A GENERAL METHOD FOR DETECTION AND RECORDING OF COMPONENT BANDS IN CHROMATOGRAPHY WITH LIQUID ELUENTS

By

L. Michael Carson

A THESIS

Submitted to the School for Advanced Graduate Studies
of Michigan State University of Agriculture and
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To Ruth

VITA

L. Michael Carson was born October 5, 1932 in Johnson City, Tennessee. He spent four years at East Tennessee State College where he received his B. S. and A. B. degrees in 1954.

He attended Michigan State University 1954-1959 where his major was Physical Chemistry and minors were Mathematics and Physics. He was a Graduate Teaching Assistant 1954-1958 and a Graduate Research Assistant 1958-1959.

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A GENERAL METHOD FOR DETECTION AND RECORDING OF COMPONENT BANDS IN CHROMATOGRAPHY WITH LIQUID ELUENTS

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ABSTRACT

A new, general method has been developed for detecting and recording the presence of solute bands in the effluent stream of a liquid-eluent chromatographic column. Since this method is based upon changes in vapor pressure which occur in the solvent due to the presence of a solute band, its applicability is not limited to specific classes of substances.

The detector developed utilizes the temperature difference which is established between a wick bathed with the effluent of a chromatographic column and a reference wick bathed with pure solvent; the wicks are enclosed in a thermostated chamber that is saturated with solvent vapor. The temperature change is detected through the difference in resistance of a pair of thermistors, one of which is in contact with the wick bathed with pure solvent, while the other is in contact with the wick bathed with the effluent solution. The thermistors constitute two arms of a Wheatstone bridge, which can be balanced when both of the thermistors are in contact with wicks bathed with pure solvent. Any unbalance in the bridge which occurs as a result of solute bands passing is continuously recorded on a recording potentiometer.

Detector response is a colligative property of the solution passing over the thermistors. A mathematical treatment has been developed and predicts a linear dependence of detector response on mole fraction of solute. The mathematical analysis is based on a condition of thermal balance, and requires suitable approximations, based on the small magnitude

of the observed changes, for derivation of the linear relationship. The kinetics of the solvent vaporization and condensation processes, as influenced by the presence of solute, are postulated to play the major role in detector response.

Further mathematical analysis of the concentration profile furnishes a relationship between the concentration giving the observed response and the initial sample concentration.

The evolution of the detector and its relationship to other means of chromatographic detection, and to related measuring techniques, are described. Qualitative results obtained in different solvent-sorbent systems for a variety of solutes are presented; the response to volatile and non-volatile solvents is discussed. Particular attention has been directed towards the analysis of the ergosterol irradiation mixture.

Some quantitative results are presented and indicate the nature and sensitivity of the response. At 33.5°C. with a sample column flow rate of 5 ml./day, a reference column flow rate of 2 ml./day, cotton thread wicks, nominal 2000-ohm thermistors with a temperature coefficient of 4.5 per cent per degree, fixed bridge resistances of 2000 ohms, and an applied bridge voltage of 6 volts, the detector has a sensitivity of 188.5 mv./M., or the capacity to detect solute in 0.01 ml. of 10^{-3} M. solutions; this corresponds to detection of 10^{-8}-10^{-9} moles of material.

The new method should complement gas chromatography as an analytical method, with its most important applications in areas where gas chromatography is weakest--analysis of high-boiling and thermally-unstable components.

It should be of particular value in biochemical, natural products, and pharmaceutical work. Physico-chemical applications of the detector include its use in determining molecular weights, evaluating activity effects, studying molecular complexing and dissociation, and obtaining fundamental information on the nature of the chromatographic process.

TABLE OF CONTENTS

continued

LIST OF FIGURES

I. INTRODUCTION

A. General

The advance of the development of accurate, rapid, and general techniques of separation and analysis is one of the best yardsticks for chemical progress. Differential migration is one of the most useful general tools of separation, including within its scope the techniques of electrophoresis, multiple partitioning, mass spectroscopy, thermal and gravitational diffusion, differential sedimentation, and chromatography.

B. Comparison of Gas and Liquid Chromatography

Chromatography is one of the most widely applicable methods of differential migration, since it is potentially useful for separation of all soluble substances. Chromatography is limited, however, by the fact that each class of separations requires a different sorbent-solvent system and detector. In liquid chromatography, the solvent may range from low-boiling organic substances to solutions of fused salts, and the sorbent may range from paper strips or strings to packed columns. The solvent in vapor chromatography does not vary as much, since it is not chosen so much for eluting power as for its applicability to the detector chosen; however, the sorbent is selected on the basis of its physical and chemical properties, in particular its vapor pressure, thermal stability, and affinity for the components to be separated. The lack of generality of any one solvent-sorbent system is almost equivalent for liquid and vapor chromatography; however, the detectors available for vapor chromatography are much more general than those required for liquid chromatography. This fact has greatly influenced the rapid development of vapor chromatography, which has undergone spectacular growth of application during the past four years.

Gas chromatography, with its various general, sensitive, rapid, convenient, and relatively inexpensive detectors, has become a standard bench and line technique for analysis and production control. Vapor chromatography is, however, encumbered with two major limitations: it can be applied only to those substances which have appreciable vapor pressures below 300-400°C, and it does not alone give adequate qualitative information about the substances separated. Even though vapor chromatography is limited, the recent trend has been to extend it beyond its natural convenient range because of the versatility of its detecting devices. Since many organic molecules, especially those of biological importance, are not thermally stable, and since most suitable sorbents have appreciable vapor pressure at elevated temperatures, this extension of gas chromatography has almost reached its practical limit.

Soluble substances with low vapor pressure may, however, be separated using liquid chromatography. The time-honored technique for following the development of a liquid column chromatogram has been to collect the effluent stream in equal fractions and analyze each fraction individually, either chemically or physically. This process is very tedious and time consuming. To achieve a parallel and complementary status to the rapidly-developing field of vapor chromatography, the field of liquid chromatography now requires a detector which will be general to a large number of solvent systems and solutes, respond sensitively to small, dilute samples, give predictable response (preferably linear response) to samples over a wide range of concentration, be simple to construct and easy to operate, and require little attention during the development of a chromatogram.

This study has been concerned with an attempt to develop a detector fulfilling most of these requirements. Before describing the present detector, however, previous approaches to the problem will be reviewed.

C. Previous Detectors for Liquid Chromatography

Kenyon *et al.* (1) have developed a detector which utilizes variation in absorption of ultraviolet light. The eluent stream from a chromatographic column is allowed to drain into a quartz, ultraviolet-absorption cell. A light source and photocell are arranged so that light is reflected from the surface of the liquid in the cell as it is filled from the column. When the effluent fills the cell, the photocell actuates the scan mechanism for a recording spectrophotometer. After the absorption spectrum of the effluent has been recorded, the absorption cell is pumped out, and the spectrophotometer is reset automatically to its initial wavelength. Thus, the absorption spectra of the solute bands are recorded intermittently during the development of the chromatogram, allowing the observer to determine the concentration and the composition of each solute band. This technique is very accurate, but is limited to substances which absorb in the ultraviolet and to solvents which are transparent; in addition to this limitation, the apparatus is very expensive to construct and data interpretation is often involved and tedious.

Baumann and Blaedel (2) have adapted high-frequency conductance measurements to the detection of solute bands in liquid chromatography. In chromatographic processes where large changes in conductance occur in the region of the solute bands, it is possible to detect their presence through high-frequency conductance measurements. These measurements allow the detection of chromatographic bands without the use of internal electrodes. This technique can locate bands before they are eluted from the column; however, it is very limited in scope and sensitivity.

Claxton (3) detects solute bands, utilizing the heat evolved accompanying the adsorption of gases and liquids on sorbent surfaces. A net heat evolution may also result from solute displacement of the adsorbed solvent at the surface of the sorbent. Detection of this net heat of adsorption with a suitable temperature-sensing device makes possible the use of this phenomenon to follow the development of a chromatogram. The major problems involved in the development of this technique are correlations of the extent of temperature rise with the nature and the concentration of the solute bands. This approach seems applicable only to those solvent-sorbent systems which have a sufficiently high surface energy.

Spackman, Stein, and Moore (4) have developed a specific application of absorption spectrophotometry for the detection of amino acids which have been separated chromatographically. The amino acids are separated by ion-exchange columns which are eluted with a solvent of continuously varying pH. The effluent is treated continuously with a standard ninhydrin reagent, allowed to react, and passed through a recording spectrophotometer. The characteristic ninhydrin absorption is observed at 570 mμ and 440 mμ, which permits convenient quantitative analysis of amino acids. This technique, which was almost 10 years in development, is so much more convenient than previously used methods that it is used today almost to the exclusion of the earlier methods.

Schultz, Bodmann, and Cantow (5) have developed a differential refractometer which has been modified by the Phoenix Precision Instrument Company (6), for continuously monitoring the effluent of a chromatographic column. The differential refractometer is potentially the most versatile technique to date for recording the development of a chromatogram, since refractive index is a general physical property, and it can be recorded continuously. The technique is not completely general for all solutes, but it is very sensitive for those solutes which have a refractive index which is appreciably different from that of the solvent used. Differential refractometry is sensitive to 1.5×10^{-6} refractive-index units or 1.8×10^{-5} milligrams per milliliter of NaCl in water. The technique is as follows. The refractive-index prism is divided diagonally into two compartments. One of the compartments contains the pure solvent, and the other holds a portion of the effluent stream, which is continuously flowing through it. A pair of photocells is arranged so as to detect the deflection of a monochromatic beam which passes through the refractive index cell. The beam deflection detected by the photocells actuates a recorder pen and a mechanism which moves the cell so as to balance the beam again. The chart-drive rate on the recorder is controlled by a mass-balance mechanism, so that the chart is driven according to the rate of flow of the solvent. This method is the most general that has been discussed so far;

however, it is limited to large preparative columns since the dead volume of the prism is too large for use with small analytical columns.

II. THE PRESENT INVESTIGATION

A. General Description of the Technique

A new technique has been developed for detecting the presence of solute bands in the effluent stream of a liquid chromatograph, utilizing a physical property different from any of those discussed in the preceding section. The detector senses a difference in temperature established between a solution and its solvent in a solvent-vapor-saturated chamber. This temperature difference arises because of the difference in the rates of evaporation from the solvent and the solution.

This method utilizes the measurement, by means of thermistors, of the temperature difference which is established between a cellulose wick saturated with the pure eluting solvent, and a similar wick which is continuously bathed with the liquid effluent from the chromatographic column; both wicks are enclosed in a thermostated chamber saturated with solvent vapor. The thermistors constitute two arms of a conventional Wheatstone bridge, which can be balanced when both the wicks are bathed with pure solvent; the temperature effect of a solute produces an unbalance which can be measured.

The temperatures sensed by the thermistors are primarily dependent upon the competition of the following processes:

1. Heat input.

    a. Electrical heating.

    b. Heat evolved by condensation of solvent on the wick.

2. Heat removal.

a. Thermal conduction of heat to the liquid flowing through the wick.

b. Heat absorbed in vaporization of the solvent (and, possibly, volatile solutes) from the wick.

c. Heat losses through conduction, convection or radiation to the interior of the thermostated chamber.

Since the electrical heating of the thermistors, the thermal conductivity of the wicks, and the condensation of solvent are not appreciably altered by the presence of the solute band on the wick, the relative rates of vaporization at the two thermistors must largely determine the thermal unbalance. The change in the vapor pressure of the solvent due to the presence of the solute band is thus indirectly detected by the thermistor, which shows an increase in temperature for non-volatile solutes, and a decrease in temperature for certain volatile solutes. The increase in temperature of the thermistor is the result of an increase in the net rate of release of the heat of condensation by the solvent condensing on the surface of the wick (or a decrease in the heat loss caused by vaporization of the solvent), and of an increase of electrical heating caused by the lowering of the resistance of the thermistor.

B. Related Techniques

Although this technique has never previously been used as a chromatographic detector, related thermometric methods have been used by several workers in the last decade for the measurement of molecular weight and the estimation of osmotic coefficients. The liquid chromatography detector was developed completely independently of these thermometric methods; however, there is striking similarity between these techniques and the new liquid chromatography detector.

Baldes (7) is credited with the first successful application of thermometric methods for the determination of molecular weight. His apparatus consisted of a pair of thermocouples enclosed in a solvent-vapor-saturated chamber. One thermocouple had a drop of solvent suspended upon it, and the other had a drop of solution. The temperature difference was observed by a galvanometer deflection due to the difference in potential from the thermocouples. This method was found to be much faster than osmotic pressure or isoteniscopy for determining molecular weight. The observed temperature rise was less than was expected, because of heat dissipation, so that the method could not be used for measuring molecular weights without calibration. However, the major limitation of this technique was the low sensitivity of the thermocouples.

Taylor and Hall (8), and Brady, Huff, and McBain (9) concurrently improved this technique by substituting thermistors for the thermocouples, substantially increasing the sensitivity of the device. The new arrangement consisted of a pair of thermistors suspended in a thermostated chamber saturated with solvent vapor. The thermistors constituted two arms of a Wheatstone bridge, supplied with an alternating potential by an oscillator. The bridge unbalance was amplified and observed on an oscilloscope. The resistance change in the variable part of the bridge was used as a measure of the temperature difference between the two thermistors. The temperature difference was correlated with the molecular weight.

Muller and Stolten (10) designed an apparatus which approaches the thermodynamically-expected temperature rise more nearly than the previous designs. This technique involved placing the thermistors in small pools of mercury, which were covered by the solvent and sample solutions. A higher temperature rise was observed for the solution, but it took longer for equilibrium to be established, more sample was required than for the drop method, and the cell had to be removed and cleaned after each determination.

Iyengar (11), and Kulkarni (12), have reported similar devices in other journals, but their modifications did not substantially alter the nature of the technique.

Higuchi <u>et</u> <u>al</u>. (13), by using an evacuated chamber and an ingenious magnetic-stirring device, have succeeded in designing, for activity coefficient measurement, an apparatus which reaches 80-90 per cent of the theoretical temperature rise, since stirring of the solvent and of the solution reduces the surface energy required for the mass exchange which produces the temperature rise. The evacuated chamber effectively reduces the time required for the maximum temperature change to be observed, and it buffers temperature changes from the thermostated bath so as to decrease thermal instability.

The striking similarity between these varied measurements and the new detector for liquid chromatography is in part deceiving, since the object in the thermometric measurements described was observation of the maximum possible temperature difference between a solvent and a solution which can be obtained thermodynamically, while the object in the design of a liquid chromatography detector is to obtain the maximum possible sensitivity for a given concentration of sample. The wick and the large bridge voltage which are used with the chromatographic detector are devices to increase this temperature change above the thermodynamically expected value, since the wick increases the surface area and alters the surface energy, and the large detector voltage causes heating, which increases the temperature difference between the thermistors, in the presence of a solute band, through increasing evaporation rates.

C. Evolution of the Present Detector

Since most of the time spent in the development of the present liquid chromatography detector involved exploratory investigation, the course of the development of the apparatus will be traced briefly from its conception to its present state.

The first detector was designed to detect the progress of the development of a chromatogram on a string column. The choice of thermistors as the sensing elements, and the use of vapor pressure as the property measured were the result of elimination of the other possible means of detection of solute bands located on a string column. A detector for string chromatography should not disturb the chromatogram, but must be able to locate the solute bands and indicate their relative concentrations. All of the commonly used techniques (14) were considered, but none was found that could be adapted to this purpose. All of the existing techniques were either too specific for one particular kind of solute, or too insensitive to small quantities of solute. The most promising of the techniques considered was differential refractometry, but it required expensive and complicated instrumentation, and it could be used only by eluting the bands off the string.

One physical property remaining to be considered was vapor pressure. The techniques commonly used for measuring vapor pressure were unsatisfactory for measuring the presence of a solute band on string, since they would require interruption of the development of the chromatogram. The idea for use of thermistors as detectors of vapor pressure was the result of the observation that colored solute bands on a string suspended in a vapor-saturated chamber appeared to spread more than might be expected from the natural spreading due to the adsorption of the components on the string. If part of the observed spreading were due to the net condensation of solvent vapor from the saturated atmosphere surrounding the string, then the region of the string occupied by the solute band should be slightly warmer than the chamber. The problem, then, was to find the proper chamber conditions and the proper detector for this temperature rise. Thermistor and thermocouple sensing devices were considered, and the thermistor was chosen because of its much greater sensitivity.

A simple thermistor detector circuit was designed, consisting of two thermistors as opposing arms of a Wheatstone bridge. The thermistors were clamped onto a string suspended on a wire frame within a bell jar saturated with water. The solvent (water) was supplied to the string by capillary siphon from a beaker at the top of the string. The unbalance of the bridge was observed with a galvanometer. The signal observed on the galvanometer was completely inconclusive until the bell jar was covered with a large, insulating cloth. The cloth served both as a temperature baffle and as a light shield. The samples were placed on the string with a long capillary and medicine-dropper. This detector arrangement gave a barely-observable response to a drop of saturated NaCl. This result, although not entirely satisfying, was positive enough to indicate that a detector might be designed using this basic principle.

The detector, string, and solvent reservoir were removed from the bell jar and placed into a silvered Dewar flask covered with a cork arranged with a hole suitable for sample injection. This system afforded a reasonably adiabatic container for the detector; however, it had several undesirable features. Samples, when injected, would disturb the equilibrium of the detector for hours; the system required hours to stabilize, but was easily disturbed with room temperature fluctuations, and sample injection was at best uncertain and unpredictable.

In spite of these difficulties, the detector showed promise in the sensitivity of its response and in its apparent linearity within the limits of sample injection. Table I is representative of the response which was obtained for successive dilutions of a saturated NaCl solution. The increasing deviation from linearity can be explained by the fact that the flow rate decreased during the time the samples were run. The flow rate was observed to decrease without apparent reason, even with a constant-head device. The solvent flow on string decreased over a period of several weeks and finally stopped. Addition of detergents or alcohols

TABLE I

REPRESENTATIVE RESPONSE FOR EARLY DETECTOR

Successive Dilutions Of Saturated NaCl	Recorder Response (Centimeter-minutes)
0.1	284
0.01	30.2
0.001	3.2

would restore the flow temporarily, but the solvent flow would decrease again until finally it could not be restored. This characteristic of string columns forced abandonment of their use with a detector. The detector was also recognized as being potentially more useful for column or paper chromatography than string, because separations on string were very limited.

Up to this stage in the development of a detector, both of the thermistors had been located on the same string. This arrangement was adopted to give an initial deflection corresponding to the total sample, which was injected just above the top thermistor, and a second positive peak or series of peaks corresponding to the solute bands separated by the column. The top thermistor was useless except for a balance element, since sample injection disturbed the equilibrium during the time that the sample passed it. The thermistor was therefore subsequently placed on a separate string, which acted as a reference wick only and more effectively served as a balance element.

Since the string had to be abandoned as a column, the emphasis of the project was shifted to designing a detector for liquid column chromatography. The string detector did not require much modification for adaptation to column chromatography, since the strings were still used as wicks to carry the effluent of the columns over the thermistors. Two chromatographic columns were mounted over the detector by boring holes in the cork covering the Dewar flask and attaching the strings to the bottoms of the columns. The signal from this detector was found to be very unsatisfactory because of a greatly increased noise level. The sources of difficulty were identified as being associated with the flow rate and the temperature fluctation of the solvent. The whole apparatus was moved to a constant-temperature room and the solvent pressure was controlled with a Cartesian manostat. These changes improved the stability of the detector, but the on-off regulation of the room temperature was observed as a sine wave on the background trace.

The background was stabilized by thermostating the detector chamber and the columns with water from a constant temperature bath controlled to $\pm 0.008^{\circ}C$. The columns and the detector were immersed into the bath, protected by a plastic bag. It was observed that the system equilibrated much more readily when the Dewar flask around the detector was replaced by a glass container covered with aluminum foil. Better equilibration with the bath temperature was obtained by using a condenser jacket around the columns. The plastic bag was eventually replaced with a long glass cylinder weighted with lead and insulated around the top with glass wool.

These steps finally sufficiently stabilized the detector that a suitable background was obtained. However, the detector sensitivity had decreased so much as to make the detector almost useless, since it was found that large concentrated samples were required to obtain appreciable signals. This lowered sensitivity was finally traced to the large solvent flow rates used. The columns (8 mm. i.d.) required flow rates of several milliliters per hour to elute samples within a reasonable length of time. These large-diameter columns were replaced by capillary columns (2 mm. i.d.), so that the flow rate could be reduced to less than 10 milliliters per day. Sample sizes were reduced to a few hundredths of a milliliter of solution.

These changes bring the detector up to essentially its present form. The nature and properties of each of the individual components of the present chromatographic system will now be considered in more detail.

D. Components of the Present Chromatographic Apparatus

1. Columns

Fundamentally the diameter and length of the column employed with this detector are not limited. Since the flow rate of solvent into the detector must be limited, however, the columns must be of small diameter if all of the eluent stream is to flow through the detector. If use of large columns is desired, a device for diverting a part of the effluent stream must be employed. The small capillary columns employed in the development of the detector are very convenient for analytical purposes, since they require only very small amounts of sample. These small columns are difficult to pack, but if handled properly they may be used indefinitely with little change in properties.

The columns packed in 2 mm. i.d., thick-walled capillary tubing were placed just above the detector chamber as shown in Figure 1. The bottom of the column extends just below the cork cover inside of the detector chamber, so that the wick does not touch the cork. The thermostating jacket must be long enough so that the solvent passing down the columns becomes equilibrated with the bath temperature. The intake for the thermostating jacket should be located at the regulator for the bath, so that the jacket temperature does not vary appreciably.

The column packing is, of course, selected for the particular type of separation which is to be made.

2. Wicks

Cotton string or thread wicks have been used throughout most of this work. The size and the weave of the wick are dictated by the flow rate of the solvent, the design of the detector, and the volatility of the solvent. Maximum sensitivity is attained with minimum wick size and maximum surface area. The wick must be large enough, in relation to the flow rate, for the solvent to flow without forming droplets or surges, and to cover as much of the thermistor surface as possible; however, since the sensitivity is decreased as wick size is increased, the optimum sensitivity is obtained with the minimum wick size which will give a smooth solvent flow.

The ideal situation might be to have the detector thermistor in contact with a large-area, uni-molecular layer of solvent. This should

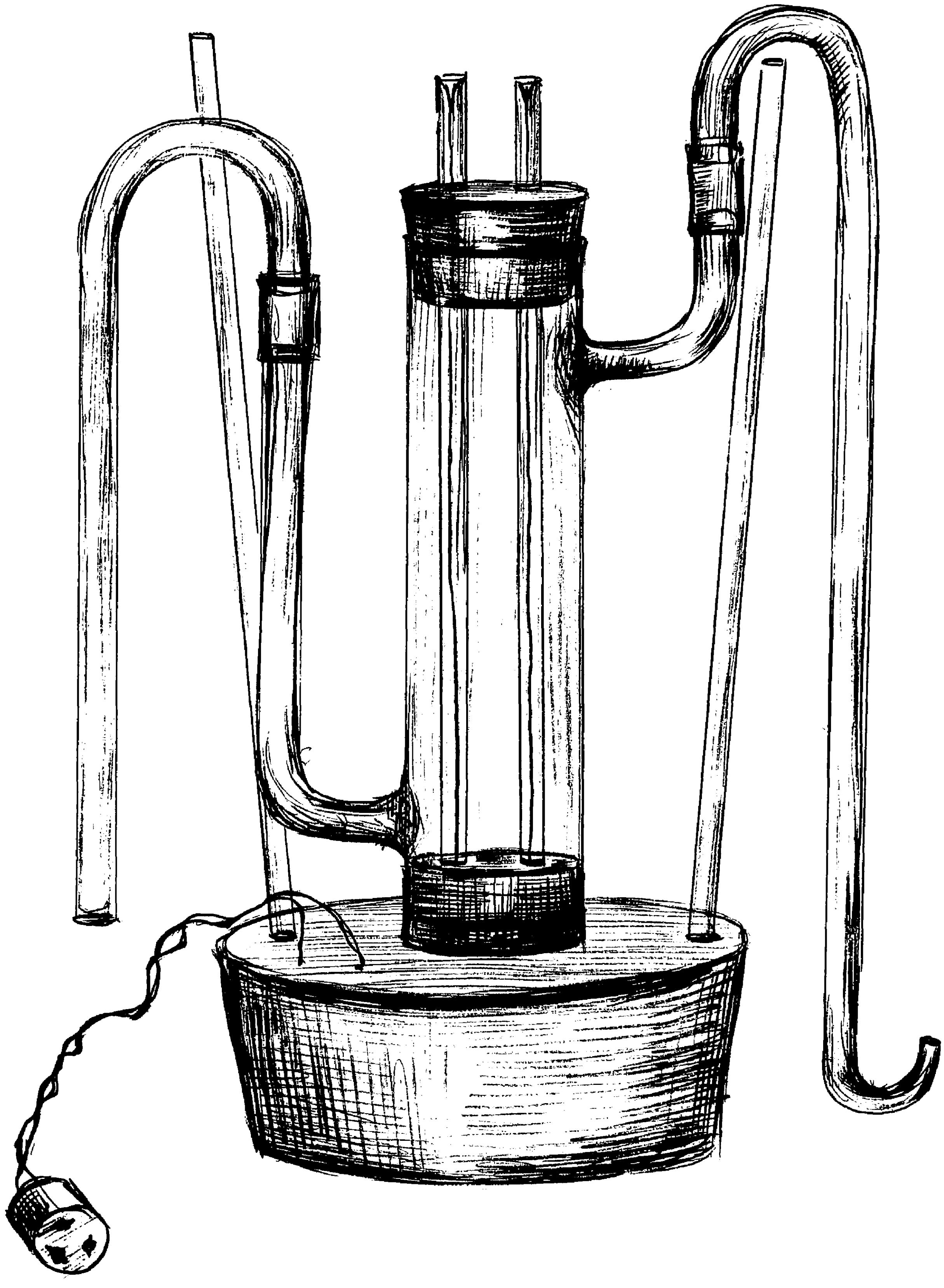

FIGURE 1. COLUMNS SHOWN MOUNTED IN THERMOSTATING JACKET ABOVE DETECTOR CHAMBER.

allow complete equilibration of solvent with the vapor phase and should give the maximum temperature change for a given amount of solute. It is impractical to achieve this situation experimentally for two reasons.

1. the flow rate would necessarily be too small, and
2. the thermistor temperature is slightly above the temperature of the saturated chamber; this increased temperature would cause the solvent to dry and cause erratic flow.

An attempt was made to construct a detector to approach this "ideal" situation as nearly as possible. The wick was eliminated, and the solvent flowed down the thermistor in a steady (though not unimolecular) stream. This detector arrangement was sensitive, but it was not stable, because the solvent stream was erratic. The presence of the wick seems to stabilize the solvent flow through the detector, and it possibly gives added stabilization because of its larger heat capacity.

The presence of a cotton wick was at one time feared to interfere with the separations on alumina and cause undue spreading of component bands. A glass-wool wick was substituted for the cellulose wick, but it did not prove as satisfactory, possibly because the larger surface area of the cotton wick permits much more rapid condensation and vaporization of the solvent vapor, giving greater response to the solute bands. The flow characteristics may also have been less stable with the glass-wool wick.

The most desirable location of the thermistors on the wick seems to be 3-4 centimeters from the mouth of the column. Placement of the thermistor too near the column does not allow time for equilibration of the solvent with the chamber atmosphere. If the thermistors are placed too far from the columns, the solute bands may be spread, due to the net condensation of the solvent on the wick at the solute band.

The end of the wick must be touching the bottom of the detector chamber or some auxiliary container, since any drop formation on the wick disturbs the flow rate of the solvent across the thermistor, causing background instability. When siphons were used to exhaust the detector chamber of excess solvent, they had to be arranged so that no drops would form even on the external outlet, since this also was found to disturb the detector.

The optimum flow rate for a given wick may be found by placing the wick in a transparent chamber saturated with solvent vapor; the solvent flow is then decreased down the wick until the wick continuously appears to be slightly damp with solvent.

3. Thermistors

The resistance of a thermistor as a function of temperature may be expressed by the equation,

$$r = r_0 \, e^{-b(T-T_0)}$$

Where r is the resistance in ohms, r_0 is the resistance at temperature T_0, b is the temperature coefficient, and T is the temperature.

The thermistors selected for a detector should be as nearly matched as possible, to minimize the instability caused by the fluctuations in the ambient temperature of the thermostated chamber and of the flowing solvent. The values of b should be within one per cent and the values of r_0 within five per cent for the two thermistors. The thermistors used had a resistance of between 2,000 and 4,000 ohms at the temperature of the thermostat. Higher resistance thermistors are less sensitive, and lower resistance thermistors cause too much electrical heating at the voltages required for sensitive operation. Since overheating of the thermistor will cause its resistance to drop, which increases the current and causes still more heating, lower resistance thermistors will be more inclined to degenerate and burn out.

Two designs of thermistors have been used, depending on the solvent and wick desired.

a. Thermistor sealed in the middle of a glass envelope (see Figure 2). This type of element was used in non-corrosive, non-conducting solvents. Its major advantage is the fact that the wick can be attached to the thermistor very securely, with a large surface area of the wick spread thinly over the surface of the thermistor. This arrangement is very close to the ideal thermistor-wick relationship, with a maximum exposed surface area of wick in contact with the thermistor. The technique for securing the thermistor to the wick is to unravel the wick up to the thermistor and allow it to rewind around the glass envelope. The strands of the wick are spread over the surface of the thermistor and tied with a fine, coarse thread, so that slight jarring will not alter the juxta-position. Care must be exercised to prevent constriction of the wick by the thread.

b. Glass probe thermistor (thermistor sealed in the end of a glass bead--see Figure 3). The thermistors for this detector were supported by a wooden lath, which was suspended in the thermostated chamber from the

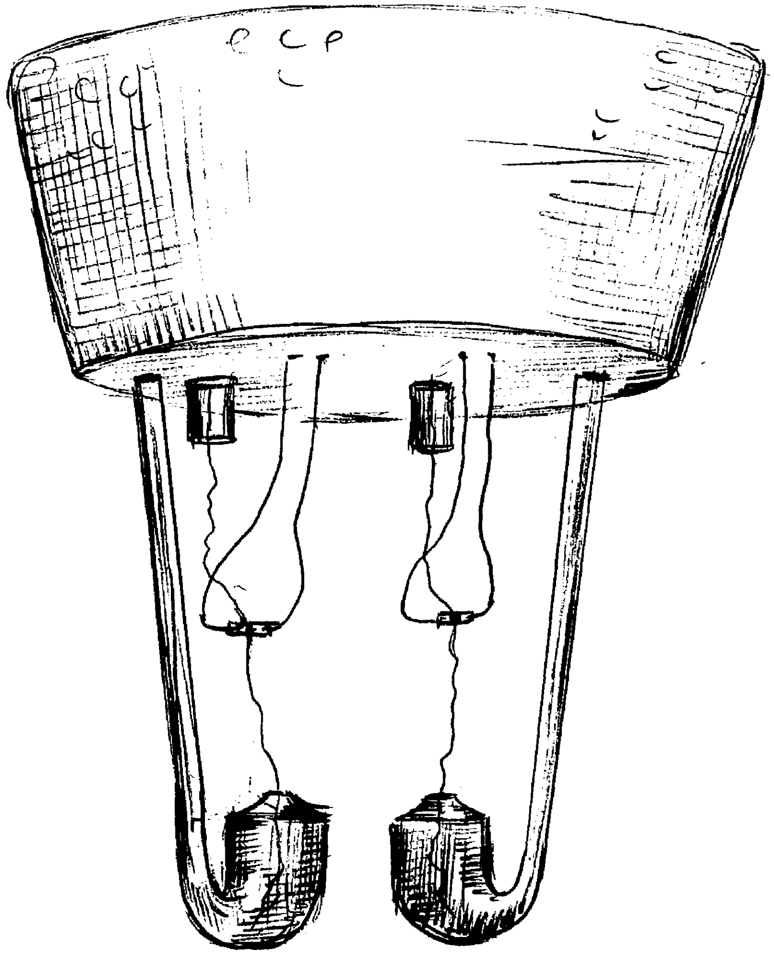

FIGURE 2. DETECTOR DESIGN USING THERMISTORS SEALED IN GLASS ENVELOPES.

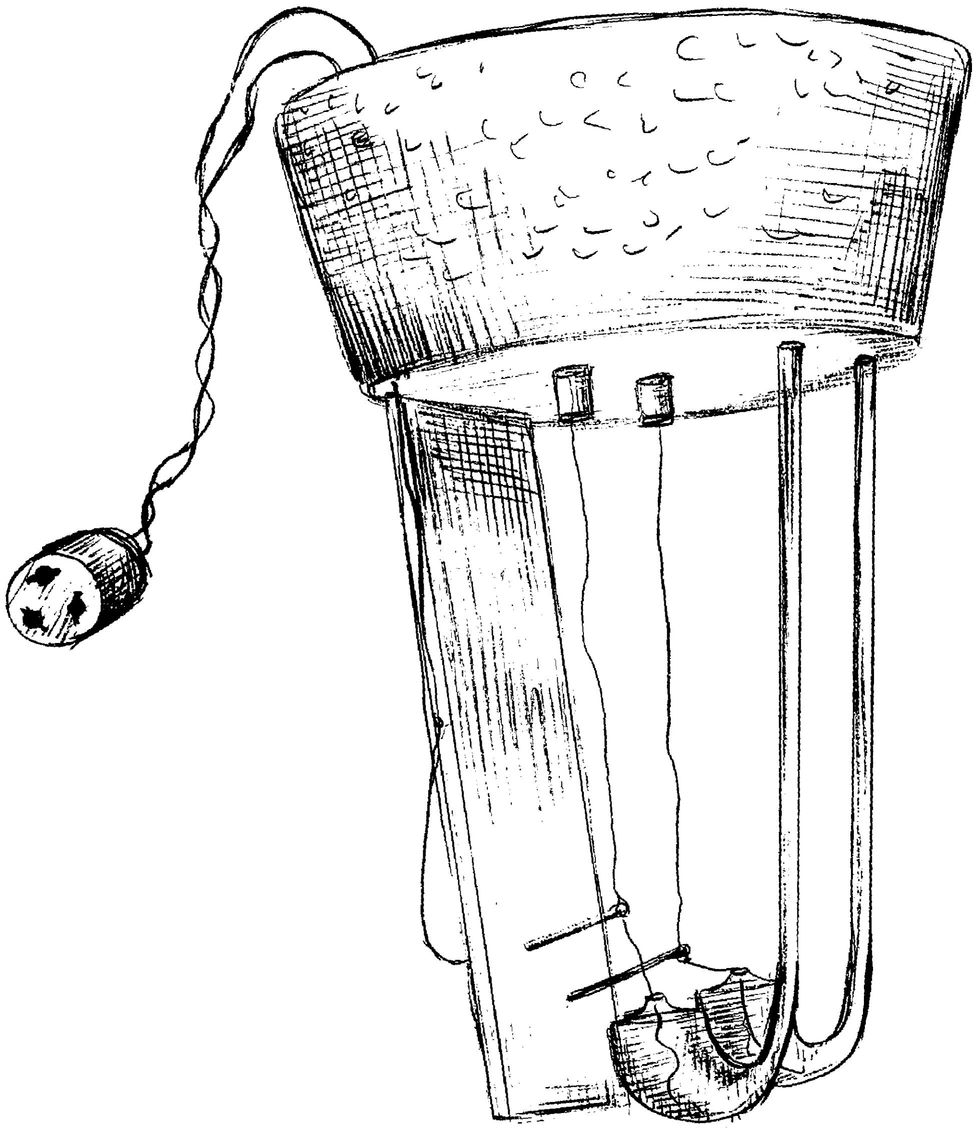

FIGURE 3. DETECTOR DESIGN FOR GLASS-BEAD THERMISTORS.

cork cover. The wick was unraveled and re-wound around the bead of the thermistor, to give a maximum exposed wick surface in contact with the thermistor. Probe thermistors are more sensitive in their response than are glass-envelope thermistors, since the probe thermistors are in closer contact with their thinner glass supports. The thermistors should be inclined slightly upward to prevent any impurities dissolved from the wooden lath from coming in contact with the wick. If the wick is improperly secured to the thermistor, the solvent may run backward down the thermistor and short the connecting leads.

4. Solvent Flow Control and Out-Gas Device

Solvent flow must be controlled very carefully in order to maintain the stability required for quantitative detection of small samples. Changes in flow rate of less than 0.1 milliliters a day may cause full-scale deflection. Attempts to control solvent flow with gas pressure and a Cartesian-diver manostat were not successful, since the pressure control was not sufficiently sensitive. Where possible, gravity furnishes the most constant source of solvent pressure. The solvent supply for the columns was a 3-liter, round-bottomed flask, which was supported by an adjustable laboratory-jack. Because of the large reservoir volume and the slow flow-rate of solvent, the level in the flask remained essentially constant over a period of several days. Slight adjustments of the solvent level were made periodically with the jack, referring the level to a fiducial mark with the aid of an eye-piece attached to the out-gassing tube.

The out-gas device is shown in Figure 4. Out-gassing is even more essential with capillary columns than with larger columns, because even very small bubbles cause solvent flow interruption in small-diameter columns.

Detector sensitivity was found to increase almost linearly as the flow rate was decreased down to 3-5 milliliters á day. The flow rate selected for most of the later work was 5 milliliters a day. A flow rate of 2.5 milliliters per day increased the sensitivity by a factor of 1.7, but the elution time became prohibitive for the size column employed. More noise was also observed at this flow rate, because the wick seemed to dry periodically.

5. Sample Injection

One of the major problems encountered in this study is the reproducible injection of a liquid sample into a moving liquid eluent. If the solvent flow is interrupted, the equilibrium in the detector chamber is disturbed. If, on the other hand, the sample is introduced directly, considerable spreading of the solute band may result because of turbulence and diffusion.

The injection arrangement used is shown in Figure 5. If the usual liquid chromatography technique is used for sample injection, the three-way stopcock is opened so as to cut the flow from the solvent reservoir and allow a long hypodermic needle to be inserted into the space just above the column. The excess solvent is removed from the column and the sample injected into the top of the capillary. The sample must be pushed

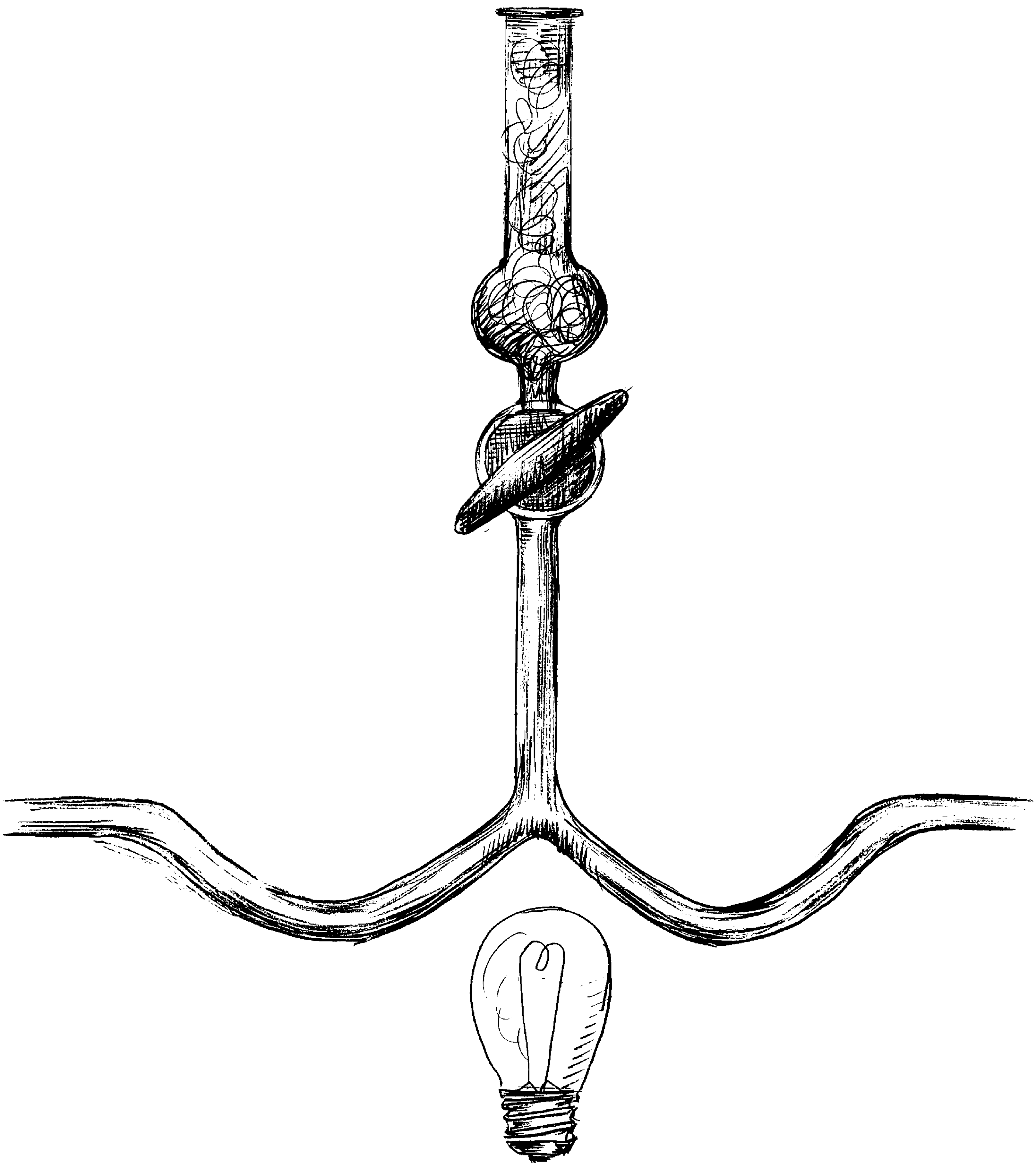

FIGURE 4. OUT-GAS DEVICE, LOCATED IN SOLVENT LINE BETWEEN RESERVOIR AND SAMPLE INJECTION PORT. HEAT OF LAMP DISPLACES DISSOLVED AIR, WHICH IS VENTED THROUGH STOPCOCK AND DRYING TUBE.

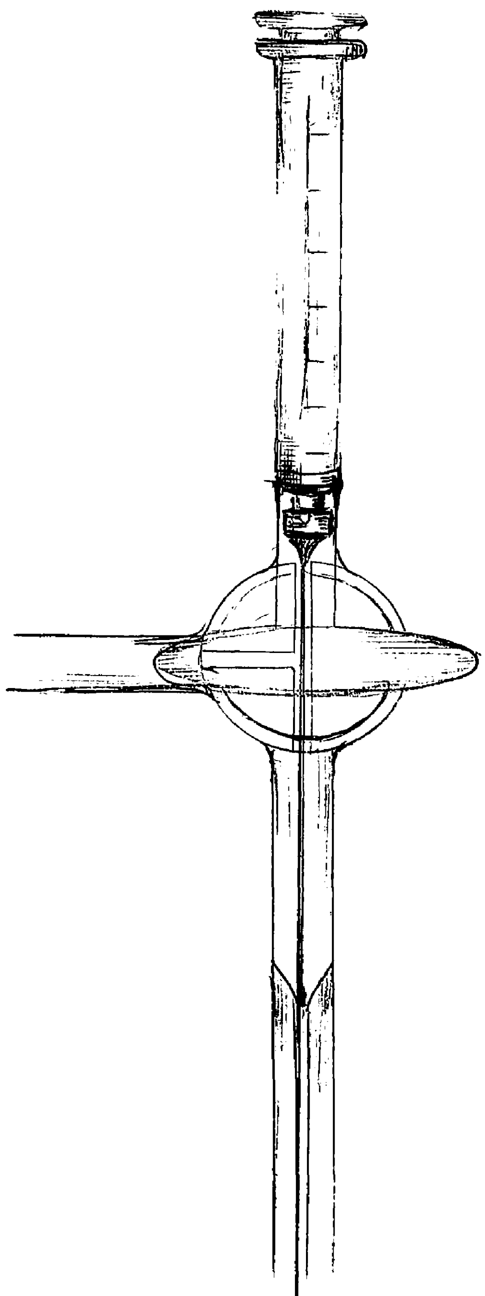

FIGURE 5. ARRANGEMENT FOR SAMPLE INJECTION.

into the capillary so as to avoid wetting the outside of the needle with sample. The needle is withdrawn as the sample fills the capillary, but it must be withdrawn slowly to avoid trapping air bubbles which would stop solvent flow. Ordinarily in column chromatography the sample is allowed to flow down into the top of the column until the top meniscus of the sample just starts onto the column, but with a capillary column the solvent level may be restored immediately, since the solute band is not spread by mixing in the capillary. It is best to restore the normal solvent pressure as soon as possible, since the detector is disturbed by the sample injection.

Surprisingly little spreading results from injection of the sample directly into the solvent stream, providing the needle used is very small and the sample is 0.01-milliliter or less. This size sample can be injected into the stream reproducibly by means of a hypodermic micro-syringe with a Chaney adaptor.

6. Thermostating and Temperature Control

Temperature control is one of the major limitations to sensitivity. The thermostat for a liquid chromatography detector should be constructed carefully with the best available equipment; however, a bath which regulates to $\pm$ 0.01°C. is adequate for detection of samples of larger concentration than 10^{-8} moles in 0.01 milliliters (10^{-3} M). The regulating elements for the bath must be isolated from the bridge circuit to prevent any electrical pick-up from the relays or heaters.

The on-off heater used was a 150-watt infra-red bulb, operated by a mercury relay switch. The relay was activated with a Fisher-Serfass Electronic Relay, controlled by a mercury micro-set thermoregulator. The steady heater and cooler (125-watt knife heater, and copper coil cooled with tap water, respectively) were adjusted so that the on-off time was 35 seconds. The detector system was insulated so that it required 195 seconds to respond to bath temperature changes.

The bath itself was insulated and covered, with the exception of a small opening for the infra-red lamp, which was set 5 inches from the water level. The water was circulated with a small centrifugal pump, which was shock-mounted to reduce vibration. The water level in the bath was maintained with a siphon.

The detector chamber was placed in the center of the bath inside a large glass cylinder. The chamber and thermostating jacket for the columns were insulated with glass wool. The glass-wool insulation also acted as a cushion to isolate the detector chamber from vibration and shock, since the suspension for the cylinder containing the chamber is supported by the wool.

7. Wheatstone Bridge

The Wheatstone bridge which is used to detect the change in resistance resulting from the difference in temperature of the thermistors need not be designed to measure the absolute resistance. This aspect of the requirement simplifies the design of the bridge considerably; however, the small signals which must be measured do require careful shielding and grounding. Much of the random noise in the circuit can be eliminated by damping, since the signals of interest are long in duration, allowing use of time constants as long as 30 seconds to five minutes.

A schematic diagram of the detector is shown in Figure 6. The thermistors (r_s and r_r) were selected as described in the thermistor section. The variable external resistances (r_r^o and r_s^o) were matched decade resistance boxes. The potential source (B) was a 6-volt storage battery which had been charged in a stepwise manner, shaken to reduce polarization, and allowed to stand two or three days before incorporation into the bridge. The storage battery, when treated in this manner, is a very stable potential source for periods of time as long as a month, provided that the top of the battery is carefully cleaned of all acid and kept dry. The unbalance of the bridge was detected with a 1.0-millivolt full-scale electronic recorder (V); the recorder used was a Brown Electronik recorder with adjustable zero and variable span from 0-1 mv. to 0-51 mv. full-scale). A change of one ohm on the bridge caused a deflection corresponding to 60 per cent of full scale on the maximum sensitivity scale (0-1 mv.) . Thus, with 2000-ohm thermistors having temperature coefficients of about five per cent per degree, a temperature difference of 0.001°C. between the thermistors could easily be discerned on the recorder.

This sensitivity is by no means the limit of the sensitivity conceivable for this type of detector. It is, however, sufficient for detection of 10^{-8}-10^{-9} moles of sample in 0.01 milliliters of solution.

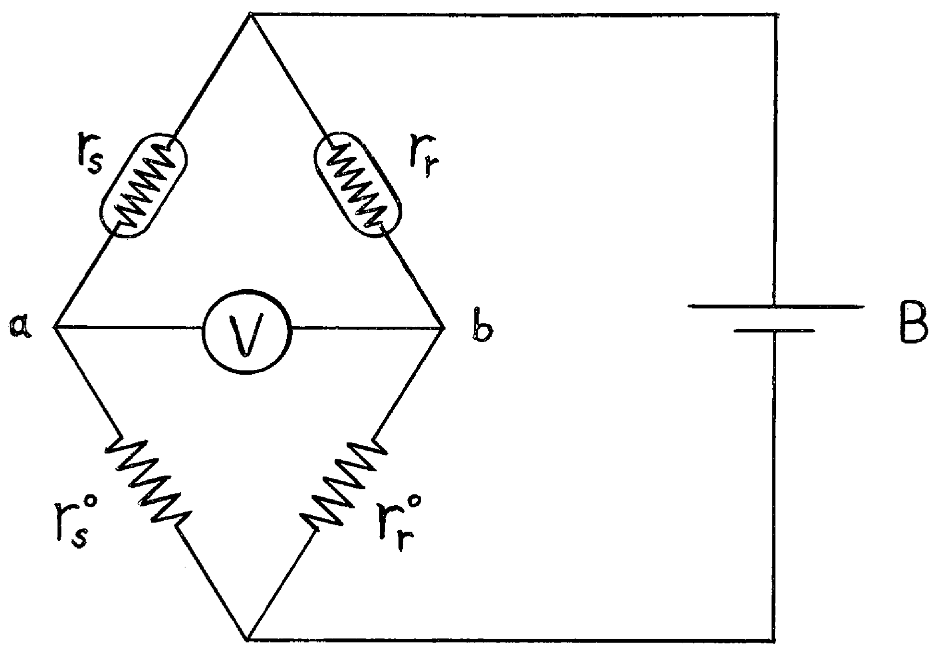

FIGURE 6. BRIDGE CIRCUIT

8. Treatment of Solvent and Preparation of Columns

One of the conveniences of gas chromatography is the fact that the same column may be used for repeated separations without significant changes in its retention times for particular components. This allows the operator to make repeated runs, using the time required for elution of a particular component band for identification of the component. In liquid chromatography a given column is usually packed for a separation and then discarded, since it may become partially deactivated. This process is very inconvenient, and it is particularly so for the new detector, since stabilization often requires several hours or longer for a new column.

The time required for stabilization of newly-packed columns was reduced considerably by equilibrating the solvent used with an excess of adsorbent. This treatment of the solvent was found to be more convenient and effective than distillation and other means of purification. When the solvent was prepared in this manner, the columns did not become deactivated over indefinite periods of time. One set of alumina columns was used for separations of ergosterol and calciferol for a period of five months with no apparent deactivation. During that time the columns were exposed to several different kinds of samples of questionable purity and became discolored to a dull yellow brown, but the retention time remained approximately the same.

Two-millimeter i.d. capillary columns were packed with an alumina slurry in the solvent used and sealed at the top and bottom with cotton plugs. The cotton plugs served two essential functions: to prevent the column from being disturbed by sample injection, and to stop solvent flow if air or dissolved gas became trapped in the capillary. These tight cotton plugs protected the columns from certain deactivation on two separate occasions when the solvent level ran down into the capillary.

9. Purification of Reagents and Preparation of Solutions

The cyclohexane (95 per cent, v./v.)-acetone (5 per cent, v./v.) solvent used for the solutions employed for molecular weight and concentration-response determinations was prepared by treatment with an excess of adsorbent. Samples of the solvent were chromatographed to determine whether the background was altered by the solvent.

Eastman White-Label Grade solutes were used directly with no further purification. The solutions were prepared by direct weighing of the solute and addition of the solvent from a graduated hypodermic syringe.

III. QUALITATIVE APPLICATIONS OF THE DETECTOR

A. General

Most of the research in the development of this detector has been directed towards devising a tool suitable for general use in liquid chromatography. The initial goal was to demonstrate its applicability to various solvent systems and to a large variety of solutes in these systems. The general exploratory results will be presented in the first three parts of the qualitative applications section.

Once the potential was clearly demonstrated, the interest shifted to two specific applications of the detector. Because of its importance to other studies being carried out in these laboratories, the analysis of the ergosterol irradiation mixture received special attention. For work with fairly dilute solutions of these high molecular weight components, it was found necessary to increase the sensitivity of the detector by a factor of 100 above its level at the time of initiation of this specific study. This new goal sparked further instrumentation to obtain the desired sensitivity.

The second of the specific applications explored has been the possible use of the detector for molecular weight determinations. This led to the mathematical and quantitative studies presented in Sections IV and V.

B. Water (Solvent)-Cellulose (Adsorbent) System

This solvent-sorbent system was employed in the early stages of the development of this detector. Since it is not very useful chromatographically, no separations were observed; however, the general nature of the detector response which was clearly established helped in mapping out the subsequent developments. The pattern of positive deflection (heating of the sample thermistor) for solutes giving solutions less volatile than the solvent, and negative deflection (cooling of the sample thermistor) for solutes giving solutions more volatile than the solvent was observed. Table II gives a list of the solutes used in this system, together with their directions of deflection.

TABLE II

SOLUTES OBSERVED WITH WATER-CELLULOSE SYSTEM

Solute	Boiling Point °C.	Direction of Deflection
Glycerol	290	positive
Ethylene Glycol	197.4	positive
Butanol-1	117	negative
Propanol-2	82.4	negative
Ethanol	78.4	negative
Sugars	non-volatile	positive
Parker Blue-black Ink		positive
Salts (Nacl, NaBr, NaI, KCl, KBr, LiCl, $CuCl_2$)		positive

The pattern of positive and negative deflection is clearly established in Table II, except possibly for butanol-1, which has a boiling point higher than that of water. The vapor pressure of a butanol-water mixture, however, is greater than that of pure water at 25°C. It is not possible to make quantitative estimations of the vapor pressure, because the concentration of the solution as it passes the thermistor is not known.

A string column was used in this preliminary work. This first detector was sensitive to moderately small samples, but, unfortunately, the reason for this sensitivity was not recognized until much later. The string column by its nature required very slow flow rates, so the quasi-equilibrium required for a temperature rise could be approached. After the string column was replaced with packed columns, several months of fruitless investigation were required to regain this sensitivity, because of the higher flow rates which resulted.

C. Butanol Saturated with Water--Cellulose System

When packed columns were first used with this system, it was possible to observe chromatographic separations for the first time with the new detector. The sensitivity of the detector was reduced because of large flow rates, so that it was not possible to observe small samples; however, it was possible to obtain some semi-quantitative results to demonstrate possible use of the detector as an analytical tool.

The most interesting, and perhaps the most important, of the observations made with the butanol-water system is the negative-peak effect due to the changes in the solvent concentration upon the introduction of a solute-containing sample on a chromatographic column. Because of its importance, this effect will be discussed in detail in a later section (Section VI-A).

Table III gives a list of the solutes used with this solvent-sorbent system, together with their deflection directions.

TABLE III

SOLUTES OBSERVED IN BUTANOL-CELLULOSE SYSTEM

Solute	Boiling Point °C.	Direction of Deflection
Glycerol	290	positive
Ethylene Glycol	197.4	positive
Mineral Oil	non-volatile	positive
Acetone	56.5	negative
Benzene	80.1	negative
Acetic Acid	118.1	negative

Partial separation of a mixture of ethylene glycol and glycerol was observed. The relative peak heights could be calibrated to give their relative concentrations within five per cent. Complete separations would have been possible using less concentrated samples or longer columns. Since the system did not afford complete separation, the individual peaks could be distinguished only when any one solute constituted no more than 70 per cent of the total solute in the sample.

D. Cyclohexane (95 per cent, v./v.) and Acetone (5 per cent, v./v.) (Solvent)--Alumina (Sorbent)

(Capillary Column--2 mm. i.d. x 23 cm.)

This solvent-sorbent system was selected because it was suitable for the separation of the ergosterol irradiation mixture. When this solvent-sorbent system was first employed, the detector was not sufficiently sensitive for direct analysis of the irradiation mixture. A large variety of different solutes, including ergosterol and calciferol, were chromatographed during the period of development of the detector sensitivity.

Table IV is a list of the non-volatile solutes run with this solvent-sorbent system.

TABLE IV

SOLUTES OBSERVED IN CYCLOHEXANE-ALUMINA SYSTEM

Mineral oil	Aroclor
Paraffin	Polystyrene
p-Dichlorobenzene	Anthracene
Naphthalene	Acridene
Camphor	Benzoic acid
Triphenyl methane	Hexamethyl benzene
Sudan red	Ergosterol
Sudan yellow	Calciferol

The saturated hydrocarbon samples (paraffin, mineral oil, and petroleum ether) which were chromatographed showed spreading due to their molecular-weight distribution, but no clear spreading of components, because the alumina column employed was too short to resolve these solutes. With longer columns, however, this system should be useful in hydrocarbon analysis for the non-volatiles.

Aromatic-hydrocarbon coal-tar derivatives, such as anthracene, are clearly separated from coal-tar heterocyclics on the short alumina column used. Asphalt samples were separated into two major components, each of which itself showed some resolution. Longer columns should resolve these samples into several peaks which would be suitable for characterization of asphalt samples.

Some solutes having solutions more volatile than the solvent were also chromatographed for comparison of the sensitivity of the detector for volatiles and non-volatiles. The sensitivity of the detector for volatile components increases as the vapor pressure increases, until the vapor pressure of the solute is so large that the solute evaporates from the wick before the sample reaches the thermistor. The maximum deflection observed showed that the detector was as much as 100 times more sensitive to volatile samples than to non-volatile samples. The maximum observed sensitivity occurred with petroleum ether as a solute in cyclohexane.

This solvent-sorbent system was employed in the later quantitative studies of detector response as functions of concentration and sample volume. These results are tabulated in the Appendix and discussed in Section V.

E. The Ergosterol Irradiation Mixture

This particular application was chosen because of its importance and because it represented a typical case of a biochemical or natural-product analysis approaching the limits of detector sensitivity. The chromatographic results could be compared with results obtained by spectrophotometric techniques recently developed in this laboratory (15), if the detector could be sufficiently developed to be able to detect very small samples of dilute solutions. Interest in this problem sparked new efforts to increase the sensitivity of the detector. In order for a chromatographically observable change in ergosterol to be induced by radiation, with the light source and monochromator available, the irradiation solution must be 10^{-3}-10^{-4} molar. If the sample size to be analyzed is to be 0.02 ml., and if direct sampling, without previously concentrating the solution is to be possible), the detector must be sensitive to 10^{-8}-10^{-9} moles of solute in a sample.

The refined instrumentation required to get the desired sensitivity was completed only shortly before the thesis was written. Unfortunately, quantitative results have not yet been obtained for this analysis; however, qualitative separations of prepared mixtures of calciferol and ergosterol have been run, and changes in ergosterol solutions subjected to sunlight have been observed.

The alumina chromatographic columns used were checked for activity with a mixture of Sudan red and Sudan yellow dyes (16). The Sudan yellow was eluted from the column and detected as a symmetrical band with a peak at 215 minutes. The Sudan red was observed as a broad diffuse band, first detected at 260 minutes. It was finally eluted completely after 420 minutes. This separation indicated adequate activity of the alumina for separation of the ergosterol irradiation mixture.

Pure samples of ergosterol and calciferol solutions were chromatographed to establish the retention times and purities. Calciferol had one peak at 205 min., while ergosterol had two peaks, one major peak at 280 min., and a minor one corresponding to the calciferol.

A 0.02-ml. sample of a 1:5 dilution of a saturated ergosterol solution irradiated with indirect sunlight for five days was chromatographed, showing a substantial decrease in the ergosterol peak and an increase in the calciferol peak. Subsequent irradiation reduced the ergosterol until it was indistinguishable from the background trace. Two other small peaks appeared between the ergosterol and calciferol during the progress of the irradiation. These peaks were not identified, but they may be tachysterol, precalciferol, or oxidation products, since in this preliminary experiment no effort was made to exclude oxygen. Figure 7 shows a representative detection of the separation of an irradiation mixture.

While only very preliminary results have been obtained with this system, there seems to be little reason to doubt that the detector can be applied to this and many other systems of biological interest.

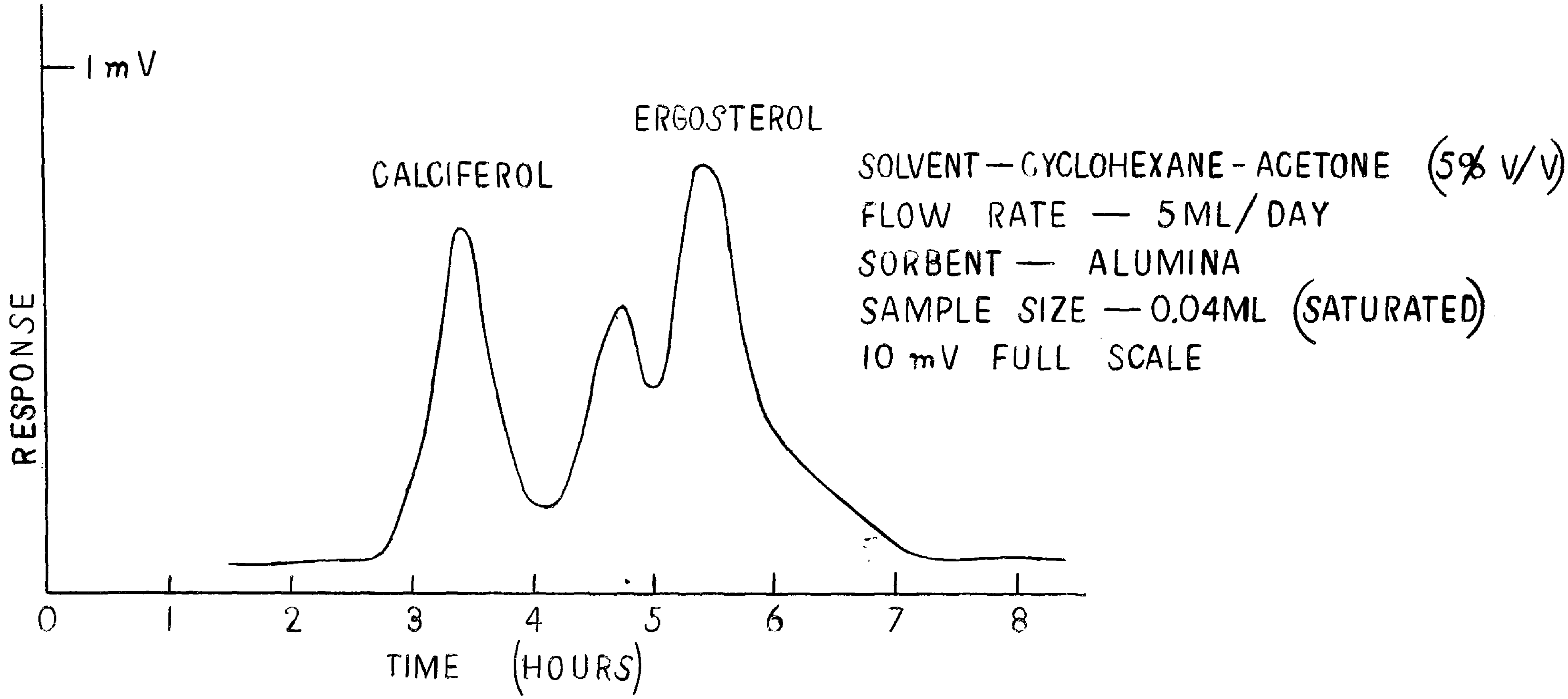

FIGURE 7. REPRESENTATIVE CHROMATOGRAM OF ERGOSTEROL IRRADIATION MIXTURE.

IV. MATHEMATICAL ANALYSIS OF THE DETECTOR RESPONSE

A. General

It is possible at the present time to propose a quantitative mathematical description of the detector response. Several simplifying assumptions are made to cast the equations into useful form; most of these assumptions can be justified because of the very small temperature unbalance involved in actual measurements. It is necessary, however, to restrict present considerations to the simplest case of a single-component solvent.

In the analysis will be considered, in that order, the electrical balance condition, the thermistor temperature-resistance relationships, the thermal balance condition for certain special cases, and the shape and size of the component peaks in the recorded chromatogram.

B. Electrical Balance Condition

The bridge circuit employed is shown in Figure 5. The battery, B, supplies a fixed voltage, E, to the bridge. The total current, i, is divided into the two paths i_s and i_r through the sample and reference thermistors, respectively. These thermistors have resistances r_s and r_r, and are in series with external resistances r_s^o and r_r^o, respectively. Then

$$(1) \qquad i = E \left(\frac{1}{r_s + r_r^o} + \frac{1}{r_r + r_r^o} \right)$$

or

(2) $$i = i_s + i_r$$

with

(3) $$i_s = \frac{E}{r_s + r_s^o}$$

and

(4) $$i_r = \frac{E}{r_r + r_r^o}$$

Since the reference arm is normally unaltered in a determination, it is convenient to define

(5) $$R_r = r_r + r_r^o$$

The recording potentiometer, V, measures the potential difference, e, between points a and b, under such conditions that current does not flow from a to b through the measuring circuit. Then

(6) $$e = i_r r_r - i_s r_s$$

or

(7) $$e = E\,\frac{r_r}{R_r} - E\,\frac{r_s}{r_s^o + r_s}$$

and

(8) $$\frac{e}{E} = \frac{r_r}{R_r} - \frac{r_s}{r_s + r_s^o}$$

Solving for r_s in terms of e gives

(9) $$r_s = \frac{\left(\frac{r_r}{R_r} - \frac{e}{E}\right) r_s^o}{\frac{r_r^o}{R_r} + \frac{e}{E}}$$

When $e = 0$,

$$(10) \qquad r_s = \frac{r_s^o}{r_r^o} r_r.$$

If $r_s^o = r_r^o$, and $e = 0$, then $r_s = r_r$. This is the condition which would ideally obtain for matched thermistors with pure solvent on each wick, equal flow rates, and identical wicks.

C. Thermistor Temperature-Resistance Relationships

The resistance of the thermistor is, of course, strongly dependent upon temperature. The normal expression for thermistor resistance as a function of temperature over a range of temperature is

$$(11) \qquad r = r_o \, e^{-b(T-T_o)}.$$

Over a very small temperature range this may be approximated by the linear expression

$$(12) \qquad r = r_o - \alpha(T-T_o),$$

where r_o is the resistance at T_o and α is the temperature coefficient of resistance. The resistance of the sample thermistor may then be expressed in terms of the resistance of the reference thermistor as

$$(13) \qquad r_s = r_r - \alpha\,(T_s - T_r).$$

It is convenient to rearrange this expression to obtain T_s in terms of r_s.

$$(14) \qquad T_s = \frac{r_r + \alpha\, T_r}{\alpha} - \frac{r_s}{\alpha}.$$

D. Thermal Balance Conditions

At each thermistor in the detector circuit, the condition of thermal balance can be expressed in terms of the heat input and heat removal factors which have been considered in Section II-A. Heat input results from electrical heating of the thermistor and from solvent condensation. Since the thermistor temperature is always higher than that of its surroundings, all other factors lead to heat removal. Most important of these is vaporization of the solvent; next most important is probably conduction of heat away by the flowing liquid. Less important, and probably negligible, heat removal occurs through radiation losses from the thermistor to the surroundings, and by thermal conduction and convection losses to the air, Non-volatile solutes have been assumed.

1. Rates of Heat Input.

a. Electrical heating of the thermistor:

(15) $$\dot{q}_E = i^2 r$$

Reference thermistor,

(16) $$\dot{q}_{Er} = i_r^2 r_r = \frac{E^2}{(r_r + r_r^o)^2} r_r .$$

Sample thermistor,

(17) $$\dot{q}_{Es} = i_s^2 r_s = \frac{E^2}{(r_s + r_s^o)^2} r_s .$$

b. Condensation of vapor:

(18) $$\dot{q}_c = k_c P_1^o A \quad \Delta H_{vap}$$

where k_c = rate constant for condensation, in moles per unit area per unit pressure per second; P_1^o = vapor pressure of pure solvent at the thermostated temperature, T_0; A = effective area of wick in contact with thermistor; and ΔH_{vap} = heat of vaporization of solvent at T_0 (assumed temperature independent). The heating due to condensation of vapor is assumed to be identical for both sample and reference thermistors, provided that A is the same for both wicks, since both are in contact with the same vapor reservoir.

2. Rates of Heat Removal.

a. Vaporization of solvent:

$$(19) \qquad \dot{q}_v = B_v \, e^{-\Delta H_{vap}/RT} \quad A \quad \Delta H_{vap} \cdot X_1 ,$$

where B_v = pre-exponential factor for rate constant for vaporization, in moles of solvent vaporizing per unit area of wick per second, and ΔH_{vap} is assumed to be the activation energy for the vaporization process. X_1 is the mole fraction of solvent at the thermistor. It has been assumed here that the solution is ideal; otherwise it is necessary to replace X_1 by $a_1 = X_1 \gamma_1$. Since sample and reference thermistors may be at different temperatures, the temperature T may be either T_s or T_r.

b. Conduction of heat by flowing liquid:

$$(20) \qquad \dot{q}_F = k_F F \, (T - T_0),$$

where k_F is the rate constant for the heat removal, in calories per second per unit flow rate per unit temperature differential between solvent, at

T_o, and wick, at T. F is the volume flow rate of the solvent down the wick (hence the flow rate of the column). F and T may differ for sample and reference thermistors, but k_F and T_o are assumed invariant.

c. Radiation of heat to surroundings:

(21) $\dot{q}_R = k_R (T - T_o)^4$.

This term is considered negligible at the small temperature differences employed.

d. Conduction and convection of heat to the surroundings through the gas phase:

(22) $\dot{q}_G = k_G A(T - T_o)$,

where k_G is the rate constant for the heat removal, in calories per second per unit area per unit temperature differential. This term will probably be negligible, but is included for completeness.

3. The Balance Conditions

All terms except radiation loss from the thermistor are included.

a. Reference thermistor:

Rate of heat input = rate of heat removal.

(22) $\dot{q}_{Er} + \dot{q}_c = \dot{q}_{vr} + \dot{q}_{Fr} + \dot{q}_{Rr} + \dot{q}_{Gr}$.

(23) $$\frac{E^2}{(r_r + r_r^o)^2} r_r + k_c P_1^o A_r \Delta H_{vap} = B_v e^{-\Delta H_{vap}/RT_r} A_r \Delta H_{vap} + k_F F_r (T_r - T_o) + k_G A_r (T_r - T_o).$$

Since pure solvent is used as the reference, $X_{1r} = 1$. During a run, the conditions at the reference thermistor are assumed constant.

b. Sample thermistor:

$$(24)\quad \dot{q}_{Es} + \dot{q}_c = \dot{q}_{vs} + \dot{q}_{Fs} + \dot{q}_{Rs} + \dot{q}_{Gs}.$$

$$(25)\quad \frac{E^2}{(r_s + r_s^o)^2}\, r_s + k_c P_1^o\, A_s\, \Delta H_{vap} = B_v e^{-\Delta H_{vap}/RT_s}\, A_s\, \Delta H_{vap} \cdot X_1$$

$$+ k_F F_s (T_s - T_o) + k_G A_s (T_s - T_o)$$

Since the resistance r_s and temperature T_s are inter-related by equation (13), equation (25) may be considered to be the relationship between T_s (or r_s) and concentration (expressed as X_1) at fixed bridge voltage, thermostat temperature, and column flow rate. Combination of this expression with equation (9) furnishes the desired relationship of observed recorder voltage, e, to concentration. Thus the problem has been solved in principle, but the equations obtained are not in readily useful form. Considerable simplification can be effected by considering the equations subject to the restriction of very small temperature difference between sample and reference thermistors, and by making other highly plausible assumptions.

E. Derivation of Response-Concentration Relationships for Ideal Solutions of Non-Volatile Solutes

The starting equations in this treatment are the thermal balance equations (23) and (25). Subtraction of equation (23) from equation (25) gives

$$(26) \quad E^2 \left\{ \frac{r_s}{(r_s + r_s^o)^2} - \frac{r_r}{R_r^{\,2}} \right\} + k_c P_1^{\,o} \;\; \Delta H_{vap} \; (A_s - A_r) =$$

$$B_v \cdot \Delta H_{vap} \; [A_s X_1 e^{-\Delta H_{vap}/RT_s} - A_r e^{-\Delta H_{vap}/RT_r}] +$$

$$+ k_F [F_s(T_s - T_o) - F_r(T_r - T_o)] + k_G [A_s(T_s - T_o) - A_r(T_r - T_o)].$$

It will be assumed that the wicks are equivalent, so that $A_s = A_r = A$. Then

$$(27) \quad E^2 \left[\frac{r_s}{(r_s + r_s^{\,o})^2} - \frac{r_r}{R_r^{\,2}} \right] = B_v \cdot \Delta H_{vap} \cdot A [X_1 e^{-\Delta H_{vap}/RT_s} -$$

$$e^{-\Delta H_{vap}/RT_r}] + k_F [F_s(T_s - T_o) - F_r(T_r - T_o)] +$$

$$k_G A (T_s - T_r).$$

It is convenient next to simplify equation (27) term by term.

The term on the left-hand side of the equation is the difference in electrical heating of sample and reference thermistors. It may be simplified by taking advantage of the relatively small difference in resistance of the two arms of the bridge.

$$(28) \quad \frac{r_s + r_s^{\,o}}{r_r + r_r^{\,o}} = 1 + \frac{(r_s + r_s^{\,o}) - (r_r + r_r^{\,o})}{r_r + r_r^{\,o}}$$

This is of the form $1 + x$ with small x, and

$$(29) \qquad (1 + x)^2 = 1 + 2x + x^2 \simeq 1 + 2x \text{ for small } x.$$

Hence

$$(30) \qquad \left(\frac{r_s + r_s^{\,o}}{r_r + r_r^{\,o}} \right)^2 \simeq 1 + \frac{2r_s}{r_r + r_r^{\,o}} + \frac{2r_s^{\,o} - 2\,(r_r + r_r^{\,o})}{r_r + r_r^{\,o}}$$

and, to this approximation,

$$(31) \qquad (r_s + r_s^{\circ})^2 = 2(r_r + r_r^{\circ})r_s + (r_r + r_r^{\circ})\,(2r_s^{\circ} - r_r - r_r^{\circ})$$

Thus

$$(32) \qquad \frac{r_s}{(r_s + r_s^{\circ})^2} = \frac{r_s}{(2r_s + 2r_s^{\circ} - R_r)R_r}$$

From equation (13),

$$(33) \qquad r_s = (r_r - \alpha T_r) + \alpha T_s .$$

Combining equations (32) and (33),

$$(34) \qquad \frac{r_s}{(r_s + r_s^{\circ})^2} = \frac{r_r + \alpha\cdot\Delta T}{[2\alpha\cdot\ \Delta T + 2(r_r + r_s^{\circ}) - R_r]R_r}$$

The electrical heating difference term then becomes

$$(35) \qquad \frac{E^2}{R_r}\left\{\frac{r_r + \alpha\cdot\Delta T}{[2(r_r + r_s^{o}) - R_r] + 2\alpha\cdot\Delta T} - \frac{r_r}{R_r}\right\} = \frac{E^2}{R_r^{\,2}}$$

$$\cdot\,\frac{2r_r(r_r^{\circ}-r_s^{\circ}) + (r_r^{\circ}-r_r)\alpha\cdot\Delta T}{R_r - 2\,(r_r^{\circ}-r_s^{\circ}) + 2\alpha\,\Delta T}$$

It is readily seen that this term is small and can be made essentially zero if $r_r^{\,o} = r_s^{\,o}$ and $r_r^{\,o} = r_r$; both of these conditions can be met experimentally. Two cases will be considered separately. In the first case, $r_r^{\,o} = r_s^{\,o}$ and $r_r^{\,o} = r_r$, so the electrical heating term drops out. In the second case, $r_r^{\,o} = r_s^{\,o}$, and the term becomes

$$(36) \qquad \frac{E^2}{R_r^{\,2}}\ \frac{(r_r^{\circ}- r_r)\,\alpha\cdot\Delta T}{R_r + 2\alpha\cdot\Delta T} \simeq \frac{E^2}{R_r^{\,2}}\ \frac{(r_r^{\circ}- r_r)}{R_r}\ \alpha\cdot\Delta T$$

where the approximation is based on the fact that $2\,\alpha \cdot \Delta T << R_r$. (For 0.1 M biphenyl, $2\,\alpha \cdot \Delta T \simeq 40$ ohms, $R_r \simeq 4000$ ohms, $\frac{2\alpha \cdot \Delta T}{R_r} \simeq 0.01$; even a 0.1 M solution is considered to be a high concentration, probably higher than would normally be encountered in chromatographic work).

The first term on the right-hand side of equation (27) represents the heat absorbed by vaporization. It can be considerably simplified by the following assumption.

$$(37)\quad \frac{e^{-\Delta H_{vap}/RT_s}}{e^{-\Delta H_{vap}/RT_r}} = e^{\frac{-\Delta H_{vap}}{R}\left(\frac{1}{T_s} - \frac{1}{T_r}\right)} = e^{\frac{\Delta H_{vap}}{R} \cdot \frac{\Delta T}{T_r T_s}} \simeq e^{\frac{\Delta H_{vap}}{R} \cdot \frac{\Delta T}{T_r^2}}$$

The exponent, $\frac{\Delta H_{vap}}{RT_r^2} \cdot \Delta T$, can be evaluated for a case studied. For 0.1 M. biphenyl in cyclohexane (95 per cent, v./v.)--acetone (5 per cent, v./v.), with $T_r \simeq 300°K$ a deflection of approximately 20 ohms was obtained; since the thermistor resistance changes about 100 ohms per degree, $\Delta T \simeq 0.2°$. For cyclohexane, $\Delta H_{vap} = 6620 \frac{cal}{mole}$ at 25°C. (17). Hence $\frac{\Delta H_{vap}}{RT_r^2} \cdot \Delta T \simeq 7 \times 10^{-3}$, for a concentration considered to be in the high range. The exponential term in equation (37) may be considered, therefore, to be of the form e^x with small x, so that

$$(38)\quad \frac{e^{-\Delta H_{vap}/RT_s}}{e^{-\Delta H_{vap}/RT_r}} \simeq e^{\frac{\Delta H_{vap}}{R} \cdot \frac{\Delta T}{T_r^2}} \simeq 1 + \frac{\Delta H_{vap}}{R} \cdot \frac{\Delta T}{T_r^2}$$

Thus the vaporization term in equation (27) becomes

$$(39)\quad B_v \cdot \Delta H_{vap} \cdot A \left[x_1\, e^{-\Delta H_{vap}/RT_s} - e^{-\Delta H_{vap}/RT_r}\right]$$

$$= B_v \cdot \Delta H_{vap}\, A\, e^{-\Delta H_{vap}/RT_r} \left[x_1 + x_1 \frac{\Delta H_{vap}}{R} \frac{\Delta T}{T_r^2} - 1\right]$$

Introducing the mole fraction of solute, $x_2 = 1 - x_1$,

$$(40)\quad B_V \cdot \Delta H_{vap} \cdot A[x_1 e^{-\Delta H_{vap}/RT_s} - e^{-\Delta H_{vap}/RT_r}]$$

$$= B_V \cdot \Delta H_{vap} \cdot A \cdot e^{-\Delta H_{vap}/RT_r} [\frac{\Delta H_{vap}}{R} \cdot \frac{\Delta T}{T_r^2} - x_2(1 + \frac{\Delta H_{vap} \cdot \Delta T}{RT_r^2})]$$

It is also convenient to rewrite the second term on the right-hand side of equation (27) to include specifically the quantity $\Delta T = T_s - T_r$.

$$(41)\quad k_F\,[F_s(T_s - T_o) - F_r(T_r - T_o)] = k_F[F_s \cdot \Delta T + (F_s - F_r)(T_r - T_o)].$$

For equal flow rates, $F_s = F_r = F$, and

$$(42)\quad k_F\,[F_s(T_s - T_o) - F_r(T_r - T_o)] = k_F \cdot F \cdot \Delta T.$$

The expressions in equations (36), (40), and (41) can now be introduced into equation (27) to obtain

$$(43)\quad \frac{E^2}{R_r^3}(r_r^{\circ} - r_r)\,\alpha \cdot \Delta T \doteq B_V \cdot \Delta H_{vap} \cdot A\ e^{-\Delta H_{vap}/RT_r}\ [\frac{\Delta H_{vap}}{R} \cdot \frac{\Delta T}{T_r^2} - x_2\,(1 + \frac{\Delta H_{vap}}{R} \cdot \frac{\Delta T}{T_r^2})]$$

$$+ k_F[F_s \cdot \Delta T + (F_s - F_r)(T_r - T_o)] + k_G A \cdot \Delta T.$$

Recombining terms, grouping those linear in ΔT, and solving for x_2 gives

$$(44)\quad x_2 = \frac{[k_F F_s + k_G A + (r_r - r_r^{o})\frac{E^2}{R_r^3}\alpha + \frac{B_V \cdot \Delta H_{vap}^2}{RT_r^2} \cdot A e^{-\Delta H_{vap}/RT_r}]\,\Delta T + k_F(F_s - F_r) \cdot (T_r - T_o)}{B_V \cdot \Delta H_{vap}\ A e^{-\Delta H_{vap}/RT_r}\ (1 + \frac{\Delta H_{vap}}{R}\ \frac{\Delta T}{T_r^2})}$$

For fixed flow rates, thermostat temperature, and bridge voltage, this is of the form

$$(45) \qquad x_2 = \frac{a \cdot \Delta T + b}{(1 + c \cdot \Delta T)}$$

where

$$(47) \quad a = \frac{k_F F_s + k_G A + (r_r - r_r^{\circ}) \frac{E^2}{R_r^3} \alpha}{B_v \cdot \Delta H_{vap} \cdot A e^{-\Delta H_{vap}/RT_r}} + \frac{\Delta H_{vap}}{RT_r^{2}} ,$$

$$(48) \quad b = \frac{k_F (F_s - F_r)(T_r - T_o)}{B_v \cdot \Delta H_{vap} \cdot A \cdot e^{-\Delta H_{vap}/RT_r}} ,$$

and

$$(49) \quad c = \frac{\Delta H_{vap}}{RT_r^{2}}$$

It will generally be true that $c \cdot \Delta T << 1$, as was shown in the discussion between equations (37) and (38), where $c \cdot \Delta T \simeq 7 \times 10^{-3}$ for 0.1 M. biphenyl, which is considered to be in the high range of concentration. Hence an error of less than one per cent will generally result from neglect of the $c \cdot \Delta T$ term, and

$$(50) \quad x_2 = a \cdot \Delta T + b$$

Equation (50) can be expressed in terms of the observed voltage, e, by use of equations (9) and (14). Then

$$(51) \quad x_2 = \frac{a}{\alpha} \quad \frac{\frac{r_r}{R_r}(r_r^{\circ} - r_s^{\circ}) + \frac{e}{E}(r_r + r_s^{\circ})}{\left(\frac{r_r^{\circ}}{R_r} + \frac{e}{E} \right)} + b$$

Setting the external resistances equal ($r_r^{\circ} = r_s^{\circ}$),

$$(52) \qquad x_2 = \frac{a(r_r + r_s^{\circ})}{\alpha} \quad \frac{\frac{e}{E}}{\left(\frac{r_r^{\circ}}{R_r} + \frac{e}{E}\right)} + b$$

Solving for the measured voltage, e,

$$(53) \qquad e = \frac{\frac{r_r^{\circ}}{R_r} E \quad (x_2 - b)}{\frac{a(r_r + r_s^{\circ})}{\alpha} - (x_2 - b)}$$

When $x_2 = 0$ (pure solvent on sample thermistor), $e = e_o$, and

$$(54) \qquad e_o = \frac{-\frac{r_r^{\circ}}{R_r} \quad Eb}{\frac{a(r_r + r_s^{\circ})}{\alpha} + b}$$

Equation (53) shows a nearly linear variation of observed voltage with the mole fraction, x_2, of solute. Deviations from linearity might be expected at higher concentrations, because of the x_2 term in the denominator of equation (53); this should cause a more-rapid-than-linear increase of e with x_2 at higher concentrations. The origin of this term can be traced back to the $\frac{e}{E}$ term in the denominator of equation (52). Since r_r°, R_r, e, and E have all been measured, the importance of this term can be estimated. The value of $\frac{r_r^{\circ}}{R_r}$ is about 0.5. The bridge voltage, E, is 6 volts. Observed values of e range from less than one millivolt to perhaps 20 millivolts at the highest concentrations employed. Hence $\frac{e}{E}$ is in general less than 3×10^{-3}, which is less than one per cent of $\frac{r_r^{\circ}}{R_r}$. Thus, except for concentrations higher than 0.1 M., equation (52) becomes

$$(55) \qquad x_2 = \frac{a(r_r + r_s^{\circ})\, R_r}{\alpha\, r_r^{\circ}} \; \frac{e}{E} + b,$$

and equation (53) becomes

$$(56) \qquad e = \frac{\alpha\, r_r^{\circ}\, E}{a(r_r + r_s^{\circ})R_r} \; (x_2 - b).$$

When $x_2 = 0$, $e = e_o$, given by

$$(57) \qquad e_o = - \frac{\alpha\, r_r^{\circ}\, E}{a(r_r + r_s^{\circ})R_r} \; b, \text{ so that}$$

$$(58) \qquad e - e_o = \frac{\alpha\, r_r^{\circ}\, E}{a(r_r + r_s^{\circ})R_r} \, x_2$$

Equation (56) or (58) may be taken as the fundamental equation for describing the detector response. Further justification for the simplification of equation (53) to the form of equation (56) can be inferred from the available data. In the quantitative study of biphenyl in cyclohexane-acetone solvent, a response of 4.15 mv. was obtained for a 0.022 M. solution, in a range where the response-concentration dependence was found to be linear. Using $\frac{r_r^{\circ}}{R_r} = 0.5$ and $E = 6$ volts, $\frac{a}{\alpha}\,(r_r + r_s^{\circ})$ is found to be 5.25 in this solution with x_2 only 2.4×10^{-3}. Thus the x_2 term in the denominator of equation (53) should be negligible up to perhaps .1 M. solutions, so that use of the linear equation (56) is clearly justified in most of the cases of interest.

It has, then, been shown that, within the framework of the assumptions made, the response of the detector should be a colligative property, dependent only upon the mole fraction of solute in the solvent. Since dilute solutions are used, the response is also a linear function of the concentration expressed in molality or molarity. Thus

$$(59) \qquad m_2 \simeq \frac{1000\ x_2}{M_1}$$

and

$$(60) \qquad c_2 \simeq m_2\ d_1$$

where m_2 = molality of solute, c_2 = molar concentration of solute, M_1 = solvent molecular weight, and d_1 = solvent density.

The assumptions which have been made are:

1) thermal balance at each thermistor,

2) equivalent wicks,

3) constant rate of condensation, determined by the solvent vapor pressure at thermostat temperature,

4) negligible radiation heat loss from the thermistors,

5) no change in composition or temperature of the solution as it descends the wick prior to reaching the thermistor,

6) no heat capacity for the wick or the thermistor,

7) small percentage changes in absolute temperature and resistance at the sample thermistor in comparison to the reference thermistor, and

8) ideal solutions of non-volatile solutes.

Each of the assumptions should almost certainly be valid except for the neglect of temperature changes in the solution as it descends the wick, and the neglect of the heat capacities of the wick and the thermistor.

The heat capacities of the wick and thermistor are probably effectively incorporated into the term $k_G A^\circ \Delta T$, but the solution temperature assumption is probably less valid. However, as shown below, the form of equations (56) and (58) is unaltered by this assumption; only the definitions of some of the parameters are changed.

Perhaps the most plausible assumption for the descending solution is that it equilibrates thermally with its surroundings through the condensation-vaporization equilibrium. Then, equating condensation and vaporization rates,

$$(61) \qquad k_c P_1^\circ = B_V \, e^{-\Delta H_{vap}/RT} \, X_1$$

where T is the temperature on the sample wick just before the thermistor. For the reference wick, $x_1 = 1$ and $T = T_o$, so

$$(62) \qquad k_c P_1^\circ = B_V \, e^{-\Delta H_{vap}/RT_o}$$

Hence

$$(63) \qquad x_1 = \frac{e^{-\Delta H_{vap}/RT_o}}{e^{-\Delta H_{vap}/RT}} \simeq 1 - \frac{\Delta H_{vap}}{R} \; \frac{(T - T_o)}{T_o^2}$$

or

$$(64) \qquad x_2 = \frac{\Delta H_{vap}}{R} \; \frac{(T - T_o)}{T_o^2}$$

and

$$(65) \qquad T = \frac{RT_o^2}{\Delta H_{vap}} \, x_2 + T_o$$

The temperature T_o in equation (25), in the second term on the right-hand side should be replaced by T. Hence

$$(66) \quad k_F F_s(T_s - T_o) \longrightarrow k_F F_s(T_s - T) = k_F F_s(T_s - T_o) - k_F F_s \cdot \frac{RT_o^2}{\Delta H_{vap}} x_2$$

The only change in the derivation is, then, the addition of the term $- k_F F_s \cdot \frac{RT_o^2}{\Delta H_{vap}} \cdot x_2$ to the right-hand side of equations (25), (26), (27), and (43). Introduction of this term would modify equation (44) to read

$$(67) \quad x_2 = \frac{\left[k_F F_s + k_G A + (r_r - r_r^{\,o}) \frac{E^2}{R_r^3} \alpha + \frac{B_V \cdot \Delta H_{vap}^2}{RT_r^2} A e^{-\Delta H_{vap}/RT_r}\right] \Delta T + k_F(F_s - F_r)(T_r - T_o)}{B_V \cdot \Delta H_{vap} \, A \, e^{-\Delta H_{vap}/RT_r} \left(1 + \frac{\Delta H_{vap}}{R} \cdot \frac{\Delta T}{T_r^2} \right) + k_F F_s \cdot \frac{RT_o^2}{\Delta H_{vap}}}$$

Equation (45) would then become

$$(68) \quad x_2 = \frac{a \cdot \Delta T + b}{1 + c \cdot \Delta T + \frac{k_F F_s \cdot RT_o^2}{B_V \Delta H_{vap}^2 \, A \, e^{-\Delta H_{vap}/RT_r}}}$$

Since $c. \Delta T << 1$, as has been discussed,

$$(69) \quad x_2 = \frac{a. \Delta T + b}{1 + \frac{k_F F_s . RT_o^2}{B_V \cdot \Delta H_{vap}^2 \, A e^{-\Delta H_{vap}/RT_r}}} = \frac{a. \Delta T + b}{\beta},$$

where

$$(70) \quad \beta = 1 + \frac{k_F F_s \cdot RT_o^2}{B_V \cdot \Delta H_{vap}^2 \cdot A e^{-\Delta H_{vap}/RT_r}}.$$

Thus

$$(71) \quad x_2 = a' \cdot \Delta T + b'$$

where

(72) $a' = \frac{a}{\beta}$ and $b' = \frac{b}{\beta}$.

The form of equation (71) is identical to that of equation (50), and equations parallel to (51) through (58) can now be written by replacing a and b by a' and b' respectively.

It has been established that the response of the detector should be a colligative property, varying linearly with mole fraction (or concentration) of solute in the solvent for ideal solutions. The colligative nature of the response leads to a high generality of applicability of the detector for chromatographic purposes, and suggests its usefulness for the determination of molecular weights and thermodynamic properties.

It is possible through use of known concentrations of samples of known molecular weight to determine the constant $\frac{a'}{\alpha}(r_r + r_s^{\,o})$, and it may then be assumed that this parameter is the same for different solutes, provided the thermostat temperature, solvent, column flow rates, and bridge voltage are unchanged. As will be shown in the next section, the linear relationship between response and concentration makes possible quantitative chromatographic analysis utilizing integrated areas of the component peaks.

The mathematical development has been based on a reasonable set of assumptions, and it leads to conclusions entirely consistent with observations thus far made.

The response per unit concentration can be seen from equation (56) to increase with bridge voltage, E, and with the temperature coefficient of resistance, α, of the thermistors used. The effect of flow rate can be seen in the constant b', which is proportional to $(F_S - F_D)$, and produces an unbalance signal with pure solvent on the sample thermistor. The flow rate also affects a', where its primary contribution enters into the numerator in a way which leads to an essentially inverse dependence of response on flow rate, as was so strikingly apparent during the evolution of the detector into its present form (see Section II-C). The effect of the heat of vaporization of the solvent is quite complex, since a high value reduces the rate of vaporization, but increases the heat effect per mole vaporized; a very high heat of vaporization would tend to diminish response. The term k_F, which includes heat capacity and thermal conductivity of the liquid, probably does not vary appreciably from one solvent to another. The effective area, A, is important in increasing the response, so wick design is of obvious importance.

F. Concentration Profile of Solute Bands

When a sample is injected onto a chromatographic column, the sample initially may be considered to have approximately a rectangular concentration profile, with a width proportional to the volume, V_O, of the sample, and a height equal to the initial concentration, C_O. When the band passes through the column, its spreading will tend to give it a Gaussian shape, provided no chemi-sorption occurs. Depending upon the relationship between V_O and the spreading, three distinct situations can arise, as represented in Figure 8. Figure 8-A shows a case in which the spreading leads to a concentration at the center of the band appreciably lower than the concentration, C_O, in the initial sample. Figure 8-B

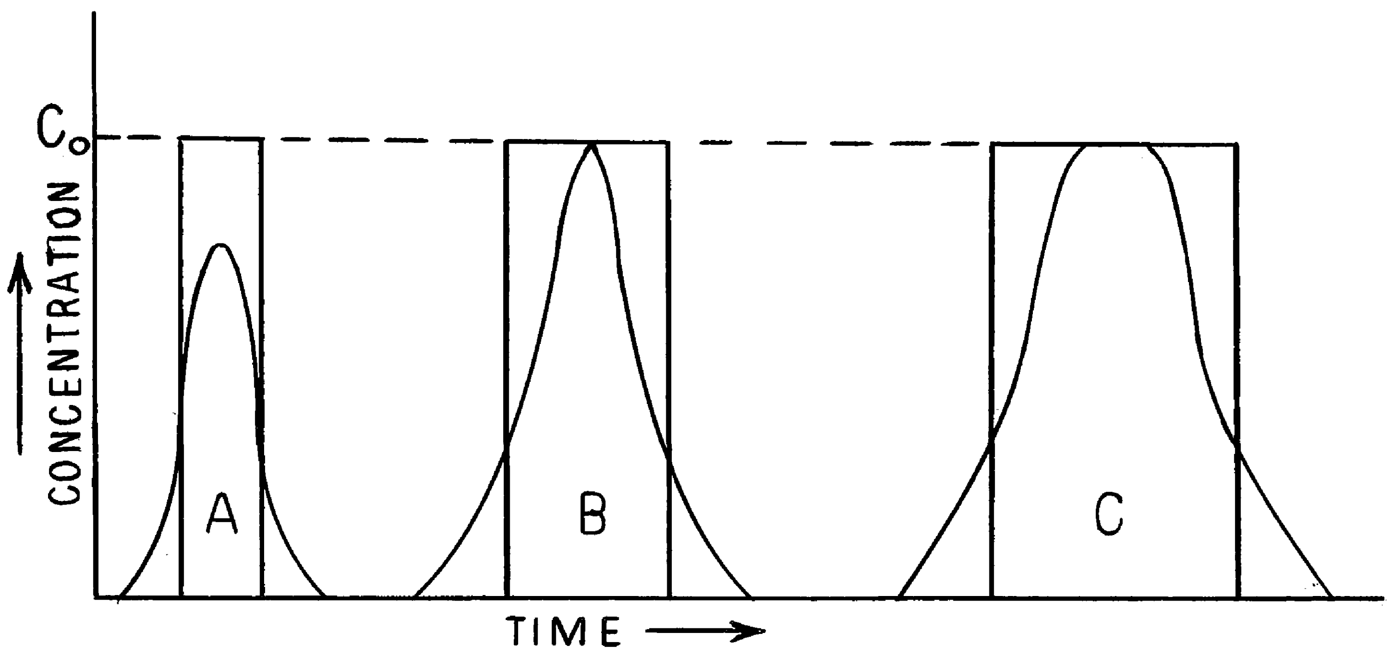

FIGURE 8. GAUSSIAN SPREAD OF CONCENTRATION FOR SAMPLE INJECTIONS OF VARYING SIZE: A, SMALL SAMPLE, $C_{MAX} < C_o$; B, LARGER SAMPLE, $C_{MAX} = C_o$, NO FLATTENING; C, STILL LARGER SAMPLE, $C_{MAX} = C_o$, WITH FLATTENING AT PEAK.

represents the special case in which the spreading and V_o are so related that the peak height is just Co, but the shape is Gaussian. In Figure 8-C is shown the case, for sufficiently large sample volumes, where the leading and tailing edges of the band are Gaussian, but where the central portion has levelled off to give a flattened peak with height C_o. Figure 8-A probably represents the situation most frequently encountered in chromatographic work, where small sample volumes are employed to provide maximum resolution of component peaks. Figure 8-C represents the case of greatest interest for molecular weight and thermodynamic studies, where it is desirable that the detector response be directly associated with a measured concentration; this case is also most useful in assessing the response characteristics of the detector itself. It is worth-while to look into the mathematics of the concentration profile to see what can be inferred from measurable properties of the component peaks.

Since the recorded chromatogram is presented in terms of millivolts of response vs. time in minutes, the mathematical treatment of the concentration profile will be set up on this basis. Assuming a Gaussian shape for the concentration profile,

$$(73) \qquad C_t = \hat{C}\, e^{-(t - \hat{t})^2/\beta^2}$$

where C_t = molar concentration of solute at time t, $\hat{C}$ = molar concentration of solute at the band peak, $\hat{t}$ = time to reach peak (from sample injection time), and β is the broadening factor, such that a high β indicates much spreading, while a low β indicates a sharp, narrow peak. If an initial volume, V_o, of sample of concentration C_o is originally injected, the total number of moles of sample, n_o, is given by

(74) $$n_o = C_o V_o .$$

This total number of moles must also be obtained by adding up the numbers of moles across the solute band--i.e., by integration of the band. For integration, the concentration expression in equation (73) must be multiplied by the volume flow rate, F, in liters per minute, and then integrated over a time interval which includes the entire band.

(75) $$n_o = C_o V_o = \int_{t=o}^{\infty} F C_t dt = F \int_{t=o}^{\infty} \hat{C} e^{-(t-\hat{t})^2/\beta^2} dt$$

Then the peak concentration is given by

(76) $$\hat{C} = C_o \frac{V_o}{F \int_{t=o}^{\infty} e^{-(t-\hat{t})^2/\beta^2} dt}$$

The definite integral can be evaluated from a table of integrals, rewriting it in the form

(77) $$\beta \int_{t=o}^{\infty} e^{-(t-\hat{t})^2/\beta^2} \, d\left(\frac{t-\hat{t}}{\beta}\right) = \beta \int_{-\infty}^{\infty} e^{-x^2} dx = \beta \sqrt{\pi}$$

Hence

(78) $$\hat{C} = C_o \frac{V_o}{F \cdot \beta \sqrt{\pi}}$$

The parameter β may be related to the band width, expressed as the half-width, $\Delta t_{\frac{1}{2}}$, at the half-height, $C_t = \frac{1}{2} \hat{C}$, from equation (73).

$$(79) \qquad \frac{C_t}{\hat{C}} = \frac{1}{2} = e^{-(t_{\frac{1}{2}} - \hat{t})^2/\beta^2} = e^{-\Delta t_{\frac{1}{2}}^2/\beta^2}$$

Thus

$$(80) \qquad \beta = \frac{\Delta t_{\frac{1}{2}}}{\sqrt{\ln 2}}$$

and

$$(81) \qquad \hat{C} = C_o \cdot \frac{V_o}{F \cdot \Delta t_{\frac{1}{2}} \sqrt{\pi / \ln 2}}$$

or

$$(82) \qquad C_o = \frac{\hat{C}\, F \cdot \Delta t_{\frac{1}{2}} \sqrt{\pi / \ln 2}}{V_o}$$

so that the initial sample concentration can be determined from the peak height, $\hat{C}$, the half-width at half-height, $\Delta t_{\frac{1}{2}}$, the volume flow rate of the column, F, and the sample volume injected, V_o.

Since it has been shown in the preceding section that the response, $e-e_o$, is proportional to concentration (equation (58)), concentration can be calculated from the observed response, if the peak is Gaussian. Furthermore, even for non-Gaussian peaks, the integrated area will be a measure of number of moles introduced, and this in combination with the injected sample volume, V_o, is sufficient to determine concentration. Thus

$$(83) \qquad e-e_o = \frac{\alpha\, r_r^o\, E}{a'(r_r + r_s^o)R_r}\, x_2 = \frac{\alpha\, r_r^o\, E}{a'(r_r + r_s^o)R_r} \cdot \frac{M_1}{1000\, d_1}\, C_2$$

or

$$(84) \qquad e-e_o = \sigma \cdot C_2 = \sigma \cdot \widehat{C}\, e^{-(t-\widehat{t})^2/\beta^2}$$

where

$$(85) \qquad \sigma = \frac{\alpha\, r_r^o\, E}{a'(r_r + r_s^{\,o})R_r} \cdot \frac{M.}{1000\, d_1} \quad \frac{\text{millivolts}}{\text{mole/liter}} \quad ,$$

a constant characteristic of the solvent system and detector under particular operating conditions. The maximum response, $(\widehat{e - e_o})$, is then

$$(86) \qquad (\widehat{e - e_o}) = \sigma\, \widehat{C} = \sigma \cdot \frac{V_o}{F.\Delta t_{\frac{1}{2}}\sqrt{\pi/\ln 2}}\; C_o$$

or

$$(87) \qquad C_o = \frac{(\widehat{e - e_o})\, F\ \ \Delta t_{\frac{1}{2}}\sqrt{\pi/\ln 2}}{\sigma\, .\, V_o}$$

The area under a Gaussian response curve is given by

$$(88) \qquad \mathcal{a} = \int_{t=o}^{\infty}(e-e_o)dt = \sigma\ \widehat{C}\ \int_{o}^{\infty} e^{-(t-\widehat{t})^2/\beta^2}\, dt$$

$$= \sigma\ \frac{V_oC_o}{F} = \sigma\ \frac{n_o}{F}$$

Thus the number of moles introduced is

$$(89) \qquad n_o = \frac{F}{\sigma} \cdot \mathcal{a},$$

and the initial concentration is

$$(90) \qquad C_o = \frac{F}{\sigma\, V_o} \cdot \mathcal{a}.$$

The conclusions regarding area are not restricted to Gaussian curve shapes, but are a consequence of the linearity of response of the detector. A general response curve can be formulated as

(91) $$e - e_o = \sigma\, \widehat{C} \cdot S(t)$$

where S(t) is the curve-shape function, which was $e^{-(t-\hat{t})^2/\beta^2}$ for the Gaussian shape. Then

(92) $$n_o = \frac{F}{\sigma} a = \frac{F}{\sigma} \int_{t=0}^{\infty} (e-e_o)dt = F\, \widehat{C} \int_{t=0}^{\infty} S(t)dt = C_o V_o$$

The relationship between $\widehat{C}$ and C_o is, of course, dependent upon the curve shape.

(93) $$\widehat{C} = C_o \frac{V_o}{F \int_{t=0}^{\infty} S(t)dt}$$

It is of interest to determine the sample volumes, V_o, which will give each of the three curve shapes shown in Figure 8. This is conveniently found by setting $\widehat{C} = C_o$ in equation (81). Then

(94) $$V_o = F \cdot \Delta t_{\frac{1}{2}} \sqrt{\pi / \ln 2}$$

is the volume which will just give the maximum, $\widehat{C}$, equal to the initial concentration, C_o. This is case B in Figure 8. Case A corresponds to $V_o < F \cdot \Delta t_{\frac{1}{2}} \sqrt{\pi / \ln 2}$, and Case C corresponds to $V_o > F \cdot \Delta t_{\frac{1}{2}} \sqrt{\pi / \ln 2}$.

G. Factors Causing Non-Linear Response

In the mathematical development of the detector response relationships, it was found that linear response is to be expected, at least up to concentrations of perhaps 0.1 - 1 M., insofar as the thermal and electrical characteristics of the detector are concerned. It is possible, however, for appreciable deviations from linearity, or apparent deviations, to occur at much lower concentrations through failure of the solutions to obey ideal solution laws, or through specific solute-solvent or solute-solute interactions which can be classed as molecular-complex formation.

In application of the detector to systems of organic solutes and solvents, in the concentration range below 0.1 M, activity effects would not be expected to be pronounced. However, it must be recognized that non-linearity can result from these effects, and it should be pointed out that the detector does indeed offer a possible tool for study of these effects.

Of more apparent concern to the present study is the possibility of molecular-compound (or complex) formation, particularly through solute-solute interaction. It is possible for complexes to form from interaction between different solute species, or from solute-solvent interaction, but the case of direct interest to the present study is dimerization of a particular solute species, and only this case will be treated in any detail.

If a solute species, A, undergoes dimerization

$$\begin{array}{ccc} C & & \\ 2A & \rightleftharpoons & A_2 \\ C-2x & & x \end{array} \quad , \tag{95}$$

an original concentration, C, will appear as a total molar concentration of $C - 2x + x = C - x$ at equilibrium. The concentration, C, and hence the dimer concentration, x, will vary across a band with the nominal shape shown in Figure 8 A or B, and the resultant shape will be complicated by the dimerization. In the case shown in Figure 8 C, however, in the flattened peak $\hat{C} \approx C_o$, and the peak height will correspond to $C_o - x$; this situation, then, is the desirable one for study of the dimerization equilibrium constant, K_d, which is given by

$$(96) \quad K_d = \frac{(A_2)}{(A)^2} = \frac{x}{(C_o - 2x)^2}$$

This equilibrium constant can be obtained readily from a response vs. concentration curve, which should approach linearity at lower concentrations. If the linear portion is extrapolated to a concentration, C_o, where dimerization occurs, the vertical depression of the actual response compared to the extrapolated response is a direct measure of σx, and a horizontal line drawn from the point obtained at C_o to the extrapolated linear response line will intersect the response line at a concentration of $C_o - x$. This method is illustrated in the next section, where it is applied to an experimental case. It must be recognized, however, that the validity of the method rests upon the assumption that the dimerization is wholly responsible for the observed non-linearity. This assumption requires more experimental data to become justified.

V. QUANTITATIVE OBSERVATIONS--CORRELATION WITH THEORY

A. General

Quantitative data became obtainable only at a very late stage of the investigation, after evolution of the detector to its present form. Earlier qualitative or crudely semi-quantitative studies had indicated a rough proportionality between concentration and response, and a quantitative study was undertaken to better define this relationship for various sample substances.

The quantitative data obtained are tabulated in complete form in Appendices I and II; all data were obtained with the cyclohexane (95 per cent v./v.) and acetone (5 per cent, v./v.) solvent-alumina sorbent system. The data obtained fall into two distinct groups: data obtained for small sample volumes with $\widehat{C} < C_0$ (corresponding to Figure 8-A), and data obtained for larger sample volumes, giving flattened peaks with $\widehat{C} = C_0$ (corresponding to Figure 8-C). The second group of data is more conveniently treated from the standpoint of detector response theory and factors causing non-linearity of response, while the first group of data requires in addition the application of the concentration profile analysis. It is more convenient to treat the flattened peaks first, and then to examine the small-sample-volume data in light of the results obtained with larger samples.

B. Large Sample Volumes, Flattened Peaks ($\hat{C} = C_o$)

Only one solute, biphenyl, has been studied extensively, with sufficiently large volumes of sample ($V_o \simeq 0.05$ ml.) at different solute concentrations to make possible the mapping-out of the concentration-response curve. A few scattered observations with hexamethylbenzene and with triphenylmethane solutions have also been obtained. The data obtained are presented in Appendix I, and the response is plotted as a function of concentration for biphenyl in Figure 9.

The data plotted in Figure 9 were not obtained under the conditions of the mathematical derivation, with $r_s^o = r_r^o$. However, essentially no change in response to sample was found to result when identical samples were injected with $r_s^o = r_r^o = 2000$ ohms, $e_o = -28.1$ mv., as compared with $r_r^o = 2000$ ohms, $r_s^o = 1949$ ohms, $e_o = 0$ mv.

Later data were obtained after the detector was opened and the positions of the wicks were altered, and these later results show a lowered response at higher concentrations, although nearly the same response was obtained in the lower concentration range (0.01-0.04 M.).

Since the deviations from linearity at higher concentrations differ from solute to solute, it appears likely that these are a function of the samples themselves, rather than of the detector. In particular, it would appear that dimerization or higher aggregation of the solute species studies could occur through π-complexing of the type reviewed by Andrews (18). The non-linearity is then ascribed to the resultant higher average molecular weight and lowered relative molar concentration at

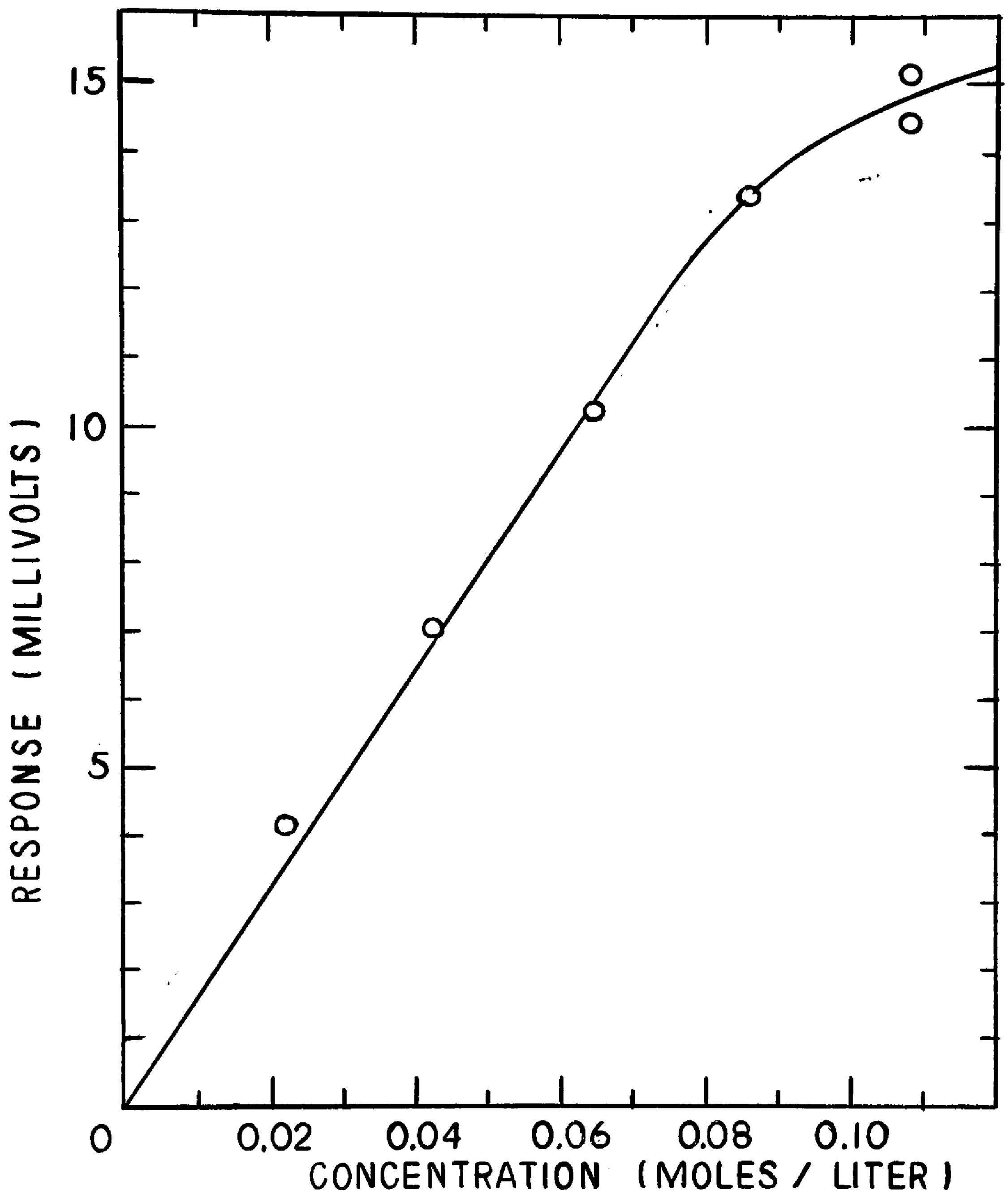

FIGURE 9. DEPENDENCE OF RESPONSE ON CONCENTRATION FOR SOLUTIONS OF BIPHENYL IN CYCLOHEXANE (95%, V/V) – ACETONE (5%, V/V) SOLVENT.

higher sample concentrations. Attempted quantitative treatment of the association would be premature on such limited data.

The three most important conclusions which can be drawn from these preliminary quantitative studies are as follows.

1) The response appears to be very nearly linear with concentration for concentrations below 0.01 M, and response to different solutes appears equivalent in this range. This suggests the predicted applicability of the detector to obtain quantitative analytical, molecular weight, and thermodynamic data.

2) Deviations at higher concentrations appear to be dependent on the nature of the solute, and probably are a measure of complexing or association phenomena.

3) From the response to low concentrations, of biphenyl, the inherent sensitivity of the detector is estimated to be 188.5 mv./M., with a peak-to-peak noise level in the present detector of about 0.1 mv. Thus the present sensitivity is such that a 0.001 M. solution is detectable at signal-to-noise of 2:1. This has been verified for an ergosterol solution.

C. Small Sample Volumes ($\hat{C} < C_o$)

Solutions of various molarities of biphenyl, triphenyl methane, hexamethylbenzene, and acridene have been injected with V_o equal to 0.01 and 0.02 ml. Peak heights, half-widths, and areas have been evaluated for these samples; the data are presented in Appendix II.

Two aspects of these data are particularly striking. First, the reproducibility of integrated areas is excellent, even when samples were introduced without first withdrawing the solvent--i.e., with simple injection below the surface of the solvent onto the column without disturbing the solvent flow. Areas are reproducible to ± 2 per cent. The integrated area should be a direct measure of the number of moles of solute in the linear range of response. Unfortunately, it was found later that these samples were run in the non-linear range of the response-concentration curve; this caused a non-linearity of area as a function of the sample volume, V_o, also, since the peak concentration depends upon V_o. Furthermore, the non-linearity led to non-equivalent areas for equivalent concentrations and sample sizes of different solutes, so that molecular weights were not obtainable from these data.

Second, the dependence of $\hat{C}/C_o$ on V_o for biphenyl solutions correlates well with the predictions of the Gaussian concentration-profile treatment and makes possible the calculation of the broadening factor, β, in equation (73); values of $\hat{C}/C_o$ were obtained from Figure 9. With sample injection beneath the solvent, $\hat{C}/C_o$ is approximately 0.3 for 0.01 ml. samples and 0.45 for 0.02 ml. samples; these give values of β of 6.4 and 8.5 min., respectively. Again the non-linearity of response is reflected in the values of β, where the half-height is lower than it should be, giving higher values of β, for the 0.02 ml. samples as compared to the 0.01 ml. samples. For 0.02 ml. samples introduced onto the column after withdrawing the solvent, $\hat{C}/C_o$ is about 0.57, indicating that the spreading factor, β, is reduced to about 7.4 min. by this technique of sample injection. These results predict a flattening of peaks to occur for V_0 greater than 0.045 ml., and this was verified experimentally in obtaining the data discussed in Section V-B and tabulated in Appendix I. The flow rate, F, was $5 \frac{\text{ml.}}{\text{day}}$.

VI. DISCUSSION AND CONCLUSIONS

A. The Pre-Peak Effect

The most interesting and useful aspect of the results obtained with the butanol and water solvent-cellulose sorbent system is, perhaps, the observed pre-peak effect. The pre-peak effect appears at the flow-through time of the solvent, and has been observed only in those solvent-sorbent systems that have a stationary phase which constitutes one of the solvent components. This occurs with a cellulose adsorbent and a water-containing solvent, since a layer of water is held on the cellulose. This layer, which may be many molecules deep, acts as the adsorbing substance binding a thin, perhaps mono-molecular, layer of butanol, the other component of the solvent.

The pre-peak has two parts: a negative deflection occurring first at the flow-through time corresponding to the time required for a solvent sample rich in butanol to be detected, and a positive peak at the time corresponding to the flow-through time of a solvent sample rich in water. Figure 10 shows a representative pre-peak.

Rationalization of the pre-peak may follow two courses which at first appear to be entirely different but are actually the same explanation from two different viewpoints.

The first, and perhaps the most straight-forward, approach is to consider the length of the adsorbent which is occupied by the solute sample when it has just completely run into the column. The solute sample

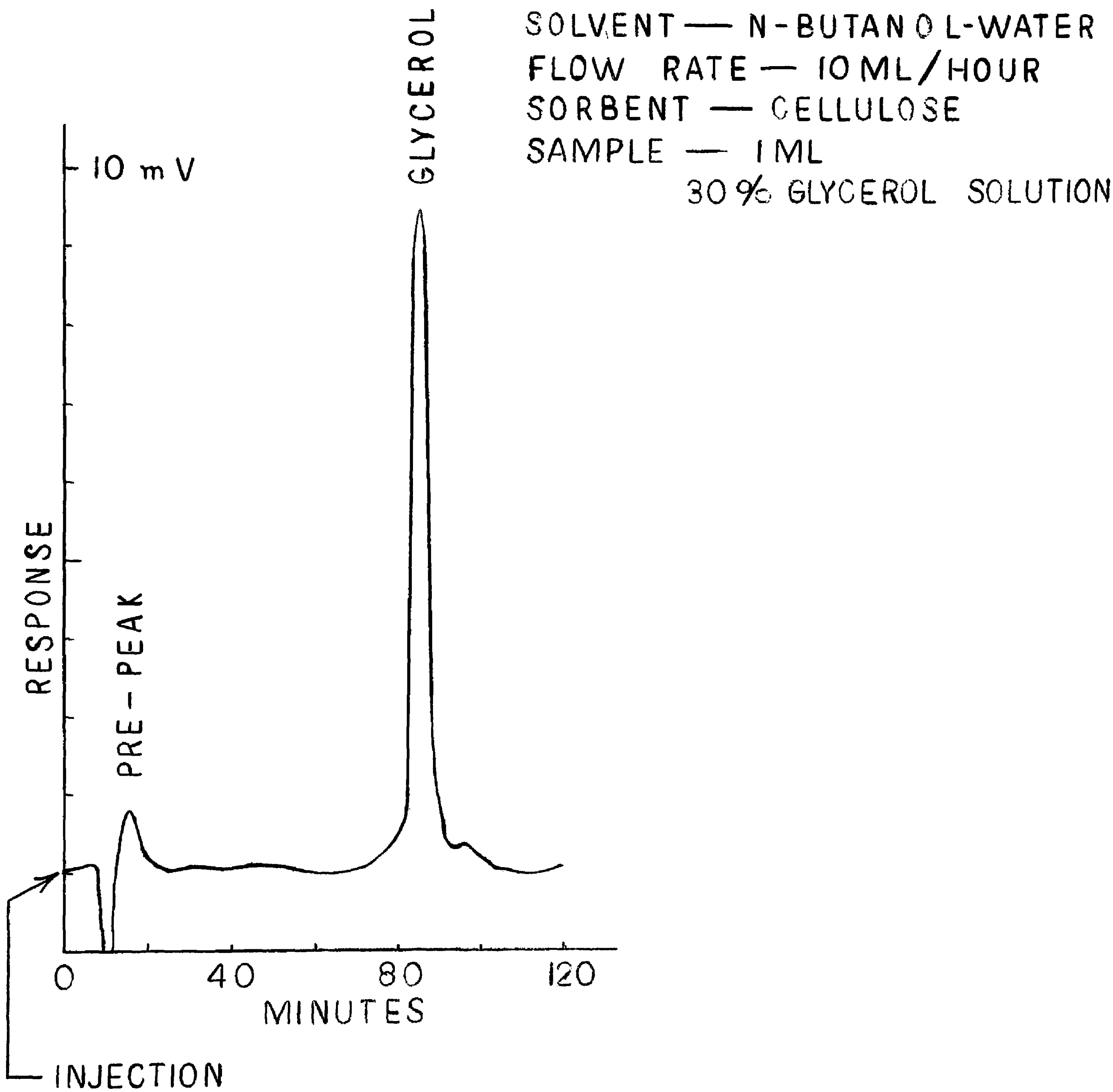

FIGURE 10. REPRESENTATIVE CHROMATOGRAM SHOWING PRE-PEAK EFFECT.

will initially displace the alcohol which is held on the water layer, so that an alcohol-rich band will flow down the column at its natural flow rate; since the solute must now be in equilibrium with the water layer adsorbed on the stationary phase, some of the water will be displaced and flow down the column at its natural flow rate. The water peak partially overlaps the alcohol peak, so that it is cancelled to a certain extent.

The alternative explanation is by analogy to gas chromatography. The phenomenon has been observed in the determination of impurities in certain of the gases usually used as carriers in gas chromatography. The phenomenon has been observed by J. P. Mickel (19) and E. Erb (20) independently in the determination of oxygen and nitrogen in hydrogen or helium, using Golay-type capillary columns.

The negative-peak effect, as it is named in gas chromatography, appears when a pure hydrogen sample is injected into a stream of impure hydrogen, which serves as the eluent stream for a capillary column. Negative oxygen and nitrogen peaks appear at the times required for elution of oxygen and nitrogen, respectively, if these gases are run in the same column with a pure hydrogen eluent. This effect can be rationalized, if one pictures bands of oxygen or nitrogen deficiency moving through the column with the same retention times as these gases would have as samples in a stream of pure hydrogen.

In liquid chromatography, one may consider the eluent as a non-compressible gas. The portion of the solvent which contains the solute could then be considered as a band which is reduced in solvent concentration. This solvent band should move through the column at the rate of flow of the components of the solvent.

This phenomenon has also been observed by B. F. Meyers (21) in the ion-exchange chromatography of amino acids, using a continuously-recording refractometer for a detector. The pre-peak, or compensation peak (as Meyers named it), was not rationalized to agree with the experimental data he obtained, and the significance of its presence was not recognized. The explanation he postulated requires the more-tightly-held component of the solvent to appear at the bottom of the column, just before the solute band appears. His recorder traces show, however, that both the components of the pre-peak appear at the retention time of the solvent. Moreover, he did not detect the amino acids at their retention times with the refractometer; he attributed this failure to the compensation of the refractive index by the changes in the solvent composition. The amino acids were, however, detected by ninhydrin reagent as much as six hours after the appearance of the pre-peak; thus, the pre-peak compensation of the refractive index by the changes in the solvent concentration could not have been due to the presence of the amino acid, but must have been caused by a solvent-deficient band moving through the column with the retention time of the solvent. His refractive-index detector apparently was not sufficiently sensitive to detect the refractive-index changes due to the amino acid, at the concentrations present in the solute bands, spread by passage through the column.

The negative peak effect, compensation effect, and pre-peak effect appear to be the same phenomenon, even though they occur in different branches of chromatography. The pre-peak may possibly be calculated for liquid chromatography to give quantitative data as to the size of the sample injected into the column, and it can be used to calculate the R_f value, which is useful in identification of component bands.

B. The Ultimate Sensitivity of the Detector

The detector, in its present state of development, is capable of detecting above the background 4×10^{-9} moles of non-volatile solute. This is by no means the ultimate in sensitivity which this detector can conceivably reach. The minimum resistance change or unbalance which can be observed with a Wheatstone bridge is limited by the following: thermal noises in the thermistors, leads, connections, and resistances; contact potentials in the connections; resistance fluctuations due to external temperature changes of the bridge elements not thermostated; noises which arise in the primary stages of amplification; voltage fluctuations in the potential source; and the thermostating of the detector.

If a Wheatstone bridge were employed using thermally-compensating elements, and if the whole bridge were thermostated, noises due to changes in the resistance of the bridge elements could be reduced below the detectable range.

Since in the development of a chromatogram several minutes are required for one solute band to pass a thermistor, thermal noises can be eliminated, because they are relatively of high frequency. If a time constant of 30 seconds is employed, the highest frequency passed will be 1/30 cycles per second. The voltage from thermal noise then would be 10^{-9} volts, which may be disregarded, even for very high amplification.

Noises due to contact potentials arising from bi-metallic junctions in the circuit may be reduced by use of special solder and leads. The remaining junction potentials, if present, can be maintained constant with thermostating of the contacts.

Carefully selected circuit components may be used in the first stages of amplification. Since most of the noise arising in the amplifier is of high frequency, as is that arising from thermal noise, these may also be reduced by damping. With proper damping and shielding, a d.c. amplifier may be designed which will have a gain of 10^3 with less than 3 per cent noise. Thus, a one-millivolt full-scale recorder, coupled with a d.c. amplifier, could detect a signal of 10^{-6} volts with a noise level of less than 5×10^{-7} volts.

Noise arising from potential changes in the voltage source may be eliminated by paralleling several storage batteries which have been designed for noise-free operation and which have been charged slowly and carefully.

Finally, bath temperature fluctuations could be reduced to $\pm 0.001^{\circ}$C., so that with thermistors matched within $\pm$ 2 per cent, bath temperature fluctuations will represent $\pm 1.2 \times 10^{-6}$ volts. This noise can be reduced still further by damping the detector chamber with insulation and reducing the on-off time of the regulator until the response time for the detector to temperature changes is longer than the on-off regulation.

If the circuit now in operation were modified as suggested in the preceding paragraphs, the detector could sense 10^{-12} moles of sample and determine quantitatively 10^{-11} moles. This is not, however, the in-principle limit of this detector, since smaller thermistors, modifications of the wick, and reduced flow rates could conceivably increase the sensitivity to detect as little as 10^{-13} or 10^{-14} moles of sample. If such an extension is possible, then this tool would become comparable with the most sensitive detectors now available in any area of chemistry.

If sensitivity of the detector is the object, then the present detector can be extended still further, since it is more sensitive to volatile substances than to non-volatile substances. For solutes having vapor pressures twice the vapor pressure of the solvent at the detector temperature, then the detector is more sensitive by a factor of 100. This would place the liquid chromatography thermistor detector already nearly on a par with the most sensitive gas chromatography detectors.

C. Suggestions for Further Work

Separations in liquid chromatography, in particular liquid-partition chromatography, are often best conducted using solvents which change in composition during the development of a chromatogram. The new detector can be extended to work in these systems if the following conditions are met: the thermistors must be carefully matched, so that solvent changes will not affect the unbalance; the flow rates of the two columns must be nearly identical; and the two thermistors should be attached in identical fashion to their respective wicks. Adaptation of the detector to these systems would greatly increase the potential usefulness of the detector.

Paper chromatography is selected in preference to column chromatography for many biochemical applications, because it is possible to use very small, dilute sample solutions, and to develop the chromatogram in more than one dimension. The use of capillary columns to some extent offsets the first advantage, but paper chromatography may still be more convenient for many applications. This detector can and should be adapted for the detection and location of solute bands in paper chromatography. No major modifications need be made for paper strips. It should be mechanically possible to devise a two-dimensional scan mechanism for the location of spots on paper. Without disturbing the progress of the chromatogram, such a device would allow the operator to determine the location, shape, and size of the spots on paper.

The contribution made by this detector would be severely limited if it were not possible to adapt it to monitor the progress of a preparative-scale chromatogram. The adaptation need only consist of a stream divider, which would monitor only a small portion of the effluent stream from a large column. This should be useful not only for laboratory-scale preparations, but also for monitoring stream concentrations in large-scale industrial processes. This detector should easily be adaptable to automation control, just as has been done with gas chromatography.

This detector is particularly well suited to exploratory research in new chromatographic systems, because of its simplicity and adaptability. The pre-peak effect also lends itself to interesting investigations into the study of the nature of adsorption and displacement. It should be possible to determine from the sizes of the pre-peaks the relative amounts of displaced components of the solvent. It should be possible to use the time of appearance of the pre-peak for determination of the R_f factors, since it embodies the solvent front.

Another important adaptation of the detector would be to the field of ion-exchange chromatography. This adaptation is perhaps the least probable of the suggested extensions, since it requires detection of subtle changes in effluent activity at a constant ionic strength.

Since the detector response has been shown to be a linear function of the mole fraction of the solution passing the sample thermistor, it may be used for determination of activity in dilute solutions. Only the preliminary studies have been completed toward this end, but the technique shows considerable promise for molecular-weight determination, association-constant determinations, and determinations of activity.

Essentially the same detector might also be used for study of the heat of reaction, if the sample thermistor were placed at the confluence of two wicks, one of which carries one reactant while the other carries the other reactant. The temperature of the chamber could be varied gradually, and a break in the curve would indicate reaction.

VII. SUMMARY

A new, general method has been developed for detecting and recording the presence of solute bands in the effluent stream of a liquid-eluent chromatographic column. Since this method is based upon changes in vapor pressure which occur in the solvent due to the presence of a solute band, its applicability is not limited to specific classes of substances.

The detector developed utilizes the temperature difference which is established between a wick bathed with the effluent of a chromatographic column and a reference wick bathed with pure solvent; the wicks are enclosed in a thermostated chamber that is saturated with solvent vapor. The temperature change is detected through the difference in resistance of a pair of thermistors, one of which is in contact with the wick bathed with pure solvent, while the other is in contact with the wick bathed with the effluent solution. The thermistors constitute two arms of a Wheatstone bridge, which can be balanced when both of the thermistors are in contact with wicks bathed with pure solvent. Any unbalance in the bridge which occurs as a result of solute bands passing is continuously recorded on a recording potentiometer.

Detector response is a colligative property of the solution passing over the thermistors. A mathematical treatment has been developed and predicts a linear dependence of detector response on mole fraction of solute. The mathematical analysis is based on a condition of thermal balance, and requires suitable approximations, based on the small magnitude of the observed changes, for derivation of the linear relationship. The kinetics of the solvent vaporization and condensation processes, as influenced by the presence of solute, are postulated to play the major role in detector response.

Further mathematical analysis of the concentration profile furnishes a relationship between the concentration giving the observed response and the initial sample concentration.

The evolution of the detector and its relationship to other means of chromatographic detection, and to related measuring techniques, are described. Qualitative results obtained in different solvent-sorbent systems for a variety of solutes are presented; the response to volatile and non-volatile solvents is discussed. Particular attention has been directed towards the analysis of the ergosterol irradiation mixture.

Some quantitative results are presented and indicate the nature and sensitivity of the response. At $33.5^{\circ}C.$ with a sample column flow rate of 5 ml./day, a reference column flow rate of 2 ml./day, cotton thread wicks, nominal 2000-ohm thermistors with a temperature coefficient of 4.5 per cent per degree, fixed bridge resistances of 2000 ohms, and an applied bridge voltage of 6 volts, the detector has a sensitivity of 188.5 mv./M., or the capacity to detect solute in 0.01 ml. of 10^{-3} M. solutions; this corresponds to detection of 10^{-8}-10^{-9} moles of material.

The new method should complement gas chromatography as an analytical method, with its most important applications in areas where gas chromatography is weakest--analysis of high-boiling and thermally-unstable components.

It should be of particular value in biochemical, natural products, and pharmaceutical work. Physico-chemical applications of the detector include its use in determining molecular weights, evaluating activity effects, studying molecular complexing and dissociation, and obtaining fundamental information on the nature of the chromatographic process.

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APPENDICES

APPENDIX I

QUANTITATIVE CONCENTRATION-RESPONSE DATA FOR LARGE-VOLUME SAMPLES (FLATTENED PEAKS)

Compound	Concentration (moles/liter)	Peak Height (mv.)
Biphenyl	0.02156	4.09
Biphenyl	0.04312	9.20
Biphenyl	0.06468	10.04
Biphenyl	0.08624	13.22
Biphenyl	0.1078	14.26
Biphenyl	0.1078	14.96
Biphenyl	0.1078	14.95
Triphenylmethane	0.1042	12.91
Triphenylmethane	0.1042	11.89
Detector removed and altered--changed response below this line.		
Biphenyl	0.04312	6.54
Triphenylmethane	0.02084	4.09
Triphenylmethane	0.04168	6.24
Triphenylmethane	0.06252	7.78
Triphenylmethane	0.08336	7.27
Triphenylmethane	0.08336	7.98
Triphenylmethane	0.08336	7.83
Triphenylmethane	0.1042	9.32
Triphenylmethane	0.1042	8.79
Hexamethylbenzene	0.06138	5.52
Hexamethylbenzene	0.1023	8.48

All data in this table were obtained at 33.5°C., using cyclohexane (95 per cent, v./v.) and acetone (5 per cent, v./v.) solvent-alumina sorbent, with an applied bridge voltage of 6 volts. Data were obtained in part with $r_s^{\circ} = r_r^{\circ} = 2000$ ohms, $e_o = -28.6$ mv., in part with $r_s^{\circ} = 1929$ ohms, $r_r^{\circ} = 1999$ ohms, $e_o = 0$, and in part with $r_s^{\circ} = 1949$ ohms, $r_r^{\circ} = 2000$ ohms, $e_o = 0$. The flow rate, F, was 5 ml./day in the sample column and about 2 ml./day in the reference column.

APPENDIX II

QUANTITATIVE CONCENTRATION-RESPONSE DATA FOR SMALL-VOLUME SAMPLES

Compound	Concentration (moles/liter)	Sample Volume V_o(ml.)	Retention Time (min.)	Peak Height (mv.)	Peak Width at Half-Height (min.)	Peak Area (mv.-min.)	$\widehat{C}/C_o$	β (min.)
Biphenyl	0.1135	0.01	90	6.69	13.7	83.9	0.32	6.4
Biphenyl	0.1135	0.01	92	5.99	14.1	79.8	0.29	6.6
Biphenyl	0.1135	0.01	93	6.53	13.0	80.8	0.32	6.1
Biphenyl	0.1135	0.01	89	6.16	13.9	77.7	0.30	6.5
Biphenyl	0.1135	0.01	95	6.04	13.7	72.6	0.30	6.4
Biphenyl	0.1135	0.01	95	6.49	13.5	80.8	0.32	6.3
Biphenyl	0.1135	0.02	95	8.78	17.9	140.2	0.45	8.4
Biphenyl	0.1135	0.02	--	8.76	18.9	146.2	0.45	8.8
Biphenyl	0.1135	0.02	96	9.18	17.5	147.3	0.48	8.2
Biphenyl	0.1135	0.02	98	8.16	19.3	144.2	0.41	9.0
Biphenyl	0.1135	0.02	92	8.89	17.9	143.1	0.46	8.4
Above this line, injected beneath solvent. Below this line, solvent removed for injection.								
Biphenyl	0.1078	0.02	98	8.98	15.8	139.1	0.49	7.4
Biphenyl	0.1078	0.02	94	9.18	15.1	129.8	0.51	7.0
Biphenyl	0.1078	0.02	96	9.53	16.3	145.2	0.53	7.6
Biphenyl	0.1078	0.02	96	10.12	16.8	144.3	0.58	7.8
Biphenyl	0.1078	0.02	92	10.03	15.8	154.4	0.57	7.4
Biphenyl	0.01135	0.02	95	0.848	16.5	11.07	0.424	7.7
Hexamethylbenzene	0.0627	0.02	90	6.77	19.8	117.7	--	9.2
Hexamethylbenzene	0.0627	0.02	88	7.76	16.6	110.4	--	7.7
Hexamethylbenzene	0.0627	0.02	92	7.34	16.6	110.4	--	7.7
Hexamethylbenzene	0.1023	0.02	93	7.58	18.9	110.4	--	8.8
Hexamethylbenzene	0.1023	0.02	92	8.34	16.8	116.6	--	7.8
Hexamethylbenzene	0.1023	0.02	92	8.27	16.1	122.8	--	7.5
Hexamethylbenzene	0.1023	0.02	94	7.58	17.0	109.5	--	7.9
Triphenylmethane	0.1042	0.02	93	8.20	17.7	130.9	--	8.2
Triphenylmethane	0.1042	0.02	94	8.44	16.5	126.7	--	7.7
Triphenylmethane	0.1042	0.02	100	8.28	17.9	125.8	--	8.3

All data in this table were obtained at 33.5°C. using cyclohexane (95 per cent, v./v.) and acetone (5 per cent, v./v.) solvent-alumina sorbent, with an applied bridge voltage of 6 volts, using r_s^o= 1929 ohms, r_r^o= 1999 ohms. The flow rate, F, was 5 ml./day in the sample column and about 2 ml./day in the reference column.