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Review

Determination of coal tar and creosote constituents in the aquatic environment

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Abstract

Creosote and its parent material, coal tar, are complex mixtures. Upon release their components fractionate into the air, water, soil/sediment and biota; as a function of their physical and chemical properties. Therefore, assessment of their fate and concentrations in the environment must consider a wide variety of both compounds and matrices. Analyses are typically complicated, consisting of sample extraction, purification and chromatography-based final characterization steps. Several new techniques have been introduced to reduce or simplify the number of steps, solvent and time required. Recently developed extraction methods include supercritical fluid, accelerated solvent, microwave and solid-phase microextraction. On-line purification and coupling of extraction and chromatography have also emerged. HPLC and GC remain the major tools for performing the final separations. Application of mass spectrometry has increased as more reliable, versatile and less expensive units have become available, such as the ion trap and mass selective detectors. Fluorescence and diode array UV, in concert with HPLC, and C-, S- and N-selective gas chromatographic detectors are also being applied.

Keywords: Creosote; Coal tar; Environmental analysis; Water analysis; Sample handling; Reviews; Polynuclear aromatic hydrocarbons

Contents

1. Introduction 80
2. Environmental contamination 80
3. Extraction of coal tar related compounds 83
   3.1. Water 83
      3.1.1. Liquid–liquid extraction 83
      3.1.2. Steam distillation 84
      3.1.3. Headspace 84
      3.1.4. Solid-phase extraction (SPE) 84
      3.1.5. In-situ sampling/extraction approaches 84
   3.2. Sediments and biota 85
      3.2.1. Aqueous caustic reflux 85

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3.2.2. Sonication/blending 85
3.2.3. Dry sample extraction methods 85
3.2.4. Column percolation 85
3.2.5. Roller or shake flask 86
3.2.6. Soxhlet-type techniques 86
3.2.7. Accelerated solvent extraction (ASE) 86
3.2.8. Microwave extraction 86
3.2.9. Supercritical fluid extraction (SFE) 87
3.3. Purification of extracts 87
3.3.1. Liquid–liquid 87
3.3.2. Thin-layer chromatography (TLC) 88
3.3.3. Low-pressure column chromatography 88
3.3.4. High-performance liquid chromatography 88
3.4. Identification and quantification of coal tar related compounds 89
3.4.1. HPLC 89
3.4.2. Gas chromatography 89
Acknowledgments 92
References 93

1. Introduction

Coal tars are generated during carbonization of coal. Today, this occurs most commonly during the production of coke. In addition, it has been estimated that more than $11 \cdot 10^9$ gallons of coal tar were generated between the early 19th and mid 20th century in the USA as a byproduct of manufactured gas generation, prior to the widespread availability of natural gas [1]. Tar composition is a function of the conditions and quality of coal used [2,3]. Creosote is a bulk distillate of coal tar. About 50% of pitch, the heavy material remaining after creosote distillation, consists of compounds with more than seven aromatic rings [4]. Creosote has been widely used as a preservative for protecting wood products such as telephone poles, railroad ties and pier pilings. Creosote applicators have recognized the synergistic toxic effects of its constituents on termites and other wood destroying pests. As noted in a commercial applicator brochure: "The true value of creosote as a wood preservative lies in the very multiplicity of toxic compounds it contains....you don't need to guess about the preserving power of creosote—just be certain it is coal tar creosote—and then use plenty of it!" [5]. Synergistic effects of coal tar components, e.g., polycyclic aromatic hydrocarbons (PAHs), on nontarget organisms have also been reported [6].

Wood creosote, derived from wood tar, is significantly different in origin and composition. It is composed mostly of phenolics and has several medicinal applications. All subsequent discussions here pertain to coal tar and coal tar-derived creosote.

2. Environmental contamination

Over 700 wood-preserving facilities have been documented in the USA alone [7]. During pressure impregnation of wood products, excess free product may be released from the treated materials. Leaching of spilled wastes from these application sites has been common. Numerous reports exist documenting coal tar and creosote contamination in and around points of large scale production and application [7–12].

It has been estimated that creosote consists of 85% PAHs; 10% phenolics and 5% other N-, S- and O-heterocyclics [7]. Some major constituents are listed in Table 1. Estimates of the total number of constituents vary, some report values as high as 10 000 individual components [13]. Most analytical efforts have concentrated on the PAHs, dominant components of coal tar and creosote. However, a number of constituents, notably the O- and N-heterocyclics, exhibit appreciable solubilities and may be transported significant distances via surface runoff or groundwater [4,9,14,15]. The low-molecular-mass heterocyclic compounds have been identified as major contributors to the acute toxicity of creosote leachates [16]. Water within sediment pores

Table 1
Partial list of compounds reported in coal tar, creosote and impacted environmental samples

Aromatic hydrocarbons	*N-heterocyclics*
Benzene	Carbazole
Toluene	Quinoline
Xylene	Acridine
Naphthalene	Indole
Methylnaphthalenes	Benzocarbazoles
Biphenyl	Anilines
Acenaphthylene	Benzoquinolines
Coronene	Phenanthridine
Acenaphthene	Methylcarbazoles
Fluorene	Cyanonaphthalene
Methylfluorenes	Benzacridines
Phenanthrene	Dibenzacridines
Anthracene	Dibenzocarbazoles
Methylphenanthrenes	Methylbenzacridines
4H-Cyclopenta[*def*]phenanthrene	Azapyrene
Acephenanthrylene	Azachrysene
Fluoranthene	Dibenzothiazole
Pyrene	Benzothiazole
Benzofluorenes	Cyanopyrene
Methylpyrenes	
Benzofluoranthenes	*O-heterocyclics*
Benzophenanthrenes	Furans
Cyclopenta[*cd*]pyrene	Phenol
Chrysene	Cresols
Benzanthracenes	Xylenols
Benzopyrenes	Dibenzofuran
Perylenes	Methylbenzofuran
Indenochrysenes	Benzonaphthofuran
Indenopyrenes	
Dibenzanthracenes	*S-heterocyclics*
Picene	Thiophenes
Benzochrysenes	Dibenzothiophene
Benzoperylene	Benzothiophene
Dibenzofluoranthene	Benzonaphthothiophenes
Dibenzopyrenes	Phenanthro[4,5-*bcd*]thiophene
Naphthopyrenes	

may also become contaminated and serve as a major vehicle for exposure of many benthic organisms [17]. The sediments themselves may form a substantial sink, as the less water soluble components associate to a high degree with organic carbon present therein [18,19]. Biotransformation of components may further complicate analyses and lead to enhanced environmental mobility. Pereira et al. reported that oxygenated azaarenes were more water-soluble and mobile in aquifers than their parent compounds [20]. Further, exposure to ultraviolet light, particularly for PAHs, may lead to photolysis, resultant enhanced mobility and altered toxicity [21,22].

Concern regarding release of creosote from treated products in the field has been voiced for many years. Shimkin et al. in 1951 detailed the occurrence of PAHs in barnacles growing on creosoted pilings [23]. Extracts of the barnacles were injected into mice and several developed subcutaneous tumors. PAHs have also been detected in mussels from treated pilings [24], lobsters held in pens constructed of creosoted timber [25] and in potable water stored in tanks sealed with coal tar-derived materials [26].

Fig. 1 illustrates some of the changes that may occur in the composition of creosote as it moves through the environment. Presented are gas chromatography–flame ionization detector (GC–FID) chromatograms of extracts of creosote-contaminated sediment; a water soluble fraction generated from this sediment; and aromatic compounds accumulated by oysters following laboratory exposure to this same water soluble fraction. Constituents in the sediment range from low- to relatively high-molecular-mass PAHs. Some reduction of the more volatile, water soluble compounds due to weathering, compared to neat creosote, has occurred. In contrast, the water soluble fraction is clearly enriched in lower-molecular-mass compounds, such as naphthalene. Compounds accumulated from this water soluble fraction by oysters appear quite similar to those originally present in the sediments, with a reduction in higher-molecular-mass compounds. In-laboratory exposure to this water-accommodated fraction increased the oysters' susceptibility to a common marine pathogen that has contributed to the significant reduction of its abundance in the Chesapeake Bay [27].

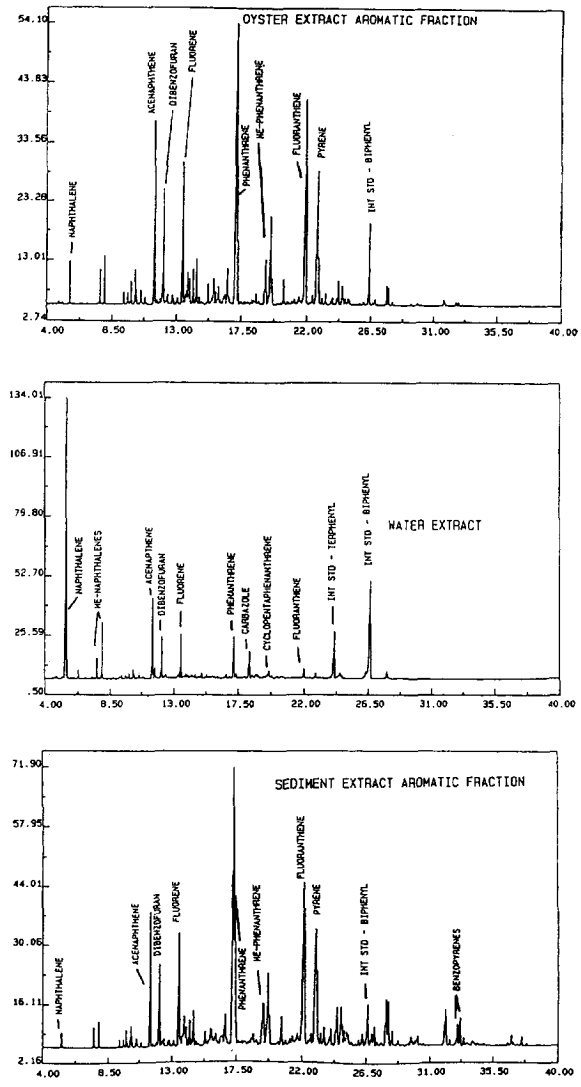

Fig. 1. GC–FID chromatograms of extracts of creosote-contaminated sediment; a water soluble fraction generated from this sediment; and aromatic compounds accumulated by oysters following exposure to the water soluble fraction. Note the preferential dissolution of lower-molecular-mass aromatics in the water and the similar profiles of the sediment and oyster extracts.

From this brief discussion it should be apparent that assessment of the extent of environmental contamination by coal tar and creosote-derived products represents a major challenge. Bulk spectroscopic analyses are generally not adequate and high resolution chromatographic techniques ultimately must be used to track the fate of coal tar related compounds in the environment. A shortcoming of most analytical approaches is the need for multiple purification steps. This increases reagent consumption and potential for analyte loss and sample contamination. Virtually all methods require repeated concentration of organic solvent volumes to: (1) minimize injection volume during subsequent chromatographic steps, reducing bandwidth and optimizing resolution; (2) provide a "weak" injection solvent for subsequent chromatographic procedures; or (3) introduce sufficient material to the detectors used. Associated evaporative losses of the more volatile components may be substantial. Losses of biphenyl and fluorene from 30% to 100% have been reported, after reduction to dryness and solvent reconstitution [28]. Effects are most severe at the lowest concentrations. Multi-step procedures may also expose light sensitive compounds to photodegradation [29]. Reduction in analytical steps may be achieved by using more selective means of extraction, combining steps (e.g., on-line extraction/purification) or eliminating use of organic solvents (as with supercritical fluid extraction, SFE). Modifications in chromatographic techniques themselves may also be beneficial.

3. Extraction of coal tar related compounds

The first step, following homogenization and subsampling, is separation of the analytes from the bulk matrix, i.e., water, sediment or tissue. The precision, accuracy and elegance of subsequent steps is immaterial, if the analytes are not quantitatively obtained during extraction. Recently, extraction methodologies have been the subject of intensive development, as they are typically the most time and reagent consuming steps [30]. Most techniques are relatively nonspecific, i.e., they remove both target and nontarget compounds, necessitating subsequent purification.

3.1. Water

Samples may consist of surface, ground or sediment-associated interstitial water. Provided a steady state situation has been reached, all coal tar/creosote constituents will be represented in the aqueous phase. Concentrations will be a function principally of water solubility, although other factors are important, such as compound stability. The presence of cosolvents or naturally occurring dissolved organic matter may affect solubility and thus the concentrations present [31,32]. This organic matter may also impact extraction efficiency [33]. Water is generally enriched with lower-molecular-mass constituents and those possessing O-, N- and S-substitutions. Often water is filtered or centrifuged, prior to extraction, to remove particulates. Particles typically are greatly enriched with PAHs compared to water.

Manipulation of sample pH, followed by extraction of the aqueous phase by liquid–liquid or solid-phase extraction is widely used. It is valuable in separating neutral (e.g., PAHs), basic (e.g., azaarenes) and acidic (e.g., phenolics) compounds [34–37].

3.1.1. Liquid–liquid extraction

This technique is very popular for the isolation of both nonpolar and moderately polar analytes. Unfortunately, it results in the consumption of large amounts of solvent, most of which must be disposed of as hazardous waste, an expensive and environmentally unsound proposition. Ong and Hites estimated that use of US Environmental Protection Agency (EPA) Method 608 alone, for the determination of polychlorinated biphenyls (PCBs) and chlorinated pesticides in water, generated 10^5 kg of dichloromethane waste annually [38]. A requirement for efficient extraction is a greater partition coefficient between the analyte and the nonpolar organic solvent used, than with water. A moderately polar solvent, such as methylene chloride, has often been favored [15,37,39]. Johansen et al. reported better recoveries of creosote-related heteroaromatic compounds using liquid–liquid extraction with methylene chloride than with solid-phase extraction [37]. Other solvents that have been employed include diethyl ether, light petroleum or mixtures thereof [14,40,41]. When hydrocarbons have been specifically targeted, nonpolar solvents such as hexane, pentane, carbon tetrachloride and chloroform have been used [42]. Solvents with densities significantly higher or lower than water facilitate phase separations. Since extract concentration is generally needed, a solvent possessing a low boiling point and toxicity is desirable.

Intimate contact between the extracting solvent and the analyte is essential. This is usually accomplished by agitation, most commonly in a separatory funnel. Unfortunately, this is labor intensive and limits the water volume extracted, often a factor when low quantitation limits are required. Use of a shaker table or magnetic stirrer may permit a degree of automation. If excessive energy is input, serious emulsions may occur. Salt addition or pH modification are sometimes employed to minimize these. After emulsion formation freezing, passage through glass wool or centrifugation may effect more complete separation. These steps extend analysis time and require additional sample handling. Alternatives include continuous extraction, wherein the solvent is recirculated by a pump or reflux [42,43]. pH adjustment of the sample, prior to extraction, may increase analyte yields [44].

While large solvent volumes are typical with liquid–liquid, several methods based on extraction with small volumes have been published. Recently, use of 8 μl of isooctane, located on the end of a PTFE rod, to extract small sample volumes was reported [45]. The sample was agitated by a magnetic stir bar in a 1 ml minivial and the extract directly injected into a GC.

3.1.2. Steam distillation

The more volatile analytes may be amenable to steam distillation [46]. Several designs are possible. In continuous extraction approaches the aqueous sample is added to one reservoir of the distillation unit and organic solvent to a second. Boiling of the solvent is initiated first, followed by that of the sample. Vapors may be condensed by a cold-finger and solvent and water extracted continuously. Both water and solvent are recirculated [47,48]. Solvent consumption is lower than with typical modes of liquid–liquid extraction, but glassware and setup are more complicated.

3.1.3. Headspace

This technique is often used for the more volatile constituents of coal tar and creosote, such as benzene, toluene, xylenes and naphthalenes. It was recently reviewed by Kuran and Sojak [46]. Analysis may be static, i.e., simply sampling the headspace over the sample with a gas syringe, after system equilibration. Alternatively, a dynamic approach may be taken by actively passing a gas over or through the sample, stripping analytes. The latter is sometimes termed purge and trap. The sample vessel may be heated to increase concentrations in the headspace and provide a constant temperature environment. After removal, analytes are trapped cryogenically or by sorption on charcoal or other material [49]. Effluent can also be transferred directly to a GC, or the trap desorbed with a small volume of liquid solvent and this material subjected to further analysis.

3.1.4. Solid-phase extraction (SPE)

SPE is an increasingly common approach. Advantages include reduced solvent consumption, elimination of emulsion formation, reduction in laboratory space required to perform extractions and greater automation potential. More selective extractions may also be obtainable, due to the variety of stationary phases now commercially available. SPE may be conducted on a column or a disc. Columns can be packed by the analyst or purchased prepacked. Water is generally passed through the column by positive pressure or vacuum. Small volumes may be forced through by centrifugation. SPE permits potential handling of large water samples, if sufficient sorbent is provided and plugging by particulates is not problematic. Extraction discs present greater cross sectional area than columns, accelerating sample passage [50]. C_{18} phases are particularly popular for removal of nonpolars [16,30,35,51]. On-line purification of analytes by selective elution of sorbed sample components is also feasible. Resins such as the XADs have also been widely used to extract large water volumes [17,42,52,53]. Polar compounds are amenable to SPE as well. Successful extraction of phenols, with and without derivatization, has been achieved using a styrene divinylbenzene sorbent [54].

Solid-phase microextraction (SPME) is a related technique. Here a fiber is coated with a stationary phase possessing characteristics facilitating preferential partitioning from the water. The fiber is immersed directly in the sample and the system allowed to reach steady state. Extent of partitioning is a function of the water–stationary phase–analyte system and may be established using standards spiked into the desired matrix [55,56]. Attainment of steady state may be hastened by sample agitation. As in the case of SPE, different stationary phases are available. A major advantage of the SPME approach is that the fiber may be placed directly into a heated GC [56,57] or a HPLC inlet [58] and desorbed, reducing sample handling dramatically. Automated systems have recently become available.

3.1.5. In-situ sampling/extraction approaches

Madsen et al. sorbed coal-tar derived materials, seeping from groundwater into surface waters, directly onto polyurethane foam plugs placed in the sediments [59]. Semipermeable membrane devices (SPMDs) have also been used as passive in-situ sampling devices. The membrane, consisting of polyethylene tubing or a dialysis bag, encloses a lipid reservoir. The lipids may be a specific material, such as triolein, or actual extracts from biota [60,61]. The SPMDs are placed in the receiving water and dissolved compounds cross the membrane and partition into the lipid phase, simulating accumulation in the lipid stores of aquatic organisms [62]. Analytes may then be extracted from the lipid by dialysis or other techniques, and typically require minimal purification prior to final chromatographic determination.

3.2. Sediments and biota

Sediments are commonly examined because of their propensity to concentrate the major constituents of coal tar and creosote. Similarly, organisms often accumulate lipophilic components to high concentrations, provided that the compounds are not easily metabolized and depurated. Unfortunately, the chemical similarity between resident organic matter in sediments, lipid in biota and the targeted analytes often leads to coextraction problems. Few techniques offer selective extraction. While some detectors possess significant selectivity, the presence of coextractives, such as lipids, may interfere with subsequent analyses. This generally necessitates one or more intermediate purification steps.

3.2.1. Aqueous caustic reflux

Caustic reflux has been used frequently for extraction of coal tar-related compounds from sediment and biological tissues. Typically, a wet homogenized sample is placed in a vessel and basic, alcoholic water added. KOH and methanol are commonly used. The vessel contents are then refluxed for several hours. The resulting saponified material is reextracted with a nonpolar solvent. This technique has been applied to the determination of PAHs in sediments [8,10] and biota [12,63]. Occasionally severe emulsions may be formed. Caustic reflux is rather labor intensive, requires handling of hazardous reagents and is difficult to automate. On the positive side, some researchers have reported that caustic treatment may free analyte residues bound to macromolecules, increasing yields [64,65]. Also glassware required is simple and inexpensive.

A variation of this involves caustic treatment, in concert with distillation. Donkin and Evans applied steam distillation to the analysis of a number of aromatic hydrocarbons in mussels [66]. Steam distillation has also been used for the determination of aromatic hydrocarbons from fish tissues [67]. Good recoveries were obtained for the lower-molecular-mass PAHs and phenol, but poor results for benzo[*a*]pyrene, a PAH of major concern.

3.2.2. Sonication/blending

Numerous methods rely on homogenization of the sample, by either mechanical or ultrasonic means, in the presence of solvent [68]. Johnston et al. utilized a sonic probe and a mixture of acetone and methylene chloride to obtain PAHs from coal tar contaminated soils [69]. A Tissumizer and methylene chloride has also been used to extract PAHs from shellfish tissues [70]. Sodium sulfate was added to the homogenized sample prior to extraction. Ultrasonic-based extractions are generally repeated with fresh solvent and filtered/centrifuged to remove particulates, as per the roller-flask methods.

3.2.3. Dry sample extraction methods

A common extraction mode for sediment or biological tissues involves use of a nonpolar organic solvent. However, substantial sample-associated water may block solvent–analyte contact. As a consequence, many extraction procedures require an initial drying step [48]. This may include air or oven drying, lyophilization or use of a chemical dessicant. The first two approaches may result in losses of the more volatile components [71]. Adding dessicant directly substantially reduces the available space for sample in the extraction apparatus and may introduce a potential source of contamination. Some dessicants release their entrained water under rigorous extraction conditions. Capangpangen and Suffet compared several approaches for drying particulates prior to PAH determination [71]. They reported increasing losses of volatile PAHs in the order: drying over dessicant in sealed jars<lyophilization<air drying. Dessication was more rapid using $CaCl_2$ than with $CaSO_4$, $MgSO_4$ or $NaSO_4$ as the drying agent. Alternatively, samples have been dewatered by initial extraction with a water soluble solvent, e.g., methanol [72]. The resulting extract may then be partitioned against water and a nonpolar solvent and the analytes back extracted. The sample may then be re-extracted with a nonpolar solvent. In some cases, the presence of small amounts of water may actually increase recoveries of some analytes. Higher recoveries of both PAHs [73] and phenols [74] from soil using SFE have been reported following addition of water.

3.2.4. Column percolation

Here, the sample is placed in a chromatographic column and a volume of solvent passed through and collected [68]. A variation of this, "matrix disper-

sion", mixes sample with a sorbent such as silica gel or alumina prior to extraction, to effect retention of unwanted co- extractives. While these procedures are straightforward, they may not exhaustively remove analytes, as the solvent is not heated and sample contact time is limited. Alternatively, "forced flow" leaching may be used. Here, pressurized solvent, near its boiling point, is pumped through a stainless steel column filled with sample [75]. Comparison of results obtained from all extraction methods should be compared to rigorous, widely accepted techniques, using matrices with "native" analyte burdens. Compounds added to samples in the laboratory to estimate method recovery may not associate with the matrix to the same degree [76,77]. Certified reference materials are available for many matrices and analytes and are excellent tools for method verification. These include reference coal tars, sediments and biological tissues [78,79].

3.2.5. Roller or shake flask

Dried sediment and biota samples may be placed in jars with solvent and agitated for several hours to effect extraction. Alternatively, some have added a dessicant directly to the wet sample in the jar. Krone et al. used a roller-based approach to extract N-heterocyclics from creosote-contaminated Eagle Harbor, WA sediments [80]. Shakers have also been used to extract PAHs and heterocyclics from sediments [81]. Following extraction, solvent is removed and filtered to remove particulates. Extractions are often repeated two to three times with fresh solvent, to ensure complete analyte removal. Multiple samples may be processed simultaneously. Equipment and glassware requirements are minimal, although the entire process is time consuming.

3.2.6. Soxhlet-type techniques

Soxhlet is considered by many as the benchmark extraction technique. Solvent path is from a boiling flask, via a side arm to an overhead condensor. Condensed solvent is then dripped onto the sample and eventually returned to the boiling flask by a siphon. This approach is often effective, but may require several hours to setup and complete. Glassware is fragile and must be carefully cleaned. Numerous researchers have applied soxhlet for the extraction of coal tar-related compounds from sediments [36,82,83] and biota [27,84]. Brilis and Marsdean reported higher recoveries of PAHs from creosote-contaminated sediments with soxhlet than sonication [85]. Some acceleration and automation of the process have been achieved with the Soxtec and Soxtherm systems. Here the sample thimble is initially immersed in boiling solvent [86].

3.2.7. Accelerated solvent extraction (ASE)

ASE is another relatively new application. Here the extraction vessel is filled with dried sample and solvent, then heated. Extraction is generally performed at pressures between 100 and 150 bar. Commonly used solvents include methylene chloride and hexane–acetone, at temperatures of 100°C–150°C. These conditions permit rapid extraction, on the order of 15 min [87,88]. Solvent consumption is reduced by up to ten-fold, compared to techniques such as soxhlet and sonication. The ASE process has been greatly automated, facilitating its use. In a commercial unit produced by Dionix the extract is forced by a N_2 stream from the vessel through a disposable filter and is collected in a septum capped vial. Both extraction and collection vessels are mounted in autosampler carousels, permitting unattended completion of multiple samples. Reagents, such as elemental copper may be placed at the bottom of the extraction vessel to provide on-line removal of sulfur coextracted from sediments.

Recently, Hageman et al. extracted PAHs from soils using subcritical water at 250°C by simply heating the samples with water in sealed vessels in a GC oven [89]. No organic solvents were required. PAHs in the water were assayed by SPME following extraction. Isotopically labeled PAHs were used as internal standards to compensate for resorption onto particulates as the water cooled after extraction.

3.2.8. Microwave extraction

Microwave-based approaches are becoming more widely used [90]. Microwave radiation is generally applied to a sample contained in a vessel filled with organic solvent. Extraction of several samples may be done simultaneously. Generally, extracts are manually removed from the vessels after treatment. Dean et al. compared recovery of PAHs from contaminated soil with microwave, soxhlet and SFE [88]. Microwave extracts were conducted with ace-

tone or methylene chloride for 20 min at 120°C. Highest recoveries were reported with microwave and SFE. Operational advantages and disadvantages were noted for the three approaches. Sample capacity, extraction time and solvent consumption were greatest for soxhlet. Operator skill and instrument cost were highest for SFE. The microwave approach was reported to fall between soxhlet and SFE for these parameters.

3.2.9. Supercritical fluid extraction (SFE)

SFE has been widely used in the food industry for the decaffeination of coffee and determination of the lipid content of fatty foods [91]. Descriptions of the SFE process, current developments and appropriate analytical strategies are available in the literature [92,93]. In SFE a supercritical fluid, commonly CO_2, is generated by pressurizing and heating a fluid above its supercritical point. CO_2 is relatively inexpensive, widely available and nontoxic. Its supercritical point (31°C and 73.8 bar) is easily reached and it is an excellent solvent for nonpolar analytes. However, other solvents have been used, e.g., nitrous oxide has been found effective for the extraction of PAHs from sediments [94]. Some of SFE's benefits are linked to the highly diffusive nature and liquid-like solvating power of supercritical fluids. Also density, and thus solvating power, may be controlled by manipulating extraction temperature and pressure. This permits the analyst a greater degree of selectivity. Following extraction, the CO_2 returns to atmospheric pressure and the gasous state and is vented. Trapping of analytes is done either by bubbling through a solvent, or collection on a solid-phase column [95,96]. The latter provides the opportunity for additional in-line purification. Elution of solid-phase traps usually requires only 1 to 5 ml of organic solvent. This reduces or eliminates the need for extract concentration, a problematic task in most trace analytical schemes. A flow restrictor maintains the solvent at supercritical pressure during dynamic extraction. In this role restrictors are vulnerable to blockage by formation of ice or coextracted lipid plugs during extraction [97]. Development of heated, automated designs has reduced these problems significantly.

A number of references are available for SFE of coal tar-related compounds from environmental matrices, especially sediments. Wright et al. examined its feasibility to remove PAHs from coal tar contaminated soils [98]. They reported similar recoveries to soxhlet, although some discrimination against high-molecular-mass PAHs was observed. Several researchers have observed that elevating extraction temperature was more effective than increasing pressure for enhancing recoveries of PAHs from solid matrices [99–101]. Addition of organic modifiers has been found to increase extraction efficiencies, particularly for high-molecular-mass and polar compounds [102]. Popular modifiers include methanol, toluene and methylene chloride. Binary modifiers to increase yields of PAHs from sediments have also been advocated [103]. Introduction of alumina and elemental copper can provide impressive on-line purification of extracts, removing considerable coextracted lipids and sulfur, respectively [94,104,105]. The combination of selective extraction, on-line cleanup potential and facile removal of extraction solvent by simple venting, has shown promise for directly coupling SFE and GC [93,106,107].

SFE has been criticized in that extraction efficiency often appears to be analyte or matrix specific, requiring considerable method validation prior to application. In reality, this criticism has also been leveled against organic solvent extraction [108]. While modifier and high temperature approaches seek to provide more "universal" extraction, utilization of these conditions may ultimately negate the selective extraction advantages of SFE. Highly automated SFE units, permitting unattended extraction and collection of multiple samples, are commercially available but are expensive.

3.3. Purification of extracts

3.3.1. Liquid–liquid

As noted previously, separation of neutral, acidic and basic compounds can be facilitated by pH adjustment of aqueous solutions. Some researchers have washed nonpolar organic solvent extracts with acidic or basic aqueous solutions to separate ionizable and neutral species [109]. Dimethyl sulfoxide (DMSO) partitioning has also been useful for separation of PAHs from interferences such as aliphatics [8] and cholesterol [12]. After partitioning into DMSO, water is added and the PAHs back extracted

with cyclohexane. Dimethylformamide–water has been partitioned against cyclohexane extracts to purify PAHs and N- and S-heterocyclics as well [110]. Alternatively, PAHs have been isolated with a N-methyl-2-pyrrolidone reagent [111].

3.3.2. Thin-layer chromatography (TLC)

Several authors have found preparative TLC useful for separating aliphatics and aromatics from polar constituents in extracts. Brumley et al. applied extracts to a preparative TLC plate and obtained fractions containing various N-heterocyclics by development with hexane and methylene chloride [83]. A similar approach was used to separate aliphatics and PAHs from creosote-contaminated Elizabeth River sediment extracts [10]. Stefanova and Lazarov eluted extracts of a coal-derived liquid from Kieselgel 60 F_{254} TLC plates with benzene [112]. N-containing functional groups were visualized by spraying the plates with tetracyanoethylene. These areas were removed from the plates and subjected to GC with mass spectrometric (MS) detection. Both one- and two-dimensional TLC approaches have been applied [113].

3.3.3. Low-pressure column chromatography

These techniques vary in their complexity, sample handling requirements, cost and automation potential. Open column adsorption and low-pressure size exclusion have been the most common approaches. A number of sorbents are valuable for the separation of aliphatic, aromatic and heterocyclic fractions. The most popular have been alumina and silica. Black separated aliphatics and aromatics by eluting 25 g of Florisil with hexane and 50% methylene chloride–hexane, respectively [8]. Commercially packed amine-based columns have also been used to separate PAHs from other components in complex extracts [114]. This application required less solvent usage and sample manipulation than DMSO partitioning approaches. West et al. obtained four different fractions by eluting an alumina column with *n*-hexane, benzene and chloroform [115]. The S-heterocylics, mainly thiophenes, were reported to coelute with the PAHs in the benzene fraction with this scheme [116]. Picel et al. eluted four polarity classes from 10 g of silica gel using 20% methylene chloride in pentane, 40% methylene chloride in pentane, ethyl ether and methanol [117]. These fractions contained: aliphatics–PAHs, neutral azaarenes and more polar constituents. They then applied alumina column chromatography to effect separation of the 4–6 ring PAHs from the aliphatics and 1–3 ring aromatics by eluting 10 g of alumina with 10% methylene chloride in pentane, followed by 60% methylene chloride in pentane. Alternatively, a column combining 20 g of silica gel over 10 g alumina, to separate PAHs and N-heterocyclics from creosote-contaminated Eagle Harbor sediment extracts, has been utilized [80]. Yu et al. placed SFE-derived extracts of coal tar-contaminated soil on a XAD-2 column and fractionated analytes according to polarity with hexane, 2-propanol and toluene [118].

Cation-exchange approaches with SP-Sephadex C-25 have been reported for separating benzacridines in creosote oils [119]. Column elution was done initially with methanol, which was discarded, and target analytes recovered by elution with an ammonia-based buffer solution.

Furlong and Carpenter eluted PAHs and azaarenes from extracts derived from Puget Sound sediments on a Sephadex LH-20 column with benzene–methanol (1:1) [120]. The two classes were then separated by partitioning against 10% H_2SO_4. The resulting acidic extract was made alkaline and reextracted with methylene chloride to obtain the azaarenes. Carotenoids and steroids from fish tissues have been removed from alumina column-derived aromatic fractions by elution of a Bio-Beads SX-12 column with methylene chloride [121]. Bio-Beads S-X8 and methylene chloride have found utility for separating large biogenic molecules from coal tar-related compounds in sediment extracts [82]. SX-3, eluted with methylene chloride–cyclohexane (1:1) has been observed to provide a more complete separation of PAHs and lipids found in sediments and biota [122]. A size exclusion chromatograph permitting automated sample injection and fraction collection was used in this latter application.

3.3.4. High-performance liquid chromatography

HPLC provides an alternative to low-pressure techniques. Advantages include greater resolution, automation potential and availability of sensitive on-line detectors to monitor performance. Variability in

stationary phase activity, as a function of moisture content, is also less of a problem than in open column techniques. A drawback is the high capital cost of equipment and columns. HPLC columns can be damaged by injection of poorly soluble sample components that may irreversibly bind to the stationary phase. Use of a disposable guard column reduces this problem, with minimal impact on resolution. Unfortunately, sophisticated HPLC systems require experienced personnel to operate and maintain them.

Fractionation of PAHs, ketones, N-heterocyclics and other compounds present in sediment extracts has been accomplished with a semi-preparative silica gel HPLC column [123]. The column was eluted with a program of hexane, methylene chloride and acetonitrile. Ramdahl separated polycyclic ketones from other constituents in environmental samples with a μPorasil semi-preparative column and a methylene chloride–hexane gradient [124]. Alternatively, a preparative aminocyano-bonded silica column has been used to purify coal tar-related compounds using a gradient of hexane and 2-propanol [82]. Motohashi et al. used a C_{18} column, eluted with acetonitrile–water, to purify benzacridines [119]. Size-exclusion chromatography with Phenogel columns has also been used to purify oyster extracts destined for PAH analysis [125].

3.4. Identification and quantification of coal tar related compounds

Although most researchers subject their extracts to multiple purification steps before final characterization, resulting fractions generally remain complex and require additional chromatographic techniques for complete resolution of components. In some cases more generic characterization techniques have been used, when detailed compositional data were not desired, or additional confirmatory information was needed. These approaches include infrared spectroscopy [126] and fluorescence [127]. On-line fiber-optic based fluorescence measurement for monitoring PAHs in the extract emerging from a SFE has also been reported [128]. TLC on silica-coated rods with flame ionization detection (FID) has been used to characterize aliphatic, aromatic and polar contributions of creosote-contaminated Canadian soils [13]. However, by far the most commonly used techniques for identification and quantification have been HPLC and GC.

3.4.1. HPLC

C_{18}-based HPLC separation approaches using either UV or fluorescence detection have been most common. These generally elute PAHs or N-heterocyclics with water–acetonitrile [8,35] or water–methanol gradients [129,130]. Fluorescence allows more selective measurements to be made than single wavelength UV, as both excitation and emission wavelengths may be optimized. Also the design of the fluorescence detector generally results in smaller backgrounds against which the analyte signal is measured. HPLC–fluorescence detection limits for some PAHs are lower than with GC–FID. Black used in-line UV at 254 nm and fluorescence (300 nm excitation/420 nm emission) to quantitate PAHs in sediments of a creosote-contaminated Michigan river [8]. A Nova-Pak, 5 μm C_{18} column has also been used to separate N-heterocyclics and various oxidation products in extracts of creosote-impacted groundwater [35]. Again, quantitation was done with fluorescence and UV detectors. HPLC–fluorescence has also been used to determine PAH metabolites in fish bile [131]. A normal-phase (amino-silica) approach has been applied to the separation of cresols [43]. Isocratic elution was accomplished with 1.5% isopropanol in hexane and fluorescence detection (277 nm excitation/300 nm emission). New approaches using diode array detectors, essentially providing UV absorption spectra for eluting peaks, and time/wavelength programmable fluorescence detectors allow enhanced selectivity and sensitivity [132,133].

3.4.2. Gas chromatography

GC has been the most popular choice for separation of individual PAHs in complex mixtures, due to the resolution possible and the availability of equipment [134]. However, HPLC on C_{18} columns does provide better resolution of some isomeric PAHs. Due to the resolution required, narrow bore (0.32 mm I.D. or less) fused-silica GC columns are generally employed. S-Heterocyclics have often been analyzed coincidently with the PAHs. Common GC stationary phases have included SE-52, SE-54, DB-5, SPB-5, OV-101, OV-1701, SBP5 and HP Ultra-2. N-

and O-heterocyclics have been examined on SE-54, CP-Sil 8 CB, SPB-5, DB-5, DB-Wax, SP-2100 and OV-101, among others. Most analyses have been conducted on 25–30 m columns, although longer columns (50–60 m) have become more popular. Injections are generally made in either the splitless or on column mode because of the sensitivity required.

MS is now one of the most commonly applied detection techniques for coal tar components. Previously, MS was expensive and often temperamental, requiring skilled operators. This limited sample throughput, necessitating the use of other GC detectors for routine samples. Lower cost, simpler and more rugged spectrometer designs have recently been developed, allowing wider deployment of this powerful technique. These instruments include mass-selective detectors [59,69] and ion traps (ITDs) [1,118,135].

Electron impact mass spectrometry (EI-MS), in the full scanning mode, can provide significant information and has often been used to identify coal tar related compounds [1,10,78,80,83,118]. However, structural assignments generated are not unequivocal when spectra alone are used. Some isomeric PAHs produce similar EI spectra at 70 eV (Fig. 2). As a consequence, additional evidence is required to confirm compound identity. Complementing MS data with GC relative retention indices has been a common approach [98]. Lee and Wright calculated retention indices for over 200 aromatics using naph-

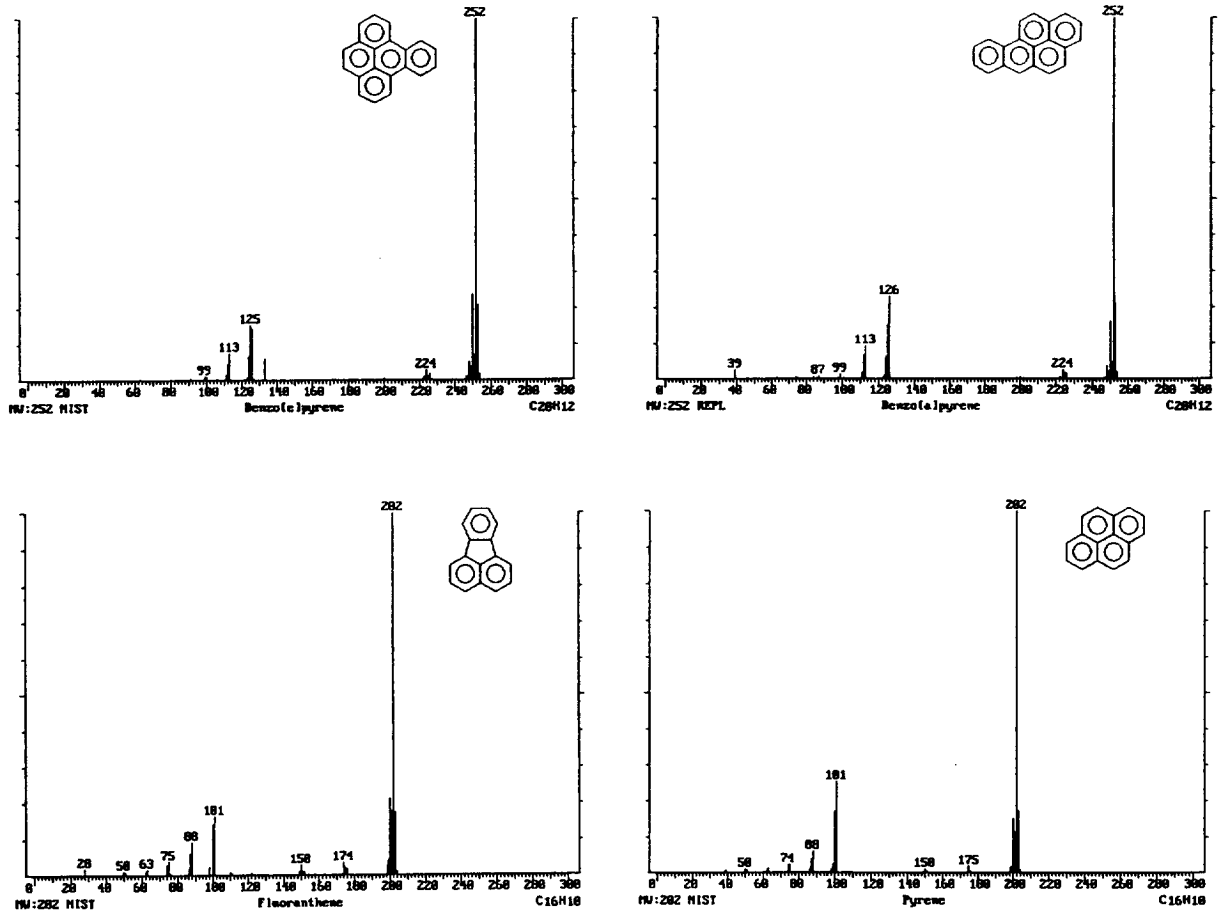

Fig. 2. EI-MS spectra from the NIST library for some important coal tar components. Note that spectra for isomeric PAHs are very similar. Thus alternative or combinations of approaches, i.e., retention indices or other MS ionization modes, are required to differentiate these compounds.

thalene, phenanthrene, chrysene and picene as markers [136]. The indices have since been were further evaluated and expanded to include additional compounds, particularly N- and S-heterocyclics [137]. Interestingly, these authors chose to use a coal tar standard for the evaluation of their retention index. Since then the US National Institute of Standards and Testing (NIST) has issued a coal tar standard reference material (SRM 1597) [78]. A PAH retention index similar to that of Lee and Wright was implemented by Bieri et al. with the addition of biphenyl and pyrene and the substitution of benzo[*ghi*]perylene for picene [82].

While providing less structural information than full scan, selected ion monitoring (SIM) mass spectrometry has become very popular, providing considerable selectivity and a facile means for quantitation [37,138]. Most researchers utilize some form of internal standard system for quantitation. Isotope dilution approaches, using deuterated or [13]C labeled PAHs, provide the researcher with a particularly accurate quantitation tool [89,139]. In addition to these, use of fluorinated PAHs for the determination of coal tar components has been proposed [140]. Standards may be added to the sample during preparation, allowing the analyst to largely compensate for losses during workup.

MS–MS is another approach that is finding increasing application [141,142]. Originally a technique beyond the reach of most laboratories, due to technical and financial reasons, introduction of ion trap MS has greatly widened its availability. In MS–MS, the analyst may select a specific ion to retain and then fragment it and monitor parent and resulting daughter ions, greatly increasing specificity. Although EI-MS is much more common, chemical ionization MS (CI-MS) has also found utility. For example, Hilpert applied negative chemical ionization (NCI-MS) to the determination of both PAHs and alkylated isomers [143]. With this approach, several PAHs with similar EI-MS spectra were easily differentiated. Other uses include application of CI-MS, with ammonia as the reagent gas, to characterize carbazoles [144].

The most common GC detection method in the laboratory is FID. Advantages of FID include its low cost, simplicity, durability and consistent response to hydrocarbons, the most targeted coal tar constituents. A FID-derived chromatogram of a coal tar SRM is shown in Fig. 3. The chromatogram is dominated by unsubstituted PAHs, with lesser contributions by alkyl, N-, O- and S-substituted compounds. Although application of a relative retention index generated identifications that agreed with those provided by NIST, care must be taken when relying on these exclusively, particularly for unknown samples. GC–FID alone must still be considered a relatively weak identification tool, even when extracts are first fractionated by polarity to reduce their complexity. One approach to increase confidence is to use multiple columns with differing stationary phases. This approach has been applied to the analysis of hydroxylated thiophenes in coal-derived liquids and coal tar [145,146].

In addition to FID, other detection methods have proven valuable. For example, electron-capture detection (ECD) has been found to exhibit some heightened sensitivity for PAHs [134]. Researchers used thermionic specific (TSD), also known as nitrogen–phosphorus (NPD) detection, for determining various N-containing aromatics in creosote, polluted harbor and river sediments [80,113,115]. Flame photometric detection (FPD) can be used to detect P- and S-containing compounds. Nishioka et al. examined S-heterocyclics in coal tar by GC–FPD and GC–EI-MS [145]. They reported that dibenzothiophene, naphtho[1,2-*b*]thiophene and benzo[*b*-]naphthothiophenes were the major S-containing compounds present. FPD was used to detect S-heterocyclics in sediments from the Black River and elsewhere [116]. The photoionization detector has also been applied, particularly to detect low-molecular-mass aromatic compounds [147]. As it is nondestructive, it may be deployed in series with other GC detectors [148]. Splitting of the GC column effluent to different detectors and ratioing their responses may also assist in the identification process.

Due to the complexity of coal-tar derived materials, the resolving power of multidimensional GC is sometimes indicated. Here columns possessing different stationary phases are connected. The entire effluent of the first column, or select portions ("heart cutting"), are transfered to the second increasing resolving power [149–151]. Direct thermal extraction has also been applied to the analysis of hydrocarbons in soils. Here the sample is placed directly in

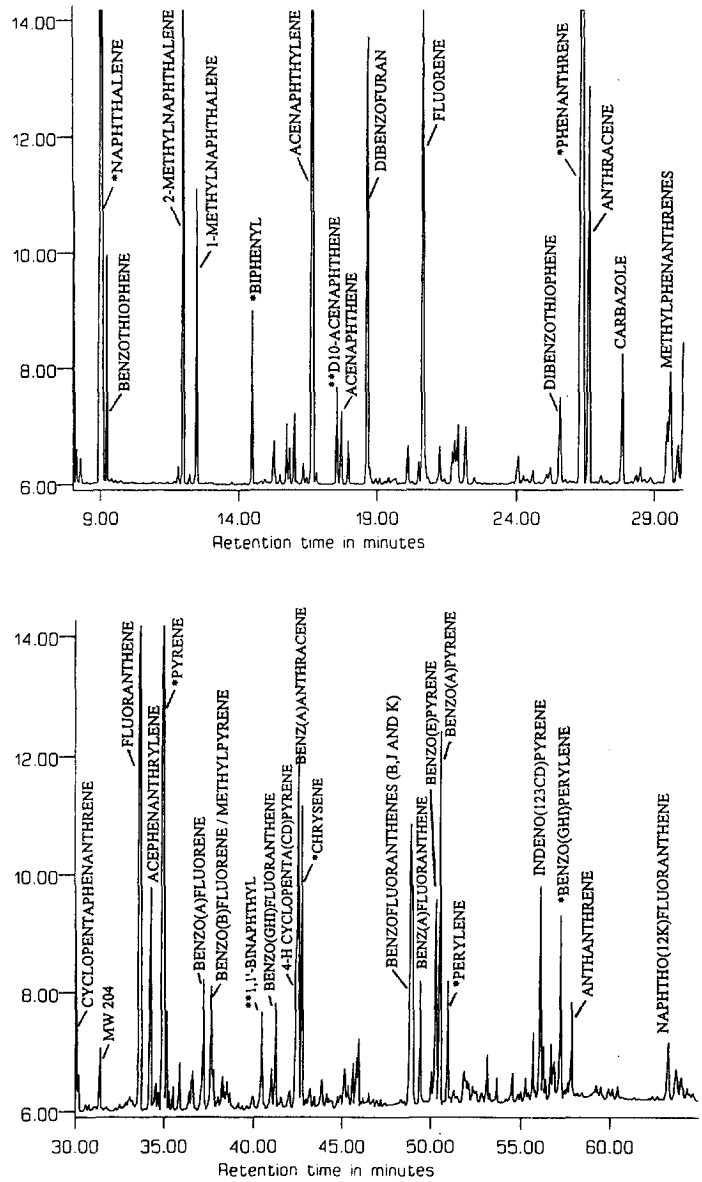

Fig. 3. GC–FID chromatogram of the NIST Coal Tar SRM 1597. A 60 m×0.32 mm I.D. DB-5 column was used. It was programmed from 90°C to 320 at 4°C/min. The carrier gas was helium. Retention index marker compounds [82] present in the SRM are denoted by "*"; surrogate standards added prior to injection are designated by "**".

the GC injector and the temperature rapidly ramped up to vaporize analytes onto the column [152]. A solvent divert and heated retention gap approaches have also permitted large volume on-column injection, minimizing the need for further sample concentration [153,154].

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