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Fundamentals of Integrated GC-MS

Part III: The Integrated GC-MS Analytical System

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FUNDAMENTALS OF INTEGRATED GC-MS

(in three parts)

Part III: The Integrated GC-MS Analytical System

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PREFACE

In the past 15 years, notable advances have been made in the analysis of drugs, pharmaceuticals, and related toxicological materials. Much of this progress can be attributed to the "coming-of-age" of integrated gas chromatography-mass spectrometry-computer controlled instrumentation for the analysis of specific organic components in complex biological specimens. The integrated GC-MS analytical system is rather unique and exceptional in that it combines the mass spectrometer's unexcelled identification potential with the gas chromatograph's separation capabilities. Since the first combination of GC and MS in 1957 by Holmes and Morrell, numerous publications have appeared in the literature by such eminent GC-MS analysts as Gohlke, Teranishi, McFadden, Watson, Biemann, Ryhage, Merritt, Karasek, and Horning; using this integrated technology for the solution of difficult analytical problems. Its achievements are now legend, having opened up new horizons or channels for analytical research in toxicology, biochemistry, pharmacology, forensics, medicine, etc. To be able to monitor a drug, its persistence and metabolic fate in biological fluids of man via mass fragmentography at picogram concentration levels provides the researcher with a tool of immeasurable significance.

The purpose of this volume is to describe in very basic terms the fundamentals of integrated GC-MS-COMP instrumentation. Therefore, by design, this volume is divided into three parts dealing with the basic theory and principles of gas chromatography, mass spectrometry, and the integrated GC-MS analytical system. It is rather evident that how one achieves maximum utility with the integrated system depends upon the analyst's understanding of each component's function (the mass spectrometer, gas chromatograph, interface, and combined GC-MS unit as well as vacuum and computer technology) and methods available for function implementation. For example, if proper gas chromatographic conditions are chosen, it follows that the selection of the operational aspects associated with optimal interface and mass spectrometer performance can be considerably expedited with minimal difficulties encountered.

To obtain the "best" results from the integrated gas chromatograph-mass spectrometer, compromises must be made to ensure that one of its component

does not interfere with the other. The column, the carrier gas, and the flow rate must be chosen in such a way that the efficiency of the separator (if one is used) is high, but the pressure in the ion source and the vacuum system relatively low. How this can be attained is the objective of this book.

In addition to the text, which is well-referenced in each section, many illustrations of actual applications and tables of data for each instrumental technique are included as aids to the analyst for his greater appreciation and understanding of the limitations as well as potentials ascribed to each method. Only by availing himself of this knowledge can the full benefit or rewards of the integrated GC-MS system be reaped. From an analytical chemist's point of view, it is hoped that this deliberate combined visual and factual approach will find acceptance by the reader who would otherwise rely only on his interpretation of the written word relative to some published procedure.

In retrospect, this volume really represents the end result of many investigations by numerous eminent scientists whose research efforts have appeared in the literature throughout the world. To them I am humbly indebted, and it is only proper that special acknowledgment be made in some cases to specific journals, publishers, and organizations for having granted special copyright permission to the authors; namely, the Journal of Chromatographic Science, Analytical Chemistry, American Laboratory (International Scientific Communications, Inc.), International Journal of Mass Spectrometry and Ion Physics (Elsevier Scientific Publishing Co.), Burgess Publishing Co., Marcel Dekker, Inc., Finnigan Corp., DuPont Instrument Products Division, Perkin-Elmer Corp., and CVC, Inc.

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Chapter 7

BASIC VACUUM TECHNOLOGY AND THE GC-MS INTERFACE

I. INTRODUCTION

In Parts I and II, the basic principles of gas chromatography and mass spectrometry, respectively, were discussed in addition to qualitative and quantitative methods of analysis. These were presented in considerable detail as a prelude to Part III, which will deal with the integration or interfacing of these two analytical techniques to provide the analyst with an extremely powerful tool for the elucidation of molecular structure. In this integrated system, the gas chromatograph can be considered as an alternative inlet to the mass spectrometer's ionization chamber via a suitable interface for the effluent emerging from the gas chromatographic column. In contrast to other static, batch-type inlets as discussed in Part II, the GC inlet is regarded as a highly dynamic system for the continuous introduction of specific components in a flowing carrier gas stream resolved from heterogeneous mixtures by the proper selection of GC column types, liquid stationary phases, and various other optimized operational parameters. However, as pointed out by Merritt [1, 2], "the development of a gas chromatographic-mass spectrometric analytical system is not a mere combining of the two techniques. There are inherent incompatibilities of operational procedures when the techniques are performed separately that must be resolved when they are combined." Moreover, the analysis ultimately requires a tremendous amount of specialized knowledge relating to the

parameters of each technique that are pertinent to the successful operation of the combined system.

This viewpoint has also been shared by other investigators, for example, McFadden [3-5], Watson [6], Ryhage [7-9], Wikstrom [9], Littlewood [10], Watson and Biemann [11, 12], Karasek [13, 14], Rees [15], Vollmin et al. [16], and Bonelli et al. [17].

Although currently the capabilities of GC-MS are being expanded by computerization, the pertinent operational aspects that one must consider for proper optimization and utilization of the integrated instrument will be discussed in this and subsequent chapters of Part III; these include (1) carrier gas-related considerations—its type, flowrate and ionization potential; (2) the sample introduction mode (direct, splitting, or via a molecular separator or concentrator); (3) the reduced pressure requirements in the ion source; (4) basic vacuum technology; (5) column type selection and separation efficiency (peak broadening); (6) sample size (load) and efficiency of sample utilization; (7) efficiency of sample concentration via a molecular separator as a consequence of the high-speed vacuum system employed; (8) choice of liquid stationary phase—its ability to separate adjacent components via solvent-solute interactions of varying magnitude and "bleed" characteristics; (9) column/molecular separator temperature control; (10) MS sensitivity, limits of component detection and scan rate as a function of resolution and sensitivity; (11) computerization of the integrated GC-MS system, and so on.

In his discussion of GC/MS/computers, Karasek [14] has placed into proper perspective the question of interfacing the GC/MS. On this subject he writes as follows:

> In 1910 Thomson first separated masses of an element, and in 1919 Aston built his mass spectrograph. Mass spectroscopy as such has a long history, but it was not until the early 1940's that analytical mass spectrometers for the chemist appeared. Initially, these instruments were used for quantitative analysis until about 1952 when gas chromatography appeared and, with its great ability to separate and quantify components of a mixture, became the preferred method. Thus freed from routine analytical work, mass spectroscopists turned their attention to developing the qualitative, molecular structure determination aspects of mass spectroscopy. Such development is due to the pioneering work done on interpretation of mass spectra by many researchers, such as Biemann [18], McLafferty [19] and Budzikiewicz et al. [20]. This interpretation is more empirical than theoretical so that structural assignments for completely unknown materials rest heavily on a strong background of empirical and semiempirical information or a comparison to established reference spectra.
>
> Interfacing a gas chromatograph to a mass spectrometer is now a widespread practice. There is a unique compatibility between these

TABLE 7.1

Detection and Identification Limits for Analytical Methods[a]

Method	Limit in grams	
	Detection	Identification
Gas chromatography	10^{-6}–10^{-12}	–
Infrared spectroscopy	10^{-7}	10^{-6}
Ultraviolet spectroscopy	10^{-7}	10^{-6}
NMR (time averaged)	10^{-7}	10^{-5}
MS (batch inlet)	10^{-6}	10^{-5}
MS (direct probe)	10^{-12}	10^{-11}
GC-MS	10^{-11}	10^{-10}

[a]From Karasek [14], courtesy of Analytical Chemistry.

two instruments: the gas chromatograph separates the components of a mixture and delivers them one-by-one to the mass spectrometer for identification. This permits the identification of compounds present in quantities as low as 10^{-6} to 10^{-10} gram. Table 7.1, comparing relative sensitivities of spectroscopic methods, shows the high sensitivity possible with the interfaced GC/MS.

The performance of the interface device determines to a large extent the type of results achieved by the entire GC/MS system [21]. The device sits at a critical point and serves a single-minded purpose. It must remove as much of the carrier gas from a GC peak as possible and transport the maximum amount of the remaining organic material into the mass spectrometer ion source. How well a given device performs these functions is measured by an enrichment factor N, indicating the amount of helium removed from a GC peak, and a yield Y, indicating the percentage of sample that actually reaches the MS ion source. The interface must also perform the function of reducing the 760 torr pressure of the GC peak to the very low pressure (10^{-3} torr or less) required by the MS ion source.

Understanding the functions and performance of interface devices is important to the overall instrumental system. Although the many interfaces reported in the literature will vary greatly in detail and design, they can generally be classified into four groups illustrated in Figure 7.1. The direct coupled uses a section of narrow-bore tubing to carry the GC column effluent into the MS ion source. Conditions

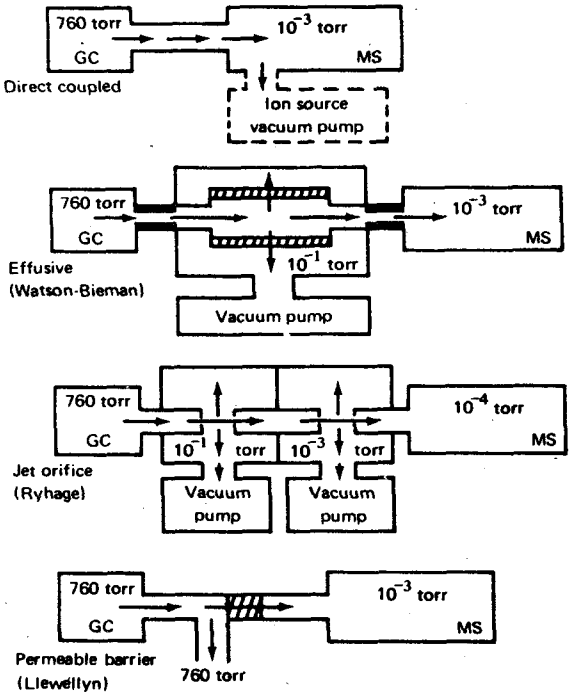

Figure 7.1. GC-MS interfaces classified into four groups. From Karasek [21], courtesy of Research/Development.

must be adjusted to have the entire pressure drop (760 torr to 10^{-3} torr) occur across this connecting tubing. Various means are used to do this: splitter valves at the GC exit, adjustment of the GC to operate under specific flow conditions, restrictors, or use of a properly sized, auxiliary vacuum pump at the MS ion source. These direct systems in particular have a number of advantages of efficiency and simplicity.

In the effusive type interface the GC effluent is forced to flow through a region where molecular flow conditions exist. This leads to selective removal of the carrier gas and creates the required pressure drop. Use is made of the fact that under molecular flow conditions the lighter molecules of the carrier gas move through orifices at faster rates. The Watson-Biemann example shown uses a porous glass fritted tube housed in an evacuation chamber. The pores in the frit, being less than 10^{-3} cm in diameter, provide the passage through

which the carrier gas is selectively moved. Constrictions at the entrance and exit to the frit section are designed to give a viscous flow to the GC effluent. Other interfaces with this principle use porous stainless steel, silver, or Teflon in place of glass.

The jet orifice interface makes use of the properties of a jet of gas expanding as it moves toward an orifice. Mathematically and conceptually, it is very similar to the effusive type. The GC effluent flows through a nozzle opening from which it emerges into a lower pressure region as an expanding jet. A short distance from the nozzle creating the jet, and directly in line, is the exit orifice to the MS through which a gas stream enriched in the heavier component passes. Because of their lower forward momentum and greater diffusivity (according to Graham's law), the lighter carrier gas molecules are removed through the action of the vacuum pumps. Both single- and two-stage jet orifice interfaces have been used.

The permeable membrane interface employs a principle of separation quite different from that of the other types. GC sample enrichment occurs because the organic component of the GC peak selectively passes through a thin elastomer membrane that exhibits a conductance to organic compounds 1000 times greater than it does to helium. The carrier gas flows over the membrane and on to the atmospheric exit, while the organic material passes through the elastomer film by a process of solution and diffusion to reach the MS ion source. High yields and efficiencies are possible with this simple interface. Single and double stages have been used. . . .

Along with a description of principles by which these interface devices function, one needs an understanding of factors that affect the results produced. In addition to yield and efficiency, such factors as time delay, inertness, GC peak distortion and the carrier gases, and GC operating conditions, are important. Table 7.2 tabulates such factors.

A simple understanding of interface operating principles and performance is deceptive. Each interface has its own limitations which must be matched and balanced against the operating parameters of different mass spectrometers and their coupled gas chromatographs. Successful use of any GC/MS interface system depends strongly upon a thorough understanding of vacuum technology. The variables of pressure and dynamic flow of gases are many and peculiar to each individual system. Chromatographs operate at a wide range of carrier flows, temperatures, and quantities of organic sample per peak. Mass spectrometers have different vacuum systems, flow conductances, ion sources, and mass selector designs. The collecting lines throughout the GC/MS system will contain valves, tubings, and orifices with individual effects on gaseous flow behavior.

TABLE 7.2

Condensed Performance of GC/MS Interfaces[a]

Classification	Efficiency, N[b]	Yield (%), Y[c]	Peak delay (sec)	Peak distortion, H[d]	Effect on peak, inert	Carrier gases
Perfect		100	0	1	Yes	All
Jet	10^2	40	1	1-2	Yes	He, H_2
Direct	1	1-100[e]	1	1	Yes	All
Effusive	10^2	50	1	1-2	Maybe	He, H_2
Permeable membrane	10^3	80-95	Variable	3	Maybe	Inorganic

[a]From Karasek [14], courtesy of Analytical Chemistry.

[b] $N = \frac{\text{conc. in MS source}}{\text{conc. in GC peak}}$

[c] $Y = \frac{\text{quantity in MS source}}{\text{quantity in GC peak}} \times 100\%$

[d] $H = \frac{\text{peak width in MS}}{\text{peak width from GC}}$

[e]Equals split ratio.

A few selected principles and details that have often gone unappreciated in the GC/MS interface problem will reveal the limitations that a particular vacuum configuration imposes on various GC analytical situations. Exact computation of vacuum system behavior is difficult, but approximate calculations can be made of an individual GC/MS system [22,23]. [From Karasek [14], courtesy of Analytic Chemistry.]

With this as an introduction to vacuum technology and the GC/MS interface considerations, such relevant subjects or topics as mean free path, viscous and molecular flow through tubes or orifices, conductance (flow resistance), pumping speed, as well as the various interfaces (direct-coupled, effusive, jet orifice, and permeable membrane) and their related problems associated with yield, enrichment, diffusion, and so on, will be discussed in this chapter.

II. FUNDAMENTALS OF VACUUM TECHNOLOGY [22-51]

A. Gas Theory: The Kinetic-Molecular Model

To appreciate more fully the various aspects of vacuum technology, there are some fundamental elements of the kinetic theory of gases that are relevant to the development of special equations of vacuum practice. The kinetic theory of gases as first proposed by Bernoulli in 1738 was an attempt to explain the properties of gases on a purely mechanical basis; through the subsequent labors of Boltzmann, Kroenig, Maxwell, Clausius, and others, the kinetic theory of gases was developed and given mathematical form.

The kinetic-molecular model for a gas is based on the following postulates:

1. Gases are composed of minute, discrete particles or molecules of the same mass and size but differing in these from gas to gas.
2. Molecules in a container (Fig. 7.2) are in continuous random motion, and collisions between molecules and molecules and the container's walls are perfectly elastic (i.e., no translational energy is lost by conversion into internal energy at a collision).

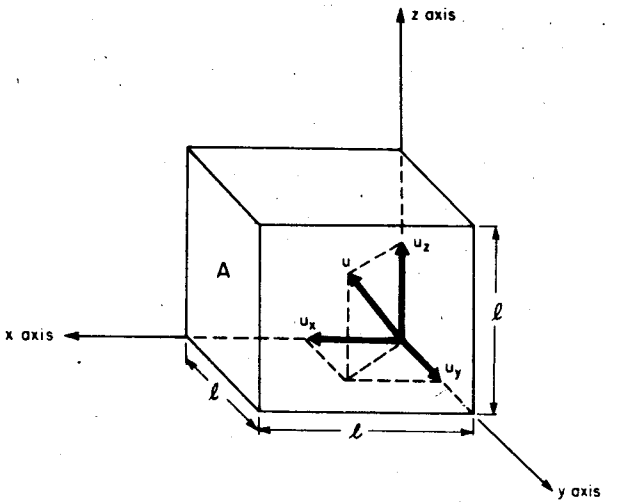

Figure 7.2. Resolution of velocity along x, y, and z axes.

3. Wall bombardment by molecules gives rise to the phenomenon called pressure. The pressure of a gas at a certain point in a given direction is defined as the time rate of transfer of momentum in the assigned direction across a unit area normal to that direction and, in the situation of a gas against a solid barrier or wall, is equivalent to the definition of pressure as the force per unit area.
4. At relatively low pressures, the average distances between molecules greatly exceed their molecular diameters. Hence, the attractive forces between molecules (dependent on the distance of molecular separation) are assumed to be negligible and, as a first approximation, the molecules can be considered to be point masses.
5. Depending on the temperature of the gas, the molecules move in straight lines with an average velocity and, since the volume occupied by the molecules is much smaller than the volume actually filled by them, a molecule can travel a considerable distance before a collision occurs with a neighboring molecule. The average distance through which a molecule travels between collisions is called its mean free path.

Based on these assumptions, one can readily derive a mathematical expression from which the gas laws can be deduced. Let us consider a cubical container as illustrated in Figure 7.2 with side l in which N molecules, each having a mass m and velocity u, are confined. As indicated in Figure 7.2, the molecular velocity can be resolved into the components u_z, u_y, and u_z, these being perpendicular to the walls of the container. In our model, the molecular velocity u is the root-mean-square velocity, or the velocity obtained from the square root of 1/N-th of the sum of the squares of the individual velocities.

$$u = (u_x^2 + u_y^2 + u_z^2)^{1/2} \tag{7.1}$$

Assuming that a molecule is moving in the x direction with velocity u_x, it will strike the yz plane with momentum mu_x and then rebound with velocity $-u_x$ and momentum $-mu_x$ since the collision is elastic. Therefore, in the x direction, the change in momentum per molecule per single collision is $mu_x - (-mu_x) = 2mu_x$. Since there are u/2l collisions per second, the change in momentum per second per molecule on the given wall is

$$(2mu_x)\left(\frac{u_x}{2 \cdot l}\right) = \frac{mu_x^2}{l} \tag{7.2}$$

A similar change in momentum will be experienced by the same molecule at the other yz plane so that the total change in momentum per molecule per second in the x direction is twice the quantity indicated in Eq. (7.2) or

$2mu_x^2/l$. Analogous changes in momentum per molecule per second occur also in the y and z directions such that:

$$\text{Total change in momentum/molecule/second} = \frac{2mu_x^2}{l} + \frac{2mu_y^2}{l} + \frac{2mu_z^2}{l} \tag{7.3}$$

$$= \frac{2m}{l}\left(u_x^2 + u_y^2 + u_z^2\right) \tag{7.4}$$

$$= \left(\frac{2m}{l}\right)u^2 \tag{7.5}$$

For N molecules in the cube, the change in momentum per second for all of them is $2Nmu^2/l$. However, since pressure is the force per unit area and the rate of change of momentum is the acting force f, it follows that

$$P = \frac{f}{A} = \frac{2Nmu^2}{l \cdot A} \tag{7.6}$$

where

P = pressure
A = total area over which the force is applied

For the cube shown in Figure 7.2, $A = 6 \cdot l^2$ so that, by substitution,

$$P = \frac{2Nmu^2}{6 \cdot l^2 \cdot l} \tag{7.7}$$

$$= \frac{Nmu^2}{3 \cdot l^3} \tag{7.8}$$

However, the volume V of the cube is l^3; therefore

$$P = \frac{Nmu^2}{3V} \tag{7.9}$$

or

$$PV = \frac{1}{3}Nmu^2 \tag{7.10}$$

Equation (7.10) is the fundamental relation of the kinetic theory of gases which states that the product for any gas should equal one-third the mass of

all the molecules (Nm) multiplied by the square of the root-mean-square velocity. This equation is applicable to a vessel of any shape whatsoever, since the total volume may be considered to consist of a large number of infinitesimally small cubes, for each of which the equation holds.

1. Deductions from the Kinetic Theory of Gases [32, 34, 38-40, 43-46, 50, 51]

a. Boyle's Law

Since there is a direct proportionality between the kinetic energy of the molecules [that is, $(1/2)Nmu^2$] and the absolute temperature such that

$$\frac{1}{2}Nmu^2 = kT \tag{7.11}$$

where k is a proportionality constant, the kinetic equation may be written in the form

$$PV = \frac{2}{3}\left(\frac{1}{2}Nmu^2\right) \tag{7.12}$$

When Eqs. (7.11) and (7.12) are rearranged, one obtains

$$PV = \frac{2}{3}kT \tag{7.13}$$

If the temperature of the gas remains constant, the kinetic energy of the gas remains constant since, according to the kinetic theory, the temperature of a system is a function only of the kinetic energy of the system. At constant temperature, Eq. (7.13) thus becomes PV = constant, or Boyle's law.

b. Charles' Law

If Eq. (7.13) is kept at constant pressure, Charles' law is obtained, which is given by

$$V = \left(\frac{2k}{3P}\right)T \tag{7.14}$$

or

$$V = k'T \tag{7.15}$$

c. Avogadro's Principle

In 1811, Avogadro suggested that equal volumes of different gases at the same pressure and temperature contain the same number of molecules.

Hence, for two different gases having equal volumes and pressure such that $P_1V_1 = P_2V_2$, one can deduce from Eq. (7.10) that

$$\frac{1}{3}N_1m_1u_1^2 = \frac{1}{3}N_2m_2u_2^2 \tag{7.16}$$

Furthermore, at constant temperature, the average kinetic energy per molecule must be the same or

$$\frac{1}{2}m_1u_1^2 = \frac{1}{2}m_2u_2^2 \tag{7.17}$$

Thus, we see that

$$N_1 = N_2 \tag{7.18}$$

which is a statement of Avogadro's principle. Recognizing that one mole of any compound contains the same number of molecules, Avogadro's number, N_0, has the value of 6.0225×10^{23}.

On a molar basis, Avogadro's hypothesis may be restated such that the same volume is occupied by 1 mole of any gas at a given temperature and pressure. Because the volume of gas at a given pressure and temperature is proportional to the number of moles, or mass, of the gas, this relationship can be expressed as

$$PV = n(\text{constant}) \tag{7.19}$$

where n is the number of moles and the constant is equal to the value of PV/T for 1 mole of the given gas. According to Avogadro's principle, this volume at 1 atm and 0°C is 22.414 liters. The constant in Eq. (7.19) is called the gas constant and is designated by R, which is the same for all gases. Thus, gases that obey Avogadro's principle, Boyle's law, and Gay-Lussac's law (where the volume of a definite quantity of gas at constant pressure is directly proportional to the absolute temperature; that is, $V \propto T$ or $V/T = \text{constant}$) can be expressed by the relation

$$PV = nRT \tag{7.20}$$

At a molecular level, Eq. (7.20) can be rewritten such that

$$PV = NkT \tag{7.21}$$

where N = the total number of molecules in a container, this number being determined from the weight (W) of gas in the container and the mass of one gas molecule so that $N = W/m$; and $k = R/N_0$, the molar gas constant R divided by Avogadro's number N_0 (k being the so-called Boltzmann constant, which is equal to 1.38×10^{-16} dyne-cm/deg).