Development of instrumental analytical methods for essential oils and aroma chemicals

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Mr. Rogers joined the analytical laboratory of FDO in 1946 in Clifton and, then, in 1949, the research laboratory. He set up the instrumental laboratory there in 1952 and in 1958 established the main instrumental laboratory at FDO Corporate Headquarters in New York. In 1964 he was made Director of Laboratories, and in 1966 Vice President and Director of Research and Development. In 1974, he undertook his present responsibilities.

Modern instrumentation for analysis of essential oils, aroma chemicals, fragrances, and flavors is more common in the laboratory today than lab balances were years ago. Few people realize how sophisticated and proficient technology has become during the few years that instrumentation has been used. Innovations in equipment and applications are daily occurrences in the laboratories of every major fragrance and flavor company. Instrumental analysis of our products affects quality control, research, development, processing, and production.

The evolution of modern analytical techniques, particularly instrumentation, has been especially significant when one considers the state of the art only twenty-five years ago when our industry first began using modern instrumental methods.

Certainly, polarimeters, refractometers, pycnometers, Nesslerimeters, Duboscq colorimeters, Lovibond color comparators, and other such instruments were used for isolated and specific analyses in our industry prior to that time. However, none of the advantages of modern instrumental analyses were present then; separations were tedious and poor, specific identifications of components were greatly limited. The chemistry of molecular structure was not in an advanced state; confirmation analysis had only been proposed a few years prior; specificity of products was hardly known.

In the fragrance and flavor industry, which products were as complex as any in existence, such data as specific gravity, optical rotation, relative color values, or functional group analyses gave as much objectivity to the important subjective odor and flavor evaluations as was then available. Under the conditions at the time, adulteration and sophistication were difficult to determine. A few chemists could wreak havoc with the work of all of the other chemists in the industry who were attempting to improve quality.

The analysis and determination of trace components for authenticity and quality were unknown. Colored spot tests might possibly determine the presence of mentha arvensis in piperita, or vice versa. The Beilstein test (copper wire test) would determine through the presence of chlorine in cassia oil that synthetic cinnamic aldehyde was present, if the synthetic cinnamic aldehyde contained chlorine as a result of impure benzaldehyde in the initial condensation reaction. However, soon after the development of this test, benzaldehyde was manufactured free from chlorine and synthetic cinnamic aldehyde could be made fairly clean—certainly clean enough to clear any analytical method of the day.

Similarly, after being duped for an unknown period, those attempting to determine the mineral oil adulteration of citronella oil developed a test using fuming sulfuric acid by which all organic components of the sample save the paraffinic hydrocarbons of mineral oil would be destroyed.

Anyone who has ever run the titrimetric test for ascaridole in wormseed oil, then a common essential oil, could never forget it. The tests required for geranium oil were extensive and time-consuming. There were several ester evaluations with two temperature saponifications separating the formate from all other esters combined. Finally, acetylation determined the total alcohol, which figure included geraniol, nerol, citronellol, and linalool.

By the mid-1940s the Essential Oil Association (EOA), had developed physical and chemical specifications which became extremely valuable. They had established ranges on physical and chemical properties of all available scientific information. Combined with odor and flavor evaluation, our products began to be reasonably described despite their complexity. The EOA Scientific Committee was responsible, in major degree, for the orderly progress of the entire industry.

Had adulteration gone unchecked in the late 1940s, the industry could have had serious credibility problems with its products and customers until instrumentation appeared on the scene in the early 1950s. If any industry and its products required the attributes of instrumental methods, it was the essential oil industry. For an

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appreciation of the contributions of speed, quality, and completeness of instrumental methods, consider the tedious methods mentioned above against the ease with which knowledge of a particular product is presented today by a simple routine gas chromatograph.

The application of instruments to the analysis of flavor and fragrance products was a spasmodic rather than gradual occurrence. In the 1930-1940 era, the organic research pioneers of the day conceded the importance of physicists and joined them to participate in a common scientific endeavor. Advances in molecular theory and pressure from war-oriented technology combined the scientific disciplines of chemistry and physics toward a common goal. Application of physical measurements to chemical components began to yield important basic knowledge.

Physical constants for instance, specific gravity had been used for many years to describe essential oils and related components. However, the physical properties measured by more modern physics/chemistry interaction were somewhat different and more specific. The absorption of infrared, ultraviolet, or visible radiation energy; the change in radio frequency when atoms are placed in a magnetic field; fragmentation of groups of atoms from a molecule; and partition co-efficients of molecules in the gaseous state are all physical properties of our products that can now be measured accurately and controlled to a degree. Physical properties of molecules are still being discovered; electronics and its auxiliary facilities, magnetic tape, for instance; integrators, computers, cathode tubes, special printing techniques, and data storage are technological advances that have been incorporated into lab equipment in different ways.

Even a thorough look at our modern instruments would not be sufficient to trace the creative ideas, engineering concepts, and effort that have brought this equipment to its current degree of perfection. A few historical facts on several of the instruments may help us understand what was involved.

Infrared instruments

Herschel discovered infrared radiation (IR) in 1800. He used a source of infrared light—in his case, the sun. He dispersed this through a prism. (His was of glass, though we now know other materials or dispersing elements are much better.) He measured the infrared region with an ordinary laboratory thermometer, which was his heat detector. His equipment at that time was substantially the same as modern infrared instruments, though only the experienced would recognize this. So much optical and electronic interaction has occurred since Herschel's beginning that it is difficult for us to see the simplified block diagram of his equipment.

In his experiments, Herschel observed two maxima in the sun's radiation spectrum, a visual intensity maximum in the yellow-green area and a heat maximum beyond the visible range. He postulated that two different types of radiation were therefore present.

In 1835, Ampere, Melloni, and Herschel (the son of the original observer) stated that visible and infrared radiation were similar light phenomena but diffracted differently by the prism. The younger Herschel was able to show infrared Fraunhofer lines of carbon dioxide and water by photographing the result of evaporation of alcohol from a soot-covered surface subjected to dispersed infrared radiation. Shortly, thermopiles were introduced as heat detectors, but these were very insensitive. In 1880, Langley introduced the bolometer, which, coupled with sensitive galvanometers, enabled diffraction gratings to be the dispersion device with resulting improvements in wavelength measurement.

Much later, in 1905, Coblentz, a physicist in Carnegie Institute, Washington, DC, produced infrared absorption spectra with equipment of his own design. He was able to record by photographic methods the absorption spectra of a few organic compounds, some of which were essential oils. He suggested some empirical correlations of molecular structure and infrared absorption peaks. This was, in effect, the first attempt to determine a specific chemical within a mixture by electromagnetic radiation methods. At that time, the chemical possibilities of the technique were still not realized, and the main efforts were directed toward academic physics research.

In 1913 physicists observed the rotational structure of molecules, and the concept of vibrating molecular bonds was proposed. By 1916 there was an intensive interest in molecular quantum mechanics. As Einstein's theories became more understood and the disciplines of engineering and mathematics became involved in physical/chemical structures, the application of infrared radiation to molecules to measure such properties as molecular bond vibrations was actively pursued in many laboratories. Over a period of time it was discovered that all organic substances possess selective absorption of certain infrared frequencies in the electromagnetic spectrum. Most of our products, of course, are organic chemicals.

By the 1930s organic chemists saw infrared as a possible tool for material identification and functional group analysis. In 1935-36, several of the major chemical companies with large research staffs began the construction of custom-built infrared spectrophotometers. It was now known that radiation absorption could be used as a method for quantitative analysis, and much theoretical work was undertaken on small molecules such as gases.

The war years of 1940-43 saw a tremendous expansion in infrared research, motivated by a series of urgent demands. The need for synthetic rubber caused the structure of natural latex to be thoroughly examined; the need for refineries, catalysts, and processes to produce more powerful, consistent, high octane rated fuel resulted in petroleum research; and the necessity for identification of the penicillin structure to speed up in-process controls of fermentations and to attempt to synthesize the active principle in penicillin increased the interest in uses of infrared analysis.

At this time, plans were formulated to produce commercial infrared instruments. Up to this point, the infrared instruments were custom-made. They were single beam instruments, operated manually, wavelength by wavelength. The detectors were based on galvanometer deflections from a thermocouple, photographically measured as the frequency of radiation was changed. Even the building which housed the instrument was important, since any vibrations such as adjacent railroad tracks, truck or auto traffic, or elevators made the equipment unuseable; also thermal stability had to be guaranteed.

The first commercial single beam instruments manufactured were the Perkin-Elmer 12-C and Beckman IR-2. Infrared gas analyses, performed by such instruments as the Baird, had limited use.

Perkin-Elmer and Beckman were double pass instruments, which meant that the infrared radiation was passed through the prism twice in order to improve the resolution of the radiation to be examined. The experts of the day saw the need for a high intensity source, a split beam with double beam optics: one beam for the sample, one for general background radiation. An amplified thermocouple readout and pen-recorded chart of some type, perhaps a percentage transmission or log percentage transmission, were also required.

In 1947 Baird Associates put a double beam infrared instrument with a small drum recorder on the market. This was quickly followed by a Perkin-Elmer with additional instrumental refinements.

Our industry became involved in the instrumental groundswell at this time. To my knowledge, the first infrared recording instruments used in the essential oil industry were purchased in 1951-52. The cost of such instruments, complete with accessories, totalled approximately $15,000. At that time, you could purchase three Cadillacs or a six-room house on a half-acre suburban site for the same price.

By 1954 the EOA Scientific Committee was studying infrared spectra to develop standards for our products. This technology, though it had been slow in becoming accepted, was soon widely applied in our industry.

Ultraviolet instrumentation

The history of ultraviolet instrumentation is not quite as complex. After its discovery by Hantzsch in 1910, interest progressed slowly until the first commercial instrument was produced by Beckman in 1930. A photographic plate was used to measure the absorption on a wavelength-by-wavelength, point-to-point basis. In 1938 the Beckman DU was commercialized. This was the prototype of all modern spectrophotometers. It was simple, though time consuming, to operate. It was extremely accurate and reproducible.

In those days, the only sources of stable voltage were dry and wet cell batteries. Modifications of the DU were rapid. Dual source units and interchangeable detector units increased the range, enabling visible spectra to be run using the same instrument. Several DU instruments were used in our industry prior to 1950. In about 1952, motor driven mechanized recording units were available; and in 1955 or so, Beckman produced its DK series of instruments, which opened up the near infrared region to special applications. Cary manufactured the precision ultraviolet instrument of the day.

Much effort went into the EOA Scientific Committee meetings to produce quantitative specifications for essential oils and components by infrared, near infrared, ultraviolet, and visible methods. The first scientific paper in the entire analytical field thoroughly describing quantitative infrared methods of analysis was published by the EOA Scientific Committee in "Applied Spectroscopy" in 1960.

Specific component quantitative analysis had rarely been available to our industry; but at this time we could measure linalool, geraniol, and citral specifically by infrared; cedrol by near infrared; piperine and CD values for citrus oils by ultraviolet; and paprika color by visible. All were invaluable contributions to the EOA methods. This was only a beginning, however. Most of these specific quantitative methods were shortly to be challenged and then replaced by a new instrumental method, gas chromatography, which was more accurate, quicker, and more specific than anything previously available.

Chromatography

Though we cannot detail the history of every modern laboratory instrument, the historical aspects of chromatography also apply to much of the modern instrumental equipment such as high pressure liquid chromatography, preparative units, and the like.

The first recorded separations that can be described as chromatography, although not so named at the time, were the discoveries of Runge, in Sweden around 1850. Using Swedish filter paper and a solvent, he separated various commercial dyes of the day. Originally the absorbent effect, according to the method used, was based on molecular weight. That particular method, known now as paper chromatography, still has wide application.

In 1906 Tswett described the use of glass tubular columns packed with a suitable absorbant to separate natural pigments in plants. The name "chromatography" (color writing) was coined at this time. Tswett used a mobile liquid to elute those colored bands on his packing material and collected the purified components as they came off the column. One wonders whether the inertness of the column material or its ability for observation led him to choose glass for this use.

In 1941 Martin and Synge suggested the use of a gas rather than a liquid solvent as the mobile phase in a chromatographic apparatus. No practical equipment was designed and no experiments carried out; the idea was a theoretical one. In 1951 Martin and James described the first apparatus; but this instrument detected only acids and bases, since the detector was in fact an automatic buret which could by titration measure the acid and bases separated. In 1954 Ray published a first chromatogram and used a thermal conductivity detector which still has wide use in gas chromatographic instruments. Within a year or so the first commercial chromatograph appeared. By 1956 many instruments of various types were available and all were rapidly being installed in the laboratories of our industry.

Here was a tool which fitted our requirements as completely as it did those of any other industry, for we were now able to separate the components of our complex mixtures. Our scientists had immediate uses in mind. The EOA instrumentalists were excellent practitioners and were responsible in part for important innovations in instrument design, such as improved injector parts and variable speed chart drive.

Advances in gas chromatography technology and equipment followed in rapid succession. Detectors and electronics improved substantially. The original thermal conductivity detectors, so-called thermistor bead detectors, were followed by hot wire detectors. Other detectors included Argon beta-ionization detectors, hydrogen flame (flame ionization) detectors, as well as detectors for special uses, such as electron capture for halogens, phosphorus and sulfur determination, and nitrogen detectors for amines, amides, and the like.

In the first commercial instruments, the oven and therefore the column temperatures were maximized at 90°C. Column packings could not tolerate higher temperatures and ovens could not be stabilized above this limit. Originally, the injector ports, columns, and detectors were all heated together in the same oven. Injector ports were separated and modified, and by 1958 programmed temperature instruments appeared. The detector and injectors were separated, heated individually, and stabilized; the columns and oven were subjected to changing rates of heating; higher boiling components would now be eluted rapidly. Temperatures could be accurately controlled to about 230°C. At the same time, cryogenic (below room temperature) instruments were also available.

Advances in column technology continued

from the initial single wide bore (¼") poorly packed columns to dual column, dual detector instruments which were available in 1960. Narrow bore packed columns (⅛") were in common use. Copper was replaced by soft stainless steel, aluminum, and glass tubing for column materials. Golay, or open tubular columns (0.01 to 0.02 inches inside diameter), greatly improved separations in 1962. In 1970 Jennings described glass capillary columns with excellent characteristics. In addition, pre-columns, molecular sieves, and back-flushing equipment could be used for specific analysis.

Column supports were improved—from ground-up fire brick, requiring harsh treatment for good performances, to specially custom-treated chromosorb of very definite particle size. (Powdered detergent and other products, even popcorn, had been used as supports on occasion in the early days.) A range of polar and non-polar liquid substrates that have excellent heat and chemical stability are now used; for instance, Carbowax 20M and Silicone OV-101.

Large bore columns in special apparatus, called preparative units, are now able to handle large size samples and give gram quantities of highly purified component from a complex mixture. In fact, commercial plant sized units now can separate quantities of up to a kilo per hour of components of mixtures of limited complexity such as a single terpene, sesquiterpene, homolog alcohol, steroids. Injectors, sampling valves for solids and gases, adaptors, septums, micropipettes, and microsyringes have each evolved into highly efficient and well-designed apparatus.

Integrators (1960-62) and computers (1970) have been incorporated into the equipment, and now a very formidable electronic/scientific machine is the common laboratory accessory.

Despite the relative perfection of gas chromatographic apparatus, this instrument, like all the others, has limitations. The gas chromatograph can separate only volatile components of mixtures, items which vaporize below 350°C at 760 mm, depending, of course, on polarity and a few other physical attributes such as sensitivity. Other commercially available equipment which would allow the separation and detection of non-volatile components is needed.

An adaptation of column chromatography has proven to be the most promising recent innovation. This instrument uses a packed column and a solvent or a mixture of solvents under high pressure (3000 psi) to move the non-volatile materials through the packed column, separate them, and elute them in pure form. The equipment is known as a high pressure liquid chromatograph. The present detector systems need improvement; but the method offers excellent advantages, and high pressure liquid chromatographs are common in the laboratories of the essential oil and fragrance/flavor industry companies.

Since excellent separation can presently be achieved by chromatography, now structural identification equipment to define the separated components must be improved as well.

Mass spectrometry and nuclear magnetic resonance

Mass spectrometry and nuclear magnetic resonance are now standard techniques in most laboratories.

Mass spectrometry has been known for quite a while; but practical, easy-to-operate instruments were a long time coming. Original equipment, circa 1950, used photographic plates and galvanometer deflection to detect the various molecular fragments caused by high energy ionization and fragmentation of the sample molecules. The stability and magnetic environmental conditions had to be satisfied. Modern equipment is much more adaptable. The combination of a gas chromatograph (which separates components), a mass spectrometer (which fragments and describes them), and a computer (which identifies and collates the information) is the modern instrumental laboratory.

Nuclear magnetic resonance is excellent for detailed structure elucidation. It describes the environment of every hydrogen atom in a molecule; thus, by assembly of information, the entire structure can usually be determined. A fourier transformation adaptor can enhance the output of nuclear magnetic resonance a thousand-fold. Even more specific instrumental techniques, such as electron spin resonance now are available.

Influence of instruments on the industry

One can see there is an interesting history in the development of instrumentation, and its acceptance and use by our industry. In looking back, it has been an exciting and invigorating experience, although there were many amusing and frustrating incidents. The first curves run on an instrument—no matter which one—on company products or on samples which were brought to the instrumental lab were extremely revealing. It was a thrill to watch an original curve being recorded, trying to predict the peaks, making identifications, imagining, theorizing, collating, and identifying.

The early instrumentalists, even in our industry, were generally well schooled in analytical and control techniques. In addition, they had to be a combination of mechanic, plumber, electrician, and electronic engineer. No doubt a basic knowledge in any of these capacities was a decided help; but most of all, patience and a good set of tools were necessary. New problems in technique and equipment were encountered daily. At the EOA scientific meetings, there were lengthy discussions on such simple problems as custom-made inks for the infrared instruments. Four or five colors were needed for superimposition of curves. Pens were of very poor construction and clogged readily. More than one curve was lost and the product identification missed because the sample evaporated from between salt plates while the pen failed to print.

After a few startup and warranty visits by the instrument manufacturer, the instrumentalists became very familiar with disassembly methods and learned to replace parts and make other modifications themselves. Instrumentalists became able to fine-tune the instrument. They could anticipate mechanical problems prior to a serious breakdown, just as a good auto mechanic is able to do. They learned how to reposition a warped glower so it would reflect a vertical image, or rethread the detector block of a new gas chromatograph to accept a larger exit port and reposition it for better sniffing and improved preparative and sample trapping work.

It was an innovative era and many modifications suggested by the practitioners were taken and incorporated into instrument design by the manufacturers. There was limited time, however, for developing techniques or making modifications, for the information that was being generated was extremely valuable in all phases of the industry. From each department of a company came requests and insistence on top priority of its projects. The "machines" were looked on by many as a panacea to their problems.

I can recall giving a talk to the American Society of Perfumers shortly after the instrumental methods were introduced into the industry. One gentleman was quite upset that we were attempting to replace the perfumers with a black box. I assured him that this could never be done, that the methods were to assist him and improve his own knowledge and techniques; I doubt very much that he was convinced. The new techniques caused anxious years before this was recognized.

Despite the technical advances, objective measurements of essential oils and aroma chemicals can only be done with much experience and extreme care to the subjective evaluations of our products. Only when the mechanisms of odor and flavor evaluation by the human senses are described more explicitly can objective methods which will correlate objective and subjective evaluations be invented and designed. Presently, instrumental methods measure physical and chemical characteristics of our products which are not related in any known way to our senses of taste and smell.

Instrumentation has been a type of equalizer in the flavor and fragrance industry. While subjective evaluation of flavor and fragrance is still a most important part of in-house technology—

and, certainly, the creative efforts of perfumers or flavorists are most important parts of the products—the analytical and research instrumental laboratories are now substantial contributors of factual, objective knowledge; the level of science in the industry has been significantly raised.

Instrumentation is an analytical tool, but its results can be applied in diverse areas. Analysis and quality control have always been the major beneficiaries of instrumentation, but the first curves developed over 25 years ago also had immediate application to research and development. Presently there is more of an instrumental effort in research than in analytical or quality control of products. For example, instruments make possible the determination and identification of trace components in natural products; these components are either occurring naturally or might be artifacts. This information is extremely important to the evaluation of the product. If on successive samples the ratio of a trace material to another changes drastically, adulteration is almost at once suspected. Once suspected, concentrated effort can readily provide proof.

With the present state of the art and the availability of equipment, the answer can almost always be found with the proper input of energy if one knows what to look for. While trace components have significant quality implications, the identification of trace components in natural products has paved the way for the development of many new products for flavor and perfume chemists. For instance, rose oxide is important to geranium analysis, but as a synthetic component it is vital to a good duplication of the natural.

Prior to instrumentation and its use in research, the development of new flavor and fragrance products depended mainly on synthesis screening, laboratory synthesis of a compound followed by evaluation for possible use. Formerly, flavor additives were not controlled to any degree by the existing agencies; now, modern government regulations have established a whole new set of ground rules around which research and development of flavor and fragrance chemicals must operate.

Chemicals like hydroxycitronellal and cyclamen aldehyde probably would not have been discovered using modern approaches. Certainly, the nitro musks and other such products would never have found their way into the fragrance area based on the type of research presently done in our laboratories. Now there is a definite trend toward nature-identical or homologous materials where information exists on usage, which should also help satisfy our initial questions regarding toxicity.

While items like hydroxycitronellal, amylcinnamic aldehyde, and the nitro musks certainly have their position in the fragrance area, they are not natural and they lack the authenticity of the real thing. Not only the perfumer or flavorist, but also the general public can recognize this natural subjective effect in flavors and fragrances, providing another reason for the trend toward nature-identical substances. The literature is full of reports and papers of advances in this area. The chemists of the flavor industry are some of the leading organic chemists in the world, and the information on natural trace substances which they develop has application to natural products of all types. Instrumentation thus has played an important part in modern approaches to synthetic flavor and fragrance products.

Summary

The state of the art in instrumentation has developed at an extremely rapid pace since its introduction into our industry approximately 25 years ago, affecting it in all its phases.

Rather than limiting subjective creativity, the years ahead offer greater challenges to our creative people because of the expansion of knowledge due to instrumentation. Perfumers and flavorists must be more creative than ever. Their total knowledge and the number of products available have increased substantially. Their creativity, therefore, has a wider base, providing more tools, new ideas, and new direction to their efforts.

In the development and manufacturing area, scientific chemical processes can now be examined in more detail. Synthesis chemists are much more aware of side reactions and the direction of their experiments. Certainly, the ease of detecting the molecular structures enables them to be much more creative in manipulating reactions. In process, raw material and finished production analyses by instruments detect problems, determine difficulties and objectives, and control quality of all phases of products.

The advances in all these technological areas have enabled the industry to improve substantially as a viable economic entity. We face with confidence the major dark clouds of unknown toxicities and greater irrelevant regulations of our products. Our instrumental technology will enable us to respond more quickly, test more thoroughly, and have confidence in the safety of our products to a greater degree.

The ultimate purpose of our products and the reason for our industry, to provide consumers with products which please their taste or smell, today and for generations to come has not changed. Safety has always been and will continue to be our most fundamental concern. We are known to respond to problems long before government regulations can be or have to be enacted. Our technology points toward a bright future for the fragrance and flavor industry.