DETERMINATION OF TRACE LEVEL HALOACETIC ACIDS, PERCHLORATES AND CHLOROPHENOLS IN WATER SAMPLES USING MICROEXTRACTION TECHNIQUES

BY

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To

My loving parents, wife, and children for their prayers & Support

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LIST OF ABBREVIATIONS

ASE	:	Automated soxhlet extraction
BET	:	Brunauer-Emmett-Teller
BJH	:	Barrett-Joyner-Halenda
BMIM[PF_6]	:	1-butyl-3-methylimidazolium hexafluorophosphate
CB-LPME	:	Cotton bud-Liquid phase microextraction
CE	:	Capillary Electrophoresis
CTAB	:	Cetyltrimethylammonium bromide
CW	:	Carbowax
DTE	:	Direct thermal extraction
EDX	:	Energy dispersive x-ray analysis
EF	:	Enrichment Factor
EME/IC	:	Electromembrane extraction/Ion chromatography
FIA	:	Flow injection analysis
GFAA	:	Graphite furnace atomic absorption spectrometry
HAAs	:	Haloacetic acids
HFM	:	Hollow fiber membrane

ICP/MS	:	Inductively-coupled plasma/mass spectrometry
LC/MS	:	Liquid chromatography/mass spectrometry
LLE	:	Liquid-liquid extraction
ESI-MS	:	Electron spin ionization-mass spectrometry
GC-ECD	:	Gas chromatography-electron capture detector
GC-MS-MS	:	Gas chromatography-tandem mass spectrometry.
LLLME	:	Liquid-liquid-liquid microextraction
LOD	:	Limit of detection
LOQ	:	Limit of quantitation
LPME	:	Liquid phase microextraction
MAE	:	Microwave-assisted Extraction
PA	:	Polyacrylate
PDMS	:	Polydimethyl siloxane
PEEK	:	Polyether ether ketone
PFE	:	Pressurized fluid extraction
PP	:	Polypropylene
Ppb	:	Parts per billion

RSD : Relative standard deviation

SDME-GC-MS : Single drop microextraction-gas chromatography-mass spectrometry

SEM : Scanning electron microscopy

SFE : Supercritical fluid extraction

SPE : Solid phase extraction

SPME : Solid phase microextraction

UPLC-UV : Ultra performance liquid chromatography-ultraviolet detector

USE : Ultrasonic solvent extraction

USEPA : United States Environmental protection agency

UV-VIS : Ultraviolet / Visible Spectroscopy

µ-SPE : Micro-solid phase extraction

HS-HFLPME-GC-ECD : Head space-hollow fiber liquid phase microextraction electron capture detector

ABSTRACT (ENGLISH)

Full Name : HAKIMU NSUBUGA

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Haloacetic acids, perchlorates and chlorophenols are chemical contaminants of mainly anthropogenic origin. These compounds are toxic, persistent and cosmically distributed environmental pollutants. The determination of these compounds in environmental matrices requires appropriate sample extraction methods since their presence is in complex matrices and at trace level concentrations. Environmental analysis is geared towards improvements in selectivity, sensitivity and automation. Notable among the recent analytical techniques are simple, affordable, faster and greener microextraction techniques. This thesis work therefore focusses on the development of solvent-minimized extraction techniques which are simple, inexpensive and use simple equipments found in most analytical laboratories. Influencing parameters and the applicability of the developed methods to environmental contaminants have all been carefully investigated and evaluated. In the first part of this thesis, the potential of micro solid phase extraction (μ-SPE) method for the enrichment of trace level haloacetic acids in swimming pool waters by ultra-performance liquid chromatography-ultra-violet detection (UPLC-UV) is

demonstrated. Novel μ-SPE sorbent is synthesized from rice husk waste material and impregnated with iron oxide via sol-gel process to improve its extractability. Extraction parameters that influence the method efficiency are investigated. When compared to what is reported in literature, our developed method proves to be more effective for trace analysis of haloacetic acids in swimming pool water. In the second part of this study, microwave assisted extraction (MAE) followed by electromembrane extraction-ion chromatography - Conductivity detection (EME/IC) is optimized and used to quantify perchlorate ions in sea food samples collected from the eastern province of Saudi-Arabia. The study attempted to include all the seafood stuffs consumed in the eastern province of Saudi Arabia. It is the first report on the combination of MAE with EME/IC and resulted to higher preconcentration and efficient sample clean-up. MAE-EME-IC is demonstrated to be a promising combination for trace level perchlorate ion analysis in seafood samples. An electromembrane extraction of the MAE extract is performed to improve the detection limits and minimize co-elution of interfering ions. In the last part of our study, a novel liquid phase microextraction approach is developed in which a piece of cotton wool acts as an extractant solvent holder for the determination of phenolic compounds in sea water. Basing on the absorptive properties of wool, the target analytes can easily be trapped within its pores which are later desorbed using a suitable solvent. Different parameters that influence the extractability of phenolic compounds are also investigated. In combination with UPLC-UV detection, our proposed method provides relatively good enrichment factors which compares quite well with those of hollow fiber membrane liquid phase microextraction. The procedure is also cost effective and easy to operate.

ABSTRACT (ARABIC)

ملخص الرسالة

الاسم نسوبوجا

عنوان الرسالة: تقدير مستويات التراكيز القليلة من حموض الهالوأسيتك، البيركلورات و الكلوروفينول في المياه بإستخدام طرق الإسخلاص الميكروني

التخصص: الكيمياء

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مركبات حموض الهالواستيك، البيركلورات والفينول هي ملوثات كيميائة منشأئها بشري وهي مقاومة التكسر، و هذه المركبات سامة تنتشر بشكل واسع كملوثات بيئية. معظم هذه المركبات ضارة للإنسان ويتوقع ان تكون مواد مسرطنة وإن كانت موجودة بتراكيز قليلة جدا في حدود جزء بالبليون **(ppb).** تقديرها في الأوساط البيئية معقد جدا حيث ان وجودها يكون في تراكيز قليلة جدا في اوساط معقدة مثل الطعام، مياه الصرف، التربة، الدم، مياه البجر والمياه العادمة. التحليل البيئي يهدف لتحسين الإنتقائة (بسبب اوساط العينات المعقدة التي تحتويها) و الحساسية (وذلك بسبب حدود التراكيز القليلة جدا للملوثات الميكرونية) و الأتمتة (وذلك لزيادة الإنتاجية). و من ابرز تقنيات التحليل الحديثة، البسيطة، السريعة و الصديقة للبيئة طرق الإستخلاص الميكروني.

العمل المتضمن في رسالة البحث هذه يركز على تطوير طريقة إستخلاص مستخدمة لكميات قليلة من المذيبات، بسيطة، غير مكلفة و تحتاج لأدوات وتجهيزات متوفرة في معظم المختبرات التحليلية.لقد تم تفحص وتقيم جميع تحسين المعايير و التوافقية للطريق المطورة للملوثات البيئية **(μ-SPE)**. وأولها تقدير تركيز احماض الهالواسيتك عن طريق الإستخلاص المرحلي الصلب الميكروني بتراكيز قليلة جدا في مياه أحواض السباحة

بأستخدام جهاز كروماتوغرافيا السائل ذات الأداء العالي المقترن بمقدر التركيز ذو الأشعة فوق البنفسجية **(UPLC-UV)** حيث انه تم تطوير المادة المدمصة في أداة **(µ-SPE)** والتي تم صناعتها من قشور الأرز بالاضافة الي أكسيد الحديد (iii) والذي تم اضافته بتقنية **(sol-gel)** والذي يزيد من مقدرة المادة المدمصة علي استخلاص احماض الهالوأستيك ولقد تم أيضاً دراسة كل العوامل التي تؤثر علي كفاءة عملية الأستخلاص وعندما تمت مقارنة النتائج المتحصل عليها والنتائج في الدراسات السابقة قد ثبت أن الطريقة المطورة أكثر كفاءة في عملية استخلاص التراكيز القليلة جداً لأحماض الهالوأستيك الموجودة في مياه أحواض السباحة.

أما الجزء الثاني من الدراسة أستخدمت فيه تقنية الأستخلاص المدعم بأشعة المايكرو ويف **(MAE)** المقترن بكل من الغشاء الكهربي وجهاز الكروماتوغرافيا الأيونية **(EME/IC)** بالاضافة الي مقدر التركيز ذو التوصيل الكهربي للأيونات ولقد تم ضبط الظروف المثلي لتحليل أيونات البيركلورات في عينات الأطمة البحرية والتي تم جمعها في اماكن مختلفة من المنطقة الشرقية في المملكة العربية السعودية . وهذه أول دراسة أجريت باستخدام **MAE** المقترن ب **(EME/IC)** لتقدير أيونات البيركلورات في عينات الأطعمة البحرية وقد ثبت ان الطريقة لها مقدرة عالية لتركيز أيونات البيركلورات قبل أدخلها لجهاز الكروماتوغرافيا الأيونية وتحليل التراكيز القليلة جداً وايضاً تم تحسين حد التقدير وتقليل المتدخلات الأيونية .

أخيراً أبتكار طريقة لقدير المركبات الفينولية في عينات مياه البحر بأستخدام قطع قطنية كمادة حاملة للمذيب المستخلص لهذه المركبات أعتماداً علي الخاصية الادمصاصية للقطن حيث يتم ادمصاص المركبات الفينولية عليه ومن ثم استخدام مذيب مناسب لاجراء عملية الأستخلاص السائل المايكروني ولقد تم تحديد كل العوامل التي تؤثر علي الأستخلاص وكفاءته ومن ثم استخدام جهاز **(UPLC-UV)** لقياس تركيز هذه المركبات والنتائج المتحصل عليها أثبتت أن هنالك تحسين جيد لهذه الطريقة مقارنة بطريق الاستخلاص الميكروني السائل باستخدام الغشاء المفرغ المصنوع من مادة الفايبر علاوة علي ذلك أستخدام مواد رخيصة الثمن والطريقة سهلة الاجراء .

CHAPTER 1

INTRODUCTION

1.1 Background of the study

To an analytical chemist, continuous improvement in the trace level analysis of toxic pollutants is a great milestone [1]. Haloacetic acids, perchlorates and phenolic compounds represent an important class of pollutants whose applications range from industrial to agricultural sectors. Some of these applications owe their importance to the pollutants' chemical inertness and stability towards oxidation or reduction. As a result, most of them are persistent in the environment; toxic and bioaccumulative in the food chain. They are harmful to humans and suspected carcinogens even at low parts per billion (ppb) levels. Several studies reveal that these compounds potentially evoke mutagenic responses that result to dermal toxicity, immunotoxicity, carcinogenicity and adverse effects on the reproductive and endocrine systems. As such, their widespread usage is of major environmental and health concern. Surprisingly, some of these pollutants have been detected in drinking water, food samples and water treatment plants. In the past decades, most effort in the analytical field has focused on the development of instruments to speed up the analysis and increase method sensitivity towards trace level analysis. Since most analytical instruments cannot handle the sample matrix directly, sample preparation is fundamental to achieve a practical and reliable method for the

analysis of complex matrices [2-4]. Experience has shown that sample preparation is often perceived as the bottleneck in any analytical method [5].

Currently, the development of solvent-minimized sample preparation techniques is of significance worth of very exciting area of research.

1.2 Problem Statement

A myriad of harmful organic and inorganic compounds have been flushed into the environment since the advent of industrialization [6]. A great many of these compounds are now under scrutiny for their possible adverse health effects. Chemical determination of these compounds in environmental samples is needed to estimate the risk that these compounds pose to humans and wildlife and to support decision-making about protection and legislation [7]. Actually most of them have been listed as priority pollutants by USEPA yet their presence in water matrices is at trace level concentrations [8]. In the past decades, most analytical efforts have focused on the development of sensitive instruments to speed up the analysis. However, most of them cannot handle the sample matrix directly. The major drawbacks encountered during environmental analysis, are the minimization or elimination of matrix components that interfere with the target compounds and the attainment of low detection limits [4-7].

Enrichment and matrix removal procedures are desirable as the conventional detection methods coupled with separation techniques do not meet the required sensitivity for trace level concentrations of these contaminants.

In this regard, miniaturization offers solutions that are cheap, faster, simpler and more environmentally appealing than conventional ones.

1.3 Justification of the study

Over the past decades, a lot of effort has been dedicated towards trace level determination of environmental pollutants in water matrices. The main problem arises from their differences in volatilities, complex matrix composition, very low concentration levels, lack or restricted reference standards, tedious and time consuming sample preparation operations [2].

Hence; recent research effort has driven towards the development of new technologies aimed at increasing the efficiency of sample preparation, improved data collection and facilitating data analysis. Conventional methods used in the extraction of these contaminants from the water matrices such as liquid–liquid extraction (LLE) and solid phase extraction (SPE) requires large amounts of hazardous high-purity organic solvents and their performance is time consuming [4-5].

1.4 Objectives of the study

The overall objective is to assess the potential formation of haloacetic acid, perchlorates and chlorophenols in the environmental water samples using miniaturized analytical techniques. The overall objective will be achieved by the following specific objectives:

i. Developing and optimizing miniaturized analytical techniques for, haloacetic acids, perchlorates and chlorophenols.

ii. Comparing the performance of the developed methods with the reported literatures in terms of extraction efficiency, robustness, portability and simplicity.

iii. Applying the developed methods to the determination of the target analytes in portable water samples and sea foods from eastern province of Saudi Arabia.

1.5 **Related literature**

1.5.1 **Sample preparation techniques**

In environmental analysis, sample preparation is often perceived as the most time-consuming step as it tends to be slow and labour-intensive. It is geared towards analyte enrichment, sample cleanup, and signal enhancement. Ease of automation is increasingly becoming a desirable attribute for sample preparation. Hence, for an efficient sample preparation, the following have to be prioritized [3, 5];

i) minimal sample loss yet with good yield of the target analyte;
ii) efficiency in removing coexisting components;
iii) convenience and fastness of the procedure;
iv) Cost effectiveness of the analytical process.

Traditionally, solid-phase extraction and liquid-liquid extraction are the most commonly used techniques for sample preconcentration and clean-up prior to chromatographic analysis [7].

However, these sample-preparation techniques have limitations such as time-consuming procedures, use of large amounts of both sample and organic solvents and difficulty to automate. Notably, with long sample preparation time, the number of samples to be analysed are reduced and the multi-step procedures involved will also result into loss of analytes. The consumption of large amounts of toxic organic solvents affects the environment, laboratory personnel and requires extra operational costs for waste management. Ideally, sample-preparation techniques should be fast, simple, inexpensive and compatible with a range of analytical instruments. Some common sample preparation techniques performed prior analyses are displayed in Table 1.

Table 1: Analytical instruments and methods typical of common pollutants

Analytes	Sample Preparation	Instruments
Organics	Extraction, concentration, Cleanup, derivatization	GC, HPLC, CE, GC/MS, LC/MS
Volatile organics	Transfer to vapor phase, Concentration	GC, GC-MS
Ions	Extraction, concentration, derivatization	IC, UV-VIS
Amino acids, fats, carbohydrates	Extraction, cleanup	GC, HPLC, CE, electrophoresis
Metals	Extraction, derivatization, Concentration, speciation	AA, GFAA, ICP, ICP/MS,IC,UV-VIS molecular absorption Spectrophotometry

Apart from the sensitivity of the analytical instrument, the accuracy and precision of any method are also dependent on sample pre-treatment, pre-concentration and clean-up. Some of the sample preparations and extraction techniques commonly used for environmental analysis are thereby discussed.

1.5.2 Methods used to extract organic analytes from aqueous samples

Aqueous samples takes up different forms which include biological fluids, waste water, natural water etc.

1.5.2.1 Liquid-liquid extraction

Liquid-liquid extraction (LLE) is a widely used and generally accepted sample pretreatment technique for most environmental contaminants. This method bases on the partitioning of two immiscible solvents in which the matrix and the target analyte has varying solubilities. Here, the extraction process is governed by two main factors namely; solubility and equilibrium. In an organic and an aqueous phase, equilibrium is established by shaking the two phases together. Suppose analyte 'i' is in the aqueous phase. The extraction process may be illustrated as shown below;

$$i_{aq} = i_{org} \qquad (1\text{-}1)$$

At equilibrium, the partition coefficient k_d for the analyte 'i' in the two-phase system is

$$K_d = \{i\}_{org}/\{i\}_{aq} \qquad (2\text{-}1)$$

$\{i\}_{org}$ and $\{i\}_{aq}$ refer to equilibrium concentration of i in the extractant and in the sample phase, respectively. The fraction of analyte extracted (f), is usually expressed as;

$$f = \frac{C_{Org}V_{Org}}{(C_{Org}V_{Org} + C_{aq}V_{aq})} \quad (3\text{-}1)$$

Or

$$f = \frac{K_d V}{(1 + K_d V)} \quad (4\text{-}1)$$

where V_{org} and V_{aq} are the volumes of the organic and aqueous phases, respectively; and V is the phase ratio $\frac{V_{org}}{V_{aq}}$. C_{org} and C_{aq} are the concentrations of the analyte in the organic and aqueous phases. To achieve some level of quantitative recoveries, two to three repeated extractions are required. Solvent selection is therefore critical in LLE.

The major criterion for selection of LLE solvent are immiscibility with water, optimum polarity to match that of the analyte, volatility (if it is to be injected directly into a gas chromatograph) and should preferably be of low toxicity and environmentally-friendly. Polar solvents are preferentially extracted in to polar solvents while uncharged solutes are more easily extracted into nonpolar organic solvents. The major problem encountered while extracting polar solutes is the miscibility of polar solvents with water, which is the main matrix for many samples. The chemical form of an analyte has a fundamental effect on the efficiency of an extraction.

For the target analyte to be extracted quantitatively from the sample and into the organic solvent; the extraction efficiency should be close to 100%.

It is closely related to both the partition coefficient (k_d) and to the volume of organic solvent used for the extraction (V_{org}) by equation (5-1).

$$E = 1/[\left(\frac{Vaq}{kdVorg}\right) + 1] \qquad (5\text{-}1)$$

Generally, high extraction efficiency (E) may be obtained by utilizing a large volume of organic solvent relative to the volume of the sample. The extractant phase (organic) is then evaporated to dryness and the resultant solid reconstituted in a small volume of a suitable solvent compatible with the analytical instrument used. This ensures high analyte enrichment.

Practically, the evaporation step for LLE is cumbersome and may cause losses of the analyte though it provides some degree of sample clean-up. To avoid contamination of the sample and formation of emulsions, great care must be dedicated to both the solvent extraction and concentration procedures. Thus LLE suffers from several limitations, such as large volume of solvent use, labor intensity, tendency for emulsion formation, and poor potential for automation [6, 9].

1.5.2.2 Solid-phase Extraction

Solid-phase extraction (SPE) is a widely used sample-preparation technique for the isolation of selected analytes, usually from a mobile phase.

It involves passing a liquid sample through small, disposable cartridge systems containing solid adsorbents as the media for retaining the compounds of interest,

followed by selective elution in a small volume of clean extract. A normal SPE sequence might involve the following four steps:

(i) Sorbent conditioning. This is aimed at solvating the functional groups of the sorbent and driving out the air in the column using suitable solvents. Normally, methanol is used as a conditioning solvent followed by water or buffer. Great care must be taken not to allow the packing to dry up.

(ii) Loading the sample. For environmental analysis, small amount of sample may be applied to the column. The retention mechanism that holds the analyte to the column includes van der waals interactions, hydrogen bonding, dipole-dipole forces, size exclusion and cation and anion exchange. During this retention step, the analyte is concentrated on the sorbent.

(iii) Rinsing the column to remove some interference and to retain the analyte.

(iv) Eluting the target analyte in a small volume of solvent. An appropriate solvent is specifically chosen to disrupt the analyte-sorbent interaction. The solvents selected are just strong enough to elute the analyte but leaving behind the more strongly bound interferents on the column.

The SPE approach offers the following advantages over LLE procedures:

a) Consumes less organic solvent;

b) Foams or emulsion hardly form;

c) Shorter sample-preparation time;

d) Can easily be automated;

e) Provides higher enrichment factors;

f) An inexpensive method.

Its limitations include low recovery, sorbents pore blockages by solid or oily components of non-volatile compounds with boiling points higher than the desorbing solvent temperature [10-11].

Hence, the demand for miniaturization in analytical systems along with use of reduced organic solvent and better automation with modern instruments have led to modifications and developments of miniaturized liquid-liquid extractions methods.

1.5.2.3 Flow Injection Analysis (FIA)

The reduction in volumes of organic solvent required for LLE, as well as subsequent automation of the extraction process can be facilitated using FIA. In this technique, solvent volumes of less than 1 mL can be used [12]. FIA is based on the injection of a liquid sample into a moving, non-segmented continuous carrier stream of a suitable liquid that forms a zone, which is later carried towards a detector [13].

From the detector, continuous record of absorbance, electrode potential and other physical parameters takes place. In comparison to the r micro-extractions techniques, FIA procedures use relatively larger volumes of organic solvents in the order of several milliliters for each analysis [14].

1.5.2.4 Microextraction techniques

Microextraction is an extraction technique in which the volume of the extracting phase is very small in relation to the volume of the sample and the extraction of analytes is non-exhaustive. Microextraction techniques (Figure 1) represent an important contribution to the improvement of sample preparation performance, which especially addresses the issues of miniaturization, automation, on-site analysis, and time efficiency [15]. The guiding principle is to utilize microliter levels of extracting phase selectively to extract or enrich target compounds from the bulk sample matrix. Partitioning is governed by physicochemical properties of the analyte, and is independent of its concentration.

Hence from absolute amount extracted, the sample concentration of the target analyte can be determined quantitatively. Depending on the extracting phase, microextraction methods can either be sorbent-based [14-20] or solvent-based [21-27] as shown in Figure 1.

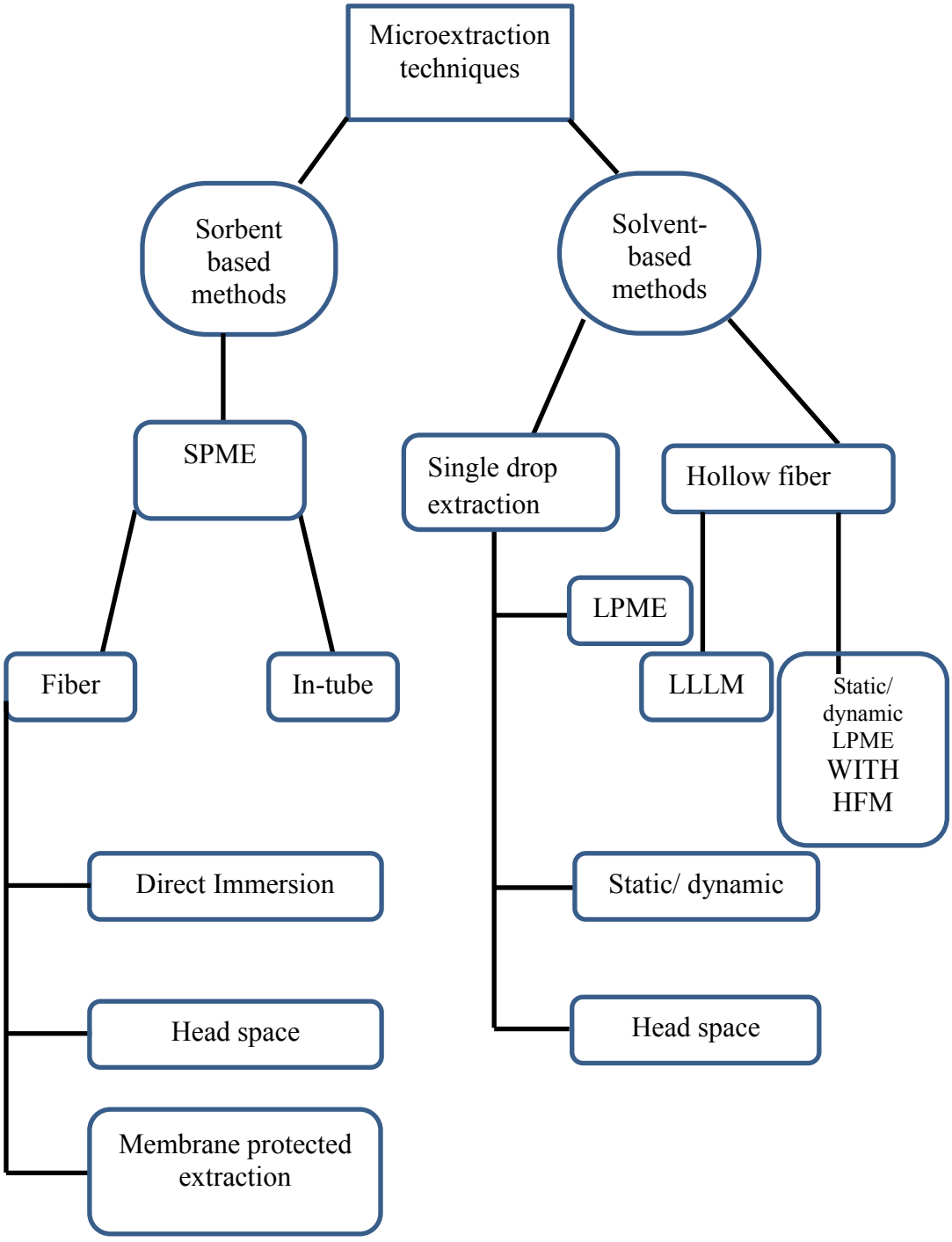


Figure 1: Microextraction techniques

1.5.2.5 Liquid-phase microextraction (LPME)

LPME is a quick, inexpensive and solvent-minimized sample-preparation technique that reduces exposure to toxic organic solvents. It is compatible with capillary electrophoresis (CE), gas chromatography (GC), high performance liquid chromatography (HPLC). It was first introduced in 1997 to describe two-phase systems in microscale LLE which involves the use of a droplet of organic solvent hanging at the end of a microsyringe needle. In this case, the organic layer is placed in an aqueous sample, and the analytes present in the aqueous sample are extracted into the organic microdrop [26-29]. If LPME is performed in a three-phase system [liquid-liquid-liquid] microextraction (LLLME)] analytes in their neutral form are extracted from aqueous samples, through an organic phase sample, and into an aqueous phase (adjusted to a different pH). Finally, the aqueous sample is withdrawn into the syringe and injected into HPLC or CE system for direct analysis [29-30]. Since inception, LPME has taken different forms all aimed at increased enrichment factors, stability of the extracting solvent as well us use of green solvents as alternative to organic ones. These include static/dynamic LPME extraction [24, 33], hollow fiber membrane-protected LPME [19, 32] and purge and trap or dynamic headspace extraction mode. The three phase system is more suitable for HPLC and CE, whereas, the two-phase LPME system is suitable for GC analysis. Pedersen-Bjergaard et al [31] demonstrated a novel method (LLLME) for concentrating methamphetamine from samples prior to CE analysis.

LLLME has been developed based on the basic principle of Supported liquid membrane (SLM) by utilizing polypropylene hollow fiber as the membrane.

The membrane was first dipped into the organic solvent (1-octanol), which filled the pores on the wall of the hollow fiber. An aqueous acidic acceptor solution (25 µl) was introduced inside the hollow fiber. The hollow fiber was then exposed to sample solution maintained at pH 13 (2.5ml). Owing to the difference in pH between the acceptor and donor phases, the analytes were extracted from the sample into the organic solvent immobilized in the pores of the hollow fibers, and further into the aqueous acceptor solution inside the hollow fiber. Figure 2 represents the experimental set up for LLLME extraction process.

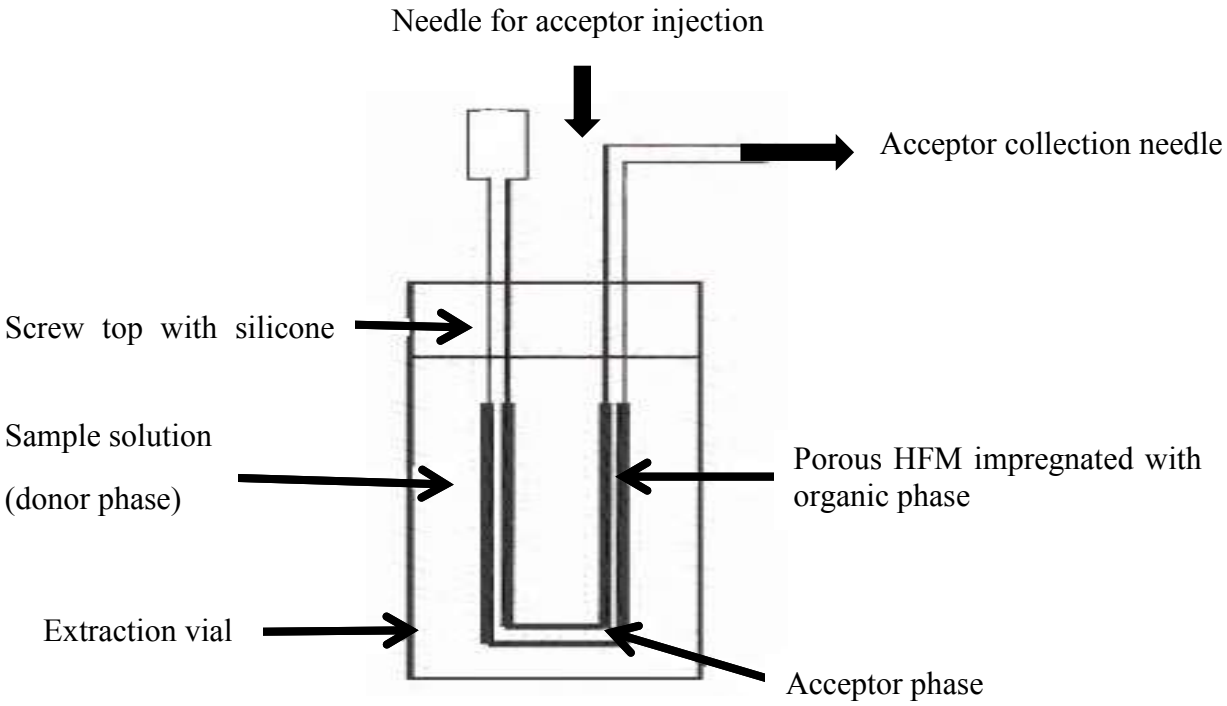


Figure 2: Experimental set-up of LLLME experiment

Considering an analyte i, the extraction equations can be expressed as;

$$i_{aq1} \overset{K_1}{\longleftrightarrow} i_{org} \overset{K_2}{\longleftrightarrow} i_{aq2} \quad (6\text{-}1)$$

Where 'aq1' represents the aqueous donor phase, 'org' represents the organic phase within the pores of the hollow fiber, and 'aq2' represents the aqueous acceptor phase. K_1 and K_2 are distribution ratios described by the equations below.

$$K_1 = \frac{C_{org,eq}}{C_{aq1,eq}} \quad (7\text{-}1)$$

and $K_2 = \frac{C_{org,eq}}{C_{aq2},eq}$ (8-1)

$C_{org,eq}$, $C_{a1,eq}$, $C_{a2,eq}$ represent the respective equilibrium concentrations of i in the organic phase, donor phase and acceptor phase. At equilibrium, the mass balance of the three-phase system at equilibrium is expressed as;

$$C_{a1,initial} = \frac{K_2 C_{a2,eq}}{K_1} + \frac{K_2 C_{a2,eq}}{V_{a1}} + \frac{C_{a2} V_{a2}}{V_{a1}} \quad (9\text{-}1)$$

where $C_{a1,initial}$ is the initial concentration of 'i' in the donor phase, V_{a1}, V_0, and V_{a2} are the respective donor, organic and acceptor phase volumes.

The enrichment factor (EF) for the LLLME system expressed as $EF = {C_{a2,eq}}/{C_{a1,initial}}$ can be calculated as;

$$EF = 1/(\frac{K_2}{K_1} + \frac{K_2 V_{org}}{V_{a1}} + \frac{V_{a2}}{V_{a1}}) \quad (10\text{-}1)$$

Since V_{org} is very small, equation can be simplified as

$$EF = 1/(\frac{1}{K} + \frac{V_{a2}}{V_{a1}}) \quad (11\text{-}1)$$

where $K = \frac{C_{a2,eq}}{C_{a1,eq}}$ (12-1)

Hence, large K and phase ratio values $\frac{V_{a1}}{V_{a2}}$ will result into a higher EF.

$$C_{a2,eq} = \frac{V_{a1}}{V_{a2}} C_{a1,initial} \quad (13\text{-}1)$$

The above condition represents complete extraction resulting to 100% recovery of analyte from the sample to the aqueous acceptor phase at equilibrium.

Pedersen-Bjergaard and Rasmussen applied the LLLME technique to the analysis of acidic drugs. In their experiment, the donor phase was acidified using hydrochloric acid, while the aqueous acceptor phase constituted sodium hydroxide solution. The experiment resulted to nearly 100% extraction efficiency. The hollow fibre served as a sieve resulting into reasonable selectivity towards extraction of drugs from plasma [19].

In another modification, Zhao et al [33] simplified the LLLME device in such a way that the microsyringe served as both a microseparatory funnel as well as a syringe for injection in to HPLC.

Their method was compatible with both CE and HPLC and was successfully applied in analysis of drugs from different matrices [35].

The main advantages of LPME over LLE techniques are capability in reducing the consumption of organic solvents, higher enrichment factors, simplicity, cost effectiveness and compatibility with the final analytical instrument.

However, LPME methods based on hanging organic drops are not stable and as a result of vigorous stirring during extraction, the extracting drop is easily dislodged from the needle tip. Additionally, biological samples, such as plasma, may emulsify substantial amounts of organic solvents, which compromise the stability of hanging drops during extraction. Therefore, hollow fiber membrane-protected LPME (Figure 2) was developed to eliminate the above problems. With this device, the extracting phase (acceptor solution) is contained within the lumen of a porous hollow fiber, either as a loop or a rod sealed at the bottom, and the extracting phase is not in direct contact with the sample solution. Mass transfer is easily accomplished across the highly porous wall of the hollow fiber, and samples may be agitated without any loss of the acceptor solution. Hence, hollow-fiber-based LPME is a more robust and reliable approach to LPME [15].

1.5.2.6 Solid phase microextraction (SPME)

This technique was developed in the late 1980's [40] as a microscale solvent-free sample preparation procedure that could serve as an alternative to traditional extraction procedures such as LLE and SPE procedures.

With SPME method, all the advantages of SPE are preserved while eliminating low analyte recovery and use of voluminous solvents. The coated fused silica SPME fiber is attached to a metal rod typical of a modified syringe. In the stand by position, the fiber is withdrawn into a protective sheath. During sampling procedure, the sheath is pushed through the septum and the plunger and thus introduces the fiber into the sample solution contained in a vial. The fiber can be immersed directly into the sample solution or held in the headspace as shown in Figure 3. As such, the SPME approaches are classified as either direct sampling SPME represented by Figure 3 B or head space sampling SPME represented by Figure 3 A. Analytes in the sample are adsorbed on the fiber. After some time, the fiber is withdrawn into its protective sheath and then inserted through the septum of a GC injector. The By pushing down the plunger, the fiber is forced into the injector where the analytes are thermally desorbed and separated on the GC column. The desorption step is usually 1-2 min. After desorption, the fiber is retracted into its protective sheath and the sheath is removed from the GC injector [36-39].

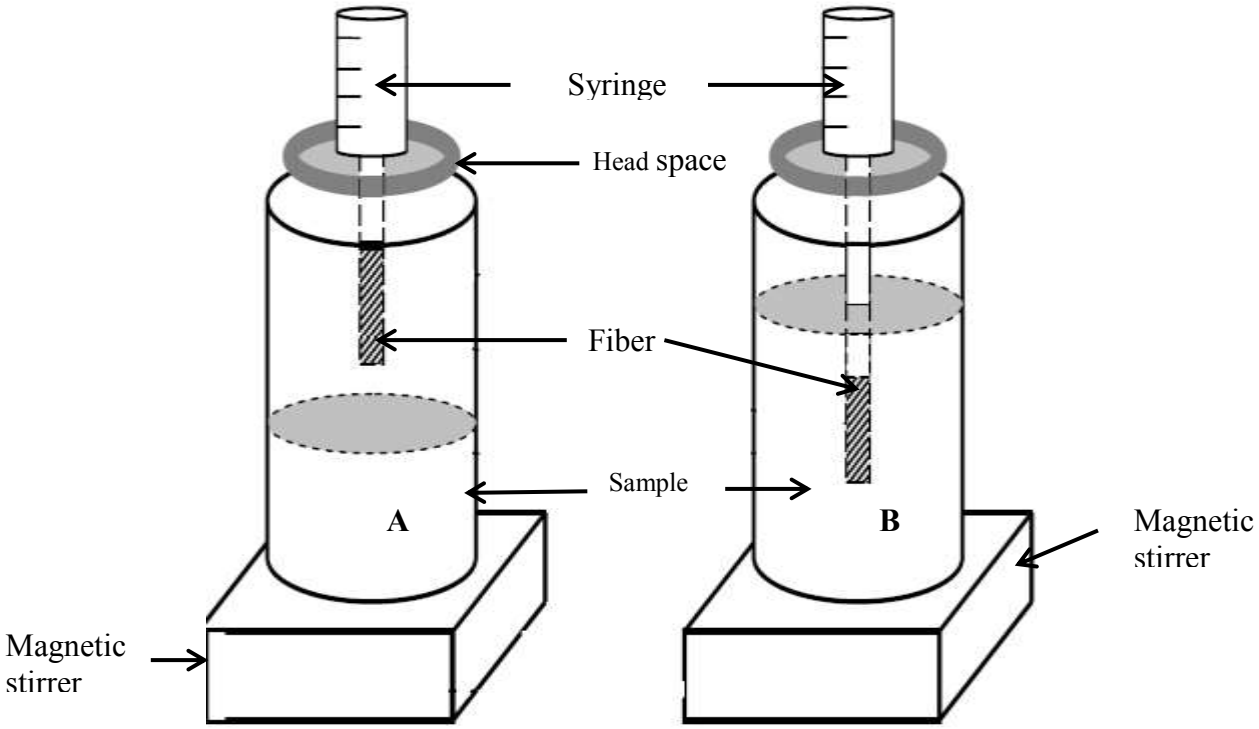


Figure 3: Different configurations of SPME method

In direct sampling approach, the fiber is inserted into the sample matrix (Figure 3 B) while in headspace sampling the fiber remains hanging over the sample (Figure 3 A).

In addition, membrane protected SPME sampling has been developed and applied in situations where the fiber is separated from the sample with a selective membrane allowing analytes through while blocking interferences. Several adsorbent polymers are commercially available for SPME applications and these include polydimethylsiloxane (PDMS), polyacrylate (PA), or mixture of polyacrylate with Carbowax (CW).

The advantages of SPME techniques in environmental analysis are [40];

- Portability of the set-up and hence suitable for field applications and large volume sampling.
- Solvent-less method thus eliminates the solvent disposal problems.
- Improved selectivity which depends on the judicious choice of the sorbent.
- Fast, simple and easily automated method.

Despite the many advantages SPME method has to offer, it still faces the following challenges;

- Only small fractions of the sample analyte are extracted by the coated fiber.
- The fiber is delicate and fragile.

- Suffer from carry over problems.
- Limited number of sorbents available for extraction.

1.5.3 Method used to extract organic analytes from solid matrices

Basically, the extraction and recovery of solutes from solid matrices involve the following main stages [41];

- Desorbing the target analytes from the active sites of the solid matrix.
- Diffusing the target analytes in to the sample matrix.
- Solubilisation of the analyte into the extractant.
- Collecting the extracted analytes.

Desorption of the analytes from their active sites within the solid matrix constitutes the rate-limiting step. This is because of the unpredictable interactions between the solute and matrix. As a result of this very realization, extraction optimization is mandated whose success entirely depends on the nature of the matrix to be extracted. The following methods have been applied in organic environmental analysis.

(i) Microwave-assisted extraction

(ii) Supercritical fluid extraction

(iii) Direct thermal extraction

(iv) Ultrasonic extraction

(v) Automated Soxhlet extraction

(vi) Soxhlet extraction and soxtec method

(vi) Pressurized fluid extraction

1.5.3.1 Microwave-assisted extraction (MAE)

MAE is a process of heating solvents in contact with microwave energy to partition compound of analytical interest from the sample matrix to the solvent. This approach combines the power of both closed-vessel microwave solvent digestions with extraction of organic analytes. The end results are rapid heating, timesaving, and large sample throughput extractions. This technique uses microwave radiation as the source of heating of the solvent–sample mixture. Due to the particular effects of microwaves on matter, heating with microwaves is instantaneous and occurs in the middle of the sample, leading to very fast extractions [42-43]. For some applications, the extraction solvent is selected as the medium to absorb microwaves.

Two technologies signify the applications of microwaves to respective samples: closed vessel system and open vessel system. Heating in closed vessel system takes place under controlled pressure and temperature, while in an open vessel system, heating is done at atmospheric pressure.

1.5.3.2 Supercritical fluid extraction

Supercritical fluid extraction (SFE) is a techniques used in environmental analysis of less volatile compounds. It operation are like that of solvent extraction and can be automated a condition that puts it on an advantage [44].

1.5.3.3 Direct thermal extraction (DTE)

In DTE both volatiles and semi-volatiles can be thermally extracted directly from solid matrix samples without the use of any solvents. Using this technique, a wide range of both volatiles and semi-volatiles can be analysed with high sensitivity. In this method, no sample preparation is required and the sampling time is small. The sample is simply weighed into the desorption tube followed by subsequent analysis [45].

1.5.3.4 Ultrasonic extraction

This depends entirely on ultrasonic vibration in ensuring intimate contact between the sample and the solvent. Extraction is facilitated by sonication and the extraction efficiency is low in relation to the other methods [46]. Ultrasonic irradiation may lead to the decomposition of some target analytes hence a strong need for method optimization. Methanol, hexane and acetone are some of the common solvents used in Ultrasonic extraction.

1.5.3.5 Automated Soxhlet extraction (ASE)

This is traditionally used as a standard for validating other extraction methods. With soxtec technique, both the extraction time and extracting solvent are significantly reduced. On average, two to six samples can be simultaneously extracted using a single soxhtec apparatus [47].

1.5.3.6 Pressurized fluid extraction

This new technique is at times referred to as accelerated solvent extraction (ASE), pressurized liquid extraction (PLE), pressurized solvent extraction (PSE) or even enhanced solvent extraction (ESE). It affords the ability to perform fast, efficient extractions due to the use of elevated temperatures, as the decrease in solvent viscosity helps to disrupt the solute–matrix interactions and increases the diffusion coefficients. Still, high temperatures favour the solubilisation of the analytes due to a change in their distribution coefficients. Lastly, the penetration of the solvent into the matrix is favoured by pressure which favors extraction return [48].

1.6 Scope of this thesis work

The Figure 4 below summarizes the different microextraction techniques used in this work for preconcentrating haloacetic acids, perchlorates and phenolic compounds from various environment matrices.

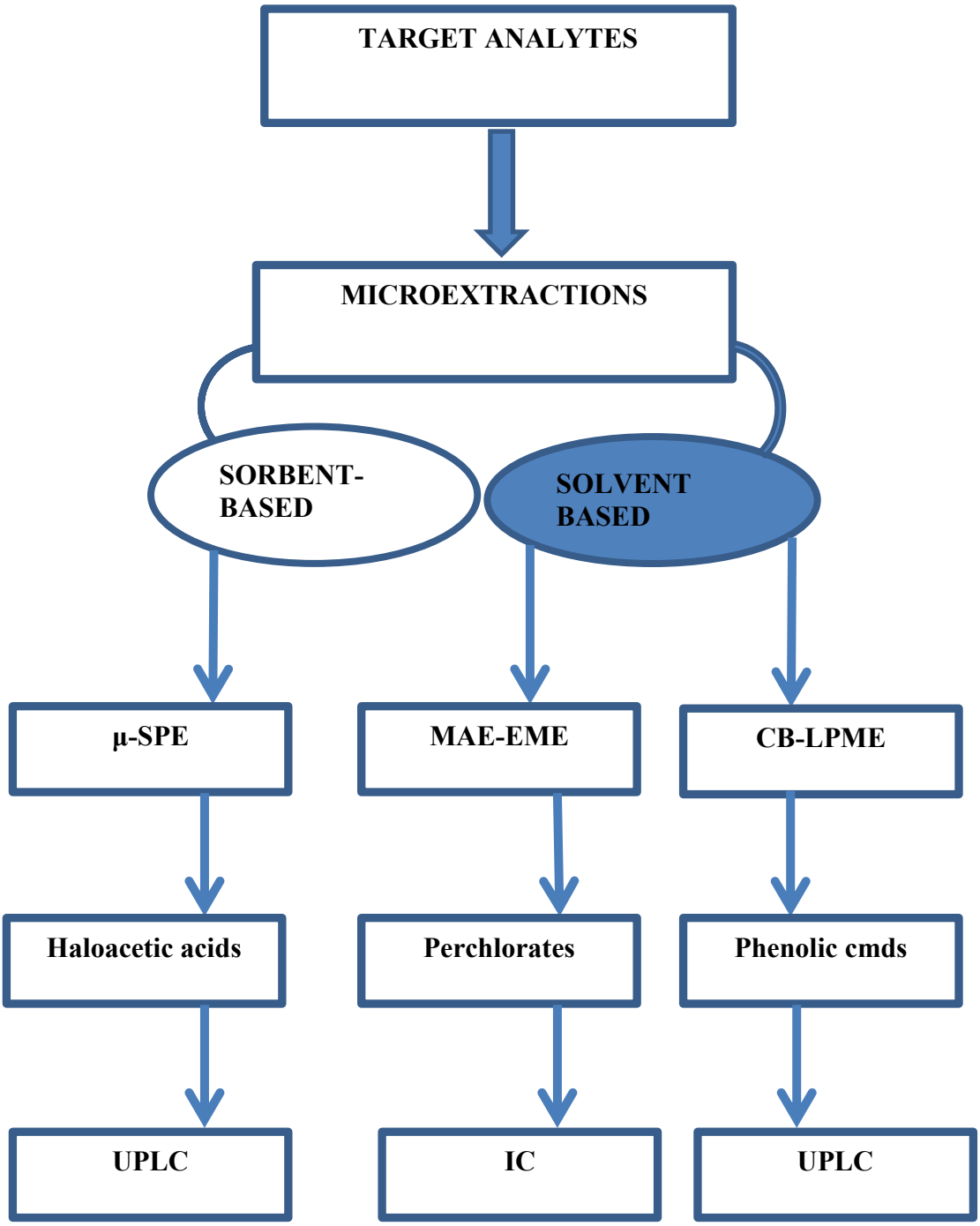


Figure 4: **Flow diagram showing the scope of this thesis work**

CHAPTER 2. MEMBRANE-PROTECTED MICRO-SOLID PHASE EXTRACTION OF TRACE LEVEL HALOACETIC ACIDS IN SWIMMING POOL WATER

1. LITERATURE REVIEW

Communities around the world are using swimming pools for recreational, physical, educational, sporting or even therapeutic activities [49-50]. This therefore demands that their water is kept safe for the health of the swimmers [49-51]. Chlorination is a common and still remains a popular choice for controlling pool water quality [52-54]. Several studies ratify that chlorination results to formation of equivocal disinfection by-products especially if the target matrix contains humic substances or bromide ions [55-57] as precursors. The nature, properties and environmental fate of these by-products have been of prime interest to environmental scientists and regulators [56, 58]. Haloacetic acids (HAAs) are the second most prevalent group of disinfection by products in chlorinated water after trihalomethanes (THMs) [59]. In recent studies, HAAs have been found to be much more relevant to the pool water since their formation has been linked to cancer [60]. They are highly water-soluble and are toxic to humans and plants [61]. Plewa et al [62] asserts that the Cyto- and genotoxic potency of HAAs is even much greater than that of THMs. Because of their potential carcinogenicity [63-64], US Environmental Protection Agency [63] (USEPA) has revised the maximum contamination level of some regulated HAAs from 0.060 mg/L to 0.030 mg/L.

World Health Organization (WHO) has also set the qualitative target levels for HAAs at 80 μg/L for dichloroacetic acid and 100 μg/L for trichloroacetic acid [49, 53]. HAAs are generally difficult to determine because of their strong acidic and hydrophilic character [65]. The current USEPA approved methods for HAAs analysis are EPA method 552.1, 552.2 and 6251 all of which involve cumbersome liquid sample preparation or even derivatization prior to GC analysis [66]. Typical analysis time for the above methods range from three to four hours [69] and few analytes are detected. Due to the ionic nature of HAAs, alternative methods that do not require derivatization including use of liquid chromatography [70-71], ion chromatography [72-73], capillary electrophoresis [74] and electron Spray Ionization-Mass spectrometer (ESI-MS) [75] have been explored. However, the detection limits of these methods have been found to be significantly greater than the GC methods [69]. Due to the complexity in composition of the pool water matrices and the trace amounts of analytes involved, an effective extraction approach prior to final analysis is significant. Conventionally, liquid-liquid extraction (LLE) and solid-phase extraction (SPE) are the most common sample preparation techniques for HAAs analysis. However, the multistep sample extraction and clean up procedures involved requires voluminous toxic solvents, are tedious, time consuming and leads to analyte loss [76]. Generally, most current sample preparation methods used in the determination of HAAs in water matrices suffer greatly from increased time for sample pretreatment and degradation of unstable species [77]. Porous membrane based liquid phase micro extraction (LPME) techniques have been explored for good analyte enrichment properties but however, solvents available for extracting both polar and semi-polar compounds are limited [78].

As a means of avoiding these limitations, sorbents have been utilized in analytes isolation and their suitability fits almost any class of analytes. In the previous few years, a sorbent-based solid phase microextraction (SPME) technique has emerged as a promising technique for preconcentrating HAAs. However, it suffers from defects like carry-over effects and fragility of the fibres [79]. Besides, it has a limited sorbent phase on the silica fibre that renders its extraction capability limited towards complex sample matrix.

To enhance the extraction sensitivity and reduce matrix interference, membrane – protected μ-SPE has been developed [80-81]. It consists of a small amount of sorbent enclosed within a membrane envelope with dimensions suitable for small sample volumes [80]. The judicious choice of the sorbent determines the selectivity of the μ-SPE device. The porous polypropylene membrane serves as a sieve and prevents particulates and humic substances that would otherwise interfere with the sorbents extraction ability [82]. Till now, sorbents have played a pivotal role in μ-SPE devices for various biological and environmental applications [83-86]. In relation to SPE method, μ-SPE offer advantages such as;

(a) Easy operation.

(b) Ability to reduce sample matrix effects and avoid blockage ie the porous membrane serves as both a pre-concentration and clean-up device thus further purification is not necessary as is the case with traditional SPE).

(c) Relatively cheap method (the envelopes are affordable and simple to prepare).

(d) Does not require frits for holding sorbent material as common in SPE columns.

(e) Carry over effects can be eliminated since μ SPE devices are ultrasonically cleaned in acetone after every extraction.

(f) The amount of organic solvent used is reduced and the final extract is compatible with ultra-performance liquid chromatography.

The applications of silica from rice husk (RH) as an adsorbent has been reported elsewhere for the removal of metal ion [87-88], 2, 4-dichlorophenol, and organic dyes [89] from water samples.

Several literatures have shown that RH contains greater than 95 % silica content [90-91] that renders it highly porous with large surface area for adsorption properties. Interestingly, silica supported metal ions have widely been used in catalytic applications but rarely as sorbent for μ-SPE extraction [93-94]. The choice of iron impregnated silica as a novel sorbent for HAAs determination is based on the substantial affinity of oxides of iron for anionic pollutants [93] from contaminated waters.

From the above premise and in a bid to fully embody and expand RH applications, we developed a μ-SPE extraction method aimed at;

(i) Reduction on the overall extraction process costs

(ii) Reduction on organic solvents usage

(iii) Minimization in the degradation of HAAs during extraction

(iv) Improving selectivity and sensitivity towards HAAs extraction.

In this work and for the first time we extracted silica from RH waste and incorporated iron oxide in to its matrix via sol-gel process to generate a novel sorbent for micro-solid phase extraction device. Subsequent analysis of HAAs in swimming pool waters was done using Ultra-High Performance Liquid Chromatography-Ultraviolet detection (UPLC-UV).

2. EXPERIMENTAL

2.1 Chemicals and Reagents

All the reagents used were of better analytical grade. HPLC –grade Organic solvents were obtained from Merck (Darmstadt, Germany). Sodium hydroxide, sodium sulfate, hydrochloric acid and Sodium dihydrogen phosphate monohydrate ($NaH_2PO_4 \cdot H_2O$) were obtained from J. T. Baker (Philips Burg, NJ). Orthophosphoric acid (85%) purity was purchased from Carl Erba (Milan Italy).Ultra-pure water was prepared using Milli-Q (Milford MA) System. The pH of the Milli-Q water was 5.6. A mixture of six HAAs standards including monochloroacetic acid (MCAA), dichloroacetic acid (DCAA), trichloroacetic acid (TCAA), monobromoacetic acid (MBAA), dibromoacetic acid (DBAA) and bromochloroacetic acid (BCAA) in methyl tert-butyl ether (MTBE) containing 2000 μg/ml was purchased from Supelco (Supelco Park, PA, USA). The structures of the model analytes are shown in Figure 5. Sulfuric acid (98%, grade for analysis) was obtained from Merck (Darmstadt, Germany).

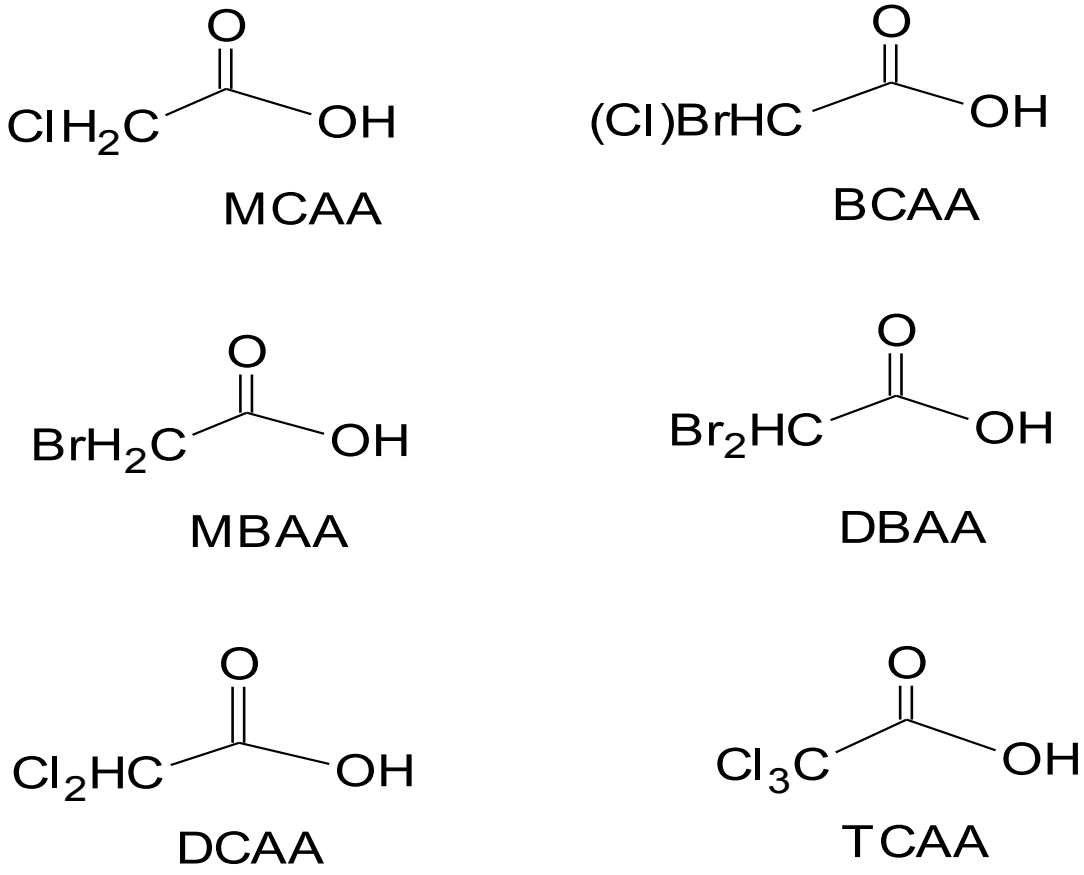


Figure 5: Structures of the model compounds used

2.2 Apparatus and Materials

Chromatographic analysis were conducted using a Waters-Acquity Ultra Performance Liquid Chromatography system (Waters corporation, Madrid Spain) using an Acquity UPLC BEH C_{18} column. The mobile phase consisted of A-20% Methanol and B-80% 15 mM Sodium dihydrogen phosphate buffer solution adjusted to pH 2.0-2.2 and at a flow rate of 0.2 ml/min.

The separated components were determined using Acquity Photo Diode Array Detector, PDA (Waters) under double channel system at wavelengths of 210 nm and 220 nm. Empower Software (Waters) was used for data acquisition and analysis.

Polypropylene sheet membrane (157 μm thickness and 0.2 μm pore size) was purchased from Membrana (Wuppertal, German). Various Sorbents including C_{18}, Graphitic carbon, Carbon-nanotubes, HayeSepA (divinylbenzene ethyleneglycodimethylacrylate), Porapak R (divinylbenzene/vinylpyrrolidinone), and DVB were obtained from Alltech (Deerfield, USA). Rice husk were obtained from a rice mill in India. The scanning electron microscope (SEM) images were recorded using an SSX-550 (Shimadzu, Japan) while Nitrogen sorption experiments were performed on an ASAP 2010 instrument (Micrometrics, USA). Surface area analysis was calculated according to Brunauer-Emmett-Teller (BET) method while the pore size and volume were evaluated using Barrett-Joyner-Halenda (BJH) isotherm method.

2.3 Preparation of standards

A working stock solution (2000 mg/L) of HAAs was prepared using HPLC grade methanol in a 10 mL volumetric flask and stored at $4^{o}C$ for subsequent usage.

The samples of required concentrations were prepared by diluting the stock solution of HAAs to respective concentrations. Wide ranges of calibration standard (1.0-150 µg/L) were prepared by spiking to different aliquots of 20 mL ultra-pure water maintained at a PH of 4.0. Hence extraction was carried out on these samples.

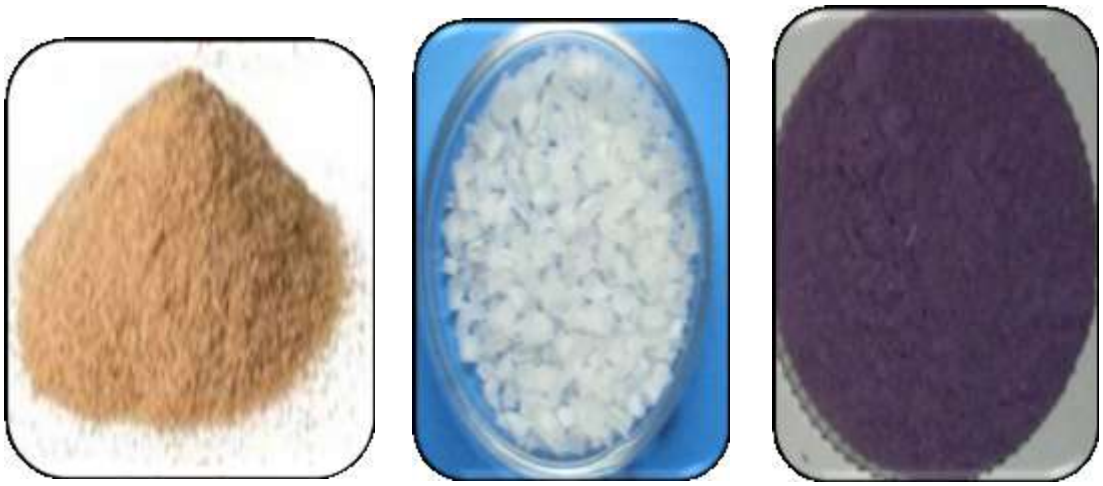
2.4 Preparation of Rice Husk Sorbent (Silica-Fe)

Rice husk material was modified to silica-Fe using sol - gel process [93-94]. Rice husk (RH) was washed with copious amount of distilled water to remove the surface impurities. The cleaned sample was then air dried and incinerated controllably to form white silica powder. The powder was treated with 1.0M HNO_3 for 24 h, filtered and washed with deionized water until a constant pH of 5.6 to form rice husk silica.

The formed silica was then oven dried at 110 $^{\circ}C$ overnight. 5.0 g of this silica was added in 250 ml of 6.0 M NaOH, stirred for 12h and filtered to remove un-dissolved material. 3.6 g of cetyltrimethylammonium bromide (CTAB) was added into the resultant sodium silicate solution and stirred till complete dissolution. The filtrate was titrated with 10 % Fe^{3+} solution [3.6 g $Fe(NO_3)_3 \cdot 9H_2O$ dissolved in 200 ml of 3.0 M HNO_3] until pH 8 was reached. Drop by drop titration followed til pH 5 was finally obtained.

The gel formed was then aged for 120 h after which filtered through suction filtration and then washed with distilled water. Finally, the product was oven dried at 110^{o}C for 24 hours and later calcined at 600 ◦C for 6 hours to produce silica-Fe. Figure 6 shows the different stages in sorbent development.

The prepared sorbents (silica, silica-Fe) were characterized by Scanning Electron Microscopy, SEM, Energy Dispersive Spectrometry, EDX, and Brunauer-Emmett-Teller, BET, Surface Area Analysis.



A B C

A-rice husk, B-rice husk silica, C- Silica-Fe

Figure 6: Different stages in RH-silica sorbent development

2.5 Preparation of μ-SPE extraction device

The porous polypropylene membrane serves as a filtering device and prevents particulates and humic substances from complex sample matrices adsorbing on the sorbent which improves the sensitivity of the extraction [82]. Fabrication procedure of μ-SPE device was discussed earlier [80-81], briefly; the membrane envelope was made from two overlapping sheets whose open edges were heat sealed. One of the two open ends was then heat-sealed. A cut glass pipette tip was used to introduce 20 mg of sorbent material through the remaining open end, which was later heat-sealed to secure the contents in a 1.5 x 0.5 cm dimension envelope. After successful packing, the devices were re-weighed to ensure consistency in weight measurements. The variations in the later measurements were found to be less than 5%. Within an interval of 15-30 minutes, several devices could easily be prepared. Each μ-SPE device can be re-used for up to 20 times after extraction if desired, following ultrasonication in (5 min) in acetone.

2.6 Extraction process

Each prepared μ-SPE device was conditioned in acetone for 10 min, dried with lint-free tissue and then placed in 20 mL of ultrapure water in a glass vial containing magnetic stirring bars. A known concentration of HAAs standard was spiked to the sample water. Without adjusting pH, temperature and salt concentration, the water sample was agitated on a vortex at a stirring speed of 750 rpm for 20 min to allow extraction take place.

After extraction, using a pair of tweezers, the μ-SPE device was removed and then fitted in to a 200 μL crimper vial for solvent desorption.

Methanol was used as desorption solvent and after desorption, 5 μL of the extract was directly injected in to UPLC for analysis. Figure 7 displays the experimental set-up.

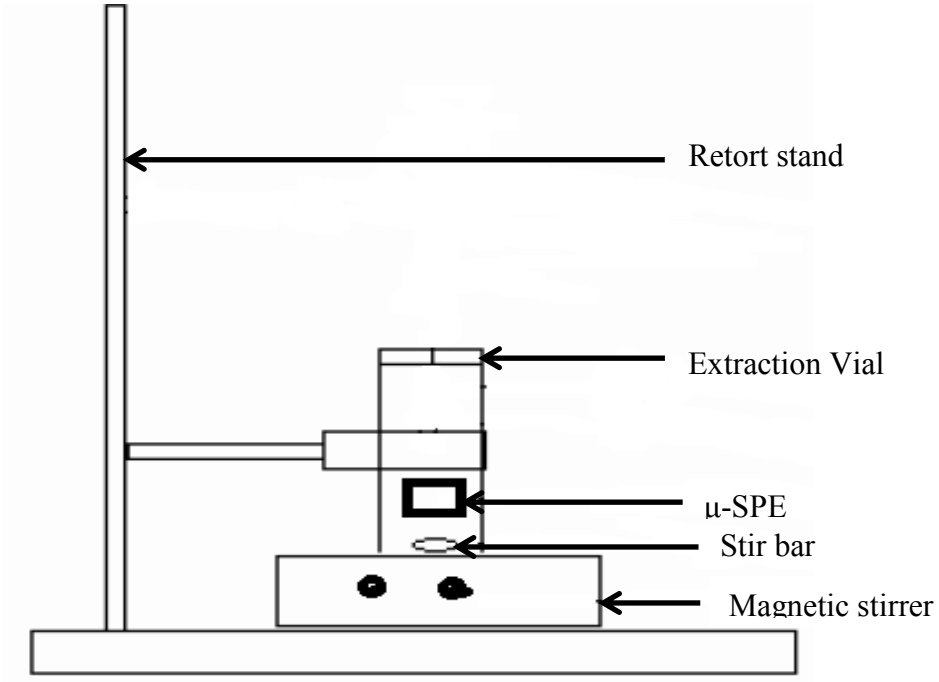


Figure 7: µ-SPE extraction set-up

2.7 Swimming pool water samples

Water samples were collected from swimming pools around King Fahd University Of Petroleum and Minerals (KFUPM). Samples were collected in screw-capped glass reagent bottles that were previously washed with acetone and oven dried overnight at 120^{o}C. The bottles were protected from light by wrapping in aluminium foil and transported under cool conditions to the laboratory for storage at 2^{o} C. Extraction and analysis was performed within 7 days of collection.

3. RESULTS AND DISCUSSION

3.1 Method development

The membrane protected μ-SPE extraction method is equilibrium based and the extraction process depends on the dynamic partitioning of analytes between the sample solution and the sorbent [80].

The main advantages of the developed method are reduced sample preparation steps, improved selectivity and sensitivity towards HAAs analysis in water matrix. Haloacetic acids are relatively polar, non-volatile and water soluble species. The method was first validated by optimizing the extraction conditions to enhance recoveries of the analytes. Using a stepwise univariate approach, factors that affect extraction efficiency were evaluated and these include, extraction time, desorption time, desorption volume, desorbing solvent, ionic strength, suitability of the sorbent and extraction pH. The

analysis was done in triplicate by spiking measured water samples with known concentrations of HAAs standards. A dilution volume of 20 mL was chosen at the start.

3.2 Suitability of the Sorbent material and Desorption volume

The efficiency of the μ-SPE device depends mainly on the nature of the sorbent material used. Six different sorbents including C_{18}, Graphitic carbon, Carbon-nanotubes, HayeSepA (divinylbenzene ethyleneglycodimethylacrylate), Porapak R (divinylbenzene/vinylpyrrolidinone) and silica-Fe were evaluated. After extraction, the extraction device was desorbed in methanol. The results are shown in Figure 8. Compared to other materials, silica-Fe shows higher extraction efficiency towards HAAs.

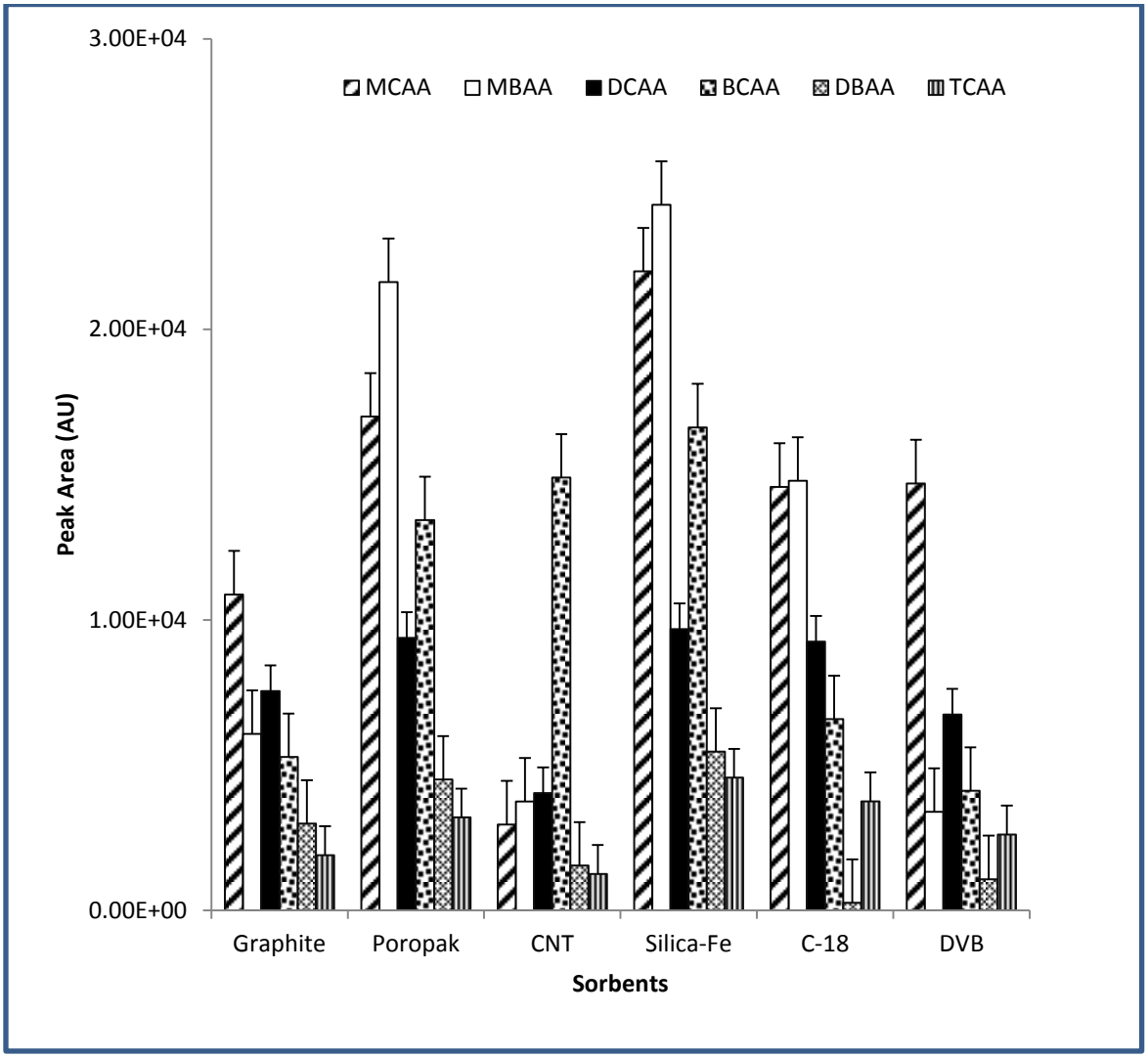


Figure 8: Suitability of various sorbents for the µSPE extraction of HAA in spiked water samples (n=3)

HAAs being polar, appear to exhibit greater interactions with silica-Fe sorbent than other sorbents. The amorphous silica and iron (iii) in silica matrix were considered as the key materials for the adsorption of HAAs.

Selection of desorption volume was done for the entire sorbents using methanol as a desorption solvent. Varying methanol volumes ranging from 50 µL to 300 µL were evaluated. Results were as anticipated as the lower volume gave higher peak areas.

100 µL was however found to be the optimum for all solvents. The selected volume was further evaluated for repeatability by carrying out the experiment in three replicates.

From the relative standard deviation calculations, the repeatability was not good. The reason was that the device could not fully immerse in the selected solvent volume. 150 µL of desorbing solvent was therefore adopted for further experiments.

3.3 Effect of sample pH on µ-SPE extraction

Haloacetic Acids are strong acidic compounds with PKa values ranging between 0.63 and 2.9 [60]. This therefore mandates the acidification of water sample before extraction. Acidification reduces the dissociation of Haloacetic acids. The effect of pH upon HAAs extractability using the proposed device was investigated by varying the sample solution pH from 2.0 to 12.0 as shown by Figure 9.

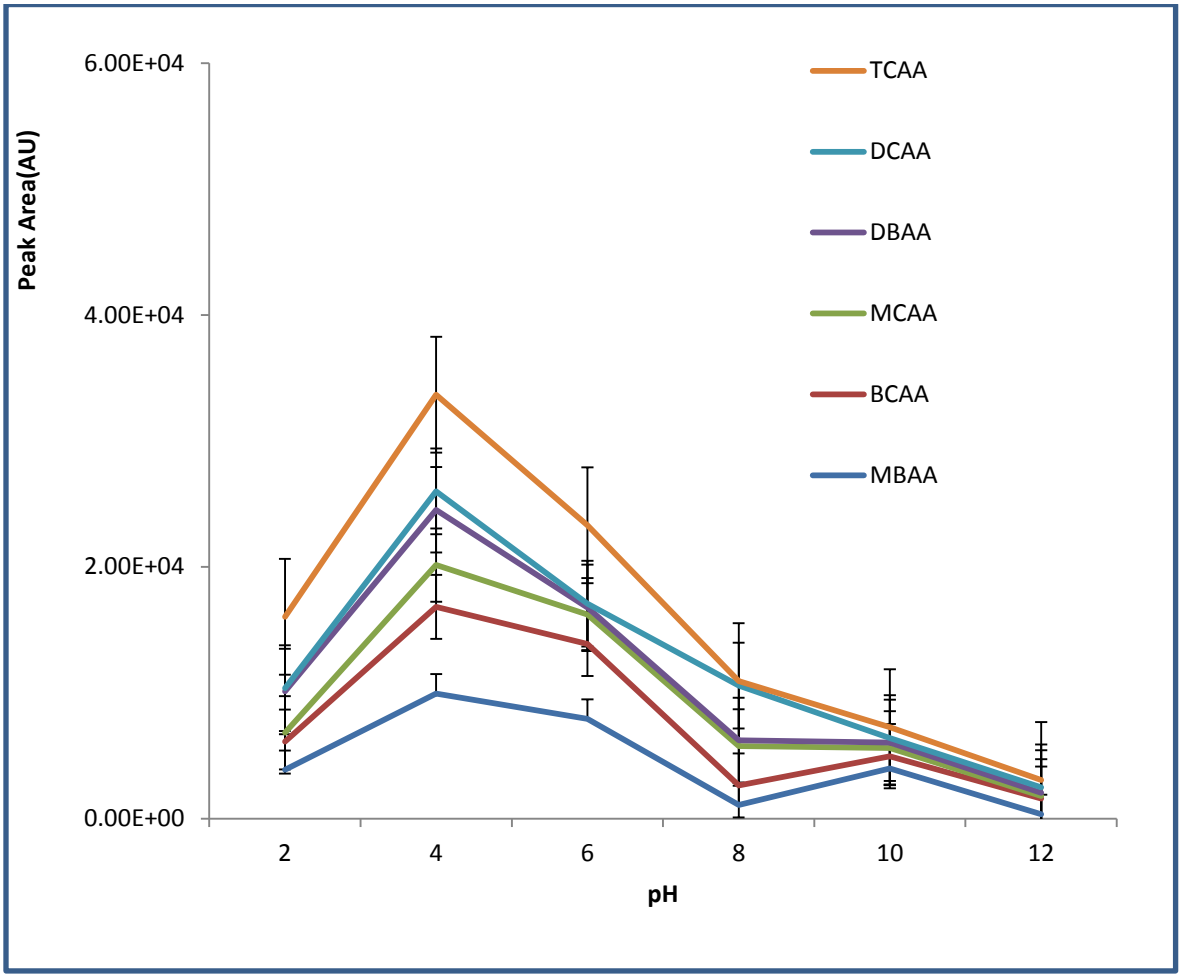


Figure 9: Effect of pH on extractability of HAAs

Best extraction efficiency for the target analytes was observed at pH 4.0 and this was applied for all subsequent analyses. At higher pH values, the recoveries for all HAAs reduced drastically possibly due to hydrolysis.

3.4 Effect of extraction time on μ-SPE extraction and carry over effects

In μ-SPE method, the amount of analyte extracted depends on rate of its mass transfer [79] from the water sample to the solid sorbent phase enclosed within the device. Sample agitation is cardinal during the extraction since the extraction efficiency is improved. This is because the contact between the analyte and the extractant is enhanced.

For this reason the effect of extraction (agitation) time using a vortex was evaluated for a range of 5 to 25 min. From the plots (Figure 10), 20 min of vortex extraction was considered as the optimum value. Above 20.0 min, there was no significant increase in extraction registered.

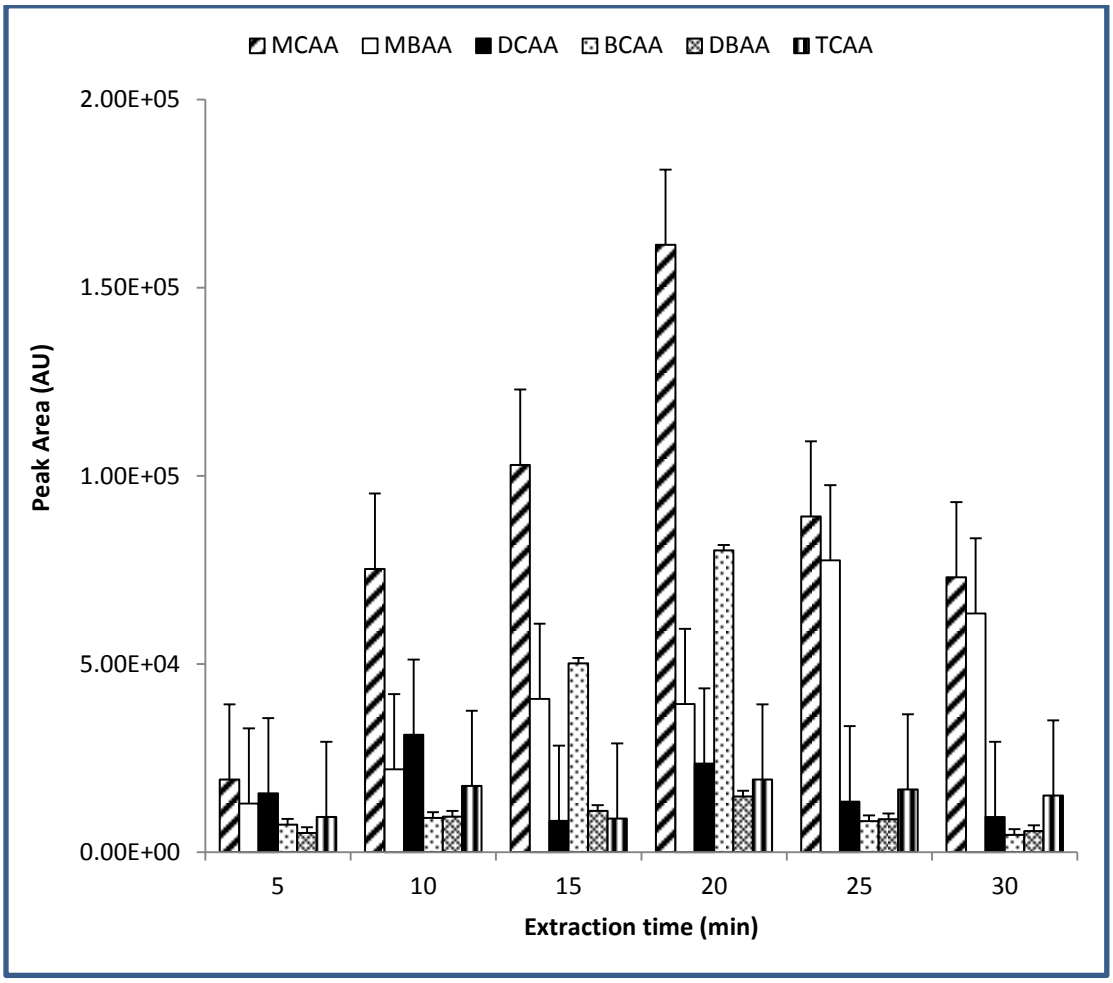


Figure 10: Effect of extraction time on µ-SPE of HAAs

After the first extraction, the device was tested for carry over effects by desorbing it in methanol for the second time. No peaks were detected. This meant the μ-SPE device was reusable after careful washing in acetone. The repeated use of the device was henceforth evaluated and results showed that it could be used for more than 20 extractions. This further proved the robustness of our device and was noted that its effectiveness depends on the durability of the protective membrane.

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The repeated use of the device was henceforth evaluated; and results showed that it could be used for more than 10 extractions. This further proved the robustness of our device and was noted that its effectiveness depends on the durability of the protective membrane.

3.5 Desorption time and the desorbing solvent

The effect of desorption time on μ-SPE was investigated over the range of 5-30 min of ultrasonication. Figure 11 shows the desorption profile of HAAs and desorption was complete within the first 20 min and no significant desorption occurred after 20 min of ultrasonication.

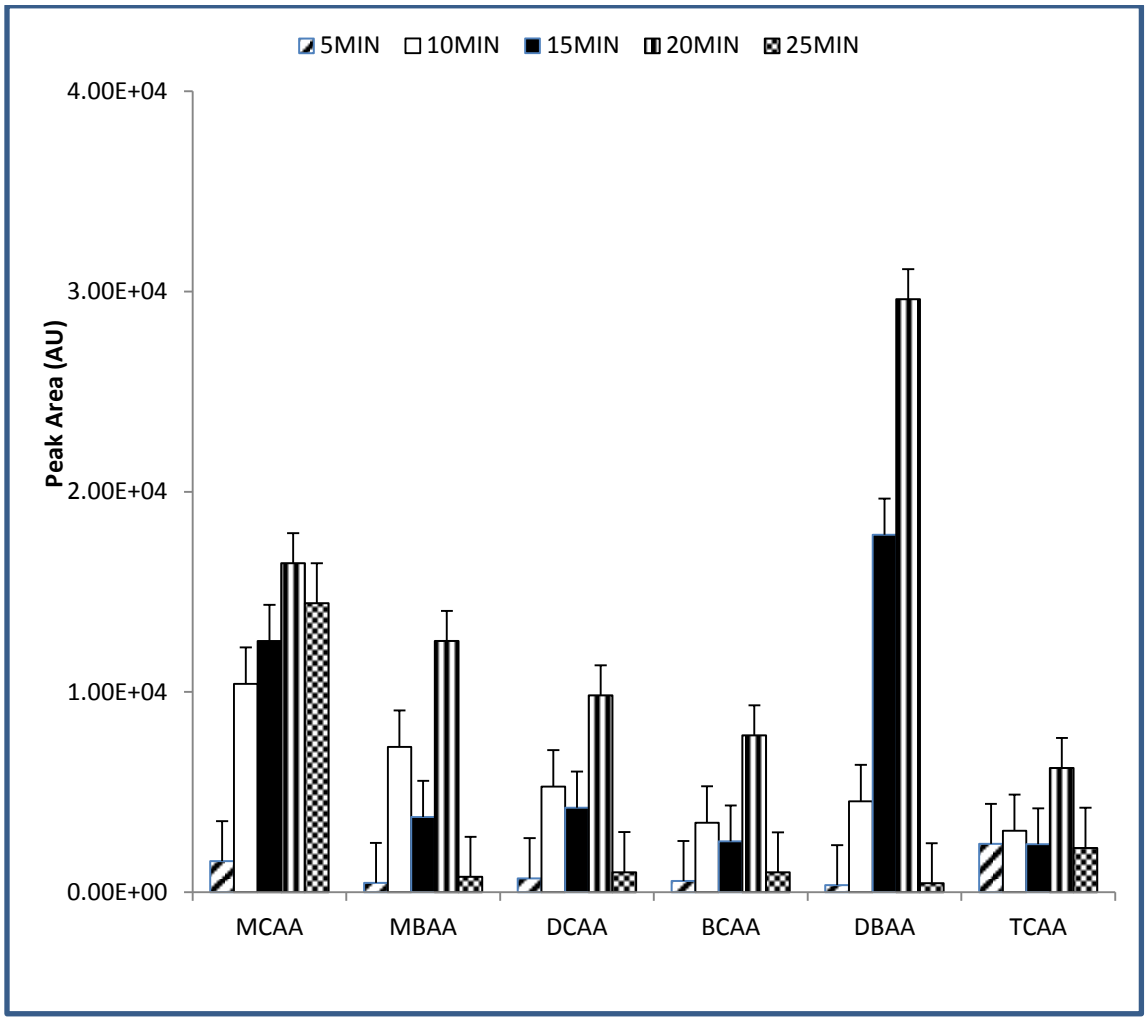


Figure 11: Effect of desorption time on µ-SPE of HAAs

After a convenient extraction, target analytes have to be eluted prior to chromatographic analysis. As a result, four different solvents (methanol, acetonitrile, acetone and 15 mM NaH_2PO_4 –pH 2.08) were evaluated as eluents and NaH_2PO_4 buffer gave the most reproducible recovery values. Owing to the polar nature of HAAs, they are preferentially desorbed by relatively polar solvents than the less polar ones.

3.6 Effect of ionic strength

For polar analytes, solubility in aqueous media generally decreases with increasing ionic strength. It was therefore expected that addition of sodium chloride would increase the μ-SPE extraction efficiency of HAAs in water sample.

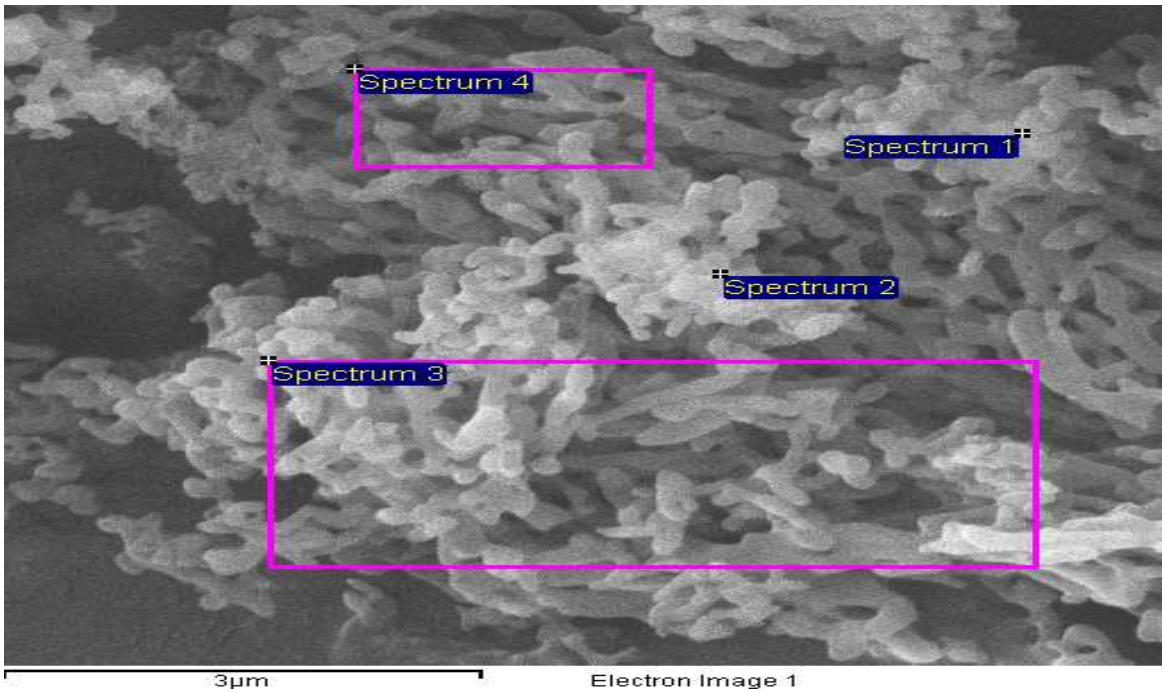
The effect of salt addition in the range of 5.0 to 30.0% W/V was evaluated. However results showed that the extraction efficiency did not increase with increasing salt addition. For polar analytes, solubility in aqueous media generally decreases with increasing ionic strength. It was therefore expected that addition of sodium chloride would increase the μ-SPE extraction efficiency of HAAs in water sample. The effect of salt addition in the range of 5.0 to 30.0% w/v was evaluated. However results showed that the extraction efficiency did not increase with increasing salt addition. Reasons could have been the influence of increasing viscosity that decreases the mass transfer processes.

3.7 Quality assurance

The scanning electron microscopic studies, SEM (Figure.12 and 13) of both sorbents reveal that rice husk silica has micropore structures of comparatively smaller size.

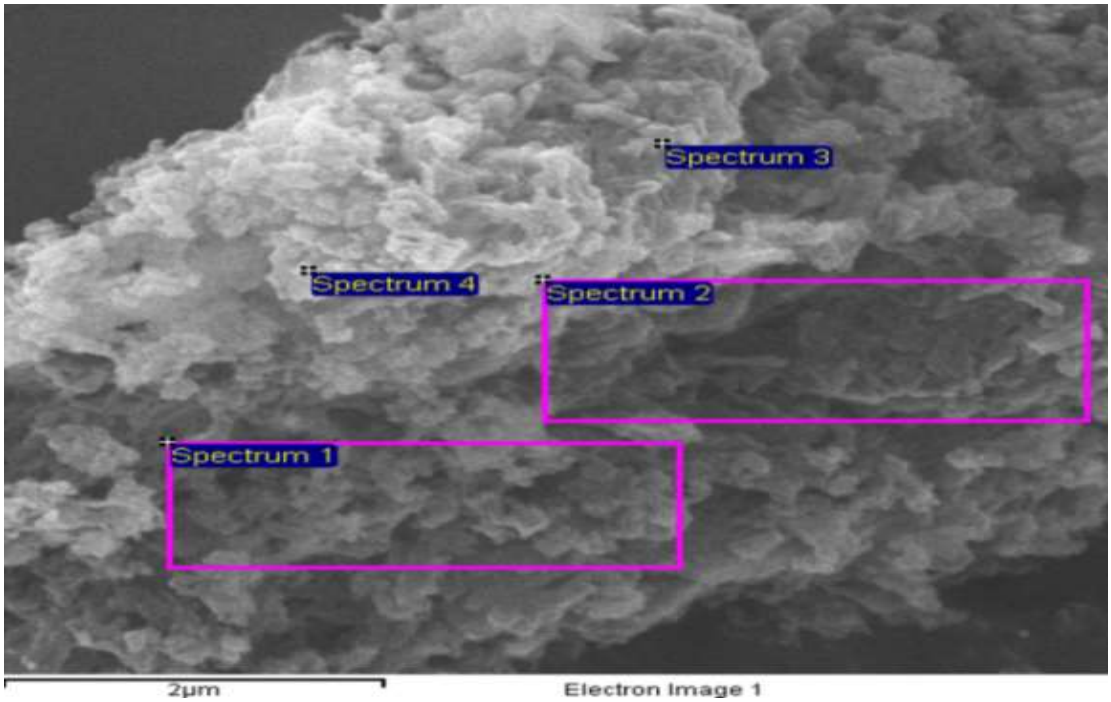
Both micrograms show a general porous morphology, however in Fig.13, pores increase both in number, size, surface area and volume.

From Figure 12, the EDX Spectra's indicate the presence of a good percentage of silicon based on the assumption that all silicon is in the form of silica. Figure 13 further indicates the incorporation of iron into the microporous structure. Generally, the adsorption of HAAs by silica-Fe sorbent is highly influenced by the sorbent's pore size, distribution, surface area and chemistry.



Processing	option: All elements analysed (Normalised)				
Spectrum	Instats.	O	Na	Si	Total
Spec.1	Yes	60	1.96	38.03	100
Spec.2	Yes	61.68	1.44	36.88	100
Spec.3	Yes	57.47	1.5	41.03	100
Spec.4	Yes	60.02	1.66	38.32	100

Figure 12: SEM micrograph for Silica



Processing option: All elements analysed (Normalized)						
Spec.	Instats.	O	Na	Si	Fe	Tot.
Spec.1	Yes	61.56	0	36.30	2.14	100
Spec.2	Yes	55.56	1.91	40.05	2.48	100
Spec.3	Yes	66.03	1.81	30.89	1.28	100
Spec.4	Yes	62.01	2.31	34.53	1.15	100

Figure 13: SEM micrograph for Silica-Fe

Incorporation of Fe (iii) in to the silica porous structure improves the adsorption capacity and selectivity for various toxic compounds through complex formation, hydrogen bonding, acid-base, electrostatic and most probably π-π interactions. During sol-gel process, some active species like the various functional groups, metal ions and metal oxides are usually incorporated in to the sorbents porous matrix and results to selective adsorption of HAAs. From the BET analysis results (Table 2), Silica-Fe shows higher specific surface area, pore volume and average pore diameter than silica. The low surface area of silica is as a result of adsorption of the water molecules to its porous matrix. However, calcination expels the water molecules and decomposes the nitrates to nitrogen dioxide which in turn leads to increased specific surface area.

Table 2: BET Analysis of Silica and Silica-Fe

SORBENT	Specific Surface Area (M^2/g)	Pore Volum Cm^3/g	Average Pore Diameter(nm)
Silica	225.4306	0.273179	4.84725
Silica-Fe	276.9548	0.422737	6.10550

Optimized μ-SPE extraction conditions were applied in measuring precision, accuracy, linearity, limits of detection (LODs), and limits of quantitation (LOQs). The results are represented in Table 3. The linearity of calibration plots was studied by spiking double distilled water over the concentration range of 1-150 μg/L. Good linearity with coefficients of determination (r^2) ranging between 0.9916 and 0.9989 was obtained.

Table 3: Calibration data for the proposed µ-SPE method (n=3)

Analyte	r^{2a}	Equation	LOD[b] (µg/L)	% RSD[c]		LOQ[d] (µg/L)
				Intra-day (n=3)	Inter-day (n=5)	
MCAA	0.9916	Y=12319X+20112	0.07	7.40	5.4	0.232
MBAA	0.9989	Y=110940X-53738	0.092	5.40	8.4	0.304
DCAA	0.9985	Y=14187X+36223	0.064	0.03	6.8	0.211
BCAA	0.9951	Y=62142X+36223	0.001	0.26	5.9	0.003
DBAA	0.9963	Y=23745X+24190	0.008	0.05	6.9	0.025
TCAA	0.9963	Y=15072X-19412	0.01	0.03	6.6	2.16

[a] Coefficient of determination, [b] Limit of quantitation, [c] relative standard deviation and [d] Limit of detection

To evaluate the accuracy of μ-SPE method, percentage recoveries at spiking levels of 10 and 15 μg/L concentrations were performed on swimming pool waters (Table 6). The corresponding chromatogram obtained after a 10 μg/L HAA standard spike is displayed by Figure 14.

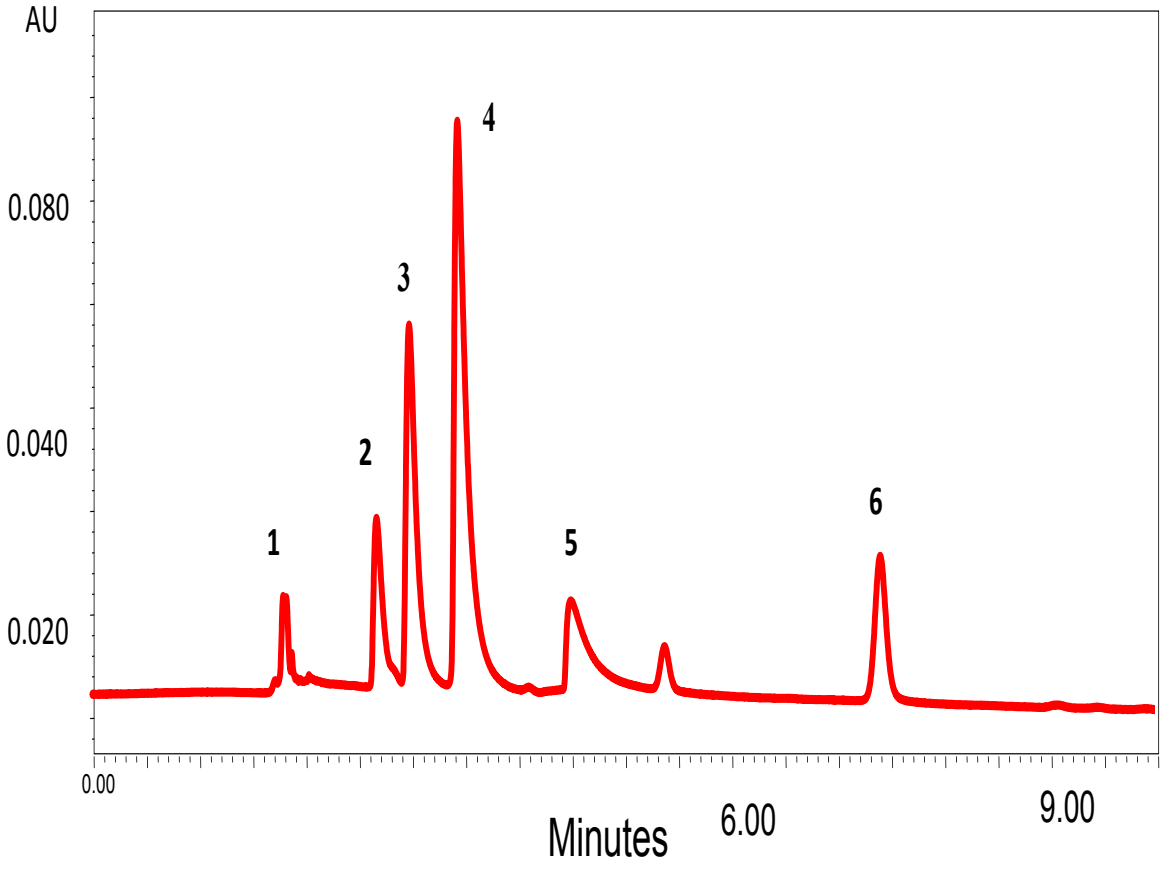


Figure 14: Chromatogram of an extracted swimming pool water sample spiked with 10µg/L concentration of HAA standard. Peak identification: 1. MCAA; 2. MBAA; 3. DCAA; 4. BCAA; 5. DBAA and 6. TCAA.

The precision of the method was expressed in terms of the relative standard deviations (RSDs). The intra-day precision of the method was determined by analysing three spiked double distilled water sample containing 10µg/L of HAAs. The %RSDs were in the range of 0.03 to 7.4. The inter-day precision of the method was determined by conducting five consecutive extractions each day for a period of four days using different µ-SPE devices. The inter-day %RSDs varied between 5.4 and 8.4.

Based on the IUPAC criteria, the limits of detection (LODs) and limits of quantitation (LOQs) were computed from the following equation:

$$LOD(LOQ) = \frac{Ck + 3(10)Sd}{b} \qquad (14-2)$$

Where 'b' is the slope of the calibration curve,

S_d is the standard deviation

C_k is the concentration of the blank.

The LOD and LOQ values are calculated basing on signal to noise ratio of 3 and 10 respectively. The LOD values range from 0.001 to 0.092 µg/L and the LOQ values were between 0.003 and 0.304 µg/L (Table 3). Results indicate appreciable LODs and %RSDs values that compare quite well with what is reported in literature (Table 4).

Table 4: Comparison of the developed method with other analytical techniques used in the determination of HAAs in water matrices

METHOD	LODs (µg/L)	RSDs (%)	Derivat. Time (min)	Sample volume (mL)	Extraction time (min)	REF.
LLE-GC-ECD (USEPA-552-1)	0.0074-0.14	7.0-59.0	30	100	12	[66]
LLE-GC-ECD (USEPA-552-3)	0.012-0.17	0.36-4.0	120	40	NA	[100]
HS-HFLPME-GC-ECD	0.10-18.00	5.0-12.0	NA	10	60	[92]
HS-SPME-GC-ECD	0.01-0.40	6.3-10.9	5	10	35	[79]
SDME-GC-MS	0.10-1.20	5.1-8.5	20	3	20	[98]
Evaporate-SPME-GC-MS	0.01-0.20	6.3-7.9	10	30	10	[101]
SLME-LC-UV	0.02-2.69	1.5-10.8	NA	20	60	[69]
LLE-ESI-MS	0.13-0.60	NA	NA	188	7	[97]
LLE-GC-MS-MS	0.025-1.00	0.9-19.9	180	1	7	[96]
UPLC-MS	0.18-71.5	NA	NA	30	NA	[99]
µ-SPE-UPLC-UV	0.001 - 0.092	0.03-7.40	NA	20	20	Current work

The developed method was tested on swimming pool water taken from two swimming pools around KFUPM. Table 5 indicates the mean concentration obtained for the six HAAs analytes. The method was able to determine HAAs analytes below the standard value set by USEPA (0.03 mg/L).

Table 5: Application of proposed µ-SPE method on swimming pool water

	Mean Conc. (µg/L) Pool A	Mean Conc. (µg/L) Pool B
MCAA	46.5	48.6
MBAA	24.6	8.6
DCAA	34.6	11.3
BCAA	6.8	7.1
DBAA	16.2	16.4
TCAA	12.6	nd

nd = not detected

To assess the matrices interference of the μ-SPE, swimming pool waters were spiked with 10 and 15 μg/L concentrations and extractions performed thereon. Table 6 shows the quantitative data obtained from the μ-SPE extraction technique.

Table 6: Mean recovery studies performed on water samples from two swimming pools A and B

		Mean recovery (%)*	
Analyte	Spiked concentrations (μg/L)	Pool A	Pool B
MCAA	10	91.6	95.1
	15	94.5	96.7
MBAA	10	66.4	74.8
	15	69.7	79.6
DCAA	10	99.9	66
	15	109	68.9
BCAA	10	87.2	100.2
	15	97.2	110.9
DBAA	10	97.5	87.5
	15	108.1	88.4
TCAA	10	74.8	76.5
	15	75.8	76.5

*RSDs in the range of 2 to7%

Results indicate that there are minimum interferences from the sample matrices.

3.8 Conclusion

Membrane-protected micro-solid phase extraction followed by UPLC-UV analysis was developed to determine six HAAs in swimming pool waters. It was simple, sensitive and relatively fast technique that did not require any derivatization.

The target analytes were directly analysed within ten min of UPLC run time. The developed method exhibited excellent precision and the detection limits were comparable to those of the standard USEPA methods.

The μ-SPE device used in the study is easy to make, inexpensive, uses a few microliters of organic solvents and does not suffer from sample carry over problems. Each device can be used for more than twenty extraction times without registering memory effects and moreover, the novel Silica-Fe sorbent used is efficient and easy to process. Some low relative recoveries observed during extractions from spiked swimming pool water samples highlighted the need for further fine-tuning of rice husk silica sorbent using different iron oxide load percentages and interestingly, it is presently being pursued in our laboratory.

CHAPTER 3. MICROWAVE ASSISTED ELECTRO-MEMBRANE EXTRACTION OF PERCHLORATES IN SEA FOOD SAMPLES FOLLOWED BY ION CHROMATOGRAPHY

1. LITERATURE REVIEW

Water contamination by the perchlorate anion (ClO_4^-) has become a significant environmental concern especially in areas surrounding aerospace operations, usage or manufacture of pyrotechnics, munitions, and other industrial applications of perchlorate salts [102-105]. Such level of contamination is of profound toxicological interest since perchlorate can potentially interfere with normal thyroid function in animals [106]. Due to similarities in size and hydration energy, perchlorate ion interferes with the uptake of iodine and affects the production of hormones in the thyroid gland. These hormones are essential for mental development and the development of hearing abilities in children. The ion is very mobile, unreactive and its salts are extremely soluble in water matrices [102, 107] a factor that makes it persistent in both ground and surface waters [108]. The inertness of perchlorate is due to its non-complexing nature, and kinetic inertness to oxidation and reduction. Perchlorate has one of the lowest hydration energies among inorganic anions, and maybe due to the delocalization of the negative charge over its four oxygen atoms. It's high polarizability and low charge density results in interactions that are similar to hydrophobic interactions.

Both aquatic and terrestrial species are prone to perchlorate contamination and Smith etal [108] in his study proclaim to have detected perchlorate anion in fish and other aquatic organisms closer to a military manufacturing site. His findings suggest that perchlorate pose an even greater health concern than previously conceived [109] and thus has prompted several related departments to established standard for perchlorate [110]. The United States' Environmental Protection Agency's (US EPA) office of Water added perchlorate to the Contaminant Candidate List in 1998 and to the unregulated contaminants monitoring rule in 1999. Up to date, suppressed ion chromatography coupled with conductivity detector has been used to quantify perchlorate to 1 μg/L method detection limit (MDL) in water matrix [111, 112]. However, this method faces more challenges with more complex background matrices and as a result, trace detection of perchlorate suffers [112, 113]. Conductivity detection works relatively well for simple matrices such as drinking water. The use of IC-ESI-MS method in providing low detection limits has been explored with marked success but the technology is rather complex and expensive [114]. This therefore demands for an extensive clean-up and or less expensive but sensitive sample preconcentrating stage of complex matrix preceding IC-Conductivity detection [114-116]. Several clean-up methods that minimize ionic interferences in these extracts have been suggested [117]. Traditionally, solid sample are extracted using soxhlet and ultrasonication methods, both of which are capable of reasonable recoveries [118]. However, the multi-step procedures involved are tedious, time-consuming, uses volumous toxic organic solvents and leads to analyte loss [118]. The disposal challenges of waste organic solvents used contradicts with modern green analytical chemistry principles.

To green the extraction process of target analytes from solid samples and speed up extraction time, the use of supercritical fluid extraction (SFE), pressurized liquid extraction (PLE), and microwave-assisted extraction (MAE), have been explored [119]. MAE results to great reduction in extraction time, solvent consumption and offers opportunities for performing multiple extractions[120]. Water has been used as an alternative solvent for MAE since it is cost effective, safe, environmentally benign and has good absorptive properties for microwave energy [121]. Because of its high permittivity and heat of vaporization in relation to other solvents, water is suitable for MAE of many polar analytes [122]. Despite the many advantages MAE has to offer; few studies promulgate its ability to quantify inorganic anions from complex matrices. Worse still, in more complicated matrices, trace detection of perchlorate using IC-conductivity detector method results to higher background noise which compromises the detection limit [113]. Typically, after MAE, it is necessary to further clean-up, pre-concentrate the target analytes from the complex sample matrices so as to minimize co-elution , purify and concentrate the extracts [120]. Microwave-assisted extraction in combination with SPE for sample clean-up has widely been used in preparation of solid samples for instrumental analysis [123]. However, the use of such integrated system involves larger volumes of toxic organic solvents. More still, there are good reasons to explore low cost alternatives aimed at reducing the usage of organic solvents resulting from such SPE system.

Currently, a membrane-based extraction technique based on supported- liquid membrane (SLM) called electromembrane extraction (EME) has emerged as a simple and cost-efficient technique in sample preparation [124]. This novel extraction method uses an electric field as driving force rather than passive diffusion.

The set-up is such that the cathode (one electrode) is placed in an aqueous donor sample, while the anode (the other electrode) is placed in an aqueous acceptor solution and the reverse. When the electrical potential is applied, there occurs mass transfer of ionic species across the SLM. After extraction, the acceptor solution is analysed directly by ion chromatography (IC), capillary electrophoresis (CE) or high performance liquid chromatography (HPLC). The working and introduction to this method appears in most recent reviews [125-127]. EME was found to be a much faster process compared with the conventional liquid phase microextraction (LPME) when it was used for extraction of basic drugs [128]. Several factors affecting extraction and enrichment have been investigated and knowledge about parameters affecting the EME performance has been improved [129-130].

In this study, a new strategy involving a microwave assisted-EME-IC-Conductivity detector is optimized and used to quantify perchlorate ions in sea food samples collected from the eastern province of Saudi-Arabia. An electromembrane extraction of the MAE extract is performed in order to introduce more clean analytes for the IC/Conductivity detector aimed at improving the detection limits and minimizing co-elution of interfering ions in tissue extracts.

2. EXPERIMENTAL

2.1 Reagents and Materials

All chemicals used were of reagent grade and deionized water with resistivity higher than 18 M Ωcm was used throughout. Sodium hydroxide, potassium hydroxide, sodium chloride, Nitric acid were obtained from J. T. Baker (Philips Burg, NJ). HPLC-grade organic solvents and room temperature ionic liquids 1-butyl-3-methylimidazolium octylsulfate ([BMIM][$OcSO_4$]), and 1-butyl-3-methylimidazolium hexafluorophosphate ([BMIM][PF_6]) and 1-butyl-3-methylimidazolium dihydrogen phosphate (BMIM[PO_4]) for EME extractions were obtained from Sigma Aldrich (Milwaukee, WI, USA) and Strem Chemicals (Newburyport, MA, USA) respectively. Water used for preparing stock solutions and as a mobile phase was generated by a Milli-Q water purification system (Millipore, Bedford, MA, USA).The DC power supply used in EME was an ES 0300 with programmable voltage in the range 0–300V and with a current output in the range of 0–450mA (Delta Elektronika BV, Zierikzee, The Netherlands). Platinum wires with a diameter of 0.5mm (K.A. Rasmussen, Hamar, Norway) were used as electrodes. Q3/2 Accurel polypropylene flat sheet membranes (157μm thickness, 0.2μm pore size) were purchased from Membrana (Wuppertal, Germany) and used in making EME membrane envelopes. A Vibramax 100 m from Heidolph (Kelheim, Germany) was used to agitate the extraction unit during the extraction. Standard stock solution of sodium perchlorate at 100 μgmL^{-1} was prepared in double distilled water and stored at -4^{o}C in a refrigerator.

From the 100 μg/mL stock solutions, working standards were prepared in 100 mL volumetric flasks.

2.2 Apparatus

2.2.1 EME system

The EME system is presented by Figure 15 and was fully described in earlier reports [124, 127, 130]. The EME system consisted of a 30 mL glass vial.

Membrane bags were prepared by cutting commercial polypropylene flat-sheet membranes into rectangular sheets with dimensions of 1.0 cm by 1.5 cm. The shorter (1.0 cm) edge was folded over to a width of 0.8cm. The edge of the fold-over flap was heat-sealed with an electrical sealer. One of the two remaining open ends was similarly heat-sealed to create an envelope. The positive electrode was inserted into the membrane envelope containing the acceptor solution. The syringe tip, the negative electrode, and the membrane envelope were all fixed on the cap of the sample vial.

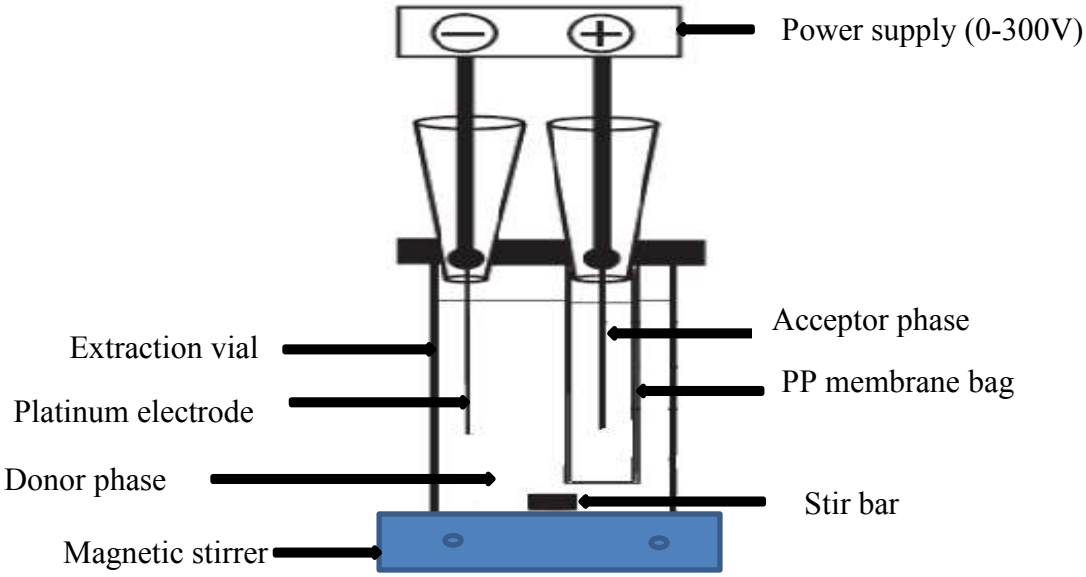


Figure 15: Schematic drawing of EME system used.

Before EME extraction, the membrane envelope was first impregnated for 10 s with 1-hexanol and then filled with 150 μL of an acceptor solution. A 50 mL Hamilton syringe was used to fill the membrane envelope with an acceptor solution and for drawing out the solution from the membrane envelope after extraction. A magnetic stir bar was placed in to the glass vial to ensure constant stirring of the donor solution (20 mL). Variable voltage 0–300 V was continuously monitored using a voltmeter.

2.2.2 MAE- System

Microwave system Multiwave 3000 (Anton Paar, Graz, Austria) with rotor HF100 and software version v1.52 was used for closed-vessel extractions. The system was equipped with 16 high pressure polytetraflouroethylene-tetraflouroethylene, (PTFE-TFM) vessels with an internal volume of 100mL (maximum pressure and temperature of 40 bars and 240°C, respectively). Before and after use, all plastic and glass utensils were thoroughly cleaned with concentrated nitric acid and then rinsed with copious amounts of Milli-Q water.

2.2.3 Ion Chromatography (IC)

Analysis was carried out using Dionex (Sunnyvale, CA, USA) ICS-2000 IC system equipped with a GS 50 gradient pump, an EG 50 eluent generator, an AS 50 auto sampler, an LC 30 chromatography oven, a CD 25 conductivity detector, a 2 mm anion self-regenerating suppressor (ASRS Ultra II), Suppressor External Regen Installation Kit for External Water Mode and Conductivity Meter (Thermo Orion).

Chromeleon 6.5 Chromatography workstation software was used to control the system. Ion separation was made with a Dionex Ion Pac AS16 Ion Pac AG16 Guard analytical column.

Conditions for the system were as follows: flow rate = 1.0 mL/min; eluent = 50 mM sodium hydroxide; injection volume =100 µL and Temperature = 30°C. Ion detection was by suppressed conductivity in the external water mode. A six-point standard curve was constructed from constant volume injections of calibration standards ranging from 1 to 125µg/g.

2.3 MAE-EME Sample preparation

Fresh sea food samples were purchased from local fish markets in Al-khobar in the eastern province of Saudi Arabia. The study attempted to include all representative sea food species within the community. Specimens were collected irrespective of their body size and weight. A minimum of two specimens from each individual species were gutted, filleted, minced and frozen prior to analysis. Examples of the sea foods used in the experiment are displayed in Figure 16.

Figure 16: Examples of seafood stuffs used in this experimental work.

The frozen samples were thawed and allowed to reach room temperature. Several 3 g sea food samples were weighed, rinsed with ultra-pure water, air-dried and introduced into a sealed PTFE vessel. 25mL volume of 100 mM HNO_3 solution followed by 100 μg/L perchlorate solution was prepared in each of the vessels. The vessels were then sealed and put into the microwave extraction system. Extractions were performed at various conditions of temperature of 100°C and irradiation power of 250W. Desired temperatures were achieved by dynamic digestion times ranging from 5 to 15 min followed by a holding time of 10 min in the static mode. After cooling, the vessels were opened and the solution transferred into an EME set up as a donor solution for further extraction. The acceptor solution constituted 150 μL of 100mM NaOH. Platinum electrodes were used during EME extraction with one electrode inserted in to the acceptor solution. The other electrode, the anode, was led directly into the sample solution. The electrodes were subsequently connected to the power supply and the predetermined voltage was turned on. The extraction was performed for 10 min and at a stirring speed of 700 rpm. Under the voltage applied, the target analytes migrated from aqueous sample to SLM, and then into the acceptor phase. After the extraction, the voltage was turned off and 120 μL of the acceptor solution was transferred into flat capped micro centrifuge tube for analysis by the IC.

3. RESULTS AND DISCUSSION

3.1 Optimization of MAE conditions

The optimization of MAE conditions has been studied in several applications. The efficiency of the process is directly related to the operation conditions selected [131].

For this work, temperature, digestion time and power were considered in the optimization process.

Preliminary experiments were performed under the same operating conditions (power, 250 W; 10 min ramp to 100°C; and solvent volume, 25 mL) to select the best extraction conditions. Water was selected as an extracting solvent basing on its excellent solubility and better microwave absorbing properties.

3.1.1 MAE Temperature

The principle of heating using microwave energy is based on the effect of microwaves on molecules by ionic conduction and dipole rotation [131]. 25ml of Water (100 mM HNO_3) acted as a medium in the extraction process. The electrophoretic migration of ions in water results into friction and heats the water [131]. The heat generated contributed to increased analyte recoveries. Because closed vessels were used, the temperatures might have reached well above the boiling points of the extracting solvents. These elevated temperatures resulted in to elevated extraction efficiencies. To determine the suitable MAE conditions, different temperatures of 60, 80, 100, 150^{o}C were evaluated. Figure 17 shows the MAE temperature profile. Since high temperatures result to higher extraction efficiency, 150^{o}C was expected to be more appropriate for this experiment but however from the graph, 100^{o}C gave an optimum extraction yield. At elevated temperatures, matrix effects from co-eluting residual matrix components result into a condition that compromises the perchlorate peak.

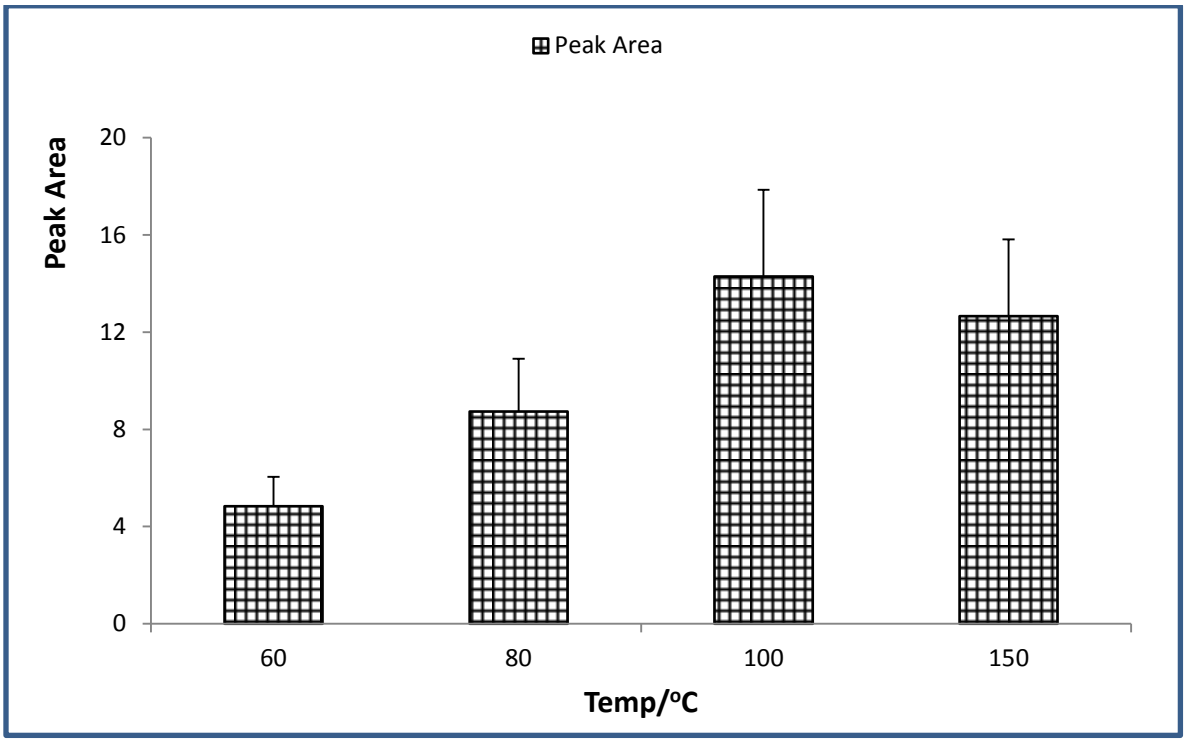


Figure 17: Effect of MAE temperature on perchlorate extractability

At higher temperatures, solvents have higher capacity to solubilize analytes. This is because increased temperature reduces surface tension and viscosity which in turn improves sample wetting and matrix penetration.

3.1.2 MAE Digestion time

In MAE the period of heating is an important factor to be considered and the extraction times are usually very short compared to conventional techniques. This is aimed at avoiding possible thermal degradation and oxidation typical of target compounds sensitive to overheating of the solute–solvent system [132]. For this reason, the effect of micro wave extraction time was evaluated for a range of 5 to 20 min.

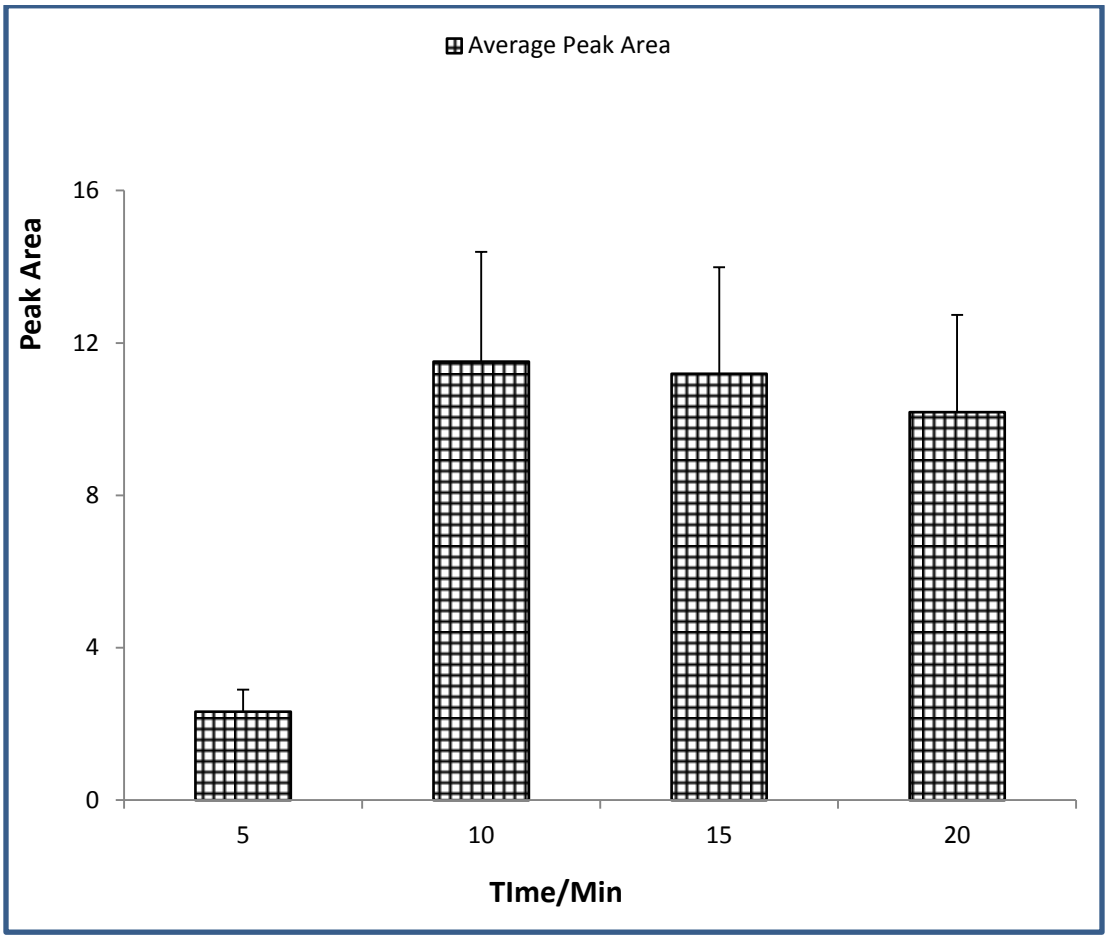


Figure 18: MAE digestion time profile

From the plots (Figure 18), 10 min of extraction was considered as the optimum value. Above 10 min, there was no significant increase in extraction registered. Irradiation time is also influenced by the dielectric properties of the solvent. Because water was used as an extracting solvent, it might have heated up tremendously on longer exposure time affecting our target analytes.

3.1.3 Microwave Power

Microwave power is directly related to the quantity of sample and the digestion time required. In the MAE set up experiment, 16 vessels were treated in a single run. Since this is a closed vessel system, the chosen power settings depends on the number of samples to be extracted during one extraction run.

The power was chosen correctly to avoid excessive temperatures, which could lead to solute degradation and overpressure inside the vessels. To determine the optimum value a, the MAE experiment was performed on four different power ratings of 100, 150, 250 and 300 W.

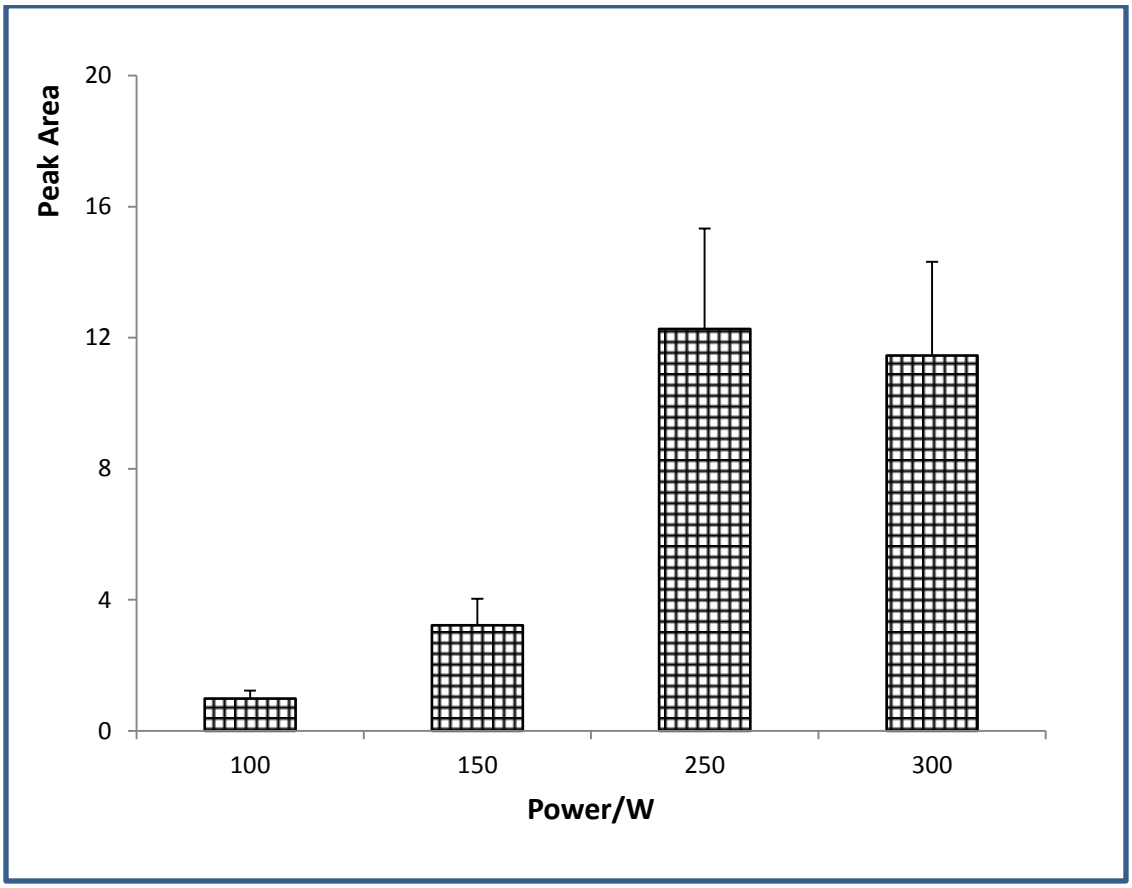


Figure 19: Effect of microwave power on perchlorate extractability

From the above Figure 19, 250W was considered as the optimum power since increasing the power to 300W resulted to slight decrease in extraction efficiency.

Microwave power and temperature are interrelated because high microwave power can bring up the temperature of the system and result in the increase of the extraction yield until it becomes insignificant or declines [133].

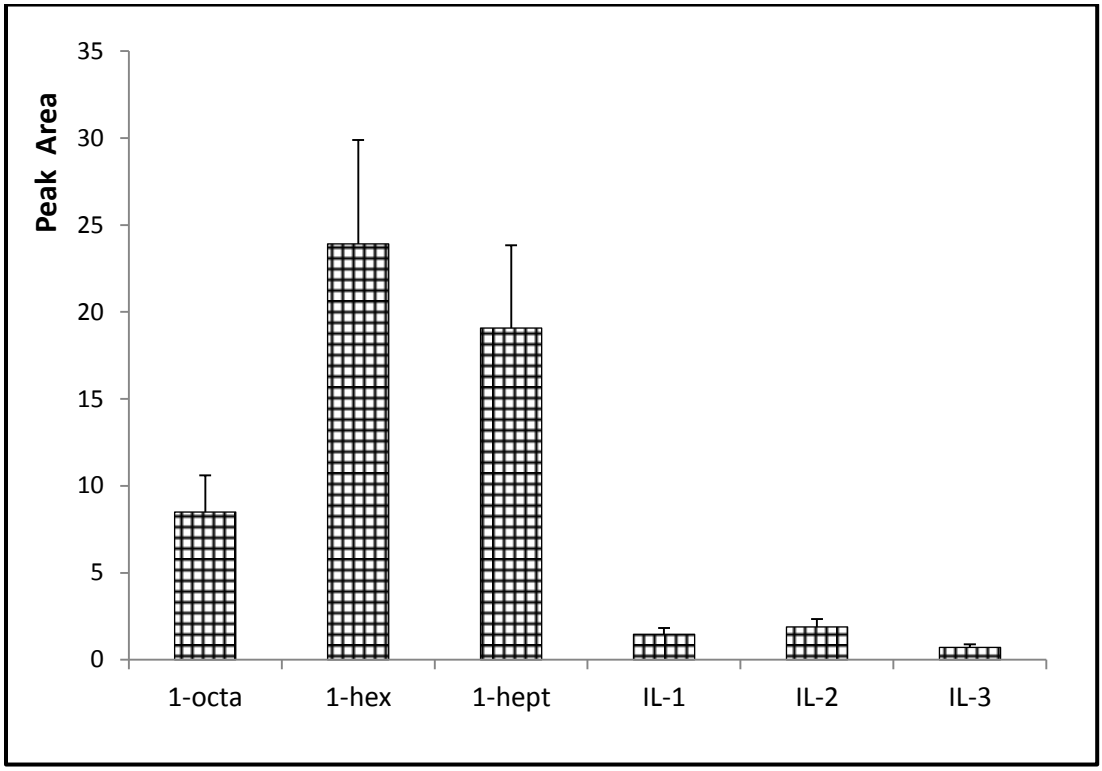
3.2 Optimization of EME conditions

Other factors were kept constant during the optimization process (optimized MAE conditions, stirring rate of 750 rpm, 24 V potential difference, and 10 min as extraction time, and 25 mL of 100 mM HNO_3 as donor and 100 mM NaOH as acceptor). For efficient EME process, SLM, pH for both donor and acceptor solutions, extraction time and applied voltage were all optimized.

3.2.1 Supported liquid membrane (SLM)

The selection of a suitable organic solvent as the SLM plays an important role in EME, as it serves as a carrier between the donor phase and the acceptor phase [134]. A suitable solvent is highly critical for electrokinetic cross-membrane extraction to succeed. The flux of analyte during extraction is affected by the gradient of analyte concentration across the SLM which once fine-tuned contributes to analyte selectivity as well as good clean-up [135-137]. The main factor considered for a suitable SLM was the stability of the currents during EME process and its extraction selectivity for the target analyte.

Hence, alcohols and ionic liquids (Figure 20) were evaluated to determine their extraction efficacy for perchlorate anion. Ionic liquids (IL) used were 1-butyl-3-methylimidazolium octylsulfate ([BMIM][$OcSO_4$]), and 1-butyl-3-methylimidazolium hexafluorophosphate ([BMIM][PF_6]) and 1-butyl-3-methylimidazolium dihydrogen phosphate BMIM[PO_4] . Results showed that 1-hexanol had higher extraction efficiency and selectivity for ClO_4^- ion.



IL-1 = [BMIM][OcSO4]

IL-2 = [BMIM][PO4]

IL-3 = [BMIM][PF6]

Figure 20: Influence of SLM on extraction performance of MA-EME extraction

The reason for the superior extraction efficacy of 1-hexanol is not clear at the moment. However, 1-octanol and ionic liquids when used as SLM gave excessive and unstable current drops.

3.2.2 Effect of pH of the donor and acceptor solutions

The charged forms of the analyte are cardinal in achieving maximum EME efficiency. This is because the electrical potential difference serves as the methods driving force generator. Due to the inertness of the SLM and analytes to the electrodes, the following are the probable electrode reactions that take place in the donor and acceptor solutions respectively.

Acceptor solution (positive electrode): $H_2O \longrightarrow 2H^+ + \frac{1}{2}O_2 + 2e^-$

Donor solution (negative electrode): $2H^+ + 2e^- \longrightarrow H_2$

From the above premise, pH values adjustments of both the acceptor and donor solutions were performed to facilitate mass transfer of analytes along the pH gradient.

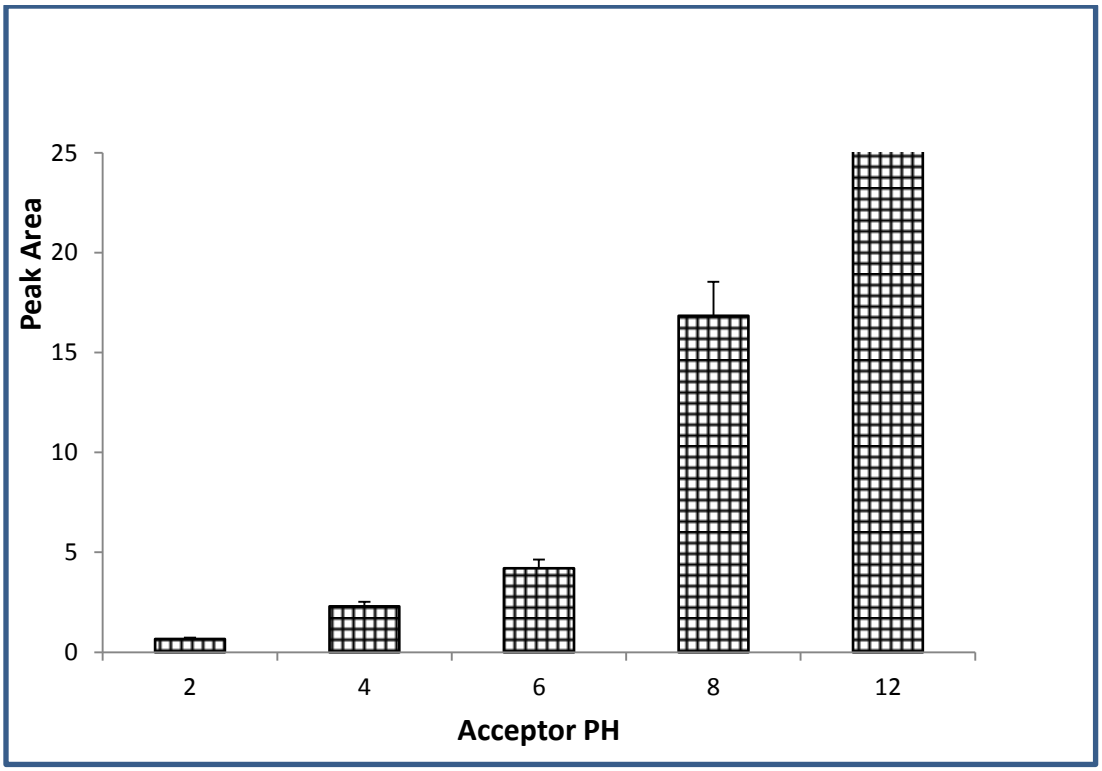


Figure 21: Effect of acceptor pH on extraction performance

Using 10 mM NaOH and 10 mM HNO_3 the pH of both acceptor and donor solutions were adjusted accordingly.

For both the donor and acceptor solution investigations were performed at pH 2, 4, 8 and 12. Nitric acid was chosen for pH modulation because the nitrate peak did not interference with our target analytes peak. Figure 21 shows pH 12 as the optimum pH of the acceptor solution. Basic pH of the acceptor solution was required for perchlorate extraction. Figure 22 shows the pH profile for the donor solution. At pH 2, the perchlorate ion was in neutral form as expected and poor extraction was eminent owing to slower migration of the analytes to the acceptor phase.

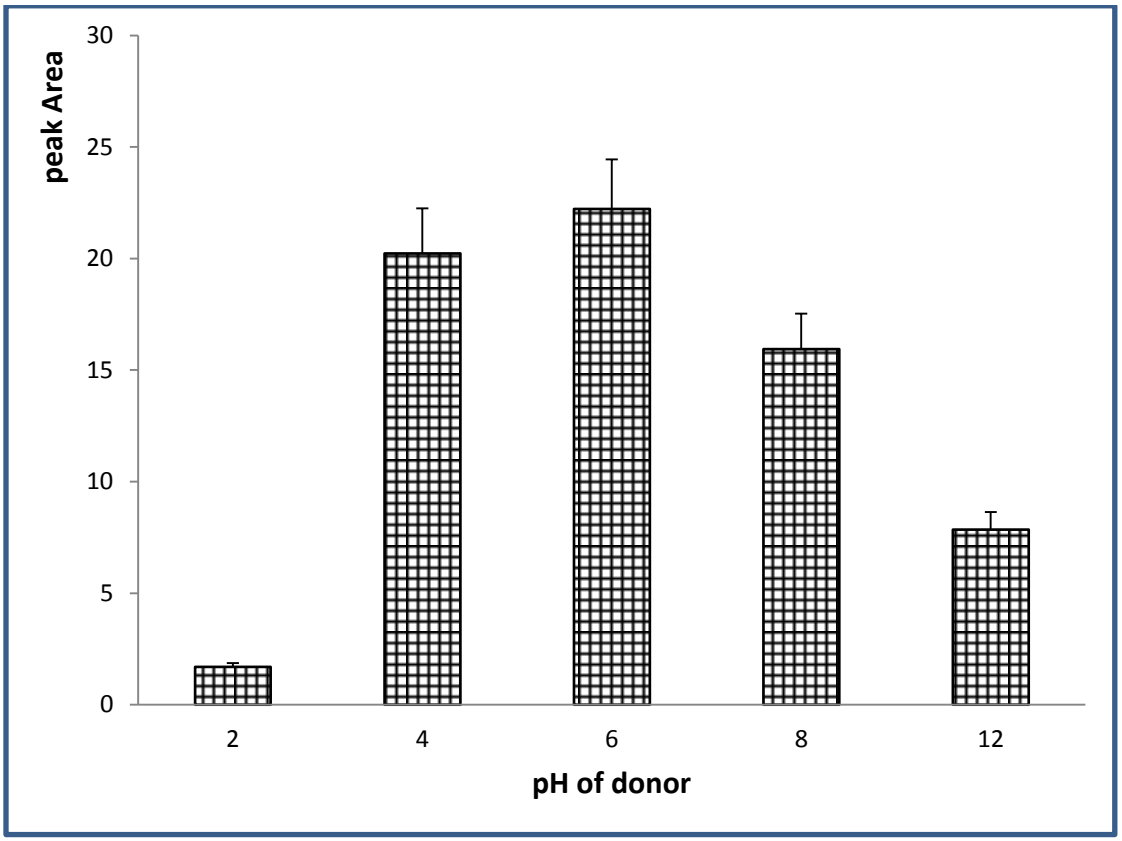


Figure 22: Effect of donor pH on perchlorate extractability

However at pH 6, there was a remarkable extraction efficacy and was considered as the optimum pH for the donor solution. Because the pH of milli Q water used was 5.8; a value closer to 6, all the proceeding experiments were performed without donor pH adjustments. At such pH, the perchlorate ion will be in an ionized form and suitable for electrical conductivity detection. However such displayed trend is in line with the theoretical understanding of the electrode processes [138].

3.2.3 EME extraction time

EME is an equilibrium based extraction and as such was demonstrated to offer fast extractions in relation to liquid-liquid extractions which are exhaustive [128]. To determine the most favorable extraction time, the experiment was conducted at extraction times of 5, 10, 15 and 25 minutes. Results in Figure 23 indicate that the EME system attained maximum extraction in 15 min however 10 min was considered optimum since the difference in extraction efficiency was very minimal.

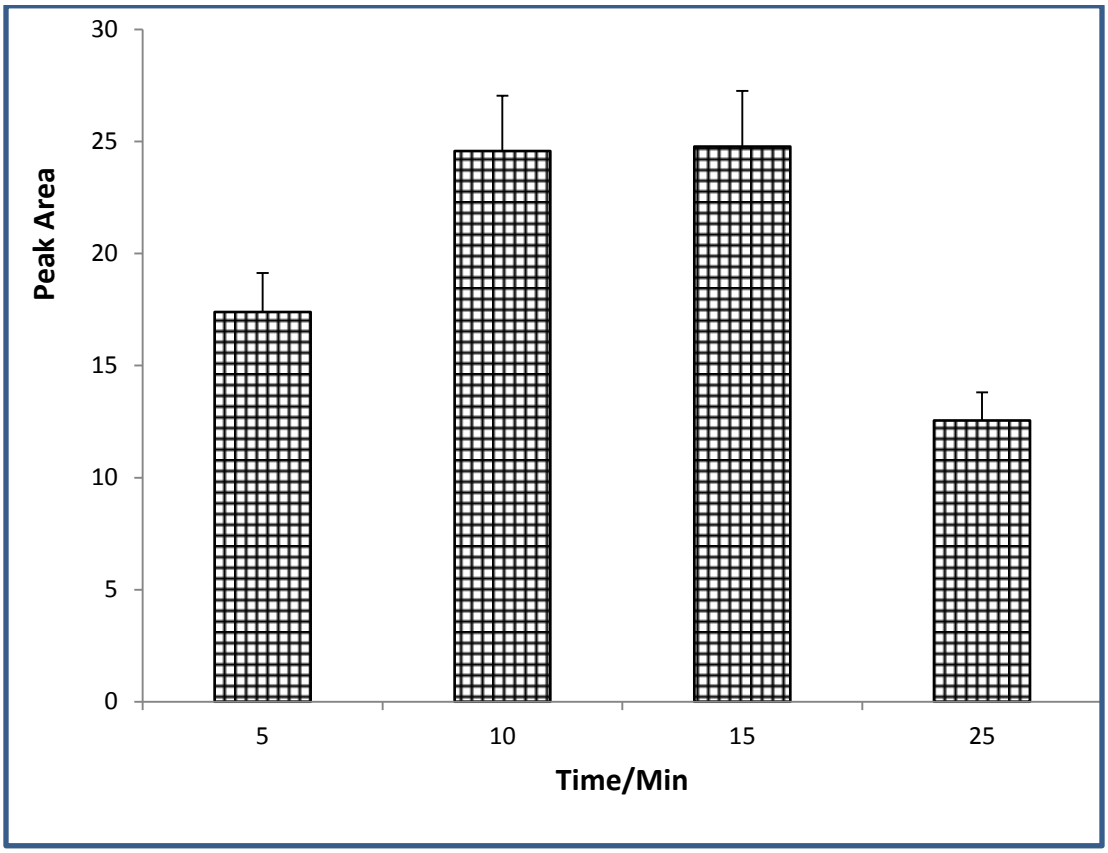


Figure 23: EME extraction time profile

At this time, the system might have entered a steady state condition resulting in to minimal gain in transfer. A similar trend has been observed in the previous reports [138].

After 15 minutes, the system registered a decrease in extraction efficiency which was attributed to small losses of polar solvent (I-hexanol) over time, resulting in back migration of the analytes and thus leading to lower preconcentration.

Also, the current became erratic possibly due to depletion of the organic solvent serving as SLM which compromised the integrity of the membrane. As a result, 10 min was chosen to be the most suitable extraction time for subsequent experiments.

3.2.4 Voltage selection

The flux of analytes varies with applied voltage [124] and in one of the EME mathematical model, it postulated that increasing voltage enhances extractability of target analytes [129]. However, when sufficient extraction time is provided, the same steady-state recoveries can be obtained [139]. Different voltages ranging from 0 to 200 V were applied to the EME system for 10 minutes as shown in Figure 24. There was almost zero transfer of perchlorate ions in to the acceptor solution observed at 0 V (without applying voltage).

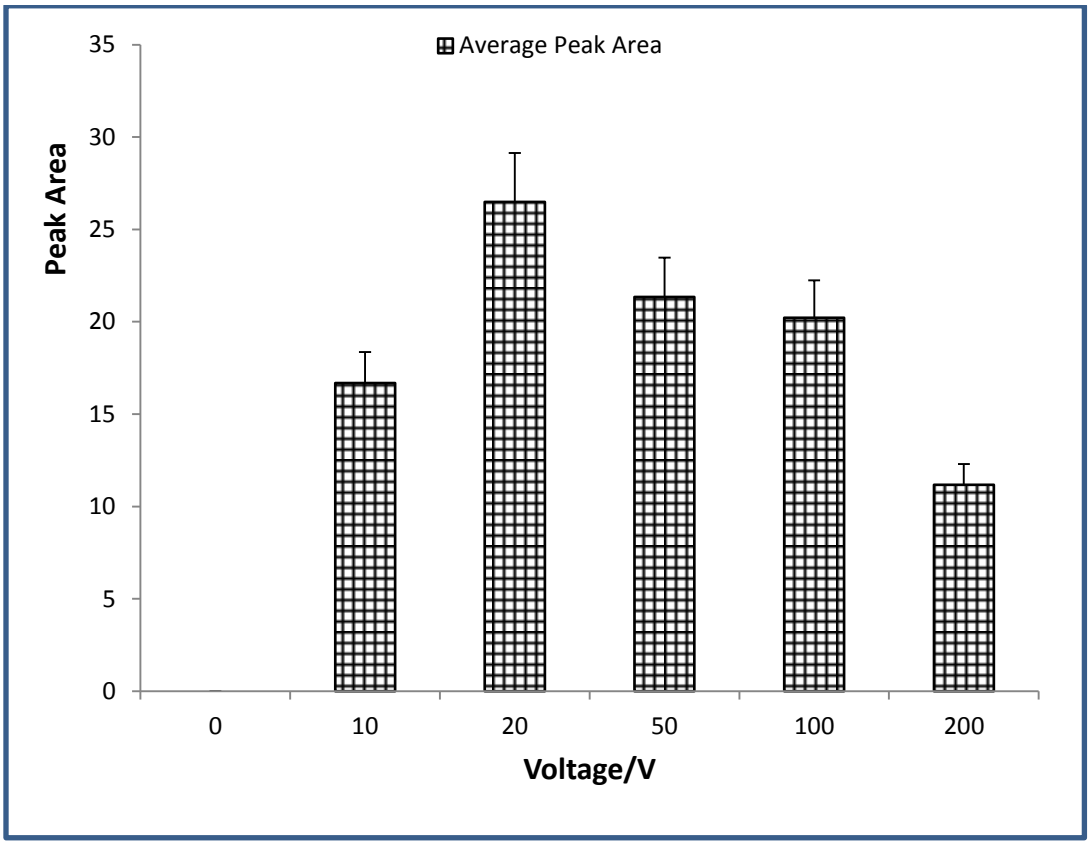


Figure 24: Selection of suitable Voltage for MAE-EME experiment

The probable reason seemed to be that diffusion was not yet forthcoming in such a shortest time. However, as voltage was applied to the EME system, the negatively charged perchlorate ions migrated to the direction of the anode across the SLM. Constant stirring of the donor solution ensured efficient replenishment and transfer of the ions in to the acceptor phase.

Voltages higher than 20 V tended to lead to current flow fluctuations resulting to excessive bubble formation (caused by the electrolysis of water) at the electrodes and this was not investigated. Extraction voltage of 20V was therefore selected for subsequent experiments.

3.3 Quantitative parameters

The study was aimed at developing an analytical method for the determination of trace level perchlorate in sea food samples.

Ideally, the method would reduce the level of interfering matrix ions without loss of perchlorate ion. The method would also offer high extraction efficiency at reduced cost. To assess the suitability and practicability of MAE-EME-IC method, various quantitative parameters such as linearity, repeatability, limit of detection and quantification were investigated. The analytical method was operated at pre-optimized conditions and results for various samples are summarized in tables below. Calibration curves were obtained by plotting the peak areas of the individual analytes with their corresponding concentrations in spiked sea food sample solutions.

Using least squares linear regression analysis, the linearity of this method was tested at five different concentration spiking levels ranging from 1 to 125 μg g^{-1}.

Analytical parameters of the developed MAE-EME-IC method for determination of perchlorate compared to MAE-LLLME-IC method. Good linearity with coefficient of determination (r^2) of ≥0.9949 was observed. Satisfactory reproducibility of relative standard deviations (RSD) 4.3% (n = 4) was obtained.

The limit of detection (LOD) for the perchlorate ion was determined based on a signal/noise (S/N) ratio of 3 and was found to be 0.04 μg/g while LOQ value of 0.1245 μg/g was obtained on a signal/noise (S/N) ratio of 10.

Table 7: Developed MAE-EME-IC method versus MAE-LLLME-IC method in terms of analytical parameters

Analyte	Linear range (µg/g)	r^{2a}	LOD[b] µg/g	LOQ[c] µg/g	MAE-EME-IC[d]		MAE-LLLME-IC[e]	
					Enrichment factor	RSD[f] %,n=4	Enrichment factor	RSD %,n=4
ClO_4^-	1-125	0.9949	0.04	0.1245	15.6	4.3	1.4	7.8

A-coefficient of determination, b-limit of detection, c-limit of quantitation

d- Microwave assisted extraction-Electromembrane extraction-ion chromatography

e- Microwave assisted extraction-(liquid-liquid-liquid microextraction) - ion chromatography f- Relative standard deviation

The enrichment factors were determined by comparing the peak areas of the analyte after optimal extraction from spiked fish tissue sample solution and the peak areas of the analytes from the same solution at zero potential (without applying voltage). These values are summarized in Table 7. To evaluate the accuracy of the proposed method, the extraction recoveries were performed on sea food samples spiked with perchlorate standard at three different concentrations.

3.4 MAE-EME-IC versus MAE-LLLME-IC method

Basing on the pre-reported optimized conditions, the performance of MAE-EME-IC was compared with microwave assisted liquid–liquid–liquid microextraction ion chromatography method (MAE-LLLME-IC).

The motivation was that EME is considered as an improved modification of LLLME with applied potential being the major differentiating factor. Applied potential enhances extraction efficiency. Exactly the same EME experimental conditions were used but for LLLME method the extraction time was adjusted up to 45 minutes. The results in Table 7 show that the enrichment factors as well as the repeatability for the proposed MAE-EME-IC were higher than those recorded by MAE-LLLME-IC method. The main driving force in EME method is the electrokinetic migration of analytes as compared to the slower passive diffusion in LLLME. Our results conformed with what is reported in literature about the faster extraction efficiency offered by EME experiments [128].

3.5 Real sample analysis

The optimized MAE-EME-IC method was applied to ten different types of sea food samples collected from local markets of Al-khobar, Saudi Arabia to examine its applicability. The results obtained are as shown in Table 8. No perchlorate anion was detected in crab samples by this method and the concentration obtained for the rest of the sea food samples were near the LOD.

To evaluate the matrices' interference of the MAE-EME-IC developed method, one sample was spiked with varying (n= 6) concentrations, and extraction recoveries calculated based on standard addition recoveries. Results in table 3 indicate that the mean recoveries in all the samples tested ranged between 85.2 to 107%. The relatively high mean recoveries indicate low matrix effect interferences. From the results, it is feasible for the developed method to detect and quantify perchlorate ions in sea food samples.

Table 8: Concentration of perchlorate in different sea food samples

English name	Concentration of ClO_4^- (µg/g)
Trivally fish	0.92 ± 0.40[a]
Stripped red mullet	0.828 ± 0.17
Barracuda fish	0.452 ± 0.61
Emperor fish	0.544 ± 0.43
Indian Mackerel	0.726 ± 2.73
solea	0.679 ± 0.93
Oyster	0.694 ± 0.02
crab	**nd**
Squid	0.514 ± 0.62
shrimp	0.864 ± 2.26

[a] = SD for three determinations

nd = not detected

3.6 Conclusion

In the current study, trace determination of perchlorates in fish and sea food samples was feasible by a combination of MAE, EME, and IC-Conductivity detector. After successful optimization, the developed method demonstrated to be an effective tool for the determination of trace perchlorate anions in complex sample matrices. The method was compared to passive diffusion in which no voltage was applied and it appeared to be much more efficient, providing satisfactory analyte enrichment in a relatively short time. Each EME disposable membrane bag was used for one analysis and this eliminated associated carry over effects.

CHAPTER 4. LIQUID PHASE MICROEXTRACTION USING COTTON WOOL AS THE EXTRACTANT PHASE HOLDER FOR TRACE LEVEL ANALYSIS OF PHENOLIC COMPOUNDS IN SEA WATER SAMPLES

1. LITERATURE REVIEW

The presence of phenolic compounds (PCs) in aquatic environment has mainly been due to industrial, agricultural and house hold production of pesticides, antioxidants, plastics, paper, drugs, dyes and even petrochemicals [140]. These compounds are of concern because of their toxicity, even at low concentration [141] Among the PCs, alkylphenols (APs) and nitrophenols (NPs) are considered highly toxic and some have been categorized as endocrine disrupting compounds (EDCs) [142] that potentially mimic /inhibit the natural action of the endocrine systems [143]. The United States Environmental Protection Agency (USEPA) classifies them as priority pollutants [144]. According to European Community (EC) directive, a legal tolerance level of 0.1μg/L for each individual phenolic compound and 0.5μg/L for the sum of all the compounds in water has been earmarked for human consumption [145-146]. In the present study, we focused on simultaneous determination of three PCs namely; 4-nitrophenol (NP-4), 2-nitrophenol (NP-2), and 4-tert-butylphenol (BP-4) as model analytes.

So far, gas chromatography (GC) [147-148] and high performance liquid chromatography (HPLC) [149] coupled to mass spectrometry are the most common techniques used for the determination of these PCs in water matrices.

However, owing to the nature of these analytes, strong need for improving chromatographic separation and sensitivity, derivatization is mandated prior to GC analysis [150].

Nevertheless, liquid chromatographic techniques are more direct and obviate the need for derivatization in the analytical process. It is therefore imperative to combine an effective sample preparation technique with an analytical process so as to realise low detection limits (ng/L). Traditionally, liquid-liquid extraction (LLE) [151] and solid phase extraction (SPE) [152] are the most commonly used methods for preconcentrating PCs from water matrices. Surprisingly, these methods are time-consuming and require voluminous toxic organic solvents.

Some solventless extraction methods like solid-phase microextraction (SPME) [153] and stir bar sorptive extraction (SBSE) [154] have been explored with marked success. However, these extraction methods are expensive, suffer from sample carry-over problems and their sorbents are fragile, and with limited life time. Alternatively, LPME based hollow fiber liquid phase microextraction (HF-LPME) [155-156], single drop liquid phase microextraction (SD-LPME) [157-159] and dispersive liquid–liquid micro-extraction (DLLME) [160] have been reported to provider higher extractability for PCs. For effective extraction, the mass transfer of analytes between the extractant phase and the bulk aqueous sample phase is commonly augmented by agitation.

As an alternative to this mode, continuous flow microextraction (CFME) was demonstrated to offer higher enrichment for trace level organic pollutants in environmental water matrices [161-162].

Organic solvents are the most commonly used extractants used in LPME methods; however, the poor reproducibility associated with volatilization has led to the use of room temperature ionic liquids (ILs). Room temperature ionic liquids (RTILs) are air and water stable salts resulting from combinations of organic cations and various anions that may be liquid at room temperature [163-164].

Ionic-liquids have been used as alternative green solvents in separation, analysis [165-166] and as additives in HPLC [167-168]. Ionic-liquid based single drop-LPME technique was demonstrated to offer best extraction efficiencies for polycyclic aromatic hydrocarbons [169], alkylphenols [170] and chloroanilines [171]. Semi and non-volatile compounds in complex samples have also been extracted using headspace single drop-LPME [169, 171]. ILs have higher viscosities, thermal stabilities and good solubility for both organic and inorganic compounds. These unique properties provide ILs with an edge of stability during the extraction process, resulting into higher enrichment factors and better extraction efficiencies. Generally, headspace extraction procedures are less sensitive than the direct immersion approach [78]. Moreover, the sensitivity and precision using single drop-LPME methods could hardly be sustained using continuous flow method of environmental sample water. One reason being the prolonged extraction time and fast stirring rate that result in drop dissolution [172]. Direct immersion using single drop-LPME is not a desirable choice for complex samples.

Use of polypropylene HFM as protective sleeves for LPME; provides efficient sample cleanup for a wide range of complex samples [121, 173]. However, the major drawbacks suffered by supported liquid membranes are:

(i) Limited life time of the liquid membrane experienced when polar organic solvents are used

(ii) The relatively low enrichment rate resulting in long enrichment time especially when large enrichment factors are required [174].

Recently, an ionic liquid based LPME method using knitting wool as an extractant phase holder before chromatographic analysis was ratified for trace analysis of ultraviolet filters in swimming pool waters. It provided good enrichment factors yet was easy to operate and cost effective [175].

The aim of this work was to demonstrate the compatibility of CFME with ionic-liquid supported cotton based liquid phase microextraction (CB-LPME) as a single step enrichment/clean-up technique, which could allow the extraction of PCs from sea water samples prior to ultra-performance liquid chromatography (UPLC) analysis. The advantage of using UPLC is that no derivatization is required. In this method a small piece of cotton wool was wound on a used GC syringe needle tip to form a cotton bud and used as the solvent holder during extraction process. In this novel method, the home made solvent-impregnated cotton wool hanging on a needle tip was placed in the sample solution and used to extract the target analytes. Factors affecting the extraction method were investigated and optimized. Cotton wool is readily available, affordable and has absorptive properties.

In comparison to HFM-LPME, the developed method is expected to offer higher enrichment factors for trace level PCs in sea water. Moreover, it is simple, fast and cost effective.

2. EXPERIMENTAL

2.1 CB-LPME experimental setup and extraction

The CB-LPME setup developed for this investigation and its microextraction unit are shown in Figure 25.

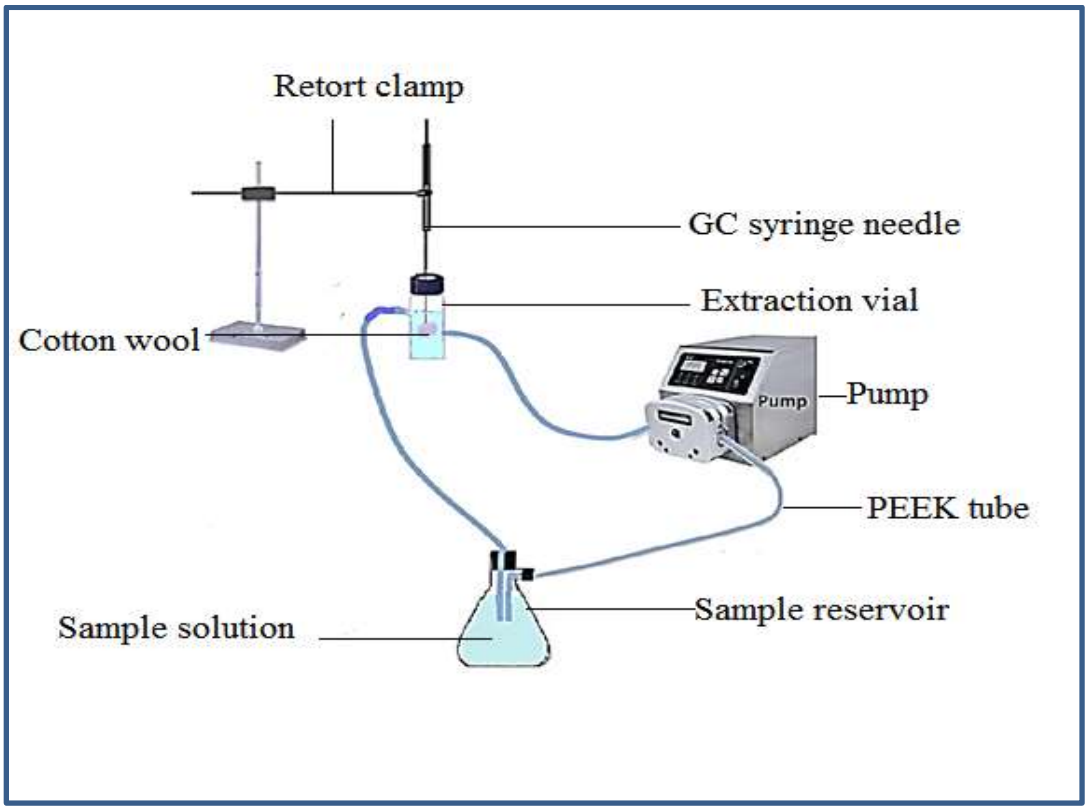


Figure 25: Diagram of the CB-LPME experimental set up

The setup of CFME includes a sample reservoir, a pump and a microextraction unit. The major part of the microextraction unit is a home-made piece of polyester cotton wool of diameter 2mm and length 0.8cm wound on a GC syringe needle tip to form a bud. Several cotton segments could be prepared within a short time. These segments were ultrasonically cleaned in acetone and air dried before use. The extraction unit was modified from a 50 mL clear glass sample vial (Supelco, Bellafonte, PA, USA) to bear two side-arms that would allow continuous flow of sample water during extraction. The PEEK tubing (Upchurch scientific, Oak Harbor, WA USA) was connected to one end of the extraction unit through the pump and from the aqueous sample solution. Another PEEK tube completes the flow by connecting back to the sample solution from an opposite end of the extraction unit.

Using a 10μL liquid chromatography (LC) syringe, 8 μL of the extraction solvent was withdrawn and injected in to the developed cotton bud attached on a needle tip held on a retort stand. The set up was then lowered in to the extraction unit. For the extraction process, the aqueous sample was continuously delivered by a pump operated at a rate of 100mL/s through the developed extraction unit. After a set time, the wool was removed and placed in a 2mL LC autosampler vial fitted with 150 μL glass insert. Thirty five microliter of acetonitrile was introduced to the glass insert to desorb the wool by sonication. Finally, the wool was removed from the sample insert and later discarded. From the extract, 10 μL of the extract were injected to UPLC system for analysis. 100 mL of the aqueous sample solution was used throughout the experiment.

2.2 Chemicals, reagents and materials

Four different room temperature ionic-liquids (>98% purity); 1-butyl-3-methylimidazolium dihydrogenphosphate ([BMIM][H_2PO_4]), 1-butyl-3-methylimidazolium tetrafluoroborate ([BMIM][BF_4]), 1-butyl-3-methylimidazolium octylsulfate ([BMIM][$OcSO_4$]), and 1-butyl-3-methylimizazolium hexafluorophosphate ([BMIM][PF_6]) were purchased from Strem Chemicals (Newburyport, MA, USA). Three alkyl phenols (2-nitrophenol, 4-nitrophenol and 4-*tert*-butylphenol) purchased from Fluka (Buchs, Switzerland) were used. The chemical structures of the alkyl phenols are shown in Figure 26.

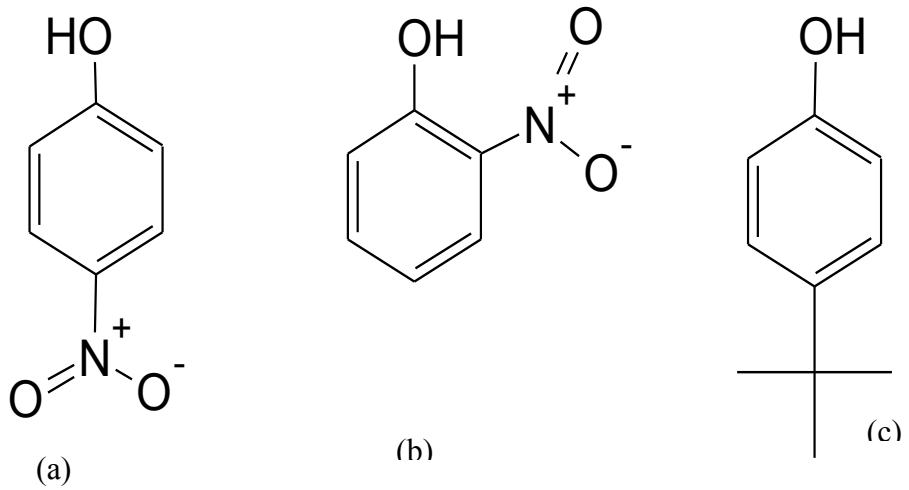


Figure 26: Chemical structures of the model analytes

HPLC-grade solvents were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Ultrapure water was produced on a Milli-Q system (Millipore, Milford, MA, USA). Separate stock solutions at 1 mg/mL concentrations were prepared in methanol. The working solution was freshly prepared daily by spiking ultrapure water with the three PCs from the stock solutions at the required concentration level (10 μg/L of each phenol). Accurel Q3/2 polypropylene hollow fiber (600 μm id, 200 μm wall thickness and 0.2 μm pore size) was purchased from Membrana (Wuppertal German). A spool of white cotton wool (polyester) with a diameter of 2 mm was secured from a local market of Al-Khobar, Saudi Arabia.

2.3 UPLC extraction conditions

An Acquity UPLC system (Waters, Milford, MA, USA) with a UV detector was used. An Acquity UPLC BEH C18 (1.7 μm, 150 x 2.1 mm i.d.), Data were collected and processed by the chromatographic Empower software. The reverse phase Spherisorb Spheris column (200× 4.6 mm × 5 μm) of ODS 2 packing material was from PhaseSep (Deeside, UK). The flow rate was 1 ml min^{-1} and the detection wavelength was set at 280 nm. An isocratic mobile phase composition of 65:35 acetonitrile (ACN): water was used for separations.

2.4 Sea water samples

Sea water samples were collected at five different locations on a gulf stretch and then transported to a laboratory in pre-cleaned glass bottles, where they were stored at 4°C in a refrigerator. The sample was filtered first through Whatman filter paper, and then through 0.45 μm Millipore membrane before analysis.The original sample pH of the sample sea water solution was 6.7 and no other physical characteristics were measured.

2.5 Blank contamination and quality control

Blank contamination is typical of PCs determination at trace level concentrations. As a result, the use of detergents and plastics was avoided so as to reduce blank problems. All the glassware was carefully washed with acetone, ultrapure water and methanol before use. Blank analysis revealed the presence of PCs at concentrations < 5 ng/L. As a result procedural blanks were regularly checked and controlled. A comparison of the procedural blank (non-spiked tap water sample (n=3)) and solvent blank (no sample) were performed to establish the possible source of PCs. The responses obtained in both cases were similar indicating that blank contamination comes mainly from experimental process. The contribution of PCs (< 5 ng/L) was then constructed from that of the samples to eliminate overestimations in the experimental results.

2.6 Quantification

The difference in peak height of the sample and blank containing corresponding concentration of IL: ACN (1:1) were used for quantification. The enrichment factor (EF) was determined using the expression below;

$$EF = \frac{{}^{H_a}/_{C_a}}{H_d} \qquad (14-4)$$

Where, H_a is the peak area determined by enriching an analyte with an initial concentration C_a (ng/ml). H_d is the peak area obtained by direct injection of 1 ng/ml in to the UPLC system.

3. RESULTS AND DISCUSSION

Experimental design is very important for the method development of microextraction techniques. The following represents the advantages of our modified LPME configuration as shown in Figure 27.

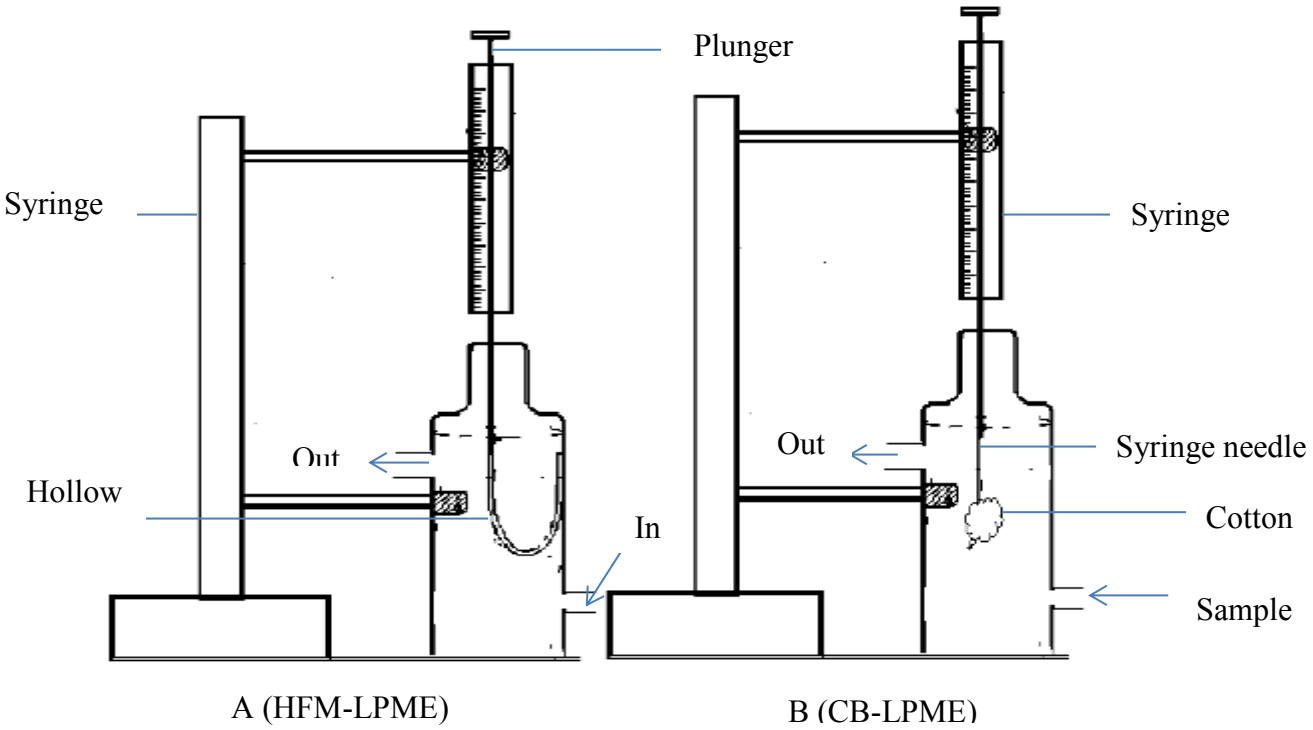


Figure 27: Comparisons in LPME setup used in this experiment

The above setup shows arrangements of the extraction vials during the experiment. The same extraction conditions and setup used in CB-LPME were applied in HFM-LPME to compare their extraction efficiencies. For HFM-LPME, six organic solvents namely ethylacetate, dichloromethane, toluene, 1-octanol, isooctane and n-nonane were investigated for their effect on enrichment as supported liquid membrane. N-nonane was found to be more suitable and was therefore used for subsequent experiments (results not indicated).

3.1 Extraction solvent

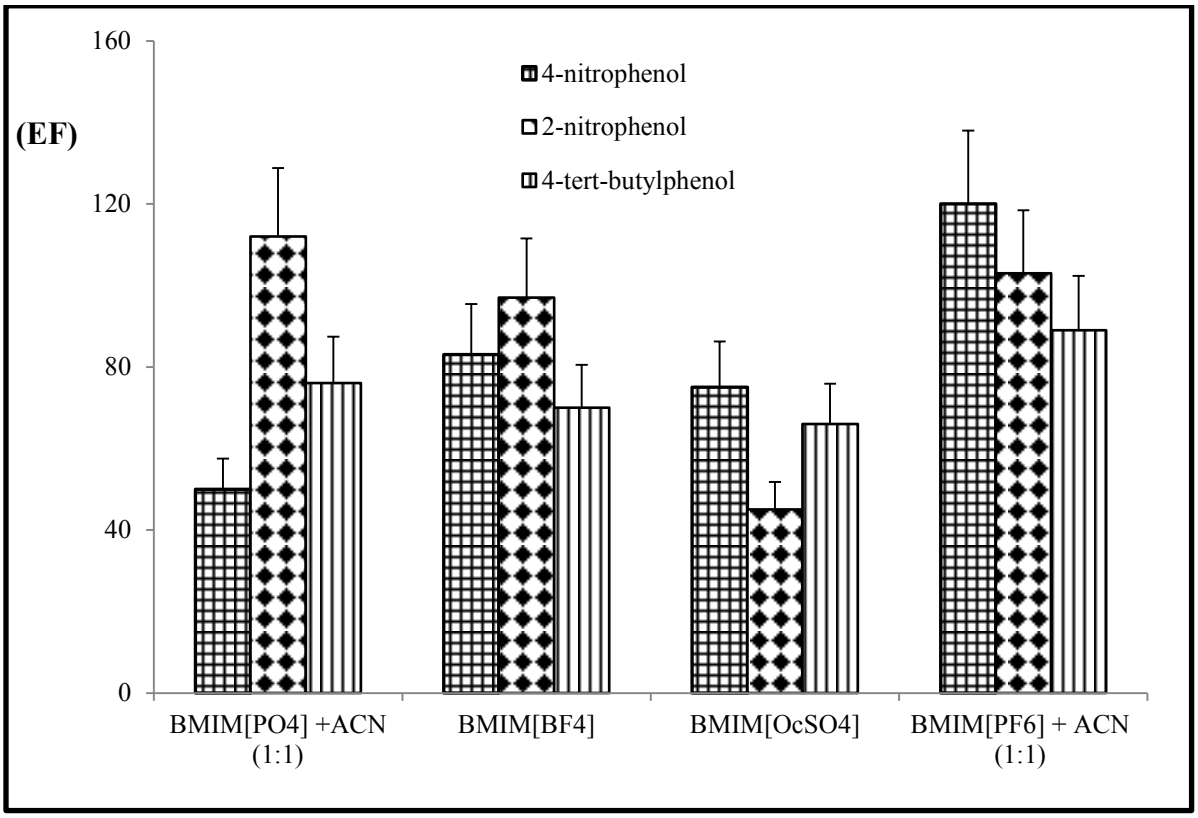
In some preliminary experiments, more attention was focused on the selection of the extracting solvent since this is cardinal in LPME experiments. To determine the suitable solvent with optimal extraction efficiency, several factors were considered. The selection process was a compromise amongst extraction ability, water solubility and evaporation rate. Since cotton wool was used as an extracting media, the extracting solvent fulfilled the following requirements:

(i) capable of being retained in the cotton wool pores, and being non-volatile, (ii) it should be immiscible with water since it serves as an intermediary between the aqueous donor and the aqueous acceptor phases and (iii) the solubility of analytes in the solvent should be higher than that in the donor phase and lower than that in the acceptor phase.

Based on the above considerations, four different ionic liquids (BMIM[BF_4], BMIM[PF_6] BMIM[PO_4] and BMIM[$OcSO_4$]) were initially evaluated for the extraction efficiency of phenolic compounds in spiked ultrapure water samples under identical extraction conditions.

BMIM[BF_4] and BMIM[PF_6] gave higher enrichment values than BMIM[PO_4] and BMIM[$OcSO_4$]. BMIM[PO_4] and BMIM[PF_6] were however more viscous, and difficult to draw into the syringe. As a result, they were diluted with ACN.

The remaining ionic-liquids had lower viscosities and were directly used in UPLC system during analysis. Figure 28 clearly shows that BMIM[PF_6] (in combination with ACN, 1:1) gave higher analyte enrichment than the rest of the ionic-liquids, and was thus chosen for further experiments.



EF= Enrichment factor (-Fold).

Figure 28: Extraction efficiency of various ionic-liquids in CB-LPME. Samples spiked at 20 µg l-1 of each analyte and 20 min extraction time

Relatively, BMIM[PF_6], selected as the extraction solvent, has a higher viscosity than typical organic solvents. ACN was used as diluent to avoid interferences with the target analytes as it was already being used as part of the mobile phase. BMIM[PF_6] was diluted with different amounts of ACN. Table 9 shows the extraction efficiency of various ionic-liquid/ACN mixtures. Dilution of BMIM[PF_6] with ACN reduces the viscosity, which increases the dielectric constant of the co-solvent (ACN) [176]. The viscosity of ionic-liquid is essentially determined by its tendency to form hydrogen bonds and by the strength of Van der Waals interactions. This could be due to the delocalization of the charge over the anion and this seems to be favored by lower viscosity, by weakening hydrogen bonding with the cation and increasing the interaction with alkylphenols [177].

Table 9 shows that BMIM[PF_6] diluted with ACN at 1:1 ratio gave higher extraction efficiency than mixtures of other ratios, and thus BMIM[PF_6]:ACN (1:1) was used for further experiments. We further evaluated the efficiency of CB-LPME in terms of enrichment factors and related it with HFM-LPME using n-nonane as a supported liquid membrane. (The results for selection of n-nonane as the best SLM are not indicated)

Table 9: Dilution of BMIM[PF6] on CB-LPME versus HFM-LPME

Analyte	Enrichment factor (-fold)			
	CB-LPME			HFM-LPME
	IL:ACN	IL:ACN	IL:ACN	IL:ACN
	2:1	1:1	1:2	1:1
4-nitrophenol	125	146	83	96
2-nitrophenol	110	120	89	83
4-*tert*-butylphenol	93	102	91	60

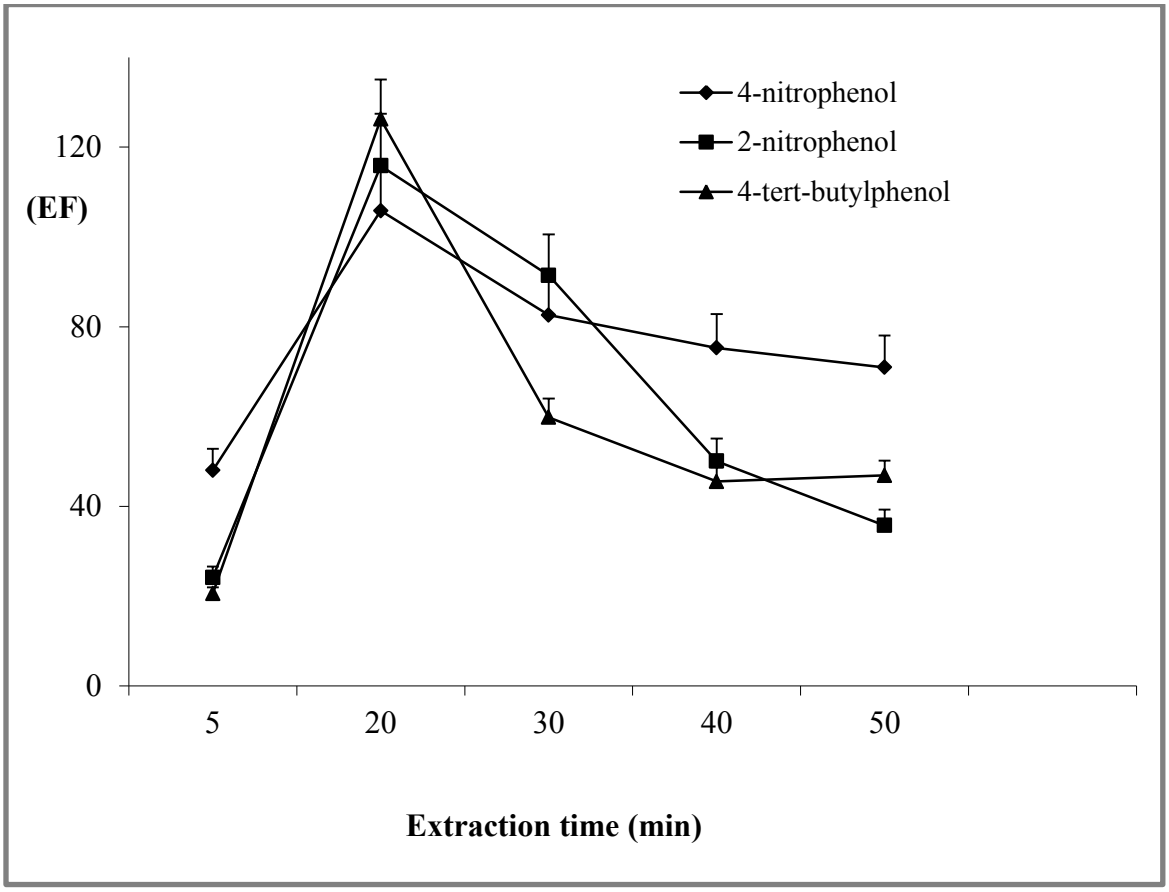
IL = ionic-liquid (BMIM[PF_6])

ACN = acetonitrile

3.2 Extraction time.

A series of extraction times from 5 to 50 min were investigated by spiking ultra-pure water with 20 μg l^{-1} of individual analytes. For all target analytes, the amount extracted increased with increasing extraction time from 5 to 20 min (Figure 29).

After 20 min, the enrichment factor decreased slightly. After reaching equilibrium, the analyte has the tendency to be extracted back to the extraction solvent (Le Chatlier's principle), resulting in enrichment factor reduction after 20 min. 20 min, therefore, appeared to be the optimum extraction time.

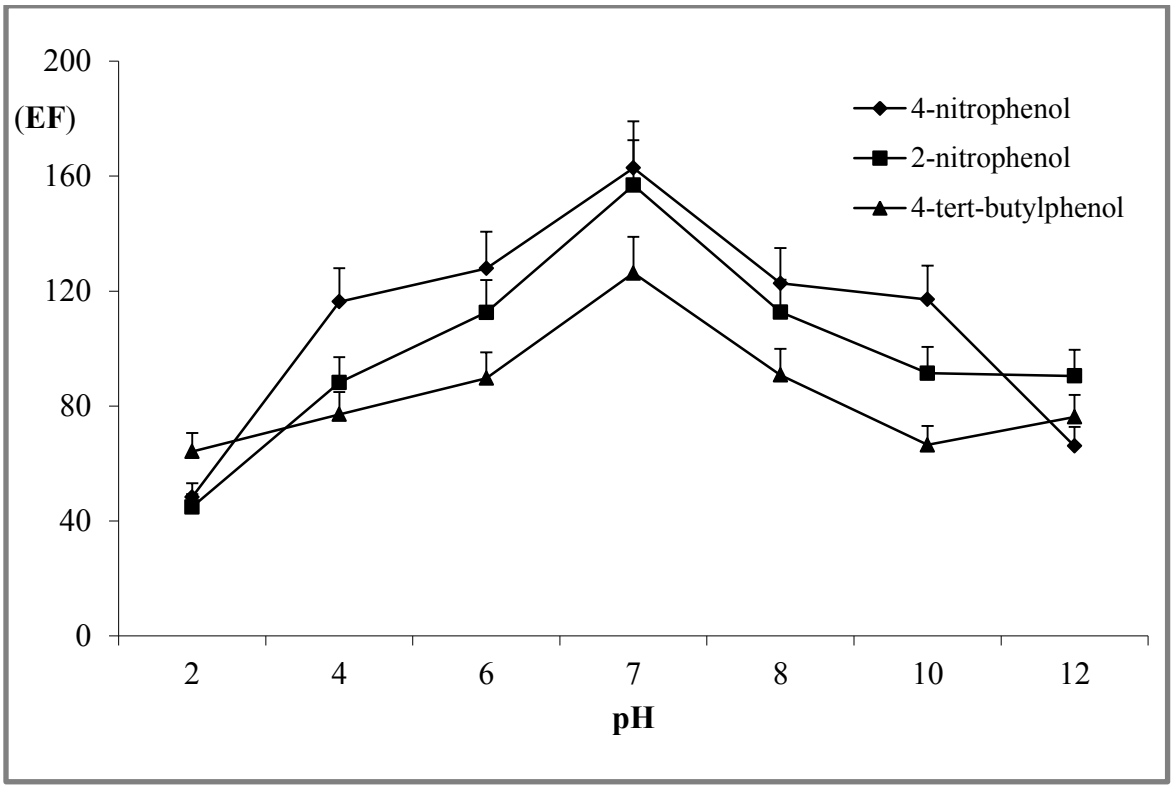


EF= Enrichment factor (-Fold)

Figure 29: Ionic-liquid CB-LPME extraction time profile of PCs. Samples spiked at 25 µg l-1 of each analyte. IL: ACN (1:1) as acceptor phase

3.3 Sample pH.

The effect of pH on CB-LPME extraction efficacy was investigated in the range of 2 to 12. The extraction profile with varying pH is shown in Figure 30. Samples at pH 7 gave higher analyte enrichment than either strongly acidic or basic conditions. For convenience, no adjustment to the pH of sea water (pH 6.7) was made before extraction.



EF= Enrichment factor (-Fold)

Figure 30: Influence of sample pH on PCs extractability

3.4 Ionic strength

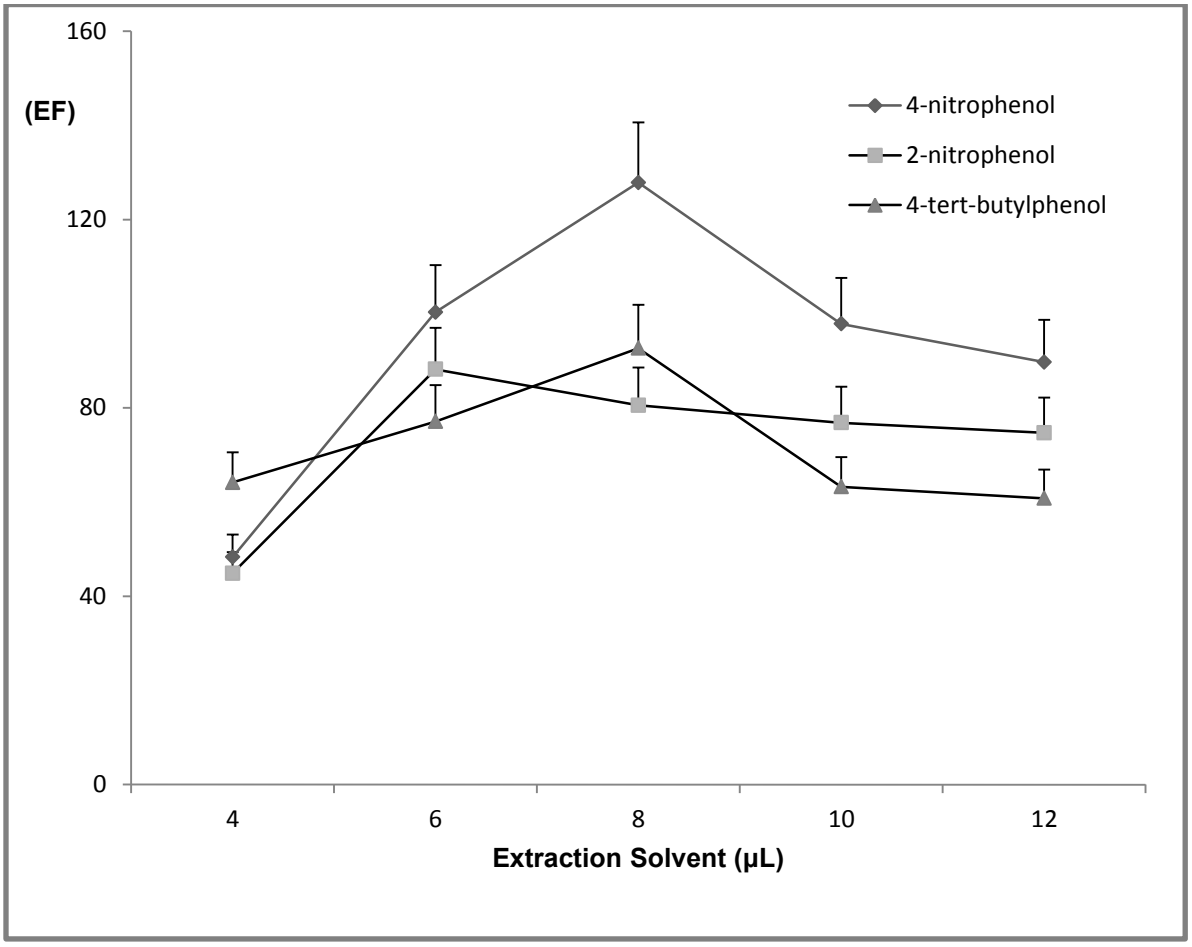
Generally, the addition of sodium chloride (NaCl) to the sample solution causes a decrease in solubility of the organic analyte and increases the distribution coefficient, which is usually used to enhance the extraction efficiency. The salting-out effect has been used commonly in LPME and significantly decreases the solubility of analytes in the aqueous sample and consequently increase their hydrophobicity [178]. In this case, fewer water molecules are available for dissolving the analyte molecules, preferably forming hydration spheres around the salt ions [179]. A series of experiments were carried out on aqueous samples containing different amounts of NaCl [(5%, 10%, 15%, 20% and 30%) (w/v)].

From the results, addition of 5-20% (w/v) NaCl increased the peak area of 4-*tert*-butylphenol but showed a decrease for the other two analytes in the study (data not shown). Moreover, addition of 30% NaCl did not show any significant increase in extraction efficiency for all the tested phenols. This could be due to the increase in the viscosity of the sample solution, which then reduced the mass transfer of the analytes to BMIM[PF_6]:ACN.

3.5 Volume of extracting solvent

The volume of solvent introduced into the cotton bud before the extraction process has a profound effect on analyte CB-LPME extraction potential. Figure 31 depicts the effect of extracting volume (BMIM[PF_6]:ACN) on the enrichment factor. Results show that; enrichment factor increased generally between 4 to 8 μL.

However, above 8 μL, there was a gradual decrease in enrichment factor possibly due to dilution effect that became predominant.



EF= Enrichment factor (-Fold)

Figure 31: Effect of extracting solvent on CB-LPME (Extraction conditions: ionic strength, 0 mg/L; pH of sample solution, 6.5; extraction time 20 min).

3.6 Method performance

The optimized ionic-liquid based CB-LPME method proved to be simple and effective for the extraction of some phenolic compounds. To evaluate the developed CB-LPME method, parameters such as linearity, precision and repeatability were determined. Calibration was performed with seven samples of ultra-pure water, each spiked with analyte concentrations ranging from 0.09 to 100 μg l^{-1}. The response was linear with coefficient of determination (r^2) values ranging between 0.9923 and 0.9962 (see Table 10). Intra-day precision was studied for 10 μg/L spiked water samples with five replicates and the relative standard deviation RSD ranged from 0.3% to 3.0%. Inter-day precision was carried out on experiments done on three consecutive days at the same concentration levels with five replicates. As can be seen from Table 10, the inter-day precision for the analysis was in the range of 4.6 and 8.2%.

Limits of detection (LODs) were calculated by progressively decreasing the analyte concentration in the spiked sample until UPLC signals were clearly discerned at S/N=3 at the final lowest concentration. LODs varied between 0.05 and 0.10 μg/L for spiked ultrapure water using CB-LPME and between 0.07 and 0.15 μg/l when HFM-LPME was used respectively. By comparing peak areas in the chromatograms, it can be seen that most of the target compounds were preconcentrated with an enrichment factor of more than 100-folds in the acceptor solution for both methods.

Table 10: Enrichment factor, linearity, and reproducibility for extraction of PCs by the proposed BMIM[PF6]:ACN (1:1) CB-LPME method

Analytes	(EF)[a]	Intra-day %,RSDs (n=5)[a]	Inter-day %,RSDs (n=6)[a]	(r^2)[a]	LOD[a] ($ngml^{-1}$)	LOD[b] ($ngmL^{-1}$)	(EF)[b]
4-nitrophenol	158	0.3	4.6	0.9962	0.05	0.07	185
2-nitrophenol	149	2.9	7.1	0.9934	0.08	0.1	200
4-*tert*-butylphenol	125	3.0	8.2	0.9923	0.10	0.15	163

EF = Enrichment factor

RSD = Relative standard deviation

LOD = Limit of detection

[a] = Quantitative parameters for CB-LPME.

[b] = Quantitative parameters for HFM-LPME.

Five different sea water samples (from different locations) were extracted under the optimized extraction conditions. Concentrations of phenolic compounds detected in the real samples are shown in Table 11. The range was from 'not detected' to 3.4 μg l^{-1}. Common components of seawater sample, such as humic acids and inorganic salts, could reduce the applicability of the method in analysis by affecting the recovery.

Therefore, to assess the matrix effects, spiked seawater samples were extracted using the present procedure and recoveries were calculated by the standard addition method.

Table 11: Concentrations of phenolic compounds detected in the seawater samples collected from Dammam, Saudi Arabia

Analyte	Concentration in ng mL^{-1} (n=3)				
	DM-1	DM-2	DM-3	KH-1	KH-2
4-nitrophenol	nd	1.9 ± 0.14	2.8 ± 0.02	1.7 ± 0.45	2.6 ± 0.05
2-nitrophenol	1.4 ± 0.01[a]	nd	2.6 ± 0.32	3.4 ± 0.30	2.3 ± 0.06
4-*tert*-butylphenol	nd	0.8 ± 0.32	0.9 ± 0.31	1.9 ± 0.04	nd

nd = not detected, [a] = SD for three determinations.

DM and KH are site locations where sea water samples were collected in Dammam and Al-Khobar along the Gulf stretch.

Table 12: Extraction recoveries obtained by BMIM[PF6]:ACN(1:1) based CB-LPME of seawater spiked samples (n=3)

Analyte	% Relative recoveries (n=3)*			
	spiked at 5 μg l^{-1}	RSDs (%)	spiked at 10 μg l^{-1}	RSDs (%)
4-nitrophenol	85	3.4	96	5.3
2-nitrophenol	105	5.1	110	7.6
4-*tert*-butylphenol	89	5.6	92	2.4

*Recoveries calculated by standard addition method

Extracted chromatograms of real sea water and spiked sea water samples at 5 μg l^{-1} and 25 μg l^{-1} of each analyte are shown in Figure 32. There was a persistent interfering ionic-liquid peak (at 0.2 min) since 1 μL of pure ionic liquid, (1 μL of BMIM[PF_6] can be carefully drawn by the syringe but 25 μL of it was impossible to draw) was directly injected into UPLC system for identification at the beginning of the experiment.

Fortunately, its retention time did not coincide with those of the alkylphenols in the study. The ionic liquid peak is not displayed on the chromatograms.

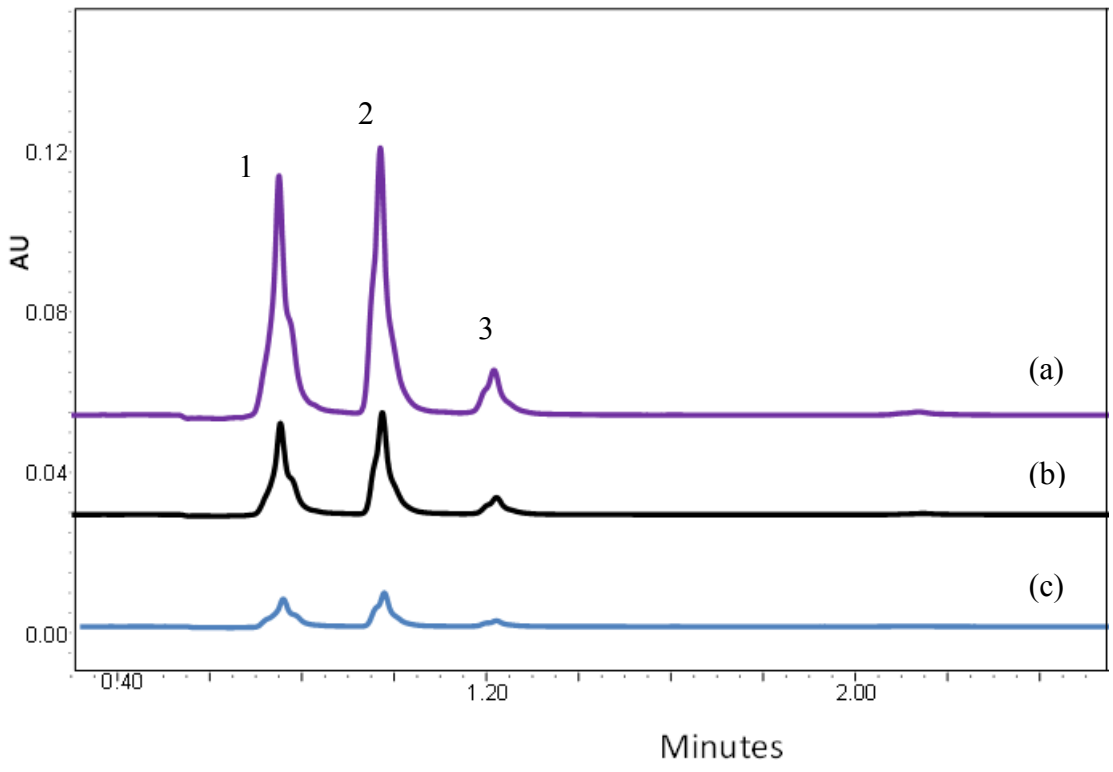


Figure 32: **BMIM[PF6]: ACN (1:1), CB-LPME-UPLC-UV chromatograms of sea water extracts. (a) Extract spiked at 25 µg l-1 of each phenol; (b) extract spiked at 5 µg l-1 of each phenol; (c) extract of real unspiked sea water sample. Peaks: (1) 4-nitrophenol, (2) 2-nitrophenol, (3) 4-*tert*-butylphenol**

Furthermore, the relative recovery of the extraction procedure, determined as the ratio of the concentrations found in real sea water and ultrapure water samples spiked at the same concentration level was also evaluated under the optimised experimental conditions. Three replicate runs of sea water samples at two different spiked concentrations (5 and 10 μg l^{-1} of each analyte, respectively) were analysed and the percentage of extracted analytes was then calculated as shown in Table 12. The recoveries of the analytes from this sea water were higher than 85% compared with that of spiked ultrapure water. This implies that the proposed method is more precise and the wastewater matrix did not have a significant effect on the extraction efficiency.

3.7 Conclusion

The present work evaluated the feasibility of combining continuous flow cotton bud-LPME as a new method for extracting phenolic compounds from sea water samples followed by UPLC analysis. The polyester cotton wool served as an extractant solvent holder and could potentially sustain high agitation speed and longer extraction time. In comparison to HF-LPME, CB-LPME offers higher extraction efficiencies as well as good linearity and repeatability.The developed CB-LPME method is fast, simple, affordable and effective.

CHAPTER 5. General conclusion and Future work

In this work, novel approaches for the solvent-minimized techniques were developed, optimised and evaluated for their effectiveness in trace level analysis of environmental pollutants. Three microextraction approaches including micro solid-phase extraction (μ-SPE), cotton bud-liquid phase microextraction combined with ultra performance liquid chromatography (UPLC-UV) and microwave-assisted electromembrane extraction (MA-EME) combined with ion chromatograph (IC-UV) were all compiled in this thesis work. Each of the three different approaches was applied to the real samples and the results obtained from this work clearly demonstrated the applicability of our approaches.

In the first part, we discussed the development of a micro-solid-phase extraction (μ-SPE) procedure using a novel sorbent prepared from rice husk material via sol-gel process. This approach was applied to the determination of haloacetic acids in swimming pool water samples. The μ-SPE devices can easily be constructed in-house and at a reasonable cost from a porous polypropylene (PP) membrane sheet. In this procedure, the porous polypropylene membrane is used as a protective sheath for the adsorbent material for extracting from dirty matrices. It serves as a filter and prevented the matrix effects. This approach was used as an alternative to traditional solid-phase extraction (SPE) techniques and it proved to be simple, cost-effective and solvent minimized approach that is sensitive, selective and reproducible given the judicious choice of the rice husk based sorbent.

In the second section, we discussed the suitability of microwave assisted-electromembrane extraction as an enrichment/clean-up approach for trace level perchlorate ions in sea food samples. The advantage of this work is that matrix effects normally encountered by other immersion-based microextraction techniques are greatly minimised. An EME procedure performed on a microwave assisted extraction extract was aimed at introducing a more clean extract in ion chromatography analytical system. Ionic liquid, 1-butyl-3-methylimidazolium hexafluorophosphate BMIM[PF_6] was used as an acceptor phase for the first time in this EME experiment. Since viscosity of BMIM[PF_6] is too high, it was mixed with acetonitrile (ACN) to facilitate the extraction. BMIM[PF_6]:ACN (1:1) was found to be the optimum extraction solvent. When this method was applied to sea food samples, it was discovered that the matrix did not have a significant effect on the extraction efficiency and recoveries of our model analytes. Moreover, the final extract could be directly injected into IC system.

In the last part of our work, we evaluated the feasibility of using an ionic-liquid based cotton bud-LPME as a new method for extracting phenolic compounds from sea water samples followed by UPLC analysis. The polyester cotton wool served as an extractant solvent holder and could potentially sustain high agitation speed and longer extraction time. In comparison to HFM-LPME, CB-LPME offers higher extraction efficiencies as well as good linearity and repeatability. The developed CB-LPME method is fast, simple and affordable. When coupled to a more sensitive detector like tandem mass spectrometer, the analytical performance of the developed method can significantly be improved.

Future Work

Rice husk material based sorbents have many unexploited potentials much more than expected, however based on the results from this work, the extractability of silica –Fe need to be evaluated further with different iron loading via sol-gel process. The developed sorbent can be applied to other environmental pollutants to evaluate its vast applications.

Possibility of automation by these developed methods also seems to be worth exciting areas for further research.

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