CHAPTER 1

INTRODUCTION

1.1 Surfactants

Surfactants are substances with molecular structures consisting of a hydrophilic and a hydrophobic part. The hydrophobic part is normally a hydrocarbon (linear or branched), whereas the hydrophilic part consists of ionic or strongly polar groups, e.g. polyglycol ether groups. Due to this characteristic structure, these compounds have a special property, namely the interfacial activity, which sets them apart from organic compounds in general [1].

Surfactants are primarily applied in aqueous solution. So surfactants can be categorised into four types according to the hydrophilic group. These surfactants are anionic surfactants, cationic surfactants, nonionic surfactants and amphoteric surfactants [2].

- *Anionic surfactants* are surface-active substances in which the hydrophilic head of the molecule carries a negative charge.
- *Cationic surfactants* are substances that carry a positively charged on the hydrophilic group.
- *Nonionic surfactants* are substances, which in aqueous solutions do not dissociate into ions. The solubility of these substances in water is provided by polar groups such as polyglycol ether groups.
- *Amphoteric surfactants* contain in aqueous solution both a positive and a negative charge in the same molecule. The charge(s) on the hydrophilic head change as a function of pH. Such surfactants must

carry a positive charge at low pH and a negative charge at high pH and may form internally neutralized ionic species (zwitterions) at an intermediate pH.

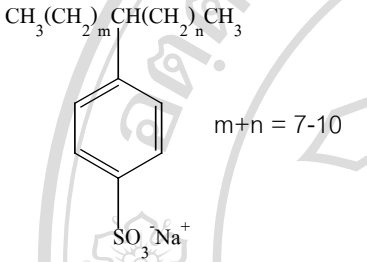
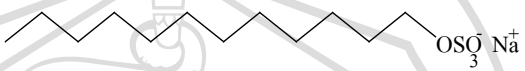
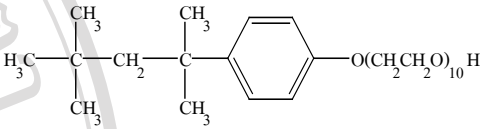
These surfactants especially anionic and nonionic surfactants are used in the large quantities and discharged into the environment, which can affect the ecological equilibrium due to the toxic nature of surfactants. Therefore, the determination of surfactants in environmental samples is of great interest, after maximum priority pollutants such as pesticides, polycyclic hydrocarbons or polychlorobiphenyls [3].

1.1.1 Anionic surfactants

1.1.1.1 Linear alkylbenzene sulfonate [4, 5]

Linear alkylbenzene sulfonate (LAS) is a mixture of closely related isomers and homologues, each containing an aromatic ring sulfonated at the *para* position and attached to a linear alkyl chain (**Table 1.1**). The linear alkyl chain has typically 10 to 13 carbon units. LAS homologues are mainly used in detergent formulations, laundry and cleaning products (~80%). LAS is commonly used in many household detergents, including laundry powders, liquid and tablets (typical concentration 3 - 22%), laundry beach additive (typical concentration 3 - 11%), dishwashing liquids (typical concentration 2 - 30%) and all-purpose cleaning powders, liquids and tablets (typical concentration 1 - 30%). LAS is also used in some industrial applications, such as textile and fibers, chemicals and agriculture.

Table 1.1 Structures and some properties of surfactants [5 - 9]

	LAS	SDS	Triton X-100
Structure			
Molecular weight	C_{10} LAS = 320, C_{11} LAS = 334 C_{12} LAS = 348, C_{13} LAS = 362	288.38	646.85
Density ($g.cm^{-3}$)	1.06	0.40	1.07
Solubility	soluble in water ($250\ g\ l^{-1}$)	soluble in water ($250\ g\ l^{-1}$ at 20°C)	soluble in all proportions at 25 °C in water, benzene, toluene, ethyl ether, trichloroethylene, ethylene dichloride, ethanol and isopropanol
Appearance	yellowish viscous liquid	white or slightly yellow powder	colorless viscous liquid

LAS degrades rapidly under aerobic conditions but it does not degrade under anaerobic conditions. LAS is transformed to sulfophenyl carboxylates (SPCs), biodegradation intermediates by microorganisms. As a result of biodegradation, the loss of interfacial activity and the toxicity towards organisms present in the environment.

In the aquatic environment, different homologues and isomers are present. Each of these components has different degree of ecotoxicity, with the shorten chain lengths being less toxic than the longer ones (**Table 1.2**). LAS toxicity increases exponentially with the carbon chain length. Moreno-Garrido *et al.* [10] reported that the toxicity of the LAS containing 13 carbon atoms to the microalgae namely *Chaetoceros gracitis* was found to be greater than that of the C_{11} LAS. For these reasons, the identification and quantification of individual LAS specie are invaluable for estimating the environmental impact and potential health effects of LAS species.

Table 1.2 Toxicity of LAS homologues [5]

Alkyl chain	Invertebrate (*Daphnia magna*) EC_{50} (mg l^{-1})	Fish (*Pimephales promelas*) LC_{50} (mg l^{-1})
C_{10}	29.5	57.5
C_{11}	21.1	21.9
C_{12}	5.9	6.6
C_{13}	2.6	1.8
Commercial LAS	5.0	2.9

1.1.1.2 Sodium dodecyl sulfate [11-12]

Sodium dodecyl sulfate (SDS) is ionic detergent that is commonly found in household products. Pure SDS is used mainly in dentifrice products, hair shampoos and emulsion polymerization. The rest is ether from used in special cosmetic formulations, e.g. for bubble baths and hair bleaches, or as fine chemical, e.g. as denaturing agent in gel electrophoresis. Technical grade SDS consists of approximately 70% SDS and 30% sodium tetradecyl sulfate. This product is generally called sodium lauryl sulfate. The molecule of SDS has a tail of twelve carbon atoms, attached to a sulfate group and giving the amphiphilic properties (**Table 1.1**).

1.1.2 Nonionic surfactant

Triton X-100, a nonionic surfactant, is often used in biochemical applications to solubilize proteins. It is effective in textile cleaning applications and is used in built formulations designed for home and industrial laundering [8, 13]. Triton X-100 is a registered trademark of octylphenol ethoxylate consisting of ethylene oxide about ten molecules (**Table 1.1**). The “Triton X” series of detergents are produced from octylphenol polymerized with ethylene oxide. The number “100” relates only indirectly to the number of ethylene oxide units in its structure.

1.2 High Performance Liquid Chromatography

Liquid chromatography is a separation technique based on a different distribution rate of sample components between a stationary and a liquid mobile phase. High performance liquid chromatography (HPLC) is the term used to describe liquid chromatography in which the liquid mobile phase is mechanically pumped through a column containing stationary phase. HPLC is widely used for separating non-volatile and thermally labile compounds because of its sensitivity, its ready adaptability to accurate quantitative determinations and its applicability to substances regarding to industry and many fields of science [14-16].

In reversed-phase HPLC, a non-polar stationary phase is used in conjunction with polar mobile phase. Almost 80% of all HPLC applications utilize this technique. Its popularity is based largely on ease of use, fast equilibration time, reproducible retention times and the basic principles of the retention mechanism can be understood easily [17].

1.2.1 Chromatographic theory [14-16, 18]

Chromatography is a separation technique where component molecules (solutes) in a sample mixture are transported by a mobile phase over a stationary phase. Each component or solute is distributed between the two phases with an equilibrium established defined by the distribution or partition coefficient (K). The distribution equation for each component between two phases is given as follows:

$$K = \frac{C_S}{C_M} \quad (1.1)$$

where C_S and C_M are the concentrations of the solute in the stationary phase and in the mobile phase, respectively.

Three basic terms are used to express the key parameters in all the chromatographic experiments, namely retention, resolution and efficiency.

1) Retention

This term is expressed as retention time (t_R) and/or in terms of capacity factor (k′). The retention time is a time for elution at the peak maximum. The capacity factor is a direct measurement of the strength of the interaction of the sample with the packing material and is defined by the expression:

$$k' = \frac{t_R - t_0}{t_0} \quad (1.2)$$

where t_0 is the time the analyte spends in the mobile phase (holdup time) as depicted in **Fig. 1.1**. Typically, k′ values between 2 and 5 represent a good balance between analysis time and resolution; however, k′ values between 1 and 10 are usually acceptable.

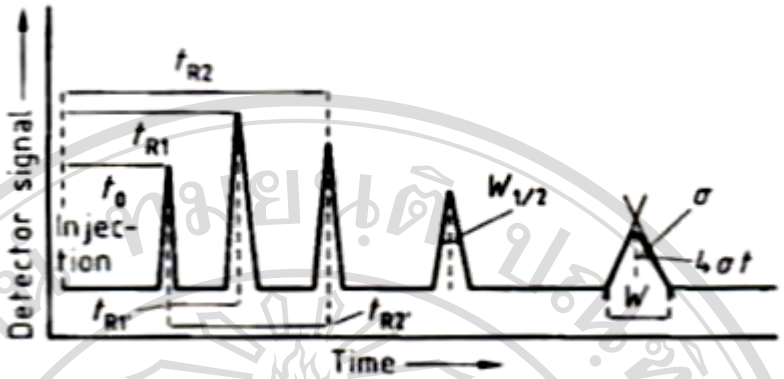


Fig. 1.1 Generalized chromatogram used to calculate chromatographic parameters [14].

2) Resolution

The resolution (R_S) of a column refers to its ability to separate two components of a sample as expressed in equation 1.3.

$$R_S = \frac{2(t_{R_2} - t_{R_1})}{(W_1 + W_2)} \qquad (1.3)$$

where W_1 and W_2 are the peak widths of components 1 and 2, respectively. A resolution of 1.5 (baseline resolution) is ideal for quantification of particular sample peaks. Yet even with a resolution of 1.0, quantification is possible because only 2% of the peak overlapping. Chromatograms with resolutions of less than 1.0 should not be used for quantitative analysis. On the other hand, an $R_s > 1.5$ is not necessary, due to the long analysis time.

In liquid chromatography (LC), factors affecting the resolution can be expressed in equation 1.4 because it combines the chromatographic parameters, i.e. separation factor (α), capacity factor (k′) and plate number (N). The effect of these parameters on resolution is illustrated in **Fig. 1.2**. The most effective way to alter resolution is to change the separation factor and the capacity factor of the column. The effect of increasing the efficiency of the column by increasing the column length or flow rate velocity is less significant, as resolution increases proportionally as the square root of the plate number. Thus, doubling the plate number by adding a second column increases resolution by only a factor of 1.4. If increased resolution is required, a column with a higher capacity factor is often choice. However, increasing the capacity factor will increase the analysis time, so a compromise must be reached between resolution and analysis time.

$$R_S = \frac{\sqrt{N}}{4}\left(\frac{k'}{k'+1}\right)\left(\frac{\alpha-1}{\alpha}\right) \qquad (1.4)$$

where $$\alpha = \frac{t_{R_2} - t_0}{t_{R_1} - t_0} = \frac{k'_2}{k'_1} \quad ; k'_2 > k'_1 \qquad (1.5)$$

The value for α can range from unity (1), when the retention times of two components are identical ($t_2 = t_1$), to infinity if the first component of interest is eluted in the avoid volume. If α = 1, no separation is possible, no matter how high the separation efficiency may be. The most powerful approach to increase α is to change the composition of the mobile phase.

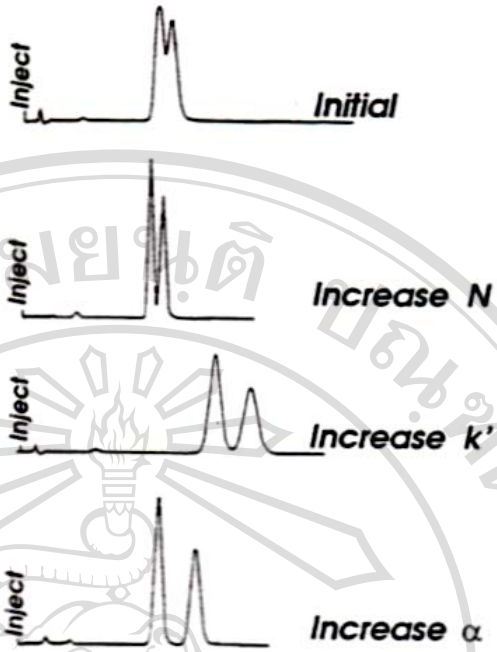


Fig. 1.2 Effect of separation factor, capacity factor and efficiency on resolution [15].

3) Column efficiency

The column efficiency is described in terms of the number of theoretical plates (N) that can be calculated from the following equations.

$$N = 16\left(\frac{t_R}{W}\right)^2 \qquad (1.6)$$

or

$$N = 5.545\left(\frac{t_R}{W_{1/2}}\right)^2 \qquad (1.7)$$

where W and $W_{1/2}$ are peak widths at its base and half-height, respectively. A large plate number system is highly efficient. In order to obtain a large plate number, the height equivalent to a theoretical plate (HETP) or plate height (H) should be the lowest value. These terms are related as follows:

$$H = \frac{L}{N} \tag{1.8}$$

where L is the length of the column. Thus, the smaller the height equivalent to a theoretical plate (HETP) or plate height (H), the greater is the efficiency of the column. In general, the H value is smaller for small stationary phase particle sizes, low mobile phase flow rates, less viscous mobile phases, higher separation temperatures and smaller solute molecule sizes.

1.2.2 Instrumentation

The basic components of a HPLC system are shown schematically in **Fig. 1.3**. The instrument consists of solvent reservoir to contain the mobile phase, pump to move the eluent and sample through the system, injection port to allow sample introduction, column to provide solute separation, detector to visualize the separated components and data processing unit to assist in interpretation and storage of results.

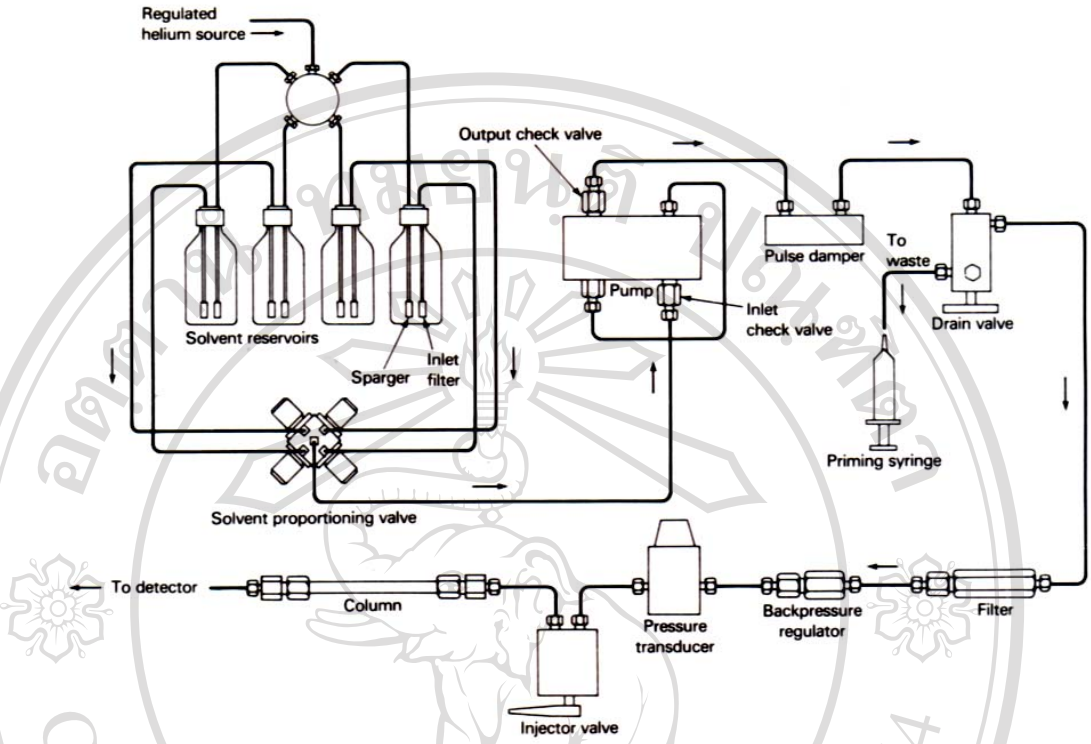


Fig. 1.3 Schematic diagram of HPLC system [16].

1.2.2.1 Mobile phase [14, 15, 18]

Retention in reversed-phase chromatography is a function of sample hydrophobicity whereas selectivity arises from the combined action of mobile phase and stationary phase on the solutes. A suitable solvent will preferably have low viscosity, be able to completely dissolve the sample without reacting with analytes and be compatible with the detection system. The mobile phase used in reversed-phase chromatography are based on a polar solvent, typically water, to which a less polar solvent such as acetonitrile or methanol is added. Properties of some mobile phases used in LC are illustrated in **Table 1.3**.

Table 1.3 Properties of some mobile phases in LC [14]

Solvent	UV cut-off (nm)	Polarity Index (P')	Solvent strength (ε^0)	Viscosity at 25°C (cP)
Heptane	195	0.2	0.01	0.40
Hexane	190	0.1	0.01	0.30
Pentane	195	0.0	0.00	0.22
Cyclohexane	200	-0.2	0.04	0.90
Toluene	285	2.4	0.29	0.55
Ethyl ether	218	2.8	0.38	0.24
Benzene	280	2.7	0.52	0.60
Methylene chloride	233	3.1	0.42	0.41
1,2-Dichloroethane	228	3.5	0.44	0.78
Butanol	210	3.9	0.70	2.60
Propanol	240	4.0	0.82	1.90
Tetrahydrofuran	212	4.0	0.57	0.46
Ethylacetate	256	4.4	0.58	0.43
Isopropanol	205	3.9	0.82	1.90
Chloroform	245	4.1	0.40	0.53
Dioxane	215	4.8	0.56	1.20
Acetone	330	5.1	0.56	0.30
Ethanol	210	4.3	0.88	1.08
Acetonitrile	190	5.8	0.65	0.34
Methanol	205	5.1	0.95	0.54
Nitromethane	380	6.0	0.64	0.61
Water	<200	10.2	Large	0.89

1.2.2.2 Stationary phase [14, 15, 19]

Reversed-phase stationary phases appear to be the most generally useful of the stationary liquid phases. The most common stationary phase in reversed-phase chromatography are those in which a functional group is chemically attached to a silica support (bonded phases). The most popular bonded phases are the alkyl groups, such as $–CH_3$, $-C_4H_9$, $-C_8H_{17}$ and $–C_{18}H_{37}$, phenyl (C_6H_5) groups, cyano [$(-CH_2)_3CN$] groups and amino [$(-CH_2)_3NH_2$] groups, with retention increasing exponentially with chain length. These packing materials, with some restrictions on the pH of the eluent used, provide good hydrolytic stability and are resistant to solvent stripping within normal column operating pressures.

Recently, chemically bonded phases are widely used in reversed-phase HPLC. They are usually made by derivatization of the silanol groups of silica. It is not possible for all the silanol groups on the silica surface to react with the functional groups, and usually only about 45% of the silanols will be bonded. Remaining unreacted acidic silanol groups can cause tailing of basic solutes such as amines. Thus, unreacted silanols are often removed by treatment with a small silating agent such as trimetylchlorosilane [$Si(CH_3)_3Cl$], a process known as end-capping. The functional group affect both of column selectivity and efficiency. The nature of the functional group controls selectivity while the chain length controls column efficiency. A list of commonly used bonded phases is given in **Table 1.4**.

Table 1.4 Commonly used bonded phases [19]

Functional group	Structure	Principle use
Octadecyl	$-(CH_2)_{17}-CH_3$	Reversed-phase
Octyl	$-(CH_2)_7-CH_3$	Reversed-phase
Propyl	$-C_3H_7$	Reversed-phase
Phenyl	$-C_6H_5$	Reversed-phase
Aminoalkyl	$-(CH_2)_n-NH_2$	Normal and reversed-phase
Cyanopropyl	$-(CH_2)_3-CN$	Normal and reversed-phase
Diol	$-CH(OH)-CH_2(OH)$	Normal-phase
Sulfonic acid	$-(CH_2)_n-SO_3H$	Strong cation exchanger
Quaternary amine	$-(CH_2)_n-N^+(CH_3)_3$	Strong anion exchanger

1.2.2.3 Detectors for HPLC [15, 18, 19]

The purpose of the detector in an HPLC system is to detect the compounds of interest in the eluent from the HPLC column. Detectors are classified as selective or universal depending on the property measurement as shown in **Table 1.5**. Selective (solute property) detectors such as ultraviolet (UV)-visible absorption detectors measure a physical or chemical property that is characteristic of the solute(s) in the mixture. Universal (bulk property) detectors such as refractive index (RI) detectors measure a physical property of the eluent. All solutes which possess a refractive index different from that of the eluent will be detected. Selective detectors tend to be more sensitive than universal detectors and they are more widely used. The choice of detector is often dictated by the chemical characteristics of the analyte species and this choice may subsequently determine which eluent is used and also possibly which stationary phase and mode of chromatography. The detector response will be related to the amount of the analyte in the column effluent though different

analytes will respond to differing extents and hence the detector must be calibrated with respect to each of the analytical species of interest.

The ideal HPLC detector should have the following characteristics [18].

- high sensitivity
- good stability and reproducibility
- linear response over several orders of magnitude
- small internal volume minimizing zone broadening
- a short response time independent of flow rate
- insensitive to changes in temperature and pressure
- high reliability and ease to use
- similar response to analytes or selective response to analyte classes

Table 1.5 Characteristics of chromatographic detectors [18]

Detector	Type	Gradient possible	Detection limit ($g\ ml^{-1}$)	Linear range
UV-VIS	selective	yes	5×10^{-10}	10^4-10^5
Photo-diode	selective	yes	$>2 \times 10^{-10}$	10^4-10^5
Fluorescence	selective	yes	$\sim 10^{-12}$	10^3-10^4
Refractive index (RI)	universal	no	5×10^{-7}	10^3-10^4
Infrared (IR)	selective	yes	10^{-6}	$\sim 10^3$
Conductometric	selective	no	10^{-8}	10^3-10^4
Amperometric	selective	no	10^{-12}	10^4-10^5
Mass spectrometry (MS)	universal	yes	10^{-10}	10^4

UV-VIS detector [14, 15, 20]

The UV-VIS detector is the most frequently used detector in HPLC. It is nondestructive and responds only to substances that absorb radiation at the wavelength of the light source.

With direct UV detection the eluent exhibits little or no absorbance at the wavelength to be monitored. When a solute is exposed to UV radiation, the radiation is absorbed by particular electronic configurations of the compound. The wavelength and the intensity of absorption depend on the presence chromophoric groups in the molecules. The common chromophores in organic compounds are unsaturated functional groups such as carbonyl, nitrile, aromatic and olefinic. These compounds absorb in the UV region (190 – 350 nm) and the intensity of absorption increases if these groups are conjugated. Colored compounds with highly conjugated chromophores and which contain easily excitable electrons, absorb visible light (350 - 700 nm). The intensity of absorption is proportional to the concentration of the analyte in the mobile phase according to Lambert-Beer law which is defined as follows:

$$A = \log\left(I_0 / I\right) = \varepsilon l C \qquad (1.9)$$

where A is absorbance, ε the molar extinction coefficient, l the optical path length, C the concentration of analyte in the mobile phase, I_0 and I the intensity of the light beam passing through the detector cell without and with the sample, respectively.

If a solute of interest does not contain a chromophore, it may be detected by indirect UV detection. Indirect detection is a technically simple and sensitive method for detection of compounds with little inherent detector response [14, 15]. In indirect UV detection a chromophore is added to the mobile phase so a continuous, positive baseline signal is generated. If an analyte having no chromophore passes the detector cell, the absorption of the mobile phase is decreased and a negative peak is recorded. The advantage of this method is that analytes without chromophores can be detected. Whereas the disadvantage is its relatively poor detection limit (between 0.1 and 1 ppm).

Mass spectrometric detector [15, 21]

The mass spectrometer has the potential to serve as the universal detector for the HPLC. It can be used to confirm the identity of a compound and will provide sufficient data to determine the structure of an unknown. A fundamental problem in coupling LC with mass spectrometry is large solvent volumes from HPLC mobile phase. Several interfaces or coupling methods (**Fig. 1.4**) have been developed for solving this problem such as moving belt, thermospray (TSP), electrospray (ES), etc.

Moving belt coupling or the particle beam (PB) interfaces are based on the selective vaporization of the elution solvent before entering the spectrometer source. Thermospray (TSP), ionspray (ISP) or the atmospheric pressure chemical ionization (APCI) can tolerate flow rates of about 1 ml min^{-1} without requiring a flow split while electrospray (ES) can be used at flow rates from 1 nl min^{-1} levels to 0.2 ml min^{-1}.

Electrospray is produced by applying a strong electric field, under atmospheric pressure, to a liquid passing through a capillary tube with a weak flux (normally 1-10 μl min^{-1}). The electric field is obtained by applying a potential difference of 3-6 kV between this capillary and the counter electrode, separated by 0.3-2 cm as shown in **Fig. 1.5**. This field induces a charge accumulation at the liquid surface located at the end of the capillary, which will break to form highly charged droplets. The solvent contained by the droplets evaporates, which causes them to shrink to the point where the repelling coulombic forces come close to their cohesion force, thereby causing their explosion as depicted in **Fig. 1.6**. Gas-phase ions are produced and modified in the atmospheric and ion-sampling regions of the mass spectrometer.

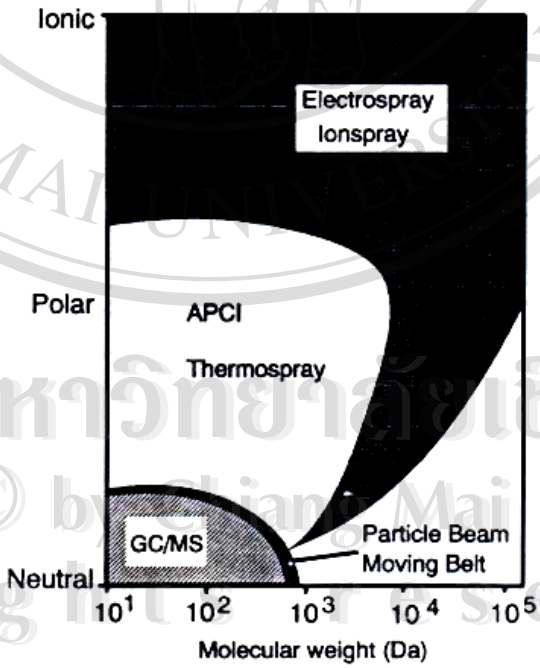


Fig. 1.4 Application range of different coupling methods [21].

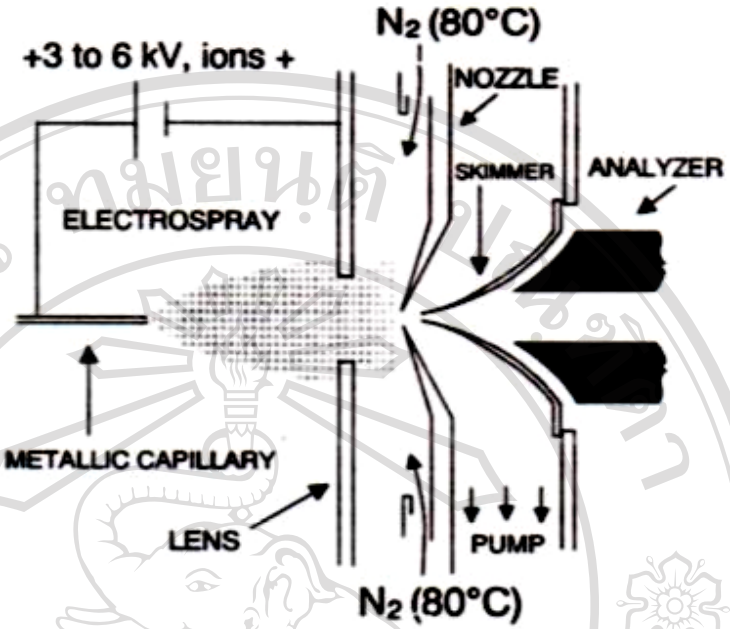


Fig. 1.5 Diagram of an electrospray source [21].

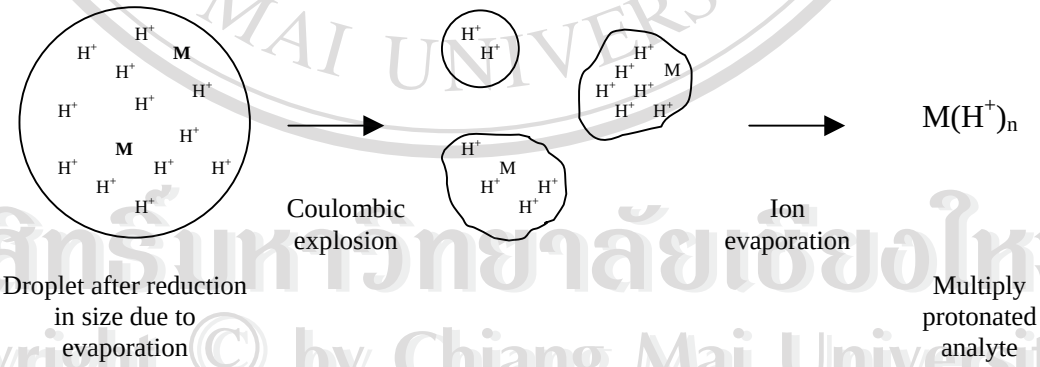


Fig. 1.6 Ionization of analyte (M) in electrospray interface [22].

1.3 Solid-Phase Extraction

1.3.1 Introduction

Sample preparation is often the most time-consuming step in a chemical analysis and the sample matrix frequently interferes with measurements. In the past, liquid-liquid extraction (LLE) has played a major role in sample clean-up and sample preconcentration. Although, LLE tends to be slow and labor-intensive and it also has to use the large amounts of organic solvent, the recovery of sample components by LLE is seldom complete. In the case of solid-phase extraction (SPE), it is easily automated, faster, and in general more efficient than LLE. The amounts of solvents used for SPE method are tremendously lower than LLE.

In SPE, solutes are extracted from a liquid to solid phase. The solid phase typically consists of small, porous particles of silica with a bonded organic phase or of an organic polymer, such as crosslinked polystyrene. The extraction can take place in a batch mode in which a solid extractant is intimately mixed with the liquid sample solution. In chemical analysis it is more common to pack the solid extractant into a small tube and pass the liquid sample through the tube (**Fig. 1.7**). Substances that have been extracted by the solid particles can be removed by washing with an appropriate solvent. For example, most organic analytes can be eluted from the cartridge with an organic solvent such as acetone, acetonitrile or methanol. Usually, the volume of solvent needed for complete elution of the analytes is much smaller than the original sample volume. Thus the concentrated analytes are achieved [23].

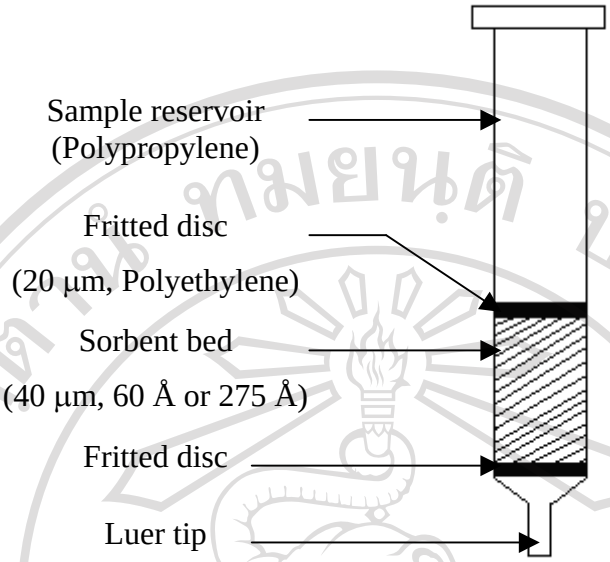
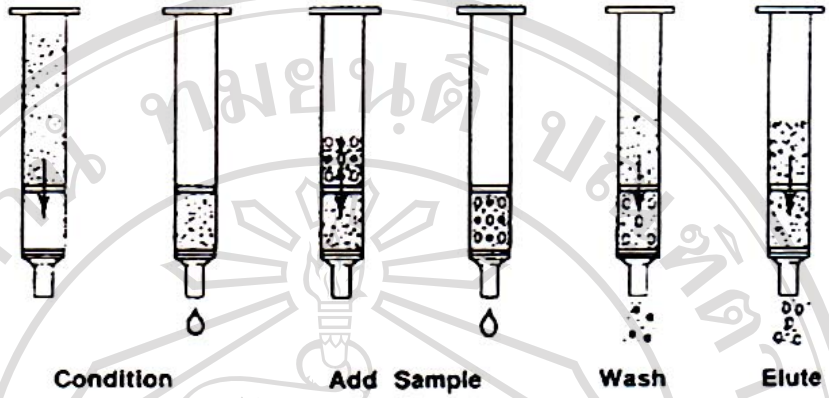


Fig. 1.7 Solid-phase extraction cartridge [23].

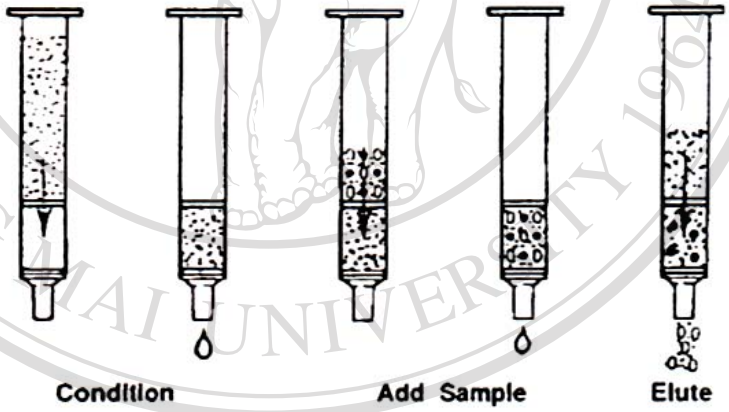
1.3.2 Steps of SPE [24]

The majority of SPE extractions are "retentive" since a sorbent retains the target analytes, while contaminants simply pass through the column to waste (**Fig. 1.8A**). On the other hand, in a "non-retentive" extraction, the sorbent has no affinity for the analytes, but a high affinity for the sample contaminants. As a result, the analyte passes directly through the column without being retained, while the contaminants are bound (**Fig. 1.8B**). Non-retentive extractions are particularly useful when the analyte is highly soluble in the sample/matrix and/or the dilution solvent and cannot be easily partitioned out of solution onto a solid or a liquid phase. They are also simpler, since there is no need for wash step.

A. Retentive



B. Non-retentive



Analyte

Contaminant

Fig 1.8 Schematic illustration of the retentive and non-retentive SPE mechanisms [24].

Sample preparation with SPE typically consists of four basic steps: conditioning, sample loading, washing and elution. Non-retentive extractions may only require two or three steps whereas retentive extractions typically consist of all four steps (**Fig. 1.8**). In each step, the sample or elution solvents are passed through the sorbent and collected as desired.

1.3.2.1 Conditioning step

Prior to loading the sample matrix, the SPE column is typically washed and wetted with methanol, isopropanol or another organic solvent(s) of intermediate polarity. This conditioning step removes trapped air and solvates or activates the ligands on the chromatographic surface, enabling them to interact more effectively with the analyte(s). This step often consists of two substeps, i.e. solvation step and equilibration step.

The equilibration step typically employs a solvent with a composition that is similar to the sample matrix in terms of the solvent ratio, ionic strength and pH. This solvent helps remove residual methanol remaining from the solvation step, and equilibrates the sorbent in a solvent that will maximize the interactions with the analyte(s) in order to promote retention. The solvation step is typically followed directly by the equilibration solvent in order to prevent the sorbent bed from drying out or losing solvation due to evaporation. In general, solvent volumes that are between 2 to 4 times the sorbent bed volume are typically necessary to ensure proper conditioning, washing and elution. Conditioning with less than 2 to 4 bed volumes increases the risk of incomplete solvation of the bed and low or irreproducible recoveries, while more than 4 bed volumes is typically unnecessary.

1.3.2.2 Sample loading step

The major goal of the sample loading step is to ensure that the analyte(s) is quantitatively retained by the sorbent. As a result, it is imperative that the sample matrix composition is adjusted in a manner that facilitates the binding of the analyte(s). The second goal of the sample loading step is to adjust the solvent conditions in order to minimized the number of impurities that are bound. This will often enhance the capacity of the sorbent for the analyte(s) and improve purity.

The degree of separation achieved during the sample loading step and chromatographic specificity in general, are highly dependent upon the solvent composition of the sample matrix (and the conditioning solvent). Changes in the polarity, aqueous:organic composition, ionic strength and/or pH of the sample matrix and the conditioning solvent can have a dramatic effect on analyte(s) recovery.

1.3.2.3 Washing step

The washing step is desired to elute impurities that are retained on the sorbent less strongly than the analyte(s) and rinse residual sample matrix components that may remain from the sample loading step. Clearly, the washing step must contain a solvent of intermediate strength, not as weak as the loading solvent and not as strong as the elution solvent. Another restriction placed on the washing solvent is that it must be miscible with the diluted sample matrix as well as the elution solvent. Optimum volumes for the washing step are typically around 1 ml for every 100 mg of sorbent or about 7 sorbent bed volumes. This volume should be sufficient to provide the consistent and effective removal of the residual impurities and to elute some of the bound contaminants.

1.3.2.4 Elution step

Once the more weakly retained contaminants have been washed from the sample, a strong solvent designed to disrupt the analyte-sorbent interactions is used to selectivity elute the analyte(s) from the sorbent. In many cases, the elution solvent must contain a mixture of several different solvents and/or chemicals (acid, base, etc.) in order to effectively break the primary interactions as well as any secondary retention mechanisms that may also be responsible for analyte binding.

Because SPE takes advantages of the same physicochemical interactions utilized in classical liquid chromatography and HPLC, the recovery and purity of the analyte(s) can be optimizes by adjusting the composition of the solvents used during each of these steps (sample loading, washing and elution).

Sorbents for SPE are available with normal-phase, reversed-phase and ion exchange packings. Typical solvents and elution orders for SPE devices with three types of packing are given in **Table 1.6**.

1.3.3 Type of silica-bonded phases [25]

The classical adsorbents that have been used include carbon, florisil, alumina or celite, macroreticular polymers and ion-exchanger resins. However, a major advance in SPE was the introduction of silica-bonded phase. The silica-bonded phases used in solid phase extraction are similar to those used in liquid chromatography (LC), except that the mean particle sizes of the silica are different. SPE irregular particles with a particle diameter ranging between 30 and 60 μm is used whereas the silica for HPLC usually has a particle diameter of 3 to 10 μm. The silica-bonded phase are synthesized by the reaction of silica surface silanol groups with

mono-, di- and trihalo or alkoxy allyl derivatives to form siloxanes. A reaction with monofunctional silanes can only result in mono- or polymer layers (**Fig. 1.9**) depending on the reaction conditions. In the synthesis, 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 end-capping with trimethychlorosilane, are shown in **Fig. 1.10**.

Table 1.6 Modes and elution characteristics for SPE devices [15]

Characteristics	Normal-phase	Reversed-phase	Ion exchange
Sorbent polarity	High such as silica, forisil, alumina, cyano	Low such as C_{18}, C_8	High; positively or negatively charged such as SAX, SCX, WAX, WCX
Typical solvent polarity range	Low to medium	Medium to high	High
Typical sample loading solvent	Hexane, toluene	Water, buffers	Water, buffers
Typical elution solvent	Ethyl acetate, acetone, acetonitrile	Water/methanol, water/acetonitrile	Buffers, salt solution
Sample elution order	Least polar components first	Most polar components first	Weakly ionized components first
Solvent change required for elution	Increase solvent polarity	Decrease solvent polarity	Increase ionic strength, change pH

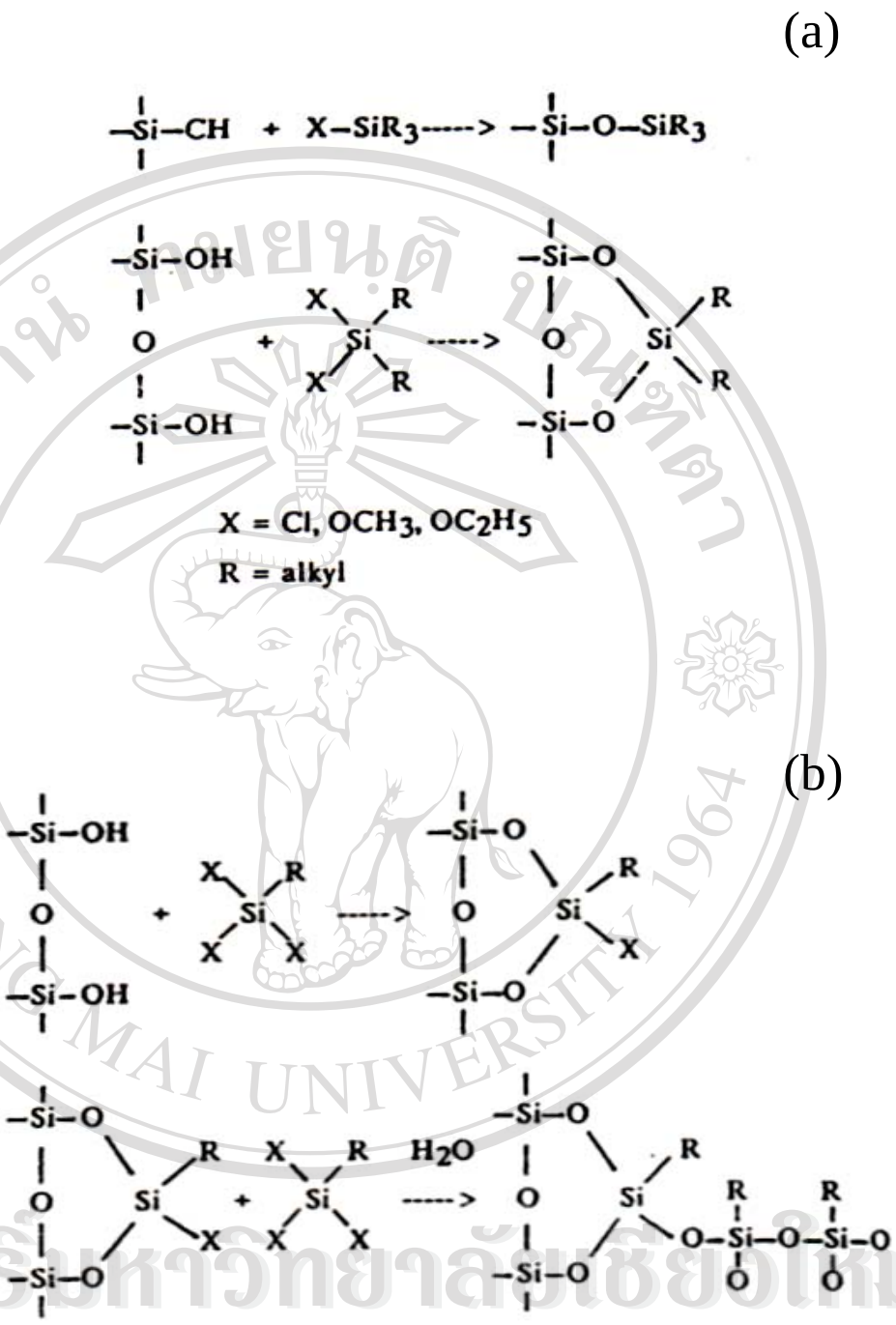


Fig. 1.9 Reaction of silica surface silanols with mono-, di- or trialkylchlorosilanes resulting in (a) monolayer and (b) polymer layers [25].

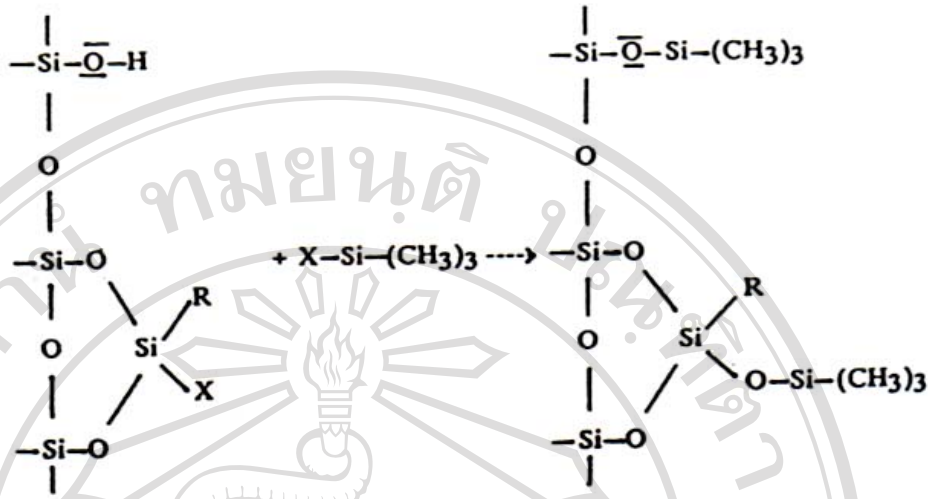


Fig. 1.10 End-capping of silanol groups with trimethylchlorosilane [25].

1.4 Methods for the Analysis of Anionic and Nonionic Surfactants

A number of analytical techniques have been used for determining surfactants in environmental samples. Chromatographic techniques like gas chromatography (GC), high performance liquid chromatography (HPLC) and capillary electrophoresis (CE) are widely used and are efficient separation methods for the analysis of surfactants matrix. Due to their low volatility, derivatization of these compounds is necessary when GC-based analytical methods are used.

The standard methylene blue method had long been used for measuring total amounts of sulfonate and sulfate based anionic surfactants in wastewater [26]. Although the method is sensitive, with a detection limit of 0.10 ppm, it is time and labor consuming and is often interfered by sample matrix. Substances such as organic sulfonates, sulfates, carboxylates, phenols, inorganic thiocyanates, cyanates, nitrates and chlorides may transfer more or less methylene blue into the chloroform phase and

result in positive interference. Negative interference can result from the presence of cationic surfactants and other cationic materials (e.g. amines) because they compete with the methylene blue in the formation of ion-pairs. The method also required a large quantity (more than 100 ml/sample) of the toxic solvent for extraction such as chloroform [27].

Surfactants are not sufficiently volatile to enable GC analysis without prior derivatization. For anionic surfactants, GC procedures require surfactants to be converted into volatile hydrocarbons through desulfonation or conversion into volatile compounds such as alkyl esters [28-30] or trifluoroethyl [31]. The use of ion-pair SPME to convert long chain fatty acids into their volatile methyl esters via in-injector derivatization was proposed by Pan and Pawliszyn [32]. The procedure of direct injection port derivatization with ion-pair reagents, also known as the pyrolysis of tetraalkylammonium (TAA) salt derivatization method, is rapid and simple alternative to conventional derivatization methods for sulfonic acids. A simple reaction of the tetrabutylammonium salt of sulfonates in the hot injection port of the GC to form the butyl esters has been used for quantitative determination of LAS [28-30]. Ding et al. [31] improved method for the determination of LAS residues in aqueous samples consisted of sequentially extracting LAS and their degradation products from the water samples by graphitized carbon black cartridges. The extract was then esterified by thionyl chloride ($SOCl_2$)-trifluoroethanol derivatization procedures. These procedures are generally time-consuming and need highly reactive reagents to convert sulfonic acids into their corresponding trifluoroethyl derivatives. For nonionic surfactants, the separation without derivatization is limited to the lower molecular weight oligomers of nonionic surfactants. Wahlberg et al. [33] demonstrated the

analysis of nonylphenol and nonylphenol ethoxylates as their pentafluorobenzoate derivatives. In this method, accurate and high sensitive analyses are possible but special apparatus is required. The higher ethoxylates of the alkylphenol ethoxylates (APE) can only be analyzed by GC after derivatization and performing at high temperature column [34]. In most cases, the analysis of anionic and nonionic surfactants by GC at trace level is limitted.

Capillary electrophoresis (CE) was increasingly applied for the analysis of ionic surfactants since 1991. The method is preferred to conventional chromatographic techniques in many applications because of its high efficiency, small sample volumes, lower solvent consumption than HPLC, short analysis time and the possibility of rapid development of the method. However, in many cases CE appears to lack sensitivity due to the short optical path associated with the on-column detection [35]. W. H. Ding and C. H. Liu [35] developed CE method with the large-volume sample stacking (LVSS) technique, one of the on-column concentration techniques, to improve its detection sensitivity for determining LAS. This method offers a reliable, sensitive and convenient analytical technique for determining LAS in commercial product samples. Nonionic surfactants are only infrequently by CE. The electrophoretic separation of neutral molecules requires the application of an ionic additive to form a pseudophase, which sodium dodecyl sulfate (SDS) is commonly used [36]. K. Heinig et al. [37] studied the separation of LAS using phosphate buffer at pH 6.8 with acetonitrile as organic modifier. The analysis of the non-absorbing alkyl sulfonates and alkyl sulfates using CE with indirect UV detection was also performed using absorbing organic buffer, i.e. salicylate buffer. In addition, CE analysis of nonionic surfactants in the groups of nonylphenol polyethoxylates using

SDS to provide a charged pseudophase and high content of organic solvent, i.e., acetonitrile was investigated. They can conclude that the high efficiency of CE in the separation of ionic surfactants could not be achieved for nonionic surfactants because of the complexity and lack of charge.

High performance liquid chromatography (HPLC) is currently the most suitable method for the determination of surfactants. The major advantages of this analytical method are its ability to separate various homologues of surfactant mixtures and the low detection limits obtained for both charged and uncharged species [38]. The HPLC separation with UV detection is the most common method in practice for the determination of surfactants in environmental samples. However, the other chromatographic detectors have been used, e.g. conductometry, fluorescence and mass spectrometry (MS).

Since LAS are anionic surfactants of major use, recent developments for the separation of LAS homologues and their isomers by HPLC have been reported. HPLC methods equipped with UV detector required the mobile phase containing either sodium chloride [39] or sodium perchlorate [40, 41] under isocratic elution and either tetraethylammonium hydrogensulfate (TEA-HSO_4) [42] or additive mixture such as triethylamine and acetic acid [43] under gradient conditions in order to resolve LAS homologues. The analysis time was carried out within 16 min when using mobile phase containing sodium chloride or sodium perchlorate, whereas the analysis times obtained using tetraethylammonium hydrogensulfate and the mixture of triethylamine and acetic acid were about 60 min and 25 min, respectively. The detection limit of LAS was closely to 0.1-0.2 ppb.

HPLC with fluorescence detection has been proved to be a successful method for the determination of LAS in water and marine samples because fluorescence detection is very specific for LAS as compared with HPLC-UV [40]. Conductivity detection has been used for determining alkyl sulfates and sulfonates surfactants, which do not possess UV absorbing properties [27, 44]. Levine et al. [27] studied the determination of anionic surfactants in wastewater by ion-pairing chromatography with suppressed conductivity detection. This method has proved specific and sufficiently sensitive for quantitative determination of sulfated and sulfonated anionic surfactants. Ion suppression method offers lower detection limits (0.8 ppb for SDS) because the suppressor reduced the mobile phase conductivity while increasing the analyte signal [44]. The disadvantage of the suppressor is that it requires an additional pump and regenerant solution for the continuous regeneration of the suppressor.

For confirmation purposes, mass spectrometry (MS) utilizing various ionization techniques such as electrospray (ES) and ionspray have been applied. Riu et al. [43] determined LAS in influent and effluent water samples from sewage treatment plants using LC-ES-MS. The method enabled unequivocal identification of LAS by monitoring the common ion at *m/z* 183 and also used for the trace determination of LAS. González-Mazo et al. [45] monitored LAS in marine environment using LC-MS with ionspray interface. The deprotonated molecules were observed when using ionspray interface in negative ion mode and applying a low extraction cone voltage of 20 V, corresponding to the four C_{10}-C_{13} LAS compounds present in the commercial mixture, the ions with *m/z* 297, *m/z* 311, *m/z* 325 and *m/z* 339 were found. Moreover an extraction cone voltage of 60 V resulted in the formation of the specific fragment ion with *m/z* 183.

Separations of ethoxylated nonionic surfactants have been attempted by HPLC with both normal-phase [13] and reversed-phase [46, 47]. In normal-phase HPLC, the ethoxylated oligomers are separated according to increasing number of ethylene oxide units. However, normal-phase HPLC has the drawback that alkyl chain homologues with common degrees of ethoxylation cannot be separated. In several studies, Triton X-100 has been analyzed by reversed-phase HPLC. For UV detection, at higher concentration of Triton X-100 (> 10 ppm), absorbance at 280 nm is employed for quantification, while at lower levels down to 1 ppm, absorbance at 200-230 nm is used to obtain a stronger response [47]. The other detection methods were also used for detected nonionic surfactants such as fluorescence [48] and MS [49]. Petrovic et al. [49] investigated the determination of alkylphenol ethoxylates (APE) in water samples using LC-MS. In this method, the determination was performed in the formation of halogenated derivatives of APE. Due to nonionic surfactants are neutral molecules, they are difficult ionized to ion species which detected in MS detectors. Consequently, methods based on liquid chromatography are also used routinely and usually employ either UV or fluorescence detection for the analysis of these surfactants.

For environmental samples, the low concentrations in which the anionic and nonionic surfactants are generally found. Therefore, it is necessary to perform an initial stage of concentration and purification of the analyte prior to its analysis [50]. The choice of the suitable extraction/enrichment technique for the recovery of surfactants in trace level from environmental samples must take into account the sensitivity, selectivity and separation capabilities of the selected analytical method [38]. The various techniques have been used such as solid-phase extraction (SPE) [41,

42, 45], Soxhlet extraction [3, 41] and solid-phase microextraction (SPME) [13, 48]. Soxhlet extraction using the large volume or organic solvents and time-consuming for the complete extraction of analyte [3]. HPLC-SPME technique eliminates the separate concentration step from SPE method because the analytes diffuse directly into the coating of SPME device and are concentrated [13]. The device is then transferred to the injection port of the HPLC, via a specially designed interface, where all the analytes are desorbed in the eluent stream and deposited at the head of the HPLC column. Among the various procedures used, SPE method is widely used because it has been shown to be a very powerful and robust alternative to the traditional methods of extraction of organic compounds from various matrices [38]. Moreover, SPE offers the advantage of immediate analyte enrichment, thus reducing analysis time and the amount of necessary solvents significantly [34].

1.5 The Scope and Aims of This Research

Linear alkylbenzene sulfonates (LAS) and sodium dodecyl sulfate (SDS), anionic surfactants, and Triton X-100, nonionic surfactant were selected to be analyzed in this work. The main objective of this research is the development of HPLC methods, particularly in respect to short analysis time and efficient analysis of these surfactants. Solid-phase extraction (SPE) was used to preconcentrate and purify these surfactants in water samples prior to HPLC-UV analysis.

The aims of this research work can be summarized as follows:

1. To optimize the HPLC-UV conditions for the analysis of LAS, SDS and Triton X-100.
2. To investigate SPE method for sample pretreatment.
3. To apply the developed methods for the determination of LAS, SDS and Triton X-100 in various water samples.