Extracted Amounts by Solid-Phase Microextraction: A Realistic Approach to the Partition Coefficient *K*

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Abstract

Because of its numerous advantages, the solventless solid-phase microextraction (SPME) sampling method coupled with an efficient chromatographic technique is used more and more to develop new analytical methods pertaining to organic molecules at low concentration in aqueous solutions, especially in the field of environmental chemistry. In a usual analytical procedure, the amount of analyte extracted by the fiber need not be determined, because the quantitation step of the analysis is mainly achieved using SPME external calibration. For some purposes, however, the determination of the partition coefficient *K* relative to a particular fiber for a specific analyte (for example) has to be calculated with accuracy. The traditional method consists of determining the response coefficient of the detector used for the analyte through a direct-injection calibration curve made from standard solutions in organic solvents and reporting it with the signal observed for the analytical sample. For the same goal, a depletion experiment method is suggested that consists of running several SPMEs from the same standard sample with the same conditions and then fitting the resulting data into an experimental regression curve, the exponential coefficient of which affords an absorption coefficient characteristic of the fiber/analyte system in a defined work-up. This self-calibrating method is revealed to be much more accurate than the previous one. Four pesticides in water solution were chosen to exemplify this study.

Introduction

Solid-phase microextraction (SPME) is a direct, multi-residue, solventless extraction method recently developed mainly by J. Pawliszyn (1) and others at the University of Waterloo, Canada. Because of its performance, it is now largely used in the field of very diluted organic molecule analysis in aqueous mediums (2–5), and one of its major applications concerns pesticide residue analysis in different types of water (6–10). The technique has also been extended to extract the same type of contaminants from food matrixes such as wine or strawberries (11–14).

In a typical analysis experiment using SPME, analytes are first absorbed onto the polymeric coating of a fused-silica fiber directly immersed into the aqueous solution sample. When the partition equilibrium is reached, the amount of each analyte absorbed on the fiber is given by the following equation:

$$n_s = KV_s/[1 + K(V_s/V_L)] \bullet C_0 \qquad \text{Eq. 1}$$

where n_s represents the amount of analyte extracted by the fiber, K is the partition coefficient relative to this equilibrium, V_s and V_L are the volumes of the polymeric coating of the fiber and the aqueous sample, respectively, and C_0 is the initial concentration of the sample for this analyte.

Then, the extracted analytes are generally thermally desorbed into the injection port of a gas chromatographic (GC) system for separation and quantitation, affording all the performance of modern chromatography to this method of analysis. The signal given by the detector is usually reported in a corresponding external calibration curve made from standard solutions of this analyte at different concentrations and extracted with the same fiber under the same conditions.

In such a procedure, the extracted amount of analyte is never calculated; in fact, its value is not necessary when the analysis is only for the determination of the analyte concentration. However, if the sensitivity of a fiber has to be characterized, in order to determine which of the commercial fibers is best suited for a defined analyte (for example), the partition coefficient K (or at least the absorption coefficient α given by the following equation) becomes a fundamental parameter:

$$n_s = KV_s/(1 + KV_s/V_L) \bullet C_0 = KV_s/n_0\,(V_L + KV_s) = \alpha n_0 \qquad \text{Eq. 2}$$

where n_0 is the initial amount of the analyte.

With such a definition, α appears as a pure number representing the proportion of the analyte extracted by the fiber in

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a specified work-up versus the analyte initially present in the sample, and it is independent of the detector used. In fact, under such conditions, α is only nature-dependent on both the polymeric coating and the analyte.

According to the assumed linearity of the detector for each analyte and the preceding equations, the 2 different parameters n_s and n_0 can be correlated to the observed signal σ given by the detector:

$$\sigma = \beta n_s = \beta\alpha n_0 \qquad \text{Eq. 3}$$

because σ is proportional to the amount of analyte detected at the end of the chromatographic system with β as the response coefficient of the detector (calculated from direct injections of standard organic solutions), and σ is also proportional to the amount n_0 of this analyte present in the initial aqueous sample from which the SPME is made, as previously indicated.

Consequently, α can easily be calculated as the ratio of the slopes of the 2 calibration curves relative to both analyte determinations (σ versus n_s and σ versus n_0), but in practice, n_s is generally obtained by dividing the measured SPME signal by β (the slope of the calibration curve drawn from direct injections). Thus far (to the best of our knowledge), this general procedure has been the basis of most of the methods used for assessing the amount n_s when it is needed, especially for determining the SPME partition coefficient K relative to such fibers in water for a particular analyte (15–16).

However, another approach to obtain n_s can be imagined, consisting in a depletion experiment realized by running several cumulative x-indexed extractions from the same sample and with the same fiber. As indicated previously for each extraction, the variation of the sample amount (which is also the extracted quantity) is proportional to the amount left by the previous extraction:

$$n_{x-1} - n_x = n_{sx} = \alpha n_{x-1} \qquad \text{Eq. 4}$$

If Equation 4 is rearranged, it yields:

$$n_x = n_{x-1}\,(1 - \alpha) \qquad \text{Eq. 5}$$

Equation 5 gives Equation 6 by extension:

$$n_x = n_0(1 - \alpha)^x \qquad \text{Eq. 6}$$

In these conditions, and as previously reported by Arthur et al. (17), n_x is decreasing in the sample according to an exponential law, and the corresponding regression curve (hereafter named the depletion curve) can easily be achieved by plotting the observed ratios σ_{x+1}/σ_1 or n_x/n_0 against x which, when treated with a computer, affords the following exponential equation:

$$\sigma_{x+1}/\sigma_1 = n_x/n_0 = e\gamma x \qquad \text{Eq. 7}$$

where n_0 (proportional to σ_1) is the initial amount of analyte in the sample before the first extraction, and e^g is the calculated value for (1 – α). In fact, this assessment of the absorption coefficient α is really independent of the response coefficient of the detector for the compound.

Actually, the comparison of the 2 curves, one obtained by depletion and the other by subtraction from $n_x - 1$ (the extracted amounts n_{sx}, each value calculated as the ratio σ_x/β), shows important differences that indicate that the slope of the calibration curve β is not suitable for an accurate determination of the amount of analyte extracted by SPME.

The work described in this paper is intended to exemplify the differences between the 2 methods for the determination of α based upon the extraction of 4 pesticide molecules (chosen as examples) onto a 100-µm polydimethylsiloxane (PDMS) fiber and finally propose the self-calibrating depletion method as a more realistic approach of the amount extracted by SPME. This should provide a more accurate experimental method for determining SPME partition coefficients K as long as the working conditions are those of the equilibrium.

Experimental

Chemicals and solvents

Pesticides were purchased as follows: diphenylamine (DPA) and procymidone (both purer than 97%) were from Aldrich (St. Quentin Fallavier, France), and carbofuran and β-endosulfan (both purer than 98%) were from OSI-RDH (Seelze, Germany). high-performance liquid chromatography (HPLC)-grade water and liquid chromatography-grade toluene were provided by Merck (Nogent sur Marne, France). Nanograde hexane and ethanol were provided by Promochem (Wessel, Germany). Helium C was supplied by Air Liquide (Paris la Défense, France).

Calibration solutions

Standard stock solutions (1 g/L) were prepared in toluene and kept in brown glass tubes at a temperature below 4°C. For direct injection calibration, standard mixture solutions containing the 4 pesticides at concentrations of 0.1, 0.5, 1, 2.5, and 5 mg/L were prepared by mixing the stock solutions and diluting them with ethanol. Accurate 1-µL injections using a plunger-in-needle syringe (SGE, France) were made in duplicate, and the observed values were averaged.

SPME procedure

A manual SPME holder was used with a 1-cm long and 100-µm thick PDMS-coated fiber (Supelco, St. Quentin Fallavier, France). The SPME fiber was conditioned as recommended by the manufacturer by heating it in the injector of the chromatograph at 250°C under a helium stream.

For each experiment, the 100-µm PDMS fiber was immersed into a 5-mL glass vial containing 4 mL of the analyzed sample stirred at 1000 rpm with a magnetic stirring bar at ambient temperature. The immersion time was varied from 5 to 70 min to realize extraction profiles (observed signal versus exposure duration) and then fixed at 30 min for subsequent experiments. After extraction, the fiber was thermally desorbed for 3 min into the glass liner of the GC injection port (270°C) in

the splitless mode and then 23 min in the split mode (60 mL/min). For the depletion experiment using SPME, working solutions at a concentration of 20 µg/L for each pesticide were prepared from an intermediate mixture at the concentration of 50 mg/L in ethanol made from the 4 stock solutions by dilution with HPLC-grade water.

Chromatographic equipment

All measurements were made from a Varian GC 3400 equipped with a Finnigan ITS 40 ion trap mass spectrometric (MS) detector and a Varian split/splitless injector (Thermo-Quest, les Ullis, France). Separations were obtained with a Supelco PTE5 column (30 m × 0.32 mm, 0.25-µm phase thickness). The temperature program of the oven was as follows: 50°C for 3 min, increased at 30°C/min to 155°C, then at 1°C/min to 175°C, then at 5°C/min to 220°C, and finally at 3°C/min to 260°C, where it was kept for 3 min. The ion trap was held at 220°C, and the interface was 250°C. The MS was tuned to FC 43 (perfluorotributylamine), and mass-to-charge ratios between 35 and 450 amu were scanned. The selective ion monitoring mode was used for quantitation, which was performed by measuring peak areas. All identifications were based on the comparison of mass spectra and GC retention times of the analyzed pesticides with those of standards.

Results and Discussion

Optimized exposure time

Extraction profiles were first realized by plotting the observed signal (area counts, arbitrary units) against the immersion duration (5, 15, 30, 45, and 70 min) of the fiber into a 50-µg/L solution of the 4 pesticides. Corresponding curves are shown in Figure 1, where the logarithmic scale was used because absorption intensity ranges were not in the same order of magnitude for all of the compounds.

For carbofuran, procymidone, and DPA, equilibration times were less than 30 min, whereas it appeared much more than 70 min for β-endosulfan. The absorption time was fixed at 30 min, and this condition was rigorously repeated for each experiment. With a sampling time shorter than that required to reach equilibrium, Ai (18) showed that the amount extracted by SPME is only a fraction of that which should be obtained at equilibrium, but all of the hypotheses and consequences suggested in the introduction of this article are still valid.

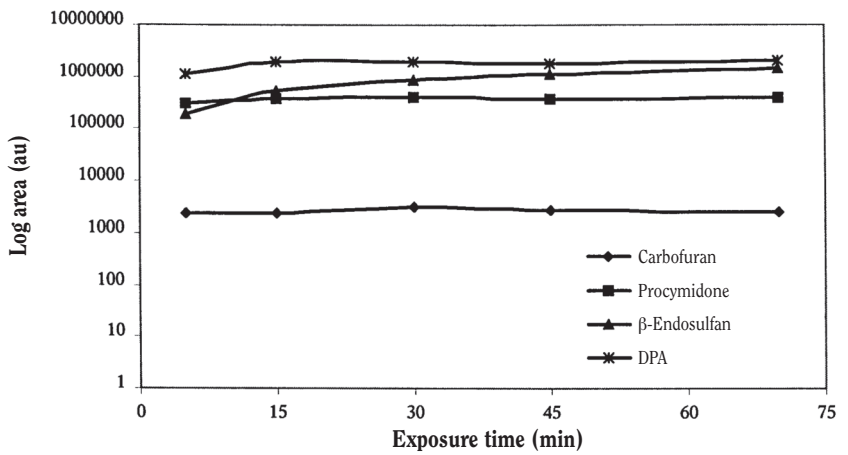

Figure 1. Extraction profiles of carbofuran (u), procymidone (n), β-endosulfan (s), and DPA (×).

Response coefficient β

The response coefficient β, as previously defined, corresponds to the slope of the direct injection calibration curve for which signals are plotted versus the amounts of analyte injected expressed in mass units. The 5-point calibration regression curves were drawn to include the origin, because no signals were observed from a blank solution free of pesticide. The corresponding values obtained for the 4 molecules (a.u./ng) are listed in Table I.

The linearity of the MS detector toward these 4 compounds is indicated by the corresponding regression coefficients, which are all close to or higher than 0.99.

The depletion experiment

Up to 15 SPME runs were performed from the same 4-mL aliquot of a solution of the 4 pesticides at a concentration of 20 µg/L with the same fiber and under the exact same conditions (exposure time, temperature, stirring, etc.). For each component, the observed signal (expressed as a percentage of the initial one for the sample) was plotted against x, the number of the run in the series. The corresponding exponential regression curve was then fitted using a computer as previously indicated. The obtained depletion curves are represented in Figure 2.

For comparison, the extracted amounts n_{sx} (estimated by dividing σ_x by β, as in the traditional procedure) were cumulated and withdrawn from n_0 to give n_x, and then the calculated n_x/n_0 ratios were plotted against x in the same diagram.

Under such conditions, it is evident that each point of the depletion curves can only be measured once, and as a consequence, their statistical validation may be poor, as in the case of carbofuran for which the regression coefficient is only 0.9592, as indicated in Table II.

The exponential and regression coefficients γ and R, respectively, relative to these depletion curves and the corresponding absorption coefficient α_{dep} calculated according to Equations 6 and 7 are also given in Table II. In addition, the ratio $\sigma_1/\beta n_0$, calculated from the first signal of each of the depletion curves and representing the traditionally determined absorption coefficients α_{tr} according to Equation 3, is also listed for comparison.

From Figure 2, it clearly appears that for none of the 4 studied compounds, the differences between the initial amount of analyte dissolved in the sample and the amount left after x number of SPME runs (which are proportional to the observed signals σ_1 and σ_x, respectively) fit with the

cumulated extracted amounts Σn_{sx}, each of them traditionally calculated from β according to Equation 3.

In this last case, the extracted amounts of β-endosulfan and DPA were obviously overestimated, because both of the resulting curves should cross the x-axis, meaning that more than 80 ng of each compound could be extracted. Based on this evidence, a symmetric underestimation of the extracted amounts could explain the corresponding curves observed for carbofuran and procymidone.

The comparison between the α values given in the two last columns of Table II (representing the proportion of each analyte extracted in each experiment according to both determination procedures) afforded another assessment of this difference, which revealed to be of the most importance in the case of carbofuran, for example.

Table I. Slopes and Regression Coefficients of Calibration Lines Obtained by the Direct Liquid Injection Method

Analyte	Response factor β (au/ng)	Linearity *R*
Carbofuran	16400	0.9978
Procymidone	38700	0.9991
β-Endosulfan	4490	0.9948
DPA	34200	0.9888

Actually, there is no doubt that the depletion curves provided a better representation of the evolution of the solution in which the analytes were dissolved than the others, and the absorption coefficients α (calculated according to Equations 6 and 7) were determined with a much higher accuracy by this method.

There is no reason for this observation to exclusively concern the 4 studied compounds, and because the linearity of the detector has been verified for each analyte, the difference between the 2 types of results could be due to some events setting between the absorption and chromatographic steps of the analysis.

In fact, the desorption step is definitely the most critical one in an analysis using the SPME–GC coupling. It consists of introducing the analytes absorbed onto the polymeric coating of the fiber into the liner of the injection port of the chromatograph. The analytes are supposed to be thermally desorbed, swept by the helium gas flow, and then introduced into the column. This description is simplified in the scheme B of Figure 3.

On the other hand, the injection of an aliquot of the sample in a solution with organic solvent proceeds very differently, as

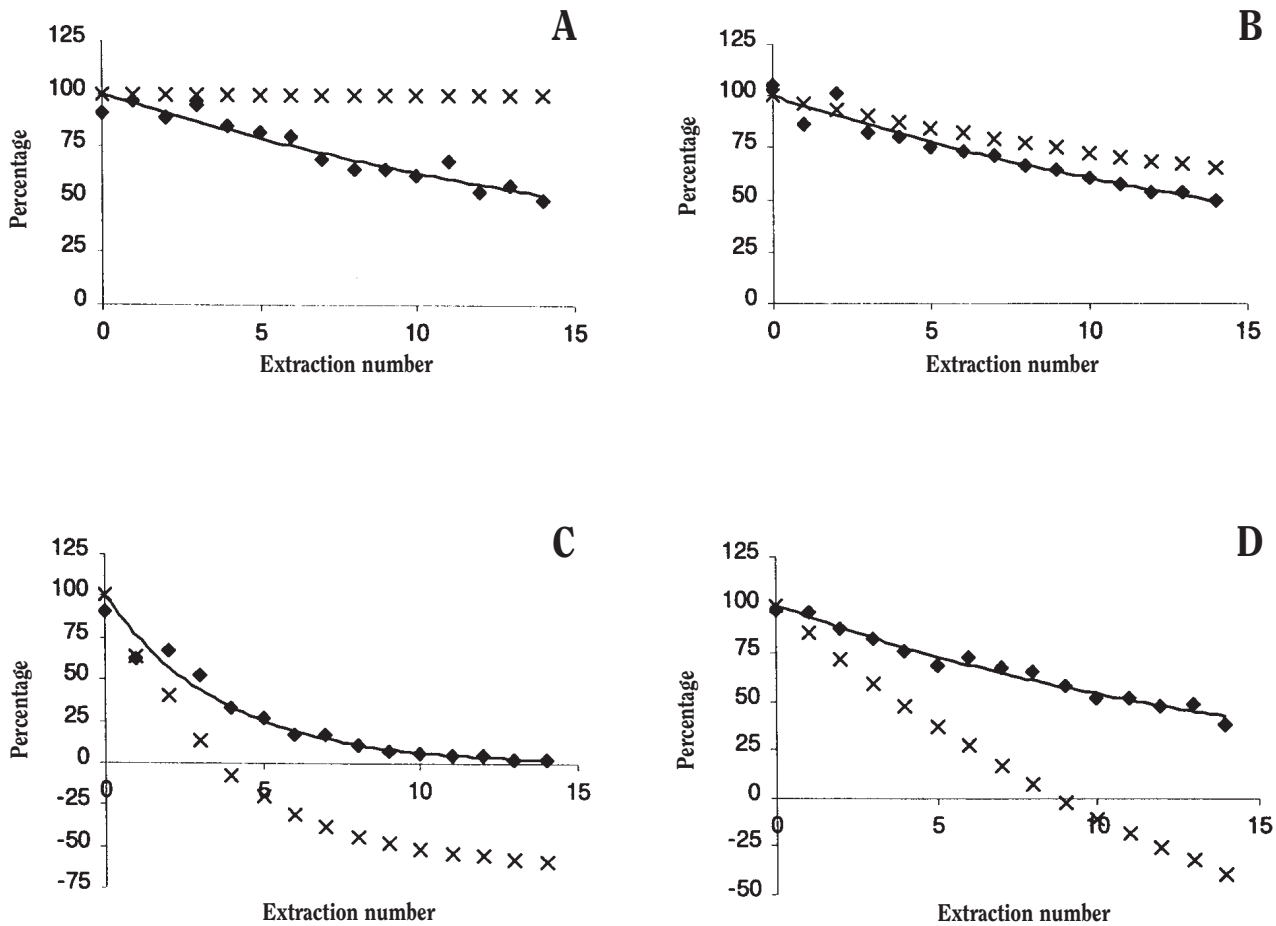

Figure 2. Computerized depletion curves (u) from successive observed signals and cumulative extracted amounts (×) according to β coefficients for carbofuran (A), procymidone (B), β-endosulfan (C), and DPA (D).

simplified in scheme A of Figure 3. In this case, the solvent is flash vaporized in the insert of the injector, transforming a 1-µL liquid sample into a gas of approximately 0.9 mL of volume (ethanol injected at 250°C). Under such conditions, it can be easily imagined how much the gas flow (initially fixed at the level of 1 mL/min) is disrupted in a 0.5-mL liner. At the same time, analytes are also vaporized, some of them rapidly, others more slowly, but they all can be partially lost because of a variety of factors (for example, the vaporization pressure pulse causing vapor overflow discrimination at the septum purge level or later during splitting).

In fact, as already described by Kaufmann (19) in a recent review about split/splitless injectors, important losses of analytes at this place may interfere during the making of standard calibration curves by direct injection. On the other hand, important heating of the vaporized analytes during the SPME desorption step without any solvent bounding protection could cause the relative degradation of compounds before their introduction into the column. In both cases, the amounts of products collected at the end of the column into the detector are different from those that were supposed to be introduced. For this reason, the direct liquid injection cannot be considered as an appropriate model for SPME injection assessment.

From a practical point of view, the determination of the absorption coefficient α by this method (using a depletion curve and calculating the exponential corresponding coefficient γ) provides a useful method for determining the correct amount of each analyte extracted by a specific SPME fiber with a defined analytical procedure. The use of such an approach should be of great interest in the determination of the partition equilibrium constant *K* relative to each molecule and for each type of fiber, as long as the partition equilibrium is reached.

The volume of the polymeric coating was calculated for the 100-µm PDMS fiber according to the description given by Arthur et al. (20). The obtained value was confirmed by the manufacturer of the fiber and in a recent publication dealing

Table II. Exponential Coefficient γ and Regression Coefficient R of the Depletion Curves: Comparison of Absorption Coefficients α_{dep} and α_{tr}

Analyte	γ	Linearity *R*	α	
			$\alpha_{dep} = 1 - e^{-g}$	$\alpha_{tr} = \sigma_1/\beta n_0$
Carbofuran	0.047	0.9592	0.046	0.00047
Procymidone	0.050	0.9805	0.049	0.035
β-Endosulfan	0.276	0.9950	0.241	0.358
DPA	0.062	0.9824	0.060	0.139

Table III. Comparison Between Extracted Amounts n_{tr} and n_{dep} and Partition Coefficients K_{tr} and K_{dep} According to Traditional and Depletion Methods

Analyte	n_{tr} (ng)	K_{tr}	n_{dep} (ng)	K_{dep}
Carbofuran	0.038	2.9	3.5	281
Procymidone	2.8	225	3.9	320
DPA	11.1	1055	4.8	417

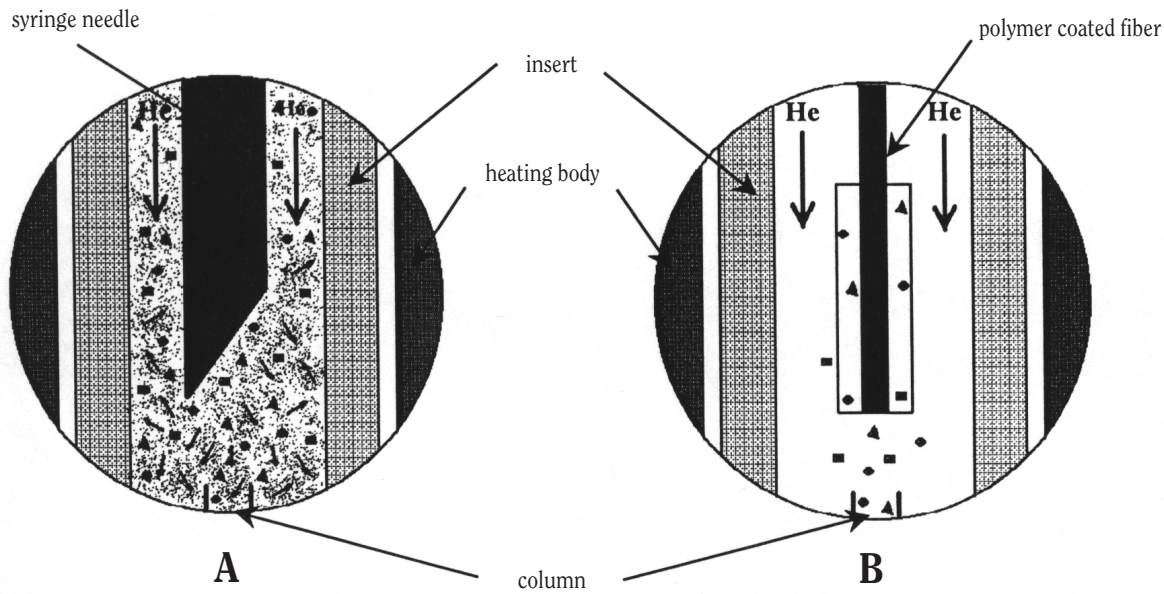

Figure 3. Direct liquid injection (A) with flash vaporization of the solvent, and SPME injection (B) by thermal desorption without solvent.

with theoretical considerations upon K_{SPME} by Górecki and Pawliszyn (21). Then, the partition coefficients were established for 3 of the studied compounds (equilibrium was not reached for β-endosulfan) by the traditional (K_{tr}) and depletion (K_{dep}) methods and compared in Table III.

Conclusion

It is clearly evident that the amount of analyte extracted by an SPME fiber cannot be calibrated with accuracy by injecting a liquid standard solution of this analyte into the split/splitless injector of a chromatographic system. Calculating the exponential coefficient of the depletion curve obtained by extracting the same sample several times under very similar conditions provides a more realistic self-calibrating method for determining the amount extracted at each run and, more generally, the corresponding extraction coefficient α relative to that fiber and analyte using a defined work-up. As long as working conditions allow the partition equilibrium to be reached, this method also provides a promising approach for determining K_{SPME} coefficients.

Considering the important difference that may be observed between the K values obtained, and because of the fundamental interest in partition coefficients for SPME, applications aiming to characterize the sensitivity of commercially available fibers for different types of analytes (such as pesticides) are presently in progress in our group.

Acknowledgments

This paper was presented at the 1999 Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy in Orlando, FL. The authors would like to thank the Fonds Européen pour le Développement Régional for financial support.

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Manuscript accepted June 30, 1999.