CHAPTER II

THEORY

2.1 Basic Theory of Solid Phase Extraction

Solid phase extraction (SPE), as a tool for sample isolation and preconcentration, is a popular and fast growing technique. Sometimes is referred to as sorbent extraction or liquid solid extraction, the technique has been applied in a variety of areas not only to sample cleanup but also to fractionation, solvent changeovers, and compound concentration. Researchers have been using SPE for many years for ion-exchange cleanup and concentration, but the modern technique had its beginning in 1978 with the commercial introduction of Sep-Pak cartridges (44).

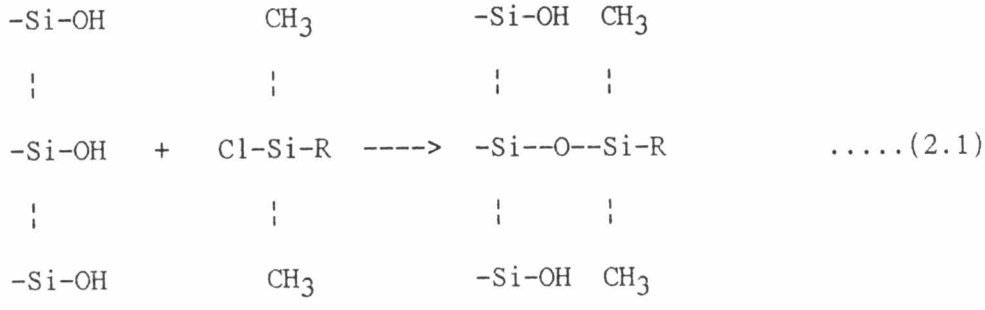
SPE is based on the separation mechanisms of liquid chromatography (LC) whereby the compound of the interest may be preferentially extracted from a more complex sample matrix. Typically, this type of separation is achieved by applying a sample to a specially prepared and specially selected sorbent column. Isolation of compounds of interest is most often realized by adsorption or retention of the analyte by the sorbent while interfering sample contaminants pass through the column. Conversely, the sample preparative scheme may be designed so that the interfering contaminants are retained by the sorbent and the analyte passes through the column. SPE may also be employed for sample concentration purposes when compound of interest

levels are too dilute for normal recovery and analysis methods (20).

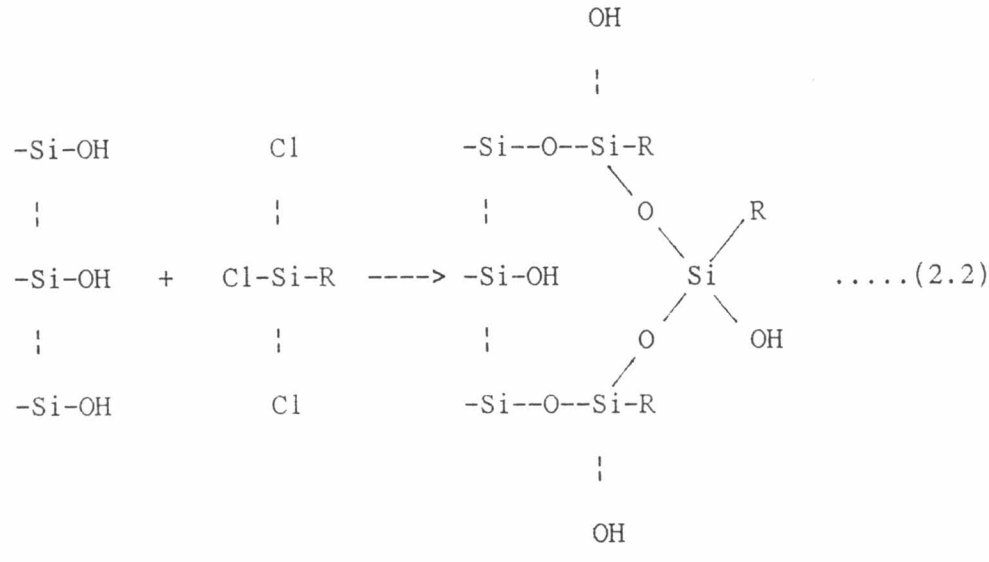
2.2 General Properties of Bonded Silica

2.2.1 Synthesis

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 (13).



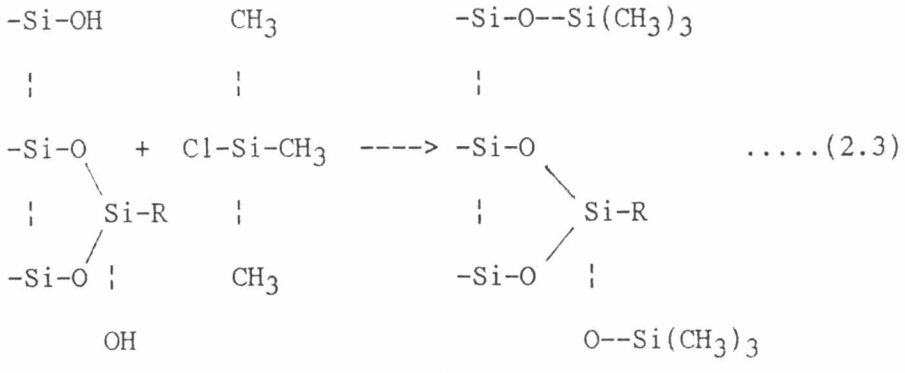
.....(2.1)



.....(2.2)

The monofunctional reaction, shown in equation (2.1), traditionally uses a silane with one reactive group (X), two dimethyl groups and one functional group (R) attached to the silane silicon atom. The surface produced by this type of reaction usually is a well-defined monolayer, where the R group on the original silane imparts the desired chromatographic characteristics to the surface. For example, for R = n-C_8H_{17}, as in the case of n-octyldimethylchlorosilane, a C_8 bonded phase surface is produced. A second method of producing a bonded phase, shown in equation (2.2), uses a polyfunctional silane that has the desired R group and two or three X groups attached to the silane silicon atom (45).

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 attached -R group and those due to the unreacted silanols. These silanol groups are deactivated by endcapping with trimethylchlorosilane as follows (15):



.....(2.3)

2.2.2 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 dissolved 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 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.

2.2.3 Physical Properties

Bonded silica sorbents are rigid materials that do not shrink or swell in different solvents, unlike many polystyrene based resins. 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.

The particle size distribution of 15-100 microns of the silicas is the most commonly used in making bonded silica sorbents. In addition, 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 most of the nominal porosity of the sorbents is 60 Angstroms adequate for compounds with molecular weights up to approximately 15000. The larger molecules 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. As a consequence, they pass through these sorbents without being retained a characteristic that can be used to advantage for removal of large molecules from samples while retaining isolates of lower molecular weight. For extraction of higher molecular weight molecules wide pore sorbents as high as 4000 Angstroms in porosity are used .

2.2.4 Solvation

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 has 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. 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. However, complete drying of a sorbent usually requires at least 30 seconds of air flow of 10 to 15 mL/min. Once the isolate retained on the sorbent, drying is usually not a problem. In fact, drying is recommended between solvent steps when the solvents are immiscible .

2.3 The Mechanics of Sorbent Extraction

2.3.1 Retention and Elution

Retention is the phenomenon where an attraction exists between the sorbent and isolate molecules, causing the isolate 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 a given isolate can, therefore, be expected to change in the presence of different solvents and sorbents.

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 attached than it is the sorbent. There is an important distinction

between the objectives of sorbent extraction and those of traditional chromatography which also employs the term retention and elution. In sorbent extraction, the goal is to retain an isolate on a sorbent strongly enough that the isolate does not remove 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.

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 μL per 100 mg of sorbent. Retention is generally strong enough when 20 bed volumes of an appropriate wash solvent (i.e., one not expected to elute the isolate) 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 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.

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.

2.3.3 Flow consideration

Another factor important to optimum sorbent extraction is flow rate of the sample and other solvents through the sorbent bed. Although the maximum acceptable flow rate is to some degree a function of how strongly an isolate is retained and the size of the sorbent bed being used, flow rates typically should not exceed 5-10 mL/min through a 100 mg sorbent bed. When isolate retention or elution is inadequate, the flow rate for sample application or isolate elution should be explored as a possible cause (13).

2.4 Type of Bonded Phase Sorbent Extraction

Bonded silica materials typically fall into three major categories based on the type of chemical interaction between the compound of interest and the bonded silica material: non-polar, polar, and ion-exchange.

2.4.1 Non-polar Extraction

Non-polar extraction columns contain silica particles bonded to octadecyl, octyl, ethyl, phenyl or other hydrophobic functional groups. These columns are used to extract hydrophobic compounds from aqueous solutions.

Non-polar extraction columns are typically prepared for and 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 passed 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

Polar extraction column contain either unbonded silica, or silica bonded with polar of 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 non-polar matrix components from the column. The compound of interest then is elute with a more polar solvent.

2.4.3 Ion-exchange Extraction

Ion exchange columns can be either cationic or anionic. It is probably the most selective extraction mechanism, and yields the cleanest extracts. The pH of the sample and all solutions should be carefully controlled; a sample with high ionic strength (over 0.2 M) should be diluted with water to enhance retention. The flow rate during sample application and elution must be relatively low (about 1-2 mL/min) for the ion exchange interactions to occur.

Cation exchange columns contain silica bonded with sulfonic or carboxylic and 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 of 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 and acidic wash to neutralize the bonded silica, or by using a basic wash to neutralize the charge on the compound of interest.

Anion exchange columns contain silica bonded with amine functional groups such as aminopropyl or quaternary amines, and are used to extract compounds which contain carboxylic acids or sulphonic acids. Again the pH of the conditioning buffer, the sample and the column rinses should be neutral to ensure ionization both the acidic compound of interest and the basic extraction column. Elution can be done by neutralizing the charge on either one (11).