Cavity Ring-Down Spectroscopy of Liquid Samples Using Standard Cuvettes at Normal Incidence

Bryan James Culbertson

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CAVITY RING-DOWN SPECTROSCOPY OF LIQUID SAMPLES USING
STANDARD CUVETTES AT NORMAL INCIDENCE

By

Bryan James Culbertson

A Dissertation
Submitted to the Faculty of
Mississippi State University
in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy
in Chemistry
in the Department of Chemistry

Mississippi State, Mississippi

May 2012
CAVITY RING-DOWN SPECTROSCOPY OF LIQUID SAMPLES USING STANDARD CUVEETTES AT NORMAL INCIDENCE

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Cavity ring-down (CRD) spectroscopy has emerged as a sensitive analytical technique. In this method, a laser pulse is injected through one of two highly-reflective mirrors which form a stable optical cavity and the rate that the light leaves the cavity is monitored by a detector placed behind the second mirror.

In this research a CRD spectrometer has been designed and constructed. The light exiting the cavity is collected via a fiber optic cable which is then directed toward a photo multiplier tube (PMT) detector. The signal is digitized and averaged by an oscilloscope and the data are transferred by an IEEE 488 interface to a personal computer where the data are analyzed. Instrument command and data acquisition are controlled by a Visual Basic computer program. A short review of several attempts to measure liquid samples using CRD spectroscopy is presented; most discuss the necessity for the incorporation of Brewster’s angle at the liquid interface. This study integrates a 1 cm standard quartz cuvette at normal incidence. It was determined that there are significant losses from scattering and reflection; however, these losses were not so large as to negate the efficacy
of the technique. The hypothesis tested here is that the light “lost” as reflections are collected by the cavity mirrors and redirected back into the cavity.

Rhodamine 6G was used as the primary model absorber in these studies. Absorbance measurements were extracted from the measured ring-down times and a detection limit was obtained. Four cavity lengths were constructed to determine the effect on the scattering losses with varying cavity lengths. The calculated detection limit for the CRD spectrometer used in this study was found to be in the range of 4-5 nM. It was found that the detection limit of the CRD spectrometer was 36 times lower than that of the commercial instrument. Aligning the cavity mirrors at longer cavity lengths proved to be more difficult; however, there were no significant additional losses observed by incorporating longer cavities.
DEDICATION

I would like to dedicate this research to my loving wife Jodi and to my parents, Bob and Susan Culbertson.
ACKNOWLEDGEMENTS

I would like to express my sincerest thanks to my major advisor Dr. Stephen C. Foster for his support and guidance throughout this research.

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CHAPTER I
INTRODUCTION

Since the inception of the scientific instrument, there has been a drive in the scientific community to improve the performance of this essential equipment. Many of the great discoveries of our time would not have been possible without the ability to perform sensitive measurements. The aim of the present work is to apply a relatively new technique to the detection of trace species in liquid samples all while being able to apply this technique to a plethora of samples and matrix environments.

The work described in this thesis represents an attempt to improve the sensitivity of UV-visible spectroscopy for liquid samples. In particular, the utility of cavity ring-down spectroscopy which uses standard 1-cm cuvettes is explored. Cavity ring-down spectroscopy (CRDS) is a highly sensitive spectroscopic technique in which extremely long path lengths are used in order to increase the sensitivity as compared to traditional spectroscopic methods. This technique uses a set of highly reflective mirrors to form an optical cavity. Numerous cavity designs have been used: traditional linear cavities\(^1,2,3,4,5\) evanescent wave cavities\(^6,7\) and fiber rings\(^8,9,10\) In each case, a light pulse is injected into the cavity and that pulse reflects back and forth between the mirrors, making many round trips through the cavity. Each time the pulse is reflected from a mirror a small portion leaks through the mirror and the intensity of the pulse in the cavity gradually falls. Using highly reflective mirrors it is possible to obtain effective path lengths of several hundred
meters in some cases. Spectra of species within the cavity are recorded by monitoring the lifetime of the pulse within the cavity. When an absorber is present, the trapped pulse loses intensity at a greater rate than it would in an empty cavity. Thus, the cavity ring-down spectrum is typically a plot of pulse lifetime versus wavelength.

There have been many uses of CRDS, most of which have been concentrated on detecting gas-phase absorbers. Gas-phase spectra are relatively easy to record. It simply requires introduction of the gas under study into the cavity. Liquid-phase studies are much less common. However, there have been several attempts to apply CRDS to the liquid phase, but such studies face additional problems. In theory the cavity could be filled with the analyte solution, but this would place liquids in direct contact with the cavity mirrors and lead to potential contamination problems. Furthermore, a filled cavity requires a large sample size which is often not available in many standard analytical applications. If a cell is introduced in the cavity to contain a liquid sample, then one must worry about cavity losses which arise because of reflection and scattering from the cell surface. Typical air/quartz reflections losses are of the order of 4% per surface and these losses could overwhelm losses from analyte absorption.

The work in this thesis describes the development of a CRDS spectrometer suitable for liquid samples confined in a 1 cm quartz cuvette. Reflection losses are minimized by placing the cuvette at normal incidence to the optical cavity. The effect of cavity length upon the method is explored and minimum detectable absorbance is calculated.
Cavity ring-down methods have been used for many years to measure the quality of highly reflective mirrors. The method was developed with the advent of dielectric coatings to accurately monitor the reflectivity of mirror coatings when they approached \( R = 1.0 \). The first use of the technique to measure spectra was reported by O’Keefe and Deacon in 1988. These two authors made the important discovery that the introduction of an absorber into the cavity would shorten the lifetime of the pulse injected into such a cavity. The weak forbidden \( b^1\Sigma_g^+ \rightarrow X^3\Sigma_g^+ \) rovibronic spectrum of gaseous molecular oxygen was recorded by monitoring the lifetime of a dye-laser pulse in a 1 m cavity.

Most of the subsequent CRDS studies have employed pulses with nanosecond durations. Shorter pulses can be used, but offer no significant advantages. Longer pulses make it difficult to record small changes in cavity lifetime. In most cases, a pulsed laser is used to generate the pulse, but there have been numerous studies involving a continuous wave (CW) laser source. If a CW laser is used it must be rapidly switched on and off to produce a short duration pulse. This is typically achieved with fast optical switches or by driving the laser out of resonance with the cavity.

As mentioned above, several cavity configurations can be envisioned, but the majority of the studies employ a pair of highly reflective (\( R > 0.999 \)) mirrors. The detector used in a CRDS system must have sufficient speed to record changes on a nanosecond or sub-nanosecond timescale. In a typical linear cavity formed between a pair of mirrors, the laser pulse is directed into the cavity through one mirror. In this configuration most of the pulse (99.9%) is reflected backward and does not enter the cavity.
cavity, but a small fraction enters and is then trapped within the cavity. As the pulse travels back and forth between the mirrors, a small portion of its intensity is lost by transmission at each mirror surface. The detector is placed behind the second mirror in order to monitor the light that leaks out of the cavity. The resultant signal is in the form of an exponential decay. The time taken for the signal to fall in intensity from its initial value ($I_0$) to its 1/e value ($I_0/e$) is called the ring-down time ($\tau$) and is indicative of what is present in the optical cavity.

CRDS is an exceptionally sensitive technique due to several factors. First, the multi-pass nature of the instrument allows for extremely long effective path lengths. Pulsed lasers, in general, are prone to large intensity fluctuations between each shot. However, in CRDS, it is not the relative intensity of the light source that is being measured, it is the rate of escape of light from the optical cavity. The initial intensity of the pulsed laser is unimportant as the rate of escape of light from the cavity for two sequential laser pulses of the same sample should be equal.

**The Beer-Lambert Law**

The Beer-Lambert law states that the absorption of light is directly proportional to the concentration of the light-absorbing species present in the sample.\textsuperscript{18} Equation 1.1 is the Beer-Lambert law where $A$ is the absorbance, $\varepsilon$ is the molar absorptivity (in M\textsuperscript{-1}cm\textsuperscript{-1}), $l$ is the path length (in cm), and $c$ is the concentration (in M).

$$A = \varepsilon l C$$ \hspace{1cm} \text{Equation 1.1}

In a traditional UV-vis spectrophotometer, the absorbance is defined as

$$A = -\log\left(\frac{1}{I_0}\right) = -\log T$$ \hspace{1cm} \text{Equation 1.2}
where $I_0$ is the incident intensity, $I$ is the transmitted intensity, and $T$ is the transmittance.\textsuperscript{18} The major limitation of traditional UV-vis spectroscopy is that a small absorbance change is being measured against a large background signal which provides little sensitivity as compared to other analytical methods.

**The Beer-Lambert Law in CRDS**

In traditional absorption spectrometry, a quantity of light is being measured. In CRDS it is not the quantity of light being measured, but the rate that light escapes from the cavity. The analysis of the data in CRDS is in the form of the Beer-Lambert Law except that it is transformed in order to account for the absorption of light by the species of interest for each pass. The Beer-Lambert law can also be written as:

$$I = I_0 e^{-\alpha d}$$  \hspace{2cm} \text{Equation 1.3}

where $\alpha$ differs from the product of the molar absorptivity and the concentration by a factor of 2.303 to account for the change from the log to the $e^x$ scale. In CRDS, multiple passes are occurring. The majority of the incident light is reflected back toward the laser while only a small percentage (based on the reflectivity of the mirror) enters the cavity. In this discussion, $I_0$ is the intensity of the laser after transmission through the first mirror. This first mirror will be called the input mirror and the light is monitored after exit through the second mirror (the output mirror) (see Figure 1.1).
The absorption of light from the first pass through the cavity can be described by Equation 1.4. As the light reflects from the output mirror, the intensity of the first reflected beam is given by:

$$I = I_0 R e^{-\alpha l}$$  \hspace{1cm} \text{Equation 1.4}

where $R$ is the reflectivity of the mirrors. After the light is reflected from the input mirror – completing one full round trip – the intensity is further reduced to:

$$I = I_0 R^2 e^{-2\alpha l}$$  \hspace{1cm} \text{Equation 1.5}

The intensity of light in the cavity after $n$ round trips is then described by:

$$I = I_0 R^{2n} e^{-2n\alpha l}$$  \hspace{1cm} \text{Equation 1.6}
After every pass through an empty cavity there will be loss of light only through the mirrors and the signal generated is then in the form of an exponential decay (Figure 1.1). The ring-down time (\(\tau\)) is defined as the time that it takes for the intensity of the incident beam to decrease to 1/e of its initial value and can be calculated as follows.\(^{20,21}\) Equation 1.6 can be rewritten as Equation 1.7 which shows the ideal behavior.

\[
I = I_0 e^{lnR^2n} e^{-2na}\text{d}
\]  
Equation 1.7

However, there are other losses that are occurring which cannot be measured independently, but also need to be included. These losses can include scattering from dust particles in the air as well as scattering from small imperfections on the mirror surfaces and are included collectively in Equation 1.8 as \(\delta_c\).

\[
I = I_0 e^{2n(lnR - \alpha l + \delta_c)}
\]  
Equation 1.8

The round trip time of a laser pulse is \(t_r = \frac{2d}{c}\) with \(d\) equal to the mirror spacing and \(c\) is the speed of light. The number of round trips made by a pulse after time \(t\) is \(n = \frac{t}{t_r} = \frac{ct}{2d}\) and thus Equation 1.8 can be rewritten as

\[
I = I_0 e^{\frac{c}{d} (lnR - \alpha l + \delta_c) t}
\]  
Equation 1.9

When \(I = I_0/e\), \(t\) equals the ring-down time, \(\tau\), and hence

\[
\frac{I_0}{e} = I_0 e^{\frac{c}{d} (lnR - \alpha l + \delta_c) \tau}
\]  
Equation 1.10

Which can be written in logarithmic form as:

\[-1 = \frac{c}{d} (lnR - \alpha l + \delta_c) \tau\]  
Equation 1.11

So that the following relationship for \(\tau\) then exists:

\[
\tau = \frac{d}{c(-lnR + \alpha l + \delta_c)}
\]  
Equation 1.12
The losses that arise from scattering from dust and mirror imperfections are significant; however, they are not so significant as to make this technique lose its value. The ring-down time for an empty cavity in this work is approximately 5 µs, whereas the ring-down time for a cavity with a cell present at normal incidence containing only water is approximately 1 µs. The reduction which occurs when an “empty” (non-absorbing) cell is present indicates that additional losses occur when the cell is inserted. A detailed description of these losses will be presented later. However, these losses occur on every transit of the laser beam through the cell and hence become an additional component included in $\delta_c$.

The ring-down time is the primary measurement of this technique, but the absorbance (and hence the concentration) of an analyte is the goal of many measurements. Throughout the course of this research, it became apparent that the ring-down time alone was not in a readily usable form and that an absorbance value needed to be calculated in order to obtain relevant calculations and observations. In order to properly calculate an absorbance value, Equation 1.12 must be rearranged to the proper form. This transformation is included in Equations 1.13 – 1.19. In the following equations $\tau$ is the ring-down time of the cavity with an absorber present and $\tau_0$ is the ring-down time with no absorber present in the cavity. For a cavity with an absorber present, the ring-down time is as shown in Equation 1.12. For an empty cavity, where no absorber is present ($\alpha = 0$), the ring-down time is as follows in Equation 1.13.

$$\tau_0 = \frac{d}{c(-\ln R + \delta_c)}$$

Equation 1.13
The absorbance can be determined by subtraction of the inverse of the solvent filled \((\tau_0)\) and sample containing \((\tau)\) cell ring-down times resulting in Equation 1.16.

\[
\frac{1}{\tau} = \frac{c}{d} (-\ln R + \alpha l + \delta_c)
\]

Equation 1.14

\[
\frac{1}{\tau_0} = \frac{c}{d} (-\ln R + \delta_c)
\]

Equation 1.15

\[
\frac{d}{c} \left( \frac{1}{\tau} - \frac{1}{\tau_0} \right) = -\ln R + \alpha l + \delta_c + \ln R - \delta_c
\]

Equation 1.16

Simplifying Equation 1.16 and solving for \(\alpha\) gives Equation 1.17. In Equations 1.17 and 1.18 \(\alpha = 2.303\epsilon C\), where \(\epsilon\) is the molar absorptivity, \(C\) is the concentration, and 2.303 is the conversion factor for natural logarithm to base-ten logarithm.

\[
\alpha = \frac{d}{cl} \left( \frac{1}{\tau} - \frac{1}{\tau_0} \right)
\]

Equation 1.17

\[
A = \epsilon l C = \frac{\alpha l}{2.303} = \frac{d}{2.303c} \left( \frac{1}{\tau} - \frac{1}{\tau_0} \right)
\]

Equation 1.18

**Brewster’s Angle and Its Application to Liquid CRDS Measurements**

Many other CRDS configurations employed to date use experimental configurations in which the sample is contained in a cell oriented at Brewster’s angle.

The reason for this was to reduce the amount of light lost due to reflections. Brewster’s angle is defined as the angle at which p-plane polarized light transmits through a medium without any reflection losses.\(^2\) Figure 1.2\(^2\) shows an example plot of the percent reflectance for a glass/air boundary as a function of angle of incidence.
Fresnel’s laws\(^2\) (Equations 1.19 and 1.20) show the calculation of the reflection for s- and p-plane polarized light that passes through two media of differing refractive index where \(n_1\) and \(n_2\) are the refractive indices of the media through which the light is passing, \(\theta_i\) is the incident angle and \(\theta_t\) is the transmitted angle. In order to calculate the angle of incidence and the angle of transmittance, Snell’s law is used (Equation 1.21).

\[
\begin{align*}
R_s &= \left(\frac{n_1 \cos \theta_i - n_2 \cos \theta_t}{n_1 \cos \theta_i + n_2 \cos \theta_t}\right)^2 \\
R_p &= \left(\frac{n_1 \cos \theta_t - n_2 \cos \theta_i}{n_1 \cos \theta_t + n_2 \cos \theta_i}\right)^2
\end{align*}
\]

Equation 1.19

\[
\frac{\sin \theta_i}{\sin \theta_t} = \frac{n_2}{n_1}
\]

Equation 1.21

In Equation 1.20, when \(\theta_t + \theta_i = 90^\circ\), the denominator goes to infinity and the reflection term goes to zero. Substituting \(90^\circ - \theta_i\) in for \(\theta_t\) into Snell’s law (Equation 1.21) gives the following.
\[
n_1 \sin \theta_i = n_t \sin (90^\circ - \theta_i) \quad \text{Equation 1.22}
\]
\[
n_1 \sin \theta_i = n_t \cos \theta_i \quad \text{Equation 1.23}
\]
\[
\frac{n_1 \sin \theta_i}{n_1 \cos \theta_i} = 1 \quad \text{Equation 1.24}
\]
\[
\tan \theta_i = \frac{n_t}{n_1} \quad \text{Equation 1.25}
\]

The angle of incidence where 100% transmission of light occurs is known as Brewster’s angle and can be calculated using Equation 1.26.

\[
\theta_B = \arctan \left( \frac{n_t}{n_1} \right) \quad \text{Equation 1.26}
\]

When the angle of incidence is 56.67\(^\circ\) or 55.59\(^\circ\) for a glass/air or a quartz/air interface, respectively, the reflection term of the p-plane polarized light is zero, allowing for 100% transmission of light through the two media.

As will be discussed in the following section, several groups have gone to great lengths in order to position a liquid sample at Brewster’s angle. This is due to the fact that at normal incidence for a glass/air or quartz/air interface \((n_{\text{glass}} = 1.52; n_{\text{quartz}} = 1.46; n_{\text{air}} = 1.00)\) there is a 4.3 % or 3.5 % reflection loss, respectively, at each boundary, but as will be shown in this work, these reflections can be recaptured by the cavity and hence not cause significant CRDS losses. A small portion of the light is reflected by the cuvette surface, but since the cell is situated at normal incidence, the light is simply reflected back to one of the highly reflective mirrors where it is then reflected back to the cavity. A hypothesis for lengthening the cavity is this: as long as the mirrors are large enough in diameter to catch the reflections from the cuvette’s surface, then there will not be a noticeable decrease in the ring-down time.
Other Liquid CRDS Experimental Configurations

The majority of attempts to minimize the losses from the cuvette interface in the cavity included placing the cuvette at Brewster’s angle\textsuperscript{24} for an air/quartz interface or through the use of a specially constructed cell\textsuperscript{25} in which the air/quartz and solvent/quartz interface angles were both set at $\theta_B$ to achieve the longest ring-down time possible.

Standard Cuvette at Brewster’s Angle

The first report of applying CRDS to liquids was performed by Xu et al.\textsuperscript{24} This technique involved a standard quartz cuvette or a double cuvette system in a cavity formed by two mirrors with a radius of curvature of 1 m spaced of 48 cm apart for a single cuvette and 70 cm apart for double cuvettes (Figure 1.3).

![Figure 1.3 Experimental setup of the single and double cuvette CRDS system employed by Xu, et al.\textsuperscript{24}](image)

The authors report a 1.2 $\mu$s ring-down time for an empty single cell, 0.8 $\mu$s for a single cell filled with hexane and 0.8 $\mu$s for the double cuvette arrangement. This cell arrangement can only be optimized for a single interface, whereas two are needed – one...
for the air/quartz boundary and one for the quartz/hexane boundary. The additional loss caused by the adoption of an intermediate compromise angle is evident in these measurements. These techniques were used to measure the C–H stretching fifth overtones in benzene and a capability of measuring absorption coefficients as small as 2–5 x 10^{-7} cm\(^{-1}\) was reported.

**Flowing Liquid-Sheet Jet**

A report of a CRDS system capable of detecting liquid samples without the use of a containment device is given by Alexander.\(^{26}\) This study employed the use of a flowing liquid sheet jet set at Brewster’s angle inside an optical cavity. Solvent was pumped from a reservoir through a pipette tip so that the resulting liquid stream was directed toward a plastic wedge. As the liquid fell from the surface of the wedge, a stable liquid sheet was formed. This liquid sheet was then situated at Brewster’s angle inside of an optical cavity. This approach removed the problem associated with multiple interface types and in theory should have generated impressive results. An illustration of the experimental setup is shown in Figure 1.4.
Ethylene glycol (EG) was used as the solvent. EG has a higher viscosity which allows for a more stable liquid surface to be produced. The ring-down time for an empty 90 cm cavity was 3.655 $\mu$s and the ring-down time for a cavity with pure EG flowing was 2.545 $\mu$s. The losses between the empty cavity and the cavity with only pure EG flowing were said to be due to scattering from the solvent as well as imperfections in the liquid surface. The limit of detection for the system was 71 nM with a linear dynamic range of 12.6 dB using malachite green dye as the analyte. These numbers suggest that this technique could be used to measure absorbance values as small as $7.09 \times 10^{-3}$ (using $\varepsilon = 9.98 \times 10^4$ M$^{-1}$cm$^{-1}$ for Malachite green). Disadvantages of this technique are the large volume (150 mL) of solvent required for these measurements, the need for a viscous solvent, and the potential problems which could arise if a volatile analyte were used.

**Liquid Filled Cavity**

Another report of circumventing a container for a liquid sample used an optical cavity in which the entire cavity was filled with solvent. Zare and co-workers$^{27}$ used a 21 cm cavity (60 mL volume) with an experimental setup shown in Figure 1.5.
Several analytes were studied here including Cu(II) acetate, LD700 laser dye, and indigo carmine. This study shows a detection limit of 1-10 pM for an absorber with a molar absorptivity of $10^5 – 10^6 \text{cm}^{-1}\text{M}^{-1}$. For comparison purposes, this corresponds to absorbance values between $1 \times 10^{-7} – 1 \times 10^{-5}$. This technique has several disadvantages. Large solvent volumes are required and most importantly the analyte is in direct contact with the high reflectivity mirrors. These mirrors are expensive and easily damaged. Any analyte adhering to the surface would quickly degrade the instrument’s performance and corrosive solvents could not be used.

**Miniature Liquid Only Cavity**

In this example of CRDS being used to detect liquid samples, van der Sneppen et al. constructed a cavity by clamping the mirrors to the sides of a silicon rubber spacer.
creating a liquid-tight seal in which the solvent can pass through for detection (Figure 1.6).

![Experimental setup of a miniaturized flow cell for liquid CRDS.](image)

Liquid CRDS was used at UV wavelengths to detect several azo dyes including direct red 10, direct violet 17, and benzopurpurine as well as three nitro-PAH compounds consisting of 6-nitrochrysene, 2-nitrofluorene, and 1-nitropyrene. The volume of the flow cell was 12 μL. The cavity length here was 2.0 mm and typical ring-down times of 20-25 ns (at 355 nm) and 70-80 ns (at 457 nm) were reported. The detection limits at 457 nm wavelength (for the azo dyes) was reported to be 28 nM for benzopurpurine ($\varepsilon = 3.6 \times 10^4 \text{ M}^{-1} \text{cm}^{-1}$) which corresponds to an absorbance of $1.01 \times 10^{-3}$, while the detection limit at 355 nm (for the nitro-PAH molecules) was determined to be between 75-150 nM (absorbance values = $7.65 \times 10^{-4} - 1.53 \times 10^{-3}$ for 2-nitrofluorene, $\varepsilon = 10.2 \times 10^3 \text{ M}^{-1} \text{cm}^{-1}$). While the results here do offer some improvements over traditional LC detection methods, major disadvantages are the short cavity length used and, again, the direct contact between the analyte and the cavity mirrors. The sensitivity of the measurement is much lower than for other techniques based solely on the short ring-down times reported.
An extremely fast digitization rate would need to be employed here in order to achieve sufficient sensitivity.

**Liquid CRDS Using a Normal-Incidence Cuvette**

The liquid CRDS experiments discussed so far all attempted to avoid using a cuvette at normal incidence to avoid reflection losses at the surface of the cuvette. However, a report in 2007\(^2\) successfully applied CRDS to the detection of liquids using normal incidence geometry and a standard cuvette. A 4-cm cavity was used here and the experimental setup is shown in Figure 1.7.

![Figure 1.7](image)

**Figure 1.7** Experimental setup for a 4 cm cavity using a normal incidence cuvette from van der Sneppen et al.\(^2\)

This technique was used at UV wavelengths (273 nm and 355 nm) to operate as an HPLC detector. The analytes separated were benzopurpurine, 2-nitrofluorene, 1-nitropyrene, and 6-nitrochrysene. The base ring-down times for this setup were 12-18 ns and 60-70 ns for 273 nm and 355 nm, respectively. The detection limit at 273 nm was 500-3000 nM (absorbance values of 0.014 – 0.084) which is approximately equivalent to the standard UV-vis detector compared in this study. The detection limit at 355 nm was...
40-80 nM (absorbance values of 0.0011 – 0.0022) which is a ~12 times lower detection limit to the UV-vis detector. The major disadvantage here is similar to the previous example in that the cavity is extremely short which results in short ring-down times that are hard to measure.

Summary of Other Liquid CRDS Experimental Configurations

Table 1.1 summarizes the studies mentioned above and presents the operating wavelengths and detection limits achieved.

<table>
<thead>
<tr>
<th>Author</th>
<th>Base Ring-down Time</th>
<th>Operating Wavelength</th>
<th>Absorbance Detection Limit</th>
<th>Concentration Detection Limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xu et al. 24</td>
<td>0.8 μs</td>
<td>590-620 nm</td>
<td>2.5 x 10^-7 cm^-1</td>
<td>–</td>
</tr>
<tr>
<td>Alexander 26</td>
<td>2.545 μs</td>
<td>628 nm</td>
<td>7.1 x 10^-3</td>
<td>71 nM</td>
</tr>
<tr>
<td>Hallock et al. 27</td>
<td>400 ns</td>
<td>620-670 nm</td>
<td>1 x 10^-7 - 1 x 10^-5</td>
<td>1-10 pM</td>
</tr>
<tr>
<td>van der Sneppen et al. 28</td>
<td>80 ns</td>
<td>425-478 nm</td>
<td>8.1 x 10^-6</td>
<td>75 nM</td>
</tr>
<tr>
<td>van der Sneppen et al. 29</td>
<td>70 ns</td>
<td>355 nm</td>
<td>4.1 x 10^-4 - 8.2 x 10^-4</td>
<td>40-80 nM</td>
</tr>
<tr>
<td>van der Sneppen et al. 29</td>
<td>18 ns</td>
<td>273 nm</td>
<td>5.1 x 10^-3 - 3.1 x 10^2</td>
<td>500-3000 nM</td>
</tr>
</tbody>
</table>

*The base ring-down time here denotes the ring-down time for solvent only measurements

CRDS Considerations for the Present Study

Many of the examples of applying CRDS to the detection of liquids presented above make an extraordinary effort to minimize reflection losses either from some sort of containment device or from the surface of the liquid itself by orientating the sample at Brewster’s angle. However, this study has determined that these efforts are unnecessary.
van der Sneppen et al.\textsuperscript{29} were the first to apply a normal-incidence cuvette to detecting liquids using CRDS. Their work successfully applied CRDS to an HPLC separation; however, the optical cavity was exceedingly short which provided little advantages over traditional detection methods. The work described in this thesis explores longer cavities, but retains a normal incidence cuvette.

Throughout the course of this research it was determined that the only requirement for applying liquid CRDS using a normal-incidence cuvette is that a stable optical cavity must be attainable and as long as the cuvette and mirrors can be precisely adjusted, there is no need to incorporate the cell at Brewster’s angle. The light reflected at a phase boundary is not actually lost, but is recaptured by the cavity mirrors.

A stable cavity is defined\textsuperscript{30} by Equation 1.27 where \( g_1 \) and \( g_2 \) are the stability parameters, \( g_1 = \left( 1 - \frac{d}{r_1} \right) \) and \( g_2 = \left( 1 - \frac{d}{r_2} \right) \). Here, \( d \) is the separation between the mirrors, and \( r_1 \) and \( r_2 \) are the radii of curvature for the two mirrors.

\[
g_1 g_2 = \left( 1 - \frac{d}{r_1} \right) \left( 1 - \frac{d}{r_2} \right) < 1 \quad \text{Equation 1.27}
\]

In the cavity used in these studies, \( r_1 = r_2 = 1 \) m so that \( g_2 = \left( 1 - \frac{d}{r_2} \right)^2 < 1 \) and the cavity is stable if \( 0 < \frac{d}{r} < 2 \) or whenever the distance separating the mirrors is less than 2 m.
CHAPTER II
EXPERIMENTAL METHODS

In the following pages a description of the cavity ring-down spectroscopy (CRDS) system constructed and the experiments performed is presented. The experimental setup as well as the light source was consistent throughout all experiments.

**Light Source**

The laser employed throughout the entirety of this research was a pulsed, Q-switched, frequency-doubled (532 nm) Nd:YAG (Continuum Minilite) laser operating at 10 Hz. This particular laser is operating with an energy of 12 mJ per pulse with a pulse width (fwhm) of 3-5 ns.

**Construction of the CRDS Apparatus**

The present work uses a standard 1 cm square quartz cuvette mounted at normal incidence centered in an optical cavity. A major component of the work described in this research was the effect of cavity length on the sensitivity of the method. Prior to this study, it was unknown whether a normally incident cell would scatter too much light from the cavity unless a very short cavity was used. Four different length cavities were arranged with the general setup as shown in Figure 2.1. The four cavity lengths constructed (mirror surface to mirror surface) were 0.1650 m, 0.2660 m, 0.4180 m, and 20
0.5205 m. The distance between the mirrors could be accurately measured with a meter stick. The distance between the front surface of the mirror mount and the reflective surface of the mirror is specified by the mount manufacturer. The problem is then reduced to a determination of the distance between the mirror mounts which could be measured with an estimated accuracy ±of 0.5 mm.

![Figure 2.1 General Setup for Liquid CRDS.](image)

The cavity mirrors were purchased from Los Gatos Research Corporation. The radius of curvature of these mirrors was \( r = 1 \) m with a greater than 99.99\% reflectivity (\( R \geq 0.9999 \)) at the design wavelength of 530 nm. The reflectivity at 532 nm was expected to be similar to this value, but no attempt was made to accurately determine the reflectivity of these mirrors. These 1” diameter mirrors were mounted in Newport (model SL25) stainless-steel gimbal mirror mounts with micrometer drives (Newport,
The optical cavity defined by the two mirrors must be precisely maintained at a fixed distance and must not be subject to any vibrations. To achieve this, the mirror mounts were mounted on vibrationally-damped Newport (model 45) posts.

The output of the frequency-doubled Nd:YAG laser was spatially filtered using a Galilean telescope with a central pinhole mounted at the focal point of the first lens. The telescope used a pair of 2.54 cm diameter quartz lenses with focal lengths of 10.5 cm and 8.0 cm, respectively. To have precise control of the direction of the beam, two Newport supermirrors were utilized to steer the beam to the center of the input mirror. Next, a focusing lens was inserted in the beam path (f = 50 cm, 2.54 cm diameter quartz) to focus the beam to the center of the cavity and to the center of the quartz cell. Light was collected from the output of the cavity by a lens (f = 5 cm, 2.54 cm diameter quartz) which was focused into a 300 µm diameter fiber-optic cable (Ocean Optics, model P300-1-SR). The fiber-optic cable was then fed into the input of a RCA C31034 photomultiplier tube (PMT) installed in an electrically and magnetically shielded housing. The output signal of the PMT was collected on a Tektronix TDS 340 digital oscilloscope. The time-dependent waveform was digitized, averaged, and stored in the oscilloscope and then transferred to a computer for subsequent processing and display using a program written in Visual Basic®. The Tektronix TDS 340 oscilloscope is equipped with a 2 megasample per second eight-bit digitizer, but it gains additional precision when waveforms are averaged inside the oscilloscope. Averaged data are stored as two eight bit words giving a $2^{16}$ (0-65536) data range.

In Figure 2.1, “PMT Detector 1” was used as the main data acquisition channel. PMT 1 was the detector which captured the light from the output of the cavity. PMT
Detector 2 was used to record when the laser fired and to generate a master trigger signal. The dielectric Newport supermirrors (model 10CM00SB.1, >99.9% reflective at 532 nm) used to steer the beam into the cavity transmit a small amount of the laser beam. A MgF$_2$-coated front surface aluminum mirror directed the weak transmitted beam into PMT 2. This procedure generated a 12-ns wide pulse which then acted as the master clock for the experiments.

The cell used in all experiments was a standard 1-cm quartz cuvette (Starna Cells, Inc., 1-Q-10) and was centered in the optical cavity for all cavity lengths. A custom cell holder was built in order to allow for precise alignment of the cell within the cavity. It was critical that the cell be perfectly normal to the optical axis of the cavity to avoid reflection losses out of the cavity and hence maximize the ring-down time. Several different cell holders were employed during the search for an adequate solution. First, a simple rotation stage was used. The rotation stage allowed for excellent maneuverability for side-to-side and up-and-down rotation, but offered little advantage to the actual mounting of the cell. The rotation stage was made for small prisms, so the cuvette was fairly unstable when mounted. Next, a holder was built in an attempt to have a reproducible location of the cell each time the cell was removed and replaced. This was also unsuccessful as the cell was required to be placed with micrometer (or better) accuracy. The solution here was to custom build a holder that afforded relative reproducibility of replacement location as well as micrometer adjustment to optimize the ring-down time.

Construction of the cell holder consisted of piecing together several pieces of aluminum together with micrometer controlled tilt platforms. First, one stainless-steel
micrometer controlled tilt platform (Newport TGN80) was mounted horizontally to a Newport 340-RC rod clamp which was clamped to a Newport model 45 post (Figure 2.2, Item 1). Next, an L-shaped piece of aluminum was mounted onto the horizontal tilt platform (Figure 2.2, Item 2). A vertically mounted tilt platform was then mounted to the L-shaped piece of aluminum (Figure 2.2, Item 3). The two tilt platforms provided the ability to tilt the quartz cell vertically and horizontally. Next, two pieces of aluminum were mounted onto the vertical tilt platform on either side of where the cell was to be located (Figure 2.2, Item 4). These two pieces of aluminum offered a support for a spring-loaded stabilizing bar to be installed. The stabilizing bar was a piece of Delrin® (poloxymethylene) with two clearance holes such that screws could capture springs and clamp the bar to the vertical tilt platform (Figure 2.2, Item 5). This ensures that when the cell is replaced, it is done as reproducibly as possible. A piece of thin Teflon® (polytetrafluoroethylene) was placed between where the cell would touch the vertical tilt platform in order to minimize the chance of scratching the optical surface of the cell. Coincidentally, there was already a hole through the center of the vertical platform to allow light to pass through, so a hole was cut in the piece of thin Teflon to match the location of the hole in the vertical tilt platform. A small aluminum platform was mounted onto the vertical platform directly below where the cell would be placed in order to be able to replace the cell at a consistent vertical position. Finally, on top of the small aluminum platform a stirring device was attached using RTV silicone (Figure 2.2, Item 6). The stirring mechanism was a Starna “Spinette” electronic cell stirrer model SCS 1.11. The cell is placed on top of the stirring mechanism and held in place by the Teflon stabilizing bar (Figure 2.2, Item 7).
Experimental Considerations

Initial experiments demonstrated the need for good signal isolation, in particular, the need to isolate the grounds between the laser firing circuits and the detector circuits. Large spikes occurred whenever the laser fired and these caused difficulty in establishing sufficient “zero” voltage levels for the experiments. The importance of establishing a quiet zero level when processing the data will be discussed in later parts of this chapter. Two optical isolators were used to isolate the grounds between the pulse generator controlling the laser flash lamp and Q-switch triggers and the laser power source. Each isolator was designed (and built in-house) to accept a TTL signal pulse which would
trigger an optical pulse which, in turn, would be received and translated back to a TTL output pulse. The optical transmitter/receiver chip is a key component of the circuit. The design is shown in Figure 2.3. In Figure 2.3, “HP 6N136” is the optical isolator chip, “Q1” and “Q2” are NPN type transistors (Motorola 2N 4400), “12 V” is a 12 V DC power source, and the resistance values are all in ohms.

![Figure 2.3 Diagram of the optical isolator circuits.](image)

The timing between the flash lamp and the Q-switch was critical to the stability and the intensity of the laser pulse. Minor timing changes were needed during the course of these experiments to maintain maximum power and stability for the laser pulses. Typical delays between firing the flash lamp and the Q-switch were between 190 and 220 μs. The timing pulses were generated by a Berkeley Nucleonics Corporation model 555 three channel pulse/delay generator. Flash lamp trigger pulses were issued every 100 ms
(followed, after 200 μs, by Q-switch triggering pulses) so that the laser operated at its design repetition rate of 10 Hz.

The laser used in these studies was acquired approximately 10 years ago and its water cooling system failed partway through these studies. The water pump and cooling fan were replaced, but the internal plastic cooling reservoir also started to leak. A container of similar size was fabricated to replace the original container which corrected the problem, but it was decided that the cooling system should be completely replaced in an attempt to generate more stable laser pulses. As designed, this low-cost laser generally took thirty minutes of continuous operation before the laser would thermally stabilize and generate reproducible pulses with good pointing stability. Given the precise alignment needed of the CRDS system, the pointing stability was vital to achieving high sensitivity. The redesigned cooling system used a 1 L glass beaker rather than a plastic container, and the beaker was placed in a water-filled temperature-controlled circulating bath (Fisher Scientific Isotemp 1016S) operating at 10 °C. The beaker contained deionized water (18 MΩ-cm) which was the cooling liquid pumped through the laser head. This deionized water container was covered with a thin plastic lid containing two holes – one for the input water line and one for the output water line. A lid was necessary to keep dust and other particulates out of the cooling water to ensure consistent laser pulses. The good thermal contact between the circulating deionized cooled water and the large thermal mass of the bathwater greatly improved the laser pulse and pointing stability and decreased the amount of time that it took for the laser to reach optimum operating temperature.
Initially, the light leaking from the CRDS cavity was directed by a lens through a short light pipe directly into PMT2. However, this design was abandoned because it was necessary to block ambient room lighting from entering the PMT input. The final configuration used to collect the data presented in this thesis used a short focal length lens to direct the output of the cavity into a 1-m fiber optic cable. The end of this cable was connected via an SMA connector to a metal plate which covered the front of the PMT housing. The SMA connector was threaded into a small hole in the plate so that the output of the fiber went directly into PMT 1. The fiber optic cable has a very small viewing angle and allows very little room light to reach the detector so that all experiments could be performed with the room lights on. The PMT voltage was also adjusted periodically in order to maintain a consistent single-exponential decay and to avoid any saturation of the PMT signal. Typical operating voltages for the PMT were between -900 V and -1100 V.

The data were transferred from the oscilloscope to the PC by an IEEE488 to USB adapter (Agilent Technologies model 82357B USB/GPIB interface). Initially, a RS-232 serial port was used to transfer the data; however, this proved inadequate as the serial port could not transfer the data to the computer fast enough. Switching to a parallel IEEE to USB adapter provided sufficient data transfer speed as to keep up with the rate at which the data were being generated.

Another consideration that proved especially difficult was the cleanliness of the solutions being prepared. Initially, distilled water from a house still was used to prepare all of the solutions and to replenish the water supply in the laser power supply. This water proved to be insufficient for the sensitive nature of this technique. Small dust
particles being stirred in the sample cell caused enough scatter losses to significantly decrease the ring-down time measurements. A trace of one such occasion is shown below in Figure 2.4.

![Figure 2.4](image)

Figure 2.4 Cavity ring-down times plotted as a function of time for distilled water. Large variability in the solvent only cell is evident and is caused by dust being stirred in the sample cell. Each data point (150 averages) represents 15 seconds elapsed time.

In an attempt to avoid contaminating the samples with dust, deionized (18 MΩ-cm) water was used to prepare fresh solutions. The output of this deionization column was not dust free and the water showed fewer, but still problematic, spikes in the baseline signal. Subsequently, another alternative was investigated. A deionized water source with a 0.2 μm filter placed at the output was utilized. After preparing new solutions a clear difference is noticed when Figure 2.4 is compared to a trace of deionized water that has been passed through a 0.2 μm filter in Figure 2.5.
Deionized (18 MΩ-cm) water that was passed through a 0.2 µm filter was used for all of the subsequent experiments. Although filtered, deionized water generated better data, there was still a moderate amount of dust in the ambient atmosphere that proved to be troublesome. All solutions were covered and special care was taken to avoid contamination of the cuvette by airborne dust particles throughout the experiments. The cuvette was held upside down when being transferred between experiments. While the cuvette was drying, it was laid on its side and a cover placed over the opening.

Initial tests of the system used the laser dye crystal violet due to its strong absorption at the 532 nm operating wavelength of the laser. However, it was apparent that this dye is very sensitive to ambient light and quickly decomposed at room temperature. A 3.68 x 10⁻⁶ M crystal violet solution was let sit at ambient conditions; a solution which had a noticeable violet color changed to a clear solution after less than 24 h. Therefore, crystal violet was replaced with Rhodamine 6G.
Calibration of Hamilton Pipettes

Many of the experiments involved the addition of small aliquots of liquid to the cuvette. These additions were performed with Hamilton adjustable-volume pipettes. Volumes of 1.00 mL and a 100 μL were used through the course of these experiments and each of the pipettes was calibrated to ensure accurate volumes were being added to the cuvette. Calibration was performed by measuring the mass of a single addition of either 1.00 mL or 100 μL of deionized water into an empty beaker. Ten separate additions were performed for each volume. Table 2.1 shows the data obtained from the measurement of the mass of seven injections of 1.00 mL of deionized water.

Table 2.1

Calibration Data for 1.00 mL Using a Hamilton Adjustable-volume Pipette

<table>
<thead>
<tr>
<th>mass of water (g)</th>
<th>volume of water (mL)</th>
<th>T = 20.8 °C</th>
<th>dH₂O = 0.9980 g/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.9988</td>
<td>1.0008</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.9978</td>
<td>0.9998</td>
<td></td>
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</tr>
<tr>
<td>0.9957</td>
<td>0.9977</td>
<td></td>
<td></td>
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<td>0.9977</td>
<td>0.9997</td>
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</tr>
<tr>
<td>0.9979</td>
<td>0.9999</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.9988</td>
<td>1.0008</td>
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<td>0.9931</td>
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</tr>
<tr>
<td>1.0040</td>
<td>1.0060</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.0009</td>
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<td></td>
</tr>
<tr>
<td>0.9993</td>
<td>1.0013</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

31
The volume of water was calculated from the measured mass and the density at 20.8 °C (room temperature). The average volume of water dispensed for 1.00 mL via the adjustable pipette was 1.0007 ± 0.0029 mL, where the error estimate is the standard deviation about the mean. Table 2.2 shows the data obtained from the calibration of the addition of 100 µL of deionized water. The average volume of water dispensed for 100 µL via the adjustable pipette was 0.0997 ± 0.0012 mL.

Table 2.2
Calibration Data for 100 µL Using a Hamilton Adjustable-volume Pipette

<table>
<thead>
<tr>
<th>mass of water (g)</th>
<th>volume of water (mL)</th>
<th>T = 20.8 °C</th>
<th>d_{H2O} = 0.9980 g/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1006</td>
<td>0.1008</td>
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<td>0.0978</td>
<td>0.0980</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1001</td>
<td>0.1003</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.0998</td>
<td>0.1000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1001</td>
<td>0.1003</td>
<td></td>
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</tr>
<tr>
<td>0.0996</td>
<td>0.0998</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Calibration of Volumetric Glassware

A 1.00 mL Class A glass volumetric pipette was also used during the course of this research. This pipette was calibrated in the same manner as the adjustable volume pipettes. Ten separate additions of 1.00 mL were added to an empty beaker and the volume was calculated from the measured mass and the density. Table 2.3 shows the
data obtained from the calibration of the 1.00 mL glass volumetric pipette. The average volume of water dispensed from the glass volumetric pipette was 1.0257 ± 0.0044 mL.

Table 2.3

Calibration Data for 1.00 mL Using a 1-mL Glass Volumetric Pipette

<table>
<thead>
<tr>
<th>mass of water (g)</th>
<th>volume of water (mL)</th>
<th>T = 22.6 °C</th>
<th>d_H2O = 0.9976 g/mL</th>
</tr>
</thead>
<tbody>
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<td>1.0216</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.9728</td>
<td>1.0255</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.9634</td>
<td>1.0355</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.9730</td>
<td>1.0253</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.9688</td>
<td>1.0297</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.9732</td>
<td>1.0251</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.9746</td>
<td>1.0236</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.9781</td>
<td>1.0199</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Crystal Violet Experiments

Initially, the dye crystal violet was used for a series of experiments in order to test the efficacy of this technique at different cavity lengths. Crystal violet, C_{25}H_{30}N_{3}Cl (FW: 407.99, Sigma-Aldrich, used without further purification) was used due to its strong absorption at the operating wavelength of the Nd:YAG laser. A UV-visible spectrum was obtained in order to verify that there was sufficient absorption at 532 nm for crystal violet and to measure the molar absorptivity at 532 nm.

First, a stock solution of crystal violet was prepared by dissolving 0.0412 g of the powder in 1 L of distilled water. The resulting concentration was 1.01 \times 10^{-4} \text{ M}. 
Dilutions were performed to attempt to make measurements by estimating the concentrations needed (using the molar absorptivity) to achieve absorbance values of 2, 1.5, 1.0, 0.5, 0.30, 0.25, and 0.10. The concentrations of crystal violet prepared (from the 1.01 x 10^-4 M stock solution) to achieve these absorbance values were 2.52 x 10^-5 M, 2.02 x 10^-5 M, 1.01 x 10^-5 M, 5.05 x 10^-6 M, 4.04 x 10^-6 M, 3.03 x 10^-6 M, and 1.01 x 10^-6 M, respectively.

Various attempts at measuring the ring-down time of pure water and then of a crystal violet solution were unsuccessful due to the fact that the cell could not be reproducibly replaced within the optical cavity. Therefore, an experiment in which the cell did not need to be moved between differing solutions was devised. These so-called “addition experiments” allow for the relative difference between pure solvent and solvent plus dye ring-down time values to be compared with confidence.

Each addition experiment started by first injecting 2.00 mL of distilled water by using two separate additions of 1.00 mL from an adjustable-volume Hamilton pipette. Prior to filling and placement of the cuvette, the outside of the cell was cleaned carefully using the drag-wipe method. The drag-wipe method consisted of folding a piece of lens paper several times, being cautious to not touch the area that would come into contact with the cell so as to prevent finger oil from contaminating the surface. Three to four drops of HPLC grade methanol were dropped onto the folded lens paper. Then, using tweezers, the methanol-soaked lens paper was dragged slowly across the surface of the cell. The drag-wipe method allows for sufficient cleaning of an optical surface without leaving any residue or streaks on the surface. Many times, the drag-wipe method was
unsuccessful and was repeated to ensure the highest possible starting value of the ring-
down time.

After the cell was thoroughly cleaned, it was placed into the custom-made cell
holder in the center of the cavity. The laser was allowed to run for 20-30 minutes, or
until a stable trace of the ring-down time was achieved. To begin data collection, the
system measured the ring-down time of pure solvent for two minutes. Following the
measurement of the solvent were successive 50 µL injections of a stock solution of
crystal violet using an adjustable-volume Hamilton pipette. Following each injection, 50
µL of the resulting solution was drawn into the pipette tip and released back into the cell
3-4 times in order to ensure sufficient mixing. A new plastic pipette tip was used for
each successive injection so as to not contaminate the stock solution. All pipette tips
used were FisherBrand SureOne non-filter pipette tips. Each successive injection was
allowed to run for two minutes before the next addition to the cell. Following the
experiment, the cell was removed and the solution was poured out. The cell was then
rinsed 10-15 times with distilled water, then rinsed three times with acetone in order to
remove as much of the water from the cell as possible before the next experiment. The
acetone rinse was essential to this process due to the fact that if there was residual water
present in the cell prior to starting the experiment, the calculations for the concentration
after each addition would have been incorrect. After noticing that the crystal violet
solutions were especially light sensitive, Rhodamine 6G was used as an alternative dye.

The first type of addition experiment was performed as follows. First, 2.00 mL of
distilled water was injected into the cell via two 1.00 mL additions using a Hamilton
adjustable-volume pipette. This was allowed to run for two minutes averaging ten shots
per data point. Next, 50 μL aliquots of a 1.147 x 10^{-5} M crystal violet stock solution were added sequentially running for two minutes each until 650 μL total volume stock solution was added. A typical set of data from this type of experiment are shown in Figure 2.6.

![Figure 2.6](image)

**Figure 2.6** An example of a typical set of data for an addition experiment involving crystal violet. Each data point (10 averages) represents one second elapsed time.

**Rhodamine 6G Experiments**

**Single Concentration Addition Experiments**

The first set of experiments utilizing Rhodamine 6G (R6G) was identical to that of the addition experiments utilizing crystal violet. Briefly, 2.00 mL of water was initially present in the cell, then, 50 μL aliquots of a stock solution of R6G were sequentially added. However, for the experiments using R6G, a new stirring mechanism was employed. A Starna “Spinette” model SCS 1.11 electronic cell stirrer was added to the custom cell holder in order to facilitate proper stirring of the mixture. Subsequently,
it was no longer necessary to draw up and release any of the solution for proper mixing. Also, the same pipette tip could now be used for every addition during a single experiment. Another difference in the procedure is that instead of distilled water, deionized water (18 MΩ-cm) that had been passed through a 0.2 μm filter was used. Filtered deionized water was not only used for the starting solvent, but it was also used to prepare fresh solutions.

The first addition experiments using R6G differed only slightly from the addition experiments involving crystal violet. Here, each concentration was allowed to run for five minutes each using a 150 shot average. Also, 50 μL aliquots of a 7.92 × 10⁻⁷ M R6G were used until the total volume added was 300 μL.

**Multiple Concentration Addition Experiments**

The next set of addition experiments using R6G utilized adding two different stock solutions of R6G. In these experiments, 2.00 mL of deionized, filtered water was initially present in the cell as before. Then, four 50 μL aliquots of a 7.92 × 10⁻⁸ M R6G solution were added sequentially allowing each concentration to run for five minutes. Directly following these additions were four 50 μL additions of a 7.92 × 10⁻⁷ M R6G solution, again allowing each concentration to run for five minutes. The stock solutions were remade several times after switching from distilled water to deionized water to filtered, deionized water; however, the scheme was held constant in that four “low” concentration additions were made immediately followed by four “high” concentration additions. This data produced nine different concentrations (including pure water). A sample data set is shown in Figure 2.7
Figure 2.7 A sample data set from the multiple concentration addition experiments using R6G. Each data point (150 averages) represents 15 seconds elapsed time.

**Single Injection Addition Experiments**

A third set of experiments were performed involving R6G which used a 1.00 mL glass volumetric pipette. For these experiments, the system was allowed to collect data for five minutes with only deionized water present in the cell. After 5 minutes, 1.00 mL of a 2.36 x 10^{-8} M stock solution of R6G was added to the cell. The final concentration present in the cell after a single addition was calculated to be 7.87 x 10^{-9} M. This single addition was allowed to run for five minutes before the experiment was stopped. A sample data set from these experiments is depicted in Figure 2.8.
Figure 2.8 A sample data set from the single-injection addition experiments using R6G. Each data point (150 averages) represents 15 seconds elapsed time.

Between each experiment, the cell was rinsed with deionized water 10-15 times, then rinsed with acetone 3 times in order to remove as much water as possible. The cell was then allowed to dry completely before refilling with water to start another experiment. The outside of the cell was cleaned in the same fashion as above using the drag-wipe method before the start of each experiment.

Data Acquisition and Processing

The light was detected by an RCA PMT detector. A Tektronix TDS 340 digital oscilloscope was used to acquire the data from the PMT tube. The specified number of waveforms was averaged (150 in most cases) by the oscilloscope, then the data from the oscilloscope were transferred to a computer by way of an Agilent Technologies model 82357B USB/GPIB interface. A Visual Basic® program written in collaboration with Dr. Stephen C. Foster was used to request averaged data, receive the data in the computer, then process the data to extract ring-down times. As discussed previously, a
ring-down signal from a well aligned cavity should be a single exponential decay. It is very important to determine an accurate baseline for the signal. In practice a ring-down waveform can be represented in the following way:

\[ V = V_{\text{baseline}}x + V_{\text{max}}e^{-\tau x} \]  

Equation 2.1

where x represents the timebase and V is the observed voltage. If the baseline voltage \( (V_{\text{baseline}}) \) equals 0 V, it is a straightforward matter to calculate the logarithm of this function and linearize the equation to Equation 2.2.

\[ \ln V = -\tau x \]  

Equation 2.2

However, the baseline is rarely at exactly 0V and may vary from shot to shot. Digital oscilloscopes have the advantage that their digitizers run continually, thus data are collected before a conventional trigger. Because of this, 10 data points were averaged immediately before each laser pulse fired, and the average of these 10 voltages was used to establish the baseline voltage for a given ring-down waveform. This baseline voltage was subtracted from each waveform data point so that the signal correctly decays to 0V, therefore, the data sets could be linearized by taking the natural logarithm of the waveform. This procedure will not correct a baseline which varies with time. The program does include a feature to record an additional 10 data points at the end of the waveform decay to fit a linear baseline correction between the starting 10 points and ending 10 points. However, we did not see a case where this additional processing improved the data collection, so this procedure was not used with the data presented in this thesis. The program generated linear least-squares fits to the logarithmic decays and generated \( R^2 \) values. Figure 2.9 shows a general flow chart for the collection of data from the oscilloscope by the computer. The graphical user interface (GUI) allows the
user to set the number of waveforms averaged in the oscilloscope (256 maximum) and
the number averaged in the computer (unlimited). A file name “root” is chosen by the
user (“filename”), the program automatically appends with an integer and a file type
(filename01.dat”), and the integer is auto-incremented after each storage event. Other
portions of the GUI allow the user to select logarithmic or linear displays of the data, to
select the oscilloscope channel for data acquisition, and to set the range over which the
waveform is fitted (by selecting cursors on the screen using mouse clicks). Figure 2.10
shows the GUI used in this work. A copy of the full Visual Basic® program is included
in the appendix to this thesis.
Figure 2.9  A general flow chart showing how the computer program collects and processes data from the oscilloscope.
The graphical user interface of the program written to facilitate the transfer of data from the oscilloscope and to further analyze the data.

Detection Limit of the CRDS System

Several methods for calculating a detection limit exist and several are discussed by Corley. The methods discussed which considers matrix effects will be ignored here due to the simple nature of the matrix used in the experiments presented. The two methods that will be discussed here are the IUPAC adopted method (also presented in Harris) and a US EPA approved method, the RMSE approach.

First, the method presented by Harris, using Equation 2.3, will be discussed.

\[ y_{dl} = y_{\text{blank}} + t \cdot s \]  

Equation 2.3

If blank samples are not available, a low concentration analyte solution may be used. The procedure to calculate the detection limit with this method is to first obtain seven measurements of a low concentration solution and calculate the standard deviation. Next,
obtain seven blank measurements (all blank measurements are zero in this work). Then, multiply the standard deviation of the seven low concentration measurements by Student’s t associated with 6 degrees of freedom for 98% confidence (t = 3.143 in this case).

The RMSE method estimates a detection limit from the generation of a calibration curve and calculating the root mean square error (RMSE). The steps for this method involve first constructing a calibration curve using concentrations that are within one order of magnitude of the estimated detection limit. Next, a calibration curve is generated and regression performed to obtain values of the slope (m), intercept (i) and $R^2$ value. Calculate the predicted response ($x_P$) from the slope and intercept values and calculate the error (E) associated with each value ($|x_P - x|$). Next, calculate the sum of the square of the errors ($\sum E^2$). The RMSE is calculated according to Equation 2.4.

$$\text{RMSE} = \left( \frac{\sum_{i=1}^{n} E^2}{n-2} \right)^{1/2}$$

Equation 2.4

The detection limit ($x_L$) is calculated according to Equation 2.5.

$$x_L = i + (3 \times \text{RMSE})$$

Equation 2.5

To convert this detection limit into a concentration, simply divide by the slope calculated from the calibration curve.

Corley states that the RMSE method provides a more reliable, easier to calculate detection limit for dynamic systems such as chromatography. The automation with which chromatography is performed regarding the integration of peaks makes the value of the standard deviation a subjective measurement. The standard deviation of a blank sample may be different at the beginning of a chromatogram as compared to the standard
deviation of the “blank” next to a peak of interest. For this reason, the RMSE method proves more reliable in this application. However, the experiments performed here were of a static system, for which the method presented in Equation 2.3 provides a better representation of the detection limit.

**Detection Limit of a Shimadzu UV-2550 Commercial UV-vis Spectrometer**

The CRDS spectrometer described in this thesis has high temporal resolution – typical ring-down times last less than 1 µs – and can thus make high speed measurements and is useful in fast kinetic experiments. Ideally it should also have high sensitivity and good linearity. As part of this study, a comparison has been made between the linearity and the detection limits of the CRDS system and a commercial Shimadzu UV-2550 UV-vis spectrometer. A series of absorption measurements were performed using the Shimadzu instrument so that its performance could be compared with the CRDS system described in this thesis.

From the previous R6G UV-vis data, the detection limit of the Shimadzu instrument was estimated to be 5.0 x 10⁻⁷ M, therefore, a target concentration (~1-7 times the detection limit) for calculating the detection limit was estimated to be 1.57 x 10⁻⁶ M R6G. First, seven absorbance measurements of 0.2 µm filtered, deionized water were performed. Next, seven absorbance measurements were obtained of a 1.57 x 10⁻⁶ M R6G solution.

In order to perform the experiments as similarly as possible to the CRDS system experiments, the 1.57 x 10⁻⁶ M R6G solution was achieved by starting with 2.00 mL of filtered, deionized water in a quartz cuvette. To the water, 1.00 mL of a 4.72 x 10⁻⁶ M
R6G solution was added to the cuvette via a 1.00 mL volumetric pipette. The cuvette was covered and mixed by inverting several times. After an absorbance measurement was performed, the cell was emptied, rinsed with copious amounts of filtered, deionized water, rinsed with acetone 3 times, then let dry. After the cell was dry, the same procedure for obtaining $1.57 \times 10^{-6}$ M R6G was followed until seven absorbance measurements were taken.
CHAPTER III

RESULTS AND DISCUSSION

The data obtained from the experiments performed using crystal violet were unimportant as many of the experimental parameters were changed during the course of experimentation. Therefore, these data will not be discussed further.

Calculation of Absorbance Values

The direct ring-down time values measured during the course of these experiments gave little information as it was very difficult to achieve an identical alignment of the cell and hence identical ring-down times for each experiment. Therefore, absorbance values were used to obtain useful insight into this technique. As described in the Introduction, the absorbance values were calculated using Equation 3.1 (previously described as Equation 1.18).

\[
A = \frac{d}{2.303c} \left( \frac{1}{\tau} - \frac{1}{\tau_0} \right)
\]

Equation 3.1

In Equation 3.1, \(A\) is the absorbance, \(d\) is the distance between the high-reflectivity mirrors, \(c\) is the speed of light (2.99792458 x \(10^8\) m/s), \(\tau\) is the ring-down time with analyte present in the cavity, and \(\tau_0\) is the ring-down time with no absorber present in the cavity.
A UV-vis spectrum of each of the five following concentrations was obtained: 1.98 x 10^{-5} M, 1.49 x 10^{-5} M, 9.90 x 10^{-6} M, 4.95 x 10^{-6} M, and 1.98 x 10^{-6} M. Figure 3.1 shows all of the spectra for the concentrations named above.

The UV-vis spectra confirmed that the value of $\lambda_{\text{max}}$ for R6G was 525 nm. This differed slightly from the value from Exciton$^{34}$ of 530 nm and is attributed to different solvents being used. Methanol was used in the evaluation by Exciton and water was used in this study. The molar extinction coefficient was measured (using the Beer-Lambert equation) to be $9.10 \times 10^{4} \text{ M}^{-1}\text{cm}^{-1}$ at the $\lambda_{\text{max}}$ of 525 nm. The molar extinction coefficient at the operating wavelength of the Nd:YAG laser (532 nm) used in this study was measured to be $7.91 \times 10^{4} \text{ M}^{-1}\text{cm}^{-1}$.
**Single Concentration Addition Experimental Data**

Figure 3.2 is a sample data trace for a single concentration addition experiment. Table 3.1 shows the concentration values for each of the sequential steps. For this particular experiment, 2.00 mL deionized water was added into the cell, then six sequential 50 μL aliquots of 7.92 x 10⁻⁷ M R6G were added to the cell under constant stirring. Ring-down times were calculated in the following way. The first five data points after each dye addition (to allow time for the dye to disperse uniformly) were discarded. The next ten points were averaged and Table 3.1 shows these values for the data presented in Figure 3.2. Standard deviations for these means are also reported in Table 3.1. This procedure was followed for all subsequent data analyses.

![Figure 3.2](image)

Figure 3.2 Sample data trace for a single concentration addition experiment. Each data point (150 averages) represents 15 seconds elapsed time.
Table 3.1
Concentration Values for Each Step in the Experiment Outlined in Figure 3.2

<table>
<thead>
<tr>
<th>Addition</th>
<th>Total Volume (mL)</th>
<th>Moles R6G added</th>
<th>Concentration (M) R6G</th>
<th>Ring-down time (ns)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.000</td>
<td>0</td>
<td>0</td>
<td>345(6)</td>
</tr>
<tr>
<td>2</td>
<td>2.050</td>
<td>$3.96 \times 10^{-11}$</td>
<td>$1.93 \times 10^{-8}$</td>
<td>214(2)</td>
</tr>
<tr>
<td>3</td>
<td>2.100</td>
<td>$7.92 \times 10^{-11}$</td>
<td>$3.77 \times 10^{-8}$</td>
<td>154(1)</td>
</tr>
<tr>
<td>4</td>
<td>2.150</td>
<td>$1.19 \times 10^{-10}$</td>
<td>$5.53 \times 10^{-8}$</td>
<td>110(1)</td>
</tr>
<tr>
<td>5</td>
<td>2.200</td>
<td>$1.58 \times 10^{-10}$</td>
<td>$7.20 \times 10^{-8}$</td>
<td>101(1)</td>
</tr>
<tr>
<td>6</td>
<td>2.250</td>
<td>$1.98 \times 10^{-10}$</td>
<td>$8.80 \times 10^{-8}$</td>
<td>86.0(6)</td>
</tr>
</tbody>
</table>

*Errors reported in parentheses represent one standard deviation in units of the last significant digit

Multiple Concentration Addition Experimental Data

There were many hurdles and setbacks to overcome while performing this research. The first to be addressed will be the use and eventual exclusion of the use of plastic pipette tips for the adjustable-volume pipettes. Figure 3.3 shows a sample data trace from a multiple concentration addition experiment using plastic pipette tips. The first five data steps represent pure water followed by four 50 μL additions of $9.20 \times 10^{-8}$ M R6G. The final four data steps are from four 50 μL additions of $9.20 \times 10^{-7}$ M R6G. Table 3.2 shows the concentration values for each data step. The values in the parenthesis in Table 3.2 are one standard deviation of the measured ring-down time.
Figure 3.3 Sample data trace from a multiple concentration addition experiment. Each data point (150 averages) represents 15 seconds elapsed time.

Table 3.2

Concentration Values for Each Step in the Experiment Outlined in Figure 3.3

<table>
<thead>
<tr>
<th>Addition</th>
<th>Total Volume (mL)</th>
<th>Total moles R6G added</th>
<th>Concentration (M) R6G</th>
<th>Ring-down time (ns)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.000</td>
<td>0</td>
<td>0</td>
<td>1261(17)</td>
</tr>
<tr>
<td>2</td>
<td>2.050</td>
<td>4.60 x 10^{-12}</td>
<td>2.24 x 10^{-9}</td>
<td>963(21)</td>
</tr>
<tr>
<td>3</td>
<td>2.100</td>
<td>9.20 x 10^{-12}</td>
<td>4.38 x 10^{-9}</td>
<td>808(31)</td>
</tr>
<tr>
<td>4</td>
<td>2.150</td>
<td>1.38 x 10^{-11}</td>
<td>6.42 x 10^{-9}</td>
<td>740(14)</td>
</tr>
<tr>
<td>5</td>
<td>2.200</td>
<td>1.84 x 10^{-11}</td>
<td>8.36 x 10^{-9}</td>
<td>680(10)</td>
</tr>
<tr>
<td>6</td>
<td>2.250</td>
<td>6.44 x 10^{-11}</td>
<td>2.86 x 10^{-8}</td>
<td>346(31)</td>
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<td>7</td>
<td>2.300</td>
<td>1.10 x 10^{-10}</td>
<td>4.80 x 10^{-8}</td>
<td>247(20)</td>
</tr>
<tr>
<td>8</td>
<td>2.350</td>
<td>1.56 x 10^{-10}</td>
<td>6.66 x 10^{-8}</td>
<td>193(10)</td>
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<tr>
<td>9</td>
<td>2.400</td>
<td>2.02 x 10^{-10}</td>
<td>8.43 x 10^{-8}</td>
<td>160(10)</td>
</tr>
</tbody>
</table>

*Errors reported in parentheses represent one standard deviation in units of the last significant digit

These data are not very useful in this form. The transformation into absorbance values has been shown earlier (Equation 3.1). Table 3.3 lists absorbance values.
calculated from the data in Table 3.2 and lists standard deviations propagated from the ring-down time data. The standard deviations are all very similar and are dominated by the error associated with the pure water $\tau_0$ value.

Table 3.3

Calculated Absorbance Values for the Experiment Outlined in Figure 3.3

<table>
<thead>
<tr>
<th>Addition</th>
<th>Concentration (M)</th>
<th>A</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>2.24E-09</td>
<td>0.000182(19)</td>
</tr>
<tr>
<td>3</td>
<td>4.38E-09</td>
<td>0.000330(37)</td>
</tr>
<tr>
<td>4</td>
<td>6.42E-09</td>
<td>0.000415(20)</td>
</tr>
<tr>
<td>5</td>
<td>8.36E-09</td>
<td>0.000503(18)</td>
</tr>
<tr>
<td>6</td>
<td>2.86E-08</td>
<td>0.001557(21)</td>
</tr>
<tr>
<td>7</td>
<td>4.80E-08</td>
<td>0.002416(26)</td>
</tr>
<tr>
<td>8</td>
<td>6.66E-08</td>
<td>0.003241(21)</td>
</tr>
<tr>
<td>9</td>
<td>8.43E-08</td>
<td>0.004040(30)</td>
</tr>
</tbody>
</table>

*Errors reported in parentheses represent one standard deviation in units of the last significant digit propagated from the ring-down times

Using the calculated absorbance data, a plot can be made of absorbance versus concentration (Figure 3.4); Beer’s Law shows that a linear relationship should exist. The slope of the linear fit to the data in Figure 3.4 is 72306 M$^{-1}$cm$^{-1}$. However, there is a clear difference in the slope of the data points resulting from the addition of the low concentration ($9.20 \times 10^{-8}$ M) R6G (plotted separately in Figure 3.5) and the slope of the data points resulting from the addition of the high concentration ($9.20 \times 10^{-7}$ M) R6G (Figure 3.6). These experiments were repeated many times and these results were consistently observed. It is hypothesized that the difference in the slope in Figures 3.5 and 3.6 is due to the use of plastic pipette tips. The plastic pipette tips are thought to
adsorb some portion of the dye molecules. Adsorption of dye molecules to the wall of the pipette tip will cause a greater percent difference in the addition of the \(9.20 \times 10^{-8}\) M R6G than in the addition of the \(9.20 \times 10^{-7}\) M R6G.

Figure 3.4 A plot of absorbance versus concentration for the experiment outlined in Figure 3.3.

Figure 3.5 A plot of absorbance versus concentration for the experiment outlined in Figure 3.3. This plot only shows the four additions of \(9.20 \times 10^{-8}\) M R6G.
Figure 3.6 A plot of absorbance versus concentration for the experiment outlined in Figure 3.3. This plot only shows the four additions of $9.20 \times 10^{-7}$ M R6G.

An attempt to counteract this adsorption affect was to soak a pipette tip in a solution of $9.20 \times 10^{-8}$ M R6G. It was hypothesized that if adsorption was the problem, soaking a pipette tip would cause the pipette tip to be completely saturated, resolving the difference of the slopes between the two concentrations. Figure 3.7 shows the results of one experiment performed identically to the experiment outlined in Figure 3.3, however, now, the pipette tip was soaked for 15 min prior to the start of the experiment. Figure 3.8 shows a plot of absorbance versus concentration for the experimental data in Figure 3.7.
Figure 3.7 Data from a multiple concentration addition experiment utilizing a pre-soaked pipette tip.

Figure 3.8 A plot of absorbance versus concentration for the experimental data in Figure 3.7.

When looking at Figure 3.8, a major improvement is noticed in the difference between the data from the addition of 9.20 x 10^-8 M and the addition of 9.20 x 10^-7 M R6G. The overall line is much straighter with a better fit of the data. However, it was also observed that instead of the slope of the first four data points being higher than the
last four data points, the slope was now lower than the first four data points. Several identical experiments with and without soaking of the pipette tips were performed with the data being very similar for each series of experiments. These differences between the data sets are consistent with dye binding to pipette tips, but here the pre-soaked (pre-saturated) tips appear to be desorbing R6G molecules into the lower concentration dye stock solution cell.

Figures 3.9, 3.10 and 3.11 are plots of concentration versus absorbance for three replicate multiple concentration addition experiments performed at 0.5205 m cavity length after pre-soaking of the pipette tips.

![Graph](image.png)

**Figure 3.9** A plot of concentration versus absorbance for a multiple concentration addition experiment at a cavity length of 0.5205 m using pre-soaked pipette tips.
Figures 3.10, 3.11 and 3.12 are plots of concentration versus absorbance for a multiple concentration addition experiment at cavity lengths of 0.5205 m using pre-soaked pipette tips.

Figures 3.10, 3.11 and 3.12 are plots of concentration versus absorbance for a multiple concentration addition experiment at a cavity length of 0.5205 m using pre-soaked pipette tips.

Figures 3.10, 3.11 and 3.14 are plots of concentration versus absorbance for three replicate multiple concentration addition experiments performed at 0.2660 m cavity length using pre-soaked pipette tips.
Figure 3.12: A plot of concentration versus absorbance for a multiple concentration addition experiment at a cavity length of 0.2660 m using pre-soaked pipette tips.

Figure 3.13: A plot of concentration versus absorbance for a multiple concentration addition experiment at a cavity length of 0.2660 m using pre-soaked pipette tips.
<table>
<thead>
<tr>
<th>Concentration (M)</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00E+00</td>
<td>0</td>
</tr>
<tr>
<td>2.00E-08</td>
<td>0.001</td>
</tr>
<tr>
<td>4.00E-08</td>
<td>0.002</td>
</tr>
<tr>
<td>6.00E-08</td>
<td>0.003</td>
</tr>
<tr>
<td>8.00E-08</td>
<td>0.004</td>
</tr>
<tr>
<td>1.00E-07</td>
<td>0.005</td>
</tr>
</tbody>
</table>

\[ y = 58082x - 8E-05 \]
\[ R^2 = 0.9973 \]

Figure 3.14 A plot of concentration versus absorbance for a multiple concentration addition experiment at a cavity length of 0.2660 m using pre-soaked pipette tips.

Figures 3.15, 3.16 and 3.17 are plots of concentration versus absorbance for three replicate multiple concentration addition experiments performed at 0.1650 m cavity length using pre-soaked pipette tips.

<table>
<thead>
<tr>
<th>Concentration (M)</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00E+00</td>
<td>0</td>
</tr>
<tr>
<td>2.00E-08</td>
<td>0.001</td>
</tr>
<tr>
<td>4.00E-08</td>
<td>0.002</td>
</tr>
<tr>
<td>6.00E-08</td>
<td>0.003</td>
</tr>
<tr>
<td>8.00E-08</td>
<td>0.004</td>
</tr>
<tr>
<td>1.00E-07</td>
<td>0.005</td>
</tr>
</tbody>
</table>

\[ y = 46903x - 0.0001 \]
\[ R^2 = 0.9956 \]

Figure 3.15 A plot of concentration versus absorbance for a multiple concentration addition experiment at a cavity length of 0.1650 m using pre-soaked pipette tips.
According to the Beer-Lambert law, the slope of a plot of absorbance versus concentration will be the molar extinction coefficient. However, the slopes in Figure 3.9 – 3.17 vary between 46576 M\(^{-1}\)cm\(^{-1}\) and 59966 M\(^{-1}\)cm\(^{-1}\). This difference is a fundamental
disadvantage to the technique used in these experiments. The fact that the cell was removed between each experiment, therefore causing differences in the base ring-down time values might be thought to explain the differences in the molar extinction coefficient values. However, the manner in which the absorbance is calculated should stop the variability in $\tau_0$ causing errors in the determination of $\varepsilon$. R6G is a light sensitive dye; therefore, degradation could have occurred in the presence of room light. It is, therefore, more likely that the changes in $\varepsilon$ observed here are due to degradation of the R6G dye. Care was taken to minimize the exposure of the dye to light (storing in a dark cabinet when not in use), but a difference in the extinction coefficients was still observed. It was also determined that differences in air flow, humidity, and the amount of dust in the air on any given day caused fluctuations in the base ring-down time. In an effort to counteract (at least to minimize) some of these influences, a box was constructed out of foam board and placed around the optical cavity. Holes were present to allow for light to enter and exit the cavity. The box helped to minimize some of these factors; however, it could not eliminate them. It seems likely that a sealed dust free cavity would show better reproducibility, but no attempt was made to confirm this in these studies.

**Single Injection Experimental Data**

In an attempt to resolve the problem of the dye binding to and/or desorbing from the plastic pipette tips, all plastic materials were excluded from subsequent experiments. The dye was stored in glass volumetric flasks and dilutions and additions were made with glass pipettes. The main reason for performing the single injection experiments was to calculate the detection limit of the apparatus at each cavity length. The detection limit
calculation was taken from Harris.\textsuperscript{32} Briefly, seven replicate absorption values were measured for the addition of 1.00 mL of $2.36 \times 10^{-8}$ M R6G to 2.00 mL of filtered, deionized water for a final concentration of $7.87 \times 10^{-9}$ M. The standard deviation of the seven measurements was calculated and the detection limit was calculated using Equation 3.2 where $y_{dl}$ is the detection limit, $y_{blank}$ is the signal for the blank (zero in our case), $t$ is student’s $t$ value for 98% confidence, and $s$ is the standard deviation.

$$y_{dl} = y_{blank} + t \cdot s$$

Equation 3.2

Seven measurements performed at a cavity length of 0.5205 m are presented in Table 3.4.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.000642(10)</td>
</tr>
<tr>
<td>2</td>
<td>0.000917(22)</td>
</tr>
<tr>
<td>3</td>
<td>0.000575(10)</td>
</tr>
<tr>
<td>4</td>
<td>0.000546(14)</td>
</tr>
<tr>
<td>5</td>
<td>0.000466(20)</td>
</tr>
<tr>
<td>6</td>
<td>0.000715(10)</td>
</tr>
<tr>
<td>7</td>
<td>0.000740(31)</td>
</tr>
</tbody>
</table>

*Errors reported in parentheses represent one standard deviation in units of the last significant digit

The standard deviation for the seven measurements in Table 3.4 was calculated to be 0.000149. Using this standard deviation and student’s $t$ value of 3.143 for six degrees of freedom at 98 % confidence gave an absorption detection limit of 0.000470. In order to transform this detection limit into a concentration, the molar absorptivity ($8.35 \times 10^{4}$...
M$^{-1}$cm$^{-1}$) was calculated from the average absorbance value (0.000657) from Table 3.4 and a concentration of $7.87 \times 10^{-9}$ M R6G to obtain a value of $5.63 \times 10^{-9}$ M for the concentration detection limit.

Seven experiments were performed identical to that of the 0.5205 m cavity for a 0.2660 m cavity length. Table 3.5 lists these measured absorption values.

### Table 3.5

Seven Absorption Measurements of $7.87 \times 10^{-9}$ M R6G at 0.2660 m Cavity Length

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.000617(37)</td>
</tr>
<tr>
<td>2</td>
<td>0.000569(63)</td>
</tr>
<tr>
<td>3</td>
<td>0.000811(41)</td>
</tr>
<tr>
<td>4</td>
<td>0.000713(20)</td>
</tr>
<tr>
<td>5</td>
<td>0.000596(46)</td>
</tr>
<tr>
<td>6</td>
<td>0.000495(13)</td>
</tr>
<tr>
<td>7</td>
<td>0.000780(33)</td>
</tr>
</tbody>
</table>

*Errors reported in parentheses represent one standard deviation in units of the last significant digit

The standard deviation was calculated to be 0.000116 which gives an absorbance detection limit of 0.000366. Again, in order to transform this value into a concentration, the molar absorptivity ($8.31 \times 10^4$ M$^{-1}$cm$^{-1}$) was calculated from the average absorbance value (0.000654) from Table 3.5 and a concentration of $7.87 \times 10^{-9}$ M R6G to obtain a value of $4.40 \times 10^{-9}$ M for the concentration detection limit.

The absorbance and concentration detection limits were also calculated using the same process as above for the 0.1650 m cavity. Table 3.6 is the absorbance data for the seven single addition experiments at 0.1650 m cavity length.
Table 3.6

Seven Absorption Measurements of $7.87 \times 10^{-9}$ M R6G at 0.1650 m Cavity Length

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.000737(73)</td>
</tr>
<tr>
<td>2</td>
<td>0.000435(30)</td>
</tr>
<tr>
<td>3</td>
<td>0.000720(95)</td>
</tr>
<tr>
<td>4</td>
<td>0.000736(67)</td>
</tr>
<tr>
<td>5</td>
<td>0.000579(35)</td>
</tr>
<tr>
<td>6</td>
<td>0.000654(38)</td>
</tr>
<tr>
<td>7</td>
<td>0.000871(99)</td>
</tr>
</tbody>
</table>

*Errors reported in parentheses represent one standard deviation in units of the last significant digit

The standard deviation was calculated here to be 0.000139 which corresponds to an absorbance detection limit of 0.000436. The molar absorptivity ($8.59 \times 10^4$ M$^{-1}$cm$^{-1}$) was calculated from the average absorbance value (0.000676) from Table 3.6 and a concentration of $7.87 \times 10^{-9}$ M R6G to obtain a value of $5.08 \times 10^{-9}$ M for the concentration detection limit.

The absorbance detection limit was measured for a 0.4180 m cavity length via the same process as for the other cavity lengths. Table 3.7 is the absorbance data for the seven single addition experiments at 0.418 m cavity length.
Table 3.7

Seven Absorption Measurements of 7.87 x 10⁻⁹ M R6G at 0.4180 m Cavity Length

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.001020(38)</td>
</tr>
<tr>
<td>2</td>
<td>0.000786(29)</td>
</tr>
<tr>
<td>3</td>
<td>0.000744(54)</td>
</tr>
<tr>
<td>4</td>
<td>0.000605(30)</td>
</tr>
<tr>
<td>5</td>
<td>0.000780(25)</td>
</tr>
<tr>
<td>6</td>
<td>0.001018(82)</td>
</tr>
<tr>
<td>7</td>
<td>0.000769(16)</td>
</tr>
</tbody>
</table>

*Errors reported in parentheses represent one standard deviation in units of the last significant digit

The standard deviation was calculated to be 0.000151 which corresponds to an absorbance detection limit of 0.000473. The molar absorptivity (1.04 x 10⁵ M⁻¹cm⁻¹) was calculated from the average absorbance value (0.000817) from Table 3.6 and a concentration of 7.87 x 10⁻⁹ M R6G to obtain a value of 4.56 x 10⁻⁹ M for the concentration detection limit.

In summary, the absorption and concentration detection limits recorded during these studies are remarkably consistent and appear to be independent of cavity length. The absorption detection limit varied between 0.00037 and 0.00047 for the four cavity lengths studied herein. This suggests that scattering losses within this system do not depend upon the mirror spacing, but this conclusion is explored in greater detail in the following section.
**Ring-down Time Versus Cavity Length**

The insertion of a surface (or multiple surfaces as with a cuvette) into a cavity will cause reflection and scattering of light with the potential to induce large light losses and hence degrade the sensitivity of a CRDS spectrometer. In this work we have studied ring-down times as a function of cavity length and can directly observe the effect of window insertion and show that these reflections and scattering losses are minimized and do not limit the use of this technique to short cavities. In working with optical cavities where there are no surfaces inserted, the ring-down time increases directly with the cavity length. As will be shown here, the ring-down time with a cell inserted at normal incidence also increases linearly with cavity length. A scatter loss parameter $\delta_c$ was included in Equation 1.13. to allow for the possibility that there may be a variation in this loss with cavity length, $\delta_c$ can be expressed as a function of $d$:

$$\delta_c = \delta_0 + \delta_1 d$$  \hspace{1cm} \text{Equation 3.3}

Insertion of Equation 3.3 into Equation 1.13 yields:

$$\tau = \frac{d}{c} \left[ \frac{1}{-lnR+\delta_0+\delta_0d} \right]$$  \hspace{1cm} \text{Equation 3.4}

Which can be rearranged to produce:

$$\frac{1}{ct} = \left[-lnR + \delta_0\right] \left(\frac{1}{d}\right) + \delta_1$$  \hspace{1cm} \text{Equation 3.5}

Equation 3.5 shows that a plot of $1/ct$ vs. $1/d$ should yield a linear result and allow $\delta_0$ and $\delta_1$ to be calculated from the slope and intercept.
Figure 3.18  A plot to determine the distance-dependent scattering losses in a variable length cavity.

In Figure 3.18, the slope was calculated to be 0.00209(46) and the intercept was calculated to be –0.0014(18) m⁻¹. Using the slope, the value for \( \delta_0 \) was calculated to be 0.00206 using \( R = 0.99997 \). The fact that the intercept and, hence, the \( \delta_1 \) value is negative is not physically meaningful and the conclusion is supported by the fact that \( \delta_1 \) is not statistically different from zero (it differs by less than 2 standard deviations from zero). Figure 3.19 is the same plot, but here the intercept is constrained to zero.
The slope in Figure 3.19 was calculated to be 0.00176(24) which gives a value of 0.00173 for $\delta_0$ (again using $R = 0.99997$) which equals $\delta_c$ in Equation 1.13 since $\delta_0$ was constrained to zero.

The data in Figures 3.18 and 3.19 show two very important conclusions. First, these data show that there is a linear relationship between the cavity length and the ring-down time, which essentially means that the cavity length used while performing experiments of this nature is relatively unimportant. A cavity length can be constructed so that the size requirements are met by the experimental demands. Another conclusion of note from the data in Figures 3.18 and 3.19 is that the scatter from the laser beam passing through the surfaces of the cell does not prevent this method being used to measure very weak absorbances. It was originally thought that by introducing a standard cuvette at normal incidence, a 4% reflection loss at each surface per pass would occur, which would subsequently make the ring-down time too small to measure accurately.$^{35}$
However, this research has clearly demonstrated that this is not the case. Scattering losses are reduced to 0.17 % (as shown by the fitted $\delta_0$ value). A stable cavity and a stable, measurable, ring-down time can be achieved by employing a standard cuvette at normal incidence in a CRDS setup. Ring-down times of >1 $\mu$s have been achieved at a cavity length of 0.5205 m.

There are a few factors to consider before choosing an operating cavity length. First, during the process of constructing and aligning the different cavities, it was found that shorter cavities required much less effort to align. Longer cavities provide the possibility of different cavity modes to be excited requiring a much more precise alignment in order to obtain a single exponential decay. However, longer cavities provide better sensitivity. Longer ring-down times result from longer cavities and give a larger dynamic range to the technique for a given digitizer rate. Low concentrations cause large changes in $\tau$, but as sample concentration increases, progressively smaller changes in $\tau$ are observed. At higher concentrations, there are relatively small differences in $\tau$ and hence high-speed digitization becomes necessary. As a consequence, longer cavities and, hence, longer ring-down times allow this technique to record higher concentrations more accurately than shorter cavity systems.

**Comparison of the CRDS Detection Limit to a Commercial UV-vis Spectrometer**

The detection limit of the CRDS apparatus described in this work was compared to a Shimadzu UV-2550 UV-vis spectrometer. The detection limit of the commercial spectrometer was calculated in the same fashion as the CRDS technique using Equation 3.2. The absorbance values of all seven measurements of the blank were -0.002 giving a
standard deviation of zero. Table 3.8 gives the absorbance values at 525 nm ($\lambda_{\text{max}}$) for seven measurements of a 1.57 x $10^{-6}$ M R6G solution.

Table 3.8
Absorbance Values for Seven Measurements of a 1.57 x $10^{-6}$ M R6G Solution Using a Shimadzu UV-2550 UV-vis Spectrometer

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.106</td>
</tr>
<tr>
<td>2</td>
<td>0.109</td>
</tr>
<tr>
<td>3</td>
<td>0.109</td>
</tr>
<tr>
<td>4</td>
<td>0.115</td>
</tr>
<tr>
<td>5</td>
<td>0.118</td>
</tr>
<tr>
<td>6</td>
<td>0.117</td>
</tr>
<tr>
<td>7</td>
<td>0.118</td>
</tr>
</tbody>
</table>

The average of the seven values from Table 3.8 was 0.113 with a standard deviation of 0.00501. Using Equation 3.2, the absorbance detection limit was calculated to be 0.0157. The calculated molar absorptivity from the average absorbance value from Table 3.8 was 7.20 x $10^{4}$ M$^{-1}$cm$^{-1}$ which corresponds to a concentration detection limit of 218 nM. The average detection limit from the four different cavity lengths of the CRDS apparatus was 0.000436. The CRDS technique described here gave a more than 36 fold smaller detection limit significantly increasing the performance as compared to the commercial UV-vis spectrometer. It is acknowledged that these two measurement techniques require different measurement times and, therefore, this comparison is not definitive.
CHAPTER IV

CONCLUSIONS AND FUTURE WORK

Conclusions

The effectiveness of applying CRDS to the detection of liquids using a normal incidence quartz cuvette at four different cavity lengths was successfully demonstrated. The major conclusion from this work is that the careful attention paid to Brewster’s angle by other research groups is unnecessary. By carefully adjusting the position of a cuvette inside an optical cavity, the majority of the reflection and scattering losses at each surface can be recaptured by the mirrors and redirected back into the cavity. The detection limit calculated for this experimental setup was between 2-9 nM for R6G. This was a 36 fold smaller detection limit than that of a commercial UV-vis spectrometer. The majority of the other liquid CRDS examples listed in the Introduction gave detection limits in the range of 20-3000 nM. The lowest detection limit reported of the studies reviewed here was by Zare’s group, where the detection limit was 1-10 pM. However, in order to achieve a detection limit this low, the analyte of interest is required to be a strong absorber ($\varepsilon = 10^5-10^6 \text{ M}^{-1}\text{cm}^{-1}$).
Future Work

There are improvements that can be made to the existing system in order to improve functionality. First, the laser can be upgraded. The laser in use here is a relatively cheap light source and, therefore, there can be some beam steering effects if the laser head is not cooled properly. Also, by employing an oscilloscope with faster digitization, the dynamic range of the response will be improved.

Day to day drift in the base ring-down time was a problem throughout this research. Differences in humidity, air currents, and the amount of dust in the air on a given day were determined to be the culprit in the drift of the base ring-down time. A box was constructed to attempt to counteract some of these effects, but drift was still present. One way to completely counteract these effects would be to use a sealed optical cavity or to use an airtight cavity with optical windows or lenses present to allow for passage of the input and output beams. The box would be fitted with a vacuum port so that a vacuum could be achieved inside the box to create a stable, reproducible atmosphere in the cavity.

Other experiments to be performed would utilize a cuvette flow cell connected to an HPLC system in order to monitor a separation, to use a CW-laser source, and to measure fast chemical kinetics. As most HPLC detection systems involve UV wavelengths, the experimental setup would be modified to use a UV light source as well as mirrors that have a maximum reflectivity in the UV range. A CW-laser will require modification of the computer control system, but once it is achieved would increase the flexibility and lower the cost of the system. Finally, the system as designed, or in which
a CW-laser is incorporated, could be used to record the concentrations of analytes as a function of time to perform accurate kinetic studies.
REFERENCES CITED


APPENDIX A

VISUAL BASIC PROGRAM FOR DATA COLLECTION AND ANALYSIS
Option Explicit
On
Imports System
Imports System.Drawing
Imports System.IO
Imports System.Math

Public Class textbox1
    Public y(1000) As Integer
    Public y_avg(1000) As Double
    Public y_real(1000) As Double
    Public y_log(1000) As Double
    Public y_save(1000) As Integer
    Public parm(2) As Double
    Public xincr As Double
    Public AqMode As String
    Public dataStream As String
    Public fields() As String
    Public charArray() As Char
    Public binHeader As String
    Public ProgName As String = "IEEETalk: ver 3.0 "
    Public ScopeChan As String = "CH1"
    Public graph_scale As Double
    Public graph_off As Double
    Public TopWinX As Integer = 1
    Public Navg As Integer
    Public num_end As Integer
    Public num_end_Str As String
    Public point1 As New Point(120, 0)
    Public point2 As New Point(120, 420)
    Public point3 As New Point(900, 0)
    Public point4 As New Point(900, 420)
    Public StoreFitPoint1 As Point
    Public StoreFitPoint2 As Point
    Public root As String
    Public space As String = " "
    Public StartFlag = False
    Public tr As StreamWriter
    Public rm As Ivi.Visa.Interop.ResourceManager
Public ioArbFG As Ivi.Visa.Interop.FormattedIO488
Public msg As Ivi.Visa.Interop IMessage
'Dim bm As New Bitmap(1160, 460)
'Dim g As Graphics = Graphics.FromImage(bm)
Protected g As Graphics
Protected a As GraphClass
Protected g2 As Graphics
Protected a2 As GraphClass
Private Sub Form1_Load(ByVal sender As Object, ByVal e As System.EventArgs) _
    Handles Me.Load
    Dim date1 As Date = Date.Now
    Dim idays As Integer = date1.Day
    Dim imnth As Integer = date1.Month
    Dim mnth As String
    Dim days As String
    g = pb1.CreateGraphics()
    a = New GraphClass(g)
    g2 = pb2.CreateGraphics()
    a2 = New GraphClass(g2)

    mnth = CType(imnth, String)
    If imnth < 10 Then
        mnth = "0" & mnth
        End If
    days = CType(idays, String)
    If idays < 10 Then
        days = "0" & days
        End If
    root = "C:\scopedata\" & mnth & days
    num_end = 0
    Me.FileNameBox.Text = filenamer(0)
    Me.TextBox2.Text = CType(point1.X, String)
    Me.TextBox3.Text = CType(point3.X, String)
    Me.Text = ProgName & " No data read"
    Me.RAvgText.Text = "10"
    Me.LAvgTextBox.Text = "2"
    rm = New Ivi.Visa.Interop.ResourceManager
    msg = rm.Open("GPIB0::1", Ivi.Visa.Interop.AccessMode.NO_LOCK, 2000, "")
    ioArbFG.IO = msg
    ioArbFG.WriteString("*CLS;:HEADER OFF") ' reset scope; set terse response
    End Sub
'******************************************************************************
' Main Start/Stop button routine.
******************************************************************************
Public Sub cbStartGet_Click(ByVal sender As Object, ByVal e As System.EventArgs) _
    Handles cbStartGet.Click
    StartFlag = Not StartFlag
    If StartFlag Then
        Me.FileNameBox.Text = filenamer(1)
        Call IEEE488set()
        Call Aquire()
        'pb1.Image = bm
    End If
    End Sub
'******************************************************************************
' Loops for a specified period of time (milliseconds)
Private Sub delay(ByVal interval As Integer)
    Dim sw As New Stopwatch
    sw.Start()
    Do While sw.ElapsedMilliseconds < interval ' Keep UI responsive
        Application.DoEvents()
    Loop
    sw.Stop()
End Sub

'***************************************************************************
' Data acquisition. Data request/transmission takes <100 ms. When averaging
' wait long enough to roll in a new set of data before transmission.
' 10 Hz laser so wait (numAvg - 1) * 100 ms
'***************************************************************************

Public Sub Aquire()
    Dim waitTime As Integer
    Dim counter As Integer
    Dim avgstop As Integer
    Dim RealAvgEnd As Double
    Dim k As Integer

    Me.Text = ProgName & " - Unsaved data"
    tr = File.CreateText(filenamer(0) & ".fit") ' Open a fit/R-squared values file
    Call WriteParameters()
    Call ButtonsOn(False) ' lock-out buttons during acquire
    If LAvgOnce.Checked Then
        avgstop = 1 ' no local average
    Else
        avgstop = CType(Me.LAvgTextBox.Text, Integer) ' local averaging
    End If
    RealAvgEnd = CType(avgstop, Double)
    waitTime = 100 * (Navg - 1)
    pb2.Image = Nothing ' reset top window to blank

    TopWinX = 0
    Do While StartFlag
        Application.DoEvents() ' Detect "stop"(startflag=false)
        counter = 1
        For k = 1 To 1000
            y_avg(k) = 0.0
        Next k
        Do While counter <= avgstop
            Call GetScopeDat() ' grab data
            For k = 0 To 1000
                y_avg(k) = y_avg(k) + y_real(k) / RealAvgEnd ' average each point
            Next k
            counter += 1
        Loop
        If LogOutRadio.Checked Then
            Call GetLog(y_avg)
            parm = lsqft(y_log, point1.X, point3.X)
            Call PlotData(y_log, Pens.White)
            Call fitOut()
        End If
    Loop
Call ShowFit(Pens.Red)
Else
  Call PlotData(y_avg, Pens.White)
End If
Call delay(waitTime)
Loop
Call ButtonsOn(True) ' reenable buttons
tr.Close()
End Sub

'******************************************************************************
' Turn-on/Lock-out buttons (true = on)
'******************************************************************************
Public Sub ButtonsOn(ByVal status As Boolean)
  If status Then
    cbStartGet.Text = "Aquire"
  Else
    cbStartGet.Text = "Stop"
  End If
  cbClear.Enabled = status
cbExit.Enabled = status
cbReplot.Enabled = status
cbSave.Enabled = status
cbRefit.Enabled = status
ReadFile.Enabled = status
ChanBox.Enabled = status
DisplayTypeBox.Enabled = status
AquisitionBox.Enabled = status
LocAvBox.Enabled = status
DataBox.Enabled = status
BaseBox.Enabled = status
FitBox.Enabled = status
End Sub

'******************************************************************************
*****
' GetScopeDat - Read and store waveforms from a TEK TDS340 oscilloscope
'******************************************************************************
*****
' RPB data (positive binary). scope: 0 (bottom of screen) to 65535 (top); 1000 points
' Data stream: 6-byte header (#42000), 2 bytes/datum (most-sig. then least sig.),
' LF terminator = 2007 bytes total. Convert to 1000 points stored in y(k).
'******************************************************************************
*****
Public Sub GetScopeDat()
  Dim k As Integer
  Dim IMAX As Integer = 2006
  ioArbFG.WriteString("*WAI;:CURV?")
dataStream = ioArbFG.ReadString()
charArray = dataStream.ToCharArray
k = 0
For i = 6 To IMAX - 1 Step 2
  k += 1
  y(k) = Asc(charArray(i)) * 256 + Asc(charArray(i + 1))
y_real(k) = CType(y(k), Double)
Next i
End Sub

'*********************************************************************************
******
' Correct baseline to zero. Calculate log(y). Baseline > Io.
'*********************************************************************************
-------
' Either (i) subtract constant baseline Ibase (1st 20 pt avg.), or (ii) subtract linear
' slope (use 1st and last 20 point averages). Calculate(ln(Ibase - I) / (Ibase - Io)).
'*********************************************************************************
******
Public Sub GetLog(ByVal ry() As Double)
    Dim ymax As Double = 0.0
    Dim ybase_start As Double
    Dim ybase_end As Double
    Dim base_slope As Double
    Dim ysum As Double
    ysum = 0.0
    For k = 1 To 20
        ysum += ry(k)
    Next k
    ybase_start = 0.05 * ysum ' avg 1st 20 points (~50,000 in dummy data)
    ysum = 0.0
    For k = 981 To 1000
        ysum += y_real(k)
    Next k
    ybase_end = 0.05 * ysum ' avg last 20 points (~50,000)
    base_slope = 0.001 * (ybase_end - ybase_start) ' avg slope ("drop"/1000 points)
    ymax = 0
    For k = 1 To 1000
        ry(k) = ybase_start - ry(k) ' remove offset (baseline = 0)
        If SlopeBackSub.Checked Then ' correct for sloping baseline?
            ry(k) = ry(k) + base_slope * CType(k, Double)
        End If
        If ry(k) <= 1.0 Then ' All data > 1 (force ln
            y_log(k) = 0.0
        Else
            y_log(k) = Log(ry(k)) ' Natural log of signal
        End If
    Next k
End Sub

'*********************************************************************************
******
' Plot data on screen. Find data extrema; scale to 5 - 410 pixel "window"
'*********************************************************************************
******
Public Sub PlotData(ByVal ry() As Double, ByVal Color As Pen)
    Dim Vmin As Double
    Dim Vmax As Double

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Dim k As Integer
Dim iy As Integer

Vmin = ry(1)
Vmax = Vmin
For k = 2 To 1000
    If ry(k) < Vmin Then
        Vmin = ry(k)
    End If
    If ry(k) > Vmax Then
        Vmax = ry(k)
    End If
Next k
If (Vmax - Vmin) <> 0.0 Then
    graph_scale = 405.0 / (Vmax - Vmin)
Else
    graph_scale = 405.0
    MsgBox("data problems: range = 0")
End If

graph_off = Vmin
For k = 1 To 1000
    iy = CType((ry(k) - graph_off) * graph_scale, Integer) - 5
    a.PlotPoint(k, y_save(k), Pens.Black) 'erase previous datum
    a.PlotPoint(k, iy, Color) 'plot new datum
    y_save(k) = iy 'store current datum
Next k

End Sub

'******************************************************
' Shut down
'******************************************************
Private Sub cbExit_Click(ByVal sender As Object, _
    ByVal e As System.EventArgs) Handles cbExit.Click
    Application.Exit()
End Sub

'******************************************************
' Clear the screen
'******************************************************
Private Sub cbClear_Click(ByVal sender As Object, _
    ByVal e As System.EventArgs) Handles cbClear.Click
    pb1.Image = Nothing
    Me.TextBox1.Clear()
    a.SetupFrame()
End Sub

Private Sub radiobutton2_Click(ByVal sender As Object, _
    ByVal e As System.EventArgs) Handles FileTypeBinary.Click
    Me.FileNameBox.Text = filenamer(0) & ""bdt"
End Sub

Private Sub radiobutton5_Click(ByVal sender As Object, _
    ByVal e As System.EventArgs) Handles FileTypeAscii.Click
    Me.FileNameBox.Text = filenamer(0) & ""txt"
End Sub

'******************************************************
' Write data set to disk
'******************************************************
Public Sub cbSave_Click(ByVal sender As Object, ByVal e As System.EventArgs) Handles cbSave.Click

If FileTypeBinary.Checked Then ' Write a binary data file
    Me.Text = filenamer(0) & ".bdt saved"
    Dim fs As New FileStream(filenamer(0) & ".bdt", FileMode.Create)
    Dim w As New BinaryWriter(fs)
    Try
        w.Write(binHeader$)
        w.Write(dataStream)
    Catch ex As Exception
        MsgBox("Empty Data Set!")
        Me.Text = ProgName & " Empty data set!"
    End Try
    w.Close()
    fs.Close()
Else ' create an ascii file
    Me.Text = filenamer(0) & ".txt saved"
    Dim sr As StreamWriter = File.CreateText(filenamer(0) & ".txt")
    Try
        For i = 1 To 1000
            sr.WriteLine("{0}", y(i))
        Next i
    Catch ex As Exception
        MsgBox("Empty Data Set!")
        Me.Text = ProgName & " Empty data set!"
    End Try
    sr.Close()
End If
Me.FileNameBox.Text = filenamer(0)
End Sub

'******************************************************************************
' Read an existing data set
'******************************************************************************

Public Sub cbRead_Click(ByVal sender As Object, ByVal e As System.EventArgs) Handles ReadFile.Click

Dim k As Integer
Dim IMAX As Integer = 2006
Dim stFileName As String = ""
Dim stFilePathAndName As String = ""
Dim openFileDialog1 As New OpenFileDialog

openFileDialog1.InitialDirectory = "C:\scopedata"
openFileDialog1.Title = "Open Binary Data File"
openFileDialog1.Filter = "Binary files (*.bdt)|*.bdt"
openFileDialog1.FilterIndex = 1
openFileDialog1.RestoreDirectory = True
If openFileDialog1.ShowDialog() = DialogResult.OK Then
    stFilePathAndName = openFileDialog1_FileName
    Dim MyFile As FileInfo = New FileInfo(stFilePathAndName)
    stFileName = MyFile.Name
    Me.Text = ProgName & stFileName
    Dim fs = New FileStream(stFilePathAndName, FileMode.Open, FileAccess.Read)
    Dim r As New BinaryReader(fs)
binHeader$ = r.ReadString()
fields = Split(binHeader, ";")
xincr = fields(4)
dataStream = r.ReadString()
r.Close()
fs.Close()
charArray = dataStream.ToCharArray
k = 0
For i = 6 To IMAX - 1 Step 2
  k += 1
  y(k) = Asc(charArray(i)) * 256 + Asc(charArray(i + 1))
  y_real(k) = CType(y(k), Double)
Next i
End If
Me.TextBox2.Text = CType(point1.X, String)
Me.TextBox3.Text = CType(point3.X, String)
a.SetupFrame()
If LogOutRadio.Checked Then
  Call PlotData(y_log, Pens.White)
Else
  Call PlotData(y_real, Pens.White)
End If
End Sub
'********************************************************
' Replot a dataset
'********************************************************
Public Sub cbReplot_Click(ByVal sender As Object, ByVal e As System.EventArgs) Handles cbReplot.Click
a.SetupFrame()
If LogOutRadio.Checked Then
  Call PlotData(y_log, Pens.White)
  If ShowFitRadio.Checked Then
    Call ShowFit(Pens.Red)
  End If
Else
  Call PlotData(y_real, Pens.White)
End If
End Sub
'********************************************************
'Refit a data set
'********************************************************
Public Sub cbRefit_Click(ByVal sender As Object, ByVal e As System.EventArgs) Handles cbRefit.Click
Dim i As Point
If point1.X > point3.X Then
  i = point1
  point1 = point3
  point3 = i
  i = point2
  point2 = point4
  point4 = i
End If
parm = lsqft(y_log, point1.X, point3.X)
Call PlotData(y_log, Pens.White)
Call fitOut()
If ShowFitRadio.Checked Then
  Call ShowFit(Pens.Red)
End If
End Sub

'*********************************************************************************
* Write Fit-file header (file tr is opened and closed in subroutine)
'*********************************************************************************

Public Sub WriteParameters()
  Dim avginfo As String
  Dim scopecount As String
  scopecount = CType(Navg, String)
  If AqMode = "SAMPLE" Then
    avginfo = "No scope averaging. "
  Else
    avginfo = CType(Navg, String) & " scope averages. "
  End If
  Try
    tr.WriteLine(avginfo & "Cursor locations: " & point1.X & " and " & point3.X & " pixels."
    tr.WriteLine(binHeader)
  Catch ex As Exception
    MsgBox("******.fit header write error!")
  End Try
End Sub

'**************************************************************
' Output Ring-down fit parameters to the text boxes and disk
'**************************************************************
Public Sub fitOut()
  Dim unit As String
  Dim Tau As Double
  Dim sTau As String
  Dim sR2 As String

  If parm(1) <> 0.0 Then
    Tau = -xincr / parm(1)
  Else
    Tau = 0.0
    MsgBox("fit error, slope = 0")
  End If
  If StartFlag Then
    Try
      tr.WriteLine(Tau & space & parm(2))
    Catch ex As Exception
      MsgBox("******.fit write error!")
    End Try
  End If
  unit = "s"
  If Tau < 1.0 Then
    Tau = Tau * 1000.0
    unit = "ms"
  If Tau < 1.0 Then
    Tau = Tau * 1000.0
    unit = space & Chr(181) & "s"
  If Tau < 1.0 Then
\[ \text{Tau} = \text{Tau} \times 1000.0 \]
\[ \text{unit} = "\text{ns}" \]

End If
End If
End If
Call PlotFitResult(Tau)
sTau = Mid(CType(Tau, String), 1, 6)
\[ \text{sR2} = \text{Mid} (\text{CType} (\text{parm}(2), \text{String}), 1, 8) \]
Me.TextBox1.Text = "\text{Tau} = " & sTau & " \text{unit} & " \text{R} & \text{Chr}(178) & " = " & sR2

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a.DrawLine(Pens.Blue, spoint4, spoint3)
a.DrawLine(Pens.Blue, spoint2, spoint1)
FitPoint1.Y = yframel - CType(graph_scale * (parm(0) + parm(1) * 
    CType(point1.X, Double)), Integer)
FitPoint2.Y = yframel - CType(graph_scale * (parm(0) + parm(1) * 
    CType(point3.X, Double)), Integer)
FitPoint1.X = point1.X + offset
FitPoint2.X = point3.X + offset
a.DrawLine(Pens.Black, offset, StoreFitPoint1, StoreFitPoint2) ' erase last fit
line
If ShowFitRadio.Checked Then
    a.DrawLine(color, FitPoint1, FitPoint2) ' draw new fit
End If
StoreFitPoint1 = FitPoint1
StoreFitPoint2 = FitPoint2
End Sub
'*********************************************************************************
** ' Move a "fit range" cursor
' Note the displacement between data (point) and plot points (spoints)
'*********************************************************************************
**
Public Sub PlotData_MouseUp(ByVal sender As Object, ByVal e As MouseEventArgs)
    Handles pb1.Click
    Dim spoint1 As Point
    Dim spoint2 As Point
    Dim offset As Integer = FrameCoord.HorizontalDisplacement
    Dim yPixelMax As Integer = 410

    spoint1.X = point1.X + offset
    spoint2.X = point2.X + offset
    spoint1.Y = point1.Y
    spoint2.Y = point2.Y
    a.DrawLine(Pens.Black, spoint1, spoint2) ' erase oldest cursor
    point1 = point3 ' move newest to oldest cursor
    point2 = point4
    Me.TextBox2.Text = CType(point1.X, String)
    If e.Button = MouseButtons.Left Then
        point3.X = MousePosition.X - Me.Bounds.Left - 75 ' generate new cursor
        point3.Y = 0
    End If
    If point3.X < 1 Then
        point3.X = 1
    End If
    If point3.X > 1000 Then
        point3.X = 1000
    End If
    point4.X = point3.X
    point4.Y = ypixelmax
    spoint1.X = point3.X + offset
    spoint2.X = spoint1.X
    spoint1.Y = point3.Y

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Spoint2.Y = point4.Y
Me.TextBox3.Text = CType(point4.X, String)
a.DrawLine(Pens.Blue, Spoint1, Spoint2)
End Sub

'*********************************************************************************
******
' Linear least squares: m = (n*Sxy - Sx*Sy) / (n*Sx^2 - Sx*Sx)  b = ymean - m
* xmean
' R-squared = (s(x-xmean)*(y-ymean))^2 / s[(x-xmean)^2] * s[(y-
* ymean)^2]
' n = no. of points, Sx^2 = sum of x^2 etc.
'*********************************************************************************
******

Public Function lsqft(ByVal Vcalc() As Double, ByVal kmin As Integer, _
ByVal kmax As Integer) As Double()

Dim i As Integer
Dim isx As Integer = 0
Dim isx2 As Integer = 0
Dim sx As Double
Dim sx2 As Double
Dim sxy As Double = 0.0
Dim sy As Double = 0.0
Dim y As Double
Dim ymean As Double
Dim xmean As Double
Dim rn As Double
Dim x As Double
Dim xerr As Double = 0.0
Dim xerrr2 As Double = 0.0
Dim xyerr As Double = 0.0
Dim yerr As Double = 0.0
Dim yerrr2 As Double = 0.0
Dim ydiff As Double
Dim xdiff As Double
Dim den As Double

If kmax < kmin Then ' ensure kmax > kmin
    i = kmin
    kmin = kmax
    kmax = i
End If
rn = CType(kmax - kmin + 1, Double)
xmean = 0.5 * CType(kmax + kmin, Double)
For i = kmin To kmax
    isx += i
    isx2 = isx2 + i * i
    y = Vcalc(i)
    x = CType(i, Double)
    sxy = sxy + y * x
    sy += y
Next i
ymean = sy / rn
sx = CType(isx, Double)
sx2 = CType(isx2, Double)
den = rn * sx2 - sx * sx
If den <> 0 Then ' protect against divide
    parm(1) = (rn * sxy - sx * sy) / den
Else
    parm(1) = 1.0
    MsgBox("1sqs failure")
End If
parm(0) = ymean - parm(1) * xmean
For i = kmin To kmax
    x = CType(i, Double)
    xdiff = x - xmean
    ydiff = Vcalc(i) - ymean
    xyerr = xyerr + xdiff * ydiff
    xerr2 = xerr2 + xdiff * xdiff
    yerr = yerr + ydiff
    yerr2 = yerr2 + ydiff * ydiff
Next i
if den <> 0 Then ' protect against divide
    parm(2) = xyerr * xyerr / (xerr2 * yerr2)
else
    parm(2) = 1.0
End If
Return parm
End Function
'******************************************************************************
******
' Maintain auto file numbering
'******************************************************************************
******
Public Function filenamer(ByVal increment As Integer)
    num_end += increment
    If num_end < 10 Then
        num_end_Str = "0" & CType(num_end, String)
    Else
        num_end_Str = CType(num_end, String)
    End If
    filenamer = root & num_end_Str
End Function
'******************************************************************************
******
' Set up channel, averaging... for IEEE data transfer
'******************************************************************************
******
Public Sub IEEE488set()
    Dim value As String
    
    If GetCH1.Checked Then
        ScopeChan$ = "CH1"
        ioArbFG.WriteString("SEL:CH1 ON;CH2 OFF;MATH1 OFF;REF1 OFF;:DAT:SOU CH1")
    Else
        If GetCH2.Checked Then
            ScopeChan$ = "CH2"
            ioArbFG.WriteString("SEL:CH1 OFF;CH2 ON;MATH1 OFF;REF1 OFF;:DAT:SOU CH2")
    End If

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Else
  If GetMath.Checked Then
    ScopeChan$ = "MATH1"
    ioArbFG.WriteString("SEL:CH1 OFF;CH2 OFF;MATH1 ON;REF1 OFF;:DAT:SOU MATH1")
  Else
    ScopeChan$ = "REF1"
    ioArbFG.WriteString("SEL:CH1 OFF;CH2 OFF;MATH1 OFF;REF1 ON;:DAT:SOU REF1")
  End If
End If
Else
  If GetOnce.Checked Then
    ioArbFG.WriteString("ACQ:MOD SAM") ' set scope to sample data
  Else
    value = Me.RAvgText.Text
    If value > 256 Then
      value = 256
      Me.RAvgText.Text = 256
    End If
    ioArbFG.WriteString("ACQ:MOD AVE;NUMAV " & value) ' set to average data
  End If
ioArbFG.WriteString("DAT:ENC RPB;WID 2;STAR 1;STOP 1000")
binHeader = ioArbFG.ReadString() & ";
fields = Split(binHeader, ";")
xincr = fields(4)
ioArbFG.WriteString("ACQ:NUMAV?;MOD?;:DAT:WID?")
value = ioArbFG.ReadString() & ";
fields = Split(value, ";")
Navg = CType(fields(0), Integer)
AqMode = CType(fields(1), String)
If AqMode = "SAMPLE" Then
  Navg = 1
End If
End Sub
Private Sub RadioButton2_CheckedChanged(ByVal sender As System.Object, ByVal e As System.EventArgs) Handles ShowFitRadio.CheckedChanged
End Sub
Private Sub GroupBox2_Enter(ByVal sender As System.Object, ByVal e As System.EventArgs) Handles DisplayTypeBox.Enter
End Sub
Private Sub GroupBox6_Enter(ByVal sender As System.Object, ByVal e As System.EventArgs) Handles AquisitionBox.Enter
End Sub
Private Sub RadioButton4_CheckedChanged(ByVal sender As System.Object, ByVal e As System.EventArgs) Handles SlopeBackSub.CheckedChanged
End Sub
Private Sub RadioButton2_CheckedChanged(ByVal sender As System.Object, ByVal e As System.EventArgs) Handles FileTypeBinary.CheckedChanged
End Sub

**********************************************************************************
******
Protected Enum FrameCoord
    X_Axis_VertLoc = 410
    HorizontalDisplacement = 50
    Y_Axis_XEnd = 1050
    Y_Axis_Start = 50
    X_Axis_Begin = HorizontalDisplacement
End Enum
Protected Enum FrameCoord2
    X_Axis_VertLoc = 110
    HorizontalDisplacement = 50
    Y_Axis_XEnd = 1050
    Y_Axis_Start = 20
    X_Axis_Begin = HorizontalDisplacement
End Enum
Public Class GraphClass
    Private Intersection As Point = New Point(FrameCoord.HorizontalDisplacement, _
        FrameCoord.X_Axis_VertLoc)
    Protected g As Graphics ' ring-down graphics
    Protected AxisColor As Color = System.Drawing.ColorTranslator.FromOle(&HFF00)
    Protected AxisPen As Pen
    Public Sub New(ByVal obj As Graphics)
        g = obj
        AxisPen = New Pen(AxisColor, 2)
    End Sub
    Public Sub SetupFrame()
        Dim pY_Begin As New Point(FrameCoord.HorizontalDisplacement, _
            FrameCoord.Y_Axis_Start)
        Dim pY_End As New Point(FrameCoord.HorizontalDisplacement, _
            FrameCoord.X_Axis_VertLoc)
        Dim pX_Begin As Point = Intersection
        Dim pX_End As New Point(FrameCoord.Y_Axis_XEnd, _
            FrameCoord.X_Axis_VertLoc)
        Drawline(AxisPen, pY_Begin, pY_End)
        Drawline(AxisPen, pX_Begin, pX_End)
        For i As Short = FrameCoord.HorizontalDisplacement + 50 To _
            FrameCoord.Y_Axis_XEnd Step 50
            Drawline(Pens.BlanchedAlmond, New Point(i, _
                FrameCoord.X_Axis_VertLoc - _
                10), New Point(i, _
                FrameCoord.X_Axis_VertLoc))
        Next
        For i As Short = FrameCoord.X_Axis_VertLoc - 50 To _
            FrameCoord.Y_Axis_Start -
Step -

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    Drawline(Pens.BlachedAlmond, _
            New Point(FrameCoord.HorizontalDisplacement, i), _
            New Point(FrameCoord.HorizontalDisplacement + 10, i))

    Next
End Sub

Public Sub PlotPoint(ByVal X As Integer, ByVal Y As Integer, Optional ByVal pn _
            As Pen = Nothing)
    Dim xp As Integer = (-Y + FrameCoord.X_Axis_VertLoc)
    g.DrawLine(pn, New Point(X + Intersection.X, xp), _
            New Point(X + 1 + Intersection.X, xp + 1))
End Sub

Public Sub PlotPoint2(ByVal X As Integer, ByVal Y As Integer, Optional ByVal pn As Pen = Nothing)
    Dim xp As Integer = (-Y + FrameCoord2.X_Axis_VertLoc)
    g.DrawLine(pn, New Point(X + Intersection.X, xp), _
            New Point(X + 1 + Intersection.X, xp + 1))
End Sub

Public Sub Drawline(ByVal P As Pen, ByVal StartPoint As Point, ByVal EndPoint As Point)
    g.DrawLine(P, StartPoint, EndPoint)
End Sub
End Class
End Class