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A study to assess the viability of novel in-situ techniques for determining chloroform in drinking water in developing countries

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Abstract

The aim of this study was to research appropriate methods for the in-situ analysis of chloroform and other disinfection by-products (DBPs) in drinking water and then assess the viability of these techniques by comparing them against the established methods. A literature search was undertaken using Web of Science and the various features contained within it to research a series of techniques in three distinct categories. These were βeta-cyclodextrin (βCD) techniques, colourimetry techniques and gas-chromatography (GC) techniques. Of the βCD techniques studied, the 2014 paper by Ncube *et al* showcased a novel method using an azo dye modified βeta-cyclodextrin epichlorohydrin polymer to measure chloroform concentration in drinking water (Ncube, Krause & Mamba, 2014). The limit of detection (LOD) obtained was lower than that of the World Health Organisation (WHO) guideline value for chloroform of 0.3 mg/L (WHO, 2008) but not lower than the U.S Environmental Protection Agency (USEPA) maximum acceptable limit for total trihalomethanes (TTHM) of 0.08 mg/L (USEPA, 2021). For the colourimetry techniques the method by Fattahi *et al* used a cotton pad soaked in reagents to quantify chloroform at LOD's lower than any of the other in-situ techniques studied (Fattahi & Shariati-Rad, 2020). These methods were compared against GC techniques in particular the method by González-Hernández *et al* (2017) which was the most inexpensive method that was still fit for purpose. In conclusion the paper by Fattahi & Shariati-Rad (2020) represented an optimal method for the in-situ analysis of chloroform and was able to compete with more expensive GC methods.

Keywords: Chloroform, disinfection by-product, beta-cyclodextrin, fluorescence, colourimetry, Fujiwara, GC-MS, GC-ECD, GC-FID

Introduction

Disinfection by-products (DBPs) or chlorination by-products (CBPs) are a series of compounds that were formed from the chlorination of drinking water supplies. This study focused on the four main DBP's, chloroform, bromodichloromethane, dibromochloromethane and bromoform (CDC, 2019) with a specific focus on chloroform. These compounds were known as total trihalomethanes (TTHM) (CDC, 2019). Chloroform was chosen as the main compound of interest in this study as it made up between 75.5% - 91.4% of TTHM (WHO, 1998). This meant that chloroform was a very good indicator of TTHM in drinking water. Although this study focused on TTHM and chloroform, as of the time of writing there were over 600 different DBPs discovered, however only a few different classes had specific limits associated with them, namely THM's and haloacetic acids (HAAs) (Richardson, 2002).

In 1974 Rook found that DBP's were formed when the chlorine in drinking water, after disinfection, reacted with naturally occurring humic and fulmic acids. DBPs containing bromine were formed when naturally occurring bromine ions competed in this process (Rook, 1974). This was confirmed in 1976 by Stevens *et al*, where they found that the rate of formation and the concentration of THMs increased as a function of the chlorine concentration, humic and fulmic acid concentration, temperature, pH and bromine ion concentration (Stevens *et al.*, 1976). A possible reaction mechanism was shown in Figure 1 below.

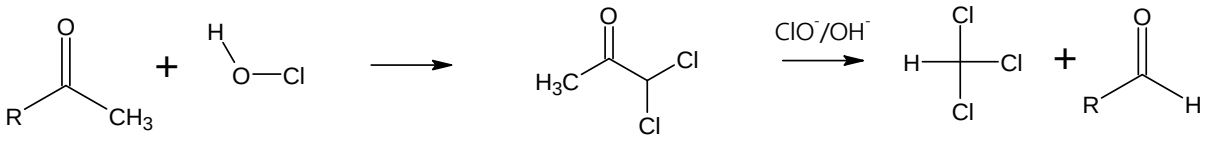

Figure 1: Possible reaction mechanism for the formation of chloroform in water adapted from (Knight et al., 2010; Wang, 2020) and drawn in Chemsketch

There were a number of known health issues associated with exposure to DBP's, with the most significant being an increased risk of cancer. The International Agency for Research on Cancer (IARC) classified chloroform and bromodichloromethane as group 2B possible human carcinogens. The chloroform classification came from a study where dogs were fed chloroform laced toothpaste for 7.5 years (WHO, 1998). Chloroform has been linked to an increased risk of bladder cancer, in the paper by Villanueva et al the authors found that the risk of developing bladder cancer was doubled after being exposed to 50 µg/L over a long time span (Villanueva *et al.*, 2006). This was consistent with various other studies on the cancer risk in different animals (IARC, 1999), there were also links to adverse foetal growth (Smith *et al.*, 2016).

In the year 2000 the Millennium Development Goals (MDGs) were devised to reduce extreme poverty by 2015. The seventh goal was to improve sustainability (UNDP, 2021), inside this target 7C was to "halve, by 2015, the proportion of the population without sustainable access to safe drinking water and basic sanitation" (The United Nations, 2008). There was a lot of controversy as to whether this was met, but the end result was that far more people had access to treated water therefore far more people were exposed to DBP's.

With the risk and prevalence associated with DBP's it was important to be able to accurately quantify these compounds in drinking water across the world, especially with the large amount of variation in water treatment processes across developing countries. The established method for this was a GC-MS method made by the USEPA (USEPA, 1979c). However, these instruments were expensive and required trained analysts to use them. The method that was currently used to analyse chloroform in water in-situ used a Fujiwara reaction which creates a coloured compound when reacted with chloroform. This method required UV-VIS spectrophotometry, was quite complicated and time consuming, while only sporting a limit of detection (LOD) of 1 µg/L (Espigares *et al.*, 2013; Pérez Pavón *et al.*, 2008). The aim of this study was to research appropriate methods for the in-situ analysis of chloroform and DBP's as a whole and then assess the viability of these techniques by comparing them against the established methods.

Beta-cyclodextrin (βCD) techniques

The initial focus of this study was on new techniques that used some form of βCD polymer. βeta-cyclodextrin was a cyclodextrin comprised of seven alpha linked D-glucopyranose units (PubChem, 2021). βCD has been used in pharmaceuticals (Gu & Wheate), HPLC chiral columns (Motoyama *et al*., 2002) and more recently molecular sensors (Ogoshi & Harada, 2008). The top part of βCD was hydrophilic due to the alcohol groups and the cavity was hydrophobic (Pereva *et al*., 2019). This cavity allowed host-guest chemistry to take place, this was when a complex was formed between 2 compounds through non covalent means. Once a fluorescent dye is attached to the molecule it could be used as a molecular sensor.

Colourimetry techniques

Colourimetry was an analytical technique used to determine the concentration of coloured compounds in solution (Housecroft & Constable, 2006). The majority of the colourimetry methods used to detect DBP's use an adapted Fujiwara reaction. This created a coloured compound that can be measured. The Fujiwara reaction was first proposed by Kyoyetsuro Fujiwara in 1914 (Naturforschende und Medizinische Gesellschaft zu, 1914). It involved the reaction of chloroform with pyridine and NaOH to form a red compound.

Gas chromatography (GC) techniques

Gas chromatography was a technique used to analyse usually volatile organic compounds. In chromatography there was a stationary phase which in the case of GC was housed in a column and a mobile phase, which for GC was a carrier gas like nitrogen. The analytes were partitioned based on their affinity for either the stationary phase or the mobile phase. This resulted in the analytes reaching the detector at different times, this was known as retention time. This could be paired with a variety of detectors like the flame ionisation detector (FID) and other techniques in tandem like mass spectrometry (MC) (Evans, 2019; Vollhardt, 2018).

Literature review

The first area to have their water supply fully chlorinated was a town called Maidstone in England in 1897. This was due to a typhoid outbreak, where they successfully isolated the contaminated supply and chlorinated it ('Epidemic of Typhoid Fever at Maidstone,' 1897). The first instance of continuous permanent

water chlorination was in Lincoln, England in 1905 again due to an outbreak of typhoid ('The Epidemic Of Typhoid Fever At Lincoln,' 1905) using calcium hypochlorite (Black & Veatch Corporation, 2010). In 1914 Kyoyetsuro Fujiwara discovered the Fujiwara reaction (Naturforschende und Medizinische Gesellschaft zu, 1914). It seemed that the first method using the Fujiwara reaction to determine chloroform was published in 1941 by Daroga & Pollard (1941), however this was used to measure chloroform in air. The next notable paper using a Fujiwara reaction was published in 1966 (Lugg, 1966). From here there were further advancements in chlorination techniques until 1974. At this time chlorination by-products were first discovered by Bellar, Lichtenberg & Kroner (1974) and Rook (1974). Rook discovered that trihalomethanes were formed by the chlorination of water in 1974 (Rook, 1974) and that same year Bellar *et al* separately made the same discovery (Bellar, Lichtenberg & Kroner, 1974; Hrudey & Fawell, 2015). A further 1974 paper by Bellar *et al* also contained one of the first methods for analysing THMs in water. They used purge and trap GC-FID and GC-MS (Bellar & Lichtenberg, 1974) which quickly became the established method.

In 1979 the USEPA finalised its maximum acceptable limit for total trihalomethanes (TTHM) at 0.1 mg/L (USEPA, 2016). That same year, the USEPA produced three methods to analyse THM's at that concentration. These were method 501.1 (USEPA, 1979b), method 501.2 (USEPA, 1979a) and method 501.3 (USEPA, 1979c). Around the same time as this, liquid chromatography – mass spectrometry (LC-MS) methods started to appear. These were mainly used for hydrophilic DBP's as they were less volatile. Theoretically LC-MS should have been able to analyse water samples without separation, however for low molecular weight compounds, the signal could not be differentiated from the baseline in older methods so derivatisation was usually necessary (Kempter, Zurek & Karst, 1999; Richardson, 2002; Richardson *et al.*, 2000). This could be overcome by using the most advanced techniques available for LC-MS, for example in the method by Tao *et al* (2020) which used ultra-high performance liquid chromatography paired with hybrid quadrupole orbitrap mass spectrometry.

Liquid chromatography techniques were not chosen for this study as the analytes of interest were volatile so gas chromatography techniques were more applicable. In 1984 seemingly the first paper that used a Fujiwara reaction to determine TTHM was published by Huang & Smith (1984). In 1987 the field of supramolecular analytical chemistry really started to take off, after the Nobel prize for chemistry was won by a group of chemists that developed molecules used for host-guest chemistry (Nobel prize, 2021). In 1990 a series of Organisation for Economic Co-operation and Development (OECD) conferences took place to form the basis for the MDGs (Hulme & Scott, 2010).

Another Fujiwara method for determining disinfection by-products in water was published in 1992 by Cherian, Raju & Gupta (1992). From here the frequency of publishing when it came to new methods to detect disinfection by-products started to increase and in 1997 the first step was taken to make βCD sensors for chloroform. Renard *et al* synthesised a high molecular weight βCD EPH copolymer, which was used as the building block for the most advanced βCD sensor for chloroform (Renard *et al.*, 1997). In 1998 the stage 1 disinfectants/disinfection by-products rule came into effect, which changed the maximum TTHM concentration to 0.08 mg/L (USEPA,

2021). In the year 2000 Allonier *et al* (2000) adapted the USEPA method 524.2 for the measurement of THMs in sea water relating to nuclear reactors. In 2001 Chen *et al* designed a system to continuously measure THM concentrations in drinking water (Chen & Her, 2001). That same year the millennium summit took place leading to the formation of the MDGs (Hulme & Scott, 2010), this lead to another increase in publishing for techniques used to detect DBPs. There were a few possible trains of thought as to why this happened. The most likely explanation was that many researchers from across the world tried to work to develop simple to use techniques to quantify DBPs in drinking water to help realise target 7C. However, a more cynical explanation could be due to extra funding received for working on projects related to the MDGs for example, the MDG achievement fund (Multi-partner trust fund office, 2021).

By 2003 purge and trap methods were starting to become outdated and headspace techniques usually paired with solid phase microextraction (SPME) started to appear in the literature (Cho, Kong & Oh, 2003). In around 2004 the World Health Organisation (WHO) published guideline values for the maximum amount of the four main disinfection by-products allowed in drinking water. These were 0.3 mg/L for chloroform, 0.1 mg/L for dibromochloromethane, 0.06 mg/L for bromodichloromethane and 0.1 mg/L for bromoform (WHO, 2008). In 2005 Chatterjee & Gupta (2005) published another Fujiwara reaction for the determination of chloroform and other colourimetry techniques started to appear. One of these other colourimetry methods to appear was published in 2007, this used the ultrasonic oxidation of methyl orange to quantify tetrachloromethane (Luo *et al.*, 2007; Wang *et al.*, 2007).

In 2010 the UN declared that target 7C was supposedly met, when according to multiple papers this was untrue, the paper by Weststrate *et al* (2019) and Satterthwaite (2016) to name a few. In 2011 Ncube *et al* published a paper where they synthesised a βCD polymer to detect chlorophenols. This acted as a precursor to a paper published in 2014 by the same authors where they synthesised a similar βCD polymer sensor that was highly specific to chloroform and was the first of its kind. This methodology was chosen as it was the initial focus of the study where everything branched out from, which was especially interesting as there are currently very few simple easy to use sensors for the detection of disinfection by-products currently employed in the field, from the literature reviewed at the time of writing. In 2015 Fong *et al* published a paper using a modified Fujiwara reaction that had been modified into a solid sensor (Fong *et al.*, 2015), this was an important step and reflected the change in thinking when it came to the detection of disinfection by-products. That same year Nie *et al* published a paper where they synthesised novel nanoparticle composites that, when used as a gas sensor, could quantify chloroform at the mg/L level (Nie *et al.*, 2015). 2015 was also the year that the MDGs were finished and replaced with the Sustainable Development Goals (SDGs) (Hulme & Scott, 2010).

In 2016 another paper was published with a technique that used nanoparticles. Rahman *et al* used nickel oxide nanoparticles suspended in carbon nanotubes to quantify chloroform (Rahman *et al.*, 2016). These types of technique were not chosen for further study, as they were not within the scope of this investigation. Headspace SPME GC techniques continued to be developed as shown in the paper

by González-Hernández *et al* (2017) which was a far more optimised version of the method by Cho, Kong & Oh (2003). The two most advanced and modern GC methods were published in late 2020 and early 2021 (Cuthbertson *et al.*, 2020; Ortega-Hernandez *et al.*, 2021). The majority of the literature for GC techniques was from between 1970 and 2010, and there was very little variation between papers. This was probably because it was an established technique and was the reason why it was chosen for this study, as it provided the strongest comparison to the new in-situ techniques that were appearing in the literature. In 2020 Shariati *et al* published a paper containing a very crude sensor that produced accurate results for use in the field (Shariati-Rad & Fattahi, 2020). Colourimetry techniques were chosen for this study as they represented the majority of the research when it came to simple to use methods of detection. They also represented all the in-situ methods that were currently in use.
The aim of this study was to compare the above techniques to assess the viability of novel in situ techniques for the analysis of disinfection by-products.

Methodology

Literature search

A systematic approach was taken to search the literature to find the most relevant papers to this study. The initial study was based solely around the βCD techniques. Specifically, the 2014 paper by Ncube, Krause & Mamba (2014), this became the point of origin for the study. From here, the references cited by this paper were checked and any relevant papers were scrutinised in more detail. The cited references in these papers were then checked and the process was repeated. The papers that cited those papers were then checked, and any relevant papers were further analysed. The whole process was then repeated for the new papers found. This was all conducted using Web of Science. After the initial search was complete, a literature search was undertaken. Firstly, a generic search string was employed for example, “determination of chloroform in water”. From here the results were filtered using the “refine results” search bar and adding various key words. The search was then reverted back to the original search query and different keywords were selected. The original search query was then altered and the process was repeated.

Analysis

When analysing and comparing the papers found, a few set criteria were used to ensure a fair comparison. These were, ease of use, limit of detection (LOD)/limit of quantification (LOQ) and cost. The ease of use was assessed based on the preparation, use and instrumentation involved. The LOD and LOQ values were compared against two values to assess the fitness for purpose of each of the methods. These were the USEPA maximum acceptable limit for TTHM in water of 0.08 mg/L (USEPA, 2021) and the WHO chloroform guideline value of 0.3 mg/L (WHO, 2008).

For the cost, the majority of the information came from places like Sigma Aldrich (Sigma Aldrich, 2021b). However, for instrumentation there was very little up to date information available as the pricing varied dramatically once negotiation took place. Furthermore, the prices weren’t listed on the websites of various suppliers of instrumentation as this was confidential information that needed to be kept out of the

hands of competitors. With all this in mind, the values for the prices of various pieces of instrumentation came from spending approximately an hour on the phone with the account manager for Plymouth with Agilent technologies for the most part (Baker, 2021).

Discussion

Beta-cyclodextrin (βCD)

Synthesis

There were a few trains of thought when it came to the structures of βCD sensors. All of them had some kind of fluorescent moiety but the type, linkage or location of this moiety changed depending on the application. For example, some authors created βCD sensors in their monomeric form and recently polymeric versions were being synthesised. There were many types of cross linking agents like epichlorohydrin (Ncube, Krause & Mamba, 2014) or acid anhydrides (Girek, Shin & Lim, 2000). There was also the question of what type of dye to use and how to attach it. There were examples of dyes being attached to the βCD structure using various different bond types.

In the paper by Ncube, Krause & Mamba (2014) they synthesised an azo dye-modified β-cyclodextrin – Epichlorohydrin (βCD-EPH) copolymer to detect chloroform, the structure of this polymer was shown in Figure 2. Their main goal was to synthesise a molecular sensor that would provide a cheap and easy method of detecting disinfection by-products. They based their synthesis on a paper by Renard *et al* (1997). This was one of the first papers to synthesise high molecular weight βCD-EPH copolymers. This was a high impact study that has been cited 305 times with the latest citation being in April 2021. The typical method used to synthesise a polymer with a βCD-EPH ratio of 10:1 was to add 5 g of the βCD to an 8 ml NaOH solution and stirred at room temperature. This formed the conjugate base of the alcohol molecules (alkoxide) attached to the outside of the structure and allowed the addition of the EPH. This was then stirred at room temperature overnight. The mixture was then heated to 300^{o}C and an excess of epichlorohydrin was then added (3,445 ml), this was likely an error as it was far larger than the size of the reaction vessel which was meant to be kept constant. There were multiple reactions that could have taken place at this point so measures were taken in this paper to limit this. The possible reactions were shown in (Renard *et al.*, 1997) and the structure of the polymer synthesised in (Ncube, Krause & Mamba, 2014) was shown in Figure 2.

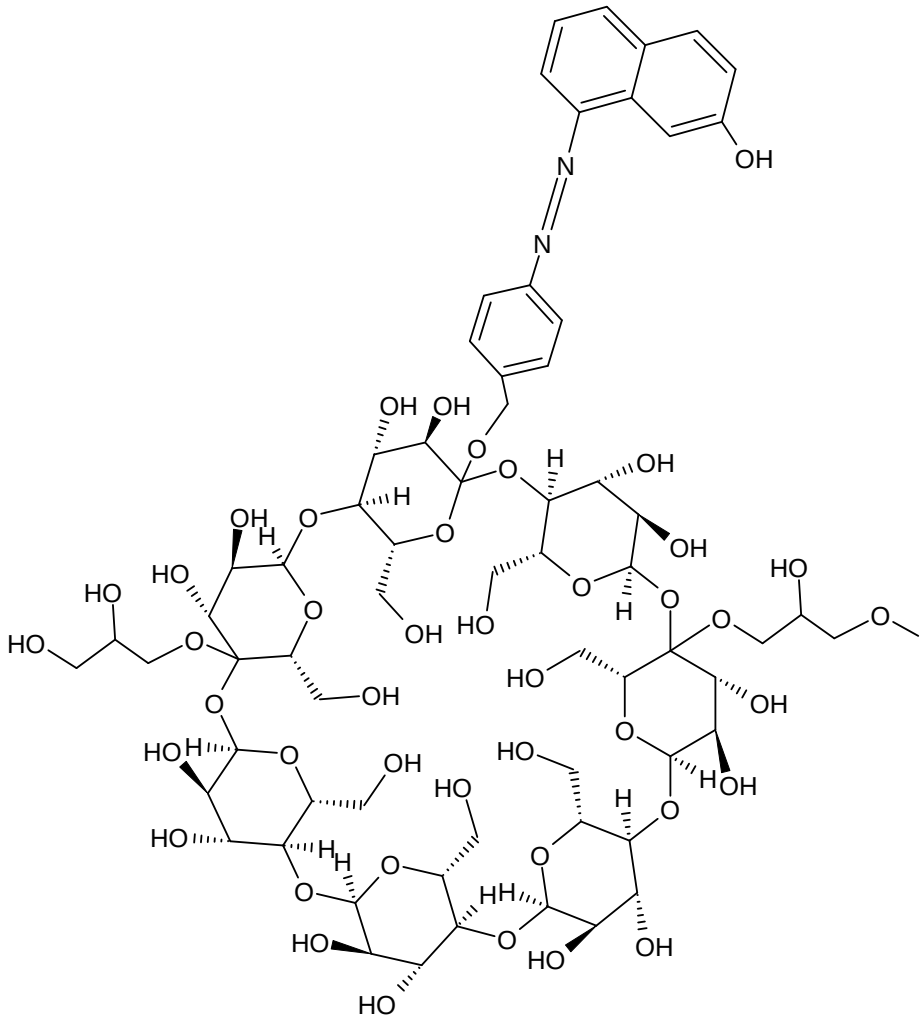

Figure 2: Polymer structure (Ncube, Krause & Mamba, 2014) drawn in Chemsketch

The conditions that were kept constant were the size of the reaction vessel (250 ml), the speed of the stirrer (600 rpm) and the volume of the reaction mixture (250 ml). This reaction was undertaken at a lower temperature to other papers, to give greater control over the reaction to stop insoluble gels forming. This was where water fills the interstitial sites in the crosslinked polymer (Osada, Ping Gong & Tanaka, 2004). The authors also investigated the effects of altering the ratio of BCD to EPH. They found that any ratio below 10:1 made water soluble polymers however this resulted in a 60-hr reaction time. For the ratios above 10:1, water soluble polymers could be obtained at a far lower yield by stopping the reaction with acetone before gelation. This took considerably less time at 3hr 50 – 4hr 40.

Osada, Ping Gong & Tanaka (2004) discovered that the optimal ratio was 5:1, as this produced the highest yield of 86% but had a 60-hr reaction time. The effect of temperature was also measured. It largely had very little effect except at the highest temperature of 90^{o}C where the resulting polymer had a higher BCD-EPH ratio. The NaOH concentration however drastically affected the polymer formation. They used a ratio of 10:1 βCD/EPH for these experiments. They found that using 50 % or above NaOH in the NaOH/water mix instantly precipitated the βCD so the polymerisation reaction couldn't take place. The lower concentrations produced water soluble polymers at much faster rates, however, at 10 % NaOH in the NaOH/water mixture

the reaction was restricted to water soluble polymers only so the yield was much larger, this came with a 60-hr reaction time. In the Ncube *et al* paper they used 10% NaOH to expose the alkoxide sites. This was an efficient choice as the 10 % concentration restricts the reaction to only synthesising water soluble polymers (Renard *et al.*, 1997). This meant that there was no need for careful observation to stop the reaction before a hard insoluble gel was formed. This concentration also led to a much higher yield. The BCD-EPH ratio used was 15:1. This reduced the reaction time to about 4 hours and due to the 10% NaOH concentration still resulted in water soluble polymers, the importance of this ratio was also shown in the paper by Zohrehvand and Evans (Zohrehvand & Evans, 2005). The method for the addition of the azo-dye was shown in the earlier paper by Ncube, Krause & Mamba (2011).

In an earlier article Ncube, Krause & Mamba (2011) synthesised an azo dye modified βCD-ethylene glycol polymer. The synthesis of the azo-dye modified polymer was the same as in the later works of Ncube *et al*, it's the diazo coupling of 4-hydroxyaniline with 2-napthol in DMF under a nitrogen atmosphere. For the actual coupling they functionalised the OH group on the βCD using the improved tosylation reaction proposed in Zhong, Byun & Bittman (1998) and (Tripodo *et al.*, 2013). The polymerisation steps were also very similar except that they use ethylene glycol ditosylate and dibutylin laurate catalyst to make an ethylene glycol linkage. The further paper published could have used epichlorohydrin to reduce the amount of compounds needed in the synthesis to make the process more sustainable and potentially easier to mass produce.

In terms of disinfection by-products Nakashima *et al* synthesised two variations of amino-β-cyclodextrin (amino-β-CDx) with a napthoamide group at two different positions on the molecule (Nakashima *et al.*, 2001; Takenaka, Higashi & Yoshida, 2002). The structures of 1 and 2 were shown in Figure 3.

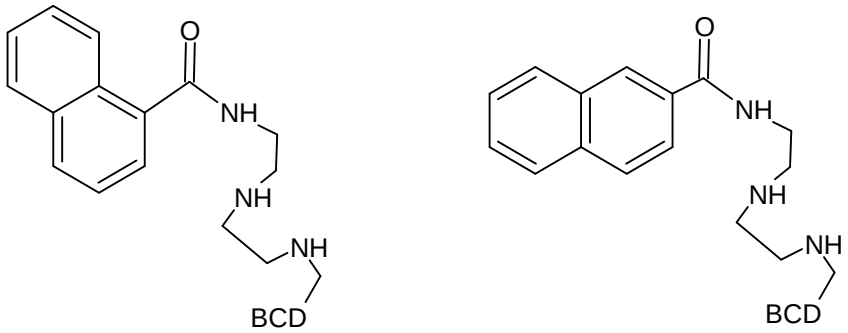

Figure 3: Monomeric βCD variants synthesised in (Nakashima *et al.*, 2001) drawn in Chemsketch

Firstly, they synthesised the monotosylated β-CD by the reaction of βCD with toluene-p-sulfonyl chloride in pyridine at room temperature for an hour and a half. This method was very old and produced a yield of 17 %, however newer methods produced a yield of 61% (Zhong, Byun & Bittman, 1998). In the paper by Ncube et al they followed the method set out in Zhong, Byun & Bittman (1998) for the functionalisation of the βCD. This used toluenesulphonic anhydride and NaOH instead which produced a much higher yield. This converted the alcohol into a tosyl group which took part in reactions. The monotosylated βCD was then dissolved in

diethylenetriamine and heated. This product (βCDxdien) was then used as a precursor to attach the fluorescent napthol moiety to. This was done by coupling 1 and 2 napthoic acid using a well-established method for creating amide bonds. This method was the N,N'-dicyclohexylcarbodiimide method. This involved the coupling of a free carboxyl group and a free amino group (Sheehan & Hess, 1955).

Temperature studies

Research by Nakashima *et al.* (2001) and Takenaka, Higashi & Yoshida (2002) focused less on the sensing properties and more on the structure of the compounds. As a result of this they investigated the effect of temperature on the fluorescence intensity. They found that for both of the structures synthesised, the fluorescence increased as the temperature decreased. They then analysed the first structure using induced circular dichroism spectroscopy and found that when the temperature was lower, the napthol moiety sat closer to the βCD structure which increased its fluorescence capability as it was closer to the guest compound, so the electron transfer was more favourable. However, the second structure in (Nakashima *et al.*, 2001; Takenaka, Higashi & Yoshida, 2002) had much less steric hindrance so upon decreasing the temperature to a point, the napthol moiety included itself in the βCD cavity. This structure was very similar to the compound made in Ncube, Krause & Mamba (2014), suggesting that this self-inclusion process could be happening there. In each of the Ncube *et al* papers, no temperature studies were undertaken, however they seem to play a large role in the fluorescence intensity of a compound. This could be due to the main goal of their papers which was to create a simple and cheap testing method for DBPs, so optimal temperature was not a concern.

Solvent effects

Nakashima *et al*. (2021) found that the level of inclusion could be controlled by changing the solvent used. With increasing concentrations of organic solvents like DMSO the structure was shifted to the high temperature version, stopping the inclusion process. Interestingly in direct opposition to the paper by Nakashima *et al* (2001), Ncube, Krause & Mamba (2011) found that DMSO, was the optimal solvent for their βCD polymer. They even found that in DMF, acetonitrile and water the fluorescence was blue-shifted which would affect any compound identification as the emitted wavelength would be different. They didn't have an explanation for this but they theorised that it was due to DMF and DMSO having bulkier methyl groups. It was then inferred that these bulkier methyl groups made the molecule more sterically hindered so it was less likely to compete with the guest molecule for the inclusion site. When determining the analyte however the polymer was dissolved in water. This was the same for Ncube *et al* 's future work (Ncube, Krause & Mamba, 2014) where no solvent studies were undertaken but water was used as the solvent for the analysis of the unknowns. This again could be due to the planned use of the sensor being in the field.

pH effects

Ncube, Krause & Mamba (2011) found that the fluorescence intensity of the monomeric azo-dye modified βCD decreased rapidly as pH increased past neutral. However, in the polymerised version the fluorescence intensity remained unchanged through this pH change. This suggested that the polymer protected the dye from being affected by pH. This was important because it showed that guest molecules in

potentially alkaline solutions would still be measured effectively, it also showed that the dye moiety was self-included in neutral conditions and lied outside the cavity in alkaline conditions. This was hard to conclude as the figure used to illustrate this was very poor quality. A similar observation was made in Nakashima *et al* (2001) and Takenaka, Higashi & Yoshida (2002). They noticed that the pH affected the inclusion process but as their βCD derivative was unpolymerised, the change in pH radically changed the fluorescence intensity due to the protolytic equilibrium of the protons on the amine groups in their dye moiety.

Sensing and fluorescence measurements

In the paper by Ncube, Krause & Mamba (2014) chloroform, dichloromethane, 1,2-dichloroethane, 1,2-dichloropropane and 1,2-dichloropropane were investigated. The resulting plot showed that chloroform had a huge quenching effect, particularly at 429 nm, compared to the other analytes, with chloroform quenching the fluorescence by approximately 35%, whereas the other analytes quenched the fluorescence by about 5%. A sensing factor was then calculated to give a quantitative measure of the polymers ability to detect chloroform. This was found to be 0.35 which was 10x higher than the sensing factor for the next molecule (1,3-dichloropropane) 0.035. An interference study was also performed. This found that even in the presence of other pollutants including chlorinated pollutants, the sensor was still highly specific to chloroform.

In the older paper by Ncube, Krause & Mamba (2011) the βCD polymer was investigated to evaluate its sensing properties towards phenol and chlorophenols. Sensitivity factors were then calculated using $\Delta I = I_0 - I$ and $SF = \Delta I / I_0$ (Becuwe *et al.*, 2008). This found that the polymer had the highest sensitivity towards 2,4-dichlorophenol, with a sensing factor of 0.35. This was identical to the sensing factor obtained from the analysis of chloroform with the βCD polymer synthesised in Ncube, Krause & Mamba (2014). They also carried out titration experiments and made a plot of concentration of 2,4-DCP against ∆I. This found that as the concentration increased the change in fluorescence intensity also increased initially. This then began to level off as the amount of free βCD sites decreased. This experiment wasn't repeated in either of the other articles but the same relationship would be shown in each case as it's a standard relationship for anything with an active site. In general for this type of sensor the fluorescence was quenched when the guest molecule binds, pushing out the self-included dye molecule thus quenching the fluorescence (Liu, Shi & Guo, 2007). The same thing was happening in this case.

In the paper by Nakashima *et al* (2001) both structures were found to be able to sense anions. However, Cl^- didn't produce a large enough change in fluorescence to be measured. Structure 1 was able to sense ClO_4^- though. This could be because it was less sterically hindered so the large chloride ion was able to be included. Interestingly this caused an enhancement of the fluorescence instead of quenching. However, the selectivity was likely low due to the fact that the sensor responded to other anions as well. There were no selectivity studies in this paper. However, in a future paper they did undertake sensitivity studies for a different guest molecule (Nakashima & Yoshida, 2006).

Colourimetry

Method

Shariati-Rad & Fattahi (2020) created a new method using a purge and trap apparatus with a reaction that created a green compound when exposed to chloroform. The apparatus used was shown in the paper by (Shariati-Rad & Fattahi, 2020). The method used was very crude, with the “bubble making device” being an aquarium filter product bought from a pet shop (Shariati-Rad & Fattahi, 2020). The system was also entirely homemade. Firstly, air was bubbled through a water sample, removing the volatile chloroform from solution and taking it into the trap. Inside the trap was a disk of filter paper that was soaked in resorcinol and NaOH. A reaction then occurred forming a green compound. The reactions were shown in Figure 4 and 5.

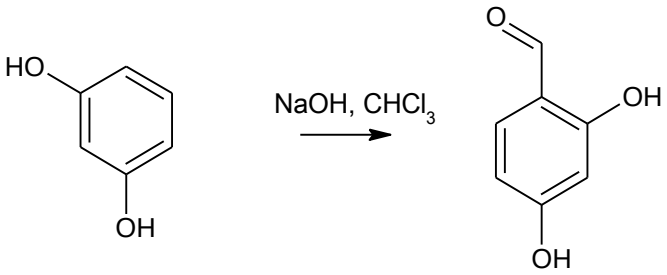

Figure 4: Reimer-Tiemann reaction (Shariati-Rad & Fattahi, 2020) drawn in Chemsketch

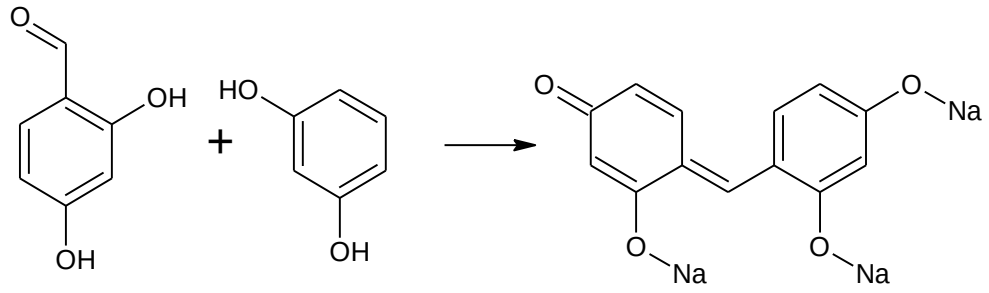

Figure 5: Seliwanoff reaction (Shariati-Rad & Fattahi, 2020) drawn in Chemsketch

Firstly, the chloroform reacted with the resorcinol under basic conditions to form 2,4-dihydroxybenzaldehyde (Figure 4). This was known as a Reimer-Tiemann reaction (Wynberg, 1960). This then reacted with another molecule of resorcinol forming the green dye (Figure 5). This was known as a Seliwanoff reaction and was most commonly seen in the Seliwanoff test for carbohydrates (Sánchez-Viesca & Gómez, 2018).

Fong *et al* (2015) used a slightly different methodology, the authors used a modified Fujiwara reaction, encapsulated in an ethyl cellulose gel and spread into a film on a thin glass sheet. They did this by dissolving ethyl cellulose in toluene and ethanol until a gel was formed. Solid 2,2-dipyridyl was then added. This was used as a less harmful alternative to pyridine, and removed the risk from the vapour (Rodman *et al.*,

2005). Liquid tetra-n-butyl ammonium hydroxide (TBAH) was then added. This was a stronger base than NaOH and removed the need for an aqueous phase (Rodman *et al.*, 2005). This was then smeared onto a glass microscope slide with a pipette tip and left to dry. An extra layer of reagents was then added and left to dry further (Fong *et al.*, 2015). The scheme for this reaction was shown in (Kofron, Kirby & Hauser, 1963; Okumura, Kawada & Uno, 1982)

Firstly, the strong base (TBAH) reacted with the halogenated hydrocarbon forming a trihalogenated anion. This then lost a halide ion forming a carbene, which reacted with the dipyridine forming a red product (Kofron, Kirby & Hauser, 1963; Okumura, Kawada & Uno, 1982).

Luo *et al* (2007), created a spectroscopic method to determine CCl_4 based on the decolourisation of methyl orange dye (MO). In their previous works they found that CCl_4 enhanced the ultrasonic oxidative decolourisation of MO (Wang *et al.*, 2007). The reactions for this were shown in (Wang *et al.*, 2007). Firstly, the ultrasound pyrolised the water, forming an OH radical and a hydrogen radical. Two OH radicals then reacted together forming hydrogen peroxide or oxidised and degraded an organic substrate (MO) (Luo *et al.*, 2007). With the addition of CCl_4 the speed of the oxidation of the MO was increased. The first reason for this was that the CCl_4 scavenged the hydrogen radicals (Zheng, Maurin & Tarr, 2005). This would push the position of equilibrium to the right, leading to more OH radicals being formed. The second reason for this was that the ultrasound broke down the CCl_4 into various oxidising species like HClO, the Cl radical, the CCl_3 radical and the CCl_2 diradical (Francony, 1996; Hua & Hoffmann, 1996; Luo *et al.*, 2007; Martire *et al.*, 2001; Rajan, Kumar & Gandhi, 1998; Wang *et al.*, 2007). Using this theory, the authors developed a spectrophotometric method to analyse CCl_4. A series of calibration standards were produced and the samples were analysed using UV-VIS. A calibration curve of rate of decolourisation against chloroform concentration was then plotted.

In the paper by Wujcik *et al* (2016), the authors used a modified Fujiwara reaction on top of an electrospun polymer. Electrospinning was the process in which a droplet of polymer solution was charged, leading to a Taylor cone effect, causing the repulsion to form the droplet into a stream, ejecting it from the vessel (Zheng, 2019), this was then collected on a plate. Specific and regulated pores can be made by altering the conditions (Chen *et al.*, 2012; Sharma *et al.*, 2015). Firstly, a solution of 4 wt% syndiotactic polypropylene (sPP) was mixed with an 8:1:1 cyclohexane/acetone/DMF solution and heated at 70 ^{o}C overnight. This was then electrospun onto aluminium foil and left to dry overnight. The parameters used for the electrospinning were shown in (Wujcik *et al.*, 2016). These were then left overnight to dry and the resulting 0.02 ± 0.006 cm thickness membranes were cut to a cross sectional area of 0.25 cm^2. This was adapted from previous works by Viswanadam & Chase (2012). The solution for analysis was then placed into a scintillation vial. The membrane holder and membrane were then placed on top. This was then heated to between 60 – 90 ^{o}C to allow the THMs to leave the solution and enter the headspace above. Pyridine and an ethanol solution saturated with NaOH were added on top of the membrane. The THMs then diffused through the membrane, leaving any water vapour still in the headspace, further preconcentrating the analytes. The THMs then reacted with the Fujiwara reagents, forming a red colour.

In the newer paper by Fattahi & Shariati-Rad (2020), a cotton pad based modified Fujiwara sensor was used to quantify chloroform. This was the simplest method studied, however it produced some of the best results. Firstly, 1500 ml of the sample was added to a 2000 ml volumetric flask, these measurements were chosen to optimise the headspace so that the chloroform was funnelled to the sensor. A cotton pad was then impregnated with 1 ml of 4 M NaOH and 1 ml of pyridine. This was then placed under the flask stopper on top of the volumetric flask and left for 40 mins. The Red Green Blue (RGB) values were then extracted using exactly the same method as the previous paper by the same authors (Shariati-Rad & Fattahi, 2020). The mechanism used for this reaction was shown in (Fattahi & Shariati-Rad, 2020).

Results

In the earlier paper by Shariati-Rad & Fattahi (2020) no analytical instrument was used. Once the coloured compound had formed a photo was taken on a Samsung Galaxy J7 phone and the image was fed into a piece of software called GetData Graph Digitizer that extracted the RGB values (Shariati-Rad & Fattahi, 2020). RGB values were numerical values of colour, used by computers and were a measure of the amount of coloured bits (Sullivan *et al.*, 2012). In the paper the authors then plotted a calibration curve of concentration against RGB, with a lower RGB value representing a darker colour and therefore higher concentration of chloroform (Shariati-Rad & Fattahi, 2020). The conditions were then optimised using this. The purging time was optimised at 40 min and the concentration of Resorcinol and NaOH were optimised at 1.8 M and 3.6 M respectively. The results and calibration curve were shown in (Shariati-Rad & Fattahi, 2020). The limit of detection values (LOD) (mg/L) were shown in Table 1. This was 0.007 mg/L. This was considerably lower than the WHO chloroform guideline in drinking water of 0.3 mg/L (WHO, 2008) and the USEPA maximum acceptable limit for TTHM of 0.08 mg/L (USEPA, 2021). This method could be made far simpler and user-friendly by conducting many experiments to produce a colour chart, linking the colour of the filter paper to an approximate concentration range. However, the method is very easy to use even in its current form.

In the paper by Fong *et al* (2015), the colour of the sensor after reacting with chloroform was measured using UV-VIS spectroscopy. They found that the LOD was 5 mg/L (Fong *et al.*, 2015). This was not suitable for the analysis of drinking water as that required a limit of detection of around 0.3 mg/L (WHO, 2008). This was due to the fact that this sensor was optimised for detecting residual chloroform in pharmaceuticals, which had a maximum acceptable limit of 60 mg/L (European medicines agency, 2019). The reason this sensor was included in this study was because of the potential of a solid test strip-like sensor that could be manufactured and used to detect chloroform with the aid of a colour chart. If this method could be adapted and altered to suit the measurement of chloroform in water, it would be a highly valuable asset, especially with the use of compact UV-VIS spectrophotometers. In a similar paper to this the authors used solid 2,2-dipyridyl and TBAH in liquid form, without encapsulation (Rodman *et al.*, 2005). This was far more efficient with an LOD of 0.17 mg/L (Table 1) for chloroform and 0.50 mg/L for CCl_4 (Table 1). This method used UV-VIS spectrophotometry, meaning it was far more in-depth than the Fong *et al* sensor (2015).

In Luo *et al* (2007), UV-VIS spectrophotometry was used to measure the decolourisation rate of MO. The LOD for this technique was 0.19 mg/L (Table 1) and the linear range was 0.4 – 20 mg/L (Luo *et al.*, 2007). The LOD was lower than the solid Fujiwara sensor which had an LOD of 5 mg/L (Table 1) (Fong *et al.*, 2015) but was higher than the LOD for the resorcinol sensor which was 0.007 mg/L (Table 1) (Shariati-Rad & Fattahi, 2020). The Luo *et al* method was also more complex. The linear range was also far bigger on this technique (Luo *et al.*, 2007). This method was used to detect CCl_4 however it could be modified to detect chloroform as well. It was stated in the paper that the authors planned to adapt this method to detect TTHM but this has yet to happen at the time of writing.

Wujcik *et al* (2016) used preconcentration. This was similar to the paper by Shariati-Rad & Fattahi (2020), except in this case, a twofold preconcentration step was employed. This meant that the LOD for TTHM was far lower than the majority of the techniques researched at 8 µg/L (Table 1) (Wujcik *et al.*, 2016). Another reason for this low LOD was that the membrane, at the temperatures employed, blocked water vapour which can interfere with the Fujiwara reaction. There was no analytical instrument employed. Similar to the purge-trap Fujiwara sensor (Shariati-Rad & Fattahi, 2020), a picture was taken and the RGB values were extracted and used as the analytical signal (Wujcik *et al.*, 2016). However, in this case it would be very difficult to make an easy-to-use colour chart, as the concentrations measured were very low and the colour changes weren't easily resolved by eye. The linear range for this sensor was also far better than the other methods studied. The linear range was 8 – 250 µg/L (Wujcik *et al.*, 2016). This meant that this sensor was able to detect in the range of both of the limits used in this study of 0.08 mg/L (USEPA, 2021) and 0.3 mg/L (WHO, 2008).

In the latter paper by Fattahi & Shariati-Rad (2020), the authors used a cotton pad-based sensor to determine chloroform in water. The calibration curve and results from this were shown in Fattahi & Shariati-Rad (2020). The LOD obtained for this method was 0.0083 mg/L (Table 1) (Fattahi & Shariati-Rad, 2020). This was very similar to the method by Wujcik *et al* (0.008 mg/L) (Wujcik *et al.*, 2016). This meant that this sensor was able to detect chloroform in the range of both of the limits studied 0.08 mg/L (USEPA, 2021) and 0.3 mg/L (WHO, 2008), as its linear range was 0.0087 – 1.5200 mg/L (Fattahi & Shariati-Rad, 2020). This made this sensor highly versatile and available for use as a standalone technique, without the use of another technique to increase the confidence in the measurement. Although the use of the RGB values as an analytical response used a simple method, it could be made easier with a colour chart. In this case the differences in colour were quite clearly defined so a colour chart would represent a modification that could be employed to increase the ease of use of this technique. In terms of the sensor itself, it was by far the easiest to use out of all the methods studied, with the cotton pad being easily prepared in-situ.

Table 1: A comparison of the reported limits of detection (mg/L) in the determination of both Chloroform/TTHM and Carbon tetrachloride using various colorimetry methods by author

Limit of detection (LOD) (mg/L)		
Chloroform/TTHM	**Carbon tetrachloride**	**Citation**
0.070	N/A	Shariati-Rad & Fattahi (2020)
5.0	N/A	Fong *et al.* (2015)
0.17	0.5	Rodman *et al.* (2005)
N/A	0.19	Luo *et al.* (2007)
0.0080	N/A	Wujcik *et al.* (2016)
0.0083	N/A	Fattahi & Shariati-Rad (2020)

GC section

Method

In the paper by Allonier *et al* (2000), the authors adapted a purge and trap gas chromatography electron capture detector (GC-ECD) method, for the analysis of chlorinated compounds in sea water. The majority of this method was adapted from a USEPA report which contained generic methods for analysing purgeable organic compounds (USEPA, 1992). For the purge and trap system the Hewlett-Packard 7695 model purge and trap concentrator was used. This model has since been discontinued. The trap consisted of a 30.5 cm x 0.312 cm column, packed with Tenax GC, silica gel and activated carbon (USEPA, 1992). This was directly coupled to the GC instrument using a transfer line and a split valve with a ratio of 1:5. Experiments were then undertaken to compare the purge and trap method with direct injection. They found that the purge and trap method was far more efficient, the optimised parameters were shown in (Allonier *et al.*, 2000; USEPA, 1992). One purge was undertaken per sample and between samples the trap was heated to 220 ^{o}C for 10 mins to remove any traces of the previous sample. The gas chromatography was performed using a HP 6890 gas chromatograph with Ni^{63} electron capture detector (ECD), the optimised parameters were shown in (Allonier *et al.*, 2000).

Cho, Kong & Oh (2003), used a headspace – SPME -GC-ECD method to analyse THM's in water. They used HS-SPME as an alternative to purge and trap. This involved placing a 85 µm carboxen/polydimethylsiloxane (85-CAR/PDMS) fibre into a syringe and suspending it in the headspace of a solution. The sample was then heated to 35 ^{o}C for 30 min and immediately after the fibre was retracted and injected into the instrument. The analytes were then desorbed at 250 ^{o}C for 4 min and analysed. The conditions used for the GC-ECD analysis were shown in (Cho, Kong & Oh, 2003).

Gonzalez-Hernandez *et al* (2017), used a HS-SPME-GC-FID method to analyse THM'S in desalinated waters. The method used was similar to the method by Cho, Kong & Oh (2003) except far more current. The fibre was exposed to the solution for 60 min at 30 ^{o}C and the desorption step occurred over 2 min instead of 4 min. The conditions for the GC-FID analysis were shown in (Gonzalez-Hernandez *et al.*, 2017).

The paper by Cuthbertson *et al* (2020) was one of the most advanced methods in the literature for the GC-MS analysis of THM's in drinking water at the time of writing. This method was very detailed, and aimed to create a few methods to analyse 61 different disinfection by-products. For the analysis of THMs the authors used a liquid – liquid extraction paired with a single quadrupole GC-MS instrument. For the liquid – liquid extraction 5 ml of methyl tert-butyl ether (MTBE) was added to 100 ml of sample along with 1 ml of concentrated sulfuric acid to bring the pH to below 1. The conditions for the GC-MS analysis were shown in (Cuthbertson *et al.*, 2020)

Results

In the paper by Allonier *et al* (2000) a purge and trap GC-ECD method was used to detect trihalomethanes. The LOD and LOQ values obtained were far superior to any of the βCD or colourimetry techniques studied. The values obtained were also far below the limits used in this study of 0.08 mg/L (USEPA, 2021) and 0.3 mg/L (WHO, 2008). However, no data regarding the linear range was given meaning that this method may be not fit for purpose. The initial USEPA document that inspired this article was frequently used by companies to promote their products. For example, in the application notes for the purge and trap apparatus from Agilent (Agilent technologies, 2021). In the application notes by Nutter, showing the use of the Atomx XYZ's moisture control system, they found that the water vapour removal was improved by 60 %, reducing peak interference and increasing the lifespan of the column (Nutter, 2018). However, the LOD values obtained were considerably higher than values in the paper by Allonier et al. The values obtained were shown in Table 2. The LOD values obtained were still fit for purpose, but again no detail of the linear range was given. This did show that the work of Allonier et al could be modernised with new technology and experiments could be undertaken to determine the linear range.

The paper by Chen & Her (2001) used a cryofocusing injector. This improved the peak resolution and narrowed the peaks obtained. A cryofocusing injector could be employed in the method by Allonier *et al* to account for any changes in peak resolution or LOD when switching out discontinued components for more modern versions. The LOD values obtained for this method were shown in Table 2. The LOD values obtained were considerably lower than the USEPA maximum acceptable limit for TTHM in water (0.08 mg/L) (USEPA, 2021) and the WHO guideline value of 0.3 mg/L (WHO, 2008). No detail of the linear range was given and it was very hard to find any information on this, as the instrument has been discontinued, similar to the method by Allonier *et al* (2000). In this case the instrument could be replaced with a new Shimadzu system like the GCMS-TQ8050 NX instrument (Shimadzu, 2021) however there are cheaper options. The range this method used for calibration was between 0.000196 – 0.025 mg/L, which was not fit for purpose. The LOD values obtained were only slightly better than the method by Allonier *et al* (2000). The approximate cost of a GC-MS instrument was £75,000 on top of the cost of a GC system (Baker, 2021). In comparison, an approximate price for a GC-ECD system was £30,000 (Baker, 2021). The difference in price was incredibly large for such a small change in LOD, especially when the LOD values in question were both fit for purpose.

In the paper by Cho, Kong & Oh (2003), the authors used a HS-SPME-GC-ECD technique for the analysis of THM's. The LOD values obtained were shown in Table

2. The LOD values obtained were far lower than the limits used in this study of 0.08 mg/L (USEPA, 2021) and 0.3 mg/L (WHO, 2008). The working range of this method was between 0.00005 – 0.08 mg/L, with a loss of linearity occurring after this point due to saturation of the fibre (Cho, Kong & Oh, 2003). This meant that the method would not be able to provide confident results for the concentration, above the USEPA limit. The conditions used for the HS-SPME step were identical to the optimal conditions found in the paper by González – Aguirre *et al* (2011).

In the paper by González-Hernández *et al* (2017), the authors used a similar method to Cho *et al* (Cho, Kong & Oh, 2003) to analyse THM's in desalinated water. The LOD and LOQ values obtained were shown in Table 2. The LOD and LOQ values obtained were lower than the limits used in this study of 0.08 mg/L (USEPA, 2021) and 0.3 mg/L (WHO, 2008). However, the values obtained were higher than that of the values obtained in the paper by Cho, Kong & Oh (2003). This was probably due to the lower selectivity of the FID detector compared to the ECD detector (Richardson, 2002).

In the method by Cuthbertson *et al* (2020) they used LLE GC-MS to analyse 61 different disinfection byproducts. They compared LLE to SPE for the extraction method, they used LLE as it was cheaper, less wasteful and produced the same relative recoveries. No LOD values were calculated but LOQ's were calculated, these were shown in Table 2. The LOQ values obtained were far below the limits used in this study of 0.08 mg/L (USEPA, 2021) and 0.3 mg/L (WHO, 2008). However, the LOQ values obtained were very similar to the LOQ values obtained in the method by Allonier *et al* (2000) and similar to the LOD values for all of the other GC methods studied. This suggests that not only is mass spectrometry not necessary for this analysis but this whole method was over-engineered and over-expensive.

The most modern and advanced method for the determination of disinfection byproducts was by Ortega – Hernandez *et al* (2021). They analysed chlorinated by-products containing nitrogen instead of THM's but their method was almost identical to the method by Cuthbertson *et al* (2020). The main difference between the two methods was the use of tandem mass spectrometry in the form of an Agilent 7000C triple quadrupole mass spectrometer. This provided far lower LOQ values which were still in the ng/L range. Explicit detail was given about the dynamic range of this method, this was three orders of magnitude (Ortega-Hernandez *et al.*, 2021). This means that the analysis would be fit for purpose to be able to detect chlorination by-products at the µg/L and the mg/L level.

Table 2: A comparison of the reported limits of detection/quantification (mg/L) in the determination of the four main DBP's using various GC methods by author

Limit of detection/quantification (LOD/LOQ) (mg/L)				
Chloroform	**Bromodichloromethane**	**Dibromochloromethane**	**Bromoform**	**Citation**
0.00005	0.00002	0.00002	0.00007	Allonier *et al.* (2000)
0.0001 *	0.00005 *	0.00005 *	0.00015 *	Allonier *et al.* (2000)
0.00015	0.00012	0.00007	0.00011	Nutter (2018)
0.00002	0.00006	0.0001	0.00012	Chen & Her (2001)
0.00001	0.000005	0.000005	0.00001	Cho, Kong & Oh (2003)
0.0116	0.0091	0.001	0.00228	Gonzalez-Hernandez *et al.* (2017)
0.0169 *	0.0118 *	0.00332 *	0.00303 *	Gonzalez-Hernandez *et al.* (2017)
0.00005 *	0.00005 *	0.00005 *	0.00005 *	Cuthbertson *et al.* (2020)

* LOQ value

Comparison of in-situ techniques

Although the UN would suggest that target 7C of the MDG's was met, the literature and data would suggest otherwise (Satterthwaite, 2016; Weststrate *et al.*, 2019). Target 7C specified that by 2015 the proportion of people without sustainable access to safe drinking water piped on premises should be halved. However, in 2010 the UN declared that target 7C had been met 5 years ahead of schedule. This was not true as the UN statistics used measured the amount of people with access to "improved" sources. The joint monitoring programme definition for "improved" spans from water piped directly into the premise to communal taps and rainwater collection. This definition also included no mention of the sustainability of the water supply or the water quality. The 2013 UN MDG report stated that "concerns about the quality and safety of many improved drinking water sources persist. As a result, the number of people without access to safe drinking water may be two to three times higher than official estimates" (The United Nations, 2013), this meant that target 7C was not met. The report also stated that 2.4 billion people with the new "improved" water systems, don't have water piped into their houses and may need to queue for water, carry heavy loads home or pay large amounts of money, all for water of very poor quality (The United Nations, 2013). This was a huge failing and highlighted the need for a simple, easy to use sensor for the determination of chloroform in particular but also other THMs. Target 7C was mostly aimed at preventing water borne diseases, but THM concentration was also a factor in water quality and with high variability in how water was treated across developing countries, it's important to be able to quantify these in areas where access to complex instrumentation or labs is scarce. In Africa for example, conventional water treatment was rare and they relied on on-site water treatment such as pit latrines, septic tanks and rain water harvesting (Wang *et al.*, 2014)

In the 2014 paper by Ncube *et al*, the authors synthesised an azo-dye modified βCD-EPH copolymer for the detection of chloroform (Ncube, Krause & Mamba, 2014). This was the main molecular sensor used for this purpose. The preparation of this sensor was far more intensive than the majority of other in-situ techniques studied. This was due to the synthesis of the modified copolymer. No explicit detail was given as to the shelf life of the polymer but it did not have to be used immediately (Ncube, Krause & Mamba, 2014) meaning that it could be produced the day before use, or with further study could be produced in bulk and used for weeks afterwards. The use of this sensor was also more complex than other in-situ sensors. It required the use of a fluorescence spectrometer and a calibration curve to be able to quantify chloroform (Ncube, Krause & Mamba, 2014). The calibration curve only needs to be produced once, as the concentration range of chloroform in drinking water should be the same, as the value is compared against a guideline value. Compact fluorescence spectrometers for use in the field can also be purchased, however they are expensive, especially when compared against some of the other in-situ techniques which don't use any instrumentation. StellarNet sell a compact spectrofluorometer for £4630 (StellarNet, 2021). This method was less versatile than other methods as it was only able to detect concentrations in the range of the WHO guideline value of 0.3 mg/L (WHO, 2008), with no detail given as to the LOD or linear range.

In terms of colourimetry techniques, three of the techniques studied stood out as potential methods for use in the field in developing countries. The first of these was

the earlier method by Shariati-Rad & Fattahi (2020). The authors used a homemade set up for the purge and trap analysis. This apparatus would be easily reproduced with the most expensive item being the 2 L volumetric flask which for example would cost £18.77 (Cole-Parmer, 2021). The actual sensor portion of this method would be very easily produced in-situ by soaking a piece of filter paper in 1 ml of a mixture of resorcinol and NaOH (Shariati-Rad & Fattahi, 2020). This can be performed at the place of measurement very easily. This method required no instrumentation and used a smart phone camera and a piece of free computer software to generate an analytical signal. The phone used was a Samsung galaxy J7 phone with a 12 MP camera (Shariati-Rad & Fattahi, 2020), any phone with a camera that is of similar or better quality would work in its place. However, with further study a colour chart could be produced that would link colour to concentration and remove the need for the camera or software. The LOD and linear range of this technique also allowed the measurement of chloroform in the range of the USEPA maximum acceptable limit for TTHM (0.08 mg/L) (USEPA, 2021) and the WHO guideline value (0.3 mg/L) (WHO, 2008). The LOD was 0.007 mg/L and the linear range was between 0.011 – 1.192 mg/L (Shariati-Rad & Fattahi, 2020)

The second method was by Wujcik *et al* (2016). This was the most complicated and expensive colourimetry method of the three potential methods which was due to the synthesis of the electrospun polymer. The first issue was that electrospinning instrumentation would be required, this would cost approximately £8000 (Spinbox electrospinning by Bioinicia, 2021). However, the biggest issue would be obtaining the syndiotactic polypropylene as in the paper the authors purchased it from Sigma Aldrich but it has since been discontinued (Sigma Aldrich, 2021a). After searching for an alternate place to purchase the polymer, no other options were found. It would be possible to synthesise the polymer as shown in the method by Miller and Bercaw but the synthesis was complicated (Miller & Bercaw, 2004). This method was able to analyse all four of the main THM's in water, this was the only in-situ technique studied that was able to do this.

The third technique that stood out was the latter paper by Fattahi & Shariati-Rad (2020). In this paper the authors developed an incredibly simple Fujiwara method to determine chloroform in drinking water. The method consisted of a cotton pad soaked in the reagents needed for a Fujiwara reaction, placed in the lid of a 2 L volumetric flask. The reagents used in this method were pyridine, NaOH and DMSO all of which were far cheaper than any of the other methods studied. The cotton pad sensor would be easily prepared at the place of measurement by soaking the pad in the reagents. This technique, similar to the previous two, also used no instrumentation and generated an analytical signal by extracting RGB values. This would normally require a calibration curve but with enough experimentation a colour chart could be produced, removing the need for this. The LOD and linear range of this sensor was 0.00083 mg/L and between 0.00087 – 1.52 mg/L (Fattahi & Shariati-Rad, 2020). This was far lower than the limits used in this study. This method was the simple enough that a chemist with minimal training or with the aid of a colour chart a non-chemist would be able to produce accurate results at LOD values far below the limits needed to determine whether a water sample abides by the various limits associated with the amount of chloroform in drinking water at an exceptionally low price compared to other techniques. It would be the optimal in-situ technique for the analysis of chloroform in drinking water in developing countries.

In-situ techniques vs lab-based techniques

Of the GC methods studied again, three methods stood out as the most efficient methods to analyse DBPs. The first of these was the method by Cuthbertson *et al* (2020). The authors used an LLE-GC-MS method to detect and quantify 61 different DBP's. The use of LLE over SPME was a step in the right direction as it was cheaper. The LOQ values obtained from this method were far lower than the limits used in this study with the LOQ for chloroform being 0.00005 mg/L. This was an order of magnitude lower than the USEPA TTHM maximum acceptable limit (0.08 mg/L) (USEPA, 2021) and the WHO guideline value for chloroform (0.3 mg/L) (WHO, 2008). This was not necessary especially when considering the fact that a single quadrupole mass spectrometer was approximately an extra £75,000 on top of the cost of a GC instrument (Baker, 2021).

The second of these methods was the method by Ortega – Hernandez *et al* (2021). This was the most advanced method to analyse DBPs at the time of writing. They used a very similar method to Cuthbertson *et al* except that they used a triple quadrupole mass spectrometer. The LOD values obtained were lower than that of Cuthbertson *et al* but within the same order of magnitude. The approximate cost of a triple quadrupole mass spectrometer was £175,000 on top of an existing GC-MS system (Baker, 2021) as this was a tandem technique. This was incredibly expensive and very few facilities in developing countries would come equipped with these techniques. The third method that stood out was the method by González-Hernández *et al* (2017). The authors used a HS-SPME-GC-FID technique to analyse THMs in water. The LOD values obtained from this method were far higher than the previous two, however they were still fit for purpose and in the same order of magnitude as the USEPA limit for TTHM of 0.08 mg/L (USEPA, 2021). The LOD values obtained for chloroform were 0.0116 mg/L. This method was considerably cheaper than the previous two, as the GC-FID system cost approximately £20,000 – £25,000 with approximately £20,000 for the headspace apparatus (Baker, 2021) If a lab-based technique was to be used, this would be the optimal technique to use out of the techniques studied.

All of the GC techniques researched had LOD and LOQ values that were far lower than what was needed to analyse disinfection by-products in drinking water as the USEPA limit and the WHO guideline used for the most part were orders of magnitude higher than the LOD and LOQ values. Also, in all but the paper by Ortega-Hernandez *et al.* (2021), no explicit detail about the dynamic range was given, although the methods should be able to analyse at mg/L and µg/L with the right sample preparation. The most important factor in the comparison of lab-based techniques was cost and as a result availability. With the minimum cost of the instrumentation alone being £40,000 without factoring in the cost to employ an analyst, reagents or electricity.

In a lot of African countries there was a lack of laboratory facilities. This was in part due to low public R&D expenditure, weak links between industry and academia, low and falling enrolment in university for science and engineering and outdated policies and institutions. This paired with graduates taking better paid jobs in industrialised countries, led to a large deficit in laboratory facilities. In an article published in the RSC newsletter it explained that complex laboratory equipment was hard to come by in Africa due to a large chain of custody leading to a much higher cost. It also went

on to explain that there was a lack of installation engineers, maintenance engineers, analysts and several other operational concerns like a lack of spare parts, support from the manufacturers, basic consumable items and bottled gases (Royal Society of Chemistry, 2018). This was the reason a simple in-situ technique for the analysis of chloroform in particular was needed to try and remove the reliance on GC techniques for this analysis.

The method by Fattahi *et al* combined LOD values that were in some cases lower than the values obtained using GC methods, with very cheap materials and with very little experience needed to use the technique and generate accurate results for the amount of chloroform in drinking water (Fattahi & Shariati-Rad, 2020). This method could be used to provide quick and accurate results for the concentration of chloroform and thus a large indicator into the concentration of DBPs to help assess the water quality regardless of the water source. It could be used in any setting, whether that's in a house with water piped directly into it, or from a communal tap. If the other three major DBP's needed to be quantified the method by Wujcik *et al* would work if the polymer could be obtained. Alternatively, the Fattahi *et al* method could be employed to quantify chloroform, and as it is the most abundant DBP it already gives a large indication into the entire DBP content, but if that measure was near or greater than the guidelines a sample could then be sent off to a facility that has GC techniques so that all of the DBPs could be quantified. However, the average concentration of chloroform in water was between 2 – 44 µg/L in treated drinking water (CDC, 1997), so this would not be a likely occurrence.

Conclusions

The aim of this study was to research appropriate methods for the in-situ analysis of chloroform and DBP's as a whole and then assess the viability of these techniques by comparing them against the established methods. This was achieved and a variety of methods were identified and assessed. The βCD methods were ultimately found to be far less effective than the colourimetry methods studied, due to being more complicated and only being able to detect chloroform in the range of the WHO guideline value of 0.3 mg/L (WHO, 2008) and the majority of GC techniques studied were found to be over-expensive and over-complicated. The GC methods studied were narrowed down to a single method by González-Hernández *et al.* (2017), which was the cheapest of these techniques but was still fit for purpose and had an LOD of 0.0116 mg/L. Of the colourimetry methods studied the method by Fattahi *et al. (2020)* provided the best results with an LOD of 0.00083 mg/L while being incredibly easy to use and very inexpensive (Fattahi & Shariati-Rad, 2020). This method was more efficient than the majority of the GC methods and would be a very good choice for a new easy to use method to analyse chloroform in drinking water in developing countries.

Future work

There are a number of further studies that could be conducted to improve upon some of the methods researched. For example, experiments could be undertaken to create colour charts from the applicable methods shown in the discussion. This would enable those methods to potentially be used by chemists with minimal training or even non-chemists. Another piece of further work could be to investigate whether changing the camera quality or settings used in the techniques that required the use of RGB values affects the analytical signal.

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