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学 位 论 文

固相微萃取技术与气相色谱—质谱联用在环境与临床毒物分析中的应用基础研究

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SOLID-PHASE MICROEXTRACTION COMBINED WITH GAS CHROMATOGRAPHY - MASS SPECTROMETRY: FUNDAMENTAL STUDIES AND APPLICATIONS IN ENVIRONMENTAL AND CLINICAL TOXICOLOGICAL ANALYSES

A Dissertation Presented
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摘　要

自从1989年Pawliszyn研究小组提出“固相微萃取”的概念后，该技术作为一种样品前处理技术得到了快速的发展。其装置的核心部分是涂渍有各种固定液或吸附材料的纤维。固相微萃取全过程包括待测物在纤维与样品（空气、水等）之间的分配或吸附以及将纤维上富集的待测物解吸到分析仪器分离检测。该技术的使用无须使用有机溶剂，装置简单，价格低廉，萃取效率高，选择性好，可以与许多其他的分析方法联用。本论文的工作主要基于气相色谱-质谱分析系统，进行各种纤维吸附材料的基础研究。选择合适的纤维萃取待测物，可以获得好的固/液分配系数，从而极大提高方法的灵敏度。

研究的目的主要有：（1）为某些固相微萃取理论的论证提供新的实验数据；（2）评估自制的纤维的吸附性能、重现性以及耐用情况；（3）应用固相微萃取技术分析环境、临床和生物样品中的各种半挥发性化合物。

全文由六章组成。第一章是文献调研，阐明了固相微萃取技术的萃取理论以及影响方法灵敏度和平衡时间的因素；介绍了用于该技术的纤维富集
材料的发展以及在各分析领域的应用。

第二章报道了在平衡态与非平衡态采用固相微萃取技术快速分析水中的致癌物硝基多环芳烃与多环芳烃。该技术被首次应用于分析水中的硝基多环芳烃，并与多环芳烃的分析结果进行比较。经实验优化后，热解吸硝基多环芳烃的温度在200°C。更高的热解吸温度会引起硝基多环芳烃的热降解，但观察不到多环芳烃热降解的现象。虽然硝基多环芳烃与其母体多环芳烃的结构相比仅多一个硝基，萃取硝基多环芳烃的平衡时间却远少于其母体多环芳烃。采用固相微萃取技术在非平衡态萃取可以定量地分析硝

基多环芳烃与多环芳烃。良好的工作曲线为非平衡态的定量理论提供了新的实验论据。此外，本章节还讨论了方法的准确度、精密度、工作曲线的线性范围、检测限以及离子强度对方法灵敏度的影响，并与美国环境保护法规中的标准方法相比较。

第三章报道了采用不同纤维估算硝基多环芳烃与多环芳烃的辛醇~水分配系数的新方法。本工作采用固相微萃取技术测定了八种硝基多环芳烃与十余种多环芳烃的固/液分配系数。实验测得的分配系数与文献报道的辛醇~水分配系数存在着线性关系。线性自由能关系理论可以成功地应用于解释该关系。与Leo碎片常数法相比，实验结果表明固相微萃取技术可以应用于估算化合物的辛醇~水分配系数，并首次报道了直接致癌物硝基多环芳烃的辛醇~水分配系数，为进一步了解硝基多环芳烃的毒性提供了重要的物理化学参数。

第四章评估了自制的涂有不同固定液或吸附材料的固相微萃取纤维，并与商品化的纤维进行比较。其中硅橡胶纤维的吸附性能可以与商品化的聚二甲基硅氧烷纤维相媲美，而且便宜、容易自制。而涂有C_{18}键合硅胶多孔层的纤维与商品化的纤维相比，由于吸附表面积大，固定液的膜很薄，有机化合物在该纤维上的质量迁移非常迅速。同时，该纤维还表现出良好的吸附性能、重现性和使用寿命。实验结果表明这两种纤维均具有良好的应用前景。

第五章报道了采用自制的涂有C_{18}键合硅胶多孔层纤维进行毒物分析的新方法。该纤维首次被应用于毒物海洛因、冰毒、可待因、和吗啡的分析。实验结果表明该方法对冰毒和可待因的分析具有良好的灵敏度、重现性和选择性。此外，采样时间、缓冲溶液以及离子强度对分析方法的影响在文中有进一步讨论。该方法被成功地应用于测定尿液中海洛因的代谢产物以及鸦片中的主要成分。

第六章报道了固相微萃取技术与顶空衍生技术相结合分析6-单乙酰吗啡，吗啡和可待因的新方法。该方法的灵敏度高于常规的吗啡试纸分析方法，且可以有效地区分含有吗啡环的不同化合物。自制的涂有C_{18}键合硅胶多孔层纤维在该实验中表现出良好的抗溶剂性。在相同实验条件下，该纤维采用顶空衍生的使用寿命至少在50次以上，而目前最具抗溶剂性的商品化纤维的使用寿命低于5次。该方法被成功地应用于滥用海洛因的鉴定分析。

关键词：固相微萃取，气相色谱-质谱，水分析，毒物分析

Abstract

Solid-phase microextraction (SPME) is a sample preparation technique which has been rapidly developing since the basic concept of SPME was first described by Belardi and Pawliszyn in 1989. It consists of two processes: partitioning of analytes between the stationary phase immobilized on a fiber and the matrix (air, water, etc.), and followed by sequent desorption of the concentrated analytes into an analytical instrument. The technique is solvent-free, simple, inexpensive, efficient, selective and compatible with a wide range of separation methods and applications. In this dissertation, research work has been focused on the fundamental studies of the properties of different fiber materials. With suitable stationary phase, the partition coefficient of an analyte between the stationary phase and the sample matrix can be dramatically improved, which results in the dramatically increase in sensitivity of the method.

The objective of this study is to (1) provide further experimental data for the refinement of SPME theory; (2) evaluate the performance of several home-made fiber coating in terms of adsorption property, reproducibility and durability; and (3) apply the technique to a variety of semivolatile organic species with different chemical properties encountered in environmental, clinical and biological samples.

The dissertation is composed of six chapters. In the first chapter, the background of the research will be given. Topics to be discussed include the theory of the different modes of extraction, factors affecting sensitivity, the effect of sampling time, the sorbent materials and areas of applications.

In the second chapter, results obtained from the application of SPME on the analysis of PAHs and NPAHs (polycyclic aromatic hydrocarbons & nitrated

polycyclic aromatic hydrocarbons) will be presented. The analytical characteristics and performance of SPME for NPAH analysis are investigated in comparison with PAHs. SPME is used for the analysis of NPAHs for the first time. After experimental optimization, the thermal desorption temperature of NPAHs is 200°C. Desorption in a higher temperature will resulted in the thermal degradation of NPAHs, which does not occur to PAHs. Though NPAHs have similar structures with their parent PAHs except for nitro, the equilibrium time of NPAHs are much less than those of PAHs. The validity and advantages of SPME sampling under the non-equilibrium situation are illustrated. The linearity of the calibration curves for NPAHs and PAHs submits new evidence for the quantitative analysis of SPME in nonequilibrium situation. Analytical parameters including the linear range, limit of detection, precision and the effect of sodium sulfate are investigated. Performance of the developed method are compared with the US EPA standard methods.

In the third chapter, a new method for estimating the octanol-water partition coefficients of NPAHs and PAHs by SPME with different fiber coatings will be described. SPME are used for the measurement of the partition coefficients of eight NPAHs and ten PAHs. The partition coefficient here is defined as the partition of the substance between the SPME coating and the water. A linear relationship between the partition coefficients (Ksw) obtained by SPME and the octanol-water partition coefficients (Kow) measured by conventional method are established for both classes of compounds. A theory of free energy relationship is used to explain the correlation. Results demonstrate that SPME provides an simpler alternative to the conventional methods for the measurement of octanol-water partition coefficients. The partition coefficients of direct carcinogens NPAHs have not previously been reported in the literature. The new information obtained here is therefore of value to those involved in the study of the biological activities of NPAHs.

In the fourth chapter, several inexpensive home-made SPME fibers are developed. The analytical performance of these fibers are evaluated against those obtained commercially. Two home-made fibers coated each with silicone rubber and C_{18} bonded silica stationary phase, respectively, show good mechanical strength and analytical performance. The silicone rubber fiber has good adsorption property the same as the commercial polydimethylsiloxane (PDMS) fiber but is considerably cheaper to make. It is suitable for the rapid screening of complex samples. The mass transfer rate of the analytes from the sample solution to the fiber coated with C_{18} bonded silica stationary phase is very fast. Thus, the analytes can quickly reach equilibrium during SPME sampling and also readily desorbed thermally. Both fibers show promising future for applications in trace organic contaminant analysis.

In the fifth chapter, new methods for the analyses of some drugs by the homemade SPME fiber coated with C_{18} bonded silica stationary phase will be present. The home-made C_{18} bonded silica particle fiber is applied to the analyses of some drugs including heroin, methamphetamine, codeine and morphine for the first time. The effects of sampling time, pH and ionic strength are investigated. These methods show good sensitivity, reproducibility and selectivity for methamphetamine and codeine. They are successfully used for the analyses of the metabolites of heroin in urinary samples and the primary compounds in a opium sample.

In the sixth chapter, A new SPME technique involves headspace derivatization is developed and successfully applied to the analyses of 6-monoacetylmorphine, morphine and codeine for the first time. The sensitivity of the method is dramatically improved over conventional method by the morphine test paper. In addition, the SPME method can distinguish the different compounds with the same morphine rings while the morphine test paper cannot. A homemade fiber coated with C_{18} bonded silica stationary phase is used in the experiment. The fiber can endure organic solvent

better than the commercial fibers. Under the same experimental conditions, the homemade fiber is much more robust and has a longer lifetime (at least 50 times) than the commercial fibers (no more than 5 times). This new method is successfully used for the detection of abused heroin.

Keywords: Solid-phase microextraction, gas chromatography - mass spectrometry, water analysis, drug analysis

1 Objectives and Background of the Research

1.1 OBJECTIVES

The objective of this study is to (1) provide further experimental data for the refinement of SPME theory; (2) evaluate the performance of several home-made fiber coating in terms of adsorption property, reproducibility and durability; (3) apply the technique to a variety of semivolatile organic species with different chemical properties encountered in environmental, clinical or biological samples.

1.2 BACKGROUND OF THE RESEARCH

1.2.1 Introduction

An analytical process typically consists of several steps, including sample preparation, sample analysis, data handling, etc., each of which is critical for obtaining accurate and reproducible results. A sample analysis flow diagram is illustrated in Figure 1.1. Over the past several decades, advances of instrumentation and microcomputer technology have led to the rapid improvement of analysis speed, resolution and automation. In contrast, there is little improvement in reducing the labor and time spent on sample preparation. Many analytical chemists still use time-consuming manual methods that have been around for decades. The results of one survey (Figure 1.2) indicate that, for all respondents, typically two-thirds of the analysis time is spent on sample preparation, considerably more than those spent on sample collection, analysis,

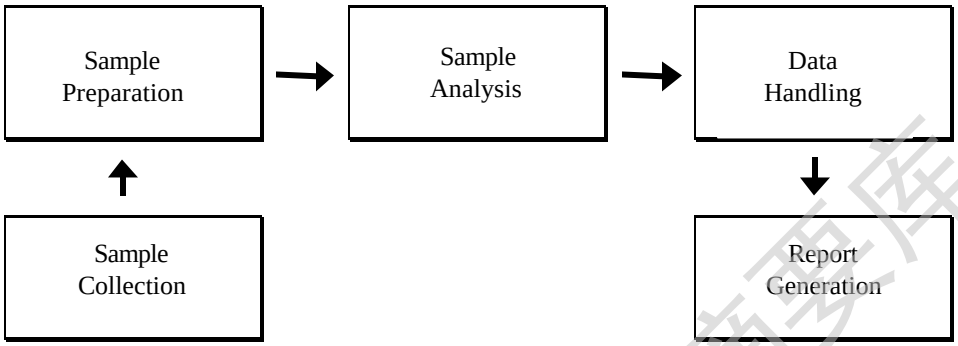

Figure 1.1. Sample-analysis flow diagram.

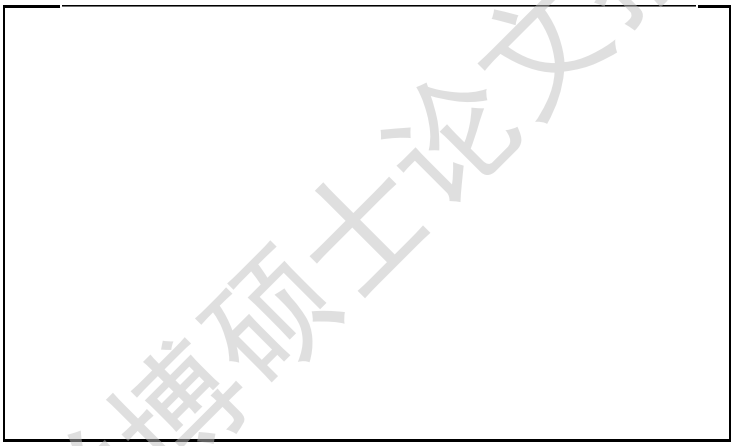

Figure 1.2. Survey results for the partition of time that analytical chemists spend on sample analysis.

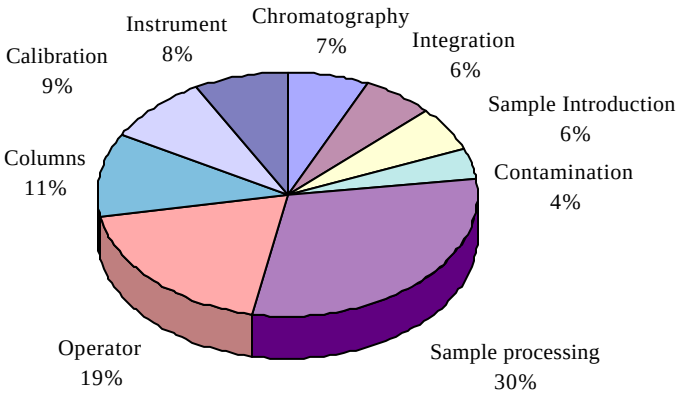

Figure 1.3. Survey results for the partition of error generated during sample analysis.

and data management[1]. In addition, survey data on error propagation for a method (Figure 1.3) indicate that typical sample processing accounts for at least one-third of the error generated during the performance of the entire analytical method; and operator-generated error is responsible for ~20%[1]. Clearly, improving and automating the sample preparation step will reduce analysis time, decrease the error and improve sample throughput.

A sample preparation step is often necessary to isolate the components of interest from a sample matrix, as well as to purify and concentrate the analytes[1]. Many sample preparation techniques are widely used in analytical laboratories, such as extraction, filtration, headspace, dialysis, derivatization, evaporation, dissolution, concentration, centrifugation, pH adjustment and others[2]. Extraction technique also consists of liquid-liquid extraction (LLE), liquid-solid extraction, solid phase extraction (SPE), supercritical fluid extraction (SFE), thermal extraction, etc. However, some commonly used sample preparation practices are still based on old technologies. For example, Soxhlet extraction (one kind of liquid-solid extraction) was developed more than 100 years ago[3]. The traditional methods are often associated with a lot of problems. They are usually time-consuming, and require a large amount of organic solvent. The complex multistep procedures involved often result in the loss of analyte during operation. This frequently makes sample preparation step the major source of error in an analysis, and prohibits integration with the rest of the analytical process. An ideal sample preparation technique should be solvent-free, simple, inexpensive, efficient, selective, and compatible with a wide range of separation methods and applications. Ideally, it should be able to perform simultaneous separation and concentration of the target species, and also facilitate opportunity for on-site extraction and analysis. Solid-phase microextraction (SPME) is a technique which can potentially offer such advantages[4].

The basic concept of SPME was first described by Belardi and Pawliszyn in 1989[5]. It consists of two processes: partitioning of analyte between the extracting phase immobilized on a fiber and the matrix (air, water, etc.), and followed by subsequent desorption of the concentrated analyte into an analytical instrument[6]. The initial work on SPME was exclusively done for SPME/GC coupling[7-11]. Then the interfaces for SPME/HPLC coupling[12-13] or SPME/CE coupling[14-15] came forth continuously. To date it has been able to combined with many analytical instruments[16-19] and widely used in differently analytical fields[20-23], especially in environmental analysis[8,24-26]. Figure 1.4 illustrates the number of published papers about SPME during the past ten years. The number nearly doubled from 1992 until 1997 but holding steady in 1998, i.e. approximate 100 papers per year.

Figure 1.4. The number of published papers about SPME during the past ten years. Published papers here are referred to those recorded by CCOD of ISI.

SPME is one kind of solvent-free techniques which attract widespread attention due to regulatory pressures to reduce the use of toxic organic solvents. In general, most of solvent-free techniques may fall into one of three categories: gas-phase, membrane and sorbent extraction. Gas-phase extraction, which belongs to non-selective phase transition techniques, also includes static headspace sampling, purge and trap and supercritical fluid extraction (SFE)[4]. However, SPME, as a sorbent extraction, is quite different from gas-phase extraction. Coated with a selective extracting phase, only a certain group of compounds can be well extracted by SPME. Purge and trap is widely used as US EPA Test Method for volatile compounds (VOCs), for example, EPA methods 502.2, 524.1, 602, 624, etc. In contrast, the inexpensive SPME method may reduce the sample preparation time and have comparable performance in accuracy, precision, detection limit, and linear range[8]. SFE is another attractive solvent-free sample preparation technique for the analysis of compounds, no matter how volatile, semivolatile or nonvolatile, because supercritical fluids possess both gas-like mass transfer and liquid-like solvating characteristics. However, SFE still requires an expensive, high-pressure delivery system and a large amount of high-purity carbon dioxide. The need for heavy equipment makes on-site field analysis much more difficult to accomplish than that by SPME[4].

Among the various extraction techniques, solid-phase extraction (SPE) is the one closely similar to SPME. It is such a significant sample preparation technique in the 1990's[27-30] that it is widely used as US EPA Test Method for semivolatile compounds (SVOCs) or non-volatile compounds, for example, EPA methods 515.2, 525.2, 608, 625, etc. Same as SPME, SPE is a phase selective technique. Many sorbents are available for the selective extraction of different compound classes due to the extensive development work done from 1970 until 1990, which may provide abundant information for the development

Table 1.1. Comparison of SPME and SPE

	SPME	SPE
basis of extraction	equilibrium	exhaustive
% of molecules extracted	< 50%	100% (goal)
% of molecules injected	100%	< 1%
time required	5 min to 1 hour	5 min to 1 hour
plugging	not possible	can occur
re-use of sorbent	> 50 times	once
solvent required	none	10~50 mL
automated (GC)	yes	no
EPA methods	none	> 20

of feasible extracting phase for SPME. A comparison of SPME and SPE is listed in Table 1.1.

Typical thickness of extracting phase on a SPME fiber is less than 100 μm, which is insufficient to exhaustively extract the compounds of interest. However, SPE allows only the analysis of 1% sample per run, while the total amount of analyte on the SPME fiber can be injected into the analyzer. Thus, SPME method actually has comparable or better than SPE for trace analysis, for example, accuracy, precision, detection limit, and linear range in the analysis of polycyclic aromatic hydrocarbons (PAHs). Although SPE consumes less labor, time and solvent in comparison with liquid-liquid extraction (LLE), a selective organic solvent is normally needed first to remove the interference, and then another solvent is needed to wash out the target compounds. In contrast, solvent usage has been completely eliminated from the sample preparation step in SPME. The compounds of interest can be readily adsorbed or desorbed through the thin extracting phase on SPME fiber because of the rapid mass transfer rate during extraction and desorption. Furthermore, SPME can be

reused more than 50 times and easily automated in GC operation while SPE cannot always.

1.2.2 Extraction

1.2.2.1 Theory

1.2.2.1.1 Establishment

The initial SPME fiber coating used was polymer, for example, polydimethylsiloxane (PDMS) or polyacrylate (PA). Thus, the dynamics of SPME extraction can be assumed as a diffusion limited process, meaning that diffusion is the only mass transport mechanism in the system[31]. No activation energy is involved in the transfer of analyte between aqueous sample and fiber coating. In addition, assuming that the diffusion only occurs in one direction (x-axis in Figure 1.5), the one-dimensional diffusion process can be described by Fick's second law[32]

(1.1)

where C(x, t) is the concentration of the analyte at position x and time t; D is the diffusion coefficient of the analyte. The total mass of the analyte adsorbed by the polymeric coating as a function of time can be calculated by[31]

(1.2)

Louch et al. derived the quantitative equation of SPME technique in equilibrium situation as the following. The simple SPME extraction system is composed of fiber coating and aqueous sample without any headspace. A mass balance equation exists before and after the extraction:

$$n_1 + C_2^{\infty} V_2 = C_2^{0} V_2 \qquad (1.3)$$