Cobalt Chloride coupled with UV/Vis Spectroscopy in the Determination of Water Content

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Abstract

The Karl Fischer equipment is an established state of the art method of determining of water content. It is established that the UV/Vis spectrometer is readily available in laboratories. This dissertation seeks to investigate the possible option of using a UV/Vis spectrometer to determine water content. A method developed by Bai and Yang (2007) to determine the water content in alcohol-based solvents, using cobalt chloride ($CoCl_2$) as an indicator and UV/Vis spectrometry to monitor changes in absorbance in relation to water content was adapted to determinate water content in tetrahydrofuran (THF). The method used different concentrations ($1.69x10^{-3}$ mol/L, $3.42x10^{-3}$ mol/L and $6.98\ x10^{-3}$ mol/L) of $CoCl_2$ in anhydrous THF. Eight dilutions were prepared, in triplicates, for each $CoCl_2$/ THF concentration by adding HPLC grade water (0.1, 0.5, 1, 2, 5, 8, 10, 15 µl) to reach a total volume of 1ml. THF has an absorbance between 200-320nm while $CoCl_2$ has an absorbance between 480-720nm. The average absorbance for each dilution was calculated at 672nm, was plotted against the wavelength and their respective polynomial equations derived. An initial increase in absorbance was unexpectedly observed with the addition of water. This unexpected increase was however followed by a decrease in absorption on further addition of water. Two hypotheses are proposed to explain the initial increase in absorbance followed by a decrease in absorbance on further addition of water. The first hypothesis is that there is an incomplete dissolution of $CoCl_2$ in THF which forms a very fine suspension escaping detection. The second hypothesis proposed is a possible incompatibility of $CoCl_2$ with residues of molecular sieves. In order to test the second hypothesis, THF was dehydrated by $MgSO_4$ and $CaCl_2$ and UV/Vis analysis was performed. The dehydrations with $MgSO_4$ and $CaCl_2$ resulted in a cloudy THF solution which brought about ambiguously high absorbance results when the solution with $CoCl_2$ was prepared and analysed by UV/Vis spectroscopy. UV/Vis absorbance of dehydrated

THF by $MgSO_4$ was lower when nano filtration was applied. The method for determination of water investigated in this research requires further investigation and elucidation before it could be considered as a possible substitute for the established Karl Fischer method.

Acknowledgements

I would like to thank my supervisor Prof. Anthony Serracino Inglott and my co-supervisor Dr. Nicolette Sammut Bartolo for their dedicated support and guidance through out this dissertation. I would like to also thank Prof. Everaldo Attard and Prof. Victor Ferrito for their valuable advice. I am thankful to the Head of Department, Prof. Lilian M. Azzopardi for her encouragement and advice, and also to all the staff at the Department of Pharmacy.

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Table of Contents

List of Abbreviations

2-MeTHF	2-methyl Tetrahydrofuran
$CaCl_2$	Calcium Chloride
$CoCl_2$	Cobalt chloride
$Co(NO_3)_2$	Cobalt nitrate
$MgSO_4$	Magnesium Sulfate
THF	Tetrahydrofuran

List of Figures

List of Tables

Chapter 1

Introduction

1.1 Background

Water content is an important factor in different sectors such as food, pharmaceutical and chemical industry. The reaction outcome and yield of chemical reactions are often influenced by water presence in solvents. The Karl Fischer titration and azeotropic distillation are two methods frequently used for water content quantification in solvents (Bai & Yang, 2007). Both methods have limitations, the Karl Fischer method has low sensitivity and the apparatus is easily disturbed while the azeotropic distillation is complicated to conduct, has a low precision in reading the volume of water and is time consuming (Fetzer, 1951; Bai & Yang, 2007). Tetrahydrofuran (THF) is used as a solvent in many reactions such as Grignard reactions and hydroboration reactions (Mc Fadden *et al.,* 2017; St. Hilaire *et al.*, 2019). This project entails the development of a simple method for water determination in THF by using cobalt chloride ($CoCl_2$) as an indicator and UV/Vis spectroscopy.

1.2 Presence of water and synthesis

The term synthesis comes from Greek which means "put together" (Chakrabarti, 2002). Synthesis is the study of how new complex molecules are constructed from smaller easily available by reacting one chemical with other chemicals (Hunt, 2009).

The long-term aim of synthesis is to develop substances and organised chemical systems and transformations (National Research Council (US), 2003). This is especially seen in medicinal chemistry where man can replicate the molecules in living organisms or create other molecules like them.

Synthesis is accomplished by carrying out chemical transformations, which can be already known or must be invented. The concept of synthesis is to invent new conversion types. These are the tools that make it possible for the invention of interesting and valuable novel substances. Synthesis involves multistep basic transformation and such understanding allows for new reactions to be possible (National Research Council (US), 2003).

1.2.1 Reaction Rate

The rate of the reaction is measured in mol dm^{-3} s^{-1} which is found by calculating the rate at which a product is formed or at which a reactant is removed. It can be calculated using equation 1 (Hunt, 2006)

$$\text{Rate} = \text{change in measured property} / \text{time} \quad \text{[Eq 1.1]}$$

i. Factors Affecting Reaction Rate

Reaction rates are affected by 7 factors. An increase of temperature increases the rate of the reaction as the proportion of molecules with enough energy increases for collision to occur (Hunt, 2014). A 10K rise in temperature, the reaction doubles. A rise in the concentration of the reactants taking part in the rate determining step also increase the reaction. Pressure in a gas state reaction, also affect the rate. When pressure is increased, the rate increases, respectively. An increase in the surface area of a solid contributes to a faster reaction rate. Photosynthetic reactions are affected by light and hence light affect the rate of these reactions (Lister & Renshaw, 2000). Free radical chain reactions are initiated by electromagnetic radiation. Thus, the more intense the radiation, the faster is the reaction rate. A catalyst, such as water, offer a different reaction mechanism with a lower activation energy and hence the reaction occurs at a faster rate (Hunt, 2014).

1.2.2 Role of Water in Reactions

Water can take part in a reaction either as a product/ reactant or as a catalyst (Akiya and Savage, 2002). There is an on-going interest for using a water medium for an organic reaction which is known as on-water reaction (Butler & Coyne, 2015). Water is present indirectly in many reactions such as in solvents.

Reaction types of when water participates in the reaction as a reactant/ product are hydrolysis, hydrogen exchange, hydration, condensation, and free-radical oxidation. Water can also catalyse a reaction by participating directly in the transition state while reducing its energy. This is important when intramolecular hydrogen transfer is involved. Water make the shape of the transition molecule more suitable for the reaction to proceed as it makes the species less strained (Akiya & Savage, 2002).

The proposed mechanism by Jung and Marcus for water catalysis is that hydrogen bonding from the free hydroxyl group transphase at the interphase to the hydrogen bond acceptor site on the water-insoluble organic compounds. It is also believed by Zuo and Qu that the organic compound must be sparingly soluble in water to react on water (Butler & Coyne, 2015). Water can act as an acid/base catalyst precursor (Akiya & Savage, 2002). On water-catalysis was described by Beattie, McErlean and Phippen (2010) as a simple acid-catalysis mechanism which is facilitated by “the hydroxide ion by-product at the oil-water interphase”.

1.3 Tetrahydrofuran

A solution is made up of two or more components, the one in excess is the solvent while the lesser is the solute (Kakabadse, 1984). Organic solvents are highly flammable compounds and can ignite spontaneously (Armarego & Chai, 2009). Solvents in the pharmaceutical industry are used in separation and purification of products of synthesis. They are further used for equipment cleaning (Grodowska & Parczewski, 2010). In this dissertation the solvent which is used is Tetrahydrofuran (THF).

THF is a cyclic ether. Being an ether, THF It is a colourless and flammable liquid with an ethereal odour (Becker *et al.*, 2012). It is less dense than water and its vapours are heavier than air. THF is miscible with alcohols, ketones, esters, hydrocarbons, ethers, and water. [1] Pure THF has a boiling point of 65-66 °C at 760mmHg pressure (Furniss *et al.*, 2003). Caution should be taken when handling THF since peroxides can form by aerial oxidation which can detonate vigorously in high concentrations and result in explosion. Accumulation of peroxides occur during distillation and it should be stopped when a quarter of the residue is left in the flask (Armarego & Chai, 2009).

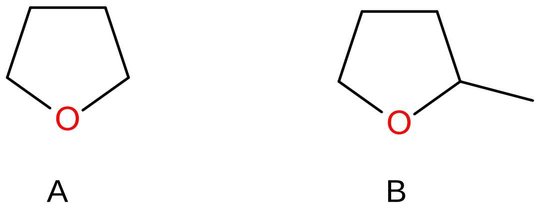

Figure 1.1: A-Tetrahydrofuran; B- 2-Methyltetrahydrofuran[1,2]

A green alternative to THF is 2-methyl tetrahydrofuran (2-MeTHF). 2-MeTHF is obtained from hydrogenation of furfural. Furfural is produced through the intramolecular cyclisation of the naturally occurring pentoses for instance from corn cobs and sugar cane, hence produced from renewable sources unlike THF which is produced from an oil derivative compound, 1,4-butanediol. Contrasting to THF, 2-MeTHF is not miscible with water and therefore can be dried more easily (Aul & Comanita, 2007).

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[1] National Center for Biotechnology Information. PubChem Compound Database. Tetrahydrofuran [Internet]. [cited 2020 July 20]. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/8028

[2] National Center for Biotechnology Information. PubChem Compound Database. 2-Methyltetrahydrofuran [Internet]. [cited 2020 July 20]. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/7301

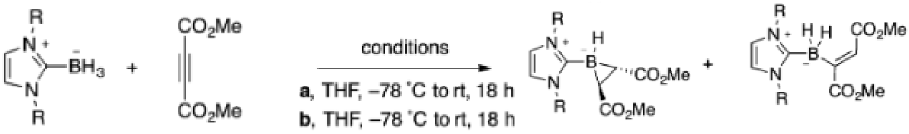
1.3.1 Reactions using THF

THF is used a solvent in many reactions such as hydroboration reactions and Grignard processes (Mc Fadden *et al.,* 2017; St. Hilaire *et al.,* 2019).

I. Hydroboration

Hydroboration is a frequently used reaction in pharmaceuticals, adhesives, polymeric material and for intermediates for example in the synthesis of BINAP (Andreou *et al.,* 2015).

An example of a hydroboration reaction is the below (Scheme 1). The hydroboration is of dimethyl acetylene dicarboxylate 6 by NHC-Boranes (Mc Fadden *et al.,* 2017).



[Scheme 1]

I **II** **III** **IV**

a) R= Me
b) R = iPr

In a, the NHC-borane is with a methyl group (compound I a). The ratio of compound III to 4 is 33 to 67 respectively from the integration of the ^{11}B NMR spectrum of the reaction mixture. The yields of compound 3 and 4 were reported 19% and 35% which were isolated by flash chromatography.

In b, the R group in the NHC-borane is an isopropyl (compound I b). The ratio of compound III to IV is 52 to 48 respectively from the integration of the ^{11}B NMR spectrum of the reaction mixture. Compound III and IV were reported to yield 14% and 29% which were isolated by flash chromatography.

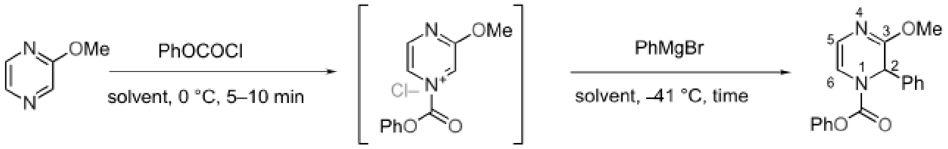
ii. Grignard Reagents

Grignard reagents are used as nucleophiles on various N-acyl-activated pyridines to produce natural products or biological active small molecules (St. Hilaire *et al.*, 2019). Grignard reagents with THF could be used to prepare magnesium battery (Yagi *et al.*, 2013). Alkaloid 205B is a product found naturally from neotropical poisonous frogs *Dendrobates* and the morphine skeleton (Tsukanov & Comins, 2014; Hiebel & Comins, 2015) use a Grignard reagent for their synthesis. Two solvent which are used for Grignard reagents are THF and diethyl ether. THF shows benefits over diethyl ether. THF allows the Grignard process to be carried out in more elevated temperature since THF has a higher boiling point than diethyl ether. The Schlenk equilibrium (Scheme 2)

is found in solutions of Grignard reagents. In diethyl ether, the equilibrium is shifted to the left while in THF all components are found substantially (Tuulmets *et al.*, 2004).

$$2RMgX \leftrightarrow R_2Mg + MgX_2 \qquad \textbf{[Scheme 2]}$$

An example of a Grignard reaction is Scheme 3 below. This is a phenyl Grignard addition to methoxy-substituted N-acylpyrazinium salts (St. Hilaire *et al.*, 2019).



[Scheme 3]

The phenyl Grignard reaction was carried out using different solvents which were dichloromethane, toluene, diethyl ether and tetrahydrofuran. The reaction was carried out by first reacting the 2-methoxypyrazine with phenyl chloroformate to synthesise the N-acylpyrazinium salt followed by addition of phenylmagnesium bromide to the intermediate in different solvents to obtain the final product, dihydropyrazine. The optimal solvent for the reaction was found to be THF in which the yield of dihydropyrazine obtained was 87%. For THF, the reaction was left to react for 30 minutes (St. Hilaire *et al.,* 2019).

Grignard reagents react with water forming hydrocarbons. In the study carried out on the effect of the presence of water on the magnesium deposition from a Grignard reagent-based tetrahydrofuran electrolyte, the resulting reaction occurs (Scheme 4).

$$\mathbf{2EtMgCl + 2H_2O \rightarrow 2C_2H_6 + Mg\,(OH)_2 + MgCl^+ + Cl^-} \qquad \textbf{[Scheme 4]}$$

With the presence of water, white turbidity was recorded indicating the presence of the Mg $(OH)_2$ precipitate. It was shown that high water content destroyed the Grignard reagent based THF and a minimal water presence reduced the magnesium deposition and dissolution reversibility from the Grignard reagent (Yagi *et al.,* 2013).

1.4 Dehydration of Solvents

Biochemical reactions, in nature, are done in water. Organic compounds which contain polar groups, such as alcohols and carboxylic acids are the most soluble in water and hence traces of hydration could be present in the solvents (Brinker & Mieusset, 2010). Organic liquids are usually dried by direct dehydration with a solid drying agent or by direct distillation. This direct distillation is especially useful when low boiling azeotropes will be formed. When the latter method is performed, guard tubes with calcium chloride or silica should be used which prevents the entry of moist air into the apparatus (Armarego & Chai, 2009).

The drying agent should not react chemically with the solvent (Williams & Lawton, 2010). The ideal desiccant should have a rapid action and effective drying capacity. It should not dissolve considerably in the solvent and economically feasible. The drying agent should have no catalytic effect in indorsing chemical reactions of the solvent, for example condensation and polymerisation (Furniss *et al.,* 2003).

The drying agent should be added in small amount while shaking after added until no further action appears to happen. If the desiccant is added largely in excess, more adsorption takes place. (Furniss *et al.,* 2003) Some common solvent of HPLC analysis are methanol and 2-propranol which are alcohols, toluene which is an aromatic compound and n-hexane which is a saturated hydrocarbon, i.e. alkane (Dong, 2006).

1.4.1 Anhydrous Calcium Chloride

Anhydrous calcium chloride ($CaCl_2.6H_2O$) is a desiccant which has a high drying capacity and is cheap. For every 1 mole of calcium chloride, it absorbs 6 moles of water, showing a high-water absorption capacity. Anhydrous calcium chloride is not fast absorbing as the atoms of calcium chloride form a blanket with a thin layer of the solution. Since anhydrous calcium chloride may contain free calcium hydroxide or basic chloride, it is not suitable to be used for dehydrating alcohols, amino acids, phenols, amines, esters, and certain aldehydes. It is suitable for saturated and aromatic hydrocarbons and ethers (Furniss *et al.,* 2003).

1.4.2 Magnesium Sulphate

Magnesium sulphate is an outstanding neutral solvent dehydrator. It is fast acting and chemically inert. It can be used for most compounds such as esters, ketones, aldehydes, amides, and nitriles to which calcium chloride is not applicable (Furniss *et al.,* 2003). One mole of $MgSO_4$ absorbs 7 moles of water showing a high-water absorption capacity. During the dehydration process, $MgSO_4$ forms agglomerates which could be restricting to be for gas transfers dehydrations (Okhrimenko *et al.,* 2017)

1.4.3 Anhydrous Calcium Sulphate

It can be produced by heating dehydrate $CaSO_4.2H_2O$ or the hemihydrate $2CaSO_4.H_2O$ in an oven at around 230°C for 3 hours or bought in the anhydrous form. The desiccant is chemically inert and rapid in the dehydrating the solvent. Calcium sulphate is insoluble in organic compounds and can be therefore be used with most organic solvents. However, as the formula shows above, it has limited ability for water absorption. Therefore, it is used where extreme dehydration is not important. Otherwise, it should be use after dehydrating the solvent with other desiccants (Furniss *et al.,* 2003).

1.4.4 Anhydrous Potassium Carbonate

Anhydrous potassium carbonate is a moderate efficient desiccant which is used to dehydrate ketones, nitriles, esters, and some alcohols. Anhydrous potassium carbonate should be avoided for the dehydration of phenols, acids, and other acidic substances as they will chemically react with the alkaline carbonate. Potassium carbonate can displace the potassium or sodium hydroxide when used with amines (Furniss *et al.,*2003).

1.4.5 Molecular Sieves

Molecular sieves are sorptive materials which can separate mixture depending on their size and molecular shape. Traditionally, molecular sieves are based on a porous material mainly consisting of zeos. Zeos are crystalline structures made up of alkaline earth cations and aluminosilicates which for a three-dimensional structure which permit new crystalline structure to be created. Newer types of molecular sieves are based on molecularly imprinted polymers which are synthesised by polymerisation. Their pores are specific to a particular target potentially to be used in the food industry (Villa *et al.* 2020).

Water usually fills up the zeo pores in molecular sieves. Upon dehydration of the zeos, these freed up space could be used by other compounds (Alothman, 2012). The freed up pores is useful for zeo to behave as a dehydrating agent since when a dehydrated zeo is introduced to a mixture of organic solvent and water, the water will be drawn into the molecular pores and dehydrating the solvent (Villa *et al.* 2020). Molecular sieves are beneficial to dehydrate water molecules from THF (Williams & Lawton, 2010).

1.5 Water Content Determination

It is important to determine and calculate the water content in different sectors such as food and pharmaceutical industry. Water content is measured in food industry as it may influence taste, texture, the shelf life, and the physical appearance of the food. Excess moisture can increase the risk of microbial growth (Appoldt & Raihani, 2017).

Moisture content influences the mechanical stress on a material during the grinding process. Materials with low water content are more brittle and plasticity is increased with increased moisture content. A material which is high in moisture content, require a higher energy utilisation during the grinding process. During the grinding process, the grinding and type of machine used depends on the water content in the material. Grinding is an important factor in the food industry for example for grinding grains and in the pharmaceutical industry for example for tablet production (Jung *et al.*,2018).

In the pharmaceutical industry, the presence of water is an important factor for both chemical and physical stability. Preparations and/or substance may be degraded. Microbial contamination is also facilitated in the presence of water (Jancic-Stojanovic *et al.,* 2012). Water could be calculated by the bipotentiometric Karl Fischer titration, pyridinium phenolate dye, azeotropic distillation and cobalt chloride with UV/Vis spectroscopy.

1.5.1 Karl Fischer

The Karl Fischer method uses either a coulometric titration or a volumetric titration. The titration is grounded on the foundation of the Bunsen reaction (Scheme 5) (Jancic-Stojanovic *et al.,* 2012).

$$I_2 + 2H_2O + SO_2 \rightarrow 2HI + H_2SO_4. \qquad \textbf{[Scheme 5]}$$

Water reacts stoichiometrically with iodine in a suitable solvent such as anhydrous methanol in the presence of an organic base for example pyridine, R'N (Jancic-Stojanovic *et al.,* 2012).

The first step is the production of the pyridinium methane sulfonate (Scheme 6)

$$CH_3OH + SO_2 + RN \rightarrow [RNH]SO_3CH_3 \qquad \textbf{[Scheme 6]}$$

With anhydrous methanol, the reaction continues as follows (Scheme 7):

$$H_2O + I_2 + [RNH]SO_3CH_3 + 2RN \rightarrow [RNH]SO_4CH + 2[RNH]I \qquad \textbf{[Scheme 7]}$$

In the volumetric titration, the iodine in the reaction is added mechanically to the sample with the solvent. The volume of reagent used in the titration indicate the water quantity present in the solvent. In the coulometric titration, iodine is generated electrochemically in situ throughout the titration. The total charged passed used in the titration indicate the water quantity present in the solvent (Tavčar *et al.,* 2012).

The volume of the titrant required for the quantification of water in solvents, is established by the endpoint of the titration. Some samples may not be suitable for the titration analysis because of their chemical interaction and/or insolubility with the reagents. Ketones and aldehydes interfere with the Karl Fischer test since they react with methanol to form water. This interference will result an incorrectly high-water content. Methanol-free reagents can be used to suppress the side reaction with methanol. The Karl Fischer titration could be used for acetonitrile and tetrahydrofuran. The Karl Fischer method is fast, accurate and precise without decomposition. It is an automated process (Tavčar *et al.*, 2012). The Karl Fischer method has low sensitivity and the apparatus is easily disturbed (Bai & Yang, 2007).

1.5.2 Pyridinium phenolate dye

Another method for determining the water content is pyridinium phenolate dye (figure 1.2). Compared to most solvents, water has a high solvent polarity. A small amount of water can enhance a solvent´s polarity. This increase in polarity can be determined by a solvent polarity probe for instance by pyridinium phenolate betaine dye. The absorption wavelength changes in UV/visabsorbance from a short wavelength region in water (yellow solution) to a longer wavelength region in acetone (green solution) up to the near infrared in solvents with low polarity (Langhals, 1990).

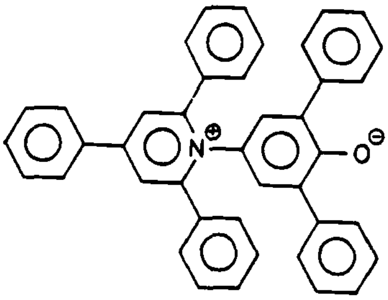


Figure 1.2: Pyridinium phenolate betaine dye

Adopted from: Langhals H. A Simple, Quick, and Precise Procedure for the Determination of Water in Organic Solvents. Analytical Letters. 1990;23(12):2243-2258.

1.5.3 Azeotropic distillation

The principle behind azeotropic distillation, is using a third constituent to separate two close-boiling constituents. An azeotropic mixture increases the variance in the boiling temperature and separation by distillation is enabled (Tryon, 1950).

The method entails the formation of an azeotrope of an immiscible organic liquid with the water, usually toluene is used. Then the azeotrope is distilled, and the water is separated as a separate phase in a graduated container. The volume of water separated is measured (Speight, 2011).

Disadvantages of azeotropic distillation include, water adheres to parts of the apparatus, a lack of precision in reading the volume of water collected because of the dimensions and shape of the graduated container for the distillation, the immiscible liquid tends to be trapped in the narrow tubes by the water and apparatus have to be clean and free from water as the immiscible liquid will be trapped by the water (Fetzer,1951).

1.5.4 Cobalt Salts

Cobalt chloride, $CoCl_2$, is frequently used to check the presence of moisture. $CoCl_2$ is an inorganic compound which forms several hydrates, $CoCl_2 \bullet nH_2O$, for $n = 1, 2, 6,$ and 9. $CoCl_2$ is sky blue in the anhydrous solid form, which turns purple in the dihydrate form and the hexahydrate is pink in colour. The anhydrous form has a molecular weight of 129.833g/mol while the hexahydrate has a molecular weight of 237.93 g/mol. It is highly hygroscopic. At 20°C, the solubility of $CoCl_2$ is 53g/mol, which is a good solubility.[3]

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[3] Cobalt (II) chloride [Internet]. Pubchem.ncbi.nlm.nih.gov. 2019 [cited 2020 August 18]. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Cobalt-chloride-_CoCl2

Upon exposure to moisture and air, $CoCl_2$ turns pink from sky blue due to the shift in the $CoCl_2$ hydrates equilibrium (Scheme 8).

$CoCl_2$	↔	**$CoCl_2 \cdot H_2O$**	↔	**$CoCl_2 \cdot 2H_2O$**	↔	**$CoCl_2 \cdot 6H_2O$**
Blue		**Blue purple**		**Mauve**		**Pink**

Scheme 8: Colour-change reaction mechanism of cobalt chloride hydrates

Adopted from: Bai H, Yang X. Spectrophotometric determination of water content in alcohol organic solvents. Journal of the Chinese Chemical Society. 2007; 54 (3): 619-624.

$CoCl_{2,}$ with UV/Vis spectrometry was used by Bai and Yang to determine the water content of Ethanol, methanol, n-propanol, iso-propanol, n-butanol (Bai & Yang, 2007). Cobalt nitrate, $Co(NO_3)_2$, was used to measure water content in ethanol, isopropanol, and n-butanol. In this method, the absorbance of $Co(NO_3)_2$ was measured with different water concentration added (Zhou *et al.,* 2010). From both researches, it was shown that as the water content increased, the absorption at the maximum decreased. Co

1.6 UV/Vis Spectroscopy

Spectroscopy is often used in physical and analytical chemistry where molecules or substances are identified depending on the spectrum emitted or absorbed. Spectroscopy is used an analytical technique for both qualitative and quantitative analysis. The data obtained from spectroscopy is a plot or a spectrum of the absorbed or emitted energy by the analyte against the energy, wavelength, and frequency of the radiation. (Thomas, 1991).

A UV/Vis spectroscopy measures the absorption of UV or visible light of the analyte. It is one of the most common detectors used in the pharmaceutical industry. This detector contains a deuterium lamp, a small flow cell and a monochromator. A monochromator allows a specific wavelength to go across. This is because it contains a movable prism or grating that allows only that wavelength (Dong, 2006). The absorbance measured by UV/Vis spectroscopy is based on the Bouger-Lambert-Beer's law.

During spectroscopy, some important conditions need to be followed, for the equation to be used. The most applicable according to Mäntele and Deniz (2017) are only monochromatic measuring light should be used, the molecules in a sample should be homogenously distributed, comprehensive measuring beam passage within the mixture sample, there should be no light scattering or photochemical reactions in the analyte, absence of absorbed light re-emission by fluorescence and adequate processing of the intensity values (Mäntele & Deniz, 2017).

1.7 Aims and Objectives

The aim of the dissertation to develop and validate a simple method to quantify the water content in THF using UV/Vis spectroscopy with $CoCl_2$ as an indicator. The developed method will be used to calculate the efficacy of dehydrating agent such as magnesium sulphate, molecular sieves, and calcium chloride to dry THF. The objectives of this dissertation are to find the absorbances of THF and THF with $CoCl_2$, and to find how the water concentration affect the absorbances of THF with $CoCl_2$.

Chapter 2

Methodology

2.1 Methodology Overview

A method developed by Bai and Yang (2007) to determine the water content in the alcohol-based solvents ethanol, methanol, n-propanol, iso-propanol, and n-butanol. The method uses cobalt chloride ($CoCl_2$) as an indicator and changes in absorbance in relation to water content is detected by UV/Vis spectrometer. The method was adapted to determine the water content in THF. The method checked the suitability of $CoCl_2$ as an indicator with THF and entailed finding and analysing the difference in absorbance in relation to different water concentration in THF and $CoCl_2$ with UV/Vis spectroscopy.

2.2 Ethics approval

Carrying out research under the auspices of the University require ethics approval. In the self-assessment form resulted in no issues being identified and the Faculty Research Ethics Committee filed the application for record and audit purposes which is attached in Appendix 1.

2.3 Materials used

- Anhydrous tetrahydrofuran by Carlo Erba Reagents S.A.S. produced in France
- Tetrahydrofuran by Honey well produced in Germany
- Cobalt chloride by Alfa Aeser produced in United Kingdom
- Calcium chloride by Carlo Erba Reagents S.A.S. produced in France
- Magnesium sulfate by Carlo Erba Reagents S.A.S. produced in France

2.4 Pilot study with cobalt chloride

A pilot study was conducted to evaluate whether $CocCl_2$ is suitable for the determination of water in THF. Cobalt chloride weighing 0.0228g ($3.51x10^{-3}$ mol/L in the anhydrous form) was transferred to a 50 ml volumetric flask. Anhydrous THF was added up to the 50ml mark. The mixture was sonicated for a few minutes to obtain a homogenous solution to enhances the dissolution of the $CoCl_2$ in THF to obtain a homogenous mixture which is an important factor in UV/Vis spectroscopy. On adding $CoCl_2$ with the mixture was stored in a desiccator to minimise THF absorbing any moisture from air. The UV/Vis absorbance of THF and $CoCl_2$ was determined to make sure that there is no interference between the two components. Vials containing 1 ml of the solution consisting of THF with $CoCl_2$ and vials containing 1ml of anhydrous THF alone were prepared in triplicates and analysed using by UV/Vis spectrometer. The absorbance spectra obtained were analysed.

2.4.1 Test Run

Vials for each water concentration were prepared in triplicates, by first measuring the HPLC grade water to be added (0.1, 1, 2, 5, 10 µl), with a micropipette, and then transferred into amber glass vials. The prepared vials were topped up by the mixture of $CoCl_2$ with THF to reach a total volume of 1 ml. Once a sample was prepared, it was labelled, capped, and stored in a resealable bag containing silica. The resealable was sealed and stored into the desiccator until all the samples were prepared to be analysed to avoid any temperature variability at the time of analysis.

2.4.2 UV/Vis analysis

For the analysis of the samples, a quartz glass cuvette (10 mm path length) and a wavelength range between 200nm to 800nm were selected. A blank cuvette was used as a reference. The apparatus used was Biochrom® WPA Lightwave II. The signal from the reference beam was subtracted from the sample signal to remove interference. The samples were shaken thoroughly before being analysed to obtain a uniform solution. Few millilitres of the sample to be analysed were used to wash the cuvette between one sample and another and the cuvettes were left to dry completely to avoid contamination of the sample to be analysed.

2.5 Analysis of different cobalt chloride solutions in tetrahydrofuran

Solutions with $CoCl_2$ and THF were prepared in 3 different concentrations: $1.69x10^{-3}$ mol/L, $3.42x10^{-3}$ mol/L and $6.98 x10^{-3}$ mol/L. This was done by first weighing the cobalt chloride which was stored in a desiccator filled with silica to avoid any humidity contaminations. The weighted $CoCl_2$ was transferred to a 50ml volumetric flask and topped up with to the 50ml mark with a newly opened anhydrous THF. The transfer of the anhydrous THF was done by a means of syringe to avoid water contamination in the bottle. The three flasks were sonicated for a few minutes to further confirm obtention of homogeneity and fully dissolved solutions. The solutions were stored in a desiccator with freshly dried silica until need for dilution preparation.

2.6 Dilutions

Vials for each water concentration were prepared in triplicates, by first measuring the HPLC grade water to be added (0.1, 0.5, 1, 2, 5, 8, 10, 15µl), with a micropipette, and transferred into amber glass vials. The prepared vials were topped up by the mixture of $CoCl_2$ with THF to reach a total volume of 1 ml. Once a sample was prepared, it was labelled, capped, and stored in a resealable bag containing silica. The resealable bag was sealed and put into the desiccator until all the dilutions for the three THF/$CoCl_2$ solutions were prepared to be analysed.

Table 2.1 Molar ratio of water to $CoCl_2$ for n-Butanol used by Bai & Yang (2007)

Amount of water used with 400uL n-Butanol (uL)	$CoCl_2$	Water
0	1	0
2	1	3.33
4	1	6.66
6	1	10
8	1	13.33
10	1	16.66

Table 2.2 Molar ratio of water to $CoCl_2$ for THF used

Amount of water used (µL)	Amount of THF used (µL)	$CoCl_2$: Water in THF solution		
		1.69×10^{-3} mol/L solution	3.42×10^{-3} mol/L solution	6.98×10^{-3} mol/L solution
0.1	999.9	1: 0.33	1: 0.16	1: 0.08
0.5	999.5	1:1.64	1: 0.81	1: 0.4
1	999	1: 3.28	1:1.62	1: 0.8
2	998	1: 6.57	1: 3.25	1: 1.6
5	995	1: 16.46	1: 8.16	1: 4
8	992	1: 26.42	1: 13.09	1: 6.42
10	990	1:33.43	1:16.56	1: 8.12
15	985	1: 49.89	1: 24.72	1:12.11

2.7 Tetrahydrofuran dehydration

Results from the anhydrous THF were not as expected. It was proposed to try with THF which is dehydrated in the lab with another dehydrating agent other than molecular sieves. $MgSO_4$ and $CaCl_2$ were chosen since they are widely used dehydrating agents in the synthesis industry.

2.7.1 Dehydration by magnesium sulfate

THF (100ml) was dehydrated with excess of $MgSO_4$, previously dehydrated in the oven. After the dehydration step, the dehydrating agent was filtered by a filter paper and filter funnel. The filtrate from the dehydration was used to prepare the 3.42×10^{-3} mol/L THF/$CoCl_2$ solution. As a control, another solution was prepared from the hydrous THF bottle with a concentration of 3.42×10^{-3} mol/L. 3 samples were taken from the dehydrated THF/$CoCl_2$ solution, 3 samples from the non-dehydrated THF/$CoCl_2$ solution and triplicate dilutions with 5µl HPLC grade water and 995µl from the dehydrated THF/$CoCl_2$ solution. The prepared samples were analysed by UV/Vis spectroscopy as described section 2.4.2.

2.7.2 Dehydration by magnesium sulfate and dilutions

THF was dehydrated by $MgSO_4$ (0.6g for 100ml THF). After the dehydration, the $MgSO_4$ was filtered by gravitational filtration. From this dehydrated THF, two THF/$CoCl_2$ solution were prepared. $CoCl_2$ was weighed and transferred to a volumetric flask and topped up with to the 50ml mark with the freshly dehydrated THF which was nano-filtered. The obtained concentration solution was of 3.42×10^{-3} mol/L. This was to be used for the dilutions. Another $CoCl_2$/THF solution with the same concentration *i.e.* 3.42×10^{-3} *mol/L* was prepared in a 10ml volumetric flask with THF freshly dehydrated without nano filtration to analyse any interactions with $MgSO_4$. The flasks were sonicated for homogeneity. From the 50ml prepared solution, the dilutions were prepared and analysed by UV/Vis spectroscopy as explain in section 2.6 and 2.4.2, respectively.

2.7.3 Dehydration by calcium chloride

It was proposed to re-test the dehydration of THF with another dehydrating agent. $CaCl_2$ was chosen. 3 different THF/$CoCl_2$ solutions were prepared, one from the THF which was not dehydrated, one from the THF dehydrated with $CaCl_2$ and another from the THF dehydrated with $CaCl_2$ which was filtered with the filter paper by gravitational filtration. From these solutions, triplicates dilution with 5µl HPLC grade water and 995µl from the respective solution were prepared. The solutions were analysed by UV/Vis spectroscopy as explained in section 2.4.2.

The bottles were left in the fume cupboard to be visually analysed the day after. It was noted that visual changes between the bottles were seen. It was proposed to use the same THF bottles and re-do UV/Vis analysis. This time, adding an additional step: nano filtration. The unexpected high results could be due to the dehydrating agent remaining in the solution even though it was filtered off using a filter paper. The method is summarised in Figure 2.1.

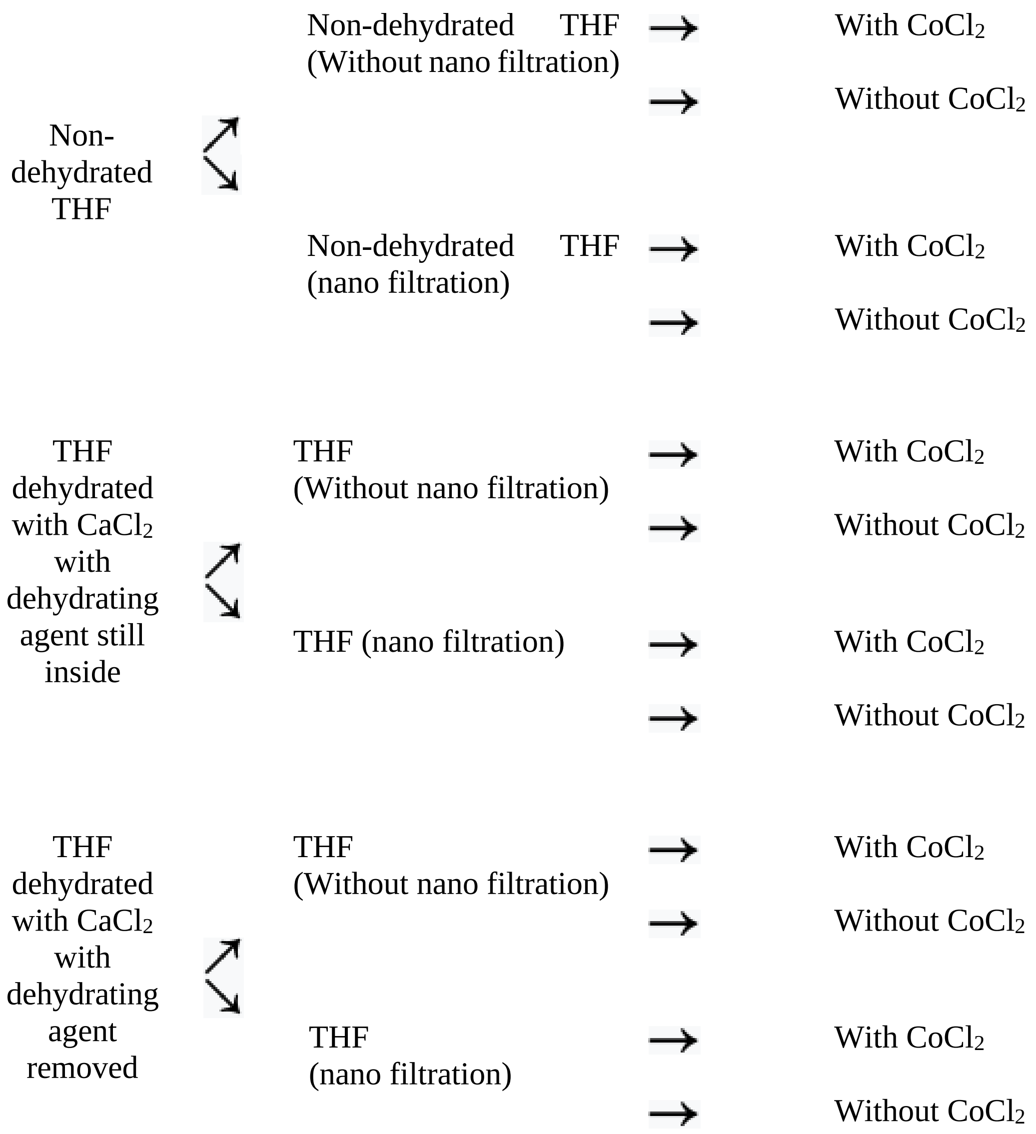

Figure 2.1 Method for THF dehydrated by $CaCl_2$

Chapter 3

Results

3.1 Suitability of cobalt chloride

Absorbance against the wavelength of THF and THF with $CoCl_2$ was plotted (Figure 3.1). THF has an absorbance between 200-320nm while absorbance in the region between 548-721nm was attributed to $CoCl_2$ with a maximum absorbance at 680nm.

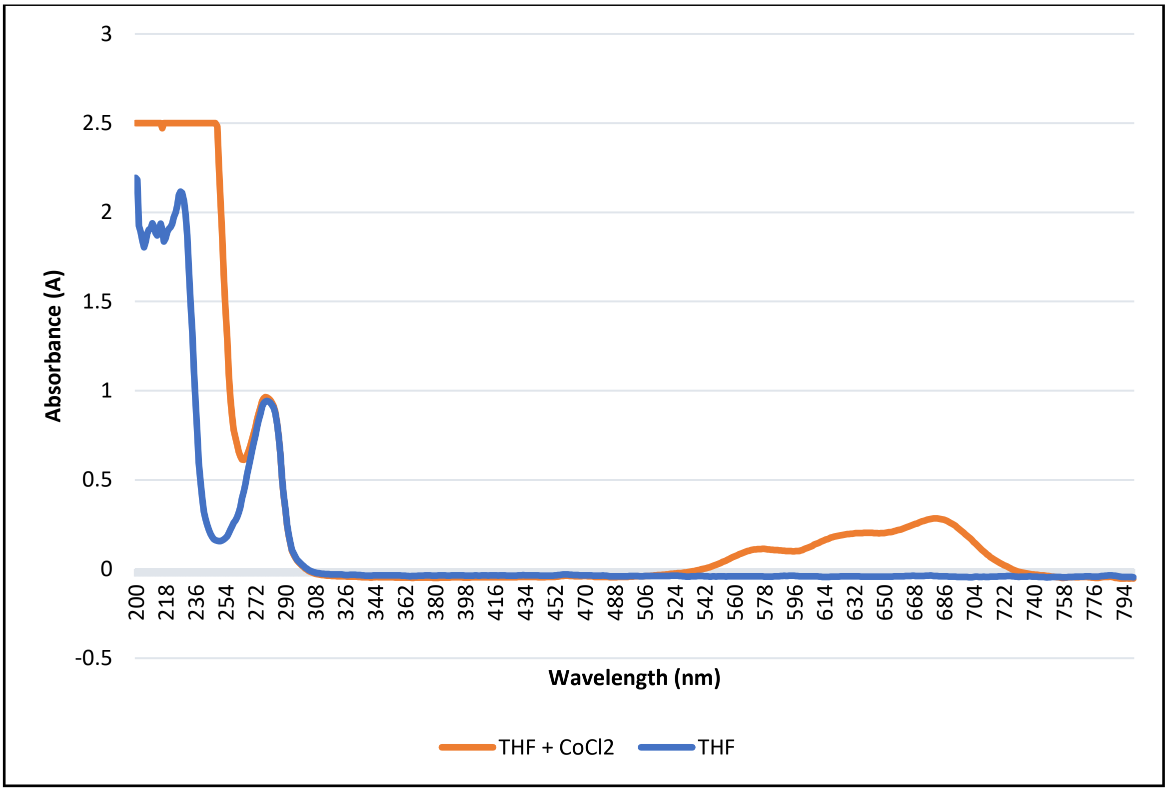

Figure 3.1 UV/Vis spectra of THF and THF with $CoCl_2$

The average absorbance for each water concentration was calculated. A graph of the average absorbance at 680nm against water added was plotted (Figure 2). It was observed that as the water concentration increased, the absorbance decreased with a polynomial second order trend.

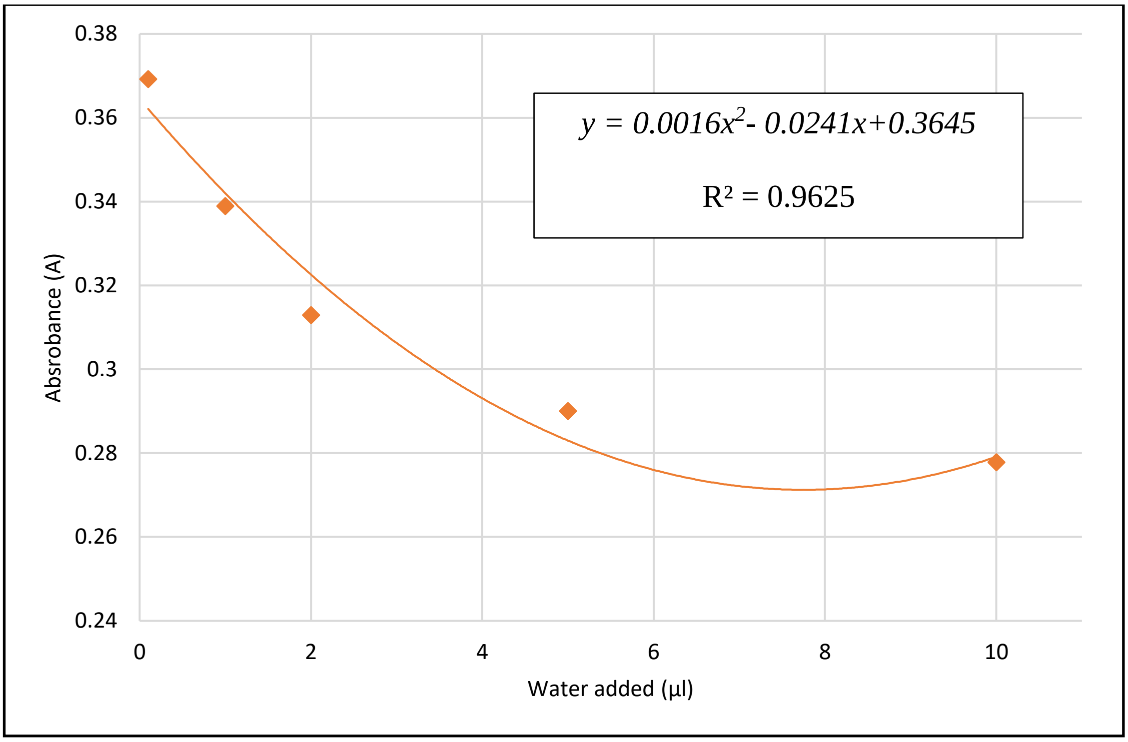

Figure 3.2 Average absorbance of THF with $CoCl_2$ at 680nm against water added

3.2 Dilutions of cobalt chloride in tetrahydrofuran

The average absorbance for each water concentration was calculated for every $CoCl_2$ /THF solution. Absorbance against the wavelength of THF and THF with $CoCl_2$ was plotted for the 3 different $CoCl_2$ / THF solutions (Figure 3.4). THF has an absorbance between 200-320nm while absorbance in the region between 480-720nm was attributed to $CoCl_2$ with a maximum absorbance at 672nm in the solutions containing molar concentrations of 1.69×10^{-3} mol/L and 3.42×10^{-3} mol/L and at 669nm in the solution containing molar concentration of 6.98×10^{-3} mol/L $CoCl_2$ in THF.

The average absorbance at 672nm against water added was plotted (Figure 3.5). Their respective polynomial second order trend lines were calculated and are shown on the graph. It was observed, as the $CoCl_2$ amount in THF doubled, the absorbance approximately doubled as shown in the Figure 3.3 and Table 3.1 below.

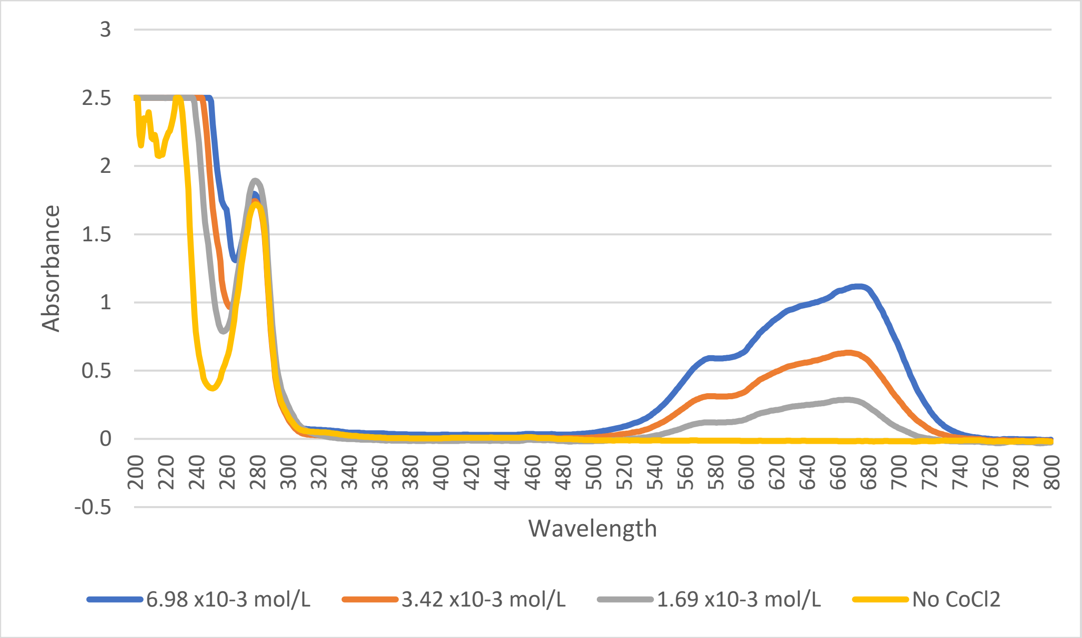

Figure 3.3 Absorbance against wavelength in different concentrations of $CoCl_2$

Table 3.1 Absorbance values with respect to the $CoCl_2$ concentration

Concentration ($x10^{-3}$ mol/L)	**Average Absorbance at 672nm**
1.69	0.28
3.42	0.63
6.98	1.12

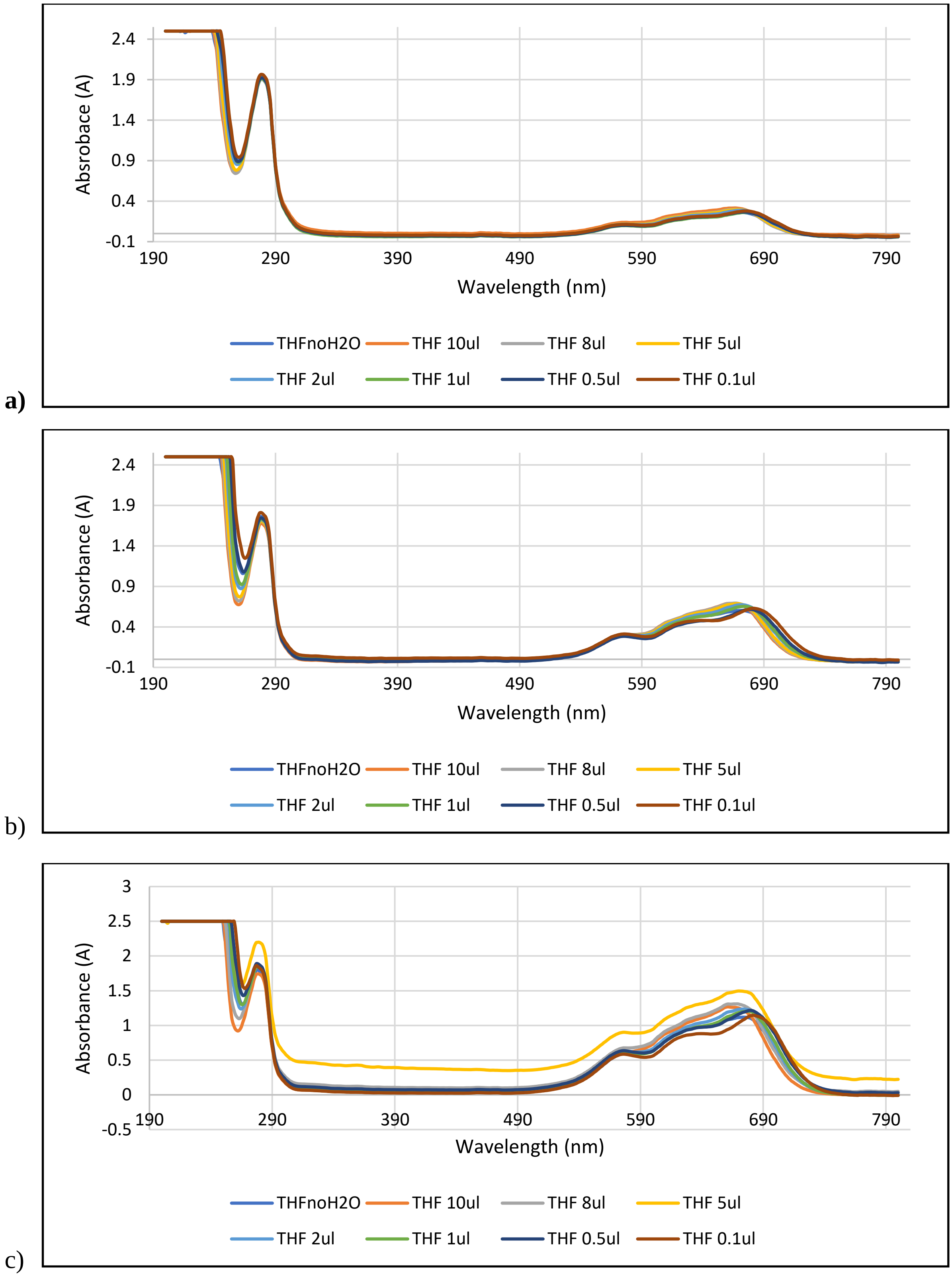

Figure 3.4 UV/Vis Spectroscopy for solution with dehydrated THF and $CoCl_2$ with water added at a) 1.69×10^{-3} mol/L, b) 3.42×10^{-3} mol/L c) 6.98×10^{-3} mol/L

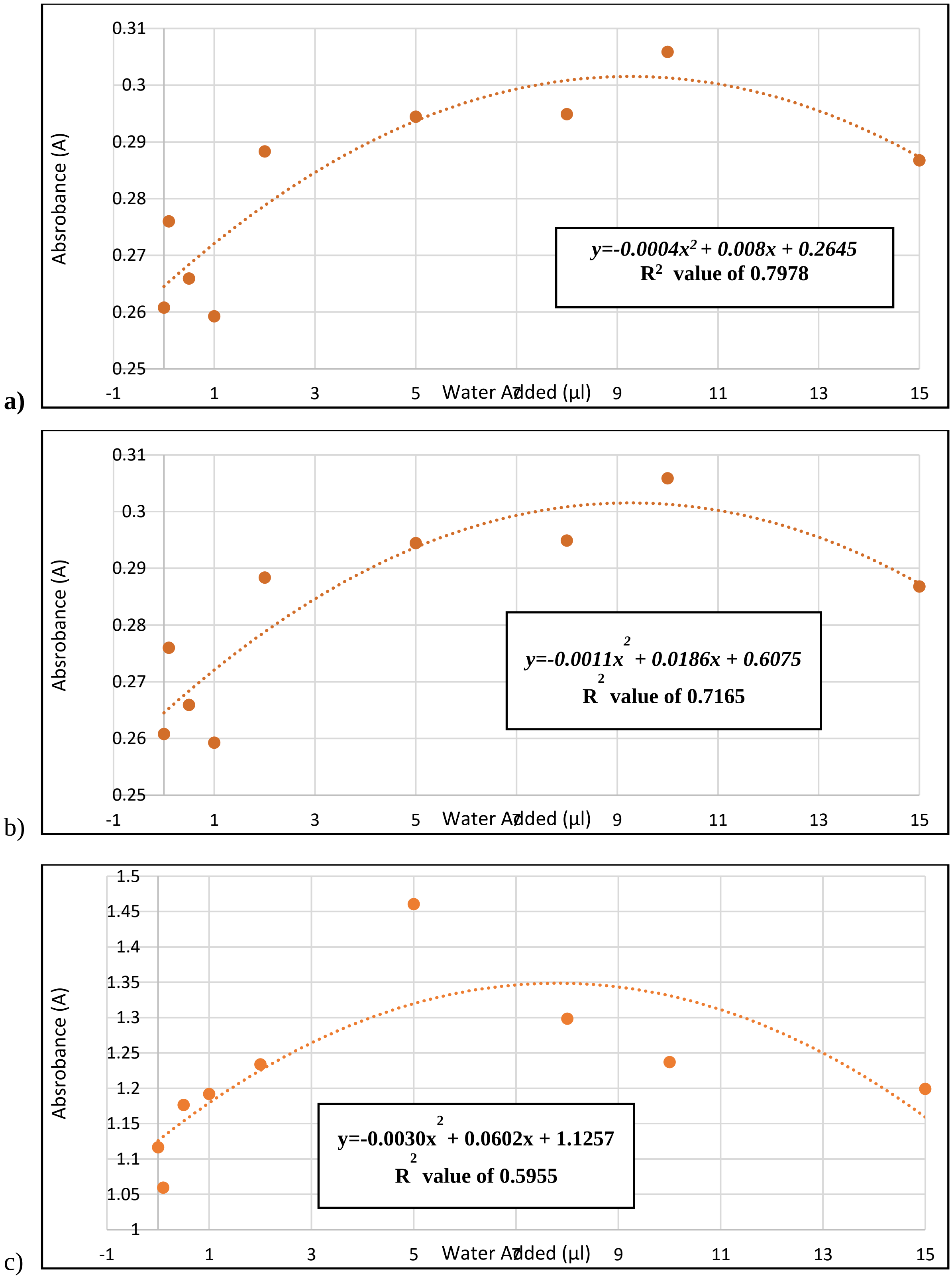

Figure 3.5 Average absorbance at 672nm against water added in a) 1.69x10-3 mol/L, b) 3.42x10-3 mol/L c) 6.98x10-3 mol/L $CoCl_2$ in THF

3.3 Analysis of tetrahydrofuran dehydrated by magnesium sulfate

$MgSO_4$ was used to directly dehydrate THF in the laboratory. On the freshly dehydrated solution, the UV/Vis analysis was performed.

3.3.1 Test run analysis

The average absorbance from each of the triplicate samples of the dehydrated THF/$CoCl_2$ solution, the non-dehydrated THF/$CoCl_2$ solution and dilution with 5µl HPLC grade water and 995µl from the dehydrated THF/$CoCl_2$ solution were calculated. A graph depicting the averages absorbances against the wavelength was plotted (Figure 3.6).

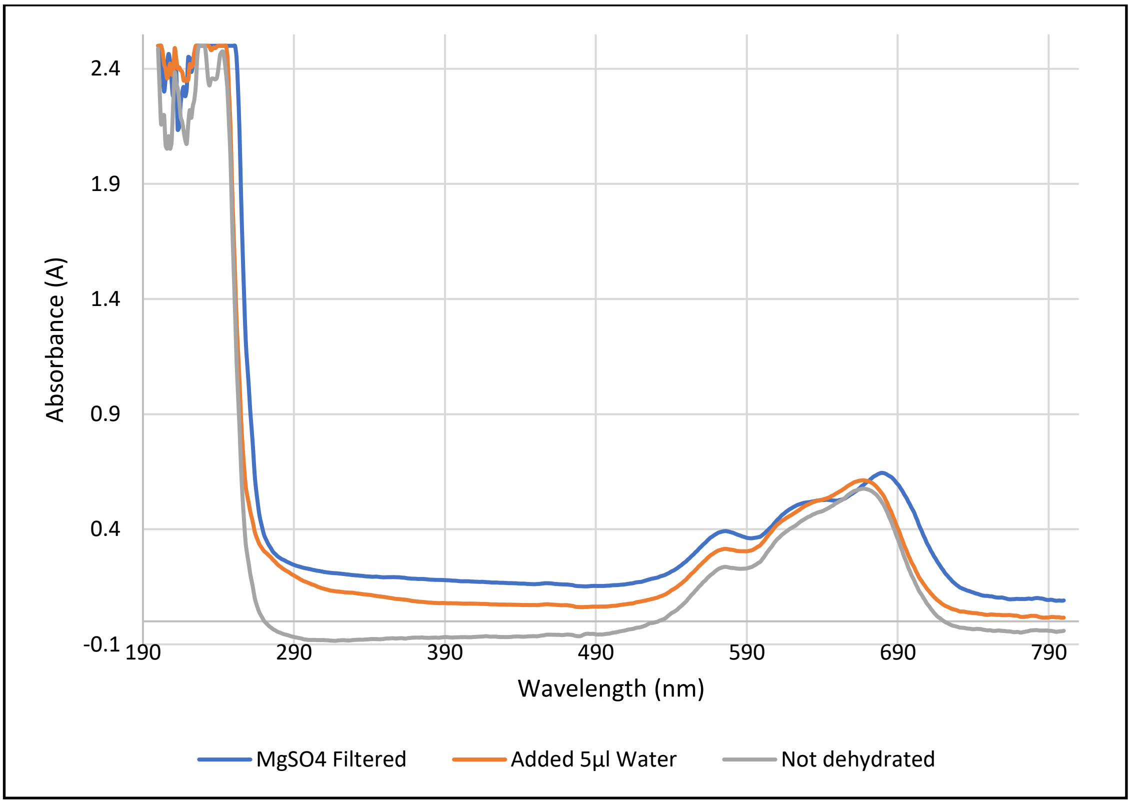

Figure 3.6 Test Run for THF dehydrated with $MgSO_4$

3.3.2 Nano filtration solution of solution

The solutions prepared with the dehydrated THF by $MgSO_4$, which one of them was nano filtered, were used to prepare two solutions containing 3.42×10^{-3} mol/L $CoCl_2$. The prepared two solutions were analysed by UV/Vis spectroscopy. The average absorbance of each of the triplicate samples were calculated and plotted (Figure 3.7). The solution which was filtered by nano filtration had a lower absorbance compared to the solution without nano filtration. There was no shift in peaks.

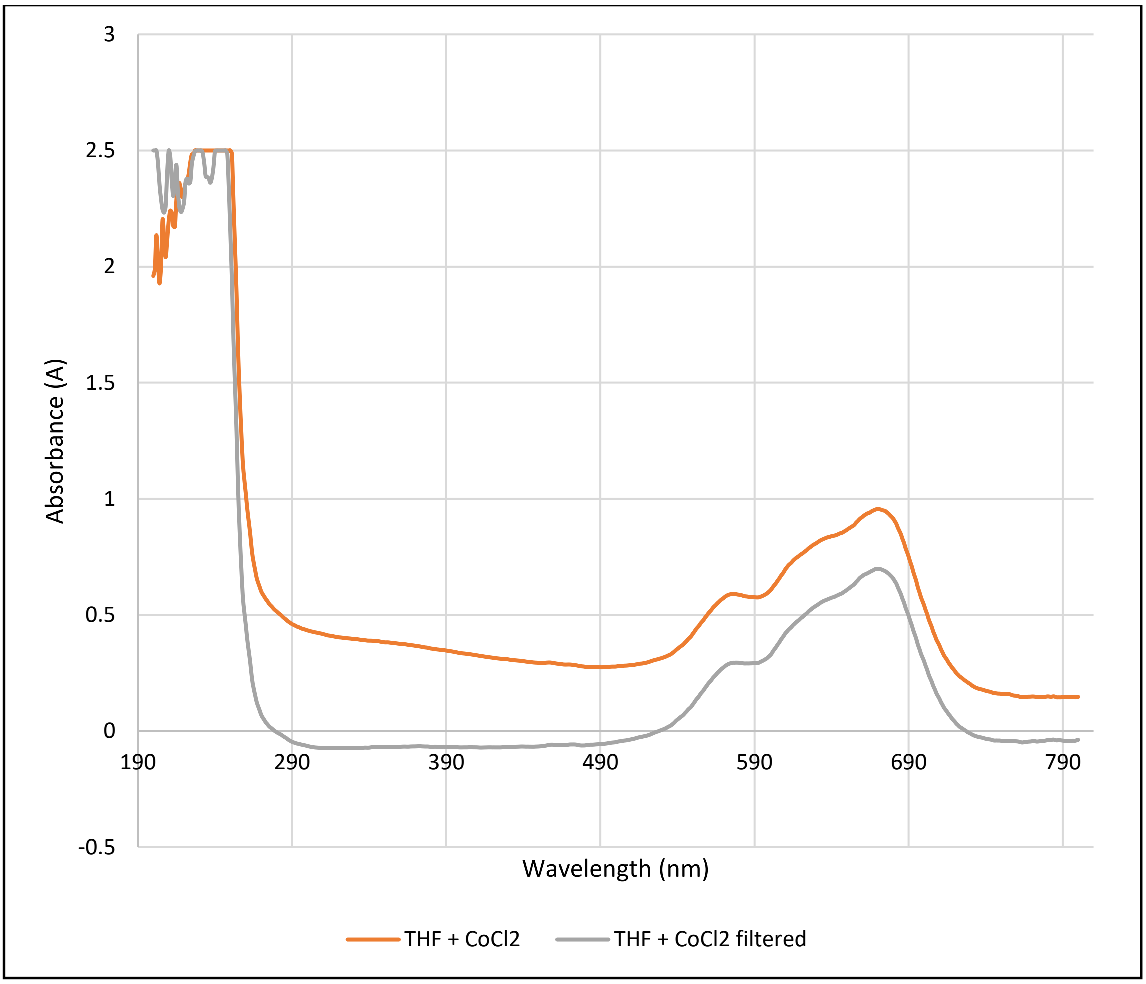

Figure 3.7 THF dehydrated by $MgSO_4$ and $CoCl_2$ (with and without nano filtration)

3.3.3 Dilutions with THF dehydrated with $MgSO_4$

The average absorbance for triplicate water concentration dilution was calculated for the $CoCl_2$/THF solution for THF dehydrated by $MgSO_4$. The obtained average absorbance against the wavelength of THF and THF with $CoCl_2$ was plotted. When focusing on the wavelength range between 400nm and 800nm, it could be seen that before the solution was nano filtered, the pre nano filtered solution had a much higher absorbance compared to the solution after nano filtered. The 15µl dilution, had the lowest absorbance level which proposes that the method could be cable to quantify water content when a high amount of water is present in THF while being ineffectual to be used for low water content determination in THF.

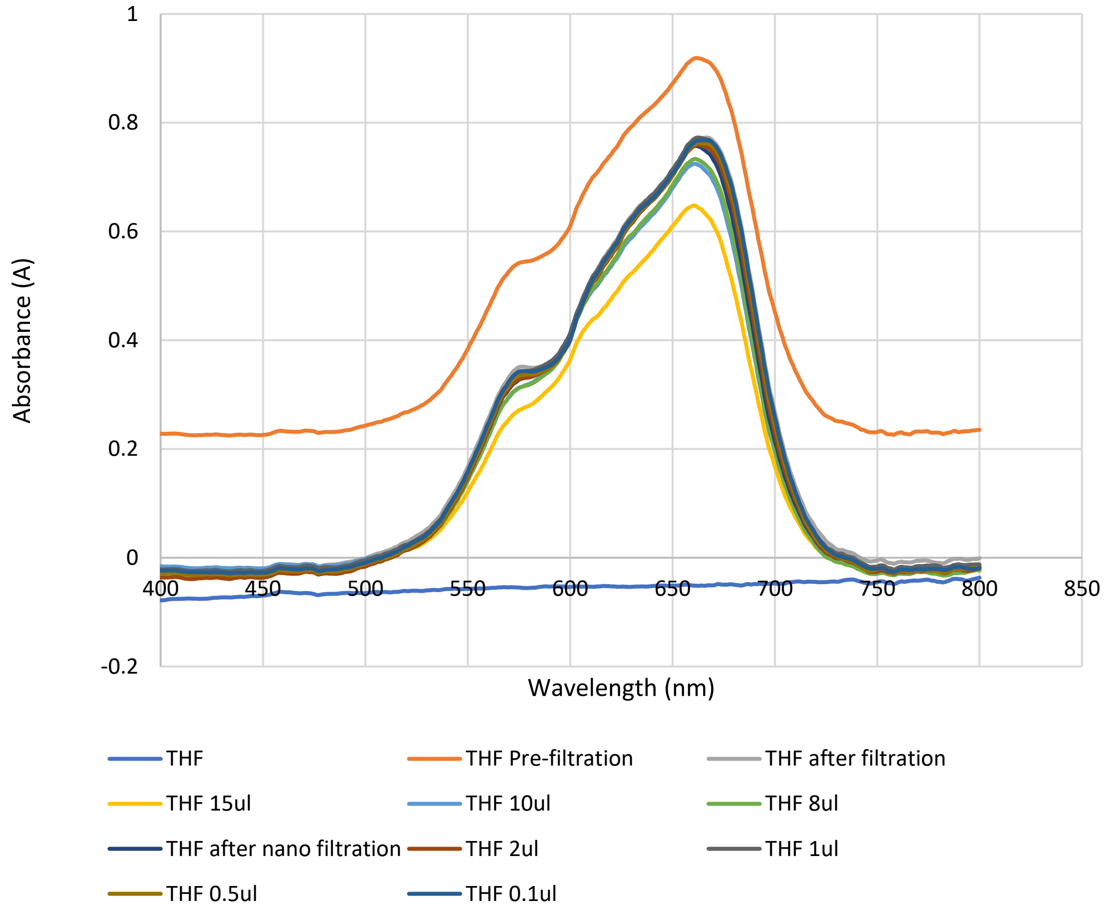

Figure 3.8 Dilutions with tetrahydrofuran with $MgSO_4$ focusing on the wavelength range 400 to 800nm

3.4 Tetrahydrofuran dehydrated by calcium chloride

The average absorbance from each of the 3 samples of dehydrated THF/$CoCl_2$ solution, the not dehydrated THF/$CoCl_2$ solution and dilution with 5µl HPLC grade water and 995µl from the dehydrated THF/$CoCl_2$ solution were calculated. A graph depicting the averages absorbances against the wavelength was plotted (Figure 3.9).

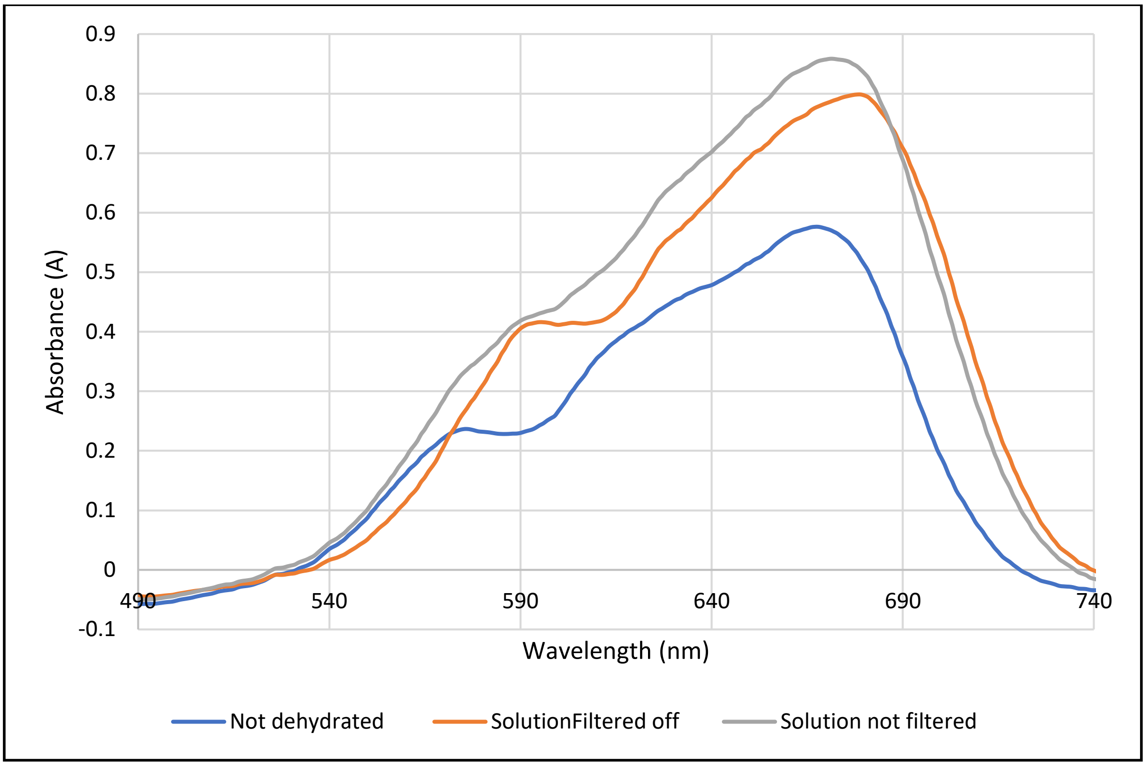

Figure 3.9 $CaCl_2$ dehydrated solution

3.4.1 Day after dehydration with calcium chloride

The bottles which were left in the fume cupboard to be visually analysed the day after are shown in Figure 3.10. The left bottles are THF dehydrated $CaCl_2$ which was removed by a filter paper, the middle bottles are of THF dehydrated by $CaCl_2$ which was left in the bottle and the right bottles of THF directly from the bottle. It is visible that the A and B bottles have a darker blue colour than that obtained from the THF not dehydrated by

$CaCl_2$. The dehydrated bottles A and B are cloudy compared to the non-dehydrated THF bottle C.

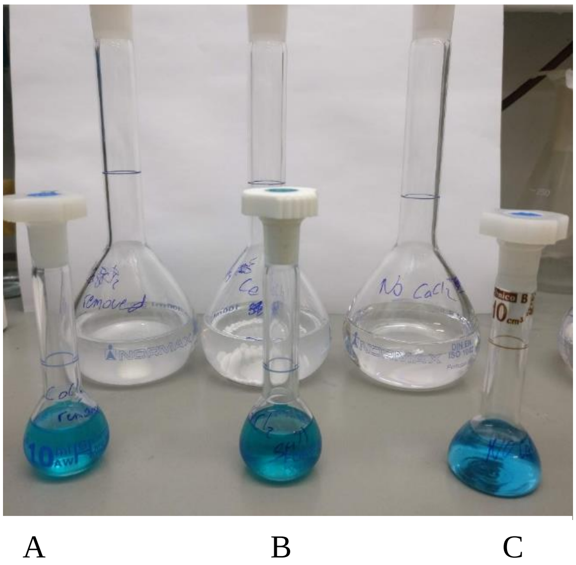

Figure 3.10 Difference between the three solution of THF dehydrated $CaCl_2$ where A is with the $CaCl_2$ removed, B with the $CaCl_2$ in the bottle and C control (without $CaCl_2$)

3.4.2 UV/Vis absorbance

The average absorbances with the bottles left for dehydration as explained in section 2.8 are shown below. The absorbance with $CoCl_2$ obtained are lower than the ones obtained from the previous day which shows that moisture was absorbed from the air lowering the absorbance. With the use of nano filtration, the absorbance levels were lower compared to the solutions without nano filtration which could be due that some particles from the dehydrating agent or $CoCl_2$ formed a suspension and with nanofiltration these particles were removed.

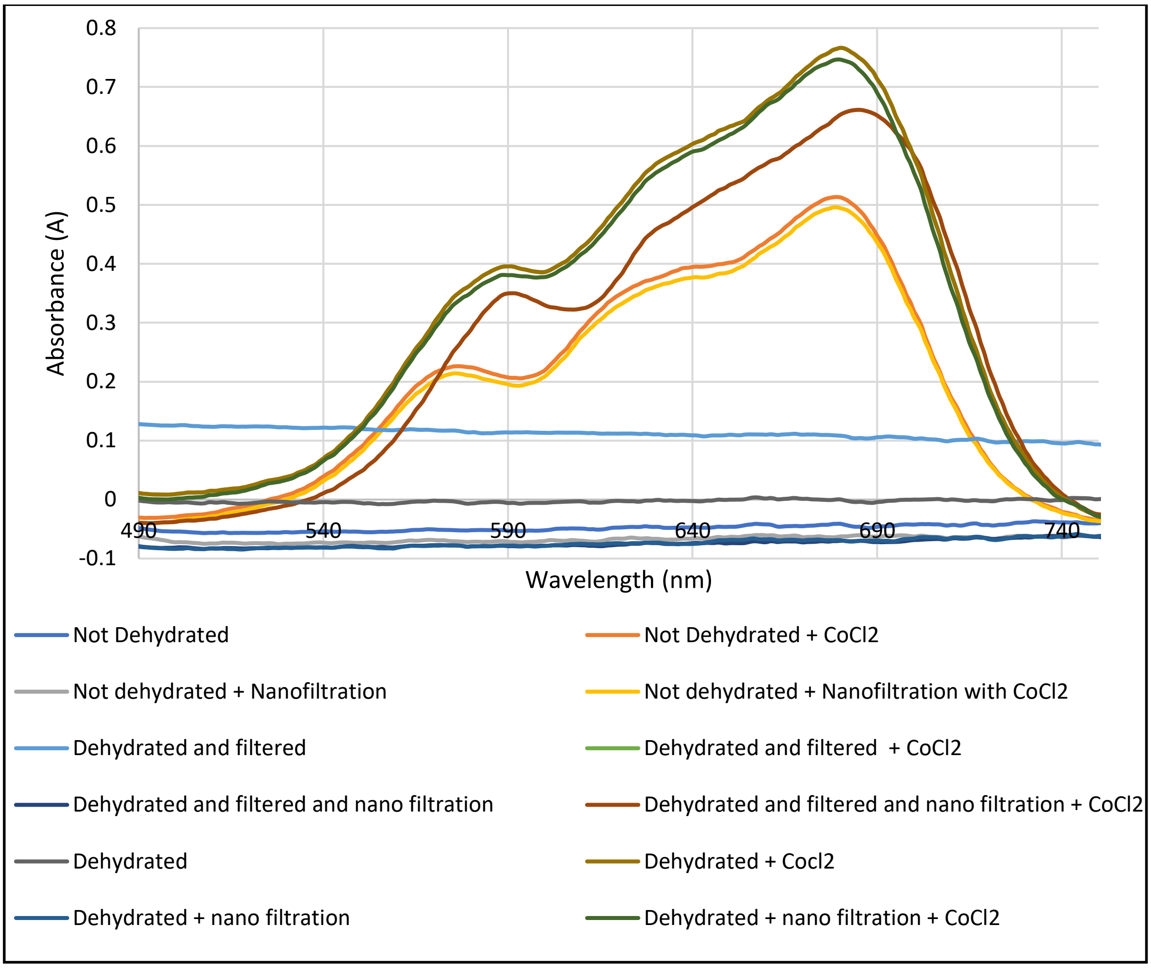

Figure 3.11 $CaCl_2$ dehydrated THF solution analysis the day after

Chapter 4

Discussion

4.1 UV/Vis Analysis

The temperature during the UV/Vis analysis was kept constant at 21°C since fluctuations of the temperature results in a difference in the absorbance levels (Bai & Yang, 2007).

The method using $CoCl_2$ as an indicator and UV/Vis spectroscopy was developed to determine the presence of water in THF. It is likely that THF would contain very small water quantities[4]. The experimental addition of water was up to a maximum of 15µl with 985 µl THF. The resultant colour change with $CoCl_2$ is inconspicuous to be visible by the naked eye.

The wavelength used for the determination of water content in samples containing THF and $CoCl_2$ was 672 nm since the maximum absorbance for $CoCl_2$ was observed at this wavelength in the $1.69x10^{-3}$ mol/L and $3.42x10^{-3}$ mol/L $CoCl_2$ in THF. The equations obtained from the plotted graphs (Figure 3.5) of the average absorbance at 672nm against water added in a) 1.69x10-3 mol/L, b) 3.42x10-3 mol/L c) 6.98x10-3 mol/L $CoCl_2$ in anhydrous THF can be theoretically used to measure water content in THF if the same concentration of $CoCl_2$ is used. In practice, their respective R^2 values are low.

The method developed by Bai and Yang (2007) resulted in a decrease in absorbance values with increasing water content with the alcohol-based solvents. In this research with THF, an initial increase in absorbance was unexpectedly observed with the addition of water. This unexpected increase was however followed by a decrease in absorption on further addition of water. Two hypotheses are proposed to explain the initial increase in absorbance followed by a decrease in absorbance on further addition of water. The first hypothesis is that there is an incomplete dissolution of $CoCl_2$ in THF which forms a very

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[4] National Center for Biotechnology Information. PubChem Compound Database. Tetrahydrofuran [Internet]. [cited 2020 April 20]. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/8028

fine suspension escaping detection. When water is added, $CoCl_2$ dissolves completely and the absorbance rises. Addition of more water could result in affecting the complex equilibrium between the various forms of hydrates. The second hypothesis proposed is a possible incompatibility of $CoCl_2$ with residues of molecular sieves.

From the results obtained by Bai and Yang (2007), there was only a downward decrease in the peaks absorbance, while in this study with THF there an initial increase followed by shift in peaks at a lower wavelength.

4.2 Dehydrating agents

From literature, it is noted that molecular sieves are one of the efficient dehydrating agents for THF which is why anhydrous THF is commonly stored under molecular sieves (Williams & Lawton, 2010). This makes the anhydrous THF stored under molecular sieves suitable for this research which safeguards that the anhydrous THF to contain minimal water or no water at all.

Results with the anhydrous THF stored under molecular sieves were different than the findings by Bai & Yang (2007). It was proposed to perform the dehydration of THF in the lab. $CaCl_2$ and $MgSO_4$ were chosen to dehydrate the THF since they are frequently used as dehydrating agents in the laboratories.

The tests to dehydrate the THF in the laboratory with $MgSO_4$ and $CaCl_2$ resulted in a cloudy THF solution. The unwanted cloudiness resulted to ambiguously high absorbance when the solution with $CoCl_2$ was prepared and analysed by UV/Vis spectroscopy. $MgSO_4$ and $CaCl_2$ are suitable desiccants to dehydrate ethers.[5] THF being a cyclic ether should be compatible to be dehydrated by $MgSO_4$ and $CaCl_2$. The cloudiness and high

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[5] Drying Agents [Internet]. UCLA College Chemistry and Biology. 2016 [cited 2020 September 4]. Available from: https://www.chem.ucla.edu/~bacher/Specialtopics/Drying%20Agents.html

UV/Vis absorbance obtained show that either THF is not suitable to be dehydrated by $MgSO_4$ and $CaCl_2$ or a different method of separation, other than gravitational filtration, is required. $MgSO_4$ and $CaCl_2$ possibly can only be suitable to dehydrate simple ether and not cyclic ethers. The difference between a simple ether and cyclic ether is their structure. An ether is distinguished by having "an oxygen atom bonded to two alkyl or aryl groups".[6]

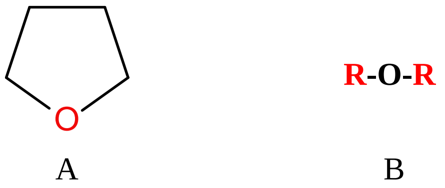

Figure 4.4.1 A) Tetrahydrofuran B) Simple Ether

After the solutions which were dehydrated by $CaCl_2$, were filtered and left to stand for a day, some deposits in the flasks were observed the next day. The deposits indicated that gravitational filtration is not enough to separate THF and the two dehydrating agents, even though this method of separation is a common practice in a laboratory setting especially during chemical synthesis. The sedimented deposits could result in undesirable reactions which can lead to a low yield of the wanted product, no reaction at all or a totally different reaction to occur. The deposits were removed by filtrating with a nano filter. A nano filter removes even the tiniest particles which may have formed during the dehydration step. This extra measure is time consuming and increases manual labour.

From the bottles which were left in the fume cupboard (Figure 3.10), it could be concluded that $CaCl_2$ may have a certain interaction THF and hence unsuitable to

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[6] Wade L. Ether [Internet]. Encyclopaedia Britannica. 2019 [cited 2020 August 16]. Available from: https://www.britannica.com/science/ether-chemical-compound

dehydrate THF. This could lead to additional unwanted impurities in the synthesised product with THF.

The UV/Vis absorbance levels obtained from the dehydrated THF solution performed in the lab with $CaCl_2$ which was left in the fume cupboard to be analysed, were lower than the ones obtained from the previous day. Lower absorptions confirm that THF had absorbed water moisture from the air and a change in the $CoCl_2$ equilibrium had occurred. This change shows that the method in principle works in THF, but a larger amount of water is needed for a shift in the $CoCl_2$ hydrates equilibrium (Scheme 8) and in turn to be effectively be quantified by UV/Vis spectroscopy.

4.3 Limitations and Recommendations for future studies

The biggest limitation of the study was the difficulty to weigh the exact amount of $CoCl_2$ each time a solution needed to be prepared. A slight change in weight of $CoCl_2$ will disrupt the $CoCl_2$ available for $CoCl_2$ equilibrium. Another problem is that $CoCl_2$ absorbs water from the air and changes the colour while weighing and hence disrupt the absorbances values. It was frequently needed to restart weighing the $CoCl_2$ since when trying to achieve the desired amount, $CoCl_2$ changed the colour from blue to mauve due moisture absorption.

It is necessary that the solubility of $CoCl_2$ in anhydrous THF is studied. This could be investigated as part of a future study. If on dilution, the decrease in absorbance is not proportional to the decrease in concentration, but even possibly higher, then it is necessary to investigate whether $CoCl_2$ is not completely dissolved in THF.

Further studies are needed for the dehydration of THF in the lab and show of which frequently used dehydrating agents are suitable to dehydrate THF. The interactions and applicability of the dehydrating agents with THF could be tackled as follows. For the dehydration part, 3 different flask (Flask I to be tested immediately after the dehydration, Flask II one to be tested after 24 hours and Flask III to be tested after 48 hours) for each concentration of dehydrating agent, in addition to one control with each type of dehydrating agent. This ensures that the ratio between the dehydrating agent and solvent to be equal through-out all the tests. If only two flasks were to be used, after each solvent sample is taken, the ratio of THF to the dehydrating agent will be increased. Different concentrations of the dehydrating agents could be used.

The method using $CoCl_2$ as an indicator and UV/Vis spectroscopy to determine the water content in THF could be used in combination to the Karl Fischer method. Using both methods for quantifications will investigate if the results obtained from the Karl Fisher and $CoCl_2$ coupled with UV/Vis spectroscopy are the equivalent.

4.4 Conclusion

An initial increase in absorbance was unexpectedly observed with the addition of water. This unexpected increase was however followed by a decrease in absorption on further addition of water. The method for determination of water investigated in this research which uses $CoCl_2$ as an indicator and UV/Vis spectroscopy requires further investigation and elucidation before it could be considered as a possible substitute for the established Karl Fischer method.

References

Akiya N, Savage P. Roles of water for chemical reactions in high-temperature water. Chemical Reviews. 2002; 102 (8): 2725-2750.

Alothman Z. A Review: Fundamental Aspects of Silicate Mesoporous Materials. Materials. 2012; 5 (12): 2874-2902.

Andreou A, Leskes M, Jambrina P, Tustin G, Grey C, Rosta E et al. Divergence from the classical hydroboration reactivity; boron containing materials through a hydroboration cascade of small cyclic dienes. Chemical Science. 2015; 6 (11): 6262-6269.

Appoldt Y, Raihani G. Determining Moisture Content, Food Quality & Safety [Internet] 2017 [cited 2020 July 20]. Available from: https://www.foodqualityandsafety.com/article/determining-moisture-content/

Armarego W, Chai C. Purification of laboratory chemicals, 6th ed. Holland: Elsevier; 2009.

Bai H, Yang X. Spectrophotometric determination of water content in alcohol organic solvents. Journal of the Chinese Chemical Society. 2007; 54 (3): 619-624.

Beattie J K, McErlean C S P, Phippen C B. The mechanism of on-water catalysis. Chemistry: A European Journal, 2010; 16: 8972–8974.

Becker K, Jährling N, Saghafi S, Weiler R, Dodt H. Chemical Clearing and Dehydration of GFP Expressing Mouse Brains. PLoS ONE [Internet]. 2012 [cited 2020 August 30] ;7(3): e33916. Available from: https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0033916

Brinker U. H., Mieusset J.L., editors. Molecular Encapsulation: Organic Reactions in Constrained Systems, 1st ed. UK: Wiley; 2010

Burns D.T., Danzer K, Townshend A. A tutorial discussion of the use of the terms "robust" and "rugged" and the associated characteristics of "robustness" and "ruggedness" as used in descriptions of analytical procedures. Journal of the Association of Public Analysts [Internet]. 2009 [cited 2020 August 30]; 37:40-60. Available from: http://www.apajournal.org.uk/2009_0040_0060.pdf

Butler R, Coyne A. Understanding “on-water” catalysis of organic reactions. Effects of $H^+$and Li^+ ions in the aqueous phase and non-reacting competitor H-bond acceptors in the organic phase: on H_2O versus on D_2O for Huisgen cycloadditions. The Journal of Organic Chemistry. 2015; 80 (3): 1809-1817.

Chakrabarti A, editor. Engineering design synthesis. 1st ed. UK: Springer; 2002.

Dong M. Modern HPLC for practicing scientists. 1st ed. USA: Wiley; 2006.

Fetzer W. Determination of Moisture by Distillation. Analytical Chemistry. 1951; 23 (8): 1062-1069.

Furniss B, Hannaford A, Smith P, Tatchell A. Vogel's textbook of practical organic chemistry. 5th ed. India: Pearson; 2003.

Grodowska K, Parczewski A. Organic solvents in the pharmaceutical industry. Acta Poloniae Pharmaceutica ñ Drug Research. 2010; 67 (1): 3-12.

Hawkins J M. Organic Chemistry: Streamlining Drug Synthesis. Nature 2015; 520: 302-303.

Hiebel A, Comins D. Novel approach toward the morphine skeleton. Synthesis of 1-(5-benzyloxy-2-bromo-4-methoxybenzyl)-6-oxo-3,4,6,7,8,8a-hexahydro-1H-isoquinoline-2-carboxylic acid phenyl ester via N-acylpyridinium salt chemistry. Tetrahedron. 2015; 71 (39): 7354-7360.

Hunt J. Complete A-Z chemistry handbook. 4th ed. UK Philip Allan; 2009.

Jancic-Stojanovic B, Vemic A, Rakic T, Kostic N, Malenovic A. Development of Karl Fischer titration method using quality by design concept. Chemical Industry. 2012; 66 (5): 659-665.

Jung H, Lee Y, Yoon W. Effect of Moisture Content on the Grinding Process and Powder Properties in Food: A Review. Processes. 2018 ;6 (6): 69.

Kakabadse G, editor. Solvent problems in industry. 1st ed. UK: Elsevier Applied Science; 1984.

Langhals H. A Simple, Quick, and Precise Procedure for the Determination of Water in Organic Solvents. Analytical Letters. 1990; 23 (12): 2243-2258.

Lister T, Renshaw J. New understanding chemistry for advanced level. 3rd ed. UK: Thornes; 2000.

Mäntele W, Deniz E. UV–VIS absorption spectroscopy: Lambert-Beer reloaded. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 2017; 173: 965-968.

McFadden T, Fang C, Geib S, Merling E, Liu P, Curran D. Synthesis of Boriranes by Double Hydroboration Reactions of N-Heterocyclic Carbene Boranes and Dimethyl Acetylene dicarboxylate. Journal of the American Chemical Society. 2017; 139 (5): 1726-1729.

National Research Council (US) Committee on Challenges for the Chemical Sciences in the 21st Century. Beyond the Molecular Frontier: Challenges for Chemistry and Chemical Engineering. Washington (DC): National Academies Press (US); 2003.

Okhrimenko L, Favergeon L, Johannes K, Kuznik F, Pijolat M. Thermodynamic study of MgSO4 – H2O system dehydration at low pressure in view of heat storage. Thermochimica Acta. 2017; 656: 135-143.

Speight J. The refinery of the future. 1st Ed. Holland: Elsevier; 2011.

St. Hilaire V, Hopkins W, Miller Y, Dandepally S, Williams A. Regioselective addition of Grignard reagents to N-acylpyrazinium salts: synthesis of substituted 1,2-dihydropyrazines and Δ5-2-oxopiperazines. Beilstein Journal of Organic Chemistry. 2019; 15: 72-78.

Tavčar E, Turk E, Kreft S. Simple Modification of Karl-Fischer Titration Method for Determination of Water Content in Colored Samples. Journal of Analytical Methods in Chemistry. 2012;2012: 379724

Thomas N. The early history of spectroscopy. Journal of Chemical Education. 1991; 68 (8): 631.

Tryon M. An improved apparatus for determining moisture in rubber by distillation with toluene. Journal of Research of the National Bureau of Standards. 1950; 45 (5): 362.

Tsukanov S, Comins D. Total Synthesis of Alkaloid 205B. The Journal of Organic Chemistry. 2014; 79 (19): 9074-9085.

Villa C, Galus S, Nowacka M, Magri A, Petriccione M, Gutiérrez T. Molecular sieves for food applications: A review. Trends in Food Science & Technology. 2020; 102: 102-122.

Williams D, Lawton M. Drying of organic solvents: Quantitative evaluation of the efficiency of several desiccants. Journal of Organic Chemistry. 2010; 75 (24): 8351-8354.

Yagi S, Tanaka A, Ichikawa Y, Ichitsubo T, Matsubara E. Effects of water content on magnesium deposition from a Grignard reagent-based tetrahydrofuran electrolyte. Research on Chemical Intermediates. 2013; 40: 3-9.

List of Publications and Abstracts

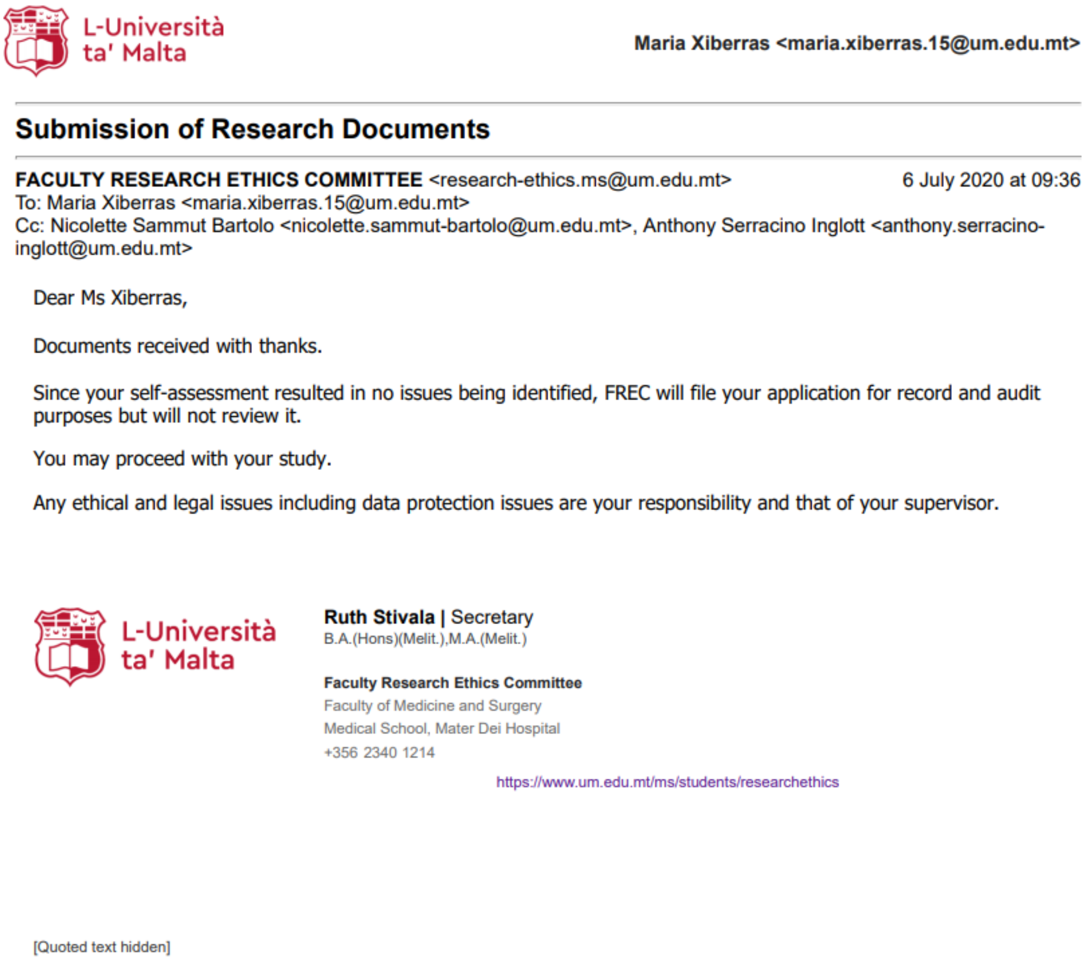
This research was published as a poster presentation at the International Pharmaceutical Federation World Congress of 2019. The poster presented is attached in Appendix 2. *Xiberras, M., Attard, E., Sammut Bartolo, N., & Serracino-Inglott, A. (2019, September). A simple method for the determination of water content in tetrahydrofuran. Poster session presented at the FIP World Congress of Pharmacy and Pharmaceutical Sciences, Abu Dhabi, United Arab Emirates.*

This research was also accepted for a poster presentation at the 12th World Meeting on Pharmaceutics, Biopharmaceutics and Pharmaceutical Technology which was postponed to 8 - 11 February 2021. The event will be held in Vienna, Austria. The accepted abstract is attached in Appendix 2.

An abstract submitted at the International Pharmaceutical Federation Congress was accepted as a poster presentation. The posters was presented during the FIP Virtual conference organised in September 2020 during a virtual gallery session. The poster that was presented is attached in Appendix 2.

Appendix 1

Ethics Approval



L-Università ta' Malta

Maria Xiberras <maria.xiberras.15@um.edu.mt>

Submission of Research Documents

FACULTY RESEARCH ETHICS COMMITTEE <research-ethics.ms@um.edu.mt> 6 July 2020 at 09:36
To: Maria Xiberras <maria.xiberras.15@um.edu.mt>
Cc: Nicolette Sammut Bartolo <nicolette.sammut-bartolo@um.edu.mt>, Anthony Serracino Inglott <anthony.serracino-inglott@um.edu.mt>

Dear Ms Xiberras,

Documents received with thanks.

Since your self-assessment resulted in no issues being identified, FREC will file your application for record and audit purposes but will not review it.

You may proceed with your study.

Any ethical and legal issues including data protection issues are your responsibility and that of your supervisor.

L-Università ta' Malta

Ruth Stivala | Secretary
B.A.(Hons)(Melit.),M.A.(Melit.)

Faculty Research Ethics Committee
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Appendix 2

Publications and Abstracts

L-Università ta' Malta
Faculty of
Medicine & Surgery
Department
of Pharmacy

A SIMPLE METHOD FOR THE DETERMINATION OF WATER CONTENT IN TETRAHYDROFURAN

Maria Xiberras[1], Everaldo Attard[2], Nicolette Sammut Bartolo[1], Anthony Serracino-Inglott[1]
[1]Department of Pharmacy, University of Malta, Msida, Malta
[2]Rural Sciences and Food Systems, University of Malta, Msida, Malta
email: maria.xiberras.15@um.edu.mt

INTRODUCTION

Water content in organic solvents used for chemical synthesis frequently influences the reaction outcome and yield.[1] Water content in solvents is usually determined using a Karl Fischer titration and azeotropic distillation. Both methods have limitations, the Karl Fischer apparatus is easily disturbed while the azeotropic distillation is complicated to conduct, time consuming and has a low precision in reading the volume of water collected. [1,2]

AIM

To develop a simple method to determine the water content in tetrahydrofuran THF using UV/Vis spectroscopy with cobalt chloride ($CoCl_2$).

METHOD

A method developed by Bai and Yang[1] to determine the water content in alcohol-based solvents using $CoCl_2$ as an indicator and a UV/Vis spectrometer as an indicator was adapted to THF.

The development of the method for the determination of water content in THF was conducted in two phases:

- In phase 1, the suitability of $CoCl_2$ as an indicator for water content in THF was investigated. Samples of anhydrous THF and anhydrous THF with cobalt chloride ($CoCl_2$) were analysed using UV/Vis spectrometry to determine the maximum absorbance of both compounds.
- Phase 2 of the study entailed finding and analysing the difference in absorbance in relation to different water concentration in THF and $CoCl_2$ with UV/Vis spectroscopy. Vials for each water concentration were prepared in triplicates, by first measuring the HPLC grade water to be added (0.1, 1, 2, 5, 10 µl), with a micropipette, and then were topped up by the mixture of $CoCl_2$ with THF to reach a total volume of 1 ml.
- The stock solution used for the method development contained 3.51×10^{-3} mol/L $CoCl_2$ in THF and for the UV/Vis spectroscopy analysis, a 0.5mm cuvette and a wavelength range between 200nm to 800nm were used.

RESULTS

- THF has an absorbance between 200-320nm while absorbance in the region between 548-721nm was attributed to CoCl2 with a maximum absorbance at 680nm. (Figure 1).
- The average absorbance of each water concentration was calculated and a graph of absorbance at 680nm against water concentration was plotted (Figure 2). It was observed that as the water concentration increased, the absorbance decreased with a polynomial second order trend.
- The equation obtained from the plotted graph (Figure 2), $y = 0.0016x^2 - 0.0241x + 0.3645$ with an R^2 value of 0.9625 may be used to measure water content in THF if the same concentration of $CoCl_2$ which was used.

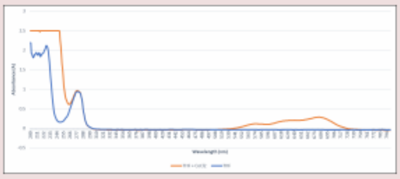

Figure 1: Uv/Vis spectra of THF and THF with $CoCl_2$

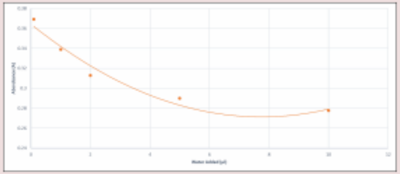

Figure 2: Average absorbance of THF with $CoCl_2$ at 680nm against water added

CONCLUSION

The developed method is simple, practical and makes use of a UV/Vis spectrometer, an apparatus which is both easy to use and available in most laboratories. The method may be used to calculate the efficacy of dehydrating agents such as magnesium sulphate, molecular sieves and calcium chloride to dry THF.

REFERENCES

[1] Bai H, Yang X. Spectrophotometric determination of water content in alcohol organic solvents. Journal of the Chinese Chemical Society. 2007;54(3):619-624
[2] Fetzer W. Determination of Moisture by Distillation. Analytical Chemistry. 1951;23(8):1062-1069.

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Determination of water content using cobalt chloride coupled with UV/Vis spectroscopy in tetrahydrofuran

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INTRODUCTION

Tetrahydrofuran (THF) is an inert, colourless solvent, which is readily miscible with water. THF is used as a solvent in various reactions such as hydroboration reactions and Grignard processes (Mc Fadden et al., 2017; St. Hilaire et al., 2019). Water content in organic solvents used for chemical synthesis frequently influences the reaction outcome and yield (Bai & Yang, 2007). The aim of the study was to devise an alternative method to determine the critical water content to the standard Karl-Fischer method.

METHODOLOGY

A method developed by Bai and Yang (2007) to determine the water content in the alcohol-based solvents ethanol, methanol, n-propanol, iso-propanol and n-butanol was adapted to THF. The method uses cobalt chloride ($CoCl_2$) as an indicator and changes in absorbance in relation to water content are detected by UV/Vis spectrometer (Bai & Yang, 2007). The study entailed determining and analysing the difference in absorbance in relation to different water concentration in THF and $CoCl_2$ using UV/Vis spectroscopy. Different concentration solutions of $CoCl_2$ in anhydrous THF containing 1.69×10^{-3} mol/L, 3.42×10^{-3} mol/L and 6.98×10^{-3} mol/L were prepared

Stock Solution

Vials for each water concentration (0.1, 0.5, 1, 2, 5, 8, 10, 15 µL) were prepared in triplicates, by first measuring the HPLC grade water to be added with a micropipette, and then transferred into amber glass vials. The prepared vials were topped up by the mixture of $CoCl_2$ with THF to reach a total volume of 1 ml. Once a sample was prepared, it was stored in a re-sealable bag containing silica. This was sealed and put into the desiccator until all the samples were prepared to be analysed..

UV/Vis Spectroscopy

For the analysis of the samples, a quartz glass cuvette (10 mm path length) and a wavelength range between 200nm to 800nm were selected. A blank cuvette was used as a reference. The apparatus used was Biochrom® WPA Lightwave II.

RESULTS

Absorbance against the wavelength of THF and THF with $CoCl_2$ was plotted (Figure 1). THF has an absorbance between 200-320nm while absorbance in the region between 480-720nm was attributed to $CoCl_2$ with a maximum absorbance at 672nm in the solutions containing molar concentrations of 1.69×10^{-3} mol/L and 3.42×10^{-3} mol/L and at 669nm in the solution containing molar concentration of 6.98×10^{-3} $CoCl_2$ in THF.

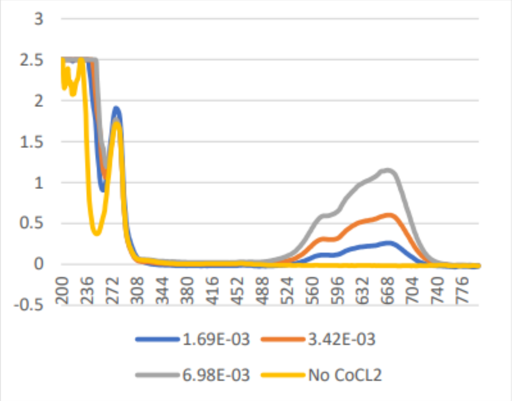

Figure 1. Absorbance against wavelength in different concentrations of $CoCl_2$

The average absorbance for each water concentration was calculated for every $CoCl_2$ concentration and a graph of the average absorbance at 672nm against water added was plotted (Figure 2,3,4). Their respective polynomial second order trend lines are shown in Table 1.

$CoCl_2$ in THF (mol/L)	Trendline	R^2 Value
1.69×10^{-3}	$y=-0.0004x^2 + 0.008x + 0.2645$	0.7978
3.42×10^{-3}	$y=-0.0011x^2 + 0.0186x + 0.6075$	0.7165
6.98×10^{-3}	$y=-0.0030x^2 + 0.0602x + 1.1257$	0.5955

Table 1. Equations for average absorbance at 672nm against water added

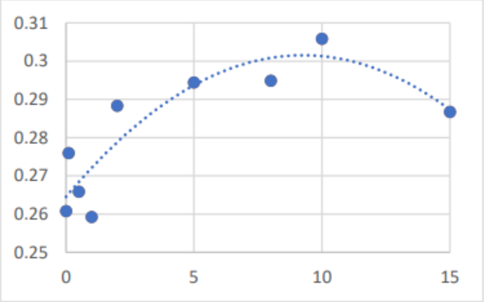

Figure 2. Average absorbance at 672nm against water added in $1.69x10^{-3}$ mol/L $CoCl_2$ in THF

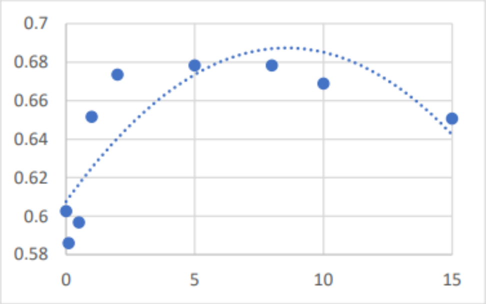

Figure 3. Average absorbance at 672nm against water added in $3.42x10^{-3}$ mol/L $CoCl_2$ in THF

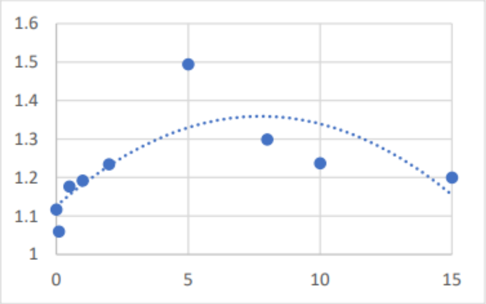

Figure 4. Average absorbance at 672nm against water added in $6.98x10^{-3}$ mol/L $CoCl_2$ in THF

DISCUSSION

This method was developed to determine the presence of water in THF. It is likely that THF would contain very small water quantities. The experimental addition of water was up to a maximum of 15µl with 985 µl THF. The resultant colour change with $CoCl_2$ is inconspicuous to be visible by the naked eye. The colour-change reaction mechanism is expressed as follows (Bai & Yang, 2007):

$CoCl_2$	↔	$CoCl_2 \cdot H_2O$	↔	$CoCl_2 \cdot 2H_2O$	↔	$CoCl_2 \cdot 6H_2O$
Blue		Blue purple		Mauve		Pink

The wavelength used for the determination of water content in samples containing THF and $CoCl_2$ was 672 nm since the maximum absorbance for $CoCl_2$ was observed at this wavelength in the $1.69x10^{-3}$ mol/L and $3.42x10^{-3}$ mol/L $CoCl_2$ in THF.

Theoretically, the equations obtained from the plotted graphs (Table 1) may be used to measure water content in THF if the same concentration of $CoCl_2$ is used. However, the respective R^2 values are low. The method developed by Bai and Yang resulted in a decrease in absorbance values with increasing water content. Yet, this was not the case with the anhydrous THF. This could be due to poor solubility of $CoCl_2$ in THF. The initial increase in absorbance followed by a decrease in absorbance on further addition of water, could be due to the incomplete dissolution of $CoCl_2$ in THF, forming a very fine suspension which escapes detection. When water is added, $CoCl_2$ dissolves completely and the absorbance rises. Addition of more water could result in affecting the complex equilibrium between the various forms of hydrates resulting in decrease in the 650nm peak and introducing a peak at 520 nm due to the tetrahydrate.

CONCLUSION

Due to these findings, it is necessary that the solubility of $CoCl_2$ in anhydrous THF is studied. This will be investigated as part of a future study. If on dilution, the decrease in absorbance is not proportional to the decrease in concentration, but even possibly higher, then it is necessary to investigate whether $CoCl_2$ is not completely dissolved in THF

REFERENCES

1. Bai H, Yang X. Spectrophotometric determination of water content in alcohol organic solvents. Journal of the Chinese Chemical Society. 2007;54(3):619-624.

2. McFadden T, Fang C, Geib S, Merling E, Liu P, Curran D. Synthesis of Boriranes by Double Hydroboration Reactions of N-Heterocyclic Carbene Boranes and Dimethyl Acetylene dicarboxylate. Journal of the American Chemical Society. 2017;139(5):1726-1729.

3. St. Hilaire V, Hopkins W, Miller Y, Dandepally S, Williams A. Regioselective addition of Grignard reagents to N-acylpyrazinium salts: synthesis of substituted 1,2-dihydropyrazines and Δ5-2-oxopiperazines. Beilstein Journal of Organic Chemistry. 2019; 15:72-78.

FIP VIRTUAL 2020 SEPTEMBER

UV/Vis spectroscopy in determining water content in solvents

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INTRODUCTION

Water content in organic solvents used for chemical synthesis frequently influences the reaction outcome and yield.[1] The Karl Fischer titration is one of the main method used to measure water content in solvents.[1] It is established that the UV/VIS spectrometer is readily available in laboratories.

AIMS

To develop an alternative method to the standard Karl Fisher method for the determination of water content in tetrahydrofuran (THF).

METHOD

A method developed by Bai and Yang[1] to determine the water content in alcohol-based solvents using cobalt chloride ($CoCl_2$) as an indicator and a UV/Vis spectrometer, was adapted for THF.

The development of the method for the determination of water content in THF was conducted in three steps.

- Three different solutions of $CoCl_2$ in anhydrous THF were prepared ($1.69x10^{-3}$ mol/L, $3.42x10^{-3}$ mol/L, $6.98x10^{-3}$ mol/L)
- Eight dilutions were prepared, in triplicates, for each $CoCl_2$/ THF concentration by adding HPLC grade water (0.1, 0.5, 1, 2, 5, 8, 10, 15 μl) to reach a total volume of 1ml.
- The prepared samples were analysed using UV/Vis spectrometry at a wavelength range between 200nm to 800nm

RESULTS

- THF has an absorbance between 200-320nm while $CoCl_2$ has an absorbance between 480-720nm (Figure 1).
- The average absorbance for each dilution was calculated at 672nm and was plotted against the wavelength. Their respective polynomial equations were derived (Figures 2-4).
- An initial increase in absorbance was unexpectedly observed with the addition of water. This unexpected increase was however followed by a decrease in absorption on further addition of water. This could be due to an incomplete dissolution of $CoCl_2$ in THF which forms a very fine suspension, escaping detection.
- Addition of more water could result in affecting the complex equilibrium between the various forms of hydrates of $CoCl_2$ resulting in a decrease in the 672nm peak and introducing lower peaks due to the formation of the hydrates.

CONCLUSION

The equations obtained from the plotted graphs of the average absorbance at 672nm against water added can be theoretically used to measure water content in THF if the same concentration of $CoCl_2$ is used. In practice, their respective R^2 values are low.

The method for determination of water investigated in this research requires further investigation and elucidation before it could be considered as a possible substitute for the established Karl Fischer method.

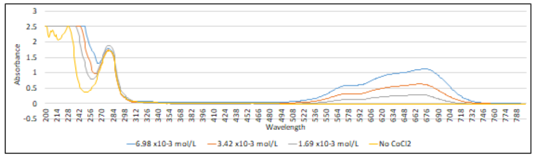
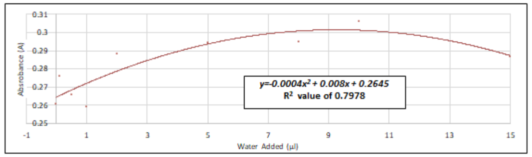
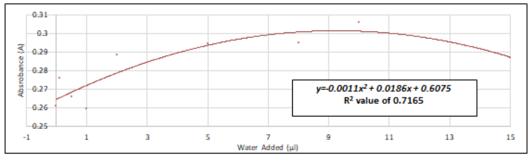


Figure 1: Absorbance against wavelength in different concentrations of $CoCl_2$



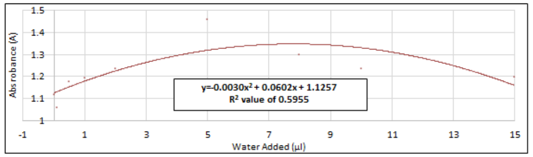
$y=-0.0004x^2 + 0.008x + 0.2645$
R^2 value of 0.7978

Figure 2: Average absorbance at 672nm against water added in $1.69x10^{-3}$ mol/L $CoCl_2$ in THF



$y=-0.0011x^2 + 0.0186x + 0.6075$
R^2 value of 0.7165

Figure 3: Average absorbance at 672nm against water added in $3.42x10^{-3}$ mol/L $CoCl_2$ in THF



$y=-0.0030x^2 + 0.0602x + 1.1257$
R^2 value of 0.5955

Figure 4: Average absorbance at 672nm against water added in $6.98x10^{-3}$ mol/L $CoCl_2$ in THF

REFERENCES

[1]Bai H, Yang X. Spectrophotometric determination of water content in alcohol organic solvents. Journal of the Chinese Chemical Society. 2007;54(3):619-624

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