QUANTITATIVE ANALYSIS OF DISINFECTION BY-PRODUCTS IN DRINKING WATER DISTRIBUTION NETWORK USING SPME AND GC

BY

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A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE DEGREE OF MASTER OF SCIENCE

IN

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CERTIFICATE

This dissertation submitted by **Ms. Romana Khan** is accepted in its present form, by the Institute of Environmental Sciences and Engineering (IESE), School of Civil and Environmental Engineering (SCEE), National University of Sciences and Technology (NUST), Islamabad as satisfying the requirement for the degree of Masters of Environmental Science.

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DEDICATED….!!!

To my family and friends

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LIST OF ABBREVIATIONS

2FI	Two-factor interaction
BDCM	Bromodichloromethane
CCl_4	Carbon tetrachloride
CDBPs	Chlorinated disinfection byproducts
$CHBrCl_2$	Dichlorobromomethane
$CHCl_2I$	Dichloroiodomethane
CHI_3	Iodoform
DBCM	Dibromochloromethane
DOC	Dissolved organic content
DoE	Design of experiments
DVB-CAR-PDMS	Divinlybenzene-carboxen-polydimethylsiloxane
GDP	Gross domestic product
HAAs	Haloacetic acids
HANs	Haloacetonitriles
HKs	Haloketones
HOCl	Hypochlorous acid
IARC	International agency for research on cancer
LOD	Limits of detection
LOF	Lact of fit
LOQ	Limit of quantification
MSD	Mass spectrometry detection
MTBE	Methyl tertiary butyl ether
NDMA	N-nitrosodimethylamine
NaOCl	Sodium hypochlorite
NHEXAS	National human exposure assessment survey
PTFE	Polytetrafluoroethylene
RF	Response factor

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ABSTRACT

Trihalomethanes (THMs) occurrence in the drinking water is a major concern in public health owing to their toxicological effects on health. These are formed during water disinfection process. This study aims at THMs monitoring in drinking water distribution network by gas chromatography with electron capture detector (GC-ECD) and TRB-1 column (30 m x 0.32 mm x 1 μm). The SPME fibre (75 μm CAR-PDMS) was found to be the most suitable for THMs extraction. A standard solution of each THM was prepared in methanol following EPA Method 551.1. Calibration of standards was carried out to obtain reproducible peaks and linear calibration curves. Response surface methodology and a central composite design (CCD) was employed for optimization of variables for THMs determination. The accuracy of the model was investigated by ANOVA. The results of RSM revealed that optimum conditions for THMs analysis were 30 min extraction time at 80 ^{o}C with addition of 3.25 g Na_2SO_4 salt and 8 min of desorption time. The optimized conditions were then used for quantification of THMs in water samples of NUST. The results achieved indicated presence of THMs in 90 % of drinking water samples collected after chlorination, with 30% sites exceeding the standard value of 80 μg/L. The most dominant THM recorded was dichlorobromomethane in almost 95 % of the samples. Iodoform was detected comparatively at low concentration (0.012 - 0.433 μg/L) in almost 45 % samples and in all the sites it was found within the threshold values (0.2 - 5 μg/L). It may be concluded that the HS-SPME technique has a great potential for the analysis of drinking water. These results show a strong link between concentration of UV_{254} absorbance and organic matter with THMs formation. The sites having high content of residual chlorine and UV_{254} exhibited comparatively larger peak signal for THMs. The potential reason for contamination at different points are due to natural organic matter and residual chlorine.

Chapter 1

INTRODUCTION

Drinking water is one of the important constituents of life support system. Management of water quality has been a key pillar of prevention and control of water-borne diseases (Moyo, 2004). Almost 50 % population in the developing countries are suffering from diseases associated with lack of clean drinking water (WHO, 2004).

Drinking water is disinfected with chlorine to inactivate the microorganisms to inhibit the spread of water borne diseases. Chlorine has been used as a preferred disinfecting agent as it is proved to be effective and relatively inexpensive (Rodriguez and Sérodes, 2001). Chlorination of water supplies began in the early 1900s and has significantly diminished the prevalence of water related diseases around the world, and thus considered as the main public health achievement of 20^{th} century. However, chlorine also combines with the natural organic matter (NOM) and other ions existing in water and produces a number of disinfection byproducts such as trihalomethanes, haloacetic acids (HAAs), haloacetonitriles (HANs) etc, with harmful long-term health effects (Calderon, 2000). The reaction of natural organic matter and chlorine can be stated as follows (WHO, 2004):

Organic matter + residual chlorine → THMs + HAAs + HANs + cyanogen-halides + other DBPs

In 1974, Rook first identified the regulated DBPs i.e. trihalomethanes (THMs) found in chlorinated drinking water (Rook, 1974). Besides regulated DBPs, there are hundreds, and thousands of compounds, which are produced from the reaction of chlorine with compounds present in the water. THMs and HAAs are the most predominant in chlorinated drinking water accounting for almost 25 % of the DBPs. Total THMs comprises of four chemicals; chloroform, bromoform, dibromochloromethane and bromodichloromethane which are frequently formed

after chlorination of water supplies. Chloroform tends to be present in the highest amount. In 1976, the U.S EPA issued the findings of a national survey that disclosed that chloroform and other THMs were abundant in chlorinated drinking water. Also in the same year, the U.S National Cancer Institute unveiled that chloroform is linked to cancer in laboratory animals. As a consequence, a significant public health issue was arised (Richardson *et al.,* 2008).

More than 600 DBPs have been recognized in drinking water to date, however only 11 DBPs (4 trihalomethanes, 5 haloacetic acids, bromate and chlorite) are currently regulated and most commonly found in chlorinated drinking water. Two major disinfectants such as, chlorine and chloramine produces substantial amounts of THMs. Brominated and chlorinated THMs are one of the most far reaching natural contaminants present in drinking water, however when iodide is available in water, iodinated THMs may also be produced (Allard *et al.,* 2012).

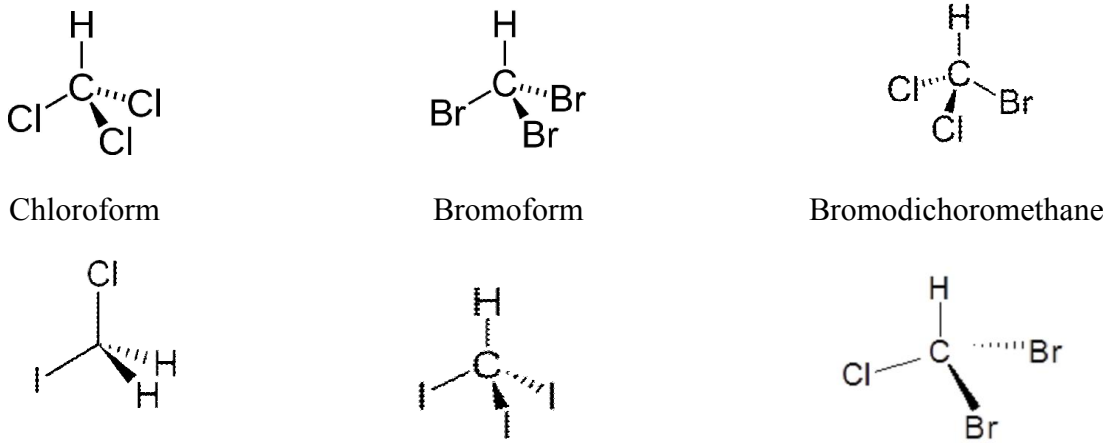

Figure 1.1: Structure of trihalomethanes (THMs)

Iodinated THMs are documented as toxicologically significant, particularly iodoform. The taste and odor threshold for iodoform ranged from 0.02 - 5 µg/L, and when surpassed may prompt organoleptic issues and consumer complaints (Allard *et al.,* 2012).

USEPA (2006) and WHO guidelines (2004) regulated trihalomethanes (THMs) and haloacetic acids (HAAs), keeping in view their potential health risk. In USEPA, (2006), sum of total THMs (i.e., chloroform, bromodichloromethane, dibromochloromethane, bromoform) is regulated at 80 µg/L and sum of five HAAs (i.e., mono-dichloro, mono-dibromo and trichloroacetic acid) is regulated at 60 µg/L. In the United Kingdom, only THMs are regulated (DWI, 1998) and a concentration of 100 µg/L at a consumer's tap has been set for TTHM. Since, organic substances are existing in drinking water and disinfection is a fundamental necessity to make the water potable, therefore, it is vital to have quality control measures for THMs prevention (Platikanov *et al.,* 2007).

Table 1.1: Guideline values for THMs of concern

THMs	Guideline values (µg/L)	
	WHO[a]	USEPA[b]
Chloroform	200	-
Bromodichloromethane	60	-
Dibromochloromethane	100	-
Bromoform	100	-
TTHMs	‡	80[c]

- Not included in regulations
‡ The sum of ratio of the concentration of each THM to its respective guideline value should not exceed 1, WHO (2004)

a. WHO, (2004)
b. USEPA, (2006)
c. 40CFR141.64 (2002)

A number of experimental designs have been employed for THMs optimization. Central composite design (CCD) with response surface modeling (RSM) are used commonly to study the effect of different variables effecting the desirable responses by changing them simultaneously. Such experimental design may reduce experiments runs as well as optimize the process for significant THMs extraction conditions (Guimarães *et al.,* 2008).

The Pakistan Council of Research and Water Resources (PCRWR) reported that 40 % of all diseases in Pakistan are water-related. It is expected that these diseases may cause annual national income losses of USD 380 - 883 M or 0.6 - 1.44 % of GDP (UNDP, 2003). In Pakistan, studies regarding DBPs identification in drinking water is very limited. Concern has been raised that these compounds may be very toxic to human health. The information on policies regarding water issues and environment do exist in Pakistan, however they are proceeding at a very slow pace.

1.1 Objectives

The study aimed to investigate the formation of DBPs, namely THMs in drinking water distribution network. The objectives are mentioned below:

i. Calibration of GC technique for trihalomethanes (THMs).

ii. Optimization of HS-SPME analytical conditions using response surface methodology (RSM) and central composite design (CCD) for THMs determination.

iii. Detection and quantification of THMs from drinking water samples using GC.

Chapter 2

LITERATURE REVIEW

Water quality assurance is always a vital issue at national as well as international level. Therefore, it is absolutely essential to regulate and treat drinking water for the benefit of a community. In fact, a major accomplishment in public health during this century has been the disinfection of public drinking water supplies. This practice has significantly reduced illness and death associated with many diseases, such as cholera, typhoid and other waterborne diseases.

2.1 Trihalomethanes (THMs)

THMs were initially recognized almost 30 years ago and uptil now DBPs have been keenly studied. Several studies regarding the knowledge of DBPs formation, existence and health risks. THMs, and to a lesser degree HAAs are presently utilized as indicator chemicals for potentially detrimental compounds formed by the chlorine addition to water. Based on this assumption, THMs and HAAs are regulated in water distribution networks in many countries. Humans are exposed to disinfection byproducts through oral, dermal and inhalational contact with chlorinated water (Backer *et al*., 2000).

Gallard *et al.* (2002) stated that there are several factors that influence THMs formation in drinking water. These includes features of the raw surface water, chlorine dosage, contact time, temperature, pH, bromide/iodide levels, the conditions in which water is being stored and supply conditions. THMs formation is found to be directly correlated with pH. Stevens *et al*. (1989) executed three studies using Ohio River water at the Cincinnati water treatment plant with various pH values. The results demonstrated that THMs production increased at higher pH. When the pH was increased from 7 to 11, there was 30 to 50% increase in THMs formation.

THMs are more commonly found in surface waters treated with chlorine that is consumed for communal drinking water purpose as stated by Golfinopoulos (2000) and Nissinen *et al*. (2002). As groundwater seldom comprises elevated concentrations of natural organic content, chlorinated private water supply network and community bores are less prone to the formation of THMs. THM formation has been revealed to be a result of many water quality parameters, such as, total organic carbon (TOC), pH, UV absorbance, temperature, bromide/iodide level and reaction/contact time (Engerholm and Amy, 1983).

Chowdhury *et al.* (2007) carried out a study on effect of various parameters using four different water supply networks in Newfoundland, Canada. They found strong associations among TOC, DOC and formation of THMs. UV_{254} is an important water quality parameter, utilizing light at UV 254 nm wavelength to determine organic matter present in water. This is due because organic compounds mostly absorb light at the UV 254 nm wavelength. According to Karanfil *et al.* (2002) specific UV_{254} provides a quantitative measure of aromatic content per unit concentration of dissolved organic matter (DOM). Natural waters with high UV_{254} values have a relatively high content of high molecular weight DOM fractions. Similarly, Korshin and his co-workers in 1997 stated that particular UV absorbance (SUVA) is a good indicator of NOM in water, which is a ratio between DOC and UV absorbance capacity. It is always important to know the organic load of the water because considerably high content of NOM will result in THMs formation as NOM is one of the most significant precursor of THMs. Temperature also showed a positive impact on THMs formation in drinking water. Stevens and his fellow workers in 1989 reported that production of THMs increased 1.5 - 2 times at every stage when the temperature was being altered (Stevens *et al.,* 1989). El-Shahat *et al*. (2001) and Hellur-Grossman *et al*. (2001) stated lesser THMs determination during the winters than during the summers. In summers, as a result of higher temperature, the reaction rates are higher resulting in

amplified rate of THMs formation. Total dissolved solids comprise of inorganic salts and slight amounts of organic matter that are dissolved in water which is indirectly related to THMs formation. High conductivity most of the time indicates addition of some pollutants and dissolved organic matter (Jayana *et al.,* 2009).

Wong and Mok (2008) examined the behaviour of two CDBPs, namely THMs and HAAs, in a treatment plant and within the water supply network of Macau. At different phases of treatment process as well as at two distinct points within the water supply network, the targeted THMs and six classes of HAAs were observed in different concentrations throughout february 2006 to january 2007. The results indicated that the overall concentration of HAA was generally lower than that of THMs in chlorinated water with a ratio ranging from around 1:1 to 2:1. The outcomes recommended that pre-chlorination and coagulation are the main stages in the treatment process that influenced CDBPs formation. Toroz and Uyak, (2005) witnessed the influence of various parameters on THMs production including seasonal and spatial variations and subsequent THMs levels were investigated in treatment plant discharges and at intervals within the drinking water supply networks.

Simpson and Hayes, (1998) determined DBPs concentration in chlorinated and chloraminated drinking water from different locations around five states of Australia. In chlorinated water, THMs were predominant while chloroform was quantified in 80 % of drinking water samples from USEPA region 5 (Illinois, Indiana, Ohio, Michigan, Minnesota and Wisconsin) as a part of the National Human Exposure Assessment Survey (NHEXAS) phase1 field study.

2.2 Public health significance

Ever since the detection of THMs in drinking water in 1970s, these compounds have been investigated keeping in view their toxicology and epidemiology. The studies carried out on

animals by King *et al.* (2000) verified that liver, kidney and intestinal tumours have a strong association with chronic consumption of THMs. Several other toxicological researches have revealed numerous THMs (e.g., bromodichloromethane, bromoform, chloroform) to be carcinogenic in research laboratory animals.

Richardson *et al.* (2008) stated that even though above 600 DBPs have been described in the literature and only few has been evaluated as health-risks studies. Epidemiological studies carried out by International Agency for Research on Cancer (IARC) in 2004 have also revealed that a lifetime exposure to chlorinated water is linked with an increased risk for cancer. The WHO (IARC) conducted research on potential carcinogens. As shown in Table 2.1, chloroform and bromodichloromethane are categorized as possible human carcinogens. The classifications of possible human carcinogens is on the basis of information from research on animals. Dibromochloromethane and bromoform are not classifiable as carcinogens, as there is insufficient research to classify them as non-carcinogenic. There is insufficient evidence of carcinogenicity in humans for all four THMs (WHO, 2004).

Table 2.1: IARC classification of THMs

THMs	Humans	Classification
Chloroform	Inadequate evidence for human carcinogenicity	Possible human carcinogen (Group 2B)
Bromodichloromethane	Inadequate evidence for human carcinogenicity	Possible human carcinogen (Group 2B)
Dibromochloromethane	Inadequate evidence for human carcinogenicity	Not classifiable as to it's carcinogenicity in humans (Group 3)
Bromoform	Inadequate evidence for human carcinogenicity	Not classifiable as to it's carcinogenicity in humans (Group 3)

Calderon (2000) summarized after several epidemiological studies that there is a strong association between ingestion of DBPs and negative reproductive as well as developmental

consequences e.g. intrauterine growth retardation, neonatal deaths, stillbirths, low birth weight, preterm delivery, petite body length and birth deficiencies such as major cardiac flaws and oral clefts. According to USEPA, (2006), several studies such as short range, high dose animal screening on separate by-products (e.g., DBCM) have also stated undesirable developmental and reproductive effects, such as whole litter resorption and decreased foetal body weight, which are analogous to those described in the human epidemiology studies. Hsu *et al*., (2001) studied probable cancer threats of THMs due to consumption of drinking water treated with chlorine in Taiwan. Lee *et al*. (2004) assessed the lifespan cancer possibility and vulnerability quotient for THMs through contact of tap water by means of statistics for human health risk assessment.

In one of the study, Richardson along with his co-workers (2008) reviewed that studies carried out over last 30 years on existence, genotoxicity and carcinogenicity of 85 DBPs, out of which 74 are thought to be emergent DBPs as a result of their restrained incidence levels and toxicological properties. These 74 comprise of halonitromethanes, iodo-acids, unregulated halo-acids, I-THMs, halomethanes, halofuranones, brominated DBPs, haloacetonitriles, tribromopyrrole, aldehydes, N-nitrosodimethylamine (NDMA) and other nitrosamines. Generally, the brominated DBPs are more genotoxic as well as cancer causing than chlorinated compounds, whereas iodinated DBPs are the utmost genotoxic of all however, they have not been verified for their carcinogenicity. Recently, new concerns were raised by Plewa *et al.* (2004) regarding human health risks, who stated that iodinated DBPs are comparatively more mutagenic and genotoxic than brominated and chlorinated THMs. I-THMs, especially iodoform (CHI_3) is formed in drinking water supplies during treatment processes.

Much of the previous research on DBPs exposure has been focused on carcinogenic or mutagenic effects on humans. However, Shafiee and Taghavi, (2012) raised new concerns by epidemiological studies about adverse developmental and reproductive effects, such as low birth

weight, intrauterine growth retardation and spontaneous abortion. As stated by Casals (2010), THMs cause harmful side effects to the human body and are considered as carcinogens. These types of cancer causing compounds have been revealed to cause DNA demage, interfere with the immune system and cell growth. There is a higher risk of asthma, eczema and eroding dental enamel when exposed to THMs in water. They are also shown to cause a higher rate of miscarriage and birth defects. This type of compound does not degrade or get digested, infact the body will store it in the fat tissues and secrete through breast milk, blood and semen.

2.3 Analytical methods for DBPs

Various approaches for the THMs and other VOCs in drinking water have been reported in the literature such as liquid liquid extraction, static headspace technique, dynamic headspace technique, solid-phase microextraction technique and direct aqueous injection,. The THMs detection in water has predominantly been done with gas chromatography (GC) equipped with electron capture detector (ECD) or mass spectrometry detector (MSD). These compounds are present at ng/L to µg/L levels in drinking water (Dewulf and Van-Langenhove, 2006).

Allard *et al.* (2012) reported that liquid liquid extraction is a basic but this is time consuming, expensive and requires the evaporation solvent and disposal of toxic chemicals. Another technique that is widely used for the extraction of volatile hydrocarbons is static headspace (HS). SPME is simple and a solvent free sampling technique that has been applied to the VOCs extraction in various matrices, including the determination of THMs in swimming pool waters. In spite of all this, some difficulties have also been reported in this method such as, stirring of sample, temperature control, limited fibre life, fibre breakage and increased fiber cost.

Charrois and Hrudey, (2007) studied DBPs analytical methods. Cancho *et al*. (2000) stated different extraction methods for the determination of I-THMs and reported that the HS-GC/ECD provides similar precision to LLE-GC methods for the detection of I-THMs. Silva *et al*. (2006)

optimized HS-SPME-GC/MS method for the analysis of dichloroiodomethane and bromochloroiodomethane in blood samples. San *et al.* (2007) analyzed different fibres; Carboxen/polydimethylsiloxane (CAR/PDMS), divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) and polydimethylsiloxane/divinylbenzene (PDMS/DVB). The PDMS-DVB fibre was selected based on it's improved detection limits, repeatability and a broader linear range.

The use of an internal standard (IS) in HS-SPME may overcome many difficulties in this technique. Stack *et al.* (2000) described the use of fluorobenzene as an internal standard for THMs extraction as described in EPA method 551.1 (USEPA, 1995). This method comprises liquid liquid extraction with MTBE, with addition of anhydrous sodium sulfate salt. The sodium sulfate salt was added to enhance the ionic strength of the solution, thus increasing the extraction of volatile compounds from aqueous phase to the headspace (HS).

2.4 Factors effecting THMs extraction efficiency

Sá *et al.* (2011) analyzed the effect of temperature ranging from 30 to 65 °C, and concluded that 55 °C was the optimum extraction temperature. The THMs extraction using the PDMS fibre has been described, however, coatings such as; CAR-PDMS or CW-DVB, have also revealed to provide improved extraction efficiencies. Cho *et al.* (2003) assessed the extraction efficiency of SPME variables, headspace and sample volume, extraction temperature, extraction time, desorption time and salt addition by using CAR-PDMS fibre. THMs detection limits between 0.005 and 0.01 µg/L were found with the CAR-PDMS fibre and electron capture detector (ECD).

2.4.1 Effect of extraction time on THM formation

Zhao *et al.* (2004) found out that the peak areas of all THMs enhanced with time of extraction upto 10 min. After that the analytes extraction showed a much slower response. Thus, in all subsequent experiments, extraction time of 10 min was used. It has been revealed that by

increasing sampling temperature reduces extraction time and recoveries. Hence increases the extraction efficiency of THMs. Ai, (1997) has discussed a model of SPME method, signifying a synergetic realtion of the analyte amount adsorbed onto the fiber and the initial concentration in the sample matrix if the stirring and time are held constant.

2.4.2 Effect of extraction temperature on THM formation

Allard *et al.* (2012) reported that increase in extraction temperature increased the rate of extraction for greater molecular weight THMs but also had the antagonistic effect of reducing the sensitivity for smaller compounds. Experiments revealed that the extraction of the small molecules ($CHCl_3$, $CHBrCl_2$, $CHBr_2Cl$) reduced with increase in temperature from 30 to 70 ^oC (Figure 2.1). However, a strong increasing trend of analytes with increasing temperature was observed. San *et al.* (2007) disclosed that bromoform was well extracted at 70 ^oC as it was the heaviest and least volatile THM and also the least soluble in water.

2.4.3 Salting-out effect on THM formation

Effect of salt addition has been used commonly in SPME and LLE. Takamatsu and Ohe, (2003) studied that the salt addition enhances the ionic strength of the solution and results in a deviation of the equilibrium state, which amplifies the volatility of analytes. As shown by Allard *et al.* (2012) the extraction of all THMs improved with the salt addition even above saturation point, except for lower molecular mass THMs (Figure 2.1).

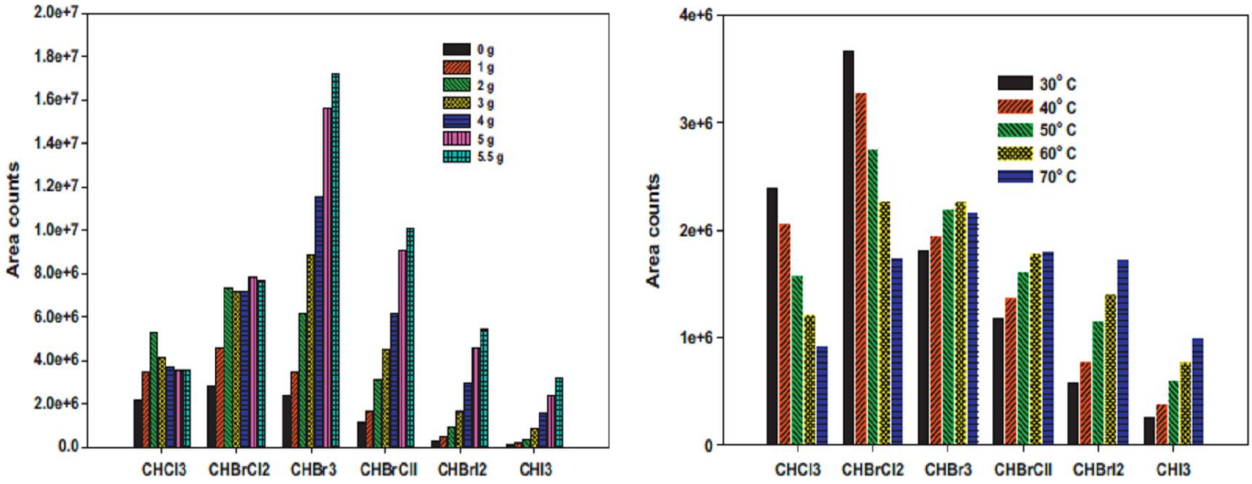

Figure 2.1: Effect of salt addition and extraction temp. on THMs extraction efficiency

2.4.4 Effect of desorption temperature on THM formation

Frazey *et al.* (1998) observed that the desorption temperature is dependent upon the stability of fiber and the analytes. Substantial iodoform thermal degradation was observed at above 200 °C. Therefore, desorption temperature of 160, 180, 200 and 220 °C in splitless mode were estimated. The extraction was observed to be comparatively analogous at each temperature except for the highest molecular mass THMs ($CHBrI_2$ and CHI_3) where an improved response was detected from 160 to 200 °C and a related response for 220 °C.

2.5 Response surface methodology

Response surface methodology was first developed by Box and Wilson in 1951. It is a collection of mathematical and statistical techniques that are suitable for design of experiments, constructing models, estimating the effect of variables and predicting optimal conditions for required responses. Main benefit of RSM is the substantial decrease of experimentals and providing adequate data for statistically effective results.

RSM has played a vital role in biotechnology and various related fields in recent years,. The RSM is a progressive methodology and its procedure can be summarized as follows. Initially, a series of experiments are performed for suitable and consistent measurement of the

response of interest. Second, develop a model of the response surface with the best fit, then determine the optimum limits of experimental parameters that yield a highest response. Finally, signify the direct and interactive effects of process variables using two and three dimensional (3D) plots (Box and Wilson, 1951).

Several classes of RSM such as central composite design (CCD), box behnken design and three level factorial design have been dicussed in the literature. Ahmad *et al.* (2009) reported that amongst the three, CCD is a more popular technique applicable for parameter optimization, evaluation and interaction of variables with least number of experiments. Khodadoust and Hadjmohammadi, (2011) employed central composite design (CCD) to study the individual and synergetic effect of the four factors towards two responses (Khodadoust and Hadjmohammadi, 2011).

Design-Expert software (trail version 9, Stat-Ease, Inc., MN) is usually used for the design of experiments (DoE) having several independent variables. The combined effect on the dependent variable may also be investigated by the selection of experimental points at which the response should be optimized (Myers and Montgomery, 2001).

Pellati *et al.* (2005) reported a proportional relation of extraction temperature and the headspace concentration of the volatile compounds. Maia *et al.* (2014) plotted the 3D response surface using CCD and it was expected that the optimal conditions were 45 °C of extraction temperature, an extraction time of 25 min and a desorption time of 5 min. Merib and co-workers (2013) generated the 3D response surfaces, which allowed the visualization of the optimal extraction conditions.

The 3D response surfaces for all THMs is shown in Figure 2.2. It can be seen from the response surface that around 60 ºC and for 40 min, maximum efficiency was acquired. The

significant efficiencies for THMs were observed when temperature was below 20 °C and extraction time was around 20 min.

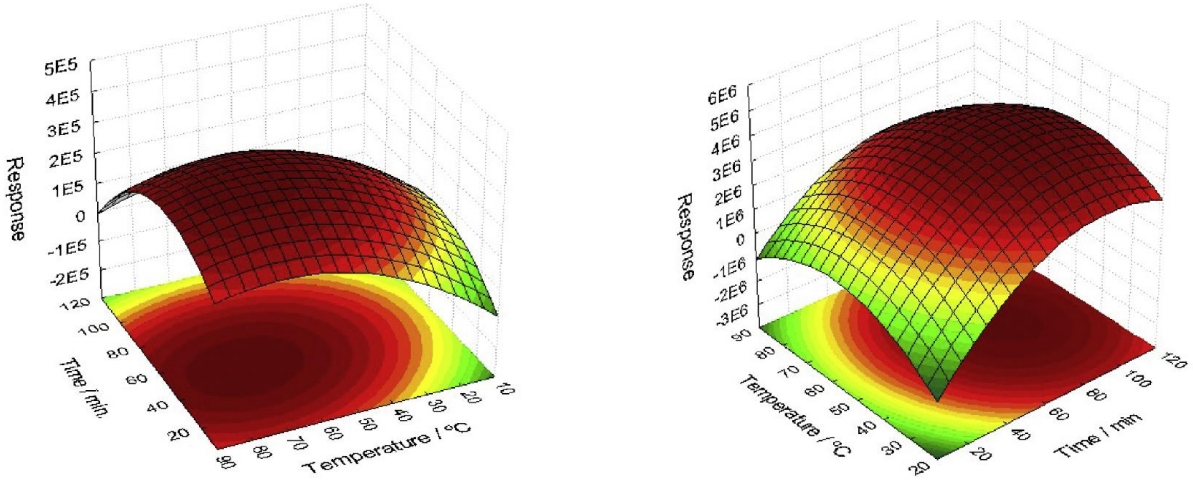

Figure 2.2: 3D surfaces showing THMs response as a function of extraction time and temperature

Chapter 3

MATERIALS AND METHODS

3.1 Sampling Area

To assess the incidence of THMs in drinking water distribution network, samples were collected from the administration blocks, academic blocks, hostel and residential areas within the National University of Sciences and Technology situated in the capital city Islamabad, Pakistan. Sampling strategy was planned in consultation with the construction and maintenance (C & M) staff, responsible for monitoring the water supply within the university. Drinking water samples were collected throughout december 2013 to april 2014 following Standard Methods (APHA, 2012).

The water supply network in the university is pumping underground water for fulfilling it's needs. The distribution network mainly consisted of tubewells (T/W), overhead (O/H) reservoirs and underground (U/G) tanks. Water is pumped from the subsurface catchments by the tubewells. There are 10 tubewells in the area out of which 9 are operational and only one is non-operational (T/W no. 3). From Location 2 and Location 3 water enters in U/G tanks then pumped to O/H reservoirs followed by all sites except hostels where water is delivered from T/W no. 8. The water flows from the O/H reservoirs to these areas under gravity. Chlorination is practiced on daily basis through sodium thiosulphate solution (2L in 200 Gallon Tank).

3.2 Cleaning of glassware

For the purpose of sample collection glass bottles (1 litre) were used with septa and polypropylene caps. The sampling bottles were washed with soapy water, soaked overnight in concentrated chromic acid solution, rinsed with distilled water and finally oven dried at 180 ^{o}C for 12 hours.

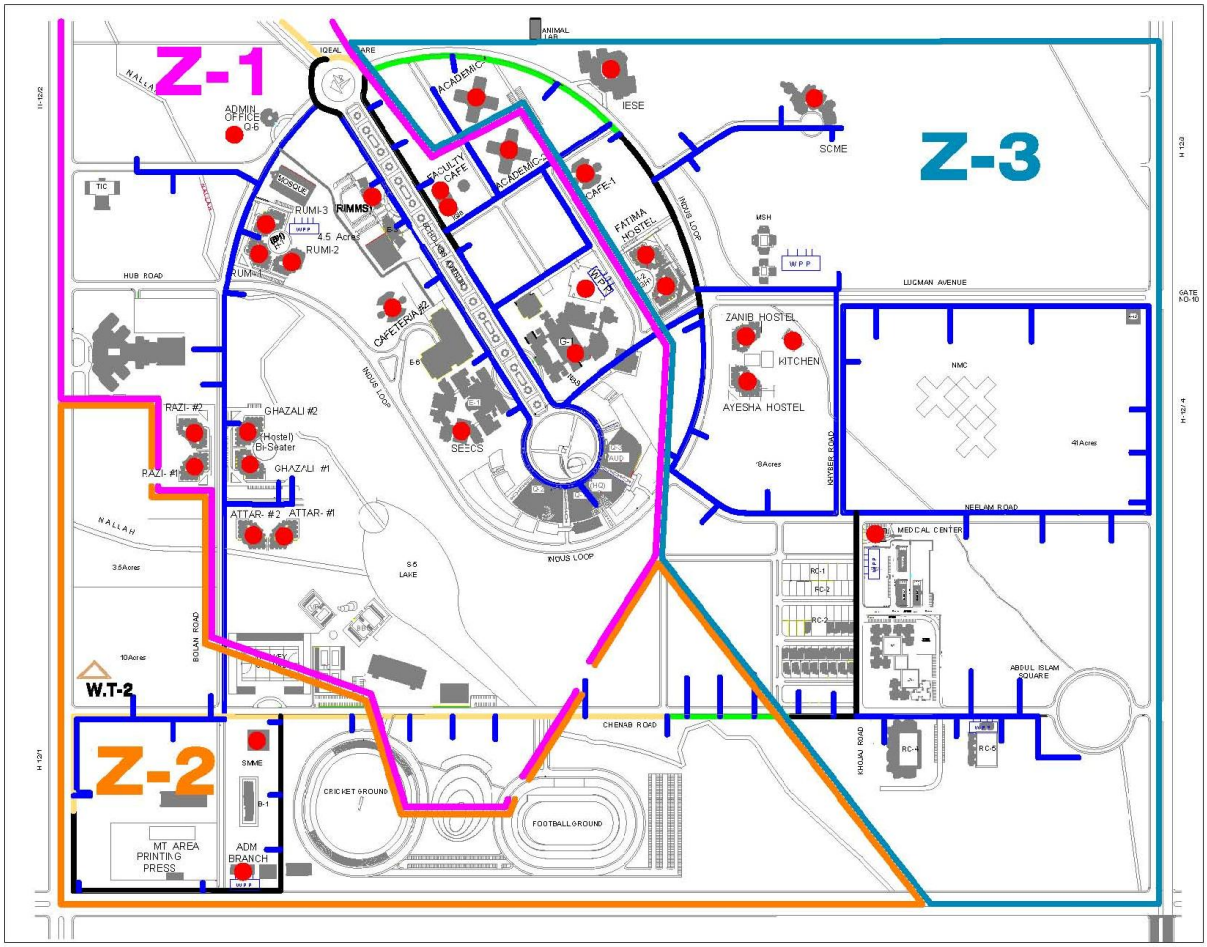

Figure 3.1: Location map of a drinking water distribution network within NUST

Table 3.1: Water storing capacity at locations within a university

Locations	Underground reservoir capacity (MG)	Overhead tank capacity (G)
Location 1	0.6	5000
Location 2	0.9	100,000
Location 3	0.1	100

3.3 Chemicals and reagents

Standard analytes were purchased from sigma-aldrich (USA) and Dr. Ehrenstorfer (Germany) with a purity of 99%. Chemical such as ethyl acetate, n-hexane and methanol were obtained from Merck (Germany). The reagent used as quenching agent for chlorine was ascorbic

acid (BDH grade). Methyl tertiary butyl ether (extraction solvent) and carbontetrachloride (internal standard) were purchased from Scharlau Spain. High purity anhydrous sodium sulphate and sodium chloride were also purchased from Scharlau Spain.

3.4 Standard solutions

Method development was based upon the EPA Method 551.1 for THMs in drinking water. Mostly THMs are volatile, light sensitive and decompose in specific organic solvents. A solution of iodoform in non polar solvent (e.g. n-pentane) rapidly turn violet because of iodine liberation (Allard *et al.,* 2012). Standard stock solutions were prepared in GC grade methanol by weighing a specific amount of analyte in 10 mL flask and stored at 4 °C. A secondary stock solution was prepared by dilution of the primary standard in methanol to make final concentration of 20, 10, 5, 1, 0.5, 0.1 and 0.05 mg/L etc. 1 µL of the stock mixture was injected into the instrument to observe the sequence of resulting peaks.

Stocks were prepared by using a 10 and 100 µL syringe and gradually adding 10 µL of standard material into the bottle just above the surface of the methanol. Care should be taken while preparation of standard stock solution in methanol.

3.5 Sample collection

Water samples were collected from drinking water source and consumer's end within the university. For the assessment of physicochemical parameters the samples were collected in clean and sterilized glass sample bottles of 1 L. The tap was allowed to run for few minutes before sample collection. Duplicate samples were collected from each site. Sample collection for THMs analysis was carried out in 40 mL clean glass vials. For headspace SPME, 20 mL of vial was filled with water sample and remaining 15 mL was left for headspace. For liquid liquid extraction glass vials were filled with water. A complete detail of sampling locations, source of water along with status of chlorination is presented in Table 3.2.

Table 3.2: Detail of sampling locations and collection source

Sr. No.	Sampling Locations	Abbreviations	Status (before or after chlorination)	Source
1.	Location # 1	L1	After Chlorination	Underground Tank
2.	Location # 2	L2B	Before Chlorination	Underground Tank
3.	Location # 2	L2A	After Chlorination	Underground Tank
4.	Location # 3	L3T	Before Chlorination	Underground Tank
5.	Location # 3	L3W	After Chlorination	Underground Tank
6.	Construction & Management	CNM	After Chlorination	Underground Tank
7.	Material Recovery Centre	MRC	After Chlorination	Underground Tank
8.	Tube Well # 8	TW8B	Before Chlorination	Tube Well
9.	Tube Well # 8	TW8A	After Chlorination	Tube Well
10.	MI Room	MI	After Chlorination	Underground Tank
11.	Iqra Apartments	IA	After Chlorination	Underground Tank
12.	Isra	Isra	After Chlorination	Underground Tank
13.	IESE	IESE	After Chlorination	Underground Tank
14.	Ghazali Hostels	GH	After Chlorination	Tube Well
15.	Rumi Hostels	RH	After Chlorination	Tube Well
16.	Attar Hostels	AH	After Chlorination	Tube Well
17.	Barrack 1	B1	After Chlorination	Underground Tank
18.	SMME	SMME	After Chlorination	Underground Tank
19.	Main Office	MO	After Chlorination	Underground Tank
20.	Admin	Ad	After Chlorination	Underground Tank
21.	IGIS	IGIS	After Chlorination	Underground Tank
22.	Concordia 1	C1	After Chlorination	Underground Tank
23.	Fatima1 Hostels	FH	After Chlorination	Tube Well
24.	Zainab Hostels	ZH	After Chlorination	Tube Well

3.6 Sample preservation

Freshly prepared 0.142 M ascorbic acid was added to each 1 L bottle prior to sampling. The ascorbic acid decreases available chlorine and prevents the additional generation of THMs. A sample volume was collected into 40 mL glass vials, and stored in dark at less than 4 ^{o}C for further analysis in GC.

3.7 On-site analysis

On-site samples were examined for pH, electrical conductivity, dissolved oxygen and free chlorine (APHA, 2012).

3.8 Lab analysis

Physiochemical analysis was performed in IESE lab following Standard Methods (APHA, 2012). Parameters along with their units and instruments used for analysis are mentioned in Table 3.3.

Table 3.3: Methods and instruments used for physicochemical parameters (APHA, 2012)

Physicochemical parameters	Units	Instruments Used
pH	-	pH meter (HACH Sens ion pH meter)
Temperature	^{o}C	Thermometer (HACH Sens ion 1 b)
Total Dissolved Solids (TDS)	mg/L	TDS meter (HACH Sens ion 5)
Turbidity	NTU	Turbidity meter (HACH Turbidimeter 2100N)
Electrical Conductivity (EC)	µS/cm	Conductivity meter (HACH Sens ion 5)
Dissolved Oxygen (DO)	mg/L	DO meter
Alkalinity	mg/L	Titrimetric analysis
Hardness	mg/L	Titrimetric analysis
Free Chlorine	mg/L	Spectroquant Colorimeter
UV_{254} Absorption	cm^{-1}	Spectrophotometer (HACH, 254 nm)

3.9 Selection of extraction technique

A series of analytical methods have been identified for the study of THMs and other volatile organic compounds in water.

3.9.1 Headspace-SPME technique

In HS-SPME, water samples (20 mL) were placed in a 40 mL EPA vials (Wheaton, USA), containing anhydrous sodium sulphate salt, internal standard and a magnetic stir bar. The sample was agitated (300 rpm) at temperature (50 ^{o}C) during the extraction process to drive THMs into the headspace. Fibre was retracted back after sometime and transferred without delay to the injection port of the GC with 220 °C desorption temperature. SPME was performed using a supleco cat. No. 57344-U fibre assembly fitted with a 75 μm (Car-PDMS) fibre. The fibre was conditioned at 250 °C for 30 min to 1 hour prior to use.

3.9.2 Liquid-liquid extraction (LLE)

Water samples (35 mL) were collected in 40 mL vials. In each sample anhydrous sodium sulfate and methyl tert-butyl ether (extraction solvent) were added. Vials were sonicated for 2 min and 500 μL of the organic layer formed was transferred into a 4 mL vial containing carbon tetrachloride. Extracts were examined within 24 hours in a TRB-1 column of GC.

3.10 Gas chromatographic analysis

The GC conditions were optimized as the injector and detector temperature are influenced by the boiling point of the analytes while the column temperature and carrier gas flow were the critical factors for eluting the analytes.

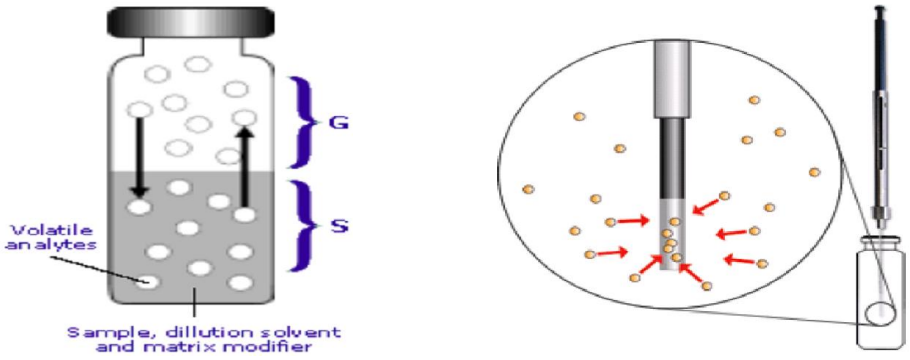

Figure 3.2: Solid-phase micro extraction fibre immersed in headspace

Analyses was performed using a Shimadzu 2010 gas chromatograph equipped with an electron capture detector. Injections were made in the split mode into a 30 m long fused silica capillary column (TRB-1), with inner diameter 0.32 mm, thickness 1 μm and filling material was 5% diphenyl-95% dimethyl-polysiloxane. THMs were analyzed as per US-EPA method 551.1 (US-EPA, 1995).

Table 3.4: Gas chromatographic conditions

Parameters	Values
1. Injector	
Pressure	48.2 Kpa
Total flow	126.9 mL/min
Temperature	220 ^{o}C
Linear velocity	24.4 cm/sec
2. Column	
Initial temperature	50 ^{o}C
Final temperature	200 ^{o}C
Temperature ramp	15 ^{o}C/min
3. Detector	
Temperature	220 ^{o}C
Current	0.03 nA
Gas flow	4 mL/min

3.11 Quantification method

The quantification of THMs in the drinking water samples was based on the internal standard (IS) calibration procedure. To use this method, carbontetrachloride (CCl_4) was used as an internal standard. Analysis of each calibration standard was done according to USEPA Method 551.1. Peak areas were tabulated for each compound and response factor (RF) was calculated using Equation 1.

$$\mathbf{RF = (As)\ (Cis) / (Ais)\ (Cs)} \quad \textbf{……} \text{ Equation 1}$$

where:

As = Analyte response

Ais = Internal standard response

Cis = Internal standard concentration (mg/L)

Cs = Analyte concentration to be measured (mg/L)

Amount of unknown analyte (Cs) was calculated using Equation 2.

$$\mathbf{Cs = (As)\ (Cis) / (Ais)\ (RF)} \quad \textbf{……} \text{ Equation 2}$$

The calibration curves and response factor should be certified on each day by measuring one or more standards.

3.12 Response surface methodology (RSM)

One of the objectives of this study was to achieve comparison and optimization of four variables used to extract THMs from drinking water i.e. salt amount, extraction time, temperature and desorption time. The experiments were carried out using a standard solution containing 1 mg/L of each THM and 1 mg/L of carbontetrachloride (internal standard). In this study optimization of variables for THMs extraction were studied using response surface methodology and central composition design (CCD) method (Myers and Montgomery, 2001).

Design-Expert software (trail version 9, Stat-Ease, Inc., MN) was used to design and analyze response surface experiments. Some phases in the RSM application are as follows:

3.12.1 Generating a design

All the selected variables with their units and ranges were entered into the software. The selection of variable levels and their high and low ranges was based on the results obtained through the previous work as well as the operational limits of the instrument. Table 3.5 was generated by the software indicating different coded values of variables.

Table 3.5: Coded variables and their low and high levels values by CCD matrix

Coded variables	Lowest (-α)	Low (-1)	Centre (-0)	High (+1)	Highest (+α)
Salt (g)	-1.25	1	3.25	5.5	7.75
Ext time (min)	-7.5	5	17.5	30	42.5
Ext temp (ºC)	5	30	55	80	105
Desorp time (min)	-4	2	8	14	20

3.12.2 Enter the response data

A CCD consisting of 30 experiments was generated by the software to optimize the levels of these variables to attain the maximum performance. At this stage the experiments were performed and responses were recorded into the run sheet (Table 3.6).

3.12.3 Evaluation of the results

The statistical analysis was done to evaluate the results. At this point Design-Expert fits linear, two-factor interaction (2FI), quadratic and cubic polynomials to the response (Table 3.7).

For each source of terms (linear etc.), if the probability falls below 0.05 significance level then the model is significant. Design-Expert indicates that the 2FI vs linear model looks acceptable ($p = 0.006 < 0.05$). These are significant terms, but adding the cubic order terms will not significantly improve the fit. Even if they were significant, the cubic terms were aliased, so

Table 3.6: Design layout of experiments and their response data

Run	Factor 1 A: Salt (g)	Factor 2 B: Ext. time (min)	Factor 3 C: Ext. temp (°C)	Factor 4 D: Desorp. Time (min)	Response THMs (mg/L)
1	5.5	5	80	2	215.891
2	3.25	42.5	55	8	150.398
3	7.75	17.5	55	8	110.23
4	1	30	30	2	54.3023
5	1	5	30	14	34.67
6	5.5	5	30	2	35
7	3.25	17.5	55	8	112.3
8	3.25	17.5	55	8	112.7
9	5.5	30	30	2	76.64
10	1	30	80	14	52.4265
11	1	30	30	14	42.5923
12	3.25	17.5	55	8	112.3
13	5.5	30	30	14	199
14	1	5	30	2	51.5
15	3.25	-7.5	55	8	101.9
16	3.25	17.5	5	8	155.468
17	1	30	80	2	35.1002
18	5.5	5	30	14	26.1645
19	5.5	30	80	14	255.98
20	3.25	17.5	55	8	112.3
21	1	5	80	2	221.651
22	3.25	17.5	55	20	174.387
23	3.25	17.5	55	8	112.7
24	-1.25	17.5	55	8	53.5
25	3.25	17.5	55	8	112.7
26	3.25	17.5	105	8	159.8
27	5.5	5	80	14	51.1482
28	5.5	30	80	2	54.6
29	1	5	80	14	225.4
30	3.25	17.5	55	-4	123.7

Table 3.7: Sequential model and sum of squares tests

Source	Sum of Squares	Df	Mean Square	F Value	p-value	
Linear vs Mean	21543.74	4	5385.94	1.29	0.3008	
2FI vs Linear	60326.45	6	10054.41	4.33	**0.0064**	Suggested
Quadratic vs 2FI	6914.50	4	1728.63	0.70	0.6054	
Cubic vs Quadratic	30174.30	8	3771.79	3.77	0.0485	Aliased

they wouldn't be useful for modeling purposes. Desired model order and terms are chosen from the list as shown below (Figure 3.3):

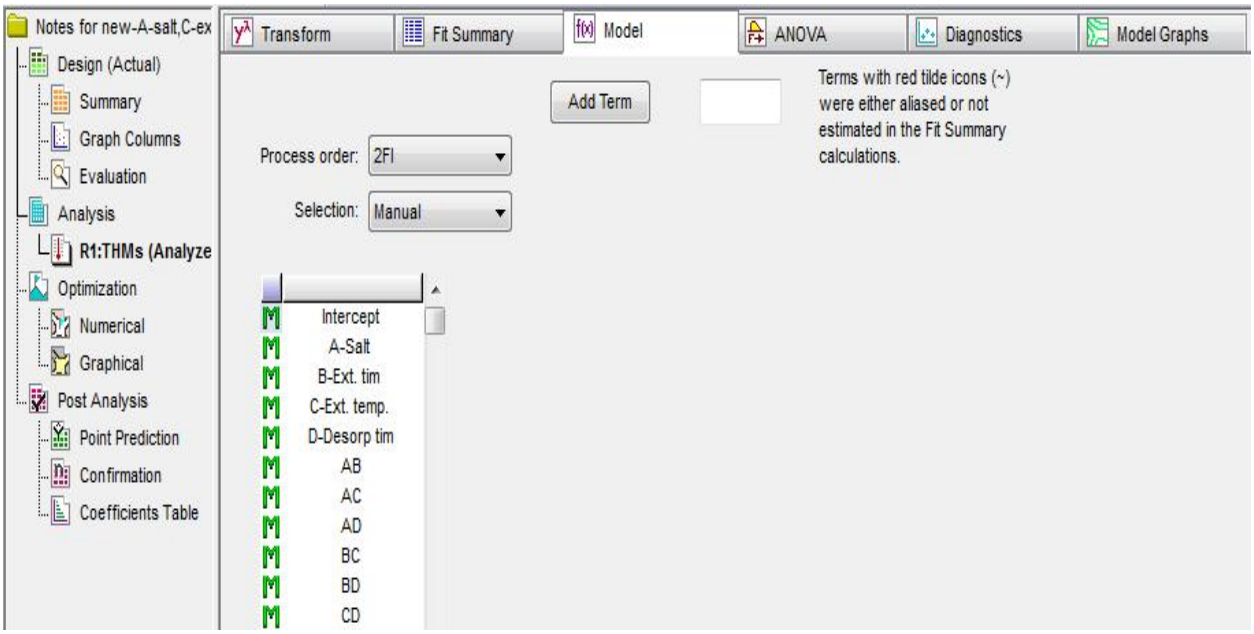

Figure 3.3: Design model order from software

ANOVA test was applied by the software to assess the significance of the model achieved for THMs. Table 3.8 shows the results of ANOVA and regression coefficients of factors that reveals the contribution of the model for THMs (significant with $p < 0.05$).

The F-value of 3.53 indicates that the model is significant. In this case C, AB, BC, BD are significant terms. While values above 0.1 indicate the model is not significant.

Table 3.8: Analysis of variance (ANOVA) for THMs optimization

ANOVA for Response Surface 2FI model						
Source	**Sum of Squares**	**df**	**Mean Square**	**F Value**	**p-value**	
Model	81870.19	10	8187.02	**3.53**	**0.0087**	Significant
A-Salt	4010.39	1	4010.39	1.73	0.2043	
B-Ext. time	1.61	1	1.61	6.93E-004	0.9793	
C-Ext. temp.	15049.64	1	15049.64	6.49	**0.0197**	
D-Desorp time	2482.10	1	2482.10	1.07	0.3140	
AB	23014.14	1	23014.14	9.92	**0.0053**	
AC	765.89	1	765.89	0.33	0.5724	
AD	1552.89	1	1552.89	0.67	0.4235	
BC	18305.01	1	18305.01	7.89	**0.0112**	
BD	16642.04	1	16642.04	7.17	**0.0149**	
CD	46.49	1	46.49	0.020	0.8889	
Lack of Fit	44087.50	14	3149.11	**6.56**	**0.2086**	Non significant

The next icon in the software is diagnostic plots to validate the model. The probability plot of actual versus the predicted response in Figure 3.4 revealed that the values fall on a diagonal line showing that experimental data is in agreement with the predicted values. This indicates good applicability of model for explanation of experimental data.

The software was then used to create 3D colored response surfaces which clearly indicates the interaction of variables with THMs formation and at the end generate the optimal conditions for THMs extraction.

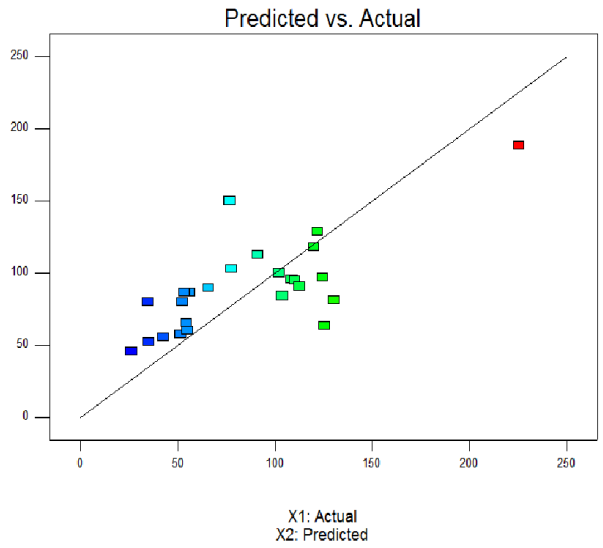

Figure 3.4: Probability plot of actual vs predicted values

3.13 Application of RSM to water samples

The optimization of HS-SPME-GC method, through a central composite design (CCD) and response surface methodology (RSM) was done to found the factors that have statistically significant impact on the THMs extraction. Therefore this design was applied for the determination of THMs from the drinking water samples of the university.

Chapter 4

RESULTS AND DISCUSSION

This section is based upon the results attained from the experiments to determine the prevalence of THMs in drinking water. Research was conducted in two phases. In the first phase drinking water samples were analyzed for physicochemical quality and then standards were calibrated using GC. In the second phase, process was optimized using response surface methodology (RSM). At the end quantitative analysis of THMs was carried out using GC.

4.1 Physicochemical quality of drinking water samples

The samples collected from the drinking water source and consumer's end within the university were analyzed for physical and chemical contamination. THMs formation has been shown to be a function of numerous water quality parameters. Previous studies reported that the rate of THMs production vary as a function of chlorine residual and TOC (Clark, 1998). The World Health Organization recommends maintenance of chlorine residual of 0.2 to 0.5 mg/L in the distribution systems under normal operating conditions (WHO, 2004). Korshin and his co-workers in 1997 stated that UV_{254} absorbance indicates the presence of NOM in water. The NOM is one of the most significant precursor of THMs development (Chang *et al.*, 2001). This correlation was considered here to develop the relationship of UV_{254} and THMs in drinking water. Table 4.1 represents the ranges and mean values along with WHO limits of the physicochemical parameters. It was found that all the values were within the permissible limits described by WHO.

Table 4.1: Water quality characteristics and their permissible limits

Sampling Locations	Physicochemical Parameters (WHO Limits)									
	pH (6.5-8.5)	Temp (Ambient °C)	EC (2500 µS/cm)	TDS (1000 mg/l)	Turbidity (5 NTU)	DO (14 mg/l)	Alkalinity (1000 mg/l)	Hardness (500 mg/l)	Residual Cl_2 (0.2-0.5mg/l)	UV_{254} (cm^{-1})
L1	7.33 7.32-7.34	20.2 23.1-23.3	739 738-740	443.4 443.3-443.5	0.59 0.58-0.6	7.7 7.6-7.8	314 313-315	326 325-327	0.23 0.22-0.24	0.066 0.065-0.067
L2B	6.9 6.8-7.0	17.1 17-17.2	854 853-855	512.5 512.4-512.6	0.118 0.117-0.119	7.2 7.1-7.3	328 327-329	516 515-517	0.20 0.19-0.21	0.062 0.061-0.063
L2A	6.66 6.65-6.67	16.7 16.7-16.9	964 963-965	578.4 578.3-578.5	0.258 0.257-0.259	7.48 7.47-7.49	324 323-325	342 341-343	0.46 0.45-0.47	0.124 0.123-0.125
L3B	6.71 6.7-6.72	19.4 19.3-19.5	894 893-895	536.4 536.3-536.5	0.395 0.394-0.396	8.0 7.9-8.1	242 241-243	278 277-279	0.19 0.18-0.2	0.057 0.056-0.058
L3A	6.6 6.5-6.7	20.4 23.3-23.5	867 866-868	520.2 520.1-520.3	0.454 0.453-0.455	8.62 8.61-8.63	234 233-235	308 307-309	0.39 0.38-0.4	0.095 0.094-0.096
IESE	7.2 7.1-7.3	19.5 19.4-19.6	750 749-751	450 449-451	0.272 0.271-0.273	8.8 8.7-8.9	332 331-333	310 309-311	0.29 0.28-0.3	0.077 0.076-0.078
CNM	6.8 6.7-6.9	16.7 16.6-16.8	855 854-856	513 512-514	0.19 0.18-0.2	5.76 5.75-5.77	314 313-315	338 337-339	0.31 0.3-0.32	0.080 0.079-0.081
MRC	6.62 6.61-6.63	16.7 16.6-16.8	820 819-821	492 491-493	0.201 0.2-0.202	10.6 10.5-10.7	300 299-301	340 339-341	0.35 0.14-0.16	0.092 0.091-0.093
TW8B	7.31 7.3-7.32	16 15.9-16.1	629 628-630	377.4 377.3-377.5	0.514 0.513-0.515	7.9 7.8-8.0	260 259-261	270 269-271	0.18 0.17-0.19	0.063 0.062-0.064
TW8A	6.87 6.86-6.88	18.5 18.4-18.6	1520 1519-1521	912 911-913	0.633 0.632-0.634	9.08 9.07-9.09	324 323-325	248 247-249	0.32 0.31-0.33	0.085 0.084-0.086
MI	7.43 7.42-7.44	16.4 16.3-16.5	796 795-797	477.6 477.5-477.7	0.71 0.7-0.72	7.02 7.01-7.03	226 225-227	338 337-339	0.22 0.21-0.23	0.071 0.07-0.072
IA	7.55 7.54-7.56	16.2 16.1-16.3	652 651-653	391.2 391.1-391.3	0.15 0.14-0.16	6.83 6.82-6.84	256 255-257	290 289-291	0.09 0.08-0.1	0.022 0.021-0.023
Isra	7.34 7.33-7.35	14.5 14.4-14.6	909 908-910	545.4 545.3-545.5	1.51 1.5-1.52	6.49 6.48-6.5	226 225-227	223 221-224	0.05 0.04-0.06	0.019 0.018-0.02
GH	6.7 6.6-6.8	19.2 23-25	785 784-786	637.3 637.2-637.5	0.72 7.1-7.3	5.90 5.89-5.91	220 219-221	333 332-334	0.19 0.18-0.20	0.066 0.065-0.067
RH	7.1 7.0-7.2	21.3 28-30	842 841-843	512.4 512.3-512.5	0.68 6.7-6.9	6.12 6.11-6.13	309 308-310	365 364-366	0.22 0.21-0.23	0.075 0.074-0.076
AH	7.9 7.8-8.0	18.9 18.8-19.0	845 844-846	497.3 497.2-497.4	0.73 7.2-7.4	8.1 8.0-8.2	318 317-319	370 369-371	0.23 0.22-0.24	0.068 0.067-0.069
B1	7.29 7.28-7.3	16.7 16.6-16.8	846 845-847	507.6 0.082	0.63 0.62-0.64	6.06 6.05-6.67	310 309-311	356 355-357	0.42 0.41-0.43	0.099 0.098-0.1
SMME	7.5 7.4-7.6	16.7 16.6-16.8	841 840-842	504.6 0.082	0.61 0.6-0.62	6.81 6.8-6.82	326 325-327	376 375-377	0.39 0.38-0.4	0.089 0.088-0.09
MO	7.28 7.27-7.29	16.7 16.6-16.8	740 739-741	444 0.816	0.49 0.48-0.5	7.7 7.6-7.8	310 309-311	324 323-325	0.2 0.1-0.3	0.069 0.068-0.07
Ad	7.45 7.44-7.46	15.9 15.8-16.0	731 730-732	438.6 0.082	0.46 0.45-0.47	8.4 8.3-8.5	370 369-371	328 327-329	0.16 0.15-0.17	0.055 0.054-0.056
IGIS	7.63 7.62-7.64	19.8 19.7-19.9	722 721-723	433.2 0.082	0.56 0.55-0.57	6.4 6.3-6.5	332 331-333	310 309-311	0.3 0.2-0.4	0.079 0.078-0.08
FH	7.28 7.27-7.29	21.2 21.1-21.3	881 880-882	528.6 528.5-528.7	0.48 0.47-0.49	8.52 8.51-8.53	329 328-330	333 332-334	0.23 0.22-0.24	0.071 0.07-0.072
ZH	7.43 7.42-7.44	21.0 22.9-23.1	876 875-877	525.6 525.5-525.7	0.65 0.64-0.66	9.09 9.08-9.10	325 324-326	228 227-229	0.13 0.12-0.14	0.052 0.051-0.053
C1	7.22 7.21-7.23	21.1 22.0-22.2	855 854-856	585.2 585.1-585.3	0.72 0.71-0.73	6.9 6.8-7.0	322 321-323	221 220-222	0.15 0.14-0.16	0.041 0.04-0.042

Upper values=mean; lower values=range

4.2 Gas Chromatographic analysis

4.2.1 Calibration of GC standards

A standard stock solution containing each THM at 1 mg/L was prepared in high purity methanol and run on GC so as to spot their retention time and signals (Table 4.2).

Table 4.2: Analytical profile of standard analytes

Analytes	Molar mass (g/mol)	Boiling Point (^{o}C)	Retention Time (min)	Peak Area ±S.D	R.S.D (%)
CHI_3	393.73	218	12.82	10934.8±776.03	7.09
CH_2ClI	176.38	108	5.196	15665.3±1568.1	10.01
$CHBrCl_2$	163.8	90	5.003	12987.1±365.36	2.81
$CHBr_2Cl$	208.28	120	6.301	46879.9±1667.5	3.56
$CHCl_3$	119.38	61.2	3.9	91367.1±1070.8	1.17
$CHBr_3$	252.73	149.1	6.9	1347.7±167.23	12.4

Figure 4.1 represents chromatogram of methanol and chloroform with retention time of 2.3 and 3.9 min respectively. GC grade methanol was used as a solvent in this study due to polar nature of the analyte. As methanol is also polar, so the standard analytes were easily dissolved in methanol. The O-H bonds in water and methanol are polar as the oxygen atom has the stronger attraction for the electron pair and attracts negative charge toward itself, thus hydrogen will gain fractional positive charge. This polarity is of great importance in interactions between molecules. The results were compared with drinking water samples in this study and water is also a strong polar compound so the behavior of the analytes is almost same in methanol and water. Chloroform has lowest boiling point of 61.2 °C among all analytes therefore it elutes earlier. Calibration curve of chloroform was established by measuring six dilutions of standard THMs. The regression coefficient was found to be 0.93 (Figure 4.2).

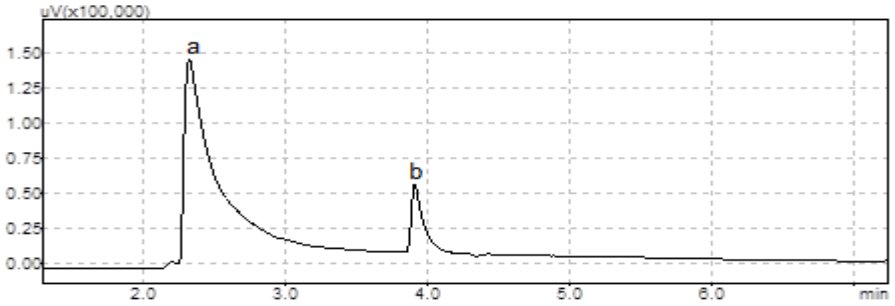

Figure 4.1: Chromatogram representing the peak signals and retention time (a) Methanol (2.3 min) (b) Chloroform (3.9 min)

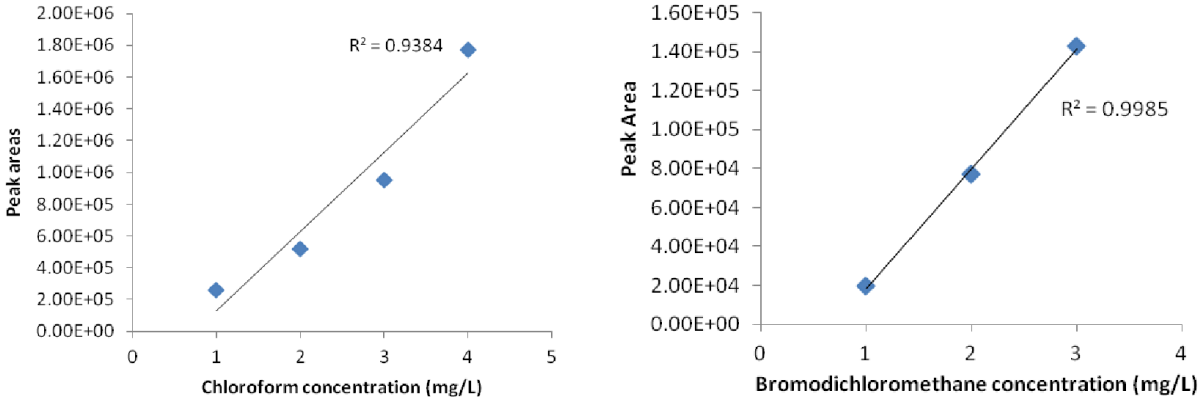

Figure 4.2: Calibration curves of chloroform and bromodichloromethane (conc. vs peak response)

The Figure 4.3 is representing two sharp peaks of methanol and bromodichloromethane. The boiling point of bromodichloromethane is 90 °C and it eluted at 5.0 min.

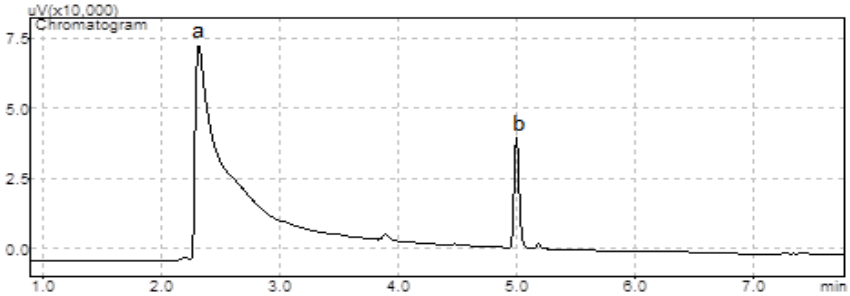

Figure 4.3: Chromatogram representing the peak signals and retention time of (a) Methanol (2.3 min) and (b) Bromodichloromethane (5.0 min)

Chloroiodomethane is a liquid halomethane, easily soluble in benzene, acetone, diethyl ether and alcohol. Its boiling point is 108 °C and it elutes at 5.196 min, showing a well identified peak even at lesser concentration. Figure 4.5 depicts a linear calibration curve with a regression coefficient value of 0.966.

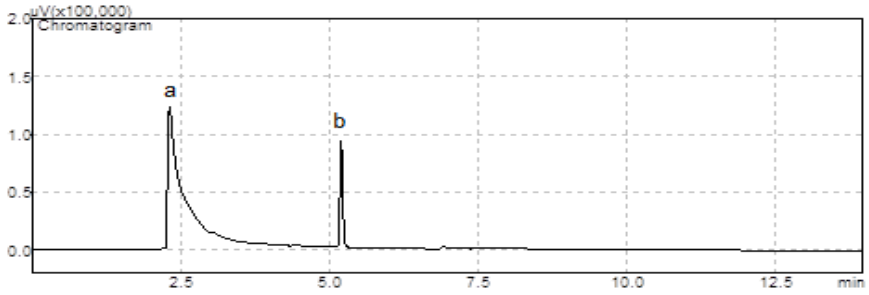

Figure 4.4: Chromatogram representing the peak signals and retention time of (a) Methanol (2.3 min) (b) Chloroiodomethane (5.196 min)

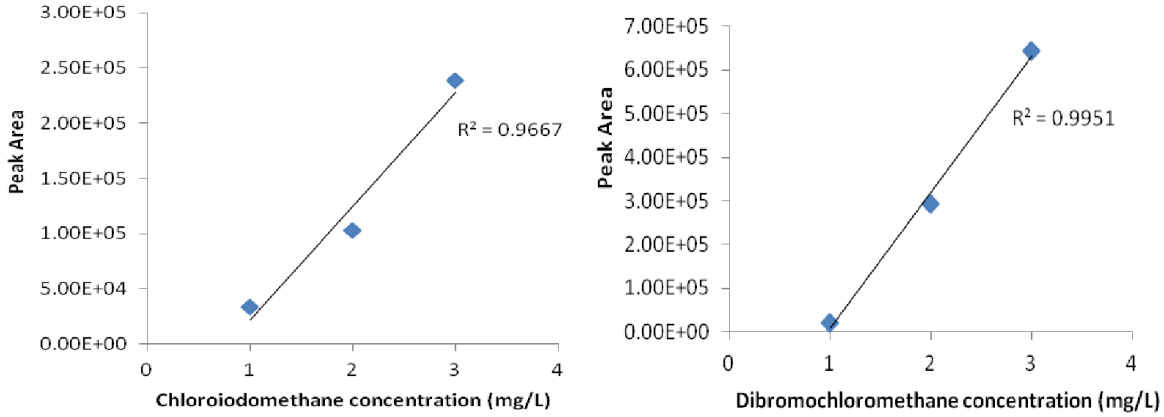

Figure 4.5: Calibration curve of chloroiodomethane and dibromochloromethane (conc. vs peak response)

Figure 4.6 is presenting two very distinct peaks of solvent and dibromochloromethane with retention time of 2.3 and 6.3 min respectively. Boiling point of dibromochloromethane is 120 °C which is higher than both chloroform, bromodichloromethane and chloroiodomethane so it elutes later ($r^2 = 0.995$).

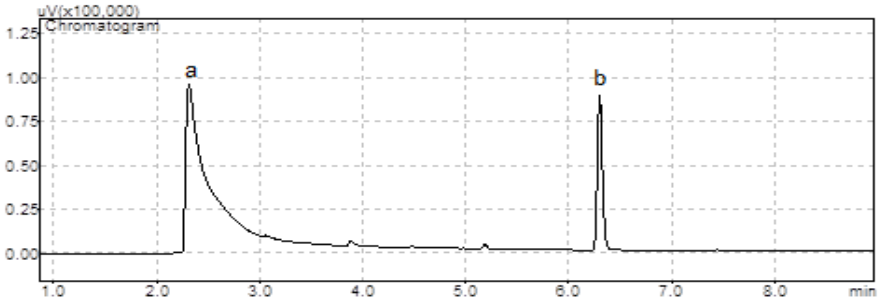

Figure 4.6: Chromatogram representing the peak signals and retention time of (a) Methanol (2.3 min) (b) Dibromochloromethane (6.3 min)

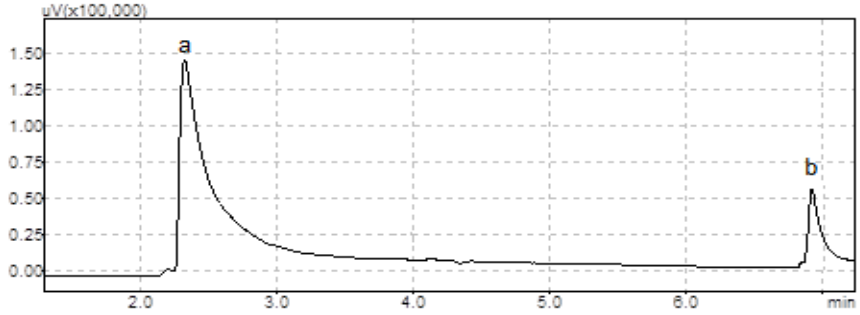

Figure 4.7: Chromatogram representing the peak signals and retention time of (a) Methanol (2.3 min) (b) Bromoform (6.9 min)

Above chromatogram represents the peak of bromoform at 6.9 min while it's boiling point is 149.1 ^{o}C. Peak area of bromoform is relatively small as compared to other analytes. Peak area actually is dependent on the fragmentation of the analyte rather than the concentration of the stock solution. So the compound that produces more fragments will illustrate high peak as it will transmit more current to the detector thus generating more signal.

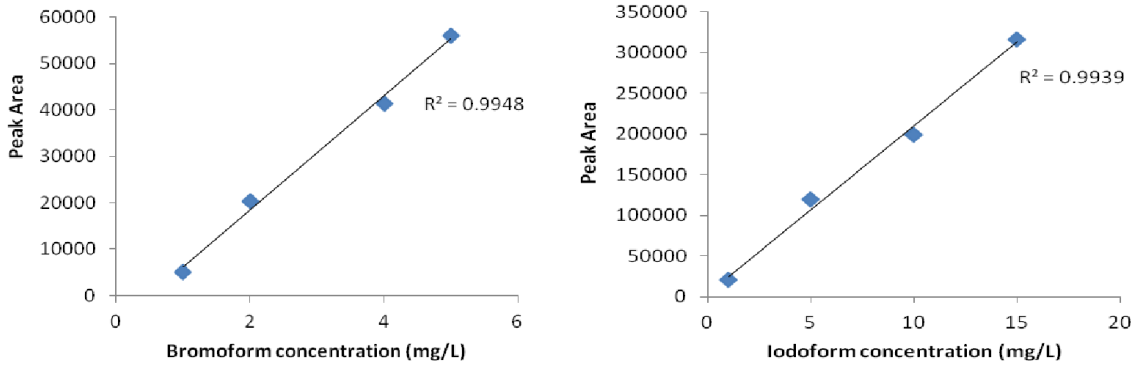

Figure 4.8: Calibration curve of bromoform and Iodoform (conc. vs peak response)

The Figure 4.8 is presenting the calibration curves for bromoform and iodoform having regression coefficient 0.9948 and 0.9939 respectively.

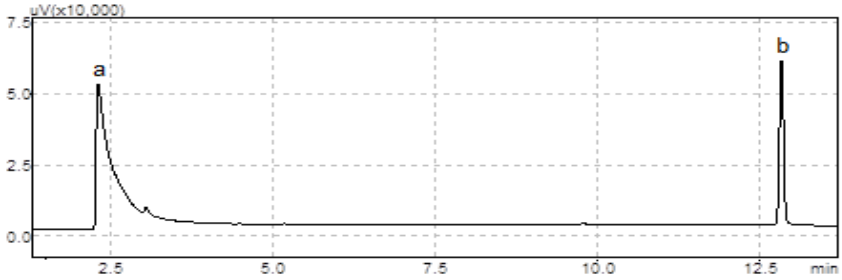

Figure 4.9: Chromatogram representing the peak signals and retention time of (a) Methanol (2.3 min) (b) Iodoform (12.8 min)

Peak of iodoform at 12.82 min shows that it has the highest boiling point among all the analyes i.e. 218 °C and it elutes at the end (Figure 4.9).

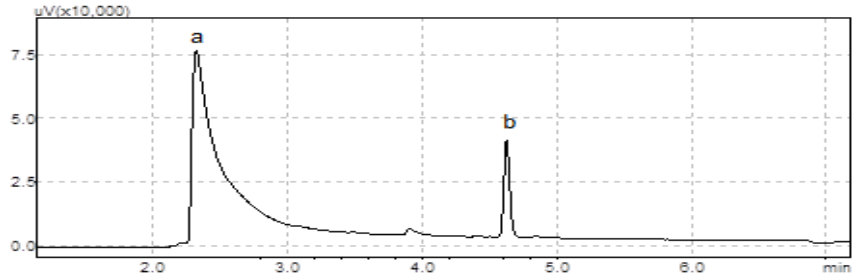

Figure 4.10: Chromatogram representing the peak signals and retention time of (a) Methanol (2.3 min) (b) Carbontetrachloride (4.6 min)

Figure 4.10 is depicting peak of methanol (2.3 min) and second well defined peak of carbontetrachloride (internal standard). It elutes at 4.6 min having 76.72 °C boiling point.

4.2.2 Mixture solution of standard analytes

A series of THMs mixtures were prepared by multiple number of trials *(Annexure I)*.

Table 4.3: Mixture composition of standard analytes

Standard analytes	Spiked concentration (mg/L)	Spiked in 10 mL MeOH (mL)
Iodoform	1	0.45
Chloroiodomethane	1	0.15
Bromoform	1	3
Chloroform	1	3
Bromodichloromethane	1	3
Dibromochloromethane	1	0.01
Carbontetrachloride	1	0.39

The stock solution mixture of analytes was prepared in such a composition that exhibited reproducible and well resolved chromatogram and that was used later for quantification. The most suitable mixture composition is shown in Table 4.3.

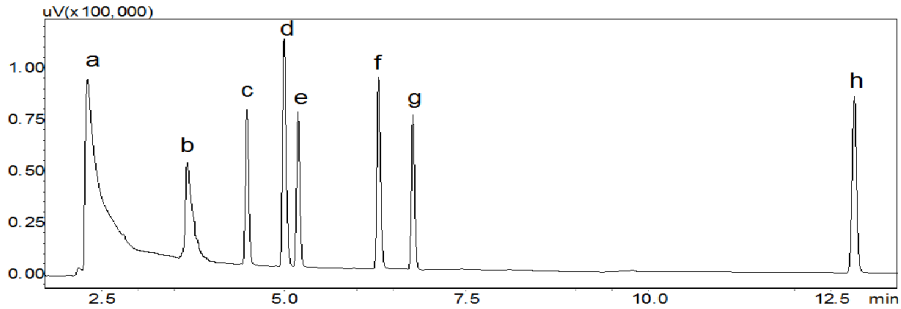

Figure 4.11: Chromatogram of THMs mixture (a) MeOH (b) $CHCl_3$ (c) CCl_4 (d) $CHBrCl_2$ (e) CH_2ClI (f) $CHBr_2Cl$ (g) $CHBr_3$ (h) CHI_3

The final stock mixture was also analyzed using SPME fiber and similar GC conditions which demonstrated even more enlarged peaks (Figure 4.12).

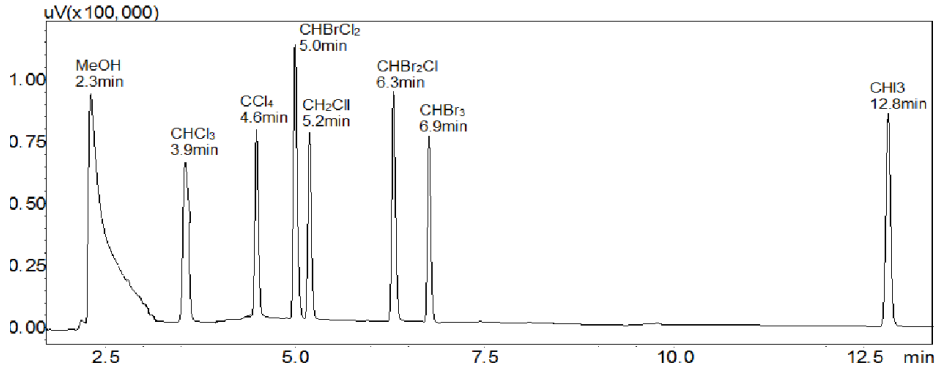

Figure 4.12: SPME stock mixture of trihalomethanes

4.3 Comparison of HS-SPME and LLE techniques

Conventional LLE-GC-ECD and HS-SPME-GC-ECD techniques were used for the comparison of experimental results. Ultrapure water was spiked with THMs mixture at 1 mg/L. Figure 4.13 shows the comparison of techniques with the help of bar chart and unpaired *t*-test. Outcomes for both procedures are given in Table 4.4. Significant differences were found between the two extraction techniques having p-values < 0.1. The results indicated that HS-SPME provides precision and improved results comparable to LLE, with added advantages of being rapid and more sensitive. Analysis of THMs in spiked ultrapure water indicated that this method may be applied to NUST water samples.

Table 4.4: Estimated concentrations and standard deviation of THMs by HS-SPME and LLE

Analytes	LLE-GC-ECD		HS-SPME-GC-ECD	
	Mean (mg/L)	± S.D	Mean (mg/L)	± S.D
CHI_3	0.855	±0.015	1.09	±0.125
CH_2ClI	0.89	±0.30	0.99	±0.0005
$CHBrCl_2$	0.87	±0.040	1.04	±0.115
$CHBr_2Cl$	0.70	±0.040	0.91	±0.01
$CHCl_3$	0.90	±0.025	1.00	±0.01
$CHBr_3$	0.72	±0.030	0.84	±0.0165

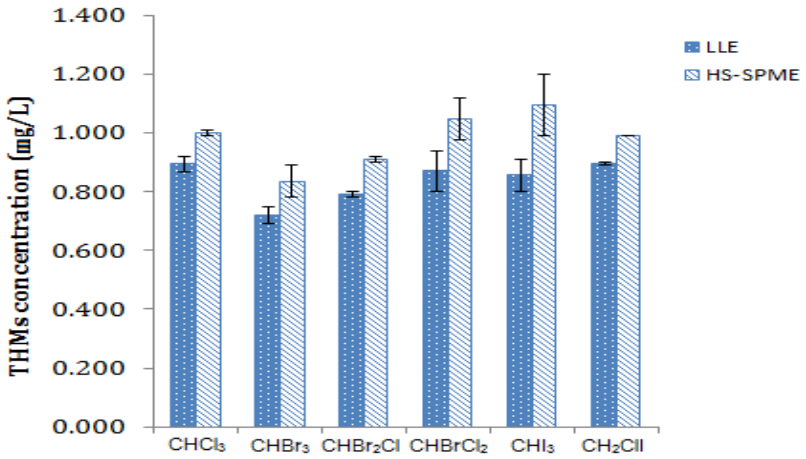

Figure 4.13: Comparison of HS-SPME and LLE technique

4.4 Effect of varying salts on THMs extraction efficiency

A sample was fortified at 1 mg/L level of each THM and salted at 1g with Na_2SO_4 and 1g NaCl whereas the third sample was not salted. The time of extraction and temperature was fixed. The addition of Na_2SO_4 and NaCl was observed to have a considerable effect on the THMs extraction. The use of sodium sulfate (Na_2SO_4) rather than sodium chloride has been recommended due to the effect of bromide ion impurities in NaCl, which were revealed to enhance sample concentrations of brominated DBPs (U.S. EPA 511.1, 1995).

Table 4.5: Effect of different salts on THMs extraction using SPME fiber

ECD peak area counts (n=3)						
Salts	**$CHCl_3$**	**$CHBr_3$**	**$CHBrCl_2$**	**$CHBr_2Cl$**	**CH_2ClI**	**CHI_3**
Control	1.00E+04	1.11E+04	2.33E+04	2.14E+04	1.01E+04	2.24E+04
NaCl	1.51E+04	1.82E+04	2.78E+04	8.95E+04	7.71E+04	3.01E+04
Na_2SO_4	5.86E+04	3.27E+04	9.78E+04	1.00E+05	1.01E+05	9.06E+04

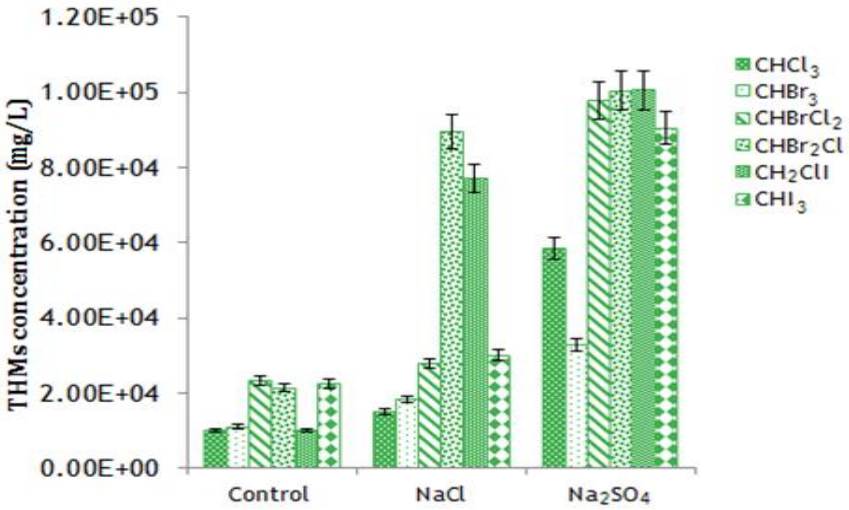

Figure 4.14: Comparison of different salts on THMs extraction efficiency using HS-SPME

The salt addition amplifies the ionic potency of the sample solution and results in dispersion of analytes into the headspace which reduces extraction times (Takamatsu and Ohe, 2003). As depicted in Figure 4.14, Na_2SO_4 was observed to have a major effect on the extraction of all analytes. It was found that with the addition of salt, peak areas of the analytes increased as compared to unsalted (control) samples.

4.5 Testing method performance

4.5.1 SPME linearity, detection limits, precision and accuracy

The precision and accuracy of the optimized HS-SPME–GC method was assessed for drinking water purposes.

The linearity of HS-SPME technique was assessed by the calibration curves. The linear ranges of calibration curves were from 5.60E+04 to 1.77E+06 and the correlation coefficients (r^2) were from 0.9948 to 0.9985 as shown in Table 4.6. Previous studies (Stack *et al*., 2000) indicated that correlation coefficients (r^2) using 100 μm PDMS fiber were 0.9920 to 0.9959 at THMs concentration ranging from 10 to 160 mg/L. In the headspace technique (Kuivinen and Johnsson, 1999), correlation coefficients were 0.996 to 1.00 at THMs concentration of 0.1 to 75 mg/L.

The sensitivity of HS-SPME technique was considered in terms of limit of detection (LOD) and limit of quantification (LOQ). In general, the LOD is taken as the lowest concentration of an analyte that may be identified, but not essentially quantified while the LOQ is the lowest concentration of an analyte under the stated conditions. Kristiana *et al.* (2010) used 100 ng/L standard for I-THMs and 1 μg/L for other THMs for determining detection limits. LOD ranged from 1 ng/L for CHI_3 to 20 ng/L for $CHCl_3$. Accordingly, LOQs ranged between 4 ng/L for CHI_3 and 68 ng/L for $CHCl_3$. In the Table 4.6, the detection limits for LOD and LOQ are 0.001 - 0.06 and 0.021 - 0.183 μg/L respectively. Results validated the proposed HS-SPME-GC/MS method is appropriate for THMs determination at μg/L levels.

The repeatability refers to the analysis of 5 samples in one day and the reproducibility refers to the analysis of 9 samples over 3 different days. The results showed excellent repeatability and reproducibility ranging from 4 to 10 % RSD which represents that the applied method is accurate.

Table 4.6: Demonstration of method performance for THMs determination

Analytes	Linearity range (µg/L)	Correlation coefficient (R^2)	LOD (µg/L)	LOQ (µg/L)	Repeatability (n=5) RSD %	Reproducibility (n=9) RSD %
$CHCl_3$	3.17E+05	0.9949	0.007	0.021	9.388	7.472
$CHBr_3$	2.39E+05	0.9667	0.010	0.030	10.684	10.582
$CHBr_2Cl$	1.43E+05	0.9985	0.060	0.183	6.734	9.809
$CHBrCl_2$	6.45E+05	0.9951	0.052	0.159	4.311	4.500
CHI_3	5.60E+04	0.9948	0.012	0.035	5.939	10.781
CH_2ClI	1.77E+06	0.9384	0.001	0.003	4.688	4.317

4.5.2 Recovery efficiencies of THMs

The percent recovery, R, of each analyte was calculated using U.S. EPA 551.1 method.

R = 100 (A-B)/C

Where,

A = total concentration in the fortified sample

B = background concentration in the unfortified sample

C = fortified concentration

Recovery efficiencies for HS-SPME and LLE methods was calculated to evaluate the appropriate method for THMs determination in water samples. THM recoveries obtained from ultrapure water are illustrated in Table 4.7. The present study exhibited acceptable recovery values between 70 and 100 % for all THMs except bromoform but HS-SPME gave the highest recoveries. The compounds meeting the criteria could be used for the analysis but the compound which does not meet the criteria must be repeated until the satisfactory performance has been achieved. Thus HS-SPME is suggested as a fast, reproducible and inexpensive method for THMs analysis in water. Cancho *et al.* (1999) determined the recovery values close to 100 % by spiking the water samples at a concentration (10, 1.5 and 0.5 mg/L) with I-THMs.

Table 4.7: Comparison of recoveries for THMs and I-THMs by using HS-SPME and LLE methods (n=5)

Extraction Techniques	THMs				I-THMs	
	$CHCl_3$	$CHBr_3$	$CHBr_2Cl$	$CHBrCl_2$	CHI_3	CH_2ClI
HS-SPME/GC/ECD						
% rec (1000 µg/L)	103.3	42.0	98.3	85.96	73.73	83.66
s.d	0.16	0.09	0.16	0.21	0.22	0.24
% r.s.d	16.2	22.08	16.32	24.02	30.48	28.95
LLE/GC/ECD						
% rec (1000 µg/L)	95.0	39.49	91.09	69.5	63.0	79.99
s.d	0.25	0.095	0.211	0.195	0.17	0.20
% r.s.d	26.32	24.06	23.16	28.05	26.98	25.01

4.6 Response surface methodology (RSM)

Response surface methodology and central composite design were used for process optimization in this study. While the software employed to design and analyze response surface experiments was Design-Expert (trail version 9, Stat-Ease, Inc., MN). The results of factor optimization for THMs detection obtained from RSM are discussed below.

4.6.1 Output model graphs

The 2D contour plot of variables comes up by default in graduated color shading. The variety of colors graduated from cool blue to warm yellow. Design-Expert contour plots are highly interactive. In the Figure 4.15, a plot of conversion as a function of salt and extraction time. As indicated by the color key on the right, the surface becomes red at higher response levels and blue for low THMs response, while the rest colors depicts the intermediate response. At any point inside the contour plot, prediction regarding the THMs extraction may also be done.

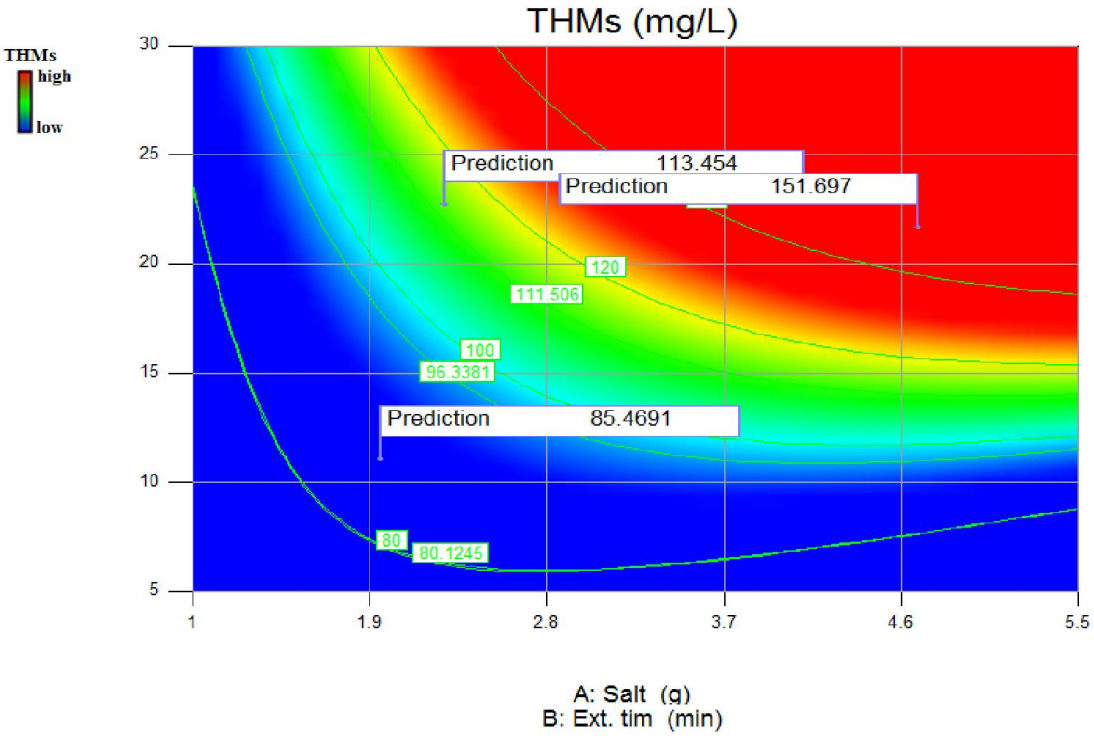

Figure 4.15: Contour plot showing THMs prediction at different regions

In order to find out the response of THMs as a function of two factors chosen for display in the software, the 3D Surface was selected from the floating graphs tool. A three dimensional response surface presented a very compelling picture of how the response can be maximized.

The response surfaces were plotted to find out the optimum values for the two evaluated factors. As depicted in Figure 4.16, the relation between extraction temperature and extraction time was significant when both variables were at high levels in their respective values keeping the other two variables fixed (salt and desorption time). In other words, for a better extraction of THMs, higher extraction temperature and extraction time should be used.

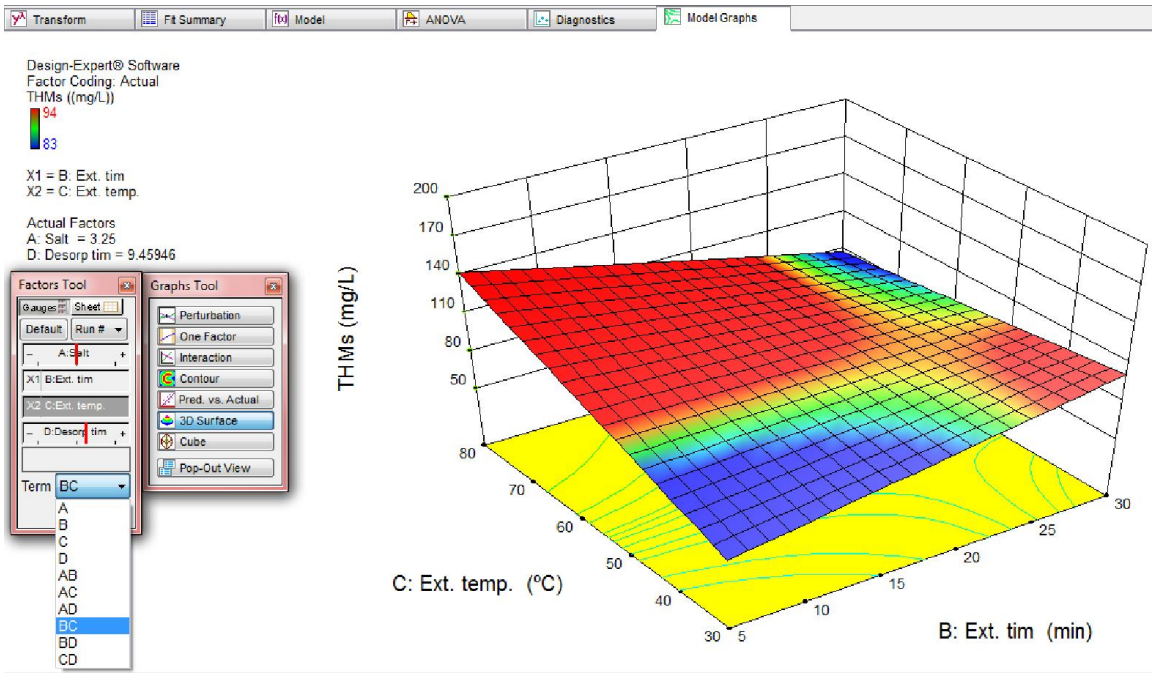

Figure 4.16: 3D response surface of THMs as a function of: extraction time and temperature

Shariati-Feizabadi *et al.* (2003) reported that by increasing the extraction temperature, the rate of THMs extraction using CAR-PDMS fibre was increased. At a higher temperature, diffusion coefficients in water and headspace are greater thus diffusion of the volatile analytes from aqueous phase to the headspace is enhanced.

Experiments done by Allard *et al.* (2012) shows that when temperature was changed from 30 to 70 ºC, the response of the small weight molecules ($CHCl_3$, $CHBrCl_2$, $CHBr_2Cl$) reduced with increase in temperature. However, an obvious increasing trend was observed for all other analytes with increasing temperature. San *et al.* (2007) indicated that bromoform was better extracted at 70 ºC as it was the heaviest, least volatile and least soluble in water (Chen and Her, 2001). The results of the present study supported this finding. Therefore, temperature of 70 ºC was selected to enhance the extraction of the THMs.

The extraction of the THMs was assessed by increasing the extraction time from 5 to 30 min (Pawliszyn, 1997). Acceptable equilibrium state for THMs in this study was achieved at 30 min as shown in Figure 4.16.

San *et al.* (2007) reported that the equilibrium phase was achieved in 5 min for the THMs having lower molecular weights such as, chloroform and bromodichloromethane using PDMS-DVB and DVB-CAR-PDMS fibres. While the extraction of these THMs using the CAR-PDMS fibre was slower, having equilibration times around 40 min. Chen and Her (2001) stated that the equilibrium time was also longer than 30 min by using a CW-DVB fibre.

The salt is added into the solution to enhance the ionic strength which results in increase of volatility of the analytes into the headspace (Takamatsu and Ohe, 2003). The extraction of all analytes were observed to increase with the salt even above the salt saturation of the solution, where a maximum was found around 3.25 g. The experiments were performed by increasing salt amount upto 5.5 g, but as the salt concentration had reached its saturation point, so it was fixed at 3.25 g.

Figure 4.17 illustrates the three-dimensional response surface plots of the THMs extraction affected by different variables. The results suggested that more salt and large extraction times lead to higher THMs.

The fiber was introduced into the GC injection port at high temperature which volatilizes the analytes and transferred into the GC column for separation, hence the injection port temperature and the desorption time are significant parameters that may affect the sensitivity of the analysis.

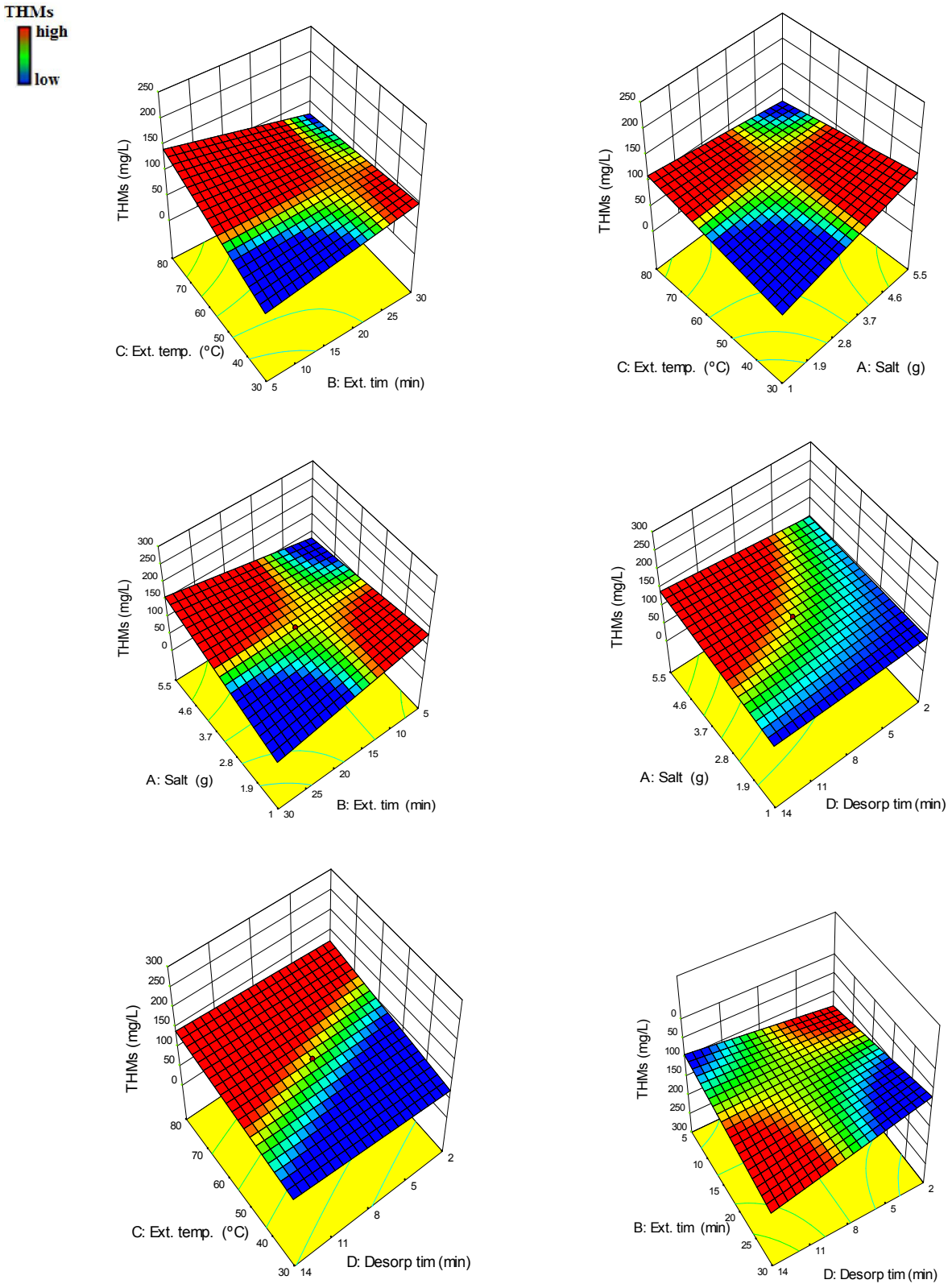

Figure 4.17 3D response surface showing the response of all THMs as a function of extraction temperature, extraction time, desorption time and salt

Desorption temperature is dependent upon the stability of the fiber and the analytes, while in this study it was fixed at 220 ºC. Frazey *et al.* (1998) observed a significant thermal degradation of iodoform beyond 200 ºC temperature.

In the evaluation of desorption time (Figure 4.17), 8 min was enough to ensure total desorption of analytes. The fiber was examined again prior to re-exposure. No peaks appeared in the resulting chromatogram, representing that this time of extraction was enough to remove all analytes from the fiber.

4.6.2 Optimization process

Design-Expert software uses an optimization method described by Myers, Montgomery and Anderson-Cook in *Response Surface Methodology*, 3rd edition, John Wiley and Sons (Myers and Montgomery, 2001). The software gives a ramps view for the optimum factor settings and the desirability of the responses. Based on the variables selected, the numerical optimization was done by the software to achieve maximum THMs extraction. It was observed that the maximum extraction efficiency was obtained when the extraction temperature was maintained at 80 ºC, the extraction time was 30 min, with addition of 3.25 g salt and 8 min of desorption time.

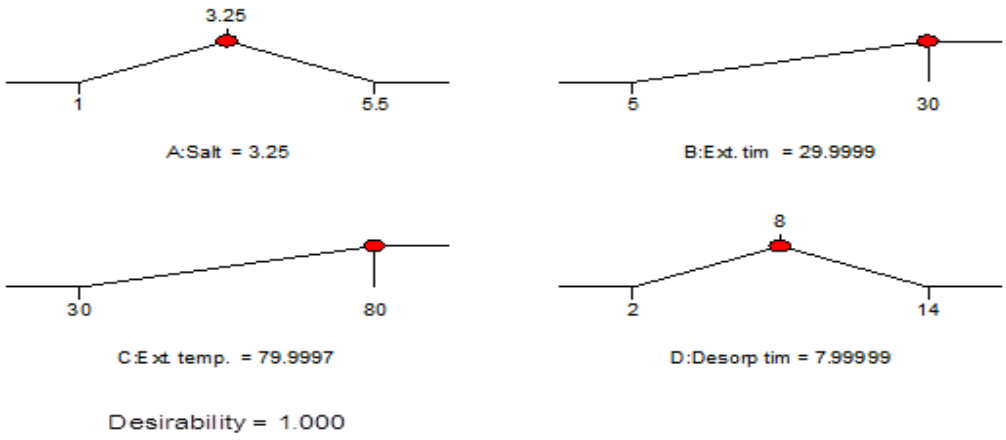

Figure 4.18: Ramp function for maximum THMs response

The ramp function (Figure 4.18) depicts the process conditions that are robust. The colored dot on each ramp reflects the variable setting or response prediction for that process.

After finding the optimum settings based on the RSM models, the next step was to confirm that they actually work. For this confirmation, a node was selected to make response predictions for any set of conditions for the process variables.

Table 4.8 shows that the Design-Expert software uses the model derived from experimental results to predict the level at which highest THMs extraction will be achieved.

Table 4.8: Optimum settings predicted by the RSM model

Confirmation Report					
Factor	**Name**	**Level**	**Low Level**	**High Level**	**Coding**
A	Salt	3.25	1.00	5.50	Actual
B	Ext. time	30.00	5.00	30.00	Actual
C	Ext. temp.	80.00	30.00	80.00	Actual
D	Desorp time	8.00	2.00	14.00	Actual

Response Table 4.9 provides a convenient comparison of the coefficients for all of the responses in terms of coded variables which demonstrates the relative effects.

Table 4.9 Response of contributing factors along p values

Response	**Intercept**	**A**	**B**	**C**	**D**	**AB**	**AC**	**AD**	**BC**	**BD**	**CD**
THMs	111.215	12.92	0.258	*25.04*	10.17	**37.92**	-6.92	9.85	*-33.8*	*32.25*	-1.70
P value		0.204	0.979	*0.019*	0.31	**0.005**	0.57	0.42	*0.011*	*0.015*	0.88
Legend		**p<.01**	*.01<=p<.05*	p >=.10							

The coefficient for AB (salt*extraction time) is 37.92, which is higher than the coefficients for factor C (25.04), BC (-33.82) and BD (32.25), where ‘C’ represents extraction temperature and ‘D’ represents desorption time. This shows that AB interaction influences THMs extraction more than factor C, BC and BD. The p values shows the significance at a glance. The bold

values indicates the significant effect of variables while italics shows less significance and the rest depicts least significance. Thus the order of significance is AB > BC, BD > AC, AD, CD which means salt (A) and extraction time (B) being the most influential factors affecting THMs extraction in drinking water with p value of 0.005 ($p < 0.01$).

The contributing factors based upon the relative degree of significance are summarized in equation 1 mentioned below:

THMs=+111.21+12.93*A+0.26*B+25.04*C+10.17*D+37.93*AB-6.92*AC+9.85*AD-33.82*BC+32.25*BD-1.70*CD **…. Equation 1**

Based upon the ANOVA results and p values given in response Table 4.9, the equation (1) reduces to equation (2) with only those factors which are statistically significant in the formation of THMs in drinking water.

THMs=+111.21+12.93*A+25.04*C+10.17*D+37.93*AB-6.92*AC+9.85*AD-33.82*BC+32.25*BD

…. Equation 2

The equations may be used to make response predictions for each factor.

4.7 Analysis of water samples from distribution network

The optimized RSM model was then applied for the analysis of real water samples from NUST. The analysis of drinking water samples demonstrated contamination at different sites. Some sampling sites in the university were showing high concentration of UV_{254} absorbance, TDS and residual chlorine, the same sites were observed to form higher THMs. These results show a clear correlation between these parameters and THMs. Mentioned below are some of the chromatograms obtained from different sites within the drinking water distribution network of a university.

Figure 4.19 signify the chromatogram acquired from drinking water sample of consumer's end located at location 2 (after chlorination) of NUST. Peaks of all THMs of interest are clearly identifiable and were confirmed by comparing the retention times of standard analytes.

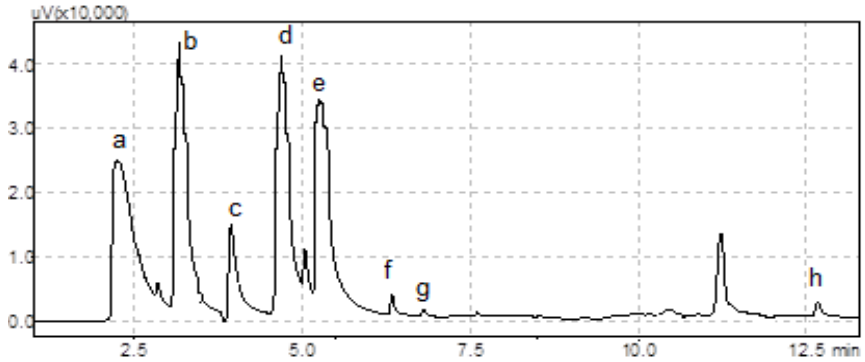

Figure 4.19: Chromatogram of drinking water sample collected from L2A site (a) MeOH (b) $CHCl_3$ (c) CCl_4 (d) $CHBrCl_2$ (e) CH_2ClI (f) $CHBr_2Cl$ (g) $CHBr_3$ (h) CHI_3

Peaks signals for bromodichloromethane (100.5 mg/L) and chloroiodomethane (9.35 mg/L) are relatively large as compared to other compounds. The contamination in consumer's tap is alarming it may be due to any organic source or anthropogenic activities. Table 4.10 shows the respective concentration of THMs in water samples. There are also some unidentifiable compounds in the sample showing peak signals but are none of our interest. The physicochemical characteristics of this site shows comparatively high content of residual chlorine (0.46 mg/L) and UV_{254} absorbance (0.124 cm^{-1}) which are directly related to THM formation. The large peak signal of chloroform shows that high content of chlorine was present at this site to react with organic matter forming THMs, as this site is located from the nearest point to the chlorination source. All the desired THMs were present and were beyond the US-EPA drinking water quality standard values, whereas only bromoform and iodoform met the standards of 100 and 5 µg/L respectively.

Past research has reported that the levels of THMs depend on their precursors particularly the NOM (principal indicators of NOM are TOC and UV absorbance at 254 nm wavelength) present in treated water before disinfection (Singer, 1994). Bergamaschi *et al.* (1999) stated that

UV_{254} exhibited linear relationship having r^2 value of 0.99 between concentration of DOM and UV_{254} absorbance in samples. The concentration of organic matter might be calculated as DOC or UV_{254} as suggested by Muller (1998).

Analysis of sample from consumer's tap of MRC site is shown in Figure 4.20. The chromatogram is showing various peaks of THM contamination. The well resolved and identifiable peaks are of chloroform, bromodichloromethane, chloroiodomethane, dibromochloromethane and iodoform. Bromoform was not detected in the sample. All the THMs except iodoform were exceeding the standard values. Baseline was also found to be unstable. The physicochemical quality showed the presence of NOM, as UV_{254} absorbance and residual chlorine were found to be comparatively higher at this site.

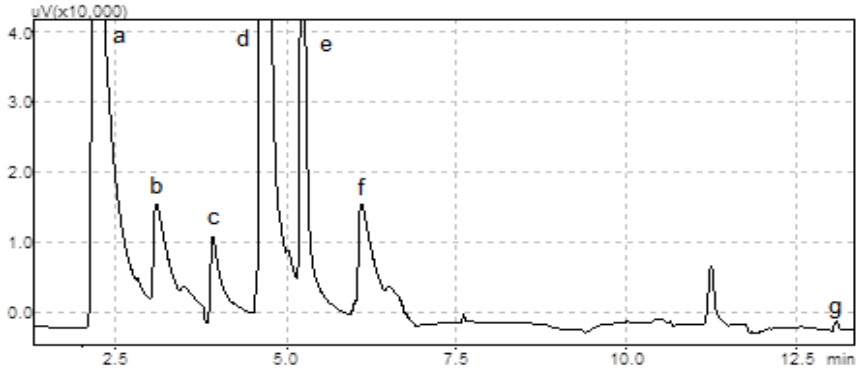

Figure 4.20: Chromatogram of drinking water sample collected from MRC site (a) MeOH (b) $CHCl_3$ (c) CCl_4 (d) $CHBrCl_2$ (e) CH_2ClI (f) $CHBr_2Cl$ (g) CHI_3

Chromatogram from SMME site is displayed in Figure 4.21. The chromatogram is showing several peaks which depicts that the site is highly contaminated with THMs. The values of residual chlorine and UV_{254} absorbance were also high which shows the presence of NOM to form THMs. Bromodichloromethane, chloroiodomethane, dibromochloromethane were present above EPA limits, while the largest peak is of chloroform (232.45 µg/L) which was above US-EPA drinking water quality standard value of 200 µg/L. Negligible amounts of iodoform (0.07 µg/L) and bromoform (0.19 µg/L) were present at this site.

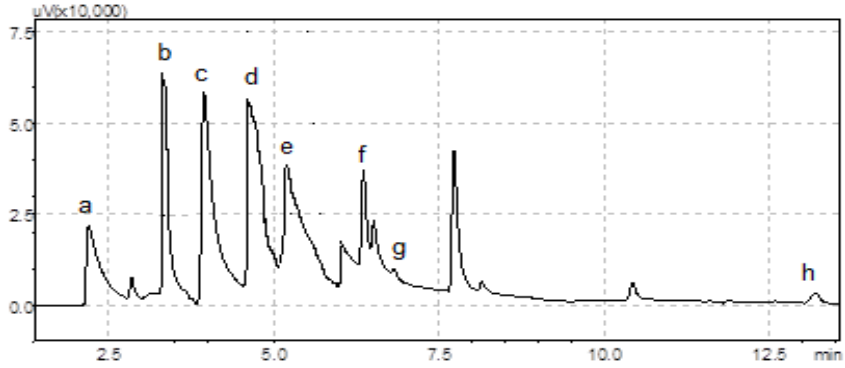

Figure 4.21: Chromatogram of drinking water sample collected from SMME site (a) MeOH (b) $CHCl_3$ (c) CCl_4 (d) $CHBrCl_2$ (e) CH_2ClI (f) $CHBr_2Cl$ (g) $CHBr_3$ (h) CHI_3

A Chromatogram of the drinking water sample collected from IA site is illustrated in Figure 4.22. The physicochemical water quality of these sites shows low concentration of UV_{254} absorbance and residual chlorine which results in low quantity of THMs formation. The well-defined and sharp peaks are of chloroform, chloroiodomethane and bromodichloromethane and were found to be within threshold levels. While rest of the THMs were not reported in the sample. Baseline of the chromatogram is also stable and showing less contamination.

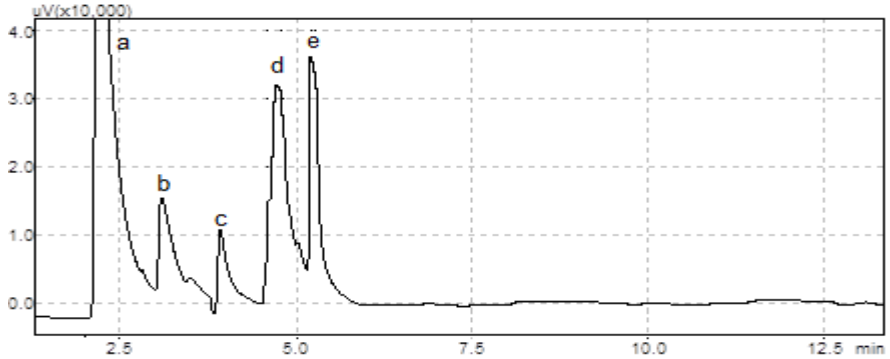

Figure 4.22: Chromatogram of drinking water sample collected from IA site (a) MeOH (b) $CHCl_3$ (c) CCl_4 (d) $CHBrCl_2$ (e) CH_2ClI

Table 4.10 demonstrates the detail of THMs (µg/L) quantified in water samples from distribution network of a university. Among 24 sites, 90 % of the samples were found contaminated with THMs while 30 % sites exceeding the standard and the threshold values.

Table 4.10 Analysis of THMs (µg/L) in drinking water distribution network of NUST

Sampling locations	Regulated THMs (USEPA limits µg/L)				Non-Regulated THMs (Threshold limits µg/L)	
	$CHBrCl_2$ (60)	**$CHBr_2Cl$ (100)**	**$CHCl_3$ (200)**	**$CHBr_3$ (100)**	**CH_2Cl (5)**	**CHI_3 (5)**
L1	***63.5**±0.35	0.865±0.075	110.2±0.25	16.7±0.04	BDL	BDL
L2B	26.0±0.1	60.5±0.5	1.3±0.1	BDL	0.035±0.15	BDL
L2A	**71.0**±0.1	**100.5**±0.5	**202.3**±1.52	82.1±0.12	**9.35**±0.15	0.21±0.15
L3B	11.2±0.5	22.3±0.59	44.8±0.2	BDL	0.014±0.01	BDL
L3A	**64.3**±0.5	**111.2**±0.55	**233.4**±0.55	46.6±0.25	**7.65**±0.06	0.12±0.1
IESE	**69.4**±0.4	2.0±0.1	0.1±0.01	BDL	**94.95**±0.15	BDL
CNM	**60.6** ±0.45	**100.2**±0.001	**200.3**±0.33	BDL	**5.54** ±0.05	BDL
MRC	**62.0**±0.1	**102.2**±0.07	**211.2**±0.01	43.4±0.06	**101.1**±0.2	0.012±0.001
TW8B	22.3±0.025	13.4±0.15	56.5±0.25	BDL	3.42±0.025	0.022±0.1
TW8A	**81.2**±0.3	**113.09**±0.1	**223.4**±0.55	0.14±0.01	**12.9**±0.4	0.433±0.12
B1	54.7±0.7	0.3±0.02	45.4±0.25	66.5±0.06	**12.1**±0.1	0.04±0.001
SMME	**64.1**±0.015	**101.5**±0.009	**232.45**±0.55	0.19±0.01	**11.70**±0.105	0.07±0.001
IGIS	0.83±0.03	2.16±0.06	**221.5**±1.5	0.9±0.1	4.8±0.1	BDL
Ad	4.02±0.02	11.55±0.25	**226**±1.0	BDL	1.15±0.15	BDL
FH	0.61±0.01	3.66±0.27	**224.3**±2.0	BDL	4.5±0.55	0.3±0.015
ZH	0.76±0.02	3.11±0.11	22.2±0.025	32.2±0.25	3.9±0.05	0.135±0.045
C1	22.3±0.025	3.34±0.25	113.95±2.05	BDL	2.3±0.4	0.14±0.15
MI	32.4±0.15	55.6±0.4	11.2±0.05	BDL	3.5±0.15	BDL
IA	11.4	BDL	101.2±0.15	BDL	BDL	BDL
Isra	35.6±0.25	5.43±0.15	BDL	BDL	BDL	BDL
GH	46.5±0.4	BDL	BDL	BDL	BDL	BDL
RH	BDL	9.87±0.06	76.8±0.35	BDL	4.3±0.05	BDL
MO	33.4±0.4	BDL	87.6±0.25	BDL	1.1±0.012	BDL
AH	55.3±0.25	44.5±0.064	86.5±0.55	BDL	BDL	0.03±0.15

*mean±standard deviation; BDL = Below Detection Limit; Bold values = Above detectable limits (ADL)

The highest mean concentration observed was 233.4 µg/L for chloroform (80 > 200 µg/L) at site L3A. Almost 90 % of the samples were found contaminated with $CHBr_2Cl$ but only 6 sites exceeded the EPA limits of 100 µg/L. $CHBrCl_2$ was above EPA limits in almost 33 % of the sites, whereas site TW8 showed highest mean concentrations for $CHBrCl_2$ (81.2 > 60 µg/L). $CHBrCl_2$ was the most dominant THM recorded in almost 95 % of the samples. CH_2ClI was found in 75 % of the samples, while 8 sites exceeded the threshold values, where highest mean value (101.1 µg/L) was observed at MRC site. CHI_3 was detected comparatively at low concentrations (0.012 - 0.433 µg/L) in almost 45 % samples and in all the sites it was found within the taste and odor threshold values (0.2 - 5 µg/L). The potential reason for contamination at different points were presence of natural organic matter (important precursor) and residual chlorine.

Chapter 5

CONCLUSIONS

The present study was designed to quantify the THMs in drinking water samples through an optimized HS-SPME technique by using GC-ECD. The outcomes of the research work are summarized as follows:

1. The concentrations of physical and chemical parameters (pH, temperature, UV_{254}, residual Cl_2, TDS, turbidity, DO etc.) of drinking water samples meet the permissible limits recommended by WHO.
2. Calibration of standards were done to get reproducible peaks and linear calibration curves. The retention times of analytes (CHI_3, CH_2ClI, $CHBrCl_2$, $CHBr_2Cl$, $CHCl_3$, and $CHBr_3$) were calculated as 12.82, 5.2, 5.0, 6.3, 3.9 and 6.9 min respectively.
3. The HS-SPME and liquid liquid extraction techniques were performed to achieve the maximum THMs response. The results showed significant ($p < 0.1$) increase in peak areas for HS-SPME, which is an excellent alternative extraction technique comparable to liquid liquid extraction.
4. The Response Surface Methodology used for process optimization in this study revealed that the optimum conditions for THMs extraction were 30 min extraction time at 80 ^oC with addition of 3.25 g Na_2SO_4 salt and 8 min of desorption time.
5. The optimized RSM model applied for the analysis of real water samples demonstrates the prevalence of THMs in the samples. The results indicated presence of THMs in 90 % of the samples, with 30 % sites exceeding the U.S.EPA standard value of 80 μg/L. Results revealed a strong link between concentration of UV_{254} and TOC with THMs formation.
6. The accuracy of the developed HS-SPME-GC/ECD method was validated by the linear range,

detection limits and precision for each analyte. Acceptable regression coefficients were found to be 0.9948 - 0.9985. Excellent repeatability and reproducibility ranging from 4 to 10 % RSD were also found. The present study exhibited highest recoveries (50 - 100 %) achieved for HS-SPME procedure, demonstrating that the method is applicable for analysis of drinking water samples and the matrix effects were negligible.

The results showed the variability of the THMs concentration in the distribution network. Emphasis may be given to decrease formation of THMs while at the same time upholding a microbiologically safe product.

5.1 Future suggestions

A baseline data for THMs specially iodinated THMs in drinking water distribution network was generated during this research study. Following are some of the future suggestions regarding the present work.

1. Epidemiological and genotoxicity studies of THMs exposure to human cells/blood may be carried out to identify toxic levels using comet assay or various other techniques.
2. A number of other factors effecting THMs formation can also be determined, such as; effect and dosage of disinfectant, distance, contact time, seasonal variation, pipe age and material etc.
3. Different methods of THMs removal from drinking water sources may be studied as they are potential human carcinogens. The strategies to control THMs concentration including; optimization of the disinfection process, reverse osmosis, enhanced coagulation, microfiltration or nanofiltration and use of granular activated carbon (GAC) filters for NOM removal before disinfection process.

Chapter 6

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Table 1: Trihalomethanes **(**THMs) mixture solution #1

Standard analytes	Spiked concentration (mg/L)	Spiked in 10 mL MeOH (mL)	Retention times (min)
Iodoform	1	0.6	12.8
Chloroiodomethane	1	0.3	5.2
Bromoform	1	2	6.9
Chloroform	1	3.3	3.9
Bromodichloromethane	1	3.3	5.0
Dibromochloromethane	1	0.1	6.3
Carbontetrachloride (I.S)	1	0.4	4.6

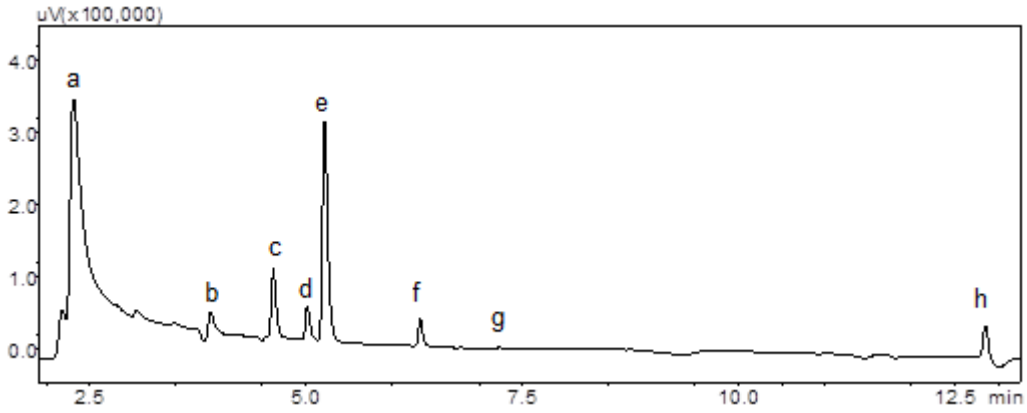

Figure 1: Chromatogram of THMs mixture #1 (a) Methanol (2.3 min) (b) Chloroform (3.9 min) (c) Carbontetrachloride (4.6 min) (d) Bromodichloromethane (5.0 min) (e) Chloroiodomethane (5.2 min) (f) Dibromochloromethane (6.3 min) (g) Bromoform (6.9 min) (h) Iodoform (12.8 min)

Table 2: Trihalomethanes (THMs) mixture solution #2

Standard analytes	Spiked concentration (mg/L)	Spiked in 10 mL MeOH (mL)	Retention times (min)
Iodoform	1	1	12.8
Chloroiodomethane	1	1	5.2
Bromoform	1	2.5	6.9
Chloroform	1	2.5	3.9
Bromodichloromethane	1	2	5.0
Dibromochloromethane	1	0.5	6.3
Carbontetrachloride (I.S)	1	0.5	4.6

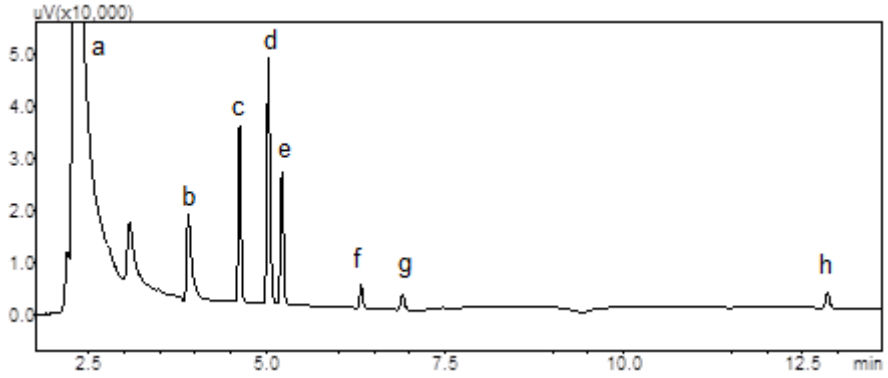

Figure 2: Chromatogram of THMs mixture #1 (a) Methanol (2.3 min) (b) Chloroform (3.9 min) (c) Carbontetrachloride (4.6 min) (d) Bromodichloromethane (5.0 min) (e) Chloroiodomethane (5.2 min) (f) Dibromochloromethane (6.3 min) (g) Bromoform (6.9 min) (h) Iodoform (12.8 min)

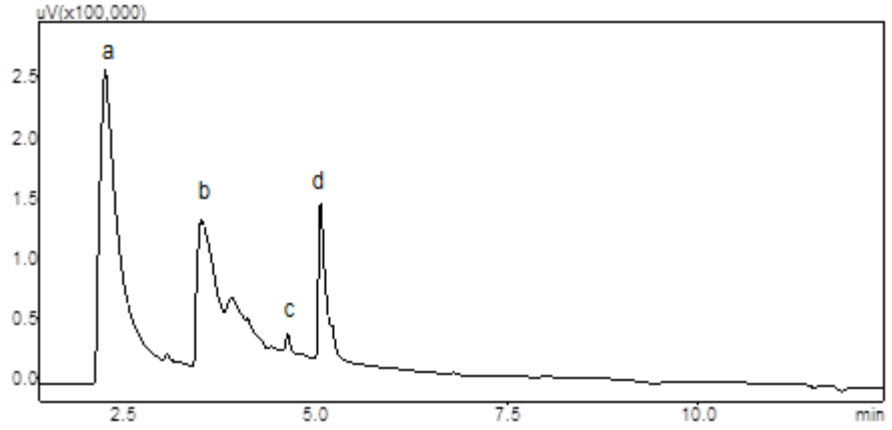

Figure 1: Chromatogram of drinking water sample collected from IGIS site (a) Methanol (2.3 min) (b) Chloroform (3.9 min) (c) Carbontetrachloride (4.6 min) (d) Bromodichloromethane (5.0 min)

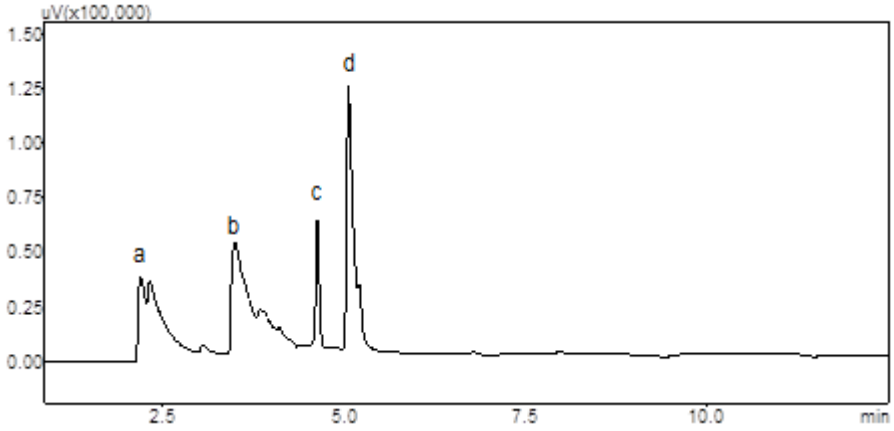

Figure 2: Chromatogram of water sample collected from FH site (a) Methanol (2.3 min) (b) Chloroform (3.9 min) (c) Carbontetrachloride (4.6 min) (d) Bromodichloromethane (5.0 min)

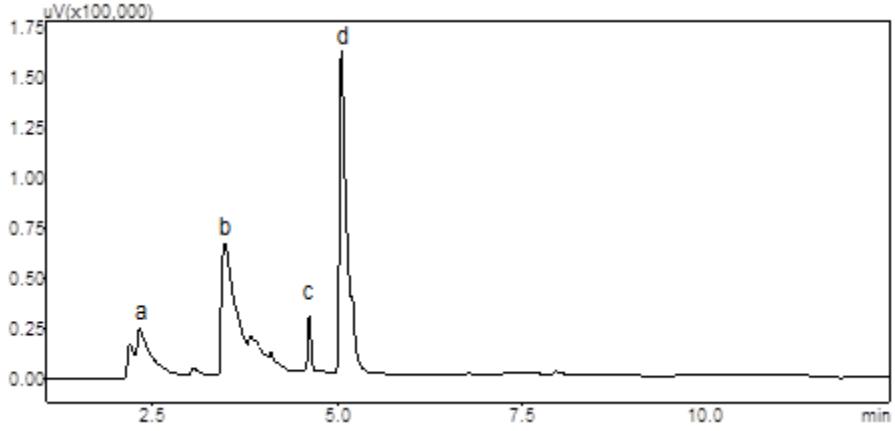

Figure 3: Chromatogram of water sample collected from Ad site (a) Methanol (2.3 min) (b) Chloroform (3.9 min) (c) Carbontetrachloride (4.6 min) (d) Bromodichloromethane (5.0 min)

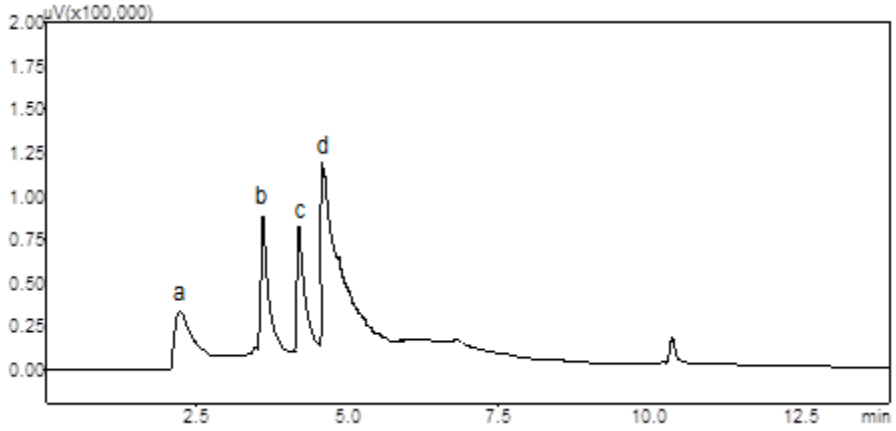

Figure 4: Chromatogram of drinking water sample collected from IESE site (a) Methanol (2.3 min) (b) Chloroform (3.9 min) (c) Carbontetrachloride (4.6 min) (d) Bromodichloromethane (5.0 min)

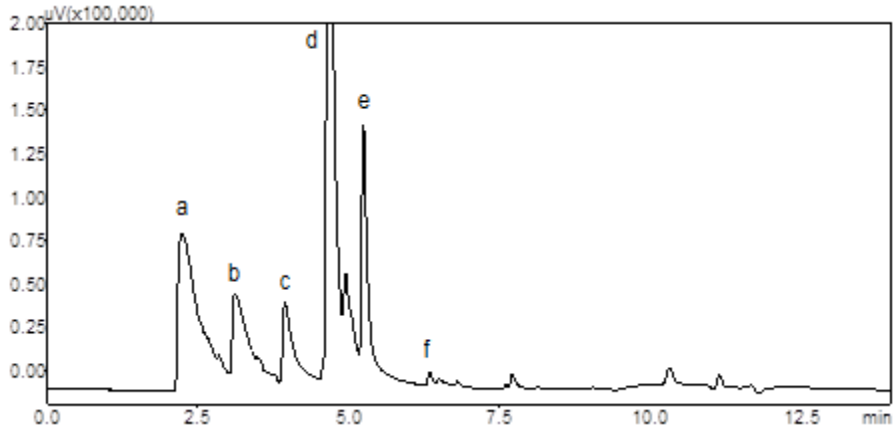

Figure 5: Chromatogram of drinking water sample collected from L3A site (a) Methanol (2.3 min) (b) Chloroform (3.9 min) (c) Carbontetrachloride (4.6 min) (d) Bromodichloromethane (5.0 min) (e) Chloroiodomethane (5.2 min) (f) Dibromochloromethane (6.3 min)