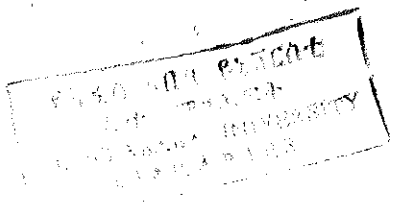
TRACE ENRICHMENT OF TRIAZINE HERBICIDES USING THE SUPPORTED LIQUID MEMBRANE EXTRACTION TECHNIQUE: APPLICATIONS TO THE STUDY OF WATERS IN ETHIOPIAN LAKES

By Negussie Megersa



A thesis submitted to the School of Graduate Studies of Addis Ababa University in partial fulfillment of the requirements for the Degree of Doctor of Philosophy in Chemistry

April 2000

To my wife
and
our parents

TABLE OF CONTENTS

LIST OF FIGURES

Fig. 2.1 Membrane Separator: (A) Aluminum Backup; (B) PTFE Block with Grooves like Archimedean Spiral; and (C) Porous PTFE Membrane with Polyethylene Backing.

Fig. 2.2 Sample collection site at Awassa Lake. A – Agricultural fields, B – Hills surrounding the lake. Numbers represent collection point (1) or approximate line followed for sample collection (2 and 3). See also text.

Fig. 2.3 Set-up of the flow system for liquid membrane extraction: (1) containers for sample solution (A) and donor buffer (B); (2) and (3) peristaltic pumps for the donor and acceptor channels, respectively; (4) PTFE tee connection; (5) mixing coil; (6) membrane extraction unit; (7) container for acidic acceptor solution; and (W) waste.

Fig. 2.4 Schematic diagram for the automated extraction of the mixture of parent compounds and degradation products of *s*-triazine herbicides. The operation is described in the text.

Fig. 3.1 Extraction efficiency versus acceptor pH. Membrane composition: 100% *n*-undecane; donor pH: 7.0; 20 min extraction of 0.5 mg/L of the herbicide compounds at donor flow rate of 1.0 mL/min. 20 μL of the enriched sample was introduced into the separation system. Symbols: (◆) - desmetryn, (■) - metoprotryn, (▲) - ametryn, (×) - prometryn, (✳) - terbutryn, (•) - dimethametryn, and (+) - dipropetryn.

Fig. 3.2 Extraction efficiency of the alkylthio-*s*-triazines versus donor pH. Acceptor pH: 0.7 and donor pH was varied as described in section 2.7. Symbols and all other conditions are the same as Fig. 3.1.

Fig. 3.3 Extraction efficiency, E, versus the donor pH for methoxy-*s*-triazine herbicides at the donor flow rate of 1.0 mL/min, 100% di-*n*-hexylether and 25 µL injection of the extracts. Symbols: (◆) -simetone; (■) - atratone; (▲) - secbumetone; and (●) - terbumetone.

Fig. 3.4 Donor flow rate dependence of E for 20 min extraction of 0.5 mg/L of each *s*-triazine compounds. (*A*) Di-*n*-hexylether membrane solvent and acceptor acid concentration of 0.5 M; Symbols: (♦) - atrazine, (■) - prometone, (▲) - propazine and (●) - terbutryn. (*B*) *n*-Undecane membrane solvent and acceptor acid concentration of 0.1 M; Symbols: (◇) - atratone, (□) - prometone, (Δ) - propazine, and (○) - terbutryn.

Fig 3.5 E_e for the sample collected from the donor waste at certain time interval. See also text. Symbols: ◆- atratone, ▲–secbumetone, and ●-terbumetone.

Fig. 3.6 Variation of the E_e with extracted sample volume for chloro-*s*-triazines (0.40 mg/l each). The acceptor solution contained 1.0 M sulphuric acid. Symbols: (◆) – simazine, (■) – atrazine, and (●) – terbuthylazine.

Fig. 3.7 Variation of E_e with extracted sample volume for aniline derivatives (0.10 mg/L each) at different concentrations of the acceptor solution: (A) 0.1 M sulphuric acid (pH ~ 1.0), and (B) 1.0 M sulphuric acid (pH ~ 0.0). Extracted compounds: (1) aniline, (2) 3-chloro-4-methylaniline, (3) 3,5-dichloroaniline, and (4) 3-methyl-5-nitroaniline.

Fig. 3.8 Variation of E_e with extracted sample volume for alkylthio-*s*-triazines (0.25 mg/L). The acceptor solution contained 0.1 M sulphuric acid. Symbols: (Δ) – dimethametryn, (○) – terbutryn, (□) – ametryn, and (◇) – desmetryn.

Fig. 3.9 Variation of E_e with extracted sample volume for simazine at different ionic strengths of the donor solution. The acceptor solution contained 1.0 M sulphuric acid. Symbols, representing the ionic strengths: (◆) – 0.23, (■) – 0.69; (●) – 1.59, and (○) – 3.1. (Similar behaviours have also been observed for atrazine and terbuthylazine).

Fig. 3.10 Chromatograms (HPLC-UV) of the methoxy-*s*-triazine herbicides used as model compounds. River water samples spiked with the standard and extracted using SPE column, 1.0 µg/L (*A*), and SLM system, 0.5 µg/L (*B*). Peaks: (1) – simetone, (2) – atratone, (3) – secbumetone, and (4) – terbumetone.

Fig. 3.11 Calibration graph for extraction of varied concentration of the sample mixture at donor flow rate of 1.0 mL/min. (*A*) di-*n*-hexylether, Symbols: ◆ - atrazine, ● - prometone, and ▲ – propazine, and (*B*) *n*-undecane membrane solvent; Symbols: ◆ - prometone, ● - terbutryn, and ▲ - propazine.

Fig. 3.12 Chromatograms (HPLC-UV) of 1.0 µg/L of the herbicide compounds studied. (i) in reagent water and (ii) spiked in river water. Samples (500 mL) were extracted at a donor flow rate of 7.0 mL/min and 50 µL of the extract were injected in each case. Peaks: (1) desmetryn, (2) metoprotryn, (3) ametryn, (4) prometryn, (5) terbutryn, (6) dimethametryn, and (7) dipropetryn. In both sets of chromatograms (a) denotes blank extraction, (b) extraction of donor buffer after sample extraction, and (c) sample extraction.

Fig. 3.13 Chromatograms (HPLC-UV) for the extracts of lake water samples: extraction conditions: donor flow rate – 5.0 mL/min; membrane solvent – di-*n*-hexylether; and sample extraction time – ten hours. Peaks (1) atrazine, and (2) terbutryn. Chromatograms for (a) extract from Awassa Lake, and (b) extract from Chamo Lake.

Fig. 3.13 HPLC-UV chromatograms for the extracts of lake water samples: extraction conditions: donor flow rate – 5.0 mL/min; membrane solvent – di-*n*-hexylether; and sample extraction time – ten hours. Peaks (1) atrazine, and (2) terbutryn. Chromatograms for (a) extract from Awassa Lake, and (b) extract from Chamo Lake.

Fig. 3.14 UV-VIS spectra for the extracts of terbutryn. (a) Extract of 0.5 mg/L standard sample of terbutryn, diluted ten times, using di-*n*-hexylether membrane solvent. (b) Extract of Awassa Lake water with the same membrane solvent.

Fig. 3.15 E versus concentration of TOPO varied in the 75% di-*n*-hexylether and 25% 6-undecanone. Symbols: (■) - DDA; (▫) - DIA; (▲) - DEA; (X)- ATOH; (∗) - PROH; (•) - TZOH; (+) - CYZN; (∘) - ATZN; (–) - PRYN; and (♦) - TRYN.

Fig. 3.16 Chromatogram (HPLC-UV) of the 0.1 mg/L of each compound spiked in river water and extracted using a di-*n*-hexylether membrane solvent with 10% TOPO. Donor flow rate – 1.0 mL/min and 200 min extraction time. Peaks: 1 – DDA; 2 – DIA; 3 – DEA; 4 – ATOH; 5 – PROH; 6 – TZOH; 7 – CYZN; 8 – ATZN; 9 – PRYN; and 10 – TRYN.

LIST OF TABLES

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to my advisors Professor Theodros Solomon and Dr B. S. Chandravanshi for their encouragement, guidance and interest in my work. The great effort of Professor Theodros in procuring the HPLC system is highly appreciated. I am also grateful to all colleagues of the Department of Chemistry, Addis Ababa University. My special thanks go to Drs. Ghirma Moges, Wendimagegn Mammo, B. Hundhammer, V. J. T. Raju, Mr. Mulugeta Desta and Mr. Gezahegn Telila.

My unreserved gratefulness is due to Professor Jan Åke Jönsson, my advisor at Lund, for the opportunity I had to work in his advanced analytical lab, for sharing his long experiences and for his special concerns for my education and my family. Thanks for the time you spent with us sailing, for extensive scientific discussions, for giving me the chance to participate in international conferences and for impressive excursions in Sweden and Southern Ethiopia. I owe you many thanks for editing and correcting my thesis.

All colleagues and friends of the Membrane, SFE and Biosensor groups are greatly thanked for the cheerful working atmosphere. I particularly appreciate Luke Chimuka for great friendship and fruitful co-operations. Above all Luke took me to the HPLC lab first, and remained a sincere friend since then. Dr Lennart Mathiasson is thanked for assistances and nice suggestions; Professor Lo Gorton for the nice Christmas party; Karin and Barbro capable people in solving all administrative problems; and Britt Johansson for endless and invaluable technical support. Tobias, Cecilia, Lotta, Pawel, Per, Eddie, Janne, Niklas and all others for hospitality, understanding and for staying good friends.

I am thankful to Dr Curt Reimann, with whom I shared an office, for nice friendship and understanding. Your plentiful help in software-related problems during writing-up of this thesis is gratefully acknowledged, besides the wonderful time at

Uppsala. Anna is also thanked for nice dinner and drinks. The invitation from Dr Malin Åkerblom to visit the environmental residue lab at Uppsala University is appreciated, and Gunborg Alex and all other colleagues in that lab are thanked for a lovely and welcoming atmosphere.

It is my greatest pleasure to thank Dr Birgitta Fredriksson for accepting us into their family and for mid-summer dinner, and Elna for your great affections. Thanks Rasmus for demonstrating the sailing techniques on Öresund. Mathias Thorslund is thanked for excellent friendship, useful first hand advice and for the last summer visit. The support of Mrs. Trunesh Namarra is greatly appreciated. I am indebted to the Ethiopian, African, Swedish and all other friends for various wonderful farewell parties and presents.

The former and present Ethiopian Ph. D students including Drs Kelemu Lemma, Amare Gessesse, Ayele Nigatu, Merid Tessema, Mr. Yirgalem Yigzaw and Mr. Gashaw Mamo have formed a nice community, a home away from home. Together we had a joyful and interesting time modifying Ethiopian dishes with Swedish ingredients.

I respectfully thank my mother, brothers and sisters, brothers- and sisters-in-law for their encouragement, support and morals. I am also thankful to many friends at home.

Finally, my heartfelt gratefulness goes to my wife, Engdawork Zewdie, for your understanding, love, patience and shouldering all the responsibilities. It is your greatest encouragement and sacrifices that brought this work to a successful end.

This study was sponsored by Addis Ababa University. The Department of Analytical Chemistry, Lund University, is greatly acknowledged for the facilities made available for part of the works done in Sweden. The financial support for the scholarship was from the Swedish International Development Agency (SIDA), and material supports from the Swedish Natural Science Research Council (NFR) are also acknowledged.

ABSTRACT

Methods for sample preparation of the *s*-triazine herbicides and their degradation products in environmental water samples using supported liquid membrane (SLM) has been developed. The herbicide compounds were selectively extracted from the flowing donor solution into a porous polytetrafluoroethylene (PTFE) membrane impregnated with *n*-undecane, di-*n*-hexylether alone or with 25% 6-undecanone and 50% of *n*-undecane. After diffusion through the hydrophobic membrane the *s*-triazine herbicide compounds were irreversibly trapped into acidic acceptor phase of lower pH. The Effects of different experimental variables governing the efficiency of the extraction process have been thoroughly studied and optimized. These include the pH of both the donor and acceptor solutions, donor flow rate, ionic strengths and the analyte trapping conditions. Furthermore, the conditions for attaining maximum enrichment factor from the membrane extraction have been investigated.

Selectivity of the membrane technique developed was compared with one of the most frequently used technique for sample preparation of organic pollutants, solid phase extraction (SPE) in off-line mode. It was observed that the chromatograms obtained using the SLM methodology were cleaner, indicating the good selectivity of the extraction method. The liquid membrane serves as a barrier for interfering compounds, and neutral molecules may not be enriched. The limit of detection for the extracts using SLM technique was also comparable to that of the SPE. The possibility of determining the extraction efficiency, and thus the enrichment factor, from the donor waste was also investigated. The results obtained from both the acceptor concentrate and the donor waste agreed well, which in turn indicated efficient permeation through the liquid membrane thereby giving minimum memory effects.

The applicability of the method for extraction of the compounds of *s*-triazine herbicides from water samples containing complex matrices was studied by spiking analyte sample solutions both in reagent water and river water. The resulting chromatograms were not differing much, which also reflects the selectivity of the membrane extraction developed.

Water samples collected from Southern Ethiopian lakes were pre-treated and extracted in the same manner. There are a number of agricultural fields in this region where herbicides are periodically applied. Thus, the lake waters certainly contain considerable quantities of these residues and degradation products. The water samples, in which the phosphate buffer was dissolved to obtain neutral pH, were extracted at high donor flow rate, 5.0 mL/min, for 10 h. Low quantities of atrazine and terbutryn were identified in Awassa Lake, while only atrazine could be seen in the extracts from Chamo Lake. In both cases the concentration range of these compounds were estimated to vary from 0.02 to 0.05 μg/L. None of these herbicides or other *s*-triazine compounds could be detected in the extracts of the water samples collected from Abbaya lake.

A mixture of the parent compounds and the degradation products of the *s*-triazine herbicides were also successfully extracted in the liquid membrane system containing carriers, e.g., trioctyl phosphine oxide (TOPO). Low detection limits were obtained for the aqueous samples spiked in river water, water containing humic acid and also in urine. Effects of the flowing donor channel, while the acceptor concentrate collected, on analyte permeation was studied in detail and better analyte results were obtained than the stagnant donor system particularly for the more polar compounds.

1. INTRODUCTION

The human environment contains countless complex chemical and biological systems that can be transferred from a particular environment to another by natural processes and by man. For example, the different water bodies in human environment including lakes, rivers, underground and surface waters, certainly contain numerous different compounds including various dissolved organic and inorganic pollutants, different minerals, salts, etc. originating from industrial wastes, agrochemical processing, municipal discharges and natural processes, e.g., decay of plants. The presence of these and several varieties of their transformation products in widely different concentrations lead to a complicated chemistry. The general deterioration of the environment thus indicates the need of continuous assessment according to certain monitoring programmes.

The use of chemical pesticides for different purposes such as forestry management, railway, protection against infections with parasites transmitted to humans by insects, and against insects and weeds in agriculture, is very common all over the world today. Considering pesticide use for agricultural purposes, it has been a known fact that their effective use has improved the quality and quantity of food, and advances in pesticide technology have increased the ability to sustain and improve the health and well-being of the ever-growing human population. Unfortunately these chemicals are potential pollutants having deleterious effects on human health and environment [1, 2] when their concentrations are higher than certain limits. In this connection, Pio and Hall [3] put the following general statement: "pollution exists when the concentration of the substances are large enough to interfere with the normal use of the environment and the well-being of biota, especially man".

Not only pesticides, but also the solvents in which they are being dissolved, and chemical substances with which they are sometimes mixed have their own toxicity [4]. There are many pathways for exposure: in drinking water from contaminated wells, in food from household pesticide use, and from residues on plants as they are picked, or machinery as they are being handled or repaired, from pesticide drift as it is being sprayed, from spills during transport and from dermal exposure during mixing and application [5]. Farm workers within agricultural sectors, mixers, loaders, applicators, and others who directly handle agricultural chemicals are, therefore, at the highest risk for pesticide-related illness [6].

Most of the pesticides released to the environment are known and reported to be toxic, and the toxicity aspects have received considerable attention. Considering the water quality, for example, stringent regulations have, in the recent years, been issued by the legislation authorities [7]. The current European Union (EU) directive dictates that the concentration of individual pesticides should not exceed a maximum admissible concentration of 0.1 μg/L in drinking water for a single pesticide and 0.5 μg/L for a total pesticides concentration. Similar regulations have been set in the United States, by USEPA; for example, 3 μg/L for atrazine and 4 μg/L for simazine [7, 8].

Often, the residues of the pesticides in the environmental samples and their degradation products produced by a combination of hydrolytic, photochemical and microbial processes are found in various complex matrices at very low concentration levels. The nature of the samples mainly requires the use of pre-concentration and clean-up techniques to bring about the concentration of the compounds of interest to detectable levels by the conventional analytical systems [9]. To this end, numerous sample preparation techniques, besides the traditional solvent extraction, have been developed.

The applications of these technologies that are commonly and frequently used in environmental analysis will be discussed in section 1.3.

In the present work supported liquid membrane (SLM) technique has been employed for sample preparation of natural water samples from various lakes located in the southern region of the rift valley of Ethiopia. One of the most commonly used herbicide family, *s*-triazines and their degradation products in the lake waters, were extracted and successfully determined. Selectivity of the membrane extraction for these compounds has been evaluated by comparing with the solid phase extraction under similar experimental conditions.

1.1 Herbicides: Classification and Fates in the Environment

1.1.1 Background History of the Herbicides. Herbicides, (from Latin herba - means herb, plant and - cida means killer, murderer), is a chemical product which can be used to destroy or inhibit the growth of weeds, or unwanted plants at a given time and space. Weeds, besides drastically reducing the crop yields and quality, harbour insect pests, and serve as alternative hosts for crop-infesting fungi [10].

Herbicides have been in use since the mid-nineteenth century. Up to Word War II, a variety of inorganic acids and salts, e.g., iron sulphate, sulphuric acid, sodium chlorate, arsenicals and copper sulphate, were applied to control unwanted weeds. In 1930s the first organic chemical herbicide, 4,6-dinitro-*o*-cresol (DNOC) was introduced [11]. DNOC was used as an insecticide before its herbicidal effect was discovered. The introduction of DNOC was followed by the appearance, in the 1940s, of the substituted phenoxy acids, e.g., chlorinated phenoxyalkanoic acids, and the substituted ureas in 1951. These acidic herbicides are still among the most commonly used herbicides, with an estimated

production of 34 x 10^6 kg/year in USA in the mid 1980s. The triazine family of herbicides, which are the second largest group used today, have also appeared in the 1950s, and since then many other classes of compounds have been added for use as herbicides [12].

The control of weeds by means of herbicides has provided many benefits. Freeing agricultural crops from weed competition results in higher food production, reduced harvesting costs, improved food quality and lowered processing costs, contributing to an abundant supply of low-cost, high quality food [13]. Not only are billions of dollars saved through increased production and improved quality, but also costs of labour and machinery for weed control reduced, livestock is saved from the effects of poisonous weeds, irrigation costs are reduced and insect and disease control costs are decreased through the removal of host weeds.

Additional benefits due to appropriate herbicide use result as millions of people are relieved of the suffering caused by allergies to pollens and exposure to poisonous plants. Recreational areas, roadsides, forests and parks have been freed of noxious weeds. Herbicides have reduced storage and labour costs and fire hazards for industrial storage yards and warehouse areas. Modern herbicides even benefit the construction industry, where chemicals applied under asphalt prolong pavement life by preventing weed penetration of the surface [11-13].

1.1.2 Environmental Fates of the Herbicides. The fate of herbicides in the environment is influenced by many chemical, biological and physical factors. The principal transport and dissipation pathways include the following processes outlined and briefly discussed below. These processes can occur simultaneously although the environmental fates of most herbicides are controlled primarily by one or two of them.

Sorption. The retention or sorption of herbicides by organic and mineral soils, sediment constituents or aquifer materials is one of the most important factors in deciding the environmental fate a particular chemical [11]. The degree to which a herbicide is retained determines its concentration in the soil solution, and thus the amount that is available to be leached. Proposed sorption mechanisms would include cation- and anion-exchange, hydrogen bonding, ligand exchange and non-polar van der Waals interaction. Sorption is most often quantified in terms of the distribution coefficient, K_d, which is the ratio of the herbicide concentration in the solid, or sorbed phase, to that in the solution phase [14].

The amount of herbicide sorbed by a given soil is influenced by the properties of both the soil and the herbicide. Important properties related to the retention ability of the soil include clay mineralogy, organic matter content, soil pH and iron and aluminium oxide content, which in turn affect the cation and anion-exchange capability [14]. Important properties of a herbicide include charge, polarity, size and flexibility. The charge is frequently influenced by the soil pH because many herbicides are ionizable [15].

Leaching. In this context leaching may be defined as the transport of chemicals in the soil profile, as a result of the action of percolating water [11]. This occurs through two distinct mechanisms; diffusion and mass flow. Diffusion results from random molecular motion and occurs from areas of high to low concentration. Movement of herbicides through dispersion, which results from the effects of differential mixing and pore water velocities in the soil matrix, is usually included with the effect of diffusion. Transport by mass flow occurs through the movement of water in which the herbicide is dissolved or by the movement of suspended soil or sediment to which the herbicide is sorbed.

The degree to which a given herbicide is leached is significantly influenced by its sorption to soils and sediments. Therefore, factors of the soil and herbicide that affect

sorption also have an influence on the degree to which the herbicide is leached [15]. However, other factors related to the movement of water through the soil also influence this process. Important contributing factors include the amount and intensity of precipitation or irrigation, the soil texture, the tillage system and the soil topology.

Runoff. Runoff can be defined as water and any dissolved or suspended matter it contains that leaves a plot of land, field or small-cover watershed in the surface drainage [16]. Processes that influence herbicide leaching also influence the degree to which a herbicide is subject to runoff loss. Those factors that encourage the leaching of a herbicide generally reduce its loss in runoff and vice versa. Specific factors known to influence the amount of herbicide lost to runoff include rainfall timing and intensity with respect to herbicide application time, herbicide application rate, herbicide solubility in water, terrain slope, vegetative cover and soil texture [16, 17]. Factors related to the sorption of herbicides, such as mobility and persistence also influence its runoff susceptibility.

Volatilization. The susceptibility of a herbicide to transport through volatilization has received much attention, due in part to the realization that herbicides in the vapour phase may be transported large distances from the point of application. Volatilization loss in some herbicides can be as high as 80 – 90% of the total applied herbicide within several days of application. The processes that control the amount of herbicide volatilized are the evaporation of the herbicide from the solution or solid phase into the air and dispersal and dilution of the resulting vapour into the atmosphere [18]. These processes are influenced by many factors including herbicide application rate, wind velocity, temperature, soil moisture content and sorption of the compound to soil organic and mineral surfaces

Properties of the herbicide that influence volatility includes vapour pressure, water solubility and chemical structure [19].

Degradation or Transformation. Degradation or transformation of a herbicide by soil microbes or by abiotic means has a significant influence not only on the fate of the herbicide in the environment but also on the compound efficiency. Herbicides that are readily degraded may have a reduced environmental impact but their herbicidal effects may be compromized.

The degradation of a herbicide by soil microbes is primarily an enzymatic process in which cellular or extracellular enzymes break down the herbicide into smaller molecules that may be used by the organism as an energy or nutrient source. Microbes may also influence the degradation of a herbicide through change in soil pH or other soil properties [11].

Degradation of a herbicide by abiotic means may be divided into chemical or photochemical pathways. Herbicides are subject to a wide array of chemical hydrolysis reactions with sorption often playing a key role in the process. Chloro-*s*-triazines, for example, are readily degraded by hydrolysis [20]. The photochemical degradation of herbicides is dependent on the ability of the herbicide to absorb light at a wavelength between 285 and 400 nm [21]. Light below these wavelengths is generally absorbed by the earth's ozone layer and does not reach the surface. Light above 400 nm does not have sufficient energy to alter chemical bonds and thus does not photo degrade herbicides. Considerable work is being conducted to investigate the possibility of utilizing photochemical reactions to degrade waste herbicides [22].

Plant or Animal Uptake. For some herbicides, uptake and accumulation by a plant species is an important dissipation pathway and moreover it is the desired outcome for the target weed species. The principal point of entry for soil-applied herbicides in seedling and adult plants is usually the root system, although this may be complicated by volatilization of the herbicide and subsequent absorption through above-ground plant organs [11]. Foliage-applied herbicides must enter the plant through the leaf cuticle or the stomata. Successful penetration through the cuticle requires several complex steps and is significantly more complicated than uptake through the plant root system [23]. Distribution of the herbicide throughout the plant after uptake is primarily dictated by the mode of action of the herbicide. Some plants, e.g., corn, have the ability to detoxify a herbicide after uptake.

The extent to which a herbicide is accumulated in living organisms is known as bioaccumulation. If a compound is transferred in the food chain, with a concurrent increase in concentration, it is called biomagnification [11]. Uptake and accumulation of a herbicide by a living organism is controlled largely by the stability of the herbicide compound in water. Herbicides that tend to bioaccumulate and biomagnify are those compounds that are not readily soluble in water. Other properties that give an indication of the tendency of a herbicide to bioaccumulate or biomagnify include the octanol – water partition coefficient (K_{ow}) and the soil sorption coefficient (K_d). The octanol – water partition coefficient indicates lipophilic nature of the herbicide and thus its tendency to accumulate in fatty tissue. The soil sorption coefficient relates to the extent to which a compound will be retained by the soil and thus potentially removed from the food chain. It should be noted, however, that even herbicides that are strongly bound to the soil might be transported to an aquatic system by runoff and become available to bottom feeding fish or benthic organisms [11-13].

1.1.3 Major Classification of Herbicides. Herbicides are classified by several methods. The major ones are the following: (1) selective and non-selective, based on their actions on the weeds; (2) pre-emergence and post-emergence, based on the basis of their applications; and (3) the chemical classification.

1. Selective and Non-Selective (Total Killer). Many materials are conveniently classified according to the properties of the active ingredient as either selective or non-selective. Selective herbicides are those that kill some members of a plant population with little or no injury to others. An example is alachlor, which can be used to kill grassy and some broad-leafed weeds in corn, soybeans and other crops. Non-selective herbicides are those that kill all vegetation to which they are applied. Examples are paraquat and glyphosate that can be used to keep roadsides, ditch banks and rights-of-way open and weed-free [13, 22].

2. Pre-Emergence and Post-Emergence. These are application methods and the classification here is based on the properties of the herbicide chemicals. Some herbicides are effective only when applied to the soil and adsorbed into the germinating seedling, and therefore are used as pre-emergence herbicides, e.g., trifluralin [22]. Others, such as diquat, exert their herbicidal effect only on contact with plant foliage and are strongly inactivated when placed in contact with soil [12, 13]. However, the distinction between pre- and post-emergence is not always clear-cut. For example, atrazine can exert its herbicidal action either following root absorption from a pre-emergence application or after leaf absorption from a post-emergence treatment, and thus it can be used with either application methods. This may be an advantage in high rainfall areas where a post-emergence treatment can be washed off the leaf onto the soil and nevertheless can provide effective weed control.

3. Chemical Classification of Herbicides. Based on the chemical structures herbicides used today can be classified into the following ten different groups. These are: (1) phenoxyacid herbicides [24-26]; (2) substituted urea and uracil herbicides [27-30]; (3) bipyridinium herbicides [31]; (4) dinitroaniline herbicides [11, 32, 33]; (5) amides [18, 33]; (6) carboxylic and benzoic acid herbicides [12, 22, 34, 35]; (7) carbamate herbicides [11, 36-39]; (8) phenols and diphenyl ether herbicides [22, 40-44]; (9) heterocyclic nitrogen containing herbicides [11-13, 25, 45-48]; and (10) miscellaneous herbicides [11, 42, 49]. Further discussion on the properties of each group of these compounds is presented in Appendix.

1.1.4 Symmetrical Triazines and Their Degradation Products. Symmetrical triazines (*s*-triazines) are one of the largest classes of agrochemicals produced and the most commonly used herbicides for pre- and post-emergence weed control for variety of crops including green vegetables. A report on world pesticide market [50] indicates that about 30% of all herbicides produced are triazines. They are synthesized by substituting 1,3,5-triazine (or symmetrical, *s*-triazines) in the 2-, 4- and 6- positions. Their properties are determined mainly by the substituents at 2-position, generally chlorine (the general name ending with – azine), methoxy- (ending – tone) and methyl(alkyl)thio- (ending – tryn). The 4- and 6-positions are usually substituted with various aminoalkyl groups [45].

General Properties of s-Triazines. Triazine herbicides are solids, with a low vapour pressure at room temperature and have water solubilities in the range 5-750 mg/L. The water solubility and other physico-chemical properties of *s*-triazine derivatives are primarily determined by substituents in the 2-position [51, 52]. The properties of chloro-*s*-triazines considerably differ from those of the other two groups, whereas the methylthio-

and methoxy- derivatives behave very similarly. Substituents in positions 4- and 6- exert substantially smaller effects on the derivative properties.

As can be seen in Table 1.1, *s*-triazine herbicides are weakly basic substances. The basicity increases with the order of substituents in 2-postion; i.e., Cl < SCH_3 < OCH_3 [9]. Other substituents have less effect on basicity. However, the size and degree of branching of the *N*-alkyl groups at 4- and 6- positions have distinct but less pronounced effects. For example, tertbutylamino derivatives are the most basic representatives of the *s*-triazine series with a common C-2 substituent. In both cases, increasing basicity parallels increasing the electron-donating power of the substituents.

A remarkable stability of the *s*-triazine derivatives can be explained by the electronic configuration of the heterocyclic ring which resembles that of benzene to a certain extent. Both ring systems are stabilized by delocalization of their π-electrons which are spread over all the six atoms in the ring systems. A partial localization of electrons in the vicinity of nitrogen atoms is due to its greater electronegativity. As a result, the aromatic character of *s*-triazine is less pronounced than that of benzene.

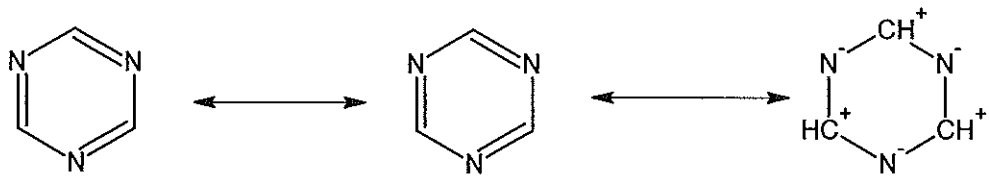
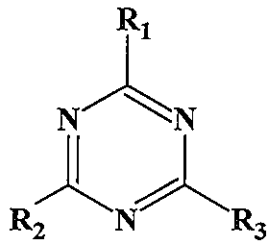


Fig. 1.1 The π-electrons in the *s*-triazine ring system.

The chemical behaviour and physical properties of the *s*-triazine derivatives are also greatly influenced by the delocalization effect in combination with inductive and mesomeric effects exerted by the substituents at C-2, C-4 and C-6 positions. The relative

Table 1.1 . Selected physical constants and substituents of the *s*-triazine herbicides [9, 45].



Common name	Substituents			Solubility in water (ppm) (20 - 25°C)	Melting point, °C	Absorption maxima, nm		Density, g/cm^3	pK$_a$ value
	R_1	R_2	R_3			λ_1	λ_2		
Simazine	Cl	NHC_2H_5	NHC_2H_5	5	225 - 227	222	263	1.302	1.65
Atrazine		NHC_2H_5	$NHCH(CH_3)_2$	33	175 - 177	222	263	1.187	1.68
Propazine		$NHCH(CH_3)_2$	$NHCH(CH_3)_2$	8.6	212 - 214	221	268	1.162	1.85
Terbuthylazine		NHC_2H_5	$NHC(CH_3)_3$	8.5	177 - 179	223	263	1.188	1.94
Cyanazine		NHC_2H_5	$NHC(CH_3)_2CN$	171	166.5 - 167			1.29	1.0
Simetone	OCH_3	NHC_2H_5	NHC_2H_5			222			
Atratone		NHC_2H_5	$NHCH(CH_3)_2$	1650		217			4.2
Prometone		$NHCH(CH_3)_2$	$NHCH(CH_3)_2$	750	91 - 92	219		1.088	4.2
Terbumetone		NHC_2H_5	$NHC(CH_3)_3$	130	123 - 124			1.081	4.6
Secbumetone		NHC_2H_5	$NHCH(CH_3)C_2H_5$	620	86 - 88			1.105	4.4

Table 1.1 Contd...

Common name	Substituents			Solubility in water (ppm) (20 - 25°C)	Melting point, °C	Absorption maxima, nm		Density, g/cm^3	pK$_a$ value
	R_1	R_2	R_3			λ_1	λ_2		
Simetryn	SCH_3	NHC_2H_5	NHC_2H_5					1.02	4.0
Ametryn		NHC_2H_5	$NHCH(CH_3)_2$	185	84 - 86	222		1.190	4.1
Desmetryn		$NHCH_3$	$NHCH(CH_3)_2$	580	84 - 86	221		1.172	4.0
Aziprotryn		N_3	$NHCH(CH_3)_2$	50	95				1.4
Dimethametryn		NHC_2H_5	$NHCH(CH_3)CH(CH_3)_2$	50	65			1.098	4.0
Metoprotryn		$NHCH(CH_3)_2$	$NH(CH_2)_3OCH_3$	320	68 - 70			1.186	4.0
Prometryn		$NHCH(CH_3)_2$	$NHCH(CH_3)_2$	48	118 - 120	223		1.150	4.3
Terbutryn		NHC_2H_5	$NHC(CH_3)_3$	58	104 - 105	223		1.115	4.3
Dipropetryn	SC_2H_5	$NHCH(CH_3)_2$	$NHCH(CH_3)_2$	16	104 - 106				4.3

electron deficiency of the ring carbon atoms makes them susceptible to nucleophilic attack, which is pronounced when electron-withdrawing substituents such as chlorine are attached to the carbon atoms. On the other hand, when the electron density of the carbon atoms is increased by electron-releasing substituents such as amino group, the rate of attack is impeded. These facts are clearly reflected by the high reactivity of cyanuric chloride (a precursor to the synthesis of all *s*-triazine herbicides), and the possibility of a stepwise substitution of its three chlorine atoms. Table 1.2 provides the important attacking reagent at the C-2 substituted chlorine of the *s*-triazine.

Herbicidally active dialkylamino-*s*-triazines behave as weak bases in aqueous solution. Protonation occurs preferably on the ring nitrogen atoms as has also been shown by ultraviolet [53] and nuclear magnetic resonance [54] studies. In general, these compounds have low solubilities in water; the 2-chloro-*s*-triazines being less soluble than the 2-methylthio and 2-methoxy analogs. Ward and Weber [55] investigated the aqueous solubility of these compounds over a wide pH range and indicated that aqueous solubility is practically independent of the pH of the solution. However, an increase in solubility is observed at pH values where strong protonation occurs [45], e.g., between pH 5.0 and 3.0 or lower for 2-methoxy and 2-methylthio-*s*-triazines, and at pH 2.0 or lower for 2-chloro-*s*-triazines.

Structural modifications of the substituents at either the 2- or the 4- and 6- positions of dialkylamino-*s*-triazines significantly affect solubility at all pH levels. Generally, increasing solubility is associated with increasing electron-donating capacity of the substituents at C-2, and decreasing size and branching of the *N*-alkyl groups in the 4- and 6- positions [45]. Differences in molecular geometry, and therefore in molecular polarity, can account for the higher solubilities of 4,6-asymmetrically substituted 2-chloro-*s*-

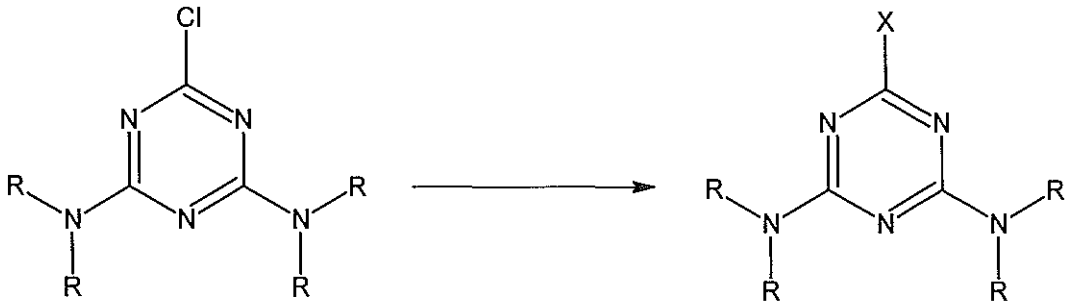
triazines such as atrazine compared to that of symmetrically substituted compounds such as simazine and propazine, Table 1.1.

Degradation Products of s-Triazine Herbicides. The *s*-triazine herbicides undergo different kinds of reactions such as hydrolysis at C-2 position, *N*-dealkylation at C-4 and C-6, and photochemical degradations at all the ring carbons. The processes governing these degradations can be either chemical, photolytic or microbial, or a combination of them.

The C-2 position, in particular, is a susceptible site for nucleophilic attack, and this effect is much pronounced for the 2-chloro-*s*-triazine family of herbicides. A number of chemical systems that can readily replace chlorine are summarized in Table 1.2. Among these, the extremely reactive group of quaternary nitrogen compounds, e.g., trimethylamine, are very important intermediates for further reaction with other nucleophilic agents to obtain the respective end product more readily than direct reactions with the 2-chloro-*s*-triazine itself. Most studies revealed that both in acidic and basic conditions a hydrolysis takes place to give the hydroxy product [56-58].

In natural waters containing sediment [59], humic and fulvic acids [57, 60], both in acidic and basic conditions, only the hydroxy product was observed as a major degradation product. It is suggested that formation of this product may be via a sediment catalysed process. Khan [61] reported that the hydrolysis of atrazine at neutral pH is slow in water containing 0.5 mg/mL of fulvic acid. Slow conversion of atrazine to the deethylated products, < 2.2%, was also noted by Geller [60] with hydroxyatrazine as the major product under these conditions.

Table 1.2. Nucleophilic reactions affecting the chlorine atom of 2-chloro-4,6-bis(alkylamino)-*s*-triazines.



R can be H or alkyl group

Attacking reagent	Resultant substituent	Attacking reagent	Resultant substituent
H^+ or OH^-	-OH	$N(CH_3)_3$	$(N(CH_3)_3)^+.Cl^-$
SH^-	-SH	NH_2NH_2	$-NHNH_2$
Alkyl-OH, OH^-	-O-alkyl	KCN	-CN
Alkyl-SH, OH^-	-S-alkyl	NaN_3	$-N_3$

Degradation in Higher Plants. The *s*-triazines are deactivated in tolerant plants by any one or all of the following three different biotransformation pathways [62], *viz.*, benzoxazinone-mediated hydrolysis, *N*-dealkylation and glutathione conjugation. In the following paragraphs a short discussion on these mechanisms will be presented.

Plants tolerance to 2-chloro-*s*-triazines is associated with displacement of the chlorine at the 2-position of the heterocyclic ring with a polar substituent. Hydrolysis of these herbicides to their hydroxy derivatives, occurring primarily in the root, has been classified as an important mechanism for detoxification [63-65]. This reaction is catalyzed by naturally occurring cyclic hydroxamic acids, found in numerous plants as glycosides and known as benzoxazinones [45, 64, 66]. The specific benzoxazinone responsible for the hydrolysis of atrazine and simazine is 2,4-dihydroxy-7-methoxy-1,4,*2H*-benzoxazin-

3(4H)-one, abbreviated commonly as DIMBOA. A model compound, Fig. 1.2, is used to explain the catalytic detoxification mechanism. Hydroxyatrazine and hydroxysimazine, thus obtained, are non-phytotoxic metabolites and they have not been reported to undergo further metabolism in plants [62-67].

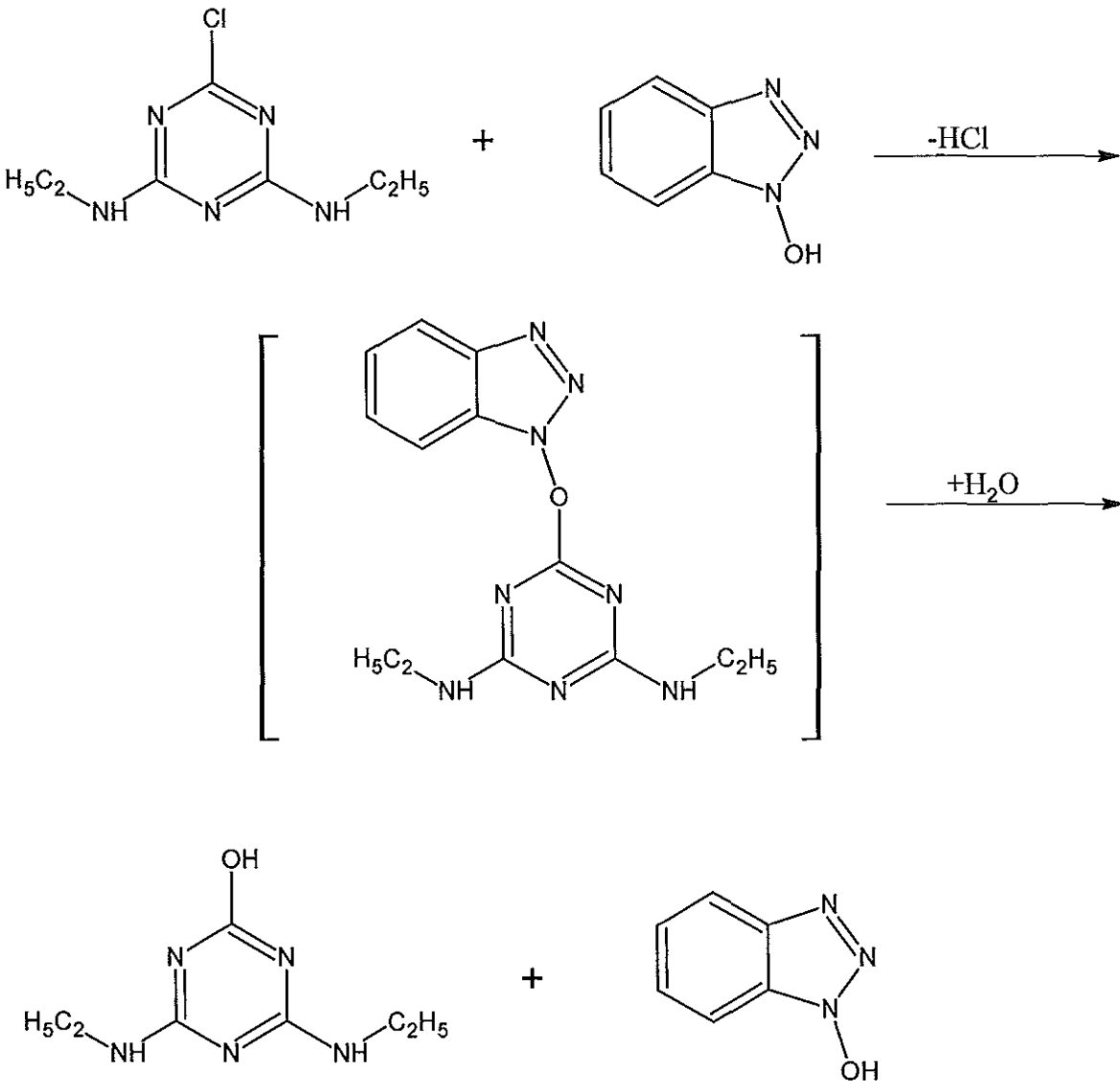


Fig. 1.2 Mechanism for detoxification of *s*-triazines in corn.

N-Dealkylation of *s*-triazine herbicides is also generally viewed as a deactivation reaction. However, it has been observed that in corn, *N*-dealkylation of the alkylamino side chains appears to be only a partial detoxification reaction, since the *N*-deethyl metabolite of atrazine is still phytotoxic [62]. *N*-dealkylation of the alkyl side chains appears to occur to some extent in all higher plants and is considered important for explaining the intermediate susceptibility of broad-leaved crops [67]. In general, *N*-dealkylated metabolites are not terminal products in plants. Subsequent conjugation or further oxidation followed by conjugation is needed for complete detoxification of the metabolites in higher plants [62].

Laboratory-scale *N*-dealkylation reaction of the *s*-triazine was carried out by heating the compound to 240-250°C. Evolution of olefins as a monomolecular thermal *N*-dealkylation was noted by way of a cyclic transition state (Chugaev Reaction) [45], Fig. 1.3. Ultrasonication of atrazine and propazine, suspended in water, also produced the corresponding olefins with ratios similar to those obtained in the thermal *N*-dealkylation [68]. Mills and Thurman [69] and others in the same group [70] have recently described results of the field dissipation studies for 2-chloro-*s*-triazines, and these results are also in agreement with this mechanism for the order of substituents removal.

Glutathione conjugation is another important mechanism of detoxification of 2-chloro-*s*-triazine herbicides in tolerant plants. The tripeptide glutathione (γ-Glu-Cys-Glu) is widely distributed in the cells of several biosystems such as mammals, insects, plants and microorganisms [71], usually present in its reduced form, abbreviated commonly as GSH. The sulfhydryl group (SH) provided by cystine is the most important structural feature in the interaction of GSH with xenobiotics.

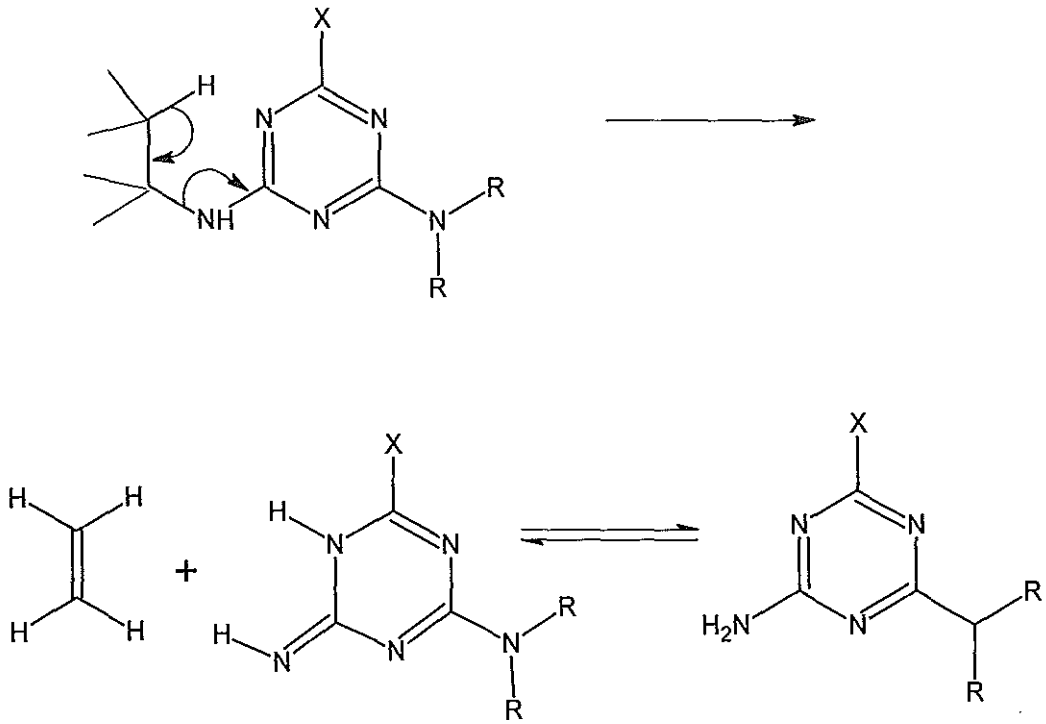


Fig. 1.3 Mechanism for *N*-deethylation of *s*-triazine herbicides containing ethyl moiety at 4- and/or 6-position(s) of the ring system.

The glutathione conjugation has been established as one of the major deactivation pathway of selected herbicides [67, 72, 73], after the first report on deactivation of atrazine by corn [71]. It should be noted that in biotransformation studies, the term conjugation describes the *in vivo* reaction of a chemically reactive metabolite of a given xenobiotic with an endogenous substrate to form a new compound (conjugate) of higher molecular weight.

The glutathione conjugates of the *s*-triazine herbicides in corn, for example, are non-phytotoxic [67]. In other plants, this conjugate is catabolized further to form a terminal product which is non-phytotoxic [62, 67].

1.2 Environmental Water Sampling Strategies

Four basic elements must be considered in solving analytical problems in environmental studies: (1) sampling; (2) sample preparation for analysis; (3) sample analysis; and (4) reporting [74, 75] (Fig. 1.4).

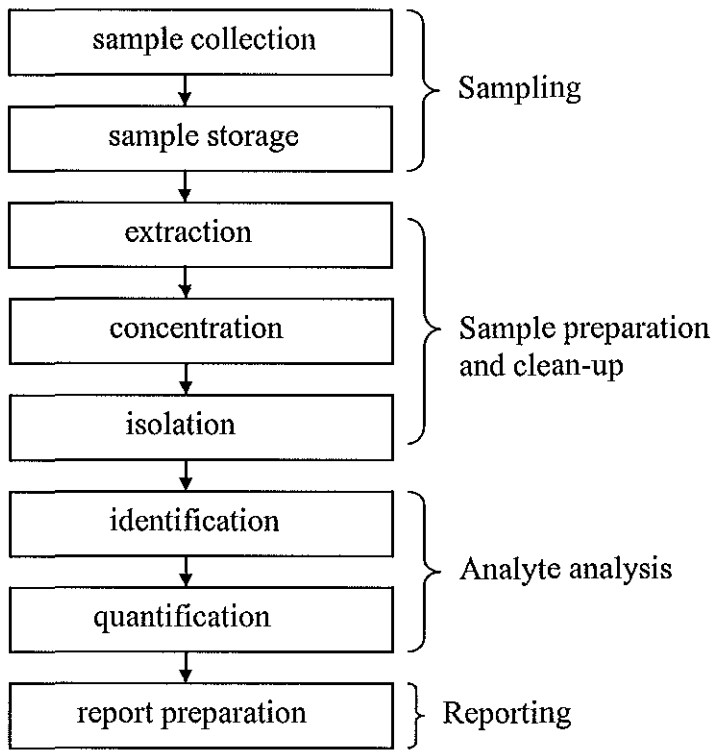


Fig. 1.4 Flow diagram of the general procedure for sample processing in environmental applications.

These elements are both independent and system interactive. Sampling, in particular, and sample preparation for analysis are often the least considered steps in solving analytical problems in spite of the fact that they may be the most significant factors and the largest sources of error. Results from badly taken samples are mainly worse than

no results, since those results may lead to incorrect conclusions and misplaced actions [76]. In this thesis, sample preparation and analysis and sample clean-up methods, with some specific applications, will be discussed in the subsequent sections, while sampling, with particular emphasis on environmental waters, is the topic of interest in this section.

Sampling, in general, can be defined as the act or process of selecting a representative portion of material, in some manner, to represent a larger body of material, for testing or analysis [76]. It is the first step in any environmental analysis, which primarily determines the quality of any data produced by analytical system. Exact sampling procedures should be based on the objectives of the analysis and the nature of the analytes. Sampling location, time of sampling and the frequency at which samples are collected are some factors that must be considered when developing sampling strategies [77].

The more general objectives of sampling is to collect representative samples of the whole so that the final results of the chemical analysis represent the entire system that it is intended to represent. This requires a detailed plan as how to carry out the sampling process. The following list includes some of the necessary criteria for effective sampling strategy in environmental analysis [78-80], (can also be used as a checklist during sampling). Some of these include: objectives of the data quality; the type of equipment required or available; the experience or training of the individual involved for the type of sampling required; list of analytes and level of detection; availability of quality control samples; the sampling approach; the data analysis method available; number of samples, sites, methods, control sites, etc.

In addition, it is also necessary to collect appropriate blank samples. Blank samples are matrices that have no measurable amounts of the analytes of interest. The ideal blank

will be collected from the same site as the sample, but will be free of the pollutants. In practice, this may not be simple.

Sample collection of natural water seems easy since it appears homogeneous. However, natural water is heterogeneous, both spatially and temporally (variation with respect to time), making it very difficult to obtain representative samples. Stratification within oceans, lakes and rivers is common with variation in flow, chemical composition and temperature. Furthermore, due to heavy precipitation (snow or rainfall) and seasonal changes, variation with respect to time can also occur.

Field sampling of natural water for analysis of pesticide residues is usually performed using either grab sampling or continuous sampling techniques. In grab sampling, a single sample increment is taken at a specific site. Such samples represent only the conditions at the time of collection, and should not be used as the sole basis for a decision about pollution abatement. However, some sources are quite stable in composition, and may be represented well by single grab samples. The quality of grab sample can be improved by collecting a series of smaller samples in a single container. The mixing of the collected sample averages the variation in sample composition. These types of samples are called composite samples. When a time factor is being taken into consideration, grab samples are collected in suitable intervals, chosen according to the expected changes. In general, composite samples reflect the average characteristics during the sampling period, and in most cases a 24 h period is a standard [79].

Continuous sampling, on the other hand, is a collection procedure where samples are constantly withdrawn from the source or parent population and accumulated for collection at a later time [81]. In principle, a continuous sampling is a technique that should give representative sample of the whole. This statement is especially true for flowing waste water when the pumping rate is proportional to the stream flow. However,

this technique generally requires large collection container, and it is also highly demanding. The instruments performing such sampling are also expensive. The analytes being collected should be very stable particularly when longer sampling period is needed.

An example of a continuous sampling has been demonstrated using supported liquid membrane extraction technology, see also section 1.6. The sampling method has been evaluated using 2-methyl-4-chlorophenoxyacetic acid [82], other phenoxyacetic acids and structurally related herbicides [83] for a sampling period of 24 hours, Fig. 1.5.

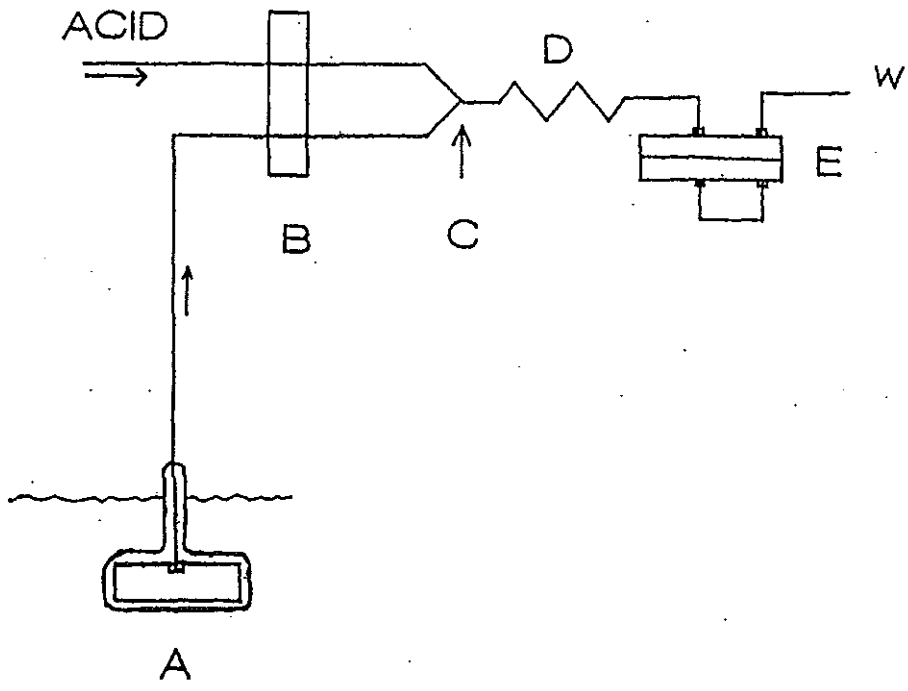


Fig. 1.5 Set-up for integrated field sampling: A - sampling compartment; B - peristaltic pump; C - confluence point of the sample stream; D - mixing coil; E - membrane separator; and W - waste [82].

The sampling procedure of the set-up is as follows: water sample from the sampling point (A), about 10 cm below the surface, is pumped using a peristaltic pump (B)

with standard PVC manifold pump tubing to the confluence point (C). The sampling compartment (A) consisted of the sampling tube immersed in a draining tube, surrounded by nylon net with 100 μm mesh size. The draining tube is fixed parallel to the stream and kept at a constant depth. The sample was then mixed with appropriate solution, an acid in the reported studies, in a mixed coil (D), and was transported through the membrane separator (E), and finally to the waste (W). Further description of the apparatus and various dimensions including the tubing are given in ref. 82.

With this sampling methodology, referred to as integrated field sampling, besides continuously sampling for a specified time, an enrichment of the sample on the other side of the liquid membrane takes place simultaneously. Moreover, the sample extracted under this condition does not require any clean-up procedure, since the liquid membrane is very selective in its action of permeation and also the analytes are separated from the possible degrading microorganisms in the water [84, 85]. The triple functions of the set-up, *viz.*, sampling, pre-concentration and sample clean-up, are straightforward to describe. It should be noted that careful selection of the sampling points, where maximum mixing occur, is crucial in order to perform optimum sampling. Multiple point sampling can also be performed to optimize the reliability of the sampling technique. The properties of the analytes to be sampled should be well known ahead to take precautions for the possible decomposition since the analytes are necessarily left in the acceptor solution for longer time.

1.3 Sample Preparation Methods with Applications to *s*-Triazine Herbicides in Environmental Waters

Sample preparation in analytical separation and identification systems is an important area, and, in recent years, it has gained relatively better attention. This may be because errors in analytical laboratories often originate from poorly designed sample preparation steps. Furthermore, since most analytical instruments are incompatible with matrices containing large constituents of interferences, and since the amount of pollutants, particularly in natural waters, are very low, sample pre-treatment and pre-concentration are unavoidable tasks. A well-prepared sample is less prone to degradation or association because of the absence of various constituents of the sample matrices. Today, there are lots of available and emerging techniques for preparation of samples from different origins, e.g., biological, environmental, foodstuff and biotechnological mixtures. The major requirements for such techniques are simplicity, speed, high recovery, precision and robustness. Due to stricter regulations concerning the use of organic solvents, such technique should use a minimum quantity of solvents. The need for handling hazardous chemicals and reducing labour require that these systems be on-line connected to the analytical instruments. These and other additional aspects of sample preparation technology have been reviewed by several workers [77, 86-91]. In this thesis some of these techniques that are relevant to environmental analysis will be discussed in some depth with special attention to their applications for sample handling of the *s*-triazine herbicides.

1.3.1 Liquid-Liquid Extraction. Liquid-Liquid Extraction (LLE) is a classical sample preparation method where the extraction sample is distributed or partitioned between two immiscible solvents in which the analytes and matrices have different solubilities. Various

reviews are available in the general literature on sample preparation using LLE [77, 78, 86, 87, 92]. Extraction is performed by vigorous shaking of the container, a bottle or separatory funnel, so as to create the possibility for a larger contact area between the solvent and the sample. During the process the analytes are transferred from the aqueous phase to the organic phase. Efficient phase separation can be achieved by allowing the extraction system to stand before collecting the organic phase, which is typically done using a suitable drying agent, e.g., anhydrous Na_2SO_4. Further clean-up and/or concentration steps are the usual practices before introduction into the analytical instruments.

LLE has an advantage in a great variety of extraction systems. It has been thoroughly studied theoretically, optimized and widely tested in practice and thus offers many possibilities for a fine-tuning of the extraction efficiency and selectivity by variation of the experimental conditions. These include adjusting of the pH of the aqueous phase, changing the organic solvent and using ion-pairing or derivatization reagents. The distribution coefficient can also be increased by addition of salt to the aqueous phase or by repeated extractions [92, 93]. Other advantages of LLE as a sample preparation technique are the simplicity of the method and requirement of minimum instrumentation and materials.

There are, however, several disadvantages with conventional LLE, which include: (i) the use of a relatively large quantity of organic solvents that are toxic not only to the individuals involved, but also to the environment. Since most of the analytical grade solvents are expensive, their use will increase the cost of analysis. (ii) It is also labour intensive, tedious and time-consuming because the extracts mainly require manual handling and further pre-concentration. (iii) Formation of emulsion is often a problem, even though this can be removed by filtering through a plug of glass wool [92], by

centrifugation, refrigeration, salting out or by addition of a small volume of organic solvent [75]. (iv) There is also a risk of loss and contamination, and (v) it is difficult to automate.

Due to these inherent limitations of the technique, much improvement has not been made to use it in a more flexible way. However, some improvements have been seen lately, and one of these is the micro-LLE, which is a miniturized version of the conventional LLE [94, 95]. For instance, micro-LLE has been applied to the extraction of 60 mL of ground water samples into 1 mL hexane to determine atrazine and acetanilide compounds [96]. Another development is the on-line coupling of LLE with flow injection for extraction of water samples [92, 97]. In this application, the water sample was segmented by extraction solvent and passed through a 6 m extraction coil. After passing a phase separator, the extract was further passed on-line, via a complicated interface, to gas chromatography (GC) for concurrent solvent evaporation injection. The possibility of coupling segmented flow extraction to a high-performance liquid chromatography (HPLC) was also investigated for extraction of the phenoxy acid herbicides and organophosphorus insecticides [98]. The drawback of the approach is the phase separation. Microporous membrane liquid-liquid Extraction (MMLLE) is a better approach since the aqueous and organic phases are separated by a support. See also section 1.6.

Sample preparation of *s*-triazine herbicides from environmental water samples commonly used dichloromethane [99] as extraction solvent. This is recommended by the US environmental protection agency, EPA, for LLE of triazines (upto 1 L water sample and pH adjusted to 7) [100]. Its replacement by a mixture of ethyl acetate and cyclohexane (1:1) was recently recommended for toxicological and ecological reasons [101]. Furthermore, LLE as sample preparation technique for the *s*-triazine herbicides in environmental waters has been documented in various papers. The parent compounds of all classes of *s*-triazines were extracted from natural water samples, and the extracts were then

separated and detected using GC-NPD (GC with nitrogen phosphorus detector) [99, 102, 103], HPLC-UV (HPLC with ultraviolet) [103, 104] and GC-MS (GC with mass spectrometry) [105, 106]. The degradation products of *s*-triazines are usually extracted from soils and sediments in a mixture of water-methanol solution [107], which is followed by concentration of the aqueous extract prior to HPLC analysis [108]. A very low detection limit, < 100 ng/L, was reported by Grandet *et al.* [109] for the GC-NPD determination of *s*-triazines and their metabolites in drinking water after LLE. The non-symmetrical triazine metribuzin, has also been extracted from soil into methanol - 0.01 M $CaCl_2$ (4:1, v/v) followed by a solid phase extraction, SPE, pre-concentration step [110].

1.3.2 Solid Phase Extraction. Solid phase extraction (SPE) is a method for sample preparation that concentrates and purifies analytes, onto a solid phase, from solutions containing complex matrices, followed by elution of the analytes with a solvent appropriate for instrumental analysis [111]. The principle of trace enrichment in SPE is based either on analyte adsorption on active carbon, graphitized carbon black and organic polymer [112-115] or on analyte partition between the sample solution and chemically modified HPLC phases, such as C8 or C18 [116-119]. The solid phases used in SPE are packed in tubes, small columns or immobilized in membrane disks [9]. The use of the latter is increasing, as the membrane disks permit higher sample flow rates and the danger of extraction of impurities from polymer tubes and frits is eliminated [9].

Extraction of trace quantities of analytes in various sample components by SPE involves the following steps [52, 111, 116]: (1) Sample pre-treatment – this may include dilution of the sample to reduce viscosity, pH adjustment, filtration, etc. It may also be required to minimize analyte-matrix interaction. (2) Column activation/pre-equilibration – prewetting the column material causes opening of the hydrocarbon chains, thus increasing

its surface area, which is followed by conditioning with small volume of HPLC grade water. (3) Sample loading – percolation of the sample solution at optimum flow rate. (4) Interference elution or washing – removal of potential interferents by washing the column with solvents of various strengths depending on the nature of separation. (5) Analyte elution or desorption – removal of the enriched analyte with a solvent which is compatible with the final analytical system.

Selection of the appropriate desorption solvent is the critical step in the SPE of *s*-triazine herbicides from environmental sources. Various solvents and their mixtures have been tested for use with C18 phase, e.g., ethyl acetate, hexane and light petroleum [120], mixtures of light petroleum with toluene at ratios of 2:1 [112] and 1:1 [121], dichloromethane with methanol (6:4) [113] or with acetonitrile (6:4) [114], pure acetonitrile [122] and methanol [123]. The highest recovery (close to 100%) has been obtained for ethyl acetate [120, 124-126].

Selective separation of the analytes from samples of varying origin can be achieved by using proper sorbents which can yield maximum recovery under given experimental conditions. Today, different sorbents are available to be used in reversed-phase, normal phase, ion-exchange or size-exclusion mode. Common sorbents for SPE and various separation modes are summarized by Hennion [87] and Thurman and Mills [111]; see also Table 1.3.

The separation has been employed both off- and on-line coupled to the analytical instruments [93, 127]. With off-line applications small disposable cartridges (100 to 1000 mg) are normally used. Before introduction to the LC-system the volume of organic solvent used for analyte desorption must be reduced. When the LC-system is connected on-line to the SPE, the mobile phase can be used for elution of the analytes from the sorbent.

Table 1.3 Common sorbents used in solid phase extraction [87, 111].

Separation mechanism	Sorbent	Structure	Application and elution solvent
Reversed phase	Octadecyl	$-(CH_2)_{17}CH_3$	Non-polar and weakly polar analytes, e.g., PCBs, PAHs and herbicides from natural water; analytes in organic solvents.
	Octyl	$-(CH_2)CH_3$	
	Ethyl	$-CH_2CH_3$	
	Cyclohexyl	$-(CH)_2$-cyclohexyl	
	Phenyl	$-(CH_2)_3$-phenyl	
	Graphatized Carbon	Aromatic carbon throughout	Non-polar to relatively polar analytes, e.g., polar pesticides in organic solvent.
	Copolymers	Styrene-divinyl-benzene	Non-polar to medium polar in organic solvent for elution
Normal phase	Cyano	$-(CH_2)_3CN$	Analytes in aqueous or organic solvents, e.g., drugs and metabolites in body fluids.
	Amino	$-(CH_2)_3NH_2$	
	Diol	$-(CH_2)OCH_2CH(OH)CH_2OH$	
	Silica gel	-SiOH	Isolation of low to moderately polar species from non-aqueous solutions.
	Florisil	Mg_2SiO_3	
	Alumina	Al_2O_3	
Ion-exchangers	Amino	$-(CH_2)_3NH_2$	Cationic and anionic organics, e.g., phenols, phenoxyacids, anilines, etc., and pH adjusted H_2O used for elution.
	Quaternary amine	$-(CH_3)_3N^+CH_3$	
	Carboxylic acid	$-(CH_2)_2COOH$	
	Aromatic sulphonic acid	$-(CH_2)_3$-phenyl-SO_3H	
Size exclusion	Wide pore hydrophic	$-(CH_2)_3CH_3$	They consist weak cation and anion exchangers, used for large biological molecules to be included in the pores for ion exchange.
	Wide pore ion exchanger	-COOH	

The sorbents in most on-line applications are packed in a small stainless steel precolumn (10 – 20 mm long with I.D. between 1 and 4.6 mm). The use of such short column in environmental samples has been described in several publications [128-132]. A typical set-up for on-line SPE is shown in Fig. 1.6 [30, 88].

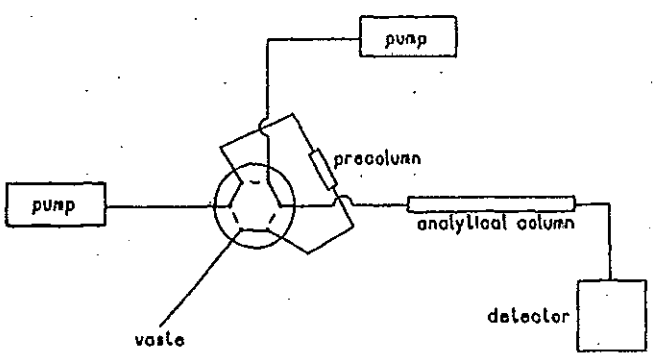


Fig. 1.6 Typical set-up for on-line SPE [88].

On-line coupling of the sample preparation by SPE to capillary-GC is an alternative approach [133]. GC-analysis is superior to LC-analysis in some aspects, because of the use of several sensitive detectors, high column efficiency and high analysis speed. However, the requirement for introduction of only small sample volumes of non aqueous solutions in capillary-GC, limits its use in environmental water analysis. The development of on-column injection techniques permitted injection of large volume of the SPE enriched sample to capillary-GC [93]. Retention gap injection technique has been used in several pesticide applications in combination with various detection systems – e.g., NPD [134] and MS [135] - yielding very low limits of detection.

The idea of the solid phase microextraction (SPME) has been coined by Pawliszyn and co-workers using sections of fused silica optical fibers, both uncoated and coated with liquid and solid polymeric active phases [136]. It was named SPME for the first time when

the coated fibers were incorporated into a microsyringe [137]. Sample extraction with SPME is performed by dipping the sampling syringe fiber into the aqueous solution containing the analytes. The analytes are then thermally desorbed directly in the GC-injector. The applications of the technique have been documented in several publications [138-141] and in the book on theoretical and practical aspects of SPME [142], recently written by the inventor of the technique.

The application of SPE has many advantages over traditional sample preparation methods for several reasons, including: its ability to pre-concentrate traces of analytes from very dilute solutions, provided that sufficiently large sample volumes are available, cost saving in terms of personnel, less risk of sampling and handling errors, low contamination risk, increased sample throughput and increased safety. Some drawbacks encountered in the SPE techniques have also been noted by several workers. The major ones are lack of selectivity, small breakthrough volumes for polar compounds and clogging of the frits in the pre-column. In particular, when SPE is applied for extraction of *s*-triazines, several factors governing the efficiency are indicated by Pacakova *et al.* [9]. When plastic cartridges are used for extraction [120] there can be a danger of contamination from possible leaching of the column, as the trapped triazines are desorbed by organic solvent and the final analysis is carried out by GC with electron capture detector (ECD). Thus, glass micro-columns have been recommended.

SPE in environmental water analysis has been reviewed by several workers [87, 116, 118, 128, 143]. Its use for trace enrichment of *s*-triazine herbicides, off- and on-line, in various environmental, biological and food samples is also available in literatures [9, 52, 143]. The technique has become rather popular in providing quantitative recovery of triazine herbicides from aqueous samples, and has been introduced into some standard methods [144].

Residues of *s*-triazine herbicide compounds have been pre-concentrated by SPE from natural (river, lake and ponds), drinking and rain water samples, and trace quantities have been determined using GC-NPD [145-147], HPLC-UV [114, 120, 130, 145, 148, 149] and GC-MS [150, 151]. In almost all these cases, water samples ranging in volume from 200 to 1000 mL have been extracted either with cartridges or membrane disks. There are also several works reporting the simultaneous extraction and determination of the parent *s*-triazine compounds and their degradation products, particularly deethylatrazine and deisopropylatrazine [70, 152-155]. For the degradation products, the recovery is often less than the expected values. The recovery decreased with increasing sample volume [70, 156] particularly in natural waters. This may be attributable to the presence of humic substances [52]. The increase in breakthrough volume by increasing the sample flow rate is also very small [150]. To improve the recovery of a SPE process for sample containing parent compounds and the degradates, use of a mixed-mode cartridge has been recommended. For example, a C18 phase and a strong cation exchanger [107], graphitized carbon black [114, 157], reversed phase material PRP-1 with Porapak G [122], etc., demonstrated better recovery. Another possibility is the use of a double trap [146], containing a LiChrospher RP-18 material in the first column, which retains all lipophilic compounds present in the sample and exhibits a very low retention for triazines, and a specifically developed reversed-phase material, e.g., Supelclean Envi-18, in the second compartment, where the triazines are pre-concentrated and subsequently eluted onto the separation column.

Additional advantages of the automated SPE for sample preparation in environmental analysis that were reported in recent reviews [9, 52] include: shortening of the analysis time by 70%, 5% better precision, increase in sample throughput by 200% and a significant reduction in the exposure of technicians to solvent fumes.

1.3.3 Supercritical Fluid Extraction. The supercritical state of a substance is reached when it is above the critical point (i.e., when the pressure is above the critical pressure and the temperature is above the critical temperature). Beyond the critical point, the densities of the gas and liquid phases become identical and the distinction between the two phases disappears; the substance becomes a supercritical fluid. The fluid is neither a gas nor a liquid but possesses properties of both. Viscosity is low and diffusivity is high, as for the gas, while the solvating power is similar to that of liquids.

Supercritical fluid extraction (SFE) is thus the technique that employs this fluid phase to effect solubilization of solutes. Several advantages have been gained by making use of the supercritical fluid because of the unique physical properties that these fluids possess. They allow more efficient mass transfer of the solutes from sample matrix than the liquid solvents, which can be attributed to the lower viscosity and higher diffusivities [158]. Their solvent power can also be adjusted, by varying the density, i.e., the pressure and temperature, so as to accomplish selective extraction of the analytes, which are mainly free from any contaminating solvent. This reduces the need for clean-up of the extracts and facilitates quantification of the target analytes. Excluding a clean-up step minimizes the analysis time and the number of error sources [159, 160]. Moreover, proper choice of the extraction fluid allows conducting the extraction at low temperatures, a feature which makes SFE particularly amenable to the treatment of thermally liable compounds [158].

A number of compounds have been used as supercritical fluid in SFE applications. CO_2, N_2O, NH_3, SF_6, chlorofluorocarbons (CFCs, "freons") and recently water [52, 160]. Most of them have some kind of drawback that includes: being reactive, toxic, and explosive and having high critical temperature and/or pressure. The most frequently used extraction fluid is supercritical carbondioxide, CO_2. It has inherent benefits such as low

cost, availability in high purity, low-toxicity and low reactivity, mild critical conditions and compatibility with detectors [52]. CO_2 is an excellent extraction fluid for non polar analytes, reasonably good for moderately polar analytes while the solubility of polar analytes is poor. It can be enhanced by addition of modifiers or co-solvents. The commonly used modifiers are ethanol [161, 162], methanol [161-163], isopropanol [163-166], and acetone and ethyl acetate [160]. Addition of the modifier can be done either by mixing with the carbon dioxide in a gas tank or by using a separate modifier pump. It can also be added directly to the sample in the extraction cell. In this case, a static extraction step must be performed to allow the modifier to be distributed in and contact the sample properly [160, 167]. For many environmental applications low concentrations of modifiers (~1%) was observed to be effective [168], since modifiers are also competing for the active sites of the packing material. Modifiers could also cause increased solvation or swelling of the stationary phase. One more drawback generally noted with addition of modifiers is that the resulting extracts are dirtier than the extracts with pure carbon dioxide [160, 169], which can be attributed to the presence of co-extracted matrix components. Thus, for such dirty extracts, additional clean-up step may be necessitated prior to the final analysis.

SFE has been applied to a wide range of samples from numerous areas of application. Three important areas of application of analytical SFE are: environmental analysis, polymer characterization and food analysis [170]. Other areas where SFE has been less frequently but successfully applied include pharmaceutical analysis [171], natural product research [172, 173] and fuel characterization and classification [170]. According to the recent report by Valcarcel and Tena [174], environmental analysis is the major area of SFE application which comprises about 41%.

The technique is primarily applied to solid samples. Liquid samples can also be extracted with supercritical fluids, but must first be immobilized on solid sorbents, e.g., in

combination with solid phase extraction precolumns [156], and disks [9, 175-177] for extraction of different pesticide groups from water samples. Membranes have also been used to immobilze liquid samples in flow systems for environmental applications [9].

SFE of *s*-triazines from solid samples has been described in several papers [159, 178-181]. The efficiency of the extraction process depends directly on their solubility in supercritical fluid, and the latter in turn depends on the fluid density, i.e., the *s*-triazine herbicides vapour pressure and on the kind and content of modifiers [9]. It has also been described in several reviews [9, 52, 182-184] that the recovery for triazines with SFE increases with increasing pressure and when methanol is added as a modifier; an increase in temperature somewhat decreases the recovery. The modifier must be added directly to the extraction cell and its effect increases with increasing polarity of the solutes. The soil type and the extraction time exert small effects.

Sample preparations of *s*-triazine herbicides in drinking and natural waters have been reported [103, 185, 186]. Alzaga *et al.* [103] spiked atrazine and simazine (0.02 – 7 ng/mL) in drinking water, stirred the content and freeze-dried. The sub-samples (2 – 3 g) of the freeze-dried residue were introduced into the 5 mL extraction cell. The final analysis was done by GC-NPD and recoveries ranging from 10 to 98% were obtained depending on the extraction conditions. Barnabas *et al.* [185] reported the selective extraction of organochlorine pesticides (OCPs) from urons and triazines, in aqueous samples. The aqueous samples were pre-concentrated on solid phase extraction media (Empore® disks) prior to SFE. It was found that with CO_2 only, the OCPs could be quantitatively extracted (recoveries ranging from 85 – 100%) with no significant loss of the other herbicide compounds. When methanol (10% v/v) - modified CO_2 was used to extract the herbicides from the same disk, quantitative recovery was obtained (87 –100% for chlorotriazines and 86 – 90% for urons). This selectivity was further enhanced by the method of separation-

detection used, i.e., GC-MSD for OCPs and HPLC-UV for both chlorotriazines and uron herbicides.

The degradation products along with the parent *s*-triazine compounds have also been extracted utilizing the SFE-technique, separation and detection of which were performed with GC-MSD [156], GC-NPD [179] and HPLC-UV [152, 159, 181]. One example will be considered here, where the sample handling of atrazine with two of its metabolites, *viz.*, 2-hydroxyatrazine and deisopropyl-deethyl-2-hydroxyatrazine (DDHA) were investigated using SFE [159]. Addition of methanol was necessary to obtain quantitative recovery for atrazine and 2-hydroxyatrazine. DDHA was only poorly recovered, however, under these conditions (20%). Addition of 2% water in methanol was found to increase the recovery of DDHA to 52%. No deleterious effect was noted on the recovery of the other two compounds.

1.3.4 Membrane-Based Sample Preparation Methods. A wide variety of membrane materials can be used for sample preparation, and all are grouped into two categories, i.e., porous and non-porous membranes. The basic principles of their categorization will be discussed in section 1.5. Here attention will be paid to the use of porous membranes in selective isolation of compounds on the basis of concentration gradient (dialysis), electrical potential difference (electrodialysis) and pressure difference (filtration), Fig. 1.7. Membrane extraction with non-porous membrane, in which case sample preparation is also based on concentration gradient, will be separately treated in section 1.6.

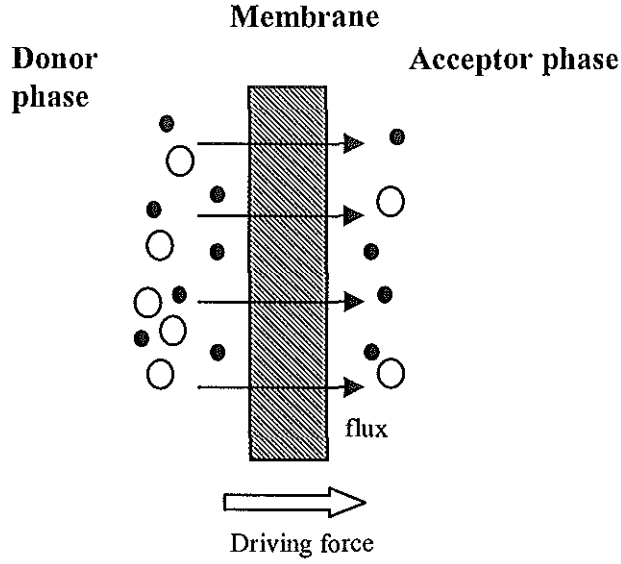


Fig. 1.7 Schematic representation of the membrane system.

In dialysis, analytes are separated from the sample matrix by their difference in size, i.e., the relative sizes of the various molecules largely determine their permeation across the porous membrane. The mass transfer occurs between the donor and the acceptor phase separated by a membrane which allows the selective penetration of molecules of low molecular weight thereby preventing penetration of the macromolecules. The driving force for the mass transfer process is the presence of the concentration gradient of the transferable solute between the two phases. Variables controlling the efficiency of the separation process include the ratio of the flow rates in the channels, the rate of mass transfer between the two phases, the dimensions of the channels, etc. Parameters such as viscosity of the sample and temperature also play significant roles in affecting the efficiency. Depending on the dimensions of the analytes, the pore size of the membrane must be critically selected, in addition to the area and thickness. The most commonly employed membranes are porous networks of synthetic polymers such as polyethylene, polysulfone and cellulose derivatives.

Depending on the movement of separated components in the acceptor phase and the sample solution in the donor phase, various dialysis processes can be distinguished. When both the donor and acceptor phases are kept stagnant, which is also known as equilibrium dialysis, analyte molecules are transported from the donor to the acceptor phase until equilibrium concentration in both phases is achieved. Transport of molecules will cease when the equilibrium is established, i.e., when concentrations in both phases are equal. If the acceptor is flowing, the molecules transported are continuously removed, and this process is known as continuous dialysis. The donor phase can be either stagnant or kept flowing continuously or in a pulsed manner. A higher concentration gradient and thus high molecular flux may be obtained when the acceptor solution is continuously flowing, and such dialysis process has been utilized in sample preparation. In all types of dialysis, the concentration in the acceptor phase can never exceed that in the donor. As a result, dilution will occur.

The two main application areas of dialysis, for sample preparation, are biomedical and food analysis. In the recent paper by van de Merbel [187], the contributions covering these areas for the last fifteen years have been reviewed. Accordingly, the on-line dialysis has been used for determination of free drugs in plasma [188, 189], in whole blood [190] and urine [191], and sugar, organic acids and alcohols in blood plasma [192], and amino acids [193] in fruit juices. Sample preparation of solid food samples in dialysis mainly requires pre-treatment such as homogenization, centrifugation and addition of water [187]. Samples prepared in this way, including egg [194] and chicken liver [195], for example, were analyzed for drug residues.

In electrodialysis, selectivity can be enhanced since mass transfer based on the charge of the molecule is introduced in addition to selectivity based on size. The advantage of this technique is that the applied potential over the ion-exchange membrane causes a net

transport of the analyte to the acceptor channel, unlike the ordinary dialysis, where back diffusion to the donor begins when the concentration on both sides of the membrane are equal. Thus, in practice analytes can almost quantitatively be transferred to the acceptor channel. The limitation in the use of this technique for complex matrices is that compounds with the same charge and size as the analytes will equally be transferred to the acceptor channel resulting in poor selectivity. On the other hand, charged macromolcules, such as plasma, can irreversibly interact with the membrane causing fouling, thereby reducing the membrane permeability [196]. Due to these and other factors, e.g., decrease in recovery with ionic strength [197], the technique so far is restricted to relatively clean samples.

On-line electrodialysis has been applied successfully to the determination of anionic and cationic analytes in aqueous environmental waters, such as ground and surfaces waters. Low levels, e.g., sub μg/L, of phenoxyacid herbicides and anilines [198], quaternary amines (diquat and paraquat) and strong acids (sulphonic acid) [197] have all been determined with enrichment factors of typically 10.

The other mode of sample preparation is filtration. Separation by means of filtration membrane is achieved when a pressure difference is applied over the membrane by vacuum or a centrifugal force. This represents a traditional off-line application where the sample is placed on one side of the membrane (the feed), and all molecules of appropriate size pass to the other side (permeate side) with applied pressure. With on-line filtration, on the other hand, the pressure is created when the sample on the feed side is pumped through a restricted outlet tubing [187]. Based on the size of the membrane pores the filtration process can be classified into three categories, i.e., hyperfiltration (0.1-1 nm) ultrafiltration (1-100 nm) and microfiltration (100-1000 nm).

The performance of the filtration process may be influenced by several parameters In addition to the applied pressure, the sample viscosity, resistance of the membrane and

membrane dimensions such as thickness, area and pore size greatly influence the recovery of the analyte of interest [199, 200]. The resistance to the mass transfer, in this case, is not only caused by the membrane itself, but also by the layer formed on the membrane surface due to the accumulation of compounds that can not pass through the membrane pores. The extent of formation of this layer, which is called concentration polarization layer, depends on the applied pressure across the membrane. Ways of minimizing of the layer have been suggested [201-203] which include use of low pressure, thinner membranes with larger pores and surface area when ever possible. The filtration membranes also suffer from fouling due to irreversible deposition of the sample components on the membrane surface causing blockage of the membrane pores.

On-line filtration is extensively applied for the removal of microorganisms and macromolecules from fermentation samples. In these applications it is coupled to an LC [201, 204] or GC [205, 206] for the final determinations. For complex fermentation broth additional sample clean-up using solid phase extraction column is recommended prior to filtrate introduction into the analytical system [202, 205].

1.3.5 Microwave-Assisted Extraction. Microwave-assisted extraction (MAE) utilizes electromagnetic radiation to desorb pollutants from their matrices. The principal advantage of the MAE is rapid extraction since the solutions are heated directly without heating the vessel [78, 207]. Thus, the rate of heating using microwave radiation is faster than in conventional methods, and there is no loss of energy due to unnecessary heating of the vessel, i.e., localized heating can occur [208]. The way in which microwaves enhance extraction is not fully understood. The main factors to consider include improved transport properties of molecules, molecular agitation, the heating of solvents above their boiling points and, in some cases, analyte selectivity [207].

The use of MAE for environmental analysis is a continuously expanding area of research at the present time. The first application of a microwave oven for the MAE of analytes from matrices using organic solvents appeared in 1986 [209]. In this work, the microwave oven was used to extract analytes from soil, seeds, foods and feed using methanol and methanol-water for polar compounds and hexane for non-polar compounds. The microwave oven was operated for 30 s duration and after cooling repeated several times. The recoveries obtained by MAE were comparable with those obtained using the traditional approaches of Soxhlet and solvent extraction [78].

The compounds mainly extracted using microwave oven are polycyclic aromatic hydrocarbons (PAHs) from contaminated environmental matrices, e.g., soil [210, 211], sediments [212] and water samples [213]. In all the works reported, the experimental variables optimized have been indicated, which include type of extraction solvent, extraction temperature, extraction time and volume of the extraction solvents. For example, the typical extraction temperature optimized for PAHs is 115°C; extraction solvents are typically hexane, acetone, dichloromethane, petroleum ether, methanol and toluene, and a typical extraction time of 15 min. In some cases, the spiked soils were aged for 24 h, 14 and 21 days for comparing the recoveries [211]. It was found that the recoveries for the aged samples usually decreased and there was more spread in recoveries with ageing [78]. Dean *et al.* [207] have described the areas of applications, particularly emphasizing the potential of MAE in environmental analysis.

The MAE technique has also been employed for sample preparation of triazine herbicide compounds, particularly atrazine and the principal degradates from soil samples [152, 214]. Molines *et al.* [214] extracted atrazine, deethylatrazine, deisopropylatrazine and simazine from 10 g soil with 40 mL of (9:1) methanol and dichloromethane at 115°C for 20 min. Extracts were dried with anhydrous sodium sulphate and concentrated first to 2

mL, which was followed by further concentration to dryness under a stream of nitrogen. The residues were reconstituted with 5 mL of methyl *t*-butylether prior to GC analysis. Recoveries ranging from 89 to 103% at a spiking level of 200 ng/kg with RSDs of 2.1 – 5.3% were obtained. These results were compared with those obtained by a conventional liquid extraction method. The authors claimed that the recoveries from MAE are at least as good as those obtained by the conventional methods, while MAE provide advantages of low solvent consumption in combination with a high sample throughput.

1.4. Sample Clean-up Methods in Environmental Analysis

Complex matrices from natural water samples, e.g., surface water, runoff water, soil water and waste water, can sometimes be removed by single extraction procedures [215]. In most cases clean-up may not be necessary for ground water and drinking water samples. However, the methods most currently used for extraction of pesticides from natural waters such as LLE and SPE are non-selective, thus yield extracts that often contain too many co-extracted and interfering compounds. These compounds should be removed before the final separation step particularly when trace level determinations are required, e.g., below microgram per liter levels [216]. The interfering compounds often generate high background peaks at the beginning of the chromatograms [217].

The need for sample clean-up depends on many factors such as the type of water sample, the concentration levels, the separation performed and the degree of selectivity in the detection mode [76, 217]. In this section of the thesis, clean-up introduced in the SPE sequence, clean-up techniques applied to extracts using adsorption and size-exclusion chromatography and chemical treatment methods will be discussed.

SPE with Non-Polar Sorbents. For determination of hydrophobic pesticides, the clean-up procedure can be included in the SPE sequence. This is performed by flushing the SPE cartridge with a small volume of water modified with organic solvent. Many of the matrix components are eluted from the hydrophobic sorbents, retaining the analytes of interest. In fact, with such flushing treatment, interferents that are more polar than the analytes can be removed [218]. Alternately, polar substances can also be removed from the sorbents by adding some organic solvents to the water sample before percolation through the sorbent. Application of this latter technique is also limited to clean-up of non polar compounds.

When clean-up can not be performed after sample application and before analyte elution, fractionation can be done by coupling different sorbents. Sample clean-up of chloro-*s*-triazines has been reported [219] using two columns, the first being packed with non-specific graphatized carbon black sorbent for trapping of many compounds, while only basic analytes being transferred and re-concentrated into a second cartridge packed with a more specific sorbent such as a cation exchanger.

Polar Sorbents (Adsorption Chromatography). Sample clean-up based on fractionation of the extracts using polar LC sorbents such as silica, alumina, Florisil (synthetic magnesium silicate) or silica chemically modified with amino group is a widely applied method. The principle that governs this process is the difference in partitioning of the analytes and co-extractives in the polar sorbents. Valls *et al.* [220] described the possibility of collection of seven fractions from the extract of urban waste and coastal waters. In this application, the extract was dissolved in hexane and percolated through the polar sorbent. Step-elution with solvents of increasing polarity allows separation into fractions, on the basis of polarity difference. The analytical procedure contains many steps and is time-consuming. The only advantage is its broad screening for the identification of unknown compounds [215]. These

polar sorbents have also been used for sample clean-up of various pesticides being packed in SPE cartridges or columns. The most common sorbent, Florisil, has successfully been used for extracts containing various pesticides [221-223]. Silica gel and alumina have also been used, the former for non-polar pesticides [221, 224], while application of the latter is limited to few pesticide compounds, e.g., organochlorine pesticides [215, 225].

Size Exclusion Chromatography. Clean-up of extracts using size exclusion (also called gel permeation) chromatography is based on separation by molecular weight. This technique primarily removes materials of high molecular weight, leaving all the pesticides and other compounds of similar molecular weight [215]. The main advantages of this technique are that the method can be fully automated and that contaminants such as more sensitive polar pesticides that are accumulated in biological tissues can be separated without being destroyed [226]. The application of the technique has been demonstrated by several workers [227, 228] for removal of animal fats in the determination of planar chlorobiphenyls (CBs). The drawback of the technique, particularly when trace organic contaminants are extracted from sediments and biota, is the difficulty to completely remove all traces of large molecules, e.g., lipids [229]. Since the larger molecules elute prior to the smaller contaminants, trace quantities will always be available in the second fraction, and this quantity will be significant when larger mass has to be removed relative to the concentration of the contaminants [226].

Dialysis, with a polyethylene (PE) film, can be used as a static size exclusion membrane [226]. The PE film was utilized to dialyse the organic extract, in order to isolate the CBs from the fat. The potential of combination of dialysis, for removal of interfering humic substances from environmental waters and trace enrichment on a pre-column packed with C18 silica for determination of polar pesticides was also investigated [230].

The applicability of the dialysis membrane, in general, depends on the material used and on the thickness and molecular weight cut-off value of the membrane [215].

Chemical Treatment. Extracts separated from animal tissues, co-extracted molecules, e.g., lipids, can be removed by saponification with 20% potassium hydroxide in ethanol [226]. The application of this treatment is, however, limited to chemically resistant contaminants. Successful application of this technique for clean-up of CBs has been reported [226], but the more highly chlorinated the CBs are, the more they are prone to loss of chlorine especially for the reactions at high temperatures, e.g., > 70°C, for long time, > 1 h. Organophosphorus pesticides are known to be hydrolysed by saponification treatment [231]. It is therefore essential to note the recovery of each compound to be determined under the specific conditions of the reaction if this technique is to be used.

The other harsh chemical method used for sample clean-up is the use of concentrated sulphuric acid. In this treatment, co-extractants and interferents are removed by dehydration and oxidation reactions. This technique is also limited to few chemical groups such as organochlorine compounds without an oxygen bridge [231, 232]. The reactions of the co-extractants with an acid are more manageable if the acid is adsorbed on silica gel. The other drawback of this method is that the columns or cartridges must be discarded after one clean-up of the extract.

Clean-up Using the SLM Extraction. In liquid membrane extraction the sample pre-concentration and clean-up are taking place at the same time. A multipurpose use of the SLM methodology including field sampling, trace enrichment and clean-up has been investigated by Mathiasson *et al.* [82] and Knutsson *et al.* [83], section 1.2 and Fig. 1.5.

Particularly complex samples from animal manures (swine, poultry and cows), that may consists of several aggressive chemicals such as organic acids, bases and sulphur compounds [233] were selectively extracted using the SLM technique. Except for the usual pretreatments such as filtration and centrifugation, no clean-up was required before the final analysis by GC [234]. In the resulting chromatograms, the only peaks appearing originated from the analytes and the derivatizing reagent, indicating that both sample preparation and clean-up can be carried out in a single step using the SLM technique.

1.5 Liquid Membranes

1.5.1 Classification of Membranes. There are many types of membranes and membrane processes that can be widely applied in sample preparation. The definition given to membranes may be related to their specific application areas. Still a general definition can be given: "a membrane is a selective barrier between two phases" [235]. Thus, the membrane acts as a barrier by restricting the movement of molecules in certain specific manner. The semi-permeable nature ensures the separation to take place while preventing intimate contact between phases (Fig. 1.7).

The large variety of membranes can be classified in different ways [236]. They are commonly classified by origin, (i.e., biological or synthetic), structure, application area or separation mechanism. In one way or another, all these classifications are interrelated, and in this thesis attention will be given to synthetic organic membranes as this type is used in most applications of sample preparations [199, 237]. From separation mechanism point of view, distinction has to be made between the following structures of membrane: porous and non-porous membranes.

Porous membranes suffer from less selectivity as any compound of appropriate size can be transported through the membrane pores. This problem can be minimized by chemical or physical modification of the membranes [238]. For example, the membrane properties of polyethylene membranes can be altered from hydrophobic to hydrophilic behaviour by introducing a cationic group, so as to exclude cationic compounds during separation.

Porous membranes are obtained as a network of a polymer such as polytetrafluoroethylene (PTFE), polypropylene or cellulose derivatives, and their structure can further be subdivided as symmetric and asymmetric. Symmetric configurations have identical geometries throughout the membrane and the resistance to mass transfer is determined by the total membrane thickness (typically 10-200 μm). Asymmetric structures, on the other hand, consist of two layers made from either the same or different materials [199]. The top layer, a thin membrane (up to 0.1-0.5 μm), is coated on a very dense layer (up to 250 μm) of rigid porous polymer. The resistance to mass transfer is determined primarily by the properties of the thin top layer. The important structural parameters in the porous membrane include the pore size, pore size distribution, shape and geometry of the pores and surface porosity.

In non-porous membranes, separation is accomplished when the molecules or group of molecules to be separated are dissolved in the membrane. One example is the supported liquid membrane. The transport of molecules is determined by physical and chemical properties of the compounds in the membrane phase, which include their solubility and diffusion coefficient. Hence, the extent of interaction between the membrane and the compounds is a more important factor to consider than the membrane pore size or molecular weight. Consequently, non-porous membranes can be regarded as relatively selective membranes. Adding carrier molecules to the membrane can further enhance the

selectivity of the liquid membrane. The carrier should have high affinity to one or a group of solutes.

1.5.2 Transport Mechanisms in Liquid Membranes. The permeation of ions or molecules through a liquid membrane can be realized to take place in stepwise manner, as also depicted in Fig. 1.8. The following five distinct steps are occurring in the process: (i) analyte diffusion from the donor bulk to the membrane surface; (ii) analyte sorption into the membrane; (iii) analyte diffusion through the membrane; (iv) desorption of analyte from the membrane, and (v) analyte diffusion from the membrane surface to the bulk of the acceptor [239].

The selective separation and thus permeation in the liquid membrane is influenced mainly by the composition of the membrane solvent, and classified as follows:

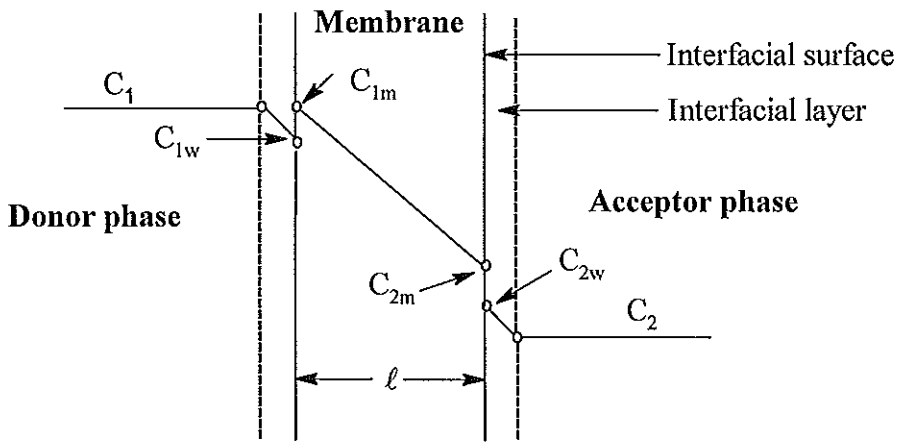


Fig. 1.8 A schematic representation of an idealized concentration profile of the analyte through the liquid membrane.

1. Passive Transport. In this process, which is also called a simple permeation or diffusive transport, the separation is based on the difference in solubility of the matrix components in the membrane phase. They diffuse into and through the membrane due to their concentration gradient. The analytes are trapped in the acceptor phase where they react with appropriate chemical species not soluble in the membrane phase. In the absence of such reaction, equilibrium is established between the three phases, resulting in distribution of solute molecules on both sides of the membrane. It is also possible to complex the analyte with a suitable complexing agent dissolved in the donor phase. After being transported through the membrane, the complex should be dissociated in the acceptor phase, and either the analyte or the complexing agent can be made to react with another species to prevent the back extraction [84].

2. Carrier Mediated Transport. The most serious problem attendant to the use of membrane separations has been insufficient transmembrane flux combined with insufficient selectivity [240]. Incorporating a carrier in a liquid membrane proved to offer reliable solutions to solving these problems, and thus have attracted the attention of analytical chemists during the last two decades. It is a powerful approach for selectively transporting the analytes through the membrane particularly when the transport based on relative solubility may be difficult to achieve, i.e., with compounds or ions of similar chemical properties or size. In a typical application, a mobile carrier, which more or less selectively binds to the analyte, is incorporated into the membrane liquid. Two requirements of the mobile carriers are of paramount importance. First, the carrier and its complex with the analyte must be soluble in the membrane phase but not in the adjacent phases (usually aqueous). Secondly, the complex formation must be strong on the donor side but weak on the acceptor side, where the carrier releases the analyte [239]. Thus, both

selectivity and rate of mass transfer will be increased. In practice, five different carrier transport mechanisms have been recognized: simple carrier mediated transport, simple carrier transport with chemical reaction in the acceptor, carrier mediated coupled co-transport, carrier mediated coupled counter transport and primary active transport. In the following section some qualitative description with specific examples will be presented [239].

(i) Simple Carrier Mediated Transport (or Facilitated Diffusion). Selective carriers are dissolved in the membrane, which can form a complex with analytes from the donor bulk. In practical applications the complex formation will increase both solubility in the membrane and speed up the diffusion process. Thus, concentration gradient across the membrane increases as compared to the simple permeation.

To illustrate the permeation process, an example of alkali metal transport by crown ethers through the hydrophobic membrane [241] will be considered. At the donor phase boundary, crown ether complexes the metal ion, and together with a counter ion the complex diffuses through the membrane. At the membrane-acceptor boundary, the crown ether releases both the metal ion and the anion, and diffuses back to repeat the cycle. This process continues with decreased rate until the condition of steady state is reached. The metal ion will be transported against its concentration gradient if the anion concentration is higher in the donor phase than in the acceptor.

(ii) Simple Carrier Transport with a Chemical Reaction in the Acceptor. The analyte transported through the membrane being associated with certain species is converted to a non-permeating compound in the acceptor phase, because of the chemical reaction taking place at the membrane-acceptor interface. The transport of acetic acid from an acidic donor

phase to an alkaline acceptor phase has been demonstrated using trioctylphosphine oxide (TOPO) as a carrier [242, 243].

(iii) Carrier Mediated Coupled Co-Transport. In such membrane processes the driving force is provided by the concentration gradient of the co-extracted compound or ion from the donor phase. For example, when dichromate, $Cr_2O_7^{2-}$, is extracted from acidic donor phase [242, 244] with triocthylamine, the complex traverses the membrane to the basic acceptor and gives up two protons, $2H^+$, in the basic acceptor phase. Dichromate is also simultaneously released and the free amine repeats the process, Fig. 1.9. Thus, extraction continues until concentration differences of the energy supplying and co-extracted proton is exhausted. It has been described by several workers [240, 242, 244] that with relatively small concentration, or pH, differences a high degree of enrichment can be achieved.

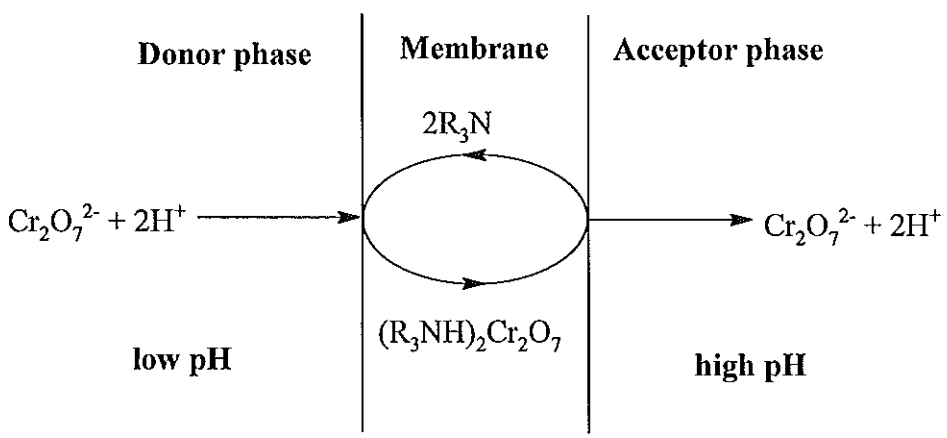


Fig. 1.9 Selective extraction of dichromate ion by coupled co-transport mechanism through the liquid membrane.

(iv) Carrier Mediated Coupled Counter Transport. The separation with this mechanism depends on reactions taking place both at the donor-membrane and acceptor-membrane interfaces. At the donor phase boundary the analyte associates with the carrier to form a

complex that can be transported through the membrane. Upon releasing the analyte the carrier combines with another species, supplied from the acceptor solution, at the acceptor phase boundary and travel back for repeating the cycle. The driving force is thus the concentration difference of the species counter transported to the donor phase.

Coupled counter transport mechanism with a liquid membrane formulation has been widely applied for recovery of copper ion, Cu^{2+}, [242, 244, 245] with the carrier usually a liquid ion exchanger, e.g., oxime. Anions such as nitrate [246] could also be transported across the liquid membrane by forming complex with tetraoctylammonium ion. Decomplexation in the acceptor phase can occur when a very high chloride concentration has been established [235]. Similar applications for enrichment of amino acids [247] and metal ion [248] concentration by coupled transported have also been reported.

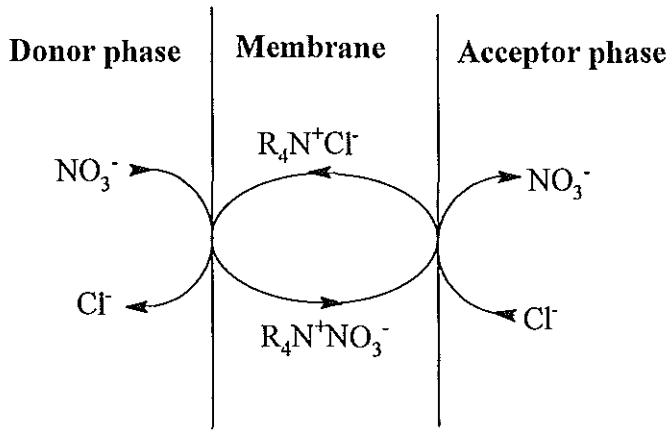


Fig. 1.10 Membrane separation of complexed nitrate ion by coupled counter transport mechanism.

(v) Primary Active Transport. In this membrane separation process electrons are co- or counter transported by making use of suitable redox chemical reaction. Application of such system is shown for extraction of picrate using a counter transport [249] and potassium ion

in a co-transport mechanisms [250]. The extraction of picrate, for example, performed using a methylenechloride liquid membrane containing N,N,N′,N′-tetramethyl-*p*-phenylenediamine (TMPD) [249]. The TMPD is oxidized to $TMPD^+$ by ferricyanide ion in the donor phase, which forms a hydrophilic ion-pair with picrate. The ion pair diffuses through the membrane and at the acceptor-phase boundary the $TMPD^+$ is reduced by ascorbic acid and picrate is released in the acceptor phase. The reduced species, TMPD, diffuses back for repeating the cycle.

1.5.3 Liquid Membrane Configurations. Liquid membranes have been known for long time as separation tools in industrial processes, e.g., for waste water cleaning and water desalination and in food industry [239]. Various aspects of the membrane processes, theory and applications have been described by several authors [235, 251]. The studies of the liquid membrane have been performed in three different formats, and thus they are categorized accordingly. The three formats are: bulk liquid membranes, emulsion liquid membranes and supported liquid membranes (SLMs). In this section the former two will be presented, and in the section that follows SLM will be dealt with separately.

(i) Bulk Liquid Membranes (BLM) – BLM is technically a very simple form of the liquid membrane consisting of two aqueous phases and one organic phase in a stirred container [252] or in a U-tube [253]. Due to the thickness of the membrane, i.e., the organic phase which causes low mass transfer rates, such membranes are mainly utilized in studies of transport mechanisms, and thus the practical applications of this type of membranes is less attractive.

(ii) Emulsion Liquid Membranes (ELM). ELMs are prepared by vigorous mixing of two immiscible phases, for example, water and oil. This results in the formation of emulsion droplets, (droplet size about 0.5-10 μm) [235], which are stabilized by addition of a surfactant. A water/oil emulsion obtained in this way is then dispersed in a third continuous phase, usually acting as a donor. For an organic liquid membrane, the so called water-in-oil-in-water emulsion consisting of two aqueous phases and an organic liquid membrane is obtained.

The extraction recovery of the ELM process is normally very low [254], but can be improved by optimization of the following parameters: the ratio of the three phases, surfactant concentration, membrane viscosity, internal phase droplet size, internal reagent utilization, contact time, etc. Therefore, the efficiency of ELM can be influenced by several factors, and production of a stable liquid membrane is largely empirical [254, 255]. After permeation, the emulsion phase is usually separated from the donor phase by sedimentation. De-emulsification is often done by centrifugation and treatment with a solvent [256], which is also followed by density separation from the reagent phase. The enrichment factor obtainable is very small (typically 20 times or less [254]), and this limits its application in trace analysis. However, many applications of the technique for recovery of different compounds in waste water and in process steams have been reported [257, 258]. Noble and Way [251] have summarized the use of ELM for industrial processes, e.g., waste water cleaning and hydrometallurgy.

1.6 Supported Liquid Membrane (SLM) Extraction

Supported liquid membrane (SLM), which is also called immobilized liquid membrane, is the most utilized format of the liquid membranes and the most flexible for structural modifications according to various needs. It is commonly prepared by immobilizing a water immiscible organic solvent within a microporous film. The first description of this technique was reported in 1967 by Ward and Robb [259]. Several materials have been tested and used since then for supporting immobilized membranes, e.g., filter paper, polytetrafluoroethylene filters, polypropyelene filters, PVC-filters, cellulose acetate membrane, polysulfone membranes and track etched polycarbon filters [260]. The main application area was in the extraction of metal ions from several matrices. Perhaps that is why it was defined [251] in one text as "a SLM is a device for selectively removing metal ions from one aqueous phase and delivering them to another aqueous phase".

Three different geometries are known and applied in various ways. For batchwise laboratory studies, the flat sheet in a diaphragm cell is the common material [261, 262], but its practical application is limited. The second form is a hollow fiber [255, 256, 263], and the advantage of this one is that it offers the highest surface area per volume of the sample. The final form is the flat-sheet membrane. The practical application of this type of SLM membranes were found to be simpler, and today most available supports are in this form [264].

As has been pointed out earlier, SLM extraction technique was frequently utilized in industrial separation of metal ions and volatile components until Audunsson [264, 265] demonstrated its use in environmental analysis for sample handling at the Department of Analytical Chemistry, Lund University in 1986. Since then, and particularly in the 1990s, the technique developed very fast, and has been successfully applied for selective

extraction of various compounds in complex matrices of environmental and biological origins.

1.6.1 Basic Principles of the Supported Liquid Membrane Extraction. Supported liquid membrane extraction technique for sample preparation combines two liquid-liquid extractions with dialysis. The technique involves the use of a porous PTFE membrane separating two aqueous solutions. The porous membrane is impregnated by soaking in a suitable organic solvent. The immobilized membrane is then mounted between two flat blocks in which grooves are machined, forming a flow channel on each side of the membrane. The whole assembly is connected to the flow system, permitting aqueous solutions to be independently pumped through each channel. See also section 2.2, in materials and methods. By proper selection of the working conditions, compounds can selectively be extracted from the flowing liquid in the donor compartment into the hydrophobic liquid membrane and subsequently extracted into the second compartment, the acceptor phase, containing the receiving solution. In a typical application, the analyte of interest, usually present in ionic form in the donor phase, will be converted to non-ionic species when associated with a suitable reagent, so as to be extracted into the organic membrane phase. The species is then transported through the liquid membrane and subsequently diffuse to the second aqueous phase. There, the chemical conditions should be such that the analytes will be converted into a non-extractable form, preventing their re-extraction into the organic phase. Analyte enrichment can thus occur since, in such arrangement, the acceptor phase is stagnant and can trap considerable fraction of the analytes of interest which were originally present in a large volume of the sample solution pumped through the donor channel [84, 215].

1.6.2 The SLM Theory. The theory of SLM extraction has been described by Audunsson [239] and thoroughly discussed by Jönsson *et al.* [266] with some additional aspects included recently [267]. The important quantitative parameter frequently utilized to express the process of extraction in liquid membrane is the extraction efficiency, E. It is defined as the fraction of the analyte extracted from the flowing donor phase into the stagnant acceptor phase, and is given by the following equation:

$$E = \frac{n_A}{n_I} = \frac{c_A V_A}{c_I V_I} \tag{1.1}$$

where n_I and n_A are the total number of moles of solutes entering the extraction system and collected in the acceptor channel, respectively, c_A is the concentration of analytes enriched in the acceptor and V_A is the final volume of the enriched sample, c_I is the concentration of the aqueous sample entering the donor channel and V_I is the total volume of the sample passing the donor channel.

E is the measure of the rate of mass transfer through the membrane and, at specified extraction time, flow rate, membrane composition and ionic strength it is constant. Alternately, E can also be calculated from the total quantity of solutes leaving the membrane as waste, using the following formula:

$$E = \frac{n_I - n_W}{n_I} \tag{1.2}$$

here, n_W is the total number of moles of analytes in the donor waste accumulated from the beginning of the experiment. E can therefore be calculated from experimentally measured

quantities using both or either of these equations. The significance of any deviation in the values of E obtained using equations 1.1 and 1.2 will be dealt with later in this section.

The extent to which the extracted molecules are enriched in the acceptor phase, i.e., the concentration enrichment factor, E_e, is given by:

$$E_e = \frac{c_A}{c_I} \tag{1.3}$$

which together with equation 1.1 leads to

$$E_e = E\frac{V_I}{V_A} = \frac{n_A V_I}{n_I V_A} \tag{1.4}$$

or with equation 1.2

$$E_e = \left(1 - \frac{n_W}{n_I}\right)\frac{V_I}{V_A} \tag{1.5}$$

The rate of mass transfer from the donor to the acceptor, and thus E, is proportional to the concentration difference, ΔC, over the membrane which can be written as:

$$\Delta C = \alpha_D c_D - \alpha_A c_A \frac{K_A}{K_D} \tag{1.6}$$

where α_D and α_A are the fractions of the analytes that are in extractable (uncharged) form in the donor and acceptor phases, respectively. c_D is the mean concentration in the donor phase and approximately equal to the mean of c_I and c_W. K_A and K_D are the partition coefficients for the analyte in the acceptor and donor phases, respectively. Usually, the

extraction conditions are set so that α_D is close to unity and α_A is a very small value. Thus, ΔC will decrease as the extraction proceeds and eventually approaches zero, which leads to the following expression for the maximum concentration factor (as c_I and c_W are then equal):

$$E_{e(\max)} = \left(\frac{c_A}{c_I}\right)_{\max} = \frac{\alpha_D K_D}{\alpha_A K_A} \tag{1.7}$$

If K_D is assumed to be equal to K_A, which is the case if the composition of two aqueous phases are similar, then:

$$E_{e(\max)} = \left(\frac{c_A}{c_I}\right)_{\max} = \frac{\alpha_D}{\alpha_A} \tag{1.8}$$

However, if the ionic strengths of the donor and acceptor phases vary significantly, this assumption may not be valid. The differences in partition coefficients may contribute significantly to the maximum enrichment factor possible [84, 267, 268]. The rate of mass transfer is constant only when ΔC is constant, i.e., when $\alpha_D c_D >> \alpha_A c_A K_A/K_D$. Thus, for the purpose of reproducible analytical application of the SLM extraction, it is necessary that α_A is very low. This condition is referred to as a complete trapping.

The conditions for the rate of mass transfer to approach equation 1.8, several parameters should be considered as detailed by Jönsson *et al.* [266], and briefly described below. If the steady-state prevails, the mass transfer of the analytes is determined by the overall mass transfer coefficient, k, and is given by:

$$\frac{1}{k} = \frac{\alpha_D}{k_D} + \frac{1}{k_M K_D} + \frac{\alpha_A K_A}{k_A K_D} \tag{1.9}$$

where k_D, k_A and k_M are the mass transfer coefficients in the donor and acceptor phases and in the membrane, respectively.

For the condition of complete tapping, i.e., α_A close to zero, the last term in equation 1.9, representing the mass transfer in the acceptor phase, diminishes and the overall mass transfer coefficient, k, is reduced to:

$$\frac{1}{k} = \frac{1}{k_D} + \frac{1}{k_M K_D} \tag{1.10}$$

or rearranging,

$$k = \frac{k_D k_M K_D}{k_D + k_M K_D} \tag{1.11}$$

It should be noted that, under these assumptions, phase equilibrium exists across both the donor and acceptor phase boundaries and other equilibria are neglected.

The rate of mass transfer can also be described in terms of the following two permeation conditions: membrane-controlled conditions and donor-controlled conditions. In the former case, the solubility in the membrane of the extractable form of the analyte is limited, and thus the mass transfer in the membrane limits the overall resistance to the rate of analyte diffusion. Thus, the rate-limiting step is the diffusion of the analyte compound through the membrane. The mass transfer coefficient, k, is then proportional to $k_M K_D/h_M$, where h_M is the thickness of the membrane [266].

On the other hand, if the solubility of the analyte in the membrane is relatively large, a considerably high mass transfer rate is typically obtained. The partition coefficient,

K_D, for the membrane/donor interface, as well as the diffusion through the membrane, is very high. The mass transfer in the donor phase is then rate-limiting, leading to the donor-controlled condition. This extraction condition prevails when K_D is greater than 10, while the mass transfer is mainly membrane-controlled when $K_D < 1$. It should also be noted that the value of the partition coefficient has no significant effect on the analyte extraction as long as it is reasonably large, i.e., in donor-controlled conditions. There have been some observations that very large partition coefficients (> 10000) are not favourable, as the transfer of the analytes out of the membrane into the acceptor phase, may then become less efficient, leading to the phenomenon which may cause the memory effect, see also the sub-section 1.6.5.

1.6.3 Selectivity, Membrane Solvents and Supports. The selectivity of the SLM extraction process depends primarily on the ability to transfer the analyte of interest between extractable and non-extractable forms in the required sequence, without making the same transfer for interfering ions.

Extraction of basic substance, B, is considered to illustrate the phenomena in selective extraction, Fig. 1.11. The sample is in extractable form when it enters the donor channel. Given enough time, virtually all basic molecules will be transported to the acceptor phase and protonated there, BH^+. Since the acceptor is kept stagnant while the donor is being pumped, the total concentration in the acceptor compartment will eventually become considerably higher than in the donor. On the other hand, interfering molecules that are charged at the experimental donor pH, e.g., A^-, and larger molecules are not extracted into the hydrophobic membrane but instead pass the donor channel to waste, Fig. 1.11A. Neutral molecules, e.g., N in Fig. 11, are freely distributed between the three phases, and thus their enrichment is unlikely to occur. Moreover, replacing the donor

solution by clean washing solution can further reduce the concentration of the neutral compounds in the stagnant acceptor phase, Fig. 1.11B.

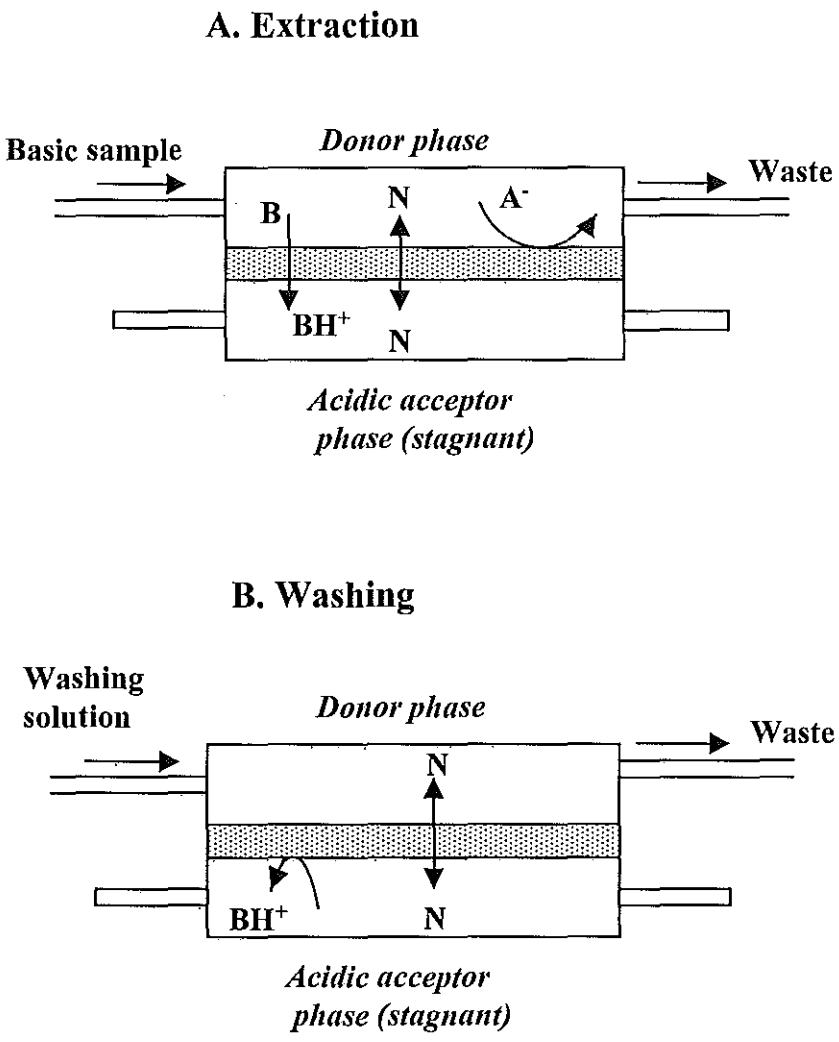


Fig. 1.11 Schematic representation of the SLM extraction of basic samples: (A) - extraction step, in the presence of acidic and neutral substances; and (B) - washing after extraction.

The selectivity can also be tuned by various means. For example, by proper selection of the donor and acceptor pH, closely related classes of compounds can be selectively separated. It has thus been possible to discriminate between aromatic amines ($pK_a \sim 5$) and aliphatic amines ($pK_a \sim 10$) [264]. In addition, by changing the composition or polarity of

the extraction solvent, it is also possible to increase the selectivity of the extraction system [269]. Polar compounds are less efficiently extracted in a non-polar membrane solvent, and thus to minimize extraction of polar molecules, the membrane solvent can be substituted by a more non-polar one.

The membrane solvent, which is immobilized in the porous membrane, plays a significant role both in increasing the analyte permeation and for the selectivity of the extraction process. The conditions or requirements for choosing the membrane liquid have been described elsewhere [264]. Here only some general qualitative aspects will be presented.

1. The solvent of choice should be as insoluble as possible in the aqueous solution in contact. When impregnated, a large surface area of thin film should come in contact with the flowing large volume of the donor sample solution. Even a slight solubility would result in loss of solvent and thus membrane leakage.

2. Solvents of low viscosity are always preferred, as the mass transfer of the analytes in the liquid membrane is inversely proportional to the solvent viscosity. However, highly volatile solvents should be avoided.

3. Partition coefficients of the analytes in the solvent of choice have to be high enough while those of the potential interferents should be as low as possible.

To fulfil the third requirement, the substance chosen as a membrane solvent should match in polarity with the analytes of interest. In SLM applications, several polar and moderately polar solvents such as di-*n*-hexylehter [270-273], 6-undecanone [274], a non-

polar solvent, *n*-undecane [265, 275, 276] and also mixtures of di-*n*-hexylether and *n*-undecane [269, 277] have been employed. Other membrane solvents studied alone or in mixture with either *n*-undecane or di-*n*-hexylether are 1-decanol, 1-dodecanol, 1-tetradecanol [277] and chlorotetradecane [278], and most of them resulted in membrane instability or insufficient extraction. The main drawback with membrane solvents of high polarity, particularly in environmental water analysis, is the decrease in selectivity and life time [279]. However, addition of reagents, e.g., trioctyl phosphine oxide (TOPO), to a moderately polar membrane solvent enabled extraction of polar compounds [269, 280] and improvement in extraction efficiencies [280]. The possibility of employing other reagents in the membrane seems a promising procedure for further improving selectivity and extraction efficiencies in environmental and biological applications.

The choice of support material is also equally important in the SLM extraction process. Way *et al.* [260] have discussed the conditions for selection of the membrane support materials, and reported the following generalization: [239] "the ideal support should be very thin (< 10 μm), should have high porosity (> 50%), a mean pore size of less than 0.1 μm and a narrow pore size distribution". Furthermore, the membrane support should be stable and easy to handle. A smooth surface of the support is also desired to minimize fouling of the membrane surface.

Several membrane materials have been tested as support [264, 273], in SLM extractions and only few resulted in good performance. Membranes with PTFE materials (type: FGLP, Millipore or TE35, Schleicher & Schuell) [273] were found suitable and used in most applications including the works presented in this thesis.

1.6.4 Donor Flow Rate Dependence of the Extraction Efficiency. The efficiency of the SLM extraction process depends on a number of parameters [84, 264, 266]. These include the flow rate, dimensions of the membrane channels, physical and chemical properties of the phases, etc. The influence of the donor flow rate on the extraction efficiency can be quantitatively described by the following equation (ignoring memory effects and assuming complete trapping, i.e., $\alpha_A = 0$, in the stagnant acceptor phase) [84, 85, 266, 267].

$$E = 1 - \exp\left[\frac{3D_D}{\pi h_D K k_M} \ln\left(1 + K k_M \sqrt{\frac{2\pi h_D}{3D_D \phi}}\right) - \sqrt{\frac{6D_D}{\pi h_D \phi}}\right] \tag{1.12}$$

where D_D is the diffusion coefficient of the analyte in the donor phase. ϕ is equal to $F_D/(Lw)$, where F_D is the volume flow rate of the donor phase, and L and w are the length and width of the donor channel (i.e., the free membrane surface), respectively. ϕ should not be considered as the linear velocity; it is rather the volumetric flow divided by the membrane area. It can be realized that the extraction efficiency, E, is a function of the three independent parameters, ϕ, D_D/h_D and $K \cdot k_M$. The latter two parameters characterize the mass transfer process in the donor phase and in the membrane, respectively.

From equation 1.12, it can be seen that the extraction efficiency decreases with increasing donor flow rate. Thus, the most efficient extractions are obtained at low donor flow rates, Fig. 1.12. In practical applications, particularly in environmental water analysis where the analytes of interest are found at trace level, it is much desirable to maximize the concentration enrichment factor, i.e., the quantity of analytes accumulated in the acceptor phase during a fixed extraction time, rather than the extraction efficiency. This demands a larger available sample volume than with low flow rates.

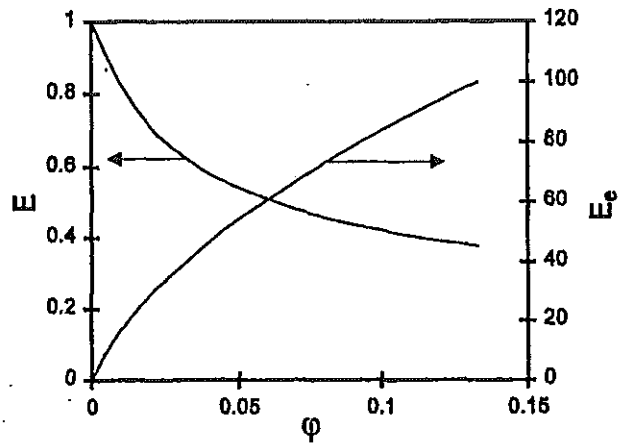


Fig. 1.12 Extraction efficiency, E, and enrichment factor, E_e, (arbitrary units) as a function of reduced flow parameter, ϕ [85].

The concentration enrichment factor obtained is related to the donor flow rate by the following expression:

$$E_e = E\frac{V_I}{V_A} = E\frac{F_D t}{V_A} \tag{1.13}$$

where, V_I is the total sample volume pumped at the donor flow rate F_D during time t. From equation 1.13 and Fig. 1.12, it is seen that the concentration enrichment factor increases with the donor flow rate for a given time. The decrease in E is then compensated for by the increased amount of the analytes allowed to pass through the system.

With limited sample volume, however, the extraction should preferably be performed under conditions of low flow rate to increase the extraction efficiency, if analyte adsorption and dispersion in the flow system is not hampering the efficiency. Extracting the same sample several times can compensate the effect due to adsorption and dispersion

during the passage. The analyte dispersion effect can also be minimized by gradually increasing the volume that may pass through the membrane, after each passage. The alternate pushing and pulling of the same sample will prolong the contact time of the analytes with the membrane; maximum fraction can be extracted from sample of small volumes.

Jönsson *et al.* [266] have described, using model calculations, the process of multiple passages of the same sample portion. Accordingly, if the extraction efficiency during the first passage is E, a fraction 1-E of the total analyte amount remains in the outlet of the donor channel. When this is extracted, $(1\text{-}E)^2$ of the initial amount remains. Thus, the overall extraction efficiency, E_n, after n passages is:

$$E_n = 1-(1-E)^n \tag{1.14}$$

It is also possible to increase the extraction efficiency of the limited amount of sample by using membranes of small channels [266, 273].

1.6.5 The Memory Effect. The values of E given in equations 1.1 and 1.2 above might not always be equal, since E in equation 1.1 indicates the amount of analytes collected in the acceptor phase, while the value obtainable in equation 1.2 measures how much of the analyte enters the membrane phase. If E in equation 1.2 is designated as E', one can define the recovery, R, as:

$$R = \frac{E}{E'} \tag{1.15}$$

If E = E', no analyte is lost in the process, and the recovery is 100%, but if E < E', which has been observed in most cases [243, 278, 279], then certain amount of the analytes may

either be adsorbed onto the apparatus or left in the membrane. The latter phenomenon causes what is termed as 'memory effect', which constitute a limitation to the SLM extraction technique. It is due to incomplete transfer of analytes from the membrane to the acceptor phase. In sequential automated extraction systems the memory effect not only causes a reduction in recovery but also leads to the carry-over effects. The extent of partitioning of the analytes between the donor and membrane phases is very important in determining the memory effect since membrane solvents giving very high partitioning coefficient might also give high memory effect, see also section 3.3.

Washing the membrane system between each extraction can minimize the memory effect [275, 278]. Conditions that enhance the extent of breaking of the complexes and ion-pairs, formed in the membrane with the analyte of interest, favour the trapping of the analytes in the acceptor, thereby lowering the memory effect. Thus, the composition of the acceptor solution, particularly when carriers are employed, plays an important role in lowering the memory effect.

1.6.6 Microporous Membrane Liquid-Liquid Extraction. Microporous membrane liquid-liquid extraction (MMLLE) is a two-phase extraction where an aqueous phase and an organic phase are separated by a hydrophobic membrane in a flow system similar to that used for SLM [281]. In MMLLE the organic solvent is the acceptor phase, which subsequently soaks the membrane material. During sample extraction the acceptor can be either stagnant of moving. The driving force for the mass transfer to the stagnant acceptor is the attainment of distribution equilibrium between the two phases. With moving acceptor phase, the mass transfer is better than the stagnant acceptor since the extracted molecules are removed continuously with a fresh solvent.

A simplified theory related to the SLM equations described above has been derived, which considers the influences of different experimental parameters on the extraction efficiency [281]. The instrumentation in MMLLE is similar to that in SLM except that the enriched extract, in the former technique, ends up in organic solvent. This behavior suggests interfacing it to either gas chromatography or normal-phase HPLC systems. In SLM, samples are enriched in aqueous solutions, which makes it compatible with reversed-phase HPLC system. Therefore, MMLLE can be seen as a complementary to the SLM extraction, permitting membrane-based sample extraction to be extended to further classes of compounds.

1.6.7 Comparison of the SLM to Other Sample Preparation Methods. Sample preparation by means of supported liquid membrane offers a number of advantages compared to other extraction methods used in trace analysis. This has been extensively discussed by Jönsson and Mathiasson [84, 85], and their co-workers [30, 238, 282]. Various particular advantages of the SLM technique and some related problems have been dealt with by Barcelo and Hennion [215] in their recent book.

The principal advantage of the SLM technique is the use of minimum organic solvent. This makes it a cost effective and environmentally friendly technique. Automation of the entire process also improves reproducibility and results in more economic analysis, since sample preparations are usually the largest source of error in analysis as well as the most time consuming and tedious part of analysis. High enrichment factors can be obtained in SLM extraction [267, 276] since very high phase-volume ratio, e.g., several thousand to one, can be handled, compared to classical LLE where ratios larger than 50:1 are difficult to handle. The possibility of emulsion formation is also eliminated by efficient contact between the various phases.

The usual problems encountered in SPE such as overloading, competitive sorption and clogging can be avoided or reduced using SLM extraction. A better selectivity has been demonstrated for trace enrichment of sulphonyl urea [271] and triazine [272] herbicides from environmental water samples with SLM extraction compared to SPE when extraction columns are used.

The more flexible nature of liquid membrane extraction allows tuning of selectivity by changing the organic solvent, changing or modifying the extraction process using carriers in various modes, adjustment of the pH in the donor and acceptor phases and changing the support materials. The use of organic solvent is also minimum (< 1 mL). In addition, since the permeation process is by analyte dissolution in the membrane chosen, breakthrough does not occur, so long as the driving force in the acceptor phase is well controlled.

The extraction time required for securing maximum concentration factor is relatively longer, and this can be one limitation to liquid membrane extraction [30, 215, 282]. The highest flow rate attainable so far reported with the larger dimension membranes is 7.5 mL/min [272], while in SPE using cartridges and membrane disks, samples can be percolated at 50 mL/min.

Comparison can also be made with dialysis, since both dialysis and the liquid membrane techniques are similar in some ways [84]. However, separation in dialysis is mainly based on size while in liquid membrane particular classes of analytes are selectively extracted. Concentration enrichment can easily be achieved with liquid membrane extraction, particularly in trace level analysis. In dialysis, however, analytes are essentially diluted.

1.6.8 Applications in Environmental and Other Matrices. The SLM technique has been actively developed and applied to variety of samples of environmental and biological origins during the last ten years. Efficient sample clean-up applications have been documented in the literature [234, 271, 272, 275, 283-285], besides the trace sample pre-concentration to bring about enrichment [234, 248, 267, 276, 281, 285-287]. These are very crucial for detecting and quantifying potential pollutants occurring at very low concentrations in the complex matrices [84, 85]. Reviews [84, 288] and a book [215] covering wide areas of applications of the technique are available.

Environmental Applications. The SLM technique has been found suitable for selective extraction of environmental pollutants of acidic and basic nature. Low concentrations of various pesticides in environmental waters, including phenoxyacids [82], sulphonylurea herbicides [268, 271], phenoxyacids and some structurally related herbicides, e.g., bentazone and dicamba [83], chlorophenols [275], aromatic surfactants [275], substituted anilines [286], and variety of alkylthio-*s*-triazines [276], chloro-*s*-triazines [270] and methyoxy-*s*-triazines [272] have successfully been extracted and analyzed. Final determinations for most of these extracts have been carried out with reversed-phase HPLC, either in off-line or on-line mode [288].

Sample pre-concentration of amine [289] and carboxylic acids [280] in air and rain water was also performed. The aqueous extracts obtained from the impinger sampling were further concentrated with SLM unit. The final separation of the extracted samples of amines was performed using GC, and ion chromatographic separation for carboxylic acids. An on-line connection to the SLM system was employed for both cases. The more polar carboxylic acids were extracted by introducing an extractant, trioctylphosphine oxide

(TOPO), in the liquid membrane to improve the efficiency of the extraction process [243, 284].

Heavy metal ions in environmental waters and other matrices have also successfully been extracted using the SLM technique [282, 288]. In most of these applications, various reagents have been utilized for sample enrichment of the metals from complex matrices. By ion-pair formation with 8-hydroxyquinoline and with thiocyanate [248], and di-2-ethylhexylphosphoric acid (DEHPA) [287, 290] in the donor phase, extraction of various toxic metals such as Cu, Cd, Co, Ni, Zn and Pb has been carried out. Stronger complexing agents, e.g., diethylenetriaminepentaacetic acid (DTPA), in the acceptor breaks the complex formed in the donor phase. In these applications, AAS was used for the final analysis of the extracts. More recently the possibility of using ion-pair chromatography [291] and potentiometric stripping analysis (PSA) [292] for determination of the metals have been described. In another application, two SLM units were serially connected in an automated system, and chromium was extracted in its two oxidation states from natural water samples [293]. For extractive separation of chromium as Cr(III) and Cr(VI) in the respective units, the ion-pair complex formation was effected using DEHPA in the first unit and methyltrioctylammonium chloride (Aliquat 336) in the second.

Biological and Biomedical Applications. There is a high demand for separation and identification of various constituents in biological samples. The sample preparation using SLM technique has been shown to satisfy some of these needs. Very complex sample matrices such as blood plasma containing basic drugs have been efficiently cleaned-up resulting in an excellent selectivity [84, 288]. The chromatograms obtained from spiked plasma are essentially identical to those of the aqueous solution, except that the peaks in the former case are lower, reflecting the protein binding of the drug [273]. Sample

preparation of various drugs in SLM that is fully automated and connected to an intelligent sample processor, ASTED (Automated Sequential Trace Enrichment of Dialysates), has successfully been utilized with HPLC in an on-line [273, 294] and capillary zone electrophoresis (CZE) in an off-line modes [274, 285]. A porous hollow fiber, low volume membrane with 1.3 μL acceptor volume was also developed for similar application in combination with capillary liquid chromatography [295]. The subsequent efforts made to couple the SLM system to CZE via a micro liquid chromatographic column [296] resulted in a satisfactory clean-up and enrichment for determination of low concentration (4 nM) of drugs in blood plasma. The SLM unit was also connected on-line to GC for applications where amines were determined in urine samples for the purpose of assessment of occupational exposure [265].

Extraction of amino acids constitutes one important area of SLM application in biological analysis. The inherent chemical properties, zwitterion formation, of amino acids makes their extraction a complicated process [288]. It is necessary to either derivatize one of the functional groups [297] or to use an ion-pairing reagent. Using the second approach, extractants such as di-2-ethylhexylphosphoric acid (DEHPA) [298] and methyltrioctylammonium chloride (Aliquat 336) [247] have been utilized and satisfactory extraction efficiency and enrichment were obtained. In the former case, the analytes were extracted from moderately acidic solution to the more acidic acceptor, and the driving force is the concentration gradient of protons. In the latter case analytes were extracted from basic donor to a neutral acceptor solution of high chloride concentration, which served as a driving force for the extraction process. In both cases, analytes are extracted by a counter co-transport mechanism, where proton and chloride ions, respectively, are moving in the direction opposite to the analytes, section 1.5. The possibilities of SLM

extraction of amino acids have also been described by making use of complexation equilibria involving, e.g., crown ethers [299, 300].

The other parallel development in liquid membrane application is the MMLLE technique. Since its first report in 1998 [281] for extraction of local anaesthetics in plasma samples, it has received importance in environmental analysis as well. Extraction of cationic surfactants [301] and vinclozolin fungicide [302] in natural waters have been recently described, the determinations of which were performed by normal-phase HPLC. A sample clean-up and enrichment of organotin compounds in complex matrices utilizing MMLLE, by off-line derivatization with tetraethylborate ($NaBEt_4$) has been reported [303]. The extracts were analysed by GC-MS in selected ion monitoring (SIM) mode. Sandahl *et al.* [304, 305] reported the combination of SLM and MMLLE for sample preparation of variety of permanently charged, ionizable and non-polar compounds at trace levels in natural water samples. In both cases reported, the extraction systems were on-line connected to the reversed-phase HPLC. In the latter case, for example, the whole extraction and analysis systems were controlled by means of four syringe pumps connected to the liquid ends of the membrane units. During the SLM extraction, the MMLLE extract was analyzed; the SLM extract was also analyzed during the MMLLE extraction [305]. This enabled processing of 22 samples per 24 hours.

1.7 Scope of the Present Study

Small-scale herbicides use has long been known in Ethiopia. Extensive applications are also noted in a number of agricultural sites, particularly in the southern rift valley regions of the country. On the other hand, these areas are known for their larger lakes, from which many hundred tons of fish are harvested each year. The agricultural farms, situated in close

proximity to these lakes, have significance in production of large quantities of cotton and maize. Smaller quantities of cereals and other products are also being cultivated mainly for household uses and domestic markets. In these regions, as is the case everywhere nowadays, a variety of pesticides have been recommended [306], and are being applied during each plantation season, for the purpose of controlling insects, weeds and herbs, and thus to promote crop production.

The residues of these pesticides can certainly enter these lakes by erosion and flood, leakage, seepage through soil, by wind and from nearby rivers, etc. Thus, the water bodies of these lakes are inevitably bound to contain considerable quantities of the pesticide residues, sufficient to affect the quality of water of these lakes. Knowledge of the level to which these residues are accumulated in the water bodies is very important. This calls for the use of certain analytical system for sample processing and quantification of their amount so as to enable one to study the various possible effects they may cause. So far no systematic studies on monitoring of the pesticide residues and/or their degradation products have been carried out.

The main objectives of the present work are thus:

1. to develop a method that can be employed in routine analysis of the basic herbicides, e.g., triazines, using supported liquid membrane technology;
2. to apply the technique to the determination of specifically recommended *s*-triazine herbicides used in the lake regions of the Southern Ethiopia; and
3. to investigate the possibility of further application of the supported liquid membrane technique for selective extraction of the degradation products of the *s*-triazine herbicides along with the parent compounds.

2. MATERIALS AND METHODS

2.1 Standard and Working Solutions

100 mg/L stock solutions of all the *s*-triazine herbicides studied (Table 1.1) were prepared in acetonitrile. The same concentrations of the degradation products were prepared as follows; deethylatrazine and deisopropylatrazine in acetonitrile, deethyl-deisopropylatrazine in reagent water, and the hydroxy products of atrazine, propazine and terbuthylazine were first dissolved in small volume, ca. 1 mL, of 1.0 M HCl, and the resulting solutions were diluted to the final volume with acetonitrile [115, 307]. Solutions of the phosphate buffer, pH 7.0, were prepared from $NaH_2PO_4.H_2O$ and $Na_2HPO_4.2H_2O$ [308]. Standard solution of humic acid was obtained by dissolving 40 mg of the solid in 200 mL reagent water, and filtering through Millipore unit. This stock solution was used to prepare a working solution of the required concentrations.

Standard solutions containing the *s*-triazine herbicides of interest were obtained by diluting the stock solution in reagent water. A series of solutions for calibration, in the concentration range of 0.1 to 2.0 mg/L, at five points, were prepared every week from the 10 mg/L standard solution. The working aqueous solution of 0.5 mg/L was obtained either by diluting the 5.0 mg/L standard aqueous solution [272, 276], or 2.5 mL of the stock solution in acetonitrile to the final volume of 500 mL with reagent water [283], which resulted in 0.5:95.5 volume ratio of the organic to aqueous composition. The resulting solution, in the latter case, was also observed suitable for extraction of the compounds, and no effects of eroding the membrane solvents was noted. Lower concentration standards and working solutions were obtained from the 10 mg/L standard solution and 0.5 mg/L aqueous solution, respectively. All stock, standard, aqueous and extracted solutions were

stored at 0°C when not in use for analysis. Standard solutions were also stable in acetonitrile for several months.

Analytical-grade organic solvents were utilized for immobilizing into the membrane support, and the ones employed for application of the works presented in this thesis were di-*n*-hexylether, *n*-undecane and 6-undecanone. Acetonitrile of HPLC grade was used both for mobile phase and for dissolving the standards. Aqueous solution of 0.05 M sodium acetate was mixed with the mobile phase to be used in an isocratic mode, and 0.035 M KH_2PO_4 for gradient system. Most of these chemicals were of analytical-reagent grade and purchased from Merck, while the standards of the herbicides were from Promochem (Wesel, Germany). Carriers included in the membrane solvents, such as diphenyl sulfoxide (DPSO), dibenzyl sulfoxide (DBSO), trioctyl phosphine oxide (TOPO) and triphenyl phosphine oxides (TPPO) were all from Sigma Chemicals (St. Louis, MO, USA). All solutions were prepared from analytical-grade reagents in high purity water obtained from a Milli Q-RO4 unit (Millipore, Bedford, MA, USA).

2.2 Experimental Set-up

The membrane holder consists of two circular polytetrafluoroethylene (PTFE) blocks, Fig. 2.1, with a diameter of 120 mm and a thickness of 8 mm, and with machined grooves like Archimedean spiral (depth 0.25 mm, width 1.5 mm and length 250 mm giving a total volume of ca. 0.95 mL). The soaked liquid membrane, when placed in the membrane separator, creates two separate channels. The rough side of the liquid membrane faces the donor channel through which the extraction sample solutions percolate. It is also equipped with an O-ring, outside of the grooves, for preventing the leakage of the sample solution. The second compartment is the acceptor channel where extracted analyte solutions will get

enriched. Both sides of the membrane holder were backed with aluminum blocks of 6 mm thickness, in which threads for the clamping screws were machined to make the assembly stable.

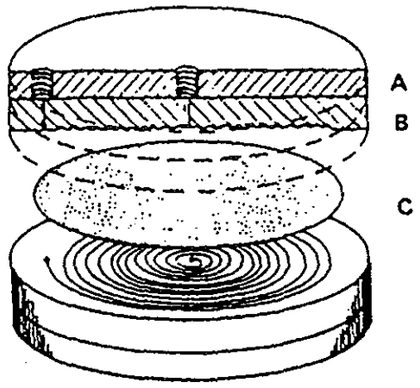


Fig. 2.1 Membrane separator: (A) aluminum backup; (B) PTFE block with grooves like Archimedean spiral; and (C) porous PTFE membrane with polyethylene backing

The liquid membrane support was porous PTFE, Millipore FG, with an average pore size of 0.2 μm, total thickness of 175 μm of which 115 μm is polyethylene backing, and a porosity of 70%. The liquid membrane was prepared by immersing the membrane support in the organic solvent to be immobilized, typically for a period of 30 min. For the cases when carriers are employed, first the required quantity of each solid substance, section 2.1, was dissolved in the membrane solvent of choice.

Sample solutions prepared for extraction were transferred to the membrane system with peristaltic pump (Minipuls III, Wilson S. A., Villiers-Le-Bel, France) using acid-resistant tubing (Acidflex, Elkay Products, Shrewsbury, MA, USA) with internal diameter of 2-mm both for the donor and acceptor channels. The sample and buffer solutions were pumped separately and merged in a PTFE tee connection at an angle of 60^{o}. Further mixing

was also performed in a coil of 1 m length. The various parts of the flow system were connected with PTFE tubing and flange-free screw fittings (Alltech Associates, Inc., Deerfields, IL, USA).

Two modes of sample transfer for extraction have been employed in the works described in this thesis. In the first case, the sample solutions are allowed to flow through the separator as mentioned above, and the enriched extracts were manually collected from the stagnant acceptor phase. The second mode of extraction used an automated system, where sequential sample extraction and collection were controlled by a home made computer programme, section 2.11.

2.3 Water Sampling from Southern Ethiopian Lakes

Lake water samples were collected three times after the herbicide application from Awassa Lake (270 km), and Chamo and Abbaya Lakes (500 km south of the capital, Addis Ababa). The first sampling was performed in August 1998, followed by November and finally in March 1999.

To obtain representative samples, a grab sampling method, section 1.2, was employed during all the sampling periods. In all cases, three variations of the collection areas were chosen. (An example of the sample collection from Awassa Lake will be considered, since similar procedures have also been followed for the rest of the sites, Fig. 2.2.) Location one is the entrance point of the tributary river called Tikur Wuha (Black Water in the local language). Sample collection for the second location covers the area from entrance of the river to about half way the length of the lake on the side of Awassa town (eastern side). Final location starts from end of the second sampling, and across the center of the water body to the other side (to the west). To obtain composite samples,

particularly in locations 2 and 3, about 200 mL of the lake water was drawn each time at a distance of about 10 m, for a minimum of 2 and 3 h, in locations 2 and 3, respectively. In location 1, sample was taken from a point of the river entrance to the lake. Lake waters were collected in polyethylene containers and brought to the laboratory in less than 48 h, and kept in the cold room below 5°C.

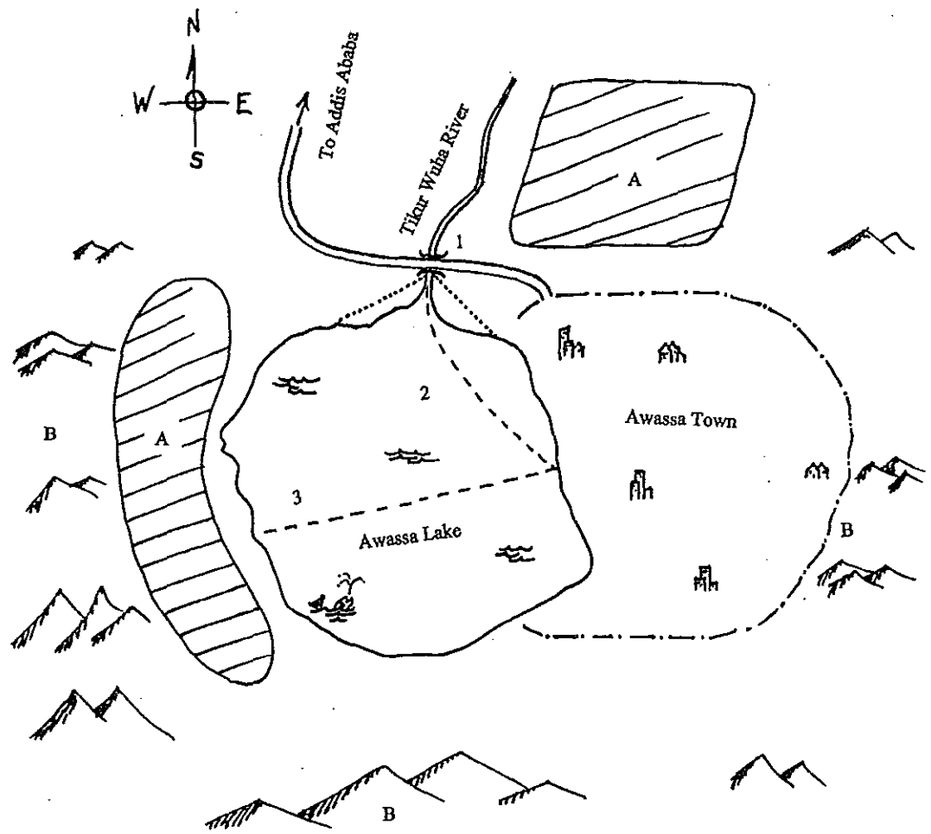


Fig. 2.2 Sample collection site at Awassa Lake. A – Agricultural fields, B – Hills surrounding the lake. Numbers represent collection point (1) or approximate line followed for sample collection (2 and 3). See also text.

Before liquid membrane extraction, the lake water samples were all filtered to remove the suspended impurities and particulate matters. The filtered samples were then

kept in the refrigerator when they were not extracted immediately. All water samples from Chamo Lake were extracted without storing for more than a day, which otherwise require re-filtering to make sure that the algae have been removed. All extractions have been carried out at ambient temperature, $20 \pm 2^{o}C$.

River waters for spiking were collected from Kävlinge [276] and Höje rivers [272], both located around Lund, 20 km north and 5 km south of Lund, Sweden, respectively.

2.4 Membrane Enrichment

In a typical application for enrichment of standard aqueous solutions, the sample containing the analytes of interest and the phosphate buffer (pH 7.0) were delivered to the liquid membrane system at a flow rate of 1.0 mL/min, Fig. 2.3. Prior to the sample extraction, the acceptor channel was filled with acidic solution, 0.1, [272, 276] 0.5 or 1.0 M [283], at a flow rate ranging from 0.2 to 0.5 mL/min, and kept stagnant. This was followed by pumping of the donor channel with the sample solution for 20 min, and for 10 more minutes with pure buffer solution to wash the flow tubing and allow all the sample solutions to pass through the liquid membrane. The system was then left to stand for ten minutes to give sufficient time for the analytes to diffuse to the acceptor acid solution where they are irreversibly trapped.

In some experiments, where samples were pumped at high flow rates, 7.0 – 7.5 mL/min, e.g., to determine the detection limit by extracting larger volume of diluted solutions, sample solutions were extracted for longer time. In all cases described above, at the end of ten minutes waiting time, the content of the acceptor channel was transferred to a 10-mL graduated glass tube, by displacing with the respective acceptor acid solution used

in the system. The resulting extracts were adjusted to pH 7.0 with diluted solution of sodium hydroxide.

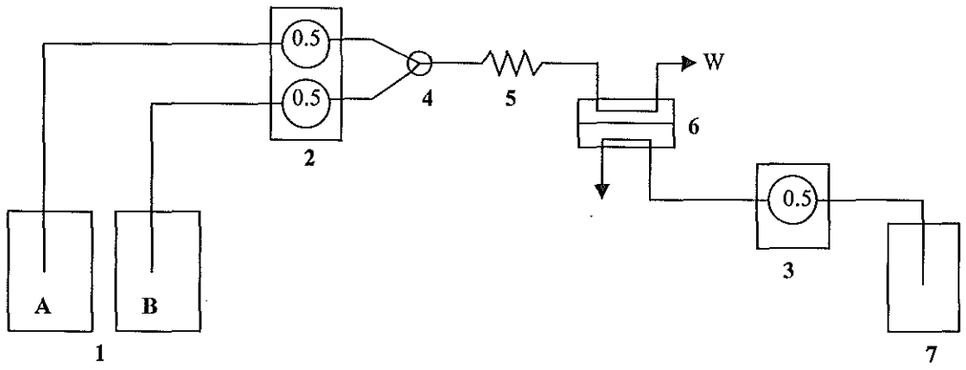


Fig. 2.3 Set-up of the flow system for liquid membrane extraction: (1) containers for sample solution (A) and donor buffer (B); (2) and (3) peristaltic pumps for the donor and acceptor channels, respectively; (4) PTFE tee connection; (5) mixing coil; (6) membrane extraction unit; (7) container for acidic acceptor solution; and (W) waste.

For lake waters, prior to extraction the sodium salts of phosphate were dissolved in the filtered water samples to obtain pH 7.0 of the sample solution. One to three litres of the processed samples were delivered to the liquid membrane, containing the appropriate membrane solvent, for extraction at a flow rate of 5.0 mL/min. When di-*n*-hexylether was used the concentration of sulphuric acid in the acceptor was 0.5 M [270], while it was 0.1 M [272, 276] for *n*-undecane membrane solvent. When sample pumping was completed, the donor channel was rinsed for 30 min with the donor buffer at the same flow rate, which was followed by 30 min standing of the membrane module. 3.0 mL of the enriched sample was transferred to a 10-mL graduated tube by purging the acceptor with the acidic solution, and pH of the enriched solution was immediately adjusted using about 0.5 mL of 6 M and 0.2 mL of 3 M NaOH for the extracts from di-*n*-hexylether and *n*-undecane membrane solvents, respectively.

2.5 Chromatographic Separation Systems

Analysis of the *s*-triazine herbicide compounds was performed using the high performance liquid chromatographic, HPLC, system, incorporating of a high pressure pump (Spectra Physics, San Jose, CA, USA) equipped with an autosampler (Waters, WISP Model 710B, Milford, MA, USA). For isocratic reversed-phase separation of the herbicide compounds a mobile phase composed of either 44% [272] or 50% [283] 0.05 M sodium acetate was utilized. In all cases, the pH was adjusted to 7.0 with 0.5 M sulphuric acid, and was then degassed by bubbling helium for at least 10 min. The mobile phase was also allowed to pass through a vacuum degasser before entering the pumping system. For rinsing of the loop of the autosampler, helium-degassed solution of 50% acetonitrile in reagent water was employed. 20 or 25 μL aliquot of the enriched analyte samples were introduced to the HPLC system, except when lower concentrations, below 5 μg/L were extracted, in which cases 50 μL were injected.

Separation of the compounds was performed on a C18 analytical column (Techsphere 5ODS 250 mm x 4.6 μm I.D.; HPLC Technology, Macclesfield, Cheshire, UK) connected to the variable wavelength UV-VIS detector (Model 757, Kartos Analytical Instruments, Ramsay, NJ, USA). The detector signals were collected and handled with a personal computer using a JCL 6000 Chromatographic Data System (Jones Chromatography Ltd., Hengoed, Mid-Glamorgan, UK). All analyses were carried out at the mobile phase flow rate of 1.0 mL/min and signals, based on the peak height, were monitored at the wavelength of 235 nm [276] or 220 nm [272, 283].

For confirmation of the identity of the *s*-triazine compounds, extracted from lake waters, a photo diode array detector (PDA 996, Waters) was used. Spectra of the

compounds identified, i.e., atrazine and terbutryn, at 210 - 300 nm from the detector were monitored using a Millennium 2.15 (Waters) chromatographic computer system.

2.6 Determination of the Extraction Efficiency

A series of aqueous sample solutions in the concentration range 0.1-2.0 mg/L was prepared from standard solution in acetonitrile, and introduced to the chromatographic system with each set of extracts. Calibration graphs for the compounds of interest were constructed based on triplicate injections. They all gave linear correlation coefficients of 0.9998 or better with insignificant intercepts at 95% confidence level. The corresponding concentrations of the extracted and enriched samples were evaluated using the calibration graph. The extraction efficiency of the technique was calculated from these results using equation 1.1.

2.7 Procedures for Optimization of the Extraction Parameters

The crucial variable in liquid membrane extraction is the concentration and/or composition of the acceptor solution. This parameter was studied by varying the concentration of the acid mainly from 0.02 to 1.0 M. The extraction, using an automated set-up, of a mixture of the degradation products and parent herbicide compounds of *s*-triazines in 1.0 M HCl, will be presented in section 2.11.

For complete transfer of the analytes through the hydrophobic liquid membrane, it is desirable that all the molecules are uncharged when entering the donor channel. To this end, the chemical behaviours of the compounds in the pH range of 3.0 - 8.0, in the donor flow system, was investigated. The donor buffers were prepared from sodium phosphate

salts [308] containing 85% H_3PO_4-NaH_2PO_4 (pH 3.0), NaH_2PO_4 (pH 4.0) and NaH_2PO_4-Na_2HPO_4 (pH 6.0-8.0). Freshly prepared buffers were utilized throughout the study.

The influence of the donor flow rate on the extraction efficiency was investigated within a wide range of donor flow rate, from 0.5 to 7.5 mL/min. For extraction of lake waters, in particular, a donor flow rate of 5.0 mL/min was utilized to transfer as high volume as three liters of the processed samples through the membrane extractor. Higher flow rates, 7.0 mL/min and 7.5 mL/min were exclusively employed for extraction of spiked river waters at low concentration levels, i.e., for determination of the detection limits.

2.8 Carry-Over Effects from the Membrane Extractions

To investigate the total quantity of the residues of molecules in the extraction system, the following procedures were followed: the sample mixture containing the analytes of interest was first extracted as described above, section 2.4. This was followed by extraction of the blank reagent water under identical conditions. The carry-over effect (COE) was evaluated from the peak heights using the following equation:

$$COE = \frac{P_b}{P_b + P_s} \tag{2.1}$$

where P_b and P_s are peak heights of the blank extraction and sample mixture, respectively.

Effects of washing time of the donor channel, after 20 min extraction and 10 min waiting was studied by rinsing the flow system for 0, 5, 10, 20 and 40 min. To study the rate of analyte transfer (waiting time), the extraction system was left to stand for 0, 2, 5, 10 and 20 min after collection of the enriched sample from the acceptor channel.

2.9 Determination of the Enrichment Factor

The extent to which the extracted molecules are accumulated in the acceptor compartment was studied either from the quantity enriched during the specified time period or by monitoring the donor waste at certain time intervals.

2.9.1 Enrichment Factor from the Acceptor Concentrate. The total number of moles of analytes accumulated from the beginning of the experiment was determined, and knowing the concentration of the sample entering the membrane unit, the enrichment factor, E_e, was evaluated using equation 1.3 or 1.5. The influence of the ionic strength on the extent of analyte permeation, and thus the effect on E_e, was studied by addition of 0.0, 0.2, 0.5 and 1.0 M Na_2SO_4 to raise the ionic strength to 0.1, 0.7, 1.6 and 3.1, respectively.

To determine the partition coefficient of the analyte between the aqueous solution and the organic solvent used in the membrane, batch solvent extractions were performed. In this study, chloro-*s*-triazine herbicides were used as model compounds [267]. Aqueous donor solutions were shaken in a separatory funnel with di-*n*-hexylether (in 5:1 volume ratio) for 15 min. The mixture was left to stand and the aqueous solution was separated. The concentration in the aqueous donor phase was measured before and after extraction using the LC system described in section 2.5. To study the effect of the ionic strength in the donor solution on the partition coefficient, this procedure was repeated but with the different ionic strengths mentioned above. The partition coefficient of the analytes from the donor solution to the membrane was calculated using the following equation:

$$K_D = \frac{\left([A]_{D(i)} - [A]_{D(f)}\right)}{[A]_{D(i)}} \qquad (2.2)$$

where $[A]_{D(i)}$ and $[A]_{D(f)}$ are the concentrations of the analyte in the aqueous donor solution before and after extraction, respectively.

To ~2 mL of the organic solution left in the funnel, 4 mL of 1 M H_2SO_4 was added and then the resultant mixture was shaken as above. To 3.0 mL of the aqueous phase was added 1.2 mL of 7 M NaOH to raise the pH to ca. 6.0, and its analyte concentration was determined by LC. This procedure was used to calculate the partition coefficient of the analytes from the acceptor solution to the organic solution according to:

$$K_A = \frac{K}{\alpha_A} \tag{2.3}$$

K is the distribution constant of the analytes between the organic and the acceptor solution. K and α_A were calculated from the following equation:

$$K = \frac{[A]_m}{([A]_a + [AH^+]_a)} \tag{2.4}$$

and,

$$\alpha_A = \frac{K_a}{([H^+] + K_a)} \tag{2.5}$$

$[A]_m$ is the equilibrium concentration of the analyte in the organic liquid. $[AH^+]_a$ and $[A]_a$ are the equilibrium concentrations of the analyte in the ionized and non-ionized forms in the aqueous acceptor solution, respectively. K_a is the dissociation constant of the analyte, and $[H^+]$ is the concentration of hydrogen ions in the acceptor solution.

2.9.2 Enrichment Factor from the Donor Waste. 0.10 mg/L of the aqueous sample solution, premixed with equal volume of the donor buffer, was extracted for 200 min at the donor flow rate of 7.5 mL/min. The donor waste was collected for one minute, at certain time intervals, to estimate the permeation of the analyte through the liquid membrane. Ten collections were performed during the whole period of extraction at 2, 5, 10, 20, 40, 70, 100, 130, 160 and 200 min. The extraction efficiency was evaluated using equation 1.2.

2.10 Comparison of Selectivity of SLM versus SPE

The aqueous mixture of the herbicides, 1.0 µg/L each of the four methoxy-*s*-triazines [272], was premixed with equal volume of the phosphate buffer prior to solid phase extraction, SPE. The SPE column used was C18 EC ISOLUTE (International Sorbent Technology Ltd., Hengoed, Mid-glamogan, UK) packed with 500 mg of the sorbent in a 6 mL polypropylene syringe barrel. Percolation of all solutions and solvents through the column was carried out using a vacuum manifold with a pressure pump. For pre-conditioning of the column, 3 mL acetonitrile was used followed by rinsing with 3 mL of reagent water for equilibration. Sample solutions, containing the four methoxy-*s*-triazines, were percolated at about 15 mL/min. The column was then flushed with 3 mL of reagent water. For elution of the sample, 3 mL (2 x 1.5 mL) of acetonitrile was used. The extracts were introduced into the separation system without any further treatment. 0.5 µg/L of the aqueous solutions prepared in identical condition were extracted using SLM at donor flow rate of 7.5 mL/min, as described in section 2.4, and the resulting chromatograms were compared for selectivity of the techniques.

2.11 Extraction of Degradation Products of *s*-Triazine Herbicides

2.11.1 Experimental Set-up. Selected degradation products of the *s*-triazine herbicides with the parent compounds were extracted in a similar SLM procedure as described in section 2.4, but using an automated extraction set-up given below, Fig. 2.4.

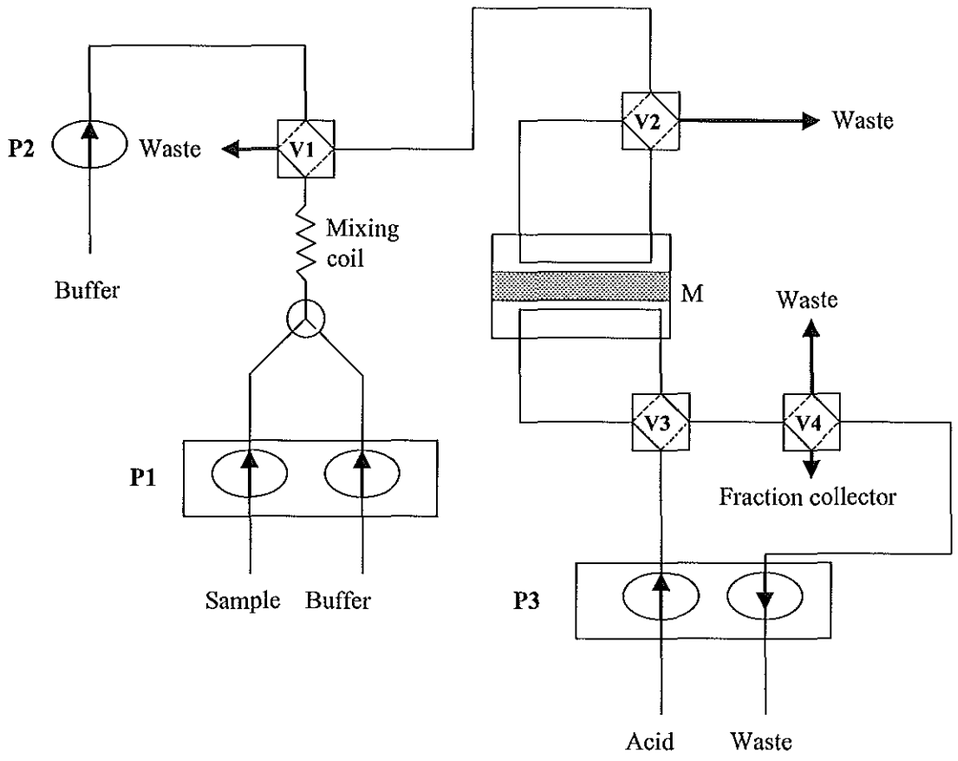


Fig. 2.4 Schematic diagram for the automated extraction of the mixture of parent compounds and degradation products of *s*-triazine herbicides using SLM technique (M represents membrane separator). The operation is described in the text.

Operation of the set-up is as follows: aqueous sample solution and the donor buffer were delivered to the membrane unit by pump P1, through valves V1 and V2. After sample extraction, a pure buffer was pumped by P2, for washing the flow system. The whole system was then left to stand to allow analyte diffusion. During analyte elution, pump P3

purged the enriched sample from the membrane unit through valves V3 and V4, and the enriched sample was collected in the fraction collector. Before subsequent extraction was started, residues in the flow system were removed by simultaneous washing of the channels with the donor buffer and the acceptor acid using pumps P2 and P3, respectively. Pump P3 was also used to empty the length of tubing from the membrane to the fraction collector before the next enrichment commences. All valves were pneumatically controlled and the operation of the valves and pumps were synchronized using a Pascal computer programme (Turbo Pascal v. 5.5 and 6.0, Borland, Scotts Vally, CA) written in the Department of Analytical Chemistry, Lund University [309]. The set-up in Fig. 2.4 is on the elute position.

2.11.2 Procedure for Sample Extraction. The aqueous sample containing a mixture of the degradation products and the parent compounds of *s*-triazine herbicides, 0.5 mg/L each, was transferred to the membrane system with an equal volume of the phosphate buffer. The sample mixture and the buffer were pumped with pump P1 separately and mixed in the tee connection. Further mixing took place in a mixing coil, Fig. 2.4. After 30 min delivery of the sample mixture, the flow system was rinsed with pure phosphate buffer for 10 min, with pump P2. The system was then left to stand for 10 min. The concentrate was collected with pump P3, by varying the acceptor flow rate, while the donor channel was either flowing or stagnant. Each time 2.0 mL of the concentrated sample was collected, in a 4-mL vial in which 300 μL of 7.0 M NaOH was added. After collection, 300 μL of 1.0 M phosphate buffer at pH 7.0 was added to obtain a constant pH of the extracted solution. Before any subsequent extraction, both channels were washed as described above.

Analysis of the extracted sample was performed using an HPLC system, similar to the ones described in section 2.5, except that a gradient system of mobile phase at a flow rate of 1.0 mL/min, was utilized. The mobile phase, in this application, was composed of 3.5 mM KH_2PO4, adjusted to pH 7.0, and acetonitrile which was pumped according to the mixing programme given below, Table 2.1.

Table 2.1 Mixing programme of the mobile phase for gradient elution of the mixture of parent compounds and degradation products of the *s*-triazine herbicides.

Time,min	% Phosphate buffer	% Acetonitrile
0.0	90	10
5.0	85	15
10.0	85	15
33.0	30	70
37.0	30	70
38.0	90	10
43.0	90	10

3. RESULTS AND DISCUSSION

3.1 Extraction Efficiency

The extraction efficiency, E, in the SLM technique is the fraction of analyte molecules recovered in the acceptor phase, and may be influenced by several parameters; see section 1.6. Among these parameters, the partition coefficient, K_p, of the analytes between the aqueous phases and the membrane plays a significant role. Its influence on E is somewhat complex [310]. It may be possible to qualitatively describe that E is in general small when the value of K_p is low, since the analytes are insufficiently extracted into the organic membrane and the mass transfer is limited by diffusion through the membrane. The most efficient extraction may be obtained at intermediate value of K_p and in this region the mass transfer is limited by the analyte permeation condition in the flowing donor phase. On the other hand, E will also decrease when K_p is very high due to the high dissolution in the membrane. The stripping of analytes in the acceptor phase thus becomes the limiting factor. It was indicated, in a recent study [311], that the most efficient extraction is obtained when the octanol - water partition coefficient is, as a rough estimate, around 10^3.

Analyte trapping in the acceptor phase is also an important factor in influencing the value of E. It will decrease with time if the trapping is incomplete, leading to less precise quantitation. The condition of incomplete trapping will be discussed in detail in conjunction to the enrichment factor, E_e, in section 3.8.

The extent of extraction of the liquid membrane, under various experimental conditions, will be quantitatively described using the values of E, from different viewpoints, equations 1.1 and 1.2.

3.2 Optimization of the Liquid Membrane Extraction

3.2.1 Composition of the Membrane Solvent. Selection of the membrane solvent for immobilization in the support material is one of the most critical steps in the SLM extraction. The solvent of choice must give high extraction efficiency and should have high affinity for the analytes of interest compared to the interferents, i.e., the K_p of the analyte molecules should be as large as possible, but not too large in which case stripping into the acceptor solution will be difficult [266, 267, 271]. Several factors that should be taken into account when the solvent is chosen have been discussed in section 1.6. Low viscosity, low solubility in water and high K_p value are some of the most important requirements.

In the present study of the extraction of *s*-triazine herbicides from different water samples, both di-*n*-hexylether and *n*-undecane membrane solvents were utilized. More polar analytes like degradation products of *s*-triazine required solvents of higher polarity, particularly when carriers were added to the membrane solvents. In these cases, 25% 6-undecanone was mixed with di-*n*-hexylether for the purpose of increasing the solubility of the carriers. The better selectivity of *n*-undecane for organic substances in natural water samples [271] and its long-term stability as a membrane solvent [265] has already been reported. This same solvent has proved both selective and stable for extraction of alkylthio-*s*-triazine herbicides spiked in natural waters. In this application more than 100 samples were processed with this membrane, without significant decrease in E. The other solvents, *viz.*, di-*n*-hexylether and its 50% mixture with *n*-undecane, were also found to be good alternatives except that their life as membrane solvents is shorter, which may be due to the gradual dissolution of the more polar solvent into the aqueous flow system. The E obtained for the alkylthio-*s*-triazines with these membrane solvents are given in Table 3.1.

Table 3.1 Extraction efficiency, E, for 20 min extraction of 0.5 mg/L of the alkylthio-*s*-triazine herbicides in various membrane solvents. Donor pH: 7.0; acceptor pH: 0.70; donor flow rate: 1.0 mL/min.

Herbicide compound	*n*-Undecane (UDC)	Di-*n*-hexylether (DHE)	UDC + DHE (50:50)
Desmetryn	0.639 (3.25)	0.584 (3.98)	0.591 (4.00)
Metoprotryn	0.620 (1.94)	0.557 (3.79)	0.561 (2.41)
Ametryn	0.650 (1.92)	0.553 (4.07)	0.540 (2.90)
Prometryn	0.572 (1.23)	0.473 (3.15)	0.470 (2.33)
Terbutryn	0.489 (0.82)	0.401 (2.71)	0.406 (2.19)
Dimethametryn	0.480 (1.09)	0.393 (2.51)	0.403 (2.11)
Dipropetryn	0.457 (0.88)	0.356 (2.90)	0.376 (2.42)

Numbers in bracket are the relative standard deviations, in %, for n = 5.

The behaviour of the *n*-undecane membrane towards change in analyte concentration, particularly at trace levels, was investigated by extracting different concentrations under the same experimental conditions. As can be seen in Table 3.2, the E values are nearly independent of the analyte concentration, see the RSD values in Table 3.1. A slight lowering of the E values for the late eluting compounds might be due to adsorption of the compounds, at lower concentrations, on the donor side.

With similar extraction condition for methoxy-*s*-triazine herbicides, pure di-*n*-hexylether exhibited better E. The same solvent was reported for extraction of chloro-*s*-triazine herbicides from natural waters [270]. The results for selected methoxy-*s*-triazines studied with different membrane solvents are shown in Table 3.3.

Table 3.2 E for samples of the herbicides at concentration levels of 1.0, 2.0 and 5.0 µg/L in both reagent and river waters. 50 µL extracts were injected and 20 µL for 500 µg/L extracted samples, n varies between 4 and 6.

Herbicide compound	**Concentration of the sample extracted/µg/L**					
	1.0 µg/L	***1.0 µg/L****	***2.0 µg/L***	***5.0 µg/L***	***500 µg/L****	***500 µg/L***
Desmetryn	0.632	0.627	0.638	0.631	0.626	0.643
Metoprotryn	0.621	0.618	0.612	0.613	0.608	0.623
Ametryn	0.637	0.626	0.643	0.643	0.645	0.651
Prometryn	0.572	0.588	0.606	0.580	0.579	0.587
Terbutryn	0.502	0.531	0.536	0.507	0.542	0.549
Dimethametryn	0.494	0.488	0.517	0.509	0.510	0.517
Dipropetryn	0.470	0.484	0.497	0.485	0.492	0.490

* Samples spiked in river water.

For samples collected from lake waters, in the vicinity of agricultural fields, both di-*n*-hexylether and *n*-undecane membrane solvents were used since more than one class of the *s*-triazine herbicides are being applied during each plantation season. The membrane solvents were used under different pH conditions in the acceptor solution; *viz.*, 0.1 M sulphuric acid for *n*-undecane [276] and 0.5 to 1.0 M for di-*n*-hexylether membrane solvents [270]. Field samples were extracted at donor flow rate of 5.0 mL/min in both membrane solvents. One important observation is that when the acid concentration was increased beyond 0.5 M, the efficiency started declining gradually, which may be due to degradation of the parent compounds [45].

Table 3.3 E for 0.5 mg/L methoxy-*s*-triazine herbicides in various membrane solvents. Donor pH: 7.0; acceptor pH: 1.0; donor flow rate: 1.0 mL/min.

Herbicide compound	Extraction efficiency, E^+, in		
	100% DHE; n = 9	*50% DHE in UDC; n = 10*	*100% UDC; n = 10*
Simetone	0.45 ± 0.05	0.32 ± 0.01	0.20 ± 0.02
Atratone	0.59 ± 0.04	0.48 ± 0.01	0.40 ± 0.04
Secbumetone	0.65 ± 0.04	0.57 ± 0.02	0.52 ± 0.05
Terbumetone	0.68 ± 0.04	0.61 ± 0.02	0.57 ± 0.02

[+]Mean ± 95% confidence interval.

3.2.2 Effect of the Acceptor pH on E. When SLM technique is applied to either acidic or basic ionizable compounds, the pH of the stagnant acceptor phase plays a significant role in controlling the degree to which the target analytes are selectively enriched. According to theoretical considerations [266], to obtain a nearly complete extraction of the basic compounds, the pH on the acceptor side should be at least 3.3 units below the pK_a of the analytes of interest. Alkylthio- and methoxy-*s*-triazine herbicides are basic secondary amine compounds having pK_a between 4.0 and 4.5 (Table 1.1) and thus can be protonated and trapped in acidic acceptor solutions of lower pH.

It was observed that the extent of enrichment of these compounds, and the E values, was dependent on the acceptor pH. The trapping capacity of the acceptor solution, in selected concentration regions, is shown in Fig. 3.1. It was also seen that E increases when the pH of the solution decreases, even though it seems to be much unaffected beyond pH of 0.7. An increase in efficiency was not observed by decreasing the pH below 0.7, Fig.

3.1, and thus a concentration of 0.1 M sulphuric acid was chosen for the extraction of alkylthio-*s*-triazine herbicides.

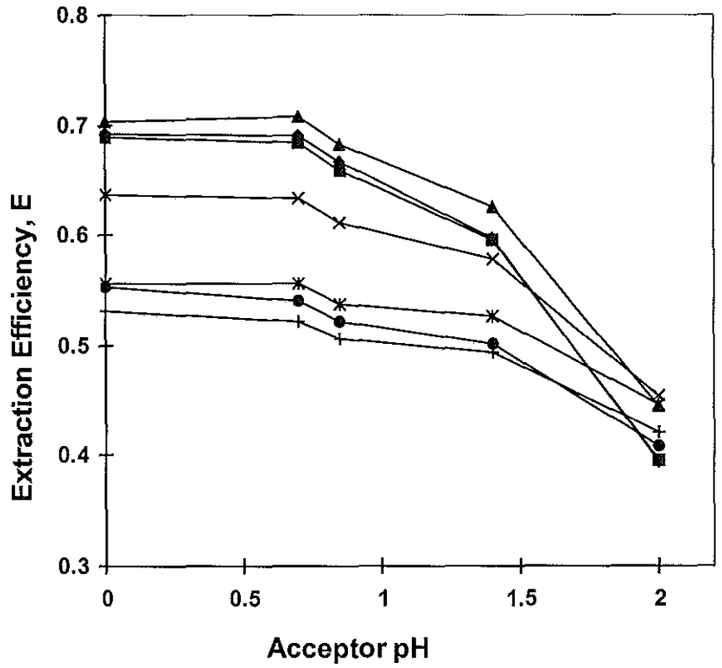


Fig. 3.1 Extraction efficiency versus acceptor pH. Membrane composition: 100% *n*-undecane; donor pH: 7.0; 20 min extraction of 0.5 mg/L of the herbicide compounds at donor flow rate of 1.0 mL/min. 20 µL of the enriched sample was introduced into the separation system. Symbols: (◆) - desmetryn, (■) - metoprotryn, (▲) - ametryn, (×) - prometryn, (✳) - terbutryn, (•) - dimethametryn, and (+) - dipropetryn.

Analytes trapping might be complete at pH 0.7, and thus the E value remained nearly constant below this pH. However, for extraction of lake waters, which were performed in the pH range from 0.7 to 0.0, the extracted solutions were adjusted to pH 7.0 immediately after collecting from the acceptor channel, to prevent possible hydrolysis of

the concentrate. In a series of experiments, this effect was thoroughly studied, and the E for prometryn and terbutryn increased by about 20% [291] and by 10% for atratone [283]. It is thus very important to note how long the extracted sample is left in acidic solution, in order to more precisely estimate the E value.

For extraction of lake water, concentrations of sulphuric acid ranging from 0.1 to 1.0 M were utilized. Both compounds identified in lakes Awassa and Chamo, were obtained when lower pHs were employed. Thus, when field samples of *s*-triazine herbicides are extracted concentrations of acids of about 0.5 M should, whenever possible, be used, unless extracts are collected in a pre-processed buffer whose pH is controlled. It was also noted that the optimum acidic condition in the acceptor phase follows the nature and composition of the membrane solvent.

3.2.3 Effect of the Donor pH on E. In the liquid membrane extraction of basic compounds, the pH of the extraction solution has to be kept 2.0 pH units more than the analytes with the highest pK_a value [266]. This is in order to keep the analytes in uncharged form and to facilitate their dissolution in the hydrophobic membrane. For alkylthio-*s*-triazines, the analytes permeation through the membrane seems to be unaffected between pH 6.0 - 8.0; see Fig. 3.2. It has been observed that around pH 8.0, results were less reproducible. This may probably be due to some chemical reaction, e.g., hydrolysis, that can possibly take place either in the donor phase or before pH adjustment.

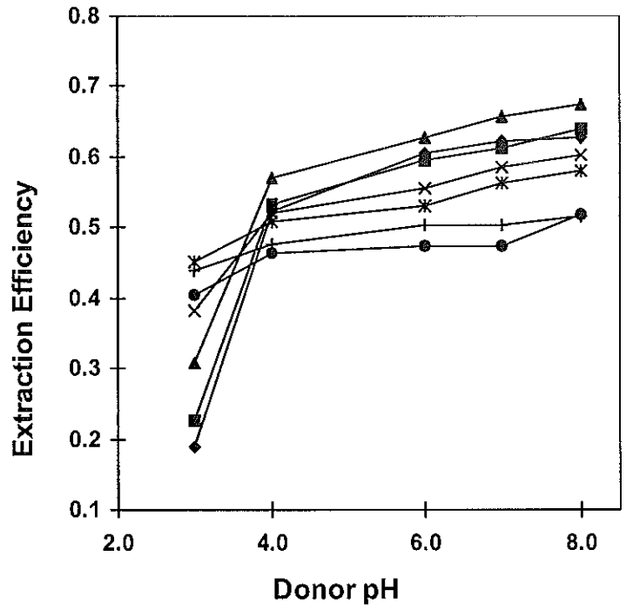


Fig. 3.2 Extraction efficiency of the alkylthio-*s*-triazines versus donor pH. Acceptor pH: 0.7 and donor pH was varied as described in section 2.7. Symbols and all other conditions are the same as Fig. 3.1.

The methoxy-*s*-triazine herbicides also exhibited good extractability at pH above their pK_a values. Highest efficiencies for both classes of the *s*-triazine herbicides were obtained at pH 7.0, and thus donor pH of 7.0 was used throughout. The decrease in E for methoxy-*s*-triazines above pH 7.0 was an unexpected observation, Fig. 3.3, which was not the case for alkylthio-*s*-triazines. This may be attributed to the greater basic character and more susceptibility to degradation of this class of *s*-triazines herbicides compared to both chloro- and alkylthio-*s*-triazines [45, 312].

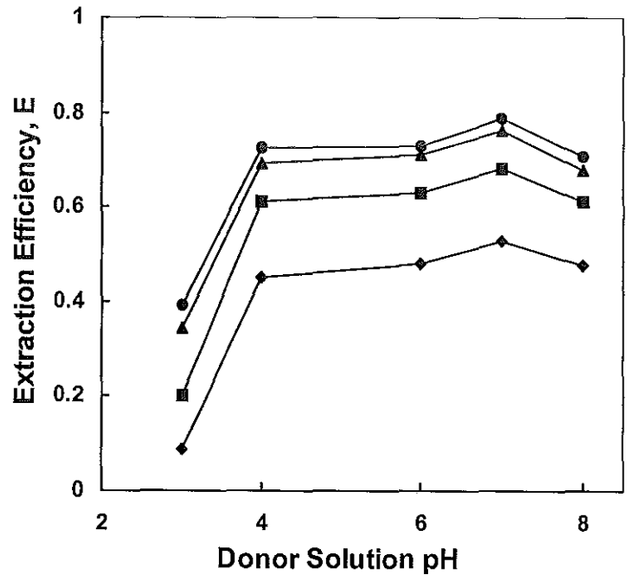


Fig. 3.3 Extraction efficiency, E, versus the donor pH for methoxy-*s*-triazine herbicides at the donor flow rate of 1.0 mL/min, 100% di-*n*-hexylether and 25 µL injection of the extracts. Symbols: (◆) -simetone; (■) - atratone; (▲) - secbumetone; and (•) - terbumetone.

3.3 Analyte Adsorption in the Extraction Systems

Whenever possible, complete extraction and quantitative transfer of the analytes in question through the liquid membrane is desired so as to obtain as high efficiency as possible. But in practical applications, notable fractions of the samples have been found in the membrane system when additional portion of the acceptor phase is taken before any subsequent enrichment [243, 278, 294]. The residual quantities of these analytes might be from one of the following two sources. One source may be adsorption of the solutes in the connecting tubes, flow system or the membrane surface on the donor side. When this portion is allowed to pass through the donor channel, in the flow system, subsequent

enrichment of adsorbed molecules will also take place. The effect due to this portion is termed the carry-over effect (COE).

To determine the COE, blank reagent water was enriched in identical manner, after collection of the concentrate from the acceptor channel. Analysis of these extracts was performed in the same way as for the analytes, as described in section 2.5, and the COE was calculated using equation 2.1. It was observed that for extraction of both the alkylthio- and methoxy-*s*-triazines, the COE quantities determined never exceeded 5%, except for dipropetryn, as shown in Table 3.4. The highest values for COE was obtained for the late eluting compounds, which are less polar and can be more adsorbed. Thus, effective rinsing of the flow system after each extraction ensures transfer of the maximum fraction of the analyte molecules to the enrichment membrane system.

Table 3.4 Carry-over effect (COE) and membrane memory effect (MME) of the herbicide compounds studied.

Herbicide compound	% COE	%MME
Desmetryn	0.55	0.11
Metoprotryn	0.74	0.22
Ametryn	0.62	0.18
Prometryn	3.00	0.29
Terbutryn	4.90	0.59
Dimethametryn	4.89	0.75
Dipropetryn	5.11	0.80

The second source of analyte residues may be from the slow diffusion through the liquid membrane to the acceptor. The effect due to this portion is termed as membrane memory effect (MME). The rate of mass transfer from the membrane to the acceptor, in

this case, may be slow. MME has also been noted in other SLM applications [243, 270], which was mainly due to slow mass transfer rate. In all cases of the present study, the MME amounts determined were all below the uncertainty of the measurement, Table 3.4.

To study the MME, the following two experimental procedures have been designed. In the first case, after collection of the enriched sample plug of the alkylthio-*s*-triazines, the system was left to stand for 20 min. Then, the acceptor channel was collected and analysed in the same way as for the samples. This same procedure was repeated three times and the mean values were calculated. A waiting time of 10 min was found enough for quantitative transfer of this class of *s*-triazines herbicides through the liquid membrane. For methoxy-*s*-triazines MME was studied by extending the waiting time before collection of the enriched sample plug. Extracts were left to stand for varied times, 0 to 20 min, and the results of this investigation are given in Table 3.5. From these results, it can be seen that both classes of the *s*-triazine compounds are quantitatively transferred during 10 min waiting time. However, when complex samples of unknown concentration, e.g., river or lake waters, are extracted, longer waiting time may be recommended as has also been reported [279]. Therefore, for extraction of lake water samples, 30 min waiting has been scheduled after extraction of either one or three litres of the processed samples.

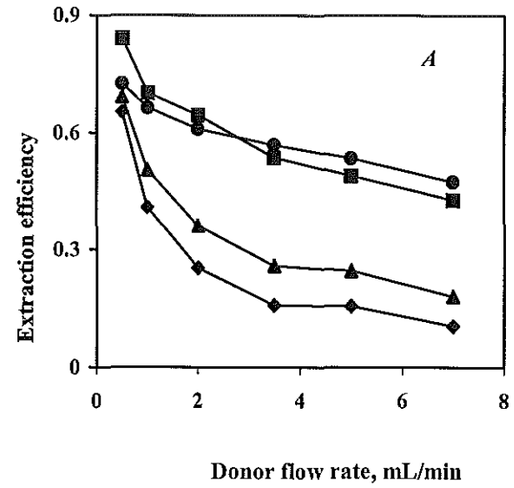
Table 3.5 Study of the effect of waiting time on E , after 20 min sample extraction and 10 min flushing of the donor stream with the donor buffer, for the mixture of the methoxy-*s*-triazine herbicides.

Herbicide compound	**E after waiting time of**				
	0 min	***2 min***	***5 min***	***10 min***	***20 min***
Simetone	0.39	0.42	0.43	0.43	0.42
Atratone	0.55	0.56	0.53	0.59	0.61
Secbumetone	0.56	0.59	0.59	0.63	0.67
Terbumetone	0.59	0.60	0.61	0.65	0.67

3.4 Donor Flow Rate Dependence of E

One of the advantages of liquid membrane extraction is the possibility to increase the amount of sample passing the donor channel per unit time, especially when larger sample volume is available. This is because one can increase the total amount of analyte accumulation in the stagnant acceptor solution during the specified period of extraction. The primary condition for attaining maximum accumulation is determined by the mass transfer in the donor channel. This is the case when the partition coefficient between the organic liquid and aqueous donor phase is relatively large ($K_p > 1$). Under these circumstances, the decrease in E with increase in the donor flow rate is small and the rate of analyte transfer and accumulation is very high. Further experimental benefits of such procedure are shortening of the extraction time and lowering of the detection limit.

For *s*-triazine herbicides, the decrease in E is very gradual, and thus the accumulation per unit time is high, Fig. 3.4. E decreases relatively faster for propazine



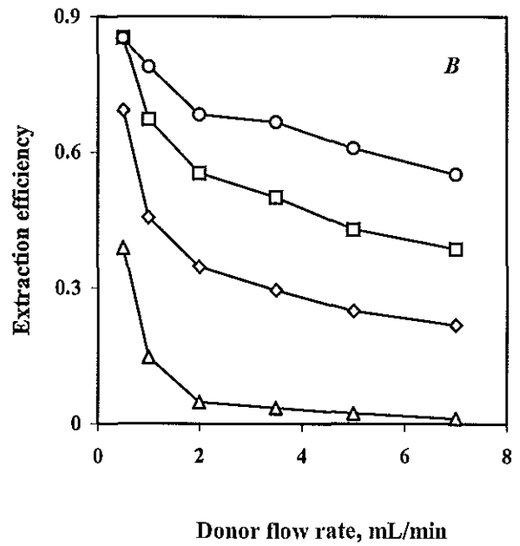


Fig. 3.4 Donor flow rate dependence of E for 20 min extraction of 0.5 mg/L of each *s*-triazine compounds. (*A*) Di-*n*-hexylether membrane solvent and acceptor acid concentration of 0.5 M; symbols: (♦) - atrazine, (■) - prometone, (▲) – propazine and (●) - terbutryn. (*B*) *n*-Undecane membrane solvent and acceptor acid concentration of 0.1 M; symbols: (◇) – atratone, (□) - prometone, (Δ) – propazine, and (○) - terbutryn.

with increase in the donor flow rate, in *n*-undecane membrane solvent, Fig. 3.4B. This is not an unexpected observation since all the chloro-*s*-triazine herbicides with lower pK_a values may have better dissolution in the more polar membrane solvents, di-*n*-hexylether, Fig. 3.4A. Methoxy-*s*-triazines were also successfully enriched in the latter membrane solvent, while both membrane solvents and their mixture proved to be suitable for alkylthio-*s*-triazines in environmental water samples.

At the expense of sample volume and also time, high analyte enrichment can best be achieved with liquid membrane extraction. Since breakthrough does not occur, analytes can be well accumulated to bring their concentration to a detectable level by conventional detection systems. The main problem associated with increased donor flow rate is the decrease in the life time of the membrane, which may probably be due to dissolution of the membrane solvent into the flowing large volume of the aqueous sample. A high pressure may also be created which hampers the analytes transfer through the membrane. For extraction of lake water samples, donor flow rate of 5.0 mL/min was chosen, which was not so high to remove the membrane liquid in limited extractions. Analytes of very low concentrations in the lake water have been enriched and quantified. When one litre of lake water samples were extracted at this flow rate, about 20 extractions can be done before the membrane starts to leak.

3.5 Determination of the Enrichment Factor

As was described above in section 2.9, determination of E_e can be done either by collecting the concentrates from the stagnant acceptor phase or by monitoring the donor waste.

3.5.1 Enrichment Factor from the Stagnant Acceptor Phase. To determine the extent to which extracted molecules are accumulated in acidic acceptor phase, one litre of spiked standard solution of herbicides, of 0.5 µg/L, was extracted at 5.0 mL/min using both di-*n*-hexylether and *n*-undecane membrane solvents. In another series of experiments three litres of spiked sample solutions, of 0.1 µg/L, were extracted in the same manner. The results are given in Table 3.6. It is seen that trace analysis using SLM is successful, and analyte accumulation seems independent of the concentration in the original sample. The performance of both membrane solvents was satisfactory, except for some chloro-*s*-triazines when *n*-undecane was used. With relatively higher sample volume the extent of

Table 3.6 Enrichment factors, E_e, for extraction of the standard samples spiked in reagent water. All samples were extracted at flow rate of 5.0 mL/min, and n = 4.

Herbicide compound	**E_e for 0.5 µg/L in one litre**		**E_e for 0.1 µg/L in three litres**	
	DHE*	***UDC****	***DHE***	***UDC***
Simazine	6.6	3.2	47	49
Atratone	170	95	327	323
Atrazine	97	29	454	467
Prometone	205	158	443	439
Propazine	6.7	2.2	10.7	nd
Terbuthylazine	18.2	16.6	78	nd
Prometryn	233	179	472	477
Terbutryn	287	225	699	740

*DHE and UDC are abbreviations used for di-*n*-hexylether and *n*-undecane, respectively.

nd – represents not detected.

enrichment with both membrane solvents is comparable, while this was lower in *n*-undecane for one litre extraction of atratone, atrazine and prometone. The reason for the discrepancy is not clear.

In other similar applications, on alkylthio-*s*-triazines, enrichment factors obtained in natural water and reagent water were compared for 500 mL samples extracted at 7.0 mL/min. To estimate the time required to obtain the same enrichment at lower flow rates, samples of the same concentrations were first extracted at 0.5 mL/min, for 20, 40, 60 and 120 min. Linear relationship was obtained for enrichment versus extraction time. By extrapolating the results for the enrichment at 7.0 mL/min, the time required to obtain the same enrichment at lower flow rate, 0.5 mL/min, was estimated, Table 3.7. These results

Table 3.7 Enrichment factors, E_e, for extraction of 500 mL of a 1.0 µg/L sample mixture, both in reagent and river waters, at flow rate of 7.0 mL/min (n = 3), and the corresponding time required if the same volume were extracted at 0.5 mL/min.

Herbicide compound	Sample in reagent water		Sample spiked in river water	
	E_e^*	*Time/min*$^+$	E_e^*	*Time/min*$^+$
Desmetryn	51	310	51	310
Metoprotryn	54	350	53	360
Ametryn	52	280	51	280
Prometryn	66	390	62	410
Terbutryn	78	490	73	460
Dimethametryn	78	480	73	510
Dipropetryn	80	490	75	530

*Enrichment factor for a 1.0 µg/L sample mixture at 7.0 mL/min, and $^+$- represents the time required to obtain the same enrichment at 0.5 mL/min.

confirm that *s*-triazines from various matrices can selectively be enriched, and using higher flow rate the analysis time can be shortened for trace level extraction of contaminants.

3.5.2 Enrichment Factor from the Donor Waste. In almost all the SLM applications so far reported, the extraction efficiency was calculated from the enriched sample collected from the acceptor. In one of the studies of this thesis, where the samples extracted at 7.5 mL/min, the donor wastes of methoxy-*s*-triazine herbicides collected during one minute, were directly transferred to the separation system without any further treatment. The number of moles in the donor waste since the beginning of the experiment was calculated using numerical integration and this was used to estimate the extraction efficiency at each interval using equation 1.2.

It was observed that during the first few minutes, E was relatively high and then becomes nearly unaffected throughout. This time may most probably be the time required for establishing a steady-state mass transfer through the membrane extraction system [264]. For routine works, in order to determine the optimal time of extraction at a given donor flow rate, it may be useful to employ this procedure during optimization of the SLM extraction. Estimation of the efficiency from the donor waste may be amenable when the compounds under study are quantitatively transferred and nearly completely trapped in the acceptor solution.

The enrichment factor, E_e, during the whole extraction period was calculated using equation 1.4 or 1.5. The problem encountered with extraction of the methoxy-*s*-triazines used as model compounds in this study was the quantification of the more polar compounds, e.g., simetone, was not reliable since it overlaps with the peaks from the molecules of the donor buffer solutions. Fig. 3.5 illustrates the increase in E_e throughout the experiment. It is also evident that since the enrichment factors calculated using

equation 1.3 agree with the values estimated using equation 1.4, the analyte accumulation effects in the membrane and thus the MME will be negligible.

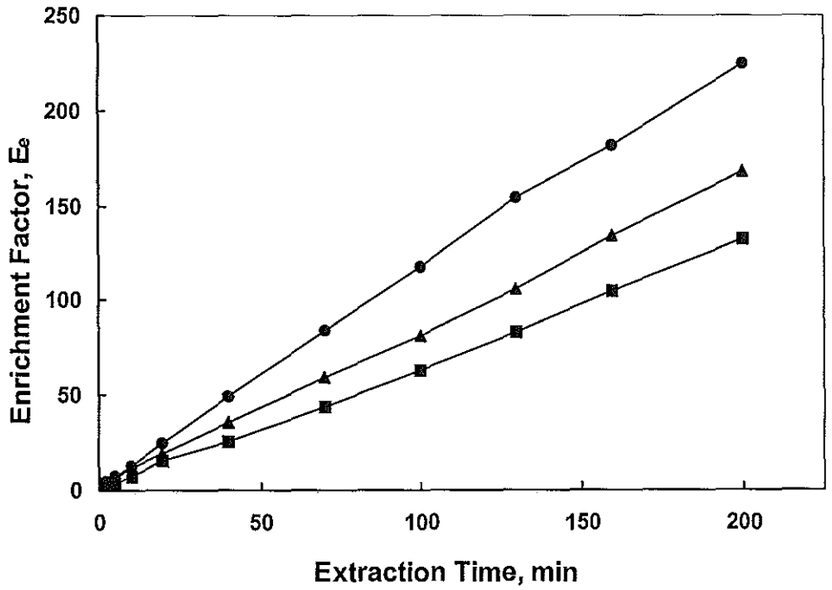


Fig. 3.5 E_e for the sample collected from the donor waste at certain time interval. See also text. Symbols: ◆- atratone, ▲–secbumetone, and ●-terbumetone.

3.6 Trapping Processes for Weak Bases in the SLM Extraction

3.6.1 Effect of the Degree of Trapping on Enrichment Factor. For basic compounds with $pK_a \leq 2$, (Table 1.1), the acceptor pH was found to be a limiting factor in the attainment of high E. This is because the condition for complete trapping in the acceptor solution, which requires an acceptor pH at least 3.3 units below the pK_a of the basic analyte [266], was not completely met. E decreased with time as the concentration of the partially ionized analytes increased in the stagnant acceptor solution.

The variation of E_e with sample volume at different concentration of sulphuric acid in the acceptor solution was studied for chloro-*s*-triazines, Fig. 3.6, and aniline derivatives, Fig. 3.7. In early extraction periods, the differences in E_e, for 0.1 M and 1.0 M H_2SO_4, is insignificant. However, the difference is more pronounced as the extracted sample volume increases, particularly for the least basic compounds, *viz.*, simazine and 3,5-dichloroaniline (Table 3.8). The results obtained are in good agreement with the developed theory that the amount of sample extracted increases with increase in the degree of trapping for basic analytes [266].

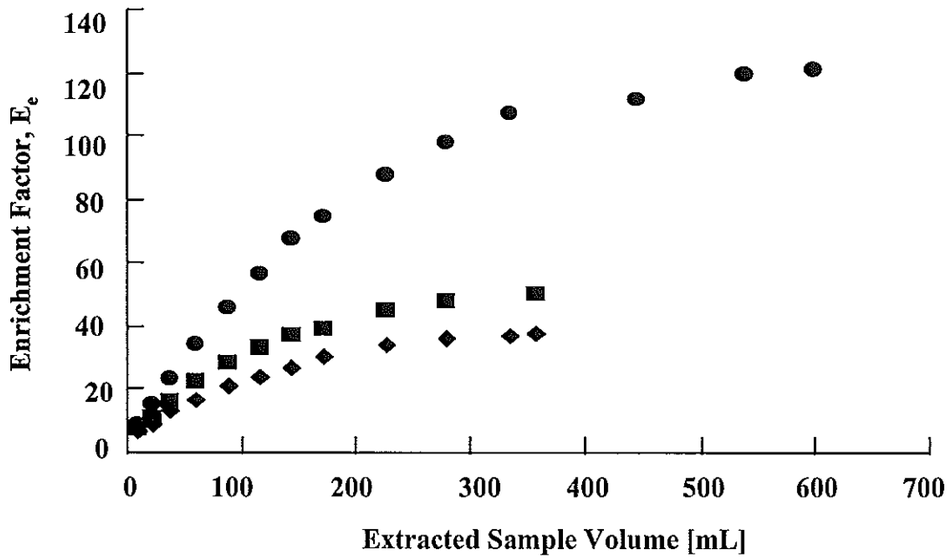


Fig. 3.6 Variation of the E_e with extracted sample volume for chloro-*s*-triazines (0.40 mg/l each). The acceptor solution contained 1.0 M sulphuric acid. Symbols: (◆) – simazine, (■) – atrazine, and (●) – terbuthylazine.

The degree of trapping could still be increases by increasing concentration of the acceptor acid, e.g., greater than 1 M H_2SO_4. The disadvantage with this approach is decreased stability for both the membrane and the analytes [9]. Furthermore, increasing viscosity and ionic strength of concentrated acceptor solution may also hamper permeation of the analytes from the membrane to the bulk of the acceptor solution.

3.6.2 The Maximum Enrichment Factor. In a co-operative study within the same group, various weak compounds were extracted [267] to verify some of the SLM theories, including the enrichment factor, developed by Jönsson *et al.* [266]. The maximum enrichment factor that can be attained may mainly be influence by the pH of the acceptor solution and the pK_a of the analyte, equations 1.7 and 2.5. To determine the maximum enrichment factor, various SLM systems were studied consisting of different types of weakly basic analytes of nearly constant ionic strength in the donor solution. These include chloro-*s*-triazines – representing an incomplete trapping; alkylthio-*s*-triazines – representing a complete trapping; and aniline derivatives – representing both of these situations.

In an incomplete trapping situation, Fig. 3.6, for simazine and atrazine the maximum enrichment factor was attained quite easily. They are more weakly basic and trapping in 1.0 M sulphuric acid is limited, which also agree well with Table 3.8, where at acceptor pH of 0.0, simazine has the highest α-value, while terbuthylazine has the least. As the extraction of analytes continues, the concentration of uncharged analytes in the acceptor builds up, which in turn decreases the flux across the membrane. Finally, the concentration of uncharged analytes in the acceptor solution and membrane equals that in the donor reaching an equilibrium and the flux ceases.

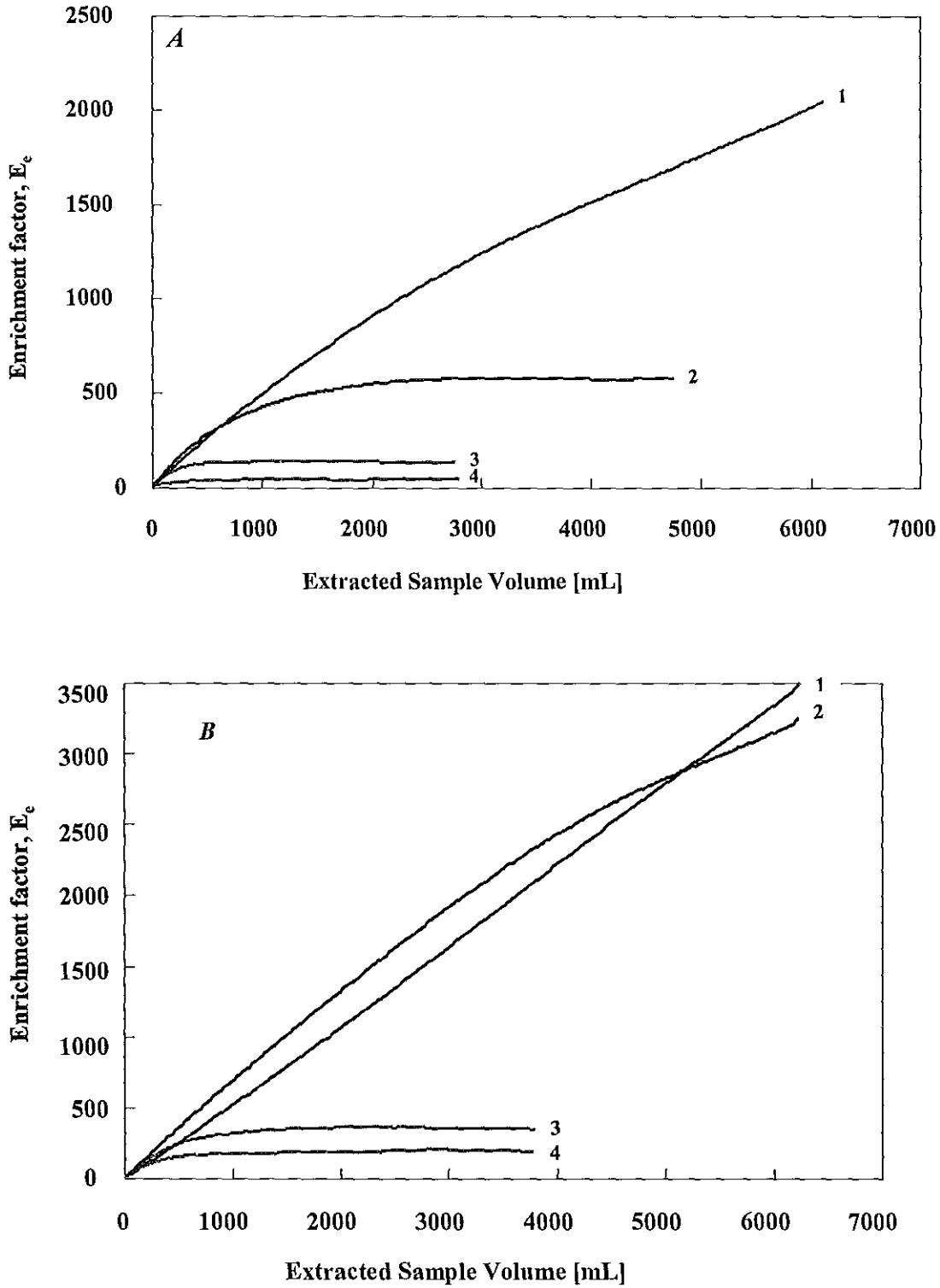


Fig. 3.7 Variation of E_e with extracted sample volume for aniline derivatives (0.10 mg/L each) at different concentrations of the acceptor solution: (A) 0.1 M sulphuric acid (pH ~ 1.0), and (B) 1.0 M sulphuric acid (pH ~ 0.0). Extracted compounds: (1) aniline, (2) 3-chloro-4-methylaniline, (3) 3,5-dichloroaniline, and (4) 3-methyl-5-nitroaniline.

Table 3.8 α_A-values at indicated pH and pK_a of the studied weak bases [9].

Compound studied	pK_a value	α_A-values at pH calculated (eqn. 2.5)		
		~1.0	*~0.7*	*~0.0*
Simazine	1.68		9.13×10^{-2}	2.04×10^{-2}
Atrazine	1.65		9.52×10^{-2}	2.13×10^{-2}
Terbuthylazine	2.0		4.57×10^{-2}	9.8×10^{-3}
Ametryn	4.1	6.82×10^{-4}		
Desmetryn	4.0	9.22×10^{-4}		
Dimethametryn	4.0	6.82×10^{-4}		
Terbutryn	4.3	4.62×10^{-4}		
Aniline	4.61	2.26×10^{-4}		2.42×10^{-5}
3-methyl-5-nitroaniline	2.34	4.21×10^{-2}		4.51×10^{-3}
3-chloro-4-methylaniline	3.97	9.86×10^{-4}		1.06×10^{-4}
3,5-dichloroaniline	2.48	2.96×10^{-2}		3.27×10^{-3}

N.B. For the aniline derivatives (the last four compounds) pK_a was calculated by ACD/pH (Advanced Chemistry Development, Inc. Toronto, Canada).

The more basic alkylthio-*s*-triazines have very low α-values at pH of about 1.0, Table 3.8, so the maximum enrichment factor was reached after longer extraction time. Furthermore, with all parameters kept constant, the compound with the smallest α-value should have the highest enrichment factor at a given extracted sample volume (Fig. 3.8). The results obtained for these compounds fulfilled this situation as seen in Fig. 3.8; desmetryn and terbutryn show the lowest and highest enrichment factors, respectively. However, ametryn is slightly more basic than dimethametryn (Table 3.8) but its enrichment factor is less than that of the latter. This apparent reverse in the trend could be attributed to differences in partition coefficients of the two compounds into the membrane

solvent. Dimethametryn is less polar than desmetryn and is therefore expected to dissolve more easily in *n*-undecane which was used as membrane solvent.

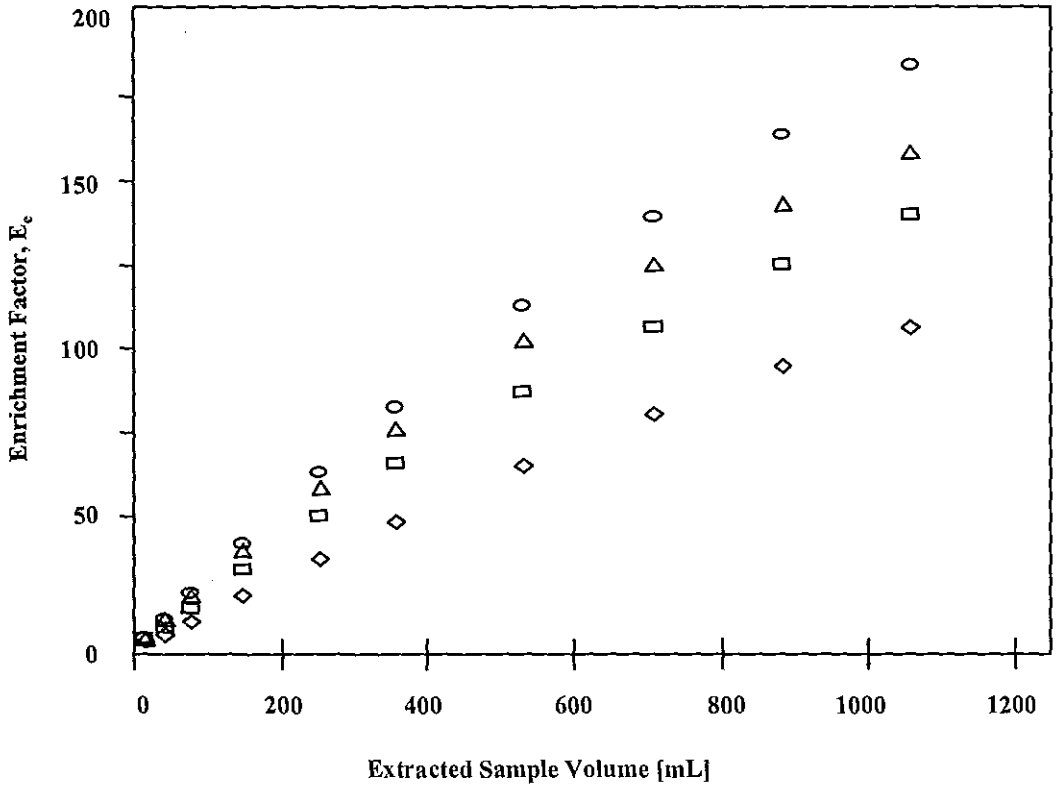


Fig. 3.8 Variation of E_e with extracted sample volume for alkylthio-*s*-triazines (0.25 mg/L). The acceptor solution contained 0.1 M sulphuric acid. Symbols: (△) – dimethametryn, (○) – terbutryn, (□) – ametryn, and (◇) – desmetryn.

The effect of pH of acceptor solution on the maximum enrichment factor was further demonstrated using some aniline derivatives extracted in 0.1 M (Fig. 3.7A), and 1.0 M (Fig. 3.7B) H_2SO_4. It can be seen that by increasing the acceptor acid concentration, up to 6000 mL of the two more basic aniline derivatives can be extracted without reaching a plateau on the curve of the enrichment factor. This observation seems attractive in the context of long time field sampling of these compounds.

3.6.3 Effect of Ionic Strength on Partition Coefficient and Enrichment Factor. One possibility for further increasing the enrichment factor of these weakly basic compounds would be increasing the ionic strength of the donor solution thereby increasing the K_D, equation 1.7. Within the ionic strength range investigated, the partition coefficient increases with ionic strength (Table 3.9). It can be noted that at constant ionic strength, the most hydrophobic compound, i.e., terbuthylazine, gave the highest partition coefficient, while for simazine it was the lowest. Increasing the ionic strength of the donor solution by addition of a salt was observed to increase the stability of the membrane liquid [246]. It suppresses the possible emulsion formation that has been noted as one of the major causes of the membrane instability.

The increase in ionic strength in the donor solution has also been seen to have a positive effect on the maximum enrichment factor (Table 3.9). The increase in enrichment factor as expected was highest at any given ionic strength with terbuthylazine. This agrees well with equation 1.7, since the enrichment factor is directly related to the partition coefficient of the analyte from the donor solution to the membrane liquid. As has been indicated in Fig. 3.9, in the initial extraction periods there are insignificant differences in enrichment factors for all the analytes at various ionic strengths. But as uncharged analytes build up in the acceptor phase, this difference becomes more evident, i.e., the concentration of uncharged analytes in the acceptor solution becomes predominant. The effect of increasing ionic strength on enrichment factor is therefore not beneficial for short times. For longer extractions it has an important analytical application since it allows processing of large volumes, especially where the analytes of interest may be present at trace levels.

Table 3.9 Comparison of maximum enrichment factors experimentally obtained from supported liquid membrane extraction ($E_{e(SLM)}$) and calculated from the measured partition coefficient $E_{e(Cal.)}$ (cf eqn. 1.7) at various ionic strengths for chloro-*s*-triazines. $K_{P(D)}$ and $K_{P(A)}$ are partition coefficients of the analytes into the organic liquid from the aqueous donor and acceptor solutions, respectively, measured as described in section 2. 9. (Each experiment was repeated at least 3 times).

	Ionic strength (M)	**Simazine**	**Atrazine**	**Terbuthylazine**
$K_{P(A)}$		13	44	300
$K_{P(D)}$	0.1	14	45	350
	0.7	21	80	610
	1.6	28	140	1120
	3.1	47	260	1990
$E_{e(SLM)}$	0.1	40	50	115
	0.7	76	92	210
	1.6	110	150	>370
	3.1	150	>147	>350
$E_{e(Cal.)}$	0.1	53	50	118
	0.7	79	90	210
	1.6	106	160	380
	3.1	180	290	680

Table 3.9, also compares the experimental maximum enrichment factor at stated ionic strengths with calculated values according to equation 1.7, using the K_A and K_D values determined. These are in good agreement. However, beyond an ionic strength of 3, the actual maximums showed indications of being less than the expected ones, suggesting

that in real SLM applications, there is an upper limit one can salt out the analytes from the donor solution to the membrane even under incomplete trapping conditions.

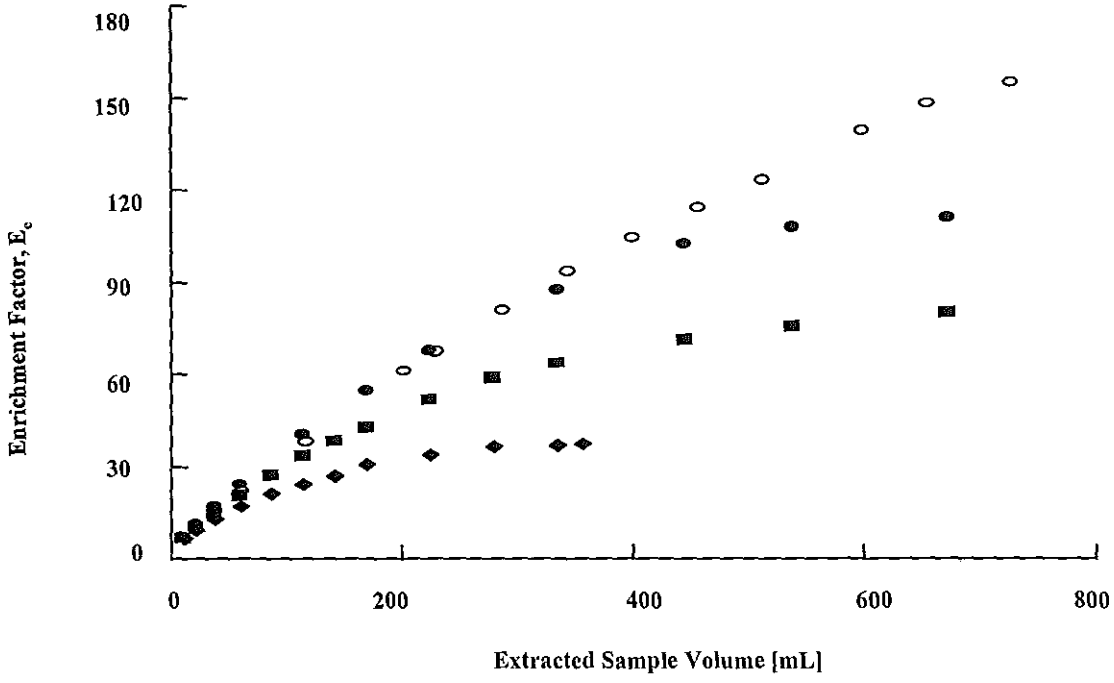


Fig. 3.9 Variation of E_e with extracted sample volume for simazine at different ionic strengths of the donor solution. The acceptor solution contained 1.0 M sulphuric acid. Symbols, representing the ionic strengths: (◆) – 0.23, (■) – 0.69; (●) – 1.59, and (○) – 3.1. (Similar behaviours have also been observed for atrazine and terbuthylazine).

3.7 Comparison of Selectivity of SLM with SPE

Aqueous sample solutions of the *s*-triazine compounds processed under identical conditions and spiked in reagent water and river water were extracted using both the liquid membrane and solid phase extraction column, to compare the selectivity of the extraction techniques at trace levels, e.g., 1.0 µg/L. The corresponding chromatograms for samples spiked in river water are given in Fig. 3.10. The methoxy-*s*-triazines have been selectively

extracted in the SLM sample preparation technique comparing with the SPE. In SPE interfering compounds from the river water were eluted along with the analytes of interest, Fig. 3.10A. In some cases, the peaks from these compounds were also made quantification of the more polar compound, simetone, difficult. To minimize the effects from these polar compounds or ions, the ionic strength of the phosphate buffer was kept to a minimum of 0.01 M. This approach also did not reduce the quantity of these peaks appearing at the early stage of the chromatogram. The chromatogram from the extracts of the liquid membrane, Fig. 3.10B, was not affected much since the membrane serves as a barrier for polar compounds and ions to enter the acceptor channel.

Improvement of selectivity of the SPE by additional clean-up, e.g., by elution of the less-retained interfering compounds with water modified with small amount of organic solvent [130, 271], is a common practice. Use of a C18 column in series with an ion-exchange column is also reported for other pesticides [153]. These experiments have not been carried out in these studies.

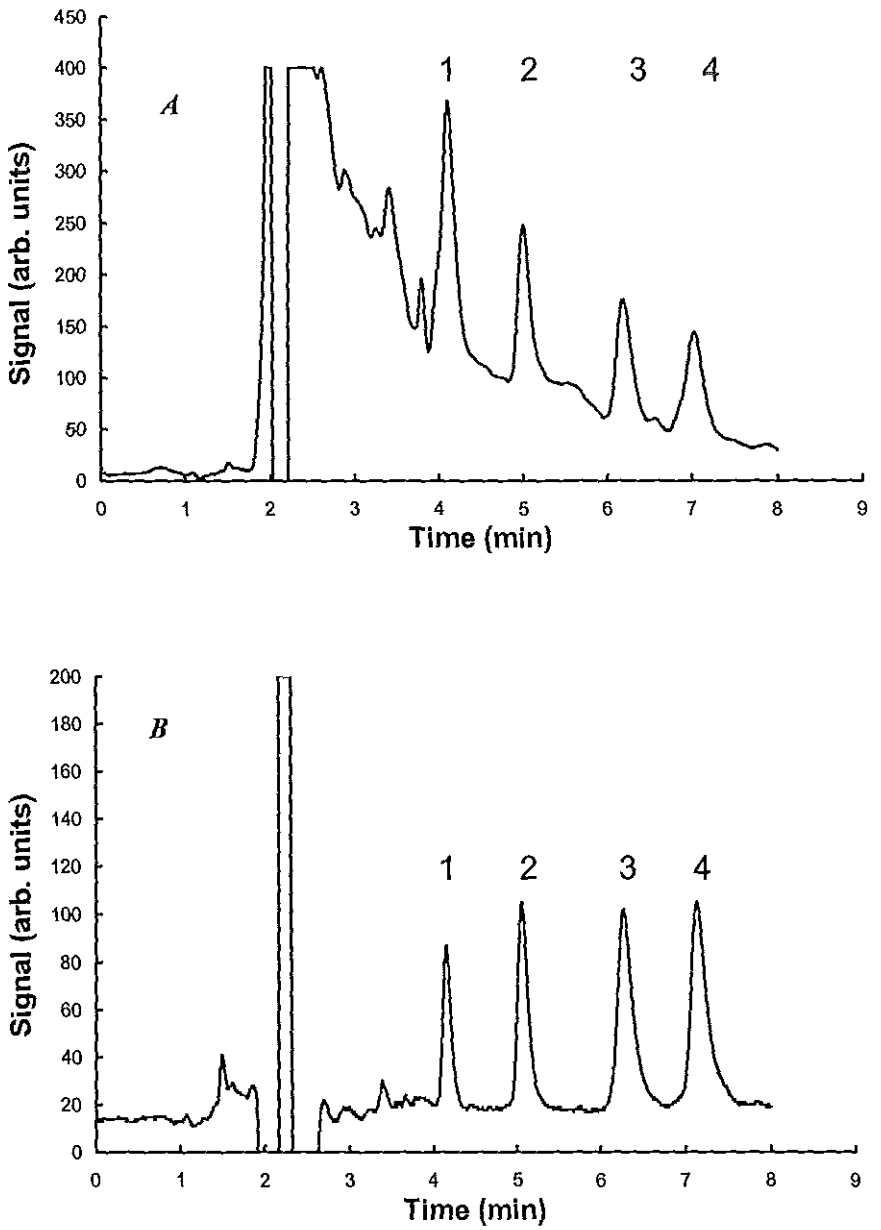


Fig. 3.10 Chromatograms (HPLC-UV) of the methoxy-*s*-triazine herbicides used as model compounds. River water samples spiked with the standard and extracted using SPE column, 1.0 μg/L (*A*), and SLM system, 0.5 μg/L (*B*). Peaks: (1) – simetone, (2) – atratone, (3) – secbumetone, and (4) – terbumetone.

3.8 Applications to the Southern Ethiopian Lake Water Samples

3.8.1 Quantification and Calibration Curve. Aqueous samples of *s*-triazine herbicides ranging in concentration from 0.05 mg/L to 0.5 mg/L, were extracted in both membrane solvents, *viz.*, di-*n*-hexylether and *n*-undecane. Concentrations of the extracted samples were quantified from standard injections of the corresponding analytes in acetonitrile. Based on the peak height measurements, calibration graphs were constructed for the extracts in both membrane solvents. All the compounds studied exhibited linear relationship of the detector response versus the concentration extracted, with insignificant intercepts and correlation coefficient of 0.998 or better. Representative curves for selected compounds are given in Fig. 3.11. The results obtained for propazine and prometone have been included in both Figs 3.11A and B, to compare the extractability of these compounds in both membrane solvents. The more polar propazine was better extracted in di-*n*-hexylether while the enrichment of prometone was well performed in both membranes. This observation may be attributed to the more basic nature of the latter, both compounds having similar structure, differing only with a substituent at the 2-position.

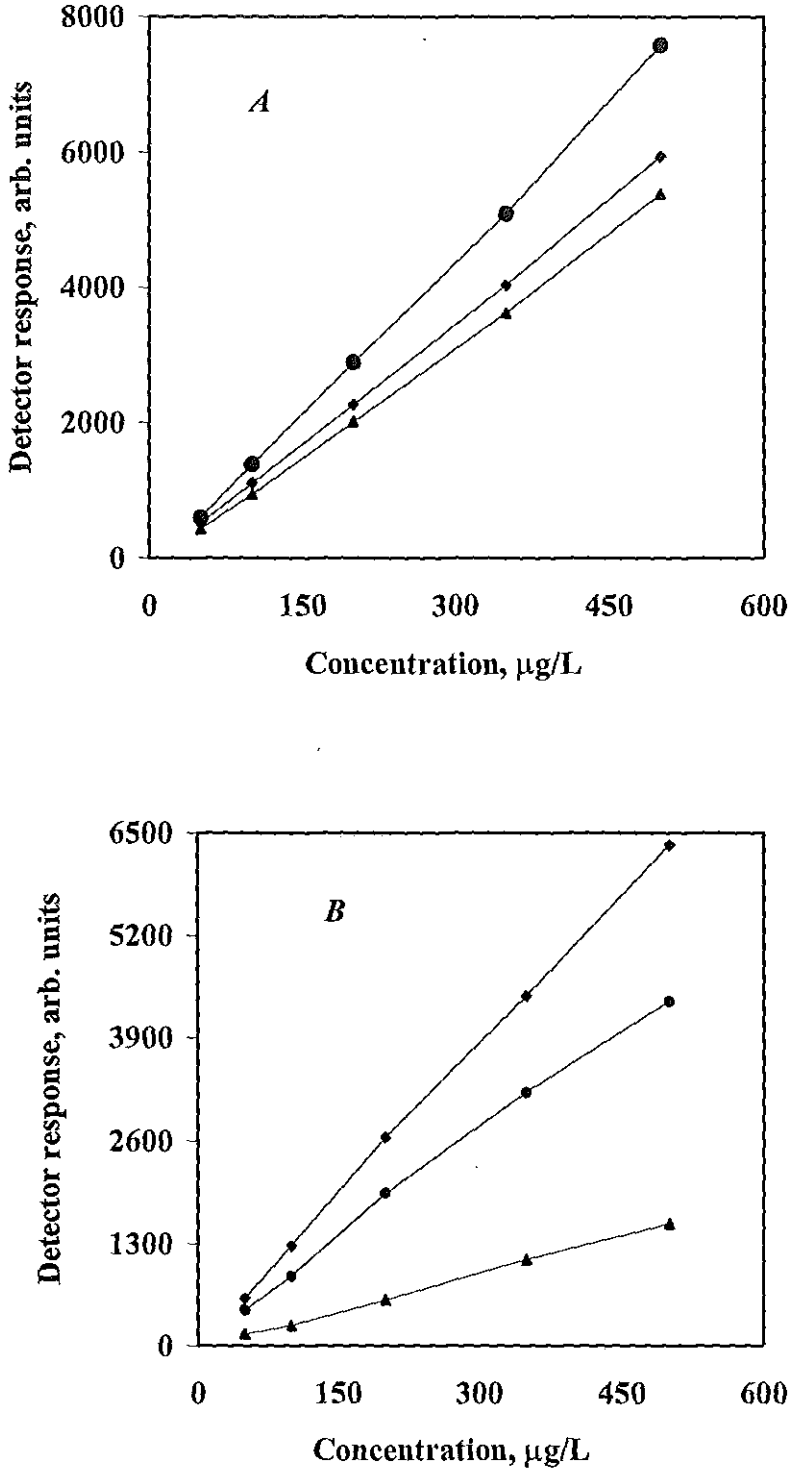


Fig. 3.11 Calibration graph for extraction of varied concentration of the sample mixture at donor flow rate of 1.0 mL/min. (*A*) di-*n*-hexylether, symbols: ◆ - atrazine, ● - prometone, and ▲ - propazine, and (*B*) *n*-undecane membrane solvents; symbols: ◆ - prometone, ● - terbutryn, and ▲ - propazine.

3.8.2 Performances and Detection Limits. The performance of the SLM extraction for trace quantities of *s*-triazine herbicide compounds from different water samples was investigated. Sample mixtures varying in concentrations at trace levels were spiked both in reagent water and river waters. To determine the limit of detection, premixed samples containing all classes of *s*-triazine herbicides, concentrations of 0.5 μg/L in one litre and 0.1 μg/L in three litres were extracted in both membrane solvents under identical experimental conditions at donor flow rate of 5.0 mL/min. The results in Table 3.10 show that for all the compounds studied, detection limits, calculated as three times the noise level, were low enough using both membrane solvents and varied trace level concentrations. This also suggests that lower sub-nanogram level of organic pollutants can be extracted and quantified using the SLM extraction technique. Similarly, low detection limits have also been quantified for specific class of *s*-triazine compounds in various matrices, and the corresponding results can be found from references 289 and 293. Typical chromatograms for trace level extracts of alkylthio-*s*-triazines are also given in Fig. 3.12.

Table 3.10 Limit of detection (LOD) for extraction of various volumes of the standard solutions at concentration levels of 0.5 µg/L and 0.1 µg/L, and donor flow rate of 5.0 mL/min.

Herbicide compound	**LOD, ng/L, for extraction of one litre standard solution**		**LOD, ng/L, for extraction of three litres of standard solution**	
	DHE*	***UDC****	***DHE***	***UDC***
Simazine	152	317	32	30
Atratone	5.9	10.5	3.4	3.4
Atrazine	10.3	34.6	2.3	2.2
Prometone	4.9	6.3	2.5	2.5
Propazine	207	*nd*	86	*nd*
Terbuthylazine	55	60.4	20	*nd*
Prometryn	4.3	5.6	2.3	2.2
Terbutryn	3.5	4.4	1.5	1.4

*DHE and UDC are abbreviations used for di-*n*-hexylether and *n*-undecane, respectively.

nd – represents not detected.

In a similar application, the limits of detection obtained from liquid membrane extractions were compared with those from the solid-phase extraction, for methoxy-*s*-triazine compounds. The results obtained for both techniques were comparable, with better selectivity for the SLM extraction as has also been discussed in section 3.7. To further assess the reliability of the liquid membrane extraction, sample mixtures spiked in reagent water and river water were extracted at lower, i.e., 1.0 mL/min, and higher, i.e., 7.5 mL/min, donor flow rates for about 270 min. Regardless of the origin of the water samples, the results obtained in both cases were similar [272, 276]. It was thus noted that the limit of detection was not affected when samples are contained in natural waters having complex

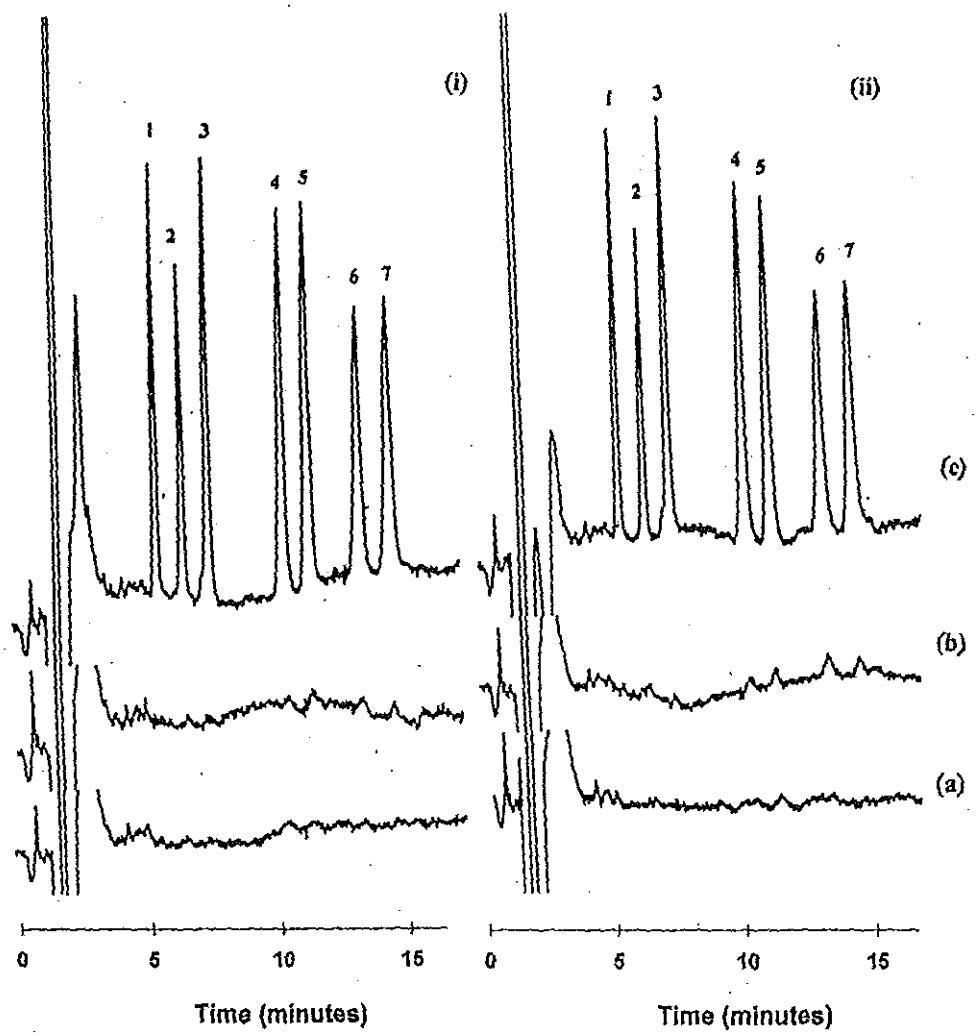


Fig. 3.12 Chromatograms (HPLC-UV) of 1.0 μg/L of the herbicide compounds studied. (i) in reagent water and (ii) spiked in river water. Samples (500 mL) were extracted at a donor flow rate of 7.0 mL/min and 50 μL of the extract were injected in each case. Peaks: (1) desmetryn, (2) metoprotryn, (3) ametryn, (4) prometryn, (5) terbutryn, (6) dimethametryn, and (7) dipropetryn. In both sets of chromatograms (a) denotes blank extraction, (b) extraction of donor buffer after sample extraction, and (c) sample extraction.

matrices of varying concentrations. Extractions at higher flow rate for fixed time improves the limit of detection twice or three times.

3.8.3 Determination of the Detection Limits in Lake Waters. The liquid membrane extraction methodology was applied to the extraction of samples in natural waters collected from Southern Ethiopian Lakes, *viz.*, Awassa, Chamo and Abbaya. In this region of the country, there is an extensive use of agrochemicals including herbicides and other pesticides, on various farms. These are sprayed by farm workers and airplane. One of the pesticides most frequently used as an active ingredient includes the *s*-triazine family, the formulation of which is prepared alone or in combination with other pesticides [306].

These agricultural fields are situated in close proximity to the lakes selected for the present study. There is a high probability for the residues of these compounds to enter the lakes by all possible means. Water bodies of the recipient lakes will thus be contaminated and may contain considerable quantity of these residues.

Water samples collected from these lakes (section 2.3) were extracted after some pre-processing steps including filtration and treatment with buffer. The quantities of *s*-triazines in these lakes have been estimated using equation 1.1. Atrazine and terbutryn were identified in Awassa Lake, while only atrazine could be quantified in Chamo Lake (Fig. 3.13). The quantities of these compounds estimated using equation 1.1 are given in Table 3.11. None of them was identified in the extracts from Lake Abbaya. Typical diode array spectra of terbutryn are given in Fig. 3.14, to confirm the identity of the compound. It is seen that these spectra, of the standard and lake water extracts, are identical. Similar spectra for atrazine were also obtained.

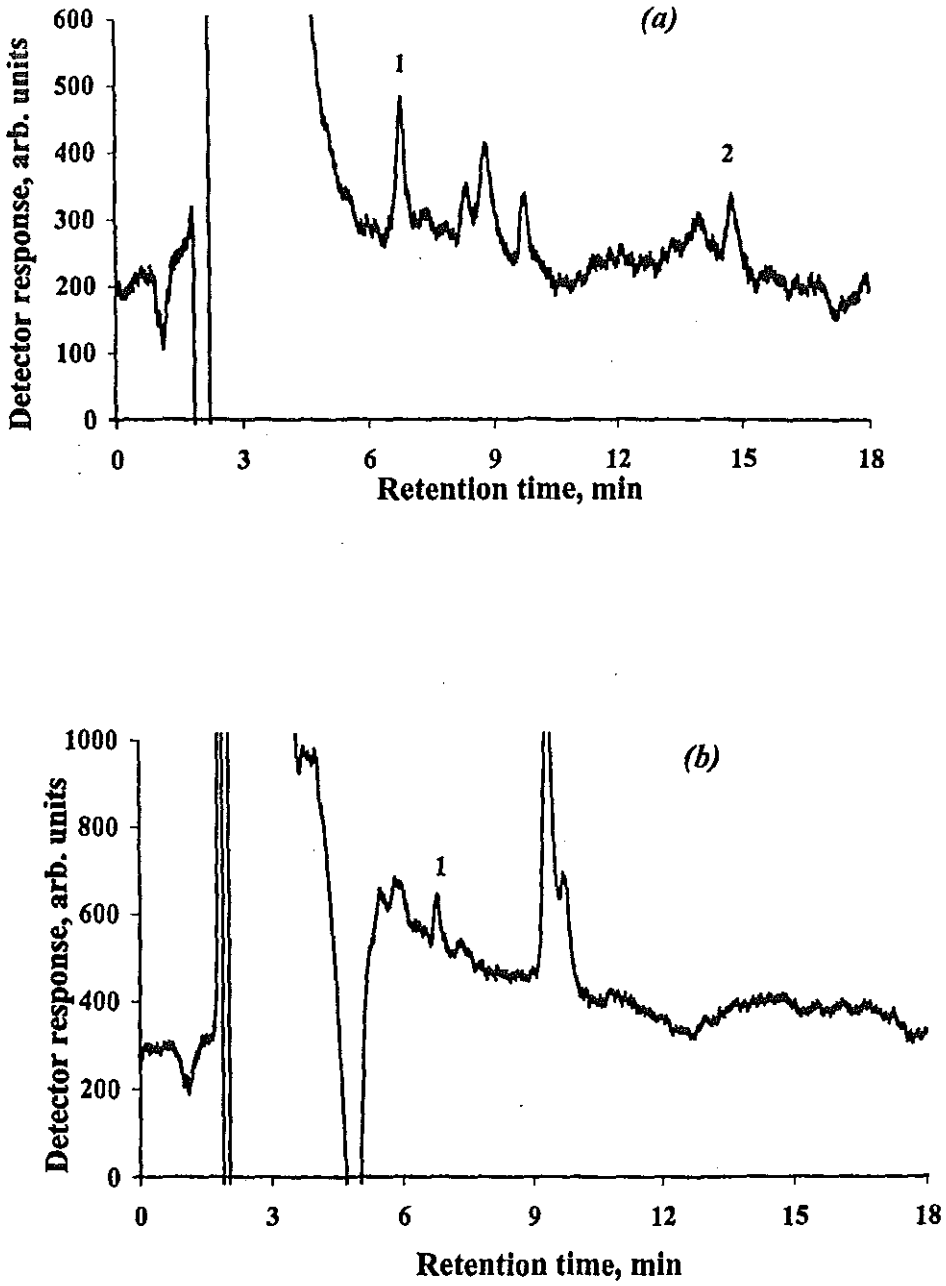


Fig. 3.13 Chromatograms (HPLC-UV) for the extracts of lake water samples: extraction conditions: donor flow rate – 5.0 mL/min; membrane solvent – di-*n*-hexylether; and sample extraction time – ten hours. Peaks (1) atrazine, and (2) terbutryn. Chromatograms for (a) extract from Awassa Lake, and (b) extract from Chamo Lake.

A closer examination of the locational variation of the herbicide distribution in Awassa Lake at different locations, for example, indicated that extracts from all sites were not always containing the residues. The samples collected from the bulk of the lake contained both atrazine and terbutryn, while only trace atrazine could be identified in the samples taken from the point of entrance of the tributary river. None of them could be seen in the sample extracts from the shore sides. This may be due to the insufficient lateral and vertical mixing of the lake water at the bank as has also been noted by Åkerblom [92]. Moreover, in none of the extracts from the third collection were the herbicides identified. This might be attributed to their degradation by various means, adsorption to plants or deposition on the sediment forming complexes with substances, e.g., metals, present in water. It can thus be generalized that the sampling, sample collection time and selection of the collection sites are very important variables when environmental monitoring experiments are to be carried out for analysis of *s*-triazine herbicides and probably residues of other pesticides in these areas.

Table 3.11 Concentrations, in μg/L, of atrazine and terbutryn determined in the extracts of waters from Awassa and Chamo Lakes applying the SLM extraction at the donor flow rate of 5.0 mL/min. DHE stands for di-*n*-hexylether and UDC for *n*-undecane.

***s*-Triazine herbicide**	**Extract from Awassa Lake*,**		**Extract from Chamo Lake*,**	
	DHE	***UDC***	***DHE***	***UDC***
Atrazine	6.7 ± 0.7 (6)	5.1 ± 2.3 (4)	5.3 ± 2.1 (3)	2.6 ± 1.9 (3)
Terbutryn	7.0 ± 3.0 (5)	8.1 ± 4.7 (4)	-	-

*Mean ± 95% confidence level for the mean values indicated in brackets.

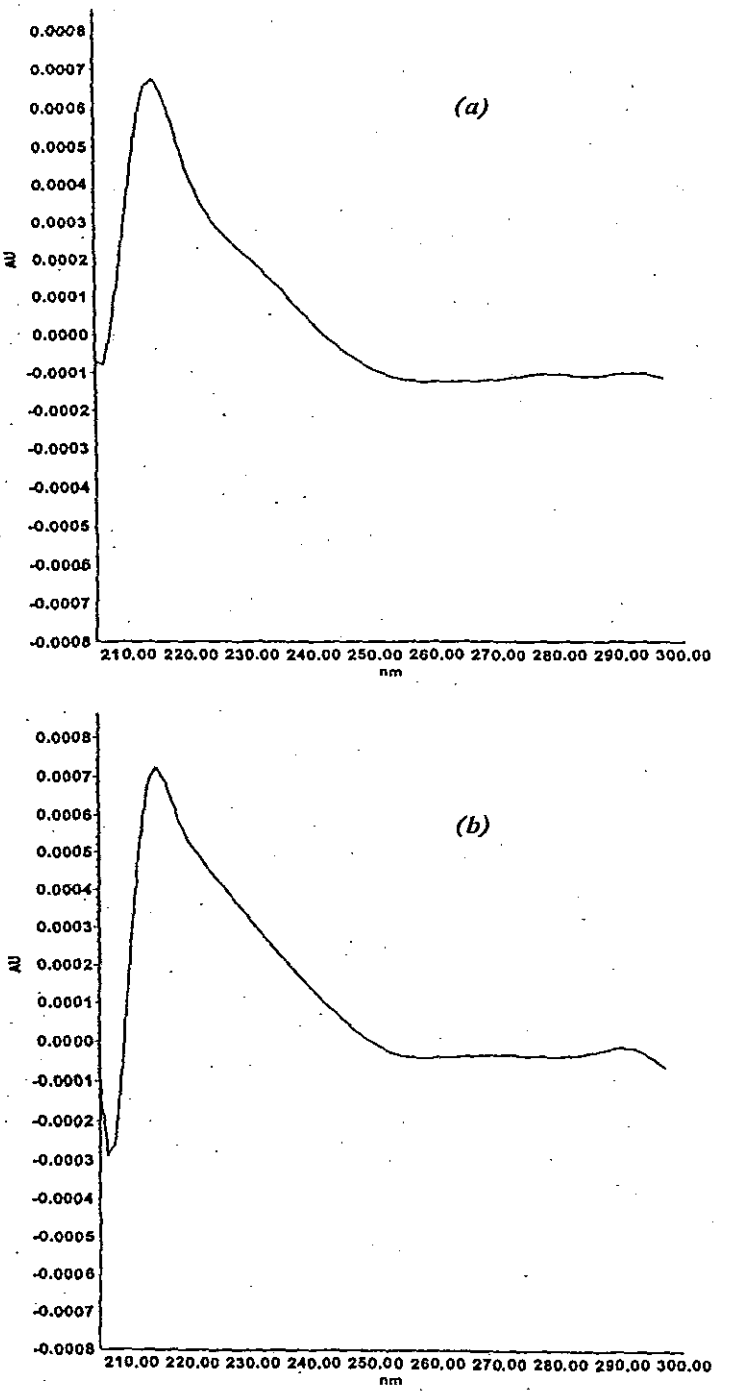


Fig. 3.14 UV-VIS spectra of the extracts of terbutryn. (a) Extract of 0.5 mg/L standard sample of terbutryn, diluted ten times, using di-*n*-hexylether membrane solvent. (b) Extract of Awassa Lake water with the same membrane solvent.

3.9 Method Development for SLM Extraction of the *s*-Triazine Degradation Products – Optimization of Parameters

3.9.1 Acceptor pH and Carriers. The degradation products and parent compounds of the *s*-triazine herbicides investigated have pK_a values ranging from 1.0 – 4.5 [9]. According to the theoretical predictions by Jönsson *et al.* [266], the acceptor solution pH must be at least 3.3 units below the lowest pK_a of the basic compounds for attaining maximum enrichment factor. This condition was not met for most of the compounds since acceptor solution of at least ~1.65 pH was required, and a pH value of ~0.0 was used (1.0 M HCl). As has also been discussed for the parent compounds in section 3.6, the condition of maximum enrichment factor may not easily be reached at trace level, and thus this condition allows processing of large sample volume [267].

A limited dissolution of polar compounds into the di-*n*-hexylether was reported to control the efficiency of the extraction process [275, 279, 286]. To alleviate this problem, TOPO was added to the membrane solvent in other applications [243]. In this study, various carriers have been employed, including TOPO, section 2.1, to enhance analyte dissolution in di-*n*-hexylether. The results obtained indicated that there were no significant differences between these carriers (Table 3.12). TOPO was chosen on the basis of its better solubility in the membrane solvent, so that its concentration could be increased further. Other carriers could not be dissolved in pure di-*n*-hexyether since they are too polar. Thus, 25% 6-undecanone was added, and even under this conditions only 1% (W/V) of the other carriers could be dissolved. Increasing the amount of 6-undecanone was not tried, since the results obtained using this membrane solvent was reported to be irreproducible, due to the stability problem [279]. Stability of the membrane, in such application, was very important since larger sample volumes should be processed for longer time.

Table 3.12 Effect of carriers on E for extraction of mixture of parent compounds and degradation products of *s*-triazine herbicides. Extraction conditions: time of extraction - 30 min; acceptor pH - ~0.0; donor pH – 7.0. UNC stands for 6-undecanone.

Compound	Membrane composition			
	DHE/UNC (75/25)%	***1% TOPO in DHE/UNC***	***1% DPSO in DHE/UNC***	***1% TPPO in DHE/UNC***
Deethyldeisopropylatrazine (DDA)	0.05 (0.45)	0.04 (0.25)	0.04 (0.29)	0.03 (-)
Deisopropylatrazine (DIA)	0.15 (0.14)	0.19 (0.19)	0.17 (0.12)	0.16 (0.13)
Deethylatrazine (DEA)	0.27 (0.15)	0.32 (0.10)	0.28 (0.11)	0.28 (0.07)
Hydroxyatrazine (ATOH	0.09 (0.29)	0.08 (0.27)	0.07 (0.30)	0.05 (0.40)
Hydroxypropazine (PROH)	0.04 (0.25)	0.05 (0.18)	0.03 (0.24)	0.02 (0.0)
Hydroxyterbuthylazine (TZOH)	0.05 (0.19)	0.08 (0.10)	0.04 (0.20)	0.02 (0.23)
Cyanazine (CYZN)	0.06 (0.54)	0.08 (0.56)	0.07 (0.34)	0.10 (0.28)
Atrazine (ATZN)	0.50 (0.09)	0.50 (0.06)	0.51 (0.10)	0.48 (0.10)
Prometryn (PRYN)	0.61 (0.18)	0.50 (0.07)	0.49 (0.07)	0.55 (0.22)
Terbutryn (TRYN)	0.59 (0.11)	0.42 (0.09)	0.40 (0.10)	0.48 (0.40)

The effects of variation in the amount of TOPO on E, both in pure di-*n*-hexylether and its mixture with 25% 6-undecanone were studied, and the results for the latter case are given in Fig. 3.15. It was seen that E increased with the amount of TOPO, except for DDA, cyanazine and the parent compounds. For DDA, since it is too polar, there was not much gain in E. Cyanazine is not as polar as DDA but it has lowest pK_a value, so the problem was incomplete trapping in the acceptor solution. Thus, increasing the amount of TOPO didn't bring about any general trend on the resulting E for this compound, i.e., relative standard deviation ranging from 28-56%. For these compounds, in general, E showed the

signs of decreasing with increase in TOPO concentration. This behaviour has also been noted by Shen *et al.* [243], and may be attributed to the slow mass transfer at the membrane and the acceptor phase interface which is associated with the more hydrophobic nature of the compounds.

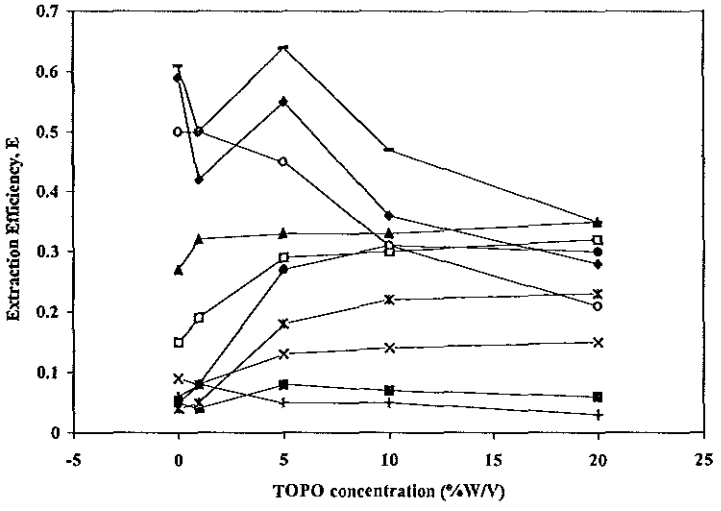


Fig. 3.15 E versus concentration of TOPO varied in the 75% di-*n*-hexylether and 25% 6-undecanone. Symbols: (■) - DDA; (▫) - DIA; (▲) - DEA; (X)- ATOH; (∗) - PROH; (•) - TZOH; (+) - CYZN; (∘) - ATZN; (–) - PRYN; and (♦) - TRYN.

3.9.2 Effect of Collection Time and Salt Addition on E. In the SLM extractions, it has been noted that allowing the membrane system to stand for some times is beneficial to allow sufficient transfer of analytes [278]. Moreover, studies by other workers [264, 313] indicate better stripping of analytes in the acceptor phase when both phases are flowing in a counter current way. For extraction of a mixture of degradation products and parent compounds of *s*-triazine herbicides, a waiting time of ten minutes was scheduled before collection of the acceptor plug. The results in Table 3.13 show that there was a general

tendency for E to increase with collection time, except for DDA and cyanazine, reflecting the degree of slow mass transfer into the acceptor phase for each compound studied. The gain in E was more pronounced with parent compounds which are more hydrophobic and therefore have slow mass transfer. For DDA since the problem is dissolution into the membrane, and not stripping into the acceptor phase, no significant gain in E was noted. Cyanazine, on the other hand, showed little gain in E and was almost not extracted at all with a stagnant donor phase while collection of the acceptor phase. The results on cyanazine suggest that enough ionization in the acceptor is perhaps the major creator of the concentration gradient across the three phases of the membrane. This is the driving force in

Table 3.13 Study of the effect of extract collection time, with donor flowing, on E. 10% TOPO in DHE was used as a membrane solvent.

Compound	**Collection time**			
	4 min, donor stagnant	***4 min, donor flowing***	***8 min, donor flowing***	***13 min, donor flowing***
DDA	0.11	0.14	0.12	0.12
DIA	0.30	0.34	0.33	0.30
DEA	0.28	0.36	0.36	0.37
ATOH	0.12	0.14	0.15	0.16
PROH	0.17	0.22	0.22	0.25
TZOH	0.24	0.29	0.30	0.33
CYZN	0.0	0.07	0.07	0.08
ATZN	0.16	0.28	0.35	0.39
PRYN	0.21	0.50	0.60	0.55
TRYN	0.18	0.49	0.54	0.54

the mass transfer since neither increased in collection time nor were positive results when both phases were flowing. The findings for DDA and cyanazine mean that the first condition to achieve high E across the membrane is enough dissolution into the membrane and sufficient trapping in the acceptor solution as previously noted [266]. For further work, a collection time of ten minutes was taken.

The effect of salt addition to enhance permeation was studied by varying the amount of sodium chloride added to the donor buffer (Table 3.14). The results reveal that for all analytes except DDA, cyanazine and hydroxy metabolites, there was slight gain in E with an increase in salt added. The increase of dissolution of analytes with salt concentration is expected in membrane extraction, and has also been observed by others [267, 314]. This may be due to increased partitioning of the analytes from the aqueous donor solution into the impregnated membrane solvent.

Table 3.14 Effect of the salt concentration in the donor solution on E. Membrane composition: 10 % TOPO in DHE, and all other conditions as in Table 3.12.

Compound	**Salt, NaCl, concentration**				
	0.0 M	***0.1 M***	***0.5 M***	***1.0 M***	***1.7 M***
DDA	0.06	0.08	0.06	0.09	0.09
DIA	0.21	0.21	0.23	0.27	0.34
DEA	0.27	0.29	0.29	0.37	0.49
ATOH	0.13	0.15	0.14	0.14	0.17
PROH	0.17	0.20	0.17	0.16	0.18
TZOH	0.24	0.26	0.24	0.22	0.24
CYZN	0.06	0.06	0.04	0.08	0.08
ATZN	0.33	0.33	0.37	0.41	0.21
PRYN	0.53	0.50	0.58	0.57	0.64
TRYN	0.46	0.39	0.50	0.50	0.56

3.10 Applications to Various Matrices Containing the *s*-Triazine Degradation Products

3.10.1 Extraction from Humic Acid and Environmental Waters. For trace level determination of micropollutants in the environment, it is important that the developed method is able to detect lower concentrations in sub μg/L levels. For environmental applications, since the sample volume is often unlimited, generally the approach is to extract larger volume.

In order to test the developed method, 3 litres of the reagent and river water both spiked at 1.0 μg/L levels of each compound were extracted at donor flow rate of 4.5 mL/min. Reagent water was also spiked with 10 mg/L humic acid and 2 μg/L of each of the analytes, and was then extracted in a similar way. No differences in enrichment factors were observed in both water samples. The results, especially for reagent water spiked with humic acid, showed that the method developed can readily be applied to surface water containing high amount of such matrices. Another important factor is the selectivity of the method towards matrices found in river water and humic acids. No significant amount of interfering peaks, originating from the matrices, were observed. This suggests the remarkable selectivity of the developed method towards humic acids despite the high sample volume extracted.

It is also of interest to see how the detection limit at various donor flow rates varies with polarity of each analyte. Ideally, with all other things equal, increased donor flow rate should be accompanied by an increase in the amount extracted per unit time and therefore lower detection limit. This is the trend observed for parent compounds which had their detection limits significantly lowered with an increase in donor flow rate [283]. For most polar metabolites, however, not much gain in detection limit resulted from increased donor

Table 3.15 Comparison of the limit of detection (LOD), in μg/L, and enrichment factor, E_e, at three donor flow rates in reagent water extracted for constant extraction time of 200 min. Membrane composition, 10% TOPO in DHE, and 120 μL injection of the extracts.

Compound	Limit of detection (LOD) and enrichment factor (E_e) at flow rate of					
	0.3 mL/min		*1.0 mL/min*		*5.0 mL/min*	
	LOD	E_e	*LOD*	E_e	*LOD*	E_e
DDA	-	-	-	-	-	-
DIA	2.8	0.9	0.6	4.5	0.8	3.2
DEA	1.1	1.6	0.3	6.9	0.3	7.2
ATOH	1.6	3.2	0.8	6.3	0.7	7.0
PROH	0.6	5.3	0.4	7.6	0.4	7.6
TZOH	0.5	7.5	0.4	8.6	0.4	9.5
CYZN	11.5	0.2	-	-	1.0	2.4
ATZN	0.4	6.2	0.2	11.2	0.07	32.6
PRYN	0.2	9.6	0.2	13.9	0.05	47.7
TRYN	0.2	9.0	0.2	9.8	0.04	40.7

flow rate (Table 3.15). The explanation for this could be that for the polar compounds, the decreased contact time and low partitioning into the membrane offsets the gain in the amount expected per unit time at higher donor flow rate. Therefore, the choice of the donor flow rate depends on the polarity of the compounds and the desired detection limits. Generally, for polar analytes, it is better to maximize E and therefore work at lower donor flow rate. For medium polar to hydrophobic compounds, the amount extracted per unit time could be maximized by working at higher donor flow rate. A Chromatogram representing the general separation condition is given in Fig. 3.16, for the river water extract spiked with 0.1 mg/L of all the compounds.

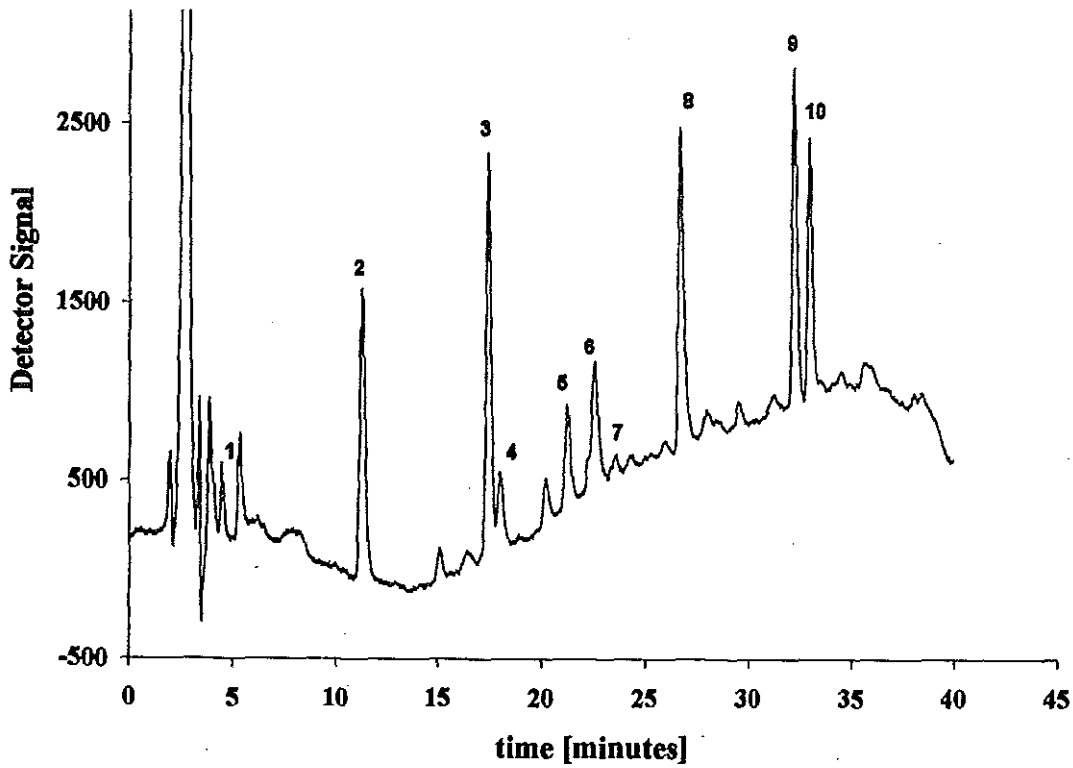


Fig. 3.16 Chromatogram (HPLC-UV) of the 0.1 mg/L of each compound spiked in river water and extracted using a di-*n*-hexylether membrane solvent with 10% TOPO. Donor flow rate – 1.0 mL/min and 200 min extraction time. Peaks: 1 – DDA; 2 – DIA; 3 – DEA; 4 – ATOH; 5 – PROH; 6 – TZOH; 7 – CYZN; 8 – ATZN; 9 – PRYN; and 10 – TRYN.

3.10.2 Extraction from Biological Samples. Sometimes organic pollutants are known to be determined in the urine of exposed workers [265]. This is because the amount that enters the body system normally does not accumulate but instead is released through the urine. It was therefore of interest to investigate whether the developed method can be used to extract these compounds spiked in urine. Urine from non-exposed people was collected, filtered, spiked with 10 μg/L of each analyte and one litre was extracted. In order to eliminate clogging of the flow system by the urine matrix, 27 nM of EDTA (ethylene

diamine tetra acetic acid) was added. Similar values of the enrichment factor and detection limit with those from other samples were obtained. This observation ensures the relative selectivity of the extraction technique. However, at the early stage of the resulting chromatograms, peaks originated from urine made detection of the more polar compound, DDA, difficult. This may be due the fact that some basic compounds, e.g., amines [265], can favourably be extracted in the membrane solvent containing TOPO. Thus, when such samples are extracted it is preferable to use the less polar solvents or di-*n*-hexylether without TOPO.

4. CONCLUSIONS

The use of supported liquid membrane (SLM) extraction technique for enrichment of trace level pollutants in environmental water samples has been demonstrated. Symmetrical triazine herbicides that are frequently occurring in natural waters were selectively extracted and a sub-nanogram quantities have been determined using HPLC-UV. The possibility of lowering the limit of detection of these compounds has been investigated by extracting lower concentrations at relatively higher flow rates. Trapping of the analytes in the acidic acceptor solution seems complete and the SLM technique is very suitable for enrichment of *s*-triazine compounds from complex matrices.

The extraction methodology is fairly selective compared to other sample preparation methods, e.g., solid phase extraction. The selectivity was further assessed by extracting analyte solutions containing the mixture of the parent compounds and the degradation products of *s*-triazine herbicides from river water, from aqueous system containing humic acid and urine samples collected from non-exposed persons. Moreover, conditions for attaining maximum enrichment factor were studied in detail to verify some of the theories of the SLM methodologies. A reasonably good enrichment has been achieved for extraction of water samples collected from Southern Ethiopian lakes. Low concentrations of the *s*-triazine herbicides contaminating the lake waters, of Awassa and Chamo, were successfully determined, even though the water samples from Abbaya Lake did not contain detectable quantities of these compounds. Thus, the SLM extraction is a powerful analytical technique for selectively enriching trace quantities of ionizable pollutants in environmental waters. However, to draw a more general conclusion about the locational and seasonal variation of these herbicides in the sites of the present study, it is desirable that a certain monitoring programme be performed. Parameters such as sampling techniques and time, collection sites and depth, and other physical and meteorological variables, etc. should be taken into consideration.

5. REFERENCES

1. V. F. Garry, J. T. Kelly, J. M. Sprafka, S. Edward and J. Griffith, *Environ. Health,* **49** (1994) 337.
2. W. J. Hayes and E. R. Laws, *Handbook of Pesticide Toxicology*, Vol. 1-3, Academic Press, San Diego, 1991.
3. C. A. Pio and A. Hall, in: *Instrument Analysis of Pollutants*, C. N. Hewitt, (Ed.), Ch.1, Elsevier Applied Science, New York, 1991.
4. G. Petrelli, G. Siepi, L. Miligi and P. Vineis, *Scand. J. Work Environ. Health,* **19** (1993) 63.
5. A. S. Rowland, *Epidemology,* **6** (1995) 6.
6. M. J. Coye, J. A. Lowe and K. T. Maddy, *J. Occup. Med.,* **28** (1986) 619.
7. M.-C. Hennion, V. Pichon and D. Barcelo, *Trends Anal. Chem.,* **13** (1994) 361.
8. F. J. Rodriguez-Plasencia, F. Navarro-Villoslada, L. V. Perez-Arribas and M. E. Leon-Gonzalez, *J. Chromatogr. A,* **760** (1997) 314.
9. V. Pacakova, K. Stulik and J. Jiskra, *J. Chromatogr. A,* **754** (1996) 17.
10. *Kirk-Othmer, Encyclopedia of Chemical Technology*, 3rd edn., Vol. 12, Wiley-Interscience, New York, 1980.
11. *Kirk-Othmer, Encyclopedia of Chemical Technology*, 4th edn., Vol. 13, John Wiley and Sons, New York, 1995.
12. P. G. McCracken and L. H. Gasper, in: *Encyclopedia of Chemical Processing and Design*, J. J. McKetta, (Ed.), Vol. 26, Marcel Dekker, New York, 1987, pp 205-222.
13. *McGraw-Hill Encyclopedia of Science and Technology*, 8th edn., Vol. 8, McGraw Hill, New York, 1997.

14. G. W. Bailey and J. L. White, *J. Agric. Food Chem.*, **12** (1964) 324.

15. R. S. C. Rao and R. E. Jessup, in: *Chemical Mobility and Reactivity in Soil Systems*, Soil Science Society of America, Madison, 1983, pp 183-201.

16. Hance, in: *Environmental Chemistry of Herbicides*, R. Grover, (Ed.), Vol. 1, CRC Press, Boca Rotan, 1980, pp 1-19.

17. R. E. Green, in: *Pesticides in Soil and Water*, W. D. Guenzi, (Ed.), Soil Science Society of America, Madison, 1974, pp 3-7.

18. A. W. Taylor and D. E. Glotfelty, in: *Rational Approach to Structure, Activity and Exotoxicology of Agrochemicals*, W. Draber and T. Fujita, (Eds.), CRC Press, Boca Rotan, 1992, pp 90-129.

19. D. E. Glotfelty and C. J. Schomburg, in: *Reactions and Movement of Organic Chemicals in Soils*, B. L. Sawhney and K. Brown, (Eds.), Soil Science Society of America, Madison, 1989, pp 305-334.

20. H. D. Skipper, V. V. Volk and R. Frech, *J. Agric. Food Chem.*, **24** (1976) 126.

21. A. J. Cessna and D. C. G. Muir, *Environmental Chemistry of Herbicides*, Vol. 2, CRC Press, Boca Rotan, 1991.

22. C. Tomlin (Ed.), *The Pesticide Manual,* 11th edn., British Crop Protection Council, UK, 1997.

23. M. D. Devine and M. H. V. Born, in: *Environmental Chemsitry of Herbicides*, A. Grover, A. J. Cessna, (Eds.), Vol. 2, CRC Press, Boca Rotan, 1991, pp 119-140.

24. A. S. Crafts, *The Chemistry and Mode of Action of Herbicides*, Wiley Interscience, New York, 1961.

25. J. D. Fryer and S. A. Evans, *Weed Control Handbook*, Vol. 1-2, Black Well, Oxford, 1968.

26. M. A. Loos, in: *Herbicides; Chemistry, Degradation and Mode of Action*, P. C. Kearney and D. D. Kaufman, (Eds.), Vol. 1, Marcel Dekker, New York, 1975, pp 2-128.

27. H. Geissbuhler, H. Martin and G. Voss, in: *Herbicides; Chemistry, Degradation and Mode of Action*, P. C. Kearney and D. D. Kaufman, (Eds.), Vol. 1, Marcel Dekker, New York, 1975, pp 209-291.

28. H. C. Bucha and C. W. Todd, *Science,* **114** (1951) 453.

29. J. A. Gardiner, in: *Herbicides; Chemistry, Degradation and Mode of Action*, P. C. Kearney and D. D. Kaufman, (Eds.), Vol. 1, Marcel Dekker, New York, 1975, pp 293-321.

30. G. Nilve, *Ph. D Thesis*, Lund University, Lund, 1992.

31. A. Calderbank and P. Slade, in: *Herbidies; Chemistry, Degradation and Mode of Action*, P. C. Kearney and D. D. Kaufman, (Eds.), Vol. 2, Marcel Dekker, New York, 1976, pp 501-540.

32. G. W. Probst, T. Golab and W. L. Wright, in: *Herbicides; Chemisty, Degradation and Mode of Action*, P. C. Kearney and D. D. Kaufman, (Eds.), Vol. 1, Marcel Dekker, New York, 1975, pp 453-500.

33. Anderson, in: *Environmental Chemistry of Herbicides*, R. Grover and A. J. Cessna, (Eds.), Vol. 2, CRC Press, Boca Raton, 1991, pp 201-298.

34. C. L. Foy, in: *Herbicides; Chemistry, Degradation and Mode of Action*, P. C. Kearney and D. D. Kaufman, (Eds.), Vol. 1, Marcel Dekker, New York, 1975, pp 399-452.

35. D. S. Frear, in: *Herbicides; Chemistry, Degradation and Mode of Action*, P. C. Kearney and D. D. Kaufman, (Eds.), Vol. 2, Marcel Dekker, New York, 1976, pp 541-607.

36. S. C. Fand, in: *Herbicides; Chemistry, Degradation and Mode of Action*, P. C. Kearney and D. D. Kaufman, (Eds.), Vol. 1, Marcel Dekker, New York, 1975, pp 323-398.

37. G. G. Still and R. A. Herrett, in: *Herbicides; Chemistry, Degradation and Mode of Action*, P. C. Kearney and D. D. Kaufman, (Eds.), Vol. 2, Marcel Dekker, New York, 1976, pp 609-664.

38. D. D. Kaufman, *J. Agric. Food Chem.*, **15** (1967) 582.

39. G. D. Paulson, M. M. Docktor, A. M. Jackobson and R. G. Zaylskic, *J. Agric. Food Chem.*, **20** (1972) 867.

40. A. H. M. Kirby, *World Rev. Pest Control,* **5** (1966) 30.

41. D. D. Kaufman, in: *Herbicides; Chemistry, Degradation and Mode of Action*, P. C. Kearney and D. D. Kaufman, (Eds.), Vol. 2, Marcel Dekker, New York, 1976, pp 665-709.

42. A. H. M. Kerby, E. L. Frick, L. D. Hunter and R. P. Tew, *Tetrahedron,* **20** (1964) 483.

43. A. S. Crafts, *Science,* **101** (1945) 417.

44. S. Matsunaka, *Residue Rev.*, **25** (1969) 45.

45. O. H. Esser, G. Dupius, C. Vogel and G. J. Marco, in: *Herbicides; Chemistry, Degradation and Mode of Action*, P. C. Kearney and D. D. Kaufman, (Eds.), Vol. 1, Marcel Dekker, New York, 1975, pp 129-208.

46. M. J. M. Wells, D. D. Riemer and W. C. Wellsknecht, *J. Chromatogr. A,* **659** (1994) 337.

47. C. W. Hubbs and T. L. Lavy, *Weed Sci.*, **38** (1990) 81.

48. M. M. Loux and K. D. Reese, *Weed Sci.*, **40** (1992) 490.

49. E. A. Woolson, in: *Herbicides; Chemistry, Degradation and mode of Action*, P. C. Kearney and D. D. Kaufman, (Eds.), Vol. 2, Marcel Dekker, New York, 1976, pp 741-776.

50. *World Pesticide Market,* FC Special Report Farm, *Chemicals,* **138** (1985) 45.

51. D. Barcelo, *J. Chromatogr.,* **643** (1993) 117.

52. J. Dean, G. Wade and I. J. Barnabas, *J. Chromatogr. A,* **733** (1996) 295.

53. T. M. Ward and J. B. Weber, *Spectrochim. Acta,* **25A** (1969) 1167.

54. G. Morimoto, *J. Chem. Soc. Japan,* **87** (1966) 790.

55. T. M. Ward and J. W. Weber, *J. Agric. Food Chem.,* **16** (1968) 959.

56. N. L. Wolge, R. G. Zepp, G. L. Baugham, R. C. Ficher and J. A. Gordon, US Environmental Protection Agency, Athens, GA, EPA - 600/3-76-067, 1976.

57. G.-C. Li and G. T. J. Felbeck, *Soil Sci.,* **14** (1972) 201.

58. N. Burkhand and J. A. Guta, *Pestic. Sci.,* **12** (1981) 45.

59. T. W. Jones, W. M. Kemp, J. C. Stevenson and J. C. Means, *J. Environ. Qual.,* **11** (1982) 632.

60. A. Geller, *Arch. Environ. Contam. Toxicol.,* **9** (1980) 289.

61. S. U. Khan, *Pestic. Sci.,* **9** (1978) 39.

62. K. K. Hatzios, in: *Environmental Chemistry of Herbicides*, R. Grover and A. J. Cessna, (Eds.), Vol. 2, CRC Press, Boca Raton, 1991, pp 141-185.

63. C. L. Tipton, R. R. Husted and F. H.-C. Tseo, *J. Agric. Food Chem.,* **19** (1971) 484.

64. H. R. Hamilton, *J. Agric. Food Chem.,* **12** (1964) 14.

65. P. Castelfrano and M. S. Brown, *Weeds,* **10** (1962) 131.

66. J. I. Willard and D. Penner, *Residue Rev.,* **64** (1976) 67.

67. R. H. Shimabukuro, in: *Weed Physiology*, S. O. Duke, (Ed.), Vol. 2, CRC Press, Boca Raton, 1985, pp 215.

68. Z. D. Tadic and S. K. Ries, *J. Agric. Food Chem.*, **19** (1971) 46.

69. M. S. Mills and E. M. Thurman, *Environ. Sci. Technol.*, **28** (1994) 73.

70. E. M. Thurman, M. T. Meyer, M. Pomes, C. A. Perry and A. P. Schwab, *Anal. Chem.*, **62** (1990) 2043.

71. P. C. Jocelyn, *Biochemistry of the SH Group,* Academic Press, New York, 1972.

72. K. K. Hatzios and D. Penner, *Metabolism of Herbicides in Higher Plants*, Burgess Publisher, Minneapolis, 1982.

73. R. H. Shimabukuro, G. L. Lamoureux and D. S. Frear, in: *Chemistry and Action of Herbicide Antidotes*, F. M. Pallos and J. E. Casida, (Eds.), Academic press, New York, 1978.

74. F. M. Garfield, *J. Assoc. Off. Anal. Chem.*, **72** (1989) 405.

75. S. K. Poole, T. A. Dean, J. W. Oudesema and C. F. Poole, *Anal. Chim. Acta,* **236** (1990) 3.

76. M. Åkerblom and J. R. Cox, in: *World Directory of Pesticide Control Organization*, G. Ekström, (Ed.), Royal Society of Chemistry, Cambridge, 1996, pp 19-41.

77. C. J. Koester and R. E. Cement, *Crit. Rev. Anal. Chem.*, **24** (1993) 263.

78. J. R. Dean, *Extraction Methods for Environmental Analysis*, John Wiley and Sons, Chichester, 1998.

79. M. Csuros, *Environmental Sampling and Analysis, Lab manual*, Lewis Publishers, New York, 1997.

80. M. J. Barcelona, in: *Principles of Environmental Sampling*, L. H. Keith, (Ed.), American Chemical Society, New York, 1988, pp 3-23.

81. J. E. Norris, in: *Principle of Environmental Sampling*, L. H. Keith, (Ed.), American Chemical Society, New York, 1988, pp 247-253.

82. L. Mathiasson, G. Nilve and B. Ulen, *Int. J. Environ. Anal. Chem.*, **45** (1991) 117.

83. M. Knutsson, G. Nilve, L. Mathiasson and J. Å. Jönsson, *J. Agric. Food Chem.*, **40** (1992) 2413.

84. J. Å. Jönsson and L. Mathiasson, *Trends Anal. Chem.*, **11** (1992) 106.

85. J. Å. Jönsson and L. Mathiasson, *Trends Anal. Chem.*, **18** (1999) 318.

86. F. I. Onuska, *J. High Resol. Chromatogr.*, **12** (1989R) 4.

87. M.-C. Hennion, *Trends Anal. Chem.*, **10** (1991) 317.

88. M. W. F. Nielen, R. W. Frei and U. A. T. Brinkman, in: *Selective Sample Handling in High Performance Liquid Chromatography, Part A*, R. W. Frei and K. Zech, (Eds.), Elsevier Science Publisher, Amsterdam, 1988, pp 5.

89. M.-C. Hennion and P. P. Scribe, in: *Environmental Analysis. Techniques, Applications and Quality Assurance*, D. Barcelo, (Ed.), Elsevier Science Publisher, Amsterdam, 1993, pp 23-77.

90. J. J. Vreuls, A. J. H. Louter and U. A. Th. Brinkman, *J. Chromatogr. A,* **856** (1999) 279.

91. M.-C. Hennion, *J. Chromatogr. A,* **856** (1999) 3.

92. M. Åkerblom, in: *Analysis of Pesticides in Ground and Surface Water*, H. J. Stan, (Ed.), Vol. 11, Springer, Berlin, 1995, pp 19-66.

93. M. Knutsson, *Ph. D Thesis*, Lund University, Lund, 1995.

94. G. R. van der Hoff, R. A. Baumann, U. A. Th. Brinkman and P. van Zoonen, *J. Chromatogr.*, **644** (1993) 367.

95. R. Heyer, A. Zapf and H.-J. Stan, *Fresenius' Z. Anal. Chem.*, **351** (1995) 752.

96. T. L. Potter, T. Carpenter, R. Putnam, K. Reddy and J. M. Clark, *J. Agric. Food Chem.*, **39** (1991) 2184.

97. E. C. Goosens, R. G. Bunschoten, V. Engelen, D. de Jong and J. H. M. van den Berg, *J. High Resolut. Chromatogr.*, **13** (1990) 438.

98. A. Farran, J. L. Cortina, J. de Pablo and D. Barcelo, *Anal. Chim. Acta,* **234** (1990) 119.

99. H. B. Lee and Y. D. Stocker, *J. Assoc. Off. Anal. Chem.,* **69** (1986) 568.

100. *US Environmental Protection Agency*, EPA 500 Series, Method 507 (Revision 2.0), 1989.

101. W. Specht, S. Pelz and W. Gilsbath, *Fresenius' Z. Anal. Chem.,* **353** (1995) 183.

102. H. Lee and A. S. Y. Chau, *J. Assoc. Off. Anal. Chem.,* **66** (1983) 651.

103. R. Alzaga, G. Durand, D. Barcelo and J. M. Bayona, *Chromatographia,* **38** (1994) 502.

104. G. Durand, V. Bouvot and D. Barcelo, *J. Chromatogr.,* **607** (1992) 319.

105. G. D. Foster, P. M. Gates, W. T. Foreman, S. W. McKenzie and F. A. Rinella, *Environ. Sci. Technol.,* **27** (1993) 1911.

106. V. Lopez-Avila, P. Hirata, S. Kiraska, M. Flanagan, J. H. J. Taylor and S. C. Hern, *Anal. Chem.,* **57** (1985) 2797.

107. H. Sabik, S. Cooper, P. Lafrance and J. Fournier, *Talanta,* **42** (1995) 717.

108. M. Schiavon, *Ecotox. Environ. Safety,* **15** (1988) 46.

109. M. Grandet, L. Weil and K.-E. Quentin, *Z. Wasser-Abwasser,* **21** (1988) 21.

110. R. M. Johnson and A. P. Pepperman, *J. Liquid Chromatogr.,* **18** (1995) 739.

111. E. M. Thurman and M. S. Mills, in: *Solid Phase Extraction: Principle and Practice in Chemical Analysis; A Series of Monographs on Analytical Chemistry and Its Application*, J. D. Winefordner, (Ed.), John Wiley and Sons, New York, 1998.

112. F. Mangani and F. Bruner, *Chromatographia,* **17** (1983) 377.

113. A. Di Corcia, M. Marchetti and R. Santperi, *J. Chromatogr.,* **405** (1987) 357.

114. M. Batissta, A. Di Corcia and M. Marchatti, *Anal. Chem.*, **61** (1989) 935.

115. M. Berg, S. R. Muller and R. P. Schwarzsnbsch, *Anal. Chem.*, **67** (1995) 1860.

116. G. Font, J. Manes, J. C. Molto and Y. Pico, *J. Chromatogr.*, **643** (1993) 135.

117. C. Crepso, R. M. Marce and F. Borrull, *J. Chromatogr. A*, **670** (1994) 135.

118. I. Liska, J. Krupcik and P. A. Leclercq, *J. High Resolut. Chromatogr.*, **12** (1989R) 577.

119. J. Sherma, *Anal. Chem.*, **59** (1987) 18.

120. J. C. Molto, Y. Pico, G. Font and J. Manes, *J. Chromatogr.*, **555** (1991) 137.

121. F. Mangani, G. Cresentini, P. Palma and F. Bruner, *J. Chromatogr.*, **452** (1988) 58.

122. S. Coppi and A. Betti, *J. Chromatogr.*, **472** (1989) 118.

123. A. Balinova, *J. Chromatogr.*, **643** (1989) 203.

124. J. Richard and G. A. Junk, *Mikrochim. Acta*, **1** (1986) 387.

125. L. M. Davi, M. Baldi, L. Penazzi and M. Liboni, *Pestic. Sci.*, **35** (1992) 63.

126. M. S. Mills, E. M. Thurman and M. J. Pedersen, *J. Chromatogr.*, **629** (1993) 11.

127. I. Liska, *J. Chromatogr. A*, **655** (1993) 163.

128. R. W. Frei, M. W. F. Nielen and U. A. Th. Brinkman, *Int. J. Environ. Anal. Chem.*, **25** (1986) 3.

129. S. Chiron, S. Papilloud, W. Haerdi and D. Barcelo, *Anal. Chem.*, **67** (1995) 1637.

130. V. Coquart and M.-C. Hennion, *J. Chromatogr.*, **585** (1991) 67.

131. E. R. Brouwer, I. Liska, R. B. Geerdink, P. C. M. Frintrop, W. H. Mulder, H. Lingeman and U. A. Th. Brinkman, *Chromatographia*, **32** (1991) 445.

132. M. W. F. Nielen, U. A. Th. Brinkman and R. W. Frei, *Anal. Chem.*, **57** (1985) 806.

133. J. J. Vreuls, G. J. de Jong, R. T. Ghisen and U. A. Th. Brinkman, *J. Assoc. Off. Anal. Chem.*, **77** (1994) 306.

134. P. J. M. Kwakman, J. J. Vreuls, U. A. Th. Brinkman and R. J. Ghijsen, *Chromatographia,* **34** (1992) 41.

135. J. J. Vreuls, A.-J. Bulterman, R. T. Ghijsen and U. A. Th. Brinkman, *Analyst,* **117** (1991) 1701.

136. R. G. Belardi and J. Pawliszyn, *Water Pollut. Res. J. Can.,* **24** (1989) 179.

137. C. L. Arthur and J. Pawliszyn, *Anal. Chem.,* **62** (1990) 2145.

138. J. Pawliszyn, *Trends Anal. Chem.,* **14** (1995) 113.

139. Z. Zhang, M. J. Yang and J. Pawliszyn, *Anal. Chem.,* **66** (1994R) 844.

140. A. A. Boyd-Boland, M. Chai, Z. Zhang, M. J. Yang, J. Pawliszyn and T. Gorecki, *Environ. Sci. Technol.,* **28** (1994) 568.

141. Z. Zhang and J. Pawliszyn, *J. High Resolut. Chromatogr.,* **66** (1993) 689.

142. J. Pawliszyn, *Sold Phase Microextraction: Theory and Practice,* Wiley - VCH, New York, 1997.

143. J. Tekel and J. Kovacicova, *J. Chromatogr.,* **643** (1993) 291.

144. J. W. Eichelberger, T. D. Behymer and W. L. Budde, EPA Method 525, Environmental Monitoring Systems Laboratory, US Environmental Protection Agency, Cincinnati, 1988.

145. M. Popl, Z. Voznakova, V. Tartar and J. Strnadova, *J. Chromatogr. Sci.,* **21** (1983) 39.

146. G. Durand and D. Barcelo, *Talanta,* **40** (1993) 1665.

147. M. Psathaki, E. Manaussaridon and E. G. Stephanou, *J. Chromatogr. A,* **667** (1994) 241.

148. P. Vitali, E. Venturini, C. Bonora, R. Calori and R. Raffaelli, *J. Chromatogr. A,* **660** (1994) 219.

149. C. C. J. Land, *LC-GC Intern.,* **7** (1994) 215.

150. M. T. Meyer, M. S. Mills and E. M. Thurman, *J. Chromatogr.*, **629** (1993) 55.

151. H. R. Buser, *Environ. Sci. Technol.*, **24** (1990) 1049.

152. T. R. Steinheimer, *J. Agric. Food Chem.*, **41** (1993) 588.

153. D. Volmer, K. Levsen and G. Wunsch, *J. Chromatogr. A,* **660** (1994) 231.

154. V. Pichon and M.-C. Hennion, *J. Chromatogr. A,* **665** (1994) 269.

155. R. N. Lerch and W. W. Donald, *J. Agric. Food Chem.*, **42** (1994) 922.

156. D. A. Cassada, W. W. Spalding, Z. Cai and M. L. Gross, *Anal. Chim. Acta,* **287** (1994) 7.

157. E. R. Brouwer, H. Lingeman and U. A. Th. Brinkman, *Chromatographia,* **29** (1990) 415.

158. T. W. King and J. E. France, in: *Analysis with Supercritical Fluids: Extraction and Chromatography*, B, Wenclawiak, (Ed.), Springer-Verlag, Berlin, 1992, pp 32-60.

159. S. Papilloud and W. Haerdi, *Chmatographia,* **38** (1994) 514.

160. T. Berglöf, *Ph. D Thesis*, Swedish University of Agricultural Science, Uppsala, 1997.

161. A. L. Blilie and J. J. Greibrock, *Anal. Chem.*, **57** (1985) 2239.

162. J. G. M. Janssen, P. J. Schoenmakers and C. A. Cramers, *J. High Resolut. Chromatogr.*, **12** (1990) 645.

163. C. R. Yonker and R. D. Smith, *J. Chromatogr.*, **361** (1986) 25.

164. C. R. Yonker, D. G. McMinn, B. W. Wright and R. D. Smith, *J. Chromatogr.*, **396** (1987) 19.

165. C. R. Yonker and R. D. Smith, *Anal. Chem.*, **59** (1987) 727.

166. S. Schmidt, L. G. Blomberg and E. R. Campbel, *Chromatographia,* **25** (1988) 775.

167. L. Karlsson, *Ph. D Thesis*, Lund University, Lund, 1993.

168. J. J. Langenfeld, S. B. Hawthorne, J. D. Miller and J. Pawliszyn, *Anal. Chem.*, **66** (1994) 909.

169. T. Berglöf, G. Jonsall and J. D. Markides, *J. Chromatogr. Sci.*, **37** (1999) 400.

170. H.-G. Janssen and X. Lou, in: *Extraction Methods in Organic Analysis*, A. J. Handley, (Ed.), CRC Press, Boca Raton, 1999, pp 100-145.

171. C. D. Bevan and P. S. Marshall, *Nat. Prod. Rept.*, **11** (1994) 451.

172. R. M. Smith and M. D. Burford, *J. Chromatogr. Sci.*, **32** (1994) 265.

173. D. M. Heaton, K. D. Bartle, C. M. Rayner and A. A. Clifford, *J. High Resolut. Chromatogr.*, **16** (1993) 666.

174. M. Vacarcel and M. T. Tena, *Fresenius' Z. Anal. Chem.*, **358** (1997) 561.

175. H. S. Ho and W. L. Budde, *Anal. Chem.*, **66** (1994) 3716.

176. I. J. Barnabas, J. R. Dean, S. M. Hitchen and S. P. Owen, *Anal. Chim. Acta*, **291** (1994) 261.

177. I. J. Barnabas, J. R. Dean, S. M. Hitchen and S. P. Owen, *J. Chromatogr. A*, **665** (1994) 307.

178. J. Janda, K. D. Bartle and A. A. Clifford, *J. Chromatogr.*, **642** (1993) 283.

179. E. G. van der Velde, M. R. Ramlal, A. C. van Beuzekom and R. Hoogerbrugge, *J. Chromatogr. A*, **683** (1994) 125.

180. V. Lopez-Zvila, C. Charan and W. F. Beckert, *Trends Anal. Chem.*, **13** (1994) 118.

181. T. R. Steinheimer, R. L. Pfeiffer and K. D. Scoggin, *Anal. Chem.*, **66** (1994) 645.

182. S. J. Lehotay, *J. Chromatogr. A*, **785** (1997) 289.

183. R. M. Smith, *J. Chromatogr. A*, **856** (1999) 83.

184. I. A. Stuart, J. MacLachlan and A. McNaughtan, *Analyst*, **121** (1996) R11.

185. I. J. Barnabas, J. R. Dean, S. M. Hitchen and P. Owen, *J. Chromatogr. A*, **32** (1994) 547.

186. J. S. Ho, P. H. Tang, J. W. Eichelberger and W. L. Buddle, *J. Chromatogr. Sci.,* **33** (1995) 1.

187. N. C. van de Merbel, *J. Chromatogr. A,* **856** (1999) 55.

188. A. T. Anderson, K. E. Rasmussen and H. E. Rugstad, *J. Chromatogr.,* **621** (1993) 189.

189. K. Johansen, K. Krogh, A. T. Andersen, A. S. Christophersen, G. Lehne and K. E. Rasmussen, *J. Chromatogr. B,* **669** (1995) 281.

190. A. T. Andersen, M. Krogh and K. E. Rasmussen, *J. Chromatogr.,* **582** (1992) 123.

191. J. D. H. Cooper, C. T. C. Fook Sheung and A. R. Buick, *J. Chromatogr. B,* **652** (1994) 15.

192. E. Verette, F. Quin and F. Mangani, *J. Chromatogr. A,* **705** (1995) 195.

193. D. Heems, G. Luck, C. Fraudeau and E. Verette, *J. Chromatogr. A,* **798** (1998) 9.

194. M. M. L. Aerts, W. M. J. Beek and U. A. Th. Brinkman, *J. Chromatogr.,* **500** (1990) 453.

195. G. Y. Eng, R. J. Maxwell, E. Cohen, E. G. Piotrowiski and W. Fiddler, *J. Chromatogr. A,* **799** (1998) 349.

196. A. J. J. Debets, W. T. Kok, K. P. Hupe and U. A. Th. Brinkman, *Chromatographia,* **30** (1990) 361.

197. A. J. J. Debets, W. T. Kok, K. P. Hupe and U. A. Th. Brinkman, *J. Chromatogr.,* **600** (1992) 163.

198. M. G. M. Groenewegen, N. C. van de Marbel, J. Slobodnik, H. Lingeman and U. A. Th. Brinkman, *Analyst,* **119** (1994) 1753.

199. N. C. van de Merbel, J. J. Hageman and U. A. Th. Brinkman, *J. Chromatogr.,* **634** (1993) 1.

200. N. C. van de Merbel, *Trends Anal. Chem.,* **16** (1997) 162.

201. T. A. Buttler, K. A. J. Johansson, L. G. O. Gorton and G. A. Marko-Varga, *Anal. Chem.,* **65** (1993) 2628.

202. N. C. van de Merbel, H. Lingeman, U. A. Th. Brinkman, A. Kolhorn and L. C. de Rijke, *Anal. Chim. Acta,* **279** (1993) 39.

203. L. H. Christensen, J. Nielsen and J. Villadsen, *Anal. Chim. Acta,* **249** (1991) 123.

204. T. Buttler, L. Gorton and G. Marko-Varga, *Anal. Chim. Acta,* **279** (1993) 27.

205. C. Filippini, J. U. Moser, B. Sonnleitner and A. Fiechter, *Anal. Chim. Acta,* **255** (1991) 91.

206. C. Mathis, M. N. Pons, J. M. Engasser and M. Lenoel, *Anal. Chim. Acta,* **279** (1993) 59.

207. J. R. Dean, L. Fitzpatrick and C. Heslop, in: *Extraction methods in Organic Analysis*, A. J. Hadley, (Ed.), CRC Press, Boca Raton, 1999, pp 166-193.

208. H. M. Kingston and L. B. Jassie (Eds.), *Introduction to Microwave Sample Preparation*, American Chemical Society, Washington DC, 1988.

209. K. Ganzler, A. Salgo and K. Valko, *J. Chromatogr.,* **371** (1986) 299.

210. I. J. Barnabas, J. R. Dean, I. A. Fowlis and S. P. Owen, *Analyst,* **120** (1995) 1897.

211. V. Lopez-Avila, R. Young and W. F. Beckert, *Anal. Chem.,* **66** (1994) 1097.

212. A. Pastor, E. Vasquez, R. Ciscar and M. Guardia, *Anal. Chim. Acta,* **344** (1997) 241.

213. F. L. Onuska and K. A. Terry, *J. High Resolut. Chromatogr.,* **18** (1995) 417.

214. O. Molines, E. A. Hogendoorn, H. A. G. Heusinkveld, D. C. van Harten, P. van Zoonen and R. A. Baumann, *Chromatographia,* **43** (1996) 527.

215. D. Barcelo and M.-C. Hennion, *Trace Determination of Pesticides and Their Degradation Products in Water*, Vol. 19, Elsevier, Amsterdam, 1997.

216. M.-C. Hennion, in: *Environmental Analysis: Techniques, Applications and Quality Assurance*, D. Barcelo, (Ed.), Elsevier, Amsterdam, 1993, pp 23-77.

217. V. Coquart and M.-C. Hennion, *J. Chromatogr.*, **553** (1991) 329.

218. G. R. van der Hoff, F. Pelusio, U. Brinkman, A. Th., R. A. Baumann and P. van Zoonen, *J. Chromatogr. A,* **719** (1995) 59.

219. A. Di Corcia and M. Samperi, *Anal. Chem.,* **62** (1990) 1490.

220. M. Valls, J. M. Bayona and J. Albaiges, *Int. J. Environ. Anal. Chem.,* **39** (1990) 329.

221. D. Barcelo, M. Sole, G. Durand and J. Albaiges, *Fresenius' J. Anal. Chem.,* **339** (1991) 676.

222. D. A. Hinckley, R. Bidlman and D. Barcelo, *Environ. Sci. Technol.,* **23** (1989) 995.

223. G. Durand, R. Forteza and D. Barcelo, *Chromatographia,* **28** (1989) 597.

224. E. M. Lores, J. C. Moore and P. Moody, *Chemosphere,* **6** (1987) 1065.

225. D. M. Swineford and A. A. Belisle, *Envion. Toxicol. Chem.,* **8** (1989) 465.

226. D. E. Wells, in: *Environmental Analysis: Techniques, Applications and Quality Assurance*, D. Barcelo, (Ed.), Elsevier, Amsterdam, 1993, pp 79-148.

227. L. G. M. T. Tuinstra, J. A. van Rhijn, A. H. Roos, W. A. Traag, R. J. van Mazijk and P. J. W. Kolkman, *J. High Resolut. Chromatogr.,* **13** (1990) 797.

228. P. Haglund, L. Asplund, U. Järnberg and B. Jansson, *Chemosphere,* **20** (1990) 887.

229. K. Grob and I. Kälin, *J. High Resolut. Chromatogr.,* **14** (1991) 451.

230. F. van der Valk and Q. T. Dao, *Chemosphere,* **17** (1988) 1735.

231. D. E. Wells and I. Echarri, *Int. J. Environ. Anal. Chem.,* **47** (1992) 75.

232. E. Storr-Hansen, M. Cleemann, T. Cederberg and B. Jansson, *Chemosphere,* **24** (1992) 323.

233. W. F. Ritter, *J. Agric. Engg. Res.,* **42** (1989) 51.

234. L. Mathiasson, M. Knutsson, G. Bremle and L. Mårtensson, *Swed. J. Agric. Res.*, **21** (1991) 147.

235. M. Mulder, *Basic Principles of Membrane Technology*, Vol. 2, Kluwer Academic Publishers, Dordrecht, 1996.

236. S. T. Hwang and K. Kammermeyer, *Membranes in Separation*, Vol. 2, John Wiley and Sons, New York, 1975.

237. R. E. Majors, *LC-GC,* **13** (1995) 364.

238. M. Sandahl, *Ph. D Thesis*, Lund University, Lund, 1999.

239. G. Audunsson, *Ph. D Thesis*, Lund University, Lund, 1988.

240. H. K. Lonsdale, *J. Membr. Sci.,* **10** (1982) 81.

241. A. Xenakis, C. Selve and C. Tondre, *Talanta,* **34** (1987) 509.

242. J. W. Frankenfeld and N. N. Li, *Recent Dev. Sep. Sci.,* **3A** (1977) 285.

243. Y. Shen, L.Grönberg and J. Å. Jönsson, *Anal. Chim. Acta,* **292** (1994) 31.

244. T. Kitagawa, Y. Nishikawa, J. W. Frankenfeld and N. N. Li, *Environ. Sci. Technol.,* **11** (1977) 602.

245. S. Schlosser and E. Kossaczky, *J. Membr. Sci.,* **6** (1980) 83.

246. A. M. Neplenbrock, D. Bargeman and C. A. Smolders, *J. Membr. Sci.,* **67** (1992) 107.

247. P. Dzygiel, P. Wieczorek, L. Mathiasson and J. Å. Jönsson, *Anal. Lett.,* **31** (1998) 1261.

248. M. Papantoni, N.-K. Djane, K. Ndung'u, J. Å. Jönsson and L. Mathiasson, *Analyst,* **120** (1995) 1471.

249. T. Shinbo, K. Kurihara, Y. Kobatake and N. Kamo, *Nature,* **270** (1977) 277.

250. J. J. Grimaldi and J. M. Lehn, *J. Am. Chem.Soc.,* **101** (1979) 1333.

251. R. D. Noble and J. D. Way, *Liquid Membranes: Theory and Application*, Americcan Chemical Society, Washington, 1987.

252. J. Rebek Jr, B. Askew, D. Nemeth and K. Parris, *J. Am. Chem. Soc.*, **109** (1987) 2432.

253. M. Newcomb, J. L. Toner, R. C. Helgeson and D. J. Cram, *J. Am. Chem. Soc.*, **101** (1979) 4941.

254. R. Murr and A. Kopp, *Int. Chem. Eng.*, **22** (1982) 44.

255. J. D. Way, R. P. Noble, T. M. Flynn and E. D. Sloan, *J. Membr. Sci.*, **12** (1982) 239.

256. S. Schlosser and E. Kossaczky, *J. Radioanal. Nuclear Chem.*, **101** (1986) 115.

257. W. Halwachs and K. Schugerl, *Int. Chem. Eng.*, **20** (1980) 519.

258. R. P. Cahn and N. N. Li, *J. Membr. Sci.*, **1** (1976) 129.

259. W. J. Ward and W. L. Robb, *Science*, **156** (1967) 1481.

260. J. D. Way, R. D. Noble and B. R. Bateman, *Polym. Mat. Sci. Eng.*, **50** (1984) 330.

261. W. C. Babcock, R. W. Baker, E. D. Lachapelle and K. L. Smith, *J. Membr. Sci.*, **7** (1980) 71.

262. T. Largman and S. Sifniades, *Hydrometallurgy*, **3** (1978) 153.

263. T. Aoki, S. Uemura and M. Munemori, *Anal. Chem.*, **55** (1983) 1620.

264. G. Audunsson, *Anal. Chem.*, **58** (1986) 2714.

265. G. Audunsson, *Anal. Chem.*, **60** (1988) 1340.

266. J. Å. Jönsson, P. Lövkvist, G. Audunsson and G. Nilve, *Anal. Chim. Acta*, **277** (1993) 9.

267. L. Chimuka, N. Megersa, J. Norberg, L. Mathiasson and J. Å. Jönsson, *Anal. Chem.*, **70** (1998) 3906.

268. G. Nilve and R. Stebbin, *Chromatographia*, **32** (1991) 269.

269. B. Lindegård, J. Å. Jönsson and L. Mathiasson, *J. Chromatogr.*, **573** (1992) 191.

270. L. Chimuka, M. M. Nindi and J. Å. Jönsson, *Int. J. Environ. Anal. Chem.*, **68** (1997) 429.

271. G. Nilve, M. Knutsson and J. Å. Jönsson, *J. Chromatogr. A,* **688** (1994) 75.

272. N. Megersa, T. Solomon and J. Å. Jönsson, *J. Chromatogr. A,* **830** (1999) 203.

273. B. Lindegård, H. Björk, J. Å. Jönsson, L. Mathiasson and A.-M. Olsson, *Anal. Chem.*, **66** (1994) 4490.

274. S. Palmarsdottir, L. Matiasson, J. Å. Jönsson and L.-E. Edholm, *J. Capill. Electrophor.*, **3** (1996) 255.

275. M. Knutsson, L. Mathiasson and J. Å. Jönsson, *Chromatographia,* **42** (1996) 165.

276. N. Megersa and J. Å. Jönsson, *Analyst,* **123** (1998) 225.

277. G. Nilve, G. Ausunsson and J. Å. Jönsson, *J. Chromatogr.*, **471** (1989) 151.

278. T. Miliotis, M. Knutsson, J. Å. Jönsson and L. Mathiasson, *Int. J. Environ. Anal. Chem.*, **64** (1996) 35.

279. M. Knutsson, J. Lundh, L. Mathiasson, J. A. Jönsson and P. Sundin, *Anal. Lett.*, **29** (1996) 1619.

280. L. Grönberg, Y. Shen and J. Å. Jönsson, *J. Chromatogr. A,* **655** (1993) 207.

281. Y. Shen, J. Å. Jönsson and L. Mathiasson, *Anal. Chem,* **70** (1998) 946.

282. N.-K. Djane, *Ph. D. Thesis*, Lund University, Lund, 1997.

283. N. Megersa, T. Solomon, B. S. Chandravanshi and J. Å. Jönsson, *Bull. Chem. Soc. Ethiop.*, (2000) (in press).

284. Y. Shen, V. Obuseng, L. Grönberg and J. Å. Jönsson, *J. Chromatogr. A,* **725** (1996) 189.

285. S. Palmarsdottir, B. Lindegård, P. Deininger, L.-E. Edholm, L. Mathiasson and J. Å. Jönsson, *J. Capill. Electrophor.*, **2** (1995) 185.

286. J. Norberg, Å. Zander and J. Å. Jönsson, *Chromatographia,* **46** (1997) 483.

287. N.-K. Djane, K. Ndung'u, F. Malcus, G. Johansson and L. Mathiasson, *Fresenius' J. Anal. Chem.,* **358** (1997) 822.

288. J. Å. Jönsson and L. Mathiasson, *Trends Anal. Chem.,* **18** (1999) 325.

289. L. Grönberg, P. Lövkvist and J. Å. Jönsson, *Chromatographia,* **33** (1992) 77.

290. N.-K. Djane, I. A. Bergdahl, K. Ndung'u, A. Schutz, G. Johansson and L. Mathiasson, *Analyst,* **122** (1997) 1073.

291. K. Ndung'u, N.-K. Djane and L. Mathiasson, *J. Chromatogr. A,* **826** (1999) 103.

292. N.-K. Djane, S. Armalis, K. Ndung'u, G. Johansson and L. Mathiasson, *Analyst,* **123** (1998) 393.

293. N.-K. Djane, K. Ndung'u, K. Johnson, H. Sartz, T. Törnström and L. Mathiasson, *Talanta,* **48** (1999) 1121.

294. J. Å. Jönsson, L. Mathiasson, B. Lindegård, J. Trocewicz and A.-M. Olsson, *J. Chromatogr. A.,* **655** (1994) 259.

295. S. Palmarsdottir, E. Thordasson, L.-E. Edholm, L. Mathiasson and J. Å. Jönsson, *Anal. Chem.,* **69** (1997) 1732.

296. S. Palmarsdottir and L.-E. Edholm, *J. Chromatogr. A,* **693** (1995) 131.

297. P. Wieczorek, J. Å. Jönsson and L. Mathiasson, *Anal. Chim. Acta,* **337** (1997) 183.

298. P. Wieczorek, J. Å. Jönsson and L. Mathiasson, *Anal. Chim. Acta,* **346** (1997) 191.

299. M. Bryjak, P. Wieczorek, P. Kafarski and B. Lejczak, *J. Membr. Sci.,* **37** (1988) 287.

300. P. Wieczorek, *J. Membr. Sci.,* **127** (1997) 87.

301. J. Norberg, E. Thordasson, L. Mathiasson and J. Å. Jönsson, (2000), submitted.

302. M. Sandahl, E. Ulfsson and L. Mathiasson, *Anal. Chim. Acta,* (2000) submitted.

303. K. Ndung'u and L. Mathiasson, *Anal. Chim. Acta,* (1999), submitted.

304. M. Sandahl, L. Mathiasson and J. Å. Jönsson, *J. Chromatogr. A,* (2000), submitted.

305. M. Sandahl, L. Mathiasson and J. Å. Jönsson, *Anal. Chim. Acta,* (2000), submitted.

306. A. Stroud, *Weed Management in Ethiopia, an Extension and Training Manual,* FAO, Technical Cooperation Programme, Rome, 1989.

307. F. Hernandez, C. Hidalgo, J. V. Sancho and F. J. Lopez, *Anal. Chem.,* **70** (1998) 3322.

308. G. D. Christian and W. C. Purdy, *J. Electroanal. Chem.,* **3** (1962) 363.

309. F. Malcus, N.-K. Djane, G. Johansson and L. Mathiasson, *Anal. Chim. Acta,* **327** (1996) 295.

310. J. Å. Jönsson and L. Mathiasson, *Advances in Chromatogr.,* (2000), submitted .

311. L. Chimuka, L. Mathiasson and J. Å. Jönsson, *Anal. Chim. Acta,* (2000), submitted.

312. P. A. Leclercq and V. Pacakova, *J. Chromatogr.,* **178** (1979) 193.

313. D. E. Barnes and J. F. V. Staden, *Anal. Chim. Acta,* **261** (1992) 441.

314. U. Cocchini, C. Nicolella and A. G. Livington, *J. Membr. Sci.,***162** (1999) 57.

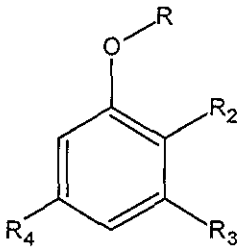
6. APPENDIX

Chemical Classification of Herbicides

1. Phenoxyacid Herbicides. The chlorine substituted phenoxyacetic acids; 2,4-D, MCPA and 2,4,5-T were introduced as selective weed killers at the end of World War II. Most herbicides of this family commonly used today and their important physical data are summarized in Table A-1. They showed high activity against many broad-leaved weeds, and in particular, 2,4-D and MCPA are commonly used for the control of weed in cereal crops and grass pastures, while many woody and broad-leaved plants are effectively controlled by 2,4,5-T [24, 25]. The 2-phenoxypropanoic acid herbicides were mainly used to control certain weed species that are not controlled by the phenoxyacetic acid herbicides. The 4-phenoxy butyric acid herbicides, on the other hand, are useful because of their low or negligible toxicity to certain crop plants, including legume species, that are damaged by even low concentration of the phenoxyacetic acids [26]. Their selectivity is in part related to the ability of plants to β-oxidize them to the corresponding phenoxyacetic acids.

Several other phenoxyalkanoic acid herbicides that are substituted by ethyl ester groups have been developed in early 1950s. One example is sesone, where R (in Table A-1) is $-CH_2CH_2-O-SO_2-ONa$, which was utilized to prevent germination of weed seeds and growth of weed seedlings in horticulture crops. It is suitable for use on deep-rooted crops where there is little chance of the crops roots absorbing 2,4-D from the surface layers of the treated soil [25]. About 20 years later five additional phenoxyethyl herbicides [26]

Table A-1 Structural formula, substituents, names and selected physical properties of the phenoxyalkanoic acid herbicides.



O-Substituent, R	Common name	Systematic name	Ring substituent			Water solubility, ppm, 25°C	Melting point, °C	pK_a value
			R_2	R_3	R_4			
$-CH_2-COOH$	MCPA	[(4-chlor-*o*-tolyl)oxy] acetic acid	$-CH_3$	-Cl	-H	1174	118-119	3.40
	2,4-D	(2,4-dichlorophenoxy) acetic acid	-Cl	-Cl	-H	725	139-139.5	2.80
	2,4,5-T	(2,4,5-trichlorophenoxy) acetic acid	-Cl	-Cl	-Cl	268	154-157	3.14
$-CH(CH_3)_2-$ COOH	MCPP (Mecoprop)	2-[(4-chloro-*o*-tolyl)oxy] propionic acid	$-CH_3$	-Cl	H	895	94-95	3.20
	2,4-DP (dichloroprop)	2-[(2,4-dichlorophenoxy] acetic acid	-Cl	-Cl	H	350	118-119	3.0
	Silvex	2-(2,4,5-trichlorophenoxy) propionic acid	-Cl	-Cl	-Cl	140	179-181	3.10
$-(CH_2)_3-COOH$	MCPB	4-[(chloro-*o*-tolyl)-oxy] butyric acid	$-CH_3$	-Cl	-H	46	102-103	4.86
	2,4-DB	4-(2,4-dichlorophenoxy) butyric acid	-Cl	-Cl	-H	46	119-120	4.58
	2,4,5-TB	4-(2,4,5-trichlorophenoxy) butyric acid	-Cl	-Cl	-Cl	42	114-115	4.78

including erbon, where R = $-CH_2-CH_2-O-CO-C(Cl_2)CH_3$ and x = y = z = Cl (Table A-1) have been introduced. Erbon was developed for use as a non-selective, persistent soil-applied herbicide in non-crop areas.

2. The Substituted Urea and Uracil Herbicides. The herbicidal properties of the substituted ureas were discovered after the end of the second World War [27]. Bucha and Todd [28] described the herbicidal properties of monuron, in 1951, whereby they stressed the toxic effect of these compounds on annual and perennial grasses. Although initially the first developed compounds of the substituted ureas, i.e., fenuron, monuron, diuron and neburon (Table A-2) were primarily suggested as industrial weed killers, in subsequent years their potential for selective application in agriculture was recognized. Then, substitutions with different moieties, aliphatic as well as cycloaliphatic, aromatic and heterocyclic urea derivatives have been prepared [27].

The type of the first technically useful phenylurea herbicides developed are indicated in Fig. A-1. Commercial representatives of this class of herbicides are fenuron, monuron, diuron, fluometuron and chlotoluron (Table A-2).

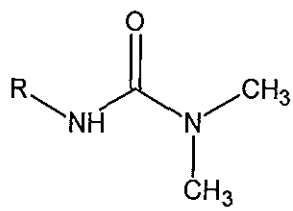
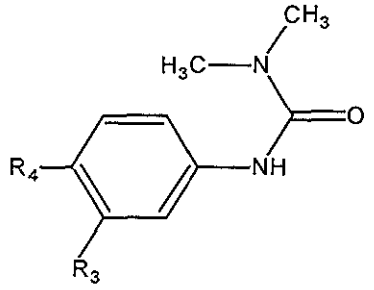


Fig. A-1 General structure of the phenylurea herbicides, where R represented a non-halogenated or a halogenated aromatic hydrocarbon moiety.

Table A-2 Physical and chemical properties, and structures of selected commercial products of phenylurea herbicides [17].



Common name	Systematic name	Ring substituent		Solubility in water, ppm, (25°C)	Melting point (mp, °C)
		R_3	R_4		
Fenuron	1,1-dimethyl-3-phenylurea	H	H	3850	133-134 (25°C)
Monuron	3-(p-chlorophenyl)-1,1-dimethylurea	H	Cl	230	176-177 (25°C)
Diuron	3-(3,4-dichlorophenyl)-1,1-dimethylurea	Cl	Cl	42	158-159 (50°C)
Clortoluron	3-(3-chloro-4-methylphenyl)-1,1-dimethylurea	Cl	CH_3	70	147-148 (20°C)
Fluometuron	1,1-Dimethyl-3-(α,α,α-trifluoro-4-tolyl) urea	CF_3	H	90	163-164.5 (20°C)
Metoxuron	3-(3-Chloro-4-methoxyphenyl)-1,1-dimethylurea	Cl	CH_3O	678	126-127 (20°C)

Substitution of a 1-methyl by 1-propyl or 1-butyl group, in the 1,1-dimethyl moiety of ureas was recognized as reducing herbicidal activity but increasing selectivity. A similar effect was obtained when substituting a 1-methoxy for a 1-methyl group and for one chlorine atom on the phenyl ring [27].

Another variation in the structural configuration was also introduced by replacing the aromatic moiety with a heterocyclic ring system. One example is the selective application of 3-(2-benzothiazolyl)-1,3-dimethyl urea (methabenthiazuron), Fig. A-2, for weed control in cereals introduced in 1965.

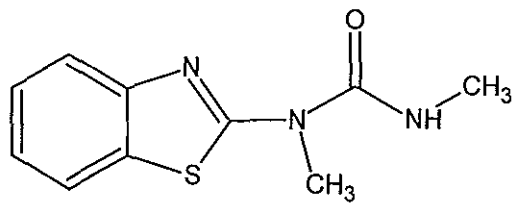
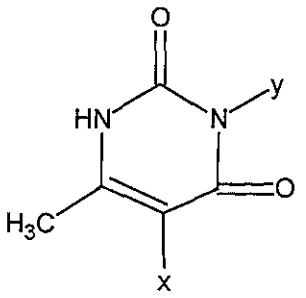


Fig. A-2 Chemical structure of Methabenthiazuron, one of the most commonly used herbicide for weed control in 1960s.

The second class of general urea compounds is the substituted uracils (see also Table A-3). The first material introduced, in 1960, on commercial scale was isocil. It was later replaced by two other compounds of uracil herbicide, *viz.*, bromocil and terbacil. These compounds are also useful in combination with substituted urea herbicides for selective control of weeds. An example is the use of bromacil with diuron for selective control of weeds in citrus and for general weed control in non-crop land areas [29].

Table A-3 Structures and properties of substituted common uracil herbicides [19].



Common name	Systematic name	Substituent		Melting point in °C	Solubility in H_2O, (ppm), at 25°C	pK_a value
		x	*y*			
Isocil	5-Bromo-3-isopropyl-6-methyluracil	Br	$CH(CH_3)_2$	158-159	2150	-
Bromacil	5-Bromo-3-sec-buthyl-6-methyluracil	Br	$CH(CH_3)CH_2CH_3$	158-159	815	9.27
Terbacil	3-Tert-buthyl-5-chloro-6-methyluracil	Cl	$C(CH_3)_3$	175-177	710	-

Sulphonyl urea herbicides introduced at the end of 1970s are a relatively new class of substituted urea compounds, generally used for selective pre- and post-emergence control of weeds in croplands. They are applied in significantly lower amount than most herbicides, and they tend to be more active against broad-leaved species than grasses. They are mainly applied in the field in doses at least 100 times smaller than for conventional herbicides [30].

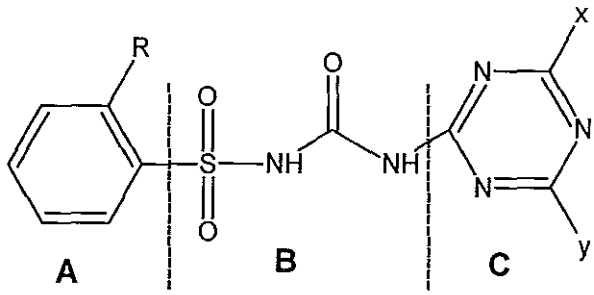
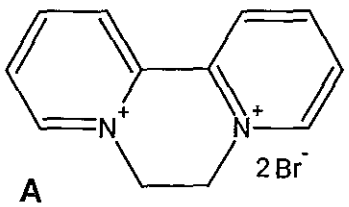


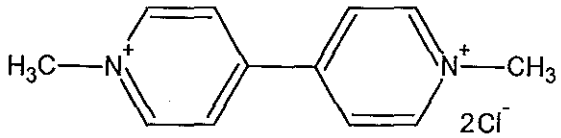
Fig. A-3 General structure of the sulphonyl urea herbicides: (x = OCH_3 and y = CH_3 for most compounds; **A**-aryl, **B**-bridge, and **C**-heterocycle) [30].

*3. **Bipyridinium Herbicides.*** The two most important compounds in a group of bipyridinium herbicides are diquat and paraquat (Fig. A-4). They are commercially available as salts, diquat as dibromide and paraquat as dichloride. The anions associated have no effect on the herbicidal activity. These herbicides are organic cations, non-selective, and quick-acting. Another common property, which is most important in use of these chemicals as non residual herbicides, is their rapid and complete adsorption onto soil particles. Any paraquat and diquat that reaches the soil is rendered unavailable to plant roots and other living organisms. The adsorption may be facilitated by the flat and highly polarizable nature of the paraquat and diquat ions [31].

Diquat and paraquat are both used as desiccants to aid harvesting of potatoes, cotton, rape and other oil seed crops. They are also of value for the control of aquatic weeds.



A



B

Fig. A-4 Chemical structures of the representative bipyridinium herbicides: dibromide and dichloride salts of diquat (**A**) and paraquat (**B**), respectively.

*4. **Dinitroaniline Herbicides.*** These herbicides are used principally for the selective pre-emergence control of annual grasses and weeds. They have little or no post-emergence activity. Prior to their use as herbicides, in 1960, the fungicidal activity of these compounds was known [32]. The unsubstituted dinitroanilines have been recognized as a dye intermediate for several decades. Of prime interest at this point is the marked general herbicidal activity of the 2,6-dinitroaniline as compared to the 2,4-or 2,3-dinitroaniline toward multiple plant species. Selective herbicidal activity was obtained by substitution on the amino group of the 2,6-dinitroaniline molecule. Selectivity was directed towards grasses rather than broad-leaved weeds and pre-emergence action rather than foliar contact.

Substitution at the 3-and/or 4-position of the ring modifies the degree but not the type of herbicidal activity. The compounds substituted at both the 3- and 4-positions also possess herbicidal activity within an acceptable range [11]. A very wide variation in molecular structure of 2,6-dinitroaniline is possible (Fig. A-5): R_1 = alkyl, haloalkyl, cycloalkyl, etc.; R_2 = alkyl, haloalkyl, cycloalkyl, H, etc.; R_3 = NH_2, Cl, H and CH_3; and R_4 = CF_3, CH_3, Cl, SO_2CH_3, SO_2NH_2, C_2–C_4 alkyl, etc.

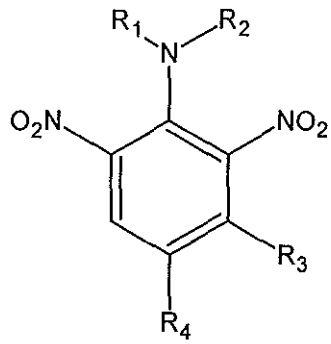
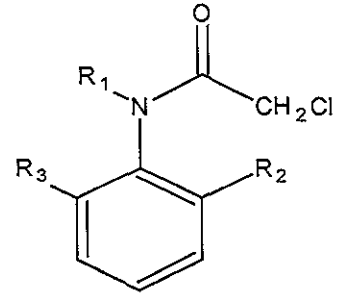


Fig. A-5 General structure of the dinitroaniline herbicides, see also text for the R substituents.

In general, dinitroaniline derivative herbicides are extremely prone to volatilization losses [33]. For this reason, they should always be incorporated in the soil immediately after application.

5. Amides. The amides compose the largest group of herbicides produced. For example, an estimated quantity of 100 million kilo grams amide herbicides is produced each year in the United States. The group contains a variety of chemical types ranging from the H-substituted acetamides to the substituted benzamides. The principal products in terms of annual production and sales are the α-halo H-substituted acetamides which includes acetochlor, alachlor, butachlor, metolachlor and propachlor (Table A-4). These chloroacetamides account for 85% of the amide herbicides. Out of these alachlor and

Table A-4 Structures, substituents and general properties of chloroacetamides.



Common name	Chemical name	Substituents			Melting point in °C	Solubility in water, ppm, 25°C
		R_1	R_2	R_3		
Acetolachlor	2-Chloro-N-(2-ethyl-6-methylphenyl)-N-(ethoxymethyl)acetamide	$C_2H_5OCH_2$	C_2H_5	CH_3	<0	223
Alachlor	2-Chloro-N-(2,6-diethylphenyl)-N-(methoxymethyl)acetamide	CH_3OCH_2	C_2H_5	C_2H_5	40.5-41.5	242
Butachlor	N-(Butoxymethyl)-2-chloro-N-(2,6-diethylphenyl)acetamide	$C_4H_9OCH_2$	C_2H_5	C_2H_5	-2.8-1.7	20
Metolachlor	2-Chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl)acetamide	CH_3OCH_2-$(CH_3)CH$	C_2H_5	CH_3	-62.1	488
Propachlor	2-Chloro-N-(1-methylethyl)-N-phenylacetamide	$(CH_3)_2CH$	H	H	77	580

metolachlor are produced in large dedicated units. The majority of the amide herbicides are applied pre-emergence or pre-plant soil incorporated. The principal use of these herbicides is the selective control of seedling grass and certain broad-leaved weeds [33]. In general, they are not considered subject to large volatilization losses. However, under special conditions, e.g., high soil moisture and low soil sorption, volatilization may be significant [18].

6. Carboxylic and Benzoic Acid Herbicides. The carboxylic acid herbicides are a general class of compounds with varied herbicidal activity. They are used both for pre- and post-emergence application both to soil and foliage [12].

One class includes the halogenated aliphatic acids which have been known for many years [34], even if their use as herbicide was known later. The major ones in order of their appearance are trichloroacetic acid (TCA), 2,2-dichloropropionic acid (dalapon), 2,2,3-trichloropropionic acid, 2,2-dichlorobutyric acid and 2,3-dichloroisobutyric acid. Alpha chlorination appears to be a major requirement for activity, with TCA, dalaphon and 2,2-diclorobutyric acid being herbicidally most active. The unsubstituted aliphatic acids, including formic, acetic, butyric, etc. are essentially inactive as herbicides.

In 1953, dalaphon was recognized as a systematic grass selective herbicide. Later, the sodium salt showed considerable promise for certain selective agricultural uses [34]. Both sodium TCA and dalaphon are somewhat selectively toxic to annual and perennial grasses in as much the same way as 2,4-D is to broad-leaved species. More recently glyphosate (Fig. A-6) was introduced and is being used widely as a principal non-selective foliar applied herbicide [12]. It is insoluble in common organic solvents, e.g., acetone, ethanol and xylene. The alkali metal and ammonium salts are readily soluble in water [22].

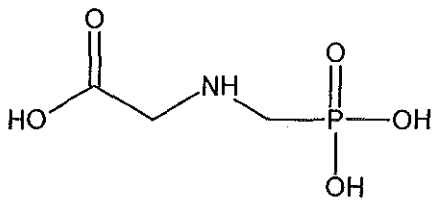


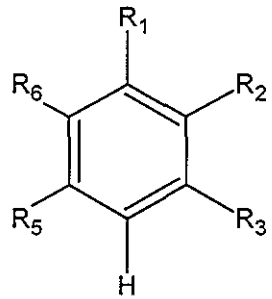
Fig. A-6 Chemical structure of glyphosate; a very common non-selective herbicide.

The halogenated benzoic acids, benzonitriles and terephthalates constitute a heterogeneous group of herbicides. They are important weed control chemicals and exhibit a variety of phytotoxic responses in higher plants. The most extensively used benzoic acid related herbicides and some important physical properties including the structure are given in Table A-5 [35].

*7. **Carbamate Herbicides.*** There are two subgroups of the carbamate herbicides: the thiocarbamates and phenylcarbamates. Most carbamates are soil applied and the compounds enter the plants through the root system and translocate to inhibit the normal plant metabolism.

The thiocarbamates have been used as herbicides since the late 1950s, s-ethyl dipropylthiocarbamate (EPTC) being the first in this group to be introduced [36]. They are particularly prone to volatilization losses and should be soil-incorporated immediately after application [11]. All compounds in this group are clear liquids with an aromatic odour. They are miscible with most organic solvents and their solubility in water is generally low. Structural information and physical properties of the selected compounds of the group are given in Table A-6.

Table A-5 Structural information and properties of the commonly used benzoic acid related herbicides.



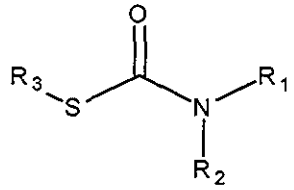
Common name	Chemical name	Substituents					Solubility in H_2O, ppm, °C	Melting point, °C
		R_1	R_2	R_3	R_5	R_6		
1,2,3-TBA	2,3,6-Trichlorobenzoic acid	COOH	Cl	Cl	H	Cl	8400 (20°C)	125-126
TIBA	2,3,5-Triiodobenzoic acid	COOH	I	I	I	H	Very slightly	224-226
Chloramben	3-Amino-2,5-dichlorobenzoic acid	COOH	Cl	NH_2	Cl	H	700 (25°C)	200-201
Dinoben	2,5-Diamino-3-nitrobenzoic acid	COOH	Cl	NO_2	Cl	H	Slightly	220-221
Dicamba	3,6-Dichloro-o-anisic acid	COOH	OCH_3	Cl	H	Cl	4500 (25°C)	114-116
Tricamba	3,5,6-Trichloro-o-anisic acid	COOH	OCH_3	Cl	Cl	Cl	Very slightly	137-139
Dichloarobenil	2,6-Dichlorobenzonitrile	CN	Cl	H	Cl	H	18 (20°C)	145-146
Chlorthiamid	2,6-Dichlorothiobenzamide	$CSNH_2$	Cl	H	Cl	H	940 (20°C)	151-152
Pronamide	N-(1,1-Dimethylpropyl)-3,5-dichlorobenzamide	CONHC-$(CH_3)_2CN$	H	Cl	Cl	Cl	-	155-157

Phenylcarbamate represents one of the two classes of carbamate herbicides. Phenylcarbamate related compounds have a wide range of pesticidal activity which includes their use as fungicide, insecticide, molluscide, etc. Their wide range of biological activity makes them useful not only as pesticides, but also as drugs and medicinal chemicals [37].

Herbicidal properties of phenylcarbamates are becoming increasingly important for several reasons. They are of low mammalian toxicity, have a relatively short residual life in soil and are readily degraded by non-target organisms [38, 39]. Furthermore, there is a broad spectrum of biological activity of the various classes of carbamates, e.g., methyl- and phenylcarbamates. Still *et al.* [37] provided the structural information of various classes and important physical and chemical properties.

8. Phenols and Diphenyl Ether Herbicides. Phenols are among the oldest known pesticides [40]. Dinitrophenols, in particular, were the first organic chemicals patented for selective weed control. A patent was issued on February 9, 1892, to Ferbenfabriken Bayer A. G. for the use of the potassium salt of 4,6-dinitro-*o*-cresol (DNOC) as insecticide [41]. Subsequently, DNOC was widely used, for example, in the United States under the name Sinox and throughout Europe under the common name DNOC [41]. Extensive investigations on structure modification which include substituent rearrangement, esterification or change in alkyl substituent size, have been done for use as more selective weed control. It was reported that both insecticidal and herbicidal activities were found to vary with alkyl chain length [40], and esterification was found to cause striking reduction in phytotoxicity of dinitroalkylphenols [42].

Table A-6 Structural formula and properties of selected thiocarbamate herbicides



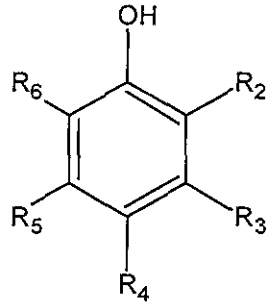
Common name	Chemical name	Substituents			Density, g/mL, (°C)	Solubility in H_2O, ppm, (°C)	Vapour press mmHg, (25°
		R_1	R_2	R_3			
EPTC	S-Ethyldipropyl-thiocarbamate	$(CH_2)_2CH_3$	$(CH_2)_2CH_3$	CH_2CH_3	0.955 (30°C)	375 (20°C)	1.55×10^{-1}
Vernolate	S-Propyldipropyl-thiocarbamate	$(CH_2)_2CH_3$	$(CH_2)_2CH_3$	$(CH_2)_2CH_3$	0.954 (20°C)	107 (21°C)	10.4×10^{-3}
Pebulate	S-Proppylbuthylethyl-thiocarbamate	C_2H_5	C_4H_9	C_3H_7	0.945 (30°C)	92 (21°C)	4.8×10^{-3}
Diallate	S-(2,3-Dichloroallyl)-diisopropylthiocarbamate	i-propyl	i-propyl	$CH_2C(Cl)=CHCl$	-	14	-
Buthylate	S-Ethyldiisobuthyl-thiocarbamte	$CH_2CH(CH_3)_2$	$CH_2CH(CH_3)_2$	C_2H_5	0.930 (30°C)	45 (22°C)	1.3×10^{-3}
Cycloate	S-Ethyl-N-ethylcyclo-hexylthiocarbamate	C_2H_5	cyclohexyl	C_2H_5	1.016 (30°C)	85 (22°C)	6.2×10^{-3}
Triallate	S-(2,3,3-Trichloroallyl)-diisopropylthiocarbamate	$CH(CH_3)_2$	$CH(CH3)_2$	$CH_2C(Cl)=CCl_2$	-	4 (25°C)	-

To improve the herbicidal activity of phenols, a number of dinitroalkylphenols were screened against representative weeds [43]. The activity was found to increase in the order DNOC (methyl) < ethyl < isopropyl < sec-butyl = pentyl. As a result of this investigation, the last two have become widely used under the recommended common name of dinoseb (DNBP) an dinosam (DNAP), respectively. Their general use has been either as oil solutions for desiccation or pre-emergence, or as amine or ammonium salts for post-emergence. They control most seedling weeds and grasses in peanuts, beans, potatoes, corn, peas, pumpkins, squash, strawberries and certain forage crops, but do not control many established perennial weeds in grasses [43]. General structure, substituents and some physical properties of the phenol herbicides are given below (Table A-7).

The other class of compounds in this group are diphenyl ethers. The introduction and development of this class of herbicides, in Japan, has a close relationship with their low toxicity to fish and shellfish. The PCP (pentachlorophenol) used prior to 1963 in rice fields caused problems with fish kills, when flowing into shallow lakes and bays after heavy rainfalls. Thus, in Japan since the early 1970 the diphenyl ether herbicides occupy a leading share of the herbicide market.

Diphenyl ether herbicides can be divided into two general groups [44]. One of these requires light for phytotoxic activity, whereas the other exhibits phytotoxic activity in the dark. The former group is characterized by having at least one substituent at the *ortho* position on one of the benzene rings, ring "A" in Fig. A-7. The "B" benzene ring usually has *p*-nitro substitution, and some compounds may also contain *p*-chloro, *p*-cyano and 3′-methoxy-4′-nitro substituents. The most common substituents on ring "A" is chlorine, except for few compounds which are substituted by bromine -Br, fluorine -F, methyl -CH_3 and trifluoromethyl -CF_3 [22].

Table A-7 Physical properties, substituents and chemical structures of common phenol derivative herbicides.



Common name	Chemical name	Substituent at position					Solubility in H_2O, ppm, (°C)	pK_a value
		R_2	R_3	R_4	R_5	R_6		
DNP	2,4-Dinitrophenol	NO_2	H	NO_2	H	H	5.6 (18°C)	4.1
DNOC	4,6-Dinitro-o-cresol	CH_3	H	NO_2	H	NO_2	130 (15°C)	3.8
Dinseb (DNBP)	2-sec-Butyl-4,6-dinitrophenol	$CH(CH_3)CH_2$ $-CH_3$	H	NO_2	H	NO_2	52 (25°C)	4.4
Dinosam (DNAP)	2-(1-methylbuthyl)-4,6-dinitrophenol	$CH(CH_3)$ $-(CH_2)_2CH_3$	H	NO_2	H	NO_2	-	-
PCP	Pentachlorophenol	Cl	Cl	Cl	Cl	Cl	20 (30°C)	4.5

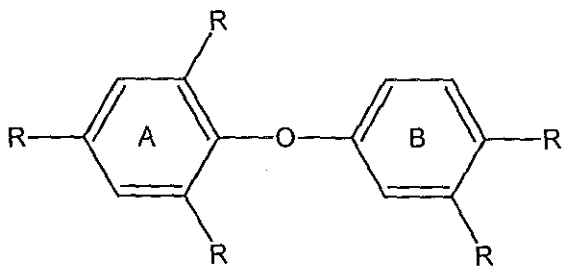


Fig. A-7 General structure for the diphenyl ether herbicides.

9. Heterocyclic Nitrogen Containing Herbicides. In this class, the following herbicide compounds containing heterocyclic nitrogen, such as triazines, pyridines, pyridazinones and imidazoles have been grouped. Except for the presence of nitrogen in the heterocylcic ring, they have different chemical and herbicidal properties, and rate of applications.

The herbicidal properties of the *s*-triazine herbicides have been discovered in the early 1950s, by the research group of J. R. Geigy Ltd., in Basel, Switzerland [45]. According to a recent report, the triazine family herbicides are the second largest group in production quantity and sales [12]. The principal triazines are the 1,3,5-symmetrical triazines (*s*-triazines), which account for over 90% of the total production for the group. The other triazines include metribuzin, metamitron and the hexazinone (1,2,4-triazinone), Fig. A-8. The properties of 1,2,4-triazines are similar to those of *s*-triazines, but their molecules are more polar [46]. Metribuzin is the major non-symmetrical triazine herbicide. All the commercial triazines are solids at room temperature and are formulated as powders or liquid suspensions. Further discussions on chemical and herbicidal properties of *s*-triazine herbicides, structural information and persistence, etc. are presented in section 1.1.4.

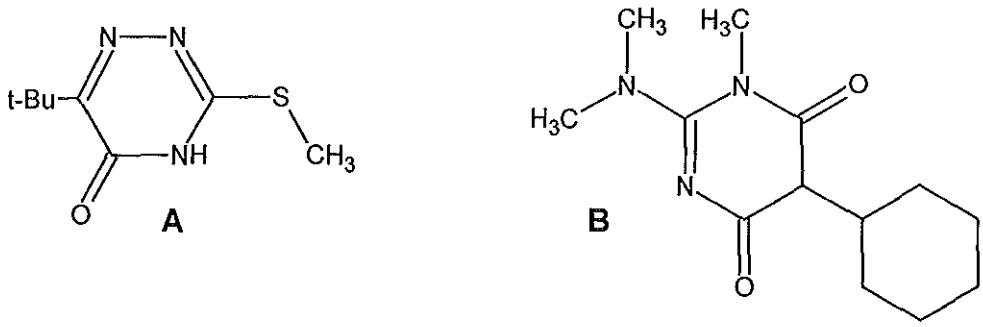


Fig. A-8 Commonly used non-symmetrical triazine herbicides; metribuzin (**A**) and hexazinone (**B**).

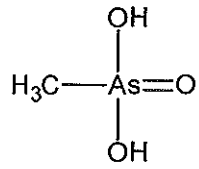
Other general compounds under this class are pyridine herbicides. These are slightly acidic in nature, strongly sorbed to soils and are readily leached. They are auxin-type herbicides, generally used for selective control of broad-leaved weeds in croplands, rangelands and non-croplands [11]. Pyridazinones are rather slightly basic and tend to be strongly sorbed in soils and do not leach readily [47]. Their use is primarily for selective pre- and post-emergence control of seedling grass and broad-leaved weeds in cotton and sugarbeet [33].

Imidazole (or imidazolinone) herbicides are amphoteric, possessing both acidic and basic functional groups [48]. An exceptional compound is buthidazole which is non ionic in nature, but in general, most of the imidazole herbicides exist as ions at typical pH values [11-13]. They are mainly used for broad spectrum, non-selective weed control of non-crop lands [11]. Many herbicidally active compounds are available in this class. Structural information, chemical and physical properties and other related data can be obtained from references 22 and 25.

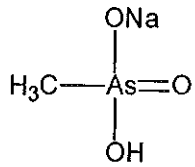
10. Miscellaneous Herbicides. Several compounds that do not appear in the general classification are significant in terms of volumes and annual sales. One notable example is bentazone. It is one of the heavily used selective contact herbicides, absorbed mainly by foliage. Various studies showed that bentazone is a persistent herbicide, the parent compound being the predominant product [12].

The other group includes one of the oldest herbicide compounds known. These are arsenical compounds: inorganics and organoarsenicals. Modern use of arsenical compounds as herbicides began prior to 1900, when sodium arsenite, $NaAsO_2$, was used as a weed killer and soil sterillant. Since then various other inorganic arsenicals have been added as herbicides. Among these are arsenic acid, $H_3AsO_4.½H_2O$, arsenic oxide, As_2O_3, and calcium arsenate, $Ca_3(AsO_4)_2$. Other inorganic arsenals such as lead arsenate, (a mixture of $PbHAsO_4$ and basic Pb salts), Paris green $[CuOAs_2O_3)_3.Cu(C_2H_3O_2)_2]$, and sodium arsenite, $NaAsO_2$, have been used as insecticides and soil sterillants. Ammonium sulfamate, $NH_2SO_3^-NH_4^+$, is another inorganic herbicide which is used for control of woody plants and herbaceous perennials [11].

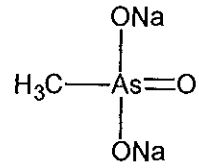
The organoarsenicals, Fig. A-9 below, are herbicides used at low application rates and have lower mammalian toxicity than the inorganic arsenicals [49]. Both these factors contribute to their being among the safest arsenical compounds in use as weed control agents. They are selective in their actions, except for cacodylic acid which is non-selective, and mainly used for post-emergence control of grass and broad-leaved weeds in croplands and non croplands.



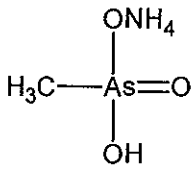
Methanearsonic acid



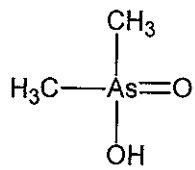
Monosodium methane-arsonic acid



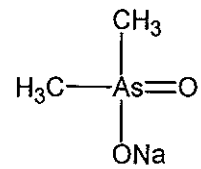
Disodium methane-arsonic acid



ammonium methane-arsonic acid



Cacodylic acid



Sodium cacodylate

Fig. A-9 Chemical structure of the organoarsenical herbicides.

There are also other miscellaneous herbicides including the trifluomethyl compounds. The herbicidal and chemical properties, specific uses as herbicide and the environmental fates of these and other miscellaneous herbicides are given in various publications [11, 12, 22].