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TECHNICAL NOTE

A METHOD OF OBTAINING ALTERNATE UNEQUAL FRACTIONS FROM A LIQUID COLUMN*

1. INTRODUCTION

THIS apparatus provides a means whereby a stream of liquid such as that issuing from a chromatogram can be divided automatically into a series of pairs of fractions. It can, for instance, be arranged to give, alternately, a very small and a much larger fraction. Subsequent analysis of the series of small fractions will then give information on which the pooling of selected groups of the larger fractions can be based. A similar result can, of course, be obtained more laboriously by taking aliquots for analysis by hand from a series of fractions of uniform size as provided by a normal type fraction collector. In addition to the saving of time compared with the taking of such aliquots by hand, two other advantages of the present apparatus should be noted. These are, firstly, that if the solution being fractionated contains any component which can become oxidised or changed in some other way on standing in the atmosphere, it may be desirable to allow the aliquots destined for analysis to fall without delay into tubes containing an inhibitor which can thus be excluded from the main bulk of the solutions. Occasionally, as when a precipitate can be expected to form on standing, aliquots must be taken immediately. Secondly, if the aliquots for analysis are allowed to fall on to planchettes for subsequent estimation of radioactivity then the time required for the drying of the samples will be saved if this process can commence as soon as the solution issues from the chromatogram.

2. METHODS

Two methods have been used, an electronic unit using "dekatron" selector tubes, and a simpler electromechanical version using count down registers.

In principle the system comprises a drop detector, the pulses from which are fed to either of two counting circuits selected alternately by a binary relay circuit. Each time a predetermined count is reached, this binary is impulsed and the fraction collector advanced.

2.1. *Method* 1 (*Fig.* 1)

As each drop passes the electrodes, conduction through the drop raises the trigger potential of the cold cathode tube V1, causing it to fire momentarily. A negative pulse from the anode triggers the monostable circuit V2, producing the required pulse for driving the first "dekatron" selector. The cathode follower V3a prevents spurious firing of V2 during reset operations. The second "dekatron" is driven from the tenth cathode of the first in the normal way.

S1 or S2 selects the appropriate cathode and a positive pulse from this cathode causes V4 to conduct, momentarily operating relay T. Contact t1 makes to operate relay A which is self-holding via a1, and simultaneously breaks the reset line, causing the dekatrons to "zero". Thereupon t1 releases to permit relay B to operate, and restores the reset line. Contact t2 advances the fraction collector one step. Contact a2 has now brought the second set of selector switches into circuit, S3 and S4, and counting proceeds until the second sample number is reached. The terminating sequence is similar except that t1 now makes to short relay A, then breaks to release relay B, thus restoring the binary to its first count condition. In many applications no more than 10 drops are required for the first count, in which case S2 need not be fitted. S5 is then useful to prevent the binary working during periods when uniform fractions in excess of 10 drops are required. Pilot lights operated by the binary relays indicate which count is in progress.

2.2. *Method* 2 (*Fig.* 2)

In the electromechanical method, the two sample counts are set up on two electrically reset registers, permitting any number of drops for either batch. The firing of the trigger tube now operates relay E and contact e_1 impulses the first counter until the preset number is reached. Contact c1 within the counter operates the relay T. Contact t1 causes the binary relays A and B to change over, thus selecting the other register for the next count. Contact t2 operates the fraction collector and t3 resets the register. Count pulses could damage the register during the reset period, and although unlikely to occur, their circuit is broken by t3 as a safeguard.

The electronic unit was tried initially since components were ready to hand, so that a prototype could be produced quickly in order to evaluate the overall system. This has proved reliable in use and adequate for many biochemical assays. The electromechanical system, using two major items, the registers, is less complex and more flexible in that it permits a wider range of ratios.

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