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

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## INTRODUCTION

A promising general method has been developed for the detection and continuous recording of component bands in chromatographic separations employing liquid eluents (paper, string or packed column chromatography). Since the method is based primarily upon colligative, rather than structural, properties, its applicability is not limited to specific classes of components or eluents.

## PRINCIPLES

The method utilizes measurements of the temperature differential 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 thermostatted chamber saturated with solvent vapor. The use of thermistors as the temperature-sensing elements permits use of a conventional Wheatstone bridge circuit, which can be balanced when both wicks are bathed with pure solvent. Since a small electrical current passes continuously through the thermistors in the bridge circuit, the temperatures sensed by the thermistors depend primarily upon competition between the following processes of heat input and heat removal.

### 1. Heat input:

- a. Electrical heating of the thermistor.
- 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.

The electrical heating and liquid flow are held essentially constant. When the column effluent liquid contains solute in a component band, the thermal conductivity of the eluting solvent is not significantly altered by the presence of solute. The vapor pressure of solvent, however, is lowered by the presence of solute, causing the ratio of the rate of vaporization to the rate of condensation to become lower on the sample

wick than on the reference wick; the sample thermistor thus seeks a new thermal balance point corresponding to a higher temperature. When the bridge unbalance is fed directly across the input terminals of a recording potentiometer, a peak is recorded for each component band eluted from the column. (If the component is more volatile than the solvent, the new balance point for the sample thermistor is at a lower temperature, rather than a higher temperature, through evaporative cooling.)

#### EXPERIMENTAL

The experimental arrangement of the detector is shown schematically in Fig. 1. The detectors,  $T_r$  and  $T_s$ , are glass-enclosed, 2000  $\Omega$ , bead-type thermistors (randomly selected, not matched) with a response of about 5% per degree Centigrade. The bridge circuit includes a 4000  $\Omega$  fixed resistor,  $R$ , in one arm and a decade resistance box in the other. The decade box possesses dials for thousands, hundreds, tens, and units ( $\Omega$ ). The bridge is operated at a 3 V potential furnished by dry cells.

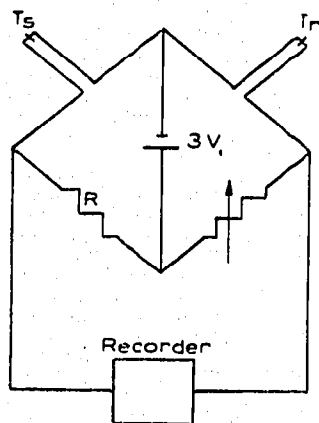


Fig. 1. Experimental arrangement of detector (schematic).

using a 1-mV full-scale recording potentiometer, it is possible to obtain a deflection of 12% of full scale for a 1  $\Omega$  change in the setting of the decade resistance box; this deflection corresponds to a temperature differential of about 0.01° between the two thermistors as compared to the balance condition. Thus changes of the order of 0.001° can be clearly seen, and even a differential of only a few ten-thousandths of a degree can be detected when the background signal is sufficiently stable.

Greater sensitivity could be obtained by use of a higher bridge voltage; a factor of three could be gained in this manner by operating at 9 V, but still higher voltages would shorten the detector life. The limiting factor at present seems to be thermostating, rather than detector sensitivity, although use of carefully matched thermistors would decrease the background signal due to bath temperature fluctuation. In the preliminary studies carried out to date, no attempt has yet been made to achieve the ultimate possible sensitivity.

The sample and reference chromatographic columns are water jacketed with force flow of jacket water from the constant temperature bath (the jacketing is essential only on the lower portion of the column, so that longer columns could be used). The columns are mounted directly above the detector, which is contained in a large glass test tube immersed in the thermostating bath. The effluent from the two columns flow into two separate small-diameter siphon tubes, which carry the liquid out of the thermostat into collecting vessels. Solvent in the sample column is forced in from a reservoir which maintains a constant head of gas pressure above the liquid. Sample is injected into the top of the column by means of a long hypodermic needle through a three-way stopcock which connects the solvent reservoir to the column and the outside. Many modifications of the columns, solvent feed, and sample injection are possible; the method which was used in this study has been in-

licated only to show the type of framework in which the detector has been utilized.

A minor modification of the experimental arrangement makes possible the recording of the derivative curves for the component peaks rather than the direct recording of the peaks themselves; this is achieved by placing both thermistors on the wick from the sample column, separated by some selected distance. When a non-volatile component first reaches the upper thermistor a deflection is obtained corresponding to its temperature rise; as the component band reaches the lower thermistor, the differential temperature recorded is a measure of the composition difference between the locations corresponding to the two thermistors. If the distance between thermistors is small relative to the width of the component band, the recorded differential temperature is nearly an instantaneous derivative of the band shape, showing a deflection in one direction as the beginning of the band reaches the upper thermistor, zero deflection as the peak passes a point halfway between the thermistors, and a deflection in the other direction as the tail of the band passes the lower thermistor. The potential advantage in the derivative method lies in its greater sensitivity in detecting poorly resolved component peaks; this sensitivity arises from the more detailed structure of a derivative curve as compared with the direct curve.

### RESULTS

The response of the detector has been investigated for a variety of solutes in three solvents: water, butanol saturated with water, and 95% ethanol. The detector has been found to respond to each solute tested. For solutes of higher volatility than the solvent, or capable of forming with the solvent, azeotropes more volatile than the solvent, the response indicates a lowering of temperature of the sample thermistor relative to the reference thermistor. For solutes of lower volatility than the solvent,

TABLE I  
RESPONSE OF DETECTOR TO VARIOUS SOLUTES

	<i>Boiling point, °C</i>	<i>Direction of deflection with water as solvent</i>	<i>Remarks</i>
Glycerol	290	+	Also + with butanol-water and 95% ethanol as solvents
Ethylene glycol	197.4	+	
Butanol-1	117	—	Forms azeotrope with water, b.p. 92.7°
Propanol-2	82.5	—	Also — with butanol-water as solvent
Ethanol	78.4	—	
Chloroform	61.2	—	
Acetone	56.5	—	
Benzene	80.1	—	
Acetic acid	118.1	+	
Sugar	non-volatile	+	
Food colors	non-volatile	+	
Ink	non-volatile	+	
Salts (NaCl, NaBr, NaI, KCl, KBr, LiCl, CuCl <sub>2</sub> )	non-volatile	+	

including non-volatile solutes, the response indicates an increase of temperature of the sample thermistor relative to the reference thermistor. Some of the results obtained are tabulated in Table I, in which a temperature increase of the sample thermistor is indicated by "+" and a temperature decrease by "-".

Examples of recorded chromatograms obtained with detector are shown in Figs. 2 and 3.

The actual magnitude of the response has been found to depend upon several factors, making comparisons of the response for different solutes relatively qualitative

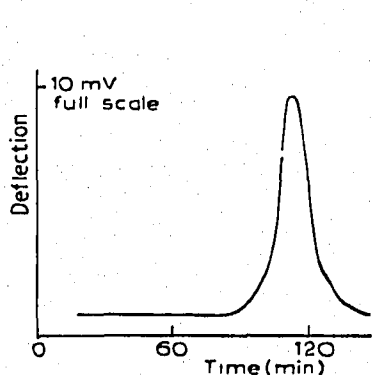


Fig. 2. 0.1 ml of 10% ethylene glycol.

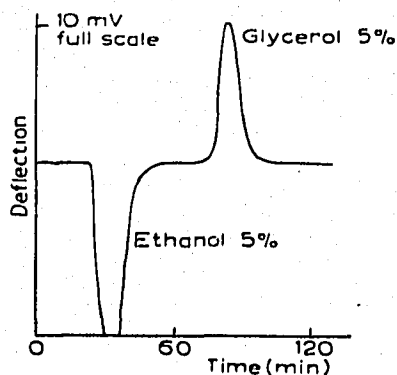


Fig. 3. 0.2 ml sample showing opposite deflection of volatile components.

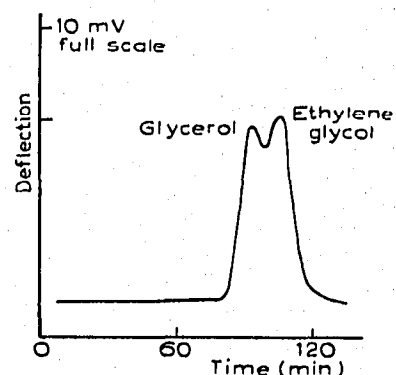


Fig. 4. 0.2 ml sample showing some resolution.

at the present time. The maximum response which has been observed thus far has been for a system employing 95% ethanol as eluent and with the detectors mounted on cotton string wicks. With this system, a sample of  $2 \mu\text{l}$  of ethylene glycol gave a peak height corresponding to  $50 \Omega$ , corresponding to a temperature differential of about  $0.5^\circ$ . Smaller responses result from the use of less volatile solvents and larger wicks.

The factors which have been found to be important in determining the response are:

- (1) the size of the sample,
- (2) the rate of flow through the column,
- (3) the size of the detector wick,
- (4) the nature of the solvent, and (in the case of volatile solutes) the nature of the solute.

Each of these factors will now be considered briefly.

### I. Sample size

The response of the detector has been found to vary directly with sample size for pure liquid samples and with sample concentration for samples introduced as solutions in the eluting solvent, with all other conditions held constant. A study of equally sized samples of a series of concentrations of aqueous solutions of sodium chloride demonstrated that both peak heights and peak areas varied directly with concentration.

### 2. *Flow rate*

The rate of flow of solvent through the detector wick is, unfortunately, a variable to which the quantitative response of the detector is extremely sensitive. Since the concentration of solute bands tends to be decreased at higher flow rates, and the solute spends less time in contact with the thermistor, peak heights and peak areas are both decreased by increasing flow rate. For comparison of results of different runs, close control of this variable is essential; better comparisons are obtained for different components in a single run.

### 3. *Size of wick*

The effect of this variable is closely dependent upon the flow rate through the column. The wick should be large enough that it does not "flood" in handling the flow rate used; drops of free liquid flowing over the detector do not permit sufficient equilibration of the liquid and vapor phases at the thermistor. On the other hand, if the wick is too large dilution of the sample bands may occur, decreasing the detector response. Both cotton string and cotton plug wicks have been used in this work.

### 4. *Nature of the solute and solvent*

While extensive quantitative data have not yet been obtained, it appears that the response to non-volatile solutes is essentially a colligative property, independent of the nature of the solute. This behavior is to be anticipated on the basis of the postulated mechanism for the detector response. The cooling of the sample thermistor observed for volatile solutes of higher volatility than (or forming more volatile azeotropes with) the solvent is in agreement with an explanation based upon relative vapor pressure lowering as the colligative property, modified by kinetic effects due to the non-equilibrium nature of the system.

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## SUMMARY

A method for the detection and recording of component bands has been described. It was found to be sufficiently general to insure its applicability to a wide variety of solvent-solute systems, and since it senses the column effluent it is independent of the type of chromatographic column used. It appears to be readily adaptable to the quantitative estimation of non-volatile solute bands for which it is based on essentially a colligative property, and it appears applicable, with specific calibration, to volatile solute bands as well.

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