14 chapter

Gas Chromatography

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14.1 INTRODUCTION

The first publication on gas chromatography (GC) was in 1952 [1], while the first commercial instruments were manufactured in 1956. James and Martin [1] separated fatty acids by GC, collected the column effluent, and titrated the individual fatty acids for quantitation. GC has advanced greatly since that early work and is now considered to be a mature field that is approaching theoretical limitations.

The types of analysis that can be done by GC are very broad. GC has been used for the determination of fatty acids, triglycerides, cholesterol and other sterols, gases, solvent analysis, water, alcohols, and simple sugars, as well as oligosaccharides, amino acids and peptides, vitamins, pesticides, herbicides, food additives, antioxidants, nitrosamines, polychlorinated biphenyls (PCBs), drugs, flavor compounds, and many more. The fact that GC has been used for these various applications does not necessarily mean that it is the best method—often better choices exist. GC is ideally suited to the analysis of **thermally stable volatile substances**. Substances that do not meet these requirements (e.g., sugars, oligosaccharides, amino acids, peptides, and vitamins) are more suited to analysis by a technique such as high-performance liquid chromatography (HPLC) or supercritical fluid chromatography (SFC). Yet gas chromatographic methods appear in the literature for these substances after derivatization.

This chapter will discuss sample preparation for GC, GC hardware, columns, and chromatographic theory as it is uniquely applied to GC. Texts devoted to GC in general [2–4] and food applications in particular [5, 6] should be consulted for more detail.

14.2 SAMPLE PREPARATION FOR GAS CHROMATOGRAPHY

14.2.1 Introduction

One cannot generally directly inject a food product into a GC without some sample preparation. The high temperatures of the injection port will result in the degradation of nonvolatile constituents and create a number of false GC peaks corresponding to the volatile degradation products formed. In addition, very often the constituent of interest must be isolated from the food matrix simply to permit concentration such that it is at detectable limits for the GC or to isolate it from the bulk of the food. Thus, one must generally do some type of sample preparation, component isolation, and concentration prior to GC analysis.

Sample preparation often involves grinding, homogenization, or otherwise reducing particle size. There is substantial documentation in the literature showing that foods may undergo changes during sample storage and preparation. Many foods contain active enzyme systems that will alter the composition of the food product. This is very evident in the area of flavor work [7–9]. Inactivation of enzyme systems via high-temperature short-time thermal processing, sample storage under frozen conditions, drying the sample, or homogenization with alcohol may be necessary.

Microbial growth or chemical reactions may occur in the food during sample preparation. Chemical reactions often will result in false peaks on the GC. Thus, the sample must be maintained under conditions such that degradation does not occur. Microorganisms often are inhibited by certain chemicals (e.g., sodium fluoride), thermal processing, drying, or frozen storage.

14.2.2 Isolation of Analytes from Foods

14.2.2.1 *Introduction*

The isolation procedure may be quite complicated depending upon the constituent to be analyzed. For example, if one were to analyze the triglyceride-bound fatty acids in a food, one would first have to extract the lipids (free fatty acids; mono-, di-, and triglycerides; sterols; fat-soluble vitamins, etc.) from the food (e.g., by solvent extraction) and then isolate only the triglyceride fraction (e.g., by adsorption chromatography on silica). The isolated triglycerides then would have to be treated to first hydrolyze the fatty acids from the triglycerides and subsequently to form esters to improve gas chromatographic properties. The two latter steps might be accomplished in one reaction by transesterification (e.g., boron trifluoride in methanol) as described in Chap. 17, Sect. 17.3.6.2, and Chap. 23, Sect. 23.6.2. Thus, many steps involving several types of chromatography may be used in sample preparation for GC analysis.

The analysis of volatiles in foods (e.g., packaging or environmental contaminants, alcohols, and flavors or off-flavors) can be achieved by GC. These materials for GC analysis may be isolated by headspace analysis (static or dynamic), simple solvent extraction, distillation, preparative chromatography (e.g., solid-phase extraction, column chromatography on silica gel), or some combination of these basic methods. Table 14.1 summarizes various methods of isolation described in some detail in Sect. 14.2.2. The procedure used for a particular food will depend on the food matrix as well as the compounds to be analyzed. The primary considerations are to isolate the compounds of interest from nonvolatile food constituents (e.g., carbohydrates, proteins, vitamins) or those that would interfere with GC (e.g., lipids). Some of the chromatographic methods that might be applied to this task have been discussed in the basic chromatography chapter (Chap. 12) of this text. Methods for the isolation of volatile substances will be covered briefly as they pertain to the isolation of components for gas chromatographic analysis.

It should be emphasized that the isolation procedure used is critical in determining the results obtained. An improper choice of method or poor technique at this step negates the best gas chromatographic analysis of the isolated analytes. The influence of isolation technique on gas chromatographic analysis of aroma compounds has been demonstrated [10]. These biases are discussed in the sections that follow and in more detail in books edited by Marsili [11] and Mussinan and Morello [12]. While these books relate to the analysis of aroma compounds in foods, the techniques for the isolation of these volatiles are the same as used in the analysis of other volatiles in foods.

14.2.2.2 *Headspace Methods*

One of the simplest methods of isolating volatile compounds from foods is by direct injection of the headspace vapors above a food product. There are two types of headspace sampling: static headspace sampling and dynamic headspace sampling.

Static (i.e., **direct**) **headspace sampling** has been used extensively when rapid analysis is necessary and major component analysis is satisfactory. In static headspace sampling, a food sample is placed into a vessel, and the vessel is closed with an inert septum. At equilibrium, the headspace of the sample is taken using a gas-tight syringe and then directly injected onto the GC. Examples of method applications include measure-

14.1 table Methods to isolate analytes from food for gas chromatography analysis

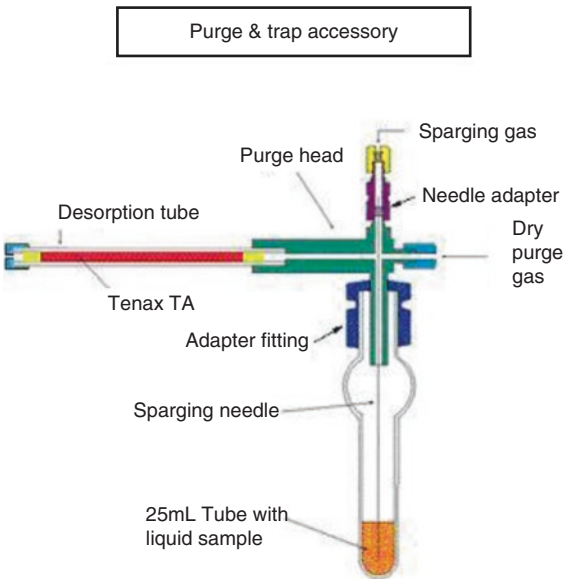
General types	*Specific methods*	*Description*	*Advantages/applications*	*Disadvantages/limitations*
Headspace sampling	Direct headspace sampling	Headspace taken from sample in closed vessel for direct injection into GC	Good for rapid analysis of very low-boiling point compounds	Low sensitivity
	Dynamic headspace sampling (purge and trap)	Sample is purged with inert gas to strip volatiles from the sample and retained by absorbent or cryogenic traps. The volatiles are then desorbed from the trap thermally or by organic solvent for GC analysis	Collects volatiles, no matter the polarity or boiling point. With adsorbent trap, no further extraction is required, and the system is readily automated	With cryogenic trap, usually must further extract with organic solvent and dry to concentrate. With adsorbent trap, get differential adsorption affinity and limited capacity
Distillations methods	Steam distillation (at normal pressure or in vacuum)	Use steam at atmospheric pressure or in vacuum to heat and codistill volatiles from the sample	Convenient and efficient	Solvent extraction is required on dilute aqueous solution collected
	Simultaneous distillation and extraction (SDE)	Product steam and solvent vapors are intermixed and condensed. The solvent extracts organic volatiles from condensed steam	Time savings due to one-step isolation and concentration. Less solvent use vs. what is needed to condense the sample in regular steam distillation	Get artifact formation due to elevated temperature
Solvent extraction	Simple batch extraction	Use typically organic solvent with, e.g., separatory funnel, to extract analytes of interest into the solvent	Efficient if multiple extractions and excessive shaking used	Need to remove nonvolatiles from the extracted sample
	Continuous extraction	Use organic solvent or CO2 with equipment to make continuous	More efficient than batch extraction	Need to remove nonvolatiles from extracted sample. Requires elaborate equipment
	Solvent-assisted flavor evaporation (SAFE)	Sample with extracting solvent are added slowly to the SAFE device. Volatiles and solvent are distilled and collected in the cold trap	High efficiency. Operate at low temperature. Minimum thermal artifact formation	Need special device and high vacuum system

14.1 table (Continued)

General types	*Specific methods*	*Description*	*Advantages/applications*	*Disadvantages/limitations*
Solid-phase extraction (SPE)			Compared to traditional liquid-to-liquid extraction: less solvent, glassware, and time required, better precision and accuracy, minimum solvent evaporation for further analysis, readily automated	
	Simple solid-phase extraction (SPE)	The liquid sample is passed through the column of filter disk with chromatography stationary phase. Solutes with affinity are retained on the phase. After rinsing the column with water or weak solvent, a strong eluent is used to elute analytes of interest		
	Solid-phase microextraction (SPME)	Stationary phase is bound to fine fused silica filament. The filament is immersed in the sample or headspace of the sample and then pulled into the metal sheath that is forced through the septum of GC. Volatiles are thermally desorbed from the filament	Simple operation. No solvent contamination or disposal. Many phases of fiber available, so analytes with wide range of polarity and volatility can be analyzed	Poor reproducibility. Lot-to-lot variation: Short life of filament. Fiber is highly selective, sample saturation, competitive absorption. Highly influenced by other volatiles in the sample
	Solid-phase dynamic extraction (SPDE)	Similar to SPME, but the polymer is coated inside a special needle used to draw headspace of food. Volatiles are absorbed to the phase and then released when the needle is injected into GC	Similar to SPME, but less issues with analyte saturation and competition	Lack of commercial device
	Stir bar sorptive extraction (SBSE)	Magnetic stir bar is enclosed in a glass coated with absorbent film. The bar spins in the sample and absorbs analytes and then the stir bar is transferred to thermal desorption unit and delivered to GC column	Higher sensitivity and accuracy than SPME. Highly automated. Can extract less volatile compounds	Limited phase available, instrumentation is expensive, high operation cost, not good for solid samples (use HS-SBSE)
Direct injection		Inject 2–3-µl sample directly into the GC column	Simple, if the sample and column allow for this	Thermal degradation of nonvolatiles. Damage to the column. Decreased separation if water in the sample. Contamination of the column and injection port by nonvolatiles

ment of hexanal as an indicator of oxidation [13, 14] and 2-methylpropanal, 2-methylbutanal, and 3-methylbutanal as indicators of nonenzymatic browning [15]. The determination of residual solvents in packaging materials also may be approached by this method. Unfortunately, this method does not provide the sensitivity needed for trace analysis. Instrumental constraints typically limit headspace injection volumes to 5 mL or less. Therefore, only volatiles present in the headspace at concentrations greater than 10^{-7}-g/l headspace would be at detectable levels (using a flame ionization detector (FID)). Furthermore, the static headspace only allows the investigation of very low-boiling compounds.

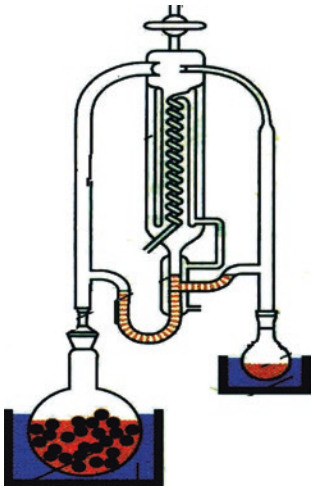
Dynamic headspace sampling (i.e., **purge and trap**) has found wide usage in recent years (Fig. 14.1). In dynamic headspace method, the sample is purged with an inert gas, such as nitrogen or helium, which strips volatile constituents from the sample. This method may involve simply passing large volumes of headspace vapors through a cryogenic trap or, alternatively, an adsorption trap. A **cryogenic trap** (if properly designed and operated) will collect headspace vapors irrespective of compound polarity and boiling point. However, water is typically the most abundant volatile in a food product, and, therefore, this distillate must be extracted with an organic solvent, dried, and then concentrated for analysis. These additional steps add analysis time and provide opportunity for sample contamination and loss. A more commonly used technique is to use adsorbent traps. **Adsorbent traps** offer the advantages of providing a water-free volatile isolate (trap material typically has little affinity for water) and are readily automated. Tenax, charcoal, or synthetic porous polymers (Porapaks® and Chromosorbs®) are frequently used trapping materials. These polymers exhibit good thermal stability and reasonable capacity. The trapped volatiles are then recovered from the trap with a suitable solvent or by thermal desorption. For automated purge-trap system, the adsorbent traps are generally placed in a closed system and loaded, desorbed, and so on via the use of automated multiport valving systems. The automated closed system approach provides reproducible GC retention times and quantitative precision necessary for some studies. The primary disadvantage of adsorbent traps is their differential adsorption affinity and limited capacity. Therefore, the GC profile may only poorly represent the actual food composition due to biases introduced by the purging and trapping steps.



14.1 figure Purge and trap accessory for headspace sampling

14.2.2.3 *Distillation Methods*

Distillation processes are quite effective at isolating volatile compounds from foods for GC analysis. Product moisture or outside steam is used to heat and codistill the volatiles from a food product. The most frequently used distillation method is steam distillation at atmospheric pressure or in vacuum. Steam distillation at normal pressure is a common method for isolating essential oils from plant materials such as hop oil. For most food analysis, this means that a very dilute aqueous solution of volatiles results, and a solvent extraction must be performed on the distillate to permit concentration for analysis. The distillation method most commonly used today is **simultaneous distillation and extraction** (SDE), modified from original Nickerson-Likens distillation head (Fig. 14.2) In this apparatus, a sample is boiled in one side flask and a small amount of extracting solvent in another. The product steam and solvent vapors are intermixed and condensed; the solvent extracts the organic volatiles from the condensed steam. The solvent and extracted distillate return to their respective flasks and are distilled to again extract the volatiles from the food. Its one-step isolation-concentration of flavor constituents allows a dramatic time saving over the separated operation and, because of their continuous recycling, a great reduction of organic solvents used. The drawback inherent in SDE is artifact formation. The elevated temperature applied during distillation may lead to lipid oxidation, Maillard browning, or Strecker reaction, which introduces errors. Also, the SDE system can be operated under vacuum, but it is not easy to regulate. Furthermore, some food aroma compounds, such as furaneol, may have low recovery by the SDE method [16]. While the distillation method is convenient and efficient, the volatile isolate can be contaminated by: (1) artifacts formed from solvents used in extraction, antifoam agents, and steam supply (contaminated water), (2) thermally-induced chemical changes, and (3) leakage of contaminated laboratory air into the system.



14.2 figure Simultaneous distillation and extraction system for sample distillation

14.2.2.4 *Solvent Extraction*

Solvent extraction is often the preferred method for the recovery of volatiles from foods. Recovery of volatiles will depend upon solvent choice and the solubility of the analytes being extracted. Solvent extraction typically involves the use of an organic solvent (unless sugars, amino acids, or some other water-soluble components are of interest).

Solvent extractions may be carried out in quite elaborate equipment, such as supercritical CO_2 extractors, or can be as simple as a batch process in a separatory funnel. Batch extractions can be quite efficient if multiple extractions and extensive shaking are used [17]. The continuous extractors (liquid-liquid) are more efficient but require more costly and elaborate equipment.

Extraction with organic solvents limits the method to the isolation of volatiles from fat-free foods (e.g., wines; some breads, fruits, and berries; some vegetables; and alcoholic beverages). Even for the fat-free food systems, a small amount of fats, waxes, or other nonvolatile compounds can be extracted. These nonvolatile materials need to be removed; otherwise, they will interfere with subsequent concentration and GC analysis.

The nonvolatile compounds can be removed using **high vacuum distillation, molecular distillation, or solvent-assisted flavor evaporation** (SAFE) distillation. SAFE is a compact and versatile distillation apparatus (Fig. 14.3) which can offer fast and reliable isolation of volatiles from complex matrices [18]. During SAFE distillation, samples with extracting solvent are introduced slowly into the flask (top left) at low temperature under high vacuum, and volatiles and solvents are evaporated instantly and condensed in a cold trap (right, typically liquid nitrogen), leaving nonvolatiles in another flask. SAFE is highly efficient and can be used to isolate volatiles from solvent extracts or even directly from foods or beverages. The drawback is that compounds with high boiling points may not be completely recovered.

14.2.2.5 *Solid-Phase Extraction*

The extractions discussed above involve the use of two immiscible phases (water and an organic solvent). However, a newer and very rapidly growing alternative to such extractions is **solid-phase extraction** (SPE) [19, 20]. In one version of this technique, a liquid sample (most often aqueous based) is passed through a column (2–10 mL vol) filled with chromatographic packing or a filter disk (25–90 mm in diameter) that has the chromatographic packing embedded in it. The chromatographic packing (i.e., stationary phase coated on silica solid supports) may be any of a number of different materials (e.g., ion-exchange resins or a host of different reversed- or normal-phase HPLC column packings).

When a sample is passed through the cartridge or filter, analytes that have an affinity for the chromatographic phase will be retained on the phase, while those with little or no affinity will pass through. The phase is next rinsed with water, perhaps a weak solvent (e.g., pentane), and then a stronger solvent (e.g., dichloromethane). The strong eluent is chosen such that it will remove the analytes of interest.

SPE has been a very popular method for sample extraction and cleanup. Many SPE columns are commercially available in different phases and formats. C18, amino, and silica gel are commonly used phases for food component analysis. C18 cartridges have high retention for nonpolar compounds, whereas highly cross-linked styrene-divinylbenzene (DVB) copolymers can extract a wide range of nonpolar and polar compounds. The new generation of polymers (Oasis® HLB, copolymer of divinylbenzene and N-vinylpyrrolidone) can even extract lipophilic, hydrophilic, acidic, and basic compounds with a single cartridge and perform fractionation based on the functional group [21].

Overall, solid-phase extraction has numerous advantages over traditional liquid-liquid extractions including: (1) less solvent required, (2) speed, (3) less glassware needed (less cost and potential for contamination), (4) better precision and accuracy, (5) minimum solvent evaporation before GC analysis, and (6) being readily automated. However, SPE method development requires tedious empirical pro-

14.3 figure Solvent-assisted flavor evaporation (SAFE) distillation system

cedures to achieve the best separation and recovery for an analyte.

Another version of this method is called **solid-phase microextraction** (SPME). This method was developed originally for environmental work [22, 23]. In this adaptation, the phase is bound onto a fine fused silica or metal filament (e.g., fiber) (approximately the size of a 10-µl syringe needle, Fig. 14.4). The fiber is immersed in a sample or in the headspace above a sample. After the desired extraction time, the fiber is pulled into a protective metal sheath, removed from the sample, and forced through the septum of a gas chromatograph where the adsorbed volatiles are thermally desorbed from the fiber (Fig. 14.5).

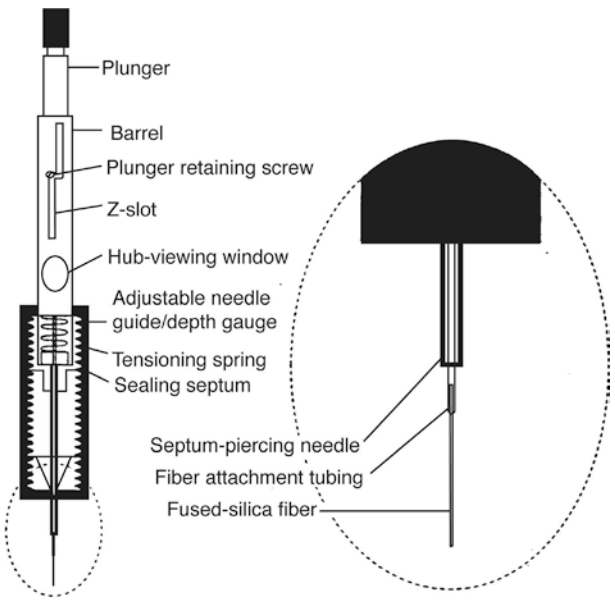
Many different phases of fiber are commercially available, and compounds with a wide range of polarity or volatility can be analyzed. PDMS (polydimethylsiloxane) is a nonpolar phase coating and can be used to extract nonpolar compounds. Polar analytes can be extracted with polar phases (e.g., polyacrylate and Carbowax® coatings). Divinylbenzene (DVB) coating is good for many volatile compounds. The coating has various film thicknesses. Thicker film fibers (100 µm) are better for volatiles, whereas thinner film fibers (7 and 30 µm) are better for larger molecules. Multiphase fibers (such as Carboxen®/PDMS, Carboxen®/DVB/PDMS) are also available to extract both polar and nonpolar compounds. A Carboxen®/PDMS fiber is good for highly volatile compounds, particularly for volatile sulfur compounds [24]. A 2-cm 50/30 DVB/Carboxen®/PDMS fiber is frequently used for volatile and semi-volatile flavor analysis in food system.

SPME has been widely used to extract volatile and semi-volatile organic compounds from environmental, biological, and food samples [25–29]. The main advantages of this technique are the simplicity of operation and no solvent contamination or disposal. With the autosampler, high precision and sample throughput can be achieved. However, depending on the fiber used, the compounds extracted can be highly selective. Furthermore, SPME has limited absorption capacity, and the fiber can be easily saturated. Thus, other volatile compounds in the sample can compete for the active site and cause competitive absorption. Other drawbacks are relatively poor reproducibility, fiber lot-to-lot variation, sensitivity to solvents, life time of the fiber, and difficulty in quantification.

Solid-phase dynamic extraction (SPDE) is another technique for volatile extraction. SPDE is similar to SPME, except the phase is coated inside a special needle. A gas-tight syringe is used for SPDE to draw the headspace of food and the volatiles are absorbed by the phase. The process can be repeated many times by moving the plunger up and down to achieve maximum absorption. The needle then can be injected into GC where the volatiles are thermally desorbed for analysis. Different phases are available. The volume of the phase is about 4.5 µl compared with only 0.6 µl for SPME, so the SPDE has less issue with analyte saturation and competition.

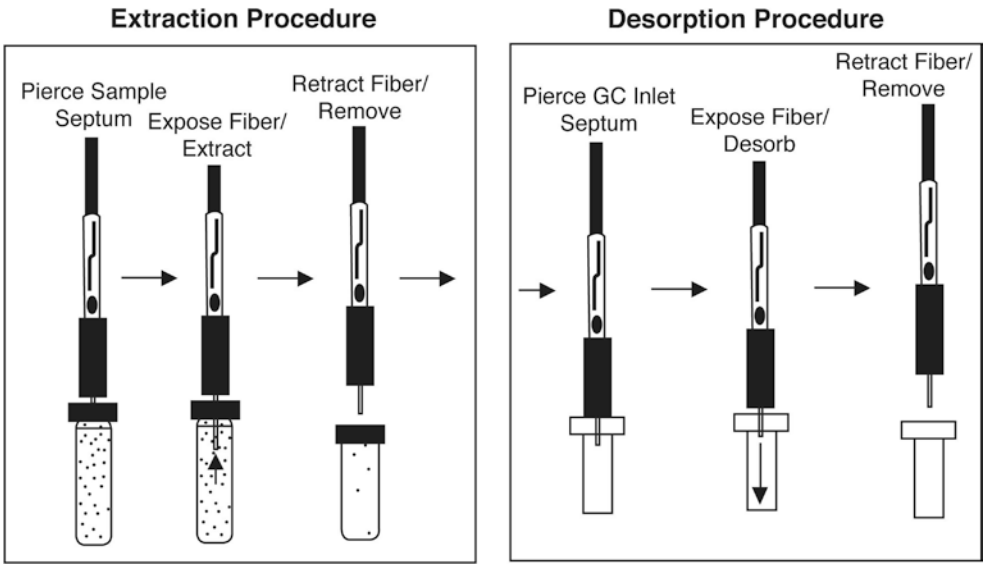
Stir bar sorptive extraction (SBSE) is a relatively new technique for volatile extraction. In SBSE (Fig. 14.6), a magnet stir bar is enclosed in a glass coated with a thick film of polymers such as polydimethylsiloxane (PDMS). The bar spins in the sample solution and absorb the analytes. Subsequently, the stir bar is transferred to a compact thermal desorption unit (TDU) mounted on a gas chromatograph (GC). The analytes are thermally desorbed in the TDU and delivered to a GC column (Fig. 14.7). The stir bar also can just hang in the headspace for volatile extraction the same way as the SPME.

The stir bar has 50–250 times more volume of absorbent than SPME. The PDMS volume is about 0.5 µl with SPME compared to 24–126 µl with SBSE. Due to the increased volume of absorbent phase, SBSE has much higher sensitivity than SPME and has minimum competition and saturation effects [30, 31]. The high sensitivity (ppt to ppg) and flexibility of PDMS-based SBSE for nonpolar and medium polar compounds make it an effective and time-saving method for extracting trace volatile compounds from complex matrices [31]. Food samples even containing fat (<3 %) or alcohol (<10 %) can be efficiently extracted with this technique.



14.4 figure

Schematic of a solid-phase microextraction (SPME) device [21] (Courtesy of Dr. Janusz Pawliszyn, Dept. of Chemistry, University of Waterloo, Waterloo, Ontario, Canada)



14.5 figure Schematic showing the steps involved in the use of a solid-phase microextraction (SPME) device (Reprinted with permission of Supelco, Bellefonte, PA.)

SBSE is considered to be superior to SPME in terms of sensitivity and accuracy for determinations at trace level in difficult matrices. The PDMS phase is robust; it does not absorb water, alcohol, or pigment; and it is very good for flavor extraction of alcoholic beverages [32, 33]. PDMS-based SBSE had been successful applications in trace analysis in environmental, food, and biomedical fields [34–37]. However, the PDMS phase is not selective for polar compounds.

In addition to PDMS nonpolar phase, polyacrylate (PA) stir bar and the ethylene glycol (EG)-silicone stir bar are also commercially available. These phases, particularly EG-silicone, extract polar compounds more efficiently than the PDMS due to their polar nature. In addition, the EG-silicone can efficiently extract nonpolar compounds due to the properties of silicone materials. The use of new phase stir bars has been applied to different analytical fields including food and wine [38].

14.2.2.6 *Direct Injection*

It is theoretically possible to analyze some foods by direct injection of the food into a gas chromatograph. Assuming one can inject a 2–3-µl sample into a GC and the GC has a detection limit of 0.1 ng (0.1 ng/2 µl), one could detect volatiles in the sample at concentrations greater than 50 ppb. Problems with direct injection arise due to: (1) thermal degradation of any nonvolatile food constituents in the injector, (2) decreased separation efficiency due to water in the food sample, and (3) contamination of the column and injection port by nonvolatile materials. Despite these concerns, direct injection is sometimes used to determine oxidation in vegetable oils [39, 40]. A relatively large volume of oil (50–100 µl) can be directly injected into an injection port of a GC that has been packed with glass wool. Since vegetable oils are reasonably thermally stable and free of water, this method is particularly well suited to oil analysis.

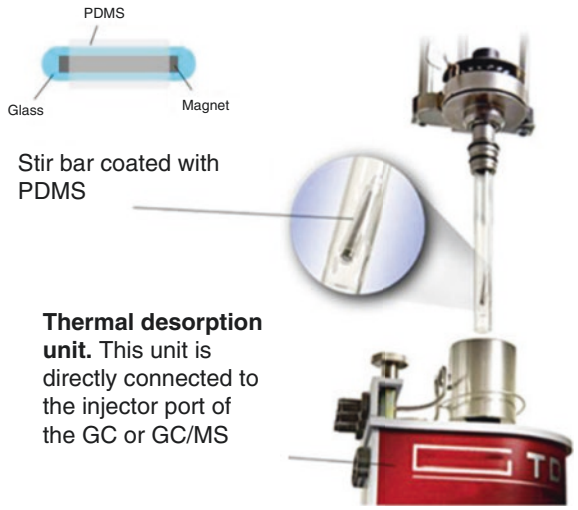
14.6 figure Diagram of stir bar sorptive extraction (SBSE) device (Courtesy of Gerstel, Inc., Linthicum, MD)

There are numerous other approaches for the isolation of volatiles from foods. Some are simple variations of these methods, while others are unique. Several review articles are available that provide a more complete view of methodology [11, 12, 41].

14.2.3 Sample Derivatization

The compounds one wishes to determine by GC must be thermally stable under the GC conditions employed. Thus, for some compounds (e.g., pesticides, aroma compounds, polychlorinated biphenyls (PCBs), and volatile contaminants) the analyst can simply isolate the components of interest from a food as discussed above and directly inject them into the GC. For compounds that are thermally unstable, too low in volatility (e.g., sugars and amino acids), or yield poor chromatographic separation due to polarity (e.g., phenols or acids), a derivatization step must

be included prior to GC analysis (see also Chaps. 19 and 23). A listing of some of the reagents used in preparing volatile derivatives for GC is given in Table 14.2. Most commonly used derivatizations are: (1) silylation for alcohols, cholesterol, and carbohydrates, (2) esterification for fatty acids, and (3) oxime formation and derivatization for aldehydes and ketones. The conditions of use for these reagents are often specified by the supplier or can be found in the literature [42]. Derivatization can be performed in liquid extract, or on an SPE extraction column, on an SPME fiber, or on SBSE, to allow automated extraction, derivatization, and GC analysis.



14.7 figure Design of a commercial stir bar sorptive extraction device (Courtesy of Gerstel, Inc., Linthicum, MD) (www.gerstel.com)

14.3 GAS CHROMATOGRAPHIC HARDWARE AND COLUMNS

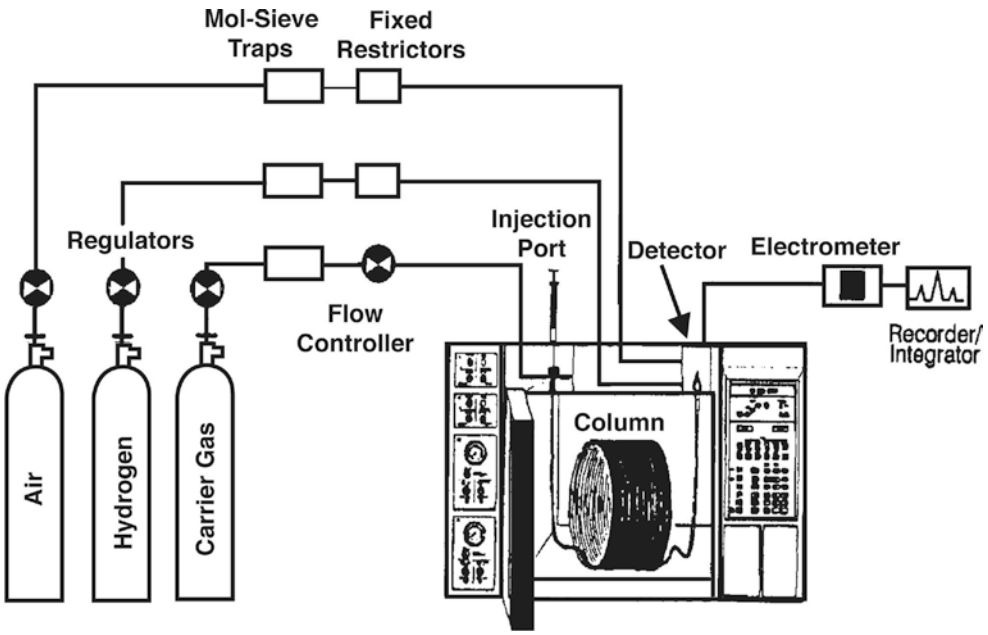
The major parts of a GC are the **gas supply** system, **injection port**, **oven**, **column**, **detector**, **electronics**, and **recorder/data handling system** (Fig. 14.8). The hardware as well as operating parameters used in any GC analysis must be accurately and completely recorded. The information that must be included is presented in Table 14.3.

14.3.1 Gas Supply System

The gas chromatograph will require at least a supply of carrier gas and, most likely, gases for the detector (e.g., hydrogen and air for a FID). The gases used must be of high purity and all regulators, gas lines, and fittings of good quality. High-quality pressure regulators must be used to provide stable and continuous gas supply. The regulators should have stainless steel rather than polymer diaphragms since polymers will give off volatiles that may contribute peaks to the analytical run. All gas lines must be clean and contain no residual drawing oil. Nitrogen, helium, and hydrogen gases are typically used as the **carrier gas** (i.e., mobile phase) to transport the analytes in the GC column. The carry gas line should have traps (moisture trap, oxy-

14.2 table Reagents used for making volatile derivatives of food components for GC analysis

Reagent	*Chemical group*	*Food constituent*
Silyl reagents	Hydroxy, amino carboxylic acids	Sugars, sterols, amino acids
Trimethylchlorosilane/hexamethyldisilazane		
BSA [*N,O*-bis(trimethylsilyl) acetamide		
BSTFA (*N,O*-bis (trimethylsilyl) trifluoroacetamide)		
t-BuDMCS (*t*-butyldimethylchlorosilyl/imidazole)		
TMSI (N-trimethylsilylimidazole)		
Esterifying reagents	Carboxylic acids	Fatty acids, amines, amino acids, triglycerides, wax esters, phospholipids, cholesteryl esters
Methanolic HCl		
Methanolic sodium methoxide		
N,N-Dimethylformamide dimethyl acetal		
Boron trifluoride methanol		
Miscellaneous		
Acetic anhydride/pyridine	Alcoholic and phenolic	Phenols, aromatic hydroxyl groups, alcohols
N-Trifluoroacetylimidizole/*N*-heptafluorobutyrlimidizole	Hydroxy and amines	Same as above
Alkylboronic acids	Polar groups on neighboring atoms	
O-Alkylhydroxylamine	Compounds containing both hydroxyl and carbonyl groups	Ketosteroids, prostaglandins



14.8 figure Diagram of a gas chromatographic system (Courtesy of Agilent Technologies, Inc., Santa Clara, CA)

gen trap, and hydrocarbon trap) in line to remove any moisture and contaminants from the incoming gas. These traps must be periodically replaced to maintain effectiveness.

14.3.2 Injection Port

14.3.2.1 *Hardware*

The injection port serves the purpose of providing a place for sample introduction, its vaporization, and possibly some dilution and splitting. Liquid samples make up the bulk of materials analyzed by GC, and they are always done by syringe injection (manual or automated). The injection port contains a soft septum that provides a gas-tight seal but can be penetrated by a syringe needle for sample introduction.

Samples may be introduced into the injection port using a **manual syringe technique** or an **automated sampling system**. Manual sample injection is generally the largest single source of poor precision in GC analysis. Ten-microliter syringes are usually chosen since they are more durable than the microsyringes, and sample injection volumes typically range from 1 to 3 µl. These syringes will hold about 0.6 µl in the needle and barrel (this is in addition to that measured on the barrel). Thus, the amount of sample that is injected into the GC depends upon the proportion of this 0.6 µl that is included in the injection and the ability of the analyst to accurately read the desired sample volume on the syringe barrel. This can be quite variable for the same analyst and be grossly different between analysts. This variability between injections and the small sample volumes injected are the reasons internal (vs. external) standards are common for GC (see Chap. 12, Sect. 12.5.3).

14.3 table Gas chromatographic hardware and operating conditions to be recorded for all GC separations

Parameter	*Description*
Sample	Name and injection volume
Injection	Type of injection (e.g., split versus splitless and conditions (injection port flow rates))
Capillary column	Phase, length, diameter, film thickness, and manufacturer
Packed columns	Solid support, size mesh, coating, loading (%)
Temperatures	Injector, detector, oven, and any programming information
Carrier gas	Flow rate (velocity) and type
Detector	Type

14.3.2.2 *Sample Injection Techniques*

The sample must be vaporized in the injection port to pass through the column for separation. This vaporization can occur quickly by flash evaporation (standard injection ports) or slowly in a gentler man-

ner (temperature-programmed injection port or on-column injection). The choice depends upon the thermal stability of the analytes. Due to the various sample as well as instrumental requirements, there are several different designs of injection ports available.

14.3.2.2.1 Split Injection

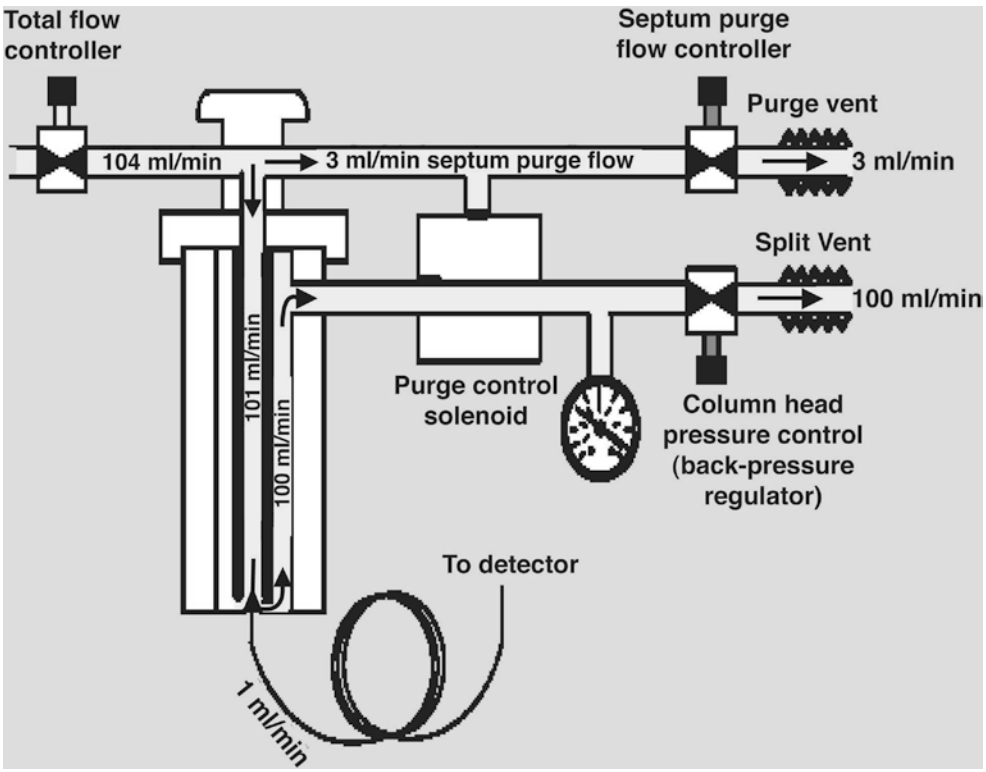
Capillary columns have limited capacity, and the injection volume may have to be reduced to permit efficient chromatography. The injection port may serve the additional function of splitting the injection so that only a portion of the analyte goes on the column (i.e., **split injection**) (Fig. 14.9, split vent valve open). The injection port is operated about 20 °C warmer than the maximum column oven temperature (commonly at 250 °C). The sample may be diluted with carrier gas to accomplish a split (1: 50–1: 100 preferred, split ratio = (column flow)/(column flow + venting flow), whereby only a small portion (1 part) of the analyte (more exactly, 1 part of gas flow) goes on the column, and the majority (49/50 or 99/100 parts) of the analytes are vented to the split vent. High split ratio typically gives a sharp, narrow peak.

14.3.2.2.2 Splitless Injection

To increase the sensitivity, a splitless injection mode can be used. In **splitless injection**, the split vent valve is closed and all of the analyte goes on the column (Fig. 14.5). Similar to the split injection, the temperature of the injector is operated at 20 °C higher than the maximum column oven temperature. Splitless injection requires to set up the initial column temperature 10–20 °C lower than the boiling point of the sample solvent, so the solvent can recondense in the column for acceptable chromatography of early eluting compounds (called solvent effect).

14.3.2.2.3 Programmed Temperature Vaporization Injection

For **programmed temperature vaporization injection** (PTV) ports, the sample is introduced into an ambient temperature port and then it is temperature programmed to some desired temperature. Since the sample is not introduced to the hot injector, the technique is desired for temperature-sensitive analytes. In addition, this technique is very useful to inject large amount sample when it is used together with split/splitless injection mode (solvent vent) to increase the sensitivity. For example, 10 µl of liquid sample can be injected at low temperature using a high split ratio to let the solvent to vent out, and then the injection mode can be changed to "splitless" as the injector is heated up to evaporate and transfer analytes onto the column.



14.9 figure Schematic of a GC injection port (Courtesy of Agilent Technologies, Inc., Santa Clara, CA)

14.3.2.2.4 On-Column Injection

On-column injection is a technique whereby the sample is directly introduced into the column for which the temperature is at that of the GC oven. The sample is then slowly volatized as the oven heats up. The initial oven temperature needs to be below the boiling point of the solvent. This technique is good for thermally labile analytes.

14.3.2.2.5 Thermal Desorption Injection

The volatiles can be introduced onto the head of a GC column for chromatographic separation directly from food samples through **thermal desorption**. The sample is heated in a thermal desorption unit, and the volatiles are carried through to a split/splitless injector. Cryofocusing with liquid nitrogen or CO_2 either in the injector or column is needed to attain sharp peaks. Alternatively, the volatiles can be retained using an absorbent such as a Tenax® trap during the purge stage and then thermally desorbed onto the column. The samples can be extracted with SPME or SBSE techniques described previously (Sect. 14.2.2.5) and then thermally desorbed onto the column for analysis. This technique has gained popularity to analyze volatile aroma compounds in foods including friuts [36, 43] and wine [45].

14.3.3 Oven

The oven controls the temperature of the column. In GC, one takes advantage of both an interaction of the analyte with the stationary phase and the boiling point for separation of compounds. Thus, the injection is often made at a lower oven temperature and is then temperature programmed to some elevated temperature. While analyses may be done isothermally, temperature-programmed runs are most common. It should be obvious that higher temperatures will cause the sample to elute faster and, therefore, be at a cost of resolution.

Oven temperature program rates can range from as little as 0.1 °C/min to the maximum temperature heating rate that the GC can provide. A rate of 2–10 °C/min is most common.

The capillary column (Sect. 14.3.4.2) also can be directly heated with an insulated heating wire based on **low thermal mass** (LTM) **technology**. A temperature sensor is mounted on the column. The column, the heating wire, and the sensor are all coiled together and wrapped with alumina foil. The column can be uniformly heated very rapidly to improve the separation and efficiency. Since the system does not have much void volume and other insulation materials, it cools very quickly. The total heating and cooling cycle is much shorter than the traditional standard GC oven, which makes it ideal for fast GC analysis. The module is available with almost any standard capillary GC column.

14.3.4 Column and Stationary Phases

The GC column may be either **packed** or **capillary**. Early chromatography was done on packed columns, but the advantages of capillary chromatography with regard to separation efficiency (see Sect. 14.4.2) so greatly outweigh those of packed column chromatography that few packed column instruments are sold any longer. While some use high-resolution gas chromatography (HRGC) to designate capillary GC, GC today means capillary chromatography to most individuals.

14.3.4.1 *Packed Columns*

The packed column is most commonly made of stainless steel or glass and may range from 1.6 to 12.7 mm in outer diameter and be 0.5–5.0 m long (generally 2–3 m). It is packed with a granular material consisting of a "liquid" coated on an allegedly inert solid support. The **solid support** is most often diatomaceous earth (skeletons of algae) that has been purified, possibly chemically modified (e.g., silane treated), and then sieved to provide a definite mesh size (60/80, 80/100, or 100/120).

The liquid loading is usually applied to the solid support at 1–10% by weight of the solid support. While the liquid coating can be any one of the approximately 200 available, the most common are silicone-based phases (methyl-, phenyl-, or cyano-substituted) and Carbowax™ (ester based).

The liquid phase and the percent loading are determined by the analysis desired. The choice of liquid is typically such that it is of similar polarity to the analytes to be separated. Loading influences time of analysis (retention time is proportional to loading), resolution (generally improved by increasing phase loading, within limits), and "bleed." The liquid coatings are somewhat volatile and will be lost from the column at high temperatures (this is dependent upon the phase itself). This results in an increasing baseline (**column bleeding**) during temperature programming.

14.3.4.2 *Capillary Columns*

The capillary column is a hollow fused silica glass (<100-ppm impurities) tube ranging in length from 5 to 100 m. The walls are so thin, ca. 25 μm, that they are flexible. The column outer walls are coated with a polyamide material to enhance strength and reduce breakage. Column inner diameters are typically 0.1 mm (**microbore**), 0.2–0.32 mm (**normal capillary**), or 0.53 mm (**megabore**).

Megabore columns (0.53 mm i.d.) were initially designed to replace packed columns without modification of instrumentation hardware. The most commonly used capillary columns are now 0.32 and 0.25 mm i.d. columns. Smaller diameters (0.10 mm and 0.18 mm i.d.) columns are used for fast GC analysis. The most common lengths of the GC column are 15, 30, and 60 m although special column can be over

100 m. Longer column will require longer analysis time. Although longer column gives improved resolution, this benefit of better separation is not particularly obvious due to already high-resolution power of capillary GC column.

As many as 200 different stationary phases have been developed for GC. As GC has changed from packed to capillary columns, fewer stationary phases are now in use since column efficiency has substituted for phase selectivity (i.e., high efficiency has resulted in better separations even though the stationary phase is less suited for the separation). Now, we find fewer than a dozen phases in common use (Table 14.3). The most durable and efficient phases are those based on polysiloxane (-Si-O-Si-).

Stationary phase selection involves some intuition, knowledge of chemistry, and help from the column manufacturer and the literature. There are general rules, such as choosing polar phases to separate polar compounds and the converse or phenyl-based column phase to separate aromatic compounds. However, the high efficiency of capillary columns often results in separation even though the phase is not optimal. For example, a 5% phenyl-substituted methyl silicone phase applied to a capillary column will separate most polar and nonpolar compounds (Table 14.4).

Liquid coating is chemically bonded to the glass walls of capillary columns and internally cross-linked at phase thicknesses ranging from 0.1 to 5 μm. Film thickness directly affects separation. Thicker films retain compounds longer in the stationary phase, thus the analytes will have longer interaction with the stationary phase to achieve separation. Generally, a thick-filmed column should be used to separate very volatile compounds. For example, an FFAP (polyethylene glycol treated with nitroterephthalic acid) column with 1-μm film thickness can effectively hold and separate hydrogen sulfide and other highly volatile sulfur compounds [24]. However, a thick film also will give higher baseline due to bleeding. A thin-film (0.25 μm) column is usually used to separate high-molecular-weight compounds; the analytes will stay in the stationary phase less time. A thin-film column also has less bleeding at high temperature, so it is used frequently for GC-MS.

Most compounds can be separated using nonpolar 5% phenyl 95% dimethylpolysiloxane-based columns (e.g., DB-5, Agilent-5, RTX-5). This type of column has a very wide temperature range (−60 °C to 325 °C) and is very stable. However, to separate very polar compounds such as alcohols and free fatty acids, a polar column is needed such as WAX (polyethylene glycol) or FFAP. A WAX type of column has superior

14.4 table Common stationary phases

Composition	*Polarity*	*Applications*[a]	*Phases with similar McReynolds constants*[b]	*Temperature limits*[c]
100% Dimethyl polysiloxane (gum)	Nonpolar	Phenols, hydrocarbons, amines, sulfur compounds, pesticides, PCBs	OV-1 SE-30	−60 °C–325 °C
100% Dimethyl polysiloxane (fluid)	Nonpolar	Amino acid derivatives, essential oils	OV-101, SP-2100	0–280 °C
5% Phenyl 95% dimethyl polysiloxane	Nonpolar	Fatty acids, methyl esters, alkaloids, drugs, halogenated compounds	SE-52 OV-23 SE-54	−60 °C–325 °C
14% Cyanopropylphenyl-methyl polysiloxane	Intermediate	Drugs, steroids, pesticides	OV-1701	−200 °C–280 °C
50% Phenyl, 50% methyl methyl polysiloxane	Intermediate	Drugs, steroids, pesticides, glycols	OV-17	60–240 °C
50% Cyanopropylmethyl, 50% phenylmethyl polysiloxane	Intermediate	Fatty acids, methyl esters, alditol acetates	OV-225	60–240 °C
50% Trifluoropropyl polysiloxane	Intermediate	Halogenated compounds, aromatics	OV-210	45–240 °C
Polyethylene glycol-TPA modified	Polar	Acids, alcohols, aldehydes, acrylates, nitrites, ketones	OV-351 SP-1000	60–240 °C
Polyethylene glycol	Polar	Free acids, alcohols, esters, essential oils, glycols, solvents	Carbowax 20 M	60–220 °C

[a]Specific application notes from column suppliers provide information for choosing a specific column
[b]McReynolds constants are used to group stationary phases together on the basis of separation properties
[c]Stationary phases have both upper and lower temperature limits. Lower temperature limit is often due to a phase change (liquid to solid) and upper temperature limit to a volatilization of phase

separation power; however, it has a narrow usable temperature range (40–240 °C). It becomes solid (lost separation power) at low temperature and bleeds highly at high temperature. It is also sensitive to residue oxygen, so it deteriorates quickly if oxygen is not removed from the carrier gas. Cyanopropyl-based columns (SP-2560, CP-Sil 88) are good for *trans*-fatty acid esters. Other specialty phase columns have been developed to improve specific resolution. Ionic liquid-based GC columns [45, 46] have very good thermal stability and can be used to analyze very polar compounds. Ionic liquid column has been used to separate fatty acid methyl esters [47]. However, their applications in food systems are still limited. The ionic liquid column cannot easily replace WAX-based column due to the strong interactions with acids (octanoic and decanoic acids), resulting in poor chromatography for these compounds. The β-cyclodextrin-based column is useful to separate chiral isomers of essential oil and other volatile compounds [48].

14.3.4.3 *Gas-Solid (PLOT) Chromatography*

Gas-solid chromatography is a very specialized area of chromatography accomplished without using a liquid phase – the analyte interaction is with a porous material. This material has been applied both to packed and capillary columns. For the capillary column, the porous material is chemically or physically (by deposition) coated on the inner wall of the capillary, and the column is called a **porous-layer open-tabular** (PLOT) column. The most popular porous materials are alumina oxide, carbon, molecular sieve, and synthetic polymer such as Poropak® or Chromosorb® (trade names of polymers based on vinyl benzene). Separations usually involve water or other very volatile compounds such as headspace gas composition (N_2, O_2, CO_2, CO) in packaged food and ethylene during fruit ripening and storage.

14.3.5 Detectors

There are numerous detectors available for GC, each offering certain advantages in either sensitivity (e.g., electron capture) or selectivity (e.g., atomic emission detector). The most common detectors are the **FID, thermal conductivity** (TCD), **electron capture** (ECD), **flame photometric** (FPD), **pulsed flame photometric detector** (PFPD), **photoionization** (PID) detectors, and **mass spectrometry** (MS) (see Chap. 11 on MS). The operating principles and food applications of these detectors are discussed below. The characteristics of these detectors are summarized in Table 14.5.

Not described in detail below with traditional GC detectors, but mentioned here is **GC-olfactory** (GC-O). In GC systems with an olfactory detector outlet, the column effluent is split so that a portion of the effluent goes to a "sniffing port" and the remainder goes to a GC detector. The sniffing port typically consists of a glass cone for the operator to use their nose to identify "aroma-active" components eluting from the column (see Chap. 35, Sect. 35.5.2.1).

14.3.5.1 *Thermal Conductivity Detector*

14.3.5.1.1 Operating Principles

As the carrier gas passes over a hot filament (tungsten), it cools the filament at a certain rate depending on carrier gas velocity and composition. The temperature of the filament determines its resistance to electrical current. As a compound elutes with the carrier gas, the cooling effect on the filament is typically less, resulting in a temperature increase in the

14.5 table Characteristics of most common detectors for gas chromatography

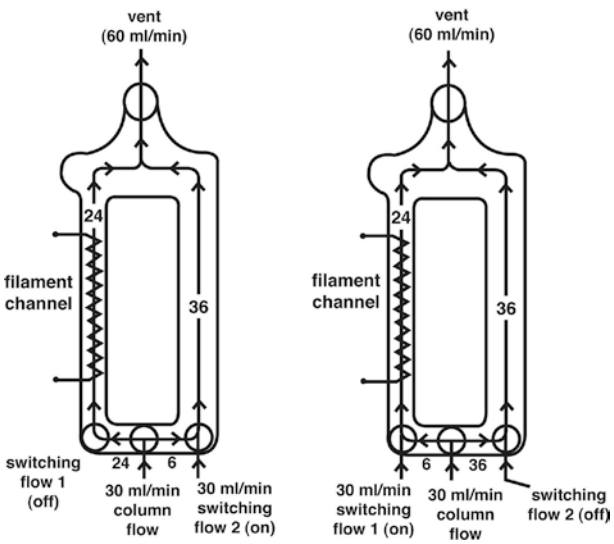
Characteristic	*Thermal conductivity detector*	*Flame ionization detector*	*Electron capture detector*	*Flame photometric detector*	*Photoionization detector*
Specificity	Very little. Detects almost anything, including H_2O. Called the "universal detector"	Most organics	Halogenated compounds and those with nitro- or conjugated double bonds	Organic compounds with S or P (determined by which filter is used)	Depends on ionization energy of lamp relative to bond energy of analytes
Sensitivity limits	ca. 400 pg. Relatively poor. Varies with thermal properties of compound	10–100 pg for most organics. Very good	0.05–1 pg. Excellent	2 pg for S and 0.9 pg for P compounds. Excellent	1–10 pg depending on compound and lamp energy. Excellent
Linear range	10^4, poor. Response easily becomes nonlinear	10^6–10^7. Excellent	10^4. Poor	10^4 for P. 10^3 for S	10^7. Excellent

filament and an increase in resistance that is monitored by the GC electronics. Older style TCDs used two detectors and two matching columns; one system served as a reference and the other as the analytical system. Newer designs use only one detector (and column), which employs a carrier gas switching value to pass alternately carrier gas or column effluent though the detector (Fig. 14.10). The signal is then a change in cooling of the detector as a function of which gas is passing through the detector from the analytical column or carrier gas supply (reference gas flow).

The choice of carrier gas is important since differences between its thermal properties and the analytes determine response. While hydrogen is the best choice, He is most commonly used since H_2 is flammable.

14.3.5.1.2 Applications

The most valuable properties of this detector are that it is *universal* in response and nondestructive to the sample. Thus, it is used in food applications for which there is no other detector that will adequately respond to the analytes (e.g., water, permanent gases, CO) or when the analyst wishes to recover the separated compounds for further analysis (e.g., trap the column effluent for infrared, nuclear magnetic resonance (NMR), or sensory analysis). It does not find broad use because it is relatively insensitive, and often the analyst desires specificity in detector response to remove interfering compounds from the chromatogram. The detector is most useful for gas composition analysis (CO_2, CO, O_2, N_2) for food packaging and fruit ripening and self-life.



14.10 figure Schematic of the thermal conductivity detector (Courtesy of Agilent Technologies, Inc., Santa Clara, CA)

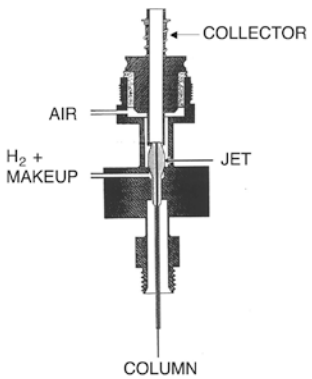
14.3.5.2 *Flame Ionization Detector*

14.3.5.2.1 Operating Principles

As compounds elute from the analytical column, they are burned in a hydrogen flame (Fig. 14.11). A potential (often 300 V) is applied across the flame. The flame will carry a current across the potential which is proportional to the organic ions present in the flame from the burning of an organic compound. The current flowing across the flame is amplified and recorded. The FID responds to organics on a weight basis. It gives virtually no response to H_2O, NO_2, CO_2, and H_2S and limited response to many other compounds. Response is best with compounds containing C-C or C-H bonds. The FID can be modified to include a methanizer to convert CO and CO_2 to methane using Ni catalyst and then detected by FID. An alternative is to oxidize all compounds to CO_2 first and then reduce to methane before FID. This approach increases the sensitivity and detectability for many carbon-containing compounds. In addition, since all compounds are converted to methane and have the same response on FID, the detector allows for the analysis of compounds without using standards.

14.3.5.2.2 Applications

The food analyst is most often working with organic compounds, to which this detector responds well. Its very good sensitivity, wide linear range in response (necessary in quantitation), and dependability make this detector the choice for most food work. Thus, this detector is used for virtually all food analyses for which a specific detector is not desired or sample destruction is acceptable (column eluant is burned in flame). This includes flavor studies, fatty acid analysis, carbohydrate analysis, sterols, contaminants in foods, and antioxidants.



14.11 figure Schematic of the flame ionization detector designed for use with capillary columns (Courtesy of Agilent Technologies, Inc., Santa Clara, CA)

14.3.5.3 *Electron Capture Detector*

14.3.5.3.1 Operating Principles

The ECD contains a radioactive foil coating that emits electrons as it undergoes decay (Fig. 14.12). The electrons are collected on an anode, and the standing current is monitored by instrument electronics. As an analyte elutes from the GC column, it passes between the radioactive foil and the anode. Compounds that capture electrons reduce the standing current and thereby give a measurable response. Halogenated compounds or those with high electrophilic compounds (diketones) give the greatest detector response. Unfortunately this detector becomes saturated quite easily and thus has a very limited linear response range.

14.3.5.3.2 Applications

In food applications, the ECD has found its greatest use in determining PCBs and pesticide residues (see Chap. 33). The specificity and sensitivity of this detector make it ideal for this application. It also is used to analyze diacetyl and other vicinal diketone in beer because ECD is very sensitive for vicinal diketones.

14.3.5.4 *Flame Photometric Detector and Pulsed Flame Photometric Detector*

14.3.5.4.1 Operating Principles

The FPD works by burning all analytes eluting from the analytical column and then measuring specific wavelengths of light that are emitted from the flame using a filter and photometer (Fig. 14.13). The wavelengths of light that are suitable in terms of intensity and uniqueness are characteristic of sulfur (S) and phosphorus (P). Thus, this detector gives a greatly enhanced signal for these two elements (several thousand folds for S- or P-containing organic molecules versus non-S or P-containing organic molecules). Detector response to S-containing molecules is nonlinear and thus quantification must be done with care.

The PFPD is very similar to the FPD. Unlike traditional flame photometric detection (FPD), which uses a continuous flame, the PFPD ignites, propagates, and self-terminates two to four times per second (Fig. 14.14). Specific elements have their own emission profile: hydrocarbons will complete emission early, while sulfur emissions begin at a relatively later time after combustion. Therefore, a timed "gate delay" can selectively allow for only emissions due to sulfur to be integrated, producing a clean chromatogram. This timed "gate delay" greatly improves the sensitivity. The PFPD can detect sulfur-containing compounds at a much lower detection limit than nearly all other methods of detection [49].

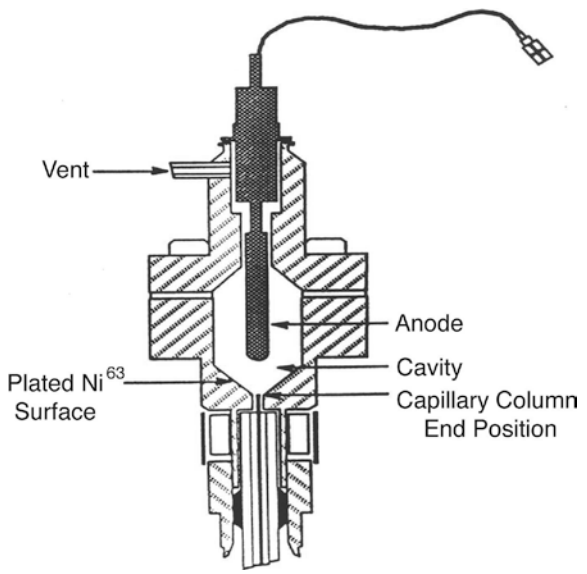
14.3.5.4.2 Applications

Both the FPD and PFPD have found major food applications in the determination of organophosphorus pesticides and volatile sulfur compounds in general. The determination of sulfur compounds has typically been in relation to flavor studies.

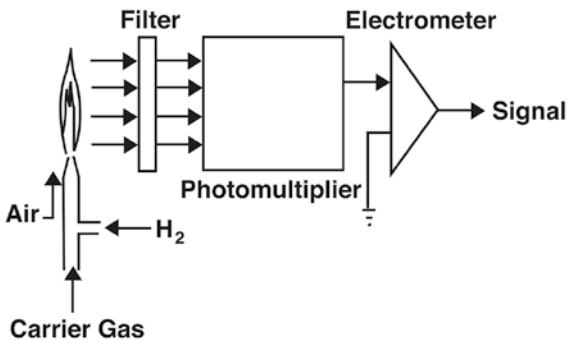
14.3.5.5 *Photoionization Detector*

14.3.5.5.1 Operating Principles

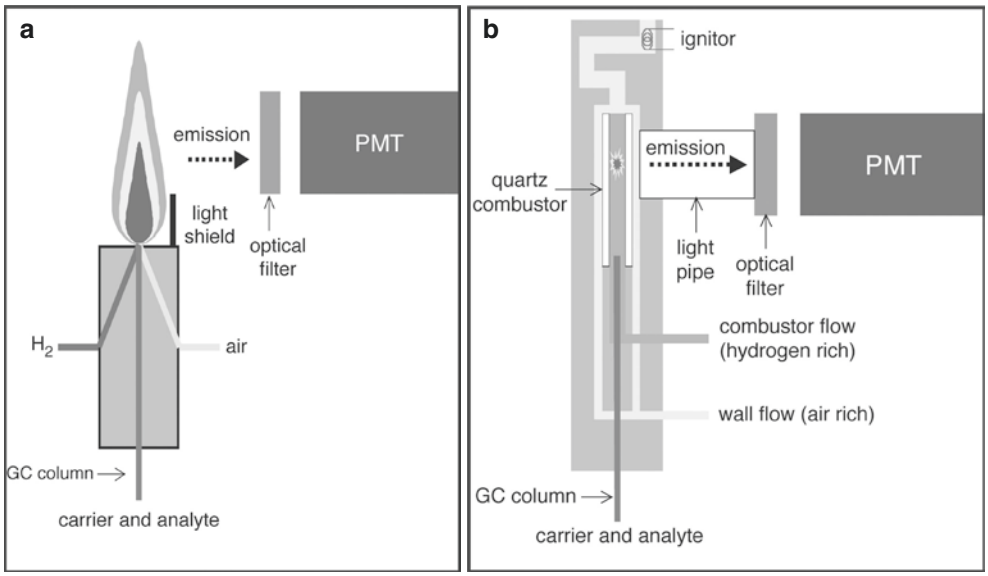
The PID uses ultraviolet (UV) irradiation (usually 10.2 eV) to ionize analytes eluting from the analytical column (Fig. 14.15). The ions are accelerated by a polarizing electrode to a collecting electrode. The small current formed is magnified by the electrometer of the GC to provide a measurable signal.



14.12 figure Schematic of the electron capture detector (Courtesy of Agilent Technologies, Inc., Santa Clara, CA)



14.13 figure Schematic of the flame photometric detector (Courtesy of Agilent Technologies, Inc., Santa Clara, CA)



14.14 figure Comparison of flame photometric detector (**a**) and pulsed flame photometric detector (**b**) (Courtesy of Varian Inc., Palo Alto, CA)

This detector offers the advantages of being quite sensitive and nondestructive and may be operated in a selective response mode. The selectivity comes from being able to control the energy of ionization, which will determine the classes of compounds that are ionized and thus detected.

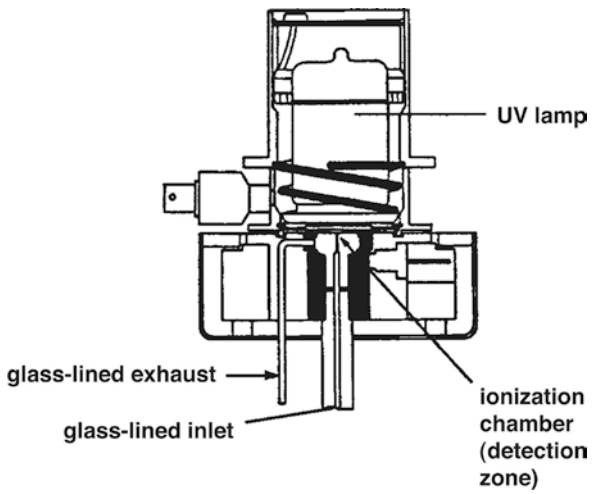
14.3.5.5.2 Applications

The PID finds primary use in analyses for which excellent sensitivity is required from a nondestructive detector. This is most often a flavor application in which the analyst wishes to smell the GC effluent to determine the sensory character of the individual GC peaks. While this detector might find broader use, the widespread availability of the FID (which is suitable for most of the same applications) meets most of these needs.

14.3.5.6 *Electrolytic Conductivity Detector*

14.3.5.6.1 Operating Principles

Compounds entering the **electrolytic conductivity detector** (ELCD) are mixed with a reagent gas (oxidizing or reducing depending on the analysis) in a nickel reaction tube producing ionic species. These products are mixed with a deionized solvent, interfering ions are scrubbed from the effluent, and the ionic analyte-transformation product is detected within the electrolyte conductivity cell. This detector can be used for the specific detection of sulfur-, nitrogen-, or halogen-containing molecules. For example, when operated in the nitrogen mode, analyte is mixed with H_2 gas and hydrogenated over a nickel catalyst at 850 °C. Acidic hydrogenation products are removed from the effluent by passage through an $Sr(OH)_2$ trap, and the NH_3 from the analyte passes to the conductivity cell where it is measured [50].



14.15 figure Schematic of the photoionization detector (Courtesy of Agilent Technologies, Inc., Santa Clara, CA)

14.3.5.6.2 Applications

This detector can be used in many applications for which element specificity is desired. Examples would be pesticide, herbicide, nitrosamine, or flavor analysis. The ELCD is very selective and quite sensitive, having

detection limits of 0.1–1 pg of chlorinated compounds, 2 pg for sulfur, and 4 pg for nitrogen.

14.3.5.7 *Thermionic Detector*

14.3.5.7.1 Operating Principles

The thermionic detector (also called the nitrogen phosphorus detector, NPD) is a modified FID in which a nonvolatile ceramic bead is used to suppress the ionization of hydrocarbons as they pass through a low-temperature fuel-poor hydrogen plasma. The ceramic bead is typically composed of rubidium that is heated to 300–350 °C. Most commonly this detector is used for the selective detection of nitrogen- or phosphorus-containing compounds. It does not detect inorganic nitrogen or ammonia.

14.3.5.7.2 Applications

This detector is primarily used for the measurement of specific classes of flavor compounds, nitrosamines, amines, and pesticides.

14.3.5.8 *Hyphenated Gas Chromatographic Techniques*

Hyphenated gas chromatographic techniques are those that combine GC with another major technique. Examples are **GC-AED** (atomic emission detector), **GC-FTIR** (Fourier transform infrared), and **GC-MS** (mass spectrometry). While all of the techniques are established methods of analysis in themselves, they become powerful tools when combined with a technique such as GC. GC provides the separation and the hyphenated technique provides the detector. GC-MS has long been known to be a most valuable tool for the identification of volatile compounds (see Chap. 11). The MS, however, may perform the task of serving as a specific detector for the GC by selectively focusing on ion fragments unique to the analytes of interest. The analyst can detect and quantify components without their gas chromatographic resolution in this manner. The same statements can be made about GC-FTIR (see Chap. 9). The FTIR can readily serve as a GC detector.

In GC-AED, the GC column effluent enters a microwave-generated helium plasma that excites the atoms present in the analytes. The atoms emit light at their characteristic wavelengths. This results in a very sensitive and specific elemental detector.

14.3.5.9 *Multidimensional Gas Chromatography*

Multidimensional gas chromatography (MDGC) greatly increases the separation ability of gas chromatography [51]. By simply coupling two GC columns, each of opposite polarity, an overall improvement in separation can be accomplished [52]. However, this tandem operation of GC columns does not actually represent multidimensionality, but rather resembles the use of a mixed-stationary phase column [51]. True MDGC involves a process known as orthogonal separation for which a sample is first dispersed by one column, and the simplified subsamples are then applied onto another column for further separation. MDGC techniques can be generally divided into two classes: (1) conventional, or "heart-cut," MDGC and (2) comprehensive two-dimensional gas chromatography (GC × GC).

14.3.5.9.1 Conventional Two-Dimensional GC

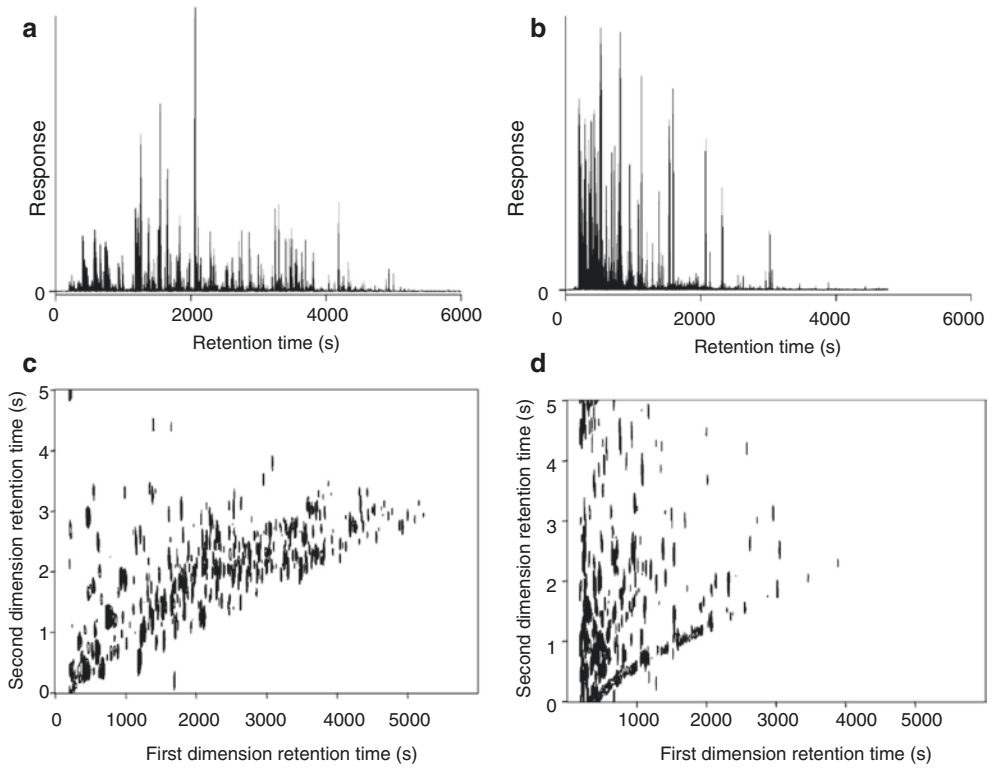
Conventional two-dimensional GC is achieved by using coupled capillary columns for which a small portion, or heart-cut, of the effluent from the first ("pre-separation") column is transferred to the second ("analytical") column. The concept of conventional MDGC is almost identical to that of preparative GC operations, for which one column is used to obtain a partially separated fraction of a complex aroma mixture, which is then reinjected onto another GC column, usually with an opposite stationary phase, for further separation. The only difference is that with MDGC there are no requirements for manual collection of the effluent obtained from the pre-separation column since the two columns are directly connected.

Because the second column in the MDGC system is only injected with a small portion of the total sample at one time, a large quantity of the sample can be injected onto the first column without the worry of chromatographic band smearing during analytical separations [53]. Therefore, trace compounds can be easily enriched for more successful detection and identification.

The MDGC technique is particularly useful to study enantiomers of flavor compounds. The interested compound can be "heart-cut" and transferred to an analytical column with an enantioselective stationary phase for good separation of targeted chiral compounds.

14.3.5.9.2 Comprehensive Two-Dimensional GC

Comprehensive two-dimensional GC is among the most powerful two-dimensional gas chromatographic techniques that have been developed to date (Fig. 14.16). Unlike conventional MDGC in which only particular segments are transferred from the pre-separation column onto the analytical column, comprehensive MDGC, or GC × GC, involves the transfer of the entire effluent from the first column onto a second column by way of a modulation interface so that complete two-dimensional data can be obtained for the entire run of the first column. The operation of the modulator involves the generation of narrow injection bands from the first column, which are continuously, but individually, sent to the secondary column for final separation.



14.16 figure Total ion chromatograms and their respective two-dimensional contour plots for an Arabica coffee extract separated by GC × GC using two different column sets: polar × nonpolar (**a**, **c**) along with nonpolar×polar (**b**, **d**) (Reprinted from Ref. [54], used with permission)

GC × GC requires that the second column can operate quickly enough to generate a complete set of data during the time that a single peak elutes from the first GC column, generally within 5 s [51, 55]. The data from both time axes are combined to create a set of coordinates for each peak so that the resultant chromatogram is actually a two-dimensional (2D) plane rather than a straight line. Peak area information can be obtained by summing the integration over both dimensions.

In GC × GC, the two columns perform independently of each other; therefore, the overall peak capacity becomes the product of the capacities for each column. Because analytes elute from the second column so quickly, data acquisition must be adequately fast enough for proper detection. Time-of-flight mass spectrometry (TOF-MS) and rapid-scanning quadrupole mass spectrometry (qMS) have both been used as effective detection methods for GC × GC to obtain mass spectral information [56, 57].

GC × GC has been used in the field of foods and beverages [58–60]. Although the instrumentation can be quite expensive, the use of comprehensive GC × GC for volatile aroma analysis has exponentially increased over the past few years as methodologies have become more established and systems have become commercially available. Overall, the application of MDGC, both conventional and comprehensive, has allowed for advanced separations of complex aromas to occur by using state-of-the-art instrumentation.

14.4 CHROMATOGRAPHIC THEORY

14.4.1 Introduction

GC may depend on several types (or principles) of chromatography for separation. The principles of chromatographic separations and chromatographic theory are discussed in Chap. 12, Sect. 12.4. For example, **size-exclusion chromatography** is used in the separation of permanent gases such as N_2, O_2, and H_2. A variation of size exclusion is used to separate chiral compounds on cyclodextrin-based columns; one enantiomorphic form will fit better into the cavity of the cyclodextrin than will the other form, resulting in separation. **Adsorption chromatography** is used to separate very volatile polar compounds (e.g., alcohols, water, and aldehydes) on porous polymer columns (e.g., Tenax^R phase). **Partition chromatography** is the workhorse for gas chromatographic separations. There are over 200 different liquid phases that have been developed for gas chromatographic use over time. Fortunately, the vast majority of separations can be

accomplished with only a few of these phases, and the other phases have fallen into disuse. GC depends not only upon adsorption, partition, and/or size exclusion for separation but also upon **analyte boiling point** for additional resolving powers. Thus, the separations accomplished are based on several properties of the analytes. This gives GC virtually unequaled resolution powers as compared to most other types of chromatography (e.g., HPLC, paper, or thin-layer chromatography).

A brief discussion of chromatographic theory will follow. The purpose of this additional discussion is to apply this theory to GC to optimize separation efficiency so that analyses can be done faster, less expensively, or with greater precision and accuracy. If one understands the factors influencing resolution in GC, one can optimize the process and gain in efficiency of operation.

14.4.2 Separation Efficiency

A good separation has narrow-based peaks and ideally, but not essential to quality of data, baseline separation of compounds. This is not always achieved. Peaks broaden as they pass through the column – the more they broaden, the poorer the separation and efficiency. As discussed in Chap. 12 (Sect. 12.5.2.2) a measure of this broadening is **height equivalent to a theoretical plate** (HETP). This term is derived from N, the number of plates in the column, and L, the length of the column. A good packed column might have $N = 5000$, while a good capillary column should have about 3000–4000 plates per meter for a total of 100000–500000 plates depending on column length. HETP will range from about 0.1 to 1 mm for good columns.

14.4.2.1 *Carrier Gas Flow Rates and Column Parameters*

Several factors influence column efficiency (peak broadening). As presented in Chap. 12, these are related by the **Van Deemter equation** (**Eq. 14.1**) (HETP values should be small):

$$\text{HETP} = Au^{1/3} + B/u + Cu \qquad (14.1)$$

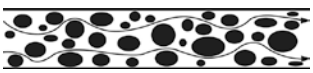
where:

HETP = height equivalent to a theoretical plate
A = eddy diffusion
B = band broadening due to diffusion
u = velocity of the mobile phase
C = resistance to mass transfer

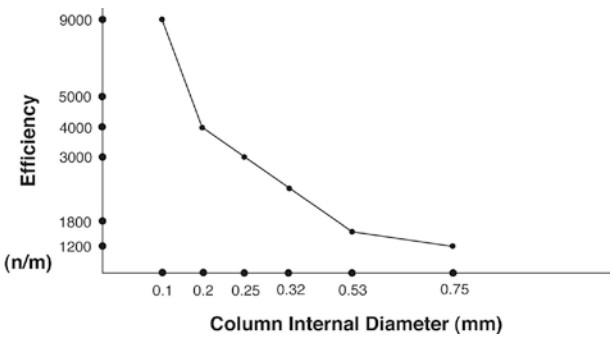
A is eddy diffusion; this is a spreading of the analytes in the column due to the carrier gas having various pathways or nonuniform flow (Fig. 14.17). In capillary chromatography, the A term is relatively very small compared to packed column chromatography. However, as the diameter of the capillary column increases, the flow properties deteriorate, and band spreading occurs. The most efficient capillary columns have small diameters (0.1 mm), and efficiency decreases rapidly as one goes to megabore columns (Fig. 14.18). Megabore columns are only slightly more efficient than packed columns. While column efficiency increases as one goes to smaller columns, column capacity decreases rapidly. Microbore columns are easily overloaded (capacity may be 1–5 ng per analyte), resulting again in poor chromatography. Thus, column diameter is generally chosen as 0.2–0.32 mm to compromise efficiency with capacity.

B is band broadening due to diffusion; analytes will go from a high to a low concentration. The term u is velocity of the mobile phase. Thus, very slow flow rates result in large amounts of diffusion band broadening, and faster flow rates minimize this term. The term u is influenced by the carrier gas choice. Larger-molecular-weight carrier gases (e.g., nitrogen) are more viscous than the lighter-molecular-weight gases (e.g., helium or hydrogen), and thus peak spreading is less for nitrogen than for helium or hydrogen carrier gases. This results in nitrogen having the lowest HETP of the carrier gases and theoretically being the best choice for a carrier gas. However, other considerations that will be discussed in Sect. 14.4.2.2 make nitrogen a very poor choice for a carrier gas.

C is resistance to mass transfer. If the flow (u) is too fast, the equilibrium between the phases is not established, and poor efficiency results. This can be visualized in the following way: if one molecule of solute is dissolved in the stationary phase and another is not,



14.17 figure Illustration of flow properties that lead to large eddy diffusion (Term A)



14.18 figure The influence of column diameter on column efficiency (plates/meter) (Courtesy of Agilent Technologies, Inc., Santa Clara, CA)

the undissolved molecule continues to move through the column, while the other is retained. This results in band spreading within the column. Another factor that influences this term is thickness of the stationary phase. Thick films give greater capacity (ability to handle larger amounts of a solute) but at a cost in terms of band spreading (efficiency of separation) since thick films provide more variation in diffusion properties in and out of the stationary phase. Thus, phase thickness is a compromise between maximizing separation efficiency and sample capacity (with too much sample, the column is overloaded and separation ability destroyed). Capillary columns with phase thicknesses of 0.25–1 μm are commonly used for most applications.

If the Van Deemter equation is plotted, giving the figure discussed in Chap. 12 (Fig. 13), we see an optimum flow rate due to the opposing effects of the *B* and the *C* terms. It should be noted that the GC may not be operated at a carrier flow velocity yielding maximum efficiency (lowest HETP). Analysis time is directly proportional to carrier gas flow velocity. If the analysis time can be significantly shortened by operating above the optimum flow velocity and adequate resolution is still obtained, velocities well in excess of optimum should be used.

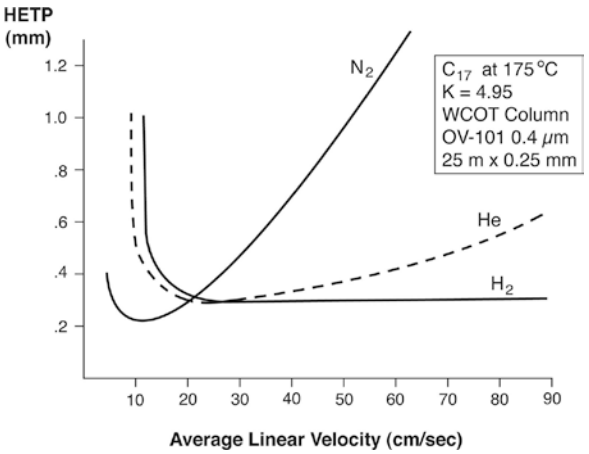
14.4.2.2 *Carrier Gas Type*

The relationship between HETP and carrier gas flow velocity is strongly influenced by carrier gas choice (Fig. 14.16). Nitrogen is the most efficient (lowest HETP) carrier gas, as discussed in Sect. 14.4.2.1, but its minimum HETP occurs at a very low flow velocity. This low mobile phase velocity results in unnecessarily long analysis times. Considering the data plotted in Fig. 14.19, nitrogen has an HETP of about 0.25 at an optimum flow velocity of 10 cm/s. The HETP of He is only about 0.35 at 40 cm/s flow velocity. This is a small loss in resolution to reduce the analysis time fourfold (10 cm/s for nitrogen versus 40 cm/s for helium). One can potentially even push the flow velocity up to 60 or 70 cm/s and accomplish separation in even shorter times.

The plots in Fig. 14.19 suggest that hydrogen is an even better choice for a carrier gas than He (i.e., has a flatter relationship between carrier gas flow velocity and HETP). However, there are some concerns about hydrogen being flammable and reports in the literature that some compounds may be hydrogenated in the GC system. Precautions should be taken when hydrogen is used as a carrier gas.

14.4.2.3 *Summary of Separation Efficiency*

In summary, an important goal of analysis is to achieve the necessary separation in the minimum amount of time. The following factors should be considered:



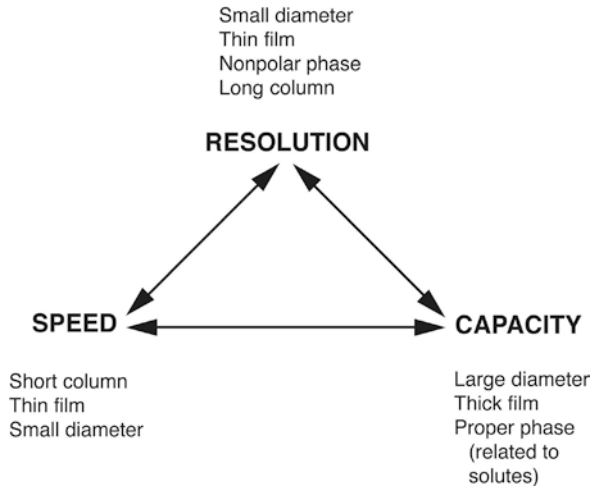
14.19 figure

Influence of carrier gas type and flow rate on column efficiency (Courtesy of Agilent Technologies, Inc., Santa Clara, CA)

1. Column diameter: In general, small diameter columns should be used since separation efficiency is strongly dependent on column diameter. While small diameter columns will limit column capacity, limited capacity often can be compensated for by increasing phase thickness. Increased phase thickness also will decrease column efficiency but to a lesser extent than increasing column diameter.
2. Column operating temperature: Lower column operating temperatures should be used—if elevated column temperatures are required for the compounds of interest to elute, use a shorter column if resolution is adequate.
3. Column length: One should keep columns as short as possible (analysis time is directly proportional to column length—resolution is proportional to the square root of length).
4. Type of carrier gas: Use hydrogen as the carrier gas if the detector permits. Some detectors have specific carrier gas requirements.
5. Flow rate: Operate the GC at the maximum carrier gas velocity that provides resolution.

The pyramid shown in Fig. 14.20 summarizes the compromises that must be made in choosing the analytical column and gas chromatographic operating conditions. One cannot optimize any given operating conditions and column choices to get one of these properties without compromising another property. For example, optimizing chromatographic resolution (small bore capillary diameter, thin phase coating, long column lengths, and slow or optimum carrier gas flow rate) will be at the cost of capacity (large bore columns and thick phase coating) and speed (thin film coating, high carrier gas flow velocities, and short columns). Capacity will be at a cost of resolution and

speed. The choice of column and operating parameters must consider the needs of the analyst and the compromises involved in these choices.



14.20 figure Relationships among column capacity, efficiency, resolution, and analysis speed

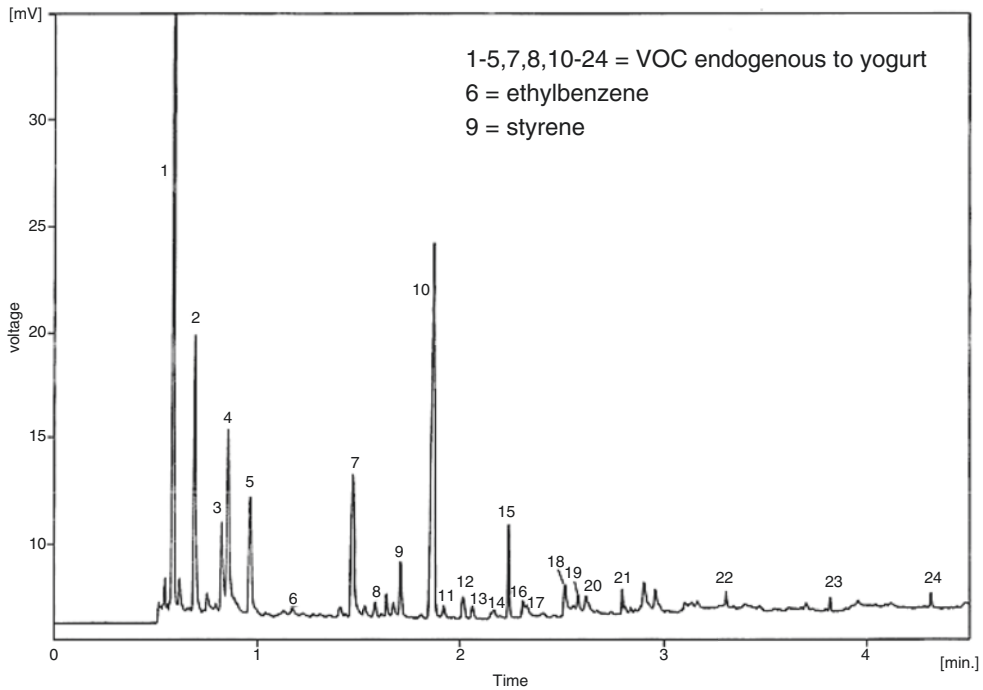
14.5 APPLICATIONS OF GC

While some detail on the application of GC to food analyses has been presented in Chaps. 19, 23, and 33, a few additional examples will be presented below to illustrate separations and chromatographic conditions.

14.5.1 Residual Volatiles in Packaging Materials

Residual volatiles in packaging materials can be a problem both from health (if they are toxic) and quality standpoints (produce off-flavors in the food). As the industry has turned from glass to polymeric materials, there have been more problems in this respect. GC is most commonly used to determine the residual volatiles in these materials [61].

The GC chromatogram presented in Fig. 14.21 illustrates the analysis of styrene and ethylbenzene monomers, from polystyrene (PS) packaging, which migrate into foodstuffs (in the current example from yogurt). Peak 6 and 9 represent ethylbenzene and styrene, respectively. The sample was extracted via HS-SPME with a divinylbenzene/Carboxen/polydimethylsiloxane fiber and analyzed by fast GC to determine the targeted VOC by headspace isolation [62].

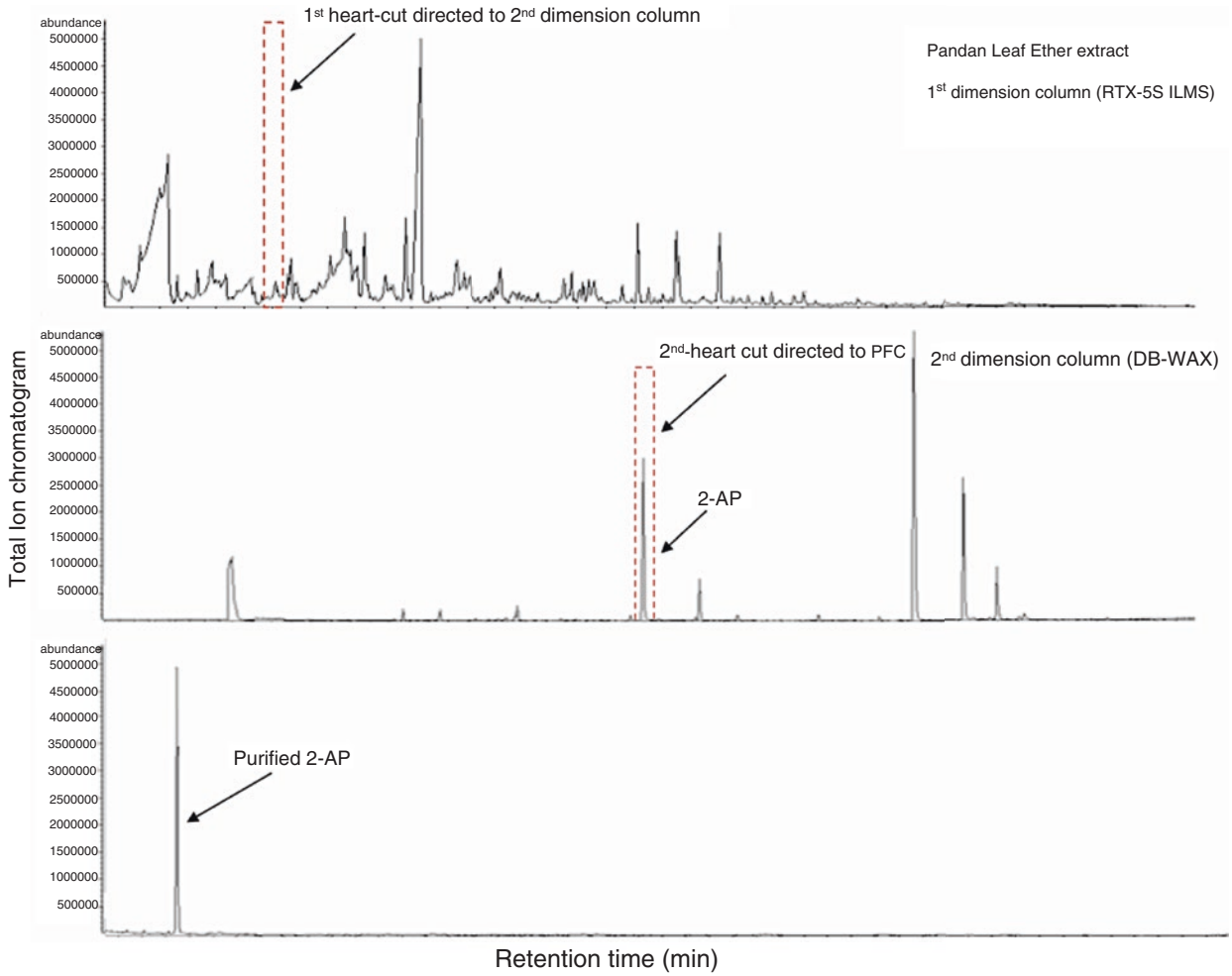


14.21 figure SPME-Fast GC determination of packaging monomers in yogurt (From Verzera et al. [62], used with permission)

14.5.2 Multidimensional GC × GC-MS for the Generation of Reference Compounds

Volatile flavor analysis is a challenging analytical task, requiring the isolation of compounds of diverse chemical species from complex matrices often present at trace concentrations. Standard reference compounds are necessary to positively identify and quantify targeted flavor compounds and furthermore define the flavor significance by subsequent sensory analysis. However, not all compounds are readily commercially available without custom synthesis, which is often an expensive and time-consuming task. Furthermore, synthetic procedures can complicate human evaluation due to food grade protocols. Multidimensional GC × GC-MS methods provide an alternative approach to develop standards needed for flavor or food analysis. For example, the potent flavor compound 2-acetyl-pyrroline (2AP), which is an important ubiquitous odorant in foodstuffs usually present in trace concentrations (ppb or less), described as having a cracker/popcorn-like odor character, is not available without custom synthesis. Figure 14.22 illustrates the application of GC × GC-MS for the isolation and purification of 2AP from a known botanical source of this compound, pandan leaf (*Pandanus amaryllifolius*):

Extraction and analysis details: An ether extract of the leaf material was purified using two-dimensional GC-MS system coupled with a fraction collector to yield a high purity compound standard. The analytical system consisted of an Agilent 6890 N GC coupled with an Agilent 5973 N MSD equipped with two Dean switch devices, two Gerstel modular accelerated column heaters (MACH), an Agilent 7683 autosampler, injector and a Gerstel preparative fraction collector (PFC). An RTX-5S ILMS (30 m × 0.25 mm × 0.25 μm) was used as a primary column, and a DB-Wax (30 m × 0.25 mm × 0.25 μm) was used as a secondary



14.22 figure

Application of multidimensional GC × GC-MS for the generation of standard compounds. Isolation of the flavor compound 2-acetyl-pyrroline (2AP) from pandan leaf extract

column. The valve-switching program for heart-cutting and isolation of 2AP was as follows: valve 1 switched on at 6.29 min, directing the flow to the secondary column, and switched off at 7.10 min; then valve 2 switched on at 15.4 min directing the flow to the cryotrap (−70 °C) of the PFC for 2AP collection and switched off at 15.75 min.

In Fig. 14.22, the top chromatogram illustrates the first dimension column separation of the pandan leaf ether extract and the first "heart-cut" region containing 2AP, which was redirected to the second dimension column. The middle chromatogram is the resultant first dimension heart-cut that was reinjected on the second dimension column; the second dimension heart-cut region containing 2AP is also shown, which was again redirected to the fraction collector. The bottom chromatogram (analyzed by GC-MS/TOF) illustrates the high purity of the final isolate of 2AP obtained.

14.6 SUMMARY

GC has found broad application in both the food industry and academia. It is exceptionally well suited to the analysis of volatile thermally stable compounds. This is due to the outstanding resolving properties of the method and the wide variety of detectors that can provide either sensitivity or selectivity in analysis.

Sample preparation generally involves the isolation of solutes from foods, which may be accomplished by headspace analysis, distillation, preparative chromatography (including solid-phase extraction), or extraction (liquid-liquid). Some analytes can then be directly analyzed, while others must be derivatized prior to analysis.

The gas chromatograph consists of a gas supply and regulators (pressure and flow control), injection port, column and column oven, detector, electronics, and a data recording and processing system. The analyst must be knowledgeable about each of these GC components: carrier and detector gases; injection port temperatures and operation in split, splitless, temperature-programmed, or on-column modes; column choices and optimization (gas flows and temperature profile during separation); and detectors (TCD, FID, NPD, ECD, FPD, and PID). The characteristics of these GC components and an understanding of basic chromatographic theory are essential to balancing the properties of resolution, capacity, speed, and sensitivity.

Unlike most of the other chromatographic techniques, traditional GC has reached the theoretical limits in terms of both resolution and sensitivity. Thus, this method will not change significantly in the future other than for minor innovations in hardware or associated computer software. New development and applications will be more related to multidimensional GC including GC X GC.

GC as a separation technique has been combined with AED, FTIR, and MS as detection techniques to make GC an even more powerful tool. Such hyphenated techniques are likely to continue to be developed and refined, especially in the area of GC X GC-TOF-MS.

14.7 STUDY QUESTIONS

1. For each of the following methods to isolate analytes from food prior to GC analysis, describe the procedure, the applications, and the cautions in use of the method:

   (a) Headspace methods
   (b) Distillation methods
   (c) Solvent extraction

2. What is solid-phase extraction and why is it advantageous over traditional liquid-liquid extractions?
3. Why must sugars and fatty acids be derivatized before GC analysis, while pesticides and aroma compounds need not be derivatized?
4. Why is the injection port of a GC at a higher temperature than the oven temperature?
5. You are doing GC with a WAX column and notice that the baseline rises from the beginning to the end of each run. Explain a likely cause for this increase.
6. The most common detectors for GC are TCD, FID, ECD, FPD, and PID. Differentiate each of these with regard to the operating principles. Also, indicate below which detector(s) fits the description given:

   (a) Least sensitive
   (b) Most sensitive
   (c) Least specific
   (d) Greatest linear range
   (e) Nondestructive to sample
   (f) Commonly used for pesticides
   (g) Commonly used for volatile sulfur compounds

7. What types of chromatography does GC rely upon for separation of compounds?
8. In GC, explain why a balance has to be maintained between efficiency and capacity. Also, give an example situation in which you would sacrifice capacity for efficiency.
9. You plan to use GC to achieve good chromatographic separation of Compounds A, B, and C in your food sample. You plan to use an internal standard to quantitate each compound. By

answering the following questions, describe how using an internal standard works for this purpose (see also Chap. 12, Sect. 12.5.3):

(a) How do you choose the internal standard for your application?
(b) What do you do with the internal standard, relative to the standard solutions for Compounds A, B, and C and relative to the food sample? Be specific in your answer.
(c) What do you measure?
(d) If you were to prepare a standard curve, what would you plot?
(e) Why are internal standards commonly used for GC?

10. A fellow lab worker is familiar with HPLC for food analysis but not with GC. As you consider each component of a typical chromatographic system (and specifically the components and conditions for GC and HPLC systems), explain GC to the fellow worker by comparing and contrasting it to HPLC. Following that, state in general terms the differences among the types of samples appropriate for analysis by GC versus HPLC, and give several examples of food constituents appropriate for analysis by each (see also Chap. 13).

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