CHAPTER II

THEORY

SOLID-PHASE EXTRACTION FOR SAMPLE PREPARATION (2,6,79-85)

In the mid 1970's, a simpler alternative approach, SPE, was introduced for the sample praparation technique. This concept, similar to low pressure liquid chromatography, is the basis for the design of a practical sample preparation technique consisting of small, disposable extraction columns filled with a variety of sorbents.

Although SPE cartridges are widely and successfully used, difficulties can arise in their routine application.

1. The columns somewhat narrow internal diameter limits usable flow rates to a range (1-10 mL/min) that necessitates long trace-enrichment times for large sample volumes.

2. A too-rapid flow through an SPE cartridge can cause kinetic effects in the bed of 40 μm particles that in turn prevent the recovery of certain analytes.

3. Relatively dirty samples (for example, those containing suspended solids) can rapidly plug the small cross-sectional area of a typical cartridge, and the increasingly slower volumetric flows can increase extraction times.

4. Channeling is caused by the inherent difficulty of packing loose particles, thus requiring the use of excess bed mass to retain the desired analyte quantitatively.

SPE disk technology provides a way around the above listed limitations of packed-bed SPE columns. Borrowing the disk configuration of membrane filters, these devices include flat disks with high cross-sectional areas that provide advantages not found in cartridges.

1. The decreased back pressure encountered with these devices makes much higher flow rates possible.

2. The decreased chance of plugging by their wide bed.

3. The channeling prevention because of new technology for embedding the stationary phase into a disk format.

4. The mass transfer improvement.

The advantages of these disks may allow them to replace SPE cartridge in many applications. For comparison, Table 2.1 presents the general characteristics and flow properties of a typical cartridges and disks have comparable packing capacities and differ only in geometry, separations achieved on cartridges often can be performed using disks containing stationary phase with similar functionality.

Table 2.1 : Comparison of a Typical Cartridge and a Typical Disk (80)

Parameter	Cartridge	Disk
Dimensions (height x diameter)	1.1 cm x 1.1 cm	0.05 cm x 4.7 cm
Cross-sectional (top) area	0.95 cm^2	11.34 cm^2
Packing weight	500 mg	500 mg
Flow at 85 kPa *	30 mL/min	100 mL/min
Linear velocity +	0.525 cm/s	0.15 cm/s

* Typical

+ At flow rate specified

TYPES OF SPE DISKS

1. Packing-impregnated PTFE

These devices consist of a PTFE fibril network that holds bonded silica particles or resin particles in place. The 8 μm particles comprise ~90% of a device's weight, and the PTFE comprises 10%. Figure 2.1 is a micrograph of this flexible, 0.5 mm thick, homogeneous material. The disks are available in sizes that fit a standard filter assembly. A 47 mm disk contains ~500 mg of sorbent, and a 25 mm disk contains ~140 mg of sorbent. The Bio-Rex chelating resin containing disk or Bio-Rex

membrane is a 40 μm resin-filled version. It may have interesting applications in the selective extraction and concentration of heavy metals in solution.

2. Packing-impregnated polyvinyl chloride

Primarily designed for protein purification, these microporous plastic sheets contain silica that is activated by a standard ion exchange or affinity chemistry. The membranes shown in Figure 2.2 have ~1 μm flow through pores that provide fast kinetics. Rapid separations can be achieved at low back pressures. The material is available in sheets, cartridges, and disks ranging in diameter from 25 mm to sizes large enough for scale-up work. Flow rates range from 20 to 80 mL/min. Examples of this type of device include Acti-Disk ,Acti-Mod products, and Fastchrom membranes.
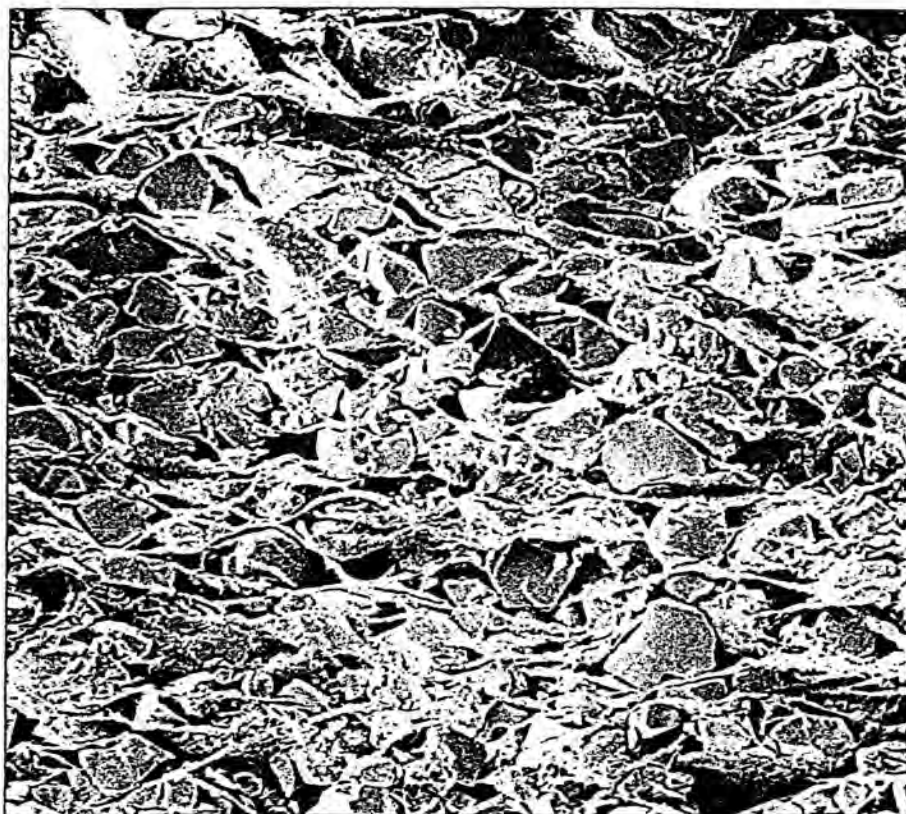

Figure 2.1 Micrograph of an Empore extraction disk. (80)

3. Derivatised membranes

Stationary-phase particles are not embedded into these membrane ; rather, the membranes are functionalised through chemical reactions. Primarily designed for biomolecule purification and separation, these devices can also be used for SPE applications. They are made of cellulose derivatised with functional groups such as diethylaminoethyl (DEAE), quaternary ammonium (QAE), sulfonylpropyl (SP), and protein A. Examples of this membrane type include MemSeps and ZetaChrom devices.

Only packing-impregnated PTFE devices are directly equivalent to typical SPE cartridge because the PTFE matrix contains the same stationary phases.
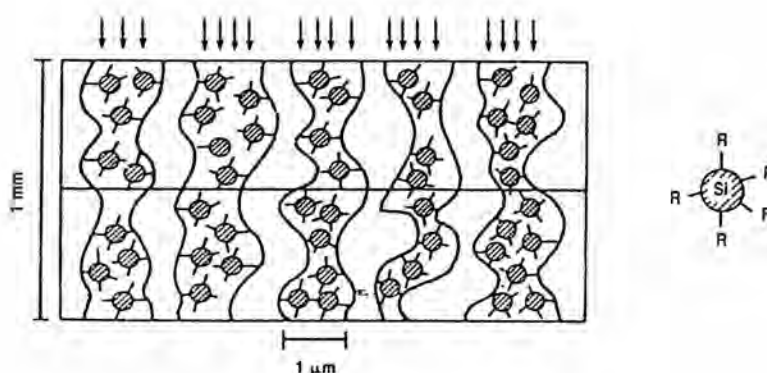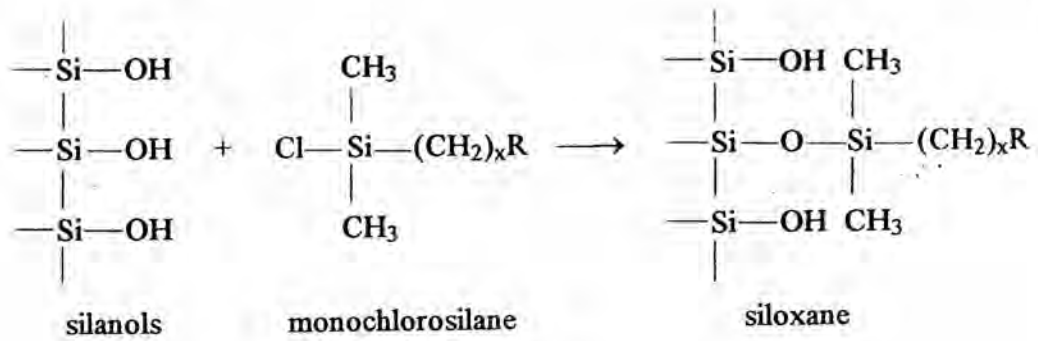

Figure 2.2 Schematic of an Acti-Disk microporous PVC-silica disk.

R = bonded functional groups. (80)

SYNTHESIS OF BONDED SORBENTS

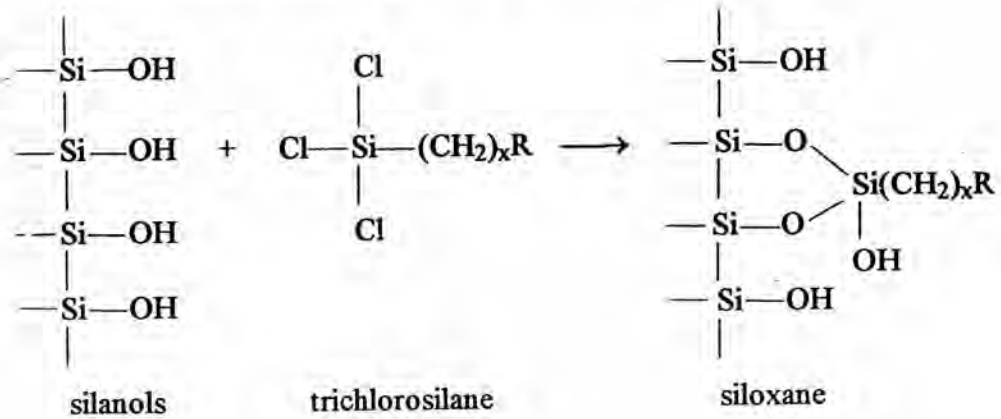In the late 1960's, the idea of treating the free silanol groups of silica with mono-, di-, and tri-halo or alkoxy silyl derivatives to form siloxanes was conceived. Although the original intent was the conversion of unbonded silica to a bonded nonpolar phase, both polar and nonpolar bonded silica are now available.
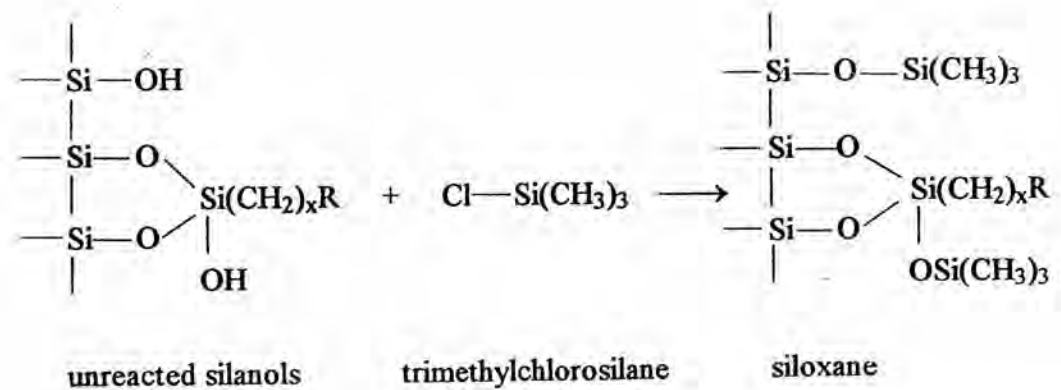
The reaction of silica with a monofunctional derivatizing agent proceeds as follows:

```
    |                               CH₃                    |
  —Si—OH                            |                    —Si—OH  CH₃
    |                               |                      |       |
  —Si—OH        +        Cl—Si—(CH₂)ₓR        ⟶         —Si—O—Si—(CH₂)ₓR
    |                               |                      |       |
  —Si—OH                            CH₃                  —Si—OH  CH₃
    |                                                      |
   silanols              monochlorosilane                  siloxane
```

A trifunctional derivative yields the following bonded phase:

```
    |                             Cl                        |
  —Si—OH                          |                       —Si—OH
    |                             |                         |
  —Si—OH        +        Cl—Si—(CH₂)ₓR       ⟶           —Si—O
    |                             |                         |        Si(CH₂)ₓR
  —Si—OH                          Cl                      —Si—O        |
    |                                                       |          OH
  —Si—OH                                                  —Si—OH
    |                                                       |
   silanols              trichlorosilane                   siloxane
```

As shown above, in both types of reactions residual unbonded silanol groups always remain after the bonding reaction. The presence of unbonded silanols causes the bonded phase to exhibit heterogeneous surface characteristics: those due to the attached -R group and those due to the unreacted silanols. These silanol groups are deactivated by endcapping with trimethylchlorosilane as follows:

```
    |                                                         |
  —Si—OH                                                    —Si—O—Si(CH₃)₃
    |                                                         |
  —Si—O                                                     —Si—O
    |        Si(CH₂)ₓR   +   Cl—Si(CH₃)₃    ⟶                 |        Si(CH₂)ₓR
  —Si—O        |                                            —Si—O        |
    |          OH                                             |         OSi(CH₃)₃

  unreacted silanols       trimethylchlorosilane             siloxane
```

The potential for competitive adsorption on the hydroxy sites of an otherwise nonpolar surface is thus eliminated or minimised.

The surface properties of the bonded sorbents depend primarily on the type of silica selected for the bonding reaction. The degree of loading and endcapping is also very important in determining the surface characteristics of a bonded phase.

SELECTION OF THE APPROPRIATE LC COLUMN FOR PAHs ANALYSIS

DIFFERENCES IN SELECTIVITY

Reversed phase LC on C_{18} stationary phases has been shown to provide excellent separations of PAHs. However not all C_{18} stationary phases provide the same selectivity (i.e., relative separation) for PAHs. The separation of the 16 priority pollutant PAHs was greatly influenced by the type of synthesis used to prepare the bonded C_{18} phase. Bonded phases that are prepared using silane modification procedures can be classified as either monomeric or polymeric phases depending on the reagents and reaction conditions used for the bonded phase synthesis. The vast majority of C_{18} phases are prepared by reaction of monofunctional silanes (e.g., monochlorosilanes) with silica to form "monomeric" bond linkages. Polymeric phases are prepared using trifunctional silanes in the presence of water which results in cross-linking to form silane polymers on the silica surface.

CLASSIFICATION OF PHASE SELECTIVITY

A simple empirical test has been developed to assess the selectivity of C_{18} stationary phases for the separation of PAHs. The test is based on the relative retention of three carefully selected PAHs solutes as shown in Figure 2.3. The retention of benzo [a]pyrene (BaP), relative to 1,2:3,4:5,6:7,8-tetrabenzonaphthalene (TBN) and phenanthro[3,4-c]phenanthrene (PhPh), provides a sensitive measure of the "polymeric" or "monomeric" character of the stationary phase. As shown in Figure 2.3, the elution order of these three solutes on phases prepared with monomeric surface modification is BaP < PhPh < TBN, whereas phases prepared with polymeric surface modification give the elution order of PhPh < TBN < BaP. A quantitative measure of the phase selectivity can be calculated to allow relative comparisons among different C_{18} phases. The selectivity factor $\alpha_{TBN/BaP}$ (defined as k'_{TBN}/ k'_{BaP}) has been
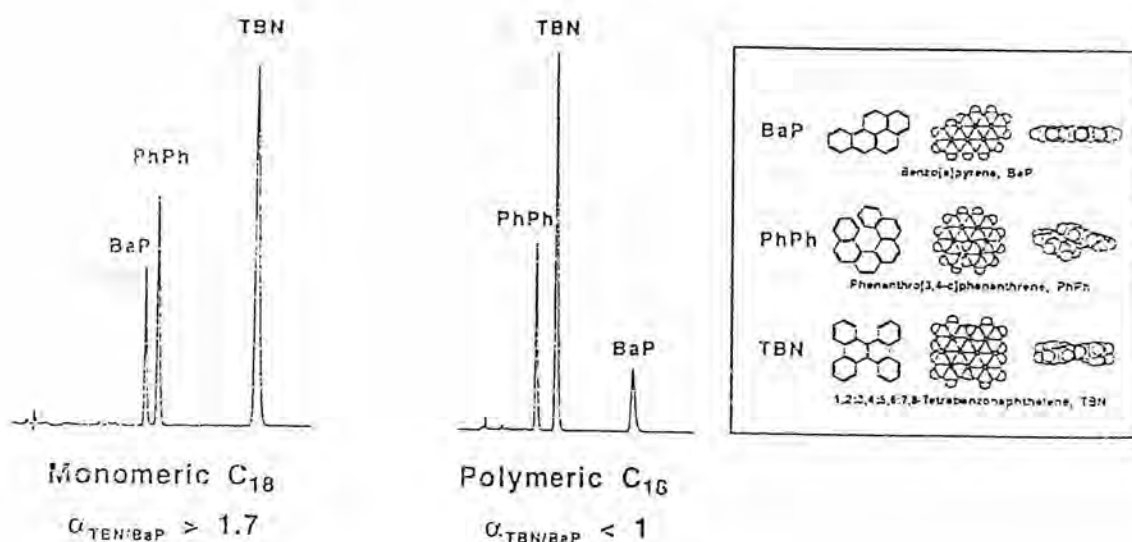
Figure 2.3 Separation of BaP, TBN, and PhPh on a polymeric and a monomeric C_{18} stationary phases. Structures of the three components in the mixture are illustrated in the box. Chromatographic conditions : mobile phase isocratic at 85% acetonitrile in water at 2 mL/min ; UV detection at 254 nm. (6)

shown to correlate with the retention behavior of PAHs and the bonded phase type. A classification scheme has been adopted based on the measurement of $\alpha_{TBN/BaP}$ values for experimental and commercial C_{18} phases. Values of $\alpha_{TBN/BaP} \leq 1$ indicate polymeric C_{18} phases, and values of $\alpha_{TBN/BaP} \geq 1.7$ indicate monomeric C_{18} phases. For values $1 < \alpha_{TBN/BaP} < 1.7$ (intermediate phases), the bonded phase synthesis is less certain and may indicate a densely loaded monomeric phase or light polymerization with di- or trifunctional reagents. The separation of the 16 priority pollutant PAHs on three C_{18} columns with different $\alpha_{TBN/BaP}$ values is shown in Figure 2.4.
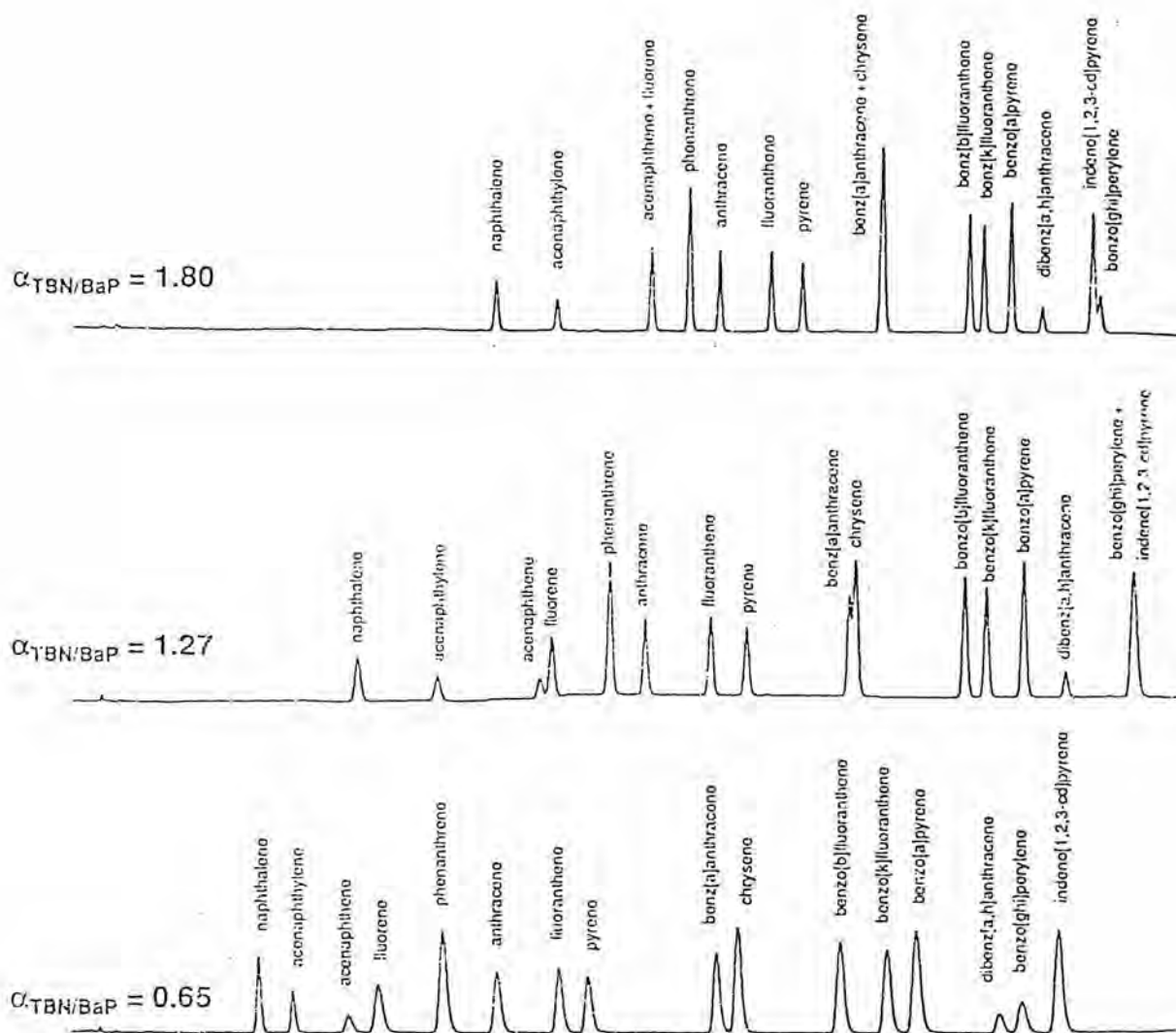
Figure 2.4 Separation of 16 PAHs on three different C_{18} columns with different $\alpha_{TBN/BaP}$ values. Chromatographic conditions : columns Zorbax ODS ($\alpha_{TBN/BaP}$ = 1.80), Bakerbond C_{18} (120 A° pore size) ($\alpha_{TBN/BaP}$ = 1.27), and Vydac 201TP ($\alpha_{TBN/BaP}$ = 0.65) ; mobile phase linear gradient from 40% acetonitrile in water to 100% acetonitrile in 30 min at 2 mL/min ; UV detection at 254 nm. (6)

GENERAL PROPERTIES OF BONDED SILICAS

1. CHEMICAL STABILITY

The bonded silica sorbent product is stable within a pH range of approximately 2 to 7.5. Above pH 7.5 the silica substrate is susceptible to dissolution in aqueous solutions. Below pH 2.0 the silyl ether linkage is labile, and the functional groups on the surface will begin to cleave, changing the sorptive properties in a non-reproducible fashion. Nonetheless, in practice bonded silicas may be used for sorbent extractions in a pH range of 1 to 14, since degradation of the sorbent is a finite process and sorbents are typically exposed to solvents for only short periods of time. Bonded silicas are chemically stable to virtually all organic solvents.

2. PHYSICAL PROPERTIES

Bonded silica sorbents are rigid materials that do not shrink or swell in different solvents. The silicas most commonly used in making bonded silica sorbents have a particle size distribution of 15-100 microns. The nominal porosity of most of the sorbents described here is 60 Angstroms, adequate for compounds with molecular weights up to approximately 15000. For extraction of higher molecular weight molecules wide pore sorbents as high as 4000 Angstroms in porosity are used.

3. SOLVATION (OR CONDITION)

Solvation of a sorbent is necessary before the sorbent will interact reproducibly with isolates. Some sorbents, particularly the most non-polar such as C_{18}, will not reproducibly retain isolates until they have been solvated. In effect, solvation is a wetting of the sorbent creating an environment suitable to isolate retention. Methanol is an effective solvating agent because it can interact with both the silanols on the silica and the carbon atoms of the bonded functional group.

THE MECHANICS OF SORBENT EXTRACTION

1. RETENTION

"Retention" is the phenomenon where an attraction exists between the sorbent and isolate molecules, causing the isolate to be immobilised on the sorbent surface as the sample solution passes through the sorbent bed. Retention is a function of three factors : the isolate, the solvent, and the sorbent. the retention behavior of a given isolate can, therefore, be expected to change in the presence of different solvents and sorbents.

2. ELUTION

'Elution' is the process by which an isolate is removed from a sorbent bed on which it has been retained. This is brought about by introducing a solvent to which the isolate is more strongly attracted than it is to the sorbent. In sorbent extraction, the goal is to retain an isolate on a sorbent strongly enough that the isolate does not move through the sorbent bed until the elution solvent is introduced. The elution solvent chosen should elute the isolate from the sorbent bed in the smallest volume possible.

3. CAPACITY

"Capacity of a given sorbent" is defined as the total mass, of a strongly retained isolate that can be retained by a given mass of the sorbent under optimum conditions. When determining the amount of a given sorbent required for an extraction procedure, consideration should be given not only to the capacity requirements for the isolate, but also for those of undesired sample components that may co-retain with the isolate. Typically, these undesired components are more important in determining capacity requirements than is the isolate.

4. SELECTIVITY

"Selectivity" is the ability of the sorbent to discriminate between the isolate and all other sample matrix components, that is, to retain the isolate exclusive of other sample components. A highly selective sorbent is one that retains only the isolate from the sample matrix. The selectivity of an extraction is a function of three parameters :

the chemical structure of the isolate, the properties of the sorbent, and the composition of the sample matrix. Maximum selectivity is achieved when a sorbent is chosen that interacts through isolate functional groups that are not common to other matrix components. Under these conditions, the isolate will retain on the sorbent and all other matrix components will pass through unretained.

SORBENT/ISOLATE INTERACTIONS

1. NON-POLAR INTERACTIONS

Non-polar interactions are those that occur between the carbon-hydrogen bonds of the isolate. These forces are commonly known as "van der Waals" or "dispersion" forces. Since most organic molecules have some non-polar structure, non-polar interactions are often used to retain isolates on sorbents offering non-polar functional groups on the surface. The most widely used sorbent for non-polar interactions is octadecyl silane bonded to the silica substrate, called C_{18}.

2. POLAR INTERACTIONS

Polar interactions are exhibited by different sorbents and functional groups on isolates. Polar interactions include hydrogen bonding, dipole/dipole, induced dipole/dipole, pi-pi, and a variety of other interactions in which the distribution of electrons between individual atoms in the functional groups is unequal, causing positive and negative polarity. This property allows an isolate molecule bearing a polar functional group to interact with a polar group on a sorbent.

3. IONIC INTERACTIONS

Ionic interactions occur between an isolate molecule carrying a charge (either positive or negative) and a sorbent carrying a charge opposite to that of the isolate. Groups on isolates and sorbents that can exhibit ionic properties allowing ion-exchange interactions can be divided into two classes : groups that can be cationic (positively charged) and groups that can be anionic (negatively charged).

4. COVALENT INTERACTIONS

Covalent interactions are those resulting in formation of a covalent bond between the sorbent and the isolate molecule. Sorbents that employ covalent bond formation are not as common as those using the other interactions, but are highly useful in that covalent bond formation is a highly selective extraction mechanism.

5. MULTIPLE INTERACTIONS

The general principles of the three most commonly employed sorbent extraction interactive mechanisms are non-polar, polar, and ionic. It is necessary to emphasise at this point that almost all available sorbents are capable of more than one of these interactions.

SOLID-PHASE EXTRACTION STEPS

1. MATRIX MODIFICATION

Prepare sample for extraction as specified in the procedure. If the sample matrix is complex or strong, matrix modification may be necessary to facilitate analyte extraction.

2. CONDITIONING

Rinse disks with elution solvent to eliminate impurities and then dry the disks. After that, condition the disks with the volumes of solvents specified. Do not allow disks to dry before the sample is applied.

3. SAMPLE ADDITION

Accurately transfer the sample to the extraction disks and draw the sample through the disks with the suitable flow rate.

4. WASHING

Wash with the specified volume of solvent. Air dry the disks if required by pulling a vacuum for the specified time.

5. ANALYTE ELUTION

Pipette the specified aliquots of eluting solvent into the extraction disks. Aspirate or force the elution solvent through very slowly and collect it for the further detection step.

POLYCYCLIC AROMATIC HYDROCARBONS (83-85)

Polycyclic organic matter (POM) can include many chemical groups as indicated by the following list developed by an EPA Task Force in 1975:

Polycyclic aromatic hydrocarbons (PAHs) or Polynuclear aromatic compounds (PNAs)

Aza arene (arenes containing a ring nitrogen)

Imino arenes (ring nitrogen with a hydrogen)

Carbonyl arenes

Dicarbonyl arenes

Hydroxy carbonyl arenes

Oxa arenes and thia arenes

Polychloro compounds

Pesticides (e.g.,aldrin, chlordane, DDT)

Chemically, any organic compound that contains two or more rings could be considered a PAH. However, of major concern are the carcinogenic PAHs and their nitrogen analogs, aza and imino arenes, which are formed during organic combustion processes. Because of their common sources, their existence in urban air, and the considerable experimental data on their carcinogenic effects, the PAHs have received the most attention.

CHEMICAL STRUCTURE

The nomenclature of PAHs compounds has suffered from considerable ambiguities in the past due to different peripheral numbering systems for American and European scientists.

The currently accepted nomenclature is that adopted by the International Union of Pure and Applied Chemistry (IUPAC) and by Chemical Abstracts Service. Of major importance are the following rules that determine the orientation from which the numbering is assigned :

(1) The maximum number of rings lie in a horizontal row ;

(2) As many rings as possible are above and to the right of the horizontal row ; and

(3) If more than one orientation meets these requirements, the one with the minimum number of rings at the lower left is chosen.

The carbons are then numbered in a clockwise fashion, starting with the first most counterclockwise carbon which is not part of another ring and is not engaged in a ring fashion. Letters are assigned in alphabetical order to faces of rings, beginning with "a" for the side between carbon atoms 1 and 2 and continuing clockwise around the molecule ; ring faces common to two rings in the parent compound are not lettered. Thus, benzo[a]pyrene would have a benzene ring fused to the "a" bond of the parent pyrene structure (Figure 2.5)
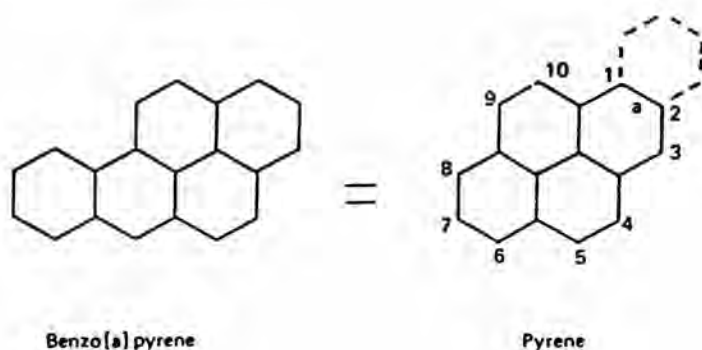

Benzo[a] pyrene   Pyrene

Figure 2.5 Accepted Nomenclature for Benzo[a]pyrene (83)

PHYSICAL PROPERTIES

Most of the PAHs compound are high melting / high boiling point solids that are extremely insoluble in water. At 25 °C, the available vapor pressure of the pure compounds vary for the individual compounds from 10^{-4} torr (3 rings) to 10^{-12} torr (7 rings). The PAHs strong absorb ultraviolet light at wavelength from 200 to 400 nm.

SOURCES OF PAHs

Combustion is the predominant end-process by which fossil fuels are converted to energy. Combustion, particularly when inefficient, is also the primary technological source of PAHs released into the environment. The need for liquid fuels to supply the transportation industry and for nonpolluting fuels for heat and power generation provide the incentive to commercialise processes to convert coal to substitute natural gas and oil. These processes represent a potentially massive new source of environmental PAHs. Apart form these sources, PAHs can emit from forest fires, volcanic activity, automobile traffic and cigarette smoke.

Not all PAHs are the result of man's activities. Biosynthesis of PAHs by plants and microorganisms is well known such as wheat and rye seeds containing only a trace of PAHs were grown in PAHs-free solutions in both the presence and absence of light and the alga *Chlorella vurgaris* is known to synthesize several PAHs.

OCCURRANCE OF PAHs

AIRBORNE PARTICULATE MATTER

Airborne particulate matter consists of a complex mixture of solid and liquid particles that contain PAHs and are emitted to the atmosphere from a variety of sources, such as the products of the combustion of fossil fuels, automotive engine exhausts, and emissions from industrial operations. PAHs, which are potently carcinogenic in nature, are liberally spread throughout the environment.

SOIL

PAHs are widely distributed in soil, and they are even found in areas remote from human habitation and industrial development. The concentration of PAHs in soil can be attributed almost exclusively to emissions from combustion processes. It has been demonstrated that plants and vegetables can absorb PAHs from contaminated soil.

WATER

PAHs are widely spread over the earth's water, and these may originate from the fallout of particulate matter transported through air, from absorption of gaseous compounds, and from polluted water. This pollution of water can represent a potential health hazard to mankind by PAHs consumption via drinking water.

SEDIMENT

PAHs are widespread in water throughout the world, and they are concentrated in the sand or bottom sediment in a manner such that concentrations can be between 2 to 5 orders of magnitude greater than in the surface water.

SEWAGE SLUDGE

The colloidal, dispersed-particle phase of wastewater can be considered to be a collector system for anthropogenous wastes not otherwise isolated (garbage) or emitted into the atmosphere. Like airborne particulate, sewage sludge thus represents pollutants entering the environmental from technical processes, households, and agricultural activities.

PETROLEUM AND ITS PRODUCTS

The presence of PAHs in petroleum and its products, e.g., lubricating oil, diesel fuel, and light heating oil, has been detected by means of UV absorption spectrometry and MS.

COSMETIC AND MEDICINAL PRODUCTS

Mineral oils and refined petroleum products used in cosmetics and medicinal products have also been shown to contain PAHs. Fully refined petroleum products with potential trace levels of PAHs are ingredients of cosmetic preparations such as cold creams, cleansing creams, baby lotions or creams, and lipsticks. Some cosmetics prepared from vegetable oils may also contain trace amounts of PAHs.

FOOD

Food can be contaminated by PAHs via air pollution, by direct flue-drying, by charbroiling, or by smoking processes. It has been demonstrated that plants and vegetables can absorb PAHs from contaminated soil. A more obvious source of carcinogenic PAHs in edible plants might be from direct deposition or absorption from the atmosphere, as approximately 10% of the total PAHs content can be removed by washing the vegetables. Plants with smaller surface areas usually contain lesser amounts of PAHs, while leafy plants, such as spinach, cabbage, or kale, contain substantially higher contents of PAHs. Apart from fresh vegetables, PAHs have also been found in edible plant products. For example, flour and vegetable oils, such as those from soybean, corn, and peanut, have been found to contain detectable PAHs contamination.

TOXICITY

1. CARCINOGENICITY

Formation of PAHs-induced cancers in laboratory animals is well documented. Experimental animal studies in which the carcinogenicity of a PAHs compound is clearly demonstrated, irrespective of administration route, have been reported. The studies show that Benzo[a]pyrene (BaP), for example, has produced tumors in mice, rats, hamsters, guinea pigs, rabbits, ducks and monkeys after administration by oral, skin and intratracheal routes. The amounts of different PAHs necessary to produce cancer in 50% of treated animals (ED_{50}) vary greatly.

2. TERATOGENICITY

Although only a small number of substances have been bioassayed for both teratogenicity and carcinogenicity, a relationship between the two is indicated by the large percentage of compounds that have produced both malformations and cancer.

A comprehensive review by DiPaolo and Kotin shows that the majority of carcinogens exert a teratogenic effect. The rapid growth, lack of differentiation, and reactivity of the cells in the developing embryo suggest that the action of chemical or biological agents will be expressed in alterations at the level of differentiation. Somatic cell division is a prerequisite for both teratogenesis and carcinogenesis. The rapid division and differentiation of fetus cells result in a sensitivity that makes the fetus especially vulnerable to teratogens.

The same compound may have different effects, depending upon the stage of development of the organism at the time of exposure. thus, a compound found to be carcinogenic to mature cells might be teratogenic to immature, embryonic cells.

3. MUTAGENICITY

The active forms of a large number of known chemical carcinogens are mutagens. The PAHs have been shown to bind to DNA. This suggests that malignant transformation can involve an alteration in the genetic constitution of treated cells. PAHs carcinogens may therefore produce mutations, some of which may involve the genes that control malignancy.

Ames, et al. propose that carcinogens that are mutagens cause cancer by somatic mutation in which flat aromatic molecules intercalate in DNA base-pair stacks. This leads, during DNA replication or repair, to an addition or deletion of base pairs in the DNA sequence. These "frameshift" mutagens are more potent if they contain a side chain that covalently reacts with DNA.

MECHANISM OF METABOLIC ACTIVATION OF PAHs

The key event of chemical carcinogenesis is the covalent binding of the carcinogen to macromolecules, especially to DNA, in the cell. Most carcinogens, especially those of the PAHs type, are, however, chemically inert. To become reactive,

they must be metabolically activated, primarily by the oxidative reactions in the body. The reactive metabolites capable of binding and initiating the carcinogenic response are known as "ultimate" carcinogens, and their immediate metabolic precursors are called "proximate" carcinogens. Jerina et al. were able to suggest that a "bay region" was the structure feature required for carcinogenic activity and that the active metabolites for many PAHs would be found to be bay region dihydrodiol epoxides, i.e., vicinal dihydrodiol epoxides, where in the epoxide ring is adjacent to a bay region (Figure 2.6)
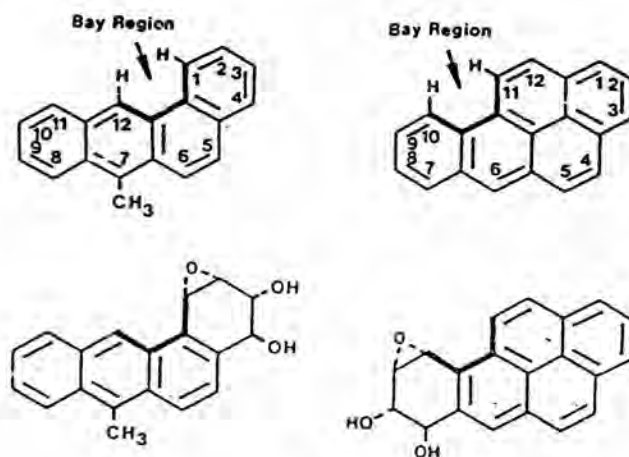

Figure 2.6 Structure of 7-methylbenzo[a]pyrene and benzo[a]pyrene showing the bay regions by thick lines, and corresponding bay region dihydrodiol epoxides. (84)

The formation of "proximate" and "ultimate" carcinogens from BaP, a model of PAHs metabolism, in cell culture or animal systems via the (1) cytochrome P-450-dependent monooxygenase system, which converts BaP into 7,8-epoxy-BaP and (2) the epoxide hydrolase, which converts the BaP epoxide into the BaP-7,8-dihydrodiol, the "proximate carcinogen" of BaP, is shown in Figure 2.7. This proximate carcinogen is then converted to BaP-7,8-dihydrodiol-9,10-epoxide, the "ultimate carcinogen" (Figure 2.7). Other carcinogenic PAHs that contain a phenanthrene structure also form bay region metabolites via similar metabolic reactions. It has been stated that dihydrodiol epoxides with the epoxy group in this region exhibit the highest biological activity of all isomeric compounds, owing to steric hindrance and increased chemical reactivity. In accordance with this concept, extensive studies from several laboratories
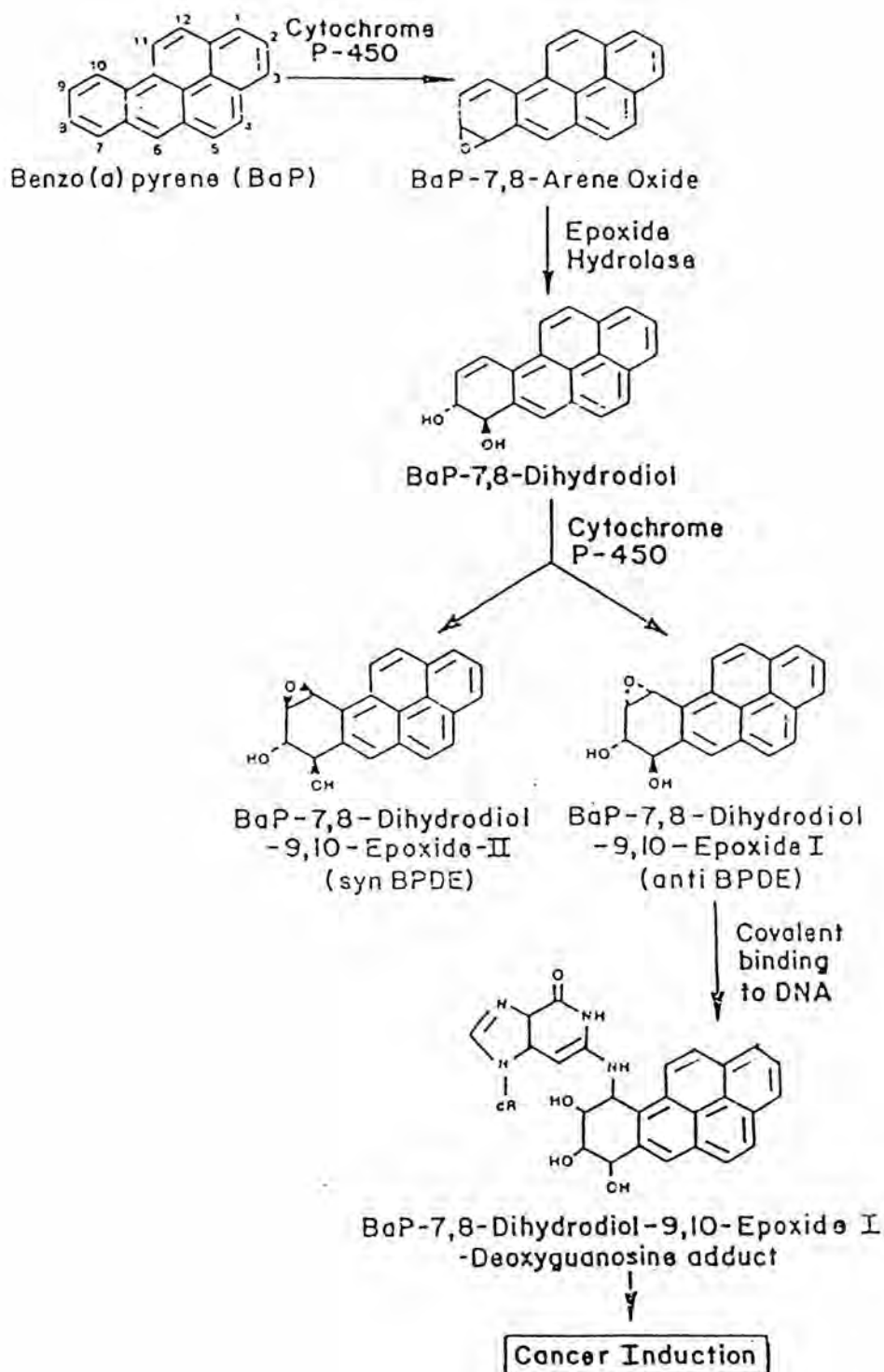
Figure 2.7 Metabolic activation of ubiquitous environmental pollutant and known

carcinogenic BaP leading to the formation of proximate and ultimate

carcinogenic metabolites and their binding to DNA, which initiates the

process of carcinogenesis. (84)

have identified a specific diol-epoxide derivative of BaP, known as 7β,8α-(+)-dihydrol-9α-10α-epoxy-BaP, as the "ultimate" carcinogen of the parent compound.