CHAPTER II

THEORY

2.1 Basic Theory of Solid Phase Extraction

Solid phase extraction (SPE) method (also referred to as sorbent extraction or liquid solid extraction) is a sample preparation tool used for the isolation, concentration, and purification of analytes from complex matrices. SPE is based on the separation mechanisms of liquid chromatography. This method is the extraction of analyte based on its partition between a liquid and a solid phase, represented by the following equibilibrium :

$$[A]_l \rightleftharpoons [A]_s \qquad (2.1)$$

where $[A]_l$ and $[A]_s$ are the concentration of the analyte in the liquid and solid phase, respectively. The partition coefficient K is defined as

$$K = \frac{[A]_s}{[A]_l} \qquad (2.2)$$

By careful selection of a solid phase and a solvent, it is possible to achieve total retention of an analyte by driving the equilibrium towards the solid phase [when K in equation (2.2) approaches infinity] or total elution by forcing the equilibrium to the liquid

phase (when K approaches zero) (65).

SPE generally consists of four basic steps (70-71):

1. Condition the column for aqueous samples. This is generally done by wetting the sorbent with a volume of methanol, which is then replaced by a volume of water. The role of this step is to secure perfect and maximum mutual contact of the liquid and solid phases. Prewetting of chemically bonded silicas causes opening of the hydrocarbon chains of the stationary phase, thus increasing its surface area. Organic solvents commonly used as prewetting media are methanol, acetonitrile, and acetone.

2. Apply the sample to the column. This results in compounds (including the compound of interest) being retained on the sorbent while others pass through unretained.

3. Rinse the column to remove interfering compounds.

4. Elute the compound of interest in a small volume of an appropriate solvent.

There are several ways in which SPE can be performed. First, it can be used for trace enrichment, in which a large volume of dilute sample is passed over the stationary phase. Phases are selected because they exhibit a very high affinity for the compound of interest and little or no affinity for the matrix. The enriched sample then can be quantitatively recovered by displacement with a small volume of higher strength eluent. In a similar manner, SPE can be used for sample isolation. The compound of interest can be selectively sorbed onto the solid phase while matrix interferences are allowed to pass through the SPE column. In the reverse manner, matrix isolation can be used to bind interferences to the solid phase, allowing the sample of

interest to pass through and be collected. Finally, SPE can be used for sample storage and transport. This approach is used in stabilizing particularly labile or volatile compounds for transport to analytical testing laboratories (61).

2.2 Sorbents Used in SPE

2.2.1 Carbon Sorbents

Carbon was the first medium used for the accumulation of organic compounds from water. The advantage of activated carbon was high sorption capacity, and high thermal stability; however, the use of heterogeneous nature of activated carbons caused problems such as irreversible sorption, affinity for some groups of compounds only, or catalytic activities of the carbon surface (70).

2.2.2 Polymer Sorbents

Polymers have been reported to use as alternative sorbents for trace enrichment, instead of carbon, since the late 1960's. Their homogeneous structure, results in a greater reproducibility of the trace enrichment experiments. The most often used types of sorbents are styrene-divinylbenzene copolymers. This group comprises, e.g., Amberlites XAD-1, XAD-2, XAD-4, Chromosorb 102, PRP-1, or Ostion SP-1. Columns packed with these materials have significant utility for certain type of separations, but they are available in a limited range of functionality. In addition, only limited separation experience

with these materials has been reported. Of particular concern are studies by Vign and co-workers that report severe nonlinear adsorption isotherms on commercial polystyrene-divinylbenzene column packing for low-molecular-weight solutes. These data suggest that isocratic retention times vary significantly with changes in sample loading, an effect that is not characteristic of silica-based, bonded-phase columns (70,79).

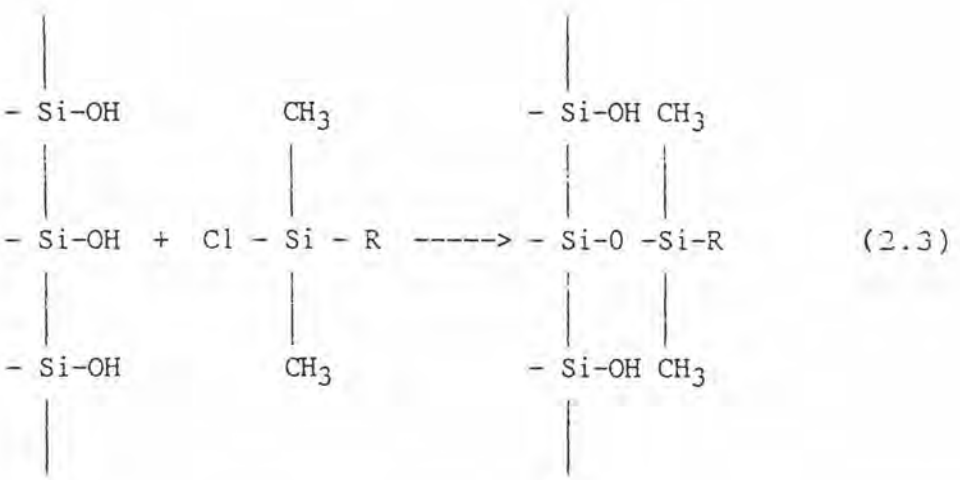
2.2.3 Bonded Silicas

These materials are the most popular and widely applied sorbents. The first attempts to use them as preconcentration media date to the middle of the 1970's. The growth in acceptance of bonded silica extraction is due to its superior selectivity resulting in very good recoveries with high purity of the compound of interest. Because of the wide variety of bonded silica sorbents available, a large selection of sorbent/solvent combination is possible. Solid phase extraction provides a rapid and effective way to cleanup and concentrate a variety of compounds for analysis. But developing a reliable extraction method depends upon understanding the properties of bonded silica materials (70).

2.2.3.1 General Properties of Bonded Silicas

2.2.3.1.1 Synthesis (64,68,79)

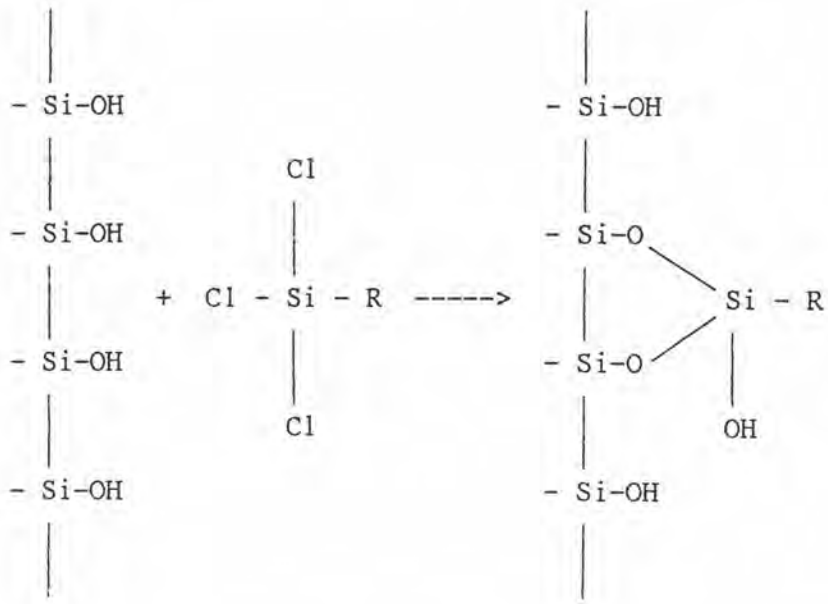
Bonded silicas are formed by the reaction of organosilanes with activated silica. The product is a sorbent with the functional group of the organosilane attached to the silica substrate through a silyl ether linkage. The reaction of silica with a monofunctional derivatizing agent proceeds in equation (2.3). The surface produced by this type of reaction usually is well defined monolayer, when the R group on the original silane imparts the desired chromatographic characteristics to the surface. For example, for R = $n\text{-}C_8H_{17}$, as in the case of n-octyldimethylchlorosilane, a C_8 bonded phase surface is produced.



(2.3)

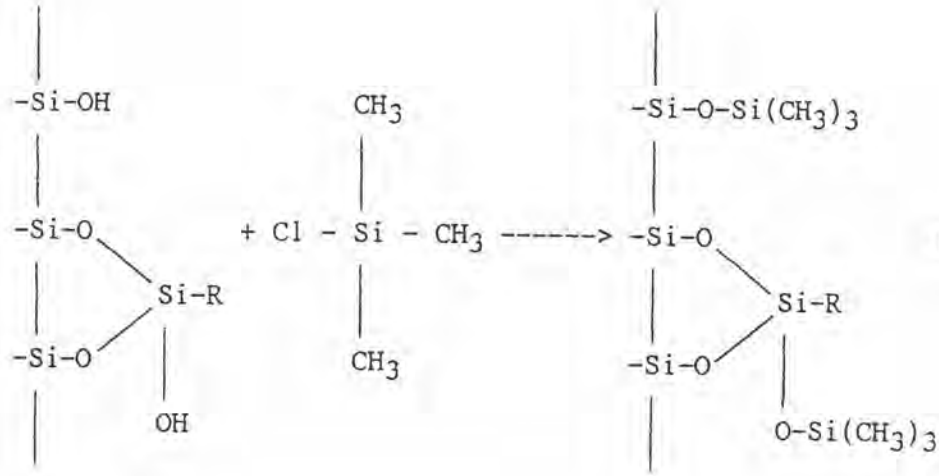
The reaction of silica with a trifunctional derivatizing agent shows in equation (2.4). Trifunctional derivatizing

agents yield phases with greater resistance to hydrolysis; these phases also possess lower silanol content.



(2.4)

As shown above, residual unbonded silanol groups may remain after the bonding reaction. The presence of unbonded silanols causes the bonded phase to exhibit heterogeneous surface characteristics: those due to the attach-R group and those due to the unreacted silanols. These silanol groups are deactivated by end capping with trimethychlorosilane as in equation (2.5). The potential for competitive adsorption on the hydroxy sites of an otherwise nonpolar surface is thus eliminated or minimized.



(2.5)

2.2.3.1.2 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 (64).

2.2.3.1.3 Physical Properties

Unlike many polystyrene based resins, Bonded silica sorbents are rigid materials that do not shrink or swell

under different solvent conditions. For this reason, bonded silicas equilibrate rapidly to new solvent conditions. This allows complex extraction procedures involving many different solvent changes to be performed rapidly. In addition, bonded silica sorbents also have advantages over polymeric resin such as XAD because they do not require extensive cleanup and there are usually fewer chromatographic interferences during analysis.

The silicas most commonly used in making bonded silica sorbents have a particle size distribution of 15-100 microns. Inaddition, the particles are irregular rather than spherical. These characteristics allow rapid solvent flow through the sorbent bed under conditions of minimal vacuum or pressure (approximately 10-15 psi).

The norminal porosity of most of the sorbents is 60 Angstroms, adequate for compounds with molecular weights up to approximately 15000. Molecules larger than this are excluded from the 60 Angstrom pores and are exposed to too little of the surface area of the sorbent for extensive interaction with the sorbent functional groups. For extraction of higher molecular weight molecules wide pore sorbents as high as 4000 Angstroms in porosity are used (64,80).

2.2.3.1.4 Solvation (64,81)

Solvation of a sorbent is necessary before the sorbent will interact reproducible with the compound of interest. Some sorbents, particularly the most non-polar such as C18, will not reproducibly retain the compound of interest until they have been solvated. In effect, solvation is a wetting of the sorbent creating an environment suitable to isolate retention. Solvation is accomplished by passing several bed volumes of a suitable solvent such as methanol through the sorbent. Once solvated, the excess methanol is removed by the solvent that prepares the sorbent to receive the sample. A small amount of methanol will remain associated with the sorbent.

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. In addition to methanol, many other solvents may be used for solvation: acetonitrile, isopropanol and tetrahydrofuran. The solvating solvent used should be miscible with the solvent used for preparing the sorbent to receive the sample.

Once solvated, the sorbent should not be allowed to become desolvated by excessive drying, specifically before application of the sample to the sorbent. If the solvated sorbent dries out, it will be necessary to solvate it again. Allowing the columns to dry out at this stage would lead to column bed channeling, resulting in poor analyte recovery. Once the compound of interest has

been retained on the sorbent, drying is usually not a problem. Infact, drying is recommended between solvent steps when the solvents are immiscible.

2.3 The Mechanics of Sorbent Extraction (64,67)

2.3.1 Retention and Elution

Retention is the phenomenon where an attraction exists between the sorbent and the compound of interest causing the compound of interest to be immobilized 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 the compound of interest can, therefore, be expected to change in the presence of different solvents and sorbents.

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

The unit of measurement most commonly used to characterize

retention and elution is bed volume. Bed volume is the amount of solvent required to fill all the internal pores and interstitial spaces of the particles in a given size sorbent bed. For 40 micron, 60 angstrom sorbents, bed volumes are on the order of 120 ul per 100 mg. of sorbent. Retention is generally strong enough when 20 bed volumes of a appropriate wash solvent (i.e., one not expected to elute the compound of interest) can be passed through the sorbent without isolate elution. Elution optimally should require no more than 5 bed volumes.

2.3.2 Capacity and Selectivity

Capacity of a given sorbent is defined as the total mass of a strongly retained the compound of interest that can be retained by a given mass of the sorbent under optimum conditions. Capacities of different bonded silica sorbents vary widely. Bonded silica ion exchange sorbents typically have capacities of 0.5 to 1.5 meq/g. For other sorbents, capacity values range from less than 1 % to as high as 5 % of the sorbent mass. If other compounds in the sample are retained by the sorbent, the capacity for the compound of interest is reduced proportionately to the amount of competing compound present. The amount of a given sorbent required for an extraction procedure should be give to the capacity requirements not only for the isolate, but also for the competing compound.

Selectivity is the ability of the sorbent to discriminate between the compound of interest and all other sample matrix components, i.e. to retain the compound of interest exclusive of other sample

components. The selectivity of an extraction is a function of three parameters: the chemical structure of the compound of interest, the properties of the sorbent, and the composition of the sample matrix. Maximum selectivity is achieved when a sorbent that retains only the compound of interest from the sample matrix is chosen.

2.3.3 Flow Rate

Flow rate of the sample and other solvents through the sorbent bed is another factor important to optimum sorbent extraction. Although the maximum acceptable flow rate is to some degree a function of how strongly the compound of interest is retained and the size of the sorbent bed being used, flow rates typically should not exceed 5-10 milliliters/minute through a 100 mg sorbent bed. If ion-exchange is used as the extraction mechanism, slower flow should be used (less than 5 mL/min). These flow rates allow sufficient time for sample compounds or other species in the solvents to diffuse through the solution to the sorbent surface.

2.4 Types Of Sorbent Extraction (67-69)

The wide variety of commercially available bonded silica materials, including nonpolar, polar, and ion-exchang sorbents, provides the versatility that makes sorbent extraction a selective and powerful extraction technique. The type of chemical interaction between the compound of interest and the bonded silica material may be described as follows:

2.4.1 Non-polar Extraction

In a nonpolar extraction, non-polar compounds in solution are partitioned onto the bonded phase through the interaction of the non-polar functional groups of both the sample and sorbent. Non-polar extraction columns contain silica particles bonded to octadecyl, octyl, butyl, phenyl or other hydrophobic functional groups. These columns are used to extract hydrophobic compounds from aqueous solution.

Non-polar extraction columns are typically prepared for an extraction process by rinsing with methanol followed by water. The hydrophobic compound of interest is retained by a hydrophobic interaction with the solid phase, and an aqueous rinse solution is pass through the column to remove the undesired polar sample components. The compound of interest is then eluted from the column with a small volume of an organic solvent.

If there are ionic groups present on the compound of interest, retention may be improved by adjusting the pH of the sample to neutralize the charge, making the compound as hydrophobic as possible. The rinse solution should be maintained at this adjusted pH to reduce the likelihood of losing the compound in the rinse step.

2.4.2 Polar Extraction

In a polar extraction, polar compounds in solution are adsorbed directly onto the active surface of unbonded silica or

partitioned onto the bonded phase through the interaction of the polar functional groups of both the sample and sorbent. Polar extraction columns contain either unbonded silica, or silica bonded with polar or hydrogen-bonding functional groups such as diol, cyanopropyl or aminopropyl. They are used to extract polar or hydrogen-bonding compounds from non-aqueous, non-polar samples.

In a polar extraction, the column is prepared by rinsing it with a non-polar solvent such as hexane or chloroform before applying the sample. The compound of interest is retained by hydrogen bonding with the solid phase. A non-polar solvent is used to rinse the undesired nonpolar matrix components from the column. The compound of interest then is eluted with a more polar solvent.

2.4.3 Ion Exchange Extraction

Ion exchange extraction is based on the principle that opposite charges attract. Extraction occurs when the charge on the analyte is opposite to that on the extraction column.

Five factors which affect ion exchange selectivity merit special discussion. The prime factor is pH, because the retention of ionic compounds is achieved by promoting ionization. The pH is lowered for basic analytes and raised for acidic ones. The second factor, counter-ion selectivity of bonded phase is also an important consideration. The selectivity of a counter-ion is the degree to which it is capable of competing with other counter-ions for the charged group on

an ion-exchange sorbent. The compound of interest is the best extracted from a sample solution with a low concentration of competing counter-ion. The compound of interest is removed from the column using an elution solution containing sufficient competing counter-ions. The third factor of important is ionic strength. Ionic strength is a measure of the concentration of all ionic species in the matrix, and it influences the retention of ionic analytes. Since ion exchange is a competitive mechanism, retention of ionic anlytes is a function of the number of other ionic molecules in the sample matrix that are capable of competing for available ionic groups on the sorbent. Thus, low ionic strengh favors retention, and high ionic strength facilitates elution. Solvents also play an important role. In some instances, the solubility of the neutral form of an acid or base is much lower in water compared to that of the ionic form. Consequently, the analyte may become insoluble when an elution solvent is used which converts it to the neutral form. A water-miscible organic solvent must be added to the elution solvent to effectively elute such compounds. Flow rate is the fifth important consideration. A flow rate of less than 5 mL/min of sample solution is suggested, since ion exchange interactions occur at a slower rate than polar or nonpolar interactions.

Ion exchange columns can be either cationic or anionic. Cation exchange columns contain silica bonded with sulfonic or carboxylic acid functional groups. These are used to extract amine-containing compounds from aqueous or non-aqueous solutions.

The columns are conditioned with methanol followed by a

buffer. The pH of the conditioning buffer, the sample and the rinse solution should be neutral, because at this pH, the amines on the compound of interest carry a positive charge and the acidic functional groups on the silica carry a negative charge, allowing retention by a strong ionic interaction. A rinse buffer can be used to remove any non-polar compounds from the column. The compound of interest may then be eluted from the column using an acidic wash to neutralized the bonded silica, or by using a basic wash to neutralize the charge on the compound of interest.

Anion exchange columns containing silica bonded with amine functional groups such as aminopropyl or quaternary amines are used to extract carboxylic acids or sulphonic acids. Again, the pH of the conditioning buffer, the sample and the column rinses should be neutral to ensure ionization of both the acidic compound of interest and the basic extraction column. Elution can be done by neutralizing the charge on either one.