

# APPLICATION OF CLOUD POINT EXTRACTION FOR THE DETERMINATION OF PYRENE IN NATURAL WATER

Siwatt Pongpiachan

Biogeochemical and Environmental Change Research Unit, Faculty of Environmental Management and National Center of Excellence for Environmental and Hazardous Waste Management-PSU Satellite Center, Prince of Songkla University, Hat-Yai, Songkhla, Thailand

**Abstract.** Triton X-114 (Triton X-114) surfactant separates into two isotropic phases at room temperature and can be successfully used in cloud point extraction for the analysis of polycyclic aromatic hydrocarbons (PAHs). We studied which type of container is the most suitable for PAHs extraction with this method and how the water affects PAH recovery. We used a generator and mini-centrifuge with a cloud point method to determine pyrene levels in water in the field. An on-site thermostat can be used along with pouring hot water from a canteen into a plastic bucket to keep the temperature stable. Significant losses of pyrene due to adsorption onto the container wall can be minimized by storing water samples in glass containers. Variation in critical micelle concentration (cmc) can be avoided by bringing the water temperature to 40°C for 5 minutes. These methods allowed pyrene to be determined in a remote tropical peat swamp area in Central Kalimantan, Indonesia.

## INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are a group of toxic organic compounds which are of anthropogenic (the result of incomplete combustion of fossil fuels and other organic materials) and natural (forest fires, volcanic activity, etc) in origin (Bjørseth, 1977; Auer and Malissa, 1990). Several PAHs have been shown to be acutely toxic. Health concerns regarding these compounds center on their metabolic transfor-

mation by aquatic and terrestrial organisms into carcinogenic, teratogenic and mutagenic metabolites, even at concentrations parts per trillion (ppt) levels (Wild *et al*, 1990). Therefore it is necessary to develop sensitive and specific methods to detect PAHs (Menzie *et al*, 1992).

Due to their extremely low concentrations, a concentration step prior to chromatographic separation has been included in the analytical methods to determine PAHs in natural water samples. The most common methods for concentration of the samples are liquid-solid extraction (Kicinski *et al*, 1989; Brouwer *et al*, 1994; Eastwood *et al*, 1994) and liquid-liquid extraction (Tavakoli *et al*, 2008). Sicilia *et al* (1999) insist that neither of these methods is ideal because of the use of large volumes of high cost organic solvents (liquid-liquid extraction) and the interference of humic acids (liquid-solid extraction) which

---

Correspondence: Siwatt Pongpiachan, Biogeochemical and Environmental Change Research Unit, Faculty of Environmental Management and National Center of Excellence for Environmental and Hazardous Waste Management-PSU Satellite Center, Prince of Songkla University, Hat-Yai, Songkhla 90112, Thailand.

Tel: +66 (0) 89 6372211; Fax: +66 (0) 74 429758

E-mail: pongpiajun@gmail.com

leads to lower recoveries than those with liquid-liquid extraction (Johnson *et al*, 1991).

Cloud point extraction using a Triton X-114 is an alternative method of concentration which has several advantages, such as high recovery of PAHs, its safety and cost benefits, easy disposal of the surfactant, ability to minimize losses due to adsorption of PAHs onto container walls and ability to determine PAHs associated with humic acid (Pinto *et al*, 1994; Ferrer *et al*, 1996).

Aqueous solutions of certain surfactants, both nonionic and zwitterionic; when heated above a certain temperature (cloud point temperature) exhibit the property of separating into two phases (Saito and Hinze, 1991; Qin *et al*, 2008; Shokrollahi *et al*, 2008). Once formed, after a given time, during which they can be accelerated by centrifugation, two transparent liquid phases are obtained; one contains most of the surfactant (the surfactant-rich phase) and the other is in an aqueous phase in equilibrium with the former, with a surfactant concentration close to the critical micelle concentration (cmc). The surfactant-rich phase can be used for the concentration of certain analytes before injection into high performance liquid chromatography (HPLC). However, some questions regarding this method have remained: which type of container is the most suitable for PAHs extraction with Triton X-114 based cloud point phenomenon, and how the water temperature affects PAHs recovery.

Without careful investigation of these two items, the reliability of the proposed methodology for the separation and concentration of PAHs at the sampling site can not be obtained. In this study, we evaluated solutions to these two problems and propose for the first time the use of either a combination of a generator and mini-centrifuge during the *in-situ* concentration step or the application of a field thermostat.

The aims of the study were to minimize the loss of pyrene during transportation by using the combination of a generator and a mini-centrifuge to conduct cloud point concentration *in-situ* and to enhance the reliability of measurements, thus providing information for researchers attempting to detect pyrene and benzo[a]pyrene in natural water.

## MATERIALS AND METHODS

### Apparatus

The chromatographic system consisted of a Hitachi HPLC pump with a fluorescence detector and a 20-ml injection loop. The stationary-phase column was a GL-Sciences Inertsil ODS-2 column. From the surfactant-rich phase obtained after cloud point concentration, 60  $\mu$ l was collected using a Hamilton syringe, of which 20  $\mu$ l was injected into the chromatographic system. A Kokusan H-122 mini-centrifuge was connected to a Yamaha EF1400 generator. For separation of the surfactant rich phase, a glass centrifuge was utilized.

### Reagents

Standard stock solutions (about 200 mg ml<sup>-1</sup>) of pyrene and benzo[a]pyrene in methanol were purchased from Aldrich and kept cool in the dark. The solution used in the study was prepared by dilution of the stock solution with acetonitrile. Acetonitrile was of HPLC quality. A Triton X-114 was purchased from Sigma and used without further purification. The mobile phase consisted of 75/25 acetonitrile/water; before use it was filtered through a 0.22  $\mu$ m membrane filter and degasified with an ultrasonic device.

### Cloud point pre-concentration

Cloud point extraction was carried out by heating a 50 ml solution of water containing 5% (w/w) Triton X-114 to 40°C for 5 minutes. In this study the water temperature was kept at 40°C because this temperature is above the cloud point temperature of Triton

X-114, which is 24°C (Purkait *et al*, 2006), the recovery of pyrene determined at 40°C is about 80% (as illustrated in Fig 2); although a higher water temperature significantly enhances the recovery of PAHs (Hung *et al*, 2007), it is fairly difficult to maintain the water temperature higher than the air temperature. Therefore, it is more realistic to keep the water temperature as close as possible to the air temperature, which is 40°C during the day time in Central Kalimantan.

Separation of the two phases was achieved by centrifugation for 5 minutes at 3,500 rpm. The supernatant aqueous phase (48 ml) was removed with a pipette. Then, the Triton X-114 containing the PAHs was dissolved in 3 ml of acetonitrile and injected into the HPLC.

#### Liquid chromatography analysis

This study was carried out with a GL-Sciences Inertsil ODS-2 column. From the surfactant-rich phase obtained after cloud point concentration, 60 µl was collected using a Hamilton syringe, of which 20 µl was injected into the chromatographic system. The separation and detection of the PAHs was carried out using a mobile phase consisting of 75:25 (v/v) acetonitrile-water (flow rate = 1.0 ml min<sup>-1</sup>), without any fluorescence wavelength programming. Each sample was injected three times. The excitation-emission wave lengths (in nanometers) used for each compound were as follows: 330-385 for pyrene and 384-406 for benzo[a]pyrene.

#### Recovery of pyrene in distilled water

Analyte losses during sampling were evaluated by adding a known quantity of a sampling efficiency standard (pyrene and benzo[a]pyrene) to the sampling container prior to sampling. The mean pyrene and benzo[a]pyrene recovery percentages (which reflect analyte losses due to both sampling and analysis) were 95% (92-97%,  $n = 10$ ) and 90% (89-93%,  $n = 10$ ), respec-

tively. The standard deviations (calculated by integrated peak's area,  $n = 10$ ) for pyrene and benzo[a]pyrene were  $\pm 5\%$  and  $\pm 7\%$ , respectively.

#### Adsorption of pyrene onto containers

The adsorption of PAHs onto the three different containers was measured by taking 10-ml aliquots of the prepared solution at selected time intervals; 0, 0.5 and 1.0% of Triton X-114 were added at the beginning, then cloud point concentration was carried out. The recovery was quantified by chromatographic analysis.

#### Effect of water temperature on recovery of benzo[a]pyrene

The effect of water temperature on recovery of benzo[a]pyrene was investigated by collecting 20 ml of each aliquot and storing at selected temperatures. The concentration of benzo[a]pyrene in each aliquot was 80 nM.

#### Determination of pyrene from natural water samples

River and lake water samples were collected, at 1-meter intervals from the surface of the water to the bottom, with a 1-1 pyrex-glass Rigo snatch bottle sampler (100 mm in diameter and 200 mm high) with a vane at the bottom. In order to eliminate other suspended particles, all water samples were passed through glass wool filters prior to pre-concentration. After filtration, 100 ml water samples was collected with a pipette and transferred to a glass bottle, where 0.50 ml (0.5%) Triton X-114 was added with a micropipette. The surfactant and water samples were mixed by shaking the glass bottle thoroughly for 30 seconds. The glass bottle was then placed for 5 minutes in a plastic bucket containing water at 40°C.

The water temperature was measured using a digital thermometer. Hot water from a canteen was mixed with the water in the plastic bucket to keep the temperature at

40°C ± 0.2°C for 5 minutes. Aliquots of 50 mL were collected from each glass bottle by pipette and transferred to a new centrifuge tube. A Kokusan H-122 mini-centrifuge was connected to a Yamaha EF1400 generator, and cloud point concentration was carried out as described previously. To control the quality and evaluate the reliability of this procedure, a standard solution of pyrene was spiked into water samples and analyzed the same way unspiked water samples were analyzed. Loss due to evaporation of pyrene and acetonitrile during transport to the laboratory was avoided by transferring all solutions into glass tubes and sealing with a gas burner. All concentration steps were carried out within 15 minutes.

#### Sampling sites

Observations and sample collection were performed during the rainy season (September 11 - 30, 1998) and dry season (April 23 to May 13, 1999) in a peat swamp of Central Kalimantan, Indonesia. We investigated water from four oxbow lakes: Lake Tahai (1°2'1" S: 113°46'56" E), Lake Takapan (1°2'8" S: 113°54'45" E), Lake Sabuah (1°2'2" S: 113°56'35" E) and Lake Tundai (2°12'43" S: 114°0'27" E) and along the Kahayan River (1°2'11" S: 113°55'45" E) with a surface area of 1.2 km<sup>2</sup> and a maximum depth of 6 m during the rainy season. Exchange of water

between lakes and river occurred vigorously during the rainy season. Lake Sunter (2°6'8" S: 106°51'44" E), located in the center of Jakarta, was selected as a typical heavy contaminated site for waste water from nearby factories and communities. The lake and river water samples were collected, using a Niskin sampler, from 3 to 4 depths between the surface and the bottom of each sampling site.

## RESULTS

#### Adsorption of pyrene and benzo[a]pyrene onto containers

Table 1 summarizes the results obtained from studying the amount of adsorption of pyrene onto the walls of polyethylene and Teflon containers containing three different concentrations of Triton X-114. The presence of Triton X-114, used for the concentration of PAHs, inhibited their adsorption onto both Teflon and polyethylene containers with no change in the signal observed for at least three days. Adsorption onto polyethylene containers was pronounced in the absence of Triton (Table 1); by 11 hours of contact, none of the pyrene could be detected. However, in the presence of Triton X-114, recovery was nearly 100%. The Teflon container appeared more suitable as a container for dissolved pyrene, since Teflon is a

Table 1  
Adsorption of pyrene onto different containers.

Container	Recovery (%)				
	0 h <sup>a</sup>	6 h	11 h	55 h	126 h
Polyethylene without TX-114	100	20.5	14.6	0	0
Polyethylene with TX-114 at 0.5%	100	50.6	55.2	67.8	48.1
Polyethylene with TX-114 at 1.0%	100	51.4	55.8	73.6	67.6
Teflon without TX-114	100	39.9	37.1	23.4	17.3
Teflon with TX-114 at 0.5%	100	55.6	56.4	46.3	41
Teflon with TX-114 at 1.0%	100	53	63.3	44	48

<sup>a</sup>h, hours

fluorocarbon solid, which is not as susceptible to London dispersion forces due to the high electronegativity of fluorine. The majority of dissolved pyrene was inhibited from adsorbing onto Teflon surfaces due to its resistance to van der Waals forces. In contrast, polyethylene has more surface adhesion sites for to adhere to than Teflon.

This ability of the surfactant to prevent PAH adsorption was also observed in three different concentrations of benzo[a]pyrene (Fig 1). The recovery of all three benzo[a]pyrene concentrations increased after 6 hours of contact. This experiment was designed to prove that glass is appropriate for collecting water samples and preserving standard solution. The recoveries of pyrene and benzo[a]pyrene, using the cloud point method, at 5 minutes, were 95% and 90%, respectively.

#### Effect of water temperature to the recovery of benzo[a]pyrene

Aqueous solutions of many surfactant micellar systems, when subjected to an increase in temperature, become turbid over a narrow temperature range, which is referred to as their cloud point. Above the cloud point temperature, such solutions separate into two isotropic phases. That is, the system will contain a surfactant-rich phase with a small amount of water (surfactant phase) in which the surfactant concentration will be approximately equal to the critical micelle concentration (cmc) of the nonionic surfactant present. Since the temperature directly affects the critical micelle concentration of Triton X-114, the concentration of PAHs in surfactant phase depends on the water temperature.

We observed a significant increase in benzo[a]pyrene fluorescent intensity with an increase in water temperature. The linear relationship between these two parameters is illustrated in Fig 2. The slope and inter-

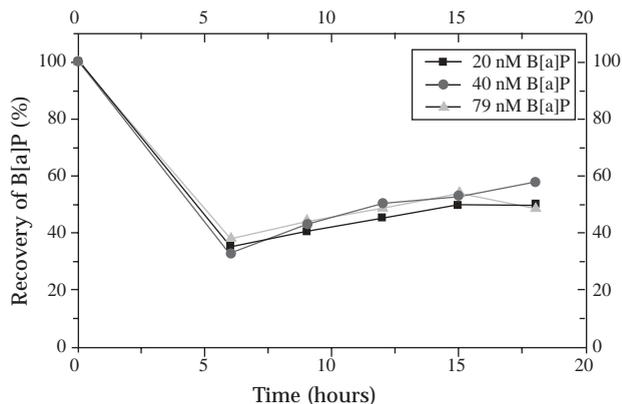


Fig 1-The recovery of three different concentrations of B[a]P.

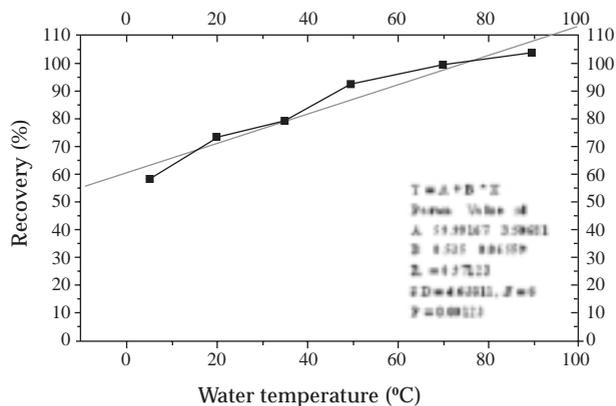


Fig 2-The effect of water temperature on recovery of B[a]P.

cept were 0.54 and 60, respectively. A strong correlation was also observed between the two ( $r^2 = 0.97$ ). We ascribe this phenomenon to the change in the structure of water molecules surrounding the hydrophilic sites of the surfactant. Interaction between solute (surfactant molecules and benzo[a]pyrene) and water molecules and the degree of benzo[a]pyrene binding upon the hydrophobic site of the surfactant is also affected by the temperature. Therefore, it is important to keep the water temperature constant during concentration.

### Determination of PAHs in water samples

In order to test the reliability of the proposed cloud point methodology for the analysis of PAHs in natural water, we collected and concentrated the water on site from three lakes (L. Takapan, L. Sabuah and L. Tahai) and one river (Kahayan River St. 1 and St. 2) in Central Kalimantan and from one lake (L. Sunter) in Jakarta, Indonesia (Table 2). The standard deviation was calculated by replicate measurement. To avoid losses during the concentration step, the standard solution of pyrene was spiked in all water samples. The retention times for pyrene and benzo[a]pyrene were 7 minutes and 12 minutes, respectively. Linear ranges were specified between the limits of quantification (calculated as ten times the standard deviation). The detection limit for the cloud point method was much greater than for the other methods found in the literature, detecting down to the level of nanograms per liter. In our study we calculated the limit of detection (LOD) by multiplying three times the standard deviation for the background noise. The LOD for benzo[a]pyrene and pyrene were  $0.6 \text{ ng l}^{-1}$  and  $1.8 \text{ ng l}^{-1}$ , respectively.

The dissolved pyrene observed in Lake Takapan varied from 77 to 271 pM and from 116 to 623 pM for Lake Sabuah (Table 2). The highest concentration of dissolved pyrene was observed from surface water in Kahayan River and Garung Canal St.1 ( $3^{\circ}2'38'' \text{ S}; 114^{\circ}12'31'' \text{ E}$ ). Kahayan River St.2 ( $2^{\circ}2'6'' \text{ S}; 113^{\circ}56'22'' \text{ E}$ ) showed the highest variances.

### DISCUSSION

We interpret the high variability in pyrene levels from Kahayan River St.1 ( $1^{\circ}2'11'' \text{ S}; 113^{\circ}55'45'' \text{ E}$ ) and St. 2, as being the result of the intermingling of surface runoff (high pyrene concentrations) and ground

water (low pyrene concentrations). The fact that the concentration of dissolved pyrene in river waters decreased exponentially with increasing depth is consistent with this interpretation. We believe the highest concentration of pyrene found in the surface river water is due to several reasons: (1) direct input from smoke from the boat diesel engine, (2) atmospheric deposition, such as airborne particulate matter containing pyrene derived from forest fires, (3) and surface runoff carry pyrene bound with dissolved humic acids. We believe the lowest pyrene concentration in the river bottom water samples is due to two reasons: (1) dilution by mixing with ground water, (2) and rapidly sinking downward by particle association.

Previous research shows that the vertical profile of dissolved pyrene in seawater was governed by planktonic bioaccumulations (McLachlan, 1995; Matti *et al*, 2000), which leads to another potential pyrene controlled factor in fresh water. In lake water, with thermally stratified conditions, the maximum concentration was found near the surface (-1 m). Near the bottom, the maximum concentrations were found in samples where sediment resuspension or transport were favored. This indicates the mechanisms, affecting dissolved pyrene in lake water are different from river water.

We observed low variance in mean pyrene values in L. Takapan, Kapuas R. ( $7^{\circ}2'59'' \text{ S}; 114^{\circ}22'10'' \text{ E}$ ) and Kahayan R. St.3 ( $3^{\circ}15'56'' \text{ S}; 114^{\circ}11'29'' \text{ E}$ ) L. Takapan, which is located about 100 km from Kapuas R. had similar variance values with Kapuas R. However, L. Tahai and L. Sabuah, which are situated relatively close together, demonstrated larger differences in variance and mean values. These results suggest the amount of dissolved pyrene is not governed primarily by geographic factors in Central Kalimantan.

The distribution of dissolved pyrene is

Table 2  
The concentration of pyrene in natural water.

Date	Place	Station	Depth (m)	PM (95% confidence interval)
990506	L. Takapan	1	0	209 ± 59
990506	L. Takapan	1	1	95 ± 61
990506	L. Takapan	1	2	77 ± 48
990506	L. Takapan	1	3	97 ± 64
990506	L. Takapan	1	5	141 ± 59
990506	L. Takapan	2	4	271 ± 53
990506	L. Takapan	3	5	132 ± 71
990506	L. Takapan	3	7	135 ± 62
990506	L. Takapan	4	5	142 ± 71
990506	L. Takapan	4	8	99 ± 65
990508	L.Sabuah	1	1	341 ± 145
990508	L.Sabuah	1	2	294 ± 133
990508	L.Sabuah	2	3	551 ± 134
990508	L.Sabuah	2	6	280 ± 109
990508	L.Sabuah	2	9	527 ± 108
990508	L.Sabuah	3	0	500 ± 101
990508	L.Sabuah	3	1	413 ± 108
990508	L.Sabuah	3	1.5	623 ± 103
990508	L.Sabuah	4	1	131 ± 65
990508	L.Sabuah	4	3	116 ± 83
990508	L.Sabuah	4	4	135 ± 91
990510	Canal in Garung	3	0.5	1,085 ± 131
990510	Canal in Garung	3	1	1,304 ± 95
990510	Kahayan R.	4	0	281 ± 131
990510	Kahayan R.	4	1	173 ± 101
990510	Kahayan R.	4	1.5	188 ± 123
990510	Kapuas R.	7	1	179 ± 70
990510	Kapuas R.	7	3	171 ± 87
990512	Kahayan R.	1	0	1,447 ± 192
990512	Kahayan R.	1	3	781 ± 208
990512	Kahayan R.	1	6	485 ± 23
990512	Kahayan R.	2	0	1,250 ± 181
990512	Kahayan R.	2	3	842 ± 269
990512	Kahayan R.	2	5	662 ± 100
990513	L. Tahai	2	0	666 ± 200
990513	L. Tahai	2	3	781 ± 170
990513	L. Tahai	2	5	539 ± 220
990524	L. Sunter	1	0	153 ± 110
990524	L. Sunter	1	2	270 ± 108
990524	L. Sunter	2	0	228 ± 58
990524	L. Sunter	2	2	117 ± 99

expected to reflect salting out processes and/or the mixing of fresh and saline waters. Fernandes *et al* (1997) demonstrated the concentration of dissolved pyrene is higher when the salinity is lower. In our study, we observed the mean concentration of pyrene in Kahayan R. St.3 was four times lower than in the Kahayan R. St.1 and St.2. The fact the Kahayan R. St.3 is the closest sampling site to the seashore supports this hypothesis. The mean concentration of pyrene in the lakes studied displayed an intermediate value between those of the Kahayan R, Garung Canal and ground water. The distribution of pyrene in the lake water samples can be described as a mixture of river water and ground water.

In conclusions, the cloud point methodology offers several advantages, such as the ability to concentrate PAHs with high recovery rates, good safety, and reasonable cost and possibility to determine PAHs associated with humic acids, which is an advantage over the conventional methods, such as liquid-liquid extraction and liquid-solid extraction. However, the most significant disadvantages of this method are the loss of PAHs due to adsorption to the container wall and variation of recovery rates due to water temperature variation during the concentration step. In our study, we found that polyethylene was not suitable to contain PAH solutions. Glass or Teflon made better containers. The effect of water temperature on the recovery of PAHs can be avoided by keeping the water temperature constant at 40°C for 5 minutes. The application of an on-site thermostat and the combination of a generator and a centrifuge enabled this method to be practical in the field.

#### ACKNOWLEDGEMENTS

The authors would like to thank the University of Palangka Raya for support

during the study in Central Kalimantan and to the National Center of Excellence for Environmental and Hazardous Waste Management-PSU Satellite Center, Prince of Songkla University for financial support of this work.

#### REFERENCES

- Auer W, Malissa H. Determination of trace amounts of polycyclic aromatic hydrocarbons in soil. *Anal Chim Acta* 1990; 237: 451-7.
- Bjørseth A. Analysis of polycyclic aromatic hydrocarbons in particulate matter by glass capillary gas chromatography. *Anal Chim Acta* 1977; 94: 21-7.
- Brouwer ER, Hermans ANJ, Lingeman H, Brinkman UAT. Determination of polycyclic aromatic hydrocarbons in surface water by column liquid chromatography with fluorescence detection, using on-line micelle-mediated sample preparation. *J Chromatogr A* 1994; 669: 45-57.
- Eastwood D, Domingues ME, Lidberg RL, Poziomek EJ. A solid phase extraction/solid state luminescence approach for monitoring PAHs in water. *Analysis* 1994; 22: 305-10.
- Fernandes MB, Sicre MA, Boireau A, Tronczynski J. Polyaromatic hydrocarbon (PAH) distributions in the Seine River and its estuary. *Mar Pollut Bull* 1997; 34: 857-67.
- Ferrer R, Beltrán JL, Guiteras J. Use of cloud point extraction methodology for the determination of PAHs priority pollutants in water samples by high-performance liquid chromatography with fluorescence detection and wavelength programming. *Anal Chim Acta* 1996; 330: 199-206.
- Hung KC, Chen BH, Yu LE. Cloud-point extraction of selected polycyclic aromatic hydrocarbons by nonionic surfactants. *Sep Purif Technol* 2007; 57: 1-10.
- Johnson WE, Fendinger NJ, Plimmer JR. Solid-phase extraction of pesticides from water: possible interferences from dissolved organic material. *Anal Chem* 1991; 63: 1510-3.

- Kicinski HG, Adamek S, Kettrup A. Trace enrichment and HPLC analysis of polycyclic aromatic hydrocarbons in environmental samples, using solid phase extraction in connection with UV/VIS diode-array and fluorescence detection. *Chromatographia* 1989; 28: 203-8.
- Matti T, Leppänen, JV, Kukkonen K. Fate of sediment-associated pyrene and benzo[a]pyrene in the freshwater oligochaete *Lumbriculus variegatus* (Müller). *Aquat Toxicol* 2000; 49: 199-212.
- McLachlan MS. Bioaccumulation of hydrophobic chemicals in agricultural food chains. *Environ Sci Technol* 1995; 30: 252-9.
- Menzie CA, Potocki BB, Santodonato J. Exposure to carcinogenic PAHs in the environment. *Environ Sci Technol* 1992; 26: 1278-84.
- Pinto CG, Pavon JLP, Cordero BM. Cloud point preconcentration and high performance liquid chromatographic determination of polycyclic aromatic hydrocarbons with fluorescence detection. *Anal Chem* 1994; 66: 874-81.
- Purkait MK, DasGupta S, De S. Performance of TX-100 and TRITON X-114 for the separation of chrysoidine dye using cloud point extraction. *J Hazard Mater* 2006; 137: 827-35.
- Qin XY, Meng J, Li XY, Zhou J, Sun XL, Wen AD. Determination of venlafaxine in human plasma by high-performance liquid chromatography using cloud-point extraction and spectrofluorimetric detection. *J Chromatogr B* 2008; 872: 38-42.
- Saitoh T, Hinze W. Concentration of hydrophobic organic compounds and extraction of protein using alkylammoniosulfate zwitterionic surfactant mediated phase separation (cloud point extraction). *Anal Chem* 1991; 63: 2520-5.
- Sicilia D, Rubio S, Bendito DP, Maniasso N, Zagatto EAG. Anionic surfactants in acid media: a new cloud point extraction approach for the determination of polycyclic aromatic hydrocarbons in environmental samples. *Anal Chim Acta* 1999; 392: 29-38.
- Shokrollahi A, Ghaedi M, Hossaini O, Khanjari N, Soylak M. Cloud point extraction and flame atomic absorption spectrometry combination for copper(II) ion in environmental and biological samples. *J Hazard Mater* 2008; 160: 435-40.
- Tavakoli L, Yamini Y, Ebrahimzadeh H, Shariati S. Homogeneous liquid-liquid extraction for preconcentration of polycyclic aromatic hydrocarbons using a water/methanol/chloroform ternary component system. *J Chromatogr A* 2008; 1196-1197: 133-8.
- Wild SR, Waterhouse KS, McGrath SP, Jones KC. Organic contaminants in an agricultural soil with a known history of sewage sludge amendments: polynuclear aromatic hydrocarbons. *Environ Sci Technol* 1990; 24: 1706-11.