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Analysis of pesticide residues in matrices with high lipid contents by membrane separation coupled on-line to a high-performance liquid chromatography system

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Abstract

Separation through membranes coupled to an HPLC system was used as a technique for the analysis of pesticide multiresidues in samples with high lipid contents. As well as the usual procedure, in the proposed system it is possible to recirculate the sample through the membrane cell, which permits the extraction system to be applied to cases in which only a very small volume of sample is available. A procedure for pesticide multiresidue analysis in egg samples was developed as a representative example of the applicability of the proposed method. To accomplish this, the analytes (dichlorvos, dimethoate, propoxur, paraoxon, pirimicarb, atrazine, ametryne, terbutryne, azinphos-methyl, folpet) were subjected to prior extraction in a Soxhlet system, after which the extract was introduced into the membrane separation device coupled to the HPLC system. This procedure afforded clean chromatograms, hence considerably facilitating determination, and at the same time was efficient in removing macromolecular compounds. For egg samples, spiked at a concentration level of 0.750 mg/kg, recoveries ranged from 60 to 98%. The detection limits varied from 0.018 mg/kg for dichlorvos to 0.002 mg/kg for atrazine.

Keywords: Membranes; Pesticides; Lipids

1. Introduction

One of the main problems involved in multiresidue pesticide analysis in real samples is the tediousness and complexity of the procedures required for the extraction, clean-up and preconcentration of the matrix analytes. These prior sample treatment steps may indeed be the most laborious part of the whole analysis, especially when the matrices are complex, such as the case of biological samples with high lipid contents. This underscores the importance and current interest in the search for new procedures that are able to simplify prior sample treatment and afford good yields and that can be readily automated.

Most pesticide residue methods described in the literature use a combination of some form of extraction with an organic solvent, with one or several clean-up steps, and purification of the extract to remove coextractants before the sample is subjected to chromatographic separation. Additionally, some solvent concentration/evaporation step is usually used with a view to reaching the desired concen-

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tration and/or the solvent is replaced by another one compatible with the chromatographic system.

Some of these stages can be combined and automated, and are coupled to the chromatographic system [1–4]. Advances in automatization in pesticide residue analysis for foodstuffs, soils and other solid samples are focused on the clean-up procedure, which involves pre-separation of the analytes from the large amount of matrix interferences.

With the development of more selective extraction techniques, such as supercritical fluid extraction (SFE) [5,6], microwave-assisted extraction (MAE) [7,8], solid-phase microextraction (SPME) [9,10], pressurized liquid extraction (PLE) [11,12] and analytical methods that are less affected by co-extracted matrix components, clean-up becomes less important than with many current methods. However, clean-up continues to be necessary in many applications, such as pesticide residue analysis in fatty foods.

Liquid–liquid partitioning [13,14], adsorption (Florisil) chromatography [15], gel permeation chromatography (GPC) [16,17] and/or solid-phase extraction (SPE) [18,19] have been widely applied for the clean-up of extracts in residue analysis. Other approaches for interference separations in sample preparation methods are dialysis [20,21], ultrafiltration and immunoaffinity chromatography [22,23], although these are not much used in pesticide residue analysis.

Analytical use of membranes in the selective separation of organic molecules [24–28] has proved to be a valid alternative to conventional procedures of liquid–liquid and solid–liquid extraction. Additionally, such techniques can be coupled to the chromatographic system [29,30] with noteworthy advantages: direct introduction of untreated samples, analyte preconcentration, elimination of interferences, changes in matrix or solvent, preservation of the chromatographic system.

Here we investigated the possibilities of using membranes in the sample treatment steps prior to HPLC analysis for the determination of pesticides in biological matrices with high lipid contents, where it is necessary to separate the analytes from other macrocomponents that interfere in the determination or may damage the chromatographic system.

The chief aim of the work was to develop a procedure that would permit a simplification of the sample treatment steps prior to chromatographic analysis. The use of separation schemes, such as those based on analyte transport through membranes, offers many perspectives in this sense since careful selection of the experimental conditions can increase both the selectivity and the sensitivity of the overall process, avoiding complicated sample treatments. On-line coupling of membranes to the chromatographic system permits a maximum reduction in the use of conventional systems for extract cleaning, affording evident economic and practical advantages.

In our laboratory (Carabias Martínez, Rodríguez Gonzalo, Paniagua Marcos, Hernández Méndez, unpublished results), we have previously performed a detailed study of the transport of different analytes (organophosphorus pesticides, carbamates, triazines, antifungal agents) through membranes, optimizing the different parameters that affect the process; i.e., membrane type and structure, preconcentration time, etc. Here we address the coupling of the separation cell to the chromatographic system and, specifically, application of this coupled system to multiresidue pesticide analysis. We also discuss the analytical characteristics of the method.

As an alternative, we propose the possibility of recirculating the sample through the membrane cell, thus allowing the extraction procedure to be applied even in cases where only very small sample volumes are available.

Application of the proposed procedure to the analysis of pesticides in eggs allowed us to check the efficiency of the procedure for the on-line removal of macromolecular compounds.

2. Experimental

2.1. Apparatus

The on-line extraction–separation system is depicted in Fig. 1. A Gilson 231-401 microprocessor-controlled autosampling injector equipped with a piston pump was used to propel the acceptor solution to the extraction cell and then to the injection loop of the chromatograph. A Gilson Minipuls-3MP4 peristaltic pump with viton tubing was used for pumping the sample to the extraction cell at a flow-rate of 0.3

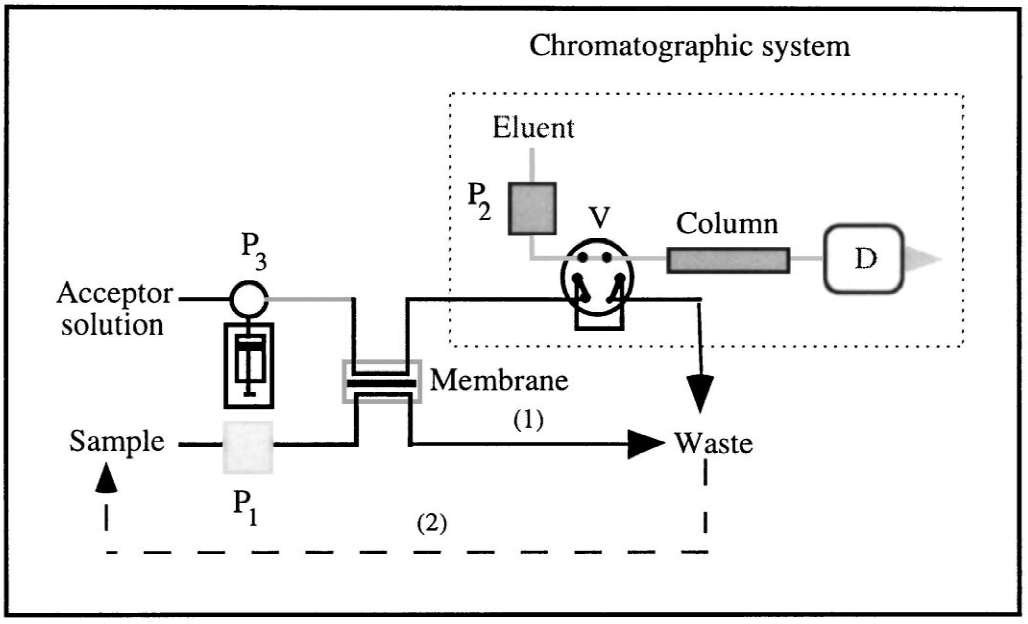

Fig. 1. Schematic diagram of the membrane cell coupled on-line to an HPLC system. P_1, peristaltic pump; P_2, chromatographic pump; P_3, microprocessor-controlled piston pump; V, chromatographic injection valve; D, UV detector; w, waste. (1) The sample circulates through the membrane cell and then goes to the waste; (2) the sample continuously recirculates through.

ml min^{-1}. All connections were of 0.5-mm I.D. poly(tetrafluoroethylene) tubing.

The extraction cell used was the standard dialyser block available in the ASTED System (Gilson); the dialyser consists of two PCTFE (Kel-F) half-blocks, with a U-shape groove, between which the separation membrane is placed. For the standard (372 mm^2) dialyser block, volumes are about 100 μl for the acceptor channel and 175 μl for the donor channel.

A reinforced silicone sheeting, Perthèse (Laboratoire Perouse Implant, France), was used throughout this work. This nonporous hydrophobic film has a thickness of 0.175 mm.

The HPLC system consisted of a Spectra-Physics Model SP-8800 ternary pump, an SP 8450 UV–Vis detector and an SP 4290 integrator. A Rheodyne injection valve with a 10-μl sample loop and a Spheri-5-RP 18 column, 250×4.6 mm, 5 μm (Brownlee Labs.) were used in all experiments. All solvents and samples were filtered through 0.45-μm pore-size nylon membrane filters (Millipore).

2.2. Reagents

All pesticides were obtained from Riedel-de Haën (Seelze-Hannover, Germany). The purities of the individual standards ranged from 97 to 99.8%. The analytes studied, listed in the order in which they appear in the chromatograms, were: dimethoate {*O*,*O*-dimethyl *S*-[2-(methylamino)-2-oxoethyl] phosphorodithioate}; dichlorvos (2,2-dichlorovinyl dimethyl phosphate); propoxur (2-isopropoxyphenyl methylcarbamate); pirimicarb (2-dimethylamino-5,6-dimethylpyrimidin-4-yl dimethylcarbamate); atrazine [6-chloro-*N*-ethyl-*N′*-(1-methylethyl)-1, 3, 5-triazine-2,4-diamine]; paraoxon (*O*,*O*-diethyl *O*-4-nitrophenyl phosphate); ametryne [*N*-ethyl-*N′*-(1-methylethyl)-6-(methylthio)-1,3,5-triazine-2,4-diamine]; azinphos-methyl {*S*-(3,4-dihydro-4-oxobenzo-[*d*]-[1,2,3]-triazin-3-ylmethyl) *O*,*O*-dimethyl phosphorodithioate}; terbutryne [*N*-(1,1-dimethylethyl)-*N′*-ethyl-6-(methylthio)-1,3,5-triazine-2,4-diamine]; folpet [*N*-(trichloromethylthio)-phthalimide].

Standard solutions were prepared in HPLC-grade hexane (Carlo Erba, Milan, Italy). Acetonitrile and methanol were also HPLC grade (Carlo Erba). All other chemicals were of analytical-reagent grade.

2.3. Procedure

2.3.1. Extraction through the membrane cell

Chromatographic determination was carried out automatically using the set-up depicted in Fig. 1(1), which included the coupling of the membrane cell to the chromatographic system. The configuration employed comprised a peristaltic pump, a programmable piston pump, the extraction cell and the chromatographic system.

The samples, solutions of the pesticides in hexane, were continuously propelled into the extraction cell by a peristaltic pump and the acceptor solution was brought to the extraction cell via a programmable piston pump. The acceptor solution, 0.01 *M* H_3PO_4 in methanol–water (70:30, v/v), was kept stopped in the extraction cell for a selected period of time (20 min). Then, the piston pump displaced a fixed volume (80 μl) to fill the injection loop of the chromatographic valve, injection into the chromatographic column being carried out automatically.

To avoid contamination among samples, the extraction cell was washed between individual samples with a 4-ml volume of a solution of methanol–water (70:30, v/v), on the acceptor side, while a solution of pure hexane was circulated across the other side of the membrane.

Alternatively, it is possible to use a procedure based on sample recirculation through the membrane cell (Fig. 1(2)).

2.3.2. Chromatographic determination of pesticides after membrane extraction

Chromatographic separation of the pesticides was performed in a C_{18} column; the mobile phase was a mixture of acetonitrile–water (45:55, v/v) in 0.01 *M* acetic acid–acetate buffer, with a flow-rate of 1.00 ml min^{-1}. The injection volume was set at 10 μl. The mobile phase was degassed by bubbling He through it. Spectrophotometric detection at 220 nm was used. Quantification was carried out using the external standard method and taking the mean peak area value of three injections.

2.3.3. Analysis of egg samples

Whole egg (without shell) was homogenised with a food blender. Twenty grams of homogenate were weighed out and 40 g of anhydrous sodium sulfate were added. The sodium sulfate–sample mixture was spiked with 0.5–2.5 ml of a standard solution of the pesticides in hexane. The pesticides were kept in contact with the matrix for a period of at least 1 h. Then, the spiked sample was placed in a Soxhlet system and extracted with 125 ml of hexane over 90 min. After extraction, the organic extract was circulated across one side of the membrane in the separation cell and the above-described extraction procedure was followed, using a preconcentration time of 20 min.

3. Results and discussion

Analyte transport through a non-porous silicone membrane has been studied previously (Carabias Martínez, Rodríguez Gonzalo, Paniagua Marcos, Hernández Méndez, unpublished results). The results showed that transport through the membrane is related to the octanol–water partition coefficients ($\log P_{ow}$) and to the solubilities of the analytes in the donor and acceptor solutions (hexane and water, respectively). On the basis of these parameters, once the conditions of the extraction system have been fixed (type of membrane, composition of the donor and acceptor solutions) it is possible to predict whether the behaviour of an analyte will be favourable or not. The pesticides studied here showed favourable behaviour for their extraction through a non-porous membrane from a solution in hexane (donor solution) to an aqueous solution (acceptor solution).

3.1. Coupling of the membrane cell to the chromatographic system

Coupling of the membrane cell to the HPLC system was accomplished as shown in the set-up in Fig. 1(1). A programmable piston pump (P_3) propels the acceptor solution to the membrane cell where it is kept for a pre-set time: this time is the preconcentration time. Then, the piston displaces a prefixed volume to fill the loop of the chromatographic valve,

injection into the column being carried out automatically.

Chromatographic separation of the analytes is carried out as specified in Section 2. Under these conditions, all 10 pesticides studied showed good separation and resolution (Fig. 2).

The first variable that must be optimised in the set-up is the volume of acceptor solution to be displaced by the programmable piston pump in order to fill the injection loop of the chromatograph with the most concentrated extract. Different methanol–water or acetonitrile–water mixtures were assayed, all of them in the presence of 0.01 *M* H_3PO_4. In a previous work (Carabias Martínez, Rodríguez Gonzalo, Paniagua Marcos, Hernández Méndez, unpublished results) it was observed that in this acidic condition the extraction of pesticides able to become protonated in the acceptor solution — pirimicarb, atrazine, ametryne and terbutryne — is favoured. Fig. 3 shows the variation in the chromatographic peak area of atrazine as a function of the volume displaced by the piston pump in the different acceptor solutions assayed. Similar results were obtained for the other analytes. Table 1 shows the optimum values of the volume to be moved by the piston pump. It may be seen that these optimum volumes depend on the composition of the acceptor solution and increase slightly with the increase in the propor-

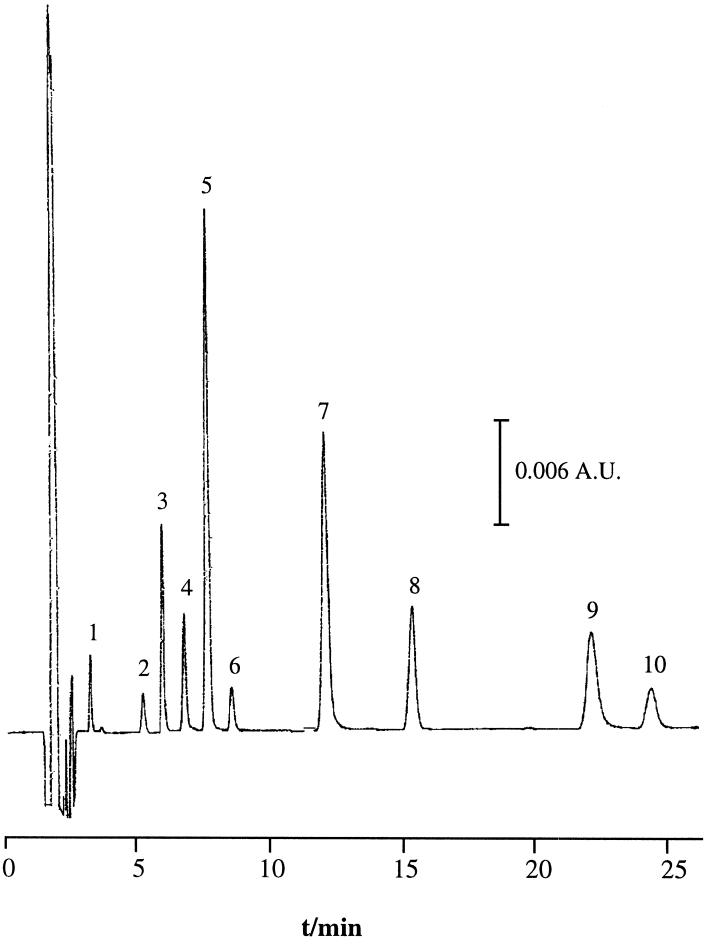

Fig. 2. Typical chromatogram of a standard solution in hexane, after membrane extraction for 20 min. Peaks: (1) dimethoate; (2) dichlorvos; (3) propoxur; (4) pirimicarb; (5) atrazine; (6) paraoxon; (7) ametryne; (8) azinphos-methyl; (9) terbutryne; (10) folpet. Acceptor solution: methanol–water (70:30, v/v) containing 0.01 *M* phosphoric acid.

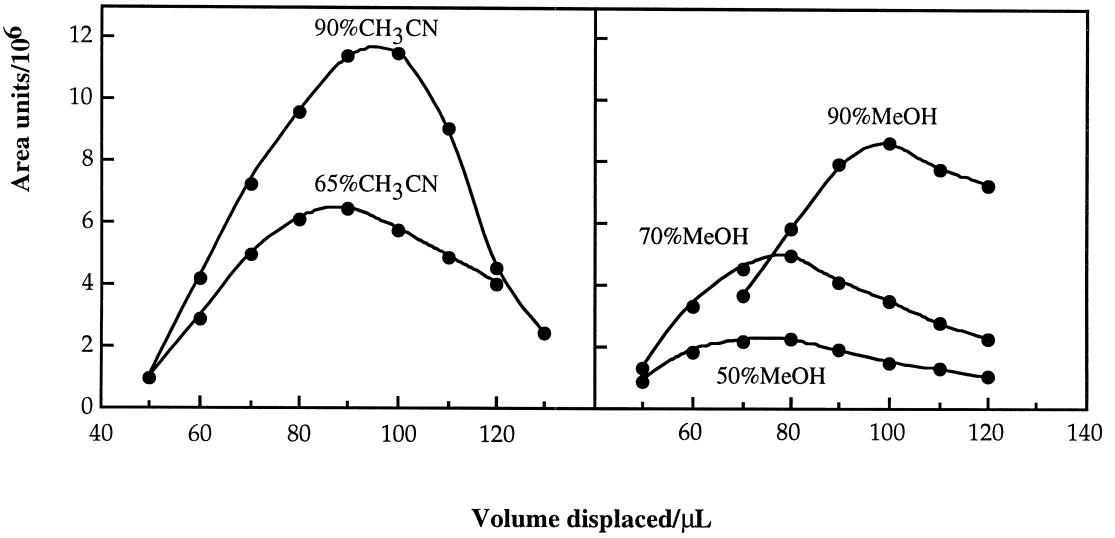

Fig. 3. Effect of volumes displaced by piston pump as a function of the composition of the acceptor solution, plotted for atrazine.

tion of organic solvent. Experimentally, the membrane is seen to undergo greater deformation in the presence of organic solvents, the volume of the extraction chamber increasing slightly.

Once the volume to be displaced to fill the injection loop with the analyte-richest fraction was known, a study was made of the effect of the preconcentration time; that is, the period of time during which the sample circulates continuously over one side of the membrane while the acceptor solution is kept stopped on the opposite side. The preconcentration time was modified between 30 and 1200 s for the different acceptor solutions assayed (Fig. 4). As expected, for all the analytes sensitivity was seen to increase with the extraction time and with the percentage of organic solvent in the acceptor solution, although such increases in sensitivity differed among the different analytes studied. In view of the different sensitivities obtained for all 10 pesticides, the preconcentration time was set at 20 min.

It was also observed that, overall, extraction was more favourable when the organic solvent was acetonitrile than when methanol was used in the acceptor solution. However, the presence of acetonitrile in the acceptor solution gave rise to a chromatographic signal close to the peak corresponding to ametryne, which interfered with its measurement. Accordingly, in later studies it was decided to use a methanol–water mixture (70:30, v/v) containing 0.01 *M* H_3PO_4 as the acceptor solution.

Table 1
Effect of volume displaced as a function of the composition of the acceptor solution

Acceptor solution	V_{opt} (μl)
Methanol–water (50:50, v/v), 0.01 *M* H_3PO_4	75
Methanol–water (70:30, v/v), 0.01 *M* H_3PO_4	80
Methanol–water (90:10, v/v), 0.01 *M* H_3PO_4	100
Acetonitrile–water (65:35, v/v), 0.01 *M* H_3PO_4	90
Acetonitrile–water (90:10, v/v), 0.01 *M* H_3PO_4	100

3.2. Sample recirculation

In this part of the study, the set-up depicted in Fig. 1(2) was used, in which the sample, after passing through the extraction cell, is not taken to the waste outlet but is circulated continuously across the membrane over a given period of time (preconcentration time). Recirculation of the sample across the separation membrane allows the procedure to be applied to samples of which only very small volumes are available.

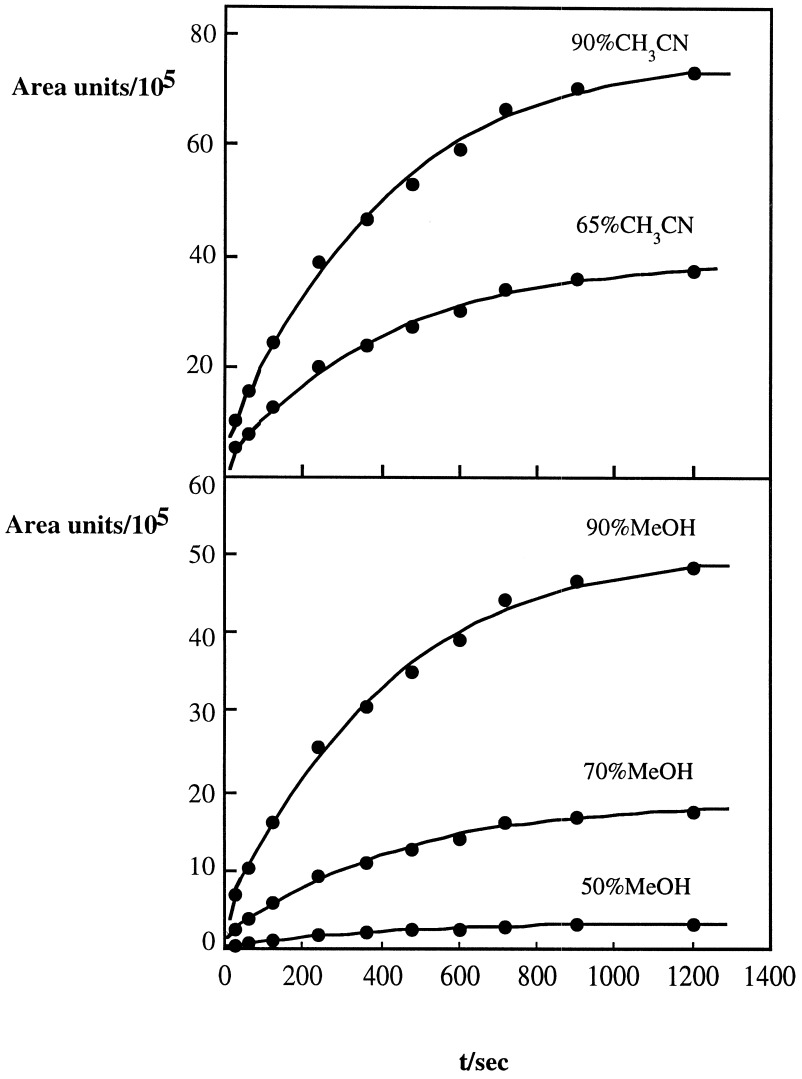

Fig. 4. Variation in the analytical signal of azinphos-methyl with the preconcentration time, as a function of the composition of the acceptor solution.

Volumes ranging between 1.0 and 5.0 ml of a sample containing approximately 5 μg/ml of each pesticide were taken and the chromatograms obtained after keeping the sample recirculating for the previously established preconcentration time (20 min) were recorded. Volumes smaller than 1.0 ml were not assayed because previously it had been calculated that the minimum volume necessary to fill the extraction chamber and the input and output lines was approximately 0.5 ml.

Table 2 shows the peak areas obtained under these conditions with those provided by the sample not subjected to recirculation. It may be seen that the peak areas obtained decrease slightly, but progressively, as the volume of recirculating sample decreases. Thus, for a sample volume of 5.0 ml, a decrease of between 0.5 and 5% is seen, depending on the pesticide in question, with respect to the area obtained for a non-recirculated sample. These differences increase up to 10–50% when the volume

Table 2
Effect of volume of recirculated sample[a]

Volume (ml)	Peak area/10^5					Peak area/10^6				
	Dmt[b]	Dcv	Pro	Pir	Pox	Atz	Amt	Azi	Tbt	Fol
No recirculation	6.0	2.6	12.4	9.7	5.3	4.9	4.2	1.8	2.9	1.3
5	6.0	2.5	11.9	9.4	5.1	4.8	4.1	1.8	2.8	1.3
4	5.4	2.5	11.3	9.3	5.0	4.7	4.0	1.7	2.8	1.3
2	4.3	2.2	10.2	8.9	4.4	4.4	4.0	1.6	2.7	1.2
1	3.2	1.9	8.5	8.1	3.6	4.0	3.7	1.4	2.6	1.1

[a] Five mg/ml of each pesticide in hexane, 20 min preconcentration time.
[b] Dmt=dimethoate; Dcv=dichlorvos; Pro=propoxur; Pir=pirimicarb; Pox=paraoxou; Atz=atrazine; Amt=amethyne; Azi=azinphos-methyl; Tbt=terbutryne; Fol=folpet.

recirculating is 1.0 ml. This decrease in sensitivity seems to be related to the progressive impoverishment of the sample during the recirculation time.

Table 3 shows the preconcentration factors obtained with both procedures, calculated as the ratio between the signal obtained on preconcentrating for 20 min, without and with recirculation, and the signal obtained with direct injection into the chromatograph of a sample at the same concentration that did not pass through the membrane system. The preconcentration factors were very similar with both procedures, although slightly lower when the recirculation procedure was used. This can be compensated by increasing the time that the sample is circulating through the system.

3.3. Analytical characteristics

The experimental relationships between peak area and pesticide concentration (Table 4) were found to be linear over the whole range tested; i.e., 1×10^{-8}–10^{-6} *M* (0.01–1.0 μg/ml). The detection limits calculated at a signal-to-noise ratio of 3 ranged between 2 ng/ml for atrazine and 27 ng/ml for dichlorvos (Table 4a). When the procedure was applied with recirculation at a sample volume of 1 ml the range of linearity was very similar, although the detection limits were slightly higher; between 3 ng/ml for atrazine and 32 ng/ml for dichlorvos (Table 4b). The relative standard deviations (RSD) for 10 replicates at a concentration level of 1.0 μg/ml are also given in Table 4.

Table 3
Preconcentration factors with and without recirculation

Pesticide	Without recirculation	With recirculation (1 ml)
Dimethoate	5.7	3.0
Dichlorvos	2.8	2.0
Propoxur	7.0	4.8
Pirimicarb	5.0	4.2
Atrazine	4.0	3.3
Paraoxon	4.5	3.0
Ametryne	3.8	3.3
Azinphos-methyl	2.3	1.7
Terbutryne	2.2	2.0
Folpet	0.7	0.6

3.4. Determination of pesticide residues in egg samples

The applicability of the proposed procedure was checked by using it to determine the pesticides in avian eggs as a representative sample of biological matrices with high lipid contents. This involved isolation of the pesticides from the egg samples by extraction with hexane in a Soxhlet extraction system and clean-up of the organic extract by membrane separation prior to HPLC analysis.

Fig. 5 shows the efficiency of the membrane clean-up procedure when applied to a non-spiked egg sample subjected to the Soxhlet extraction procedure. Fig. 5a shows the chromatogram obtained when an aliquot of the hexane extract was injected directly into the chromatograph, and Fig. 5b shows the chromatogram corresponding to another aliquot of the same hexane extract when the clean-up procedure was applied; i.e., when it was passed through the

Table 4
Analytical characteristics: calibration straight line, relative standard deviation and detection limit

Pesticide	Intercept	Slope (AU/mol l^{-1})	Corr. coef.	RSD (%)	DL (μg/l)
(a) Without recirculation:					
Dimethoate	$(0.5\pm2.1)\times10^{3}$	$(3.38\pm0.09)\times10^{10}$	0.995	11.2	8
Dichlorvos	$(0.3\pm3.6)\times10^{2}$	$(12.6\pm0.2)\times10^{9}$	0.999	7.0	27
Propoxur	$(0.2\pm0.2)\times10^{4}$	$(82.0\pm0.9)\times10^{9}$	0.999	4.4	5
Pirimicarb	$(-0.2\pm0.4)\times10^{4}$	$(6.6\pm0.2)\times10^{10}$	0.996	3.9	8
Atrazine	$(0.04\pm0.3)\times10^{3}$	$(22.2\pm0.1)\times10^{10}$	1.000	5.9	2
Paraoxon	$(-0.2\pm0.1)\times10^{4}$	$(31.8\pm0.6)\times10^{9}$	0.998	5.9	23
Ametryne	$(0.05\pm0.3)\times10^{4}$	$(22.8\pm0.2)\times10^{10}$	1.000	4.9	5
Azinphos-methyl	$(-0.05\pm0.2)\times10^{4}$	$(11.6\pm0.2)\times10^{10}$	0.998	8.5	11
Terbutryne	$(-0.2\pm0.2)\times10^{4}$	$(8.4\pm0.1)\times10^{10}$	0.999	9.6	17
Folpet	$(0.4\pm0.2)\times10^{4}$	$(9.1\pm0.1)\times10^{10}$	0.999	7.6	20
(b) With recirculation (1 ml):					
Dimethoate	$(0.2\pm0.2)\times10^{4}$	$(16.2\pm0.6)\times10^{9}$	0.993	14.9	13
Dichlorvos	$(-0.7\pm0.6)\times10^{3}$	$(10.5\pm0.3)\times10^{9}$	0.995	8.7	32
Propoxur	$(-0.08\pm4.74)\times10^{3}$	$(6.3\pm0.2)\times10^{10}$	0.991	5.9	6
Pirimicarb	$(-0.2\pm0.2)\times10^{4}$	$(46.3\pm0.8)\times10^{9}$	0.998	5.4	11
Atrazine	$(0.3\pm4.9)\times10^{3}$	$(17.0\pm0.2)\times10^{10}$	0.999	8.0	3
Paraoxon	$(-0.3\pm1.8)\times10^{3}$	$(25.1\pm0.9)\times10^{9}$	0.994	5.5	27
Ametryne	$(-0.3\pm0.7)\times10^{4}$	$(17.8\pm0.3)\times10^{10}$	0.998	8.1	5
Azinphos-methyl	$(-0.1\pm0.3)\times10^{4}$	$(10.0\pm0.2)\times10^{10}$	0.997	12.7	12
Terbutryne	$(-0.2\pm0.4)\times10^{4}$	$(6.0\pm0.2)\times10^{10}$	0.996	8.0	19
Folpet	$(0.4\pm2.2)\times10^{3}$	$(9.0\pm0.1)\times10^{10}$	0.999	14.5	20

AU, area units; RSD, relative standard deviation ($n=10$) for 1 ppm of each pesticide; DL, detection limit ($3N/m$, where N is the noise and m is the slope of the calibration graph).

membrane cell prior to HPLC. This clean-up step through the membrane afforded chromatograms that were much more free of interferences, especially in the zone of long elution times where the appearance of low polarity species, extractable in hexane together with the pesticides, is expected to occur.

In order to optimise the Soxhlet extraction procedure, the effect of the extraction time on the recovery of the different analytes was studied. To do so, different 20-g samples of beaten egg were spiked at a concentration level of 0.750 mg/kg by the addition of 1.0 ml of a standard solution of the pesticides in hexane. Analyte extraction was accomplished using a 125-ml volume of hexane, modifying the extraction time between 300 and 60 min. Table 5 shows the recoveries of the pesticides from spiked eggs for the different extraction times assayed.

It may be seen that the most suitable time for total extraction of the analytes is between 90 and 120 min, for which the recoveries range between 60 and 110%. For shorter times, extraction is insufficient while for times longer than 120 min low extraction yields are obtained, probably due to the fact that losses occur through decomposition or volatility.

For the analysis of pesticides in egg samples it is also possible to apply the procedure with sample recirculation, for which volumes of only about 1 ml are required. The time that the sample is kept recirculating depends on the sensitivity required.

3.5. Analytical data for the determination of pesticide residues in eggs

Egg samples were fortified at concentration levels of ca. 0.050–0.750 mg/kg of each pesticide. The pesticides were extracted with 125 ml of hexane in a Soxhlet extraction system over 90 min. Then, the hexane solution was propelled to the membrane cell coupled to the chromatographic system and analysed according to the described procedure, using a preconcentration time of 20 min. This hexane solution, as obtained from the Soxhlet extraction procedure, is named ‘direct extract’.

The experimental relationship between peak areas

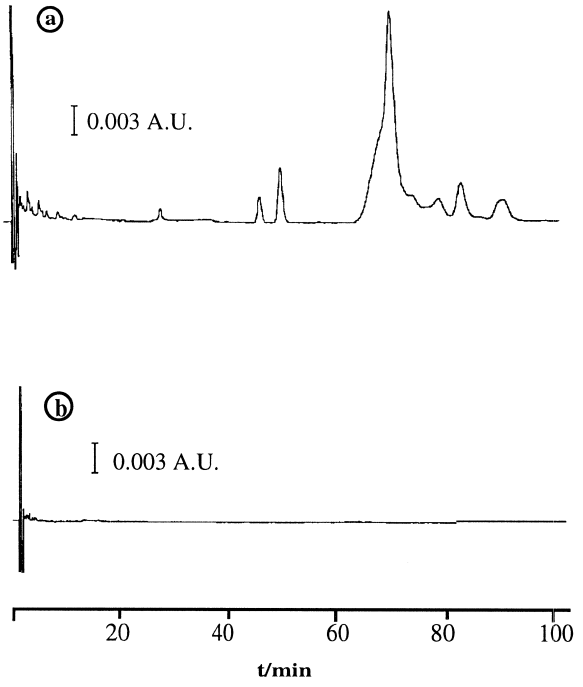

Fig. 5. Chromatogram of a non-spiked egg sample: (a) injected directly into the chromatograph without passing through the membrane cell; (b) after passing through the membrane.

and pesticide concentrations in the samples were found to be linear over the whole range tested (Table 6a). As can be seen, the values of the calibration slopes obtained in egg samples are not very different from those obtained when hexane standards were used (Table 4a), showing that, under these conditions, no significant matrix effect occurs. In egg samples, it is not possible to determine pesticides folpet and dimethoate; the former undergoes a hydrolysis reaction to yield phthalimide during the Sohxlet extraction procedure, and dimethoate cannot be determined as it elutes at short times where a greater number of interferences was observed. The carbamate propoxur cannot be quantified accurately because an interferent co-elutes with it.

The detection limits, calculated at a signal-to-noise ratio of 3 varied considerably, depending on the pesticide in question, and was in the 0.013 mg/kg (atrazine) and 0.20 mg/kg (dichlorvos) range.

The WHO/FAO Codex Alimentarius Commission has set the maximum residue limits (MRLs) for some pesticides in foodstuffs of animal origin: for organophosphorus pesticides in eggs, the MRLs are in the 0.05–0.2 mg/kg range. The European Union (EU) has published a directive setting the MRLs for foodstuff of animal origin [31], in agreement with the Codex recommendations.

One way of increasing the sensitivity of the method and improving the detection limits is to include a new preconcentration step in the procedure based on concentration of the organic extract obtained in the Soxhlet extraction. Thus, after applying the Soxhlet extraction procedure, an aliquot of 100 ml of the hexane extract is evaporated and the dry extract is redissolved in 5 ml of hexane. This 'concentrated extract' is placed in the separation cell and the clean-up procedure through the membrane is applied before injection into the chromatograph. In this case, since the volume of concentrated extract is 5 ml, the sample remains recirculating through the membrane cell for 20 min.

Table 6b shows the calibration fits obtained using

Table 5
Pesticide recoveries from egg samples as a function of the extraction time in the Soxhlet system

t (min)	Recovery (%)							
	Dcv	Pro	Pir	Atz	Pox	Amt	Azi	Tbt
60	55	35	51	49	36	49	42	54
90	63	95	102	84	85	84	49	95
120	60	84	110	95	88	95	76	110
180	35	68	107	88	86	88	57	94
300	23	53	105	86	76	86	28	88

Table 6
Calibration fits for multiresidue pesticide analysis in egg samples

Pesticide	Intercept ($\times 10^{-3}$)	Slope (AU/mol l^{-1})	Corr. coef.	RSD (%)	DL (mg kg^{-1} egg)
(a) Direct extract:					
Dichlorvos	0.03±0.04	$(6.3\pm0.2)\times10^{9}$	0.999	–	0.200
Pirimicarb	−0.4±0.4	$(4.1\pm0.2)\times10^{10}$	0.996	4.4	0.044
Atrazine	−0.8±0.7	$(1.7\pm0.4)\times10^{11}$	0.999	4.9	0.013
Paraoxon	−0.2±0.2	$(2.0\pm0.1)\times10^{10}$	0.993	7.9	0.119
Ametryne	−2±1	$(1.7\pm0.7)\ x10^{11}$	0.997	6.5	0.019
Azinphos-methyl	0.1±0.6	$(8.3\pm0.4)\ x10^{10}$	0.997	12.4	0.056
Terbutryne	0.4±0.7	$(8.6\pm0.4)\times10^{10}$	0.998	9.5	0.063
(b) Concentrated extract (5 ml):					
Dichlorvos	−0.3±0.5	$(3.1\pm0.4)\times10^{9}$	0.996	6.8	0.018
Pirimicarb	−2.0±0.7	$(2.5\pm0.6)\times10^{10}$	1.000	1.1	0.004
Atrazine	−0.4±3.3	$(4.0\pm0.3)\times10^{10}$	0.998	4.3	0.002
Paraoxon	0.6±0.2	$(8.8\pm0.2)\times10^{9}$	1.000	5.7	0.012
Ametryne	−1±3	$(5.8\pm0.2)\times10^{10}$	0.999	3.1	0.003
Azinphos-methyl	−0.6±2.1	$(3.1\pm0.2)\times10^{10}$	0.998	6.0	0.007
Terbutryne	−1.8±0.9	$(3.9\pm0.1)\times10^{10}$	1.000	3.3	0.007

AU, area units; RSD, relative standard deviation ($n=3$). Fortification level: 20 $\mu g l^{-1}$ (0.125 mg/kg egg) of each pesticide; DL, detection limit ($3N/m$, where N is the noise and m is the slope of the calibration graph); –, not detected.

these concentrated extracts. As in the previous case, linear ratios were obtained throughout the concentration range assayed — 0.005–0.75 mg/kg in egg samples, corresponding to a concentration range of 0.016–1.60 mg/l in the concentrated extract (5 ml). In this case, the calibration slopes obtained were very different from those obtained when calibration was accomplished using hexane standards and from those obtained when the direct extract was used (Table 6a), being lower by at least one order of magnitude.

This decrease in sensitivity seems to be related to a strong matrix effect exerted by co-extracted matrix components present in the concentrated extract (5 ml) at a higher concentration than in the direct extract (125 ml). These co-extractants produce a considerable increase in the density and viscosity of the final hexane extract, hindering the diffusion of the analytes towards and across the membrane.

The detection limits obtained under these conditions (Table 6b) are much lower than the MRLs set by the WHO/FAO Codex Alimentarius Commission for organophosphorus pesticides in eggs, where the MRL is 0.05 mg/kg. The relative standard deviation obtained for three replicates at a concentration level of 0.125 mg/kg lie within an acceptable range for trace analysis (Table 6b). Fig. 6 shows representative chromatograms of an egg sample fortified at a concentration level of 0.25 mg/kg egg.

4. Conclusions

Use of a membrane separation cell coupled on-line to an HPLC system permits a clean-up procedure that is simple and effective for the on-line removal of macromolecular compounds before the sample is subjected to a chromatographic separation. Application of this procedure to the determination of pesticide residues in egg samples shows that the membrane clean-up step is a valid alternative for samples with high lipid contents for the elimination of substances that interfere in analyte detection or that may damage the chromatographic system.

Additionally, the proposed system allows recirculation of the sample through the membrane cell, facilitating its use with small samples volumes, even as low as 1.0 ml.

For the determination of pesticide residues in fortified egg samples, the detection limits found are

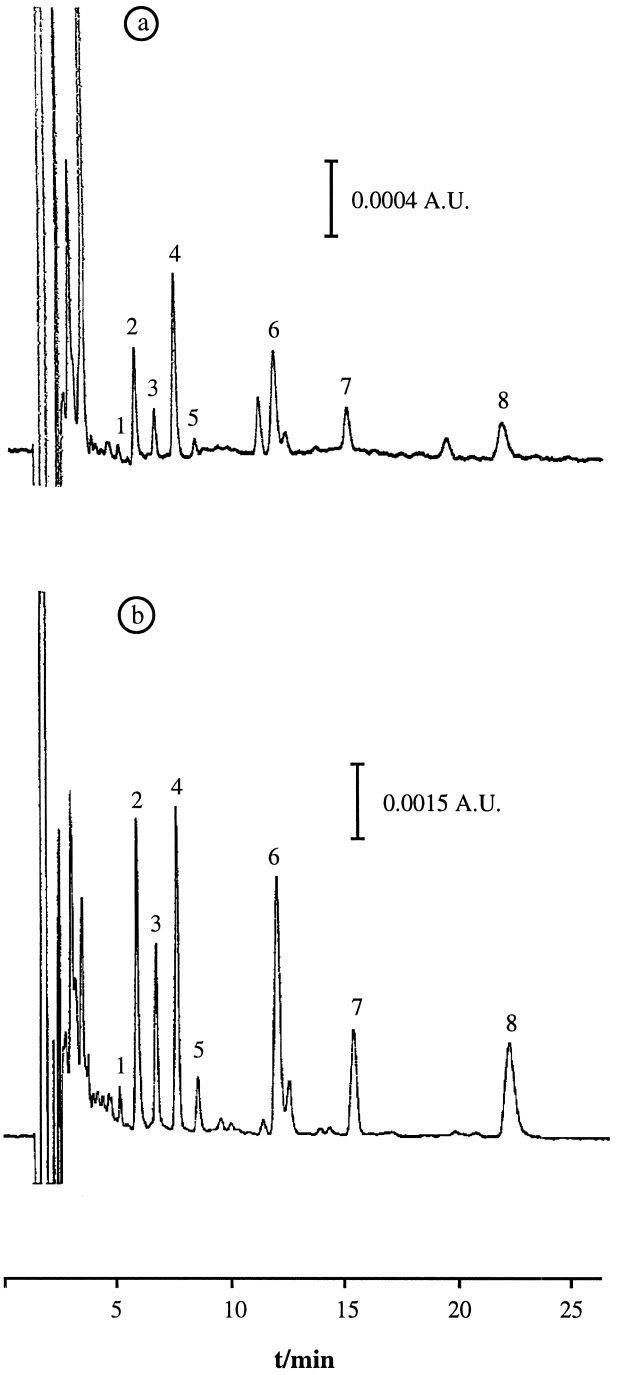

Fig. 6. Representative chromatograms of an egg sample spiked at a level of 0.25 mg/kg egg. (a) Direct extract; (b) concentrated extract. Peaks: (1) dichlorvos; (2) propoxur + interference; (3) primicarb; (4) atrazine; (5) paraoxon; (6) ametryne; (7) azinphos-methyl; (8) terbutryne.

sufficiently low for the method to be applied at levels 2–20 times lower than the MRLs set by the Codex Alimentarius Commission for organophosphorus pesticides in eggs.

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