COMPARISON OF DIFFERENT TYPES OF COATINGS IN HEADSPACE SOLID PHASE MICROEXTRACTION FOR THE ANALYSIS OF PESTICIDE RESIDUES IN VEGETABLES AND FRUITS

Chai Mee Kin[1*] and Tan Guan Huat[2]

[1]*Dept. of Science and Mathematics, College of Engineering, Universiti Tenaga Nasional, Km 7, Jalan Kajang-Puchong, 43009 Kajang, Selangor.*
[2]*Dept. of Chemistry, Faculty of Science, Universiti Malaya, Lembah Pantai, 50603 Kuala Lumpur.*

**Corresponding author: Mkchai@uniten.edu.my*

Abstract

Despite the continuing development of solid-phase microextraction (SPME) fiber coatings, their selection presents some difficulties for analytes in choosing the appropriate fiber for a particular application. There are many types of SPME coatings available commercially. The most widely used for determination of pesticide residues in vegetable and fruits are polydimethylsiloxane (PDMS) and polyacrylate (PA). A headspace solid phase microextraction (HS-SPME) procedure using these two commercialized fibers (PDMS and PA) is presented for the determination of selected groups of organochlorine and organophosphorus pesticides. The extraction performances of these compounds were compared using these two fibers. The optimal experimental procedures for the adsorption and desorption of pesticides were determined. An explanation for the extraction differences is suggested based on the different thickness, polarity of the polymeric film of fibers and the different extracting matrices. In addition, the higher detector response of the pesticides after addition of aliquots of water and an organic solvent to the vegetable and fruit samples are also discussed. The SPME fibers were re-usable until a maximum of 120 extractions. Finally, the optimized procedures were applied successfully for the determination of these compounds in vegetable and fruits samples. Mean recoveries for all pesticides were between 75.0-97% with RSD below 7%.

Keywords: HS-SPME, GC-ECD, GC-MS, pesticide.

Introduction

The current developments of analytical technologies to detect pesticide residues in fruits and vegetables have mostly focused on the simplification, miniaturization and improvement of the sample extraction and cleanup methods with universal microextraction procedures [1]. Solid-phase microextraction (SPME), developed by Pawliszyn and co-workers and has been marketed since 1993 by Supelco in an attempt to redress limitations inherent in SPE and LLE [2]. It can integrate sampling, extraction, concentration and sample introduction into a single uninterrupted process, resulting in high sample throughput. Its important features are its simplicity, low cost, rapidity, selectivity and sensitivity. SPME has been applied to analysis in various fields, such as environmental chemistry, forensic chemistry, pharmaceutical, food, beverage and flavours. [3-6]. Nowadays, a large number of fiber coatings are available, namely poly dimethlysiloxane (PDMS), polyacrylate (PA), PDMS-divinylbenzene (DVB), carbowax-DVB, Carboxen-PDMS and DVB-Carboxen-PDMS coated fibers. However, the majority of studies concerning the determination of pesticide residues are performed using manual SPME, PDMS or PA fiber, and direct immersion. [7-11]. Although direct SPME applications for the determination of pesticides in food samples such as juices [12], honey [5,10] and fruit samples [1] have been reported, only a few references on the headspace (HS) SPME approach for the determination of pesticides in fruit [13-14] or vegetable [15-16] samples can be found.

The present study was carried out to evaluate two different fibers, PDMS and PA in the extraction of 8 organophosphorus and organochlorine pesticides in fruit and vegetable samples using HS-SPME.

Experimental

Chemicals and Reagents

All solvents used were HPLC grade. Pesticide standards (diazinon, chlorothalonil, malathion, chlorpyrifos, quinalphos, profenofos, α-endosulfan, β-endosulfan) were > 95% pure and obtained from AccuStandard Inc. New Haven CT, USA. Stock standard solutions of each pesticide at different concentration level, 5-400 mg/kg

were prepared in methanol and stored at 4 ^{o}C. Working standard solutions of pesticides mixture were prepared daily by volume dilution in distilled water. In the calibration and quantitation studies, an internal standard, 1-chloro-4-fluorobenzene, 200 μg/L which is effective as a surrogate to compensate the data of all the 8 pesticides was added to each sample prior to GC analysis.

SPME Procedure

SPME holder and fiber assemblies for manual sampling were obtained from Supelco (Bellefonte, PA, USA) and used without modification. The fiber coatings assayed were polydimethylsiloxane (PDMS 100 μm) and polyacrylate (PA 85 μm). Before measurements the PDMD fiber was conditioned in the injector to fully remove any contaminant which may cause high baseline noise and large ghost peaks. Then the fibers were repeatedly injected into the GC until the interfering peaks disappeared.

Preliminary experiments were carried out to evaluate the HS-SPME process by comparing two coating materials with different polarities and thickness. After that, the optimization of the main parameters affecting the SPME of the pesticides from aqueous solution (i.e. extraction time and temperature, desorption time and temperature, the effect of salt addition and stirring rate) were carried out. In these studies, distilled water samples spiked with the appropriate amount of the standard solution was used. In contrast, the spiked vegetable and fruit samples were used for the study of the effects of dilution and organic solvent.

Fruit and Vegetable Samples

All determinations were performed using the PDMS 100 μm fibers. Initially, 1.0 g of the homogenized fruit and vegetable sample was placed in a 15 mL clear glass vial and added with 100 μL of a mixture of methanol/acetone (1:1), and topped up with distilled water containing 10% NaCl to 5.0 g. The samples were added with the internal standard. The PDMS fiber was exposed to the headspace above the sample for 30 min at 60 ^{o}C. Quantification of pesticides in the samples was carried out by a five point-calibration in the matrix using spiked samples by comparing the ratio the peak area of the analyte against the peak area of the internal standard versus the concentration of the analytes. Each sample was analyzed in triplicates.

Blanks were run periodically during the analysis to ascertain the absence of any contaminant pesticides.

Gas Chromatography – Electron Capture Detector (GC-ECD)

A Shimadzu GC 17A version 2.21 gas chromatograph with an electron capture detector (ECD) was used. A SGE BPX5, 30m x 0.32 mm id capillary column with a 0.25 μm film was used in combination with the following oven temperature program: initial temperature 120 ^{o}C, then heated at 7 ^{o}C/min to a final temperature of 250 ^{o}C, and then held for 4.5 min. The total run time was 23.07 min. The splitless mode was used for the injection. The injector temperature was at 240 ^{o}C and the detector temperature was at 300 ^{o}C. Nitrogen gas (99.999%) was used as the carrier gas with a gas flow at 24.4 cm/sec linear velocity and the pressure at 94 kPa.

Results and Discussion

Selection of SPME coating

The choice of an appropriate coating is essential for the SPME method. The sensitivity of each fiber is different depending on the molecular mass and the polarity of the analytes to be extracted. The performance of PDMS and PA were compared by determining the detector response (peak area) of the selected OCPs and OPPs insecticides.

From Table 1 and Table 2, it was observed that compounds with the higher octanol-water partition coefficient (log K_{ow}) and low solubilities in water, such as chlorpyrifos, α-endosulfan and β-endosulfan were the more extensively adsorbed when the PDMS fiber is used due to the higher affinity to the non-polar fiber coating. In contrast, when the PA fiber is used the less polar insecticides were less effectively extracted with a decrease adsorbed amount of 20-30 % when compared to PDMS fiber. Compounds with higher polarities such as malathion and diazinon were adsorbed at a higher percentage (65-80%) by PA in relation to PDMS fiber. Generally, the PDMS fiber gives high extraction efficiency than the PA fiber which can be explained not only by the nature of the fiber or compounds, but by the slightly larger volume of the PDMS fiber with respect to the others and hence the larger capacity to adsorb the analytes. α-Endosulfan presents the best limit of detection due to the high ECD response as a consequence of having six chlorine atoms in its molecule

Table 1: Physicochemical properties of the selected pesticides molecular formula, molecular weight, water solubility, vapor pressure and Log K_{ow} [17]

Name	Molecular Formula	Molecular Weight	Water Solubility (mg/L) at 25 °C	Vapor Pressure (mm Hg)	Log K_{ow}
Diazinon	$C_{12}H_21N_2O_3PS$	304.35	40	9.02×10^{-5}	3.30
Chlorothalonil	$C_8Cl_4N_2$	265.92	0.6-1.2	5.7×10^{-7}	3.05
Malathion	$C_{10}H_{19}O_6PS_2$	330.36	130	3.94×10^{-5}	2.75
Chlorpyrifos	$C_9H_{11}C_{l3}NO_3PS$	350.62	2	2.02×10^{-5}	4.69
Quinalphos	$C_{12}H_{15}O_3N_2PS$	298.18	22	2.6×10^{-6}	4.44
Profenofos	$C_{11}H_{15}Br_{Cl}O_3PS$	373.60	28	6.23×10^{-6}	4.74
α-Endosulfan	$C_9H_6Cl_6O_3S$	406.96	0.32	3.0×10^{-6}	3.83
β-Endosulfan	$C_9H_6Cl_6O_3S$	406.96	0.32	5.96×10^{-7}	3.83

Table 2: Mixture concentration, detector response and LOD of the selected pesticides.

Compounds	Mixture Conc. (μg/L)	PA		PDMS		Peak Area ($\frac{PA}{PDMS}$ x 100%)
		Peak Area	LOD (ng/L)	Peak Area	LOD (ng/L)	
Diazinon	160	51600	50	77855	10	66.28
Chlorothalonil	80	20910	50	59586	10	35.09
Malathion	160	32350	100	40677	50	79.53
Chlorpyrifos	4	213738	5	813108	1	26.29
Quinalphos	160	29911	100	43403	50	68.92
Alpha-Endo	2	150010	1	695171	0.2	21.58
Profenofos	20	28592	5	53532	1	53.41
Beta-Endo	4	109042	5	419156	1	26.01

Parameters influencing the HS-SPME process

HS-SPME is an equilibrium process that involves the partitioning of analytes from aqueous phase to gas phase and eventually into the polymeric phase according to their partition coefficients K_d [2]. The optimization of parameters that influence the partition of analytes between the headspace and the solution are thus extremely important. Temperature, appropriate time period for the extraction, memory effect, stirring rate and ionic strength are the main parameters that should be taken into account. The optimization of above parameters was checked with both types of fibers.

Effects of Extraction Temperature

Extraction temperature should be optimized first since it plays the most important role in the extraction process by controlling the diffusion rate of analytes into the coating. The effect of temperature in the extraction yield was investigated by varying the temperature between room temperature (25 °C) and 95 °C with a constant extraction time of 30 min.

In relation to the expected behavior of the pesticides, increasing the temperature improves the mobility of the pesticides through the liquid and gas phase and better recoveries were obtained up to 60 °C. At higher temperatures the ability of the SPME fiber to adsorb the tested pesticides begins to decrease. This is because adsorption is an exothermic process and therefore, disfavored at high temperatures. Thus increasing the temperature would cause the distribution constant at equilibrium to decrease [18]. Moreover, the decrease of the extraction yield could be due to the enhanced hydrolysis of OPPs at elevated temperatures. Besides, an increase in water vapor pressure is another cause of decrease in the sensitivity of HS-SPME when the extraction

temperature exceeds 60 ^{o}C. Thus, the optimum extraction is achieved at 60 ^{o}C and this temperature was selected for the subsequent experiments.

Effects of Extraction Time

Since the HS-SPME technique is an equilibrium process of the analytes between the vapor phase and the fiber coating, it is important to determine the time required to reach equilibrium. When analytes have low values for Henry's constant, low concentrations at the vapor pressure are expected, thus translating to a small concentration gradient and this results in longer periods to reach the equilibrium. Furthermore, analytes with high molecular masses are expected to require longer equilibrium times, due to their lower diffusion coefficients (the equilibrium time is inversely proportional to the diffusion coefficient) [19]

Under the above observed optimum conditions, adsorption-time profiles for PDMS and PA fibers were generated for each pesticide and are presented in Figs. 1A and 1B, respectively. For the PDMS fiber, the equilibrium time of most analytes is shorter and almost reached after 60 min (Fig 1A), whereas, all the analytes need 90 min to reach equilibrium for PA fiber (Fig 1B). This is because PDMS coating is a viscous liquid polymer and the diffusion coefficient of the analyte in it will be orders of magnitude higher than its diffusion coefficient in a solid polymer of PA. Therefore, since the dynamics of mass transport in a well-stirred solution is controlled by the diffusion coefficient of analyte in the coating, the extraction time required with a liquid polymer coating will be considerably less than that required with a solid-phase polymer [2]. Thus the longer equilibrium time for the PA coating can be explained. Another limitation of PA for the extraction of organophosphorus and organochlorine pesticides is the more polar character of its coating.

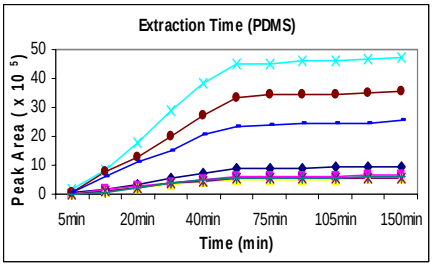

Fig 1A: Extraction Time of PDMS

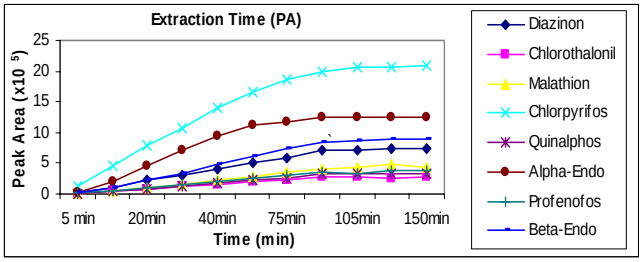

Fig 1B: Extraction Time of PA

Effects of Stirring Rate

The results showed that the response increases if the stirring speed is increased which agrees with the fact that SPME is a technique based on equilibrium and that good diffusion through the phases is essential to reach equilibrium faster. Although the equilibrium time progressively decreases with increasing agitation rate, the amount of analyte extracted decreases at the maximum speed. This is because at the maximum speed the stirring bar begins to vibrate and agitation of the sample is not uniform. This faster agitation tends to be uncontrollable and the rotational speed might cause a change in the equilibrium time and poor measurement precision. Thus, a constant gentle stirring speed was selected in this study to increase the rate of extraction.

Effects of Ionic Strength

In SPME procedure the salting out effect can be employed to modify the matrix by adding a salt, e.g. NaCl to increase the ionic strength of the matrix so as to decrease the solubility of analytes and release more analyte into the headspace, thereby, contributing to enhanced adsorption on the fiber [19]. The increase in solubility of analtyes in water, will increase the influence on adsorption by the addition of a salt. Thus, with reference to the PDMS fiber the compounds with higher water solubility such as diazinon and malathion showed an increase in extraction yield by increasing the NaCl concentration until 30% (w/v). However, no effect or even a slight decrease in extraction yield was observed for compounds of low water solubility after 10% (w/v). For the PA fiber, similar behavior was observed. Salt contents of 10% were selected for the PDMS and PA fibers.

Effects of Desorption Temperature

In SPME techniques, a significant amount of the analytes often remain adsorbed on the fiber after the desorption step in the GC injection system. This problem becomes more serious when low volatility compounds are analyzed. For both fibers, desorption at 200 and 230 ^{o}C was not capable of desorbing completely the analytes; they were completely removed from the coating at 240 – 300 ^{o}C and not much significant differences were observed within this range of temperature. Hence a temperature of 240 ^{o}C for PDMS and 260 ^{o}C for PA were selected since high temperatures can shorten the coating lifetime and can result in the bleeding of the polymer, causing problems in the separation and quantification [20].

Effects of Desorption Time

After investigating several desorption times between 1 to 15 min the results showed that a 6 minute-period was sufficient to desorb pesticides in the GC injector port; but the fiber remained for another 4 min to eliminate all residues on the fiber to guarantee a reproducible desorption.

Effects of Water and Organic Solvent

The influence of adding water on the samples in order to favor the release of analyte from the matrix was established by using different amounts of water. The results showed that the detection response of all pesticides was enhanced with the addition of water and decreased when the amount of water added exceeded a certain level. The HS-SPME process is affected by the suspended matter and dissolved compounds (sugar, pectins etc) contained in the vegetable and fruit samples which could adsorb the analytes, forming micelles and thus making it difficult for the analytes to reach the fiber (interfering with diffusion) [21]. Since the analytes were analyzed by HS-SPME, the addition of higher amounts of water would dilute the concentration of the analytes and increase the diffusion barrier of pesticides from aqueous phase to gaseous phase. Moreover, the increase or decrease in average recovery (%) obtained was compound and structure-dependent. The average recovery (%) of chlorpyrifos was significantly increased when the amount of water added reached the optimum level. This is because chlorpyrifos has low water solubility (2 mg/L) and high vapor pressure (2.02×10^{-5} mmHg). The desorbed pesticides will be easily released from aqueous solution to gaseous phase. However, malathion which has a relatively high water solubility (130 mg/L) and low vapor pressure (3.94×10^{-5} mmHg) to be released from the sample matrix will be retained in aqueous solution, and subsequently not much significant increase in the recovery (%) will be obtained when the amount of water added is increased.

The addition of hydrophilic solvents can also promote the release of organic compounds from the vegetable and fruit samples. However, the presence of a high concentration of an organic solvent would lead to a significant decrease in the extraction efficiency of the analytes [21]. Therefore, only a small amount of solvent is recommended for use as the additive. In this study, 2% (vol/weight) of organic solvent was added to the vegetable and fruit samples. From the results obtained, an average percentage recovery (%) obtained using a mixture of methanol/acetone (1:1) was much higher compared to that using the other organic solvents. Besides the extraction efficiency, a mixture of methanol/acetone (1:1) was selected because it is relatively non-toxic, easy to volatilize and readily obtainable in the laboratory.

Coating lifetime

A coating lifetime is important for practical application (changes of efficiency with number of analyses). The coating is damaged mainly during the extraction due to interference between the matrix of samples and the fiber. This effect is more pronounced when the sampling is performed directly from the aqueous solution (immersion SPME). In contrast, in the HS-SPME mode the fiber is suspended in the headspace above the liquid layer of the samples and there is no interference between the matrix of samples and the coating. Thus the coating is protected and the lifetime is increased. In conventional SPME process (immersion technique) each fiber can be re-used for approximately 30 times for surface water samples and 27 times in run-off water [18,22]. However, in this study using the headspace technique the fibers can be re-used for up to100 – 120 times.

Recoveries

The addition of aliquots of water and organic solvent yielded extraction recoveries ranging between 75% and 97% for all the selected pesticides in all the vegetable and fruit samples studied. The relative standard deviations for all the experiments studied were less than 7% and linear calibration curves resulted for all the investigated range with correlation coefficients better than 0.9900. Table 3 showed that the linear range, LOD, mean relative recoveries and RSD values by using the optimized HS-SPME procedure. This method also tested on actual vegetable (tomato) and fruit (guava) samples and it was found that the pesticide residues of these two

types of samples were below the Maximum Residue Level as stated in the Malaysian Food Act 1983 which is from 1 to 50 μg/L.

Table 3: Recovery test on vegetable and fruit samples by using the optimized developed procedure.

Pesticide	Linear range (μg/L)	LOD (μg/L)	Tomato Recovery (%) (n=3)	Guava Recovery (%) (n=3)
Diazinon	1000-10	0.2	91 ± 1.3	82 ± 3.3
Chlorothalonil	1000-10	0.2	92 ± 1.4	84 ± 0.8
Malathion	5000-50	1.0	94 ± 1.8	95 ± 0.8
Chlorpyrifos	50-0.5	0.02	82 ± 2.5	94 ± 0.5
Quinalphos	5000-50	1.0	95 ± 0.3	92 ± 1.9
Alpha-Endo	20-0.1	0.01	93 ± 0.7	92 ± 1.2
Profenofos	100-1	0.1	90 ± 1.4	94 ± 0.8
Beta-Endo	100-1	0.1	81 ± 0.8	97 ± 0.9

Conclusion

This comparative study of SPME procedures using different types of fibers showed that the use of these coatings is very useful for the determination of organophosphorus and organochlorine pesticides in fruit and vegetable samples. The differences in selectivity provided by the different coating can be used not only for quantification purposes, but also for identification of these compounds in complex matrices. Optimization of the parameters affecting the method sensitivity should be carefully developed in order to enable substantial increase in the amount extracted of most analytes and to improve the limit of detection. The developed method of HS-SPME with GC-ECD is precise, reproducible and linear over a wide range.

Acknowledgement

The authors acknowledge the financial support provided by the Malaysia Toray Science Foundation and Universiti Tenaga Nasional to pursue the research work. The authors would like to thank University of Malaya for providing the opportunity and facilities to undertake the research.

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