Available online at www.sciencedirect.com
SCIENCE DIRECT
Talanta 69 (2006) 443–449
Talanta
www.elsevier.com/locate/talanta

Mass distribution for the microextraction of polycyclic aromatic hydrocarbon metabolites investigated with fluorescence spectrometry

Matthew Marlow, Robert J. Hurtubise *

Department of Chemistry, University of Wyoming, Laramie, WY 82071, USA

Received 26 July 2005; received in revised form 5 October 2005; accepted 5 October 2005
Available online 9 November 2005

Abstract

Mass-balance data were acquired using fluorescence spectrometry for 2-naphthol and three polycyclic aromatic hydrocarbon (PAH) metabolites using liquid–liquid–liquid microextraction and liquid–liquid microextraction systems. The PAH metabolites are very important biomarkers, and there has been no previously reported mass-balance data on these compounds with microextraction systems. In addition, the effects of two solvent systems used in the preparation of donor and acceptor phases were investigated. The effects of the solvent systems on the partitioning process were compared. The mass balance results showed that significant amounts of the hydrophobic metabolites were held in the pores of the microfiber in both the three-phase and two-phase microextraction systems. Based on the mass-balance data, a new enrichment factor was defined as the concentration of the solute trapped in the pores of the fiber divided by the initial concentration of the solute in the donor phase. Because of the relatively large amount of the solute in the pores, it is envisioned that the solutes could be easily extracted from the pores, the extraction solvent concentrated, and further separated by capillary electrophoresis or characterized by solid-matrix phosphorescence, solution fluorescence, or mass spectrometry.

Keywords: Liquid–liquid–liquid microextraction; Liquid–liquid microextraction; Polycyclic aromatic hydrocarbon metabolites; Fluorescence spectrometry; Complete mass balance

1. Introduction

The metabolites of polycyclic aromatic hydrocarbons are important biomarkers for exposure to polycyclic aromatic hydrocarbons. Many of the polycyclic aromatic hydrocarbons are potent carcinogens and mutagens and are most often excreted in the urine [1,2]. Frequently, the metabolites appear in complex biological matrices and are present only at trace levels. Therefore, developing methods for sample clean-up, preconcentration, and detection in complex matrices is of major concern for determining exposure levels to these potentially carcinogenic compounds. The matrices most often encountered are fluids such as plasma, blood, and urine. Much of the work regarding polycyclic aromatic hydrocarbon metabolites has focused on 1-hydroxypyrene since it is the principle metabolite of pyrene [3].

Recently, the technique of liquid-phase microextraction has been developed to concentrate small amounts of sample, to separate small solutes from larger species, such as DNA, and to reduce the use of toxic organic solvents used in more conventional extraction measures, for example, soxhlet extraction. Several types of liquid-phase microextraction techniques have been developed. Single-drop microextraction [4–7], liquid–liquid–liquid microextraction (LLLME) [8–11], hollow fiber protected liquid-phase microextraction [12,13], and stir bar sorptive extraction [14–17] are some of the techniques that have been developed for the extraction and preconcentration of analytes from various matrices.

LLLME is a three-phase microextraction technique which incorporates a porous polypropylene hollow fiber as a membrane that separates the donor phase from the acceptor phase [8,9] with an organic phase impregnating the pores of the hollow fiber. LLLME can extract ionic hydrophobic compounds from aqueous samples and concentrate them for detection. The aqueous phase outside the fiber is the donor phase, and the phase inside the lumen of the fiber is the acceptor phase. The analyte partitions from the donor phase into the organic phase, and it further partitions into the acceptor phase where the solute is concentrated for further detection. Typically, the acceptor phase

* Corresponding author. Tel.: +1 307 766 6241; fax: +1 307 766 2807.
E-mail address: Hurtubis@uwyo.edu (R.J. Hurtubise).

0039-9140/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2005.10.009

is a small volume of solvent that is in the microliter range [8,9]. The resulting difference in phase ratio of the acceptor and donor phases allows for substantial enrichment of the analyte. The small pores of the hollow fiber also prevent large matrix components in the donor phase from entering the acceptor phase, thus permitting the clean-up of the sample. If the acceptor phase is changed from an aqueous phase to an organic phase (1-octanol), then a two-phase microextraction system results.

The work discussed in the literature primarily considers the applications of three-phase and two-phase microextraction. Several fundamental studies have been reported for the mass transfer in three-phase and two-phase microextraction systems. Ma and Cantwell [18] have considered several aspects of three-phase microextraction. For example, they developed a solvent microextraction technique to perform simultaneous forward- and back-extraction across a microliter-size organic liquid membrane. Also, they developed a kinetic model to describe the extraction process and verified the extraction process experimentally. Several other groups have reported on the theoretical aspects of three-phase microextraction [19–25]. Ho et. al [26] have further developed some of the theoretical aspects of three-phase microextraction and two-phase microextraction. They provided a mathematical description for extraction, recovery, and enrichment for two-phase and three-phase microextraction systems.

A more detailed understanding of the basic aspects of three-phase and two-phase microextraction is important for improving extraction efficiency and enrichment of analytes of interest. In this paper, fluorescence spectrometry was used to determine the masses in the donor and acceptor phases, and the pores of the hollow fiber for several hydroxyl polycyclic aromatic hydrocarbon metabolites. Detailed mass data were obtained for both three-phase and two-phase microextraction systems. Solution fluorescence spectrometry proved to be very effective in determining the masses of the solutes in the various phases. Comparisons were made between the masses in three-phase and two-phase microextraction systems, two different solvent systems, and two different time periods. Also, based on the mass-balance data a new enrichment factor was introduced.

2. Experimental

2.1. Liquid–liquid–liquid microextraction

The microextractions were performed with the Accurel Q3/2 polypropylene hollow fiber (Membrana, Wuppertal, Germany). The length of the fiber was 6.5 cm. The inner diameter of the fiber was 600 μm. The wall thickness was 200 μm and the pore size was 0.2 μm. The procedure for liquid–liquid–liquid microextraction and for liquid–liquid microextraction were published previously with the exception that in this work the donor and acceptor phases were aqueous phases for some of the experiments rather than methanol:water (30:70) [27].

2.2. Reagents

The donor solution used for this work was 0.01 M HCl. To prepare the donor phase, aliquots of the appropriate amount of sample were added to a 4 mL vial and then diluted to a total volume of 4 mL. The acceptor phase was 25 μL of 0.1 M NaOH, or 25 μL of 1-octanol, and it was added to the lumen of the hollow fiber with a 50 μL syringe. The NaOH was obtained from J.T. Baker Chemical Co. (Phillipsburg, NJ). 1-Octanol was obtained from Aldrich Chemical (Milwaukee, WI). For several of the experiments, the donor and acceptor phases were prepared in methanol:water (30:70). In several other experiments, the donor and acceptor phases were prepared in HPLC grade water. The HPLC grade methanol and water were purchased from EM Science (Gibbstown, NJ).

The compounds investigated in this work were 2-naphthol, 1-hydroxypyrene, 3-hydroxybenzo[a]pyrene, and tetrol II-2. 3-Hydroxybenzo[a]pyrene and tetrol II-2 were purchased from the NCI Chemical Carcinogen Reference Standard Repository, Midwest Research Institute (Kansas City, MO). 2-Naphthol was obtained from Aldrich (Milwaukee, WI). These compounds were 99% pure or greater. Methanol and water were of HPLC grade and purchased from EM Science (Gibbstown, NJ).

2.3. Mass balance

Experiments were preformed to remove the metabolites and 2-naphthol from the pores of the polypropylene hollow fiber and determine the mass of the solute trapped in the organic phase using fluorescence spectrometry. After the microextraction procedure was performed, the acceptor phase was collected and the hollow fiber was removed and lightly blotted with a Kimwipe Kimberly-Clark Corp. (Roswell, GA). Furthermore, the fiber was then placed in 4 mL of HPLC grade methanol and stirred for 5 min to remove the trapped analyte in the pores of the fiber. The fluorescence intensities of the metabolites and 2-naphthol were obtained using the Perkin-Elmer LS 50-B luminescence spectrometer. The concentration present in the pores of fiber was determined using standard fluorescence calibration curves. The solutions for the calibration curves were prepared in pure HPLC grade methanol. The concentrations of solute in the acceptor and donor phases were determined as discussed earlier [27].

The complete mass balance was obtained in duplicate for each metabolite and 2-naphthol. In one set of experiments, the complete mass balance was obtained where the donor and acceptor phases were prepared in methanol:water (30:70). In another set of experiments, the complete mass balance was obtained in which the donor and acceptor phases were prepared in water.

2.4. Spectroscopy with 1-octanol

A standard 4 mL glass vial with a screw cap was used for this experiment. 1-Octanol was stored under nitrogen. The stock solutions of 1-hydroxypyrene were prepared in HPLC grade methanol and stored in a refrigerator. Sodium hydroxide solutions were prepared in HPLC grade water. For this experiment, 1 mL of the 0.1 M NaOH stock solution was added to a 4 mL glass vial, and then 1-hydroxypyrene was added in microliter amounts to the 1 mL of the 0.1 M NaOH that was added to the glass vial. Then, 1 mL of 1-octanol was added to the glass vial

Table 1
Mass data for 2-naphthol and several PAH metabolites using three-phase microextraction at 30 min (the donor and acceptor phases were prepared in water[a])

Compound[b]	Donor (%)	Pores (%)	Acceptor (%)	Total (%)
1	21.4 ± 2.2	40.0 ± 3.6	39.7 ± 2.7	101.1 ± 5.0
2	17.0 ± 1.8	82.4 ± 0.59	5.0 ± 0.21	104.4 ± 1.9
3	47.5 ± 0.3	49.2 ± 12.1	0.99 ± 0.14	97.7 ± 12.1
4	43.8 ± 1.2	61.8 ± 5.5	0.31 ± 0.055	105.9 ± 5.6

1. The initial mass of 2-naphthol was 125.2 ng. 2. The initial mass of 1-hydroxypyrene was 16.5 ng. 3. The initial mass of 3-hydroxybenzo[a]pyrene was 100.0 ng. 4. The initial mass of tetrol II-2 was 200.0 ng.
[a] Average of nine experiments.
[b] The number refers to the initial mass and name of the compound investigated.

Table 2
Mass data for 2-naphthol and several of the PAH metabolites at 30 min (the donor and acceptor phases were prepared in methanol:water (30:70)[a])

Compound[b]	Donor (%)	Pores (%)	Acceptor (%)	Total (%)
1	59.5 ± 4.6	43.1 ± 4.5	13.6 ± 2.0	116.2 ± 6.7
2	38.5 ± 2.4	53.5 ± 8.9	8.5 ± 0.8	100.5 ± 9.3
3	33.9 ± 1.6	56.0 ± 2.2	0.62 ± 0.068	90.5 ± 2.7
4	71.6 ± 1.1	18.1 ± 0.6	0.25 ± 0.027	90.0 ± 1.3

1. The initial mass of 2-naphthol was 129.6 ng. 2. The initial mass of 1-hydroxypyrene was 20.0 ng. 3. The initial mass of 3-hydroxybenzo[a]pyrene was 105.2 ng. 4. The initial mass of tetrol II-2 was 216 ng.
[a] Average of nine experiments.
[b] Number refers to the initial mass and name of the compound investigated.

and shaken. After 15 min, the 1-octanol phase was removed and fluorescence spectra were collected for 1-hydroxypyrene.

2.5. 1-Octanol volume

The volume of 1-octanol in the pores of the hollow fiber was determined by weighing the hollow fiber with no 1-octanol present and then immersing the hollow fiber in octanol for 15 s and blotting the excess 1-octanol away with a Kimwipe. The fiber was weighed again. Using the density of 1-octanol, the volume of 1-octanol in the pores of the fiber could be determined. The volume of octanol present in the pores was determined to be on average 23 ± 0.95 μL. This experiment was performed 10 times. The density of 1-octanol used for the calculation of the volume was 0.83 g/mL.

3. Results and discussion

3.1. Masses at 30 min for three-phase microextraction

In studying three-phase microextraction, it is important to obtain the mass distribution of the solute in the three phases including the mass of the solute trapped in the pores of the hollow fiber to assess the effectiveness of the microextraction system. Therefore, the mass-balance data were obtained for several of the metabolites and 2-naphthol with two different solvent systems. In one set of experiments, the donor and acceptor phases were aqueous. The second set of experiments were performed with donor and acceptor phases prepared in methanol:water (30:70). The methanol:water (30:70) solvent was investigated because this solvent was important in the solid-matrix phosphorescence of benzo[a]pyrene–DNA adducts and tetrol I-1 [28–30]. The mass-balance data would not only give the distribution of the solute in the phases, but also permit conclusions to be made about the effects of the two different solvent systems on the mass distributions. The results of the mass balance experiments in aqueous and methanol:water (30:70) solvents are given in Tables 1 and 2, respectively, at 30 min. Experiments showed that at 30 min extraction equilibrium was not reached [27]. However, 30 min was used because the microextraction was very effective over this time period and 30 min could be used in more routine experiments.

The mass-balance data for the hydroxyl PAH metabolites and 2-naphthol showed that a substantial amount of the solute gets trapped in the 1-octanol in the pores of the fiber for the four solutes investigated and for both solvent systems (Tables 1 and 2). However, the amount trapped in the pores changes with solvent composition. For example, for 1-hydroxypyrene, the percent mass trapped in the pores of the fiber was 82.4% of the initial mass when aqueous phases were used (Table 1). The percent mass trapped was lower when methanol:water (30:70) was used as the solvent. Table 2 shows that 53.5% was trapped in the pores of the fiber. Furthermore, the partitioning of the three metabolites to the acceptor phase from the 1-octanol phase was relatively small when using either solvent system. However, for 1-hydroxypyrene, as Tables 1 and 2 show, the amount in the acceptor phase was higher (8.5%) when using the methanol:water solvent system compared to the aqueous phases (5.0%). For 3-hydroxybenzo[a]pyrene and tetrol II-2, the transfer to the acceptor phase was slightly better with aqueous phases. It should be emphasized that the amount trapped in the organic phase within the pores of the hollow fiber is much greater than the amount transferred to the acceptor phase using either solvent system (Tables 1 and 2).

The quantity of 2-naphthol transferred to the acceptor phase was greater than for the metabolites using either solvent system. This most likely results because of the lower $\log K_{ow}$ (2.7) value of 2-naphthol, and fewer aromatic rings, as compared to the hydroxyl PAH metabolites. The term K_{ow} is the partition coefficient of a solute distributed between 1-octanol and water. It is widely used as a measure of the hydrophobicity of a solute. The PAH metabolites have significantly higher $\log K_{ow}$ values because of the greater number of aromatic rings. We have discussed previously the relationship between $\log K_{ow}$ and the microextraction of the PAH metabolites in the three-phase microextraction system [27]. The higher the $\log K_{ow}$ the less efficient was the extraction because a large fraction of the solute remained in the 1-octanol in the pores. It is also apparent that adding methanol to the solvent system inhibited three of the four model compounds from leaving the donor phase as is observed by comparing the data in Tables 1 and 2 for the percentage of masses remaining in the donor phase after extraction. A clear example of this is tetrol II-2 where 71.6% of the starting mass (216 ng) remained in the donor phase when using methanol:water (30:70) phase (Table 2). For tetrol II-2, with

Table 3
Mass data for 2-naphthol and the PAH metabolites at 180 min (donor and acceptor phases prepared in MeOH:water (30:70)[a])

Compound[b]	Donor (%)	Pores (%)	Acceptor (%)	Total (%)
1	31.2 ± 2.5	44.9 ± 1.9	19.0 ± 2.5	95.1 ± 4.0
2	3.8 ± 1.7	86.2 ± 4.3	8.1 ± 0.25	98.1 ± 4.6
3	2.1 ± 0.23	66.0 ± 1.1	0.41 ± 0.29	68.5 ± 1.2
4	71.7 ± 1.5	19.2 ± 1.6	0.34 ± 0.12	91.2 ± 2.2

1. The initial mass of 2-naphthol was 138.0 ng. 2. The initial mass of 1-hydroxypyrene was 16.0 ng. 3. The initial mass of 3-hydroxybenzo[a]pyrene was 80.0 ng. 4. The initial mass of tetrol II-2 was 216.0 ng.
[a] Average of four experiments.
[b] Number refers to initial mass and name of the compound investigated.

Table 4
Mass data for two-phase microextraction of hydroxyl-PAH metabolites at 30 min[a]

Compound[b]	Donor (%)[c]	Pores (%)	Acceptor (%)[d]	Total (%)
2	26.1 ± 1.5	36.2 ± 2.4	13.8 ± 1.7	76.1 ± 3.3
3	23.4 ± 1.9	29.8 ± 1.5	10.9 ± 1.5	63.9 ± 1.9
4	70.8 ± 1.9	17.9 ± 2.7	6.8 ± 1.2	95.5 ± 3.5

2. The initial mass of 1-hydroxypyrene was 18.8 ng. 3. The initial mass of 3-hydroxybenzo[a]pyrene was 80.0 ng. 4. The initial mass tetrol II-2 was 210 ng.
[a] Average of nine experiments.
[b] Number refers to initial mass and name of the compound investigated.
[c] The donor phase was 0.01 M HCl in methanol:water (30:70).
[d] The acceptor phase was 0.025 mL of 1-octanol.

aqueous phases, only 43.8% remained in the donor phase. Amazingly 61.8% of the initial mass of tetrol II-2 (200 ng) was trapped in the pores of the hollow fiber with aqueous donor and acceptor phases (Table 1). Only 18.1% of the tetrol II-2 was in the pores with methanol in the donor and acceptor phases (Table 2). The exception was 3-hydroxybenzo[a]pyrene with methanol present. The methanol aids in removing the 3-hydroxybenzo[a]pyrene from the donor phase into the 1-octanol phase, but does not assist in the partitioning to the acceptor phase (Table 2). For 3-hydroxybenzo[a]pyrene, 33.9% of the initial mass remained in the donor phase when methanol:water (30:70) was present, but 47.5% remained in the donor phase when the solvent was aqueous.

3.2. Mass balance at 180 min for three-phase microextraction

The mass-balance data for three-phase microextraction of 2-naphthol and the hydroxyl-PAH metabolites at 180 min is given in Table 3. At 180 min, the microextraction system should be at extraction equilibrium. The conditions for three-phase microextraction are the same for Table 2 with all the phases prepared in methanol:water (30:70). However, the initial concentrations of three of the analytes are different in Table 3 as compared to Table 2. Nevertheless, the initial masses are close to one another. Because the results are reported in percentages in Tables 2 and 3, the results in these tables are directly comparable. Tetrol II-2 had the same initial concentration for both experiments. The mass transferred to the acceptor phase is similar to the mass transferred at 30 min for 1-hydroxypyrene. For example, 8.1% is present in the acceptor phase as compared to the 8.5% in Table 2 at 30 min. In addition, the results for tetrol II-2 are almost identical to the results in Table 2 where a majority of the mass for tetrol II-2 remained in the donor phase at about 72%. There is however a slightly better transfer of the mass to the acceptor phase (0.34%) at 180 min for tetrol II-2 as compared to the mass (0.25%) transferred at 30 min (Table 2). The complete mass-balance data at 180 min demonstrate that the mass in the donor phase decreased more significantly for all the compounds investigated except tetrol II-2. At 30 min 1-hydroxypyrene has 38.5% remaining in the donor phase, whereas at 180 min the percentage remaining was only 3.8%. In addition, there was no corresponding increase in mass to the acceptor phase at 180 min with the exception of 2-naphthol, where 19.0% was found in the acceptor phase compared to 13.6% as shown in Table 2 at 30 min. The results in Tables 2 and 3 demonstrate that at 180 min the percentage of solute trapped in the pores of the hollow fiber is greater compared to the mass trapped in the pores at 30 min. For 1-hydroxypyrene, an amazing 86.2% was trapped in the pores at 180 min, whereas 53.5% was trapped at 30 min.

The total mass found experimentally was low for 3-hydroxybenzo[a]pyrene at 180 min for three-phase microextraction with methanol:water (30:70) phases (Table 3). Only 68.6% was recovered for the three-phase system. 3-Hydroxybenzo[a]pyrene has five aromatic rings and could have adsorbed to the surface of the stir bar or glass container. Large molecular weight polycyclic aromatic hydrocarbons are known to adsorb to glassware and stir bars as has already been reported [31].

3.3. Mass balance at 30 min for two-phase microextraction

In Table 4, the mass data for two-phase microextraction for the metabolites are presented. In this experiment, the donor phase was prepared in methanol:water (30:70). With 1-octanol as the acceptor phase, a substantial amount of the mass still remained trapped in the pores of the hollow fiber, and a significant amount of the mass also remained in the donor phase. The mass of tetrol II-2 remaining in the donor phase was 70.8% of the initial mass present (210 ng). However, with two-phase microextraction, a major improvement of transfer to the acceptor phase was observed in contrast to three-phase microextraction (Tables 1 and 2). This resulted because 1-octanol was the acceptor phase. The major driving force for tetrol II-2 is its solubility in the 1-octanol. Tetrol II-2 cannot ionize in the water or methanol:water (30:70) acceptor phases. Thus, the percentages of tetrol II-2 in the previous acceptor phases is small (Tables 1 and 2). There was an improvement by factors of 22 and 27, respectively, as compared to the masses obtained with three-phase microextractions in Tables 1 and 2. For example, with two-phase microextraction, 14.3 ng of tetrol II-2 were transferred compared to 0.62 and 0.53 ng, respectively, for tetrol II-2 in Tables 1 and 2. It should be noted in Table 4 that the percent total mass found is low for 1-hydroxypyrene and 3-hydroxybenzo[a]pyrene. No specific explanation can be given for this other than the general loss due to the small initial sample

Table 5
The enrichment factor ($E_{f(pore)}$) determined for the solute trapped in the pores of the fiber compared to the basic acceptor phase for three-phase microextraction

Compound	E_f for basic acceptor phase[a]	$E_{f(pore)}$ aqueous phase[b]	$E_{f(pore)}$ (30:70) MeOH:water phase[b]	$E_{f(pore)}$ (30:70) MeOH:water phase[c]	$E_{f(pore)}$ two-phase extraction[b,d]
1	40.2 ± 1.6	69.6 ± 6.2	75.1 ± 7.9	78.2 ± 3.3	–
2	10.9 ± 2.0	143.0 ± 10.2	92.9 ± 15.3	150.0 ± 7.5	15.7 ± 1.1
3	0.3 ± 0.06	85.5 ± 21.0	97.5 ± 3.9	114.8 ± 1.9	12.9 ± 0.65
4	1.1 ± 0.9	107.4 ± 9.6	31.1 ± 1.1	33.4 ± 2.8	7.8 ± 1.2

1. 2-Naphthol. 2. 1-Hydroxypyrene. 3. 3-Hydroxybenzo[a]pyrene. 4. Tetrol II-2.
[a] Values taken from reference [27] for methanol:water (30:70) at 30 min for three-phase microextraction.
[b] Values determined at 30 min.
[c] Values determined at 180 min.
[d] The donor phase solvent was 0.01 M HCl in methanol:water (30:70). Name and number of the compounds investigated.

sizes. However, the adsorption of the solutes to the glass vial and the stir bar may also be a factor for the loss observed in Table 4.

3.4. Pore enrichment factor

The hydrophobicity of the hydroxyl PAH metabolites and 2-naphthol is an important parameter to be considered in microextraction. Several of the metabolites have log K_{ow} values greater than 4.0 [27]. The large values of log K_{ow} demonstrate the high degree of hydrophobicity of the PAH metabolites and their strong preference for 1-octanol. The metabolites are only slightly polar, and therefore the hydrophobicity of the metabolites will be one of the main factors influencing both the three-phase and two-phase microextraction of these compounds. In addition, the large hydrophobicity of the hydroxyl PAH metabolites results in a sizeable amount of these compounds being retained in the pores of the fiber. Based on the results in Tables 1–4, we define a new enrichment factor given by Eq. (1), where C_2 and C_0 are the concentrations of the solute trapped in the pores of the hollow fiber and the initial concentration present, respectively.

$$E_{f(pore)} = \frac{C_2}{C_0} \quad (1)$$

Table 5 compares the newly defined pore enrichment factor obtained under several conditions with the enrichment factors obtained with the normal three-phase microextraction system using a basic methanol:water (30:70) acceptor phase at 30 min. The enrichment factors in the second column of Table 5 are defined as the ratio of the concentration in the basic acceptor phase divided by the initial concentration in the donor phase. The pore enrichment factors (Eq. (1)) were determined for several of the metabolites and 2-naphthol using the mass data in Tables 1–3 for both aqueous and methanol:water (30:70) phases. The enrichment factors for the solutes trapped in the pores were also determined from the two-phase microextraction data in Table 4.

The pore enrichment factors (Eq. (1)) were considerably higher compared to the enrichment factors determined from the basic acceptor phase (Table 5, column 2). Using 1-hydroxypyrene as the example for the data in Table 5, the enrichment factor for the analyte trapped in the pores was 143.0 when aqueous phases were used at 30 min. The pore enrichment factor was 92.9 when methanol:water (30:70) phases were used at 30 min. In contrast, the normal enrichment factor with the basic acceptor phase from the three-phase microextraction was 10.9 (Table 5). It is interesting to note that in Table 5 the largest increase in the pore enrichment factors for 2-naphthol was only by a factor of 1.9 with methanol:water (30:70) phases at 180 min. Because of the high degree of hydrophobicity of the PAH metabolites, they would tend to stay in the pores and 2-naphthol would spend less time in the pores.

Comparing the pore enrichment factors (fourth column in Table 5) when methanol:water (30:70) phases were used with enrichment factors obtained with a basic acceptor phase using methanol:water (30:70) at 30 min extraction (second column in Table 5), the pore enrichment factors improved by factors of 1.9, 8.5, 325.0, and 28.3 for 2-naphthol, 1-hydroxypyrene, 3-hydroxybenzo[a]pyrene, and tetrol II-2, respectively. The pore enrichment factors for 1-hydroxypyrene and tetrol II-2 can further be improved if aqueous phases are used. For example, the pore enrichment factor for tetrol II-2 was improved by a factor of 97.6 at 30 min. The pore enrichment factor for 1-hydroxypyrene was improved by a factor of 13.1 with aqueous phases as compared to the basic acceptor phase in Table 5.

Finally, the pore enrichment factors for two-phase microextraction were determined for 1-hydroxypyrene, 3-hydroxybenzo[a]pyrene, and tetrol II-2 (last column in Table 5) at 30 min. For 1-hydroxypyrene, the pore enrichment factor was 15.7; for 3-hydroxybenzo[a]pyrene it was 12.9; and for tetrol II-2 it was 7.8. These values show that less mass is trapped in the pores of the hollow fiber compared to the amount trapped in the pores of the three-phase microextraction systems (compare columns 3 and 4 in Table 5).

Based on the results in Table 5, it is proposed that using the fiber without the acceptor phase and with only 1-octanol in the pores of the hollow fiber and a aqueous donor phase would prove to be a very efficient means for the microextraction of the PAH metabolites. Work has been undertaken to explore this possibility, and the initial results are very encouraging.

3.5. Anion in the organic phase

Because solution fluorescence spectrometry was very effective in obtaining the masses of the solutes in the two-phase and

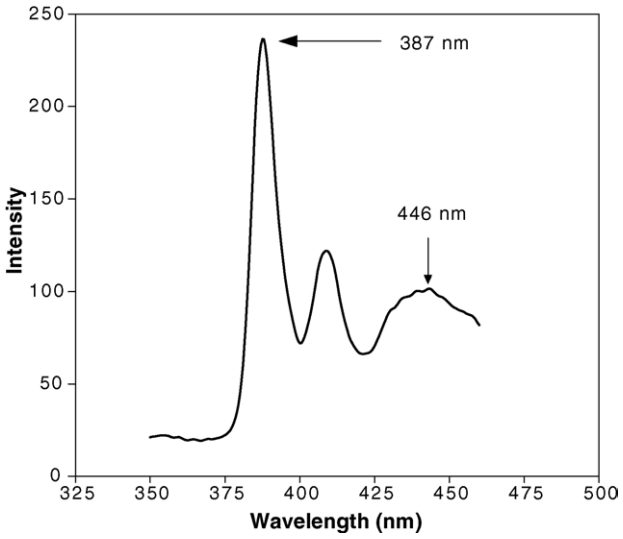

Fig. 1. Emission spectrum for 1-hydroxypyrene in 1-octanol after the 1-octanol was in contact with an aqueous solution of 0.1 M NaOH.

three-phase microextraction systems, preliminary experiments were performed with fluorescence spectrometry to identify the components in 1-octanol. The species in the 1-octanol in the pores would have an influence on the distribution of the solutes in the microextraction systems.

The mass-balance data in Tables 1–4 indicate the complexity of the solute distribution in the three-phase and two-phase microextraction systems. In considering models for the mass transfer of the metabolites for three-phase microextraction, it is important to determine the species present in each phase. For three-phase microextraction, it is assumed that in the 0.01 M HCl donor phase the hydroxyl aromatics are in their neutral form. Also, it is assumed that upon entering the 0.1 M NaOH acceptor phase that the compounds are completely ionized and form the anions. However, what is present in the 1-octanol organic phase in the pores of the fiber is not as clear. Experiments were performed using fluorescence spectroscopy where the 1-octanol was sampled to determine what species were present in the 1-octanol. However, the 1-octanol was not sampled directly from the pores of the hollow fiber because of the small volume of 1-octanol in the pores. A 1-hydroxypyrene stock solution was prepared in an aqueous 0.1 M NaOH to form the anion. Subsequently a small aliquot (10 μL) was removed from the stock solution and added to 3 mL of 1-octanol, and the sample was shaken vigorously and allowed to stand for 15 min before collecting spectral data from the 1-octanol solution. In Fig. 1, it was observed that the molecular form (387 nm) of 1-hydroxypyrene appeared in the 1-octanol as well as the anion (446 nm).

The results in Fig. 1 show that the conjugate base of 1-hydroxypyrene reacted with a proton in the 1-octanol to give the neutral form of 1-hydroxypyrene. It is proposed that the proton was removed from the water that dissolves in the 1-octanol when performing the extraction. It has been reported that a substantial amount of water is able to dissolve in 1-octanol [32,33]. The pK_a of 1-hydroxypyrene in water is 8.81[34]. Thus, its conjugate base would be a strong base and would have a strong tendency to react with water. It is probable that during the microextraction of the metabolites that both forms of the metabolites are present in the 1-octanol phase. The results observed in Fig. 1 suggests that even if the metabolite is ionized in the acceptor phase, the metabolite can partition back out of the acceptor phase into the 1-octanol in the pores of the hollow fiber and subsequently decrease the enrichment factor. The results in Fig. 1 indicate that more detailed investigations are needed in the future to understand the mechanistic aspects of three-phase microextraction of large molecular-weight hydroxyl aromatics and that fluorescence spectrometry would be very useful in characterizing the species.

4. Conclusions

To our knowledge, this is the first mass distribution data that have been obtained for solutes in three-phase or two-phase microextraction systems. Also, fluorescence spectroscopy was shown to be a very useful tool in the determination of the very small concentrations of hydroxyl aromatics distributed in the various phases. The data in Tables 1–4 show the complexity of microextraction and indicate that several factors are involved in the transfer of the solute to the acceptor phase. The hydrophobicity of the hydroxyl aromatic metabolites is one of the main factors in determining the partitioning of these compounds in the microextraction systems. The results for the two solvent systems show that the solvent used to prepare the donor and acceptor phases can have an important influence on the distribution of the solute. However, the trends on the distribution with the different solvents are not completely obvious. For example, more solute appears in the acceptor phase when aqueous phases are used for three-phase microextraction, except for 1-hydroxypyrene. For 1-hydroxypyrene, more solute appeared in the acceptor phase for three-phase microextraction with methanol:water (30:70).

Because of the substantial amount of PAH metabolites in the pores of the fiber, a new enrichment factor was defined that is based on how much solute appears in the pores of the microfiber. This enrichment factor would be very important for hydrophobic compounds. In particular, with aqueous phases, a very sizeable amount of solute remains in the pores. By simply removing the compound from the pores with a small volume of methanol, evaporating the methanol, and dissolving the residue in a very small amount of solvent, the solute could further be characterized with techniques such as capillary electrophoresis, solid-matrix phosphorescence spectroscopy, mass spectrometry or fluorescence spectroscopy. It has been reported that solid-matrix phosphorescence spectroscopy is useful for the detection of hydroxyl aromatic metabolites in urine fractions [29]. For 1-hydroxypyrene, the range for human samples is 459 to 39,000 fmole/mL [29]. By using the two-phase microextraction system without the acceptor phase, as discussed in this paper, with the urine factions, the concentration of the hydroxyl aromatic metabolites would be enhanced considerable and thus make detection easier. Also, large molecular-weight species from the urine fractions would not pass into the acceptor phase.

Acknowledgement

Financial support for this project was provided by the Chemical Sciences, Geosciences and Biosciences Division, Office of Basic Energy Sciences, U.S. Department of Energy (DE-FG02-04ER15545).

References

[1] P.J. Lioy, J.M. Waldman, A. Greenberg, R. Harkov, C. Pietarinen, Arch. Environ. Health 43 (1988) 304.
[2] J.P. Bulter, G.B. Post, P.J. Lioy, J.M. Waldman, A. Greenberg, J. Air Waste Mange. Assoc. 43 (1993) 970.
[3] C.-T. Kuo, H.-W. Chen, J.-L. Chen, J. Chromatogr. B 805 (2004) 187.
[4] P. Das, M. Gupta, A. Jain, K.K. Verma, J. Chromatogr. A 1023 (2004) 33.
[5] Y. He, H.K. Lee, Anal. Chem. 69 (1997) 4634.
[6] H. Liu, P.K. Dasgupta, Anal. Chem. 68 (1996) 1817.
[7] M.A. Jeannot, F.F. Cantwell, Anal. Chem. 68 (1996) 2236.
[8] S. Pedersen-Bjergaard, K.E. Rasmussen, Anal. Chem. 71 (1999) 2650.
[9] K.E. Rasmussen, S. Pedersen-Bjergaard, M. Krogh, H.G. Ugland, T. Gronhaug, J. Chromatogr. A 873 (2000) 3.
[10] X. Wen, C. Tu, H.K. Lee, Anal. Chem. 76 (2004) 228.
[11] X. Jiang, S.Y. Oh, H.K. Lee, Anal. Chem. 77 (2005) 1689.
[12] G. Shen, H.K. Lee, Anal. Chem. 74 (2002) 648.
[13] C. Basheer, H.K. Lee, J. Chromatogr. A 1057 (2004) 163.
[14] M. Kawaguchi, K. Inoue, M. Yoshimura, R. Ito, N. Sakui, N. Okanouchi, H. Nakazawa, J. Chromatogr. B 805 (2004) 41.
[15] C. Blasco, M. Fernandez, Y. Pico, G. Font, J. Chromatogr. A 1030 (2004) 77.
[16] M. Kawaguchi, Y. Ishii, N. Sakui, N. Okanouchi, R. Ito, K. Saito, H. Nakazawa, Anal. Chim. Acta 533 (2005) 57.
[17] M. Kawaguchi, N. Sakui, N. Okanouchi, R. Ito, K. Saito, H. Nakazawa, J. Chromatogr. A 1062 (2005) 23.
[18] M. Ma, F.F. Cantwell, Anal. Chem. 70 (1998) 3912.
[19] L. Chimuka, N. Megersa, J. Norberg, L. Mathiasson, J.A. Jonsson, Anal. Chem. 70 (1998) 3906.
[20] S. Pedersen-Bjergaard, T.S. Ho, K.E. Rasmussen, J. Sep. Sci. 25 (2002) 141.
[21] D. Kou, S. Mitra, Anal. Chem. 75 (2003) 6355.
[22] K. Kuosmanen, M. Lehmusjarvl, T. Hyotylainen, M. Jussila, M.-L. Riekkola, J. Sep. Sci. 26 (2003) 893.
[23] A. Bjorhovde, T.G. Halvorsen, K.E. Rasmussen, S. Pedersen-Bjergaard, Anal. Chim. Acta 491 (2003) 155.
[24] L. Hou, H.K. Lee, Anal. Chem. 75 (2003) 2784.
[25] K.E. Rasmussen, S. Pedersen-Bjergaard, Trends Anal. Chem. 23 (1) (2004) 1.
[26] T.S. Ho, S. Pedersen-Bjergaard, K.E. Rasmussen, J. Chromatogr. A 963 (2002) 3.
[27] M. Marlow, R.J. Hurtubise, Anal. Chim. Acta 526 (2004) 41.
[28] M. Li, R.J. Hurtubise, A. Weston, Anal. Chem. 71 (1999) 4679.
[29] B. Smith, R.J. Hurtubise, Appl. Spectrosc. 54 (2000) 1357.
[30] P.B. Steinbach, R.J. Hurtubise, Appl. Spectrosc. 54 (2000) 287.
[31] A.H. Ackerman, R.J. Hurtubise, Talanta 52 (2000) 853.
[32] D.C. Scott, J.W. Clymer, Pharma. Technol. 11 (2002) 30.
[33] S.A. Margolis, M. Levenson, Fresen. J. Anal. Chem. 367 (2000) 1.
[34] X. Xu, R.J. Hurtubise, J. Liq. Chromatogr. Rel. Technol. 22 (5) (1999) 669.