GAS CHROMATOGRAPHIC STUDIES:
THE APPLICATION OF GAS CHROMATOGRAPHY
TO FLAVOR ANALYSIS

by

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A B S T R A C T

The application of gas chromatography to flavor research is reviewed. New methods for the preparation of capillary columns and long narrow-bore packed columns are described. Long narrow-bore columns having 500-700 plates per foot were found to be a suitable substitute for capillary columns in high resolution gas chromatography. The achievement of high sensitivities with flame ionization detection (FID) systems is evaluated. The modifications and the methods used to achieve sensitivities of the order of 10^{-12} gram/sec of ethyl acetate are described. Eight different FID systems were tested and compared. A new method for single column temperature programming at relatively high sensitivities and for elimination of background interference in a gas chromatograph-mass spectrometer system is described. Methods for decontamination of 1-μl syringes, fraction collection and detection of trace peaks appearing on the slope of large ones were developed. A loss of chromatographic resolution of up to 50% due to "unswept pockets" was found to occur in the vacuum system of a gas chromatograph-mass spectrometer system. Retention data of homologous series of alcohols, esters, aldehydes and ketones on seven liquid phases are presented. More than 150 constituents were detected in a grape fusel oil distillation residue using temperature programmed capillary columns. Twenty-eight of these were tentatively identified using the standard addition method. Selected examples of the application of the systems and the methods developed to the direct analysis of the flavor constituents of alcoholic beverages and fermentation by-products are given. Preliminary results of analysis of grape fusel oil distillation residue by dual channel gas chromatography are shown.

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PART I

GENERAL INTRODUCTION AND SCOPE

The fundamental aspects and the understanding of the sensations of smell and taste by human beings and animals is considered to be one of the most neglected fields of scientific enquiry (1). An explanation and perhaps support of this observation can be given. Man's interest in the flavor and aroma of the world surrounding him is probably very old. It has only been within the past decade, however, that he has developed sufficiently sensitive techniques to enable him to determine the chemical composition of flavors and odors (2). Revealing the chemical composition of flavors is an essential step for understanding the underlying processes in the sensations of smell and taste. Apart from its importance in the basic research of odor, revealing the composition of specific flavors has gained new dimension as an indispensible part of applied research in the widely diversified areas of the advanced technology of today. In many branches of modern life and particularly in the world's huge food and beverage industries, understanding the mysteries of flavors, their objective evaluation and control are of utmost importance. The objective characterization of flavor and aroma of a product in the food industry must be logically preceded by a comprehensive analysis of its chemical composition.

By their nature, food flavors offer formidable difficulties to the invesigator of their chemical composition. Although it is difficult

to generalize, the majority of food flavors appear to share certain characteristics:

1. They usually consist of complex mixtures of many components. For instance, Teranishi et al (3) detected more than 150 constituents in a strawberry flavor oil and Keulemans (4) reported the presence of over 200 constituents in a coffee flavor concentrate.

2. Flavors are the minor constituents of the foodstuffs and beverages in which they are present and exert their strong influence at extremely low concentration levels (2,5). The concentration of flavor constituents is frequently well below the limits of detectability of ordinary chemical methods of analysis (2) and their presence can be detected only by applying costly and tedious concentration procedures (6,7). For instance, the human nose can detect a concentration of 10^{-16} gram/ml of ionone in water (5). Presently the most sensitive detection devices existing can detect 10^{-12} gram of substance and the most sensitive wet chemical methods rarely approach the 10^{-9} gram level. In order to obtain sufficient material for a study of the composition of orange juice flavor Hall and Wilson (6) started with 39,000 liters of orange juice. Haagen-Smith (7) investigated volatile flavor constituents of pineapple starting with 4,000 kg of fruit.

3. The exact molecular configuration of the constituents composing

> flavors is highly specific, i.e., slight changes of molecular configuration of the compound in question such as isomerisation or rearrangement produce large changes in odor (2,8). For example, the molecular structures of menthol and isomenthol differ only in conformation but this difference is sufficient to produce a pleasant taste in the case of menthol and an unpleasant taste in the case of isomenthol (8).

In view of these examples it is evident that only the extremely sensitive chemical techniques can offer any possibility of success in divulging the constitution of food flavors (2).

Of the vast array of tools and methods that the chemist can use as aids for flavor analysis, gas chromatography plays a central role (5,9-11). It has rapidly become the primary means in flavor analysis and has greatly enhanced our capabilities and potentialities in this field. The impact of gas chromatography on flavor research has been clearly expressed in the scientific literature. Weurman (9) in his article on recent development in food odor research methods,writes: "Odor research got its second start with the arrival of gas chromatography...." Bavisotto et al (10) write: "The common denominator behind the impetus in flavor research has been the progress made in the refinement of instrumental methods, with particular emphasis on gas chromatography." Bidmean (11) writes: "The technique (gas chromatography) is admirably suited to the separation of small amounts of volatile materials in a complex flavor mixture."

The factor which has the greatest influence on flavor is odor (12). Odor is a sensation produced upon contact of vapors of volatile compounds with the olfactory receptors. It is only natural, therefore, that gas chromatography, which is now the most powerful technique for analysis of volatile compounds, would become the major tool in flavor research.

Gas chromatography was introduced by James and Martin in 1952 (13), and has since grown to become one of the most versatile and widely used research tools ever invented. Voluminous literature on the subject in the form of books, monographs and symposia proceedings has become available and over 10,000 scientific articles dealing with the development and applications of gas chromatography have appeared. Abstracting services are also being conducted. Widely differing gas chromatographs, ranging from large preparative units which use columns 25 cm. in diameter (14) to miniature models for space exploration (15), have been developed to suit the requirements of specific applications. The range of applications of gas chromatography and its potentialities are rapidly expanding through the development of advanced instrumentation and techniques. The application of gas chromatography in flavor analysis exemplifies this statement. Before the development of the sensitive detectors, i.e., during the 1953-1959 period, gas chromatography became widely accepted in flavor research but it did not bring basic changes in the approaches to the problem. Only recently, after the development of the highly sensitive ionization detectors the situation became different and the introduction of these detectors in odor research is now fundamentally changing the approaches by enabling direct and head space analysis (3,9). The direct, or headspace methods of analysis of flavors, are important not only in eliminating the need for tedious and costly concentrations, but mainly in making it possible to determine directly the true composition of the natural flavor (9). Furthermore, this approach minimizes the possibility of creating artifacts which has always been a problem in flavor analysis (2).

Several schemes for detailed analysis of flavors using gas chromatography as the major tool have been proposed (5,9). In general, however, the

chemical analysis of flavors almost always involves three principal stages (2):

1. A concentration stage, in which enrichment of the concentration of flavor ingredients in the sample is achieved.

2. A fractionation or a separation stage in which the individual flavor components of the original mixture are isolated (not necessarily collected) in a pure form.

3. The identification stage, in which the molecular structure of the individual compounds is determined.

Gas chromatography offers a possibility to carry out these three stages in a single step; however, it is not equally successful for all three of them. The concentration stage can be either bypassed with considerable success by using high sensitivity detection devices (9) or performed by preparative gas chromatography (5). Gas chromatography is ideally suited for the fractionation stage (3,5,9-11). The identification stage can be performed with gas chromatography only to a limited extent. It should be remembered, however, that when very small quantities of a flavor constituent are present in the original mixture (10^{-10} to 10^{-12} gram) gas chromatography becomes the only tool that can provide information on the identity of this constituent through its retention behavior. Weurman (9) advocates the use of pyrolysis as an addtional aid for the identification of flavor constituents by gas chromatography. Bayer (5), on the other hand, recommends microscale reactions combined with gas chromatography as a means of identification . Mass spectrometry is the most sensitive and the most powerful tool for identification of the pure flavor constituents separated by gas chromatography (2,3,5,16). The high cost of mass spectrometers, however, prevents their widespread use in flavor research.

Until the last two or three decades the production of alcoholic beverages throughout the world was carried out on a traditional basis. A rather amusing expression of the traditional approach to production of alcoholic beverages (Chem. and Eng. News, March 15, 1965) is given in a sign which hangs outside the Stitzel-Weller distillery in Louisville, Kentucky, which reads: "No Chemist Allowed! Nature and the old time "know how" of a Master Distiller gets the job done here. Because traditional Kentucky Whiskey is a natural product, we disdain synthetics, scientists and their accompanying apparatus. This is a Distillery - not a whiskey factory".

The consumption of alcoholic beverages (beer, wine and distilled beverages) in the world is enormous. To supply the huge demand for alcoholic beverages, the industry has had to scale up production. In a large scale production, relying on the traditional rules only can lead to great uncertainty and frequent pitfalls. Adopting a scientific approach to the problems of alcoholic beverage production becomes inevitable. Of the many aspects of alcoholic beverage production, the control of the flavor of the final products is perhaps the most difficult and naturally the most important one. Strict control of the production variables does not necessarily secure a uniform high quality product. Routine organoleptic evaluation of the product throughout the production cycle is not highly reliable (17) and does not give clues as to the cause or source of trouble, if any. A comprehensive chemical analysis of the flavors involved combined with their organoleptic appraisal is considered as a most desirable approach (18-20) to the control of flavor in production. Due to the difficulties in chemical analysis of flavors mentioned earlier such an approach could not be easily performed in the past. With a development effort, gas chromatography combined with organoleptic testing can offer the possibility of routine

control and diagnosis of flavor formation throughout the production cycle of alcoholic beverages.

Development of sensitive methods for analysis of the flavor congeners in alcoholic beverages would assist in preventing the formation of undesirable flavors due to incorrectly operated stages in the production cycle but it cannot prevent the occurrance of such flavors if their precursors are present in the raw material, i.e.,grain. Securing high quality starting material is therefore essential for production of high quality beverage. Unfortunately, no rigid formula of what is high quality grain for production of alcoholic beverages exists. It would be correct to state that high quality grain for alcoholic beverage production would be grain that contains a high percentage of the precursors of desirable flavors constituents and a low percentage of the precursors of undesirable flavor constituents. To determine which constituents have desirable and which undesirable effects on the flavor, the alcoholic beverages must first be analysed in the greatest possible detail. By correlating the composition of a series of samples with their corresponding organoleptic definition, one can presumably conclude which constituents are desirable and which undesirable for the flavor of the final product. By carrying out research to determine the precursors of the desirable and undesirable consitituents, one would acquire the information required to conduct plant breeding aimed to produce varieties of grain which are enriched in precursors of desirable constituents and contain a minimum of precursors of undesirable ones (20). The levels of the various precursors in the different varieties of grain would be monitored by suitable analytical methods and used to guide the plant breeder in his program. Availability of suitable methods for analysis of the trace flavor constituents in alcoholic beverages, however, is the first prerequisite of the above mentioned scheme for development of high quality grain.

The work described in this thesis was initiated with the purpose of developing sensitive gas chromatographic techniques for analysis of alcoholic beverages and fermentation by-products. While pursuing this aim, contributions to the special aspects of flavor analysis by gas chromatography were made by introducing improvements in the existing techniques and apparatus, collecting data and studying the problems involved in the achievement of high sensitivity and high resolution. One of the most powerful methods for identification of the constituents occurring in complex mixtures is the one stage combination of gas chromatography and mass spectrometry. It was therefore our aim to assemble such a system and investigate its limits of applicability in the identification of constituents emerging from a gas chromatographic column. The compilation of retention data of the compounds of interest and investigation of the selectivity of various liquid phases towards such compounds were considered to be essential for the project and has been given considerable attention.

PART II

SPECIAL PROBLEMS IN THE APPLICATION OF GAS CHROMATOGRAPHY TO FLAVOR RESEARCH

Chapter 1

Defining the Special Performance Requirements of the Gas Chromatography System

The applications of gas chromatography in food and beverage flavor research and the specific problems encountered in each are too numerous to be reviewed here with any detail. They range from analysis of the flavor constituents of coffee (21), honey (22), cheese (23), coconut oil (24), peaches (25) and grapes (26) to the study of the effect of cooking on flavor components of beef (27), investigation of volatile autoxidation products of sunflower oil (28) and flavor deterioration in fried chicken (29), covering practically the entire range of industries and products. Hundreds of references pertaining to the direct or indirect application of gas chromatography in the food and beverage flavor research can be found in bibliographies on gas chromatography (30, 31).

Different performance features of the gas chromatograph are essential for different applications. For instance, high sample capacity of the column and efficiency of the fraction collection are the features required in preparative gas chromatography (14); compactness, low weight and special sampling equipment in space applications (15), and automatic sampling combined with frequent repetition of the analysis cycle in process analysis (32). Similarly the special problems encountered in flavor

analysis (Part I) impose certain performance requirements on the gas chromatograph. In order to produce superior results in flavor research in general, or in some particular aspect of flavor analysis (detection, resolution or identification) it becomes necessary to improve the performance of the gas chromatographic system and develop methods which are especially suited to the specific needs of the problem.

Resolution and sensitivity. Resolution which depends on the column and sensitivity which depends on the detector are important in optimizing any application of gas chromatography (33). In trace analysis, however, these factors become by far more critical and must be given thorough consideration (33).

The basic function of gas chromatography in food and beverage flavor research is in revealing the composition of the mixtures of volatile constituents with special emphasis on trace constituents. This involves three stages ;

a. resolving the individual constituents of the mixture into well defined zones which are eluted out of the chromatographic column in succession,

b. detecting each of the individual constituents resolved even when present in very small quantities,

c. determining the identity of the constituents.

Obviously, high resolution, high sensitivity and capability to provide evidence for identification are the major performance requirements of the application of gas chromatography to flavor analysis. Resolving the components of flavor mixtures is a prerequisite for revealing their composition. Securing high resolution is, therefore, essential.

Detecting the presence of trace flavor constituents emerging in a pure form from a high resolution column is entirely dependent upon the sensitivity of the detection system used. It would therefore be correct to state that in flavor research the achievement of high sensitivities is a fundamental aspect (9). A careful analysis of the above mentioned basic requirements shows that they are of opposing character and that achieving very high values for one must be done by sacrificing another.

Sample capacity. High resolution can, for instance, be achieved with columns of low sample capacity such as capillary columns (34, 35). However, even when a high sensitivity detection system is used, trace constituents may remain unrevealed due to the small amount of sample permitted. Developing columns that offer sufficiently high sample capacity and high efficiency is therefore of special interest in flavor analysis. Certain compromise between capacity and efficiency must be found and columns that provide increased capacity as compared to the "classic" high resolution capillary columns while still offering a sufficiently high number of plates and acceptance of a wide variety of liquid phases should be preferred. High sample capacity is essential when the range of concentrations of the constituents of the mixture varies over more than three orders of magnitude. In flavor research one frequently deals with ranges of concentrations extending over five to nine orders of magnitude (5, 9). As the range of concentrations increases, more and more resolution and sample capacity are needed to facilitate high detectability.

Range of molecular structures. As stated earlier, a common characteristic of food and beverage flavors is the fact that they are present as complex mixtures (3, 4). To reveal the composition of a complex mixture of unknown constituents is a formidable task (2, 5, 9). The selec-

-tivity of the liquid phase or solvent efficiency plays a major role in achievement of high resolution (36-38). When the nature of the substances present in the mixture is known, high resolution can be provided by using high selectivity liquid phases (36). However, since in most of the cases the mixtures consist of several groups of compounds (alcohols, esters, aldehydes, ketones, etc.) one cannot usually provide equally high resolution for all the members of all the groups simultaneously. One is therefore confronted with a problem the solution of which requires a choice between four different approaches;

a. preliminary fractionation into separate groups (alcohols, esters, ketones,etc.),

b. repeated analysis with different liquid phases,

c. searching for a suitable general purpose liquid phase,

d. development of special multi-column gas chromatographic system.

The disadvantages of approaches a and b are that the procedures are more complicated and more sources of errors and artifacts exist. A major design effort is required for approach d which would require the development of new concepts and new components. Approach c is most suitable for rapid routine monitoring of the levels of the trace flavor constituents in a given product; however, it requires a somewhat tedious empirical search for suitable liquid phases and relies more on column efficiency.

Range of concentrations. The quantitative composition of the sample is directly related to the problem of achieving resolution and associated with sample capacity of the column (33). When the sample consists of a mixture in which the range of concentrations of flavor

constituents extends over two orders of magnitude only, high resolution combined with sufficiently high sensitivity may secure high detectability without the need for high sample capacity. When the composition of the mixture extends over a wide range of concentrations, i.e., five to nine orders of magnitude (5, 9), revealing the presence of the trace constituents becomes extremely difficult due to severe overlapping, and due to lack of clues as to their presence. If, for example, under most of the major peaks appearing in a typical 100-peak flavor chromatogram a trace constituent of 1 ppm of a compound is hidden, exposing the mixture through different liquid phases may only hide these traces in another place under another compound (5). It can, therefore, be concluded that the narrower the range of concentrations of the sample the easier it becomes to reveal its composition by gas chromatography. A glance at the "Glueckauf plot"(33) supports this statement directly. Unfortunately, the composition of mixtures encountered in food and beverage flavor research usually extends over a wide range of concentrations (5, 9,). In direct analysis of alcoholic beverages, for instance, one is confronted with mixtures which extend over 9 - 10 orders of magnitude.

Range of boiling points. Additional difficulties in revealing the composition of flavor mixtures are encountered when constituents of widely differing boiling points are present. Usually, as a solution to this problem, temperature programming is performed (3, 39). However, liquid phases that exhibit selectivity towards low boiling constituents are usually not stable at higher temperatures and cannot be used for revealing the composition of the high boiling point constituents. On the other hand, liquid phases that are stable at high temperatures are usually solids at low temperatures and cannot be used for the separation of the

low boiling point constituents. In addition to the question of stability of the stationary phase at high temperatures or its viscosity at low temperatures (39), the continuously increasing vapor pressure of the liquid phase occurring during temperature programming causes severe problems when working at high sensitivity. The widely adopted dual column - dual detector approach to the problem (39) is applicable only for low or moderate sensitivities or only when programming through ranges of temperatures at which the vapor pressure of the liquid phase is negligible. A new,simple method for temperature programming at high sensitivities will be described in this thesis. It therefore can be concluded that the narrower the range of boiling points of the constituents composing the mixture, the easier it becomes to find a suitable liquid phase.

Chapter 2

Chromatographic Resolution and Methods for Achievement of High Resolution

The principles and theory of the process of chromatographic separation have recently been presented in a detailed and an exceptionally clear way by Giddings (37, 38). Earlier, the theories of gas chromatography have been presented in all the textbooks on gas chromatography (33, 40 - 44) and its special techniques (34, 35, 39). The presentation of formulae here is therefore restricted to a minimum and introduced only to clarify the text.

Guide rules for selection of optimum parameters for achievement of a gas chromatographic separation have been described in the literature (33, 35 - 37, 40, 44). These rules apply to flavor analysis in a general way. They usually deal with optimizing the operating parameters and deriving the minimum number of theoretical plates required to achieve the separation of an isolated pair of substances. However, in the application of gas chromatography to flavor analysis we deal with detection of trace constituents in complex mixtures under complicated sets of circumstances. The general rules for the achievement of high resolution or high column efficiency cannot therefore be blindly followed.

To succeed in performing the complex gas chromatographic function of resolving multicomponent mixtures, one must optimize the use of theory, experience and intuition, according to the character of the problem on hand. It is the purpose of this chapter to mention briefly the general rules on increasing column efficiency and resolution in view of the special problems involved in determining the composition of flavor mixtures.

2.1. Principles and Guide Rules for Achievement of High Resolution

2.1.1. Column efficiency

Chromatographic separation is achieved in principle due to the differences in the velocities of component zone migration and limited zone spreading. No separation is achieved when zone spreading overpowers the difference between the component velocity of zone migration. Resolution is a measure of the degree of separation of zones (38). The degree of zone spreading is a measure of column quality or column efficiency. It is expressed in terms of the "Height Equivalent to a Theoretical Plate", H.E.T.P. (33 - 34). Column efficiency is therefore one of the two major factors involved in the achievement of high resolution. It is therefore of interest to review here briefly the theoretical guide rules for the achievement of high column efficiencies.

By assuming additive but independent contributions of the underlying processes responsible for zone spreading, van Deemter, Zuiderweig and Klinkenberg (45) developed the rate theory of gas chromatography. The principle outcome of their theory is the well known van Deemter equation:

$$H = 2\lambda d_p + 2\frac{\gamma' D_{gas}}{u} + \frac{8}{\pi^2} \cdot \frac{k'}{(1+k')^2} \cdot \frac{d_f^2}{D_{liq}} u. \qquad [1]$$

where :

d_p = solid support particle diameter

$k' = K \frac{F_{liq}}{F_{gas}}$

K = distribution (partition) coefficient

λ = packing constant

γ' = tortuosity constant

D_g = diffusion coefficient of the component in the gas phase

D_1 = diffusion coefficient of the component in the liquid phase

d_f = liquid phase film thickness

u = linear velocity of the carrier gas

F_{gas}; F_{liq} = volume fraction of gas and liquid in the column.

It immediately becomes apparent from equation 1 that by decreasing the particle size, decreasing the liquid phase film thickness, using uniform packing, employing a heavier gas (lower gas diffusion coefficient) and optimizing the flow rate, a decrease of H, i.e., increase of column efficiency, will be achieved.

In a simpler form, the van Deempter equation can be rewritten as follows :

$$\bar{H} = A + B/\bar{u} + C_1\bar{u} \qquad [2]$$

where : $\bar{H}$ = the average plate height

$\bar{u}$ = the average linear velocity of the carrier gas

A = eddy current or multiple path term

B = molecular diffusion term

C = resistance to mass transfer term

Jones (46) showed that additional terms are required in the van Deempter equation to account more accurately for the processes taking place in a gas chromatographic column and derived a six term equation which can be written in the simplified form as follows :

$$\bar{H} = A + B/\bar{u} + C_g\bar{u} + C_1\bar{u} + C_2\bar{u} + C_3\bar{u} \qquad [3]$$

The terms A and B are identical to those of equation 1. The liquid mass transfer term C_1 differs only slightly in the numerical coefficient 2/3 instead of $8/\pi^2$. The new terms in Jones' equation account for resistance to mass transfer in the gas phase ($C_1\bar{u}$), velocity distribution ($C_2\bar{u}$) and

"correlation" ($C_3\bar{u}$). Giddings (37) derived a different modification of the classic van Deempter equation :

$$H = \frac{2\ D_g}{u} + gR(1 - R)\frac{d^2u}{D_1} + \omega\frac{d_p^2u}{D_g} \qquad [4]$$

where : g = configuration factor, a constant depending on the shape of the units of liquid phase (generally equal 1/4)

R = retention ratio (peak velocity to gas velocity)

ω = proportionally constant

In the simplified form this equation can be written as follows :

$$H = B/u + C_l u + C_g u \qquad [5]$$

By using the modified version of the van Deempter equation a somewhat more refined prediction of guide rules for optimizing column parameter can be made as shown by Giddings (37). Equations 1, 2, and 3 provide a considerable amount of information regarding the possible ways for achieving high column efficiencies. Keulemans (40), Purnell (42), Dal Nogare (33) and Giddings (37) presented an excellent analysis of the practical approaches for minimizing H. Giddings' approach (37) will be followed here in a condensed way with added comments of interest in flavor analysis.

Column length, L. From equations 1, 2, and 3, one gathers that H is independent of column length and that the total number of theoretical plates should be directly proportional to the length of the column. This seems to be true only when the pressure drop across the column is small (44). With longer column elution times increase. Since analysis time is not of great importance in research, using long columns is one of the easiest ways to improve resolution. However, because both peak spreading and differential migration take place, the overall resolution

increases only with $\sqrt{L}$ rather than with L. The maximum practical column length is limited by difficulties created in preparing such columns, by difficulties in operating at high inlet pressures and by the decrease of sensitivity occuring due to the broadening of the peaks. Before these limits are reached, however, there is a wide range of column length that can be used without serious difficulties. The practical limits of maximum column length can be extended provided special methods for preparation of such columns and operation at high pressures are developed. Long packed columns are of special interest in flavor analysis because they combine higher sample capacity and high efficiency. A method for preparation of long columns in single lengths was developed and described here and the applicability of such columns as substitutes to capillary columns was investigated (section 2.3).

Carrier gas flow velocity. For maximum resolution the flow velocity of the carrier gas has to be adjusted to make H minimal. Mathematically the optimum flow velocity, u_{opt}, is found by setting the derivative dH/du equal to zero (33, 36, 37, 40, 42, 44) and solving for u. In practice, however, it is easier to find u_{opt} by plotting H versus u and noting the location of the minimum. The u_{opt} can be also found by injecting a pair of closely related substances under different flows and measuring the resolution obtained in each case. Chromatographing isothermally at u_{opt} flow rates for the resolution of complex mixtures with a wide range of boiling points may give extremely long elution times for the heavy constituents and cause lowering of peak heights to the levels of baseline fluctuations. As a rough general rule u should, therefore, be kept between 1 to 2 times u_{opt}. The recently developed technique of flow programming (47) seems to be particularly suitable for work with complex mixtures.

Pressure. Flow velocity in any point of the column is dependent

on the inlet and outlet pressures set by the operator. The optimum flow velocity can be achieved for various inlet and outlet pressures. To optimize the absolute values of the inlet and outlet pressures in addition to the flow velocity it is necessary to consider how the various terms appearing in the complete equations depend on the pressure. The term B in equations 2 and 3 is proportional to the gaseous diffusion coefficient D_g which is inversely proportional to p. Working at higher pressures therefore improves the efficiency. On the other hand the term C_g appearing in equation 5 is inversely proportional to D_g and thus proportional to p. By combining the appropriate expressions into the equation that gives H_{min} one observes that the smallest value of plate height occurs as $p \longrightarrow \infty$. However, the actual gain of resolution obtained by increasing the average pressure $\bar{p}$ depends on the relative magnitude of C_g and C_l. Further increases of pressure are of no value when $C_g \gg C_l/\bar{p}$. As far as the pressure is concerned there are no special rules to be followed in flavor analysis.

Solid support. Difficulties in the application of the guide rules for high column efficiency are caused by solid support effects. The theory of gas chromatography assumes that the solid support is an inert material which acts only as a scaffold to hold the liquid phase in a highly dispersed configuration. The commonly used diatomaceous earth is far from being inert and causes considerable loss of column efficiency due to adsorption on the liquid-solid interface. Various treatments for reducing the surface activity of solid supports tend to decrease its surface area thus causing an undesirable decrease of the dispersion of the liquid phase. The structure of solid support is of the utmost importance for the achievement of high efficiencies and directly influences the term d_f^2 in equation 4. Smaller pores are more desirable for smaller H.

The pink ground firebrick with numerous pores in the 1 μ range is superior to the white form of diatomaceous earth. Unfortunately the pink firebrick exhibits stronger adsorption effects and its use is almost prohibited for analyses of substances which contain free active groups such as -OH,-COOH and $-NH_2$.

Particle diameter. Equation 1 shows that H is proportional to d_p. In equation 4, H is proportional to d_p^2. Using smaller particle diameter is therefore beneficial for obtaining smaller H. As particle diameter is decreased, the pressure drop across the column increases and so do experimental inconveniences. With particular interest to flavor analysis one must optimize column length, particle diameter and inlet pressure. The gain of column efficiency by extra column length with somewhat bigger particles seems to be more promising than shorter fine mesh columns for the same inlet pressure. Long narrow bore packed columns (30 to 100 feet 1/8" O.D.)which are very promising for high resolution applications cannot be easily used with the 100 - 120 mesh packing.

Column internal diameter. The plate height equations do not indicate any relationship between H and the diameter of the column. It is well known, however, that large diameter preparative columns are much inferior to analytical columns. The reason for extra zone spreading in large diameter columns originates in non-uniform velocity profiles across the column cross section. It is therefore desirable to work with columns of small internal diameter. There is a recent trend towards the use of small diameter columns (1/8" or 3/16" O.D. instead of the standard 1/4" O.D.) although the overall gain of efficiency when detectable is not high.

Carrier gas. The gas phase diffusion coefficient, D_g, appears in two terms of the plate height equation 4. The molecular diffusion term B is proportional to D_g while the term C_g (gas phase resistance to mass transfer) is inversely proportional to D_g. The term B becomes smaller when a carrier gas with a small D_g is used but then the term C_g becomes larger. At low flow velocities the term B is dominant and D_g should be small while at high carrier gas velocities D_g should be large. The application of this guide rule to flavor analysis must be evaluated in terms of the use of long packed columns. With such columns the use of carrier gases of small D_g and relatively low carrier gas velocities is desirable but may lead to long elution times and necessitates the use of high inlet pressures because the gases that have larger D_g usually have higher viscosities. Helium is most unsuitable for this purpose because it combines large D_g and relatively high viscosity (higher than nitrogen). Nitrogen is, of course, suitable for medium length columns (30 - 50 feet) but not for extra long columns (50 - 150 feet) because it will require excessive inlet pressures. Hydrogen appears to be most suitable for this range. The relative merits of carrier gases in conjunction with long narrow bore packed columns are further discussed in section 2.3 of this chapter.

2.1.2. Resolution

The factors that influence the resolution of any given pair of substances are best analysed from the separation function (37).

$$S.F. = \frac{(\Delta K)^2 L}{16H(\beta + K)^2} \qquad [6]$$

where : ΔK = the difference in the partition coefficients

K = the mean partition coefficient

H = plate height

L = column length

β = ratio of free gas volume to liquid volume in the column.

The factors which affect column efficiency, H, were discussed in the previous section of this chapter. Obviously, smaller H results in better resolution. The effect of column length has also been discussed earlier. In the following the effect of various parameters on K and β will be discussed.

Solvent efficiency or selectivity. In most of the cases the term selectivity refers directly to ΔK and seems more correct than the term polarity for describing the properties of liquid phases (48). Giddings (37, 38) defined the quantity $\Delta K/K$ as "relative selectivity". Equation 6 shows that resolution can be achieved only when $\Delta K \rightarrow 0$, and that liquid phases that exhibit larger ΔK for a given pair of substances achieve the separation more easily. The bulk of published data on properties of liquid phases and their selectivity is the only source of information for a scientist who is attempting to achieve a particular separation. The difference in the partition coefficients of two compounds expresses differences in interaction forces between the molecules of the solvent (liquid phase) and the molecules of the solutes. Unfortunately, the theory of these interactions is extremely complex and selectivity cannot be predicted (37, 40). The search for high selectivity liquid phases is therefore entirely empirical and guided only by experience and chemical intuition.

Selectivity is not the only property of the liquid phase that influences the resolution. The liquid phase diffusion coefficient D_1 (equations 1 and 4) is one of the most important single terms influencing

column efficiency. Unfortunately, very little data on D_1 values are available. This is due to experimental difficulties in measuring this quantity. Pioneering work on the measurement of diffusion coefficients was carried out by Evanoff and Harris (49) using polarographic techniques. The use of some high selectivity liquid phases may in cases become prohibitive due to extremely small values of D_1 of the compounds of interest in these phases or due to adsorbtion of these compounds on the gas-liquid interface.

Temperature. The effect of temperature on K is very strong (exponential). In most of the cases an increase of 25-35°C will reduce K to half of its original value and double the rate of component migration. The relative selectivity $\Delta K/K$ decreases as the temperature is increased. At sufficiently high temperatures ΔK becomes so small that the values of H and L are no more sufficient to provide resolution. Lowering column temperatures obviously enhances the resolution as far as $\Delta K/K$ is concerned. It also decreases H which brings further enhancement of the resolution. However, there is a limit to how much the operating temperature can be lowered while still gaining in resolution because D_1 becomes too small. At lower operating temperatures elution times increase thus decreasing the detectability.

Liquid phase loading. The effect of the liquid phase loading percentage on the resolution is expressed in equation 6 through β. The quantity β appears both directly in this equation and indirectly through H (R in equation 4 equals $\beta/(\beta+K)$). The resolution appears to increase with β becoming smaller, i.e., increasing the percentage of liquid phase. However, H suffers a prominent increase for liquid

loading exceeding 30% which limits the maximum permissable liquid loadings to about 30%. Below 30% loading H does not vary greatly. The elution time is strongly influenced by changes of the liquid phase percentage and although it does not appear in the equation, high liquid loadings automatically limit the column length L which in turn limits the resolution. In optimizing L and β the elution time and loss of detectability must be taken as limiting factors. In flavor analysis by gas chromatography detectability cannot be sacrificed. High resolution should, therefore, be achieved by using longer columns with lower percentages of liquid phase provided there is a net gain of resolution.

2.1.3. Capillary columns

Capillary columns, which were introduced by Golay in 1957 (50), have repeatedly proven indispensible in high resolution gas chromatoraphy (3,4,51-53). They are, therefore, of special interest in flavor research. Because of their low sample capacity (34,35), however, capillary columns are in principle not suitable for trace analysis (34). They are usually used in flavor research for analysis of preconcentrated samples (3,4). Due to the large β values of capillary columns they need more theoretical plates to perform, given separation than packed columns (54). However, due to the low pressure drop per unit length, capillary columns can offer a very large number of theoretical plates by using appropriate column lengths, without the need of extra high pressures and without causing excessive increase of elution times. Furthermore, due to the low flow rates (0.5-5cc/min.) used in capillary columns, single column temperature programming can be readily performed. These features of the capillary columns make them particularly attractive for the analysis of

complex mixtures. Two monographs (34,35) give a detailed account on theory, technology and application of capillary columns. Our contribution in this field deals with the development of an improved method for preparation of such columns.

2.2. An Improved Method for Preparation of Capillary Columns.

2.2.1. Introduction

Capillary columns have repeatedly been proved excellent for high resolution in gas chromatography (3,4,51-53). Due to difficulties involved in their preparation and use, however, the application of capillary columns is presently not as wide as it should be, considering their merits (55). Special care should be taken to select solvents of suitable polarity for the coating solutions (34); the wetting properties of the stationary phase towards the material of the capillary tubing should be checked (34,56), and means should be made available for forcing the coating solution through the column at a low and constant velocity (35). This latter condition is difficult to achieve even when precision needle valves are used to control the gas flow (35).

The existing methods for preparation of capillary columns have been reviewed by Kaiser (34) and Ettre (35). Although the importance of careful control of the coating process has been stressed by these authors, the simplified approach of merely forcing the coating solution through the column by a constant gas pressure still seems the prevalent method in the literature. In our laboratory, this simplified approach gave less satisfactory results. The method of Kaiser (34), using an electrolytic cell

for producing a low and controlled gas flow, gave satisfactory results.

We developed a novel method for coating capillary columns, based on stabilization of the plug velocity and gas velocity in the column by the use of a flow restriction device placed downstream from the column to be coated. This device was designated a "liquid brake".

2.2.2. Experimental

Apparatus. A relatively simple glass apparatus was used (Fig. 1), consisting of gas source, mercury manometer (A), inlet system (B-E), capillary column to be coated (F), volumetric collector tube for the coating-plug (G), plug-velocity stabilizing system (H-K), and soap bubble flow meter (M). The heavy, solid crosslines on Figure 1 (marked B, C, etc.) represent interconnections in the glass system; these were made of short pieces of Tygon tubing, 1/16" I.D., and provided with metal screw clips for opening and closing of the flow lines. The coating solution did not come in contact with these tube connections with exception of the one marked D. Other connections between glass tubings, and between glass tubing and metal capillary tubing, were made as butt joints, using a sleeve of Tygon tubing and taking care to join butt ends closely so as to minimize the deposition of coating liquid at these locations.

A nitrogen cylinder with pressure reducing valve provided the carrier gas and pressure required to force the coating solution through the column.

The inlet system was made from a graduated 1-ml pipette (E), 2 mm I.D., by cutting off the pointed tip. The coating solution was introduced at location C either by disconnecting the Tygon tubing and

Figure 1

Coating Apparatus (schematic)

A Mercury manometer

B-E. Inlet system

F Capillary column to be coated

G Volumetric collector tube for the coating plug ("liquid brake").

H-K Plug-velocity stabilizing system

M Capillary soap bubble flow meter

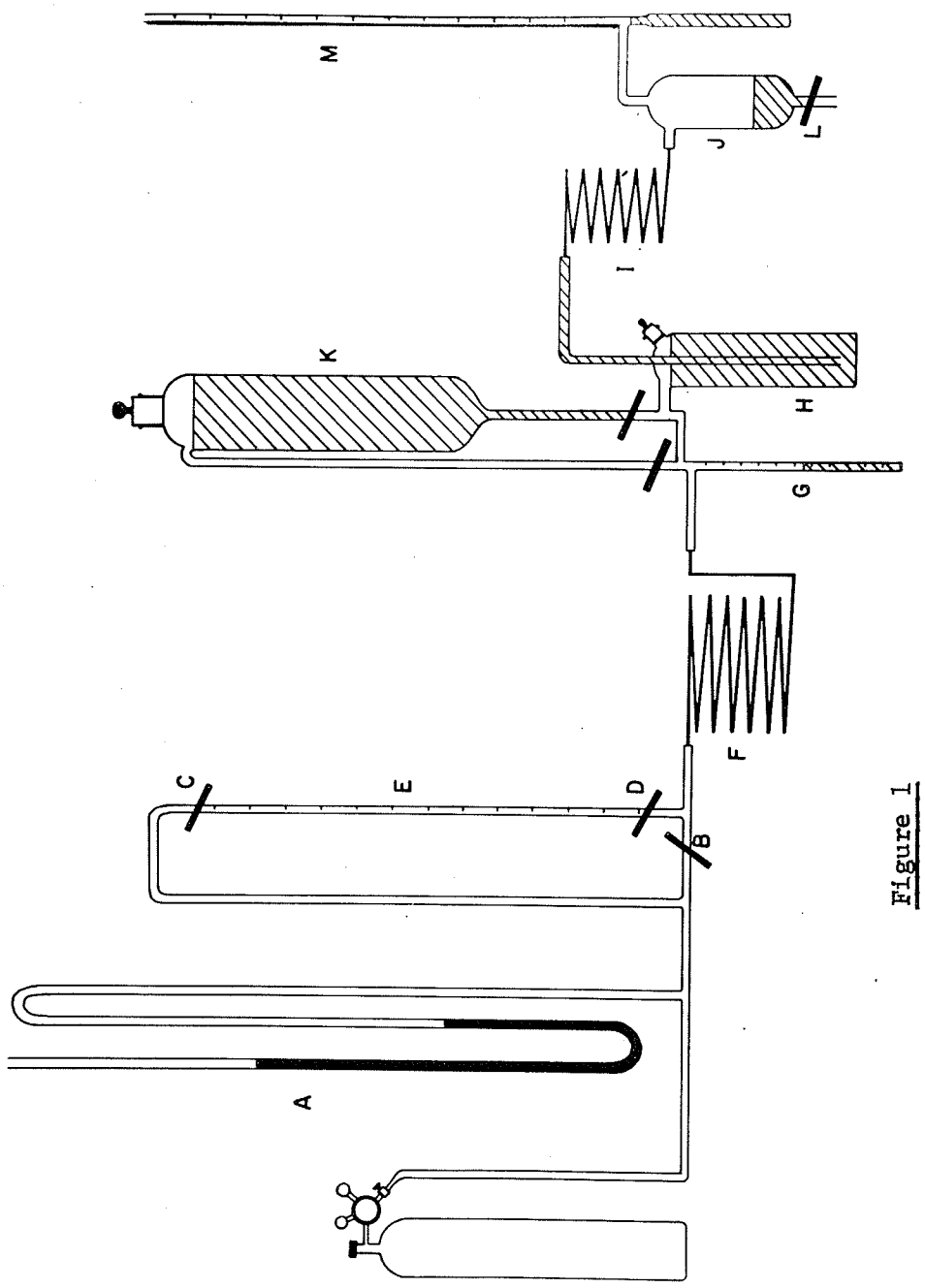


Figure 1

adding the solution by a pipette, or by injecting the solution by a hypodermic syringe through a rubber septum arrangement at location C (not shown in the figure).

The volumetric collector tube (G) for the coating plug was made from a section of a graduated 1-ml pipette sealed at the lower end. It functioned as a measuring reservoir for the unconsumed part of the coating plug emerging from the capillary tubing after coating. To minimize deposition of coating solution in the interconnections between column and collector tube and between column and inlet system, these interconnections were made as short as possible.

The plug-velocity stabilizing system, designated the liquid brake, consisted of two reservoirs (H, K) for the "brake" liquid, a restricting capillary tubing (I), and a collector flask (J) for the brake liquid with an outlet tube (L). Reservoir H and collector flask J were of approximately 10 ml volume. The dimensions of the restricting capillary tubing and the viscosity of the brake liquid were chosen so that the pressure required to force the brake liquid through the restricting capillary tubing was considerably higher than the pressure required to force the coating plug through the column to be coated. A restricting capillary tubing of 10 ft length and 0.01 inch I.D. (stainless steel) was satisfactory for the coating of columns of 0.02 inch I.D. (irrespective of column length). Distilled water was used as brake liquid. The purpose of reservoir K was to refill reservoir H, if required, without changing the pressure in the system.

The soap-bubble flow meter (M) was made from a thick-walled glass capillary tubing, 3 ft x 1 mm I.D.

A modified version of the apparatus (Fig. 2) was occasionally

Figure 2

Modified Version of the Coating Apparatus

for Accurate Determination of

Film Thickness

Shows the capillary column connected to two plug volume measuring capillaries (Teflon).

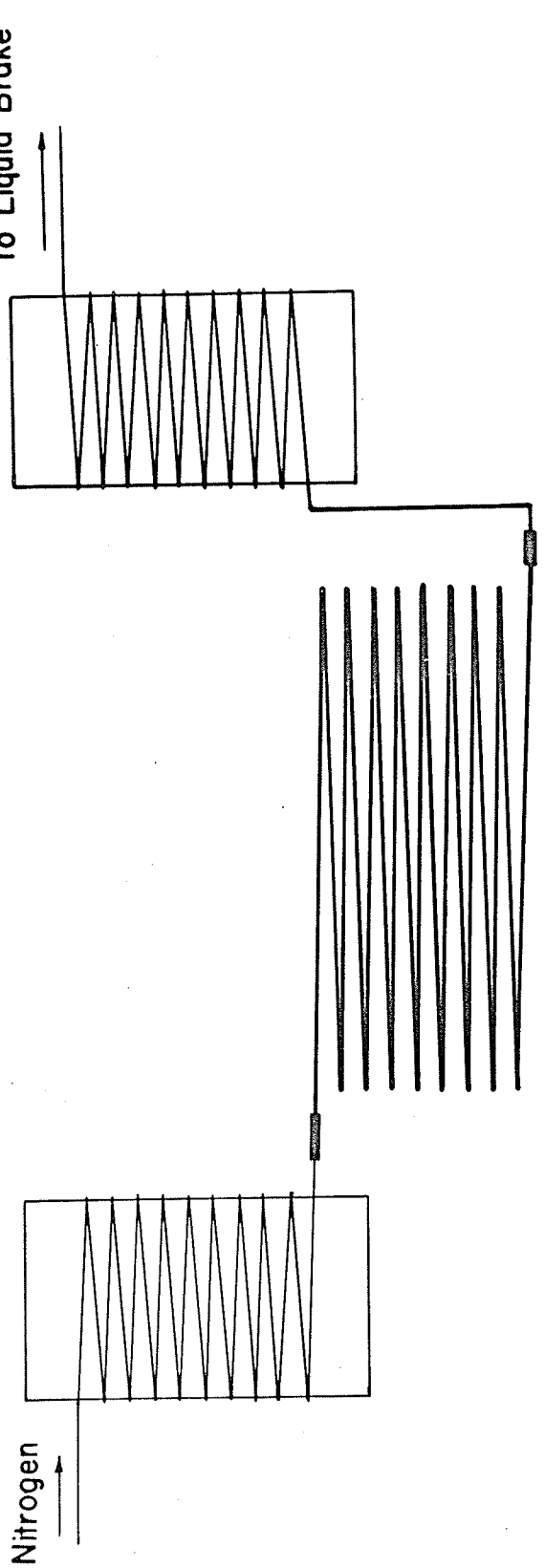


Figure 2

used for more accurate estimation of the film thickness. In the modified version, two transparent and non-wettable capillary tubings (Teflon) were inserted between the capillary column and the inlet system and, on the outlet side, between the capillary column and the liquid brake system. The Teflon tubing diameter (0.022 inch I.D.) was slightly larger than the diameter (0.02 inch I.D.) of the column to be coated.

Procedure. The column to be coated was freed from possible organic material deposited on the inner wall during its production or otherwise by passing through it a series of pure solvents followed by drying as described by Kaiser (34) and Ettre (35). Subsequently, the volume of pure coating solvent consumed for wetting the inner wall of the column at a certain plug speed was measured in order to estimate the concentration of coating solution required to give the desired film thickness (34). The column was again dried before being coated.

The desired amount of coating solution was introduced into the graduated pipette E at location C, using a 1-ml syringe, with clip D closed. Clip C was then closed and (with clip B open, the two clips for reservoir K closed, and reservoir H filled with distilled water) a pressure of approximately 15 psi was applied. The resulting low flow of nitrogen through the column was measured with the flow meter (M). Clip B was closed and clips C and D opened; the coating solution slowly entered the capillary column with no change in the flow rate. The flow rate at different intervals during the plug travel was measured with the flow meter. The time for the coating plug front to pass through the column was measured. The volume of unconsumed coating solution collected in tube G was recorded.

After the coating plug had emerged from the column, the flow of gas was continued for approximately 4 hr at unchanged flow rate to

evaporate the solvent in the column. The gas pressure was then gradually reduced to zero; the liquid brake system was disconnected from the column and the column and the flow meter was connected directly to the column. A higher flow rate of about 2 ml/min was further maintained for about 10 hr by applying a gas pressure of 5-10 mm Hg (above atmospheric). The column was then conditioned as described by Kaiser (34).

The plug velocity was obtained at different times during the coating process by measuring the flow rate and converting it to linear velocity of the plug (the coating plug, the brake liquid in capillary I, and the air displaced from collector flask J all have identical flow rates). The average velocity of the plug through the column was obtained by measuring the time for the coating plug front to pass through the column.

The film thickness was estimated from the volume of coating solution consumed in the column (the difference between the volumes measured in E and G), the concentration of the coating solution, and the area of the inner wall of the capillary column (34).

More accurate estimations of film thickness were obtained using the modified version of the apparatus (Fig. 2). The volume of coating solution consumed in the column was obtained from measurements of the length of the plug as it travelled through the Teflon capillaries before and after coating.

2.2.3. Results and discussion

The described procedure for coating of capillary columns provides for a rigid control of the plug velocity during its travel through the column and of the gas flow during the subsequent drying process. The difficulty in obtaining this rigid control with many of the common coating procedures in use is associated with the continually changing

resistance to flow that occurs when a coating plug travels through a capillary tubing. This difficulty has been overcome in the present system by introducing a constant and high resistance to flow, the liquid brake, in the system downstream from the column to be coated. In principle, this secondary flow restrictor is constructed to give considerably higher flow resistance than that occurring in the column during the coating process. The resulting flow rate in the system will thus depend on the secondary flow resistance and be virtually independent of the varying flow resistance in the column. By maintaining a constant resistance to flow in the secondary restrictor, the flow rate in the column will remain constant during the entire coating process.

In the present system, the secondary flow restrictor consisted of a short capillary tubing and a water supply that was being continually forced through the capillary tubing to create the required flow resistance.

The results of coating a capillary column with and without the use of the liquid brake system were compared in terms of the plug velocity and the gas velocity as recorded at regular intervals during the coating process (Fig. 3). The column in these experiments was 100 feet long and of 0.02 inch I.D.; the gas pressure from the nitrogen tank was maintained at 760 and 40 mm Hg when coating with and without the liquid brake, respectively; a coating plug of 0.5 ml volume was introduced into the column.

When the liquid brake system was used (Fig. 3, A), the flow rate remained constant at a linear velocity in the column of 1.35 cm/sec during the entire coating process, i.e., before, during and after the travel of the plug through the column.

When the liquid brake system was omitted (Fig. 3, B), the flow rate varied considerably. At the introduction of the plug into the column

Figure 3

Plug and Gas Velocity in the Column

as a Function of Time During

the Coating Process

A. With liquid brake

B. Without liquid brake

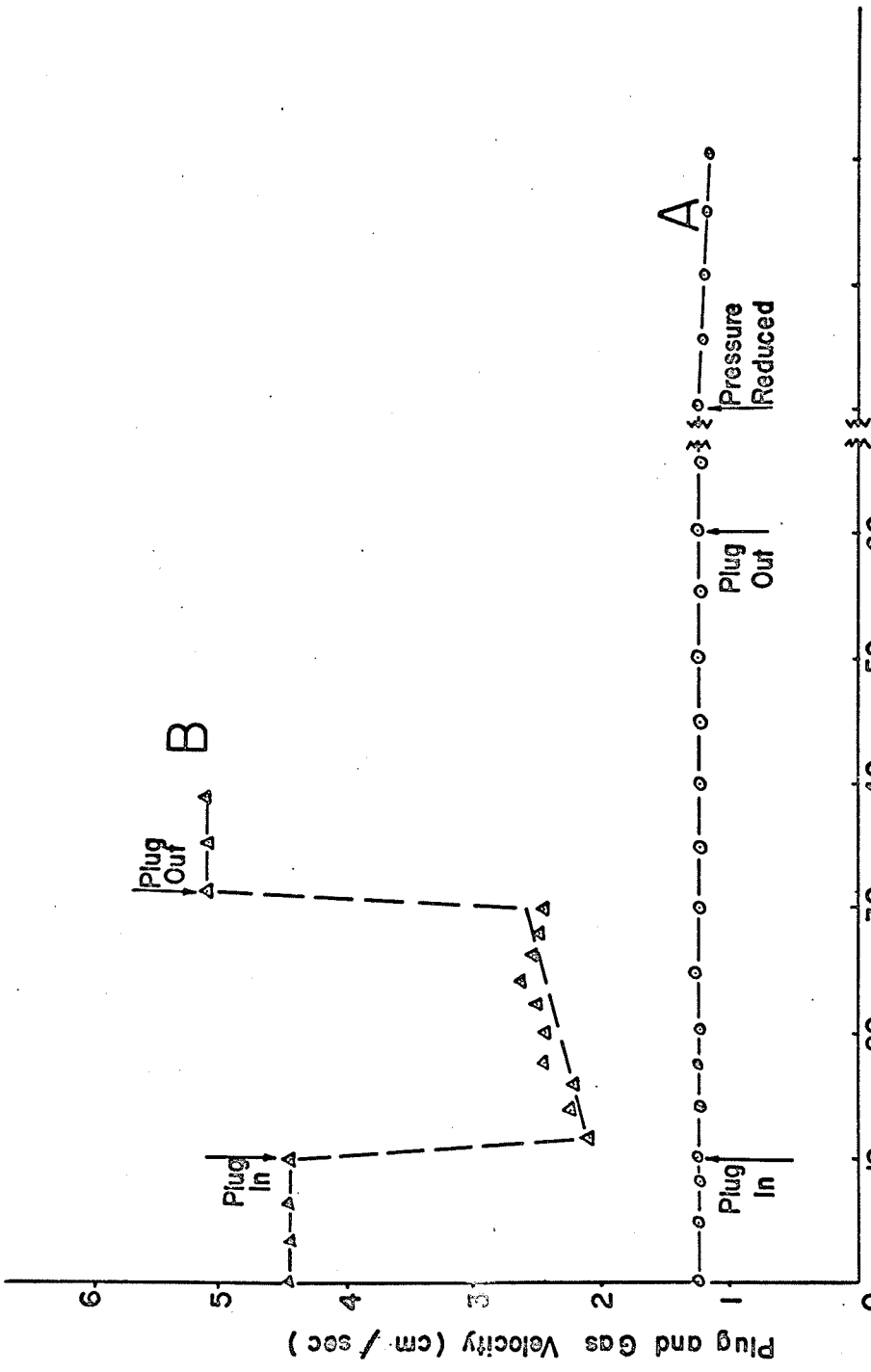

Plug and Gas Velocity (cm / sec)
Time (min)
Plug In
Plug Out
Pressure Reduced
A
B

Figure 3

the linear velocity in the column decreased abruptly from 4.4 to 2.1 cm/sec. During the coating, the plug velocity was irregular and, furthermore, it increased gradually from 2.1 to 2.6 cm/sec, i.e., a relative increase of 24 per cent. At exit of the plug from the column, the gas velocity in the column increased abruptly from 2.6 to 5.1 cm/sec.

The flow pattern obtained without use of the liquid brake (Fig. 3, B) probably illustrates the conditions prevailing in the more commonly used dynamic coating procedures. Under such conditions, the formation of a uniform film is severely hampered by different factors. As the film thickness under certain conditions has been found proportional to the plug velocity (34), the gradual increase in plug velocity during the coating process should result in a gradually increasing film thickness along the column. The rapid acceleration of the plug during its exit from the column should result in a considerable increase in film thickness in this region of the column. The abrupt increase in gas flow that occurs after emergence of the coating plug may result in the formation of small new plugs of coating solution at various locations in the column; these plugs would travel at considerable velocity and result in an uneven film thickness along the column. Finally, a slow and uniform rate of evaporation of the solvent after exit of the coating plug, which is considered an important factor for obtaining a uniform film (34), is difficult to obtain under such conditions.

As pointed out by Bernhard (57) and Zlatkis (58), a certain degree of "smoothing" of film irregularities undoubtedly occurs during the conditioning of the column, particularly if the highest permissible temperature for the liquid phase is used. One might expect, however, that the smoothing is effective only in localized areas in the column

and that it might be difficult by this procedure to correct for a gradually increasing film thickness throughout the entire column as caused by plug acceleration during coating. Also, the beneficial effect of the smoothing upon column quality may not be entirely predictable.

The flow pattern obtained with use of the liquid brake (Fig. 3, A), however, should greatly facilitate the deposition of a uniform film as the plug velocity remains constant during coating, and the gas flow after plug emergence remains constant and at the same low velocity as that of the coating plug.

The flow stabilization principle provides a coating procedure that is well defined, reproducible, and simple to perform. It should contribute to alleviate the present situation in coating procedures as recently described by Desty (10) : "Coating procedures are still fairly unreliable, particularly in inexperienced hands ... There is great need for careful systematic work ... if we are to improve on the 'bash it through and hope' method widely practiced."

Other types of flow restrictors, such as a narrow-bore capillary tubing through which the nitrogen gas would flow and create the required back-pressure, could probably with equal effect replace the liquid brake system here described, provided the restrictor were placed downstream from the column to be coated.

2.3. Preparation and Performance of Long Narrow-Bore Packed Columns

2.3.1. Introduction

High resolution is attainable in gas chromatography by the combined effects of high solvent (liquid phase) and column efficiencies, small β values and sufficient column length (33,37). Capillary columns offer the use of practically unlimited column lengths and high column efficiencies. However, they have large values for β and small sample capacity. Furthermore, their application is limited only to stationary phases of suitable wetting properties (56,57,59). Unfortunately, liquid phases of high solvent efficiency towards polar substances frequently exhibit poor wetting properties (57).

Long narrow-bore packed columns of low liquid phase loadings (60-64) offer an attractive alternative to capillary columns in high resolution gas chromatography. They offer relatively high column efficiencies, compatibility with any liquid phase, intermediate values of β, sufficiently wide range of useful column lengths and low cost. These features, together with a most valuable increase in the sample capacity, make the long packed columns suitable for flavor analysis.

The conventional method for packing gas chromatographic columns (vertical filling and tapping) is inapplicable for packing long narrow-bore columns. In order to obtain the desired column length Spencer (61) interconnected 10-ft sections of 1/8" O.D. packed columns. Interconnecting short column sections by Swagelok unions increases the dead volume of the column. Kargers and Cooke (62) found that the inability to pack long single length columns efficiently outweighs the disadvantage of

the slight increase of the dead volume occuring when short sections are interconnected. A similar opinion was expressed by Amos and Hurrel (60). The development of a suitable method for packing of long narrow-bore columns in a single length is desirable for the elimination of dead volumes, for cutting down the cost of the column and for simplifying their preparation. It was our purpose to develop such a method and investigate the applicability of long narrow-bore packed columns in flavor analysis as an alternative to capillary columns.

2.3.2. Experimental

Columns up to 50 ft lengths were packed by suspending the tubing vertically, straight or in U shape using specially designed tools which are shown in Figure 4a. These tools consist of a kit of grooved clamps made of brass which were used to fasten the column ends at the upper end of the stairwell and to serve as a weight on the lower part of the U shape as shown in Figure 4b. The packing material was poured batch-wise into the two ends of the column through small funnels and packed by light tapping (vertical and horizontal) on the clamps and on the column. The column was then coiled with a minimum radius of curvature of 2.25" and preconditioned. Columns 1 and 2 (Appendix 1) were prepared according to this procedure. Most of the work was carried out with column 1 which contained the same liquid phase as a 100 ft, 0.02" I.D. capillary column (column 3). Several test mixtures containing C_1 to C_6 alcohols (Poly-science Corporation, Evanston, Illinois, Qual. Kit No. 13) and a 1:1 mixture of 2-pentanol and 3-pentanol were run with columns 1,2,3 and 4 under comparable conditions. An Aerograph model 660 gas chromatograph (FID system 6., section 3.7.1.) was used in conjunction with the long narrow-bore packed columns. A Barber Coleman Selecta 5000 gas chromatograph,

Figure 4

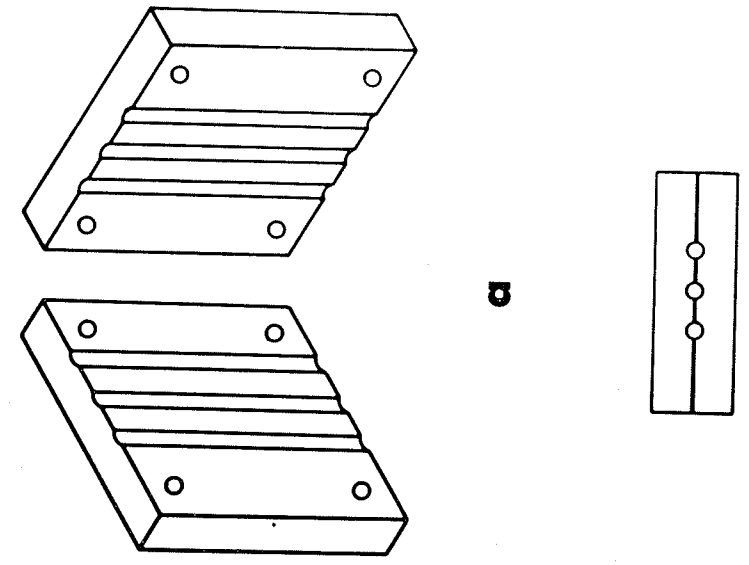
Grooved Plates for Packing

Long Narrow-Bore Columns

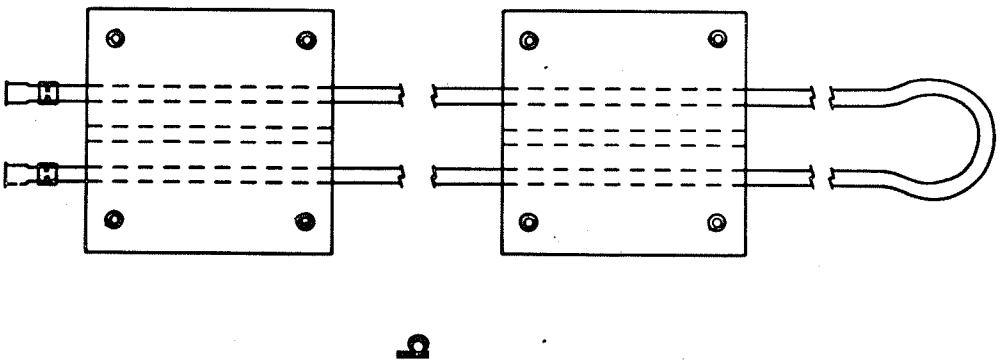
in Single Lengths.

A. The grooved plates (exploded view).

B. Illustration of the mode of use of the grooved plates for packing of a long U shaped column.



a



b

Figure 4

(FID system 4 section 3.7.1.) and a "scavenger" gas arrangement to eliminate dead volumes in the outlet of the column (similar to the arrangement shown in Figure 17) was used in conjunction with the capillary column. The mixture of pentanols was run at 100°C with columns 1 and 3. The nitrogen inlet pressure of column 1 was set at 40, 50, 60, 70, 80, 90, 100 and 120 psi and the mixture of pentanols was run twice at each pressure. The highest resolution was observed for inlet pressure of 70 psi. The mixture of pentanols was run with column 3 at various split ratios 1:50 to 1:300 with nitrogen flow rates ranging between 2.5 and 7.0 ml/min. The optimum flow rate (u_{opt}) was found to range between 2.5 and 3.5 ml/min. Above split ratios of 1:200 no improvement of the resolution was observed. For comparison of the time required to achieve given separation, the Qual. Kit No. 13 mixtures (five) were run with column 3 at 100°C adjusting the flow rate so that baseline separation of all the constituents is achieved (approx. 7 ml/min). These mixtures were also run with column 1 at 100°C with 110 psi inlet pressure and at 128°C with 130 psi inlet pressure. A mixture of C_5 - C_{10} normal paraffins was run with column 2 at 107°C with inlet pressure of 110 psi. The Qual. Kit No. 13 mixtures were also run on column 4 which is an ordinary 1/4" O.D. analytical column. The permeability of the long narrow-bore columns to different carrier gases was investigated by plotting the flow rate versus the inlet pressure for column temperatures of 95 or 100°C.

2.3.3 Results and discussion

Preparation. The merits of packing long columns in single lengths relative to those of packing short sections which are later

interconnected should be considered in view of the application and in view of the loss or gain in column performance relative to one another. The columns packed in single lengths using the tools shown in Figure 4 exhibited sufficiently high efficiencies to permit the following conclusion to be made: the improved method of packing long narrow-bore columns described here enables the preparation of equally efficient long columns in a single length as compared with the efficiencies of similar columns packed in short sections reported in the literature (60,61). With no sacrifice or gain of column efficiency, packing of long columns in single lengths as described here has the advantage of considerably lower cost and expedient preparation.

Performance. Column 1 (37 ft, 1/8" O.D.) exhibited 20,000 theoretical plates for 3-pentanol at 100°C and at u_{opt}, i.e., 540 plates per foot for an alcohol. At about twice u_{opt}, column 1 exhibited 13,800 theoretical plates for 3-pentanol and at the same temperature. Column 2 (50 ft, 1/8" O.D.) exhibited 20,000 theoretical plates for heptane at 100°C and at approximately twice u_{opt}. From the number of theoretical plates observed at u_{opt} and 2 u_{opt} with column 1 it was estimated that column 2 can be expected to exhibit about 35,000 theoretical plates at u_{opt} for heptane at the same temperature. This would give about 700 plates per foot for a hydrocarbon. Amos and Hurrell (60) obtained about 500 plates per foot for a hydrocarbon on a 40 ft, 1/8" O.D. column packed in short sections. Spencer (61) obtained 650 plates per foot for heptane for a 100 ft, 1/8" O.D. column. Obviously, the efficiency of the columns 1 and 2 prepared in single length by the method described here is equal if not better than the efficiency of similar columns

packed in short sections. Comparing the chromatograms of Polyscience mixture 13-4 obtained with column 1 (Fig. 5b) and column 4 (Fig. 5c) reveals the superior performance of column 1 over column 4 (ordinary 1/4" analytical column) but considerably less pronounced than may be expected.

Comparison with capillary columns. The chromatograms of the 2-pentanol and 3-pentanol mixture obtained at 100° C and optimum flow conditions with column 1 and 3 are shown in Figure 6. Visual comparison of these chromatograms shows that the resolution was only slightly higher for the capillary column (Fig. 6a) than for the packed column (Fig. 6b). Trace impurities, however, were more clearly detected with the packed column. The elution time was twice as long for the packed column (12 min) as for the capillary column (6 min). Using the Polyscience Qual. Kit 13 test mixtures the two columns were compared at the same temperature (100°C) but with higher than optimal inlet pressures (flow rate) so as to minimize the analysis time without losing baseline separation for adjacent major peaks. In Figure 5 the chromatograms of Polyscience test mixture 13-4 are shown. The overall resolution obtained was similar for the long packed column (Fig. 5b) and the capillary column (Fig. 5a). Minor peaks, however, were again better revealed with the packed column. The total elution time was three times as long for the packed column (20 min) as for the capillary column (6 min). In order to compare the resolution of the two columns at almost equal elution times the temperature and the inlet pressure for the packed column were increased (128°C; 130 psi) to give a shorter total elution time. Under these conditions the chromatogram of test mixture Polyscience 13-4 (Fig.5d) shows a considerable decrease of the resolution as compared to the resolution obtained at 100°C and 110 psi (Fig. 5b). Also, an adsorption effect of the solid

Figure 5

Comparison of Long Narrow-Bore Packed,

Capillary and Regular Columns

Using Test Mixture 13-4

Test mixture 13-4 (Qual. Kit.13, Polyscience Corp.) consists of seven alcohols (plus impurities) which are listed according to their peak numbers in chromatograms a-d :

1. n-propyl alcohol
2. isopropyl "
3. 3-methyl-2-butanol
4. isoamyl alcohol
5. 2-methyl-1-pentanol
6. 3-hexanol
7. n-hexyl alcohol.

The chromatographic conditions for chromatograms a-d were as foll

Chromatogram a

Column 3 (100 ft, 0.02" I.D.)
Col. temp. 100°C
Split ratio 1 : 100
Inlet pressure 250 mm Hg
Sample size 0.05 µl

Chromatogram b

Column 1 (37 ft, 1/8" O.D.)
Col. temp. 100°C
Inlet pressure 110 psi
Sample size 0.05 µl

Chromatogram c

Column 4 (10 ft, 1/4" O.D.)
Col. temp. 100°C
Inlet pressure 20 psi (60 ml/min)
Sample size 0.5 µl

Chromatogram d

Column 1
Col. temp. 128°C
Inlet pressure 130 psi
Sample size 0.05 µl

Carrier gas N_2

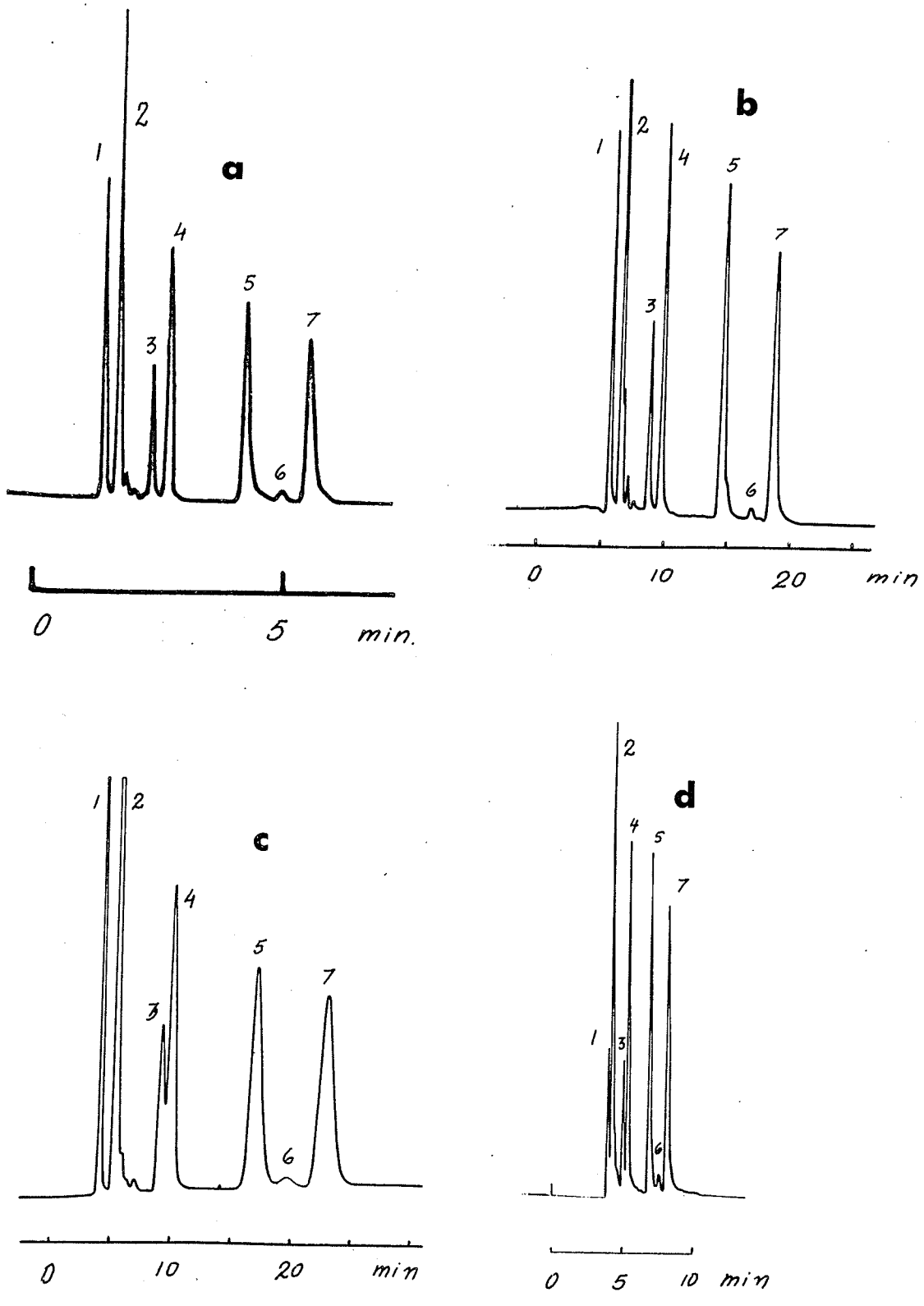

a
1
2
3
4
5
6
7
0
5
min.
b
1
2
3
4
5
6
7
0
10
20
min
c
1
2
3
4
5
6
7
0
10
20
min
d
1
2
3
4
5
6
7
0
5
10
min

Figure 5

Figure 6

Comparison of Long Narrow-Bore Packed and Capillary Columns at Optimum Flow Rates (U_{opt}).

A test mixture of 2-pentanol and 3-pentanol was used to compare the resolution of these columns at the same column temperature (100°C) and using the same liquid phase (Tergitol NP-35) in both columns.

Chromatogram a

Column 3

Split ratio 1 : 200

Inlet pressure 135 mm Hg

Sample size 0.05 µl

Chromatogram b

Column 1

Inlet pressure 70 psi

Sample size 0.05 µl

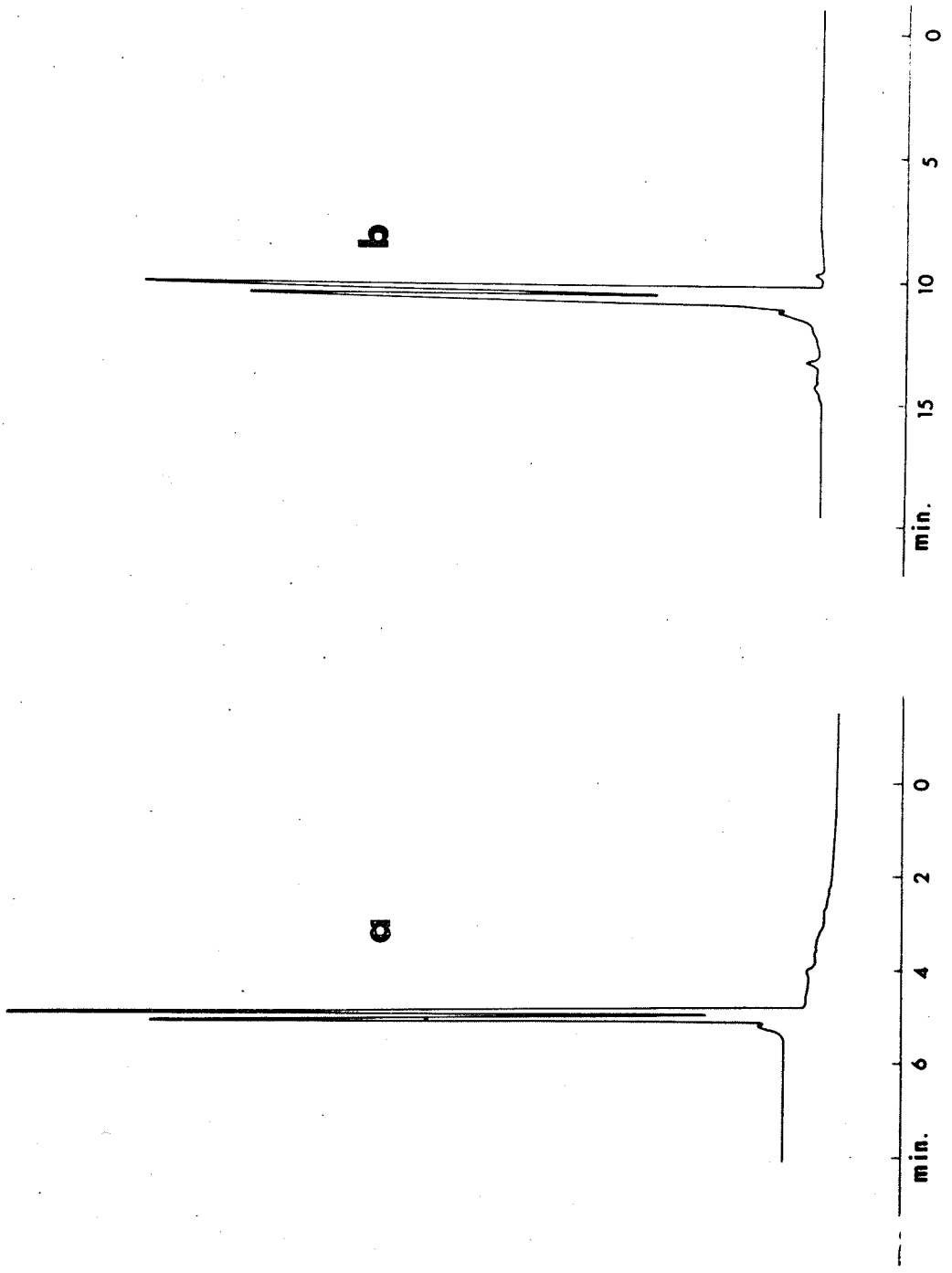


Figure 6

support (65) was indicated by the difference in the relative peak heights obtained at 128°C (Fig. 5d) and at 100°C (Fig. 5b).

Selection of carrier gas. Since operating at high pressures creates experimental difficulties and in fact limits the maximum column length that can be used without special sampling equipment, it was of interest to examine the pressure-flow curves of these columns with different carrier gases. In Figure 7 the pressure-flow curves of column 1 and 2 for nitrogen, helium and hydrogen are shown. The flow rates of each of these gases at given pressure are inversely proportional to their viscosities. At 100°C the viscosities of nitrogen, helium and hydrogen in micropoises are 208, 228 and 103, respectively (37). From these values and the curves shown in Figure 7 it becomes obvious that helium is most undesirable for use with long narrow-bore columns particularly when taking into consideration the fact that for practically all compounds the D_g term in helium is large (section 2.1.1). In hydrogen the D_g is even larger but hydrogen has very low viscosity which is essential in eliminating the need for very high inlet pressures. In view of the fact that D_g is inversely proportional to the pressure, hydrogen can be used at relatively high inlet pressures without considerable loss of efficiency due to the large D_g values of organic compounds in it because a considerable length of the column is under sufficiently high pressure. It can be concluded that the use of hydrogen would be more suitable with columns longer than 50 ft while the use of helium should be avoided altogether. Nitrogen should be most suitable for columns shorter than 50 ft.

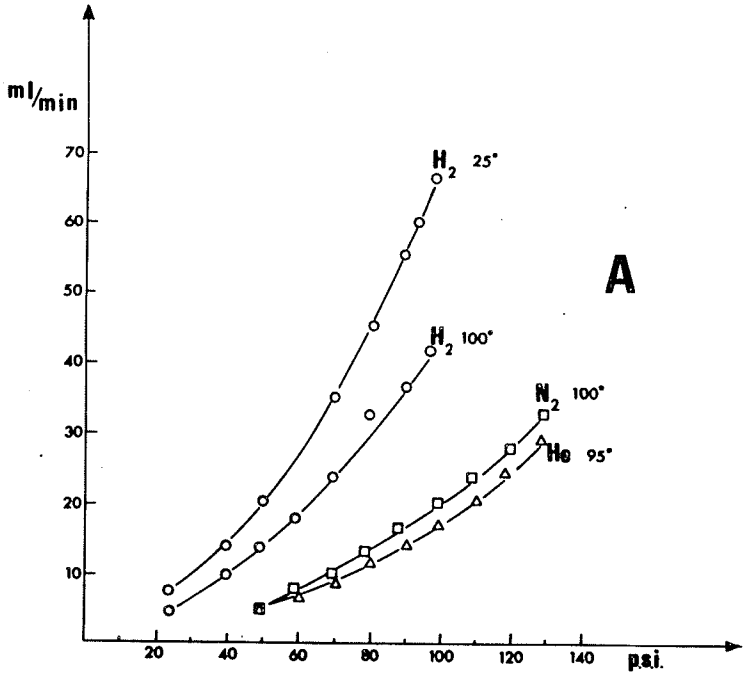
Conclusions. Long narrow-bore packed columns were found to be successfully applicable as substitutes to capillary columns in high resolution gas chromatography. They offer resolution similar to capillary

Figure 7

The Flow as Function of the Pressure for Long Narrow-Bore Packed Columns Obtained with Different Carrier Gases.

A. Pressure-flow plots for column 1 (37 ft, 1/8" O.D.) with hydrogen, nitrogen and helium.

B. Pressure-flow plots for column 2 (50 ft, 1/8" O.D.) with hydrogen, nitrogen and helium.


ml/min
70
60
50
40
30
20
10
H2 25°
H2 100°
N2 100°
He 95°
A
20
40
60
80
100
120
140
p.s.i.

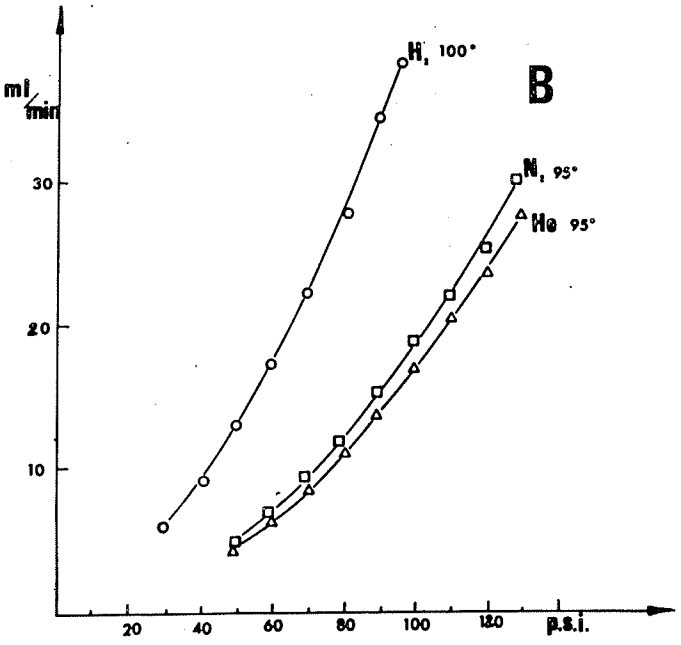

ml/min
30
20
10
H2 100°
N2 95°
He 95°
B
20
40
60
80
100
120
p.s.i.

Figure 7

columns, about 100 times higher sample capacity, and compatability with any liquid phase. This is achieved at the expense of considerably longer elution times, pronounced adsorption effect of the solid support and difficulties in injection.

Chapter 3

Flame Ionization Detection Systems and Methods for Achivement of High Sensitivities

3.1. Introduction.

The flame ionization detector (FID) was introduced in 1958 almost simultaneously and independently by McWilliam and Dewar (66,67) and by Harley, Nel and Pretorius (68). Since then, it has been given considerable attention in the literature (30,31) and has become the most widely used detection device in gas chromatography. The introduction of the high sensitivity flame (66) and argon ionization (69) detectors represent two of the most important developments in gas chromatography which greatly enhanced its scope of applications. A large fraction of the applications of gas chromatography mentioned in the literature (30,31) are in fact based on the availability of the highly sensitive detection devices in general, and on the flame ionization detector in particular. Regardless of the practical achievements made through the use of FID it is difficult to speak about its optimal design parameters and its ultimate sensitivity since so far the mechanism of ion formation is not fully understood. Nevertheless, sufficient data are now available to permit the introduction of further improvements in its overall performance.

Because of the immediate need for a high sensitivity detector at the time when FID was first introduced, and the relative ease with which simple operating models of flame ionization detectors can be assembled, such detectors were produced and marketed on a purely empirical basis and

with surprisingly little knowledge of the principles involved (70). Many of these units are still in use and, what is unfortunate, some are still being manufactured.

The literature on this remarkable detector was briefly reviewed by Schomburg (71) in the beginning of 1962. An excellent discussion of the practical problems encountered in the application of FID and its "micro" version in capillary column gas chromatography was given by Kaiser (34) in 1962. The most comprehensive recent volume on ionization detectors was given by Krugers (72). He reviews thoroughly the literature on mechanisms of ion formation in flames up to 1964 and includes discussions of other aspects of FID systems with considerable detail.

The methods for achieving high sensitivity with FID systems have not been reviewed systematically. Availability of high sensitivity detectors is one of the most important aspects of the application of gas chromatography in flavor research (9). A review of the literature is therefore included here emphasizing the practical problems encountered in the application of high sensitivity FID systems to flavor research problems.

Detecting and recording the presence of an organic compound in a hydrogen flame involves four principal processes:

1. Formation of ions
2. Recombination of ions
3. Collection of ions
4. Measurment of ion currents.

The overall sensitivity of a FI detector depends on the efficiency of each of these processes. Some of the factors which have an effect on the

above mentioned individual stages are common to more than one process but do not necessarily contribute in the same direction for each of them. For instance, geometry of the jet may affect the formation and collection of ions but not the measurement of ion currents whereas the mechanical properties and the shape of the electrodes may influence the recombination (73), the collection (73) and the measurement of ion currents (74) but not the formation of ions. The factors that affect the overall sensitivity of a FID system can be classified as follows (74) :

1. Geometry
2. Electrical effects
3. Operating conditions
4. Nature and purity of the materials used for construction of the FID.

3.2. Formation of Ions in Flames

The processes which lead to formation of ions in flames have been the subject of continuous study for many years before the invention of the flame ionization detector (75). Unfortunately, a full explanation of this phenomenon does not exsist as yet. Calcote (75-78) established that chemi-ionization, not thermal ionization, is responsible for the formation of ions in flames. Comprehensive reviews on the principles underlying the operation of FID were given by Sternberg et al (70) in 1961 and by Krugers (72) in 1964. Sternberg et al (70) summarized the evidence presented by Calcote (75-78) which rejects the possibilities that ionization is due to the formation of solid carbon particles or due to thermal ionization of impurities present in flames. Krugers (72)

systematically analysed the basic reactions responsible for the formation of ions in flames and the possible behaviour of groups of organic compounds under the conditions existing in flames. Unfortunately, experimental work with flames is beset by many practical difficulties, and much conflicting evidence has resulted (79). For this reason no particular hypothesis has gained general acceptance. However, it is becoming clear that the reaction (I) originally proposed by Calcote (75) has gained the most support (80) as the basic reaction which leads to formation of ions in flames.

$$CH^{\bullet} + O^{\bullet} \longrightarrow CHO^{+} + e \qquad (I)$$

This reaction requires CH radicals and atomic oxygen. The CH radicals are formed by pyrolysis in the inner oxygen-free regions of the flame (70,72) and atomic oxygen is formed by dissociation of molecular oxygen and a number of other free radical reactions. Calculated from the ionization efficiency measurements (81) this reaction occurs with probability of about $1:10^5$. An increase in the probability of ion formation will lead to increased sensitivity of the FID system. Changes in the hydrodynamical structure of the flame (70,82,96), its burning velocity (34,72), its temperature (83), the composition of the gases issuing from the jet (70), the rate of supply of oxygen (70,72,84,85) and the thermal conductivity of the carrier gas (86) affect the overall ionization efficiency and the probability of ion formation. In the following paragraphs of this section the factors which affect the probability of ion formation are briefly reviewed.

3.2.1. Premixed versus diffusion flames

There are two principal types of flames, premixed flames and diffusion flames. In premixed flames the fuel and the oxydant gas are

mixed before entering the flame and issue from the jet as a homogeneous mixture (Bunsen flame). In diffusion flames there is no premixing, the fuel issues from the jet alone and comes in contact with the oxydant which is supplied only from outside the flame by diffusion through its various reaction zones. The diffusion flames differ considerably from premixed flames due to the fact that the fuel is in continuous contact with the oxydant throughout the entire process and reaction rates are not limited by the rate of supply of the oxydant. Krugers (72), however, compared various parameters of premixed and diffusion flames and concluded that these two types of flames differ less than might be expected. Unfortunately, diffusion flames have been subjected to considerably less quantitative study than premixed flames (70). The flame ionization detector is usually operated with a diffusion flame (72) which is more suitable since the pyrolysis of organic molecules is achieved in the oxygen free zone of the flame and probably yields more CH radicals. Sternberg (70) made measurements with diffusion and premixed flames and found that the yield of ions is lower in premixed flames.

3.2.2. The effect of the oxygen

The effect of the flow rate of air (oxygen) on the yield of ions from a diffusion flame depends on whether the total quantity of oxygen supplied to the boundaries of the flame is larger or smaller than the quantity which can diffuse per unit time into the reaction zone of the flame. When this quantity is smaller the yield of ions, i.e., sensitivity, will be dependent on the rate of supply of oxygen to the boundaries of the flame (flow rate of air) and will increase until the rate of supply of oxygen becomes equal to its diffusion rate to the reaction zone of the flame. From this point on the yield of ions ceases to be

dependent on air flow rate and remains constant when further increase of air flow rate is made. It should be remembered, however, that high flow rates of air may induce turbulence in the flame and cause a decrease in the yield of ions. The actual quantities of air needed to operate a given FID unit in the "plateau" region is largely dependent upon its dimensions and on the geometry of the parts which lead the air to the flame (87,88). In most of the cases, however, flow rates of 150 to 400 cc/min of air should be sufficient to keep the response at least at the beginning of the plateau region. Using pure oxygen or oxygen enriched air has been proven to give higher sensitivities (70,84,85,89) due to the higher concentration of oxygen on the boundaries of the flame. Bruderreck et al (89) found the response of their FID to be six times higher when operated with pure oxygen instead of air. Under such conditions, however, the temperature of the jet tip may rise to glowing temperatures and cause a considerable increase of the noise. Accessibility of the air (oxygen) to the flame and particularly to its base (the edge of the jet) (73) is another aspect of oxygen supply to the flame. Certain rather massive jets (90) may restrict access of air from below the orifice of the jet as compared with hypodermic needle type jets (73). Cone shaped jets of the type described by Kaiser (34) and Bruderreck et al (89) may provide a favorable compromise between access of oxygen and the mass of metal needed to serve as a heat sink.

The fact that no substantial increase of sensitivity is observed with more than 30% oxygen (72),can be explained if assumed that the formation of atomic oxygen is dependent on the regime of the flame but not on diffusion rate of oxygen. In other words up to a certain level the process is limited by diffusion and above this level by the rate of formation of

atomic oxygen.

3.2.3 Hydrogen to inert gas ratio

The effect of the nitrogen to hydrogen ratio of the fuel gas issuing from the jet and air flow rate on the yield of ions from given FI detector is the most frequently studied aspect of FID systems as far as formation of ions is concerned (34,70,72,89-95). In most of the cases, the total flow rate of the fuel gas issuing from the jet ranges between 0.5 - 2.5 ml/sec (30 - 150 ml/min). The fuel gas in FID systems consists of a mixture of an inert gas (He, N_2, A, CO_2) with hydrogen. The composition of the fuel gas, i.e., hydrogen to inert gas ratio, has a great influence on the overall behaviour of the flame and exhibits a strong effect on the sensitivity of the FID unit (95). For any jet geometry and dimensions there will always be an optimum hydrogen to inert gas ratio and optimum flow rate of the fuel gas for maximum sensitivity. This optimum must be empirically determined. Manufacturers of gas chromatographs sometimes provide information or the actual curves which show the optimum range of hydrogen to inert gas ratios required for highest sensitivity. Seibel (95) presented an empirical equation which enables us to calculate the hydrogen flow rate required for highest sensitivity when the helium flow rate through the column is known. Although this equation is not valid in cases where nitrogen is the carrier gas it can be used as a guide or a starting point in the determination of the real optima. Such empirical equations can be derived by plotting the hydrogen flow rate required to give maximum response at given inert gas flow rate versus the inert gas flow rate. For each jet diameter a linear relationship is observed.

Total fuel gas flow rate. For each jet diameter there is an optimum of total flow rate of the fuel gas for highest sensitivity. Siebel (95) showed that with a 0.016" I.D. jet highest sensitivity is obtained for total fuel gas of about 175 ml/min and that the sensitivity at this flow rate was twice as high as the sensitivity at 40 ml/min of fuel gas. For a 0.025" I.D. jet, on the other hand, it was found that almost equal sensitivity was obtained for fuel flows ranging from 173 to 340 ml/min. It is quite possible that the variation of the sensitivity with variation of fuel mixture flow rate is not caused by variation of the probability of ion formation alone but is a complex function of several parameters.

Nature of carrier gas. The effect of the nature of the carrier gas (the inert gas of the fuel mixture) on the sensitivity of FID units was investigated by Sternberg et al (70) and by Hoffmann and Evans (86) and interpreted in terms of the differences in the physical properties. Sternberg et al found that argon gives higher sensitivity than helium and nitrogen when the oxydant atmosphere is ordinary air. Premixing the fuel gas with known percentages of oxygen was found to produce increased sensitivity with helium but decreased sensitivity with argon and nitrogen. The response curve for helium with 4 ml/min premixed oxygen almost coinsides with the response curve of argon without premixed oxygen. Hoffmann and Evans (86) found that the sensitivity of the FI detector is inversely proportional to the thermal conductivity of the carrier gas. When maximum sensitivity is desired, a carrier gas with lowest possible thermal conductivity should be chosen.

3.3 Recombination of Ions

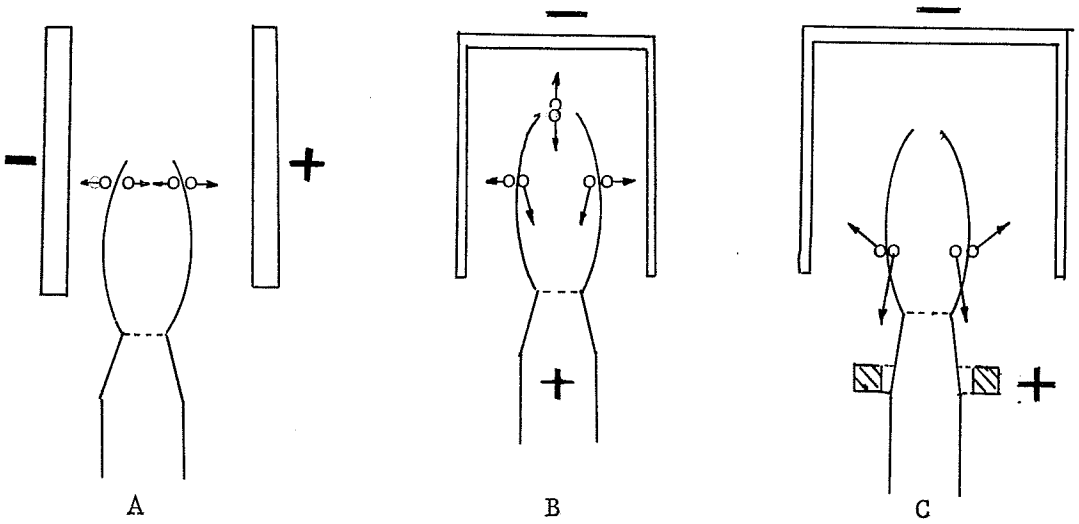
It is presumed that ions are formed in a very thin reaction region at the inner core of the flame (70,73,81). Above, or even within the reaction zone positive and negative ions tend to recombine (77,81). The rate of this recombination is directly proportional to the product of the positive ion concentration $[N^+]$ and the electron concentration $[N^-]$ (81),i.e.,

$$-\frac{dN}{dt} = \alpha[N^+][N^-]$$

in which α is the recombination coefficient. Calcote (77) used the decay of ion concentration behind the flame front (above the reaction region) for measurement of the ion recombination coefficient. He found that even with subatmospheric pressure flames the ion concentration above the reaction zone drops rapidly, indicating that recombination of ions in flames is fast and must be prevented. Dewar (73) carefully examined the problem of charge separation which is essential in order to eliminate recombination. He concluded that a narrow cylindrical electrode positioned above a hypodermic needle jet produces a sharp gradient of the electrical field right along the surface of the reaction zone cone of the flame and enables efficient separation of charges at the site of ion formation. Some workers (70,81,95) tend to assume that by applying sufficiently high polarizing voltages to the flame, recombination is entierly eliminated since a plateau is observed in the current-voltage characteristics of the FID. Such assumption cannot be made since residual recombination of ions, the extent of which depends on the several factors, may always take place. Alterations of the flame variables will undoubtedly cause variation of α which means that changes in the sensitivity cannot

be attributed to production of ions only. Ongkiehong (81) expressed the assumption that the longer it takes the ions and electrons to reach the electrodes the more are lost by recombination and concluded that the longer the distance between the electrodes the greater the loss of ions due to recombination. Ongkiehongs's conclusion is probably true only for some electrode configurations but certainly does not apply to the cases where the jet is used as an electrode. If, for instance, the jet serves as a positive electrode the electrons move towards the jet; the positive ions would continue their journey to the collector passing through electron free region of the flame. Therefore, the time it would take for the ions to reach the electrode should not alter their total number.

The effect of electrode position on ion recombination. The paths of a particular pair of a positive ion and an electron towards their respective electrodes depend upon the site of "birth" of this particular pair and the relative position of the electrodes. The three principle possibilities of electrode positions are shown schematically in the following illustration.



Configuration A represents the group of FID units which do not use the jet as an electrode (section 3.5.1) and which have two symetrically positioned electrodes (95). The probability for recombination with this configuration seems to be higher than in configurations B and C. We must remember, however, that although the concentration of ions is very low for minute quantities of organic compounds, and so is the probability for collisions, the electrical forces of attraction between opposite charges may lead to recombination even when their paths to the electrodes do not exactly cross. Furthermore, at higher concentrations of sample in the flame this effect may become more of a problem and cause a decrease of the linearity .

3.4 Collection of Ions

Loss of sensitivity due to low collection efficiency can be quite easily diagnosed and eliminated. However, few workers seem to realize that the ion collection efficiency of FID units changes continuously depending on the history of the column and the samples run through it. The ion collection efficiency of a FID unit is dependent upon several factors which are discussed in the following parts of this section.

Polarizing voltage. The magnitude of the polarizing voltage applied across the electrodes of the FID unit is the most important single factor which affects the efficiency of ion collection. The current-voltage characteristics of FID units which are frequently reported in the literature (70,73,81,95) show three distinct regions :

1. Low polarizing voltages region (10-70 volts), in which the response of the detector is proportional to the magnitude of the polarizing voltage.

2. Intermediate polarizing voltages region (60-300 volts) in which

the response is independent of the magnitude of the polarizing voltage, i.e., a plateau region.

3. Region of high polarizing voltages (above 300 volts); the response again depends on the magnitude of the polarizing voltage due to ion multiplication.

The plateau region of the current-voltage characteristic of FID units gives the useful range of operating voltages. The lowest voltage of the plateau region, however, depends very much on the quantity of substance used to determine the current-voltage curve (96). This is a result of the fact that the voltage applied less the voltage drop across the input resistor gives the effective polarizing voltage applied to the electrodes (96). This aspect of the operation of a FID affects the linearity of the detector only and will not be discussed further. The current-voltage curves of FID units produced under various conditions have been used by a number of workers as a tool for diagnosis, for understanding its properties and for optimizing its geometry (70,73,81,87,90,92,95-98).

Geometry of the electrodes. Electrodes of great variety of shapes have been used by various workers for construction of FID units (73). For instance, the use of rods (90), wires (88), rings (99), gauzes (81,96), plates (95), cylinders (73) and "bells" (70) have been described. The shape and dimentions of the electrodes are largely dependent on the mode of interconnecting the FID unit with the electrometer and on their potential relative to ground. Certain electrode shapes, such as rings or rods, cannot be used when both the enclosure of the unit and the collector electrode are at ground potential due to the danger of pronounced competitive collection. The effect of electrode geometry on efficiency of ion collection

was thoroughly discussed by Dewar (73). The most important aspect of Dewar's work is in determining the electrode geometry which provides the most effective charge separation by concentrating the voltage gradient of the electric field on the surface of the reaction cone of the flame. He showed (73) that a narrow cylindrical electrode is superior to a vertical rod or flat horizontal electrodes. The narrow cylindrical electrode is more promising from the sensitivity point of view since it not only assures complete collection of ions but permits better separation of charges which decreases the probability of recombination. It should be remembered, however, that the narrow cylindrical electrode would be most useful when a hypodermic needle jet is used. The most suitable polarity and mode of connection for narrow cylindrical electrode are ground potential and direct connection of the electrode to the input of the electrometer. Dewar (73) pointed out that this arrangement has faster response which is of particular interest in capillary column gas chromatography. Frequently the jet is used as one of the electrodes. The shape of the jet, however, is always designed to satisfy the requirements of the flame rather than the requirements for efficient collection of ions or electrons. The most suitable place for positive ion or electron collection by the jet is in its center which is empty. Because of the small internal diameter of jets used in FID units it is quite difficult to place a thin rod or a wire in the middle of the jet. Carroll (100) experimented with several electrode shapes including a cylindrical gauze and found it less suitable than flat screen with a hole in the center positioned above the jet. His experiments, however,are not representative of the cylindrical shape because the overall arrangement of the electrodes in his experiment was very poor. Janak (101) found that with two electrodes of equal size,

mounted in a position lateral to the flame, the response can drop abruptly with increasing mass flow rate. He called this phenomenon "the inversion effect". This effect is probably nothing else but failure of the electrodes to "extract" the ions and the electrons from a flame which has high linear velocity.

Contamination. The surface of the electrodes can become contaminated with inorganic or even organic residues from the process of combustion. Even when present in very small quantities these contaminants can cause a sharp drop of the sensitivity. For instance formation of a very thin film (few molecular layers) of silicon oxides on the surface of the electrodes will be sufficient to cause drastic change of the surface properties of the electrode metal. Frequent cleaning of the electrodes becomes essential particularly when working with trimethylsilyl derivatives or with columns which contain silanized solid supports.

3.5 Measurements of Ion Currents

The ion currents produced in FID units are in the 10^{-7} to 10^{-13} ampere range. The internal resistance of the FID cell is in the 10^{8} to 10^{14} ohm range. Measurement and recording of currents of this magnitude from sources of such high internal resistance cannot be made without amplification. Regular amplifiers or recording devices, however, have relatively low input impedances and cannot be used for measurement of such currents. Electrometer amplifiers, which have distinctive characteristics, have been introduced in the past especially for the purpose of measuring signals produced by high impedance sources. Their basic properties are high input impedances, low current offsets and d-c (low frequency a-c) amplification. While there are many kinds of electrometer amplifiers,

those that have vacuum tube inputs offer the best compromise of input impedance, low current offset, frequency response, immunity to over-voltages, durability and cost (102). Vacuum tube electrometers are, therefore, the most widely used type of amplifiers for measurement and amplification of FID ion currents.

Electrometer amplifiers have been developed for and are used extensively in different fields of scientific research such as pH, surface contact, and ionization chamber measurements, mass spectrometry and photometry. Most of the electrometers, developed to a high degree of sophistication for application in these areas, can be directly used in FID systems. High quality general purpose electrometers can also be directly used with FID units with great success (74,103). A number of special problems, however, are encountered in the application of electrometers to FID gas chromatography.

A flame ionization detection system consists of a FID unit, a source of polarizing voltage and an electrometer amplifier. These three components of the system can be interconnected in a surprisingly large number of ways. Selecting a proper mode of interconnection between the components of the system for a specific FID design is one of the most critical factors for the successful operation of a FID system at high sensitivities. The mode of interconnection depends largely on the design features of the FID unit and is very much related to the problem of ion collection (section 3.3). A series of practical problems arises when one attempts to measure very small currents. Many factors may interfere with the operation of such systems. It is therefore of special interest in high sensitivity applications of FID systems to recognize the potential sources of interference and eliminate them.

3.5.1. Modes of assembling the components of FID systems

The possible modes of interconnecting the FID electrodes, the source of polarizing voltage and the electrometer can be divided into two groups:

A. FID systems in which the flame jet functions as one of the electrodes,

B. FID systems in which the flame jet is not used as an electrode.

Each of these two groups can be further subdivided into two different arrangements;

a. both electrodes insulated from the body and from ground,

b. one electrode not insulated from the body.

The various modes of assembling the components of a FID system are as follows :

Group A

1. Both the jet and the collector are insulated from ground. The collector is kept at ground potential. The polarizing voltage is connected between the jet and ground.
   (a) jet positive
   (b) jet negative.

2. Only the collector is insulated from ground and kept positive. The polarizing voltage is connected between the amplifier and the collector.

3. Only the collector is insulated from ground and kept positive. The polarizing voltage connected between ground and amplifier thus elevating the amplifier to the polarizing voltage level.

4. Only the jet is insulated from ground and kept at negative potential. The polarizing voltage is connected between the jet and the amplifier.

Group B

1. The electrodes are identical in shape and size and are both insulated and symmetrically located relative to the jet. The polarizing voltage can be connected to each one of the electrodes without causing any change of its performance.

2. Both electrodes are insulated from ground but not of equal size and not symmetrically located relative to the jet. The small electrode in form of a ring is located in the vicinity of the jet and connected to the polarizing voltage. The polarizing voltage connected between the small electrode and ground.
   (a) The electrode located near the jet is kept at positive potential,
   (b) The electrode located near the jet is kept at negative potential.

3. Exactly as in B-2 but the small electrode is kept at ground potential and the collector is kept positive. The polarizing voltage is connected between the collector and the amplifier (applicable only when the jet is made of non-conducting material).

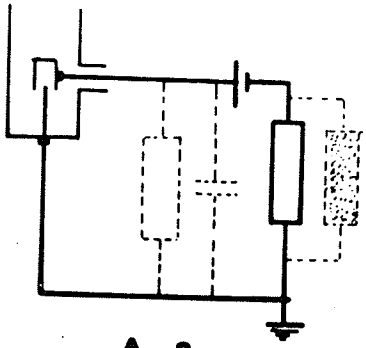
The most important modes for assembling FID systems listed above are schematically shown in Figure 8. Connecting the electrometer directly to an insulated jet and placing the polarizing voltage between ground and the

Figure 8

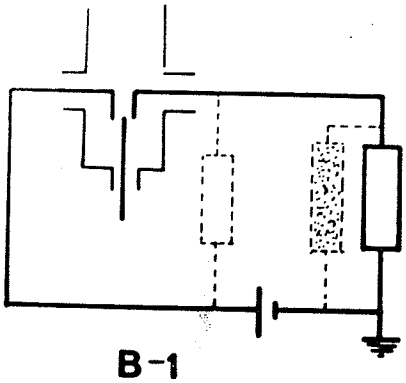
Examples of Various Modes for Assembling the Components of FID Systems.

Heavy lines - The components of the FID systems.

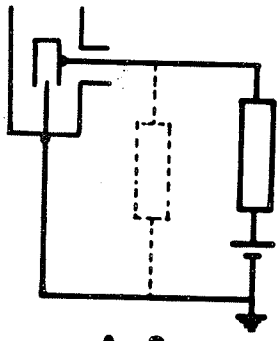
Dotted lines- "Leakage resistances" and extra capasitance. "White resistances" are those that increase the standing current and "stippled resistances" are leakage resistances which are parallel to the input resistor.



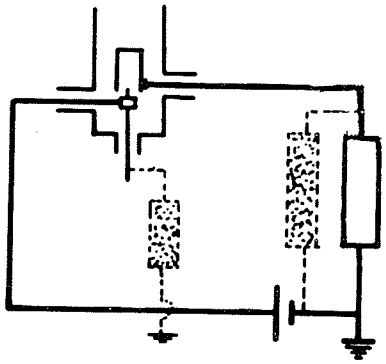
A-2



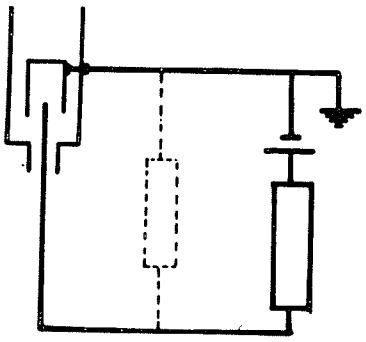
B-1



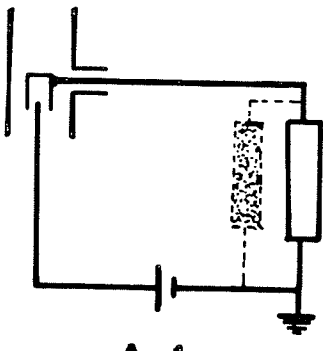
A-3



B-2



A-5



A-1

Figure 8

electrometer (Fig. 8, A-5), so that the electrometer is at elevated potential (positive), is an additional mode of combining the components of the FID system which can be classified under group A and which has not been mentioned in the literature.

The most widely used modes for assembling FID systems are A-1, A-2, B-1, B-2 (Fig. 8). Each one of these and the rest have some characteristic advantages. The possible sources of trouble, i.e., leakage of currents and undesirable capacitance are shown in Figure 8 with dotted lines. Leakage of current between the points indicated in Figure 8 occurs due to inadequate insulation or failure to maintain the high insulation under the operating conditions. This is apparently one of the most common causes for loss of sensitivity in FID systems. With arrangement A-2 which is one of the most widely used in gas chromatography, the extra capacitance in the high resistance loop (created because of the presence of the polarizing voltage in this loop) usually causes loss of response speed and is susceptible to mechanical vibrations which in turn cause loss of sensitivity.

3.5.2. Insulation

Securing very high resistance insulation in the input circuit of a FID system is one of the first requirements for high sensitivity measurements. A number of high resistance insulating materials are available, however, the real difficulty is to find high resistance materials which can maintain their properties under the extremely unfavorable conditions existing in the FID units during operation. In measuring very small ion currents by measuring the voltage drop across a very high resistace, the insulation resistance of the FID electrodes, leads and

cables must be several orders of magnitude greater than the resistance of the input resistor of the electrometer. Input resistors of 10^{10} to 10^{12} ohm are used for high sensitivity measurements. It is therefore necessary to use insulating materials which have resistances of 10^{14} ohms at least. Finding materials which can maintain such resistace in a water saturated atmosphere and at temperatures up to 300°C is a formidable task (34).

Insulation materials. Teflon is the most satisfactory insulation material for the resistance levels required in high sensitivity work with vacuum tube electrometers. It has high volume resistivity and a surface on which water vapor films do not readily form. Teflon is quite inert chemically and can be readily cleaned. It is also easily machined. Teflon's principal shortcoming is that it cannot be used above 220°C and that, when deformed, internal charges appear causing spurious voltages and currents (104). Virgin teflon must therefore be used. Finger oils and other contamination can be expected to decrease its resistance and cause loss of sensitivity. Special care must be taken to avoid contamination of teflon parts during their construction in the machine shop. Polystyrene, Kel-F and polyethylene are good general purpose insulators which can safely be used in the interconnecting cables between the FID unit and the electometer but not as insulating material for the electrodes of the FID, since at elevated temperatures polyethylene and polystyrene may give off small quantities of organic material to which the FID responds. Various glasses and ceramics are frequently used as insulators in construction of FID units. Kaiser (34), however, reported that spark plug ceramic becomes unusable at 140°C and that very few porcelain electrode holders retain the insulation quality required for FID at 160°C. Another disadvantage of ceramic or glass is that they are difficult to machine and have very poor surface

properties at high humidity (104). Sapphire is considered as one of the best insulators available (104). It is especially suitable for FID units since it is thermally stable up to temperatures far beyond the operating temperatures encountered in gas chromatography. Unfortunately, the use of sapphire is limited because of its high cost and the fact that it is difficult to machine.

Selecting the most suitable insulating material for construction of electrode holders is only part of the problem of securing highly insulated electrodes. Under the operating conditions of a FID unit the position of the insulator relative to the jet is of great importance. For an electrode holder positioned right above the flame jet (66,81,87,89,90) it would be much more difficult to maintain the high resistance than for electrode holder positioned underneath the level of the jet flame (105,106).

Interconnecting cables. The ion currents produced in the FID unit must be fed to the electrometer input without allowing any loss to occur in the interconnecting cables. Special care must, therefore, be taken in the selection of suitable cables. Short screened cables which offer best possible insulation must be used (34,104). The cables must also have high mechanical stability to prevent variations of the input capacitance which occurs as a result of mechanical vibrations (34). Teflon coaxial cable is satisfactory and desirable. However, when it is possible to bring the electrometer close to the FID unit and connect its input by a rigid screened conductor it should be preferred over the use of cables. The cables must be kept as clean as possible (107) to prevent leakage of current through the contamination layers.

3.5.3. Humidity

Formation of thin films of water on the surface of various components of an FID system can seriously reduce their effective resistance. The FID unit and the electrometer are especially susceptible to high humidities. Within the FID unit water is continuously generated whereas only environmental humidity affects the electrometer.

Humidity within the FID unit. Combustion water is continuously generated in the FID unit and unless the whole unit is kept at temperatures above 100°C, water vapors will condense on all of its parts. Certain FID units are built so that in order to have all the remote parts above 100°C the unit itself must be kept at very high temperatures. For instance, the Barber Coleman Selecta 5000 gas chromatograph has the electrode and the ignitor arms projected out of the detector oven and condensation of water at the edge of one of the electrodes was observed even when the detector oven was kept at 150°C. Furthermore, even when no condensation occurs monomolecular layers of water may form in the water saturated atmosphere of the unit and cause loss of sensitivity. Two FID units (74,106), which were independently developed, solved this problem by sweeping the electrode arm with air thus preventing diffusion of water vapors into the cooler areas of the unit, i.e., the electrode holder.

Environmental humidity. Although mentioned in the literature (108), environmental humidity is not always recognized as a factor which can affect the sensitivity of the electrometer. It is probably due to the fact that high quality electrometers are usually protected from contact with the external atmosphere by housing their high resistance components (the input resistors and the electrometer tube) in hermetically sealed

enclosures. Also, some electrometers are built with input resistors which are coated with thin films of special water repelling silicones. A third point is the fact that in many geographic locations in the world the relative humidity is usually well below the levels that can affect the electrometer. Nevertheless electrometers used in high sensitivity applications must be continuously protected from high humidity in order to secure consistent results.

3.5.4. Special features of the electrometers used in FID systems

Kaiser (34) presented an exellent and comprehensive description of the performance specifications that would qualify an electrometer amplifier for use in a FID system. The purpose of this paragraph is to discuss the special problems encountered when using electrometers in FID systems,i.e.,the special features required which are usually not available with general purpose electrometers or electrometers produced for other applications.

The need to suppress rather large background currents is the most important special factor in the application of electrometers as part of FID systems. Many commercially available general purpose electrometers lack provisions for suppression of background currents and cannot be used in FID systems. Others are provided with capabilities of suppressing only small background currents. These electrometers are limited for use in FID systems only in conjunction with very stable liquid phases and only at relatively low temperatures.

Dual column temperature programming is a very useful technique for analysis of mixtures which contain components of wide range of boiling points (39). This technique was extended several years ago to dual-column, dual- flame temperature programming (39). The continuously

changing background current occurring during the programming of the temperature is balanced by the current of the reference flame (67). Several methods for combining the two FID units have been used. Some of the dual flame systems are based on especially designed dual input single output electrometers. The provision of dual input (when necessary) for the compensation of the column bleed in temperature programming is the second special requirement in the application of electrometers to FID systems.

Background current suppression. The background currents can be compensated (suppressed) either at the input or at the output of the amplifier. When relatively volatile stationary phases are used background currents as high as 10^{-9} amperes must be suppressed and currents produced due to the presence of the sample in the flame as small as 10^{-12} amperes (or less) be measured accurately. The latitude of the zero compensation of most general purpose electrometers is very small because it is based on compensation at the output of the electrometer. Such electrometers cannot therefore be used without additional equipment (picoampere source) or modification. Most of the electrometers designed for gas chromatography (87,91,109-111) use the input suppression method. For high sensitivity work this is the only acceptable method. If no input suppression is used the high background currents will saturate the linear portion of the input tube and,even if suppressed at the output, the amplifier will not be operable with high input resistances. The use of such resistances is essential for the achievement of high sensitivities. The application of a general purpose picoampere source cannot solve this problem entirely because the resistors used in the picoampere source must have resistances of at least two orders of magnitude higher than the input resistors of the electrometer

in order to avoid ion currents passing through them. In order to produce the relatively high suppressing currents with resistors 100 times higher than the input resistor of the electrometer, as required, one must apply high voltages. For instance, in order to suppress a 10^{-9} ampere current when 10^{10} ohm resistor is used at the input of the electrometer one must use at least a 10^{12} ohm resistor in the picoampere source and apply 1000 volts. Commercially available picoampere sources (Keithley Instruments Model 261) do not provide such high voltages. The conclusion is that electrometers of special design are required for FID systems particularly when high background currents must be suppressed.

Special requirements for temperature programming. When the electrodes of two separate FID units or a dual jet FID unit are interconnected as suggested by McWilliam and Dewar (67), ordinary electrometers can be used for dual column operation without any modification. However, in arrangements which use a different approach, also described by McWilliam and Dewar (67), the ion currents generated in the two FID units are separately fed through two matched input resistances and the voltage drop created in them applied to the grids of two matched electrometer tubes. These are the dual input single output electrometers. Although the most difficult problem in dual-column dual-flame temperature programming is to achieve perfectly matched columns the matching of the performances of the two electrometer inputs is also a problem. The application of a special electrometer tube which actually contains two electrometer tubes in one enclosure and which uses only one filament was suggested by Gesser (112). A successful experiment with single column temperature programming at moderate or high sensitivities is described in section 4.2, Part II.

3.6. Sources of Noise in FID Systems

Detector sensitivity, expressed in terms of the minimum detectable quantity, is defined as the quantity of substance that produces a signal-to-noise ratio of 2. The sensitivity of a FID system is obviously dependent upon the noise. In a most general way noise can be defined as the fluctuation of the baseline. Some of the factors that can cause fluctuations of the baseline are; fluctuations of the column temperature, fluctuations of the carrier gas flow rate, fluctuations of the hydrogen flow rate, mechanical vibrations of the electrodes, mechanical vibrations of the interconnecting cables, purity of the gases, turbulence of the flame caused by unstabilized flow of the combustion air, temperature of the flame jet tip, construction materials of the FID unit components, gas leaks and contamination.

In order to achieve high sensitivities with FID systems, all the factors that were mentioned in the previous four sections of this chapter, and the above mentioned sources of noise must be given thorough consideration.

3.7. Experimental

3.7.1. Description of the FID systems used.

Altogether four different FID units were used with five different electrometers and four gas chromatographs. In addition, a slightly modified FID unit of the Barber Coleman Selecta 5000 gas chromatograph was tested with a Cary model 31 vibrating reed electrometer.

System 1. Micro-Tek (M-T) original FID system. Consists of a Micro-Tek model 2500 research gas chromatograph equipped with a multi-range electrometer and a FID unit. The FID unit is provided with two insulated

electrodes. The jet is not used as an electrode (mode of assembling B-2, section 3.4.1). The collector electrode is of a narrow cylindrical design as shown in Figure 9. The collector is always at ground potential. The second electrode can be maintained either positive or negative but was always operated at +300 volts.

System 2. M-T original FID unit combined with a Keithley 410 electrometer. Consists of the original FID unit of the M-T model 2500 gas chromatograph directly connected to the Keithley 410 electrometer. The collector electrode was directly connected to the input of the electrometer via a 1.5 ft coaxial cable and suitable Amphenol connectors. The second electrode was directly connected to the special +216 volt polarizing voltage outlet of the Keithley electrometer. This system was also used with the second electrode directly connected to the original metal jet (which was also insulated).

System 3. M-T modified FID unit combined with a Keithley 410 electrometer. Consists of a modified version of the original M-T FID unit (Fig. 9), directly connected to the electrometer as described in the foregoing. In Figure 9 the second electrode of the FID unit is shown connected to the metal plated tip of the glass sleeve which is not connected to the jet. This corresponds to mode of assembling B-2 (section 3.4.1). The modified FID unit was also further slightly altered by connecting the second electrode to a hypodermic needle (gauge 18) which was used as a jet and which was insulated from ground by mounting it on a small teflon cone. This altered arrangement corresponds to mode of assembling A-1a.

System 4. Barber Coleman (B-C) original FID system. Consists of

Figure 9

Modified FID Unit

Features two highly insulated electrodes and arrangement for protecting the electrode holders from water vapors and contamination.

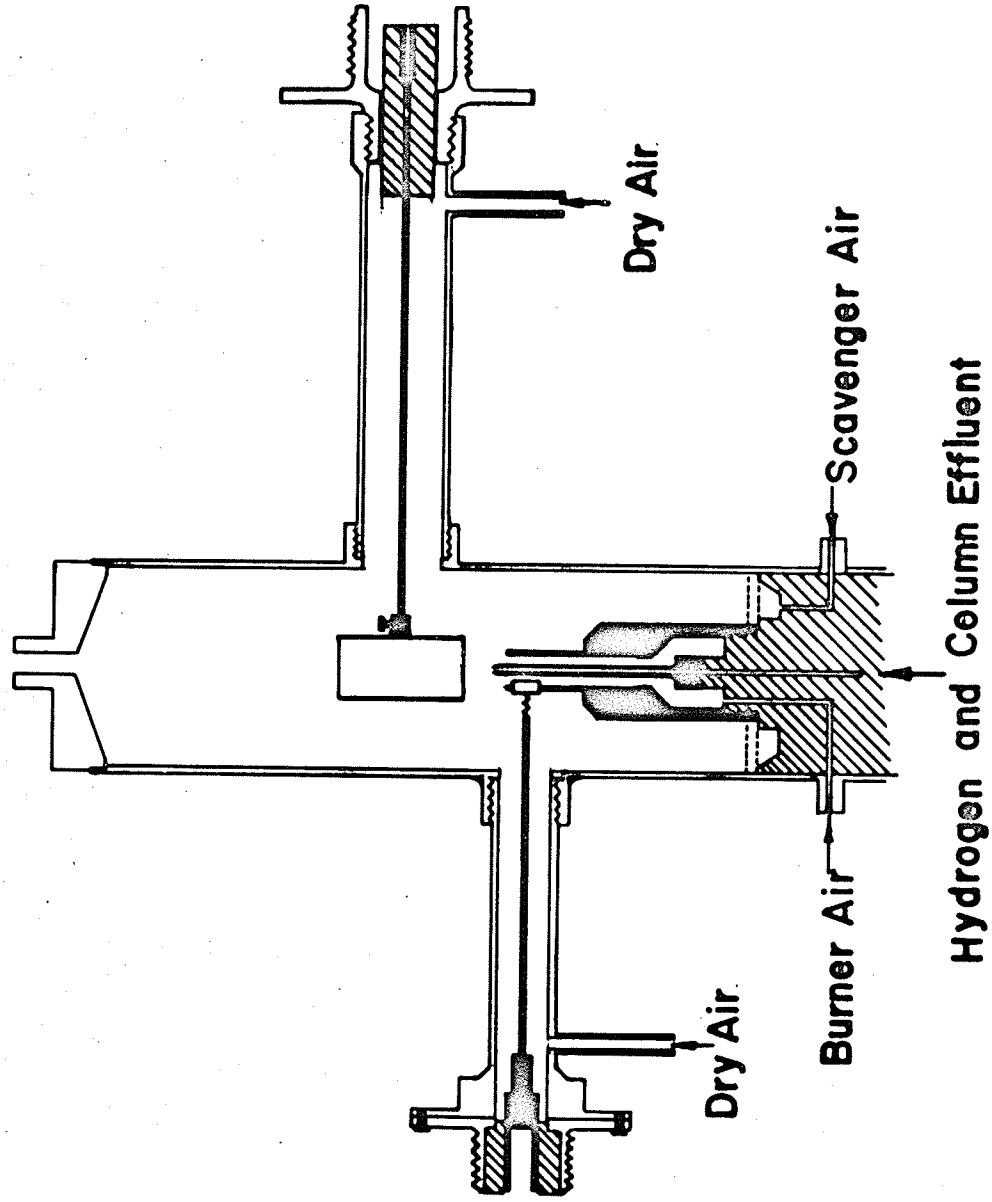

Dry Air
Scavenger Air
Hydrogen and Column Effluent
Burner Air
Dry Air

Figure 9

the components of the Barber Coleman Selecta 5000 gas chromatograph which include a dual input single output electrometer, two 240 volt U-160 Burgess batteries for the polarizing voltage and a stainless steel FID unit which has only one insulated electrode. The system is assembled according to mode A-2. The dry cell batteries which provide the polarizing voltage are mounted within the electrometer and connected to the collector electrodes of the individual FID units via 3.5 ft heavy coaxial cables (screened).

System 5. B-C original FID unit combined with a Keithley 610B electrometer. Since the original B-C FID unit provides only one insulated electrode, only mode of assembling A-2 can be used. Special boxes to house the polarizing battery were constructed in order to facilitate the connection of the Keithley model 610B electrometer to the FID unit. A Burgess U-160, 240 volt dry cell battery was used to provide the polarizing voltage and connected with the positive lead to the collector electrode of the FID unit and with the negative lead to the electrometer via 1 ft coaxial cables on each side of the box.

System 6. Aerograph models 660 and 705 gas chromatographs' original FID systems. Consist of a gold plated FID unit (which has two insulated electrodes, a quartz jet and a wide cylindrical gauze collector) and an electrometer which has a built-in polarizing voltage power supply.

System 7. Aerograph models 660 and 705 original FID unit combined with a Keithley 610B electrometer. The FID unit was directly connected to the Keithley electrometer by connecting the collector electrode to the input of the electrometer using the original cable of the instrument since the inputs of both the original electrometers of these gas chromatographs

and the Keithley electrometer use the same type of Amphenol connector. The polarizing voltage power supply of the gas chromatograph was used and therefore the original Aerograph electrometer was turned on during operation. A proper ground connection between the two electrometers must be secured in order to close the circuit between the FID unit, the Keithley electrometer and the polarizing voltage power supply. This system corresponds to mode of assembling B-2.

System 8. M-T original or modified FID unit combined with a Keithley 610B electrometer. Combining a Keithley 610B electrometer to the M-T original or the modified FID units was done exactly as with the Keithley 410 electrometer except for the need to add a source of polarizing voltage, because the Keithley 610B electrometer does not have a built-in polarizing voltage. A special box made of aluminium similar to the one previously mentioned was used to house a 240 volt U-160 Burgess battery which provided the polarizing voltage. The positive terminal of the battery was connected to the hypodermic needle jet (which was used as one electrode) via a coaxial screened cable. The negative terminal of the battery was connected to the aluminium box. The circuit was closed via the screen of the cable.

3.7.2. Voltage dividers

The Keithley electrometers (models 410 and 610B) have outputs of 5 volt and 3 volt respectively for full scale deflection and cannot be directly connected to 1 mV potentiometric recorders. For this reason two simple voltage dividers were made as follows : A 5000 ohm 2 watt resistor connected in series to a 100 ohm 5 watt variable resistor was used to short the output of the electrometer. The outer and the middle terminals of the 100 ohm resistor were connected to the 1 mV recorder.

Varying the position of the middle terminal of the 100 ohm resistor feeds to the recorder various percentages of the electrometer output with a maximum of $\frac{100}{5100} \times 100 = 2\%$. About one percent of the output was usually used and was measured from the reading of the panel meter of the electrometer and the corresponding deflection of the recorder. A second voltage divider similar to the first one which uses two variable resistors and which provides two outlets for high and low sensitivity recording was made using a 10,000 ohm 2 watt resistor, a 100 ohm 5 watt variable resistor and a 20 ohm 5 watt variable resistor.

3.7.3. The modified M-T FID unit.

A modified version of the original M-T FID unit consisting of a brass enclosure on which two electrode arms were mounted (Figure 9) was constructed. The electrode arms were made of 0.5" brass tubing on which teflon insulated electrode holders were mounted and inlets for dry air sweeping of the electrode arms were soldered as close as possible to the electrode holders. A five inch long 1/16" in diameter stainless steel rod which was silver soldered to an Amphenol connector at one end was used to support the collector electrode. A similar arrangement was used for the second electrode. The remaining parts of the FID unit were those of the original M-T FID unit except that for part of the experiments the original jet was replaced by a gauge 18 stainless hypodermic needle the tip of which was cut to a symmetric circular opening and which was shortened to total length of 1 inch.

3.7.4. Measurement of the resistances of the FID electrodes.

The FID unit was removed from the gas chromatograph and placed on a wooden table. The electrode was connected to the input of the electrometer

(Keithely 610B) by a coaxial cable the screening of which was not allowed to make contact with the body of the FID unit. The positive terminal of a 240 volt battery was connected to the body of the FID unit and the negative terminal to the ground of the electrometer. The current passing through the insulation was then measured and the resistance of the insulation calculated using Ohm's law ($R = \frac{V}{I}$).

3.7.5. Stability of a FID system as a function of the polarizing voltage.

The Micro-Tek original FID unit was connected to a Keithley 410 electrometer and to a Keithley model 240 regulated high voltage power supply as shown in Figure 10a. The high voltage power supply was set on "positive" and polarizing voltages increasing in 10 volt increments were applied to the electrode positioned near the jet. The output of the electrometer was monitored on a Honeywell recorder and 5 μl of line natural gas were injected after each change of the polarizing voltage. The signal of the detector resulting from the injection of line gas did not increase above polarizing voltages of 40 volts indicating that the plateau of the current-voltage characteristics of this unit begins between 40-50 volts. The baseline stability at each polarizing voltage up to 180 volts was recorded for 5 to 10 minutes. The measurements were made by operating the electrometer at the 3×10^{-10} ampere range with 1% of the output fed to the recorder. The stability was qualitatively evaluated from the relative magnitude of the baseline fluctuations and schematically plotted versus the polarizing voltage as shown in Figure 10b.

3.7.6. The effect of environmental moisture.

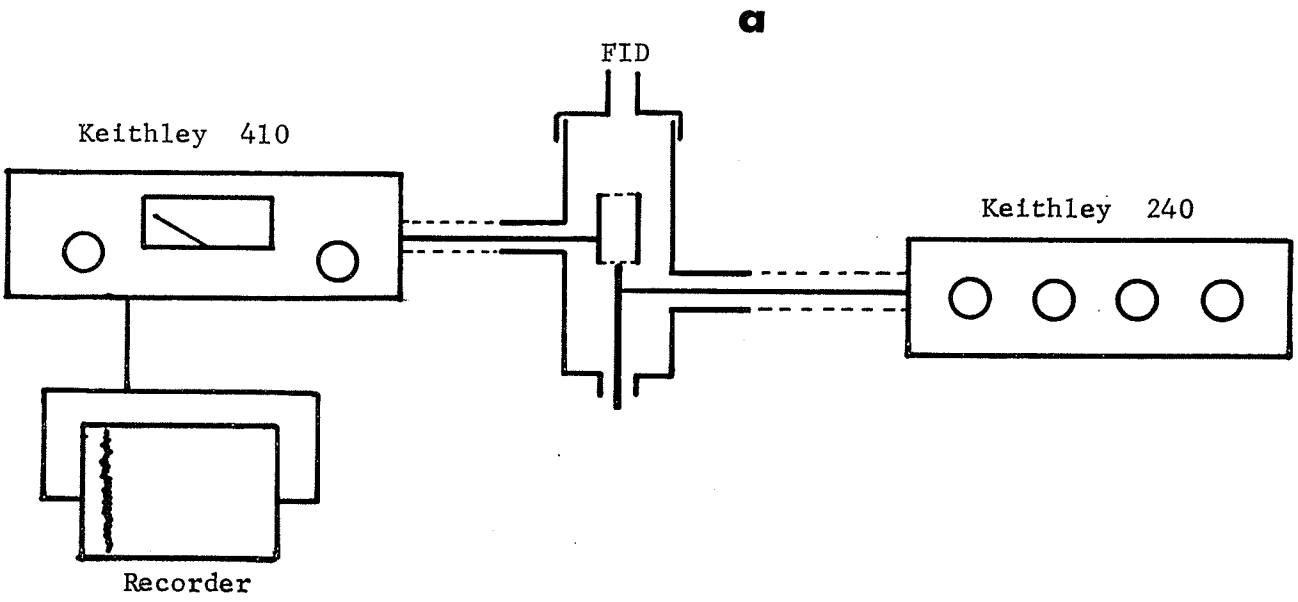
Two experiments were carried out to find the effect of environmental moisture on the sensitivity of electrometers used in FID systems.

Figure 10

The Effect of the Magnitude of the Polarizing Voltage on the Stability of a FID System.

a. Layout of instruments used in the experiment (schematic).

b. The baseline stability as a function of the magnitude of the polarizing voltage.


a
FID
Keithley 410
Keithley 240
Recorder

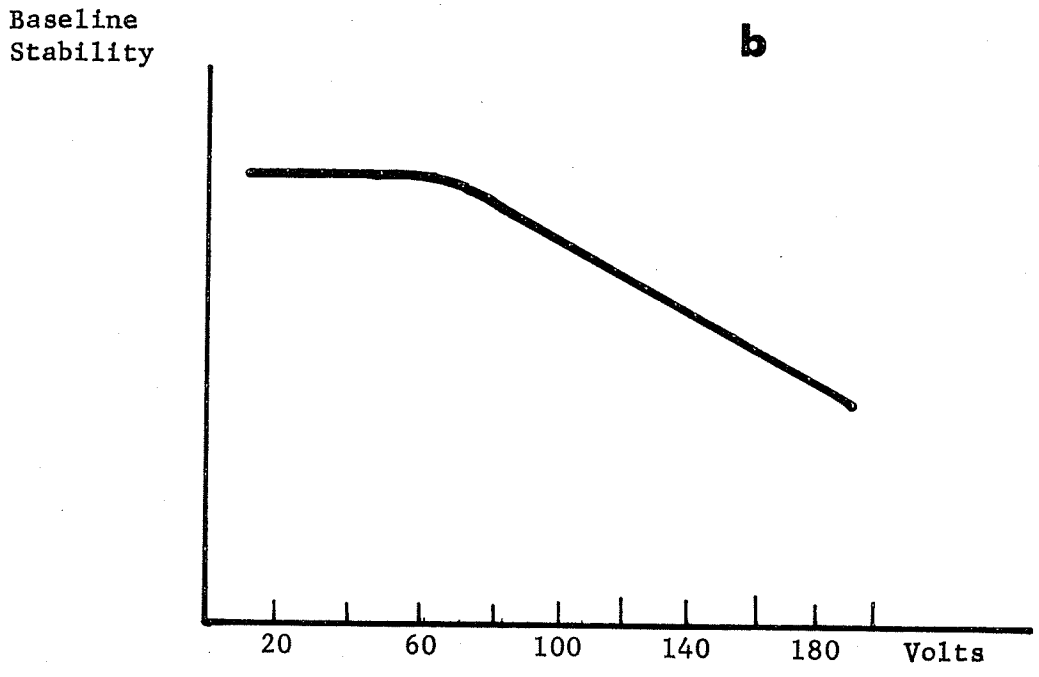

Baseline
Stability
b
20
60
100
140
180
Volts

Figure 10

The first experiment was carried out at the Israel Institute of Technology using a home-made electrometer almost identical to the one described by Andreatch and Feinland (113). The second experiment was carried out at the University of Manitoba using the M-T model 2500 original electrometer.

The home-made electrometer which was connected to a flame ionization gas chromatograph (114) was wrapped with a polyethylene bag of suitable size. About 150 grams of activated silica gel were placed inside the bag which was then carefully sealed to avoid any further contact with environmental moisture. Samples of air (50 μl) which contained several parts per million of methane were injected into the gas chromatograph in half hour intervals and the response recorded on a 55 mV Fisher Recordall potentiometric recorder. After three days the polyethylene bag was removed and 50 μl of the methane containing air were again injected in intervals. A plot which shows the methane peak height versus time is shown in Figure 11.

The electrometer tube and the input resistors of the M-T model 2500 original electrometer are enclosed by the manufacturers in a special box which presumably protects them from contact with environmental moisture. It was obvious, however, that this box was not built properly and could not protect the sensitive parts from contact with moisture. The following experiment was therefore performed : A piece of copper tubing (5" x 1/8" O.D.) was soldered to a hole made in the top plate of the box and connected to a 3-4 feet coiled 1/8" O.D. copper tubing which was placed in a Dewar flask. The Dewar flask was filled with liquid nitrogen and nitrogen gas at a rate of about 20 ml/min was passed through the box for several hours. Samples of Rum of equal size were injected before and after the "dry nitrogen" treatment.

Figure 11

The Effect of Environmental Humidity

On the Sensitivity of a Home-Made

Electrometer.

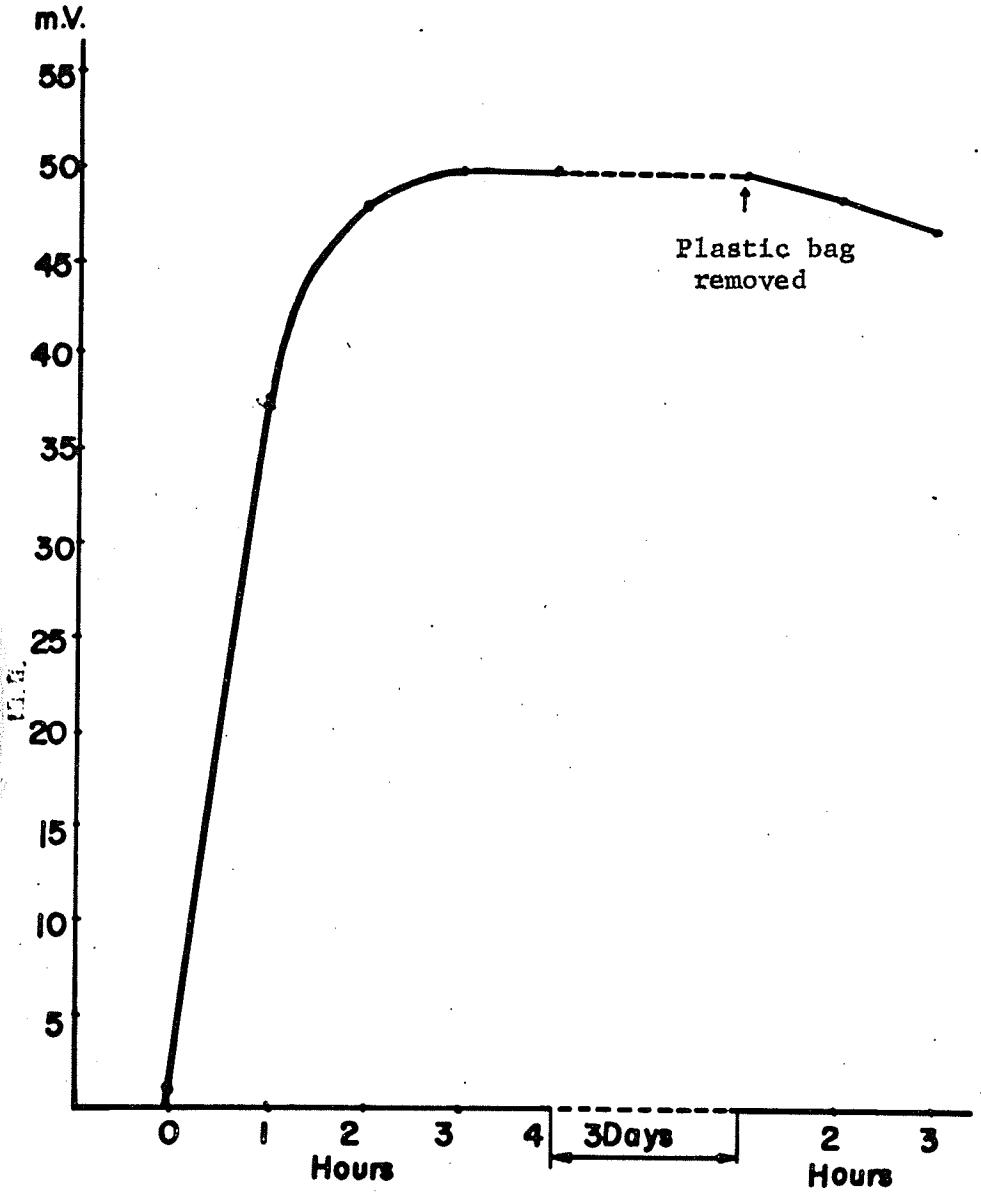

m.V.
55
50
45
40
35
30
25
20
15
10
5
Plastic bag
removed
0
1
2
3
4
3Days
2
3
Hours
Hours

Figure 11

3.7.7. Optimum operating flow conditions of various FID systems

The various FID systems mentioned earlier were operated under many different flow conditions. However, for each jet there was always a particular set of carrier gas (nitrogen or helium) to hydrogen flow rate which gave best overall performance in terms of stability and sensitivity and which was found on the basis of the accumulated experience with each of the systems tested.

The optimum flow rates for the various systems were found to be :

FID unit	carrier gas ml/min.	hydrogen ml/min.	air ml/min.
M-T original jet	45-60	25-40	(15-20 psi)
M-T modified, hypodermic needle jet	40-65	25-40	200
B-C Barber Coleman original	60-80	35-50	250
Aerograph original, quartz jet	35-50	18-25	20(oxygen)

3.7.8. Calculation of the absolute and the relative sensitivity.

The absolute sensitivity of four FID systems in terms of the minimum detectable quantity of substance was semiquantitatively measured and calculated as follows: The peak area of ethyl acetate appearing in a sample of Rum (Rum B) run with the system in question at the maximum operating sensitivity was measured. Knowing the volume of the sample injected and the fact that ethyl acetate is present in Rum in quantities ranging from 300 to 700 ppm (115), the total quantity of ethyl acetate injected was calculated assuming that the sample contained the 700 ppm of ethyl acetate. Using this value, the area of the peak and the area of the smallest detectable peak (a triangle of 4 mm base and

4 mm height was taken as the smallest detectable peak of elution time similar to ethyl acetate.) the minimum detectable quantity of ethyl acetate was calculated.

The relative sensitivity of various FID systems was estimated by comparison of the peak heights or peak areas of the corresponding peaks of the samples run separately with each of the systems being compared (usually under similar operating conditions) and by taking into account the sample size. For instance, 0.5 and 0.05 μl of Rum A and B were run with systems 1 and 3 and their relative sensitivity was estimated from the areas of one of the corresponding peaks. Similarly 0.2 and 0.05 μl of grape fused oil were run with systems 3 and 4.

3.8. Results and Discussion

3.8.1. Performance of the FID systems tested

From the very beginning of this work it became obvious that the commercially available gas chromatographs do not offer sufficiently high sensitivities for direct analysis of flavor constituents in alcoholic beverages. One of the main objectives of our work, therefore, was to develop a high sensitivity FID system. This objective was materialized by assembling and testing eight different FID systems under various operating conditions, by making modifications in the design of FID units, changing the mode of assembling and eliminating the undesirable effects of various factors which cause loss of sensitivity.

The evaluation of the relative sensitivity of the systems tested and their performance is based on the results from over 2500 chromatograms obtained throughout this work with the systems described. These chromatograms provided also the basis for qualitative rating of the FID

systems in terms of their stability, susceptibility to fluctuations of the operating conditions, reproducibility and flexibility. The sensitivity of four FID systems was also estimated in terms of the minimum detectable quantity of ethyl acetate and found as follows :

system 1	2.2×10^{-8} gram
System 2	5.6×10^{-11} gram
System 3	5.6×10^{-12} gram
System 4	4.0×10^{-9} to 4.0×10^{-10} gram

The sensitivity of system 3 (5.6×10^{-12} gram) is equal to the highest sensitivity of flame ionization detectors reported in the literature so far (34,81,87,96). No such FID sensitivities have been reported in the analysis of alcoholic beverages and by-products of alcoholic fermentation.

Selected illustrations of the great improvement of the sensitivity of the systems developed in our laboratory over the commercially available ones and relative to each other are shown in Figures 12 and 13. Comparing the peak areas of ethyl acetate in the chromatograms of Rum B recorded with systems 1 and 3 (Figure 12) which were obtained with the highest possible sensitivity and by taking into account the quantities of material injected in each case, an increase of sensitivity of about 3900 times for system 3 as compared to system 1 is observed. The chromatograms of 0.05 and 0.2 μl of grape fusel oil obtained at maximum operating sensitivity with systems 3 and 4 correspondingly are shown in Figure 13. Comparing the indicated portions of these chromatograms reveals that system 3 exhibits about 100 times higher sensitivity as compared to the sensitivity of system 4. The special features of system 3 are the high quality of the electrometer (high signal-to-noise ratio and high overall gain), a very high insulation of the electrodes, a desirable mode of assembling

Figure 12

Comparison of the Performance of FID Systems 1 and 3 at their Highest Operable Sensitivity.

A. Chromatogram of 0.5 μl of Rum B* obtained with column 5 using FID system 1. Col. Temp. programmed from 80°C to 100°C, a rate of 2 deg/min.

B. Chromatogram of 0.05 μl of Rum B* obtained with column 6 using FID system 3(Col. Temp. 65°C, isothermal).

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* One of two samples of Rum received from Seagram's Research Laboratory, LaSalle, Quebec, for comparative analysis.

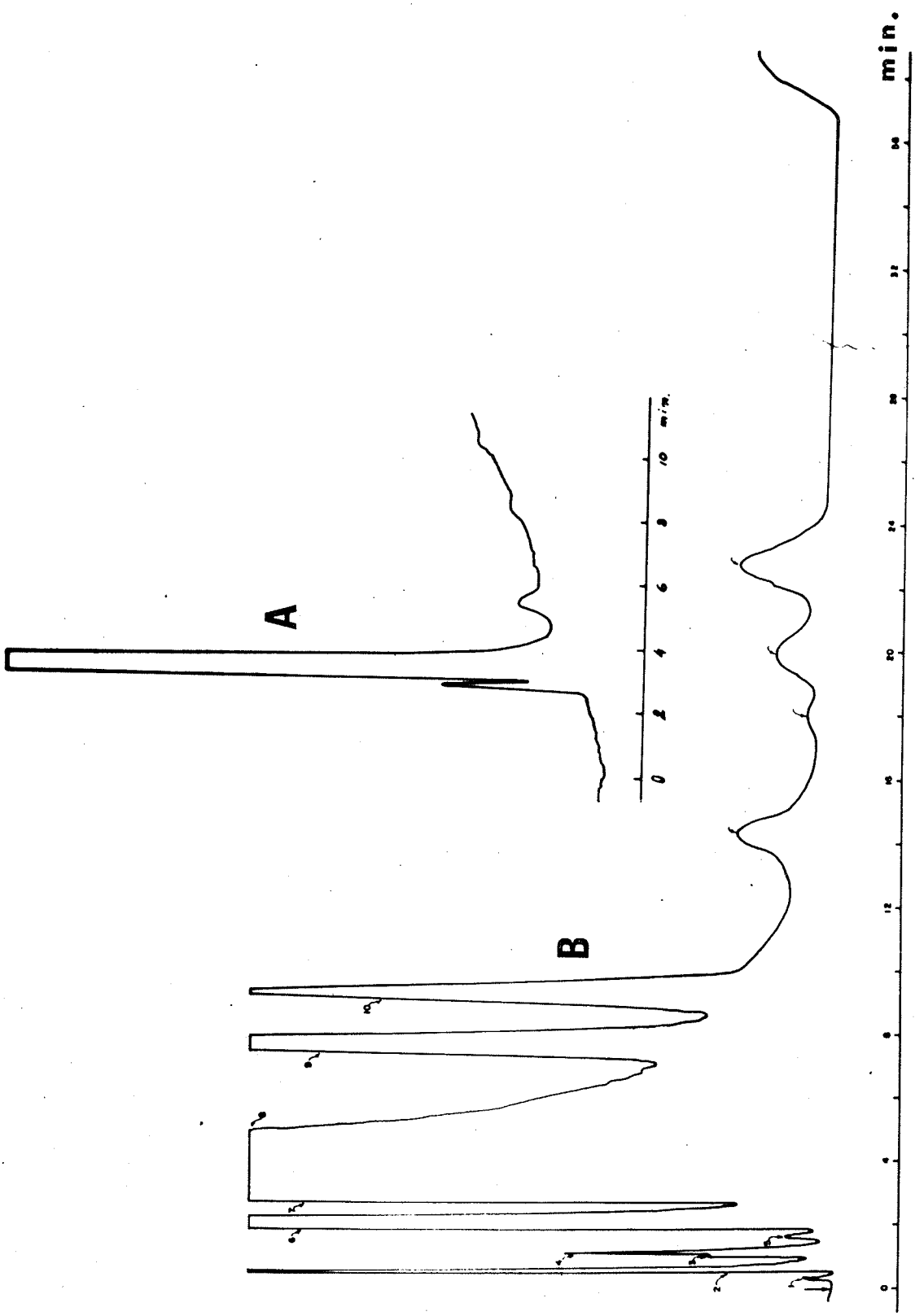

Figure 12

Figure 13

Comparison of the Performance of FID Systems 3 and 4 at their Highest Operable Sensitivity.

A. Chromatogram of 0.2 μl of grape fusel oil distillation residue I* obtained with column 6 using FID system 4. Col. temp. 89°C, flow rate 20 ml/min (nitrogen).

B. Chromatogram of 0.05 μl of grape fusel oil* obtained with column 6 using FID system 3. Col. Temp. 65°C, flow rate 12 ml/min (nitrogen).

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* Section 2.1, Part III.

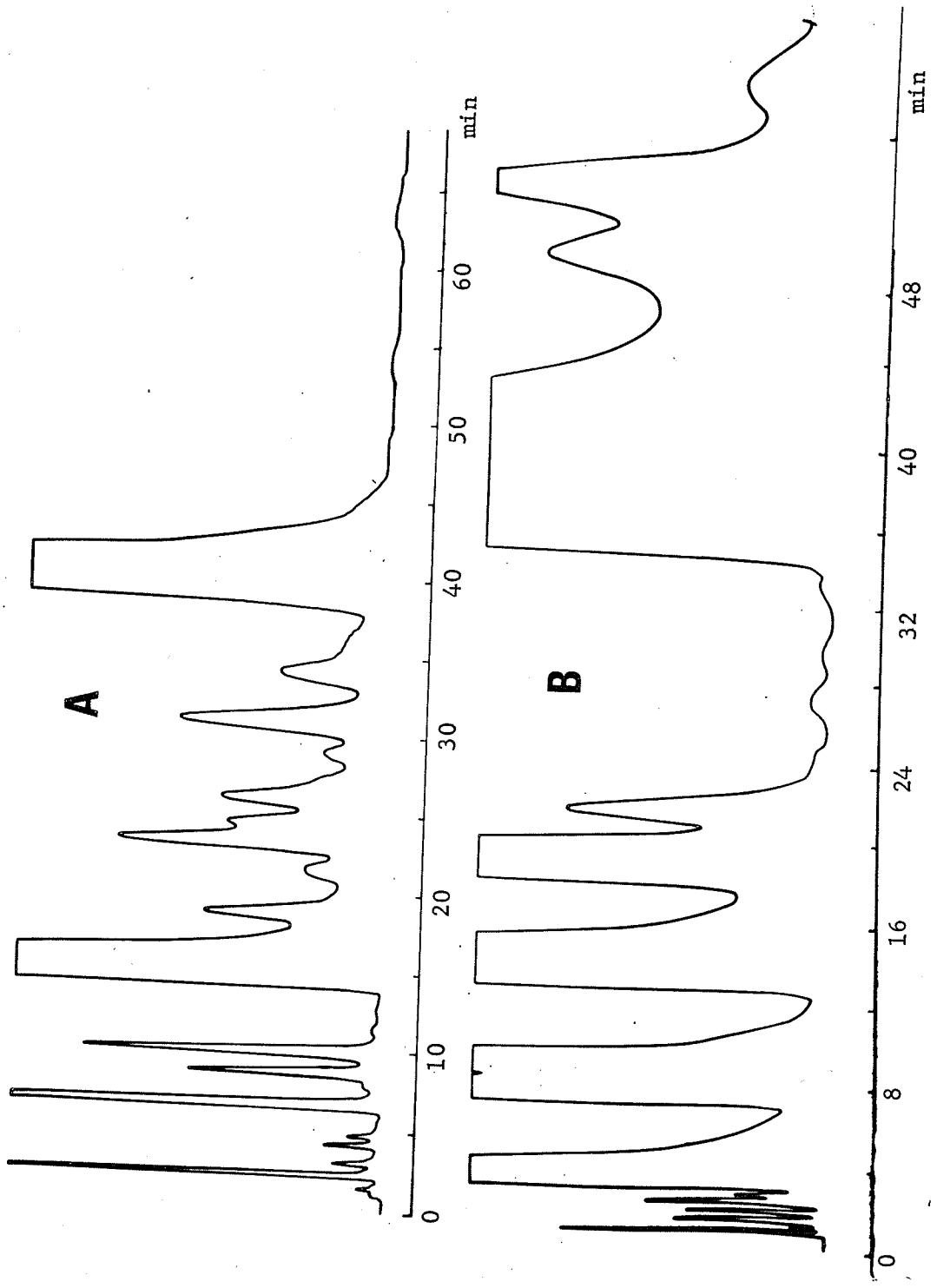


Figure 13

(A-1) and electrode geometry which permits an efficient charge separation at the site of their formation (73). Since the same electrometer is used both in system 2 and 3 the net sensitivity gain of system 3 over system 2 can be attributed to the higher electrode insulation of the modified FID unit and the mode of assembling used. The insulation of the electrodes of system 2 and 3 was measured as described in section 3.7.4. It was found to be as follows: The insulation of the collector electrode of the M-T original FID unit at room temperature was between 10^9 and 10^{11} ohms depending on the actual position of the electrode holder (rotation of the electrode holding rod on its axis gave different readings). The insulation of the second electrode was 10^8 ohms. The resistance of both electrodes of the modified FID unit was higher than the measuring capability of our system, i.e., higher than 10^{14} ohm. The sensitivity of system 3 in terms of minimum detectable quantity was found to be 5.6×10^{-12} gram, i.e., of the order of 10^{-12} g./sec. This value is now generally accepted as the highest sensitivity obtainable with FID systems (34,81,87,96). When operated at its highest sensitivity (3×10^{-11} ampere range) system 3 was not sufficiently stable for routine applications. At such high sensitivities the system becomes sensitive to practically all the possible sources of interference and noise. Although these can be carefully eliminated certain desirable functions such as attenuation during the chromatographic run and temperature programming could not be performed when system 3 was operated at its highest sensitivity. Attenuation at the midst of a chromatographic run could not be performed because only input attenuation was available and any switching of the input resistance caused loss of balance for several minutes. Temperature programming at such high sensitivities becomes impossible

or limited to only a narrow range of temperatures (approx. 20^{o}). Compared to system 3, system 2 offered sensitivity of only 10 times lower which is adequate for many purposes and can be rated second best of the systems tested. In terms of sensitivity systems 4,5,7 and 8 together can be rated at third place after system 3 and 2 with some of them (systems 5 and 7) close to the sensitivity of system 2. The qualitative properties and the overall performance of some of the systems (susceptibility to mechanical vibrations, long term stability, sensitivity, etc.) can be attributed to specific characteristics of these systems. For instance, the susceptibility of system 3 to mechanical vibrations can be explained on the basis of the vibration of the electrode supporting rod of the FID unit in case of system 3 (Fig. 14) and on the basis of the fluctuations of the input capacitance in the input cable in the case of system 4.

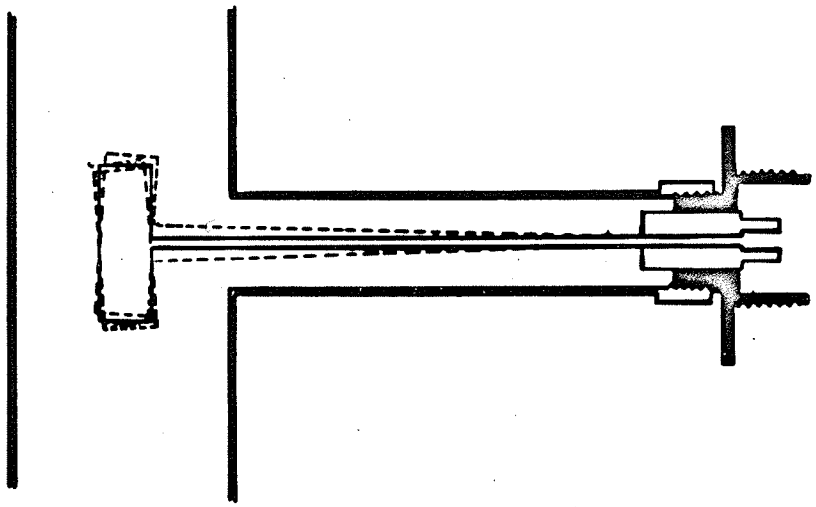
3.8.2. The effect of selected factors on the sensitivity of FID systems.

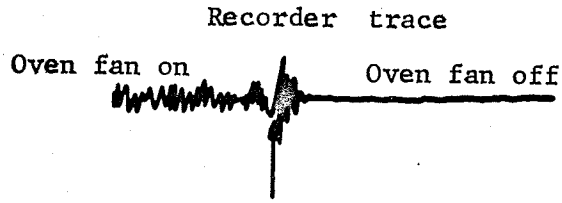
Electrode insulation. The importance of electrode insulation for the achievement of high sensitivities has been emphasized in the literature (34,72,89,104,106). Maintaining the high insulation during the operation of a FID unit is a difficult problem which was solved independently by Karmen (106) in 1965 and by us in 1964. Our design is shown in Figure 9. Practically all FID units described in the literature have their collector electrodes and their holders positioned above the level of the jet. For several reasons it is more convenient to do so. The insulation of an electrode holder mounted on cooler parts of the FID unit or on parts which are projected out of the oven can in principle be maintained since it is not subjected to high temperatures. On the other hand, it is being continuously contaminated by non volatile combustion products (106) and submitted

Figure 14

Effect of Mechanical Vibrations of the Collector Electrode on the Noise of a FID System 3.

The recorder trace obtained during the operation of the oven fan and after the oven fan was stopped illustrates the effect of the mechanical vibrations on the noise of the system.





Oven Fan Stopped

Figure 14

to the high humidity produced by the water of combustion. Karmen (106) solved this problem by placing the collector electrode holder well below the level of the flame and supplying the combustion air through the metal tube which encloses the electrode holder. We solved the problem by removing the electrode holder from the vicinity of flame (Fig. 9) and preventing diffusion of water vapors or other combustion products to the electrode holder by making provisions for dry air to sweep the electrode arms continuously. The same overall effect is achieved both in our and Karmen's designs. Our design, however, has the advantage of achieving the same effect without the need of making changes in the construction of the gas chromatograph and the disadvantage that air in addition to the combustion air must be supplied.

Modes of assembling the components of the FID system. Six of the most common and interesting modes of combining the components of a FID system are schematically shown in Figure 8. The possible locations of current leakage or extra capacitance are shown with dotted lines. Systems 1,2,6 and 7 were assembled according to mode B-2, systems 3 and 8 according to mode A-1 and systems 4 and 5 according to mode A-2. Krugers (72) was the first to report the use of mode A-3. Mode A-5 is suggested here as a new promising mode of assembling.

When the insulation of an electrode holder or other components of the FID system is insufficient (less than 10^{13} ohm) the circuit of the FID system behaves as if an additional resistance or resistances have been connected either in parallel to the input resistor of the electrometer or across the electrodes of the FID unit (shorting). These can be called "leakage resistances". In Figure 8, leakage resistances which

short the electrodes of the FID unit are shown with dashed lines (left white). "Parallel" resistances are shown with dashed lines which are stippled. Leakage resistances which short the electrodes of the FID unit actually close the circuit and cause an increased flow of current through the input resistor. When the leakage current is relatively small this causes serious interference but not loss of sensitivity. When such leakage currents are large they cause complete saturation of the linear range of the electrometer amplifier and drastic loss of sensitivity. The second type of current leakage is caused by "parallel" resistances. These cause direct loss of sensitivity by "shorting" the path of the current through smaller resistances, i.e. less ion current flows through the input resistor of the electrometer.

Of the circuit tested,mode A-1 proved to be the best and provided the highest sensitivity. Generally this mode of assembling promises good ion collection (only electrons move against the flow of gases issuing the jet), minimum of recombination due to separation of charges at the site of ion formation (73) and minimum competitive collection. Mode A-2 is widely used in gas chromatography although it has some distinct disadvantages : It is susceptible to mechanical vibrations, it has slower response due to the increased capacitance in the high resistance loop, the "heavy" positive ions must travel against the flow of gases issuing from the jet and no effective separation of charges can take place (73). Furthermore, when the jet becomes overheated it can readily emit electrons which will cause a high background current and noise. The advantages of such mode are simple construction and no competitive collection. Systems 4 and 5 were assembled according to mode A-2 and exhibited satisfactory performance at moderate sensitivities. Since most of the troubles with these systems arise due to the presence of

the polarizing battery in the high resistance loop, the performance can be considerably improved by eliminating the use of interconnecting cables (by assembling the FID unit, the polarizing voltage, and the electrometer as close as possible). Systems 1,2,6 and 7 are based on mode B-2 although they differ in geometry of the electrodes and in that a metal jet is used in systems 1 and 2 and a quartz jet in systems 6 and 7. From the results obtained with these systems it can be concluded that this mode of assembling performs well and can be used for moderate and high sensitivity work. The disadvantages of this mode are; it does not offer efficient separation of charges, in some configurations (systems 6 and 7) one of the electrodes is placed in the vicinity of the flame which can be a serious source of noise and when an insulated metal jet is not used as an electrode it can act as a competitive collector (if the resistance of its insulation is not sufficiently high). Also, it can accumulate charge and cause distortion of the electric field. When a metallic jet is used, however, the system can be converted to mode A-1 without any modifications, which is an advantage. Mode B-1 shown in Figure 8 has not been tested in this work. It has been found by Novak and Janak (101) to produce the "inversion effect" at higher flow rates. Seibel (95), however, described an all ceramic unit especially designed for this mode of assembling which exhibits surprisingly attractive characteristics. Mode A-3 was also not tested in our experiments since it requires a special type of electrometer. Krugers (72) found this mode to perform better than the other modes tested by him. This can be expected because the problem of input capacitance can be entirely eliminated by the special guarding arrangements that can be made with such electrometers (103). Mode A-4 has not been reported yet in the literature and was not tested in our work, again because it requires

a special electrometer as mentioned earlier. There are several good reasons which make it possible to predict that such a system would be very suitable for high sensitivity work. Usually rapid response is achieved when the collecting electrode attracts electrons rather than the sluggish positive ions. When the jet is positive,higher collection efficiencies can be expected under a wide range of carrier gas flows since the mobile electrons can easily reach the jet. The whole body of the detector can be used as the second electrode thus entirely eliminating competitive collection. The only serious disadvantage of this mode would probably be the difficulty to obtain highly insulated jets.

The effect of environmental moisture. When the concentration of water vapors in the atmosphere of the laboratory reaches certain level, monomolecular or multimolecular layers of water can be formed on the surface of the input resistors of the electrometer and on the surface of the electrometer tube. The presence of such water layers on the surface of the most sensitive components of the electrometer reduces its overall input resistance considerably and causes loss of sensitivity very much in the same way as caused by leakage of current through "parallel resistances" (Fig. 8). A semi-quantitative experiment which demonstrates the effect of environmental moisture on the sensitivity of a home-made electrometer was carried out as described in section 3.7.6. The response of the electrometer increased about 50 times after being "dried" by silica gel (Fig.11). The effect of environmental moisture on the sensitivity of unprotected electrometers is particularly strong in high humidity areas of the world. However, even in dry areas electrometers must be protected from moisture if high sensitivity is to be secured and dependence of the sensitivity upon their distance from the tea kettle is to be avoided. Smith (108) solved this

problem by mounting his electrometer within a desiccator. The electrometer of the Beckman GC-4 gas chromatograph is maintained at 50°C in order to prevent formation of water layers. Some commercial electrometers of higher quality (Keithley 410) have their input resistors and electrometer tube enclosed in hermetically sealed boxes. A slight improvement of the sensitivity of the Micro-Tek original electrometer was achieved by flushing its input resistors area with dry nitrogen as described in section 3.7.6. When samples of Rum of equal size were injected before and after the dry nitrogen treatment, an increase in the peak heights of the pre-ethanol peaks of about 3 times was observed for samples that were injected after the drying treatment.

Effect of the polarizing voltage on stability. A rather strange dependence of the signal-to-noise ratio of a FID system upon the magnitude of the polarizing voltage was detected and qualitatively measured as schematically shown in Figure 10a and as described in section 3.7.5. When this system was operated at polarizing voltages above 70 volts the stability, i.e., signal-to-noise ratio decreased proportionally to the magnitude of the polarizing voltage as shown schematically in Figure 10b. No explanation can be given of this phenomena since no further experimentation was carried out. It is possible, however, that the noise produced by mechanical vibrations of the collector electrode is proportional to the strength of the electric field.

Chapter 4

Experimental Difficulties Encountered in High Sensitivity Gas Chromatography and Methods for their Elimination

4.1. Introduction

The factors which have little or tolerable influence on the performance of a gas chromatograph when operating at moderate or low sensitivities become critical and limiting when the instrument is operated at high sensitivities. Unless the effect of these factors is eliminated, reduced or carefully controlled, the useful range of sensitivities of the instrument or its range of applications is reduced. Frequently the development of improved methods of control or operation is required to eliminate the interference of these factors. For instance, small fluctuations of the column temperature do not affect the baseline stability at low sensitivities; however, at high sensitivities they may cause baseline fluctuations of considerable magnitude which interferes with detection of trace constituents and limits the usefulness of the high sensitivity. The interference caused by this factor can be eliminated by providing accurate temperature control. Similarly, the fluctuations occurring in other parameters of the system must be eliminated.

Apart from the need for precise control of the variables, serious difficulties in performing functions such as temperature programming, detection of trace constituents appearing on the shoulder of large peaks, eliminating minute contamination, etc., are encountered. The methods that were developed for performing these functions at high

sensitivities are described in the following sections of this chapter.

4.2. A New Method for Temperature Programming at High Sensitivities .

4.2.1. Introduction

Programming the column temperature so that it continuously rises (linearly or non linearly) throughout the chromatographic run is a widely used technique in gas chromatography which is particularly suited in analysis of mixtures containing constituents of wide range of boiling points (39). Such mixtures are frequently encountered in flavor research (39) which makes PTGC a very valuable technique for the investigator of flavors. High sensitivity is, however, a prerequisite in flavor research. Temperature programming can therefore be of full value in flavor analysis only if it can be performed at high sensitivities. Recording a meaningful high sensitivity chromatogram of a temperature programmed run using an ordinary gas chromatograph is practically impossible. The continuously increasing vapor pressure of the liquid phase rapidly brings the response of the detection system off scale. The dual column technique (39) was introduced with the intention to solve this problem. However, it appears to be applicable only for low or moderate sensitivities. For dual column temperature programming at high sensitivities the two columns and all the associated components of the "dual" system must be perfectly matched in their behavior. Preparing perfectly matched columns was found in our laboratory to be unobtainable even when special packing procedure was used.

At high sensitivities any small difference in the behavior of the corresponding components of the dual system, including the columns,

exhibits a strong effect on the position of the baseline because it is amplified 100 to 10,000 times more than when programming at low or moderate sensitivities. Gas solid chromatography or use of the recently developed porous polymer beads, permit temperature programming at high sensitivities. Unfortunately, these approaches are presently applicable only for separation of low boiling substances (117). We wished to find a general solution to this problem and,as a result, a very simple idea was tested and found successful for single column temperature programming at sufficiently high sensitivities.

4.2.2. Principle.

Programmed temperature gas chromatography is most difficult when liquid phases of limited temperature stability must be used (39). Such liquid phases usually consist of polymers of low average molecular weight (400-600). The method developed makes use of the fact that the rate of migration of the vapors of such liquid phases in another column of a highly stable liquid phase is extremely slow at any of the temperatures at which the more volatile liquid phase is useful. This fact is used in the following way : When an ordinary analytical column containing a liquid phase which can be used only up to 160°C is connected to a second short column (3-8 inches) containing a liquid phase which can be used up to 280-300°C, the rate of migration of the vapors of the first liquid phase into the second column is very slow at 160°C as compared to the rate of migration of the constituents which are being analysed. In other words the method makes use of the difference in the rates of migration of the vapors of the liquid phase and the compounds analysed in a "bleed absorbing column" containing a highly stable liquid phase.

The vapors of the more volatile liquid phase do not reach the detector during the one programming cycle. Only the vapors of the stable phase reach the detector. However, the change of the vapor pressure of the stable phase during the programming cycle is negligable at any of the useful temperatures of the more volatile phase.

4.2.3. Experimental

Column 1 was mounted on an Aerograph 660 single column gas chromatograph. The column effluent (30 ml/min.) was split in approximately 2:1 ratio and fed to a FID unit and to the inlet of a Hitachi-Perkin Elmer RMU-6D mass spectrometer as described in section 5.2.2. and schematically shown in Figure 18. The temperature of the column was then manually programmed up to 160°C starting at 50°C, at an average rate of 4 deg/min without injecting any sample. The output of the FID and the total ion monitor (TIM) systems were simultaneously recorded at the highest operable sensitivity (attenuation 1 x 10 on the FID system and 0.1 x 10^{-9} ampere on the TIM system). An eight inch column 1/8" O.D. packed with 15% wt/wt of Carbowax 20M-TPA on Anachrom ABS 60-80 mesh (preconditiond at 245°C for 2 hours) which was connected between the outlet of the column and the stream splitter was used as a bleed absorbing column. The temperature of the column 1 and the bleed absorbing column was then again programmed (from 50° to 196°C) under identical conditions. The oven was kept at 196°C for 4 minutes and then cooled to 100°C. Starting from 100°C the programming of the oven temperature was then repeated at an average rate of 15 deg/min up to 192°. In order to check the effect of the bleed absorbing column on the retention times of compounds eluted from the analytical column a mixture of C_{10},C_{11}, and C_{12}

normal paraffins was run before and after the connection of the bleed absorbing column under identical flow and temperature conditions.

4.2.4. Results and discussion

The superimposed column bleed profiles obtained with the FID system without and with the column bleed absorbing attachment are shown in Figure 15. The liquid phase used in column 1 (Tergitol NP-35) was earlier found to be applicable with FID systems only up to 160°C. The column bleed profile obtained after the attachment of the bleed absorbing column shows that the maximum operating temperature of this liquid phase is extended to 190°C. Figure 15 shows that the absorbing attachment effectively eliminates the exponential column bleed profile in the 50-185°C range and enables single column temperature programming at relatively high sensitivities. The bleed profile of the repeated temperature programming carried out after the attachment of the bleed absorbing column is shown in Figure 15 with a dashed line. The fact that this profile almost matches the first one indicates that the rate of migration of the vapors of Tergitol NP-35 in the bleed absorbing column is negligible up to 190°C. The bleed absorbing column was found to cause only a slight increase, (10%), of the retention times of the C_{10}, C_{11} and C_{12} normal paraffins at 125°C as compared to their retention times obtained on the analytical column alone. The additional retention caused by the absorbing column is probably dependent upon several factors and could be reduced by adjusting the length, the percentage of the liquid phase and the temperature of the absorbing column. These variables probably could be optimized for minimum retention at given absorbing capacity. The temperature of the absorbing column does not have to be the same as the temperature

Figure 15

Superimposed Column Bleed Profiles Obtained by Single Column Temperature programming at Relatively High Sensitivities.

A. Column bleed profile of column 1.

B. First column bleed profile of column 1 with a freshly activated bleed absorbing column attached to it.

C. Second column bleed profile of column 1 with the bleed absorbing column without reactivation of the later.

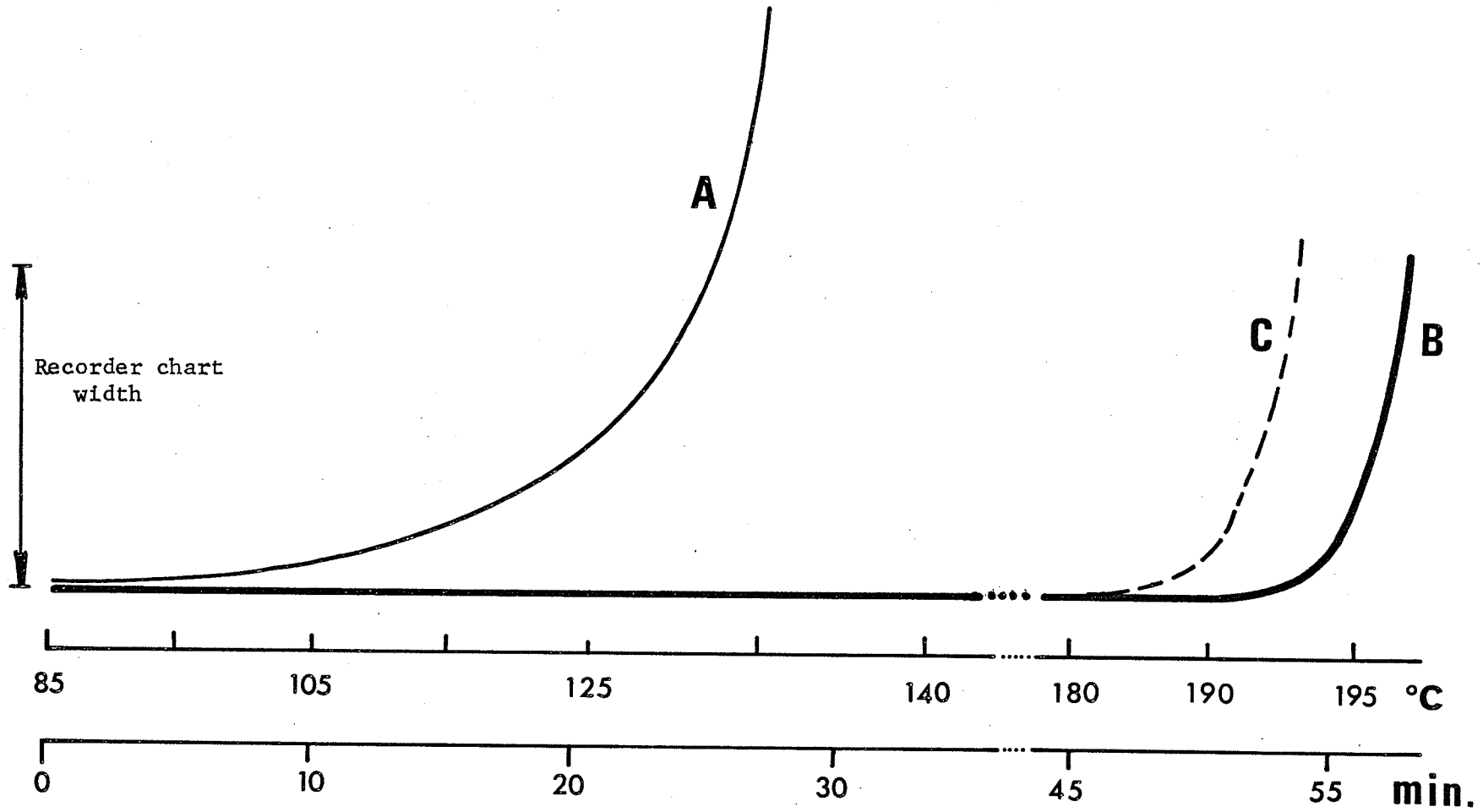

A
B
C
Recorder chart
width
85
105
125
140
180
190
195
°C
0
10
20
30
45
55
min.

Figure 15

of the analytical column. Furthermore, since the absorbing column must frequently be "reactivated" it would be of great advantage to assemble the absorbing column into a suitable compartment which can be independently heated even without any special control of the temperature. Such a compartment can easily be installed within the column oven because it requires only a limited space. From the results obtained with the eight inch absorbing column it can be concluded that much shorter column (1-3 inch cartridges) can be used. In order to avoid loss of resolution in the bleed absorbing column the liquid phase used in it must be carefully selected to match the properties of the liquid phase used in the analytical column as far as the order of elution is involved.

4.3. Wide Range Zero Shifting for the Detection of Trace Constituents.

4.3.1. Introduction

Trace flavor constituents in alcoholic beverages frequently exhibit retention behavior similar to the retention time of ethanol, the matrix component of these beverages. Even when the retention time of some of these constituents is 30-50% longer than that of ethanol they appear only on the shoulder of the ethanol peak which under high sensitivity conditions extends over many times the full scale range of the recorder. Under such conditions most of the shoulder of the ethanol peak remains unrevealed in its "off scale" portion. Trace constituents appearing on this shoulder remain therefore undetected. If the sensitivity of the detection system or sample size is decreased so that a greater portion of the ethanol peak would appear on the chart, the detection of the trace peaks would again not be possible because the size of these peaks

would also decrease proportionally and frequently become smaller than the minimum needed for detection. This problem was solved using a method which we called "wide range zero shifting".

4.3.2. Experimental.

Apparatus. The modified FID unit shown in Figure 9 combined with a Keithley 410 electrometer as described in section 3.7.1. (system 3) were used at 3×10^{-10} ampere range with about 1% of the output fed to a 1 mV Honeywell recorder.

Procedure. Slightly after the ethanol peak reaches "off scale" the baseline position is shifted from +0.1 mV (which is the original position of the baseline) to about -10 mV. This brings the range which without the shift is ten times "off scale" into the range of the 1 mV recorder. One millivolt portion of the ethanol peak shoulder is then recorded. The baseline position is immediately shifted to -9 mV and the portion of the peak which, without the shift, would be nine times "off scale" is then recorded. This procedure is repeated until the baseline reaches its original position.

4.3.3. Results and discussion.

Samples of Gin, Coriander seed distillates and Juniper berries distillate were analysed gas chromatographically using the zero shifting method. The results obtained are shown in Figure 16. Peaks 11, 12 and 13 remain undetected when the sample is run without zero shifting. A large peak (No. 10) remains undetected even with the zero shifting because its retention time is too close to that of ethanol. When the sensitivity of the system is decreased 10 times so that greater portion of the

Figure 16

Detection of Trace Peaks

by Wide Range Zero Shifting.

All the chromatograms were obtained under identical conditions :

FID system 3 at 3 x 10^{-10} amps. range

Column 5

Col. temp. 69°C

Carrier gas He

Sample size 1.9 μl

The individual chromatograms were obtained with the following samples :

Chromatogram	1	Coriander seed distillate	I
"	2	" " "	II
"	3	" " "	III
"	4	Gin B_1	
"	5	" A_1	
"	6	" A_2	
"	7	Alcohol for Gin	
"	8	Juniper Berries distillate.	

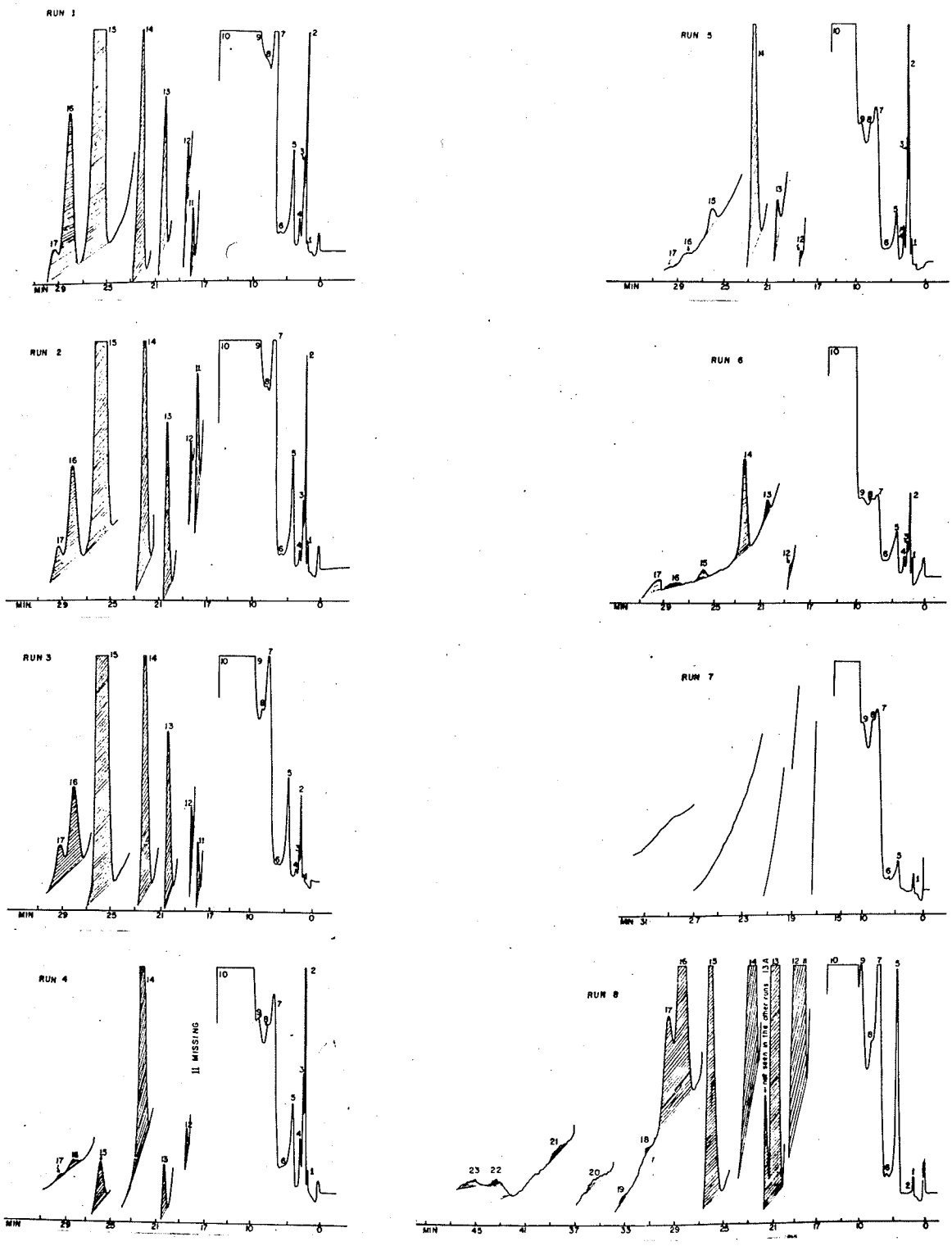


Figure 16

ethanol peak shoulder can be seen on the chart, peaks 11,12 and 13 in most of the samples remained undetected because of insufficient sensitivity.

4.4. Monitoring Contamination and Decontamination of Microliter Syringes.

4.4.1. Introduction.

Quantities of material of the order of 10^{-11} gram can be detected in high sensitivity gas chromatography (9). It is easy to visualize that cross contamination of the syringe used for injection of samples to the gas chromatograph would become a critical problem at such sensitivities. After a microliter syringe has been used with a given sample it is usually repeatedly flushed with solvent or with the new sample before it is reused, assuming that a number of such "rinsing" actions (between 5-10) decontaminates the syringe. However, it was found (118) that the "memory" of the syringes to previous samples is sufficiently large and that repeated ordinary rinsing cannot remove the contaminants.

Ott et al (118) described a continuous extraction method for decontamination of 10 μl or larger syringes. They found that it is necessary to keep the syringes for 15 hours in the extraction apparatus in order to remove all the detectable contamination. Decontamination of 1 μl Hamilton syringes is considerably more difficult than decontamination of 10 μl syringes and cannot be done with the extraction method due to their different design. A simple and a very efficient method for monitoring contamination and decontaminating microliter syringes was developed using a flame ionization detection system and a modified mode

of interconnecting the inlet and the outlet of a dual column gas chromatograph.

4.4.2. Experimental.

The "dual" injection port of a Barber Colman Selecta 5000 gas chromatograph was connected to one flame ionization detector as shown in Figure 17. One injection port leads the carrier gas to the FID unit via the column used for separation and the second injection port leads the carrier gas at flow rate of 35 ml/min to the same FID unit via empty tubing. The syringe to be decontaminated is inserted in the second injector and, after about one second, vapors from the contaminated syringe reach the detector and generate a signal which is proportional to the quantity of the contaminants. The plunger of the syringe is then moved in and out so that vapor entrapped within the needle is pushed out. This is done until no detectable signal is generated.

4.4.3. Results and discussion

One microliter syringes are very useful in high sensitivity gas chromatography although they are less accurate than the 10 μl syringes. However, their design is such that their complete decontamination by repeated rinsing is practically impossible. In most applications of gas chromatography contaminations of the order of 10^{-10} or 10^{-11} gram per injection may remain undetected. In analysis of flavors at high sensitivities, however, such quantities of contamination can cause confusion and creation of artifacts. The method described was found to solve this problem completely for 1 μl Hamilton syringes. The method is considerably less suitable for decontamination of 10 μl syringes.

Figure 17

Arrangement for Decontamination

of Microliter Syringes.

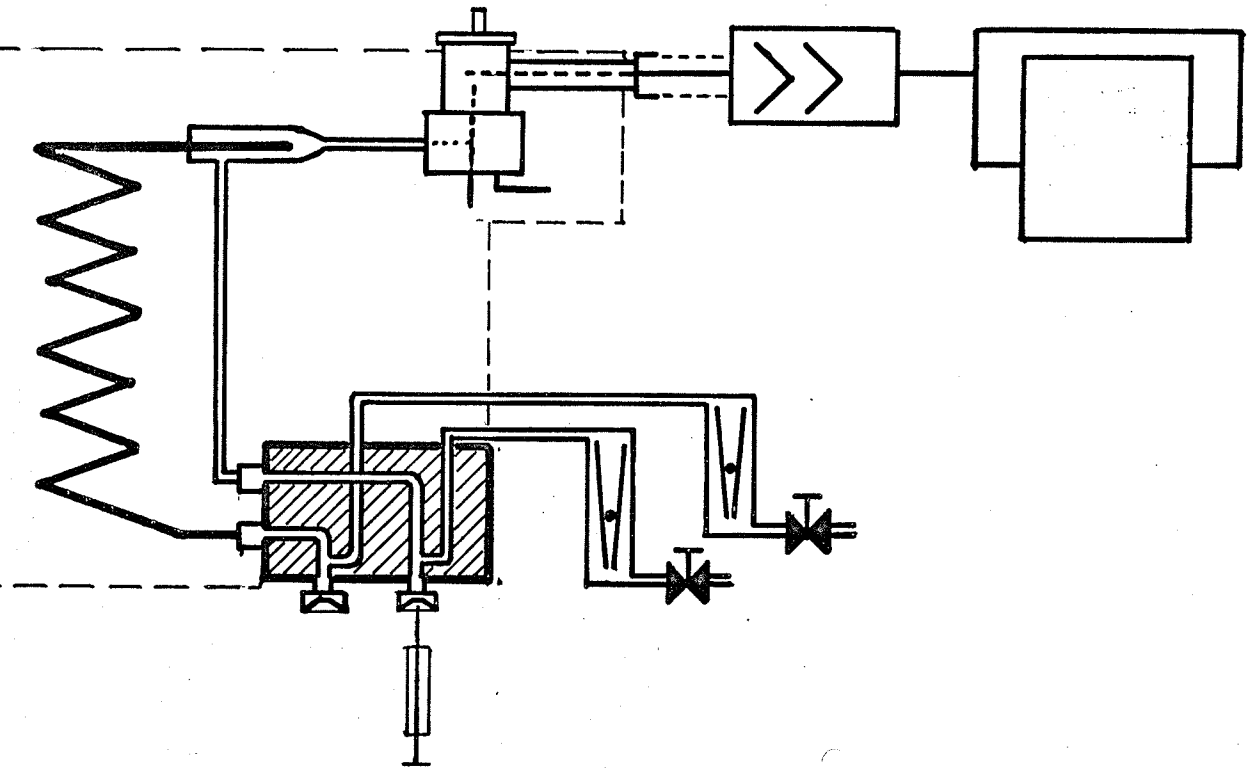


Figure 17.

Chapter 5

The Identification of Flavor Constituents Detected by Gas Chromatography.

5.1. Introduction

The identification of gas chromatographic peaks is well recognized as a difficult task (119,120), especially when dealing with a complex mixture of natural products. A large portion of the literature on gas chromatography (30,31) deals with the various methods for peak identification or their application to specific problems. The different approaches for identification of gas chromatographic peaks can be classified into five categories :

1. Retention behavior.
2. Multichannel or gas density balance detection.
3. Auxiliary detection.
4. Isolation for subsequent study.
5. Precolumn or post-column reactions and pyrolysis.

A somewhat different classification was given by Crippen and Smith (120). Frequently, two or more methods must be applied simultaneously or consecutively for the solution of more difficult problems. The selection of suitable methods and the scheme to be followed in the identification of certain peaks depend largely on the quantity of substance represented by the peak, on the available instrumentation and on any foreknowledge of the sample. For instance, when a gas chromatographic peak represents quantities of the order of 10^{-10} gram the only applicable methods for the identification of this peak are those based on its retention

behavior. On the other hand, when sufficiently large quantities of pure substance (10^{-4} to 10^{-7} gram) can be readely collected, a variety of methods, both classical and instrumental, can be applied. In the identification of flavor constituents one rarely deals with sufficiently large quantities of substance. As a result only the most sensitive methods for identification are directly applicable in flavor analysis. Of the methods available, those based on the retention behavior and on multichannel detection are the most sensitive. Unfortunately, peak identification based on retention data obtained from one column lack the reliability necessary for unambiguous identification (121) offered by the spectrometric methods (122). Nevertheless the methods based on retention behavior are most widely used for the identification of gas chromatographic peaks. These methods are particularly successful when combined with high resolution gas chromatography (34) and when some knowledge on the nature of the constituents of the mixture under investigation is available.

One of the most powerful tools for identification of organic compounds is the high resolution mass spectrometer or mass spectrograph (121,122). Medium resolution mass spectrometers are equally successful when substances of known mass spectra are being identified. In addition, the mass spectrometer is also one of the most sensitive instruments used in chemical analysis. It can produce interpretable spectra from quantities as small as 2×10^{-8} gram of substance (125). The capabilities and the sensitivity of the mass spectrometer are therefore ideally suited for the identification of flavor constituents (2,16) both when directly connected to the gas chromatograph (3,16,39,123-125) or when used independently (126). Unfortunately, the high cost of mass spectrometers prevents their

universal use in the identification of gas chromatographic peaks. The minimum quantities of material required to obtain IR, UV or NMR spectra of a gas chromatographic peak are considerably larger than the quantities needed to obtain mass spectra. Their applicability is therefore limited to the identification of peaks which represent the more abundant constituents of flavor mixtures.

Trapping gas chromatographic peaks for further investigation is a widely used approach for peak identification (127-140). The problem of efficient fraction collection, however, is by far more difficult than it may seem to be. The fact that fraction collection has been the subject of numerous studies and publications (127-140) supports this statment. None of the methods for fraction collection described in the literature seem to be universally applicable. The difficulty of collecting the substances represented by chromatographic peaks, particularly when present in small quantities (121), has limited the application of subsequent instrumental identification and promoted the development of interface devices for direct connection of gas chromatographs to other instruments (121,125). In this work the scheme used for peak identification involved three somewhat independent approaches :

1. Use of retention data for tentative identification and aiming to confirm it by mass spectrometry.

2. Tentative identification from the retention behavior which is further confirmed or found wrong by the standard addition method (34,120).

3. Collection of single peaks or groups of peaks for long term storage and further examination by mass spectrometry, or high resolution gas chromatography.

Approach 1 is applicable mostly for trace peaks of intermediate size (10^{-7} to 10^{-8} gram), approach 2 for trace peaks of small size (10^{-9} to 10^{-11} gram) and approach 3 for all peaks which represent more than 10^{-7} gram of substance. By using preparative scale enrichments of the trace constituents appearing in various regions of a chromatogram the constituents which are present in 10^{-9} to 10^{-8} gram level can be concentrated to the levels necessary for approach 3. Peak identification is recognized as a project for a team of qualified chemists. Sjostrom and Cairncross (141) suggested a team of 2 to 5 scientists at the Ph.D. level (supported by assistants) for physicochemical separation and identification studies in the flavor field. In this work some progress and contributions were made in each of the above mentioned approaches. These are described in the following sections of this chapter and should be considered as the initial stages in development of a general scheme.

It was hoped that the combined gas chromatograph mass spectrometer (GC-MS) system which is described in section 5.2, would enable expedient identification of many peaks. However, due to random fluctuations of the accelerating voltage of the ion source, no positive mass identification could be made on the spectra recorded even when the spectra of known hydrocarbons were taken shortly before or after the experiment under identical operating conditions. In addition, it was found that the bandwidth of the amplifier was not sufficiently wide to permit truthful recording of the mass spectra at the scan rate of 3.0 seconds for full mass range which

is the slowest acceptable in GC-MS for avoiding falsification of the pattern. Such falsification is caused by the change of the sample concentration occurring during the period of the scan. This finding was later confirmed by a representative of the Perkin Elmer Corporation. The work with the GC-MS system, however, produced interesting information which is described in sections 5.2 and 5.3.

5.2. Loss of Chromatographic Resolution in the Vacuum line of a Gas Chromatograph Mass Spectrometer (GC-MS) System

5.2.1. Introduction

The tandem operation of a gas chromatograph with a mass spectrometer has rapidly emerged as a most powerful analytical method. Numerous references pertaining to the development or the application of such systems can be found in recent bibliographies on gas chromatography (142,143) and a recent review on mass spectrometry (144). Instruments which combine both a gas chromatograph and mass spectrometer in a single unit are already commercially available from one company (LKB-Instrument AB, Stockholm, Sweden) and other companies which produce mass spectrometers offer especially designed models and optional accessories for the same purpose.

A variety of interface devices (123,124,145-148) and methods (149-152) have been developed to facilitate the interconnection of gas chromatographs to mass spectrometers and achieve enrichment of the sample to carrier gas ratio. Other specific problems such as scan rate considerations (153), obtaining high resolution mass spectra (123,124,148,154), the effect of column bleed (155) and change of sample concentration during

the recording of the mass spectra of gas chromatographic peaks (149), have been studied with considerable detail. When the column outlet is directly connected to the inlet of the mass spectrometer so that the total column effluent enters in the vacuum line, loss of chromatographic resolution may occur due to the need to maintain part of the column at low pressures and very high linear velocities of the carrier gas. This aspect of the "total effluent" mode of operation, was first discussed by Varadi and Ettre (156) and was later more thoroughly investigated by Teranishi et al (155). These works dealt with capillary column only because the vacuum system of a mass spectrometer cannot tolerate the total column effluent issuing from packed column without the use of interface devices. When the column outlet is kept at atmospheric pressure and only a fraction of the effluent is allowed to leak into the mass spectrometer or when interface device are used, no loss of resolution should occur if the vacuum line of the mass spectrometer or the interface device can maintain the chromatographic resolution. Henneberg (148) showed that this is feasible but expressed the opinion that only some of the interface systems described in the literature can meet this requirement. In addition, we suspected that dead volumes or unswept pockets present in the vacuum line of the mass spectrometer may cause loss of resolution much in the same way as dead volumes in the gas chromatograph do. Dead volumes in the vacuum line of the mass spectrometer are bound to occur particularly when general purpose mass spectrometers are being modified for interconnection to gas chromatographs. It was our intention to use a modified general purpose mass spectrometer. We therefore investigated the effect of dead volumes in the vacuum system of the mass spectrometer.

5.2.2. Experimental

Apparatus. A Hitachi - Perkin Elmer model RMU-6D all glass inlet mass spectrometer was modified for interconnection with a gas chromatograph using an interface accessory kit modelled after the helium separator described by Watson and Biemann (123,124). This kit was made available and installed by representatives of the Perkin Elmer Corporation. We assembled the components of the gas chromatograph mass spectrometer system (Fig. 18) which consisted of an Aerograph model 660 gas chromatograph, a microvolume stream splitter, an FID system (system 6, section 3.7.1) a 5 ft heated capillary (0.01" I.D.) which connects the stream splitter to the mass spectrometer, a helium separator, a mass spectrometer equipped with a total ion monitoring (TIM) electrode and a Keithley model 610B electrometer (used in conjunction with the TIM electrode). A Leeds and Northrup 1 mV potentionetric recorder was used with the FID system and a Honeywell 1 mV potentiometric recorder with the TIM system. A Welch, Duo-Seal model 1405H vacuum pump (not shown in Fig. 18) was used in conjunction with the helium separator. The helium separator was originally connected by glass blowing to the inlet system of the mass spectrometer at a point which was halfway between the pinhole leak and the ion source (Fig. 19a). After a series of experiments the interconnection between the helium separator and the inlet of the mass spectrometer was made as close as possible to the pinhole leak (Fig. 19b).

Procedure. The GC-MS system was operated as a FID-TIM dual channel system. The FID system was used to record the chromatographic resolution at the outlet of the column and the TIM system to record the chromatographic resolution at the ion source of the mass spectrometer.

Figure 18

A Gas Chromatograph - Mass Spectrometer System (Schematic)

P.L. Pinhole Leak

H.S Helium Separator

I.S. Ions Source

FID Flame Ionization Detector

TIM Total Ion Monitor

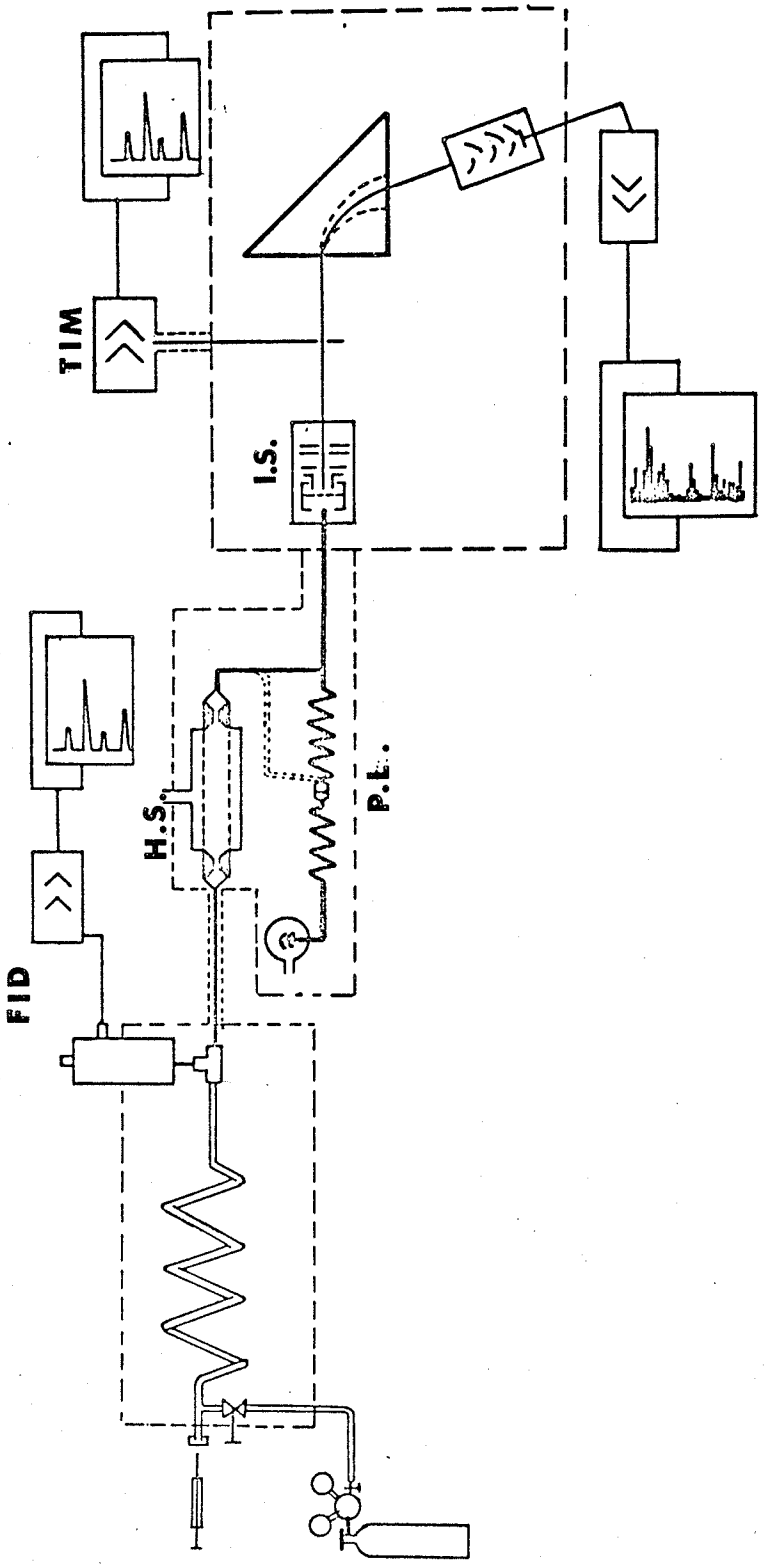


Figure 18.

Figure 19.

Points of Connection of the Helium Separator to the Inlet System of the Mass Spectrometer

a. Point of connection at half-way between the pinhole leak and the ion source.

b. Point of connection made as close as possible to the pinhole leak.

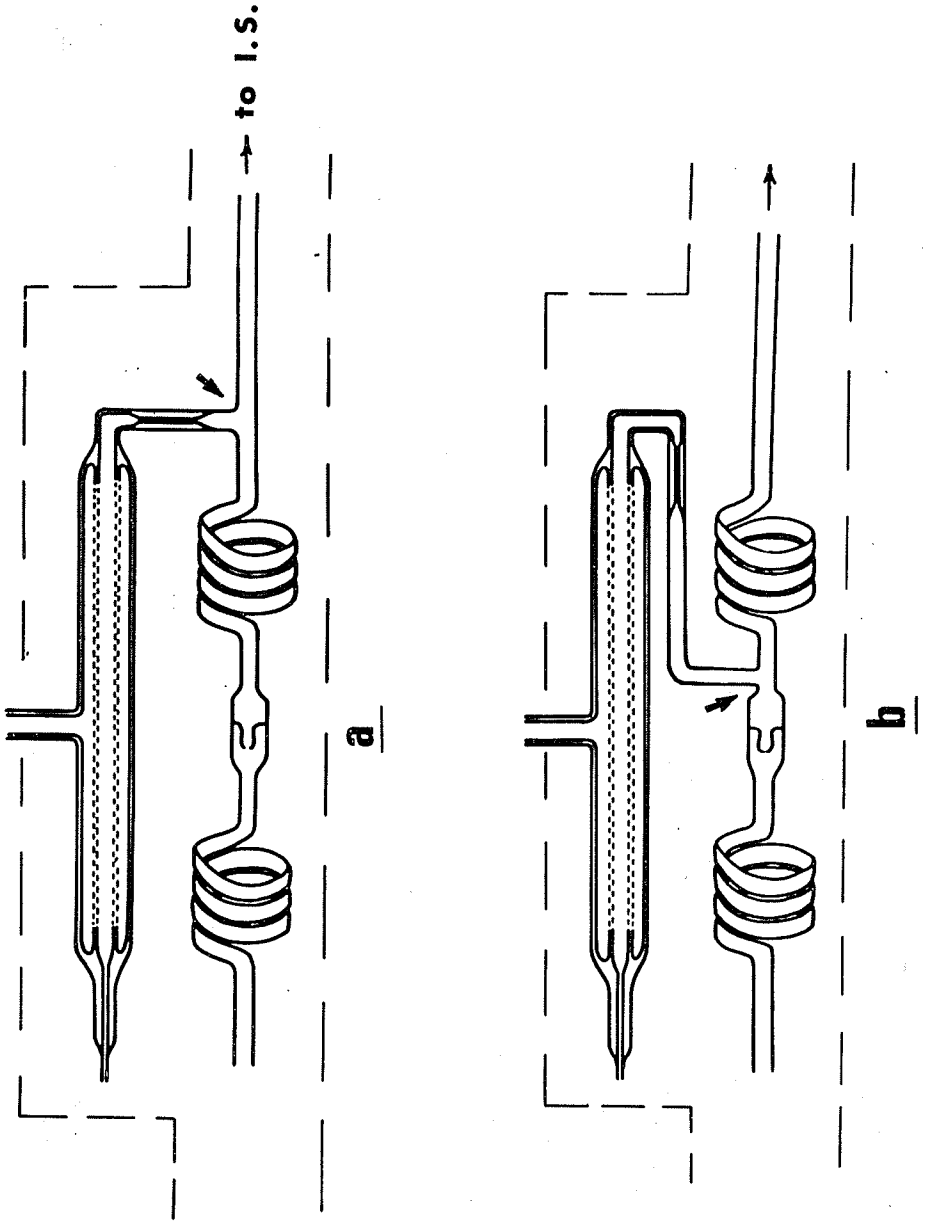


Figure 19.

The column effluent reaches the stream splitter at the rate of 30 ml/min. When kept at 200°C the interconnecting capillary allowed about 10 ml/min of helium to pass into the helium separator (according to the specifications). This means that the column effluent was split at 2:1 ratio with 20 ml/min flowing to the flame and 10 ml/min to the helium separator. The high vacuum section of the mass spectrometer was kept between 3×10^{-6} to 5×10^{-6} torr (the reading of the ion gauge). Total ion monitor (TIM) and flame ionization (FI) chromatograms were simultaneously recorded during each chromatographic run, and the percent loss of chromatographic resolution in the vacuum line of the mass spectrometer was calculated by comparing the corresponding peak parameters and resolution appearing in the TIM and FI chromatograms obtained before and after changing the point of connection of the helium separator. Columns 1 and 7 (Appendix 1) were used under operating conditions listed in Figures 20 and 21. Samples of grape fusel oil (as specified in Figures 20 and 21) were run before and after the point of connection.

5.2.3. Results and discussion.

The FI and the TIM chromatograms obtained with the original GC-MS system (Fig. 18), in which the helium separator was connected to the mass spectrometer at a point midway between the pinhole leak and the ion source (Figure 19a), show loss of resolution in the TIM chromatogram of 53% between peaks 2 and 3 and 42% between peaks 10 and 11 (Fig.20), as compared to the resolution between these peaks observed in the FI chromatogram. The original point of interconnection between the helium separator and the mass spectrometer (Fig. 19a) was the shortest possible. However, with this arrangement the volume between the pinhole leak and

Figure 20.

Loss of Chromatographic Resolution in the Mass Spectrometer Vacuum Line

Shows the corresponding portions of the FID and TIM chromatograms of grape fusel oil distillation residue II obtained with column 1 at 120°C.

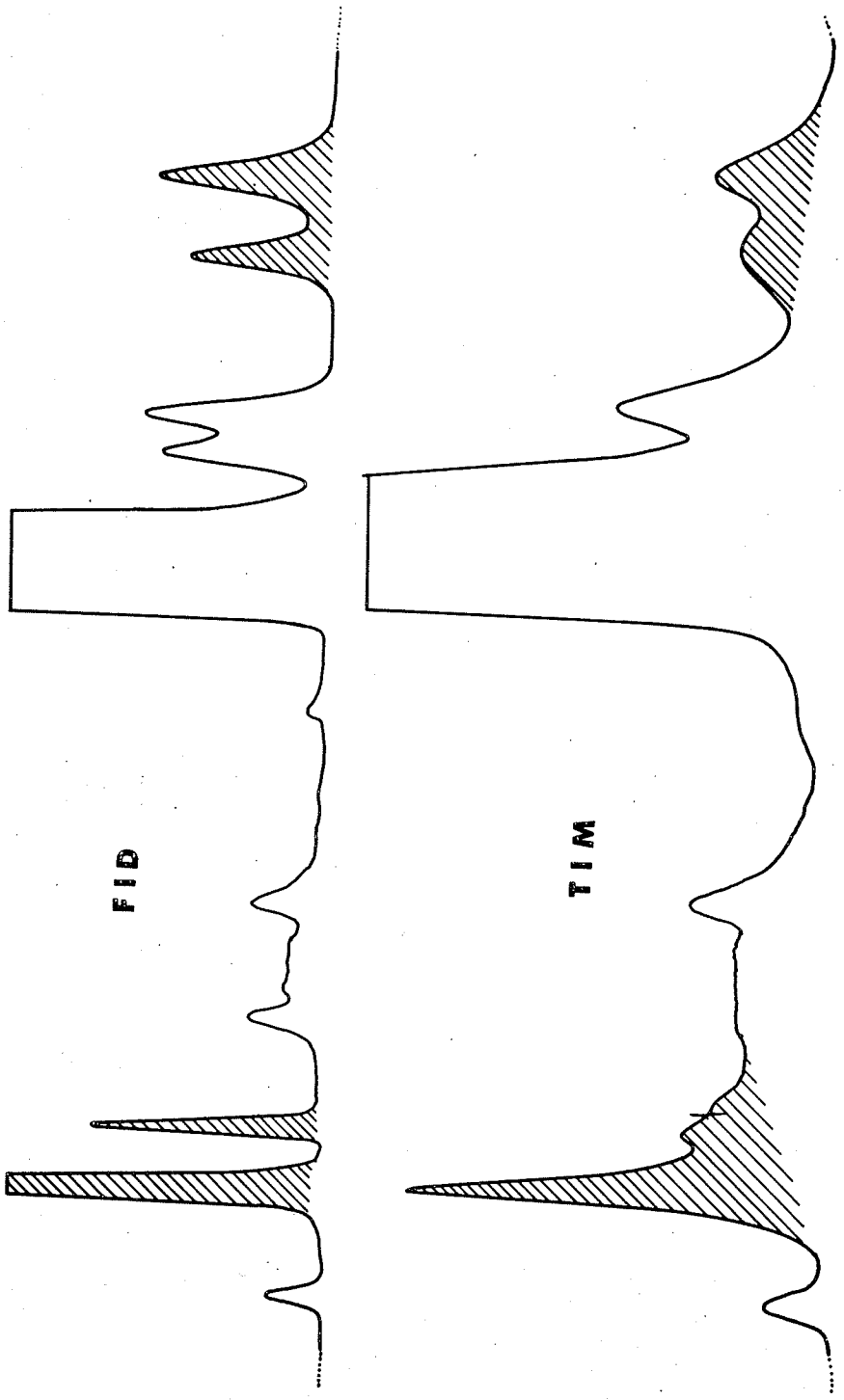


Figure 20.

the point of connection represents an "unswept pocket" which causes the observed loss of resolution.

In the FI and TIM chromatograms obtained with the modified GC-MS system in which the helium separator is connected next to the pinhole leak (Fig. 19b), no loss of resolution between peaks 30 and 31 is observed (Fig. 21). However, some trace constituents appearing on the shoulder of larger peaks which are detected in the FI chromatogram are not seen in the TIM chromatogram indicating that in the case of small peaks following large ones loss of resolution still takes place. This is probably due to some hold-up by the sintered glass helium separator. With the point of interconnection between the helium separator and the mass spectrometer positioned next to the pinhole leak an extra volume of 20 ml and extra length of 40 cm were added to the path of the gases to the ion source. A comparison of the resolution in the TIM chromatograms relative to the FI chromatograms obtained before and after the change of the point of interconnection shows that the presence of unswept volumes in the high vacuum line of the mass spectrometer causes serious loss of chromatographic resolution whereas extra volume has little or no effect on the resolution.

5.3. Temperature Programming with a GC-MS System. Elimination of the Continuous Change and Interference of the Background Spectra.

Teranishi *et al* (155) pointed out that the sensitivity of a GC-MS system is limited by the extent of column bleeding and showed that only liquid phases that do not obscure the mass spectral patterns of the material being analysed can be used. This situation limits the

Figure 21.

Comparison of FID and TIM Chromatograms after Elimination of the "Unswept Pocket" in the Vacuum Line.

Shows the corresponding FID and TIM chromatograms of grape fusel oil distillation residue II obtained with column 7 (temperature programmed).

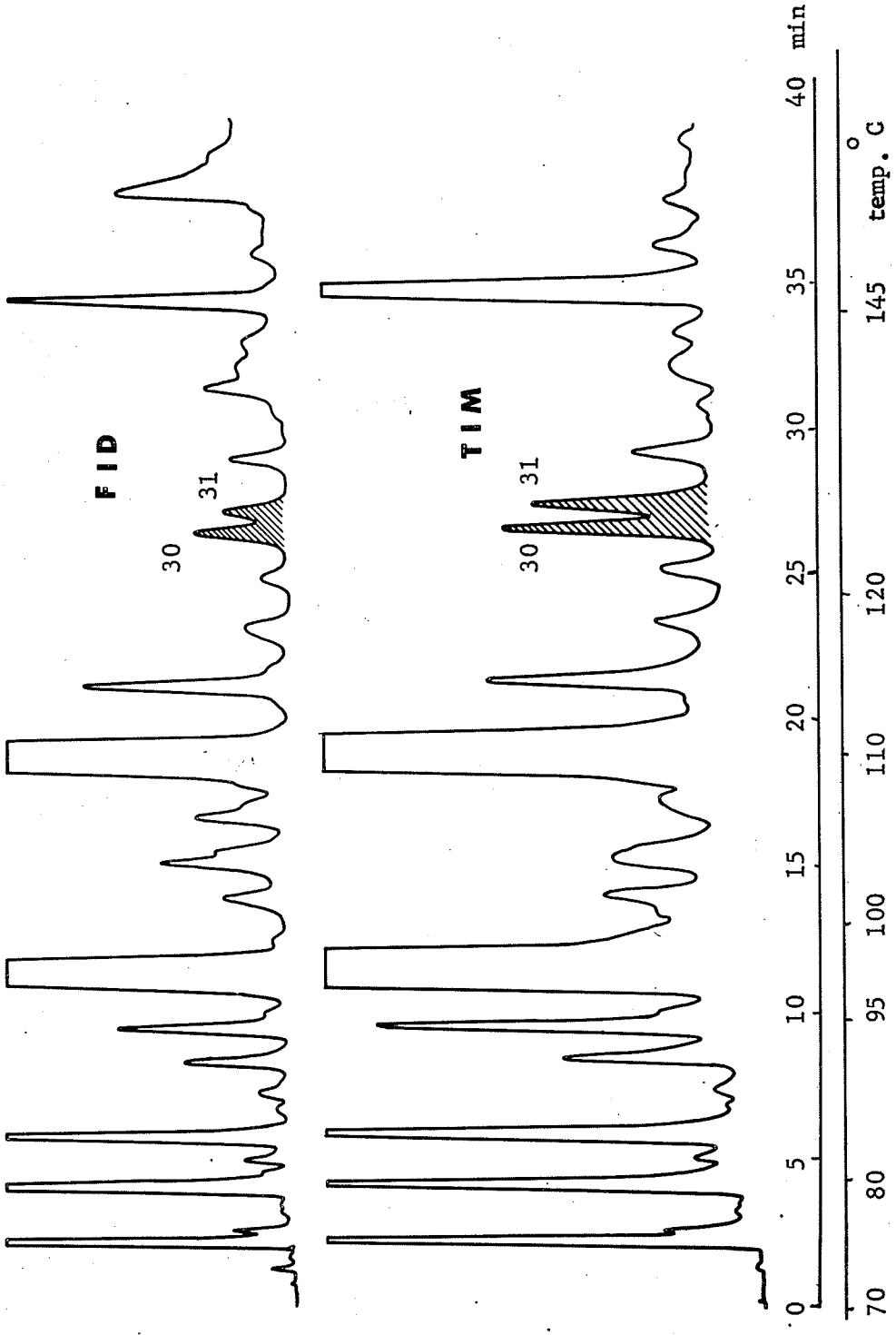

FID
30
31
TIM
30
31
0
5
10
15
20
25
30
35
40
min
70
80
95
100
110
120
145
temp. C
°

Figure 21.

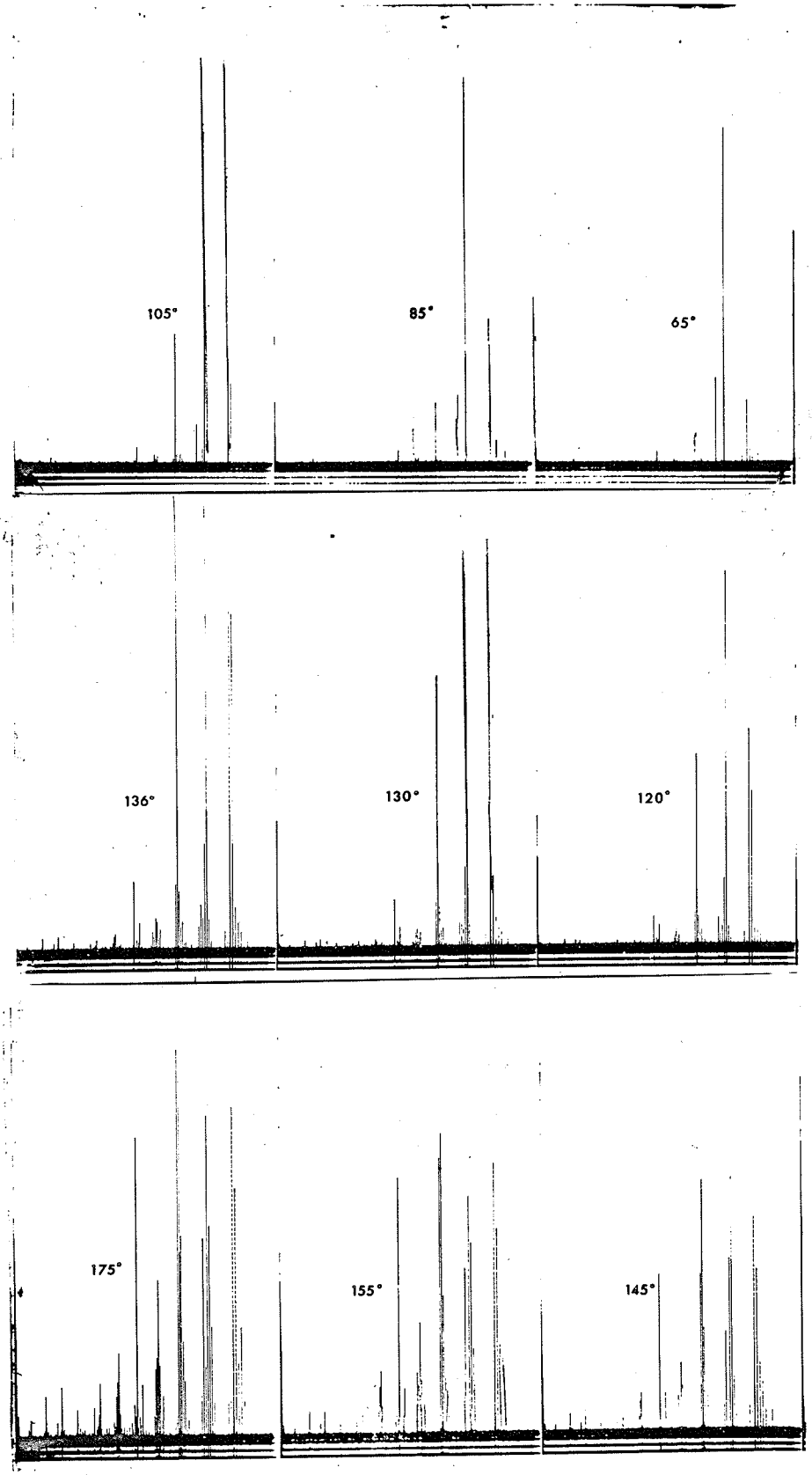
maximum operating temperature of the gas chromatograph and drastically reduces the possibilities in the selection of liquid phases. In temperature programmed operation of a GC-MS system, the continuously increasing rate of column bleeding causes a continuous increase in the magnitude and change in the pattern of the background spectra. This complicates the procedure of extracting the sample spectrum from the raw data and necessitates repeated recording of the background spectra during the temperature programmed run. However, when complex multicomponent mixtures are being analysed, resolved or partly resolved, constituents of the mixture are eluted from the column in succession without a pause which prevents the recording of the background spectra at frequent intervals. Naturally, the column temperature can be programmed again without injecting the sample and background spectra at the appropriate temperatures recorded but this is time consuming and still leaves one with the laborious job of subtracting the background spectra at each temperature from the spectra obtained for each peak. These difficulties seriously limit the applicability of temperature programming with GC-MS systems. We found that the method for single column temperature programming at high sensitivities described in section 4.2 is directly applicable for the solution of this problem and that it successfully eliminates the interference of the column bleeding. The system shown in Figure 18 was used exactly as described in section 5.2.2. The column temperature was first programmed without the bleed absorbing column and background spectra at various temperatures were recorded (Fig. 22). A continuous increase of the intensity of the background spectra is observed (Fig.22), indicating that the sensitivity of the GC-MS system is inversely proportional to the column temperature (155). Column temperature was programmed again after the connection of the bleed absorbing column and background spectra

Figure 22.

Background Mass Spectra of Column 1

at Various Temperatures.

Figure 22.



recorded at various temperatures (Fig. 23). A constant background spectra of low intensity are observed throughout the temperature programming indicating that the column bleed is effectively absorbed and its interference eliminated.

5.4. Identification of Gas Chromatographic Peaks Based on their Retention Behavior

5.4.1. Compilation of gas chromatographic retention data

Voluminous gas chromatographic retention data have accumulated in the last decade. It is found both scattered in articles (157,158) and collected in form of books (159,160). Unfortunately, it is not represented in a unified way and the numerous liquid phases used hamper its widespread applicability. A more important source of difficulties and inaccuracy of the inter-laboratory use of retention data originates from solid support effects (161,162). Scholz and Brandt (162) showed that the support effects include tailing, changes in retention time with changes both in sample size and in the sequence in which solutes are injected, and inversions of elution sequence caused by varying the amount of liquid phase or sample size.

Solid support effects towards certain groups of compounds (saturated hydrocarbons, alkyl halides etc,) are negligible and retention data of these compounds are reliably used. Unfortunately, oxygenated hydrocarbons and particularly alcohols which were of major interest in this work exhibit strong interaction forces with the solid support originating in hydrogen bonding (161,162). In view of Scholz and Brandt's findings (162) it becomes obvious that retention data available in the literature

Figure 23.

Background Mass Spectra of Column 1 + Bleed Absorbing Column at Various Temperatures.

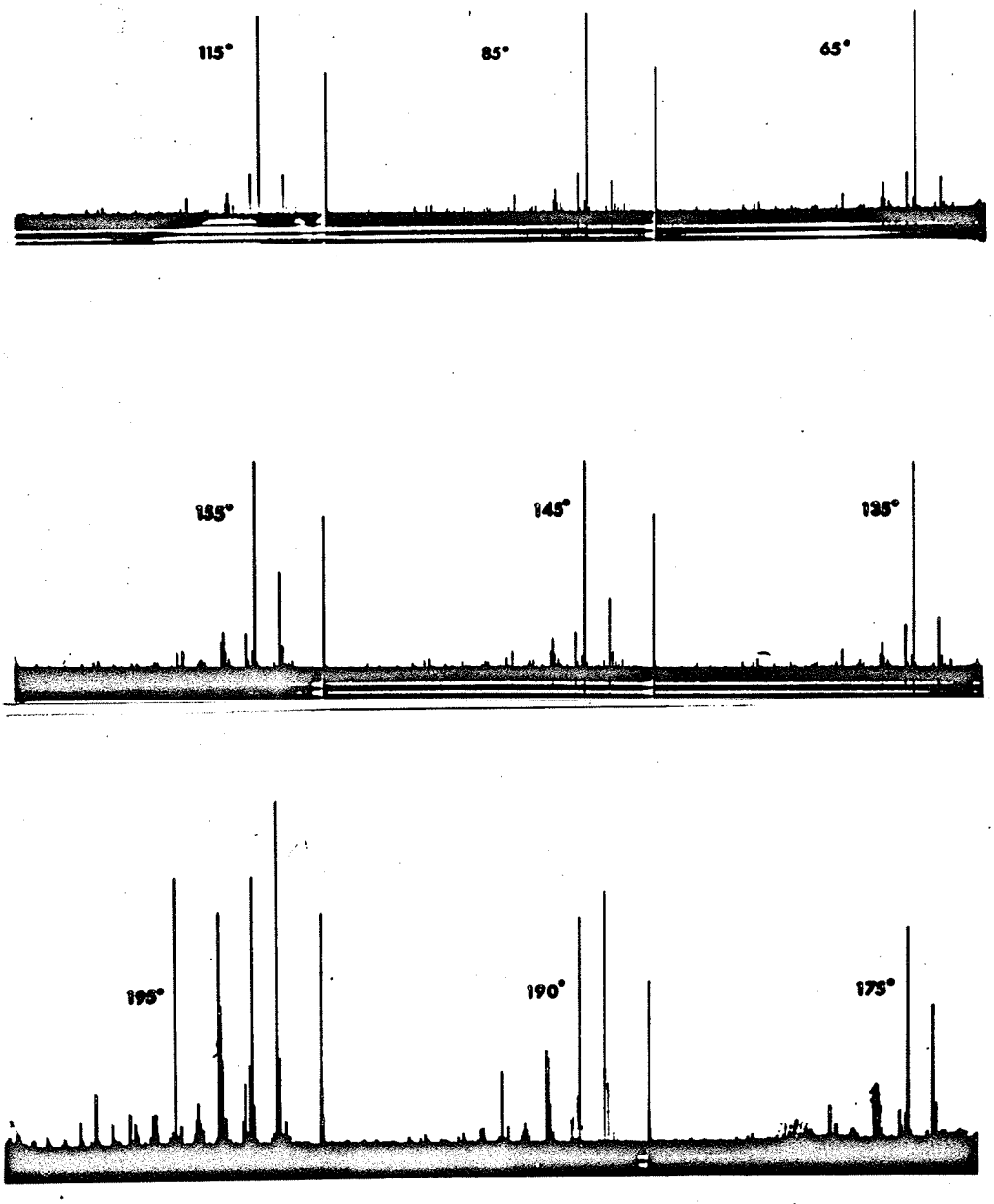


Figure 23

cannot be reliably used for the identification of the compounds of interest in this work. It was concluded that only retention data produced on the same column would be successfully applicable and the compilation of such data on the liquid phases of interest was therefore undertaken. It was of special interest to obtain the retention behavior of various homologous series of compounds in order to compensate for the incompleteness of the available "library" of reference compounds. Eighteen test mixtures listed in Appendix 2 containing homologous series of alcohols, esters, aldehydes, ketones and normal paraffins were chromatographed on eighteen columns (Appendix 1) at two or three column temperatures. Since FID systems were used (systems 1,4 and 8, section 3.7.1) hold-up times were calculated as follows : Each column was separately connected to a Carle microvolume thermal conductivity detector and operated as an ordinary gas chromatograph using helium as carrier gas. About 20 μl of air was injected through an injection port arrangement which was free of unswept pockets and elution times were measured with a stopwatch. Knowing the hold-up time, the flow rate and the inlet and outlet pressures, the free volume of the column, V_{gas}, was calculated using equation 7 (40).

$$t_h = \frac{2}{3} \frac{V_{gas}}{F} \frac{(\frac{Pi}{Po})^3 - 1}{(\frac{Pi}{Po})^2 - 1} \qquad [7]$$

where : t_h = gas hold-up time

V_{gas} = column free volume

Pi,Po= inlet and outlet pressures

F = flow rate at column temperature.

Knowing the V_{gas} of the column, the flow rate at the operating temperature, the inlet and outlet pressures, we calculated the hold-up times for the

conditions used during the compilation of the data and used it for obtaining the corrected retention times. Retention indices (163) were calculated from the data obtained. However, the mixture of n-paraffins used for obtaining the retention indices was frequently eluted considerably faster than expected and since it did not contain normal paraffins of sufficiently high carbon number the retention indices of a number of compounds could not be calculated. The results obtained are summarized in Table 1. Relative retention times were purposely not calculated because it is easier to convert the retention times to relative retention times for different reference compounds than to convert relative retention times from one reference compound to relative retention time of another reference compound.

5.4.2. Standard addition

When the identity of certain peaks is suspected (either from their retention times or from foreknowledge of the composition of the sample), standard addition (34,120) offers a simple and reliable method for positive identification. The applicability of the method, however, is limited only to the identification of compounds which are available for addition to the mixtures analysed. When combined with high resolution columns (34) standard addition is the only applicable method for the identification of peaks which represent less than 10^{-10} gram of substance. Standard addition was found to be particularly suitable for the tentative identification of trace constituents in grape fusel oil revealed by wide range temperature programmed capillary columns. Twenty-eight peaks in the chromatogram of a grape fusel oil distillation residue (Fig. 24) were tentatively identified (Table 2) using the following approach : The

Table 1

Retention Data

Compound	Col. 11 Carbowax 20M				Col. 6 Hall. Carb.		Col. 12 Arm. SD		Col. 13 Hall. M18-ol		Col. 14 Zonyl		Col. 15 SAIB		Col. 16 Di(eth.-hex.)	
	78°		110°		70°		50°		85°		85°		85°		80°	
	R.T.	R.I.	R.T.	R.I.	R.T.	R.I.	R.T.	R.I.	R.T.	R.I.	R.T.	R.I.	R.T.	R.I.	R.T.	R.I.
Methyl Alcohol	30	868			47	652	37	579	25	630	8	653	27	687	13	-
Ethyl -	36	907			73	713	59	634	34	667	14	761	38	737	21	-
n-Propyl -	71	1019			175	814	149	737	79	775	21	844	87	851	44	664
n-Butyl -	304	-			854	999	565	877	448	994	74	1066	423	1062	253	877
n-Amyl -	594	-			1156	-	1207	959	1019	1297	134	1188	926	1166	600	977
2-Propanol	35	902	15	853					444	700						
2-Butanol	62	996	23	951					96	800						
3-Pentanol	111	1095	35	1055					194	888						
2-Hexanol	251	-	67	1190					472	1001						
2-Heptanol	481	-	118	1297					1025	-						
2-Octanol	940	-	201	1393					2229	1195						
2-Nonanol	1819	-	344	1497					-	-						
Iso Propyl Alcohol	36	903			83	728	69	652	46	704	16	787	48	769	29	612
Iso Butyl -	101	1039			313	883	280	802	144	850	28	906	136	910	80	739
Iso Amyl - / Act. Iso Amyl -	228	-			845	998	874	924	356	965	60	1026	334	1030	195	847
Methyl Formate	16	715			14	-	9	-	14	-	9	675	19	638	10	-
Ethyl -	22	800			25	559	20	524	22	610	18	818	34	720	17	-
n-Propyl -	35	898			62	692	57	629	39	686	33	936	70	822	37	642
n-Butyl -	61	994			149	795	149	736	80	776	64	1061	194	958	87	750
Methyl Acetate	21	790			25	559			18	-	26		37	731	20	-
Ethyl -	32	880			47	654			28	642	40		59	797	35	636
n-Propyl -	52	968			108	758			59	737	69		188	954	70	677
n-Butyl -	97	1073			256	858			130	837	124		250	991	159	823
n-Amyl -	187	-			622	962			287	938	233		541	1095	245	873
n-Hexyl -	348	-			1454	-			637	1038	415		1151	1196	-	-
Iso Propyl Acetate	35	804			64	697	70	653	40	606	49	1015	75	830	46	672
Iso Butyl -	71	1019			176	818	209	772	91	793	98	1134	179	947	121	792
Iso Amyl -	139	1133			440	922	557	876	212	899	192	1258	344	1052	297	895
Iso Propyl Propionate	48	956			137	785	170	-	72	761	68	1073	125	900	104	772
Iso Butyl -	108	1092			381	906	522	-	185	882	148	1206	310	1023	303	897
Iso Amyl -	216	-			922	-	1361	-	420	986	283	1336	703	1130	726	-
Methyl Butyrate	49	945			121	770	137	-	63	746	64	1061	120	894	89	753
Ethyl -	77	1034			216	839	270	-	109	816	93	1126	196	959	166	828
n-Propyl -	139	1133			494	936	670	-	234	912	167	1229	399	1054	382	924
n-Butyl -	268	-			1136	-	1706	-	514	1011	308	1353	830	1153	900	-
n-Amyl -	462	-			2623	-	-	-	1183	1109	559	-	1759	-	2078	-
Ethanal	11	610			10	-	10	-			7	600	10	-	10	-
Propanal	15	698			22	542	25	543			13	745	21	650	18	-
Butanal	28	853			49	659	59	634			27	893	47	767	36	639
Pentanal	49	958			126	776	144	933			55	1036	110	882	86	749
2-Propanone					28	576			19	-	27	906	31	709	17	-
2-Butanone					58	683			36	674	43	995	62	804	35	636
3-Pentanone					125	775			71	760	68	1073	111	884	82	743
3 Heptanone					520	941			257	923	178	1243	374	1046	355	916
4 Heptanone					610	960			294	940	207	1273	442	1068	417	935
2-Octanone					1384	-			693	1048	-	-	1118	1192	1065	-
Methyl Propionate							59	634	35	671	40	978	63	807	36	642
Ethyl -							117	712	56	729	59	1024	102	872	68	720
n-Propyl -							288	805	116	823	103	1134	213	970	151	818
n-Butyl -							719	903	256	923	186	1252	447	1069	353	915
n-Amyl -							1841	-	566	1023	336	1371	953	1170	819	-
Flow rates	36.4		34.6		35.5		35.6		34.9		32.5		34.0		36.4	

RT - retention time in seconds

RI - retention index

Figure 24.

Identification of Peaks

By Standard Addition

Illustrates the identification of small peaks in a complex chromatogram of 0.5 µl grape fusel oil distillation residue II obtained with temperature programmed capillary column* (column3). Ethyl heptanoate (F2), ethyl nonanoate (H4) and phenethyl acetate (I9) are shown to increase in peak height (dotted peaks) after addition of these compounds to the mixture.

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* Chromatographic conditions as in Figure 28.

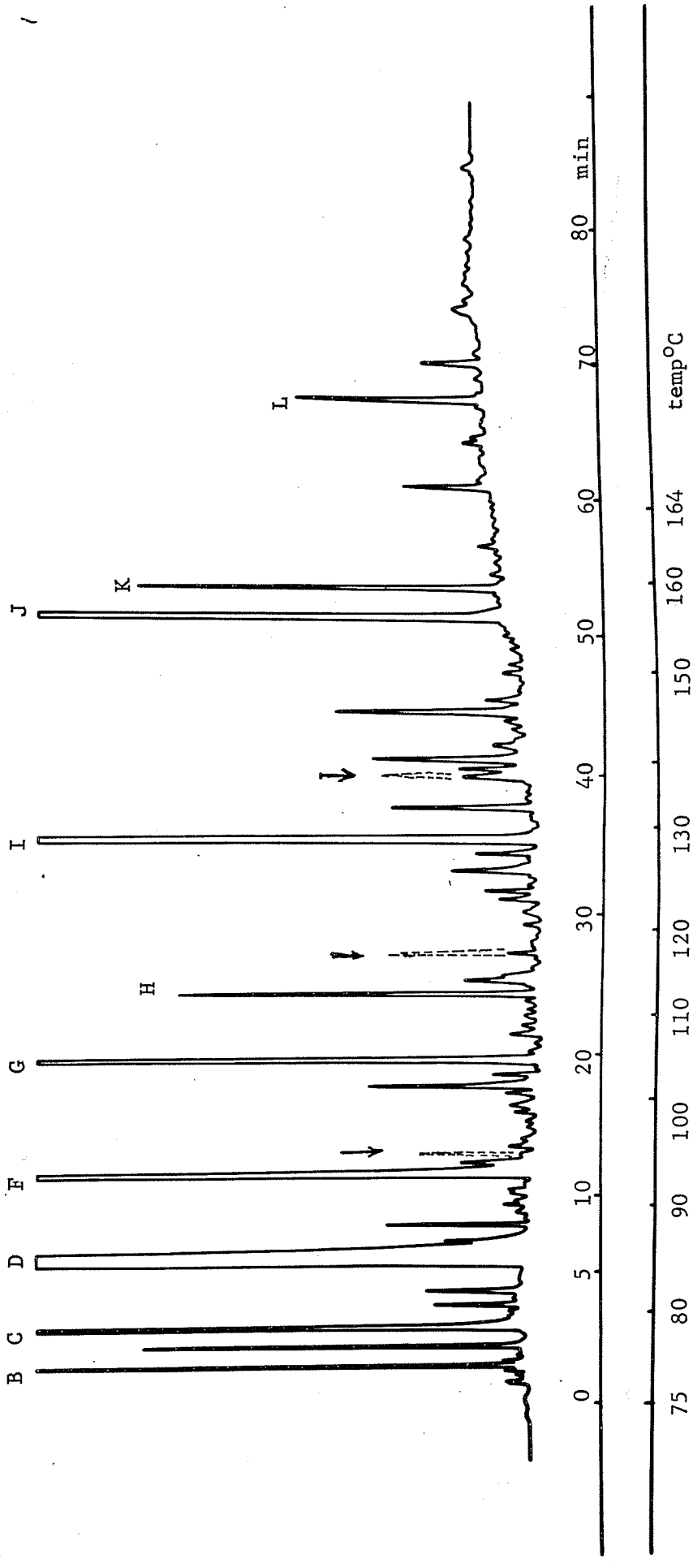
B
C
D
F
G
H
I
J
K
L
0
5
10
20
30
40
50
60
70
80 min
75
80
90
100
110
120
130
150
160
164
temp°C

Figure 24

Table 2

Tentative Identification of Peaks in Grape Fusel Oil (Distill. Res. I, II)

Chromatograms by Standard Addition.*

Peak Designation**	Compound	Chromatogram number
F2	Ethyl Heptanoate	BC 3027
Go	Ethyl Octanoate	" "
H4	Ethyl Nonanoate	" "
Io	Ethyl Decanoate	" 3028
Jo	Ethyl Laurate	" "
C4	n-Butyl alcohol	" "
F6	n-Heptyl acetate	" 3029
G4	n-Octyl acetate	" "
H10	n-Nonyl acetate	" "
I5	n-Decyl acetate	" "
C6	Isoamyl acetate	" 3030
G4	Isoamyl octanoate	" "
I4	Isoamyl nonanoate	" "
Ko	Isoamyl decanoate	" "
Do	2-Hexanol	" 3037
D8	2-Heptanol	" "
F9	n-Heptyl alcohol	" "
F12	2-Octanol	" "
H2	n-Octyl alcohol	" "
H18	n-Nonyl alcohol	" "
I9	Phenethyl acetate	" "
Mo	Ethyl palmitate	" 3039
I6	Ethyl phenacetate	" "
B2	Propyl acetate	" 3038
E1	Isoamyl propionate	" "
F1	Isoamyl butyrate	" "
Lo	Ethyl myristate	" "
Fo	n-Hexyl alcohol	" "

* On temperature programmed capillary column.

** Figure 24

original grape fusel oil sample was chromatographed under the conditions described in Figure 24; then, to approximately 10 μl of the original mixture 0.01 to 0.05 μl of one or more compounds suspected to be present were added and the mixture re-run, under the same conditions. The chromatograms obtained with and without the added substances were compared. Peaks which increased in relative height without increase of their width at half height were considered as tentatively identified. Using this approach,eight high boiling compounds and four low boiling constituents appearing in the chromatograms of samples of distilled spirits (received from Seagram's Research Laboratory, LaSalle, Quebec) were tentatively identified.

5.5. Collection and Long Term Storage of Gas Chromatographic Fractions

The difficulties encountered in trapping minor constituents emerging from a gas chromatographic column have repeatedly been pointed out in the literature (134,137,139,140,164). Amy et al (137) recently described an excellent method for the collection of micro-or submicrogram fractions. Their method consists of collecting the substance of interest on a small quantity of column packing which is placed in a melting point capillary. This method is applicable only in conjunction with a solid sample probe of a mass spectrometer. Unfortunately, the use of such a probe is restricted to samples of low volatility (more volatile substances would evaporate and be pumped out even before the probe is inserted into the ion source). In addition, upon direct sealing of the melting point capillaries,decomposition of small quantities of substance may occur. Also, the method described by Amy et al (137) does not secure gas tight connection between the collecting capillary and the outlet of the gas chromatograph.

A somewhat improved method for fraction collection which is applicable for wider range of quantities and boiling points of the collected substances, particularly suitable for long term storage and compatible with several methods for subsequent analysis, was developed. A special set of tools was designed for use with one particular size of thin wall glass tubing and assembled as shown in Figure 25. The unique features of this system for fraction collection consist of a heating arrangement which prevents condensation of the sample on the tubing edges so that sealing without decomposition can be performed and a special cooling arrangement which permits control of the temperature of the collection tube by adjusting the flow rate of air passing through the cooling jacket. The collection tubes (8" long, .197$\pm$.013" I.D.) were custom made by Friederich & Dimmock Inc., Millville, New Jersey.

An O-ring arrangement permitted gas tight connection between the collection tube and the outlet of the gas chromatograph. The collection tubes were made so that they can be filled with any type of packing (solid adsorbents, column packing and glass beads). The sealing of the collection tubes for long term storage was done with a small glass blowing flame so that they can later be magnetically broken in the inlet system of a mass spectrometer. After sealing and storage the collection tubes were cooled and centrifuged. The material collected was thus brought to the narrow end of the tube and could be removed for subsequent analysis by cutting and using a 1 or 10 μl syringe. Using the method described collection efficiencies of up to 85% for medium and low boiling ethyl esters were obtained for 2-5 μl of substance injected.

Figure 25.

Fraction Collection Apparatus

(Schematic)

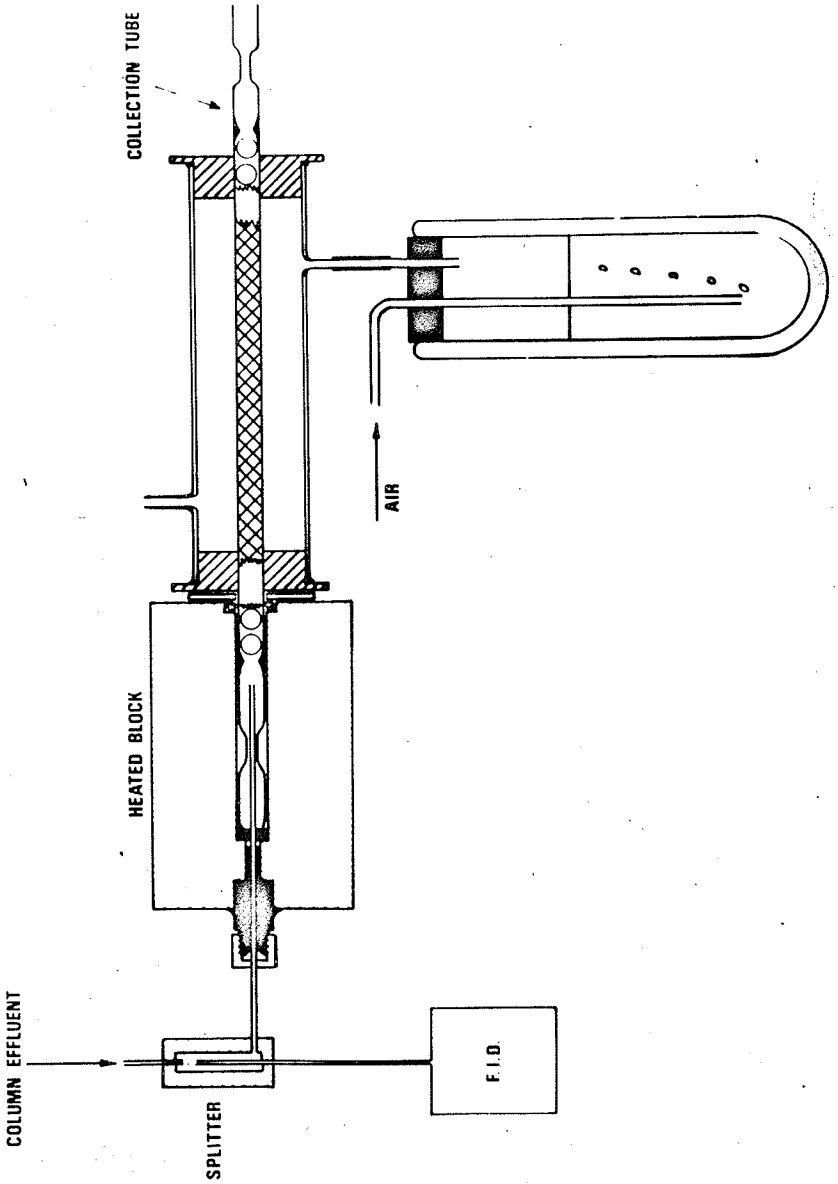
COLLECTION TUBE
AIR
HEATED BLOCK
COLUMN EFFLUENT
SPLITTER
F.I.D.

Figure 25.

P A R T III

THE APPLICATIONS OF GAS CHROMATOGRAPHY TO THE ANALYSIS OF ALCOHOLIC FERMENTATION BY-PRODUCTS

Chapter 1

Introduction and Brief Review of the Literature

Water solutions of pure ethyl alcohol lack a particular odor and cannot be tasted (12). Even in fairly high concentrations it has only a rather faint sweet taste (12). The flavor of alcoholic beverages must, therefore, be attributed to many known and unknown trace constituents present in these beverages. It can be expected that a comprehensive chemical analysis of the composition of the trace flavor constituents in alcoholic beverages would be related to its flavor definition. However, in spite of much work done on the chemical analysis of alcoholic beverages, the overall effect of the compounds found on the sensory perception has not as yet been elucidated (165), i.e., the findings of chemical analyses frequently cannot be correlated with the flavor definition of the samples. A possible explanation to this situation can be found in the fact that our senses are in some cases 10^2 to 10^4 times more sensitive than the most sensitive detectors (5,166). This means that a more detailed analysis is required to permit a flavor-composition correlation to be made.

Gas chromatographic analysis of fusel oils and alcoholic beverages has been the subject of numerous reports in the literature (167-192). Analysts working on alcoholic beverages and related products began to become aware of the potentialities of gas chromatography in 1958, after

the first reports on the subject appeared (167-173). These reports demonstrated that even with the low sensitivity of the instruments available at that time,gas chromatography held great promise. Van der Kloot and Wilcox (168) recognized that higher sensitivity is essential for extending the spectrum of components that can be determined by gas chromatography. Bavisotto and Roch (169) demonstrated the applicability of gas chromatography in analysis of volatiles in beer during its brewing,fermentation and storage. Fouassin (173) determined quantities of nine trace flavor constituents in forty-seven different brands of alcoholic beverages. Using gas chromatography, Baraud (174) determined and compared the quantities of nine alcohols and fourteen esters in the fusel oils of several products showing that the quantitative composition varies greatly. Bouthilet and Lowrey (175) presented gas chromatographic data on the major fusel oil constituents of grape brandies and attempted to correlate the results with those obtained by colorimetric procedures. Austin and Boruff (176)described a method for concentration of congeners of grain spirits and their analysis by gas chromatography. They expressed the belief that ultimately gas chromatography may serve for controlling the distillation streams. Mecke et al (177) developed a method for extraction and concentration of the flavor constituents of wines for further gas chromatographic analysis of the concentrates. They detected and determined about fourteen flavor constituents in a number of different wines but could not correlate their composition with the differences in their organoleptic tests. Webb and Kepner (178) investigated the properties of selected liquid phases for the gas chromatographic separation of the major constituents of fusel oils.

Kepner and Webb (179) studied the composition of muscat raisin fusel oil using both preparative and analytical gas chromatography. They detected about forty constituents and identified thirty of them mostly by using retention data.

Using a flame ionization gas chromatograph Maurel (180) determined the composition of ten different alcoholic beverages monitoring nine constituents and analysed five different fusel oils for the eight main constituents. Sihto et al (165) discussed in detail the problems and the potentialities of gas chromatography in the analysis of alcoholic beverages. They obtained good results by using a FI detector at apparently low or moderate sensitivity but concluded that the availability of more sensitive detectors will promote research on alcoholic beverages. In 1962, Hirose et al (181) reported the results from a comprehensive study of the composition of corn, barley and sweet molasses fusel oils. Starting with fractional distillation of twenty liters of fusel oil and proceeding with adsorption chromatography and finally gas chromatography, they isolated about sixty-five components and identified fifty-nine of them. Hirose et al (181) expressed the view that different fusel oils consist of many common compounds present in varying quantities. In 1963, Pfenninger published a comprehensive review of the literature on fusel oils (182), a description of gas chromatographic procedures for the determination of the relatively low boiling constituents appearing in fusel oils (183) and the results on the analysis of seven constituents of fusel oils of various sources (184). In a commercial bulletin Kabot and Ettre (185) reported the analysis of various alcoholic beverages by flame ionization gas chromatography and a specially developed column (column 6, Appendix 1). In a series of articles Kayahara et al (186-188) reported the methods

and the results in their studies on the flavor components of whiskey using gas chromatography in conjunction with an elaborate scheme for extraction and fractionation of the flavor components. They showed marked differences in the composition of various brands of whiskey. Bober and Haddaway (189) used the gas chromatographic pattern of various alcoholic beverages for the identification of their brand or type. Later (190) they developed an interesting procedure for the concentration of congeners in alcoholic beverages by using stationary phases as extracting liquids. Kepner et al (191) showed that when high sensitivity ionization detectors are used, direct analysis of head space vapors of alcoholic beverages, which gives a precise measure of the aroma composition of the sample, can be successfully performed. Singer and Stiles (192) compared the colorimetric and gas chromatographic methods for the determination of the higher alcohols in alcoholic beverages. They found the gas chromatographic data to agree with the method of A.O.A.C. In his paper "Quality and Flavor by Gas Chromatography", Bayer (5) showed impressive multicomponent chromatograms of the pentane extracts of alcoholic beverages obtained on capillary column. The main working stages of the scheme proposed by Bayer (5) for analysis of flavors involve extraction, preparative gas chromatography for removal of the solvent and analysis of the enriched sample by high resolution capillary columns.

Chapter 2

Selected Examples of Gas Chromatographic Analysis of Alcoholic Fermentation By-Products

2.1. Analysis of Fusel Oils.

Fusel oil is defined in the Encyclopedia Britanica as ".... the mixture of volatile oily liquids of characteristic odor and taste produced during alcoholic fermentation processes". In other words, fusel oil can be defined as a complex mixture of by-products of alcoholic fermentation which consists mostly of isoamyl, active amyl, isobutyl and n-propyl alcohols. The amount of fusel oil produced during alcoholic fermentation is comparatively small and is dependent on the raw material used and on the conditions of fermentation. In distilleries, ethyl alcohol is recovered from the fermented liquor by distillation, leaving most of the fusel oil as a residue. This residue can be considered as a concentrated form of the flavor constituents which have passed with ethyl alcohol into the distillate. As such it is ideally suited to function as a model mixture in the development of gas chromatographic methods for the analysis of alcoholic beverages. Most of the constituents present in the fusel oil can be expected to be present in the distilled spirits, although many of them may remain undetected due to their extremely low concentrations. Indirectly the fusel oil represents the flavor spectrum of the distilled spirits. The development of gas chromatographic methods for detailed analysis of fusel oils was therefore considered as an important stage in this work. Most of the experiments were carried out with grape fusel oil which was received from Seagram's Research Laboratory, LaSalle Quebec. Grain fusel oil received from the same source was also studied.

In order to obtain further enrichment of the trace flavor constituents, 10 ml of grape fusel oil was distilled up to 133°C leaving a 1.5 ml of residue which was designated "grape fusel oil distillation residue I" and used for gas chromatographic analysis. A second distillation was carried out up to 140°C starting with 50 ml of grape fusel oil and leaving 5 ml of residue. This residue was designated "grape fusel oil distillation residue II" and used for gas chromatographic analysis.

The original grape and grain fusel oils and the grape fusel oil distillation residues were extensively analysed by gas chromatography. Due to limitations of space, however, only few selected examples are presented here.

2.1.1. Preliminary gas chromatographic investigation of grape and grain fusel oils.

The alcohols of fusel oils which contain six or less carbon atoms and esters which contain ten or less carbon atoms can be considered as the low boiling point constituents of fusel oil. In the preliminary analysis of the low boiling constituents of fusel oil, grape and grain fusel oils were directly chromatographed under identical conditions on two different liquid phases, Carbowax 20M and THEED, using four different levels of detector sensitivity. The superimposed chromatograms of grape and grain fusel oil for each sensitivity are shown in Figure 26. Twenty-six components were detected on column 5 (Fig. 26c) and fourteen on column 8 (Fig.26d). One of the components separated on column 8 (peak 5, Fig. 26d) was not separated on column 5, thus bringing the total number of separated different components to twenty-seven. Eleven constituents listed in Figure 26 were tentatively identified by utilization of published retention data (193, 194). From the chromatograms shown in Figure 26 it becomes apparent that

Figure 26.

Superimposed Chromatograms of

Grape and Grain Fusel Oils

a. Low sensitivity

Dotted line - Grape fusel oil (f.o.)
Solid line - Grain f.o.
FID system 1
Column 8
Col. temp. 100°C
Flow rate 35 ml/min
Sample size 0.2 µl
Attenuation 10^{-8} (3% of the output)

b. Low sensitivity

Dotted line-Grain f.o.
Solid line -Grape "
Flow rate35 ml/min
FID system 1
Column 8
Col. temp. 100°C
As in a exept:
Attenuation 10^{-8} (11% of the output)

c. High sensitivity

Solid line-Grape f.o.
Dotted line-Grain f.o.
FID system 2
Column 5
Col temp. 81.5°C
Flow rate 35 ml/min
Sample size 0.2 µl
Attenuation 3 x 10^{-10} amps.
(1% of the output)

d. Medium sensitivity

As in C
exept
Column 8
Attenuation 1 x 10^{-8} amps.
(1% of the output)

Peaks identified in chromatogram C.

Peak No.

2. Acetaldehyde
3. Acetone
4. Ethyl acetate
5. Ethyl alcohol
7. n-Propyl alcohol
8. isoButyl alcohol
10. n-Butyl alcohol
12. isoAmyl acetate
13. isoAmyl alcohol
14. n-Amyl alcohol
16. Unidentified
17. "
18. "
19. Hexyl alcohol

Peak No. 5 in chromatogram d was identified as 2-butanol.

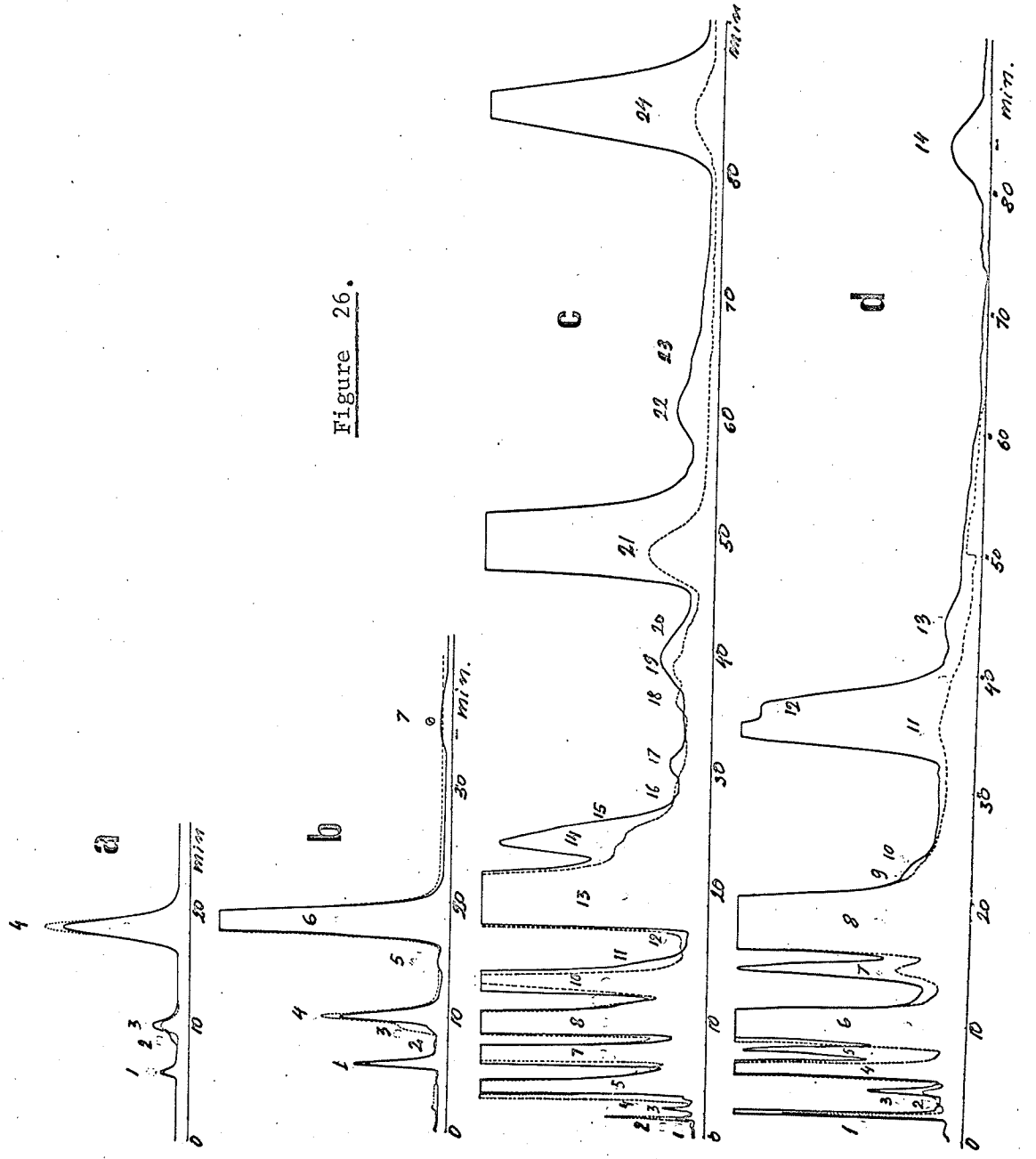

Figure 26.

grape and grain fusel oils consist mostly of common constituents (181); however, their quantitative composition differs largely. Most of the published gas chromatographic investigations of fusel oils have been carried out with thermal conductivity detectors (174,178,179,183,185) or with flame ionization detectors at moderately high sensitivity (165,180). Many trace components may thus have escaped detection. The fact that twenty-seven constituents were detected by exposing only the low boiling portion of the fusel oils indicates that the composition of this portion has been revealed with considerable detail through the use of high sensitivity.

2.1.2. Revealing the complexity of grape fusel oil by temperature programming and high sensitivity using packed columns.

Most of the published data on gas chromatographic analysis of fusel oils has been carried out isothermally at relatively low temperatures (165,174,183,184). However, the compounds known to be present in fusel oils (181) cover a wide range of chemical structures, concentrations and boiling points. Their detailed analysis therefore necessitates the application of either lengthy fractionation procedures (179,181) or more advanced gas chromatographic techniques such as temperature programming and high sensitivity. The gas chromatographic approach offers speed, simplicity and applicability in routine monitoring of the levels of the flavor constituents. By applying the dual column wide range temperature programming (60^{o}-240^{o}C) method to the analysis of grape fusel oil distillation residue using columns 9 and 10 in conjunction with a thermal conductivity detector at least sixty-two constituents were detected (Fig. 27a). Combining this information with the information of three more

Figure 27.

Chromatograms of Grape Fusel Oil at

Various Sensitivities.

A. Dual column wide range temperature programmed run of grape fusel oil distillation residue II.

Thermal conductivity detector (M-T).

Columns 9 and 10

Col. temp. programmed (60 - 240°C)

Flow rate - 20 ml/min (helium)

Sample size 4 μl

B. Isothermal run of grape fusel distillation residue I at moderate sensitivity

FID system 4

Column 6

Col. temp. 95°C

Flow rate 20 ml/min (nitrogen)

Sample size 0.3 μl

C. As in B exept :

Col. temp. 89°C

Sample size 0.2 μl

D. Isothermal run of grape fusel oil at high sensitivity

FID system 3

Col. temp. 65°C

Flow rate 12 ml/min (nitrogen)

Sample size 0.05 μl

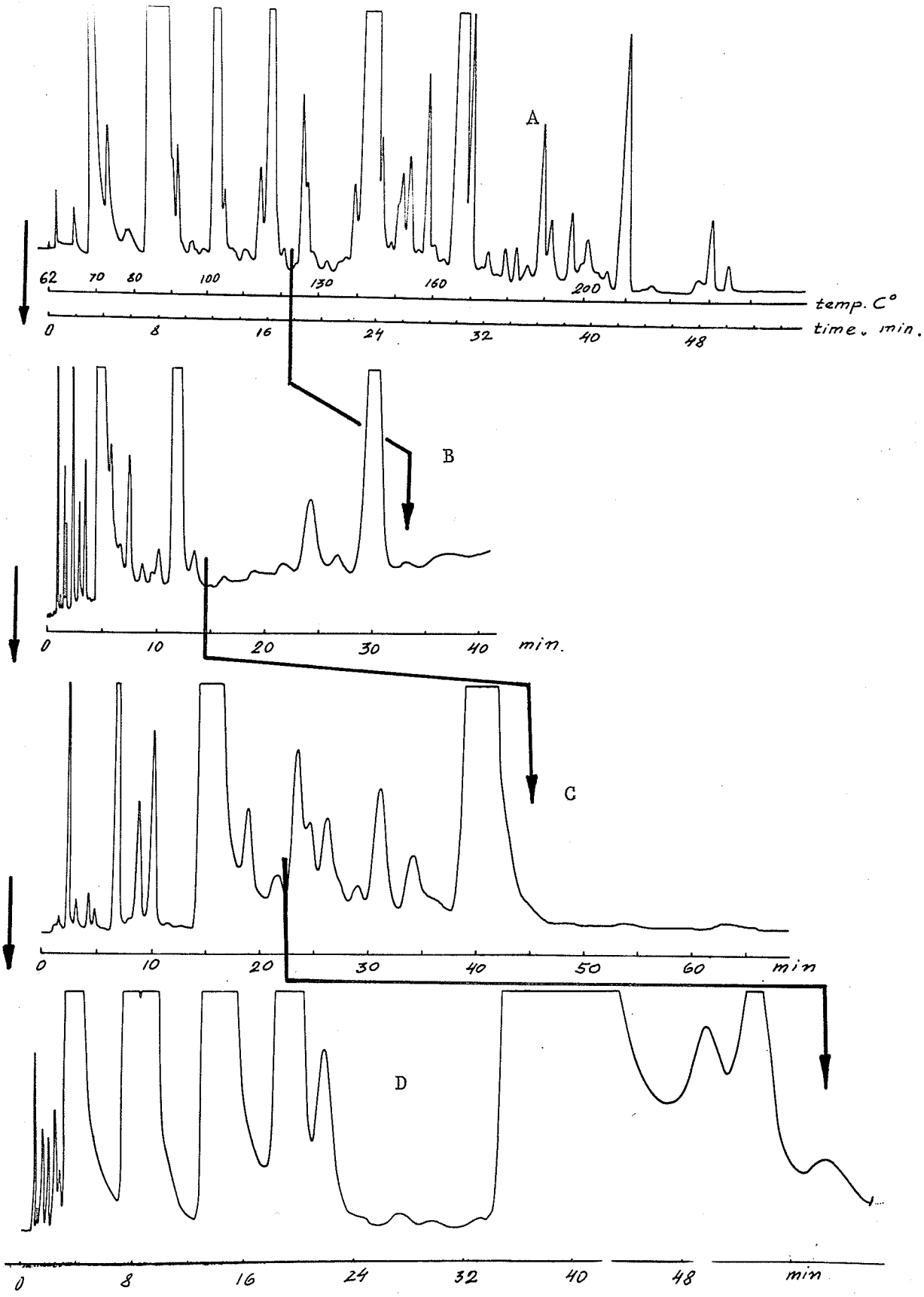
A
62 70 80 100 130 160 200
temp. C°
0 8 16 24 32 40 48
time. min.
B
0 10 20 30 40 min.
C
0 10 20 30 40 50 60 min
D
0 8 16 24 32 40 48 min

Figure 27.

detailed chromatograms of the low boiling constituents using column 6 in conjunction with FID systems 3 and 4 (section 3.7.1, Part II) as shown in Figure 27, a total of eighty two constituents were detected.

Hirose et al (181) who performed the most comprehensive analysis of fusel oils reported the presence of sixty-seven constituents starting with 20 liters of fusel oil and using a tedious fractionation procedure. From the results obtained in this work it can be concluded that high sensitivity and temperature programmed gas chromatography can substitute for or produce superior results to lengthy fractionation procedures in a detailed analysis of fusel oil.

2.1.3. Revealing the complexity of grape fusel oil by temperature programmed high resolution gas chromatography.

Fusel oils have not been investigated with capillary columns. The complex nature of the fusel oil mixtures, however, requires the application of high resolution columns and temperature programming for a detailed analysis. Furthermore, the results obtained with packed columns (section 2.1.1 and 2.1.2) indicated that many trace constituents appearing between major ones (Fig. 27) remain unresolved and undetected due to the low column efficiency of ordinary packed columns. It was therefore expected that high efficiency capillary columns would further improve the results. Column 1, which is a capillary column (100 ft 0.02" I.D.) containing Tergitol NP-35 as liquid phase was used in this study. A column of 0.02" I.D. was preferred in this case over the more frequently used 0.01" I.D. columns since the 0.02 I.D. columns offer higher sample capacity thus providing higher detectability. At 100°C column 1 exhibited an average of 30,000 theoretical plates for 3-pentanol (average of 3 measurements of

the same chromatogram). Grape fusel oil distillation residues I and II were chromatographed on column 3 while programming the temperature from 65°C to 170°C at low program rate (1.5 deg/min.). Two of the chromatograms obtained are shown in Figures 24 and 28. These chromatograms reveal the complexity of the fusel oil mixture with the greatest detail obtained so far. By combining the information from several chromatograms obtained at slightly different operating conditions and sample sizes,the presence of at least 150 compounds was revealed. Due to the limited stability of the liquid phase the column temperature could not be raised more than 170°C. This means that the chromatograms shown in Figures 24 and 28 represent only the low and medium boiling point constituents of the grape fusel oil. It can therefore be expected that by using more stable liquid phases an additional increase of the number of detectable constituents will be observed at higher column temperatures. Using the standard addition method (section 5.4.2, Part II) twenty eight of the peaks appearing in the chromatograms shown in Figure 24 and 28 were tentatively identified (Table 2). In addition to these the identity of at least six other peaks of the well known constituents of fusel oil (ethyl acetate, ethanol, n-propyl alcohol, isobutyl alcohol, isoamyl alcohol and n-hexyl alcohol) is recognizable from their position in the chromatogram and their relative size. The chromatograms shown in Figures 24 and 28 reveal the presence of at least sixty compounds previously unknown as fusel oil constituents.

2.1.4. Preliminary investigation of fusel oil by dual channel gas chromatography.

Dual channel gas chromatography and particularly the combination of a flame ionization (FI) with an electron capture (EC) detector offers

Figure 28.

Chromatogram of Grape Fusel Oil Distillation Residue II

Obtained with a Temperature Programmed Capillary Column *

Conditions :

FID system 4

Column 3

Col. temp. programmed (60 - 180°C)

Inlet pressure 250 mm Hg

Split ratio 1 : 100

Program rate 1.5 deg/min

Sample size 0.7 µl

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* Peak designation as in Table 2

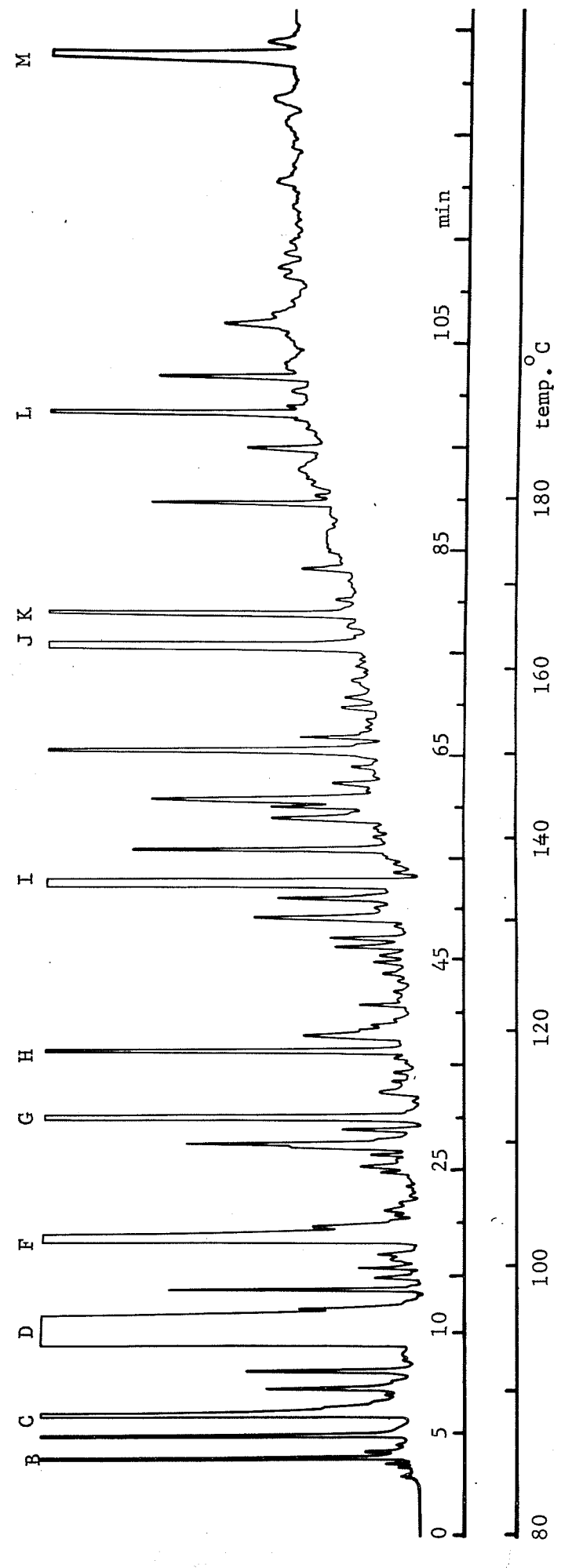
B
C
D
F
G
H
I
J K
L
M
0
5
10
25
45
65
85
105
min
80
100
120
140
160
180
temp.°C

Figure 28

additional possibilities in high sensitivity analyses and identification (195). In analysis of complex mixtures FI-EC dual channel operation enables the detection of heavily overlapped and unresolved peaks. The application of dual channel gas chromatography to the analysis of fusel oil has not been reported in the literature. A dual channel gas chromatographic system consisting of the original Aerograph 660 electron capture system and FID system 7 (section 3.7.1, Part II) was assembled using column 7 and a microvolume stream splitter. Nitrogen was used as the carrier gas at a total flow through the column of 40 ml/min which was split in 2:1 at the outlet, feeding two parts to the EC detector (27 ml/min) and one part to the FI detector. Column temperature was manually programmed at a rate of approximately 3 deg/min. The outputs from the two detectors were fed to two 1 mV potentiometric recorders (Texas and Leeds & Northrup). In order to secure synchronization of the FI and the EC chromatograms, 12 marks were made on both charts throughout the chromatographic run. A dual channel chromatogram of grape fusel oil distillation residue II is shown in Figure 29. In many points where the FI record shows no peak the EC record shows strong signals. Dual channel seem to hold promise as a tool for obtaining a more detailed picture of the composition of fusel oils and other complex mixtures of natural products.

2.2. Analysis of Alcoholic Beverages.

The ability of gas chromatography to differentiate between samples of alcoholic beverages of different flavor definition has been proven (172, 173,180,189,190). However, it may frequently occur that:

1. Part of the differences in the chemical composition are not

Figure 29.

Dual Channel Chromatogram of Grape

Fusel Oil Distillation Residue II

FI - Flame Ionization

EC - Electron Capture

FID system 7

EC system - Aerograph 660 original

Column 7

FI to EC split ratio 1 : 2

Flow rate 40 ml/min (helium)

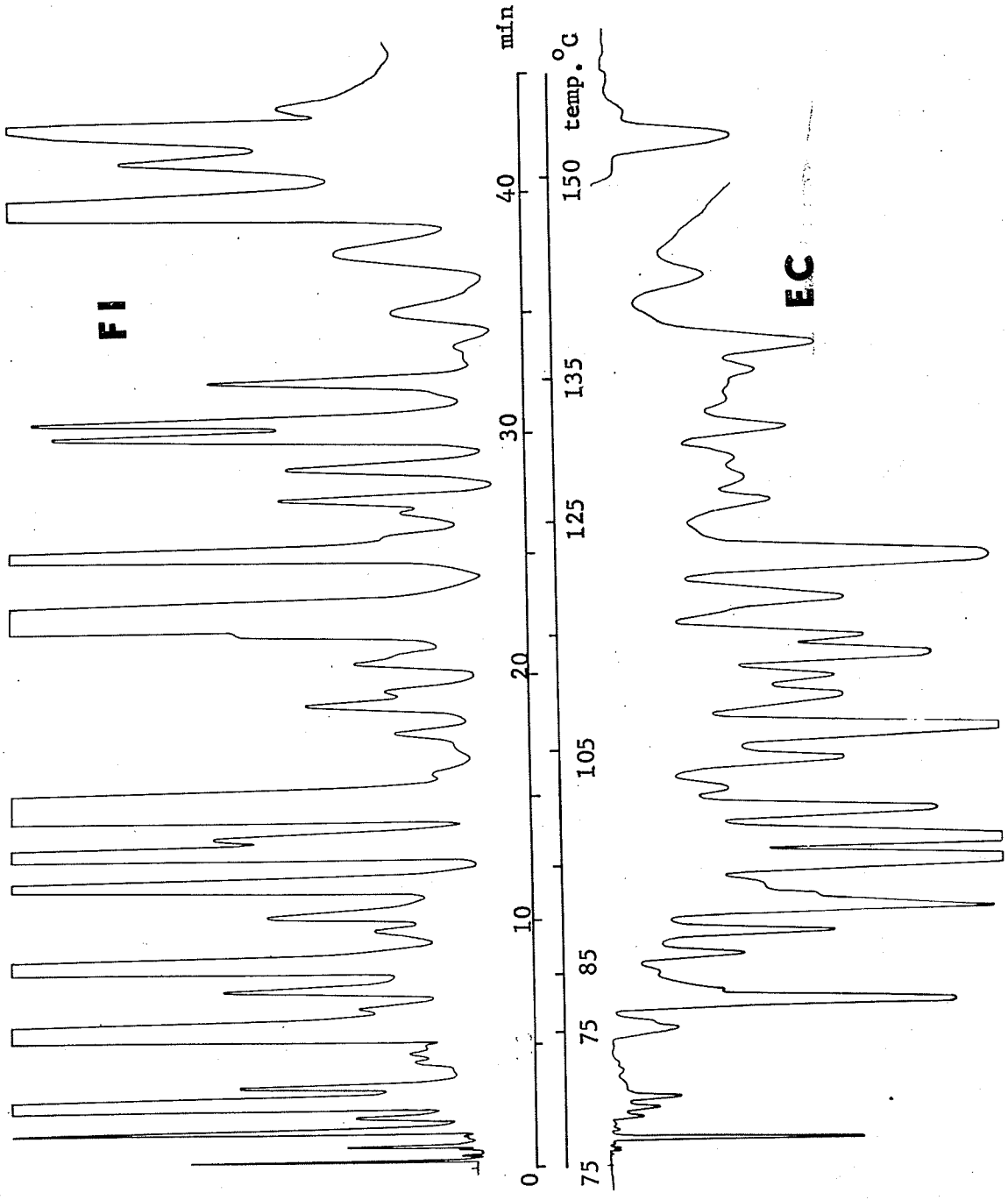

Figure 29.

related to the differences in flavor (5).

2. The differences in the chemical composition do not explain the differences in flavor (165).

In the first case the information needed to explain the differences in flavor is obscured by useless information. In the second case the information needed to explain the differences in flavor has not been obtained and more detailed analysis is required in order to reveal it. In order to reveal which of the compounds present are related to a particular flavor definition the crude information produced by the gas chromatograph must be processed and analysed in terms of accumulated information on many similar samples. The comparison of the chemical composition of two isolated samples of different flavor may never yield clues as to the reasons for this difference unless the influence of each of the constituents on the flavor is well known. A graphic approach for processing of the raw gas chromatographic data using the results obtained with a series of samples of organoleptically defined spirits was therefore devised. Thirty-two samples of distilled spirits were isothermally chromatographed under identical conditions on column 5 using FID system 2 (Section 3.7.1 , Part II). The peak heights of six of the flavor constituents detected were measured in all the chromatograms and arranged in order of increasing magnitude, which gives a plot of the type shown in Figure 30. Most of the graphs thus obtained show "plateau" regions indicating that a group of samples contain the same quantity of this flavor constituent. The numbers of the samples representing a "plateau" group were then recorded and the organoleptic definition checked to see whether there is anything in common. Also it was checked whether some of the same sample

Figure 30.

Illustration of a Graphic Method for Flavor-Composition Correlations.

The graph represent a plot of the peak heights of a peak(No. 5) appearing in the chromatograms of series of samples versus the sample number and shows "plateau region".

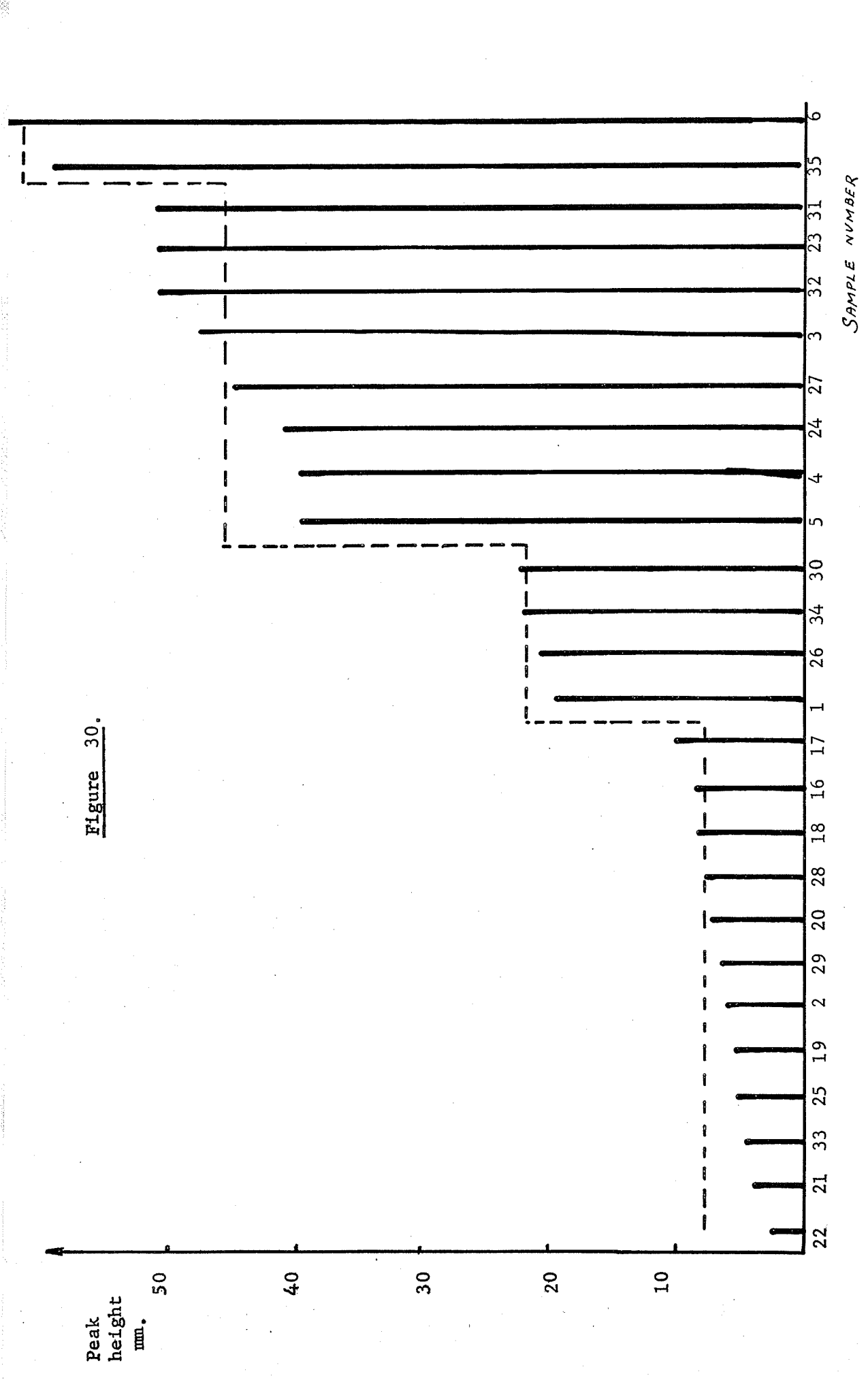
Peak height mm.
50
40
30
20
10
22 21 33 25 19 2 29 20 28 18 16 17 1 26 34 30 5 4 24 27 3 32 23 31 35 6
SAMPLE NUMBER

Figure 30.

numbers appear together in the same plateau of more than one graph. The only correlation that could be made was that the samples found to contain the highest quantities of ethyl acetate were found to include "fruity" or "apple" flavor characteristics in their definitions.

2.3. Gas Chromatographic Analysis of Gins and Related Samples.

The only example of gas chromatographic analysis of gin that could be found in the literature (185) shows practically nothing but ethyl alcohol, even though the analysis was carried out with a flame ionization detector. Three samples of Gins (A_1, B_1 and A_2), three Alcoholic Distillates (I, II and III) from coriander seed, an Alcoholic Distillate from Juniper Berries and Alcohol for Gin were received for comparative studies from Seagram's Research Laboratories, LaSalle, Quebec, and analysed under identical conditions using FID system 2 (section 3.7.1, Part II) and the wide range zero shifting method described in section 4.3, Part II. The chromatograms obtained are shown in Figure 16. All the chromatograms shown in Figure 16 were simultaneously recorded at two sensitivities (the lower sensitivity record is not shown) thus permitting measurement of the peak areas of peaks which are off scale at the higher sensitivity. Part of the above mentioned samples were also analysed on different dates under different chromatographic conditions (using the same column).
The results of these analyses together with the results obtained from the chromatograms shown in Figure 16 can be summarized as follows :

1. Eighteen flavor constituents in the Gins and the Distillates from coriander seed were detected.

2. Changes in the composition of Gins A_1 and B_1 upon storage were detected by comparison of chromatograms obtained on the dates approximately 38 days apart.

3. From the peak areas of the constituents appearing in the chromatograms of the Distillates (I, II, III) from coriander seed, it may be possible to calculate the percentages of the different coriander seeds (I, II, III) to be mixed in order to obtain uniform levels of the major flavor constituents.

4. Linalool was tentatively identified in the Distillates from coriander seed (using retention times).

5. Geraniol was tentatively identified in the Distillate from Juniper Berries (using retention times).

6. From several chromatograms of the Distillate from Juniper Berries, obtained at two temperatures, a total of 40 flavor constituents were detected.

These results could be produced only with a high sensitivity FID system developed in this study. Attempts to analyse these samples on a Barber Coleman Selecta 5000 gas chromatograph failed to produce any useful results.

2.4. Gas Chromatographic Analysis of Distillates from Fermentation-Time Study.

A study of the effect of the duration of the fermentation process on the composition of flavor constituents was carried out by Seagram's Research Laboratory, LaSalle, Quebec, and the direct gas chromatographic

analyses for determining the changes occurring with time were made with the systems developed. These analyses were carried out in the following stages :

1. Preliminary gas chromatographic separations under different conditions for exploratory purposes.

2. Relatively low sensitivity runs of most of the samples under identical conditions for separation and measurement of the major constituents.

3. High sensitivity low temperature runs of all samples under identical conditions for monitoring the variation in composition of the highly volatile "pre-ethanol" flavor constituents.

4. Medium sensitivity higher temperature runs of all samples under identical conditions for monitoring the variation in composition of two less volatile "post-isoamyl alcohol" constituents.

All the analyses were carried out on column 5 (Carbowax 20M) using FID system 3 (Section 3.7.1, Part II). Peak heights of eight peaks were measured in all the samples and plotted versus time of fermentation (sample number). The resulting graphs are shown in Figure 31. Certain peaks show definite changes with time of fermentation and some tentative correlations between the changes in composition and the changes of the flavor characteristics of the samples were made. For instance, "slightly ethereal" which is part of the description of Bourbon samples 1 and 2 might be interpreted to be a result of the high content of Peak No. 4 in these samples.

Figure 31.

Graphic Presentation of Variation of the Levels of Flavor Constituents with Time of Fermentation.

Shows the variation of peak heights of eight peaks appearing in the chromatograms of eleven samples of distilled spirits fermented for different periods of time versus the sample number (time of fermentation).

1. Corn high wine peak 4
2. " " " " 6
3. " " " " 8
4. " " " " 19
5. Bourbon peak 4
6. " " 19
7. " " 21
8. Corn high wine peak 21

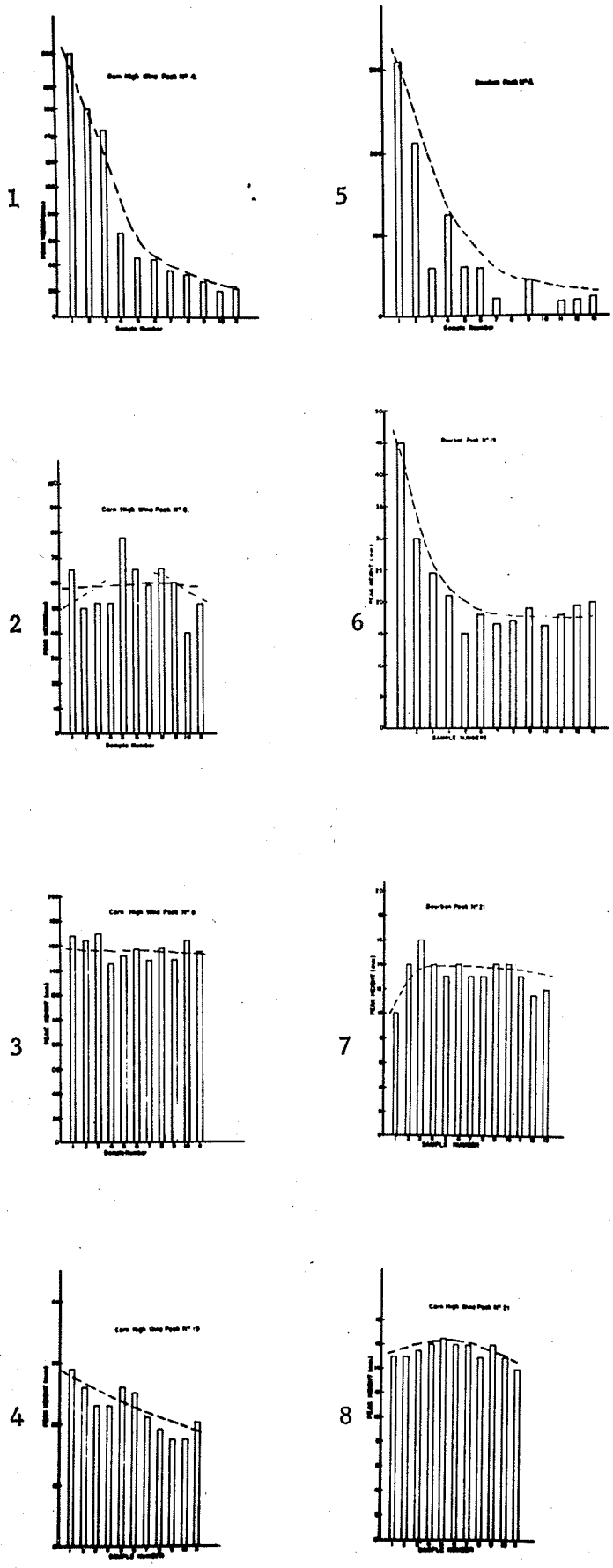

Figure 31.

SUMMARY AND CONTRIBUTION TO KNOWLEDGE

I. Flavors consist of complex multicomponent mixtures which exert their strong influence on our senses at extremely low concentrations. Their detailed analysis is therefore a difficult task. Gas chromatography has been shown to be ideally suited for analysis of flavors. Sensitive gas chromatographic methods were developed for the analysis of alcoholic beverages with the view to control the production variables and for securing high uniform quality. The combination of such methods with plant breeding programs is proposed for the development of new varieties of grain.

II. High resolution and high sensitivity are the basic requirements for application of gas chromatography to flavor analysis. High resolution was achieved by the combined effect of high column and liquid phase efficiencies.

1. Capillary columns have proven very successful in high resolution gas chromatography. A new method for preparation of such columns which provides the conditions for highly constant flow of the coating solution is expected to deposit highly uniform films of liquid phase on the walls of the capillary.

2. For higher detectability, columns of higher sample capacity are desirable in high resolution gas chromatography of flavors. Long narrow-bore packed columns were found to offer about 100 times higher sample capacity as compared to capillary columns. A method

for preparation of such columns in a single length was found to produce comparable results to columns prepared in short sections. A 37-ft narrow-bore packed column was found to perform comparably to a 100-ft 0.02" I.D. capillary column.

3. The sensitivity of FID systems is dependent on many factors including the design of the FID unit, the performance of the electrometer and the mode of assembling the components of the system. Sensitivities of the order of 10^{-12} gram/sec were achieved by modifiying a commercial FID unit, using a high quality electrometer and selecting a desirable mode of assembling. It was shown that environmental humidity can drastically reduce the sensitivity of FID systems.

4. Special problems are encountered when using high sensitivity detection systems for the analysis of flavors. The column bleed restricts the use of temperature programming to only low or moderate sensitivities; the trace constituents appearing on the slope of the matrix component cannot be detected and cross contamination of the samples during injection becomes a serious problem. New methods for eliminating the effects of these factors were developed and tested.

5. The identification of flavor components detected as gas chromatographic peaks is a difficult task because of the limited amount of material that can be isolated (if any). Mass spectrometry and retention data are the most sensitive methods available for the identification of organic compounds. The one stage combination

of gas chromatography and mass spectrometry is a powerful method for analysis of flavors. Such a system was assembled and "unswept pockets" in the vacuum line of the mass spectrometer were found to cause severe loss of chromatographic resolution. A new method for the elimination of the interference of the background spectra in a temperature programmed gas chromatograph-mass spectrometer system was developed. This method increased the sensitivity of such systems and will permit the use of practically all liquid phases. Strong solid support effects prevent the inter-laboratory use of retention data of polar compounds such as alcohols. The compilation of retention data of alcohols, esters, ketones and aldehydes was undertaken as a preliminary stage in the systematic identification of flavor components of alcoholic beverages. The standard addition method was found particularly suitable for the identification of trace constituents of complex mixtures revealed by temperature programmed capillary columns.

III. The application of the high sensitivity systems developed to the analysis of alcoholic fermentation by-products produced superior results to the results reported in the literature for similar samples.

1. Grape fusel oil was found to contain at least 150 compounds whereas only about 90 compounds have been reported in the literature.

2. The chemical composition of different alcoholic beverages is not easily correlated with their flavor. A graphic method which is intended to aid the correlation of the chemical composition of series of samples to their organoleptic definitions is proposed.

3. The direct analysis of samples of gin and related products with a high sensitivity FID system revealed the presence of 18 flavor constituents in gin and 40 constituents in a distillate from Juniper berries.

4. Dual channel gas chromatography combining electron capture and flame ionization detectors was found to be highly promising for the analysis of a complex fusel oil mixture.

APPENDIX 1

Col. No.	DIMENSIONS Length ft.	O.D in.	Tube mater.	SOLID SUPPORT Type	Mesh Range	Treatment	LIQUID PHASE Kind	Wt %	Method of coating
1	37	1/8	Cu	Chromosorb G	60-80	DMCS	Tergitol NP-35	1.0	Filter
2	50	"	"	"	"	"	Triton X-305	1.2	"
3	100	1/16 (I.D .02	S.S.	-	-	-	Tergitol NP-35		Coat plug
4	10	1/4	Cu		60-80	DMCS	Triton X-305	6.3	Filter
5	6	"	"	Chromosorb	"	Silanized	Carbowax 20M	9.1	Tray
6	6	1/8	"	Diatoport S	"	HMDS	Hallcomid M18+ Carbowax 600	9.5 1.3	Rot. Evap.
7	6	3/16	"	"	"	"	DEGS	9.1	Tray
8	6	1/4	"	Chromosorb W	"	"	THEED	20.0	Rot. Evap.
9	7	1/8	"	Diatoport S	"	"	Triton X-305	5.0	
10	7	1/8	"	"	"	"	"	"	"
11	7	"	"	"	"	"	Carbowax 20M	5.0	"
12	7	"	"	"	"	"	Armeen SD	10.0	"
13	"	"	"	Gas Pack F	"	Perfluoro-carbon impreg.	Hallcomid M-18-OL	5.0	"
14	6	"	"	Diatoport S	"	HMDS	Zonyl E-7	10.0	"
15	7	"	"	"	"	"	ZAIB	5.0	"
16	"	"	"	"	"	"	Di-(2 ethylhexyl) selacate	5.0	"

Description of Columns

APPENDIX 2

Test Mixtures Used for Compilation of Retention Data.

Mixture 1.
methyl alcohol
ethyl "
n-propyl "
n-butyl "
n-amyl "

Mixture 2.
n-amyl alcohol
n-hexyl "
n-heptyl "
n-octyl "
n-nonyl "
n-decyl "
n-undecyl "
n-dodecyl "
n-tetradecyl "

Mixture 3.
2-propanol
2-butanol
3-pentanol
2-hexanol
2-heptanol
2-octanol
2-nonanol

Mixture 4.
isopropyl alcohol
iso butyl alcohol
iso amyl "
act. amyl "

Mixture 5.
methyl formate
ethyl formate
n-propyl formate
n-butyl formate

Mixture 6.
methyl acetate
ethyl "
n-propyl "
n-butyl "
n-amyl "
n-hexyl "

Mixture 7.
n-amyl acetate
n-hexyl "
n-heptyl "
n-octyl "
n-nonyl "
n-decyl "

Mixture 8.
isopropyl acetate
isobutyl "
isoamyl "

Mixture 9.
Methyl propionate
ethyl "
n-propyl "
n-butyl "
n-amyl "

Mixture 10.
isopropyl propionate
isobutyl "
isoamyl "

Mixture 11.
methyl butyrate
ethyl "
n-propyl "
n-butyl "
n-amyl "

Mixture 12.
ethyl heptanoate
" octanoate
" nonanoate
" decanoate
" dodecanoate
" pentanoate
" hexanoate
" undecanoate

Mixture 13
ethyl dodecanoate
" tetradecanoate
" pentadecanoate
" hexadecanoate
" octadecanoate
" undecanoate

Mixture 14.
formaldehyde
acetaldehyde
propanal
butanal
pentanal

Mixture 15.
2-propanone
2-butanone
3-pentanone
3-heptanone
4-heptanone
2-octanone
2-nonanone

Mixture 16
n-hexane
n-heptane
n-octane
n-nonane
n-decane
n-dodecane

Mixture 17
n-decane
n-dodecane
n-tetradecane
n-hexadecane

Mixture 18.
n-pentane
n-hexane
n-heptane

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