Fundamentals of Integrated GC-MS

Part II: Mass Spectrometry

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(in three parts)

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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 components

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.

July 1976 Benjamin J. Gudzinowicz
Providence, Rhode Island

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Chapter 5

MASS SPECTROMETRY: GENERAL THEORY, PRINCIPLES, AND INSTRUMENTATION

I. INTRODUCTION

Any device featuring electrical detection and having the ability to separate gaseous positive ions according to their mass-to-charge ratio (m/e) is usually referred to as a mass spectrometer, whereas a mass spectrograph is an instrument in which the focused ion beams are recorded on a photographic plate. In principle, the conventional mass spectrometer is an electronic, high-vacuum instrument used for the analysis of gases, liquids, or volatilized solids by means of the dissociation of molecules by electron impact, chemical ionization, or field ionization bombardment and the subsequent separation of the positive ions according to their mass-to-charge ratio. How this separation is achieved depends entirely on the spectrometric instrumentation employed (magnetic deflection, radio frequency, quadrupole, time-of-flight, etc.). The charged positive ion beams are resolved in sequence and simultaneously monitored by an ion collector with suitable electrometer circuitry. The signals resulting from this ion-scanning process are amplified and recorded using one of three common modes: (1) an oscillographic recorder, (2) an analog magnetic tape, or (3) a photographic plate. In either mode, a "mass spectrum" of the dissociated, ionized molecule is achieved.

If a limited region of a mass spectrum is of particular interest to the analyst, its spectral range can be repeatedly scanned and displayed on a cathode-ray tube. By maintaining constant the operational conditions of the

Figure 5.1. Mass Spectrum of sarpagine. From Spitteler [1].

mass spectrometer, the dissociated fragments will always occur in the same relative abundance for any given substance, thus providing a specific "fingerprint" spectrum or "cracking pattern" for each compound as noted in Figure 5.1, where the ion currents due to the molecular ion and its ionized fragments appear as discrete peaks provided that the instrument has sufficient mass resolution. Thus, it is possible to differentiate between the two isomers based on their respective mass spectra where the only variance noted might be that due solely to the strength of a single chemical bond.

In Figure 5.2, the basic components of a mass spectrometer are shown. By reference to Figure 5.2, the theory of operation of the mass spectrometer can be briefly summarized. Under normal operating conditions, the vacuum systems associated with the instrument maintain it at the low pressures required to avoid intermolecular and interionic reactions. Whether the compound in question is a gas or a volatilized liquid or a solid, the heated inlet system allows the material to enter the highly evacuated ionization chamber through a molecular leak whose conductance is preferably in

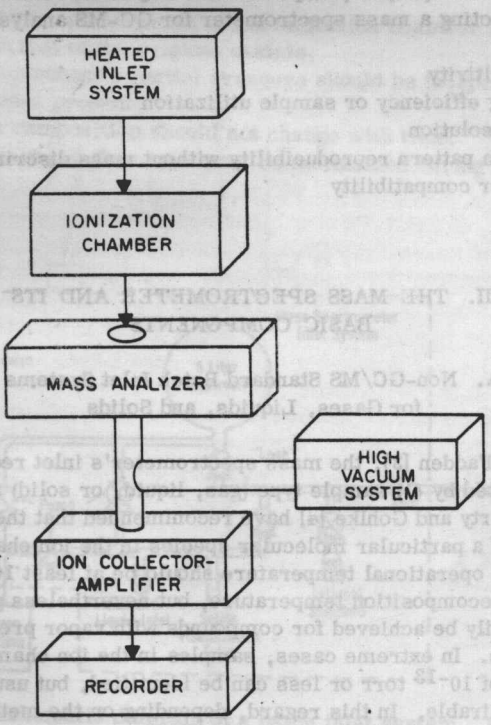

Figure 5.2. Basic components of a mass spectrometer.

the 0.1 to 0.4 cc/sec range. Although the leak attempts to maintain sample flow constant, conductance will substantially decrease for compounds with increased molecular weight since the molecular flow rate is inversely proportional to the square root of the molecular weight of the component. The gaseous molecule is bombarded in the ionization chamber and the positive ion fragments (which are more abundant than the negative ions by several orders of magnitude) are accelerated from the ion source electrostatically and then resolved or separated according to their mass-to-charge ratio (m/e) by the mass analyzer. These mass-separated ions of a particular m/e impinge sequentially on an ion collector electrode, causing an electrical current which is amplified 10^3 to 10^8 times with virtually no noise into an electrical signal that is proportional to ion abundance and compatible with fast recording devices.

In subsequent sections, common types of mass spectrometers used in integrated gas chromatography-mass spectrometry (GC-MS) will be discussed, focusing specific attention on the interrelationships of mass spectrometer components and GC-MS analytical requirements. McFadden [2] has ably placed into proper perspective the important features to be considered in selecting a mass spectrometer for GC-MS analysis:

1. MS sensitivity
2. Pumping efficiency or sample utilization
3. Mass resolution
4. Spectrum pattern reproducibility without mass discrimination
5. Computer compatibility

II. THE MASS SPECTROMETER AND ITS BASIC COMPONENTS

A. Non-GC/MS Standard Batch Inlet Systems for Gases, Liquids, and Solids

As noted by McFadden [3], the mass spectrometer's inlet requirements are primarily dictated by (1) sample type (gas, liquid, or solid) and (2) volatility. McLafferty and Gohlke [4] have recommended that the available vapor pressure of a particular molecular species in the ion chamber at a specified, controlled operational temperature should be at least 10^{-2} torr while still below its decomposition temperature, but nevertheless good qualitative spectra can easily be achieved for compounds with vapor pressures in the 10^{-7}-torr range. In extreme cases, samples in the ion chamber with partial pressures of 10^{-13} torr or less can be identified, but usually 10^{-4} to 10^{-7} torr is desirable. In this regard, depending on the method of sample introduction into the spectrometer's dynamically pumped vacuum system,

sample size requirements will vary. For example, using the mass spectrometer as a gas chromatographic detector, the approximate detection limits for an instrument operated in a (1) batch inlet, (2) GC-MS, or (3) multiple detection (MID) mode is 10^{-7}, 10^{-11}, or 10^{-12} g, respectively [2].

A schematic diagram of a typical heated glass inlet system is shown in Figure 5.3 which consists of a sample introduction probe, a molecular leak for metering the vaporized sample to the ionization chamber, a diffusion pump, and a micromanometer (not shown) for determining the amount of sample introduced. In general, there are two main problems associated with sample introduction, as pointed out by Tanner [5]. The first is to obtain a truly representative small sample of the gas or liquid to be analyzed. The second is to determine accurately the pressure of the sample when it is in the sample expansion volume.

In this regard, to avoid serious errors in quantitative studies, Inghram and Hayden [6] specify four conditions for the ideal operation of a sample inlet system:

1. Gas mixture composition in the ionization chamber should be identical with that of the original sample.
2. Each component's partial pressure should be independent of other compounds present.
3. Sample composition should not change with time.
4. A constant gas flow rate must be maintained during the analysis.

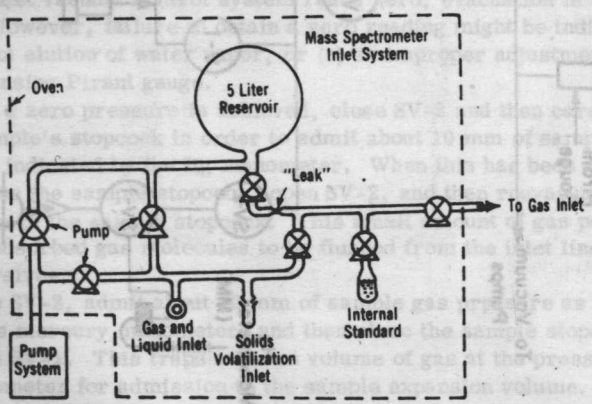

Figure 5.3. Schematic diagram of a batch inlet system. From McLafferty and Gohlke [4], courtesy of Chemical & Engineering News.

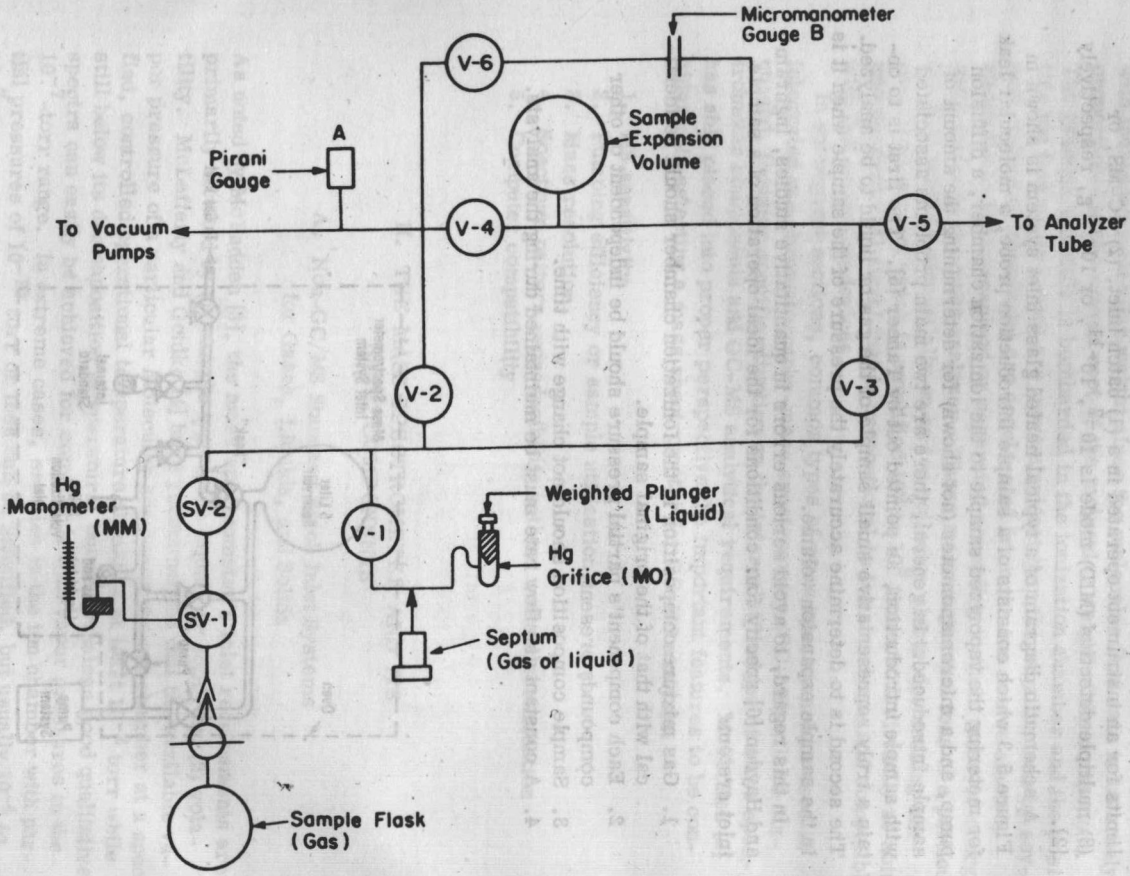

Figure 5.4. Block diagram of MS gas-liquid inlet systems.

All gases admitted into the ionization chamber are first introduced into the expansion volume (1 to 2 liters) shown in Figure 5.4, which represents a block diagram of a mass spectrometer inlet system. Normally, the pressure in this volume chamber is about 50 μm, though samples can be examined at either higher or lower pressures. As indicated, gases or liquids having a vapor pressure of at least 100 mm Hg at room temperature can be introduced directly into the expansion volume by means of the gas/septum or mercury orifice/septum inlet systems, respectively. On the other hand, higher-boiling liquids in the 150 to 300°C range may be introduced into the heated chamber either by hypodermic syringe injection through a moderately temperature-resistant silicone septum or by means of a stainless steel injection dipper.

1. Introduction of Gaseous Samples

When gas is stored at atmospheric pressure or less in a sample flask, the introduction of gases into the metering volume from a gas bulb is usually a fairly simple matter. The sample flask may be a one- or two-stopcock bottle, or it may be a metal bomb having a high vacuum valve. If a flask is used, it is attached to the gas inlet line via glass joints or Tygon or Neoprene tubing. The procedure for introducing the sample is as follows:

1. Prior to attaching the sample flask to the inlet as shown in Figure 5.4, close valves V-1, V-3, V-4, V-5, SV-1, and SV-2, but open valve V-2.
2. With the sample flask in position, open SV-1 and SV-2 and evacuate the system to the sample stopcock. When the high-sensitivity Pirani gauge (A) for the inlet vacuum control system reads zero, evacuation is considered complete. However, failure to obtain a zero reading might be indicative of (a) a leak, (b) elution of water vapor, or (c) an improper adjustment of the pressure-sensing Pirani gauge.
3. When a zero pressure is achieved, close SV-2 and then carefully open the sample's stopcock in order to admit about 10 mm of sample gas pressure as indicated by the Hg manometer. When this has been accomplished, close the sample stopcock, open SV-2, and then reevacuate the system again to the sample stopcock. This small amount of gas permits previously absorbed gas molecules to be flushed from the inlet line and SV-designated valves.
4. Close SV-2, admit about 50 mm of sample gas pressure as determined by the mercury manometer, and then close the sample stopcock.
5. Close SV-1. This traps a known volume of gas at the pressure of the Hg manometer for admission to the sample expansion volume.
6. Open V-3 and V-4 and zero the micromanometer gauge (B). With the gauge zeroed, close V-2 and V-4.

7. Open SV-2 to expand the gas in the trapped volume into the expansion volume. To isolate the expansion volume from the other parts of the inlet system, close V-3.

8. Measure the pressure of the gas in the expansion chamber by readjusting the micromanometer gauge (B) to zero by means of a balancing voltage switch and pressure dial; the pressure dial reading is then converted to microns of pressure from a calibration chart. After the sample volume pressure has been determined, valve V-5 is opened to admit the gas through the molecular leak into the ionization chamber.

Using leak-tight, calibrated hypodermic syringes with stainless steel needles, gas samples can be introduced into the MS inlet system through an injection port containing a silicone rubber septum as shown in Figure 5.5a.

With mercury-covered frits (Fig. 5.6) operated in conjunction with a room-temperature inlet system, a constant-volume gas pipette (Fig. 5.7a) provides the analyst with another method for introducing a calibrated amount of gas sample. It can be used as an integral part of a mercury-sealed gas storage system (Fig. 5.7b), where the gas is sealed off with a mercury-covered frit. When the capillary of the gas pipette is filled entirely with mercury, the pipette is filled by contacting its tip with the upper surface of the gas storage system's frit. A release of pressure on the other side of the mercury gas pipette permits gas to flow into the pipette. By submerging the gas-filled pipette below the surface of the mercury-sealed orifice sample

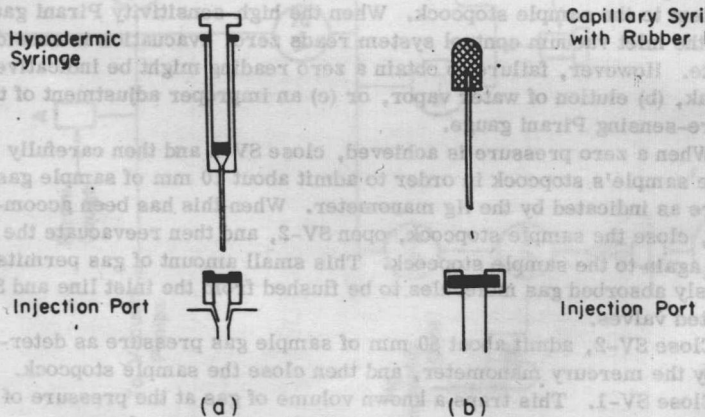

Figure 5.5. Sample introduction systems for gases (a) or liquids (a, b).