DOUBLE-PHASE LIQUID MEMBRANE EXTRACTION FOR THE ANALYSIS OF PESTICIDES

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Abstract

A simple and solvent minimized sample preparation technique based on two-phase hollow fiber-protected liquid-phase microextraction was investigated for HPLC analysis of selected pesticides in water samples. Four pesticides (procymidon, methidation, quinalphos, and vinclozolin) were considered as target analytes. Parameters such as extraction solvent, salt concentration, stirring speed, extraction time, and pH value were optimized using spiked deionised water samples. The analytes were extracted from 12 mL water samples through organic solvent (n-hexane and isooctane) immobilized in the pores of a porous polypropylene hollow fiber into 50 µL acceptor phase present inside the hollow fiber. Excellent separations of analytes were obtained on C18 column using acetonitrile-water ratio of 55:45 v/v at elevated flow rate of 0.8 mL/min.

Keywords: Membrane extraction, Liquid-phase Microextraction

Introduction

The increasing production and application of pesticides for agricultural and non-agricultural purposes has caused the pollution of air, soil, ground, and surface water which involves a serious risk to the environment and as well as human health due to either direct exposure or through residues in food and drinking water. In the world, alarming levels of pesticides have been reported in air, water, soil, as well as in foods and biological materials. The analysis of pesticides poses special problems for the chemist, since the pesticides belong to different groups of chemical substances, having a broad range of polarity and acidic characteristics. Most pesticides are volatile and thermally stable, and therefore are amenable to GC with specific detectors or with mass spectrometry (MS). In contrast to GC, LC methods have the advantage of being suitable for thermally unstable and polar/ionic pesticides, as these compounds require derivatization prior to GC analysis.

Thus, interest in the field of pesticide analysis is focusing on improving methodologies, with regard to how rapidly, accurately, and sensitively the chemicals can be detected. This field is highly dependent on the development of new analytical instruments or techniques**.**Despite the valuable advances in separation and quantification, traditional liquid/liquid extraction (LLE) is still among the most popular procedure in routine sample preparation. LLE is recognized as an attractive method for screening tests of unknown pesticides not only because of its simplicity, robustness, minimal operator training, efficiency, and a wealth of available analytical data, but also because of its wide acceptance in many standard methods. However, this age-old technique requires large-volumes of high-purity solvents, which are hazardous, yields small sample-to-solvent volume ratios, and is time-consuming. Although automation has overcome some of its shortcomings, multistage operation and problems of emulsions formation counteract this capability.

There have been several publications on miniaturizing of LLE in analytical chemistry. The major ideas behind this were to facilitate automation and effectively to reduce the consumption of organic solvents. Miniaturised LLE, or LPME, was introduced in 1996, and involved the used of a droplet of organic solvent hanging at the end of a micro-syringe needle [1]. In these systems, analytes were extracted from aqueous samples and into the organic droplet based on their distribution constants, and after extraction, the organic droplet was retracted into the syringe and then injected directly into a gas chromatograph (GC) [2].

In addition, LPME was performed in a three-phase system where ionic analytes in their neutral form were extracted from aqueous samples, through a thin layer of an organic solvent on the top of the sample, and into an aqueous micro droplet (microextract) placed at the tip of a micro syringe. In the latter, pH was selected to ionise

the analytes to maximise partition coefficients and to prevent back-extraction into the organic phase again. In the three-phase system providing an aqueous micro extract, high performance liquid chromatograph (HPLC) was typically used in the final chromatographic analysis [3].

As an alternative to the microdrop concept, we recently introduced an inexpensive and disposable hollow-fibre based device for static LPME both in a two- or three-phase system [4]. This technique; liquid-phase microextraction (LPME) is based on porous hollow polypropylene fibre. When using two-phase LPME the analytes are extracted from an aqueous sample matrix into an organic acceptor phase, which is similar to two-phase LLE. The acceptor phase can then be analysed by gas chromatography (GC) or high performance liquid chromatography (HPLC) in the normal phase mode. Three-phase LPME involves extraction from an aqueous sample matrix, through an organic phase in the pore of the hollow fibre, immiscible with water and back into a new aqueous phase inside the lumen of the hollow fibre. This process is similar to LLE with back extraction. Analytical techniques such as reversed phase HPLC and aqueous capillary electrophoresis (CE) can be used for the analyses of the aqueous acceptor phase in three-phase LPME [5].

The polypropylene fiber used for LPME is less expensive compared to commercial SPME fibers, and a fresh piece is used for each extraction to avoid contamination. In addition, hollow fiber-supported-LPME provides a high enrichment factor, and can also be used as a good clean up device for complex matrices. LPME is generally compatible with GC, capillary electrophoresis, high-performance liquid chromatography (HPLC) and analytical results with low detection limits and good reproducibility can be obtained [6].

The LPME technique is a further development of the supported liquid membrane technique (SLM) and of microporous membrane liquid-liquid extraction (MMLLE) [4]. The chemistry of hollow fibre-based LPME is similar to the chemistry used for extraction with supported liquid membranes (SLM), but the techniques differ significantly in terms of instrumentation and operation. SLM is a flowing system with a pump, which continuously feed the membrane with fresh sample. Thus, SLM is an instrumental sample preparation technique, and each membrane is normally used for a large number of extractions. On the other hand, in hollow fibre-based LPME, both the sample and the extracting phase are stagnant, the membrane (hollow fibre) is used only for a single extraction, and no instrumentation like pumps are required for the sample processing. Thus, with LPME, a large number of samples may be processed simultaneously for instance in a 96-well system [3].

Recent work in our laboratory, have successfully introduced the new extraction technique which involves two-phase liquid phase microextraction combined with micro-liquid chromatography (micro-LC) for the analysis of selected pesticides in water. In this novel procedure, a cone-shaped Nylon membrane is used to protect the extracting solvent, thus permitting extraction only on the surface of the solvent immobilized in the membrane pores. This technique provides both preconcentration and sample clean-up because of the selectivity of the membrane, and the extract can be directly injected into GC or HPLC. The results demonstrated that the procedure is inexpensive, simple to operate and provide stable and acceptable extraction efficiency [7].
The main objective of this paper was to investigate the two-phase (liquid-[membrane]-liquid) liquid membrane extraction mechanism based on phase equilibrium and analyte partition distribution theory using non-ionizable compounds like methidation, vinclozolin, quinalphos, and procymidon with special emphasis on the optimization of parameters in water samples. This was done by studying various parameters which might the extraction recovery.

Experimental

Reagents

Methidation, procymidon, quinalphos, vinclozolin, and profenofos (internal standard) were obtained from Dr. Ehrenstorfer (Augsburg, Germany). HPLC grade acetonitrile and *n*-hexane were obtained from J. T. Baker (USA). Double-distilled deionized water of at least 18 MΩ was purified by Nano ultra pure water system (Barnstead, USA). Stock standard solutions (1000 µg/mL) of the pesticides were prepared in acetoitrile and were stored in the freezer at about -15 ^{o}C. An acetonitrile solution (5mg/L) of profenofos was prepared and used as the internal standard (I.S.).

Equipment and conditions for liquid chromatography with UV-detection (HPLC-UV)

Analysis of extracted pesticides from water samples was performed on an Agilent 1100 Series HPLC equipped with a model Agilent 1100 pump, degasser and detector. Separations and determination of prepared pesticides

mixture were carried using mobile phase acetonitrile-water (55:45) (v/v) at flow rate of 0.8 ml/min. The prepared mixture was injected onto the column triplicate. UV detection of analytes was at 200nm.

Liquid-phase microextraction (LPME)

The homemade coil-shaped setup of HF-LPME device is illustrated in Figure 1. Accurel Q3/2 polypropylene tubular membranes (Membrana, Wuppertal, Germany) with a wall thickness of 200 µm (0.2 µm pore size) and an internal diameter of 600 µm were cut in pieces of 10 cm length for LPME experiments. Each piece of fibre was employed only once to avoid any possibility of carryover. In the optimised method, the fibre pores were impregnated in *n*-hexane or isooctane for 5 s and the excess of this solvent was removed by ultrasonicating the fibre in ultrapurewater for 15 s. After this, the fibre was immersed into the sample (12mL) in a 15mL vial with screw tops and silicone septum by making sure that the whole fibre is totally immersed in the water phase but above the magnetic stirrer bar, so it would not be damaged during the stirring. The fibre was supported in the coil-shape configuration by two medical syringe needles (0.6 cm outer diameter) and it was filled with 50 µL of acceptor solution (*n*-hexane or isooctane). Two syringe needles were inserted through the silicon septum; one served to introduce the accepter solution into the hollow fiber prior to extraction while the second needle was utilized for collection of the acceptor solution after extraction. The vials are placed on a multiple-station magnetic stirrer for 45 minutes at approximately 500 rpm and covered with a small piece of parafilm, in order to avoid contamination, and to reduce evaporation. After extraction, the acceptor solution was flushed into 200 µL vial/insert for the analyte-enriched solvent (50 µL) was withdrawn and transferred into a 1.5mL cone-shaped vial, the solvent was dried with gentle flow of nitrogen and redissolved with 100 µL of acetonitrile solution containing 5 ppm profenofos (I.S.). A 1 µL of solvent was withdrawn into a syringe and injected into HPLC system for analysis.

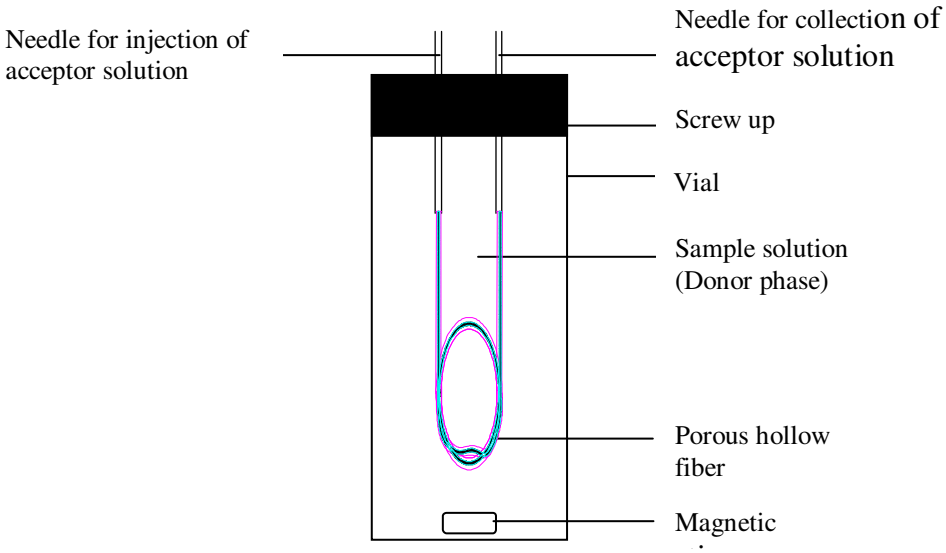

Fig. 1: Two-phase LPME System

(Cross section of the hollow-fiber inside the aqueous sample during two-phase LME)

Calculation of enrichment factors and extraction recoveries

The enrichment factor (EF) was defined as the ratio between the final analyte concentration $C_{a,final}$ in the sample extract (acceptor phase) and the initial concentration of analyte $C_{s,initial}$ within the sample:

$$EF = C_{a,final}/C_{s,initial} \quad (1)$$

The enrichment factor was determined by peak area measurements and by calibration with standard solution containing the [8] pesticides which the concentration level are higher than the concentration in the samples. The

extraction recovery (ER) was defined as the percentage of the total analyte amount $n_{i,initial}$ (originally present in the sample) which was transferred to the extract (acceptor phase) at the end of the extraction ($n_{a,final}$):

$$ER = (n_{a,final}/ n_{i,initial}) \times 100\%$$
$$= (V_a/V_s)\, EF \times 100\% \qquad (2)$$

where V_a and V_s are the volumes of acceptor solution and the sample solution (donor solution), respectively. The extraction recovery was determined from EF, V_a and V_s as shown in Eq. (2).

Results and Discussion

Basic principles

The disposable LPME device is illustrated in Fig.1. The sample solution was filled into a 15 mL sample vial and 50 µL of the acceptor solution was immobilized into the hollow fibre. The analytes were extracted from an aqueous sample solution through a porous hollow fibre (organic phase) into an acceptor phase. During extraction, each LPME device was vibrated to promote analyte extraction.

Provided there were high partition coefficients from the sample to the acceptor phase, analyte enrichment occurred due to high volume ratio between the sample and the acceptor solution. Disposable LPME devices eliminated the possibility of carry-over effects and there was no need for regeneration of the porous hollow fibre [9].

Optimization of liquid chromatography with UV-detection (HPLC-UV)

The standard pesticides were analyzed using HPLC-UV. Excellent separations of analytes were obtained on C18 column using acetonitrile-water ratio of 55:45 v/v at elevated flow rate of 0.8 mL/min. Fig.2 shows the results of direct injection analysis of standard pesticides.

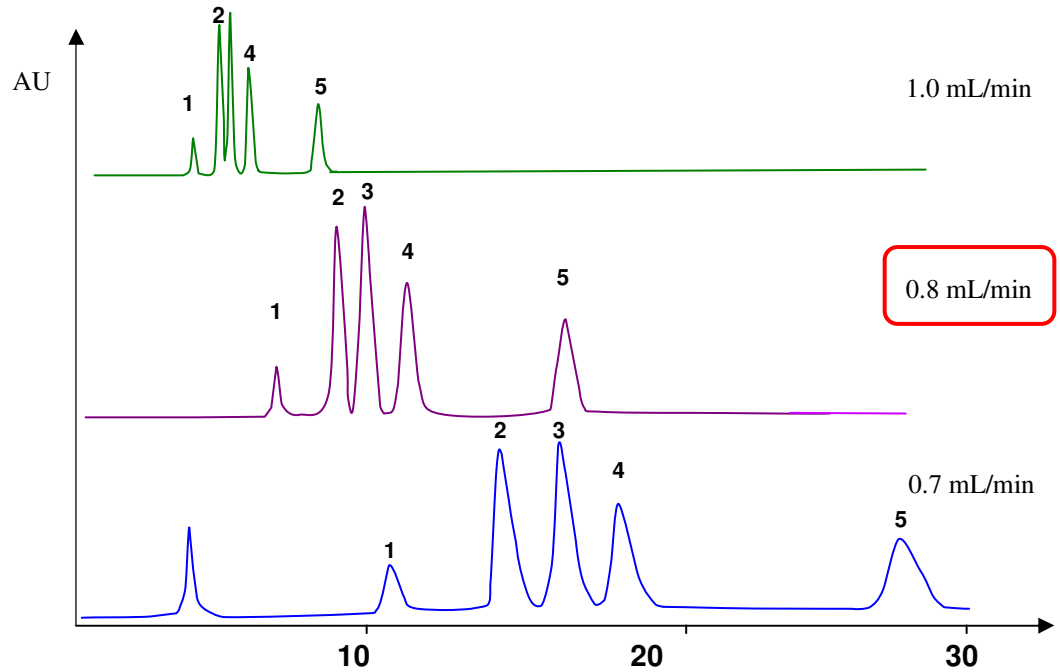

Fig. 2: Chromatogram of standard pesticides studied from direct injection at differences flow rates. Peaks identification: (1) Methidation, (2) Procymidon, (3) Vinclozolin, (4) Quinalphos, (5) Profenofos (IS). HPLC conditions: mobile phase, acetonitrile:water (55:45) (v/v); detection, 200nm; injection volume, 1 µL.

Peaks identification of standard pesticides

From individual direct injection, all of the pesticides were well separated. All the five pesticides were eluted within 20 minutes. Based on the chromatograms obtained, methidation was eluted first followed by procymidon, vinclozolin, quinalphos, and profenofos. The retention time of each pesticides obtained from the chromatogram is presented in Table 1.

Table 1: Retention time of each pesticide studied

Pesticides	Retention time, t_R (min)
Methidation	6.455
Procymidon	8.472
Vinclozolin	9.394
Quinalphos	10.778
Profenofos (IS)	16.192

Optimization of LPME procedure

In order to optimize the liquid-phase microextraction of pesticides using water sample, analytical factors that potentially affect sample extraction were studied. Such factors include solvent type, extraction time, stirring rate, sample pH, acceptor phase type, and salt addition to the donor phase. In this stage, all experiment procedures will be assayed in triplicate. Thus, my future work is optimizing the parameters that affect the LPME.

Conclusion

In the present work, attention was focused on separation of the standard pesticides using HPLC. The best condition of HPLC was analyzed. This work also demonstrated the successful extraction of whole pesticides by HPLC within 30 min. A two-phase (liquid-[Membrane]-liquid) and triple phase (liquid-liquid-[membrane]-liquid) liquid membrane extraction (LME) based on the simple phase equilibrium and analyte partition distribution theory will be elucidated. The surface area effect will be studied and correlated with the overall efficiency of the extraction, which in turn is related to the analyte diffusion rates.

This research will lead towards advancement of knowledge and comprehensive theory on analyte mass transfer mechanism based on phase equilibrium and partition distribution. A detailed understanding on the theory will open new doors to exploration of the extraction mechanism and introduction of novel simple extraction methods in the future.

References

1. K. E. Rasmussen, S. Pedersen-Bjergaard, Trends in Analytical Chemistry. 23 (2004) 1.
2 T. S. Hoa, T. Vasskog, T. Anderssen, E. Jensen, K. E. Rasmussen, S. Pedersen- Bjergaard, Analytica Chimica Acta. 592 (2007) 1–8.
3. S. Pedersen-Bjergaard, K.E. Rasmussen, J. Chromatogr. B. 817 (2005) 3–12.
4. T. S. Ho, S. Pedersen-Bjergaard, K. E. Rasmussen, J. Chromatogr. A. 963 (2002) 3–17.
5. H. G. Ugland, M. Krogh, L. Reubsaet, J. Chromatogr. B. 798 (2003) 127–135.
6. C. Basheer, H. K. Lee, J. Chromatogr. A. 1057 (2004) 163–169.
7. M.M. Sanagi, H.H. See, W.A.W. Ibrahim, A.A. Naim, J. Chromatogr. A. 1152 (2007) 215-219.
8. T. G. Halvorsen, S. Pedersen-Bjergaard, K. E. Rasmussen, J. Chromatogr. B. 760 (2001) 219–226.
9. H. G. Uglanda, M. Krogha, K. E. Rasmussena, J. Chromatogr. B. 749 (2000) 85 –92.