A PHOTOELECTRICALLY-PROGRAMMED ELECTROLYTIC GRADIENT GENERATOR*

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Since the introduction of gradient-elution chromatography by Derreux[1] many devices for the generation of gradients have been described[2–11]. Most of these use a mixing chamber containing a solution representing one gradient limit fed from a reservoir containing a solution representing the other limit. Specific gradient curves may be obtained either by using specially-shaped vessels or multiple mixing chambers[12].

During experiments on the chromatography of proteins on DEAE cellulose filter paper (Whatman D.E. 20) it became evident that no existing device could create reproducible gradients within very small volumes (1–2 ml) and at low accurately controlled flow rates (1–2 ml/h). The possibility of using a photoelectrically controlled gradient generator using electrolytic gas production to pump the buffers was therefore investigated.

The application of this device to paper chromatography has already been described[7] and this paper gives details of the final design which can produce flow rates of up to 40 ml/h and has provision for the programmed selection of buffer vessels.

General description

The gradient generator consists of 4 units.

(1) The programmer,
(2) A differential amplifier,
(3) Buffer vessel selector,
(4) The electrolysis vessels.

Constructional detail

(1) *The programmer.* The programming unit is shown in detail in Fig. 1. The gradient scanner light source is a 6 V, 4 W lamp ((1)) supplied from an accumulator. The vertical image of its filament is focussed on a 20 mm–2 mm slit ((2)) by means of a simple lens (a microscope ocular) ((3)). Behind the slit is a channel guiding a strip of 35 mm film on which is photographed the gradient function. The light passing

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through the slit is split into two beams by means of a "Y" shaped "Perspex" conductor (4). This is cut out of a length of 3 cm cylindrical "Perspex" rod. On each arm of the "Y" is located a Philips ORP90 cadmium sulphide photo-conductive cell (5,6). The buffer vessel programming scanner consists of a 2.5 V lamp (7) focussed on a

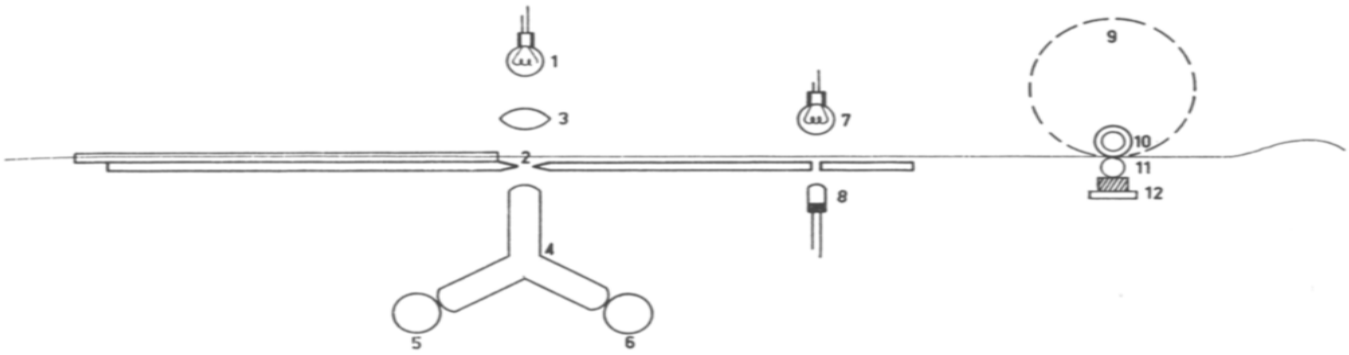

Fig. 1. Mechanical and optical arrangement of gradient generator.

1	= 6 V, 4 W lamp	7	= 2.5 V, 2 W lamp
2	= 20 × 2 mm slit	8	= OCP71 phototransistor
3	= Lens	9	= Synchronous clock motor
4	= Perspex conductor	10	= Film drive roller
5, 6	= ORP90 photo-conductive cells	11	= Idle roller
		12	= Polyurethane foam loading pad

The photocells and phototransistor are housed in a light-proof box attached to the panel bearing the slit and film guides.

OCP71 phototransistor (8) through a 2.5 mm diameter hole aligned with the "sound track" edge of the film.

The film is driven by a Sangamo 1 rev./h synchronous motor (9). A rubber covered 4 mm diameter brass roller (10) is sweated onto the motor shaft and supported at its distal end by a bearing. The film is held between this and a brass idler roller (11) which is loaded by a 7 mm pad of polyurethane foam (12).

A typical length of film is shown in Fig. 2. It is made by photographing a series of

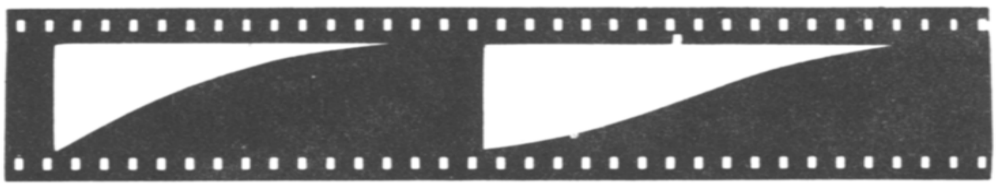

Fig. 2. Typical gradient functions photographed on 35 mm film. Note that the buffer vessel change-over cue spot is displaced from the function change by a distance equal to that between the scanning slit and phototransistor hole (3.5 cm in our instrument).

blocked-in graphs of the gradient function on Kodak "Microfile" film. A camera which leaves little space between the frames (a Voigtlander "Vito") was used and the spaces blacked out with retouching ink.

(2) *The electronic circuits.* As the generator was to operate in a temperature controlled cold room solid-state electronic components were used without temperature compensation circuits. The complete schematic circuit is shown in Fig. 3. One of the

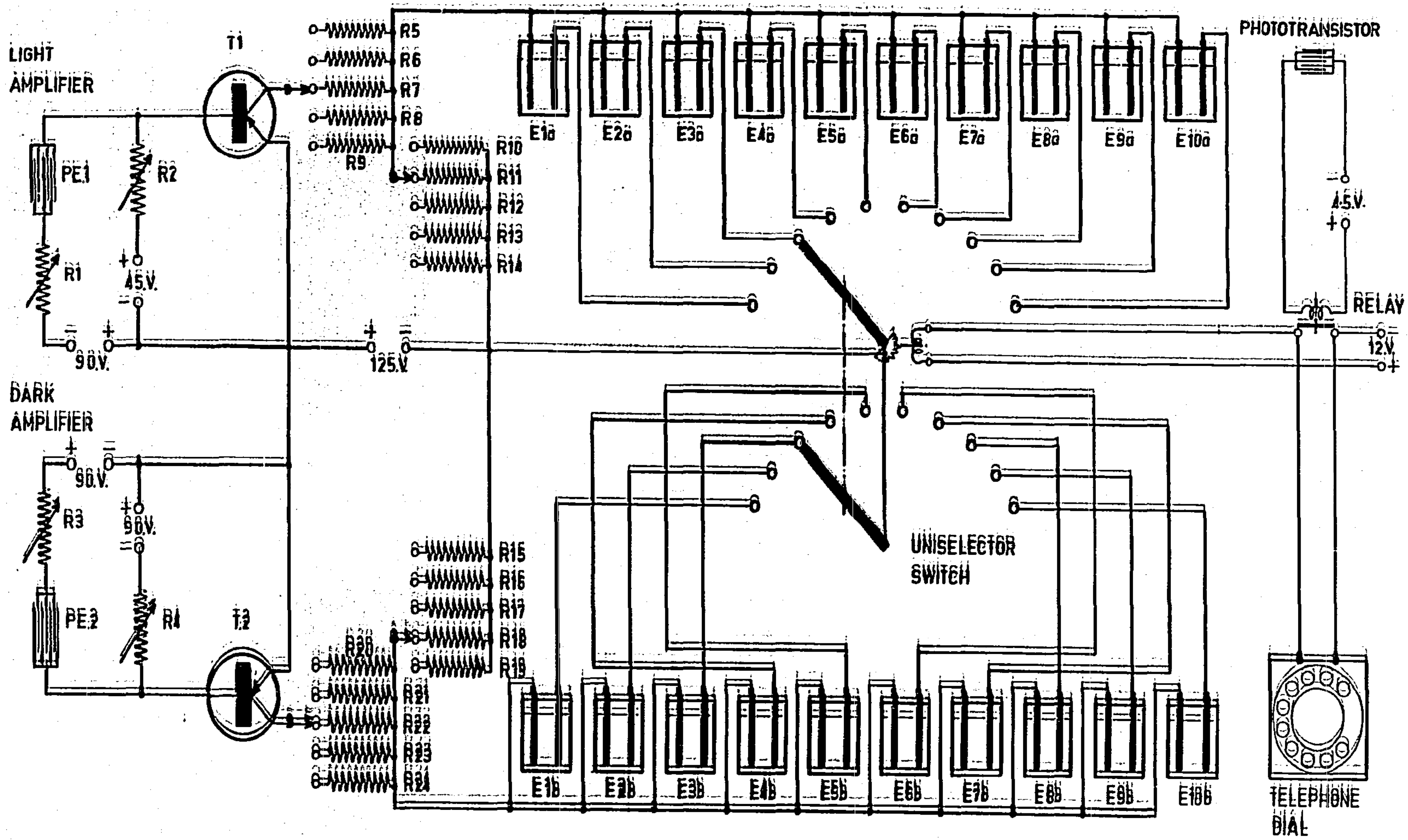

Fig. 3. Schematic circuit of gradient generator. P.E.1, P.E.2 = Mullard ORP90 cadmium sulphide photocells; T.1, T.2 = Mullard OC28 germanium power transistors.
R1 = 50,000 Ω; R2 = 75,000 Ω; R3 = 50,000 Ω; R4 = 100,000 Ω (all high stability carbon potentiometers);
R5 = 10 Ω; R6 = 175 Ω; R7 = 350 Ω; R8 = 460 Ω; R9 = 670 Ω; R10 = 7,000 Ω; R11 = 3,100 Ω; R12 = 700 Ω; R13 = 140 Ω; R14 = 35 Ω; R15 = 10 Ω; R16 = 175 Ω; R17 = 350 Ω; R18 = 460 Ω; R19 = 670 Ω; R20 = 7,000 Ω; R21 = 3,100 Ω; R22 = 700 Ω; R23 = 140 Ω; R24 = 35 Ω (all 10 W wire wound resistances);
The values given are for a 700 Ω electrolysis vessel resistance. E_a, E_{2a} etc. = "light" electrolysis vessels; E_b, E_{2b} etc. = "dark" electrolysis vessels. Phototransistor = Mullard OCP71 phototransistor. The relay, uniselector switch and telephone dial were obtained from Commonwealth of Australia, Post Master General's Department Disposals. Each of the 90 and 45 V supplies were obtained from separate dry batteries. The 125 V supply was obtained from a transformer and rectifier rated at 150 mA.

ORP90 photocells is biassed so that the end of it connected to the base of the OC28 transistor is positive. Increasing light on this tube increases the current through it, which opposes the negative bias current set by R_{11}. This reduces the collector current of the OC28 which is flowing through one of the electrolysis vessels. The other ORP90 photocell is biassed so that the end of it connected to the base of the other OC28 transistor is negative. Increasing light on this photocell increases the negative flow of current through the base-emitter circuit of the transistor, hence increasing the collector current flowing through the other electrolysis vessel. Darkening of the photocells leads to a reversal of these processes. A differential pair of currents is produced and hence differential rates of electrolytic gas production which cause differential flow rate of the two buffers. The resistance network in the collector circuits enables the rate of electrolysis to be varied, maintaining constant load upon the transistors.

(3) *Buffer vessel selector.* The buffer vessel selection system uses a standard four pole 25 position telephone exchange uniselector. These are normally available in a wide range of pole and position numbers. The coil of our selector was rewound to operate at 12 instead of 50 W. Whenever a light area in the sound-track edge of the film is interposed between the phototransistor and lamp a current flows through the relay, which closes the circuit energising the uniselector coil. The selector switch is therefore advanced one position. This switches the collector currents of the two amplifiers to another set of electrolysis and buffer vessels.

As the unit is intended for use with both cationic and anionic exchange columns and upper one row of paired contacts on the selector serves a set of electrolysis and buffer vessels of increasing ionic strength and decreasing pH. The other row serves a set of vessels of increasing ionic strength and increasing pH. The change-over is made by a two position switch. The 25 position selector provides a wide choice of buffer combinations.

The initial buffers for any run are selected by means of the telephone dial, which was considered to be the most fool-proof and convenient means of shifting the selector externally. So that the condition of the selector may be determined at a glance the contacts also operate a set of indicator lights.

(4) *Electrolysis and buffer vessels.* These are 1 l "Soluvac" saline bottles, fitted with No. 7 rubber stoppers. Great care must be taken to see that these are a gas-tight fit. The electrodes are 6 mm carbon rods held 2–5 cm apart by glass spacers and the electrolyte is 3 N H_2SO_4. The upper ends of the carbon rods are coated with petroleum jelly to prevent "creep" of the electrolyte up the rods. The rods are connected into the circuit by small "alligator" clips. At the maximum electrolysis current, 150 mA, the resistance of the vessel remains constant for prolonged periods because the principal factor in the resistance is electrode polarisation. Constancy of resistance is also favoured by the large volume of electrolyte, whose concentration is changed only slightly by the loss of water due to electrolysis.

The electrolysis vessel is connected to its buffer vessel by 3 mm PVC cannula and the buffer vessel line is a 5 mm PVC cannula which is connected to a glass manifold fitted to the top of the column. An earlier version of the generator used a micro-

mixing chamber at the junction of the buffer lines. This consisted of a 4 mm glass tube containing a piece of PVC coated clock spring vibrated by a 50 c/s electromagnet. At higher flow rates (10–15 ml/h) this seems to be unnecessary and has been retained for paper chromatography only.

SUMMARY

A chromatography elution gradient generator is described which is based upon a new principle. A photograph of the gradient function is scanned by two photocells so that the changing light and dark areas of the function are converted into varying electric currents which produce gas by electrolysis and pump the gradient limit buffers at rates corresponding to the function.

The device has the advantages of a high degree of reproducibility in the production of complex gradient shapes. It is also possible, by varying the overall rate of electrolysis, to produce the same gradient function in a volume as small as 2 ml at a flow rate of 1–2 ml/h or in a volume as large as 1 l flowing at 40 ml/h; this makes it possible to conduct pilot experiments on small columns or ion exchange paper and then to apply the results to large preparative columns.

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