CHAPTER 3

EXTRACTION AND ENRICHMENT OF PCBs, CHLOROBENZENES AND HCH INTO A THIN LAYER OF A LOW-DIELECTRIC SOLVENT ASSISTED BY WATER

3.1 BASIS OF THE METHOD

The method developed here is found to be effective in recovering all analytes chosen, and minimizing solvent consumption. It is simple and rapid as far as sample preparation is concerned. MAE in general, minimizes solvent consumption; however this is still in tens of milliliters, and if further improvement is done, the solvent requirement could still be lower. The present approach is based on the fact that microwave generates heat through a good dielectric medium like water in which the solid sample is dispersed or covered completely. MW heating can then release analytes from the hot swollen solid samples, especially the water insoluble non-polar and volatile analytes rise to the surface where the solvent trap is available to capture and dissolve the analytes. The swelling of samples in water medium was discussed in the publications of Budzinski et al. (1999); Eskilsson and Björklund (2000) and Fahmy et al. (1993). Many solid samples like textiles, soils, and leather were successfully analyzed by this procedure in this study. The solvent requirement is only for trapping the analytes by dissolution and up to 5 ml were tried with 1 ml being sufficient for many cases. This procedure was mainly developed for solid sample extractions; however even liquid samples tried ensured convincing
results. Interestingly, water samples as high as 100 ml volumes responded well with good recoveries by this approach.

MAE finds application only with solvents of high dielectric strength. Actually, low dielectric solvents are not MW conducive and hence applications with hydrocarbons are limited as discussed in the reference by Budzinski et al. (1999). But hydrocarbon solvents are claimed as good solvents for the chosen analytes and n-octane is reported Numata et al. (2003) to be the best for the recovery of PCBs, which is also confirmed by this study, as it is lighter floats on the surface of water. There are three different techniques available to use a low dielectric solvent for the extraction in MW analysis. The first approach is to use co-solvents like acetone along with low dielectric extractants as reported by some earlier studies by Luque-Garcia and Luque de Castro (2003); Budzinski et al. (1999); Eskilsson and Björklund (2000); Enders and Schwedt (1996); Weichbrodt et al. (1999); Lopez-Avila et al. (1995); Punt et al. (1999); Chee et al. (1996) and Barnabas et al. (1995). The second technique for the same purpose involves the use of a chemically inert fluoropolymer (Carbofon disk, CEM, USA; Weflon disk, MLS, Germany) which is discussed earlier by Eskilsson and Björklund (2000); Vetter et al. (1999) and Hummert et al. (1996), where the polymeric material absorbs MW energy and transfers heat to a low dielectric solvent. The third approach is to use water at some specific proportion by deliberate addition or use the moisture originally present, to effect extraction of the analyte on to a MW transparent solvent as described in the following publications by Numata et al. (2003); Budzinski et al. (1999); Garcia–Ayuso et al. (1998); Eskilsson and Björklund E. (2000); Punt et al. (1999); Onuska and Terry (1993) and Molins et al. (1997). Water vapours are used to evaporate PCBs by MW heating, as reported by Xiong and Pawliszyn
(2002). In another recent publication by Numata et al. (2003). MW extraction and distillation of PCBs are detailed in which n-octane is used in the range of 20 ml and heated with 3 ml water. The present study is to find the possibility of extracting and enriching persistent organic pollutants such as PCBs and chlorobenzenes with a smaller volume of a MW transparent solvent constituting a thin layer. n-Octane and other hydrocarbons are MW inactive, but when water medium picks up heat due to MW absorption it is easily relayed to n-octane layer which is floated over water for trapping hydrophobic semi-volatiles. There are only a few such studies in MW analyses involving two immiscible solvents, to effect the recovery of an analyte. In an earlier attempt, Daghbouche et al. (1997), successfully extracted grease and oils using 10 ml carbon tetrachloride from 25 ml water and then using 7 ml of the same solvent to effect extraction from 100 ml wastewater following pressurized MW heating. Onuska and Terry (1995) of their two publications, one for the extraction of PCBs from water samples using a solvent and the other for the extraction of chlorinated benzenes from water sample claimed 1000 folds pre concentration of the analytes in the latter. Likewise, triazine pesticides were recovered by Steinheimer (1993) from water samples. In our present study sodium chloride has been used, with sample and 1 ml of 10% sulphuric acid. The inclusion of sodium chloride should be beneficial as Camel (2000) mentioned in a review paper that the addition of sodium chloride to the sample enhanced the extraction because of faster heat transfer. As the concentration of PCBs in many samples is very low normally, the small solvent volume also helps in enrichment and improves the detectability. The distinct advantages of the proposed technique are the solvent minimization, speed of analysis and good recovery. This technique offers enrichment similar to solid phase extraction since hydrophobic analytes can be extracted and enriched through a solvent layer of a few
milliliters (without resorting to matrix cleaning). Compared to SPE technique, this method is faster and is suitable for the extraction of non–polar analytes of semi to non-volatile category from solid and aqueous samples with enrichment.

3.2 EXPERIMENT

Aroclors 1248, 1260 and florisil were procured from Supelco, Bellefonte, PA, USA. Isooctane, HPLC grade was from E-Merck. n-Octane, analytical reagent grade was procured from S.D.fine chem. Ltd., Mumbai, India. 1,4-Dichlorobenzene (DCB) and Hexachlorobenzene (HCB) were purchased from Lancaster, Lancashire, England. γ-Hexachlorohexane (HCH) was from Dr.Ehrenstorfer, Augsburg, Germany.

3.2.1 Equipments

The MW extractor system used for this study has FMW design; model Soxwave 100 along with a programmer for operation, procured from Prolabo, Fontenay-sous-Bois, France. The programmer is used to select the experimental conditions of MW power in percentage and time duration in minutes. This model works at atmospheric pressure and has 300 watts capacity with an available power range 30W-150 W. MW radiation is of the frequency 2450MHz. Extraction vessel is open and of borosilicate glass fitted with a solvent collector and an overhead Graham’s water condenser. Julabo cooling water circulator of model FE1800, from Seelbach, Germany was used to support Graham’s water condenser. Water of HPLC grade purity was prepared using Milli-Q model of Millipore, Billorica, MA, USA. Gas Chromatography of model, Auto system XL equipped with Electron Capture Detector (ECD) and
temperature programmable injector operated with Turbo Chrome Navigator workstation was procured from Perkin-Elmer (Norwalk, USA). DB-17, a mid-polar capillary column of 30m-length, 0.32 mm I.D. and 0.25μm-film thickness, procured from J&W Scientific, Folsom, CA, USA was used for GC separation.

Stock solution preparation of PCBs: Aroclor 1260 standard of 100μg/ml was prepared as stock solution. From this, 1-10μg/ml concentrations were prepared using HPLC grade MeOH. In a similar way Aroclor 1248 was prepared.

Stock solution preparation of chlorobenzenes and γ- HCH: Stock solutions of 1000 μg/ml were prepared for 1,4 Dichlorobenzene, hexachlorobenzene and γ-hexachlorohexane in MeOH. A mixture of standard solutions of individual concentration of 1,4-DCB, HCB and γ-HCH in the range of 1-10μg/ml was prepared using HPLC grade MeOH.

3.2.2 Extractions of samples
3.2.2.1 Preparation of Soil Sample

The soil free from the analytes investigated, was air-dried, pulverized, and sieved to a grain size of 2 mm. 25 g soil was mixed with acetone until it was completely soaked to form slurry. Then, an appropriate volume of the standard solution equivalent to 5 μg of analyte was spiked and mixed well for over 3 hrs. Similar approach in spiking was followed individually for Aroclors 1248, 1260, chlorobenzenes and HCH. The bulk of the solvent was slowly evaporated at room temperature by thorough manual shaking. This sample was kept for 48 hours in a fume hood to dry out completely and aged for 1 week at
room temperature. The prepared soil sample was stored in a refrigerator at 4°C until taken up for the analysis.

3.2.2.2 Preparation of other solid samples

Leather and textile (cotton) samples were shredded into approx. 100 mesh fineness and used for the extractions as detailed further. The standard solution equivalent of 1μg of analyte was spiked into each of these samples (3 g) and taken for analysis. Spiking was done with appropriate volume of standard solution to the weighed sample and left for overnight drying at room temperature and taken for analysis the next day.

3.2.2.3 Extraction of solids

5 g of soil sample was weighed and transferred into the MAE vessel. In the case of leather or textiles, as they are voluminous, 3 g of samples were taken. To the solid sample, 25 ml of water, 1 ml of 10% sulphuric acid and 0.1 g of sodium chloride were added. Over this, 1 ml of n-octane was allowed to float. MWP was rotated from 10-40% for 1-15 min duration for the extraction. The experiment was repeated exactly as discussed above except for n-octane volume of 2 ml and for 5 ml. The same extraction method as detailed above was followed for Aroclors 1248, 1260, chlorobenzenes and HCH.

3.2.2.4 The alternate extraction method by MAE employing higher volume of the solvent (n-octane)

5 g of the spiked soil sample was taken in the vessel along with 25 ml of water, 1 ml of H₂SO₄ and 0.1 g of NaCl. 10 ml of n-octane was added over this and MWP of 20% was applied for 10 min (decided from the optimized
MAE studies). It was left to cool for 30 min. After 30 min, the condenser was dismantled and the solvent and other contents were transferred into a 100 ml separating funnel (filtered through Whatman 41 paper). The aqueous portion was drained; the solvent layer dried over anhydrous Na₂SO₄ and made up to 10 ml before GC analysis. Leather and textile samples were extracted by a similar approach but with 3g quantities. The method was followed exactly in the same way for all the solid samples spiked with chlorobenzenes and HCH mixture.

3.2.2.5 Soxhlet extraction

This technique was carried out for the solid samples as a referral technique. This approach detailed here is common for all the analytes investigated. 5 g of the sample was taken along with dried Na₂SO₄ and 100 ml hexane –acetone mixed solvent (1:1) in 150 ml extractor vessel. Extraction was carried out for 16 hours at 10 cycles per hour. After the extraction, the extract was cleaned-up by a florisil column, then concentrated by a rotary evaporator and subsequently under a gentle stream of nitrogen to near dryness. The residue was made up to 1 ml with iso-octane.

3.2.2.6 Preparation of liquid samples

MAE of PCBs: 1 ml PCB standards (Aroclor 1248 and 1260) at 1 μg/ml were spiked into 25, 50, 75 and 100 ml volumes of Milli-Q water individually and shaken gently to ensure thorough mixing. Each of the spiked water samples was taken in a 250 ml MAE vessel, to which 1 ml of 10% sulphuric acid and 0.1 g of sodium chloride was added. Over this 1 ml of
n-octane was allowed to float. MWP was rotated from 10-40 % for 1-5 min duration for extraction. The experiment was repeated by varying only the n-octane volume to 2 ml and 5 ml.

Extraction of chlorobenzenes, and HCH: The approach is the same as used for PCBs but with the spiking of mixture containing individual concentration of DCB, HCB and HCH at 1μg.

Note: In all the MAE, after completion of extraction of a sample, nearly 30 min was left, after which the condenser was dismantled and the extraction vessel drawn out. The condenser was not rinsed as that would contribute to some more solvent usage and the experiment was tried for finding the analytes only in the solvent trap. The solvent was drawn out from the top with a long slender pipette and then dried with anhydrous Na₂SO₄ before GC analysis.

3.2.2.1 Liquid-Liquid Extraction

LLE technique was followed as a referral technique for the aqueous samples and the approach was common for all analytes under the study. Extraction was carried out with a 100 ml sample and 25 ml dichloromethane, made acidic by adding 10% sulphuric acid in a 250 ml separatory funnel, and the process was repeated twice with fresh solvent of same quantity. The extracts were combined and after the addition of 1 ml of isooctane, evaporated to approx. 1 ml using a rotary evaporator. The extracts were filtered, dried over Na₂SO₄, and the solvent changed to n-hexane. The extracts were again concentrated in a rotary evaporator, followed by a florisil column cleanup. The
extracts were then concentrated by a rotary evaporator and subsequently under gentle stream of nitrogen to near dryness. The residue was made up to 1 ml with iso-octane.

3.3 RESULTS AND DISCUSSION

3.3.1 Selection of solvent for enrichment of PCBs

The main criterion for selection of the solvent involved identifying a good solvent for analytes, like PCBs, chlorobenzenes and HCH and also stable at MAE conditions, n-Octane is reported by Numata et al. (2003) to be the best for the extraction of PCBs in terms of recovery. The main extraction is done by water heated by MW, when volatiles, semi volatiles and even non-volatiles (water insoluble) are released from solid matrices and rise to the top where the solvent trap is laid. Hence, the trapping solvent should be lighter than water to float on top otherwise the top becomes an escape route. n-Octane satisfies this requirement as well and also suffers only indirect heating by water. n-Octane is therefore a useful liquid trap as is also a good solvent for PCBs, chlorobenzenes and HCH while it causes no emulsion formation.

3.3.2 Optimisation of MAE for the extraction of PCBs and other chloroorganic compounds

3.3.2.1 Solid samples

Optimisation of MW energy and the duration of the extraction to achieve the best recovery of analytes chosen are discussed. The optimal extraction conditions are found to be different for solid and liquid samples. The difference was prevalent even for different analytes. In general, extractions of
solid analytes require higher power and longer duration (that is, the extraction is extended by 3-8 min) compared to liquid samples of even 3 or 4 times higher volumes. The study on optimisation of aqueous phase is discussed separately under the specific heading. For extractions from solid samples, 25 ml water and 1 ml n-octane were used. The optimisation study involved rotating MWP from 10-50% for different durations from 3 to 15 min for solid samples. All solid samples (spiked at the same level of 1 µg/5 g soil of Aroclor 1260) of equal weights were tried for extraction, which led to the observation that among the solids chosen, soil required comparatively a longer time of extraction. Cellulosic (textile) and proteinaceous (leather) matrices which undergo swelling; hence these matrices release the analytes at a faster rate. For all the recovery studies only spiked analytes (both solid and liquid samples) were used. Regarding the recovery of PCBs in solids, the extraction recovery of Aroclor 1260 was found to be 98.6% (averaged for all the PCB congeners and the recovery is referred that way for Aroclors 1248 and 1260 in this report) from textile and 97.9% from leather with 10% MWP (microwave power) applied for 8 min duration while from soil the recovery was 96.3% at the same 10% MWP, but in 10 min. The recoveries of Aroclor 1248 were 99.2% from textile and 99.1 from leather with 10% MWP applied for 8 min duration and the recovery from the soil was 98.2% in 10 min at the same power. The recovery trend for PCBs (Aroclor 1260) from soil on varying MW heating duration is shown in Figure 3.1. Aroclors 1260 and 1248 followed a similar trend. Since recovery of Aroclors was found to be different for soil, leather and textile, it is inferred that the extractability of the same analyte differs with matrix. Recovery of Aroclor from spiked leather is shown in Figure 3.2. In the case of recovery of chlorobenzenes and HCH from solid samples, DCB gave the best recovery of 97.4% from the soil at 10% MWP in 5 min. In the case of leather the recovery
of DCB was 95.1% at 10% used for 3 min and it was 96.6% for cotton textile at 10% MWP in 3 min. But for DCB alone 2 ml n-octane was employed. Prolonged heating beyond 10 min even at 10% MWP caused loss of the highly volatile DCB. Losses of HCB and HCH were observed significant at 10% MWP if extraction exceeded 15 min or at 20% MWP when application exceeded 8 min. The recovery of HCB in the soil was 97.9% at 10% MWP in 10 min or at 20% MWP in 5 min (the spiked recovery of HCB from soil is shown in Figure 3.3). From the leather sample, the recovery of HCB was 98.9% at 10% MWP in 8 min and from the textile it was 97.6% at 10% MWP in 10 min. The other analyte HCH in the mixture was found to give the maximum recovery of 99.1% from soil at 10% MWP in 10 min, while the same power helped to get the maximum figures of 98.7% recovery from leather in

![Figure 3.1 Recovery trend for a soil spiked with Aroclor 1260 at 200 ng/g found when MWP was varied from 10-40% over a duration of 1-10 min. Conditions:- volume of aqueous phase: 25 ml and n-octane volume :1 ml](image-url)
Figure 3.2 Recovery trend (%) of PCBs (Aroclor 1260) from leather on varying MW heating duration (min)

Figure 3.3 Recovery trend (%) of HCB in soil on varying MW heating duration (min)
8 min and 97.1% from textile in 8 min. The recovery of HCH from a textile sample is shown in Figure 3.4. In general, using 30% MWP beyond 3 min and 40% MWP even for a minute were not suitable for recovering this kind of volatile analytes. Hence beyond 40% MWP the extraction was not attempted. Optimised condition for solids can be summarised as follows:

10% MWP for 10 min is the suitable condition for the extraction of Aroclor 1260, Aroclor 1248, HCB and HCH from soil samples while it is 8 min, for the extraction of same analytes from leather and textile samples. Only for highly volatile analyte like DCB, a milder conditions compared to the above mentioned is required which is 10% MWP for 5 min for the extraction of DCB from soil samples, and for extraction from leather and textiles, 10% MWP for 3 min is recommended.
3.3.2.2 Liquid samples

In the case of liquid samples the extraction actually depended on the volume of aqueous phase and its influence on recovery is discussed under relevant heading. In studying the power optimisation for aqueous samples, water samples of various volumes (25, 50, 75 and 100 ml) spiked with Aroclors 1248 & 1260 were employed. Methanolic preparations of standards enabled their complete miscibility in water. While MWP was rotated from 10%-50%, extraction duration was varied for 1-10 min. As mentioned earlier, 1 ml n-octane was used as trap throughout this study (except for DCB in which case it is 2 ml). In the extraction of PCBs from the largest volume of 100 ml aqueous samples, 10% MWP for 5 min duration gave the recovery of 96.9% for Aroclor 1260 and even a better recovery of 99.8% at 20% MWP in 5 min. For Aroclor 1248, the recovery was 98.6 at 10% MWP and 98.5% at 20% MWP from 5 min extraction. Further increase of MWP to 30% also ensured recovery exceeding 90% in both cases in 1 min but remained the same till 2 min. However, at 40% MW power the recoveries dropped considerably on increasing the time from 1 to 5 min and the values for both Aroclors 1260 and 1248 dropped to around 70% even with 1 min. extraction. In Figure 3.5, the dependency of recovery of Aroclor 1248 extracted from 100 ml aqueous sample, on MWP (%) and duration of extraction is shown. In the extraction of chlorobenzenes and HCH from 100 ml aqueous sample, DCB gave 100.7% recovery at 10% MWP in 5 min and produced good results between 85 and 90% range, at 20% MWP in 2 min and also at 30% MWP in 1 min but heating beyond that affected the analyte recovery. Figure 3.6 shows the dependency of the recovery of DCB from 100 ml aqueous sample on varying MWP (%) and duration. For HCB, the recovery was 97.1% at 10% MWP in 5 min and 96.2 %
at 20% MWP in 3 min while HCH showed 100.4% recovery at 20% MWP in 3 min and 98.4% at 10% MWP in 5 min. At 30% MWP, HCB was stable only till 1 min while HCH was till 2 min. The lowest MWP of 10% was found to be the best option when applied for 5 min for achieving good recoveries of all the analytes studied whereas for DCB 3 min was found to be sufficient.

**Figure 3.5** The dependence of recovery of Aroclor 1248 (spiked at 1 μg) from 100 ml water on MWP varied from 10-40%.

*Conditions: - n-octane volume: 1 ml and duration: 5 min.*
Figure 3.6 The dependence of recovery of volatile analyte DCB from 100 ml water on MWP varied from 10-40%.

Conditions: - n-octane volume: 1 ml and duration: 3 min.

When the lowest volume of liquid sample, namely 25 ml was tried with 10% MWP, for 1 min, it gave a good recovery of 93.1% for Aroclor 1260 while studies at 20% power also resulted in the recovery of 94.5 % in 1 min duration. Similarly Aroclor 1248 gave good response with 92.9% recovery at 10% MWP in 1 min. HCH gave 97.2% at 10% MWP in 1 min while HCB gave a recovery of 96.8% at 10% MWP in 1 min. At 30% MWP, extraction for even 1 min, affected the recovery and hence no trial was attempted beyond this power. For 25 ml water samples even 10% MWP was too powerful to serve for DCB even if applied just for a minute, as the analyte is volatile and is easily lost in such a low volume of water, which gets heated excessively even by a MWP
of 10%. As mentioned in the case of solids, solvent rinsing of the condenser was avoided. However to verify that if a rinsing of the condenser improves the recovery, it was carried out twice with a total of 1 ml quantity of solvent when the recovery improved as expected. However this rinsing was not effective with DCB. This shows that these analytes are not trapped well, for which reduction of MWP or duration of extraction would help. Increased quantity of solvent employed for the extraction also would help which is discussed separately.

Unlike solids, the trend was the same for PCBs, chlorobenzenes and HCH in aqueous matrices, as there were no noticeable differences in the extraction conditions except for DCB, which was poorly recovered using 1 ml n-octane from 25 ml. However for the same analyte and all the other analytes involved in this study from water samples of 50 and 75 ml, 1 ml n-octane supported well, as revealed from the recoveries of 91.2% and 95.6% respectively for DCB when 10% MWP was used for 3 min. The study shows that 10-20% MWP as the appropriate range for extraction from water samples and to a small extent 30% MWP and nothing beyond.

3.3.3 Influence of volume of aqueous phase

In the case of solid samples, aqueous phase was required only to cover the solid sample and also to fulfill minimum level of the extractant in the extraction vessel. The volume required to cover most solid samples was between 15 and 20 ml, and to ensure some excess of water above the sample, 25 ml of water was used for the extraction of solids. Volume of water lower than 25 ml was difficult to deal with, as the analytes were easily lost (due to excessive heating by MW). The extent of recovery gradually improved from 15 to 25 ml; 30 ml did not give any better recovery than 25 ml and hence 25 ml
was used for extraction. To study the effect of variation of aqueous phase on the extraction of solid samples, soil sample spiked with Aroclor 1248 and 1 ml n-octane were used. The recovery trend of Aroclor 1248 from soil on the variation of aqueous sample volume (from 15-30 ml) is shown in Figure 3.7A.

For studying the extraction of aqueous samples, 1µg equivalent of Aroclor 1248 was taken in 25, 50, 75 and 100 ml water samples separately. As the volume of the aqueous phase increased the efficiency of MW decreased. However it was possible to find the MAE condition to overcome this shortcoming. The higher volume of water led to the decreasing analyte recovery but extending the application of MWP for slightly longer duration offset that the influence of varying volume of water from 25-100 ml on the recovery of an analyte (Aroclor 1248) is shown in Figure 3.7B. In the case of water samples, analytes are in free state hence a slight increase of MWP or extending the duration of extraction helped to preserve the recovery of analytes on increasing volume. The interest in coping with the increasing volume is to achieve the best enrichment.

In order to clarify if the analyte spiked just floats (only in case of water samples) without mixing thoroughly leading to easier extraction, samples were drawn from different levels of the vessel containing spiked solutions and subjected to HPLC analysis using photometric detection. The container used was the MW vessel of 250 ml with 35 mm dia. The spiked solutions were poured into this and after gentle shaking samples were drawn from top, middle and bottom portions of the solution. No solvent for trapping was added at this stage. Separately spiked solutions of Aroclors 1248, 1260, chlorobenzenes and HCH mixture were analyzed by HPLC, which gave matching peak areas for
Figure 3.7 The influence of variation of aqueous phase volume on the recovery of spiked Aroclor 1260 in correlation to MWP, using n-octane 1 ml and duration 5 min. A. From a soil sample; B. From a water sample
samples drawn from different regions in the MW vessel, thus proving that analytes are distributed well in the sample. It is inferred that the analytes on MW heating migrated from the entire bulk to the surface where they are trapped by the solvent. It is observed from Figure 3.7A and B that from 25 ml water sample, the recovery was higher initially which decreased on increase of MWP from 20-40% whereas from solids, with 25 ml water the recovery was initially (10% MWP) lower which reaches a maximum with 20% MWP and then decreases. This could be due to the fact that from solids the analytes are released from the interior pores and the amount released depends on MWP and time while from water sample the initial release is higher which reduces with time and or MWP. The optimal conditions of water samples are summarized below:

For water samples of volume 100 ml (suitable for 50-100 ml) the 10% MWP for 5 min was found suitable for all the analytes. For 25 ml water volume 10% MWP for 1 min was found sufficient for the extraction for all the analytes. Except for DCB, in all other cases 1 ml n-octane has served very well as the trappent whereas for DCB 2 ml or 5 ml n-octane has done well.

3.3.4 Influence of volume of extracting solvent

The main aim of this study is to minimize solvent for extraction as the role of solvent is only to trap the analytes. n-Octane as solvent trap was tried from 1 to 5 ml range; with the maximum volume (5 ml) being only one fourth of the amount (20 ml) used for a similar purpose reported earlier by Numata et al. (2003). Solvent levels lower than 1 ml could not be dealt conveniently for which some modification is required in the design of MW vessel. The study was done to find if there was any change of recovery on varying extractant volume in the range 1-5 ml for solid and aqueous samples.
At the end of the experiments, any loss of the extracting solvent was verified, as n-octane loss raises the fear over loss of any analyte. But the quantity of n-octane used for extraction was found to be intact (volumetrically verified) at the end of the experiment under the conditions prescribed for the extraction of solids and aqueous samples. 1 ml n-octane constituted 1 mm thickness in the MAE vessel of dimension 35 mm dia. It was found that 2 ml n-octane had thickness of 2.1 mm, and 5 ml had 5.2 mm. 25 ml aqueous sample had a length of 26 mm, 50 ml had 52 and 100 ml had 101 mm. Extraction into a smaller volume (thin layer) of organic solvent from a comparatively larger volume of aqueous phase or solid sample could help in achieving enrichment. In the case of liquid samples of 25, 50, 75 and 100 ml with 1 to 5 ml n-octane, the recoveries confirmed enrichment of 5 to 100 folds. For 50, 75 and 100 ml volumes of aqueous samples in the cases of all the analytes, 1 ml n-octane was found to be effective. As the aqueous phase volume decreased heat pick-up due to MW irradiation increases leading to the loss of analytes and it is serious in the case of volatile analytes like DCB when extracted from 25 ml water. In such cases, trapping is improved by a thicker cover of the solvent that is, with increased volume. In this study, 2 ml and higher volume of 5 ml were employed which responded with better recoveries for DCB even from 25 ml aqueous phase. Similar is the situation when higher MWP (from 30%) is opted for the extraction, the excessive heat generated demands thicker solvent pad. Once 30% MWP usage exceeded 5 min and 40% and higher MWP tried even for a minute, the analytes were lost with 1 ml n-octane trap while 2 ml and higher volume of n-octane were effective. However, extractions from solids (except for DCB) were successful with 1 ml n-octane and 25 ml aqueous volume, the probable reason being that the analyte
is not in free state and its release from the matrix takes longer time. The main influence noticed with the variation of solvent volume was that in the extraction of solids, at MWP higher than 30%, volume of n-octane higher than 2 ml, was only effective and less than this could not effect trapping of analytes. The variation of volume of extracting solvent did not produce any significant change in the analyte recovery with MWP less than 30% for an extraction time upto 5 min except in the case of highly volatile DCB. For DCB, only 2 ml and preferably 5 ml could help as is shown in Figure 3.8. It was produced by changing the volume of n-octane from 1,2 and 5 ml for the recovery of DCB (a volatile case) and Aroclor 1260 (a semi volatile case) when extracted from 25 ml aqueous sample at a constant extraction time of 3 min. It is clear from the Figure 3.8 that just 1 ml was good enough upto 20% MWP for Aroclor 1260 and it is the same experience in this study for all other analytes chosen.

3.3.5 Influence of weight of the sample

Influence of weight of the sample was studied using Aroclor 1260 because it is the difficult to extract analyte especially from a soil sample (since it is the difficult matrix).

1 to 5 g of soil spiked with Aroclor 1260 was dispersed in 25 ml water phase and over which 1 ml n-octane floated for analyte enrichment. 10% MWP was used for durations of 3 min, 5 min and 10 min. The trend is shown in Figure 3.9. Lower weight (1 g) has shown good recovery of 80% just by 5 min but with 10 min duration, the recoveries are leveled to a maximum.
Figure 3.8 The influence of variation of n-octane on the recovery of a volatile analyte, DCB and a semi volatile analyte, Aroclor 1248 in correlation to MWP.

Conditions: volume of aqueous phase: 25 ml; duration: 3 min.

Figure 3.9 The influence of weight of sample is shown with Aroclor 1260 extraction from soil sample, weight varied from 1-5 g @ MWP 10%
3.3.6 Analysis of samples (real and spiked) and comparison of the data obtained by other methods

Several real solid and water samples were screened to validate this procedure. Soil samples from a nearby industrial site were collected and analyzed by the proposed procedure and also by the Soxhlet procedure. Consumer goods like textiles and leather articles were also taken for screening. Similarly water samples from a lake near an industrial site were collected in polypropylene containers. All real samples were stored at 4°C until taken for the analysis. The real samples were not found to contain PCB residues and hence the same were spiked with Aroclors 1248 and 1260. Since environmental samples are normally found to contain PCBs at much lower levels, (these low levels of spiking for samples were done separately) spiking was done with varying low levels of PCBs at 25 ng and 50 ng/g of soil samples and extracted by the proposed method. Because this procedure deals with an extractant of 1 ml it does not involve a concentration step for solvent elimination and also the detection is by GC-ECD, detectability of 18-20 ng/g of Aroclors 1248 (18 ng/g) and 1260 (20 ng/g) for solid samples was the best possible. Whereas the same analytes could be detected down to 0.8-1.0 ng/ml in water samples because water sample volumes as high as 100 ml could be handled with the use of 1 ml n-octane and thus 100 times enrichment is possible. The results revealed that the spiked levels of 25 ng and 50 ng responded well. The real solid samples were not found to carry any DCB, HCB and HCH, which led to the spiking of the same into these samples. Spiking of chlorobenzenes and HCH into soil samples at 10 ng and 50 ng/g, were successfully detected except for DCB. The method detection limit (LOD) observed for DCB was 50 ng/g and hence its spike levels were 100 and 250 ng/g which were successfully achieved. The recovery data
and LOD for all the analytes from soil samples are given in Table 3.1. Screening for chlorobenzenes and HCH in real water samples showed that these samples were free of DCB, HCB and HCH except for one lake water sample, which contained HCH. Analyses of water samples (for all the analytes) were carried out by spiking quantities equivalent to 5 and 10 ng/ml. In the case of water samples, spiked experiments with Aroclor 1248 and 1260 even at 5 and 10 ng/ml responded successfully. LOD for DCB in water sample was 3.1 ng/ml, hence DCB along with HCB and HCH responded at the spike level of 5 and 10 ng/ml. The recovery data for these spiked analytes from water samples and LOD for analytes are given in Table 3.2. For the purpose of comparing the different procedures detailed in experimental section, a soil sample spiked with Aroclor 1260 at 200 ng/g (as only at this concentration detection of the analytes by all the extraction methods become possible) was extracted by the proposed procedure, which gave a recovery of 101% (% RSD 3.4 from 3 replicates). The same sample was also subjected to analysis by the alternate MAE method using 10 ml n-octane (recovery of 102% with % RSD 3.1). The recovery from Soxhlet extraction for the same sample was 98.9% (% RSD 2.9). The chromatograms of this soil with 200 ng/g spike of Aroclor 1260 (only those from MAE) are produced in Figure 3.10, in which (A) shows that of the sample under similar conditions but using 10 ml n-octane and (B) shows the same done using 1 ml n-octane. A lake water sample was found to contain HCH residue at 4 ng/ml (%RSD 3.1 from 3 replicates). Figure 3.11, represents the chromatograms for real aqueous sample containing HCH residue (from MAE) and in this overlay of chromatograms (A) is with 10 ml n-octane and (B) is with 1 ml n-octane, under similar experimental condition. Both these chromatograms reveal that in this analytical approach, enrichment is a major benefit and the use of 1 ml over 10 ml gives 10 times enrichment. Figure 3.12 is
shown for the spiked recovery of DCB (250 ng/g), HCB (50 ng/g) and HCH (50 ng/g) from a soil and this is achieved using 2 ml n-octane. Figure 3.13 produced for Aroclor 1248, represents spiked (5ng/ml) recovery from water sample of 100 ml extracted and enriched using 1 ml of n-octane. Results by alternate methods agreed well as shown in Table 3.3 with the proposed method (involving even 1 ml n-octane), thus proving the validity of the procedure.

3.4 ANALYTICAL SEPARATION OF PCBs

3.4.1 GC Instrumental conditions for PCBs

B-17 capillary column was used. The injection port and detector (Electron Capture detector) temperatures were 200°C and 375°C respectively. Column oven conditions for PCBs: The initial oven temperature was 140°C, held for 2 min and increased to 275°C at 5°C min⁻¹ and held for 20 min. 2 μl was injected in splitless mode.

3.4.2 GC Instrumental conditions for chlorobenzenes and HCH

Column oven conditions: The initial oven temperature was 45°C, held for 5 min, increased to 50°C at 1°C min⁻¹ and then increased to 150°C at 10°C min⁻¹ held there for 5 min. It was further elevated to 280°C at 10°C min⁻¹ and held for 2 min. 2 μl was injected in splitless mode.
### Table 3.1 LOD and % Recoveries (ng/g) for spiked analytes of soil samples

<table>
<thead>
<tr>
<th>Analyte</th>
<th>LOD</th>
<th>Spiked level</th>
<th>% Recovery</th>
<th>%RSD (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aroclor 1260</td>
<td>18</td>
<td>25</td>
<td>93.5</td>
<td>5.4</td>
</tr>
<tr>
<td>Aroclor 1260</td>
<td>18</td>
<td>50</td>
<td>91.8</td>
<td>3.9</td>
</tr>
<tr>
<td>Aroclor 1248</td>
<td>20</td>
<td>25</td>
<td>94.1</td>
<td>4.8</td>
</tr>
<tr>
<td>Aroclor 1248</td>
<td>20</td>
<td>50</td>
<td>95.3</td>
<td>4.1</td>
</tr>
<tr>
<td>DCB</td>
<td>50</td>
<td>100</td>
<td>90.3</td>
<td>4.7</td>
</tr>
<tr>
<td>DCB</td>
<td>50</td>
<td>250</td>
<td>98.9</td>
<td>2.6</td>
</tr>
<tr>
<td>HCB</td>
<td>5</td>
<td>10</td>
<td>90.4</td>
<td>3.9</td>
</tr>
<tr>
<td>HCB</td>
<td>5</td>
<td>50</td>
<td>92.2</td>
<td>3.8</td>
</tr>
<tr>
<td>HCH</td>
<td>5</td>
<td>10</td>
<td>93.2</td>
<td>4.2</td>
</tr>
<tr>
<td>HCH</td>
<td>5</td>
<td>50</td>
<td>90.9</td>
<td>3.9</td>
</tr>
</tbody>
</table>

# NA-Not applicable.

### Table 3.2 LOD and % Recoveries (ng/g) for spiked analytes of water samples

<table>
<thead>
<tr>
<th>Analyte</th>
<th>LOD</th>
<th>Spiked level</th>
<th>% Recovery</th>
<th>%RSD (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aroclor 1260</td>
<td>0.8</td>
<td>5</td>
<td>89.7</td>
<td>4.2</td>
</tr>
<tr>
<td>Aroclor 1260</td>
<td>0.8</td>
<td>10</td>
<td>95.8</td>
<td>2.6</td>
</tr>
<tr>
<td>Aroclor 1248</td>
<td>1.0</td>
<td>5</td>
<td>93.5</td>
<td>4.8</td>
</tr>
<tr>
<td>Aroclor 1248</td>
<td>1.0</td>
<td>10</td>
<td>93.8</td>
<td>2.6</td>
</tr>
<tr>
<td>DCB</td>
<td>3.1</td>
<td>5</td>
<td>91.7</td>
<td>4.5</td>
</tr>
<tr>
<td>DCB</td>
<td>3.1</td>
<td>10</td>
<td>93.5</td>
<td>3.4</td>
</tr>
<tr>
<td>HCB</td>
<td>0.3</td>
<td>5</td>
<td>91.8</td>
<td>2.7</td>
</tr>
<tr>
<td>HCB</td>
<td>0.3</td>
<td>10</td>
<td>95.8</td>
<td>3.1</td>
</tr>
<tr>
<td>HCH</td>
<td>0.3</td>
<td>5</td>
<td>93.9</td>
<td>2.4</td>
</tr>
<tr>
<td>HCH</td>
<td>0.3</td>
<td>10</td>
<td>95.1</td>
<td>2.9</td>
</tr>
</tbody>
</table>

# NA-Not applicable.
Figure 3.10 The GC-ECD chromatograms of spiked Aroclor 1260 to a soil A. using 10 ml n-octane; B. using 1 ml n-octane, showing the enrichment. Sample extracted (under identical conditions except for n-octane)
Figure 3.11 The GC-ECD chromatograms of a real water sample found to contain γ-HCH (under identical conditions except for n-octane) A. using 10 ml n-octane; B. using 1 ml n-octane, showing enrichment
Figure 3.12  GC-ECD chromatogram of a soil sample spiked with DCB, HCB and HCH extracted (trap) using 2 ml n-octane.

Figure 3.13  GC-ECD chromatogram of spiked recovery Aroclor 1248 from 100 ml water using 1 ml n-octane.
Table 3.3 Comparison of the recovery values obtained for the spiked samples analyzed by various methods. %RSD (n=3) is given in parenthesis

<table>
<thead>
<tr>
<th>Nature of sample (Spiking solids at 1μg/5g; and liquids at μg/100 ml)</th>
<th>Soxhlet method (hexane/acetone 1:1) μg/5g</th>
<th>MAE using 10 ml n-octane * 1μg/5g ** μg/100 ml</th>
<th>LLE using n-octane (only for water samples) μg/100 ml</th>
<th>MAE (proposed procedure) * 1μg/5g ** μg/100 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Textile A*</td>
<td>HCB</td>
<td>0.96(3.7)</td>
<td>1.01(2.4)</td>
<td>NA*</td>
</tr>
<tr>
<td>-do- B*</td>
<td>Aroclor 1260</td>
<td>0.97(4.1)</td>
<td>0.98(2.9)</td>
<td>NA</td>
</tr>
<tr>
<td>Leather A*</td>
<td>Aroclor 1248</td>
<td>0.95(3.4)</td>
<td>0.98(3.7)</td>
<td>NA</td>
</tr>
<tr>
<td>-do- B*</td>
<td>HCH</td>
<td>1.00(2.5)</td>
<td>1.02(2.1)</td>
<td>NA</td>
</tr>
<tr>
<td>Wood*</td>
<td>HCH</td>
<td>0.93(1.9)</td>
<td>0.92(1.6)</td>
<td>NA</td>
</tr>
<tr>
<td>Soil A*</td>
<td>HCH</td>
<td>0.99(2.3)</td>
<td>0.96(2.0)</td>
<td>NA</td>
</tr>
<tr>
<td>Soil B*</td>
<td>DCB</td>
<td>0.95(2.9)</td>
<td>0.95(2.2)</td>
<td>NA</td>
</tr>
<tr>
<td>Soil C*</td>
<td>Aroclor 1260</td>
<td>0.97(3.8)</td>
<td>1.02(3.1)</td>
<td>NA</td>
</tr>
<tr>
<td>Water A**</td>
<td>Aroclor 1260</td>
<td>NA</td>
<td>0.98(2.9)</td>
<td>0.97(3.4)</td>
</tr>
<tr>
<td>Water B**</td>
<td>HCH</td>
<td>NA</td>
<td>1.01(1.5)</td>
<td>0.94(2.9)</td>
</tr>
</tbody>
</table>

# NA - Not applicable