Solid Phase Microextraction
A Practical Guide

SOLID PHASE MICROEXTRACTION

A PRACTICAL GUIDE

edited by

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SOLID PHASE MICROEXTRACTION

Preface

Analytical labs worldwide demand high sample throughput, fast reporting of results, reduced operating costs, and instruments that occupy minimum benchspace. Surprisingly, many chromatography analyses consume less time than preparing the sample for analysis. In fact, two-thirds of analysis time is typically spent on the sampling and sample preparation steps because most procedures are based on nineteenth century technologies that are time- and labor-intensive, contain multiple steps that can lose analytes, and use toxic organic solvents. To illustrate, a recent survey of HPLC and GC users stated that 90% of the respondents use two or more preparation techniques per sample [1]. As a result, integrating several sample preparation and separation methods is very difficult without some kind of human intervention. And, of course, the possibility for error (human, systematic, or contamination) occurs with each additional step.

The goal of sample preparation is to produce samples with the highest analyte concentration possible and the lowest level of contamination, thereby maximizing the analyte signal while minimizing interferences in the subsequent analysis. Obviously, this goal should be achieved with the easiest to reproduce and least costly procedure. Solid Phase Microextraction (SPME), extracts the analytes of interest without additional sampling or technician time, and consequently minimizes the chance for human or systematic error. Moreover, SPME does not require additional solvents or benchspace. It can be used for field sampling, such as streams, air, and fire residue, or it can be easily automated using a single fiber to sequentially sample from numerous vials, then desorb the sample into a gas or liquid chromatograph. It fulfills the laboratory requirements of productivity and reduced costs; therefore, it is an attractive sample preparation technique to replace the traditional techniques of static headspace, purge and trap, liquid/liquid extraction, and Sohxlet extraction.

SPME has been commercially available for only five years and new applications are being developed and published rapidly. Nevertheless, as with any rapidly developing technique, chemists have had to conduct their own literature search to determine if the technique may apply to their work. This comprehensive

reference will assist readers in determining whether SPME can replace their current sample handling techniques. It is the first text to provide a full spectrum of proven SPME methods for laboratories performing routine analyses from authoritative research and methods development chemists. Readers will benefit from understanding the technique and comparing it to traditional methods for use in their own laboratories. In addition, proven SPME methods and practical tips for developing new methods will directly assist the chemist, thereby saving methods development cost and time.

This book covers three areas. The first chapters present SPME theory, a methodical approach to developing new SPME methods, and a thorough description of available fibers and the classes of compounds to which they apply. Second, specific application chapters on pharmaceutical, environmental, foods and flavors, and forensic and toxicology methods provide in-depth discussions of Solid Phase Microextraction used in these disciplines. These discussions include how SPME meets the day-to-day challenges that the analytical chemist faces and the regulatory agencies' requirements. For example, environmental laboratories must achieve the low minimum detectable quantities that are outlined in EPA or other environmental regulatory agencies' methods; moreover, laboratories require fast sample turnaround to remain competitive. For SPME to meet their needs, it must provide equivalent or better analysis time and results than their current methods. Therefore, each chapter illustrates how SPME meets specific industry requirements for individual applications. Third, Professor Janusz Pawliszyn, the inventor of the technique, and a member of his research team describe new developments in the technology and recommendations for new applications. Professor Pawliszyn and his researchers have shown vision in numerous analytical disciplines and continue to innovate new technologies for sample preparation and analyses.

The contributing authors are research scientists who initially developed the technique and industry scientists who have further developed practical methods. The contributors have pioneered the use of SPME in their specific application fields; furthermore, they frequently present and publish their work with SPME. This combination of university researchers, manufacturers' research chemists, and industry scientists balances the book's content between theory and practical applications.

Unlike much of the published material on the technique, this book emphasizes practical applications and methods development using commercially available fibers. The contributors have provided valid and reproducible applications in their area of expertise, explained SPME versus other techniques for the applications, and documented their work with supporting chromatograms, graphs, tables, and references. This book is intended for analytical chemists and laboratory managers who are looking for faster and less expensive methods to perform their analyses. Moreover, it assumes that readers are already knowledgeable in sample handling techniques used in chromatography. Potential readers include chemists who have not tried SPME and want to determine if it will work for their applications. Additionally, experienced users will value this comprehensive reference on

this relatively new technique. Furthermore, the book can be used as a graduate level textbook in a sample preparation course.

I sincerely thank all contributors for their outstanding research and dedication to this project. Their efforts have stimulated the scientific community to rethink how sample preparation is performed. I would like to especially remember Mr. Brian MacGillivray, author of the environmental applications chapter, who passed away last year. Brian's enthusiasm for this project was vital in making it a reality, and his talents will be greatly missed. I thank Dr. Stephen Scypinski, coauthor of the pharmaceutical applications chapter, who provided tremendous help early in my career while helping me to maintain my sense of humor. Steve was also instrumental in initially designing the book's contents. I am grateful to my colleagues at Varian Chromatography Systems, who provided a stimulating environment to create this book. I am also indebted to Professor Barry Eckhouse of St. Mary's College in Moraga, CA, who renewed my fondness for writing. Most of all, I thank my husband, Peter Wercinski, and my parents, Charles and Rosemary Scheppers, for their encouragement, patience, and love. It is to them that I dedicate this book.

Sue Ann Scheppers Wercinski

1. Ronald E. Majors. Trends in Sample Preparation. LC-GC Magazine, Vol. 14, No. 9, 1996, pp. 754-766.

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Contents

Preface *iii*

Contributors *ix*

1
Solid Phase Microextraction Theory 1
Sue Ann Scheppers Wercinski and Janusz Pawliszyn

2
Method Development with Solid Phase Microextraction 27
Zelda Penton

3
SPME Fibers and Selection for Specific Applications 59
Robert E. Shirey

4
Pharmaceutical Applications 111
Stephen Scypinski and Ann-Marie Smith

5
Environmental Applications 131
Brian MacGillivray

6
Food and Flavor Applications 177
Xiaogen Yang and Terry L. Peppard

7
Forensic and Toxicology Applications 203
José R. Almirall and Kenneth G. Furton

8
New Developments in SPME 217
Ralf Eisert and Janusz Pawliszyn

Appendix *243*

Index *245*

1

Solid Phase Microextraction Theory

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INTRODUCTION

An understanding of Solid Phase Microextraction (SPME) theory provides not only insight into the technique, but more importantly, assistance when developing and optimizing methods. First, this chapter describes the SPME components and sampling procedure to explain how the technique is performed. Second, SPME theory is highlighted to support how sensitivity is achieved and how the extraction and desorption times are determined for both direct and headspace sampling. The theory is applied for general guidelines on increasing sensitivity and decreasing extraction times. In addition, specific techniques are described in the subsequent chapters on methods development, fiber selection, and applications. Finally, great care has been taken to emphasize practical aspects of the theory throughout the chapter for realistic laboratory conditions and applications. This should assist an individual's understanding of how the technique applies to their own specific applications.

SPME COMPONENTS AND SAMPLING PROCEDURE

Solid Phase Microextraction (SPME) utilizes a short, thin, solid rod of fused silica (typically one cm long and 0.11 mm outer diameter), coated with an absorb-

ent polymer. The fiber is the same type of chemically inert fused silica used to make capillary GC columns and it is very stable even at high temperatures. The coated fused silica (SPME fiber) is attached to a metal rod, and both are protected by a metal sheath that covers the fiber when it is not in use. For convenience, this assembly is placed in a fiber holder and, together, the system resembles a modified syringe (Figure 1.1).

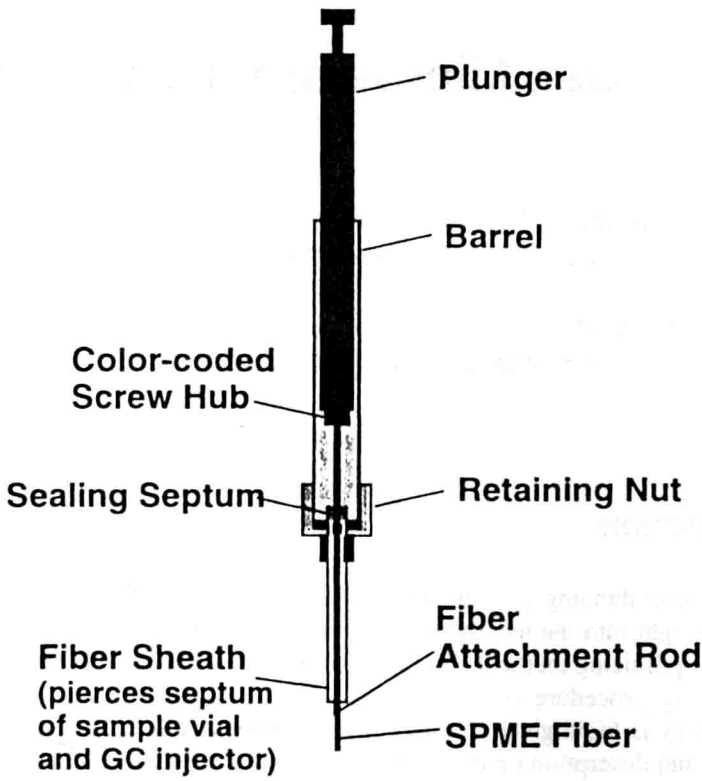

Figure 1.1 SPME fiber with holder. (Courtesy Z. Penton, Varian Associates, Inc.)

The SPME extraction technique consists of two processes: (1) analytes partition between the sample and the fiber coating, and (2) the concentrated analytes desorb from the coated fiber to an analytical instrument. To perform the extraction, an aqueous sample containing organic analytes or a solid sample containing volatile organic analytes is placed in a vial, then closed with a cap and septum. To sample, the SPME protective sheath pierces the septum then the plunger is lowered to either immerse the fiber directly into the aqueous sample or expose it to the sample headspace (Figure 1.2). The target analytes are subsequently extracted

from the sample matrix into the fiber coating. After a pre-determined absorption time, the fiber is withdrawn back into the protective sheath, then the sheath is pulled out of the sampling vial. The sheath is immediately inserted in the GC or HPLC injector and the plunger is again lowered to expose the fiber. This time, the fiber is exposed to a high temperature in the injector liner (GC) where the concentrated analytes are thermally desorbed and, consequently refocused onto the GC column (Figure 1.3). In HPLC, solvents are used to desorb the analytes from the fiber. Afterwards, the fiber is withdrawn into the protective sheath and it is removed from the injector.

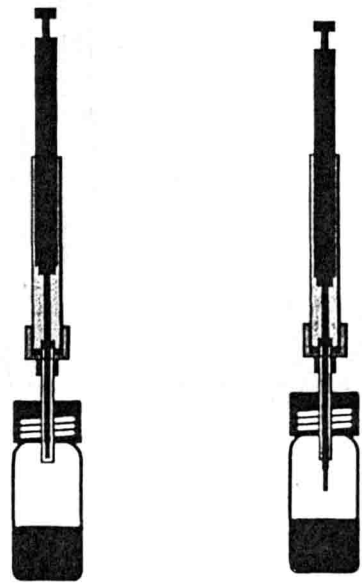

Figure 1.2 SPME headspace sampling. (Courtesy Z. Penton, Varian Associates, Inc.)

Different types of sorbents will extract different groups of analytes; therefore, many different fiber coatings have been developed. Similar to selecting an analytical GC column where "like dissolves like," a fiber is chosen based on its selectivity for certain target analytes and their volatility ranges. Nonpolar coatings (e.g., poly(dimethylsiloxane)) retain hydrocarbons very well. In contrast, polar fiber coatings (e.g., polyacrylate and carbowax) extract polar compounds such as phenols and carboxylic acid very effectively. The affinity of the fiber coating for target analytes is crucial in SPME sampling because both the matrix and fiber coating are competing for analytes. For example, a polar coating chosen to extract polar compounds from water must have a stronger affinity for the analytes than water in order for them to be extracted. The process of choosing a fiber coating for specific analyses is discussed in Chapter 3.

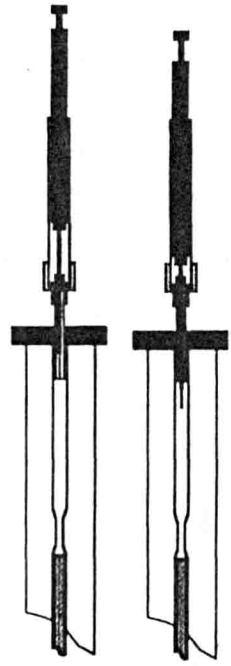

Figure 1.3 SPME desorption into a GC injector. (Courtesy Z. Penton, Varian Associates, Inc.)

SPME SENSITIVITY

Solid Phase Microextraction is an equilibrium technique; therefore, analytes are not completely extracted from the matrix. The liquid polymeric fiber coatings (e.g., poly(dimethylsiloxane)) provide a non-exhaustive liquid-liquid extraction with the convenience of the "organic phase" being attached to the fiber [1]. When a sample is placed in a closed vial, an equilibrium forms between three phases: (1) the fiber coating to the aqueous phase, (2) the headspace to aqueous phase, and (3) the fiber coating to headspace (Figure 1.4). The analyte recovery expected from SPME is related to the overall equilibrium of the three phases present in the sampling vial.

Of course, the total amount of analyte does not change during the extraction. Moreover, the distribution among the three phases after equilibrium is represented as follows:

$$C_o V_s = C_h^{\infty} V_h + C_s^{\infty} V_s + C_f^{\infty} V_f \tag{1.1}$$

Where: C_0 is the initial concentration of the analyte in the aqueous solution; C_h^∞, C_s^∞, and C_f^∞ are the equilibrium concentrations of the analyte in the headspace, aqueous solution, and fiber coating, respectively; and V_h, V_s, and V_f are the volumes of the headspace, aqueous solution, and fiber coating, respectively [2]. If no headspace exists in the closed vial, then $C_h^\infty V_h$, the headspace term, is omitted and the equilibrium is formed only between the aqueous solution and fiber.

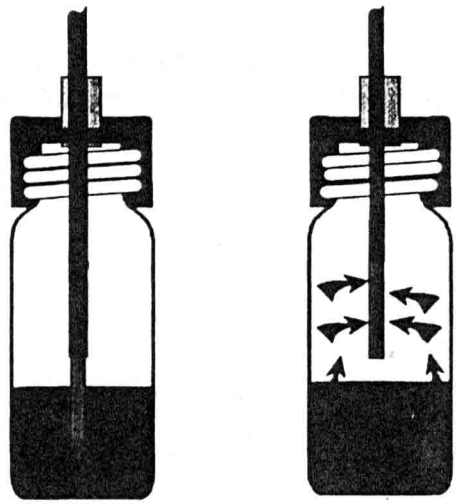

Figure 1.4 SPME is a three-phase system between the solid or aqueous solution, $(C_s^\infty V_s)$, the headspace above the liquid or solid, $(C_h^\infty V_h)$, and the fiber coating, $(C_f^\infty V)$. Direct sampling (left) and headspace sampling (right) are illustrated. The partition coefficients between the three phases are $K_{fh} = \frac{C_f}{C_h}$, $K_{hs} = \frac{C_h}{C_s}$, and $K_{fs} = \frac{C_f}{C_s}$. (Courtesy Z. Penton, Varian Associates, Inc.)

In this section, the theory developed for fibers with liquid polymeric coatings is discussed. This rationale is also applicable to the more recently developed fibers, e.g., porous solid materials. First, the principles of direct, liquid sampling, i.e., immersing the fiber directly into the aqueous sample, will be discussed. Then, the principles of headspace sampling will be presented.

Direct Liquid Sampling

The partitioning between the fiber coating (stationary phase) and the aqueous phase is described by the distribution constant, K_{fs}:

$$K_{fs} = \frac{C_f}{C_s} \tag{1.2}$$

Where: C_f is the concentration of analyte in the fiber coating and C_s is the concentration of analyte in the aqueous phase [3]. This is a characteristic parameter that describes the fiber coating's properties and its selectivity toward a specific analyte, versus other matrix components.

The partition ratio, k' is:

$$k' = \frac{C_f V_f}{C_s V_s} = \frac{n_f}{n_s} = K_{fs} \frac{V_f}{V_s} \tag{1.3}$$

Where: n_f and n_s are the number of moles in the fiber coating and aqueous phases, respectively, and V_f and V_s are the volumes of the fiber coating and water solution. Because the coatings used in SPME have strong affinities for organic compounds, K_{fs} values for targeted analytes are quite large, which means that SPME has a very high concentrating affect and leads to good sensitivity [4].

However, K_{fs} values are not large enough to exhaustively extract most analytes in the matrix. Instead, SPME, like static headspace analysis, is an equilibrium sampling method and, through proper calibration, it can be used to accurately determine the concentration of target analytes in a sample matrix. Two different equations are used to determine the amount absorbed by the fiber, depending on the sample volume. For large sample volumes (>5 mL), the amount of analyte absorbed by the fiber coating at equilibrium is directly proportional to the initial aqueous concentration, C_o. The following equation is used when the volume of the aqueous sample, V_s, is much larger that the stationary phase volume; that is, its volume is relatively infinite to the fiber volume ($V_s >> K_{fs}V_f$).

$$n_f = K_{fs} V_f C_o \tag{1.4}$$

Where: n_f is the amount extracted by the fiber coating. Here, the sample volume does not need to be known (it is relatively infinite), which is ideal for field sampling and simplifies laboratory operations.

On the other hand, when sampling from a finite sample volume, such as 2–5 mL, the sample can be significantly depleted, and the amount absorbed becomes: