Assessment of polycrystalline graphites as sorbents for solid phase microextraction coupled to high performance liquid chromatography

By

Rocio Aranda Rodriguez

M.Sc. Chemistry (Carleton University)

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Abstract

The prime objective of this thesis was to develop novel solid supports for solid phase microextraction (SPME) for the application with HPLC analysis. In this work, three polycrystalline graphites (PG) were tested: pencil lead, glassy carbon and carbon fibers. Methods of determination of two groups of compounds were developed. Triton X-100 and pentachlorophenol were used as model analytes. The method for the determination of nonionic surfactants consisted of direct SPME followed by HPLC-fluorescence detection at λ_{ex}=230 nm and λ_{em}=310 nm. The results showed that PG performed equally well as the commercially available polymeric fibers (PDMS/DVB and Carbowax/TR). The limit of detection (LOD) found was 0.5 μg/L with a linear dynamic range (LDR) of three orders of magnitude (0.5-150 μg/L), and a precision of 10 % RSD. In the case of pentachlorophenol, the method consisted of direct SPME using polished glassy carbon followed by HPLC-UV at 225 nm. The LOD was 0.5 μg/L, with a LDR of three orders of magnitude (0.5-100μg/L) and a precision of better than 7%. Furthermore, this method was successfully applied to the determination of pentachlorophenol in spiked river samples and model solutions containing fulvic acids (0-100 mg/L).

It was found that the limit of detection was determined mostly by incomplete extraction, however, incomplete desorption also limits the amount of analyte detected. It was concluded that the analytes adsorbed onto the PG surfaces rather than absorption as observed with the commercially available liquid polymeric fibers.

The second part of this project comprised the development of derivatization procedures by SPME-HPLC using commercially available fibers and the PG. This

procedure is of importance in the analysis of primary alcohols. Two different methods for the determination of a polyethoxylated linear alcohol (Brij 56, a nonionic surfactant) were developed.

In the first, Brij 56 was determined by extraction followed by on-line derivatization using 1-naphthoyl chloride in presence of a catalyst and fluorescence detection at λ_{ex}=228 nm and λ_{em}=366 nm. The method had a LOD of 0.1 mg/L and a LDR of two orders of magnitude (0.1-10 mg/L), with a reproducibility of 10% RSD. It is apparent that the derivatization procedure limited the LOD of the method, since only 0.1 % of what was extracted was transformed to the derivative.

In the second, simultaneous derivatization-extraction followed by HPLC-fluorescence detection was employed. It was found that the LOD was improved by two orders of magnitude over the first method. In addition, the high chemical stability of the PG allowed adsorption of the derivatizing agent without further dilution. This is considered a great advantage since PG can provide a customized solid support for SPME-derivatization.

A disadvantage was incomplete desorption, however this was overcome by cleaning the PG prior to analysis. The main advantage is the chemical and mechanical resistance of PG compared to polymeric fibers as no damage of the surface was observed using solvents or derivatization reagents. The lower cost of PG compared to the polymeric fibers is also important.

Partition coefficients were calculated based on the Langmuir isotherm model and on partition data from solution. Although comparison between the two could not be

done, the trend with respect to carbon type was the same in both cases, i.e. higher for pencil leads coated than for polished glassy carbon.

Experiments were carried out which involved chemical modification of the surface of carbon fibers and the effects on the adsorption/desorption process. Better desorption efficiency was observed using untreated carbon fibers. No significant differences were found among treated carbon fibers (nitric acid, nitrogen and hydrogen at 350°C). Results suggested that treatment with nitrogen and hydrogen decreased the C/O ratio on the surface and that carbon fibers reoxidized with time. Morphological changes, such as crevasses, were observed for hydrogen treatment.

To the memory of my beloved father

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Publication and Presentations

The following publications and presentations have resulted from this work

Refereed Contributions

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Nomenclature

Abbreviations

AE	Alcohol polyethoxylate
ACN	Acetonitrile
APEO	Alkylphenol polyethoxylate
BET	Brunauer, emmet and Teller equation
BTEX	Benzene, toluene, ethylbenzene and xylene isomers
CE	Capillary electrophoresis
CF	Carbon fibers
CFA	Nitric acid treated carbon fibers
CFH	Hydrogen treated carbon fibers
CFN	Pyrolysis treated carbon fibers
DMAP	4-(dimethylamino)pyridine
DVB	Divinylbenzene
ECD	Electron capture detector
FA	Fulvic acids
GC	Gas chromatography
GCar	Glassy carbon rod
GC-FID	Gas chromatography with flame ionization detector
GC-MS	Gas chromatography with mass spectrometer
GCU	Glassy carbon (untreated)
GCP	Glassy carbon polished
HOPG	Highly oriented pyrolytic graphite
HPLC	High performance liquid chromatography
HS	Humic substances
HA	Humic acids
LDR	Linear dynamic range
LLE	Liquid-liquid extraction
LOD	Limit of detection

MS	Mass spectrometry
1-NC	1-Naphthoyl chloride
NPEO	Nonylphenol polyethoxylate
OPEO	Octylphenol polytethoxylate
PAH	polycyclic aromatic hydrocarbons
PAN	Polyacrylonitrile
PCBs	Polychlorinated biphenyls
PDMS	Poly(dimethylsiloxane)
PDMA	Pyrenyldiazomethane
PFBHA	o-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine
PG	Polycrystalline graphites
PLC	Pencil lead coated
PLU	Pencil lead (untreated)
PCP	Pentachlorophenol
RSD	Relative standard deviation
SFC	Supercritical fluid chromatography
SFE	Supercritical fluid extraction
SPE	Solid phase extraction
SPME	Solid-phase microextraction
SSA	Specific surface area
TEPA	tetraethylenepentamine
XPS	x-ray photoelectron spectroscopy

Symbols

a_o	Amount of Triton in solution
a	Fused silica inner radius
$[A]$	C_w
$[A_{ad}]$	Amount of analyte in the surface at equilibrium
b	Fiber coating outer radius
$C_{s\,\max}$	Maximum concentration of the active sites in the coating
C_w	Equilibrium concentration of the analyte in the sample
C_s	Equilibrium concentration on the analyte in the fiber
d	Vial inner radius
D_w	Analyte diffusion coefficient in the sample
k	Rate constant
K	Equilibrium partition coefficient
K_{ds}	Equilibrium partition coefficient based on surface area
K_{ow}	Octanol-water partition coefficient
L	Fiber coating length
Γ_{obs}	Surface coverage observed
Γ_{sat}	Surface coverage at saturation point
M_s	Amount of analyte in the coating
M_w	Amount of analyte in the sample
V_s	Coating volume
V_w	Sample volume
S_s	Coating surface area
$[S_o]$	Total concentration of active sites on the surface
ϕ	Surface area
Θ_{obs}	Fraction of surface covered
x	Amount of Triton adsorbed

Chapter 1

1 Introduction

Chapter 1

1 Introduction

1.1 Conventional analytical methods for water samples

An analytical method consists of several steps including sampling, sample preparation, separation, identification, quantitation and analysis of the data. Each step is important since each can affect the accuracy and precision of the final result.

The main objective of sample preparation is to extract the analyte from the sample matrix and to transfer it into a phase that is compatible with the instrumental method. In this process, the effects of the matrix and interfering compounds are minimized, and sample enrichment is achieved.

In the last two decades, powerful analytical instrumentation such as GC/MS and LC/MS has been developed. In contrast, some of the sample preparation methods reported in the 19th century are still in use, e.g. liquid-liquid extraction and Soxhlet extraction. Such methods are manual and labor-intensive; therefore, the time spent in sample preparation is roughly two-thirds of the overall time of analysis.

Methods such as liquid-liquid extraction (LLE), solid phase extraction (SPE), headspace analysis, supercritical fluid extraction (SFE) and solid phase microextraction (SPME) have been reported in the literature, and are briefly described below.

Liquid-Liquid Extraction (LLE)

Liquid-liquid extraction is one of the most popular methods. It consists of the partitioning of organic compounds between water and a water immiscible solvent. The partitioning efficiency depends on the affinity of the solute towards the extracting solvent and the number of extraction steps.

Despite the simple and inexpensive equipment required for LLE, there are many drawbacks, such as formation of emulsions, non-selectivity and the requirements for large quantities of high purity solvents. Moreover, many solvents do not readily extract some polar compounds.

Not only are the purchase and disposal costs of solvents of concern, but also the environmental issues; halogenated solvents such as dichloromethane are used in LLE. Recent awareness of the hazards of halogenated solvents has resulted in international initiatives to eliminate the production and the use of such organic solvents. The phasing out of organic solvents is prompting a major change in analytical methodology in the near future.

Solid phase extraction (SPE)

Solid phase extraction has become quite popular, and is considered an alternative to LLE for many environmental samples. In SPE, the analyte transfers from the gas or liquid phase to a solid phase. A wide variety of formats, including cartridge, disks, 96-well SPE plates, and fibers has expanded the application of SPE. Selectivity has been achieved through the use of novel bonded phases and molecular imprinting phases (Major et al., 1997).

Although the most popular format for SPE is the cartridge, the disks allow higher flow rates because of their higher cross-sectional areas and shorter bed depths. Because of disk construction characteristics, particles are held tightly in the poly (tetrafluoroethylene) (PTFE) or fiberglass supporting materials and show none of the channeling that can occur in cartridges. Under a typical set of operating conditions, the disks operate with lower linear velocities, which provide better contact time. However, disks are more expensive and fewer phases are available.

Compared to LLE, the most important feature of SPE is the concentration enrichment of the analytes by the sorbent. In natural waters where the concentration of the analyte is very small, large sample volumes can be processed in order to obtain a convenient analyte concentration. SPE also has the advantages of lower costs since a small amount of solvent is required and due to simpler and reduced processing procedures. It can be automated and used in the field.

The main consideration when using SPE is that analyte-matrix interactions affect the extraction efficiency. For effective sorption, the analyte-matrix and sorbent-matrix interactions should be weak and the analyte-sorbent interactions strong. Desorption of accumulated organic compounds can be carried out by elution with a suitable solvent or solvent mixture, or by increasing temperature (thermal desorption).

Problems encountered when using SPE for analytical work are related to large variations in the physical and chemical properties of different sorbents. Organic solvents are also required, though in smaller quantities that in LLE.

Supercritical fluid extraction (SFE)

Supercritical fluid extraction is also considered an environmentally friendly technique. Supercritical carbon dioxide ($SFCO_2$) is the most commonly used solvent because of its chemical inertness and low toxicity. $SFCO_2$ has properties of a liquid and a gas. Because of low viscosity and large diffusion coefficients, mass transfer is rapid. Varying the fluid pressure or temperature can change properties such as density thus solvent power. In practice, the choice of the supercritical fluid depends on the polarity of the analyte, the solvent strength and selectivity required, the thermal stability of the analytes at the operating temperatures, and the instrumental limitations.

Supercritical fluid extraction has been very popular for extraction of nonpolar compounds from solid samples. However, extractions from aqueous solutions are limited by instrumental factors since the phase separation must be done at high pressure. More disadvantages include poor extraction of trace levels of polar analytes, since $SFCO_2$ is relatively nonpolar. This can be somewhat overcome by the use of a modifier. SFE equipment is not portable and relatively expensive compared to other techniques.

Solid phase microextraction (SPME)

Another important technique is solid phase microextraction (SPME). This technique has been developed to be solvent-free, easy-to-use, relatively inexpensive and portable. It consists of a fused silica fiber coated with a polymer. The analyte is partitioned between the sample matrix (air or water, usually) and the polymer. Although the volume of the polymer is very small as compared to the volume of the solid phase used in SPE, all the analyte which partitions into the solid phase is transferred to the detection instrument; therefore higher sensitivity is achieved. SPME has been

successfully coupled to gas chromatography (GC); however, applications to liquid chromatography analysis have been limited by the nature of the commercially available fibers.

1.2 Research Objectives

The purpose of this study is to expand the applicability of solid phase microextraction coupled to high performance liquid chromatography (SPME/HPLC). In particular, the objectives are:

1. To develop new solid supports for solid phase microextractions. Polycrystalline graphites (PG) were chosen for this purpose and they include pencil lead, glassy carbon and carbon fibers. These PG were used for the direct extraction of a nonionic surfactant (Triton X-100) and pentachlorophenol form aqueous solutions.
2. To develop methods of SPME-HPLC-derivatization procedures and to apply them to the determination of an alcohol ethoxylate (nonionic surfactant represented by Brij56). For this purpose two different derivatization approaches were considered:

a) extraction following on-line derivatization was explored using a commercially available SPME fiber (PDMS/DVB). Derivatization took place in the SPME/HPLC interface in the presence of a catalyst.

b) simultaneous extraction-derivatization in the aqueous sample was investigated using glassy carbon and pencil lead. This was achieved by doping the polycrystalline rod with the derivatization reagent and immersing it into the aqueous solution.

3. To investigate the mechanism of adsorption and desorption using the aforementioned solid supports.
4. To study the effect of chemical modification on the surface of carbon fibers in the adsorption/desorption process.

Chapter 2

2 Solid phase microextraction

Chapter 2

2 Solid phase microextraction

2.1 Introduction

Solid phase microextraction (SPME) was introduced in the early 90's (Arthur et al., 1990) as a solvent-free and easy-to-use sample preparation technique. SPME consists of a fused silica fiber coated with a stationary phase. In the earliest stage, this stationary phase was a viscous liquid polymer, similar to those used in the GC columns. In fact, an SPME fiber could be seen as a GC column inside out. To date, the development of additional fibers of different polarities and properties has increased the number of SPME applications (Boyd-Boland et al., 1994).

2.2 Theoretical considerations

2.2.1 Thermodynamics

Solid phase microextraction, unlike liquid-liquid extraction, is not exhaustive. Instead, it is an equilibrium process in which the analyte is partitioned among the existing phases, i.e. gas, liquid and SPME coating.

In a two-phase model (aqueous sample (w) and solid phase (s)) the target analyte will distribute among the two phases. This partitioning can be expressed as (Pawliszyn, 1997):

$$K = \frac{C_s}{C_w} \qquad (2\text{-}1)$$

Here, C_s and C_w are the equilibrium concentrations of the analyte in the fiber coating and aqueous phase, respectively, and K is the equilibrium partition coefficient. Figure (2-1) shows a graphical representation of a SPME/sample system.

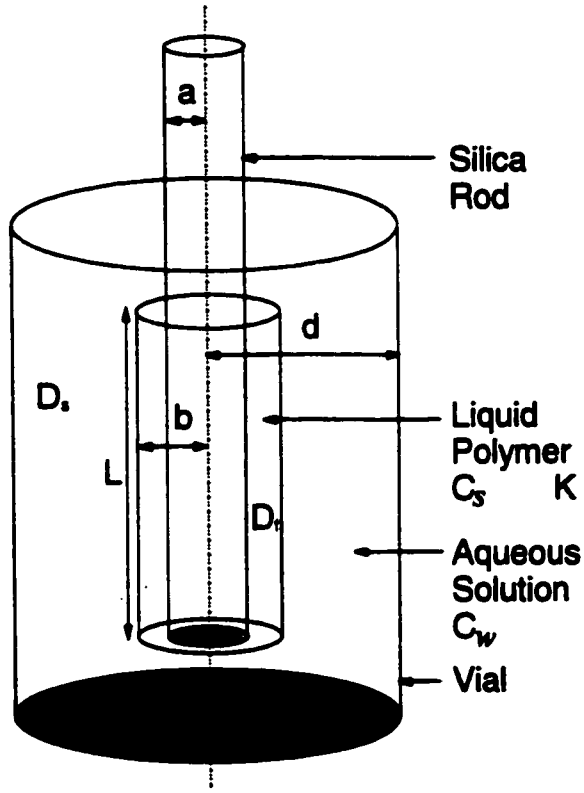


Figure (2-1). Graphic representation of SPME/sample system configuration. Here *a*, fused silica inner radius; *b*, fiber coating outer radius, *L*, fiber coating length; *d*, vial inner radius; C_w analyte concentration in the sample, D_w, analyte diffusion coefficient in the sample; C_s, analyte concentration in the coating (Pawlizsyn, 1997).

At equilibrium, the initial amount of the analyte (C_0V_w) is equal to the amount distributed in the coating (s) and aqueous phase (w), which can be described by the mass balance as (Pawliszyn, 1997):

$$C_0V_w = C_sV_s + C_wV_w \qquad (2-2)$$

Where V_w and V_s are the volumes of the sample and fiber, respectively.

Substituting equation (2-1) in equation (2-2), the amount of analyte adsorbed by the SPME polymer film in moles (n) is expressed as, (Pawliszyn , 1997):

$$n = \frac{C_0V_sV_wK}{KV_s + V_w} \qquad (2-3)$$

At first, the theory developed for SPME assumed that the above equation could be simplified since the volume of the fiber was very small (nL) compared to the volume of the sample (mL); thus the KV_s term could be neglected. In this case, the amount of the analyte extracted by the fiber coating at equilibrium would be independent of sample volume, which is described as (Pawliszyn , 1997):

$$n = KV_sC_0 \qquad (2-4)$$

However, this assumption has been shown to be incorrect even for those analytes whose partition coefficient values were very small or for large sample volumes (Gorecki et al., 1997). This misinterpretation also led to miscalculation of the partition coefficient values.

As an example, Gorecki et al. (1997) reported that for K values of the order of 10,000, the error is around ±20% for a 2 mL sample and 100 μm thick fiber. It is thus not surprising that there is disparity in the values of K reported in the literature (Gorecki et al., 1997). Therefore, in order to avoid these errors, the volumes of samples and solutions used for calibration should be consistent or the sample used must be very large.

Nonetheless, equation (2-4) is important as it shows the proportionality among the initial amount of the analyte, the value of K and the coating volume. This linear relationship allows the quantification of the analyte present in a given sample. At higher concentration, the linearity is no longer followed as overload of the fiber is expected. Equation (2-4) also implies that the sensitivity can be improved by increasing the volume of the stationary phase, usually by increasing the thickness of the polymer. Thicker coatings, however, slow down the mass transfer.

2.2.2 Kinetics

In direct SPME, the analyte is transferred from the bulk of the aqueous solution to the surface of the polymer to finally diffuse onto it. This continuous mass transfer occurs until equilibrium is reached.

This mass transfer can be described by Fick's second law of diffusion, which for a one-dimensional process can be expressed as (Pawliszyn, 1997):

$$\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial x^2} \qquad (2-5)$$

Here, C is the concentration and D the diffusion coefficient of the analyte through the fiber. If a cylindrical geometry is considered, the following expression is obtained:

$$\frac{\partial C}{\partial t} = D\frac{1}{r}\left[\frac{\partial}{\partial r}\left(r\frac{\partial C}{\partial r}\right)\right] \qquad (2-6)$$

In a perfectly agitated system, the speed of absorption is limited by the diffusion of the analyte in the coating. However, in experimental conditions this is not achievable. As a result, an unstirred thin layer of water remains next to the fiber, which limits the rate of absorption since analytes must first diffuse across this static layer. Therefore it is important to maintain a constant rate of stirring during the procedure.

The equilibrium time depends on the partition coefficient K. When K is large, more analyte has to diffuse through the static water layer, therefore, the equilibrium times will be even longer. This is particularly true when dealing with compounds of larger molecular weights in a homologous series.

In practice, this is obtained as an extraction profile graph, which is obtained by plotting the amount of analyte in the coating versus time. The typical graph shows that the amount of analyte in the fiber increases until it reaches equilibrium, which is observed as a plateau.

2.3 Adsorption versus absorption

Because the original SPME coatings were based on liquid polymers, it was assumed that the analyte was absorbed into the coating. The partition coefficient, K, based on the model of absorption is described by equation (2-1), which is expressed in terms of volume as (Yang et al., 1998):

$$K = \frac{M_s / V_s}{M_w / V_w} \qquad (2-7)$$

Here M is the amount of analyte and V volume of the coating (s) and aqueous sample (w).

Figure (2-2) illustrates the mechanisms of absorption into liquid polymer, and adsorption in two pore sizes. In this mass transport process, the analyte diffuses from the bulk of solution or headspace to the surface of the solid support. The analyte is then adsorbed onto the surface. In liquid polymers, the analyte migrates through the polymer at a rate limited by its diffusion coefficient. In the case of adsorption, the analyte remains on the surface of the solid support.

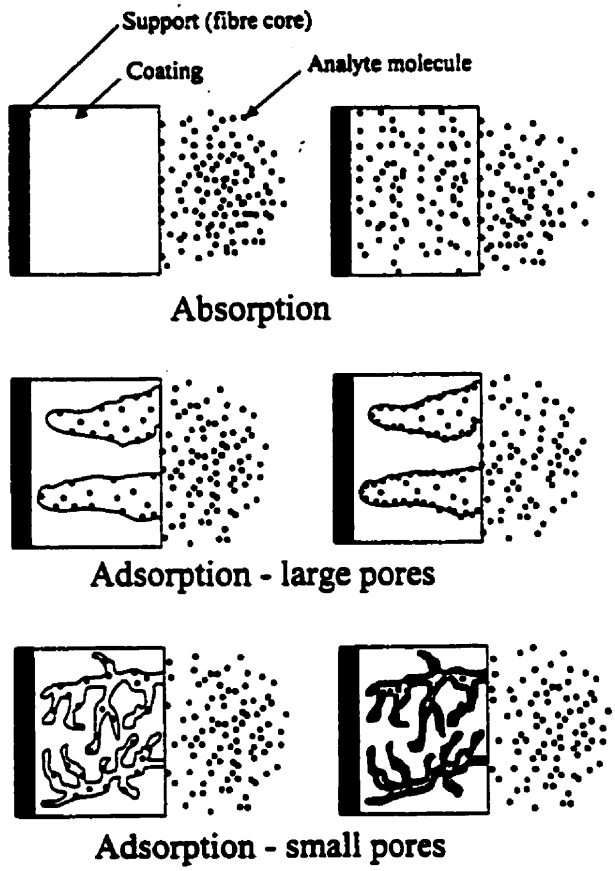


Figure (2-2). Comparison of absorption and adsorption extaction mechanism at the initial stage (left) and steady state (right) (Gorecki et al., 1999).

Yang et al. (1998) first suggested that adsorption onto the mixed phases was the primary mechanism as suppose to absorption. Thus the process was better described by the partition coefficient, K_{ds}, based on surface area (S_s) rather than volume (V_s) of the coating:

$$K_{ds} = \frac{M_s / S_s}{M_w / V_w} \qquad (2\text{-}8)$$

As seen in equation (2-8) the numerator is expressed in terms of surface area of the fiber.

Yang et al. calculated the K and K_{ds} of polychlorinated biphenyls (PCBs) ranging from di- to decachlorobiphenyls; the values were correlated with the corresponding octanol-water partition coefficient, K_{ow}, values. Two PDMS fibers (7 and 100μm thickness) were assessed. By definition, the value of K is independent of the thickness of the coating.

The K ratios ranged from 3 (the lower PCB) to 22 (for the larger molecular weight). The K_{ds} ratios between 7 μm and 100μm were very close to unity (0.28-2.3). It was thus concluded that sorption of high molecular weight PCBs on PDMS fibers is better described as adsorption phenomena. It is important to point out that PDMS is a liquid polymer, and it may be that the diffusion coefficient of these PCBs in the polymer is very small, thus they are more likely to remain on the surface.

This theory of adsorption rather than absorption for fibers such as Carbowax/DVB and PDMS/DVB was further explored by Gorecki et al. (1999). This question arose due to the results obtained using these fibers indicating incomplete desorption and carryover.

Gorecki et al. assumed that a Langmuir adsorption isotherm describes equilibrium analyte extraction by PDMS/DVB and Carbowax/DVB coatings. In this case, weak interactions between the porous polymer and the analyte are predominant, and the available sites to be occupied by the analyte molecules are limited. As a result, any other molecule will compete for those available sites and in some cases will displace the already occupied sites if the affinity is higher. This suggests that the linear dynamic

range of the fiber will be small and that the matrix composition will affect the amount adsorbed by the porous polymer.

The amount of analyte found in the surface at equilibrium in mol cm^{-2}, $[A_{ad}]$, can be expressed as (Gorecki et al., 1999):

$$[A_{ad}] = [S_o]\frac{K_A[A]}{1+K_A[A]} \qquad (2-9)$$

Where $[S_o]$ is the total concentration of active sites on the surface in mol cm^{-2}, K_A is the adsorption equilibrium constant, and $[A]$ is the concentration of A in the sample matrix (mol cm^{-3}).

As noted, the concentration of the analyte is expressed in terms of moles per cm^2, which is impractical. If it is assumed that the sorbent has a uniform size distribution, then each term of equation (2-9) is multiplied by the ϕ/V_s, where ϕ is the surface area (cm^2). In this case, the surface concentration is replaced by bulk concentration (moles cm^{-3}) thus the equation obtained is:

$$\frac{\phi}{V_s}[A_d] = \frac{\phi}{V_s}[S_o]\frac{K_A[A]}{1+K_A[A]} \qquad (2-10)$$

If the maximum concentration of the active sites in the coating is given by

$$C_{s\max} = [S_o]\frac{\phi}{V_s} \qquad (2\text{-}11)$$

and the concentration of the analyte on the fiber is expressed as:

$$C_{sA} = [A_{ad}]\frac{\phi}{V_s} \qquad (2\text{-}12)$$

then $[A]$ can be expressed as C_{wA}:

$$C_{wA} = [A] \qquad (2\text{-}13)$$

Substituting (2-11,12 and 13) in (2-10), we obtained:

$$C_{sA} = \frac{C_{s\max} K_A C_{wA}}{1 + K_A C_{wA}} \qquad (2\text{-}14)$$

The mass balance for this purpose can be expressed as:

$$C_{oA} = C_{SA} V_s + C_{wA} V_w \qquad (2\text{-}15)$$

From eqn. (2-14), the equilibrium concentration of the analyte is:

$$C_{wA} = \frac{C_{sA}}{K_A\ (C_{s\max} - C_{sA})} \qquad (2\text{-}16)$$

By combining equation (2-15) and (2-16) we finally obtain:

$$n = C_{sA} = \frac{K_A C_{oA} V_w V_s \left(C_{s\max} - C_{sA}\right)}{V_w + K_A V_s \left(C_{s\max} - C_{sA}\right)} \qquad (2\text{-}17)$$

Note that C_{sA} appears in both sides of the equation (2-17).

As mentioned before, the adsorption process is a competitive process in which the presence of other compounds affects the amount of the target analyte that is being extracted. This dependency can be expressed as:

$$C_{sA} = \frac{C_{s\max} K_A C_{wA}}{1 + K_A C_{wA} + K_B C_{wB}} \qquad (2\text{-}18)$$

Where K_B is the adsorption equilibrium constant for compound B and C_{wB} is the equilibrium concentration of B in a sample.

Figure (2-3) shows the calibration curves for i-propanol in the presence of methyl-isobutyl ketone (PDMS/DVB fiber, headspace sampling). The upper line represents methyl-isobutyl ketone at a concentration 10x lower than the i-propanol concentration and circles are at equal concentrations.

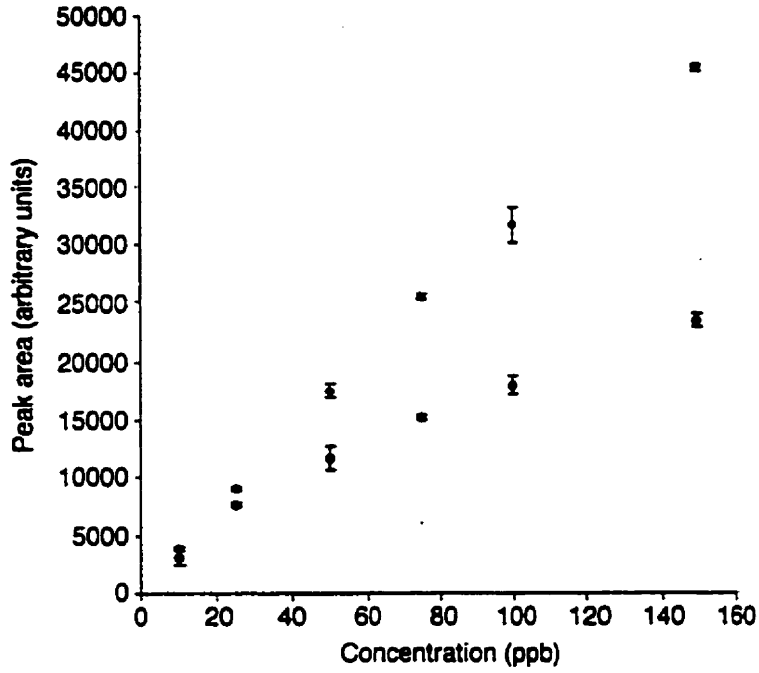


Figure (2-3). Calibration curves for i-propanol in the presence of methyl-isobutyl ketone (Gorecki et al., 1999).

From Figure (2-3) it can be concluded that the presence of interfering compounds can affect both the amount extracted and the linear range of the method for porous polymer fibers.

It was also observed that the presence of additional compounds, which are co-extracted from the sample, reduces the amount of analyte extracted by the fiber, unless those compounds are present at very low concentrations in the sample at equilibrium.

2.4 SPME Applications

2.4.1 Desorption

In a well-agitated solution, the desorption process is the inverse of absorption if the initial concentration in the fluid is zero and the linear flow rate surrounding the fiber is high. In order to quantify the amount extracted by the fiber, SPME can be hyphenated to different instrumentation systems such as GC, HPLC, CE, for further separation and detection, providing a suitable interface is available (Figure 2-4).

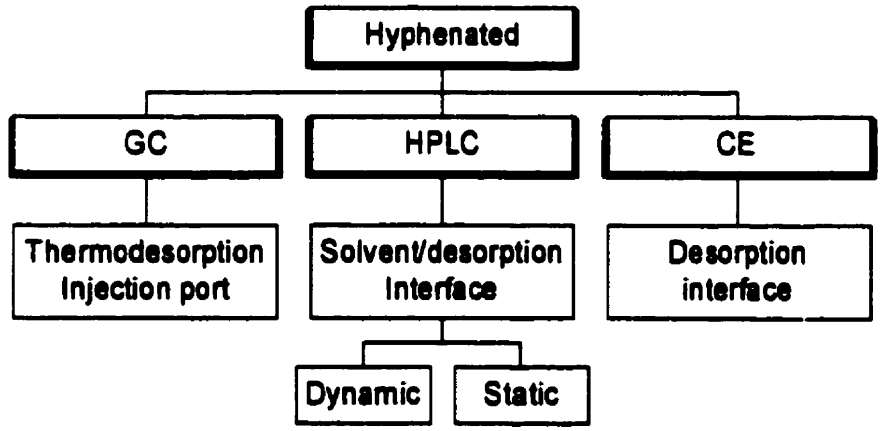


Figure (2-4). Desorption modes for SPME.

In SPME-GC, analytes are desorbed in a modified injection port of the GC and directed to the analytical column; thus SPME is endowed with the merit of being a solvent-free technique. In order to obtain a quantitative and fast desorption, the temperature must be the highest amenable for the target analyte. GC-injector port desorption was the first reported for SPME and it has been extensively investigated in more than 500 articles. The only limitation is the instability of the fiber at high

temperatures, e.g., it was reported that PDMS fibers start bleeding at 200°C (Chong et al., 1997).

In SPME-HPLC, desorption is attained by the use of a solvent. During this process, the affinity of the analyte towards the solvent must be larger than towards the coating; thus the choice of solvent is of paramount importance. Moreover, complete desorption should be obtained by a small volume of solvent because both the flow rate and the dimensions of the interface in HPLC are very small. The later is due to the chromatographic requirement of low dead volume.

The main disadvantage of this method is the limited number of fibers which are compatible with organic solvents (PDMS/DVB, CW/TR and PA only, Table 2-1). Moreover, as discussed later, there is often an incomplete desorption. It is not surprising that, since its introduction, less than 20 articles have been published on SPME-HPLC.

2.4.2 Types of solid phases

As mentioned before, liquid polymers such as PDMS were initially used to coat the silica rod. Supelco currently offers a wide variety of coatings for different applications (Table 2-1), which includes liquid polymers and porous solids. In the latter, DVB micro-spheres are held onto the fiber by a glue or Carbowax (Pawliszyn, 1997). The selection of the appropriate coating is crucial in the sensitivity of SPME since it determines the K values for a target analyte. Also listed in Table (2-1) are those fibers that have been applied to HPLC analysis.

Table (2-1). SPME fibers supplied by Supelco.

SPME Fibers	Thickness (μm)
Poly(dimethylsiloxane) PDMS	7, 30, 65
Poly(acrylate) PA	85
Poly dimethyl siloxane/divinylbenzene (PDMS/DVB) (HPLC)	65, 60
Poly (ethylene glycol)/poly(divinylbenzene) (Carbowax/DVB),	65
Poly(ethylene glycol)/template poly(divinylbenzene) resin Carbowax/TR (HPLC)	50
Carboxen/divinylbenzene	65
Carboxen/polydimethylsiloxane	75

Late advances in SPME technology incorporate new solid supports whose applications have been focused only at the research level. Examples of these new coatings include ethoxy-dimethylsiloxane (Ligor et al., 1999), graphitized carbon black (Mangani et al., 1995, Djozan et al., 1997), Carboxen-PDMS, C_8 fiber coating (Popp et al., 1999), sol-gel coating (Chong et al., 1997 and Zhou, et al., 1999) and C_8 porous layer (Liu et al., 1997).

Of importance to the work in this thesis are reports of carbon based phases. Mangani et al. (1995) made silica fibers coated with graphitized carbon black for the analysis of benzene, toluene, ethylbenzene and xylene isomers (BTEX) and some other volatile organic compounds. The behavior of the fibers was determined by sampling gaseous mixtures in a calibration apparatus. Extractions were performed by sampling the headspace above the solution containing the target analytes. Thermodesorption was

achieved at 240°C for 1 min. LOD were of the order of pg/mL and LDR of two orders of magnitude.

Chai et al. (1995) described the analysis of environmental air samples by SPME using two carbon-based coatings of unknown thickness, Carboxen, a porous molecular sieve, and Carbopack B, a graphitized carbon coating, compared to a 100 μm PDMS fiber. In this case the PDMS fiber had the greatest response for all compounds used whereas the Carboxen coating absorbed a larger quantity of organic compounds than the Carbopack B coating.

Djozan et al. (1997) coated a silica rod with extra-fine powdered activated charcoal (100 μm thickness) to analyze BTEX by GC-FID. Headspace extractions above stirred solutions (25-75°C) were performed. Desorption took place in the GC injection port held at 280°C. The main advantages of this coating were its chemical, mechanical and thermal stability. It also exhibited faster mass transfer, obtaining adsorption equilibration times of a few minutes. They obtained LOD 1.5-2.0 pg/mL and linear dynamic range of 5 to 10^4 pg/mL for the BTEX.

Several authors developed coatings using sol-gel coating technology (Chong et al., 1997 and Zhou et al., 1999). Such coatings possess higher thermostability and larger surface areas due to the porous structures. In contrast to Supelco's fibers, sol-gel coatings are chemically bound to the substrate providing stronger adhesion, thus chemical and mechanical resistance. Memory effects were not reported for the use of these fibers.

2.5 SPME-HPLC

2.5.1 SPME/HPLC interface

The first SPME/HPLC interface was developed in 1995 (Chen et al., 1995); it was modified and is now commercially available from Supelco. The interface consists of a tee port connected to a six port valve in the place of the sample loop (Figure 2-5). The interface is composed of three parts: a stainless steel body (internal volume of ca. 65 μL), a stainless steel cap, and a double tapered ferrule which is placed in between them. The septum-piercing needle rests at the top of the ferrule; when the plunger is pressed, the fiber slides through the ferrule. The design of the interface is such that the coating of the fiber is positioned just below the inlet of the mobile phase. By closing a clamp, the SPME fiber is compressed by the ferrule just above the coating. The system is then sealed and it can withstand high pressures.

Two different desorption modes are possible using this interface. In the static mode, the interface is previously filled with a solvent, and then the fiber is soaked for a period of time before switching the valve to flush the solvent and analyte onto the HPLC column. In the dynamic mode, the fiber is flushed by fresh mobile phase for a certain period of time.

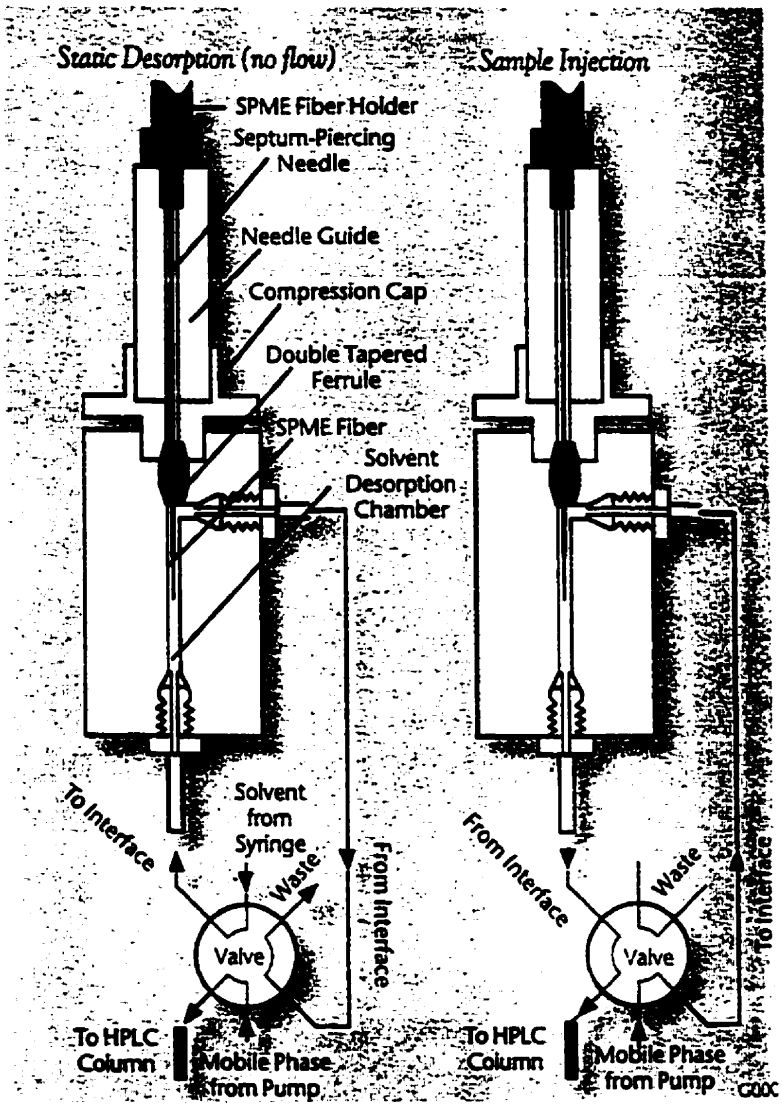


Figure (2-5). SPME/HPLC Interface (Supelco).

In 1995, Pawliszyn's group described the first interface for HPLC analysis for the determination of polycyclic aromatic hydrocarbons (PAH's) (Chen et al., 1995). Since then, only a limited number of articles have been published in the literature (Table 2-2a and 2b).

Table (2-2a). Summary of the work on SPME/HPLC (modified from Wu et al., 1999)

ANALYTE	FIBER	LOD (ng/mL)	DESORPTION CONDITIONS					
			Mode	Duration	Solvent	Flow rate (mL/min)	Desorp. (min)	REF
PAH's	PDMS	N/A	Dynamic	0	ACN:.H_2O (90:10)	0.2	N/A	Chen et al., 1995.
Triton X-100	PA, PDMS, CW/TR, CW/DVB, PDMS/DVB, PDMS/TR	~2	Dynamic	0	B/A (3:97)	1.5	1-50	Boyd-Boland et al., 1996
Proteins	PA	N/A	Static	5s	ACN	N/A	N/A	Liao, et al., 1996
Pesticides	PA	5-10	Static	30 min	Methanol	N/A	N/A	Jinno et al., 1996
Phenylurea and carbamate pesticides	Various GC capillary column	3-4	Dynamic	0	Methanol	N/A	N/A	Eisert et al., 1997
Corticosteroids	PDMS/DVB, PA, CW/DVB, CW/TR	4-30	Static	5 min	Methanol/ H_2O (50:50)	Linerarly increased form 0.2 to 1 mL/min during the first 2 min	Conti-nuosly exposed	Volmer et al., 1997
PAH's	PDMS, PDMS/DVB, CW/DVB, CW/TR	N/A	Dynamic	15s, 180s	ACN/H_2O	Isocratic	Two successive desorption	Daimon et al., 1997

Table (2-2b). Summary of the work on SPME/HPLC (modified from Wu et al., 1999)

ANALYTE	FIBER	LOD (ng/mL)	DESORPTION CONDITIONS					
			Mode	Duration	Solvent	Flow rate (mL/min)	Desorp (min)	REF
Benzo-diazepines	PA, CW/TR, sol-gel C_{11} PDMS	N/A	Static	30min	ACN/ buffer	50 µL/min		Jinno et al., 1998
Aromatic amines	PA, PDMS/DVB, CW/DVB, CW/TR	0.3-2	Static and dynamic	0-5min	Varied comp. ACN/acetate buffer	0.2 and 1	1-5min	Chao et al., 1999
Explosives	CW/TPR	N/A	Dynamic	30 min	Methanol/ H_20, 50:50	1.3 and 0.2 mL/min	30min	Wu et al., 1999
Hydroxy-aromatic compounds	PDMS/DVB, PA, CW/TR	0.66-3.2	Static Dynamic	 1-2min	ACN/acetate buffer	0.1-0.2 mL/min	1-2 min	Wu et al., 1999
Diethyl-phthalate	PDMS/DVB, PDMS/TPR, CW/TPR	1	Static	30s	ACN/H_20	0.5mL/min		Kelly et al., 1999
Antibiotics	PDMS/DVB, CW/TPR, CW/DVB	4-40	Static	5 min	ACN/H_20	1mL/min	Continuosly exposed	Lock et al., 1999
Polar pesticides	CW/TPR, CW/DVB, PA	0.1-50	Static	5 min	Methanol/ gradient elution	0.2 ml/min	2 min	Moder et al., 1999

Tables (2-2a and2b) are an updated summary of the work done in SPME/HPLC. It shows the analyte, type of fibers under investigation and limit of detection. Also it describes desorption condition such as desorption mode, duration and solvent.

The following section will be devoted to highlight those contributions to the SPME/HPLC analysis such as relevant experimental considerations. In addition drawbacks such as desorption and chromatographic implication will be discussed.

2.5.2 Relevant experimental considerations

As mentioned before, Chen et al. (1995) published the first application of SPME/HPLC for the analysis of 13 PAH's using a PDMS fiber. In this work, swelling of the polymers by the solvents was reported. It was estimated that desorption was achieved in less than 1 s, and only 0.2 μL of solvent was needed. It was found that, under experimental conditions, desorption was complete.

Boyd-Boland et al. (1996) described a method applied to nonionic surfactants, reporting detection limits of the order of parts per billion. Desorption took place in a dynamic mode for 1 min or 50 min with the mobile phase. In addition, the efficiencies of methanol, acetone and dichloromethane as desorption solvents were tested using a static mode for 1 min. Similar results were obtained with both modes and all the solvents tested. A carryover of 10% was also observed even at 50 min desorption.

Volmer et al. (1997) described a method for the determination of corticosteroids in urine. Static desorption mode was applied for 5 min followed by dynamic mode using the mobile phase throughout the analysis. Because of a higher chemical resistance, CW/TR was selected for further experiments.

It is important to remember that in the early stages of SPME-GC, the desorption procedure was optimized in terms of time and desorption temperatures. In SPME/HPLC the experimental variables that can be explored are desorption solvent, mode and time. In addition, some authors have examined the effect of temperature in the desorption process.

Daimon et al. (1997) first examined the effect of the temperature in order to enhance the amount of analyte desorbed from the fiber. Daimon et al. described an interface coiled with a heating wire to obtain temperatures of 60, 90 and 180°C. These temperatures were obtained by adjusted the voltage of a power supply. Another approach was the use of capacitive discharge reaching temperature as high as 430°C (Figure 2-6).

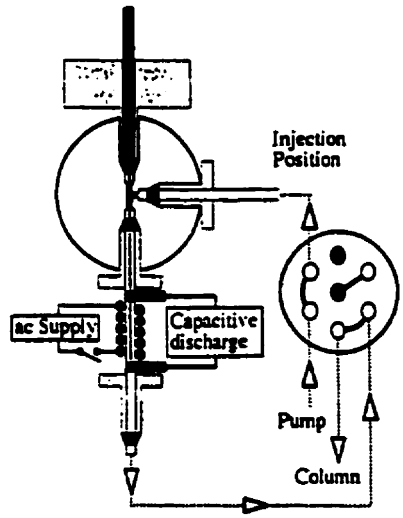


Figure (2-6). Schematic diagram of the temperature controlled SMPE/HPLC interface (Daimon et al., 1997).

Carryover was decreased to less than 10 % by increasing the time of desorption (15 s vs. 180 s) at room temperature, but peak broadening occurred.

The percent of carryover after 15 s decreased with increasing of temperature (32-43 % at 22°C and 2-5% at 180°C). However, capacitive discharge desorption was not as efficient (carryover of 17-28%) which was attributed to the large internal diameter of the desorption chamber, thus the fiber was not heated as fast. The precision of the method was between 5-30 %RSD without heating and 3-13 with heating at 90°C.

Despite the enhancement in the desorption efficiency by an increase in temperature, this procedure defeats the purpose of using HPLC in the first place, since many of the compounds analyzed by HPLC decompose with heat.

Volmer et al. (1998) described the use of SPME/HPLC/MS/MS for N-methylcarbamate pesticides in water. The LOD obtained was between 0.3-1.9 μg/L and precision between 4-13 % RSD and LDR of 2-2000 μg/L. Evaluation of the fibers showed that Carbowax-TR was more mechanical and chemical durable than PDMS/DVB and PA.

Wu et al. (1999) described a method for the analysis of aromatic amines. Moreover, desorption parameters such as desorption mode, composition of the desorption solvent and time of desorption were optimized. The fibers under study were PDMS/DVB and CW/TR. They studied both static and desorption mode at different solvent composition of acetonitrile (ACN)/H_2O (40 to 80% ACN) at constant time of desorption. They found no statistical differences among the modes and fibers.

To study the effect of time of exposure using dynamic mode, the fiber was exposed to the mobile phase for 2 and 5 min at 0.2 mL/min flow rate. The peak area of

the analyte increased with increasing desorption period using CW/TR fiber whereas for the PDMS/DVB 92-97% of desorption was obtained in a 2 min period.

It was also found that flow rate has an effect in the peak broadening of the analytes. In general, carryovers ranged from 0.3-3 %. The detection limits obtained ranged from 0.66-1.5 ng/mL using CW/TR and 0.33-2.4 ng/mL using PDMS/DVB fiber.

Analysis of tetracycline antibiotics was described by Lock et al. (1999). They observed deterioration of the fibers during the conditioning process, in which the fibers were exposed to the mobile phase gradient flow for 30 min. This includes pitting of the polymer coating and loss of the solid phase material.

L. Wu et al., (1999) described a different SPME/HPLC system. The main difference was the use of a C_8 refocusing unit that was connected to a ten port valve (replacing the six port valve). Two pumps (analytical and desorption) were also connected to the system.

After the extraction procedure, the fiber was placed in the SPME/HPLC interface. Desorption took place inside the desorption chamber, with a desorption pump directing the solvent (0.2 mL/min) through the interface and transferring its content to the C_8 refocusing unit. An analytical pump then directed the mobile phase (1.3 mL/min) through the refocusing unit desorbing the explosives and transferring them to the analytical column for separation and detection. Moreover, it was suggested that the refocusing unit eliminated the potential problem of extracolumn dispersion caused by a large sample volume.

2.5.3 Main drawbacks in the use of SPME/HPLC

The aforementioned papers described the most important aspects of the development of the SPME/HPLC procedure. In most of the cases, the authors agreed that the main shortcomings of this application are:

1) The limited number of fibers commercially available (PDMS/DVB, CW/TR and PA).
2) Lack of durability of the fiber. Under experimental conditions, it was reported that the coating can be destroyed by the solvents causing pitting of the polymer or total loss of it (Volmer et al., 1999, Lock et al., 1999, Wu et al., 1999). In most of the cases, CW/TR was the most robust to the analysis conditions.
3) Memory effects. Diverse percents of carryover have been reported (from 1-10%). Carryover is more intense for polar analytes using a Carbowax fiber due to strong absorption (Möder et al., 1999).
4) Chromatographic implications. Desorption mode seems to affect the chromatographic performance. Long dynamic desorption times caused peak broadening (Daimon et al., 1997) as did high flow rates (Wu et al., 1999). The results are however inconsistent. Results also differ from one fiber to another, for instance, differences between static and dynamic mode were insignificant using CW/TR, however this was different for PDMS/DVB (Wu et al., 1999). This discrepancy is attributed to differences in the partition coefficients between the fiber and the solvent.

2.6 Derivatization/SPME techniques

2.6.1 Introduction

An increasingly popular way to enhance the sensitivity and selectivity of detection of the analytes is a derivatization procedure by which target moieties are reacted with a suitable chromophore, fluorophore or electrophore. These derivatization techniques have been extensively used in GC and HPLC analysis, since chromatographic performance and detectability are improved.

Moreover, some properties such as water solubility and vapor pressure of the analytes are affected. For instance, conversion of polar into less polar derivatives can be achieved reducing analyte water solubility and increasing its vapor pressure. Extraction procedures such as LLE, SPE, SPME have been benefited from derivatization schemes.

Improvements of the dectectability of a compound are achieved by the use of derivatization reagents that enhance the response in the detector (use extensively in this work). In GC analysis, for example, the fluorinated anhydride derivatives are used primarily for electron capture detection (ECD) (Supelco Catalogue). In HPLC, derivatization is applied when the target compounds lack chromophores. This is important, since the absorbance of underivatized compound near 200 nm cannot be used due to absorbance of the mobile phase (Toyo'oka et al., 1995).

Further advantages can be obtained, e.g. derivatization provides specificity based on functional groups which at the same time could be used as a confirmatory test (Martos et al., 1998).

The selection of derivatization reagent is of paramount importance. An ideal derivatization reagent should possess the following criteria:

1) complete and quantitative conversion to a single conjugate under mild reaction conditions,
2) performance of the reaction in either aqueous or non-aqueous solvents,
3) minimal side reactions
4) the reagent as well as the conjugate should possess reasonable stability at room temperature and
5) chromatographic separation of the reagent and the conjugate should not be difficult.

2.6.2 SPME/Derivatization schemes

Derivatization for SPME applications has been developed for the same reasons as given above. A general view of the strategies has been described (Pawliszyn, 1997, Pan et al., 1997) and is shown in Figure (2-7).

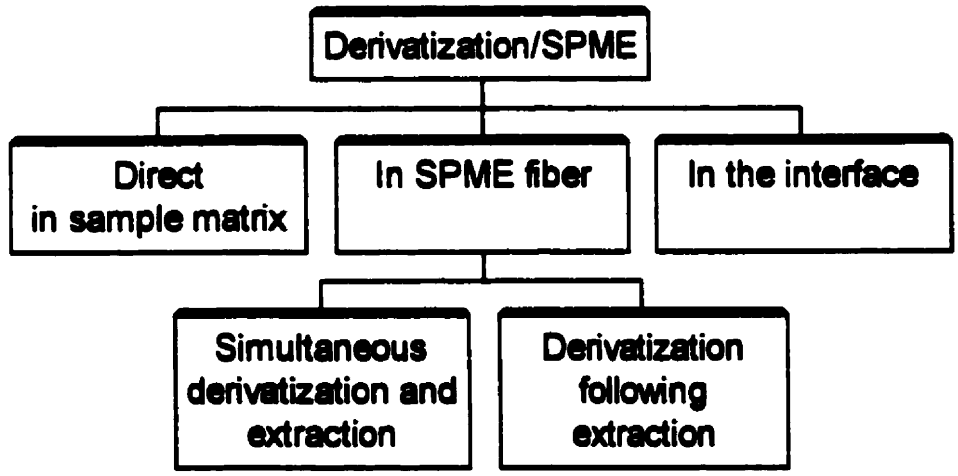


Figure (2-7). Derivatization/SPME modalities (Pan et al., 1997).

Direct derivatization in sample matrix

Direct derivatization in the sample matrix is an extension of solvent extraction. Derivatization takes place in the sample matrix and facilitates the extraction, as the partition coefficient, *K*, of the derivative is larger than the non-derivatized analyte. This method is recommended when the reagent and products are stable in aqueous solution.

Another approach was aqueous-phase derivatization prior to headspace extraction. This method was applied to the determination of ionic mercury species, where derivatization in the sample vial was obtained by adding an ion-pair derivatization (sodium tetraethylborate) and subsequent headspace extraction with a PDMS fiber (Cai et al., 1995).

Nilsson et al. (1998) developed a method of aqueous-phase derivatization followed by SPME for the analysis of phenoxyacetic acids (PAA). The procedure included the addition of benzyl bromide and phosphate buffer to a solution containing PAA. The effect of pH was studied (3, 4, 5, 6.3, and 7.4). It was found that carboxylic acids could be derivatized in aqueous solutions, significant amount of derivatives was extracted by the PDMS fiber. LOD were in the order of 0.1-1 μg/L and a RSD of 14-32%.

In-fiber derivatization

The more advantageous of the procedures is in-fiber derivatization. The polymeric material can be seen as an organic medium where the reaction takes place. Once the fiber is doped with the derivatization reagent, it is immersed in the sample where simultaneous extraction and derivatization take place. There are many doping approaches; some of them include the absorption of the derivatizing reagent in the

headspace such as analysis phenoxyacid herbicides (Nilsson et al., 1998) and formaldehyde (Martos et al., 1998).

In the analysis of phenoxyacid herbicides, a PDMS fiber was exposed to the vapors of benzyl bromide for 10 min, then placed in the headspace of a aqueous solution containing the analyte and NaCl at pH=0 (Nilsson et al., 1998). This procedure did not succeed since low amounts of PAA were transferred to the fiber due to the low vapor pressure of the PAA even at low pH.

Determination of gaseous formaldehyde (Martos et al., 1998) was obtained by on-fiber derivatization with o-(2,3,4,5,6,-pentafluorobenzyl)hydroxylamine (PFBHA). SPME fibers (PDMS, PDMS/DVB, CW/DBV, PDMS/DVB and Carboxen 1006/PDMS) were placed in the headspace of a solution containing PFBHA-HCl. The fibers were then placed in a headspace of hair gel and particle board contaminated with formaldehyde. The criteria of fiber selection were based on the highest loading and yet high desorption yield, plus good stability of the PFBHA on the fiber with time. PDMS/DVB fulfill the aforementioned criteria.

Another way of doping the fiber is by dissolving the derivatization reagent in an organic solvent (Pan et al., 1997), in which case, the affinity of the derivatization reagent towards the fiber must be larger than towards the solvent. In addition, chemical resistance of the fibers towards the solvent and derivatization reagent is required.

This method was applied to the analysis of fatty acids (Pan et al., 1997) in waste water. PA fiber was dipped into a solution containing 5 mg/mL of pyrenyldiazomethane (PDAM) in hexane for 60 min. The doped fiber was transferred to the headspace of aqueous solution containing the fatty acids and saturated NaCl , pH 1.5. Equilibration

was reached after 30 min. This procedure was also applied to the determination of fatty acids in milk samples. Here extractions were performed for 2 h and at room temperature. This procedure achieved linear dynamic range of 0.005-5 μg/L and a LOD of 6-10 ng/L.

The same procedure was applied to the analysis of fatty acids in fecal samples using PDAM, and headspace (Mills et al., 1999).

Derivatization following extraction

The second in-fiber derivatization modality is derivatization following extraction. After extraction, the fiber is placed in the headspace of a vial containing the reagent. This is a more complicated system, since the derivatization reagent vapour pressure and fiber/air partition coefficient must be large. This method was applied to in the analysis of steroids (Okeyo et al., 1998).

Acidic herbicides were extracted from aqueous solution via SPME (Lee et al., 1998). The fiber was then placed in an N-methyl-N-nitro-N-nitrosoguanidine (MNNG)-diazomethane kit from Aldrich Chemical Co. heated at 50°C. The limits of detection using PA fiber were 10-900 ng/L and 30-1500 ng/L using PDMS.

Derivatization in the GC injection port

Finally, derivatization in the GC injection port is an extension of normal GC derivatization. Pan et al. (1995) carried out this procedure using an ion pair reagent tetramethylammonium hydrogen sulfate and tetramethylammonium hydroxide for carboxylic acids.

2.6.3 Theory

The following kinetics theory was described by Martos et al. (1998) and considers in-fiber derivatization when the fiber is doped with the derivatization reagent. It is then assumed that the coating contains a high concentration of derivatization reagent allowing simultaneous derivatization and trapping of the analyte in the fiber.

The adsorption of the reagent (R) on the fiber can be described as:

$$\text{Fiber (S)} + \text{Reagent (R)} \xrightarrow{k_1} \text{R-S} \quad \text{(adsorption)}$$

$$\text{R-S} \xrightarrow{k_{-1}} \text{R} + \text{S} \quad \text{(desorption)}$$

Where $K_A = k_1/k_{-1}$ is the equilibrium constant for absorption of the reagent.

Once the fiber is in contact with the sample, some analyte molecules can be adsorbed onto the available sites. This process is described by:

$$\text{Analyte (A)} + \text{Fiber (S)} \xrightarrow{k_2} \text{A-S} \quad \text{(adsorption)}$$

$$\text{A-S} \xrightarrow{k_{-2}} \text{A} + \text{S} \quad \text{(desorption)}$$

Here $K_B = k_2/k_{-2}$ is the equilibrium constant for adsorption of the analyte. However, it is expected that molecules of the reagent occupy most of the sites.

The reagent, R, will then react with the analyte to form product P and is expressed as:

$$\text{A} + \text{R-S} \xrightarrow{k^*} \text{P-S} \quad \text{(reaction)}$$

It is assumed that k^* is rate limiting as opposed to the rate of the diffusion of the analyte toward the solvent. In order to keep pseudo first-order kinetics of reaction, the amount of reagent must be in excess, so that reagent concentration remains constant throughout the extraction process. Thus, the overall rate of reaction is dependent only on the concentration of the analyte.

Desorption of the product either in the GC injection port of in the SPME/HPLC interface is the final step. It is then desired to have a fast desorption.

$$\text{P-S} \xrightarrow{k_3} \text{Product} + \text{S} \quad \text{(desorption)}$$

Martos et al. (1998) described the above mechanism of in-fiber derivatization for the analysis of formaldehyde with PFBHA to form the oxime. They proposed that the mechanism for this system is a Langmuir-Rideal, which assumes that the reaction takes place on the surface between an adsorbed molecule and a gaseous-phase molecule. The velocity of oxime formation (weight/time) is proportional to the concentration of gaseous formaldehyde (C_{HCHO}), the rate of reaction between PFBHA (k^*) and formaldehyde and the total number of sorbent sites occupied by PFBHA (θ).

$$v = C_{HCHO} k^* \theta \qquad (2\text{-}19)$$

Finally, a direct relationship between the velocity of the reaction and the reaction rate as a function of the analyte concentration can be expressed as:

$$v = C_{HCHO} k^{*} \qquad (2-20)$$

This relationship is important since it is expected that quantitative analyses of unknown amount of formaldehyde is possible using this empirically determined first-order rate constant when the amount of PFBHA is negligibly consumed. Figure (2-8) shows the results obtained by Martos et al. The values of the inverse of the reaction velocity (s/ng) of the product at different concentration of formaldehyde are plotted. The inverse of the slope represents the apparent first-order rate constant (0.00297 ng/ppb) which can be used to quantify unknown concentration of formaldehyde. The main advantage is that no calibration curve is required for quantification.

The developed method was found to have a precision of 12%RSD and LOD of 4.6 ppbv.

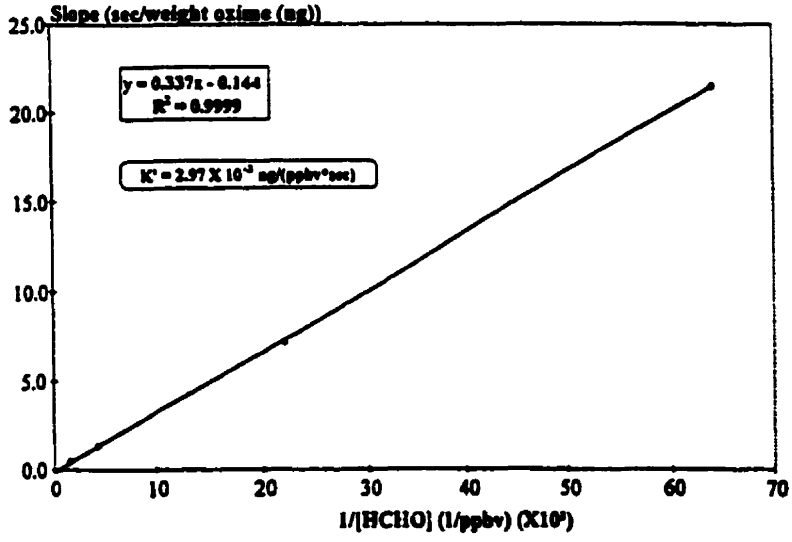


Figure (2-8). Plot of the inverse of the reaction rate versus the inverse formaldehyde concentration (Martos et al., 1998).

Chapter 3

3 Polycrystalline graphites

Chapter 3

3 Polycrystalline graphites

3.1 Introduction

Despite the rapid development of new fibers for SPME, such as mixed phases and sol-gel fibers, the application of SPME to HPLC analysis has been limited due to the nature of the commercially available fibers (Chapter 2). Therefore, a new solid phase is required that overcomes most such drawbacks.

Silica fibers coated with graphite were developed for the analysis of volatile organic compounds. These graphite-coated fibers offered many advantages, such as chemical, mechanical and thermal stability, in addition to high surface area. They were only applied to GC analysis, however (Mangani et al., 1995, Djozan et al., 1997).

It was found that some graphite-like materials such as glassy carbon and carbon fibers maintain some graphite characteristics. They could be used to advantage for SPME-HPLC applications due to their rigid nature.

Such materials are named polycrystalline graphites (PG) (also included are carbon blacks and pyrolytic graphite), and are obtained by carbonization of polymeric materials. They do not graphitize readily even at high temperatures. Therefore, PG are aggregates of graphite crystallites which vary in size, orientation, degrees of porosity and purity. As

a consequence, their properties and use vary enormously as discussed in the next sections.

3.1.1 Graphite

Graphite, strictly speaking, is considered an ideal material with a perfect crystal structure. In the real world such material does not exist; however it is important to describe graphite's characteristics in order to understand polycrystalline graphites, containing a graphitic structure but with a number of structural defects.

In graphite, each carbon atom is bonded to three neighbors in the sample plane. This gives σ bonds framework of planar hexagons bound by sp^2 hybridization (Figure 3-1). Each atom also has one 2p orbital available to form delocalized π orbitals.

The σ bond has a length of 0.141 nm and high strength (524 kJ/mole); the spacing between layers is 0.335 nm and the interlayer strength 7 kJ/mole. Thus graphite is stable and has a high electric conductivity due to the delocalized π electrons. As the layers are held together by van der Waals' forces it requires little energy to slide them, hence graphite is a good lubricant.

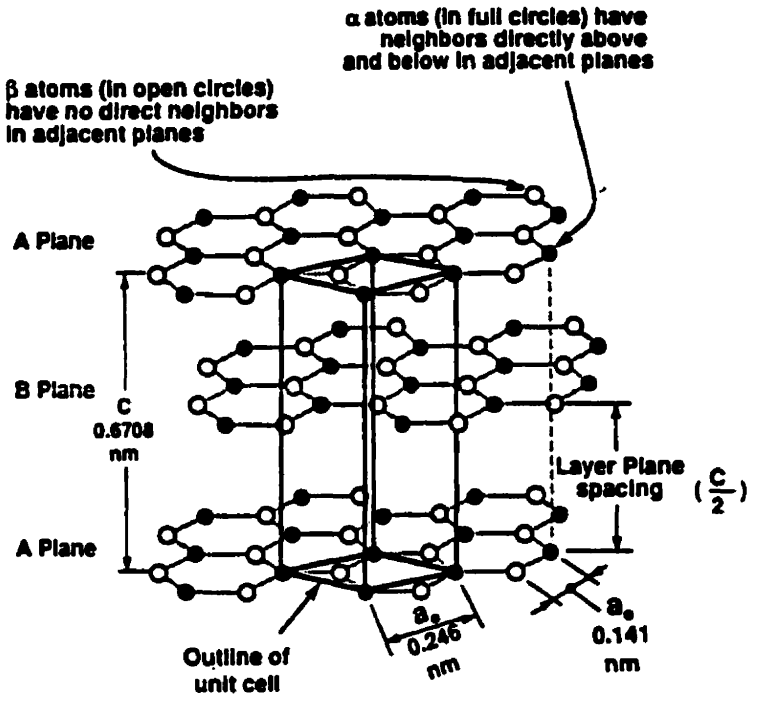


Figure (3-1). Crystal structure of graphite (Pierson, 1993).

Table (3-1). Physical properties of graphite (Pierson, 1993).

Crystalline form	Hexagonal
Lattice parameters	a_o= 0.246 nm b_o=0.671 nm
Color	Black
Density at 300 K, 1 atm	2.26 g/cm^3

Chemical properties

Pure graphite is one of the most chemically inert materials. However, some imperfections confer some catalytic effect, increasing chemical reactivity in proportion to the surface area. At high temperatures, graphite oxidizes with water, and oxygen. It has been suggested that functional groups such as C-O or C-O-H are present in the surface of graphite (Hirohata et al., 1994).

3.1.2 Glassy carbon

Glassy carbon has been used in electrochemistry as a working electrode (Ryan et al., 1994) and as stationary phase in chromatography (Larkins et al., 1993). Industrial applications include vessels for chemical processing, and acid battery electrodes (Pierson, 1993).

Vitreous or glassy carbon (GCar) is obtained by carbonization of organic polymers such as polyfurfuryl alcohol, phenolics, polyimide, polyacrylonitrile (PAN) and cellulose. It does not graphitize readily and has characteristics and properties that are isotropic. Its structure is more closely related to glassy material with non-crystal arrangements. It has been suggested that sp^3 bonds are present, explaining the poor graphitization, and the high strength and hardness (Figure 3-2).

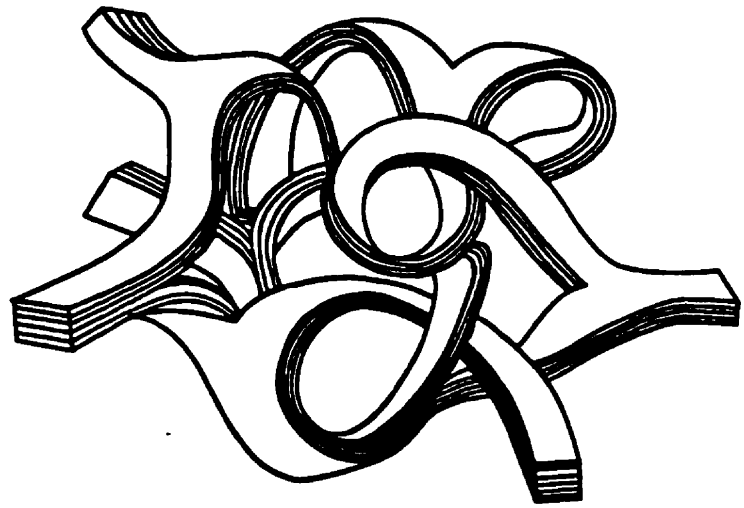


Figure (3-2). Proposed model of the ribbon structure of glassy carbon (Pierson, 1993).

Carbonization is achieved by slowly heating the polymer in a reducing or inert environment (rate of heating 1-5°C/min to 1000°C), and no mesophase is reached. GCar does not graphitize to any extent. Heating to 1800°C produces a material with an interlayer spacing (d) of 0.36 nm and a crystal size (Lc) of 1.5 nm.

Glassy carbon contains small randomly oriented crystallites. Within each crystallite, the interatomic distances deviate from those of the ideal graphite by 5 %. GCar has a low density (approximately two thirds that of the ideal graphite), which implies that it has high porosity; however helium permeability is low, therefore the pores are extremely small, typically 0.1- 0.3 nm in diameter.

Glassy carbon's suitability as a chromatographic stationary phase relies upon its chemical stability and high surface area (1.62 m^2/g) (Pierson, 1993). Like graphite, GCar is a hydrophobic, highly polarizable solid. The highly ordered planar surface, on the molecular level, produces unique structural selectivity, with an amorphous macro structure that forms the porous particles necessary for high surface area. The main disadvantage is that GCar is highly retentive and higher molecular weight solutes may not elute (Rittehnhouse et al., 1996).

Table (3-2). Physical and Mechanical Properties of GCar and other carbon materials at 25°C (Pierson, 1993).

Properties	GCar	Carbon (Graph.)	Pyrolitic graphite
Density g/cm^3	1.54	1.42	2.10-2.24
Flexural strength, MPa	210	260	80-170 (c)
Compressive strength, MPa	580	480	

3.1.3 Carbon fibers

Carbon fibers have been used as reinforcements in composite materials based on a polymeric matrix. They have been widely used in aerospace, as well as in the area of high technology products (Lee et al., 1997).

The production process of PAN-based carbon fibers includes spinning the PAN co-polymer to form a fiber. As a result of stretching, a high strength fiber is obtained with 500–1300 % elongation.

The structure of PAN-based carbon fibers is turbostatic and is composed of small two-dimensional fibrils or ribbons. Structural models proposed two critical parameters: Lc, the stack height of the ribbon, and the crystallite size La, and the mean length of a straight section of the fibril. La is less than 20 nm (Figure (3-3)).

The interlayer spacing never shrinks to less than 0.344 nm, indicating a poor alignment of the basal planes and the presence of defects, stacking faults and dislocation.

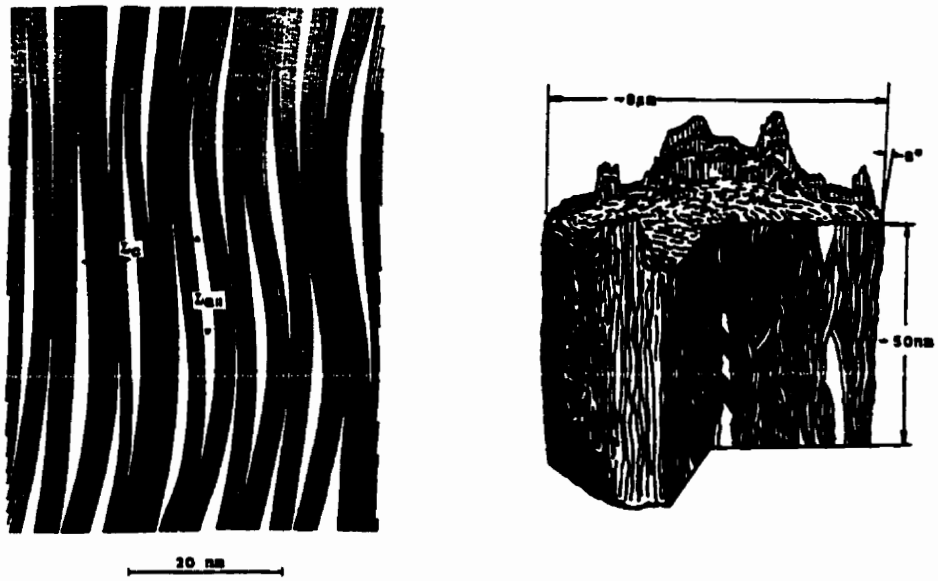


Figure (3-3). a) Two-dimensional schematic representation, and b) three-dimensional representation of the structure of carbon fibers (Peebles, 1995).

3.2 Adsorption onto solid surfaces from liquids

3.2.1 Introduction

Adsorption onto solid surfaces from liquids has been studied for dilute solutions. The thermodynamic and kinetic behaviour of an adsorption process generally varies from one adsorbent to another and depends upon the nature of the solute adsorbed (Mutlu et al., 1997). In order to determine the mechanism of adsorption, many variables must be considered (Partiff et al., 1983): a) the surface, b) the solute, c) the solvent, d) the nature of the interactions between surface and adsorbent solute, e) the structure of the adsorbed layer.

Surface. It is important to consider the characteristics of the surface, such as its chemical nature and the presence of contamination. It has been observed that surface

heterogeneity in active carbon affects dramatically the adsorption phenomena. Chemical heterogeneity includes the presence of functional groups, whereas geometrical heterogeneity comprises the existence of pores or defects (Heuchel et al., 1995). Moreover, a chemical modification on the surface will lead to a different surface reactivity.

Solute. It is important to consider the nature of the solute and its interaction with the solvent as the physicochemical properties of the solute will affect the interactions with the solvent. Solubility is important, and is related to the chain length and the presence of functional groups.

Solvent. The properties of the solvent will affect its interaction not only with the solute but also with the adsorbent and the adsorbent layer.

Interaction between solute and surface. Adsorption from solutions by solids has been studied for dilute solutions. The interaction between the surface and adsorbed species may be either chemical (chemisorption) of physical (physisorption).

Structure of the adsorbed layer. Generally speaking, a monolayer is postulated in adsorption isotherms. However, some solutes will change orientation at the surface and there can be formation of multimolecular layers.

3.2.2 Adsorption Isotherms

An adsorption isotherm is a mathematical expression that relates the concentration of adsorbate at the interface to its equilibrium concentration in the liquid phase. There are many types of isotherms, such as Langmuir, Temkin, Freundlich, which differ in one or more of the assumptions made in deriving the expression for the surface coverage. A

type of adsorption isotherm commonly observed in adsorption from solutions of surfactants is the Langmuir isotherm, expressed by (Gorecki et al., 1999):

$$C_{sA} = \frac{C_{s\max} K_A C_{wA}}{1 + K_A C_{wA}} \qquad (3-1)$$

Where C_{sA} is the surface concentration at equilibrium, $C_{s\max}$ is the maximum concentration of the active sites on the surface, C_{wA} is the concentration of component A in the liquid phase at adsorption equilibrium and K is a constant. The assumptions of this model (Rose, 1989) include that the 1) the adsorbent is homogeneous, 2) both solute and solvent have equal molar surface area, 3) there are not interactions, such as solute-solute or solute-solvent interaction in either phase, and 4) the adsorption is a monolayer.

To determine whether adsorption is following the Langmuir isotherm, the equation can be transformed into a linear form by inverting it as (Gorecki, et al., 1999):

$$\frac{1}{C_{sA}} = \frac{1}{C_{s\max}} + \frac{1}{C_{s\max} K_A C_{wA}} \qquad (3-2)$$

A plot of $1/C_{sA}$ versus $1/C_{wA}$ should be a straight line with a slope of $1/C_{s\max}K_A$ and an intercept of $1/C_{s\max}$.

In the adsorption of surfactants some of the restrictions are not met, however, they still show Langmuir-type behaviour. The fact that the data fit this isotherm does not mean that the assumptions are followed. For instance, adsorption of nonionic surfactants

is highly dependent on temperature, molecular structure of the adsorbate due to the solute-solvent, solute-solute interactions, such as micelle formation (Parfitt, 1983).

Adsorption of nonionic surfactants will be further discussed in Chapter 6.

3.3 Adsorption onto graphite

The surface of graphite, as an ideal adsorbent material, is considered homogeneous. It is therefore not surprising that many authors have studied adsorption onto this surface. However, there is a lot of potential misunderstanding of the materials used for such studies, since they often used polycrystalline graphites. Some examples are graphitized carbon black (Subramanian et al., 1993), pyrolitic graphite (McDermott et al., 1994 and Aviram et al., 1995) and activated carbon (Kochkodan et al., 1996), which in some cases are heterogeneous.

As mentioned before, the characteristics of the surface have an effect in the adsorption phenomena. The overall heterogeneity of these polycrystalline graphites consists of both chemical and geometrical heterogeneity. Chemical heterogeneity includes surface impurities as well as functional groups present on the surface. Geometrical heterogeneity includes surface defects and pores (Heuchel et al., 1995).

Many authors have considered that impurities present in the surface of graphite dominate the interaction between the adsorbant and sorbent. Subramanian et al. (1993) investigated the adsorption of dextrines onto graphite as received and leached with HCl. They concluded that purification of graphite by leaching reduces the content of metallic impurities and increases graphite hydrophobicity, therefore leached samples showed less adsorption than original.

MacDermott et al. (1994) hypothesized that the kinetics of adsorption depend on specific chemical sites which exist only at defects on highly oriented pyrolytic graphite (HOPG). Results were compared to those obtained using glassy carbon. It was found that adsorption of quinones onto HOPG followed Langmuir isotherms, and that it was dependent on defect density as measured by scanning tunneling microscopy.

Kim et al. (1995) investigated adsorption of anionic naphthalene derivatives at the graphite-aqueous solution interface. Adsorption isotherms were obtained by contacting graphite with a solution containing the derivative in the presence of inert electrolyte at constant agitation and temperature (25°C). An increase in hydrophobicity of a surfactant promotes its adsorption onto graphites surfaces. It is generally understood that a benzene ring strongly affects adsorption onto the hydrophobic surface from aqueous solutions, through both a reduction in solubility as well as strong π-π bond. The actual areas occupied by the individual surfactants, as revealed by the adsorption isotherms, provide some indication of their possible surface orientation.

Kochkodan et al. (1996) studied the effect of temperature on the adsorption of nonionic surfactants onto acetylene carbon black and activated carbon. They suggested that the structure of adsorbed layer is temperature dependent.

3.4 Effects of surface modifications

Adsorption is highly dependent on the nature of the surface. As already mentioned, the interaction of the solute with the interface will depend upon the functional groups present on the surface. The defects in the crystallites of graphite will confer some reactivity; moreover, impurities also could change the adsorption characteristics.

The surface of polycrystalline graphites have been chemically modified and will be explained below.

Modification of glassy carbon

Glassy carbon has been used as a working electrode in electrochemistry. Thus chemical modifications were done to improve the electrochemical behaviour. In addition, glassy carbon has been used as stationary phase in HPLC.

As mentioned before, CO and COH groups are present in the surface of graphite and they are also expected to be in the surface of glassy carbon. Gomathi et al. (1995) characterized the surface of glassy carbon (freshly cut, polished, chemically pretreated and electrochemically pretreated). The reactivity depended heavily on the treatment of the surfaces as observed by measuring the cyclic voltammetric response. The numbers of oxygen containing groups, possibly of phenolic nature, were higher in chemically and electrochemically treated surfaces.

Maeda et al. (1996) characterized the surface of a glassy carbon anodized in a primary alcohol to elucidate the surface structure by measuring its wetability and capacitance. They showed that it was possible to modify the surface of the glassy carbon by covalently bonding primary alcohols. It was proposed that the terminal hydroxyl groups on electrodes are likely to be oxidized to carboxyl groups during the anodic modification. Depending on the alcohol, the electrode can be more hydrophilic or hydrophobic.

Knox et al. (1996) modified porous graphite for ion exchange chromatography. They used polyethylene imine $(CH_2\text{-}CH_2\text{-}NH\text{-})_n$ which, being polymeric, is expected to be strongly adsorbed on to graphite. They proposed three different treatment methods,

dynamic coating, insoluble monolayer and cross-linked coating. Monolayer coating was found to give better chromatographic performance. In addition, monolayer coating was stable over 8000 column volumes of eluent.

Modification of pyrolytic graphite

Some studies have demonstrated that the surface of graphite can be oxidized to form carboxylic and hydroxilic groups therefore the surface will be more polar (Aviram et al., 1995). Chemical modification of the surface of highly oriented pyrolytic graphite (HOPG) has been carried out by oxidation with acetic acid and CrO_3 (Aviram et al., 1995). Further chemical modification included reacting the oxidized surface with $(CH_3)_2$-N-$(CH_2)_{21}$-CH_3). Auger electon spectra showed that the signal due to oxygen first increased due to oxidation, and then after 30 min of surface treatment did not change.

Modification of carbon fibers

In the early development of carbon fibers, the adhesion between the fibers and matrix resin was poor. This is the reason why many commercial carbon fibers are available in surface-treated form. Coating or sizing is sometimes applied after fiber surface treatment. Epoxy without a hardener is often used as sizing for the epoxy polymer matrix to improve handling of the tow and to decrease surface damage of the fibers during manufacturing of the composite (Hage et al., 1997).

Different surface treatments of carbon fibers have been found to be efficient because they promote the removal of surface contaminants, which may inhibit wetting. Treatment also removes weak boundary layers from the fiber, roughens the fiber surface to increase the contact area, and produces chemically or physically active sites to bond

with the polymer matrix. It has been proven that treatment of the fiber with nitric acid introduces functional groups such as carboxyl and phenolic onto the fibers (Pittman et al., 1997).

Modifications of functional groups at the carbon fiber surface are based on the application of classical reactions of organic chemistry. The reactions most commonly used are esterification of strongly acidic carboxylic groups, and selective or non-selective reduction of carbonyl and carboxylic groups.

In the early-1970s, carbon fibers were used for the adsorption of gaseous compounds. Researchers combined the knowledge of carbon fibers and activated carbon to develop activated carbon fibers. As a result, activated carbon fibers were used in adsorption of SO_2/NO_x and volatile organic compounds from air, in addition to catalysis. Activated carbon fibers provide a faster rate of adsorption, high adsorption capacity, and faster fluid transport to and from micropores and mesopores which improves their regeneration (Burchell, 1999).

Zielke et al., 1996 modified the surface of carbon fibers by reaction with methanol/HCl (esterification) and selective reduction of carbonyl groups with $NaBH_4$ and non-selective reduction with $LiAlH_4$. Studies by X-ray photoelectron spectroscopy showed that the conversion of carboxyl groups to esters was incomplete and selective and non-selective reduction were also incomplete. However, it has been reported that BET-measurements showed both increased surface area and porosity of the surface oxidized fibers.

Lee et al. (1997) reported oxidation of the PAN-based carbon fibers. Sizing was removed by heating the fibers at 300°C for 4 h whereas heating at 700°C and 950°C

oxidation of the surface was accomplished. Morphological changes were not observed by scanning electron microscopy at 5000 magnification, however they were observed by atomic force microscopy (AFM). More importantly, changes in porosity and surface are were observed. The oxidation with air followed by oxidation in an inert atmosphere increased the surface area by 20 fold (from 1.1 m^2 g to 20 m^2 g).

Pittman et al. (1997) carried out oxidation of PAN-based carbon fibers with nitric acid (at different times) followed by reaction with tetraethylenepentamine (TEPA). The quantity of surface bound acidic and basic functions on these modified fiber surfaces was measured by NaOH and HCl uptake experiments. Then, methylene blue and metanil yellow dye adsorption experiments were employed to provide a measure of the surface area of both the surface density and steric availability of the surface acidic and basic functions. It was estimated that 52 % of the acidic groups reacted with TEPA, and 2.6 amino groups were introduced for each acidic group. More relevant for this work, Pittman et al. found that the surface areas increased with increasing the oxidation treatment due to formation of crevasses and pits as shown in Figure (3-4).

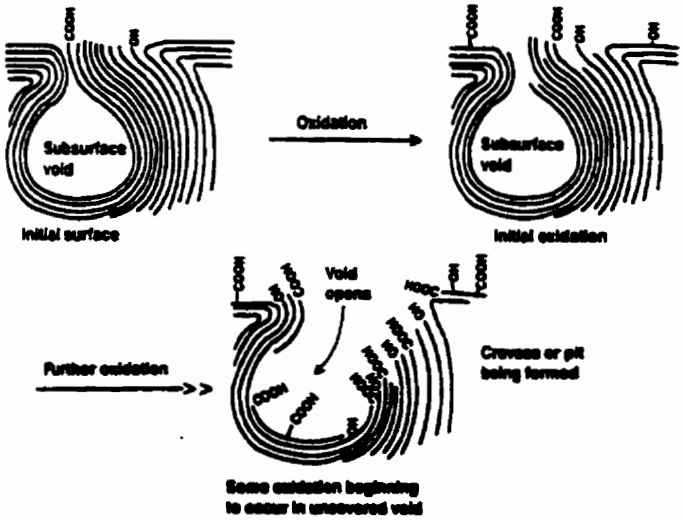


Figure (3-4). Introduction of acidic functions during nitric acid oxidation of carbon fiber surfaces which causes an increase in surface area (Pittman et al., 1997).

As already mentioned, the purpose of surface modification of carbon fibers is to improve the interaction with the resins. Apart from some articles describing the use of activated carbon fibers for adsorption of gases in air, information on the effects of such modification on the adsorption/desorption process was not found, albeit an exhaustive literature search was attempted.

Chapter 4

4 Direct SPME

Chapter 4

4 Direct SPME

4.1 Application to a nonionic surfactant

4.1.1 Introduction

Nonionic surfactants are those which do not have a charged functional group. The most common nonionic groups are hydroxyl (ROH) and ether (R-O-R'). The water solubilizing properties of a hydroxyl group or an ether groups are low compared with sulfate or sulfonate groups. Surfactants showing desirable properties are therefore obtained by using multiple hydroxyl groups or multiple ether groups. After anionic, nonionic surfactants are the most important. Among nonionic compounds, those having one or more polyoxyethylene groups are the most widely used. The length of the chain makes the compounds more or less water soluble.

The problem with the synthesis is the polydispersity and formation of undesired products (Miszkiewicz et al., 1996), surfactants can therefore be complex.

The products therefore contain several homologues with different polyoxyethylene chain lengths (Scullion et al., 1996). Moreover, hydrophobic reagents, e.g. alcohols or alkylphenols, can consist of several components. The distribution of molecular weights depends on the catalyst used and conditions of ethoxylation. In most

cases, the products contain very small quantities of the starting alcohol, but the distribution of the higher ethoxylates approaches a Poisson distribution.

The most important nonionic surfactant is described as the alkylphenol polyethoxylate (APEO). An estimated 500 000 tones per year of APEOs are currently used in the US, Europe and Japan (Renner, 1997). However, due to their poor biodegrability, their use will be phased out by the end of 2000 (Kiewiet et al., 1996). As a result, in 1995 there was a voluntary ban in northern Europe, and APEOs were replaced in all household products by alcohol ethoxylates (AE). In the future, AE will be, by far, the most important nonionic surfactant used (Figure 4-1). AEs are highly biodegradable and their use in Germany is estimated to be 50 000 tones per year. Despite the restriction in the use of APEO in domestic detergents, they may still be used in industrial products (Matthijs et al., 1991).

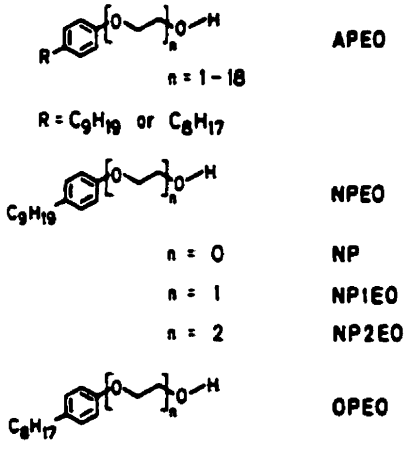


Figure (4-1). Structures of different nonionic surfactants, Alkylphenol polyethoxylates (APEO), Nonylphenol polyehtoxylates (NPEO), octylphenol polyethoxylates (OPEO) (Marcomini et al., 1987).

Detection of nonylphenol and its lower ethoxylates (NPEO) in treated wastewater sludges, river waters and sediments in Europe led to their removal from cleaning products and to the publication of a Chemical Hazard information Profile by the U.S Environmental Protection Agency (Naylor et al., 1992). Moreover, in 1984 it was discovered that APEO breakdown products are weakly estrogenic (Renner, 1997).

Although NPEO have been found in environmental samples, their concentration varied over a wide range (Naylor et al., 1992). Data reported range from 0.64-16 μg/L in rivers and up to 900 μg/L in sediments. Concentrations of AE in untreated municipal sewage are in the 0.5-5 mg/L concentration range and in wastewater after activated sludge treatment in the range 0.015-0.040 mg/L (Marcomini et al., 1996). Therefore, the analytical methods for analysis of surfactants in water must have detections limit of the order of 10 to 100 μg/L.

4.1.2 Determination of nonionic surfactants

Separation of surfactants from matrixes can be achieved by different methods, such as solvent sublation, liquid-liquid extraction, solid phase extraction, precipitation and dialysis.

In solvent sublation, the surfactant is transferred from the aqueous phase to constant flow of a bubbled innert gas that rises to an organic phase (ethyl acetate) above the aqueous solution. The solvent is then collected and concentrated for further analysis. The major disadvantage is the sample size required (4 L) and time of bubbling (3h).

Because it is easy to perform, liquid-liquid extraction is the most commonly used method. It has unavoidable drawbacks, such as the large amount of solvents required,

and formation of emulsions. Moreover, fatty acids are coextracted; thus extra cleaning steps must be included (Schmitt et al., 1990).

Solid phase extraction includes the use of activated charcoal and cation exchange resins. Extraction using C_{18} disks have also been reported, but they are not selective and elution of the surfactant is difficult (Schmitt et al., 1990).

Separation and quantification of surfactants are achieved by GC, HPLC and CE. The major limitation for GC is the lack of volatility of the surfactants. This problem can be overcome by transforming the alcohol moiety to its methyl ester. The drawback is that the instrument has to be run at higher temperatures that may cleave the molecule. Some compounds may even decompose at these higher temperatures. The main advantage is that GC separates on the basis of the hydrocarbon chain length and ethoxy chain length.

The major advantage of HPLC is its ability to separate and quantitate the various homologues and oligomers by length of the alkyl and ethoxylate chains. Reverse-phase HPLC provides information about the alkyl chain length whereas normal-phase resolves the ethoxylate oligomers (Figure 4-2).

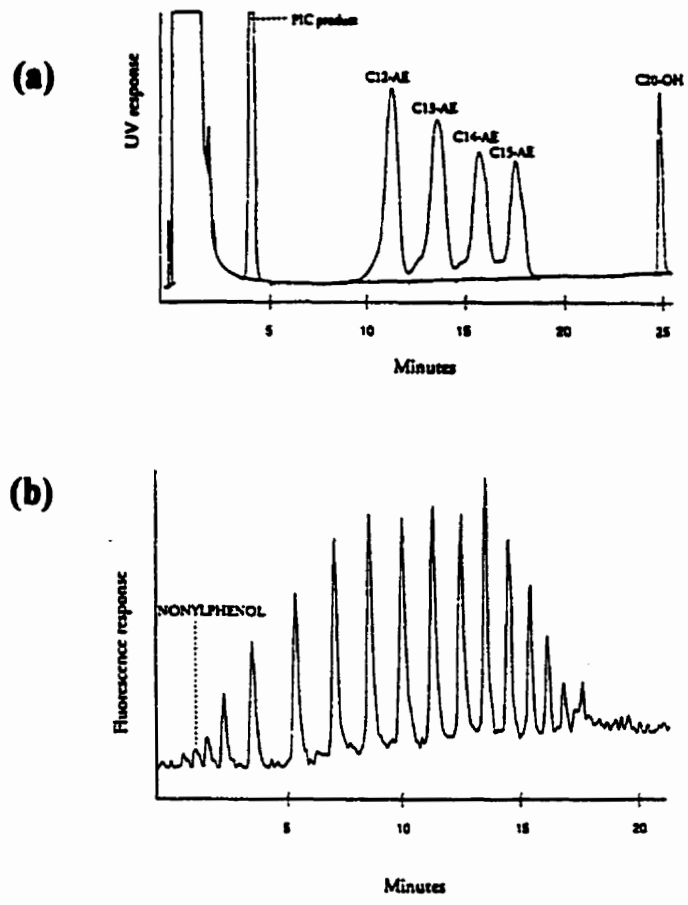


Figure (4-2). (a) Reversed-phase chromatographic separtion of alkyl ethoxylate homologues and (b) Normal-phase chromatographic separtaion of APE oligomers (Kiewiet et al., 1996).

The sequence of elution on normal-phase depends on the length of the polyethylene chain; retention time increases with the length of the chain. Interactions between the column packing and alkylaryl and alkyl groups of ethoxylated alkylphenols, alcohols and acids are all much less important. All oligomers with the same polyethylene chain length have similar retention times under the same conditions, irrespective of the length of the alkyl and alkylaryl chain, i.e. p-nonyl and p-octylphenol are eluted as a single peak (Miszkiewicz et al., 1996).

A method described by Scullion et al. (1996) utilizes a trimethylsilyl column to provide separation of anionic (alkylbenzene sulphonates) and nonionic (alkyl phenol) surfactants. The mobile phase composition was water/acetonitrile with an ammonium acetate buffer, detection was done with fluorescence. The method was applied to the analysis of surface water, and some matrix interference was observed. Desbèn et al. (1996) obtained separation of AE surfactants on a C_8 column using a mobile phase of acetonitrile/water and refractometer detection.

4.1.3 Research Objectives

Solid phase microextraction (SPME) has become an important sample preparation technique mainly for the extraction of organic compounds from aqueous, air and solid samples (Eister et al., 1997). The advent of new fibers has increased the scope of SPME not only in analysis by GC, but also by CE (Eister et al., 1997) and HPLC (Eister et al., 1997, Chen et al., 1995, Boyd-Boland et al., 1996, Aranda et al., 1998). Boyd-Boland et al. (1996) described a method to determined nonionic surfactant using SPME-HPLC-UV detection.

The use of SPME/HPLC, however, has been limited due to the poor chemical resistance of commercially available fibers, carryover of the analyte from one analysis to the next, and stripping of the coating. **Therefore a new solid phase is required.** Graphite could be a suitable candidate since it provides a high surface area, high adsorption capacity and, most importantly, high chemical resistance.

The use of pencil leads as sorbents for SPME-GC analysis was first reported for the determination of lindane, methyl parathion and 2-chlorophenol (Wan et al., 1994). The authors achieved detection limits of parts per trillion, but carryover from one analysis

to the next was reported. A different approach consisting of electrodeposition of diamines onto the pencil leads followed by GC analysis with detection limits of the order of parts per billion was reported by Conte et al. (1996).

Glassy carbon has been mainly used as a material for working electrodes in electrochemistry. Because of its mechanical and chemical stability, and its high surface area, it has been used as a stationary phase for HPLC (Knox et al., 1996) and supercritical fluid chromatography (Larkins et al., 1993, Rittenhouse et al., 1996).

Polycrystalline graphites have not been used for SPME/HPLC. The aim of this work was to develop and study the applicability of two polycrystalline graphites (pencil lead and glassy carbon rod) as sorbents for SPME of a nonionic surfactant. Among the nonionic surfactants, alkylphenol ethoxylates (represented in this work by Triton X-100) are the most important and extensively used (Figure 4-1).

4.2 Experimental

4.2.1 Analytical system

The HPLC system consisted of a Varian 9050 autosampler connected in series to a six port Valco valve (Figure 4-3). The sample loop in the valve was replaced by the SPME/HPLC interface (Supelco, Bellefonte, USA). A pump was used to deliver methanol into the SPME/HPLC interface for static desorption experiments (Aranda et al., 1999). The mobile phase was delivered by a gradient pump (Varian 9010) to an ODS-Zorbax (250 mm x 4.6 mm x 5 μm particle size) column and a ODS-Zorbax guard column (4.6 mm x 1.25 cm). The mobile phase was composed of 60% A and 40% B. (A: methanol and B: 30:70 water:acetonitrile) at a total flow rate of 2.0 mL/min. Detection

(Linear Instruments Co. LC 304 fluorescence detector) was done at λ_{ex} = 230 nm and λ_{em} = 310 nm. Standard solutions in methanol were automatically injected (20 μL) by means of the autosampler into the HPLC column.

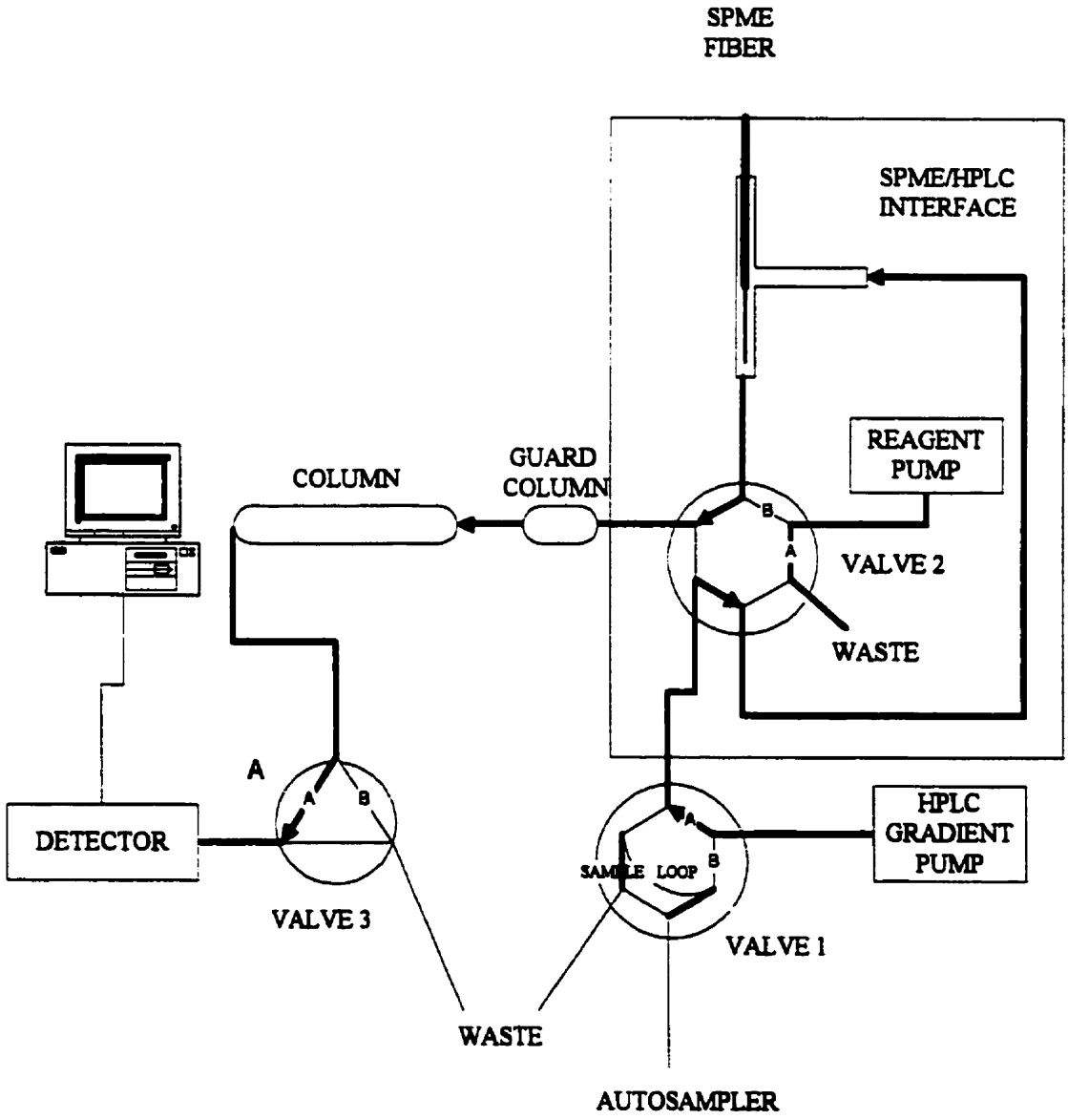


Figure (4-3). Schematic diagram of the SPME-HPLC system used in this study.

4.2.2 Solid supports

Polycrystalline graphites

Pencil leads (60 mm x 0.5 mm), H grade, were obtained from Pentel (Japan). These were used as received (PLU) or after coating with graphite by carbon evaporative coating (ca. 5 nm thickness) (PLC). Both PLU and PLC were conditioned prior to analysis by immersion in acetone for 1 h. The optimum conditioning time was found by performing blanks after this procedure. This was carried out by static desorption at the interface with methanol.

Glassy carbon rods (GCar) (50 mm x 1 mm) were obtained from Alfa ÆSAR (Ward Hill, MA, USA). They were used as received (GCU), or after a light polishing between extractions with 1000 grit sandpaper followed by sonication in deionized water for 10 min (GCP). Both were cleaned by immersion in acetone for 20 min. The conditioning time was obtained by performing blanks, it was found that 20 min was optimum as compared to 60 min for pencil lead.

SPME Fibers

SPME fibers, 50 μm Carbowax/TR-100 and 60 μm Polydimethylsiloxane-divinylbenzene (PDMS/DVB) were obtained from Supelco (Bellefonte, USA). These fibers were conditioned by immersing them in acetonitrile with stirring for 1 h, followed by methanol for 1 h. The optimum times for conditioning the fiber were found by performing a dynamic desorption at the SPME/HPLC interface.

4.2.3 Reagents

Triton X-100 was obtained from Aldrich Chemical Co. (Milwaukee, WI, USA) and used as received. All solvents used were Optima grade (Caledon Laboratories, ON, Canada). A stock standard solution was prepared in methanol (500 mg/L) and diluted to obtain concentrations in the range of 0.010 to 10 mg/L which were used to calibrate the response of the detector. Aqueous model solutions were prepared by serial dilution from a stock solution containing 100 mg/L of Triton X-100 in deionized water (Milli-Q Plus water purification system, 18MΩ).

4.2.4 Procedure

Determination of Triton X-100 using SPME fibers

Extraction. Six mL aliquots of aqueous solutions with Triton X-100 concentrations from 0.005 to 0.150 mg/L were transferred to 7 mL vials sealed with Teflon lined septa, which could be pierced by the needle of the SPME device. The solution was vigorously mixed with a magnetic stirrer, the speed of which was kept constant for all extractions. Extractions were performed at room temperature (23 ± 2°C) for 60 min.

Desorption. After extraction of the analyte from the aqueous sample, the SPME device was transferred to the interface, the clamp was closed and the system was sealed (Figure 4-4a). Valve 1 (Figure 4-3) was switched to the inject position for two minutes to perform a dynamic desorption.

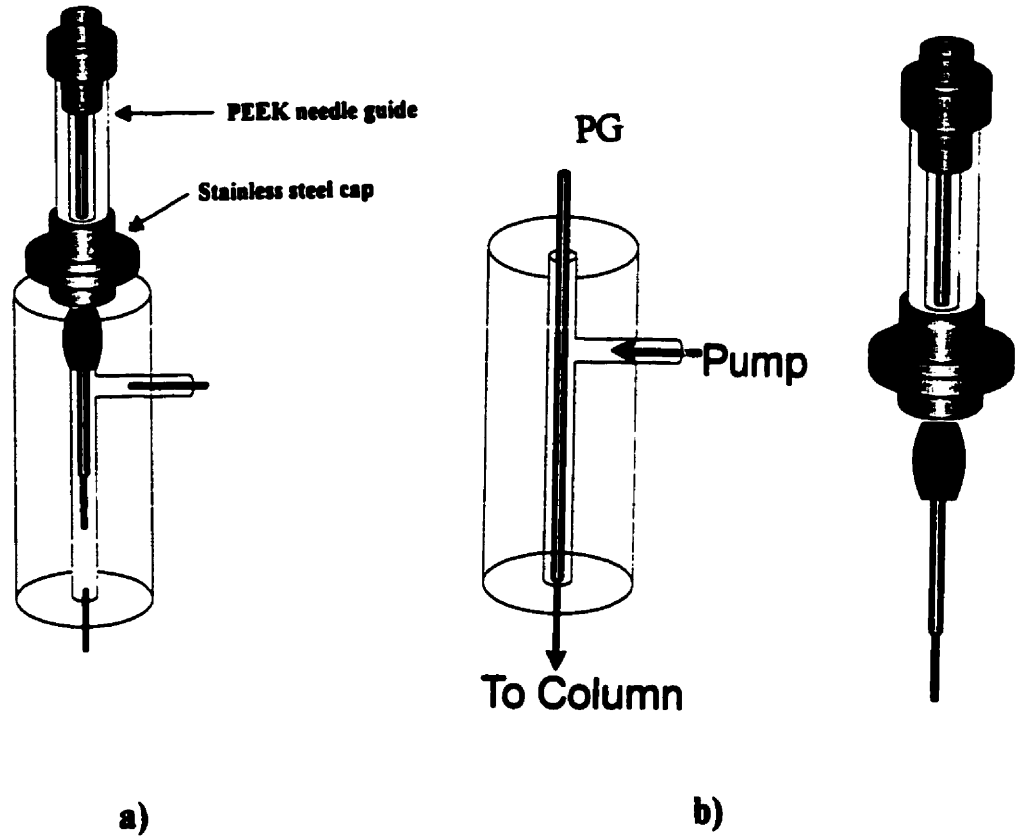


Figure (4-4). (a) Cross sectional diagram of the SPME/HPLC interface using a conventional SPME fiber, (b) use of the interface for graphite rods.

Determination of Triton X-100 using polycrystalline graphites

Extraction. Six mL aliquots of aqueous samples containing Triton X-100, at concentrations from 0.005 to 0.150 mg/L, were transferred to 7 mL vials without caps. PL and GCar rods were mounted in a pencil holder (Figure 4-5) allowing at least 4 cm of the rod to be exposed to the environment (solution headspace); however, only 1.0 ± 0.1 cm length of the carbon rod was immersed into the stirred solution. Extractions were performed at room temperature (23 ± 2°C) for 1h.

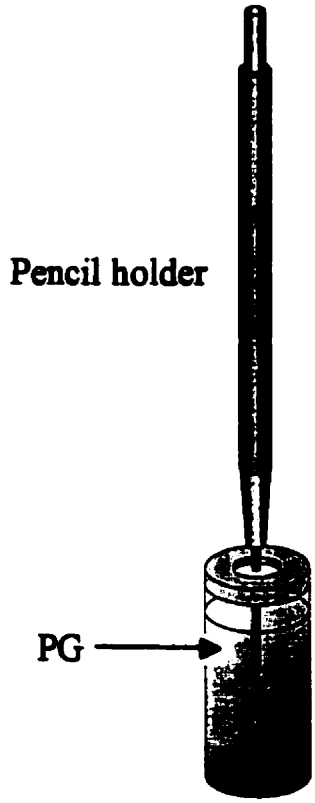


Figure (4-5). SPME device for extraction using glassy carbon and pencil lead.

Desorption. Desorption took place in the static mode in the SPME/HPLC interface. The system was first sealed as above, using an SPME fiber without a coating, to allow filling of the interface with methanol. This was done just prior to the end of the extraction time (Figure 4-4a). The presence of the fiber during filling ensured that when the carbon rod was inserted in its place, the volume of methanol was sufficient to fill the interface without any overflow. The stainless steel cap, PEEK needle guide and SPME fiber were then withdrawn together (Figure 4-4b). The carbon rod with adsorbed analyte was transferred into the interface. Approximately 4 cm of the rod, including the 1-cm

exposed to the aqueous solution, was then inside the body of the interface. After allowing time for desorption, the rod was withdrawn, the system sealed as above, and the mobile phase then flushed the contents of the desorption chamber onto the HPLC column.

4.2.5 Scanning Electron Microscopy

The scanning electron micrographs of the surfaces of the carbon rods were obtained using a JSM 6400 system (JEOL, Japan).

4.3 Results for Triton X-100

A typical chromatogram is shown in Figure (4-6). In this study, a C_{18} column was used. It was found that the best chromatograms were obtained using a mobile phase composed of methanol/acetonitrile:water composition, as peak tailing was then small.

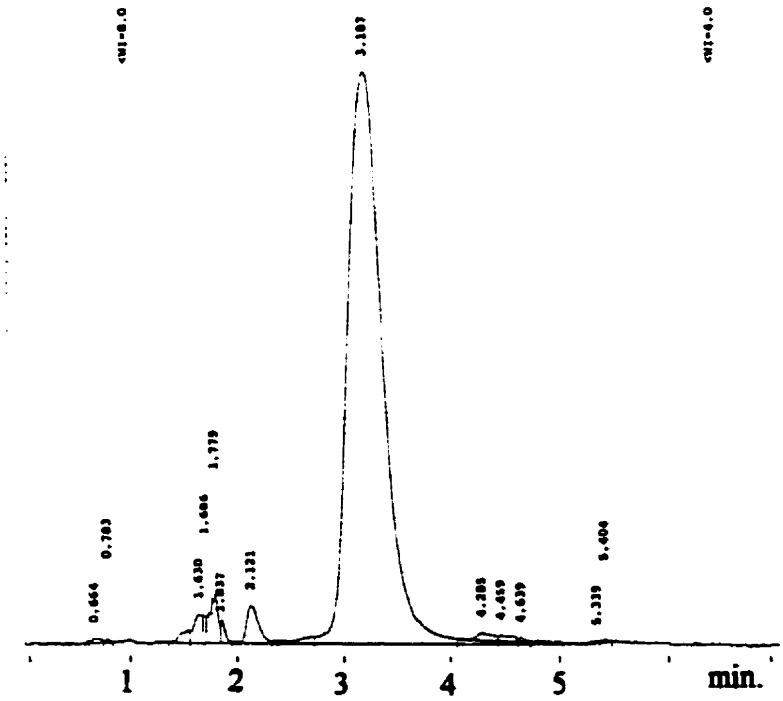


Figure (4-6). Typical chromatogram of Triton X-100.

4.3.1 Optimization and evaluation of solid supports

Preliminary experiments were performed at room temperature with aqueous solutions containing 0.100 mg/L of Triton X-100 using a 60 min extraction and 60 min static desorption with methanol.

Pencil Lead

Experiments performed using a single PLU rod showed poor reproducibility (74 % RSD, n = 7). The amount of Triton X-100 detected decreased with the number of extractions performed with a single rod, which indicates incomplete desorption and consequently saturation of the surface. The results are illustrated in Figure (4-7), which is a plot of the amount detected vs. the number of consecutive extractions using 5 different pencil leads. Using a single pencil lead, the amount of Triton X-100 decreased by 5 fold after 7 extractions. However, for the first extraction using 5 different PLU, better precision was attained (13 % RSD, n = 5). The results suggest that the composition of different pencil leads is consistent; therefor a new pencil lead could be used for each sample.

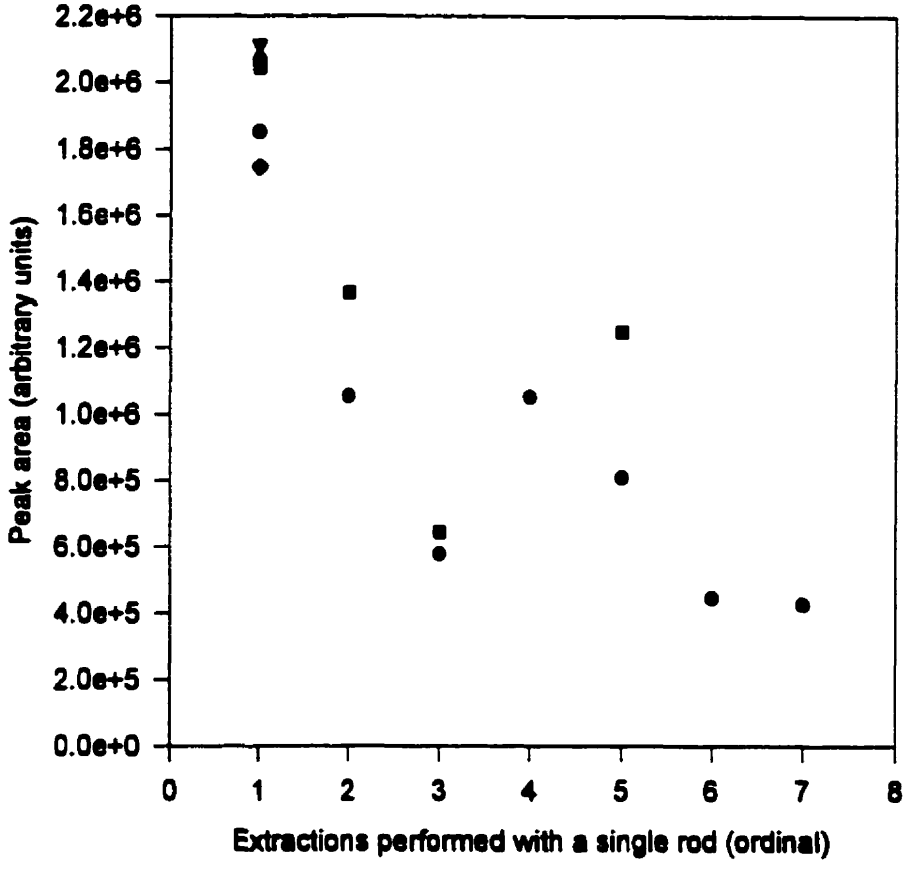


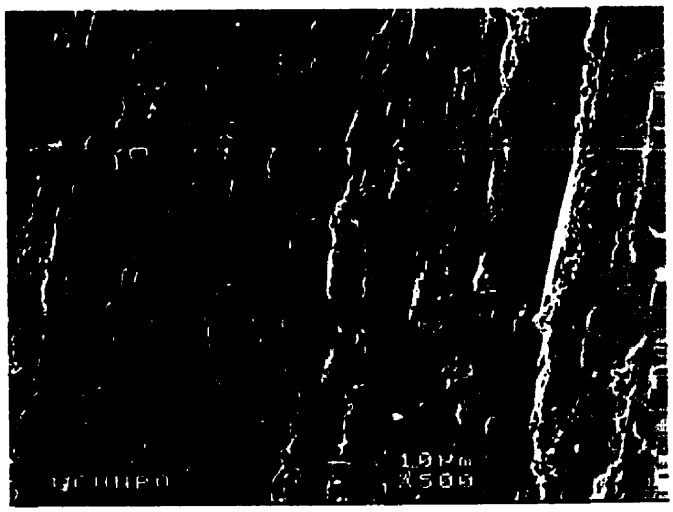
Figure (4-7). Peak area of Triton X-100 using a single PLU rod for successive extrtactions.

Pencil lead is a mixture of fine graphite powder and resins. Because its exact composition is unknown and in order to provide a more homogeneous surface, pencil lead rods were coated with graphite by carbon evaporative coating (ca. 5 nm thickness). Significantly better precision (4 % RSD, n = 5) was obtained using these carbon coated rods. This suggests that the carbon coating produces a homogeneous layer of graphite surface allowing for a better adsorption-desorption process.

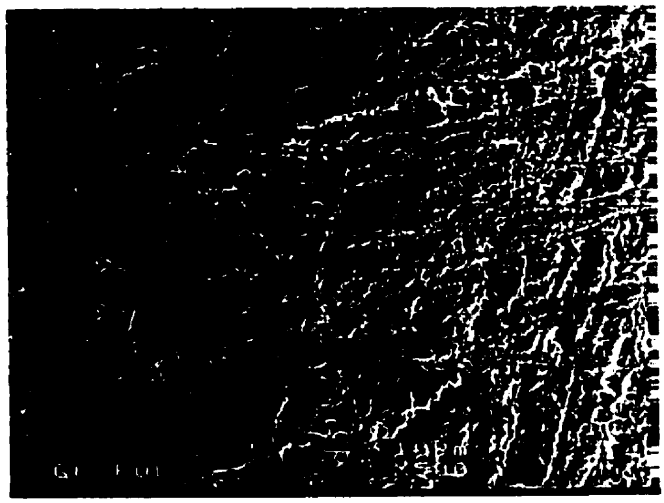
Glassy Carbon

The results obtained using PL coated with graphite gave better results, mainly due to the homogeneity of the surface, it was of our interest to find a better surface for this purpose, therefore glassy carbon was chosen. Properties such as high strength, high resistance to chemical attack and low helium permeability make it a good candidate. Moreover, glassy carbon has been used in electrochemistry as working electrode with a high versatility. It has low density and a uniform structure which is generally free of defects (Pierson, 1993) providing a homogeneous surface.

The precision obtained by using the same GCU for successive extractions was 16 % RSD (n = 4). Greater adsorption was expected using GCU because of its larger geometrical surface area (32.2 vs. 15.9 mm^2). However, this was not the case. This may be due to the apparently low porosity of the GCU surface as evidenced by the electron micrograph in Figure (4-8a). The glassy carbon surface was then sanded followed by cleaning in ultrasound to detach any loose particles, producing polished glassy carbon rods. Sanding of the surface likely increased the surface area (see the relatively porous surface shown in Figure (4-8b)). When this was done between successive extractions, it provided a fresh surface free of the analyte.



(a)



(b)

Figure (4-8). Scanning Electron Micrograph of (a) glassy carbon rod as received (GCU), and (b) glassy carbon rod after sanding (GCP), both at 500x magnification.

The time of extraction profile (from 1 to 90 min) was obtained for GCP using Triton X-100 at 0.100 mg/L with a 60 min desorption time with methanol (Figure 4-9). It was observed that analyte was being extracted even after 1.5 hours of exposure. A time of 60 min was selected as an optimum adsorption time for the remainder of the experiments. Further discussion on adsorption and desorption is provided in Chapter 6.

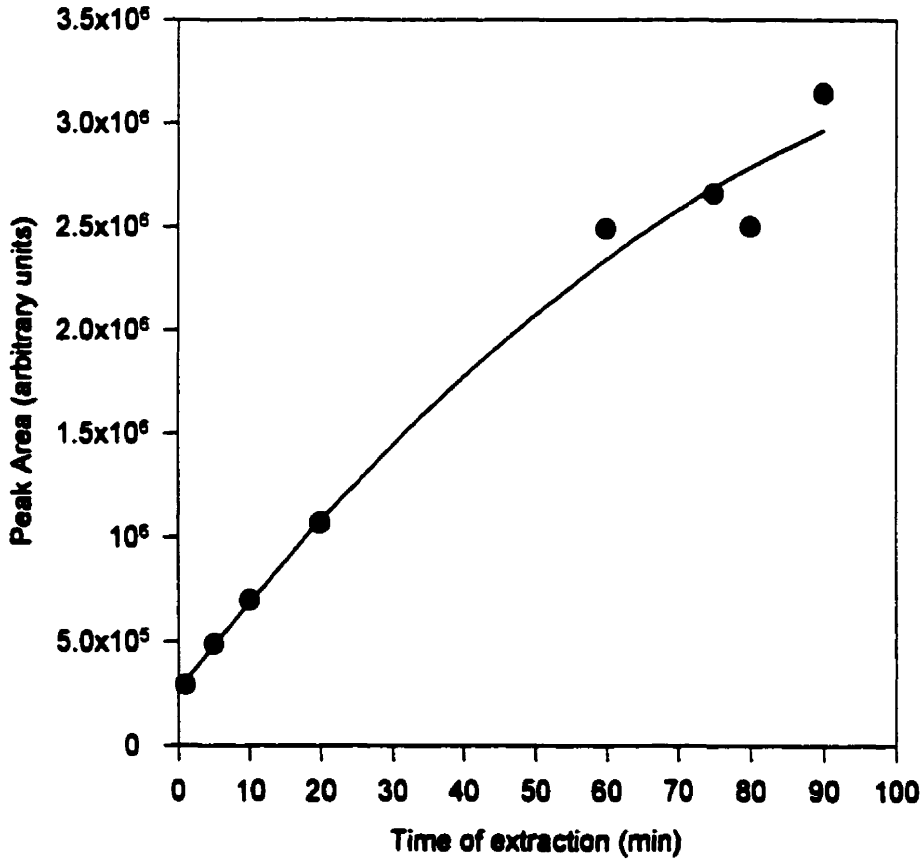


Figure (4-9). Peak area of Triton X-100 versus time of extraction on a glassy carbon rod.

4.3.2 Desorption

Desorption from SPME fibers

Efficient desorption is of paramount importance since it affects the accuracy of the results and sensitivity of the method.

A problem of carryover (up to 10%) has been reported in the literature (Boyd-Boland et al., 1996, Möder et al., 1999). Desorption from the SPME fibers was done dynamically as outlined above. A second desorption showed that carryover was present (6 % of the amount obtained in the first desorption). Therefore the fibers had to be cleaned by immersing them in methanol for 30 min prior to the next extraction. Blanks between extractions then showed no carryover of Triton X-100.

Although the carryover (6%) was measured at one concentration, the excellent linear dynamic range (Figure A1-2, Appendix 1) indicates that carryover is not a problem, as long as the cleaning procedure between successive runs is used. Swelling of the polymeric coating of SPME fibers frequently led to stripping of the coating from the silica fiber when the assembly was withdrawn through the double sided ferrule.

Desorption from polycrystalline graphite

As discussed previously, static desorption failed to completely desorb Triton X-100 from PLU and GCar even after a 60 min desorption time. Data plotted in Figure (4-10) shows that the amount desorbed increases rapidly with time up to 60 min, and possibly decreases after 60 min. It was observed that methanol in the interface is lost by capillary action and evaporation which likely caused some analyte to be readsorbed in dry areas. Sealing the system could solve this problem.

After cleaning PLU and GCU rods with methanol for 30 min, the rods were placed in the SPME/HPLC interface and small amounts of Triton were still detected. Using a new rod for each extraction can solve this problem. This is feasible because of their low cost and good reproducibility. In the case of GCU, carryover can be overcome by sanding the surface. Blanks were performed between extractions using these GCar and no analyte was found.

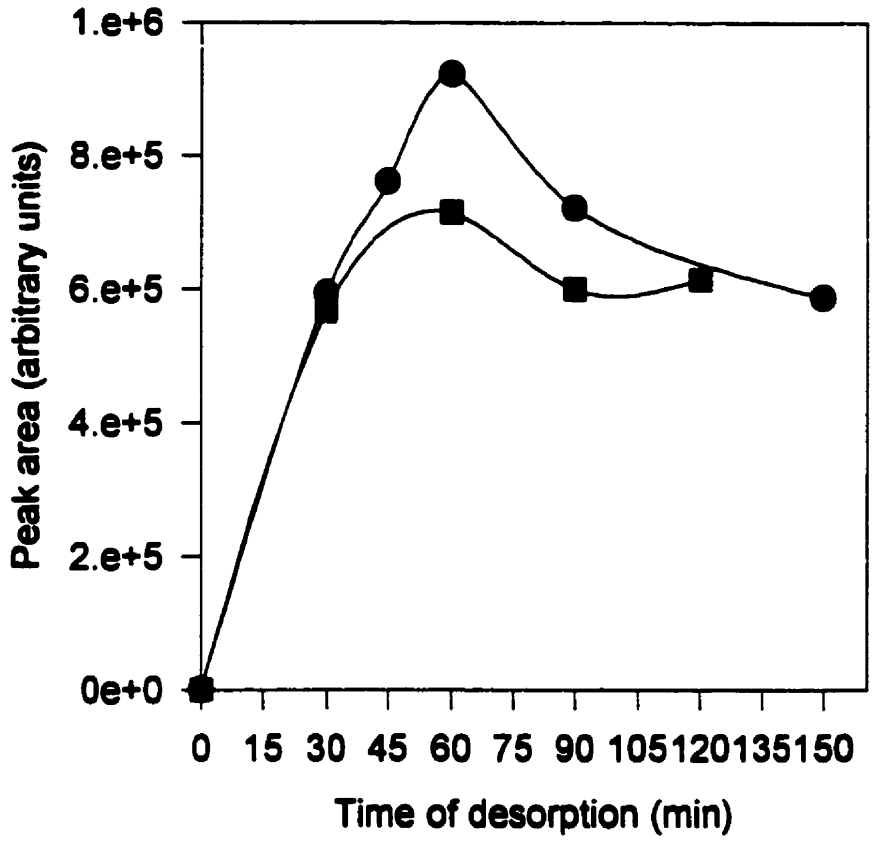


Figure (4-10). Desorption profile of Triton X-100 from pencil leads. Static desorption using methanol.

4.3.3 Assessment of polycrystalline graphites as sorbents for analytical purposes by comparing their performance with SPME fibers

In order to obtain the analytical figures of merit, the extraction time was set to 60 min and desorption with methanol to 60 min.

The results obtained for each type of SPME fiber (Carbowax and PDMS/DVB) and polycrystalline graphite (PLU, PLC, GCU and GCP) are shown in Table (4-1). The

analytical procedure for each solid support is presented in Table (4-2). The results are compared to those obtained by Boyd-Boland (1996); the experimental conditions differ from our experiments only in the separation column and detection system used.

Limit of detection (LOD) is defined as the minimum concentration of analyte that gives a signal statistically different from the background noise, usually given as a pre-set confidence interval. The LOD in this work is defined as the minimum concentration of analyte that can be extracted and detected whose signal is five times larger than the baseline noise.

Recapitulating, the use of a single PLU rod for calibration and analysis was not suitable because of carryover. The LOD using a single PLU was 5 μg/L. Because the exact composition of PLU is unknown, and in order to provide a homogeneous surface, PLU rods were coated with a 5 μm thick graphite layer (PLC). The precision improved to 4% RSD and the detection limit by one order of magnitude to 0.50 μg/L. Improved detectability is attributed to more efficient desorption, since there was no carryover when using PLC. It is believed that coating the pencil lead provides a more homogeneous surface.

The LOD for GCU was 50 μg/L. It was improved by two orders of magnitude to 0.50 μg/L when the surface was sanded (GCP). This is attributed mainly to the increase of the surface area since sanding roughens the surface (see Figure 4-8). The precision was essentially unaffected by the roughening.

Also presented in Table (4-1) are data from the use of conventional SPME fibers. Using a Carbowax/TR fiber resulted in a detection limit, linear dynamic range and precision that are no better than those obtained using the carbon rods. Our results are

similar to those obtained by Boyd-Boland et al. who reported limit of detection of 1.57 μg/L, and a linear dynamic range of three orders of magnitude.

Table (4-1). Figures of merit of the analysis method of Triton X-100 using different solid supports: pencil lead uncoated (PLU), coated PLC, glassy carbon untreated (GCU), sanded (GCP) and SPME fibers.

	LOD (μg/L)	LDR (μg/L)	RSD (%)[1]
Uncoated (PLU), one rod use repeatedly	5	ND	74
Uncoated (PLU) new rod use for each extraction	5	ND	13
Coated (PLC)	0.50	0.50 – 150	4
GCU	50	50-	16
GCP	0.50	0.50-150	14
PDMS/DVB fiber	0.50	0.50-100	13
Carbowax fiber	0.50	0.50-100	15
PDMS/DBV and Carbowax[2]	1.57	100-100000	2-15

[1]At least 4 replicates at 100 μg/L of Triton X-100 in water
[2]Boyd-Boland et al., 1996

Table (4-2). Experimental conditions used during the acquisition of the analytical figures of merit.

	Extraction time (min)	Desorption mode	Column	Detection
Pencil lead	60	Static with methanol, 60 min	C_{18}	Fluorescence
Glassy carbon	60	Static with methanol, 60 min	C_{18}	Fluorescence
SPME in this study	60	Dynamic with mobile phase, 2 min	C_{18}	Fluorescence
SPME (Boyd-Boland et al.)	60	Dynamic with mobile phase, 1 min	Normal phase	UV (220nm)

4.3.4 Summary for Triton X-100

The performances of two polycrystalline graphites were assessed for analysis of a nonionic surfactant. Preliminary results showed that pencil leads and glassy carbon performed as well as SPME fibers (PDMS/DVB and Carbowax/TR) in terms of limit of detection, linear dynamic range and precision. However, a longer desorption time was necessary using the carbon rods. Carryover was present in all the sorbents; this can affect the limit of detection and reproducibility. Problems with both were overcome by using a new pencil lead for each extraction or by sanding the surface of glassy carbon rods between extractions (which allows for about 100 extractions per rod). We have found the advantages of carbon, notably its chemical resistance and low cost, outweigh these disadvantages. It is believed that polycrystalline graphites can be more versatile since any derivatization reagent could be sorbed onto their surface (more on this in Chapter 5). This would permit the analysis of compounds that require derivatization prior to detection such as alcohols and amines.

4.4 Application to pentachlorophenol

4.4.1 Introduction

For evaluation of the performance of polycrystalline graphites, Triton X-100 was selected to represent the nonionic surfactant class. As a surfactant, Triton X-100 has a hydrophilic and hydrophobic moiety, which confer special properties to the molecule. As a result, unique properties at interfaces are observed, e.g. at liquid-solid interfaces. In addition, it was observed that glassy carbon gave the best results in terms of detection limit and linear dynamic range. Moreover, about 100 extractions can be performed using a single glassy carbon rod.

It was therefore interesting to assess glassy carbon's applicability for SPME-HPLC for other analytes. Pentachlorophenol (PCP) was chosen as target analyte for various reasons. PCP is polar and thus more difficult to extract from water than many other analytes, it is still an important analyte environmentally, and it is a weak acid.

In addition, in order to enhance extraction efficiency the sample must be acidified. It has been reported that commercially fibers are not resistant to low pH or even to acidic vapours (Aikawa et al., 1996).

Pentachlorophenol

Chlorinated phenols (CP's) are widespread in the environment. Those with three or more chlorine atoms have been used as pesticides and as synthesis intermediates of other pesticides and dyes. PCP is used to control mold, mildew and termites in wood, and it has also been used as a herbicide in pineapple and sugar cane fields. Other

important sources of CP's are desinfection plants and, pulp and paper mills where CP's are produced by chlorination of lignin.

Although the use of PCP has been restricted in Canada since 1993, the amount of PCP released in the effluents from pulp and paper sectors in 1993 was about 32 kg/year. The concentrations in the effluents were less than 10 times the Provincial Water Quality Objective and the highest concentration found was 3μg/L (Socha et al., 1993).

Nowadays CP's are of concern because of the direct effects on aquatic ecosystems and the indirect effects on human beings and animals as a result of their entry into the food chain. Chlorophenols photodecompose and are degraded by soil bacteria. An important aspect is the evaluation of the fate of the chlorophenols in the environment and their uptake into plants as well as their accumulation and metabolism (Roy et al., 1994). Fruits, vegetables and grains contaminated with PCP account for 99.8% of the exposure in non occupational persons.

In 1993, the Ontario Ministry of Environment and Energy released a list of candidate substances for bans, phase outs or reduction. The compounds to be included in the list were identified based on their presence in Ontario's environment and their potential hazards. The risk involved in the use of these substances was not assessed since exposure data were lacking. The list included PCP, 2,3,4,5-tetrachlorophenol and 2,4,5-trichlorophenol (Socha et al., 1993).

Determination of pentachlorophenol

The current standard methods of phenol analysis in wastewater, such as U.S. Environmental Protection Agency (EPA) method 604 Phenols and the acid-extractable section of EPA method 625m are based on liquid-liquid extractions. They require

extensive cleanup procedures that are time-intensive and involve expensive and hazardous solvents, which are undesirable for health and disposal reasons.

Solid phase extraction of PCP has been achieved using Supelclean ENVI-Chrom P following analysis by GC (Supelco, 1999).

Methods using SPME/GC using polyacrylate fiber (Bulchholz et al., 1994) and SPME-GC-FID using PDMS, PA and CW-DVB fibers (van Doorn et al., 1998) have been also reported.

Humic substances

Organic carbon is composed of both dissolved and particulate forms. Total organic carbon is often calculated as the difference between total carbon and total inorganic carbon. In water, organic carbon is principally composed of humic substances.

Natural waters contain a diversity of dissolved and particulate inorganic and organic constituents. Lakes and rivers have approximately 10 times more dissolved inorganic matter than organic matter, whereas groundwater has 100 times more inorganic matter than organic matter (CWQG, 1987).

Dissolved and particulate matter are operationally separated with the use of a 0.45 μm pre-size filter. Material passing through is considered dissolved, and the remainder particulate. Both of these classes of matter consist of organic (e.g. proteins, fats, carbohydrates and related compounds) and inorganic material (e.g. suspended minerals, rock particles, clays, salts).

Humic substances (HS) represent a significant fraction of the bulk of organic matter in most soils and water. HS contain molecules with large molecular weights (3,000-30,000 daltons). They contain functional groups such as hydroxyl, phenolic and

carboxyl groups conferring to the HS a hydrophilic and acidic character (Figure 4-11). Because of their polyelectrolytic nature, HS have the ability to bind to various inorganic and organic compounds.

HS may be classified into three fractions based on their solubility, which is pH dependent. Structurally they are believed to be similar; however, they differ in molecular weight and functional content. The three fractions are:

- Humic acids, which are soluble in alkaline solutions but precipitate in acidic solution;
- Fulvic acids, which are soluble over the entire pH range due to their lower molecular weight and higher acidity;
- Humin, which is the fraction that is insoluble at all pH levels.

Only recently has the importance of dissolved organic matter been recognized. The distribution of organic compounds in water is governed, in part, by the extent of the interactions between them and the HS. It has been reported that HS (the major part of dissolved organic matter (DOM)) enhance the apparent solubility of non polar compounds (Suffet et al., 1989) modifying their bioavailability and toxicity. Wershaw et al. (Suffet et al., 1989) found that the apparent water solubility of DDT increased more that 200 times in an aqueous solution containing HS. Maguire et al. (1995) reported that DOM could affect the recoveries of organic compounds by LLE and SPE.

HS can also exert a competitive effect on the adsorption of volatile organic compounds on activated carbon during treatment of drinking water, and adversely affect the efficiency of oxidative water treatment process.

The aim of these studies is to assess the performance of glassy carbon as sorbent for SPME of PCP from aqueous samples. Optimization of the method, such as time of

extraction and desorption will be given. Moreover, the developed method will be applied to real samples and model solutions containing fulvic acids (FA) at various concentrations.

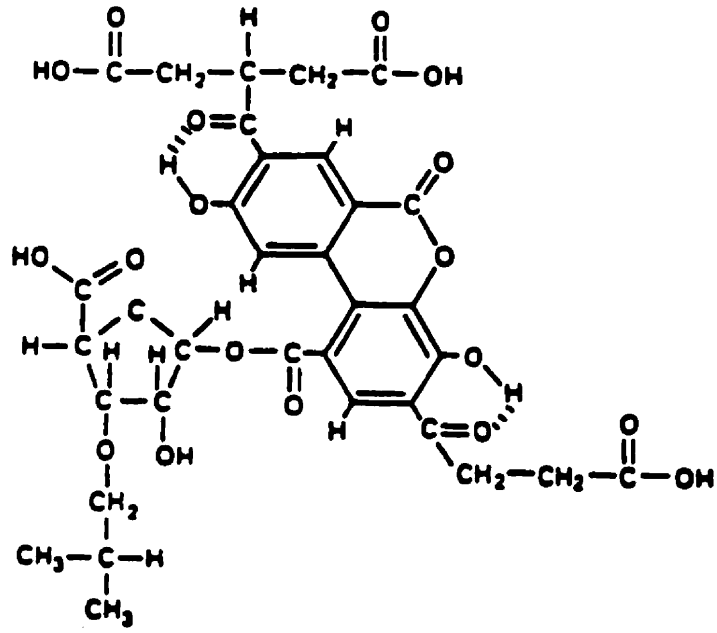


Figure (4-11). Structure of fulvic acids (Stevenson, 1994).

4.4.2 Experimental

Instrumentation

The analytical system described in section 4.2.1 was employed. The mobile phase was composed of 95 %A and 10 % B. (A:methanol and B: 1% acetic acid in water). Total flow rate of 1.5 mL/min. Detection (Variable-wavelength UV-VIS, Varian 9050) was done at 225 nm. Standard solutions in methanol were automatically injected (20 μL) by means of the autosampler into the HPLC column.

Sample preparation

Standard solutions of PCP were prepared in methanol at concentration from 0.05 to 20 mg/L. These standards were used to calibrate the response of the detector and to spike the aqueous solutions. These methanol solutions were prepared at such concentrations that the volume of methanol was constant in all the model solutions. Model solutions were prepared by spiking deionized water (Milli-Q-Plus water purification system, 18.2 MΩ) with PCP to obtain a concentration range of two orders of magnitude. Each solution was acidified to pH 1 using HNO_3.

A 2 L sample of Rideau River surface water was collected form a site at Carleton University using a precleaned Teflon water sampler. This surface water had a pH of 8.0 and dissolved organic carbon of 6.5 mg of C/L (Mandal et al., 1999). Samples of 200 mL were spiked with PCP in methanol to give concentrations from 0.005 to 0.100 mg/L. The solutions were mixed overnight with a magnetic stirrer. Each solution was acidified to pH 1.0 using HNO_3.

The fulvic acid used in this study was obtained from the Department of Water and Environmental Studies in Lipköping, Sweden. Solutions containing 5, 10, 50 and 100 mg/L were prepared by weighing the appropriate amount of FA in 200mL volumetric flask. Each flask was spiked with the proper volume of PCP standard in methanol and mixed for 2 days in the dark.

Solid phase microextractions using glassy carbon

Solid phase microextractions using glassy carbon (GCP) were performed as described in section 4.2.4.2. Methanol was used as a desorption solvent. Previous studies showed incomplete desorption, even after 3 desorption steps. The glassy carbon

rod was therefore placed in an oven at 310°C for 15 min prior to extraction. This treatment was sufficient to clean the rod between extractions.

4.4.3 Results

It has been demonstrated that pH affects the extraction of acidic compounds from water by LLE, SPE, SFE and SPME. For acidic compounds, the partition coefficient, K, between the fiber and water increases as the pH decreases below the value of pKa. of those compounds (Pan et al., 1995, van Doorn, et al., 1998).

Pentachlorophenol has a pKa of 4.35 and is thus relatively acidic. The concentration of the neutral form increases as the pH decreases below the value of pKa. Its solubility, on the other hand, decreases increasing the attraction of PCP to the glassy carbon surface.

Therefore, prior to extraction, the pH of the aqueous PCP solution was lowered to 1.0 with HNO_3. The necessity of this was observed in preliminary experiments in which the amount of PCP extracted was small and the reproducibility was poor when the pH was not lowered.

Although the effect of pH on the extraction efficiency in solvent extraction is well known, the application of low pH to SPME is limited due to the instability of the fibers at low pH. Moreover, deterioration of the fiber is observed even in contact with acidic vapours (Pan, 1996, Aikawa et al., 1997). In addition, the vapours could destroy the epoxy glue, detaching the silica rod from the syringe. In contrast, glassy carbon is quite resistant to low pH and concentrated acids.

Optimization

In the search for the most favorable conditions, optimization extraction time , temperature and desorption time were investigated.

Optimization of extraction time was carried out between 1 to 90 min using an aqueous solution containing 0.10 mg/L of PCP. The exposure time profile is shown in the Figure (4-12) (one value per point). It is observed that the curve levels off after 60 minutes.

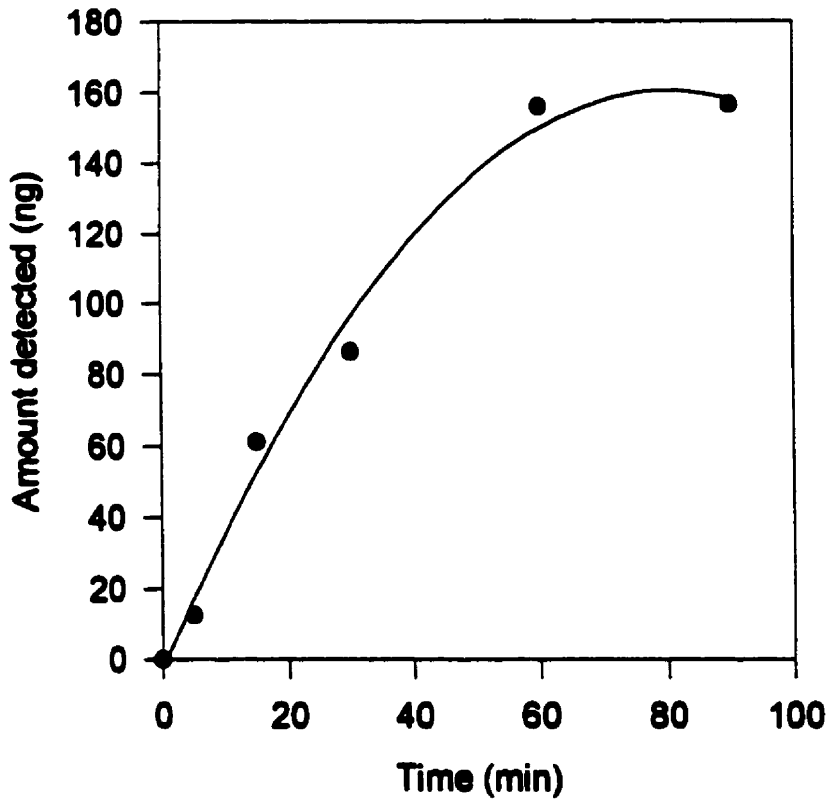


Figure (4-12). Extraction time profiles for 600 ng of pentachlorophenol using glassy carbon polished.

The time of desorption was optimized, after extracting for 60 min from aqueous solutions containing 0.10 mg/L of PCP. The process was repeated in the interface at two different temperatures: 25°C and 38°C, for up to 50 min using methanol. The results, shown in Figure (4-13) illustrate that this change in temperature has no effect after 5 min of desorption. However, at longer times, the amount desorbed at 25°C remains constant, while it increases at higher temperature (38°C). It may be possible that the difference in temperature (13°C) is small to obtain a considerable change in desorption.

Incomplete desorption can be considered a drawback in the use of glassy carbon as SPME solid support, especially if quantitative analysis is being considered. This is discussed further in Chapter 6.

The experiments hereafter were thus carried out under the following experimental conditions: 60 min extraction from a solution acidified to pH 1.0, followed by 5 min static desorption in the interface at room temperature.

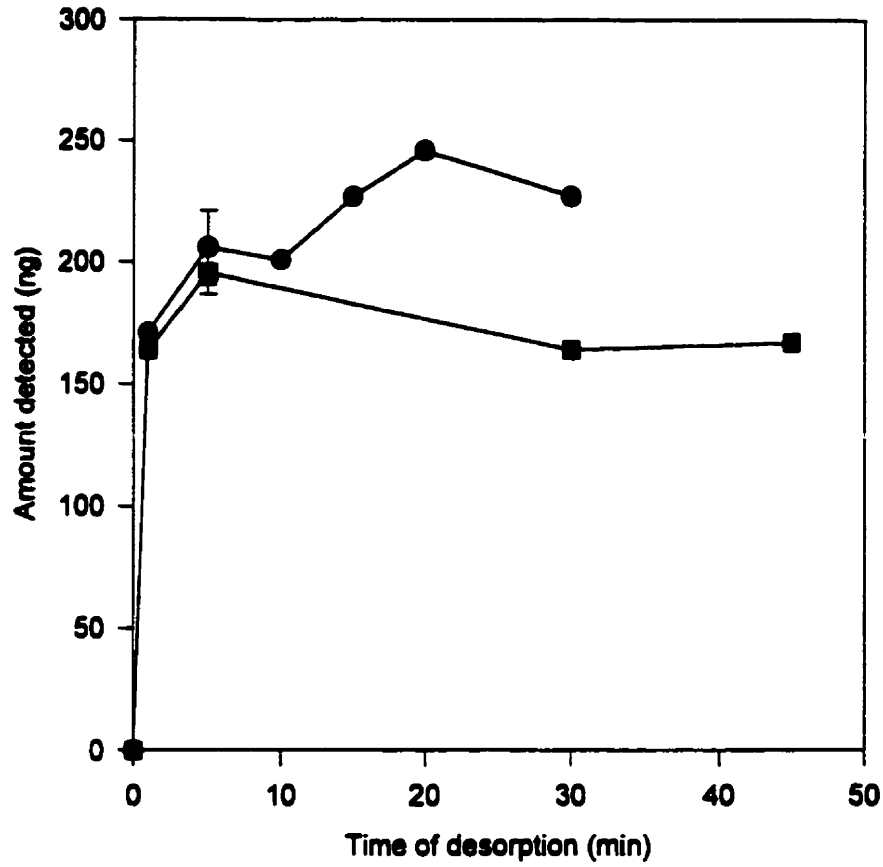


Figure (4-13). Desorption time profiles of pentachlorophenol at 25°C and 38°C. One value per point except for 5 min which shows the mean of 7 replicates and 1 standard deviation.

Application to real samples.

To date, SPME has been successfully applied to simple samples such as model solutions or drinking water samples. For more complicated matrices such as soils and sludge, headspace analysis may alleviate some interferences. Nevertheless, this is limited to volatile analytes and to those samples where interactions between the target analytes and the matrix are negligible. Thus, it would be interesting to study the applicability of glassy carbon as sorbent for SPME of river samples with organic matter content. The presence of humic substances has a negative effect in the extraction procedures. They

can lead to emulsion formation in LLE, plugging and overloading of the cartridge in SPE, and retention of the analyte in the aqueous phase. In SPME, it is observed that these humic substances are adsorbed onto the surface of the coating, hindering the absorption of other compounds (Porschmann et al., 1998). Moreover, there is evidence that some organic compounds bind to humic substances. As a result, the apparent solubility is enhanced and their bioavailability decreased.

In order to see the effect of humic substances on the performance of glassy carbon, river water samples were spiked with PCP to obtain concentrations from 0.005 to 0.1 mg/L. It is observed that 6.5 mg of C/L in the sample does not affect the determination of PCP, since the precision, linear dynamic range and sensitivity are the same for both samples (Table (4-3)). This is also exemplified in Figure (4-14).

Table (4-3). Analytical figures of merit for the analyis of penthachlorophenol in water using glassy carbon and values reported in the literature.

	Glassy Carbon	Poly(acrylate)[1]	CW/DVB[2]
LDR (μg/mL)	0.005-0.1[3]	0.008-8	0.02
	0.005-0.1[4]	0.007-0.7	
LOD μg/L)	0.5	1.4	0.5
	0.5	0.11	
RSD (%)	1-7		12

[1] Buchholz et al. 1994
[2] van Doorn et al. 1998
[3] This work for model solutions
[4] This work for Rideau River samples

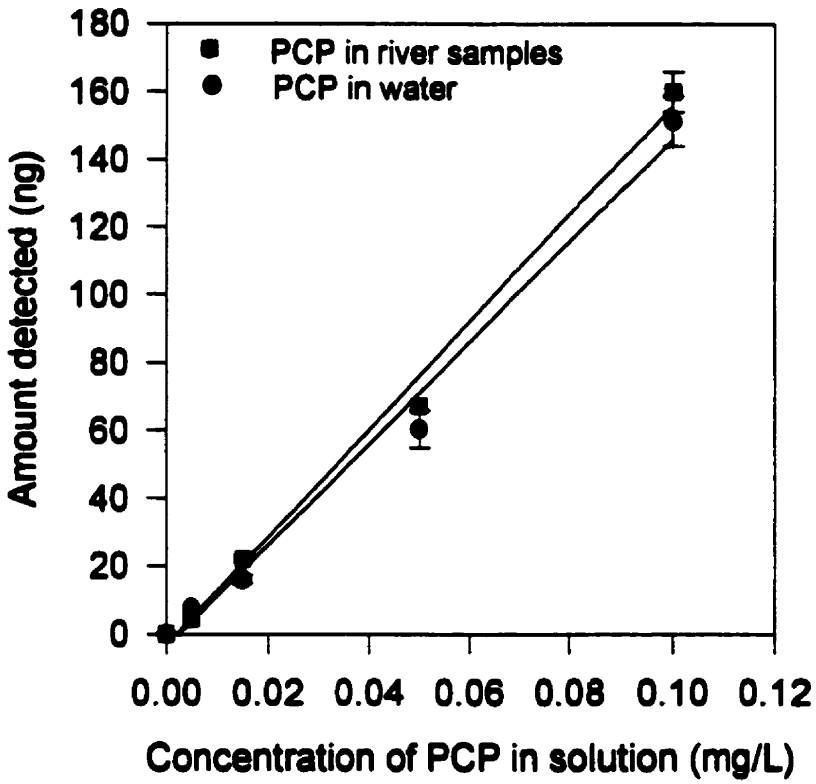


Figure (4-14). LDR of PCP spiked on Rideau River water sample and deionized water. Extraction 60 min, desorption with MeOH for 5 min. Minimum 3 replicates %RSD 5-10%.

Our results are comparable with those reported in the literature (Table (4-3)). Buchholz et al. (1994) reported limits of detection of the order of parts per billion using GC-FID and GC-MS and LDR of 2-3 orders of magnitude. Van Doorn reported a LOD of 0.5 μg/L using GC-FID, whereas those reported here are 0.5 μg/L using HPLC-UV. The main advantage of the method presented herein, is that derivatization is not required.

Effects of fulvic acids

As demonstrated in the previous experiments, the presence of organic matter at a concentration of 6.5 mg of C/L did not affect the sensitivity of the method. It was hypothesized that an effect would be seen at higher concentrations.

Fulvic acids were chosen to exemplify the organic matter present in real samples. Although fulvic acids are only a fraction of the total organic matter, they were selected as they are soluble over the entire pH range. Model solutions containing different amount of fulvic acids were prepared. Each sample was allowed to equilibrate for 48 hours in the dark. It is important to note that the sample was not acidified at this point because it is hypothesized that in doing so, both fulvic acids and PCP will be in the protonated form making them more hydrophobic. This could enhance their interaction with each other and decrease the free PCP in solution. As a result, the amount of PCP extracted would be low, due to analyte-matrix interaction and not to the extraction procedure. Therefore, prior to extraction the sample was acidified with HNO_3 to pH 1.0 and mixed for 2 min. The extractions took place for 60 min and desorption for 5 min at room temperature.

It was observed that fulvic acids at concentrations up to 10 mg/L do not seriously affect the extraction efficiency (Figure 4-15). However, at 50 mg/L, the amount of PCP detected decreased by about 50%. This could be attributed to two phenomena. PCP may have bound to the FA, and been made more soluble in effect. The other is that fulvic acids are adsorbed to the surface of the glassy carbon obstructing the adsorption of the PCP.

Lee et al. (1998) found that the extraction efficiency was reduced at concentrations of fulvic acid exceeding 5 mg/L. The decrease in factors for extracting

herbicides ranged from 2-10 for herbicides in 10 mg/L humic acid solutions. The experimental set up was using 25 μg/L of mixed herbicide aqueous solution spiked with 1.25 to 10 mg/L of humic substances. HS in aqueous solution would inhibit the adsorption of herbicides onto the fiber.

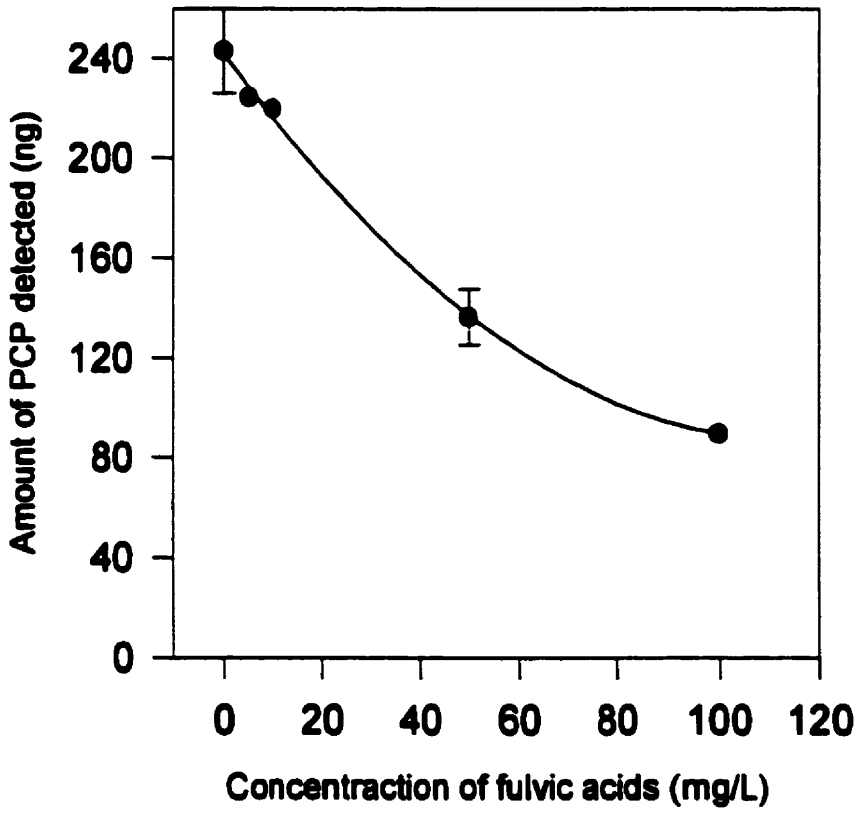


Figure (4-15). Effect of fulvic acid concentration in the extraction efficiency of pentachlorophenol.

Summary

Determination of pentachlorophenol using SPME-HPLC UV detection was achieved with glassy carbon as the sorbent. The limit of detection and linear dynamic range were comparable to those obtained by SPME-GC-FID and SPME-GC-MS. The main drawback is the incomplete desorption which affect the LOD and LDR since the surface is overloaded with analyte at higher concentration.

Glassy carbon was suitable for the extraction of PCP from samples with low content of dissolved organic carbon (6.5 mg/L of C). This was observed by comparing the sensitivity of spiked deionized water and spiked river water, which was unchanged.

Further experiments demonstrated that content of fulvic acids up to 10 mg/L does not affect the extraction. However, at higher concentrations (50 and 100 mg/L) the amount extracted decreases significantly.

Chapter 5

5 SPME/HPLC-Derivatization

Chapter 5

5 SPME/HPLC-Derivatization

5.1 Application to alcohol ethoxylates

5.1.1 Introduction

Nonionic surfactants are used worldwide as defoamers, emulsifiers and in some pesticide formulations. Because of health and environmental concerns, the use of APEO in household products has been banned in some European countries (Renner, 1997). As result, alcohol ethoxylates (AE) will be used to a greater extent. The levels of AE in untreated municipal sewage are in the 0.5-5 mg/L concentration range. Much lower concentrations (0.015-0.040 mg/L) are expected and found in wastewater after activated sludge or trickling filter treatments as well as in natural waters, depending on the extent of the biotic and abiotic removals of AE (Marcomini et al., 1996).

5.1.2 Analysis of alcohol ethoxylates

Analysis of nonionic surfactants has been well documented (Kiewiet et al., 1996, Marcomini et al., 1996, Miszkiewicz et al., 1996) and were described in Chapter 4. They include solvent sublation, liquid-liquid extraction, solid-phase extraction, solid phase microextraction, precipitation and dialysis.

The most suitable method for the analysis of surfactants is HPLC since separation based on the homologues and oligomer series can often be achieved, depending on the separation column. In the past years, capillary electrophoresis (CE) has been building its niche in the analysis of surfactants (Heining et al., 1996).

The detection of nonionic surfactant, in either HPLC or CE, is accomplished by refractive index (Desbène et al., 1996) flame ionization or evaporated light scattering; however, poor sensitivity is obtained in all cases. The presence of the benzene ring in the APEO allows detection by UV absorbance and by fluorescence detection (Holt et al., 1986). In contrast, due to the lack of chromophors, AEs require derivatization prior to detection by UV or fluorescence.

Figure (5-1) outlines the sample preparation and analysis of AE by derivatization with phenyl isocyanate. The procedure requires isolation and cleanup procedures prior to off-line derivatization.

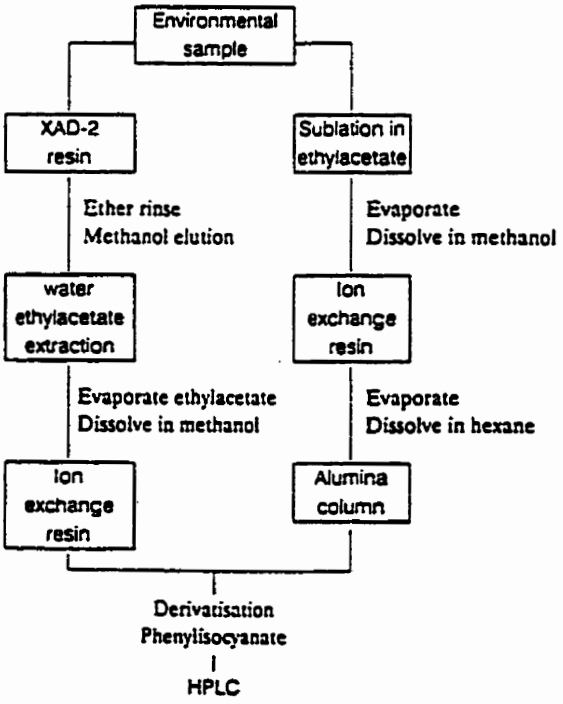


Figure (5-1). Sample preparation and derivatization scheme for the determination of AE in environmental samples (Matthijs et al., 1991).

Since alcohol ethoxylates surfactants posses at least one alcohol group, they will undergo nucleophilic substitution (SN2 reactions). Methods based on esterification have been widely explored, and some derivatization reagents and products are shown in Figure (5-2). A review of derivatization procedures is given by Marcomini et al. (1996).

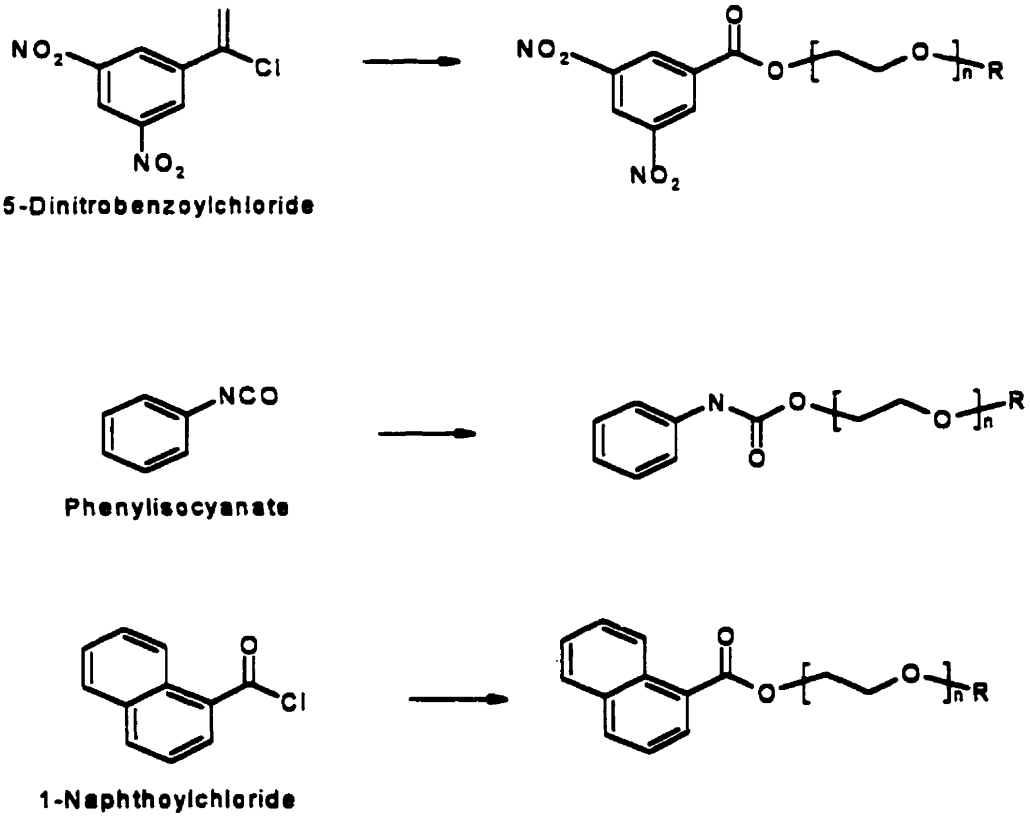


Figure (5-2). Fluorescent and/or UV-absorbing reagents proposed for AE derivatization (Marcomini et al., 1996).

Some of the reaction procedures are shown in Table (5-1), which includes derivatizaton reagent, solvent and reaction conditions.

Nozawa et al. (1980) used 3,5-dinitrobenzoyl chloride for derivatization of polyoxyethylene-monododecylether on a large scale. The HPLC separation took place in a reverse phase column and no limit of detection was reported.

A reasonable limit of detection (0.05 mg/L) was obtained by using a polyaromatic derivatizing compound such as 1-anthroylnitrile (Kudoh et al., 1984) which unfortunately is not commercially available. Moreover, it was reported that 1-anthroylnitrile reacted with water, therefore drying steps were included in the procedure.

A method for routine analysis of influent and effluent of sewage treatment plant was described by Kiewiet et al. (1995). The linear alcohol polyethoxylates were reacted with phenyl isocyanate after tedious isolation and cleanup steps. Analysis was peformed by HPLC-UV. The LOD for the effluents was 3.0 μg/L.

Further procedures for the analysis of AEs and polyethylene glycols in environmental samples by derivatization with 1-napththoyl isocyanate (NIC) and 1-naphthoyl chloride (1-NC) were proposed by Zannete et al. (1996). The conditions are presented in Table (5-1). The limit of detection using NIC was 5 ng and with 1-NC was 10 ng.

Table (5-1). Derivatization procedures for alcohol ethoxylates.

Surfactant	Reagent	Solvents	Condition	Ref	LOD
0.1g polyoxyethylene -monododecyl ether	0.2g 3,5-dinitro beonzylchlori de	20 mL pyridine	65°C for 30min	Nozawa et al., 1980	ND
1-250μg alcohol ethoxylated	5μg 1-anthhoylnitril e	5 mL 0.2% ACN in triethylamine	45°C for 2h. Diluted in mobile phase	Kudoh et al., 1984	50-200 μg/L
1 mL of a fatty alcohol polyethoxlate in acetonitrile	50 mL 4-nitrobenzoyl chloride or benzoyl chloride	10 μL pyridine	80°C for 30min	Zanette tl al., 1996	
Standard in acetonitrile	10 μL naphthhoyl isocyanate (NIC)	After evaporation redissolve in 100μL dimethylfora mide and 10 μL	35°C for 30min	Zanette et al., 1996	5 ng
Standard in acetone	20μL 1-naphthoyl chloride (NC)	After evaporation redissolved in 100 μL of ACN and 10μL pyridine	80°C for 15min	Zanette et al., 1996	10 ng

ND: Not determined

5.1.3 Research objectives

Chapter 2 includes SPME-derivatization procedures which have been applied to GC analysis. To date, no SPME-HPLC derivatization work has been published. The aim of this work was to develop a method for the determination of alcohol ethoxylates (Brij 56) in water samples by means of derivatization/SPME/HPLC with fluorescence detection

1) A model compound (1-hexadecanol) was chosen to investigate the reaction conditions during derivatization with 1-naphthoyl chloride. Moreover, the effect of temperature and presence of a catalyst were investigated.

2) Solid phase microextraction-derivatization was investigated using PDMS/DVB fibers for on-line derivatization of the alcohols with 1-naphthoyl chloride. In addition, the use of polycrystalline graphites for simultaneous in-fiber derivatization was investigated.

3) Finally, the effect of a second compound in the extraction-derivatization of the alcohol ethoxylate was investigated. The second compound was 1-hexadecanol.

5.2 Experimental

5.2.1 Reagents

The following chemicals were obtained from Aldrich Chemical Co. (Milwaukee, WI, USA): Brij 56 ($C_{16}H_{33}(OCH_2CH_2)_{10}OH$), and 1-hexadecanol (99%), the derivatization reagent 1-Naphthoyl chloride (97%), and 4-(dimethylamino) pyridine (DMAP) (99%). All of them were used without further purification. All solvents used were HPLC grade or better (Fisher Scientific, Nepean, CA). All aqueous solutions were prepared with deionized water (Milli-Q systems).

Stock standards were prepared by weighing the appropriate standards (1-Hexadecanol or Brij 56) and dissolving in pyridine, water or acetone.

5.2.2 Solid supports

SPME fibers

SPME fibers (60 μm film thickness polydimethylsiloxane-divinylbenzene; PDMS/DVB) were purchased from Supelco (Bellefonte, PA, USA) and conditioned by successively immersing them for 1 h in 4 mL of each of stirred acetonitrile, methanol and the HPLC mobile phase.

Polycrystalline graphites

Pencil leads (60 x 0.5 mm) H grade were obtained from Pentel (Tokio, Japan). These were used as received (PLU) or after coating with graphite by carbon evaporative coating (ca. 5nm thickness) (PLC). Both PLU and PLC were conditioned prior to analysis by immersion in acetone for 1 hour.

Glassy carbon rod (5 0 x 1mm) was obtained from Alfa ESAR (Ward, Hill, MA, USA). It was used as received after cleaning by immersion in acetone for 20 min (GCU).

5.2.3 Instrumentation

The HPLC system consisted of a Varian 9050 autosampler, a Varian 9010 ternary gradient pump and a Linear Instruments Co. LC 304 fluorescence detector. The system was coupled to the SPME/HPLC interface (Valco valve, internal volume 60 μL, Supelco, Bellefonte, USA) by connecting the autosampler valve (Valve 1) in series with the SPME/HPLC (Figure 4-3). In order to avoid overloading the detector with an excess of reagent, a three port valve (Valve 3) was placed between the column and the detector. This valve was opened at the beginning of the HPLC run to allow excess reagent to be discarded.

An ODS-Zorbax column (250 mm x 4.6 mm x 5 μm particle size) and an ODS-Zorbax guard column (4.6 mm x 1.25 cm) were used. The elution program was as follows; 60 % A for 10 min, ramped to 95 % A at 10 min and 95% A thereafter. Phase A was methanol and phase B was 30:70 water:acetonitrile. The flow rate was 2.0 mL/min. Detection was at λ_{ex}=228 nm and λ_{em}=366 nm.

5.2.4 Derivatization

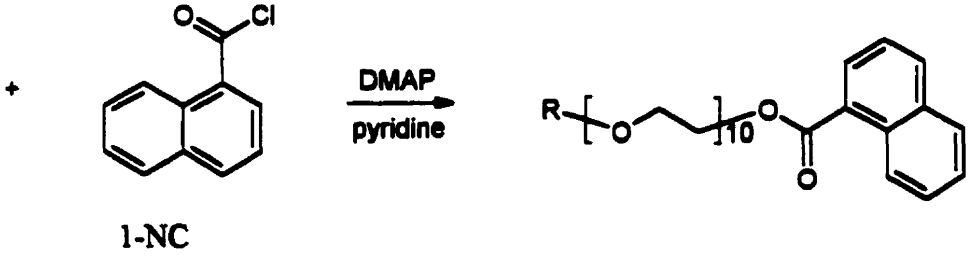
Reaction in organic solvents

Derivatization reactions were first tested on a relatively large scale in organic solvents. Solutions of nominal concentrations of 68 g/L of Brij 56 or 24 g/L of 1-hexadecanol were placed in round bottom flasks and approximately 0.01 g of DMAP was

added to each followed by the equivalent of a 4:1 molar ratio of 1-NC to active hydrogen. The reaction scheme is shown in Figure (5-3).

The solution was stirred at 80°C for 24 h. Aliquots were taken at 1, 5, 15, 30, 60 min. and 24 h. and diluted with acetonitrile. The solutions were injected (20 μL) into the HPLC. Derivatization was carried out in the presence of DMAP at 22, 36 and 80°C and for 1-hexadecanol in the absence of DMAP at 80°C as well. Product formation was confirmed by GC-MS for 1-hexadecanol only (Appendix 2).

Brij 56



1-NC

Figure (5-3). Reaction schemes of the derivatization of Brij 56 with 1-naphthoyl chloride in presence of DMAP as catalyst.

For external calibration, aliquots of Brij 56 solution in acetone were made in the concentration range from 0.2 –500 mg/L. 500 μL of a pyridine solution containing 2.5 mg 1-NC and 0.5 mg DMAP were added to each vial, and the reaction was allowed to take place at 80°C for 2 h. 500 μL of acetonitrile was added to each vial, and the solutions were injected into the HPLC.

SPME/derivatization in the interface (On-line derivatization)

The experimental procedure for the pre-column derivatization following SPME extraction is shown in Figure (5-4). Solid phase microextractions were performed by exposing the fiber to stirred solutions of Brij 56 (6.0 mL) for 1 h. The fiber was then air-dried in the headspace for 5 min and transferred to the SPME/HPLC interface. This was previously filled (ca 65μL) with a pyridine solution containing 500 mg/L of each DMAP and 1-NC which had been delivered by means of an HPLC pump connected to Valve 2 (Figure (4-3)) in position B. After reaction, Valve 2 was switched to position A for 2 min to inject the sample and Valve 3 to position B for the first 5 min. to reject excess derivatizing reagent. Experiments to determine the effect of reaction time were performed by analyzing 10 mg/L solutions of Brij 56 in deionized water.

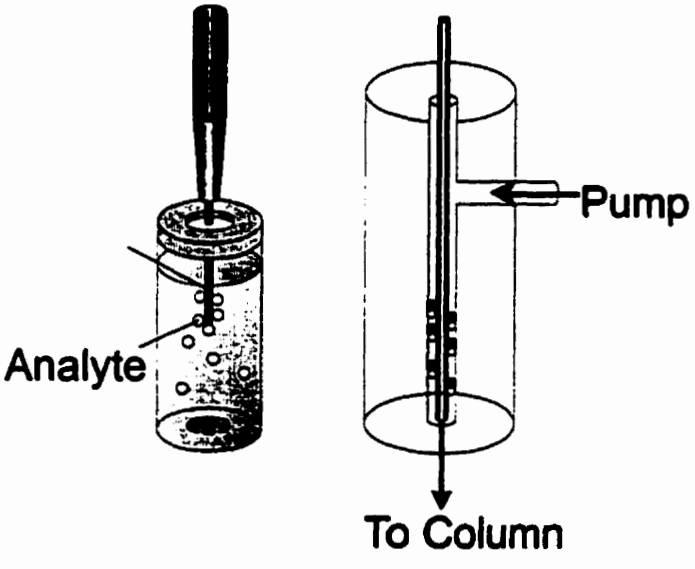


Figure (5-4). Schematic of on-line derivatization/SPME

In order to study product formation as a function of concentration, aqueous solutions containing 0-10 mg/L of Brij 56 were extracted for 60 min and derivatizated for 30 min.

The dependence of adsorption and derivatization processes of Brij 56 on the concentration of 1-hexadecanol (as an interfering compound) were evaluated. Aliquots of 100 mg/L of 1-hexadecanol in acetone were transferred to 7 mL vials to give final concentrations of 0-100 mg/L; the acetone was evaporated by blowing nitrogen. An aliquot of 6.0 mL of 10 mg/L Brij 56 were added to each vial and mixed for 5 min. Extraction and on-line derivatization were performed as described above.

Simultaneous in-fiber derivatization/SPME using PGC

Simultaneous in-fiber derivatization/SPME was performed using pencil lead and glassy carbon. The set-up of this technique is shown in Figure (5-5).

A six mL sample of aqueous solution containing Brij 56 (0.10-0.20 mg/L) was transferred to a 7 mL vial. Approximately 5 mg of DMAP was added, and the solution was stirred for 2 min. Prior to derivatization, one centimeter of the pencil lead rod was dipped directly into 1-NC for 1 min. Then the rod was immersed in a stirred aqueous solution containing Brij 56. The analytes were allowed to partition into the carbon rod and react with the derivatizing reagent at 25°C. After 60 min, the rod was transferred to the SPME/HPLC interface (previously filled with methanol), acetonitrile or acetone for a specific period time period.

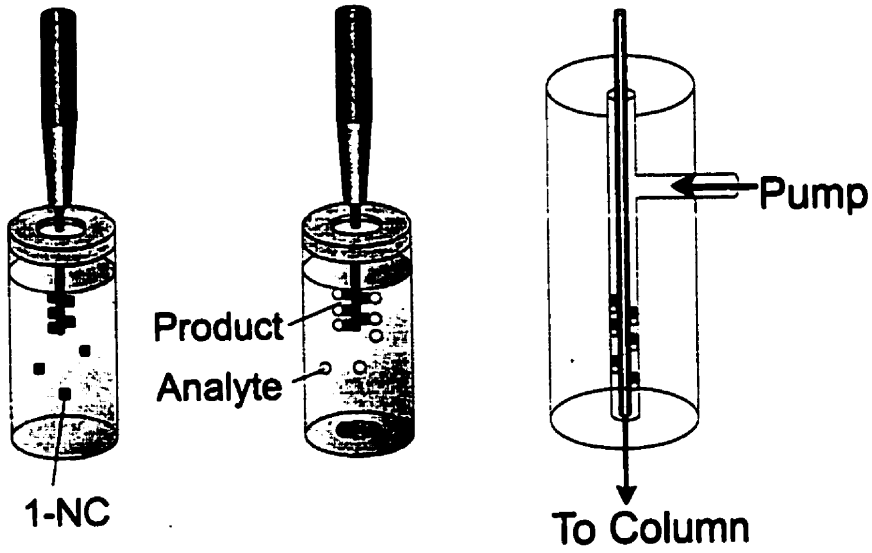


Figure (5-5). Schematic of simultaneous derivatization/extraction.

5.3 Results

5.3.1 Derivatization in organic solvents

As an alcohol polyethoxylate, Brij 56, posses a hydroxyl group and it will undergo nucleophilic substitution reaction (SN2 reactions) and esterification reactions are the most widely used. Derivatization with 1-NC was optimized using 1-hexadecanol as a model compound.

Figure 5.5 shows the reaction time profile for the derivatization of 1-hexadecanol and Brij 56. The presence of DMAP improved the yield of the reaction of 1-hexadecanol by 4 fold at 80°C. Moreover, the yield of derivative obtained at 80°C without DMAP was

similar to those obtained at 36°C and 22°C with DMAP. Significant product was obtained after 1 min and did not increase after 5 min (22 and 36°C). The same trend was observed for Brij 56. In spite of the greater yield obtained at 80°C with DMAP, subsequent reactions in the SPME/HPLC interface were performed at room temperature so that no heating device was required. Moreover, the amount of product remained unchanged after 30 min of reaction.

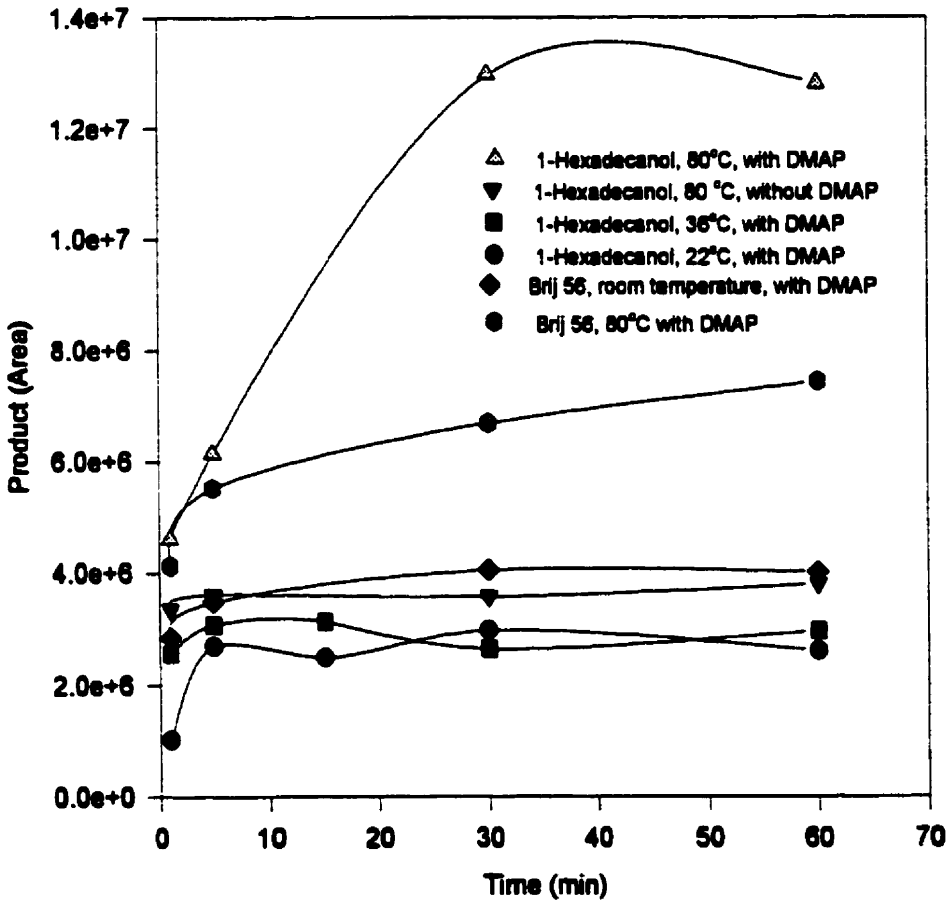


Figure (5-6). Derivatization of 1-hexadecanol and Brij 56 with 1-NC as functions of time and temperature (one repetition).

One of the characteristics of an SN2 reaction is that it shows second-order kinetics and follows the rate law (McMurry, 1992):

$$Rate = k[A][B]$$

Therefore, the rate of reaction is dependent on both the amount of Brij 56 and 1-NC.

If the concentration of 1-NC is much larger than that of Brij 56, then pseudo-first-order kinetics can be assumed with respect to the Brij 56.

This is important since even if the reaction does not go to completion, the reaction yield should be consistent after some fixed reaction time. Thus, the amount of derivative detected will be proportional to the concentration of the analyte in the sample.

It was thus important to confirm that the product formation responded linearly to the amount of Brij 56. For this purpose an excess of 1-NC and a constant amount of DMAP were added. It is shown in Figure (5-7) the linear dependence of the production formation and concentration of the Brij 56 in the vial. Repetitions (n=3) were performed in only two points of the plot and are shown as the mean ± 1 standard deviation.

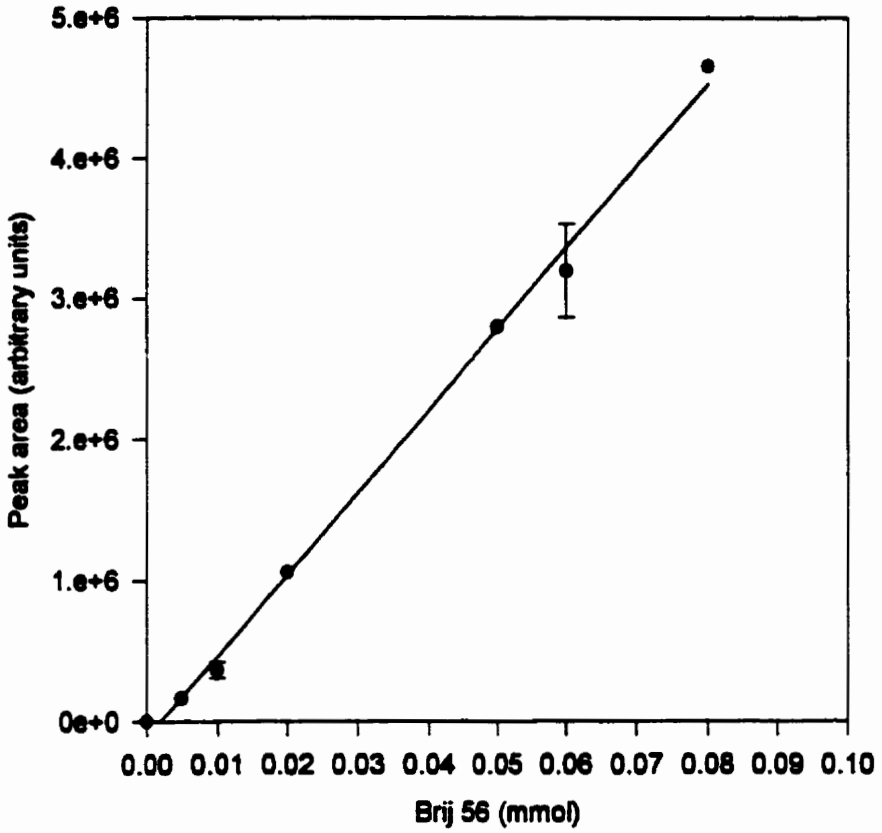


Figure (5-7). Product formation as function of amount of Brij 56, Linear from 0.005-0.08 mmol with a $R^2 = 0.996$

5.3.2 SPME/derivatization in the interface (On-line derivatization)

Extraction followed by on-line derivatization was carried out using PDMS/DVB fiber. Derivatization after extraction was chosen in this work for various reasons. The poor resistance of the fiber towards 1-NC prevents simultaneous derivatization and extraction. Due to the low vapour pressures of alcohol ethoxylates or their esters, headspace extraction is difficult to perform. The desorption chamber in the SPME/HPLC interface allows a static desorption and pre-column derivatization. The chosen scheme

was therefore extraction of the analytes from water using PDMS/DVB, followed by derivatization and desorption of the derivative in the SPME/HPLC chamber.

It is hypothesized that the reaction is most likely to occur in the pyridine and not on the fiber. This was observed by performing derivatization in a micorvial containing ca 20 μL of 1-NC/pyridine where, product was subsequently detected by HPLC. This necessitated an excess of 1-NC in the pyridine, which would overload the detector. The addition of valve 3 (see Figure (4-3)) partially solved the problem by allowing diversion of the early eluting 1-NC to waste. Despite avoiding overloading the detector, the excess of 1-NC affected the chromatographic performance as excessing peak tailing of the analyte was observed (Figure 5-8).

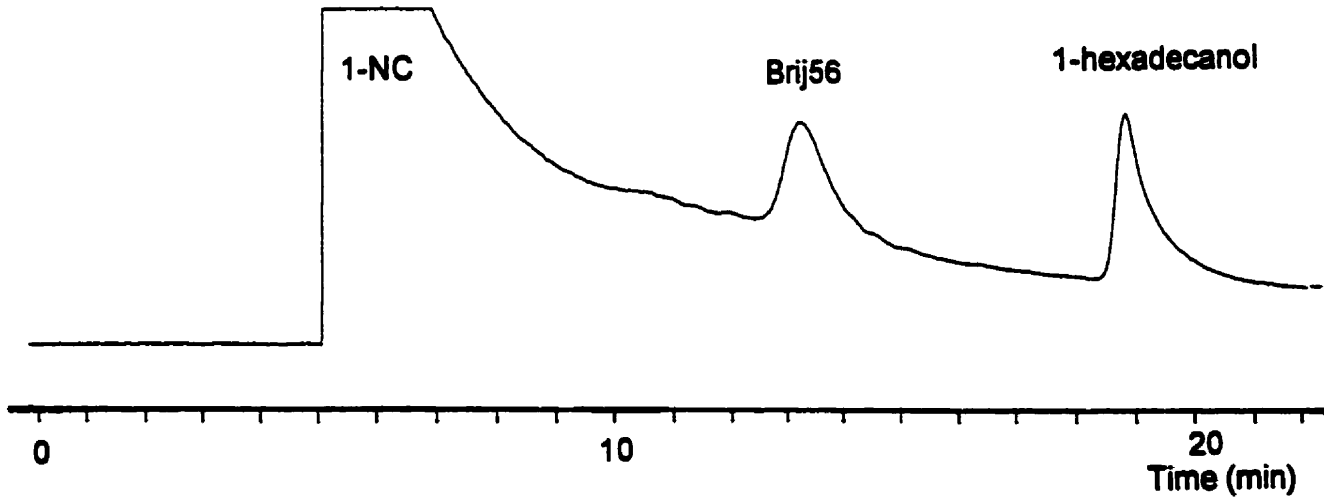


Figure (5-8). Chromatogram of the 1-NC derivatives obtained by on-line derivatization of Brij 56 and 1-hexadecanol (10 mg/l each)

Reaction in organic solvent showed that the esterification reaction proceeds at room temperature, and that the product could be obtained even after 1 min. However, extractions of 10 mg/L of Brij 56 from water followed by reaction in the SPME/HPLC desorption chamber failed to produce detectable product after 1 min. Better results were obtained by increasing the time of reaction up to 30 min. This time profile is shown in Figure (5-9) and the error bars are 1 standard deviation of at least three replicates. Therefore, subsequent experiments were performed as follows; time of extraction 60 min (with stirring), time of reaction 30 min.

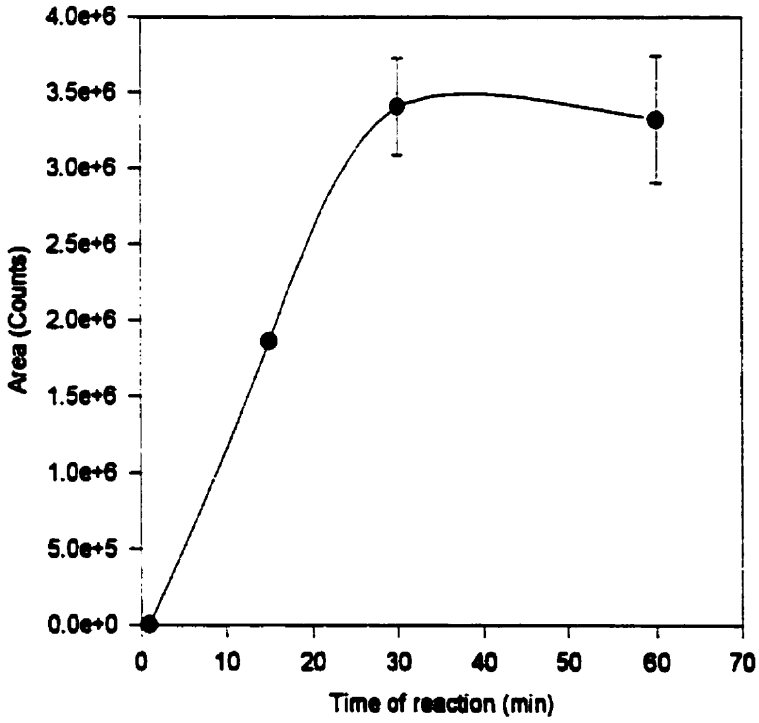


Figure (5-9). Effect of derivatization reaction time on detector signal. Samples contained 10 mg/L of Brij 56. Extraction time 60 min.

The linearity of the method was obtained by extracting Brij 56 from water over the range of 0.1 to 10 mg/L (Figure 5-10). The correlation coefficient was 0.991. The excess of 1-NC created a sloping baseline (Figure 5-8), producing a negative effect on the limit of detection (0.1 mg/L) and linear dynamic range (two orders of magnitude) which is shown in Figure (5-10). Moreover, this may also be responsible for the somewhat broad peaks observed, due to modification of the HPLC stationary phase by the 1-NC.

The sensitivity of this method depends on several equilibria such as partitioning of the analyte onto the fiber, desorption of the analyte into pyridine and finally reaction of the analyte with 1-NC in the presence of DMAP. The results indicate that the latter is the limiting factor in this method. If it is considered that 15-20 % of the Brij 56 present in water is extracted by the PDMS/DVB fiber (this was calculated from the results obtained in the direct extraction of Triton X-100 from water, see Chapter 4), then only about 1 % was converted to the derivative in these experiments. Reaction yield can be improved by heating the desorption chamber, which however was not attempted since deterioration of the fiber was expected.

The precision of the method (approximately 10 %, as shown in Figure (5-9)) is remarkable in view of how many equilibria must be attained:

1) Extraction equilibrium, in which the Brij 56 partitions between the fiber and the aqueous sample.
2) Desorption equilibrium in the interface, where Brij 56 is desorbed by the reagent solvent (pyridine containing 1-NC and DMAP).

3) Reaction between Brij 56 and 1-NC. In this case, adsorption of the product onto the PDMS/DVB fiber is negligible. This was corroborated by a second desorption with the mobile phase and blanks between extractions.

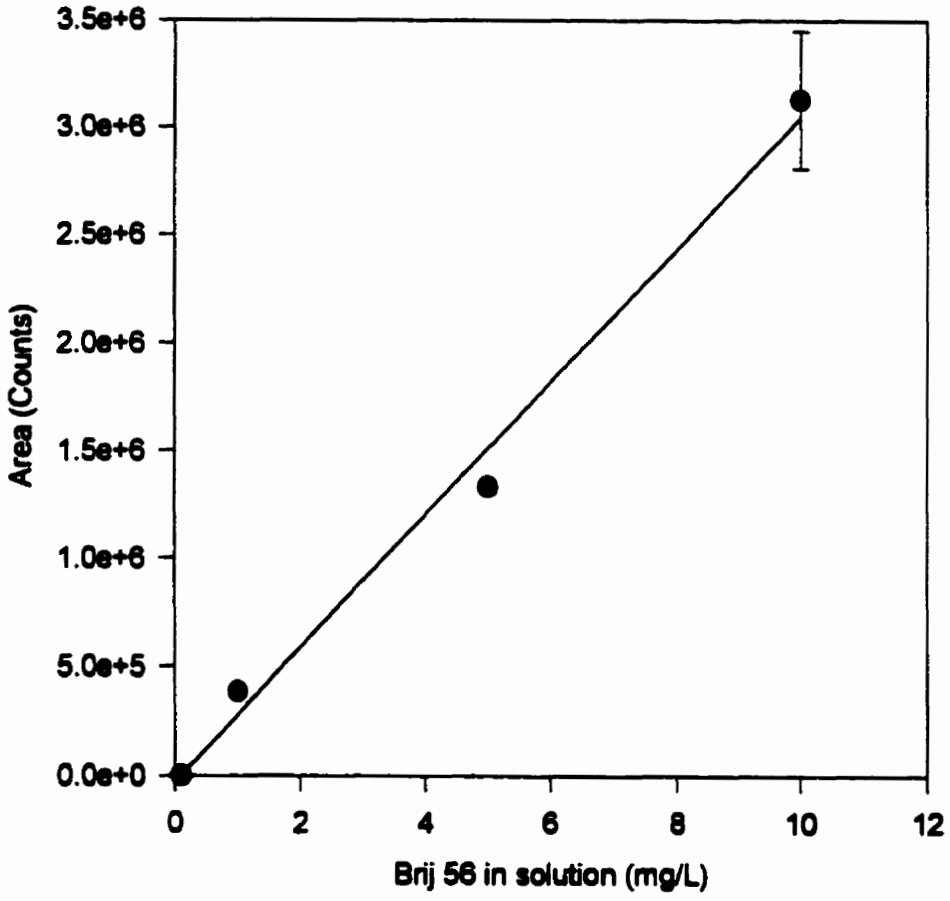


Figure (5-10). Linear dynamic range of Brij 56 (0.1-10 mg/L), three replicates were performed at 10 mg/L only.

Effect of alcohol concentration on on-line derivatization

In order to observe the effect of 1-hexadecanol on the determination of Brij 56, samples containing 10 mg/L of Brij 56 and 1-hexadecanol ranging from 0-100 mg/L were prepared. Extraction and derivatization were performed as described in section 5.3.2.

Results indicated that the presence of 1-hexadecanol decreased the amount of Brij 56 extracted and derivatized as observed in Figure (5-11).

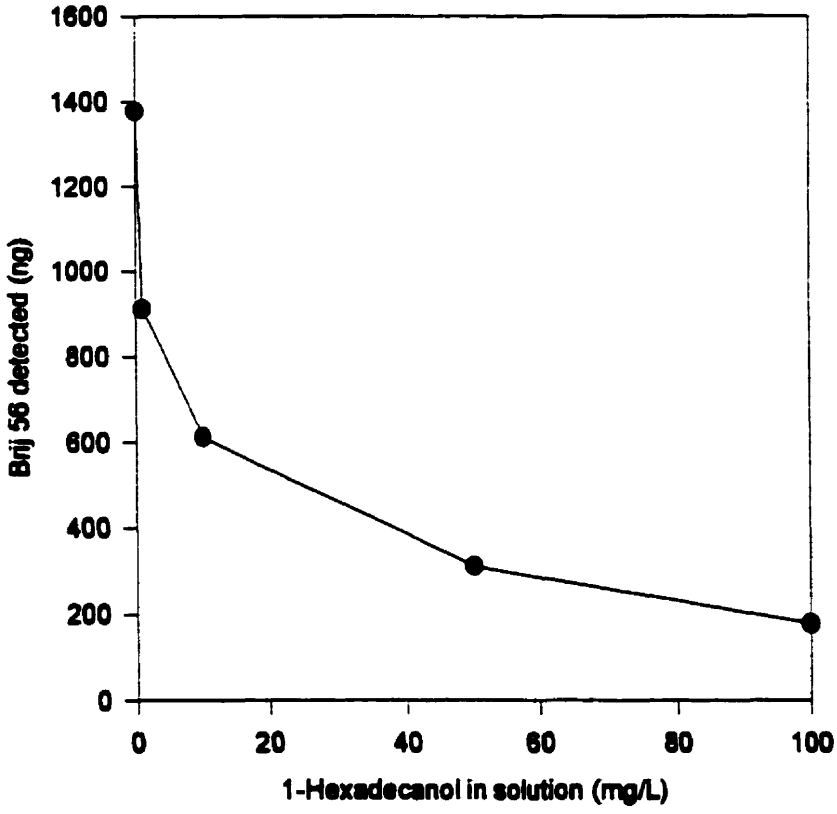


Figure (5-11). Extraction of Brij 56 as a function of 1-hexadecanol concentration. Extraction with PDMS/DBV fiber for 60 min. On-line derivatization, reaction took place for 30 min. Desorption 2 min. (one experiment per point).

Yang et al. (1998) and Gorecki et al. (1999) proposed a mechanism of extraction using PDMS/DVB fibers. They suggested that adsorption better describes the extraction process and as such, the presence of additional compounds will compete for the adsorption sites, reducing the amount of Brij 56 extracted by the fiber. It was observed, that at low concentration of 1-hexadecanol (1 mg/L), the amount of Brij 56 detected decreased by 33 % and at 100 mg/L by 87%. This is in accordance with the results presented by Gorecki et al., which showed that the amount of isopropanol extracted by a PDMS/DVB fiber decreased by 50% when the concentration of a MIBK was equal to that of isopropanol (Figure (2-3)). In this case, when the concentration of 1-hexadecanol was 10 times larger than Brij 56 (1 mg/L), the amount detected decreased by 33%.

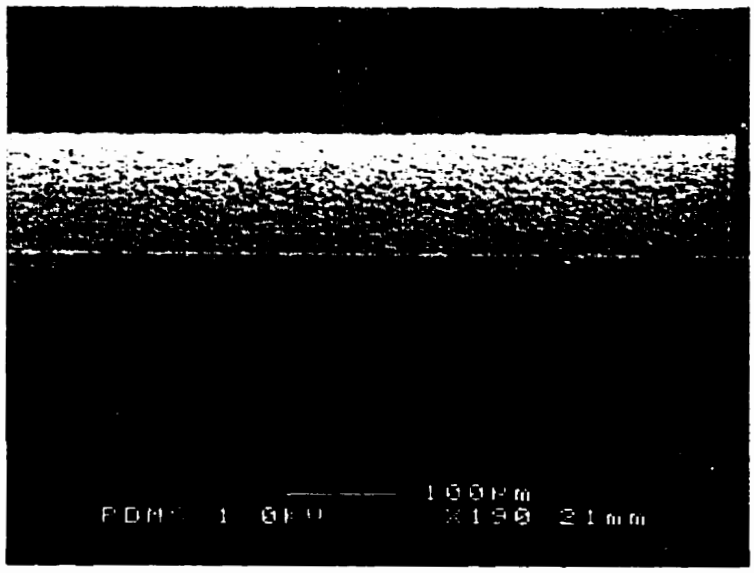
The derivatization reaction is unlikely to be responsible for the lower Brij 56 signal. The amount of 1-NC used up during the derivatization reaction must be negligible compared to the amount remaining. This will ensure that the reaction is not limited by the 1-NC. The maximum amount of 1-NC present in the interface is 2.6 mmol of 1-NC and assuming that 20% of the 1-hexadecanol is extracted by the fiber and desorbed, then the mole ratio of 1-NC:hexadecanol is 5000:1. Therefore, competition for the 1-NC between 1-hexadecanol and Brij 56 is unlikely.

Another explanation is the effect of 1-hexadecanol in the behaviour of the surfactant at the interface and in the bulk solution. Despite the presence of the hydroxyl group in the molecule of 1-hexadecanol, its solubility in water is limited because of the long alkyl chain. Enhancement of the solubility of nonpolar compounds (1-hexadecanol in this case) in water by surfactants has been documented (Kile et al., 1989, Jafvert et al., 1994). Thus, we might expect that nonpolar compounds may have an effect on the

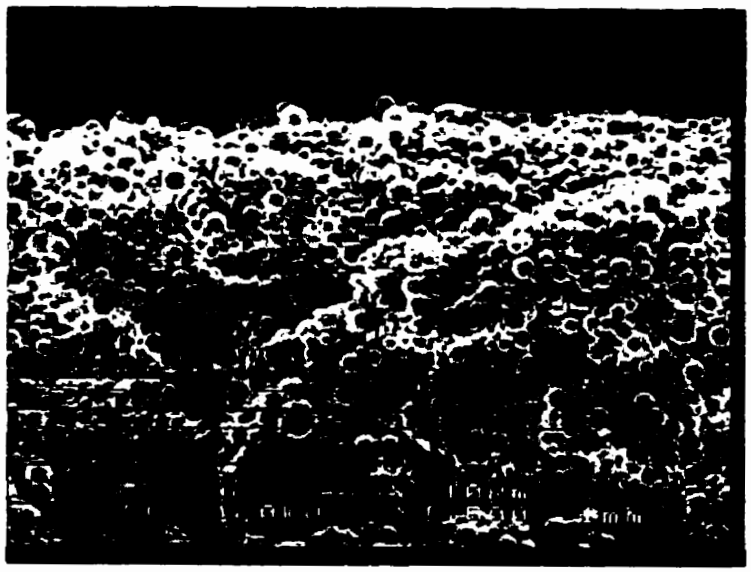
adsorption properties of surfactants, and so the amount of surfactant extracted could vary as the concentration of 1-hexadecanol varies. These changes in the bulk of the solution may affect the adsorption of surfactants.

5.3.3 Simultaneous in-fiber derivatization/SPME using PGC

SPME/HPLC derivatization is limited by the availability of the fibers and their poor resistance towards some chemicals and even towards the HPLC mobile phase. In previous interface derivatization experiments using commercial SPME fibers, it was observed that after a few extractions the polymer suffered some damage. Figure (5-12a,b) shows a PDMS/DVB fiber after 10 extraction followed by derivatization in the interface (1-NC and DMAP in pyridine). At 190x magnification (Figure (5-12a)), cracks in the polymer can be seen. Figure (5-12b) shows that the PDMS/DVB fiber is composed of microspheres (1500x magnification). Therefore a new solid phase is required.



(a)



(b)

Figure (5-12). Scanning electron micrograph of a PDMS/DBV fiber after 10 extractions followed by derivatization in the SPME/HPLC interface at (a) 190 x magnification and (b) 1 500x magnification.

In this section, Brij 56 is determined by simultaneous derivatization-extraction and analysis by HPLC-fluorescence detection. The sorbents used were two polycrystalline graphites: pencil lead and glassy carbon. The time profile of simultaneous extraction and derivatization were performed using PLC and it is illustrated in Figure (5-13). After 60 min the amount of derivative detected started to level off.

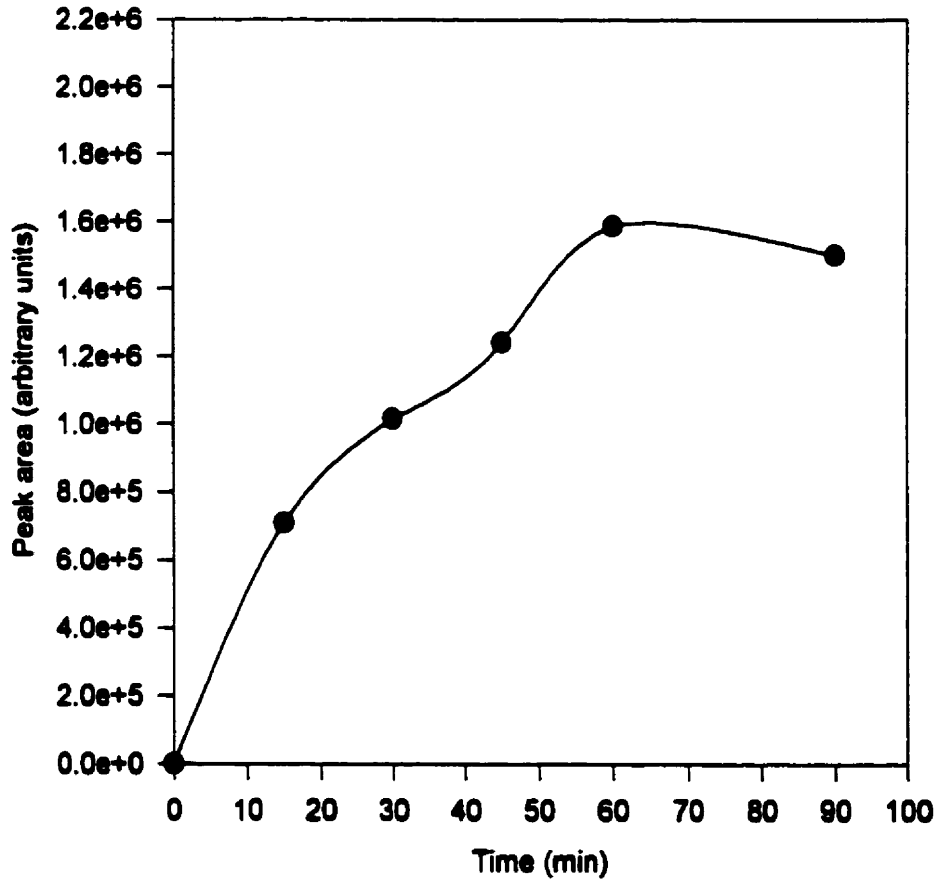


Figure (5-13). Simultaneous derivatization-extraction time profile of aqueous solutions containing 0.10 mg/L Brij 56. One minute desorption in the interface. (One repetition).

Figure (5-14) shows the results of simultaneous derivatization-extraction of Brij 56 in water at concentration ranges from 0.01 to 0.2 mg/L of Brij 56 for three polycrystalline graphites: PLU, PLC and GCU.

The results showed a sigmoidal curve in which, at low concentration, formation of the ester is small (or its adsorption or desorption are hindered), whereas for higher concentration the response increases exponentially. It is important to note that the upper limit using polycrystalline graphites is in the same order of magnitude as the LOD using PDMS/DVB fibers (0.1 mg/L).

The small range of detection (0.01mg/L) is also attributed to the excess of derivatizing reagent, since the polycrystalline graphites were doped with pure 1-NC. This causes the baseline background to be higher, and the detector is overloaded. However, lower amounts can be detected (0.01 mg/L).

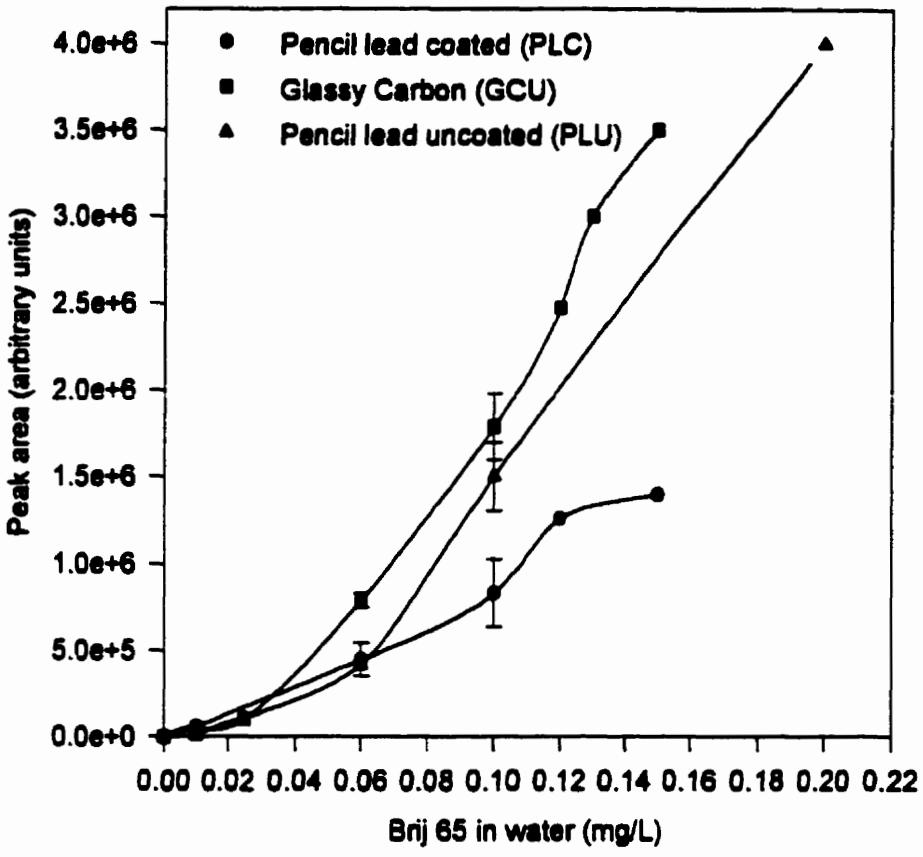


Figure (5-14). Simultaneous derivatization-extraction as a function of concentration of Brij 56 in solution. One standard deviation represents the error bars of a minimumof 3 repetitions.

At low concentrations, the amounts of derivative detected using the three different PG are in the same range, where as higher concentration the differences are more evident. GCU followed by PLU and PLC obtains the highest amount of derivative, At 0.2 mg/L PLU gave a higher result, however only one data point was obtained. This is related to the surface area, since more analyte reaches the surface and reacts with 1-NC.

The formation of the naphthoyl derivative is a multistep mechanism as described in section 2.6.2:

1) adsorption of 1-NC onto the surface of the PL. It is believed that some NC will diffuse into the pores and some will form a thin film coating on the PL surface
2) adsorption of Brij 56 from solution onto the PL of more likely 1-NC layer. There are few sites available, so is unlikely that molecules of Brij 56 would be adsorbed onto the surface of the polycrystalline graphite. Considering that 1-NC is in excess, then all the sites on the surface of polycrystalline graphite would be occupied by molecules of 1-NC.
3) reaction between Brij 56 and 1-NC.
4) desorption of the product from the surface of the polycrystalline graphite.
5) Desorption of the product from the surface and diffusion out of the pores of polycrystalline graphite.

5.4 Summary

Analysis of Brij 56 was achieved by solid-phase microextraction followed by on-line derivatization-fluorescence detection. A low esterification reaction yield and chromatographic problems due to the excess of derivatizing reagent cause the high limit of detection (0.1 mg/L) and narrow linear dynamic range (two orders of magnitude) (Table (5-2)). However, it is evident that SPME/HPLC with on line derivatization can be done.

This is a promising method that opens possibilities in the analysis of primary alcohols and other compounds requiring derivatization for detection. The main problem

at present is deterioration of the fibers. Detection limits could be improved by the use of fibers that are resistant to attack by the 1-NC.

The presence of 1-hexadecanol as a model interfering compound had a negative effect in the determination of Brij 56. The extraction-derivatization of Brij 56 is inversely proportional to the amount of 1-hexadecanol present in the sample. This is important to consider when environmental samples are analyzed, since it indicates the adsorption of the analyte onto the fiber is influenced by the presence of other compounds.

Simultaneous derivatization-extraction was achieved using pencil lead and glassy carbon. Better detection limits were obtained (0.01mg/L). However, a linear response was not obtained, restricting their use for quantification purposes. The main advantage is their chemical resistance towards the 1-NC which could be adsorbed to the surface without further dilution.

Table (5-2). Summary of the two methods developed for the determination of Brij 56 in aqueous solutions.

	Solid	LOD (mg/L)	LDR (mg/L)	Precision (%RSD)*
Extraction followed by derivatization	PDMS/DVB	0.1	0.1-10	10
Simultaneous derivatization extraction	PLU	0.01	0.1	22
	PLC	0.01	0.1	12
	GCU	0.01	0.1	5

* Minimum three repetitions

Chapter 6

6 Adsorption and desorption considerations

Chapter 6

6 Adsorption and desorption considerations

6.1 Introduction

6.1.1. General considerations

In the previous chapters, polycrystalline graphites were evaluated in terms of their performance as solid supports for solid phase microextraction. The analytical figures of merit of glassy carbon and pencil lead were obtained; these were further compared to those from polymeric fibers. The results proved that both of GCar and PL could be used as sorbents for SPME. The results showed that the target analytes **adsorbed** onto the solid support. This is different from liquid-polymers, where **absorption** is the mechanism. In addition, the effectiveness of desorption limited the sensitivity of the method.

In this section, further analysis of the data will be presented. In an attempt to obtain information on adsorption and desorption mechanisms, adsorption isotherms and mass balances will be considered. Moreover, partition coefficients will be calculated.

In the initial stage of the investigation, carbon fibers were also considered. However, as it will be shown, their use was limited mainly due to the inadequacy of the

SPME interface. Nonetheless the results obtained will be described in this section, since they add to the information on carbon adsorbents.

The aim of this chapter is to investigate the mechanism of adsorption and desorption of Triton X-100 from polycrystalline graphites. The following approaches will be considered.

1) Determination of mass balance. For this purpose, after extraction, the amount of Triton X-100 remaining in the supernatant was obtained by spectrofluorometry. The amount adsorbed and desorbed was determined by HPLC/fluorescence detection. Special attention was pointed toward desorption efficiency and its kinetics.
2) Partition coefficients for Triton X-100 using PG were obtained from adsorption isotherms (using the Langmuir mode) and from the surface-based partition coefficient definition (K_{ds}).
3) Effect of surface modification of carbon fibers in the adsorption/desorption process was assessed.

6.1.2 Adsorption of nonionic surfactants on liquid-solid interface

Adsorption of nonionic surfactants is rather a complex system and is affected by the concentration of the surfactant and temperature. This is reflected in the shape of the isotherm, e.g. micelle formation flatterns the curve, heterogeneity of the solid yields isotherms with higher slopes for adsorption onto higher energy sites than adsorption onto low-energy sites (Rosen, 1989).

Figure (6-1) illustrates the most likely orientation changes that a nonionic surfactant will undergo when adsorbed from aqueous solution. Figure (6-2) shows the expected adsorption isotherms for each case. At low concentration (I), the solute-solute

interactions are negligible and the molecule is adsorbed by the hydrophobic moiety (in an aqueous solvent); however, the ethoxy chain may have some interaction with the surface. As the concentration increases, the surface is close to monolayer saturation. This is observed as a decrease on slope on the adsorption isotherm. At still, higher concentrations, solute-solute and solute-adsorbent interactions determine what will happen at the interface. If the hydrophobic moiety-surface interaction is stronger, the hydophilic part will be displaced from the surface by other adjacent molecules (IIA) and the opposite case is shown in IIC. The intermediate case is shown in IIB. As the concentration of the surfactant approaches the critical micelle concentration, the molecules will be vertically oriented and a larger amount of surfactant will be adsorbed onto the surface. This can be seen in the isotherms A and C, whereas in the case of B, no more molecules can be accommodated on the surface.

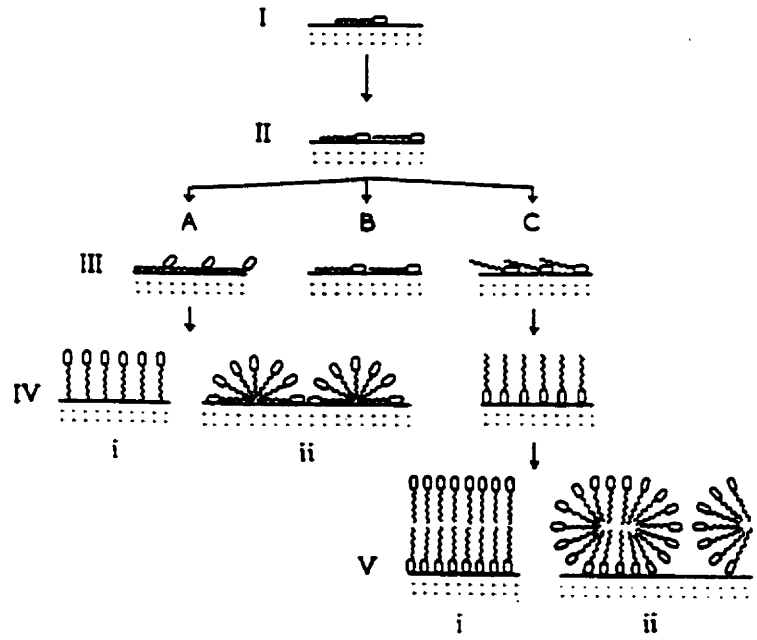


Figure (6-1) Model for the adsorption of nonionic surfactants, showing the orientation of surfactant molecules at the surface (Parfitt et al., 1989).

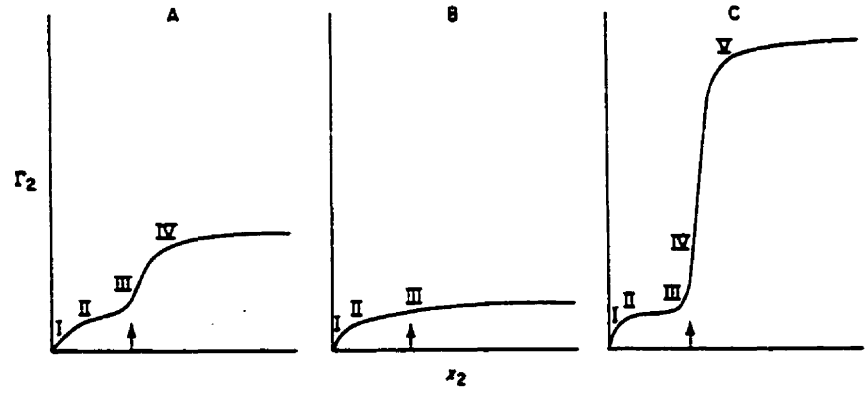


Figure (6-2). Adsorption isotherms corresponding to the three adsorption sequences illustrated in previous figure; critical micelle concentration is indicated by an arrow (Parfitt et al., 1989).

6.2 Experimental

6.2.1 Solid supports

Glassy carbon rods

Glassy carbon rods (50 x 1 mm, Type 2) were obtained form Alfa ÆSAR. They were treated and conditioned as described in section 4.2.2.

Carbon fibers

Carbon fibers were Hercules Type AS4, 3000 filament bundles. Many commercial fibers are sold with a matrix-compatible coating or sizing to improve

adhesion and to resist fiber degradation on composite formation. In order to remove this coating, and to chemically modify the surface, the carbon fibers were subjected to acid treatment, hydrogen reduction, or heating in an inert atmosphere.

Carbon fibers treatments

Acid treatment (oxidation) (CFA)

The acid treatment was done by exposing approximately 0.4 g of fibers to 70% nitric acid at 115°C for 3 h (Pittman et al., 1997). They were then washed with distilled water until the pH value was the same as the distilled water. The fibers were then soaked in 2.0 N NaOH solution for 30 min, rinsed thoroughly with deionized water and acetone, dried, and finally stored under nitrogen.

Reduction with hydrogen (CFH)

The hydrogen treatment was performed by exposing the fibers to flowing hydrogen gas at 350°C for 1 h. A 3000 filament bundle was placed inside 1/4" copper tubing held inside an oven. Hydrogen gas was flowed through the tube for ca. 2 h. The oven was ramped to 350°C in 20 min, kept at that temperature for 1 h, and then cooled down. The treated fibers were stored under nitrogen.

Pyrolysis (CFN)

Other filament bundles were heated at 350°C for 1 h under N_2 atmosphere using the same procedure as for the hydrogen treatment.

6.2.2 Specific surface area determination (SSA)

The specific surface area was determined with an Asap 2010 surface area analyzer (Micromeritics Instrument Corp). The analyses were performed by Régent Dutrisac at the National Research Council of Canada.

6.2.3 Studies by X-ray photoelectron spectroscopy (XPS)

The XPS analyses were performed with a Kratos Axis XPS using monochromated Al K α x-radiation and charge neutralization. They were performed by Geral Pleizier at the Institute for Chemical Process and Environmental Technology at National Research Council of Canada.

Table (6-1). Physical characteristics of the different sorbents used.

Rod	Geometrical surface area* (mm^2)	Specific surface area m^2/g	Density g/cm^3	Treatment
Glassy carbon (GCP)	32.2	8.2	1.42	Sanded
Pencil lead (PLC)	15.9	4.5		Coated
Carbon fibers (CF)		1.3	1.7	
Single filament	0.219			
3000 filament	657			As received, oxidation, reduction or pyrolysis

* 1 cm length exposed to solution

6.2.4 Reagents and sample preparation

Triton X-100 was obtained from Aldrich Chemical Co. (Milwaukee, WI, USA) and used as received. All solvents used were Optima grade (Caledon Laboratories, ON, Canada). A stock standard solution was prepared in methanol and diluted to obtain concentrations in the range of 0.010 to 10 mg/L, which were used to calibrate the response of the HPLC detector.

Aqueous model solutions were prepared by serial dilution from a stock solution containing 100 mg/L of Triton X-100 in deionized water (Milli-Q Plus water purification system, 18 MΩ). A set of standards (0.01-0.2 mg/L) were prepared and used to calibrate the response of the fluorescence spectrometer.

6.2.5 Extractions and desorption

Figure (6-3) illustrates the experimental approach for each polycrystalline graphite.

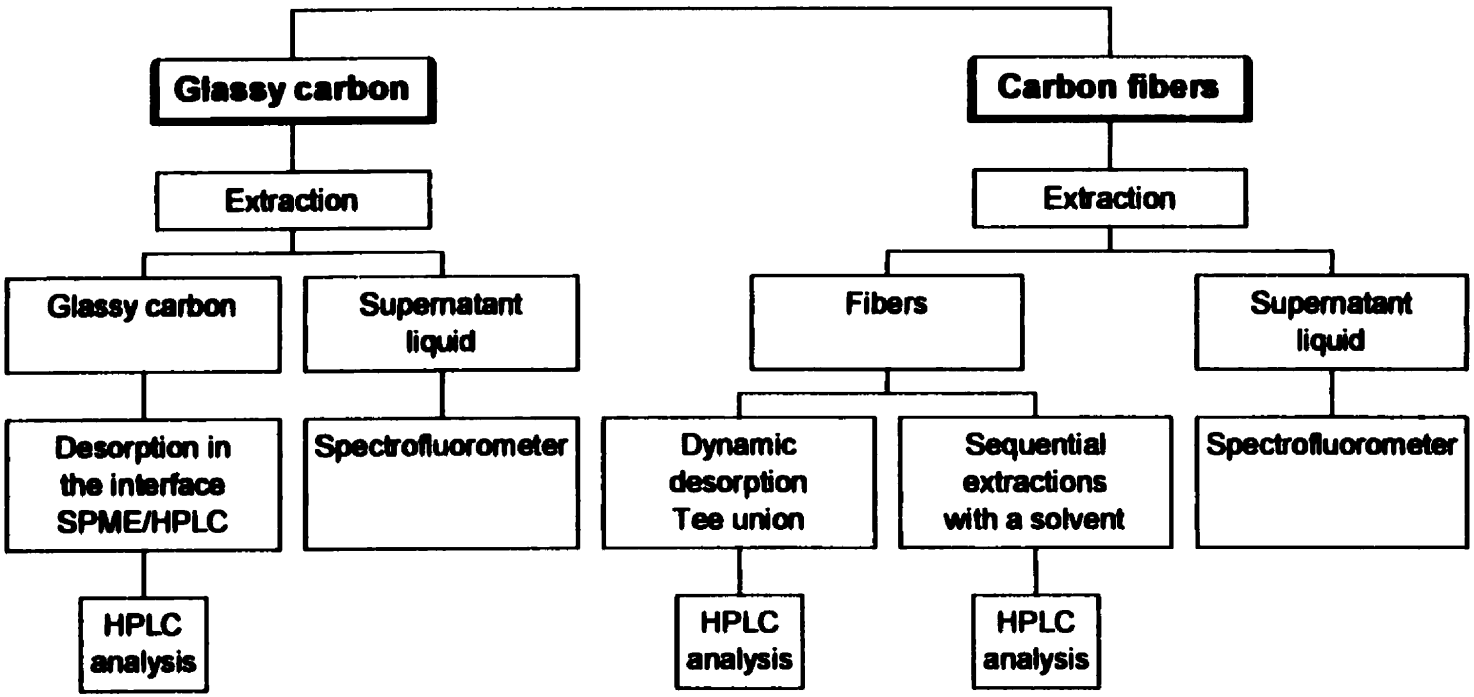


Figure (6-3). Experimental approach for determination of mass balance

Glassy carbon

Extractions and desorption of Triton X-100 using glassy carbon were performed as described in section 4.2.4.

Carbon fibers

Extraction of Triton X-100 using carbon fibers was performed as follows. Carbon fiber bundles (3000 fibers) were chopped to 1 cm length (ca. 0.0026g) and the fibers were transferred to a 7 mL vial to which a 6.00 mL aliquot of an aqueous solution of Triton X-100 was added. The solution was then shaken for 60 min. The carbon fibers settled to the bottom, after which the supernatant solution was transferred to an empty vial for fluorescence readings.

Desorption

Two different methods were performed for desorption of Triton X-100 from carbon fibers. These will be described in following paragraphs.

Sequential extraction. The fibers left in the vial were sequentially extracted with three portions of 1 mL of methanol or acetonitrile. The solvent was then concentrated by evaporation under N_2, and 20 μL were injected into the HPLC for quantification.

Desorption in a new interface

For this purpose, a stainless steel union steel union tee (Swagelok, 1/16") was connected to the 6 port valve in place of the SPME/HPLC interface. After extraction, the fibers were inserted into the union tee (previously filled with solvent) through one of the ends, and the tee was then reconnected to the valve. After 5 minutes, the valve was switched for 0.5 min to the inject position by which the solvent was directed through the

tee to the HPLC column. The valve was then switched three times every 10 min for 0.5 min to sequentially desorb and quantify any remaining analyte.

6.2.6 Determination of the amount of Triton X-100 remaining in solution

A stock standard solution of Triton X-100 was prepared in deionized water (Milli-Q Plus water purification system, 18MΩ) and diluted to obtain concentrations in the range of 0.01-0.2 mg/L. These standards were used to calibrate the response of the detector. The fluorescence of standards and samples after extraction were determined using a fluorescence spectrometer (Perkin-Elmer, 204-A) set at λ_{ex}= 230 nm and λ_{em}= 310nm.

6.3 Results

6.3.1 Desorption considerations, mass balance.

As mentioned in Chapter 2, desorption in a well-agitated system is the inverse of adsorption. The assumptions are that the initial concentration in the fluid is zero and that the flow rate surrounding the fibers is high. However, in HPLC the latter is not always possible since the solvent flow rate is small. In desorption with the help of a solvent, the following is observed. The target analyte partitions between the solvent and the solid phase, in this case polycrystalline graphite, which is significantly porous. In addition the process is inefficient.

Desorption efficiency was determined for the various polycrystalline graphite types. For this purpose, as well as for calculation of how much was retained on the surface, the solutions before and after extraction were analyzed by spectrofluorometry. The results shown in Table (6-2) reveal that about 75% of analyte were adsorbed onto the surface. Only 24% and 3% of the initial amount (GCP and CF respectively) were desorbed in the interface. Therefore, it can be estimated that the desorption efficiency is about 32% and 4% for GCP and CF respectively.

The differences between the % desorbed form carbon fibers using different desorption modes are discussed later in this section.

Table (6-2). Adsorption and desorption recovery for glassy carbon and carbon fiber. % Desorbed refers to the percent of the amount initially in solution.

	% Adsorbed obtained by fluorimeter	% Desorbed Obtained by HPLC	Desorption mode
GCP	75	24	
CF	75	3	SPME interface
		24	Extraction with MeOH
		19*	New interface

* Large RSD

Figures (6-4) and (6-5) show the amount detected versus the initial amount of Triton X-100 in solution, and the concentration left in the vial versus the initial amount of Triton X-100 in solution, for polished glassy carbon (GCP) and carbon fibers (CF), respectively. Figure (6-4) shows that both, the amounts remaining in solution and the amount detected, are linear in the range of study (0-900 ng). This is important with regards to potential analytical applications. Even though there is an incomplete desorption the amount detected is linearly related to the initial concentration.

The linear dynamic range obtained for glassy carbon was 0.50-150 μg/L (section 4.3.3). If adsorption onto the surface of glassy carbon is the mechanism, it is expected that the LDR would be limited by the surface area.

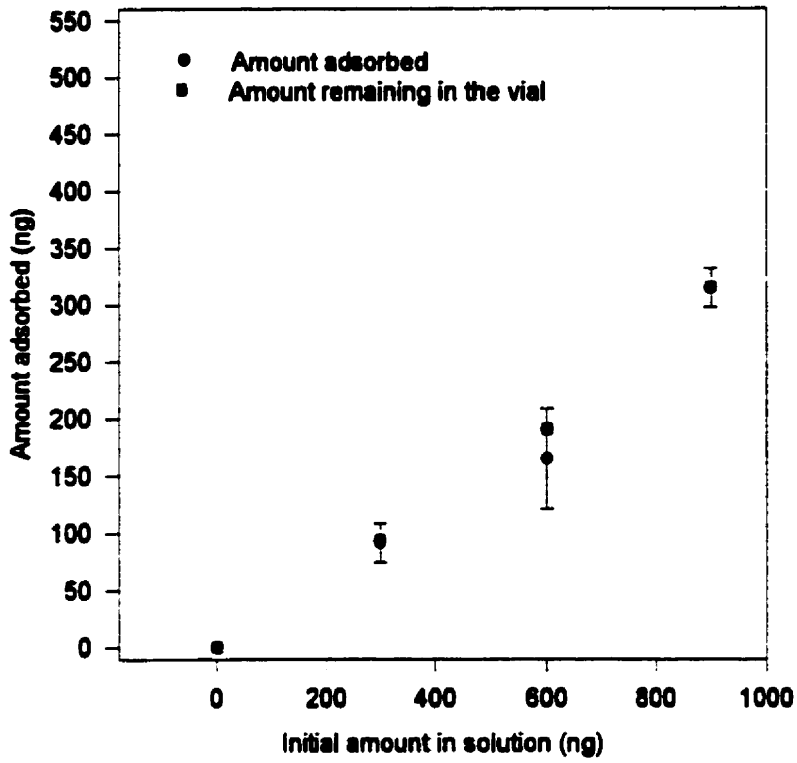


Figure (6-4). Mass balance of Triton X-100 using polished glassy carbon rod. Mean and one standard deviation of three repetitions.

In addition, at low concentrations an exhaustive extraction does not occur since 25 % is left in the vial, regardless of the initial concentration. These observations are important for the use of this solid support for analytical purposes.

Data plotted in Figure (6-5) show that for CF, saturation is obtained at lower concentration since the amount left in the vial is close to the initial concentration, i.e. very little is adsorbed. But it is noted that the amount detected is linearly proportional to the initial concentration nevertheless.

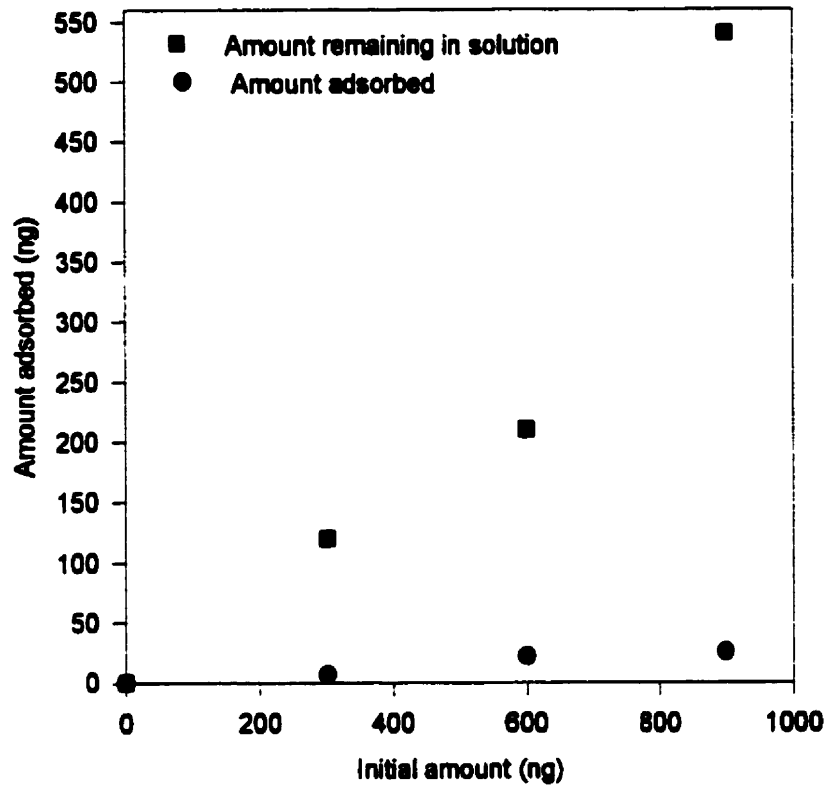


Figure (6-5). Amount of Triton X-100 adsorbed and desorbed from carbon fibers (CF). Desorption took place in the SPME/HPLC interface. The values are the average of three repetitions.

Desorption process for carbon fibers

In initial experiments with CF, it was observed that as desorption time increased, the amount detected decreased. These unexpected results can be explained by the fact that the solvent was lost by capillary action out of the interface; consequently the amount remaining in the interface decreased.

Experiments were carried out to try to prove that the low detectable amount was due to this problem, and not due to poor desorption. After the extraction procedure

described in section 6.2.3, three sequential extractions with methanol were performed on carbon fibers. After evaporation, the solvent was injected into the HPLC. The results were similar to glassy carbon (24% desorbed). In order to see the effects of the solvent in desorption, sequential extractions were also performed with acetonitrile, but no differences from the methanol were found. It is thus concluded that poor desorption from CF was due to loss of solution as described above and not to poor desorption by desorption solvent.

Dynamic desorption using the new interface. The dynamic desorption using the three way tee improved desorption as much as 19 %. The results were however not better than when using sequential extraction (24 %). This can be explained by the fact that the fibers were exposed to methanol for 5 min, and then for 10 min with the mobile phase, which contained water, thus decreasing the solvent power.

Kinetics of desorption

The desorption profile shown in Figure (4-10) illustrates that the amount desorbed after 30 min does not increase when using PLC; a complete desorption seems thus to be achieved. However, blank runs after each extraction demonstrated that desorption of Triton X-100 from polycrystalline graphites was incomplete.

The desorption rate constant was obtained for Triton X-100 using PLC assuming a first order kinetics, the values shown in Figure (4-10) were used to obtained Figure (6-6). The rate constant determined from the slope was 0.0036 min^{-1} (6.0×10^{-5} s^{-1}).

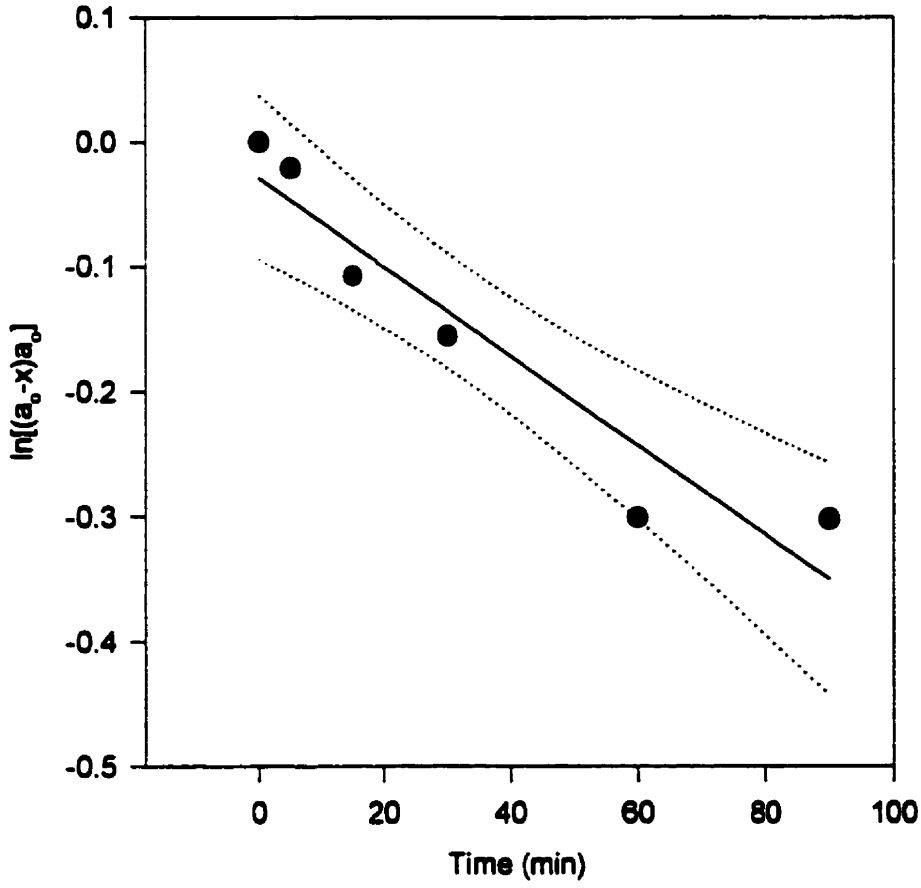


Figure (6-6). Kinetics of desorption of Triton X-100 from PLC. Data were obtained from Figure (4-10), Chapter 4. Linear regression is shown (y = -0.02 – 0.0036x) with 95% confidence intervals.

6.3.2 Calculations of partition coefficients from adsorption isotherms and surface-based partition coefficients

Calculation of partition coefficient from adsorption isotherms

The adsorption isotherms of Triton X-100 on GCP and PLC are illustrated in Figure (6-7). It is apparent that the adsorption of Triton X-100 onto GCP is higher than

on PLC, which could be associated with the higher surface area of GCP. The adsorption isotherms were obtained at very dilute concentrations below the critical micelle concentration 150 mg/mL (Sigma product information). Although the data can be described by any of the isotherms shown in Figure (6-1), it is more likely that if a monolayer is formed, Triton X-100 adsorption will fit the type A isotherm, as the surface of polycrystalline graphites is considered hydrophobic. Hence the alkylphenol part of the molecule of Triton X-100 will be adsorbed onto the surface of the GCP and PLC.

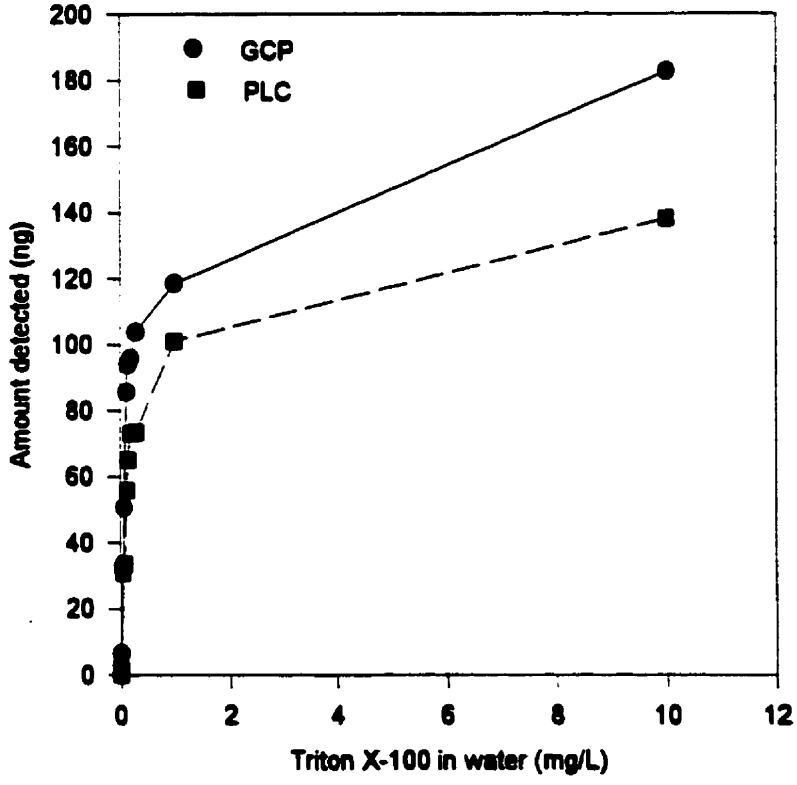


Figure (6-7). Adsorption isotherm of Triton X-100 for glassy carbon polished (GCP) and pencil lead coated (PLC). Each point corresponds to an average of two repetitions.

Moreover, surface saturation is observed at a concentration of approximately 1 mg/L of Triton X-100. Despite the fact that the surface area limited the amount adsorbed, a linear dynamic range of three orders of magnitude (0.5-150 μg/L) was obtained for both polycrystalline graphites (Chapter 4).

It has been suggested that adsorption of surfactants on carbon black follows a Langmuir isotherm (Kochkodan et al., 1996). The data presented in Figure (6-7) were plotted according to equation (3-2), in which $1/C_{sA}$ versus $1/C_{wA}$ should be a straight line with a slope of $1/C_{s\max}K_A$ and an intercept of $1/C_{s\max}$ (Figure 6-8). The values of C_{sA} were obtained using the data presented in Table (6-3).

Table (6-3). Physical description of the surface of PLC, GCP and CFU.

	Specific surface area (m^2/g)	Sample size (g) ± 0.0001	Area exposed to solution (cm^2)±	Pore size* (Å)
PLC	4.5 ±0.9	0.0045	198 ± 40	65
GCP	8.2 ±1.6	0.0105	861 ± 172	16
CF	1.3 ±0.3	0.0026	35 ± 7	33

*Pore size refers to the average of the pore distribution obtained by the BET method.

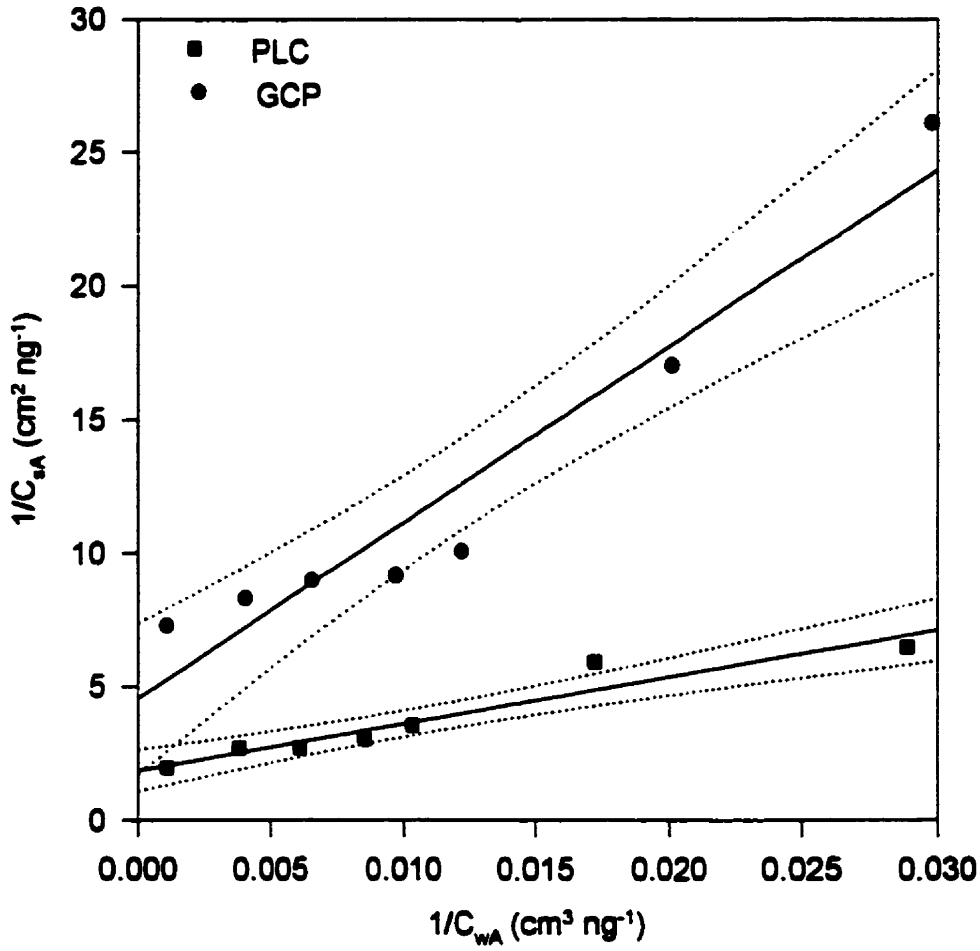


Figure (6-8). Plot of $1/C_{sA}$ versus $1/C_{wA}$ for both, pencil lead (PLC) and glassy carbon (GCP). Linear regression is shown with 95% confidence intervales (dotted lines).

From these plots, the values of $C_{s\,max}$ and K were determined for both types of carbon and are summarized in Table (6-4).

Table (6-4). Values of equilibrium constants and maximum surface coverage for GCP and PLU obtained from the Langmuir isotherm.

	$C_{s\,max}$ (ng/cm^2)	K_A (ng^{-1} cm^3)	R^2
GCP	0.22± 0.08	0.007 ± 0.001	0.942
PLC	0.57± 0.17	0.010 ± 0.001	0.952

Although there are large uncertainties in these values, it is apparent that the maximum adsorbable amount ($C_{s\,max}$) on the PLC is greater that that on GCP. The specific surface area of GCP is higher than PLC (see Table 6-1), however GCP pore size is smaller than PLC (16 vs. 33). The actual surface that is exposed to solution may be smaller for GCP than PLC.

The values of K_A were different by more than one standard deviation. This is expected, since K_A is an equilibrium constant for the adsorption of the surfactant on carbon in both cases, however, pencil lead contains resins which may affect the apparent value of K_A.

Calculation of surface-based partition coefficient, K_{ds}.

A partition coefficient of the analyte on the PG and sample can be calculated accordingly to equation (2-8) which considers the surface of the solid support instead of volume. Partition coefficients of Triton X-100 were calculated with the values shown in Table (6-3) and the data shown in Figure (6-4). The values are shown in Table (6-5) as well as the values of K_A (obtained from the adsorption isotherms). Despite the differences in the units, the same trend is observed, the values of the partition coefficient are larger for PLC followed by GCP. Because the chemical properties of the PG surface

are similar, the values of the partition coefficient constants are in the same order of magnitude.

Table (6-5). Surface-based PG/water partition coefficients (K_{ds}) for Triton X-100 obtained.

PG	K_{ds} (cm^{-1})	Adsorption isotherm K_A (ng^{-1} cm^3)
PLC	0.091	0.010 ± 0.001
GCP	0.021	0.007 ± 0.001

Yang et al. (1998), calculated the values of K_{ds} for PCB using PDMS fibers (100μm and 7μm thickness) considering their geometrical surface area. The values range form 1.6 for PCB-209 to 76 for PCB-8. However, the values cannot be compared since units of K_{ds} are not provided. It is important to note that the values of K are larger than the values of K_{ds} by 10^3-10^5 orders of magnitude. Thus the assessment of the effectiveness of the PG as solid support based on the values of K_{ds} may not be realistic since the LOD are similar to those obtained using PDMS/DVB and Carbowax/DVB fibers.

Kinetics of adsorption

The extraction profile using GCP was shown in Figure (4-9). Using this data, and assuming first-order kinetics, Figure (6-9) is obtained. The rate constant, k, calculated from the slope of the curve is of 0.0043 min^{-1} (7.0×10^{-5} s^{-1})

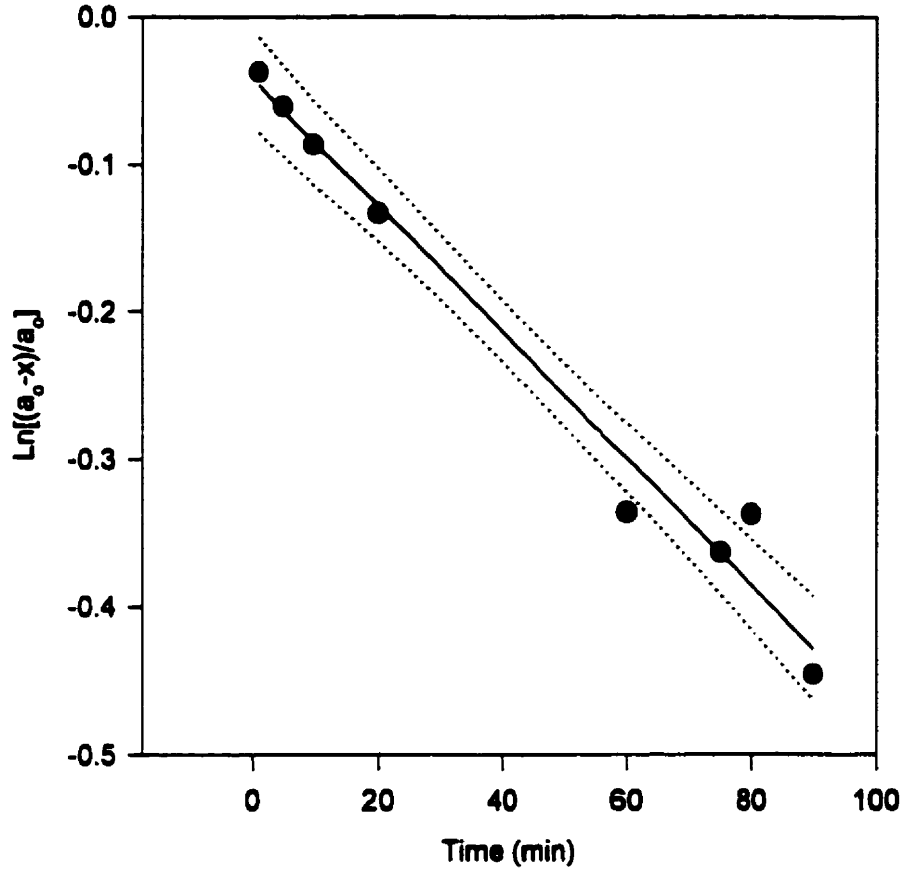


Figure (6-9). Kinetics of adsorption of Triton X-100 onto glassy carbon polished. The data were obtained from Figure (4-9). y=-0.04 – 0.0043x.

6.3.3 Effect of modification of carbon fiber surfaces on the adsorption/desorption efficiencies.

It was hypothesized that a change in the functional groups present on the surface of carbon fibers could modify the adsorption/desorption process due to the change in polarity of the surface. Nitric acid treatment introduces carboxylic and phenolic functional groups onto the fibers (Pittman, 1997), conferring some polarity to the surface. Further treatment such as reduction with hydrogen may confer hydrophobicity to the surface. Moreover, it has been demonstrated that oxidation either with nitric acid or air increases the surface area by forming crevasses in the surface (Pittman et al., 1997).

Nonionic surfactants appear to adsorb more efficiently onto hydrophobic surfaces than onto hydrophilic ones (Rosen, 1989). Nevertheless, it has been reported that nonionic surfactants adsorbed onto silica by hydrogen bonding between SiOH groups on the surface and the oxygen in the ethoxy chain such as in Figure (6-1) case IIIC.

It is believed that oxidation will promote the interactions between the hydrophilic moiety of the surfactant and the carbon as shown in Figure (6-1) case IIIC, whereas hydrogen treatment will promote hydrophobic interactions (case IIIA), which also will determine what moiety is towards the aqueous solution. In addition, the effect of the desorption solvent will be different in case A and case C thus the desorption efficiency.

Previous results suggested that desorption was a limiting factor in the analysis, since only 24 % of Triton X-100 adsorbed was desorbed.

The data in Table (6-6) show the amount of Triton X-100 adsorbed by carbon fibers calculated by measuring the amount remaining in solution after removal of the fiber. The amount adsorbed obtained after successive extractions from CF followed by

HPLC analysis represents the amount that is desorbed by this procedure. The desorption efficiency (% desorption) was calculated as the ratio between the amount desorbed and adsorbed.

Surface treatment improves the amount adsorbed by the carbon fiber (1.5×10^{-4} untreated and 2.1×10^{-4} treated) with no difference among treatments. It was observed that untreated fibers gave higher desorption efficiencies, but the differences are not significant. Also, the H_2 reduction of the surface gives lower desorption efficiency, which could be explained by the higher affinity of the nonionic surfactant towards non-polar interfaces which reduced the amount desorbed. However, the numbers are not significantly different from the other treatments.

Table (6-6) Effect of surface treatment on carbon fibers in the adsorption and desorption process. Numbers in parenthesis refer to %RSD of at least three repetitions.

Treatment	Amount adsorbed (μg ads/μg CF)		% Desorption
	By fluorimetric determination of the supernat	By successive extraction followed by HPLC	
Untreated (CFU)	1.5×10^{-4}(8)	9.3×10^{-5}(2)	62 (8)
Oxidation with HNO_3 (CFA)	2.1×10^{-4}(11)	1.1×10^{-4} (20)	54 (16)
Oxidation with HNO_3 followed by reduction with H_2 (CFH)	2.1×10^{-4}(11)	9.2×10^{-5}(6)	46 (16)
Heating in an N_2 atmosphere at 350°C (CFN)	2.1×10^{-4}(0)	1.1×10^{-4} (nd)	52 (nd)

Surfaces of the treated fibers were characterized by x-ray photoelectron spectroscopy. Several lengths of fibers were cut and attached to the analysis stub with carbon tape. Two areas were analyzed on each sample and the table reports the average of the two results in atomic % (Pleizier, 1999). The results are shown in Table (6-7).

Table (6-7). XPS results of the CF chemically treated. The values is the average of two areas analyzed per sample in atomic %.

Sample	CFN(1)	CFA(1)	CFH(1)	CFN(2)	CFH(2)
Total C	73.0	87.7	81.9	90.2	90.9
Total Oxygen	22.2	8.4*	14.6	7.4	6.7
Nitrogen	2.8	3.2	2.4	2.4	2.4
Sodium	2.0		1.1		
Total C/O ratio	3.3	10.4	5.6	12.2	13.6

* One peak at 532.8 eV

The last two columns represent two CF bundles treated three days prior to the XPS analysis whereas the first three were treated one year prior to the analysis. The ratio of the total C/O ratio is presented in the last row. It is observed that the surface gets reoxidized with time as the ratio decreased from 12.2 to 3.3 for the CFN and 13.6 to 5.6 for CFH. The value of CFA may not be significant since the total oxygen was obtained from one peak at 532.8 eV. It is also observed that treatment of the fibers either by pyrolysis with N_2 (CFN) or reduction by H_2 (CFH) does reduce the amount of oxygen on the surface of the fibers. Nevertheless the changes are not significantly different from one treatment to another. This is in agreement with the adsorption/desorption results, in which significant differences were not seen.

The surfaces of the treated carbon fibers were observed by SEM at 3000 magnification and are shown in Figure (6-10a-d). Figure (6-10a) shows a fiber untreated. No apparent effects from the acid treatment or heating at 350° C in an inert atmosphere (Figure 6-10c, d) were observed. Treatment of the fibers with hydrogen changed the surface morphology as observed by some crevasses (Figure 6-10e), however, such alteration did not change the adsorption/desorption efficiency.

These morphological changes have been also reported in the literature in which the surface was modified after heating the fiber at 500°C for 1 h under an air atmosphere (Lee et al., 1997) of after nitric acid oxidation (Pittman et al., 1997).

It is believed that a truly reduced surface is not possible since reoxidation could occur during manipulation prior to extraction which explains the similarity in the results.

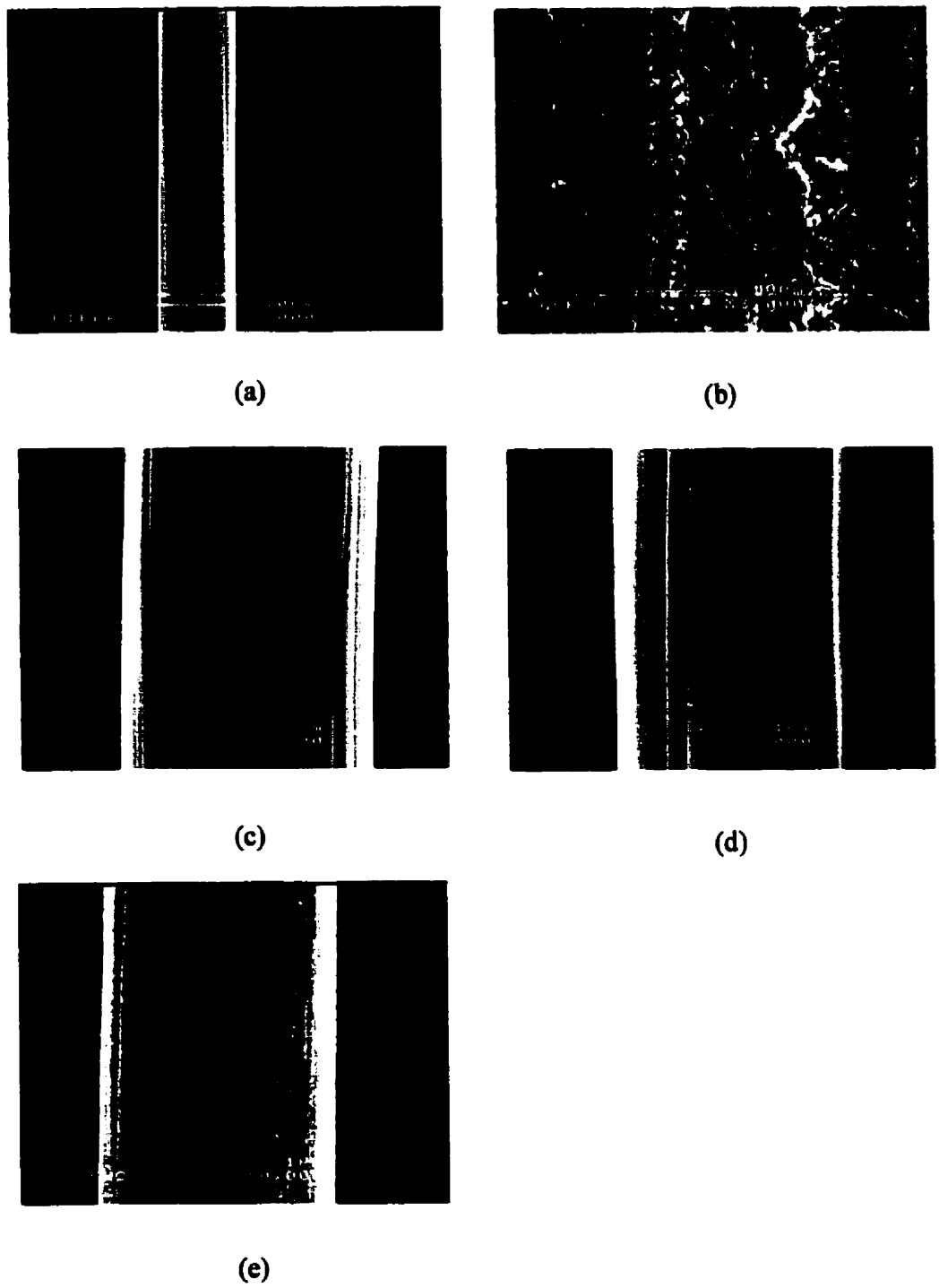


Figure (6-10). Scanning electron macrographs of carbon fibers chemically treated. (a) untreated and (b) GCP at 3000 x magnification; (c) nitric acid treatment, (d) pyrolysis and (e) hydrogen reduced at 10 000 x.

6.3.4 Surface coverage

It has been proposed that adsorption of compounds onto graphite occurs through physisorption and that dispersive interactions are the predominant interactions (Larkins, et al., 1993). This distinguishes extractions with polycrystalline graphites from normal SPME fibers. Saturation of the surface was observed at 150 μg/L of surfactant for both PL and GC.

It is possible to estimate surface coverage by Triton X-100 molecules. Theoretical molecular areas for Triton X-100 were calculated using HyperChem molecular modelling software, which calculates the smallest possible box that can enclose the molecule. Triton X-100 is a mixture of molecules varying only in the number of ethoxy units (n=0-20); the following calculation sets n=10. Two conformations were considered, the hydrophilic part elongated (Figure 6-11a) and in a coil conformation (Figure 6-11b). The dimensions of the box are presented in Table (6-8). The areas occupied by the molecule in different axes were calculated assuming flat orientation and considering two axes.

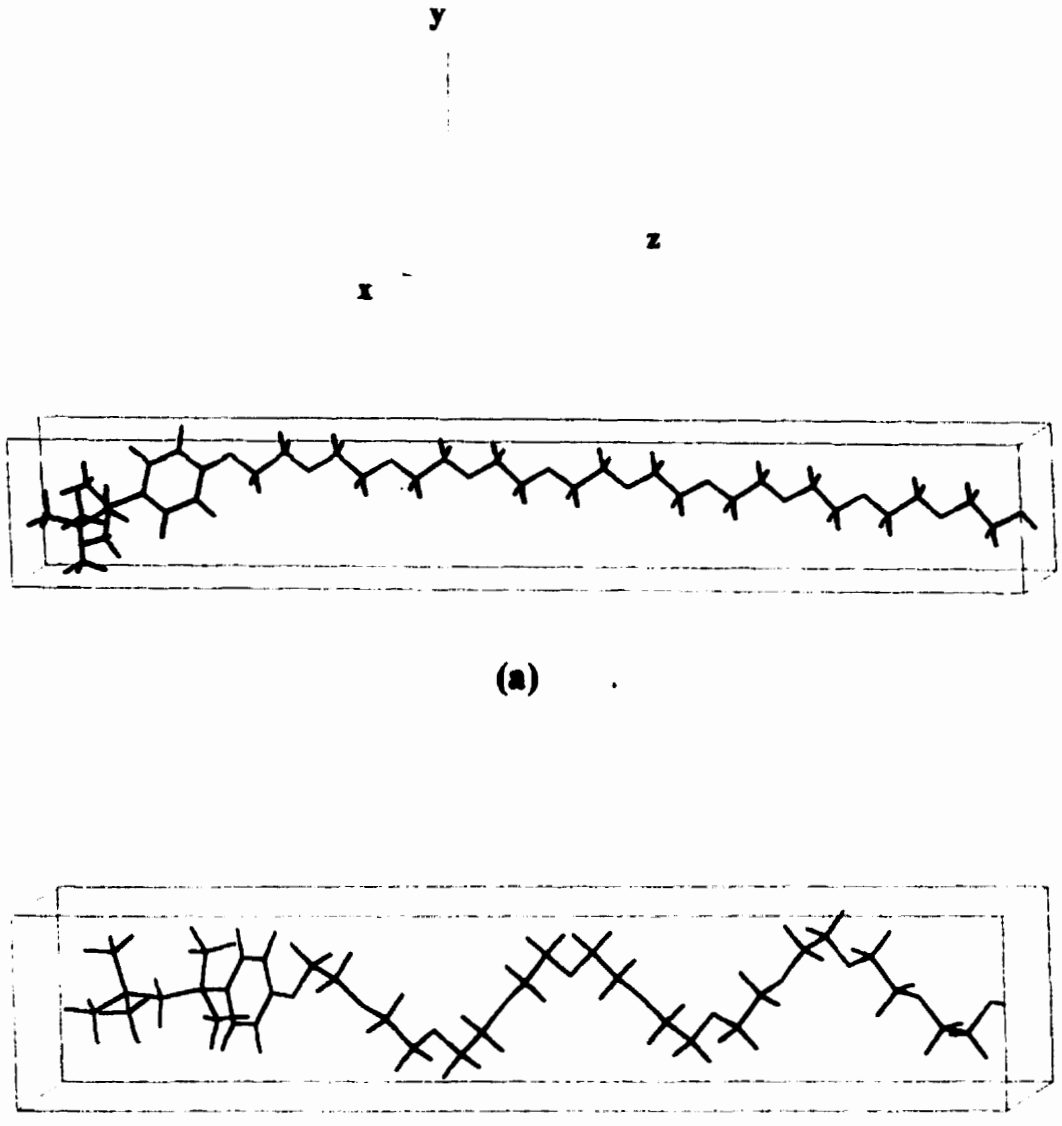


Figure (6-11) Triton X-100 molecule obtained by HyperChem program. Two conformations are shown one with the hydrophilic part elongated (a) and in a coil conformation (b).

Table (6-8). Dimensions of the box (Å) which enclosed the molecule of Triton X-100

Axes	Elongated (Figure 6-8a)	Coil (Figure 6-8b)
X	6.51	8.78
Y	5.77	5.67
Z	45.41	33.81

The saturation coverage Γ_{sat} was calculated taking into account the area occupied by Triton X-100 at 0.1 $\mu g/cm^3$ and assuming one of the orientations shown in Figure (6-10). It was assumed that the molecules adsorbed in one of the three orientations shown in Figure (6-11) and that there were no interactions between them.

The surface coverage Γ_{sat} of the carbon by the Triton X-100 was calculated assuming a monolayer coverage, for each of the three orientations. The coverage varied from 52 $pmol/cm^2$ for the case where the molecules were extended and lying flat (6-11a); to 87 $pmol/cm^2$ for the case where the molecules were in a coiled configuration lying flat (Figure 6-11b), to 334 $pmol/cm^2$ for molecules in the coiled conformation with alkylphenol oriented to the surface and 441 $pmol/cm^2$ with the alkylphenol oriented to the surface but with the ethoxy chain elongated.

These results can be compared to the actual surface coverage Γ_{obs}, which at an aqueous concentration of 0.1 $\mu g/cm^3$ was found to be 2.0 $pmol/cm^2$ of exposed surface for GCP and 26 $pmol/cm^2$ of exposed surface for CF. The values of Θ_{obs} ($= \Gamma_{obs}/\Gamma_{sat}$) provide an indication of the fraction of the surface covered.

In the case of GCP, the values of Θ_{obs} ranged from 3.4×10^{-3} to 1.9×10^{-2}. This may indicate that the surface is in fact quite porous and that the monolayer coverage is

not achieved. For the CF the values of Θ_{obs} ranged from 7.9 x 10^{-2} to 4.7 x 10^{-1}, indicating essentially a non-porous surface or less than monolayer coverage.

The values of Θ_{obs} can be corroborated with the scanning electron micrographs shown in Figure (6-10), where GCP and CF are shown at same magnification (3000x). GCP shows larger porosity than the carbon fibers and more importantly to the measured surface areas in Table (6-1), which are in agreement with this trend.

Table (6-9) Adsorption data for Triton X-100 onto glassy carbon rod and carbon fibers

Adsorbant	Area ($Å^2$)	Γ_{sat} (pmol/cm^2)	Γ_{obs} (pmol/cm^2)	Θ_{obs}
GCP	295.6[1]	56	1.99	1.9 x 10^{-2}
	191.7[2]	87		1.2 x 10^{-2}
	49.8[3]	334		3.4 x 10^{-3}
CF	295.6	56	26	4.7 x 10^{-1}
	191.7	87		3.1 x 10^{-1}
	49.8	334		7.9 x 10^{-2}

Theoretical molecular area assuming flat orientation 1(x,z elongated), 2(y,z coiled) and 3(xy, coiled).

Our results can be compared to those obtained by Bossoletti et al. (1995) who studied the adsorption of nonionic surfactants (Nonylphenyl polyethoxylates (NP) and polyoxyethylene (20) cetyl ether (Brij 58)) at the carbon-black water interface. It was concluded that the molecules were adsorbed by the alkyl chain, and the ethoxy units were directed to the aqueous medium in a coil conformation, stabilizing the molecules.

Chapter 7

7 Conclusions

Chapter 7

7 Conclusions

7.1 Summary of conclusion

7.1.1 Direct SPME using polycrystalline graphites

Application to the determination of nonionic surfactants

Alternative solid phases were tested for SPME/HPLC analyses that replace the conventional polymeric fibers currently used. The solid supports were polycrystalline graphites, such as glassy carbon and pencil lead.

A method for the determination of a nonionic surfactant (represented by Triton X-100) was developed. It included a direct SPME extraction from aqueous samples coupled to HPLC-fluorescence detection. The analytical method was evaluated by comparing the results with those obtained using PDMS/DVB and CW/TR fibers. The LDR was of three orders of magnitude (0.5-150 μg/L) and the LOD was 0.5 μg/L for both glassy carbon and pencil lead. Problems encountered using these phases were related to incomplete desorption which limited the limit of detection of the method. This however did not prevent their use as SPME solid phases, since each one of them could be cleaned prior to the next analysis. The main advantage was the chemical and physical resistances of the carbon rods, since they remained intact even after a hundred extractions per rod.

Determination of pentachlorophenol using glassy carbon for direct SPME/HPLC

A method for the determination of pentachlorophenol from aqueous solutions using glassy carbon for SPME/HPLC-UV detection was developed. The results obtained were comparable to those reported in the literature using SPME/GC-FID and SPME/GC-MS. The LDR was 0.5-100 μg/L and the LOD 0.5 μg/L. The method was applied to spiked river samples (which contained 6.5 mg of C/L) and model solutions containing fulvic acids in the concentration range of 5-100 mg/L. Results showed that the presence of fulvic acids did not affect the extraction efficiency at a concentration up to 10 mg/L, i.e. no matrix effect was observed in the real samples. Therefore, glassy carbon can be used as a solid support for extraction of pentachlorophenol in aqueous solutions maintained at a pH as low as 1.0 (which is not recommended for polymeric fibers). No derivatization was necessary.

7.1.2 SPME/HPLC derivatization using PDMS/DVB fiber

A novel method for the determination of a nonionic surfactant of the linear alcohol ethoxylated class was developed. The method consists of direct extraction using a PDMS/DVB fiber and on-line derivatization using 1-naphthoyl chloride-fluorescence detection. A linear dynamic range of two orders of magnitude (0.1-10 mg/L) was obtained which was attributed to the low esterification yield. Moreover, it was found that the presence of another alcohol diminished the amount detected. This was attributed to the limited amount of surface available.

7.1.3 SPME/HPLC derivatization using polycrystalline graphites

Another derivatization approach was developed for the determination of Brij 56. This method uses polycrystalline graphite as sorbent to perform a simultaneous derivatization-extraction in an aqueous sample. The method proved to have a lower LOD than previously described methods. A simgoidal response was observed, however, the response was linear in the range of 0.01 to 0.1 mg/L. This response was observed for glassy carbon and pencil lead. The main advantage is the resistance of the glassy carbon towards derivatization reagents such as 1-naphthoyl chloride.

7.1.4 Adsorption and desorption considerations

Direct SPME from aqueous samples using polycrystalline graphites was successfully applied to the determination of Triton X-100 and pentachlorophenol. It was assumed that the analyte was adsorbed onto the surface of the polycrystalline graphite. The data were fit to a Langmuir isotherm model, and the monolayer concentration of Triton X-100 on the surface was calculated to be 0.22 and 0.57 ng/cm^2 for GCP and PLC, respectively. The values of K_A for both were in the same order of magnitude (0.007 and 0.01 cm^3/ng respectively).

A surface-based partition coefficients, K_{ds}, were also obtained, the values were 0.021 and 0.091 cm^{-1} for GCP and PLC, respectively. Although the values of K_{ds} cannot be compared with those obtained for K_A, they followed the same trend with respect to carbon type.

The rate constants of adsorption and desorption, assuming first order kinetics were obtained. The values using glassy carbon glassy carbon were 0.0043 min^{-1} for adsorption and 0.0037 min^{-1} for desorption.

7.1.5 Surface modifications

Carbon fibers were chemically modified by three different methods: oxidation with nitric acid, heating the fibers to 350°C under N_2 and heating the fibers to 350°C under hydrogen. Adsorption/desorption of Triton X-100 on the different fibers showed that adsorption was improved when the surface was treated. However, Triton X-100 desorbed more efficiently from untreated fibers (62 ± 5 %) than from those subjected to oxidation with acid (54 ± 6 %) or reduction (46 ± 7 %). The values however were not significantly different.

Chemical modifications did however change the morphology of the fiber as evidenced by the scanning electron micrographs, which showed presence of crevasses on the surface. No changes were observed at 10 000x magnification for fibers treated by the other methods.

7.2 Future contributions

7.2.1 Use of polycrystalline graphites for direct extractions

The use of polycrystalline graphites was successfully applied to the determination of a nonionic surfactant and pentachlorophenol by HPLC. Thus, it would be interesting to apply these polycrystalline graphites to the determination of compounds within a wider range of polarities such as PAH, carboxylic acids and amines.

7.2.2 SPME-HPLC derivatization

Derivatization procedures are commonly applied in HPLC analysis. Therefore, coupling SPME/HPLC with derivatization will expand the application of SPME to HPLC analysis. Derivatization schemes could be developed for carboxylic acids, alcohols and amines. As an example, haloacetic acids and fatty acids using florescent reagents such as PDMA or proteins using dansyl cadaverine (Haugland, 1996).

Although PG has high chemical and mechanical resistances, its application to derivatization is currently limited due to the low LDR. It is thus important to decipher the mechanism of simultaneous derivatization-extraction using PG so as to obtain a wider linear response.

Moreover, from the commercial point of view, PG could be used as an SPME support containing a derivatization reagent. This would be an extension of SPE cartridges containing derivatization reagents for the determination of ketones and aldehydes.

7.2.3 Surface modification

Despite the poor performance of carbon fibers, their flexibility, chemical and mechanical stability are advantageous to HPLC applications. It is of interest to further investigate surface modifications such as attachment of different groups that will change the adsorbent properties of the surface and expand its applicability. It is foreseen that low detection limits could be obtained by increasing the exposed surface area of carbon fibers to the sample. This could be achieved by increasing the number of filaments, since carbon fibers are commercially available of bundles containing up to

12 000 filaments, thus the sensitivity of the method using them will be greater that using the polymeric fibers.

Desorption was improved up to 50% by sequentially extracting the fibers with methanol. The design of a SPME/HPLC interface which could be applied to carbon fibers will expand their use in analytical chemistry since dynamic desorption could then be used.

References

Aikawa, B., Burk, R. C., Determination of chlorinated acetic acids in drinking water by in-situ derivatization and solid phase microextraction. Intern. J. Environ. Anal. Chem., 66 (1997) 215-224.

Aranda, R., Burk, R.C. Determination of a non-ionic surfactant by solid-phase microextraction coupled with high performance liquid chromatography and on-line derivatization. J. Chromatogr. A., 829 (1998) 401-406.

Arthur, C.L., Pawliszyn, J. Solid phase microextraction with thermal desorption using fused silica optical fibers. Anal. Chem., 62, (1990) 2145-2148.

Aviram, A., Li, L.,. Pomerantz, M., Roland, P. A., Schrott, A. G. A simple and specific method of attaching molecules to graphite. Langmuir, 11 (1995) 2049-2053.

Bossoletti, L., Ricceri, R., Gabrielli, G. The adsorption of polystyrene sulfonate and ethoxylated non-ionic surfactants at carbon black-water interface. J. Dispersion Science and Technology, 16(3&4) (1995) 205-220.

Boyd-Boland, A. A., Chai, M., Luo, Y. Z., Zhang, Z., Yang, M.J., Pawliszyn, J. B., Gorecki, T. New solvent-free preparation techniques based on fiber and polymer technologies. Environ. Sci. and Tech., 28(13) (1994) 569A-574A.

Boyd-Boland, A. A., Pawliszyn, J. B. Solid phase microextraction coupled with high-performance liquid chromatography for the determination of alkylphenol ethoxylate surfactants in water. Anal. Chem., 68 (1996) 1521-1529.

Buchholz, K. D., Pawliszyn, J. Optimization of solid phase microextraction conditions for determination of phenols. Anal. Chem., 66 (1994) 160-167.

Burchell, T., D. Carbon Materials for advance technologies. Elsevier Science Ltd. UK. First edition. ISBN 0-08-042683-2, 1999. 95-118.

Cai, Y., Bayona, J. M. Determination of methylmercury in fish and river water samples using in situ sodium tetraethylborate derivatization following by solid-phase microextraction and gas chromatography-mass spectrometry. J. Chromatogr. A, 696 (1995) 113-122.

Chai, M., Pawliszyn, J. Environment. Sci. Technol. 29 (1995) 693.

Chao, Y., Huang, S. D. Solid phase microextraction coupled with high performance liquid chromatography for the determination of aromatic amines. Anal. Chem., 71 (1999) 310-318.

Chao, Y., Huang, S. D. Determination of hydroxyaromatic compounds in water by solid phase microextraction coupled with high-performance liquid chromatography, J. Chromatogr. A, 835 (1999) 127-135.

Chen J., Pawliszyn, J. B. Solid phase microextraction coupled to high-performance liquid chromatography, Anal. Chem., 67, (1995) 2530-2533.

Chong, S. L., Wang, D., Hayes, J.D., Wilhite, B. W., Malik, A. Sol-Gel Coating Technology for the preparation of solid-phase microextraction fibers of enhanced thermal stability. Anal. Chem., 69, (1997) 3889-3898.

Conte, E. D., Miller, D. W. A solid phase microextraction-electrodeposition device for the determination of putrescine and cadaverine by high resolution gas chromatography. J. High Resol. Chromatogr., 19 (1996) 294-297.

Daimon, H., Pawliszyn, J. Effect of heating the interface on chromatographic performance of solid phase microextraction coupled to high-performance liquid chromatography. Analytical communications, 34. (1997) 365-369.

Desbène, P. L. Portet, F. I., Goussot, G. J. Quantitative trace analysis of surfactant mixtures by reversed-phase high-performance liquid chromatography with refractometric detection. J. Chromatogr. A, 730 (1996) 209-218.

Djozan, D., Assadi, Y. A New porous-layer activated-charcoal-coated fused silica fiber: Application for determination of BTEX compounds in water samples using headspace solid-phase microextraction and capillary gas chromatography. Chromatographia, 45, (1997) 183-189.

Eisert, R., Pawliszyn, J., New Trends in solid-phase microextraction. Critical Reviews in Analytical Chemistry, 27(2) (1997) 103-135.

Gomathi, H., Chandra, N., Sharma, L. R., Rao, G. P. Glassy carbon-surface characterization by electrochemical and spectral methods. Bulletin of Electrochemistry, 11(5) (1995) 248-252.

Gorecki, T., Pawliszyn, J. Effect of sample volume on quantitative analysis by solid-phase microextraction. Part 1. Theoretical considerations. Analyst, 122, (1997) 1079-1086.

Gorecki, T., Yu, X., Pawliszyn, J. Theory of analyte extraction by selected porous polymer SPME fibers. Analyst, 124, (1999) 643-649.

Hage, E., Costa, S. F., Pessan, L. A. Modification of the carbon fiber surface with a copper coating for composite materials with epoxy. J. Adhesion Sci. Technol., 11(12) (1997) 1491-1499.

Heinig, K., Vogt, C., Werner, G. Separation of ionic and neutral surfactants by capillary electrophoresis and high-performance liquid chromatography. J. Chromatogr. A. 745 (1996) 281-292.

Heuchel, M., Jaroniec, M. Comparison of energy distributions calculated for active carbons from benzene gas/solid and liquid/solid adsorption data. Langmuir, 11 (1995) 1297-1303.

Hirohata, Y., Ikita, M., Hino, T., Yamashina, T. Desorption behavior for H_2O from isotropic graphite dipped in water. Carbon, 32(2) (1994) 369-372.

Holt, M. S., McKerrell, E. H., Perry, J., Watkinson, R. J. Determination of alkylphenol ethoxylates in environmental samples by high-performance liquid chromatography coupled to fluorescence detection. J. Chromatogr., 362 (1986) 419-424.

Jafvert, C. T., Van Hoof, P. L., Heath, J. K. Solubilization of non-polar compounds by non-ionic surfactant micelles. Wat. Res., 28(5) (1994) 1009-1017.

Jia, C., Luo, Y., Pawliszyn, J. Solid phase microextraction combined with HPLC for determination of Metal Ions using crown Ether as Selective extracting reagent. J. Microcolumn Separations, 10(2), (1998) 167-173.

Jinno, D., Taniguchi, M., Sawada, H., Hayashida, M. Microcolumn liquid chromatography coupled with solid phase microextraction (SPME/micro-LC) for the analysis of benzodiazepines in human urine. Microcolumn separation techniques developing in Japan. Analusis Magazine, 26(5), (1998) M27-M30.

Kelly, M. T., Larroque, M. Trace determination of diethylphthalate in aqueous media by solid phase microextraction-liquid chromatography. J. Chromatog. A., 841 (1999) 177-185.

Kiewiet, A. T., van der Steen, J. M. D., Parsons, J. R. Trace analysis of ethoxylated nonionic surfactants in samples of influent and effluent of sewage treatment plants by high-performance liquid chromatography. Anal. Chem., 67 (1995) 4409-4415.

Kiewiet, A. T., de Voogt, P. Chromatographic tools for analyzing and tracking nonionic surfactants in the aquatic environment. J. Chromatogr. A, 733 (1996) 185-192.

Kile, D. E. Chiou, R. Effect of some petroleum sulfonate surfactants on the apparent water solubility of organic compounds. Environ. Sci. Technol., 24 (1990) 205-208.

Kim, B. S., Hayes, R. A., Ralston, J. The adsorption of anionic naphthalene derivatives at the graphite aqueous solution interface. Carbon, 33(1) (1995) 25-34.

Knox, J. H., Wan, Q. H. Surface modification of porous graphite for ion exchange chromatography. Chromatographia, 42 (1996) 83-88.

Kochkodan, O. D., Klimenko, N. A., Karmazina, T. V. Thermodynamic characteristics of adsorption of nonionic surfactants onto acetylene carbon black and AG-3 activated carbon. Colloid journal, 58(3) (1996) 330-334.

Kudoh, M., Ozawa, H., Fudano, S., Tsuji, K. Determination of trace amounts of alcohol and alkylphenol ethoxylates by high-performance liquid chromatography with fluorimetric detection. J. Chromatrogr., 287 (1984) 337-344.

Laidler, K. J. Chemical Kinetics. Third edition.Harper & Rox, publishers, New York. ISBN 0-06-043862-2, 1987, 18-53.

Larkins, W. C., Oleski, S. V. Importance of molecular shape in supercritical fluid chromatography using a porous glassy carbon adsorbent stationary phase. J. Microcol. Sep., 5 (1993) 543-550.

Lee, J. S., Kang, T.J. Changes in physico-chemical and morphological properties of carbon fiber by surface treatment. Carbon, 35(2) (1997) 209-216.

Lee, M. R., Lee, R. J., Lin, Y-W., Chen, C. M., Hwang, B. H. Gas-phase postderivatization following solid-phase microextraction for determining acidic herbicides in water. Anal. Chem., 70, (1998) 1963-1968.

Ligor, M., Scibiorek, M., Buszewki, B. New coating surfaces of fibers for solid-phase microextraction. J. Microcolumn Separation, 11(5), (1999) 377-383.

Liu, Y., Shen, Y., Lee, M. L. Porous layer solid phase microextraction using silica bonded phases. Anal. Chem., 69, (1997) 190-195.

Lock, C. M., Chen, L., Volmer, D. A. Rapid analysis of tetracycline antibiotics by combined solid phase microextraction/high performance liquid chromatography mass spectrometry. Rapid commun. Mass Spectrom., 13 (1999) 1744-1754.

Maeda, H., Itami, M., Yamauchi, Y., Ohmori, H. Surface characterization of glassy carbon electrodes anodized in 1-alkanols by their wettability and capacitance. Chem. Pharm. Bull., 44 (1996) 2294-2299.

Maguire, R. J., Batchelor, S. P., Sullivan, C. A., Environmental Toxicology and Chemistry, 14 (3) (1995) 389-393.

Majors, R. E., Raynie, D. E. Sample preparation and solid-phase extraction. LC-GC. 15(12) (1997) 1106-1117.

Mandal, R., Sekaly, A. L. R., Murimboh, J., Hassan, N. M., Chakrabarti, C. L., Back, M. H., Grégoire, D. C., Schroeder, W. H. Effect of the competition of copper and cobalt on the lability of Ni (II)-organic ligand complexes, Part II: in freshwaters (Rideau River surface waters). Analytica Chimica Acta, 395 (1999) 323-334.

Mangani, F., Cenciarini, R. Solid phase microextraction using fused silica fibers coated with graphitized carbon black. Chromatographia, 41(12/12) (1995 678-684.

Marcomini, A., Giger, W. Simultaneous determinations of linear alkylbenzenesulfonates, alkylphenol polyethoxylates, and nonylphenol by high-performance liquid chromatography. Anal. Chem., 59(13) (1987) 1709-1715.

Marcomini, A., Zanette, M. Chromatographic determination of non-ionic aliphatic surfactants of the alcohol polyethoxylate type in the environment. J. Chromatogr. A, 733 (1996) 193-206.

Martos, P.A., Pawliszyn, J. Sampling and determination of formaldehyde using solid-phase microextraction with on-fiber derivatization. Anal. Chem., 70, (1998) 2311-2320.

Matthijs, E., Hennes, E. C., Determination of surfactants in environmental samples. Tenside Surf. Det., 28 (1991) 35-45.

McMurry. Organic Chemistry. Third Edition, Brooks/Cole Publishing Company. ISBN 0-534-16218-5, 1992.

McDermott, M. T., McCreery, R. L. Scanning tunneling microscopy of ordered graphite and glassy carbon surfaces: electronic control of quinone adsorption. Langmuir, 10 (1994) 4307-4314.

Mills, G. A., Walker, V., Mughal, H. Headspace solid-phase microextraction with 1-pyrenyldiazomethane in-fibre derivatisation for analysis of faecal short-chain fatty acids. J. Chromatogr. B, 730 (1999) 113-122.

Miszkiewicz, W., Szymanowski, J. Analysis of nonionic surfactants with polyoxyethylene chains by high performance liquid chromatography. Critical reviews in Analytical Chemistry, 24(4) (1996) 203-246.

Möder, M., Popp, P., Eisert, R., Pawliszyn, J. Determination of polar pesticides in soil by solid phase microextraction coupled to high-performance liquid chromatography-electrospray/mass spectrometry. Fresenius J. Anal. Chem., 363 (1999) 680-685.

Mutlu, M., Hizarciogly, N., Gökmen, V. Patulin adsorption kinetics on activated carbon, activation energy and heat of adsorption. Journal of food Science, 62(1) (1997) 128-130.

Naylor, C., Mieure, J. P., Adams, W, J., Weeks, J. A., Castaldi, F. J., Ogle, L. D., Romano, R. R. Alkylphenol ethoxylates in the environment. JAOCS, 69(7) (1992) 695-703.

Nilsson, T., Baglio, D., Galdo-Miguezm, I., Madsen, J. O., Facchetti, S. Derivatisation/ Solid phase microextraction followed by gas chromatography-mass spectrometry for the analysis of phenoxy acid herbicides in aqueous samples. J. Chromatogr. A, 826 (1998) 211-216.

Nozawa, A., Ohnuma, T. Improved high-performance liquid chromatographic analysis of ethylene oxide condensates by their esterification with 3, 5-dinitrobenzoyl chloride. J. Chromatogr. 187 (1980) 261-263.

Okeyo, P.D., Snow, N. H. Analysis of estrogens and anabolic steroids by SPME with on-fiber derivatization and GC/MS. J. Microcolumn Separations, 10(7) (1998) 551-556.

Pan, L., Adams, M., Pawliszyn, J. Determination of fatty acids using solid-phase microextraction. Anal. Chem., 67, (1995) 4396-4403.

Pan, L. Strategies for the analysis of polar analytes using solid-phase microextraction. Ph.D. Thesis, (1996), Waterloo, Canada.

Pan, L., Pawliszyn, J. Derivatization/Solid-phase microextraction: New approach to polar analytes. Anal. Chem., 69, (1997) 196-205.

Parfitt, G. D., Rochester, C. H. Adsorption from solution at the solid/liquid interface Academic Press Inc. (Londond) Ltd.. ISBN –12—5444980-1, 1983, 105-152.

Pawliszyn, J. Solid phase microextraction, Theory and Practice. Wiley-VCH, Inc. ISBN 0-471-19034-9, 1997, 247p.

Peebles, L. H. Carbon fibers: formation, structure, and properties. CRC Press, Inc. ISBN 0-8493-2450-5, 1995, 203p.

Pierson, H. O. Handbook of carbon, graphite, diamond and fullerenes. Properties, processing and applications. Noyes Publications, 1993, 399.

Pittman, C. U., He, G.-R., Wu, B, Gardner, S. D. Chemical modification of carbon fiber surfaces by nitric acid oxidation followed by reaction with tetraethylenepentamine. Carbon, 35 (1997) 317-331.

Popp, P. Paschke, A. Efficiency of Direct solid-phase microextraction from water-comparison of different fiber types including a new C8-coating. Chromatographia 49(11/12), (1999) 686-690.

Pörschmann, J., Kopinke, F.-D., Pawliszyn, J. Solid phase microextraction for determining the binding state of organic pollutants in contaminated water rich in humic organic matter. J. Chromatogr. A, 816 (1998) 159-167.

Renner, R. European bans on surfactant trigger transatlantic debate. Environmental Science & Technology. 31(7) (1997) 316A-320A.

Rittenhouse, C. T., Olesik, S. V. High performance liquid chromatographic evaluation of a low-temperature glassy carbon stationary phase. J. Liq. Chrom. & Rel. Technol., 19 (1996) 2997-3022.

Rosen, M. J. Surfactants and interfacial phenomena. Second edition, John Wiley and Sons Inc., ISBN0-471-83651-6, 1989, 33-107.

Roy, S., Hanninen, O. Pentachlorophenol: uptake/elimination kinetics and metabolism in an aquatic plant, *Eichhornia crassipes*. Environmental Toxicology and Chemistry, 13 (5) (1994), 763-773.

Ryan, M. D., Bowden, E. F., Chambers, J. Q. Dynamic electrochemistry: methodology and application. Anal. Chem., 66 (1994) 360R-427R.

Schmitt, T. M., Allen, M. C., Brain, D. K., Guin, K. F., Lemmel, D. E., Osburn, Q. W. HPLC determination of ethoxylated alcohol surfactants in wastewater. JAOCS, 67(2) (1990) 103-109.

Scullion, S. D., Clench, M. R., Cooke, M., Ashcroft, A. E. Determination of surfactants in surface water by solid-phase extraction, liquid chromatography and liquid chromatography- mass spectrometry. J. Chromatogr. A, 733 (1996) 207-216.

Socha, A. C., Aucoin, R., Dickie, T., Angelow, R. V., Kauss, P. Candidate substances or Bans, Phase-outs or reductions, multimedia revision. Ministry of Environment and Energy, Ontario, (1993).

Stevenson, F. J. Humus Chemistry genesis, composition, reactions, 1994, Second edition. John Wiley & Sons, Inc. 1994

Subramanian, S., Laskowski, J. S., Adsorption of dextrin onto graphite. Langmuir, 9 (1993) 1330-1333.

Suffet, I. H., MacCarthy, P., Aquatic Humic substances, Influence on fate and treatment of pollutants, 1989, American Chemical Society, Washington.

Supelco Cataloge, 1999 chromatography products.

Toyo'oka, T. Use of derivatization to improve chromatographic properties and detection selectivity of physiologically important carboxylic acids. J. Chromatogr. B, 671 (1995) 91-112.

Tsubokawa, M. Hosoya, J. Kurumada, Reactive & functional Polymers, 27 (1995) 75-81.

Van Doorn, H., Grabanski, C. B., Miller, D. J. Hawthorne, S. B. Solid-phase microextraction with pH adjustment for the determination of aromatic acids and bases in water. J. Chromatogr. A, 829 (1998) 223-233.

Volmer, D.A., Hui, J. P. M. Rapid SMPE/LC.MS/MS, Analysis of N-Methylcarbamate pesticides in water. Arch. Environ. Contam. Toxicol., 35, (1998) 1-7.

Wan, H. B. Chi, H., Wong, M. K. Mock, C. Y. Solid phase microextraction using pencil lead as sorbent for analysis of organic pollutants in water. Anal. Chim. Acta, 298 (1994) 219-223.

Wu, L., Almirall, J. R., Furton, K. G. An improved interface for coupling solid-phase microextraction (SPME) to high performance liquid chromatography (HPLC) applied to the analysis of explosives. J. High Resol. Chromatogr., 22(5) (1999) 279-282.

Wu Y.C., Huang, S-D. Determination of hydroxyaromatic compounds in water by solid-phase microextraction coupled to high-performance liquid chromatography. J. Chromatr. A, 835 (1999) 127-135.

Yang, Y., Hawthorne, S. B., Miller, D. J. Adsorption versus absorption of polychlorinated biphenyls onto solid-phase microextraction coatings. Anal. Chem., 70, (1998) 1866-1869.

Zanette, M., Marcomini, A., Marchiori, E., Samperi, R. High-performance liquid chromatographic-fluorescence determination of aliphatic alcohol polyethoxylates and poly(rhyleneglycol)s in aqueous samples. J. Chromatogr. A. 756 (1996) 159-174.

Zhou, Z.P., Wang, Z. W., Wu, C. Y., Zhan, W., Xu, Y. Sol-gel Method for the preparation of solid-phase microextraction fibers. Analytical letters, 32(8), (1999) 1675-1681.

Zielke, U., Huttinger, K. J., Hoffman, W. P. Surface oxidized carbon fibers: II. Chemical modification, Carbon, 34(8) (1996) 999-1005.

Appendix 1

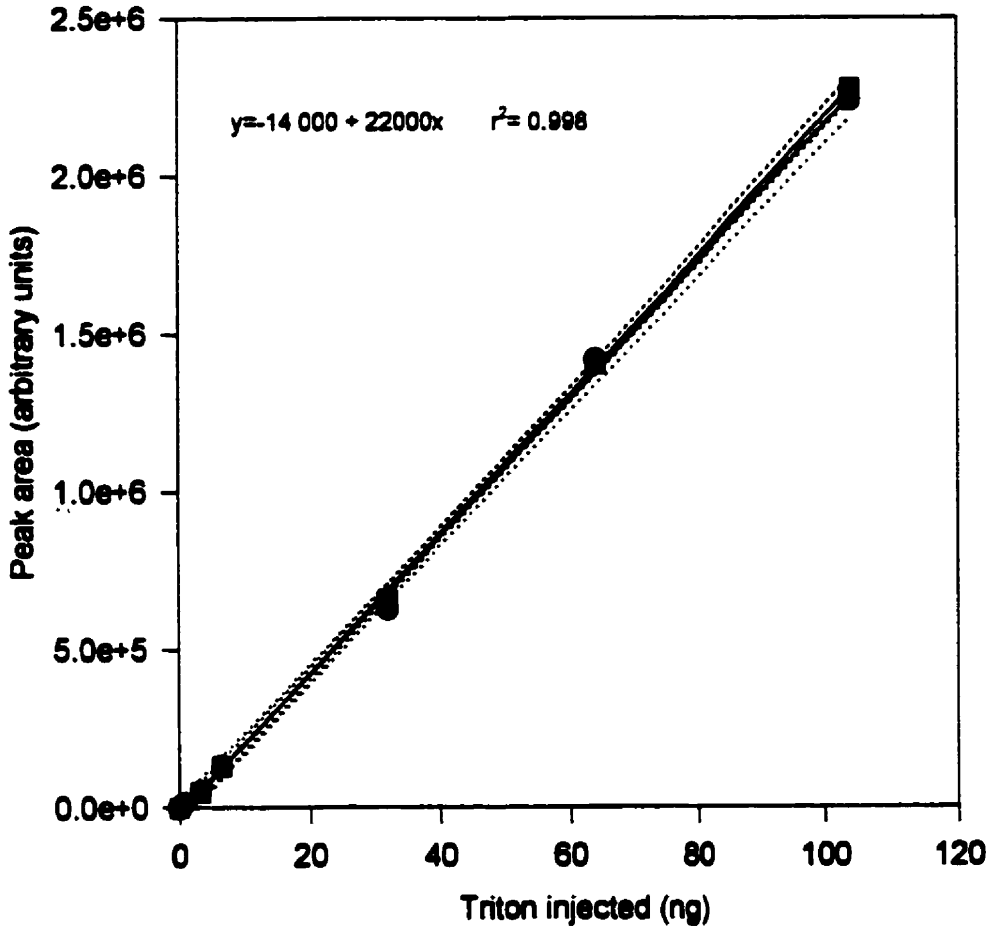


Figure (A1-1). Typical analytical calibration curve for Triton X-100 in methanol. Analysis was performed by RP-HPLC-fluorescence detection at λ_{ex}= 230 nm λ_{em}=310 nm.

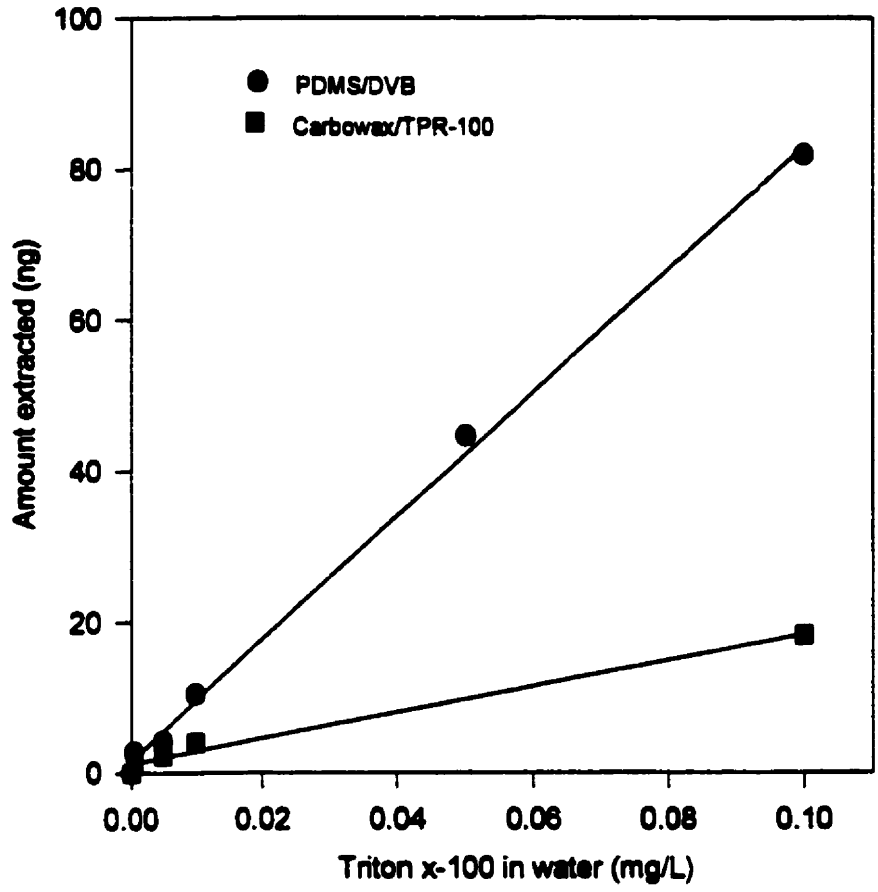


Figure (A1-2). Linear dynamic range of PDMS/DVB and Carbowax/TR fiber for Triton X-100. At 60 min extraction and 5 min dynamic desorption in the SPME/HPLC interface.

Appendix 2

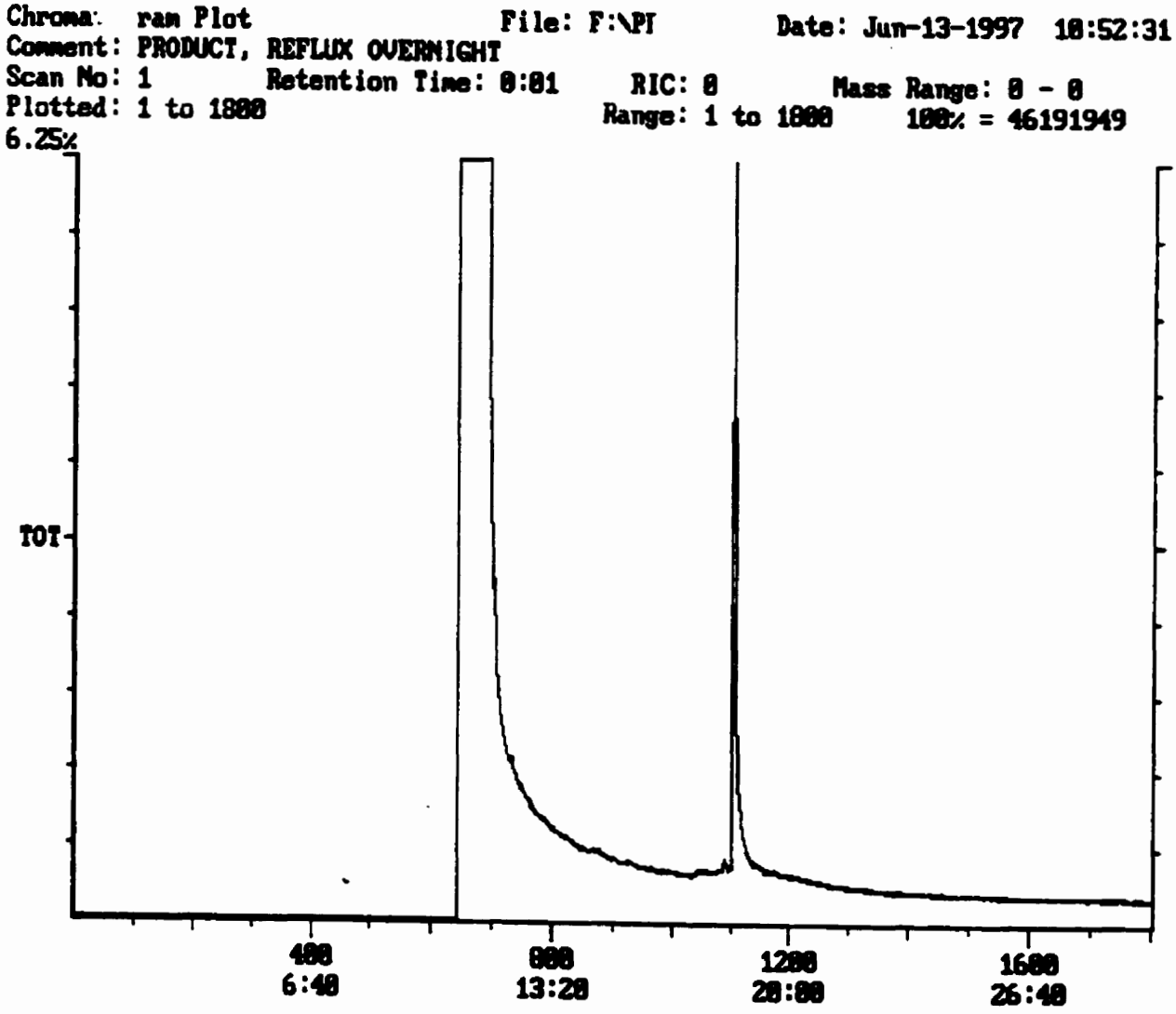


Figure (A2-1). Chromatogram of Napththoyl ester derivative. Analysis was performed by GC-MS. It was carried out by using a Varian Saturn II ion trap (electron impact), equipped with a J&W DB-5 capillary column (30 m x 0.25 mmx 0.25μm), (Chromatographic Specialties, Brockville, ON, CA). The GC oven was held at 60 °C for 2 min, ramped at 15 °C/min to 320°C, and held for 30 min. The mass Spectrometer was operated in full scan mode from 50-650 amu. Solvent delay 10 min.

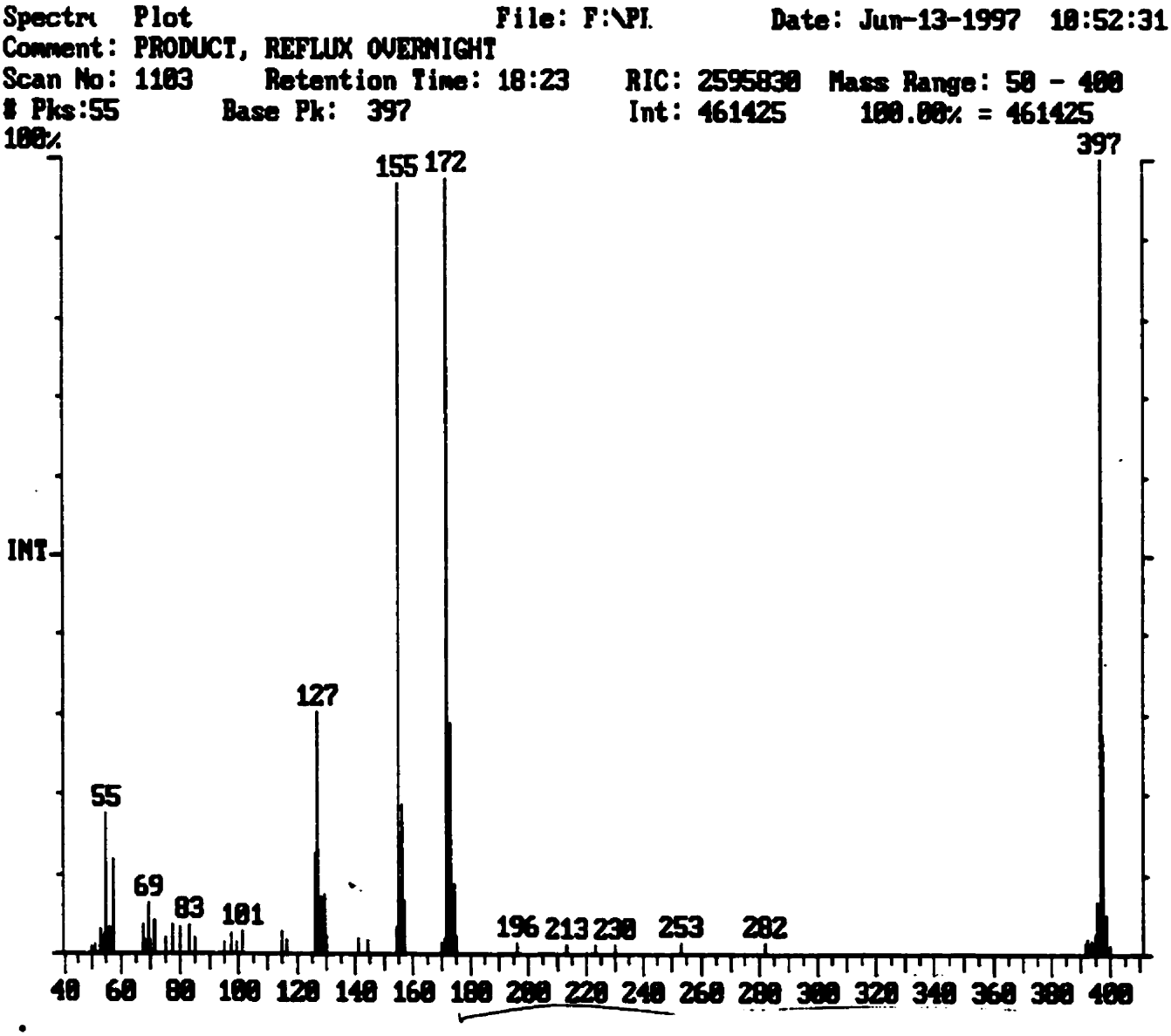


Figure (A2-2). Mass spectrum of the derivative. The mass spectrum of the ester showed a molecular ion at m/z 397 and a fragment ion at m/z 155. Molecular mass of the naphthoyl hexadecanoate is 396. The molecular ion at m/z 397 may be attributed to protonation in the ion trap.

Appendix 3

INSTITUTE for CHEMICAL PROCESS and ENVIRONMENTAL TECHNOLOGY

XPS Analysis Report DATE: March 30, 2000.

From: Gerald Pleizier.
For: Carleton University
Project Code: 5742SURF
Sample ID: Three carbon fiber samples: pyr(N2), pyr(H2) and Acid dig
Sample Coated: no

Comments:

Several lengths of fibers were cut and attached to the analysis stub with carbon tape. Analysis was by Kratos Axis XPS using monochromated Al K α x-radiation and charge neutralization. A survey scan was first taken to determine which elements XPS can detect, then each element is run at high resolution for peak shape, position and for quantitative results. Two areas were analyzed on each sample and the table reports the average of the two results in atomic %. Keep in mind that XPS cannot detect hydrogen and 100 % does not include hydrogen.

XPS Analysis Results.

Sample	Pur (N2)	Acid dig	Pur (H2)	CF N2	CF AS4
Date	28-3-00	28-3-00	29-3-00	3-4-00	3-4-00
Total Carbon	73.0	87.7	81.9	90.2	90.9
C 1s at 285.0 eV.	45.2	59.5	56.1	59.2	59.4
286.5	11.6	14.4	11.5	14.2	15.4
288.2	8.7	4.9	5.8	6.7	5.8
289.5	4.7	3.6	4.4	4.3	4.2
291.1	2.7	3.2	2.4	3.6	3.6
292.6		2.3	1.8	2.3	2.4
Total Oxygen	22.2	8.4	14.6	7.4	6.7
O 1s at 531.5 eV.	12.7	One peak	8.5	1.6	1.5
533.5	7.1	At 532.8	4.2	5.8	5.2
535.9	2.5	EV.	1.6		
538.6			0.3		
Nitrogen	2.8	3.2	2.4	2.4	2.4
Sodium	2.0		1.1		
Silicon		0.6			