

**SOLID PHASE MICROEXTRACTION BASED ON
POLYPYRROLE FILMS**

By

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Abstract

Polypyrrole (PPY, with different *N*-substituents or with different counter ions) films were prepared and applied for solid-phase microextraction (SPME). The extraction properties of the new films to volatile organic compounds were examined using an SPME device coupled with GC/FID. A PPY coated capillary was applied for in-tube SPME to evaluate its extraction efficiency towards less volatile compounds and ionic species. The porous surface structures of the films, revealed by scanning electron microscopy (SEM), provided high surface areas and allowed for high extraction efficiency. Compared with commercial SPME stationary phases, the new phases showed better selectivity and sensitivity toward polar, aromatic, basic and anionic compounds, due to their inherent multifunctional properties. In addition, introducing a new functional group, such as a phenyl group in poly-*N*-phenylpyrrole (PPPY), or incorporating an appropriate counter ion into the polymer could modify the extraction efficiency and selectivity of the films for SPME. For in-tube SPME, the PPY coated capillary showed superior extraction efficiency to commercial capillaries for a variety of compounds, demonstrating its potential applications for a wide range of analytes when coupled with HPLC. The sensitivity and selectivity of the films for SPME could also be tuned by changing the film thickness. The preliminary results of a study on electrochemically controlled SPME demonstrated that the unique electroactivity and reversible redox property of polypyrrole films could be used to further improve the selectivity and sensitivity of SPME by controlling the applied electrochemical potentials. These results are in line with both the theoretical expectations and the results obtained by other methods, which indicate not only that PPY films can be used as new stationary phases for SPME, but also that SPME method may provide an alternative tool for studying materials like polypyrrole.

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List of Abbreviations

AAS	Atomic absorption spectrometry
AES	Atomic emission spectrometry
CV	Cyclic voltammetry
DEP	Diethyl phthalate
DMP	Dimethyl phthalate
DL	Detection limit
DS	Dodecylsulphate (sodium salt)
EC-SPME	Electrochemically controlled SPME
ES	Electrospray
FIA	Flow injection analysis
GC	Gas chromatography
HPLC	High performance liquid chromatography
ICP	Inductively induced plasma
LC	Liquid chromatography
MS	Mass spectrometry or Mass detection
PCbPY	Poly- <i>N</i> -(2-carboxyethyl)pyrrole
PCPY	Poly- <i>N</i> -(2-cyanoethy)pyrrole
PMPY	Poly- <i>N</i> -methylpyrrole
PPPY	Poly- <i>N</i> -phenylpyrrole
PPY	Polypyrrole
PSS	Poly(sodium 4-styrenesulfonate)
RSD	Relative standard deviation
SEM	Scanning electron microscopy
SPME	Solid-phase microextraction
TBAP	Tetrabutylammonium perchlorate
TS	<i>p</i> -Toluenesulphonate (sodium salt)

CHAPTER 1

INTRODUCTION

1.1. Introduction to solid-phase microextraction

Solid-phase microextraction (SPME) is a sample preparation and sample introduction method that consists of two processes: analyte extraction and desorption (or release). First, a small amount of extracting phase coated on a solid support is placed in contact with the sample matrix for a predetermined period of time, and the target analytes are extracted from the sample matrix into the extracting phase by partition or adsorption. The extracting phase with concentrated analytes is then transferred to an instrument or interface for analyte desorption or release, followed by separation and quantification. Analyte extraction can be carried out from headspace (for gaseous or volatile analytes) or by direct immersion of extracting phase in the sample solution. Volatile and thermally stable analytes can be desorbed thermally by directly transferring the extracting phase with extracted analytes to a heated gas chromatography (GC) injector. Less volatile and /or thermally labile compounds or ions can be released from extraction phase by a suitable solvent or mobile phase used in high performance liquid chromatography (HPLC). Since there is no extra step involved between analyte extraction and desorption, these two processes (sample preparation and introduction) can often be integrated into a single step, especially in an automated process with an autosampler.

Two main approaches of the SPME technique have been explored to date, which are shown in Figure 1-1. One is the traditional approach using polymer-coated fibers (fiber SPME) and the other is the so-called in-tube SPME developed. The main advantages of using a coated fiber for SPME are its flexibility and portability, which make this approach well suited for field applications, especially when the analysis is carried out on site. The coated fiber can be exposed directly to the medium being analyzed, such as river water or ambient air, without the need to collect a sample. Analysis can then be performed using field portable instrumentation, or the fibers can be transported to the lab for analysis. In-tube SPME, using a coated open tubular capillary (GC column) as the extracting phase, has been used mainly

for extraction of less volatile and /or thermally labile compounds or ions from aqueous samples. In-tube SPME can be easily coupled on-line to a standard autosampler or an automated HPLC system for automated extraction and analysis. With this technique, target analytes in aqueous samples are directly extracted from sample matrix onto the inner surface coating of a capillary, and then desorbed by introducing a moving stream of mobile phase or static desorption solvent when the analytes are more strongly absorbed to the coating.

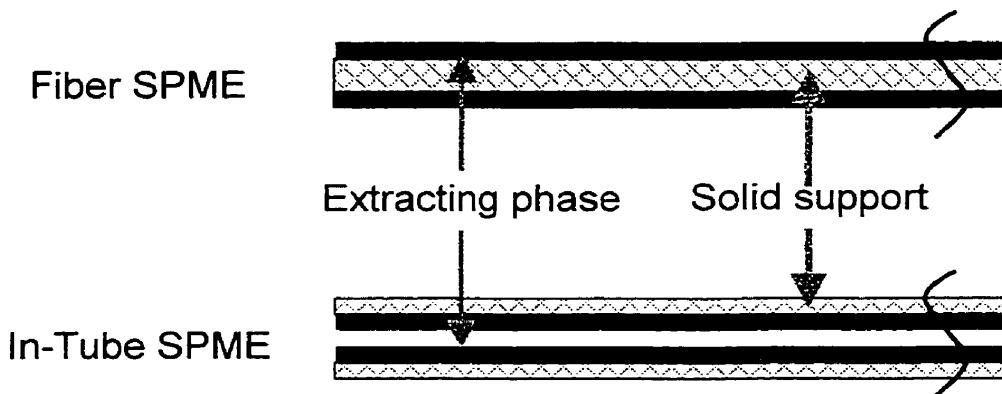


Figure 1-1. Fiber SPME and in-tube SPME

Generally, the amount of analyte extracted in SPME increases with the extraction time until equilibrium is established (extraction time profile). However, the theories behind the equilibrium extraction processes are different for different types of coatings. There are mainly two distinct SPME coating types available commercially. Coatings of the first type, including poly(dimethylsiloxane) (PDMS) and poly(acrylate) (PA), extract analytes by absorption. This extraction process is non-competitive, therefore in most cases the amount of an analyte extracted by such coatings from a sample is independent of the matrix composition. No displacement effect occurs. The amount of an analyte extracted from a sample is linearly dependent on its initial concentration, provided that other variables such as temperature, extraction time and agitation conditions are kept constant. The theory for coatings of this type is well understood and has been described in detail in the literature [1-3]. For a two-phase system (coating/sample matrix), the amount of an analyte (A) extracted by coatings of this type at equilibrium can be expressed as:

$$n_A = K_A V_f V_s C_A^0 / (K_A V_f + V_s) \quad (1-1)$$

where n_A is the amount of the analyte A extracted by the coating at equilibrium, V_s and V_f are the volumes of the sample solution and coating, respectively, C_A^0 is the initial concentration of the analyte A in the sample, and K_A is the distribution coefficient. The effect of sample volume (V_s) on quantitative analysis by SPME was studied in detail previously [4,5]. In most cases, V_s is much larger than the coating capacity ($V_s \gg K_A V_f$), therefore the amount of an analyte extracted by this type of coatings is directly proportional to the initial analyte concentration.

$$n_A = K_A V_f C_A^0 \quad (1-2)$$

This feature, combined with the advantages (flexibility and portability) of fiber SPME, makes fiber SPME well suited for on-site field sampling and analysis.

Coatings of the second type, including poly(dimethylsiloxane)/divinylbenzene (PDMS/DVB), CarbowaxTM/divinylbenzene (CW/DVB) and CarbowaxTM/template resin coatings, extract analytes by adsorption. This extraction process is limited to the surface of the coating. It is competitive, which means that a molecule with higher affinity to the coating can displace a molecule with lower affinity. Since the number of active sites on the surface of this type of coatings is limited, a linear response for these coatings can be expected only when the concentrations of all the compounds extracted from a sample are low. A theory suitable for coatings of this type has been described recently based on Langmuir's adsorption theory [6]:

$$n_A = K_A V_f V_s C_A^0 (C_{f\max} - C_{fA}^\infty) / [V_s + (K_A V_f (C_{f\max} - C_{fA}^\infty))] \quad (1-3)$$

where $C_{f\max}$ is the maximum concentration of active sites on the coating, and C_{fA}^∞ is the equilibrium concentration of the analyte on the coating. The other terms in equation (1-3) have the same meanings as in equation (1-1) with the exception of the distribution coefficient K . In equation (1-3), K is defined as the adsorption equilibrium constant, but it is the partition coefficient in equation (1-1).

The main difference between Equation (1-1) and Equation (1-3) is the presence of the coating concentration term ($C_{f\max} - C_{fA}^\infty$) in the numerator and denominator of Equation (1-3). For very low analyte concentrations on the coating, it can be assumed that $C_{f\max} \gg C_{fA}^\infty$.

For this condition to be fulfilled, analyte concentration in the sample and /or its affinity towards the coating must be low. Under these circumstances, a linear dependence should be observed. Otherwise, non-linear adsorption relationship will be obtained (see the section on extraction mechanism in Chapter 4).

1.2. The development of SPME technique and the challenges faced

Since its introduction, SPME has become a popular sampling method for a variety of volatile and semi-volatile compounds due to its simple, solvent-less, reliable, and flexible properties. Reviews and books on the theory, method development and applications have been published [1-9]. The SPME devices and a variety of fiber assemblies with different coatings are commercially available from Supelco (Bellefonte, PA). In most cases, however, the compounds that can be analyzed successfully are either nonpolar or of medium polarity. In the development of the SPME technique, it has been a challenge to extract polar and /or ionic analytes from water samples because of the less polar properties of the commercial SPME coatings and the stronger interactions between water and polar analytes. A solution to improve the extraction ability for these analytes is to converting them to less polar, nonionized forms by pH adjustment or derivatizations [1-2]. However, derivatizations are often complicated processes that require a great deal of time and reagent. Perhaps the best solution is to develop polar and ion exchange coatings for direct extraction of the target species from sample matrices. Recently, several new SPME coatings, which showed higher extraction efficiency to polar compounds, have been developed and commercialized, including PDMS/DVB and CW/DVB. However, only a few reports have been found in literature on direct SPME of ionic species [10-12]. In all of these studies, custom-made ionic coatings were used rather than commercial fibers. Up to now, the most successful applications of SPME are for analyses of volatile and semi-volatile organic compounds by coupling SPME with GC. Little work has been carried out on non-volatile or thermally labile compounds which are best separated by HPLC, even though SPME can be coupled directly with HPLC [13-15] and LC-MS relatively easily [16,17]. These compounds include most pharmaceutical products, drugs, peptides, nucleic acids, proteins, some pesticides, organometallic compounds and ionic species. One of the main difficulties limiting the wide

application of SPME/LC is the absence of suitable SPME stationary phases that not only have high extraction ability for the analytes but are also stable in various sample matrices and desorption reagents. In addition, to extract analytes directly from sample matrix, especially from complicated sample matrix such as biological samples, highly selective and stable coatings are required. Therefore, the development of SPME in this area will depend on the advances of material science and technology. One of our goals is to develop new polymeric phases for SPME to extend its applications to polar and ionic compounds.

1.3. Polypyrrole and functionalized polypyrroles in analytical sciences

The interest in conducting and electroactive polymers has increased rapidly in the past two decades due to their multifunctional properties and potential applications as novel materials, such as ion exchangers, energy-storage materials, corrosion-resistant coatings, catalysts, conductive textiles and fabrics, and materials for separation, actuators, chemical sensors and biosensors, electronic nose and tongue, and controlled (drug) delivery systems [18-51]. Among various conducting polymers studied so far, the most widely used polymers are based on polypyrrole, polyaniline and polythiophene (Figure 1-2). Of these three classes of materials, polypyrrole (PPY) and its derivatives have been the most widely used and intensively studied over the past twenty years, because pyrrole and some of its derivatives are available commercially, and their stable polymer films can be conveniently prepared on various substrate materials from organic or aqueous media by electrochemical or chemical methods. Books and reviews on the evolution of conducting polymers and their applications have been published [39-51].

Since the first report on the electrochemical synthesis of PPY in 1979 [52], great effort has been made to utilize the versatile materials in analytical sciences. Due to its inherent multifunctional properties, such as anion exchange, permeability (porous structure), and the ability to form hydrogen bond, acid-base, π - π and hydrophobic interactions, PPY has been used in analytical sciences as ion exchanger, membrane and electrode materials for selective anion electrodes (determination of chloride in blood serum [53, 54]), membrane separations (of gas mixtures [55,56], ionic [57,58], and neutral molecules [59,60]), gas sensors (for alcohol vapors [61-63] and ammonia [63-64]) and electronic nose [41,65,66].

One unique advantage of conducting, electroactive polymers (CEP) such as PPy over conventional materials is that their physical and chemical properties can be changed or controlled electrochemically. For a simple PPy system, the redox states can be controlled as shown in Figure 1-3.

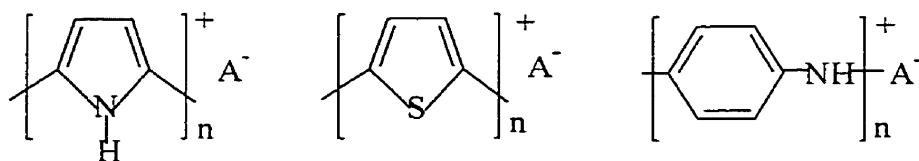


Figure 1-2. Structures of polypyrrole, polythiophene and polyaniline.

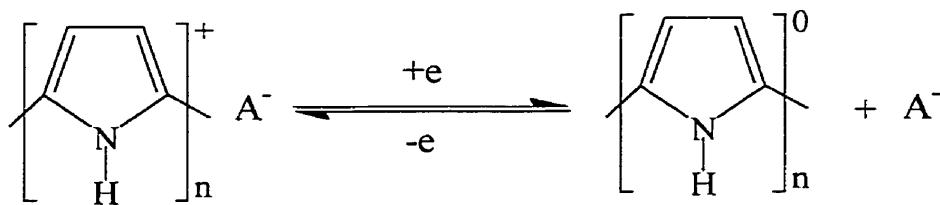


Figure 1-3. Redox properties of polypyrrole.

The reversible redox and anion-exchange properties of the regular PPy have been used to advantage in the development of electro-controlled release devices [67-69], especially for drug release at a controlled rate [67,68]. A simple approach is the incorporation of an anionic reagent such as a drug as counter ion (A^-) in an oxidized PPy film. The anionic reagent is released from the polymer structure upon reduction. This approach has been successfully demonstrated in the case of doping and release of glutamate anions, a drug of interest for neuroscience [67]. A number of groups have utilized the redox properties of PPy in membrane and chromatography separation systems as well as sensor studies. It has been found that PPy films in different redox states demonstrate remarkably different separation efficiency and sensor responses [31-35, 63,70]. In particular, electrochemically controlled chromatographic systems with PPy as stationary phases have been used for separation of anions and neutral organic compounds [31-35, 70]. Electrochemical detectors for HPLC have

also been developed based on the same principle [71,72]. An alternative to using electrochemical control of polymers during chromatography is to use redox reagents to control the redox states of the polymer and hence its retention characteristics. For example, the effect of a reducing reagent on retention of amino acids on PPy coated column has been investigated using simple concentration gradients of sodium sulfite as a reductant [73]. In addition, the permeability of PPy films can be altered by appropriately controlling the electropolymerization conditions so that films with effective size-exclusion selectivity can be prepared [74].

In addition to the inherent functionalities of PPy, a range of predetermined functional groups can be incorporated into PPy to introduce specific molecular interactions (*i.e.* functionalized PPy can be prepared). Owing to the electroactivity and enhanced molecular recognition ability, functionalized polypyrroles have attracted great interest in the area of electroanalysis, separation sciences and biosensors [42-46].

PPY can be prepared either chemically or electrochemically. PPy is formed in its oxidized form, carrying approximately one positive charge in every three to four pyrrole rings which is counter-balanced by an anion (counter ion, A⁻) from the electrolyte (Figure 1-4).

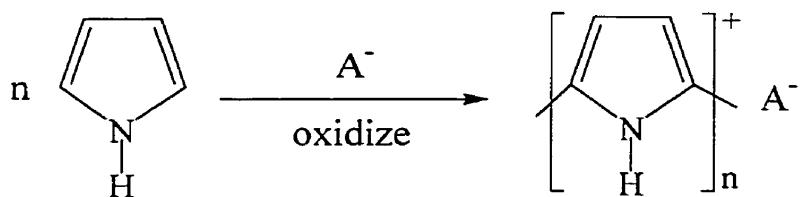


Figure 1-4. Oxidative polymerization of pyrrole.

Introducing functional groups into PPy via counter ions

The counter ions play an important role in the synthesis of PPy and provide a convenient way to introduce molecular recognition sites. Because PPy can be polymerized from both organic and aqueous media with neutral pH at low anodic potential (while

polyaniline can only be polymerized from acid and polythiophene from organic solvents. In addition, much higher electrochemical potential is required for preparation of polythiophene), a wider range of counter ions (A^-) including bioactive ions can be incorporated into PPy compared with other conducting polymers, such as polyaniline and polythiophene. The nature and size of counter ion have a significant effect on the properties of PPy. It has been shown that the ion-exchange selectivity series is determined by the counter ions incorporated during synthesis [45,75]. For example: for PPy-Cl, $\text{Br}^- > \text{SCN}^- > \text{SO}_4^{2-} > \text{I}^- > \text{CrO}_4^-$; and for PPy- ClO_4 , $\text{SCN}^- > \text{Br}^- > \text{I}^- > \text{SO}_4^{2-} > \text{CrO}_4^-$. In addition, the nature of ion exchange can be changed depending on the size of the counter ion used. For example, PPy films incorporated with small anions, such as Cl^- , ClO_4^- or glutamate anion, have anion exchange properties. The anions can be released upon reduction of the films as shown in Figure 1-3. However, the rate of such a release is higher with small anions. The anion mobility decreases with the increase of counter ion size. In extreme cases, incorporation of larger and more hydrophobic molecules such as dodecyl sulfate or multicharged polyelectrolytes (PEs) such as poly(styrene sulfonate) (PSS) will totally eliminate the anion exchange property. In contrast, PPy films incorporating these large anions showed cation exchange and redox properties as shown in Figure 1-5. Because the counter ions cannot be released or rejected from the polymer structure upon reduction due to their bulky structure, cations (C^+) in solution are incorporated into the PPy films to maintain electrobalance. When the films are reoxidized, these cations can be released. The cation exchange and redox properties of PPy-PSS have been utilized to develop novel extraction systems for cationic species such as actinide and lanthanide ions [76,77] and other cationic ions [78-80]. Electrochemically controlled binding and release of cationic drugs such as dopamine can also be realized using PPy-PSS film [80]. Incorporation of PEs also results in the production of more open, porous polymer structure, allowing the extraction and release of larger molecules such as proteins [81]. When intermediate size organic counter anions such as *p*-toluene sulphonate (TS) are incorporated into PPy films, both anion and cation exchange property of the films have been observed in the redox processes [70,82]. The physical properties of PPy films such as morphology, porosity, mechanical property and thermal stability are also influenced by the counter ions used. For example, it has been shown that improved mechanical and thermal properties have been achieved for PPy films incorporating sulfonated aromatic counter ions [39,40,50].

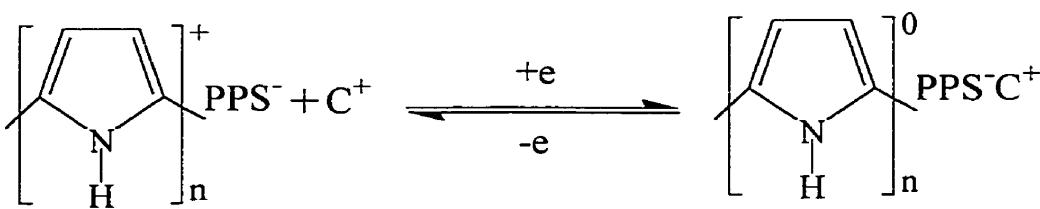


Figure 1-5. Redox properties of PPY incorporating larger counter ions (PPS⁻).

More importantly, specific molecular interactions or molecular recognition sites can be introduced as counter ions into PPY films. For examples, PPY films capable of metal recognition have been prepared by incorporating metal-complexing reagents as counter ions [83-88]. These materials have been utilized to develop electrochemical sensors with improved selectivity for the target metals. For examples, calcion reagent has been used for calcium analysis [84]; Alizarin Red S, bathocuproine sulfonate, sulfosalicylic acid, and Pyrocatechol Violet (PVC) have been used for the extraction and selective determination of Cu (II) and Cu(I) species [85-87]. An on-line derivatization device has been designed for post-column spectrophotometric detection of the separated metal ions based on the principle of electrochemically controlled release of dithiocarbamate ligand from a PPY film [88]. Bioactive groups such as enzymes [89], antibodies [90-92] and living cells [93] have been incorporated into PPY for the development of biosensors. An interesting example of using PPY functionalized with bioactive groups for analytical separation was the development of a novel form of affinity chromatography. In this case, the antigen was incorporated into PPY and it was demonstrated that the potential applied to the column had a dramatic effect on the binding capacity [94]. Optically active counter ions such as camphorsulfonate can also be incorporated into PPY films to induce chirality in the resultant materials [95,96]. The multifunctional nature of polyelectrolytes also facilitates incorporation of electroactive groups such as polyvinylsulfonate (ferrocene) [97] or biofunctional groups such as heparin or dextran sulfate [98].

Functionalized PPy prepared by covalent attachment of functional groups

Functionalized PPy films can also be prepared by polymerizing pyrrole derivatives with functional groups in the nitrogen or the 3-position. Although regular PPy can act as an anion-exchange material, its capacity is restricted to one positive charge per three pyrrole rings and this binding capacity vanishes when it is reduced. Anion-exchange properties of PPy can be dramatically improved by incorporating cationic substituents into the PPy backbone. PPy films with permanent anion exchange capacity, which are independent of the applied potentials, have been prepared using pyrrole monomers bearing alkylammonium or pyridinium groups, and their enhanced anion exchange properties have been exploited to bind a variety of organic and inorganic anions [24,46,48]. Functionalized PPy that have permanent cation exchange properties, such as poly(3-methylpyrrole-4-carboxylic acid) and poly(4-(pyrrol-1-ylmethyl)benzoic acid), have also been synthesized and studied [28,35]. Poly(3-methylpyrrole-4-carboxylic acid) has been used as HPLC stationary phase for separation of amines and proteins [35]. Crown ether or polyether ligands can be incorporated into PPy to form macrocyclic cavities for cationic metal ion recognition [99-105]. Both 3-substituted and *N*-substituted pyrroles have been prepared for this purpose; however, 3-substituted PPy films showed better electroactivity and metal recognition properties [99]. The electroactivity and cation binding ability of *N*-substituted PPy can be improved using an electroactive crown ether monomer such as pyrrole *N*-substituted by ferrocene crown ether [103,104]. In addition, anionic crown ethers such as tetrasulfonated dibenzo-18-crown-6 can be incorporated into PPy as counter ions [105].

Thompson and co-workers have published a series of papers on the application of various *N*-substituted PPy to organic vapor sensing [18]. Eight different PPy films prepared from pyrrole and seven *N*-substituted pyrrole derivatives were characterized by electrochemical and surface analytical methods. The vapor sorption properties of these films were examined using thickness-shear mode (TSM) acoustic wave sensors. The results have shown that both the surface and electrochemical properties and the vapor sorption properties are different for different PPy films. A significantly different sensing response was also observed by Janata and co-workers in their potentiometric gas sensor study [106]. In this case, PPy film incorporated with nitrotoluenes exhibited higher selectivity to aromatic compounds than regular PPy film due to the increased π - π interactions.

Inherently conducting chiral polymers are of considerable interest because of their potential applications such as chiral electrodes for electrochemical asymmetric synthesis and as novel chiral stationary phases for chromatographic separation of enantiomers. For these purposes, chiral PPy films have been prepared using optically active pyrrole monomers and their enantioselective recognition properties have been studied [107-110].

DNA diagnostics has become an important area of molecular biology and biotechnology studies. To realize specific DNA recognition, electrochemical genosensors (or DNA biosensors) have been prepared by either adsorption of DNA on the surface of PPy coated electrode due to electrostatic attraction or by copolymerization of pyrrole and pyrroles substituted with various oligodeoxynucleotides (ODNs) [111,112]. Microelectronic devices such as DNA chips and peptide chips (from pyrrole-peptide monomers) have also been prepared based on an electro-directed copolymerization approach [113,114].

Metalloporphyrins have the potential to be used to mimic enzymatic systems or as biosensor materials. Metalloporphyrins can be incorporated into PPy by different approaches [48]. One approach is to use anion-substituted metalloporphyrins as counter ions. For example, tetrasulfonated and tetracarboxy-substituted metalloporphyrins and metallophthalocyanines have been introduced into PPy films as counter ions during formation of the polymers. Another approach is to polymerize pyrrole-substituted metalloporphyrins. The third approach is to immobilize charged metalloporphyrins into PPy films by ion exchange or electrostatic binding. In this approach, PPy films bearing anion exchange groups such as poly[(pyrrolealkyl)ammonium] polymers have been used.

Only a few examples have been found on direct *N*-functionalization of PPy after its polymerization, such as the derivatization of PPy films by ferrocene, glucose oxidise and dithiocarbamate moieties [115-117]. Dithiocarbamate functionalized PPy has been used for designing metal sensors [117].

Overoxidized PPy films

It has been found that PPy can be overoxidized in the presence of nucleophiles when sufficiently positive potentials are applied to the polymer [118]. It is believed that attack of

the pyrrolic ring by hydroxide or water results in loss of conductivity and conversion of the polymer from an electronic/ionic conductor into a non-electronic but purely ionic conductor [118]. Characterization of the polymer films by Fourier transform IR [119] and x-ray photoelectron spectroscopy (XPS) [120] revealed that overoxidation results in addition of carbonyl and carboxylic groups into the polymer backbone. Therefore, overoxidized PPy films display cation permselectivity (uptake of cations and repulsion of anions) [120-127]. One fruitful application of these films has been the electrochemical determination of cationic neurotransmitters such as dopamine in physiological media in which the effect of interference anions such as ascorbate can be reduced dramatically [121-125]. The cation permselective and preconcentraing properties of the films have been found to increase with the increase of PPy film thickness [124]. In addition, during overoxidation, the counter ions are expelled from the films, resulting in porous structures in the films. The larger the counter ion used for PPy preparation, the higher the porosity of the films and therefore the higher the permeability. For example, improved cationic selectivity has been reported for films prepared with bulky counter anions [124,125]. An interesting study has been reported recently on molecularly imprinted PPy [127]. In this work, overoxidized PPy films templated with an L-amino acid have been utilized for potential-induced enantioselective uptake / release of targeted amino acids.

Conducting PPy composites or copolymers

The initial conducting polymers such as regular polypyrroles are generally insoluble, intractable and nonmelting (and thus not processible) and had relatively poor mechanical properties. However, in recent years, remarkable progress has been made in the direction of improving the mechanical and thermal properties as well as the processibility of these materials. As a result, a variety of composite films and membranes or copolymers of PPy with other materials have been synthesized [43,51,128,129]. Soluble PPy composites [128-130], PPy micro/nanotubules [21,47], PPy/proteins colloids [131] and PPy/hydrogels [132] are also prepared and they maintain the ion exchange property and electroactivity of PPy. In addition to the improvement in their physical properties, specific chemical interactions or functional groups including biomolecules can be incorporated into the composite materials

during their preparation processes. For example, to improve the efficiency of enzyme immobilization into PPy films, polyanions have been used to form polyanion-enzyme conjugates, which can then be incorporated into PPy as counter ions [133]. Soluble composites consisting of PPy-poly(vinyl alcohol) and PPy-poly(vinylpyrrolidone) have been prepared and their stabilities and anion uptake properties have been studied [130]. By incorporating L-aspartic acids into PPy-poly(vinyl alcohol) composite as a chiral selector, enantioselective uptake of amino acids can be achieved [130]. A chiral selective and electroconductive adsorbent has been developed for chromatographic separation of amino acids [134]. The adsorbent was prepared using a molecular imprinting technique by modifying the resin surface with PPy coating. The chiral selectivity of the adsorbent is based on ligand exchange of coordinated copper (II) complexes of D- or L-amino acids. An interesting research based on PPy microtubules has been conducted to develop on-line bioreactors that can encapsulate the derivatising reagents such as enzymes [21].

In recent years, interest in the application of conducting polymers (CPs) to membrane technology has grown; the main attraction being that the transport properties of the membranes can be dynamically and reversibly altered. Although stand-alone PPy membranes have been used for separation / transport of gas molecules [55,56], simple ions [57,58], and small organic molecules [59,60], their mechanical properties are not satisfactory. A more suitable approach is to coat the layers of CPs on to the conventional membranes as supports. If the membrane is first (sputter) coated with a layer of metal, e.g., Pt, then excellent electrical control can be achieved, allowing efficient electrocoating of the PPy and electrochemical control of the separations [135,136]. For example, using PPy coated, platinised poly(vinylidene) fluoride membranes, the transport properties of proteins across the composite membranes have been investigated and the selective separation of human serum albumin from a protein mixture has been achieved [135]. Highly selective membranes that involve immobilizing apoenzymes within a microporous composite have also been developed [136]. These composite membranes consist of a microporous polycarbonate filter that is sandwiched between two thin films of PPy. The apoenzyme is physically trapped within the pores of the membranes by the PPy films. Apoenzyme is also entrapped within the PPy films. This study shows that enantioselective membranes can be made for chiral separation and a fivefold difference between the transport rates of L- and D-amino acids has

been achieved. A similar sandwich membrane (PPY-nylon membrane-PPY) has been prepared by a chemical oxidation method and it has been used for electrokinetic separation of lysine and aspartic acid [137]. A further example of the possible applications of PPY composite membranes involves their use as an interface to a mass spectrometer [138].

1.4. Objectives of this work

The main objectives of this work are to prepare PPY films (or coatings) and to examine their potential applications for SPME. PPY films with different *N*-substituted functional groups and PPY films with different counter ions have been prepared by electrochemical and chemical polymerization methods (Chapter 2). The interactions between these films and target analytes and the properties of these films as SPME extracting phases have been investigated by coupling SPME with chromatographic methods.

In the first part of this research (Chapter 3 to Chapter 8), the inherent multifunctionalities of PPY have been characterized by studying the interactions between the polymer film and the selected analytes using an in-tube SPME method. A PPY coated capillary and several commercially available capillaries are used as the extracting phases. Compared with commercial SPME extracting phases, the PPY coated capillary has shown better extraction efficiency and selectivity toward aromatic, polar, basic compounds and anionic species, due to the anion exchange, acid-base, π - π and hydrophobic interactions, as well as the interactions through polar functional groups between PPY and analytes.

In Chapter 3, the anion exchange property of the polymer has been evaluated by its extraction ability towards inorganic anions. The π - π interaction, hydrophobic interaction and the interactions due to the polar functional groups between PPY and analytes have been studied (see Chapter 4) using three groups of aromatic compounds. In this chapter, the surface properties of PPY coatings, the effect of PPY coating thickness on extraction efficiency and selectivity, and the possible extraction mechanism have also been investigated. In Chapter 5, sample matrix and pH effects on the extraction efficiency of PPY coating are discussed using a group of aromatic amine compounds (stimulants). Due to the polar and weakly basic (pK_a 9-10) property of these compounds, it is difficult to extract them efficiently from aqueous solution without pH adjustment. Chapter 6 demonstrates the

application of PPY coated capillary in-tube SPME for speciation of organoarsenic compounds and further discusses the anion exchange property of PPY. Chapter 7 shows the effect of organic solvents such as ethanol in sample matrix on extraction and further demonstrates the high extraction efficiency of PPY coating for polar organic compounds such as pesticides. In Chapter 8, a simple and convenient approach to enhance the extraction efficiency of in-tube SPME has been demonstrated using PPY modified commercial capillary columns.

As described in the previous section, functionalized PPY films can be prepared using monomers with different functional groups. In Chapter 9, PPY and four of its *N*-substituted derivatives have been prepared on the surface of a metal (Pt, Au, or stainless steel) wire, respectively by electrochemical polymerization. The coated wires have been used with an SPME device coupled with GC/FID to examine their extraction properties to the volatile organic compounds. The results show that these coating have different selectivity toward the groups of compounds studied. For example, poly-*N*-phenylpyrrole (PPPY) has better selectivity to aromatic compounds than PPY, indicating that the selectivity of the film can be modified by introducing a new functional group (phenyl in PPPY) into the polymer.

The unique electroactivity and the reversible redox properties of PPY can be utilized to achieve electrochemically controlled SPME for charged species. This will be demonstrated in Chapter 10 by our preliminary results.

One of the advantages of using conducting polypyrroles for SPME is that functionalized PPY coatings can also be formed using different counter ions, and therefore the properties of the coatings for SPME can be modified accordingly. This will be described in Chapter 11.

Based on the results obtained in this research, some conclusions and recommendations have been given in Chapter 12.

The experimental results are in good agreement with both the theoretical expectations and the results obtained by other methods. This demonstrates not only that PPY films can be applied for SPME of a wide range of analytes but also that SPME may provide a useful method in studying materials like polypyrroles, in addition to its application as a sample preparation and introduction technique.

1.5. References

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CHAPTER 2

PREPARATION OF POLYPYRROLE FILMS

2.1. Introduction

Polypyrrole films can be prepared on various substrate materials with an oxidative reaction (see Figure 1-4) by chemical or electrochemical polymerization methods. An oxidizing reagent such as ferric chloride (FeCl_3) or ferric perchlorate [$\text{Fe}(\text{ClO}_4)_3$] is often used in chemical polymerization of pyrroles [1-3], and the Cl^- or ClO_4^- can therefore be incorporated into PPY films as the counter ion. In electropolymerization, PPY films are generally deposited onto a supporting electrode surface by anodic oxidation of the corresponding pyrrole monomer in the presence of an electrolyte solution (counter ion). Compared with the chemical method, electropolymerization is generally preferred because it provides a better control of film formation process (thickness and morphology) and enables small electrode (such as microelectrode) substrates to be coated [1-7]. In addition, cleaner and more uniform polymer coatings can be obtained because a chemical oxidizing reagent is not needed [7]. More importantly, *via* a electrochemical method, a variety of substituted pyrrole monomers can be polymerized and a wide range of counter ions can be incorporated into PPY films as described in the previous chapter, therefore, PPY coatings with different functionalities can be prepared on electrodes.

Different electrochemical techniques can be used for polymerization of pyrroles, including potentiostatic (constant-potential or controlled potential), galvanostatic (constant-current or controlled current) and potentiodynamic (potential scanning *i.e.* cyclic voltammetry) methods [1,2,4,7]. Using the potentiostatic method, the potential can be controlled to ensure that the integrity of the component to be incorporated (such as the counter ion or a functional group) can be accurately maintained during polymer film formation, which is important to prevent overoxidation of polymer films and decomposition of bioactive material. Using a galvanostatic technique ensures that the rate of polymerization is controlled and constant throughout the deposition process. Because of these features, these two techniques are more commonly used than the potentiodynamic technique in PPY film

preparation. The latter method is mainly used to obtain qualitative information about the redox processes involved in the polymerization reactions, and to examine the electrochemical behavior of the polymer film after electrodeposition.

The choice of solvent and electrolyte is of particular importance in the electrochemical method since both solvent and electrolyte should be stable at the oxidation potential of the monomer and provide an ionically conductive medium [7]. Acetonitrile is the commonly used organic solvent since it has a large potential window and allows a good dissociation of the electrolyte and thus a good ionic conductivity [7]. Since pyrrole has a relatively good solubility in water and a low oxidation potential [1,2], electropolymerization of pyrrole can also be carried out in aqueous electrolytes, which provides a great opportunity to incorporate a wider range of counter ions into PPY films.

Over the past twenty years, polypyrrole has appeared as the most extensively studied conducting polymer. However, despite the volume of work already done in this area, there has been little progress made on the mechanism of polypyrrole synthesis, especially concerning the most efficient method, electropolymerization. Although several mechanisms have been proposed [7], the mechanism proposed by Diaz and co-workers [8] is the one most commonly referred to in the literature. According to this mechanism, the polymerization proceeds via a radical cation coupling, involving the release of two electrons and two protons per monomer. The resulting polymer mainly contains structural units of 2,5-linkage. The polymer is formed in its oxidized form, which has high electroactivity and conductivity, carrying approximately one positive charge in every three to four pyrrole rings which is counter-balanced by an anion (counter ion) from electrolyte (Figure 1-4). The polymer films can be electrochemically cycled between the oxidized, conducting state and the neutral, insulating state due to their reversible redox properties (Figure 1-3).

2.2. Chemicals and reagents

Pyrrole (98 %), *N*-methylpyrrole (99%) and *N*-(2-cyanoethyl)pyrrole (99%) were obtained from Aldrich (ON, Canada) and distilled before use. *N*-phenylpyrrole (99 %) (Aldrich) and tetrabutylammonium perchlorate (TBAP) (electrochemical grade, Fluka, Buchs, Switzerland) were used as received. *N*-(2-carboxyethyl)pyrrole was prepared by Dr.

Deng using a literature method [9]. Lithium perchlorate (LiClO_4), sodium *p*-toluenesulphonate (TS), sodium dodecylsulphate (DS), poly(sodium 4-styrenesulfonate) (PSS, 100 % sulfonated, MW 70000) were obtained from Aldrich and used as received. Ferric perchlorate ($\text{Fe}(\text{ClO}_4)_3 \cdot 6\text{H}_2\text{O}$) and perchloric acid (70%) were purchased from BDH (Toronto, ON Canada). All other chemicals were obtained from Aldrich and used as received. All the chemicals used in this study were of analytical-reagent grade or HPLC grade. Deionized water was obtained from a Barnstead/Thermodyne NANO-pure ultrapure water system (Dubuque, IA, USA).

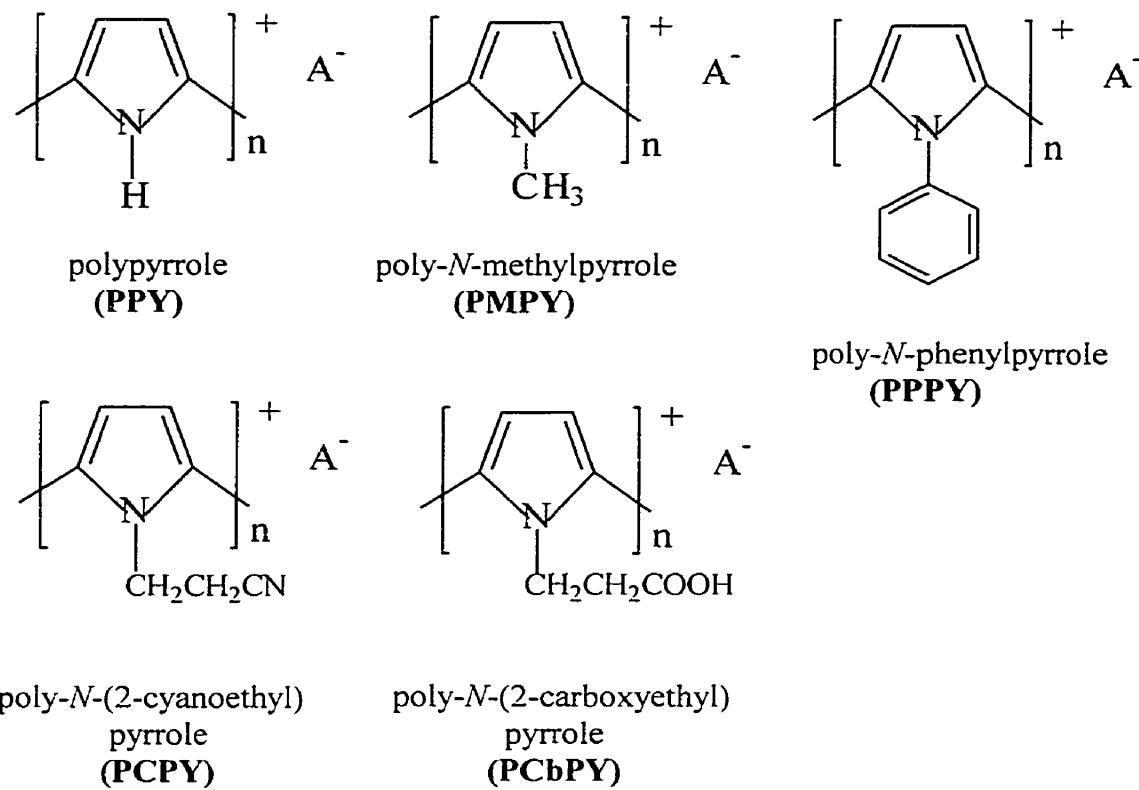


Figure 2-1. Structures of polypyrrole and four of its *N*-substituted derivatives (A^- is the counter ion, ClO_4^-).

2.3. Electrochemical deposition of PPY films

Electropolymerization was performed using a three-electrode system [10]. A metal (Pt, Au, or stainless steel) wire was used as a working electrode and a platinum wire wound into cylindrical shape was utilised as a counter electrode. An Ag/AgCl electrode or a saturated calomel electrode (SCE) was employed as a reference electrode. Polypyrrole (PPY), or one of its *N*-substituted derivatives as shown in Figure 2-1, was directly deposited onto the surface of a working electrode from a solution of 0.1 M electrolyte and 0.1 M corresponding monomer in acetonitrile or water (see Table 2-1). A constant potential deposition was carried out using a potentiostat (model RDE2, Pine Instrument Company) or a potentiostat/galvanostat (Model 273, EG&G Princeton Applied Research). The electrolyte solutions were degassed by bubbling nitrogen through them for 20 min prior to electrodeposition. The working electrode was polished with 400-grit Sic sandpaper, cleaned with pure water and acetone, and then electrolyzed at a potential +0.5 V for 5 min to remove any impurity. An anodic potential (see Table 2-1) was then applied to the working electrode for polymerization. The metal wires coated with PPY films were cleaned with methanol and dried under N₂. The polymerization conditions are listed in Table 2-1.

Table 2-1. Polymerization conditions and polymer density (g/cm³)

Polymer	Monomer	Solvent	Electrolyte	E _{applied} (V)	Density*
PPY-ClO ₄	Pyrrole	Acetonitrile	TBAP	0.7 (1.0)*	1.48
PMPY-ClO ₄	N-methylpyrrole	Acetonitrile	TBAP	0.8 (1.0)*	1.46
PCPY-ClO ₄	N-(2-cyanoethyl)pyrrole	Acetonitrile	TBAP	1.0 (1.2)*	1.40
PCbPY-ClO ₄	N-(2-carboxyethyl)pyrrole	Acetonitrile	TBAP	1.0 (1.2)*	1.40
PPPY-ClO ₄	N-phenylpyrrole	Acetonitrile	TBAP	1.0 (1.2)*	1.42
PPY-Cl	Pyrrole	Water	NaCl	0.7 ~ 0.8	
PPY-ClO ₄	Pyrrole	Water	LiClO ₄	0.7 ~ 0.8	1.51
PPY-TS	Pyrrole	Water	TS	0.7 ~ 0.8	1.37
PPY-DS	Pyrrole	Water	DS	0.7 ~ 0.8	
PPY-PSS	Pyrrole	Water	PPS	0.8 ~ 0.9	

* Values for E_{applied} (in parentheses) and density are taken from references [1,11].

2.4. Preparation of PPy coated capillary by chemical polymerization

PPY film was coated on the inner surface of a fused silica capillary (60-cm long, 0.25-mm i.d.) by chemical polymerization. The capillary was cleaned with acetone and dried with N₂ before it was coated. As shown in Figure 2-2, the PPy coating was prepared by first passing the monomer solution (pyrrole in isopropanol, 50% v/v) through the capillary with the aid of N₂, to form a thin layer of monomer on the capillary inner surface, and then allowing oxidant solution (0.2 M ferric perchlorate in 0.4 M perchloric acid) to flow through the capillary in the same way as for the monomer. The polymer was formed by oxidative reactions when the oxidant reagent reached the monomer in the capillary. The above procedure was referred to as one PPy coating cycle, which can be repeated several times (1 to 4 times in our case) to increase the thickness of PPy film. During polymerization, the color of the capillary changed gradually from yellow to black, indicating the formation of PPy film on the inner surface of the capillary. The PPy coated capillary was then washed with methanol and dried by purging with N₂. Finally, it was coupled to HPLC and conditioned with a mobile phase before use.

Different silica capillaries were tested for the possibility of making PPy film on the inner surface, including untreated silica tubing and treated GC precolumns (polar, intermediate, and nonpolar). Although PPy film can be easily formed by chemical polymerization, the film can only be coated firmly on the inner surface of polar treated GC capillary. There may be certain chemical interactions between the polar surface (poly(ethylene glycol)) and PPy film because it was found recently that PPy could be coated much more easily on the inner surface of Omegawax or Supelcowax capillary columns which have the same polar inner phase as the polar treated precolumn.

Other monomers such as *N*-methylpyrrole (99%) and *N*-phenylpyrrole were also tried using the above method. *N*-methylpyrrole could be polymerized on the inner surface of a capillary as easily as pyrrole. However, polymer film could not be formed on the inner surface of a capillary using *N*-phenylpyrrole, probably due to the higher oxidation potential (1.2 V) required for polymerization of this monomer or low polymerization rate as compared to that of pyrrole.

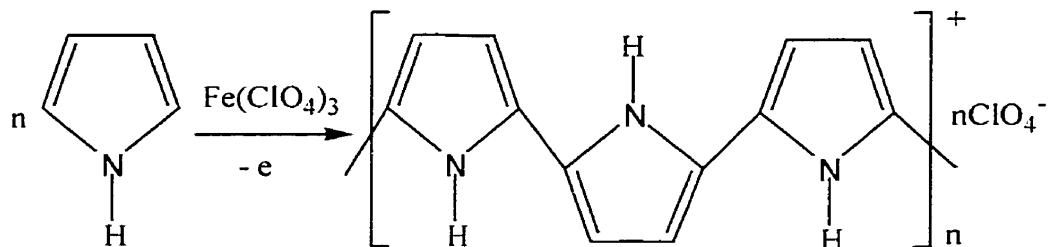
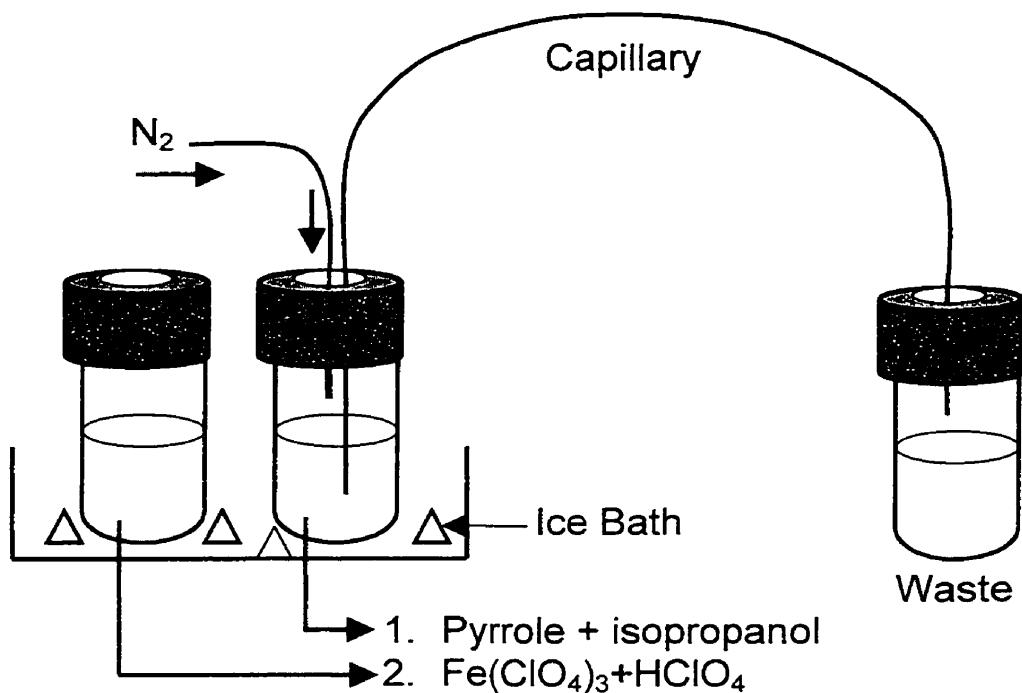


Figure 2-2. Schematic illustration of chemical polymerization of pyrrole on the inner surface of a capillary.

2.5. Scanning electron microscopy of the polymer surfaces

Each of the PPy coated metal wires or capillaries was cut into a 1-cm long piece, coated with gold film and then analyzed using a HITACHI S-570 scanning electron microscope (15 kV accelerating potential) or a LEO 1530 electron microscope. The results are discussed in the following chapters.

2.6. References

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CHAPTER 3

SPME OF INORGANIC ANIONS

3.1 Introduction

To date, commercial SPME fibers have been designed to extract only compounds that do not carry a charge. For direct SPME of ionic species (*i.e.* without derivatization or conversion of the ionic analytes to non-ionic forms), only a few reports have been published [1-3]. In all of these studies, custom-made ionic coatings were used rather than commercial fibers. One of particular interest reports extraction of arsenate using conducting polymer polythiophene with controlled electrochemical potential [3]. Ion chromatography (IC) is an ideal and powerful tool for separation and detection of ionic analytes. However, no research on coupling SPME to IC has been reported. This is likely due to the lack of suitable stationary phases for SPME of ionic compounds or the difficulty in preparing such phases as films or coatings on certain inert substrates (such as fused silica fiber or capillary) for SPME.

In this work, the inherent anion exchange property of conducting polypyrrole (PPY) (Figure 3-1) has been examined by both fiber SPME and in-tube SPME methods (see Experimental). The preliminary results demonstrate that PPY coated capillary can be utilized for direct in-tube SPME of anionic species from aqueous solutions without derivatization. This in-tube SPME process can be easily automated using an autosampler. A fully automated process including SPME, ion chromatographic separation (IC) and conductivity detection (CD) can be realized by coupling in-tube SPME on line with IC/CD. This work presents the first example of coupling SPME to ion chromatography (IC).

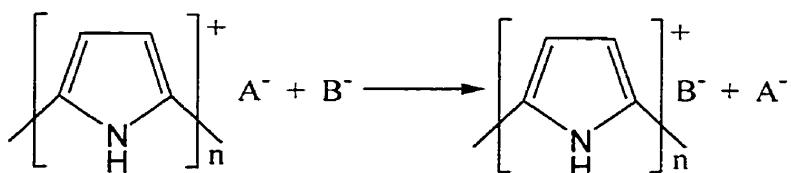


Figure 3-1. Schematic illustration of anion exchange process.

3.2 Experimental

3.2.1 Chemicals and reagents

Sodium selenite (Na_2SeO_3) was obtained from Aldrich (ON, Canada). Sodium selenate (Na_2SeO_4) and arsenic acid (potassium salt, KH_2AsO_4) were purchased from Sigma (ON, Canada). An ion chromatographic (IC) standard solution containing fluoride (100 ppm), chloride (200 ppm), bromide (500 ppm), nitrate (500 ppm), phosphate (1000 ppm), and sulfate (1000 ppm) was obtained from the Center for Groundwater Research (Waterloo, ON Canada). Standard solutions (100 ppm) for selenite, selenate and arsenate were prepared with deionized water. IC standards for these ions (containing 10 ppm for each ion) were prepared by mixing the above individual standard solutions with deionized water. All solutions were serially diluted with deionized water for analysis. Tap water was analyzed directly without any treatment. The chemicals used in this study were of analytical-reagent grade. The following commercial fibers and capillaries from Supelco (Bellefonte, PA, USA) were examined for their extraction ability toward inorganic anions: Fibers, polydimethylsiloxane/divinylbenzene (PDMS/DVB), polyacrylate, carbowax/templated resin (CW/TPR). Capillaries, polar silica tubing (0.25-mm i.d.) which was also used as the host capillary for preparing PPy coated capillary, and Omegawax 250 (0.25- μm film thickness, 0.25-mm i.d.). The PPy coated Pt wire and PPy coated capillary were prepared according to the method described in chapter 2.

3.2.2. Fiber SPME and in-tube SPME

The methods of coupling fiber SPME and in-tube SPME to IC (or HPLC) are described below. In order to achieve effective extraction, separation and detection of anionic species, a PPy coated anionic exchange fiber (metal wire) or capillary and the IC/CD system were used. Although commercial fibers or capillaries were also evaluated initially, they did not show good extraction ability for the ions studied, thus no further studies on them were carried out.

Fiber SPME coupled to IC

A modified syringe assembly has been used as an SPME device as shown in Figure 3-2 (A), the metal wire (Pt, Au, or stainless steel) coated with PPy film at one end is inserted

into the needle of a Hamilton syringe and attached to the plunger. The plunger moves the metal wire in and out of the needle to protect the coating. As shown in Figure 3-2 (B), the commercial SPME device and a used fiber assembly from Supelco (Bellefonte, PA, USA) can also be utilized by replacing the fiber with a PPY-coated metal wire.

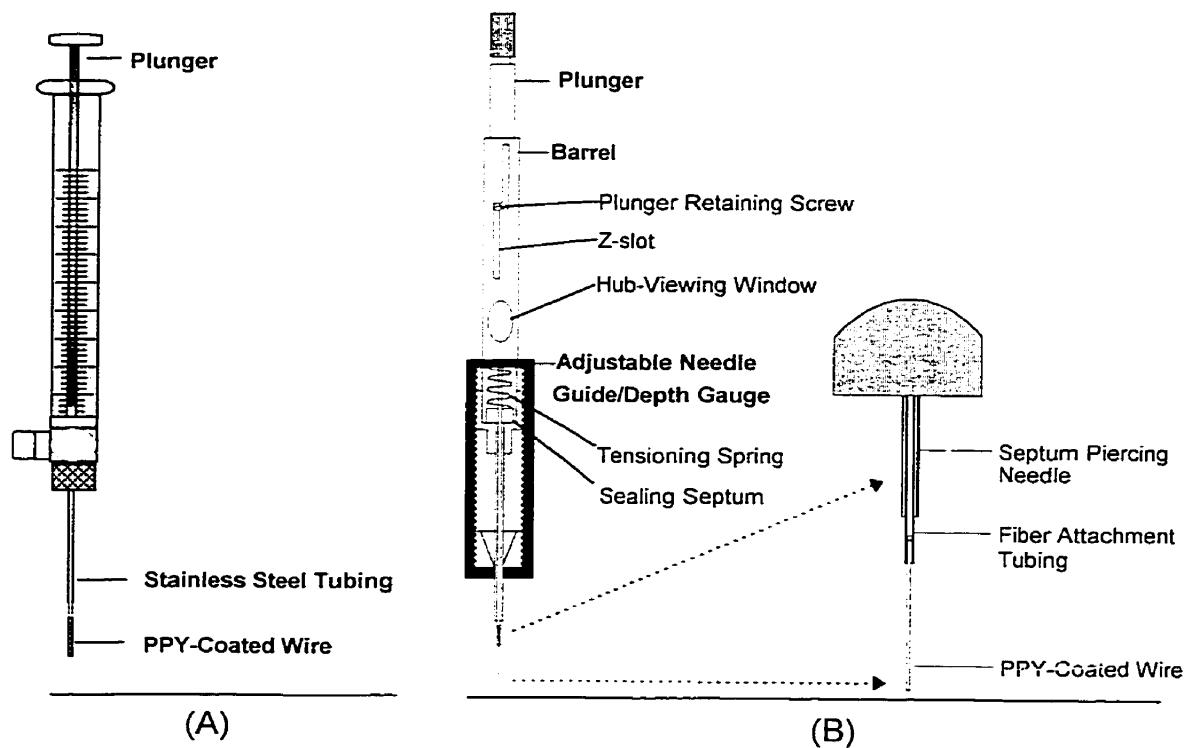


Figure 3-2. Fiber SPME devices. (A) A modified syringe assembly; (B) A modified commercial SPME device.

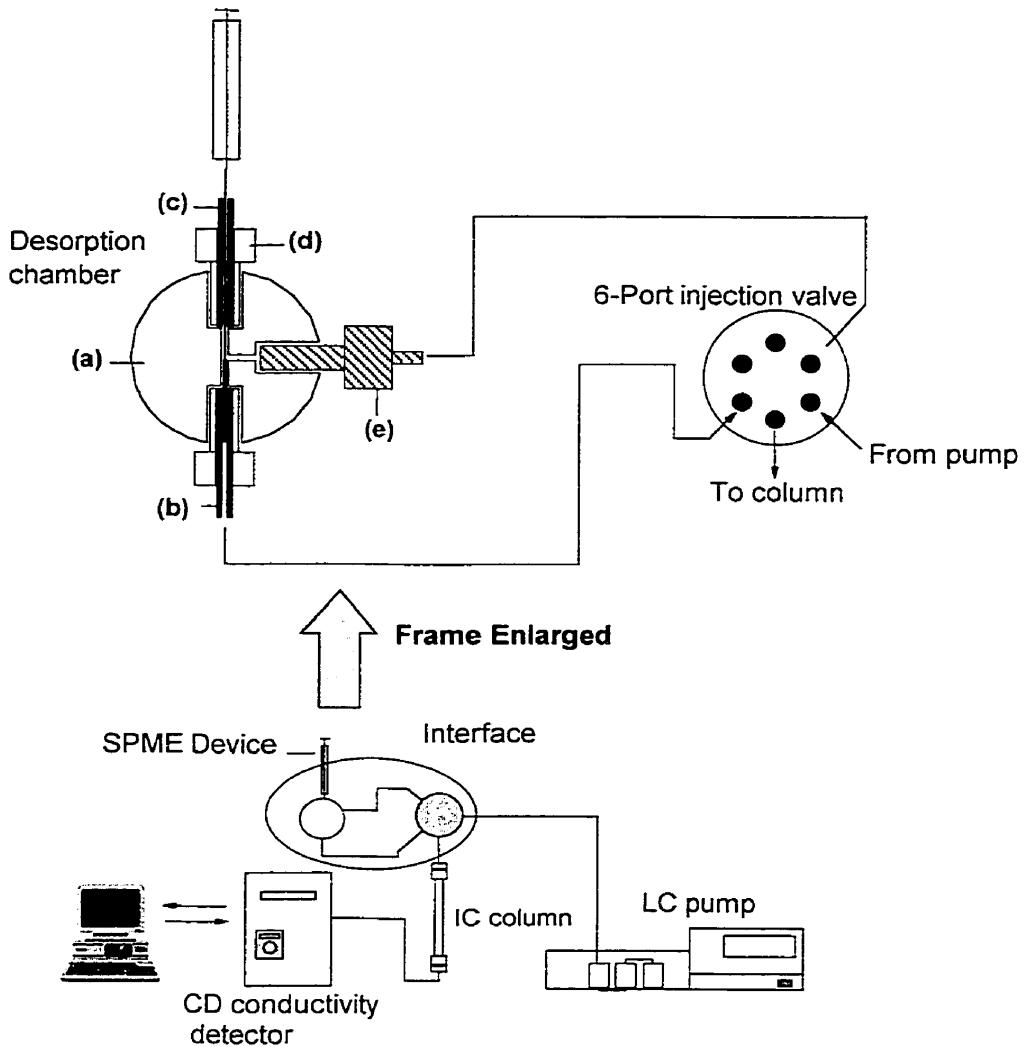


Figure 3-3. SPME -IC system: (a) stainless steel (SS) 1/16-in. tee joint (Valco, Houston, TX); (b) 1/16 in. SS tubing (Supelco, Bellefonte, PA); (c) 1/16 in. poly (ether ether ketone) (PEEK) tubing (0.02 in. I.D.); (d) two-piece finger-tight PEEK union; (e) PEEK tubing (0.005 in I.D.) with a one-piece PEEK union. All PEEK products were obtained from Sigma-Aldrich (Milwaukee, WI). The upper portion of PEEK tubing in part c was enlarged to fit the needle of the syringe. The SS rod in the SPME device can be tightly sealed by the PEEK tubing and union, withstanding solvent pressures as high as 4500 psi. The desorption chamber is placed in the position where the injection loop normally resides on the Rheodyne valve. When the valve is at “load” position, the desorption chamber is at ambient pressure so that the fiber can be placed into the chamber.

For fiber SPME, a PPY coated metal wire was first conditioned in mobile phase solution for 30 min. It was then exposed to a stirred water sample spiked with analytes for 30 min. To transfer the ionic analytes from the extracting phase to IC system, an SPME-LC interface was used [4]. This interface included a desorption chamber which was connected to a six-port Rheodyne 7161 injection valve. The construction of the SPME-IC system is shown in detail in Figure 3-3. Before transferring the PPY coated wire into desorption chamber, the injection valve was turned to LOAD position. The coated metal wire was then introduced into desorption chamber by pushing the syringe plunger. The two-piece PEEK union was closed tightly. The valve was then switched to the INJECT position, and analytes extracted were desorbed by mobile phase flow and carried to the IC column.

In-tube SPME coupled to IC

For automated in-tube SPME, a 60 cm section of PPY coated capillary was mounted in the Famos autosampler (LC packings, Amsterdam, The Netherlands) replacing the non-coated capillary, which is usually installed there for transferring sample to the HPLC loop for direct injection. The total internal volume of the capillary was about 29 μL . The autosampler software was programmed to control the extraction and desorption processes of the in-tube SPME. The detailed instrumental setup is illustrated in Figure 3-4 and in reference [5]. After conditioning the capillary with mobile phase for 30 min, a sample volume of 25 μL (total volume of the syringe) was aspirated from a sample vial at a flow rate of 63 $\mu\text{L}/\text{min}$. Then the same sample volume was dispensed back into the same vial. These two steps were repeated several times (usually 10 times, but this can be optimized experimentally) to achieve good extraction efficiency. After the extraction step, the six-port valve was set to the LOAD position. A 38- μL of mobile phase was aspirated from a solvent vial and transferred to the injection loop (40 μL , made of a 56-cm long PEEK tubing with 300 μm i.d.) for desorption of the extracted analytes from the capillary coating. The six-port valve was then switched to the INJECT position, and the sample was transferred from the loop to the IC column by the mobile phase flow.

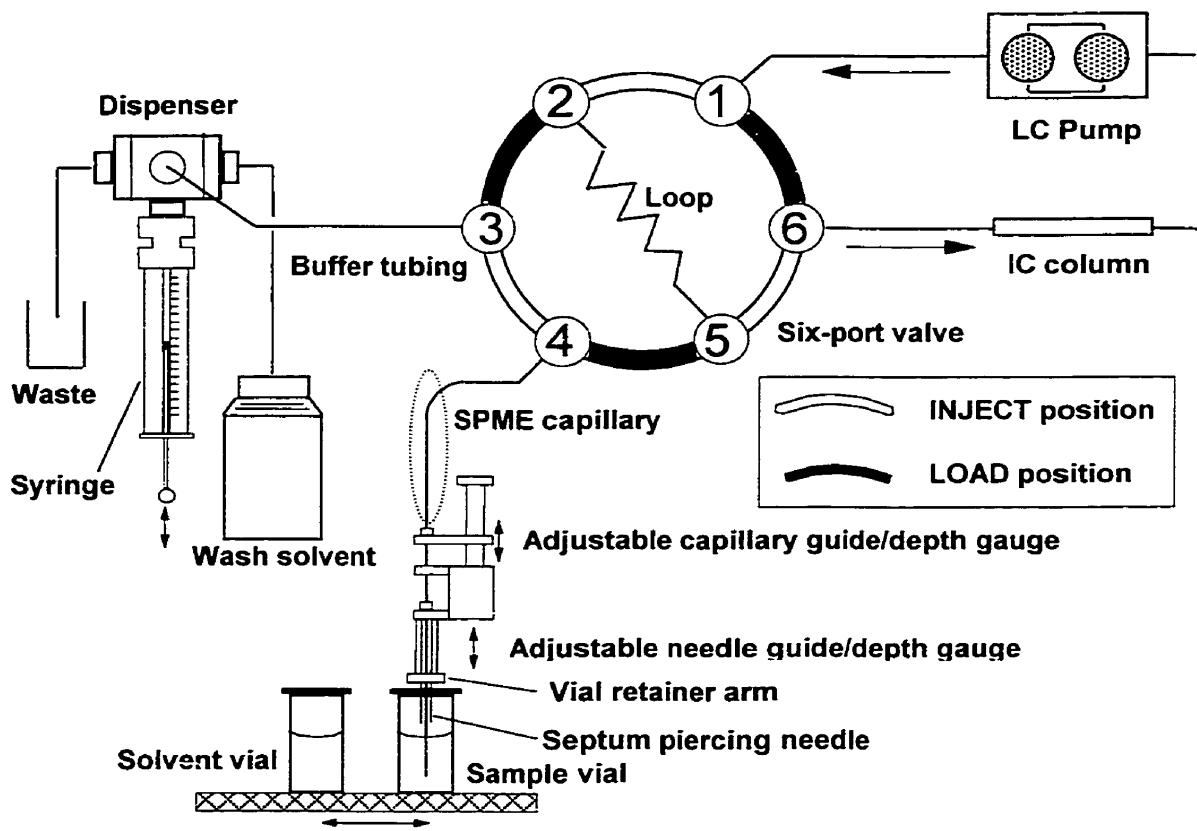


Figure 3-4. Instrument setup of the automated in-tube SPME-IC system: A piece of PPY coated capillary (in-tube SPME device) resides in the position of former needle capillary. The aqueous sample is repeatedly aspirated from the sample vial through the SPME capillary and dispensed back to the same sample vial (INJECT position) by movement of the syringe. After the extraction step, the six-port valve is switched to the LOAD position for desorption of the analytes from the SPME extracting phase by flushing a solvent (the mobile phase in this work) from a solvent vial through the SPME capillary. The desorbed sample solution is then transferred to the injection loop. After switching the valve to the INJECT position, a mobile phase flow carries the analytes to the IC column where separation is performed.

When an autosampler is not available, an off-line manual in-tube SPME process can be performed. First, the capillary was conditioned for 30 min by passing the mobile phase solution through the capillary with the aid of N₂. Then, a sample solution was allowed to pass through the capillary for extraction by the same method as the above. This extraction step was repeated several times to achieve good extraction efficiency. The sample solution was pushed out of the capillary at end of the last extraction step with N₂. After extraction, the six-port injection valve was turned to the LOAD position, and the capillary was installed in the position of the sample loop. Connection of the capillary to the injection valve was facilitated by the use of 2.5-cm sleeves of 1/16-inch polyetheretherketone (PEEK, 330 µm in i.d.) tubing, a stainless steel HPLC nut and a ferrule at each end of the capillary. Finally, turning the valve to the INJECT position, the sample was desorbed and carried to the column by mobile phase flow.

3.2.3. Separation and detection

A Dionex CD20 conductivity detector was used in combination with an Anion Self-Regenerating Suppressor (ASRS-ULTRA4-mm) for suppressed conductivity detection in AutoSuppression external water mode. Two Waters Model 510 LC pumps were used, one for delivering mobile phase flow and the other for supplying external water flow for the Suppressor. A Dionex anion exchange column, IonPac® AS14 (4 × 250 mm), was used for separation of all the anions studied. An aqueous solution containing 3.5 mM Na₂CO₃/1.0 mM NaHCO₃ was used as mobile phase. Flow rate was 1.0 mL/min. Chromatograms and data were acquired with a Varian Star system.

3.3. Results and discussion

3.3.1 Comparison of different SPME methods

The conventional fiber SPME method provides the advantages of simplicity, flexibility and portability, which is particularly suitable for field sampling. One advantage of using PPY film to extract anions is that problems of analyte loss by desorption prior to analysis are eliminated, because the extraction is based on ion exchange rather than adsorption or absorption and the analytes are non-volatile ions. Therefore, extra devices and

materials to store SPME fibers and protect from sample loss during storage and transportation are not required. However, our results showed that the capacity of the film prepared as described for fiber SPME was not large enough to achieve sufficient extraction. Only a small surface (1 cm at the tip) of a thin metal wire was coated with polymer film. The currently used fiber SPME assembly and interface do not allow the use of longer fibers and thick film. In addition, the film was easily damaged (stripped), when it was drawn back into the needle from solution after extraction and when it was inserted into the desorption chamber for desorption, probably due to polymer swelling. This also resulted in poor reproducibility. To solve these problems in the future, the currently used SPME device and interface should be modified.

One advantage of using an open tubular capillary for SPME (in-tube SPME) instead of the conventional SPME fiber is that high extraction efficiency can be easily achieved by using a longer capillary coated inside with a suitable stationary phase (PPY in this study). Another advantage of in-tube SPME is that it allows convenient automation of the extraction process, which not only reduces the total analysis time and labor required, but also provides better precision relative to manual techniques. However, an autosampler is required in order to realize automation. When the autosampler is not available, in-tube SPME can still be carried out by using an off-line manual method described in detail in the experimental section. Although it is possible to obtain reproducible results by precisely controlling the parameters in extraction processes (such as sample volume, sample flow rate, extraction times and etc.), the manual off-line method is time and labor consuming. Therefore, the following discussion will focus only on the results obtained using automated in-tube SPME method.

3.3.2. Automated in-tube SPME of inorganic anions

Two groups of inorganic anions were used to examine the extraction ability of PPY-coated capillary toward anionic species by coupling the automated in-tube SPME with ion chromatography (IC). The peak areas for each ion obtained by standard liquid injection and SPME methods were compared in Table 2-1. The high extraction efficiency of PPY coated capillary for anionic species was demonstrated by the much larger area counts obtained by SPME compared with those obtained by standard method. A typical chromatogram of an

inorganic anion standard obtained by this coupled in-tube SPME/IC method is shown in Figure 3-5 (A). One example of using this method for the analysis of water sample is shown in Figure 3-5 (B). The estimated concentrations for chloride and sulfate in tap water were at ppm level.

Although the natural ion exchange property of PPy can be utilized for SPME of anions as shown in this paper, its capacity is limited because there is only one positive charge per three or four pyrrole units and this property will disappear when PPy is reduced [6]. The ion exchange property can be markedly improved by incorporating cationic substituents to the polypyrrole skeleton. Films with permanent anion exchange capacity have been synthesised by using pyrrole monomers substituted with alkylammonium or pyridinium groups, and their enhanced anion exchange properties have been exploited to bind a variety of organic and inorganic anions [6-9].

3.4. Summary

The ability and potential application of PPy coated capillary to extract anionic species from aqueous solutions have been demonstrated, which indicates the importance of developing new coating materials for SPME to extend its application range. This study also demonstrates that SPME can be a useful method to examine the properties of polymers, such as the ion exchange property of PPy. Since SPME can be easily coupled with chromatographic methods, this coupled technique provides the advantage of examining the selectivity of the polymer to different compounds simultaneously.

Although this work is focused on SPME of anions, it is important to point out that functionalized polypyroles that have permanent cation exchange properties, such as poly(3-methylpyrrole-4-carboxylic acid) [10,11] and poly(4-(pyrrol-1-ylmethyl)benzoic acid) [9], have been synthesised and studied. Composite or copolymer films that possess the features of both conducting polymer and cationic exchanger have also been studied, including polypyrrole-polyestersulfonic acid, polypyrrole-polyvinyl sulfate, poly-*N*-methylpyrrole-polystyrene sulfonate, and polypyrrole-polyacrylic acid [6-9]. These novel materials might have the potential to be used as coatings for SPME of cationic species.

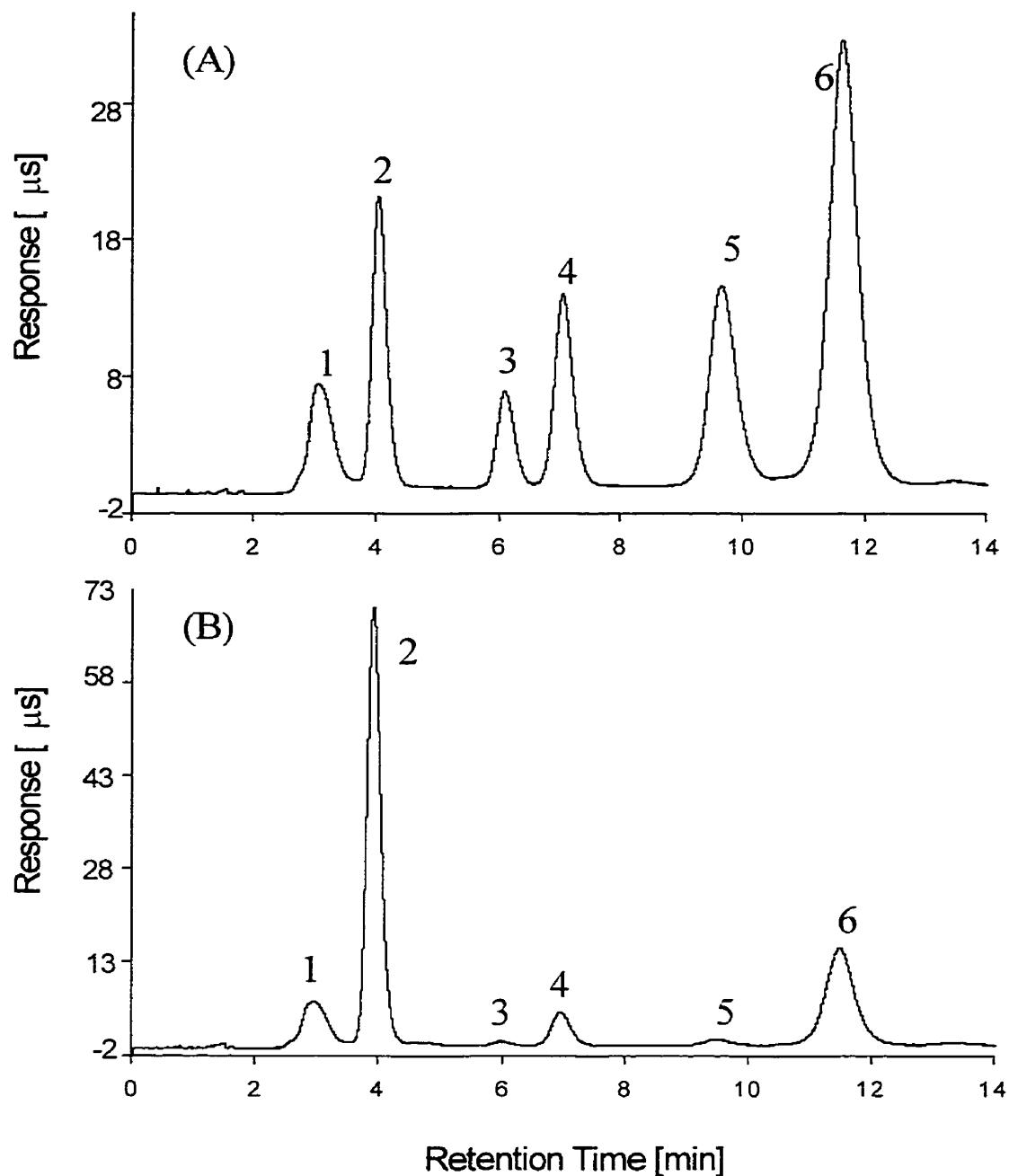


Figure 3-5. Chromatograms of (A) an anion standard mixture and (B) tap water sample obtained by PPy coated capillary in-tube SPME method. Peak identification and concentration for (A): 1. fluoride (1 ppm), 2. chloride (2 ppm), 3. bromide (5 ppm), 4. nitrate (5 ppm), 5. phosphate (10 ppm), and 6. sulfate (10 ppm). Peak labels for (B) are the same as those in (A). Conditions: see experimental section.

Table 3-1. Comparison of the responses obtained by standard injection and PPY coated capillary in-tube SPME of anionic species

	Peak area (counts)						Anion mixture (2) ^b		
	Anion mixture (1) ^a			Anion mixture (1)			Anion mixture (2)		
	fluoride	chloride	bromide	nitrate	phosphate	sulfate	selenite	selenate	arsenate
Standard injection (25 µL)	7864	13226	13183	16250	16754	46566	3559	8802	12422
PPY In-tube SPME	120004	211809	84592	189453	290398	667326	33353	80863	105386
Response increase (fold) ^c	15	16	6	12	17	14	9	9	8

^a Anion mixture (1) contains: 1 ppm fluoride, 2 ppm chloride, 5 ppm bromide, 5 ppm nitrate, 10 ppm phosphate, and 10 ppm sulfate.

^b Anion mixture (2) contains: 1 ppm of each anion.

^c Response (area counts) increase (fold) was calculated by comparing the result of in-tube SPME with that of standard injection method. For example, the response (area counts) increase = 120004/7864 = 15 for fluoride.

3.5. References

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CHAPTER 4

IN-TUBE SPME OF AROMATIC COMPOUNDS

4.1. Introduction

The analysis of both polar and non-polar aromatic compounds in aqueous samples has become an important topic due to the ever increasing environmental and health concerns as a result of the carcinogenic and mutagenic properties of these compounds. To determine these compounds at a low concentration level, it is important to use suitable sample preparation methods for the extraction and concentration of trace analytes from water samples. On-line techniques are often preferred to achieve fast analysis and automation. The traditional techniques used for extraction and concentration of aromatic compounds from water samples are solvent extraction and solid phase extraction [1,2]. These methods require large volumes of toxic organic solvents, and are often time-consuming and labor-intensive since they are mainly off-line manual techniques. Solid-phase microextraction (SPME), which has obtained widespread acceptance in many areas recently (see chapter 1), can overcome the problems of traditional methods by eliminating the use of organic solvents and by integrating sample extraction, concentration and introduction into a single step. This technology is more rapid and less expensive than the traditional methods, and it can be easily automated. In-tube SPME is a relatively new version of SPME, which can be easily coupled on-line with HPLC for the analysis of less volatile and /or thermally labile compounds [3,4]. As described in chapter 3, in-tube SPME uses a coated open tubular capillary as an extraction device instead of the conventional SPME fiber, which allows for convenient automation of the extraction process. Automated in-tube SPME not only saves analysis time but also provides better precision relative to manual techniques. However, one of the main difficulties limiting the wide application of SPME-LC is the absence of suitable SPME stationary phases, that not only have high extraction ability for the analytes, but are also stable in solutions of various matrices. In the development of the SPME technique, it has been a challenge to extract polar and /or ionic analytes from water samples because of the less polar properties of the commercial SPME coatings and the stronger interactions between water and polar analytes. A solution to improve the extraction ability for these analytes is to convert them to less polar,

nonionized forms by pH adjustment or derivatizations [5,6]. However, derivatizations are often complicated processes that require a great deal of time and reagent. Perhaps the best solution is to develop polar and ion exchange coatings for direct extraction of the target species from sample matrices. One of our objectives is to prepare such new coatings that can be used for both polar and non-polar compounds.

In this study, in-tube SPME based on a PPy coated capillary was investigated for the extraction of aromatic compounds from aqueous solutions. The PPy coated capillary was coupled on-line to HPLC to achieve automated in-tube SPME and HPLC analysis. Three groups of aromatic compounds were examined, which included a group of model compounds containing both polar and non-polar aromatics, a group of 16 polycyclic aromatic hydrocarbons (PAHs) and a group of 6 heterocyclic amines. The results demonstrated that PPy coating had higher extraction efficiency than the currently used commercial capillary coatings, especially for polycyclic aromatic compounds and polar aromatics due to the π - π interactions, interactions from polar functional groups, and hydrophobic interactions between polymer and analytes. In addition, the porous surface structure of the PPy coating provided a high surface area allowing for improved extraction efficiency. It was found that, under the same extraction conditions, the extraction efficiency and selectivity could be greatly enhanced by using a thicker coating. The preliminary study on extraction mechanism indicated that analytes were extracted onto PPy coating mainly by an adsorption mechanism. The method was applied for the extraction and analysis of both polar and non-polar aromatics in water samples.

4.2. Experimental

4.2.1. Chemicals and reagents.

Benzene and phenol were purchased from BDH (Toronto, ON, Canada). Toluene, dimethyl phthalate (DMP) and diethyl phthalate (DEP) were obtained from Aldrich (ON, Canada). Naphthalene was obtained from Supelco (Bellefonte, PA USA). A standard solution (1 mg/ml) for each model aromatic compound (benzene, toluene, naphthalene, phenol, dimethyl phthalate (DMP) and diethyl phthalate (DEP)) was prepared in methanol. A mixture of these model compounds was prepared in acetonitrile which contained 20 μ g/mL

of phenol, 50 µg/mL of benzene, 50 µg/mL of toluene, 10 µg/mL of DMP, 10 µg/mL of DEP and 10 µg/mL of naphthalene. Finally, the mixture was spiked to water for the extraction experiments. A PAHs standard containing 16 components (2000 µg/ml in CH₂Cl₂ / benzene (50/50)) was obtained from Supelco (Bellefonte, PA USA). The solution was first diluted to 20 µg/ml with a mixture of CH₂Cl₂ and benzene (50:50), then to 2 µg/ml with acetonitrile, and finally to the low concentration with water for analysis. The six aromatic amines (which were kindly provided by Dr. H. Kataoka, University of Okayama, Japan) are 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx), 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline (4,8-DiMeIQx), 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), 2-aminodipyrido[1,2-*a*:3'2'-*d*]imidazole (Glu-P-2), 3-Amino-1,4-dimethyl-5*H*-pyrido[3,4-*b*]indole (Trp-P-1) and 3-Amino-1-methyl-5*H*-pyrido[3,4-*b*]indole (Trp-P-2). Each amine was dissolved in methanol to make a stock solution at a concentration of 0.3 mg/mL, and used after dilution with water. Solvents used were of analytical-reagent or HPLC grade. Water was obtained from Barnstead/Thermodyne NANO-pure ultrapure water system (Dubuque, IA). The PPy coated capillaries were prepared according the method described in chapter 2.

4.2.2. Automated in-tube SPME

In-tube SPME and the technique of coupling automated in-tube SPME to LC were described in chapter 3. However, since a different autosampler was used in this work, the operation detail and coupling of in-tube SPME to HPLC were slightly different from that described previously. A schematic diagram of the in-tube SPME-HPLC system is illustrated in Figure 4-1. Briefly, a coated capillary (60 cm long) was used as the in-tube SPME device, and placed between the sample injection loop and injection needle of the autosampler. An autosampler was programmed to control the extraction, desorption and injection processes. Vials (2 mL) were filled with 1 mL of sample for extraction, and set into the autosampler. In the extraction process, a 40-µL sample was drawn from the sample vial into the capillary at a flow rate of 100 µL/min. The same volume of sample was then ejected back into the same sample vial. These two steps together are referred to as one draw/eject cycle, which can be repeated through programming of the autosampler software. In this work, the extraction of analytes onto the capillary coating was performed by 15 repeated draw/eject cycles of sample

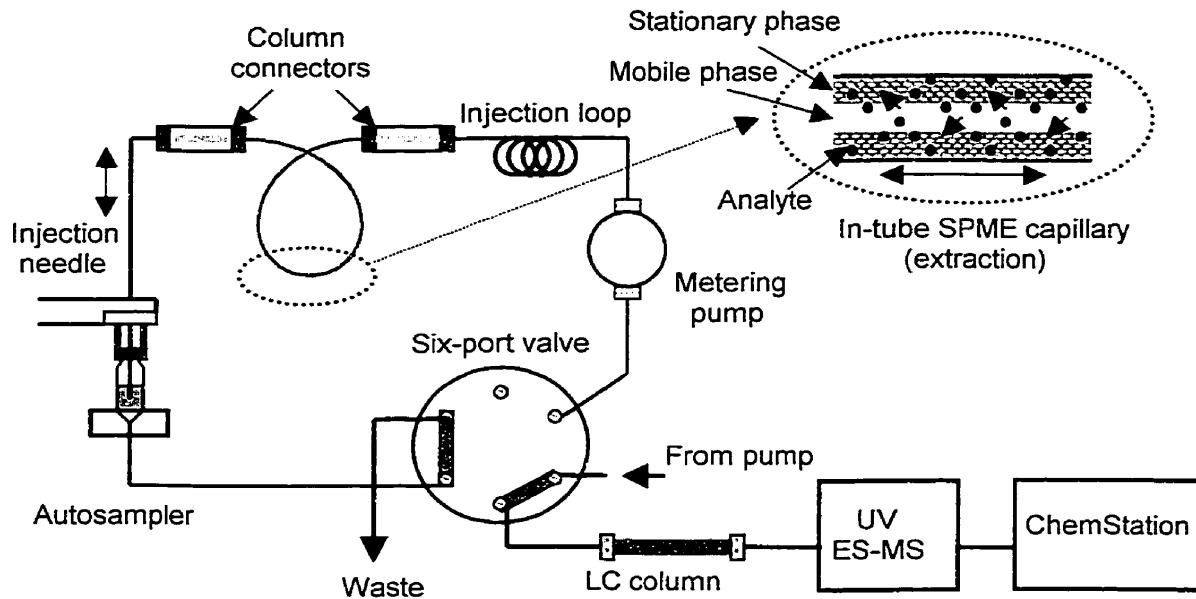
at a flow rate of 100 $\mu\text{L}/\text{min}$ with the six-port valve in the LOAD position. Since the total internal volume of each capillary was around 30 μL and the volume of the injection needle was 10 μL , the optimal sample volume for each draw/eject step was 40 μL to fill the coated capillary. In addition, 1.5 mL each of methanol and pure water in 2-mL vials were set on the autosampler for SPME capillary washing and conditioning. They were carried out by 2 repeated draw/eject cycles of these solvents prior to extraction (for each draw/eject cycle, 40 μL solvent was drawn into the capillary and then the 40 μL solution was ejected back to the same solvent vial). Most of the extracted analytes could be desorbed from the capillary coating with mobile phase flow by simply switching the six-port valve to the INJECT position. However, some analytes, such as the aromatic amines studied in this work, had stronger interactions with the extraction phase and thus could not be desorbed easily by mobile phase. Therefore, a 40 μL methanol was drawn into the capillary to assist desorption of these analytes before switching the valve to the INJECT position.

In order to compare the extraction efficiencies of different capillary stationary phases, a PPY coated capillary and the following commercial capillaries (from Supelco, Bellefonte, PA) were examined under the same conditions: Omegawax 250 (0.25 μm film thickness, 0.25 mm i.d.), SPB-1 (0.25 μm film thickness, 0.25 mm i.d.), SPB-5 (0.25 μm film thickness, 0.25 mm i.d.), and a retention gap capillary (a polar silica tubing, 0.25 mm i.d., which was also used as host capillary to make PPY coated capillary). Figure 4-2 shows the structures of the capillary stationary phases and some of the compounds studied.

4.2.3. Separation and Detection

The HPLC system used was a Model 1100 series LC consisting of a binary pump, an on-line vacuum degasser, an autosampler, a thermostated column compartment, a variable-wavelength UV detector and an atmospheric pressure (AP) – electrospray (ES) mass spectrometer (Agilent Technologies, Palo Alto, CA, USA).

(A) Extraction (LOAD position)



(B) Desorption (INJECT position)

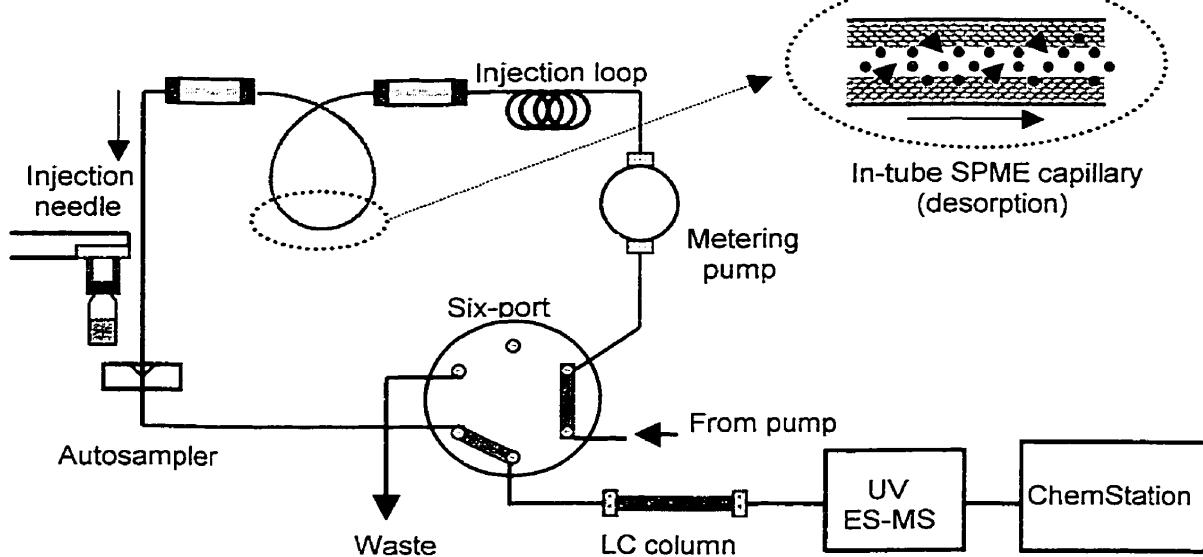
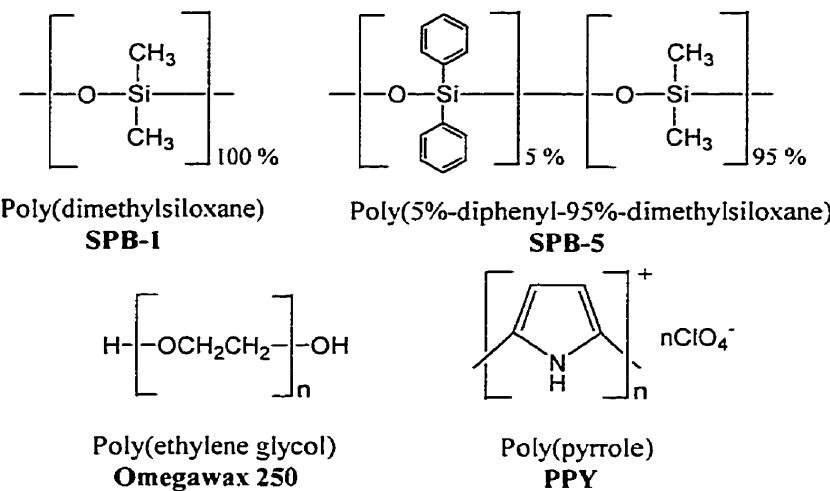
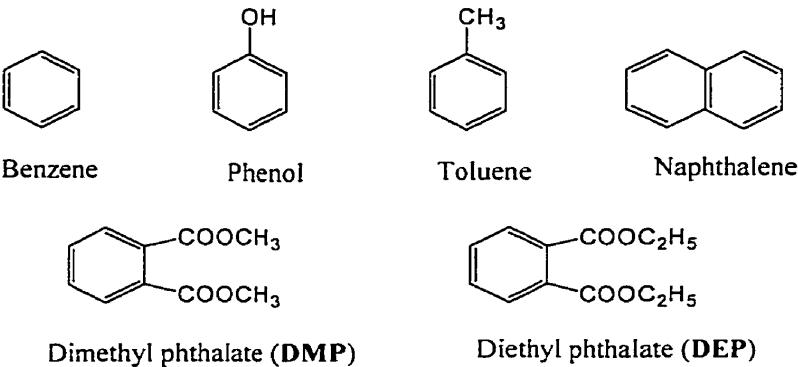


Figure 4-1. Schematic diagrams of the automated in-tube SPME-HPLC system: (A) extraction and (B) desorption.

A. Capillary stationary phases



B. Model Aromatic Compounds Studied



C. Aromatic Amines Studied

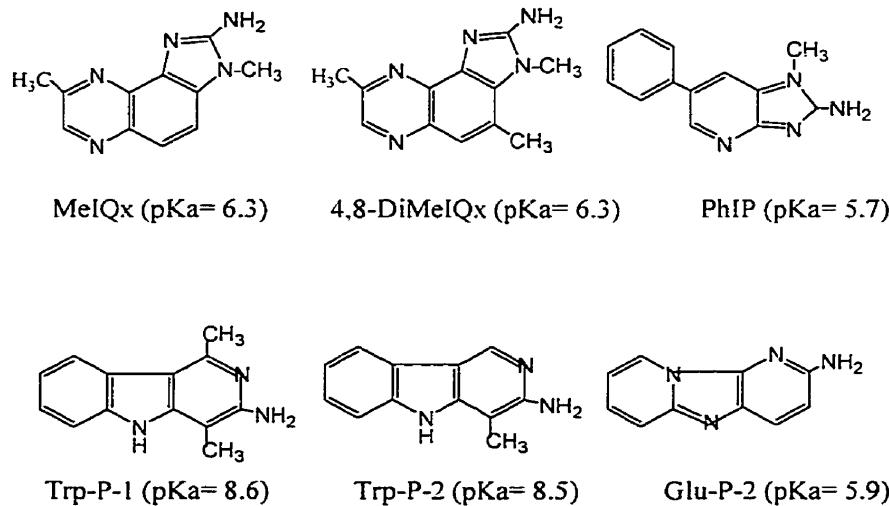


Figure 4-2. Structures of the capillary stationary phases and some of the compounds studied.

For the model aromatic compounds, separation was performed using a Hypersil BDS C₁₈ column (5.0 cm × 2.1 mm i.d., 3 µm particle size) from Agilent Technologies under room temperature. The mobile phase consisted of acetonitrile and water (40:60) with a flow rate of 0.2 mL/min. An UV detector, set to 200 nm, was used for the first 7 min, and then changed to 219 nm for the rest of the run as shown in Figure 4-3.

For the separation of 16 PAHs, a SUPELCOSIL LC-PAH column (5 cm × 4.6 mm, 3 µm particle size) from Supelco (Bellefonte, PA USA) was used at ambient temperature. Mobile phase: initially, CH₃CN: water (50:50) was kept for 5 min, then the component of CH₃CN were increased linearly and reached 90 % at 20 min and held at this ratio for the rest of the run. Flow rate was kept at 0.5 mL/min. UV detection was performed using a wavelength program to optimize signal intensities as shown in Figure 4-4.

For separation of the 6 aromatic amines, the same C-18 column was used as for separation of the model aromatic compounds. Mobile phase was a mixture of A (CH₃CN: CH₃OH = 1:3) and B (ammonium acetate, 100mM) with a ratio of 50: 50; flow rate was increased linearly from 0.2 to 0.5 mL/min within 20 min. ESI-MS detection: nebulizer gas, N₂ (40 psi); drying gas, N₂ (10 L/min, 350 °C); capillary voltage, 1500 V; fragmentor voltage, 90 V; ionization mode, positive; mass scan range, 100-300 amu; selected ion monitoring (SIM), m/z 214 (MeIQx), 228 (4,8-DiMeIQx), 225 (PhIP), 184 (Glu-P-2), 198 (Trp-P-2), and 212 (Trp-P-1).

4.2.4. Sample preparation

Drinking water and lake water samples collected from local areas were prepared by spiking three different amounts of analytes in sample solutions, respectively. Each (1 mL) of these spiked sample solutions was shaken thoroughly and allowed to stand for 2 to 5 min, then set into the autosampler and analyzed by the method developed. The results were compared with those of non-spiked samples and pure water samples analyzed by the same method and under the same conditions to obtain the recoveries.

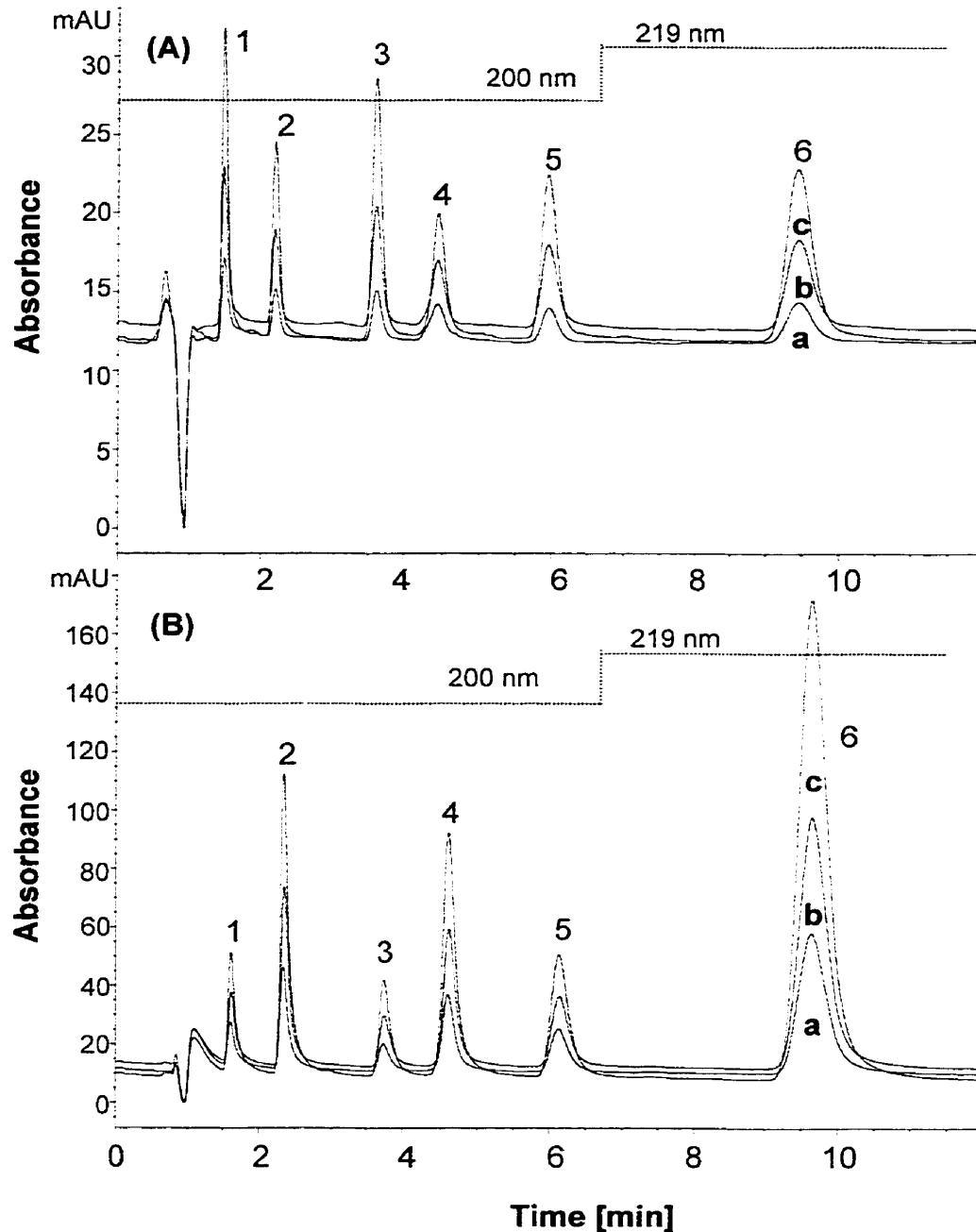


Figure 4-3. HPLC/UV chromatograms of the 6 model aromatic compounds by (A) standard injection ($10 \mu\text{L}$) and (B) PPy coated capillary in-tube SPME. Peak identification and concentration: (1) phenol (a: 200 ng/mL , b: 400 ng/mL , and c: 800 ng/mL), (2) DMP (a: 100 ng/mL , b: 200 ng/mL , c: 400 ng/mL), (3) benzene (a: 500 ng/mL , b: 1000 ng/mL , c: 2000 ng/mL), (4) DEP (a: 100 ng/mL , b: 200 ng/mL , c: 400 ng/mL), (5) toluene (a: 500 ng/mL , b: 1000 ng/mL , c: 2000 ng/mL), and (6) naphthalene (a: 100 ng/mL , b: 200 ng/mL , c: 400 ng/mL).

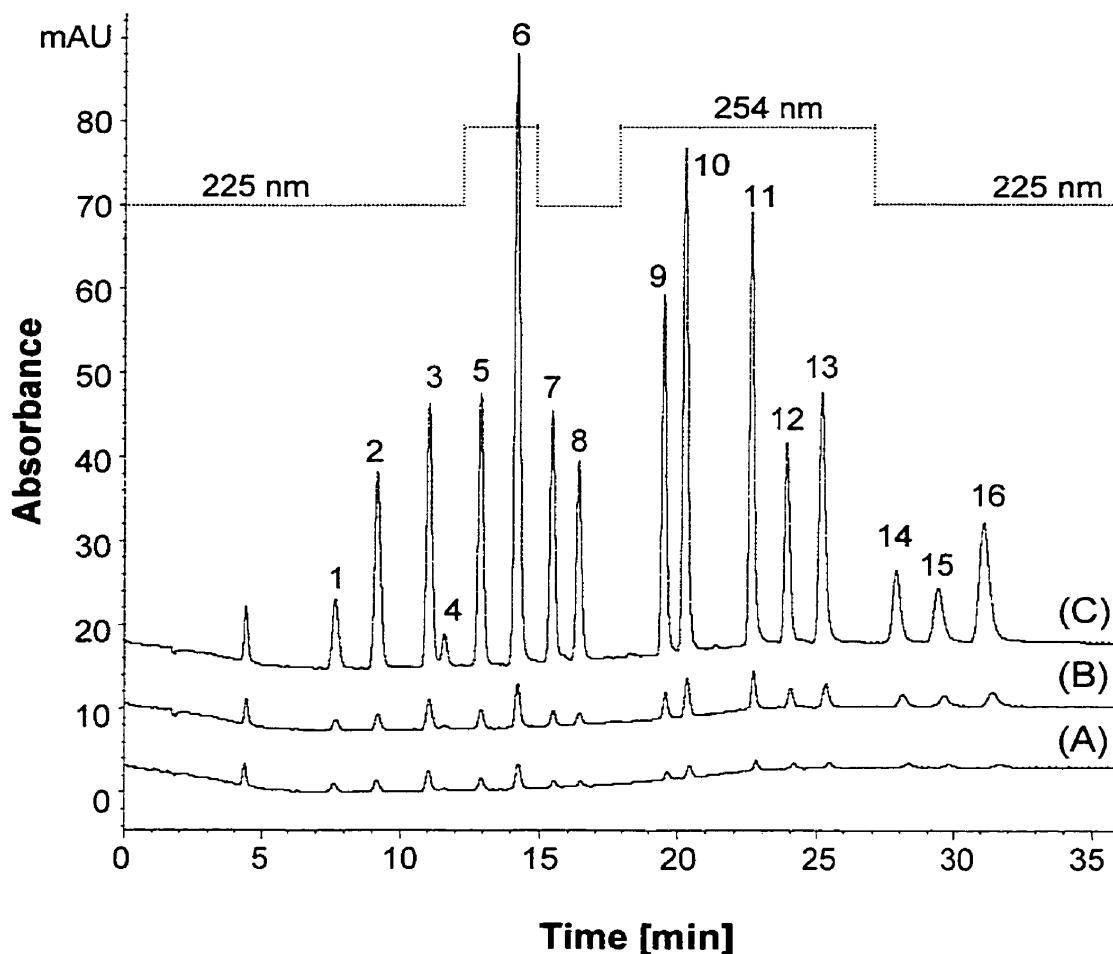


Figure 4-4. Separation of a PAH mixture (each 100 ng/mL) with solvent gradient by (A) standard injection (10 μ L) and (B) host capillary in-tube SPME, and (C) PPy coated capillary in-tube SPME. Peak identification: 1. Naphthalene, 2. Acenaphthylene, 3. Acenaphthene, 4. Fluorene, 5. Phenanthrene, 6. Anthracene, 7. Fluoranthene, 8. Pyrene, 9. Benzo[a]anthracene, 10. Chrysene, 11. Benzo[b]fluoranthene, 12. Benzo[k]fluoranthene, 13. Benzo[a]pyrene, 14. Dibenzo[a,h] anthracene, 15. Benzo[g,h,i]perylene, and 16. Indeno[1,2,3-cd]pyrene.

4.3. Results and discussion

4.3.1. Separation and detection conditions

All three groups of aromatic compounds could be separated under the conditions listed in the experimental section. UV detection conditions were optimized by selecting appropriate wavelengths at which most compounds had better signal intensities, as shown in Figure 4-3 and Figure 4-4. Under these wavelengths, different sample matrices (buffer solutions) did not show significant effects on the UV detection (data not shown). For the group of amine compounds, mass detection method was used due to their weak UV signals. For each amine, mass spectra under positive ion mode were initially analyzed by liquid injection. Each amine gave a simple spectrum in the mass range m/z 100-300, with $[M+1]^+$ ion as the base ion. These base ions were selected for the analyte quantification. Optimization of the mass detection conditions included capillary voltage, fragmentor voltage, nebulizer pressure, drying gas flow rate and temperature. The results were summarized in the experimental section.

4.3.2. Optimization of in-tube SPME conditions

In SPME, the extraction efficiency and selectivity of a coating to the analytes depend on the interactions between the analytes and the stationary phase, which include hydrogen bonding, acid-base, $\pi-\pi$, dipole-dipole, dipole induced-dipole, and dispersion (hydrophobic interaction) forces. Ideally, the extraction ability of a coating should be evaluated by the distribution coefficient K of the analyte between the coating and sample matrix. Selectivity should be judged by the selectivity factor (α) defined as $\alpha_{j,i} = K_j / K_i$, where K_j and K_i are the distribution coefficients of compounds j and i between the same coating and sample matrix. However, for some compounds or coatings, K values are not available or difficult to measure accurately. Therefore, the extraction efficiency is often evaluated by the amount of analyte extracted by the coating, which, for an absorption-based SPME coating, can be expressed as:

$$n_A = K_A V_f V_s C_A^\circ / (K_A V_f + V_s) \quad (1-1)$$

For porous coatings that extract analytes by adsorption, the equation that takes into account the active extraction sites on the porous surface can be expressed as follows:

$$n_A = K_A V_f V_s C_A^\circ (C_{f\max} - C_{fA}^\infty) / [V_s + (K_A V_f (C_{f\max} - C_{fA}^\infty))] \quad (1-3)$$

The two equations were discussed in chapter 1. Although some terms in the two expressions are different, the extraction efficiencies of different coatings for the same sample can be compared by the amount of analytes extracted (or extraction yield) under the same conditions, especially when the coatings have the same thickness or volume. For practical purposes, even when the data on thickness for some coatings are unknown, the comparison of the amount of analyte extracted by different coatings can still provide useful guidance for the coating selection.

When the initial concentrations of all the analytes in a sample are the same, the selectivity of a coating to different compounds in a sample can be evaluated by the selectivity factor defined as $\alpha_{ji} = K_j / K_i = n_j / n_i$.

One of the advantages of using the amount of analytes extracted, n_A , to evaluate the extraction efficiency and selectivity (by $\alpha_{ji} = n_j / n_i$) is that n_A can be easily obtained from experimental measurements with the following expression:

$$n_A = F \times A = (m / A_d) \times A \quad (4-1)$$

where n_A is the amount (mass) of analyte extracted by SPME, F is the detector response factor which can be calculated by comparing the amount of analyte (m) injected to the area counts (A_d) obtained by liquid injection, A is the response obtained by SPME.

In this work, several parameters of in-tube SPME were optimized to achieve the best extraction efficiency for the aromatic compounds studied. These parameters include capillary coatings, coating thickness, capillary length, extraction time profile (the number of draw/eject cycles for each extraction, the sample volume and flow rate for each draw/eject cycle), sample matrix and pH, and desorption conditions. A detailed theoretical description and discussion on some of these parameters was reported previously [3].

Table 4-1. Comparison of the Extraction Properties for the Model Compounds by Different Capillaries

compounds*	<i>F</i> ^a	analyte extracted (ng) ^b				extraction yield (%) ^c				selectivity factor ($\alpha_{A/benzene}$) ^d						
		Host	SPB-1	SPB-5	Omeg	PPY	Host	SPB-1	SPB-5	Omeg	PPY	Host	SPB-1	SPB-5	Omeg	PPY
phenol	0.059	3.6	3.9	1.0	3.9	7.2	1.8	1.9	0.5	2.0	3.6	0.8	0.9	0.1	0.9	1.2
DMP	0.048	3.0	1.9	0.8	3.6	13.9	3.0	1.9	0.8	3.6	13.9	1.4	0.9	0.2	1.6	4.5
benzene	0.138	10.9	10.3	18.1	11.1	15.5	2.2	2.1	3.6	2.2	3.1	1.0	1.0	1.0	1.0	1.0
DEP	0.039	2.6	1.7	1.5	3.5	13.8	2.6	1.7	1.5	3.5	13.8	1.2	0.8	0.4	1.6	4.4
toluene	0.124	10.8	10.0	32.7	11.6	32.2	2.2	2.0	6.5	2.3	6.4	1.0	1.0	1.8	1.0	2.1
naphthalene	0.013	2.6	3.8	10.3	9.4	18.8	2.6	3.8	10.3	9.4	18.8	1.2	1.8	2.8	4.2	6.0

* Compound concentrations in the sample: phenol (200 ng/mL), DMP (100 ng/mL), benzene (500 ng/mL), DEP (100 ng/mL), toluene (500 ng/mL), naphthalene (100 ng/mL). ^a A 10- μ L sample was directly injected to obtain *F* (detector response factor for each analyte, see equation (4-1)). ^b A 1-mL sample was analyzed by in-tube SPME, the amount of analyte extracted (*n*_A) was calculated by equation (4-1).

^c The extraction yields (%) are the percentages of extracted amount of analytes per initial amounts of analytes in a 1-mL sample solution.

^d Selectivity factors were calculated by comparing the extraction yield of a analyte relative to that of benzene. A 4-PPY-cycle coating was used.

Table 4-2. Comparison of the Extraction Efficiencies for the PAHs by Different Capillary Coatings

PAH compounds	detector response ^a	amount of analyte extracted (ng) ^b	or extraction yield (%) ^c
naphthalene	0.058	0.9	1.7
acenaphthylene	0.046	0.6	1.8
acenaphthene	0.027	0.7	1.8
fluorene	0.213	1.2	2.1
phenanthrene	0.050	0.8	2.5
anthracene	0.024	0.8	2.6
fluoranthene	0.090	1.0	3.2
pyrene	0.115	1.1	3.1
benz(a)anthracene	0.082	1.5	4.8
chrysene	0.052	1.4	4.4
benzo(b)fluoranthene	0.072	1.9	6.3
benzo(k)fluoranthene	0.107	1.5	5.8
benzo(a)pyrene	0.079	1.8	6.0
dibenz(a,h)anthracene	0.098	1.4	4.9
benzo(ghi)perylene	0.099	1.6	5.2
Indeno(1,2,3-cd)pyrene	0.088	1.9	7.3

^a F was obtained by injecting 10 μL of 1 $\mu\text{g/mL}$ solution (each PAH 10 ng was injected, see equation (4-1)).

^b A 1-mL sample (100 ng/mL for each PAH) was analyzed by in-tube SPME, the amount of analyte extracted (n_A) was calculated by equation (4-1); the extraction yield (%) = $n_A \times 100 / M$, M is the total amount of each analyte in the 1 mL solution. Since the concentration of each analyte is 100 ng/mL, M = 100 ng. ^c Selectivity factors were calculated based on n_A relative to n_1 (naphthalene).

Table 4-3. Comparison of the Extraction Efficiencies for the Aromatic Amines by Different Capillaries.

compounds	m/z (M+1)	detector response ^a		analytes extracted (ng) ^b			extraction yield (%) ^c		
		$F \times 10^{-6}$		Host	Omeg	PPY	Host	Omeg	PPY
MeIQx 4,8-	214	1.71		3.3		18.3	1.3	3.3	18.3
	228	1.97		1.3	4.5	18.3	1.3	4.5	18.3
DiMeIQx									
PhIP	225	1.27		1.1	5.0	15.9	1.1	5.0	15.9
Glu-P-2	184	4.33		0.9	9.4	15.7	0.9	9.4	15.7
Trp-P-2	198	1.44		1.4	10.7	23.1	1.4	10.7	23.1
Trp-P-1	212	2.48		1.6	14.5	28.9	1.6	14.5	28.9

^a Detector response factors (F) were obtained by liquid injection 10 μL of 1 $\mu\text{g}/\text{mL}$ solution (each amine 10 ng was injected, see equation (4-1)). ^b A 1-mL sample containing each analyte 100 ng/mL was analyzed by in-tube SPME, the amount of analyte extracted (n_A) was calculated by equation (4-1). ^c The extraction yields (%) are the percentages of extracted amount of analytes per initial amounts of analytes in a 1-mL sample solution. 15 extraction cycles were performed by in-tube SPME.

4.3.2.1. Comparisons of PPy coating with commercial coatings for in-tube SPME

Previous studies [3,4] showed that Omegawax was the best capillary for in-tube SPME of polar compounds among the commercial GC capillaries tested, including SPB-1 and SPB-5 (see Figure 4-2). In this work, a PPy coated capillary, a host silica capillary (without coating) and the three commercial capillaries described above were evaluated for their extraction ability. Three groups of aromatic compounds were selected, representing several functionalities. The first group, the so-called model compounds, includes both non-polar (benzene, toluene, and naphthalene) and polar compounds (phenol, DMP and DEP) containing one or two π rings. The second group contains 16 polycyclic aromatic hydrocarbons (PAHs). The third group consists of 6 aromatic amines representing a wide range of basic heterocyclic aromatics.

For the model compounds, the extraction efficiency and selectivity of PPy coating can be clearly observed from Figure 4-3 and Table 4-1. In these experiments, larger amounts of phenol, benzene and toluene, relative to DMP, DEP and naphthalene, were used to increase their responses. For liquid injection as shown in Figure 4-3(A), peaks 1, 3 and 5 are higher than peaks 2, 4 and 6 due to their larger concentrations. However, the opposite trends were obtained when the same samples were analyzed by the PPy coated capillary in-tube SPME as shown in Figure 4-3(B). The above opposite trends and the selectivity factors listed in Table 4-1 illustrated that PPy coating had better selectivity towards the polar compounds DMP and DEP, and the two π -ring naphthalene. These results can be explained by considering the structures of PPy and the analytes. Since PPy contains a conjugated π structure, it will extract aromatics by π - π interactions as well as hydrophobic interactions, and these interactions will increase accordingly with increasing aromatic rings, such as for naphthalene and polycyclic aromatics (PAHs). The results shown in Figure 4-3 and Table 4-1 agree well with the above expectation. The high selectivity of PPy coating for polar aromatics such as DMP and DEP is possibly due to the additional interactions between the polar components of the polymer and analytes. However, the PPy coating did not show a high extraction ability for phenol, probably due to the weak interaction between the PPy and the undissociated acidic molecules (since PPy is a weak acid itself) [7]. The effect of PPy coating on the extraction is also easily observed when comparing the results of PPy coated

capillary with those of the non-coated host capillary as listed in Table 4-1. These results are consistent with those obtained by Wallace and co-workers in their studies on using PPY coated stationary phases for HPLC separation of the similar compounds [7,8].

Compared with other coatings tested under the same conditions, as shown in Table 4-1, PPY coating demonstrated the best extraction efficiency for most of the compounds studied. SPB-1 and SPB-5 contain non-polar coatings and they showed better selectivity to non-polar compounds such as benzene, toluene, and naphthalene. However, compared with SPB-1, the extraction ability of SPB-5 to non-polar aromatics was significantly increased due to the π - π interactions introduced by the phenyl group (5 %) in the polymer. Omegawax did not show good extraction efficiency for the compounds studied, but it did show a better extraction selectivity to DMP, DEP and naphthalene relative to benzene and toluene due to its polar property.

To examine the compatibility of the in-tube SPME with solvent gradient conditions and to further evaluate the extraction efficiency of PPY coating towards polycyclic aromatic compounds, a 16 PAH mixture was analyzed by both liquid injection and in-tube SPME methods. As shown in Figure 4-4, the retention times of PAHs by liquid injection agree well with those obtained by SPME, which illustrates that SPME sampling does not affect the retention of the analytes under solvent gradient conditions. Compared with other coatings studied, PPY showed again the highest extraction efficiency for the PAHs studied (Table 4-2). In addition, the extraction efficiency increased with the increase of molecule size due to the increased π - π and hydrophobic interactions. However, for PAHs larger than benzo[b]fluoranthene, the hydrophobic interactions became dominant and a slight decrease in extraction efficiency was observed. These trends were also found in a previous study on porous coating SPME of PAHs [9]. Due to the high extraction ability of PPY coating, the PPY coated capillary in-tube SPME-HPLC method could be applied to detect low concentration of PAHs (up to 0.5 ng/mL), which were not detectable with the liquid injection method (detection limit, $DL = 10$ ng/mL). The extraction yield for naphthalene was smaller in Table 4-2 relative to that in Table 4-1, possibly due to a sample matrix effect, which will be discussed later.

Six aromatic amines that are target mutagens or carcinogens were also examined. A previous study showed that Omegawax was better in extraction of these compounds than SPB-1, SPB-5 and a non-polar precolumn [4]. However, as illustrated in Table 4-3, even a thin PPy coating (two coating cycles, see the next section for coating thickness effect on extraction) showed higher extraction efficiency for these compounds than Omegawax and other coatings tested. The high extraction ability of PPy to these compounds is due to the increasing interactions of polar functional groups (such as hydrogen bonding, base-acid and dipole-dipole) between polymer and analytes. Table 4-3 does not include the results obtained by SPB-1 and SPB-5 because they were discussed previously [4].

4.3.2.2. Effects of PPy coating thickness and surface property on extraction

Previous in-tube SPME studies did not consider the effect of coating thickness (an important parameter) on extraction properties, due to the expensive cost in testing commercial coatings of different thickness and the low availability for some commercial coatings of different thickness. In this work, since the thickness of PPy coating can be controlled easily by changing the number of PPy coating cycles (see Chapter 2, preparation for PPy coated capillary), this provides the opportunity to systematically study the effect of coating thickness on in-tube SPME. It can be predicted from equation (1-1) and (1-3) that the amount of analytes extracted will increase when the coating thickness (and thus the volume of the coating, V_f) increases. Thus, the extraction efficiency and selectivity can be manipulated by controlling the coating thickness. This expectation was proved by experimental results in this work. For example, the extraction efficiency of the PPy coating to the group of model compounds studied increased gradually with an increase in PPy coating thickness (the number of PPy coating cycles) as shown in Table 4-4. Meanwhile, the selectivity of the coating to polar aromatic compounds such as DMP and DEP, and to polycyclic aromatics such as naphthalene was also increased relative to benzene and toluene. For a porous coating, in which the active surface area controls its extraction ability, it is more important to consider the total surface area than the coating volume. This is because the surface area of a porous coating is larger than that of a non-porous coating even though it has the same or even smaller coating volume relative to a nonporous coating due to the high

porosity of the porous coating [9-11]. If a porous coating layer can be treated as an idealized bed consisting of uniform spherical microparticles, the total surface area can be expressed as

$$S_t = \pi L [(D/2 + a)^2 - (D/2)^2] d\rho s = a\pi L (D + a) d\rho s \quad (4-2)$$

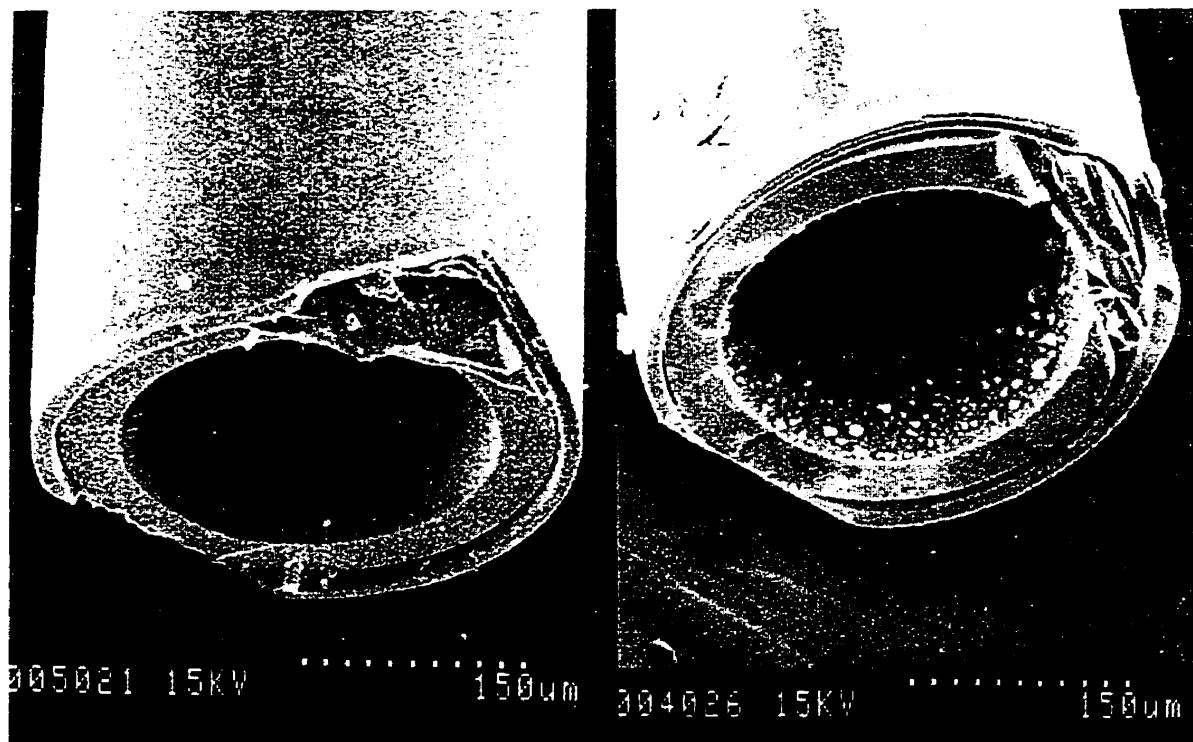
where S_t is the total surface of the porous coating, a is the coating thickness, L is the length of the capillary, D is the inner diameter of the coated capillary (after coating), d is the density of the PPy particles, ρ is the porosity of the porous coating, and s is the specific surface area. Therefore, when the coating thickness and polymer particle density increases, the coating surface area and hence the extraction efficiency will increase. Scanning electron microscopy (SEM) of the PPy coated inner surfaces confirmed this prediction as shown in Figures 4-5 and 4-6. The increase in extraction selectivity with an increase of coating thickness is due to the enhancement of specific interactions ($\pi-\pi$ and polar functional groups) relative to non-specific interactions (hydrophobic). The porous inner surface characteristics of PPy coated capillaries with 1 to 3 coating cycles can be seen clearly when comparing them with the surface of non-coated host capillary (Figure 4-6). The SEM image on the inner surface of a 4-cycle PPy coating is not included in this figure, because it does not show significant difference compared with that of a 3-cycle coating. The estimated thickness of a 4-cycle PPy coating is less than 0.5 μm , according to the SEM study and the calculation based on the density [12] and mass of PPy. To our knowledge, this is the first systematic SEM study on the PPy coated capillary inner surface with different thickness.

As expected, the extraction efficiency for the group of PAHs and amine compounds were also increased with an increased coating thickness (data not shown). Actually, the extraction ability of a thick PPy coating toward aromatic amines was so strong that these compounds could not be desorbed easily by the mobile phase. To overcome this problem, a thinner PPy coated capillary (2 coating cycles) had to be used for amines rather than a thicker coating (4 coating cycles, which was used for other analytes). However, even a thin PPy coating could extract more analytes from the sample than other coatings, as shown in the previous section (Table 4-3). The enhanced extraction efficiency of thinner porous SPME coatings was also demonstrated in the recent studies on porous SPME coatings [9,10] and Sol-gel SPME coatings [11].

Table 4-4. Effect of PPy Coating Thickness on the Extraction Efficiencies for the Model Aromatic Compounds

compound*	F ^a	amount of analyte extracted (ng) ^b					extraction yield (%) ^c					selectivity factor ($\alpha_{A/benzene}$) ^d				
		0-PPY	1-PPY	2-PPY	3-PPY	4-PPY	0-PPY	1-PPY	2-PPY	3-PPY	4-PPY	0-PPY	1-PPY	2-PPY	3-PPY	4-PPY
phenol	0.059	3.6	4.9	5.7	6.6	7.2	1.8	2.5	2.9	3.3	3.6	0.8	1.1	1.1	1.1	1.2
DMP	0.048	3.0	5.3	8.7	11.6	13.9	3.0	5.3	8.7	11.6	13.9	1.4	2.4	3.4	3.9	4.5
benzene	0.138	10.9	11.3	12.7	15.0	15.5	2.2	2.3	2.5	3.0	3.1	1.0	1.0	1.0	1.0	1.0
DEP	0.039	2.6	6.1	9.2	11.9	14.4	2.6	6.1	9.2	11.9	14.4	1.2	2.7	3.6	4.0	4.4
toluene	0.124	10.8	13.9	19.3	26.5	32.2	2.2	2.8	3.9	5.3	6.4	1.0	1.2	1.5	1.8	2.1
naphthalene	0.013	2.6	5.8	9.3	13.4	18.8	2.6	5.8	9.3	13.4	18.8	1.2	2.6	3.7	4.5	6.0

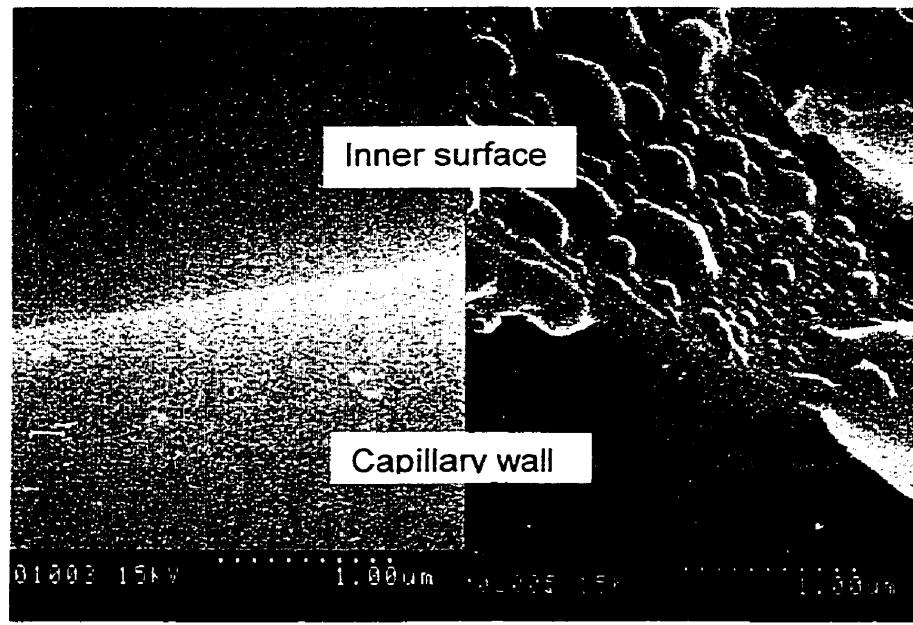
* Compound concentrations and other conditions (including the notes a, b, c and d) are the same as in Table 4-1. The thickness of PPy coating increases from 0-PPY (coating cycle, without coating) to 4-PPY (coating cycles). 15 extraction cycles were used for in-tube SPME.



(a) host capillary

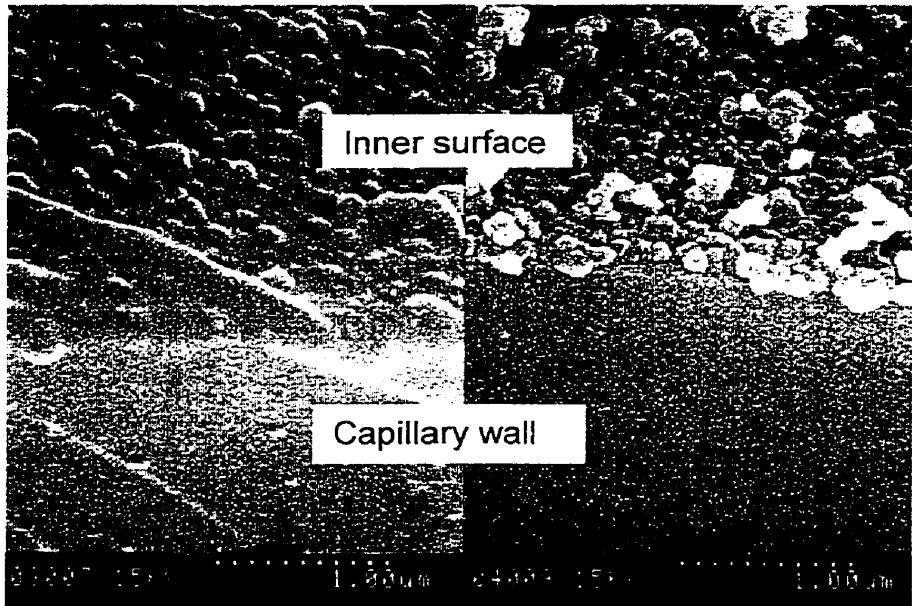
(b) PPy coated

Figure 4-5. Scanning electron micrographs of the PPy coated capillary and the host silica capillary. (a) Cross sectional view of the host capillary. (b) Cross sectional view of the PPy coated capillary (4 cycle coating).



(c) host capillary

(d) 1 cycle PPY



(e) 2 cycle PPY

(f) 3 cycle PPY

Figure 4-6. Scanning electron micrographs of the PPY coated capillaries and the host silica capillary. The enlarged inner surface views for the host capillary (c), and for the PPY coated capillaries with 1 PPY coating cycle (d), 2 PPY coating cycles (e), and 3 PPY coating cycles (f).

Table 4-5. Effects of the Number of Extraction Cycles on the Extraction Efficiency of PPy to PAH Compounds.

PAH compounds*	F detector response ^a	analytes extracted (ng) or extraction yield (%) ^b				Selectivity ^c α_{M1}
		5 cycles	10 cycles	15 cycles	20 cycles	
naphthalene	0.058	3.0	4.5	5.9	6.7	7.5
acenaphthylene	0.046	3.9	5.9	7.6	8.8	9.9
acenaphthene	0.027	3.9	5.5	6.7	8.1	8.9
fluorene	0.213	5.7	6.8	8.1	9.2	10.1
phenanthrene	0.050	5.7	8.6	11.1	13.2	14.6
anthracene	0.024	6.2	9.3	12.8	14.0	15.4
fluoranthene	0.090	7.9	12.7	17.5	21.0	23.6
pyrene	0.115	8.2	13.2	18.0	21.9	24.7
benz(a)anthracene	0.082	8.3	14.4	20.7	25.7	30.0
chrysene	0.052	7.2	12.9	18.7	23.0	26.9
benzo(b)fluoranthene	0.072	8.7	15.4	24.3	30.7	34.7
benzo(k)fluoranthene	0.107	5.5	10.3	15.7	19.3	22.7
benzo(a)pyrene	0.079	6.1	12.3	18.7	23.6	26.2
dibenz(a,h)anthracene	0.098	3.2	6.9	10.7	13.4	14.7
benzo(ghi)perylene	0.099	3.2	7.2	10.8	13.6	15.6
Indeno(1,2,3-cd)pyrene	0.088	5.5	11.4	16.0	20.0	23.9

* Compound concentrations and the experimental conditions (and the notes a, b and c) are the same as in Table 4-2.

^c Selectivity factors were calculated only for the 25 extraction cycles based on n_A relative to n_1 (naphthalene).

4.3.2.3. Extraction time profile and capillary length.

In the extraction process as shown in Figure 4-1 (A), a 40- μ L sample was drawn from the sample vial into the capillary at a flow rate of 100 μ L/min. The same volume of sample was then ejected back into the same sample vial. The two steps together are referred to as one draw/eject cycle, which can be repeated through programming of the autosampler software. As shown in Table 4-5, the amounts of analytes extracted increase greatly when the number of draw/eject cycles increases from 0 to 15. After this number of extraction cycles, the amounts of analytes extracted continue to increase but with smaller slopes. However, a further increase in the extraction cycles increases the analysis time, which is not desirable for routine analysis. Therefore, to achieve sufficient extraction efficiency within a short period of time (10 min), 15 draw/eject cycles were used in this work. The sample volume used for each draw/eject cycle can also be optimized. It was found that the larger the volume, the higher the amount of analytes extracted. However, the maximum volume that could be used in each step was 40 μ L, which was determined by the inner capillary volume (30 μ L) and the volume of the needle assembly (10 μ L). The flow rate in each draw/eject step is also an important factor. It was found that the extraction efficiency was higher with a higher flow rate as a result of improved agitation. However, very high flow rates affected the precision due to the formation of air bubbles at the edges of the capillary. A capillary of 60 cm long was the optimal for in-tube SPME. Below this level, extraction efficiency was reduced, and above this level, peak broadening was observed.

4.3.2.4. Sample matrix and pH

The effects of sample matrix and pH on in-tube SPME were examined using several buffer solutions with pH 3.0-10.0. For the 6 model compounds and the group of 16 PAHs studied, no significant effects on the extraction efficiency were found under the matrices and pHs tested, although the extraction ability increased slightly when a salt (NaCl) was added to the solution. However, sample pH showed great effects on the extraction of aromatic amines due to their basic properties. Similar to a previous study on using an Omegawax capillary for extraction of the same type of compounds [4], the extraction efficiency of PPy coating to these amines increased with increasing sample pH. Tris-buffer at pH 8.5 was used for the amines in this study.

4.3.2.5. Desorption of analytes from capillary

Most of the analytes studied could be desorbed from the extraction capillaries with mobile phases by simply switching the six-port valve to the INJECT position as shown in Figure 4-1 (B). However, some analytes, such as the aromatic amines studied in this work, had stronger interactions with the extraction phase and thus could not be desorbed easily by mobile phase. Therefore, a 40 μL aliquot of methanol was drawn into the capillary to assist desorption of these analytes before switching the valve to the INJECT position.

4.3.3. Extraction mechanism for PPY coating SPME

Porous coatings extract analytes mainly by adsorption processes. The theory of analyte extraction by porous SPME coatings was developed recently based on Langmuir adsorption isotherm (see chapter 1, equation 1-3) [13]. According to this theory, the number of effective surface sites where adsorption can take place is limited. When all such sites are occupied, no more analyte can be extracted. This suggests that analyte extraction is a competitive process in which a molecule with higher affinity for the surface can replace a molecule with lower affinity. In other words, the amount of analyte A extracted (n_A) from a sample mixture must be lower than that (n_A) obtained from a sample containing only analyte A.

The large difference in extraction yields (%) for naphthalene obtained by PPY coating from a sample containing 6 model compounds (Table 4-1) and a sample having 16 PAHs (Table 4-2) indicates that the PPY extraction is mainly based on adsorption mechanism. This was further confirmed by the results shown in Figure 4-7. At high concentration ranges, the slopes of the calibration curves obtained from the sample mixtures are smaller than those obtained from the samples containing only a single analyte, due to the larger competing effects on extraction in sample mixtures. However, these phenomena were hardly observed at low concentration ranges (lower than 400 ng/mL) where the linear calibration curves have almost the same slopes. This is because the number of unoccupied active surface sites on the coating is relatively larger in diluted solutions and thus there are fewer competitions (replacements) in the extraction process. Since the amount of analyte extracted from a sample can be significantly affected by sample matrix composition as shown above, appropriate calibration methods must be applied in quantitative analysis with adsorption-based coatings.

In addition, diluted solutions should always be used to reduce competing effects on extractions.

It must be pointed out, however, that conducting polymers like PPy are complicated systems. Further studies are needed for better understanding of the interaction mechanism between analytes and the polymers. The adsorption of organic vapors on PPy films was studied recently [14]. A mechanism considering both adsorption and absorption was also proposed [15,16].

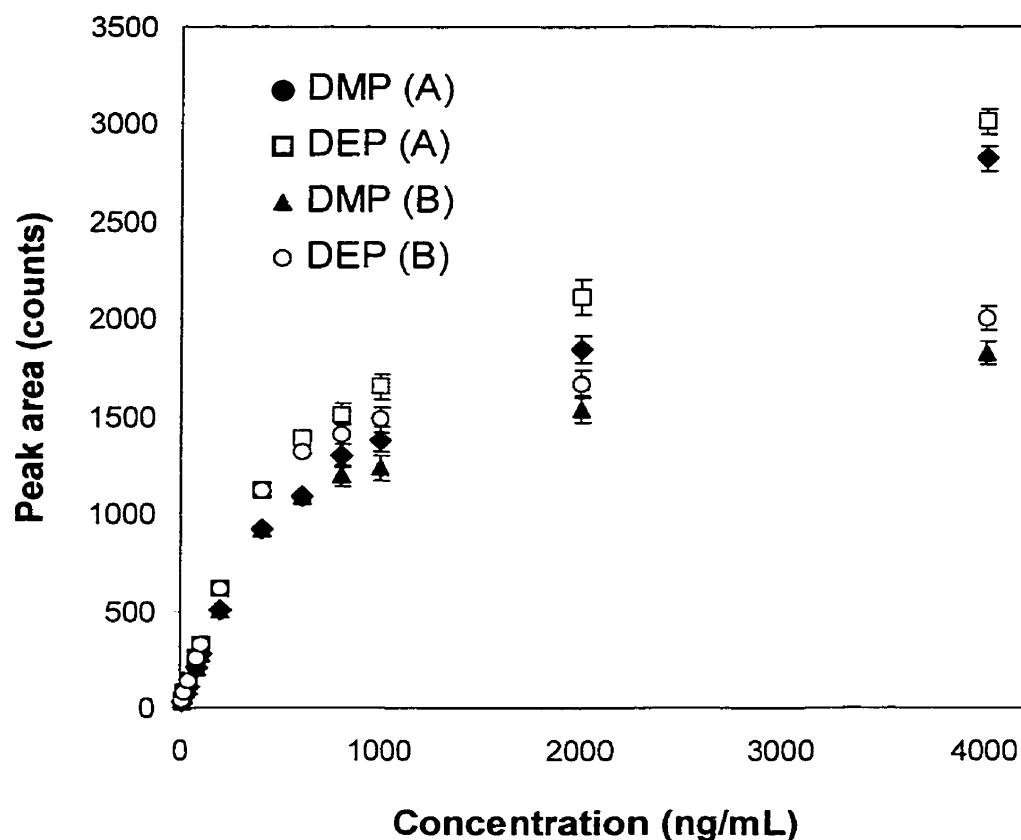


Figure 4-7. Calibration curves for the DMP and DEP obtained under the conditions of (A) sample solutions contain only DMP or DEP, and (B) sample solutions contain all 6 model compounds with increased concentrations as shown in Table 4-6.

4.3.4. Precision, limit of detection and linearity

The precision of the method varies between 1.8 to 7.2 % RSD ($n = 7$), depending on the compounds and concentrations studied. Due to the higher extraction efficiency of PPY coating, lower detection limits ($S/N = 3$) can be achieved for most of the analytes compared with commercial coatings. Under current experimental conditions, the detection limits are at low ng/mL levels for most of the compounds studied. Calibration curves (peak area counts against analyte concentrations) are linear within at least two orders of magnitude. For example, for the model compounds, good linear relationships were obtained in the listed concentration ranges as shown in Table 4-6. The in-tube SPME method can generally enhance sensitivity more than 10 times for most of the compounds studied relative to liquid injection method (10- μ L injection). The extraction efficiency can be further increased by using a relatively larger volume of sample, and by increasing the extraction time.

Table 4-6. Linear Regression Data and Detection Limits (DL) for the Model Compounds

compound	linear range (ng/mL)	linear regression equation ^a	correlation (R^2)	DL (ng/mL)
Phenol	20 ~ 1000	$y = 0.3964x + 19.275$	0.9984	2.0
DMP	2 ~ 500	$y = 2.3258x + 15.103$	0.9967	0.6
Benzene	40 ~ 2500	$y = 0.1960x - 2.5944$	0.9983	10.0
DEP	2 ~ 500	$y = 2.7897x + 25.055$	0.9978	0.4
Toluene	20 ~ 2500	$y = 0.3866x + 13.788$	0.9955	5.0
Naphthalene	1 ~ 500	$y = 11.889x + 7.9823$	0.9994	0.1

^a Obtained from calibration curves by plotting the peak area counts of each compound against corresponding concentrations; number of data points: 11 points (3 repeats for each point).

4.3.5. Stability of in-tube PPY coating

The stability of PPY coating for in-tube SPME is comparable to or better than commercial coatings tested, because PPY is stable in most of the mobile phases used for HPLC. No significant changes in its extraction performance were observed after hundreds of extractions over several months during this study. More importantly, PPY is stable over a pH range of 1.5 to 10.0. This quality of a coating provides an advantage for manipulation of the

extraction efficiency and selectivity based on the acid / base property of the analytes, especially for basic, acidic, ionic compounds and amphiprotic species. The pH effects on extraction efficiency of PPy coating will be discussed in detail in the next chapter.

4.3.6. Analysis of water samples

Tap water, lake water, and deionized water samples spiked with analytes at three different concentrations (50, 100 and 200 ng/mL, respectively for the model compounds; 10, 50 and 100 ng/mL for both PAHs and aromatic amines) were analyzed. The results were compared with those of non-spiked water samples. The recoveries of the analytes from sample matrices compared with a pure water matrix were between 89.6 to 96.4 %. No analytes were found in drinking water and lake water samples. However, a small peak at the retention time of DEP was detected from a deionized water sample. More importantly, the intensity of this peak increased significantly for a deionized water sample stored in a plastic bottle for about 3 months (data not shown). This result suggests that this compound might be leaching from the plastic bottle. Further studies to identify the peak and the sources of the compound are necessary, although a similar result was also obtained by other chemists [17].

4.4. Summary

In this study, a PPy coated capillary has shown higher extraction ability towards polycyclic aromatic compounds than mononuclear aromatics due to the increased $\pi-\pi$ and hydrophobic interactions between polymer and analytes. The extraction efficiency of PPy to polar aromatics is higher than that to non-polar ones because of the additional interactions between polar components of the polymer and analytes. These results are consistent with the expectations from the structure of PPy and its interactions with analytes. On the other hand, these results also indicate that SPME can be a simple and useful method for studying the properties of materials like polypyrrole by using compounds with known properties.

The higher extraction efficiency of PPy coating compared with commercial coatings demonstrated in this study highlights the importance of developing new coating materials for SPME to extend its application areas. Due to the multifunctional properties of PPy, this in-

tube SPME method can be extended to other groups of analytes as will be discussed in the following chapters.

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CHAPTER 5

ANALYSES OF STIMULANTS IN URINE AND HAIR SAMPLES

5.1. Introduction

Amphetamine (AP), methamphetamine (MA) and their methylenedioxy derivatives are a major class of central nervous system stimulants and are often abused by athletes, drug addicts and recreational users [1]. Stimulant abuse is generally verified by examining urine samples [2]. However, the period during which drugs can be detected in urine samples is usually short, about 10 days after their use [3-4]. To detect the long-term abuse of drugs, hair analysis has attracted great interest recently because drugs can be incorporated into hair and remain there for several months or even years [3-6]. In fact, for practical purposes, the two tests complement each other. Urine analysis provides short-term information of an individual's drug use, whereas long-term histories are accessible through hair analysis.

A number of chromatographic methods have been applied for the determination of stimulants in biological samples, including gas chromatography (GC), high performance liquid chromatography (HPLC), gas chromatography -mass spectrometry (GC-MS) and HPLC-MS [2-8]. Among these methods, GC-MS and HPLC-MS have been the most powerful methods because of their high sensitivity and selectivity, as well as the ability for analyte identification from mass spectra [2,6-8].

For the analysis of a small amount of stimulants in biological samples, sample treatments such as extraction, preconcentration and clean-up steps are often required to improve the sensitivity and selectivity. However, traditional off-line procedures such as solvent extraction, solid phase extraction and other techniques, are time-consuming and labour-intensive, require large volumes of sample and solvent, and need additional instrumentation to automate. SPME can overcome the problems of traditional methods by eliminating the use of organic solvents and by integrating sample extraction, concentration and introduction into a single step [9-11]. Recently, SPME coupled with GC has been used for the analysis of stimulant compounds [5,12-15]. However, due to the polar and ionic (base-acid equilibrium) property of these compounds, in order to achieve sufficient extraction

efficiency, long extraction times and pH adjustment are required even for the relatively polar commercial coatings such as PDMS/DVB [14,15]. In addition, the PDMS/DVB coating was not stable under the extraction conditions tested (degraded after a small number of extractions) [14]. Therefore, it is necessary to develop more stable and efficient coatings for extraction of these basic compounds. As demonstrated in chapter 4, PPY coating not only has high extraction efficiency for aromatic amines but is also stable in solutions of a wide pH range. It is expected that PPY coating will show good extraction efficiency to the stimulants studied and the extraction efficiency will be influenced by the sample pH, since the possible interactions between PPY and the studied stimulants include base-acid, π - π , dipole-dipole, and hydrophobic interactions, hydrogen bonding and ion exchange. To confirm these expectations experimentally, in-tube SPME studies of these stimulants were performed in this work using a PPY coated capillary and several commercially available capillaries. As expected, the PPY coated capillary showed the best extraction efficiency for the compounds studied among all the capillaries tested. Therefore, an automated in-tube SPME technique was developed based on the PPY coated capillary, and was coupled on-line with HPLC-MS for the determination of stimulants in spiked human urine and hair samples.

5.2. Experimental

5.2.1. Chemicals and reagents

Standard solutions of (+)-amphetamine (AP), (+)-methamphetamine (MA), (\pm)-3,4-methylenedioxyamphetamine (MDA), (\pm)-3,4-methylenedioxymethamphetamine (MDMA), and (\pm)-3,4-methylenedioxyethylamphetamine (MDEA) were purchased as 1-mg/mL standard solutions in methanol from Radian International (Austin, TX, USA). Each stimulant was used after dilution with a suitable buffer and water to the required concentration. For other chemicals and reagents, see the previous chapters. The chemical structures of the stimulant compounds studied are shown in Figure 5-1.

5.2.2. Instrument and analytical conditions

All experiments were carried out on an Agilent 1100 series LC-MSD system (see chapter 4). A Supelcosil LC-CN (3.3 cm \times 4.6 mm i.d., 3 μ m particle size,) from Supelco

(Bellefonte, PA, USA) was used for the LC separation. LC conditions were as follows: column temperature, 25°C; mobile phase, acetonitrile/50 mM ammonium acetate (15:85); flow rate, 0.4 mL/min. The optimized ES-MS detection conditions were as follows: nebulizer gas, N₂ (40 psi); drying gas, N₂ (12 L/min, 350°C); fragmentor voltage, 30 V for AP and MDA, and 50 V for MA, MDMA, and MDEA; capillary voltage, 1000 V; ionization mode, positive; mass scan range, 100-250 amu; scan time, 0.67 sec/cycle; selected ion monitoring (SIM), m/z 136 (AP), 150 (MA), 180 (MDA), 194 (MDMA) and 208 (MDEA).

The preparation for the PPy coated capillary and the operation details for the automated in-tube SPME-HPLC have been described in chapters 2 and 4, respectively.

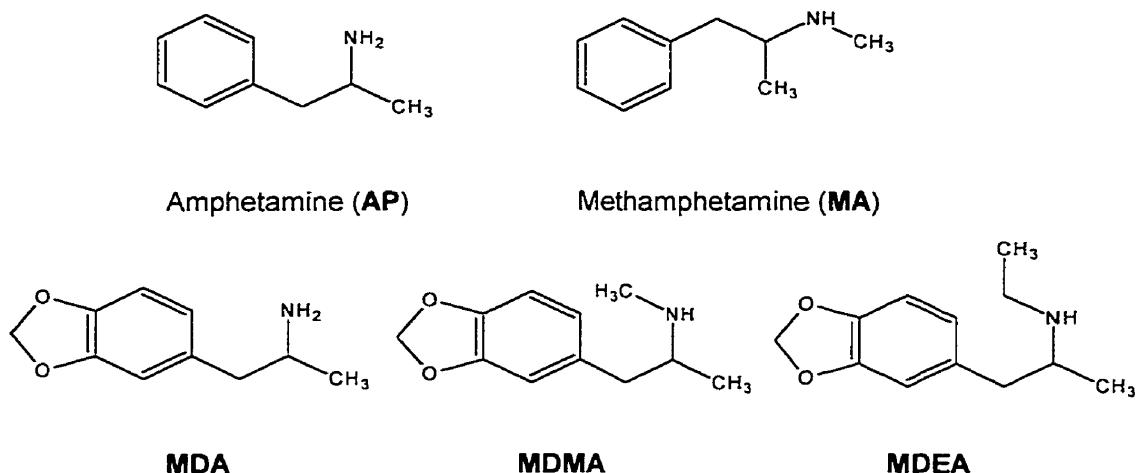


Figure 5-1. The structures of the stimulant compounds studied

5.2.3. Sample preparation

Drug-free urine and hair samples were collected from a healthy volunteer. Urine samples were diluted 10 times with water and used for analysis either without filtration or after filtration (syringe microfilter, 0.45 µm, Gelman Science) if necessary. An aliquot of the sample or filtrate was pipetted into a 2-mL vial and 0.2 mL of 0.5 M sodium carbonate buffer (pH 10.0) was added. After the total volume was made up to 1 mL with water, the vials were

set on the autosampler. Recovery data and calibration curves for urine analysis were obtained by spiking stimulants to the urine samples with the method of standard addition.

Hair samples were treated using a reference method [4]. Briefly, each (10 mg) of the hair samples was cleaned, dried, cut to small pieces (0.5 cm long) and then placed into a 15-mL sample vial containing 5-mL mixture of methanol and 5 M HCl (20:1,v/v). After spiking a certain amount of stimulants in these sample vials, the samples were sonicated for 1 hour and then allowed to stand at room temperature overnight. After the hairs were filtered off, the filtrate was evaporated to dryness under a nitrogen stream. The residue was dissolved with 1 mL of 100 mM carbonate buffer (pH 10.0) for analysis. To build the calibration curves for hair analysis and to obtain the recovery data, different amounts of stimulants were spiked to the hair samples by a standard addition method.

5.3. Results and discussion

5.3.1. LC separation and mass detection

LC separation of stimulants was performed using a Supelcosil LC-CN column as described in the experimental section.

To select the monitoring ion for each of the compounds, electrospray (ES) mass spectra under positive ion detection mode were analyzed by flow injection analysis (FIA) with liquid injection. As shown in Figure 5-2, each compound gave a very simple spectrum in mass scan mode for the range of m/z 100-250 at low fragmentor voltages. Each of the drugs gave a protonated molecular ion $[M+H]^+$ as the base ion, although fragment ions corresponding to $[M-NH_2]^+$, $[M-NHCH_3]^+$ or $[M-NHC_2H_5]^+$ were also observed, especially at higher fragmentor voltages (data not shown). Each base ion accounted for above 80 % of the total ion current. These results indicate that these base ions can be used for quantification in single ion monitoring of each stimulant (*i.e.* m/z 136 for AP, m/z 150 for MA, m/z 180 for MDA, m/z 194 for MDMA and m/z 208 for MDEA). For these selected ions, the following mass detection conditions were optimized: fragmentor voltage, capillary voltage, nebulizer gas pressure, drying gas flow rate and temperature.

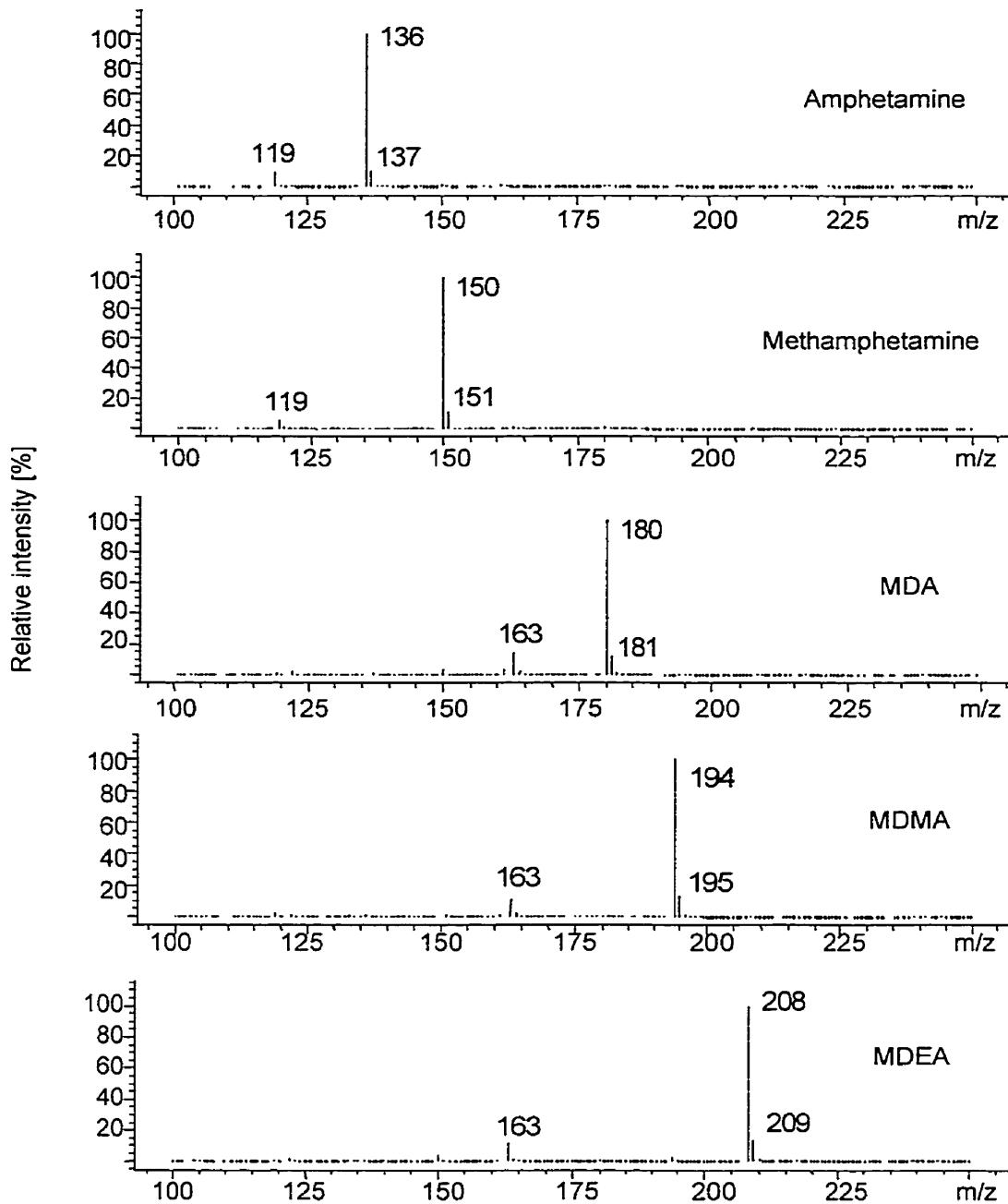


Figure 5-2. Mass spectra of the stimulant compounds obtained under mass scan mode.

Concentration used for each compound: 1 $\mu\text{g/mL}$; injection volume: 10 μL . Fragmentor voltage: 30 V for m/z 136 (AP) and m/z 180 (MDA); 50 V for m/z 150 (MA), m/z 194 (MDMA) and m/z 208 (MDEA). Other conditions see experimental section.

A schematic illustration of the ES-MS system was given in Figure 1 of reference 16. The fragmentor voltage (or collision energy) is the potential difference applied between the capillary and the skimmer (Figure 1 of ref. 16). Fragmentor voltage influences the fragmentation of the charged species and the transmission of ions. As shown in Figure 5-3, at the lower energy levels, the signal intensity for each ion increased with the increase of fragmentor voltages. The $[M+H]^+$ ion signals for AP (m/z 136) and MDA (m/z 180) reached the highest intensity at about 30 V, while for MA (m/z 150), MDMA (m/z 194), and MDEA (m/z 208), the $[M+H]^+$ ion signals arrived the highest at around 50 V. When fragmentor voltages were larger than 50 V, $[M+H]^+$ ion signal intensity decreased due to acceleration of fragmentation.

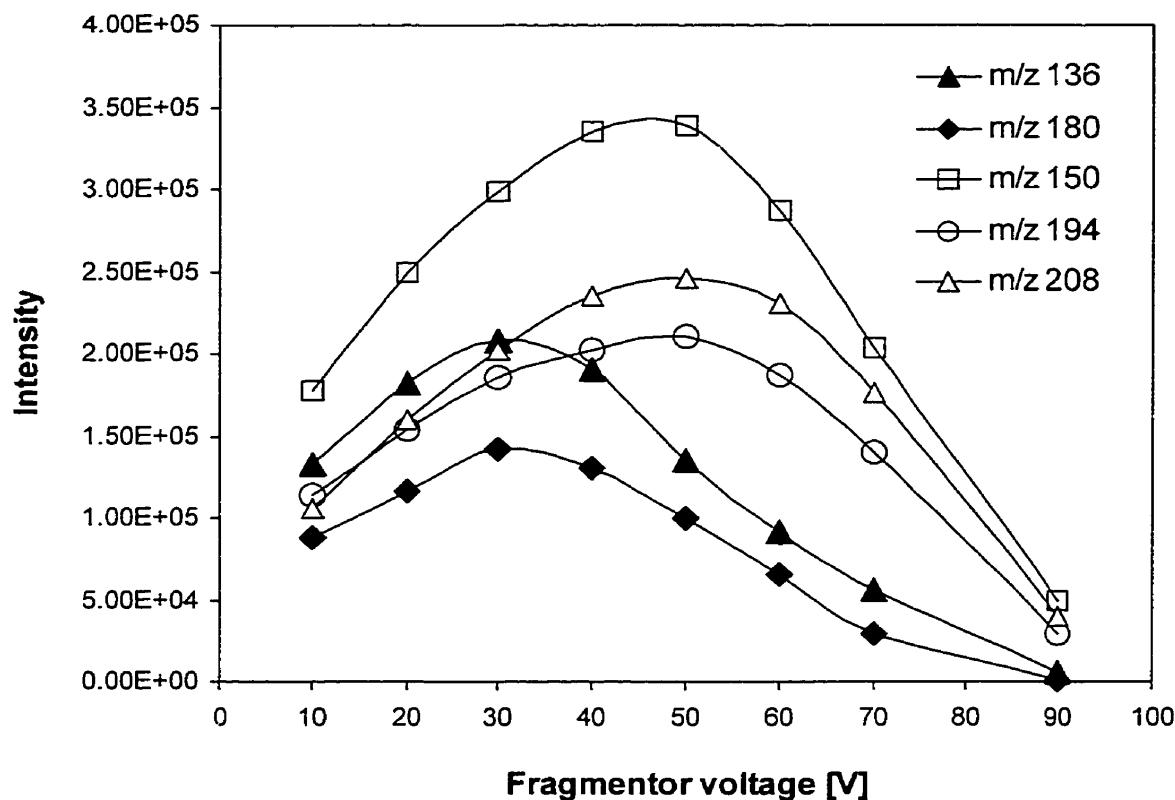


Figure 5-3. Effect of fragmentor voltage on the signal intensity obtained under mass scan mode. Concentration used for each compound: 1 $\mu\text{g/mL}$; injection volume: 10 μL . Other conditions: see experimental section.

The capillary voltage is applied to the entrance of the capillary that connects the spray chamber with the first vacuum stage (Figure 1 of ref. 16). The capillary acts as a counter electrode for the grounded spray chamber and nebulizer needle. The polarity of the capillary is, therefore, opposite to the polarity of the ions analyzed. The effect of capillary voltage on signal intensity is shown in Figure 5-4. For each ion, the signal intensity decreased when capillary voltage was increased from 1000V to 6000V, therefore, 1000 V was used in this study instead of the 3500 V as used in a previous study [17].

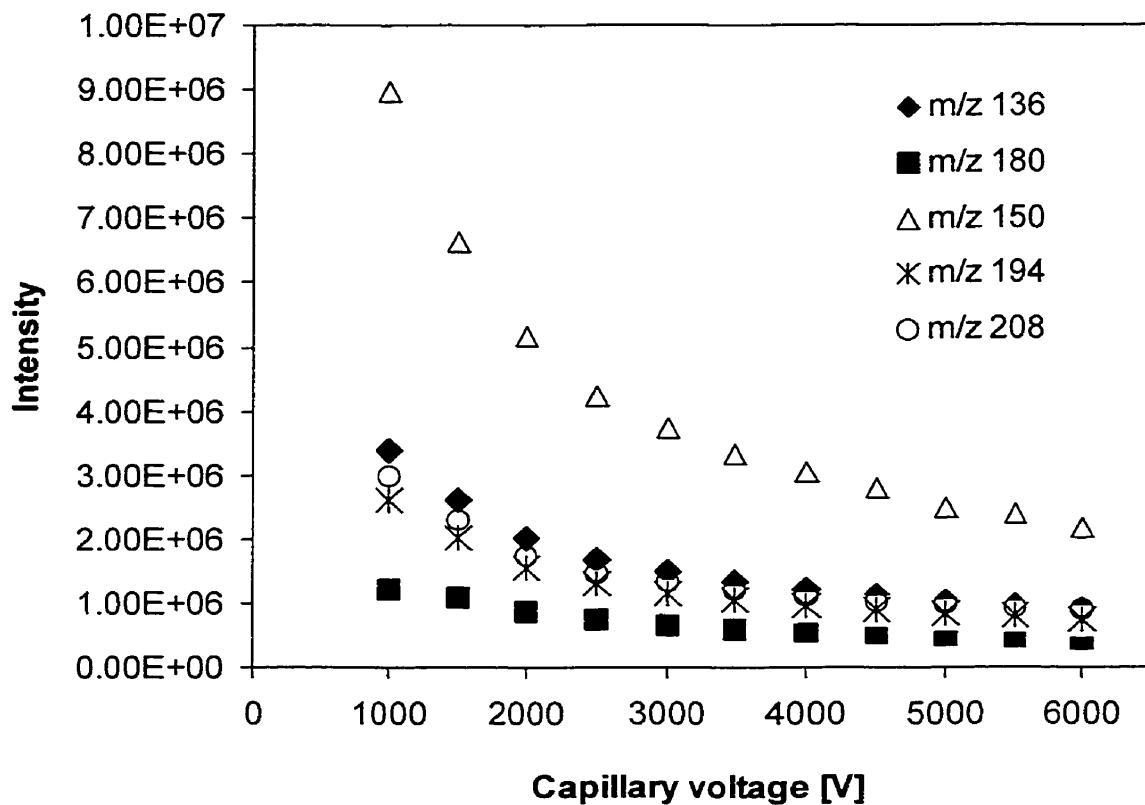


Figure 5-4. Effect of capillary voltage on the signal intensity obtained under selected ion monitoring (SIM) mode. Concentration used for each compound: 100 ng/mL; injection volume: 10 μ L. Fragmentor voltage: 30 V for m/z 136 (AP) and m/z 180 (MDA); 50 V for m/z 150 (MA), m/z 194 (MDMA) and m/z 208 (MDEA). Other conditions: see experimental section.

The effects of nebulizer pressure, drying gas flow rate and gas temperature on mass detection were also investigated. The optimized mass detection conditions were summarized in the experimental section. Due to optimization of the mass detection conditions, the signal intensity for each compound increased about two times as compared to the results obtained using the mass detection conditions of a previous study [17], in which the mass detection conditions were not optimized [18].

5.3.2. Optimization of in-tube SPME conditions

The extraction efficiency in SPME can be evaluated by determining the amount of analyte extracted as described in the previous chapter. In this study, to optimize the in-tube SPME conditions for the stimulant compounds, several parameters were investigated by coupling in-tube SPME with HPLC/ MS. These parameters include the stationary phase of the SPME capillary, coating thickness, extraction time profile (the number of draw/eject cycles for each extraction), sample matrix and pH, and desorption conditions.

Table 5-1. Comparison of the extraction efficiencies of different capillary coatings

Compound ^a	Amount of analyte extracted (ng) ^b					Extraction yield (%) ^c				
	SPB-1	SPB-5	Omeg	PPY	Host	SPB-1	SPB-5	Omeg	PPY	Host
AP	0.51	0.50	2.15	8.34	1.69	1.02	1.00	4.30	16.68	3.38
MDA	0.59	0.72	3.24	9.01	1.74	1.18	1.44	6.48	18.02	3.48
MA	0.82	0.71	4.70	11.14	2.08	1.64	1.42	9.40	22.28	4.16
MDMA	1.66	2.01	5.50	13.13	2.62	3.32	4.02	11.00	26.26	5.24
MDEA	2.01	2.05	6.47	13.26	2.74	4.02	4.10	12.94	26.52	5.48

^a A 10-µL (1.00 ng) sample containing 100 ng/mL of each stimulant was directly injected.
A 1-mL sample containing 50 ng/mL of each stimulant in 50 mM carbonate buffer solution (pH10.0) was extracted by in-tube SPME.

^b For each analyte, the amount of stimulant extracted was calculated by equation (4-1).

^c Extraction yields (%) are the percentages of extracted amounts of stimulants per initial amounts (50 ng) in the 1-mL sample solution using in-tube SPME.

Capillary coating selection. The effect of different stationary phases on the extraction efficiency to stimulants was evaluated using a PPY coated capillary and four commercial capillaries. The amounts of analytes extracted by in-tube SPME with different capillaries

under the same extraction conditions were calculated according to equation (4-1) described in chapter 4. As shown in Table 5-1, Omegawax capillary showed better extraction efficiency than SPB-1 and SPB-5 toward the compounds studied because poly(ethylene glycol) coating used in Omegawax is relatively more polar than the coatings used in SPB-1 and SPB-5 (see Figure 4-2, chemical structures of the stationary phases). However, the extraction efficiency of the PPy coated capillary was much higher than that of Omegawax. Due to its high extraction efficiency toward the analytes and its good stability as an in-tube SPME extracting phase as described in chapter 4, the PPy coated capillary was selected for further study.

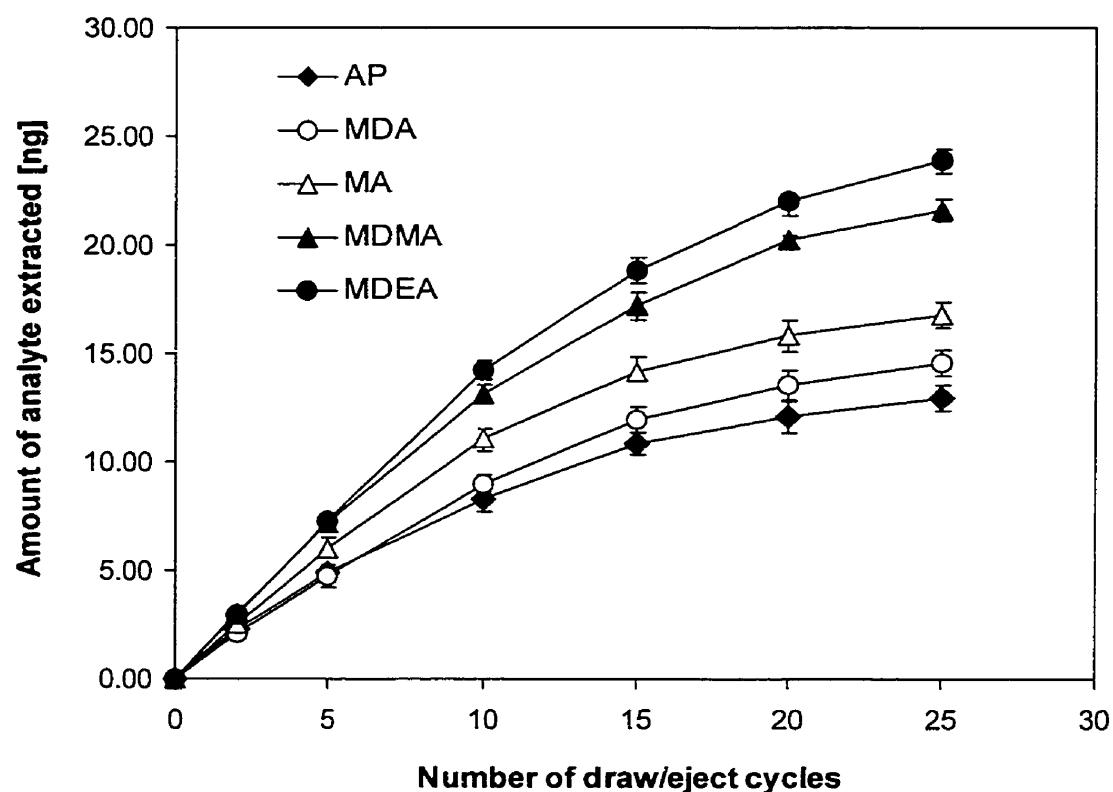


Figure 5-5. Extraction-time profiles for stimulants obtained by the PPy coated capillary in-tube SPME. Samples were prepared in 100-mM carbonate buffer solutions, concentration of each stimulant was 50 ng/mL, and other conditions see experimental section.

Extraction time. The extraction time profile was evaluated by examining the effect of the number of extraction cycles on extraction efficiency. As shown in Figure 5-5, the

amounts of analytes extracted increased greatly when the number of extraction (draw/eject) cycles increased from 0 to 10. In contrast to a previous study [17] in which the extraction equilibrium was reached with a Omegawax capillary after 15 extraction cycles, in this study with the PPY coated capillary, the extraction equilibrium was not reached even after 25 extraction cycles. Further increase in extraction cycles continued to increase the amount of analyte extracted, but it also increased the extraction time. Therefore, to achieve sufficient extraction efficiency within a short period of time, 10 draw/eject cycles are used in this work. More extraction cycles could be used with PPY coating to realize higher extraction efficiency. In this study, all the results were obtained by using a 60-cm long capillary with 10 draw/eject cycles (40 μ L for each cycle at a flow rate of 100 μ L/min).

Sample matrix and pH effects. The effects of sample matrix and pH on the extraction of stimulants were evaluated using several buffer solutions with pH 3.0-11.5. As shown in Figure 5-6, the extraction efficiency of PPY coating to the analytes increased significantly when the sample pH increased from 3.0 to 10.0. Therefore, a 100-mM carbonate buffer with pH 10.0 was selected in this work. These results can be easily understood when considering the acid-base equilibrium of the analytes (their pK_a 's are between 9.5 to 10.0 [19]) in the solutions of different pH values. The extraction efficiency of a coating to the analytes depends on the inter-molecular interactions between the coating and analytes, including base-acid, π - π , dipole-dipole, and hydrophobic interactions, hydrogen bonding and ion exchange. Because the PPY coating is positively charged in its oxidized form (a weak acid) and the analytes also had positive charges at low pH solutions (the acid forms), the electrostatic repulsion between the coating and analytes led to the lower extraction efficiency in the low pH sample solutions. With the increase of pH in the sample solutions, the positive charges on the analytes were reduced, the base forms of analytes were increased, and the attractive inter-molecular interactions between analytes and PPY became dominant, therefore the extraction efficiency increased. Similar to a study on β -blockers [20], sample matrix also showed significant effect on the analyte extraction. For example, adding salt (NaCl) or using buffers (buffer 2 and buffer 3) in the sample solutions could enhance the analyte extraction relative to the pure water matrix (although these solutions had similar pH values). In a previous SPME-GC work on amphetamine (AP) and methamphetamine (MA) [14], an approximately linear relationship was found between salt concentration and analyte recovery, up to NaCl

saturation. Thus, to achieve reproducible analyte extractions and recoveries, it is crucial to maintain constant pH and ionic strength in the samples using a buffer solution.

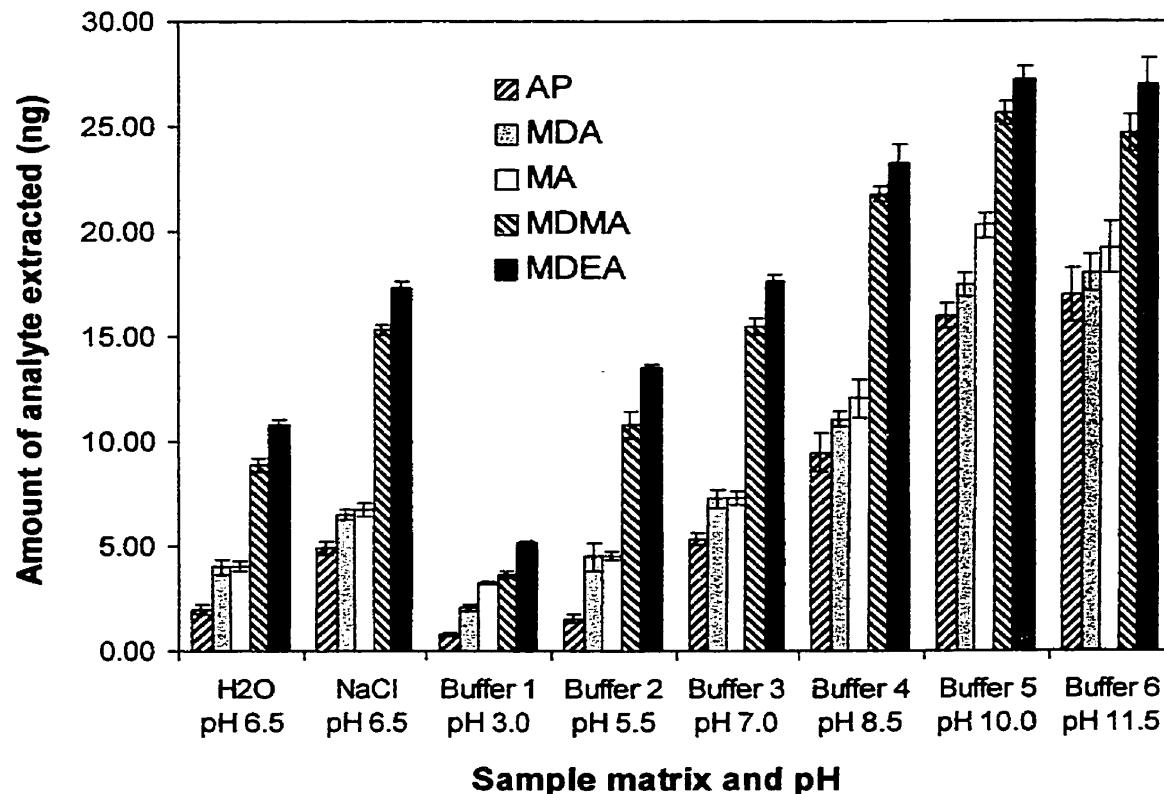


Figure 5-6. Effects of sample matrix and pH on the extraction efficiencies of PPy coating to the studied stimulants. Sample matrix and pH: (1) deionized water (pH 6.5), (2) NaCl saturated water solution (pH 6.5), (3) buffer 1 (Gly-HCl, pH 3.0); (4) buffer 2 (sodium acetate, pH 5.5), (5) buffer 3 (sodium phosphate, pH 7.0), (6) buffer 4 (Tris-HCl, pH 8.5), (7) buffer 5 (sodium carbonate, pH 10.0), and (8) buffer 6 (sodium phosphate-NaOH, pH 11.5). Buffer concentrations in all samples were 100 mM. Concentration of each compound in all the samples was the same: 100 ng/mL.

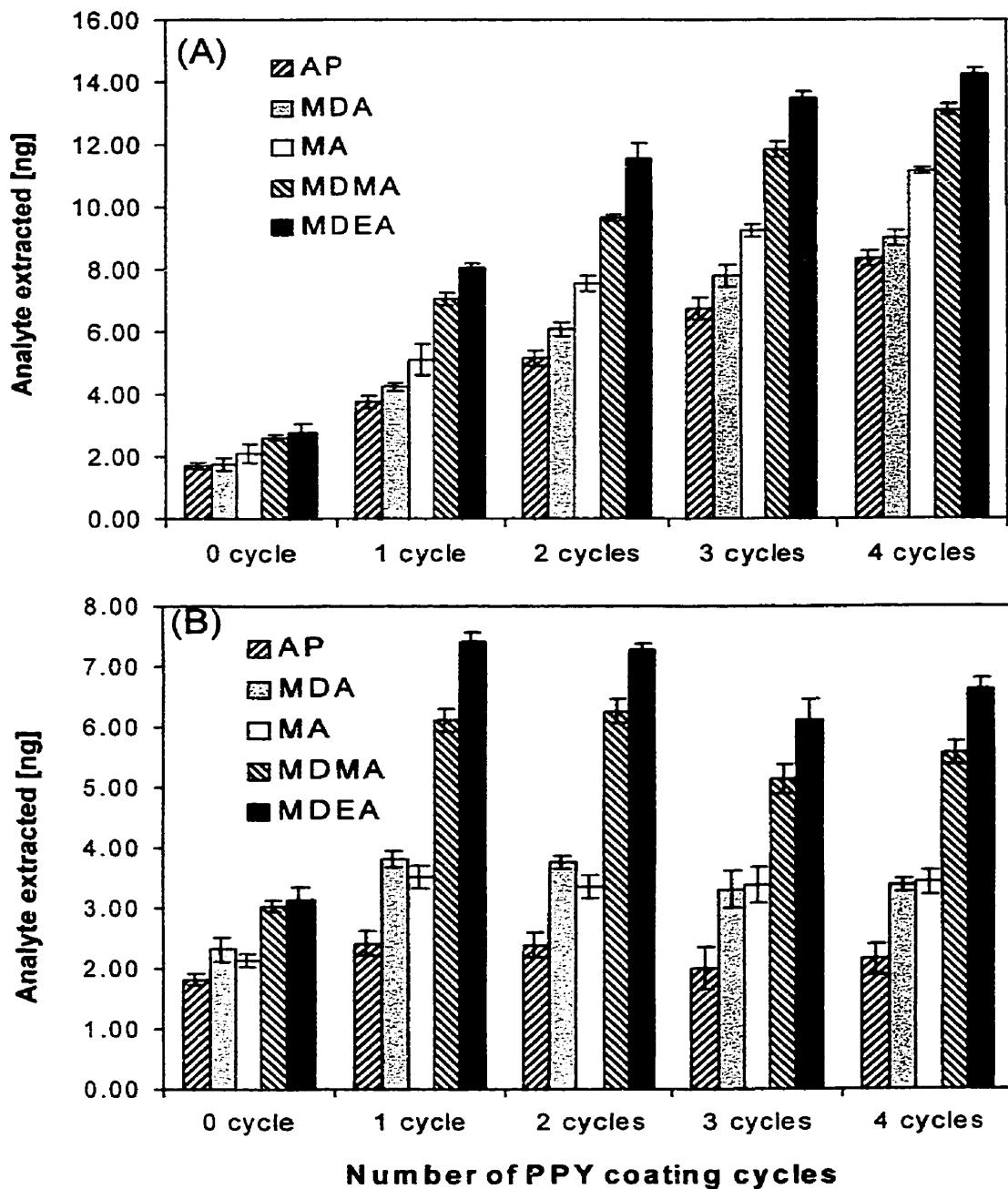


Figure 5-7. Effect of the PPy coating thickness on the extraction efficiency for the stimulants. (A) Each sample was prepared using a carbonate buffer with pH 10.0. (B) Each sample was prepared using deionized water (pH around 6.5). Concentration of each compound in all samples was the same: 50 ng/mL.

Effect of PPy coating thickness. The effect of coating thickness on extraction is one of the most important parameters for SPME [9-11]. For porous coatings such as PPy coating, increasing the coating thickness means not only an increase of the total coating volume (V_t), but also an increase of the total surface area ($C_{f\max}$). Therefore, it is expected from equation (1-3) described in chapter 1 that the amount of analyte extracted will increase when the coating thickness increases. This expectation has been confirmed experimentally by PPy coating SPME of aromatic compounds as described in chapter 4. However, in chapter 4, the effect of PPy thickness on extraction was examined only under the optimized pH conditions. In this work, the effect of coating thickness was evaluated for samples of two different pH values, one is higher and another is lower than the pK_a of the analytes. As shown in Figure 5-7 (A), at optimized sample pH (10.0), the extraction efficiency for all the studied compounds increased almost linearly with the increase of the PPy coating thickness. However, as seen in Figure 5-7 (B), a totally different trend was observed when the same experiments were performed for a low pH sample (sample was prepared directly in the deionized water, pH around 6.5). As discussed above, there were two main opposite interactions between the analytes and the PPy coating in the low pH samples, the electrostatic repulsion forces and the attractive inter-molecular interactions ($\pi-\pi$, dipole-dipole, hydrophobic and hydrogen bonding). The two opposite forces might cancel each other to certain degrees although both of them increased with the increase of coating thickness. Therefore, after an initial increase in extraction efficiency as compared with the non-coated host capillary, no significant changes in the extraction efficiency (actually, a slight decrease in extraction) were observed with further increase of coating thickness (Figure 5-7 (B)). These results demonstrate that when there are base-acid (or electrostatic) interactions between the analytes and the extraction phase in addition to the other inter-molecular interactions, the extraction properties of a coating toward the analytes will depend on both the sample pH and the coating thickness.

Analyte desorption. To recover the analyte from the extraction capillary, several solvents and buffer solutions were tested. The results showed that the best desorption solution was the mobile phase, because desorption of analytes with a mobile phase solution could be achieved easily by simply switching the six-port valve to the INJECT position. Although injecting 40 μL of methanol or acetonitrile could also recover the analyte from the capillary, it introduced an extra step.

Analysis time and analyte carryover effect. The extraction time for a 10-cycle extraction was about 8 min. Therefore, the whole analysis for the stimulants including extraction, desorption, separation and detection could be accomplished automatically within 20 min by the developed method. The analyte carryover (or memory) effect was not observed because the stimulants were desorbed completely by the mobile phase flow. In addition, the capillary was washed and conditioned by draw/eject cycles of methanol and pure water prior to each extraction (see Chapter 4 for in-tube SPME experiment).

Stability of in-tube PPy coating. In this work, one single PPy coated capillary was used for all the extraction experiments (except for the coating thickness experiments), and no significant changes in its extraction performance were observed after hundreds of extractions during the whole study period (about two months). More importantly, PPy coating is stable in solutions over a wide pH range. This quality of SPME coating provides an opportunity to manipulate the extraction efficiency and selectivity by adjusting the sample pH based on the acid/base property of the analytes.

5.3.3. Method performance

Due to the optimization of both mass detection and in-tube SPME conditions, a highly sensitive method for the determination of stimulants has been developed with detection limits ($S/N = 3$) of 8–56 ng/L for the analytes (Table 5-2). These sensitivities are at least one order of magnitude higher than that of a previous in-tube SPME study for the same compounds [17]. Increasing the number of extraction cycles and using a more sensitive and selective detection method can further increase the sensitivity of the method. The precision of the method varies between 0.8 to 6.4 % RSD ($n = 5$), depending on the analytes and their concentrations (Table 5-2).

To test the linearity of the calibration curves, various concentrations of stimulants ranging from 0.05 to 400 ng/mL were analyzed. The calibration curves were constructed by comparing peak area counts against analyte concentrations. As shown in Table 5-2, a linear relationship was obtained for each analyte in the concentration range of 0.1 – 100 ng/mL (11 point calibrations, 3 repeats for each point).

Table 5-2. Linear regression data, precision (RSD) and detection limits (DL) for stimulants in water samples

Analytes	Regression line ^a		Correlation	DL (ng/L)	RSD % (n = 5) ^b	
	Slope ($\times 10^5$)	Intercept ($\times 10^5$)			within-day	between-day
AP	0.7515	1.8632	0.9986	23	3.3	6.4
MDA	0.2674	0.6061	0.9987	56	2.6	4.5
MA	2.5318	5.3341	0.9996	8	1.2	3.2
MDMA	0.6125	0.7502	0.9991	34	0.8	2.4
MDEA	0.7372	0.7483	0.9989	22	1.7	2.5

^a Obtained from calibration curves by plotting the peak area counts against analyte concentration in the range of 0.1 – 100 ng/mL; and number of data points: 11.

^b Obtained from the samples containing each analyte 50 ng/mL.

5.3.4. Analyses of urine and hair samples

Chromatograms for the analyses of urine and hair samples are shown in Figures 5-8 and 5-9. No interference peaks were detected from the non-spiked urine sample (a) as shown in Figure 5-8(A). Only a small peak from non-spiked hair sample was detected at retention time 3.6 min, which co-eluted with the first analyte peak in the spiked hair samples as shown in Figure 5-9. Although the chromatographic separations for the analytes are not perfect, the mass detector under SIM can selectively detect and quantify each of the 5 stimulants in the sample mixtures as shown in the selected ion monitoring chromatograms (SIM), Figure 5-8 (B) and Figure 5-9 (B).

The initial recovery experiments for the spiked urine samples showed that the analyte recoveries (79–86 %) from urine samples without filtration were better than those (70–80 %) obtained from filtered samples, implying that filtration may cause loss of small amount of analytes. In addition, the recoveries of analytes from urine samples were lower as compared to those (close to 100 %) obtained from water samples. This is likely due to the sample matrix (the organic and inorganic components in urine), which may affect the extraction and mass detection processes. To validate the method for urine analysis, the calibration curves were reconstructed by spiking the urine samples (each 10- μ L) with various concentrations of stimulants ranging from 0.1 to 400 ng. As shown in Table 5-3, a linear relationship was obtained for each analyte in the concentration range of 0.5–100 ng/mL (9-point calibration, 3

repeats for each point). However, the slopes of these lines are smaller (7–20 % lower) than those obtained from water samples (Table 5-2). Similar results were also obtained for hair samples as shown in Table 5-4. A linear relationship was obtained for each analyte in the concentration range of 1–100 ng/mL (8-point calibration, 3 repeats for each point). However, the slopes of the calibration curves reconstructed from hair samples were even smaller than those from urine samples (Table 5-3), which indicated a larger matrix effect for hair samples. Therefore, in order to obtain accurate results, a standard addition or internal calibration method should be performed for the analysis of urine and hair samples to effectively nullify the matrix effect. As shown in Table 5-5, excellent analyte recoveries were achieved from spiked urine samples by applying the standard addition method to the samples. By this method, similar results were also obtained for hair analysis (data not shown). The quantification limits of stimulants in urine and hair samples, as shown in Table 5-3 and Table 5-4, were calculated as signal-to-noise ratio of 3 in comparison of peak heights of non-spiked samples and the spiked samples of the lowest detectable concentration [17]. The within-day and between-day variations for analyses of spiked urine and hair samples are also listed in these Tables.

Table 5-3. Linear regression data, precision (RSD) and quantitation limits (QL) for stimulants spiked in urine samples

Analytes	Regression line ^a		Correlation R^2	QL (ng/mL urine)	RSD % (n = 3) ^b	
	Slope ($\times 10^5$)	Intercept ($\times 10^5$)			within-day	between-day
AP	0.6004	1.7008	0.9987	13	5.0	5.3
MDA	0.2181	0.6012	0.9985	28	3.6	6.8
MA	2.0095	5.0091	0.9993	4	2.2	3.0
MDMA	0.5503	1.1087	0.9997	15	2.7	4.2
MDEA	0.6876	0.7763	0.9993	12	5.5	5.5

^a Obtained from calibration curves by plotting the peak area counts against analyte concentration in the range of 0.5 –100 ng/mL; and number of data points: 9.

^b Obtained from the spiked urine samples containing each analyte 50 ng/mL.

Table 5-4. Linear regression data, precision (RSD) and quantitation limits (QL) for stimulants spiked in hair samples

Analytes	Regression line ^a		Correlation R^2	QL (ng/10 mg hair)	RSD % (n = 3) ^b	
	Slope ($\times 10^5$)	Intercept ($\times 10^5$)			within-day	between-day
AP	0.4672	1.2643	0.9988	0.60	5.4	6.7
MDA	0.1813	0.5803	0.9984	0.85	6.3	8.0
MA	1.5788	5.5121	0.9995	0.25	0.8	2.5
MDMA	0.4612	0.8529	0.9996	0.65	2.1	4.4
MDEA	0.5557	0.7399	0.9998	0.52	3.5	3.8

^a Obtained from calibration curves by plotting the peak area counts against analyte concentration in the range of 1–100 ng/mL; and number of data points: 8.

^b Obtained from the spiked hair samples containing each analyte 50 ng/mL.

Table 5-5. Recoveries of stimulants spiked to urine samples

Analytes ^a	Recovery (%), mean \pm SD ^b (n = 3)		
	0.5 µg/mL urine	5 µg/mL urine	10 µg/mL urine
AP	92.4 \pm 5.2 (5.6)	94.6 \pm 4.7 (5.0)	100.7 \pm 1.7 (1.7)
MDA	89.5 \pm 3.9 (4.4)	102.3 \pm 3.7 (3.6)	97.9 \pm 4.3 (4.4)
MA	94.7 \pm 2.6 (2.7)	95.9 \pm 2.1 (2.2)	99.6 \pm 3.5 (3.5)
MDMA	92.2 \pm 4.3 (4.7)	98.9 \pm 2.7 (2.7)	105.1 \pm 4.1 (3.9)
MDEA	93.0 \pm 6.6 (7.1)	95.0 \pm 5.2 (5.5)	94.3 \pm 6.0 (6.4)

^a 10 µL of urine was used for each analysis, see experimental section for details. ^b

Relative standard deviations (%) are shown in parentheses.

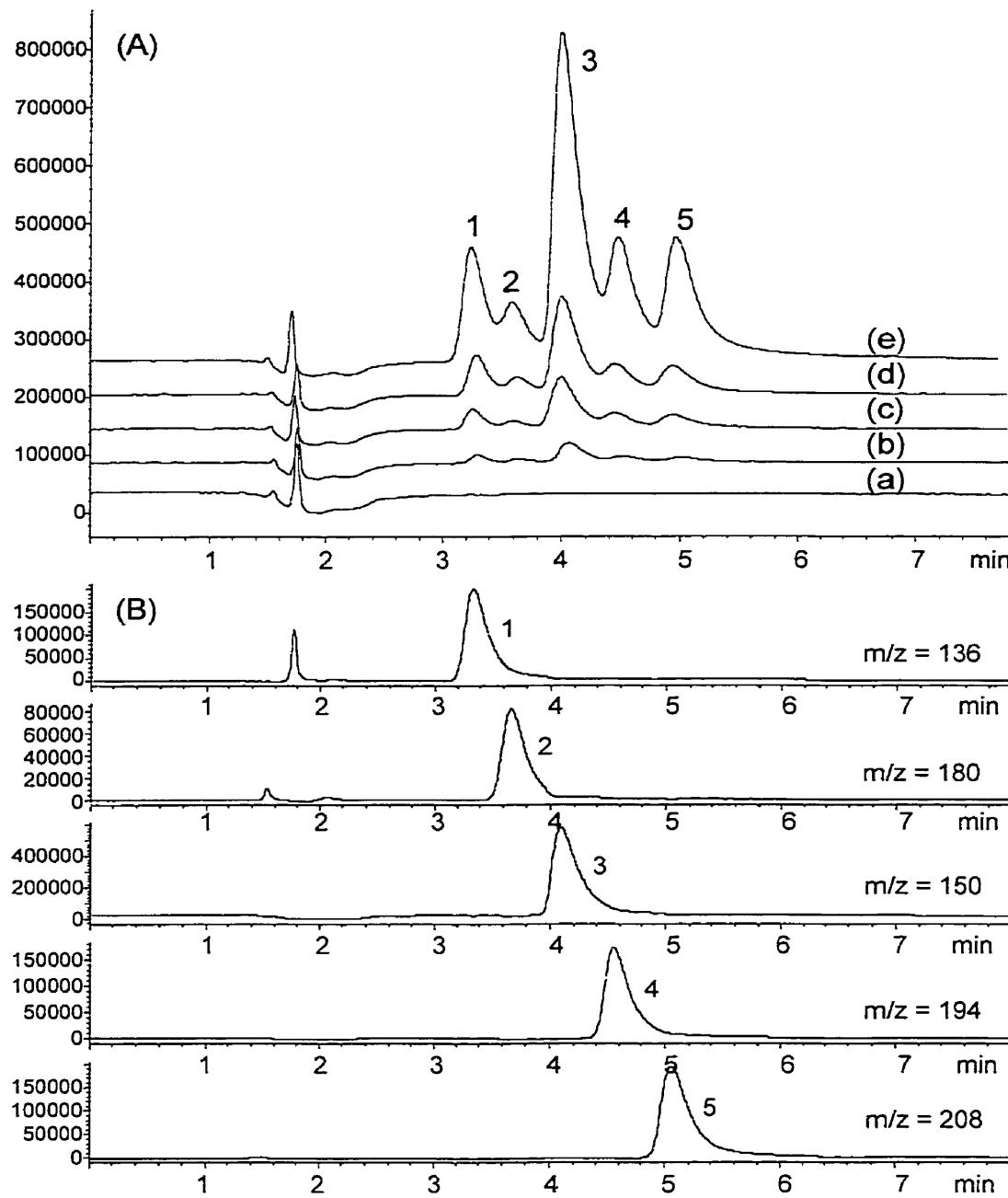


Figure 5-8. Total ion current (TIC) and selected ion monitoring (SIM) chromatograms obtained from urine samples by in-tube SPME-HPLC-MS. (A) TIC chromatograms obtained from urine and spiked urine samples. Each of the spiked urine sample chromatograms is offset by 10 % for clarity. (B) SIM chromatograms obtained from the spiked urine sample (e). The amounts spiked for each compound in the urine samples are: (a) 0 ng/mL (sample blank), (b) 1 ng/mL, (c) 5 ng/mL, (d) 10 ng/mL and (e) 50 ng/mL. Peak identification: 1. AP (m/z 136), 2. MDA (m/z 180), 3. MA (m/z 150), 4. MDMA (m/z 194), and 5. MDEA (m/z 208).

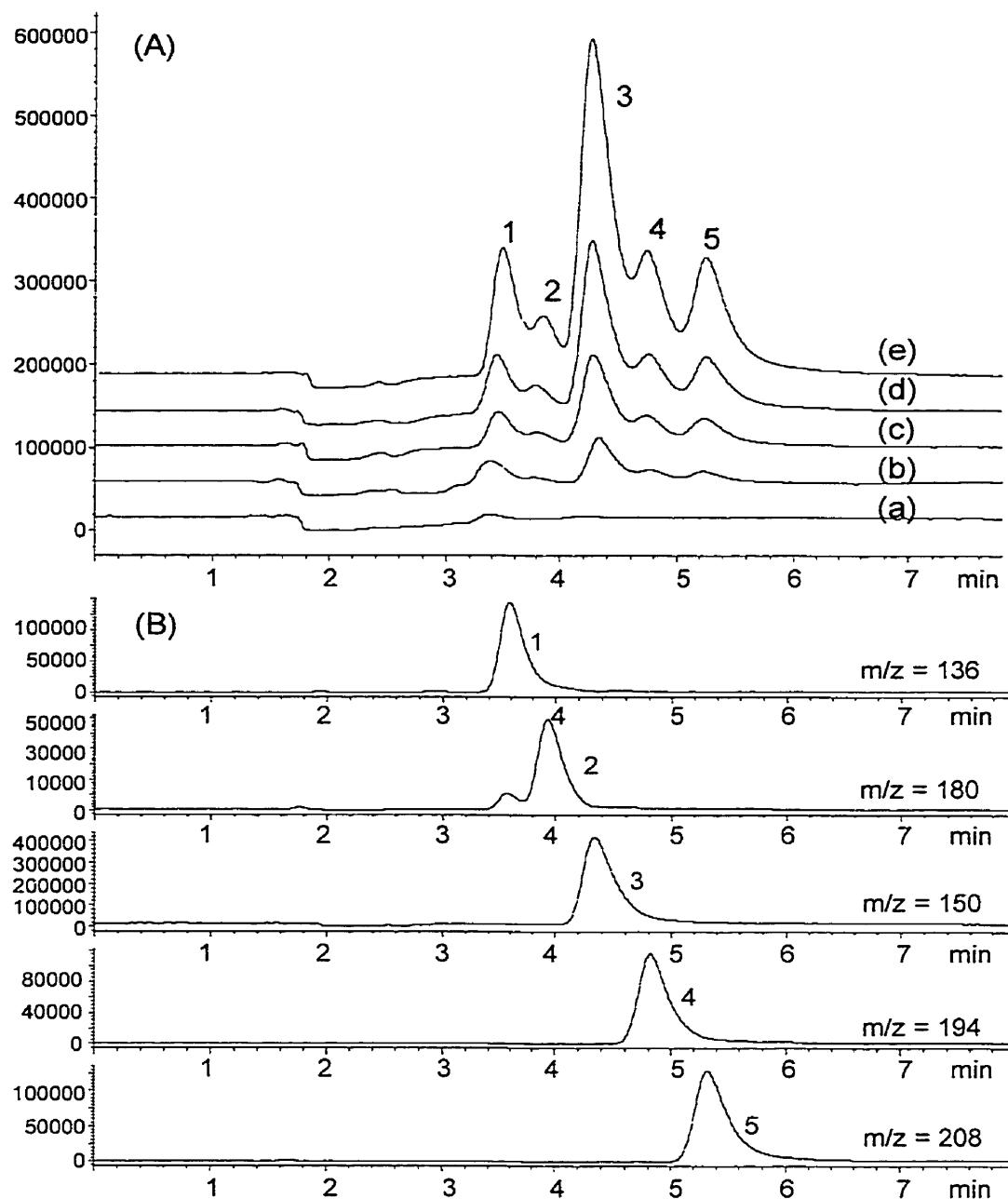


Figure 5-9. Total ion current (TIC) and selected ion monitoring (SIM) chromatograms obtained from hair samples by in-tube SPME-HPLC-MS. (A) TIC chromatograms obtained from hair and spiked hair samples. Each of the spiked hair samples is offset by 10 % for clarity. (B) SIM chromatograms obtained from the spiked hair sample (e). The amounts spiked for each compound in the hair samples are: (a) 0 ng/mL (sample blank) (b) 5 ng/mL, (c) 10 ng/mL, (d) 20 ng/mL and (e) 50 ng/mL. Peak identification: see Figure 5-8.

5.4. Summary

The ability and potential application of the PPy coating for in-tube SPME of stimulants from biological matrices have been demonstrated. Compared with the in-tube SPME method using commercial capillary coatings, higher sensitivity can be achieved using the PPy coated capillary due to its higher extraction efficiency to the stimulants. The extraction efficiency and selectivity of the PPy coating towards basic, acidic and amphiprotic compounds are pH dependent due to the different base/acid properties of the compounds and the coating in various pH solutions. Since the PPy coating is stable in solutions over a wide pH range, it is possible to achieve high extraction efficiency and selectivity by using a suitable sample pH. In addition, the PPy coating thickness has a significant effect on analyte extraction for in-tube SPME.

The PPy coated capillary in-tube SPME method developed in this work can be extended to extract other stimulants or drugs from biological samples. For examples, nine β -blockers in human urine and serum samples have been determined recently by coupling the PPy in-tube SPME with HPLC-MS [20].

5.5. References

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CHAPTER 6

SPECIATION OF ORGANOMETALLIC COMPOUNDS

6.1. Introduction

Chemical speciation has attracted great interest since the toxicity and mobility of an element, and its biological and environmental importance are highly related to the chemical forms of the element in the sample. It is well known that the inorganic species of arsenic such as arsenite (As(III)) and arsenate (As(V)) are more toxic than the organoarsenicals such as monomethylarsonic acid (MMAs) and dimethylarsinic acid (DMAs), and arsenobetaine (AsB) and arsenocholine (AsC) are relatively non-toxic [1-2]. The opposite trend is true for the tin species, *i.e.* the organic species of tin are more toxic than the inorganic forms, following the order of tributyltin (TBT) > dibutyltin (DBT) > monobutyltin (MBT) [3-5]. For organoarsenic species, AsB has been the most extensively studied owing to the high levels of this compound found in many edible fish, shellfish and seafood products [6]. TBT has been one of the most investigated organotin compounds because of its use in antifouling paints and the increasing concern for its release into the aquatic and sedimentary environments [3,4]. TBT is toxic to shellfish in water at ng of Sn /mL levels [5]. The accumulation of TBT in shellfish and other organisms in water as well as in the sediment materials may also lead to toxic effects in human. Therefore, highly sensitive and selective techniques are required for analysis of TBT.

For the speciation of both organotin and organoarsenic compounds, the coupling of chromatographic separation with element-specific spectrometric detection has proven to be useful. In particular, high performance liquid chromatography (HPLC) with inductively coupled plasma mass spectrometry (ICP-MS), atomic absorption spectrophotometry (AAS), inductively coupled plasma atomic emission spectrometry (ICP-AES), and atomic fluorescence spectrometry (AFS) have played important roles in organoarsenic [7-16] and organotin [17-19] species speciation studies. Although these techniques, especially HPLC-ICP-MS, offer the advantages of high selectivity and sensitivity, chemical species identification is based entirely on the separation condition (retention time) since only

elemental information is provided by these detection methods. The difficulty and uncertainty for chemical speciation increase when analysing real samples, especially for samples with complicated matrices, due to the possibility of peak shift and the presence of unknown components [6,10]. Recently, there has been an increasing interest in using electrospray (ES) as an ionization source for mass spectrometric analysis of organometallic compounds [6, 20-31]. Electrospray mass detection (ES-MS) provides the unique opportunity for analysis of molecular forms of species, because ES is a ‘soft’ ionization technique, which produces gas-phase analyte ions (which preserve the molecular or complex structure of the analytes) directly from pre-existing ions in solution. This detection technique also offers the possibility to observe both the molecular and elemental signals of the metal species when used as a dual detector [6,20,30]. The efficiency for analyte speciation can be improved greatly by coupling HPLC with ES-MS because both analyte retention and analyte molecular information can be obtained using this hyphenated technique.

For the analysis of trace organometallic compounds in complex matrices, sample treatments such as extraction, preconcentration and clean-up steps are often required to improve the sensitivity and selectivity. However, traditional off-line procedures such as solid phase extraction, solvent extraction and other techniques [17-19], are time-consuming and labour-intensive, and require large volumes of sample and solvent. Although several papers have been published for the on-line preconcentration of inorganic arsenic ions, none of them has included organoarsenic compounds [9,32].

As has been shown in the previous chapters, in-tube SPME can be easily coupled on-line with HPLC to achieve automated extraction and concentration of analytes from water samples. The extraction efficiency and selectivity of in-tube SPME can be improved by using an appropriate coating based on the properties of the analytes. In this chapter, in-tube SPME of organoarsenic and organotin compounds from aqueous solutions have been studied, respectively, using a PPY coated capillary and several commercial capillaries. Compared with commercial capillaries, the PPY coated capillary has demonstrated higher extraction efficiency for most of the organoarsenicals studied, especially for the anionic species monomethylarsonic acid (MMAs) and dimethylarsinic acid (DMAs). However, for cationic species such as AsC and TBT, a Supel-Q PLOT capillary (one of the commercial capillaries tested) provides better extraction efficiency. The results are in good agreement

with the theoretical expectation obtained from the structure of PPy and the analytes studied (see Figure 6-1). The higher extraction efficiency of PPy to neutral organometallic compounds such as AsB is due to the hydrophobic interactions and interactions from polar functional groups between polymer and analytes. The much higher extraction efficiency of PPy to anionic organoarsenic compounds but lower extraction efficiency to cationic species is due to the additional electrostatic interaction or anion exchange property of PPy. Since the PPy coated capillary showed better extraction efficiency to most of the arsenic compounds studied, it was used for in-tube SPME and speciation of organoarsenic compounds when coupled with HPLC-MS. While the Supel-Q PLOT capillary was selected for analysis of TBT when coupled with HPLC-MS.

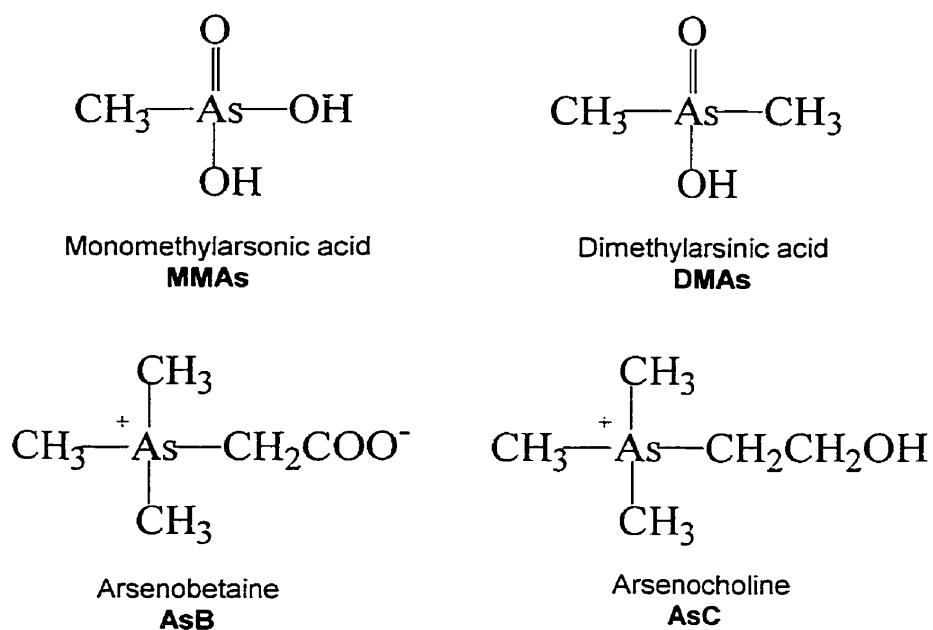


Figure 6-1. Structures of the organoarsenic compounds studied.

6.2. Experimental

6.2.1. Chemicals and reagents

Arsenic compounds studied include sodium methylarsonate (MMAs, Chemical Service, West Chester, PA, USA), dimethylarsinic acid (DMAs, sodium salt, Sigma Chemical Co., St. Louis, MO, USA), arsenobetaine (AsB), and arsenocholine (AsC). AsB

and AsC were synthesized using literature methods [33,34]. A standard solution (1mg/ml) for each arsenic compound was prepared in pure water, from which a chromatographic standard (mix) was prepared and diluted gradually with water when necessary.

Tributyltin chloride (Bu_3SnCl or TBT, 98%), dibutyltin dichloride (Bu_2SnCl_2 or DBT, 95%) and butyltin trichloride ($BuSnCl_3$ or MBT, 95%) were obtained from Alfa Aesar (Johnson Matthey, Ward Hill, MA, USA). Stock solutions of these compounds were prepared in methanol and were refrigerated when not in use. A mixture of these compounds was prepared by mixing a certain amount of the above individual solutions in methanol. Analytical solutions were prepared by spiking a certain amount of the mixture in mobile phase solution or deionized water.

The dogfish muscle reference material, DORM-2, and the harbour sediment reference material, PACS-2, were obtained from the CNRC (Canadian National Research Council, Ottawa, Ontario, Canada). Other chemicals were obtained from Aldrich (ON, Canada). All solvents used in this study were of analytical reagent-grade or HPLC grade. Water was obtained from a Barnstead/Thermodyne NANO-pure ultrapure water system (Dubuque, IA, USA).

6.2.2. Instrument and analytical conditions

The HPLC system used was a Model 1100 series LC-MS (see Chapter 4). An anion exchange chromatographic method was used for the separation of organoarsenic compounds. Separation conditions: column, SUPELCOSIL LC-SAX1 (25 cm × 4.6 mm i.d., 5 μ m particle size) from Supelco (Bellefonte, PA USA); mobile phase, 30 % methanol + 70% aqueous solution (100 mM ammonium acetate, 0.6% acetic acid); flow rate, 0.5 mL/min. The optimized ES-MS detection conditions for arsenic compounds: nebulizer gas, N_2 (50 psi); drying gas, N_2 (10 L/min, 350°C); fragmentor voltage, 60 V for MMAs and DMAs, and 70V for AsC and AsB; capillary voltage, 1200 V; ionization mode, positive; mass scan range, 100-300 amu; selected ion monitoring (SIM), m/z 165 (AsC), 179 (AsB), 141 (MMAs), and 139 (DMAs).

The separation conditions for organotin compounds: column, SUPELCOSIL LC-18 (5 cm × 4.0 mm, 5 µm particle size) from Supelco (Bellefonte, PA USA); mobile phase, 80 % methanol + 20% aqueous solution (0.1% trifluoroacetic acid, TFA); flow rate, 0.5 mL/min. The optimized ES-MS detection conditions for TBT: nebulizer gas, N₂ (55 psi); drying gas, N₂ (9 L/min, 350°C); capillary voltage, 2500 V; ionization mode, positive; mass scan range, 100-300 amu. Variable fragmentor voltages were used for different selected ions: 40 V for m/z 291 (Bu_3Sn^+), 60 V for Bu_2SnH^+ (m/z 235), 80 V for BuSnH_2^+ (m/z 179), 120 V for SnH_3^+ (m/z 123).

The preparation for the PPy coated capillary was described in Chapter 2. In-tube SPME and the technique of coupling automated in-tube SPME with HPLC were described in Chapter 4.

6.2.3. Sample preparation

For the analysis of organoarsenicals in tap water samples, each of the tap water samples (final sample volume: 1 mL) spiked with 0, 20, 50, and 100 ng/mL of the analytes, respectively, was set into the autosampler and analyzed by the developed method. The results were compared with those of non-spiked water samples. For the analysis of organoarsenic compounds in the DORM-2 reference material, sample treatment was performed according to the method reported previously [35,36]. In brief, 0.5 g of DORM-2 samples was placed in each of the four 30-mL glass centrifuge tubes. Employing the method of standard additions, appropriate spikes were added to each tube as well as 10 mL of methanol-water (1:1 v/v) solution. Each tube was sonicated for 30 min, and centrifuged at 3200rpm for 10 min. The extracts were removed by a Pasteur pipette. The extraction process was repeated three times for each sample and the extracts were combined, evaporated to dryness at room temperature with the aid of nitrogen, dissolved in 10 mL deionized water, diluted further with water if necessary, and finally analyzed by in-tube SPME-HPLC-MS.

For the analysis of organotin compounds in lake water samples (collected from a local area), each of the water samples (final sample volume: 1 mL) spiked with different concentrations of the analytes (including MBT, DBT and TBT) was set into the autosampler and analyzed by the developed method. The results were compared with those obtained from

non-spiked lake water sample and the pure water samples spiked with the same amounts of analytes. For the analysis of TBT in a sediment reference material (PACS-2), TBT was extracted from the sample matrix by the 1-butanol method given by Siu *et al.* [28]. Briefly, 4 g of PACS-2 samples was placed in each of the three 50-ml glass centrifuge tubes. Using the method of standard additions, appropriate spikes were added to each tube as well as 8 ml of 1-butanol. The mixtures were sonicated for 1 h, and then centrifuged at about 3200 rpm for 10 min. The 1-butanol phase was removed from each centrifuge tube. A 100 μ L of the 1-butanol solution was first diluted to 1 ml with the mobile phase solution, then a 100 μ L of this diluted solution was used for TBT analysis after another 10 times dilution with deionized water.

6.3. Results and discussion

6.3.1. ES-MS detection and chromatographic separation

6.3.1.1.Organoarsenic compounds

To select the monitoring ion for each of the arsenic compounds studied, mass spectra under positive ion mode were analyzed by liquid injection. As shown in Figure 6-2, very little fragmentation was observed for each compound. For compounds MMAs, DMA_ns and AsB, the most intense ions (base ions) observed were their protonated molecular ions $[(M+H)^+]$; for AsC which exists as a cation in solution, the most intense ion was its molecular ion (M^+). Therefore, these base ions were chosen for mass detection in selected ion monitoring (SIM) mode (i.e. m/z 165 for AsC, m/z 179 for AsB, m/z 141 for MMA_ns and m/z 139 for DMA_ns). As seen in Figure 6-2, the mass spectra for MMA_ns and DMA_ns showed a number of ions at m/z values greater than that of the protonated molecular ions. These ions are probably adducts and polymeric compounds of the analytes or matrix components (such as Na⁺). For example, the second intense ion with m/z 163 in MMA_ns spectrum may correspond to the sodium cationized molecule ion $[(M+Na)^+]$. Similar mass spectra were also obtained in a recent ES-MS study on these arsenic compounds in which the possible sources for these ions were discussed [25]. To optimize the mass detection conditions, several instrumental parameters were evaluated and were discussed elsewhere [26]. The optimized

mass detection conditions for the studied arsenic compounds were summarized in the experimental section.

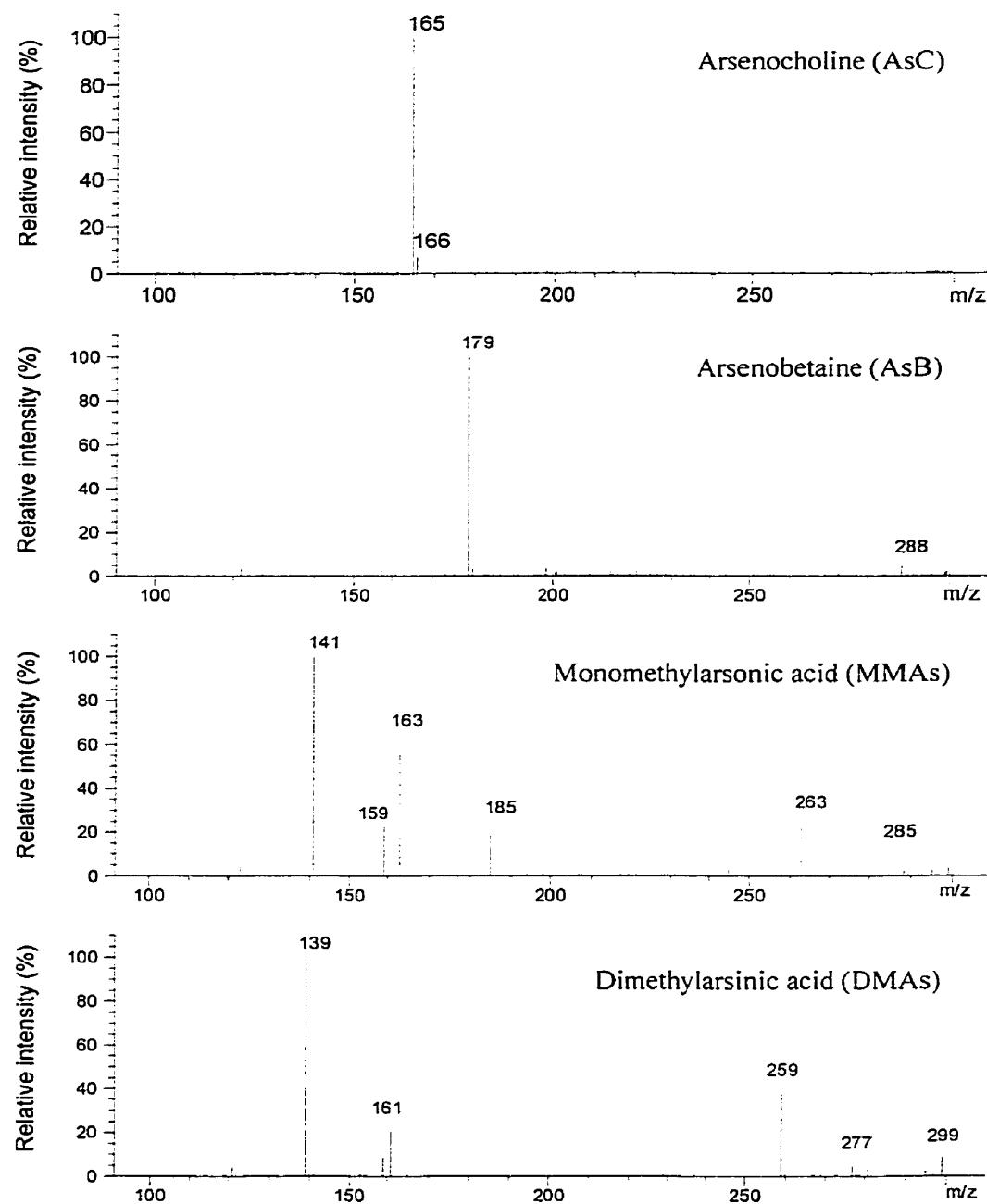


Figure 6-2. Mass spectra of the arsenic compounds obtained under scan mode at fragmentor voltage of 60 V. Concentration used for each compound, 10 µg/mL; Injection volume, 20 µL. Other conditions see experimental section.

The phosphate mobile phase, often used for anion exchange separation of arsenic compounds, is not compatible with ES-MS detection due to its low volatility [8]. In this study, a mobile phase of 30% methanol and 70% aqueous solution (100 mM ammonium acetate, 0.6 % acetic acid) was used to achieve good separation and sensitive detection for the four organoarsenic compounds studied.

6.3.1.2.Organotin compounds

The ES mass spectra of tributyltin chloride at different fragmentor voltages are shown in Figure 6-3. At fragmentor voltages lower than 35 V as shown in Figure 6-3 (a), only the isotopic distribution of the molecular ion Bu_3Sn^+ (or TBT $^+$) was observed with m/z 291 as the most abundant TBT isotope. At higher fragmentor voltages as shown in Figure 6-3 (b) to (d), the fragmentation of Bu_3Sn^+ occurred. The fragment ions, Bu_2SnH^+ (m/z 235), BuSnH_2^+ (m/z 179), and SnH_3^+ (m/z 123), were formed via loss of one, two, and three butyl groups from the parent Bu_3Sn^+ ion. As shown in Figure 6-3 (e), at fragmentor voltage 150V or higher than 150V, SnH^+ (m/z 121) and elemental ion Sn^+ (m/z 120) were detected.

For dibutyltin (DBT) dichloride, however, only very weak mass signals (with intensity less than 3% of that of TBT signal) were observed, representing the singly charged chloride adduct ion Bu_2SnCl^+ or $(\text{DBT-Cl})^+$ with m/z 269. No mass signals of $\text{Bu}_2\text{Sn}^{2+}$ or DBT^{2+} were observed under current mass detection conditions. For monobutyltin (MBT) trichloride, no useful mass spectral information related to this compound was obtained using the solvent and ionization conditions in this work, only very weak signals from solution matrix were detected. The reason for the weak mass signals observed for DBT and MBT is unclear. Similar results were also obtained by other researchers [28,29,37].

By detecting the selected ions that correspond to TBT, it was found that the presence of DBT and MBT in the sample matrix did not influence the determination of TBT when they were not separated completely from each other. Nevertheless, the conditions for separating TBT from the possible impure components and MBT and DBT were investigated using a reverse-phase chromatographic system. Figure 6-4 shows the total ion chromatogram (A) and the selected ion monitoring chromatogram (B) of a mixture containing TBT, DBT and MBT. By comparison with the chromatograms obtained from the individual analyte

solution (data not shown), it was found that the TBT peak (peak 2, with the highest intensity) was located at retention time 2.16 min. The smaller peaks (peak 1 and 3) at 1.38 and 2.72 min were probably impurities in the solutions as these two peaks were observed in both sample blanks and the three individual samples (TBT, MBT, or DBT) at m/z 121. However, no signals corresponding to the other four ions (m/z 291, 235, 179 and 123) were observed from the individual chromatograms of DBT and MBT. Therefore, to avoid possible interference from m/z 121 ion, only the four ions with m/z 291, 235, 179 and 123 were used in analysis of TBT.

For the analyses of TBT, the following mass detection conditions were further optimized: fragmentor voltage, capillary voltage, nebulizer gas pressure, drying gas flow rate and temperature. Since these parameters and their influences on the electrospray processes were described and discussed in detail in the previous studies [26,30], only the effects of the two important parameters, fragmentor voltage and capillary voltage, on mass signals of the target compound were addressed in this study. The effects of fragmentor voltage on the intensities of the studied ion signals are shown in Figure 6-5. Among all the ions detected, the Bu_3Sn^+ or TBT^+ ion (m/z 291) showed the highest intensity at a 40 V fragmentor voltage. Other ions showed their peaks at different voltages: 60 V for Bu_2SnH^+ (m/z 235), 80 V for BuSnH_2^+ (m/z 179), 120V for SnH_3^+ (m/z 123) and 135 V for SnH^+ (m/z 121). These results suggest that it is possible to achieve the highest sensitivity by simultaneous monitoring all these ions under their peak fragmentor voltages, since the intensity of total ion signal is the sum of the intensities of all the individual ion signals. Fortunately, the current mass detector can do this work by a fast switching of the fragmentor voltages. As shown in Figure 6-6, the optimum capillary voltage for the analysis of TBT was found between -2000 and -3000 V. Since the trends of capillary voltage effects on all the detected ions were quite similar, only the changing trend of the total ion signal with capillary voltages is shown in Figure 6-6. The optimised mass detection conditions were summarized in the experimental section.

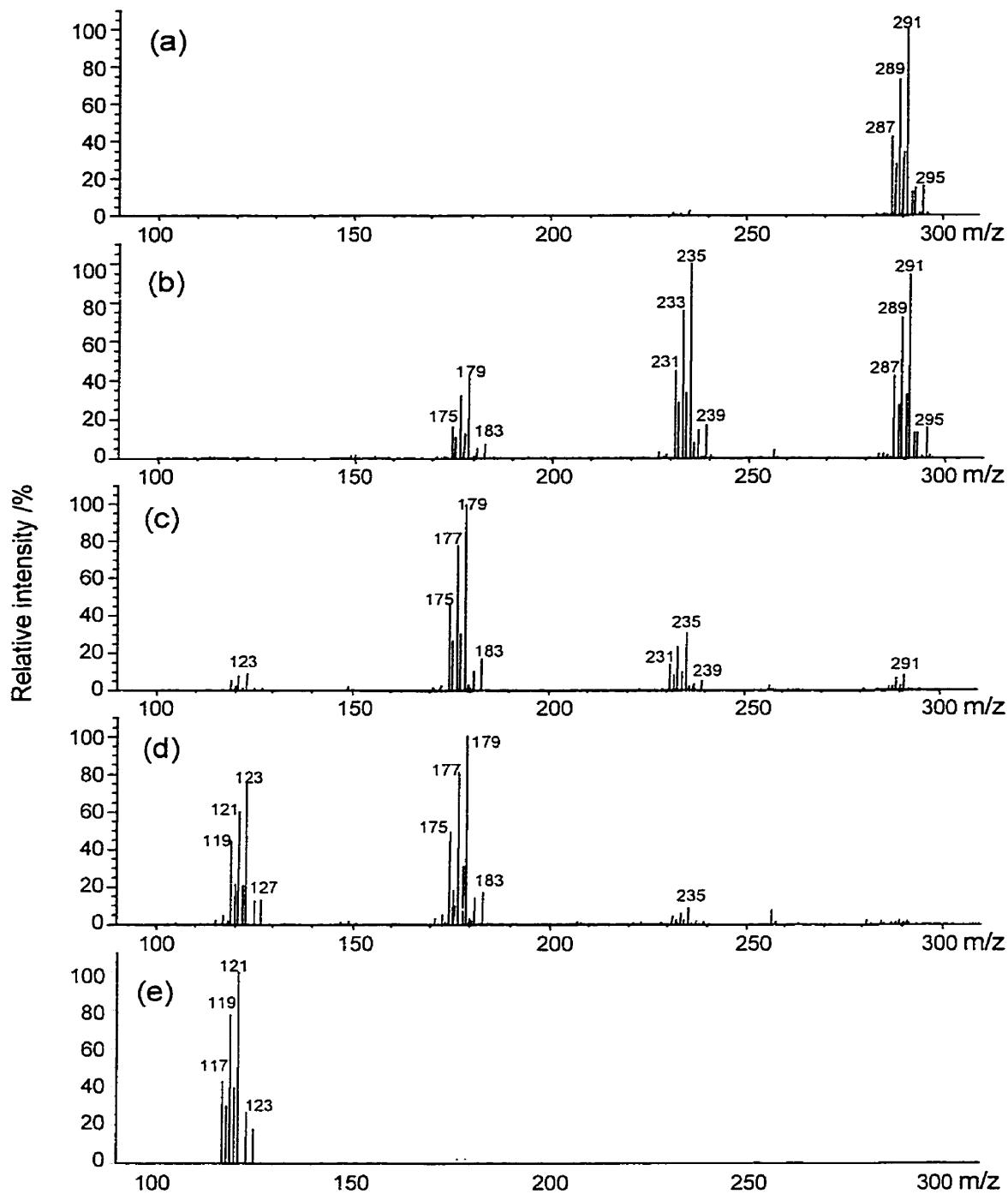


Figure 6-3. ES mass spectra of tributyltin chloride obtained under mass scan mode at different fragmentor voltages. (a) 30V, (b) 60V, (c) 80V, (d) 100V and (e) 150V. Concentration of tributyltin chloride: 10 µg/mL. Injection volume: 20 µL.

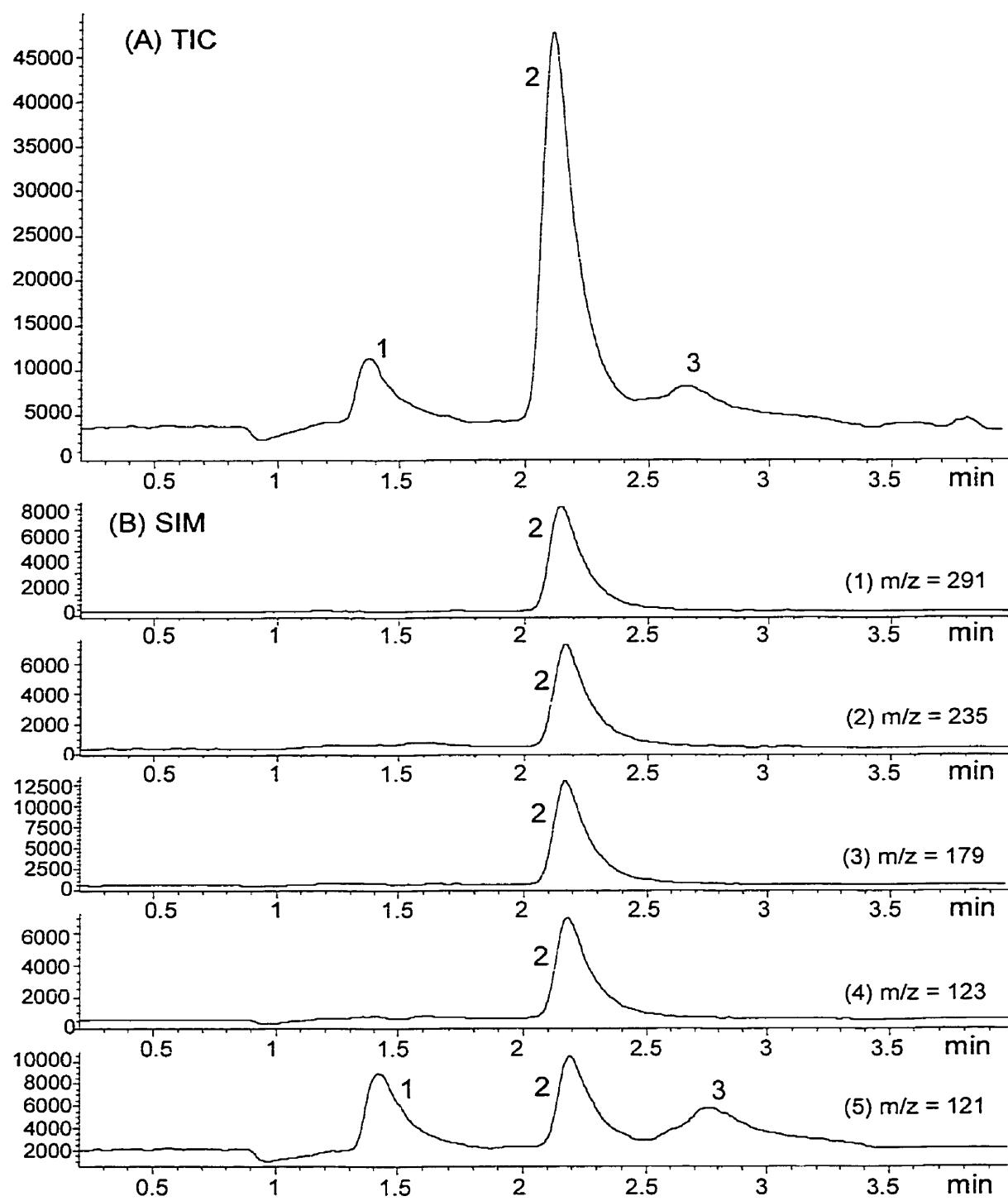


Figure 6-4. Chromatograms obtained by in-tube SPME-HPLC-ES-MS from an aqueous mixture, which contains 10 ng/mL of TBT and 50 ng/mL each of DBT and MBT. Peak identification: 1,3: impurities; 2: TBT. DBT and MBT were not detected.

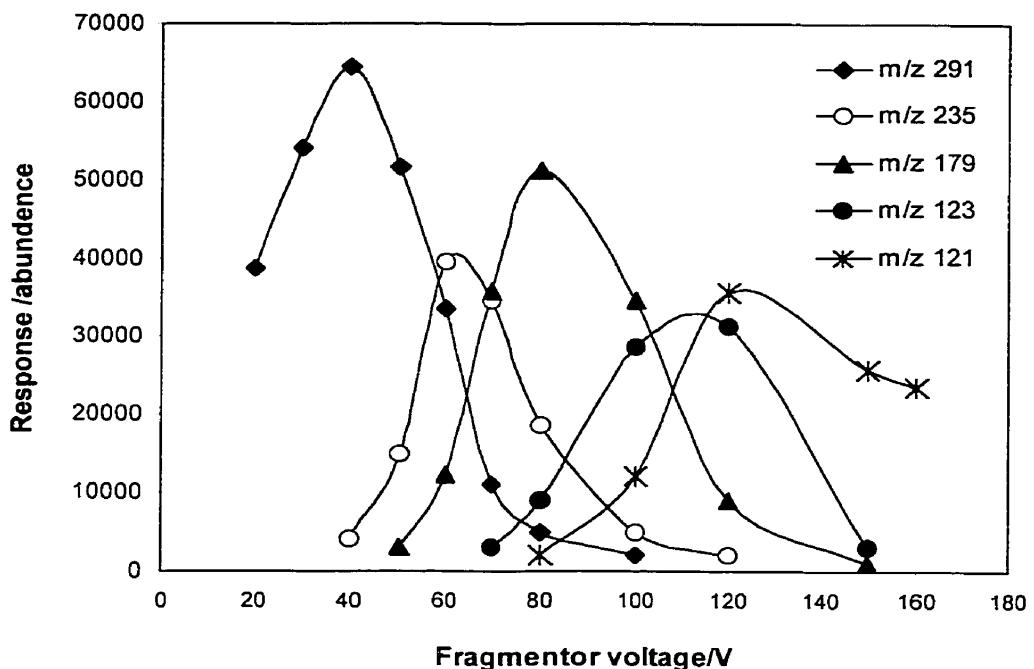


Figure 6-5. Effect of fragmentor voltage on signal intensity of the fragment ions obtained from a 10 μ g/mL TBT sample under mass scan mode. Other mass detection conditions: see the Experimental.

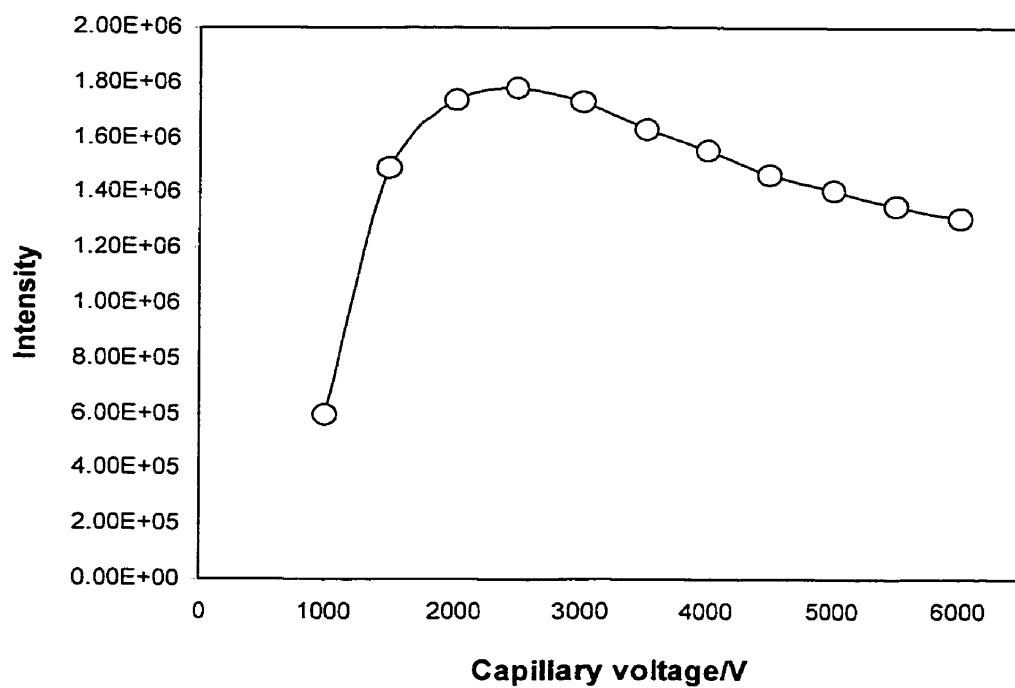


Figure 6-6. Effect of capillary voltage on the total ion signal intensity of a 50 ng/mL TBT sample obtained under selected ion monitoring mode. Other mass detection conditions: see the Experimental.

6.3.2. In-tube SPME

As discussed in the previous chapters, to optimize the extraction efficiency of in-tube SPME, several parameters such as capillary coatings, capillary length, coating thickness, extraction time profile, sample matrix and pH, and desorption conditions should be evaluated by coupling in-tube SPME with HPLC-MS.

The results obtained from the studies of capillary length effect and extraction time profile are similar to those described in chapters 4 and 5, therefore they will not be discussed. In this work, all the results were obtained using a 60cm long capillary with 15 draw/eject cycles ($40\text{ }\mu\text{L}$ for each draw/eject step at a flow rate of $100\text{ }\mu\text{L/min}$). The mobile phase was used for desorption of analytes from the extraction capillary.

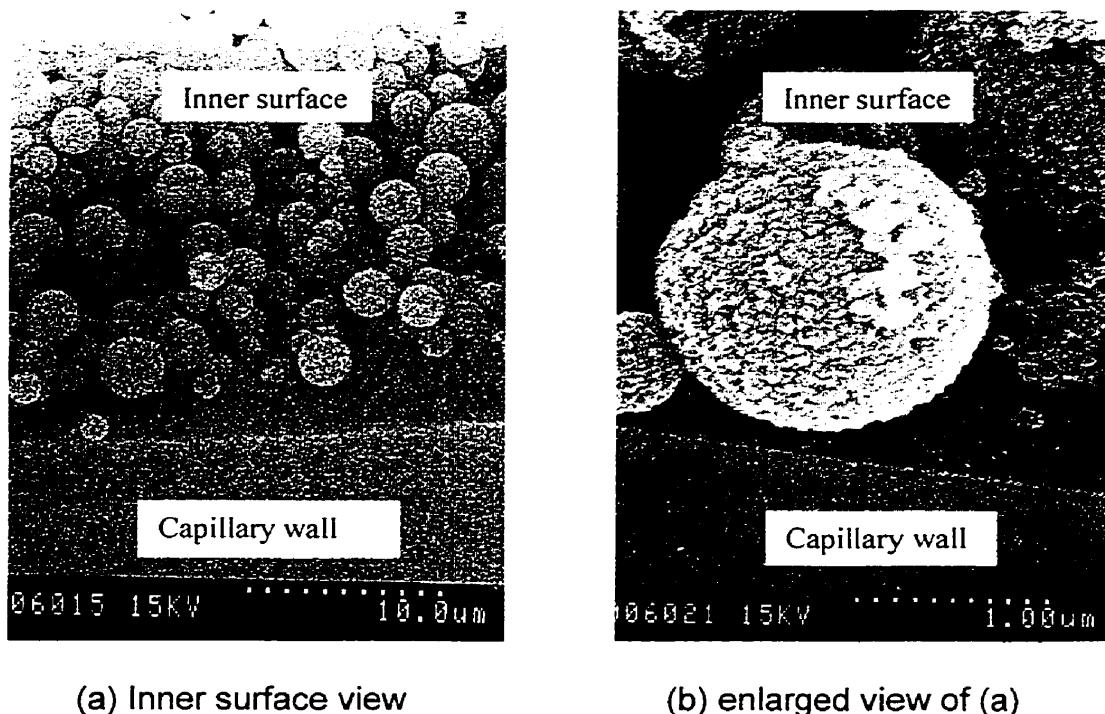


Figure 6-7. Scanning electron micrographs of a Supel-Q PLOT capillary. (a) Inner surface view, (b) enlarged inner surface view.

6.3.2.1.Capillary coating selection

The effects of different capillary stationary phases on extraction efficiency for the compounds studied were evaluated using a PPY coated capillary and five commercially available capillaries. The chemical structures and properties of the coatings were discussed in the previous chapters. The scanning electron microscopic (SEM) study showed that the coating surface structures of PPY (chapter 4) and Supel-Q PLOT (Figure 6-7) capillaries are porous, while other capillary coatings are non-porous (data not shown). The pore sizes of PPY coating are smaller than that of Supel-Q PLOT. However, the exact porosity of the coating is hard to measure accurately.

Extraction of organoarsenic compounds. As illustrated in Figure 6-8, among all the capillary coatings tested, a PPY coated capillary gave the best extraction efficiency towards all the compounds except for AsC. The hydrophobic interactions and the interactions from polar functional groups represent the main interactions between analytes and the commercial capillary coatings. For the PPY coating, however, another interaction — the electrostatic interaction — between the charged analytes and the positively charged PPY coating should also be considered. The extraction efficiency of the PPY coating for the four organoarsenic compounds decreases in the order of MMAs > DMAs > AsB > AsC. The dramatic difference in extraction abilities is mainly due to the difference in electrostatic interaction between PPY and the compounds, since in water solution, MMAs and DMAS are negatively charged anions, AsB is a neutral species, while AsC is a positively charged cation. Due to the same reason, the PPY coated capillary extracted less AsC compared with Supel-Q PLOT capillary, but it extracted significantly more of the anionic species such as MMAs and DMAs compared with all the commercial capillaries as shown in Figure 6-8. Among the commercial capillaries tested, Supel-Q PLOT showed the best extraction ability for all of the compounds studied. Therefore, the amounts of analytes extracted by the PPY coated capillary and the Supel-Q PLOT under the same experimental conditions were calculated according equation (4-1) and are listed in Table 6-1. The extraction efficiencies (extraction yields) of the PPY coated capillary toward MMAs and DMAs are much higher than for AsC, demonstrating clearly the selectivity of the PPY coating to anionic compounds.

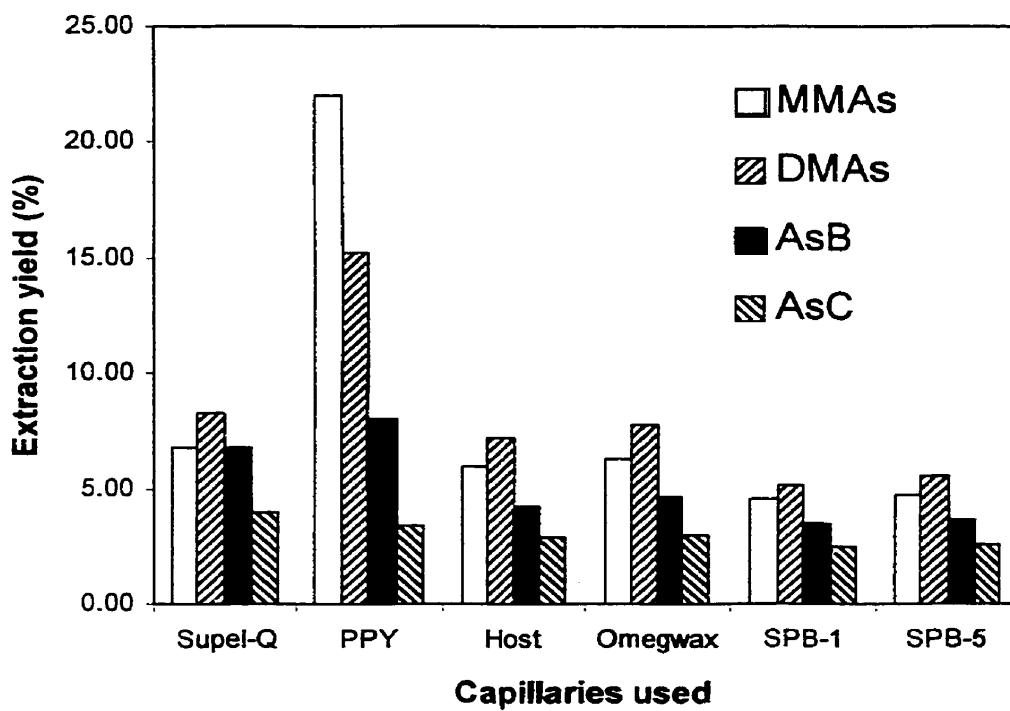


Figure 6-8. Comparison of the extraction efficiencies for arsenic compounds obtained by in-tube SPME with different capillaries. Concentration of each compound in the sample: 20 ng/mL.

Table 6-1. Comparison of the extraction efficiencies obtained by Supel-Q PLOT and the PPY coated capillary in-tube SPME

Analytes*	Amount extracted (ng) ^a		Extraction yield (%) ^b		Selectivity factor ($\alpha_{A/AsC}$) ^c	
	Supel-Q	PPY-coated	Supel-Q	PPY-coated	Supel-Q	PPY-coated
MMAAs	1.36	4.40	6.79	22.01	1.69	6.44
DMAAs	1.65	3.04	8.24	15.20	2.05	4.44
AsB	1.36	1.60	6.80	8.00	1.70	2.34
AsC	0.80	0.68	4.01	3.42	1.00	1.00

* The sample solution contains 20 ng/mL of each analyte. A 20- μ L sample containing 0.40 ng of each analyte was directly injected; A 1-mL sample was used for in-tube SPME. ^a For each analyte, the amount extracted (ng) was calculated according to equation (4-1). ^b Extraction yield (%) for each analyte was calculated by comparing the amount of analyte extracted (m) with the initial amount (M) of each analyte in the 1-mL sample solution. Yield (%) = m × 100/M. Since the initial amount of each analyte in the solution is 20 ng, Yield (%) = m × 100/20. ^c The selectivity factor ($\alpha_{A/AsC}$) is defined as the ratio of the amount extracted for the analyte A relative to that for AsC.

Extraction of TBT. As shown in Figure 6-9, among all the capillaries tested, the Supel-Q PLOT capillary (with a porous divinylbenzene polymer coating) gave the best extraction efficiency towards TBT probably due to its larger pore size (Figure 6-7), which was compatible to the bulky TBT molecules. While the PPY coated capillary showed the lowest extraction efficiency for TBT due to the electrostatic repulsion. The amounts of analytes extracted by different capillaries (as shown in Figure 6-9) under current experimental conditions were calculated according to equation (4-1) and the results obtained by Supel-Q PLOT are listed in Table 6-2.

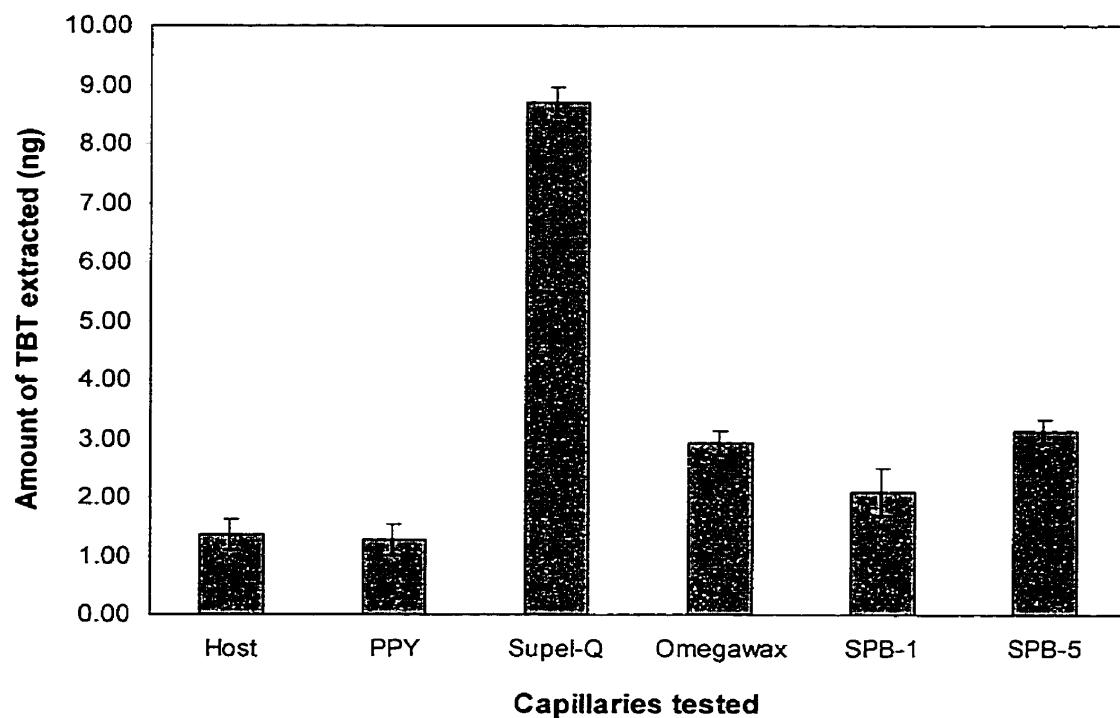


Figure 6-9. Comparison of the extraction efficiencies obtained by in-tube SPME with different capillaries. Concentrations of TBT in the tested solutions were all the same (20 ng/mL).

Table 6-2. Comparison of direct injection and in-tube SPME on the extraction of TBT

Peak area counts ($\times 10^5$)	Detector response	Amount of TBT extracted ^d		
Direct injection ^a	In-tube SPME ^b	$F^c (\times 10^{-5})$	TBT extracted (ng)	Extraction yield (%)
0.35	6.99	1.15	8.1	40.5

^a A 20- μ L (0.4 ng) solution (20 ng/mL TBT) was directly injected. ^b A 1-mL sample (20 ng/mL TBT) was extracted by in-tube SPME with a Supel-Q PLOT capillary, and desorbed with mobile phase flow. ^c Detector response factor (F) was calculated by comparing the amount injected (0.4 ng) and the peak area, $F = 0.4/(0.35 \times 10^5)$. ^d The amount of TBT extracted was calculated by equation (4-1); extraction yield (%) is the percentage of extracted amount of TBT per initial amount (20 ng) in a 1-mL sample solution.

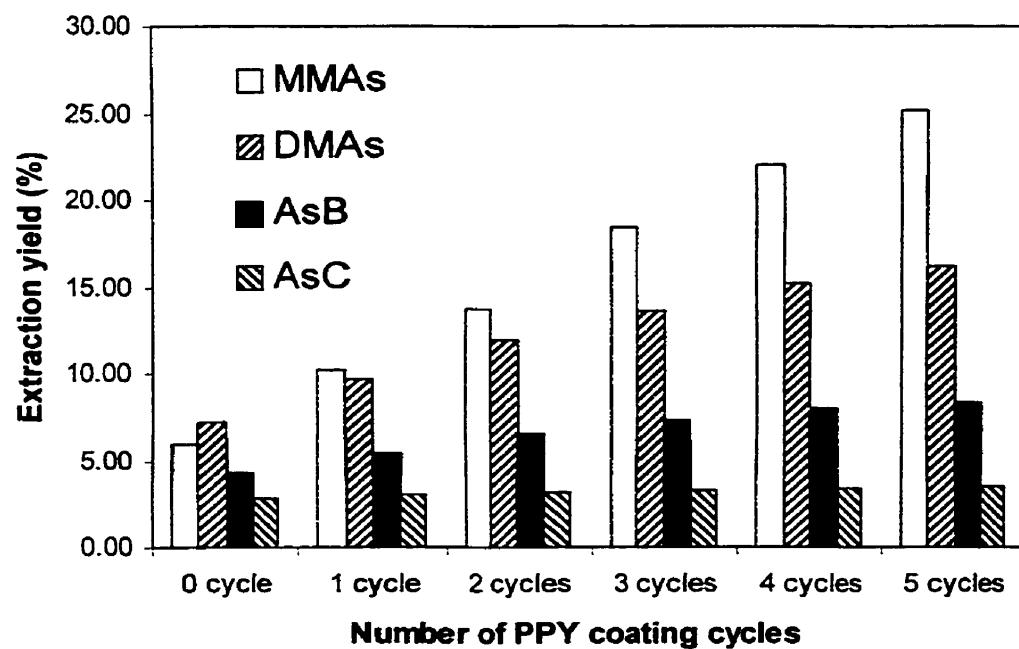


Figure 6-10. The effect of PPY coating thickness on the extraction efficiency for arsenic compounds. Concentration of each compound in the sample was the same as in Figure 6-8.

6.3.2.2. The effect of PPY coating thickness on its extraction ability

The effect of PPY coating thickness on its extraction efficiency towards arsenic compounds is illustrated in Figure 6-10. With the increase of PPY thickness, the extraction efficiencies for the compounds increased almost linearly. However, the slopes of the increases for different analytes are significantly different, following the order of MMAAs >

DMAs > AsB > AsC. As a result, the extraction selectivity (see also Table 6-1) of the PPy coating toward the anionic analytes was enhanced with the increase of coating thickness.

6.3.2.3.The effects of sample matrix and pH

It was shown in the previous chapters that sample matrix and pH had significant effects on the extraction of analytes. In this study, the effects of sample matrix and pH on the extraction of organoarsenic compounds and TBT were tested using several buffer solutions with pH 1.5-10.0. The results showed that no significant effects on the extraction efficiency were found from the studied buffer solutions towards the studied analytes, except for the saturated salt (NaCl) solution in which the extraction efficiency was lower than that in other solutions. However, much larger matrix effects on the mass signals were observed from the standard liquid injection method as discussed below.

In ES-MS detection, sample matrix has a great effect on the signal to noise ratio, and therefore the sensitivity of the method. Generally, the sensitivity of a method for the analytes in a complex sample matrix, such as most of the biochemical samples, will decrease relative to the pure water matrix due to the large ‘chemical noise’ or interference created by the sample matrix [20,29]. One of the advantages of SPME sampling over standard liquid injection is that there is less matrix effect on ES-MS signal. This is because, for liquid injection, the entire sample matrix was introduced into HPLC-ES-MS system, while for in-tube SPME, mainly the analytes extracted were introduced to the separation and detection system, since the sample solution (matrix) was ejected back to the sample vial after completing the extraction. As shown in Figure 6-11, using liquid injection, the matrix effect of a saturated NaCl solution on TBT signal (2a) was so large that it not only created higher noise, but also delayed the TBT peak compared with the pure water matrix (1a and 1b). However, this effect was much smaller for the in-tube SPME method (2b). The similar effects were also found in the pH 10.0 buffer solution (5a and 5b). For the pH 3.0 buffer solution (3a and 3b), using in-tube SPME sampling (3b), the signal to noise ratio (S/N) was greatly improved relative to liquid injection (3a). Similar phenomena were also observed for the pH 7.0 buffer solution (4a and 4b) and other buffer solutions (such as the sodium acetate buffer at pH 5.5 and the Tris-HCl buffer at pH 8.5, data not shown). Another advantage of the in-tube SPME over liquid injection is that a more diluted sample solution (at least 10

times more diluted) could be used (due to its high preconcentration factor for the analytes); thus the matrix effects on mass detection could be further reduced. Since sample matrix is complicated and ion strength is high in most of biological samples, the effective analyte-matrix separation and desalting achieved by in-tube SPME should be beneficial to the ES-MS studies of biological samples.

For arsenic compounds, the effects of sample matrix and pH on extraction and detection were similar to those of TBT.

6.3.3. Precision, Limit of Detection and Linearity

The precision obtained for arsenic compounds using the PPY coated capillary in-tube SPME varies between 3.6 to 7.3 % RSD ($n = 7$), depending on the compound and concentration studied. Similar RSD values are also obtained for TBT using Supel-Q PLOT in-tube SPME (for instance, for a 20 ng/mL sample, the RSD for the detected ions within 7 repeated measurements are between 2.5 to 5.6 %).

Due to the optimization of the extraction and detection conditions, low detection limits ($S/N = 3$) were achieved for most of the analytes studied. Using PPY coating, the detection limits of the method for arsenic compounds were at low ng/mL levels (Table 6-3). A detection limit of 0.05 ng/mL could be achieved for TBT using Supel-Q PLOT capillary in-tube SPME coupled with HPLC-MS (Table 6-4). Increasing the number of extraction cycles or using a highly sensitive and selective detection method could further increase the sensitivity of the methods.

To test the linearity of the calibration curves, various concentrations of analytes ranging from 0.1 to 200 ng/mL were examined. The calibration curves were constructed by comparing peak area counts against analyte concentrations. The linear regression data obtained by the developed methods are listed in Table 6-3 and Table 6-4.

For each arsenic compound, as shown in Table 6-3, a good linear relationship was obtained in the concentration range of 5 to 200 ng/mL (8-point calibration, 3 repeats for each point).

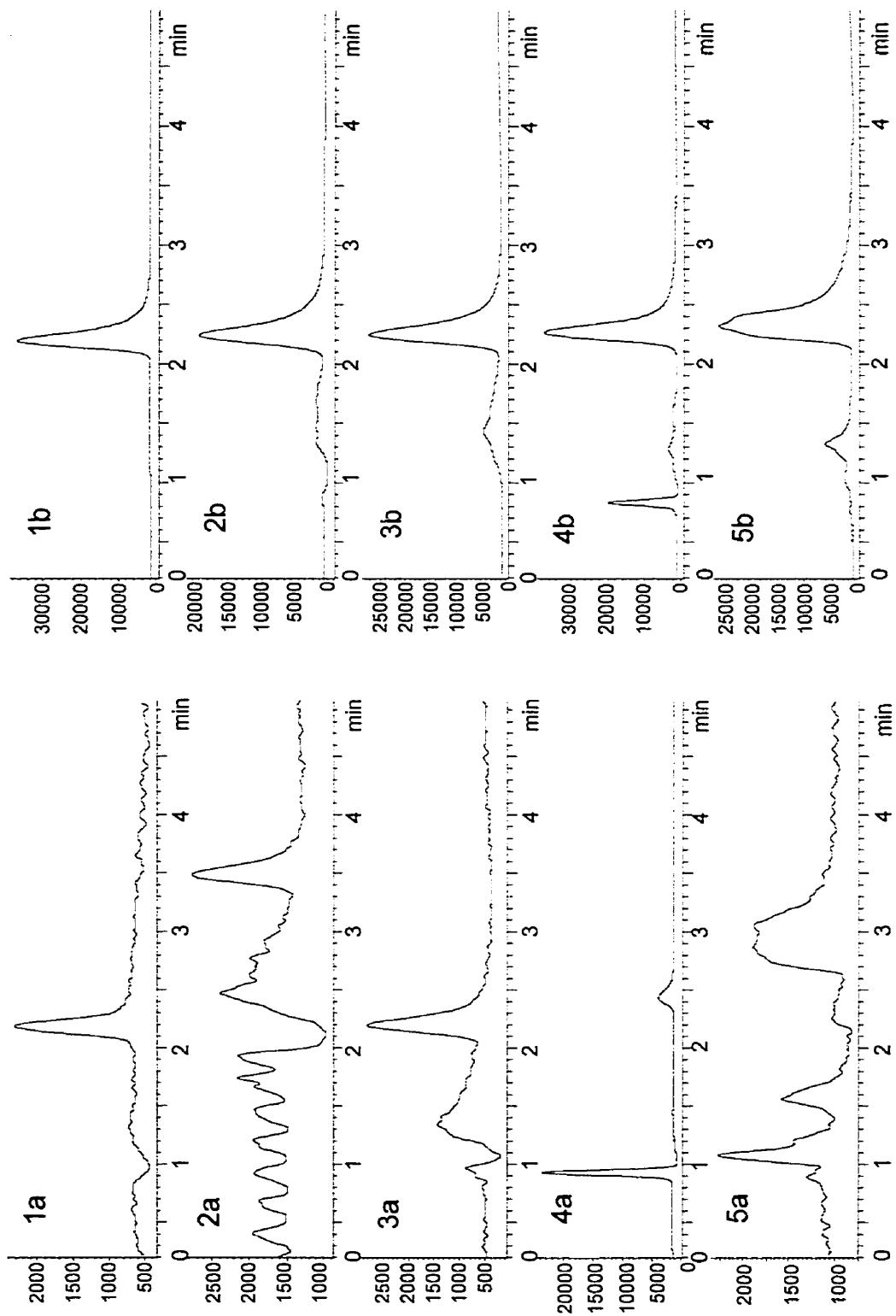


Figure 6-11. Comparison of the sample matrix effects on mass detection signals (TIC) obtained by standard liquid injection ($20 \mu\text{L}$) (a) and in-tube SPME (b). 1.Water solution; 2.NaCl saturated water solution; 3.Gly-HCl buffer (pH 3.0); 4.Sodium phosphate buffer (pH 7.0) and 5.Sodium carbonate buffer (pH 10.0). Concentration of TBT in each of the samples was 10 ng/mL . Buffer concentrations in all samples were 100 mM . Other conditions; see Experimental.

Table 6-3. Linear regression data and detection limits (DL) obtained for arsenic compounds by in-tube SPME with the two different capillaries listed

Analyte*	PPY coated capillary in-tube SPME			Supel-Q PLOT in-tube SPME		
	Regression equation	Correlation (R ²)	DL (ng/mL)	Regression equation	Correlation (R ²)	DL (ng/mL)
MMA _s	y = 5428x + 6840	0.9973	1.25	y = 1809x + 5724	0.9987	4.15
DMA _s	y = 11005x + 1582	0.9991	0.54	y = 6073x + 2141	0.9993	1.12
AsB	y = 21537x + 6999	0.9995	0.18	y = 18364x + 17021	0.9979	0.26
AsC	y = 19432x + 1233	0.9997	0.25	y = 22795x - 8527	0.9986	0.12

* Regression equations were obtained from calibration curves by plotting the peak area counts against corresponding concentrations, number of data points: 8 points (n = 3 for each point). Detection limits (DL) were determined with S/N = 3.

Table 6-4. Linear regression data and detection limits (S/N = 3) for the selected ion and total ion mass signals of TBT

Ion detected	Linear regression equation ^a	Correlation coefficient (R ²)	Detection limit (ng/mL) ^b
Total ion	y = 34292x + 16918	0.9995	0.05 (1.5)
Bu ₃ Sn ⁺ (m/z 291)	y = 9691x + 3833	0.9998	0.10 (2.5)
Bu ₂ SnH ⁺ (m/z 235)	y = 6555x + 4503	0.9996	0.16 (3.8)
BuSnH ₂ ⁺ (m/z 179)	y = 11936x + 5794	0.9993	0.08 (2.3)
SnH ₃ ⁺ (m/z 123)	y = 6110x + 2789	0.9989	0.18 (4.5)

^a Obtained from the calibration curves by plotting the peak area counts of each selected ion or total ion signal against the TBT concentration ranging from 0.5 to 200 ng/mL; number of data points: 9 points (n = 3 for each point). ^b Detection limits obtained by direct liquid injection (20 µL) are shown in the parentheses.

For TBT, as illustrated in Table 6-4, a good linear relationship was obtained for both the total ion of TBT and each selected ion of TBT in the concentration range of 0.5 – 200 ng/mL (9-point calibration, 3 repeats for each point). Since all the selected ions could be detected simultaneously, each of the selected ions and / or the total ion signal could be used for quantification of TBT, and the highest sensitivity could be achieved using the total ion mass signal. In addition, each of the ions could also be used for inter-calibration to check the possible interference to some of the ions detected. For example, with the method of standard

additions, if the amount of analytes determined using the m/z 123 ion is significantly different from the results obtained using other ions, it obviously indicates that there are interferences for the m/z 123 ion.

6.4. Applications

6.4.1. Analysis of organoarsenicals in tap water samples

Tap water samples spiked with different amounts of arsenic compounds were analyzed and compared with non-spiked water samples. As shown in Figure 6-12, no signals for the tested compounds were detected from non-spiked tap water samples. The recoveries of the compounds spiked in the tap water samples using standard additions were in the range of 91.0 to 108.6 % (Table 6-5).

Table 6-5. Recoveries of organoarsenic compounds spiked into tap water samples

Compounds	Recovery (%), mean \pm SD ^a (n = 3)		
	Spiked 20 ng/mL	Spiked 50 ng/mL	Spiked 100 ng/mL
MMA _s	98.4 \pm 4.7 (4.8)	101.6 \pm 6.7 (6.6)	92.7 \pm 1.7 (1.8)
DMA _s	96.7 \pm 3.9 (4.0)	94.9 \pm 2.7 (2.8)	102.1 \pm 2.3 (2.2)
AsB	93.5 \pm 3.7 (3.9)	98.4 \pm 4.5 (4.6)	95.4 \pm 3.4 (3.6)
AsC	106.0 \pm 2.6 (2.4)	96.0 \pm 5.2 (5.4)	99.4 \pm 6.6 (6.6)

^a Relative standard deviations (%) are shown in parentheses.

6.4.2. Determination of AsB in a certified dogfish reference material

To evaluate the performance of the method in analysis of seafood products, determination of organoarsenic compounds in a certified dogfish reference material (DORM-2) was carried out. As shown in Figure 6-13, the only arsenic compound detected was AsB. The method of standard addition was required to determine the concentration of AsB and the recoveries of spiked AsB in the samples due to the sample matrix effects discussed previously. The analysis of DORM-2 by this new method provided an AsB content of 16.0 ± 0.6 mg/kg As, in agreement with both the certified value (16.4 ± 1.1 mg/kg As), and the results obtained by other workers using other methods [6,11,36]. The recoveries for the spiked amounts of AsB in the samples are in the range of 92.7 to 102.4 %.

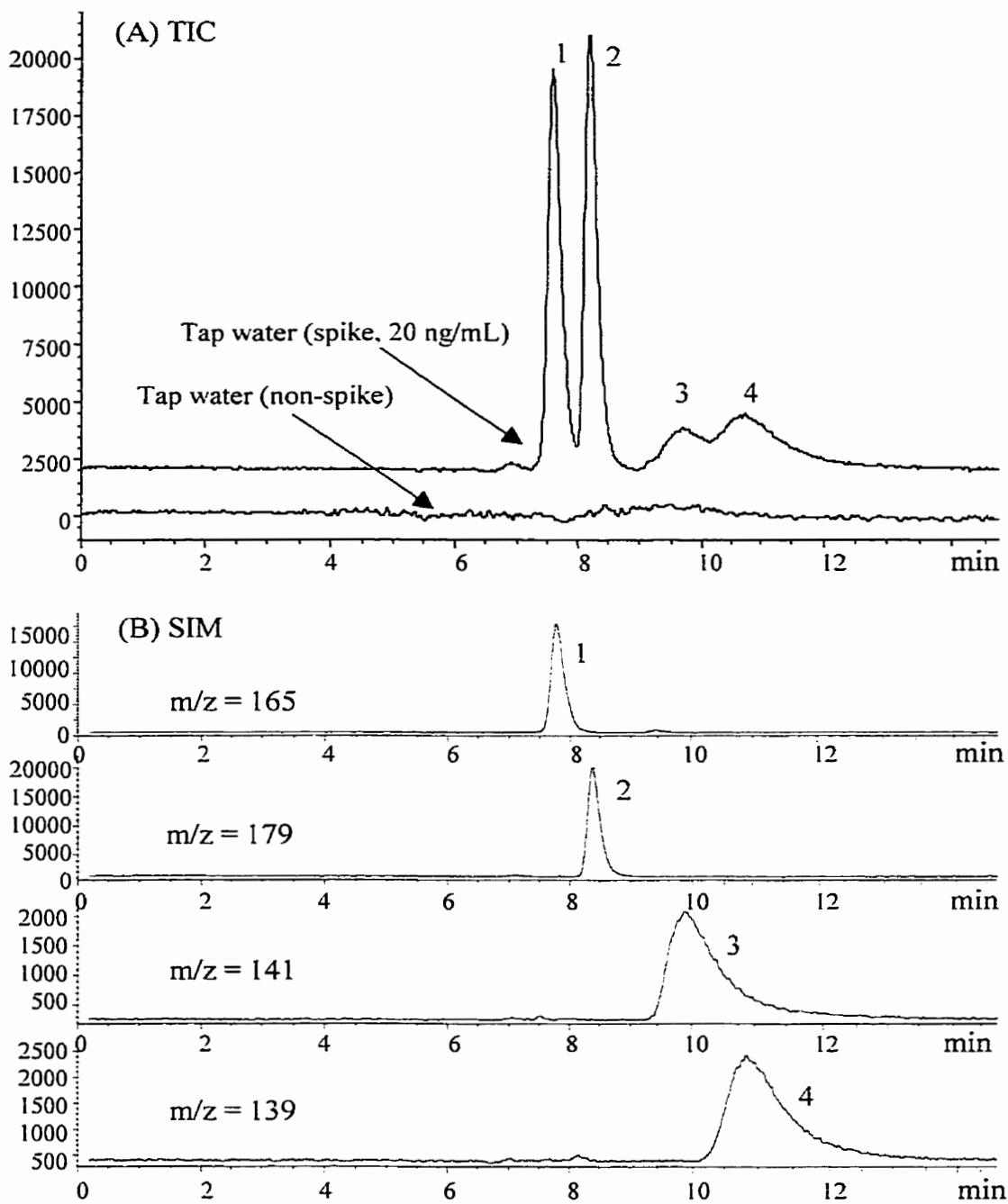


Figure 6-12. Total ion current (TIC) and selected ion monitoring (SIM) chromatograms obtained from tap water samples by PPY coated capillary in-tube SPME-HPLC-MS. (A) TIC chromatograms obtained from tap water and spiked tap water samples. The spiked water trace is offset by 10 % for clarity. (B) SIM chromatograms obtained from spiked water sample. The amount spiked for each compound in the water sample is 20 ng/mL. Peak identification: 1. AsC, 2. AsB, 3. MMAAs, and 4. DMAAs.

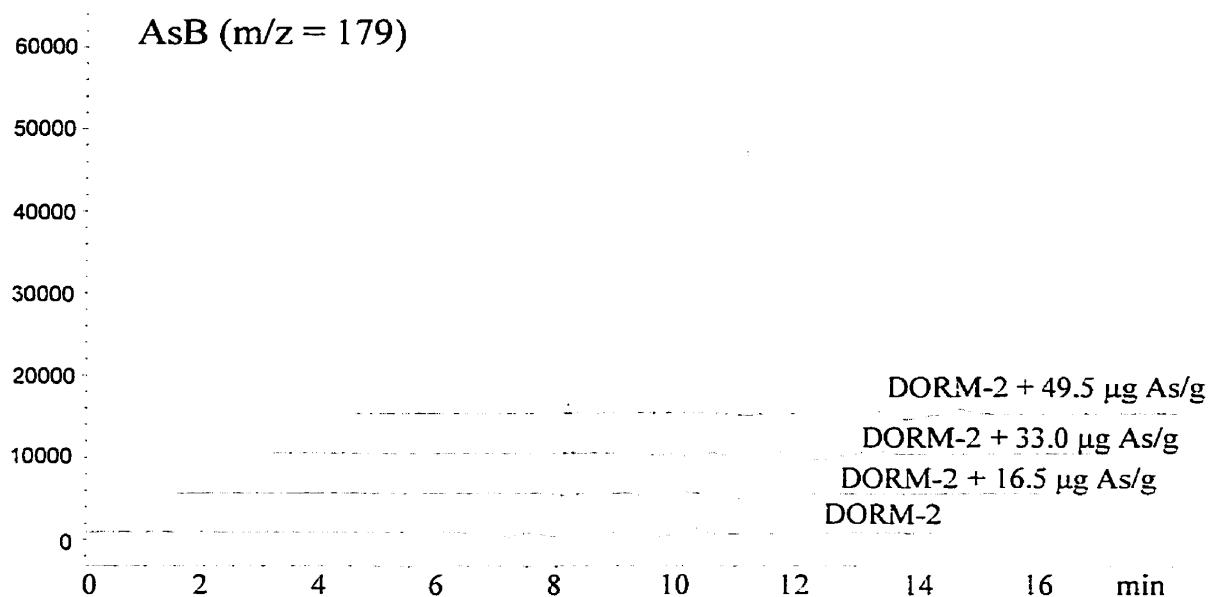


Figure 6-13. SIM chromatograms obtained by PPY in-tube SPME-HPLC-MS from a certified DORM-2 sample solution, and the DORM-2 sample solutions spiked with different amounts of AsB. The spiked samples are offset by 10 % in both x and y for clarity.

6.4.3. Analysis of TBT in lake water samples

The proposed method was applied to the analysis of lake water samples spiked with different amounts of the studied organotin compounds. As shown in Figure 6-14, no analyte signals were found in the non-spiked sample within the detection range of the method. No MBT and DBT could be detected from the spiked samples by this method. The recovery data of three parallel measurements of TBT from the water samples are listed in Table 6-6.

6.4.4. Determination of TBT in a certified sediment reference material

To further evaluate the performance of the method, determination of organotin compounds in a certified sediment reference material (PACS-2) was carried out. As shown in Figure 6-15, the only tin compound detected by this method was TBT although other tin species such as MBT and DBT were also determined by other methods such as LC-ICP-MS [17]. The method of standard additions was applied to determine the concentration of TBT and the recoveries of spiked TBT in the samples. The analysis of PACS-2 by this method

provided a TBT content of $1.03 \pm 0.15 \mu\text{g Sn/g}$, in agreement with the certified value ($0.98 \pm 0.13 \mu\text{g Sn/g}$). The recoveries for the spiked TBT in the samples are in the range of 94.2 to 103.4 %.

Table 6-6. Recoveries of tributyltin (TBT) spiked into lake water samples

Ion detected	Recovery (%), mean \pm SD ^a (n = 3)		
	Spiked 2 ng/mL	Spiked 5 ng/mL	Spiked 10 ng/mL
Total ion	92.1 ± 3.8 (4.1)	93.9 ± 4.6 (4.9)	95.7 ± 2.8 (2.9)
Bu ₃ Sn ⁺ (m/z 291)	93.4 ± 3.4 (3.6)	94.6 ± 6.1 (6.5)	101.7 ± 3.7 (3.6)
Bu ₂ SnH ⁺ (m/z 235)	90.9 ± 2.7 (3.0)	92.6 ± 3.9 (4.2)	93.1 ± 2.9 (3.1)
BuSnH ₂ ⁺ (m/z 179)	95.4 ± 4.7 (4.9)	98.4 ± 4.5 (4.6)	95.8 ± 2.4 (2.5)
SnH ₃ ⁺ (m/z 123)	88.5 ± 3.7 (4.2)	90.5 ± 6.4 (7.1)	92.4 ± 5.2 (5.6)

^a Relative standard deviations (%) are shown in parentheses.

6.5. Summary

In-tube SPME is a simple, efficient and integrated tool for sample extraction, preconcentration and introduction. The extraction efficiency and selectivity of in-tube SPME can be optimized by selecting suitable extraction conditions, including using an appropriate extraction phase. As demonstrated in this chapter, a commercially available Supel-Q PLOT capillary was selected for extraction of TBT, while a custom-made PPY coated capillary was used for extraction of organoarsenicals. The technique of HPLC-ES-MS provides both analyte retention and molecular structure information. Therefore, coupling the in-tube SPME with HPLC-ES-MS can facilitate the development of sensitive and selective methods for speciation of organometallic compounds. The results obtained from this study and our previous studies on organometallic compounds [26,30,31] demonstrate the capability and advantages of the in-tube SPME-HPLC-ES-MS method in speciation of organometallic compounds.

The multifunctional properties of the PPY coating, especially the anion exchange property, have been examined further in this work. The ability and potential application of the PPY coating for extraction of organoarsenic compounds have been demonstrated. The results are consistent with the theoretical expectations from the structure of PPY and its interactions with the analytes.

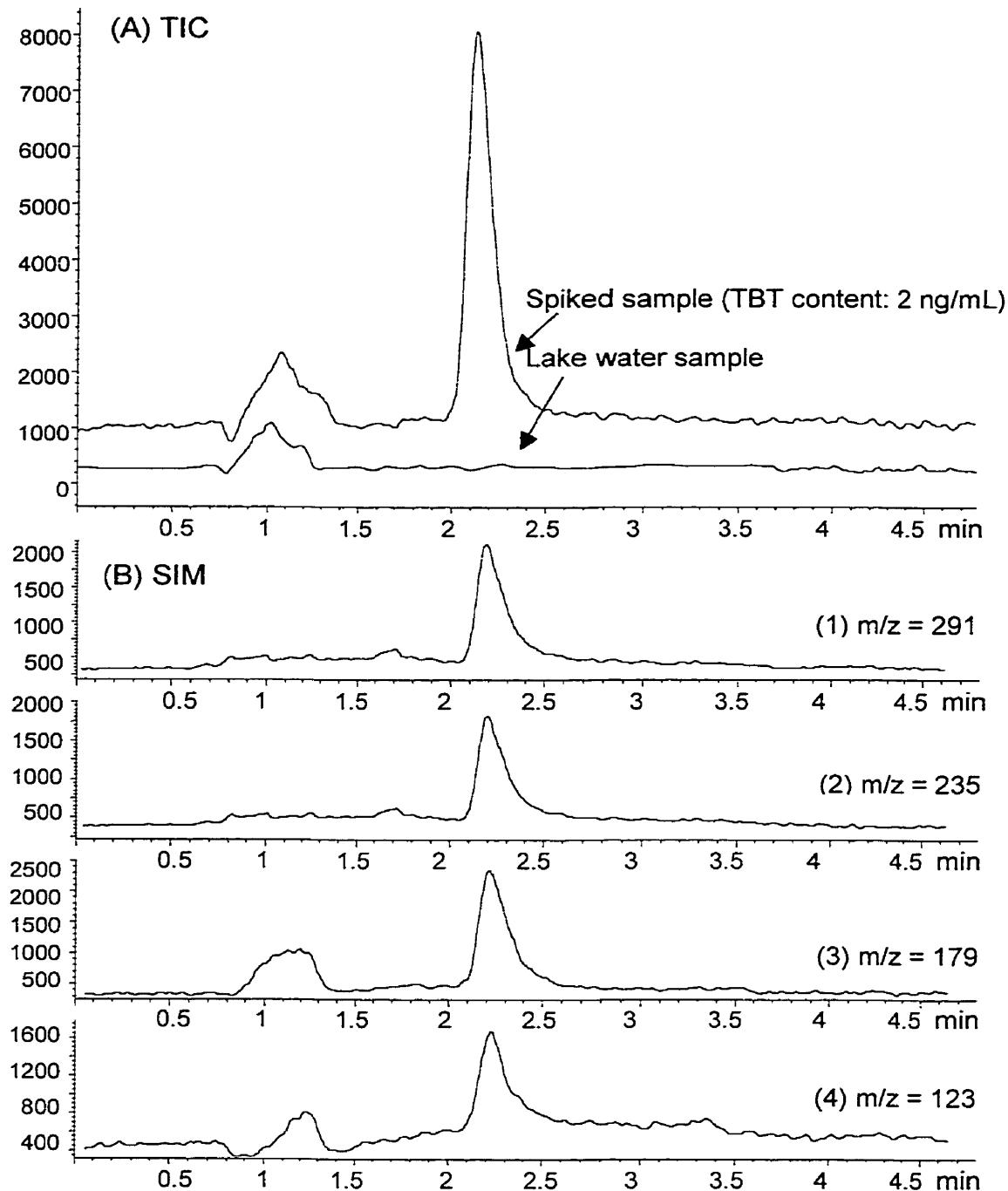


Figure 6-14. Chromatograms obtained from the analysis of lake water samples by Supel-Q PLOT capillary in-tube SPME-HPLC-ES-MS. (A) TIC chromatograms from a lake water sample and a spiked lake water sample. The spiked water trace is offset by 10 % for clarity. (B) SIM chromatograms from the spiked water sample. The final concentration of each of the compounds (TBT, DBT and MBT) in the spiked sample solution is 2 ng/mL.

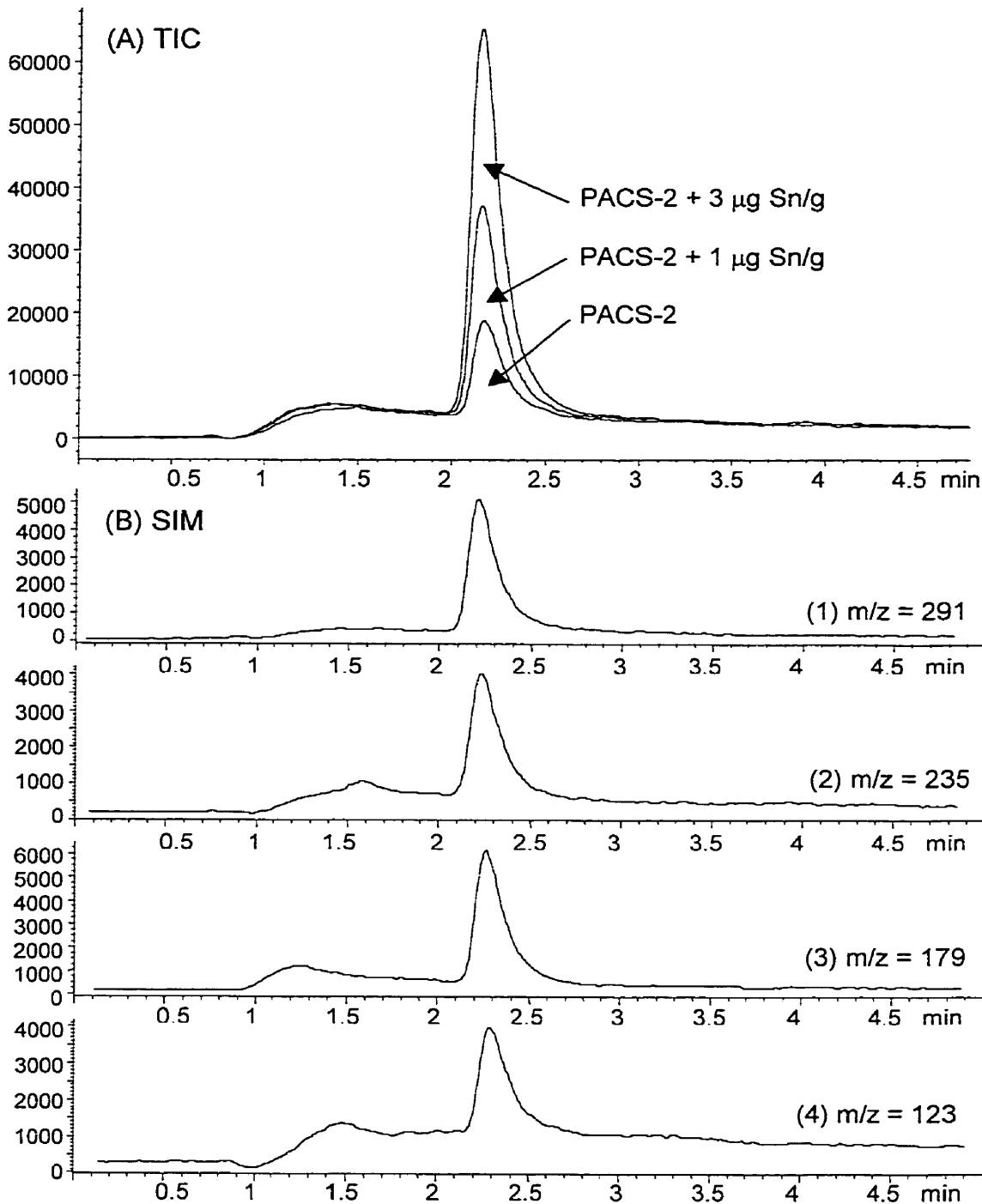


Figure 6-15. (A) TIC chromatograms obtained by Supel-Q PLOT capillary in-tube SPME-HPLC-MS from the solution containing the certified reference material PACS-2 and the TBT-spiked PACS-2 sample solutions. (B) SIM chromatograms obtained from the PACS-2 sample solution.

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CHAPTER 7

ANALYSIS OF PESTICIDES IN WATER AND WINE SAMPLES

7.1. Introduction

Pesticide analysis in environmental and biological samples has received great attention for many years due to the wide use of pesticides in agricultural and household applications as well as their environmental impact [1-5]. Most analytical methods for pesticide analysis are based on chromatographic techniques, by both gas chromatography (GC) and high performance liquid chromatography (HPLC). For polar, less volatile and thermally labile pesticides such as phenylureas and carbamates, HPLC is obviously the preferred approach [2, 4-9]. A variety of detection methods have been used in HPLC analysis of pesticides, including common UV [1], diode-array [4,6], electrochemical [7] and fluorescence [8]. In spite of the high sensitivity of fluorescence detection with post-column derivatization or the robustness of UV detection, mass detection has become widely accepted as the preferred technique for the identification and quantification of pesticides and other polar and thermally liable compounds because of its high sensitivity and selectivity [2,5,9].

In addition to the use of a highly sensitive and selective detection method, selecting an appropriate sample preparation technique is very important for determining small amounts of pesticide residues in complex sample matrices. Several sample preparation techniques, mainly liquid-liquid extraction (LLE) [3] and solid-phase extraction (SPE) [4,5], have been applied for extraction of pesticides from water and other sample matrices. Solid phase microextraction (SPME), due to its simple, fast, and solvent-less features, has received growing interest for its applications in pesticide analysis (see a recent review [1]). As demonstrated in the previous chapters, in-tube SPME is a simple and efficient microextraction and preconcentration technique, which can be easily coupled on-line with HPLC for the analysis of less volatile and /or thermally labile compounds. In-tube SPME coupled with HPLC-UV has been applied for the analysis of pesticides in water samples [10-13]. However, the sensitivity of the method was limited by the UV detector and the commercial capillary (Omegawax 250) used for extraction [11-13]. Although the use of a

capillary LC with an on-column focusing technique could increase sensitivity, this required a much longer analysis time compared with the conventional HPLC analysis [13].

The selection of a suitable coating is key in the optimization of an in-tube SPME method. Omegawax 250 was selected in the previous pesticide studies due to its higher extraction ability for polar compounds compared with other commercial capillaries tested [10-13]. However, it has been demonstrated in the previous chapters that a PPY coated capillary has a superior extraction efficiency toward polar compounds, aromatic compounds, and anionic species compared with the commercial capillaries used, due to the inherent multifunctional properties of the polymer (base-acid, π - π and dipole-dipole interactions, ion-exchange, and hydrogen bonding). Therefore, in this study, in-tube SPME of pesticides was re-investigated using a PPY coated capillary and several commercially available capillaries. In addition, a more sensitive and selective detection method (ES-MS) was used together with the UV detection method. To determine pesticides in wine and ethanol-water mixtures, the influence of the ethanol content on the performance of in-tube SPME was also studied.

7.2. Experimental

7.2.1. Chemicals and sample preparation

Six phenylurea pesticide standards (diuron, fluometuron, linuron, monuron, neburon, and siduron) and six carbamates (barban, carbaryl, chlorpropham, methiocarb, promecarb, propham) were obtained from Chem Service (West Chester, PA, USA). They were of $\geq 98\%$ purity and used as received. Acetonitrile, ethanol and methanol (HPLC grade) were obtained from EM science (Gibbstown, NJ, USA). Pure water was obtained from a NANO-pure ultrapure water system described previously. Surface water was obtained from Laurel Creek, Waterloo, ON. Private residential well water was obtained from Cambridge, ON. Municipal tap water was taken from the tap in our lab (Waterloo, ON). A white wine (Hochtaler, Andres wines Ltd.) was purchased from a local liquor store.

Individual standards for each compound with concentrations of 2 mg/mL were prepared using methanol as a solvent. A standard stock mixture with a concentration of 0.2 mg/mL for each compound was prepared in methanol. Water samples (spiked with 20, 40,

and 100 ng/mL of each compound) were freshly prepared before experiments by spiking the standard stock solution or diluted solutions into pure water, surface water, well water, and tap water. The aqueous samples for calibrations, limit of detection, and linearity tests were prepared by spiking the standard mixture or diluted mixtures into pure water or water samples to reach appropriate concentration levels. Methanol concentration was kept equal to or lower than 1% in the solutions. A wine sample was analyzed directly or after 10-fold dilution with pure water. The results obtained from spiked wine samples were compared with those of non-spiked wine samples and water samples. The structures of the studied pesticides are given in Figure 7-1.

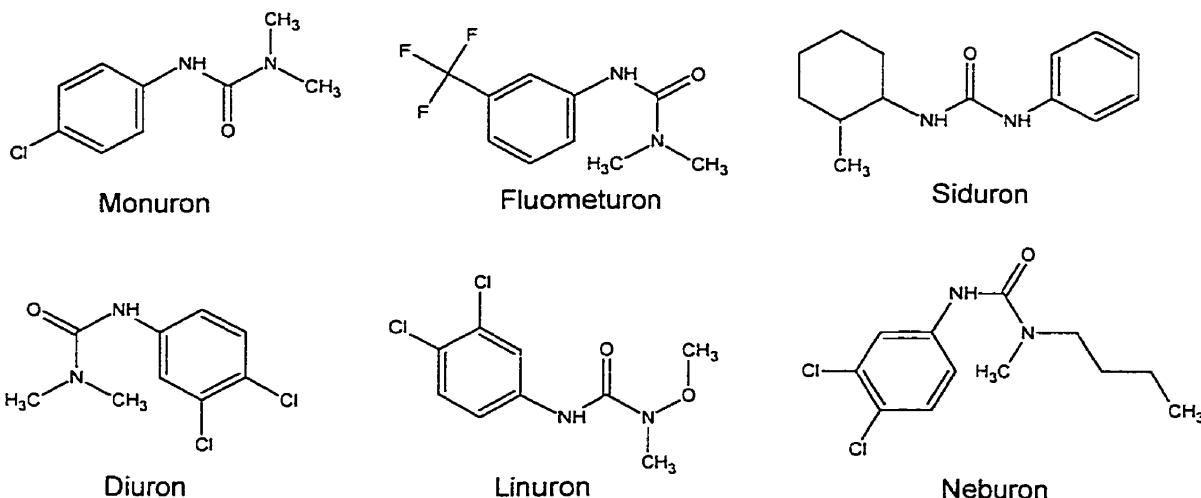
7.2.2. Instrument and analytical conditions

PPY or poly-*N*-methylpyrrole (PMPY) film was coated on the inner surface of a fused silica capillary (60 cm long, 0.25-mm i.d.) by a chemical polymerization method described in chapter 2. The structures of PPY and PMPY were shown in chapter 2. In-tube SPME and the technique of coupling automated in-tube SPME with HPLC were described in detail in chapter 4.

All experiments were carried out on a Agilent 1100 series HPLC coupled with an atmospheric pressure (AP)-electrospray ionization (ES) mass spectrometer and a variable-wavelength UV detector (see chapter 4). A Supelcosil LC-18 column (5 cm × 4.6 mm, 5 µm particle size) from Supelco (Bellefonte, PA, USA) was used for the separation under room temperature. Mobile phases were composed of acetonitrile and H₂O (each contained 0.05% formic acid). For the separation of phenylurea pesticides, the ratio of acetonitrile/H₂O was kept at 40/60 for the first 4.5 min and then the content of acetonitrile was increased linearly to 60% at 7 min and held at this ratio for the rest of the run. The flow rate was 0.5 mL/min. For the separation of carbamates, mobile phase ratio of acetonitrile/H₂O was kept at 55/45 and flow rate was 0.5 mL/min. The wavelength used for UV detection was 245 nm for phenylureas and 225 nm for carbamates according to the previous studies [10-13]. The optimized ES-MS conditions were as follows: nebulizer gas, N₂ (30 psi); drying gas, N₂ (10 L/min, 350°C); capillary voltage, 4500 V; ionization mode, positive; mass scan range, 50-350 amu; fragmentor voltage, variable depending on the ions selected. The monitoring ions

selected for each analyte and the corresponding fragmentor voltages used are listed in Table 7-1.

A. Phenylurea pesticides



B. Carbamate pesticides

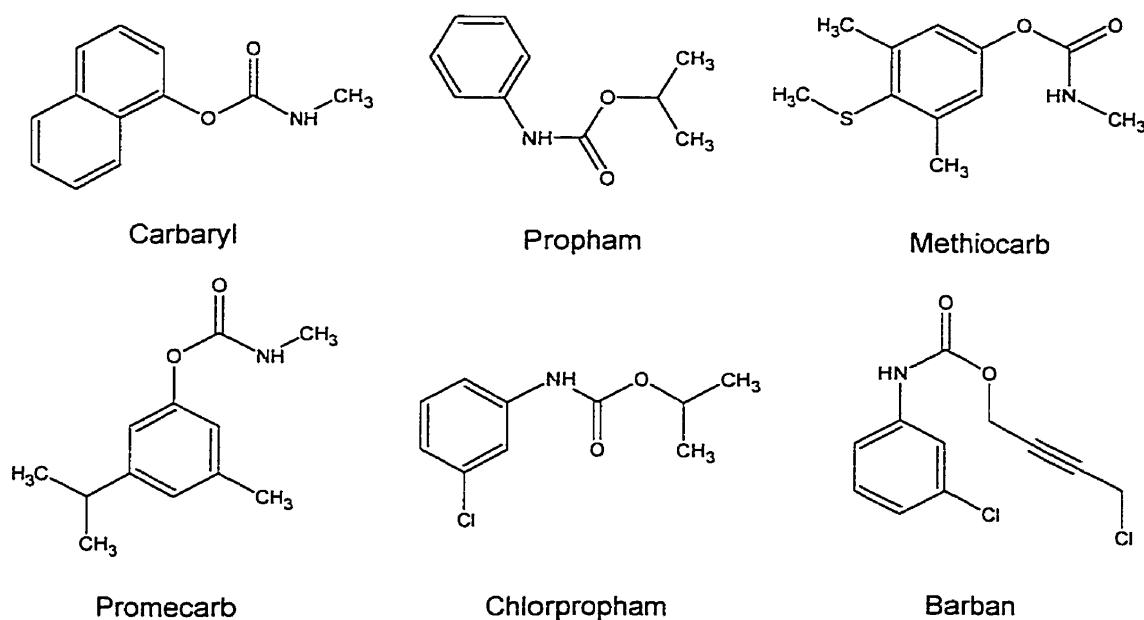


Figure 7-1. Structures of the pesticides studied.

7.3. Results and discussion

7.3.1. Separation and detection

The results showed that both pesticide mixtures could be separated under the conditions listed in the experimental section. To select the monitoring ion for each of the compounds, ES mass spectra under positive ion detection mode were analyzed by liquid injection. The following mass detection conditions were optimized and are summarized in the experimental section, including fragmentor voltage, capillary voltage, nebulizer gas pressure, drying gas flow rate and temperature. Table 7-1 lists the ions selected for each compound and the corresponding fragmentor voltages used for the selected ions.

Table 7-1. Ions selected for each pesticide and the corresponding fragmentor voltages (V_f) used in ES-MS detection

Carbamate	MW	m/z and ions selected	V_f (V)	Phenylurea	MW	m/z and ions selected	V_f (V)
Carbaryl	201	202 [M+H] ⁺	30	Monuron	198	199 [M+H] ⁺	60
		145 [M+H-CH ₃ NCO] ⁺	60			221 [M+Na] ⁺	70
Propham	179	120 [C ₆ H ₅ NCO+H] ⁺	90	Fluometuron	232	72 [C ₃ H ₆ NO] ⁺	100
		138 [M+H-C ₃ H ₆] ⁺	60			233 [M+H] ⁺	60
Methiocarb	225	226 [M+H] ⁺	30	Diuron	232	72 [C ₃ H ₆ NO] ⁺	100
		169 [M+H-CH ₃ NCO] ⁺	60			233 [M+H] ⁺	60
Promecarb	207	208 [M+H] ⁺	30	Siduron	232	72 [C ₃ H ₆ NO] ⁺	100
		151 [M+H-CH ₃ NCO] ⁺	60			233 [M+H] ⁺	60
Chlorpropham	214	154 [M-C ₃ H ₇ OH] ⁺	90	Linuron	248	255 [M+Na] ⁺	120
		172 [M-C ₃ H ₆] ⁺	60			249 [M+H] ⁺	60
Barban	258	258 [M] ⁺⁺	30	Neburon	274	275 [M+H] ⁺	50
		178 [M+H-81] ⁺	60			297 [M+Na] ⁺	70

7.3.2. Optimization of in-tube SPME conditions

To optimize the in-tube SPME conditions for pesticide analysis, the following parameters were investigated by coupling in-tube SPME to HPLC, including the stationary phase of the SPME capillary, coating thickness, extraction time profile, sample matrix and pH, and desorption condition. However, only the influence of capillary coatings and coating thickness on extraction are discussed here, because the results obtained for other parameters in this work are similar to those obtained and discussed in the previous chapters. In this

work, a 60-cm long capillary was selected and an extraction time-profile of 15 draw/eject cycles (40 µL for each cycle at a flow rate of 100 µL/min) was used in all in-tube SPME experiments. No buffer solution or salt was used since they did not influence extraction efficiency significantly towards the studied pesticides. The analytes were desorbed with mobile phases by switching the six-port valve to the INJECT position.

Capillary coating selection. The previous in-tube SPME work on pesticide analysis showed that Omegawax 250 was the best capillary among the commercial GC capillary columns tested [10-13]. However, it has been shown in our studies (see Chapter 3-6) that a PPY coated capillary has better extraction efficiency than the Omegawax towards aromatic compounds, polar compounds and anionic species. Therefore, the effect of different capillary coatings on pesticide extraction was evaluated further in this work, using the custom-made PPY and PMPY capillaries and the commercially available capillaries. The amounts of analytes extracted by in-tube SPME with different capillaries under the same conditions were calculated by equation (4-1) and are listed in Table 7-2. As in the previous pesticide studies [10-13], the three commercial capillaries, SPB-1, SPB-5 and Supelcowax, did not show good extraction efficiency for the pesticides studied, therefore, their results are not included in Table 7-2. For phenylurea pesticides, PPY, PMPY and Supel-Q PLOT capillaries showed better extraction efficiencies than Omegawax 250 as shown in Table 7-2, indicating their potential application for in-tube SPME. Similar results were also found for carbamates (data not shown). The high extraction efficiencies of these coatings might be explained in part by the high surface areas of their porous surface structures (see SEM figures in Chapter 4 and Chapter 6). Since the PPY coated capillary had the best extraction efficiency for the analytes among all the tested capillaries, it was selected for further study.

Effect of PPY coating thickness on SPME. The effect of coating thickness on extraction is one of the most important parameters for SPME. As shown in Table 7-3, the extraction efficiency for phenylurea pesticides increased gradually with the increase of the PPY coating thickness, which is consistent with the results obtained previously. Similar results were also obtained for the carbamate pesticides (data not shown).

Table 7-2. Comparison of the extraction efficiencies for phenylurea pesticides obtained by different capillary coatings

Compound*	$F (\times 10^{-5})^a$	Amount of analyte extracted (ng) ^b					Extraction yield (%) ^c				
		Host	PMPY	PPY	Supel-Q	Omeg	Host	PMPY	PPY	Supel-Q	Omeg
Monuron	0.27	1.4	35.5	52.9	52.3	6.5	0.7	17.7	26.5	26.2	3.3
Fluometuron	0.21	2.6	33.6	48.6	45.5	6.8	1.3	16.8	24.3	22.7	3.4
Diuron	0.63	2.4	48.8	66.8	63.9	21.3	1.2	24.4	33.4	31.9	10.7
Siduron	0.17	2.2	43.7	53.8	52.1	12.8	1.1	21.8	26.9	26.0	6.4
Linuron	1.44	1.3	42.3	53.4	36.4	15.0	0.7	21.1	26.7	18.2	7.5
Neburon	0.52	4.4	50.9	67.1	46.0	36.8	2.2	25.5	33.6	23.0	18.4

* Sample contains 200 ng/mL each of the analytes. ^a A 10- μ L (2- μ g) sample was directly injected to obtain the detector response factor F (see equation (4-1)). ^b A 1-mL sample was used for in-tube SPME. The amount of each pesticide extracted was calculated by equation (4-1). ^c Extraction yields (%) are the percentages of extracted amounts of pesticides per initial amounts (200 ng) in the 1-mL sample solution using in-tube SPME.

Table 7-3. Effect of PPY coating thickness on the extraction efficiency for the phenylureas

Compound	Amount of analyte extracted (ng) ^{a,b}					Extraction yield (%) ^{a,c}				
	0-PPY	1-PPY	2-PPY	3-PPY	4-PPY	0-PPY	1-PPY	2-PPY	3-PPY	4-PPY
Monuron	1.4	6.4	15.0	35.5	52.9	0.7	3.2	7.5	17.7	26.5
Fluometuron	2.3	5.3	15.3	33.6	48.6	1.2	2.6	7.7	16.8	24.3
Diuron	2.4	12.1	26.4	48.8	66.8	1.2	6.1	13.2	24.4	33.4
Siduron	2.2	9.7	22.3	43.7	53.8	1.1	4.9	11.2	21.8	26.9
Linuron	1.3	6.7	14.5	42.3	53.4	0.7	3.3	7.2	21.1	26.7
Neburon	4.4	16.1	27.6	50.9	67.1	2.2	8.0	13.8	25.4	33.6

^a The coating thickness of PPY increases from 0-PPY (coating cycle, without PPY coating) to 4-PPY (coating cycles). Detector response factor (F) and other conditions (and notes ^b and ^c) are the same as in Table 7-2.

Effect of ethanol content on extraction efficiency. To determine pesticides in wine or other ethanol/water containing food products, the effect of ethanol contents in sample solutions on extraction was studied. As shown in Table 7-4, the amount of pesticides extracted decreases with the increase of ethanol content in the solutions. For example, the extracted amount (mass) for each pesticide was decreased up to 90 % in a solution containing 40 % ethanol compared with a solution having no ethanol. Similar results were also obtained

in a recent study on the determination of pesticides in food simulants using a fiber SPME method [14]. Because of this effect, the content of ethanol or methanol (which has a similar effect as ethanol) in the sample solutions should be kept to the lowest level or at least kept at the same level in order to get reproducible results. In addition, internal calibration or a standard addition method must be used for quantification.

Table 7-4. Effect of ethanol contents in sample solutions on the extraction efficiency for the phenylurea pesticides

Compound ^a	Amount of analyte extracted (ng) ^b at the following ethanol contents (%)				Extraction efficiency decrease (%) ^c at the following ethanol contents (%)			
	0	10	20	40	0	10	20	40
Monuron	52.9	41.4	33.0	5.1	0	22	38	90
Fluometuron	48.6	43.5	32.1	6.2	0	10	34	87
Diuron	66.8	57.9	50.6	10.1	0	13	24	85
Siduron	53.8	41.0	33.5	6.1	0	24	38	89
Linuron	53.4	46.6	40.8	8.9	0	13	24	83
Neburon	67.1	55.8	50.0	14.4	0	17	26	79

^a Detector response factor (*F*) and other conditions (including note ^b) are the same as in Table 7-2.
^c The percentages of the decreased amount of analyte extracted in ethanol containing solutions as compared with the amount of analyte extracted in a solution having no ethanol.

Analysis time and carryover effect. The extraction time for a 15-cycle extraction was about 12 min, therefore, the whole analysis for the pesticides including extraction, desorption, separation and detection (UV and MS) could be accomplished automatically within 25 min by the developed method. The analyte carryover (or memory) effect was not observed because analytes were desorbed completely by the mobile phase flow and the capillary was washed continually during analysis.

7.3.3. Method performance

Due to the high sensitivity of mass detection and the high extraction power of PPy coating, lower detection limits ($S/N = 3$) were obtained for all the pesticides studied (Table 7-5 and Table 7-6) compared with the previous in-tube SPME studies [11-13]. For most of the compounds, the sensitivities of the PPy in-tube SPME-MS method are more than one order

of magnitude higher than those of the previous in-tube SPME-HPLC-UV studies [10-12]. In this work, UV detection was carried out together with MS detection for comparison. The detection limits obtained by UV detector are also listed in these tables, which are lower than those of the previous in-tube SPME method due to the higher extraction efficiency of PPY compared with Omegawax. The precision of the method varies depending on the analytes tested, their concentrations and the detectors used (see Tables 7-5 and 7-6). The calibration curves were constructed by comparing peak area counts against analyte concentration ranging from 0.5 to 200 ng/mL. A linear relationship was obtained for each analyte in the concentration range of 0.5 to 200 ng/mL for MS detection and 2 to 200 ng/mL for UV detection, as shown in Tables 7-5 and Table 7-6.

In this work, one single PPY coated capillary was used for all the extraction experiments (except for the coating thickness experiments), and no significant changes in its extraction performance were observed after hundreds of extractions during a two-month period of study. The reproducibility obtained from five different capillaries coated with the same thickness of PPY was satisfactory, with the differences in their extraction efficiency ranging from 3 to 10 % for a sample containing 100 ng/mL phenylureas.

7.3.4. Analysis of pesticides in water and wine samples

Tap water, surface water and well water samples spiked with different amounts of the pesticides were analyzed by the method and the results were compared with those of non-spiked water and pure water samples. No pesticides studied were detected from the non-spiked water samples using this method. As shown in Figure 7-2, by detecting the selected analyte ions (Table 7-1) under selected ion monitoring mode (SIM), MS detection method provided greater selectivity for the carbamates studied compared with the UV detection method. For tap water and surface water samples, the UV detector recorded large impurity peaks in front of the analyte signals. These peaks might influence the determination of pesticides when a complete separation could not be achieved. Although MS detection did not provide significant improvement (compared with UV) in sensitivity for chlorpropham (peak 5) and barban (peak 6), it provided much higher sensitivity than UV detection for most of the carbamates studied. Higher sensitivity and selectivity were also obtained in phenylurea

pesticide analysis. Therefore, only the results obtained by MS detection method was shown in Figure 7-3. Using the PPY in-tube SPME-HPLC method, the recoveries of the spiked analytes from well water samples are close to those obtained from pure water samples (95% – 104%). However, the analyte recoveries are 10 to 18 % lower from tap water and surface water samples compared with the results obtained from pure water samples. This is likely due to the sample matrices. Therefore, standard addition method was used in all the analysis. The sample matrix effect became more obvious when analyzing wine samples directly, because the spiked analyte signals from wine samples were much lower compared with those from pure water samples. There are significant concentrations of other components as well as 11 % ethanol in the wine samples, which influence not only the extraction efficiency of SPME but also the ES-MS and UV detection to the analytes. After appropriate dilutions of the sample (10 times dilution, for an example), sample matrix effects could be reduced as described in Chapter 6. In this work, therefore, wine samples were analyzed after a 10-fold dilution. As shown in Figure 7-4, MS detector again produced better sensitivity and selectivity than UV detector for analysis of the spiked phenylureas in wine samples. Similar results were also obtained for carbamates (data not shown). The analyte recoveries from spiked wine samples are between 89.2 to 96.9 %.

7.4. Summary

The high extraction efficiency of the PPY coating for polar pesticides from water and wine samples has been demonstrated. Compared with the previous studies of in-tube SPME-HPLC-UV method, higher sensitivity and selectivity have been achieved by the method developed in this work, due to the combination of the high extraction efficiency of PPY and the high sensitivity and selectivity of ES-MS. This PPY coated capillary in-tube SPME-HPLC-MS method has been extended to the analysis of other polar compounds such as caffeine and catechins in tea and other beverages [15].

Table 7-5. Linear regression data, detection limits (DL) and precision (RSD) obtained for phenylureas by SPME with two detection methods*

Compounds	PPY coated capillary in-tube SPME-HPLC-ES-MS				PPY coated capillary in-tube SPME-HPLC-UV			
	Regression equation	Correlation (R ²)	DL (ng/mL)	RSD (%)	Regression equation	Correlation (R ²)	DL (ng/mL)	RSD (%)
Monuron	y = 89149x + 89042	0.9993	0.03	3.1	y = 0.965x + 1.866	0.9996	2.1	2.1
Fluometuron	y = 86567x + 140045	0.9998	0.03	3.3	y = 0.673x + 0.390	0.9999	4.5	4.7
Diuron	y = 40971x - 44166	0.9974	0.08	4.5	y = 0.730x - 1.114	0.9987	3.7	3.6
Siduron	y = 121358x + 70201	0.9992	0.01	2.6	y = 0.684x - 0.600	0.9993	4.2	4.4
Linuron	y = 14990x - 8951	0.9995	0.32	5.3	y = 0.959x + 0.505	0.9979	2.2	2.6
Neburon	y = 88949x + 17039	0.9957	0.03	4.2	y = 0.833x + 2.524	0.9986	3.1	3.7

* Regression equations were obtained from calibration curves by plotting the peak area counts against analyte concentrations, number of data points: 8 (n = 3 for each point). Detection limits were determined with S/N = 3. RSD (%) was calculated from a sample containing each analyte 20 ng/mL (n = 5).

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Table 7-6. Linear regression data, detection limits (DL) and precision (RSD) obtained for carbamates by SPME with two detection methods*

Compounds	PPY coated capillary in-tube SPME-HPLC-ES-MS				PPY coated capillary in-tube SPME-HPLC-UV			
	Regression equation	Correlation (R ²)	DL (ng/mL)	RSD (%)	Regression equation	Correlation (R ²)	DL (ng/mL)	RSD (%)
Carbaryl	y = 66753x + 232642	0.9993	0.04	2.7	y = 4.498x + 17.985	0.9983	0.38	2.3
Propham	y = 32888x + 107280	0.9998	0.08	4.3	y = 0.623x - 4.015	0.9995	4.6	3.8
Methiocarb	y = 59018x + 213654	0.9974	0.05	3.6	y = 0.724x + 2.700	0.9985	3.4	2.9
Promecarb	y = 144595x + 491057	0.9992	0.01	3.4	y = 0.393x + 0.737	0.9996	8.0	5.0
Chlorpropham	y = 17246x + 221013	0.9995	0.41	5.8	y = 0.431x + 4.101	0.9935	7.4	4.2
Barban	y = 5603x + 10710	0.9957	1.2	6.3	y = 0.366x + 0.617	0.9997	8.2	4.6

* Regression equations were obtained from calibration curves by plotting the peak area counts against analyte concentrations, number of data points: 8 (n = 3 for each point). Detection limits were determined with S/N = 3. RSD (%) was calculated from a sample containing each analyte 20 ng/mL (n = 5).

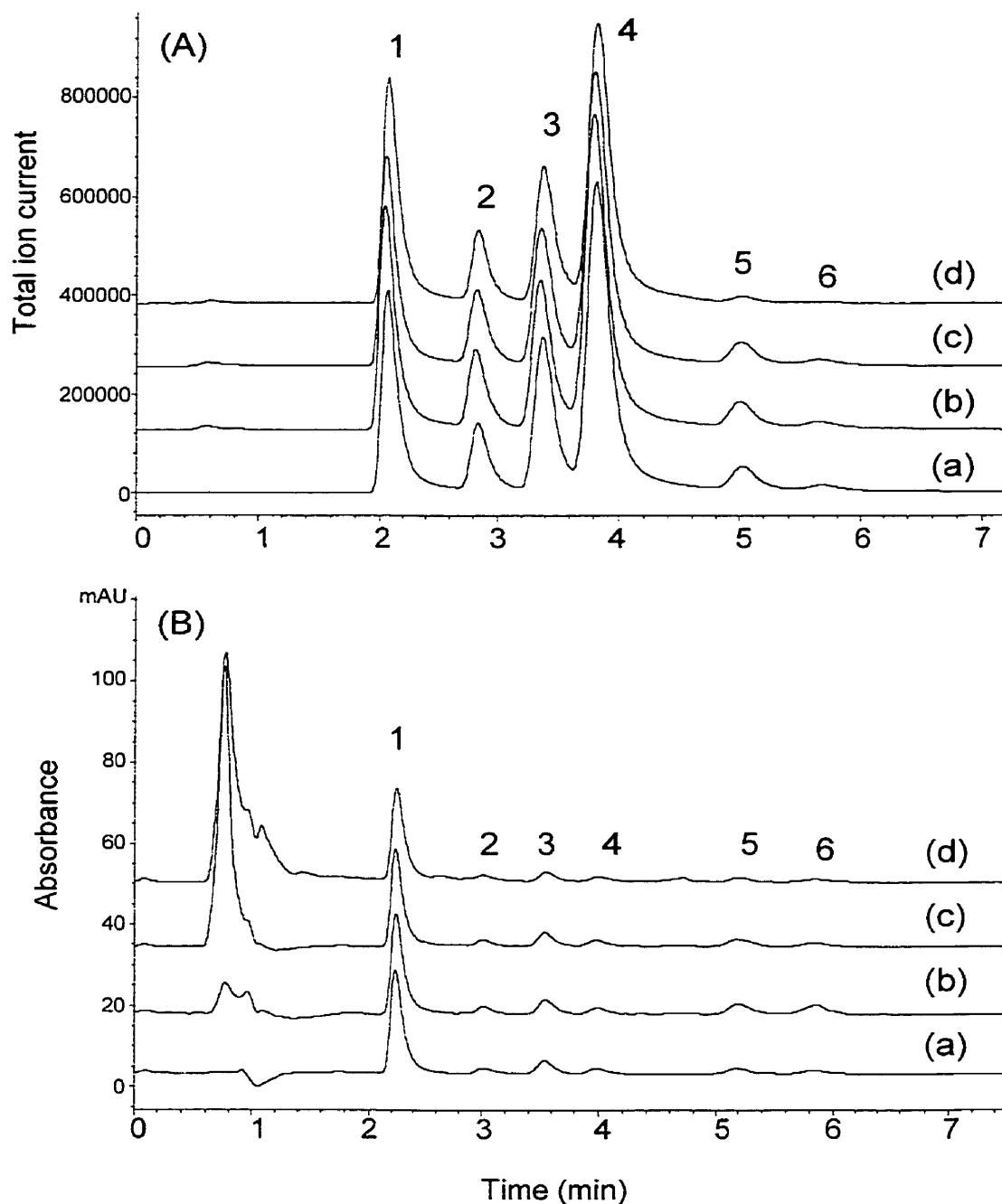


Figure 7-2. Chromatograms of the six carbamates spiked into the different water samples obtained by (A) in-tube SPME-HPLC-ES-MS and (B) in-tube SPME-HPLC-UV. 20 ng each of the pesticides was spiked in each of 1-mL water samples. Water samples: (a) pure water; (b) well water; (c) tap water; and (d) surface (lake) water. Peak identification: 1. Carbaryl, 2. Propham, 3. Methiocarb, 4. Promecarb, 5. Chlorpropham, and 6. Barban.

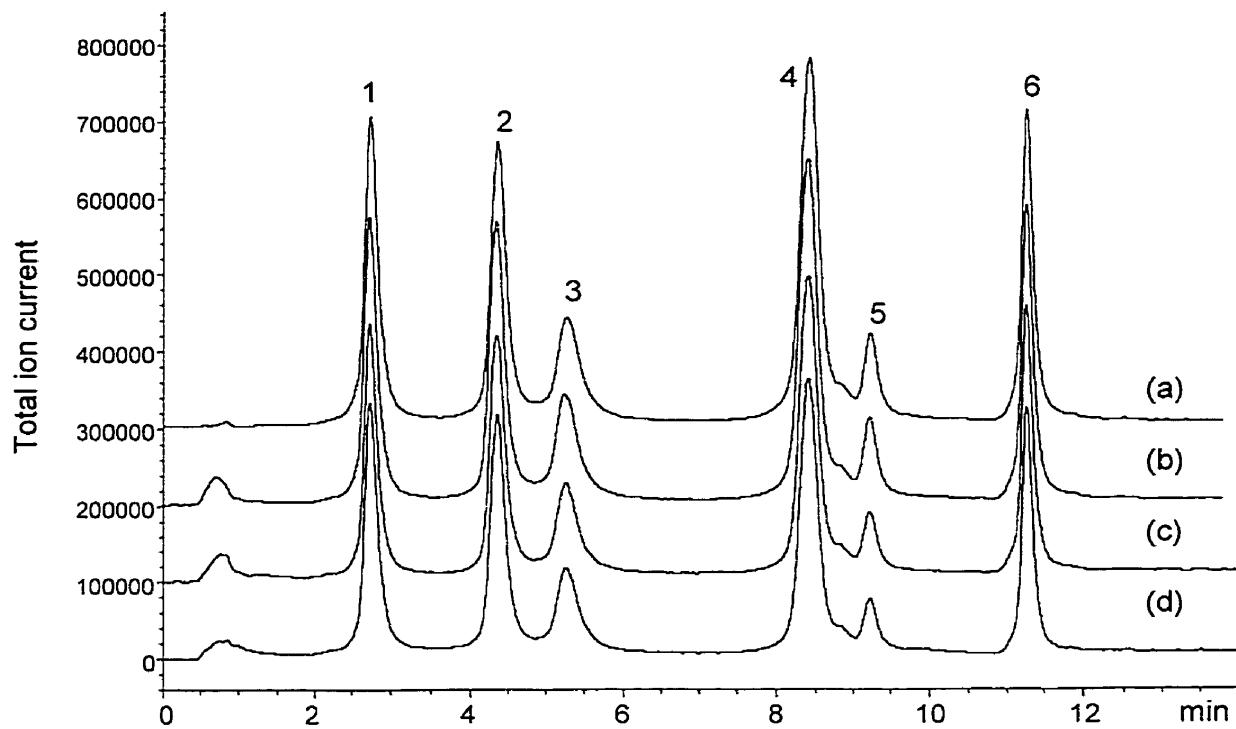


Figure 7-3. Chromatograms of the six phenylureas spiked into the various water samples obtained by in-tube SPME-HPLC-ES-MS. 20 ng each of the pesticides was spiked in each of the 1-mL samples. Water samples: (a) pure water; (b) well water; (c) tap water; and (d) surface (lake) water. Peak identification: 1. Monuron; 2. Fluometuron; 3. Diuron; 4. Siduron; 5. Linuron; 6. Neburon.

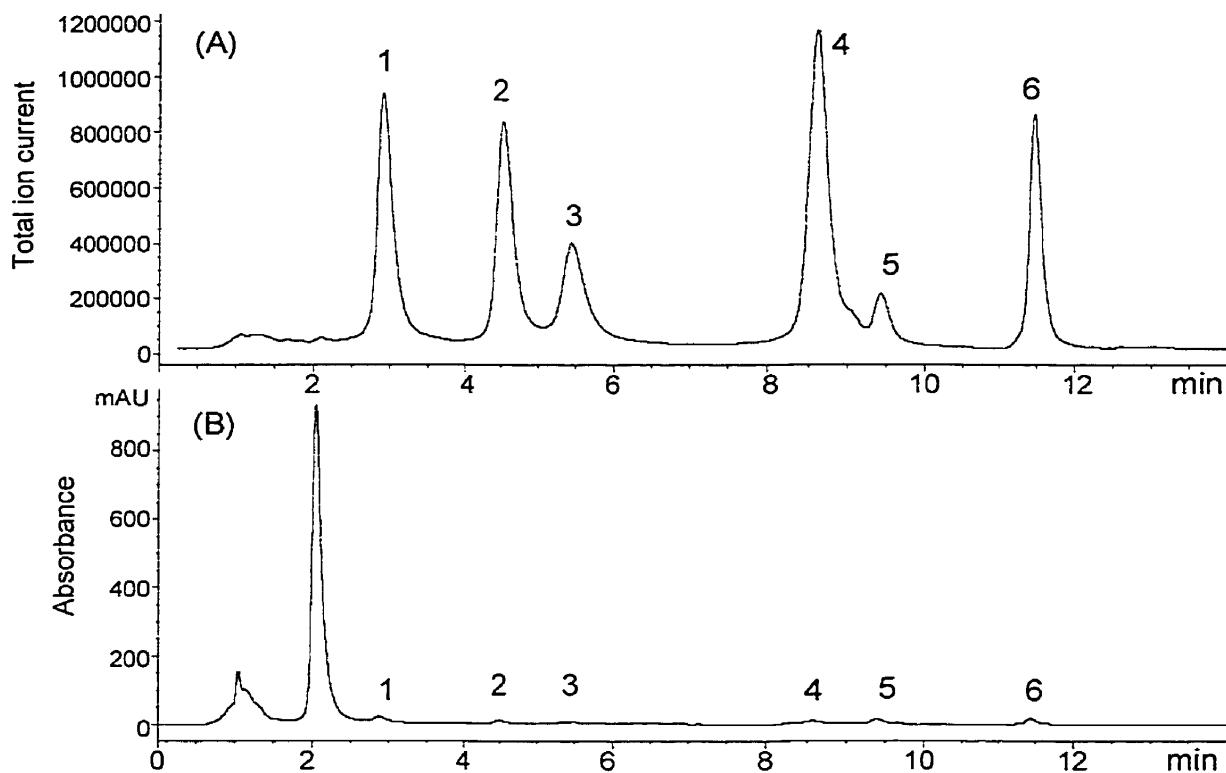


Figure 7-4. Chromatograms of the six phenylureas spiked into a 10 dilute wine sample obtained by (A) in-tube SPME-HPLC-ES-MS and (B) in-tube SPME-HPLC-UV. 80 ng each of the pesticides was spiked into the 1-mL dilute sample. Peak identifications are the same as in **Figure 7-3**.

7.5. References

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CHAPTER 8

IN-TUBE SPME BASED ON PPY MODIFIED COMMERCIAL CAPILLARY COLUMNS

8.1. Introduction

It was demonstrated in the previous chapters that PPY coated capillary had better extraction efficiency than the commercial capillaries tested for most of the compounds studied. It was also found that the PPY coating could be prepared on the inner surface of a polar capillary column such as Omegawax 250 more easily than on the non-coated silica capillary. Therefore, in this study, the commercially available polar GC capillary columns such as Omegawax 250, Supelcowax and Supel-Q PLOT were modified by coating a thin film of PPY on their inner surfaces and then tested for in-tube SPME. The performance of the modified and the original capillaries in extraction of the compounds with different functional groups was evaluated by coupling the in-tube SPME with HPLC. Compared with the original commercial capillaries, the PPY modified capillaries showed significantly different extraction selectivity and better extraction efficiency for most of the compounds studied, especially to aromatic, polar and basic compounds and anionic species. The reasons for the improvement in SPME performance after modification are due to the introduction of new functional groups on the coating surface and the changes in the surface properties (porosity and surface area). Since the PPY modified capillaries can be easily prepared and are more stable in most of the solvents used, they can be applied for in-tube SPME of a wide range of analytes.

8.2. Experimental

8.2.1. Chemicals and reagents

Nine β -blockers were purchased from Sigma Chemicals (St. Louis, MO, USA), including acebutolol hydrochloride, alprenolol hydrochloride, labetalol hydrochloride, metoprolol tartrate, nadolol, oxprenolol hydrochloride, pindolol, (S)-(-)-propranolol

hydrochloride and timolol maleate. These drugs were dissolved in methanol to make stock solutions at concentrations of 1 mg/mL. The solutions were stored at 4°C and used after dilution with an aqueous buffer solution (Tris-HCl, pH 8.5) to the required concentration [1]. The L-amino acids studied were also obtained from Sigma (St. Louis, MO, USA), including alanine (Ala), leucine (Leu), phenylalanine (Phe), tyrosine (Tyr), tryptophan (Trp), methionine (Met), threonine (Thr), asparagine (Asn), glutamic acid (Glu), arginine (Arg) and histidine (His). Each of these amino acids was dissolved in pure water to make separate stock solutions at 0.1 mg/mL. A standard mixture of 10 µg/mL for each amino acid in water was prepared from the stock solutions. The solutions were stored at 4°C and used after dilution with water to the required concentration. The structures of the β-blockers and amino acids are shown in Figures 8-1 and 8-2.

Other chemicals such as pesticides, aromatic compounds, stimulants and organoarsenic compounds are the same as those used in the previous chapters.

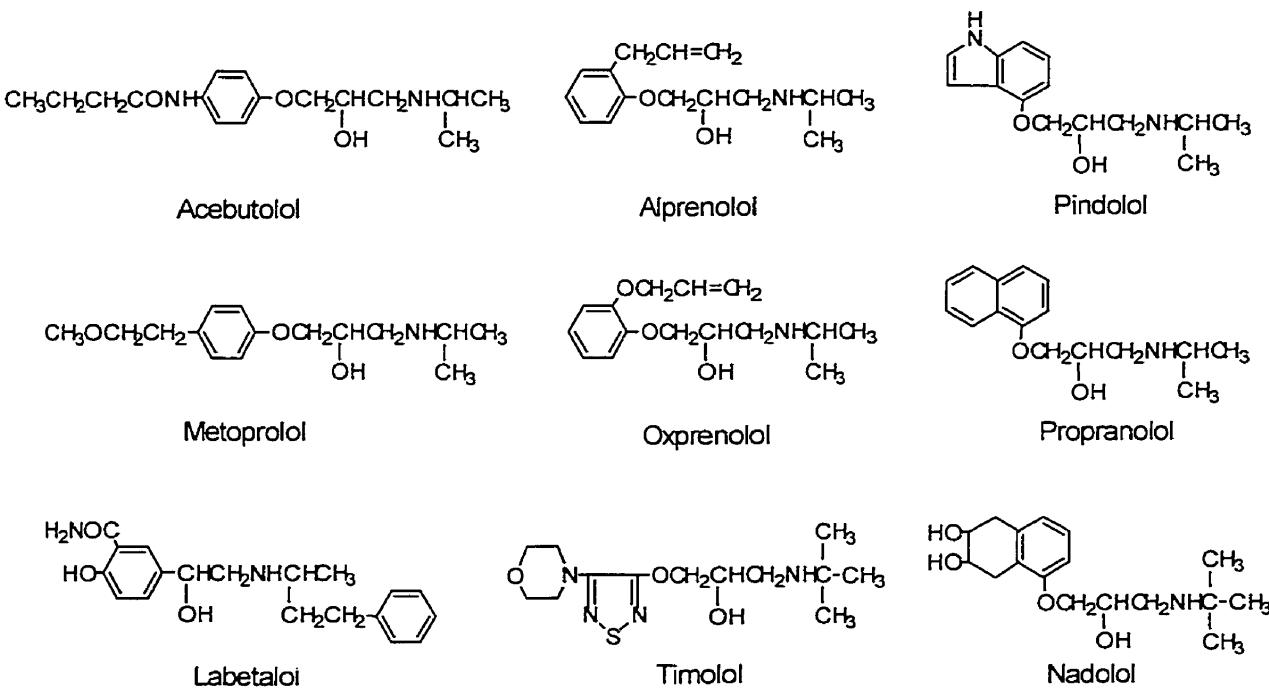


Figure 8-1. Structures of the β-blockers studied

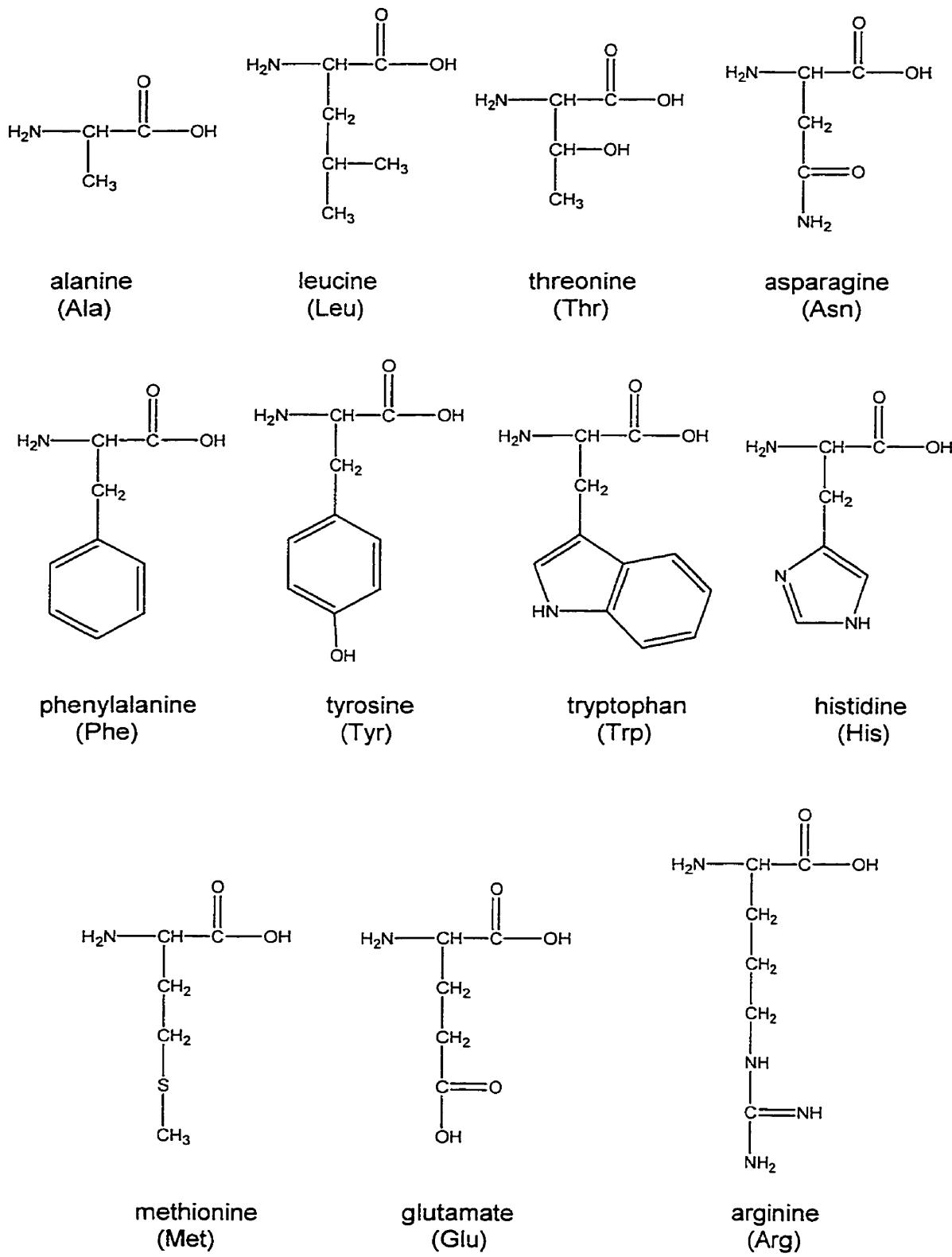


Figure 8-2. Structures of the amino acids studied.

8.2.2. Instrument and analytical conditions

The PPY modified commercial capillary columns were prepared according to the method described in Chapter 2 (see **2.4. Preparation of PPY coated capillary**). In-tube SPME operation and the technique of coupling in-tube SPME with HPLC were described in Chapter 4. All experiments were carried out using a Model 1100 series HPLC system described in detail in Chapter 4.

For the separation of nine β -blockers, A Hypersil BDS C₁₈ column (5.0 cm \times 2.1 mm i.d., 3 μ m particle size) from Agilent Technologies (Palo Alto, CA, USA) was used. LC conditions were as follows: column temperature, 25°C; mobile phase, acetonitrile/methanol/water/acetic acid (15:15:70:1, pH 4); flow rate, program from 0.2 to 0.45 mL/min for a 25 min run. ES-MS conditions were as follows: nebulizer gas, N₂ (40 psi); drying gas, N₂ (10 L/min, 350°C); fragmentor voltage, 70 V; capillary voltage, 4500 V; ionization mode, positive; mass scan range, 100-400 amu; selected ion monitoring (SIM), m/z 249 (pindolol), 250 (alprenolol), 260 (propranolol), 266 (oxprenolol), 268 (metoprolol), 310 (nadolol), 317 (timolol), 329 (labetalol) and 337 (acebutolol).

For the eleven L-amino acids, the following separation conditions were used: column, Supelcosil LC-18 column (5 cm \times 4.0 mm, 5 μ m particle size) from Supelco (Bellefonte, PA USA); mobile phase, 25 % acetonitrile + 75% aqueous solution (0.1% trifluoroacetic acid, TFA); flow rate, 0.2 mL/min. ES-MS detection conditions were as follows: nebulizer gas, N₂ (35 psi); drying gas, N₂ (10 L/min, 350°C); capillary voltage, 5000 V; fragmentor voltage, 40 V; ionization mode, positive; mass scan range, 50-300 amu; selected ion monitoring (SIM), m/z 90 (Ala), 132 (Leu), 166 (Phe), 182 (Tyr), 205 (Trp), 150 (Met), 120 (Thr), 133 (Asn), 148 (Glu), 175 (Arg), and 156 (His).

For the six model aromatic compounds (benzene, toluene, naphthalene, phenol, dimethyl phthalate (DMP) and diethyl phthalate (DEP)), separation was performed using a Supelcosil LC-18 column as was described above. The mobile phase ratio of acetonitrile/water (43/57) was kept constant for the first 7 min, then the proportion of acetonitile was increased linearly to 55 % at 15 min. Mobile phase flow rate was 0.35 mL/min. An UV detector, set to 200 nm, was used for the first 11 min, and then changed to 219 nm for the rest of the run.

The separation and detection conditions for pesticides and organoarsenic compounds were the same as those described in the previous chapters.

8.3. Results and discussion

8.3.1. Preparation of PPy modified capillary and SEM study of the inner surface

As described in Chapter 2, although pyrrole could be easily polymerized chemically, PPy film could only be formed firmly on the inner surface of a silica capillary treated with a polar-deactivated reagent (poly(ethylene glycol)). It was also found recently that PPy film could be coated much more easily on the inner surfaces of polar GC capillary columns such as Omegawax, Supelcowax and Supel-Q PLOT, indicating that there might be certain chemical interactions between the polar inner surfaces of the columns and the PPy film. Further studies should be conducted in the future to elucidate these interactions. The inner surface coating of Omegawax or Supelcowax is poly(ethylene glycol), while the coating of Supel-Q PLOT is divinylbenzene (DVB) polymer. Due to the similarity of Omegawax and Supelcowax in their chemical components and performance for SPME before and after PPy modification, only the results obtained using Omegawax and Supel-Q PLOT are discussed in this chapter.

The inner surface characteristics of the capillary columns were investigated by scanning electron microscopy (SEM, see Chapter 2 for experimental detail). As shown in Figure 8-3, Omegawax has a uniform non-porous surface (a). However, after modification with PPy film, the surface becomes porous, and the porosity of the surface increases when the thickness of PPy coating increases as shown in Figure 8-3 (b)-(d). The porous surface structures should significantly increase the effective surface areas of the coatings, and therefore higher extraction efficiency can be expected compared with the non-porous coatings such as the coating of unmodified Omegawax. In addition, the extraction efficiency and selectivity of the modified capillary should increase with the increase of PPy coating thickness due to the enhancement of the surface area as demonstrated in chapter 4. Supel-Q PLOT has a porous inner surface coating as illustrated in Figure 8-4 (a). However, the pore size of the coating is much larger than that of PPy so that the PPy film can be coated both on the surface of the particles and inside the pores as shown in Figure 8-4(b)-(c). Therefore,

after modification with PPy film, the pore size and porosity of the porous surface structure have been changed, and thus changes in its extraction selectivity and efficiency can be expected due to the size exclusion effect [2].

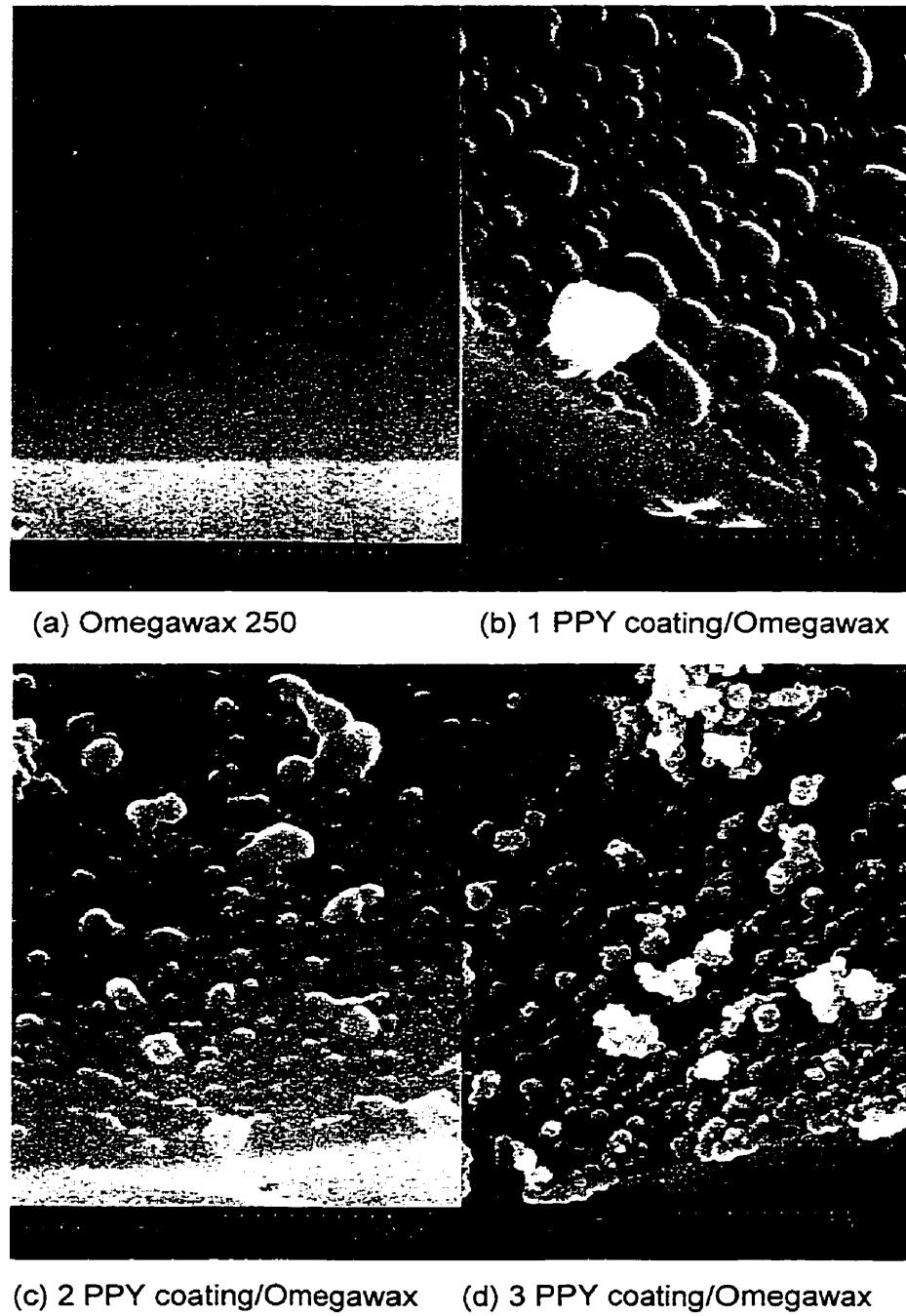


Figure 8-3. Scanning electron micrographs of an Omegawax and the PPY modified Omegawax capillary columns. (a) The inner surface view of a Omegawax column, (b) to (d) the inner surface views of PPY modified columns with 1 PPY coating cycle (b), 2 PPY coating cycles (c) and 3 PPY coating cycles (c)

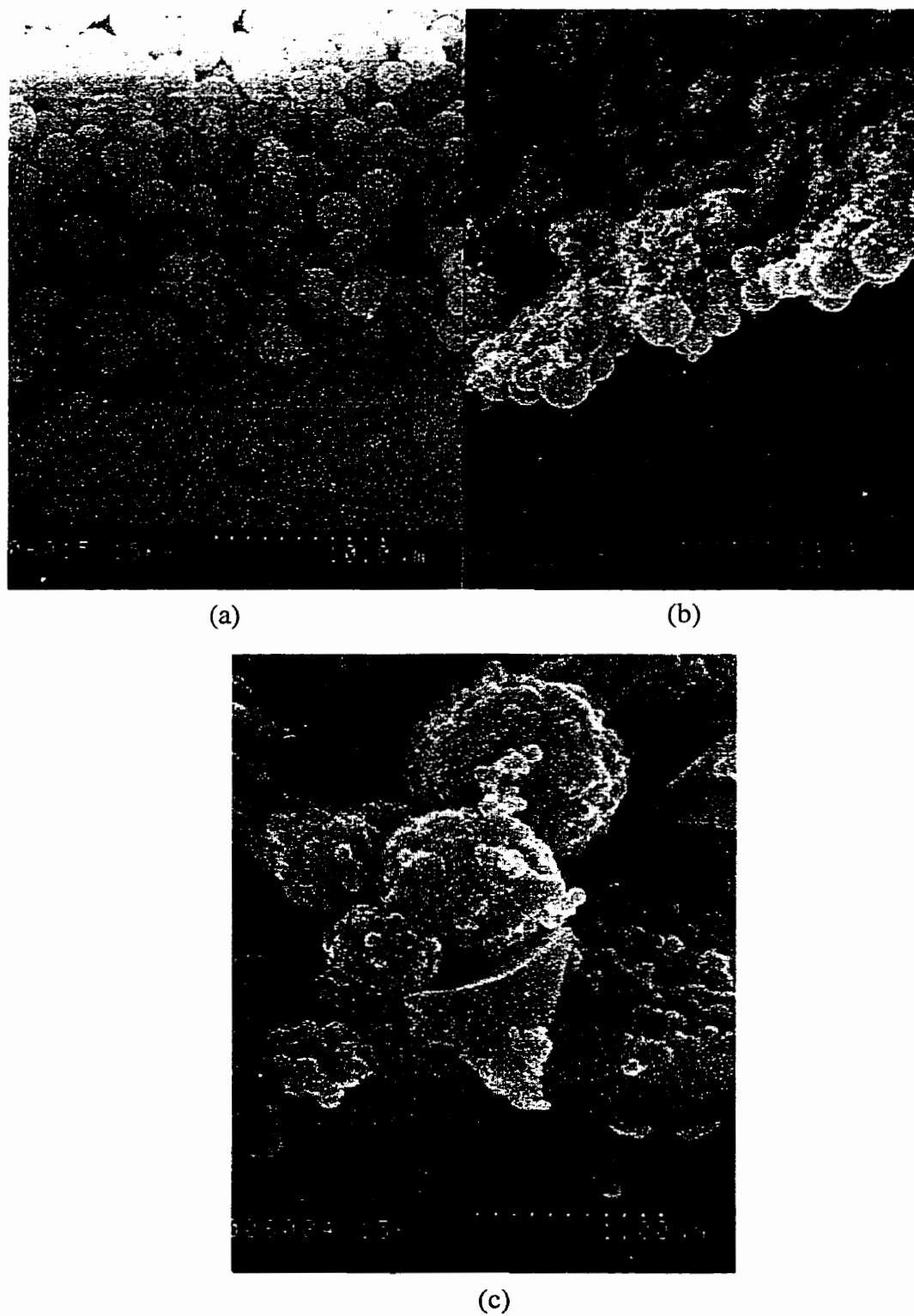


Figure 8-4. Scanning electron micrographs of a Supel-Q PLOT and the PPY modified Supel-Q PLOT capillary columns. The inner surface views of (a) a Supel-Q PLOT column and (b) a Supel-Q PLOT column modified with 1 PPY coating cycle. (c) The enlarged view of one section of the inner surface shown in (b)

8.3.2. Separation and detection

To select the monitoring ion for each of the β -blockers and amino acids, ES mass spectra were initially analyzed by direct liquid injection. Each compound gave a very simple spectrum, with $[M+H]^+$ ion as the base ion. Each base ion accounted for above 70% of the total ion current, therefore, these base ions are used for quantification in selected ion monitoring of each β -blocker and amino acid. Optimized ES-MS conditions for β -blockers and amino acids are summarized in the experimental section.

As shown in Figure 8-5, although β -blockers could not be separated completely under the current chromatographic conditions, all of the β -blockers could be separated by the mass detector using selected ion monitoring mode (SIM) as shown in Figure 8-6. Similar results were also obtained for amino acid analysis in this work (figures not shown), indicating the advantages of mass detection over other detection methods.

As shown in Figure 8-7, the six model aromatic compounds were completely separated using the chromatographic conditions listed in the experimental section. The separation and detection conditions for other compounds were discussed previously.

8.3.3. Extraction properties of the modified capillaries for in-tube SPME

In this work, a 60-cm long capillary column was selected and an extraction of 15 draw/eject cycles (40 μ L for each cycle at a flow rate of 100 μ L/min) was used in all in-tube SPME experiments. The analytes were desorbed by mobile phases as described previously. To evaluate the effect of PPY coating on extraction efficiency, the extraction performances of the original and PPY-modified capillary columns are compared by coupling in-tube SPME with HPLC. As shown in Figure 8-5, compared with the original columns, after modification with only one PPY coating cycle (see chapter 2, preparation of PPY coated capillary), the extraction efficiencies of the columns toward β -blockers were increased significantly, especially for the Omegawax column. Similar results were also obtained for other basic drugs (data not shown) such as the stimulants discussed in chapter 5. It can also be seen from Figure 8-5 (A) that the selectivity of the Supel-Q PLOT column towards more polar compounds (compounds 1 to 5) relative to less polar compounds (compounds 6 to 9) has been improved after PPY modification.

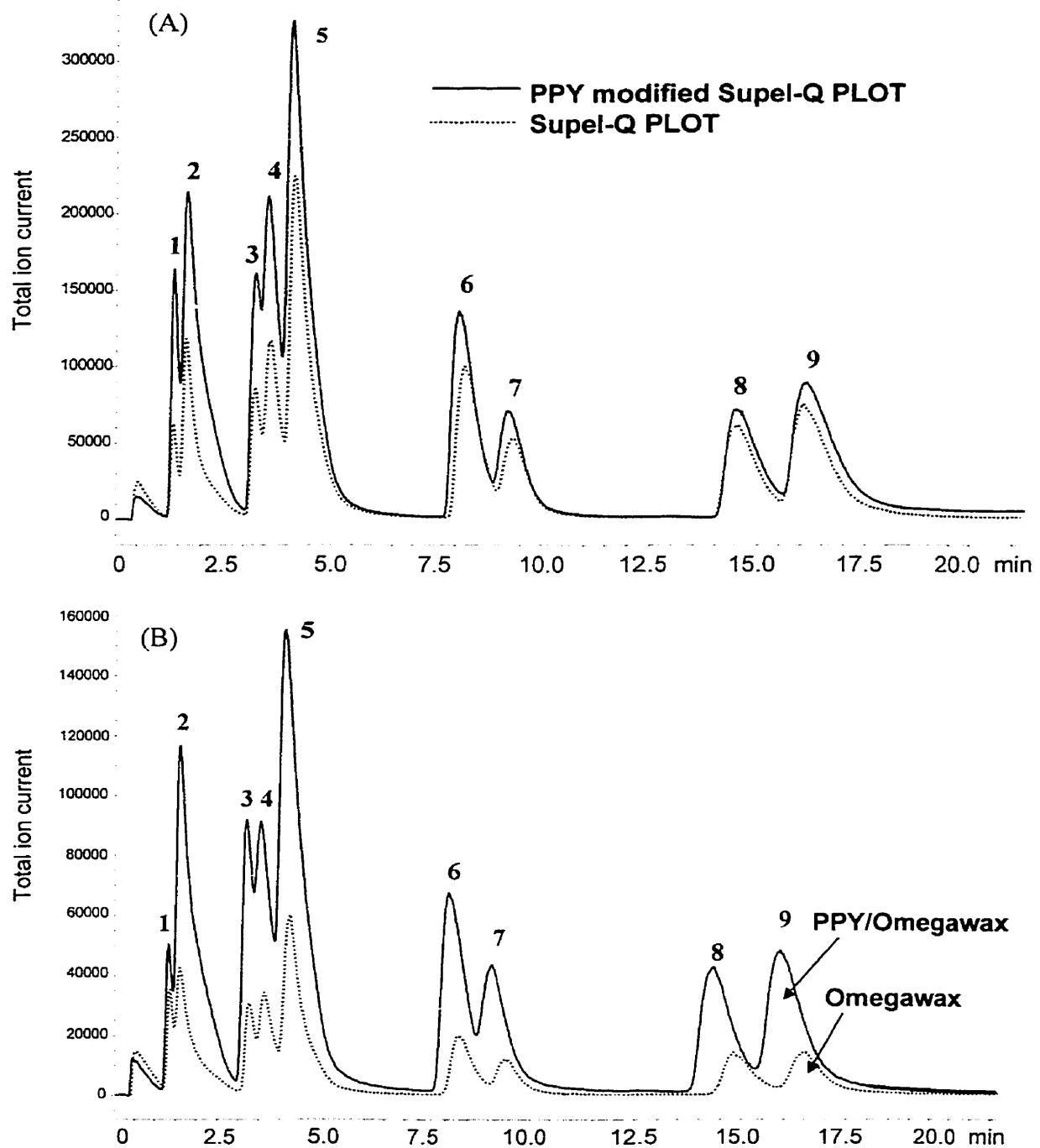


Figure 8-5. Comparison of the chromatograms obtained for β -blockers by in-tube SPME-HPLC-MS with (A) Supel-Q PLOT capillary columns and (B) Omegawax columns before and after PPY modifications. Concentration of each β -blocker: 100 ng/mL. Peak identification: 1, nadolol; 2, pindolol; 3, acebutolol; 4, timolol; 5, metoprolol; 6, oxprenolol; 7, labetalol; 8, propranolol; and 9, alprenolol.

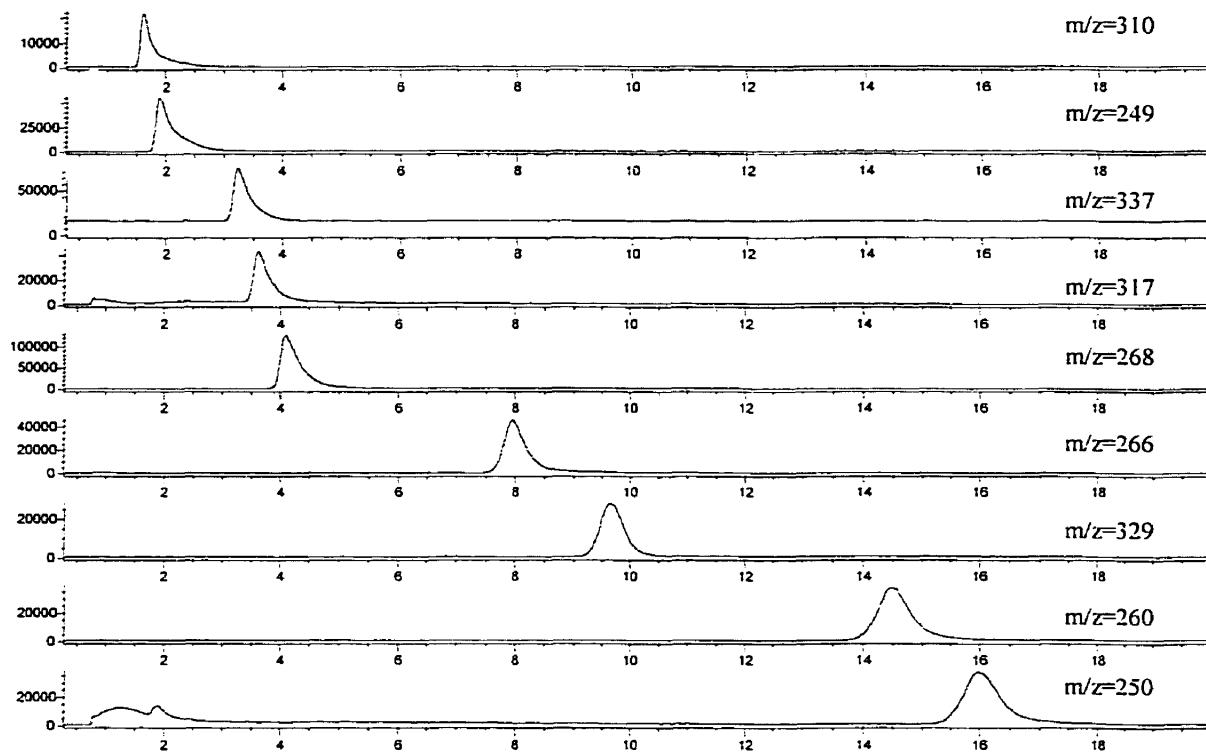


Figure 8-6. Selected ion monitoring (SIM) chromatograms of β -blockers obtained by in-tube SPME-HPLC-MS with Omegawax. Analyte concentrations and peak identifications are the same as in Figure 8-5.

The thickness of PPy film also has a significant effect on the extraction properties of the modified columns. This effect was studied initially for Omegawax columns using a group of model aromatics which contained both polar (phenol, DMP and DEP) and non-polar (benzene, toluene and naphthalene) compounds. As illustrated in Figure 8-7, with the increase of PPy film thickness, the extraction efficiencies (corresponding to the peak areas) of the PPy modified columns to naphthalene (having two π rings) and to polar compounds DMP and DEP increased significantly relative to less polar compounds benzene and toluene. The enhancement in extraction selectivity toward polar and polycyclic aromatic compounds with an increase of the PPy film thickness is due to the increase of specific interactions ($\pi-\pi$

and polar functional groups) and / or the changes in the surface properties as discussed previously.

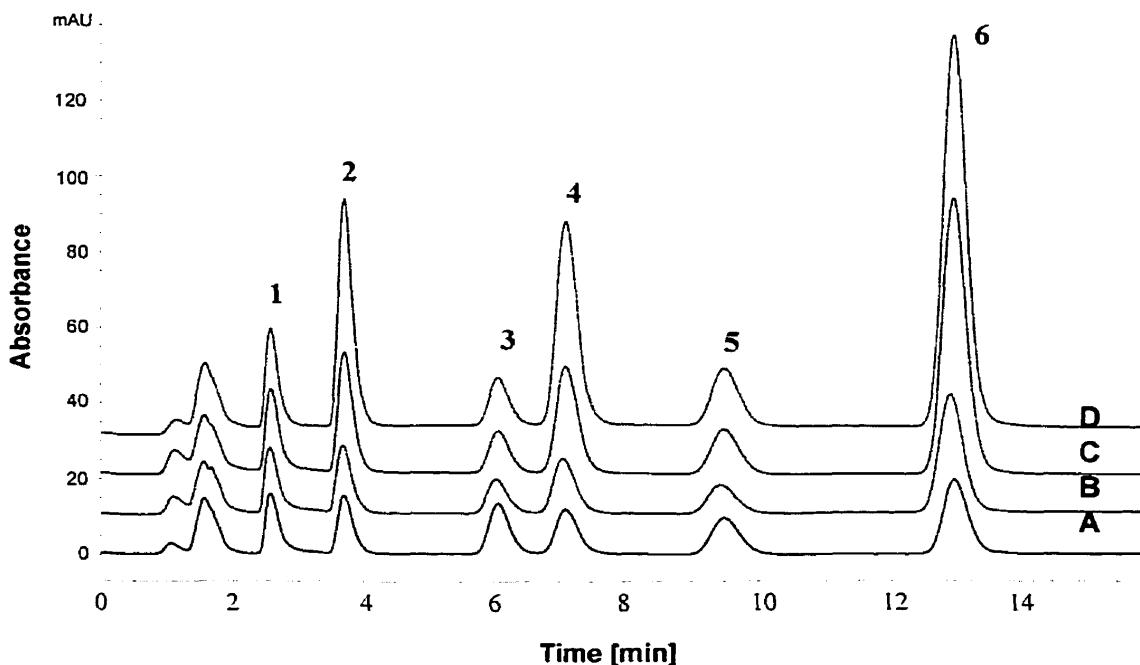


Figure 8-7. Chromatograms of the six model aromatic compounds obtained by in-tube SPME-HPLC-UV with an Omegawax column (A) and PPy modified Omegawax columns (B, 1 PPy coating cycle; C, 2 PPy coating cycle; and D, 3 PPy coating cycle modifications). Peak identification and analyte concentration: 1: phenol (2 ppm), 2: DMP (1 ppm), 3: benzene (5 ppm), 4: DEP (1 ppm), 5: toluene (5 ppm), and 6: naphthalene (1 ppm).

The extraction performances of different capillary columns for the selected amino acids are compared in Figure 8-8. Surprisingly, a Supel-Q PLOT column, which showed higher extraction ability than Omegawax and other commercial capillaries for many polar compounds such as β -blockers (Figure 8-5), gave the worst extraction efficiency for the studied amino acids among the tested columns. However, after modification with PPy film (3 coating cycles), the extraction efficiency of Supel-Q PLOT and Omegawax changed dramatically. In particular, the extraction efficiency for tryptophan (Trp), phenylalanine (Phe) and tyrosine (Tyr) were increased greatly compared with the unmodified columns, possibly due to the enhanced π - π interactions and porous inner surface structures introduced

from PPy coating. In addition, these changes in extraction selectivity and efficiency could be enhanced when the thickness of PPy film was increased as illustrated in Figure 8-9.

The enhanced extraction efficiency of PPy modified capillary columns for other polar compounds such as pesticides (Figure 8-10) were also observed. However, for cationic species, such as arsenocholine (AsC) in Figure 8-11, the modified column showed worse extraction efficiency compared with the original column due to electrostatic repulsion discussed in chapters 5 and 6.

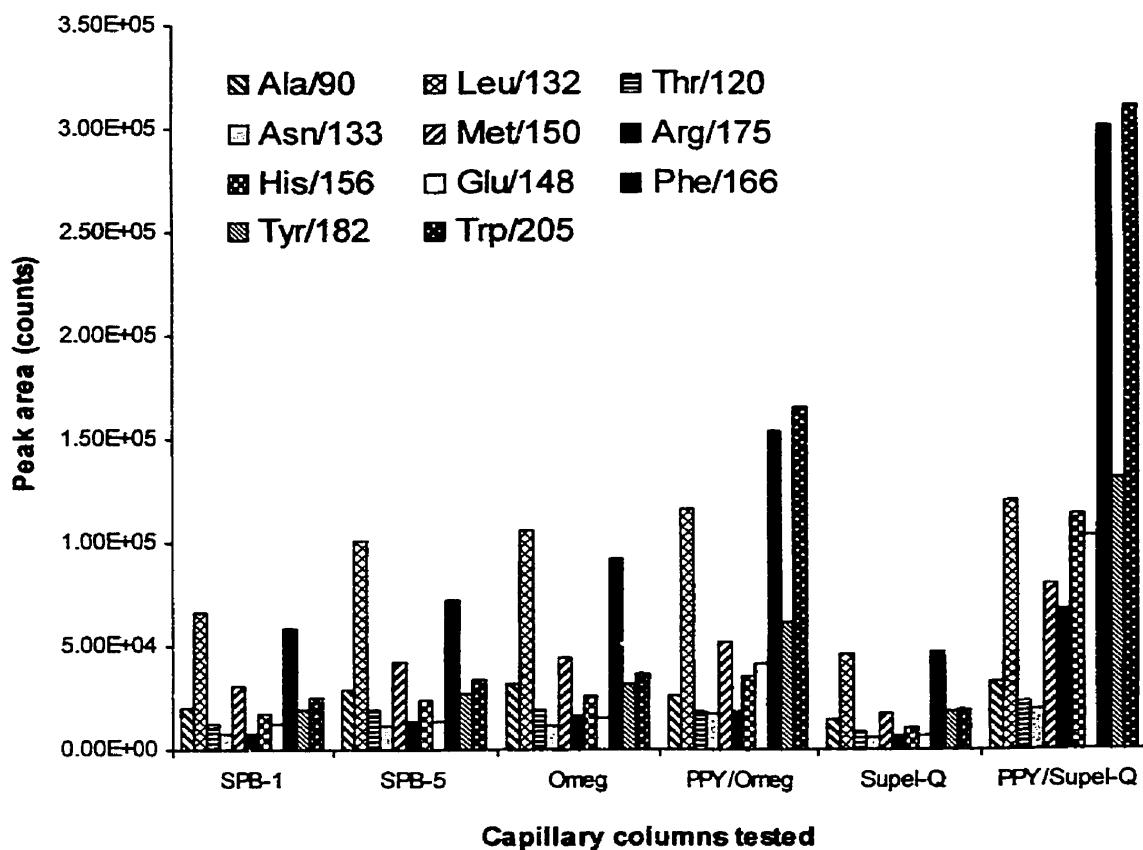


Figure 8-8. Comparison of the responses (peak areas) obtained by in-tube SPME-HPLC-MS with different capillary columns. PPy/Omeg and PPy/Supel-Q refer to Omegawax and Supel-Q PLOT columns modified with 3 PPy coating cycles, respectively. Analyte concentration: each 100 ng/mL.

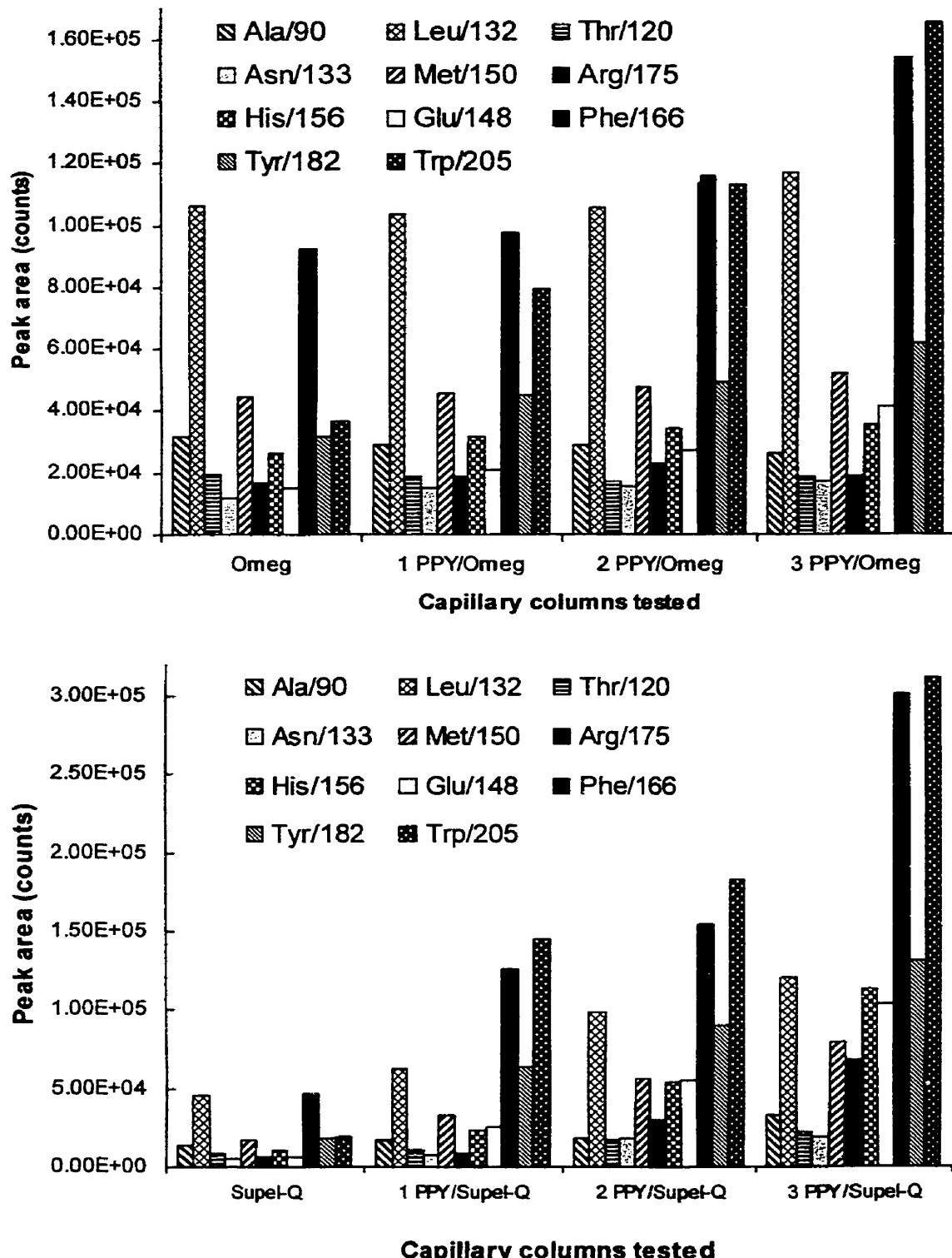


Figure 8-9. Effect of PPy film thickness on the extraction performances of in-tube SPME. Analyte concentration: 100 ng/mL for each amino acid.

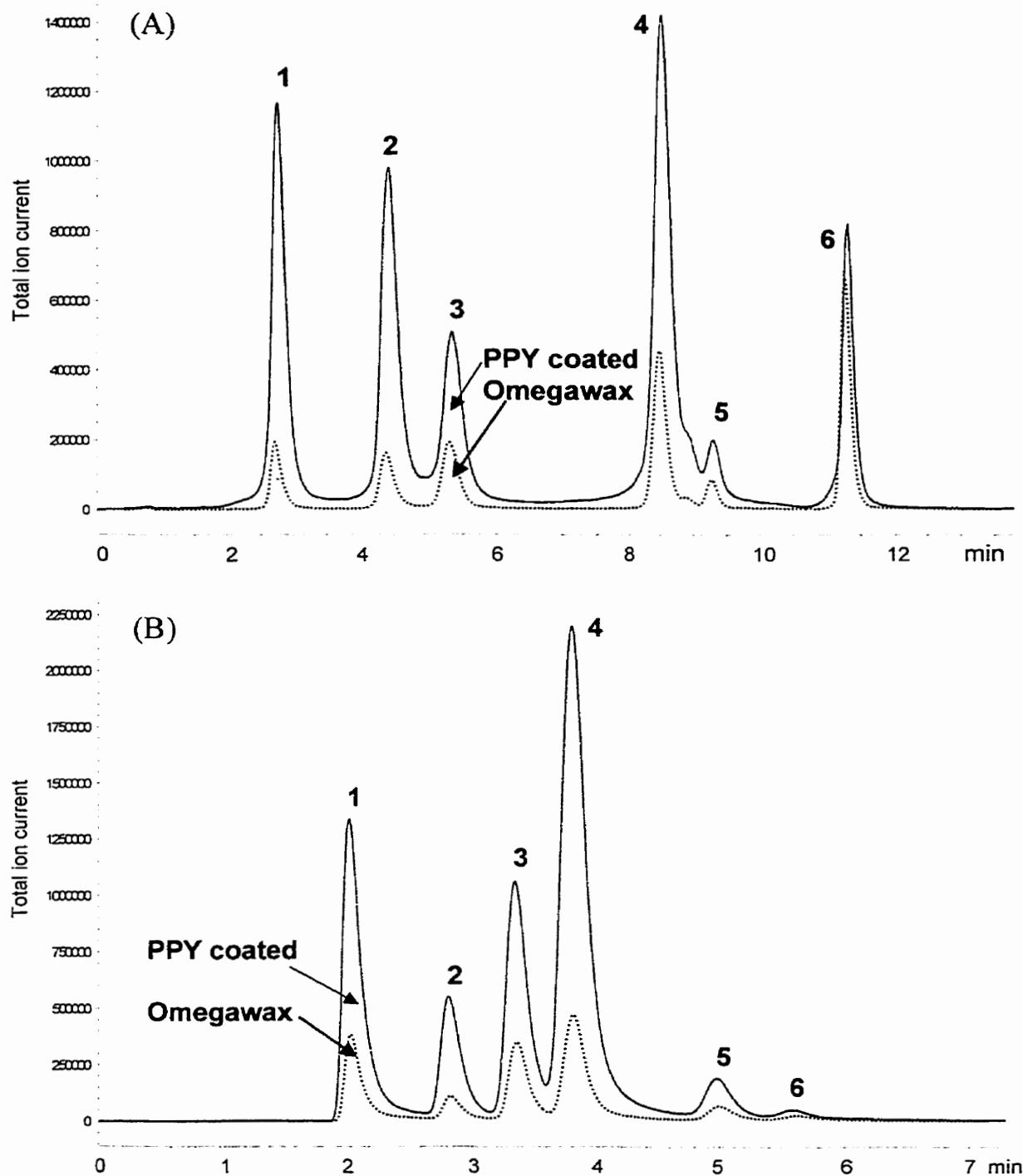


Figure 8-10. Chromatograms of phenylurea and carbamate pesticides obtained by in-tube SPME-HPLC-MS with Omegawax and PPy (3 PPy coating cycles) modified Omegawax columns. Analyte concentration: each 100 ng/mL. (A) Phenylureas: 1. monuron, 2. fluometuron, 3. diuron, 4. siduron, 5. linuron, and 6. Neburon. (B) Carbamates: 1. carbaryl, 2. propham, 3. methiocarb, 4. promecarb, 5. chlorpropham, and 6. barban

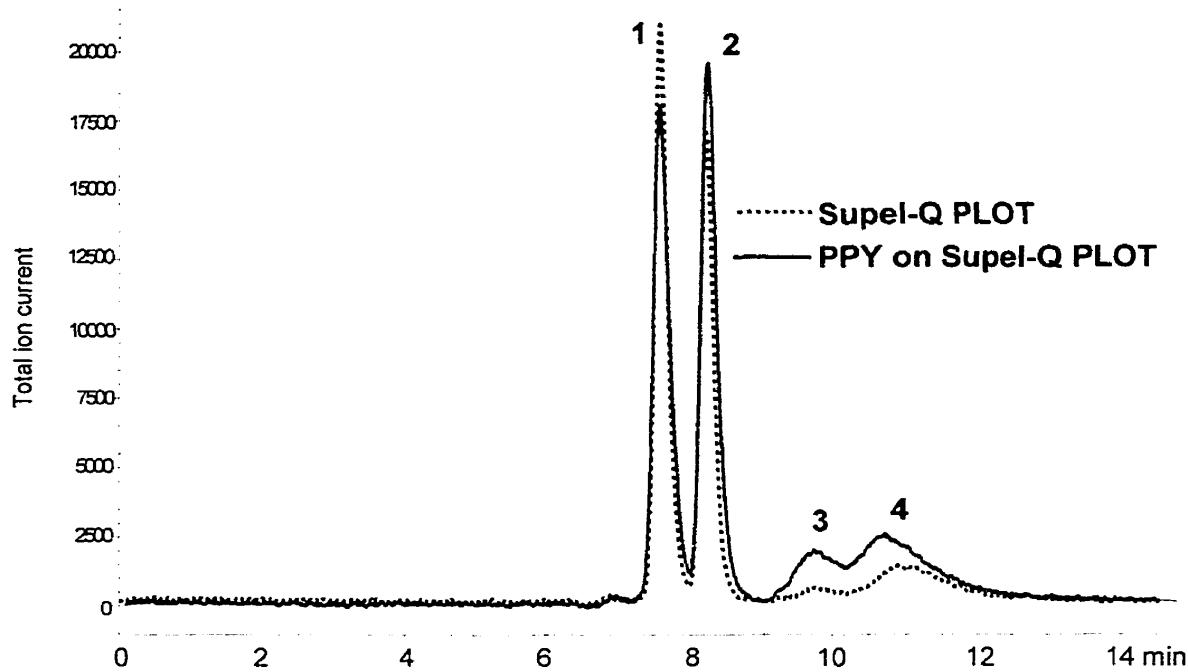


Figure 8-11. Chromatograms of organoarsenicals by in-tube SPME-HPLC-MS with Supel-Q PLOT and PPY (1 PPY coating cycle) modified Supel-Q PLOT columns. Concentration of each analyte: 50 ng/mL. Peak identification: 1. arsenocholine (AsC), 2. arsenobetaine (AsB), 3. monomethylarsonic acid (MMA_s) and 4. dimethylarsinic acid (DMA_s).

8.3.4. Stability of the modified capillary columns

It was found in our studies that Supel-Q PLOT coating was easily degraded when used in some of the HPLC mobile phases. However, after modification with one or two cycles of PPY film, its stability in these solutions was increased dramatically due to the stability of PPY film in most of the solvents or buffer solutions used in HPLC as discussed in the previous chapters. Improvements in stability and performance for other materials, when used in a composite film with PPY, has been reported previously [3-4].

8.4. Summary

Polar GC capillary columns, such as Omegawax, Supelcowax and Supel-Q PLOT, can be easily modified chemically by coating a thin film of PPY on their inner surfaces. The modified columns have shown significantly better extraction efficiency than the original columns toward aromatic compounds, polar compounds and anionic species, due to the new functionalities introduced on the surfaces and the changes in the surface properties (increase in surface area and porosity). Since these modified capillary columns can be easily prepared by a chemical polymerization method and are more stable than the original columns to the solvents used in HPLC, they are well suited for in-tube SPME applications of a wide range of analytes when coupled to HPLC.

8.5. References

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2. Wang, J.; Chen, S-P.; Lin, M.S. *J. Electroanal. Chem.* **1989**, **273**, 231.
3. Lewis, T.W.; Wallace, G.G.; Smyth, M.R. *Analyst*, **1999**, **124**, 213.
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CHAPTER 9

SPME OF VOLATILE ORGANIC COMPOUNDS

9-1. Introduction

Solid-phase microextraction (SPME) has become a popular sampling method for a variety of volatile and semi-volatile organic compounds due to its simple, solvent-free, reliable, and flexible properties [1-3]. However, for the analysis of polar compounds, derivatization and pH adjustment are often necessary to achieve good extraction efficiency and sensitivity. In addition, the extraction of polar compounds out of a non-polar matrix is still a challenging task since most of the coatings available commercially are non- or slightly polar and they extract the non-polar matrix components overwhelmingly or severely swell in the matrices [4]. Recently, a Nafion coated fiber has been used for the analysis of polar compounds (alcohols) in nonpolar matrices [4]. However, the use of a Nafion fiber has some disadvantages, such as a poor fiber blank and large memory effect due to the strong binding between the analytes and the Nafion fiber.

The capability and potential applications of PPY film for SPME of less volatile compounds from aqueous samples have been demonstrated in the previous chapters. In this chapter, PPY and four of its *N*-substituted derivatives have been prepared by electrochemical polymerization. The extraction properties of these new coatings towards several groups of volatile organic compounds have been evaluated by coupling fiber SPME to gas chromatography (GC). The preliminary results have shown that these coatings have different selectivities to various organic compounds depending on their chemical structures and functional groups. In general, compared with commercial SPME coatings, these new coatings have shown better selectivity toward polar compounds due to the interactions of polar functional groups between analytes and the coatings. More importantly, the selectivity and sensitivity of the coatings can be modified by incorporating a new functional group into the polymer, as indicated in this work by poly-*N*-phenylpyrrole (PPPY) coating which has better selectivity to aromatic compounds (compared with PPY coating) due to the phenyl group incorporated into the polymer. In addition, the extraction of polar analytes from

nonpolar matrices has been performed using PPY and PPPY coatings, and the results have been compared with those obtained by a Nafion fiber.

9.2. Experimental

9.2.1. Chemicals and reagents

The organic compounds benzene, 1-butanol, 2-pentanone, 1-nitropropane and pyridine (analytical-reagent grade, BDH, Toronto, ON, Canada) were used as received. All other chemicals (analytical-reagent grade, Aldrich) were used as received. The commercial SPME device and fibers were obtained from Supelco, Inc. (Bellefonte, PA). Commercial fibers coated with 30 µm thick poly(dimethylsiloxane) (PDMS), 65 µm poly(dimethylsiloxane) / divinylbenzene (PDMS/DVB), 85 µm poly(acrylate) (PA), 65 µm carbowax® / divinylbenzene (CW/DVB) and 75 µm carboxen™ / poly(dimethylsiloxane) (CAR/ PDMS) were used. The chemicals and the electrochemical polymerization conditions for coating PPY and its derivatives on metal wire have been described in chapter 2. The chemical structures of PPY and its *N*-substituted derivatives have been given in Figure 2-1 (Chapter 2).

9.2.2. Scanning electron microscopy (SEM) study of the polymer surfaces

All the SEM studies on the polymer-coated surfaces were carried out according the conditions described in chapter 2.

9.2.3. SPME of volatile organic compounds

For the new coatings, a modified syringe assembly was used as an SPME device as described in chapter 3 (see Figure 3-2 (A)). A metal wire (platinum or stainless steel) was inserted into a Hamilton syringe and the polymer coating was deposited on the surface of the wire tip by electrochemical polymerization. When the plunger is retracted, the metal wire with coating can be drawn into the syringe needle, thus protecting the polymer coating when the syringe needle is used to pierce the septum during extraction and desorption in the GC injector. The plunger can then be pushed down to expose the polymer coating to the sample for extraction or to the GC injector for thermal desorption. The commercial SPME device and a used fiber assembly could also be used by replacing the fiber with the polymer-coated

metal wire (see Figure 3-2 (B)). Before they were used for SPME, the coated metal wires were dried under N₂ and then preheated at 100 °C for 30 min and finally conditioned at 180 ~200 °C in a GC injection port under helium protection until a clean blank was obtained.

Gas samples were prepared by injecting 2 or 5 µL of each of the organic compounds into silanized 1-L gas standard bulbs (Supelco, Mississauga, Canada) and then allowing them to stand in ambient temperature for an hour or longer to reach full evaporation and equilibration. Extraction processes were performed by standard headspace SPME [1-3]. In this work, all SPME extractions were performed at room temperature. A 20-min period was found suitable for extraction and 2 min for desorption (at 200 °C).

9.2.4. GC separation conditions

All the GC analyses were carried out using an SPB-1 column (Supelco), 30 m × 0.25 mm, 1 µm film thickness, equipped with a 1 m deactivated fused-silica precolumn. A Varian 3400 CX gas chromatograph (GC) equipped with septum programmable injector (SPI) and a flame ionization detector (FID) was used. All the GC experiments were carried out with helium as carrier gas (helium pressure: 20 psi) and the GC data were acquired with a Varian Star system.

The GC method for BTEX (benzene, toluene, ethylbenzene and p-xylene) analysis was the same as the reported previously [5].

The following conditions were used for the McReynolds probe compounds (i.e. benzene, 1-butanol, 2-pentanone, 1-nitropropane and pyridine): the column was maintained at 60 °C for 2 min and then ramped to 100 °C at a rate of 10 °C/min. For syringe injection, the injector was at 40 °C initially, ramped to 180 °C at 250 °C/min, and then maintained at that temperature for the rest of the run. For SPME injection, the coating was desorbed at 220 °C for 2 min. The FID detector temperature was 275 °C for all analytes.

The analyses of polar compounds (alcohols) in nonpolar matrixes (hexane or gasoline) were carried out according to the literature method [4].

For the following three groups of compounds, the GC conditions employed were the same and they were: For SPME injection, column temperature program 40 °C (2 min), 20 °C/min to 140 °C, and hold at 140 °C for 2 min; injector SPI, 200 °C; detector FID, 250 °C.

For direct injection, the same conditions were used except that the injector was temperature programmed (69 °C, 200 °C/min to 180 °C). Group one compounds were methanol, acetone, hexane, tetrahydrofuran (THF), benzene, toluene, ethylbenzene and p-xylene; group two compounds were ethylamine, acetonitrile, dichloromethane, hexane, benzene, toluene, ethylbenzene, and p-xylene. Group three compounds (10 alcohols) were methanol, ethanol, 2-propanol, 1-propanol, 2-butanol, 1-butanol, 2-pentanol, 1-pentanol, 2-hexanol and 1-hexanol.

9.3. Results and Discussion

9.3.1. Cyclic voltammetry (CV) and scanning electron microscopy (SEM)

Following polymer film formation, the cyclic voltammogram for each polymer film was obtained at a scan rate of 50 mV/s versus a SCE electrode using 0.1 M tetrabutylammonium perchlorate (TBAP) in acetonitrile without a monomer as the supporting electrolyte. Well-defined, broad anodic and cathodic peaks were obtained for all the polymers studied, reflecting the slow rate of counter ion transfer in and out of the films. As the chain length of the *N*-substituent increases, both the anodic and the cathodic peak potentials become more positive relative to the parent PPy film. Since the CV results are similar to those previously reported [6], they will not be discussed in detail in this work.

It was previously observed that surface morphology could have an effect on the response of the polymer-coated sensors to different organic vapours [6-10]. PPy films are frequently described as having a ‘cauliflower-like’ appearance, which arises from the nucleation and phase growth mechanism of the electropolymerization process. The scanning electron micrographs obtained in this work for the five studied polymers are similar to those reported previously [6-10], and therefore, they will not be duplicated here. Generally, films of the *N*-substituted pyrrole derivatives show similar morphologies to the parent PPy film, although there are some differences. For example, both poly-*N*-methylpyrrole (PMPY) and poly-*N*-phenylpyrrole (PPPY) films exhibit a much more granular and compact surface than PPy film, while poly-*N*-(2-cyanoethyl)pyrrole (PCPY) and poly-*N*-(2-carboxyethyl)pyrrole (PCbPY) films show similar topography but with deep, open channels running into the bulk of the polymers. These differences in morphology may have a significant influence on the

response of the coated vapour sensors [6-10] and the performance of the coatings for SPME of gas samples. This occurs firstly through variations in effective surface area, which determines the extraction efficiency, and secondly through the film porosity, which affects both the efficiency and selectivity of SPME and sensors.

9.3.2. Gas-phase extraction of volatile organic compounds

9.3.2.1. SPME method development

A group of (BTEX) compounds including benzene, toluene, ethylbenzene, and *p*-xylene was chosen first to study the suitability of the new coatings for SPME because these compounds have been studied and well characterized previously using PDMS fiber [5]. Experiments on extraction time and desorption time profiles were performed for both the new coatings and commercial fibers. The extraction time of 20 min was chosen for all the coatings and fibers since the amount of analytes extracted or the detector responses (peak areas) remained constant after 10 min extraction (20 min should be long enough to ensure equilibrium sorption). The desorption time chosen was 2 min because no carryover (or memory effect) was found at the injection temperatures used after 2 min desorption. The method precision for the determination of BTEX (5 ppm) was carried out with the new coatings by 7 replicate measurements. The relative standard deviations (RSDs) fall in the range of 2.5 % to 8.8 %. For the other groups of compounds discussed below, RSD values are within the range of 1.8 % ~ 11.2 % for three replicate measurements.

9.3.2.2. Comparison of the new coatings with the commercial fibers

In order to examine the extraction properties of the newly prepared coatings and compare them with the commercial SPME fibers, the following four groups of compounds with various functionalities were tested by coupling SPME with GC-FID.

Group one compounds. This group of compounds includes methanol, acetone, hexane, tetrahydrofuran (THF), benzene, toluene, ethylbenzene, and *p*-xylene. The extraction processes were carried out in gas phase. The selected chromatograms obtained by SPME-GC are illustrated in Figure 9-1 and Figure 9-2. The results obtained by SPME-GC methods with the different commercial fibers and the new coatings are shown in Figure 9-3. It can be seen from these results that the extraction properties of the new coatings are remarkably different

from those of the commercial fibers. For example, the new coatings have better selectivity towards polar compounds such as methanol while the commercial fibers respond more sensitively to the less polar aromatic compounds. It is not surprising that PPy and its derivatives are sensitive to methanol and other polar gaseous compounds given their structures which are likely to exhibit hydrogen bonding and acid-base interactions. These results are in line with those studies published previously on gas sensors and ‘electronic noses’ [6-13].

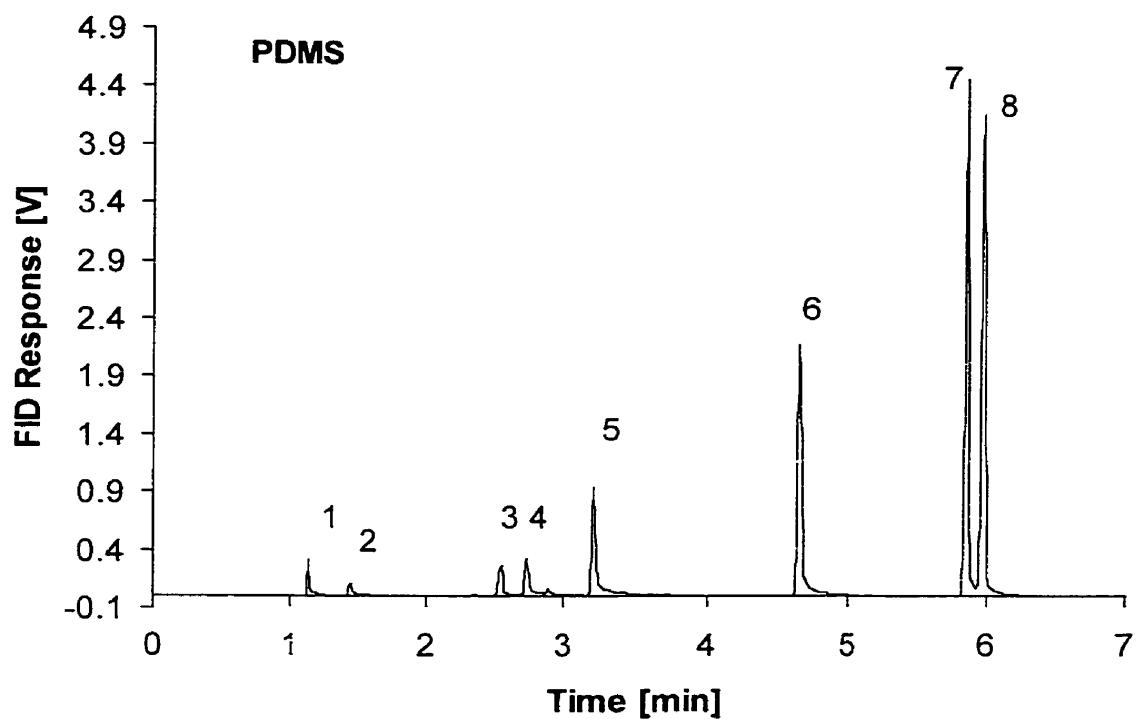


Figure 9-1. Chromatogram of group one compounds obtained by PDMS fiber SPME coupled with GC. Peak identification: 1. methanol, 2. acetone, 3. hexane, 4. tetrahydrofuran (THF), 5. benzene, 6. toluene, 7. ethylbenzene, and 8. *p*-xylene.

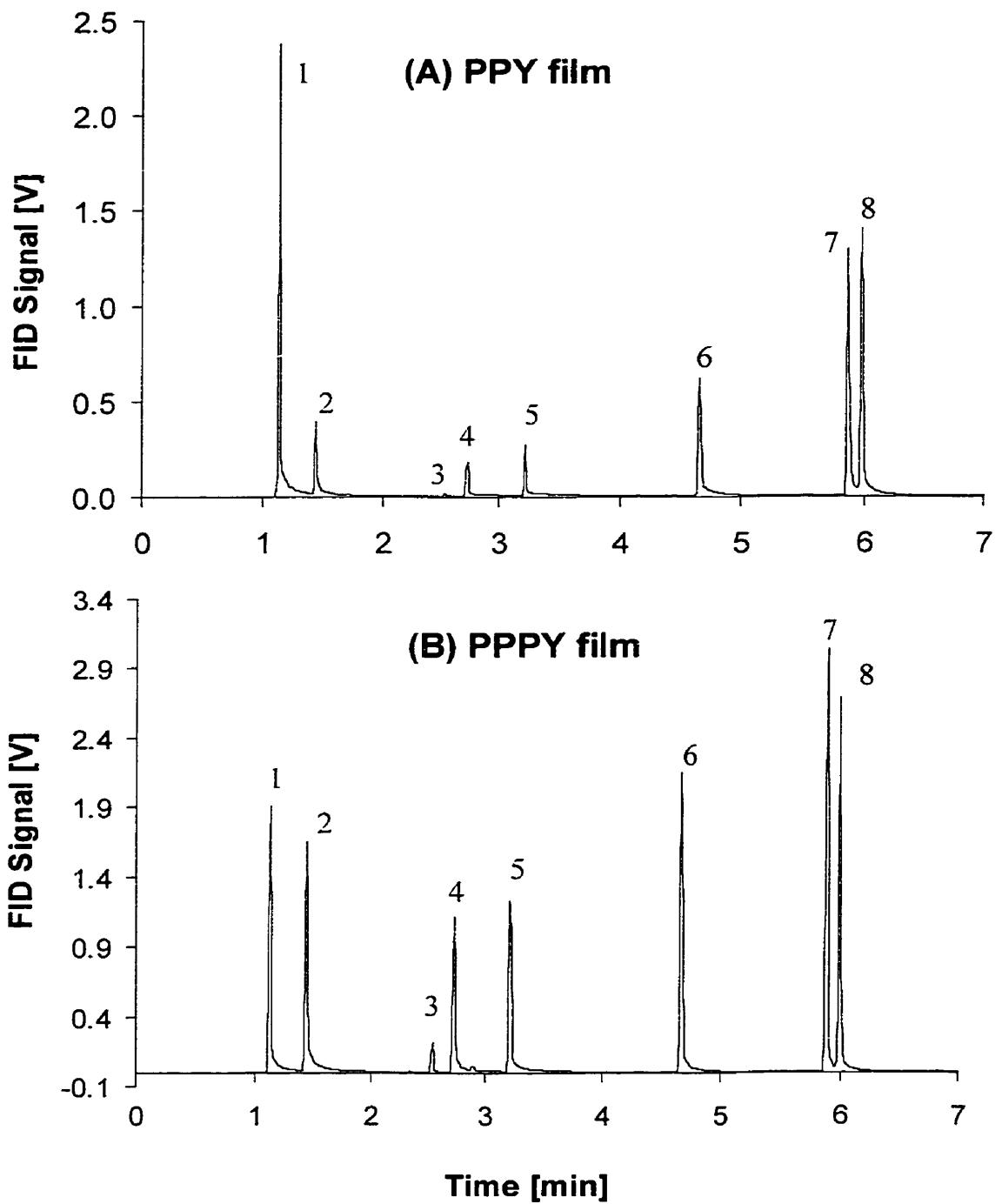


Figure 9-2. Chromatograms of group one compounds obtained by (A) PPy and (B) PPPY film SPME coupled with GC. Peak identification and analytical conditions are the same as in Figure 9-1.

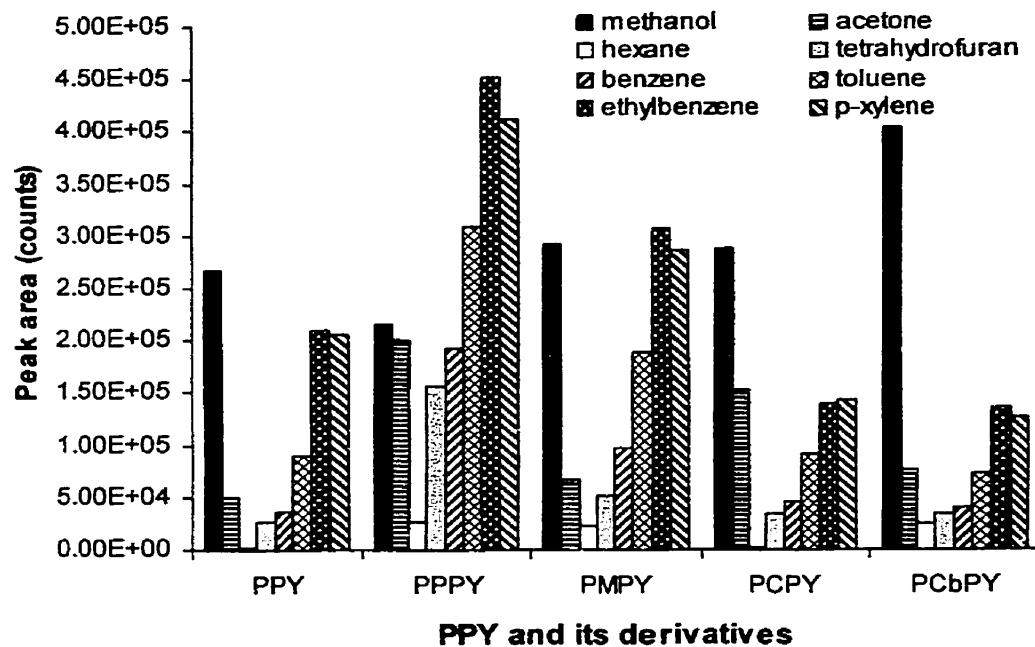
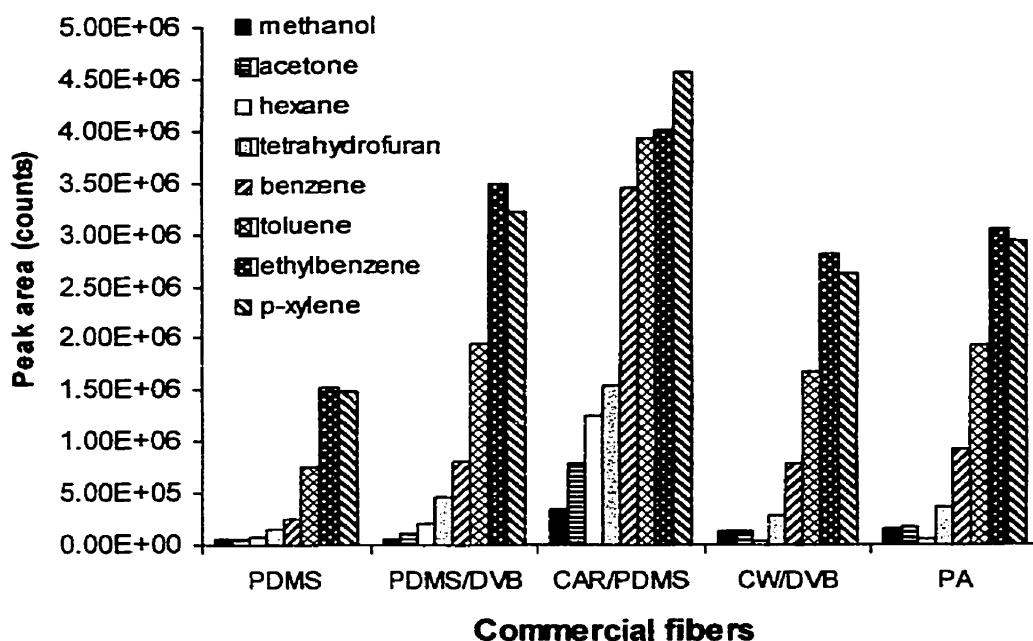


Figure 9-3. Responses of different commercial SPME fibers (upper) and the new coatings (below) towards group one compounds. Conditions: see the Experimental section.

More importantly, significantly different extraction properties have been observed among the new coatings toward the compounds studied (Figure 9-3). For example, all the new coatings show high responses to methanol, but the PPPY coating has better extraction ability than PPy and other coatings toward the less polar aromatic compounds due to the introduction of phenyl groups (π - π interactions) in the polymer. The PPPY coating also shows better sensitivity to acetone relative to other coatings, indicating its potential application for this type of analyte. The differences in the extraction properties among the new coatings are possibly due to the differences in their chemical structures (functional groups) and surface properties (effective surface area and pore size). The increased sensitivity of PPPY coating to the less polar aromatic hydrocarbons is probably due to the increased π electronic interactions and hydrophobic interactions between the compounds and the PPPY polymer. Similar results have also been reported for the different responses of gas sensors based on PPy and its copolymer with nitrotoluenes [11]. The PCbPY coating, on the other hand, shows the poorest extraction ability to the less polar aromatic compounds due to its polar functionality. These results demonstrate that the selectivity and sensitivity of SPME can be modified by incorporation of additional functional groups into the polymer.

Group two compounds. This group of compounds includes ethylamine, acetonitrile, dichloromethane, hexane, benzene, toluene, ethylbenzene, and p-xylene. The results (see Figure 9-4) were similar to those found for group one compounds, i.e. the new coatings have better selectivity for polar compounds compared with the commercial fibers studied. The extraction properties of the new coatings are also different from each other for these compounds likely due to the similar reasons as discussed above. The low extraction ability toward nonpolar compounds (e.g. hexane) suggest that these new coatings can be utilized for the analysis of polar compounds in nonpolar matrixes (e.g. gasoline, see the following section). In addition, these new coatings, especially PPPY and PMPY, have shown high sensitivity toward acetonitrile, indicating their potential application for this type of analyte.

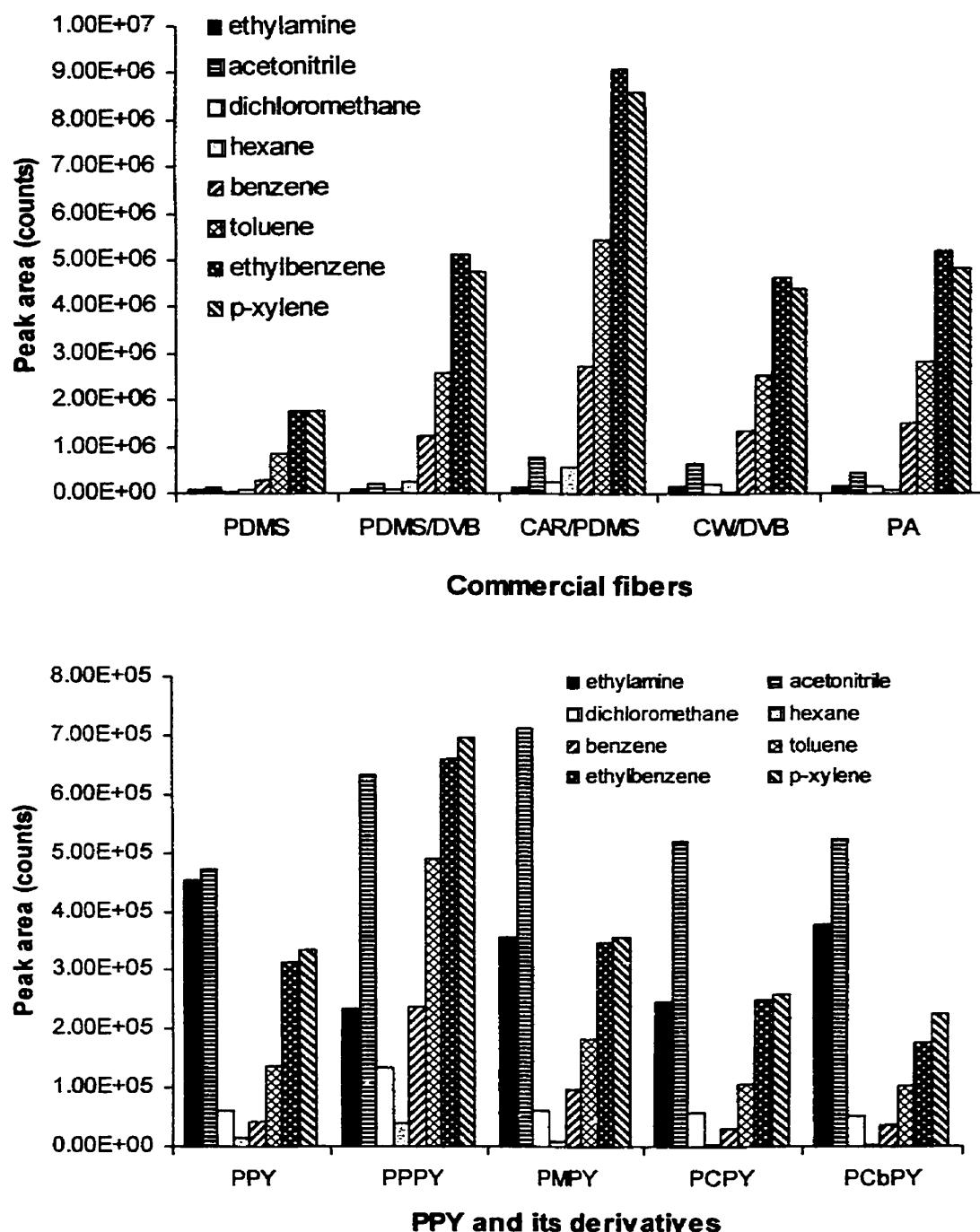


Figure 9-4. Responses of different commercial SPME fibers (upper) and the new coatings (below) towards group two compounds. Conditions: see the Experimental section.

McReynolds test compounds. The compounds used in this group are the McReynolds test solutes for GC stationary phases, including benzene, 1-butanol, 2-pentanone, nitropropane, and pyridine. Each of these probe compounds represents a distinct group of organic compounds. The responses obtained by SPME coupled with GC with different coatings are illustrated in Figures 9-5. The results illustrate that the new coatings respond more sensitively toward 1-butanol and pyridine relative to the non-polar benzene, while PDMS fiber responds more selectively to benzene and pyridine relative to other compounds. The selectivity of polar commercial coatings such as PDMS/DVB and CW/DVB are similar to that of the new coatings. These differences and similarities can also be explained by considering their structures and functionality.

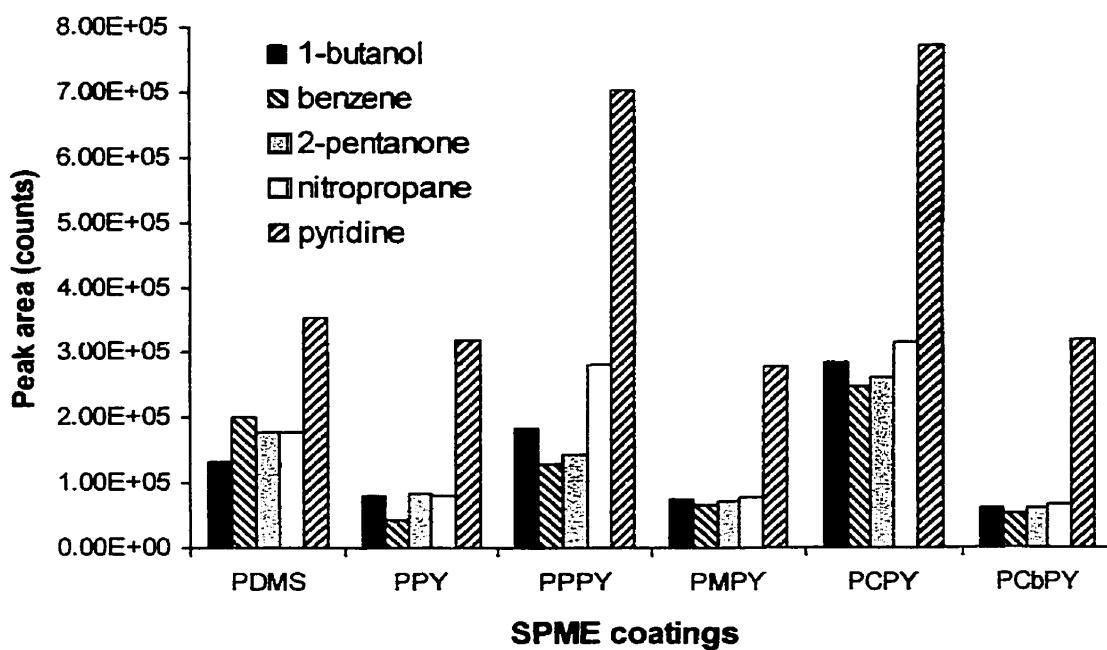


Figure 9-5. Responses of different SPME coatings towards McReynolds compounds.

An alcohol mixture. To further study the extraction ability of the new coatings to polar compounds, a mixture containing the same amount (mass) of each of the 10 alcohol compounds was tested. As shown in Figure 9-6, with the increase of carbon numbers or boiling points of the aliphatic alcohol, a nearly linear increase in signal intensity was observed when using PDMS fiber for SPME. This result is easy to understand because the PDMS fiber has the same chemical composite as the GC column used in this study. Both contain the nonpolar liquid poly(dimethylsiloxane) stationary phase, which retain or extract analytes by absorption based on hydrophobic interaction. For a homologous series of compounds, there exists a linear relationship between the number of carbons (or boiling points) and their retention power on this stationary phase (linear retention index). However, a remarkably different response pattern was observed for each of the new coatings when performing SPME for the same sample mixture under the same conditions. As shown in Figure 9-7 (A), PPy coating extracts methanol more efficiently than other alcohols, mainly due to its hydrogen bonding ability; with an increase of carbon chain number, its extraction ability decreases first and then increases due to the increase of hydrophobic interactions between PPy and analytes. An interesting discovery was found for PPPY coating in extraction of these compounds. As shown in figure 9-7 (B), with the increase of carbon chain number for the alcohol molecules, a nearly linear increase in signal intensity was observed for the *n*-alcohols. However, the PPPY coating extracted *n*-alcohols more selectively than their isomers. This selectivity to *n*-alcohols is possibly due to the steric hindrance of phenyl group in PPPY on hydrogen bonding and / or the small pore size on the coating surface, since the main interactions between the alcohol molecules and the polymer coatings include not only the hydrophobic interaction but also hydrogen bonding. This selectivity to *n*-alcohols or size exclusion effect to their isomers was also observed for the other *N*-substituted pyrrole polymer coatings and for the commercial CAR/PDMS fiber (Figure 9-8).

9.3.3. Analysis of polar compounds in nonpolar matrixes

Based on the results obtained from a recent work [4], none of the commercial coatings performed well for the extraction of polar analytes from nonpolar matrixes (e.g., alcohols in gasoline), due to either coating swelling in nonpolar solvents or low sensitivity toward target analytes (analyte signals were buried in the baseline due to the dominant responses of these fibers to nonpolar solvents). Up to now, no data has been published on

this type of potentially important application except for one study on a Nafion fiber [4]. Obviously, new coatings that will be suitable for this kind of analyses should be prepared and studied.

As illustrated above, PPy and PPPY coatings have greater extraction ability to alcohols than nonpolar compounds such as hexane. Therefore, the analysis of polar compounds in nonpolar matrixes was performed using alcohols in hexane as an example. The results are similar to those obtained by a Nafion fiber except that cleaner baselines and chromatograms are obtained using the new coatings (Figure 9-9 in this work in comparison with Figure 1 of ref.[4]), possibly due to the better stability of the new coatings after conditioning compared with the Nafion fiber. Gasoline samples were also analyzed using PPPY coatings and the results (data not shown) are similar to those obtained by a Nafion fiber [4].

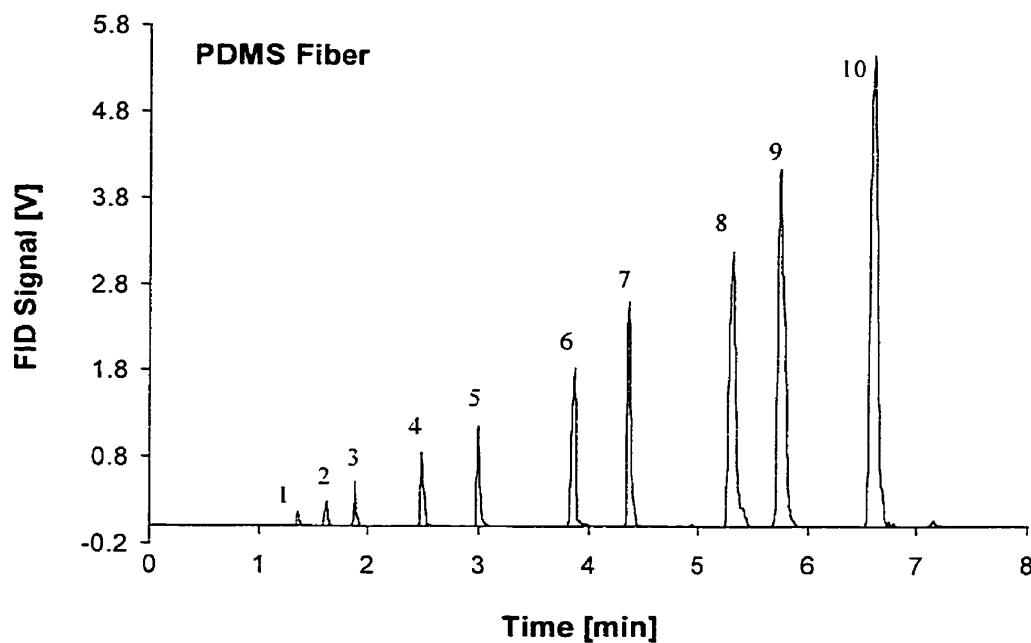


Figure 9-6. Chromatogram for a mixture of 10 alcohols by SPME-GC. 1: methanol, 2: ethanol, 3: 2-propanol, 4: 1-propanol, 5: 2-butanol, 6: 1-butanol, 7: 2-pentanol, 8: 1-pentanol, 9: 2-hexanol and 10: 1-hexanol. Conditions: see Experimental section.

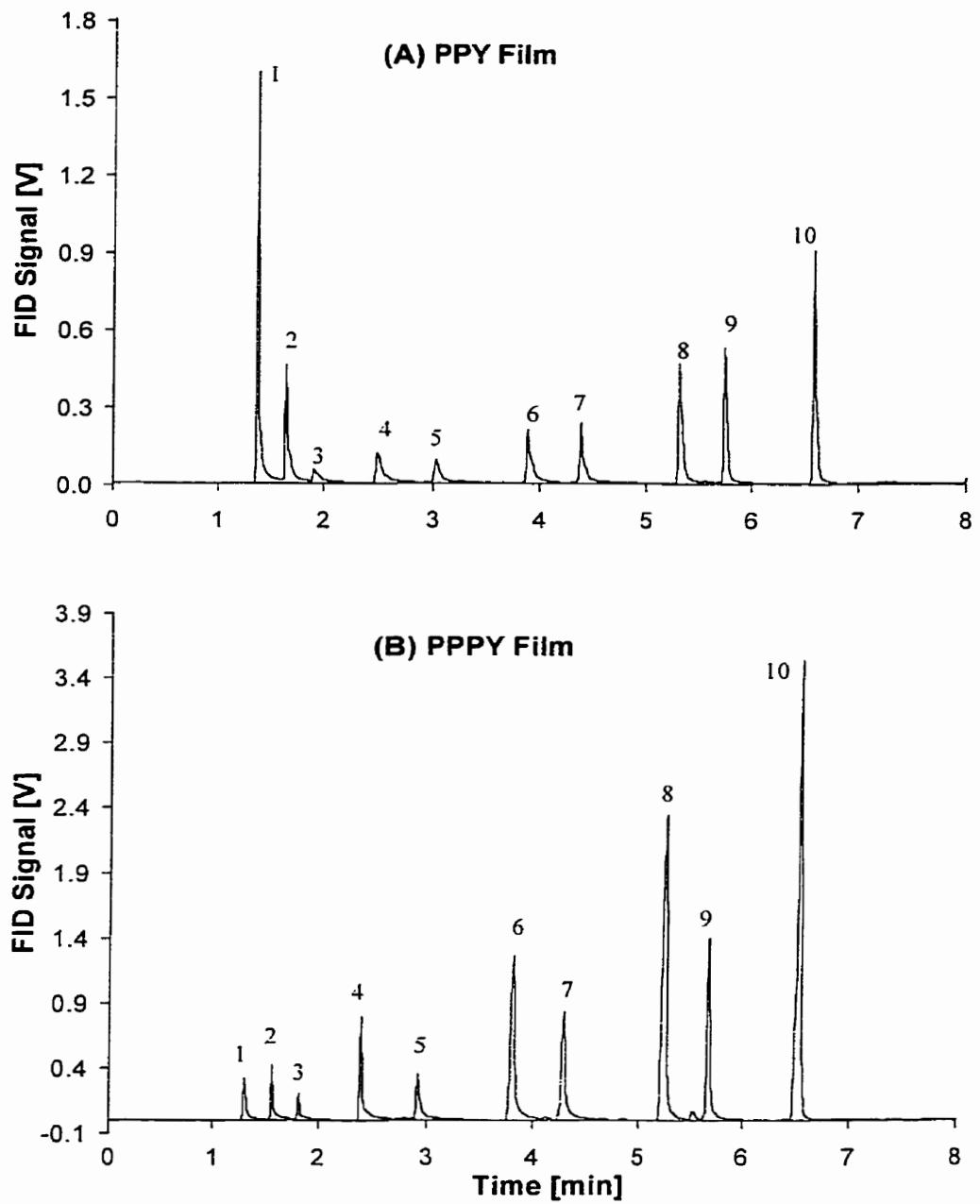


Figure 9-7. Chromatograms for a mixture of 10 alcohols by SPME-GC with (A) PPy and (B) PPPY films. 1: methanol, 2: ethanol, 3: 2-propanol, 4: 1-propanol, 5: 2-butanol, 6: 1-butanol, 7: 2-pentanol, 8: 1-pentanol, 9: 2-hexanol and 10: 1-hexanol. Conditions: see Experimental section.

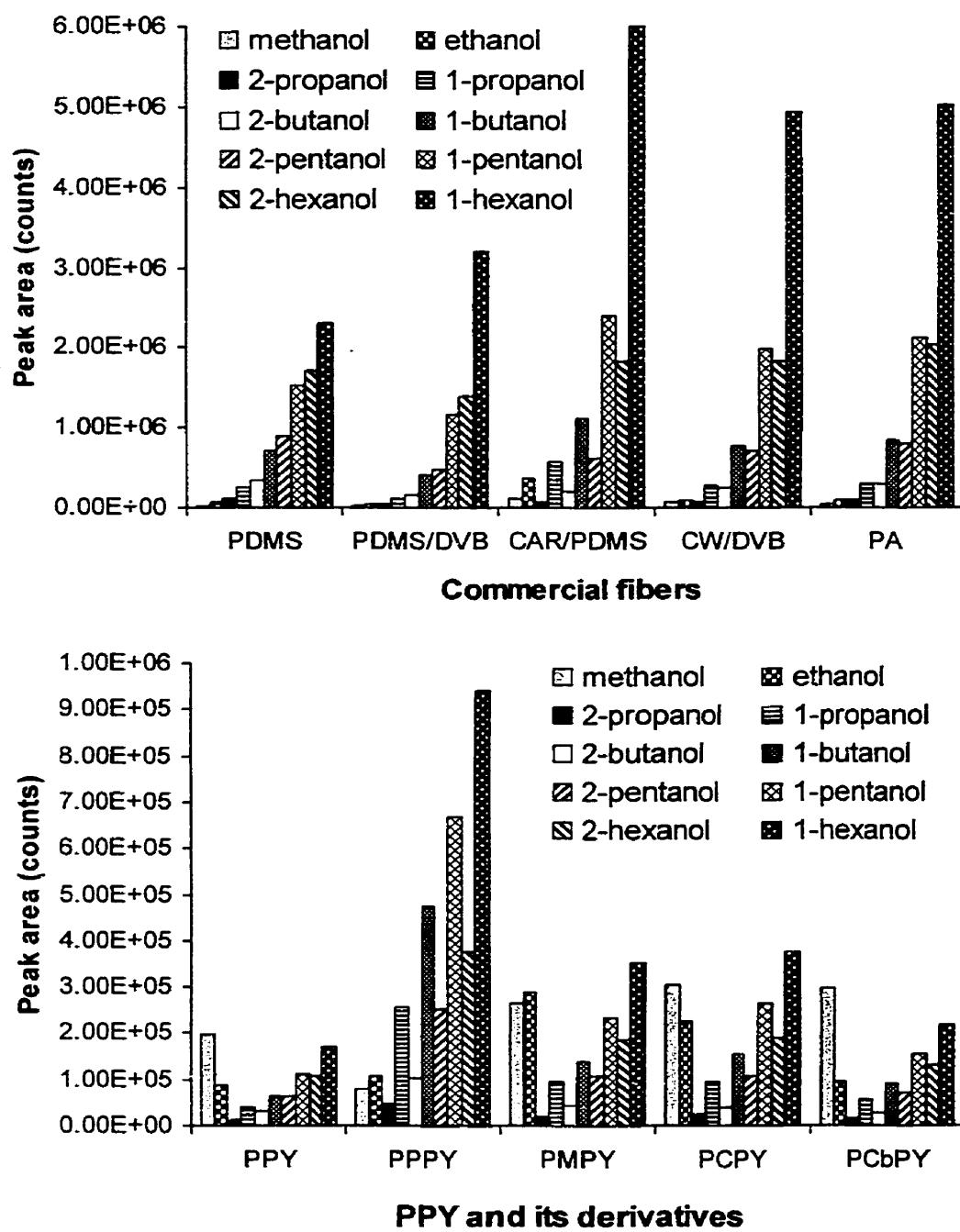


Figure 9-8. Responses of different commercial SPME fibers (upper) and the new coatings (below) towards 10 alcohols. Conditions: see the Experimental section.

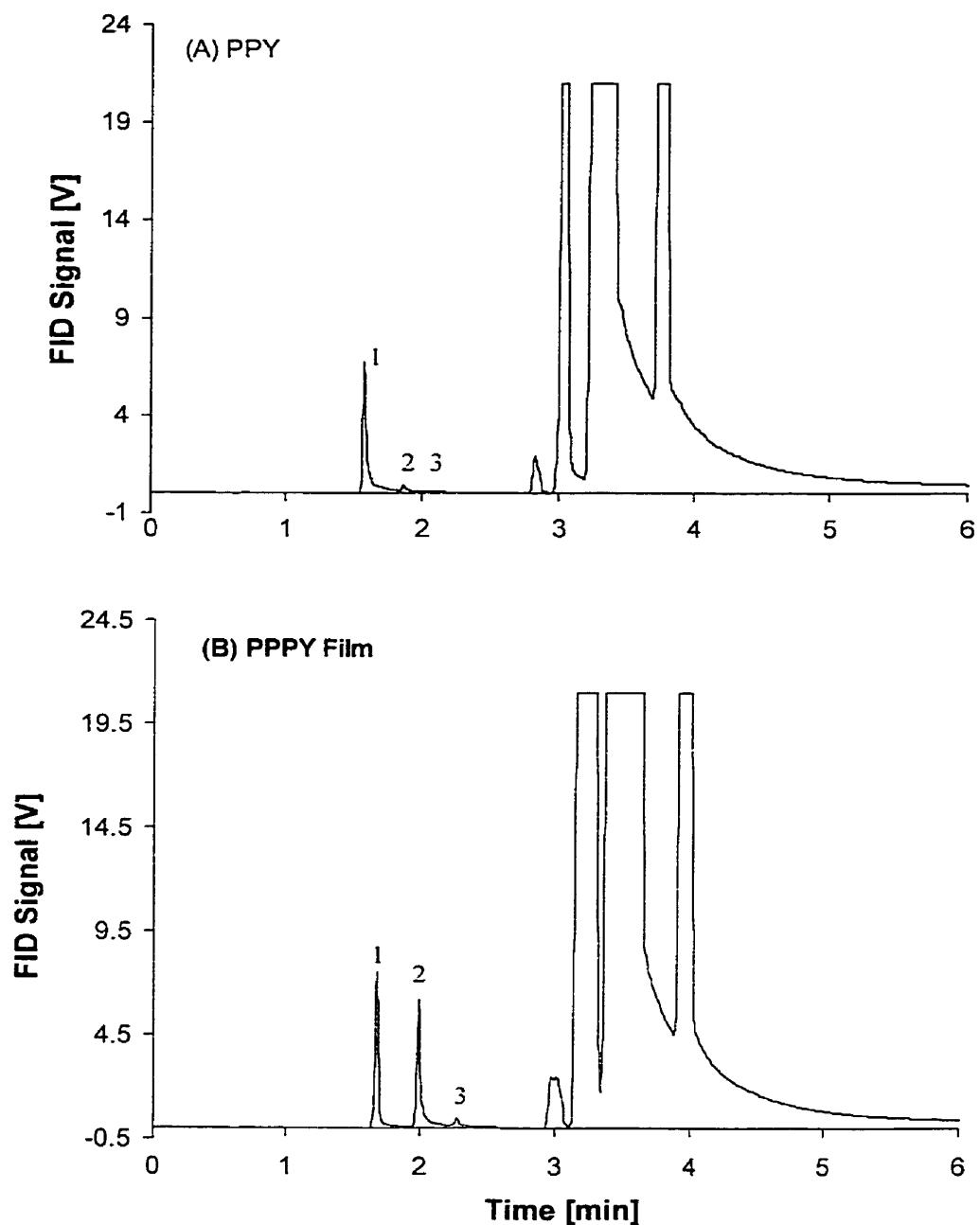


Figure 9-9. SPME-GC analysis of 40 ppm methanol (1), ethanol (2), and 2-propanol (3) in hexane with (A) PPy and (B) PPPY films.

9.4. Summary

In this work, PPY and its four derivatives have been employed for the first time as coatings for SPME. By comparing the responses (peak areas) obtained by SPME using the new coatings with those of commercial SPME fibers under the same extraction conditions, it can be seen that the new coatings have better selectivity toward polar compounds such as alcohols, amines and aromatic *N*-heterocyclics due to their inherent functionality. In addition, the different responses among the new coatings to various compounds suggest that the selectivity and extraction efficiency of the coatings for SPME can be improved or modified by introducing new functional groups into the polymer. Analyses of polar analytes in nonpolar matrices have been performed with the new coatings and the results are better than those obtained with a Nafion perfluorinated resin.

9.5. References

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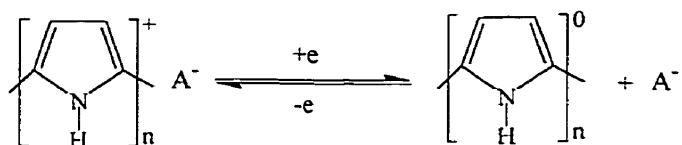
CHAPTER 10

ELECTROCHEMICALLY CONTROLLED SPME

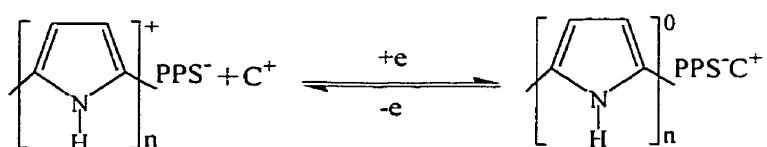
A PRELIMINARY STUDY

10.1. Introduction

Due to their electroactivity and reversible redox properties, conducting polymers, especially polypyrrole (PPY) and its derivatives, have attracted great interest in the development of electrochemically controlled delivery devices and separation systems for charged species (see Chapter 1). Electrochemical switching of the polymer is accompanied by the movement of counter ions in and out of the polymer film for charge balance. Based on the redox and anion exchange properties of PPY (Scheme 10-1), a variety of anions such as ferrocyanide and adenosine triphosphate (ATP) have been electrochemically entrapped into PPY films during oxidation and released during reduction [1-5]. In addition, PPY films with cation exchange and redox properties have been synthesized using bulky and ‘immobile’ poly(electrolytes) such as poly(styrenesulfonate) (PSS) as counter ions during electropolymerization. Since the counter ions cannot be released from these polymers upon reduction, cations (C^+) in solution are incorporated into the PPY films to maintain electroneutrality. When the films are reoxidized, these cations can be released (Scheme 10-2). These properties of PPY films have been utilized to extract cationic species such as metal ions [6-8] and drugs [9,10]. The aim of this work is to study the capability and potential application of electrochemically controlled SPME (EC-SPME) based on PPY films.



Scheme 10-1



Scheme 10-2

10.2. Experimental

10.2.1. Chemicals and reagents

Sodium methylarsonate (MMAs) was obtained from Chem Service (see chapter 6). Lithium perchlorate, sodium glutamate, dopamine (3,4-dihydroxyphenethylamine) hydrochloride and other chemicals were purchased from Aldrich (ON, Canada). Stock solutions of each analyte were prepared in pure water at 1 mg/mL. Analytical solutions were prepared by diluting the corresponding stock solutions with water. All chemicals were analytical-reagent grade.

10.2.2. Instrument and analytical conditions

All the electrochemical experiments were carried out on a potentiostat/galvanostat (Model 273, EG&G Princeton Applied Research). PPy films including PPy-Cl, PPy-ClO₄ and PPy-PSS were deposited on platinum wires (working electrodes, a 200 μm diameter × 1.5 cm long section of Pt wire was covered by PPy films) from the corresponding aqueous electrolytes by a potentiostatic technique described in chapter 2 (Table 2-1). After PPy film formation, cyclic voltammetric (CV) experiments were performed in 0.1M corresponding electrolytes. Electrochemically controlled SPME (EC-SPME) was performed according to the procedures described below.

EC-SPME of anions. To extract anions with PPy-Cl or PPy-ClO₄ film, the film was first reduced (by a cathodic potential!) to eject counter ions, washed serially with 0.01 M NaCl solution and pure water, and then transferred to a corresponding analyte solution (50 mL). The extraction of anionic species was realized by applying an anodic potential for a predetermined period of time (15 min). After extraction, the electrode was washed with the 0.01 M NaCl solution and water, and then transferred to a small volume vial (2-mL) containing 1 mL of 0.01 M NaCl solution where the analyte was released by applying a 2-min cathodic pulse.

EC-SPME of cations. To extract cations with PPy-PSS film, the film was first washed with 0.01 M NaCl solution and water, then transferred to an analyte solution for extraction upon reduction of the film (15-min). After extraction, the electrode was washed with the 0.01 M NaCl solution and water, and then transferred to a small volume vial (2-mL)

containing 1 mL of 0.01 M NaCl solution where the analyte was released by applying a 2-min anodic potential.

The above processes could be repeated in order to increase the amount of analyte extracted. The conditions for extraction and release of the studied compounds are summarized in Table 10-1.

Determinations of the released species were carried out by flow injection analysis (FIA) using an Agilent 1100 series HPLC system coupled with a ES-MS detector (see Chapter 4). A 10- μ L aliquot of the released analyte solution was injected. A Supelcosil LC-18 guard column (3 cm \times 4.6 mm, 5 μ m particle size) from Supelco (Bellefonte, PA, USA) was used in the system. The mobile phase used was methanol/water (1:1). The main mass detection conditions are also listed in Table 10-1. Other detection conditions for all the analytes are the same: detection mode, positive; nebulizer gas, N₂ (40 psi); drying gas, N₂ (10 L/min, 350°C); mass scan range, 100-550 amu; scan time, 0.67 sec/cycle.

Table 10-1. Conditions for the analyte extraction, release and determination

Compounds	Polymer film	$E_{\text{extraction}}^{\text{a}}$	$E_{\text{release}}^{\text{a}}$	MS detection conditions (SIM) ^b
MMA	PPY-ClO ₄	0.2 V	-1.0 V	V _f = 60 V; V _{cap} = 1200 V; m/z = 141
Glutamate	PPY-ClO ₄	0.2 V	-1.0 V	V _f = 40 V; V _{cap} = 4500 V; m/z = 148
Perchlorate	PPY-Cl	0.5 V	-1.0 V	V _f = 40 V; V _{cap} = 1500 V; m/z = 99
Dopamine	PPY-PSS	-0.6 V	0.4 V	V _f = 40 V; V _{cap} = 2500 V; m/z = 172

^a $E_{\text{extraction}}$ and E_{release} are the potentials applied during extraction and release of analyte by EC-SPME. ^b SIM, selected ion monitoring mode; V_f, fragmentor voltage; V_{cap}, capillary voltage.

10.3. Results and discussion

10.3.1. Preparation of PPY coated Pt electrodes

Scanning electron micrographs (SEM) of the prepared polymer films are shown and discussed in the next chapter. The cyclic voltammograms of these films obtained in this study are shown in Figures 10-1 to 10-3.

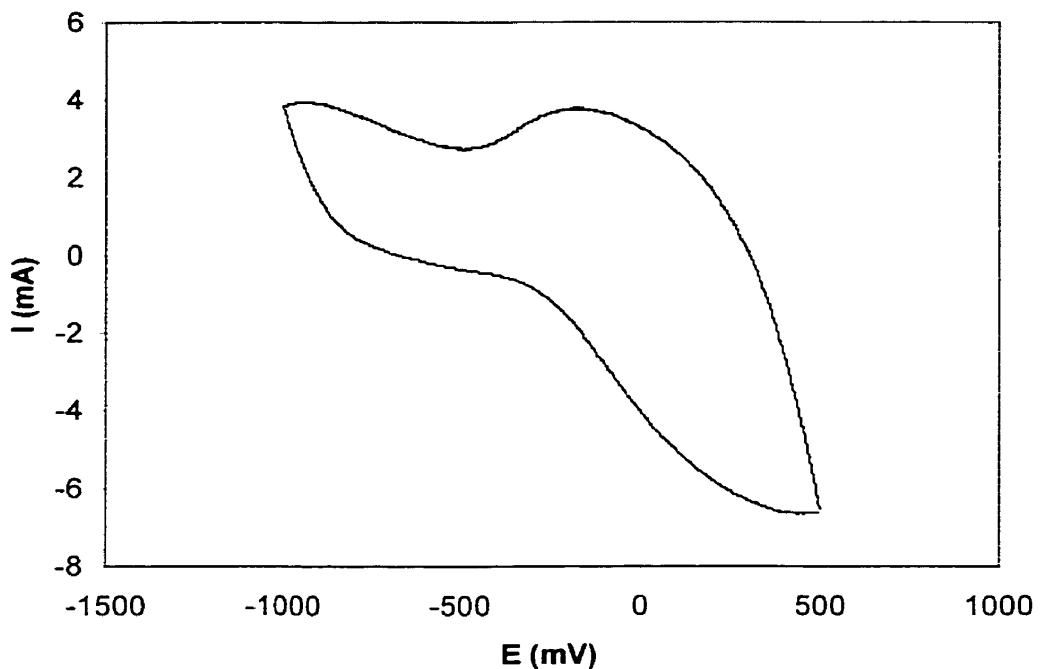


Figure 10-1. Cyclic voltammogram of PPy-Cl film coated Pt electrode in a 0.1 M NaCl aqueous solution at a scan rate of 50 mV/s.

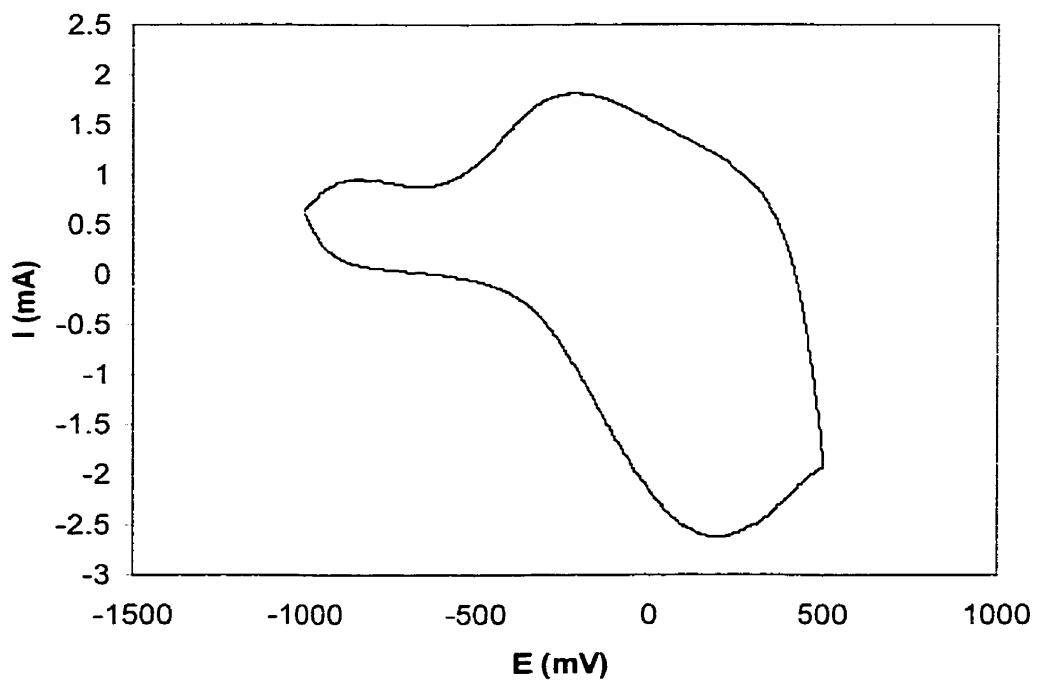


Figure 10-2. Cyclic voltammogram of PPy-ClO₄ film coated Pt electrode in a 0.1 M LiClO₄ aqueous solution at a scan rate of 50 mV/s.

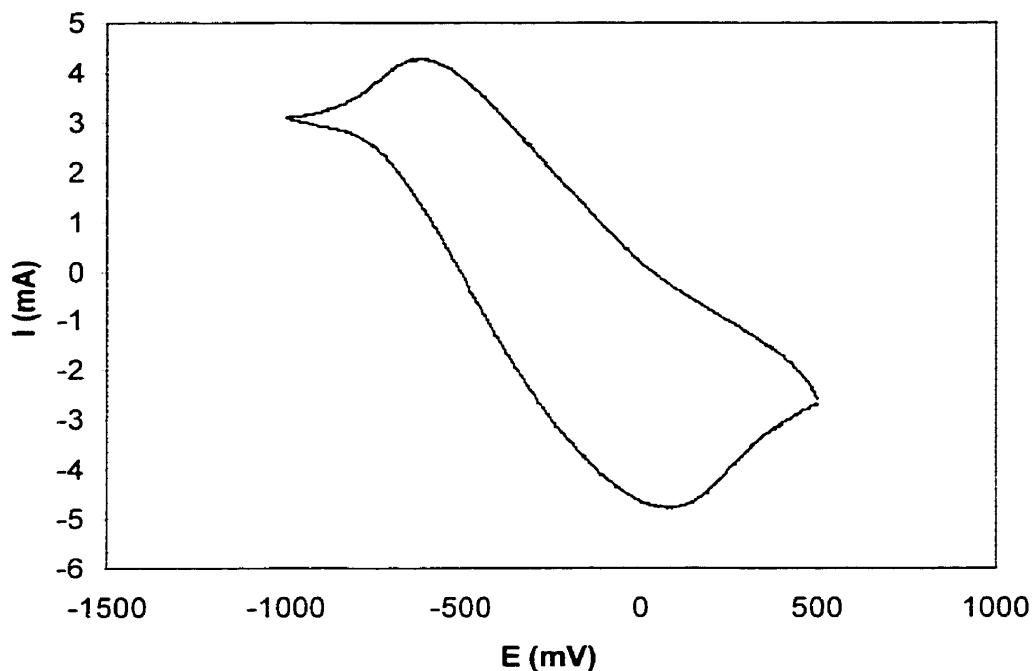


Figure 10-3. Cyclic voltammogram of PPy-PSS film coated Pt electrode in a 0.1 M PSS aqueous solution at a scan rate of 50 mV/s.

10.3.2. EC-SPME and FIA

It was difficult to detect the analyte signals obtained by only one extraction-desorption process during EC-SPME due to the small amount of PPy coatings used for SPME and the relatively large volume of desorption solvent (1 mL), especially after washing the films with NaCl and water (see experimental section). Therefore, the EC-SPME process described above were repeated 5 to 10 times in order to obtain analyte signals which were strong enough to be quantified.

The results for MMAs obtained by EC-SPME (A) and by SPME without applying electric potential (B) are shown in Figure 10-4. MMAs is a negatively charged ion in neutral aqueous solution. After a 10 extraction-desorption process, a large signal corresponding to

MMAs (m/z 141) was observed by EC-SPME as shown in Figure 10-4(A). However, no MMAs signal was detected when potentials were not applied during the SPME process as illustrated in Figure 10-4 (B). Similar results (figure not shown) were also obtained for glutamate anions, a drug of interest for neuroscience. These results indicate the capability and potential advantages of EC-SPME in improving the selectivity and efficiency of SPME techniques for charged species.

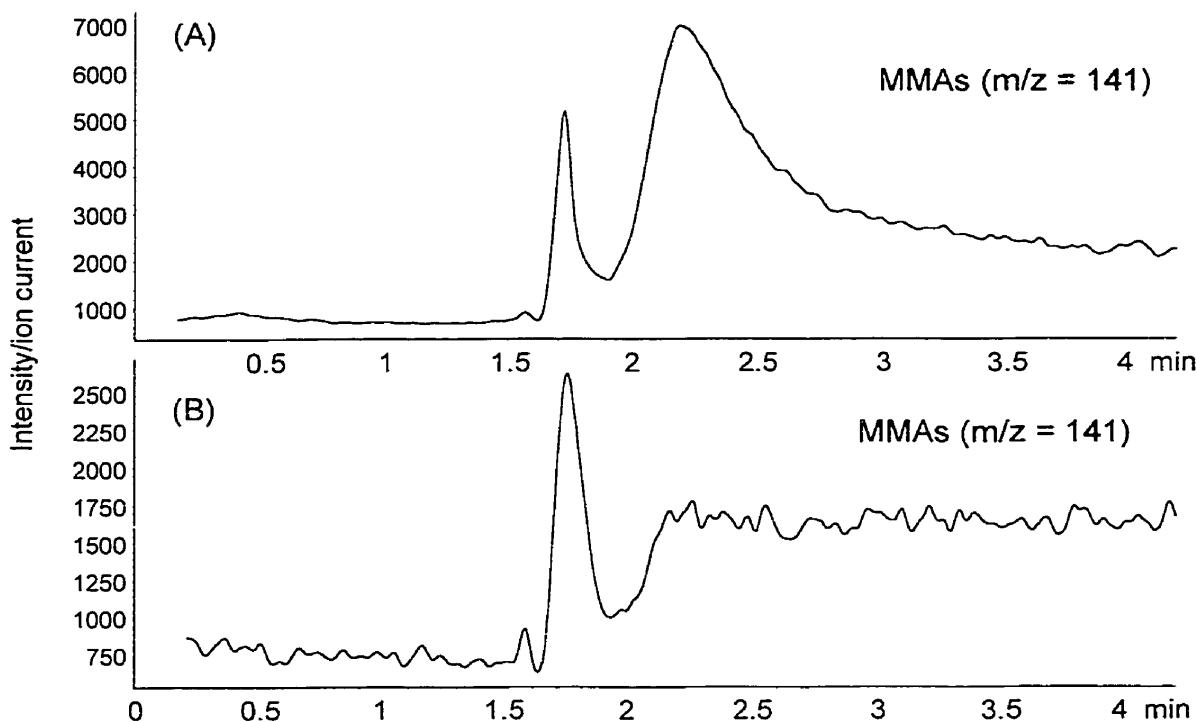


Figure 10-4. Comparison of the results for MMAs obtained by EC-SPME coupled with LC-MS (A) and by SPME coupled with LC-MS when potentials were not applied during the SPME process (B). A 10 extraction-desorption process was performed for both (A) and (B). Concentration of MMAs: 100 ng/mL. A 10- μ L aliquot of the released analyte solution was injected.

Analysis of perchlorate in water samples has received great attention due to the increasing environmental concern of its leaching to various water resources [11-14]. Recently, several papers were published on determination of perchlorate by ES-MS methods [11-14]. Ion-pair or complexing reagents were used in these studies to increase the efficiency in sample preparation and sensitivity in detection [11-13]. In this work, a PPY-Cl film coated platinum electrode was used to extract ClO_4^- from a spiked water sample by EC-SPME (see Table 10-1 for analytical conditions). As illustrated in Figure 10-5, after 10 extraction-desorption cycles, analyte signals corresponding to 100 ng/mL ClO_4^- (m/z 99) were detected by flow injection analysis (FIA) with ES-MS detection under selected ion monitoring mode (SIM).

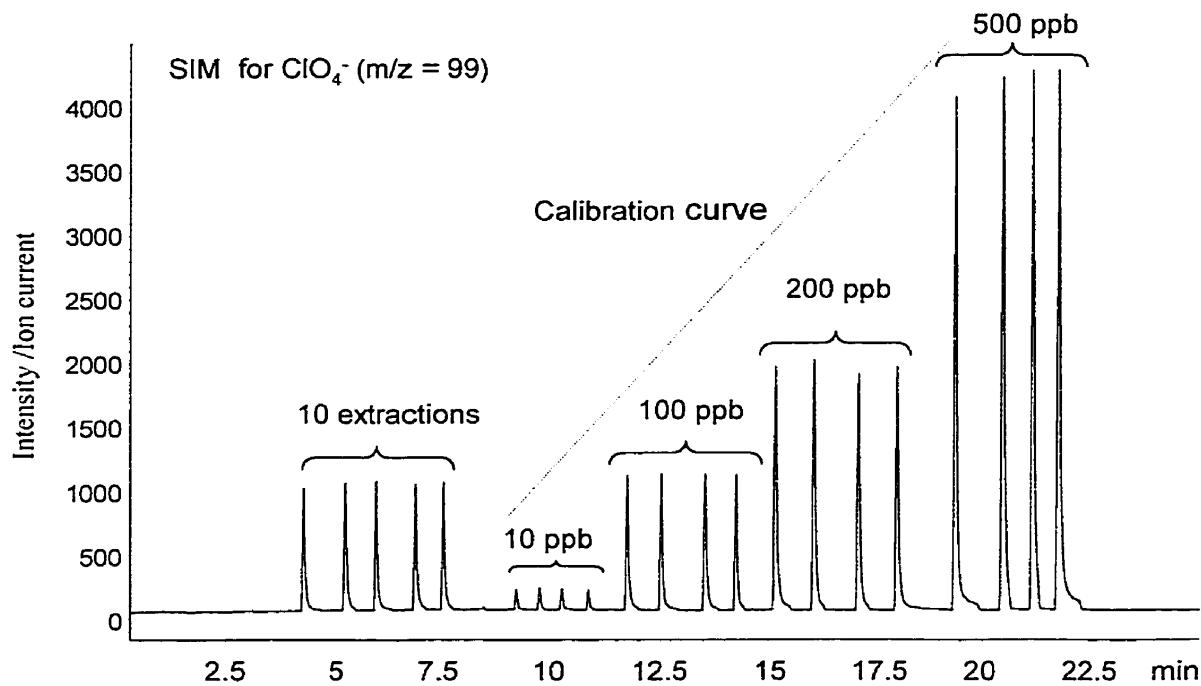


Figure 10-5. Analysis of ClO_4^- by EC-SPME with ES-MS detection. The concentration of ClO_4^- in the sample was 50 ng/mL. A 10- μL of the desorbed analyte solution was injected. The calibration curve shown in the right side of the figure was determined by liquid injection (10 μL) of the standard analyte solutions (concentrations were shown on the Figure).

Cationic species such as dopamine can also be extracted by EC-SPME using a PPy-PSS coated platinum electrode. As shown in Figure 10-6, the amount of dopamine extracted (corresponding to the peak area counts) was increased almost two times after a 10 extraction-desorption process compared with that of a 5 extraction-desorption process, indicating that the extraction efficiency could be enhanced significantly by increasing the EC-SPME (extraction-desorption) cycles. Since many drugs are actually protonated amines like dopamine, success in EC-SPME of dopamine would indicate that the method could be applicable to a large class of medicinal compounds. However, it should be noted that anodic dopamine release demands careful potential control to avoid oxidation or decomposition of dopamine. Specifically, the anodic potential of the PPy film must be controlled to $E \leq 0.60$ V [9] to avoid oxidation.

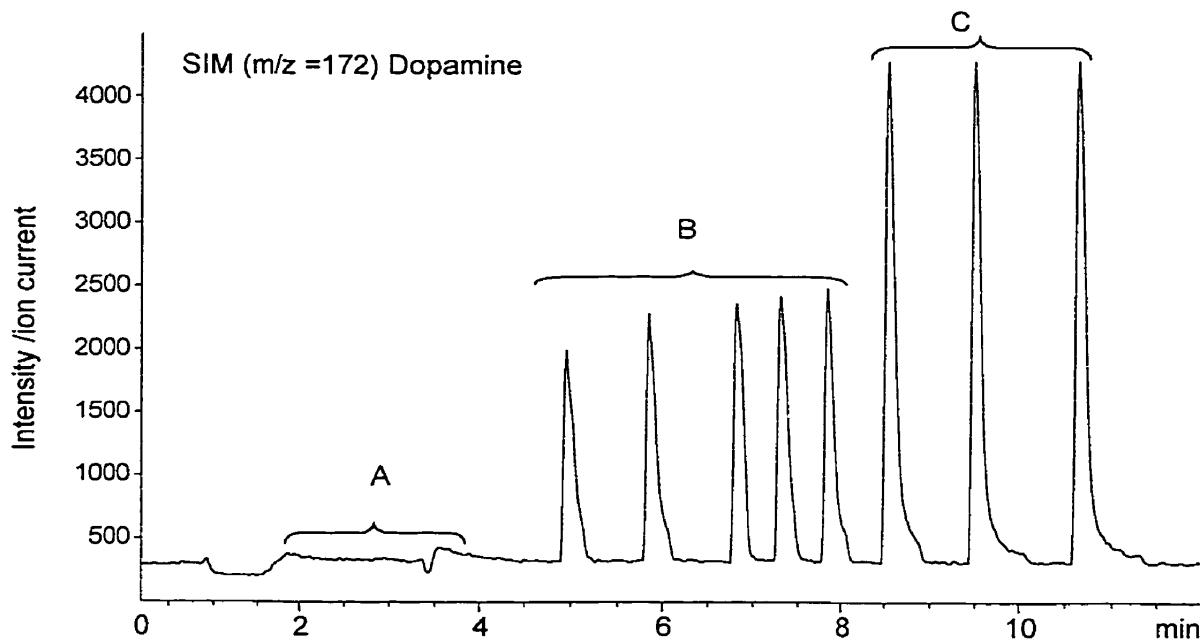


Figure 10-6. Determination of dopamine in an aqueous solution by EC-SPME with ES-MS detection. (A) 0.01 M NaCl solution blank, (B) after 5 extraction-desorption cycles and (C) after 10 extraction-desorption cycles. Concentration of dopamine: 50 ng/mL; injection volume: 10 μ L.

10.4. Summary

The preliminary results obtained here demonstrate the capability and potential applications of EC-SPME based on PPY films. The possible advantages of this technique are the enhanced extraction selectivity and efficiency achieved by applying appropriate electrochemical potentials during extraction and desorption processes. It might be possible to extract a group of compounds simultaneously or a single analyte from a mixture based on the electrochemical properties of the charged analytes. In addition, the sensitivity of the method could be improved greatly if an on-line electrochemically controlled desorption chamber with a small volume (microliter or smaller) could be designed and utilized in the FIA system.

However, to achieve EC-SPME, a high quality electrochemical instrument such as a potentiostat is required to precisely control the potentials applied. This means that extra expenses are needed compared with the conventional SPME method. In addition, the analytes of interest and /or the PPY films must be stable in the applied potential range to avoid decomposition or changes in their properties. For real samples that have complex matrices, the situation becomes more complicated. Therefore, further studies are required in order to extend this technique to applications for real samples.

10.5. References

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CHAPTER 11

SPME BASED ON PPy FILMS WITH DIFFERENT COUNTER IONS

A PRELIMINARY STUDY

11.1. Introduction

For conducting polymers such as polypyrrole (PPy), the counter ions (A^-) not only play an important role in the synthesis of PPy but also provide a convenient way to introduce functional groups into the polymer films (see Chapter 1 for a brief review). Since PPy can be polymerized from both organic and aqueous media with a neutral pH at a low anodic potential, a wide range of counter ions (A^-) can be incorporated into PPy films. For example, PPy films capable of metal recognition have been prepared by incorporating metal-complexing reagents as counter ions [1-6]. These materials have been utilized to develop electrochemical sensors with improved selectivity for the target metals. Bioactive groups such as enzymes and antibodies have also been introduced into PPy films for the development of biosensors [7-10]. In addition, the size of the counter ion has a significant effect on the properties of PPy. For example, PPy films with small counter anions, such as Cl^- and ClO_4^- , have anion exchange properties. The anions can be released upon reduction of the films as described in scheme 10-1 (Chapter 10). However, the rate of such a release is higher with small anions. The anion mobility decreases with the increase of counter ion size. In extreme cases, incorporation of larger and more hydrophobic anions such as the multicharged polyelectrolytes (PEs) will totally eliminate the anion exchange property. In fact, PPy films incorporating large anions such as poly(styrenesulfonate) (PSS) showed cation exchange and redox properties as demonstrated in Chapter 10. The physical properties of PPy films such as morphology, porosity, mechanical strength and thermal stability are also influenced by the counter ions used. It has been shown that improved mechanical and thermal properties have been achieved for PPy films incorporating sulfonated aromatic counter ions [11,12].

In this chapter, PPy films with the following counter anions have been prepared: Cl^- , ClO_4^- , *p*-toluenesulphonate (TS), dodecylsulphate (DS) and poly(styrenesulfonate) (PSS).

The extraction properties of these PPy films in SPME of volatile and non-volatile compounds have been evaluated by coupling PPy coated Pt wire SPME to GC and HPLC.

11.2. Experimental

11.2.1. Chemicals and reagents

The reagents and conditions for the preparation of PPy films with different counter anions (Cl^- , ClO_4^- , DS, TS and PSS) have been described in Chapter 2 (see also Table 2-1). The volatile organic compounds examined in this work are the same as those studied in Chapter 9. Sample preparations for stimulants (amphetamine (AP) and methamphetamine (MA)) and dopamine have been described in Chapters 5 and 10, respectively.

11.2.2. Instrument and analytical conditions

SPME-HPLC analysis of organic cations. The extraction properties of PPy- ClO_4 and PPy-PSS toward amine-containing drugs were evaluated and compared by coupling SPME to a FIA or HPLC-MS system. SPME was performed according to the procedure illustrated in Figure 11-1. To increase the extraction efficiency, a 2-cm long section of a Pt wire (250-mm diameter) was bent into a “U shape” and was coated with PPy film to increase the coating surface. The coated Pt wire was washed with pure water and then transferred to an analyte solution (50-mL) for extraction. After a 30-min extraction, the analytes were desorbed (or released) into a small sample vial (2-mL) containing a 1 mL mixture of 0.01 M NaCl and methanol (1:1, v/v). The desorbed analytes were determined by flow injection analysis (FIA) or HPLC-MS using an Agilent 1100 series HPLC system coupled with a ES-MS detector (see Chapter 4 for detail). A 10- μL aliquot of the released analyte solution was injected. For FIA, the mobile phase used was methanol/water (1:1) solution. For the separation of amphetamine (AP) and methamphetamine (MA), a Supelcosil LC-CN column (3.3 cm \times 4.6 mm i.d., 3 μm particle size,) from Supelco (Bellefonte, PA, USA) was used. Mobile phase was acetonitrile/50 mM ammonium acetate (15:85) with a flow rate of 0.4 mL/min. The optimized ES-MS detection conditions for AP and MA were as follows: nebulizer gas, N_2 (40 psi); drying gas, N_2 (12 L/min, 350°C); fragmentor voltage, 50 V; capillary voltage,

1000 V; ionization mode, positive; selected ion monitoring (SIM), m/z 136 (AP), 150 (MA). MS detection conditions for dopamine were the same as those used in Chapter 10.

SPME-GC analysis of volatile organic compounds (see chapter 9)

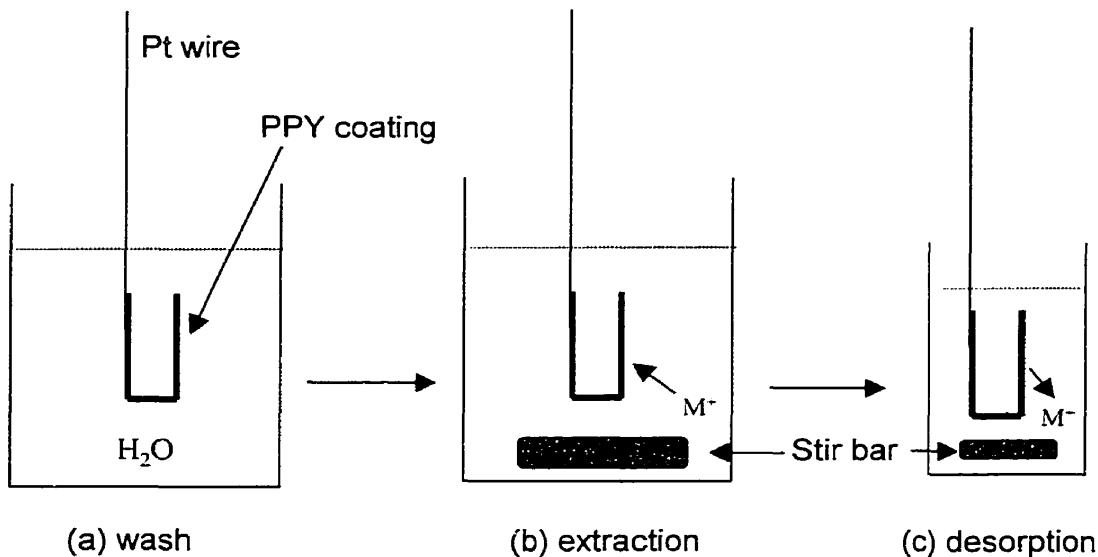


Figure 11-1. SPME of organic cations. (a) Wash with pure water; (b) extraction of analyte (M^+) by PPy coating; and (c) analyte desorption with a solution of NaCl (0.01 M) and methanol (1:1).

11.3. Results and discussion

11.3.1. SEM study of the PPy coated Pt surfaces

The porous surface structure of PPy- ClO_4 , which is similar to that of PPy-Cl, can be observed in Figure 11-2(A) and Figure 11-3(A). PPy-DS also has a porous surface as shown in Figure 11-2(B) and Figure 11-3(B), but the particles are smaller and more compact than those of PPy-Cl and PPy- ClO_4 . The SEM picture of PPy-TS is quite interesting, the particles have ‘elephant-nose’ shapes as shown in Figure 11-3(C). However, as illustrated in Figure 11-2 (D) and Figure 11-3 (D), the surface of PPy-PSS is much smoother than the other PPy films studied in this work and its porous structure can be observed only under much larger magnifications (Figure 11-4).

coated Pt surfaces

Figure 11-2. Scanning electron micrographs of (A) PPy-CIO₄, (B) PPy-DS, (C) PPy-TS and (D) PPy-PSS

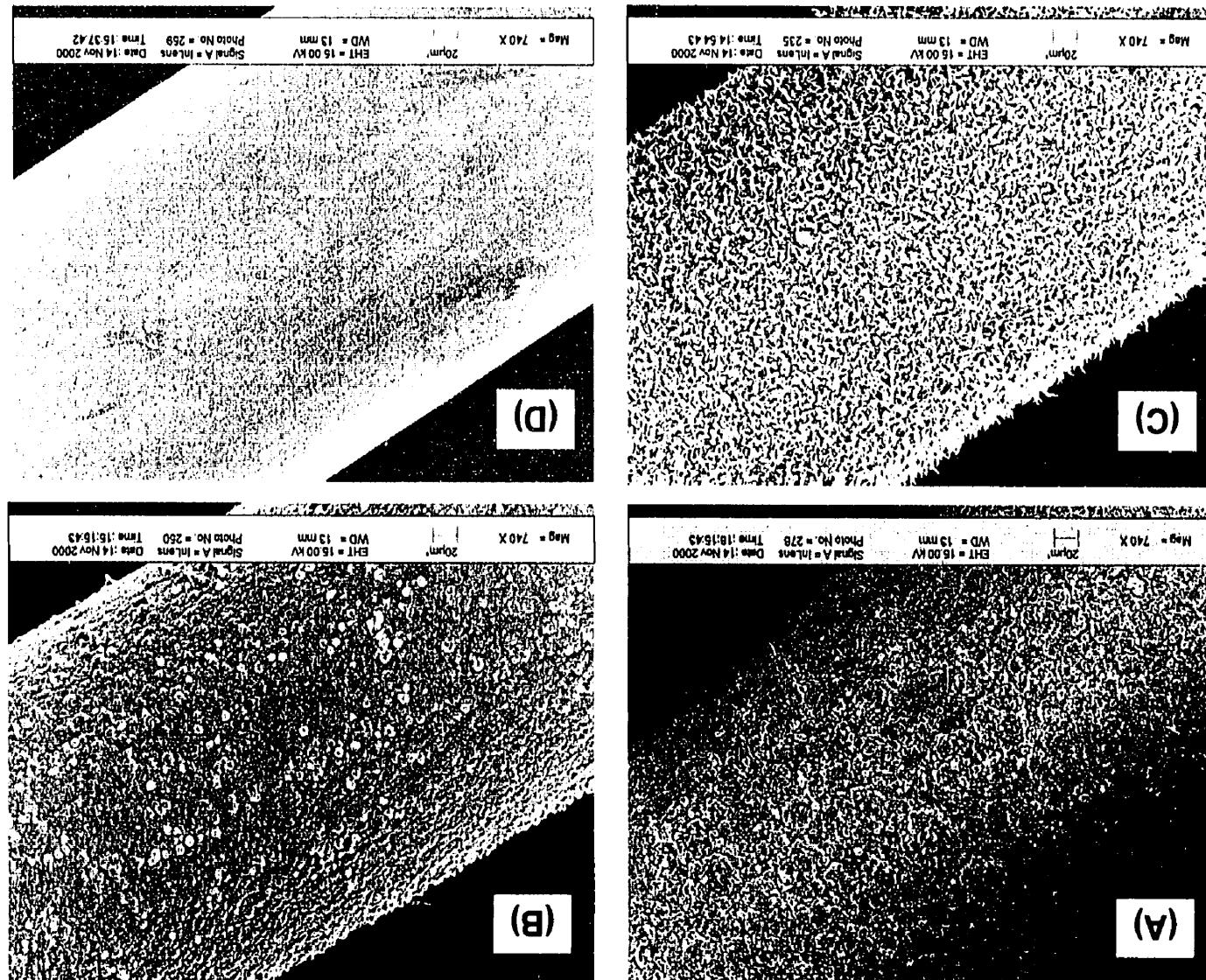
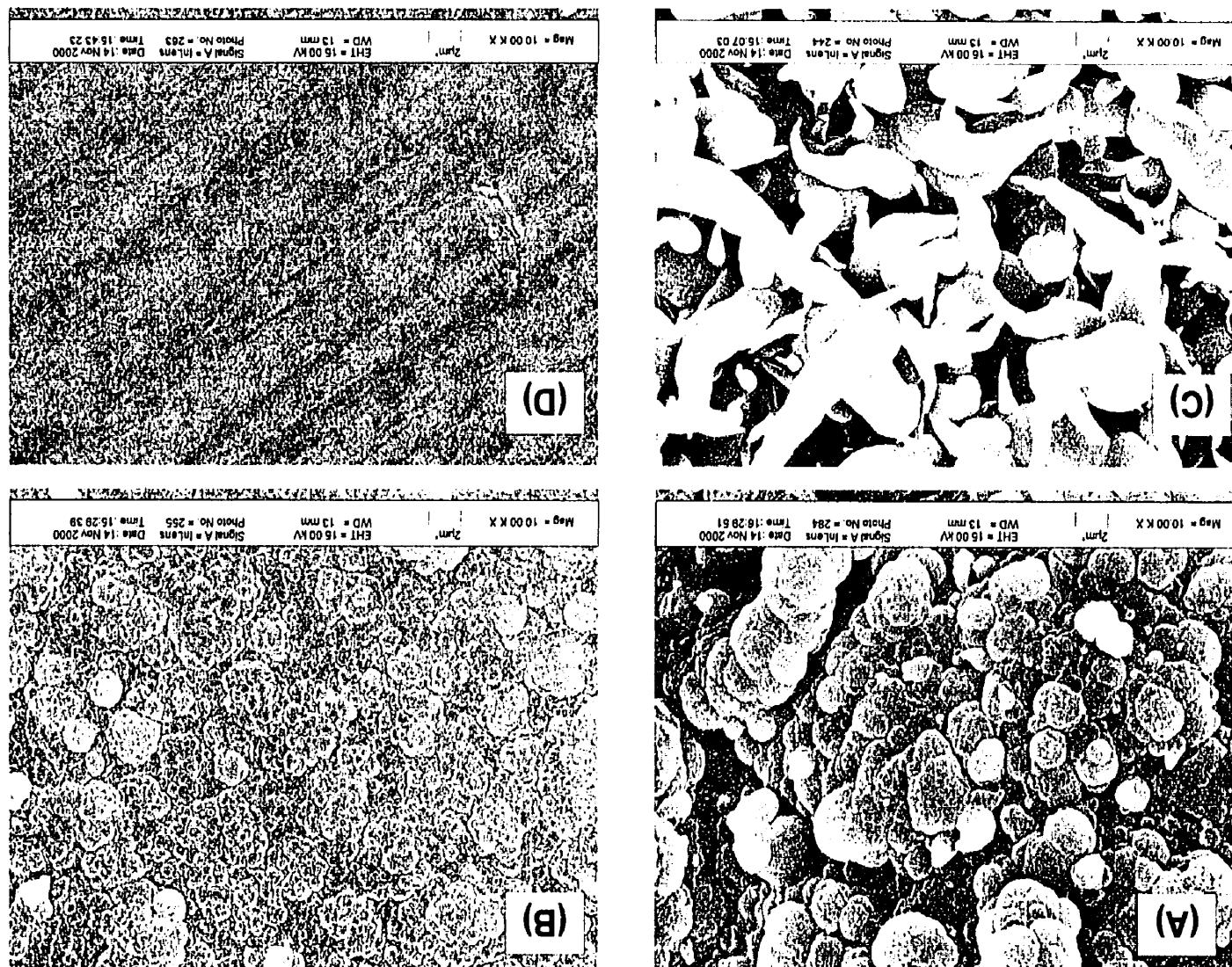


Figure II-3. Enlarged SEM images of the PPy coated surfaces, (A), (B), (C) and (D) as shown in Figure II-2.



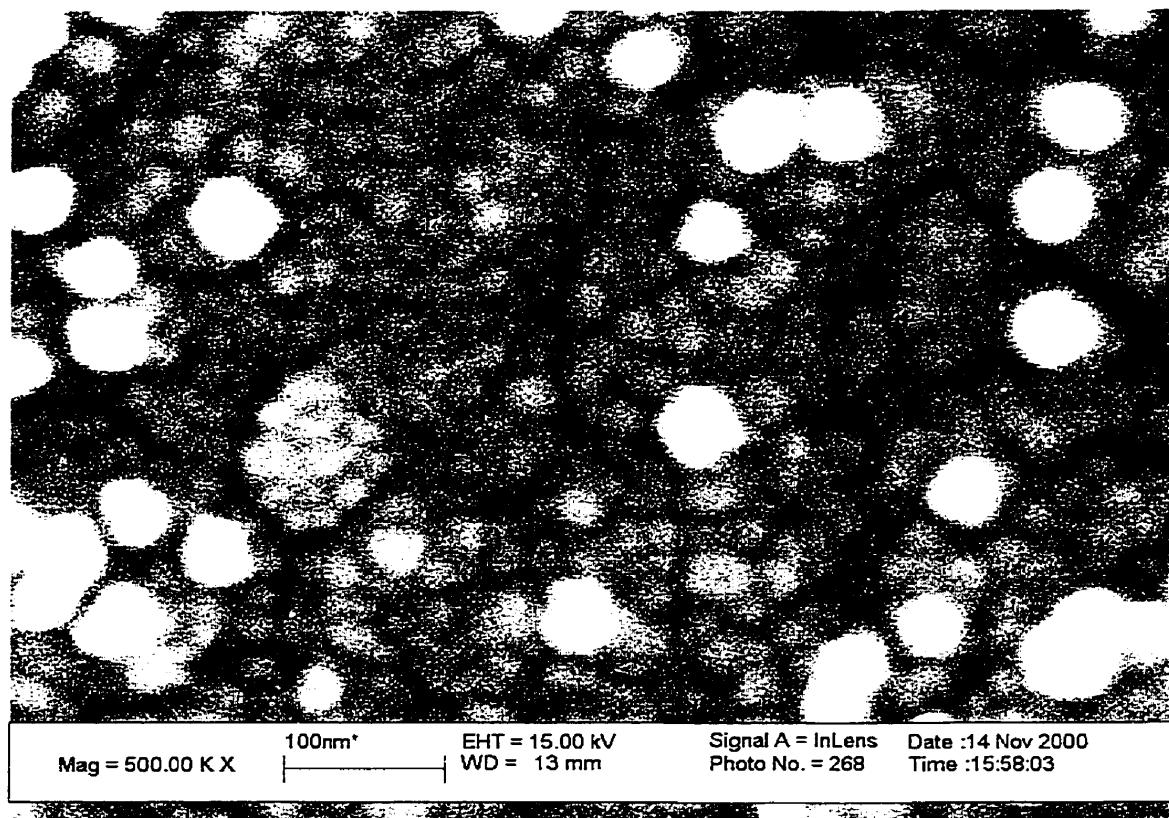


Figure 11-4. Scanning electron micrograph of PPY-PSS coated surface, the same surface as shown in Figure 11-3 (D), but obtained with much higher magnification.

11.3.2. SPME-HPLC analysis of organic cations

PPY films with small counter anions such as Cl^- or ClO_4^- have anion exchange properties, which can be used to extract anionic species from aqueous solution as discussed in Chapter 3 and Chapter 6. However, when larger counter anions such as poly(styrenesulfonate) (PSS) were incorporated into the PPY films, cation exchange properties can be observed possibly due to the bulky anions on the surface, which cannot be exchanged by other anions. As shown in Figure 11-5, since dopamine is a cation in neutral aqueous solution, it was extracted much more effectively by PPY-PSS film than by PPY- ClO_4 film. Similar results were also obtained for stimulant drugs such as amphetamine (AP) and methamphetamine (MA) as seen in Figure 11-6. The two

compounds can be easily separated and analyzed by coupling this SPME technique to HPLC-MS as demonstrated in Figure 11-7. Because many drugs are protonated amines like dopamine and amphetamine in aqueous or biological media, the high extraction efficiency of PPy-PSS film indicates its potential application in SPME for a large class of medicinal compounds.

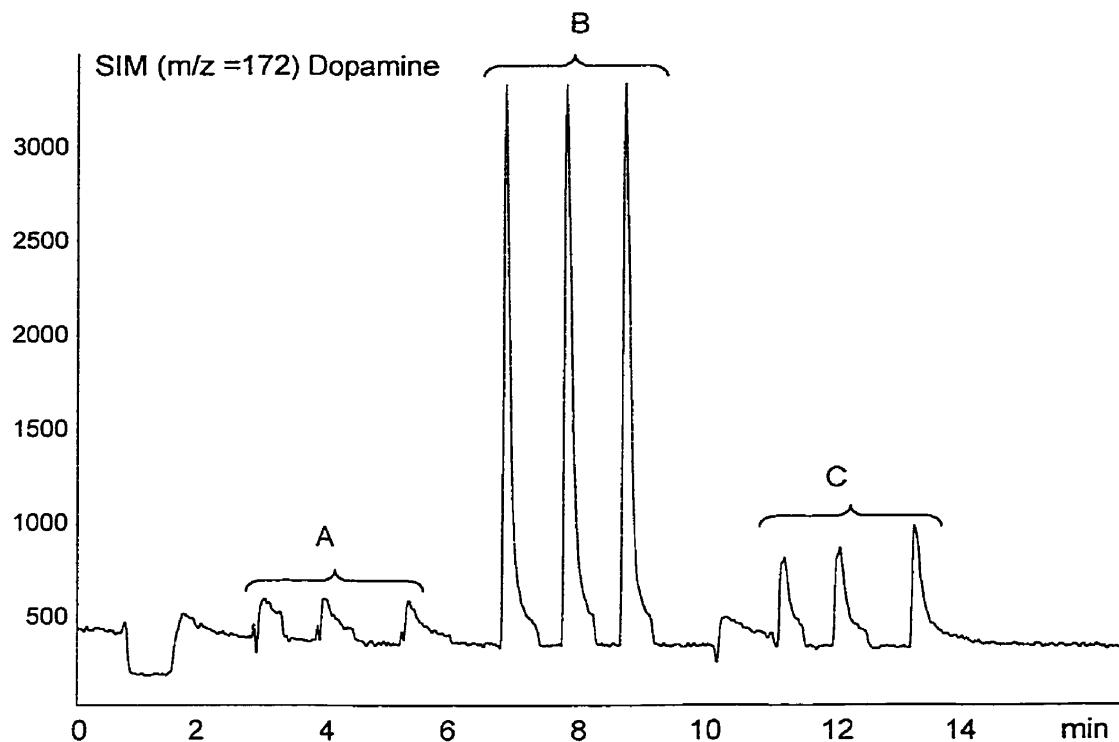


Figure 11-5. Comparison of the signals obtained with FIA-MS detection by injections of $10 \mu\text{L}$ of (A) desorption solution (blank), (B) the desorption solution in which the dopamine was desorbed from PPy-PSS coating and (C) the desorption solution in which the dopamine was desorbed from PPy- ClO_4 coating. The concentration of dopamine is 50 ng/mL . Desorption solution is a 1:1 mixture of 0.01 M NaCl and methanol. 2 extraction-desorption cycles were used.

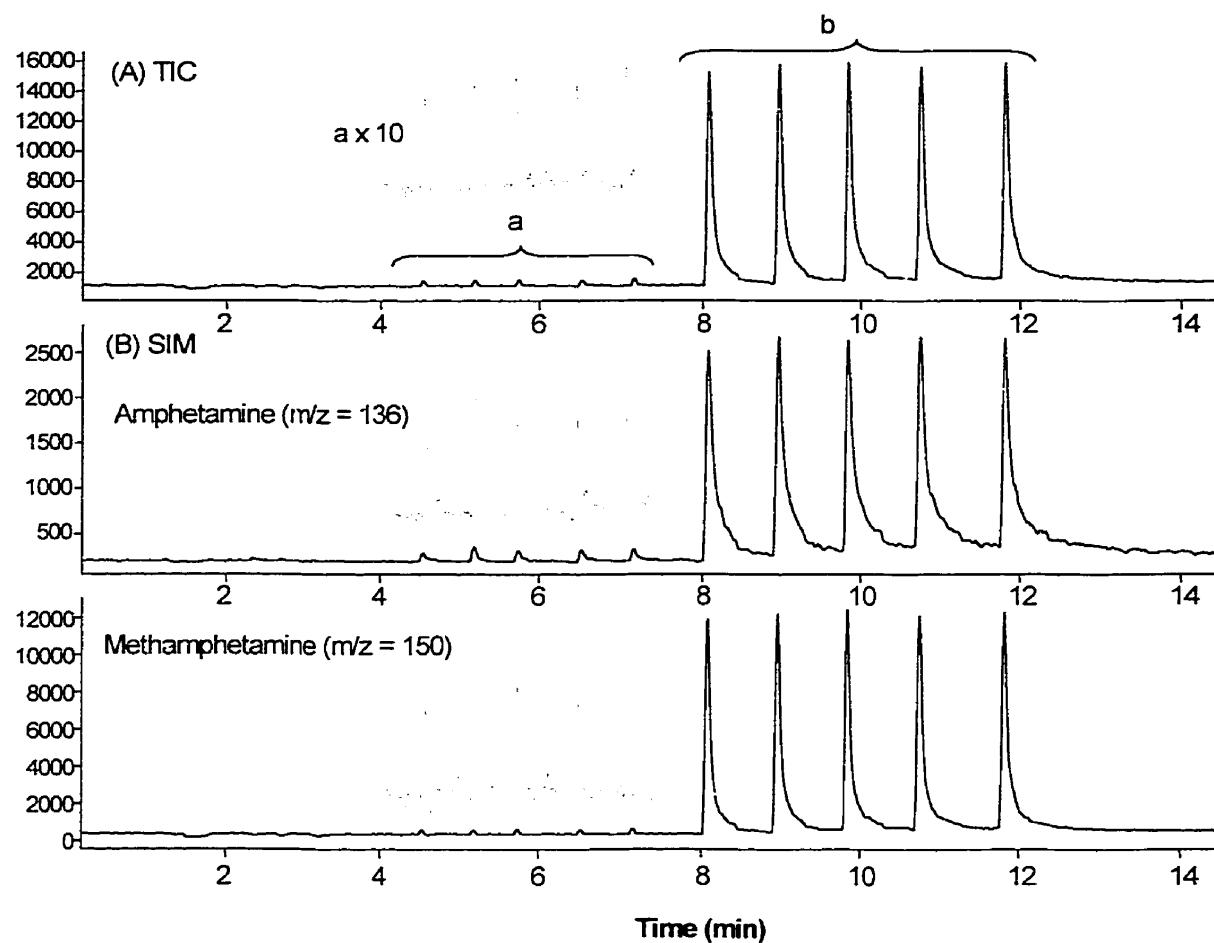


Figure 11-6. Comparison of the signals obtained with FIA-MS detection by injections of 10 μ L of (a) the desorption solution in which the dopamine was desorbed from PPy-ClO₄ coating and (b) the desorption solution in which the dopamine was desorbed from PPy-PSS coating. AP and MA concentrations: each 50 ng/mL. Desorption solution is a 1:1 mixture of 0.01 M NaCl and methanol. 2 extraction-desorption cycles were used.

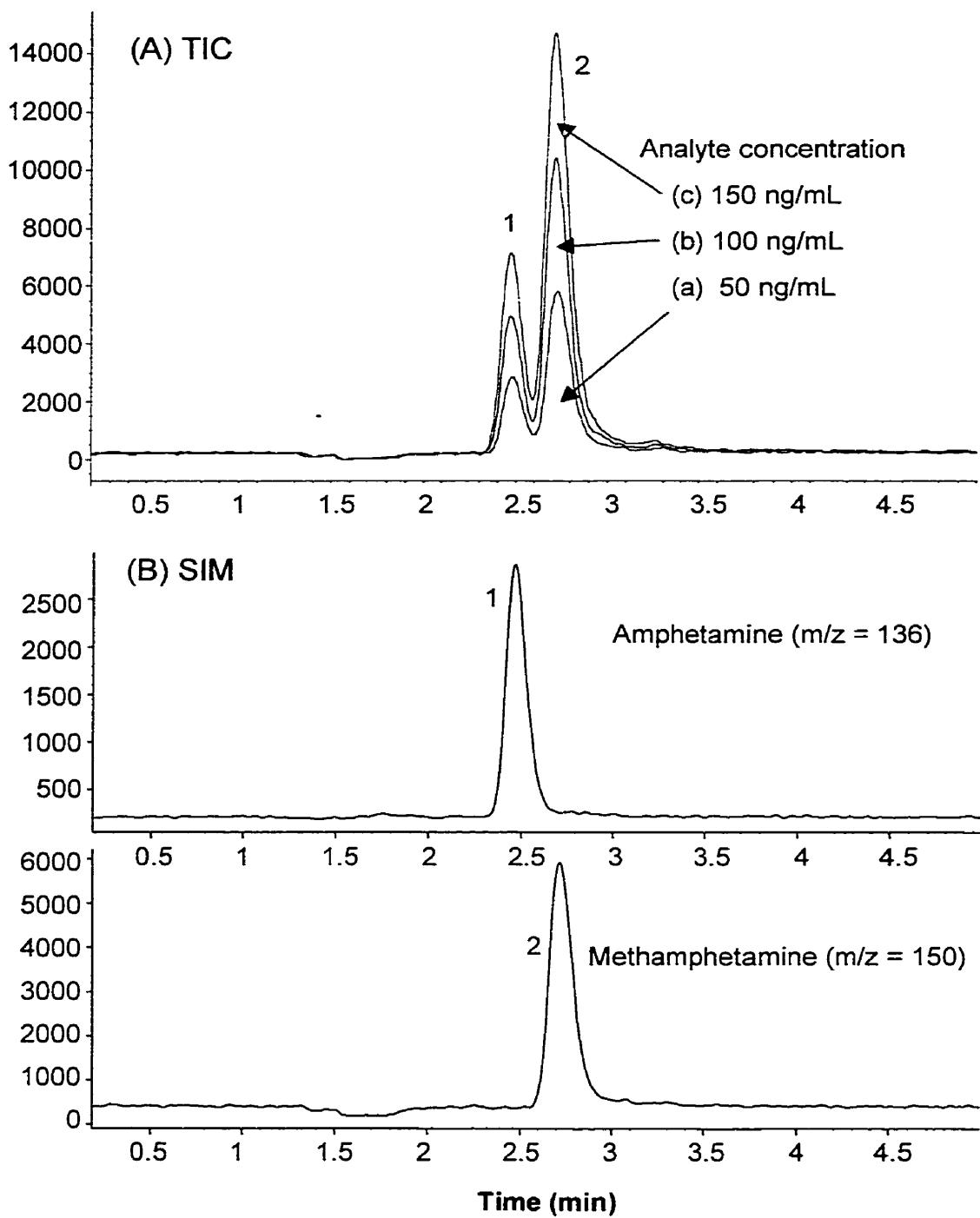


Figure 11-7. Chromatograms obtained by PPY-PSS coating SPME coupled with HPLC-MS. (A) TIC chromatograms. (B) SIM chromatogram obtained from a solution containing each analyte: 50 ng/mL; injected sample volume: 10 μ L.

11.3.3. SPME-GC analysis of volatile organic compounds

Three groups of volatile organic compounds were employed to test the extraction properties of the PPY films bearing different counter ions. Because the PPY-Cl coating could be easily stripped from Pt wire surface during extraction process, the results obtained with this coating were not reproducible, and therefore, their results are not discussed here. The PPY films with ClO₄, TS, DS or PSS as the counter ion, respectively, have better mechanical and thermal stability (especially for the organic counter ions, TS, DS, PSS) and could be used successfully for gas phase extraction of volatile organic compounds. As illustrated in Figure 11-8 and Figure 11-9, all these new coatings showed better extraction efficiencies towards polar compounds such as methanol and acetonitrile compared with the commercial SPME coatings (see Chapter 9). Among these new coatings, their extraction properties towards these compounds are not significantly different. However, due to the increasing hydrophobic interactions, the PPY-DS coating showed better extraction ability towards less polar compounds compare with PPY-ClO₄ coating.

The extraction properties of the new coatings towards a group of alcohol compounds are given in Figure 11-10. The PPY-ClO₄ and PPY-TS coatings showed similar extraction selectivity for these compounds. However, the PPY-DS and PPY-PSS coatings had better extraction efficiency for the alcohols of longer hydrophobic carbon chains. An interesting and perhaps very important discovery is that the PPY-DS coating has higher extraction selectivity towards *n*-alcohols than their isomers, probably due to its compact surface property or the smaller pore size, which created size exclusion effect or steric hindrance for the isomers.

11.4. Summary

In this chapter, the effects of counter ions on the SPME properties of PPY films have been investigated. Compared with the PPY-ClO₄ coating that showed anion exchange property (Chapter 3), the PPY-PSS coating demonstrated superior extraction efficiency to organic cations due to the cation exchange property of the bulky sulphonate anions on the coating surface and the increasing hydrophobic interactions between analytes and the PPY coating.

All the new coatings showed good extraction ability for polar organic compounds such as the commonly used organics, methanol and acetonitrile. The hydrophobic property was increased when the larger counter ion such as DS or PSS was introduced into the PPY film; therefore, their extraction ability towards less polar compounds could be enhanced. Compared with other coatings studied in this work, the PPY-DS coating showed significantly different extraction selectivity towards *n*-alcohol molecules and their isomers. These results demonstrated that the surface properties of the coatings might also have an important effect on their SPME performance. Compared with the PPY-ClO₄ or PPY-Cl coatings, it was found that the mechanical and thermal stability of the PPY coatings could be improved greatly when the organic counter ions such as DS, TS and PSS were incorporated into the polymer.

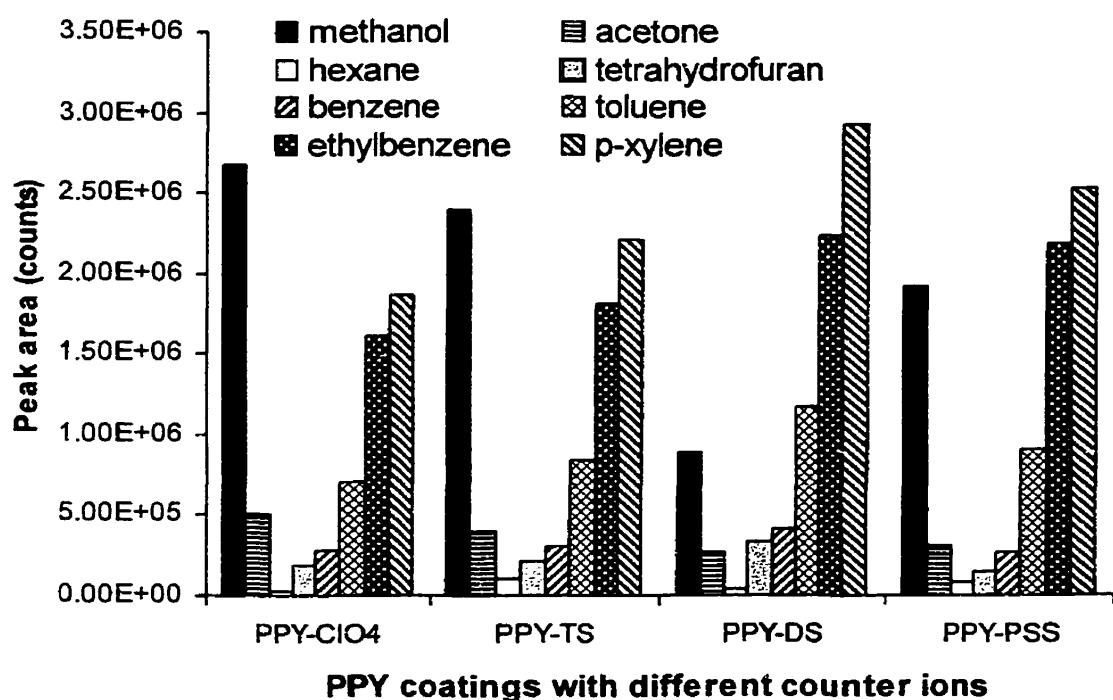


Figure 11-8. Comparison of the extraction abilities of PPY coatings for the group one compounds

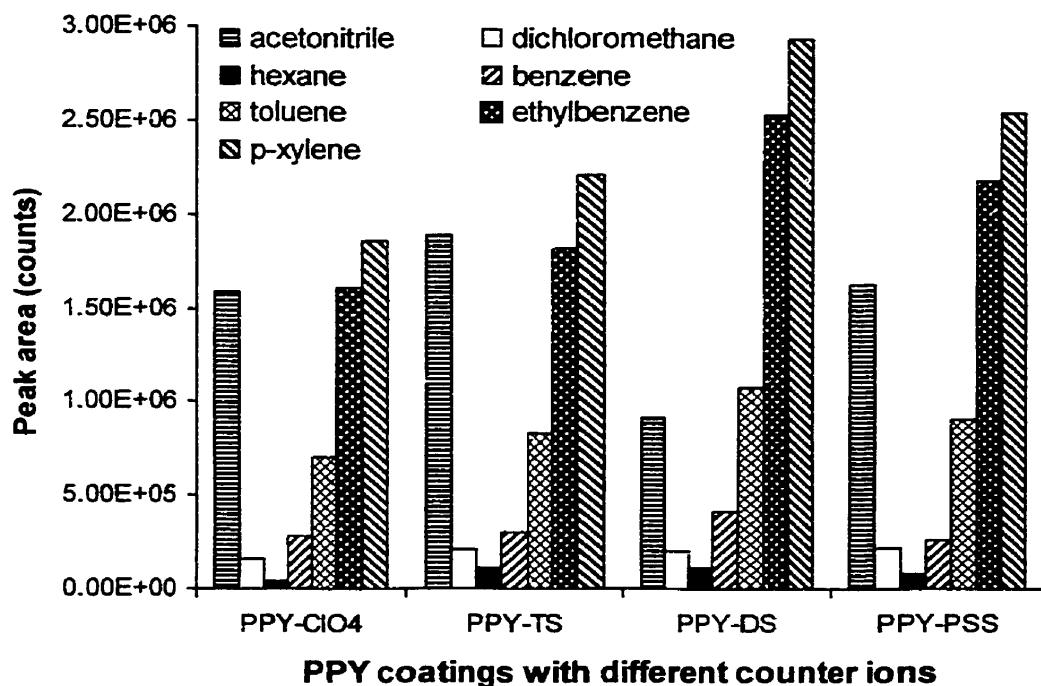


Figure 11-9. Comparison of the extraction abilities of PPY coatings for the group two compounds

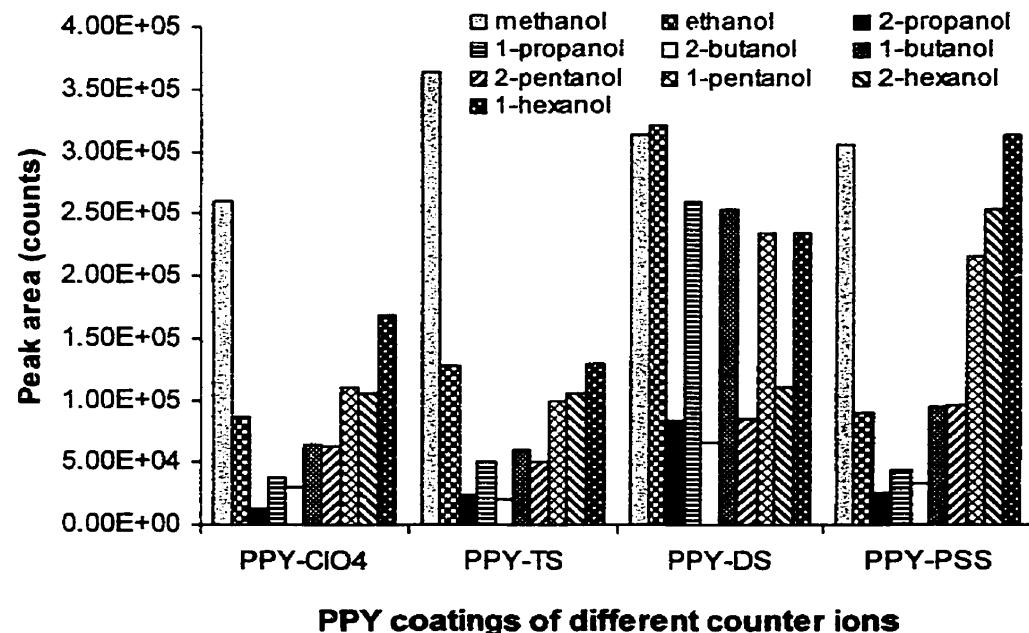


Figure 11-10. Comparison of the extraction abilities of PPY coatings for the alcohol compounds

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CHAPTER 12

CONCLUSIONS AND RECOMMENDATIONS

In this work, polypyrrole films (or coatings) with different *N*-substituted functional groups and with different counter ions have been prepared by electrochemical and chemical methods. The surface morphology of these films has been studied by scanning electron microscopy. The interactions between these films and the target analytes and the potential applications of the new films as SPME extracting phases have been investigated by coupling SPME technique with chromatographic methods.

The inherent multifunctional properties of polypyrrole (PPY) are characterized first using an in-tube SPME method, by studying the interactions between the polymer film and the selected analytes. Compared with commercial SPME extracting phases, the PPY coated capillary has shown better extraction efficiency and selectivity toward aromatic, polar, basic compounds and anionic species, due to the anion exchange, acid-base, π - π and hydrophobic interactions, as well as the interactions through polar functional groups between PPY and analytes. Therefore, the PPY-coated capillary in-tube SPME method, when coupled with HPLC, has been successfully applied for the analyses of aromatic compounds [1, 2], polar compounds [3], basic drugs [4, 5], organometallic compounds [6, 7] and anionic species [7, 8].

Functionalized PPY films can be prepared using monomers with different functional groups. In this work, the films of PPY and four of its *N*-substituted derivatives prepared by an electrochemical method have been used to extract various groups of volatile organic compounds. The preliminary results have shown that these new coatings have different selectivities toward the groups of compounds studied due to their differences in chemical structures and functional groups. For example, poly-*N*-phenylpyrrole (PPPY) has better selectivity for aromatic compounds than PPY due to the phenyl group incorporated into the polymer film. These results demonstrate that introducing a new functional group into the polymer can modify the selectivity of the film.

One of the advantages of using conducting polypyrroles for SPME is that functionalized PPy films can also be formed using different counter ions, and therefore the properties of the coatings for SPME can be modified accordingly. The preliminary results obtained in this work have indicated the potential applications of PPy films of different counter ions for SPME. However, further studies are required to realize their practical applications. In addition, other counter anions, especially those that can introduce specific interactions or molecular recognition sites such as metal complexing reagents and bioactive functionalities (see Chapter 1), should be considered in the development of highly selective SPME coatings for special applications.

The unique electroactivity and reversible redox properties of PPy can be utilized to achieve electrochemically controlled SPME for charged species as illustrated by our preliminary results. However, much work is needed to improve the current technique. For example, in order to improve the sensitivity and realize online extraction and desorption, it is desirable to design a small (microscale) flow through cell that introduces a smaller dilution factor and dead volume but is still capable of being controlled electrochemically.

The research work reported in this thesis can be treated as a starting point. More exciting work and results in this area can be and should be expected in the near future. Nevertheless, the results obtained in this work are in good agreement with both the theoretical expectations and the results obtained by other methods. This demonstrates not only that PPy films can be applied for SPME of a wide range of analytes but also that SPME may provide a useful method in studying materials like polypyrroles, in addition to its application as a sample preparation and introduction technique.

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2. Wu, J.; Pawliszyn, J. "Preparation and applications of polypyrrole films in solid phase microextraction." *J. Chromatogr. A*, **2001**, 909, 37-52.
3. Wu, J.; Xie, W.; Pawliszyn, J. "Automated in-tube solid phase microextraction coupled with HPLC/ESI-MS for the determination of catechins and caffeine in tea and other beverages." *Analyst*, **2000**, 125, 2216-2222.

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1. "Preparation and applications of polypyrrole films in solid phase microextraction". CRESTech 2000, **September 28, 2000**. Toronto, Canada.
2. "Using polypyrrole coatings for solid phase microextraction", *the 2000 Pittsburgh Conference on Anal. Chem. and Appl. Spectro.*, New Orleans, LA. USA. **March 12~17, 2000**. Paper No. 645.
3. "Solid phase microextraction based on polypyrrole films", CSC 2000 Canadian Society for Chemistry National Meeting & Exhibition, Analytical Chemistry Division, AN1. Separation Science. Calgary, Alberta, Canada. **May 27~31, 2000**.
4. "Applications of polypyrrole coated capillary in-tube SPME coupled with HPLC-ESI-MS", CSC 2000 Canadian Society for Chemistry National Meeting & Exhibition. Analytical Chemistry Division, AN3. Bioanalytical Chemistry. Calgary, Alberta. Canada. **May 27~31, 2000**.
5. "In-tube solid phase microextraction based on polypyrrole modified capillary columns" *the 2nd symposium on extraction technology (Extech 2000)*, Waterloo, Ontario, May 1-3, **2000**.
6. "Polypyrrole and poly-N-phenylpyrrole for solid phase microextraction", *the first symposium on extraction technology (Extech 99)*, Waterloo, Ontario, **May 1-3, 1999**.
7. "Photoelectron Spectra of Trimethylphosphine Substituted Tungsten Carbonyls". *The 80th Canadian Society for Chemistry Conference and Exhibition, Physical and Theoretical Division, I Chemical Applications of Synchrotron Radiation*. University of Windsor, **June 1~4, 1997**.
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