Chapter

Sample Preparation Techniques for Gas Chromatography

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Abstract

In gas chromatography (GC), the sample is vaporized and injected onto the head of a chromatographic column. Elution is brought about by the flow of an inert gaseous mobile phase such as helium, argon, nitrogen, carbon dioxide, and hydrogen. In GC, the mobile phase does not interact with molecules of the analyte, and it only transports the analyte through the column. In two general kinds of GC, *gas-solid* chromatography (GSC) and *gas-liquid* chromatography (GLC), the mechanisms of analyte retention in the column are thoroughly different. In GLC, the analyte has been participated between a gaseous mobile phase and a liquid stationary phase. While in GSC, the retention of analytes is the consequence of its physical adsorption onto a solid stationary phase. In comparing of GLC and GSC, more widespread use of GLC has been found in all fields of science. This is mainly due to the semipermanent retention of active or polar molecules and the severe tailing of elution peaks, which is a consequence of the nonlinear character of adsorption process, in GSC. In GC, column efficiency requires that sample be of suitable size and be introduced as a plug of vapor. So, the sample preparation is a very important step in GC. The sample should be injected into a flash vaporizer port located at the head of the column, and its temperature is about 50°C above the boiling point of the least volatile component of the sample. So, the components of the sample should be easily vaporized in this temperature, and they should have high heat resistance not to be decomposed. Both of liquid and solid samples can be introduced to the column. But solid samples are ordinarily introduced as solutions or sealed into thin-walled vials that can be inserted at the head of the column and punctured or crushed from the outside. In order to separate and analyze the gaseous, liquid, and volatile solid samples directly, GC is a suitable analytical equipment. When the analyte sample is nonvolatile, the derivatization and pyrolysis GC techniques are crucial. Gas chromatography can be applied to the solution of many problems in various fields such as drugs and pharmaceuticals, environmental studies like air and clinical samples, petroleum industry, pesticides and their residues, and foods. On the other hand, most samples are not ready for direct introduction into instruments. For organics and volatile organics, the sample preparation procedures can be named as extraction, cleanup, derivatization, transfer to vapor phase, and concentration. So, the basic concept of a sample preparation method is to convert a real matrix into a sample in a format that is suitable for analysis by a separation or other analytical techniques. The goals of sample treatment step are as follows: (1) The capability of using smaller amounts of initial sample, especially for trace analysis. (2) Achieving higher specificity and selectivity in analytical determinations. (3) To improve the potential for automation or online methods and minimize the manual operations. (4) The usage of no or small volumes of organic solvents in order to approach the green chemistry techniques with less wastes and more friendly environment. On the other hand,

different samples possess a variety of sample treatment methods, for example: (1) In order to treat solid samples and separate a purpose analyte, some enhanced solvent extraction methods include pressurized liquid extraction, microwave- and sonic wave-assisted extraction, supercritical fluid extraction, and superheated water extraction. (2) For analytes in solution, the sample preparation can be attributed to the analyte trapping methods such as -phase extraction, solid-phase microextraction, and stir bar extractions. (3) Also, the extraction of the analytes into a liquid phase can be achieved by other methods like membrane extraction, single-drop microextraction (SDME), and purge and trap. (4) For separation of analytes in the gas phase, trapping analytes from vapor samples and headspace analysis are used. As a result, sample preparation is not only a critical step but also possesses different ways to treat and convert matrix into a suitable sample to inject GC.

Keywords: sample treatment, green chemistry, extraction methods, solid-phase extraction, cleanup

1. Introduction

Most samples are not ready to introduce directly into the column of gas chromatography (GC) instrument [1–7]. So, the sample preparation is the most important step prior to GC determination of an analyte. There might be several processes within sample preparation which depend on the complexity of the sample; the analyte concentration level in the sample and its level need to be analyzed by the GC instrument. On the other hand, sample preparation is often a severe process that accounts for the complexity of the analyte analysis. Instance for organics and volatile organics, the sample preparation procedures can be mentioned such as extraction, cleanup, derivatization, transfer to vapor phase, and concentration.

Before analyzing a sample by GC, the sample preparation procedure should be reviewed to some important constraints such as accuracy, precision, cost, the amount of available laboratories, the analysis time consumption, and the possibility of method automation. Since analytical instruments, like GC, have become quite sophisticated and then provide high levels of accuracy and precision, sample preparation step has been accounted for the majority of the variability. For instance, the sample preparation might involve several discrete steps and also manual handling and take some days, whereas the GC analysis can be performed in a matter of minutes. Therefore, typically two-thirds of the time in GC analysis can be spent on sample preparation. It is worth noting that better improvement in the GC analysis can be brought by significantly simpler sample preparation processes. Some suitable approaches to reducing uncertainty during sample preparation are minimizing the number of steps and using appropriate techniques. On the other hand, the greater the number of steps, the more error there are. So, if it is possible, one or more sample preparation steps should be eliminated. Also, the choice of an appropriate method for sample preparation can improve precision.

The goal should be to choose a combination of sample preparation and GC instrumentation to reduce both of the number of sample preparative steps and relative standard deviation (RSD) and/or increasing precision. Sample preparation step can affect some other quantitative statistical parameters such as limit of detection (LOD), limit of quantitation (LOQ), limit of linearity (LOL), and linear dynamic range (*LDR*). Limit of detection (LOD) is defined as the lowest concentration or weight of analyte which can be determined at a specific confidence level. The lowest concentration level at which a measurement is quantitatively meaningful is called limit of quantitation (LOQ). For all practical purposes,

the upper limit of quantitation is the point where the calibration curve becomes nonlinear. This point is called the limit of linearity (LOL). The range of analyte concentration which possesses linearity toward instrumental signal is called linear dynamic range (LDR). Considering all these, the recovery in sample preparation method is an essential parameter which affects quantitative issues such as detection limit, sensitivity, LOQ, and LOL. The sample preparation methods which enhance performance result in larger recovery, higher sensitivity, and lower detection limits. Also, other important parameters in choosing an appropriate sample preparation method include higher speed procedures or use of online methods, low cost, and less reagent consumption or use of greener sample preparation methods.

Before a new sample preparation procedure is used, it must be validated. The different figures of merit should be determined during the validation process. A typical validation process includes the following steps:

1. Determination of the random and systematic errors in terms of precision and bias.

2. Determination of the detection limit for each analyte in the sample.

3. Determination of the accuracy and precision at the concentration range where the GC method is used.

4. Measurement of the linear dynamic range and the calibration sensitivity.

Generally, method validation provides not only a comprehensive picture of merits of a new sample preparation method but also a useful comparison with other existing methods [2].

As mentioned above, the main concept of a sample preparation method is to convert a real matrix into a sample format which is suitable for analysis by a separation or other analytical methods. This can be approached by using a wide range of techniques that have a common list of aims such as [3]:

1. The removal of serious interferences from the sample in order to increase the selectivity of the both separation and detection stages.

2. The increase of analyte concentration and sensitivity.

3. To convert the analyte into a more suitable form to detect, determine, and/or separate.

4. To apply more reproducible techniques which do not depend on the variations of the sample matrix.

Although some traditional sample preparation techniques are still in use, the trends in recent years have been toward to [3]:

1. Using smaller initial sample sizes even for trace analyses.

2. Achieving higher specificity and selectivity.

3. To reduce manual operations and to improve potential for automation or online techniques.

4. To approach to a more environmentally friendly methods (green chemistry) with less or no use of organic solvents and less waste production.

On the other hand, different samples possess a variety of sample treatment methods. Therefore, in this chapter, by paying attention to the type of sample matrix, information required (quantitative or qualitative), and sensitivity required, the sample preparation methods used before GC analyses are discussed.

2. Extraction techniques

The earliest sample preparation method is extraction, in which the analyte of interest is separated from a sample matrix with an optimum yield and selectivity. Two major kinds of extraction include solid-phase extraction (SPE) and liquid-liquid extraction (LLE). In SPE, the analyte can be separated from a solid sample, and in LLE, it is extracted from sample solutions [7]. The solvents, in which the analyte is extracted, may be organic liquids, supercritical fluids, and superheated liquids [3]. Also, the extractor solvent may be bonded to a polymeric support, as in membrane extractions [4]. By optimizing the extraction conditions such as temperature, pressure, and pH of the solution and also appropriately using additives and reagents, the selectivity and yield of the extraction process will be improved.

The basic purpose of all extraction methods is to concentrate the analyte selectively in one phase. Each analyte is distributed between two phases according to the distribution constant, temperature, and relative volumes of the phases. In many of these methods, there is a conflict between the analytes of interest and the other soluble interferences to be quite extracted into the extractor phase. Exhaustive extraction techniques, like Soxhlet extractions, are often designed to provide thorough extractions regardless of the matrix. So, this kind of extraction can be applied to a range of samples such as different soil types but limits selectivity [3].

In order to enhance the selectivity of the extraction process, the supercritical fluid extraction (SFE) was introduced. In comparison with organic solvents, the carbon dioxide solvent is a weaker eluent and more selective extractor medium.

2.1 *Solid-phase* extraction (SPE)

In general, SPE involves four steps:

1. Column preparation or prewash step.

2. Sample loading or the retention of the analytes of interest on the cartridge and/or sorbent.

3. Column postwash to remove undesirable contaminants. In reality, the compounds of interest are retained on the sorbent, while interferences are washed away.

4. Analyte desorption from the cartridge. The adsorbed analytes are recovered by an appropriate eluting solvent.

SPE sorbents are commercially available in three formats:

1. Cartridge.

2. Columns fashioned like syringe barrels.

3. Disks.

Also, sorbent phases can be purchased, and typical column housings are manufactured of polypropylene or glass. In order to contain the column with the sorbent phase, porous frits made of polyethylene, stainless steel, or Teflon can be used [2].

There are some examples for applying SPE as sample preparation step before GC detection of different analytes in a variety of samples. Some of them are pointed below.

Lee et al. reported the determination of endocrine-disrupting phenols, acidic pharmaceuticals, and personal care products in sewage by solid-phase extraction and gas chromatography-mass spectrometry [8]. In this work, an anion exchanger was used as a solid-phase extractor, and a multiresidue method was developed and optimized for the extraction of 21 phenols and acids in sewage. The phenols and acids were then selectively eluted in separate fractions and were converted into volatile derivatives, by suitable reagents, for GC-MS determination.

Stajnbaher et al. studied a multiresidue method for determination of 90 pesticides in fresh fruits and vegetables using solid-phase extraction and gas chromatography-mass spectrometry [9]. In this study, a SPE on a highly cross-linked "*poly(styrene-co-divinylbenzene)*" column was used for cleanup and preconcentration of the pesticides from the water-diluted acetone extracts, and then the pesticides were determined by GC-MS.

2.2 Solid-phase microextraction (SPME)

Miniaturization of analytical processes into microchip platforms designed for micro total analytical systems is a new and rapidly developing field. Solid-phase microextraction (SPME) is a modern technique that consists in direct extraction of the analytes with the use of a small-diameter fused silica fiber coated with an adequate polymeric stationary phase [10]. On the other hand, in two designs of SPME, a thin layer of sorbent is coated on the outer surface of fibers (fiber design), and the inner surface of a capillary tube (in-tube design) is covered. The fiber design can be used as an interface in both GC and HPLC, but in-tube design has been just applied as an easier approaching interface with HPLC. In fiber design, a thin film of liquid polymer or mixture of a solid sorbent with a liquid polymer has covered on the surface of a fused silica core fiber.

The properties of extraction process, by SPME, are as follows [2]:

1. By SPME, samples are analyzed after equilibrium is reached or at a specified time prior to achieving equilibrium.

2. Exhaustive extraction of analyte from the sample matrix is not achieved by SPME.

3. So, SPME operationally encompasses non-exhaustive, equilibrium and preequilibrium, batch, and flow-through microextraction techniques.

4. SPME is directly applicable for field applications in air and water sampling.

5. It does not require continuation of extraction by SPME until equilibrium is reached.

6. A quantitative extraction may be obtained by careful control of time and temperature.

7. SPME is a solventless sample preparation procedure.

8. SPME is compatible with chromatographic analytical systems, and the process is easily automated [11].

9. In conventional SPE, the analyte can be extracted exhaustively (>90%) into the solid phase from a sample medium, while small amount of sample (1–2%) has been introduced into the analytical equipment. But in SPME, although the analyte extraction is non-exhaustive and its small portion has been extracted into the solid phase (about 2–20%), all sample can be injected into the analytical instrument. So, besides high concentration ability and selectivity, SPME possesses another advantage in the ability of using trace analyses [12].

10. SPME facilitates unique investigations, such as extraction from very small samples (i.e., single cells).

11. In SPME, changes in the sample matrix may affect quantitative results (disadvantage).

12. SPME can be used to extract semivolatile organics from environmental waters and biological matrices as long as the sample is relatively clean. Since extraction of semivolatile organics by SPME from dirty matrices is difficult, one strategy for doing it is to heat the sample to drive the compound into the sample headspace for SPME sampling [13].

SPME can be conducted in three modes [2]:

1. Direct extraction, in which the coated fiber is immersed in the aqueous sample.

2. Headspace configuration, for sampling air or the volatiles from the headspace above an aqueous sample. However, headspace techniques are more applicable to volatile organics than to the semivolatile organic compounds.

3. Membrane protection configuration, in which the coated fiber is protected with a membrane, for analyzing the analytes in too much dirty samples.

The SPME procedure is performed through two separate steps:

a. At first, the solid sorbent is immersed into the sample medium for a specific period of time. This step is used for both of fiber and in-tube designs.

b. Then, the solid sorbent, either fiber or in-tube design, is interfaced with GC and HPLC (or capillary electrophoresis) instruments for thermal and solvent desorption processes, respectively.

As discussed before, SPME has been introduced as a solventless extraction method, in which a fused silica fiber has been coated with a *thin* film of sorbent, to separate volatile analytes of interest from a matrix sample. Usually, the fiber is placed into a syringe needle which is protected for easy penetration into the sample and GC vial septa. Analyte extraction and analysis depend on the fiber type and its thermal desorption into a GC inlet. There are two approaches to SPME sampling of volatile organics:

1. Direct sampling. In this approach, the fiber is placed directly into the sample matrix.

2. Headspace sampling. In this approach, the fiber is placed in the headspace of the sample.

Choosing between direct immersion and headspace SPME is relatively straightforward. Direct immersion SPME is warranted for liquid and solution samples which are used in solid-phase and liquid-liquid extraction methods. Headspace SPME is considered for extraction of volatile specious, with normal boiling point less than 200°C, from solid and liquid samples. For higher boiling point analytes, direct immersion SPME is probably necessary. Headspace is more preferred for especially complex or dirty samples due to fouling the fiber coating in a direct immersion process.

SPME fibers have different coatings, and there is no single coating for extraction and separation of all volatile organics from a sample. Therefore, different types of coatings with different polarities are applied on SPME fibers. Currently, three types of fiber coatings are commercially available: (1) nonpolar, (2) semipolar, and (3) polar coatings. There are several SPME fiber coatings commercially available. These range in polarity from polydimethylsiloxane (PDMS), which is nonpolar, to "*carbowax-divinylbenzene*" (CW-DVB), which is highly polar. The nonpolar fibers are more commonly used for headspace SPME as the majority of volatile analytes tend to be nonpolar or slightly polar. The advantage of using different fiber polarities is that using a matched polarity fiber, as polar coated for a polar analyte, makes extraction selectivity be enhanced. On the other hand, there is less of a chance of extracting interfering compounds along with the analyte of interest, and an organic matrix is not a problem.

Fiber coating thickness is a second parameter that should be considered to select a fiber for both direct immersion and headspace SPME. The PDMS coating is commercially in hand in three thicknesses: 100, 30, and 7 μm. The 100-μm-thick fiber is generally applied for highly volatile compounds or when a larger organic matrix volume is used. The 7-μm-thick fiber is used for less volatile compounds.

Once the fiber is chosen, extraction conditions must be optimized. There are many variables such as (1) extraction time, (2) sample volume, (3) agitation, (4) temperature, and (5) sample matrix.

1. As extraction time is increased, a plateau in peak area is reached. So, this represents the time required for the system to reach the equilibrium and is the optimized extraction time. Most headspace SPME methods are completed in less than 5 min, while direct immersion SPME may require more than 30 min. Also, direct immersion SPME is highly matrix dependent.

2. The sensitivity of a SPME method is proportional to the number of moles of analyte recovered from the sample. As the sample volume increases, analyte recovery increases too. But in very dilute samples, larger sample volume results in slower kinetics and higher analyte recovery.

3. In many extraction methods, the agitation method affects both the extraction time and efficiency. In direct immersion SPME, agitation is often accomplished with a magnet and a stirrer. So, the stirring rate should be optimized and constant during the extraction process. Also, the fiber should be off-centered in the vial so that liquid is moving quickly around it. Agitation can also be achieved by physical movement of the fiber or by movement of the sample vial and/or sonication.

4. Extraction temperature can also be an important factor, especially in headspace SPME analyses. Despite of GC headspace analysis, increasing the temperature in SPME makes the extraction sensitivity decrease.

5. By modifying the sample matrix, the extraction recovery can be improved. There are two ways to modify the sample matrix: (a) adjusting the sample pH

or its salt content and (b) dissolving the solid sample in a proper solvent like water or a strongly aqueous solution. In similarity with classical liquid-liquid extractions, modifying the pH can change the extraction behavior.

Also, the SPME-GC injection system must be optimized. When the SPME interfacing GC is used, the GC injection system is typically done under splitless conditions. Since there is not any solvent and accommodation of the sample solvent, there is no need of specific small internal diameter glass liners, which are often used [2]. The main consideration is to transfer the analytes in the shortest possible time out of the fiber coating and in focusing the analytes into the sharpest bands possible. For semivolatile compounds, inlet optimization is very simple, and classical splitless inlet conditions can be used. A typical condition would be a temperature of about 250°C; a sufficient head pressure can maintain optimum GC column flow and an initial column temperature at least 100°C below the normal boiling point of the analyte. For volatile analytes, the optimization of the inlet is more difficult. So, keeping the initial column temperature at enough low level to refocus these analytes is often not possible, without cryogenics. The inlet must therefore be optimized to provide the fastest possible desorption and transfer to the GC column, while the GC column is maintained as cool as possible to achieve any focusing that is possible.

There are some examples for applying SPME as sample preparation step before GC detection of different analytes in a variety of samples. Some of them are pointed below.

Goncalves et al. studied solid-phase microextraction-gas chromatography-(tandem) mass spectrometry as a tool for pesticide residue analysis in water samples at high sensitivity and selectivity with confirmation capabilities. In this study, for SPME extraction a *"poly(dimethylsiloxane)-poly(divinylbenzene)"*-coated fiber was selected [14].

Yonamine et al. studied solid-phase microextraction-gas chromatography-mass spectrometry and headspace-gas chromatography of tetrahydrocannabinol (THC), amphetamine, methamphetamine, cocaine, and ethanol in saliva samples. In this study, at first saliva samples were submitted to an initial headspace procedure for ethanol determination by a GC-flame ionization detector. Then, two consecutive fiber solid-phase microextractions were carried out: THC was extracted by submersing a polymeric fiber, and amphetamine, methamphetamine, and cocaine were subsequently extracted after alkalinization [15].

2.3 Molecularly imprinted polymer (MIP) adsorbent in SPE and SPME

Molecularly imprinted polymer (MIP) is an alternative kind of sorbent which can be applied for *solid-phase extractions* and solid-phase microextractions. MIP is a polymeric sorbent which is produced in the presence of a target analyte, as a molecular template. Once the template is washed and removed through the polymer, some selective recognition sites has been remained in the polymeric sorbent for selective extraction of the analyte target. By using MIP as the sorbent, the surface contact area between the sorbent and the sample is much greater than in the coated fiber or coated inner surface tubing SPME procedures described earlier [2]. MIP inherent advantages include reusability, simplicity, low cost, high affinity and selectivity for target molecule, and physical and chemical stability over a wide range of experimental conditions and solvents [16].

Some of those applications are discussed below.

Djozan et al. studied preparation and evaluation of solid-phase microextraction fibers based on monolithic molecularly imprinted polymers for selective extraction of diacetylmorphine and analogous compounds. The main purpose of this research

was to develop a technique for fabrication of a monolithic and robust solid-phase microextraction on the basis of MIP interfacing with GC and GC-MS analysis for selective extraction and structural analysis of diacetylmorphine, respectively. On the other hand, a very simple approach has been developed for the fabrication of SPME fiber from diacetylmorphine-imprinted polymers which were subsequently used for extraction of diacetylmorphine and then analyzed with GC and GC-MS [16].

Rehim studied new trend in sample preparation [17]: online microextraction in packed syringe for liquid and gas chromatography applications and determination of local anesthetics in human plasma samples using gas chromatography-mass spectrometry. In this study, local anesthetics in plasma samples were used as model substances, and the method was developed and validated for microextraction in packed syringe (MEPS) online with GC-MS. MEPS and SPE procedures have some differences. In MEPS method, the sorbent packing is placed directly into the syringe, not into a separate column, as it is done in SPE. So, a separate robot does not need for applying the sample into the separation phase, as it is done in SPE. Also, the packed syringe can be applied several times for different samples [17].

2.4 Stir bar sorptive extraction (SBSE)

Stir bar sorptive extraction (SBSE) is used for the extraction of trace amounts of organics from aqueous food, environmental, and biological samples. A stir bar has been covered with a sorbent phase and placed into the sample solution to separate the analyte of interest. Although SBSE procedures are not exhaustive, more quantitative extractions can be achieved than those of SPME procedures.

The coated stir bar is usually used to stir the sample solution for a specialized period of time, depending on the sample volume and stirring speed, until approaching equilibrium.

SBSE improves on the low concentration capability of in-sample solid-phase microextraction (IS-SPME). Also, SBSE can be applied to headspace sorptive extraction (HSSE) [2].

Some of SBSE applications with GC analysis are discussed below.

Nakamura et al. studied simultaneous determination of alkylphenols and bisphenol A in river water by stir bar sorptive extraction with in situ acetylation and thermal desorption-gas chromatography-mass spectrometry. In this study, SBSE was used for the sample enrichment of seven alkylphenols and bisphenol A in river water. Also, in situ derivatization in aqueous samples was performed with acetic acid anhydride as acetylation reagent [18].

The extraction phase on the stir bar in SBSE is critical for the performance of both extraction and thermal desorption. The sol-gel coating technology possesses the potential to prepare thermally stable coatings [19].

Guan et al. studied determination of organophosphorus pesticides in cucumber and potato by stir bar sorptive extraction. In this study, organophosphorus pesticides (OPPs) in vegetables were determined by SBSE and capillary GC with thermionic specific detection (GC-TSD). Hydroxy-terminated polydimethylsioxane (PDMS) prepared by sol-gel method was used as extraction phase [19].

2.5 Soxhlet extraction

Soxhlet extraction was accepted as a standard method for the extraction of semivolatile and nonvolatile organics by the US Environmental Protection Agency (EPA 3540C0) and also the extraction of fat in cacao products by the Association of Official Analytical Chemists (AOAC 963.15). Soxhlet extraction was *introduced by Franz Ritter von Soxhlet in 1879*. It had been the most extensive applied technique

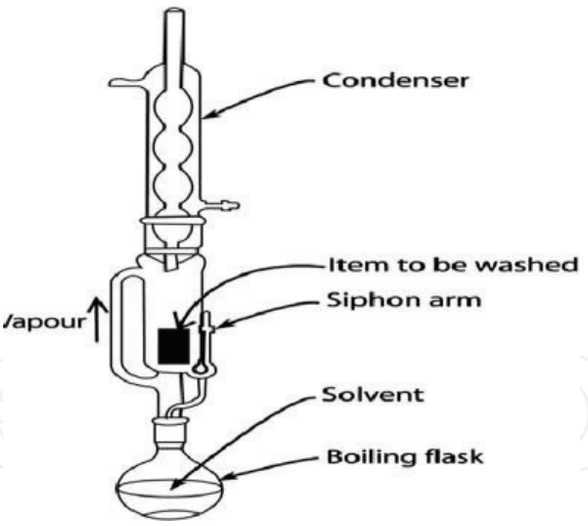

Figure 1.
The schematic diagram of Soxhlet apparatus.

till the other modern extraction methods were developed in the 1980s. Nowadays, Soxhlet is still applied for the extraction of semivolatile organic compounds from solid samples. Soxhlet extraction is a classical method which is operated under atmospheric pressure, in high temperature or under ultrasonic irradiation. In this technique, relatively large volumes of organic solvents are usually used, and it is a time-consuming technique [2].

Soxhlet apparatus has three components, and its schematic diagram is shown in **Figure 1** [2]:

1. The top part is a solvent vapor reflux condenser.

2. The middle part is a thimble holder with a siphon device and a side tube.

3. The bottom part is a round-bottomed flask which connects to the thimble holder.

4. A porous cellulous sample thimble is filled with sample solution and inserted into the sample thimble holder. Usually, 300 ml of solvents is introduced to flask for 10 g of a sample. The flask is heated slowly on a heating mantle, and the solvent vapor goes toward the reflux condenser and, after condensing, drips back to the thimble chamber. When the analyte reaches the top of the sample thimble holder, it is transferred back into the bottom flask via a siphon device. This cycle is repeated many times for a predetermined period of time. Since the boiling points of analytes are usually higher than those of solvents, the analytes accumulate in the flask and the solvents recirculate. Finally in each cycle, the analyte can be extracted with fresh solvents.

The properties of Soxhlet extraction are as follows [2]:

1. In Soxhlet extraction, the extraction is slow and can take between 6 and 48 h. On the other hand, it is a time-consuming technique (its drawback). It is mainly due to the analyte that is extracted with cooled condensed solvent.

2. The extract volume is relatively large (its drawback). So, the evaporation step is usually needed to concentrate the analytes before the analysis.

3. The sample size is often *10 g* or more, and multiple samples can be extracted on separate Soxhlet units.

4. Soxhlet is a rugged and well-established technique.

5. Relatively large solvent consumption (its drawback).

An automated Soxhlet extraction (Soxtec) was approved by the EPA (EPA 3541) in 1994 for the extraction of semivolatile and nonvolatile organic compounds [2]. Automated Soxhlet extraction is relatively faster than Soxhlet extraction, with lower consuming organic solvents [2]. In this method, the extraction is performed in three stages:

- In the first stage, a thimble containing the sample is immersed in the boiling solvent for about 60 min. Since the contact between the solvent and the sample is more vigorous and the mass transfer in a high-temperature boiling solvent is more rapid, extraction here is faster than in Soxhlet.

- In the second stage, the sample thimble is placed above the boiling solvent. Then, the condensed solvent drips into the sample and extracts the organics and falls back into the solvent reservoir as well. This stage is similar to traditional Soxhlet and takes usually 60 min.

- In the third stage, the solvent is evaporated, and a concentration step happens for 10–20 min.

Li et al. studied the determination of organochlorine pesticide residue in ginseng root by orthogonal array design Soxhlet extraction and gas chromatography. In this study, a method involving four-factor-three-level orthogonal array design was developed. The orthogonal array designs included extracting solvent component, particle size, solvent overflow recycle, and time needed for the optimization of extracting nine organochlorine pesticides from ginseng root, followed by capillary GC-electron capture detector and MS detector [20].

2.6 Ultrasonic extraction

Ultrasonic extraction, also known as sonication, uses ultrasonic vibration to ensure intimate contact between the sample and the solvent. Sonication is relatively fast, but the extraction efficiency is not as high as some other techniques. Also, ultrasonic irradiation may decompose some of organophosphorus compounds.

Before the sonication is used for real sample, the selected solvent system and optimum conditions for adequate extraction of the target analytes from reference samples should be investigated.

A typical sonication device can be equipped with a titanium tip. The sample is usually dried with anhydrous sodium sulfate and mixed with a certain volume of selected solvent. The disruptor horn tip is positioned just below the surface of the solvent, yet above the sample. Extraction can be carried out in duration as short as 3 min. After extraction, the extract is filtered or centrifuged, and also some form of cleanup is needed before analysis [2].

The ultrasonic extraction (USE) is a very versatile technique due to the possibility of selecting the solvent type or solvent mixture that allows the maximum extraction efficiency and selectivity. In USE, several extractions can be done

simultaneously, and no specialized laboratory equipment is required (advantage). But it is not easily automated (disadvantage) [21].

Goncalves et al. studied the assessment of pesticide contamination in soil samples from an intensive horticulture area, using ultrasonic extraction and gas chromatography-mass spectrometry. In this study, the application of an USE method combined with GC and GC-MS for the analysis of some pesticides in soil samples was investigated. The USE technique was used to separate the pesticides from the soil samples [21].

2.7 Supercritical fluid extraction (SFE)

In supercritical fluid extraction (SFE), supercritical fluids possess specific properties which make them facilitate the extraction of organics from solid samples. Two configuration of SFE operations are on- or off-line mode. In the online operation, SFE is matched directly to an analytical instrument like GC, supercritical fluid chromatography (SFC), and HPLC. Off-line SFE, as its name implies, is a stand-alone extraction method independent of the analytical method to be applied. Off-line SFE is more flexible and easier to perform than that of the online procedure. It allows the extract to be available for analysis by different techniques [2].

A supercritical fluid (SF) is a substance above its critical temperature and pressure. Also, it is an interface between gas and liquid. In fact it is not a liquid and or a gas, it is a SF.

CO_2 has a low supercritical temperature (31°C) and pressure (73 atm). It is nontoxic and nonflammable and also is available at high purity. So, carbon dioxide has become the solvent of interest for most SFE applications. Supercritical CO_2 is nonpolar and without permanent dipole moment; therefore, it can be utilized to extract nonpolar and moderately polar compounds from matrices. For the extraction of polar compounds, supercritical N_2O and $CHClF_2$ are more efficient. But these SFs are not environmentally friendly and they are not used in routine analysis [2].

SFE has gained increased attention as a good candidate instead of conventional liquid solvent extraction. This is mainly due to significant properties of supercritical fluids (SFs) such as their high diffusivity and low viscosity which make them extract selectively different chemicals without additional cleanup steps and so use little sample amounts [22].

Rissato et al. studied the supercritical fluid extraction for pesticide multiresidue analysis in honey and determination by gas chromatography with electron-capture and mass spectrometry detection. In this study, SFE procedure was used to separate some pesticides from honey samples, and it was compared with liquid-liquid extraction method [22].

2.8 Accelerated solvent extraction (ASE)

The other names of accelerated solvent extraction (ASE) are pressurized fluid extraction (PFE) and pressurized liquid extraction (PLE). Conventional solvents are used in ASE at high temperature (100–180°C) and pressure (1500–2000 psi) to increase the extraction percentage of organic compounds from solid samples.

Supercritical fluid extraction is matrix dependent and usually needs the addition of organic modifiers. ASE was developed to overcome these limitations. Although it was expected that conventional solvents would be less efficient than supercritical fluids, the results turned out to be quite the opposite. In many cases, extraction was faster and more complete with organic solvents at elevated temperature and pressure than with SFE [2].

The elevated pressure and temperature used in ASE affect the solvent and sample properties and their interactions as well. ASE properties include the following [2]:

1. Under higher pressure, the extraction would be performed at higher temperature values. This is mainly due to the increase of the solvent boiling point.

2. At higher pressures, the solvent penetration into the sample medium would be increased, and so the extraction of the interested analyte may be facilitated from the matrix.

3. At higher temperatures, the mass transfer and solubility of the analyte are enhanced.

4. The elevated temperature can reduce the power of analyte-sample bonds like dipole, hydrogen, and van der Waals interactions.

5. High temperature decreases the solvent viscosity and surface tension and so enhances solvent penetration into the matrix medium.

6. Therefore, faster extractions and better analyte recoveries can be achieved by ASE procedures.

ASE process has some steps mentioned below:

1. The extraction cell is filled with the sample medium.

2. Then, the solvent is entered in.

3. And, the cell temperature and pressure are increased to the desired level. The necessary time to enhance the temperature can be between 5 and 9 min (for up to 200°C).

The above steps are referred to the prefill method. If before addition of solvent the sample is warmed, the process is mentioned as preheat method. In comparison of the two procedures with each other, the prefill method is usually preferred [2].

A. Pastor et al. studied the determination of PAHs in airborne particles by accelerated solvent extraction and large-volume injection-gas chromatography-mass spectrometry. The procedure included extraction of some PAHs by accelerated solvent extraction (ASE) followed by gel permeation chromatography (GPC) cleanup and GC-MS detection of PAHs. In this study, the hexane-acetone mixture (1:1 v/v) gave the best recoveries when ASE parameters were fixed at 125°C and 1500 psi and a total time of 10 min [23].

2.9 Microwave-assisted extraction

It should be noted that microwave-assisted extraction (MAE) is different from microwave-assisted acid digestion. The former uses organic solvents to extract organic compounds from solids, while the latter uses acids to dissolve the sample for elemental analysis with the organic contents being destroyed. MAE is applied for the extraction of semivolatile and nonvolatile compounds from solid samples.

In general, organic extraction and acid digestion use different types of microwave apparatuses, as these two processes require different reagents and experimental conditions. The basic components of a microwave system include a microwave generator (magnetron), a waveguide for transmission, a resonant cavity, and a power supply. There are two types of laboratory microwave units:

1. Closed extraction vessels under elevated pressure.

2. Open vessels under atmospheric pressure.

In the liquid and solid states, molecules do not rotate freely in the microwave field, despite of gaseous molecules; therefore, no microwave spectra can be observed. Liquid- and solid-state molecules respond to the radiation differently, and this is where microwave heating comes in. During microwave heating procedure, electromagnetic energy would be changed to heat. This is mainly due to the ionic conduction and dipole rotation of the molecules which are imposed. Ionic conduction is concluded from the ion mobility in a solution under an electromagnetic field, and then, the heat is produced. Dipole rotation means that the directions of dipole rotations are changed under microwave irradiation. When a polarized molecule is imposed in an electromagnetic field, it can rotate around its axis at a rate of 4.9×10^9 times per second. So, with the larger molecular dipole moments, the more vigorous oscillations of molecules are obtained under a microwave field.

The proper choice of solvent is the key to successful extraction in MAE. In general, three types of solvent system can be used in MAE:

1. Solvent(s) of high dielectric coefficient.

2. A mixture of solvents of high and low dielectric coefficient.

3. A microwave transparent solvent used with a sample of high dielectric coefficient.

Zhou et al. studied the microwave-assisted extraction followed by gas chromatography-mass spectrometry for the determination of endocrine-disrupting chemicals in river sediments. In this study, the most efficient extraction (>74%) of the analyte was achieved by choosing methanol as the solvent, 110°C and 15 min, as the extraction temperature and time, respectively. The cleanup step was performed by passing the extracts through a non-deactivated silica gel column [24].

2.10 Headspace extraction

From an analytical point of view, volatile organic compounds (VOCs) are organic materials whose vapor pressures are greater than or equal to 0.1 mmHg at 20°C. Many VOCs are environmental pollutants, and in most cases of their analyses, the analytes are transferred to a gas-vapor phase and then analyzed by GC techniques [2].

Generally, the analysis of pure volatile compounds is simple, and the volatile analyte can be injected directly into a GC column [25]. However, the challenge is to extract the analytes from the matrix samples such as soil, food, cosmetics, polymers, and pharmaceutical raw materials. Headspace extractions are approaches to this and are divided into two categories: static headspace extraction (SHE) and dynamic headspace extraction (purge and trap) [2].

Static headspace extraction is known as equilibrium headspace extraction or simply as headspace. This technique has been available more than 30 years, so its instrumentation is both mature and reliable. In this technique, the extraction method includes the following [2]:

1. A sample, either solid or liquid, is put in a headspace autosampler (HSAS) or vial.

2. The sample vial is brought to a constant temperature and pressure, and the volatile analytes diffuse into the headspace vessel.

3. When the analyte concentration in the headspace part of the vessel reaches to an equilibrium level with respect to its concentration in the sample, the vial is connected to the GC column head, and then, a portion of the headspace is introduced into a GC for detection. This analyte transfer is due to a pressure drop between the vessel and the GC inlet pressure.

4. The vial is again isolated. For automated systems, this sampling procedure can be repeated by the same or the next vial.

The advantage of static headspace extraction is the ease of initial sample preparation. Usually for qualitative analysis, the sample can be placed directly into the headspace vial and analyzed with no additional preparation procedures. But for quantitative analysis, it may be vital to know the optimized matrix effects to gain good sensitivity and accuracy.

For large solid samples, it may be needed to change the physical state of the sample matrix. Two approaches in differentiating the sample state are to powder the solid sample and to disperse it into a liquid.

By crushing the solid sample, the surface area available for the volatile solute to distribute into the headspace phase is enhanced. So, the solute is distributing between a solid and the headspace phases. But in the second procedure, dispersing the solid into a liquid is preferred because the analyte partitioning process into the headspace often reaches the equilibrium faster. Therefore, by choosing a suitable solvent with high affinity toward the volatile analytes, the problems with sample and standard transfer from volumetric flask to headspace vials can be eliminated [2]. Some experimental factors affecting SHE should be optimized to improve extraction efficiency, sensitivity, quantitation, and reproducibility. These experimental variables include vial and sample volume, temperature, pressure, and the form of the matrix itself.

For the analysis of trace amounts of analytes, or where an exhaustive extraction of the analyte is required, purge and trap or dynamic headspace extraction (DHE) is more preferred than SHE. This technique is used for both solid and liquid samples. The samples can be biological, environmental, industrial, pharmaceutical, and agricultural. In DHE, there is no equilibrium between its concentration in the gas and matrix phases. Instead, they are removed continuously from the sample by a gas flow. This provides a concentration gradient between two mentioned phases which makes the exhaustive extraction of the volatile analytes.

A typical purge and trap system consists of the following:

1. A purge vessel.

2. A sorbent trap.

3. A six-port valve.

4. Transfer lines.

A purge and trap cycle consists of several steps: (1) purge, (2) dry purge, (3) desorb preheat, (4) desorb, and (5) trap bake. Each step is synchronized with the operation of the six-port valve and the GC [or GC-MS (mass spectrometer)]. The mentioned steps in a purge and trap cycle can be explained as follows:

1. An aqueous sample is introduced into the purge vessel.

2. The valve is set to the purge position. A purge gas (typically, helium) breaks through the sample continuously and sweeps the volatile organics to the trap, where they are retained by the sorbents. Then, the gas is vented to the atmosphere.

3. The purging step consists of purge, dry purge, and preheating. However, the purge step takes about 10–15 min, and the flow rate of helium is about 40 ml/min. After purging, while the trap is at the ambient temperature, the purge gas is transferred directly into the trap without passing through the sample. This step is called dry purge. The main objective of this step is to remove the water which has been accumulated on the trap. Dry purging often takes place between 1 and 2 min. Then, the purge gas is turned off, and the trap is heated to about 5–10°C below the desorption temperature. Preheat makes the subsequent desorption faster.

4. When the purging step is complete, the trap is heated, from 180 to 250°C, to desorb the analytes into the GC column to be analyzed. On the other hand, it is back-flushed with the GC carrier gas. So, the preheat temperature is reached, and the six-port valve is rotated to the desorb position to initiate the desorption step. Desorption time is about 1–4 min and depends on the carrier flow rate in GC instrument. For instance, the trap desorption time is short at the high flow rate, and so, a narrowband injection is achieved. The flow rate of the desorb gas should be selected in accordance with the type of GC column used. On the other hand, the operational conditions of the purge and trap must be compatible with configuration of GC system. With a packed GC column, higher carrier gas (desorb gas) flow rates can be applied. Usually, the optimum flow rate is about 50 ml/min. Capillary columns require lower flow rate and are often preferred over the packed one for better resolution.

5. In the trap baking step, after the desorption step, the valve is readjusted in the purge position. The trap condition is adjusted at desorption temperature, or 15°C upper than it, for 7–10 min. The objective of this step is to remove possible contaminants and eliminate sample transport.

6. After the trap baking step, the trap temperature is diminished and the next sample can be extracted. In each step, the conditional parameters such as temperature, time, and flow rate should be the same for all of the samples and calibration standards.

The trap is usually a stainless steel tube 3 mm in inside diameter (ID) and 25 mm long packed with multiple layers of adsorbents, and it should do the following steps:

1. Retain the analytes of interest, but do not introduce impurities.

2. Allow rapid injection of analytes into the GC column.

The sorbents are often arranged in layers to increase the trapping capacity. During purging process, the purge gas reaches the weaker sorbent at first, and only less volatile organics are retained. But more volatile compounds just pass through this layer and then are trapped by the other stronger adsorbent layers. During

desorption process, the trap is heated and back-flushed with the GC carrier gas. However, the less volatile compounds have never been in contrast with the stronger adsorbents, and so, the reversible adsorptions can be achieved.

To trap volatile organic compounds, the substances such as Tenax, silica gel, activated charcoal, graphitized carbon black (GCB or Carbopack), carbon molecular sieves (Carbosieve), and Vocarb are usually used [2]. Tenax is not only a porous but also a hydrophobic polymer resin based on 2,6-diphenylene oxide, with low affinity for water. So, highly volatile and polar compounds are seldom adsorbed on Tenax. Tenax should not be heated to temperatures upper than 200°C, because of its decomposition under high temperatures. The two types of Tenax are Tenax TA and Tenax GC. The former has higher purity and is more preferred for trace analysis. Silica gel is hydrophilic and is an excellent candidate for trapping polar compounds. Also, it is a stronger sorbent than Tenax. The problem is that water can be retained on the gel. Charcoal, as another stronger sorbent than Tenax, is hydrophobic and is mainly used to trap very volatile compounds such as dichlorodifluoromethane, a.k.a. Freon 12. These compounds can break through Tenax and silica gel. Conventional traps like Tenax, silica gel, and charcoal are usually used in series. If the boiling points of the analytes are above 35°C, Tenax itself will be suitable, and so, silica gel and charcoal can be ignored. Graphitized carbon black (GCB), as an alternative sorbent to charcoal and silica gel, has both the hydrophobic property and the trapping capacity similar to Tenax. Also, it is often used along with carbomolecular sieves and can trap highly volatile compounds. Vocarb is a highly hydrophobic activated carbon which can diminish water trapping and be purged fast. Vocarb is usually operated with an ion-trap mass spectrometer, which can be affected by trace levels of water or methanol. GCB, carbon molecular sieves, and Vocarb possess high thermal stability and can be operated at higher desorption temperatures than those that Tenax can be done [2].

The transfer line between the trap and the GC column is often made of nickel, deactivated fused silica, and silica-lined stainless steel tubing. By using these inert materials, the active sites which can interact with the analytes are eliminated. On the other hand, the transfer line is kept at a temperature higher than 100°C to avoid the condensation of water and the volatile organics. Also, the six-port valve which controls the gas flow path is also heated above 100°C to avoid condensation.

2.10.1 Interfacing purge and trap

As noted above, the operational conditions of purge and trap must be adaptable with the GC system configuration. For example, megabore capillary columns (0.53 mm ID or larger) are typically used at a flow rate of 8–15 ml/min. Since desorption process is slower at such flow rates, the column is usually cooled to 10°C or less temperatures at the stating of the GC run to retain the very volatile compounds. Sub-ambient cooling may be eliminated by using a long column (60–105 m) with a thick film stationary phase (3–5 μm). However, this flow rate is still too high for a GC-MS analyzer. So, a GC-MS interface like a jet separator or an open-split interface should be applied to decrease the carrier flow rate in the mass detector. However, an open-split interface makes a reduction in the analytical sensitivity due to entering just a portion of analyte into the detector [2].

Narrow-bore capillary columns (0.32 mm ID or smaller) with MS detector are commonly operated at lower flow rates (less than 5 ml/min). There are two ways to couple purge and trap with this type of columns:

1. To desorb the trap at a high flow rate and, then, with a split injector, split the flow into the GC instrument. So, a fast injection is obtained without significant loosing of the analytical sensitivity.

2. To desorb or refocus the analytes on a second trap and use a low desorb flow rate. At this flow rate, the time of desorption is too long to achieve a narrow bandwidth injection. A cryogenic trap is often used as a second trap and made of a short piece of uncoated fused silica capillary tube. It is cooled to −150°C by liquid nitrogen to refocus the analytes. After refocusing the analytes, the cryogenic trap is heated quickly to 250°C to desorb the analytes into the GC column.

A moisture control device is another interface which must be used. The purge gas, which is coming from purge vessel, is saturated with water, and so water can be collected on the trap and later released into the GC column during trap heating. Water decreases column efficiency and interferes with some certain detectors such as PID and MS. The column can also be plugged by ice if cryogenic trapping is used. Therefore, water requires to be removed before entering the GC. Two water management techniques are ordinarily applied [2]:

1. To have a dry purge step prior to the desorption process. However, by this approach, some hydrophilic sorbents such as silica gel are not compatible.

2. To use a condenser between the trap and the GC instrument. The condenser is made of inert materials such as a piece of nickel tube. During desorption, the condenser is maintained at ambient temperature, and water is condensed and removed from the carrier gas. After completing the desorption process, the condenser is heated and the water is vented.

Lacorte et al. studied an automated technique based on purge and trap coupled to gas chromatography with mass spectrometric detection for the trace determination of five of the most important water odorants. Analytes were purged from 20 ml of water sample containing sodium chloride at ambient temperature and trapped on a Tenax sorbent by a flow of He. The desorption step was done with helium, purge gas, and temperature programming. The desorbed analytes were directly transferred to a gas chromatograph with a mass spectrometer detector for separation and determination [26].

2.11 Membrane extraction

Among a wide variety of separation methods, membrane extraction and/or transport of analytes through the membranes is a powerful technique for their concentration, separation, and recovery. In this method, the sorption and desorption steps are combined into a one-step process, and, because of its simplicity, low cost, and high efficiency, it possesses an important role in biology, chemistry, and separation sciences; therefore, the efforts for developing of these types of sample treatment methods are increased [5]. In membrane transport, the sample is in contact with one side of the membrane, which is referred to the feed (or donor) phase. Also, the membrane phase serves as a selective barrier. The analytes pass through the membrane phase toward its other side, which is referred to the permeation (or acceptor) phase. Sometimes, the permeated analytes are swept by another phase like either a gas or a liquid. Its schematic diagram is shown in **Figure 2**.

A membrane can be accompanied with an instrumental analysis for online analysis (its advantage). Specially, a mass spectrometer or gas chromatograph can be applied as the detector device. Once a membrane is coupled to the mass spectrometry (MIMS), the membrane can be put in the vacuum compartment of the mass spectrometer. The permeated analytes are directly introduced into the

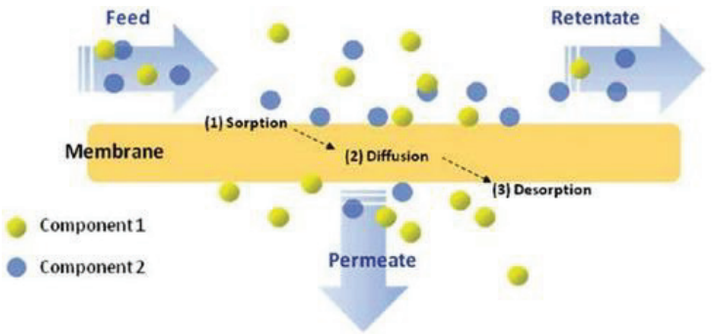

Figure 2.
The schematic diagram of membrane extraction.

ionization chamber of the MS instrument. In membrane introduction gas chromatography, a sorbent trap is interfaced between the membrane and the GC. Then, the permeated analytes are carried by a gas stream to the trap for preconcentration step. After completing the trap or preconcentration step, the trap is quickly heated to desorb the analytes into the GC column, as a narrowband injection. For instance in a GC connection, an aqueous sample from the loop of a multiport injection valve is injected into the hollow fiber membrane module by a N_2 stream. The gas pushes the sample through the membrane fibers, and so the organic analytes permeate to the acceptor phase. Then, they are swept to a micro-sorbent trap by a countercurrent nitrogen stream. After completing the extraction of the analytes on the trap, during a predetermined period of time, the trap is electrically heated to desorb the analytes into the GC column [27].

For matrix samples, GC has gained a good potential of choice, because of its excellent separation ability. Tandem MS has been introduced as a faster alternative technique to GC separation, but such these instruments make higher costs. In membrane-based methods, limit of detections are especially in the parts per thousand (ppt) to parts per billion (ppb) range.

The main drawback in membrane extraction coupled with a GC instrument is the slow permeation through the polymeric membrane and the aqueous boundary layer. This problem is much less than it in membrane introduction mass spectrometer (MIMS). The reason is that the vacuum in the mass spectrometer makes a high partial pressure gradient for mass transfer.

The time needed to complete the permeation process is mentioned as lag time. Another disadvantage of membrane extraction is that the lag and/or transport time can be significantly longer than the time of sample residence in the membrane phase. This is mainly due to the boundary layer effects. When the carrier fluid is an aqueous stream, a static boundary layer is formed between the membrane and the aqueous phase. Since the analytes are being stuck in the boundary layer, the gradient for mass transfer decreases and the transport time enhances. Sample dispersion is another cause of the long lag time in flow injection techniques where an aqueous carrier fluid is used. Axial mixing of the sample with the carrier stream causes dispersion. So, the sample volume increases, and longer residence time in the membrane phase is obtained. Dilution reduces the concentration gradient across the membrane, which is the driving force for diffusion [2, 5].

Membrane pervaporation (permselective "evaporation" of liquid molecules) is the term used to describe the extraction of volatile organics from an aqueous matrix to a gas phase through a semipermeable membrane. The extraction of volatiles from a gas sample to a gaseous acceptor across the membrane is called permeation, which is the mechanism of extraction from the headspace of an aqueous or solid sample. In pervaporation process, the organic analytes of interest move from the bulk aqueous

sample solution into the membrane phase and dissolve into it. Then, the analytes diffuse across the membrane phase and permeate into the acceptor or permeate phase and evaporate into the gas phase, as well. An additional step is occurred in headspace sampling mode, and the analytes transport into the headspace phase from the bulk aqueous phase. In both cases, the concentration gradient across the membrane is the driving force for the analyte transport across the membrane. Its schematic diagram is shown in **Figure 3**.

2.11.1 Membrane modules

Membranes can be categorized both based on its structure in two kinds, porous and nonporous, and based on its geometry in two types, flat sheet and hollow fiber. Membranes which are applied in pervaporation and gas permeation are especially hydrophobic and nonporous silicone (polydimethylsiloxane or PDMS) membranes. Aqueous organics dissolve into the membrane phase and are extracted, while the aqueous contaminants are unextracted into the membrane. The microporous membranes in pervaporation are usually made of polypropylene, cellulose, or Teflon. The disadvantage of this membrane is to permit the passage of large quantities of water. Usually, water must be removed before it enters the analysis instrument.

As understood the name, flat-sheet membranes are flat, like a sheet of paper, and can be made as thick as less than 1 mm. However, the typical holders are necessary to hold them in place. In-tube hollow fiber membranes are 200–500 mm in diameter and also allow fluids to flow both inside and outside. Hollow fibers are self-supported and offer the advantage of larger surface area per unit volume and high packing density. A large number of parallel fibers can be packed into a small volume.

2.11.2 Optimization of membrane extraction

Several factors, which affect the extraction efficiency and sensitivity by the membrane, such as temperature, membrane surface area, membrane thickness, geometry, sample volume, and sample flow rate, should be optimized for specific applications. Higher temperature has two opposite effects on the extraction efficiency. On the other hand, it facilitates mass transfer by increasing diffusion coefficient and, on the other hand, decreases analyte partition coefficient in the membrane. So, the temperature of the membrane module should be controlled to avoid fluctuation extraction efficiency and sensitivity. Another effective parameter

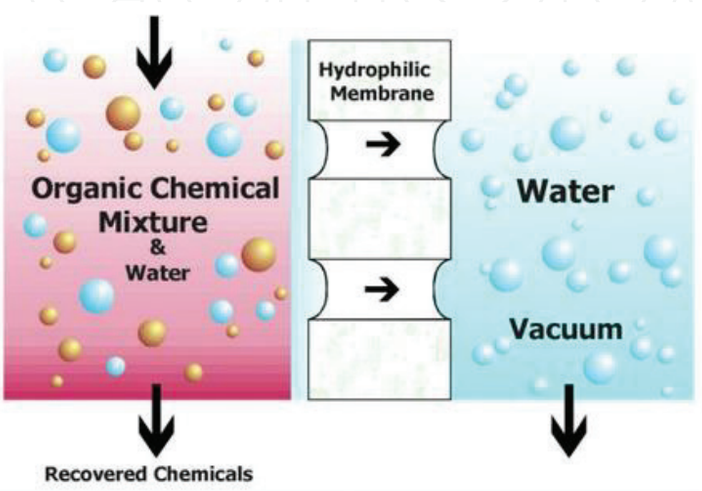

Figure 3.
The schematic diagram of membrane pervaporation.

is the membrane thickness. Faster mass transfer is achieved by using thinner membranes, and in the case of hollow fibers, using longer membranes and multiple fibers is better. Also by using the larger volume of the sample, higher sensitivity can be obtained. However, larger volumes take longer to extract, but this lower sample flow rate makes the extraction efficiency increase.

3. Concentration techniques for reducing the solvent volume

Once the analytes are diluted in the presence of a large volume of solvents during the extraction processes, they should be concentrated to analyze by instrumental methods as GC. If the amount of solvent to be removed is not very high and the analyte is nonvolatile, the solvent can be vaporized by a gentle stream of nitrogen gas flowing either across the surface or through the solution. But when a large volume of solvent should be removed, a rotary vacuum evaporator is used. In this case, the solution is placed in a round-bottomed flask which put in a heated water bath. A water-cooled condenser is attached at the top of flask to condense the evaporated solvent, and it distils into a separate container. Then, the flask is rotated continually to expose maximum liquid surface to evaporation. It should be noted that evaporation should stop before the solution reaches dryness.

For achieving smaller volume, e.g., less than 1 ml, a Kuderna-Danish concentrator is used. In this case, the solution is slowly heated in a warm water bath until the necessary volume is obtained. Also, an air-cooled condenser provides the solvent reflux [2].

4. Cleanup techniques

Sample cleanup is especially important for analytical separations such as GC, HPLC, and electrophoresis. Often, many solid matrices, as soil, biological materials, and natural products, contain hundreds of interferences at higher concentrations than those of the analytes. So, a cleanup step is vital to separate the trace amount of analyte from interferences. On the other hand, some high-boiling materials can cause a variety of problems such as the adsorption of analyte in the injection port or in front of a GC or HPLC column. Therefore, some positive and negative errors can be observed in the retention time of the analyte.

Some other cleanup techniques include gel permeation chromatography (GPC), acid-base partition cleanup, solid-phase extraction (SPE), and column chromatography, which are discussed in the following step [3].

Gel permeation chromatography (GPC) is a size-exclusion method which contained organic solvents (or buffers) and porous gels to separate macromolecules larger than analytes of interest. GPC is used to eliminate lipids, proteins, polymers, copolymers, natural resins, cellular components, viruses, steroids, and dispersed high-molecular-weight compounds from the sample. This method is suitable for both polar and nonpolar analytes [2]. On the other hand, GPC is usually used to remove high-boiling materials which condense in the injection port of a GC or the front of the GC column [28].

Acid-base partition cleanup is a liquid-liquid extraction procedure to separate acids such as organic acids and phenols from the base or neutral analytes like amines, aromatic hydrocarbons, and halogenated organic compounds, by adjusting pH. Also, this cleanup method is applied for petroleum waste prior to analysis [2].

Solid-phase extraction cartridge is a traditional column chromatography which is applied to clean up the biological, clinical, and environmental samples. Some of the SPE application examples are as follows [29]:

1. The cleanup of pesticide residues and chlorinated hydrocarbons.

2. The separation of nitrogen compounds from hydrocarbons.

3. The separation of aromatic compounds from an aliphatic-aromatic mixture.

4. The cleanup of steroids, esters, ketones, glycerides, alkaloids, and carbohydrates.

5. The cleanup of cations, anions, metals, and inorganic compounds.

As discussed in previous sections, the sufficient amount of a sorbent, which is loaded with the sample extract, has packed the SPE cartridge. Then, the analyte of interest is eluted through the column by an efficient eluting solvent, and the other contaminants are remained on the cartridge. The packing compound may be an inorganic material like either Florisil or one of many stationary phases which are commercially available [30].

5. Chemical derivatization analysis

Gas chromatography of volatile or nonpolar compounds may be done without derivatizing the sample; indeed, derivatives of compounds such as hydrocarbons or halogenated hydrocarbons cannot easily be prepared. It is possible to analyze polar compounds such as carboxylic acids and amines, without prior derivatization, on polar GC phases such as those based on polyethylene glycol. However, derivatization is useful in many instances where it may [31]:

1. Increase the volatility and decrease the polarity of polar compounds.

2. Stabilize compounds which are unstable at the temperatures required for GC.

3. Improve the separation of groups of compounds on GC column.

4. Yield information with regard to the number and type of functionalities present in mixtures of unknown compounds.

5. Improve the behavior of compounds toward selective detectors such as electron capture or nitrogen-selective detectors and mass spectrometry.

However, there are some drawbacks in using derivatization process before GC analysis:

1. The derivatizing agent may be difficult to remove and interfere in the analysis, and this is particularly disadvantageous when the purity of a compound is being assessed by GC.

2. The derivatization conditions may cause unintended chemical changes in a compound, for example, dehydration.

3. The derivatization step increases the time required for analysis.

For these reasons, GC with derivatization is less frequently employed in quality control applications, where the purity of a single substance or the components in a formulation are being determined.

Derivatization reactions are usually simple chemical reactions which are likely to occur in nearly quantitative yield such as acylation, alkylation, and silylation. In silylation reactions, some derivatives like trimethylsilyl (TMS) and tertiarybutyldimethylsilyl (TBDMS) can be prepared from a wide range of functional groups including hydroxyl, carboxylic, amine, amide, thiol, phosphate, hydroxide, and sulfonic. In acylation processes, acetate formation of the analytes is prepared by some derivatizing agents such as acetic anhydride, trifluoroacetic anhydride (TFAA), pentafluoropropionic anhydride (PFPA), and heptafluorobutyl anhydride (HFBA). Alkylation reactions may be used to derivatize carboxylic acids, amines, sulfonic acids, phosphonic acids, phosphates, barbiturates, uracils, purines, penicillins, thiols, and inorganic anions [31].

Derivatization reactions require relatively simple apparatuses:

1. Sample container and reaction vessel.

2. Heating and evaporation apparatuses.

3. Sample and reagent handling systems.

4. Removal systems for the exiting of the derivatizing reagents.

The certain standard procedures in derivatization are the following:

1. When volumes of reagent are small, reactions are carried out in 0.3 or 1 ml capacity Reacti-Vials or V-Vials. When volumes of solvents or reagents are greater, such as in aqueous phase reactions, then 3.5-ml screw-top sample tubes with aluminum-lined caps are used.

2. The reagents or solvent are evaporated under a stream of nitrogen gas with the sample maintained at 60–80°C in a heating block. Obviously, less volatile reagents require heating at higher temperatures for their efficient removal. If the sample is volatile, evaporation at a low temperature for a longer time may be required, or it may be better to inject it without removing the reagents.

3. Drying is carried out by passing the sample through ca. 3 cm of anhydrous sodium sulfate contained in a Pasteur pipette plugged with cotton wool. Anhydrous magnesium sulfate may be used too.

4. Dissolution of the derivatized sample prior to analysis is done by treating the sample with 2 ml of solvent for capillary column GC using the splitless injection mode (the volume may be adjusted if a split injection is used) or 100 μl for packed column GC. Since in most circumstances the derivatized compound should be clearly observed in relation to any interfering peaks from reagent residues, in injection 1 μl of product solution, 200 μg of material can be chosen as a proper starting point for the development of a method.

5. Removal of excess reagents is carried out by passage through a short column of Sephadex LH20. The sample is passed through a short column prepared by introducing Sephadex LH20 suspended in EtOAc/hexane (1:1, V/V) into a Pasteur pipette plugged with cotton wool and allowing the solvent to drain out to leave a pluge of ca. 3 cm of the adsorbent [31].

However, in some cases, the derivatization leads to sharper peaks and therefore to better separation and higher sensitivity. But the derivatization procedure requires

more time and effort. Assadi et al. studied the determination of chlorophenols in water samples using simultaneous dispersive liquid-liquid microextraction and derivatization followed by gas chromatography-electron-capture detection [32]. In this research, dispersive liquid-liquid microextraction (DLLME) and derivatization coupled to gas chromatography-electron-capture detector (GC-ECD) was simultaneously applied for quantitative investigation of chlorophenols (CPs) in water sample. In this method, 500 μl of acetone, as disperser solvent, containing 10.0 μl of chlorobenzene, as extracting solvent, and 50 μl of anhydride acetic acid, as derivatizing reagent, was quickly injected into 5.00 ml of water sample containing CPs (analytes) and K_2CO_3 (0.5%, w/v) by a syringe. So, during a few seconds of time, the analytes were both derivatized and extracted simultaneously. Then, the mixture was centrifuged, and 0.50 μl of precipitated phase containing concentrated analytes was analyzed by GC-ECD instrument [32].

6. Superheated water extraction

When the temperature of liquid water is increased under pressure, between 100 and 374°C, its polarity is reduced significantly, and so, it can be applied as an extracting solvent for a wide variety of analytes. Its most interested application has been to determine PAHs, PCBs, and pesticides from environmental samples. Although it gives comparable results to Soxhlet extraction, the organic solvent consumptions have been significantly decreased, and quicker extractions were achieved. Unlike supercritical fluid extraction (SFE), unless the pressure is decreased and steam is applied, n-alkanes cannot be extracted. Other superheated water applications include the separation of required oils from plant substances where it preferably extracts the more important natural oxygenated compounds than steam distillation. The aqueous extract can be enriched via different methods such as solvent extraction, SPE, SPME, and extraction disk. On the other hand, the extraction can be coupled to LC or GC instruments, as online methods. In many cases the superheated water extraction is cleaner, faster, and cheaper than the conventional extraction methods [33].

The pressures, which are needed to keep a condensed state of water, are moderate in 15 bar at 200°C and 85 bar at 300°C. At any pressure, if the pressure falls below the boiling point of liquid water, superheated steam is produced. This superheated state possesses a significantly lower dielectric constant than that of the liquid state and also has gas-like diffusion velocity and viscosity properties. Consequently, superheated water behaves completely different from an extraction liquid solvent.

Superheated water has been widely used as an analytical extraction solvent. The changes in the polarity of water with increasing temperature have been also exploited in superheated water chromatographic methods [34].

Ozel et al. studied the analysis of volatile components from *Ziziphora taurica* subsp. *taurica* by steam distillation, superheated water extraction, and direct thermal desorption with GC·GC-TOFMS [35]. In this research, volatile compounds from the leaves of *Ziziphora taurica* subsp. *taurica* have been separated by steam distillation, superheated water extraction, and direct thermal desorption methods. The volatile constituents were analyzed by a perfect two-dimensional gas chromatography-time-of-flight mass spectrometry instrument. Some other researchers reported that superheated water is a powerful alternative extractor for separation of essential oils, because of its ability in working at low temperatures and obtaining higher speed extractions. Therefore, this makes the decomposition of volatile and heat-sensitive analytes be avoided. Extra advantages of the use of SWE are its simplicity, low cost, and friendly environment [36].

7. Single-drop microextraction

Single-drop microextraction (SDME) has witnessed incessant growth in the range of applications of sample preparation for trace organic and inorganic analysis. In SDME, a Teflon rod (or needle of a syringe) with a spherical recess at its one end is loaded with 8 μl of organic solvent (n-octane) containing the internal standard (n-dodecane) and immersed in aqueous sample taken in a 1 ml vial for a known period of time while being stirred. Thereafter, the rod is exited from the solution, and with a GC syringe, 1 μl of extract is injected into the GC column for analysis [37]. The stirrer rate of donor aqueous phase affects the solvent extraction speed and homogeneity of the obtained extract. SDME is comparable to SPME in terms of speed, precision, and sensitivity. But it is much cheaper than SPME and provides narrower peaks because in SDME, the solvent evaporation is faster than the analyte desorption from the fiber in SPME. However, in SDME, just little portion of extract is used to inject the GC column. By using a GC syringe instead of Teflon rod, the inconvenience of its filling can be eliminated. So, 1 μl of extract can be retracted back into the syringe after extraction process and injected directly into the GC column. Thus, the GC microsyringe can be used without any modification, and all other devices are general laboratory equipment. The GC microsyringe with a bend tip can hold the organic drop in place at controlled stirring rate. So, a number of instrumental analysis methods can be coupled to single-drop microextraction procedures.

There are two modules in SDME: direct immersion single-drop microextraction (DI-SDME) and headspace SDME. Their schematic diagrams are shown in **Figure 4**.

The direct immersion SDME is just applied for liquid samples containing nonpolar or relatively polar analytes. To stabilize solvent drop during the extraction process, any insoluble and special materials must be removed from the sample medium, and a proper organic solvent with the least solubility in water, high boiling point, and high affinity to extract the analyte of interest should be chosen. Also at a moderated stirring rate, the drop must not be dislodged. However, DI-SDME is more favorable to match with GC method because of using water-immiscible solvent in the

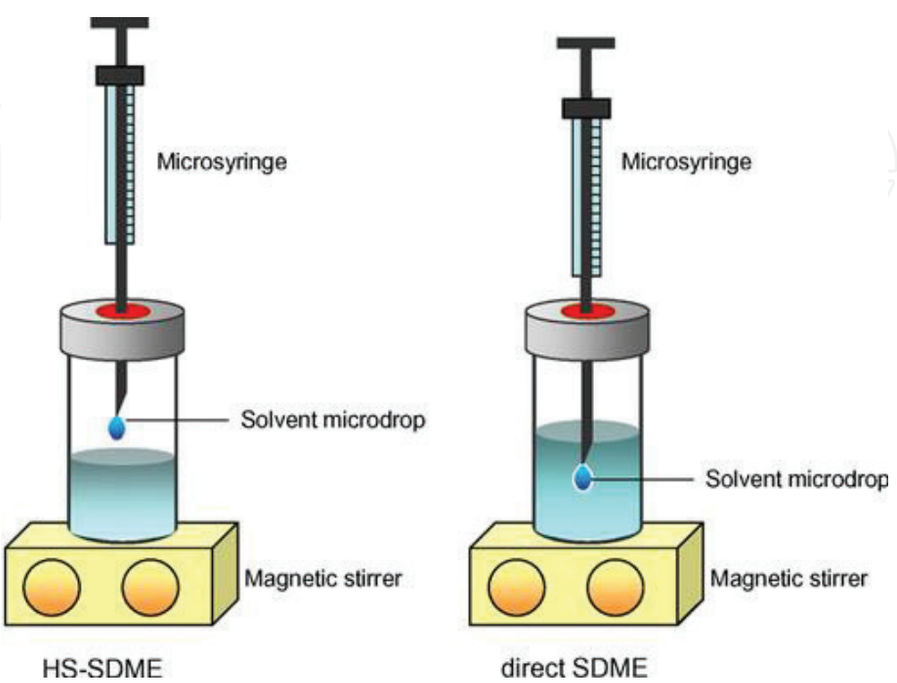

Figure 4.
Schematic diagrams of two modules in SDME.

drop. The searches are shown that N-octane and toluene possess the best extraction efficiency for nonpolar substances, while chloroform is more favorable to extract polar alkaloids, and then they can be analyzed by GC techniques [37]. One limitation of direct immersion SDME is the instability of the droplet at high stirring rates. Although high stirring rates enhance the extraction efficiency, to avoid the problem caused by elevated stirring speeds, a 1-μl microsyringe (instead of a more common 10-μl one) with some modification of its tip was used by Ahmadi et al. [38].

Ionic liquids have been established as alternative to organic solvents because of their high boiling point and viscosity which allow production of larger and more reproducible extraction drops. HPLC is a preferred method for analyzing ionic liquid extract, but their nonvolatility causes them unsuitable for GC analysis. To couple ionic liquid-based SDME to GC instrument, the extract is introduced via a removable interface which prevents entering of ionic liquid into the GC column, while the analytes can be entered quantitatively into the capillary column [37].

HS-SDME in which the organic droplet is held above the aqueous sample solution is most suitable for the consideration of volatile or semivolatile analytes [39]. The advantages of HS-SDME include the following: (1) Headspace SDME permits quick stirring of the sample solution with no concerning on the droplet stability. (2) The effects of nonvolatile matrix interferences are reduced, even if they are not eliminated. (3) In this mode, the analytes are distributed between three phases: the aqueous sample, headspace, and organic droplet. Since an elevated stirring rate of the sample solution enhances the mass transfer between the three phases. (4) In comparison with HS-SPME, HS-SDME shows to have the same precision and rate of analysis as HS-SPME. However, HS-SDME procedure possesses two special advantages over HS-SPME. At first, the approach of choosing solvents is wider. Second, the solvent cost (on the basis of several microliters) is negligible in comparison with the cost of commercially available fibers in SPME [39]. Alternatively, the use of SDME for headspace analysis seems relatively difficult, because of the requirement of the higher boiling point solvents. Although the most suitable solvents for gas chromatography should have relatively high vapor pressures or low boiling points, the limit of these solvents is obvious: they would evaporate too quickly in the headspace during extraction. Therefore, the select of suitable solvents should be the first decision in HS-SDME techniques.

8. Conclusions

Many methods are available for the treatment of volatile substances prior to instrumental analysis. In this chapter, the major methods which are leading to GC analysis have been explained. It has been observed that yet the classical techniques such as purge and trap, static headspace extraction, and liquid-liquid extraction act as important roles in chemical analysis of all sample types. New methods, such as SPME and membrane extraction, possess some advantages like convenient in automation and field sampling and reduction of solvent consumption, as well. If the analyst may be confronted with every difficulty, there is an appropriate available method to solve and face it. As a consequence, the main and primary analytical problem is to select the best sample preparation technique.

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