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SAMPLE CLEAN-UP, ENRICHMENT AND DETERMINATION OF *S*-TRIAZINE HERBICIDES FROM SOUTHERN ETHIOPIAN LAKES USING SUPPORTED LIQUID MEMBRANE EXTRACTION

Negussie Megersa[1,2], Theodros Solomon,[1] B.S.Chandravanshi[1] and Jan Åke Jönsson[2*]

[1]Department of Chemistry, Addis Ababa University, P.O. Box 1176, Addis Ababa, Ethiopia
[2]Department of Analytical Chemistry, Lund University, Box 124, S-221 00, Lund, Sweden

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ABSTRACT. The liquid membrane extraction method has been employed for selectively extracting trace quantities of *s*-triazine herbicides in environmental waters collected from lakes Awassa, Chamo and Abbya, located in close proximity to the agricultural farms in Southern Ethiopia. In liquid membrane extraction, the uncharged triazine compounds from the flowing donor solution diffuse through a porous poly(tetrafluoroethylene) (PTFE) membrane, containing a water immiscible organic solvent. The *s*-triazine molecules are then irreversibly trapped in the stagnant acidic acceptor phase since they become protonated. Using both di-*n*-hexylether and *n*-undecane membrane solvents, *s*-traizine herbicides were extracted and low detection limits of about 1 ng/L have been obtained by extraction of three liters of sample solution spiked with 0.1 μg/L of each triazine. Residues of atrazine and terbutryn ranging in concentration from 0.02 to 0.05 μg/L have been successfully determined.

INTRODUCTION

Triazine herbicides are the derivatives of symmetrical (*s*-) triazine whose chemical characteristics may be determined primarily by the substituents in position 2, commonly chlorine (the commercial name ending with -azine), methoxy (ending with -tone) and methylthio (ending with -tryn). Positions 4 and 6 are usually substituted by various alkylamino groups [1, 2]. These compounds were synthesized about forty years ago by J.R. Geigy, in Switzerland, and currently they are popular as the major types of herbicides on worldwide scale for selective pre- and post-emergence control of broad-leaved and grassy weeds in corn, soyabeans and other field crops including green vegetables [3-5]. Because of their intensive use and higher application rates, residues of these compounds are eventually transported to surface water, ground and lake waters, and rivers by various mechanisms such as non-point source run-off, ground water discharge or atmospheric deposition [6].

This family of herbicide, together with their degradation products, is among the classes of chemical pollutants more heavily monitored, and detected in environmental waters usually at levels higher than the maximum amount allowed [7-10]. According to the current European Union (EU) directives, for example, the maximum admissible concentration (MAC) in drinking water is limited to 0.1 μg/L for single pesticides and 0.5 μg/L for the sum of pesticides including toxic transformation products [7, 11-12]. This poses a strong demand for development of new techniques to extract large volumes of water samples containing trace quantities of micropollutants. Furthermore, in the effort to develop analytical methodologies capable of determining potential pollutants in water samples, minimizing the effects of matrix interferences by selectively extracting the target compounds, should be given foremost attention [13].

Sample preparation of *s*-triazine herbicides is frequently based on liquid-liquid extraction (LLE) which is a labour intensive and time consuming technique besides the use of large volume of expensive organic solvents. Recent reviews, e.g., by Dean *et al.* [14], and Pacakova *et al.* [15] summarized the various techniques employed for sample preparation of *s*-triazines which include LLE, solid-phase extraction (SPE), solid-phase microextraction (SPME), supercritical fluid extraction (SFE) and microwave assisted extraction (MAE).

An alternative sample preparation method using a hydrophobic membrane, separating phases, has been developed and being used for more than a decade for pre-treatment and enrichment of various samples containing complex matrices [16]. The principle of this technique, called supported liquid membrane (SLM) extraction, is based on selective extraction of the analyte molecules from the flowing aqueous donor phase to the hydrophobic membrane followed by re-extraction into the second aqueous phase filled with appropriate solution for irreversibly trapping the extracted analytes. SLM was found suitable for selective extraction and enrichment of polar compounds, such as organic acids and bases, charged compounds and metal ions [17]. A complementary technique for extraction of trace non-polar molecules from environmental and biological samples using microporous membrane liquid-liquid extraction (MMLLE) has also been recently developed in our group [18]. The MMLLE involves a two-phase extraction, aqueous/organic, where the two phases are separated by a hydrophobic membrane in a similar flow system used for SLM. The principles and applications of both methods have been reviewed recently by Jönsson and Mathiasson [19, 20].

Symmetrical triazines have been extracted from environmental water samples using liquid membrane technology. All classes of *s*-triazines; *viz.*, chloro-*s*-triazines [21], alkylthio-*s*-triazines [22] and methoxy-*s*-triazines [23] have been selectively enriched in the methods developed using the SLM, and the final analysis were carried out using HPLC-UV system. Similarly, Martinez *et al.* [24] have extracted the *s*-triazines from oil samples with membrane barriers followed by flow injection analysis (FIA) or HPLC in an on-line mode.

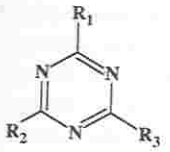
In the present work, a method for selectively enriching all classes of triazines in a complex mixture has been employed to extract the residues of *s*-triazines in natural water samples collected from Awassa, Chamo and Abbaya Lakes, found in the rift valley of Southern Ethiopia. Triazine herbicides have been in use extensively for long time in agricultural farms situated in close proximity to these lakes.

EXPERIMENTAL

Chemicals. The standards of *s*-triazine compounds, including simazine (99%), atratone (99.5%), atrazine (98.4%), prometone (98.2%), propazine (98.6%), terbuthylazine (99.8%), prometryn (99.2%) and terbutryn (99.5%) were purchased from Promochem (Wesel, Germany). Structural information and other related physical parameters are given in Table 1.

Organic solvents used for immobilization into the membrane support were di-*n*-hexylether and *n*-undecane (Sigma, St. Louis, Mo, USA). Acetonitrile of HPLC grade for the mobile phase, sodium dihydrogen phosphate and disodium hydrogen phosphate used as a buffer solution, in the extraction system, were from Merck (Darmstadt, Germany). All solutions were prepared from analytical-grade reagents in a high purity reagent water obtained from a MilliQ-RO4 unit (Millipore, Bedford, MA, USA).

Table 1. Substituents and selected physical constants of the *s*-triazine herbicides under study [2, 15, 22-23].



Compound common name	Substituents			Solubility*	Melting point, °C	pK_a value	Absorption maxima, nm		Density, g/cm^3
	R_1	R_2	R_3				λ_1	λ_2	
Simazine	Cl	NHC_2H_5	NHC_2H_5	5	225-227	1.65	222	263	1.302
Atratone	OCH_3	NHC_2H_5	$NHCH(CH_3)_2$	1650	-	4.20	217	-	-
Atrazine	Cl	NHC_2H_5	$NHCH(CH_3)_2$	33	175-177	1.68	222	263	1.187
Prometone	OCH_3	$NHCH(CH_3)_2$	$NHCH(CH_3)_2$	750	91-92	4.20	219	-	1.088
Propazine	Cl	$NHCH(CH_3)_2$	$NHCH(CH_3)_2$	8.6	212-214	1.85	221	268	1.162
Terbuthylazine	Cl	NHC_2H_5	$NHC(CH_3)_3$	8.5	177-179	1.94	223	263	1.188
Prometryn	SCH_3	$NHCH(CH_3)_2$	$NHCH(CH_3)_2$	48	118-120	4.10	223	-	1.150
Terbutryn	SCH_3	NHC_2H_5	$NHC(CH_3)_3$	58	104-105	4.30	223	-	1.115

*Solubility in water (mg/L) at 20-25°C.

Sampling and sample pre-treatment. Natural water samples were collected three times after the herbicide application from Awassa Lake, (270 km), and Chamo and Abbaya Lakes, (500 km from Addis Ababa), located in the rift valley region, Southern Ethiopia. Triazines, as a pre- and post-emergence weed control, are applied between April to July depending on the duration, length and intensity of the rain during the season. Thus, the first sampling was performed in August 1998, followed by the second in November 1998 and finally in March 1999. Samples from Awassa Lake, for example, were taken from different locations, i.e., from entrance point of the tributary river (Locally called Tikur Wuha -meaning black water), from the shore side and from the center (bulk) of the lake water. Samples from Chamo Lake were also collected from the bulk and the shore on the side of the Sille farm, while arbitrary sampling has been performed for samples from Abbaya Lake, due to the overflow of the lake water during sampling periods. In each case, composite samples were obtained by collecting approximately 200 mL lake water at about 10 m distance for a minimum of two hours. These samples were brought to the laboratory in less than 48 h and kept in the cold room below 5°C.

Before liquid membrane extraction the water samples were all filtered to remove the suspended impurities and particulate matters. The filtered samples were then kept in the refrigerator when they are not immediately extracted. All water samples from Chamo Lake were extracted without storing for more than a day, which otherwise require refiltering to make sure that the algae have been removed. All extractions have been carried out at ambient temperature, 20 ± 2°C.

Experimental set-up. The porous PTFE -poly(tetrafluoroethylene)- membrane (pore size 0.2 µm, total thickness 175 µm of which 115 µm is polyethylene backing, porosity 0.7, FG Millipore Bedford, MA, USA) was prepared by immersing into the appropriate membrane solvent (C, Figure 1) for at least half an hour. The impregnated membrane was then placed between the two PTFE blocks in the membrane holder.

The membrane holder consists of two circular PTFE blocks, (B, Figure 1), with a diameter of 120 mm and a thickness of 8 mm, with machined grooves like Archmedean 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 membrane faces the donor channel through which the extraction solutions percolate. It was also equipped with an O-ring, outside of the grooves, for preventing the outflow of the sample solutions. The second compartment is the acceptor channel where extracted samples will get enriched. The whole construction, including the aluminium blocks, (A, Figure 1), of 6 mm backing the membrane holder on both sides, were clamped together with six stainless screws to make the set-up stable. Excess membrane solvent on the membrane surfaces was removed by flushing both channels for about ten minutes each with reagent water.

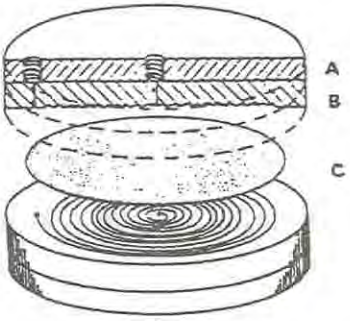


Figure 1. Membrane separator. A: Aluminium backup, B: PTFE block, and C: Immobilized liquid membrane.

Sample solutions prepared for extraction were transferred to the membrane system with peristaltic pump (Minipuls III, Gilson 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°. Further mixing was performed in a coil of 1 m length. Various parts of the flow system were connected with PTFE tubing and flange-free screw fitting (Alltech Associates, Inc., Deerfields, IL, USA).

Preparation of the standard and working solutions. 100 mg/L stock solutions of the parent *s*-triazine compounds were prepared in acetonitrile. A standard solution, containing mixture of the eight compounds was obtained by diluting the stock solution in reagent water. A series of solutions for calibration, in the range from 0.1 to 2.0 mg/L, at five points, were prepared every week from the 10 mg/L standard mixture. The working solution of 0.5 mg/L was obtained by diluting 2.5 mL of the stock solution in acetonitrile to 500 mL with reagent water, which resulted in 0.5:99.5 volume ratio of the organic to aqueous composition. The resulting solution was observed suitable for extraction of the compounds and had no effect on the membrane solvent. Lower concentration standards and working solutions were obtained from the 10 mg/L standard and the 0.5 mg/L aqueous solutions, respectively. All stock and standard solutions were stored at 0°C when not in use for analysis, and are stable in acetonitrile.

Membrane enrichment. For analyte enrichment, the standard solution and phosphate buffer (pH = 7.0 and ionic strength of 0.2) were delivered to the liquid membrane system at donor flow rate of 1.0 mL/min. Prior to sample extraction, the acceptor channel was filled with an acidic solution at 0.5 mL/min and closed. This was followed by pumping the donor channel with the sample solutions for 20 min, and for 10 more minutes with the 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 sample to diffuse to the acceptor acid solution where they are irreversibly trapped.

For lake waters, prior to extraction the sodium salts of phosphate buffer were dissolved in the filtered lake water samples to give a pH of 7.0. One liter and three liters of the processed lake water samples were transferred to the liquid membrane, containing 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 kept at 0.5 M [21], while it was 0.1 M for *n*-undecane membrane solvent [22]. When sample pumping was completed, the donor channel was rinsed for 30 min with the donor buffer at the same donor flow rate. Then, the membrane system was left to stand for 30 min. 3.0 mL of the enriched sample was collected by pumping the acceptor with acidic solution, and the pH adjustment has been carried out immediately 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.

Chromatographic separation system. Analysis of the *s*-triazine compounds has been performed using the high performance liquid chromatographic system consisting a high-pressure pump (HPLC pump 422 Kontron Instruments, Milan, Italy) equipped with an autosampler (HPLC Autosampler 460 Kontron Instruments, Milan, Italy). For isocratic reversed-phase separation of the *s*-triazines in the present study, a mobile phase was prepared from the mixture containing 50% actonitrile and 50% aqueous sodium acetate buffer, 0.05 M. The pH was adjusted to 7.0 with 0.5 M sulphuric acid, and was then degassed by bubbling helium for about 10 min. The mobile phase was also passed through a vacuum degasser (Cambridge Scientific Instruments Ltd., CSI 6150, London, UK) before entering the pumping system. For washing the loop of the autosampler helium degassed solution of the 50% acetonitrile in reagent water was used.

Separation of the compounds was performed on a C18 analytical column (Techsphere 5ODS, 250 mm X 4.6 µm id; HPLC Technology, Macclesfield, Cheshire, UK), and detected with a UV detector (Model 757, Kratos Analytical Instruments, Ramsey, NJ, USA). The detector signals were collected and handled with a personal computer using the 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 a wavelength of 220 nm.

For confirmation of identity of the compounds a photo diode array detector (PDA 996, Waters) was used. Spectra of the compounds identified were monitored using the Millennium 2.15 (Waters) chromatographic system, between 210 - 300 nm.

RESULTS AND DISCUSSION

The SLM extraction may be considered as a three-phase extraction system, with an organic phase sandwiched between two aqueous phases. The process of analyte enrichment is therefore the combination of extraction into the organic solvent followed by a back-extraction

into the second aqueous phase [17, 19]. The theories applied to the mass transfer process in SLM extraction have been described elsewhere [25, 26]. In general, the rate at which analyte molecules are transported may be explained by the following two conditions. (i) Donor-controlled extraction - with this process the mass transfer is limited by the diffusion in the donor phase and donor flow conditions, and (ii) membrane-controlled extraction - in this case the rate-limiting step is the diffusion of the analytes through the membrane.

With donor-controlled extractions the distribution coefficients, K_p, is larger than 10, while $K_p < 1$ with membrane-controlled extractions. It should be noted that the value of the distribution coefficient has no significant effect on the extraction efficiency as long as it is reasonably large.

Liquid membrane extraction parameters

(i) Extraction efficiency. The extraction efficiency, E, is the fraction of the analytes extracted from the flowing donor phase into the stagnant acceptor phase [27, 28], and E is calculated using the following equation:

$$E = \frac{n_a}{n_d} = \frac{C_a V_a}{C_d V_d} \qquad (1)$$

where n_a and n_d are the number of moles of solutes collected in the acceptor solution and entering the extraction system, respectively. C_a is the concentration of the analytes enriched in the acceptor and V_a is the volume, after pH adjustment, of the enriched samples collected from the acceptor phase. C_d is the concentration of the aqueous sample entering the donor phase and V_d 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. In earlier works on extraction of *s*-triazines using liquid membrane methodology [21-23], it has been observed that extraction is fairly complete, and amounts of molecules quantified in blank extraction varied only from 0.5 to 5.0% of the extracted sample. The mass transfer was also examined [22] by allowing the extraction system to stand still to give time to the molecules retained in the membrane to diffuse. The amount of analytes quantified in a series of experiments was below the uncertainty of the measurement. Thus, it can be concluded that the mass transfer of these compounds is controlled only by their diffusion to the membrane (donor controlled condition [17]) and the process of analyte transfer seems faster, i.e., complete in 20 min.

(ii) Membrane solvent. Choice of the organic solvent is a critical step in membrane extraction since the mass transfer solely depends on the composition of the membrane solvent. The solvent of choice must give high extraction efficiency and should have high affinity for the analytes of interest comparing to the interferents, i.e., the partition coefficient, 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 [19, 25-26].

Both di-*n*-hexylether and *n*-undecane have been reported for extraction of *s*-triazine herbicides from environmental waters. The fairly polar compounds like chloro-*s*-triazines [21] and methoxy-*s*-triazines [23] have been well extracted in the more polar solvent, di-*n*-hexylether. The problem with polar membrane solvents is the decrease in physical stability owing to the greater solubility in the water samples, particularly at very high donor flow rate.

Thus, there must be a compromise between the donor flow rate and the extraction efficiency. For alkylthio-*s*-triazines, it has been demonstrated that both membrane solvents can be used, and the decrease in E for *n*-undecane after extraction of more than 100 samples is negligible [22].

In the present work both membrane solvents have been employed since more than one class of *s*-triazines were applied in the agricultural fields in the area of the study. The membrane solvents were used under different pH conditions in the acceptor solution; *viz.*, 0.1 M sulphuric acid for *n*-undecane and 0.5 M for di-*n*-hexylether membrane solvents. The results for 20 min extraction of 0.5 mg/L of the compounds at donor flow rate of 1.0 mL/min are given in Tables 2 and 3. As it can be seen from these results, *s*-triazine compounds are well enriched using both membrane solvents, except for the chloro-*s*-triazines whose enrichment seem to depend mainly on the concentration of the acceptor acid. One important observation is that when acid concentration was increased beyond 0.5 M, the efficiency starts declining gradually which may be due to analyte degradation [2].

Table 2. Effect of the acceptor pH on E, by changing the concentration of sulphuric acid, with di-*n*-hexylether as a membrane solvent. Numbers in bracket are relative standard deviation for triplicate extractions.

Compound	E at varied molar concentration of sulphuric acid				
Common name	0.02 M	0.05 M	0.10 M	0.50 M	1.0 M
Simazine	0.041 (0.12)	0.074 (0.17)	0.105 (0.57)	0.339 (1.21)	0.442 (0.36)
Atratone	0.595 (1.31)	0.658 (0.41)	0.662 (1.90)	0.667 (1.70)	0.631 (0.57)
Atrazine	0.078 (0.10)	0.140 (0.32)	0.192 (0.80)	0.427 (1.52)	0.501 (0.10)
Prometone	0.685 (0.26)	0.745 (0.73)	0.746 (2.30)	0.750 (1.76)	0.703 (0.85)
Propazine	0.084 (1.62)	0.186 (0.27)	0.262 (1.08)	0.518 (1.60)	0.549 (0.99)
Terbuthylazine	0.090 (2.36)	0.217 (0.78)	0.321 (2.18)	0.539 (1.54)	0.554 (0.71)
Prometryn	0.711 (1.72)	0.776 (0.12)	0.768 (1.74)	0.763 (1.86)	0.692 (1.49)
Terbutryn	0.703 (1.01)	0.753 (0.90)	0.780 (3.78)	0.717 (1.40)	0.632 (0.28)

Table 3. Effect of the acceptor pH on E, by changing the concentration of sulphuric acid, with *n*-undecane as a membrane solvent. Numbers in bracket are relative standard deviation for triplicate extractions.

Compound	E at varied molar concentration of sulphuric acid				
Common name	0.02 M	0.05 M	0.10 M	0.50 M	1.0 M
Simazine	0.030 (0.16)	0.053 (0.17)	0.094 (0.21)	0.283 (1.49)	0.319 (1.25)
Atratone	0.478 (1.96)	0.526 (1.88)	0.464 (2.57)	0.508 (2.31)	0.481 (0.95)
Atrazine	0.035 (0.21)	0.057 (0.07)	0.108 (0.17)	0.381 (1.79)	0.459 (0.86)
Prometone	0.674 (3.04)	0.743 (2.48)	0.678 (2.04)	0.717 (3.72)	0.667 (1.25)
Propazine	0.055 (0.12)	0.108 (0.20)	0.174 (0.31)	0.463 (2.66)	0.527 (1.21)
Terbuthylazine	0.109 (0.16)	0.217 (0.26)	0.298 (0.84)	0.562 (3.67)	0.563 (1.38)
Prometryn	0.730 (2.80)	0.812 (2.14)	0.756 (2.57)	0.768 (4.35)	0.692 (1.67)
Terbutryn	0.745 (3.25)	0.825 (2.02)	0.751 (2.15)	0.736 (4.18)	0.644 (1.70)

(iii) Effect of the donor and acceptor pH on E. One of the most important factors governing the SLM extraction of *s*-triazines is the acceptor pH.

The influence of the donor pH on the extraction efficiency of the *s*-triazine herbicides is not very crucial so far as the pH of the donor solution is kept 2 pH units more than the highest pK_a of the compounds [25], to facilitate their dissolution in the membrane. E for *s*-triazines is not affected when the donor pH is between 6.0-8.0 [22-23]. Thus, using the phosphate buffer, ionic strength in the final solution is 0.2 [29], the donor pH was adjusted to 7.0, both in the standard as well as in the field samples.

The extent of extraction of the *s*-triazine is rather more dependent on the acceptor pH. Using dilute aqueous sulphuric acid solution, this effect was controlled so as to obtain maximum extraction efficiency by minimizing the possibility of degradation of the compounds. Their hydrolysis in acidic solutions to yield the hydroxy derivatives has been reported [2]. When the pH of the extracted solution was adjusted to 7.0, immediately after the extract collection, the possibility of degradation is less likely. For example, in the present study, extraction efficiency of prometryn and terbutryn has been increased by 20% and about 10% for atratone [22-23], by careful control of the possibility of degradation as stated above. Therefore, it is very important to note how long the extracted sample is left in acidic solution to estimate the extraction efficiency. Two more compounds; *viz.*, propazine and prometone, whose extractabilities in SLM have not been investigated, are included in this work, and they have also exhibited fairly good efficiencies (Tables 2 and 3). Propazine, having lower pK_a value, as all other chloro-*s*-triazines, is better extracted in higher acidic conditions. Thus, in general, the optimum acidic condition in the acceptor phase follows the nature of the membrane solvent, and whenever possible concentrations of acids less than 0.5 M should be used unless the extract is collected in a pre-processed buffer whose pH is controlled.

Donor flow rate. Extraction efficiencies close to 100% can be achieved if the donor flow rate is low enough. However, when large sample volumes are available, e.g., natural water, extractions at higher flow rate are preferred since the enrichment of the analytes per unit time increases. The application of such experiment is useful for compounds having large partition coefficient between the liquid membrane and the aqueous donor phase. As it has also been shown in Figures 2a and 2b, the decrease in extraction efficiency for *s*-triazine compounds is gradual and thus the accumulation per unit time is high. The extraction efficiency decreases very fast for propazine as the donor flow rate incerases in the *n*-undecane membrane solvent, (Figure 2b). This is not unexpected observation since all the chloro-*s*-triazines [21] with lower pK_a values may have better dissolution in the more polar membrane solvent, di-*n*-hexylether, (Figure 2a).

At the expense of sample volume and 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 membrane liquid into the flowing large volume aqueous sample [22]. In the present work, for extraction of *s*-triazine compounds from lake waters, donor flow rate of 5.0 mL/min was chosen, which is not very high to remove the membrane liquid in few extractions. Analytes of very low concentration in the lake water have been enriched and quantified. About 20 extractions can be done before the membrane is leaking when one liter water sample is extracted at this flow rate.

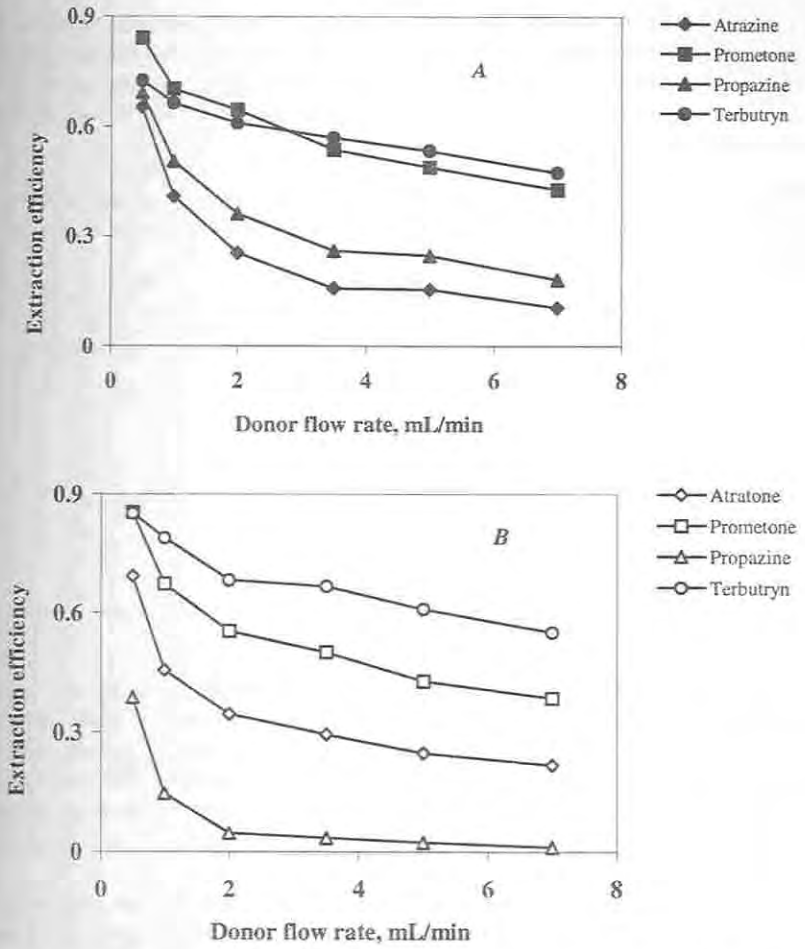


Figure 2. Donor flow rate dependence of the extraction efficiency for the extracts of 0.5 mg/L of each of the *s*-triazine compounds. Duration of extraction: 20 min and donor pH of 7.0. *(A)* Di-*n*-hexylether membrane solvent and acceptor acid concentration of 0.5 M, and *(B)* *n*-undecane membrane solvent and acceptor acid concentration of 0.1 M.

Enrichment factor. The extent to which the extracted molecules are accumulated in the acceptor compartment has been studied at the donor flow rate of 5.0 mL/min, using both membrane solvents. First, one liter spiked standard solution of 0.5 µg/L, with respect to all the *s*-triazine compounds, was extracted. In another series of experiments three liters of spiked sample solutions, whose concentrations were 0.1 µg/L were extracted in the same manner to investigate the enrichment of the compounds at trace levels. In both cases, enrichment factor, E_e, was evaluated using the following equation:

$$E_e = \frac{C_a}{C_d} \qquad (2)$$

where C_a is the concentration of the enriched sample, and C_d is that of the solution entering the donor channel to be extracted. The results obtained are given in Table 4. This may proves that trace analysis with SLM technique for *s*-triazine herbicides is successful, and accumulation of the compounds seems independent of the concentration in the original sample, regardless of the sample origin.

Table 4. Enrichment factors, E_e, for extraction of the standard samples spiked in reagent water. All samples were extracted at the donor flow rate of 5.0 mL/min, and the values are mean of four extractions.

Compound common name	E_e for 0.5 µg/L in one liter		E_e for 0.1 µg/L in 3 liters	
	Di-*n*-hexylether	*n*-Undecane	Di-*n*-hexylether	*n*-Undecane
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

nd – not detected.

Quantification. Calibration graphs for the eight *s*-triazine compounds under the present study have been constructed after liquid membrane extraction of samples in reagent water ranging in concentrations from 0.05 mg/L to 0.5 mg/L, using the donor flow rate of 1.0 mL/min in both membrane solvents, di-*n*-hexylether and *n*-undecane. All the compounds exhibited linear relationship of the detector response and the concentration extracted, with insignificant intercept and correlation coefficient of 0.998 or better. Representative examples of the calibration graphs are given in Figure 3.

The applicability of the liquid membrane extraction technique was further assessed by extracting trace level concentrations of the standard solution for determination of the detection limit. Sample concentrations of 0.5 µg/L in one liter, and 0.1 µg/L in three liters, were extracted in both membrane solvents under identical experimental conditions at the donor flow rate of 5.0 mL/min. Samples were premixed with equal volume of the donor buffer. The results obtained from both experiments are summarized in Table 5. The detection limits, calculated as three times the noise level, were compared in both membranes and varied trace level concentrations. Earlier reports with liquid membrane extraction of *s*-traizines indicated that detection limits can be lowered up to 30 ng/L [22] and 15 ng/L [23] for alkylthio- and methoxy-*s*-traizine herbicides, respectively, in environmental waters. In this work, where all classes of *s*-triazine herbicides have been analysed together in a single extract, detection limits down to sub-nanogram per liter have been determined for most compounds, and this proves the successful application of the SLM technique for extraction of trace and ultratrace quantities of micropollutants in environmental water samples.

Applications. The liquid membrane extraction methodology was applied for extraction of samples of 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 insecticides, to various farms sprayed by farm workers and air plane. One of

the pesticides most frequently used as an active ingredient is the *s*-triazine family, the formulation of which is prepared alone or in combination with other pesticides. For example, atrazine is mixed with alachlor, metolachlor and bentazone in various proportions [30, 31].

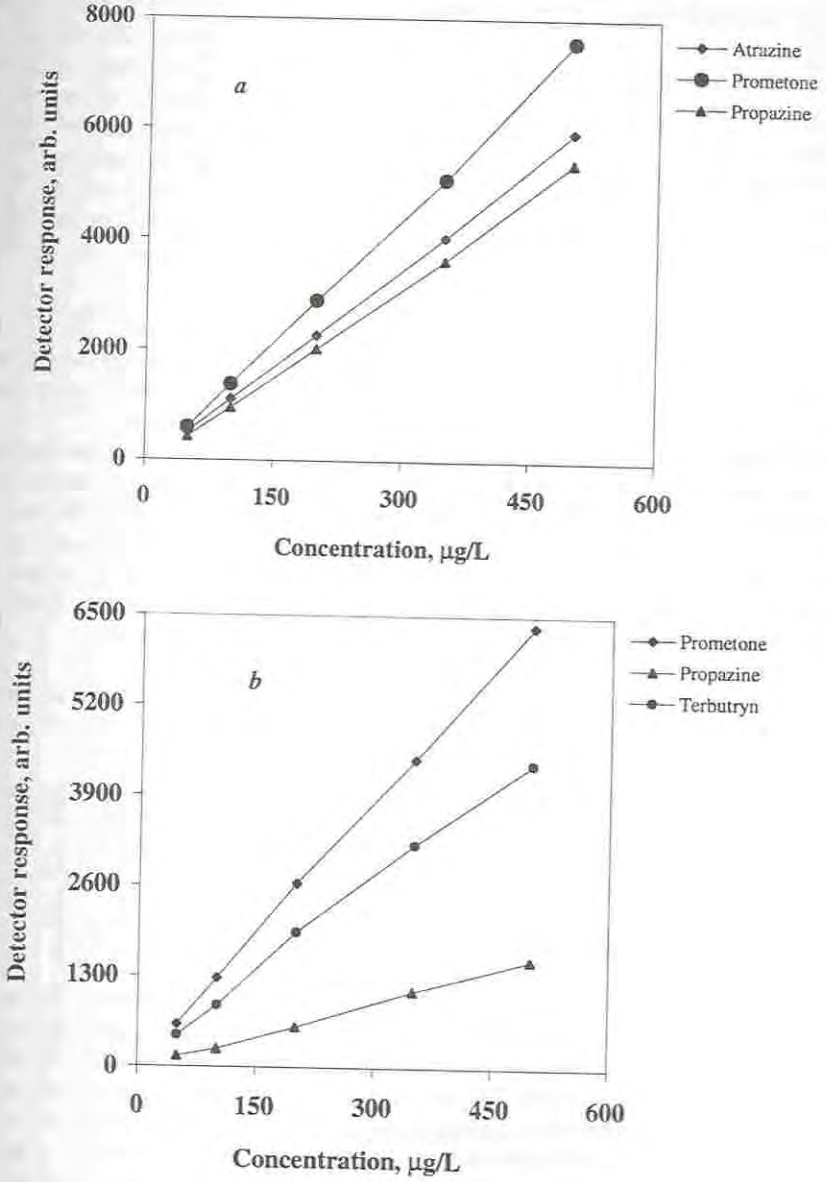


Figure 3. Calibration graphs for extraction of varied concentrations of the sample mixture at donor flow rate of 1.0 mL/min. *(a)* Di-*n*-hexylether, and *(b)* *n*-undecane membrane solvents.

Table 5. 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.

Compound common name	LOD, ng/L, for extraction of one liter standard solution		LOD, ng/L, for extraction of three liters standard solution	
	Di-*n*-hexylether	*n*-Undecane	Di-*n*-hexylether	*n*-Undecane
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

nd – not detected.

These agricultural fields are situated in close proximity to the lakes selected for the present study. There is a high possibility for the residues of these compounds to get their ways to the lakes by surface runoff, wind, cattle hooves etc. Water bodies of the recipient lakes will thus be contaminated and may contain considerable quantity of these residues.

Water samples collected from these lakes were extracted, after some pre-processing steps including filtration and treatment with buffer. The extraction process follows the same procedure as the extraction of trace level standard extraction for determination of the detection limit. The amounts of *s*-triazines in these lakes have been estimated using the extraction efficiencies at 5.0 mL/min, equation 1. Atrazine and terbutryn were identified in Awassa Lake, while only atrazine could been found in the enriched extracts of Chamo Lake, (Table 6). None of them could be identified in the extracts from Abbaya Lake.

Table 6. Concentrations, in µg/L, of atrazine and terbutryn in the enriched extract from Awassa and Chamo Lakes, applying 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 compound	Extract from Awassa Lake*		Extract from Chamo Lake*	
	DHE	UDC	DHE	UDC
Atrazine	6.6 ± 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.

The quantities of atrazine estimated in Awassa Lake by liquid membrane extraction when di-*n*-hexylether was used as a membrane solvent were about 0.05 µg/L and 0.04 µg/L in *n*-undecane membrane solvent. Slightly lesser quantities of atrazine were found in Chamo Lake; 0.04 µg/L with di-*n*-hexylether and 0.02 µg/L with *n*-undeacne membrane solvent. Terbutryn was also identified and quantified in Awassa Lake, and the quantities estimated were 0.03 µg/L and 0.04 µg/L in di-*n*-hexylether and *n*-undecane membrane solvents, respectively. Chromatograms for the extracts of lake water samples enriched using supported liquid membrane are given in Figure 4. Typical spectra of terbutryn are shown in Figure 5. It can be seen that both the spectra, for 0.5 mg/L extracted sample and diluted ten times and that from the extract of lake water, are identical.

A closer examination of the seasonal variation of the herbicides distribution in Awassa lake 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 atrazine could be identified in the samples taken from the point of entrance of the tributary river. None of them could be observed 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 [32]. Moreover, in none of the extracts from the third collection were the herbicides identified. Probably, in due time they have been either degraded, adsorbed to the plants or settled down by forming complexes with various substances e.g., metals. It can thus be generalized that sampling, sample collection time and collection sites are very important variables when environmental monitoring experiments are to be carried out, for analysis of herbicide residues in these areas.

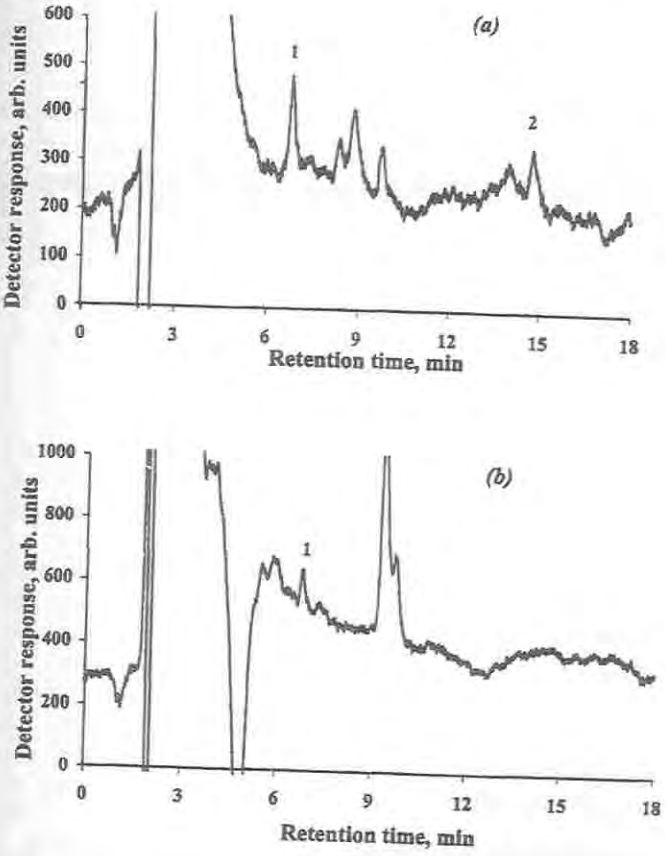


Figure 4. HPLC-UV chromatograms for the extracts of lake water samples: Extraction conditions: donor flow rate - 5.0 mL/min; membrane solvent - di-*n*-hexylether; and sample extraction time - ten hours. Peaks (1) atrazine and (2) terbutryn. Chromatograms for *(a)* extract from Awassa Lake and *(b)* extract from Chamo Lake. 50 µL of the enriched extracts were introduced to the separation system.

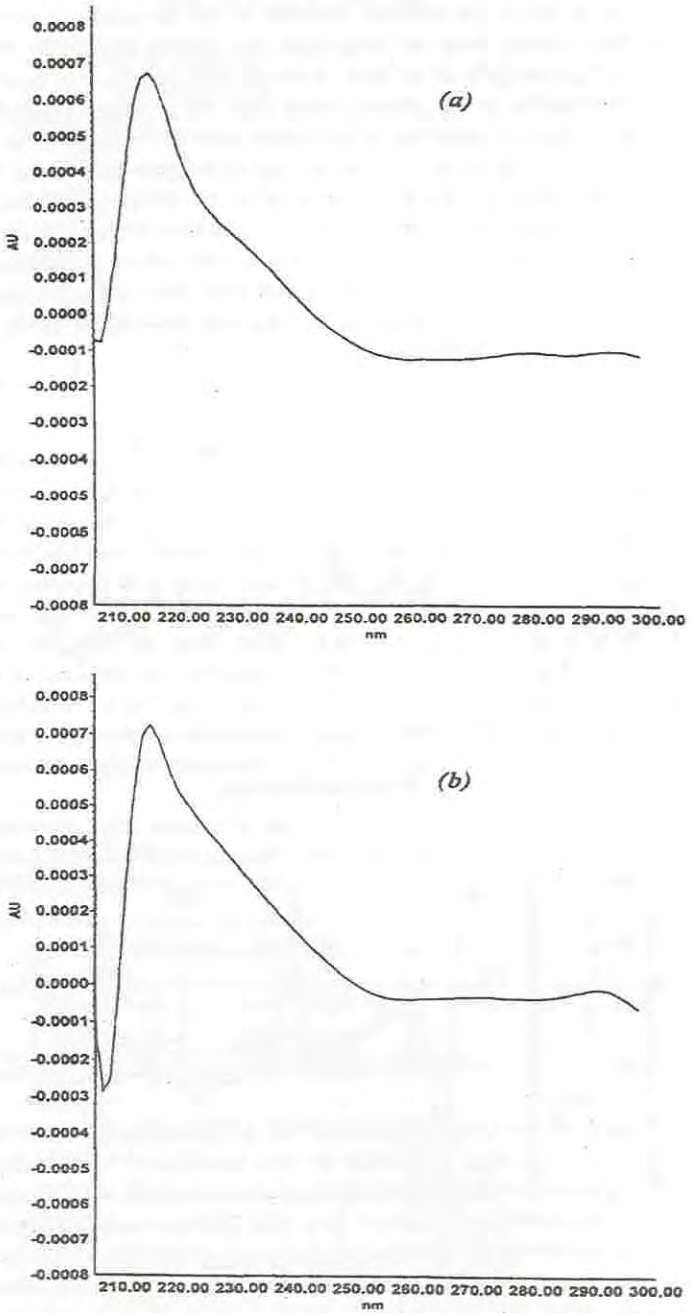


Figure 5. UV spectra for the extracts of terbutryn. *(a)* Extract of 0.5 mg/L standard sample of terbutryn, diluted ten times, using di-*n*-hexylether membrane solvent. *(b)* Extract of lake water in identical conditions as in *a*.

CONCLUSIONS

In this work, the possibility of applications of the supported liquid membrane technique for extraction of the natural water samples containing complex matrices has been presented. In addition to the extensive investigation of the enrichment process, the extraction conditions of two more *s*-triazine compounds; *viz.*, propazine and pormetone, have been studied. The results obtained from lake water extraction confirmed the presence of the herbicide compounds, and these were lower in concentration than the admissible limits given by some authorities, e.g., EU directives [11]. It may also be important to examine the seasonal variation of these parent compounds by performing certain monitoring programme, taking the sampling technique and time, collection sites and depth, and other physical and meteorological parameters into consideration. Equally important is the selective enrichment and determination of the breakdown products of these compounds, and further investigations in this area is currently in progress.

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