GAS CHROMATOGRAPHY

IRWIN HORNSTEIN

UNITED STATES DEPARTMENT OF AGRICULTURE

A major contributor to food flavor is odor. Odors are produced by volatile materials which impinge on the olfactory centers. The acuteness of this sense is fantastic; man can detect concentrations as low as fractional parts per billion of some substances and man has rather a poor sense of smell as compared to other members of the animal kingdom. The volatile flavors of foods are usually a complex mixture of organic compounds which vary in amount, stability and volatility. The classical approach by flavor chemists to isolate and identify these materials has been to use micro distillation techniques in conjunction with methods of chemical analyses. Separations by distillation even with the most elaborate setups and maximum precautions give incomplete resolution of complex mixtures and higher boiling materials are often badly decomposed. The introduction of chromatographic methods has been as great a boon to the flavor chemist as to the biochemist, and with the development of gas chromatography progress in the flavor field should move on apace.

Chromatography is a method of separating a mixture into its components by repeating many, many times the unit step of partitioning a compound between two phases. One of these phases is stationary and, if in column form, composed of granules of very large surface area; the other is a fluid that flows through this stationary phase.

In gas chromatography, the moving fluid, as the name implies, is a gas; the stationary phase may be either a liquid or a solid. Gas-liquid chromatography is the more useful form for most purposes and will be discussed here.

In the first slide, a schematic diagram of equipment normally used is shown. We have a tank of gas under high pressure, followed by a precision gas regulating valve and a pressure gauge. For liquid samples, the most commonly used method of sample introduction is to insert the needle of a micro syringe through a rubber septum cap and quickly inject the sample into the gas stream. For gaseous samples, a known volume is introduced from a calibrated chamber by the carrier gas. The tube itself may be glass or metal and may vary in shape, length, and diameter. A typical column might be made of copper, 6' in length and 1/4" O. D. The inert granules should be of uniform size; their function is to act as the support for the stationary liquid phase with which it is coated. The column operating temperature is a function of the boiling points of the materials to be separated. The vapor pressure of the stationary phase must be negligible at this column temperature. The gas is the eluent, and depending on the detector is usually helium, nitrogen, or argon. The rate of flow of a given component through the column depends on its normal vapor pressure as well as the extent of its interaction with the liquid phase. The components emerge in the form of

bands separated by zones of carrier gas. The composition of the emerging gas stream is constantly monitored by a suitable detector and the information continuously recorded. A typical chromatogram is shown in the next slide. The peak areas represent a momentary change in a physical property of the gas stream when it contains a foreign material.

The ever-increasing use of gas chromatography is a result of its inherent advantages over distillation and other forms of chromatography. The separations are similar to those obtained by distillation, but with tremendously increased resolution, and they are carried out on micro amounts of material. Compared with other forms of chromatography, the low viscosity of the gas permits the use of longer columns with a corresponding gain in efficiency. It is easier to detect changes in composition of a gas than in a liquid stream, and separations are effected much more rapidly. The major areas of research in the development of gas chromatography have been finding the appropriate conditions and liquid phases for carrying out a given separation and in developing more sensitive detection devices.

Just as the heart of the gas chromatograph is the column, the brain is the detector. Since changes in the effluent gas stream may be minute and rapid, the detector must have a fast response, high sensitivity and good stability. Many detectors have been developed for use in gas chromatography; those most commonly used are flame, ionization, Beta-ray ionization, and thermal conductivity. Thermal conductivity, in part because historically it was the first really practical detector, is the most widely used although the ionization type detectors are more sensitive. In the hot-wire thermal conductivity detector, a filament heated by an accurately controlled electric current is held in the center of a small tube through which the carrier gas passes. All else constant, the thermal conductivity of the gas determines the temperature of the wire and consequently its resistance; this is the property measured. When a foreign substance enters with the gas stream there is a change in the thermal conductivity of the gas and consequently a change in the resistance of the wire. In practice 2 gas channels and 2 wires that are as nearly alike as possible are built into a cell. Pure carrier gas flows through one channel and an equal quantity of gas, which has passed through the column, through the second. Any differences in resistance of the two wires due to the effect of volatile components in the effluent are recorded. This is conveniently done by means of a Wheatstone Bridge circuit. For most organic compounds the change in thermal conductivity for a given weight of compound is a maximum when hydrogen is the carrier gas. However, hydrogen has its drawbacks. Catalytic reaction at the hot wire may take place, and there is some hazard in its use. Helium has almost as high a thermal conductivity as hydrogen and none of its drawbacks and is the preferred choice for the carrier gas.

The principal of the Beta-ionization detector can briefly be summarized as follows. A source of Beta rays of relatively low energy is present in the detector; tritium dissolved in titanium is a convenient source. A portion of the carrier gas, which in this case is argon, is raised to a high energy metastable state by this radiation on entering the detector. This excitation level is higher than the ionization potential of most organic vapours. Organic vapours present in the carrier gas are ionized

on collision with excited argon atoms as they enter the detector; this causes a considerable increase in current through the cell, and it is this ionization current that is amplified and recorded. At first glance helium would appear to be a better choice for the carrier gas but its metastable state is so energetic that impurities normally present in the gas are ionized, rendering it unstable for use. The sensitivity here is about 300 times that of the thermal conductivity cell. However, not all materials will ionize under these conditions, and it is therefore not as universal as the thermal conductivity detector.

The operation principles of the flame ionization detector are as follows. Sample components are swept by the stream of carrier gas, usually nitrogen, into a burner assembly where it is mixed with hydrogen gas. The mixture burns in an atmosphere of air or oxygen. The temperature of the hydrogen flame causes a complex ionization to take place. There is a ring-type collector electrode above the flame and the ion current between the burner and the collector is amplified and measured. The magnitude of the output signal is proportional to the number of carbon atoms passing through the flame; in essence, this is a carbon atom counter. The sensitivity here may even exceed that of the Beta ionization detector. Again, not all materials will be detected; for a given analysis this may or may not be advantageous.

A few words about the columns - Packed columns may vary in length from, say, 3 to 60 feet; usually a 6 to 8 foot column will be satisfactory. Increasing the length of the column improves the separatory power, but not proportionately. The column width is usually about 1/4 inch. The inert support may be diatomaceous earth or ground fire-brick frequently, 30 - 60 mesh size. A practical rule for the choice of stationary liquid phase is that a similarity in the chemical nature of the liquid phase and the material to be separated should exist. Capillary columns were introduced rather recently. Here the column length may run to 100 feet. The I. D. is about .01 inch and the stationary liquid phase is coated directly on the inner wall of the capillary. The resolution on these columns is higher than that of packed columns. Their capacity is however low and they must be used in combination with the more sensitive ionization type detectors. Probably the most efficient and sensitive separation technique currently available is this combination of capillary column and ionization detector.

I wish to emphasize at this point that gas chromatography is essentially a separation technique. A completely unknown mixture placed on a GLPC column may be separated but no information concerning the chemical nature of the compounds will be obtained. It is necessary to trap out the eluted components and to identify them either by classical chemical methods or by physical measurements such as infra-red and ultra-violet spectroscopy or mass spectrometry or by a combination of methods depending on the ingenuity of the chemist. However, should one have a mixture of homologous materials, then gas chromatography can be used both to separate and identify.

I would now like to describe just such an application of gas chromatography in which this technique was used to separate, identify, and quantitatively determine the free fatty acids present in pork and beef fat. Our interest in this problem arose from the observation that the differences

in the flavor of these meats apparently resided in the fat, the lean of these meats contributing an identical basic "meaty" flavor and the fat the distinguishing flavor overtones. It was our thought that the free fatty acids might in part contribute to flavor directly or act as precursors for the formation of flavor compounds such as carbonyls.

The problem resolved itself into finding a satisfactory method of quantitatively analyzing a mixture of the pure free fatty acids and in developing an adequate cleanup procedure for the separation of small amounts of the fatty acids from large amounts of fat and subsequently converting the acids to their methyl esters. The methyl esters, because of their lower boiling points, can be separated at lower column temperatures than the corresponding acids. Even so, column temperatures in the neighborhood of 200°C. are required to effect separation, thus limiting the materials that can be successfully used as the "liquid" phase. Our choice of liquid substrate was a commercially available polyvinyl acetate of 1500 molecular weight. The base line drift in all experiments was negligible. The Gas chromatograph used was a Beckman GC-2. The detector is of the thermal conductivity type connected to a 1-mv. recorder. In the next slide the retention times and separation factors are reported for commonly found C_{12} through C_{18} fatty acid methyl esters. The operating conditions were helium flow rate at exit 83 ml./min., temperature 205°C., column packing 15% polyvinylacetate on Chromosorb "R", column 1/4" O. D. and 8' long. The retention time represents the time required for a given component to be eluted from the column at the operating conditions described. Retention times are determined by the use of authentic samples. The separation factors are the ratios of the retention times for two components and measure the resolving power of the column. The farther apart the peaks, the greater the deviation of this value from unity. In this instance, the separation factors are measured to a C_{17} reference acid.

The column support is theoretically inert, but in our studies this hasn't been so. The results just shown were obtained with one batch of Chromosorb "R". The next several batches gave poorer results. Eventually, we used Chromosorb W. More recently we have used the 30-60 mesh fraction obtained by sieving Celite 545. This fraction was acid washed, dried at 120°C., and then exposed to vapors of dichlorodimethylsilane. The polarity of the Celite surface is markedly decreased by this treatment and columns prepared with the support have shown improved separation factors, sharper peaks, and even lower retention times.

The analytical procedure developed involves the adsorption on a strong anion exchange resin of the free fatty acids from a solution of fat in petroleum ether to which a known amount of the C_{17} straight chain saturated acid has been added, then, decanting the fat plus petroleum ether and preparing the methyl esters directly on the resin by treatment with anhydrous methanol - HCl. The esters are, after appropriate cleanup, obtained in a petroleum ether solution and chromatographed. The areas under the peaks are directly related to the quantity of ester present. Peak areas are taken as one-half peak width times peak height. Calibration factors are obtained by initially chromatographing standard solutions of authentic esters. The C_{17} acid added to an unknown is used as an internal standard. The per cent recovery of the C_{17} acid carried through the procedure gives a correction factor for all acids found. Absolute recoveries range from 60-95%. In the

next slide, recovery data for a known mixture of fatty acids covering a concentration range similar in ratio to that found in lard is shown. Quantitative results without the internal standard would be unobtainable. It is interesting that with the thermal conductivity detector 2 γ can be readily detected and 25 γ determined quantitatively. In general, 1/4 to 1/2 milligram is an adequate sample size.

Free fatty acids in beef and pork fat were determined prior to and after heating in air at 100°C. After heating for 4 hours at 100°C. in the presence of air, the amounts were roughly doubled but no new acids were found. The quantitative results are shown in the next slide. Of interest is the greater amount of free unsaturated acid in pork.

Beef free fatty acids are shown in the next slide, the peaks labeled tetradecenoic, pentadecenoic, hexadecenoic, and heptadecenoic are assigned on the basis of their retention volume rather than by comparison with authentic samples. The retention volume is the retention time multiplied by the flow rate.

It has been shown that by plotting the log of retention volume against the number of C atoms for a homologous series, straight lines are obtained. Thus, fatty acid esters with no double bond fall on one line, those with one double bond on another, and so on. In the next slide, this plot is shown for the peaks in the preceding slide. Assigned structures are black dots, circles are for known compounds. The good agreement with prediction renders probable the assignments made. The chromatogram for pork free fatty acids is shown in the next slide. The major qualitative difference is the absence of the C_{14} unsaturated acid and the presence of linolenic acid.

Gas chromatography can also be useful for quality control. The extreme sensitivity of the capillary column and ionization detector could be used to sample the air space above the contents of a canned meat product. One characteristic chromatogram for the trace amounts of volatiles might mean a satisfactory product, another type a defective product. In this instance, neither qualitative or quantitative knowledge of the separated compounds is important, but the information gained is valuable.

Gas chromatography may ultimately unravel the tangle of odor compounds that contribute to meat flavor, and an artist-chemist (he's a better chemist than artist, I hope) at our Laboratory has suggested that the eventual chromatogram of beef flavor may resemble that shown in the next and final slide.

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CHAIRMAN PEARSON: Thank you, Dr. Hornstein, particularly for the enlightening material on beef flavor.

Our next speaker this afternoon is from the Pioneering Research Division, Quartermaster Research and Engineering Center in Massachusetts.

He has long been doing work on meat flavor and is well known, particularly for his work on irradiated meat. At this time we are going to hear from Dr. Charles Merritt, Jr., who will speak to us on the use of Mass Spectroscopy as a tool for separating and identifying meat flavor components. Dr. Merritt.

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