Automatic Fraction Collectors and a Conductivity Recorder

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AUTOMATIC FRACTION COLLECTORS

In recent years a variety of designs of automatic fraction collectors have appeared (Brimley & Snow, 1949; Cuckow, Harris & Speed, 1949; Edman, 1948; Phillips, 1949; Stein & Moore, 1948).

The collectors described here possess the advantages that they are simple and cheap to construct and have given reliable service over long periods. The three machines all operate on the same principle, involving a siphon set on a balance arm which, in filling and emptying, operates a mechanical escapement. The siphon and its delivery arm are caused to rotate by means either of a spring, a twisted cord or a small weight.

The first two single-row machines differ only in arrangement of siphon, the second being the more robust. The third machine has several rows of tubes and is consequently more compact. The movement from one row to the next is accomplished with the aid of a second escapement.

Fraction collector Model 1

The apparatus described here can be readily constructed in any laboratory workshop. It consists of a circular rack of 114 test tubes opposite each of which a brass pin projects radially inwards. The siphon is suspended, by a twisted polyvinylchloride cord, from a balance beam furnished with adjustable counterweights, over the centre of the rack. The siphon empties into the test tubes through a long arm which carries a loop with overlapping ends made from glass rod (Fig. 2). The ends of the loop and the radial pins constitute an escapement which operates as follows. When the siphon is empty the radial pin presses against the upward-pointing end of the loop, and when it has become about half full the beam tilts and the loop descends, the pin passing between its ends. The arm, now free, rotates under the torque of the twisted suspending cord, and comes to rest with the downward-pointing end of the loop touching the next radial pin. When the siphon empties into the corresponding tube, the beam tilts back and the upward-pointing end of the loop comes to rest against this pin as in its starting position, the delivery arm having moved forward a distance equal to that separating the two ends of the loop. This distance, however, is small so that liquid continues to empty into the same tube in spite of the movement.

The last pin is long enough to project beyond the back of the loop and prevents it from moving further. A piece of glass tube may be slipped over any pin to act as a stop, the test tube in the corresponding position being replaced by a funnel leading to a receiver beside the rack.

Details of construction. The rack is constructed from a 30 in. square of 0·25 in. 'Tufnol' sheet (Tufnol Ltd., Perry Barr, Birmingham), to take $\frac{5}{8}$ in. test tubes at 3° intervals. The support for the beam prevents six positions from being used. The balance beam is pivoted on a pin through a slit in an upright rod set 5 in. from the centre of the rack, and carries on it two sliding weights for coarse and fine adjustments. Fixed to the rod is a cross-piece with two screws to limit the excursion of the beam. It also carries a Perspex block with two mercury pools into which a staple on the beam dips, to record the operation of the siphon when conductivity (see below) or some other property of an effluent is simultaneously being recorded.

The siphon, delivery arm and escapement loop are constructed wholly of glass. The adjustable siphon illustrated in Fig. 3 contains a hollow plunger, loosely fitting the outer tube, retained in any desired position by a wire ring. The bore of the centre tube of the plunger is equal to that of the siphon tube, so that the delivery volume is unaffected by the surface tension or density of the liquid. Tubes of 2 mm. diameter are suitable for flow rates under 30 ml./hr. For higher flow rates or relatively viscous liquids larger-bore tubes should be used. A vent pipe is provided between the siphon and the delivery arm. Because of the length of the latter it is impossible without the vent to prevent air and liquid siphoning over continuously. A small amount of liquid remains in the delivery arm, but this is reasonably constant from cycle to cycle. A tail attached to the receiver hangs vertically down below the point of support and fits loosely into a bearing just above the base board, to steady the siphon as the delivery arm fills with liquid.

Setting up apparatus. The pivot holes should be along the axis of the beam and the siphon and balance weights be symmetrical above and below so that with the siphon half full the centre of gravity of the beam is at the central pivot. The counterweight of the siphon is adjusted until the tail,

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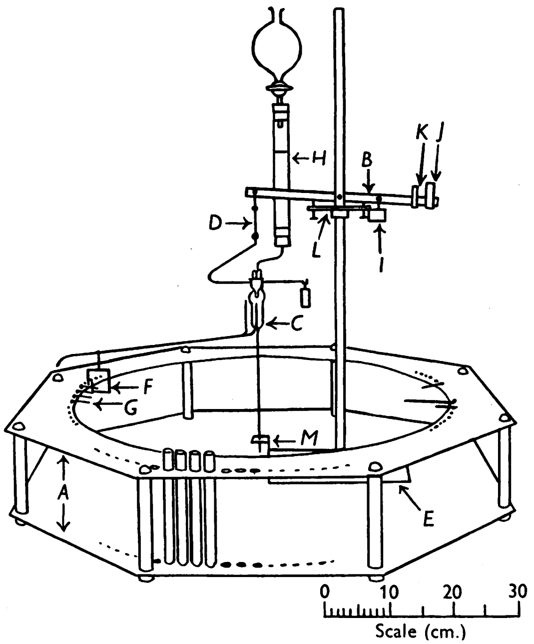

Fig. 1.

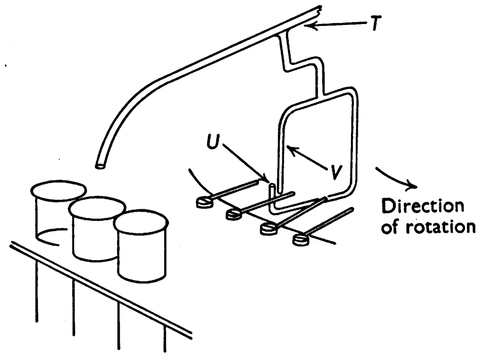

Fig. 2.

Fig. 1. Automatic fraction collector Model 1 (isometric view). *A*, rack for 114 test tubes; *B*, counter-balanced beam; *C*, adjustable siphon; *D*, twisted cord; *E*, base plate; *F*, escapement loop; *G*, escapement pins; *H*, chromatograph tube; *I*, mercury cup switch; *J*, *K*, counter-balancing weights; *L*, adjusting screws to limit beam movement; *M*, tail bearing.

Fig. 2. Detail of escapement mechanism of Model 1. *T*, delivery arm; *U*, upward-pointing end of loop; *V*, downward-pointing end of loop.

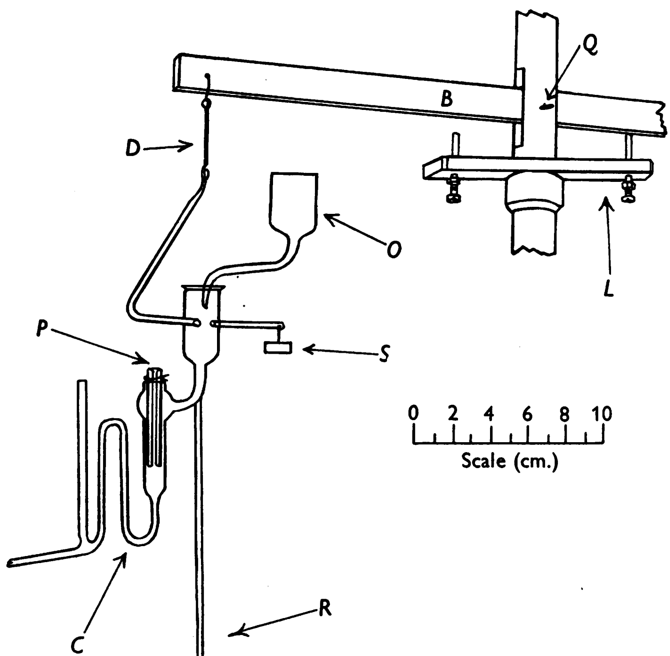

Fig. 3. Detail of beam and siphon assembly of Model 1. *B*, counter-balanced beam; *C*, adjustable siphon; *D*, twisted polyvinylchloride cord; *L*, plate carrying adjusting screws to limit beam movement; *O*, collecting cup; *P*, adjustable iner tube of siphon; *Q*, beam pivot; *R*, tail; *S*, siphon-counter-balancing weight.

removed from its bearing, is vertical. The escapement loop is adjusted until the delivery arm is directly over the tubes at both positions of the escapement. The suspension cord is given two or three turns and the tail replaced in its bearing. The balance limit screws are adjusted to give an excursion of the siphon of 5 mm. Finally, the counter-balancing weights are adjusted so that the siphon moves when half to two-thirds full. The minimum volume for satisfactory operation is about 2 ml.

This apparatus is in the Research Laboratories of Boots Pure Drug Co. Ltd., Nottingham.

a link surrounding the central pivot. This link carries two screws to adjust the excursion of the beam. The torque to rotate the beam is provided by a helical spring of about ten turns of Chromel wire around the central pivot attached at one end to the block and at the other to an adjustable collar. The siphon is connected to the delivery arm with a collar of heavy rubber tubing.

Setting up apparatus. For satisfactory operation the centre of gravity of the beam with the siphon one-half to two-thirds full must lie at the point of the pivot. Adjustment of the centre of gravity vertically and along the beam can be

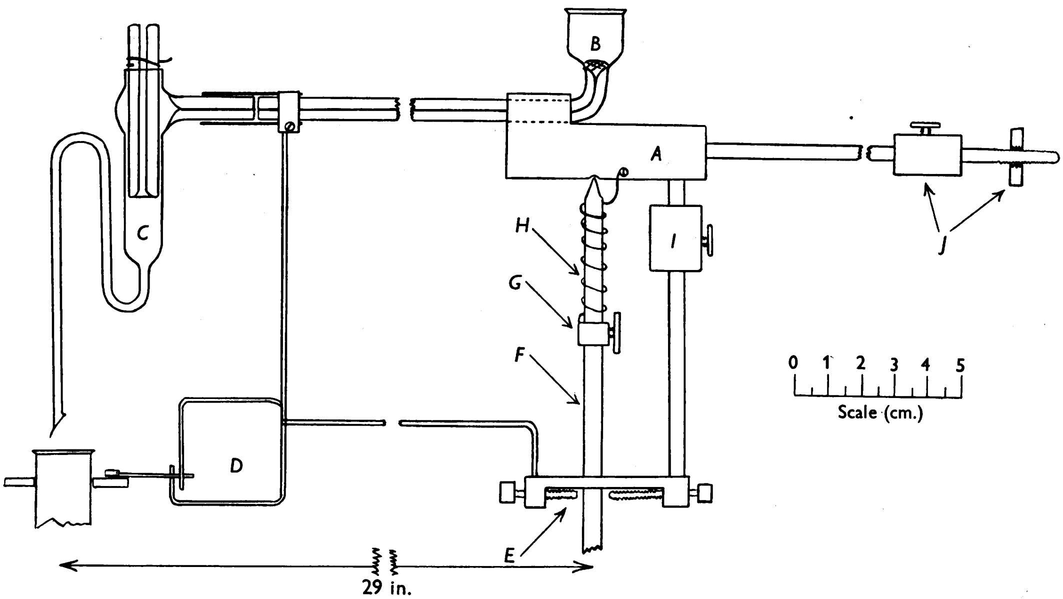

Fig. 4. Side elevation of fraction collector Model 2. *A*, brass bearing block; *B*, collecting cup; *C*, adjustable siphon; *D*, wire escapement loop; *E*, excursion-limiting screws; *F*, central pivot; *G*, spring adjustment; *H*, spring; *I*, vertical sliding weight; *J*, coarse- and fine-adjustment horizontal sliding weights.

effected by moving the weights; but at right angles to the beam it must be correct before leaving the workshop, otherwise the friction of the link against the pivot is excessive. The spring is then given several turns and adjusted until no vertical force is applied to the block. This spring could well be replaced by a thread and weight as in Model 3.

A small plug of cotton wool is pushed into the bottom of the collecting cup to prevent ingress of air bubbles. The minimum volume this apparatus will handle satisfactorily is about 0·5 ml.

This apparatus is in the Biochemistry Department, The Lister Institute of Preventive Medicine, Chelsea Bridge Road, London, S.W. 1.

Fraction collector Model 2

Perhaps the chief drawbacks of the model described above are the fragility of the siphon assembly and the limitation of sensitivity imposed by friction at the balance pivot. By placing the siphon at the end of the delivery arm and utilizing this as the beam, the apparatus could be made more robust and the whole could be supported on a single point, thus considerably increasing the sensitivity. The siphon delivering directly into the test tubes empties more cleanly.

Details of construction. The rack is made from 0·25 in. aluminium sheet with 120 $\frac{5}{8}$ in. holes arranged at 3° intervals. Fig. 4 shows the arrangement of the siphon assembly. A brass block has two arms at right angles carrying adjustable weights. The point of the central steel pivot bears in a depression made by a centre punch in the under side of the brass block. A delivery arm, of thick-walled 1 mm. glass capillary tube with a collecting cup at the centre, is fixed in the block with sealing wax. The escapement is fixed both to the delivery arm, and to the vertical rod projecting from the block through

Fraction collector Model 3

In this model (Fig. 5) the size of the rack is diminished by the use of several concentric circles of tubes. To reduce friction at the link on the central pivot, the main beam rocks on two points, whose bearings are attached to a glass tube itself pivoted on the central support. This glass tube also carries two arms, one hollow containing the weight and cord

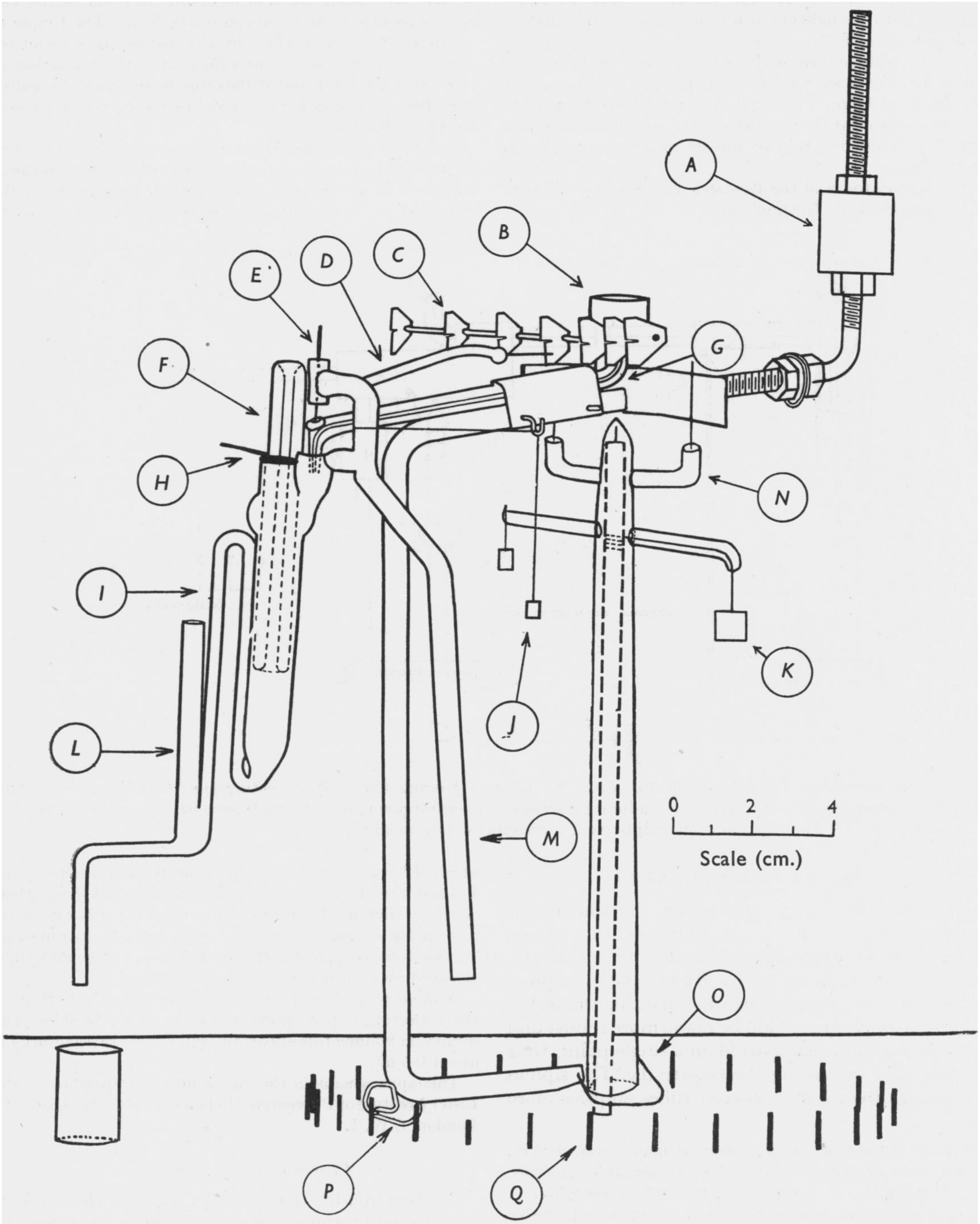

Fig. 5. Side elevation of fraction collector Model 3. *A*, centre of gravity adjustment weight; *B*, collecting cup; *C*, fins of siphon escapement; *D*, siphon arm; *E*, siphon pivot; *F*, adjustable siphon plunger; *G*, delivery arm; *H*, siphon plunger retaining wire; *I*, adjustable siphon; *J*, weight and cord to operate siphon escapement; *K*, weight and cord to operate beam escapement; *L*, siphon vent tube; *M*, siphon counter-balancing arm; *N*, main beam pivot; *O*, beam link; *P*, main escapement; *Q*, main escapement pins.

responsible for the torque necessary for the beam movement and the other supporting a counterweight which ensures that the glass tube hangs symmetrically on the central pivot. The link is replaced by a C-shaped piece of wire attached to the base of the beam to limit the excursion of the beam. In this way rubbing contact of the link and the central pivot is eliminated. The beam and delivery arm are similar to those of Model 2 except that the siphon, now pivoted at the end of the delivery arm, by the operation of an escapement on the beam once per revolution of the beam, moves outwards from one circle of holes to the next. It is a convenient geometrical fact that equal numbers of circles in con-

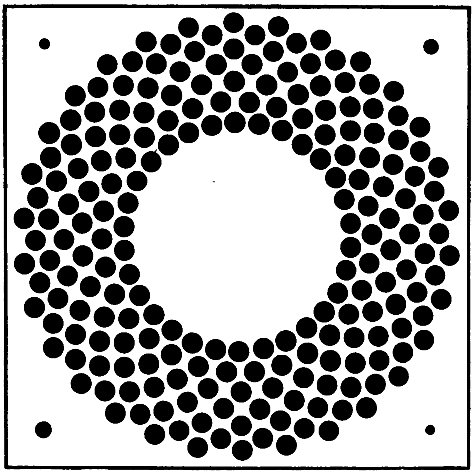

Fig. 6. Plan of closely packed arrangement of holes in test tube rack of Model 3.

centric rings packed as closely as possible lie also on arcs of circles whose centres lie on the circumference of the innermost ring and whose radius is equal to that of the innermost ring (Fig. 6). If, therefore, at the end of a pivoted beam of length equal to the radius of the innermost ring and with an escapement allowing delivery of liquid into each tube of the innermost ring, is fitted a pivoted siphon provided with a similar escapement and of the same length as the beam, delivery into each tube of successive rings is possible. The angle between successive positions of both escapements is the same. The beam escapement consists of a loop of wire (Fig. 7) as in the previous models except that the ends of the loop are nearly horizontal (in fact normal to the line joining the loop with the pivot on which the beam turns) instead of vertical as before. The loop contains also a horizontal portion at right angles to the ends of the loop. These engage with vertical pins in the rack as in the previous models, but instead of the upward excursion of the beam being limited by the link surrounding the central pivot, the horizontal portion of the loop rests against the escapement pins except in the case of a single short pin. In this case, the only difference is that the beam moves up further than usual, and its movement is finally limited by the link against the central pivot. The purpose of this larger excursion of the beam is to operate the siphon escapement which is mounted on the beam itself.

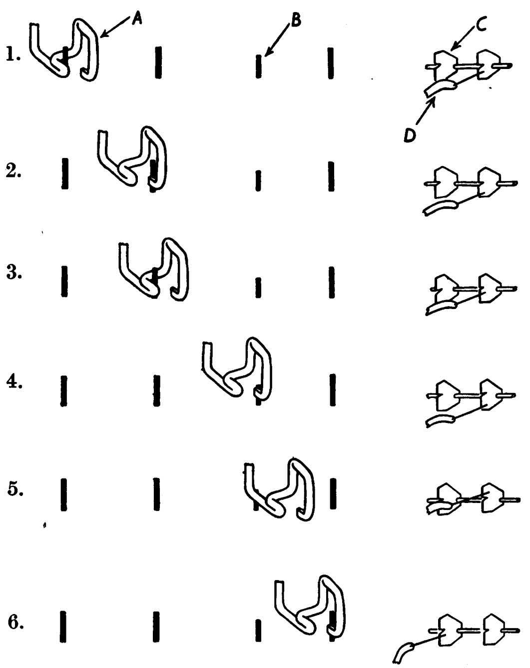

Fig. 7. Diagram of main beam escapement and siphon escapement in successive positions. *A*, main escapement; *B*, vertical pins; *C*, pins of siphon escapement; *D*, siphon arm. 1, siphon has emptied, beam tilted up. Siphon arm on lower part of fin. 2, siphon has partly filled, beam has fallen, escapement has disengaged and beam has rotated to next pin. 3, siphon has emptied, escapement still engaged. 4, siphon has filled, beam has fallen, escapement is now engaged by short pin. 5, siphon has emptied and short pin has passed under rear of escapement. Siphon arm has now passed to upper part of fin. 6, siphon has filled, beam has rotated on to next pin and the siphon arm has slipped through the slit in the fin and has rotated until arrested by the next fin. The siphon delivery arm is now over the next ring of tubes.

This escapement is in principle similar to the others, but in this case the pin is the moving member and is attached to an arm at right angles to the plane of symmetry of the siphon. The loops, seven in number, made from sheet-metal fins split horizontally with the upper half displaced sideways enough to admit the pin on the siphon arm, are mounted on the beam on an arc whose centre lies at the siphon pivot.

During the normal operation of the beam escapement the pin on the siphon arm rests against the lower half of a fin. But when the beam escapement reaches the short pin, the beam rises more than usual and the siphon pin moves from the bottom to the top half of the fin. As the siphon fills it drops and the siphon pin passes between the leaves of the fin and the siphon delivery tube moves over to a new ring of tubes as the beam moves on to the next position (Fig. 7).

Details of construction. The rack consists of a 15 in. square of 0·25 in. 'Tufnol' sheet drilled out to take 210 16 mm. test tubes; the radius of the inner circle of thirty tubes is 3·7 in. The ring of vertical pins (height $\frac{3}{8}$ in.) has a radius of 3 in., the short pin being 0·25 in. high. The beam is constructed of 0·25 in. aluminium rod supported on a vertical pivot 0·25 in. diameter and 7·5 in. high. The delivery arm is attached to the beam by a sheet-metal staple held in position with two pins, one of which is formed into a hook to support the thread and weight coming from the siphon arm. The siphon escapement is also attached to the staple. The horizontal and vertical portions of the beam are threaded, the former to allow adjustment of the weights acting as the fine adjustment for balancing the beam. The vertical threaded portion provides adjustment of the counter-balancing weights by means of which the centre of gravity can be brought to the central pivot.

Setting up apparatus. Similar adjustments to those described for Model 2 are necessary. The smallest possible weights should be used to provide the torque necessary for the beam and siphon arm rotations. The minimum volume this instrument will handle satisfactorily is about 1 ml.

A CONDUCTIVITY RECORDER

The change in electrical conductivity in the effluent from a column provides in many cases a convenient method for following its operation. This technique is, however, only applicable where the effluent is aqueous. When the stationary phase is aqueous the

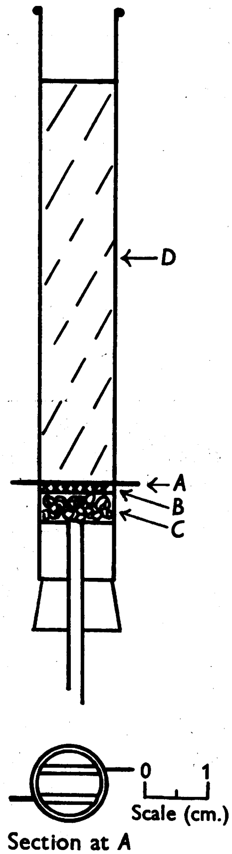

Fig. 8. Diagram of chromatogram tube fitted for conductivity measurement. *A*, platinum electrodes; *B*, cloth disk; *C*, glass wool; *D*, chromatographic filling.

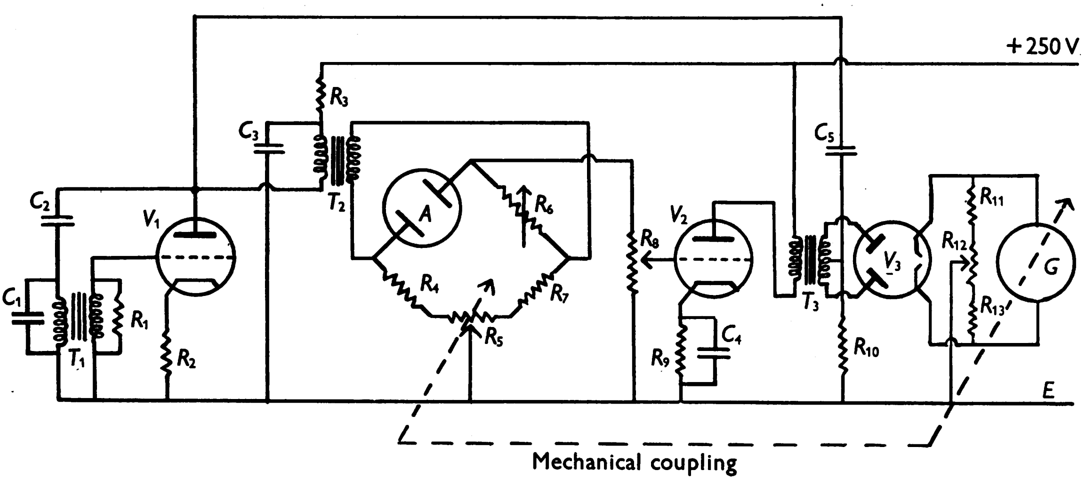

Fig. 9. Circuit diagram of conductivity recorder. Component values: condensers C_1, C_2, 0·01 μF.; C_3, 8 μF.; C_4, 50 μF.; C_5, 0·1 μF.; resistances, R_1, R_6, 20 KΩ; R_2, 500 Ω; R_3, 100 KΩ; R_5, 120 Ω; R_8, 0·5 MΩ; R_9, R_{12}, 1 KΩ; R_{10}, R_{11}, R_{13}, 10 KΩ; transformers, T_1, T_2, 3:1 ratio; T_3, 1:2 ratio, centre-tapped; valves, V_1, ML4; V_2, MH4; V_3, 2D4B. R_4 and R_7 are multiplying resistances; the scale is multiplied one, two, five and ten times with values of respectively 0, 60, 240 and 540 Ω. *A*, conductivity cell. *G*, galvanometer, mechanically coupled to R_5 in recording apparatus.

conductivity of the column itself can conveniently be measured between two horizontal electrodes of flattened platinum wire sealed into the column within 1 cm. of the bottom (Fig. 8).

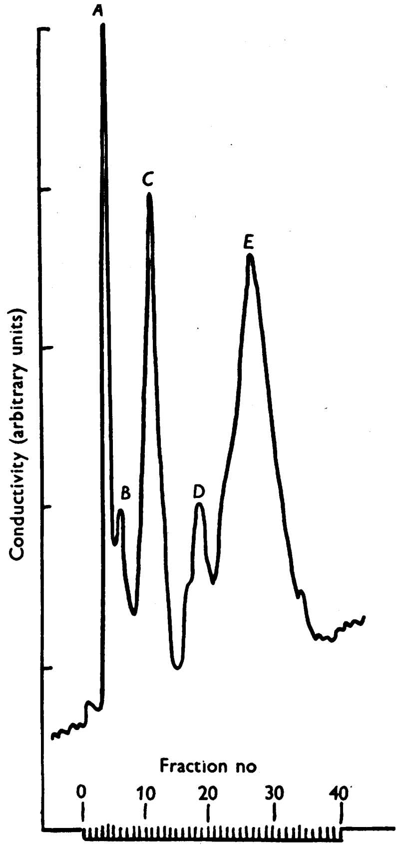

Fig. 10. Fractionation of a penicillin mixture. Tracing from conductivity-recorder chart. *A*, non-penicillin acids; *B*, *n*-heptyl-; *C*, *n*-amyl-; *D*, *n*-pent-2-enyl-; *E*, benzylpenicillin.

A commercial recording potentiometer in which the galvanometer and slide wire were mechanically coupled was used in the circuit shown in Fig. 9 to provide an automatically balanced bridge to record changes in conductivity of the column. The valve oscillator provides 1000 cyc./sec. at 0·5–1·0 V. to the bridge, whose output is amplified and fed to a phase-distinguishing circuit consisting of a bridge with two matched diodes. The d.c. output from this second bridge is fed to the recording galvanometer which automatically corrects the adjustment of the main bridge.

An additional pen is fitted to the recorder whereby the excursions of the beam of the fraction collector are marked on the same chart. The application of this technique to the analysis of a mixture of penicillins is described below.

Example of use of conductivity recorder

'Hyflo-Supercel' kieselguhr (5·0 g.) (Johns-Manville Ltd., London, S.W. 1) was thoroughly mixed with 2·5 ml. of pH 5·5 sodium citrate buffer (0·1 M), which had previously been equilibrated with a 1:1 (v/v) mixture of ethyl ether and di-*iso*propyl ether. It was made into a thick slurry with the mixed ethers and then packed into a 1 cm. diameter chromatogram tube, 15 cm. long, fitted with platinum electrodes. The tube was set up in the automatic collector with a 3·75 ml. receiver and the electrodes were connected to the conductivity recorder. The mixed ether solvent was run through the column from a separating funnel until the conductivity was steady. Then, without interrupting the flow of solvent, a solution of 10 mg. of crude penicillin acids (1000 i.u./mg.) in 1 ml. of the mixed ethers was carefully run on to the surface of the kieselguhr from a long capillary pipette inserted down through the separating funnel.

Fig. 10 is a photographic reproduction of the record obtained. Forty fractions were collected, non-penicillin acids being present in fractions 3–5, *n*-heptylpenicillin in 6–8, *n*-amylpenicillin in 9–14, *n*-pent-2-enylpenicillin in 15–20 and benzylpenicillin in 21–35.

SUMMARY

1. Three types of automatic fraction collector working on essentially the same principles are described, all of which can be easily constructed in any laboratory workshop.

2. A conductivity recorder is described that provides automatic registration of the changes in electrical conductivity of the stationary aqueous phase of a partition chromatogram together with the volume of effluent passing through the column.

3. An example of the application of an automatic fraction collector and the conductivity recorder to the analysis of a mixture of penicillins is described.

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