dyes is to study their intrinsic properties in the gas-phase. Using mass spectrometry, the various ionic forms of a chromophore can be isolated and studied. This restricts ambiguity arising from the equilibrium that exists for different ionic forms of rhodamine B and eliminates the complexity introduced by intermolecular interactions with the solvent and by comparison with solution-phase studies a better understanding of the specific effects of solvent interactions can be achieved.

Here we report the intrinsic photophysical properties of protonated rhodamine B (RBH\(^+\)), a well-known xanthene-based dye. Rhodamine B is used as the active medium in pulsed and continuous wave lasers, as a staining fluorescent dye in biology, and as a tracer dye to track the movement of water. Rhodamine B was synthesized by Maurice Ceresole in 1887 and in aqueous solution was first studied by Holmes, who determined that the absorption spectra could be explained by a dynamic equilibrium between the two forms of rhodamine B.

Rhodamine B exists in either a protonated, zwitterionic, or a colorless lactone form (Scheme 2.1) with the pK\(_a\) of equilibrium between the zwitterionic and cationic form of rhodamine B being \(\sim 3.2\).
Scheme 2.1: Structure of Rhodamine B (a) cation, RBH$^+$; (b) zwitterion, RB$^\pm$; and (c) lactone RB.

The absorption and emission properties of rhodamine B are highly sensitive to the solvent environment and although numerous studies have been carried out to study rhodamine B in the condensed phase, its photophysics continues to be subjects of significant debate$^{7,9-12,25-27}$. Just like other rhodamine dyes, rhodamine B has a high molar absorptivity ($\varepsilon$) ($\sim$106,000 M$^{-1}$cm$^{-1}$ in ethanol at 543 nm$^{28}$). Rhodamine B has been reported to have a fluorescence quantum yield
(Φf) ranging from 0.3 - 0.52, depending on the solvent\textsuperscript{9-11,29}. As the polarity and viscosity of the solvent mixture increases, the quantum yield of rhodamine B increases\textsuperscript{9-11}. In methanol the absorption and emission maxima for the cationic form of rhodamine B are 552 nm and 577 nm, respectively, whereas in water, there is a small red-shift of the respective maxima to 557 nm and 580 nm\textsuperscript{11,26}. This red-shift has been attributed to a greater stabilization of the highly polarizable excited state with respect to the ground state due to more favorable solvent interactions which leads to a decrease in the S\textsubscript{0}→S\textsubscript{1} energy level gap\textsuperscript{17, 19, 30}. The solvent environment also influences the fluorescence lifetime of cationic rhodamine B. In methanol, the lifetime was determined to be 2.3 ns compared to just 1.6 ns in water\textsuperscript{9}.

Reports on the properties of gas-phase rhodamine B are sparse. The absorption and emission spectra for the neutral form of rhodamine B transferred into the gas phase by heating, were shown to occur at significantly shorter wavelengths (435 nm and 525 nm, respectively) compared with the corresponding bands in solution (540 nm and 575 nm, respectively)\textsuperscript{30}. Very recently, Zenobi and coworkers have studied the neutral form of a different rhodamine, rhodamine 19 (R19), by forming complexes with metal cations (R19 + M\textsuperscript{+}), in the gas-phase\textsuperscript{31}. As expected, a blue shift was observed for the absorption spectra of the complexed neutral form of R19 in the gas-phase.

In this work, we investigate the intrinsic properties of gaseous RBH\textsuperscript{+} using a trapping mass spectrometer which has been modified to enable laser excitation and fluorescence detection of the trapped ions. Fluorescence emission and excitation spectra, as well as the fluorescence lifetime of gaseous cationic rhodamine B (RBH\textsuperscript{+}) are reported and compared with pre-existing theoretical calculated values\textsuperscript{32} as well as literature solution-phase data. By forming rhodamine B metal cation complexes (RB + M\textsuperscript{+}), where M\textsuperscript{+} = Li\textsuperscript{+} or K\textsuperscript{+}, the zwitterionic form of rhodamine B (RB\textsuperscript{±}) was also studied and the data were compared to literature solution-phase data.
2.2 Experimental Methods

Rhodamine B (Scheme 2.1) was supplied by Sigma-Aldrich Canada Ltd. (Oakville, ON) and used without further purification.

Solutions for electrospray ionization were prepared by dissolving the dye in 50:50 methanol/water solutions to a concentration of 0.1 μM. The ESI solutions for complexes of rhodamine B with alkali metal cations were generated by the addition of 100 μM of metal salts (CH₃COOLi or CH₃COOK) to 0.5 μM rhodamine B dissolved in 50:50 methanol/water solutions. Mass spectra were recorded using a modified commercial quadrupole ion trap (QIT) mass spectrometer (Bruker Esquire 3000+, Bruker Daltonik, Germany) equipped with an electrospray ionization (ESI) source. The solution was infused at a flow rate of 2.5 μL/min with a concurrent flow of N₂ nebulizing gas (11-28 psi) through the electrospray emitter. Countercurrent drying gas (also N₂) was fixed at 3.5 L/min at a temperature of 300 °C. In the Bruker ESI source, the ESI emitter is held at ground while the entrance of the capillary inlet to the mass spectrometer was adjusted to −(3000 - 3200) V. The ion accumulation time (t_{acc}) was set to maintain an ion charge control (ICC) value of ~1×10⁶ for all fluorescence experiments. This ICC value corresponds to an estimated ion number of ~5×10⁴ (16). Ions are held in the QIT by an electrodynamic trapping field at trapping parameter, q_z of 0.59, and are subject to (cooling) collisions with ~2.6 × 10⁻³ mbar of helium bath gas. Collision induced dissociation of rhodamine B was also investigated via positive mode ESI ion trap tandem mass spectrometry.

RBH⁺ ions generated by ESI were mass-selected and stored in the QIT where they were irradiated with the frequency-doubled output of a mode-locked Titanium: Sapphire laser (Tsunami, Spectra-Physics, Mountain View, CA pumped by a 10W Millenia Pro; 80 MHz repetition rate, ~130 fs pulse duration). Upon second harmonic generation, visible light in the range of 345 - 540 nm can be generated from this laser source. The laser beam enters and exits through two holes in the ring electrode of the QIT, thus intersecting the cloud of trapped, mass-selected ions. Fluorescence is collected through a third hole in the ring electrode which is orthogonal to the path of the laser beam. The collected fluorescence light is directed through a high-pass filter (Chroma Technology Corp., Rockingham, VT) and focused on the slit of a
spectrograph (Shamrock303i, Andor Technologies, Belfast, Ireland) which disperses the light onto an electron-multiplied charge-coupled device detector (Newton EM-CCD, Andor Technologies, Belfast, Ireland). The EM-CCD was cooled and operated with electron multiplying features activated. More detailed information about this experimental set-up can be found elsewhere.\textsuperscript{16,33}

2.2.1 Gas-phase LIF for protonated rhodamine B (RBH\textsuperscript{+}):

Fluorescence spectra were measured by irradiation of the isolated ion population with laser power (2 - 31.5 mW) over a range of wavelengths from 420 – 532 nm, for 2-20 sec. The number of RBH\textsuperscript{+} ions stored in the trap (ICC) was changed by adjusting the ion accumulation time (t\textsubscript{acc}) from 0 – 220 seconds. Following the irradiation period, the ions were scanned out of the QIT and a mass spectrum was recorded. The laser power and irradiation time used were selected to ensure fragmentation of the precursor ion, which would be visible in the recorded mass spectra, did not occur. Emission spectra were summed at each excitation wavelength. The emission spectra were background-subtracted using spectra recorded under identical conditions but without ions in the trap. The fluorescence emission spectrum of RBH\textsuperscript{+} ions was measured using 500 nm laser excitation with a 515 nm long pass (LP) filter. The spectrum was processed with a 1st-order, 25 point, Savitzky-Golay digital filter and is the red trace shown in Figure 2.2-a. The experimental conditions for all fluorescence measurements are summarized in Table 2.1.

The excitation spectrum was produced by measuring the fluorescence intensity of the RBH\textsuperscript{+} ions irradiated at a series of excitation wavelengths, stepping in 5-10 nm intervals, using a band pass (546 – 610 nm) filter in the detection path. The fluorescence intensity at each excitation wavelength was taken as the area under the curve of the emission spectra from 550 – 610 nm. The excitation spectrum shown is the average of four separate measurements of the fluorescence intensity as a function of excitation wavelength. The error shown corresponds to ± one standard deviation of four replicate measurements.

For lifetime measurements, the (filtered, 515 nm LP filter) fluorescence light was redirected toward an infinity corrected objective (RMS10X, Thorlabs, Newton, NJ) which focused the light
onto a Single-Photon Avalanche Diode (SPAD – PDM Series, Micro Photon Devices, QC). Concurrently, the laser beam exiting the QIT was sent onto a fast photodiode (Thorlabs, Newton, NJ) and the signals from both the SPAD and photodiode detectors were sent to a Time-Correlated Single-Photon Counting (TCSPC) card, model TimeHarp 200 (PicoQuant GmbH, Berlin, Germany) to record the fluorescence decays. A pulse picker was used in the excitation beam path to pick one out of three pulses in a train pulses thus increasing the time between laser pulses from 12.5 ns to 37.5 ns. Fluorescence decays for RBH$^+$ were obtained by irradiating multiple populations of trapped, mass-selected ions, each with 4 mW at 510 nm (515 nm LP filter) for 5 seconds irradiation times, to a total of 1200 seconds. The reported error indicates one standard deviation from three replicate measurements.

2.2.2 Gas-phase LIF for neutral, zwitterionic rhodamine B (RB$^$):

Fluorescence emission spectra for RBH$^+$ and [RB-M]$^+$ were measured by irradiation of the isolated ion population with 2 mW laser power at 490 nm with a 500 nm LP filter, for 2 sec. Following the irradiation period, the ions were scanned out of the QIT and a mass spectrum was recorded. The laser power (2 mW) and irradiation time (2 sec) used were selected to ensure that there was no fragmentation of the precursor ion, which would be visible in the recorded mass spectra. Twenty, 2 sec emission spectra were summed at each excitation wavelength. The emission spectra were background-subtracted using spectra recorded under identical conditions but without ions in the trap. For these set of experiments, the ion accumulation time was set to maintain an ion charge control (ICC) value of ~ 6.5×10$^5$, which corresponds to ~ 32.5 ×10$^4$ ions in the trap.

For all fluorescence measurements, the experimental conditions are summarized in Table 2.1.
Table 2.1: A summary of the experimental parameters for the RBH$^+$ fluorescence experiments.

<table>
<thead>
<tr>
<th>PD Mass Spectra</th>
<th>Emission Spectra</th>
<th>Excitation Spectra</th>
<th>Fluorescence Lifetime Measurement</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICC (counts)</td>
<td>4 x 10^4</td>
<td>1.3 x 10^6</td>
<td>1.3 x 10^6</td>
</tr>
<tr>
<td>$q_z$</td>
<td>0.27</td>
<td>0.59</td>
<td>0.59</td>
</tr>
<tr>
<td>$\lambda_{ex(max)}$ (nm)</td>
<td>510</td>
<td>500</td>
<td>420-532</td>
</tr>
<tr>
<td>Power (mW)</td>
<td>12.5</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>$t_{excitation}$ (s)</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>$t_{total}$ (s)</td>
<td>-</td>
<td>2 x 20</td>
<td>2 x 20</td>
</tr>
<tr>
<td>Filter</td>
<td>-</td>
<td>515 LP</td>
<td>515 LP</td>
</tr>
<tr>
<td>$\Delta P_{He,\text{trap}}$ (mbar)</td>
<td>2.6 x 10^{-3}</td>
<td>2.6 x 10^{-3}</td>
<td>2.6 x 10^{-3}</td>
</tr>
</tbody>
</table>

2.2.3 Gas-phase LIF for cationic rhodamine B (RBH$^+$) compared to cationic rhodamine 575 (R575$^+$):

The relative brightness of these two rhodamine dyes was compared by measuring the fluorescence emission spectra for each under similar experimental conditions, as shown in Table 2.2.

Table 2.2: Experimental conditions to collect fluorescence emission spectra for RBH$^+$ and R575$^+$.

<table>
<thead>
<tr>
<th></th>
<th>RBH$^+$</th>
<th>R575$^+$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solution</td>
<td>0.1 μM in 50:50 MeOH/H$_2$O</td>
<td></td>
</tr>
<tr>
<td>P (mW)</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>ICC</td>
<td>1 x 10^6</td>
<td></td>
</tr>
<tr>
<td>$q_z$</td>
<td>0.59</td>
<td></td>
</tr>
<tr>
<td>$t_{excitation}$ (s)</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>$t_{total}$ (s)</td>
<td>5 x 30</td>
<td></td>
</tr>
<tr>
<td>$\Delta P_{He,\text{trap}}$ (mbar)</td>
<td>2.6 x 10^{-3}</td>
<td></td>
</tr>
<tr>
<td>$\lambda_{ex}$ (nm)</td>
<td>510</td>
<td>470</td>
</tr>
<tr>
<td>Filter</td>
<td>515 LP</td>
<td>480 LP</td>
</tr>
</tbody>
</table>
2.3 Results and Discussion

2.3.1 Photodissociation and Collision-induced Dissociation Mass Spectra

Figure 2.1 compares the photodissociation (PD) mass spectra ($\lambda_{\text{ex (max)}} = 510$ nm; $P = 12.5$ mW; $t_{\text{ex}} = 2$ sec; $q_z = 0.27$) and the collision-induced (CID) mass spectra of the protonated form of rhodamine B (RBH$^+$).

Both forms of dissociation result in the formation of the same fragment ions from the precursor ion, RBH$^+$ ($m/z$ 443). The dissociation mass spectra were recorded at a low $q_z$ (0.27) and with fewer trapped ions (ICC ~ 40, 000) in order to observe most fragments as well as to avoid any space-charge effects. Space charge limits are reached when too many ions are stored in the trap and result in a poor mass resolution.

The main fragment ion formed via PD and CID is at $m/z$ 399 which is due to a loss of $m/z$ 44. This corresponds to a loss of CO$_2$ from the carboxyphenyl group in rhodamine B. When this fragment ion was isolated by tandem mass spectrometry (MS$^3$) and irradiated with the laser ($\lambda_{\text{ex}} = 510$ nm), it did absorb light and photodissociate. This means the fragment ion at $m/z$ 399 contains the complete xanthene ring and has an appreciable absorption cross-section.
Figure 2.1: Dissociation products from protonated rhodamine B (RBH$^+$) in a quadrupole ion trap (QIT) mass spectrometer. Mass spectrum (a) was measured using photodissociation with a visible laser and (b) was measured from collision-induced dissociation in the QIT.
2.3.2 Fluorescence Excitation and Emission Spectra

i) Gas-phase Fluorescence Spectra:

The excitation and emission spectra for cationic rhodamine B are shown in Figure 2.2. For gas-phase RBH⁺, the maximum excitation and emission wavelengths are $\lambda_{\text{ex (max)}} = 531$ nm and $\lambda_{\text{em (max)}} = 542$ nm, respectively. The Stokes shift, the energy difference between the emission and excitation maxima, is 380 cm⁻¹. The emission and excitation spectra have a distinct mirror-image like quality. There is a shoulder, presumably due to a strong vibronic transition between $S_0$ and $S_1$, at ~1400 cm⁻¹ from the maxima in the emission spectrum and this is mirrored by a shoulder present at ~ +1570 cm⁻¹ above the excitation maximum. The small Stokes shift and the mirror-image quality of the fluorescence excitation and emission spectra indicate that the geometry change between the ground and electronically excited states of rhodamine B in the gas-phase is small.

Unexpectedly, we found that gaseous RBH⁺ is approximately two-fold brighter than rhodamine 575 ($\Phi_f = 0.82$, $\varepsilon = 82,000$ M⁻¹cm⁻¹ in ethanol)³⁴, which means that it is brighter than the well-known rhodamine 6G by ~ 50%¹⁵. While part of this difference in brightness may be due to differing absorptivities, the magnitude of the difference ($\text{R575H}^+ : \text{RBH}^+ \sim 1:1.5$) suggests that the quantum yield of gaseous RBH⁺ may be higher than that of gaseous R575H⁺. This would be the reverse of their relative quantum yields in solution, in which rhodamine B has a significantly lower quantum yield (0.3 – 0.52)⁹⁻¹¹,²⁹ than rhodamine 575. If this is the case, the implication is that interactions between RBH⁺ and solvent molecules provide significantly more possible deactivation pathways than those between the monoethylamino rhodamines, such as, R575 and R6G.
Figure 2.2: Fluorescence excitation/absorption (black) and emission (red) spectra for cationic rhodamine B in the gas-phase (RBH$^+$)

### ii) Comparison of Gas-phase Spectra to Solution-phase Fluorescence Spectra:

The absorption and emission maxima of rhodamine B in solution have been found to be $\lambda_{\text{abs (max)}} = 552$ nm and $\lambda_{\text{em (max)}} = 577$ nm in methanol$^{11}$ and $\lambda_{\text{abs (max)}} = 557$ nm and $\lambda_{\text{em (max)}} = 580$ nm in water$^{26}$.

The most obvious difference between the experimental gas-phase data and literature solution phase data is that the solution phase fluorescence absorption and emission maxima are red-shifted compared to the gas-phase fluorescence excitation and emission maxima (by -721 cm$^{-1}$)
and -1119 cm\(^{-1}\), respectively in MeOH solutions and -884 cm\(^{-1}\) and -1209 cm\(^{-1}\), respectively in aqueous solutions). This presumably reflects a larger \(S_0 \rightarrow S_1\) energy gap in the gas-phase than in methanol or water solutions, which is due to the highly polarizable excited state being better stabilized than the ground state due to solvent interactions\(^{17,19,30}\). Similar red shifts in the absorption of rhodamine cations upon solvation have also been reported in previous experimental and computational studies\(^{14-16,27,31,35}\), and by Pappalardo and Ahmed who studied vapour phase neutral RB\(^{\pm}\)\(^{30}\).

Setiawan et al. have examined solvent effects on the spectroscopy of RBH\(^+\) computationally using time-dependent density functional theory (TD-DFT) and several different levels of theory\(^{32}\). For these calculations, the solvent effects are included via a conductor-like polarizable continuum model (CPCM). Calculations at the BLYP/6-311G\(^+\) level of theory found the \(S_0 \rightarrow S_1\) transition to lie at 512 nm in the gas-phase and at 528 nm in water\(^{32}\). The predicted solvent shift (-613 cm\(^{-1}\)) is in the same direction as seen by experiment, but it is somewhat smaller than what is actually observed (-880 cm\(^{-1}\)). While the BLYP density functional was identified as being the most accurate in that report, since it gave an excitation maximum in water (528 nm) that was close to the literature value (557 nm),\(^{26}\) it is interesting to note the other methods used (B3LYP, BH&HLYP, and HF) gave excitation maximum values that were much higher in energy (463 nm – 361 nm) than experimental values, but the predicted solvent shifts were closer to the experimental solvent shift (863 cm\(^{-1}\), 939 cm\(^{-1}\), and 891 cm\(^{-1}\), respectively).

The observed fluorescence Stokes shift for gaseous RBH\(^+\) (382 cm\(^{-1}\)) is approximately half that of RBH\(^+\) solvated in methanol (785 cm\(^{-1}\)), consistent with a smaller change in molecular geometry from \(S_0 \rightarrow S_1\) in the gas phase than in solution. This is not surprising, given that solvent relaxation is expected to occur around the excited RBH\(^+\), which would result in further geometrical changes in the excited solvated dye.

### 2.3.3 Fluorescence Lifetime

The measured fluorescence decay for gas-phase RBH\(^+\), with the corresponding fit by an exponential decay convoluted with a Gaussian instrument response function is shown in Figure 2.3. The fluorescence decay of RBH\(^+\) in the gas-phase is well fit by a single exponential with a
lifetime of $\tau_{\text{gas}} = 5.97 \pm 0.12$ ns. This is significantly longer than that of the dye in methanol (2.3 ns) or water (1.6 ns)\(^9\). Other cationic rhodamine dyes, as well as other fluorophores including fluoranthene and BODIPY-TMR, have also been found to have longer lifetimes in the gas-phase than in solution\(^{36-41}\).

![Fluorescence decay and fitted lifetime for gas-phase cationic rhodamine B (RBH\(^+\)).](image)

**Figure 2.3:** Fluorescence decay and fitted lifetime for gas-phase cationic rhodamine B (RBH\(^+\)).

The increase in the fluorescence lifetime for rhodamine B in vacuum must correspond to a decrease in the radiative ($k_r$) and/or non-radiative ($k_{nr}$) rate constants (Equation 2.1).

$$\tau = \frac{1}{k_r + \Sigma k_{nr}}$$  \hspace{1cm} (2.1)
In solution, the radiative rate decay constants for RBH\(^+\) have been calculated to vary little \((1.7 – 2.3 \times 10^8 \text{ s}^{-1})\), regardless of the type of solvent (i.e. in water or a series of alcohols) \(^7, 9, 11, 42\). On the other hand, the non radiative rate (attributed primarily to internal conversion) is much more sensitive to the solvent, ranging from \(1.7 – 4.6 \times 10^8 \text{ s}^{-1}\) in the same series of solvents \(^9, 11, 42\). The non-radiative rate has been found to decrease steadily with decreasing dielectric constant and with an increasing \(S_1-S_0\) energy gap\(^9\).

The increase in the fluorescence lifetime when going from the condensed phase to vacuum is in part due to the decrease in the radiative \((k_r)\) decay constant because of the change in the index of refraction from solvent to vacuum\(^43\). This effect can be estimated using a simple model (Equation 2.2) which links the gas- and solution-phase radiative rates \((k_r)\) by \(^43-44:\)

\[
k_r(\text{soln}) = n^2 \frac{\varepsilon_{\text{soln}}(\lambda)}{\varepsilon_{\text{gas}}(\lambda)} k_r(\text{gas}) \tag{2.2}
\]

Where \(n\) is the refractive index of the solvent and \(\varepsilon\) is the molar absorptivity in solution or in vacuum at a particular wavelength. Unfortunately, the gas-phase molar absorptivity of trapped ions \((\varepsilon_{\text{gas}})\) cannot be measured in our experimental set-up because of the low ion density.

Assuming that the molar absorptivity of RBH\(^+\) does not change from solution to vacuum, the above model can be simplified to give Equation 2.3:

\[
k_r(\text{gas}) \sim \frac{k_r(\text{soln})}{n^2} \tag{2.3}
\]

The refractive index in solution is higher \((n_{\text{MeOH}} = 1.329)\)\(^45\) than that of vacuum \((n_{\text{gas}} = 1)\), so this simple model suggests that the radiative rate decay constant in the gas-phase should be lower \((\sim 1.3 \times 10^8 \text{ s}^{-1})\) than that in solution phase \((\sim 2.3 \times 10^8 \text{ s}^{-1} \text{ in MeOH})\)\(^9\), which explains in part the longer fluorescence lifetime of RBH\(^+\) in the gas phase.

The increase in the fluorescence lifetime from the condensed phase to vacuum is also due to a decrease in the non-radiative \((k_{nr})\) decay constant (Equation 2.1). The non-radiative rates in
vacuum can be estimated from Equation 2.4, which results from rearrangement of Equation 2.1 followed by substitution by Equation 2.3. Using literature values for \( k_{r\text{(soln)}} \) and \( n_{\text{soln}} \) and the experimental gas-phase lifetime of \( \tau_{\text{gas}} = 5.97 \text{ ns} \), we arrive at a range of values for \( k_{nr\text{(gas)}} \) of \( \sim 0.4 - 0.7 \times 10^8 \text{ s}^{-1} \). These values are 4 – 6 times lower than the reported solution phase radiative rates \((1.7 - 4.6 \times 10^8 \text{ s}^{-1})\) \(^9, 11, 42\) which further explains the longer fluorescence lifetime in vacuum. The substantial decrease in non-radiative rates is consistent with our speculation that the relative brightness of RBH\(^+\) results from a fluorescence quantum yield that is significantly higher in the gas phase than in solution.

\[
\Sigma k_{nr\text{(gas)}} = \frac{1}{\tau_{\text{gas}}} - k_{r\text{(gas)}} = \frac{1}{\tau_{\text{gas}}} - \frac{k_{r\text{(soln)}}}{n_{\text{soln}}^2}
\]  

(2.4)

### 2.3.4 Relative Brightness of Rhodamine B

In order to determine the relative brightness of rhodamine B to other rhodamine dyes (rhodamine 575, rhodamine 590, and rhodamine 6G) \(^15\) that have been studied in our lab, the fluorescence emission spectra of rhodamine B and rhodamine 575 were recorded under similar experimental conditions (Figure 2.4). Previous studies done in our lab have found that the relative brightness in the gas-phase is as follows, R575 (1.00) < R590 (1.15) < R6G (1.29) \(^15\). From Figure 2.4, it is evident that RB is more than twice as bright as R575, therefore the relative brightness of these four dyes is now assigned as R575 (1.00) < R590 (1.15) < R6G (1.29) < RB (~2).
2.3.7 Neutral Rhodamine B (Metal Ion Complexes)

In solution, rhodamine B can exist in either the protonated (acidic) form or the zwitterionic (basic) form, depending on the pH of the solution. The structures of both forms of rhodamine B are shown in Scheme 2.1. Although information can be obtained about the protonated form of rhodamine B (RBH\(^+\)) in the gas-phase, the neutral form of rhodamine B cannot be trapped in a mass spectrometer for examination. However, metal complexed forms which have an overall net charge can be trapped, providing a route to probe the intrinsic properties of the neutral
rhodamine B by complexing it to alkali metal cations ([RB-M]^+). Similar work has been done by Zenobi et al., who studied rhodamine 19 anions, cations, and metal-complexed neutrals \(^{31}\).

The absorption and emission properties of the rhodamine B cation (RBH^+) differ from those of the zwitterion (RB\(^\pm\)) in solution. Reported literature excitation and emission maxima of rhodamine B in acidified methanol solution are \(\lambda_{\text{abs (max)}} = 552\) nm and \(\lambda_{\text{em (max)}} = 577\) nm \(^{11}\). There is a blue shift in the absorption and emission maxima, of 233 cm\(^{-1}\) and 225 cm\(^{-1}\), respectively, for the non-acidified solution of rhodamine B, RB\(^\pm\) (\(\lambda_{\text{abs (max)}} = 545\) nm and \(\lambda_{\text{em (max)}} = 569.6\) nm)\(^{11}\). This shift is attributed to the difference in the protonation state of the carboxylic acid group of the dye (Scheme 2.1). Fleming and Sadkowski found that the radiative rates for both forms of the dye are the same, within experimental error, suggesting that there is little interaction between the xanthene chromophore and the carboxylic acid group \(^{12}\). The Stokes shifts for the cationic and neutral forms of rhodamine B are quite similar in solution (785 cm\(^{-1}\) and 793 cm\(^{-1}\), respectively).

In order to study zwitterionic rhodamine B in the gas-phase, it was complexed to lithium and potassium ([RB-Li]^+ and [RB-K]^+) and the fluorescence emission spectra of the metal-ion complexes were collected and compared to the cationic rhodamine B (RBH^+) under the same experimental conditions (Figure 2.5).

There is a blue shift in the fluorescence emission maxima when going from the gas-phase cationic rhodamine B to the metal-ion complexes with zwitterionic rhodamine B. The shift is in the expected direction based on solution data, that is, the protonated form lies furthest to the red. However, the results show that the effect is not quite as simple as a protonated versus a zwitterionic form of rhodamine B, because as the size of the cation increases, the blue shift increases (280 cm\(^{-1}\) and 710 cm\(^{-1}\) for lithium and potassium, respectively). Calculations are underway to further explore this phenomenon.
Figure 2.5: Fluorescence emission spectra of RBH⁺ (red), [RB-Li]⁺ (blue), and [RB-K]⁺ (black) collected under the same experimental conditions.
2.4 Conclusions

The intrinsic (gas-phase) properties of cationic rhodamine B were determined using a quadrupole ion trap mass spectrometer coupled with laser-induced fluorescence spectroscopy. The measured excitation and emission maxima for RBH⁺ lie at higher energy in the gas-phase than in the solution phase and the fluorescence lifetime for the cationic form of rhodamine B in the gas-phase is much longer than in solution phase.

Some preliminary work on the zwitterionic form of rhodamine B (RB±) in the gas-phase was also conducted by complexing rhodamine B to two metal cations. Complexation of RB± with Li⁺ results in a shift of the emission maximum to a higher energy than that of RBH⁺. The emission maximum of [RB-K]⁺ lies even further to the blue. Further work on this is still required, that is, a wider range of metal cations should be examined. Computational work should also be done in order to determine the metal-ion coordination sites in each of these complexes. Measurement of metal-ion complex excitation spectra will also provide a useful system for testing computational methods to calculate absorption spectra.

These experiments show how the optical properties of fluorophores are affected by the environment they are in and provide a baseline from which to better understand solvent effects.
2.5 References

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Chapter 3

The Optical Activity of Gas-phase Fluorescein Ions Studied Using Photodissociation in a Quadrupole Ion Trap Mass Spectrometer

In this chapter, kinetics and power dependence photodissociation studies for the three prototropic forms of fluorescein are discussed. This project was initially started by Peter McQueen, a previous Master’s student in the Jockusch lab. Peter found that fluorescein does not fluoresce to a significant extent when isolated in the gas-phase; therefore, he carried out photodissociation ‘action spectroscopy’ studies in the quadrupole ion trap to determine the intrinsic properties of the three ionic forms of fluorescein in the gas-phase\(^1,2\).

To support the action spectroscopy studies, I carried out photodissociation kinetic and power dependence experiments on the three isolated charge states of fluorescein. These results were incorporated into a paper, on which I am the second author\(^1\). I present them in greater detail in this thesis chapter.
3.1 Introduction

Fluorescein, like the rhodamine dyes, is a xanthene dye which has been extensively studied. It was first synthesized in 1871 by Adolf von Baeyer\(^3\). Fluorescein is used in a wide range of applications and is one of the most commonly used fluorescent probes in the biosciences\(^4\). Due to its high molar absorptivity, large fluorescence quantum yield, and high photostability, fluorescein is a useful and sensitive fluorescent label\(^5\). Fluorescein was the first fluorescent dye used as a tracer dye in water and is still used for visual qualitative studies for the underground contamination of wells\(^6-7\). Fluorescein dyes have also been used extensively for imaging\(^8\) as well as diagnostic tools\(^9-10\).

The fluorescence properties of fluorescein are highly solvent and pH dependent and this is why its behavior in solution is very complicated\(^3-5, 11-13\). This chromophore can exist in up to seven different forms (Scheme 3.1), each of which possesses their own unique spectral properties. Although fluorescein’s sensitivity to its environment can be exploited with chemical and biological sensors, its sensitivity complicates the interpretation of results obtained for many biological investigations. The fluorescein dianion, monoanion, and cation have fluorescence absorption maxima of 490 nm, 470 nm, and 435 nm respectively in water\(^5\). The fluorescein dianion has the highest fluorescence quantum yield of about 0.93 where as the anion and cation have much lower fluorescence quantum yields of 0.30 and 0.18, respectively\(^5\). All three prototropic forms of fluorescein, the dianion, monoanion, and cation, have high molar absorptivities of 76 900 M\(^{-1}\)cm\(^{-1}\), 29 000 M\(^{-1}\)cm\(^{-1}\), and 53 000 M\(^{-1}\)cm\(^{-1}\), respectively, at their respective maximum absorption wavelengths\(^5\).

Although fluorescein is highly fluorescent in solution, it has recently been found to not fluoresce to a significant extent in the gas-phase, indicating that other pathways are more favourable for the de-excitation of these ions\(^1\). Studies done in our lab on fluorescein show that the fluorescein dianion, which has the highest quantum yield in solution, does not fluoresce significantly in the gas-phase because it undergoes electron photo detachment (ePD)\(^1\), while the gas-phase fluorescein monoanion does show a weak fluorescence signal\(^14\). The fluorescein cation also does not fluoresce in the gas-phase. This is not surprising considering it has a relatively low quantum
yield in solution and it is believed to fluoresce in solution through deprotonation in the excited state, resulting in the formation of the fluorescent excited monoanionic species. 

**Scheme 3.1:** Chemical structure of seven pH-dependent prototropic forms of fluorescein.
Currently, the intrinsic properties of the fluorescein monoanion are being investigated in detail as well\textsuperscript{14}. Photodissociation action spectra were measured and compared to solution phase absorption spectra\textsuperscript{1}. The excitation maxima for the fluorescein cation, monoanion, and dianion were found (by Peter) to be at 430 nm, 520 nm, and 500 nm, respectively\textsuperscript{1}. A recently measured full action spectrum of the fluorescein cation shows that the actual excitation maximum is at 425 nm.

Here, the optical properties of gas-phase fluorescein ions were explored using a quadrupole ion trap coupled with a tuneable laser source. The photodissociation kinetics and power dependence of the gas-phase fluorescein ions (dianion, monoanion, and cation) were investigated. These initial experiments provide a better understanding of the properties of each of the fluorescein ions, which will aid in interpretation of future studies using step-wise hydration to regain fluorescence in gas-phase fluorescein. We aim to not only have a better understanding of the intrinsic properties of fluorophores in varying micro-environments but also of how to manipulate fluorescence through non-covalent interactions.

### 3.2 Experimental Methods

Fluorescein was obtained from Sigma-Aldrich (Oakville, ON, Canada) and was used without further purification. Solutions for electrospray ionization were prepared by dissolving fluorescein in methanol/water solutions to a concentration of 1.5 μM for the cation and monoanion, and 2 μM for the dianion. To form the cation and monoanion, a 70:30 methanol/water solvent was used while the fluorescein dianion was obtained from a 30:70 methanol/water solution. Some ammonium hydroxide was added to these dianion electrospray solutions to improve the stability of the doubly negative charged ion.

Mass spectra were recorded using a modified commercial quadrupole ion trap (QIT) mass spectrometer (Bruker Esquire 3000+, Bruker Daltonik, Germany) equipped with an electrospray ionization (ESI) source\textsuperscript{15}. The solution was infused at a flow rate of 2.5 μL/min with a concurrent flow of N\textsubscript{2} nebulizing gas (15 - 28 psi) through the electrospray emitter. Counter-current drying gas (also N\textsubscript{2}) flow rates ranged from 2 - 5 L/min at a temperature of 300 °C. In the Bruker ESI
source, the ESI emitter is held at ground while the entrance of the capillary inlet to the mass spectrometer was adjusted to ± (2500 - 4000) V. The ion accumulation time was set to maintain an ion charge control (ICC) value of 40,000 ± 5,000 for all photodissociation experiments.

Trapped fluorescein ions generated by ESI were mass-selected and stored in the QIT where they were irradiated with the frequency-doubled output of a mode-locked Titanium: Sapphire laser (Tsunami, Spectra-Physics, Mountain View, CA pumped by a 10W Millenia Pro, 80 MHz repetition rate, ~130 fs pulse duration). Upon second harmonic generation, visible light in the range of 345 - 540 nm can be generated from this laser source. The laser beam enters and exits through two holes in the ring electrode of the QIT, thus intersecting the cloud of trapped, mass-selected ions. The laser power is adjusted using a neutral density filter and monitored with a Solo 2 power meter (Gentec Electro-Optics Inc., Quebec City, QC) equipped with a XLP12-1S-H2 detector (power-noise level ±0.5μW). More detailed information about this experimental set-up can be found elsewhere\textsuperscript{15-16}.

Reaction kinetics and power dependence photodissociation curves for each of the fluorescein ions were produced by measuring photodissociation mass spectra of fluorescein irradiated at a series of irradiation times and laser powers, respectively. The excitation wavelength used for each charge state is near the maximum determined from the action spectroscopy\textsuperscript{1}.

Gas-phase fluorescein ions, of the desired charge state, were accumulated, isolated, and stored in the QIT at an ICC of ~ 40,000. Following a cooling delay of at least 50 ms, the isolated ions were irradiated for at least 50 ms with the laser at a specific power. Table 3.1 summarizes the cooling delay period ($t_{\text{cooling}}$), irradiation time ($t_{\text{irradiation}}$), $q_z$, excitation wavelength ($\lambda_{\text{ex}}$), helium pressure ($P_{\text{He,ex}}$), and laser power used for the photodissociation studies of each prototropic form of fluorescein.

Photodissociation mass spectra of the isolated fluorescein ions were collected for ~ 2 minutes with the laser on. Control mass spectra were recorded under identical experimental conditions, except the shutter is kept closed (laser off), for ~ 1 minute before and after the photodissociation mass spectra were obtained. For each measurement these collection times correspond to a total of ~ 50 – 100 photodissociation mass spectra for each fluorescein charge state. The precursor ion yield at each irradiation time / laser power was calculated by dividing the average precursor ion
intensity \((I_p)\) during photodissociation (laser on) with the average precursor ion intensity without photo-excitation (laser off), (Equation 3.1).

\[
\text{Precursor ion Yield} = \frac{I_p(\text{laser on})}{I_p(\text{laser off})}
\]

(3.1)

**Table 3.1:** Experimental conditions for photodissociation and collision-induced mass spectra, power dependence, and kinetics, for the three charge states of gas-phase fluorescein.

<table>
<thead>
<tr>
<th>Photodissociation Mass Spectra</th>
<th>ICC (Counts)</th>
<th>tc (ms)</th>
<th>tirrad (ms)</th>
<th>Power (mW)</th>
<th>qz</th>
<th>λex (nm)</th>
<th>PHe,ex (mbar)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cation</td>
<td>40,000</td>
<td>700</td>
<td>20</td>
<td>0.18</td>
<td>430</td>
<td>1.5 × 10⁻⁶</td>
<td></td>
</tr>
<tr>
<td>Monoanion</td>
<td>40,000</td>
<td>500</td>
<td>2</td>
<td>0.18</td>
<td>520</td>
<td>1.5 × 10⁻⁶</td>
<td></td>
</tr>
<tr>
<td>Dianion</td>
<td>40,000</td>
<td>100</td>
<td>0.3</td>
<td>0.18</td>
<td>500</td>
<td>1.2 × 10⁻⁶</td>
<td></td>
</tr>
</tbody>
</table>

**Power Dependence**

| Cation                        | 40,000      | 50      | 50         | 0-42.5     | 0.27| 430| 1.5 × 10⁻⁶ |
| Monoanion                     | 40,000      | 50      | 50         | 0-12       | 0.27| 520| 1.5 × 10⁻⁶ |
| Dianion                       | 40,000      | 50      | 0.3        | 0.27       | 500| 1.2 × 10⁻⁶ |

**Kinetics**

| Cation                        | 40,000      | 50      | 0-1000     | 20         | 0.27| 430| 1.5 × 10⁻⁶ |
| Monoanion                     | 40,000      | 50      | 0-500      | 2          | 0.27| 520| 1.5 × 10⁻⁶ |
| Dianion                       | 40,000      | 50      | 0-300      | 0.3        | 0.27| 500| 1.2 × 10⁻⁶ |

### 3.3 Results and Discussion

#### 3.3.1 Photodissociation and Collision-induced Dissociation Mass Spectra

Figure 3.1 compares photodissociation (PD) and collision-induced dissociation (CID) mass spectra of the fluorescein cation ([F + H]⁺), fluorescein monoanion ([F - H]⁻), and fluorescein dianion ([F – 2H]⁻²). The photodissociation mass spectra were obtained using the QIT under the experimental conditions listed in Table 3.1. The ICC for all experiments was kept low (~ 40,000) to maximize mass resolution. The \(q_z\) was also kept low (0.18) so that any low mass fragment ions that are formed can be detected.
The fluorescein cation ($m/z$ 333) has the most complex mass spectra with a range of fragment ions forming in the mass range $m/z$ 325 – 175. The PD mass spectrum (Figure 3.1-a) and the CID mass spectrum (Figure 3.1-d) show similar fragment ions from the precursor ion. The main fragment ion formed at $m/z$ 287 corresponds to the loss of 46 Da which is probably due to the loss of formic acid ($\text{CO}_2\text{H}_2$) from the benzoic acid moiety. The other low mass fragment ions formed indicate the dissociation of the xanthene moiety of this chromophore.

The PD (Figure 3.1-b) and CID (Figure 3.1-e) mass spectra for the fluorescein monoanion ($m/z$ 331) are much less complex than those for the fluorescein cation. The same fragment ions ($m/z$ 287 and 286) are formed with similar intensities by PD and CID. The fragment ion at $m/z$ 287 is formed due to the loss of carbon dioxide (-44 Da), most likely from the benzoic acid group.

The fluorescein dianion ($m/z$ 165) PD mass spectrum (Figure 3.1-c) shows that the main fragment ion is a radical monoanion ($m/z$ 330) formed due to the loss of an electron from the dianion (Equation 3.2).

$$[F - 2H]^{-2} + h\nu \rightarrow [F - 2H]^- + e^-$$  \hspace{1cm} (3.2)

Because the dianion is multiply charged and rather small, it isn’t surprising that it readily loses an electron. Due to high inter-electron repulsions, multiply charged anions are known to be much more fragile and more susceptible to electron loss and fragmentation in the gas-phase than in the condensed phase\textsuperscript{17}.

The CID mass spectrum (Figure 3.1-f) for the dianion was measured in a Fourier transform ion cyclotron resonance mass spectrometer (FT-ICR-MS) where the isolated precursor ion fragmented by sustained off-resonance irradiation, collisionally activated dissociation\textsuperscript{1}. Unlike the PD mass spectrum, there is no fragment ion formed at $m/z$ 330 which shows that the dianion only loses an electron via activation by the visible laser light. The only fragment ion formed by CID for the fluorescein dianion is at $m/z$ 285. This is likely due to the loss of carbon dioxide from the benzoic acid moiety. This fragment ion is also observed, at a very low intensity, in all the PD mass spectra of the dianion.
Figure 3.1: Photodissociation (a-c) and collision induced/activated dissociation (d-f) mass spectra of the fluorescein cation \([F+H]^+\), monoanion \([F-H]^-\), and dianion \([F-2H]^2-\) measured using the QIT (all photodissociation mass spectra and CID spectra of the cation and monoanion) and a FT-ICR-MS for the fluorescein dianion CID spectra.
3.3.2 Photodissociation Kinetics Studies

The dissociation kinetics of all three fluorescein ions were studied by measuring the respective precursor ion intensity (Equation 3.1) as a function of the irradiation time under the experimental conditions listed in Table 3.1.

The fluorescein cation kinetics plot (Figure 3.2-a) follows a first order decay, that is, the data fits a single exponential decay (Equation 3.3). This suggests that only a single structural conformation of the fluorescein cation exists in the gas-phase.

\[ [F^+] = [F^+]_0 \cdot e^{-kt}, R^2 = 0.9972 \quad (3.3) \]

The kinetics plot for the fluorescein monoanion (Figure 3.2-b) is best fit as a sum of two exponentials (Equation 3.4), which is consistent with the existence of two non-interconverting conformers of the monoanion, \( F_1^- \) and \( F_2^- \), which have different dissociation rate constants (\( k_{d1} \) and \( k_{d2} \)). The relative amplitudes of \( F_1^- \) and \( F_2^- \) are 62:37, suggesting conformers existing in a ~ 62:37 ratio with ~ 1% of the isolated population of \( m/z \) 331 which does not dissociate which may mean that a contaminant of mass 331 is present that does not dissociate under the experimental conditions set for the quadrupole ion trap.

\[ [F^-] = ([F_1^-]_0 \cdot e^{-k_{d1}t}) + ([F_2^-]_0 \cdot e^{-k_{d2}t}) + y_0, R^2 = 0.9995 \quad (3.4) \]

The fluorescein dianion kinetics plot (Figure 3.2-c) is similar to the fluorescein cation, in that it also follows a first order decay (Equation 3.5) consistent with the existence of only a single form of the fluorescein dianion in the gas phase.

\[ [F^{-2}] = [F^{-2}]_0 \cdot e^{-kt}, R^2 = 0.9989 \quad (3.5) \]
Figure 3.2: Photodissociation kinetic plots of gas-phase fluorescein a) cation, b) monoanion, and c) dianion. The ln(precursor ion intensity) as a function of the irradiation time (ms) plots are shown on the left and the corresponding precursor ion intensity as a function of the irradiation time (ms) plots are shown on the right.
3.3.3 Photodissociation Power Dependence Studies

The photodissociation of all three fluorescein ions were also studied by measuring the precursor ion intensity (Equation 3.1) as a function of the laser power under the experimental conditions listed in Table 3.1. The results are shown in Figure 3.3. These experiments show that the most stable ionic form of fluorescein is the cation, since dissociation of [F+H]^+ is complete at 42 mW; followed by the monoanion ([F-H]^-) which is dissociated by 12 mW; and the dianion is very fragile, requiring less than 2 mW to dissociate.

The photodissociation of [F+H]^+ (Figure 3.3-a) shows a higher order decay that is non-linear with power. A third order polynomial fit (Equation 3.6) is shown on the left hand side of Figure 3.3-a. The right hand side of Figure 3.3-a shows the corresponding plot of [F+H]^+ intensity directly as a function of the laser power, to emphasize the initial induction period during which ions are heated to sufficient levels for photodissociation to become apparent, that is, a multiple photon process.

\[
\ln[F^+] = -(\sigma_1 P^2 + \sigma_2 P^3), \ R^2 = 0.9961 
\]  \hspace{1cm} (3.6)

Dissociation of the fluorescein monoanion ([F-H]^-) also shows a complicated dependence on laser power (Figure 3.3-b). The data can be fit by a sum of two exponentials (representing the two populations), where each have a second order dependence on power (Equation 3.7). Fitting the data with Equation 3.7, results in a ratio of [F_1^-]:[F_2^-] = 33:62 ratio, which is similar to that found from the kinetics experiments (Figure 3.3-b) and consistent with the existence of two non-interconverting populations. The existence of a complex multiple photon process is suggested by the non-zero coefficients of the second order term for the power, that is, the \(\sigma_n\) term in Equation 3.7. Approximately 5% of the isolated population of m/z 331 does not dissociate which suggests that a contaminant of mass 331 is present that does not dissociate under the experimental conditions set for the quadrupole ion trap.

\[
[F^-] = ([F_1^-]_0 * e^{-(\sigma_1 P + \sigma_2 P^2)}) + ([F_2^-]_0 * e^{-(\sigma_3 P + \sigma_4 P^2)}) + y_0, \ R^2 = 0.9990 
\]  \hspace{1cm} (3.7)
Figure 3.3: Photodissociation power dependence plots of gas-phase fluorescein (a) cation, (b) monoanion, and (c) dianion. The $\ln$ (precursor ion intensity) as a function of the laser power (mW) plots are shown on the left and the corresponding precursor ion intensity as a function of the laser power (mW) plots are shown on the right.
Electron photon detachment of the fluorescein dianion ([F-2H]^-2) is linear with laser power with zero-intercept (Figure 3.3-c), which indicates that it is a simple single photon process, that is, removal of an electron results from the absorption of a single photon (Equation 3.8).

\[ \ln[F^-2] = -\sigma P \quad , R^2 = 0.9998 \]  (3.8)
3.4 Conclusions

The optical activity of the three prototropic forms of fluorescein in the gas-phase were determined via photodissociation experiments using a quadrupole ion trap mass spectrometer coupled with a tunable Ti:Sapphire laser source. This information provides a better understanding of the effects of the solvent on the photophysical properties of the different ionic forms of fluorescein.

The photodissociation kinetics and power dependence studies for the fluorescein cation indicate that it undergoes multiple photon dissociation from a single population. The fluorescein monoanion photodissociation results were more complicated and the kinetics and power dependence studies suggest it is a multiple photon dissociation process and two conformers of the monoanion which have different dissociation rate constants exist in ~ 60:40 ratio. Electron photo-detachment from the dianion shows first order kinetics and is linear with power with a zero intercept, thus indicating a single photon process for a single population.

Although fluorescein is highly fluorescent in solution, it has recently been found to surprisingly not significantly fluoresce in the gas-phase. Future work on this system will involve determining how many non-covalent interactions are required to restore fluorescence to gaseous fluorescein. The trapping mass spectrometer used for these experiments is advantageous as it will allow the desired fluorescein clusters with a known number of solvent molecules to be mass isolated and stored, thus making it possible to perform spectroscopic studies on trapped clusters of known composition. In addition to experimental studies, electronic structure theory calculations will be needed to examine the role that water molecules play in the stabilization of fluorescein as well to determine the modes of water molecule binding.

These experiments aim to not only have a better understanding of the intrinsic properties of fluorophores in varying micro-environments but also of how to manipulate fluorescence through non-covalent interactions.
3.5 References


2. P.D. McQueen, MSc Thesis, University of Toronto, 2009.


Appendix I: Fluorescence Accumulation Time and ICC Dependence

Figure I.1: Fluorescence intensity of cationic rhodamine B (RBH⁺) as a function of (a) the accumulation time and (b) the corresponding ion-charge control value.
The ion accumulation time (\(t_{\text{acc}}\)) is adjusted in order to obtain the desired ion population which is measured by the ion charge control (ICC) under the experimental conditions listed in Table I.1. Figure I.1 shows the dependence of the fluorescence intensity on the accumulation time, which was adjusted from 2 – 220 seconds, as well as the corresponding ICC.

**Table I.1:** A summary of the experimental parameters for the fluorescence accumulation time and ICC dependence experiments.

<table>
<thead>
<tr>
<th></th>
<th>Fluorescence Accumulation Time and ICC Dependence</th>
</tr>
</thead>
<tbody>
<tr>
<td>(ICC) (counts)</td>
<td>(1.2 \times 10^6)</td>
</tr>
<tr>
<td>(q_z)</td>
<td>0.59</td>
</tr>
<tr>
<td>(\lambda_{\text{ex (max)}}) (nm)</td>
<td>490</td>
</tr>
<tr>
<td>Power (mW)</td>
<td>4</td>
</tr>
<tr>
<td>(t_{\text{excitation}}) (s)</td>
<td>3</td>
</tr>
<tr>
<td>(t_{\text{total}}) (s)</td>
<td>(3 \times 20)</td>
</tr>
<tr>
<td>Filter</td>
<td>480 LP</td>
</tr>
<tr>
<td>(\Delta P_{\text{He, trap}}) (mbar)</td>
<td>(2.6 \times 10^{-3})</td>
</tr>
</tbody>
</table>

As seen in Figure I.1-a, the measured fluorescence signal increases linearly with accumulation time until \(t_{\text{acc}} \sim 25\) ms but after this, the fluorescence signal still increases linearly with accumulation time but at a slower rate. This increase in fluorescence signal with accumulation time corresponds to an increase in the number of ions, which fluoresce, trapped in the QIT. Figure I.1-a clearly shows that the ion density increases linearly with accumulation time up to a certain point. As the number of ions trapped in the QIT is increased, the ion cloud expands due to larger space-charge effects\(^1\).

The decrease in the amount of fluorescence collected with respect to the accumulation time, after 25 ms, is either due to a decrease in the efficiency of being able to accumulate ions in the trap when the number of trapped ions is high, or due to a decrease in the overlap between the laser excitation beam and the expanding ion cloud\(^2\).
The measured fluorescence signal was also plotted as a function of the ICC value, as shown in Figure I.1-b. The ICC is calculated from the product of accumulation time with the total ion current (TIC) signal from the mass spectrometer (Equation I.1).

\[
ICC = t_{acc} \times TIC
\]  

(I.1)

This value provides an estimation of the number of ions trapped in the QIT. In Figure I.1-b as the ICC value increases, the fluorescence signal increases linearly ($R^2 = 0.99318$) until the ICC value is ~ 1 million which corresponds to an accumulation time of 45 ms. At ion accumulation times greater than 45 ms, the fluorescence intensity of trapped ions continues to increase but the ICC initially increases at a slower rate and then levels off. This occurs due to the saturation of the ion detector thus making the mass spectrum an unreliable way of determining the number of trapped ions in the QIT.

The non-linearity of fluorescence signal with accumulation time in Figure I.1-b occurs at an accumulation time that is slightly longer (45 ms) than that observed from the accumulation time alone (25 ms, Figure I.1-a) which suggests that with time, ions can no longer be trapped in the QIT efficiently. Similar results have been observed for other rhodamine dyes by our group.

Appendix II: Fluorescence Power Dependence

**Figure II.1:** (a) Fluorescence intensity of RBH\(^+\), integrated emission spectra between 520 – 620 nm, as a function of the excitation power at two buffer gas pressures (blue = \(2.1 \times 10^{-3}\) mbar and green = \(1.4 \times 10^{-3}\) mbar); (b) Emission spectra at ten different laser powers with \(\Delta P_{\text{He,trap}} = 2.1 \times 10^{-3}\) mbar.
Figure II.1-a shows the dependence of the fluorescence intensity on the laser power at two different helium pressures ($\Delta P_{\text{He,trap}} = 2.1 \times 10^{-3} \text{ mbar}$ and $1.4 \times 10^{-3} \text{ mbar}$) measured under the experimental conditions shown in Table II.1.

**Table II.1:** A summary of the experimental parameters for the fluorescence power dependence.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICC (counts)</td>
<td>$1.2 \times 10^6$</td>
</tr>
<tr>
<td>$q_z$</td>
<td>0.59</td>
</tr>
<tr>
<td>$\lambda_{\text{ex (max)}}$ (nm)</td>
<td>510</td>
</tr>
<tr>
<td>Power (mW)</td>
<td>2 – 31.5</td>
</tr>
<tr>
<td>$t_{\text{excitation}}$ (s)</td>
<td>20</td>
</tr>
<tr>
<td>$t_{total}$ (s)</td>
<td>$20 \times 5$</td>
</tr>
<tr>
<td>Filter</td>
<td>515 LP</td>
</tr>
<tr>
<td>$\Delta P_{\text{He,trap}}$ (mbar)</td>
<td>$2.1 \times 10^{-3}$, $1.4 \times 10^{-3}$</td>
</tr>
</tbody>
</table>

At low powers, the fluorescence intensity increases linearly with the laser power, until ~ 12.5 mW. At powers greater than this, the fluorescence signal decreases. The decrease of fluorescence intensity at higher powers is due to increased photodissociation of the RBH$^+$ precursor ion and this is observed in the mass spectra, that are simultaneously being recorded, which show the appearance of product ions from the precursor ion. As the pressure is decreased, there is an overall decrease in fluorescence intensity at higher powers only and the photodissociation starts at a lower power, thus shifting the maximum fluorescence intensity to a lower power (15 mW to 12.5 mW). Work done in our group$^{1,2}$ as well as by Sassin and co-workers, who were studying the fluorescence of rhodamine 575 cations in a QIT$^3$ have shown similar results.

As the helium pressure is decreased, the amount of photodissociation increases which therefore leads to a decrease in the fluorescence intensity from the trapped ions. The effect of an increased pressure basically provides better collisional cooling of the excited trapped ions’ and this reduces the rate of photodissociation thus preventing the population of fluorescent precursor ions from being decreased$^1$. 

Figure II.1-b shows the fluorescence emission spectra of RBH$^+$ collected at $\lambda_{\text{ex (max)}} = 510$ nm with powers ranging from 2–31.5 mW. When going to powers greater than 20.5 mW, the emission spectra change (get broader) and the emission maxima show a slight red shift. This suggests that either the fragment ions produced also fluoresce, and produce their own distinctive emission spectra, or there is continuous heating of the fluorescing ion population$^4$.