ELSEVIER

Journal of Chromatography A, 878 (2000) 27–33

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Multiple solid-phase microextraction

E.H.M. Koster*, G.J. de Jong

Department of Analytical Chemistry and Toxicology, University Centre for Pharmacy, A. Deusinglaan 1, 9713 AV Groningen, The Netherlands

Received 13 August 1999; received in revised form 5 January 2000; accepted 27 January 2000

Abstract

Theoretical aspects of multiple solid-phase microextraction are described and the principle is illustrated with the extraction of lidocaine from aqueous solutions. With multiple extraction under non-equilibrium conditions considerably less time is required in order to obtain an extraction yield that is equal to that of one extraction at equilibrium. On the other side, the extraction yield can be increased if multiple extraction is performed with the same total time as is needed for one extraction at equilibrium time. The effect of multiple extraction is strongly dependent on the value of the partition constant and for practical use the length of the desorption time is important. A good agreement between theoretical and experimental data has been obtained. Chromatograms are presented showing the potential of multiple solid-phase microextraction.

Keywords: Multiple extraction; Solid-phase microextraction; Lidocaine

1. Introduction

Solid-phase microextraction (SPME), introduced by Arthur and Pawliszyn [1], has been applied to various types of analysis, such as environmental, food, biological and pharmaceutical analysis. SPME is an easy-to-handle sample pretreatment method which combines sample clean-up, concentration and introduction into the separation system [2]. Its principle is based on the partition of an analyte between the sample matrix and the coating on a fused-silica fiber. The coated fiber can be directly immersed into the sample or placed into the headspace above the sample, where the analytes of interest are extracted. After the sorption process, the analytes can be directly transferred to a gas chromatograph [3] or a liquid chromatograph [4,5].

The time used to reach the partition equilibrium depends on parameters, such as sample matrix, agitation of the sample, temperature and properties of the coating and analyte. An equilibrium time of 30–60 min is very common for direct immersion SPME. Extraction time can be shorter than the equilibrium time but this results in lower extraction yields and therefore higher detection limits, i.e., there is a compromise between extraction time and yield.

In this paper, multiple extraction with a relatively short extraction time is introduced. Multiple extraction seems interesting because of the exponential relationship between the time and the extracted amount and, so, multiple SPME can result in either an increase in extraction yield or a decrease in extraction time. To our knowledge this is the first

*Corresponding author.

0021-9673/00/$ – see front matter
PII: S0021-9673(00)00161-8

paper on multiple SPME. In order to demonstrate the potential of multiple SPME under non-equilibrium conditions, the theoretical aspects of this procedure are discussed and a practical example of the combination of multiple SPME with liquid chromatography (LC) is shown.

2. Theoretical aspects

In the literature several formulas have been proposed to describe the relationship between time and amount of analyte absorbed by a coated fiber [3,6–9]. The amount of analyte extracted by immersing a fiber in a sample for a specific time, $n_{f,t}$, can be calculated according to:

$$n_{f,t} = n_{f,e}\left[1 - e^{(-at)}\right] = \frac{KV_f n_s^0}{KV_f + V_s} \cdot \left[1 - e^{(-at)}\right] \quad (1)$$

Here, $n_{f,e}$ is the amount of analyte extracted by the fiber at equilibrium, a is the parameter describing how fast the partition equilibrium can be reached, t is the extraction time, K is the partition constant, V_f and V_s are the volumes of the fiber coating and sample, respectively, and n_s^0 is the amount of analyte in the sample before extraction.

Arthur et al. [10] derived an equation, which describes the amount of analyte extracted from a sample after i extractions to show the depletion of analyte in the sample. However, this equation is only valid for extractions at equilibrium. Using Eq. (1) for the calculation of the amount of analyte extracted after a specific time t, the amount of analyte extracted after i extractions (at that specific time) can be determined mathematically as follows. After the first extraction at a specific time, the amount of analyte left in the sample for a next extraction, n_s^1, is:

$$n_s^1 = n_s^0 - n_{f,t} \quad (2)$$

Combining Eqs. (1) and (2) and rearranging results in:

$$n_s^1 = \frac{n_s^0\left[V_s + KV_f e^{(-at)}\right]}{KV_f + V_s} \quad (3)$$

Similarly, the amount of analyte in the solution after i extractions at the same specific time can be calculated by:

$$n_s^i = \frac{n_s^0\left[V_s + KV_f e^{(-at)}\right]^i}{(KV_f + V_s)^i} \quad (4)$$

The amount of analyte extracted by the fiber at the ith extraction with a specific time, $n_{f,t}^i$, can be expressed as:

$$n_{f,t}^i = \frac{KV_f n_s^{i-1}}{KV_f + V_s} \cdot \left[1 - e^{(-at)}\right] \quad (5)$$

Substituting Eq. (4), for n_s^{i-1}, into Eq. (5) results in:

$$n_{f,t}^i = \frac{KV_f n_s^0\left[1 - e^{(-at)}\right] \cdot \left[V_s + KV_f e^{(-at)}\right]^{i-1}}{(KV_f + V_s)^i} \quad (6)$$

After each extraction the fiber is desorbed into the analysis system, i.e., the sorption–desorption cycle is repeated as many times as wanted and the extracted amounts are collected into the analysis system. Finally, the cumulative extracted amount is analyzed. The total or cumulative extraction yield of the multiple extraction at a specific time, n_{total}, can be expressed as follows:

$$n_{total} = \sum_{i=1}^{i} n_{f,t}^i \quad (7)$$

Fig. 1A shows the theoretical cumulative extraction yield after multiple extraction at 40, 20, 10, 5 and 2 min versus the number of extractions. The total number of extractions at a specific time has been chosen so that the total extraction time is 40 min. This example is based upon the extraction of lidocaine from buffer (500 ng/ml) with a 100 μm polydimethylsiloxane (PDMS)-coated fiber, i.e., the partition constant and a-value were determined from an experimental time–sorption profile (see Fig. 2).

The a-parameter does not influence directly the gain in yield or the time reduction that can be obtained by multiple extraction. In contrast, the partition constant which could be related to the a-parameter [8] influences the effect of multiple extraction, because for large K-values samples are more depleted during the first extraction. This means that there is not sufficient analyte left to obtain a relatively high extraction yield in the following extractions. Therefore the effect of multiple extraction on time and yield are diminishing with an

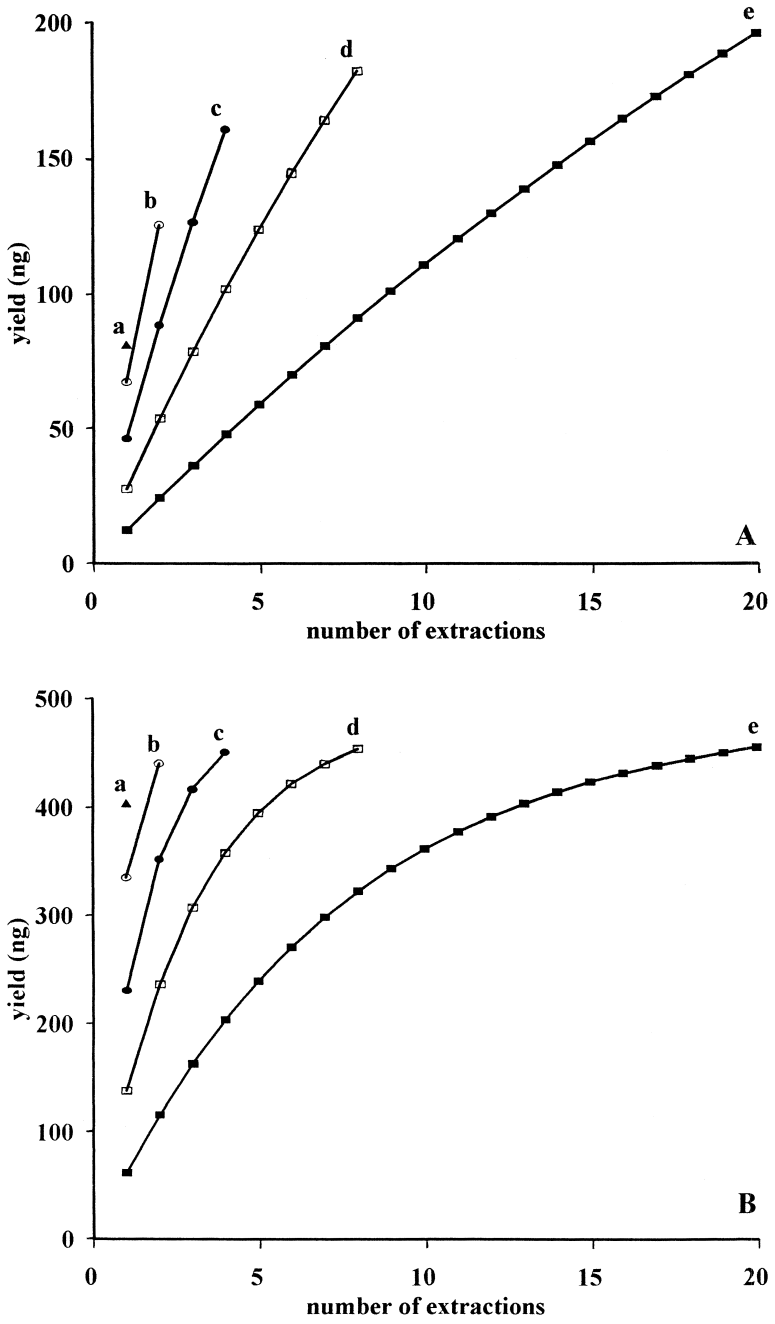

Fig. 1. Theoretical cumulative yield versus number of extractions at (A) $K=330$ and (B) $K=10\,000$: (a) one extraction of 40 min, (b) two extractions of 20 min, (c) four extractions of 10 min, (d) eight extractions of 5 min, (d) 20 extractions of 2 min.

increasing partition constant. For K-values $\geq 10\,000$ the yield of a single extraction at equilibrium is $\geq 90\%$ of the cumulative yield that can be obtained with multiple extraction with a 100 μm PDMS-coated fiber and 1 ml sample (see Fig. 1B). However, the volume of the coating (V_f) and sample (V_s)

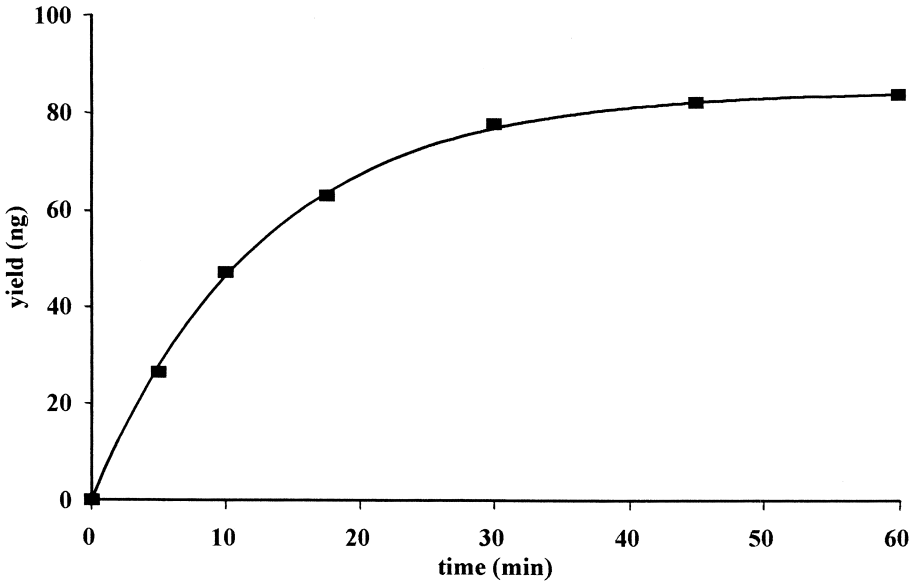

Fig. 2. Time–sorption profile for the extraction of lidocaine from borate buffer, pH 9.5 (1 ml, 500 ng/ml) with a 100 μm PDMS-coated fiber.

also influence the effect of multiple extraction, i.e., if V_f is increased or V_s is decreased the sample is more depleted during the first extraction and so the gain in time or yield is diminished. On the other side, this means that if a smaller coating volume or a larger sample volume is used, *K*-values $\geq$10 000 still can result in a favorable effect of multiple extraction.

Furthermore, it should be noted that the desorption of the fiber after each extraction should be complete, because then the amount desorbed from the fiber after the first extraction has no influence on the second and, if wanted, following desorptions. This means that if SPME is combined with LC, a large *K*-value is required for the desorption of the fiber in the SPME–LC interface.

3. Experimental

3.1. Apparatus and chemicals

The SPME fiber holder for autosampler use, and 100 μm PDMS-coated fiber ($V_f = 0.628$ μl) were obtained from Supelco (Bellefonte, PA, USA). Stock solutions of lidocaine hydrochloride, purchased from Holland Pharmaceutical Supply (Alphen a/d Rijn, The Netherlands), were prepared in ultrapure water. Ultrapure water was obtained by using an Elga Maxima Ultrapure Water (Salm & Kipp, Breukelen, The Netherlands) purification system. For extraction, 0.1 *M* buffer solutions, pH 9.5 were prepared by dissolving boric acid, purchased from Merck (Darmstadt, Germany), in ultrapure water and adjusting the pH with 1 *M* sodium hydroxide.

LC analysis was performed with an ATI Unicam 4880 liquid chromatography system (ATI Unicam, Cambridge, UK), containing a UV–Vis detector which was used at 210 nm, and a 125 mm×4.0 mm I.D. RP-C8 column (Merck). The SPME–LC interface obtained from Supelco was coupled to the six-port Rheodyne injection valve of the LC system [11]. The mobile phase consisted of a 25 m*M* phosphate buffer, pH 4.0 containing 15% acetonitrile (Lab-Scan, Dublin, Ireland) and 0.025% triethylamine (Sigma, St. Louis, MO, USA).

3.2. SPME procedures

The fiber was checked daily for impurities by putting the fiber into the desorption chamber of the

SPME–LC interface, where it was statically desorbed for 10 min with mobile phase. Subsequently the chamber was flushed with mobile phase. A 100 m*M* borate buffer containing 0.30 g/ml sodium chloride (Merck) of pH 9.5 was prepared containing 500 ng/ml lidocaine. A 1-ml volume of this standard solution was transferred to a 1.5-ml vial containing a magnetic stirring bar (7×2 mm) and capped immediately. Extraction is performed by direct-immersion of the fiber into the buffer solution for a specific time. Samples were agitated with a magnetic stirrer (IKA, mini-mr, Straufen, Germany) during extraction. After the extraction, the fiber was statically desorbed for 10 min in the desorption chamber filled with mobile phase. If needed extraction and desorption are repeated. The desorption chamber was subsequently flushed with mobile phase for 15 s to inject the analyte into the LC system [11].

4. Results and discussion

First a time–sorption profile for the extraction of lidocaine from an aqueous solution was measured in order to obtain a good approximation of the partition constant, which was found to be about 330, and the *a*-parameter (0.0792) which describes how fast the equilibrium can be reached under certain conditions. Fig. 2 shows that an extraction time of 45 min is sufficient to obtain more than 95% of the maximum yield. Subsequently multiple extraction was performed and the yields were compared with the values obtained with Eqs. (6) and (7). These equations can also be used to calculate the extraction time needed for a fixed number of extractions to obtain a yield that is equal to that of one single extraction.

Fig. 3A depicts the chromatogram of the SPME–LC analysis under conditions that would normally be used for analysis, i.e., the sample was extracted for 45 min and the fiber was desorbed for 10 min in the desorption chamber filled with mobile phase before the chamber was flushed with mobile phase to inject the extracted lidocaine into the LC system for analysis. In the second experiment the sample was extracted twice for 17.5 min, the fiber was desorbed two times into the chamber and the total extracted amount was injected into the LC system (Fig. 3B). In this way the same total sample preparation time of 55 min (sum of sorption and desorption times) was needed as in the first experiment. In the third experiment the sample was extracted twice for 9 min (Fig. 3C). Theoretically this should result in the same total extraction yield as in the first experiment. Extraction of blank borate buffer showed no compound(s) which interfere with lidocaine. As Fig. 3 shows, impurities can also be enriched by multiple extraction as is normally true for preconcentration methods. If the *K*-value of the impurity is low, a considerable gain in yield can be obtained as discussed in the theoretical section. It should be noted that the gain in yield of the impurity can be substantially different from that of the compound of interest due to differences in *K*-values. The increase in peak height of the impurity at the retention time of 2.5 min is about the same after two-fold extraction at 17.5 and 9 min (see Fig. 3B and C, respectively). A possible explanation for this is that the equilibrium time of the impurity is relatively short. In previous studies with urine and plasma samples [11,12] also no interfering compounds were found in the blank, which indicates that multiple extraction could also be applied to these biological samples. Moreover, another detection system can be used (e.g., mass spectrometry) in order to enhance selectivity.

Table 1 summarizes the results and demonstrates that the experimental and theoretical extraction yields are in good agreement with each other, as the average standard deviation of the multiple extraction is 0.96%. For the same total sample preparation time a considerable increase in extraction yield was found for a double extraction. Furthermore, the total time can be reduced about 17 min without a decrease of the yield. It should be noted that in these experiments a relative long desorption time was needed to remove the lidocaine from the fiber which means that the total time can even be more reduced if a shorter desorption time could be applied. In order to obtain an impression about the number of extractions that are useful in multiple SPME the sum of extraction and desorption time, instead of only the extraction time, is the best criterion.

5. Conclusions

With multiple SPME, sample preparation time can

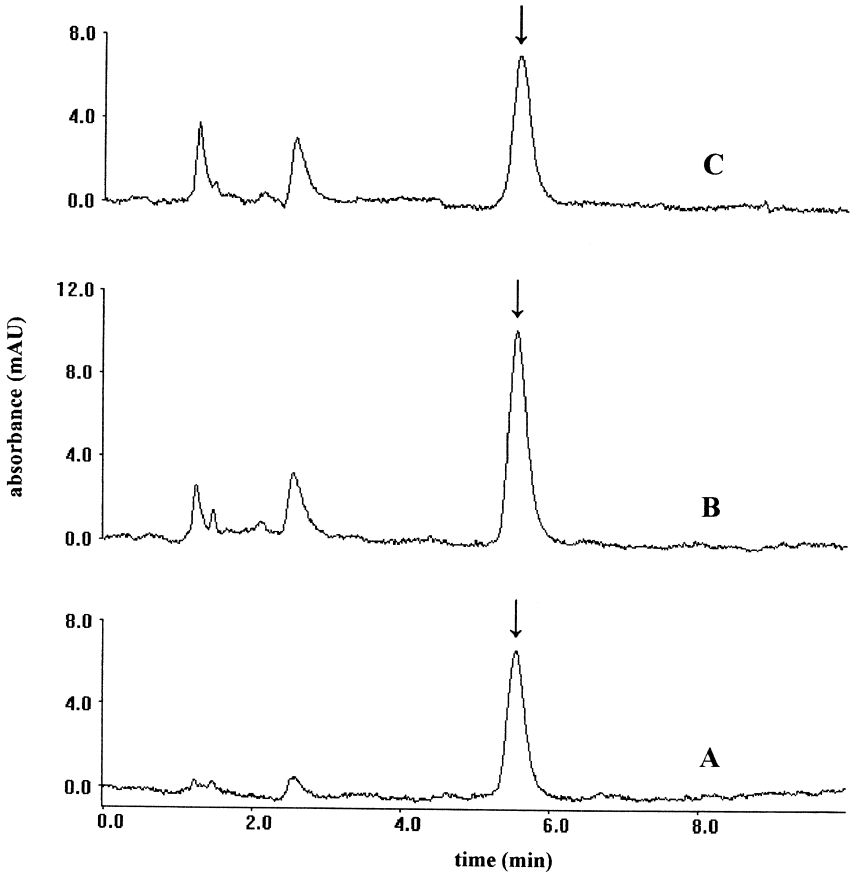

Fig. 3. Chromatograms of the SPME–LC analysis of lidocaine in borate buffer, pH 9.5 (1 ml, 500 ng/ml) after multiple extraction with a 100 μm PDMS-coated fiber: (A) one extraction of 45 min, (B) two extractions of 17.5 min, (C) two extractions of 9 min.

be decreased without a loss in sensitivity, i.e., the same sensitivity can be obtained in a shorter time. It is also possible to achieve higher extraction yields without increasing the total sample preparation time, i.e., sorption on the fiber and desorption into the desorption chamber. Preliminary experimental data have shown that the theoretical model presented for multiple extraction seems reliable. A considerable gain in time or yield has been obtained for the extraction of lidocaine from aqueous solutions. Theoretically, for compounds with lower partition constants a larger effect is predicted. In principle

Table 1
Experimental and theoretical extraction yields[a]

No. of extractions	Extraction time (min)	Total time[b] (min)	Experimental yield (%)	Theoretical yield (%)
1	45.0	55	16.8	16.9
2	17.5	55	24.8	24.3
2	9.0	38	17.7	16.9

[a] Experimental conditions: see Fig. 3.
[b] After each extraction the fiber was desorbed for 10 min.

multiple extraction can also be combined with gas chromatography (GC). The analytes desorbed after the first extraction should then be focused at the front of the column during the subsequent extraction. Probably, this can be achieved by a relatively low oven temperature. However, prevention of peak broadening or even peak splitting seems critical. In GC the analytes are thermally desorbed from the fiber in a relatively short time, which is favorable for the effect of multiple SPME.

For routine analysis of many samples, the reduction of the time of the SPME step is very important. Especially, this is true if a relatively fast separation (GC or LC) is used. If the SPME procedure is automated [10] multiple extraction does not mean an increase of manpower. Moreover, automation can improve the reproducibility of the extraction under non-equilibrium conditions. In the future, the application of multiple SPME to real samples will be investigated and the practical usefulness will be demonstrated.

References

[1] C.L. Arthur, J. Pawliszyn, Anal. Chem. 62 (1990) 2145.
[2] Z. Zhang, M.J. Yang, J. Pawliszyn, Anal. Chem. 66 (1994) 844a.
[3] D. Louch, S. Motlagh, J. Pawliszyn, Anal. Chem. 64 (1992) 1187.
[4] J. Chen, J. Pawliszyn, Anal. Chem. 67 (1995) 2530.
[5] A.A. Boyd-Boland, J. Pawliszyn, Anal. Chem. 68 (1996) 1521.
[6] W.H.J. Vaes, E. Urrestarazu Ramos, H.J.M. Verhaar, W. Seinen, J.L.M. Hermens, Anal. Chem. 68 (1996) 4463.
[7] W.H.J. Vaes, C. Hamwijk, E. Urrestarazu Ramos, H.J.M. Verhaar, J.L.M. Hermens, Anal. Chem. 68 (1996) 4458.
[8] J. Ai, Anal. Chem. 69 (1997) 1230.
[9] J. Dewulf, H. Van Langenhove, M.J. Everaert, J. Chromatogr. A 761 (1997) 205.
[10] C.L. Arthur, L.M. Killam, K.D. Buchholz, J. Pawliszyn, J.R. Berg, Anal. Chem. 64 (1992) 1960.
[11] E.H.M. Koster, N.S.K. Hofman, G.J. de Jong, Chromatographia 47 (1998) 678.
[12] E.H.M. Koster, C. Wemes, J.B. Morsink, G.J. de Jong, J. Chromatogr. B 739 (2000) 175.