# Scientific Instrumentation in Research

The introduction of modern scientific instrumentation has had a dramatic effect on research and development in all scientific disciplines in academic as well as in industrial establishments. The Beckman Model G pH meter was the first instrument to use electronics as a primary tool for chemical investigation, a harbinger of the revolution in instrumentation that was to follow. The Beckman DU UV-vis spectrometer followed the Beckman Model G, introducing the vast potential spectrophotometric techniques to routine chemistry.

Before the introduction of the Beckman DU, chemical instrumentation was specialized equipment in the hands of a few labs that were either exceptionally skilled in optics and engineering or unusually well-funded. The DU made analytical instrumental analysis available to scientists not interested in or capable of making their own instruments at an affordable price. The result was a standardization of techniques and measurement that can be extrapolated across all disciplines of chemistry. For the first time, any lab could directly compare results with literature or other labs without having to replicate whatever homemade instrument was used originally. The introduction of almost every modern spectrometers can be traced back to the introduction of the DU, which in turn can be traced to the Model G pH meter. The introduction of modern chemical instrumentation made the stunningly powerful tools of electronic and electro-optics analyses available to almost every chemist, dramatically increasing the pace, breadth, and ease of chemical investigation.

As an organic chemist, today I shall focus my lecture on the application of instrumentation on chemical research.

To appreciate the role of scientific instrumentation in scientific research, I shall briefly touch on spectroscopy.

Spectroscopy is a type of chemical analysis done by shining light on a sample to determine what is inside. Chemists commonly measure the absorbance, how much light is absorbed by the sample, or the transmittance, how much light passes through the sample. In a chemical analysis, many different kinds (wavelengths or energies) of light (a spectrum) are shone through a sample. Some of the light is absorbed. By knowing what wavelengths of light are absorbed by the sample, we know what is inside. But, if we are looking for a specific molecule or characteristic and shine the wrong wavelengths of light through a sample, no matter how much light we put through, we will never learn anything about the sample. Because different molecules and characteristics of molecules absorb at different energies of light, there is a need for different forms of spectroscopy.

Each different form of spectroscopy uses a different part of the electromagnetic spectrum, shown below, to investigate specific characteristics of a sample. For example, infrared spectroscopy (IR) is used to investigate how the molecules in a sample vibrate, while ultraviolet spectroscopy (UV) is used to investigate how certain chemical bonds in a molecule are arranged. Remember, IR spectroscopy can not be used to investigate the information that UV provides and vice-versa.

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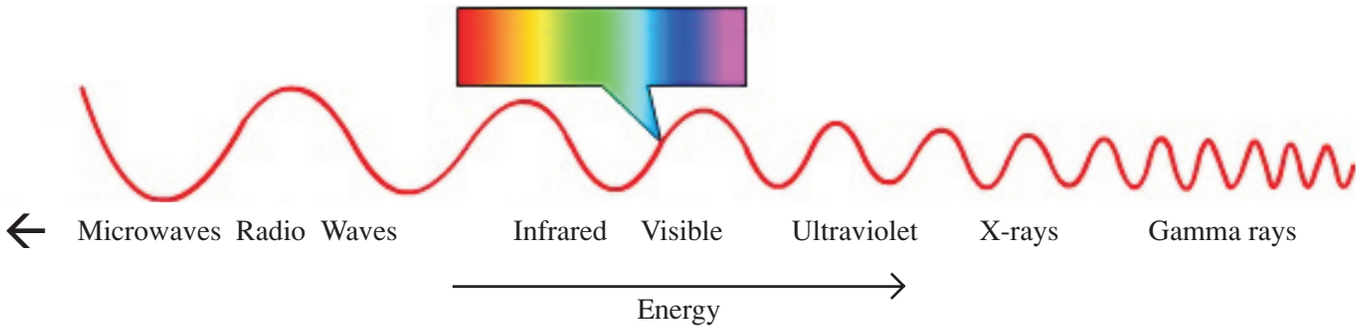
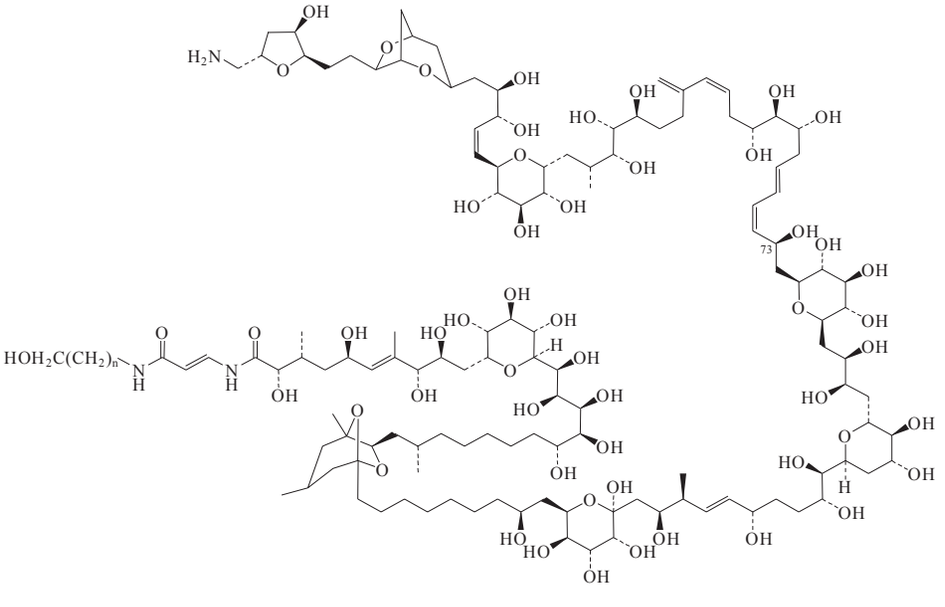
* บทบรรยายพิเศษเรื่อง Scientific Instrumentation in Research (เครื่องมือวิทยาศาสตร์กับงานวิจัย) สมเด็จพระเจ้าลูกเธอ เจ้าฟ้าจุฬาภรณวลัยลักษณ์ อัครราชกุมารี ทรงบรรยายในการเปิดสัมมนาทางวิชาการ เรื่อง การใช้และดูแลเครื่องมือวิทยาศาสตร์ที่ซับซ้อนราคาแพง ซึ่งสำนักวิทยาศาสตร์ แห่งราชบัณฑิตยสถาน สำนักงานคณะกรรมการวิจัยแห่งชาติ และสมาคมธนาคารกระดูกและเนื้อเยื่อประเทศไทย ในพระอุปถัมภ์สมเด็จพระเจ้าพี่นางเธอ เจ้าฟ้ากัลยาณิวัฒนา กรมหลวงนราธิวาสราชนครินทร์ ร่วมกันจัดขึ้น ณ โรงแรมมิราเคิล แกรนด์ คอนเวนชั่น กรุงเทพฯ เมื่อวันที่ ๑๗-๑๘ สิงหาคม พ.ศ. ๒๕๔๘




Four primary spectroscopic techniques have been in use since the early 1960s. They are *nuclear magnetic resonance (NMR), infrared (IR), ultraviolet-visible (UV-VIS),* and *mass spectroscopy (MS).* All of these methods are used daily by most organic chemists. They require a minuscule amount of material and for the most part are nondestructive. The use of NMR, IR, UV-VIS, and MS can be replaced by another well-known tool, *X-ray crystallography,* but only for the analysis of well-formed crystals.

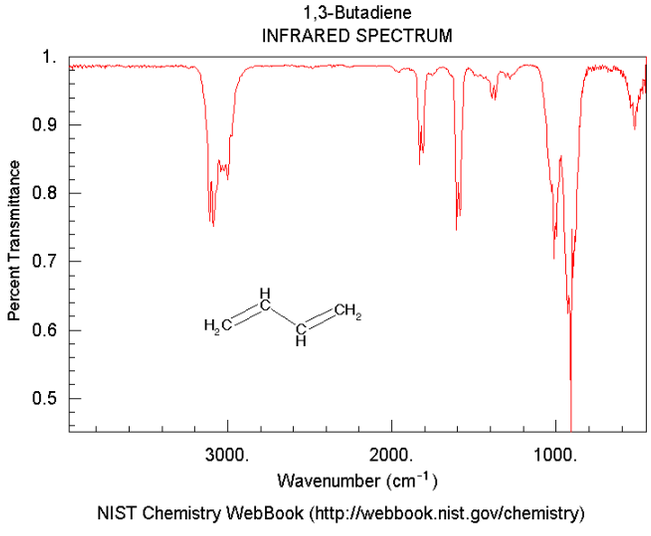
Compounds with molecular weights of under 1,000 amu are excellent candidates for study by the methods of organic spectroscopy. In recent years, advances in NMR and MS instrumentation along with experimental strategies have even made it possible to tackle structural elucidation of very complex compounds. For example, the intricate structure of the natural product palytoxin structure as shown of molecular weight 2677 amu and molecular formula $C_{129}H_{223}N_3O_{54}$ was proposed based on spectroscopic data. But the process of collecting the appropriate data, making unambiguous interpretations, and reweighing the data took some twenty years!




To be successful in the task of organic structure analysis, especially when an exotic compound is in hand, requires several attributes. In addition to chemical common senses it is helpful to have experience

in five other areas. First, a general knowledge about principles of organic structures is a must. You should also have an understanding about common functional group. Second, an organized and systematic approach must be applied. Third, understanding of how to interpret a spectral trace is essential. Fourth, appreciating the dangers of using negative or unreliable data to make positive conclusions about a molecular structural feature is important Fifth, being able to maintain a proper perspective in situations where conflicting data sets seem to be in hand is necessary, and when this occurs, being able to draw on experience gained from past success in problem solving is helpful. Finally, simultaneously using data from NMR, MS, IR, UV-VIS and looking repeatedly for multiple pieces of data to support individual conclusions might be considered as a universal goal.

**IR Spectroscopy**



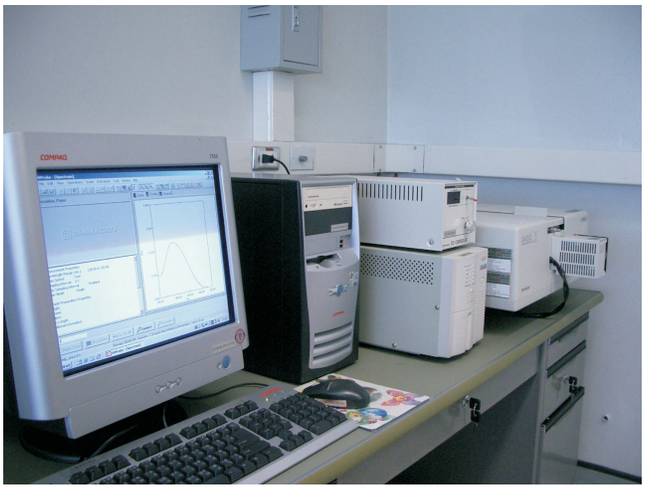

Infrared spectroscopy measures the vibrations of molecules. Each functional group, or structural characteristic, of a molecule has a unique vibrational frequency that can be used to determine what functional groups are in a sample. When the effects of all the different functional groups are taken together, the result is a unique molecular "fingerprint" that can be used to confirm the identity of a sample.

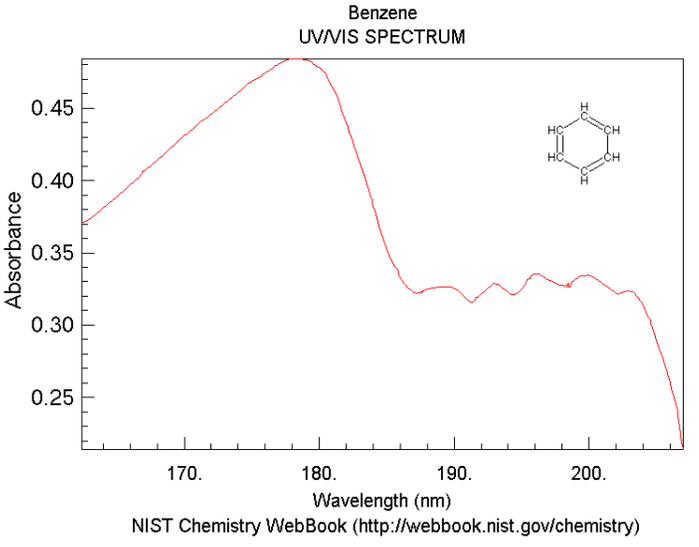
Butadiene was the essential chemical ingredient of the synthetic rubber program during World War II. Butadiene molecules were linked in long polymer chains to produce synthetic rubber. Knowing the concentration and purity of butadiene was essential to controlling rubber quality, and infrared spectroscopy was the only technique that could accurately gauge these properties.

Finding the concentration and purity of hydrocarbons is only the very tip of what infrared spectroscopy can do. Because of its versatility and convenience, IR spectroscopy is now an essential in almost every branch of chemistry.

Modern infrared spectrometers are very different from the early dispersive instruments that were introduced in the 1940s. While there is still a need for dual-beam dispersive instruments in high-precision work, most instruments today use a Fourier Transform infrared (FT-IR) system.

## UV-vis Spectroscopy






UV-vis spectroscopy probes the electronic transitions of molecules as they absorb light in the UV and visible regions of the electromagnetic spectrum. Any species with an extended system of alternating double and single bonds will absorb UV light, and anything with color absorbs visible light, making UV-vis spectroscopy applicable to a wide range of samples.

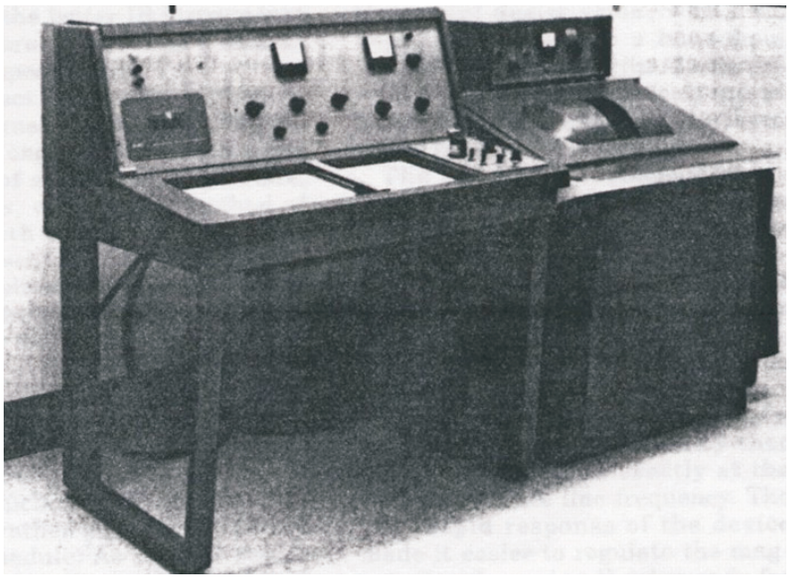
A UV-vis spectrum of benzene is shown. The boom in ultraviolet spectroscopy began with vitamin A, but benzene is one of the most common chemicals that can be analyzed with UV-vis spectroscopy. In fact, a spectrum of benzene was included in the first article written about commercial UV-vis instruments. (Cary and Beckman, 1941)

Today, UV-vis spectroscopy is one of the truly routine techniques in modern biochemistry, biology, and pharmaceutical research.

UV-vis spectroscopy is used every day in thousands of labs around the world. Modern UV-vis instruments are quite different than when the DU was introduced in 1941, but they all operate on the same basic principles. The movie below explains the workings of the two most popular types of UV-vis spectrophotometers for research use today: the dual-beam and diode-array UV-vis spectrophotometers

## Nuclear magnetic resonance (NMR)

Nuclear magnetic resonance (NMR) is one of the most powerful tools in a synthetic or structural chemist's arsenal. By probing the magnetic properties of spin-active nuclei, chemists can learn more from an NMR spectrum than almost any other analytical technique. The application of NMR to chemistry is different from pH, UV-vis, and IR spectroscopy in that instrumentation was produced before applicable chemical problems were known. The Beckman Model G and Beckman DU were all instruments designed to answer specific questions asked by chemists of the time, but Varian began development of its line of NMR spectrometers well before commercially-viable applications were known. The result was an unusually short gestation period from the discovery of nuclear magnetic resonance to the introduction of the first widely successful instrument, the Varian A-60.



Today very high field NMR spectrometers are produced and at CRI we have one 600 MHz NMR as shown. We also have the 400 MHz NMR machine at CRI. I am very proud to say that all the high resolution NMR machines that we have at CRI are acquired from the grants I have obtained from German Government.

**400-MHz NMR**
**(Bruker AM 400)**


**600-MHz NMR**
**(Bruker AVANCE 600)**

**Mass spectrometry**

Mass spectrometry involves the generation of ionic species from an organic molecule subjected to an electric or magnetic field. The fragmentation data obtained by this process are invaluable for deriving molecular formulas (or partial formulas). Techniques have been developed to facilitate visualizing a MS peak containing the


molecular formula information. For research work the mass spectrometry is usually coupled to gas chromatography commonly known as GC/MS.

**Flash Animation of GC/MS**

The movie shows a short series of steps for the process of a single analyte (already separated from the other analytes in the chromatographic mixture) denoted as ABC exiting the chromatographic column and:

- the analyte (A-B-C) undergoing ionization and fragmentation
- the charged fragments ($A^+$ $B^+$ $C^+$) being separated by mass
- the fragments which are focused on the mass filter's exit slit passing into the detector
- and the charged ions being detected.

In this example, the lightest fragment is $B^+$; the heaviest $A^+$. The last frame of the movie is a mass spectrum displaying only these three fragments. Their relative mass to charge ratios are specified by their relative position on the x axis (low mass/charge to left, high mass/charge to right). The relative amounts (commonly called peak intensity) of each of these fragments determined during the mass analyzer's scan is reflected by the y axis.

The suite of gas chromatographic detectors includes: the flame ionization detector (FID), thermal conductivity detector (TCD) or hot wire detector), electron capture detector (ECD), photoionization detector (PID), and others. Another GC detector that is also very expensive but very powerful is a scaled down version of the mass spectrometer. When coupled to a GC the detection system itself is often referred to as the mass selective detector or more simply the mass detector. This powerful analytical technique belongs to the class of hyphenated analytical instrumentation (since each part had a different beginning and can exist independently) and is called gas chromatograhy/mass spectrometry (GC/MS).

Placed at the end of a chromatographic column in a manner similar to the other GC detectors, the mass detector is more complicated than, for instance, the FID because of the mass spectrometer's complex requirements for the process of creation, separation, and detection of gas phase ions. A capillary column most often used in the chromatograph because the entire MS process must be carried out at very low pressures (~$10^{-5}$ torr) and in order to meet this requirement a vacuum is maintained via constant pumping using a vacuum pump. It is difficult for packed GC columns to be interfaced to an MS detector because they have carrier gas flow rates that cannot be as successfully pumped away by normal vacuum pumps; however,

capillary columns' carrier flow is 25 or 30 times less and therefore easier to "pump down". That said, GC/MS interfaces have been developed for packed column systems that allow for analyte molecules to be dynamically extracted from the carrier gas stream at the end of a packed column and thereby selectively sucked into the MS for analysis. For one type interface, using a silicone membrane, the selectivity for organic molecules (the analyte) over helium (the carrier gas) is 50,000.

I have previously mentioned the analytical technique belongs to the class of hyphenated analytical instrumentation and cited gas chromatograhy/mass spectrometry (GC/MS) as an example. Recently this technique has been used extensively especially the coupling of HPLC with other instruments including NMR, UV, MS and commonly known as LC-hyphenated techniques.

HPLC originally stands for High Pressure Liquid Chromatography which is in common use today for the separation of various compounds. The technique is evolved from the low pressure liquid chromatography and medium liquid chromatography. Today the word HPLC is commonly referred as High Performance Liquid Chromatography. HPLC or commonly called LC nowadays is a very versatile separation technique thus the instrument is coupled with other instruments for identification of organic compounds especially natural products.

LC-hyphenated techniques are playing an increasingly important role as a strategic tool to support phytochemical investigations. Indeed, these techniques provide a great deal of preliminary information about the content and nature of constituents of crude plant extracts. This is very useful when large numbers of samples must be processed since unnecessary isolation of known compounds is avoided. Once the novelty or utility of a given constituent is established, it is then important to process the plant extracts in the usual manner, to obtain samples for full structure elucidation and biological or pharmacological testing.

The recent introduction of LC/NMR for crude plant extract screening will probably make another breakthrough in the on-line structural determination of natural products. This hyphenated method allows the recording of precious complementary on-line structure information when LC/UV/MS data are insufficient for unambiguous peak identification. Indeed, LC/NMR has proven to be very effective in obtaining 1-D spectra on both flowing and non-flowing samples, as well as stop flow 2-D spectra. However, compared with DV or MS, NMR remains a rather insensitive detection method and the need for solvent suppression in conventional LC/NMR restricted the observable NMR range.

LC/MS analysis of crude plant extracts is not straightforward due to the great variety of their constituents. No interface allows an optimum ionisation of all the metabolites within a single crude plant extract since the response is compound dependent. Often, different ionisation modes or different interfaces are necessary to obtain a complete picture of the extract composition.

With the full set of spectroscopic information obtained by LC/UV, LC/MS and LC/NMR, the phytochemist will be able to characterise rapidly the main constistuents of a given plant and to choose carefully which metabolites are to be isolated for indepth structural or pharmacological studies. The chemical screening of

extracts with such hyphenated techniques generates a huge amount of information. In order to rationalise this approach and to use it efficiently with a high sample throughput, the next challenge will be to find a way to centralise all these data for rapid pattern recognition by reference to standard compounds. With such an analytical system, phytochemists will then be able to concentrate their efforts on finding new biological targets. This aspect still remains the more difficult problem to solve when searching for new leads.

The recent advances in electronics and computer hardware and software made the today expensive machines highly sophisticated and thus very sensitive and need careful maintenance. We have to look after these expensive machines with "Tender Loving Care" like our children. Moreover these highly sophisticated and sensitive machines often break down like these two quotations.

WARNING : THIS MACHINE SUBJECT TO BREAKDOWNS DURING PERIODS OF CRITICAL NEEDS.

KEEP COOL AND SAY NICE THINGS TO THE MACHINE: NOTHING ELSE SEEMS TO WORK.