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SAMPLE PREPARATION BY MEANS OF A SUPPORTED LIQUID MEMBRANE FOR THE DETERMINATION OF CHLOROPHENOXYALKANOIC ACIDS

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SUMMARY

A sample preparation system for the determination of chlorophenoxyalkanoic acids by liquid chromatography was investigated. The technique permits enrichment and sample clean-up in a flow system, and is used on-line with the liquid chromatograph. The acidified sample comes in contact with a liquid membrane into which the analytes are extracted. On the other side of the membrane, acidic constituents may be trapped by dissociation in an appropriate buffer solution. The accumulated fraction is transported to the injection loop of the liquid chromatograph. The technique has been used for determinations of chlorophenoxyalkanoic acids in solutions containing humic substances.

INTRODUCTION

Chlorophenoxyalkanoic acids (CPAs) are widely used for weed control in crops. These acids show auxin-like activity[1], as they produce uncontrolled proliferation of portions of the plant, resulting in death because it can no longer feed itself. In Table I some common CPAs are listed. In Sweden, MCPA is the most commonly used of these acids; its consumption in Sweden in 1987 was 1540 tons/year and that of 2,4-D was 60 tons/year. The use of 2,4,5-T has been prohibited in Sweden since 1977, as it is often contaminated with traces of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Because of the extensive use of CPAs, contamination of rivers and lakes can be expected and has actually been confirmed[2].

CPAs are commonly determined by gas chromatography (GC) after derivatization to more volatile compounds[3–5]. Diazomethane, in spite of its toxicity and safety hazards, is often used as a derivatization agent[2,6–9]. Pentafluorobenzyl bromide has been used as a reagent to obtain increased sensitivity in GC with electron-capture detection[10–13]. 2-Chloroethanol[14,15] and (2-cyanoethyl)dimethyl(diethylamino)silane[16] are other reagents that have been applied. The need for derivatization, which is time consuming and may introduce errors in the analytical method, has made liquid chromatography (LC) an attractive alternative for the determination of these compounds[17–20].

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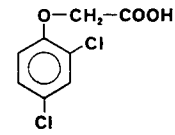
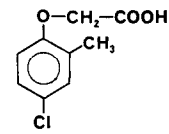
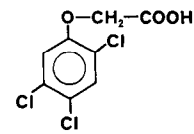
TABLE I

CHLOROPHENOXYALKANOIC ACIDS (CPAs) USED IN THIS WORK

Compound	*Abbreviation*	*Structure*
2,4-Dichlorophenoxyacetic acid	2,4-D	
2-Methyl-4-chlorophenoxyacetic acid	MCPA	
2,4,5-Trichlorophenoxyacetic acid	2,4,5-T	

LC detection is usually carried out by means of UV-absorbtion measurements[19,21–23], but LC–mass spectrometry has been used in some instances to increase the selectivity of detection[24–26]. In the analysis of environmental samples for pollutants, sample enrichment and clean-up are almost always necessary. With CPAs, the most frequently used sample preparation technique is liquid–liquid extraction[3,4,6,8,9,12–14]. After a first extraction, back-extraction followed by another extraction and/or clean-up of the derivatized acids on columns are often performed to obtain low background signals[27]. Solid-phase extraction has also been used for sample preparation[2,7,16,19,22,28,29]. Liquid–liquid extraction and column techniques have been compared for the analysis of soil[30] and water[31].

The liquid membrane technique developed by Audunsson[32] has been shown to have excellent properties for the concentration and clean-up of amines in urine[33]. The application to acidic compounds is a matter of choosing the appropriate pH in the donor and acceptor phases. The membrane system was originally coupled directly to a gas chromatograph, but the replacement of the gas chromatograph by a liquid chromatograph is straightforward, as shown in this work. The system performs extraction and back-extraction in a single step, with simultaneous sample clean-up of the concentrated analytes. The sample preparation is performed in a closed system, which minimizes the risk of losses and contamination due to sample handling. For the analysis of natural waters there is also a possibility of on-site enrichment, which makes transportation of large volumes of water unnecessary. The process can also be easily automated, which is valuable if a large number of samples are to be analysed and, additionally, increases the quantitative precision.

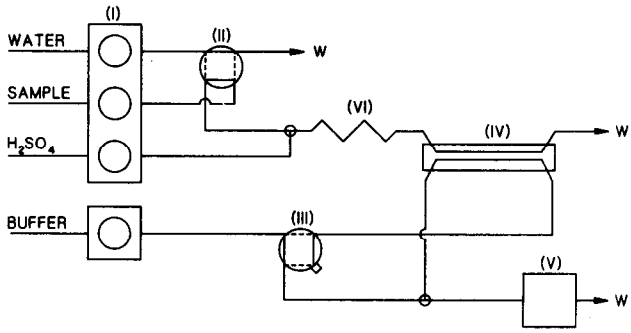


Fig. 1. Schematic diagram of the experimental set-up.

EXPERIMENTAL

Equipment

A schematic diagram of the experimental setup is shown in Fig. 1. Two peristaltic pumps (I) (Minipuls 2; Gilson Medical Electronics, Villiers-le-Bel, France) with standard PVC manifold pump tubing (Elkay Products, Shrewsbury, MA, U.S.A.) were used to control the donor and acceptor flow-rates independently. The various parts of the flow manifold were connected with 0.5 and 0.3 mm. I.D. Teflon tubing and Altex screw fittings. The confluences, where the channels meet at an angle of 60°, were made of PTFE. Both the sample inlet valve (II) and the switching valve (III) were pneumatically actuated four-way Kel-F slider valves [Cheminert; Laboratory Data Control (U.K.)]. The membrane separator (IV) was machined from blocks of PTFE by cutting two U-shaped grooves on the opposite faces of the blocks. The grooves were 0.25 mm deep, 1.5 mm wide and 150 mm long, giving a geometric volume of each groove of *ca.* 56 μl. The membrane was clamped tightly and evenly between the surfaces of the blocks by ten screws. To make the membrane separator more rigid, the PTFE blocks were backed up with aluminium blocks (6 mm thick) in which the threads for clamping screws were machined. The liquid membrane support was Fluoropore FG (average pore size 0.2 μm, total thickness 175 μm, of which 115 μm is polyethylene backing, porosity 0.70; Millipore, Bedford, MA, U.S.A.). The liquid membrane was prepared by immersing the membrane in the chosen solvent for about 15 min. After installation in the separator, excess solvent on the membrane surface was removed by pressing water through both channels.

Operation of the system

The sample is introduced by valve II. The sample volume is determined by time and flow-rate. The sample is acidified by mixing with acid in the mixing coil (VI) (100 cm × 0.5 mm I.D.). Non-ionized species in the donor phase will be extracted into the liquid membrane. After traversing the membrane, acidic constituents may be trapped in the acceptor by dissociation. During sample introduction, valve III is in its bypass position, *i.e.*, the acceptor phase in the membrane separator is stagnant. After the sample has passed the separator on the donor side, valve III is switched and the accumulated analyte is transferred to the detector (V). The CPA peaks were mon-

itored at 285 nm by a Spectra-Physics Model 770 spectrophotometer (Schoeffel Instrument U.S.A.) equipped with a Servogor 210 recorder (Goerz Electro, Austria).

The chromatographic separations were performed with a Spectra-Physics SP 8000 liquid chromatograph equipped with a Valco loop injector (100 μl), and a 150 × 4.6 mm I.D. ODS-2 C_{18} reversed-phase column (Phase Separations, Queensferry, U.K.). Spectrophotometric detection was carried out with an LDC Spectromonitor III varaible-wavelength UV detector (LDC, Riviera Beach, FL, U.S.A.).

Chemicals

The organic solvents used were undecane (Merck) (pro analysi), 1-decanol (Riedel-de Haën) (pro analysi), 1-dodecanol (Fluka) (puriss), 1-tetradecanol (Merck) (purum), and di-*n*-hexyl ether (Sigma). The analytes were 2,4-D (Janssen Chimica) (pract.), MCPA (Fluka) (purum) and 2,4,5-T (Janssen Chimica) (purum) (see Table I). Humic acid (Fluka) (pract.) was used as an interferent. All other chemicals were purchased from Merck and were of analytical-reagent grade. Water was purified with a Milli-Q/RO-4 unit (Millipore, Bedford, MA, U.S.A.).

RESULTS AND DISCUSSION

The aim of this work was to apply the liquid membrane configuration for the sample preparation of CPAs in water containing humic substances with subsequent analysis by LC. The parameters studied were the choice of solvent in the liquid membrane, interferences from humic substances and the direct coupling of the sample preparation system with the LC system.

Unless stated otherwise, the sample flow-rate was 0.25 ml/min and the acid flow-rate was 0.25 ml/min, resulting in a total donor flow-rate of 0.50 ml/min, 0.1 *M* in sulphuric acid. Further, the acceptor flow-rate was 0.25 ml/min, 0.1 *M* in phosphate buffer (pH $\approx$ 7), and the analyte used as a model substance was 2,4-D in water.

Choice of membrane liquid

To evaluate the membrane performance, the enrichment factor is plotted as a function of sample volume introduced. The enrichment factor is expressed as $C_p^{max}/C_{o,s}$, where C_p^{max} is the maximum concentration in the peak measured by the detector and $C_{o,s}$ is the concentration of analyte in the introduced sample.The enrichment factor per unit sample volume, $C_p^{max}/(C_{o,s}V_s)$, is the slope of the resulting regression line.

The desired properties of the solvent in the liquid membrane have been discussed elsewhere[32,34]. *n*-Undecane was shown to be suitable for the extraction and sample clean-up of amines in urine samples[33]. However, *n*-undecane could not be used for the CPAs as the distribution ratio between the donor phase and the *n*-undecane in the liquid membrane was too small to give a sufficient transport rate of the analytes, leading to an enrichment factor per unit sample volume of only 0.4 ml^{-1}. A marked improvement was obtained with a more polar liquid in the membrane. For 1-decanol, the enrichment factor per unit sample volume was 3.6 ml^{-1}. It should be noted that the enrichment factors are strongly dependent on the donor flow-rates[32,33].

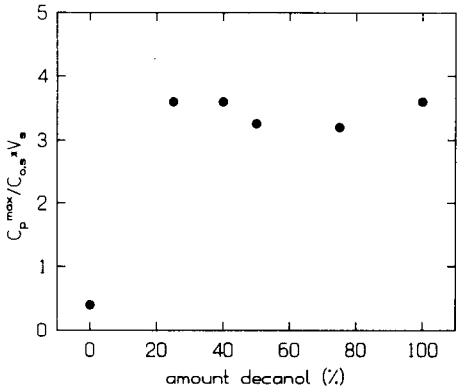


Fig. 2. Enrichment per unit sample volume as a function of the amount of 1-decanol in the liquid membrane.

The use of 1-decanol as the immobilized liquid decreases the selectivity of the technique as polar interferents will be coextracted with the analytes. In order to increase the selectivity of the membrane, mixtures of 1-decanol and *n*-undecane were used. In Fig. 2 the enrichment factor per unit sample volume as a function of the amount of 1-decanol in the membrane is illustrated.

The initial increase in enrichment with 1-decanol is due to an enhanced transfer rate from the donor interfacial layer into the membrane, which is proportional to the distribution ratio between the phases. At the same time, the transfer rate from the membrane phase into the acceptor interfacial layer decreases as it is inversely proportional to the distribution ratio between these phases. The difference in the distribution ratio of the analyte on each side of the membrane is due solely to the different ionic strengths in the donor and acceptor phases. Thus the total permeability in the membrane increases with an increased distribution, reaching a plateau for large distribution ratios. The mechanism for this behaviour has been studied theoretically and experimentally elsewhere[34]. As can be seen in Fig. 2, this plateau of mass transfer is reached for an immobilized liquid containing 25% 1-decanol in *n*-undecane and further increases in the amount of 1-decanol do not change the enrichment factor. This independence of mass transfer rate on the distribution ratio at the plateau makes the sample workup much less sensitive to the matrix interferences which usually affect the distribution ratio.

Increasing the polarity of the membrane solvent will not only decrease the selectivity towards polar interferents, but will also decrease the physical stability of the liquid membrane, owing to an increased solubility of the solvent in water. As a thin film of large area of the solvent comes into contact with a large volume of water, the solubility in water must be very low and will determine the lifetime of the membrane.

In Fig. 3, the enrichment factor per unit sample volume as a function of operation time of the membrane is shown for three different liquid membranes. Curve a represents a membrane with a liquid consisting of 25% 1-decanol in *n*-undecane. After 20 h the enrichment factor per unit sample volume has decreased to about 75% of the value at 10 h. This is primarily due to losses of 1-decanol from the liquid

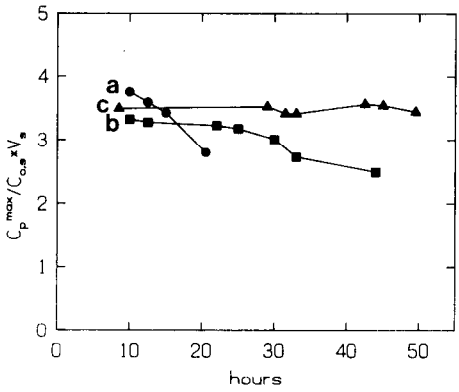


Fig. 3. Comparison of the stability of the membranes expressed as the enrichment factor per unit sample volume for different operation times. (a) 25% 1-decanol in *n*-undecane; (b) 5% 1-dodecanol in *n*-undecane; (c) 5% 1-tetradecanol in *n*-undecane.

membrane. Curve b illustrates the behaviour of a membrane consisting of 5% 1-dodecanol in *n*-undecane. This membrane is stable for 20–25 h, after which the enrichment factor decreases markedly. These curves confirm the fact that the enrichment factor per unit sample volume is virtually independent of the distribution ratio once the mass transfer limit has been reached. As the amount of alcohol in the membrane is lowered, a critical value is reached after which a further decrease in the amount of alcohol, and hence the distribution ratio, will result in a decreased enrichment factor. Curve c represents a membrane with a liquid consisting of 5% 1-tetradecanol in *n*-undecane. This membrane was stable for at least 50 h with an enrichment factor per unit sample volume of 3.5 ml^{-1} and a variation of 2% relative standard deviation (R.S.D.) during this time, which is slightly higher than the R.S.D. for the measurement at each point (1.3%). Each point represents the slope of a line where the concentration in the acceptor is measured as a function of sample volume for five different volumes between 0.5 and 3.5 ml.

The 5% 1-tetradecanol membrane was used for the enrichment of large sample volumes of low concentrations. On injecting volumes greater than 4 ml a negative deviation from linearity appeared, as can be seen in Fig. 4. This deviation is probably due to ester formation in the membrane, but this has not been confirmed. This deviation from linearity with injection volume is not satisfactory, as enrichment factors for large sample volumes are not easily predictable. However, if a constant injection volume is used, the acceptor concentration will vary linearly with the concentration of the injected sample.

To circumvent the problem of deviation from linearity, a membrane with 50% dihexyl ether in *n*-undecane as the immobilized liquid was examined. This membrane did not cause deviation from linearity for injection volumes up to 7 ml and gave an enrichment factor per unit sample volume of 3.8 ml^{-1} for 2,4-D. In order to increase the selectivity towards polar interferents, a solvent consisting of 25% dihexyl ether in *n*-undecane was chosen, which resulted in a decrease in the enrichment factor to 2.6 ml^{-1}. The amount of dihexyl ether needed to reach the plateau of mass transfer is therefore larger than that for the alcohols. This is probably due to the greater ability

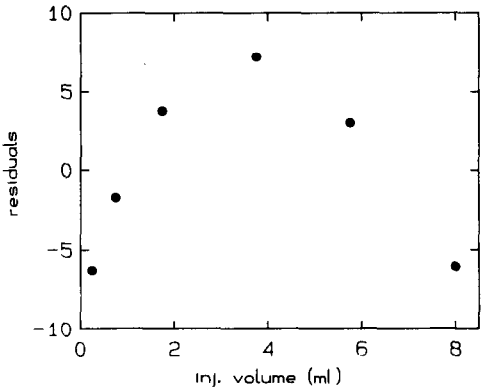


Fig. 4. Differences between the regression line and the experimental points for different injection volumes. Liquid membrane consisting of 5% 1-tetradecanol.

of the alcohols to dissolve by hydrogen bonding, which makes them more efficient as solvents for extraction of CPAs.

Using the membrane of 50% dihexyl ether in *n*-undecane, the enrichment factors per unit sample volume for MCPA and 2,4,5-T were 4.2 and 3.6 ml^{-1} respectively. This membrane was stable for several weeks and was used in the subsequent experiments.

Interferents

In the analysis of natural waters, problems arising from humic substances are almost always encountered. Humic substances consist of species with molecular weights ranging from a few hundred to several millions. The chemical structure is undefined and includes an unusually large number of functional groups[35].

Humic substances are detrimental to both GC and LC columns and must be removed prior to injection. The processing of 1.5 ml of a 350 ppm humic acid solution, in the same way as for CPAs, results in a 99% reduction in the absorbance at 285 nm. Using an *n*-undecane membrane instead of the 50% dihexyl ether membrane is considerably more effective.

However, as can be seen in Fig. 5, there is a fraction of humic acid absorbing at 285 nm that is enriched. This enriched fraction could not be washed back into the donor side by means of a sample-free donor stream as described previously[33]. Hence the fraction is ionized at pH 7 but partially ionized or non-ionized at pH 1. In the experiments discussed below, no adverse effects related to this fraction were observed.

On-line coupling of the flow system to liquid chromatography

The coupling of the sample preparation system discussed above to reversed-phase LC is straightforward. The enriched sample plug in the acceptor is transported to the injection loop of the liquid chromatograph. The pneumatically controlled injector automatically injects a major part of the plug into the chromatographic set-up.

The chromatographic column and eluent system chosen in this work were used previously for the separation of CPAs[22,23]. Throughout, the chromatographic sep-

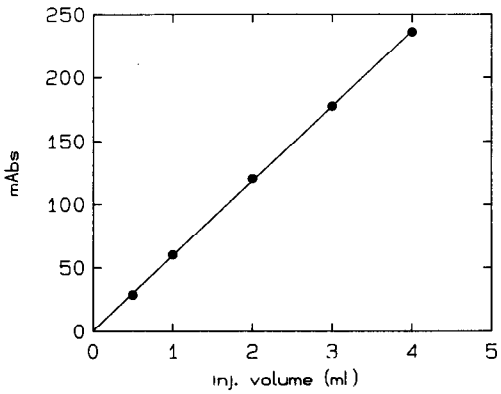


Fig. 5. Detector signals obtained for injections of a 350 mg/l humic acid solution with a membrane of 50% dihexyl ether.

arations were performed with a reversed-phase C_{18} column and methanol–1% acetic acid (3:2) as the mobile phase.

The concentration profile of the enriched sample reaching the injector of the liquid chromatograph is not rectangular, leading to an inhomogeneous concentration in the injector loop. Therefore, comparison of the chromatographic signals on injecting enriched samples and on injecting standard solutions into the chromatograph gives an observed practical enrichment factor which is smaller (about 50%) than the concentration-based enrichment factor described above $[C_p^{max}/(C_{o,s}V_s)]$.

By using a lower flow-rate of a more concentrated sulphuric acid solution on the donor side of the membrane, the total flow-rate passing the membrane could be lowered. This resulted in a larger practical enrichment factor owing to the longer contact time of the sample with the membrane, which means that the recovery is increased. By using a 1.3 *M* sulphuric acid solution at a flow-rate of 0.04 ml/min instead of a 0.2 *M* solution at a flow-rate of 0.25 ml/min, the practical enrichment factor of 2,4-D was increased from about 2 to 3.5 ml^{-1}.

Determination of CPAs in water samples

The detection limit in the chromatographic step, taken as the concentration that gives a signal-to-noise ratio of 2, is about 0.1 ppm. The enrichment factor needed for the determination of a given concentration can be calculated and thence the sample volume or alternatively the time required for the sample preparation.

As an example, if a 1 ppb sample is to be analysed, this will demand an enrichment factor of 100, which will require a 30 ml or take 120 min with the following typical flow-rates: sample, 0.25 ml/min; sulphuric acid stream, 0.04 ml/min; and acceptor, 0.15 ml/min.

Standard solutions of CPAs in water were analysed and the signal was plotted as a function of concentration. The enrichment time was 10 min and the second sample preparation is performed while the first one is being chromatographed. The time required for each analysis is 11–15 min with continuous use of the system.

Solutions containing the same amount of CPAs in 350 mg/l humic acid were also analysed in the same manner. As shown in Table II, no differences at a 95% level

TABLE II

REGRESSION PARAMETERS OF CALIBRATION GRAPHS (PEAK HEIGHT *VS.* CONCENTRATION IN ppm) FOR THREE DIFFERENT CPAs

Experimental conditions as in Fig. 6A. Four concentrations were measured ranging from 0.05 to 1 ppm.

Compound	*Slope*[a]	Intercept[a]
2,4-D	56.4 ± 3.0	0.4 ± 1.7
MCPA	42.8 ± 2.4	0.4 ± 1.4
2,4,5-T	38.4 ± 2.7	0.3 ± 1.5
2,4-D[b]	58.1 ± 1.8	0.0 ± 1.0
MCPA[b]	44.0 ± 1.4	0.1 ± 0.8
2,4,5-T[b]	39.4 ± 1.0	−0.2 ± 0.6

[a] Limits for 95% confidence intervals.
[b] In a solution of 350 mg/l humic substances.

of significance were found between the slopes of the calibration graphs for the determination of the CPAs, whether they are in pure water or in solutions containing humic acid. Fig. 6 shows separations of 10 and 50 ppb CPAs in 350 mg/l humic acid solution.

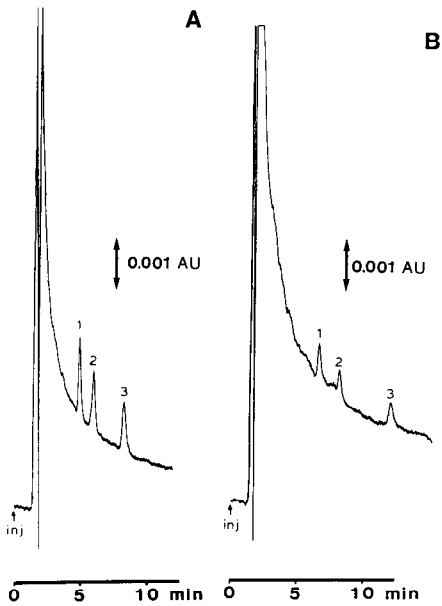


Fig. 6. Chromatograms showing the separations of (1) 2,4-D, (2) MCPA and (3) 2,4,5,-T. (A) Concentration, 50 ppb of each CPA; injection volume, 2.5 ml; chromatographic system as decribed in the text. (B) Concentration, 10 ppb; injection volume, 7.5 ml; eluent, methanol–acetic acid (58:42); other parameters as described in the text.

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