CHAPTER 1

INTRODUCTION

The large majority of the organic compounds in water and wastewater found on the priority pollutants list are more amenable to concentration by liquid-liquid or solvent extracion procedures which are devided into two techniques (1) ,i.e., macroextraction and microextraction techniques. However , the macroextraction technique is not attractive since it requires large sample volumes, normally uses 1000.00 to 2000.00 mL of the water samples which are not always available when the sample must be collected in the feild as well as a large volumes of the extracting organic solvent, as many as three times with 200.00-250.00 mL each time. The combined extract is then dried and concentrated by evaporation using Kuderna-Denish apparatus (2) to achieve the sensitivity prior to analysis. However, concentration is time-consuming and can introduce errors from losses of analyte due to volatilization (3). The impurities in the solvent are also concentrated so that the resulting solvent contains both the original impurities in water and in solvent. In addition to such drawbacks, the macroextraction approach to wastewater analysis is often the source of difficulties , e.g., emulsions ,

interferences and false positives. Therefore , microextraction technique is developed from the first technique in order to diminish the problem facing in the macroextraction method that the procedures involve a single equilibration of 10.00-100.00 mL of aqueous sample using sample-to-solvent ratio on the order of 100:1.

The microextraction technique has a number of practical advantages over the macroextraction technique as follows (3,4,5) :

1. Solvent concentration is not necessary ; thus, volatile materials can be analyzed in the same extract as the semivolatile.

2. The method can be conveniently and rapidly analyzed ; there is no need for a "sample processing crew". Errors and accidents (spill, etc.) do not pose serious problems because a new extract can be prepared immediately.

3. Formation of emulsions which is generally the major problem encountered in macroextraction method is not a problem since only an analytical portion of extract (1.0 - 5.0 µL) is required.

4. Since microextraction is able to extract and concentrate pollutants simultaneously in one step , so column cleanup of extracts is not usually required.

5. Minimal use of glassware , solvent and sample reduces cleaning problems and minimizes sample contamination.

6. The method is advantage for situation when the sample size is very limited ,i.e.,the sample must be collected outside the laboratory.

7. The cost of analysis decreases because small amount of solvent, glassware are used and the concentration step does not add to the time per analysis.

The microextraction not only fulfills the practical requirements but also gives reliable data. Because of it is easy to perform, flexible and simple sample handling, so it is used for analysis many class of priority pollutants.

1.1 THE PURPOSE OF THE STUDY

Microextraction technique was developed to analyze phenol and some derivatives including 2-nitrophenol, 2,4-dichlorophenol, 2,4,6-Trichlorophenol and 4-chloro-3-cresol in water samples. Therefore,the various effects on the percent recovery of the phenol and some derivatives were carried out in this study and there were :

1. The pH effect , i.e., 1,2.....,9.

2. The shaking time effect ,i.e., 5,10,15,20, 25,30, min.

3. The effect of organic solvents ,i.e., methylene chloride, carbon disulfide, and hexane.

4. The salting out effect with sodium chloride and anhydrous sodium sulfate.

5. The effect of sample-to-solvent ratios, i.e., 9:1, 5:5, 2:8.

Moreover, the accuracy and precision of the developed technique was also evaluated under the optimal condition found from the previous studies.

The entire investigation was carried out by the gas chromatograph equipped with flame ionization detector (GC-FID) and the internal standardization method was performed for the entire study.

1.2 HISTORICAL

Phenolic compounds have been shown to be a toxic to aquatic life (6) and human health at parts per million (ppm) level (7). They have a marked corrosive effect on any tissue, especially phenol, bad odour and they may also cause the liver and kidney damage (7). One of the phenolic compounds , 2,4,6 - trichlorophenol, has been shown to be a carcinogenic in the test animals which an additional lifetime cancer risk of 1 in 100,000 occurs at a level of 12.0 µg/L (7,8) and these are based on organoleptic effects. Besides, chlorophenols have the ability to impart tastes and odours to drinking water supplies, edible aquatic life at parts per billion (ppb) levels. The taste and odour properties of certain phenol derivatives have been the rationale for setting such low criteria for phenol . The World Health Organization (WHO) , European and international limit for phenol in water is 1.0 µg/L (9). Similarly, the Russian limit for phenol in water is 1.0 µg/L (9). These chemicals have been included on the U.S. Environmental Protection Agency (U.S. EPA) list of priority pollutants (10,11). For the protection of human health, the U.S. EPA interim draft water quality criteria for a variety of phenolic compounds are respectively 300.0 , 70.0 , 0.3, 0.0 µg/L for phenol, 2-nitrophenol, 2,4-dichlorophenol and 2,4,6-trichlorophenol and however , there is no

criteria set for 4-chloro-3-cresol (7).

Phenolic pollutants are of environmental concern and can pollute into the environment from a variety of sources (6,12). Phenolic compounds occur widely in nature; They are building unit of plants and are formed as product in metabolic processes of plants. They are also important raw materials in petrochemical and chemical industries,e.g., in the manufacture of phenol-formaldehyde resins, lacquers, binders, dyes, paper and pulp mills as well as in the production of pharmaceuticals , insecticides , herbicides , and fungicides, etc (6,12). Many phenolic compounds are used for protection of foodstuff, drugs and other materials, especially those containing oils or fats, against oxidation (6,12). They are contained in wastewater from coking plants and brown coal distillation plants , industrial effluents and in pesticides , herbicides , and fungicides. Routine disinfection of drinking water by chlorination can give rise to chlorinated phenols (6,13).

With the growing occurence and utilization of phenolic compounds , an over increasing emphasis is placed on their rapid and sensitive analysis. Detection and determination of phenolic compounds have been studied for several decades. The analyst was confronted with many problems in the determination of phenolic compounds in water sample as the following :

1. Due to the low concentration (generally 75.0 µg/L or less) in river water (14,15) and surface water samples (16), the preconcentration step necessitates to raise the concentration to the level at which the identification and the quantitative analyses can be determined.

2. Serious errors, that the concentration of the solute is in the range less than 1.00 ppm, can be occured owing to the handling solution, contamination or loss in the sampling step and in any steps of analytical process.

3. Phenolic compounds may have as little as 0.01% of the organic fraction present in the water samples. Thus, the analytical method should be devised so that the phenolic compounds can be analyzed without any interference from the other pollutants.

As phenolic compounds must often be analyzed in complex mixture, the method for the determination of trace phenolic pollutants in aqueous samples are generally carried out in two steps. The first step is a clean-up or extraction and preconcentration in order to enhance sensitivity. The second one is qualitative and quantitative analyses of phenolic compounds. There are many extraction methods and preconcentration steps which are mostly used for separation of phenolic compounds from various organic impurites.

1. Liquid-Liquid Extraction Technique (2,3,17-22)

Liquid - liquid extraction is based on equilibrium of the organic compounds between aqueous phase and organic phase which is an traditional method for separation and concentration of organic compounds from water.

2. Adsorption Technique (23-25)

The adsorption technique is made by taking the water sample and passing directly through the column with an adsorbent such as macroreticular resin (26 - 28), graphitized carbon black (29-31) , or charcoal (32), etc. and eluting the sorbed compounds from the column by an organic solvent. Finally, the eluted solution is analyzed by suitable instrument.

3. Direct Aqueous Injection Technique (33,34,35)

It is the simplest technique of analysis the organic priority pollutants in water. Nevertheless, this technique usually has high detection limit and in some case, it has not had a high sensitivity enough to analyze an organic compounds in very low concentration.

4. Steam Distillation Technique (36,37)

Steam distillation is a means of separating and purifying organic compounds. Essentially the operation consists in volatilising a substance by passing a

steam into a mixture of the compounds and water. Provided the organic compounds have an appreciable vapour pressure (at least 5-10 mm at 100 ^{o} C), they will distill with the steam. Steam distillation takes place at a temperature below the boing point of water and hence,in numerous, well below the boing point of organic substances.

A great variety of qualitative and quantitative analyses have been used for phenolic compounds and the optical method are more often employed. The standard method for the determination of phenolic compounds is based on the coloured derivative formed by coupling with 4-amino-antipyrine (33,34). This method is very sensitive , and however , it permits only the determination of total phenols. The spectroscopic methods, Raman and IR spectroscopy, microwave adsorption spectroscopy and mass spectrometry (MS) (8,38,39) are used for identification purposes and chiefly for elucidation of the structure of phenolic compounds and their bonding arrangement.

Paper and thin-layer chromatography are simple and readily accessible techniques, suitable for detection of phenolic compounds, as these substances absorb in the UV spectral region and form many coloured derivatives. These methods are still successfully used in many laboratories, especially in the analysis of natural phenolic compounds in the dye industry. At

present , gas chromatography (GC) (19-28,30-35) , gas chromatography-mass spectrometry (GC-MS) (2,40-43) ,and high performance liquid chromatography (HPLC) (44-47) are most extensively used, as they are faster and more efficient than the above mothods.

Gas chromatograph belongs among the most popular methods for the analysis of phenolic compounds due to its high separation efficiency, speed of analysis and detection sensitivity. Since the high polarity and low vapor pressure of phenolic compounds, a derivatization steps has often been used in analysis of these compounds to achieve improved chromatographic performance and,sometimes,more efficient extraction from aqueous samples. Derivatizing reagents such as acetic anhydride (19,21,48-49) , chloroacetic anhydride (50) , pentafluorobenzyl bromide (51-52) , heptafluorobutyryl imidazole (53) ,silanizing reagents (54), 1-fluoro-2,4-dinitrobenzene (55) and diazomethame (56), etc , are always used in preparing derivatives of phenolic compounds. Lower molecular mass and more volatile phenol derivatives can be separated directly without pretreatment (12).

The factors which must be considered in comparing and evaluating the various analytical techniques are

1. The separation efficiency should include

complete isolation of the phenolic compounds from the other organic pollutants and the resolution of the phenolic fraction into its various compounds.

2. The lower detection limit must be sufficient to resolve and identify the phenolic compounds.

3. Ease and speed of analysis.

The microextraction was first reported by Rhodes and Millar (57) and it was used to determine the volatile organic compounds in the fruits.

Grob et al. (17) attempted to define the role of GC in the investigation of organic substances in water, which was important due to the handling of water samples before GC analysis depended entirely on the imformation expected from the subsequent separation, identification and quantitation. A rapid and simple liquid extraction method, based on shaking 1000.00 mL of water with a small volume (0.50-1.00 mL) of solvent and subsequent high-resolution GC analysis of the extract was described. The qualitative and the semiquantitative information at the pptr (parts per trillion) level was easily obtained.

Murray (3) developed an extraction flask similar to the rapid liquid extraction used by Grob et al. The flask which contain 980.00 mL and 0.20 mL was

shaken manually for 2 minute. By tilting the extraction flask and carefully adding water through the side arm , the solvent layer was hold in the center portion and finally displaced into the capillary tube. About 50.0 µL were recovered and were suitable for direct analysis by the GC. A comparison of the microextraction procedure was made with the extracts from two macroextraction methods , i.e. , continuous steam distillation extraction and continuous solvent extraction for the analysis of chlorinated pesticides, alkanes and phthalates in samples of tap water.

Junk et al. (5) analysed halocarbons , herbicides ,insecticides , and aromatic compounds in a variety of natural and wastewater by microextraction technique using sample-to-solvent ratios 100 : 1 and 500:1 and then determined by both GC-FID and GC-ECD .

The U.S. EPA method which was applicable to the determination of phenolic pollutants in municipal and industrial discharges was the macroextraction method (18). A normally achievable limit of detction , using FID , was 0.50 to 1.00 ppm in the solution actually injected into the gas chromatograph.

Mathew and Elzerman (19) reported a GC micromethod for the determination of trace amounts of some chloro- and nitrophenols in aqueous solution by extracting with methylene chloride and using

sample-to-solvent ratios ,i.e.,1:1 ,10:1,20:1 and 50:1.

Bengtsson (20) described a GC microextraction technique for the analysis of a variety of substituted phenolic compounds from water samples which was designed for situation when the sample size was very limited (0.50 to 10.00 mL). The effect of sample-to-solvent ratios,i.e., 1:1 , 20 :1 , and 100:1 , the extracting solvent ,i.e., acetone / hexane , methylene chloride , ethyl acetate , toluene , triethylamine / toluene , acetic anhydride / ethyl acetate , and butyric anhydride / ethyl acetate were studied.

Coutts et al. (21) analysed trace phenolic compounds in aqueous solution by microextraction technique. The method was carried out by using a sample-to-solvent ratios,i.e.,1:1,10:1,20:1 ,and 50:1 and a methylene chloride as an extracting solvent and then determined by GC-FID.

Abrahamsson and Xie (22) studied the micromethod for analysis of chlorophenols in drinking water, sea water and waste water from sulfate pulp mill by using two different water-to-solvent ratios. For high concentration more than 1.0 µg/L of chlorinated phenols , a 5:1 ratio was used , and for low concentration less than 1.0 µg/L of chlorinated phenols , a 200:1 was used.

Rhodes and Nulton (58) studied a methodology

of microextraction in which the ratios of solvent-to-wastewater ranged from 1:40 to 1:100 for the analysis of priority pollutants, volatile aromatics, phthalates, polynuclear aromatics and phenolic compounds. The results obtained during an U.S. EPA verification analysis of a variety of industrial process waters and effluents were discussed with respect to precision, percent recovery of analytes, specific analytical problems encountered, and the potential for extending this approach to other compound classes.

Thrun et al. (59) investigated various effects on extraction efficiencies when using a microextraction technique to extract benzene, toluene, ethylbenzene, and o-xylene from water into pentane. The effect of sample-to-solvent ratios,i.e., 20:1 and 100:1, salting out with sodium sulfate, and the present of other organic substances in the matrix were all evaluated.

Mieure (60) presented the rapid and sensitive method which it was suitable for determination of the organohalides in drinking water, natural water, and effluent water. The method based on the microextraction technique subsequent with GC-ECD determination. The effect of sample-to-solvent ratios and salting out effect were studied.

Richard and Junk (61) described the rapid microextraction method for the determination of

halomethanes in water. The procedure involved vigorous shaking 10.00 mL of sample with 1.00 mL of pentane with subsequent GC-ECD analysis. Less than 0.1 µg/L of halomethanes in a 10.00 mL water sample was easily detected.

Reunanen and Kroneld (62) presented a microextraction method for the quantitative determination of volatile halocarbons in raw and drinking water ,human serum, and urine by GC-ECD. The sample-to-solvent ratios were compared between 20:1 and 100:1.

Thielen et al. (63) used microextraction and capillary column GC techniques to plant discharge streams for repetitive wastewater discharge permit analysis by using 2.00 mL of hexane to extract 200.00 mL of sample and compared the result with purge-and-trap for volatiles and semivilatiles.

Henderson et al. (64) developed a microextraction method for the determination of halomethanes in ppb level by using 3.00-5.00 mL of pentane to extract 117.00-115.00 mL of water samples in 120.00 mL serum bottles capped with a teflon-coated rubber septum and sealed by crimping the aluminium septum retainer over the lip of the bottle.

Glaze et al. (65) presented a second and a third generation liquid liquid extraction method which

was more convenient and rapid for routine trihalomethanes analysis and was capable of the analysis of other purgeable organics when coupled with capillary GC-ECD or GC-FID analysis by using sample-to-pentane ratios ,i.e.,19:1 and 99:0.5. Extensive data on matrix effects, changes in pH and ionic strength were also evaluated.

Murray and Lockhart (66) used a GC microextraction method for analysis of selected petroleum hydrocarbon in water and fish tisue . The most effective sample-to-solvent ratios were studied by using hexane 0.25 , 0.50, 0.75, 1.00 mL to extract 950.00 mL of water samples.

Suparnpongs and Leepipatpiboon (67) studied the microextraction technique for determination of halogenated alkanes in water samples by using GC-ECD. The various effects including sample-to-solvent ratios, extracting organic solvents , shaking times and salting out with sodium chloride and sodium sulfate on percent recoveries of these compounds were evaluated.

Tiyanon and Leepipatpiboon (68) developed the microextraction technique for extracting trace polynuclear aromatic hydrocarbons in water samples before determination by GC-FID. The factors effected on the percent recoveries ,i.e., extracting organic solvents , sample-to-solvent ratios , shaking times and

salting out with sodium chloride and sodium sulfate were studied.