THE QUANTITATIVE ESTIMATION OF SUBSTANCES ON PAPER CHROMATOGRAMS

III. ELECTRONIC SYSTEMS FOR THE PHOTOMETRIC SCANNING OF PAPER CHROMATOGRAMS

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INTRODUCTION

In the two preceding papers of this series, two principal pieces of equipment required for the accurate quantitative estimation of substances on paper chromatograms have been described[1,2]. The first section of the apparatus treats paper chromatograms automatically with chemical reagents; the second part scans the resulting strips photometrically for light-absorbing or fluorescent peaks.

In this paper we shall describe in more detail the latest electronic systems which have been devised to obtain the best performance from the second part of the apparatus. These include a number of items which may be of value to workers who wish to construct similar apparatus of their own, but any of them can be used in isolation or in different combinations from that described below. The apparatus to be described includes the following items:

(1) A solid-state SWEET circuit[3,4] providing outputs of the order of 10 to 50 mV from a standard photomultiplier (RCA 1P21) with typical chromatographic zones,

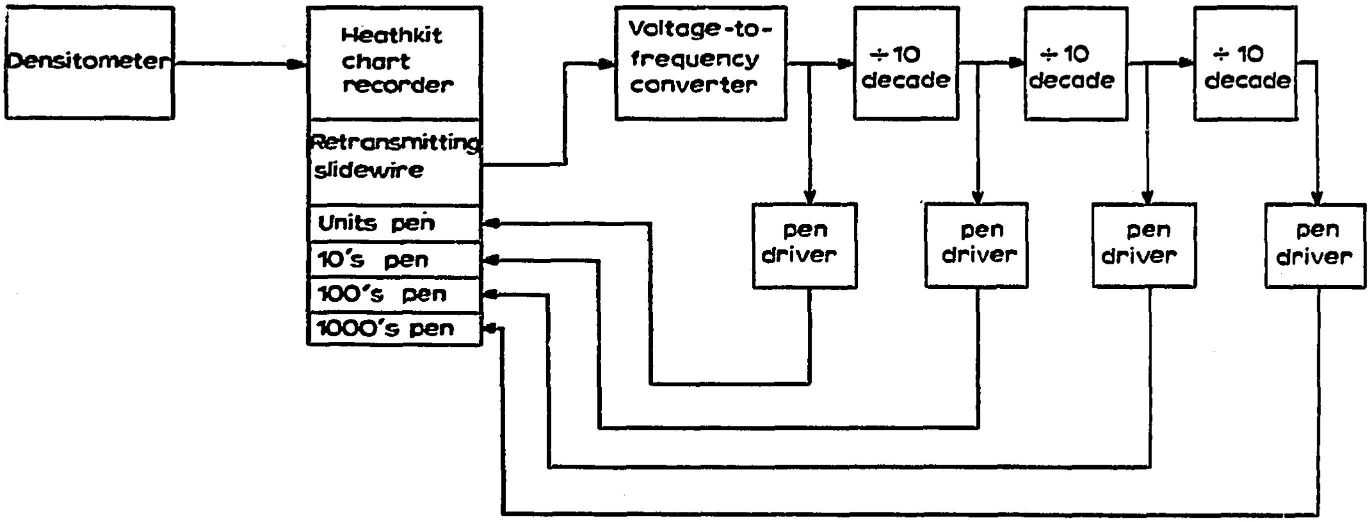


Fig. 1. Block diagram of scanning system with chart-recorder and 4-channel integral recorder.

and suitable for driving a variety of commercially available potentiometric chart recorders.

(2) Retransmitting slidewire attachments to two commercially available potentiometric recorders providing a 0 to —10 V output suitable for driving two different sorts of digital integrators.

(3) A voltage-to-frequency converter with a chain of decade counters providing, via Schmidt-type triggers, a digital output suitable for driving a 4-pen marker unit.

(4) A 4-pen marker unit providing a 4-decimal digit integral record on the chart alongside the scanning record which greatly facilitates the calculation of the areas of peaks on the chromatogram.

(5) A stabilized light source using a tungsten filament lamp which is suitable for the visual range of wavelengths.

The overall system is shown in block form in Fig. 1 for a configuration providing a 4-decimal integral record alongside the chart record of the photometric signal.

EXPERIMENTAL

Solid-state densitometer

The main circuit is identical with the silicon-transistor version of SWEET's circuit[3] described by GORDY, HASENPUSCH AND SIEBER[4]. The output section was modified to provide a continuously variable sensitivity, with a maximum response of approximately 8 mV per increment of 0.1 optical density unit; and also a continuously variable backing-off voltage to bring the background signal to zero (*i.e.* the optical density level of filter papers: approximately 2.8[2]).

Fig. 2 shows Fig. 5 from GORDY *et al.*[4] with the modifications described above. As shown by SWEET[3] the voltage response ΔV of this type of photometer is given by the equation

$$\Delta V = K \ln(I_0/I)^n \qquad (1)$$

where n is close to 1.0 for many types of photomultiplier. If n can be brought exactly to 1.0, a linear relationship between the voltage response and changes in optical density will be obtained. In practice, a photomultiplier design giving the best approximation to a linear form of eqn. 1 (*i.e.*, $n = 1.0$) is selected (*e.g.*, RCA 931-A or 1P 21) and the residual non-linearity is corrected by using a biassed silicon-diode chain in the final output of the instrument. Fig. 3 shows the relationship obtained with this circuit at first trial and the final linear relationship after adjustment of the setting of potentiometers R28 through R31 of Fig. 2. These adjustments were made by trial and error using a standardized neutral wedge filter and the stabilized tungsten light sources described below to obtain optical densities over the range 0.0–3.0.

Amplified output via retransmitting slidewires

A standard retransmitting slidewire was fitted to a Heathkit EUW-20 A potentiometric chart recorder. It consisted of a 3-turn 1000 Ω potentiometer (Heathkit, Type 11–48) connected coaxially to the spindle of the main servo-potentiometer of the recorder itself. This potentiometer is the same as used for the servo-potentiometer itself. This potentiometer was fed from a stabilized power supply of 10 V, thus

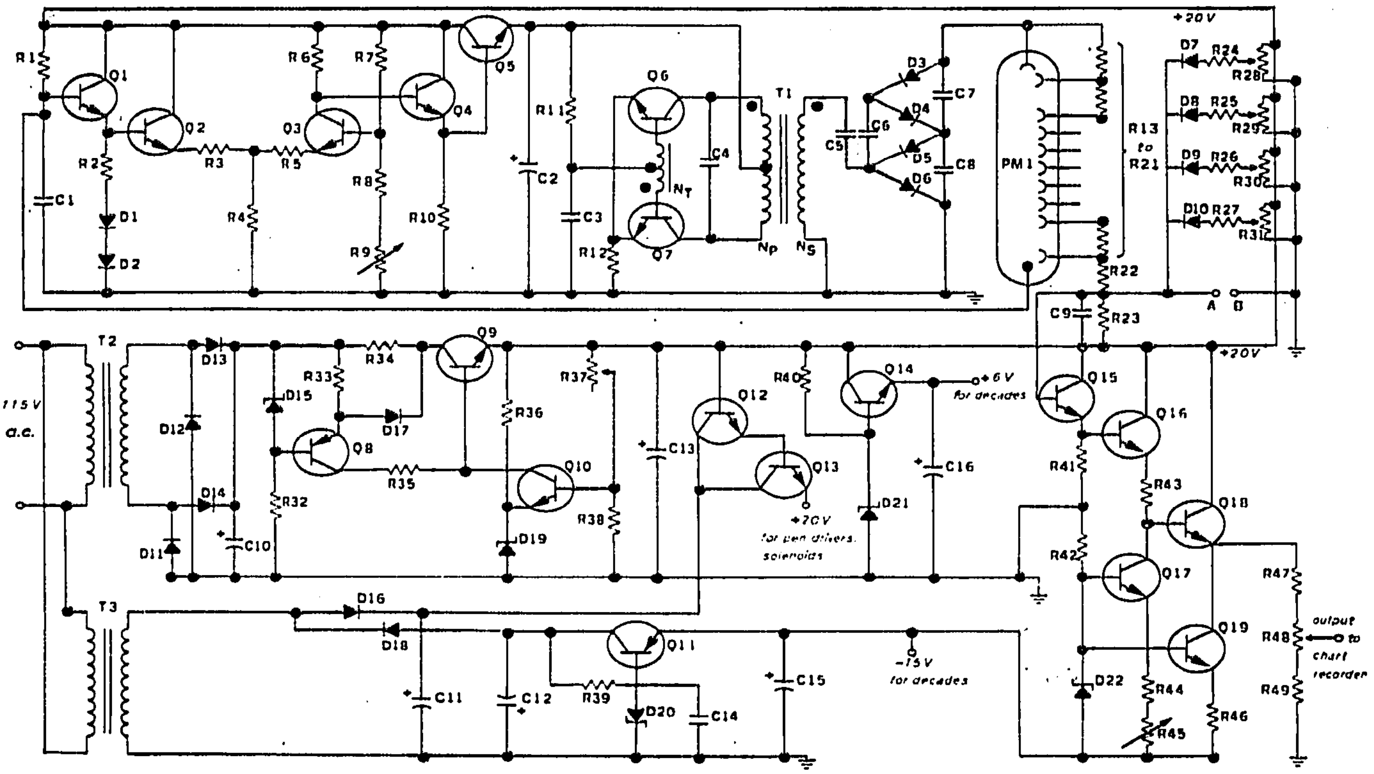


Fig. 2. Circuit diagram of SWEET-type logarithmic photometer after GORDY *et al.*[4], modified to provide continuously variable gain, and backing-off voltage. The upper block is directly taken from Fig. 5 of GORDY *et al.*[4]. The right-hand section of the lower block (Q15-Q19, etc.) is the modification providing a larger and continuously variable gain. The rest of the lower block is the power supply for the densitometer and other parts of the system. Components: C1 = 0.01 μF, mylar; C2 = 5.0 μF @ 50 V d.c.; C3, C9 = 0.1 μF; C4 = 0.00068 μF; C5, C6, C7, C8 = 0.01 μF @ 1 kV; C10 = 2000 μF @ 50 V d.c.; C11 = 4000 μF @ 50 V d.c.; C12 = 500 μF @ 50 V d.c.; C13 = 100 μF @ 35 V d.c.; C14 = 0.01 μF; C15 = 20 μF @ 35 V d.c.; C16 = 10 μF @ 50 V d.c.; D1, D2, D11, D12, D13, D14, D16, D17, D18 = 1N 4002; D3, D4, D5, D6, D7, D8, D9, D10 = 1N 4006; D15, D19, D21, D22 = 1N 753; D20 = 1N 4745; R1 = 20 MΩ 1/2 W carbon film; R2 = 100 kΩ; R3, R5, R35, R38 = 1 kΩ; R4, R32, R42 = 4.7 kΩ; R6 = 200 kΩ; R7 = 15 kΩ; R8 = 470 Ω; R9 = 500 Ω pot.; R10 = 100 kΩ; R11, R33, R36 = 2.2 kΩ; R12 = 33 Ω; R13 through R22 = all 475 kΩ 1/2 W carbon film; R23 = 60 kΩ 1/2 W carbon film; R24 = 180 kΩ; R25 = 120 kΩ; R26 = 82 kΩ; R27 = 56 kΩ; R28, R29, R30, R31 = 10 kΩ 20-turn pot.; R34 = 33 Ω 2 W; R37 = 2.5 kΩ pot.; R39 = 1 kΩ 1 W; R40 = 1.5 kΩ; R41 = 200 kΩ; R43 = 5.6 kΩ; R44 = 3.3 kΩ; R45 = 10 kΩ pot.; R46, R47 = 5.6 kΩ; R48 = 100 Ω 10-turn knobpot.; R49 = 22 Ω. T1 = Ferroxcube type. N_T = 4-turn center tapped No. 26 nyclad wire; N_P = 20-turn center tapped No. 26 nyclad wire; N_S = 200-turn No. 32 nyclad wire; T2, T3 = 115 V a.c. primary, 24 V a.c. @ lamp secondary; Q1, Q4, Q15, Q16, Q18 = 2N 3390; Q2, Q3, Q6, Q7, Q17, Q19 = TI 416; Q5, Q10, Q12, Q14 = 2N 3053; Q8 = 2N 3638A; Q9 = 2N 3054; Q11 = 2N 1540; Q13 = 2N 3055. All resistors are 0.5 W, 5%, unless otherwise stated.

providing an output varying between 0 and —10 V over the full-scale deflection (f.s.d.) of the chart recorder. This could be used to drive a variety of analogue-to-digital (A-to-D) conversion and integrating devices.

A slightly different form was used with an Esterline Angus Lab. Graph Series 601-S potentiometric recorder. This is a very fast instrument, and was fitted with a linear 500 Ω retransmitting slidewire by the manufacturer. The low power-rating of this slidewire required the use of an operational amplifier (Type Nexus SQ-10A) to provide the output voltage (Fig. 4).

The only disadvantage of this method of amplifying the photometric signal for

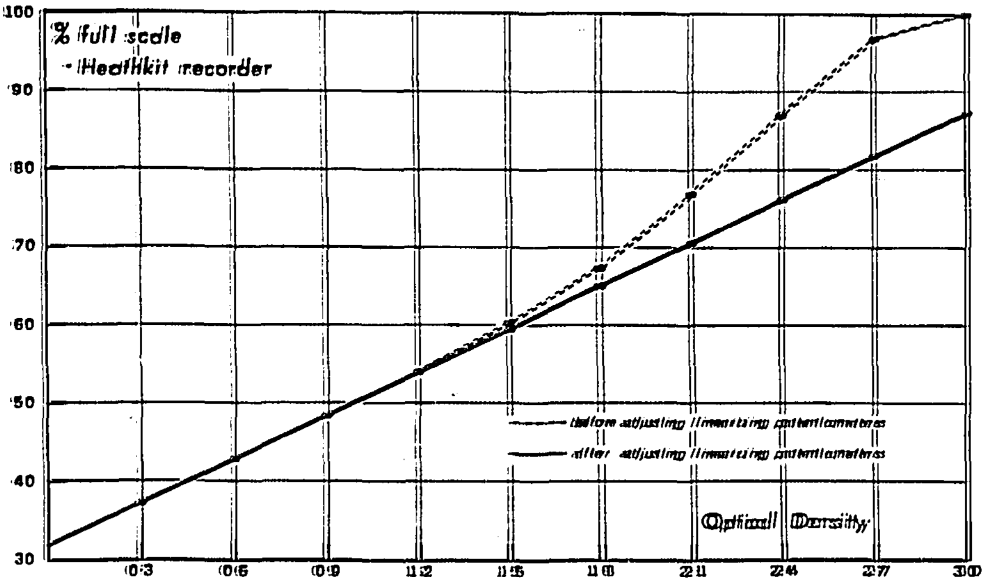


Fig. 3. The relationship between illumination of the photomultiplier and the output of the densitometer circuit before and after trimming the linearizing potentiometers of Fig. 2 (R24–R31). Illumination was varied by a rotary variable density neutral filter between a stabilized tungsten lamp and the photomultiplier.

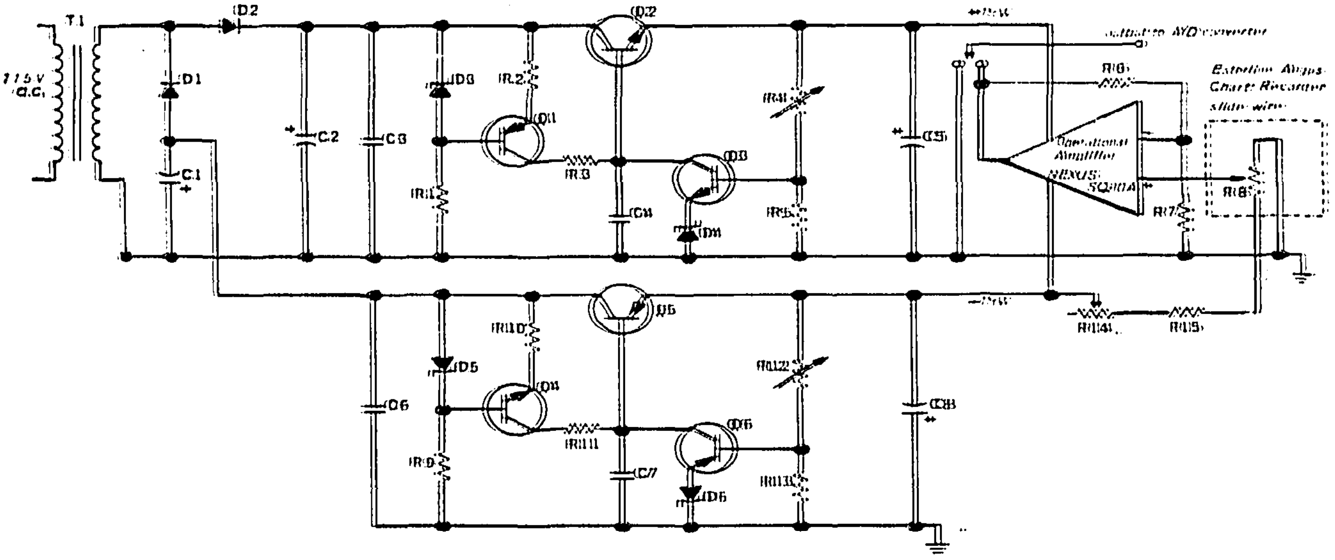


Fig. 4. Circuit providing amplified output for voltage frequency conversion via a retransmitting slidewire on the chart recorder. The system shown is that for a 500 Ω, low-wattage slidewire on the Esterline Angus recorder. The 1000 Ω, 5 W slidewire on the Heathkit recorder was capable of providing sufficient current without the use of an operational amplifier, the output being taken directly from the −15 V line via a potential divider. Components: C1, C2 = 250 μF @ 50 V electrolytic; C3, C4, C6, C7 = 0.02 μF ceramic; C5, C8 = 20 μF @ 50 V electrolytic; D1, D2 = 1N 4002; D3, D4, D5, D6 = 1N 753; R1, R9 = 4.7 kΩ; R2, R10 = 2.2 kΩ; R3, R5, R7, R11, R13 = 1 kΩ; R4, R12, R14 = 2.5 kΩ potentiometers; R6 = 20 kΩ; R8 = 500 Ω Esterline Angus recorder slidewire; R15 = 15 kΩ; T1 = 115 V a.c. primary, 24 V a.c. @ 1 A secondary. All resistors are 0.5 W, 5%, unless otherwise stated.

purposes of integration or A-to-D conversion is that it is completely dependent upon the characteristics of performance of the recorder. It is, however, a very cheap, stable and robust method which we have found to be extremely reliable in practice. It has the advantage of providing complete electrical separation of the circuits used in the photometer and those used in integrating or data-processing devices. The disadvantage was negligible with the high-speed Esterline Angus recorder, which has an f.s.d. time of $\leqslant$ 0.2 sec. The output of both systems gave a voltage swing of 10 V for full-scale deflection which is suitable for driving a wide variety of analogue-to-digital converters.

Voltage-to-frequency converter

This unit is designed to convert the amplified voltage from the retransmitting slidewire of the chart recorder to a continuous train of pulses whose frequency is instantaneously and directly proportional to the level of the photometric signal. The pulses are then recorded on the chart alongside the scanning record of the optical density along the strip chromatogram, thus providing a cumulative, digital integral record from which areas under peaks can rapidly be calculated by counting[2].

The circuit is based on the voltage-to-frequency converter described by HOWARD[5] and is shown in Figs. 5 and 6. It is important to note that the unijunction transistor oscillator gives a linear response to input *current* and *not* to voltage; the circuit is in effect a voltage-to-current-to-frequency converter. HOWARD's circuit is effectively linear in response over the range 3×10^3 to 13×10^3 c.p.s. and needed modifying in order to provide a linear output over a range beginning near 0 c.p.s. The device we adopted was to run the main oscillator over the range of frequencies in which linearity is obtained and connect it in parallel with a "subtracting" oscillator via a coincidence gate and inverter to the output (Figs. 5, 6). The gating oscillator was run at a frequency of 300 c.p.s., thus providing an eventual output pulse frequency range of 0–500 c.p.s. This produced slight random irregularities at very low frequencies (0 to 5 c.p.s. output) but they were of no consequence in practical use.

The output of this device consisted of pulses of —20 V and 10 μsec duration. These were fed to four-pen drivers via three decade-dividers. Standard commercially available units (Nuclear Chicago, Type 826992) were used as decade dividers and the circuit for the pen drivers is given in Fig. 7. The latter gave pulses of 24 V $\times$ 20 m/sec to the pen solenoids.

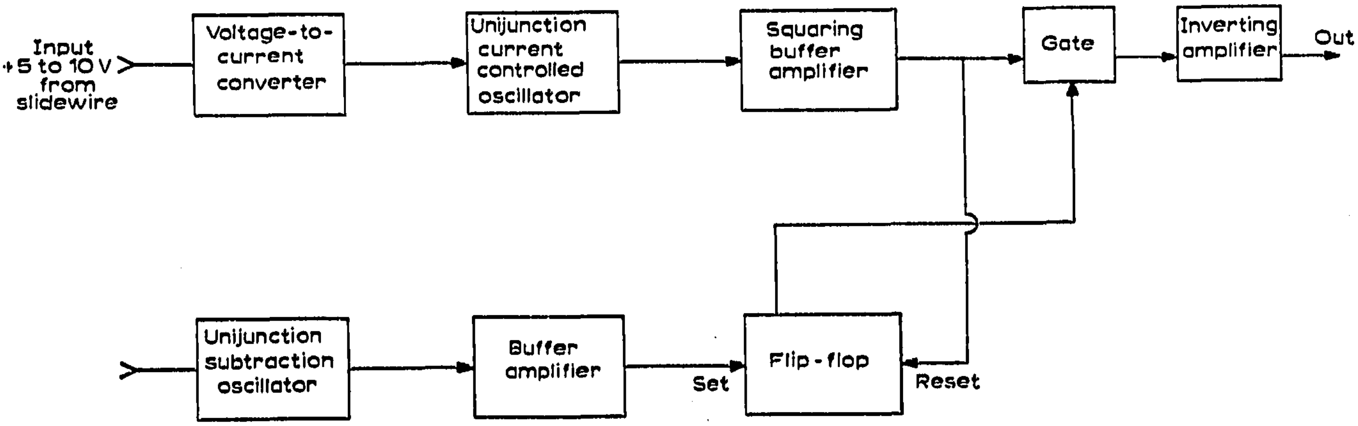


Fig. 5. Block diagram of voltage-to-frequency converter for continuous integration via the 4-channel pen unit.

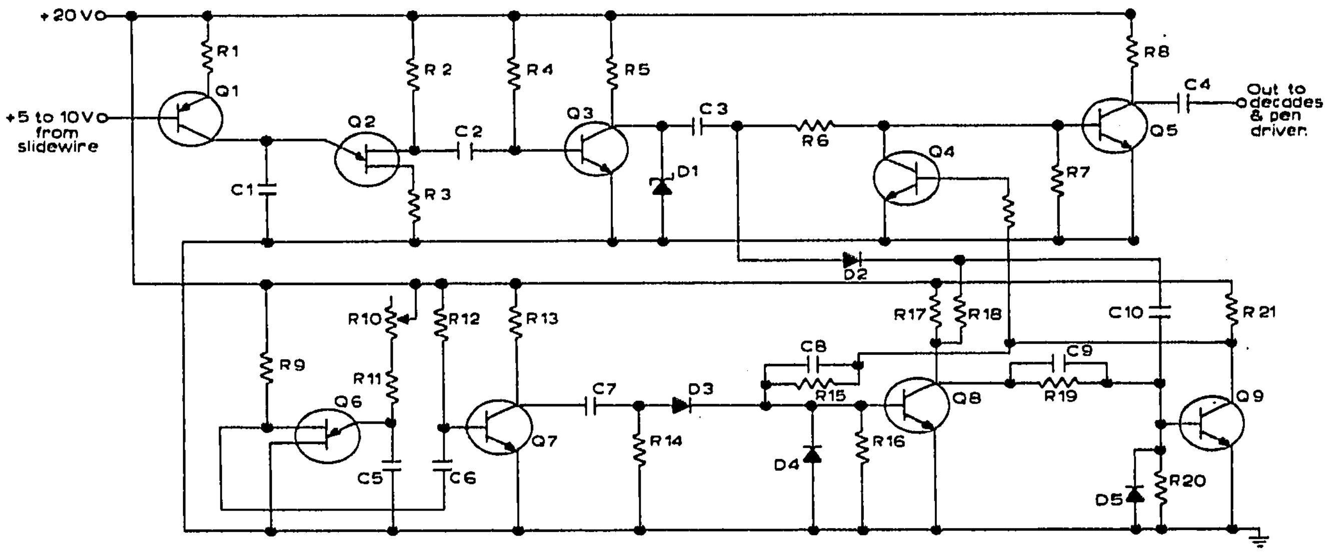


Fig. 6. Circuit diagram of voltage-to-frequency converter. Q1 is a voltage-to-current conversion stage; Q2 the unijunction oscillator; Q3 the buffer amplifier. Q4–Q9 function as a subtraction-inversion gate with variable subtraction to obtain appropriate firing-rates for the 4-pen integral recording unit. Components: C1 = 0.1 μF 200 V mylar; C2, C3, C4, C6, C8, C9, C10 = 0.001 μF; C5 = 0.47 μF 200 V mylar; C7 = 0.0005 μF; D1 = 1N 753; D2, D3, D4, D5 = 1N 459; R1, R4, R7, R14, R16, R20 = 20 kΩ; R2, R9 = 1 kΩ; R3 = 51 Ω; R5, R8, R13 = 3.3 kΩ; R6, R11, R18 = 12 kΩ; R10 = 20 kΩ 20-turn potentiometer; R12 = 33 kΩ; R15, R19 = 10 kΩ; R17, R21 = 2.2 kΩ; Q1 = 2N 3638A; Q2, Q6 = 2N 1671B; Q3, Q4, Q5, Q7, Q8, Q9 = 2N 3053. All resistors are 0.5 W, 5 %, unless otherwise stated.

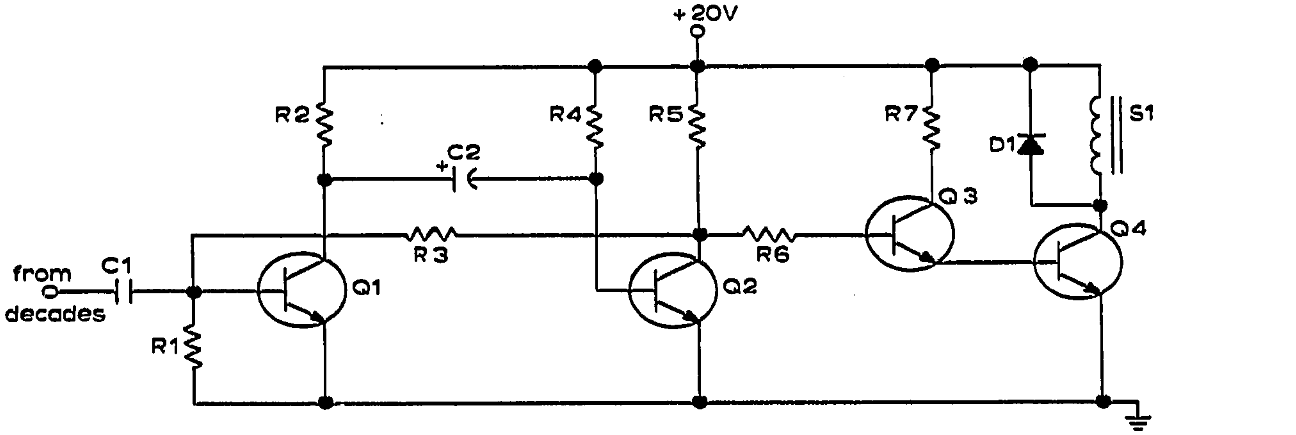


Fig. 7. Circuit diagram of pen drivers. S1 is the solenoid of a modified Potter-Brumfield relay used to actuate each pen (see text and Fig. 10). Components: C1 = 0.001 μF 600 V; C2 = 1.0 μF 50 V electrolytic; D1 = 1N 4002; R1 = 2.2 kΩ; R2, R5 = 2.2 kΩ; R3 = 20 kΩ; R4 = 56 kΩ; R6 = 910 Ω; R7 = 330 Ω, 2 W, 5 %; S1 = modified Potter-Brumfield relay (see text); Q1, Q2, Q3 = all 2N 3053; Q4 = 2N 3054. All resistors are 0.5 W, 5 %, unless otherwise stated.

The performance of this system was tested in three ways corresponding to the three main sections contributing potentially independent errors to the system as a whole. First, the small pulses of the voltage-to-frequency converter were counted electronically using a Baird Atomic counter (modular design) and a variable stabilized voltage supply as input. Plots of output frequency *versus* voltage at 10–20 points over the range 0.05 to 10 V were linear to within ± 0.1 %. Next, the linearity of the retransmitting slidewire was checked by connecting it to the input voltage-to-frequency converter and obtaining output counts at different settings of the recorder pen. These were obtained by manually positioning the pen with zero input to the Heathkit EUW-20A recorder. Plots of frequency against chart-setting of the pen over the range 30.0 to 95.0 on the chart were obtained. The results were analyzed by linear regression using a FORTRAN program on a PDP-8 computer (Digital Equipment Corporation, Maynard, Mass., U.S.A.) and are summarized in Table I and in Fig. 8a.

Finally, the linearity of the whole system was checked by connecting the pens of the four-pen integrator unit (*vide infra*) to the outputs of the drivers. The chart was run with zero input to the recorder and the pen set manually to various chart settings as above. The calibration curve thus obtained was linear to within ± 0.1 % or less and is shown in Figs. 8b and 8c.

TABLE I

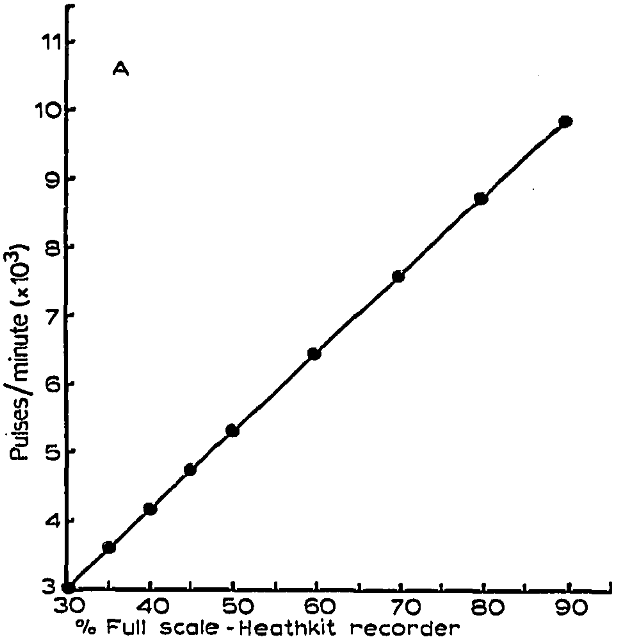
STATISTICAL ANALYSIS BY LINEAR REGRESSION OF THE PERFORMANCE OF THE VOLTAGE-TO-FREQUENCY CONVERTER

Voltage setting (x) (*Heathkit scale*)	*Pulses/sec* (y)	*Linear regression analysis*
30.0	6822.0	$\Sigma(x - \bar{x})^2 = 5687.50$
35.0	8052.0	$\Sigma(y - \bar{y})^2 = 3.5564 \times 10^8$
40.0	9299.0	$\Sigma(x - \bar{x})(y - \bar{y}) = 1.4223 \times 10^6$
45.0	10546.0	
50.0	11798.0	for $y = bx + c$
55.0	13049.0	
60.0	14307.0	Sum of squares of residuals = 768.00
65.0	15567.0	$b = 250.07 \pm 0.10608$
70.0	16805.0	$c = 697.56 \pm 6.9662$
75.0	18070.0	
80.0	19307.0	Correlation coefficient = 0.999999
85.0	20574.0	
90.0	21775.0	
95.0	23073.0	

95 % confidence limits as percent of estimate of y:
at x = 30.0 ± 0.570 %
at x = 600 ± 0.255 %
at x = 90.0 ± 0.174 %

Four-pen integral marker

The unit is shown in Figs. 9 and 10. Four solenoid relays (Potter Brumfield, Type KRP-11D, rated at 6 V, 1.5 W) were taken apart and the solenoid unit modified as shown in Fig. 10. The armatures were fitted with steel wire driving pins by soldering



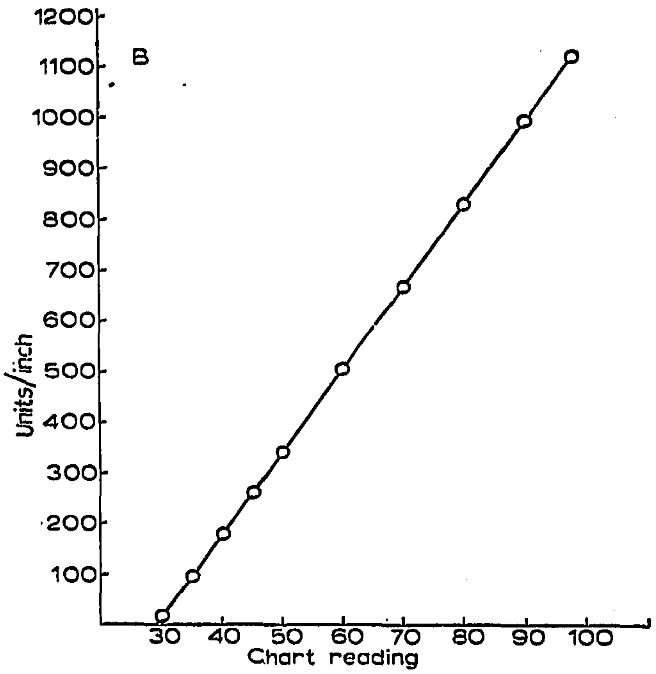


Fig. 8a. Frequency–voltage relationship of the voltage-to-frequency converter and retransmitting slidewire on the Heathkit recorder. The pen of the recorder was set manually to the position shown on the abscissa with the recorder itself switched off. Pulses were counted on a Baird Atomic Modular Scaler.

Fig. 8b. Frequency–voltage relationship of the complete recording system. The pen of the Heathkit recorder was set to various positions of the scale by varying the zero control with zero input, and the chart run for 3–5 in. (7.5–12.5 cm) at each setting. The number of pulses recorded over the middle 2–4 in. (5.0–10.0 cm) of each section of the record was counted, using a magnifying lens when necessary.

appropriately shaped pieces of wire (gauge 18*) to them. A slight rotary movement in the case of the lower unit (No. 2) was taken up by soldering a small length of steel tubing (hypodermic syringe, gauge 14*) to the armature to act as a bearing for the steel wire driving pin. Four levers of stainless steel (gauge 20*) mounted on needle bearings transmitted the movement to the lightly mounted pens which were made from pieces of hypodermic syringe needles (gauge 26*) mounted on steel shim strips (0.005 in. thick, approximately 0.13 mm) rotating on needle bearings.

The pens were connected via fine polyethylene tubing (I.D. approximately 0.5 mm) to four separately adjustable polyethylene ink-wells. When not in use, the whole unit was tilted back on its bearing-attachment to the cross-rod of the Heathkit recorder to drain the pens by siphoning into the wells. The pens were filled for use by light pressure on the ink-wells while closing their open tops with one finger.

This system was found to be remarkably reliable in routine use. Clear records with little or no blocking or splashing were obtained and the pens never blocked if they were drained while not in use. It was considerably less messy than the use of open pens or of non-adjustable ink-wells. To make room for the 4-pen unit and its record on the lower part of the chart, the zero-setting of the recorder pen was set to 30 % f.s.d. as base-line.

* Gauges are American Standard wire gauges.

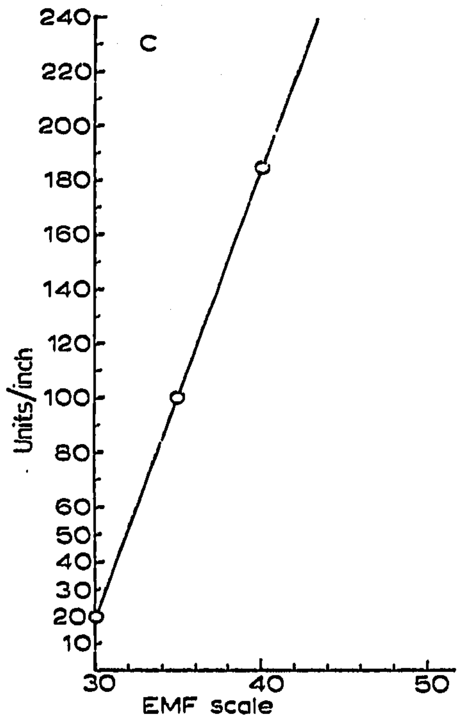


Fig. 8c. Expanded section of Fig. 8b, which is used to obtain an accurate estimate of the background rate of firing for subtraction from peak areas during normal use of the apparatus. The backing-off voltage is usually adjusted to set the background reading from the chromatogram in the range 35–45 on the chart scale (see Fig. 11).

The solenoids were capable of firing discretely at up to 20 to 25 pulses/sec; at higher frequencies the response is fused, usually with the solenoid in an intermediate or closed position. At the chart speeds we use (approximately 1 cm/sec), this is of no consequence since the record cannot be read at densities of greater than approximately 15 to 20 strokes/cm, and the count is taken from the next higher decade whenever one decade pen has seized through over-driving. This defect of the pen-response is in fact advantageous since the integral record is clarified by the attention of the observer being automatically directed to those parts of the record which are most easily and accurately counted. A record illustrating these points is shown in Fig. 11. The calculation of peak areas from such records has been described previously in detail[2].

Power supplies and stabilized light sources

Stabilized power supplies are crucial to the trouble-free operation of photometric chromatogram scanners of the type considered here. Since the present scanner[2] is not a null or differential instrument, it is important to avoid drift in both the supply to the light source and to the recording and integrating system.

The main power supply circuits are shown in Figs. 2 and 12. These supplies have been found to be extremely stable in routine use. About 30 min "warming-up" is required for optimal performance of the Heathkit recorder (and most of the others we have used), but the lamp and other elements require no observable warm-up time for fully satisfactory performance. If the recorder is left on with a blank strip of paper stationary in the scanner and minimally damped so that an oscillation of approxi-

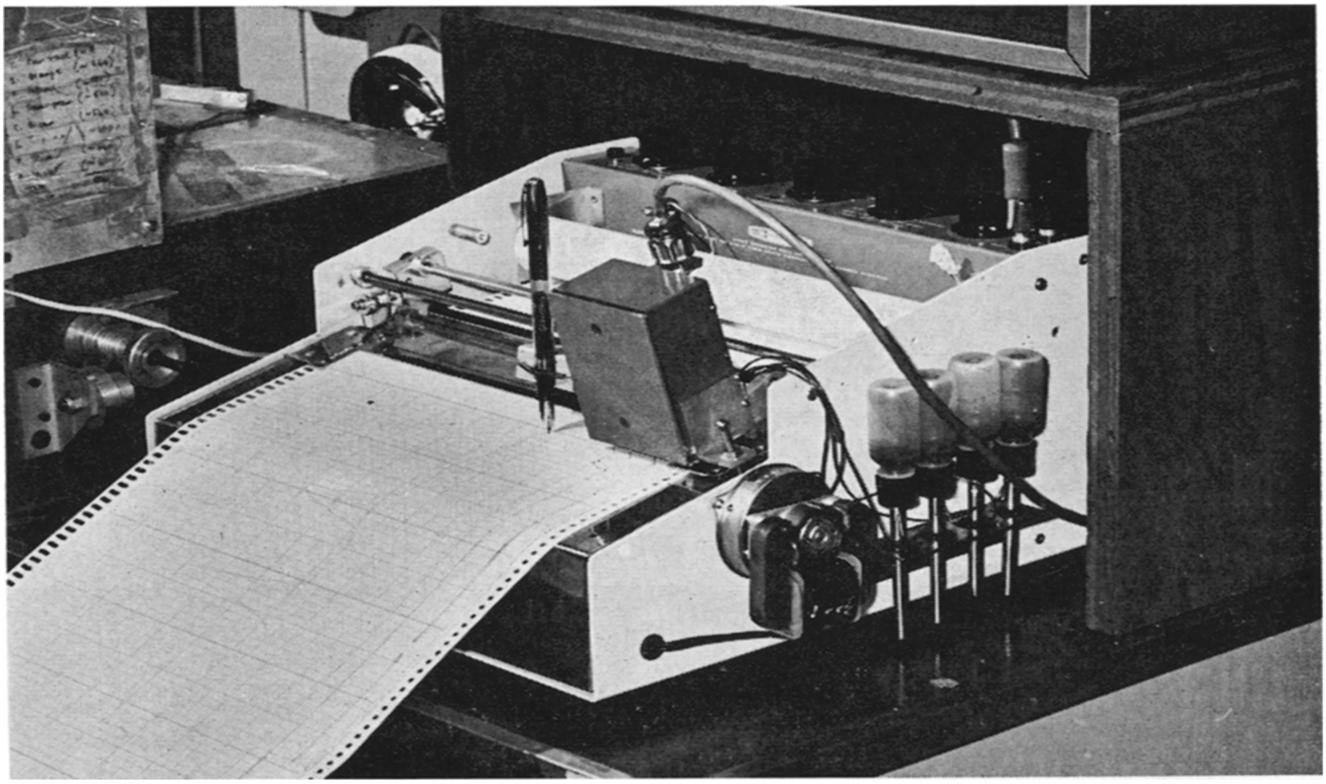


Fig. 9a.

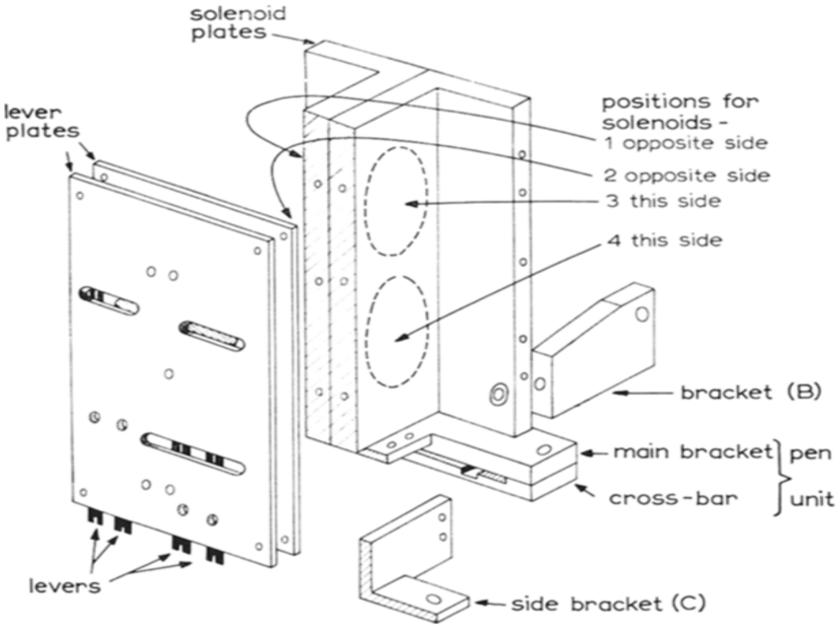


Fig. 9b.

Fig. 9. Four-pen event marker for 4-digit integral record.

(a) General view of unit attached to main cross-bar of Heathkit recorder. The zero of the recorder is set to 30% full-scale deflection to make room for the integral record. At the right are four variable-level ink-wells supplying the pens by polyethylene tubes.

(b) Exploded diagram demonstrating the construction of the unit. The levers actuating the pens are of steel, the rest is of aluminium. The unit rides on the cross-bar of the Heathkit recorder by the holes in the bracket(s), B. The level of the pens of the unit is controlled by a screw through the hole in the side-bracket, C (see also Fig. 9a). The main parts are screwed together through the holes shown.

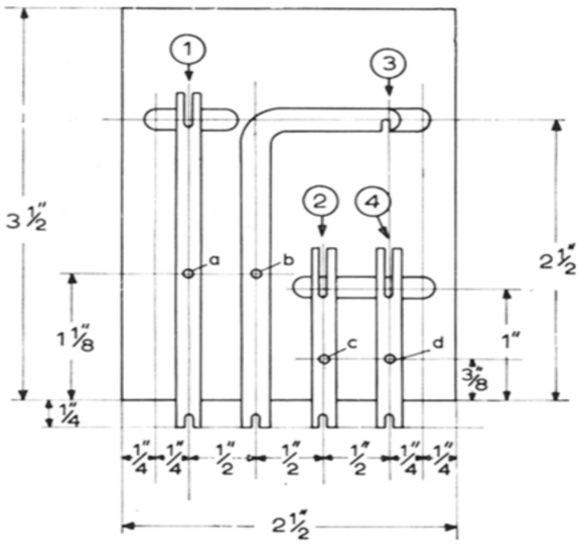


Fig. 9c.

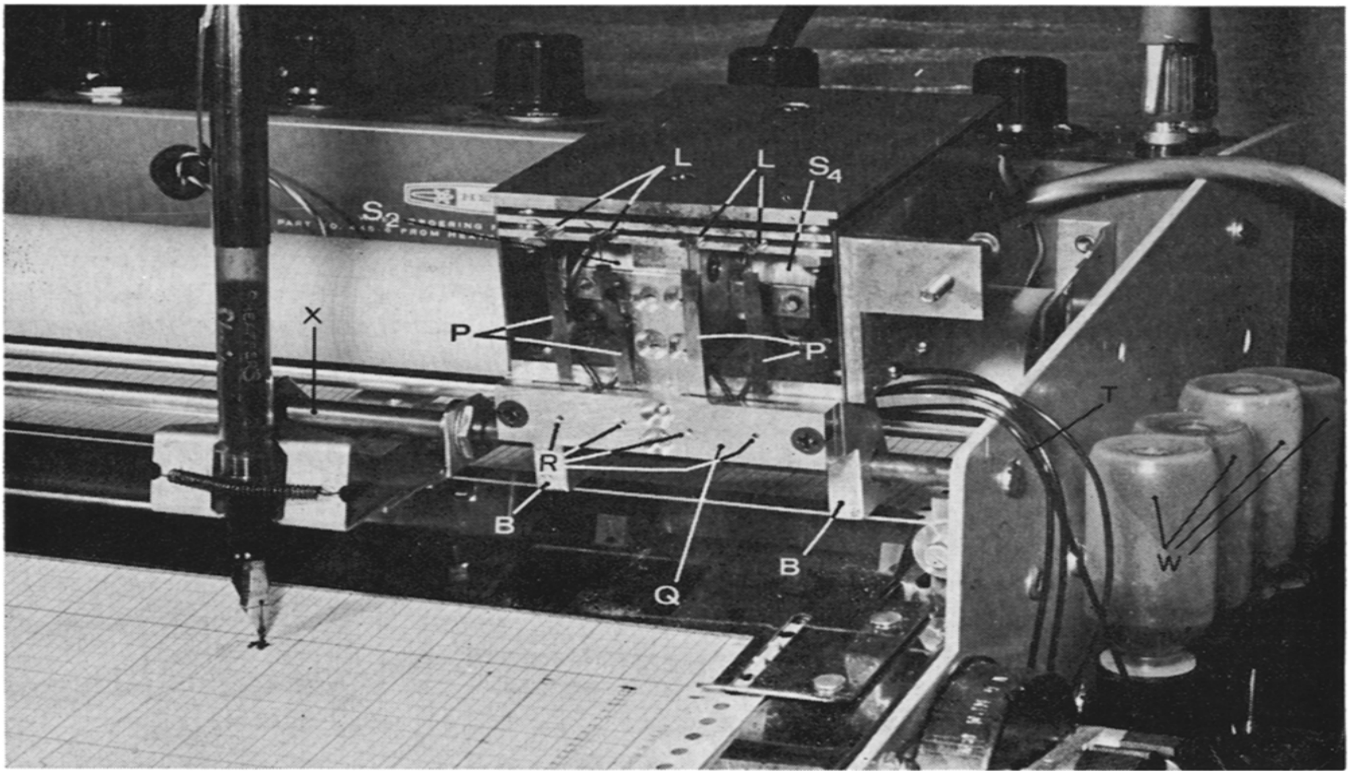


Fig. 9d.

(c) Detail of the levers and the mounting plate. The diagram is drawn from the front with the front plate removed. The levers rotate on five steel pins a, b, c, d, and are actuated by driving pins attached to the armatures of the solenoids 1, 2, 3, 4. The driving pins from the solenoids 1, 3 and 4 are attached directly to the respective solenoid armature. The driving pin from solenoid no. 2 (See Fig. 10, D2) is longer and rotates in a tube soldered to the solenoid armature. The pens (26-gauge hypodermic needle tubing) fit into the notches at the two lower ends of the levers by a light spring action (see Fig. 9d). Dimensions are given in inches and fractions (1 inch = 1" = 2.54 cm).

(d) The unit shown tilted up off the chart to demonstrate the brackets (B) securing it to the main cross-bar (X) of the recorder and the pens (P). These are of thin strip steel and move on pins in the bearings (R) in the cross-beam (Q) of the unit. Solenoids 2 and 4 are seen (S2, S4) and the emerging (driving) ends of the levers (L) in which the pens are engaged. (T), tubing from the ink-wells (W) to the pens.

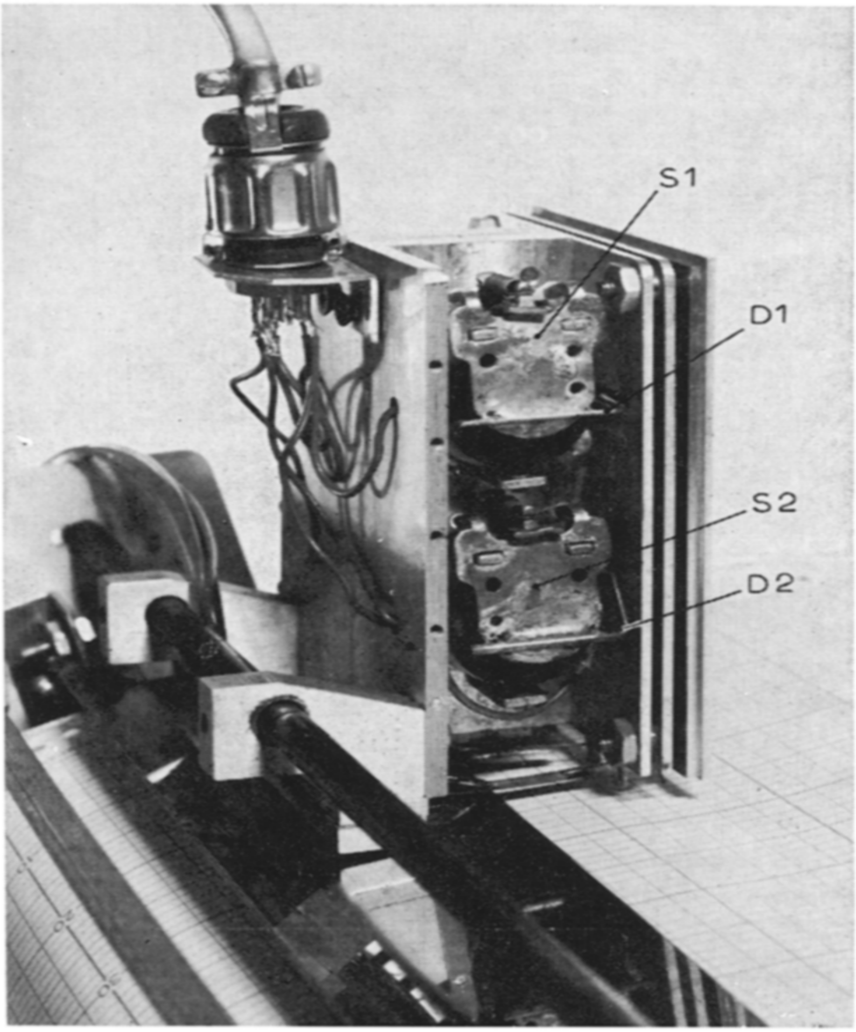


Fig. 10. Four-pen marker for four-digit integral record. This shows the detail of solenoids 1 and 2 (S1, S2) and the arrangement of the driving pins (D1, D2) attached to the armatures.

mately 0.5 % f.s.d. is obtained, drift is less than 1 % of f.s.d. for periods of an hour or more under ordinary laboratory working conditions.

When using xenon arcs with the scanner, the standard unstabilized sources and circuits recommended by the manufacturers have been used. Performance has been satisfactory when a diffuse image has been used to illuminate the inlet slit of the scanner's monochromator[2] but not as good as had been obtained in the visual range using stabilized tungsten sources. Good results were obtained with a special current-stabilizing source for limited periods of time after which failure of one or more components occurred. A new stabilizer is presently being tested and will be described in full in a subsequent paper if its performance continues to be satisfactory.

DISCUSSION

The electronic apparatus described above is the end result of the use and trial of many similar systems over the last eight years, in conjunction with the scanner described in an earlier paper[2]. While it will be superseded shortly by equipment

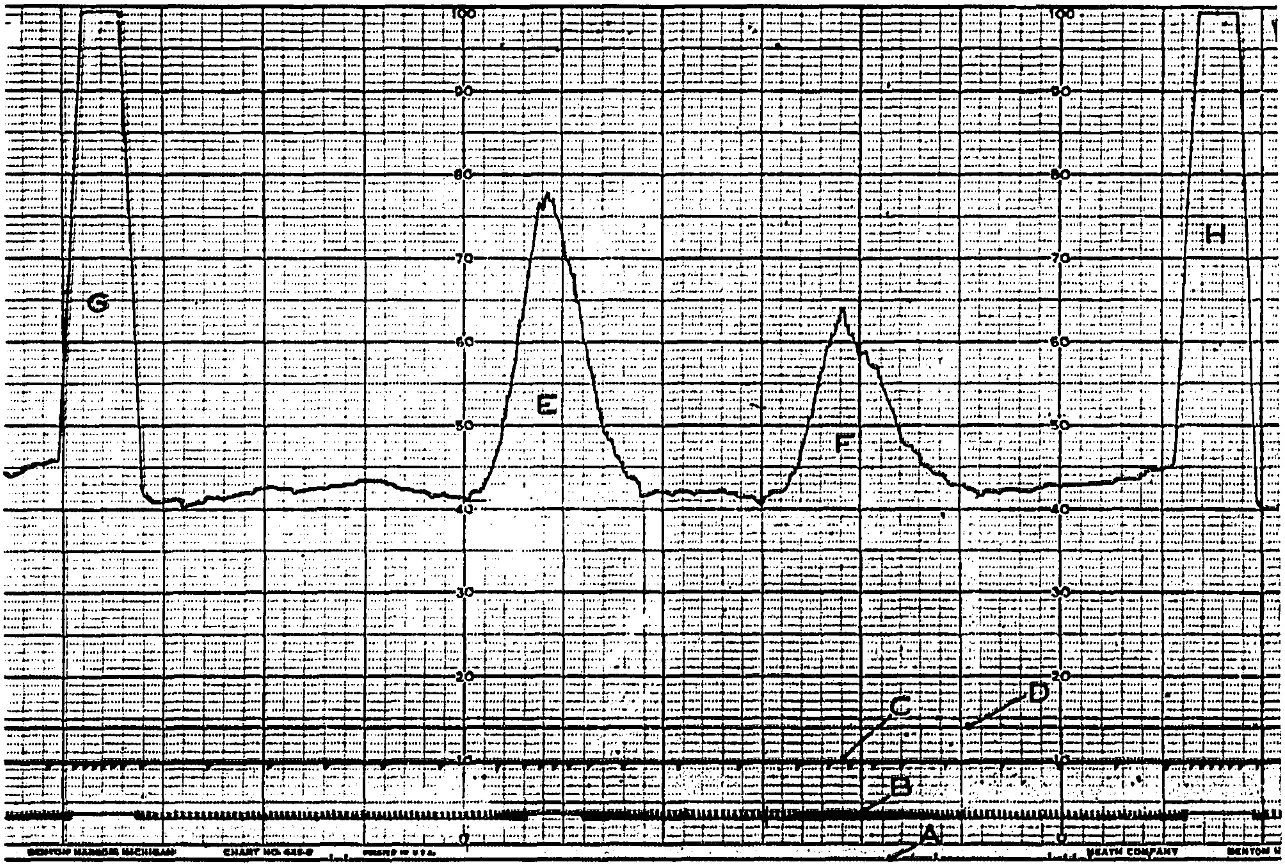


Fig. 11. Typical chart record provided by the apparatus. The 1's pen (A) is seized as the background has been set rather high and an occasional artefact is seen. Above come the traces of the 10's (B), 100's (C) and 1000's (D) pens. E and F are peaks on the chromatogram. G and H are peaks given by opaque adhesive tape (this was one of a train of sections of chromatograms linked by tape for continuous scanning of a large batch).

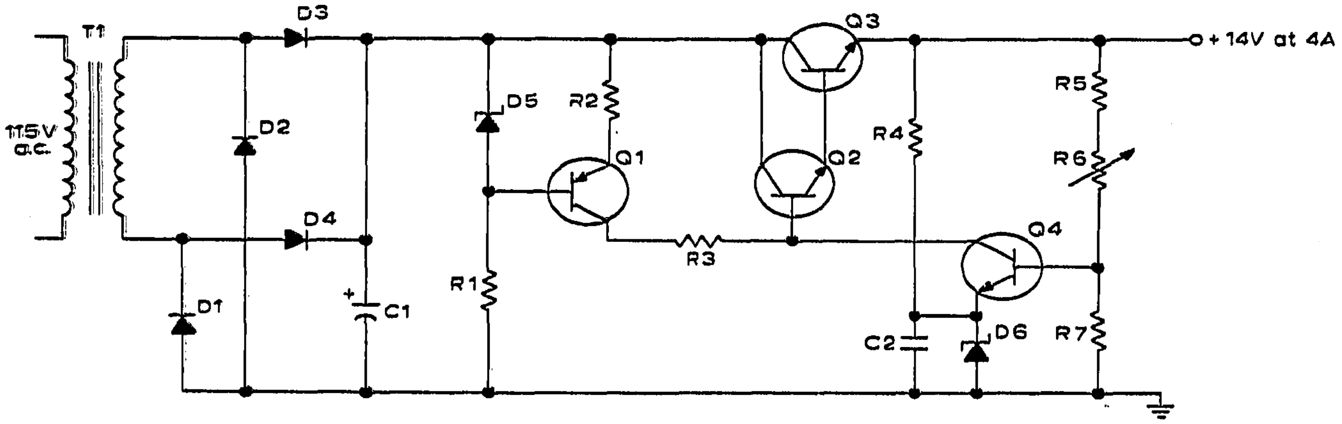


Fig. 12. Circuit diagram of stabilized d.c. power supply for a tungsten light source. A standard 14 V automobile headlamp was used. Components: T1 = Triad 4-48 U, 18 V @ 6 A; D1, D2, D3, D4 = MR 1121; D5, D6 = 1N 753; Q1 = 2N 3638A; Q2 = 2N 3054; Q3 = 2N 3055; Q4 = 2N 3053; R1 = 3.3 kΩ; R2 = 2.2 kΩ; R3 = 1 kΩ; R4 = 5.6 kΩ; R5 = 470 Ω; R6 = 2.5 kΩ potentiometer; R7 = 1.2 kΩ; C1 = 5500 mμF @ 50 V d.c., electrolytic; C2 = 0.01 μF @ 600 V. All resistors are 0.5 W, 5%, unless otherwise stated.

designed for use with a new and improved scanner, it represents a stable, reliable, and accurate set of equipment for use with scanners of the relatively simple type described so far[2].

While there is little novelty in most parts of the equipment described here, its use of solid-state circuitry and its proven reliability in routine laboratory use suggested that its description and publication in detail might be of use to other workers wishing to embark on methods of this sort.

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SUMMARY

The design, construction, and testing of electronic circuits useful for chromatogram scanners is described. These include a stabilized tungsten lamp source, sources and amplifiers for retransmitting slidewires to provide outputs for analog-to-digital conversion equipment, a voltage-to-frequency converter driving a 4-pen integral recorder, and a solid-state logarithmic densitometer circuit of the SWEET type.

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