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# Porous Polymer Monolith Microextraction Platform for Online GC-MS Applications

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## 1. Introduction

One of the key steps in chemical analysis is sample preparation. It is a very important step prior to analysis, otherwise the analyte signal could be suppressed by sample matrix interference. In most cases sample preparation entails some form of extraction of the analyte(s) of interest from the interfering species. Conventional extraction techniques include liquid-liquid extraction (LLE) and solid phase extraction (SPE). LLE involves mixing two immiscible solvent together, and based on solubility equilibria the analyte or the interfering species partitions preferentially in the organic solvent. Ideally, the partition coefficient is very high to allow for efficient extraction (Park et al., 2001). In SPE, analytes of interest are extracted from solution by passing the liquid through a solid phase, such as a C-18 SPE cartridge. Based on different physical and chemical properties, the desired solute either is adsorbed onto the solid phase, from whence it can later be eluted, or remains in solution while impurities are retained on the solid phase (Körner et al., 2000). The LLE and SPE extraction methods are laborious and demand the use of copious amounts of samples and solvents, therefore driving up the cost of chemical analysis. For LLE, emulsion formation can be a nuisance. These methods are also limited by factors like cartridge clogging in SPE, single use of the SPE and the need for toxic and polluting solvents (such as halogenated solvents like chloroform and dichloromethane) in LLE.

Other emerging extraction techniques that are based on miniaturization of LLE in effort to minimize solvent use and simplify the sample preparation process include: single-drop microextraction (SDME), hollow fibre liquid-phase microextraction (HF-LPME) and dispersive liquid–liquid microextraction (DLLME). Briefly, SDME, introduced by Cantwell and Jeannot in 1996, entails exposing a microdrop of extractant placed on the end of a Teflon rod to the sample solution or sample headspace. The HF-LPME uses two phase sampling mode, a hydrophobic hollow fibre is used to support the extractant and the fibre is exposed to the sample and the analyte is extracted in the organic extractant. On the other hand, the DLLME is based on rapidly injecting a few microliters of high density solvents mixed with a disperser solvent into the aqueous sample. The turbulence from the rapid injection causes large interfacial area emulsion droplets to form and distribute in the sample solution. Analytes partition into this emulsion droplets; and after centrifugation, they sediment at the bottom of a vial and then are sent for analysis. In general these miniaturized LLE techniques are convenient, efficient (given a high partition coefficient), inexpensive and requires very

little solvent. They are also easily coupled to gas chromatography (GC) (Jeannot & Cantwell, 1996). An extensive discussion of these methods is available and covered in the review by Pena-Pereira et al., 2009. An obvious disadvantage with these methods is that only a few organic solvents are usable; and so the methods lack versatility and are only applicable to the extraction of a limited number of analytes.

Solid phase microextraction (SPME), developed by Pawliszyn in the 1990s (Arthur & Pawliszyn, 1990), surmounts some of the limitations of the SPE, LLE and miniaturized LLE. SPME method employs a fused silica fiber coated with a polymeric coating to extract organic compounds from their sample matrix and directly transfer analytes into GC, eliminating offline sample extraction steps (Z. Zhang & Pawliszyn, 1995). Different polymer stationary phases have been used in SPME for the analysis of many different analyte classes. Common commercially-available SPME stationary phases include polyacrylate, polydimethylsiloxane (PDMS), divinylbenzene (DVB)/PDMS, carboxen/PDMS, and DVB/Carboxen/PDMS. However, SPME fibres are generally very fragile and cases of them breaking off in the GC port are very common. Therefore they have to be used with extreme care, and sometimes they have to be replaced fairly often, which makes them expensive. Another disadvantage of SPME is the limited variety of available stationary phases, which limits the range of analyte classes that can be extracted. There have been publications of novel SPME stationary phases being developed to address this.

Second and third generation variants of SPME have been developed, such as the needle-trap devices. The need-trap extraction method involves packing a needle with polymer beads or carbon based sorbents. A gaseous sample is drawn through the needle housing the sorbent plug and the analyte of interest is adsorbed and then directly injected and desorbed into GC (Ueta et al., 2010). The needle-trap extraction method possesses the benefits of microscale extraction (*i.e.* low cost, high efficiency, low-volume solvent use) while overcoming SPME's problem of fragility issues. However, this method is only applicable to headspace extraction of volatile samples and so can only be used to extract a limited range of analytes.

A new class of microextraction technique is polymer monolith microextraction (PMME) introduced by Feng and coworkers in 2006. Feng and coworkers fabricated their device using a regular plastic syringe (1 ml) and a poly (methacrylic acid-co-ethylene glycol dimethacrylate) monolith entrained in a silica capillary (2 cm × 530 μm I.D.) (Zhang et al. 2006). PPME shares the advantages of SPME while solving most of its limitations. In general the most important component of the PPME device is the porous polymer monolith usually formed in the confines of a silica capillary, although other materials such as polyether ether ketone (PEEK) and titanium tubes can used (Wen et al. 2006).

Porous polymer monoliths are relatively new materials synthesized by carrying out free radical (radicals generated by heat or ultra-violet radiation following destabilization of azo initiators) polymerization of cross-linking and monovinyl monomers, in the presence of suitable porogenic solvents. The generated radicals initiate a rapid polymer chain growth at what become nucleation sites, which continue to grow as the reaction proceeds. As polymer molecular weight increases, the solubility decreases and a two-phase system of solid polymer and liquid solvent results. The resulting monolith microstructure consists of an agglomeration or globules, whose size directly impacts resulting pore size distribution.

Globule size is influenced by many factors, including the number of nucleation sites present, monomer concentration, solubility, and degree of cross-linking. Thus, polymer microstructure can be controlled by rate of reaction, monomer/porogen ratio, type of porogenic solvents and fraction of cross-linking monomers. Detailed descriptions of various chemistries, and characterization of resulting monoliths can be found in references (Gibson et al. 2008 & Svec, 2010).

The polymer monoliths used in making the PPME platforms can easily be made from different types of monomers with different polarities depending on the analytes of interests. The large surface area provided by the tailor-able macropores in monolith structure may help to improve the extraction efficiency. The convection flow provided by the flow-through channel within the monolith also helps in accelerating mass transfer. Furthermore, PPME device is very easy to prepare, which also makes it a better choice than particle packed extraction cartridges.

There have been numerous literatures reporting on the application of this PMME method for extraction and coupling to GC and LC, with the latter being more common. For example, Wen et.al detected and quantified a series of sexual hormones (testosterone, methyltestosterone and progesterone) in liquid cosmetics extracted by a poly (methacrylic acid-co-ethylene glycol dimethacrylate) monolithic capillary. By simple dilution and filtering after extraction, the samples were directly injected into HPLC system. The limit of detection was 2.3 to 4.6 μg/L. Calibration curves showed good linearity in the concentration range of 10 to 1000 μg/L with $R^2$ above 0.996 (Wen et al., 2006).

A variation on conventional PPME involves the production of molecularly-imprinted polymers (MIPs) to make up the extraction monolith (Bravo et al., 2005; Sanbe & Haginaka, 2003; Schweitz, 2002; Zhou et al., 2010). The imprints left by template molecules act as "artificial receptor-like binding sites" which are selective to the analyte of interest. Zhou and coworkers synthesized a molecularly imprinted solid-phase microextraction monolith (MIP-PPME) for selective extraction of pirimicarb in tomato and pear. They prepared a pirimicarb MIP monolith in a micropipette tip using methacrylic acid as the functional monomer, ethylene dimethacrylate as the cross-linker and the mixture of toluene-dodecanol as the porogenic solvent. The dynamic linear ranges were from 2.0 to 1400 μg/kg for pirimicarb in tomato and pear (Zhou et al., 2010).

Until now polymer monolithic materials used in PMME have mainly been poly-(methacrylic acid-co-ethylene dimethacrylate) (MAA-co-EDMA) (Liu et al., 2011) and most documented applications have been for LC. Our group has demonstrated the use of many other types of acrylate, epoxy-based, and acrylamide polymer monoliths for PMME and have shown that these simple PPME devices can be fabricated for online-GC applications. A schematic set up on how they are coupled to GC-MS is shown in Figure 1, which is similar to a commercial SPME operation. The fabrication and application of these newly developed PMME materials will be discussed in this chapter. In particular we shall demonstrate the application of PPME to the extraction of caffeine, polyaromatic hydrocarbons (PAHs) and hormones in water. We have also evaluated the use of porous layer open tubular (PLOT) monoliths as PPME platforms. The potential advantage of PLOT (otherwise will be referred in this chapter as open tubular polymer monolith) is the faster mass transfer and consequently faster extraction than PPME. These materials are still being evaluated in our research group.

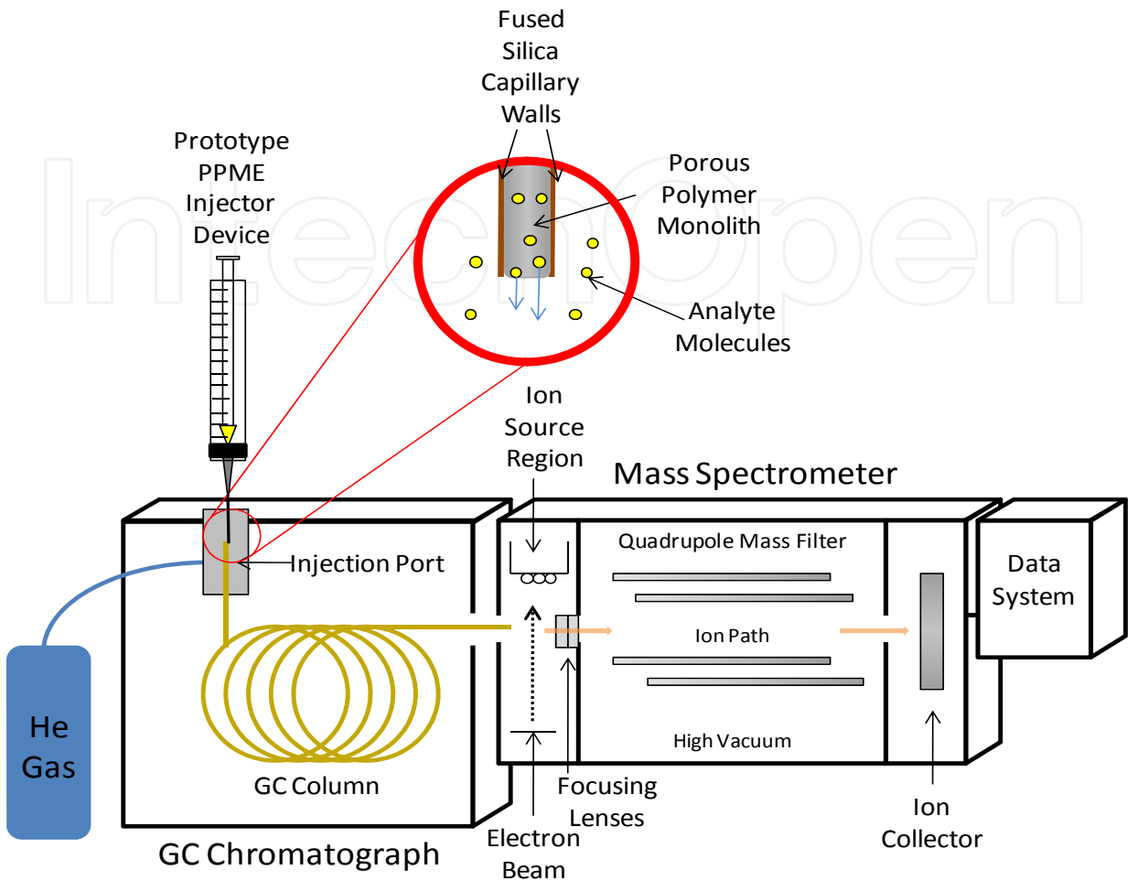


Fig. 1. Schematic of a PPME at the Injection Port of a GC-MS system.

## 2. Chemicals and materials

Acrylamide (99%, AA), styrene (>99%), 4-vinylpyridine (99%, 4-VP), glycidyl methacrylate (97%, GMA), ethylene glycol methacrylate (98%, EDMA), divinylbenzene (80%, DVB), 4,4'-azobis(4-cyanovaleric acid) (75+%), 1,1'-carbonyldiimidazole, 3-(trimethylsilyl) propyl methacrylate (98%), cyclohexanol (97%), 1-dodecanol (98+%), 3-aminopropyltriethoxylsilane (99%, APTES), ethanol (85%), naphthalene (≥99.7%), 2,6-dimethylnaphthalene ( 99%), phenanthrene (≥99.5%), HPLC grade acetonitrile, fluorene ( ≥99.0%), caffeine, isotopically-labelled (trimethyl $^{18}C$) caffeine, megestrol acetate, 17β-estradiol, acetic acid (≥99%) and methanol were purchased from Sigma-Aldrich ( St. Louis, MO, USA). Sodium acetate (99+%) was purchased from Alfa Aesar (Ward Hill, MA, USA). 99.7% NaOH and citric acid monohydrate were purchased from Fisher Scientific (Fair Lawn, NJ, USA).

The structures of analytes selected for extraction and selected reagents used for making the polymer monoliths are shown in Figure 2. All through the experiment deionized (D.I.) water used was prepared using a Millipore Elix 10 water purification system.

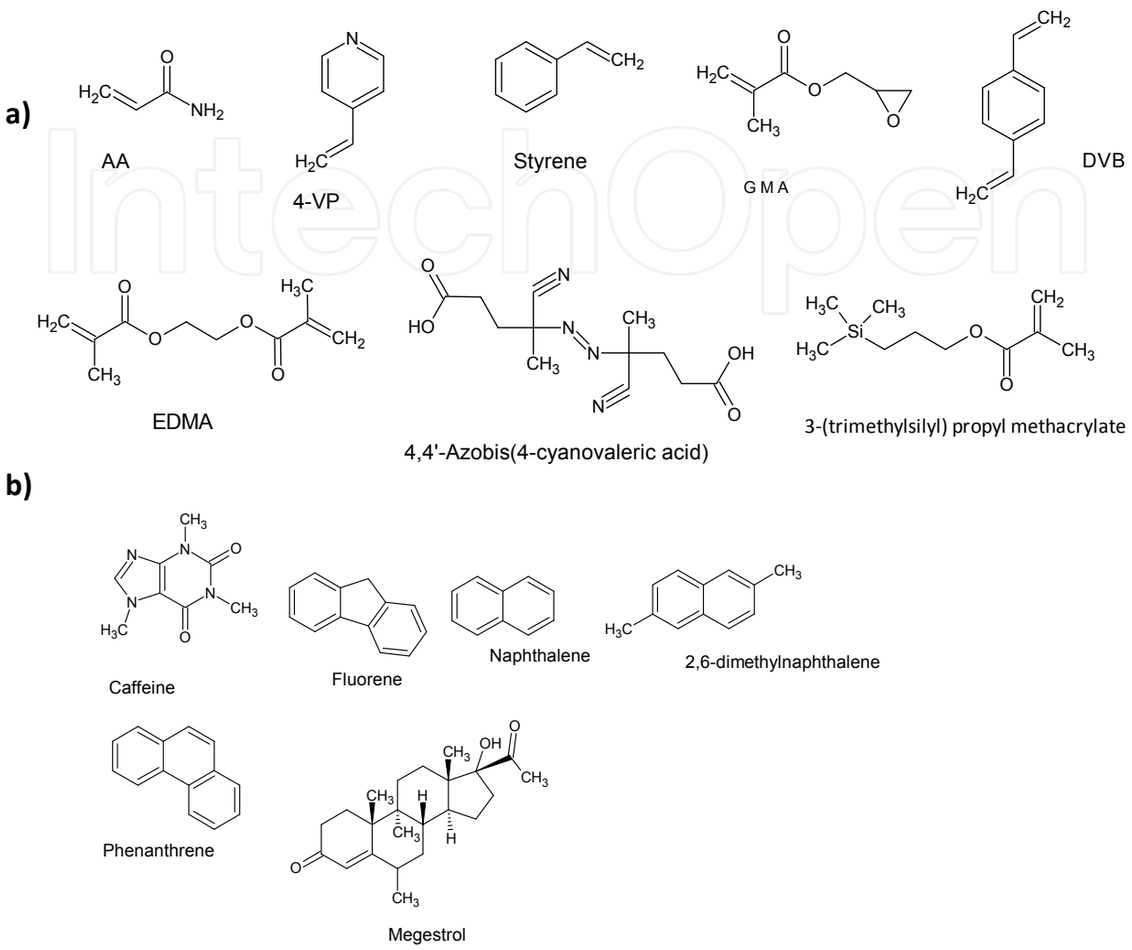


Fig. 2. a) Structures of some reagents used to make the polymer monoliths and, b) structures of some of the analytes that were tested.

## 3. Methods

### 3.1 Formation of porous polymer monoliths on fused silica capillary

#### 3.1.1 Poly(Acrylamide-co-EDMA)

The poly(acrylamide-co-EDMA) monolithic capillary was formed inside a polyimide-coated fused silica capillary (10 cm x 250 µm, i.d., Polymicro Technologies, USA) by heat-initiated polymerization. To begin, the capillary was conditioned by flushing (using a Harvard Apparatus PHD Ultra syringe pump) it with 1.0 M NaOH at a flow rate of 10 µL/min for 3 h, 0.1 M HCl for 1 h, washed with deinionized (D.I.) water for 1 h, then dried with a flow of

nitrogen. Next, the capillary was flushed at 10 µL/min with 20% (v/v) 3-(trimethoxysilyl)propyl methacrylate in toluene overnight (17 h), then infused (for 5 minutes at 5 µL/min) with the pre-polymer mixture, consisting of 13.0 mg acrylamide, 179 µL EDMA, 7 mg 4,4′-azobis(4-cyanovaleric acid) initiator, and 500 µL 1/1 (v/v) ethanol/D.I. water. Both ends of the capillary were capped and the capillary was placed in a 70˚C oven overnight (16 h). Once monolith formation was complete, the capillary was flushed with 1/1 (v/v) methanol/D.I. water to remove any unreacted reagents.

### 3.1.2 Poly (Styrene-co-Divinylbenzene)

The poly(styrene-co-divinylbenzene) monolithic capillary was formed the same way as the preparation of poly(acrylamide-co-EDMA) monolith except the composition of prepolymer mixture, which consists of 200 µL styrene, 80 µL divinyl benzene, 600 µL ethanol, and 5 mg 4,4′-Azobis(4-cyanovaleric acid) initiator.

### 3.1.3 Poly (4Vinylpyridine-co-EDMA)

The poly (4Vinylpyridine-co-EDMA) monolith was formed the same as documented above for the others. The pre-polymer mixture, consisted of 179 µL of EDMA, 13 µL of 4-vinylpyridine, 7 mg of 4,4′-Azobis(4-cyanovaleric acid), 250 µL of ethanol, and 250 µL of D.I. water.

### 3.1.4 Poly (Glycidylmethacrylate-co-EDMA)

The poly (glycidylmethacrylate-co-EDMA) monolith was formed by polymerization of a mixture of 80 µL of EDMA, 240 µL of glycidyl methacrylate, 7 mg of 4,4′-azobis(4-cyanovaleric acid), 400 µL of cyclohexanol, and 180 µL of dodecanol.

### 3.1.5 Poly (styrene-co-divinylbenzene) PLOT

The poly(styrene-divinylbenzene) PLOT capillary was formed inside a 250 µm i.d., 10 cm long fused silica capillary as outlined schematically in Figure 3. This method was adapted from the literature (Schweitz, L. 2002). Briefly, the capillary was conditioned by flushing with 1.0 M NaOH for 3 h at a flow rate of 10 µL/min. This was followed by a D.I. water wash for 1 h, then dried with a flow of nitrogen. Next, the capillary was filled with 20% (v/v) APTES in toluene overnight (16 h), then rinsed with acetone for 1 h and then dried. The initiator solution, consisting of 8.4 mg of 4,4′-azobis(4-cyanovaleric acid) initiator, 9.7 mg of 1,1′-carbonyldiimidazole, 1.5 mL of methanol, and 1.5 mL of 50 mM sodium acetate-acetic acid buffer (pH ~5.5) was prepared and the pH of the final solution was adjusted to 5.5 using acetic acid. The capillary was infused at 10 µL/min with the initiator solution overnight (16 h), then washed with 1/1 (v/) methanol/D.I. water for 30 minutes and dried with a flow of nitrogen. Next, the prepolymer mixture, consisting of 200 µL styrene, 200 µL DVB, and 600 µL ethanol, was prepared and infused into the capillary for 5 min at 5 µL/min. Both ends of the capillary were capped and placed in a 70˚C oven overnight (16 h) for polymerization to ensue. The PLOT column was washed with 1/1 (v/v) methanol/water.

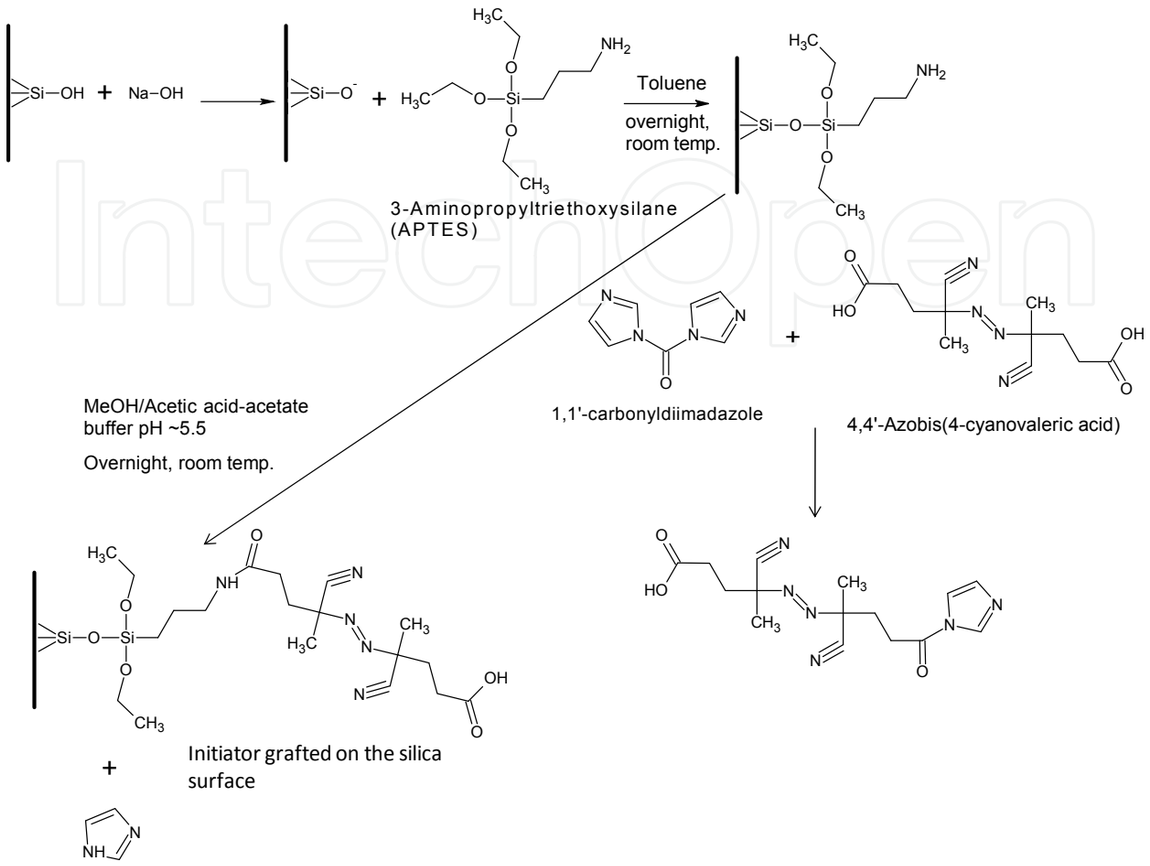


Fig. 3. Reaction schematic for grafting initiator onto the capillary wall for open tubular monolith formation

### 3.2 PPME device fabrication

A prototype PPME device was prepared using the porous polymer monolith described above, disposable plastic syringes with removable needles, micropipette tips, and fast-drying epoxy. A disposable plastic syringe was dismantled by removing the inner plunger. The rubber stopper was removed from the end of the plunger and the tip of the stopper was trimmed off, creating a sort of rubber washer. A 10-100 µL yellow micropipette tip was trimmed so that its wide end fits snugly over the end of the hard plastic plunger, the stem of which had previously been trimmed to fit inside the pipette tip. The pipette tip was filled with thoroughly-mixed epoxy glue then secured onto the trimmed plunger stem. A capillary containing the desired porous polymer monolith was then inserted into the narrow end of the pipette tip attached to the plunger and the rubber washer was slipped over the capillary and secured to the base of the pipette tip with epoxy. The prototype was left to dry overnight then reinserted into the syringe outer barrel. With the PPME capillary withdrawn into the syringe barrel, a needle was attached to the syringe. This enables the PPME device to puncture a GC septum effectively before the PPME capillary is injected for desorption. A schematic of the in-house fabricated PPME device is shown in Figure 4.

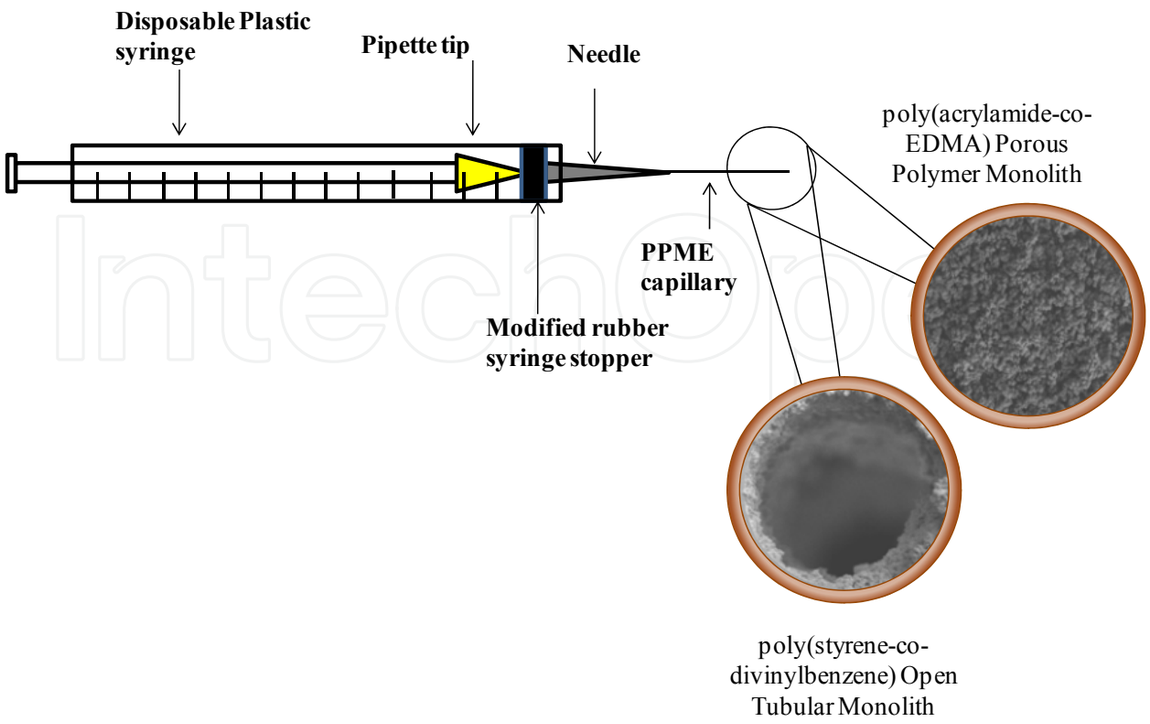


Fig. 4. A schematic of the in-house fabricated PPME device with an inset of SEM images of poly(acrylamide-co-EDMA) and poly (styrene-co-divinylbenzene) PLOT platforms.

### 3.3 Extraction of caffeine and PAHs using PPME and analysis by GC-MS

#### 3.3.1 Caffeine extraction

3 mL of caffeine standards with the concentration of 100, 200, 300, and 400 µg/mL were prepared in 4 mL vials and 75 µL of the internal standard, isotopically-labelled (trimethyl $^{13}C$) caffeine, was added. The poly(acrylamide-co-EDMA) PPME capillary was chosen for this extraction as it posses fairly high polarity stationary material that could preferentially adsorb caffeine. The PPME was conditioned by inserting it in the GC injection port for 30 minutes at 270˚C, then cooled at room temperature. Starting with the 100 µg/mL caffeine standard, the vial was placed on a stir plate and stirred at a controlled rate. The PPME capillary was then immersed in the standard solution for 7 minutes to allow caffeine to adsorb onto the polymer monolith, then the PPME was injected into the GC port and desorbed at the GC injector for 3 minutes at 200˚C. This procedure was repeated for the 200, 300, and 400 µg/mL standards with the PPME capillary being cooled at room temperature between runs. For comparison, the above procedure was also repeated using a commercially available DVB/Carboxen/PDMS StableFlex™ SPME fibre (Supelco).

#### 3.3.2 Polyaromatic hydrocarbon (PAH) extraction

1.5 mL of PAH standards containing naphthalene, 2,6-dimethylnaphthalene, and phenanthrene with the concentration of 20, 50, 100, and 200 µg/mL were prepared in 2 mL vials with 75 µL of the internal standard fluorene (480 µg/mL) added. Based on polarity

matching considerations, the poly (styrene-co-divinylbenzene) PPME device was conditioned in the GC injection port for 8 minutes at 280˚C then cooled at room temperature. Starting with the 20 µg/mL standard, the vial was placed on a stir plate and stirred at a controlled rate. The PPME capillary was immersed in the standard solution for to allow the PAHs to adsorb onto the polymer monolith. The adsorption times that were evaluated included 3 minutes, 1 minute, and 30 seconds. After extraction the PPME was injected into the GC injection port (200˚C) for 3 minutes' desorption. This procedure was repeated for the 50, 100, and 200 µg/mL standards with the PPME capillary being cooled at room temperature for 8 minutes between runs.

### 3.3.3 Evaluation of capability of the porous monoliths for hormone extraction

Hormones in the environment emerging contaminants of scientific interest. Conventional analysis approach is to extract the hormones by a C-18 SPE cartridge and elute with an appropriate solvent followed by analysis by HPLC-DAD-MS. Analysis of hormones by GC-MS often demands a derivatization process. Different derivatization reagents have been used including silylating reagent usually, N,O-bis(trimethylsilyl)trifluoroacetamide and Heptafluorobutyric acid. Our goal was to explore the use of PPME to extract the hormones and carry out on fiber derivation followed by injection of the PPME into the GC. This has been demonstrated largely on acrylate SPME fibers by several authors (Yang et al. 2006, Pan et al. 2008).

Before the attempt to carry out on-porous polymer derivatization, it was crucial first to determine which porous polymer monolith was best suited for the extraction of hormones. Megestrol standards with the concentration of 0.1, 1, 10, and 20 ppm in D.I. water were prepared. Poly(GMA-co-EDMA) and poly(4VP-co-EDMA) porous polymer monoliths were chosen to evaluate their ability to extract the hormone. Both monoliths were preconditioned by washing with 1:1 (v/v) methanol/D.I. water at 25 µL/min for 40 minutes then drying with nitrogen. Starting with the 0.1 ppm megestrol standard, each porous polymer monolith in a capillary (10 cm long) was infused with the hormone solution at 25 µL/min for 40 minutes and the eluate solution was collected in a 2 mL vial. The monoliths were then dried with nitrogen and eluted with 9:1 (v/v) methanol/D.I. water at a flow rate of 25 µL/min. The eluate was collected in a 2 mL vial. This procedure was repeated for the 1, 10, and 20 ppm hormone standards and the collected solutions were analyzed by HPLC-DAD for both the monoliths.

## 4. Instrumentation

### 4.1 GC-MS instrumentation conditions

GC analysis was carried out on an Agilent 6890N gas chromatography system with an Agilent 5975C VL MSD (Agilent Technologies, USA). Analytes were separated on a 5% phenyl methyl siloxane capillary column (30 m x 250 µm I.D., 0.25 µm film thickness, Agilent Technologies, USA). The column oven temperature was initially set at 180˚C for 3 minutes and then was increased to 205˚C at 5˚C/min. This temperature was held till the end of the analysis. The MS detector temperature was set at 200˚C. The injection port temperature was set at 280˚C with a split-less injection mode. Helium was used as carrier gas set at the flow rate of 24.1 mL/min.

### 4.2 HPLC -DAD Instrumentation and conditions

Analysis of megestrol eluted from the porous polymer monolith were performed on a Thermo LCQ fleet HPLC-DAD system consisting of a LC pump, an autosampler, a 6-port injection valve with a 20-µL injection loop, and a diode array detector (DAD) with the scan range from 250 to 400 nm. The temperature of column oven was set at 30 °C. The temperature of autosampler tray was set at 6 °C to prevent degradation of the hormones. An Agilent Zorbox Eclipse PAH RPLC column (250 x 3.0 mm I.D., 5 µm) (Agilent, Santa Clara, CA, USA) was used for the separation of hormone. The mobile phase used was acetonitrile (C) and 0.1% formic acid in $H_2O$ (D) at a flow rate of 500.0 µL/min. The eluent gradient setting is shown in Table 1.

| %M.P. / Time(min) | Mobile phase C % | Mobile phase D % |
|---|---|---|
| 0 | 20 | 80 |
| 5 | 55 | 45 |
| 6 | 95 | 5 |
| 10 | 95 | 5 |
| 12 | 20 | 80 |
| 15 | 20 | 80 |

Table 1. HPLC gradient setting for the separation of antibiotics/hormones.

## 5. Results and discussion

### 5.1 Monolithic structure of PPME and PLOT

The structure of different PPME and PLOT was investigated by scanning electron microscope (SEM), shown in Figure 3. As can be seen in Figure 3 (inset picture consist of SEM image of poly(acrylamide-co-EDMA) and poly (styrene-co-DVB) PLOT), there were many macropores and flow-through channels in the network skeleton , which can provide the high porosity and good mass transfer desired for a microextraction platform. The SEM image of poly (styrene-DVB) PLOT confirms that using the initiator capillary wall grafting procedure outlined in Figure 3, the polymer monolith only formed around the walls of the capillary. Using this procedure many other different types of open tubular polymer monoliths can be fabricated. It is proposed that the PLOT structure could potentially result in a faster mass transfer and efficient analyte extraction compared to the conventional polymer monolith. At present, research is still being conducted to confirm and test this hypothesis.

### 5.2 Applications of PPME

#### 5.2.1 Caffeine extraction

Different concentrations of caffeine standards from 100 to 400 µg/mL, with isotopically-labelled (trimethyl $^{13}C$) caffeine was used as the internal standard, were extracted using a poly (acrylamide-co-EDMA) PPME. The calibration curve obtained is shown in Figure 5 a). The y-axis on the calibration curve is the peak area ratio of caffeine (m/z 194) to the internal standard (m/z 197), obtained from extracted ions chromatogram. The linear regression coefficient ($R^2$) achieved with the PPME was 0.9771. This satisfactory $R^2$ indicates that this

in-house built poly (acrylamide-co-EDMA) PPME is a suitable analytical tool for extracting caffeine in a wide range of concentration. We compared the extraction efficiency of the in-house fabricated PPME with the commercial SPME (2 cm-50/30µm DVB/Carboxen™/PDMS StableFlex ™ purchased from Supelco (Bellefonte, PA, USA)). The linear regression coefficient for the commercial SPME was 0.999 (as shown in Figure 5 b), which certainly rivals the in-house made PPME. This could partly be due to potential better mass transfer for the commercial SPME due to the thin film thickness (30 µm) of the stationary phase. However, it is expected by fabricating open tubular polymer monolith based PPME the mass transfer effect could improve and the results could compare with the commercial one. However, the results obtained with the PPME herein shows good usability especially with possible benefits over the commercial SPME, such as better sample capacity and less prone to breakage and thus can be reused for a much longer time.

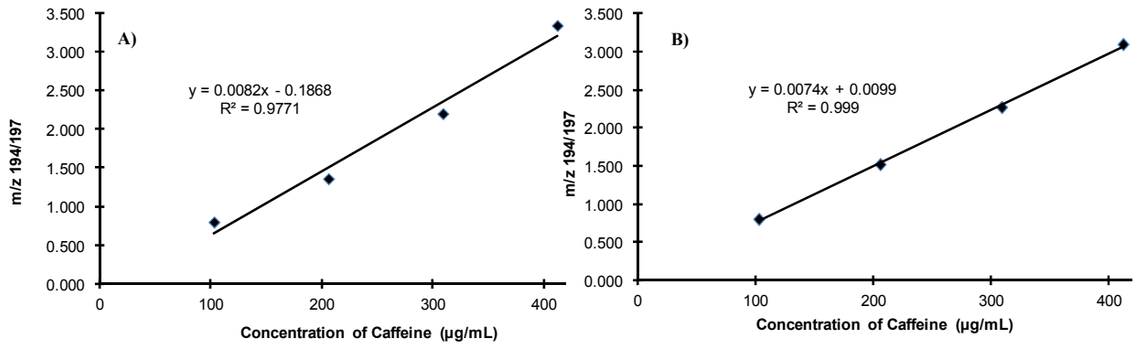


Fig. 5. Calibration curve of caffeine extracted using a) Poly (acrylamide-co-EDMA) PPME and b) commercial SPME.

The successful extraction of caffeine by PPME suggests an obvious extension of this extraction method to analysis of other compounds such as, theobromine, and theophylline and other compounds in the methylxanthine class. Theobromine is found in chocolate, tea leaves, and the cola nut, while theophylline is found in cocoa beans as well as in therapeutic drugs for respiratory disorders. Because of their presence in food and drugs it is clear that an effective and efficient extraction method for methylxanthines would be a relevant application. Using a multi-step extraction, which included the use a $^{18}$C extraction tube SPE followed by filtration, Aresta's group reported the determination of caffeine, theobromine, and theophylline, among other compounds, in human milk by HPLC-UV (Aresta & Zambonin, 2005). Since it has been shown that PPME is a viable method for caffeine extraction, it is reasonable to suggest that PPME may also be used to analyze other methylxanthines, and other polar like compounds of interest in pharmaceutical, nutraceutical and environmental fields. Moreover, PPME may be more efficient, reusable, could reduce analysis cost by reducing the steps needed in analysis process and in reducing the solvents required.

### 5.2.2 Extraction of polyaromatic hydrocarbons

With the successful extraction of caffeine using PPME, the extraction of another class of analytes, polyaromatic hydrocarbons (PAHs) using poly(styrene-co-DVB) PPME (a fairly

non polar material whose polarity could potentially march the PAHs) were also attempted. A mixture of four different PAHs at different concentrations were tested. The PAHs tested included naphthalene, 2,6-dimethylnaphthalene, phenanthrene and florene, where florene was also used as an internal standard. While good results were obtained at 3 min, 1 min adsorption time, it was found that even with a 30-second adsorption, satisfactory extraction of the PAHs was still achieved. Figure 6 showed the GC-MS chromatogram of four PAHs extracted by poly(styrene-co-DVB) PPME with 30- second adsorption time. Good baseline separation was achieved as attested in the chromatogram, with enough resolution to allow separation of more PAHs. This potential is important in environmental samples which can have numerous PAHs present. The PAHs peaks on the PPME showed some tailing, possibly attributable to analytes slow mass transfer. We propose this could be alleviated by the use of a PPME fabricated poly(styrene-co-DVB) PLOT platform. This work is in progress.

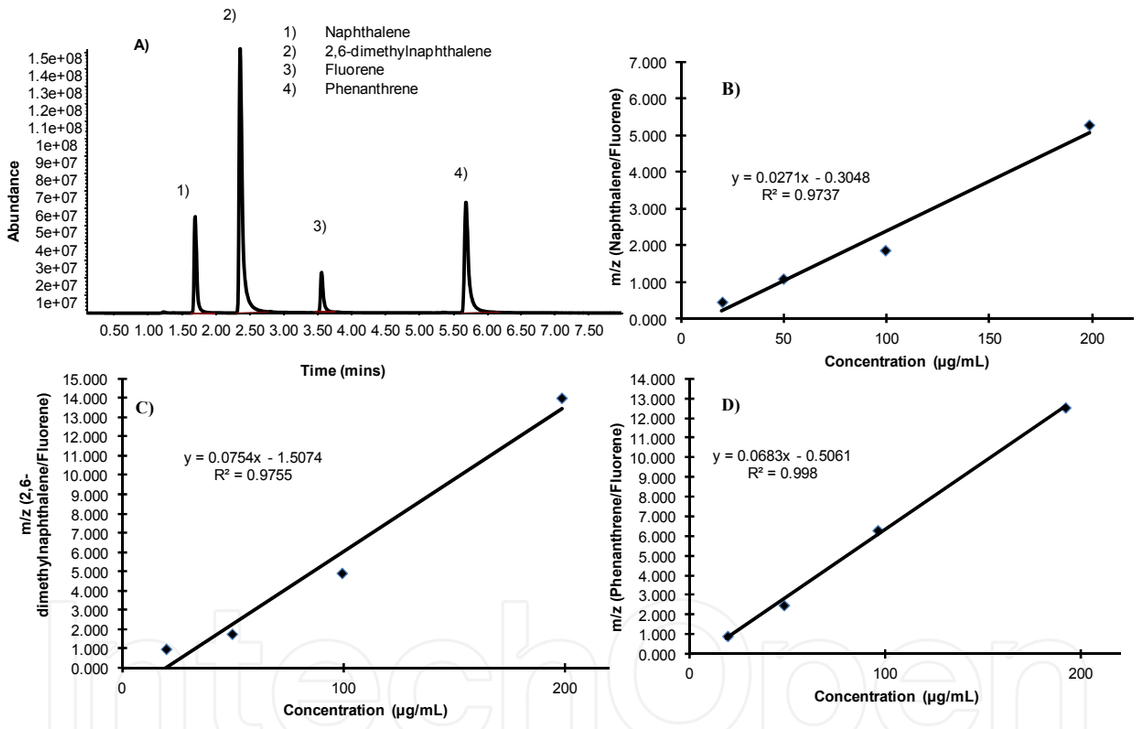


Fig. 6. A) GC-MS chromatogram showing separation of 100 µg/mL of three PAHs including; 1) naphthalene, 2) 2,6-dimethylnaphthalene, 3) fluorene, and 4) phenanthrene extracted by poly(Styrene-co-DVB) PPME; Calibration curves of B)naphthalene; C) 2,6-dimethylnaphthalene; D) phenanthrene extracted by poly(Styrene-co-DVB) PPME with 30 second adsorption and fluorene as an internal standard.

Calibration curves of the three PAHs extracted by poly(Styrene-co-DVB) PPME are shown in Figure 7 b,c,d, with analytes concentration range from 0 to 200 µg/mL. All the three PAHs showed satisfactory linear calibration curves with $R^2$ higher than 0.997 ($R^2$ = 0.9737 for naphthanlene, 0.9755 for 2,6-dimethylnaphthalene and 0.998 for phenanthrene). Clearly, the

results obtained using the in-house fabricated poly (styrene-co-DVB) PPME revealed several things. First, the well-defined peaks in chromatograms obtained from GC-MS analysis showed that PAHs, even in low concentrations, are readily extracted and desorbed from the PPME capillary, making PPME a suitable method for PAH extraction. Second, this method showed feasibility for environmental monitoring of PAHs in bodies of water such as lakes, rivers, and ponds. The PPME, being more robust and rugged than conventional SPME fibers, can be taken to monitoring sites, which could make sampling process much easier. Third, this method can be extended to the analysis of any hydrophobic anyalytes immersed in an aqueous solution, since the analytes would very readily adsorb onto the hydrophobic polymer . With this knowledge, a plethora of polymers can be fabricated, each with a specific targeted analytes class to be extracted from a given sample matrix.

### 5.2.3 Extraction efficiency of megestrol by the polymer monoliths

There is growing public concern for the presence of hormones in the ecosystem, and the possible pathways by which these substances can enter into human and animal food chains. The concern focuses particularly on the common use of these substances in animal husbandry especially as growth promoting agents. Due to their stability most of the hormones are excreted unchanged or as active metabolites. Most commonly used processes are based on the extraction of hormones from a sample matrix and analysis by liquid chromatography (Mitani et al., 2005; Yi et al., 2007). Analysis by GC-MS could however be faster, but the hormones require derivatization to make them more volatile. This has been actualized typically by extraction of the hormones from sample matrix by C-18 cartridges, followed by pre-concentration, derivatization and then GC-MS analysis. A new method called on-fiber derivatization allows the hormones to be extracted, derivatized on the solid-phase and subsequently analyzed directly by GC-MS. Carpinteiro and coworkers combined SPME and on-fiber derivatization to detect and quantify estrogens in water by GC-MS. Samples were first extracted by SPME, and then were silylated on the headspace of a vial containing the derivatization agent. The MS signal intensity were dramatically increased by the derivatization step. The study of matrix effects demonstrated that the method is applicable for the determination of estrogens in both surface and sewage water (Carpinteiro et al., 2004). This on-fiber derivatization method eliminates the further sample preparation step before injecting into GC-MS. We propose PPME could have even better versatility in carrying out extraction and on-porous polymer derivatization of the hormones.

To test the hypothesis, two types of monoliths, poly( 4VP-co-EDMA) and poly (GMA-co-EDMA) were first tested for their extraction efficiency of megestrol. We evaluated the % recovery of megestrol using HPLC-DAD analysis. Both poly( 4VP-co-EDMA) and poly (GMA-co-EDMA) were found to have very good adsorptive capacity for megestrol with calculated % recovery of 98% and 107% respectively. An overlapped chromatogram indicating megestrol peak for poly( 4VP-co-EDMA) , poly (GMA-co-EDMA) and directly injected 10 ppm megestrol standard is shown in Figure 8. These two polymers were found to be good stationary phases for analysis of hormones.

At the point of publication of this chapter, the demonstration of the on-fiber derivatization of the hormones using PPME fabricated from poly( 4VP-co-EDMA) and poly (GMA-co-EDMA) was ongoing.

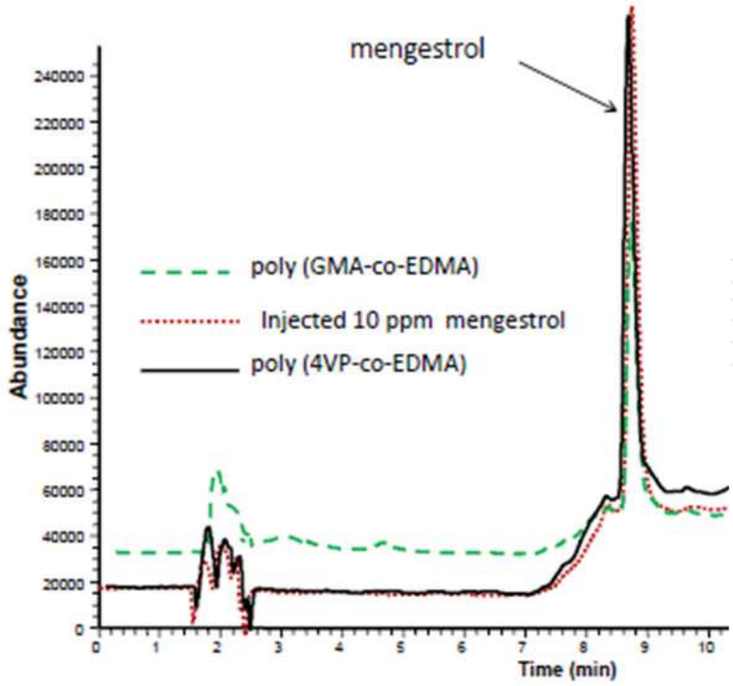


Fig. 8. Overlapped chromatogram indicating megestrol peak for poly( 4VP-co-EDMA) and poly (GMA-co-EDMA) and directly injected 10 ppm megestrol standard.

## 6. Conclusions

Polymer monolith microextraction (PPME) has been demonstrated to be feasible, facile and versatile platform for analysis, preconcentration and extraction of analytes and for online coupling to GC-MS. The monoliths that have been tested including poly (styrene-co-divinylbenzene), poly(acrylamide-co-EDMA), poly (4-vinylpyridine-co-EDMA) and poly (glycidyl methacrylate-co-EDMA, have been found to work well when matched with analytes of corresponding polarity. The polymer monoliths based materials provided for satisfactorily fast mass transfer and thus good extraction efficiency. Good linear calibration curves were achieved in a wide concentration range when using poly(acrylamide-co-EDMA) for analysis of caffeine, poly (styrene-co-divinylbenzene) for analysis of PAHs. Both poly (4-vinylpyridine-co-EDMA) and poly (glycidyl methacrylate-co-EDMA were tested and found to be good sorbents for extraction of hormones such as megestrol with good % recoveries of 98% and 107% respectively, which suggests the potential of applying this PPME method to a wider selection of hormones and to a more complicated sample matrix.

An analogous PLOT polymer monolith was successively prepared and is being tested as a microextraction platform.

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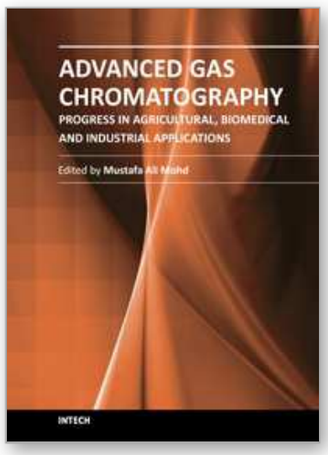


**Advanced Gas Chromatography - Progress in Agricultural, Biomedical and Industrial Applications**

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Progress in agricultural, biomedical and industrial applications' is a compilation of recent advances and developments in gas chromatography and its applications. The chapters cover various aspects of applications ranging from basic biological, biomedical applications to industrial applications. Book chapters analyze new developments in chromatographic columns, microextraction techniques, derivatisation techniques and pyrolysis techniques. The book also includes several aspects of basic chromatography techniques and is suitable for both young and advanced chromatographers. It includes some new developments in chromatography such as multidimensional chromatography, inverse chromatography and some discussions on two-dimensional chromatography. The topics covered include analysis of volatiles, toxicants, indoor air, petroleum hydrocarbons, organometallic compounds and natural products. The chapters were written by experts from various fields and clearly assisted by simple diagrams and tables. This book is highly recommended for chemists as well as non-chemists working in gas chromatography.

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