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A DETECTOR SYSTEM FOR USE IN LIQUID COLUMN CHROMATOGRAPHY

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SUMMARY

A liquid chromatography detector based on heat of adsorption is described together with its ancillary electronic equipment. The system may be added to any column and used to detect sub-microgram amounts without the need for thermostatic control except under extremely adverse laboratory conditions.

INTRODUCTION

Liquid chromatography as an analytical method has not been applied as widely as gas chromatography mainly because it has not possessed detectors comparable as regards sensitivity, stability and general applicability with those available to the latter technique.

The detector described here uses heat of adsorption as the basis of its operation and is, therefore, not novel, but detailed attention to design has produced a system in which both sensitivity and stability are of a very high order. The applicability of a heat of adsorption detector to a particular analytical problem depends on whether the compounds to be detected are adsorbed from the eluant stream by the detector packing, and, if they are adsorbed, whether their heat of adsorption differs from that of the eluant. Since in general not only the eluant but also the detector packing may be selected it is often possible to choose a favourable combination which will give good sensitivity for detection of components in a particular mixture.

PRINCIPLE OF DETECTION

In the simplest form of this detector the eluant stream from a chromatographic column is passed through two chambers in series, the first being packed with small glass beads, the second with silica gel; each of the chambers contains a thermistor of equal resistance and these thermistors are combined with two resistors of equal value to give a balanced electrical bridge. A component appearing in the eluant stream and being adsorbed by the silica gel in the second chamber causes an exo-

thermic reaction and a temperature rise of the thermistor in this chamber resulting in a corresponding drop in resistance value of this arm of the bridge and the appearance of an out-of-balance voltage at the bridge output. This voltage may be applied directly to a suitable potentiometric recorder or, if it is too small, it may be fed to an amplifier prior to the recorder, resulting in either case in a record of the eluted compound. In practice when attempting to use this type of detection system at microgram levels a number of factors must be considered.

DESIGN FOR LOW LEVEL DETECTION

It has been calculated[1] that adsorption of a $1 \mu g$ sample by a silica gel packed detector will give rise to a temperature difference in the range 10^{-3} to 10^{-4} °C between the first and second chambers. Thus spurious temperature differentials between the two chambers must be considerably less than 10^{-4} °C if microgram levels of detection are to be attempted using a two thermistor detector.

Differentials may occur as a result of temperature variations of eluant or as a result of external thermal influences on the body of the detector; when both causes are operating these differentials show on the record as periodic variations superimposed on a steady drift of the signal; even with extensive thermal insulation of the eluant reservoir, column and detector, several hours elapse before equilibrium is established sufficiently to allow detection of microgram amounts of solute. For a detector that can be used with normal chromatograph columns in the open laboratory, a compensating device is required to off-set the effects of thermal changes caused by such external factors. This effect was achieved by using four accurately-matched thermistors sited in three chambers as shown schematically in Fig. 1. With this
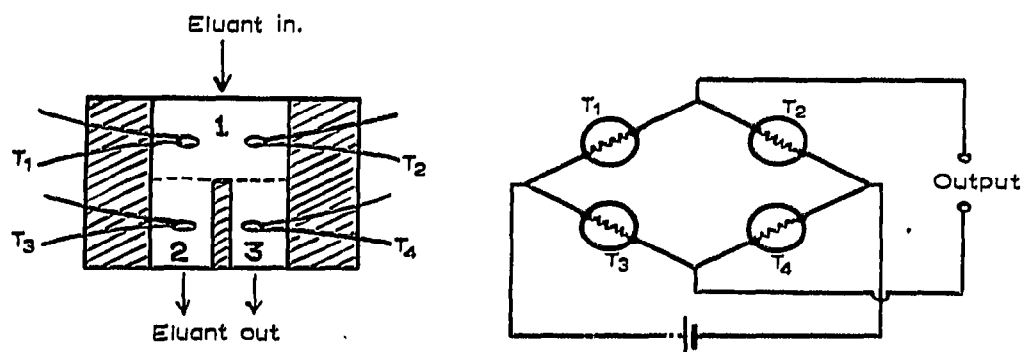

Fig. 1. Arrangement of matched thermistors in detector to give temperature compensation.

arrangement either a vertical or horizontal temperature gradient leaves the balance of the bridge unaltered since in both cases two thermistors in the same arm of the bridge will be equally affected. Adsorption of a compound by silica gel in chamber 2 will, however, result in a resistance drop in thermistor T3 and a corresponding output from the bridge.

In common with all detector systems noise limits the sensitivity of the thermal detector.

In a representative thermistor bridge driven from a 1 V source a temperature rise of 10^{-4} °C at one thermistor will result in a bridge output of 10^{-6} V. Experience

has shown that the minimum signal-to-noise ratio permissible in practice is 2:1 so that if the passage of $1 \mu g$ of sample is to be detected the maximum noise which can be tolerated is 5×10^{-7} V.

Noise from the thermistor bridge is a combination of intrinsic thermistor noise (Shot, Flicker and Thermal effects) and noise consequent on the dissipation of energy in the bridge. Intrinsic noise is purely a function of absolute temperature and since this is fixed within relatively narrow limits by chromatographic considerations, there is little scope for reduction of intrinsic noise.

This, however, is not the case when considering bridge dissipation. Heating of the thermistors by the bridge current will produce convection currents in the eluant surrounding them, thus causing fluctuations in the rate at which energy is dissipated. In consequence there will be random variations in the temperature and resistance of each thermistor resulting in a noise output from the bridge. This noise output may be reduced by lowering the bridge supply voltage so that the thermistors dissipate less energy. Such a reduction will reduce the signal output also but whereas signal will decrease linearly with voltage, energy dissipation will decrease as its square, so that a voltage may be found which gives an optimum signal-to-noise ratio.

In the present detector a dissipation of approximately $30 \mu W$ per thermistor was found to be satisfactory, corresponding to a bridge supply of 0.5 V. As noted previously a noise level of 5×10^{-7} V should not be exceeded at the bridge output (amplifier input) if detection at the microgram level is to be made but a solid state D.C. amplifier with noise level less than 5×10^{-6} V at the input does not appear to be available commercially. Valve amplifiers can improve on this by a factor of 10 but are, in their D.C. form, very subject to drift which would render them unsuitable for our purpose. This disadvantage may be countered, however, by supplying the thermistor bridge with an A.C. voltage and using an A.C. valve amplifier. An additional point in favour of A.C. amplification is that, if necessary, the circuit may be tuned to amplify preferentially at the signal frequency, thus enabling an effective increase in signal-to-noise ratio to be made. This has not been utilised in the present apparatus since a further increase in sensitivity would only be found useful if the thermal stability of the detector could be increased even over that provided by the four thermistor design.

It seems possible, however, that a combination of tuned amplification with a temperature controlled eluant reservoir, column and four-thermistor detector, could be used to extend sensitivity by at least a further factor of 10.

CONSTRUCTION DETAILS

Detector

The exploded diagram and the complete assembly (Fig. 2) are self-explanatory in conjunction with the following notes. Unshaded parts are constructed in Fluon (PTFE), cross-hatched parts in stainless steel, dotted parts in aluminium and the eight connecting studs (to which are soldered the thermistor leads and terminations of the miniature five core shielded cable) are brass 12 B.A. bolts secured through the flanged periphery of the central Fluon disc. Three 6 B.A. bolts pass through the end plates and the three central Fluon discs (in which are drilled the chambers housing
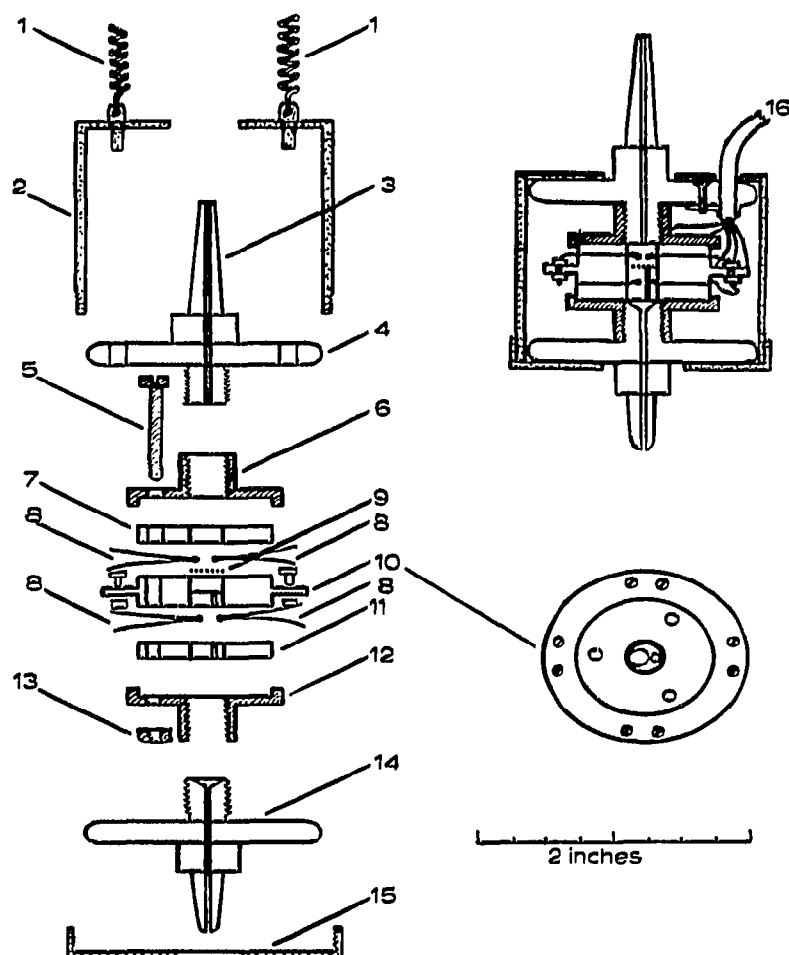
Fig. 2. Diagram of the detector. 1 = Retainer spring. 2 = Aluminium case. 3 = B.5 taper cone. 4 = Fluon end flange. 5 = 6 B.A. bolt. 6 = Stainless steel end plate. 7 = Upper Fluon disc. 8 = Bead thermistor (Fenwal Type No. GB32J1). 9 = Disc of stainless steel mesh. 10 = Flanged central Fluon disc. 11 = Lower Fluon disc. 12 = Stainless steel end plate. 13 = 6 B.A. nut. 14 = Fluon end flange. 15 = Aluminium case screw cap. 16 = Miniature 5 core screened cable.

the thermistor beads) and when tightened compress the discs sufficiently to prevent eluant leaks along the thermistor leads or between the disc interfaces.

The threaded portion of each Fluon end flange is approximately 0.005 in. longer than the corresponding threaded section of the stainless steel end plate so that when screwed fully home a liquid-tight joint is made with the face of the Fluon disc. The two studs forming spring anchorages on the upper face of the polished aluminium case extend downwards to pass through clearance holes in the upper Fluon end flange. This arrangement enables the aluminium case to be rotated by hand when fitting the B.5 cone to the corresponding socket at the base of the chromatograph column without affecting the liquid-tight joints in the assembled detector.

Chambers No. 1 and 3 are packed with fine glass beads, chamber No. 2 is packed with silica gel and a ¼ in. diameter circle of stainless steel gauze fitting tightly into the base of No. 1 prevents any intermingling between the contents of the three chambers. Thin plugs of silica wool are used to close the top of chamber No. 1 and the bottoms of chambers No. 2 and 3. The diameter of chamber No. 3 is kept small

in comparison with that of No. 2 so that the greater part of the eluant passes through the silica gel packed chamber. The four thermistors each have a nominal resistance at 25 °C of 2000 Ω but are matched to each other within 0.5%.
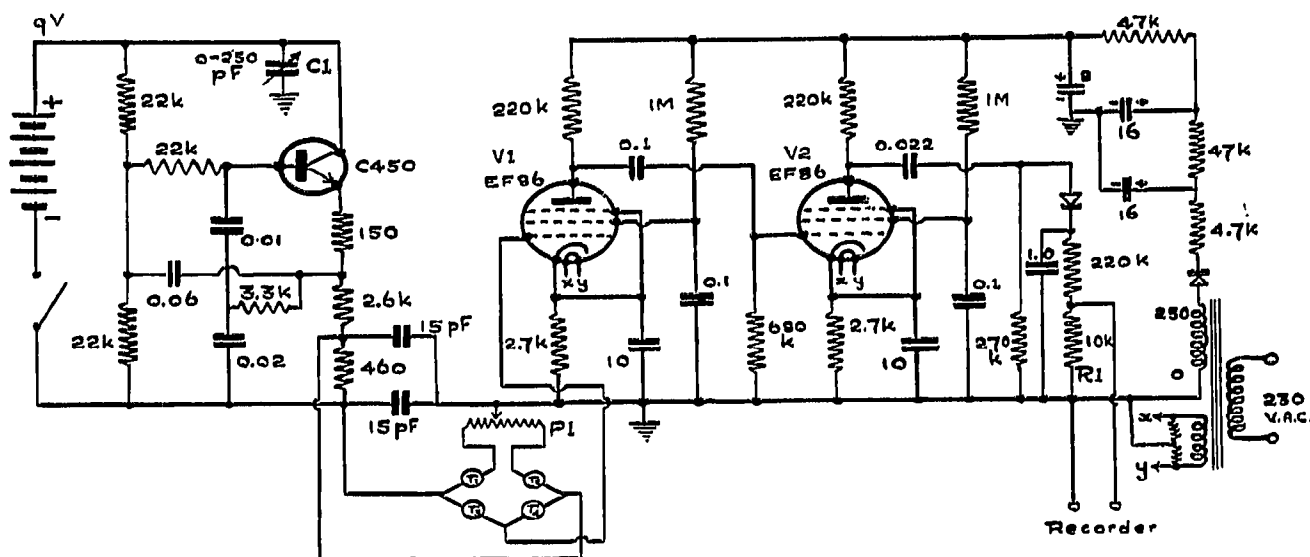

Fig. 3. Circuit diagram of the oscillator and amplifier.

Oscillator and amplifier

A twin-T resistance/capacitance oscillator (Fig. 3) gives an output of 4 V at a frequency of 1 kHz and a potential divider reduces this to 0.5 V before it is applied to the thermistor bridge. As the oscillator is floating (not directly grounded to the amplifier chassis) and feeding a high-gain amplifier it must be well shielded. This is adequately accomplished by assembling the complete oscillator on a 2 in. square of "Veroboard" and mounting this inside the amplifier chassis at a point adjacent to both the amplifier input and the point of entry of the shielded 5 core cable from the detector.

Stray unbalancing capacities between oscillator and ground, and between detector leads and ground, occur but these may be permanently balanced out by a 0–250 pF capacitor, the adjustment of which will be detailed later.

The amplifier (Fig. 3) uses two EF86 low-noise tetrodes operating under conditions designed to give a total gain of 2.7×10^4. Rectification of the 1 kHz signal is by a germanium diode and a condenser/resistor combination giving a time constant of about 0.2 sec which helps to damp response to any mains-propagated disturbances without interfering with adsorption signals. In practice it was found convenient to use a multi-range recorder providing F.S.D. over a range of 1 mV–20 V but if it is desired to use a recorder without this facility, R1 (Fig. 3) may be replaced by a suitable chain of resistors combined with a multi-way switch to provide a suitable range of outputs. The complete circuit shown in Fig. 3 may be comfortably accommodated in an instrument case measuring $9 \times 5 \times 4$ in.

SETTING UP AND OPER

The B.5 cone detector inlet is inserted in the corresponding socket at the end of the column and the retention springs secured over hooks on the column.

After establishing a steady eluant flow rate through the column and detector a few minutes should be allowed for equilibrium to be established before clipping the input lead from a cathode ray oscilloscope to the anode of V2 (Fig. 3).

The C.R.O. time base and sensitivity controls should be set to give a convenient size of picture of the 1 kHz output from the amplifier and adjustment of both P1 and C1 (Fig. 3) made until the trace is reduced to a straight line. Complete balance of the bridge is ensured by repeated adjustment of P1 and C1 as the C.R.O. sensitivity is steadily increased. Once balancing is completed there is no further need to adjust C1 and for this reason it should preferably be mounted in a normally inaccessible position within the instrument case.

The control P1 (Fig. 3), a Beckman precision 10 turn Helipot, is now set so as to give about half-scale deflection on the recorder and the detector is ready for use.
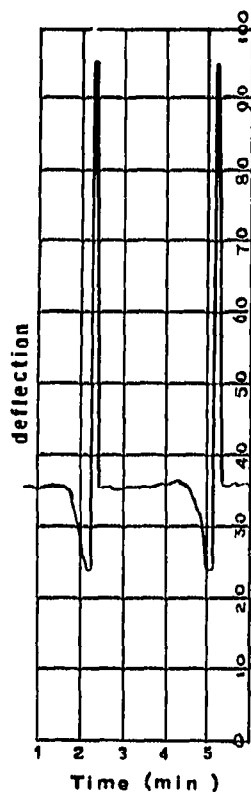
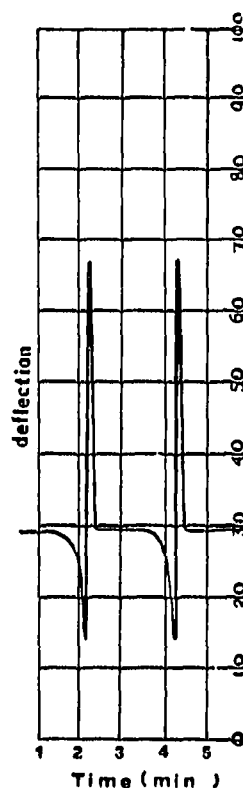Fig. 4. Duplicate injections of 0.5 µl of 1% n-butanol in toluene.

Fig. 5. Duplicate injections of 0.5 µl of n-butanol.

PERFORMANCE

Sensitivity tests have been carried out using a 0.2 cm bore glass column packed with glass beads, eluant being A.R. toluene flowing at a rate of 1 ml/min and samples being injected by Hamilton micro-syringe. Figs. 4 and 5 show duplicate runs obtained

by injecting 0.5 μl of 1% *n*-butanol in toluene (approx. 4 μg *n*-butanol) and 0.5 μl of neat *n*-butanol respectively, the latter results being obtained at 100 times reduced sensitivity. From Fig. 4 it would appear that for this system the detection limit is of the order of 0.1 μg, but it will be realised that detection limits will inevitably vary widely with different eluants and samples.

CONCLUSIONS

The system which has been described provides an inexpensive and robust means of detecting μg-quantities of chemical compounds which are being eluted from liquid chromatograph columns, with potential applicability to a wide range of chemical problems.

REFERENCE

1 K.-P. HUPE AND E. BAYER, *J. Gas Chromatog.*, 5 (1967) 197.