CHAPTER II

THEORY

Milk and dairy products are mostly analyzed for two main reasons. One is for nutritional or clinical purposes and the other is for toxicity and chemistry.

The Composition of Milk (69)

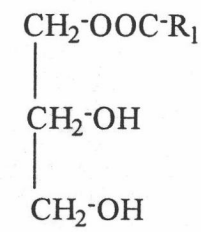
Milk has six major constituents : water, lipids, proteins, citric acid, carbohydrate and trace minerals. All constituents in milk are shown in Table 2.1

Table 2.1 : The Constituents in Milk

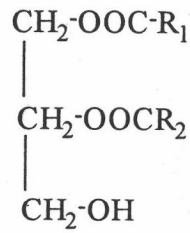
Constituent	percentage
Water	87
Carbohydrates	
- glucose	trace
- lactose	4.9
Proteins	
- caseins	2.9
- β- lactoglobulin	0.32
- α-lactalbumin	0.13
- immunoglobulin	0.05
- serum albumin	0.07
Lipids	3.7
- glycerides	0.1
- phospholipids	0.18
Citric acidMinerals	
- Calcium	0.12
- phosphorus	0.10
- sodium	0.05
- potassium	0.15
- chloride	0.11

Lipids

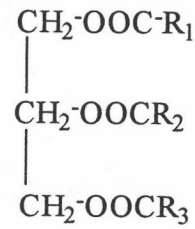
Lipids are the only organic compounds of biological origin that are insoluble in water. There are two main classes, saponifiable and non-saponifiable. Saponifiable lipids such as neutral fat (triglycerides of fatty acid, di-, mono-), glycolipids, phospholipids, cholesterol esters are the major lipids in milk. The other are non-saponifiable lipids, e.g., stearoids, carotenoids and vitamins.



monoglyceride

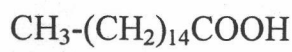


diglyceride

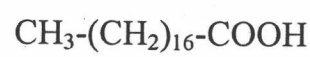


triglyceride

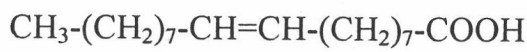
Palmitic acid, stearic acid and oleic acid are the most abundant fatty acid in milk. Phosphoglycerides are minor ingredients of milk fat, but they are crucial to its physical structure. They are found almost exclusively in cell membranes and help stabilize the fat globules. Most phosphoglycerides contain an alcohol component linked to the phosphoric acid so that it creates a polar hydrophilic head.



Palmitic acid



Stearic acid



Oleic acid

This explains the emulsifying capacity. In the physiological state, the milk fat is dispersed as an immiscible emulsion in the aqueous phase of milk plasma. The fat globules are distributed in sizes ranging from 0.1μm to larger than 22 μm depending on the mammal species, its breed and state of lactation. Most of the fat globules are between 1-4 μm.

Stability of fat emulsion is attributed to a stabilizing membrane surrounding. The nucleus of the fat globules by the encapsulating material, consist of a mixture of protein and lipid. The fat globules are shown in Figure 2.1.

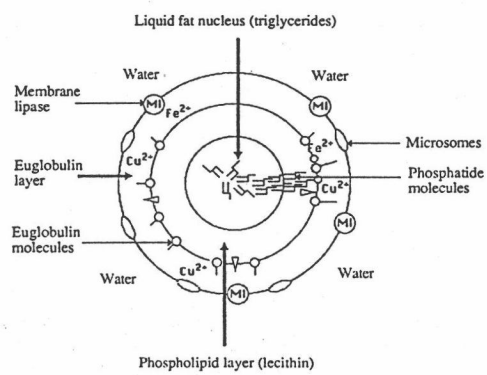


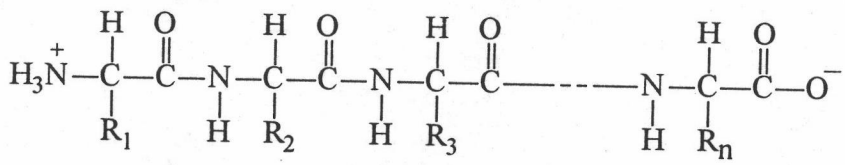
Figure 2.1 The fat globules

The lipid fraction is approximately half of the total membrane material. Approximately 40% of lipid fractions are the hydrophilic (mono-, di-glyceride) and the rests are triglycerides (30%), cholesterol and cholesterol esters (30%). The glycoprotein and lipoprotein which is comprising the fat globule membrane are sensitive to the usual denaturating factors such as heat and mechanical action.

Proteins

Chemical and physical properties of proteins

Amino acids are the basic structure units of proteins. An amino acid consists of a central atom, "α", sided by an amino group, a carboxyl group, a hydrogen and substituent group. The carboxyl group of one amino acid is bonded to the α-amino group of another amino acid by elimination of water forming a peptide bond as shown below.



Caseins are the major class of milk proteins and have few serine and threonine residues. In general, proteins consists of one or more polypeptide chains that are held together by disulfide bonds or by noncovalent forces. Protein has four types of structure:

1. primary structure has a specific sequence of successive amino acid residues.

2. secondary structure is a specific conformation of the polypeptide chain.

3. Tertiary structure has a unique manner in which the polypeptide chain is folded.

4. Quaternary structure has an individual packing arrangement for several polypeptide chains. Ultimately, it is the amino acid sequence that determines the three-dimensional conformation of protein molecules.

At neutral pH, amino acids are predominantly dipolar ions (Zwitterions) rather than nonionized molecules. This ionization state varies

with pH. The isoelectric pH value is the point of absence of a net electrical charge on the molecule, which is the pH of zero electrophoretic mobility. Among milk proteins, the isoelectric point ranges from 3.3 (lowest) to 6.0 (highest). Protein molecules can be extremely changed in structure by a change of pH or temperature. This is known as denaturation and expressed by decreasing solubility. Denaturated proteins are unfolded from the original multi-dimensional structure.

Milk proteins are traditionally divided between caseins and whey proteins.

Caseins are the group of proteins precipitated from skim milk at pH 4.6 at 20°C. The proteins which are soluble which are under this condition are called whey proteins or milk serum proteins. Casein is not a chemically homogeneous single protein, but a mixture of different proteins that have distinctive properties and as such can be separated. In milk, the main amount of caseins is present in the form of an aggregate with calcium phosphate, in large spherical micelles varying in size from 30 to 300 nm in diameter, with a distribution centered around 130 nm and molecular weight of 10^7-10^9. Micelles contain 92% proteins (α_{s1},α_{s2},β,κ casein), and the rest is calcium, phosphate and small amounts of magnesium and citrate ions. The total calcium content of skim milk has been estimated to be 30 mM and the calcium content of serum is only 2.9 mM. Thus more than 90% of calcium content in milk is associated with casein micelles, either as amorphous calcium phosphate and citrate ion or bound to the protein at the orthophosphate group. The light scattering induced by the colloidal casein explains the "milky" appearance of milk.

Casein is the 80% of total milk protein and the rest is whey protein. It has five types, α_{s1}, α_{s2}, β, κ, γ .

α_{s1}-casein is a single chain polypeptide that contains eight sites with phosphate residues as esters of serine. These phosphoseryl residues carry a net

negative charge that relative to the remaining hydrophobic segment creates a dipole. This peptide is insoluble in an aqueous medium which contains more than 5 mM calcium ions. .

α_{s2}-casein possesses similar features.

β-casein is also a single protein with one phosphoserine, like the α_{s1} -casein that give an insoluble complex. But this protein is inversely related to the temperature; at 1°C this complex is soluble even in the presence of 400 mM calcium ions.

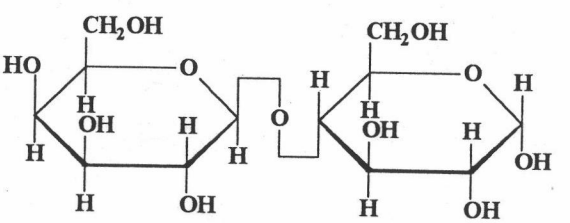
κ-casein contains up to two phosphate side and does not bind as much calcium as α -casein. In contrast to α_{s1} and β-casein, κ -casein is soluble in water. It is the only casein, component that also contains a carbohydrate (galactose, galactoseamine and neuraminic acid) moiety that is linked to a serine or threonine residue by a glycoside bond. para-κ-Casein that is the product of κ -casein after attach by the enzyme chymosine on the proteolytic enzyme, is insoluble in water. Furthermore, that enzyme can destabilize the colloid, exposing the insoluble calcium salts of α_s -casein and β-casein to the aqueous medium, causing coagulation to occur.

About 3-10% of total milk protein does not exist in micellar form. They are subdivided between heat-labile proteins (lactabumins, lactoglobulins and immunoglobulins) which precipitate at pH 4.7 after previous heat treatment at 95-100 °C for 30 min. and protease-peptone which remains in solution even after this treatment but it can be precipitated by 12% trichloroacetic acid.

β-lactoglobulins is the serum protein which contains the major amount of cysteine residues.

Lactose

Lactose is one of the most common natural disaccharides and occurs almost exclusively in milk and contains glucose and galactose moieties. Lactose is a disaccharide form from a molecule of galactose linked at carbon one to carbon four of glucose molecule. It exists in both α and β configurations. Both α and β isomers of lactose exist in equilibrium in aqueous solutions, due to mutarotation. The ratio at equilibrium state is slightly affected by temperature. Lactose is less sweet than sucrose and the β-isomer is sweeter than the α-isomer. Lactose and casein are the two principle reactants in the browning reaction of dairy products. The reaction between sugar and protein is irreversible.



α-Lactose

Minor components of Milk

1. Enzymes

Enzymes are complex proteins identified by their abilities to catalyze chemical reactions with a high specificity for substrate. The enzymes in milk are lipase, alkaline phosphatase, lactoperoxidase and catalase.

- Lipase catalyzes the hydrolysis of milk fat to glycerol and fatty acids.

- Alkaline phosphatase catalyzes the cleavage of phosphoric acid esters into phosphoric acid and the corresponding hydroxylic compounds, alcohols or phenols.

- Lactoperoxidase catalyzes the transfer of oxygen from peroxides to other readily oxidized substances.

- Catalase is an enzyme that promotes the decomposition of hydrogen peroxide to water and molecular oxygen.

2. Non protein nitrogeneous substances

Most nitrogen in milk is localized in protein (more than 95%), and the rest is small molecules : urea, uric acid, amino acids, amines, ammonia etc.

3. Vitamins

Milk contains both fat-soluble and water-soluble vitamins that are shown in Table 2.2

Table2.2: The amount of fat-soluble and water-soluble vitamins

Vitamin	mg/100mL
Fat-soluble vitamins	
A	0.08
D	0.00006
E	0.1
K	0.005
Water-soluble vitamins	
B vitamins	
B_1(thiamine)	0.05
B_2(riboflavin)	0.17
B_6(pyridoxine)	0.06
B_{12}	0.00045
Biotin	0.003
Niacin	0.1
Pantothenic acid	0.35
Folic acid	0.006
Vitamin C	2.1

4. Citric acid

Citric acid is found in milk at about 0.2% that is in soluble form. It is a valuable substrate for fermentation.

5. Inorganic elements(Minerals)

In milk, the mineral salts are chloride, phosphate citrate potassium, sodium, calcium and magnesium. These inorganic elements play an important role in maintaining stability of protein micelles in milk.

6. Gases

Milk contains natively dissolved gases (approximately 5 % by

volume) which consist of carbon dioxide, nitrogen and oxygen.

Physical properties of milk

Milk is a true aqueous solution of lactose, salts and a few other minor compounds, which is emulsified with fat and supported a colloidal dispersion of proteins. The physical properties of milk (cow milk) are shown:

pH value (at 25°C)	6.6
Acidity	0.018% lactic acid
Specific gravity (20°C)	1.032
Freezing Point	-0.540 °C
Boiling Point	100.17 °C
Osmolality	275 mOs/Kg
Viscosity	1.6314 cpoise
Surface tension	50 dynes/cm.
Electric conductivity	48×10^{-4} $ohm^{-1}cm^{-1}$
Refractive index (20°C)	1.3440-1.3485

The Sample Preparation of Milk and Dairy Products (70-71)

The analysis of milk and dairy products has been reports. For the nutritional and clinical purposes, milk is analyzed in many ways. One purpose of analytical method is to determine milk composition, safe dairy foods, energy content, favor, etc. The sample preparations of milk sometime complicate the analysis because of its matrix so that it takes many steps. It can be seen that the milk composition has three major components (except water) which are lipids, proteins and carbohydrates. Thus, the analytical purposes have two aspects.

The first is determination of the nutrient in milk and the other is determination of substance or organic molecules in milk. The preparation method before detection will be described here.

1. Centrifugation or Ultracentrifugation

Centrifugation is one way to isolate or purify macromolecules. Any molecules or particles are subjected to a centrifugal field by being spun in a centrifuge rotor, it can be said that they are subjected to a centrifugal force. For a particle of mass (m) this force is given by:

$$F_{sedimentation} = m(1- \bar{\nu} \rho)\omega^2 r$$

r = distance of the particle from the center of the rotor.

$(1- \bar{\nu} \rho)$ = Buoyancy factor ; $\bar{\nu}$ = specific volume of the particle (mL/g)

ρ = solution density (g/mL)

ω = angular velocity

Since different-sized particles or molecules will differ in sedimentation velocity so it can use for separation. Therefore, we usually use this method to separate fractions in milk. Ultracentrifugation is centrifugation but it was developed in 1923 to attain high speed, up to 80,000 rpm.

2. Precipitation

The solubility of milk is changed depending on four major effects : the concentration of dissolved salts, polarity of the solvent, pH and temperature.

2.1 Effect of Salt Concentrations

The solubility of the components of milk especially proteins are a sensitive to the concentrations of dissolved salts. The salt concentration is expressed in terms of the ionic strength, I, which is defined.

$$I = 1/2 \sum C_i Z_i^2$$

C_i : molar concentration of i ionic species

Z_i : ionic charge of i ionic species.

There are two effects of salt concentrations, salting in and salting out. The solubility of a protein at low ionic strength generally increases with the salt concentration. It has been shown that the "salting in" phenomenon when the salt concentration of the protein solution increases, the additional counterions more effectively shield the protein molecules multiple ionic charges and thereby increase the protein's solubility. At high ionic strength, the solubilities of proteins decrease. This is known as "salting out". Salting out occurs because of the competition between the added salt ions and the other dissolved solute for molecules of solvation. So that, at high salt concentration, so many added ions are solvated that the amount of bulk solvent available becomes insufficient to dissolve other solutes. Therefore, solute-solute interactions become stronger than solute-solvent interaction and the solute precipitates. Salting out is the basis of one of the most commonly used purification procedures. Some ions, notably, I^-, ClO_4^-, SCN^-, Li^+, Mg^{2+}, Ca^{2+} and Ba^{2+} increase the solubility of proteins rather than salting them out but these ions tend to denature proteins.

2.2 Effect of organic solvents

Water-miscible organic solvents, such as acetone and ethanol are generally protein precipitants because they have low dielectric constants.

They are lower the solvating power of their aqueous solutions for dissolved ion such as proteins. This techniques can be effectively combined with salting out.

2.3 Effect of pH

At the isoelectric point (pI), the protein molecule carries no net charge and its solubility of protein is at a minimum. The change in pH will affect the shape of a protein. It is resulting in solubility behavior, which is shared by most protein (because of the net charge not balance to zero). To a first approximation, they should not be subject to salting in at pI. Conversely, as the pH is varied from its pI, causing it's net charge to increase, it should be increasingly subject to salting in because the electrostatic interaction between neighboring molecules increase.

2.4 Effect of temperature

Temperature is usually not used in the preparation steps in milk. Therefore, it makes the denaturation of milk but the temperature is used for incubation or preparation of antibiotic in milk analysis.

3. Dialysis (Molecular filtration)

Dialysis is used routinely to remove small-molecule contaminants. The process of dialysis separates molecules according to size through semipermeable membranes containing pores of less than macromolecular dimensions. Cellophane (cellulose acetate) is the most commonly used dialysis material although many other substances such as nitrocellulose and collodion are similarly employed. A macromolecular solution is sealed inside a dialysis bag that is immersed in a relatively large volume of the new solvent. Then, it will be stirred for several hours.

Dialysis gives two benefits.

- To separate small solutes by passage through the membrane.

This method is the one method that can separate ions, and antibiotic drug residues and etc. from milk.

- To concentrate macromolecule in the semipermeable membrane bag. This is similar to ultrafiltration, but uses pressure.

4. Extraction technique (72)

Extraction is the original preparation for every sample. Liquid-liquid extraction and liquid-solid extraction have been used. According to the basis of "like dissolves like", it has been used to extract organic substances from matrix samples. A suitable solvents must be selected for the substance in the matrix. The solvent of choice should be :

- easily recoverable
- nontoxic and not highly flammable
- volatile
- stable
- transparent to UV so that UV detector can be used for HPLC
- minimal emulsion formation

Salting out is sometimes combined with extraction to aid the transfer from aqueous to solvent phase.

5. Chromatographic method for clean up step

Chromatography is a routine preparative method nowadays. Milk and dairy products usually pass this preparative technique especially "clean up step". Many popular chromatographic techniques are used with milk and dairy products. Most of them are used to separate organic substance from the milk samples.

5.1 Gel Filtration Chromatography

Gel Filtration Chromatography or size exclusion/molecular seive chromatography, in which molecules are separated according to their size and shape. The stationary phase consists of spongelike material containing pores that span a relatively narrow size range of molecular dimensions. The separation will occur because the big molecules will be excluded from the pores. On the other hand, the smaller molecules will pass through the pores. Molecules with molecular masses ranging below the exclusion limit of a gel will elute from the gel in the order of their molecular masses with the largest eluting first. With this technique, we can choose the suitable size range of the stationary phase. For milk samples this technique is usually used for clean up of lipids. The most commonly used materials for making chromatographic gels are dextran, agarose and polyacrylamide.

5.2 Adsorption Chromatography

This technique is also usually used to separate nonpolar substances from the milk sample. In adsorption chromatography, molecules are physically adsorbed on the surface of insoluble substances such as alumina, chacoal, silica gel and florisil, through Van Der Waals and hydrogen bonding. The molecules are the eluted from the column by pure or mixed solvents.

5.3 Reversed-Phase Chromatography

Like adsorption chromatography, Reversed-phase chromatography separates nonpolar substances from milk samples. Reversed-phase chromatography (RPC) is a form of liquid-liquid partition chromatography in which the stationary phase consists of a nonpolar and the mobile phase is a more polar liquid. It is the first technique that was developed to separate liquid or nonpolar substances from the milk sample. Furthermore, it can be effective in separating other polar substances.

We can conclude that the preparation of milk and dairy product samples is in three principle steps.

1. Aseptic storage and preparing sample before extraction.
2. Extraction and concentration.
3. Clean up procedure; Sample clean up is required when impurities in the sample matrix interfere.

Empore ™ Technology (5, 73)

Empore ™ is a tradename of an extraction disk that is a new generation of solid phase extraction devices. It is a device that was produced or developed to provide advantage over the original solid phase extraction cartridge (column).

Solid Phase Extraction (SPE)

For many years, sample dissolution followed by liquid-liquid extraction has been popular for sample preparation procedure. Traditional liquid-liquid extractions that were performed in separatory funnels, are tedious, time consuming and costly. These methods not only require several sample handing steps but may also present some problems:

- phase emulsions
- impure and wet extracts
- large solvent volumes
- non quantitative and irreproducible extracts

In the mid 1970's a simpler alternative approach, solid phase extraction was introduced. Similar to low pressure liquid chromatography, this uses small, disposable extraction columns filled with a variety of sorbents. Pressure can use to force the solution through the columns. Solid phase extraction or sorbent extraction is a physical extraction process that involves a liquid and a solid phase. Sorbent/ Solid phase has a greater attraction for the isolate than the solvent in which the isolate is dissolved. As the sample solution

passes through the sorbent bed, the isolate concentrates on this surface while the other sample components pass through the bed. Very selective extraction resulting in highly purified and concentrated isolate can be acheived by choosing sorbents with an attraction for the isolate but not for the other components.

A variety of bonded silica is commercially available, used as a sorbent, offering a wide range of selective properties for extraction.

General properties of bonded silica

1. Synthesis - Bonded silica is formed by the reaction of organosilanes with activated silica. The product is a sorbent with the functional group of the organosilane attrached to the silica substrate through a silyl ether linkage.

- Another reaction calls endcapping in which some remaining silanol groups on the silica are deactivated.

2. Stability The bonded silica sorbent product is stable with a pH range of approximately 2-7.5.

- Below pH 2.0, the silyl ether linkage is labile, and the functional groups on the surface will begin to cleave. In some case, the sorbent can be used at pH 1-14 if it is carried out for a short period.

- Above pH 7.5 the silica substances are susceptible to dissolve in the aqueous phase.

All organic solvents can be used with bonded silica.

3. Physical properties Bonded silica sorbents are rigid materials that do not shrink or swell in different solvents. The silica most commonly used in making bonded silica sorbents has a particle size are irregular rather than spherical. The nominal porosity of most of the sorbent is 60 Å, suitable for compounds with molecular weight up to approximately 15000. In the higher molecular weight, wide pore sorbent as high as 4000 Å in porosity is used.

4. Solvation Solvation of a sorbent is necessary before the sorbent will interact reproducibly with the isolate. Some sorbents, especially non-polar such as octadecyl (C_{18}) will not reproducibly retain the isolate until they have been solvated. Solvation is accomplished by passing several bed volumes of a suitable solvent such as methanol through the sorbent. Methanol is an effective solvating agent because it can interact with both the silanols on the silica and the carbon atoms of bonded functional groups.

The Mechanics of Sorbent Extraction

1. Retention and elution

" Retention " is the phenomenon where an attraction exists between the sorbent and the isolate molecule, causing the isolate to be immobilized on the sorbent surface as the sample solution passes through the sorbent bed. The sorbent /isolate interactions occur among:

1.1 Non-polar interations

Non-polar interactions are occurred between the carbon-hydrogen bonds of the sorbent functional groups and the carbon-hydrogen bonds of the isolate. These forces are commonly known as "Van Der Waals" or "dispersion" forces. Nearly all of the isolate species have a potential for non-polar interactions. The most widely used sorbent for non-polar interactions is octadecyl silane bonded to silica substrate. C_{18} is a very non selective sorbent but it is a very effectiveness for isolating groups of compounds.

1.2 Polar interactions

Polar interactions include hydrogen bonding, dipole/dipole, induced dipole/dipole, and pi-pi.

1.3 Ionic interactions

Ionic interactions occur between an isolate molecule carrying a charge and a sorbent carrying a charge.

1.4 Covalent interactions

Covalent interactions are those resulting in formation of a covalent bond between the sorbent and the isolate molecule.

1.5 Multiple interactions

It is necessary to emphasize at this point that almost all available sorbents are capable of more than one interaction.

"Elution" is the process by which an isolate is removed from a sorbent bed which it has been retained. This is caused by introducing a solvent which the isolate is more strongly attracted than it is to the sorbent. The elution solvent chosen should be eluted the isolate from the sorbent bed in the smallest volume possible. For 40 μm, 60 Å sorbents, the bed volume is 120 μL per 100 mg.

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. The capacity for 100 mg of sorbent might retain as much as 5 mg of a strongly retained isolate.

- 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.

3. Flow consideration

Flow rate of the sample and solvents through the sorbent bed is the important factor to optimum sorbent extraction. Flow rate through a 100 mg sorbent bed should not exceed 5-10 mL/min.

Types of Sorbents

1. Normal Phase (Polar Bonded Sorbents)

Normal phase is a bonded phase where the R group of the silyl derivatizing agent is a polar functional group such as cyano, amino or diol group. In normal phase, the sample is dissolved in a solvent of low solvent strength passed through the sorbent surface, and partitioning of the analyte with polar sorbent will occur.

2. Reversed Phase (Nonpolar bonded sorbent)

Nonpolar bonded sorbents are less polar than the mobile phase or sample solution. Examples are octadecyl, octyl, cyclohexyl, butyl, and phenyl groups that are bonded with silica. These bonded phases have been used extensively for the trace enrichment of organic material from aqueous matrices in clinical and environmental samples.

3. Ion-exchange (Charged Bonded Sorbents)

The most popular bonded sorbents contain $-SO_3^-$ and $-N^+(CH_3)_3$ ionic functional groups are used to charge sorbents. These functional group are a strong exchanger for extraction of ions ($-SO_3^-$ for extraction of basic analytes and $-N^+(CH_3)_3$ for extraction acidic analytes). The elution step will be done with high ionic strength and varied pH.

4. Wide-Pore Bonded Sorbents

Wide-pore bonded sorbents (40 μm, 275 Å) find utility in the isolation of high molecular weight compounds (compounds with molecular weight above 2000).

5. Size Exclusion

This is controlled by pore size. Higher molecular weight compounds are excluded from the pores and are eluted with the eluent.

Solid phase extraction has been used in three modes, sample clean up, sample concentration and matrix removal.

- Sample clean up : the sorbent will retain the analyte from a small volume of sample solution and allow impurities to pass through the sorbent.

- Sample concentration : it is the trace enrichment that the analyte is in the dilute of sample solution.

- Matrix removal : the impurities are retained but the analytes pass unretained through the sorbent with the eluent.

The modern technique SPE has its beginning in 1978 with the commercial introduction of Sep-Pak cartridges (Water Associate). It usually includes a plastic tube (most often a polypropylene syringe barrel), frits at both ends, and 100-500 mg of 40 μm stationary-phase particles in the middle.

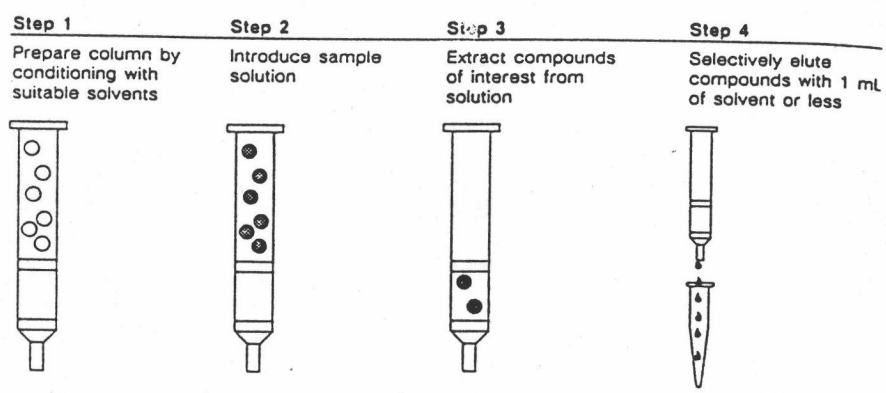


Figure 2.2 Solid Phase Extraction cartridge

Although the SPE cartridge is 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), thus along time is required for large sample volumes.

2. Rapid flow through an SPE cartridge can cause kinetic effects in the bed of 40 μm particles that prevents the recovery of certain analytes.

3. Dirty samples can rapidly and easily plug the small cross-sectional area of the cartridge.

4. Long extraction time

5. Bed channeling may be occurred.

To overcome this problem, a new generation of SPE devices has recently been presented, borrowing the disk configuration of membrane filters.

- Flat disks with high cross-sectional area
- Decrease back pressure (higher flow rates are possible)
- Decrease the chance of plugging
- Prevent channelling
- Improve mass transfer

Types of SPE disks

1. Packing-impregnated polytetrafluoroethylene (PTFE)

These devices consist of a PTFE fibril network that holds bonded silica particles or resin in place. The 8 μm particle comprises ~ 90% of the weight of the device and the PTFE comprises ~ 10%.

A: Chemically bonded silica particles

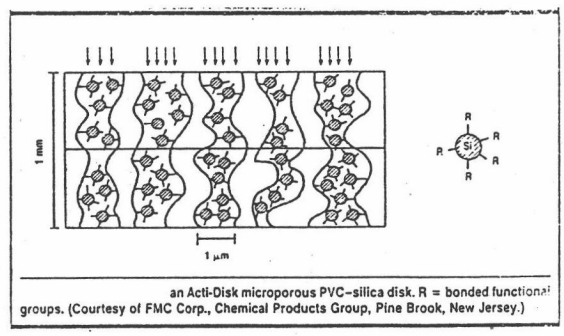
B: The individually particles suspended in a densely woven of micro-PTFE fibril

Figure 2.3: The Micrograph of SPEM

Figure 2.3 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 disks are manufactured by 3M and distributed by Varian Sample Preparation Products and J.T.Baker Inc. Bio-Lad Laboratories produces 40 μm resin-filled version. The Bio-Rex chelating resin-containing disk have interesting application in the selective extraction and concentration of heavy metals in solution.

2. Packing-impregnated polyvinyl chloride

This is primarily designed for protein purification. These microporous plastic sheets contain silica activated by a standard ion exchanger or affinity chemicals. The membranes shown in figure 2.4 have ~ 1μm distance flow through pores that provides fast kinetics.



an Acti-Disk microporous PVC–silica disk. R = bonded functional groups. (Courtesy of FMC Corp., Chemical Products Group, Pine Brook, New Jersey.)

Figure 2.4: The polyvinyl chloride SPEM

The material is available in sheets, cartridges and disks ranging in diameter from 25 mm to size large enough for scale-up work. Flow rate ranges from 20-80 mL/min. An example of this type of device includes Acti-Disk and Acti-Mod products.

3. Derivatized membrane

Stationary phase particles are not embedded into membranes. Rather, the membranes are functionalized through chemical reactions. They are made of cellulose derivatized with functional groups such as DEAE (diethylaminoethyl), sulfonylpropyl etc.

Only packing-impregnated PTFE devices are directly equivalent to the typic SPE cartridges because the PTFE matrix contains the same stationary phase. The comparison between disk and cartridge are shown in Table 2.3.

Table 2.3: The comparison of cartridge and disk parameter

parameter	cartridge	disk
dimension (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

The Empore™ extraction Disk usage step

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. Disk Conditioning

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

3. Sample Addition

Accurately transfer the sample to extraction disks. Adjust the flow rate to be suitable.

4. Disk Wash

Wash with the specified volume of solvent. This step is required to remove impurities or interferences so that it can be done if the analytes must not elute in this time. Air dry the disks, then use vacuum if require.

5. Elution

Optimum volumes of selected solvents are passed through the disk for elution the analyte (or impurities in matrix removal mode) and collected for the further detection step.