

# LECTURE # 7

## Properties of good Supporting Materials

- Large capacity to hold stationary phase.
- It must be inert to mobile phase.
- The support may be one which holds polar solvent or non-polar solvent as the stationary phase.
- The retention of solvents depends upon the nature of supporting material.
- The supporting material has large surface area which is available for the solvents.
- The important property is that it has large porosity.
- The activity of the supporting material- they can easily activated. They can easily be activated. They must have degree of activation.
- Like resins they can be regenerate to again behave as active supporting material.

## **Supporting material:**

- Rubber
- Diatomaceous earth
- Glass powder
- Poly-vinyls
- Di-vinyls
- Resins

## **Stationary Phases and Their Properties in HPLC**

### **➤ Normal Phase Material For Adsorption Chromatography**

- **Silica**

The most used adsorbent material is silica. The silica particles are made by sol-gel processes, starting with either sodium silicate or alkoxysilane and resulting in spherical porous beads of even particle size.

Normal pore sizes vary from 6 to 30 nm. The silica particles are rigid and non-compressible, which is one reason why many HPLC packing materials are based on a silica structure. The silica surface contains silane groups with a  $pK_a$  from 6 to below 3. Thus, silica is acidic, causing extra strong retention of analytes with amino groups.

Silica is used for adsorption chromatography with organic solvents as mobile phases. Typical mobile phases are hexane mixed with a more polar solvent, such as dichloromethane or ethyl acetate, often with 0.1-0.5% of methanol or acetonitrile. Acetone, otherwise with many good properties, cannot be used in HPLC with UV detection due to high UV cutoff.

Alkanes are weak solvents in adsorption chromatography, while alcohols are strong solvents.

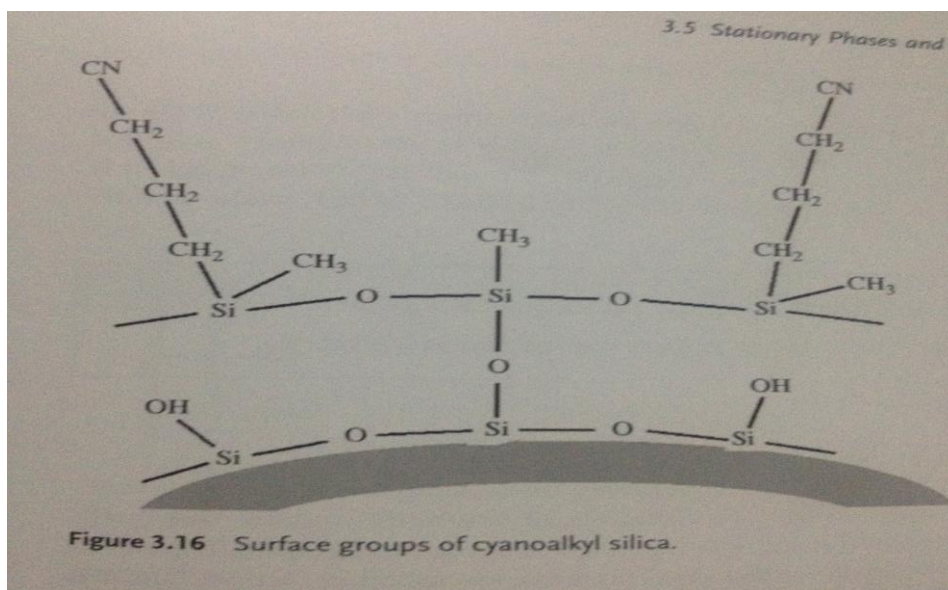
Analytes with polar groups (OH, NH, SH, CO, NO, SO, etc) are more retained than analytes without polar functions.

Silica is a good stationary phase for the separation of position isomers.

- **Silica with Bounded Polar Functional Groups**

Due the many good properties of silica as a chromatographic material, several polar organic functions have been bounded to the surface to avoid the unwanted Silone interactions, including hydroxyl, cyano, amino, and ion exchange functions. Such materials act as polar adsorbents and can even be used for gradient elution, but the application area is usually not very wide.

Solvents similar to the solvents used on silica can be used, but the materials can also be combined with aqueous solvents, such as for hydrophilic interaction chromatography.



- Hydrophilic Interaction Liquid Chromatography (HILIC)

HILIC materials can, in principle, be any one of the polar stationary phases, from silica to ion exchangers, making use of polar interactions between the analyte and the stationary phase with mobile phases consisting of a mixture of water and acetonitrile or methanol or ethanol. Zwitterionic materials (ZIC-HILIC) constitute a prominent type of HILIC columns.

The HILIC mode starts with a high amount of organic solvent in the mobile phase; hydrophobic analytes are eluted first, while hydrophilic (polar) analytes are retained. By increasing the water content of mobile phase, the polar analytes will be eluted.

A HILIC gradient may start with 80% and go down to 20% acetonitrile in a 50 mM aqueous buffer.

- **Carbon Materials**

Carbon has a strong affinity to high molecular mass substances and has traditionally been used for purification/decoloration in synthetic organic chemistry. The porous graphitic carbon (PGC), which is used for chromatography, behaves as hydrophobic adsorbent, stronger than reversed-phase materials, but with strong dipolar and electron lone pair interactions for additional retention of polar analyt.

Porous graphitic carbon particles are used with aqueous mobile phases, but are probably more valuable for solid-phase extraction in small columns than in ordinary HPLC columns.

Typical mobile phases with PGC are mixtures of alcohols and aqueous buffers, as with reversed-phase materials.

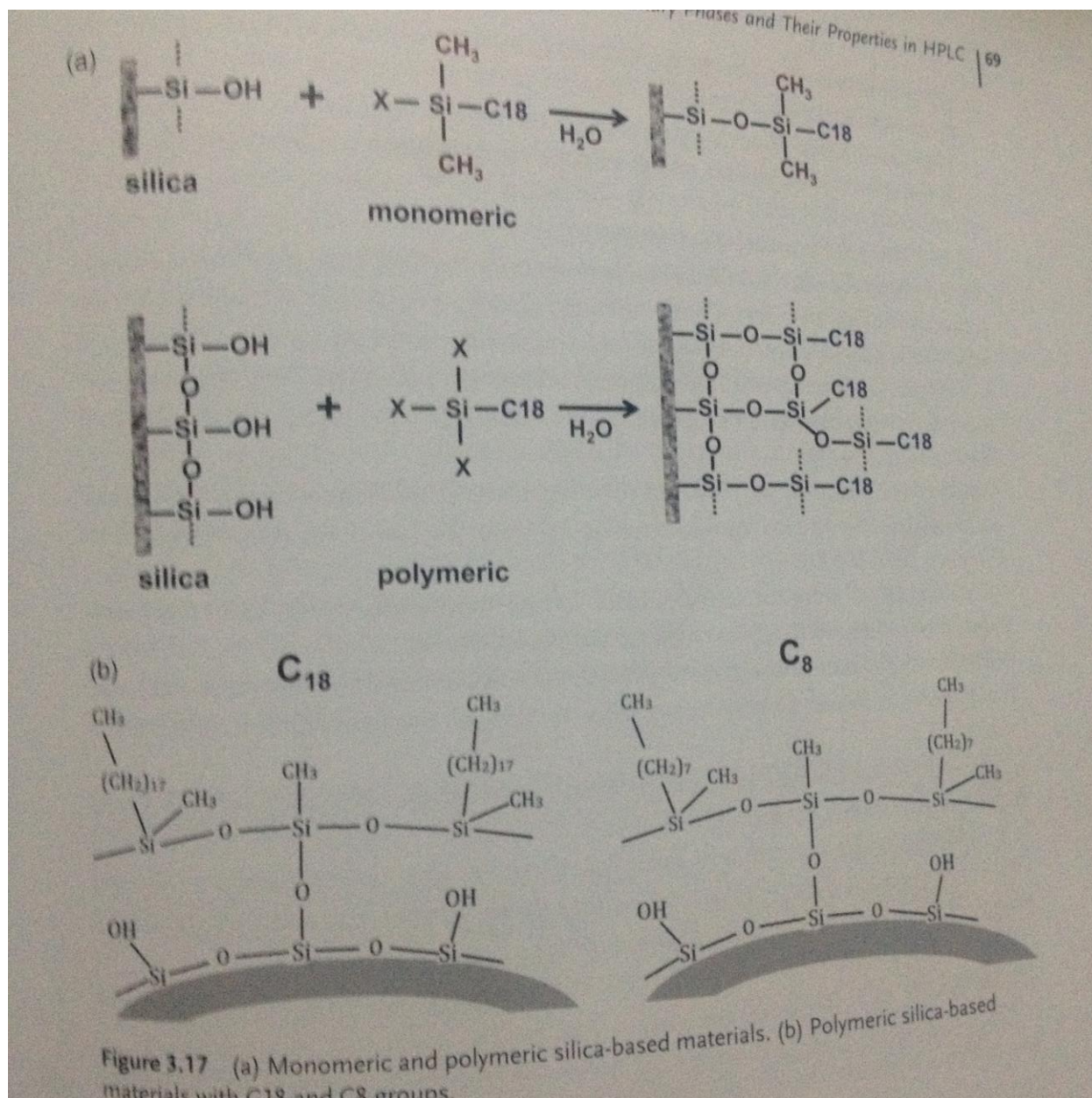
## ➤ **Reversed-phase Materials**

### **Separation Principles**

Reversed-phase (RP) materials have received this name since the elution order is approximately reversed compared to adsorption chromatography on polar materials. This means that nonpolar analytes are more retained than polar analytes on a nonpolar stationary phase.

Reversed-phase materials are mainly used with buffered aqueous mobile phases (containing a mixture with a miscible organic solvent), but non aqueous mobile phases can also be used for many hydrophobic molecules (such as lipids). Ordinarily, the mobile phase consists of mixtures of acetonitrile-water or methanol-water, most often with buffers or acids for pH control.

Solvents gradient start typically with 5% acetonitrile or methanol and then gradually increase the amount of organic solvent. If silica-based C18 materials are started with 0% organic solvent, the C18 groups extending from the surface are at risk of being flattened, resulting in lower loading capacity and reduced retention.



Reversed-phase chromatography is the most widely used separation principle in HPLC.

- **Silica-based Reversed-phase Materials**

The silica-based reversed-phase materials are made from silica particles by bonding C18 or other alkyl chain silanes, with different protective groups, including hybrids with small carbon chains, into particles with different properties depending on the chemistry that has been performed. By reaction of silanol groups with alkoxy or chlorosilane, -O-Si-O- bonds are created for the common reversed-phase materials. Depending on the silane substituents and the reaction conditions, the stationary phase obtains a character as monomeric or polymeric. A monomeric phase is made by reaction a monochlorosilane with silica. A polymeric phase is made by reaction with a trichlorosilane.

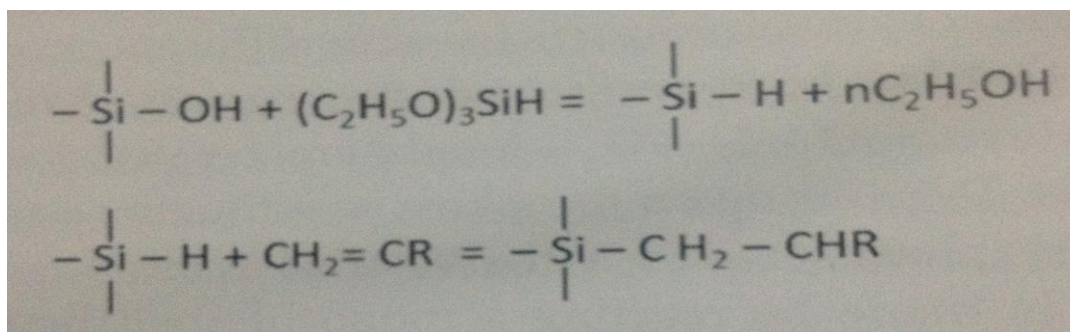
The silica-based materials, in general, are very rigid and are stable at pressures even higher than 1000 bar.

- **Hybrid Materials and Hydrosilated Materials**

Hybrid materials are made as polymers of alkyl silanes and organic monomers, with fewer accessible silanol groups while still maintaining the rigidity of silica, hybrid materials often allow higher pH of the mobile phase,

Hydrosilated materials are made by replacing the Si-O bonds on the silica surface with Si-C bonds:





More than 95% of the silanol groups can be replaced by the stable Si-C bonds.

## Increased Performance

### ➤ Speed

Higher speed and shorter analysis time can be obtained by a combination of smaller particles or core-shell particles, shorter columns, and increased temperature.

### ➤ Efficiency

Higher column efficiency can be obtained by longer/coupled columns, by smaller particles, and often by increased temperature.

### ➤ Resolution

Higher resolution can be obtained by longer/coupled columns. Smaller particles have limited effect on the resolution.

### ➤ Detection

Narrow-bore columns increase sensitivity with concentration-sensitive detectors.

### ➤ Column Lifetime

Protecting the columns with guard columns increase the lifetime.

## Limitation of HPLC

| Perceived Limitations  |
|--|
| Lack of an ideal universal detector                          |
| Less separation efficiency than capillary gas chromatography |
| Relatively difficult for novices                             |
| Still arduous for regulatory testing                         |
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