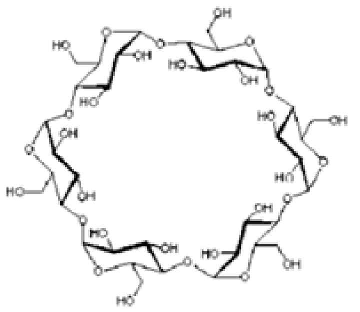
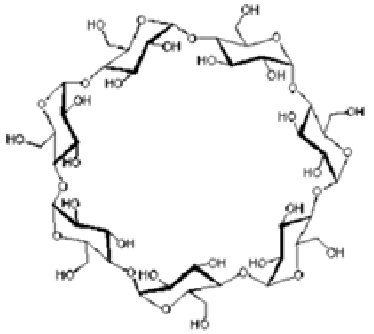
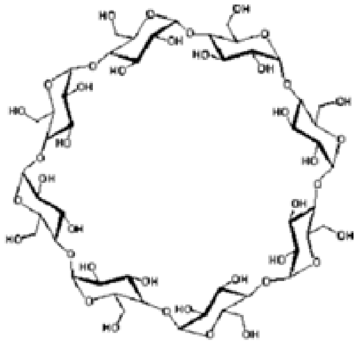
# Detection of heavy metals with dithizone-cyclodextrin complex


α-cyclodextrin


β-cyclodextrin


γ-cyclodextrin

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# Abstract

Heavy metals are recognized toxic elements and their release into waters causes harmful effects on human health and the environment, since they are not biodegradable (Jaishankar et al, 2014). A way to detect heavy metals in organic solutions is with dithizone and its ability to form coloured solutions of dithizone-metal complexes (Fourmentin et al, 2018). This study investigates the detection of five different heavy metals (Cd, Co, Cu, Pb and Zn) in water solutions using dithizone as a detecting reagent and cyclodextrin to increase the solubility of dithizone in water by spectrophotometry. Two cyclodextrin (HP-β-cyclodextrin and β-cyclodextrin) were tested and compared with the solubility ability of acetone and chloroform Milli Q water mixtures. β-cyclodextrin-Milli Q water have been shown to be the best solvent for increasing the solubility of dithizone. Five different heavy metals (Cd, Co, Cu, Pb and Zn) was tested in a dithizone-cyclodextrin aqueous solution. The results for the detection of heavy metals was mostly inconclusive, as the absorbance of most metals decreases with increasing concentrations of the metals. It was possible to detect zinc-dithizone-β-cyclodextrin complex by spectrophotometry. It can be concluded that colorimetric detection of heavy metal via dithizone-β-cyclodextrin is not a viable technique for detection of Cd, Co, Cu and Pb, but this technique can be utilized to detect Zn in an aqueous solution.

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# 1 Introduction

Many metals are found naturally in the nature in various compositions depending on the environments and concentrations. Some of those metals are called heavy metals. A lot of the heavy metals are daily released into the environment through smoke, sewage and waste all over the world by humans. The most commonly found heavy metals in water are arsenic, cadmium, chromium, copper, lead, nickel, and zinc (Jaishankar et al, 2014).

Because of our increasing consumption, the releasing of the heavy metals are only getting worse. Many heavy metals are very dangerous for the environment and living organisms, even in small doses. Some of them are vital for our survival, such as iron, nonetheless all heavy metals in large doses can be toxic. The releasing of the heavy metals into the environment is becoming a critical topic of discussion as they are non-degradable. Thus meaning that even if small doses are presented in the environment, they can accumulate in living organisms, which can then be introduced to humans through consumption (Jaishankar et al, 2014).

The world is already seeing the effects of heavy metal consumption. There are some places in Africa, where it can be difficult to live near a mine facility, because a lot of the heavy metals from the mine often gets into their drinking water and give the people problems with their health (Liu et. al, 2014). An example is in Kabwe city in Zambia, which has a high soil concentration of lead (759 mg/kg), cadmium (22.3 mg/kg) and zinc (106 mg/kg), the report states that this are attributed to the closed lead and zinc mine (Yabe, et al 2010). Exposures and deaths due to heavy metals can be decreased by developing simple methods for heavy metal determination people can apply to their drinking water (IUG, 2009).

Now days heavy metals are detected by using heavy equipment, like NMR, mass spectrometry, capillary electrophoresis (CE), etc. (National Research Council, 2003). These methods usually need a lab with electricity and are not that easy to carry around in the field. Therefore, it is needed to develop a method, that are easy to use, lightweight and in no need of electricity.

In past studies, multiple different organic solutions were used to investigate the effects of dithizone and its ability to form colored solutions of dithizone-metal complexes. Such solutions (i.e chloroform, methanol) are toxic compounds to humans and the environment, therefore it would be ideal to utilize a solution which is effective as well as less harmful to its surroundings. One group of less harmful compounds that has been investigated to increase the solubility of hydrophobic compounds in aqueous solutions is cyclodextrins. Cyclodextrins are a group of cyclic oligosaccharides that can be used to increase the solubility of compounds that are not soluble in aqueous solutions, due to them having a hydrophobic interior and a hydrophilic exterior (Fourmentin et al, 2018).

In this project we investigate how cyclodextrin increase the solubility of dithizone and dithizone-metal complexes in a water solution. The utilization of cyclodextrins in comparison to other compounds were used to conclude its capability in portraying the colorimetric capacities of dithizone and of its different metal complexes.

## 1.1 Problem definition

Does the addition of cyclodextrins affect the solubility of dithizone and its ability to form different dithizone-metal complexes?

### 1.1.1 Sub-questions

- How do the HP-β and β-cyclodextrin solutions compare to acetone and chloroform solutions?
- Moreover, what are the limitations/abilities of using cyclodextrins to detect different heavy metals in water?

# 2 Theory

In order to be able to conduct experiments it is important to understand the basic aspects of the materials in use. Based on our problem formulation, the basis of this project will be on dithizone, its solubility and its ability to form metal complexes. The solubility for dithizone is dependent on the solutions that it is dissolved in, specifically for this project the interests is in cyclodextrins. As there a quite a few variations of cyclodextrins all which are relatively new in the research field, it is important to understand the basic role cyclodextrins play when concerning the changing of the solubility of solids (particularly dithizone). Likewise, it is necessary to distinguish how each variation of cyclodextrins, more precisely HP-β and β, effect the solubility of dithizone. Creating a solution of dissolved dithizone allows one to see if water contains heavy metals. This is of importance as heavy metals are quite dangerous to the environment and humans. As dithizone is a titrant, in other words it changes colour when it forms dithizone-metal complexes, the absorbance spectra of a dithizone metal solution will be able to determine the (relative) amount of Heavy metals present. To be able to collect the absorbance spectra data a spectrometer is used.

## 2.1 Dithizone

Most heavy metals are natural occurring substances that are present in the environment. They are found in plants, animals, water and even air thus it's impossible to not be exposed to some level of heavy metals. At low dosages they are relatively harmless but in large amounts, they can be potentially life threatening that's why it's important to be able to measure the concentration of heavy metals in the environment. One way to find the presence and concentration of heavy metals in a solution is to react it with dithizone (Martin & Griswold, 2009). At room temperature 1,5-Diphenylthiocarbazone can be found in a solid state, its melting point is 168 degrees Celsius, its molecular formula is $C_{13}H_{12}N_4S$ and its molecular weight is 256.33 g/mol 5). It is black in colour, has an acidic nature and has a solubility of 31.8 ul/ (Ntoi, Buitendach & Eschwege, 2017). Dithizone it known to be unstable in light as well as temperatures that are above 35 degrees. Instability in dithizone also arises when it is introduced to heavy metals and oxidation. Deteriorated dithizone or dithizone solutions with impurities, such as small amounts of aldehydes and ketones, can be depicted by a yellow solution. (Thiagarajan & Subbaiyan, 1992)

Dithizone also known as 1,5-Diphenylthiocarbazone, is widely used to determine the amount and the type of metals in a solution (Khan, Ahmed & Bhanger, 2005)(Snyder, 1947). Dithizone is more widely known as a colorimetric reagent of the first class, that is to say that it can react with minute concentrations' (about 0.2 ppm) of metals in a solution and create a coloured solution (Clyde, 1986). Dithizone can also be used as a titrant because it has been shown that its applicable as an amperometic reagent for titrations, that is to say it can be used to find the equivalence point in a titration by measuring the electrical currents produced in the titration (Clyde, 1986).

There is a wide range of metals that can react with dithizone and there are multiple ways to measure the amount of metal-dithizone complexes formed. Firstly, as dithizone (refer to figure 1) and dithizone-metal complexes are insoluble in water and therefore to determine the amount of metal in the solution the dithizone is required to be dissolved in an organic compound. In most experiments dithizone is dissolved in chloroform that results in a green coloured solution.

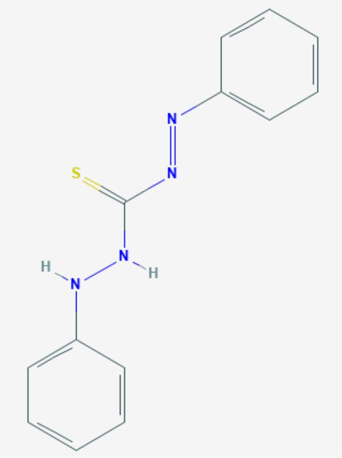

*Figure 1: Chemical composition of dithizone in keto-formation (PubChem, 2018).*

When metals are introduced they form metal complexes that that render the chloroform solution to change to a different colour. Copper (Cu), silver (Ag), gold (Au), zinc (Zn), cadmium (Cd), mercury (Hg), thallium (T1) (ous), tin (Sn) (ous), lead (Pb), bismuth (Bi), magnesium (Mn) (ous), cobalt (Co), Ni (nickel), lead (Pd), and platinum (Pt) are all metals that will form complexes with dithizone, their reactions are said to be rather distinctive. Their reaction towards dithizone can be put in 4 groups: “(A) Pd, Au, Hg, Ag, and Cu are the metals reacting in an acid solution; (B) in a slightly acid solution, buffered to a pH of about 6, Zn is most sensitive to dithizone (C) in a slightly alkaline solution containing CN', Sn (ous), T1 (ous), Pb, and Bi are reactive; and (D) in a strongly alkaline solution containing tartrate ion, Co, Ni, and Cd are affected.” (White & Fischer 1936). The colour changes exhibited by the the dithizone-metal complexes are caused by the a intramolecular proton transfer, in other words a sequence of oxidations and reductions are able to occur. This is due to the delocalization of pi electrons along the base of the dithizone (Ntoi, Buitendach & Eschwege, 2017). Some metals form a two dithizone complex, for example lead- dithizone complex (refer to figure 2) this dithizone-rich complex is known as the keto form. Whereas most metals form a single dithizone complex for example zinc-dithizone complex this metal rich complex is known as the enol form (refer to figure 3) (Irving & Bell, 1952).

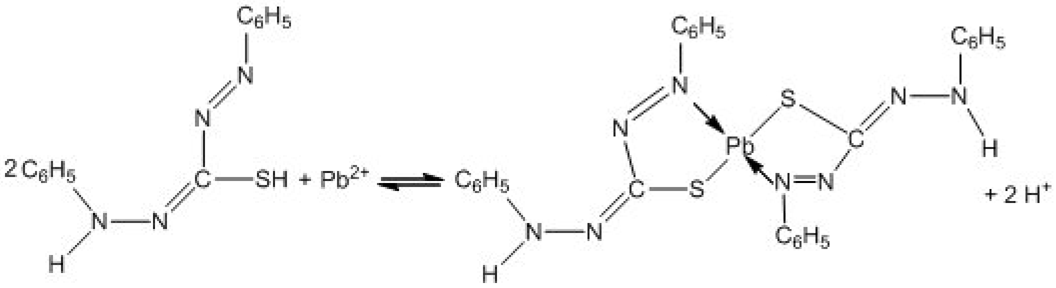

*Figure 2; formation of lead-dithizone complex (Nur, et al,2017)*

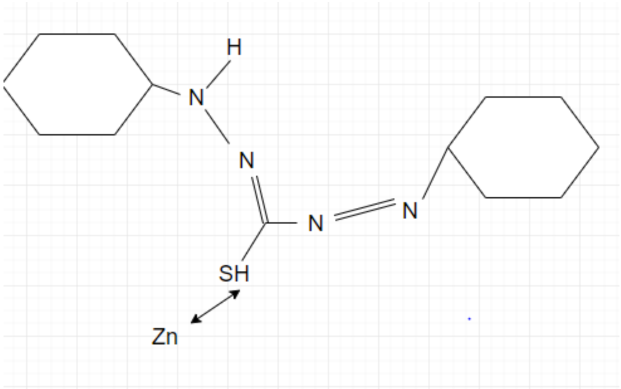

*Figure 3; formation of zinc(+2)-dithizone complex (selfmade).*

These two different forms can be seen through its absorption at different wavelengths. The keto formation can be seen at a wavelength of 600 nm and the enol form can be observed at a wavelength of 470 nm (refer to figure 4) Dithizone is able to form complexes by replacing the hydrogen atom bonded to the sulfur with a metal and by having a double bonded nitrogen form a bond with the metal (Irving & Bell,. 1952).

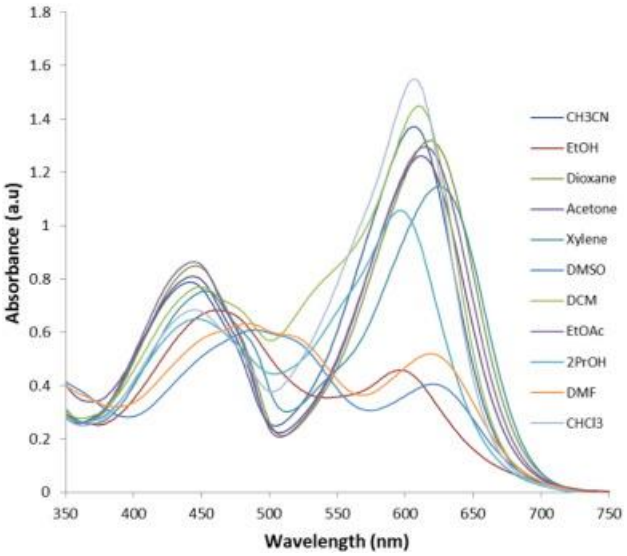

*Figure 4; Absorbance graph of dithizone in multiple different solvents (Rauf, et al. 2015)*

## 2.2 Cyclodextrin

Cyclodextrins (CD) are cyclic oligosaccharides that were first described in 1891 by the French pharmacist Antoine Villiers and was then called “cellulosines”. But it wasn’t until by the end of the 1940s that they were described as “cyclodextrins” by the German chemist Friedrich Cramthat (Fourmentin, et al, 2018). Cyclodextrins are produced by enzymatic degradation of starch, and depending on the type of starch, reaction conditions and the type of enzyme, different cyclodextrins can then be produced. Cyclodextrins contains six or more α (1-4) linked D-glucose (D-glucopyranoside) units. Due to the chair conformation of the glucose units, cyclodextrin has a truncated cone shape with a hollow hydrophobic interior and a hydrophilic exterior (Jambhekar & Breen, 2016). The most common types of cyclodextrins are α-cyclodextrin which contain six glucose units, β-cyclodextrin (β-CD) which contain seven glucose units and γ-cyclodextrin which contain eight glucose units (refer to figure 5 and 6) (Fourmentin, et al, 2018). ). As can be seen in Table 1, cyclodextrins, especially β-cyclodextrin, have a relatively low solubility in water. α-, β-, and γ-cyclodextrins have a relative low solubility in water due to intermolecular bonding via their hydroxyl groups (Fourmentin, et al, 2018). To increase the solubility of cyclodextrins in water, cyclodextrins has been modified to increase their solubility. One of the ways to increase the solubility is by substitution of hydroxyl groups with more hydrophilic groups, and thus increase their solubility in aqueous solutions. One of the cyclodextrin derivatives is hydroxypropyl-β-cyclodextrin (HP-β-CD), where some of the hydroxyl groups has been substituted by hydroxypropyl groups, as can be seen in figure 6 (Jambhekar & Breen 2016). As can be seen in Table 1 hydroxypropyl-β-cyclodextrin has a higher solubility compared to β-cyclodextrin.

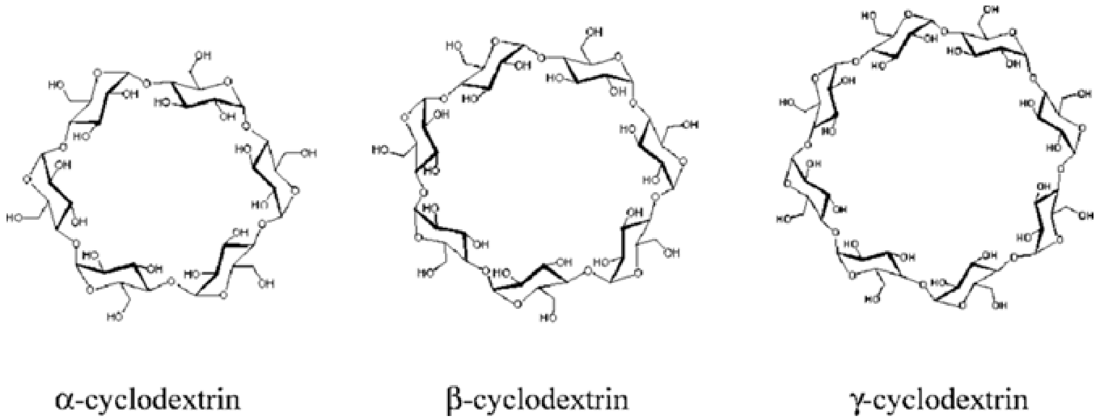

*Figure 5; The structure of the cyclodextrins ʎ , β and □ (Li, et al. 2007).*

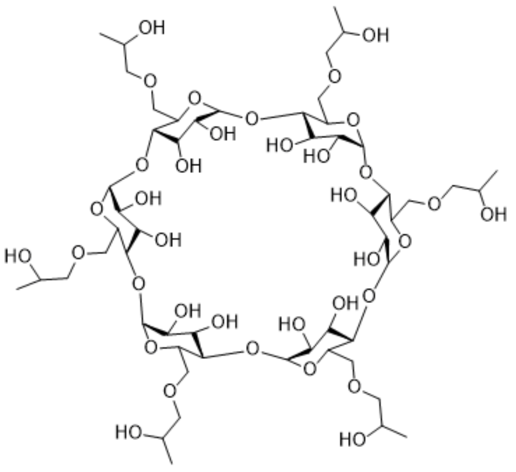

*Figure 6: The structure of hydroxypropyl-β-cyclodextrin (HP-β-CD) (Li, et al. 2016)*

Because of cyclodextrins molecular shape and structure, cyclodextrins have the ability to trap a guest molecule in their hydrophobic interior and thus form inclusion complexes with other molecules. The driving forces behind the formation of inclusion complex are dipole-dipole forces, van der Waals interactions, hydrogen bonds, hydrophobic interactions, solvent effects and the release of enthalpy-rich water from the interior (Fourmentin, et al, 2018). Depending on the size of the guest molecule and on the size of the cyclodextrins, one guest molecule can form complex with one (1:1 complex) or two cyclodextrin molecules (2:1 complex)(refer figure 7) or two guest molecules can form complexes with one cyclodextrin molecule (1:2 complex) (Fourmentin, et al, 2018). According to *Mohamad S. and Mohd Kamal S.N.R.*, the inclusion complex that dithizone-β-cyclodextrin forms with a host-guest ratio of 2:1, whereas the zinc-dithizone-β-cyclodextrin inclusion system forms with a host-guest ratio of 1:1 (refer figure 8) (Mohamad & Mohd Kamal, 2013)

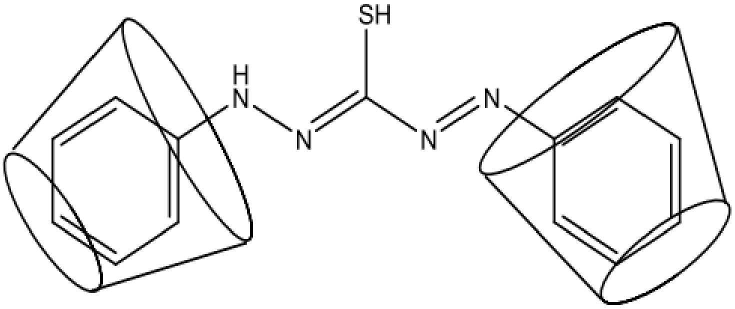

*Figure 7; A 2:1 cyclodextrin, dithizone compound (Siti, et al, 2011).*

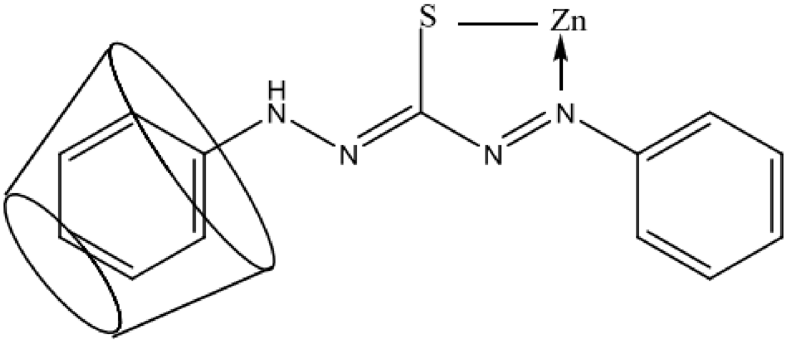

*Figure 8; A cyclodextrin bonded to a dithizone-zinc metal complex (Siti, et al, 2011).*

**Table 1: Characteristics and properties of α-CD, β-CD, HP-β-CD and γ-CD.**

Adapted from (Fourmentin, et al, 2018; Jambhekar & Breen, 2016)

| Cyclodextrin | α-cyclodextrin | β-cyclodextrin | HP-β-cyclodextrin | γ-cyclodextrin |
|---|---|---|---|---|
| D-glucose units | 6 | 7 | 7 | 8 |
| Molecular formula | $C_{36}H_{60}O_{30}$ | $C_{42}H_{70}O_{35}$ | $(C_6H_9O_5)_7(C_3H_7O)_{4.5}$ | $C_{48}H_{80}O_{40}$ |

| | | | | |
|---|---|---|---|---|
| Avg. molecular weight (g/mol) | 972 | 1135 | 1400 | 1297 |
| Solubility in water at 25 °C (g/L)(Fourmentin et al 2018; Jambhekar and Breen 2016) | 145 | 18.5 | >600 | 232 |

Due to their relative non-toxicity and their ability to form inclusion complexes, cyclodextrins have many practical applications including: enhancing the water solubility of hydrophobic molecules, enhancing the bioavailability of drugs, masking undesired odor and taste, decrease the rate of degradation of drugs from exterior factors such as light, temperature, oxidation and hydrolysis and thus increase the stability of drugs. Cyclodextrins have many applications in many fields and industries including but not limited to: pharmaceutical industry, biotechnology, analytical chemistry, textile industry, cosmetics industry, food, and agriculture industries (Fourmentin et al 2018).

## 2.3 Heavy Metals

Heavy metals are natural processes of the Earth's crust, they are non-degradable and cannot be destroyed. Any toxic compound that has a high density (greater than 5 g/cm3) in their element and high atomic weight (relative atomic mass) can be considered as heavy metals, an example for cadmium (Cd), mercury (Hg), chromium (Cr), arsenic (As), thallium (Tl), and lead (Pb). Heavy metal pollution has become really a concern all over the world, due to their harmful effects on humans, they enter our organism via food, air and drinking water (Duruibe, Ogwuegbu & Egwurugwu, 2007). Not all metals have the same environmental effect. Some are important trace elements, and some are potent toxins, and for some the ecotoxicological properties are not well known (Mahurpawar, 2015).

Heavy metals such as Pb, Hg, As and Cd are known human carcinogens. These metals are among the severe pollutants under natural conditions because of their persistent and bioaccumulation properties, they are also classified as priority pollutants and can be toxic even in small concentrations (Järup, 2003). The second category includes metals that are required for living organisms, and includes these most widely used in society, such as Fe, Cu and Zn. These metals are major constituents of the so called Technosphere. The last category consists of metals such as platinum (Pt), rhodium (Rh) and iridium (lr), metals that are increasingly used in catalysts and electronic components, but whose effects on the environment are not well known (Morais, Costa & Lourdes Pereir, 2012).

Poisoning by heavy metals could result, from higher ambient air concentrations close to emission sources, from consumer waste, industrial, or from acidic rain breaking down soils and releasing heavy metals into lakes, streams and groundwater. In general pollution in river and streams is due to industrialization. Industries such as textiles, recycle facilities, fertilizer and drug industries located besides, the river or lakes are the principal causes for heavy metal pollution (McLaughlin et al., 2000).

Over time heavy metals are unsafe because they tend to bioaccumulate meaning that there is an increasement in the concentration of a chemical in a living organism, compared to the chemical's concentration in the environment. River sediments become the storage of heavy metals, which in turn becomes the potential source of metal pollution to the linked aquatic systems. The processes by which heavy metals are deposited in the river sediments are precipitation, adsorption and hydrolyzation (McLaughlin et al., 2000).

The pH is an important factor controlling chemical form of metals. pH affects both solubility of metal hydroxide minerals and adsorption - desorption processes. Most metal hydroxide minerals have very low solubility under the pH conditions prevailing in natural water. Because the concentration of hydroxide ion is directly related to pH, the solubility of dissolved metal hydroxide minerals increases with decreasing pH (Chuan, Shu & Liu, 1996), and more dissolved metals become potentially available for incorporation in biological processes as pH decreases. Ionic metal species also are the most toxic form to aquatic organisms. Temperature exerts a significant influence on metal speciation, because most chemical reaction rates are highly sensitive to temperature changes (Calmano et al., 1993).

### 2.3.1 Heavy Metals in the experiments

In the experiment only some heavy metals are examined. There is used cobalt (Co), copper (Cu), zinc (Zn), lead (Pb) and cadmium (Cd). These metals properties and effects on humans and the environment will be elaborated in the following sections.

#### 2.3.1.1 Cobalt

Cobalt (Co) is number 27 in the periodic table, with an common oxidation state of either +2 or +3 and often magnetized and alloyed with aluminium and nickel to make very powerful magnets. Cobalt is found in cobaltite, skutterudite and erythrite. Cobalt metals are sometimes used in electroplating and cobalt salts is often used to produce the colour blue in paint, glass etc. In some countries the radioactive cobalt-60 is used to treat cancer. Cobalt function as a trace element and is a part of the active site of vitamin B12 (Royal Society of Chemistry, 2018).

Cobalt is essential for preserving human health, it is very helpful in the treatment of  diseases such as anemia because it stimulates the production of hemoglobin (red blood cells). Human organism may also use cobalt in place of zinc. Cobalt also helps to stimulate and regulate the production the production of some enzymes, such as thyroxine, a thyroid hormone (Leyssens et al., 2017).

#### 2.3.1.2 Copper

Copper (Cu) is number 29 in the periodic table with an common oxidation state +2 and is one of the oldest metals to be worked by people. Copper occur naturally and is obtained from treatment of chalcopyrite and bornite. It had been used as armor and for coins, but now a days it is used in electrical equipment because of its ability to lead electrons (Royal Society of Chemistry, 2018).

Copper is essential for hemoglobin synthesis and in catalysis of metabolic reactions. It plays a crucial role in many biological enzyme systems that catalyze oxidation and reduction reactions. However, it is very toxic to aquatic organisms at relatively high concentrations in the environment. (Stern, et al., 2007).

#### 2.3.1.3 Zinc

Zinc (Zn) is number 30 in the periodic table with a common oxidation state +2 and are mostly used to galvanize other metals, such as iron. Zinc is found in several ores, like calamine. Galvanized steel, which zinc is in, is used for car bodies, suspension bridges etc. It is also used to produce die-castings, and manufacturing of many product such as paints, rubber, plastics etc. (Royal Society of Chemistry, 2018). Zinc is very essential for the life of human being and animals, it is essential for male reproductive activity. Its presence in many enzymes involved in significant physiological functions like protein synthesis. The excess amount of zinc can cause system dysfunctions, cause impairment of growth and reproduction, the clinical signs of zinc toxicosis have been reported as vomiting, diarrhea, bloody urine, liver failure, kidney failure and anemia (Chasapis et al., 2012).

#### 2.3.1.4 Lead

Lead, also called palladium (Pd), is number 46 in the periodic table it is found with a common oxidation state of either +2 or +4 and is mainly used in catalytic converters for cars. Lead is extracted as a by-product of nickel, copper or zinc refining. It is used in a lot of products today, such as jewelry, dental fillings, laptops and mobile phones (Royal Society of Chemistry, 2018). Lead is a well-known neurotoxin. Damage of neurodevelopment in children is the most serious effect. Exposure in uterus, during breastfeeding and in early childhood may all be responsible for the effects. It has been suggested that lead on a molecular and cellular level may enhance carcinogenic events involved in DNA damage, DNA repair, and regulation of tumor suppressor and promoter genes (Tchounwou et al., 2012).

#### 2.3.1.5 Cadmium

Cadmium (Cd) is number 48 in the periodic table with the symbol Cd with a common oxidation state +2. Cadmium is often used for industrial purposes (electroplating, batteries, pigments, alloys etc.) and its presence in the environment is due to human activity. The use of cadmium in batteries has shown significant development in recent years, but in response to environmental concerns its commercial use has failed in developed countries. Cadmium is known to be toxic for human health and very pollutant for the environment. The main exposure to cadmium is mostly via the respiratory or gastrointestinal area  causing gastrointestinal area erosion, hepatic or renal injury and coma, pulmonary depending on the way of harming (Johri, Jacquillet & Unwin, 2010). The contamination is mainly by drinking water or food. Cadmium can be damaging the lungs, liver, kidney, testis, brain etc. Significant inhalation of Cd can occur from tobacco smoke. Cereals, especially rice, also tend to accumulate Cd from the soil, resulting in foodborne exposure. Amyotrophic lateral sclerosis, optic nerve damage, striatal damage and peripheral polyneuropathy were observed as long term neurotoxic consequences (Tchounwou et al., 2012)

## 2.4 Absorption

Absorption is a process, where gas, liquid or energy penetrates a substance and gets absorbed. In this section is there a focus on how ultraviolet (UV) light affects molecules and how the process can be observed. When a source of energy (light) penetrates atoms or molecule, the energy gets absorbed causing the atom to go from a low energy state (called ground state) to a higher energy state (called the excited state). The molecule’s transition in energy is caused by the movement of an electron from an occupied orbital- usually an orbital closer to the nuclei- to an unoccupied orbital -an orbital further away from the nucleus. The change in energy is the change of potential energy of the electron, in other words if it is closer to the nuclei then it has a low potential energy and vice versa. This transition is often from the highest occupied molecular orbital (HOMO) to the lowest unoccupied molecular orbital (LUMO) (Pavia, et al, 2015).

Some principles of absorption spectroscopy were developed by Lambert Beer. He stated that the absorbance is proportional to the traveling length. Later, he discovers that the absorbance also is proportional to the concentration. From this he develops an empirical expression for absorption and wavelength called Lambert Beer’s Law,

$$A = \log\left(\frac{I_0}{I}\right) = \varepsilon \cdot c \cdot l$$

Where $A$ is absorbance, $I_0$ is the intensity of the light incident upon sample cell, $I$ is the intensity of the light leaving sample cell, $c$ is the molar concentration of solute, $l$ is the length of sample cell in cm and ($\varepsilon$) is the molar extinction coefficient. The molar extinction coefficient is not a variable parameter involved in preparing a solution, but a property the molecule goes through under an electronic transition. The transition in molecules penetrated by light can be observed by using a spectrophotometer that makes an absorption spectrum. (Pavia, et al, 2015) From the equation can either absorbance (A) or concentration (c) be calculated, if epsilon ($\varepsilon$) and length (*l)* are known. To obtain ($\varepsilon$) and length (*l)* can be done by plotting A versus c for a serie of solutions. Here will the equation give a straight line, where the slope is epsilon ($\varepsilon$*)* x length (*l*). (Zumdahl & DeCoste 2017)

The spectrum for UV and visible light are composed in a broad range of radiation wavelength 400- 800 nm. An absorption spectrum for molecules that absorbs in the UV usually consist of sharp lines, because of the quantized process between two discrete energy levels. The UV absorption for molecules often occurs over a wide range of wavelengths because molecules at room temperature have many excited modes of vibration and rotation. The transitions that are observed on the spectrum differ only slightly, because there are so many possible way for the electron crossing orbital. The spectrophotometer cannot resolve the transitions, because of the lines for the transitions are placed so closely together. Therefore, the spectrophotometer traces the pattern for a short period, which gives an absorption spectrum for different wavelength. At a major transition, sees a broad band of absorption near a specific wavelength. (Pavia, et al, 2015)

The spectrophotometer used in the experimental part is a multichannel diode-array spectrophotometer. The spectrophotometer works by sending a visible light and a UV light out from the tungsten and the deuterium lamp. The light passes through the first lens, and then the cuvette that spreads the light. The light that gets through the cuvette then passes through a second lens that makes the light hit a grating. The grating spreads the light, so different wavelengths of the light hits different parts of the diode array, refer to figure 9 (Agilent, 2018).

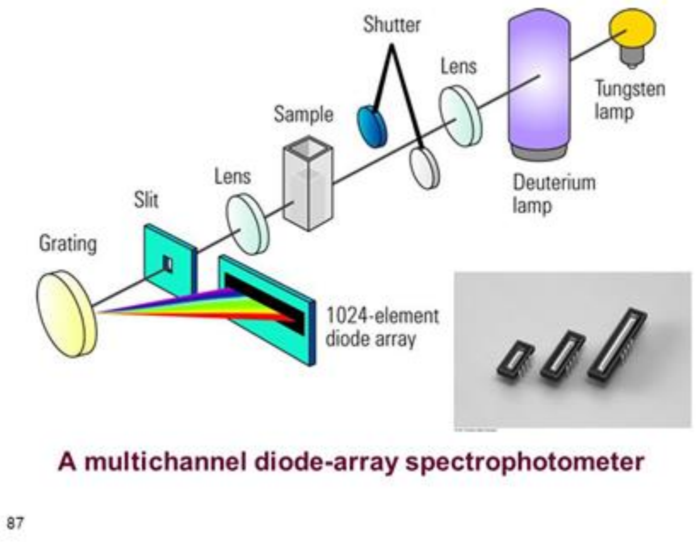

*Figure 9: A sketch of how the spectrophotometer function. The spectrophotometer sends a visible light and a UV light out from the tungsten and the deuterium lamp. The light passes through the first lens, and then the cuvette that spreads the light. The light that passes through the cuvette then goes through a second lens that makes the light hit the grating. The grating spreads the light, so different wavelengths of the light hits different parts of the diode array (Agilent, 2018)*

The relationship between the light that passes through and the reflected light depends on the colour of the solution. Most of the mixed wavelength is absorbed, but the remaining light will then assume the complementary colour to the wavelengths absorbed. This relation is indicated by the colour wheel shown in figure 10. In the colour wheel are the complementary colours diametrically opposite of each other. An example is the absorption of 430-480 nm light renders a substance yellow/orange colour (Reusch, 2013).

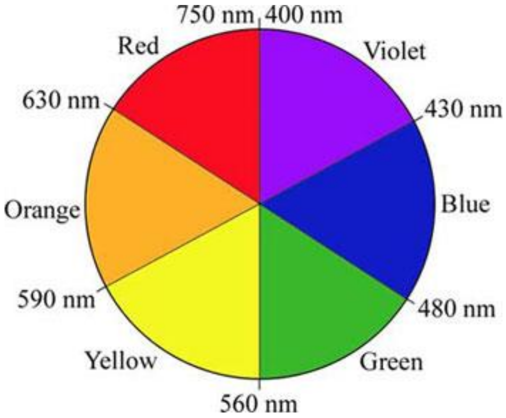

*Figure 10: The colour chart show the complementary colours and at which wavelength they are expected to show in the spectrum (Hayek, 2015).*

# 3 Methods/ experiments

In this experiment study, the experiments were focused on studying the effects of dithizone and dithizone-metal complexes when introduced to a cyclodextrin Milli Q water solution. Multiple other tests included dithizone, heavy metals and acetone mixtures as well as investigations on the effects of different cyclodextrin mixtures. The basis of collecting data was absorbance spectra graphs of different dithizone solutions. To study the ability to detect heavy metals in water solutions using dithizone and cyclodextrins, the experiments was conducted in five main areas: a comparison of the solubility of dithizone in chloroform and in cyclodextrin, detection of metals in a dithizone-acetone solution,  detection of metals in a dithizone-cyclodextrin water solution, determination of the complex formation constant of dithizone-cyclodextrin-metal complex and determination of the stability of the dithizone-metal complexes over time.

## 3.1 Materials

### 3.1.1 Chemicals

- Dithizone (1,5-Diphenylthiocarbazone) 43820 from Sigma-Aldrich,
- MilliQ water
- 10 mM β-cyclodextrin
- 40 mM HP-βcyclodextrin
- Chloroform ($CHCl_3$) Assay (GC) 99.8% F.W 119.38 CAS 67-66-3 EINECS 200-66-8.
- Aceton rein ($CH_3COCH_3$) Assay >99% M.W 58.08 ART.:2659.500 Batch No 26.280120.

### 3.1.2 The heavy metal solutions

**Zinc solution:** 0.2450 g of zinc nitrate hexahydrate ($Zn(NO_3)_2 \cdot 6H_2O$) was dissolved with milliQ water in a 50 mL volumetric flask to make a solution with Zinc concentration:1078 ppm. Zinc solution (c:10.78 ppm) made by diluting Zinc solution (c:1078 ppm) by a factor of 100 in a 100 mL volumetric flask.

**Cobalt solution**: 0.2028 g of cobalt(II)chloride hexahydrate ($CoCl_2 \cdot 6H_2O$) was dissolved with milliQ water in a 50 mL volumetric flask to make a solution with Cobalt concentration:1004 ppm. Cobalt solution (c:10.04 ppm) made by diluting Cobalt solution (c:1004 ppm) by a factor of 100 in a 100 mL volumetric flask.

**Copper solution:** 0.1896 g of copper(II)nitrate trihydrate ($CuN_2O_6$ * 3 $H_2O$) was dissolved with milliQ water in a 50 mL volumetric flask to make a solution with Copper concentration:1026 ppm. Copper solution (c:10.26 ppm) made by diluting Cu solution (c:1026 ppm) by a factor of 100 in a 100 mL volumetric flask.

**Cadmium solution:** 0.0828 g of cadmium chloride ($CdCl_2$) was dissolved with milliQ water in a 50 mL volumetric flask to make a solution with Cadmium concentration:1021 ppm. Cadmium solution (c:10.21 ppm) made by diluting Cadmium solution (c:1078 ppm) by a factor of 100 in a 100 mL volumetric flask.

**Lead solution:** 0.0817 g of lead(II)nitrate ($Pb(NO_3)_2$) was dissolved with milliQ water in a 50 mL volumetric flask to make a solution with lead concentration:1022 ppm. Lead solution (c:10.22 ppm) made by diluting lead solution (c:1022 ppm) by a factor of 100 in a 100 mL volumetric flask.

**Equitments:** Glass beakers, glass volumetric flasks 25, 50 and 100 mL, balance, volumetric pipettes 5, 10 and 20 mL, funnels, magnetic stirrer, magnets, tin foil, *Red Tide USB650* spectrometer from Ocean Optics, graduated cylinders, test tubes, laboratory centrifuge, micropipettes from 2 µL to 1000 µL, cuvettes, pipettes.

## 3.2 Constants throughout the experiment

- All beakers containing dithizone were covered in tin foil to assure there was no light exposure.
- All mixing was done via magnetic stirrer set at 500-750 rpm to assure that the dithizone in every solution was mixed properly.
- All dithizone samples and stock solutions were centrifuged at 2600 rpm for 2 minutes to assure that the absorbance value acquired by the spectrometer was of the dissolved dithizone.

### 3.2.1 Setting for the spectrometer

All absorbance values were acquired with a *Red Tide USB650* spectrometer from Ocean Optics. The average was changed to 10 and the diode (ms) to 80. A reference dark correct in raw was captured using just air as the sample. The dark correct was then disabled and the spectrometer was set to absorbance. A reference sample was made using a cuvette with water.

## 3.3 Comparing chloroform/dithizone and cyclodextrin/dithizone

The purpose of this experiment was to see which solute can dissolve dithizone better. Many of the research papers written about dithizone mix it with chloroform to see a colour change to green (White, W.E 1936). Therefore, in this investigation, dithizone will be mixed with same concentrations of chloroform, HP-β and β-cyclodextrin.

### 3.3.1 Procedure

In three 50 mL beakers, 20 mL of Milli Q water, 0.03g of dithizone and 50 μL of copper (10.26 ppm) was added. 56 μL of chloroform, 12.5 μL of HP-β-cyclodextrin and 50 μL of β-cyclodextrin were separately added in the different beakers. After a time increments of 16, 32, 64 minutes, a (3 mL) test tube was filled and centrifuged at 2600 rpm for 1-2 minutes, or until the mixture was a clear pale yellow coloured liquid. A sample of the liquid was isolated and place it into a cuvette. The sample was then placed in a spectrometer and the absorbance of the sample was measured.

The procedure was redone with 50 μL of zinc (C:10.78ppm) instead of copper.

### 3.3.2 Solubility of dithizone in an aqueous cyclodextrin solution.

The purpose of this experiment was to investigate the solubility of dithizone in an aqueous cyclodextrin solution and using the results to calculate an estimation of ε for dithizone-β-cyclodextrin complex and using the result to estimate the concentration of dithizone in an aqueous solution containing β-cyclodextrin.

### 3.3.3 Procedure.

In a glass beaker covered in tin foil and on a magnetic stirrer, 20 mL Milli Q water and 0.0274g dithizone was added and an absorbance was measured. Afterwards 10 uL of 10 mM β-cyclodextrin was added to the solution and a new measurement was taken. Afterwards 10 uL of β-cyclodextrin was added, so the total volume of β-cyclodextrin was 20 uL and a new absorbance was measured. This process was repeated and absorbances was measured when the total volume of β-cyclodextrin in the solution was: 0 uL(control), 10 uL, 20 uL, 50 uL, 100 uL, 200 uL , 500 ul, 1000 uL, 1500 uL, 2000 uL, 2500 uL and 3000 uL.

## 3.4 Dithizone in an acetone solution

The purpose of this experiment was to see dithizones ability in form metal complexes in other organic solutions. This in turn will be compared to the metal complexes formed with a dithizone cyclodextrin solution. Acetone was used because out of the three organic solutes readily available for use (ethanol, methanol and acetone) acetone, water and dithizone mix created a bolder colour change. In this investigation a dithizone acetone and Milli Q water stock solution was created, then different amounts of metals were added.

### 3.4.1 Stock solution

0.05 g of dithizone, 200 mL of Milli Q water and 70 mL of acetone was added into a 300 mL beaker. Then centrifuge the solution in order to separate the solid dithizone from the clear yellow liquid. With a pasteur pipet, the liquid was carefully pipetted into a different 300 mL beaker.

### 3.4.2 Dithizone-metal complex in an acetone solution

10 mL of stock solution was transferred into five 50 mL beakers.1000, 200, 50, 10 μL of cobalt (C: 10.04 ppm) was added to 4 of the 5 beakers respectively. In 5, 10, 20, 40, 60 min time intervals a sample of each solution was transferred to a cuvette and the absorbance was measured.

The procedure was redone with copper (C: 10.26 ppm).

### 3.4.3 Stability of dithizone acetone solution

This experiment was conducted to see why the absorption of dithizone metal complex decreases over time. When acquiring data for the dithizone metal complexes, it was noticed that the absorption will decrease after a certain point.  It was hypothesized that this was due to light exposure. The absorbance of a solution of acetone, dithizone and Milli Q water was measured once right after zinc was added, and then after 40 minutes in darkness.

#### 3.4.3.1 Procedure

10 mL of stock solution was transferred into a 75 mL beaker where 200 µL of zinc (C: 10.78 ppm) was then added. Absorption reading were done right after the addition of the zinc and the second absorption reading after 40 mins of mixing. It is important to note that the mixture should not be exposed to any light before the 40 min reading.

## 3.5 Experiments with dithizone, β-cyclodextrin and heavy metals

The purpose of the experiments was to investigate whether is it possible to detect five different metals (Cadmium, Cobalt, Copper, Lead and Zinc) in a dithizone-β-cyclodextrin aqueous solution.

### 3.5.1 Stock solution

20 mL milliQ water + 27 mg dithizone + 100 µL β-cyclodextrin. Was mixed in a beaker using a magnetic stirrer for 30 min in darkness. Afterwards the solution was centrifuged to remove excess dithizone.

### 3.5.2 Procedure

The experiment was conducted by adding 10 mL (or 20 mL when specified in the captions of data plots) of the stock solution using a volumetric pipette, that was prepared beforehand, to each beaker. A new stock solution was prepared each time a new experiment was conducted. Afterwards, a known volume (10 µL, 20 µL, 50 µL, 100 µL, 200 µL 500 µL and 1000 µL) of metals (Cadmium, Cobalt, Copper, Lead or Zinc) was added to each beaker. The solution in the beakers was stirred using a magnetic stirrer. Absorbance was measured after 5, 10, 20, 40 and 60 min after a known volume of a specific metal (Cadmium, Cobalt, Copper, Lead or Zinc) was added.

## 3.6 Complex formation ratio of β-cyclodextrin-dithizone-zinc complex

The purpose of this experiment was to investigate the complex formation, that is to say how many metal atoms bond to the dithizone molecules.

### 3.6.1 Procedure

In a 500 mL vol flask 110 mg zinc was added and filled to top with Milli Q water, the solution was mixed until zinc was fully diluted into the water. Then 25 mg of dithizone, 850 µL of β-cyclodextrin and 500 mL of Milli Q water were added into a 600 mL beaker and mix for 10 min in darkness.  2, 5, 7, 10 and 12.5 mL of dithizone mix was pipetted into 25 mL volumetric flasks and filled up to the mark with zinc solution. 2, 5, 7, 10 and 12.5 mL of zinc solution was pipetted into 25 mL tin foil wrapped volumetric flasks and filled up to the mark with dithizone solution. The absorbance of all 10 solutions were then measured along with a sample of the dithizone solution and the zinc solution.

# 4 Results/Analysis

This section is a summary of our results from the experiments, that are explained in the previous section. All the data are gathered with a spectrometer, where the absorption for the different solutions are saved. Most of the results are plotted into graphs, to get a better overview of the data points. Some graphs will show the absorption over time of different solvent at a specific wavelength in order to compare their relative solubilities of dithizone. Additional graphs show the absorbance over wavelength at a specific time to demonstrate a which wavelengths dithizone-metal complexes are identified as well as to see the progression of dithizone-metal complex formation. Other graphs are plotted with different heavy metals concentrations over wavelength, at the same time point to see if the concentration of  has an impact on the absorption. In addition the molar extinction coefficient is calculated for one of the experiments to find the concentration of dithizone in a solution.

## 4.1 Compare the HP-β-cyclodextrin, the β-cyclodextrin, chloroform and acetone

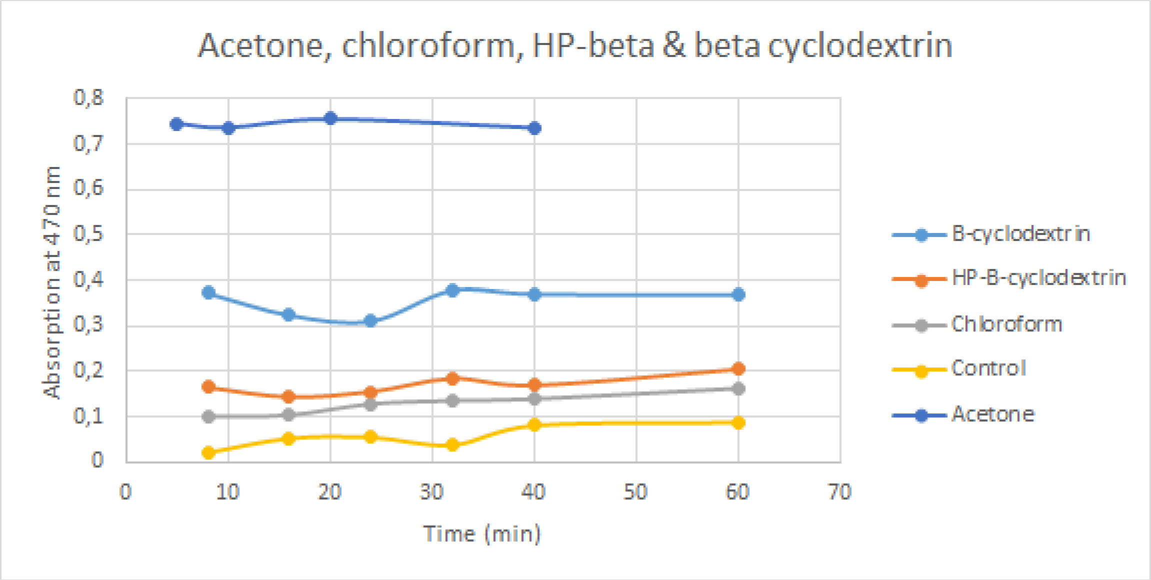

*Figure 11; Absorbances at 470 nm of 56 μL of chloroform, 12.5 μL of HP-β-cyclodextrin and 50 μL of β-cyclodextrin + a solution of 0.03g dithizone and 20 mL of Milli Q water over time (min). Absorbances at 470 nm of 70 mL of acetone in 200 mL of Milli Q water and 0.05g of dithizone over time (min).*

Figure 11 shows the relative solubility abilities of water, chloroform, HP-β-cyclodextrin and β-cyclodextrin when diluting 0.03g of dithizone, with the exception of acetone. The absorption of acetone is around 0.75 because it has a much higher concentration compared to the other solutions. For an example, in this figure the mole ratio of acetone to cyclodextrins/chloroform is 9799: 1 which would then place the solubility of acetone near the control. Thus, it can be concluded that the β-cyclodextrins have the highest solubility. The absorptions for the control, chloroform and HP raised by approximately 0.5 whereas the absorptions for β and acetone remained constant (all with the exception of stray data points) throughout the 60 minutes.

## 4.2 Absorption decrease over time

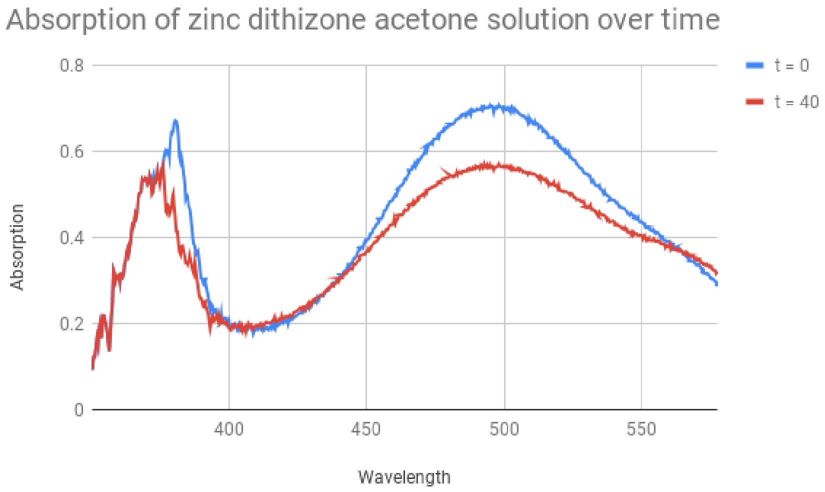

*Figure 12; Absorption spectra of 0.05 g of dithizone, 200 mL of Milli Q water and 70 mL of acetone at 0 minutes and 40 minutes.*

Figure 12 exhibits the change in absorption of zinc-dithizone complex in an acetone solution at 0 and 40 minutes. As the spectra at t=40 is smaller than the one at t=0 it can be concluded solution becomes more clear over time. Furthermore, when inspecting the wavelength at 560-570 nm it can be seen that the absorption of t=40 has become bigger than the one at t=0. This could signify that some amount of dithizone (which is exhibited by the absorption at 470 nm) was used to form dithizone-zinc complexes (seen at 560-570 nm). But as there is no data for wavelengths bigger than 570 nm, more cannot be concluded.

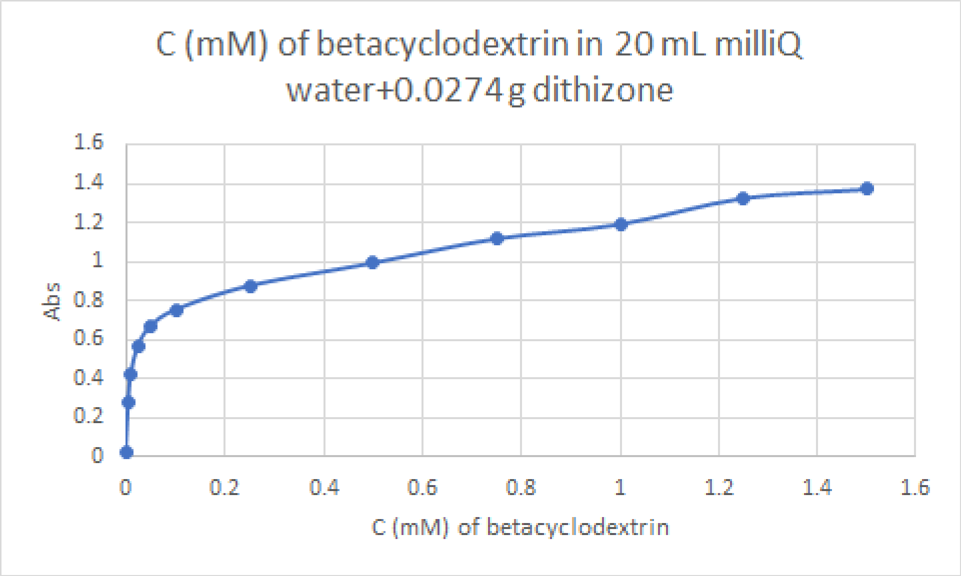

*Figure 13: Absorbances of different C (mM) of β-cyclodextrin in 20 mL stock solution. Absorbances measured after 20 mL of solution contains C (mM) of β-cyclodextrin: 0 mM(control), 0.005 mM, 0.01 mM, 0.025 mM, 0.05 mM, 0.1 mM, 0.25 mM, 0.5 mM, 0.75 mM, 1.0 mM, 1.25 mM and 1.5 mM. Solution: 20 mL milliQ+0.0274g dithizone*

Figure 13 shows the absorbances at 471 nm of different concentrations of β-cyclodextrin in a 20 mL dithizone-water solution. As can be seen in figure 13, the absorbance increases with higher concentrations of β-cyclodextrin. From these results and from section and 2.3, it is possible to approximately calculate the molar extinction coefficient of dithizone-β-cyclodextrin complex at 471 nm.

**Tabel 2: Calculated molar extinction coefficient**

*The molar extinction coefficient was calculated at each data point (refer to tables in appendix for table with calculated molar extinction coefficients for all concentrations of β-cyclodextrin*

| Absorbances at 471 nm subtracted from C(β-cyclodextrin):0 mol/L. Abs:0.0286 | Molar extinction coefficient M^-1*cm^-1 |
|---:|---:|
| 0.5429 | 21716 |
| 0.6431 | 12862 |
| 0.7265 | 7265 |
| 0.8493 | 3397.2 |

| | |
|---|---|
| 0.9659 | 1931.8 |
| 1.0899 | 1453.2 |
| 1.1634 | 1163.4 |
| 1.2972 | 1037.76 |
| 1.3426 | 895.0667 |

Average molar extinction coefficient: 9433.8 $M^{-1}*cm^{-1}$.

## 4.3 Copper and zinc in different solvents over time

### 4.3.1 Copper

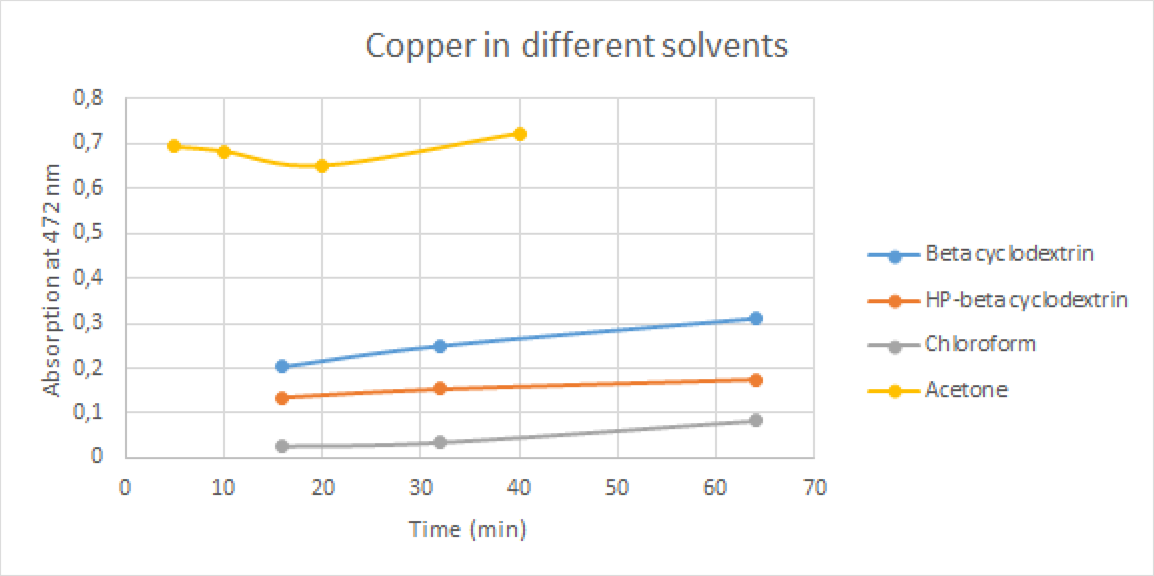

*Figure 14; Absorbances at 472 nm of 56 µL of chloroform, 12.5 µL of HP-β-cyclodextrin and 50 µL of β-cyclodextrin + a solution of 0.03g dithizone, 0.0513 ppm of copper (C:10,26) and 20 mL of Milli Q water over time*

Figure 14 shows the ability that Acetone, Chloroform, HP-β-cyclodextrin and β-cyclodextrin has in forming in dithizone -copper complexes. All absorbance reading were done at a wavelength of 472 nm because that was the location of the max absorbance for all the spectras. The absorption of acetone is around 0.75 because it has a much higher concentration compared to the other solutions. For an example, in this figure the mole ratio of acetone to cyclodextrins/chloroform is

9799 : 1 which would then place the solubility of acetone near the control. Thus, it can be concluded that the β-cyclodextrins have the highest ability to form dithizone-copper complexes. The absorptions of the chloroform, HP-β and acetone solutions were raised by approximately 0.5 whereas the absorptions for β was raised considerably by 1.2 throughout the 65 minutes.

### 4.3.2 Zinc

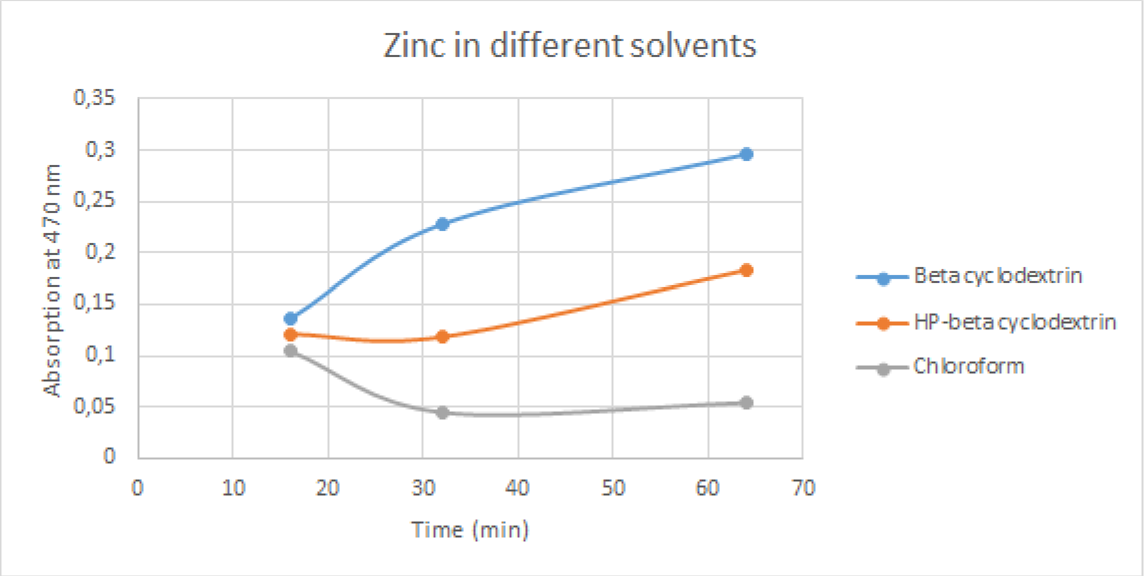

*Figure 15; Absorbances at 470 nm of 56 µL of chloroform, 12.5 µL of HP-β-cyclodextrin and 50 µL of β-cyclodextrin + a solution of 0.03g dithizone, 0.02695 ppm of zinc (C:10.78) and 20 mL of Milli Q water over time*

Figure 15 shows the ability of Chloroform, HP-β-cyclodextrin and β-cyclodextrin has in forming in dithizone -zinc complexes. All absorbance reading was done at a wavelength of 470 nm because that was the location of the max absorbance for all the spectra. What is particularly interesting is that the absorbances of all solutions start around the same value then fan out. That is to say the absorption of chloroform is decreasing whereas the absorption of the two cyclodextrins are increasing, β has a much bigger slope than HP-β. It can be concluded that the β-cyclodextrins have the highest ability to form dithizone-zinc complexes.

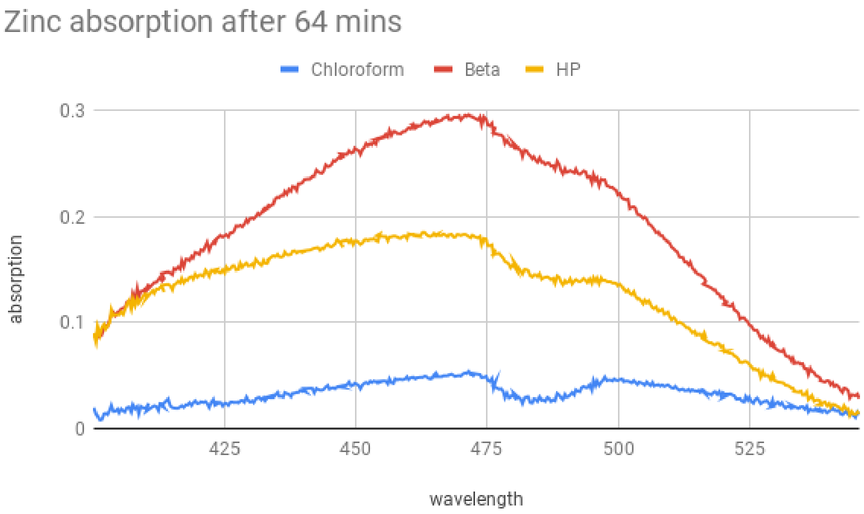

*Figure 16; the absorption spectra of 56 μL of chloroform, 12.5 μL of HP-β-cyclodextrin and 50 μL of β-cyclodextrin + a solution of 0.03g dithizone, 0.02695 ppm of zinc (C:10.78) and 20 mL of Milli Q water at 64 minutes.*

Figure 16 depicts the absorbance spectra of 0.02695 ppm of zinc in a chloroform, HP-β cyclodextrin and β-cyclodextrin solutions at 64 minutes. This figure shows how the formation a dithizone- zinc complexes create a peak at 500 nm along with an absorbance at 470 nm which are dithizone molecules.

## 4.4 Cobalt in a dithizone acetone solution

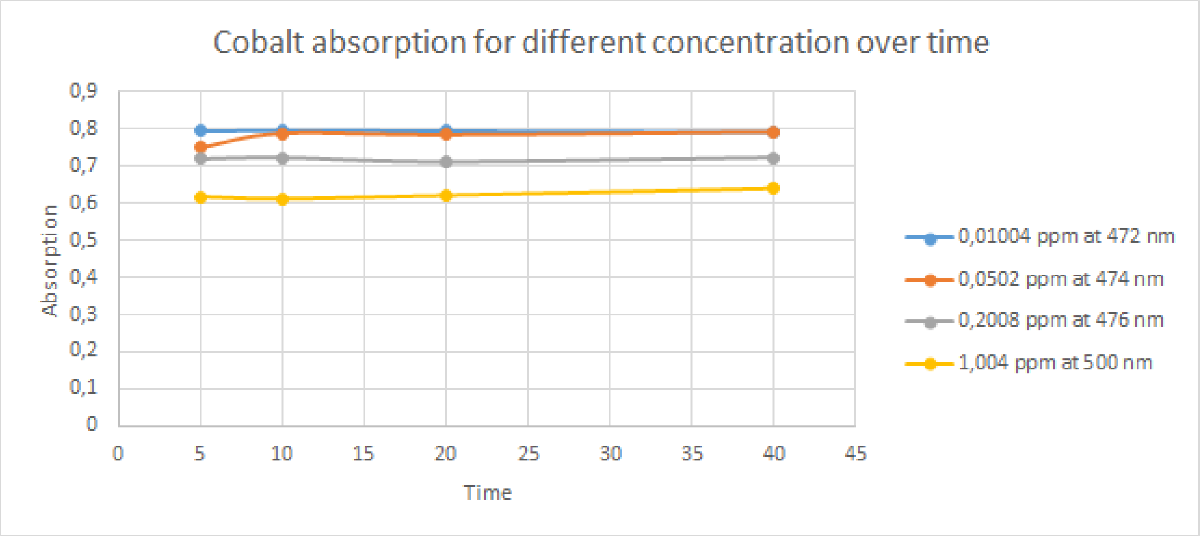

*Figure 17; 0.01004, 0.0502, 0.2008 and 1.004 ppm of cobalt + a solution of 0.03g dithizone and 20 mL of Milli Q water over time. Absorptions readings were done at their max peak, which were 472, 474, 476 and 500 nm respectively.*

Figure 17 shows the change in absorbance of 0.01004, 0.0502, 0.2008 and 1.004 ppm of cobalt in a acetone solution over time. It should be stated that the absorbance readings for each different concentration was done at a different wavelength. These wavelengths were chosen because they were wavelength at which the max absorbances were observed. It can be seen that the absorptions remain constant throughout time, with the exception of 1.004 ppm of cobalt which increases by 0.2.

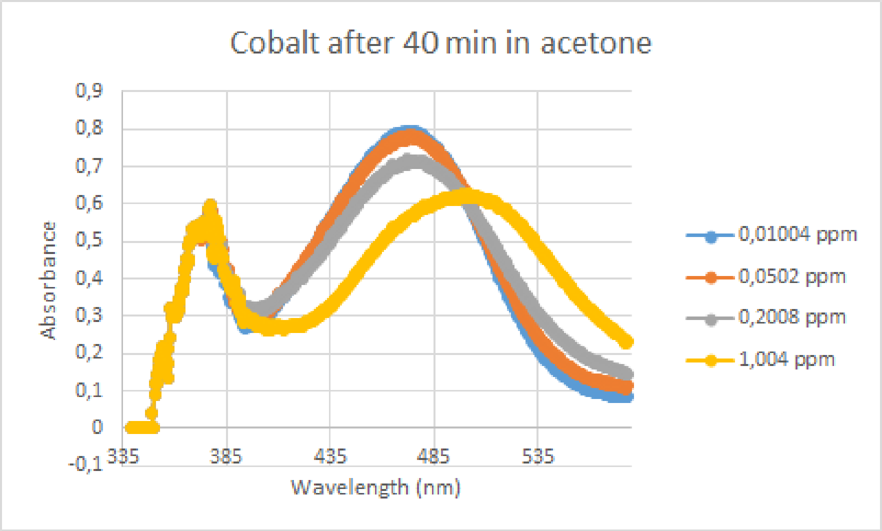

*Figure 18; Absorption at t=40 of 0.01004, 0.0502, 0.2008 and 1.004 ppm of cobalt + a solution of 0.03g dithizone, 70 mL of acetone and 20 mL of Milli Q water.*

Figure 18 depicts the absorbance spectra of different concentrations of cobalt in a acetone solution at t=40. This figure shows how the formation a dithizone- cobalt complexes shift the max absorbance from 470nm a pale yellow colour towards 500nm a pinker colour (for translations of wavelength reading to visible colour refer to figure 10 in section 2.4). It should be noted that absorbances at 470 nm are dithizone molecules.

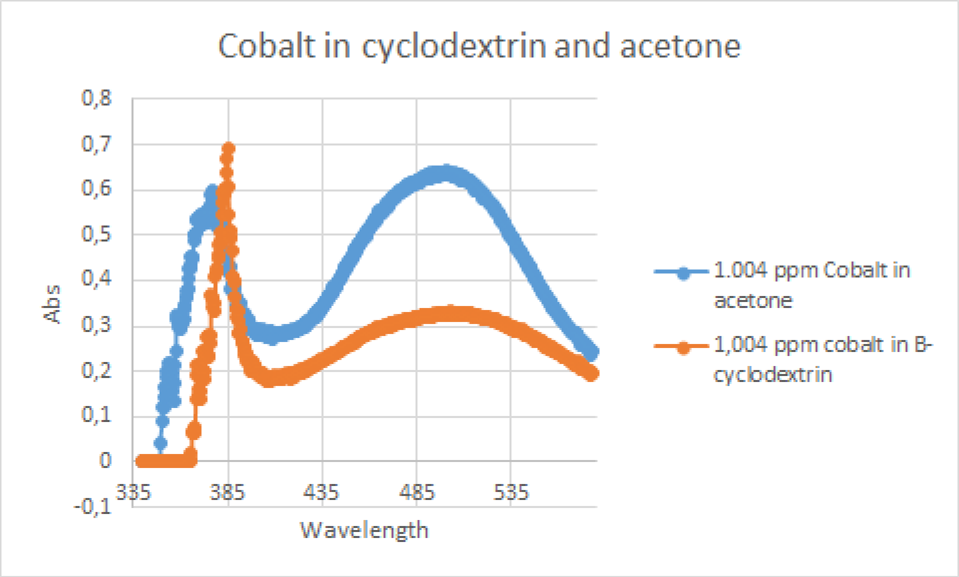

*Figure 19; absorption at t=40 mins of 1.004 ppm cobalt in a cyclodextrin stock solution of 200 mL milliQ water+0.2755 g dithizone+1000 µL 10 mM β-cyclodextrin. As well as 1.004 ppm cobalt in a acetone stock solution of  of 0.03g dithizone, 70 mL of acetone and 20 mL of Milli Q water*

Figure 19 depicts the different absorbance spectra of 1.004 ppm of cobalt in a acetone solution or B-cyclodextrin solution at t=40. Although acetone has a max absorbance of 0.65 because it has a much higher concentration compared to the other solutions. In this figure the mole ratio of acetone to cyclodextrins/chloroform is 489.95: 1. Therefore dithizone has a much higher solubility in β-cyclodextrins than in acetone. What is also noticeable is that in both acetone and cyclodextrin solutions the max absorbance occurs at 500 nm.

## 4.5 Experiments with dithizone, β-cyclodextrin and heavy metals

Results from the experiments as described in section 3.5: Detection of five different metals (Cadmium, Cobalt, Copper, Lead and Zinc) in a dithizone-β-cyclodextrin aqueous solution. Maximum absorbance of dithizone-β-cyclodextrin complex in a aqueous solution was measured at 471 nm as presented earlier and the maximum absorbance of dithizone-β-cyclodextrin complex at 471 nm varied between 0.4 and 0.6.

### 4.5.1 Cadmium (Cd)

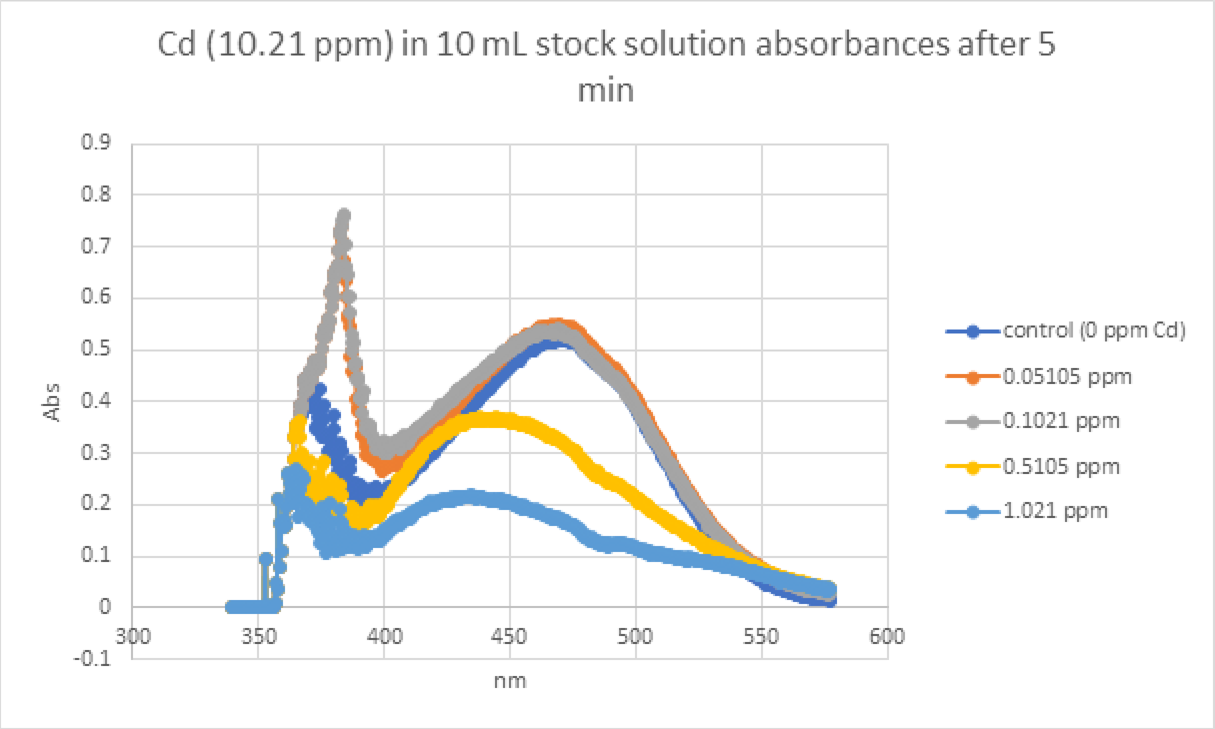

*Figure 20: Absorbances of different C (ppm) of Cd in 10 mL stock solution. C (ppm) of Cd in 10 mL stock solution: 0 ppm (control), 0.05105 ppm, 0.1021 ppm, 0.5105 ppm and 1.021 ppm. Absorbances measured 5 min after addition of Cd. Stock solution:200 mL milliQ water+0.2755 g dithizone+1000 µL 10 mM β-cyclodextrin.*

Figure 20 shows the absorbances of different concentrations of cadmium in a 10 mL dithizone-β-cyclodextrin aqueous solution. As can be seen in figure 20, the absorbance decreases with higher concentrations of cadmium and the max absorbance shifts towards 434 nm. It can be concluded from the results as presented in figure 20, that it is not viable to use a dithizone-β-cyclodextrin aqueous solution and subsequently spectrophotometric measurements in the visible region as a method to detect cadmium.

### 4.5.2 Cobalt (Co)

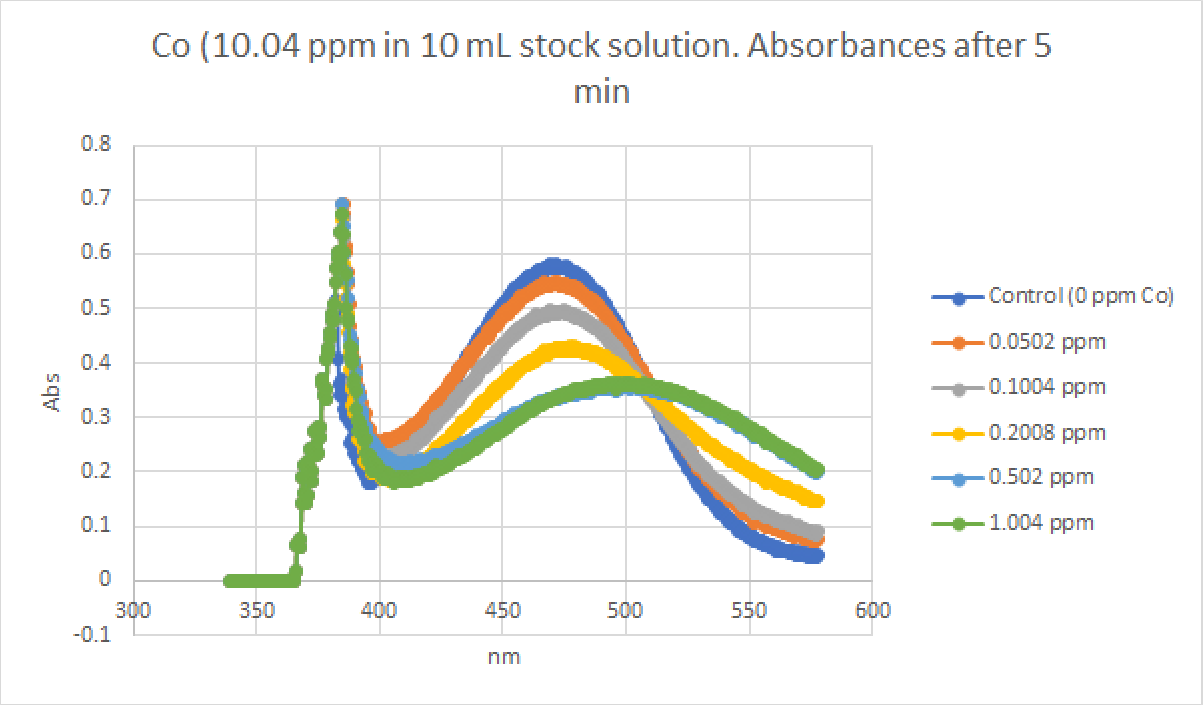

*Figure 21: Absorbances of different C (ppm) of Co in 10 mL stock solution. C (ppm) of Co in 10 mL stock solution: 0 ppm (control), 0.0502 ppm, 0.1004 ppm, 0.2008 ppm, 0.502 ppm and 1.004 ppm. Absorbances measured 5 min after addition of cobalt. Stock solution:200 mL milliQ+0.2761 g dithizone+1000 µL 10 mM β-cyclodextrin.*

Figure 21 depicts the absorbances of different concentrations of cobalt in a 10 mL dithizone-β-cyclodextrin aqueous solution. As can be seen in figure 21, the absorbance decreases with higher concentrations of cobalt and the max absorbance shifts towards 500 nm. It can be concluded from the results as presented in figure 21, that it is not viable to use a dithizone-β-cyclodextrin aqueous solution and subsequently spectrophotometric measurements in the visible region as a method to detect cobalt.

### 4.5.3 Copper (Cu)

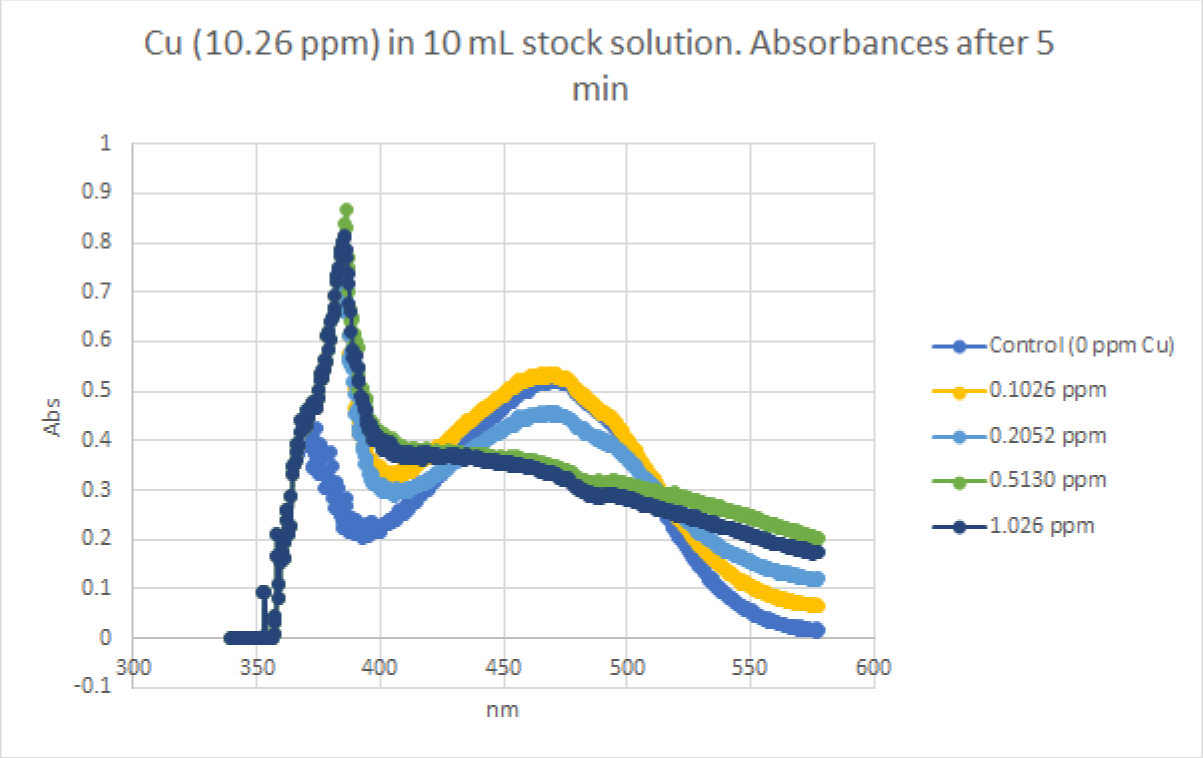

*Figure 22: Absorbances of different C (ppm) of Cu in 10 mL stock solution. C (ppm) of Cu in 10 mL stock solution: 0* ppm *(control), 0.1026 ppm, 0.2052 ppm, 0.5130 ppm and 1.026 ppm. Absorbances measured 5 min after addition of Cu. Stock solution: 200 mL milliQ+0.2755 g dithizone+1000* µ*L 10 mM β-cyclodextrin.*

Figure 22 depicts the absorbances of different concentrations of copper in a 10 mL dithizone-β-cyclodextrin aqueous solution. As can be seen in figure 22, the absorbance decreases with higher concentrations of copper at 470 nm wavelength. Although it can be seen that there is a absorption increase at 570 nm wavelength. However, as the spectrometer data ends at 570 nm it is not known if there is higher absorbance values in higher wavelengths. It can be concluded from the results as presented in figure 22, that it may not viable to use a dithizone-β-cyclodextrin aqueous solution and subsequently spectrophotometric measurements in the visible region, as a method to detect copper.

### 4.5.4 Lead (Pb)

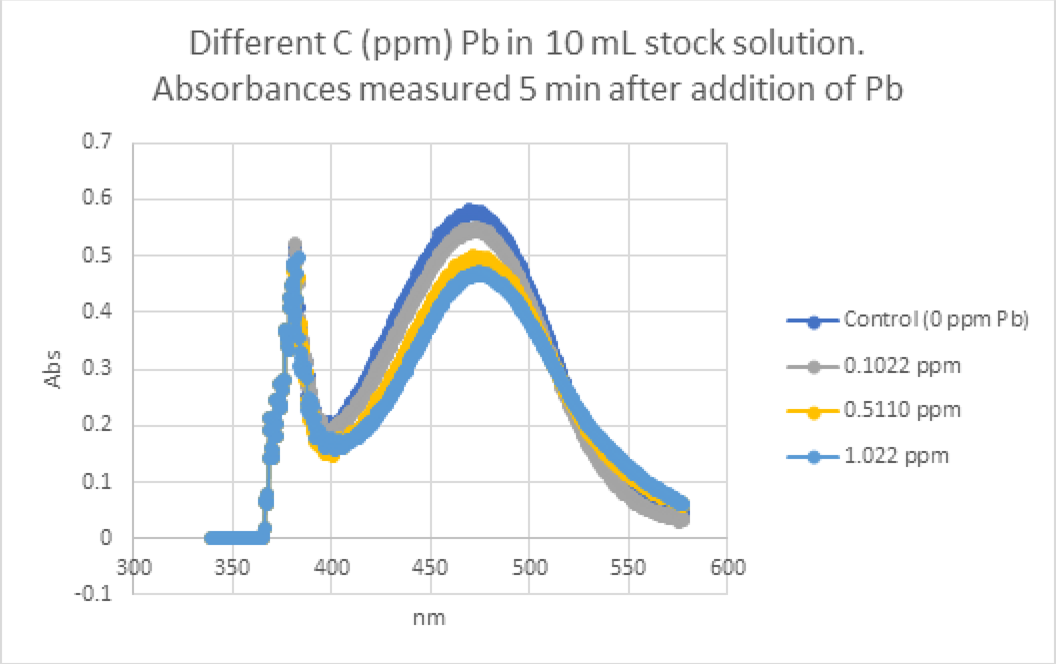

*Figure 23: Absorbances of different C (ppm) of Pb in 10 mL stock solution. C (ppm) of Pb in 10 mL stock solution: 0 ppm (control), 0.1022 ppm, 0.5110 ppm and 1.022 ppm. Absorbances measured 5 min after addition of Pb. Stock solution: 200 mL milliQ+0.2761 g dithizone+1000 µL 10 mM β-cyclodextrin.*

Figure 23 depicts the absorbances of different concentrations of lead in a 10 mL dithizone-β-cyclodextrin aqueous solution. As can be seen in figure 23, the absorbance decreases with higher concentrations of lead. It can be concluded from the results as presented in figure 23, that it is not viable to use a dithizone-β-cyclodextrin aqueous solution and subsequently spectrophotometric measurements in the visible region, as a method to detect lead.

### 4.5.5 Zinc (Zn)

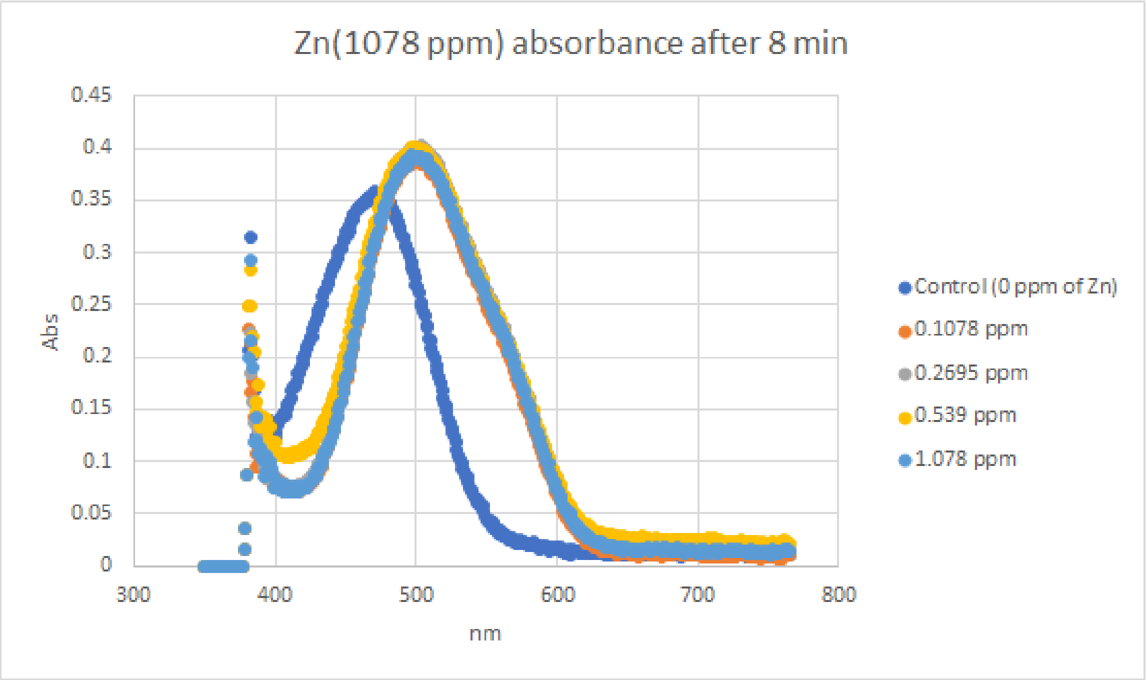

*Figure 24: Absorbances of different C (ppm) of Zn in 20 mL stock solution. C (ppm) of Zn in 20 mL stock solution: 0 ppm (control), 0.1078 ppm, 0.2695 ppm, 0.539 ppm and 1.078 ppm. Absorbances measured 8 min after addition of Zn. Stock solution: 20 mL milliQ+0.027 g dithizone+100 µL 10 mM β-cyclodextrin.*

Figure 24 depicts the absorbances of different concentrations of Zinc in a 20 mL dithizone-β-cyclodextrin aqueous solution. As can be seen in figure 24, the absorbance increases with higher concentrations of Zinc and the max absorbance shifts from 471 nm towards 503 nm. It can be concluded from the results as presented in figure 24, that it is viable to use a dithizone-β-cyclodextrin aqueous solution and subsequently spectrophotometric measurements in the visible region, as a method to detect zinc.

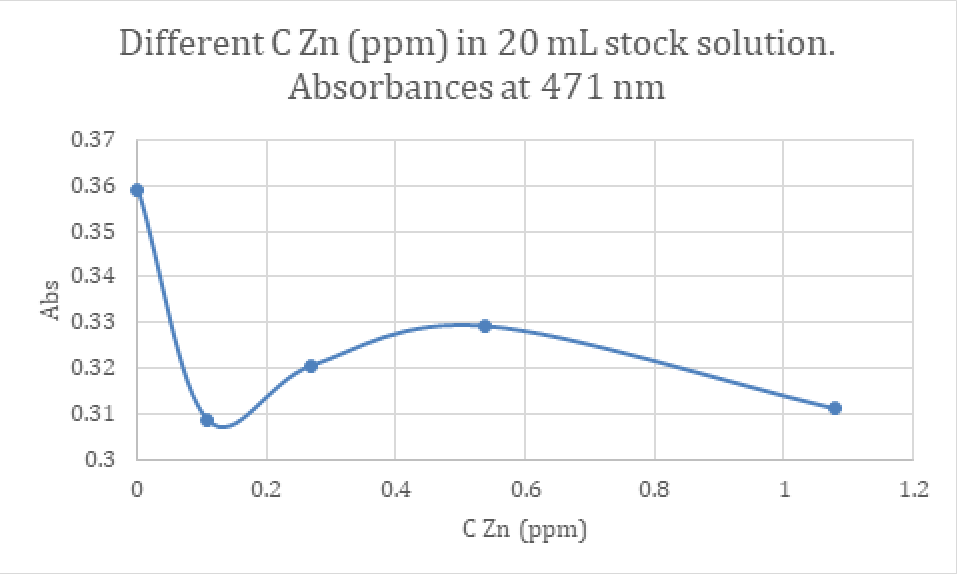

*Figure 25: Absorbances at 471 nm with different C (ppm) of Zn in 20 mL stock solution. C (ppm) of Zn in 20 mL stock solution: 0 ppm (control), 0.1078 ppm, 0.2695 ppm, 0.539 ppm and 1.078 ppm. Absorbances measured 8 min after addition of Zn. Stock solution: 20 mL milliQ+0.027 g dithizone+100 µL 10 mM β-cyclodextrin.*

Figure 25 depicts the absorbances at 471 nm of different concentrations of Zinc in a 20 mL dithizone-β-cyclodextrin aqueous solution. As can be seen in figure 25, the general trend is that the absorbance at 471 nm decreases with higher concentrations of Zinc.

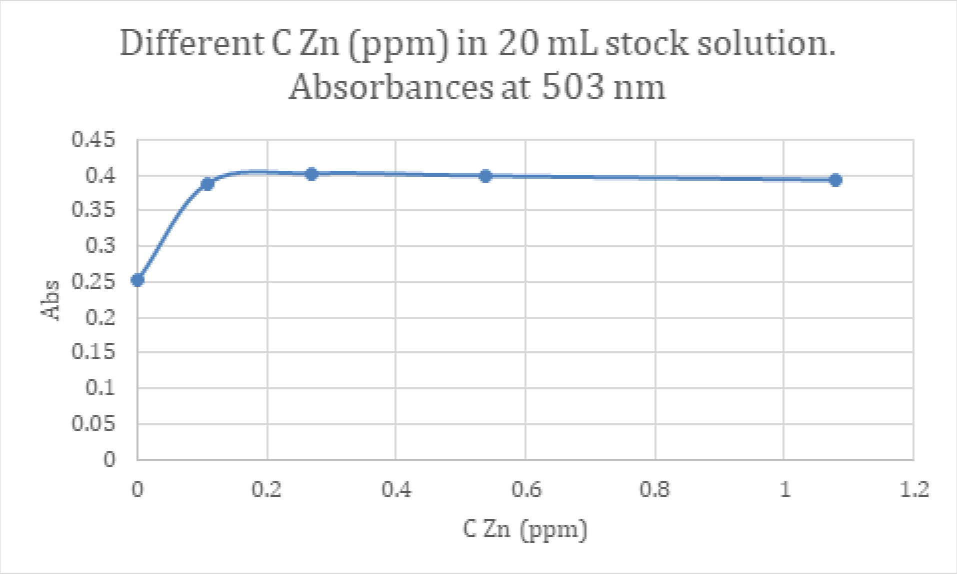

*Figure 26: Absorbances at 503 nm with different C (ppm) of Zn in 20 mL stock solution. C (ppm) of Zn in 20 mL stock solution: 0 ppm (control), 0.1078 ppm, 0.2695 ppm, 0.539 ppm and 1.078 ppm. Absorbances measured 8 min after addition of Zn. Stock solution: 20 mL milliQ+0.027 g dithizone+100 µL 10 mM β-cyclodextrin.*

Figure 26 depicts the absorbances at 503 nm of different concentrations of Zinc in a 20 mL dithizone-β-cyclodextrin aqueous solution. As can be seen in figure 26, the absorbance at 503 nm increases with higher concentrations of Zinc, until it reaches a saturation point at a Zinc concentration of 0.1078 ppm.

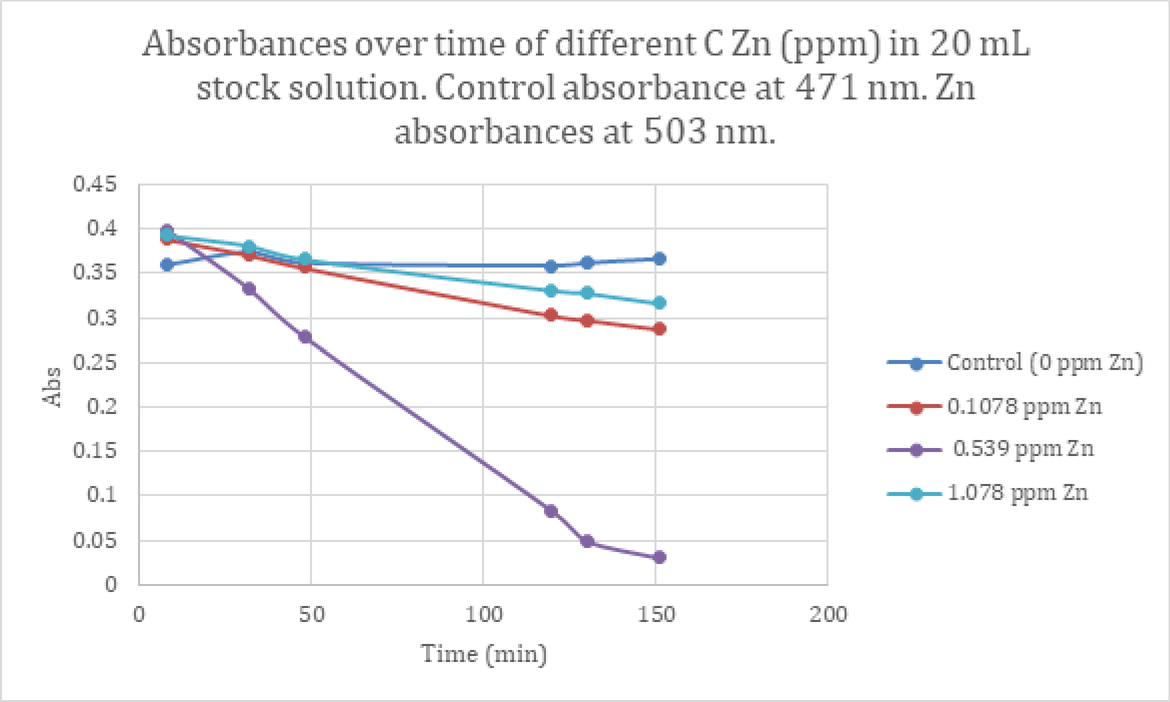

*Figure 27: Absorbances over time (min) with different C (ppm) of Zn in 20 mL stock solution. Absorbances of control measured at 471 nm. Absorbances of different concentrations of Zn (ppm) in stock solution measured at 503 nm.  C (ppm) of Zn in 20 mL stock solution: 0 ppm (control), 0.1078 ppm, 0.539 ppm and 1.078 ppm. Stock solution: 20 mL milliQ+0.027 g dithizone+100 µL 10 mM β-cyclodextrin.*

Figure 27 depicts the stability of the dithizone-β-cyclodextrin and the zinc-dithizone-β-cyclodextrin complexes over time (min). The absorbances of different solutions with different concentrations of Zinc in a 20 mL dithizone-β-cyclodextrin aqueous solution was measured over time (min). The absorbances were measured at 471 nm for the control and at 503 nm for solution containing Zinc. As can be seen in figure 27, the absorbances of the solutions containing Zinc decreases slightly over time, with C (Zn:0.539 ppm) exhibiting the largest decrease in absorbance. The dithizone-β-cyclodextrin complex exhibits the smallest decrease in absorbance over time (min) and thus is shown to be the most stable.

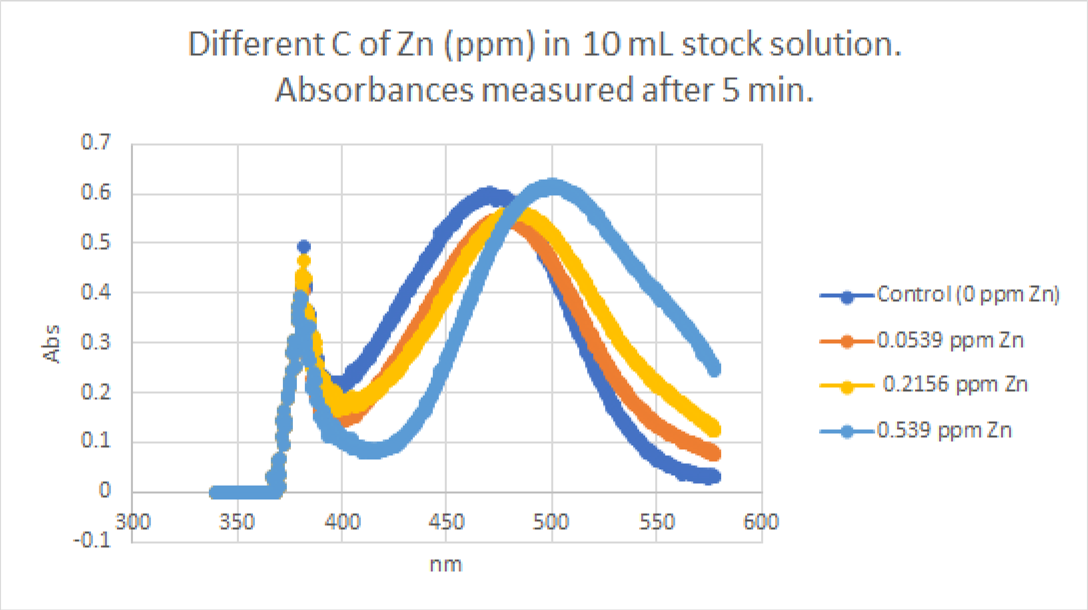

*Figure 28: Absorbances of different C (ppm) of Zn in 10 mL stock solution. C (ppm) of Zn in 10 mL stock solution: 0 ppm (control), 0.0539 ppm, 0.2156 ppm, 0.539 ppm. Absorbances measured 5 min after addition of Zn. Stock solution: 20 mL milliQ+0.027 g dithizone+100 µL 10 mM β-cyclodextrin.*

Figure 28 shows the absorbances of different concentrations of Zinc in a 10 mL dithizone-β-cyclodextrin aqueous solution. As can be seen in figure 28, the absorbance increases with higher concentrations of Zinc and the max absorbance shifts from 471 nm towards 503 nm.

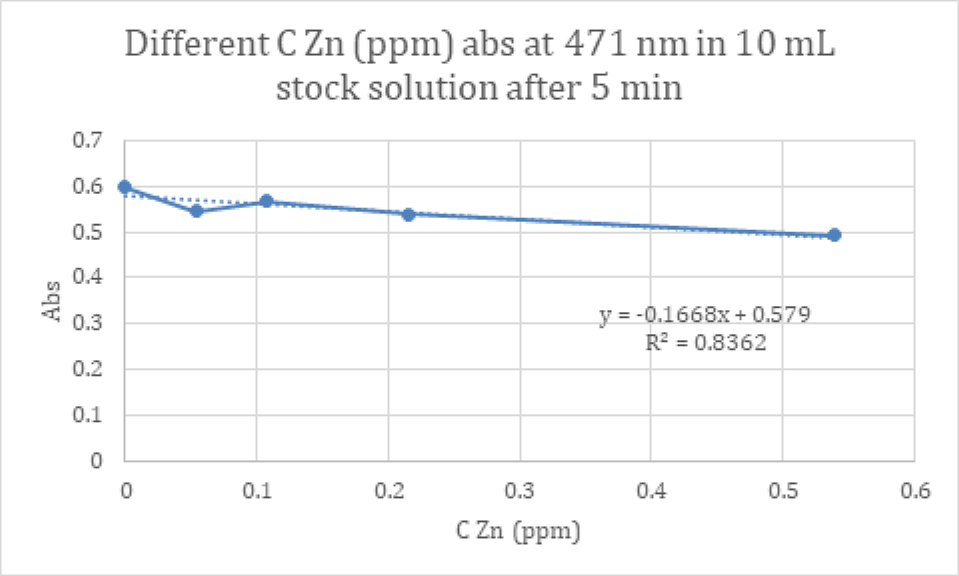

*Figure 29: Absorbances at 471 nm with different C (ppm) of Zn in 10 mL stock solution. C (ppm) of Zn in 10 mL stock solution: 0 ppm (control), 0.0539 ppm, 0.1078 ppm, 0.2156 ppm 0.539 ppm. Absorbances measured 5 min after addition of Zn. Stock solution: 20 mL milliQ+0.027 g dithizone+100 µL 10 mM β-cyclodextrin.*

Figure 29 shows the absorbances at 471 nm of different concentrations of Zinc in a 10 mL dithizone-β-cyclodextrin aqueous solution. As can be seen in figure 29, there is a linear correlation between an increase in Zinc concentration and a decrease in absorbance at 471 nm. The linear correlation follows the equation y=-0.1668x+0.579.

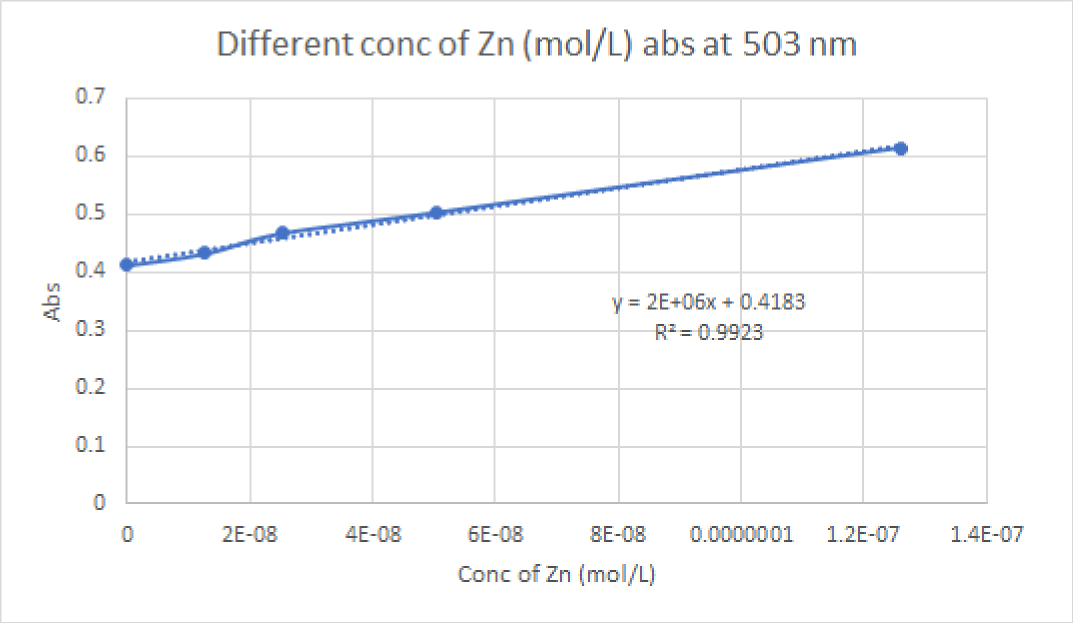

*Figure 30: Absorbances at 503 nm with different concentrations (mol/L) of Zn in 10 mL stock solution. C (mol/L) of Zn in 10 mL stock solution: 0 mol/L (control), 1.2609*10^-8 mol/L, 2.5219*10^-8 mol/l, 5.04381*10^-8 mol/L and 1.2609*10^-7 mol/L. Absorbances measured 5 min after addition of Zn. Stock solution: 20 mL milliQ+0.027 g dithizone+100 µL 10 mM β-cyclodextrin.*

Figure 30 shows the absorbances at 503 nm of different concentrations of Zinc in a 10 mL dithizone-β-cyclodextrin aqueous solution. As can be seen in figure 30, there is a linear correlation between an increase in Zinc concentration and a increase in absorbance at 503 nm. The linear correlation follows the equation $y=2*10^6x+0.4183$. This linear correlation can be used to estimate the molar extinction coefficient of the zinc-dithizone-cyclodextrin complex. The molar extinction coefficient is estimated to be $2.0*10^6$ $M^{-1}*cm^{-1}$

## 4.6 Complex formation ratio of β-cyclodextrin-dithizone-zinc complex

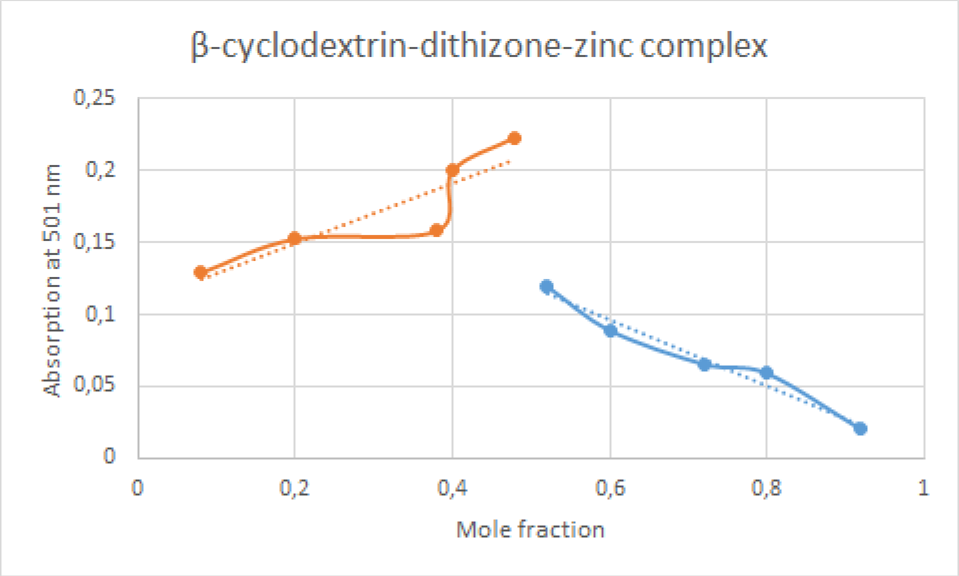

*Figure 31: Absorption at 501 nm of 2, 5, 7, 10 and 12.5 mL of dithizone mix ( 25 mg of dithizone, 850 μL of β-cyclodextrin and 500 mL of Milli Q water) added to 23, 20, 18, 15 and 12.5 mL of zinc solution respectively (red line). 12.5, 10, 7, 5, 2 mL of zinc (C: 220.0 ppm) added to 12.5, 15, 18, 20, 23 mL of dithizone mix respectively (blue line)*

Figure 31 attempts to show the equilibrium constant for complex formation ratio of dithizone-metal complexes. Ideally the red line and the blue line should meet at a point, that point would be the complex formation ratio. Nonetheless this graph or any conclusions reached from this graph cannot be used as evidence due to its unreliability.

# 5 Discussion

In this section different aspects of the project will be discussed. These aspects include the amelioration of methods/ experimentation along with in depth interpretation of results and analysis. Some of the conditions in which the experiments are conducted are discussed as the data collected could been improved. Analysis was used to introduce hypothesis on the solubility of dithizone in different solutions as well as which solution had the greatest solubility and why. Some questionable results such as the deviations in the expected colour of solutions will also be discussed. In addition, the β-cyclodextrin-dithizone complex ability to detect heavy metals in a aqueous solution will be discussed along with the use of method in the field.

## 5.1 Limitation

Dithizone is known to be light sensitive, it directly degraded when exposing to light, thus experimental data collected can be subject to error. Therefore, it is recommended that the solution is kept in constant darkness to prevent the degradation of dithizone. In the experiments, tin foil was used to cover all the beakers but there are uncertainties on light contact while manipulating the product.

During the experiments the stock solutions of dithizone were all yellow, it was hypothesized to be caused by either: the decomposition of dithizone due to water, too small concentration of dithizone and/or the effects of cyclodextrins on the dithizone molecule (refer to section 7 for possible solutions). Therefore, it will be suggested to conduct experiments (3.3, 3.5 and 3.6) where water was replaced with another solvent that does not decomposed dithizone. As for experiment 3.4 a higher concentration of acetone as well as a higher concentration of dithizone may affect the colour of the solution to the expected blue/green colour.

Another limit are the glasses beakers currently used for experiments in labs, because of its many qualities i.e. it can be heated to moderately high temperature, nonetheless it may also contain a small amount of old residue. The use of plastic beakers might have been better, as it could lead to a lower probability of contamination. Some other limit are the cyclodextrins, they usually are mixed with a buffer solution, for this reason there is uncertainty in the purity of the cyclodextrins which will also affect the certainty of the experimental data.

Normally for a good complex formation, the pH should be the same for both solutions. In the mole fraction experiment the pH was not the same, that is to say the pH for the zinc solution was lower than the pH for the dithizone solution, that could be the reason why the obtained result is not as expected, refer figure 31.

## 5.2 Solubility

Figure 13 was used to try and calculate the molar extinction coefficient of dithizone in a aqueous cyclodextrin solution. As there was no linear correlation between concentration of β-cyclodextrin, no definitive molar extinction coefficient could be calculated by linear regression. Instead the molar extinction coefficients were calculated at each measurement. Because of that the molar extinction coefficients varied widely, the lowest values reported to be around 800 $M^{-1}$*cm $^{-1}$ whereas the highest were calculated to be 49800 $M^{-1}$*cm $^{-1}$. The coefficient values at low concentrations were determined to be unprecise and molar coefficient values calculated when the absorbance was more than 1 were both not included in the calculation in the average molar extinction coefficient. Henceforth, the results stated in section 4.2 specifically figure 13 cannot be used as definitive results but can be used as an approximation.

Different solutes were tested to observe which were better to dissolve dithizone. In figure 11, acetone got the highest absorbance which remained constant throughout the experiment. Although this may be true, the absorbance of acetone was much greater as it had a much higher acetone concentration (refer appendix). Compared to the other solvents, the molar availability of acetone was 9799: 1. The much higher concentration were necessary to see a colour change when mixed with dithizone and water, which mean that there was a need for more acetone than cyclodextrins to observe the same colour change. It was concluded that the β-cyclodextrin had the highest solubility and is most constant. Whereas the absorptions for the control, chloroform and HP-β-cyclodextrin raised, if the experiment were performed for a longer period, the absorptions would maybe get higher. This hints that the solubility for the control, chloroform and

HP-β-cyclodextrin is lower than β-cyclodextrins as it takes more time for the dithizone to reach the saturation point.

Even though it was hypothesized that HP-β-cyclodextrins would have a higher solubility as there is a hydrogen bonding hydroxyl group surrounding the lipophilic exterior resulting in a dramatic improvement in the molecules solubility (figure 7) (Jambhekar & Breen, 2016), it was surprising to see that the β-cyclodextrins had a higher ability to dissolve dithizone than HP-β-cyclodextrins. It was hypothesized that the β-cyclodextrin had a greater ability to dissolve dithizone because the dithizone molecules had a higher rate of forming complexes with them. As the HP-β-cyclodextrins had the additional hydroxyl group, the thiol group on the dithizone might be hydrogen bonded to the RN2H2R section of the dithizone molecule. This in turn might inhibit the ability dithizone has to form a inclusion complex with cyclodextrin as cyclodextrins, upon formation of a inclusion complex, encapsulates one of the benzene rings of dithizone.

After the addition of dithizone, all of the solutions turned yellow even though the expected colour change was green, as described in some of the articles (White & Fischer, 1936). As all the mixtures were diluted with a lot of water, the yellow colour could be a result of the dilution factor. In some smaller experiments, pure acetone or chloroform were added in a test tube each with dithizone, here the colour changed into a very dark green. So, to sum up, the addition of water changes the colour of the dithizone from green to yellow. Unfortunately, the experiment was not tried with pure cyclodextrin, because it is originally a powder. Another reason to the yellow colour could be the lack of keto formation, which can be caused by the deterioration of dithizone or the buffer used in the cyclodextrin (Irving & Bell, 1952).

## 5.3 Detection of heavy metals in a dithizone-cyclodextrin aqueous solution

In section 9.4, multiple pictures of the colours produced by different metal-dithizone complexes are shown. It was noticed that when different metals were added to different solvents (β-cyclodextrin-water solution, acetone) they produced different colours. However, when the stock solution of acetone-water was used to form complexes, the resulting colours were similar to the ones found in cyclodextrin-water mix. This may be caused by the deterioration of dithizone or the buffer used in the cyclodextrin (Irving & Bell, 1952).

In comparison of the different molar extinction coefficient for the β-cyclodextrin-dithizone complex and dithizone, the average that were found is much lower compared to the found values in literature for dithizone. The one found in the project is 9433,8 $M^{-1}$ $*cm^{-1}$, whereas the values from the literature is 19450 $M^{-1}$ $*cm^{-1}$ at 450 nm and 22500 $M^{-1}$ $*cm^{-1}$ with $H_2O$ at 475 nm (Sommer, 1989). Whereas the found the molar extinction coefficient for the β-cyclodextrin-dithizone-zinc complex is higher ($2.0*10^6$ $M^{-1}$ $*cm^{-1}$) than the molar extinction coefficient found in literature at $2.20x10^4$ $M^{-1}$ $*cm^{-1}$(Mohamad & Mohd Kamal, 2013).

In the figures 20-30 in section 4.5, the heavy metals lead, copper, cadmium and cobalt, their absorbance decreases as the concentrations get higher, whereas the only one increasing is zinc. Even though the cyclodextrin can form different complexes, depending on the size of the guest molecule and on the size of the cyclodextrins, it seems that the complex formation between β-cyclodextrin-dithizone-zinc is more favorable than the other metals.

As presented in section 4.5, it was not possible to properly detect Cd, Co, Cu and Pb in an aqueous solution via dithizone-cyclodextrin complex, due a decrease in absorbance in the four different solutions containing metal, dithizone and cyclodextrin. A less intense colour of the solutions was also observed when compared to dithizone-cyclodextrin solutions (see appendix section 9.4.2). There are multiple factors that could influence our result. According to previous research, many of the metals that were used in our experiment form a ligand-metal complex ratio of 2:1. Research of the dithizone-metal complex ratios include: According to *Khan et al. 2005* the ratio of dithizone-Pb(II) complex is 2:1, according to *Kumar, B. et al.* the ratio of dithizone-Cu(II) complex is : 2:1, according to *Duncan, J. F.; Thomas, G. F.* the dithizone-Co(II) complex ratio is either 2:1 or 3:1 and according to *Khan et al. 2007* the dithizone-Cd(II) complex ratio is 2:1 (Khan et al. 2005. ; Kumar, B. et al. 1991. ; Duncan, J. F.; Thomas, G. F. 1960.; Khan et al. 2007). But according to *Mohamad & Mohd Kamal* the dithizone-Zn(II) complex ratio is 1:1 (Mohamad & Mohd Kamal, 2013). Due to the complex ratios of 2:1 of the different dithizone-metal complexes, it could then be possible that cyclodextrins could not form inclusion complex with dithizone due to steric hindrance. It could also be possible that upon formation of the dithizone-metal complexes in the aqueous solutions, the dithizone-metals complexes precipitated due to their low solubility in aqueous solutions when compared to dithizone. Another factor could also have an influence on the low solubility of dithizone-metal complexes. When the experiments were conducted as in section 3.5, the dithizone was not dissolved in an organic solution beforehand, thus the only compound that could increase the solubility of dithizone was cyclodextrin. When comparing to previous research introduced above and our results, it can then be concluded that β-cyclodextrin cannot alone increase the solubility of dithizone-metal complexes for colorimetric detection. It can then be concluded that it is not viable to implement this exact technique to detect Cd, Co, Cu and Pb using dithizone, but this technique can be implemented to detect Zn in an aqueous solution.

The complex formation with the heavy metals could also be affected by the pH, because each heavy metal have a more favorable pH solution to react with dithizone, as described in (refer 2.1) (White & Fischer, 1936). But it is difficult to conclude, because the pH was not observed throughout all the experiments and it is unsure if it changed. In the experiment where it was observed the pH were around 7.

# 6 Conclusion

In this project the addition of cyclodextrins and its effect the solubility of dithizone, as well as its ability to form different dithizone-metal complexes were investigated. Furthermore, the HP-β and β-cyclodextrin solutions where compared to acetone and chloroform solutions. On top of the limitations/abilities of using cyclodextrins to detect different heavy metals in an aqueous solution.

It can be concluded that out of all solvents used, β-cyclodextrin had the greatest ability to dissolve dithizone. When different metals were mixed into the cyclodextrin solution, different absorption spectra were recorded. It was seen that copper complexes increased absorbance in the 570 nm wavelengths (refer to figure 22). This was seen primarily in the dithizone-cyclodextrin solution as in the dithizone-acetone solution there was no absorption shift. Cobalt complexes shift the wavelengths towards 500 nm but as the absorption continuously decreases cannot be concluded that cobalt is readily detectable in either a dithizone-acetone solution or dithizone-cyclodextrin solution. Cadmium complexes have been found to shift the wavelength to 434 nm in the dithizone-cyclodextrin solution, but as the absorption continuously decrease with each higher concentration of cadmium no conclusion as to the utilization of cyclodextrins and its ability to form dithizone metal complexes could be reached. Lead complexes had no change in its absorption spectra other than the decrease in absorbance at 470 nm. Zinc-dithizone complexes has been shown in both solutions can be detected and have been found to give the best absorbance spectra out of all metal complexes. This was due to a increase in absorbance at a wavelength of 503 nm. From the experiments conducted, the molar extinction coefficient for the β-cyclodextrin-dithizone-zinc complex was found to be $2.0*10^{6}$ $M^{-1}$ $*cm^{-1}$ and for the β-cyclodextrin-dithizone complex an average molar extinction coefficient was determined to be 9433,8 $M^{-1}$ $*cm^{-1}$ . Overall the method could only detect zinc and could not detect cadmium, cobalt, copper or lead in a dithizone-cyclodextrin solution. Further investigation, development and improvement of the method is needed if this method should be utilized for detection of heavy metals in aqueous solutions.

It can be concluded that due to the limited conditions in the lab, the experiments were not optimally conducted, and the data is a bit faulty. This was easily seen in the different absorbance spectra of the different dithizone-cyclodextrin control solutions made. In other words, when a new stock solution was made using the same materials and amounts, the max absorbance for the control solution changed value, although it was always detected at 470 nm. The yellow coloured solutions observed are either due to the dilution factor, the keto formation and/or the deterioration of dithizone or the buffer used in the cyclodextrin as there should had been a peak at 600 nm. Overall the utilization of cyclodextrins does increase the solubility of dithizone but does not provide an accurate method of detecting different heavy metals in a water solution.

# 7 Perspective

For further research in this area some of the following points should be improved for a better and accurate results:

- There are many heavy metals, but this study considered only five metals (lead, copper, cadmium, zinc and cobalt) which from the literature are more and less prevalent in soils and water. A further study should be done to include other heavy metals like, arsenic, nickel, and mercury also present in the environment to determine their contamination.
- The study covered only two cyclodextrins (β and HP-β), different cyclodextrins such as gamma and alpha-cyclodextrin could have been used to see if their ability to dissolve dithizone was on par with β-cyclodextrins.
- Acetone was the only organic solvent used in the control experiment, more experiments could have been done with methanol and ethanol to compare their effectiveness to the cyclodextrins solutions.
- As mentioned in section 5.1 additional experiments focused on the effects of cyclodextrins on the dithizone molecule should also be conducted. These additional experiments might include further analysis on how the cyclodextrins bond with dithizone. Investigations on if/how the cyclodextrins affect the dithizone molecule, specifically dithizones ability to form complexes with metals, possible deformations of the dithizone molecule.
- Additional analysis on the effects of cyclodextrins on colorimetric reagents could be done by experimenting with a molecule similar to dithizone. 1,5-Diphenylcarbazide is a compound with the abilities analous to dithizone. It can be used as a coulometric titrant that has the ability to form complexes with chromium, cadmium, mercury, magnesium,

aldehydes, and emetine. As it is a phenylhydrazine it is insoluble in water, therefore it is proposed to implement cyclodextrins in order to augment its solubility in water, as done in this project. It would be intriguing to see if the effects of cyclodextrins on 1,5-Diphenylcarbazide is similar to the ones seen with Diphenylthiocarbazone (Dithizone) (PubChem, 2012).

- Further experimentation on the effects of β-cyclodextrin bonded with dithizone could be done. To clarify, solutions of β-cyclodextrin-dithizone and β-cyclodextrin-dithizone-metal complexes could have been subject to pH and thermal changes.

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