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Supercritical fluid extraction of organochlorine pesticides from an aqueous matrix

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Abstract

Supercritical fluid extraction (SFE) conditions were optimised for the removal of organochlorine pesticides (OCPs) from water. OCPs were collected and extracted from solid-phase extraction disks (Empore) and also directly from a water sample using a modified extraction cell. High recoveries (>90%) were obtained for two of the three OCPs with Empore disks. Despite the good solubility of OCPs in pure CO_2, the analyte recoveries decreased when they were extracted directly from water. Three different flow-rates were used in the direct SFE with no apparent change in recovery, indicating that extraction was diffusion-controlled. The effect of increasing the ionic strength of the aqueous sample on analyte recovery was investigated.

1. Introduction

There has been increasing interest in supercritical fluid extraction (SFE) as a sample preparation technique in analytical chemistry. SFE has increasingly replaced more conventional extraction methods, such as Soxhlet and solvent extraction with organic solvents [1–3]. Supercritical fluids possess physical properties that are intermediate between those of liquids and gases. These unique properties lead to increased diffusion rates and low viscosity which ensure rapid extraction of analytes is possible. Solvent strength is related to supercritical fluid density which can be altered by changing the pressure and temperature of the fluid. This enables selective extractions to be performed [4].

Uncertainty about the long-term environmental effects of organochlorine pesticides (OCPs) has led to voluntary or compulsory control of their use in most countries [5]. However, because of their highly persistent nature and known mammalian toxicity residue analysis is still continued. All of the OCPs studied (lindane, dieldrin and aldrin) are found on the Department of the Environment's "Red-List" [6] of dangerous substances in water.

The majority of literature published on the supercritical fluid extraction of pesticides is concerned with removal from solid matrices with little or no water content [7–9]. There are several problems associated with extraction of analytes from aqueous solution. For direct extraction, the nature of the sample necessitates the use of an extraction cell different in design to the conventional "flow-through" type to retain

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the sample in the cell. However the main problem is the relatively high solubility of water in supercritical carbon dioxide, approximately 0.3% [10]. This can cause restrictor plugging by ice during the supercritical fluid adiabatic expansion and carry over of water into the collection solvent and ultimately into the chromatographic detection system.

Initial studies on SFE from water matrices used "closed loop stripping", where the supercritical fluid was recirculated by a pump back into the water sample. After equilibration a sample is taken via an injection loop and analysed by supercritical fluid chromatography (SFC) [11,12]. Another method incorporates a sandwich type phase separator to remove the aqueous phase from the supercritical carbon dioxide before analysis by on-line SFC [13].

The recent introduction of solid-phase extraction (SPE) disks (Empore) has led to direct extraction of trace organics from aqueous samples. SPE allows more rapid extraction than is possible using conventional methods. The disks comprise a PTFE membrane impregnated with C_{18} bonded silica. They are used in a conventional filtration apparatus where the sample is filtered under vacuum [14]. The large surface area of the disk ensures the high flow-rates used do not cause break-through of sample. Preconditioning of the disk prior to use is necessary to activate the sorbent sites [15]. Since the eluting solvent is carbon dioxide only methanol and water are used for preconditioning. The disks were used to isolate the OCPs from the water matrix prior to SFE. After filtration of sample, the disks are dried, loosely rolled and placed in a conventional 10-ml extraction cell for SFE with carbon dioxide.

The cell used for direct extraction of OCPs from the aqueous phase is shown in Fig. 1 [16]. The cell is made from stainless steel and has an internal volume of 50 cm^3. The inlet tube has a conventional HPLC solvent filter attached to it to aid mixing of the sample with the supercritical carbon dioxide. The 'head-space' configuration of the cell allows the supercritical fluid to pass through the aqueous sample before exiting via an outlet frit into the restrictor to be collected.

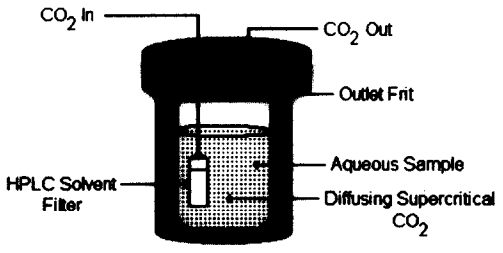

Fig. 1. Schematic diagram of the "headspace" extraction cell.

1.1. Mechanism of pesticide extraction from water

The hydrides of small non-metallic elements are gaseous at room temperature. Water is the sole exception and its existence in condensed phases is due to the strength of the O-H···O hydrogen bonds and to the fact that each water molecule can form four such bonds acting as both a hydrogen donor and receptor. As a consequence, the structure adopted by ice is a tetrahedral one with unfilled space left within the crystal. Pure water has a similar tetrahedral arrangement [17].

Hydrophobic substances are defined as those that are readily soluble in many non-polar solvents, but only sparingly soluble in water. In fact the attraction of non-polar groups for each other plays only a minor role in the hydrophobic effect. The hydrophobic effect primarily arises from the strong attractive forces between water molecules, which being isotropically arranged, must be disrupted or distorted when any solute is dissolved in water. If the molecule is ionic or contains polar groups, it can form strong bonds to water molecules, which more than compensate for the disruption or distortion of the bonds existing in pure water; and ionic or polar substances will tend to be soluble in water. No such compensation occurs with non-polar groups and their solution in water is accordingly resisted.

When a hydrophobic pesticide is introduced in the water matrix there is a disruption in the ordering of the structure. The removal of the molecule is entropically favoured and it will be

partitioned at the supercritical CO_2–water interface. Extraction from a solid matrix tends to be initially equilibrium controlled (due to interaction of the supercritical fluid with the analyte on the surface of the matrix) followed by diffusion (kinetic controlled) through the matrix as the extraction proceeds. However, extraction from water differs from this since water is sparingly soluble in supercritical CO_2. This implies that extraction will only be kinetically controlled as the supercritical CO_2 diffuses through the aqueous matrix.

Addition of salt to the water sample causes an increase in the ionic strength of the solution which in turn increases the solvent–solvent interactions. The relative hydrophobicity of the pesticides are enhanced and the molecules tend to aggregate together to reduce the disruption to the water structure. These larger pesticides structures can then be more easily removed from the aqueous matrix by the diffusing supercritical CO_2. Once the concentration of pesticide falls below the "aggregation point" the effect of ionic strength on the recovery will be reduced. The addition of salt to the aqueous sample should allow pesticide molecules to be more efficiently removed in the earlier stages of extraction until the "aggregation point" is reached. No additional effect on recovery will be noted once the pesticide concentration is below the "aggregation point".

2. Experimental

2.1. Reagents

Lindane, aldrin, dieldrin (purities 99.5%) and hexabromobenzene (99.2%) were obtained from Promochem (St. Albans, UK). Methanol, hexane (HPLC grade) and acetone (AnalaR) were obtained from various sources. Carbon dioxide (SFC grade) was supplied from Air Products (Sunderland, UK). Celite, 60–80 mesh, (used as an inert matrix for initial optimisation experiments) was purchased from BDH (Merck, Poole, UK). Solid-phase extraction disks (Empore) and Bond Elut extraction cartridges were both obtained from Phase Separations (Clwyd, UK).

2.2. Apparatus

The optimisation and SPE were performed on a Carlo Erba SFE 30 system (Carlo Erba, Milan, Italy) using a 150-ml syringe pump (SFC 300, Fig. 2). All SFE was performed in off-line mode through a heated metal restrictor maintained at 150°C with the analytes being collected in a suitable solvent (hexane). Static and dynamic extraction were achieved by means of an air actuated pneumatic valve. All extractions were performed at constant pressure (between 15 and 45 MPa) and temperature (between 40 and 150°C). Optimisation experiments were carried out using a 1.67-ml Keystone extraction cell (Mettler-Toledo, Halstead, Essex, UK). The size of the solid-phase extraction disks necessitated the use of a larger extraction cell. A 10-ml cell supplied by Jasco (Mettler-Toledo) was used for these extractions.

Due to the large size (50 ml) of the 'headspace' extraction cell used for direct analysis of water samples a Jasco SFE system was used. The Jasco SFE system has been described in detail elsewhere [16] The collection system was modified to ensure effective trapping of the pesticides which could be lost due to aerosol formation during the violent depressurisation of the CO_2. The modified system uses a 25 cm^3 glass vial, containing 6–7 ml of solvent. A PTFE-coated rubber septum cap is pierced by the 1/16 in. (1 in. = 2.54 cm) stainless-steel tubing from the

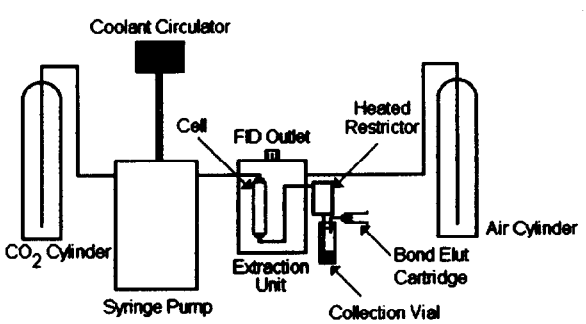

Fig. 2. Schematic diagram of the Carlo Erba SFE apparatus.

back-pressure regulator (BPR) and by a syringe needle to which a Bond Elut C_{18} cartridge is attached. The needle allows the depressurized CO_2 to escape while the cartridge traps any analyte that may be carried with the aerosol and can be subsequently back flushed with a small amount of collection solvent into the vial.

Analysis of the extracts was by a Perkin Elmer 8420 gas chromatograph (Buckinghamshire, UK) with electron-capture detection (ECD) and split injection (50:1 ratio). A 0.5-μl volume was injected onto a 12 m × 0.25 mm I.D. BP-5 fused-silica column (SGE, Ringwood, Australia). The oven was maintained at 240°C throughout the analysis with the injector and detector temperatures 250 and 350°C, respectively. Nitrogen was used as both carrier and make-up gas. Hexabromobenzene was used as an internal standard. A typical chromatogram is shown in Fig. 3.

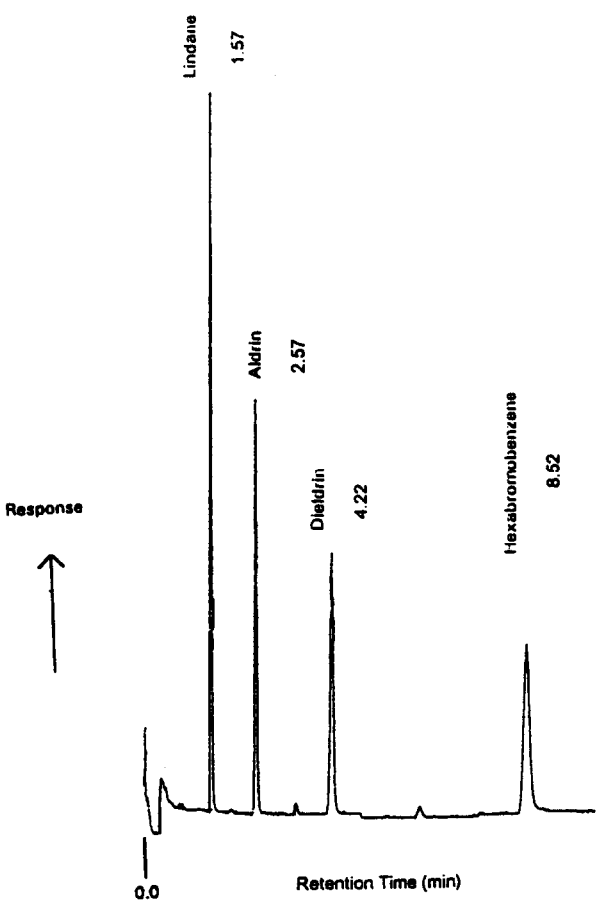

Fig. 3. A typical GC–ECD chromatogram.

2.3. Procedure

Optimisation

Pressure and temperature variables were initially optimised by extracting the OCPs from an inert matrix (Celite). A known amount of pesticide was spiked onto 0.2 g of Celite and the solvent allowed to evaporate. A simple experimental design approach was used to combine various pressure/temperature combinations. Extraction time was not used as a variable because the fixed restrictor on the Carlo Erba SFE dictates that the fluid flow-rate is not constant. The amount of CO_2 passing through the extraction cell was kept constant and therefore extraction time varied during each experiment. The optimum conditions for extracting the three OCPs from Celite were found to be 300 atm (1 atm = $1.01 \cdot 10^5$ Pa) and 50°C (density 0.85 g ml^{-1}).

Solid-phase extraction

These conditions were then used to extract the OCPs that had been trapped on a solid-phase extraction disk. A sample consisting of 200 ml of distilled water to which 10 μg of each OCP had been added was used for the extractions. The sample was then pre-treated prior to filtration by adding 5 ml of methanol and adjusting the pH to 2 with concentrated hydrochloric acid [15].

The disks were first pre-treated by activating with 10 ml of methanol for 3 min and then passing through air for 1 min. A further 5 ml of methanol was added and allowed to soak for another 3 min followed by 10 ml of distilled water and the sample which was filtered in approximately 5 min (care must be taken not to allow the disk to become dry during this state). The disk was then air dried for 10 min, placed in an oven (45°C, 20 min) for further drying and then rolled and placed in the 10-ml extraction cell. A 30-min static extraction was then carried out under the optimum conditions followed by a

dynamic extraction where two lots of 30 ml of CO_2 were passed through the cell. The extracts were then combined and analysed as before.

Direct extraction from water

Direct analysis of a water sample containing OCPs was evaluated using the "headspace" extraction cell (Fig. 1). The extraction conditions used in the SFE of the solid-phase extraction disks were used to extract lindane, aldrin and dieldrin (10 μg each in acetone) from 45 ml of distilled water. The effect of flow-rate on recovery was investigated by extracting at 0.7, 1.0 and 1.5 ml min^{-1}. Extraction times were varied from 15 min to 2 h and the extracts analysed by GC–ECD.

The effect of adding sodium chloride (8 g) to the sample (45 ml) prior to extraction was investigated. Salt is commonly used in solvent extraction [18] as it increases the ionic strength of the solution. This increases the relative hydrophobicity of the non-polar OCPs and therefore will aid their removal from the matrix. The amount of salt used in the experiment corresponds to the ratio used in the EPA method [18].

3. Results and discussion

In the initial stages of developing a SFE method the supercritical fluid composition, pressure and temperature must all be considered. This can be achieved by extracting the analytes from an inert matrix such as Celite to prevent matrix effects. This can also be useful in determining the trapping efficiency of the collection device. Preliminary studies on such spiking experiments indicated that the conventional collection devices on both the Jasco and Carlo Erba instruments were unsatisfactory at trapping the extracted analytes. This led to the development of the modified collection vessel now used incorporating a Bond Elut cartridge (Fig. 2).

3.1. Optimisation of SFE conditions for OCPs

The optimum conditions for extraction of OCPs by pure CO_2 were determined by dynamically extracting from Celite at various pressure/temperature combinations using a simple factorial design. It must be noted that the extraction conditions may have to be altered when the OCPs are extracted from a real matrix.

3.2. SPE–SFE of aqueous samples

The optimum conditions for SFE of OCPs from Celite were found to be a dynamic extraction at 300 atm and 50°C. These conditions were used in extracting OCPs which had been previously trapped on a SPE disk. However, a 30-min static extraction, at the same pressure and temperature, was performed prior to the dynamic extraction to successfully remove all of the trapped OCPs from the disks. The recoveries of the OCPs are shown in Table 1.

It is seen that quantitative recoveries are possible for aldrin and dieldrin using a combined SPE–SFE method. These results are comparable to extraction of OCPs from spiked sand [8]. The recoveries for lindane are lower than expected although it is not possible to determine whether this is due to poor retention on the SPE disk or to the actual SFE of the disks. The R.S.D. values on four extractions are high; however this

Table 1
Combined solid-phase extraction–supercritical fluid extraction of organochlorine pesticides from an aqueous sample

Extraction number	% Recovery (10 μg)		
	Lindane	Aldrin	Dieldrin
1	65.3	103.0	80.0
2	78.8	89.9	96.6
3	88.5	96.8	99.6
4	75.5	104.4	90.2
Average	77.0	98.5	91.6
% R.S.D.	12.4	6.7	9.5

is an accumulated error generated over three separate stages *i.e.* SPE, SFE and GC.

3.3. Direct analysis of aqueous samples

SFE of OCPs from an aqueous sample was carried out directly, at different flow-rates of supercritical CO_2 using the "headspace" extraction cell (Fig. 1). The same conditions were used as in the combined SPE–SFE experiment. The effect of increasing the ionic strength of the solution by adding sodium chloride to the sample was also investigated. The percentage recoveries of the extractions at various extraction times were determined. The results are shown in Figs. 4 and 5. As expected the recovery increases with increased extraction time as more CO_2 is allowed to pass through the cell. However it would not be practical to extend extraction time beyond 2h because of the amount of water carry over observed at long extraction times. The number of cell volumes swept for the three flow-rates studied (0.7, 1.0, 1.5 ml min^{-1}) and at a typical extraction time of 60 min are 0.9, 1.3 and 1.9, respectively. It can be seen from the graphs that within experimental error (see below) flow-rate has little effect on the recoveries of OCPs. Although the cell volumes swept is more than double, at flow-rates between 0.7 and 1.5 ml min^{-1}, the recoveries do not show a marked increase. The curves obtained increase rapidly and then gradually plateau indicating that the extraction is kinetically controlled [19]. Diffusion from the aqueous matrix or slow desorption kinetics limiting the rapid extraction of analytes.

Eventhough the effect of "salting out" is well known and is frequently used to assist extraction [18] the results reported in Fig. 5 indicate that salt has no significant effect on the recovery of the analytes. This may be due to the much increased water carry over observed when salt is added to the extraction cell (even at short extraction times) causing problems in detecting the OCPs by GC–ECD. It therefore appears impractical to use salt to assist the extraction of hydrophobic molecules by supercritical CO_2.

Fig. 6 shows the percentage recoveries for all the OCPs with respect to flow-rate and salt addition and the variation in data obtained. It is concluded that all the results reported fall within an extraction "envelope" and that this represents the actual limits of the method to extract OCPs directly from water. The deviation in results observed at longer extraction times may be due to the increased amount of water carry over observed which becomes more noticeable at higher flow-rates (1.5 ml min^{-1}). This observation combined with a removal of the majority of collection solvent by the violent depressurisation of CO_2 from the BPR may cause an increased error in the overall analytical procedure.

A repeatability study ($n = 5$) was undertaken on a 15-min extraction at 300 atm, 50°C and 1 ml min^{-1}. The % R.S.D. for lindane, aldrin and dieldrin was found to be 6.7, 7.3 and 8.2%, respectively. These recoveries compare favourably with those reported for the combined SPE–SFE method.

The overall recoveries obtained for direct extraction from water are in the order of 20% lower than the extractions involving prior trapping of the analytes onto C_{18} solid-phase extraction disks using the combined SPE–SFE method. The lower recoveries of OCPs obtained by direct extraction may be indicative of poor diffusion of the supercritical CO_2 through the aqueous matrix. However, the inclusion of a modified cell with HPLC solvent filter to increase the diffusion of the supercritical CO_2 should allow extraction from within the bulk aqueous sample and not just the CO_2–water interface [16]. The lower recoveries of OCPs obtained may however be acceptable in qualitative analysis particularly as no preconcentration step is required.

4. Conclusions

The extraction of OCPs from an aqueous matrix has been achieved by two different methods. The techniques are simple to undertake and are faster than conventional solvent extraction. Solid-phase extraction disks are shown to be efficient at trapping OCPs and give quantitative results with supercritical CO_2. This leads to the

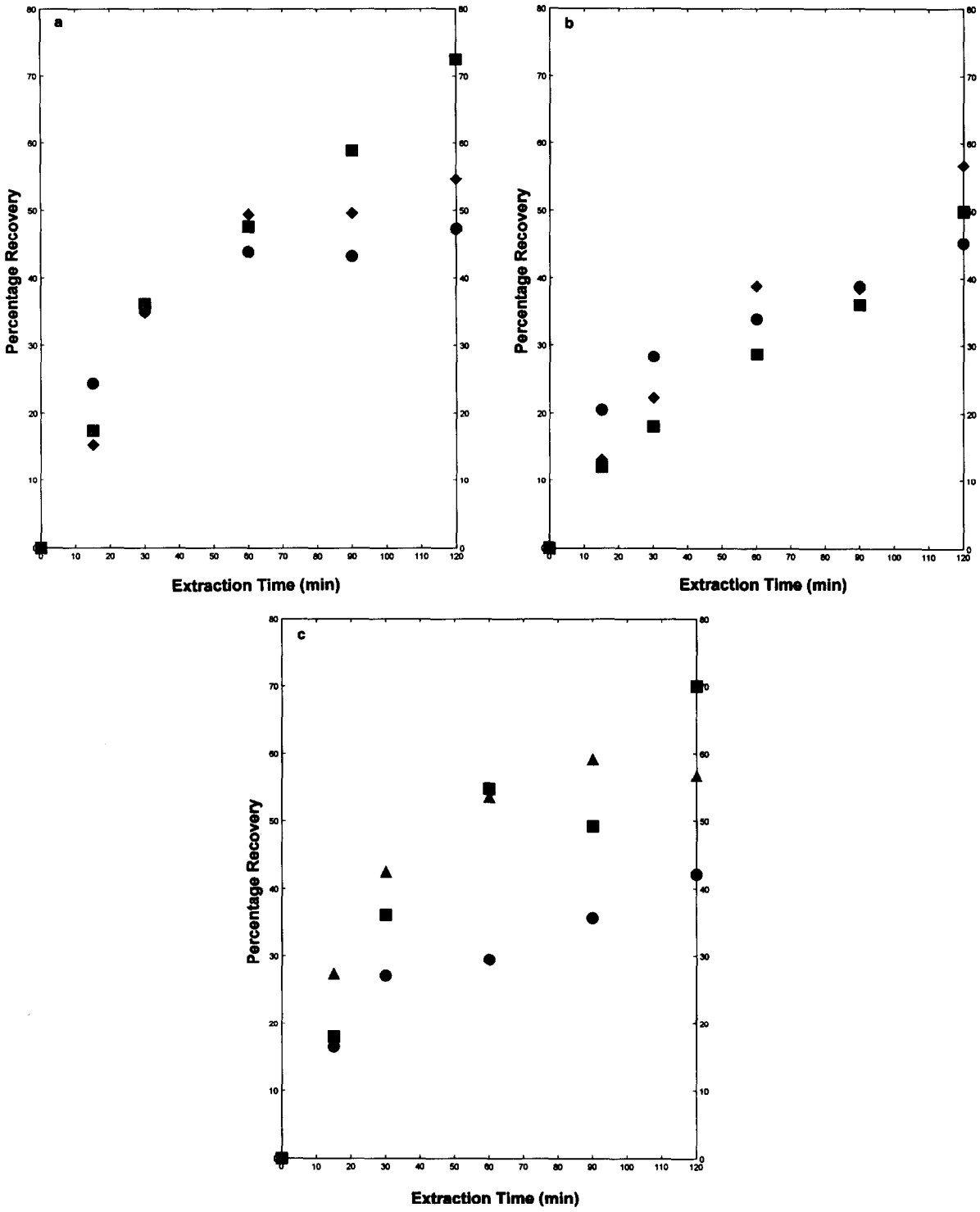

Fig. 4. Percentage recovery *versus* extraction time. (a) Lindane, (b) aldrin, (c) dieldrin. Flow-rates: ● = 0.7; ■ = 1.0; ◆ (a and b) = 1.5, ▲ (c) = 1.5 ml/min.

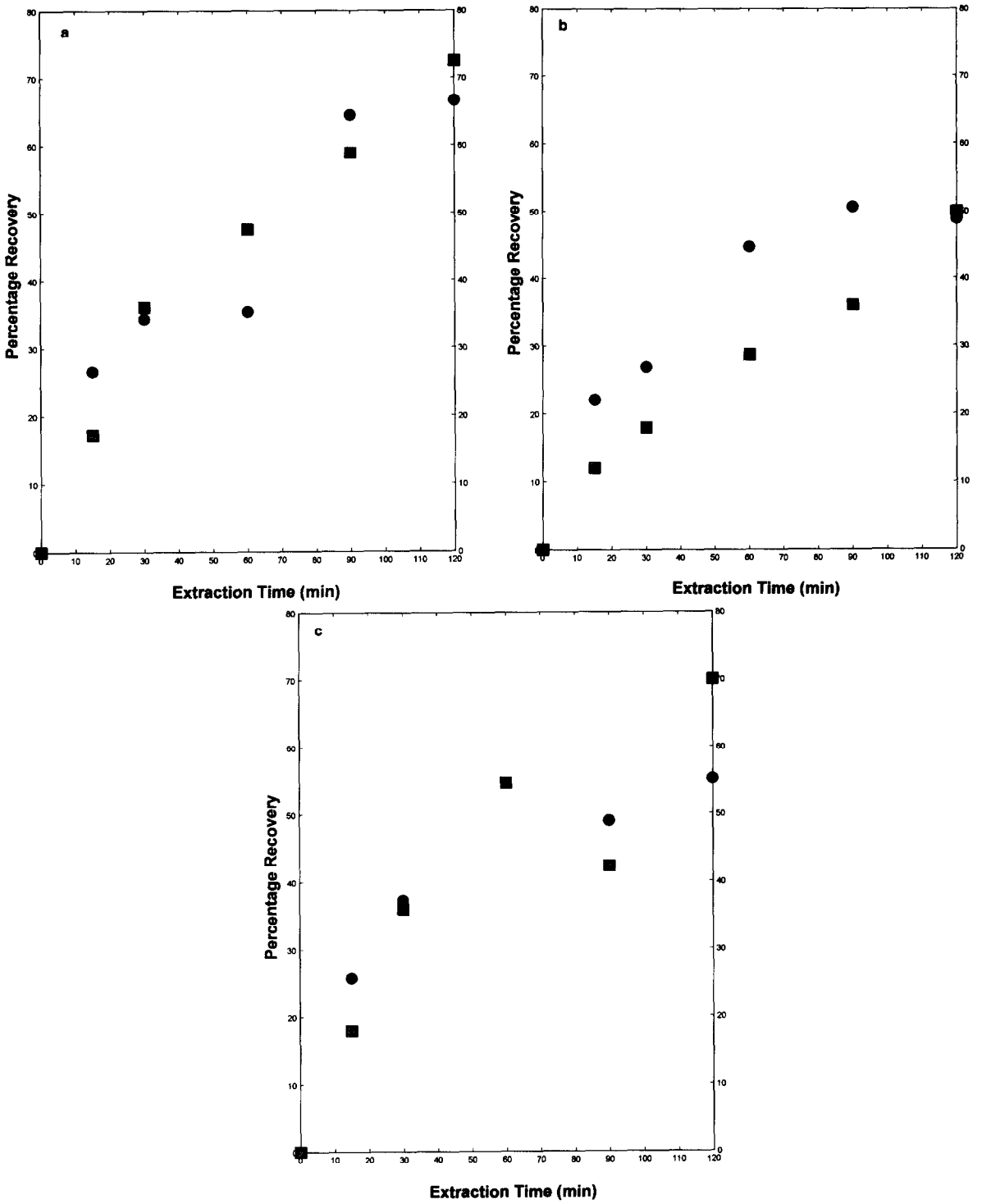

Fig. 5. Percentage recovery *versus* extraction time. Effect of salt for (a) lindane at 1.0 ml/min, (b) aldrin at 1.0 ml/min, (c) dieldrin at 1.0 ml/min. ● = With salt; ■ = without salt.

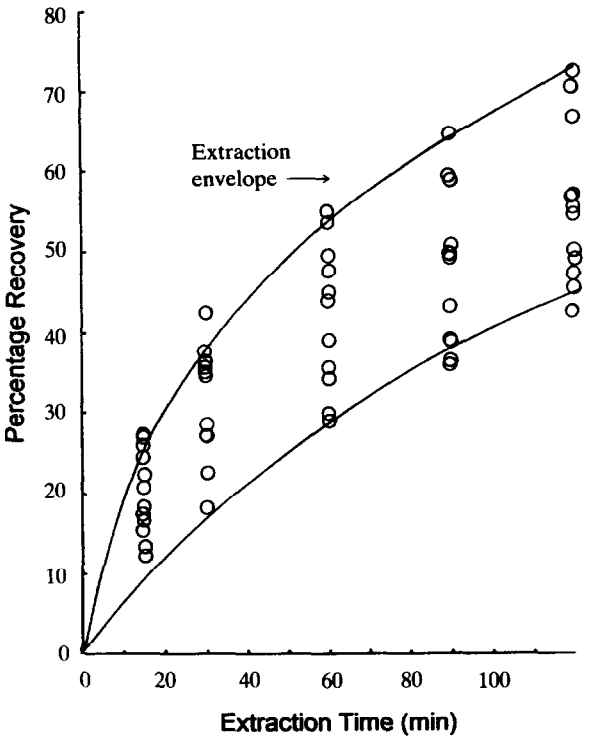

Fig. 6. A plot of percentage recovery data for aldrin, dieldrin, lindane at the three flow-rates studied plus the data when salt was added (1.0 ml/min only).

possibility of selectively extracting pesticides from the disks by using SFE rather than conventional solvents. Direct extraction from water was not affected by the addition of salt to the matrix although this did have an adverse effect on detection at longer extraction times. Flow-rate appears to have little effect on the recoveries of OCPs direct from water indicating that the process is kinetically limited by diffusion through the aqueous matrix. The method can potentially be used for trace analysis of pesticides in waste waters without the need for a preconcentration step which is usually required in solvent extraction.

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