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Title: QUANTITATIVE GAS CHROMATOGRAPHIC ANALYSIS OF

SYNTHETIC DAIRY CULTURE FLAVORS
_____

Abstract approved_____
                          Dr. R. C. Lindsay

The development of synthetic culture flavors for use in dairy

products such as sour cream, cottage cheese, butter, and butter-

milk has created the need for a rapid quantitative analysis for the

synthetic flavor concentrates. An internal standard gas chromato-

graphic method has been developed which is capable of $\pm 10\%$ accuracy

for each flavor compound in single sample analyses. The separation

of water, acetaldehyde, dimethyl sulfide, acetic acid, diacetyl,

propylene glycol, and acetone (the internal standard) was performed

with a column consisting of a 1/8 inch OD section of stainless steel

tubing 10 feet in length packed with 120-150 mesh Porapak Q plus a

stainless steel precolumn 1/8 inch OD and 10 inches in length packed

with 120-150 mesh Porapak R. The column was operated isother-

mally at $155^{\circ}C$ with a flow rate of 44 ml per min of nitrogen carrier

gas. A flame ionization detector was used.

In the course of the investigation, Porapak Q (available from

Waters Associates Inc., Framingham, Mass.) was evaluated. The

optimum mesh size under the conditions employed was the 120-150

mesh range. The efficiency of Porapak Q columns, as reflected by

the number of theoretical plates per foot for n-propanol and the reso-

lution of n-propanol and iso-propanol, was found to increase when

the column diameter was increased from 1/16 inch OD to 1/8 inch

OD to 1/4 inch OD.

Quantitative Gas Chromatographic Analysis
of Synthetic Dairy Culture Flavors

by

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QUANTITATIVE GAS CHROMATOGRAPHIC ANALYSIS
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INTRODUCTION

The formulation of synthetic culture flavor concentrates for use in dairy products has created the need for a rapid, quantitative analytical method to ensure the desired uniform flavor in the final products. Such a method is also desirable for studies of the influence of storage time and conditions on the stability of the flavor concentrates (with respect to degradation or chemical reaction of the flavor compounds) and the flavored products.

Gas chromatography possesses the required speed and sensitivity and is well suited to the quantitative analysis of mixtures of volatile compounds. Until recently, however, a column packing material which would provide adequate separation of the constituents of a typical synthetic culture flavor was not available. Porapak, a porous polymer bead packing available from Waters Associates, Inc., was recently introduced as a column packing material and found to possess unique properties which appeared promising for the analysis of synthetic culture flavor concentrates.

The purpose of this investigation was to evaluate the porous polymer packing materials for possible applications in flavor analysis and, specifically, to develop a method for rapid quantitative analysis of synthetic dairy culture flavor concentrates.

REVIEW OF LITERATURE

Synthetic Culture Flavor Concentrates

Extensive work on the separation and identification of the volatile flavor components of high quality butter cultures (19) has led to the development of synthetic flavor concentrates to be added to various dairy products including buttermilk, sour cream, cottage cheese, and butter (20). Interest in these synthetic flavors is increasing because of the difficulty involved in manufacturing and marketing uniform cultured products with the pleasing, well-balanced flavor and aroma of high quality cultures. Flavor compounds included in the formulations are acetaldehyde, dimethyl sulfide, acetic acid, diacetyl, and lactic acid in a solution of propylene glycol and water. These concentrates have been shown by taste panel studies (20) to impart desirable culture flavors to the appropriate products. However, the concentration, both relative and absolute, of these flavor compounds has been shown (18, 19, 20) to be very critical in maintaining the desired flavor balance and intensity. The flavor formulations for the various products as determined by Lindsay, Day, and Sather (20) are shown below.

Compound	Cultured butter	Sour cream	Cottage cheese	Buttermilk
	Concentration--mg/kg of product			
Lactic Acid	250.0	-	-	-
Acetic Acid	30.0	30.0	30.0	1,250.0
Acetaldehyde	0.2	0.2	0.2	0.5
Dimethyl sulfide	0.08	0.025	0.025	0.025
Diacetyl (low level)	0.5	1.0	1.0	1.0
(high level)	2.0	2.0	2.0	2.0
pH of product	5.2	4.6	5.2	4.6

Survey of Available Quantitative Methods

Acetic Acid. Acetic acid may be easily determined by titration with standard base provided that no other acids are present. In the butter flavor formulation, where lactic and acetic acids are both present, both can be separated and determined by the method of Wiseman and Irvin (32) which employs a saturated sucrose stationary phase on a Celite column with internal indicator. The column is eluted with acetone-skellysolve B solutions and the fractions titrated with standard base. This method was used successfully by Bills and Day (2) for the determination of the lower free fatty acids of cheddar cheese.

Acetaldehyde. The 3-methyl-2-benzothiazolone hydrazone colorimetric method originally described by Sawicki et al. (27) and as modified by Lindsay and Day (7) for determination of acetaldehyde in lactic starter cultures may be used when the synthetic culture concentrate is diluted to an appropriate acetaldehyde concentration.

Bills and Day (3) have used a gas chromatographic analysis employing the on-column trapping technique of Morgan and Day (24) for the determination of acetaldehyde and ethanol in lactic cultures. Their column was 0.318 cm OD by 305 cm long, packed with 20% 1,2,3-tris(2-cyanoethoxy)propane on 80-100 mesh Celite 545, and operated at 50°C.

Diacetyl. The Owades and Jakovac method (25) as modified by Pack et al. (26) for use in mixed strain cultures may be employed utilizing the purge system as described; or, preferably, an aliquot of the flavor concentrate may be added directly to the buffered hydroxylamine and the ferrous iron-dimethylglyoxime complex (λ_{max} = 530 mμ) formed in the normal manner. The superiority of this method over others frequently used has been demonstrated by Pack et al. (26).

Dimethyl Sulfide. Dimethyl sulfide is known to form coordination-addition complexes with bromine or iodine and the halides of mercury, platinum, and palladium which are used qualitatively for its characterization (5). Its quantitative determination, however, is not well documented. Toan, Bassette, and Claydon (29) have reported the determination of dimethyl sulfide by gas chromatographic analysis of headspace gas in water and milk, and Miers (23) reported its estimation in tomato products by trapping the dimethyl sulfide in concentrated sulfuric acid and later releasing it for gas chromatographic headspace analysis by diluting the solution to 5% acid.

Propylene Glycol. Propylene glycol may be determined iodometri-
cally (8) without interference from the other constituents in the
flavor concentrate. This method depends on the periodate oxidation
of propylene glycol to formaldehyde and acetaldehyde (31) along with
the formation of iodate ion. With potassium iodide, the excess of
periodate and the generated iodate form triiodide ion which is ti-
trated with standard potassium thiosulfate solution. The amount of
periodate used, and, hence, the amount of propylene glycol is calcu-
lated on the basis of the difference in the amount of titrant required
for the sample and a periodate blank.

Gas Chromatography

Quantitative Techniques

Quantitative gas chromatographic data is obtained by some
measurement of peak height or peak area, or an integration of the
analog detector signal. McNair and Bonelli (22) have shown that
electronic digital integration is superior to area calculation by tri-
angulation, cutting and weighing the chart paper, and ball and disc
mechanical integration. Unfortunately, the cost of such electronic
integrators is quite high, so the other methods are still in wide-
spread use.

In a quantitative gas chromatographic analysis, some type of

calibration must be used to relate the recorder response to the actual weight of material being eluted. Three methods of achieving this calibration are direct calibration, area normalization, and the use of an internal standard (6, 22).

To calibrate directly, blends of components in question are prepared, chromatographed, and the values of the peak heights or areas are then plotted against the known weight of sample injected. The unknown sample is then injected and its peak height or area used to determine the weight of material from the standard curve. This method is capable of high accuracy, but precise control must be maintained on all column and detector variables such as carrier gas flow rate, hydrogen and air flow rates (hydrogen flame detector), column and detector temperatures, and sensitivity of electronics (electrometers, recorder, power supplies, etc.). The method of sample injection must be controlled so that the precise amount injected is known, and the column must be quite stable over a period of time.

The area normalization method gives only semi-quantitative results and is based on the assumption that the ratio of the area under the peak of a given substance to the total area under all peaks in the chromatogram, multiplied by 100, is equal to the percentage of the substance present. The method may be refined considerably by the determination and application of correction factors (7) which

take into account the relative detector response for each component.

The internal standard method nullifies most apparatus variables by using peak height or peak area ratios referred to an internal standard compound. The standard peak should be completely resolved, should be eluted close to the components of interest, and should be nearly the same size as the other peaks. Calibration with an internal standard is performed by preparing and chromatographing known weight ratios of the component in question and the internal standard compound. The component/standard area ratios are then plotted against the component/standard weight ratios. An unknown sample is handled in the same way; a known amount of internal standard is added to a given volume, and the mixture is chromatographed. Comparison of the sample component/standard area ratios to the curve gives the component/standard weight ratio from which the amount of the component may be calculated. The internal standard technique has the advantages that sample size need not be accurately known and reasonable accuracy may be achieved without exact reproduction of operating conditions from run to run.

Carroll (4) suggested utilizing the product of peak height times retention time as a quantitative estimation of peak areas in calculations. This method was used by Kepner, Maarse, and Strating (16) in connection with an internal standard for the determination of components in the headspace of beer and rum and was reported to give ±5% reproducibility.

The Development of Porous Polymer Packings

Early in 1966, Hollis (13) reported the development and testing of a number of porous polymers as gas chromatographic column packings. The polymers were synthesized from monomers including styrene, tertiary-butylstyrene, and ethylvinylbenzene crosslinked with divinylbenzene. The polymer beads had a very rigid and porous structure and were reported to possess the partition properties of a highly extended liquid surface without the problems of ordinary support polarity, liquid phase volatility, or freezing point which are normally encountered in gas-liquid chromatography. It was assumed that the solute molecules partitioned directly from the gas phase into the solid amorphous polymer. Successful separations reported for mixtures of C_1 to C_9 alcohols, C_1 to C_6 hydrocarbons, glycols, volatile organic acids, and fixed gases indicate the versatility of the new material. Other reported applications are the determination of trace amounts of water (14) and the determination of trace impurities in ethylene (33).

The characteristics of the polymer beads are altered by the addition of polar monomers in the synthesis, and there are currently six available types of Porapak[1] which vary in polarity. These are

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[1] Porapak is the registered trademark of Waters Associates, Inc., Framingham, Massachusetts, for its porous polymer bead packings.

designated as types P, Q, R, S, T, and N. Information concerning

the uses of the various types is available as a technical information

booklet from the manufacturer (10). Other similar materials now

available are Chromosorb 102 (Johns-Manville) (15) and Polypak

(F and M Scientific Division of Hewlett-Packard) (12).

Column Comparisons

A widely accepted method of comparing gas chromotographic

columns is the comparison of the number of theoretical plates ex-

hibited toward a specified solute under defined conditions of tempera-

ture, carrier gas flow rate, and sample size (1, 6). This value, N,

may be readily calculated from the formula $N = 16 \left(\dfrac{t_r}{W}\right)^2$, where

t_r is the retention time or distance and W is the base width of the

peak in the same units as t_r. The resolution between two closely

spaced peaks is another indication of efficiency. The resolution, R,

is determined from the relationship

$$R = \frac{2(t_{r2} - t_{r1})}{W_1 + W_2} \qquad (1, 6).$$

Also important in obtaining the maximum efficiency from a

column is the adjustment of the flow rate to its optimum value. This

is reflected by the effect of the flow on the height equivalent to a

theoretical plate, $H = \dfrac{L}{N}$ (1, 6), where L is the column length and N is the number of theoretical plates. Usually, when H is plotted against the flow rate or linear velocity, a value for the flow will exist for which H is a minimum. This is the optimum flow rate or linear velocity for a given set of conditions.

EXPERIMENTAL

Evaluation and Comparison of Porapak Columns

Column Preparation and Conditioning

Porapak Q, advertised as the most universal of the available

types, was obtained from Waters Associates, Inc. in the following

mesh sizes: 80-100, 100-120, 120-150, and 150-200 mesh. The

material was in the form of granular white beads which were free

flowing and easily packed into columns. Unless otherwise specified,

all columns used were packed in 0.125 inch OD x 0.085 inch ID type

304 stainless steel tubing which had been thoroughly cleaned by

drawing solvents through it with an aspirator. The solvents, listed

in the order of their use, were 150 ml petroleum ether, 100 ml con-

centrated nitric acid, 1000 ml distilled water, 100 ml very dilute

ammonium hydroxide, 2000 ml distilled water, and 150 ml acetone.

The tubing was dried; one end was loosely plugged with glass wool;

and the columns, supported inside an appropriate length of two inch

galvanized pipe, were packed with light tapping.

Initially, the manufacturer's recommended procedure of con-

ditioning newly packed columns for one hour at 230°C with gas flow

was utilized. Later in the work, however, changing retention times

were noted, and a two day conditioning period at 200°C with gas flow

was adopted to minimize the possibility of thermal degradation of

the polymeric packing material (advertised maximum temperature

250°C).

Optimum Conditions and Comparisons

The objective of this phase of the study was to determine

optimum conditions for the use of Porapak columns. The columns

prepared and tested are shown in Table 1 with the results of the

various tests. The optimum flow rate was determined for each

column in an Aerograph Model A-100 thermal conductivity gas

chromatograph at 150°C by the following procedure. The helium

flow, regulated by a two stage tank regulator, was adjusted to 5 to

10 ml per min and the flow rate measured with a soap film flowmeter

and stopwatch. After the flow rate had stabilized, 0.4μl of ethanol

was injected and chromatographed. After running a duplicate

sample, the flow was increased by 5-10 ml per min and the flow

measurement and injection repeated. In this manner a series of

injections were made over a range of flows which were normally

from 5 to 50 ml per min. Graphic determination of the optimum

flow rate required measurement of the retention time and base

width of the ethanol peaks so that the number of theoretical plates,

N, could be calculated for each flow rate where $N = 16 \left(\dfrac{t_r}{W}\right)^2$.

The height equivalent to a theoretical plate, H, was found by dividing

the column length in centimeters by the number of plates. These values of H were then plotted against their corresponding flow rates to give a curve from which the flow rate corresponding to the minimum value of H was taken as the optimum. This process was repeated for each column listed in Table 1.

The resolution values were determined using the optimum flow for each column at $180^{\circ}C$ for the n-propanol-isopropanol pair and at $230^{\circ}C$ for the mixture of 2-butanone and 3-methyl-2-butanone. The resolution was calculated from the equation

$$R = \frac{2(t_{r2} - t_{r1})}{W_1 + W_2} \quad .$$

Analysis of Synthetic Culture Flavor Concentrates

Gas Chromatographic Procedure

Preliminary observations with samples of a synthetic flavor concentrate for cottage cheese and sour cream showed that a Porapak Q column resolved all the components except dimethyl sulfide and acetic acid. This problem was overcome by installing a short precolumn of Porapak R which is more polar than Porapak Q. The Porapak R precolumn increased the retention time of acetic acid sufficiently to resolve it from the more non-polar dimethyl sulfide. The Swagelok union connecting the two columns was also packed with

Porapak R to eliminate dead volume.

The column used for the quantitative analysis consisted of a 1/8 inch OD ten foot long section of stainless steel tubing packed with 120-150 mesh Porapak Q with a stainless steel precolumn 1/8 inch OD and 10 inches in length packed with 120-150 mesh Porapak R. The column operating temperature was $155^{\circ}C$, and the flow rate measured at that temperature was 44 ml per min of nitrogen carrier gas. The injection port temperature was $190^{\circ}C$, and the detector oven was at $210^{\circ}C$. The instrument employed was an Aerograph Model 204-1B dual hydrogen flame detector gas chromatograph with the readout on an Esterline Angus Model E1101S recorder using a chart speed of 0.5 inches per min. The recorder zero and electrometer balance were carefully checked and adjusted, if necessary, before each run.

Internal Standard Preparation. Acetone was chosen as the internal standard because it was readily available in high purity and was resolved from components of the flavor mixture. Since preliminary observations by repeated chromatographic comparison with solutions of diacetyl had shown the apparent concentration of dilute solutions of both acetone and isopropanol to decrease slightly with storage, the internal standard solution was prepared fresh daily. The preparation consisted of weighing 0.0200 g of acetone in a capillary tube

as described below and making up to 100 ml with distilled water.

For accurately weighing 5 to 50 mg quantities of volatile liquids, a glass melting point capillary with one end drawn out to a fine tip was used. The empty tube, wiped free of lint and finger prints, was weighed on an analytical balance. The tube, held with a piece of glassine paper, was removed from the balance and filled by capillary action. The excess liquid was wiped from the surface of the tube, and after evaporation of the last traces of liquid from the outer surface, it was placed on the balance. Excess liquid was withdrawn (a few milligrams or tenths of milligrams at a time) from the tube by touching the fine tip lightly with a tissue until the desired weight was reached. The tube with its contents was carefully placed in a volumetric flask nearly filled with distilled water and crushed with a glass stirring rod. The stirring rod was then rinsed while being withdrawn and the solution made up to volume.

Sample Preparation and Injection. The solution to be sampled and the internal standard solution were cooled in ice water to lower the vapor pressures of the solutions and to minimize loss of volatiles in subsequent handling. One hundred μl of each solution was taken with separate 100 μl syringes (Hamilton Co.) and placed in a cooled 3 ml conical centrifuge tube with a Kel F stopper. The tube was then shaken for fifteen seconds on a vortex mixer to thoroughly mix the contents, and a sample of 0.5 to 0.6 μl was withdrawn with a 10 μl

syringe (Hamilton Co.) and injected into the gas chromatograph.

Preparation of Standard Curves. The standard curves were pre-pared for each component in the flavor concentrate. Peak area esti-mates were obtained by the method of Carroll (4) used by Kepner, Maarse, and Strating (16). This involved multiplying the peak height by retention distance (measured from the water peak on the Porapak column) for each component. The standard curves were obtained by plotting the area ratio (component/standard area estimates measured at predetermined attenuation factors) versus the concentration of the component in grams per liter. The average of at least two deter-minations was used for most data points on the standard curves.

Solutions of acetaldehyde (Eastman grade, Distillation Products Industries) were prepared by adding the cooled acetaldehyde to 10 ml of ice water in a 20 ml beaker on an analytical balance, weighing as quickly as possible, and rinsing the contents into a 100 ml volumetric flask, then making to volume with distilled water. The concentra-tions prepared were 0.958, 2.045, 2.908, 4.115, and 6.131 grams per liter. These solutions were sampled and chromatographed with the internal standard as described above to yield the standard curve for acetaldehyde shown in Figure 1.

Solutions of dimethyl sulfide (Eastman grade, Distillation Products Industries) were prepared in distilled water by weighing

the cooled liquid in glass capillary tubes as outlined above for the

internal standard preparation. The concentrations prepared were

0.150, 0.250, 0.350, 0.520, 0.613, 0.801, and 0.943 grams per

liter. These solutions were chromatographed in the usual manner

to yield the standard curve for dimethyl sulfide shown in Figure 2.

Solutions of acetic acid (reagent grade glacial acetic acid,

Du Pont) were prepared by weighing the appropriate amount directly

into a 100 ml volumetric flask on a triple beam balance and then

making up to volume with distilled water. The concentrations pre-

pared were 199.6, 307.0, 400.0, 500.4, and 600.0 grams per liter.

These solutions were chromatographed to yield the standard curve

for acetic acid shown in Figure 3.

Solutions of diacetyl (Eastman grade, Distillation Products

Industries) in distilled water were prepared in the same manner as

described for acetaldehyde. Concentrations prepared by this

technique were 40.00, 35.00, 30.00, and 25.00 grams per liter.

Concentrations prepared by volumetric dilution of the above solutions

were 20.00, 15.00, 10.00, 5.00, and 1.00 grams per liter. The

standard curve for diacetyl is shown in Figure 4.

Solutions of propylene glycol (Matheson Coleman & Bell) in

distilled water were prepared by weighing the viscous liquid directly

into a 100 ml volumetric flask on a triple beam balance, then making

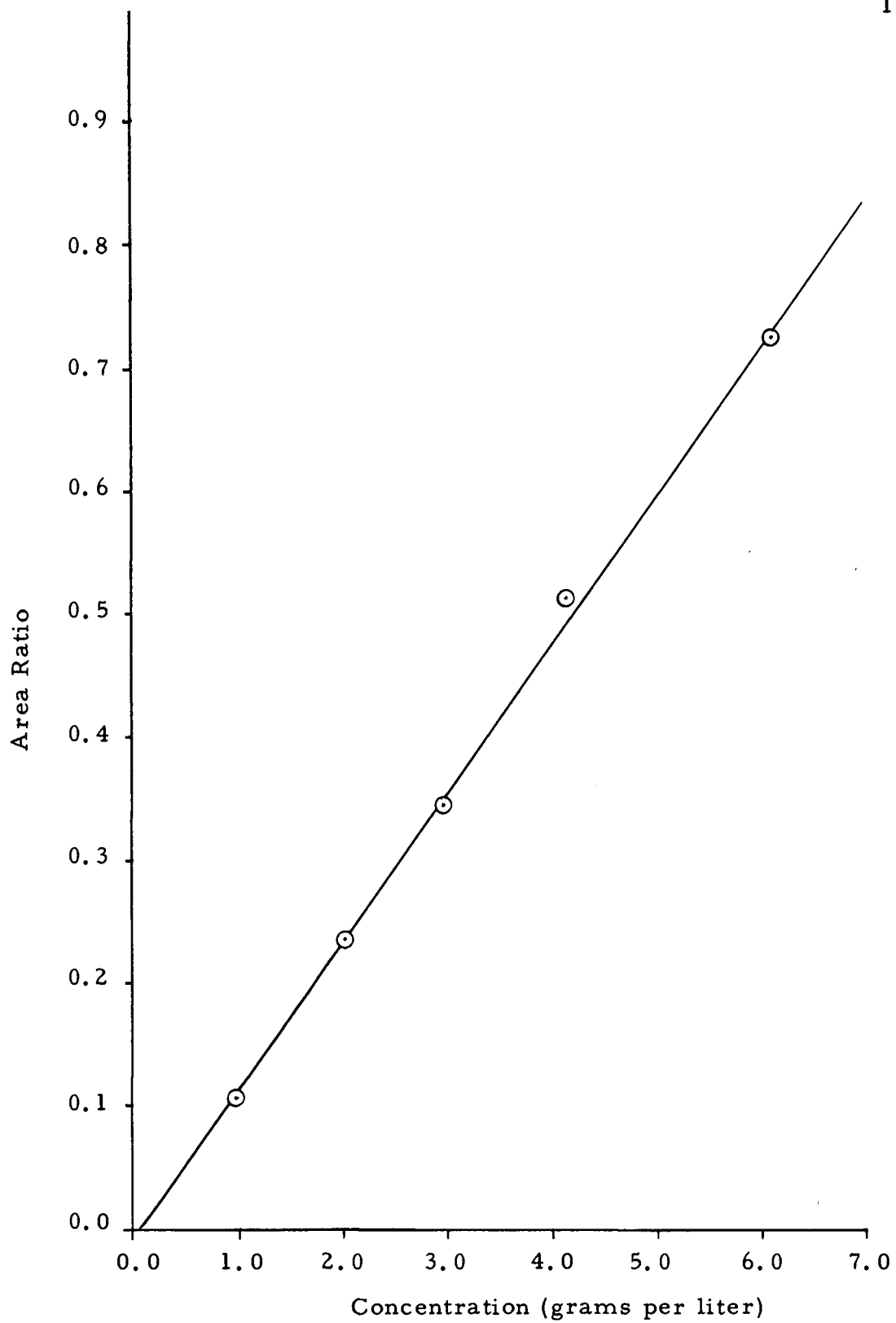up to volume. Figure 5 shows the propylene glycol standard curve.

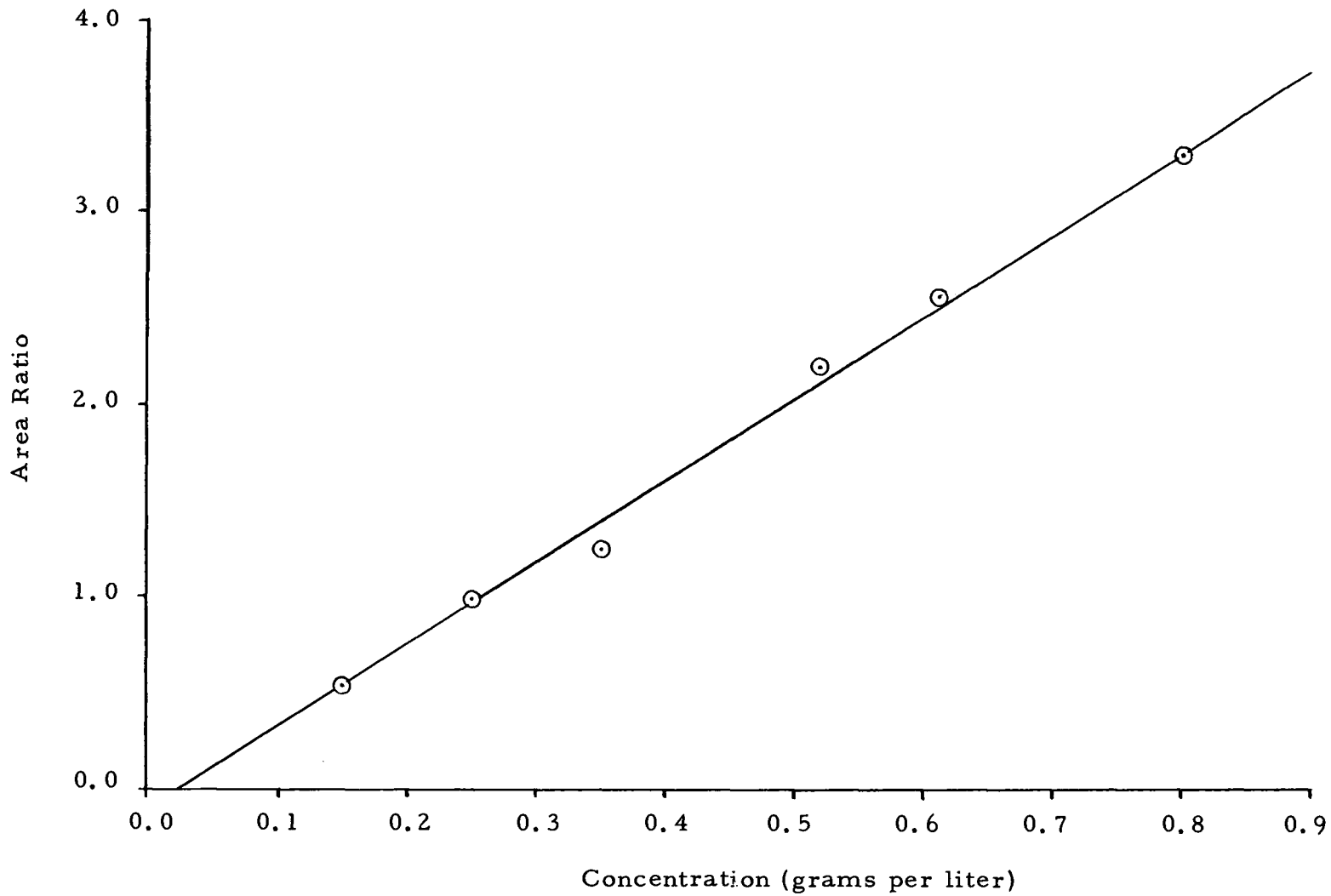Figure 1. Standard Curve for Acetaldehyde.

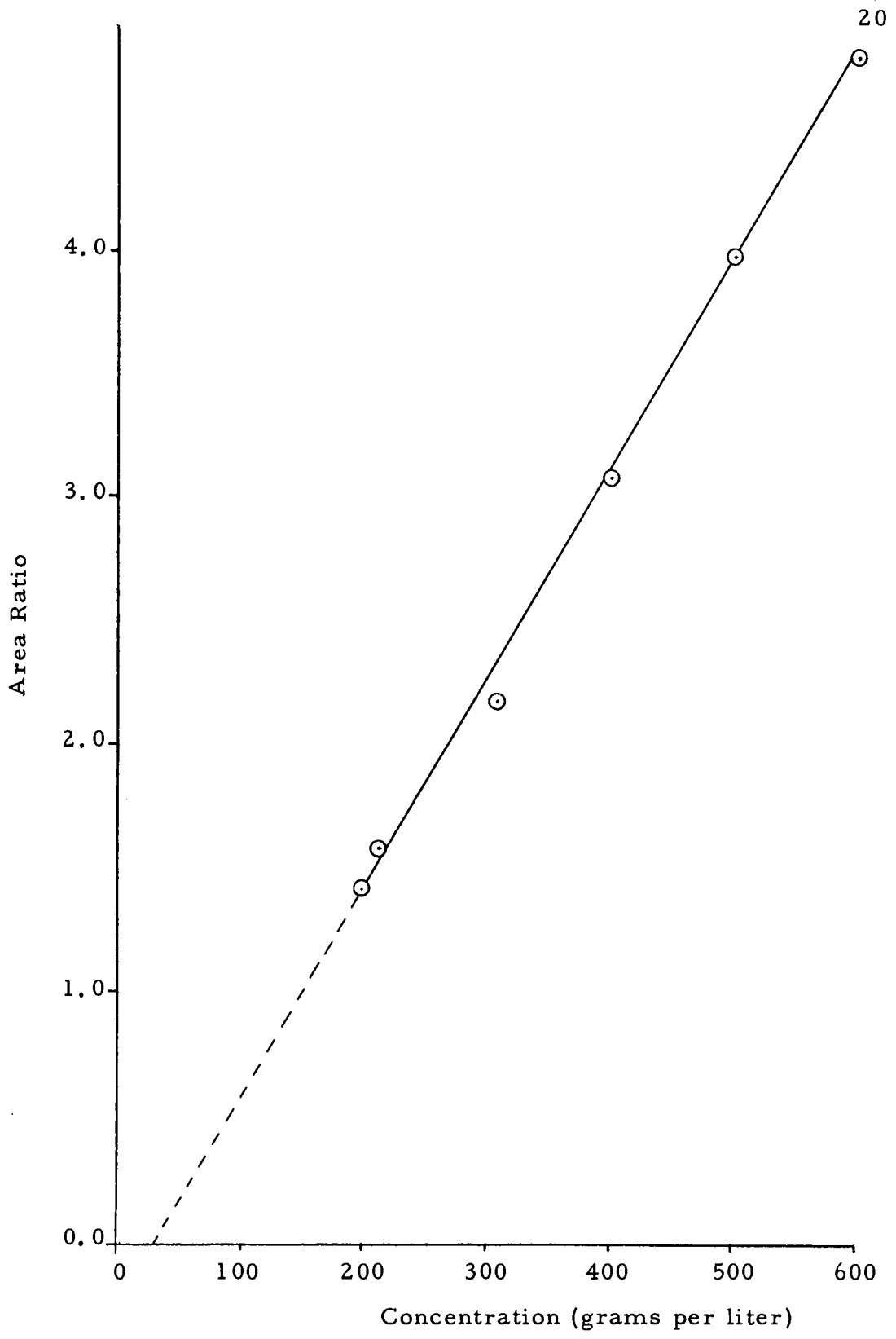Figure 2. Standard Curve for Dimethyl Sulfide.

Figure 3. Standard Curve for Acetic Acid.
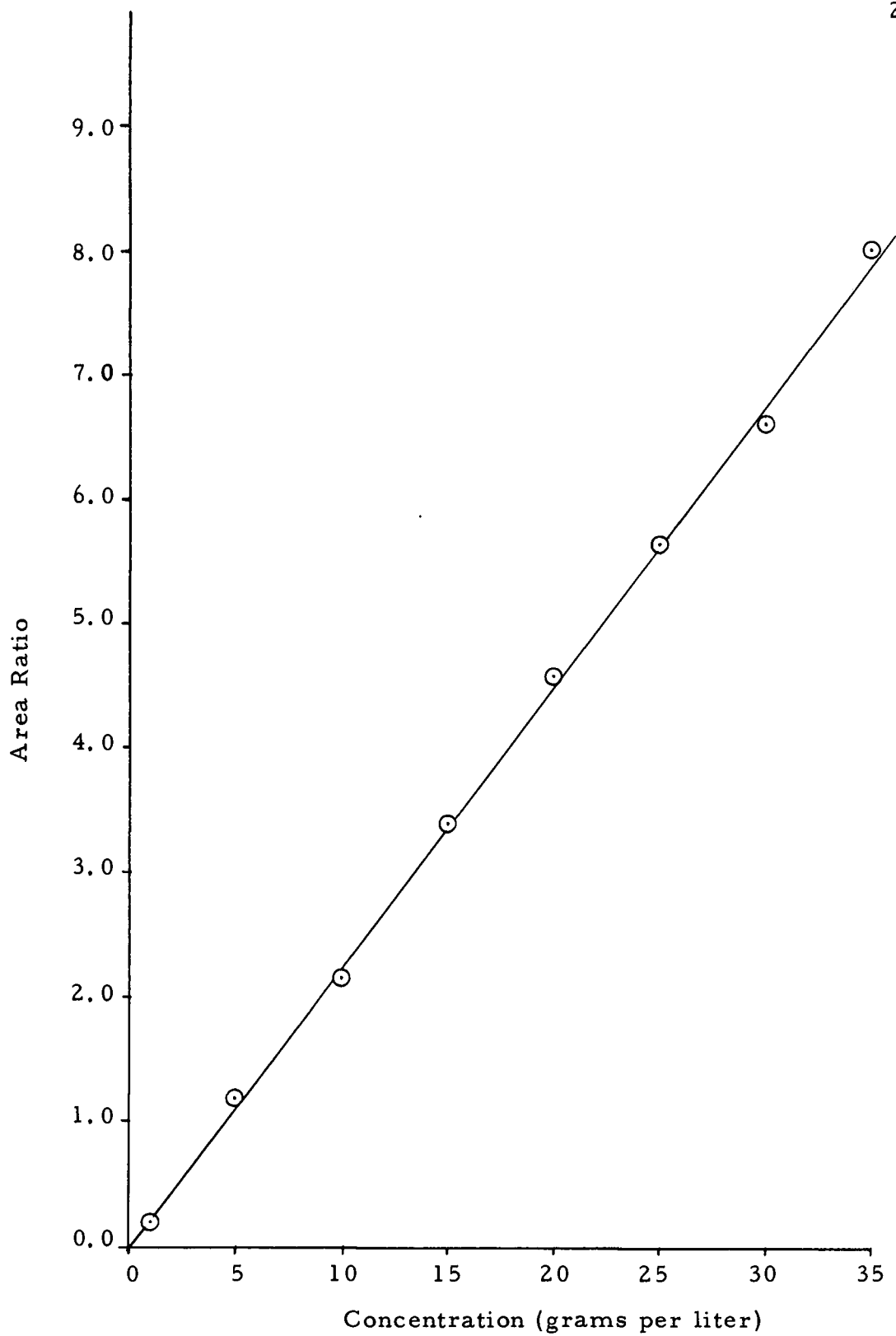
Figure 4. Standard Curve for Diacetyl.
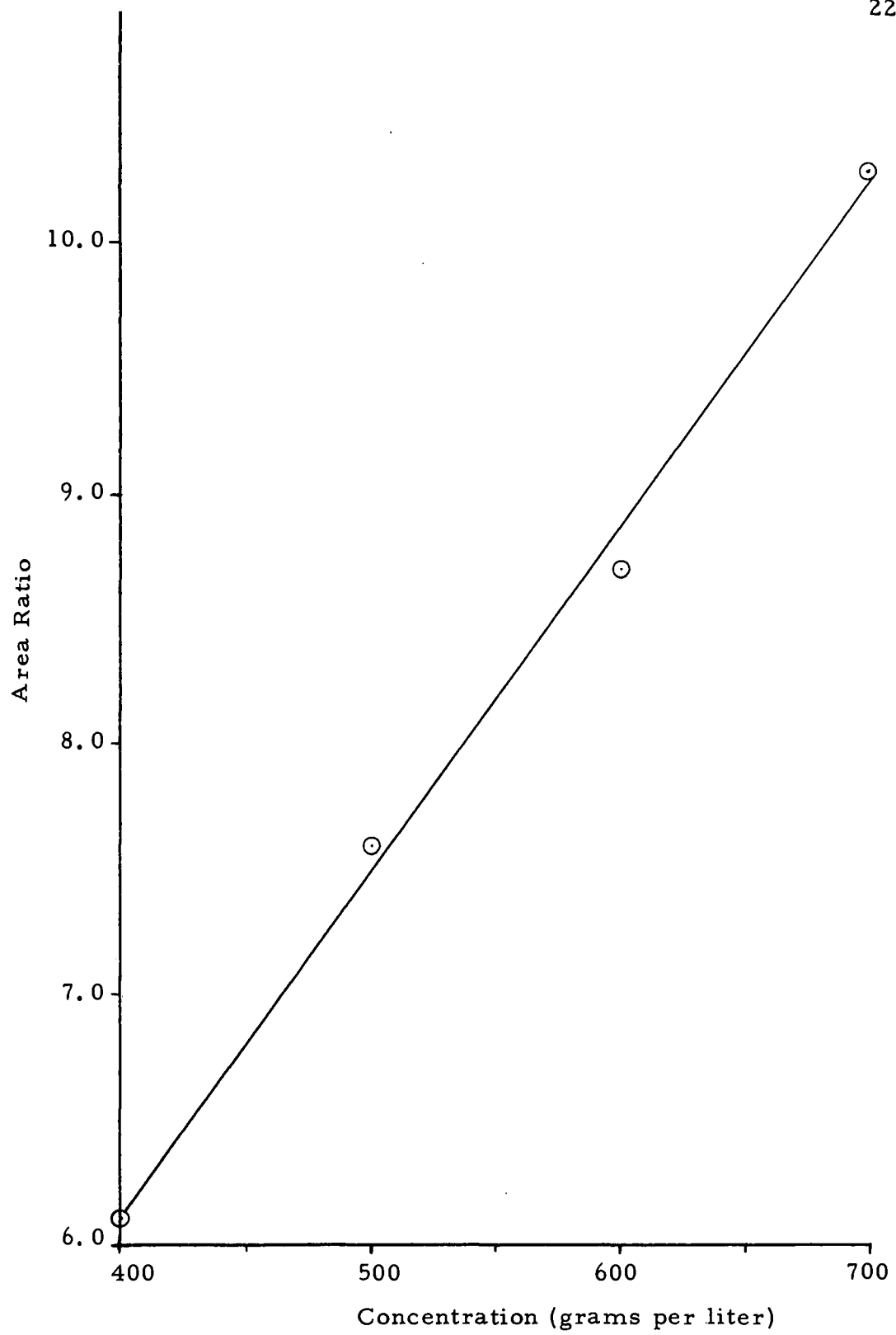
Figure 5. Standard Curve for Propylene Glycol.

Samples Analyzed. Six lots of synthetic culture flavor concentrate, including five for cottage cheese or sour cream (A, B, C, D, E) and one for butter (F), were available for analysis.

Sample	Months of storage	Comments
A	0	Freshly prepared
B	1.5	Stored at 5°C
C	15	Stored at 5°C
D	15	Stored at room temperature (21-23°C)
E	26	Stored at room temperature
F	11	Stored at 5°C

The samples ranged from a freshly prepared sample to one stored for more than twenty-six months. Four of the samples had been refrigerated and two stored at room temperature. The six samples were analyzed by the internal standard gas chromatographic method described above. The component/standard area ratios were calculated as previously described, and the corresponding concentration of each component was determined from its standard curve. The lactic acid in the sample of butter culture flavor was flushed from the column by reversing the gas flow after the analysis had been completed. The same samples were also analyzed for diacetyl, acetaldehyde, and acetic acid utilizing chemical methods as described below.

Chemical Methods

Diacetyl Determination. The iron-dimethylglyoxime color reaction of the Owades and Jakovac (25) method for diacetyl determination was used. The reagents and procedures were as described by Pack et al. (26), except that an aliquot of the concentrate, diluted to contain 100 μg or less of diacetyl, was added directly to the buffered hydroxylamine solution instead of utilizing the purging process. Suitable controls were run to insure that the other components in the flavor concentrate did not influence the results.

Acetaldehyde Determination. The 3-methyl-2-benzothiazolone hydrazone method of Sawicki et al. (27) was used as described by Lindsay and Day (17), except that an aliquot of the flavor concentrate calculated to contain 5 to 50 μg of acetaldehyde was substituted for the cultures in the purging apparatus. Control tests showed that the flavor concentrate could not be analyzed by direct addition to the reagents as in the case of the diacetyl determination because propylene glycol gave rise to color forming products when present during the ferric chloride oxidation.

Acetic Acid Determination. Acetic acid was determined in the five samples for cottage cheese or sour cream. One hundred μl aliquots (100 μl Hamilton syringe) were diluted in 25 ml of water and titrated

to a phenolphthalein endpoint with 0.1299 N NaOH which was pre-
pared, standardized, and stored as directed in the Food Chemicals
Codex (8). A 10 ml burette graduated to 0.05 ml was used for the
titration.

Propylene Glycol Determination. Because of its insignificance as a
flavor compound, no effort was made to precisely determine the
propylene glycol content of the flavor concentrates. The iodometric
determination of propylene glycol as outlined by the Food Chemicals
Codex (8) was carried out on two 600 g per liter solutions of propy-
lene glycol in water. The same solutions were analyzed by the gas
chromatographic procedure for comparison.

RESULTS AND DISCUSSION

Preliminary Considerations

The need for a rapid quantitative analysis for the synthetic culture flavor concentrates is evident when one considers the difficulty involved in accurately preparing and storing solutions of extremely volatile compounds such as acetaldehyde (B. P. 21°C) and dimethyl sulfide (B. P. 37°C). Furthermore, an undetected error in formulation could result in large volumes of poorly flavored products reaching the market with the attendant economic consequences. The diversified nature of the components in this flavor mixture places stringent requirements on a gas chromatographic analysis. Common column packing materials could probably have been used for this analysis, but much more experimentation with various combinations and conditions would surely have been necessary. The ability to separate a wide variety of volatile low molecular weight compounds at easily maintained temperatures and still allow the elution of compounds such as acetic acid and propylene glycol under similar operating conditions is a valuable characteristic of the porous polymer packings. Furthermore, duplication of columns should be much simpler where no coating of a solid support is involved and no liquid phase is present to bleed from the column. Although some problems with Porapak were encountered in this study, these appear to have been satisfactorily solved.

Evaluation and Comparison of Porapak Columns

Column Conditioning

Early in the work with the synthetic culture flavor concentrate,

an increase in retention times from day to day was observed with

the Porapak columns. These increases seemed to continue indefinite-

ly and eventually rendered the columns completely unusable. When

one of these discarded columns was unpacked, the Porapak was found

to be a light tan color and possessed an unpleasant odor. Correspon-

dence with the manufacturers (21) disclosed similar results in their

laboratories. The manufacturers had traced the problem to the

presence of free diethylbenzene (not completely removed in the

manufacturing process) in the polymer. The manufacturer recom-

mended washing the Porapak four or five times with liquid acetone,

then heating the columns packed with the dried, washed material

while purging with inert gas for a period of 48 to 72 hours. This

procedure was tried, together with keeping the conditioning tempera-

ture and maximum operating temperature of the columns below

210°C, and was found to yield columns with excellent stability for at

least the period tested (about three months). This treatment also

greatly reduced column bleeding as evidenced by the reduced back-

ground signal from the detector and the small amount of baseline

drift when temperature programming.

Optimum Conditions and Column Comparisons

Since the porous polymer packing materials had not been widely
used, little information was available concerning the optimum mesh
size, column dimensions, and operating conditions. It was thus
deemed desirable to attempt to define some of the properties of
Porapak columns and the conditions which would produce high effici-
ency.

While the method used for the determination of the optimum
flow rates is recommended for normal packed columns, in most
cases only a minimum desirable flow rate could be firmly established
for Porapak columns. As can be seen in the sample plot in Figure
6, a minimum value of H was not sharply defined, although it is
clearly desirable to use a flow greater than 30 ml per min. In prac-
tice the flow could not be increased indefinitely since the maximum
operating pressure for the apparatus employed was 100 psi. This
pressure was limiting for columns packed with the finer mesh materi-
als. For example, the maximum flow attainable for a 1/8 inch x 5
foot 150-200 mesh Porapak column was about 40 ml per min at 150°.

Due to thermal expansion, the Porapak columns exhibit a con-
siderable decrease in flow at constant pressure as the temperature
is increased (13). This decrease is greater than normally encoun-
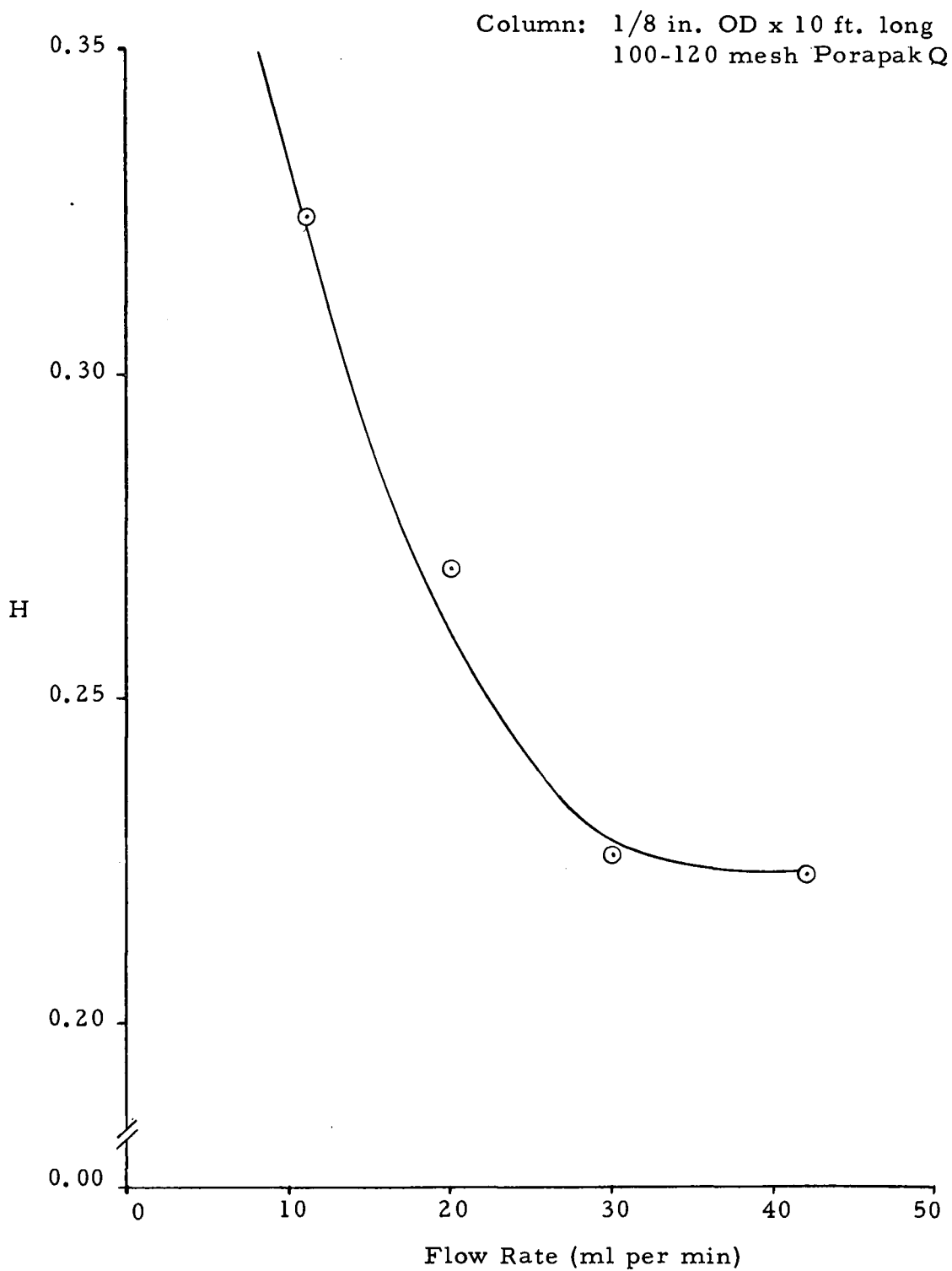tered with other column packings. For this reason, when using

Figure 6. Typical H vs. Flow Rate Plot.

Porapak columns, it is desirable to measure the flow rate at the operating temperature. The results of the optimum flow determinations for each column, along with the number of the theoretical plates and the resolution values for each, are shown in Table 1. The resolution values are strictly of relative significance in comparing the columns under given conditions, as are the numbers of theoretical plates. These methods of comparison have been considered inadequate by some authors (9, 10) for the comparison of different types of columns (open tubular versus packed columns, for example), but they are still valuable for the comparison of columns of the same type.

The two mixtures, propanol+isopropanol, and 2-butanone+3-methyl-2-butanone were chosen for the study of resolution value because they were closely-eluted pairs and were representative of types of flavor compounds which have been encountered in dairy products. Any other closely eluted pair of compounds could have served equally well for the comparative study.

From the results in Table 1, it is quite clear that the 120-150 mesh size of Porapak was the most efficient. The greatest total number of theoretical plates determined for propanol was 3,000, and was exhibited by the 1/8 inch x 10 foot 120-150 mesh column. Only the 1/4 inch x 5 foot 120-150 mesh column showed a greater efficiency in terms of plates per foot (304 vs. 300). A 1/8 inch x 10 foot

Table 1. Comparison of Porapak Q Columns.

Column	Optimum flow ml/min	Resolution Propanol-isopropanol	Theoretical plates Propanol	Resolution 2-butanone-3-methyl-2-butanone	Theoretical plates 3-methyl-2-butanone	Plates per foot - propanol
1/8" x 5' 80-100 mesh	40	1.87	790	2.80	815	158
1/8" x 10' 80-100 mesh	23	2.50	1210	3.93	1490	121
1/8" x 2 1/2' 100-120 mesh	45	1.25	309	1.80	297	123
1/8" x 5' 100-120 mesh	35	1.76	720	3.01	770	144
1/8" x 10' 100-120 mesh	42	2.50	1560	5.09	2600	156
1/8" x 2 1/2' 120-150 mesh	51	1.24	285	1.68	290	114
1/8" x 5' 120-150 mesh	40	2.06	1014	3.15	907	207
1/16" x 5' 120-150 mesh	21	1.44	400			80
1/4" x 5' 120-150 mesh	26	2.55	1520			304
1/8" x 10' 120-150 mesh	38	3.33	3000	5.61	3100	300
1/8" x 2 1/2' 150-200 mesh	38	1.62	550	2.54	617	220
1/8" x 5' 150-200 mesh	39	2.00	894	3.57	1110	179
1/16" x 5' 150-200 mesh	13	1.98	816	4.07	1720	163

column packed with 120-150 mesh Porapak was chosen for the synthetic culture flavor analysis since this was the most efficient combination obtained.

The differences in the number of theoretical plates, resolution values, and plates per foot for the three 120-150 mesh 5 foot columns 1/16 inch, 1/8 inch, and 1/4 inch OD show increases in efficiency with increasing column diameter. This is in contrast to a decrease in the maximum number of theoretical plates and plates per foot with increasing diameter for typical columns as published by Baumann and Gill (1). Perhaps this discrepancy can be explained by the physical difference in column packings. Hollis (13) suggested that the partition process with porous polymers is different from that in conventional column packings in that the components to be separated apparently partition directly into the solid amorphous polymer, while partition in conventional gas chromatography is from a gas to a solid adsorbent surface (gas-solid chromatography) or from the gas into a thin film of liquid on an inert solid support (gas-liquid chromatography). Partition into porous polymer beads appears to involve the entire packing particle as opposed to conventional thin film phenomena which involve only a very small portion of each particle. As the porous polymers are studied more closely, there will undoubtedly be modifications of current gas chromatographic theories to account for their unique behavior.

The relative retention data shown in Table 2 were kindly supplied by a colleague (28). These compounds are representative of a typical flavor mixture, and their separation illustrates the ability of a Porapak column to resolve mixtures of volatile, low molecular weight compounds. This mixture was also chromatographed on Apiezon L and Carbowax 20-M packed columns, and the advantage of Porapak was superior resolution of the more volatile components at somewhat higher and more easily regulated temperatures. A limitation of the Porapak column was the extremely long retention times of higher molecular weight compounds as compared to the other types of columns. In an unknown mixture there may be higher molecular weight compounds which would not be eluted from the column. For this reason it may be advisable to make preliminary runs with an unknown mixture on some other column type and reserve the use of a Porapak column for separations of the more volatile fractions.

The early elution of water in symmetrical peaks is a characteristic of Porapak columns. This prevents interference with the elution of other compounds and simplifies the quantitative determination of water in mixtures when using a thermal conductivity detector (14).

Table 2. Relative Retention Times on a Porapak Q Column.

Compound	t_R/t_R [a]	Compound	t_R/t_R
Acetaldehyde	0.044	Ethanol	0.513
Acetone	0.631	Propanal	0.634
Ethyl Formate	0.703	Formic Acid	0.725
Ether	0.762	2-Methyl Propanal	0.839
Acetic Acid	0.942	Butanone	0.950
Butanal	0.953	2-Methyl Propanol	0.962
Diacetyl	0.976	Ethyl Acetate	1.000
3-Methyl Butanal	1.206	Propionic Acid	1.227
2-Pentanone	1.251	Pentanal	1.275
Ethyl Propanoate	1.276	2-Pentanol	1.305
3-Methyl Butanol	1.369	Furfural	1.545
Ethyl Butyrate	1.573	2-Hexanone	1.582
Hexanal	1.609	Furfurol	1.612
2-Hexanol	1.640	2-Heptanone	2.054
Heptanal	2.107	2-Heptanol	2.161
Ethyl Hexanoate	2.730	2-Octanone	2.851
Octanal	2.925	2-Octanol	3.004
Phenethyl Alcohol	3.534	2-Nonanone	4.181
Nonanal	4.296		

[a] t_R/t_R = Relative retention time based on ethyl acetate = 1.000, elution time 20.5 min. Stainless steel column 1/8 inch OD x 10 feet in length packed with 80-100 mesh Porapak Q; Column temperature: 100° - 225°C programmed at 4°C per min; nitrogen flow: 25 ml per min at 100°C; Aerograph 204-1B.

Analysis of Synthetic Culture Flavor Concentrates

Gas Chromatographic Procedure

In a good quantitative gas chromatographic analysis, it is necessary for all the components to be completely resolved. Porapak Q alone could not resolve dimethyl sulfide and acetic acid, but the addition of a short precolumn of a more polar material, Porapak R, retarded the acetic acid sufficiently to effect the separation. The use of a precolumn was observed to contribute less to peak broadening than mixing the same ratio of Porapak R with Porapak Q prior to packing the column. Once the separation was achieved, the flow rate and temperature were adjusted to reduce the analysis time as much as possible.

Internal Standard Preparation. The chief advantage of the internal standard method is that it nullifies the influence of most apparatus variables such as small temperature or flow rate variations and changes in detector and recorder sensitivities. In addition, the absolute injection volume need not be precisely known or reproduced. Requirements of the method for maximum accuracy are that the internal standard be eluted near the components of interest, be completely resolved from all components of the mixture, and be present in about the same concentration as the components of interest.

Acetone was found to be completely resolved from the constituents of the flavor mixture, and its ready availability in high purity made it a logical choice for the internal standard.

In an early investigation to check the reproducibility of a diacetyl standard curve, the same solution of internal standard was used on three successive days, and each of the three successive standard curves was displaced above the previous one. This implies that the concentration of the acetone solutions was decreasing. The displacement of the curves did not occur when the internal standard was freshly prepared each day. Considering the possibility of evaporation, several different types of containers for the acetone solutions were tried. These included test tubes with teflon lined screw caps, 20 ml screw cap bottles with a 1/8 inch thick silicone rubber cap liner and a 3/16 inch hole through the cap through which a syringe could be inserted for liquid withdrawal, polyethylene bottles, and sealed 1 ml glass tubes which were broken open just prior to an analysis. All these containers seemed to perform in a similar manner, showing an apparent decrease in acetone concentration with time. Isopropanol solutions were also tested and similar results observed.

The method of preparing solutions of volatile liquids described in the experimental section was adopted as a rapid and convenient method of daily standard preparation. In the hands of the author,

this method has been at least as accurate as weighing a larger amount of acetone or other volatile material, then transferring and diluting to the desired concentration. This is presumably due to the evaporation which occurs while such solutions are being weighed and transferred.

The 100 μl Hamilton syringes were used for handling the samples because the high viscosity imparted by propylene glycol prevented accurate pipetting of the solutions.

Preparation of Standard Curves. Solutions for construction of standard curves were prepared in distilled water by weighing several different concentrations for each instead of merely making volumetric dilutions of a single solution prepared by weighing. The purpose of this procedure was to aid in cancelling any weighing errors, especially with the more volatile compounds. An amount of each component of the flavor concentrate was also weighed carefully in the same manner as the solutions for the standard curve and made up in propylene glycol instead of distilled water to check for a possible matrix effect (6). When these solutions were chromatographed, their area ratios fell on the standard curves constructed from the water solutions.

When the electrometer was properly balanced and zeroed, the attenuation ranges on the instrument employed proved to be sufficiently linear to allow corrections to be made for peaks measured at

different attenuations without noticeable deviations from the standard

curve.

Results of Analysis

Table 3 shows the analytical results. The five samples of

synthetic culture flavor concentrate for cottage cheese and sour

cream (A-E) were analyzed to determine the effects of storage time

and conditions on the levels of the flavor compounds present. The

sample of butter flavor concentrate was analyzed to demonstrate that

the analysis could be conducted and the lactic acid backflushed from

the column. Presumably, the amount of lactic acid could be esti-

mated by difference from the amount of acetic acid determined by

gas chromatography and the milliequivalents of standard base re-

quired to titrate an aliquot of the concentrate.

The chemical analyses for acetic acid, acetaldehyde, and

diacetyl were conducted to test their applicability to the flavor con-

centrate analysis and provide a basis for the comparison of the gas

chromatographic results. The results for acetaldehyde imply that

there are other components in the mixture which are purged into the

reagent and react to give high values in the 3-methyl-2-benzothia-

zolone hydrazone test. Propylene glycol was shown to be readily

oxidized to color-forming products when present during the ferric

chloride oxidation step. A possible explanation for the apparently

Table 3. Results from the Analysis of Six Synthetic Culture Flavor Concentrates.

Sample	Age (mo.)	Gas Chromatographic Analysis (grams per liter)					Chemical Methods (grams per liter)		
		Acetaldehyde	Dimethyl Sulfide	Diacetyl	Acetic Acid	Propylene Glycol	Acetaldehyde[a]	Diacetyl[b]	Acetic Acid[c]
A	0	1.38	0.232	19.6	312	657	1.63	17.6	297.5
B	.1.5	0.41	0.264	19.9	310	648	1.52	16.6	279.6
C	15	0.24	0.20	7.8	182	479	1.45	4.4	184.8
D	15	0.24	0.154	7.0	139	420	1.38	5.8	139.4
E	26	0.22	0.38	6.05	140	524	1.29	3.6	138.1
F	11	0.39	0.075	4.4	108	0	0.49	5.2	

Sour Cream and Cottage		Desired Concentrations (grams per liter)							
Cheese (A - E)		2.0	0.25	20.0	300.0	---			
Butter (F)		0.4	0.16	6.0	60.0	0			

[a] Determined by the 3-methyl-2-benzothiazolone hydrazone method.

[b] Determined by the iron-dimethylglyoxime colorimetric method.

[c] Determined by titration with 0.1299 N NaOH.

low diacetyl values by the iron-dimethylglyoxime colorimetric

method may be that the 15 min reaction time allowed between the

diacetyl and hydroxylamine reagent was not sufficient to convert all

the diacetyl to dimethylglyoxime.

Figure 7 shows a typical chromatogram obtained in the analysis

of sample A. The numbered peaks are identified as follows:

1 air

2 water

3 acetaldehyde

4 acetone (internal standard)

5 dimethyl sulfide

6 acetic acid

7 diacetyl

8 propylene glycol

A large peak, roughly 75% as large as the acetic acid peak, was

also observed to elute about 35 minutes after propylene glycol in the

aged samples but was not present in the more freshly prepared

samples A and B. It is speculated that this peak may represent a

condensation product which would account for some of the decreases

in acetic acid, diacetyl, and acetaldehyde observed on storage of the

flavor concentrates as shown in Table 3.

The relative errors found for single determinations by the

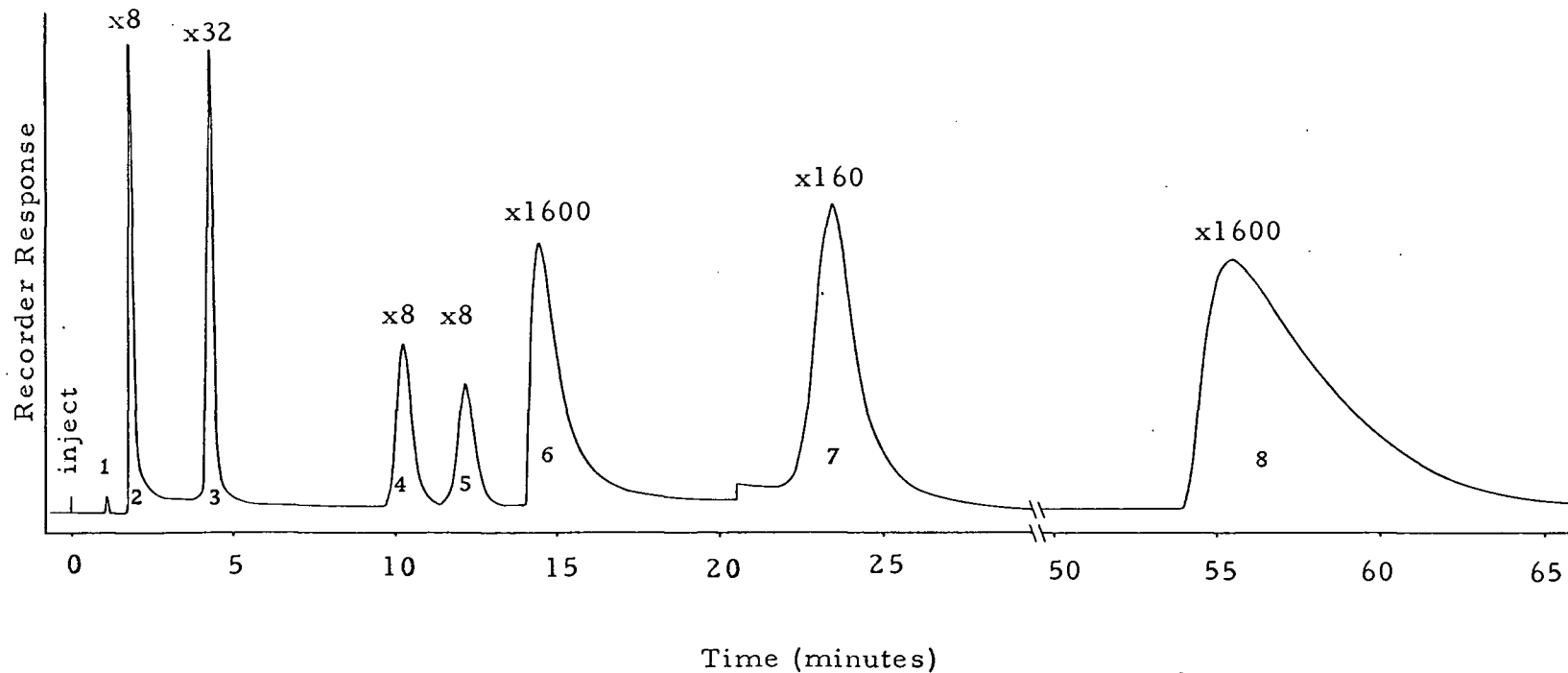quantitative gas chromatographic method utilized were between 5 and

Figure 7.   Chromatogram of Synthetic Culture Flavor Concentrate, Sample A.

10% for acetaldehyde, dimethyl sulfide, diacetyl, and acetic acid, but varied from 5 to 15% for propylene glycol. While gas chromatography is reported to be capable of greater accuracy (22), it was not deemed necessary for this application. For example, the difference threshold of diacetyl in cottage cheese has been shown (11) to be 1.4 ppm which would correspond to an error of about 7% since the concentrate is diluted by a factor of ten thousand in the finished product.

The large concentration and long retention time of propylene glycol compared to the internal standard make its determination less precise than the others. If one desired to know the propylene glycol concentration more precisely, the sample could be run at a higher temperature, and if a thermal conductivity detector were used, water could also be determined. In preliminary investigations the conclusion was reached that the available thermal conductivity detectors were not sufficiently sensitive to give accurate analyses of the entire flavor mixture without overloading the columns with respect to acetic acid and propylene glycol. Ideally, one could use an instrument which had both a thermal conductivity detector and a flame ionization detector. In this way the water content of a sample could be determined in the same analytical run as the other components.

SUMMARY AND CONCLUSIONS

Porapak, a porous polymer packing material for gas chromato-
graphic columns, was evaluated for possible application to flavor
analysis. The optimum flow rate for each column prepared was
determined. The 120-150 mesh size was found to produce the most
efficient columns, and column efficiency was shown to increase with
increasing column diameter. Porapak was found to be outstanding
in its ability to separate extremely volatile low molecular weight
organic compounds which are difficult or often impossible to separate
with columns of other materials. Many higher molecular weight
compounds, however, were found to have extremely long retention
times even at high temperatures. In some cases very short Porapak
columns may be used to effect some of these separations.

A quantitative gas chromatographic analysis was developed to
be used for synthetic dairy culture flavor concentrates composed of
acetaldehyde, dimethyl sulfide, diacetyl, acetic acid, and lactic acid
in a solution of propylene glycol and water. The analytical column
consisted of a combination of Porapak Q and Porapak R; and an in-
ternal standard method, with acetone as the standard, was employed.
The accuracy was found to be better than $\pm 10\%$ for each flavor com-
ponent. This was considered sufficient for quality control and storage
studies. Since the components are all well separated, this method

should prove valuable for detecting compounds arising from degradation or interaction of the flavor compounds. The analysis is simple enough to be carried out in any laboratory possessing an isothermal gas chromatograph equipped with a flame ionization detector, and no other special equipment or highly trained personnel would be required.

The following conclusions were drawn from the findings of the investigation:

1. Porapak must be washed free of all residual chemicals from its manufacture and conditioned at least 48 hours to provide stable columns.

2. Porapak of the 120-150 mesh size was found to give the most efficient columns.

3. Column efficiency was found to increase with increasing column diameter with Porapak, in contrast to the more commonly used column packings.

4. Porapak columns offer very good resolution of volatile low molecular weight compounds and early elution of water with symmetrical peak shape compared to columns of other materials.

5. Since higher molecular weight compounds that would be eluted from conventional columns may be retained by Porapak columns, care should be exercised with unknown

samples.

6. Dilute aqueous solutions of isopropanol and acetone were found to decrease in concentration with time, even when stored in sealed glass containers.

7. An internal standard quantitative gas chromatographic analysis using a Porapak column was developed for synthetic dairy culture flavor concentrates.

8. Six samples of synthetic culture flavor concentrates were analyzed to demonstrate the usefulness of the method for detecting concentration changes due to aging.

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