THE DEVELOPMENT AND CHARACTERIZATION OF A SUPERCRITICAL STATE CHROMATOGRAPHIC SYSTEM WITH A NEW MICRO ADSORPTION DETECTOR

A Dissertation

Presented to

the Faculty of the Department of Chemistry

College of Arts and Sciences

University of Houston

In Partial Fulfillment

of the Requirements for the Degree

Doctor of Philosophy

---

by

Jesse Lee Cashaw

January 1970

DEDICATION

To Helen and Debora

ACKNOWLEDGEMENT

The author wishes to thank Professor A. Zlatkis for suggesting the thesis problem and for his enthusiastic interest and advice throughout the course of this investigation. The author is also grateful to Dr. H. O. Gerlach and Dr. H. Pauschmann for suggestions in the initial phase of this work.

THE DEVELOPMENT AND CHARACTERIZATION OF A SUPERCRITICAL STATE CHROMATOGRAPHIC SYSTEM WITH A NEW MICRO ADSORPTION DETECTOR

An Abstract of

A Dissertation

Presented to

the Faculty of the Department of Chemistry

College of Arts and Sciences

University of Houston

In Partial Fulfillment

of the Requirements for the Degree

Doctor of Philosophy

---

by

Jesse Lee Cashaw

January 1970

ABSTRACT

The use of fluids above their critical temperatures and pressures in chromatography offers many intriguing possibilities. Supercritical fluid chromatography (SFC) combines some of the advantages of both gas and liquid chromatography. High molecular weight compounds can be separated rapidly by utilizing SFC. A complete system for SFC is described. The system employs a metering pump, relief valve, damping device, constant pressure regulator, sample injection valve, oven, flow controller and a heat of adsorption detector. The detector is composed of multi-thermocouples (thermopile) with a suitable adsorbent embedded in one end which becomes the hot junction, and the opposite end serves as the cold junction. The temperature changes resulting from the heats of adsorption and desorption of compounds eluted from the column are measured. The detector is unique in that by the proper selection of the mobile phase and the absorbent all substances separated can be detected making it of universal application. The maximum sensitivity of the detector for samples passing through the average column is in the submicrogram range. The operation of the SFC system is demonstrated by showing several examples of separations and detections. The relationship of detector sensitivity to flow rate is also discussed.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

I.

INTRODUCTION

INTRODUCTION

Supercritical fluid chromatography (SFC) is chromatography in which the mobile phase is a supercritical fluid. A supercritical fluid is formed when a liquid is heated to its critical temperature and a pressure equal to or greater than its critical pressure is applied.

The use of fluids above their critical temperatures and pressures in chromatography offers many intriguing possibilities. SFC combines many of the advantages of both gas and liquid chromatography. There are two striking advantages of SFC over gas chromatography. First, SFC offers a means of chromatographing macromolecules. Second, in certain cases it eliminates the laborious task of making suitable derivatives for gas chromatography. The major advantages of SFC over liquid chromatography are (1) Solvent power is a function of pressure which can be varied throughout the system. In liquid chromatography it is necessary to change the solvent composition to change the solvent power; and (2) Also, the relatively low viscosity and high diffusivity of dense gases should speed the separation of large molecules.

Although the application of supercritical fluids to chromatography is relatively new, studies on the solubility of solids in supercritical fluids were reported in the latter half of the last century. Hanney and Hogarth (1,2) reported solubility studies using such solvents as alcohol (ethyl and methyl), ether, carbon disulfide and

tetrachloride, paraffins, and olefins, and such solids as sulphur, chlorides, bromides and iodides of metals, and organic substances such as chlorophyll and the aniline dyes. In all cases studied, the solids remained in solution, or rather in diffusion when the temperature was raised above the critical point.

The solubility of solids in gases has been reviewed by Rowlinson and Richardson (3) and, independently, by Booth and Bidwell (4). The reviews indicate that the solubility of the solid is not discontinuous at the critical point of the solution. The compressed gases seem capable of dissolving solids to about the same extent as a liquid of the same density. Further, from spectroscopic studies (3,4) it appears that in all reported instances of enhanced solubilities in a compressed gas the mixture is a true molecular solution and not a colloidal dispersion. Thus it is easier to conceive for this solubility a critical region where there is no clear distinction between liquid and vapor than a critical point which suggests a sharp discontinuity.

The feasibility of utilizing supercritical fluids in chromatography was first demonstrated by Klesper, Corwin and Turner (5), who studied the enhanced solubility of some porphyrins in dichlorodifluoromethane at pressures up to 2000 pounds per square inch (p.s.i.) and temperatures from 150-170°C. Increased solubility was not observed with monochlorotrifluoromethane, trifluoromethane, and nitrogen. Dichlorodifluoromethane showed no effect from 1-600 p.s.i. Above 1,000 p.s.i. with dichlorodifluoromethane and above 1,400 p.s.i. with monochlorodifluoromethane, vaporization increased with increasing

gas pressure. Two porphyrins, Ni etioporphyrin and Ni mesoporphyrin IX dimethylester, were separated on a column packed with 33 percent polyethylene on chromosorb W at 1,600 p.s.i. using dichlorodifluoromethane as the mobile phase. Later the work of Klesper was extended to include separations of various types of metalloporphyrins (6,7) and various metallic chelates of acetylacetone.

In 1966, Sie, van Beersum, and Rijnders (8) reported the separation of C_7 to C_{13} n-paraffins in 90 minutes on a column filled with 25 percent squalane on Sil-O-Cel with carbon dioxide at 40°C and 1150 p.s.i. Benzene, toluene, p-xylene and cis-decalin were separated in 130 minutes and toluene, n-octane, cumene and sec-butylbenzene in 60 minutes on the same column with carbon dioxide at 730 p.s.i. They also noted that at lower temperatures solute volatility is decreased, but the increased non-ideal gas phase interactions at high pressure, which enhance volatility, compensate for the temperature effect. The versatility for the separation of solutes of high molecular weight was demonstrated by the separation of phenanthrene, anthracene, fluoranthene, pyrene, and other unidentified compounds of higher molecular weight from coal tar (9). The separations were effected on an alumina column using isopropanol as the mobile phase at 245°C and 45 kg/cm.^{2} Sie and co-workers also effected a separation between 1,2-benzpyrene (b.p. 493°C) and 3,4-benzyrene (b.p. 495°C) on an alumina column using pentane as the mobile phase at 200°C and 39.8 kg/cm^2 pressure. This separation is noteworthy in that 1,2-benzopyrene and 1,4-benzopyrene have widely different carcinogenic properties and occur in particulate air pollutants.

Recently McLaren, Myers, and Giddings(10) working at pressures up to 2,000 atmospheres, described the enhanced solubility and differential migration of various polymers and biomolecules in compressed carbon dioxide at 40°C and in ammonia at 140°C. Their report included the migration of species with molecular weights up to 400,000. Carotenoids, amino acids, carbohydrates, and several polymers were caused to migrate and separate, using NH_3 and CO_2 as the carrier gases, just above their respective critical points. The workers also studied the relative migration rate of squalane and silicone gum rubber in CO_2 with respect to pressure using Apiezon-L as stationary phase. The squalane started eluting at 300 atm and showed a linear increase in its rate of migration above 1000 atm. The silicone gum rubber (SE-30) did not migrate until the pressure exceeded 1,200 atm and the rate of migration remained constant with increase in pressure.

Although several investigators have reported on the use of SFC, many problems must be solved before it can be used with the same facility as gas chromatography. A driving force for flow must be developed and the flow rate must be variable, independent of pressure. A constant pressure must be maintained on the column which must be kept at a constant temperature. Injection of samples at high pressure and high temperature creates a unique problem. Conventional syringes that are commonly used in gas chromatography will not suffice and any injection valve constructed should ideally withstand high temperature as well as high pressure.

Even more critical in the development of critical state systems is the problem of component detection. Klesper and co-workers (5) removed their columns from the SFC system and used X-ray to identify the bands along the column. They later included trapping the components in alumina and visible-ultraviolet adsorption as a means of detection. Sie and co-workers (8) were able to use a flame ionization detector when carbon dioxide was used as the critical fluid but when they used less volatile mobile phases (isopropanol and pentane) they employed an ultraviolet absorption detector which necessarily restricted their studies to compounds which absorbed in the ultraviolet-visible range. Giddings and co-workers (11), working at 500 atm, with carbon dioxide as the carrier gas, reported an unsuccessful attempt to use the flame ionization detector commonly used in gas chromatography.

If a SFC system, with all of the above problems surmounted, could be constructed, it is conceivable that the system could be operated with equal facility as gas chromatography. The development and the utilization of a SFC system with a new micro heat of absorption detector is described in this communication.

II.

THEORETICAL CONSIDERATIONS

THEORETICAL CONSIDERATIONS

The effect of pressure on solubility in the critical region has been the subject of many recent papers (12-16). Of special interest is the effect of gas pressure on the partition coefficient which has been described by Sie (8,17) and, independently, by Rowlinson (1,3). The partition (distribution) coefficient k in GLC is defined as

$$k = \frac{\text{Amount of solute per unit volume of stationary liquid phase}}{\text{Amount of solute per unit volume of gas phase}}$$

Thus, any change in carrier gas pressure may alter k in three ways:

1. The interaction between carrier gas molecules and solute molecules;
2. The effect of pressure on the stationary liquid;
3. The solution of carrier gas in the stationary liquid.

Although the last effect is easy to visualize, it is the most difficult to describe quantitatively. Fortunately, for SFC it is of less importance than the other two effects which allow a thermodynamic approach. Such an approach leads to the equation

$$\ln k_p = \ln k_{p+} + \frac{P - P^+}{RT} (2B_{1,2} - V_2)$$

where k is the partition coefficient, P the pressure, P^+ a reference pressure (e.g., a pressure at one atmosphere or less which can be regarded as ideal), R the gas constant, and T the absolute temperature, V_2 the partial molar volume of solute in the liquid and $B_{1,2}$ the second virial cross coefficient of the system carrier gas solute.

At temperatures below the Boyle point, $B_{1,2}$ is negative and V_2 is generally positive. Therefore, an increase in pressure in the gas phase and in the liquid phase reinforce each other in shifting the equilibrium in a sense which may be looked upon as an enhancement of volatility.

For actual experimental considerations, the quantity measured, k' (a modified partition coefficient, often called the capacity ratio), is related to the true partition coefficient k by

$$k' = k \frac{V_L}{V_G}$$

V_G and V_L are the volume of the gas phase and the liquid phase, respectively. The elution volume V_R of a solute can be written

$$V_R = V_G + kV_L = (1 + k')V_G$$

At constant flow rate through the column, elution volumes are proportional to elution times, hence

$$t_R = (1 + k')t_G$$

k' is thus found by measurement of the elution time of a solute (t_R) and the holdup time of an inert gas (t_G).

The relation of temperature to k' is of special interest. At low pressure volatility decreases at lower temperatures and correspondingly k' is larger, but at high pressures the reverse is true. In this case volatility increases with decreasing temperature because molecular

interactions in the gas phase are more pronounced at the greater density accompanying the lower temperature and correspondingly k' is smaller.

Myers and Giddings (18) have given an exhaustive mathematical treatment to the van Deemter equation to determine the effect of high pressure on the constants of the van Deemter equation. Sie and Rijnders (19), although less rigorous in their approach, have given a qualitative and semiquantitative approach to the effect of pressure on these parameters.

Figure 1 represents a plate-height curve under normal gas chromatographic conditions. The classical form of the van Deemter equation is given by

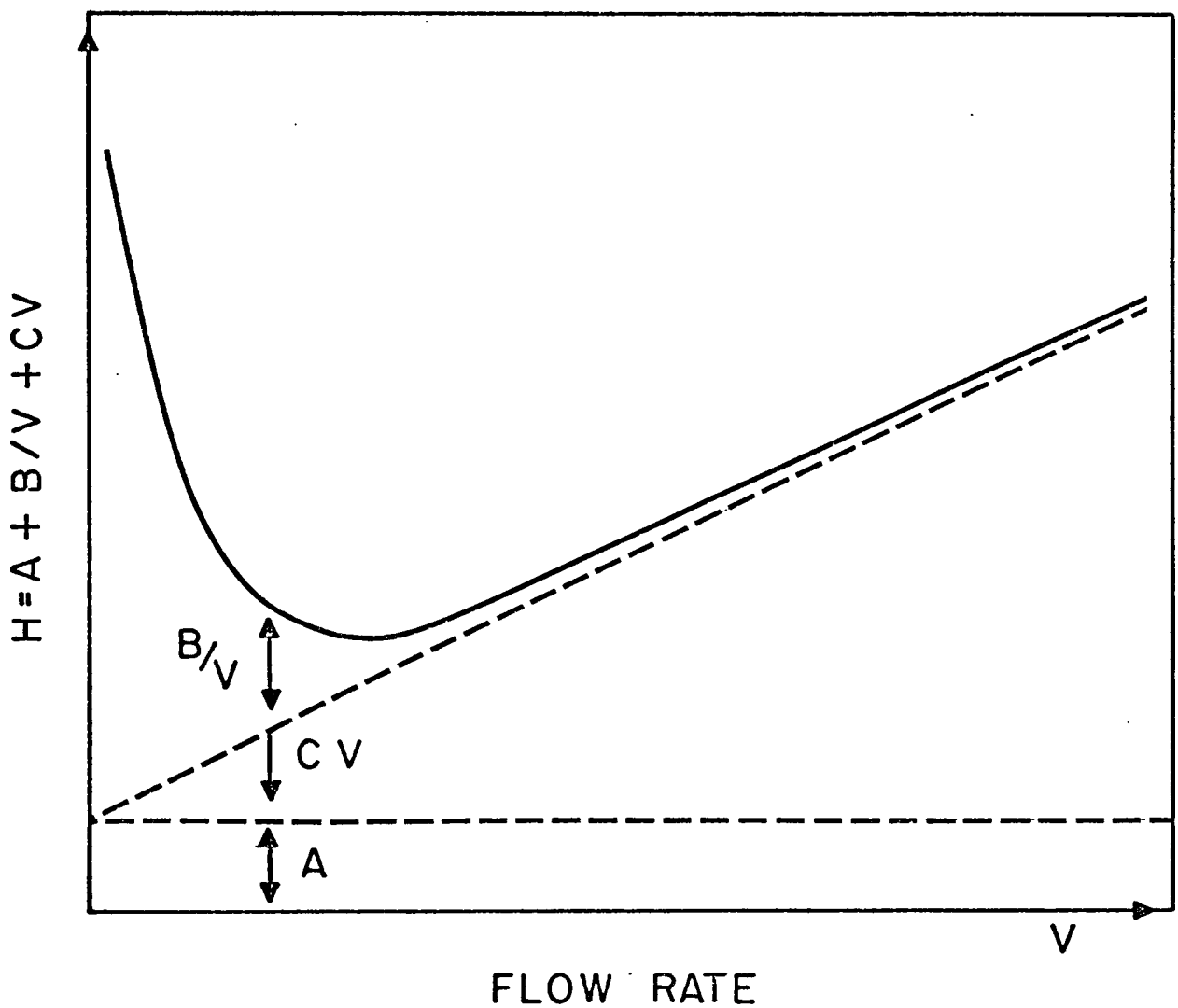
$$H = A + \frac{B}{V} + CV$$

where H is the plate height, and A, B, and C are the terms for Eddy diffusion, longitudinal diffusion and nonequilibrium, respectively. The term, V, is the linear gas velocity.

The A term, i.e., the contribution by Eddy diffusion, is a characteristic of the column packing diameter and packing irregularities. This term is approximately zero under normal gas chromatographic conditions. This can be shown from Figure 1 if the Eddy diffusion contribution is obtained as the intercept of the "straight" part of the plate-height curve on the H axis (as is commonly done). Sie and Rijnders demonstrated that when this approach is applied to systems at higher pressure, e.g., 50 kg/cm^2, the A term is not zero. Their

Figure 1

THEORETICAL PLOT OF
VAN DEEMTER'S EQUATION



observation is at variance with the classical idea that Eddy diffusivity (spreading due to statistical variations of velocity within the packed bed) contributes as an additive constant which only depends on packing geometry and particle dimensions.

The B term in the van Deemter equation is equal to $2\gamma D_g$ where γ is a tortuosity factor and D_g is the molecular diffusivity in the gas phase. Since the latter diminishes with pressure, the B term must become smaller at higher pressures. Correspondingly, the minimum plate height shifts to lower velocity as the pressure is increased. Further, under high pressure, the contribution of longitudinal molecular diffusion is relatively unimportant and can, for all practical purposes, be neglected.

The C term (i.e., the mass transfer or nonequilibrium term) is given by

$$\frac{8}{2} \frac{k}{(1 + k')^2} \frac{d_f^2}{D_l} V,$$

where d_f is the film thickness and D_l is the molecular diffusivity in the liquid phase. The effect of pressure on k', the capacity ratio, was discussed earlier. The C term is allowed to be split into the respective contributions of the two phases (C_l and C_g) by varying D_g, either by changing average column pressure or the nature of the carrier gas. In the low pressure region, D_g can be taken to be inversely proportional to pressure, while changes in partition coefficients, liquid volume and liquid diffusivity (caused by dissolved

carrier gas) are still negligible. Since the effect of pressure on k', the capacity ratio, was discussed earlier by this writer, and since the effect of pressure on the other variables of the C term has been discussed by other authors (18,19), only some conclusions drawn from these works will be mentioned here. The deterioration of column efficiency with increasing pressure is very rapid at low pressure, but slackens off at higher pressure. Further, the non-equilibrium contribution increases with increasing pressures and becomes by far the most important factor in determining column efficiency at high pressures and velocities.

III.

EXPERIMENTAL CONSIDERATIONS AND PROCEDURE

EXPERIMENTAL CONSIDERATIONS AND PROCEDURE

Unlike conventional gas chromatography, fluids above their critical temperatures and pressures place severe demands on the component parts of a chromatographic system. In addition to being able to withstand high temperatures and pressures, the SFC system must resist corrosion which is commonly encountered at elevated temperatures and pressures. Additional consideration must be given to the problems of constant flow, damping, pressure regulation and sampling. Further, since the molecules of dense gases are very close, they have a tendency to "swamp" conventional detectors used in gas chromatography; thus the construction of a detector that can function in liquids or dense gases is imperative for an operational SFC system.

The Supercritical State System

A diagram of the SFC system is shown in Figure 2. The mobile fluids (supercritical fluids) used in this study were pentane and isopropanol. The pentane (commercial grade from W. H. Curtin, Inc., Houston, Texas) was dried over phosphorus pentoxide before it was used. The isopropanol (Fisher Certified from Fisher Scientific Co., Fairlawn, New Jersey) was used without further purification.

The metering system consists of a laboratory feed pump, relief valve, damping system, pressure regulator, sampling valve, and a flow control system that was independent of pressure. In the initial stages of this work some components had to be constructed in our laboratory

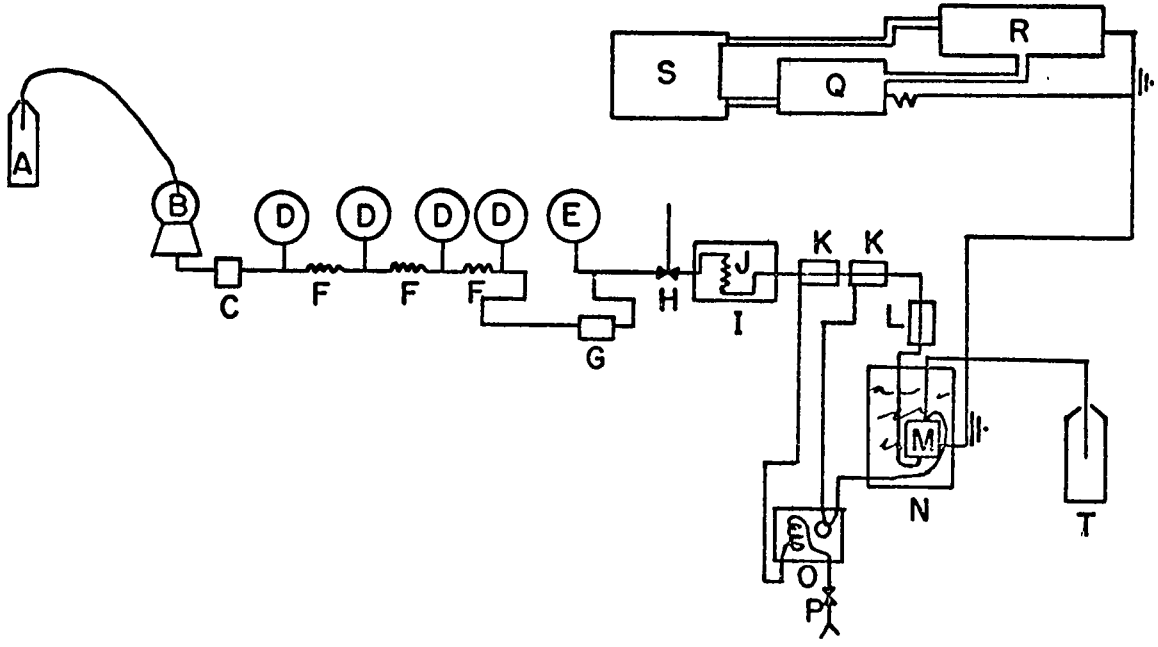
FIGURE 2

SCHEMATIC DIAGRAM OF APPARATUS

FOR CRITICAL STATE SYSTEM

A	Resevoir	K	Condenser
B	Pump	L	Micrometering valve
C	Relief valve	M	Detector
D	Bourdon gauge, 0-5000 p.s.i.	N	Dewar flask
E	Bourdon gauge, 0-1500 p.s.i.	O	Lauda bath
F	Pneumatical resistance	P	Tap water
G	Pressure regulator	Q	Amplifier
H	Injection valve	R	Constant voltage regulator
I	Oven	S	Recorder
J	Column	T	Receiving flask

Figure 2



because component parts were not available for high pressure-low flow systems.

The laboratory feed pump (Whitey Model LP10 from Whitey Research Tool Co., Emery, Calif.) employed was a diaphragm pump with a stainless steel head. The pump had a maximum working pressure of 5000 p.s.i. Since the flow rate and the working pressure is maintained independently of the pump, a pump with a diaphragm had to be used. The pump operates at a constant speed and therefore displaces a constant volume each cycle. If the volume of fluid normally displaced by the pump cannot be forced through the system, the stainless steel diaphragm, which is flexible, retains the excess fluid and prevents the pump from "building up" excess pressure. The diaphragm essentially functions as an internal relief valve and permits the pump to operated at a preset rate for a long period of time.

Although the diaphragm in the pump serves as an internal relief valve which will retain small volumes, an external relief valve was required in the system to protect the diaphragm from excess volumes and pressures. The first relief valve used in the system was constructed from parts taken from two regulators. The pressure required to actuate the valve could be varied from 1000 to 5000 p.s.i. by adjusting a screw in one end of the valve. During later stages of the study relief valves for small flow systems were available from commercial sources. A stainless steel relief valve (Sage Model No. 214-2A-4CC, Sage Engineering and Valve Company, Houston, Texas) which was preset to open at 4500 p.s.i. was installed in the system. The valve had a teflon

seat which greatly decreased the differential between operating and set pressure.

Since the pump employed was a piston pump, it was necessary to construct a damping system to damp the pulsation caused by the pump. This is generally done by the use of a reservoir and a needle valve down stream from the pump. The reservoir serves as a condenser and the valve serves as a resistor. Since our detector (which will be discussed later) was extremely sensitive to pulsation, we employed four 0 to 5000 p.s.i. stainless steel gauges (Model 9400, 4 1/2" in diameter, from Rawson Company, Houston, Texas) in series to serve as condensers and the gauges were connected by 30 inches of .005" I.D. x 1/16" O.D. stainless steel tubing which served as resistors. While the single reservoir system removes about 90 percent of the pulsation, the multiple damping system employed here removes more than 99 percent of the detectable pulsation.

Even at this writing, the problem of maintaining constant high pressure in small systems remains unresolved. A diagram of a conventional pressure regulator is shown in Figure 3. The liquid enters the inlet chamber and flows through the orifice to the outlet chamber. The size of the orifice is controlled by the position of the diaphragm, which is balanced between the pressure of the outlet fluid beneath it and the force of the spring above it. This type of regulator operates well at low pressure (under 500 p.s.i.), but at high pressures the spring is unable to maintain the balance. There are two common approaches to the solution of this problem. One approach is to apply

FIGURE 3

SCHEMATIC DIAGRAM OF A LOW-PRESSURE REGULATOR

A Inlet chamber

B Poppet

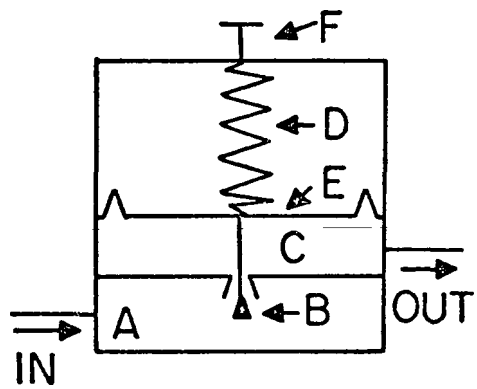
C Outlet chamber

D Spring

E Diaphragm

F Adjusting screw

Figure 3



a pressure, from an external source, i.e., an air or nitrogen cylinder, to the top side of the regulator such that the spring is working against a small differential pressure. This method is called external loading. The second method, which is the one employed by this writer, requires the use of the mobile fluid in the top side of the regulator. This method is known as internal loading. Two internal loading regulators built in our laboratory are shown in Figure 4. Figure 4A shows a regulator loaded from the upstream side of the regulator and Figure 4B shows a regulator loaded from the downstream side of the regulator. Although both regulators could be operated at pressures up to 5000 p.s.i., neither was stable for a long period of time and thus required constant watch. After several months of operation, a regulator (Grove Model No. 16 LH, from Grove Valve and Regulator Company, Oakland, Calif.) was obtained with a maximum working pressure of 2000 p.s.i. Although this regulator does not operate with the efficiency of the low pressure regulator, it functions reasonably well with the SFC system.

The sampling system incorporated a liquid sampling valve (Biotron Model LS-2, obtained from the Biotron Company, Houston, Texas) with an interchangeable sample loop. The valve had a maximum working pressure of 1500 p.s.i. A 10 microliter sample loop was used for most of the study. The valve was connected to the column by 2 inches of 0.15" I.D. stainless steel tubing. Sample solutions were supplied to the valve by means of a 2 cc hypodermic syringe equipped with a No. 24 gauge needle inserted through one end of a piece of teflon tube

FIGURE 4

A. PRESSURE REGULATOR LOADED FROM UPSTREAM SIDE

A Inlet chamber

B Poppet

C Outlet chamber

D Spring

E Diaphragm

F Needle valve

G Bourdon gauge, 0-5000 p.s.i.

B. PRESSURE REGULATOR LOADED FROM DOWNSTREAM SIDE

A Inlet chamber

B Poppet

C Outlet chamber

D Spring

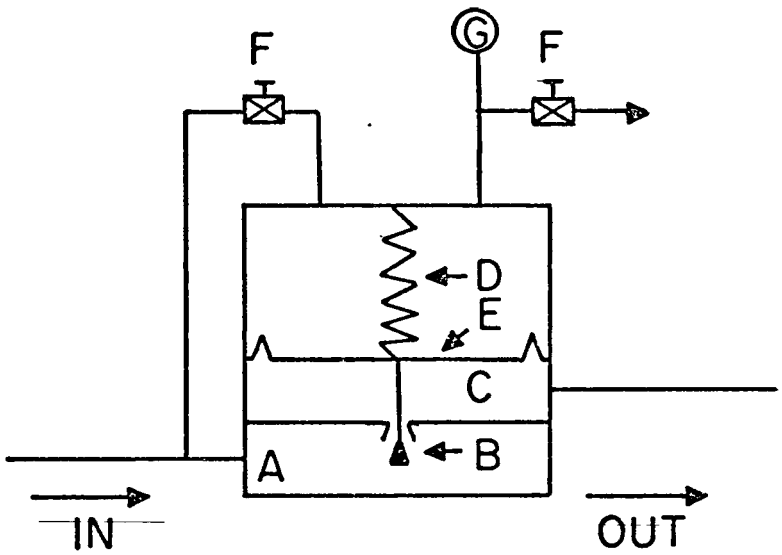
E Diaphragm

F Needle valve

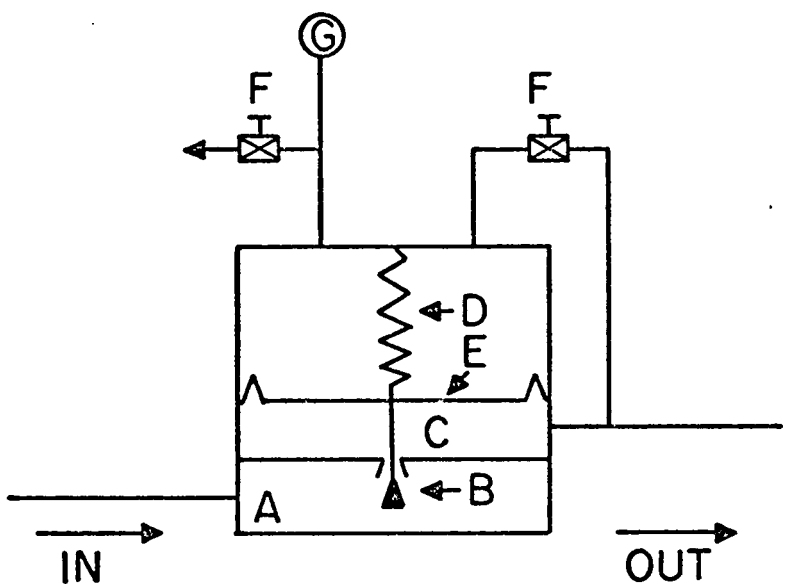
G Bourdon gauge

Figure 4

A.



B.



attached to the inlet arm of the valve. The use of the sampling valve afforded peaks which were reproducible to within 1 percent of the positive peak area. The sampling system was ideal for evaluating the detector which will be described in the next section.

Packed columns were used exclusively in this study. Granulated adsorbents and porous polymers were used as the stationary phases. Some use was also made of the recently reported durapaks (20,21) which have the stationary liquid phases chemically bonded to the support. All columns used in this study were constructed of stainless steel. The columns were 1/4" O.D. x 4.5 mm I.D. and were of varying lengths.

Before packing, each column was coiled and cleaned. The cleaning procedure consisted of passing 100 ml. concentrated hydrochloric acid through the column under vacuum and subsequently rinsing the column with 500 ml. of tap water followed by 500 ml. of distilled water. The column was dried in an oven for 2 hours at 150°C and allowed to cool in a desiccator. For packing, one end of the column is fitted with a stainless steel filter disc (Perkin-Elmer Co. No. 154-1156, Perkin Elmer Corporation, Norwalk, Conn.) and attached to a vacuum. A glass funnel is attached to the opposite end and placed in an upright position. The vacuum was turned on and the packing material was added through the funnel under constant vibration, e.g., by holding a commercial vibrator against the column. After the packing had been completed, the funnel was removed and the end was fitted with a filter disc. The column ends were connected to the corresponding parts of the instrument by Swagelok fittings.

The connection at each end of the column consisted of a Swage-lok cap (Part No. 400-C, 1/4", Crawford Fitting Co., Solon, Ohio) through which a 1/16" hold had been drilled and 3 inches of 1/16" O.D. x 0.15" I.D. stainless steel tubing soldered therein. This design gave minimum dead volume. The pre-column end is connected to the sampling valve by means of a 1/16" G.C. Swagelok union, and the exit end is connected to the metering system by the same type of union. The direction of flow through the column was, in all cases, the same as the direction of packing.

The column which was placed in an environmental oven (Statham Model SD-6, Statham Instruments, Inc., Los Angeles, Calif.) for accurate temperature control during chromatographic procedure, was conditioned 15°C above the operating temperature. The extent of column bleed could be monitored by the needle valve in the metering system. The material from the column had a tendency to coat the needle valve in the metering system and caused a decrease in flow through the system.

A diagram of the flow-controlling system is shown in Figure 5. The two major demands of a flow controlling system for SFC are that it functions well at high pressure and that it does not introduce excessive dead volume. Conventional metering valves that are designed for high pressure are also designed for high flow and they have very high dead volumes. Low dead volume metering valves that are used in conventional gas chromatography work well at low pressure but they are unable to function at high pressure due to the high pressure drop across the valve. Since the operation of a fine metering

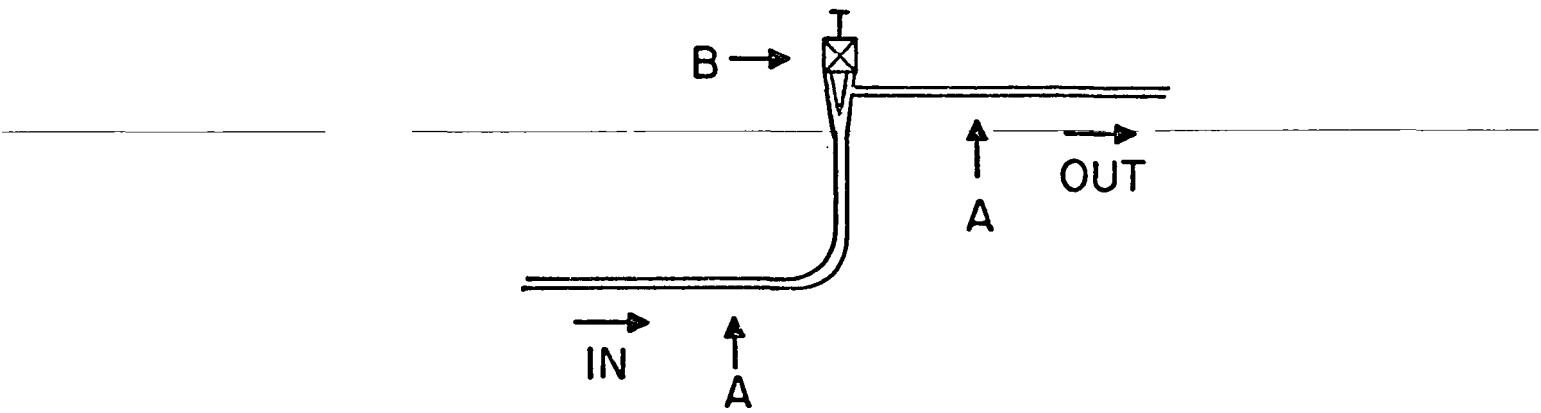
FIGURE 5

SCHEMATIC DIAGRAM OF FLOW CONTROLLING SYSTEM

A Stainless stell capillary tubing

B Stainless steel micrometering valve

Figure 5



valve depends not on the total pressure but on the pressure drop (differential pressure or Δp) across the valve, it was conceivable that a fine metering valve could be placed in a high pressure system at some point where the differential pressure would be small. Working on the basis of differential pressure rather than total pressure, we attached 2 feet of 1/16" O.D. x .01" I.D. stainless steel tubing to the exit end of the column. A fine metering valve (Nupro IS, Nupro Company, Cleveland, Ohio) was connected to the capillary tubing and a second 2 foot section of the capillary tubing was coiled, one end attached to the exit end of the metering valve and the opposite end was connected to the detector. The function of the second capillary is twofold. It prevents any significant pressure drop across the metering valve and it serves as a cooling reservoir which allows the mobile phase to come to constant temperature before entering the detector.

The Detection System

A diagram of the detection system used in this study is shown in Figure 6. The detection system consists of a constant voltage supply, amplifier, heat of absorption detector and a constant temperature source.

The amplifier (Model 4A, Applied Research Associates of Texas, Inc., Austin, Texas) used in the study is a low noise, low-level DC amplifier with a DC input resistance of 4,000 ohms and an output of 10 millivolts at 500 ohms. The signal from the amplifier was recorded on a Speedomax G recorder (Leeds and Northrup Co., Houston, Texas) with a variable

FIGURE 6

DIAGRAM OF THE DETECTION SYSTEM

A Detector

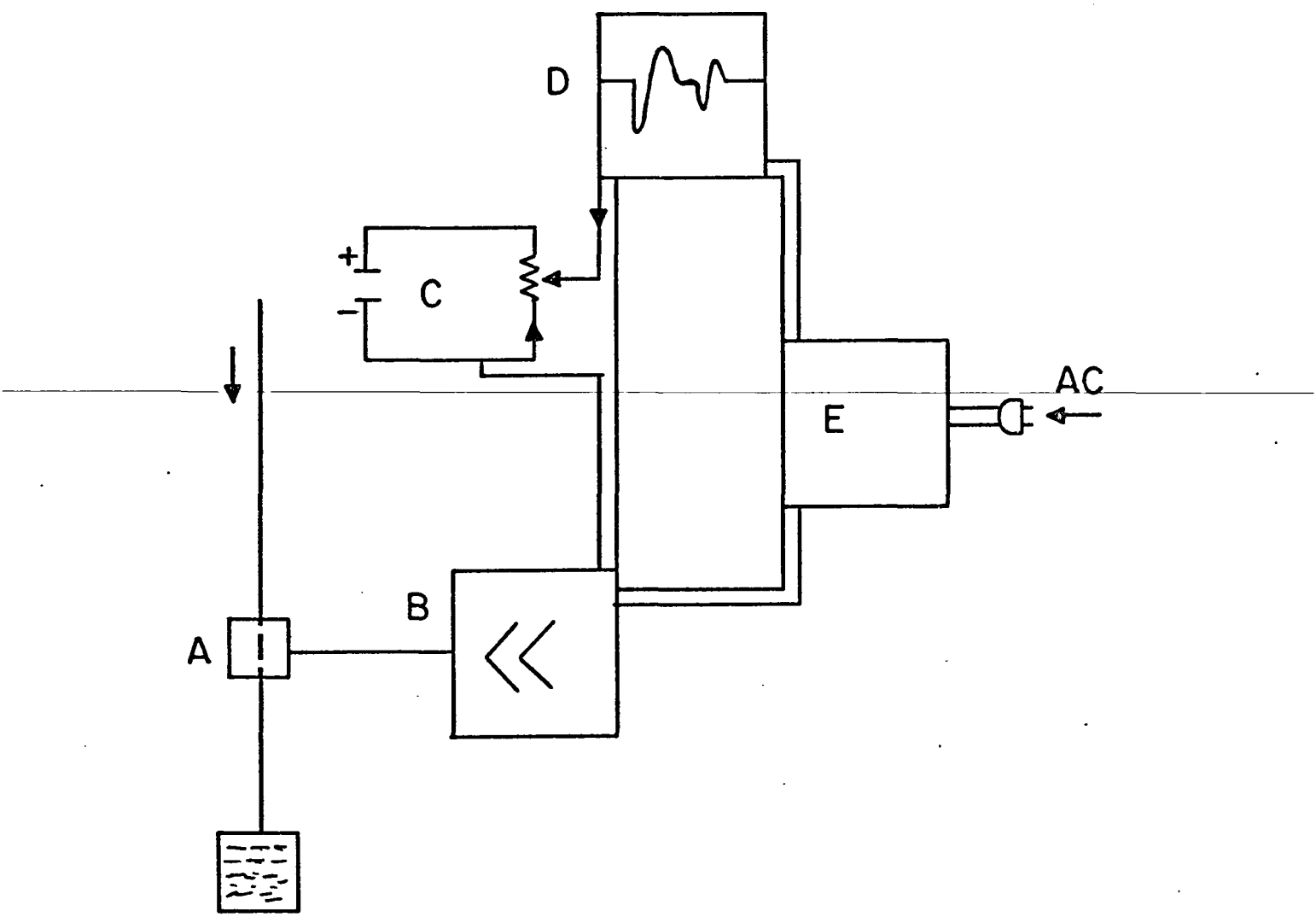
B Amplifier

C Balance control

D Recorder

E Constant voltage supply

Figure 6



span. The recorder was operated at a span of 10 millivolts to equal the output of the amplifier. The electrical power for the recorder and the amplifier was furnished by an AC voltage regulator (Model 500S, Sorenson Co., South Norwalk, Conn.).

Since the amplifier used in this system is a general purpose amplifier and was not specifically designed for this purpose, it was necessary to install a 10-turn potentiometer in the electronic system between the amplifier and the recorder in order to adjust the base line on the recorder. The electronic arrangement is shown in Figure 6.

A diagram showing the component parts of the detector, which had to be kept at a constant temperature, is shown in Figure 7. The detector is composed of multi-thermocouples (thermopile) with a suitable adsorbent embedded in one end which becomes the hot junction, and the opposite end serves as the cold junction. The differential temperature resulting from the heats of adsorption and desorption of compounds passing through the hot junction of the detector were amplified and recorded. In the initial experiments the thermopiles were prepared in our laboratory. Iron and constantan, insulated with teflon, were used as the thermoelectric base wires. The wires were held parallel and wound around a plexiglass board 13 mm wide and 3 mm thick such that the iron and constantan wires alternated. The position of the wires was fixed by covering them with a sodium silicate solution and allowing the solution to dry. After drying, the teflon was removed from the wire along the thickness of the board on each side with a razor blade and subsequently the wires were cut

FIGURE 7

DIAGRAM SHOWING THE COMPONENTS OF THE DETECTOR

The teflon discs are 35 mm in diameter.

A Top teflon disc

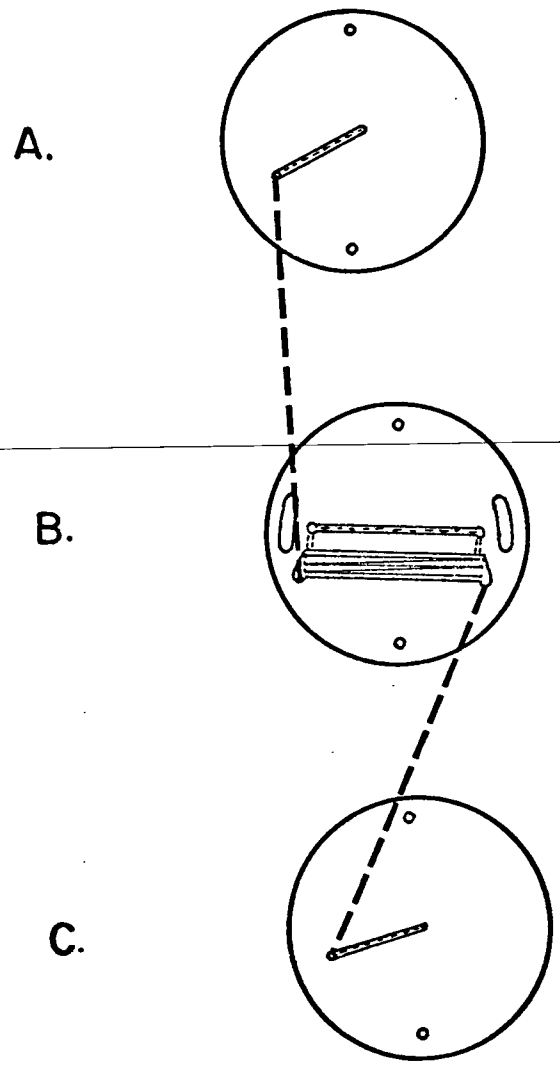
B Mounted thermopile

Size of thermopile compartment: 3 mm x 3 mm x 20 mm

Total length of thermopile: 17 mm

C Bottom teflon disc with filter

Figure 7



along the thickness of the board such that 1.5 mm of wire was exposed at each end. The exposed ends were welded together to form a group of thermocouples which comprised the thermopile. Two thermopiles were obtained from each operation and the number of thermocouples in each thermopile is equal to the number of winds made around the plexiglass board.

A diagram of the welding apparatus is shown in Figure 8. It consists of a 0-1500 volt power supply (Kepco part #188·0030, New York), rectifier and filters. The actual voltage necessary for welding the thermocouples, which were made from 1.00 mm wires was 16 to 18 volts. The welding was accomplished with the aid of a microscope.

The thermopile was mounted in a teflon disc, 3 mm in thickness, and soldered to teflon-insulated leads which were connected to the input terminals of the amplifier. The mounted thermopile is placed between two teflon discs, each 6 mm thick, and the three discs which form the sensing element are held together by two stainless steel pins. Each disc has a 1.00 mm grove extending from the center to a 1.0 mm bore through the disc such that the mobile phase enters the detector and flows across the cold junction of the thermopile and exits after flowing across the hot junction. A stainless steel filter is mounted in the teflon disc on the exit side of the thermopile to retain the absorbent embedded in the exit end of the thermopile. The detector is situated between two steel flanges containing cavities for the centering of the discs. The flanges are held together by means of 4 Allenhead screws, which also serve to seal the system

FIGURE 8

ELECTRONIC DIAGRAM OF WELDING APPARATUS

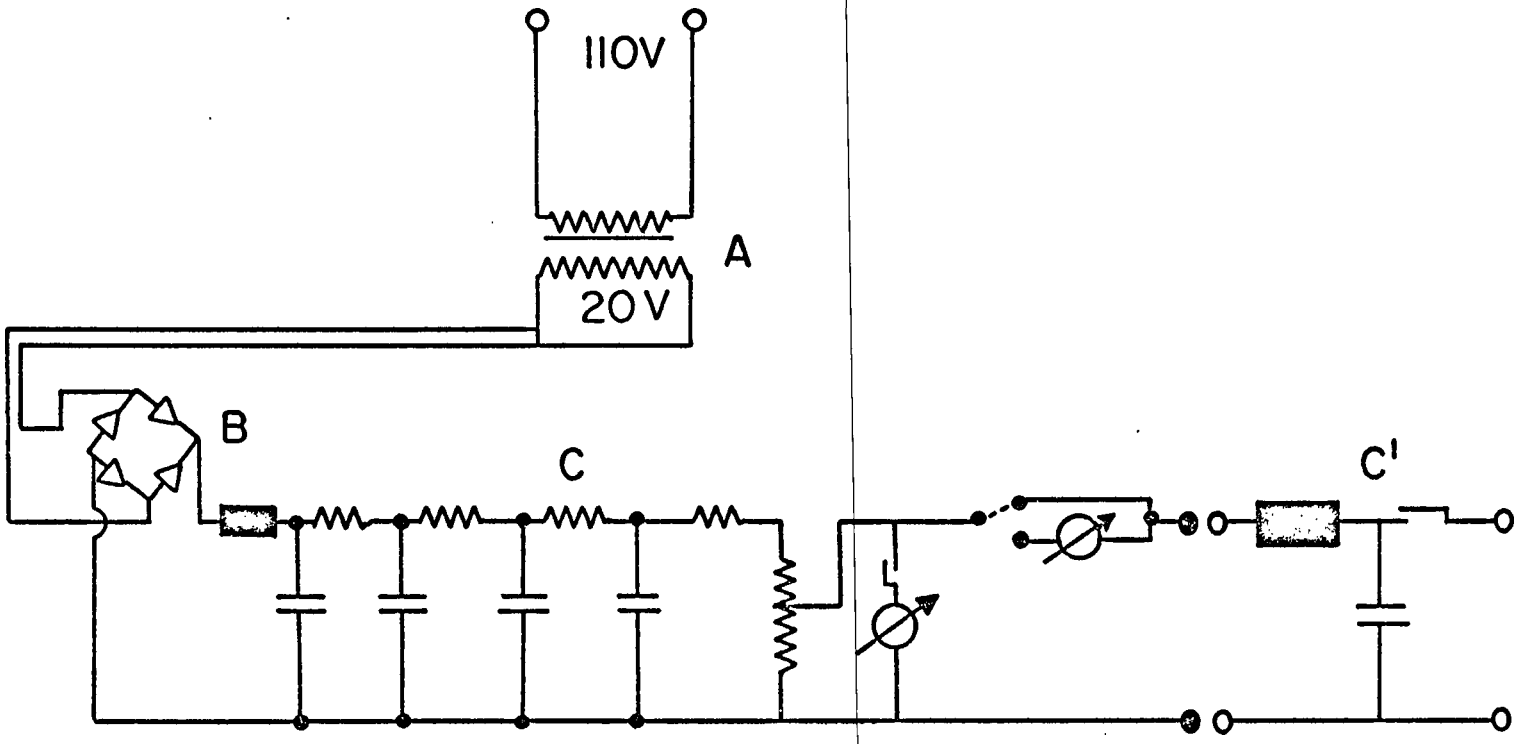
A Transformer

B Rectifier

C Filter

C' Filter

Figure 8



externally. Each flange contains two additional holes through which the teflon discs may be forced out of the cavities in which they rest. Each flange has a 1/8" x .027" I.D. stainless steel tube 1.0" in length soldered in the center which serves to connect the detector to the column and to the eluate container. All connections were made with Swagelok fittings.

After operating the SFC system for a period of time, thermopiles were available from a commercial source (Science Products Corp., Dover, New Jersey). The thermopiles, constructed from 36 gauge (.005" diameter) wire insulated with teflon, were further insulated with teflon tape and mounted in the teflon disc as described for the initial experiments.

A special cooling system was designed to bring the supercritical fluid to ambient temperature and to maintain the detector at a constant temperature. A diagram of the cooling system employed in this study is shown in Figure 9. For the first part of the cooling system, a water jacket (condenser) was constructed around the capillary tubing at the exit end of the column using 1/4" O.D. stainless steel tubing and the necessary Tees and Swagelok fittings. Tap water was allowed to flow briskly through the condenser to cool the mobile phase to ambient temperature. A second condenser was arranged around the capillary tubing near the entrance to the metering valve and connected to the thermostating system described below. The second part of the cooling system consisted of a circulating bath (Lauda Model K-2, Brinkman Instruments, Inc., Westbury, New York), a Dewar flask which

FIGURE 9

SCHEMATIC DIAGRAM OF COOLING AND THERMOSTATING SYSTEM

A Mobile fluid exit

B Thermostating bath

C Detector

D Magnetic stirrer

E Micrometering valve

F Water from circulating bath

G Condenser

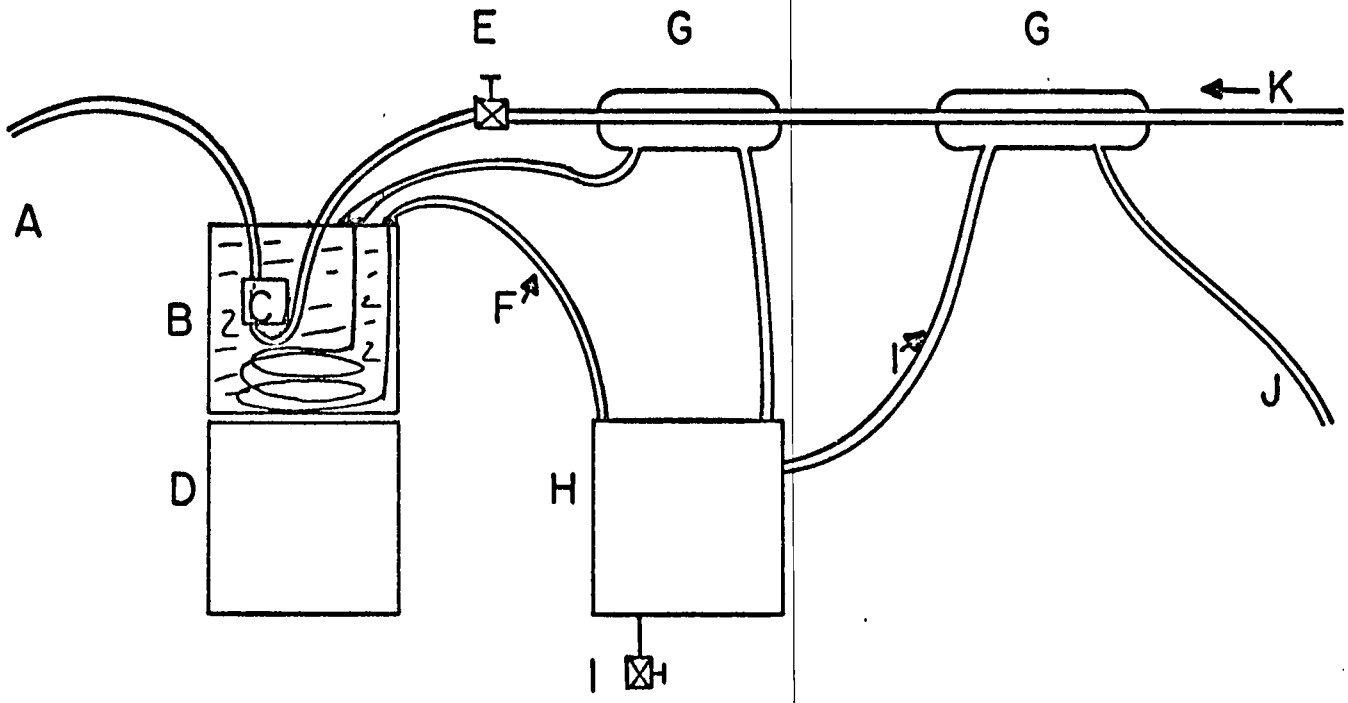
H Circulating bath

I Tap water

J To sink

K Mobile fluid from column

Figure 9



housed the detector and a magnetic stirrer. The Dewar flask was filled with water and mounted on the magnetic stirrer. The temperature in the flask was kept uniform by continuous stirring and kept constant by circulating water from the circulating bath through a 1/4" O.D. stainless steel coil tube placed in the Dewar flask. The water leaving the Dewar flask was allowed to flow through the second condenser described above before returning to the circulating bath. The circulating bath has a control accuracy of $\pm$0.02°C and the temperature in the Dewar flask has even less variation. The circulating bath was operated at 27°C and the temperature of the Dewar flask was 25°C.

IV.

RESULTS AND DISCUSSION

RESULTS AND DISCUSSION

From theoretical considerations, the optimum column efficiency in conventional gas chromatography is obtained when the ratio of inlet-to-outlet pressure is less than 3 and ideally about 1.5. Since the product of pressure and gas velocity is virtually constant along the column, the gas velocity will be more uniform at a low than a high inlet-to-outlet pressure ratio, and it will be possible to operate the entire column closer to the linear gas velocity corresponding to the optimum efficiency. The required gas rate is obtained by applying a pressure difference between column inlet and outlet. Generally the outlet pressure used is the prevailing atmospheric pressure and the inlet pressure is controlled from a valve leading to a cylinder containing the carrier gas. If the atmospheric pressure were used for the outlet (assuming the same optimum inlet-to-outlet pressure ratio as gas chromatography) pressure in a SFC system, the inlet-to-outlet pressure ratio would be far greater than 3 and the column efficiency would be seriously altered. In the SFC system described in this study, the flow-controlling system described earlier also served as an outlet pressure control. A study of the ratio of inlet-to-outlet pressure was made on a column packed with alumina (100-200 mesh) and the results are shown in Table I. The column inlet pressure was varied from 370 to 1120 p.s.i., while maintaining the column temperature at 225°C and from 435 to 1150 p.s.i. while maintaining the column at 24°C. The pressure drop in either case did not exceed 30 percent. Pentane was used as the mobile phase.

TABLE I

PRESSURE DROP ACROSS COLUMN AT DIFFERENT INLET PRESSURES*

Inlet Pressure	Outlet Pressure	Δp**	Percent Pressure Drop
A			
1120	950	170	15.2
970	800	170	17.5
930	760	170	18.3
730	560	170	23.0
600	450	150	25.0
370	225	115	31.0
B			
1150	980	170	14.8
930	790	160	17.2
810	700	110	13.6
640	550	90	14.1
550	475	75	13.6
435	375	60	13.7

Column 6 ft x 4.5 mm I.D.; Packing, alumina (100-200 mesh)
Pentane flow rate, 3 ml/min

*Pressure is expressed in pounds per square inch
**Difference between inlet and outlet pressure = Δp

A Temperature, 225°C; B Temperature, 24°C

Conventional gas chromatography is also limited to the use of coarse-grained absorbents and supports which maintain low pressure gradients and low pressure ratios. In the SFC system, the inlet-to-outlet pressure ratio was unaltered when a column containing very fine aluminum oxide (200-325 mesh) was placed in the system at 240°C using isopropanol as the mobile phase and working at pressures up to 1100 p.s.i. The separation of compounds should be greatly enhanced in the SFC system by using fine supports and adsorbents while maintaining the flow along the entire column near the optimum flow rate.

Evaluation of the Detector

The micro adsorption detector evaluated in this study contained a thermopile consisting of 25 thermocouples of copper/constantan wire and had silica gel (200-325 mesh) embedded in one end to form the hot junction. The detector functions as a micro column and with each step of adsorption and desorption there is associated both an evolution and an uptake of heat whose values are of equal but of opposite signs. The heats of absorption and desorption are proportional to the concentration of the substance in the stationary phase. Therefore, a change in the concentration of the substance in the eluent will cause a change of temperature, provided the eluted substance and the eluent have differing solubilities in the stationary phase.

If the detector was perfectly insulated, the change in concentration of a substance in the eluent would be continuously indicated

by changing the temperature. That is to say, a Gaussian concentration profile would show a Gaussian concentration curve. The rise in temperature on reaching the maximum of the peak would be equal to the following fall in temperature and the original temperature would be attained. However, there is a continuous exchange of heat from the detector with the surroundings and as the form of a peak passes through the detector, a portion of the heat evolved will be transported away so that the temperature expected under adiabatic conditions will not be reached. When the end of the peak passes through the detector, the heat dissipates and the temperature will sink below the ideal starting temperature. The original temperature of the detector will once again be restored by the surroundings. Figure 10 shows a typical temperature profile as measured by the micro adsorption detector under SFC conditions.

Since the response of the detector is based on the difference in solubility of the eluent and the substance in the stationary phase, a substance having greater solubility in the stationary phase than the eluent would be expected to give a positive peak, i.e., the positive "lobe" would appear first, and the substances with less solubility would be expected to give a negative peak. Figure 11 shows peaks from two compounds that are more soluble and two compounds that are less soluble in the stationary phase than the eluent. These peaks also demonstrate the versatility of the detector. For instance, a compound that is only sightly soluble in the stationary phase would give a large signal with an eluent that is highly soluble in the stationary

FIGURE 10

TYPICAL TEMPERATURE PROFILE AS MEASURED BY

MICRO ADSORPTION DETECTOR

4-Hydroxyundecanoic Acid (10 μg) eluted under SFC conditions:

CD = positive peak width

DE = negative peak width

CE = total peak width

BD = positive peak height

DA = negative peak height

AB = total peak height

Figure 10

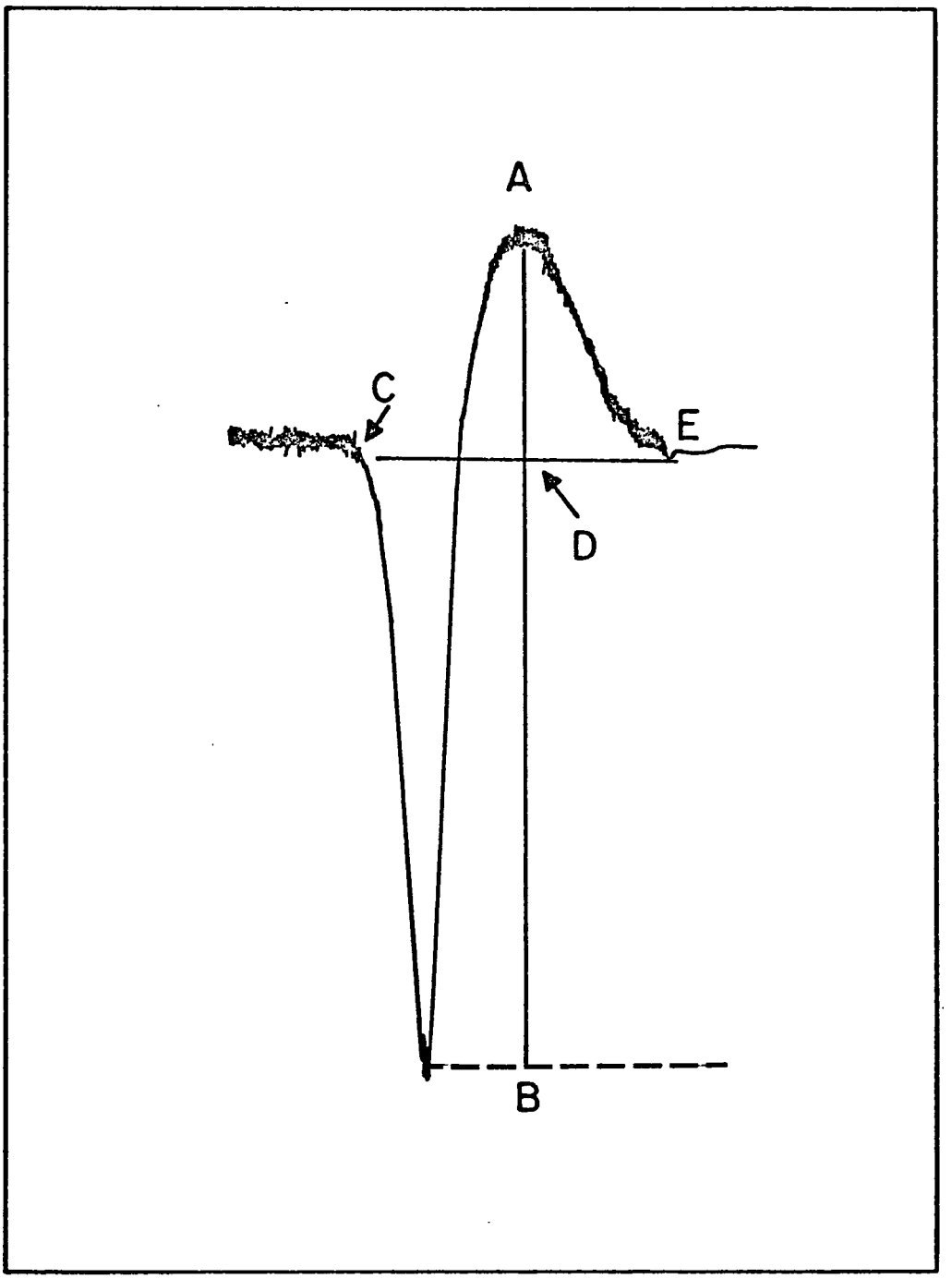


FIGURE 11

PEAK PROFILES EXHIBITED BY DIFFERENT COMPOUNDS

ELUTED FROM THE MICRO ADSORPTION DETECTOR

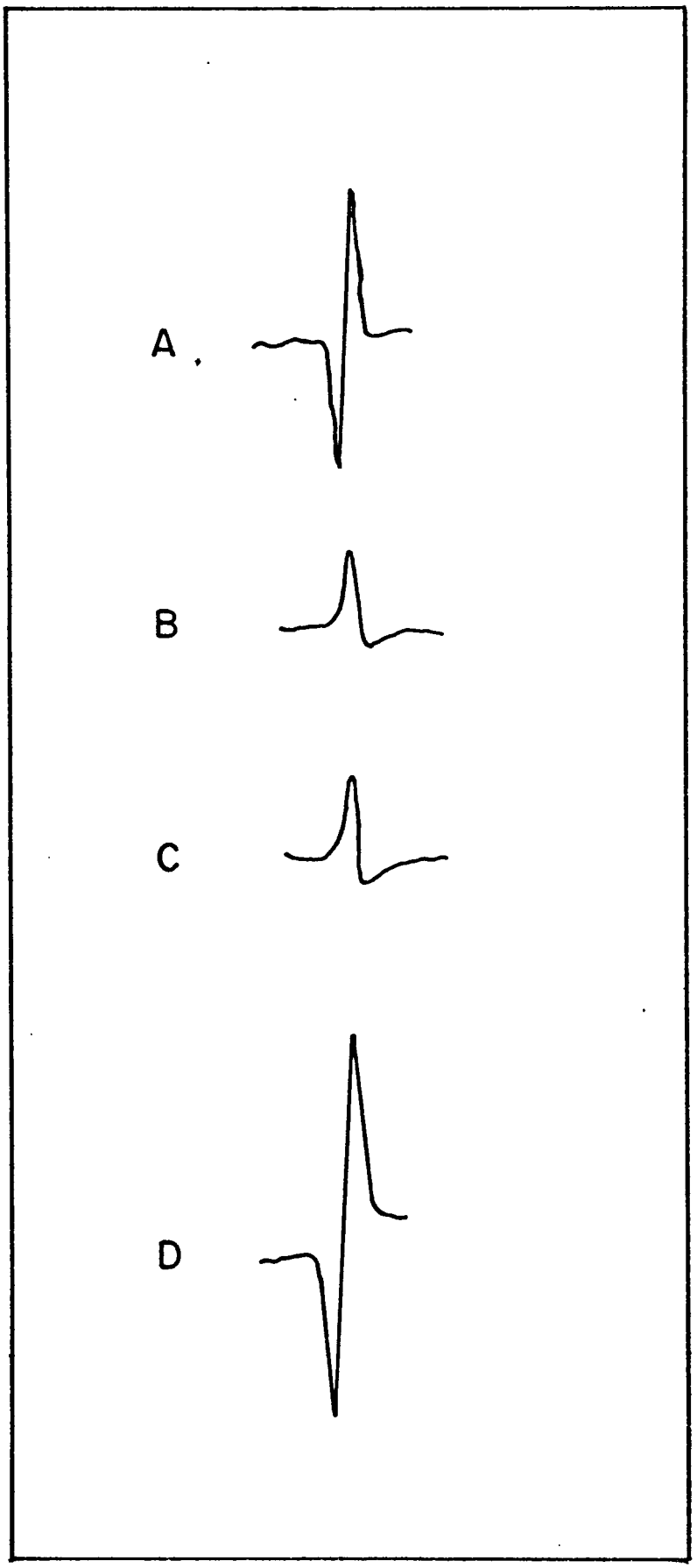
A Acetone

B trans-Decahydronaphthalene

C cis-Decahydronoaphthalene

D Dypnone (ϕ-C(ϕ)=CH-C(=O)-ϕ)

Figure II



phase and a compound that is highly soluble would give a large signal with an eluent that is only slightly soluble. Therefore, by the proper selection of the mobile and stationary phase, all substances separated can be detected making the detector of universal application.

The response time of the detector is obtained from Figure 12. Samples of n-butanol (4 μg) and acetone (2 μg) were injected alternately into the system while varying the flow rate of the eluent pentane from 0.75 to 10 ml and the peak area versus flow rate was plotted. The peak area became constant at a flow rate of 8 to 10 ml per minute for each sample. The detector's minimum response time in each case was 4 seconds. It is noteworthy that on varying the flow rate from 0.75 to 10 ml., the height of the acetone peak only increased from 114 to 133 mm while the width of the peak decreased from 28 to 4.6 mm. Similarly, the height of the n-butanol peak only increased from 118 to 140 mm while the width decreased from 29 mm to 4.6 mm. The change in peak width caused by changing the flow rate is comparable to the change in peak width caused by band broadening on a column due to long elution time. The small changes in peak heights associated with changes in flow rate indicate that the micro adsorption detector is self-compensating for loss of sensitivity normally caused by band broadening due to long retention times.

The sensitivity and quantitative response of the detector are shown in Figure 13. The micro adsorption detector had a lower limit of detection of less than 250 nanograms. The signal to noise ratio on injection of 250 nanograms of n-butanol was greater than 3:1. The

FIGURE 12

PLOT OF PEAK AREA VERSUS FLOW RATE

Solvent: Pentane

Adsorbent: Silica gel (200-325 mesh)

Figure 12

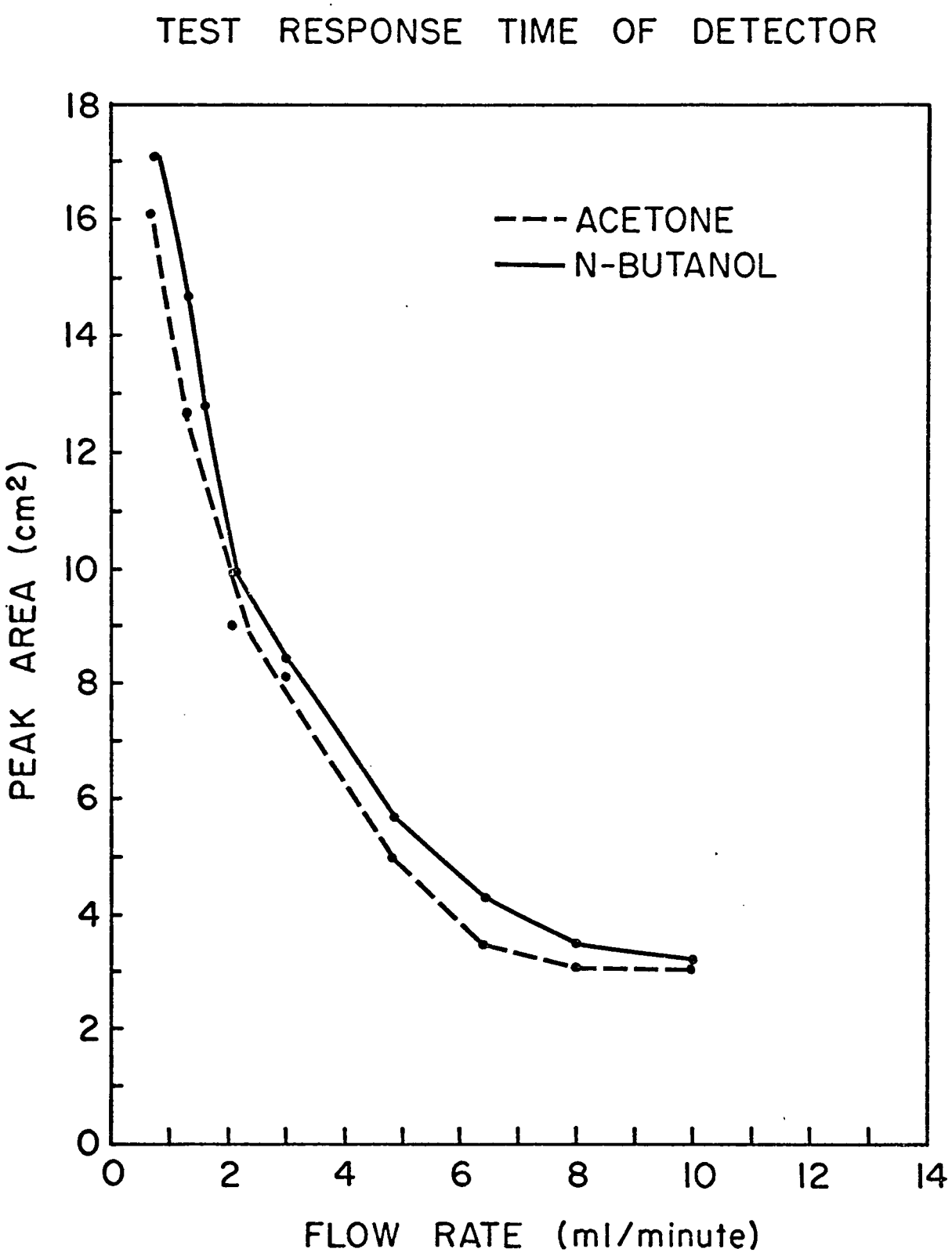
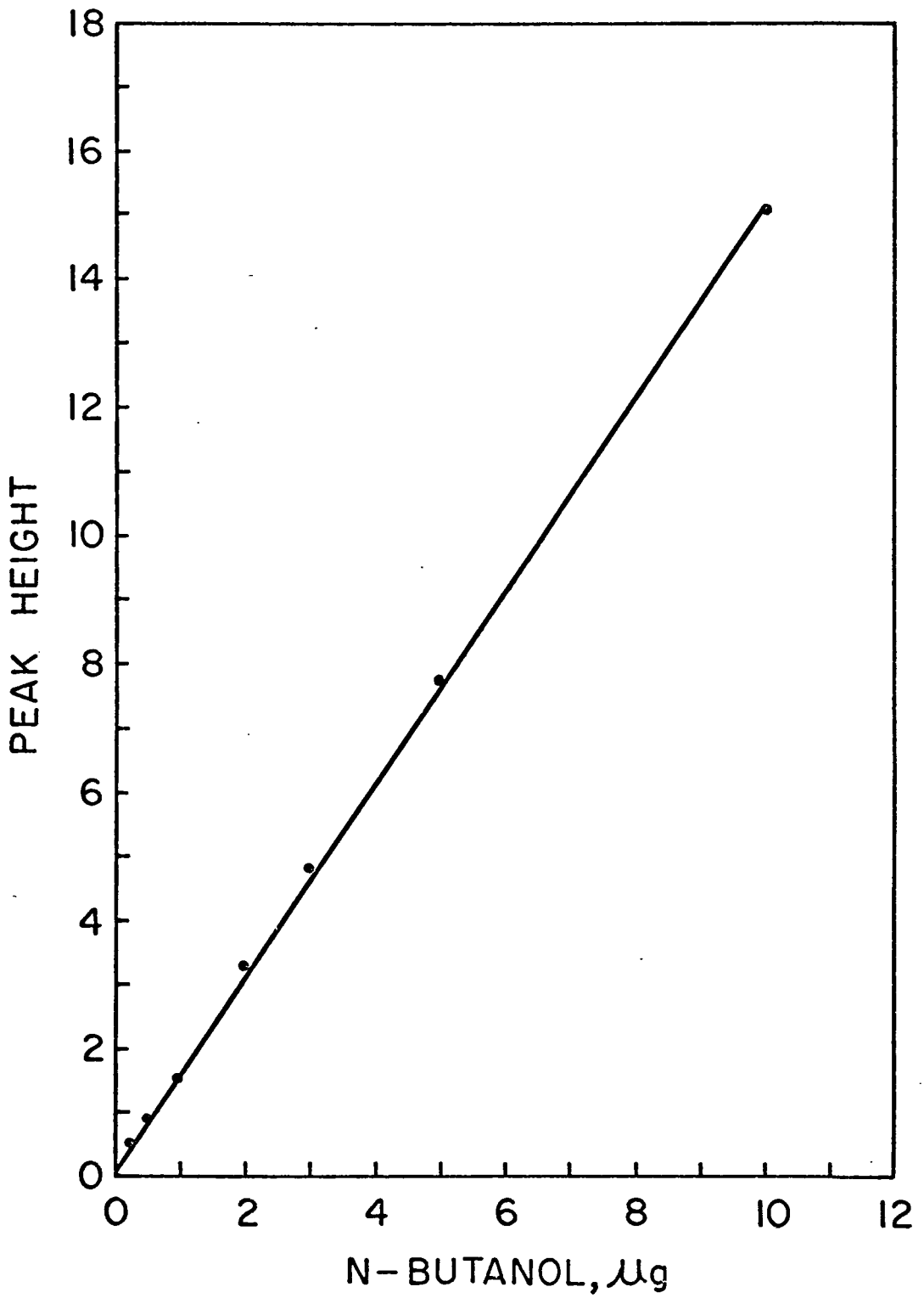

TEST RESPONSE TIME OF DETECTOR
ACETONE
N-BUTANOL
PEAK AREA (cm²)
FLOW RATE (ml/minute)
0
2
4
6
8
10
12
14
16
18

FIGURE 13

LINEARITY OF DETECTOR RESPONSE

Positive lobe height versus amount of n-butanol injected in the range 0.25 - 10 μg. Eluent: pentane, Adsorbent: silica gel (200-325 mesh).

Figure 13



linearity of the response was obtained by plotting the peak height of the positive lobe versus the concentration of the compound. Hupe et al. (22,23) and later Burtis and co-workers (24), using a micro adsorption detector with thermistors as the sensing elements in a low-pressure liquid chromatographic system, suggested using peak to peak height (Figure 10, Line AD) to produce a calibration curve. For calibration under actual SFC conditions, the area of the positive peak lobe is preferred because there is a gradual decrease in flow rate associated with SFC systems which causes a decrease in peak height. Further, peaks which slightly overlap tend to reduce the total peak height while having a very minimal effect on the positive peak lobe. Quantitative measurements of positive peak heights indicated that the micro adsorption detector is linear over a concentration range of at least 2 power of 10 for all substances tested.

The sensitivity of the micro adsorption detector employed in this study should be markedly enhanced by increasing the number of thermocouples. Although increasing the number of elements in series will necessarily increase the resistance of the thermopile, the signal-to-noise ratio will be improved significantly because the signal is directly proportional to the number of elements in series while the noise is proportional to the square root of the number of elements in series. Further, the detector should function well with capillary columns due to its high sensitivity and low dead volume.

Separations and Plate Height

Figure 14 shows some plate height versus velocity curves for a SFC column with n-pentane as the carrier gas. Three compounds, n-butanol, n-octanol, and n-dodecanol, were selected for this study because of the difference in their retention times. The column (5 ft. x 4.5 mm I.D.) was packed with Poropak Q (150-200 mesh). The operating temperature was 210°C and the pressure was 600 pounds per square inch. The flow rate was varied from 1.1 to 5.6 ml. All three curves approximate a straight line except for the anomaly at 1.6 ml. which is more striking in the case of n-butanol. Although no explanation of this anomaly was apparent, the plate heights were plotted on an expanded scale and with the exception of n-butanol, the values at 1.6 ml. may fall well within experimental error.

The same conditions described for the plate height study were employed to separate some normal alcohols. Figure 15 shows the separation of methanol, n-butanol, n-hexanol, n-decanol, and n-dodecanol at a flow rate of 1.0 ml. per minute. Although the compounds were well separated the typical symmetrical profile that is common in gas chromatography was not observed with the micro adsorption detector. The detector gives an adsorption and a desorption peak for each compound. Therefore, the base line is positioned near the center of the chart paper. The base line noise is consistent with the high sensitivity of operation.

Figure 16 shows the separation of the normal alcohols repeated at a flow rate of 1.5 ml. per minute. This time the separation is achieved

FIGURE 14

RELATIONSHIP BETWEEN PLATE HEIGHT AND FLOW RATE

Column:	4.5 mm I.D. X 5 ft. (stainless steel)
Stationary phase:	Porapak Q (150-200 mesh)
Mobile phase:	Pentane
Temperature:	200° C
Pressure:	600 p.s.i.
Flow rate:	1.0 ml per minute

Figure 14

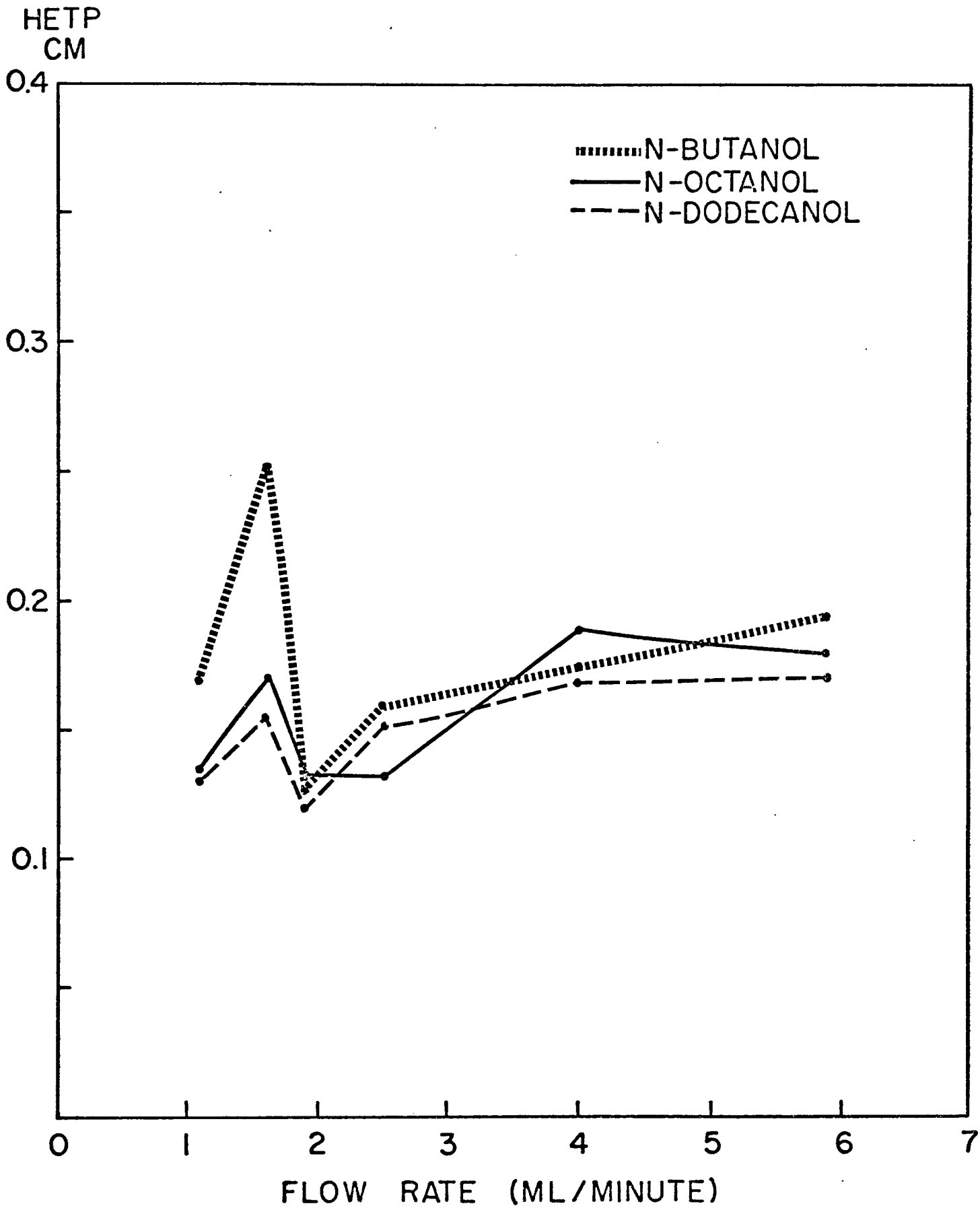

HETP
CM
0.4
0.3
0.2
0.1
N-BUTANOL
N-OCTANOL
N-DODECANOL
0
1
2
3
4
5
6
7
FLOW RATE (ML/MINUTE)

FIGURE 15

SEPARATION OF SOME NORMAL ALCOHOLS

Column:	4.5 mm I.D. X 5 ft. (stainless steel)
Stationary phase:	Porapak Q (150-200 mesh)
Mobile phase:	Pentane
Temperature:	200° C
Pressure:	600 p.s.i.
Flow rate:	1.0 ml per minute

A Methanol, 1 µg

B n-Butanol, 1 µg

C n-Hexanol, 3 µg

D n-Decanol, 15 µg

E n-Dodecanol, 15 µg

Figure 15

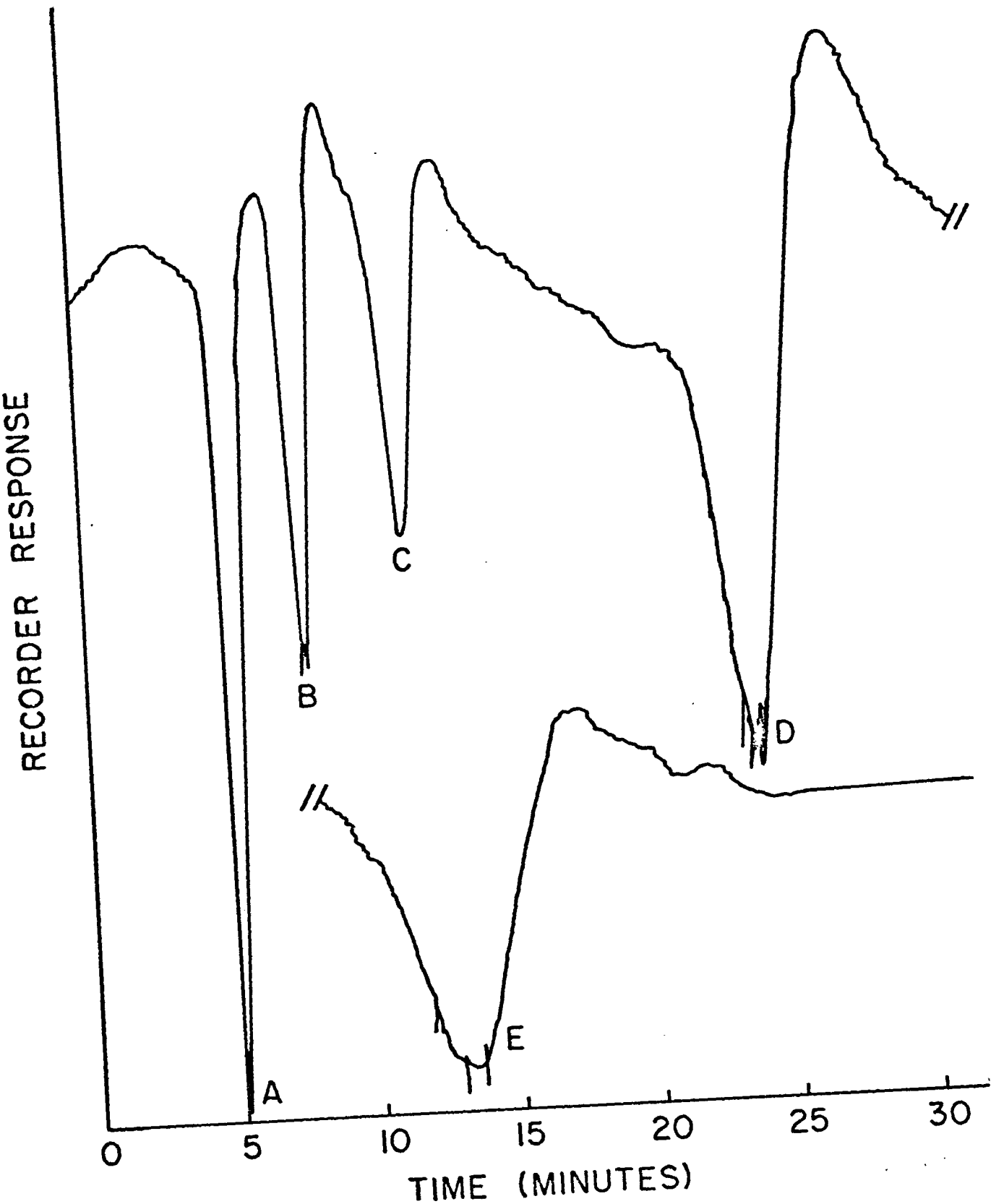


FIGURE 16

SEPARATION OF SOME NORMAL ALCOHOLS

Column:	4.5 mm I.D. X 5 ft. (stainless steel)
Stationary phase:	Poropak Q (150-200 mesh)
Mobile phase:	Pentane
Temperature:	200° C
Pressure:	600 p.s.i.
Flow rate:	1.5 ml per minute

A Methanol, 1 µg

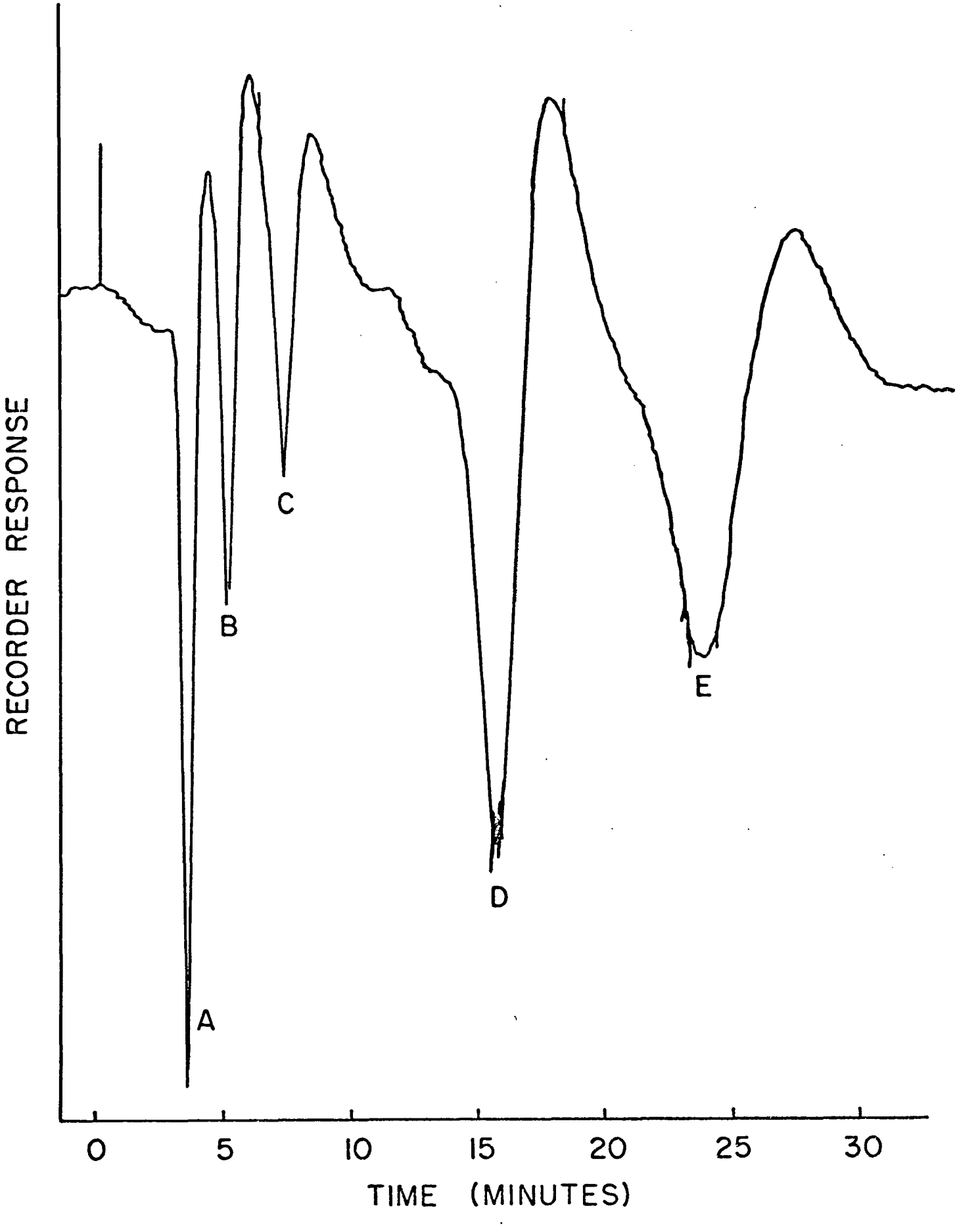
B n-Butanol, 1 µg

C n-Hexanol, 3 µg

D n-Decanol, 15 µg

E n-Dodecanol, 15 µg

Figure 16



in a shorter time although the sequence is the same.

The separation of 4-hydroxyundecanoic acid and octyl butyrate is shown in Figure 17. The recently reported chemically bound stationary phase, Durapak (N-Octane/Porasil C), is used to effect this separation. The facility with which the hydroxyacid is eluted from the column gives support to the premise that SFC could eliminate the laborous task of making derivatives of compounds containing hydroxy and carboxy groups for gas chromatography.

Generally the compounds of biological interest that require the formation of derivatives for gas chromatography are those containing hydroxy, carboxy and amino groups. Amines were eluted in the SFC system with the same facility as other compounds but the detection of amines by the micro adsorption detector in its present form has a serious drawback. Apparently the amines react with the exposed ends of the thermocouples and a very broad peak is produced. This problem can be eliminated by coating the ends of the thermocouples with teflon although some loss in sensitivity would be experienced. This decrease in sensitivity probably could be compensated for by increasing the number of thermocouples in the detector.

The unusual eluting ability of supercritical fluids was further demonstrated by the elution of fatty acids, phospholipids and other high boilers some of which are used for stationary phases in gas chromatography. The retention times for some of these compounds are listed in Table II.

FIGURE 17

SEPARATION OF A HYDROXY-ACID FROM AN ESTER

Column:	4.5 mm I.D. X 6 ft.
Stationary phase:	Durapak (N-Octane/Porasil C, 120-150 mesh)
Mobile phase:	Pentane
Temperature:	201° C
Pressure:	600 p.s.i.
Flow rate:	1.5 ml per minute

A Octyl Butyrate, 10 µg

B 4-Hydroxyundecanoic Acid, 10 µg

Figure 17

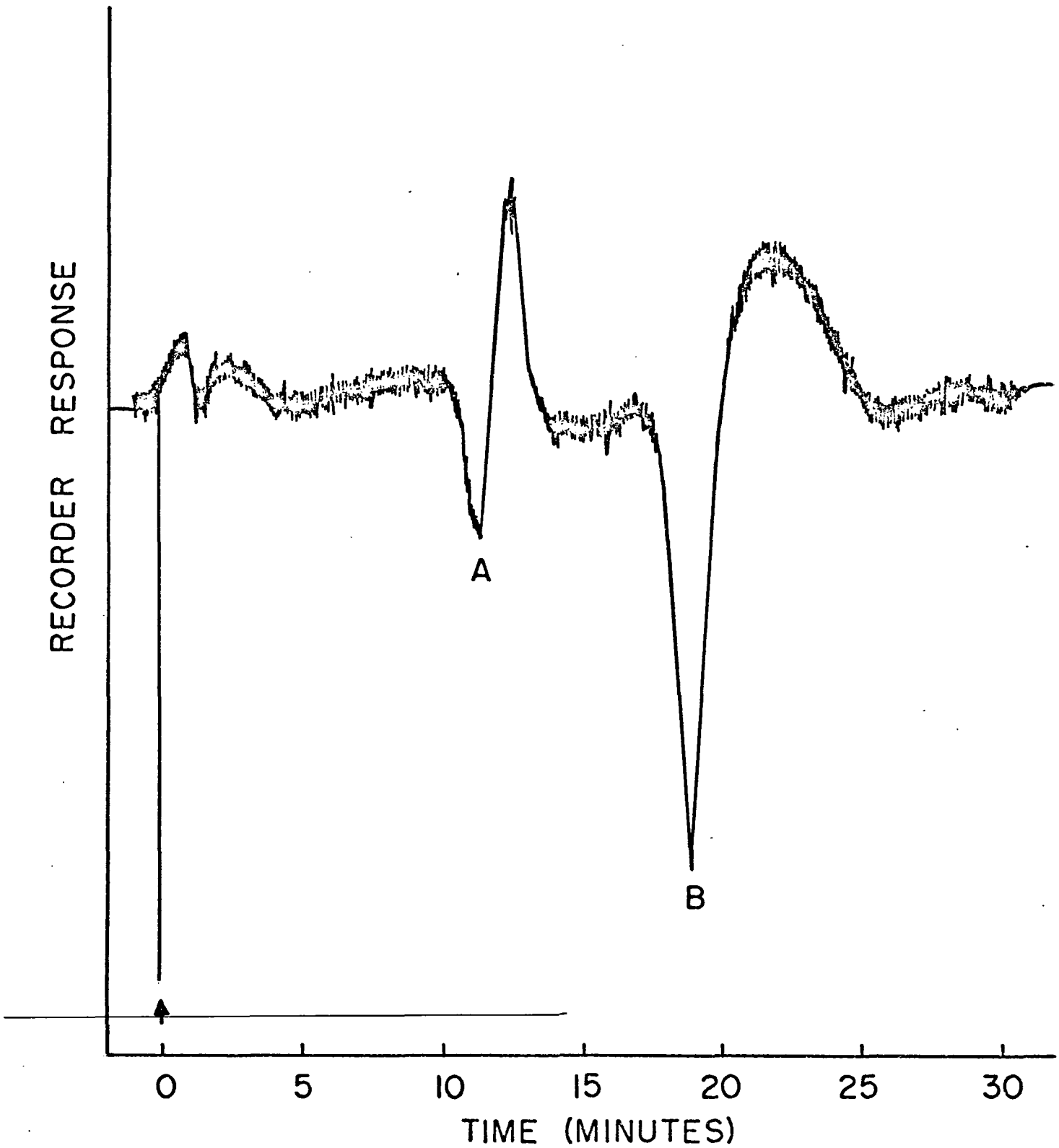


TABLE II

RETENTION TIME OF SOME HIGH BOILING COMPOUNDS

ELUTED UNDER SFC CONDITIONS

Compounds	R_t (Minutes)
A	
Butanoic Acid	8.28
Hexanoic Acid	8.85
Octanoic Acid	9.69
Dodecanic Acid	13.5
Hexadecanoic Acid	15.9
Octadecanoic Acid	16.8
Lecithin (synthetic)	48.1
Cephalin (synthetic)	53.7
B	
Tripalmitin	14.4
Squalene	14.4
Glycol-bis-cyanoethylether	75.5
C	
Ethyleneglycol	15.0
Triethyleneglycol	18.1

A. Conditions: column, 5 ft X 4.5 mm I.D. stainless steel tube containing Poropak Q (150-200 mesh); pressure, 680 p.s.i.; flow rate, 1.5 ml per minute; adsorbent in detector, silica gel (200-325 mesh); mobile phase, pentane.

B. Conditions: column, 6 ft X 4.5 mm I.D. stainless steel tube containing Durapak (octane/porasil C, 120-150 mesh). Other conditions were the same as in A.

C. Conditions: column, 6 ft X 4.5 mm I.D. stainless steel tube containing Durapak (carbowax/porasil C, 80-100 mesh). Other conditions were the same as in A.

The fatty acids were eluted in increasing order of their molecular weights. Band broadening, which is commonly associated with the elution of fatty acids in gas chromatography, was not observed. The successful use of a packed column for the elution of free fatty acids under SFC conditions suggests a method for the study of free fatty acids occurring in biological materials. This method would not only eliminate the necessity of preparing esters from the acids, but it would eliminate the possibility of side reactions during esterification.

Tripalmitin (glyceryl tripalmitate) was eluted from a packed column with greater facility in SFC than has been achieved by gas chromatography, although partially acetylated glycerides derived from natural sources have been eluted at very high tempeartures.

Since ionized substances ordinarily cannot be volatilized and separated by gas chromatography, studies involving the isolation of the intact molecules of phospholipids have been confined to liquid chromatography. The elution of lecithin (phosphatidylcoline) and cephalin (phosphatidylethanolamine) from a packed column at 200°C with pentane could be a highly significant step in the development of a method for the determination of phospholipids. Elution of the phospholipids could be facilitated by increasing the pressure, using a polar mobile phase or by changing the packing material in the column.

Although this preliminary work demonstrates that phospholipids can be eluted by SFC, the actual determination of phospholipids in biological materials would require further study.

V.

CONCLUSIONS

CONCLUSIONS

The development of an SFC system that can be operated with reasonable facility affords a rapid means of analyzing polar and high molecular weight compounds. It eliminates the necessity of preparing derivatives of compounds containing hydroxy, carboxy, and amino groups which are prevalent in biological materials. Further, it offers the possibility of analyzing complex molecules that can not be analyzed by gas chromatography.

The development of the micro adsorption detector, which is sensitive in the submicrogram range, gives considerable latitude to the operation of the SFC system. Components in low concentration can be detected. Also, components separated from a mixture can be collected separately and recovered unchanged. Further, since the SFC system employs a liquid metering system and since the components are detected in the liquid phase, the SFC system can be operated with equal facility as a liquid chromatograph.

Additional experimentation is necessary to obtain maximum efficiency from the SFC system. In order to determine optimum column conditions, uniform small particles, which are not commercially available, should be employed as absorbents and supports. In order to realize the maximum sensitivity of the detector, the maximum number of thermocouples that will perform as a unit should be determined.

REFERENCES

1. Hannay, J. B. and Hogarth, J., Proc. Roy. Soc. London, 30, 324 (1879).

2. Hannay, J. B. and Hogarth, J., ibid., 30, 170 (1880).

3. Rowlinson, J. S. and Richardson, M. J., Advan. Chem. Phys. 2, 85 (1959).

4. Booth, H. S. and Bidwell, R. M., Chem. Rev. 44, 477 (1949).

5. Klesper, E., Corwin, A. H., and Turner, D. A., J. Org. Chem. 27, 700 (1962).

6. Karayannis, N. M. and Corwin, A. H., Anal. Biochem. 26, 34 (1968).

7. Karayannis, N. M., Corwin, A. H., Baker, E. W., Klesper, E. and Walter, J. A., Anal. Chem. 40, 1736 (1968).

8. Sie, S. T., Van Beerum, W. and Rijnders, G. W. A., Separation Sci., 1, 459 (1966).

9. Sie, S. T. and Rijnders, G. W. A., ibid., 2, 755 (1967).

10. McLaren, L., Myers, M. N. and Giddings, J. C., Sci., 159, 197 (1968).

11. Giddings, J. C., Myers, M. N., McLaren, L. and Keller, R. A., ibid., 162, 67 (1968).

12. Fisher, M. E., J. Math. Phys. 5, 944 (1964).

13. Fisher, M. E., Rept. Progr. Phys. 30, 615, The Institute of Physics and the Physical Society, London, 1967.

14. Heller, P., Rept. Progr. Phys. 30, 731, The Institute of Physics and the Physical Society, London, 1967.

15. Kadanoff, L. P., Rev. Mod. Phys. 39, 395 (1967).

16. Mountain, R. D., Rev. Mod. Phys. 38, 205 (1966).

17. Sie, S. T. and Rijnders, G. W. A., Anal. Chim. Acta, 38, 31 (1967).

18. Myers, M. N. and Giddings, J. C., Anal. Chem. 37, 1453 (1965).

19. Sie, S. T., and Rijnders, G. W. A., Separation Sci. 2, 699 (1967).

20. Abel, E. W., Pollard, F. H., Uden, P. C. and Nickless, G., J. Chromatog. 22, 23 (1966).

21. Halasz, I. and Sebastian, I., Angew. Chem. Internat. Edit., 453, 8 (June 1969).

22. Hupe, K.-P. and Bayer, E., Fourth Int. Sym. on Advances in Gas Chromatog., p. 174 (1967), A. Zlatkis, ed.

23. Hupe, K.-P., and Bayer, E., J. Gas Chromatog. 5, 1 (1967).

24. Burtis, C. A., Gere, D., Gill, J. M. and MacDonald, F. R., Fifth Int. Sym. on Separation Methods: Chromatog. p. 5 (1969), E. Kovats, ed.