

GAS CHROMATOGRAPHIC TRACE ANALYSIS OF WATER
IN ETHANOL AND ITS APPLICATION TO A
PROBLEM IN PAPER CHROMATOGRAPHY

by

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INTRODUCTION

Chromatography is a spatial separation of the components of a mixture brought about by repeated exchange of solute species between two physical phases. One of these phases, the immobile or stationary phase, is fixed in space, while the other, the mobile phase, moves relative to it. By way of an illustration, assume a vertical tube is packed with a powdered solid and that a multi-component solution is added to the column at the top. This solution flows downward through the powder and is followed by pure solvent which gives uninterrupted flow through the column. It is presumed that each solute shows an affinity for the solid and tends to adhere to it. This affinity is a function of the identities of the solute, the adsorbent, and the solvent. In any one adsorbent-solvent combination, it is dependent on the nature of the solute only. The measure of this affinity is the distribution coefficient, i.e., the equilibrium ratio of the concentration of the solute in one phase to that in the other. As the solution encounters solid devoid of any solute, there is a net transfer of solute from the fluid to the solid which continues until equilibrium is established. This reduces the amount of solute in the solution. As this solution passes on to contact more solid in its advance through the bed, the process is repeated. The solution is depleted of solute by deposition on the solid at the advancing fluid front. At the back of the solution zone

where solvent is being added to the column, the solution bearing solute in equilibrium with solute in the immobile phase is displaced by the solvent devoid of solute, and solute leaves the stationary phase to enter the solvent. The total effect is a deposition of solute in the immobile phase at the head of the advancing band and extraction at the back of the zone. In this way the various solutes migrate at different rates through the column. Ideally, the solutes are separated into clearly distinct zones on the column or appear in the washings from the column as successive solutions of individual solutes. The problem in chromatography is the determination of the factors which affect this separation. Use of a liquid as the mobile phase is designated by the term liquid chromatography. In the above illustration where the solutes are distributed between a liquid and a solid surface, the terms employed are solid-liquid or adsorption chromatography. If the flowing fluid is a gas, the system is referred to as gas-solid chromatography (GSC) or gas-adsorption chromatography.

A liquid may first be adsorbed on a solid so that the latter is viewed as merely a support for the liquid and renders it immobile. Here it is thought that the primary process involved is a liquid-liquid partition of solute molecules between an immobile and a mobile liquid phase. This has been called liquid-liquid chromatography, liquid partition chromatography, or simply partition chromatography. If the mobile phase is a gas, the process is called gas-liquid chromatography (GLC). It is preferable in any system of nomenclature to use a combination of words designating the physical

state of the phases involved rather than to imply any mechanism by which the separation occurs, e.g., adsorption or partition, since chromatography is often a combination of these. The extent and effect of the contribution of each is not clearly understood nor are they separable experimentally. The investigations reported here are concerned with paper chromatography where it is presumed that the interlocking network of cellulose fibers acts as a support for an imbibed, stationary aqueous phase. Gas chromatography is used as an analytical tool to study phenomena associated with paper chromatography.

In paper chromatography, the extent of migration of a solute in any chromatographic system is routinely reported in terms of the R_F -value which is the distance traveled by the solute zone center divided by the distance traveled by the mobile fluid front. Martin and Synge (1) derived

$$R_F = \frac{1}{1 + \alpha (A_S/A_M)} \quad (1)$$

where A_S is the cross-sectional area of the stationary liquid held by the inert support, A_M is the cross-sectional area of the mobile fluid phase and α is the equilibrium liquid-liquid distribution coefficient, i.e., c_S/c_M where c_S is the solute concentration in the stationary liquid and c_M is the concentration in the mobile phase. Their derivation is based on the assumptions that the composition of the liquid phases does not change during chromatography, that A_S and A_M are constant throughout the length of the paper traversed by the

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mobile fluid, that α is not concentration dependent, that equilibrium of solute between the two liquid phases is attained very rapidly, and that molecular diffusion of the solute in the direction of flow is negligible. Giddings and Keller (2), following a suggestion of Martin (3), consider R as the fraction of solute molecules in the mobile solvent at any one time and derive the same expression for R as given for R_F in equation (1). Indeed, the validity of the statement $R = R_F$ is subject to the assumptions of Martin and Synge plus the additional condition that the velocity of the mobile phase at the zone is the same as the solvent velocity at the liquid front. In a sense, this is not an additional independent assumption, for if (A_S/A_M) is constant, then no velocity gradients will exist in liquid-liquid systems. In gas chromatography (A_S/A_M) may be constant and there may still be velocity gradients by virtue of the compressibility of the mobile fluid. Giddings, Stewart, and Ruoff (4) have shown that the amount of mobile fluid per unit area of paper shows an appreciable increase from the solvent front to the solvent source, i.e., A_M is far from constant along the path of fluid flow. The velocity at the fluid front exceeds the fluid velocity at the solute zone by ten to twenty per cent (2). There are few remarks to be found in the literature concerning any variation of A_S for liquid-liquid systems. The effect of nonuniform distribution of immobile liquid phase and a subsequent variation of A_S along the chromatographic column has been investigated in gas-liquid chromatography by Keller, Bate, Costa, and Forman (5). Keller and Stewart (6) concluded that as long as α of equation (1) is constant, then a column with a nonuniform distribution of liquid partitioner

behaves the same as a column with the same amount of liquid uniformly distributed over the support throughout the column as far as zone position is concerned. The conclusion they reach is that there is an average value of A_3 which can be used to describe the extent of zone migration. The heart of the matter rests in the definition of such average values, the determination of when these average values should be used, their calculation and permissible deviation. There is every indication that such averages cannot be used in the calculation of column efficiencies since these seem to depend on a detailed knowledge of the distribution of partitioner (7). The question as to whether this approach is applicable to liquid-liquid systems is still subject to experimental test although theory would indicate this to be the case. Indeed the tremendous success of chromatography may rest upon behavior depending on such average values rather than upon the particular details of the physical situation.

The usual procedure in paper chromatography, where the immobile phase is imbibed water, is to saturate the liquid to be used as the mobile fluid with water, to place some of this in the chromatographic chamber to saturate its atmosphere, and to suspend the papers in this atmosphere for a period of time prior to performing the chromatography. During this equilibration period, the water of the paper has an opportunity to absorb this vapor so that the mobile and immobile phases both reach an equilibrium composition. Then, during the actual chromatography, there should be no net exchange of liquid between the two phases. Improper equilibration may lead to anomalous results such as the appearance of two zones for a single

solute species as was pointed out by Keller and Giddings in their review (8). Suppose that the mobile phase is not saturated with the immobile phase. It may be presumed that as the fluid migrates down the paper, it extracts immobile phase until it reaches an equilibrium concentration and in so doing dehydrates the paper so that A_g varies in the direction of fluid flow. The observations of Keller, et al (5) bear this out for gas chromatographic columns. Here liquid partitioner evaporates from the packing until the carrier gas is saturated. The net effect is to introduce a discontinuity in partitioner concentration within the column, i.e., it suddenly changes from a low value to the concentration of liquid on the original packing which may be quite high (a change from five per cent liquid load to thirty per cent). Beyond the discontinuity, the carrier is saturated and no further evaporation occurs. Consider, for example, anhydrous butanol flowing down a strip of paper. As the alcohol encounters the water phase, it will extract water from the paper dehydrating the paper and reducing A_g . Upon becoming saturated, extraction stops and A_g assumes its original value. This introduces a discontinuity in the amount of immobile phase closely analogous to the situation in gas chromatography. However, an additional effect in liquid-liquid systems is that the mobile phase in advance of the discontinuity is saturated with water while back of the discontinuity it is anhydrous or nearly so. Hence, a discontinuity in mobile phase composition is introduced, and the partition coefficient may assume different values in these two regions. Add to this the variation in A_M and there is indeed a complicated system full of the promise of

strange behavior.

Another effect which manifests itself is that no support is completely inert. The support must, by definition, hold a liquid phase strongly enough to render it immobile. It is presumed that this affinity would also be exhibited toward the solutes. Keller and Stewart (6) and Bate (9) prefer to write equation (1) as

$$R = \frac{1}{1 + \alpha (A_L/A_M) + \beta (A_S/A_M)} \quad (2)$$

where $A_L = A_S$ of equation (1), A_S is the cross-sectional area of the solid support, and β is a distribution coefficient for solid adsorption. The term $\beta (A_S/A_M)$ is a measure of the extent of participation of the solid in retention of the solute. Depending upon the values of α and β , this term is negligible if $A_L \gg A_S$. If, however, A_L is reduced so that the effect of liquid partition no longer overwhelms retention at the solid-liquid interface, then the solid surface term must be included. Keller and Stewart pointed out that if $A_S = kA_L$ then equation (2) takes the form

$$R = \frac{1}{1 + \alpha' (A_L/A_M)} \quad (3)$$

where

$$\alpha' = \alpha + k\beta$$

and includes partition and adsorption. In the region where the quantity of immobile phase has been reduced, R is governed by α' or by α and β , whereas in the unaffected region, R is governed by α .

It is also possible that the immobile phase will imbibe the mobile phase in the non-equilibrium situation.

In summary, a non-equilibrium between the fluid phases may introduce a discontinuity in the distribution of the immobile phase and a discontinuity in the value of α brought about by a change in the composition of the mobile phase and an increase in the participation of the support in retention by solid adsorption.

Much of this difficulty can be surmounted by proper equilibration of the two phases. This is easily done with mobile fluids which are immiscible with water by presaturation. This presumes that the equilibrium composition of mobile component is the same toward bulk water as it is toward the adsorbed water of cellulose, an assumption that has not been tested. It is known, however, that perfectly good chromatograms can be formed with mobile fluids which are miscible with water. As Lederer (10) has pointed out, it is difficult to understand the mechanism of such chromatograms using a model based on the distribution of solute between two immiscible liquids. Martin (11, 12) has proposed that the water held by the cellulose is a gel which differs from bulk water (13) and may be considered to be immiscible with solvents which, in the bulk, are completely miscible with water. Although intuitively satisfactory, Martin's liquid-gel model leads to conflicting ideas. It implies that the value of α for the distribution of a solute between the gel and an immiscible solvent such as chloroform, is identical with the α for the distribution between bulk water and chloroform as it might be determined in the laboratory with a separatory funnel. Yet,

it must also be assumed the gel is so different from bulk water that this imbibed water is immiscible with something like ethanol. There is complete ignorance of the composition of an ethanol-water solution which would be in equilibrium with this gel. Keller (11) found very poor paper chromatograms of the triphenylmethane series of dyes using absolute ethanol as the mobile phase. There was severe tailing and solute retention at the point of application. As the water content of the mobile phase was increased, tailing was reduced, the peaks became gaussian, and there was less retention at the origin. He proposed that the concentrated solutions of ethanol were dehydrating the paper. No direct experimental proof of this was ever given.

If indeed, the mobile phase extracts water from the paper, this should be detectable either by examining the water content of the paper after chromatography or by attempting to detect the water in the mobile phase which drips from the end of the paper. The first approach seemed difficult since it is a problem to determine the total water content of paper. The tenacity with which the water is held depends upon the amount present, and it is difficult to reproducibly dehydrate successive samples of paper to the same level. Also, the liquid remaining on the paper after chromatography is mobile phase held interstitially and both it and the immobile phase probably contain alcohol. There is no guarantee that total liquid loss from the paper after a chromatographic run is a measure of immobile aqueous phase remaining. Analysis of eluent fractions seemed easier, although it is necessary that a few per cent water in ethanol be determined and

that the analysis of 0.2 mls. or less be performed. This last condition is necessary since there is very little water in a strip of paper and an appreciable alcohol sample is likely to have extracted most, if not all of the water. Thus, subsequent samples will show a constant composition. Gas chromatographic analysis immediately suggested itself since it is rapid and can be performed on very small samples.

The development of a gas-liquid chromatographic method for the quantitative analysis of small amounts of water in the presence of alcohol involved several steps. These were the following: (1) the selection of a satisfactory support for the partitioning liquid; (2) the selection of a liquid for the immobile phase and its amount; (3) a decision as to the appropriate column length, operating temperature, flowrate, and sample size; (4) the correlation of measurements made from the chromatographic record with the composition of known samples; (5) arrival at an estimate of the precision of the analysis.

These questions are answerable only if considered with the nature of the analyses to be performed and the limitations of the equipment employed. The problem is to analyze representative samples of the solutions dripping from the end of a paper strip or column of cellulose pulp. Such samples are small, duplicate analyses are desirable, and the proportion of water is very small.

The record obtained from the gas chromatograph is a series of peaks registered on a strip of paper by a recorder (15). The properties of the peaks are to be correlated with sample composition. These peaks ought to be well separated, fairly sharp, and symmetrical

with a minimum of tailing, and yet broad enough to permit the measurement of areas. If the components appear too soon at the outlet end, peaks will be very high and likely to go off the record making area measurements impossible. Even if kept on the recorder scale, they are likely to be so narrow that their areas are difficult to measure with any degree of reproducibility. A problem with the analysis is that if the sensitivity of the machine is set so that the alcohol peak is on the scale, the water peak, if seen at all, is so small that it cannot be measured accurately. If, on the other hand, the sensitivity is changed to magnify the water peak, the huge alcohol peak is off scale. The equipment used has no automatic circuit attenuation device to reduce the response of the recorder for large signals (16), so that a unique necessity of the analysis is that the alcohol and water peaks be sufficiently separated so that there is time to change the sensitivity settings on the equipment and establish a new base line between the appearance of the peaks. Hence, appreciable retention times are necessary. A limit to extension of this procedure is the observation that the longer the material resides on the column, the flatter and broader the peak. This is not serious with the principal component, alcohol, but very small water peaks can easily be lost completely if the peak becomes so low and broad that it is not distinguishable from the random background circuit noise. Peak tailing adds to the peak broadness and tailing with water is notorious.

In gas-liquid chromatography, it is important that the support be inert toward the solute, i.e., $\beta (A_S/A_M)$ of equation (2) should be

negligible. This is especially difficult to achieve with polar solutes, e.g., compounds containing hydroxyl, carboxyl, amine groups, etc. Adsorption isotherms for solid surfaces are generally non-linear, i.e., β of equation (2) or α' of equation (3) are concentration dependent. This leads to very broad asymmetrical peaks with extensive tailing or "bearding" (3) and it is almost impossible to obtain reproducible quantitative results with such peaks (17). It has also been observed that with supports in the Chromosorb family, the peak height of the water increases with successive identical samples. Ottenstein (18) proposed that an active support tenaciously adsorbs the water as it passes through the column and reduces the peak. Later samples are depleted to a lesser extent so that the peaks are larger. Eventually the support should be saturated with water so that reproducible results are obtained. However, little faith can be placed in the consistency of this procedure since the adsorption is not completely irreversible. Ottenstein himself reported that if the calibration is repeated after an hour of standing, the first water peaks are again low. He concluded that the water was picked up and then slowly released. There are other examples of this in the literature (17).

There appears to be at least two different kinds of active sites on supports made of diatomaceous earth which includes the Chromosorb group (19). One type is acidic and attracts basic compounds, while the second type is thought to involve hydrogen bonding. Deactivation of this type of support may be attempted either by removal of these sites with different washes or by covering them. An

acid wash removes some iron and aluminum (1.6 per cent Fe_2O_3 and 4.4 per cent Al_2O_3 in Chromosorb-F (20)), and an alcoholic solution of a base is used to remove a "so-called" soluble layer (19, 21). The effect of treatment with alkali is not understood (22). This procedure is, at best, only partially effective in reducing participation by the support when polar compounds are chromatographed. Some workers (23) cover the active sites with gold or silver. Others add a small amount of material, appropriately called a "tailing reducer", to the immobile liquid; it is pictured as being more strongly adsorbed on the support than any solute chromatographed (17, 24). Supports may be first covered with a film of material such as a silicone before applying the partitioner (25, 26). Knight (27) reduced tailing of some compounds by saturating the carrier gas with water. It is difficult to see how this last technique could be applied to the analysis of aqueous samples.

The immobile liquids used in gas-liquid chromatography are generally arranged in some order of "polarity". The least polar are the saturated hydrocarbons, e.g., n-octadecane, while the polar liquids contain such functional groups as hydroxyl or ether linkages, e.g., polyethylene glycols. Although there is no good definition of "polarity", there is an intuitive feeling for the term among workers in the field. It has been observed that participation of the support is common when polar compounds, e.g., alcohols, acids, amines, are chromatographed on active supports coated with a non-polar liquid (28, 29). As the polarity of the partitioner is increased, the activity of the support is reduced. Apparently partitioner and solutes compete

for the active sites and the effect of partitioner on the solutes is reduced. It seemed that best results might be attained if a polar liquid were applied to a support of minimal surface activity, a feature inherent in the nature of the support or achieved by some deactivation process. Recent interest has been shown in fluorocarbons, e.g., powdered Teflon (18, 30), as representing minimal activity. Staszewski and Janak (31) have recently reported that Teflon is a suitable support for the chromatography of polar substances on non-polar stationary liquids. Symmetrical peaks were obtained for highly polar solutes, e.g., the lower alcohols. Ettre (32) does not recommend Teflon as a support except for highly polar solutes, a fact which argues against its general applicability. In the work reported here, a comparison was made between deactivated Chromosorb and a fluorocarbon, and indeed the latter was found superior, although difficult to manipulate. Even here, however, it is probably safer to assume that there is a combination of liquid solution and solid adsorption since, for water, tailing was never completely absent even at small sample sizes.

Guiding principles for the selection of a liquid partitioner to achieve specific, desired separations are still largely qualitative (33, 34, 35, 36, 37), although attempts have been made to arrive at a quantitative classification (38). As a general rule, polar liquids should be used to separate polar solutes. As the polarity of the liquid is reduced, polar solutes pass through the column more as a group, and there is a loss of resolution of the individual components. Water and ethanol are two very polar compounds which have similar

properties. Gas chromatographic analysis, particularly quantitative, for water in various samples has proved a challenge. Drawert (39, 40) categorically stated that the analysis of aqueous alcohol solutions cannot be accomplished by gas chromatography alone. He converted the water in the sample to hydrogen by passing the sample through a reactor containing calcium hydride at the inlet of the column. Kung, Whitney, and Cavagnol (41) used calcium carbide to produce acetylene. Ettre and Brenner (42) indicated that in some cases a molecular sieve can be used to entrap water in the sample before the mixture is passed onto the column.

Methods of eliminating water in the sample have been discussed by Cacace, Ikram, and Stein (43). Keulemans (15) recommended immobile phases in the glycerol and polyglycol family. Partitioners in this group have been closely examined by Adlard (34). Aqueous solutions of the low molecular weight ethers and alcohols have been resolved by Bodnar and Mayeaux (44) on triethylene glycol and polyethylene glycol-400. Murata and Takenishi (45) analyzed aqueous solutions of low concentrations of alcohols, nitriles, and aldehydes on polyethylene glycol or glycerol and obtained probable error of five per cent for the alcohols. Fox (46) determined micro amounts of alcohol in blood using glycol columns. Wesselman (47) examined the resolution of ethanol, water, and acetone on columns holding polyethylene glycol-400, diglycerol, dinonyl phthalate, Ucon 75-H-90000, and tricresyl phosphate and obtained the best results with a six foot column of 30 per cent (w/w) polyethylene glycol-400 on 30-60 mesh Chromosorb at 100°C. Van der Kleet and Wilcox (48) analyzed the volatile components in beer in

the parts per million range using eight foot columns of glycerine and Carbowax-1500 supported by acid and alkali washed 30-60 mesh Chromosorb of 1:4 (w/w) liquid load at 60°C. Kryacos (49) used two foot columns of 40 per cent (w/w) polyethylene glycol-400 on 30-60 mesh C-22 fire-brick at 60°C. for his determination of water in the products of the cool-flame combustion of hydrocarbons. Taira (50) used a one meter column of Carbowax-400 at 45° and 60°C. in the analysis of the products of alcoholic fermentation. Kamibayashi, Miki, and Ono (51) used a combination column of one meter of glycerine and two meters of silicone oil DC 200 or polyethylene glycol-1500, both on C-22 fire-brick, in their quantitative determination of small amounts of alcohols in water. Dimick and Corse (52) used 10 foot columns of Carbowax or silicone at 150°C. Swoboda (53) used a dual column scheme incorporating 10 per cent polyethylene glycol-400 and 20 per cent diglycerol on Celite combines with a back-flushing technique whereby the water was never permitted to enter the lower part of the column where alcohol resolution occurred. Zientara and Owades (54) used four meters of Carbowax-1500 in their analysis of beer volatiles. Salo (55) used a combination column of 2.24 meters of paraffin oil and 0.6 meters of polyethylene glycol-1500 on Chromosorb to separate isopropanol, ethanol, methanol, and water at 47°C. Morrison (56) analyzed 20 per cent by volume solutions of ethanol in water quantitatively using an eight foot column of 20 per cent Carbowax on 60-80 mesh Teflon at 72°C. Stevens (57) used a six meter column of three parts glycerol on 10 parts 40-60 mesh Celite 545 at 78°C. for alcohols, aldehydes, esters, and water.

Other liquids have been used in aqueous analyses. Dinonyl

phthalate has been investigated by Abraham and coworkers (58) and by Cvetanovic and Kutsche (59). Tailing is severe. Whitham (60) rejected both paraffins and dinonyl phthalate in preference to polyethylene glycol-600. Zilberman and Lazaris (61) used 30 per cent dibutyl phthalate while Haskins and coworkers (62) used 30 per cent di-(2-ethylhexyl)-phthalate at 65°C. and Perkin-Elmer "B" packing at 90°C. Kamibayashi, Miki, and Ono (63) completely resolved water and twelve alcohols on two meter columns of silicone oil DC 200 on Celite at 55°C. and di-(2-ethylhexyl)-sebacate on Chromosorb at 80°C. Yamamoto and Saito (64) used a 70 cm. column of 25:100 (w/w) octadecyl alcohol on Celite 408 at 60°C. with a three per cent relative error in analysis. Kelker (65) used a 4.9 meter column of 30 per cent Citroflex A (o-acetyl-tri-ethylhexyl citric ester) on Embacel at 122°C. Lower temperatures gave a loss of resolution and asymmetry. McReynolds (66) used sucrose octaacetate supported by Teflon in his investigation of alcohols, aldehydes, ketones, and other oxygenated compounds including water and found they had lower efficiencies than if Celite was used. Sandler and Strom (67) supported the surfactant extracted from "Tide", a commercial detergent, on Fluoropack and C 22 firebrick in his resolution of aqueous solutions of oxygenated organics. Matubara and Kinoshita (68) tested various liquid loadings on various supports of polyethylene glycol, tricresyl phosphate, glycerol, triethanol amine, and Tween-80. Best results were obtained with columns of 20 per cent liquid load of 3:1 (w/w) Tween-80-glycerol mixture on C 22 firebrick at 90°C. where resolution of acetone, isopropanol, ethanol, n-butanol, and water was achieved in 17 minutes.

Zarembo and Lysyj (69) reported favorably on the use of 22 foot columns of 33 per cent Armeen SD, a mixture of high molecular weight normal primary amines, on 30-60 mesh Chromosorb at 88°C. in the quantitative analysis of mixtures of low molecular weight alcohols containing 25-75 per cent water. Water was reported as having a small amount of tailing. Smith (70), in his investigation of water solutions, tested polyethylene glycol-200, polyethylene glycol-400, glycerol plus tricresyl phosphate, diglycerol, hexantriol, triethanolamine, Tween-80, and glycerol monooleate. He reported the detection of 0.6 per cent water in ethanol on a one meter column of 20 per cent triethanolamine on 60-100 mesh Celite 545 at 100°C.

In the past the polyethylene glycols have been popular liquids. Liquid loads ranged from 20 to 30 per cent and they were generally supported by either Chromosorb or a fluorocarbon. Column lengths employed were 0.6 to 6.0 meters with an average length of about 2.5 meters. Temperatures ranged from 45° to 150°C. with an average of 74°C., and there was a direct relationship between column length and temperature.

Ottenstein (18) recommended THEED (tetrahydroxyethyl-ethylenediamine)



be employed as an immobile phase in the determination of water in ethanol. It has a polarity similar to the polyethylene glycols (71) and is particularly noted for giving symmetrical peaks. It is be-

lieved that the large polar functional groups block out the active sites of the support, thus reducing tailing. THEED is thermally unstable at high temperatures and the recommended maximum operating temperature is 135°C.

Ottenstein (18) predicted that a two per cent liquid load on a fluorocarbon would give excellent results. There are very good reasons for this severe reduction in liquid load. Theoretically, the efficiency of a packing material depends upon the film thickness (72) and this has been borne out by experiment (73, 74). The Chromosorbs are very porous materials (75) with surface areas of about 4.5 m²/gm. (20). Teflon, on the other hand, has a surface area of 0.64 m²/gm. (75) to 2 m²/gm. (73). A statement that the liquid load should be reduced by a factor of 0.14 to 0.5 to maintain the same film thickness, and hence the same efficiency, on Teflon relative to Chromosorb would be strictly true only if a gram of each contained the same number of particles of the same size; but it can be said that a reduction of load is indicated by the difference in porosities. Retention times are reduced for low loads (76, 77). In practical terms, this means that shorter columns or lower temperatures may be used with low load columns and the same resolution is obtained as with a column of higher load (78). It is desirable that the column used have a sufficient lifetime (5) to allow the completion of the program. By using a lower liquid load, short columns may be used at lower temperatures for a longer period of time without appreciable changes in behavior. Low liquid loads, however, are an invitation to participation by the support (20, 77, 79). As a compromise, a load of

nearly ten per cent as recommended by Nadeau and Oaks (80) was decided upon. This loading gave separations superior to loads below ten per cent.

The quantitative composition of the sample is found by measurements of some property of the peaks obtained on the chromatographic record. The discussion here is restricted to thermal conductivity detectors. As far as the instrumentation is concerned, characteristics of the record will depend upon the design of the detector, the sensing circuit, and the recorder. It is not widely appreciated that the recorder circuit is a source of non-linear response (81, 82, 83). The response, as well as the precision and accuracy of the analysis are dependent upon the nature of the carrier gas. Nitrogen and carbon dioxide are inferior to hydrogen and helium in these respects (84, 85, 86, 87).

Early workers assumed that the response of thermal conductivity detectors depended upon the concentration of solute vapor in the sensing channel of the detector and not upon the identity of the solute. This was shown to be false (88, 89). Calibration of response as a function of solute identity and concentration is a necessity.

The most popular peak properties are height and area. These two methods have been compared (90, 91, 92) and for quantitative analyses, peak areas are preferred. Peak heights are more reliable than areas for solutes having short retention times, i.e., narrow peaks (93, 94). Peak height ratios were found to be independent of sample size and, with a few exceptions, of carrier flowrate. They

depend upon retention time, temperature, and pressure (93, 95, 96, 97). Heights have been reported as both linear (98, 99) and non-linear (100) with respect to concentration. Tenney and Harris (99) reported ± 0.89 to 0.78 per cent at the 95 per cent confidence limits for peak heights while Dietz (101) reported two per cent at this level. Green (97) reported an error of 1.5 per cent.

With either peak heights or peak areas, a decision must be made as to the units of concentration to be used. A correction factor is then included to compensate for the difference in response of the solutes. Assuming a linearity in detector response with concentration, a linear equation should result which relates the peak property to the amount of solute. There is much indecision in the literature as to the proper concentration units. Janak, Komers, and Sima (86) and Schomburg (102) compared peak areas to both mole and weight per cents. Satisfactory use of mole percentages have been reported (103, 104, 105, 106) with errors as low as one per cent (107). Elsey and Wagner (108) reported an error of 18 per cent using weight per cent and 9 per cent using mole per cent. Others (69, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118) enthusiastically proclaim weight per cents to be superior. Even the theoretician seems confused since Feish and Engelhardt (119) and Mecke and Zirker (120) claimed to have theoretically justified the correlation of peak areas with weight per cent while Hoffman (121) stated that the correlation can be either to weight per cent or mole percent, depending upon the identities of the carrier gas and solutes. The literature dealing with the use of areas indicated that a relation between area per cent and composition per

cent is independent of minor variations in operating conditions. Peak areas are sensitive to temperature but not to flowrate (122). Some workers combined peak areas with retention times or volumes in their quantitative analyses (123, 124, 125). Areas may not be linear in concentration (124). Another group of authors felt that volume per cent is the proper expression (126, 127, 128, 129) in relating chromatograms to sample composition.

Numerous methods are employed for the determination of peak areas. They may be determined automatically and recorded on the chart by electronic or mechanical integrators (83, 118, 130, 131, 132). Orr (83) indicated that integrators can introduce error. A planimeter (104, 133), the weight of the cut out peak, or tracing (134, 135), and quadrature of the peak, which involves use of the peak height and the peak width at one-half the peak height, (136, 137, 138, 139, 140) are the most common methods used to measure areas. Comparative studies of these various methods have been made (86, 141, 142, 143, 144). Janak found all integrators to be more accurate than planimeters or quadrature methods (86, 141), and that for all methods, errors in measuring were one to three per cent for areas greater than 100 mm.², about 10 per cent for areas of 51 to 100 mm.², and 15 to 25 per cent for areas of 0 to 50 mm.². He found no one method to be universally superior. Schwenk, Hackenberg, and Schneck (132) found planimeters and integrators to be comparable in accuracy. Chino, Kasamatsu, and Suzuki (145) felt that area measurements were the major source of error in quantitative evaluation. The literature indicated that, on the average, one may expect 0.5 to 5 per cent error in quantitative analysis

by gas chromatography.

EXPERIMENTAL

Reagents used and their sources are listed in Table 1 in the Appendix.

Carbowax - Firebrick Column

A 5 ft commercial column consisting of untreated firebrick coated with Carbowax 400 was tested in the separation of ethanol and water. Even at temperatures above 100°C the water peak exhibited extreme tailing (Fig. 1). In samples containing less than 5% water, the water peak is broad and extremely flat.

THEED - Chromosorb Column

Chromosorb was treated with 3 M hydrochloric acid and rinsed with distilled water until wash showed no reaction with silver nitrate. The Chromosorb was then treated with 3 M potassium hydroxide in methanol and rinsed with distilled water until wash showed no reaction with phenolphthalein. It was then washed with acetone and dried. Enough THEED to give approximately a 27% by weight coating was dissolved in acetone and then stirred with Chromosorb until the acetone evaporated. After drying, the 30 to 60 mesh fraction was separated. The THEED coating of a sample dried over phosphorus pentoxide and then extracted was found to be 24.5% by weight. When the coated Chromosorb was dried in an oven at 100°C, the THEED partially decomposed and a constant weight could not be obtained either before or after extraction.

Copper tubing, 8.0 ft by 0.25 in. (O.D.), was packed with 27.33 g of the treated Chromosorb. The column was conditioned 7.5 hr at 100°C at a helium flow rate of 7.28 mL/min. Operating this column at 75°C gave a retention time for ethanol of approximately 51 min. Water did not come off at the end of 2.5 hr. At 100°C, the retention time for ethanol was approximately 21 min; the retention time for water was approximately 70 min. Peaks on the chromatographic record were not symmetrical. The water peak, especially, exhibited a great deal of tailing (Fig. 2).

This column was shortened to 6 ft by cutting 2 ft off the inlet end. The chromatograms obtained were still unsatisfactory, especially in the degree of tailing of the water peak (Fig. 3). The retention time for ethanol was approximately 11 min; the retention time for water was approximately 36 min.

THEED - Neutraport T Column

On the recommendation of Ottenstein, Neutraport T, a fluorocarbon, was used as an inert support. The Neutraport T was not pretreated prior to the addition of the immobile phase. Enough THEED to give approximately a 10% by weight coating was dissolved in acetone and then stirred with chilled Neutraport T until the acetone evaporated. The chilling was to reduce the amount of static charge on the support. After drying, the 30 to 60 mesh fraction was separated. The THEED coating of a sample dried over phosphorus pentoxide and then extracted was found to be 10.5% by weight. When the coated Neutraport T was dried in an oven at 100°C, the THEED partially decomposed, and a constant weight could not be obtained.

either before or after extraction.

Copper tubing, 3 ft by 0.25 in. (O.D.), was packed with 12.06 g of the treated Neutraport T. The exact weight was difficult to determine as THEED apparently is very hygroscopic and the sample weight increased constantly during the weighing operation. The column was conditioned approximately 7-hr at 100°C at a helium flow rate of 23.3 ml/min. This column at 75°C gave an ethanol retention time of about 9.5 min and a water retention time of about 35 min. At 84°C, the retention time for ethanol was approximately 5.0 min; the retention time for water was 24 min. After this column was used about 9 months, the ethanol retention time had reduced to about 3.5 min and the water retention time was about 18 min. Retention times fluctuated slightly with variations in sample size, flow rate, and temperature. Peaks on the chromatographic record were fairly symmetrical, especially in the lower sample size range (Fig. 4).

Equipment and Instruments

Analyses were made with a Cenco No. 70130 Vapor Phase Analyzer (Central Scientific Co., Chicago, Illinois) equipped with a thermal conductivity detector. Column, detector, and sample injection unit were all at the same temperature. Concentration profiles were recorded on a Leeds and Northrup Speedomax Model S, variable range, variable sensitivity recorder of 1 sec response time and 30 in./hr chart speed. Samples were introduced into the gas chromatograph with a 100 μ l hypodermic syringe. Driving pressure was measured by a mercury manometer placed at the source of the carrier gas, and flowrates were measured by means of a soap-film flowmeter and stopwatch.

The gas chromatograph was equipped with a sensitivity adjustment which could be varied discretely over an 8 unit interval, while the recorder had a range finder which could be varied continuously from 1 to 20 millivolts. Both controls were used to attenuate the peak heights during analyses.

The paper chromatographic chamber consisted of a 19.4 cm by 7.0 cm (I.D.) glass tube that was fitted at both ends with rubber stoppers. A chromatographic boat was suspended from the top stopper. Eluent was added to the boat through a small hole in this stopper. The bottom stopper contained a thistle tube to collect drippings from the paper. Samples were collected in calibrated 2 ml test tubes fitted to the thistle tube with a cork.

The paper strips employed were 22.6 by 4.1 cm. The strips were folded at 1.5 and 3.0 cm to fit the boat. At 20.2 cm, the tip was tapered.

The cellulose powder column was an 8.0 in. by 0.8 in. (I.D.) glass tube fitted with a sintered glass plate. A dropping funnel fitted with a calcium chloride drying tube was attached to the top of the column. Samples were collected in calibrated 2 ml test tubes.

Preparation and Storage of Samples

Samples of ethanol and water mixtures prepared for calibration purposes are listed in Table 2 with their corresponding weight per cents. These samples were stored in glass bottles with tight fitting rubber caps. Absolute ethanol stored in this type of bottle remained anhydrous for 6 months.

It was found that the best way to store unknown samples was to

place the stoppered collection tubes in a tightly closed bottle. Refrigeration caused any moisture in the air column above the sample to condense. Samples that stood for several days before analysis had a larger water content than expected, presumably because the sample picked up moisture from the air.

Paper Chromatograms

The chromatographic cylinder was saturated with absolute alcohol vapors overnight. An especially prepared paper strip was hung from the boat, and 25 ml of absolute ethanol, the eluent, was added to the boat.

The paper strip was pretreated in various ways before the chromatograms were run. When the paper strip was allowed to equilibrate overnight in alcohol vapors, slightly more than 2 hr was required for the first drop to be delivered off the bottom. About the same time was required for a nonequilibrated strip. From 1.5 to 1.8 hr was required for the collection of 0.2 ml eluent. When analyzed, eluent from the equilibrated strip showed no appreciable water content. A very slight amount of water was found in the eluent from the paper strip that had not been equilibrated with ethanol.

Another experiment employed a paper strip equilibrated overnight in a water atmosphere. After drying 5 hr at room temperature, it was equilibrated with ethanol in the chromatographic cylinder overnight. This time the eluent moved slightly faster. About 2 hr elapsed before the first drop was delivered off the bottom of the paper. About 1.5 hr were required for collection of 0.2 ml samples.

As a last variation, a paper strip was equilibrated in a

water atmosphere, dried, but not equilibrated in an ethanol atmosphere. The chromatogram was run as soon as the strip was placed in the system. The eluent again moved faster. The time required for the appearance of the first drop was about 1 hr. About 1.2 hr were required for the collection of the 0.2 ml samples. This latter procedure proved to be the most satisfactory for analyses (Table 3).

In order to determine the location of the water peaks in the paper chromatograms, a paper strip was dyed with crystal violet dissolved in water. This strip was dried at room temperature for about 11 hr. The strip was then placed in the chromatographic cylinder and 25 ml absolute ethanol was used to run the chromatogram. A dark zone on the front of the strip became evident as the run proceeded. At the completion of the run, the dye at the top of the strip was almost completely extracted, while the bottom of the strip was appreciably colored. The 0.2 ml samples collected were diluted with 95% ethanol and the per cent transmission was recorded with a Bausch and Lomb spectrophotometer (Table 4). The greatest amount of crystal violet appeared right after the solvent front and the concentration soon reduced to a low value.

Cellulose Powder Chromatograms

Columns of different lengths were employed to determine if the amount of water picked up by absolute ethanol is dependent on the length of the column as well as the length of time the chromatogram requires. Cellulose powder was packed in the column in 2 to 4 cm batches. In a 17 cm column, 22 min were required for the first drop

to appear after the addition of the eluent. In a 9 cm column, 5 minutes elapsed before the appearance of the first drop. In both cases, the time required for the collection of 0.2 ml was slightly under one minute.

The cellulose powder in the 17 cm column weighed 18.2115 g. Since a typical paper strip weighed 0.8029 g, the amount of pulp in this column would have made a paper strip about 460 cm long (excluding length of the tip). The 9 cm column weighed 10.7215 g, which would be equivalent to a paper strip approximately 284 cm long. Figs. 5 and 6 and Tables 5 and 6 show the results of the analyses of these chromatograms.

Calibration of the Gas Chromatograph

Chromatograms of the standard samples indicated that the THEED - Neutraport T column was applicable over the complete range of water-ethanol mixtures. The column was calibrated in the region of 10% water and below. The two calibration curves that were necessary to cover this region are in Figs. 7 and 8 and Tables 7 and 8. For samples containing about 2.3 to 10% water, sample size was 20 μ l, the sensitivity readings for ethanol and water being 5 and 7 respectively. The millivolt readings for ethanol and water were 20 and 5 respectively. These readings gave chromatograms with reduced ethanol peaks and enlarged water peaks. For samples containing less than 2.3% water, the sample size was 70 μ l, the sensitivity readings were 3 and 7, and the millivolt readings were 20 and 5. Greater magnification for the water peak was prevented by the magnification of random background noise.

DISCUSSION

The column employed for the analytical analyses of ethanol-water samples consisted of TREED on Neutraport T. This column was capable of analyzing samples over the entire ethanol-water range. An extensive calibration of the column was carried out only in the region of ten per cent or less water. In this region, the eluent from the cellulose powder columns and paper strips was analyzed. Two different calibrations were necessary to cover this area; one calibration was for 70 microliter samples, and the other one was for 20 microliter samples. In samples containing 70 microliters, the influence of the ethanol peak was still appreciable at the start of the water peak (Fig. 4, D, E). Since the very end of the water peak did not tail reproducibly, and the beginning of the water peak was masked by the end of the ethanol peak, the area of the water peak was measured from its apparent starting point, rather than extrapolating the water peak to the base line, and straight across according to the dotted line in Figure 4, D. This measurement gave reproducible results. Even though the entire amount of water present was not measured, calibration of the chromatograms cancelled out this omission. The effect of the alcohol peak tailing into the water peak was less noticeable in the 20 microliter samples than in 70 microliter samples. In sample sizes up to about ten microliters, this overlapping problem did not exist.

Samples containing 0.50 per cent water gave chromatograms with small, but measurable peaks. In samples containing 0.35 per cent water,

peaks did not register on the chromatogram; however, the tail of the ethanol peak was higher and flatter than it would have been had no water been present (Fig. 4, E). In samples containing 0.43 per cent water, there was a slight indication of a peak whose area was not measurable. In analyzing chromatograms of water content around 0.43 per cent and less, comparisons were made with calibration chromatograms. In performing these comparisons, tracings of the unknown samples were superimposed on the calibration chromatograms.

Measurable peak area would place the lower detectability limit of water for the column at about 0.50 per cent; however, by using this method of comparison, the analysis could be extended down to about 0.35 per cent water. The difference in the appearance of the curves at these various per cent levels permitted pinpointing water composition to within ± 0.1 to ± 0.2 per cent. Use of this method of course required extensive calibration in this region.

The range of the deviation of the per cent water between duplicate, and at time triplicate, analyses in the 81 samples of eluent from the cellulose powder columns was from 0.00 to 2.00. There were only six values over 1.00, and these deviating values could be attributed in part, if not all, to the fact that the ethanol in the test tubes absorbed moisture from the air between analyses. The average of all of the deviations fell at 0.44. A simple interpretation for deviations was not possible because of effects introduced by such factors as the following: the inherent error in the planimeter, which was found to be dependent on area size in agreement with Janak's report (141); the extreme differences in attenuation between the ethanol peak and the water

peak, coupled by the fact that this attenuation varied with sample size; the conversion of area to per cent area, and then to per cent water, a procedure that employed use of two nonlinear curves, each geared to a particular sample size. The analyses of samples containing water in the vicinity of 2.5 per cent tended to have a narrower spread of deviation values than those containing water in the higher per cent regions. Repeated planimetry was employed for each analysis, and results indicated that, in agreement with the theory of Chino et al (145), if a more accurate method of measuring areas were available, more precise analytical results could have been obtained.

Since the retention time of the ethanol and water peaks was found to decrease with the number of hours the column operated, calibration samples were run interspersed with the samples to be analyzed. Calibrations made four to six months apart were found to give curves parallel to each other. This fact suggested a redistribution of the immobile phase, as was pointed out by Keller et al (5) and Keller and Stewart (6). Sample sizes differing in magnitude by less than 20 per cent gave similar per cent areas. Outside of this permissible deviation range, sample size was found to have a definite effect on per cent area. Therefore, sample size was maintained as constant as possible for the different calibration runs and for the analyses of unknown samples.

As the erratic behavior in Figures 5 and 6 indicates, sample storage presented a problem. In the analysis of the nine centimeter cellulose powder column, samples 37 through 41 were analyzed after samples one through five. A slight interruption was noticeable in the curve after sample five and a greater one before sample 35. By the time

the analysis of sample 16 was begun, several days had elapsed since the samples were obtained. With samples 28 through 34, erratic behavior is marked. This disturbance has been ascribed to two causes. The minor cause was the evaporation of the ethanol as shown by the decrease in the volume of the sample. However, the decrease in sample volume was not enough to account for the increase in the amount of water shown to be present by analyses. This increase in per cent water was therefore primarily attributed to the absorption of water vapor from the atmosphere. This fact further illustrated ethanol's strong dehydrating power.

Both the cellulose powder columns and the dyed paper strip indicated that the water absorbed by the ethanol came off in a peak after the solvent front. The fact that the water did not show this abrupt peak when the eluent from the paper strips was analyzed was attributed to two causes, a dilution effect and an evaporation effect. In the length of time required for collection of these samples, the water peak on the paper could have been affected by the ethanol atmosphere condensing and diluting the peak. The secondary effect was attributed to the evaporation of some of the water into the atmosphere. By running cellulose powder columns, both these effects were eliminated, and the water appeared in its predicted peak following the solvent front.

The azeotropic mixture of ethanol and water did not appear as a distinct feature in any of the analyses. There was not even a leveling of the curves in the vicinity of this area. In Figures 5 and 6, it was interesting to note that when the eluent, absolute ethanol, contained between 8 and 9 per cent water, the dehydrating power of ethanol seemed

to level off. Dehydration was probably still occurring, but it was greatly reduced. It could be assumed that at this point, the ethanol was no longer a strong enough dehydrating agent to remove the water held by the cellulose support. This was supported by the fact that in the nine centimeter column, most of the water appeared in the first milliliter of eluent and showed a per cent composition of water between eight and nine, while in the 17 centimeter column, most of the water appeared in the first two milliliters of eluent and showed similar per cent composition. This suggested that if ethanol, employed as an eluent, were mixed with enough water to give about ten per cent water by weight, no noticeable dehydration of the cellulose support should occur.

APPENDIX A

TABLE 1

REAGENTS EMPLOYED AND THEIR SOURCE

<u>Reagent and Supplies</u>	<u>Manufacturer</u>
Carbowax 400	Wilkins Chemical Company
THIRD	Applied Science Laboratories State College, Pennsylvania
Chromosorb	Johns Manville
Neutraport T	Micro Tek, Distinal Research Service
Crystal Violet	Allied Chemical and Dye Corporation
Cellulose Powder	Whatman and Company
Chromatographic filter paper No. 1	Whatman and Company
Absolute ethanol reagent quality	U. S. Industrial Chemicals Company
Hamilton Syringe No. 701 N	Micro Tek, Distinal Research Service
Helium	Matheson Company, Incorporated

TABLE 2
CALIBRATION SAMPLES

Approximate Total Weight of Samples - 20 Grams

Sample No.	Weight Per Cent H ₂ O	Weight Per Cent CH ₃ CH ₂ OH
B 1	55.0	45.0
B 1a	55.5	44.5
B 2	40.1	59.9
B 2a	39.7	60.3
B 3	31.6	68.4
B 4	21.9	78.1
B 5	9.44	90.6
B 5a	9.91	90.09
B 5b	10.7	89.3
B 6	4.98	95.02
B 6a	5.41	94.59
B 7	64.7	35.3
B 8	75.6	24.4
B 9	84.2	15.8
B 10	95.1	4.9
B 10a	94.8	5.2
B 11	97.8	2.2
B 12	2.28	97.72
B 13	1.16	98.84
B 13a	1.17	98.83
B 14	0.58	99.42
B 14a	0.59	99.41
B 15	0.35	99.65
B 15a	0.34	99.66
B 16	0.43	99.57
B 17	0.50	99.50

TABLE 3

ANALYSES OF ELUENT FROM PAPER STRIPS

CH₃CH₂OH Sensitivity 3; Millivolt Range 20H₂O Sensitivity 7; Millivolt Range 5Sample Size Analyzed in Gas Chromatograph 70 μ l

Paper Strip A, 0.2 MI Eluent per Sample, non-equilibrated

Paper Strip B, 0.1 MI Eluent per Sample, non-equilibrated

Paper Strip C, 0.2 MI Eluent per Sample, H₂O equilibrationPaper Strip C, 0.1 MI Eluent per Sample, H₂O equilibration

Sample	::: ::: ::: :::	Average Per Cent H ₂ O
1 A		0.42
2 A		0.35
3 A		0.35
4 A		0.35
5 A		0.35
6 A		0.35
7 A		0.35
1 B		0.34
2 B		0.34
3 B		0.34
4 B		0.34
1 C		0.42
2 C		0.35
3 C		0.35
4 C		0.35
5 C		0.35
6 C		0.35
7 C		0.35
1 D		0.43
2 D		0.35
3 D		0.34
4 D		0.35

TABLE 4

SPECTROPHOTOMETER RECORDINGS FOR CRYSTAL VIOLET SAMPLES

Sample Size 0.2 ml
Original Concentration of Crystal Violet 0.628g/250 ml; $6.45 \times 10^{-3} M$
Concentration of Samples 1-9 0.2 ml/250 ml
Concentration of Samples 1a-4a 0.2 ml/500 ml

Sample No.	:	Per Cent Transmittance
1	:	0.0
2	:	6.5
3	:	46.0
4	:	73.5
5	:	81.0
6	:	83.5
7	:	83.0
8	:	85.0
9	:	87.0
1a	:	41.0
2a	:	77.0
3a	:	93.0
4a	:	98.5

TABLE 5

ANALYSES OF ELUENT FROM 17 CM CELLULOSE POWDER COLUMN			
Sample No.	Sample Size : Collected	Total Volume : Collected	Average Per Cent Water
1	0.2 ml	0.2 ml	8.21
2	0.2	0.4	8.58
3	0.2	0.6	8.43
4	0.2	0.8	7.94
5	0.2	1.0	8.30
6	0.2	1.2	8.35
7	0.2	1.4	8.37
8	0.2	1.6	8.34
9	0.2	1.8	8.39
10	0.2	2.0	8.00
11	0.2	2.2	7.47
12	0.2	2.4	7.55
13	0.2	2.6	7.19
14	0.2	2.8	7.14
15	0.2	3.0	7.05
	2.0	5.0	discarded*
16	0.2	5.2	5.03
17	0.2	5.4	4.72
18	0.2	5.6	3.89
	2.0	7.6	discarded
19	0.2	7.8	3.01
20	0.2	8.0	3.25
21	0.2	8.2	3.31
	2.0	10.2	discarded
22	0.2	10.4	2.63
23	0.2	10.6	1.98
24	0.2	10.8	2.47
	4.0	14.8	discarded
25	0.2	15.0	1.70
26	0.2	15.2	0.77
27	0.2	15.4	1.88
	5.0	20.4	discarded
28	0.2	20.6	1.64
29	0.2	20.8	2.34
30	0.2	21.0	1.71
	5.0	26.0	discarded
31	0.2	26.2	1.59
32	0.2	26.4	1.47
33	0.2	26.6	3.09
	5.0	31.6	discarded
34	0.2	31.8	2.15
35	0.2	32.0	3.04
36	0.2	32.2	2.57
	5.0	37.2	discarded
37	0.2	37.4	3.09
38	0.2	37.6	2.80
39	0.2	37.8	2.65
	10.0	47.8	discarded
40	0.2	48.0	3.69

*Samples were discarded in favor of a broader range.

ANALYSES OF ELUENT FROM 9 CM CELLULOSE PULP COLUMN

Sample No.	Sample Size : Collected	Total Volume : Collected	Average Per Cent Water
1	0.2 ml	0.2 ml	8.95
2	0.2	0.4	8.60
3	0.2	0.6	8.37
4	0.2	0.8	7.29
5	0.2	1.0	6.99
6	0.2	1.2	7.54
7	0.2	1.4	6.44
8	0.2	1.6	6.61
9	0.2	1.8	5.43
10	0.2	2.0	4.88
11	0.2	2.2	4.53
12	0.2	2.4	4.62
13	0.2	2.6	3.59
14	0.2	2.8	3.73
15	0.2	3.0	3.83
	2.0	5.0	discarded*
16	0.2	5.2	2.76
17	0.2	5.4	2.39
18	0.2	5.6	2.83
	2.0	7.6	discarded
19	0.2	7.8	2.03
20	0.2	8.0	2.73
21	0.2	8.2	2.40
	2.0	10.2	discarded
22	0.2	10.4	1.84
23	0.2	10.6	1.98
24	0.2	10.8	1.80
	4.0	14.8	discarded
25	0.2	15.0	2.10
26	0.2	15.2	1.31
27	0.2	15.4	1.83
	5.0	20.4	discarded
28	0.2	20.6	2.56
29	0.2	20.8	2.63
30	0.2	21.0	2.41
	5.0	26.0	discarded
31	0.2	26.2	2.37
32	0.2	26.4	1.96
33	0.2	26.6	2.66
	5.0	31.6	discarded
34	0.2	31.8	2.59
35	0.2	32.0	3.29
36	0.2	32.2	3.07
	5.0	37.2	discarded
37	0.2	37.4	0.43
38	0.2	37.6	0.46
39	0.2	37.8	0.35
	10.0	47.8	discarded
40	0.2	48.0	0.69
41	0.2	48.2	0.71

*Samples were discarded in favor of a broader range.

TABLE 7

DATA FOR CALIBRATION GRAPH PLOTTED IN FIGURE 7

Sample Size 20 μ l
 CH₃CH₂OH Sensitivity, 5; Millivolt Range, 20
 H₂O Sensitivity, 7; Millivolt Range, 5

Sample	Actual Per Cent H ₂ O	Per Cent H ₂ O Area
B 13	1.16	8.5
		9.3
		Average 8.9
B 12	2.28	13.3
		11.8
		17.6
		14.0
		13.0
		15.1
Average 14.1		
B 6a	5.41	31.2
		33.9
		31.2
		29.9
		Average 31.6
B 5b	10.66	54.8
		55.6
		55.8
		57.6
		Average 56.0

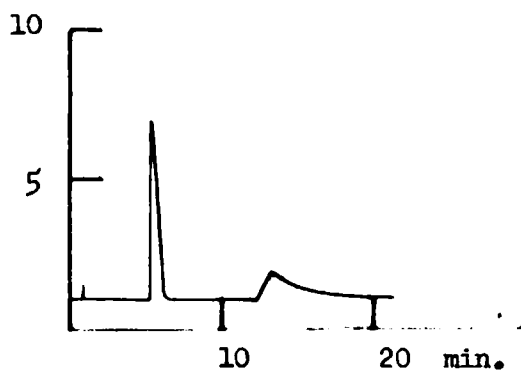
TABLE 8

DATA FOR CALIBRATION GRAPH PLOTTED IN FIGURE 8

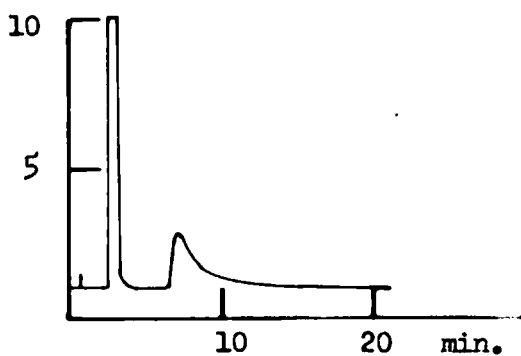
Sample Size 70 μ l
 CH₃CH₂OH Sensitivity, 3; Millivolt Range, 20
 H₂O Sensitivity, 7; Millivolt Range, 5

Sample	Actual Per Cent H ₂ O	Per Cent H ₂ O Area
B 14a	0.59	8.4
		4.3
		5.8
		Average 6.2
B 13a	1.17	21.2
		19.3
		20.8
		18.7
		Average 19.6
B 12	2.28	38.6
		43.3
		39.4
		Average 41.4

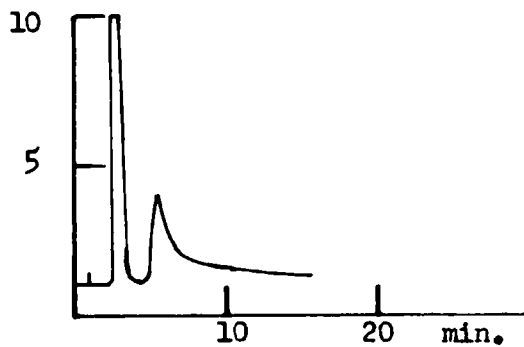
APPENDIX B



A. Sample B 1
Operating Temp. 85°C
Sample Size 3 μ l

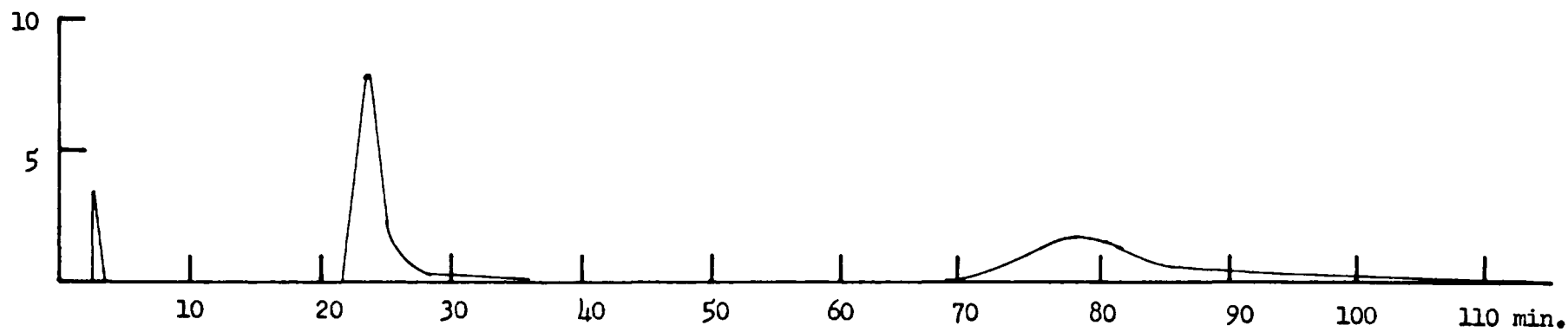


B. Sample B 1
Operating Temp. 113°C
Sample Size 3 μ l



C. Sample B 1
Operating Temp. 120°C
Sample Size 4 μ l

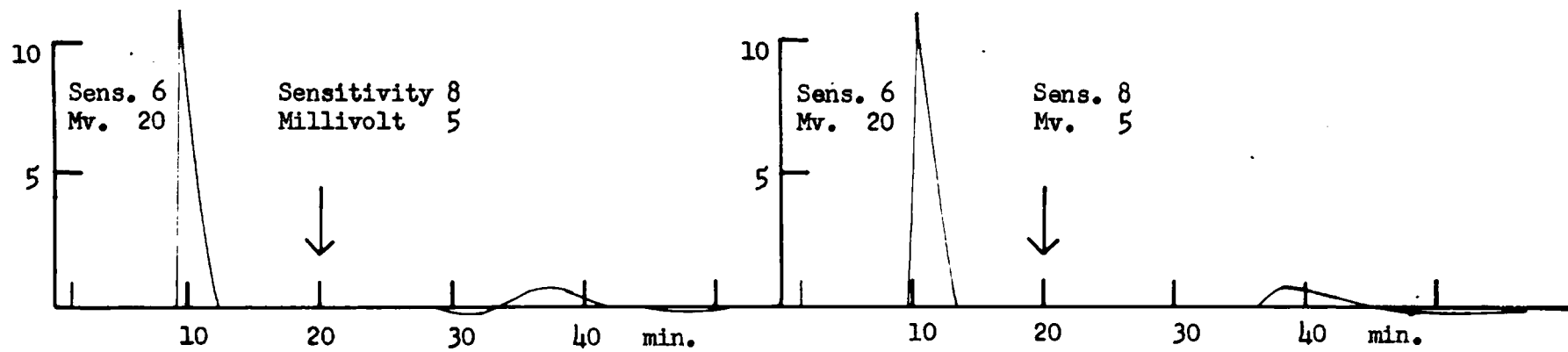
Figure 1. Carbowax 400 - Firebrick Chromatograms.



Sample B 1
Operating Temp. 100°C
Sample Size 10 μ l

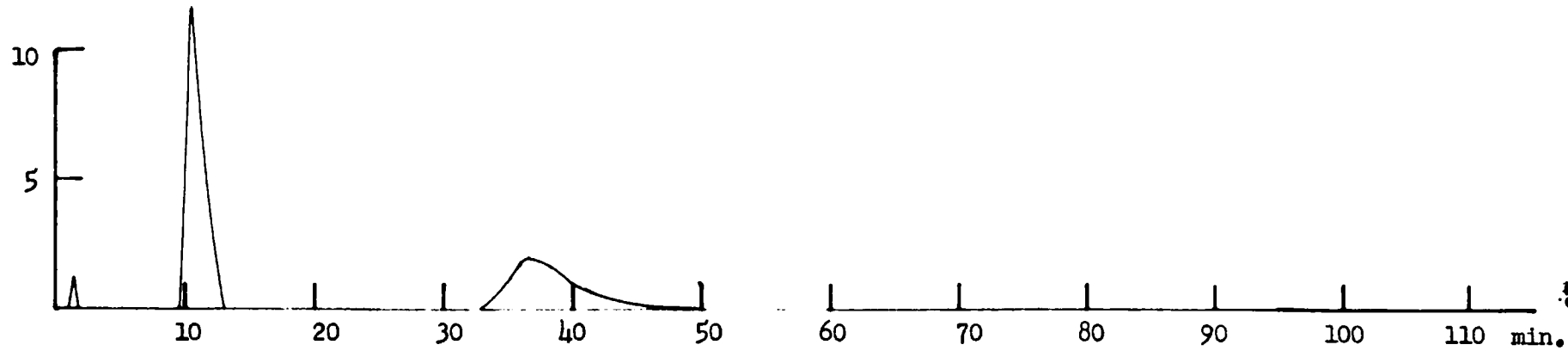
Figure 2. THEED - Chromosorb Chromatogram for 8 ft. Column.

Figure 3. THEED - Chromosorb Chromatograms for 6 ft. Column.



B. Sample B 6
Operating Temp. 100°C
Sample Size 8 μ l

C. Sample B 6
Operating Temp. 100°C
Sample Size 8 μ l



A. Sample B 1
Operating Temp. 100°C
Sample Size 5 μ l

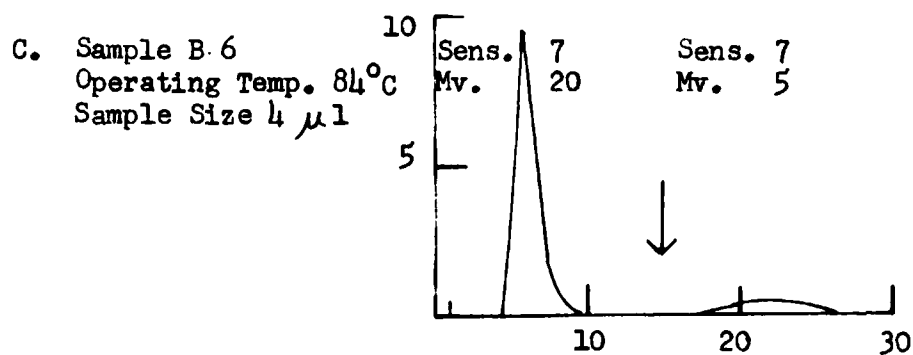
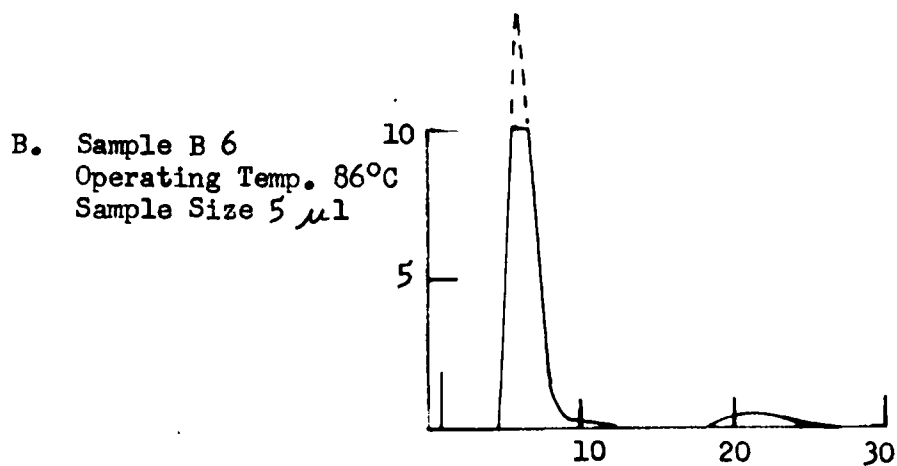
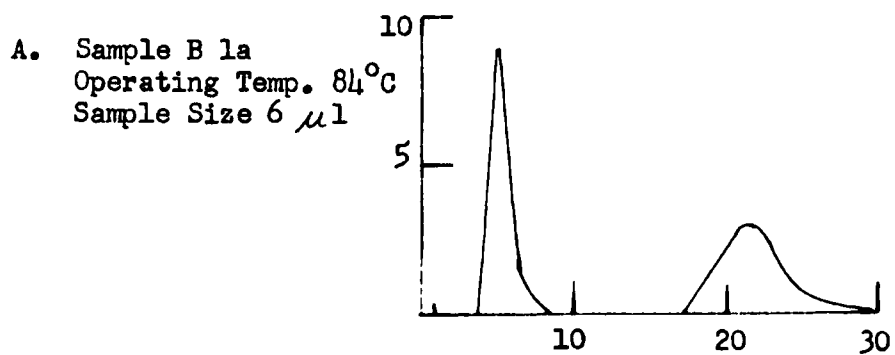


Figure 4. THEED - Neutraport T Chromatograms.

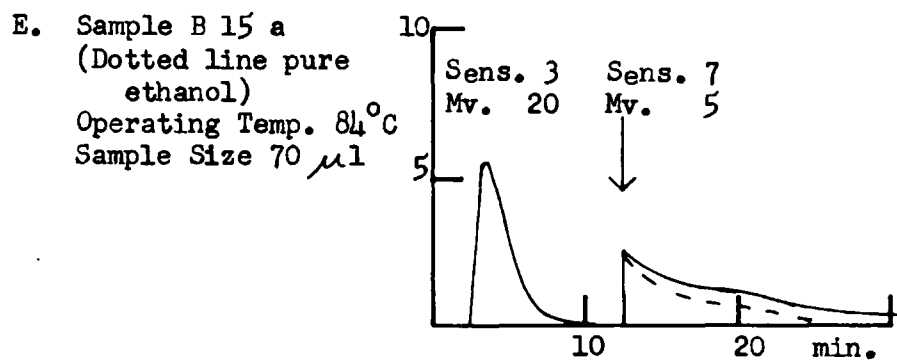
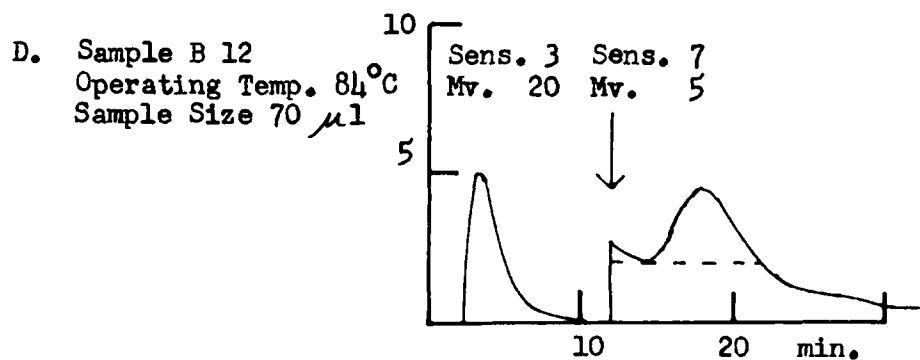


Figure 4. (Continued) - THEED - Neutraport T Chromatograms.

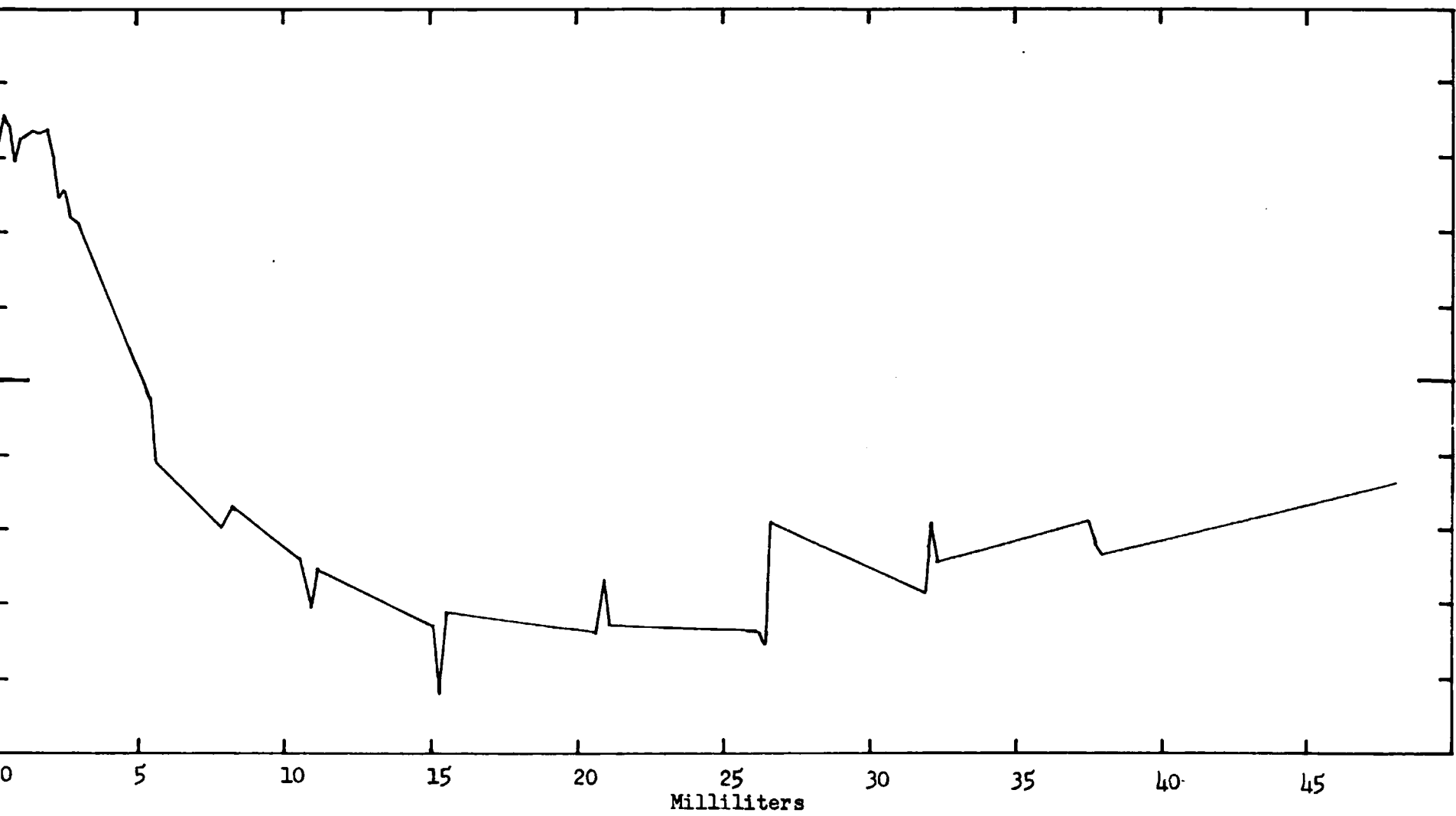


Figure 5. Per Cent Water Found in 17 cm Column.

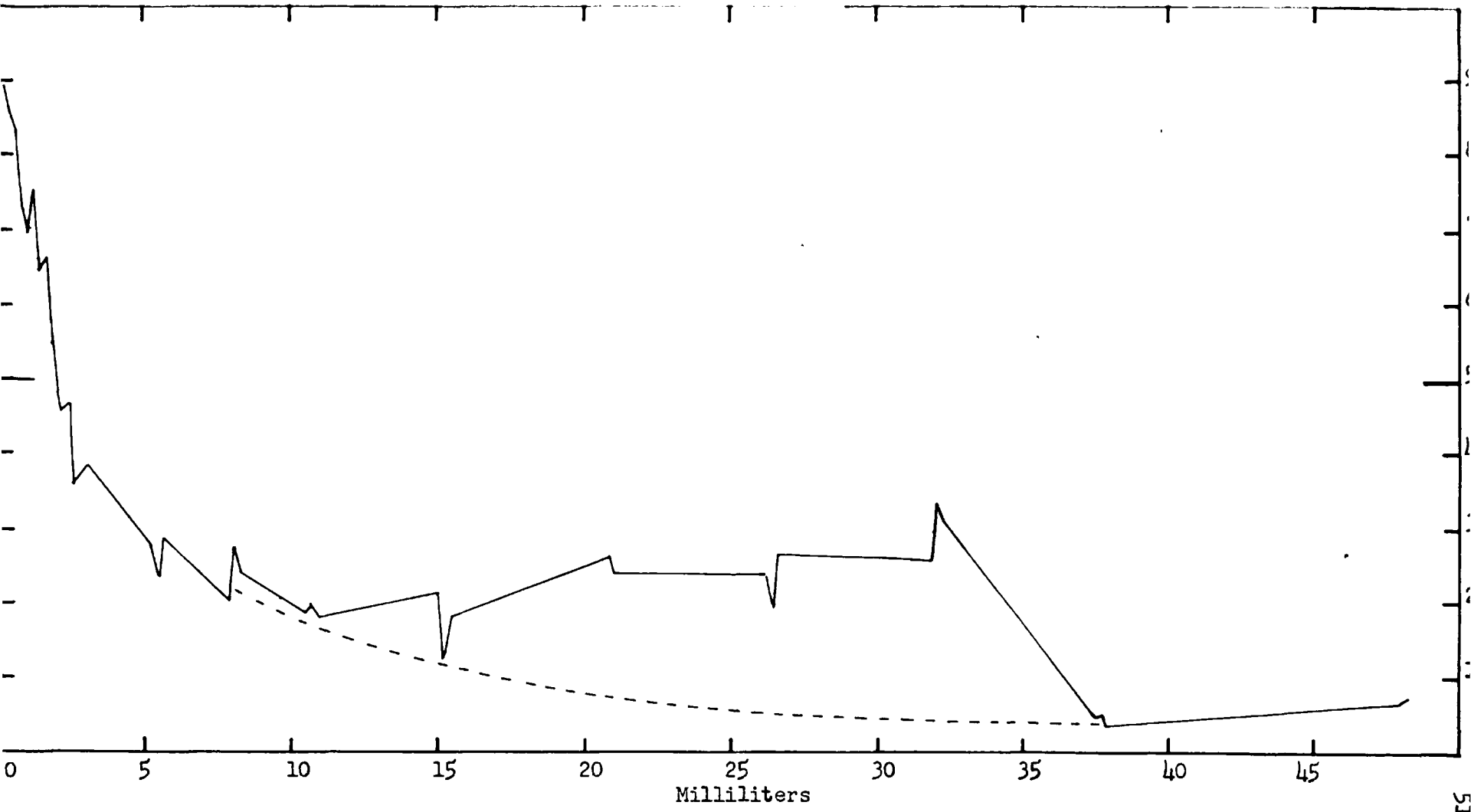
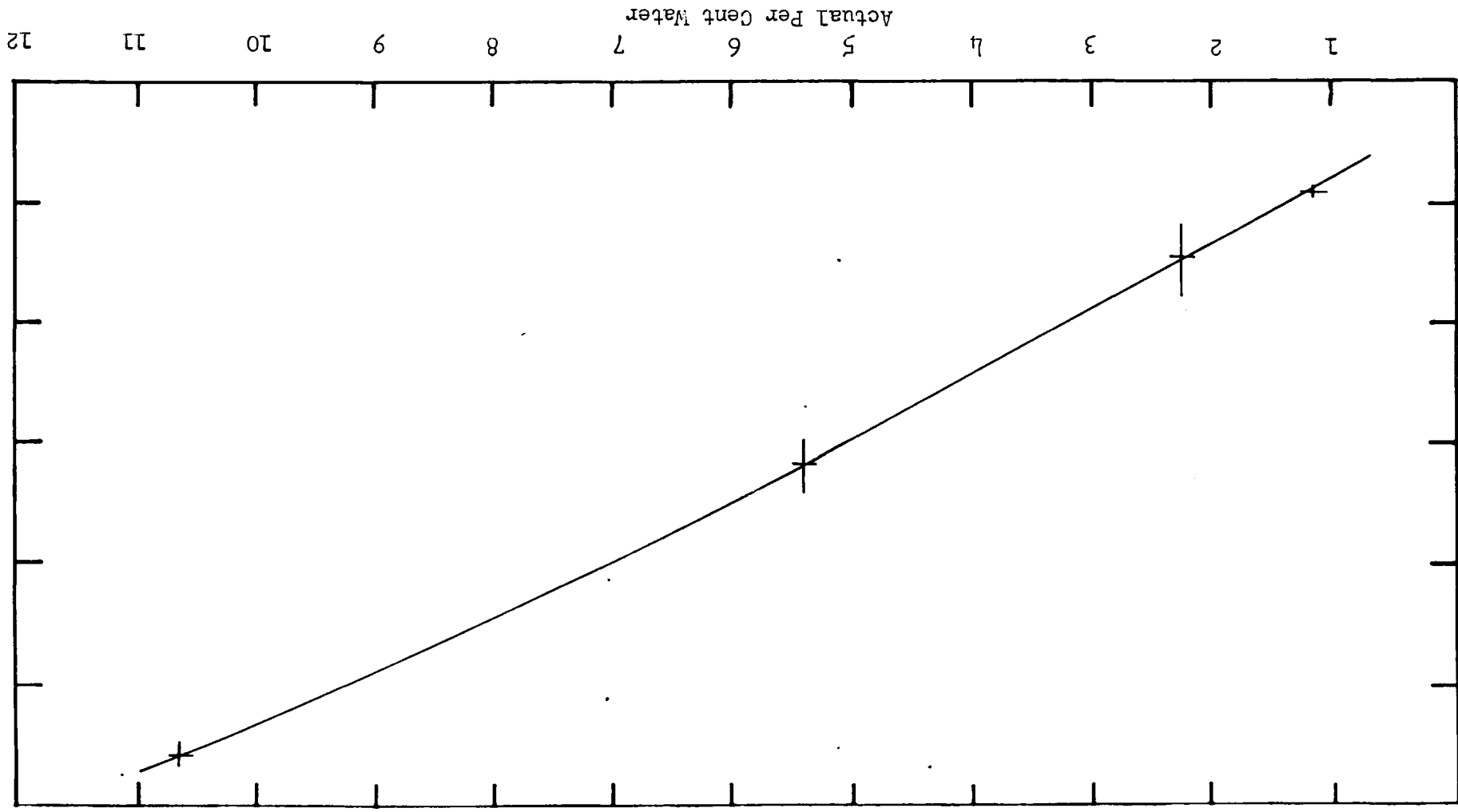


Figure 6. Per Cent Water Found in 9 cm Column.

Figure 7. Calibration Curve for 20 μ l Samples

Per Cent Area



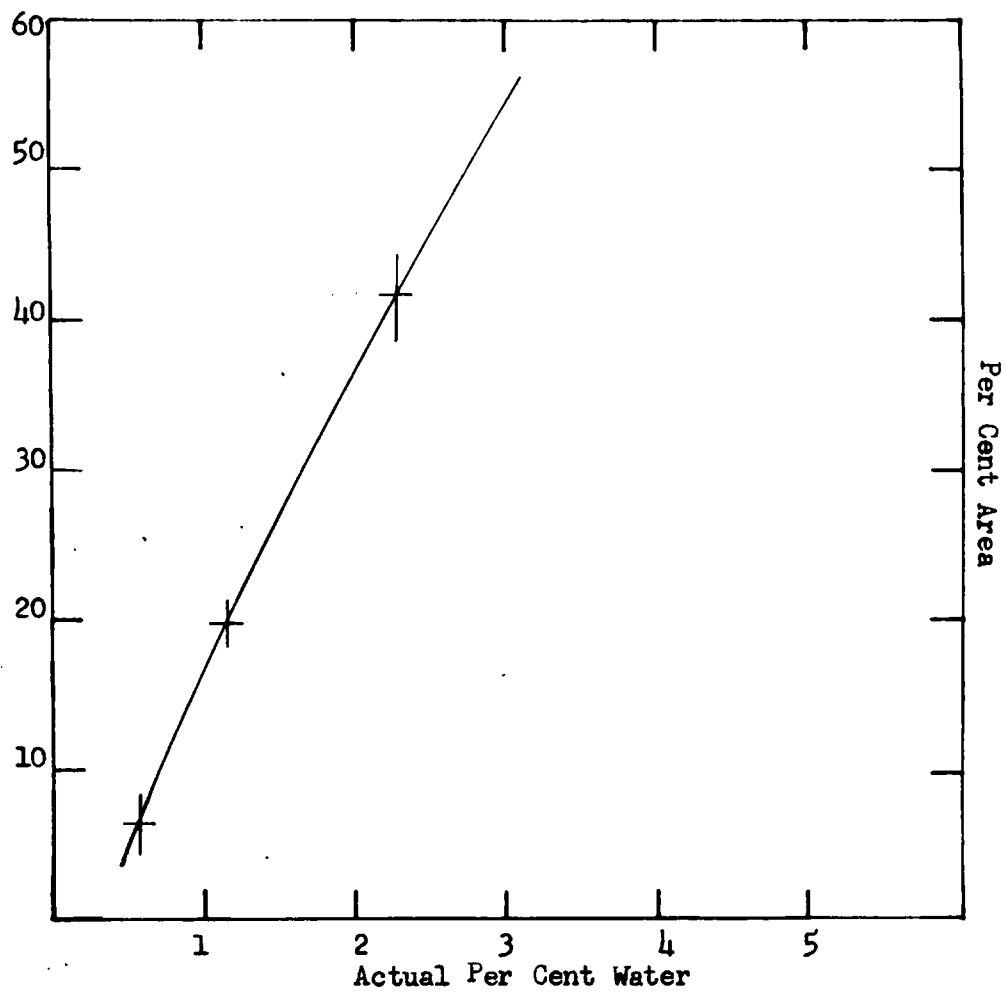


Figure 8. Calibration Curve for 70 μ l Samples.

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