Determination of mercury(II) concentration in water using dithizone.

Master level subject module project in chemistry House 28 - RUC 2017

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Abstract

This project uses UV-VIS spectroscopy to quantify the con concentration of mercury(II) ion in aqueous solutions. This is done by extracting the ions with dithizone in chloroform and measuring the absorbance of the formed dithizone and mercury coordination complexes in chloroform. And comparing this absorption with an external calibration curve. It was possible to determine mercury(II) concentrations as low as $\approx$ 40 ppbs. The molar absorptivity coefficients for pure dithizone in chloroform and the dithizone mercury(II) complex, in chloroform, were determined to be $37.3 \cdot 10^3$ and $68{,}8 \cdot 10^3$ $(M^{-1} \cdot cm^{-1})$ respectively.

Contents

Introduction

In Bolivia and other countries in south America, there is a lot mining activities, and where there is mining there might be pollution. In rural areas there might not be any governmental organs to oversee the amounts of wastes produced by the mining companies. A cheap and simple method for on-site determination of various heavy metals, in the water, could facilitate local high school level students in alerting officials to a possible contamination. The ambition is the be able to detect mercury level as low as 10 ppb, using the procedures described in the project.
Originally the intention were to make a kit, consisting of various pre-made solutions of dithizone in chloroform and buffers in the relevant pH ranges. Such that determination could be done with a portable uv-vis detector and laptop. The scope of this task were too much for time we had available. Instead this will be a collection of the fundamental knowledge necessary to understand the principles behind uv-vis spectroscopy, buffer solutions and complex formation.

1.1 Problem statement

Which procedures should be followed when using dithizone to determine concentrations of heavy metals in the ppb ranges, using portable equipment?

Theory

2.1 Lambert Beer's law

The color of a substance is determined by which wavelengths, of light, the substance absorbs. In the visible range of light, 400 - 780 nm, light rays, called photons, excites an electron in a molecule, the photon is then said to be absorbed, by the molecule. Beer-Lambert's law is derived from this phenomenon, by analyzing the ratio of the intensity of light shone into the sample, over what can be detected on the other side. This form the basis of spectrophotometry which can be used to determinate concentrations of various species, dissolved in a liquid.

When working with spectrophotometry, the concept of transmittance needs to be introduced, since older articles uses this. Transmittance, T is the ratio between the intensity of the light shone into the sample and the intensity recorded on the other side of the sample. $T = \frac{I_1}{I_0}$, where $I_0 > I_1 \geq 0$, that is the intensity of the light never increases, and defined to be non-negative.

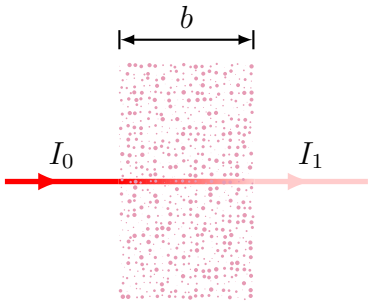

Figure 2.1: The intensity of the light diminishes when it travels through the sample.

Figure 2.1, illustrates the setting for deducing Beer-Lambert's law. The law stipulates that there is a relationship between I_0 and I_1. There are two factors

governing this relationship, both are related to the number of molecules in the sample. The concentration of the sample and the width of the vessel, containing the sample. A more concentrated sample, will have more molecules, per mL, which will absorb more photons, than a sample with a lower concentration. If the width of the sample, corresponding to "b" on figure 2.1, is doubled, the light will collide with twice as many molecules. Let the concentration of the sample be denoted by c, and the width by b. Then the above mentioned criteria can be expressed as $I_1 \propto c \cdot b \cdot I_0$, this proportionality can be exchanged with equality if a proportionality constant β is included, yielding $I_1 = \beta \cdot c \cdot b \cdot I_0$.

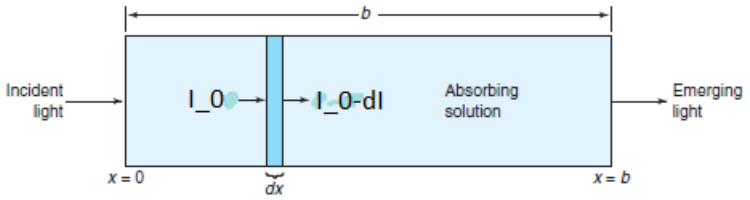

Figure 2.2: The change in intensity, when the light has traveled an infinitely short distance, dx. Figure from [2]

In the above it has been assumed that the contribution of c and b are linear. That is the effect of widening the pathway, could also be obtained by increasing the concentration. The intensity can be described as a function of either concentration or path length. This means that you can find the change in intensity, when the light has traveled a short distance, dx, through the sample, see figure 2.2. Let $I(x)$ denote the intensity as a function of distance traveled. Thus a small change in intensity, $dI(x)$ can be described as $dI(x) = -\beta \cdot c \cdot dx \cdot I(x)$. The minus sign is included, since a change in in intensity must be a reduction, according to inequality $I_0 > I_1 \geq 0$. An equation as this one, is called a differential equation, where the change in intensity, $dI(x)$, in respect to the change in position, dx, is dependent on its

intensity at a previous stage, $I(x)$. This differential equation can be solved by a method called separation of the the variables. The first step is to separate the variables, in this case $I(x)$ and x

$$\frac{1}{I(x)}dI(x) = -\beta \cdot c \cdot dx$$

Now everything dependent on x is on the left side and dx is on the right side. The next step is to integrate both sides;

$$\int_{I_0}^{I_1} \frac{1}{I(x)}dI(x) = \int_0^b -\beta \cdot c dx$$

The limits of integration were chosen by the following reasoning, the maximum change in intensity, would be from no change, I_0 to the recorded intensity when the light has passed the sample I_1. Similarly the light can travel from $x = 0$ to $x = b$.
The next step is to evaluate the primitives at the limits of integration.

$$[\ln I(x)]_{I_0}^{I_1} = [-\beta \cdot c \cdot x]_0^b \Leftrightarrow$$

$$\ln I_1 - \ln I_0 = -\beta \cdot c \cdot b - (-\beta \cdot c \cdot 0) = -\beta \cdot c \cdot b$$

By multiplying both side by -1, and collecting the logarithm the following is obtained.

$$\ln \frac{I_0}{I_1} = \beta \cdot c \cdot b$$

The convention is to express this in term of $\log_{10}$, and not the natural logarithm, this can be done by using that the functions 10^x and $\log_{10} x$ are each others inverses. Thus $\log_{10} 10^x = x$ or $10^{\log_{10} x} = x$.

$$\ln 10^{\log_{10} \frac{I_0}{I_1}} = \beta \cdot c \cdot b \Leftrightarrow$$

$$\ln 10 \log_{10} \frac{I_0}{I_1} = \beta \cdot c \cdot b \Leftrightarrow$$

Since β was an arbitrary proportionality constant, both sides can be divided by $\ln 10$, and name $\frac{\beta}{\ln 10} = \epsilon$. This leads to

$$\log_{10} \frac{I_0}{I_1} = \epsilon \cdot c \cdot b$$

which is Beer-Lambert's law, where $\log_{10} \frac{I_0}{I_1}$ is called the absorbance, A. ϵ is called the molar absorptivity coefficient.

$$A = \epsilon \cdot c \cdot b \tag{2.1}$$

Beer's law relates absorbance, to the concentration, this is what spectrophotometry utilizes. This will be elaborated shortly, but first a few notes regarding the formula.
The absorbance can not be negative, this would require the fraction $\frac{I_0}{I_1} < 1$, this would mean that the light should gain intensity when passing through the sample. If a sample does this, it violates premises for Beer's law to be valid. The range of absorbance, $A = (0,1.3)$ corresponds to 0 to 95 % absorbance.
When a spectra is obtained for a sample, the absorbance vary over the different wavelengths, since the absorbance vary, (the left side of 2.1), this must result in a proportional change of the right side, the only quantity able to vary is ϵ. A more correct way to state 2.1 would be to indicate the dependency on wavelength.

$$A_\lambda = \epsilon_\lambda \cdot c \cdot b$$

The absorbance, A is related to transmittance, T, by $A = -\log_{10} T$.

Application of Beer's law

The main processes of the spectrophotometer are shown in figure 2.3. Light of all wavelengths, are sent through the sample, these wave length are split up by diffraction grating. And each wave length are then recorded on a diode array. The intensity of the light, I_0, are the same for all wavelengths when it leaves the the light source. The width of the sample, b, are the same for all the measurements. When obtaining a spectrum of a sample, with fixed concentration, the absorption is recorded and plotted against wavelength. From equation 2.1, it is then given that, if you repeat the process, and prepare a new sample, with the same concentration, it should have the same

absorbance. And a difference in absorption at different wave length, stems from the difference in molar absorptivity coefficients.

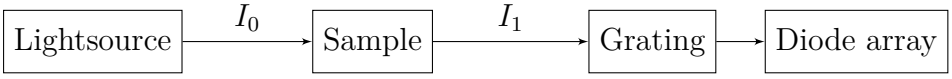

Figure 2.3: The components in a spectrophotometer

2.2 Acids, bases and Buffer systems

Acids and Bases In order to understand buffer solutions, and complex formation, a basic understanding of acids and bases is needed. The following sections will contain a brief run through with emphasis on the basic concepts.

The fundamental concepts of a an acid is that when acid is added to water, it increases the hydronium ion concentration $[H_3O^+]$. And when a base is added to water it decreases the $[H_3O^+]$ by increasing the hydroxide ion concentration, $[OH^-]$. There a two main ways of defining acid and bases, one is the Brønsted-Lowry definition, here acids are defined as proton donors and bases are defined as proton acceptors. Another definition is the Lewis definition, an Lewis base is a substance that can donate a pair of electrons and a Lewis acid is substance that can accept a pair of electrons.

The reaction between an acid and a base also results in the products that are classified as an acid and a base.

$$H_3PO_{4(aq)} + H_2O_{(l)} \rightleftharpoons H_3O^+_{(aq)} + {H_2PO_4}^-{}_{(aq)} \tag{2.2}$$

Where H_3PO_4 is the acid and its conjugated base ${H_2PO_4}^-$, is the first acid/base pair. The second acid/base pair is H_3O^+ and its conjugated base H_2O. This reaction like many other chemical reactions happens in a equilibrium between reactants and products. Which means the forward reaction happens at the same rate as the reverse reaction. This do not mean that there

are the same amount of reactants and products. The equilibrium constant K for the reaction:

$$a\mathrm{A} + b\mathrm{B} \leftrightharpoons c\mathrm{C} + d\mathrm{D} \tag{2.3}$$

Is determined by this equation:

$$K = \frac{[\mathrm{C}]^c \cdot [\mathrm{D}]^d}{[\mathrm{A}]^a \cdot [\mathrm{B}]^b} \tag{2.4}$$

pH scale

Autoprotolysis is a self ionization which water undergoes, this causes water to act as both an acid and a base.

$$\mathrm{H_2O_{(l)}} + \mathrm{H_2O_{(l)}} \rightleftharpoons \mathrm{H_3O^+_{(aq)}} + \mathrm{OH^-_{(aq)}} \tag{2.5}$$

The equilibrium constant for the autoprotolysis reaction is called K_w, and express by:

$$K_\mathrm{w} = [\mathrm{H_3O^+}][\mathrm{OH^-}] \tag{2.6}$$

And K_w have the value $K_\mathrm{w} = 1.00 * 10^{-14}$ at 298K. Next the pH scale is introduced by rewriting equation 2.6:

$$K_\mathrm{w} = [\mathrm{H_3O^+}][\mathrm{OH^-}] \tag{2.7}$$

$$\log K_\mathrm{w} = \log[\mathrm{H_3O^+}]\log[\mathrm{OH^-}] \tag{2.8}$$

$$-\log K_\mathrm{w} = -\log[\mathrm{H_3O^+}] - \log[\mathrm{OH^-}] \tag{2.9}$$

$$pK_\mathrm{w} = \mathrm{pH_3O^+} + \mathrm{pOH^-} \tag{2.10}$$

$K_\mathrm{w} = 1.00 * 10^{-14}$ at 298K, which equals to $-\log K_\mathrm{w} = 14.00$ at 298K.

$$14.00 = \mathrm{pH_3O^+} + \mathrm{pOH^-} \tag{2.11}$$

This provides a important understanding of the acid and base relationship in aqueous solutions. Since $\mathrm{pH_3O^+} = \mathrm{pH}$, then we can make the assumption: $\mathrm{pH} = -\log[\mathrm{H^+}]$. And pH is used to define the acidity of solutions. A solution is acidic if $[\mathrm{H^+}] > [\mathrm{OH^-}]$, neutral if $[\mathrm{H^+}] = [\mathrm{OH^-}]$ and basic if $[\mathrm{H^+}] < [\mathrm{OH^-}]$.

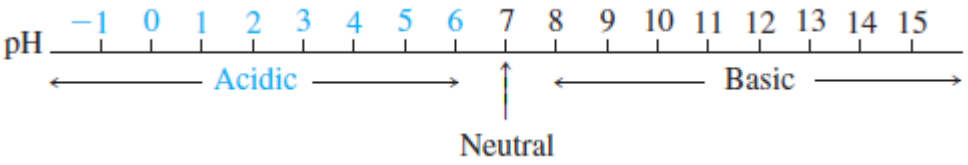

Figure 2.4: The pH scale at 298K. Note that the neutral point will change with temperature as K_w have different values depending of the temperature. From [2]

Strong and weak acids and bases

Acid and bases are classified after their ability to completely or partially dissociated in aqueous solutions into H^+ and OH^-:

$$HCl_{(aq)} \rightleftharpoons H^+_{(aq)} + Cl^-_{(aq)} \tag{2.12}$$

$$KOH_{(aq)} \rightleftharpoons K^+_{(aq)} + OH^-_{(aq)} \tag{2.13}$$

HCl and KOH are a strong acid and base respectively which means that they both have high equilibrium constants for the reactions and HCl have a negative pK_a value HCl. While KOH have a negative pK_b value. A weak acid is defined as an acid that do not fully dissolve in water and have a pK_a value between 1 and 14. A weak base is defined as a base that do not fully dissolve in water and have a pK_b value between 1 and 14.

Buffer solutions Buffer solutions have the ability to resist minor pH changes if small quantities of acid or base is added. The buffer solution must also contain a relative large amount of acid and base in order to react with any added H^+ or OH^-. It is also a criteria that the acid and base components of the buffer must not undergo a neutralization reaction and consume each other. Therefore, is a buffer solution made of a weak acid and its conjugate base or a weak base and its conjugate acid.

The buffering capacity is an expression for the effectiveness of the buffer, which means the ability to resist pH changes. The buffering capacity is proportional with the concentrations of the weak acid and its conjugate base.

So larger amounts of buffer components means greater buffering capacity of the solution.

Preparation of a buffer solution:

In order to make a buffer solution with a specific pH, it is needed to write the equilibrium constant as:

$$K_{\mathrm{a}} = \frac{[\mathrm{A}^-][\mathrm{H}^+]}{[\mathrm{HA}]} \tag{2.14}$$

Then the equation is rearranged:

$$[\mathrm{H}^+] = \frac{K_{\mathrm{a}}[\mathrm{HA}]}{[\mathrm{A}^-]} \tag{2.15}$$

By taking the negative logarithm on both sides of the equation, this expression is obtained:

$$-\log[\mathrm{H}^+] = -\log K_{\mathrm{a}} - \log\frac{[\mathrm{HA}]}{[\mathrm{A}^-]} \tag{2.16}$$

Which is rearranged to:

$$-\log[\mathrm{H}^+] = -\log K_{\mathrm{a}} + \log\frac{[\mathrm{A}^-]}{[\mathrm{HA}]} \tag{2.17}$$

Therefore $-\log[\mathrm{H}^+]$ can be expressed as pH:

$$\mathrm{pH} = pK_{\mathrm{a}} + \log\frac{[\mathrm{A}^-]}{[\mathrm{HA}]} \tag{2.18}$$

This is called the Henderson-Hasselbalch equation, which is more commonly expressed as :

$$\mathrm{pH} = pK_{\mathrm{a}} + \log\frac{\text{conjugate base}}{\text{acid}}$$

Where pH is the pH of the buffer solution. The pK_{a} value is a constant for the specific weak acid, the fraction part of the equation is the equilibrium of the acid and its conjugate base. In situations where the concentrations of the acid and its conjugate base are approximately equal, [acid]≈[conjugate base], then:

$$\log\frac{[\mathrm{conjugatebase}]}{[\mathrm{acid}]} \approx 0 \tag{2.19}$$

This gives pH $\approx pK_a$. So in order to make a buffer that functions properly. The acid and its conjugate base have to be in comparable amounts, which means in relative equal concentrations and not [acid] $\ggg$ [conjugate base] or the reversed. This is ensured by picking a acid with a pK_a fairly close to the desired pH, usually $pK_a = \text{pH} \pm 1$, this is sometimes referred to as the buffering range.

2.3 Complexes

In the experimental section of this project, coordination complexes are used to quantify the amount of specific heavy metals in water samples. The following section will outline the basic theory of metal complexes' structures and chemical properties. Subsequently, there will be a description of the structure of the lead, mercury and dithizones' chemical properties.
A coordination complex consists of a central atom or ion that is surrounded by one or more molecules or ions. These are known as ligands, and act as a Lewis base by donating an electron pair to the central atom. The central atom functions as a Lewis acid by receiving the electron pair. The bonds in a coordination complex can be regarded as covalent bonds [3]. The number of bonds the central atom can make in the complex is called the coordination number. This can vary from 2 to 12 bonds depending on the atom. Even though a given atom has a coordination number of 6, it does not necessarily make complexes with 6 bonds. This depends, among other things, on the size of the ligand and the radius of the atom so there can be a sterical hindrance [5]. Ligands are classified according to the number of possible donor electron pairs. Monodentate ligands can only donate one electron pair and only makes a single bond to the central atom. Multidentate ligands can donate two or more electron pairs, and therefore make multiple bonds to the central atom. Each electron pair is donated from different atoms in the molecule, these atoms are called donor atoms. Complexes containing multiple ligands

are a special class called chelates. Complex formation occurs on the basis of thermodynamics and kinetics. A complex with a multidentate ligand, is as the main rule more favorable than two monodentate ligands. As this reduces entropy by creating more order. This is called the chelating effect [5] [3]. This is shown in the following reaction where M is the central atom, l is monodentate ligands and L multidentate ligands [5]:

$$\mathrm{Mll} + \mathrm{L} \longrightarrow \mathrm{ML} + \mathrm{l} + \mathrm{l}$$

Complex formation is equilibrium between the complex, ML, central atoms, M, and ligands,L:

$$\mathrm{M_{(aq)}} + \mathrm{L_{(aq)}} \longleftrightarrow \mathrm{ML_{(aq)}}$$

The stability of the complex is given by the stability constant, K, which is expressed by the equation:

$$K = \frac{[\mathrm{ML}]}{[\mathrm{M}] \cdot [\mathrm{L}]}$$

The formation of complexes with several ligands happens through stepwise reactions between several equilibriums. The following reaction is an example of an atom with the coordination number of 4, which forms a complex with 4 monodentate ligands [3]:

$$\begin{aligned}
\mathrm{M_{(aq)}} + \mathrm{L_{(aq)}} &\longleftrightarrow \mathrm{ML_{(aq)}} \\
ML_{(aq)} + L_{(aq)} &\longleftrightarrow ML_{2(aq)} \\
ML_{2(aq)} + L_{(aq)} &\longleftrightarrow ML_{3(aq)} \\
ML_{3(aq)} + L_{(aq)} &\longleftrightarrow ML_{4(aq)}
\end{aligned}$$

Each reaction have their own stability constant [3]:

$$\begin{aligned}
K_1 &= \frac{[\mathrm{ML}]}{[\mathrm{M}] \cdot [\mathrm{L}]} \\
K_2 &= \frac{[\mathrm{ML_2}]}{[\mathrm{ML}] \cdot [\mathrm{L}]} \\
K_3 &= \frac{[\mathrm{ML_3}]}{[\mathrm{ML_2}] \cdot [\mathrm{L}]} \\
K_4 &= \frac{[\mathrm{ML_4}]}{[\mathrm{ML_3}] \cdot [\mathrm{L}]}
\end{aligned}$$

In complexes with multiple ligands, the stability constant is the product of several stability constants. The stability constant for the overall complex is sometimes written as β_n, where n is the number of ligands [3]:

$$\beta_4 = K_1 \cdot K_2 \cdot K_3 \cdot K_4$$

A more general equation for the stability constant of the reactionβ_n is expressed [2]:

$\mathrm{nL^-_{(aq)}} + \mathrm{M^{n+}_{(aq)}} \rightleftharpoons \mathrm{ML_{n(aq)}}$

$$\beta_n = \frac{[\mathrm{ML_n}]_{\mathrm{aq}}}{[\mathrm{M^{n+}}]_{\mathrm{aq}} \cdot [\mathrm{L}]^{\mathrm{n}}_{\mathrm{aq}}} \tag{2.20}$$

2.3.1 Extraction and separation of metal ions

This project aims to quantify different metal ions in aqueous solutions. A method to do this is by separating the ions with a organic ligand that form neutral charged complexes, such as dithizone which will be examined in a following section. These complexes are extracted to a non polar phase. The organic ligand functions as weak acid, HL, which loses a proton when it makes a bond to a metal ion: $\mathrm{HL_{(aq)}} \rightleftharpoons \mathrm{H^+_{(aq)}} + \mathrm{L^-_{(aq)}}$
The equilibrium constant for this reaction:

$$K_{\mathrm{a}} = \frac{[\mathrm{H^+}]_{\mathrm{aq}} \cdot [\mathrm{L^-}]_{\mathrm{aq}}}{[\mathrm{HL}]_{\mathrm{aq}}} \tag{2.21}$$

The ligands can form complexes with different metal ions, but some selectivity can be accomplished by regulating the pH. An equation can derived for the distribution coefficient, D of the concentration of metal in the polar and non polar phase. With the assumption that most of the metal in the organic phase is in the complex form ML_n and most of the metal in the aqueous phase is in the ion form M^{n+}. The partition coefficients for the ligand and complex are defined as:

$$HL(aq) \rightleftharpoons HL(org),\ K_L = \frac{[HL]_{org}}{[HL]_{aq}}$$

$$ML_n(aq) \rightleftharpoons ML_n(org), K_M = \frac{[ML_n]_{org}}{[ML_n]_{aq}} \tag{2.22}$$

The distribution coefficient can be expressed as:

$$D = \frac{[\text{totalmetal}]_{\text{org}}}{[\text{totalmetal}]_{\text{aq}}} \approx \frac{[\text{ML}_\text{n}]_{\text{org}}}{[\text{M}^{\text{n+}}]_{\text{aq}}} \tag{2.23}$$

By combining equation 2.22 and 2.20 $[ML_n]_{org}$ can be expressed as:

$$[ML_n]_{org} = K_M[ML_n]_{aq} = K_M\beta_n[M^{n+}]_{aq}[L^-]^n_{aq} \tag{2.24}$$

Then the expression for $[L^-]_{aq}$ from equation 2.21 gives:

$$[ML_n]_{org} = \frac{K_M\beta_n[M^{n+}]_{aq}K^n_a[HL]^n_{aq}}{[H^+]^n_{aq}} \tag{2.25}$$

Then this expression of $[ML_n]_{org}$ is put into equation 2.23, which gives:

$$D \approx \frac{K_M\beta_nK^n_a[HL]^n_{aq}}{[H^+]^n_{aq}} \tag{2.26}$$

It is assumed that most of HL is in the non polar phase, so $[HL]_{aq} = \frac{[HL]_{org}}{K_L}$ is inserted in the previous equation to make this equation for distribution of metal-chelate complex between phases:

$$D \approx \frac{K_M\beta K^n_a[HL]^n_{org}}{K^n_L[H^+]^n_{aq}} \tag{2.27}$$

From 2.27 its apparent that the distribution coefficient for metal ion depends on pH and ligand concentration. Since different metals have different partition coefficients, K_M, it is possible to make a selective extraction of certain metal ions. If a pH is chosen where D is small for one metal and large for another metal. Figure 2.5 shows that it is possible to separate Hg^{2+} from Pb^{2+} if a pH between 2.3-5.9 is chosen. By having the pH under 5.9, no lead will no extracted, while 100 % of the mercury would be extracted.

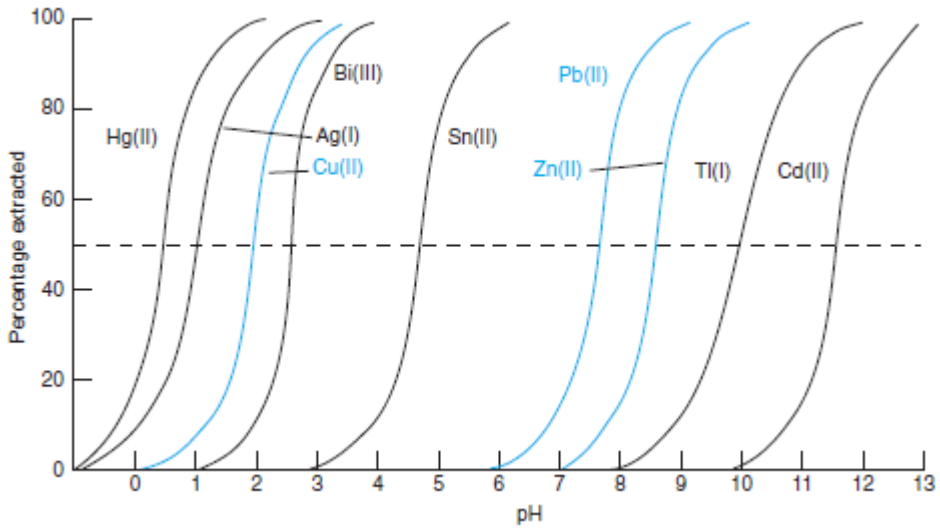

Figure 2.5: The pH dependency of the degree extraction of various metals. From [2]

2.3.2 Dithizone

In this project all the extractions of chelate complexes are done with dithizone, DZ, which is an abbreviation for diphenylthiocarbozone. Dithizone has the molecular formula $C_{13}H_{12}N_4S_1$ and is known by IUPAC name; (1E)-3-anilino-1-phenylimino-thiourea. Dithizone is an organic molecule which is green and soluble i non polar solvents. Dithizone is only soluble in aqueous solutions with a pH above 7, where it is converted to an yellow ion. Dithizone is good ligand due to its complex formation and high stability constants with many different di- and trivalent metals, including mercury and lead ions. Dithizone is a multidentate ligand which can donate 2 pairs of electrons were sulphur and nitrogen are the donor atoms. The formed coordination complex consists of 2 dithizone molecules surrounding a single metal ion, see figure 2.6. The most acidic proton, is the one on the sulphur.

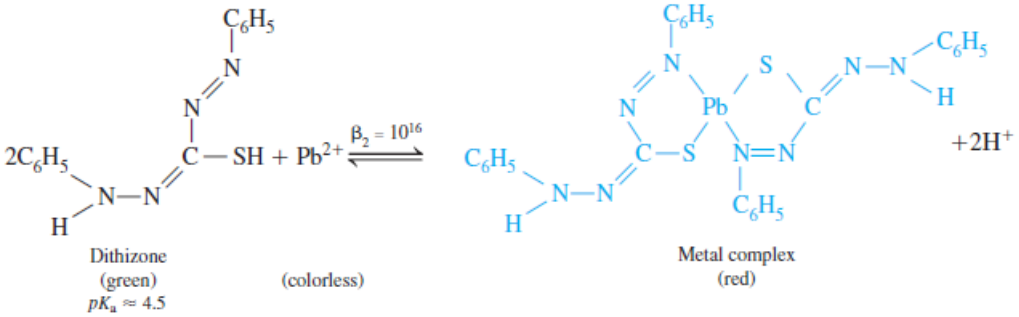

Figure 2.6: The complex formation between DZ and lead. From [2]

Method

3.1 Choice of method

In this project we have chosen to use the external standard method, when measuring the concentration of metal ions. This was chosen over standard addition, as that method requires that you make more measurements, in the field. Standard addition would also require that know concentrations of the metal ions are present, in order to make the additions.
The advantages of the external standard method, seams evident. The external standard curves can be prepared beforehand, for each of the metal complexes, thus the only thing needed to preform a quantitative analysis are solutions of know concentrations of DZ in chloroform and a laptop with a spectrophotometer.
The following procedure was followed in construction of the calibration curves. Firstly six calibration points were gathered, where dithizone were in excess, three times the number of moles mercury present in the sample with the highest concentration. The range of concentration, in the samples of known analyte were, devised as follows $[M^{2+}concentrationinterval] = \frac{17}{60} \cdot [DZ]$. When the preliminary calibrations curves were made, it was found that the linear range, only were made up of points where $[M^{2+}]_{min} = \frac{1}{20} \cdot [DZ]$ and $[M^{2+}]_{max} = \frac{1}{3} \cdot [DZ]$.
A blank sample spectra of pure DZ were recorded and used to correct the recorded peaks of the calibration points, by subtracting the pure sample times a factor, which takes into account how much DZ was complexed. The

factor was calculated by assuming complexation ratio of 1:2 between [M^{2+} and DZ, (this was later confirmed by experiment), another assumption was that all the metal formed complex'. The theoretical amount of DZ tied up in a complex were then subtracted starting amount this was then divided by initial amount. There are overlaps in the peaks of the spectra of pure DZ and the metal complex', thus the mentioned method of correcting. Finally three sample measurements were were recorded.

The known concentrations were plotted against the corrected absorptions in a Excel data sheet. The calibration curve were made by linear regression. Data points which were far off the curve, were discard in order to optimize the calibration curve. Samples were discarded if they showed signs of contamination or gave false readings due to cuvette misplacements in the UV-VIS machine. This was a problem because the cuvette had room enough to be placed at an wrong angle which was not perpendicular to the laser beam. This problem was solved by squeezing the cuvette in place with a couple of plastic strips on each side. The figure 3.1 below, show what happens when the cuvette is placed wrong; firstly the light scatters because it comes at an angle through the glass. This is a problem because one wavelength is passed through the selector at the time, but when it hits the glass it shatters to multiple wavelengths. Secondly the light has to travel a little further trough the sample. Both factors causes a misreading on UV-VIS spectra, so the sample has to be redone, if possible, or discarded.

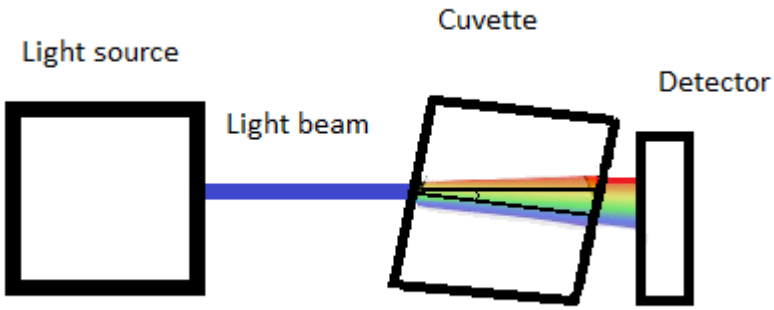

Figure 3.1: The figure show the scattering of light when it enter the glass at an wrong angle

It was assumed that the calibration curve should comply to Lambert-Beer's law and follow linearity, which was checked by looking at the graphs residual plot. If the calibration curve was linear, then follows it Lambert-Beer's law.

Experiments

4.1 Molar absorptivity

During the course of this project, multiple calibration curves were made of pure dithizone in chloroform, in order to obtain the molar absorptivity for the two maximum peaks at λ_{440nm} and λ_{605nm}. In order to filter out noise on the spectra, the average of the absorptions of the 5 wavelengths, around the maximum wavelength, were used. An alternative method could be to do polynomial regression around the the peak, and then calculate the absorption from the resulting equation. This first option were chosen, do to a time consideration.
A calibration curve were constructed, for a range of dz from 26 ppb to 6745 ppb, in order to determine the linear range of the curve. If a point is outside the linear range, the solute does not follow Beer's law at that concentration. If Beer's law is not applicable then the point can not be used to determine the molar absoptivity.

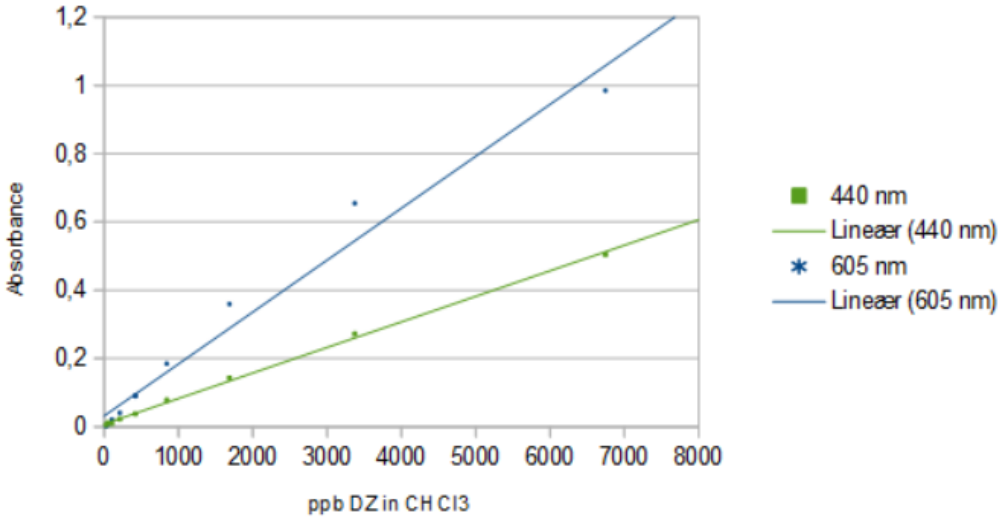

Figure 4.1: Calibration curve for DZ, at it two maximums at λ_{440nm} and λ_{605nm}. DZ concentration range [26.3-6750 ppb]

Figure 4.1 shows the two calibration curves, where the curve for λ_{440nm}, seems linear, as opposed to the curve for λ_{605nm}. For a concentration of 6745 ppb, the absorbance is outside the linear range and is therefore disregarded. This could mean that the spectrophotometer has trouble recording over 90 % absorbance.

A second calibration curve were drawn up, so it could be determined if a DZ concentration of 3375 ppb, had a absorbance in the linear range. This is shown in figure 4.2 form visual inspection it seems that the absorbance corresponding to lies a bit low, it is therefor also considered to be outside the linear range. A third and final plot was drawn up, where the mentioned point were omitted and the units on the x-axis, were change from ppb to M ($\frac{mol}{L}$). The change in units were made in order to have the molar absorptivity as the slope of the regression line see figure 4.3.

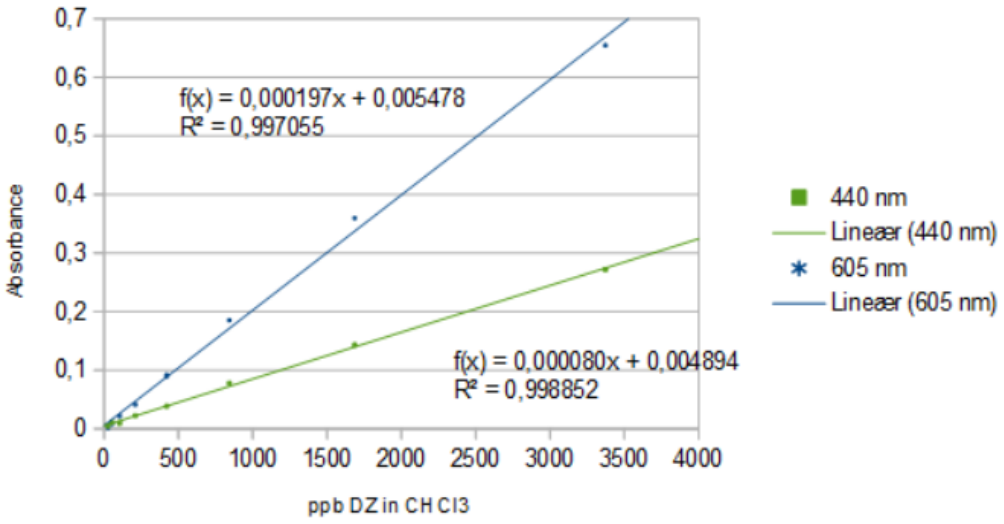

Figure 4.2: Calibration curve for DZ, at it two maximums at λ_{440nm} and λ_{605nm} with regression equations. DZ concentration range [26.3-3375 ppb]

For comparison the following molar absorptivity have been found in the literature $(40.6 \pm 0.5) \cdot 10^3$ at λ_{606nm}, [4]. 40800 at λ_{605nm} and 16,400 at λ_{440nm} [6]. While they were found to be $(41.5 \pm 0.5) \cdot 10^3$ at λ_{605nm} and $(16 \pm 0.2) \cdot 10^3$ at λ_{440nm}, by [1].

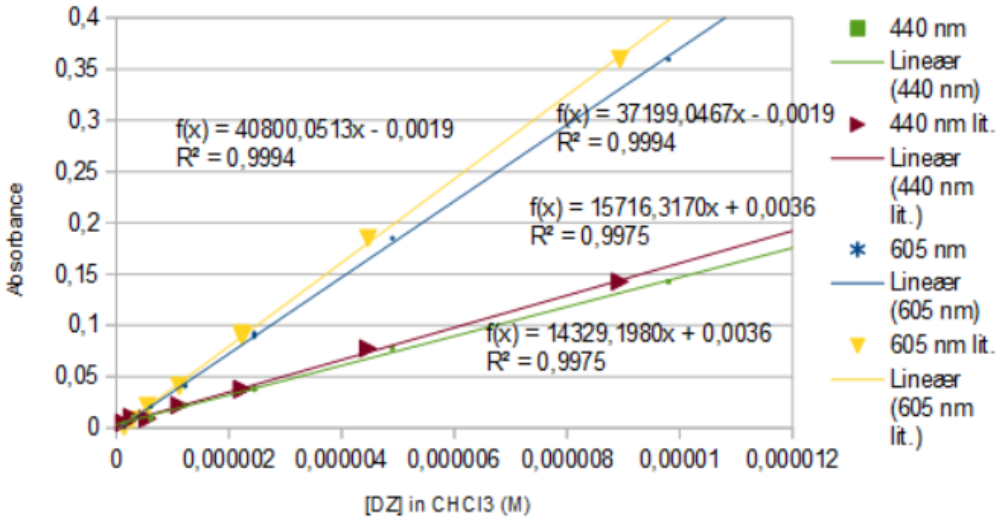

Figure 4.3: Calibration curve for DZ, at it two maximums at λ_{440nm} and λ_{605nm} with regression equations. DZ concentration range [26.3-1678 ppb]. The curves denoted with the prefix lit. were made on the basis of molar absorptivity coefficients found in literature.

From figure 4.3 we see that, in this instance, we found the molar absorptivity coefficients to be$37{,}2 \cdot 10^3$ at λ_{605nm} and $14{,}3 \cdot 10^3$ at λ_{440nm}. This determination were done a total of 3 times, and the results were;

Experiment	1	2	3
$\epsilon_{\lambda_{440nm}}$	$16{,}8 \cdot 10^3$	$14{,}3 \cdot 10^3$	$14{,}4 \cdot 10^3$
$\epsilon_{\lambda_{605nm}}$	$36{,}2 \cdot 10^3$	$37{,}2 \cdot 10^3$	$36{,}3 \cdot 10^3$

Table 4.1: Experimentally found molar absorptivity coefficients, in units of $M^{-1} \cdot cm^{-1}$. Note that the first value were recorded before we started using a vortex mixer.

4.2 Storage of the dithizone solution

In order to determine lights effect on a DZ stock solution, to experiment were preformed. In both the solutions of dithizone were kept at the same temperature, 25 degree Celsius, but one was covered by aluminum foil and kept in a dark place, the other were left on out on the table.

	Kept in dark		Kept in light	
Number of day	$\epsilon_{\lambda_{605nm}}$	$\epsilon_{\lambda_{440nm}}$	$\epsilon_{\lambda_{605nm}}$	$\epsilon_{\lambda_{440nm}}$
0	$36.2 \cdot 10^3$	$16.8 \cdot 10^3$	$36{,}2 \cdot 10^3$	$16.8 \cdot 10^3$
21	$34.9 \cdot 10^3$	$15.8 \cdot 10^3$	$31.9 \cdot 10^3$	$13.0 \cdot 10^3$

Table 4.2: First experiment to determine light influence on the molar absorptivity coefficients, duration 21 days, in units of $M^{-1} \cdot cm^{-1}$.

In the first experiment the there were a decrease in both peaks, the decrease were greatest for the solution kept in the light. The on e kept in the dark showed at decrease of 3,6 % at λ_{605nm} and 6 % at λ_{440nm}. Whereas the one exposed to light showed decreases of 12 % at λ_{605nm} and 23 % at λ_{440nm}. The experiment were repeated, but over shorter period of time.

	Kept in dark		Kept in light	
Number of day	$\epsilon_{\lambda_{605nm}}$	$\epsilon_{\lambda_{440nm}}$	$\epsilon_{\lambda_{605nm}}$	$\epsilon_{\lambda_{440nm}}$
0	$37{,}2 \cdot 10^3$	$14{,}3 \cdot 10^3$	$37{,}2 \cdot 10^3$	$14{,}3 \cdot 10^3$
10	$37{,}2 \cdot 10^3$	$14{,}5 \cdot 10^3$	$33{,}3 \cdot 10^3$	$13{,}1 \cdot 10^3$

Table 4.3: Second experiment to determine light influence on the molar absorptivity coefficients, duration 10 days coefficients, in units of $M^{-1} \cdot cm^{-1}$.

In the second experiment there were no decrease in absorbance, for the sample not exposed to light. The sample exposed to light had a fall in absorbance by 10 % at λ_{605nm} and 8 % at λ_{440nm}.

4.3 Separation funnel

When working with two-phase systems, it is easy to separate the phases using a separation funnel. In order to establish guidelines for working with the separation funnel, a few experiments were carried out, to determine an optimal shaking period. The shaking experiments were done with different shaking times and at both low and high concentrations in order determine if the higher concentrations required longer shaking times. The procedure and concentrations can found in the procedure section of the project.
The shaking times showed to have little influence on the absorbances of the complexes. As seen in figure 4.4 and 4.5 the spectra looked similar from 15 seconds to 180 seconds, the small variations are assumed to come from other sources of errors than the shaking periode.

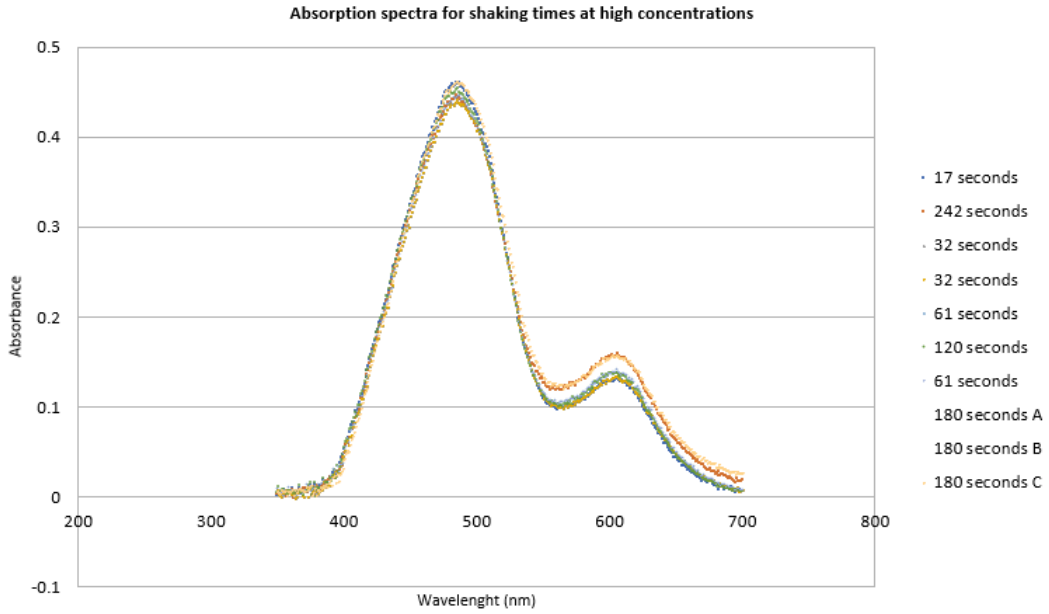

Figure 4.4: Complex formation with 1.33 ppm Hg^{2+} and 3.35 ppm DZ, after varies periods of shaking.

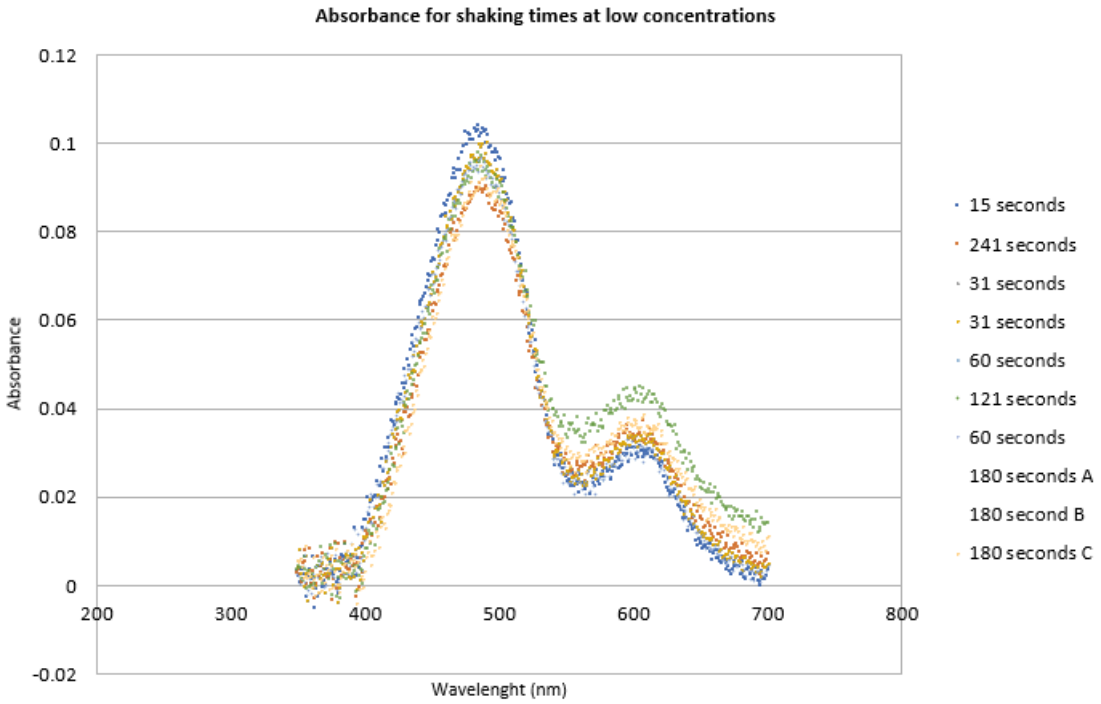

Figure 4.5: Complex formation with 244 ppb Hg^{2+} and 670 ppb DZ, after varies periods of shaking.

4.4 Sonication experiment

During our work we observed a 19 % decrease in absorbency in the uv-vis spectra for pure DZ, at the maximum peak around λ_{605nm}, when the DZ solution was put in sonic stirring, for a concentration of DZ of 202 ppb.
An experiment was set up, where 5 mL solution of DZ in chloroform, 606 ppb, were placed in 6 test tubes, and then left in the sonic bath for respectively 1, 5, 10, 15, 20 and 25 minutes.

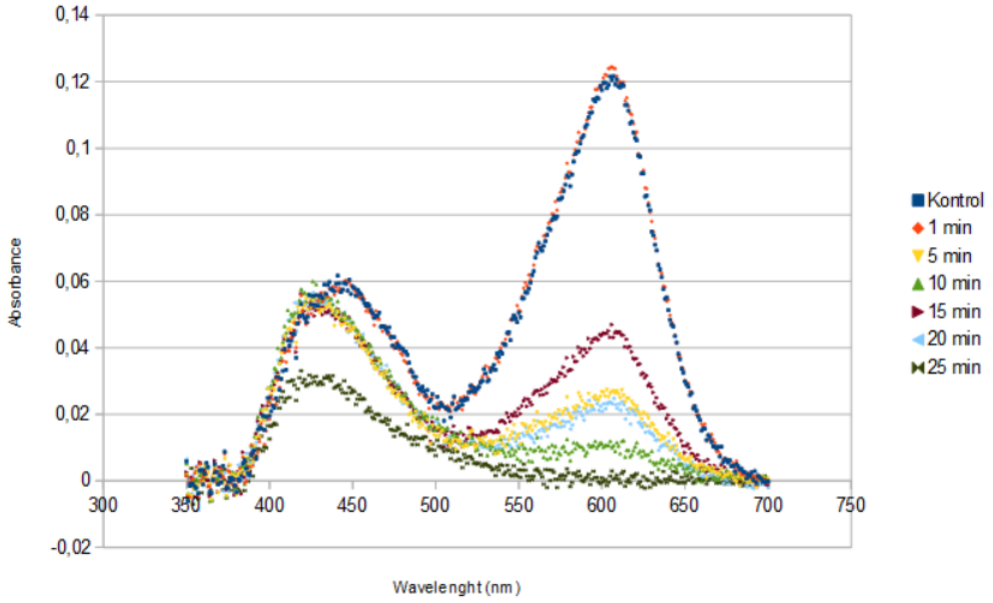

Figure 4.6: Absorption spectra, for pure DZ in chloroform, 606 ppb.

A trend was observed in the spectra in figure 4.6, the maximums at λ_{440nm} and λ_{605nm} showed a decreased in absorbency. No clear correlation between time in the sonic mixer, and level of decrease in absorbency, can be concluded. The control sample and the sample that spent 1 minute, had the same spectra. The samples that were in stirred for 5 to 20 minutes, showed the same narrowing of the peak interval at λ_{440nm}, but varied without a clear connection to the time stirred at λ_{605nm}. The sample that were stirred for 25 minutes had no peak at λ_{605nm}, and were flattened at the peak λ_{440nm}.

A second experiment were set up, where the concentration of DZ were increased to 6,75 ppm, and pure chloroform were included.

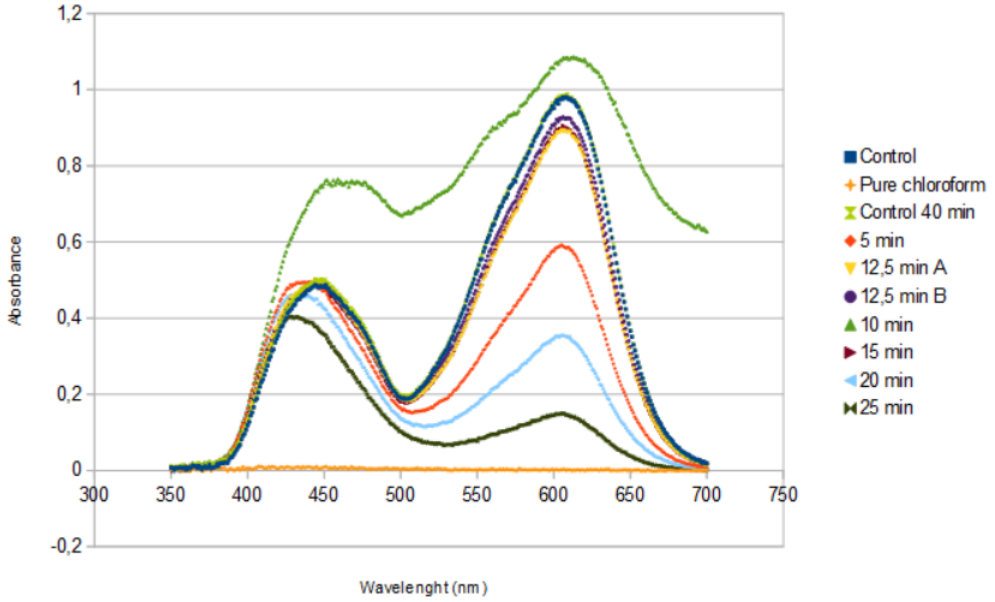

Figure 4.7: Absorption spectra, for pure DZ in chloroform, 6,75 ppm. Left in sonic stirring bath for different periods of time.

The spectra from the this experiment is shown in figure 4.7. The two control samples spectra were overlapping, which could be expected. The sample left stirring for 10 minutes showed an increase in absorbency at both peaks, witch would mean an increase in concentration of DZ, this is not possible. This sample must be discarded.

The pure chloroform did not show any change that could account for the observed changes.

The samples 12,5 A, 12,5 B and 15, showed a small decrease in absorbency from 0,9748 to 0,8890, 0,9248 and 0,8975 respectively, corresponding to 5 to 9 %, at λ_{605nm}. For these samples there were no deviation from the the control sample at λ_{440nm}.

The samples, 5, 20 and 25 min, show decreased absorbency at the peaks λ_{440nm} and λ_{605nm}. The decrease is proportional to the amount of time stirred.

Both of the previous experiments showed change in absorbency, when pure DZ in chloroform were stirred by ultra sound. These changes showed no clear correlation to time spent in the sonic bath. A third experiment were carried out, where in a solution of DZ in complex with Hg^{2+}, were left in the sonic, from 1 to 15 minutes. The initial concentrations of DZ, in chloroform, were 6,75 ppm and the concentration of Hg^{2+} in aqueous solution were 3,2 ppm.

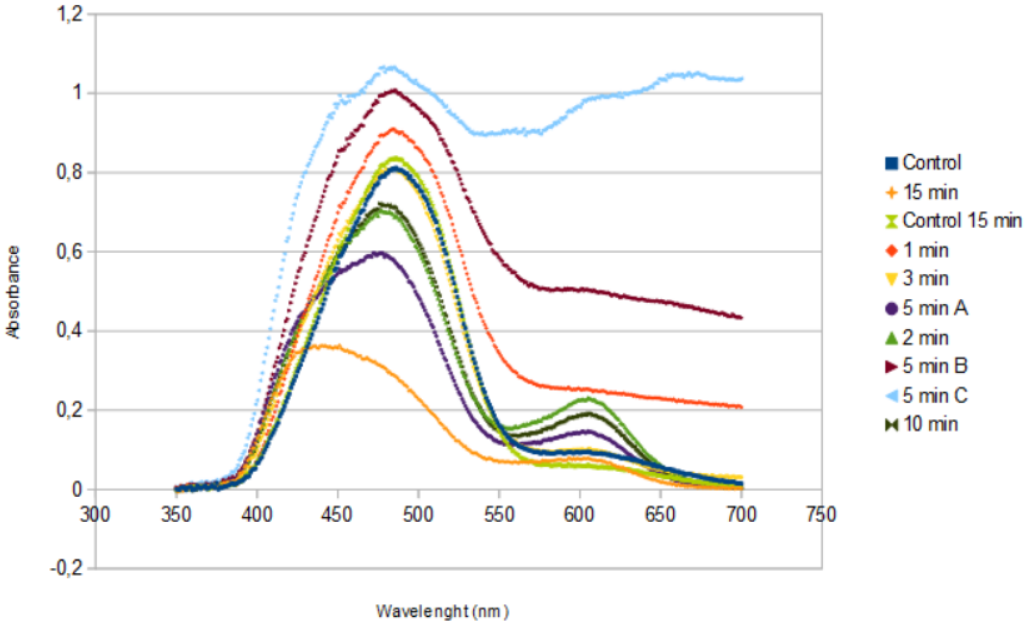

Figure 4.8: Absorption spectra, for DZ in chloroform, 6,75 ppm, in complex with Hg^{2+}, 3,2 ppm. Left in sonic stirring bath for different periods of time.

The control sample showed a slight increase in absorbency at λ_{485nm}, from 0,81 to 0,84, and a decrease at λ_{605nm} from 0,94 to 0,059. This could be as a result of a degree of complexation.
The 3 minute sample is overlapping the control sample.
The samples 1 min, 5 min B and 5 min C, show abnormal behaviour, there

might have been some contamination of the samples.
The samples 2 and 10 min, show a shift in maximum peak from λ_{485nm} to λ_{477nm}. Sample 10 min have a slightly higher maximum λ_{477nm} at , and lower maximum at λ_{605nm}, than sample 2.
The sample 5 min A, showed maximums at λ_{605nm}, the absorbency is less than the previous samples.
The sample 15 min, has maximums at λ_{440nm}, the same as DZ in chloroform, and λ_{605nm}.

The samples, from experiment 3 were left in the test tubes, covered by para film for 36 hours, figure 4.9 shows the obtained spectra.

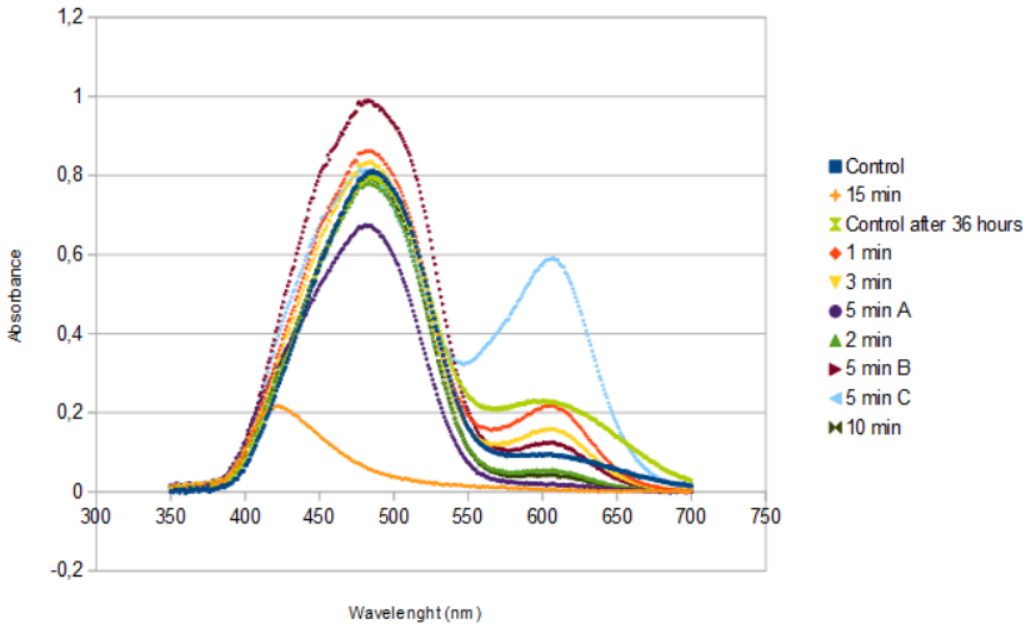

Figure 4.9: Absorption spectra, for DZ in chloroform, 6,75 ppm, in complex with Hg^{2+}, 3,2 ppm. Left in sonic stirring bath for different periods of time. After letting them settle for 36 hours.

All but three samples stabilized at the DZ-Mercury(II) maximum peak at λ_{485nm}, around 0,8 absorbance.

It was observed in figure 4.9 that the curve for 15 minutes was notably lower at the λ_{485nm} point and could possibly be an outlier. Therefore, a Grubbs test for an outlier was preformed using this equation:

$$G_{calculated} = \frac{|questionablevalue - \bar{x}|}{s} \tag{4.1}$$

The Grubbs value,$G_{calculated}$ for 15 minutes was calculated to be 2.280. The critical value for G_{table} with 10 observation is 2.176, which means that $G_{calculated} < G_{table}$ and datum must be considered an outlier with a 95 % confidens limit.

4.5 Continue variation method

This project work with an assumption that dithizone forms complexes with mercury in a ratio of 2:1. To test this hypothesis a continuous variation experiment were devised. First there will be a short presentation of the main points in the method. This method is used to identify the stoichiometry of the predominant coordination complex, if there is multiple possible complexes:

$$\mathrm{M} + \mathrm{L} \rightleftharpoons \mathrm{ML}$$
$$\mathrm{M} + 2\,\mathrm{L} \rightleftharpoons \mathrm{ML_2}$$
$$\mathrm{M} + 3\,\mathrm{L} \rightleftharpoons \mathrm{ML_3}$$

The procedure is to hold the volume and the total concentration [M]+[L] constant. And only make samples with different the ratios of [M]:[L]. First the absorbance of the samples is measured in the UV-VIS at $\lambda_{\max}$ for the complex. Then a graph is produced with absorbance plotted against the mole fraction of L. The absorbance must be the corrected absorbance:

$$\text{Corrected absorbance} = \text{measured absorbance} - \varepsilon_{\mathrm{M}} b \mathrm{M_T} - \varepsilon_{\mathrm{L}} b \mathrm{L_T} \tag{4.2}$$

Where b is the path length, M_T and L_T are total concentrations of M and L, and ε_M and ε_L are the molar absorptivities of pure M and L. The maximum absorbance should be observed at mole fraction x_i of the predominant complex.

4.5.1 Determination of dithizone complex ratio

As mentioned in the procedure for the experiment, samples with different mole ratios was prepared. Which was plot against the corrected absorbance in a so called Job's plot. It was later discovered that some crucial mole fractions were not prepared, for example the 0.667 x_i which would have been the mole fraction of the complex ML_2. Instead a 0.700 x_i sample was prepared, which gave the maximum absorbance in the Job's plot of the experiment:

Figure 4.10: Job's plot of dithizone and mercury ion complex

Unfortunately the Job's plot indicate that the ratio should be 2.33:1 (or more precise 7:3) for maximum at 0.700 x_i. But it is more likely to make the assumption that the correct ratio is 2:1, because 0.700 $x_i \approx$ 0.667 x_i.

This assertion is further confirmed when the Job's plot is done with 2 linear regressions:

Figure 4.11: The same Job's plot with two linear regressions to determine a possible intercept

It is observed that the maximum have move to a slightly lower x_i value. In order determine a new x_i value, the intercept was found by solving two equations with two unknowns. The intercept was calculated to be (0.685,0.347), which is better value for the maximum because the previous maximum from figure 4.10 was (0.700,0.333). The new x_i of 0.685 is closer to the expected 0.667, but the experiment should be redone with the correct molar fractions and repeated multiple times in order to determine the correct complexation ratio or to analyse if there is any statistical difference between the observed values and the hypothesis.

4.6 External standard curve

In order to be able to determine unknown heavy metal concentrations in water samples. A set of three external standard curves were produced for Hg^{2+}

in complex with dithizone. The three curves are made from experiments using different concentrations of dithizone and mercury. This is to optimize precision in different ranges because preliminary experiments showed increased uncertainty in the low ppb ranges. Furthermore is was found impossible to determine reliable low (<20 ppb) metal concentration with a disproportional large concentration of dithizone. Figure 4.12 and 4.13 clearly displays there is relative more noise or disturbance in low range then the medium range and small changes in the absorbance have greater impact in the lower range.

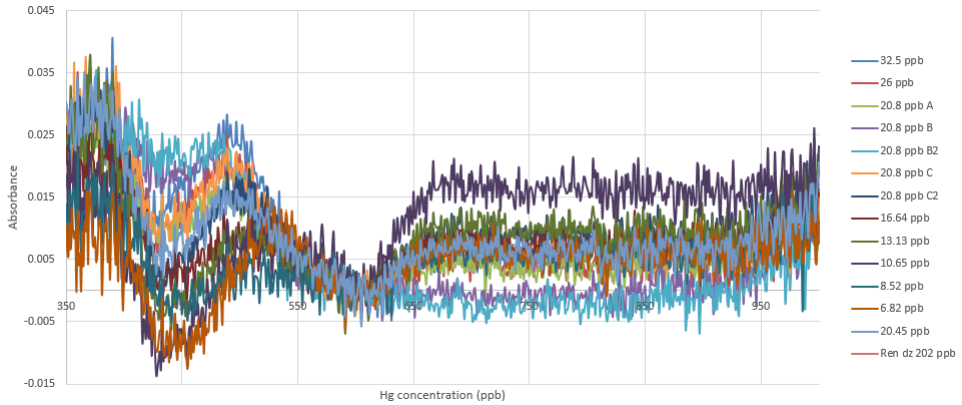

Figure 4.12: Absorption spectra for the Dz 202 ppb standard curve

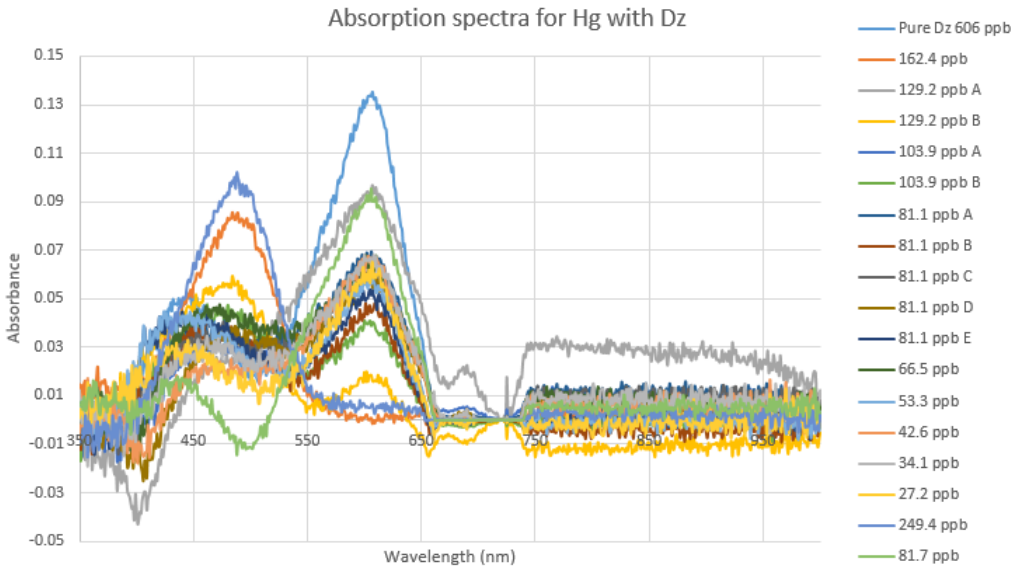

Figure 4.13: Absorption spectra for the Dz 606 ppb range standard curve

The first external standard curve, see figure 4.14, which is called the Dz 202 ppb, where made to measure Hg^{2+} in 5-35 ppb. Statistics such as standard deviation, detection limit, confidence limit and more were calculated for the standard curves using Excel. The detection limit for the model was calculated to be 2.12 ppb, together with a 95 % confidence limit of $\pm$ 1.97 ppb.

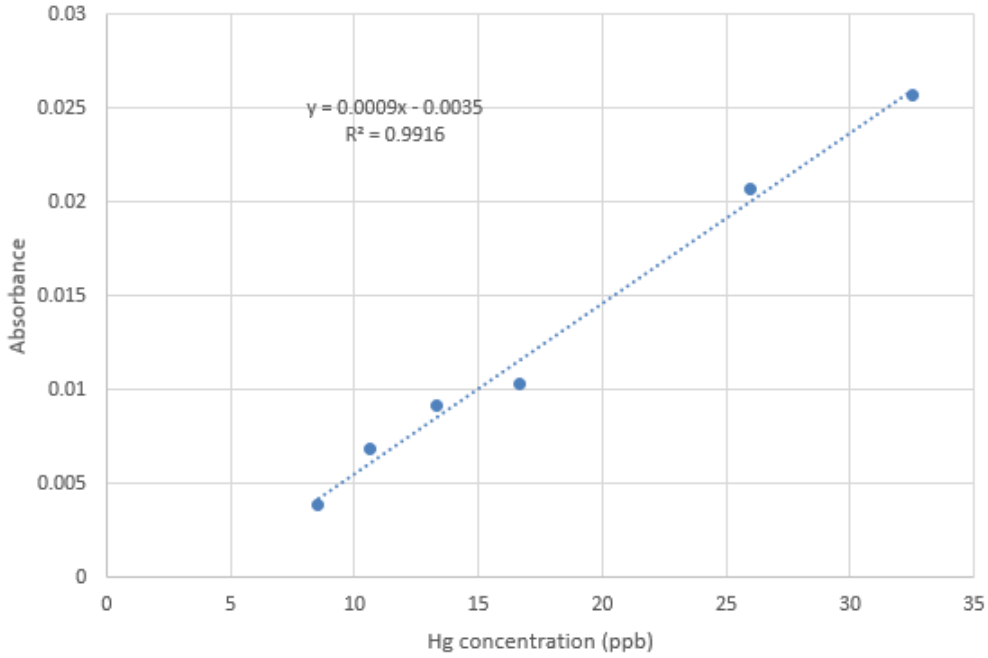

Figure 4.14: The Dz 202 ppb standard curve

The second external standard curve, see figure 4.15, called the Dz 606 ppb, where made to measure Hg^{2+} in 30-200 ppb. This curve showed less linearity then the 2 other curves . The detection limit of the model was calculated to be 46.41 ppb with a 95 % confidence limit of ± 36.56 ppb.

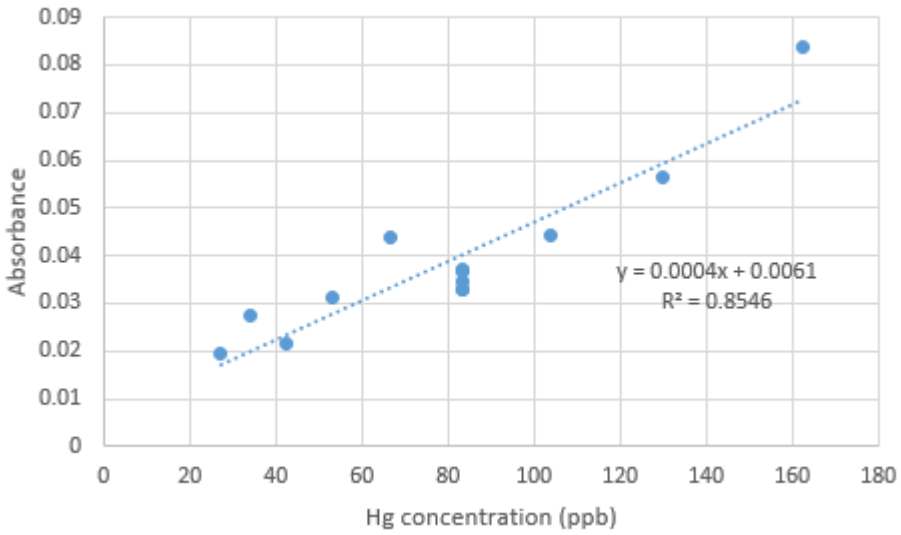

Figure 4.15: The Dz 606 ppb standard curve showed less linearity the other curves due to some unseen sources of errors

The third external standard curve, see figure 4.16, called the Dz 4022 ppb, where made to measure Hg^{2+} in 200-1200 ppb range. The detection limit of the model was calculated to be 41.28 ppb with a 95 % confidence limit of ± 34.40 ppb.

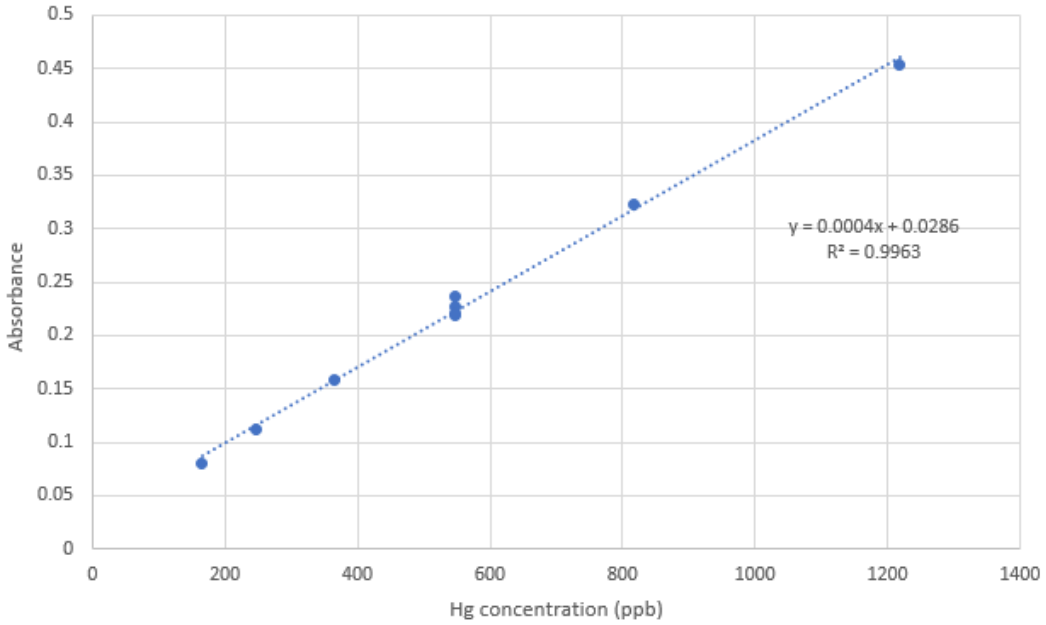

Figure 4.16: The Dz 4022 ppb standard curve

When the 3 standard curves are plotted in the same graph, 4.17, its shows that Dz 606 have Dz 4022 remarkable similar a and b values. Where Dz 202 had a significant higher value for the slope. The data for Dz 202 had a low standard deviation and high linearity. So the curve is still usable in ranges below 35 ppb Hg^{2+}, but becomes critical in the high ranges. The slope from Dz 606 and 4022 is assumed to the correct slope since it is reproduced in 2 experiments. The molar absorptivity ε_0 for the dithizone mercury ion complex is calculated to be $154 \cdot 10^3$ in Dz 202 and $68.8 \cdot 10^3$ in both Dz 606 and Dz 4022. This futher enhances the assumption that standard curves for Dz 606 and Dz 4022 are more correct. Because these values corresponds to similar values found in litterature. The molar absorptivity ε_0 for the complex was found to be $57.7 \cdot 10^3$ in dichloromethane and $71.2 \cdot 10^3$ in tetrachlorid [6]. Therefore, it is expected that the value for the complex in chloroform, which this project uses as solvent, should lie somewhere in between their values. The three models were plotted in the same coordinate system, shown on figure 4.17. The model Dz 202 should not be used for predicting values for

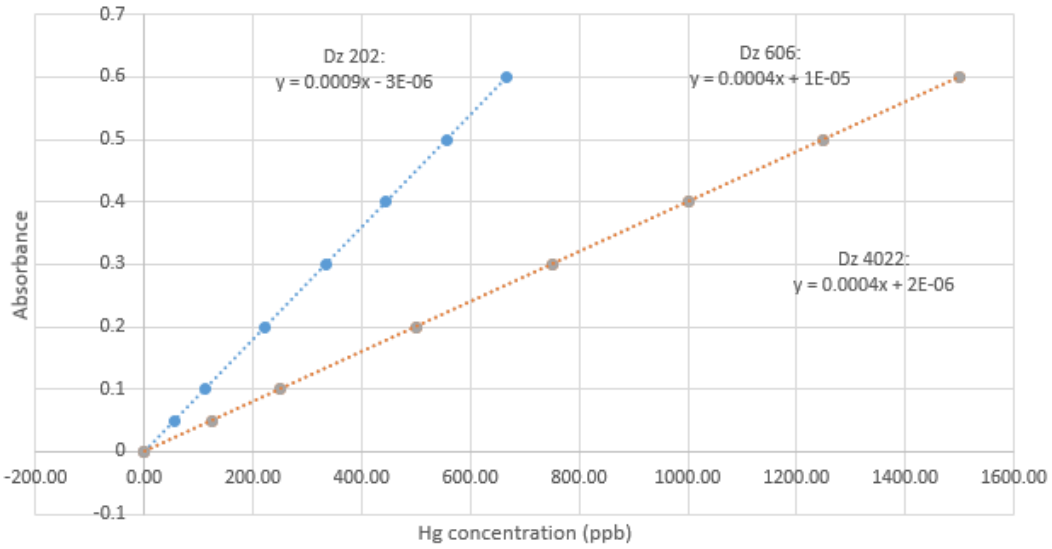

Figure 4.17: A plot of all 3 standard curves, where the curve Dz 202 has been extrapolated.

absorbances over 0.025. The models Dz 606 and Dz 4022, are in agreement with each other.

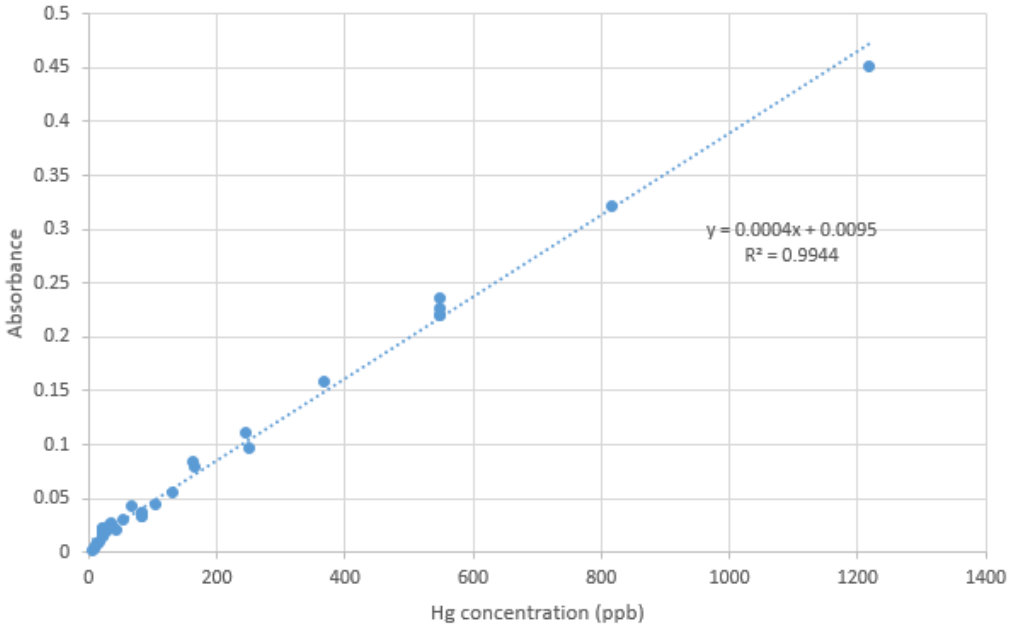

Figure 4.18: Linear regression on the data from all 3 standard curves.

4.7 Determination of Hg^{2+} in unknown samples

The accuracy of this projects method was tested by using previous data from former samples, which were not used in the making of the standard curves. This were done by measuring the absorbance and calculating Hg^{2+} concentrations with the models. Figure 4.19, shows the results. The difference between the values were then checked to see if they lay within the 95% confidence limit of the models, see figure 4.20. The dithizone solution used was 945 ppb with chloroform as solvent. The Hg^{2+} samples used range from 6.4-406 ppb at pH 1.8. For comparison it was claimed that, by [6], that the method used in this project could determine metal concentrations in the ppm range.

Absorbance at mean 488	0.1475684	0.0526566	0.021886	0.012497	0.001365
Actual Hg2+ ppb	**406**	**102**	**51**	**25.5**	**6.4**
Dz 202 pbb	163.97	58.51	24.32	13.89	1.52
Dz 606 ppb	368.93	131.65	54.72	31.25	3.42
Dz 4022 ppb	368.95	131.67	54.74	31.27	3.44
Combined	368.93	131.65	54.72	31.25	3.42

Figure 4.19: A table showing the actual concentrations with the observed concentrations calculated from the different standard curves

Difference in Hg concentration from observed and actual						
Actual Hg2+ ppb	**406**	**102**	**51**	**25.5**	**6.4**	95% CL
Dz 202 pbb	242.03	43.49	26.68	11.61	4.88	± 1.97
Dz 606 ppb	37.07	-29.65	-3.72	-5.75	2.98	± 36.56
Dz 4022 ppb	37.05	-29.67	-3.74	-5.77	2.96	± 34.40
Combined	37.07	-29.65	-3.72	-5.75	2.98	
Green is inside the model's 95% CL		**Red is outside the model's 95% CL**				

Figure 4.20: The table displays the model's ability to determine the concentrations within a 95% confidence limit. A green box symbolises yes, a red symbolises no

4.8 Procedures

Separation funnel:

Preparation of solutions: The experiment was done at high and low concentrations to analyze if a change in concentration could have an impact on the absorption of the samples. Therefore, dithizone and mercury ion solutions were made with high and low concentrations. The high concentration dithizone solution (3.36 ppm), was made with 1 mL stock solution (672.2 ppm) which was diluted with chloroform to 200 mL. The low concentration dithizone solution (672 ppb), was made with 10 mL dithizone solution (3.36 ppm) which was diluted with chloroform to 50 mL. The high concentration mercury ion solution (1.22 ppm), was made with 1 mL Hg2+ solution (40.6 ppm) which was diluted with buffer to 100 mL. The low concentration mercury ion solution (244 ppb), was made with 10 mL Hg2+ solution (1.22 ppm) which was diluted with buffer to 50 mL.

Procedure: The experiment was first done with the high concentrations of dithizone (3.36 ppm) and mercury (1.22 ppm). The UV-VIS computer was set to calculate the absorption with an average of 10 samples with a sampling length of 60 ms. Pure chloroform was used as reference. First, the absorption of the pure dithizone solution (3.36 ppm) was measured. Next dithizone solution (3.36 ppm, 5 mL) and mercury ion solution (1.22 ppm, 5 mL) were added to a separation funnel and then shaken for different amounts of time. Excess chloroform gas was let out of the funnel every 15-20 seconds to decrease the pressure inside the funnel. Some of the dithizone phase (approx. 2 mL) was extracted to a glass cuvette and the absorption spectra of the sample was measured, in the UV-VIS, 2 minutes after the shaking was ended. The experiment was redone with shaking times of 15 seconds, 30 seconds, 1 minute, 2 minutes, 3 minutes and 4 minutes. The glass cuvette and the separation funnel were emptied and rinsed between each sample. Later the experiment with low concentrations dithizone (672 ppb) and mercury (244 ppb) were done following the same procedure.

Experiment 1: From a stock solution of DZ in chloroform, 675 ppm, 1 mL was put in a 100 mL flask, by a glass pipette, and filled to the mark with chloroform. Then the solution were stirred on a whirl mixer, on medium setting for 10 seconds, this is marked solution A. 9 mL of this solution were placed in a 100 ml flask and filled to the mark with chloroform.
Portions of 5 mL, of this solution were placed in 6 test tubes. The test tubes were labeled in placed in a sonic bath, a timer was set and the samples were taken out at the indicated times. After the sample were taken out, the spectra were obtained after 2 minutes.

Experiment 2: The same as above, but the 5 mL portions came from solution A.

Experiment 3: 50 mL of solution A were put in a separation funnel, to this were added, 50 mL Hg^{2+} dissolved in a hydrogen chloride, potassium chloride buffer, with pH 1,8.
The mixture were shaken by hand for 1,5 minutes, then the chloroform phase were distributed in 5 mL portions, in 8 test tubes. The test tubes were labeled in placed in a sonic bath, a timer was set and the samples were taken out at the indicated times. After the sample were taken out, the spectra were obtained after 2 minutes.
The sonic bath were a Branson 1210, from Buch and Holm A/S, and it was filled with demineralised water.

Procedure of the continue variance experiment

Preparation of solutions. A dithizone solution (2756 ppb, 100 mL) was prepared from the stock solution. The dithizone solution was then diluted to 9 samples, containing different moles fractions, x_i, of dithizone in the range from 0.1 to 0.9 mole fractions. A mercury ion solution (3248 ppb, 100 mL) was also prepared from stock solution. The mercury ion solution was then diluted to 9 samples, containing different mole fractions of mercury ions in the range from 0.1 to 0.9 mole fractions. Both dilutions were done by measuring weight on a Mettler PM480 Deltarange.

Procedure. The UV-VIS spectres of the samples were measured with a Red Tide USB650 from Ocean Optics and a computer. The UV-VIS spectrometer was set to calculate the absorption with an average of 10 samples with a sampling length of 60 ms. Pure chloroform was used as reference. The dithizone and mercury ion samples are paired so the sum of the moles are constant. First, the absorption spectre of pure dithizone (0.9 x_i, approx. 2 mL) solution was measured. Next, dithizone solution (0.9 x_i, 5 mL) and mercury ion solution (0.1 x_i, 5 mL) were added to a separation funnel and then shaken for 120 seconds. Excess chloroform gas was let out of the funnel every 30 seconds to decrease the pressure inside the funnel. Then, some of the dithizone phase (approx. 2 mL) was extracted to a glass cuvette and the absorption spectra of the sample was measured 2 minutes after the shaking was ended. The experiment was redone with same procedure for each sample pair. The separation funnel were emptied and rinsed with Milli Q water between each sample.

Procedure of mercury standard curve

Preparation of solutions 3 dithizone solutions were prepared from the stock solution. A 4022 ppb (100 mL) solution, A 606 ppb (100 mL) solution and a 202 ppb (100 mL) solution A mercury ion solution (1218 ppb, 100 mL) was also prepared from stock solution. The mercury ion solution was then diluted to 6 samples, containing different concentrations of mercury ions in the range from (406 ppb - 6.4 ppb).

Procedure. The UV-VIS spectres of the samples were measured with a Red Tide USB650 from Ocean Optics and a computer. The UV-VIS spectrometer was set to calculate the absorption with an average of 10 samples with a sampling length of 60 ms. Pure chloroform was used as reference. First, the absorption spectre of the pure dithizone (4022 ppb, approx. 2 mL) solution was measured. Next, the dithizone solution (4022 ppb, 4 mL) and a mercury ion solution (6.4 ppb, 4 mL) were added to a separation funnel

and then shaken for 90 seconds. Excess chloroform gas let out of the funnel every 30 seconds to decrease the pressure inside the funnel. Finally , some of the dithizone phase (approx. 2 mL) was extracted to a glass cuvette and the absorption spectra of the sample was measured 2 minutes after the shaking was ended. The experiment was redone with same procedure for every mercury ion solution, some of the concentrations were measured multiple times for calibrations. The separation funnel were emptied and rinsed with Milli Q water between each sample. The same procedure were also used for the other 2 dithizone solutions.

Procedure of light effects on dithizone

Preparation of solutions Two dithizone solutions (6722 ppb, 100 mL) were prepared from stock solution. Next, one solution was left in the fume hood, so it would be exposed to both daylight and darkness. The second solution was put in a cupboard and was only taken out when needed for samples, therefore it was mainly exposed to darkness. The solutions were kept for several weeks and were analysed during the project to see if light had a degenerate effect on dithizone.

Procedure. First, two dilution series were made from both the "light" and "dark" solutions in the range (6722 ppb - 53 ppb). Next, the dithizone dilution were shaken on VF2 Janke and Kunkel vortex mixer for 10 seconds. And then extracted (approx. 2 mL)to a glass cuvette and the absorption spectra of the sample was measured approx. 1 minute after the shaking was ended. The UV-VIS spectres were measured with a Red Tide USB650 from Ocean Optics and a computer. The UV-VIS spectrometer was set to calculate the absorption with an average of 10 samples with a sampling length of 60 ms. Pure chloroform was used as reference. The experiment was redone with same procedure for every dithizone dilution, some of the concentrations were measured multiple times for calibrations. The separation funnel were emptied and rinsed with Milli Q water between each sample.

Discussion

5.1 Reflections on the molar absorptivity experiment

The values listed in table 4.1, differ from the ones found in literature, a possible explanation could be that our dithizone solution were of a lower concentration, than calculated. From figure 4.3, the linear fits can be compared, the 605 nm lit (the yellow one) and 605 nm (the blue one), would be overlapping if the concentrations use our curve all decreased by roughly 9 %. The 9 % comes from the ratio $\frac{37{,}2\cdot 10^3}{40{,}8\cdot 10^3} = 0{,}91$. But in order to determine this, we should have made more experiments, to demonstrate higher accuracy.
In [1] the coefficient were obtained, by adding a aqueous solutions of know concentrations of silver(I) to a solution with dithizone in chloroform this was shaken, the before and after absorbance recorded. The idea behind their method were as follows, from the assumption that all of the silver were in complex, in a 1 to 1 ratio with dithizone, the molar apsorptivity could be found by $\epsilon_0 = \frac{\Delta A}{[\mathrm{Ag}^+]}$. The strength of this method, lies in the fact that the concentration of dithizone solution can be unknown.
One point on the method applied by [1] is that they used rather high concentrations of dithizone, their start concentration were approximately 6 ppm. Our data suggest that concentrations over 2 ppm outside of the linear range. In the overall scheme of things it is not important, since our calibration curves for dithizone mercury complex, is independent of the exact dithizone

concentration.

5.2 Reflections on the storage experiment

The two experiments indicate that there are some degree of deterioration over time, for a solution dithiozone in chloroform. There were an indication that exposure to light accelerated the deterioration, this is in line of what is stated in [6].
It was deemed that a dithizone stock solution could be used for up to ten days if kept in the dark.

5.3 The separation funnel experiment

The experiments did not find any optimal shaking time or that the absorbance is dependent on the shaking time during the time periods which was measured. It must be assumed that there is a shaking time minimum, which lie below 15 seconds. At both high and low concentrations the equilibrium is established before 15 seconds of shaking. It is concluded just be consistent the same shaking period in future experiments to minimize a source of errors and eliminate a variable, we chose 90 seconds of shaking time.

5.4 Reflections on the sonication experiment

The experiment showed that the sonic bath caused significant changes at the λ_{605nm} peaks of pure dithizone in chloroform, without any sensible correlation between time spend in the sonic bath and decrease in peak height. The peak at λ_{440nm} were less affected. We theorize that the peak at λ_{605nm} comes from the S-H bond, and that ultrasound is so energetic that bound breaks. This often seen with indicator that the loss of a proton comes with a change in color.
For the peaks around λ_{485nm} there were large differences in absorption when

exposed to ultrasound, for the mercury-dithizone complex. Even though they eventually stabilized, we chose to use a separation funnel, since this enabled us to obtain the spectra immediately.

5.5 The continuous variation experiment

This experiment were in agreement with the complex formation ratio of 2:1 between dithiozone and mercury. This is consistent with the result in [1].

5.6 Reflections on the external standard curve- and the unknown sample

The Dz 202 standard curve was the most inaccurate, in the both the lower and higher ranges. This was unexpected, because it was supposed to be the most precise in 5-35 ppb range with a low detection limit and and narrow confidence limit. This precision could maybe be obtained if the low ppb Hg^{2+} samples were remeasured again with a ≈200 ppb dithizone solution. The Dz 606 ppb, Dz 4022 ppb and the combined standard curves gave almost the same observed values for the different samples. They also came closer to the actual concentrations of the samples. It was unexpected that they also showed the higher accuracy in the low ppb range, even if some of the actual concentrations were well below the model's detection limits. The Dz 606 ppb and Dz 4022 ppb standards determined 4 of 5 concentrations within the 95% confidence limit, both missing the higher Hg^{2+} concentration (406 ppb) with a few ppbs. This was also unexpected as it was assumed that these models would be better to determine the higher concentrations.

Conclusion

We were able to create two models that could determine Hg^{2+} concentrations in the ppb ranges, by measuring absorbance using a portable uv-vis detector. It was verified that the complex formation ratio is 2:1, between dithizone to mercury(II). The complex equilibrium is reached within 15 seconds of shaking, using a separation funnel, with dithizone in chloroform with equal volume of aqueous solution containing mercury(II) ions. The usage of sonic baths should be avoided, due to its degenerating effects on dithizone and that it scrambles the absorption spectra of the dithizone mercury complex. It was found that when storing dithizone in chloroform it should be kept from exposure to light in order to slow down the deterioration.

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