AN EVENT MARKING SYSTEM FOR USE WITH FRACTION COLLECTORS AND EFFLUENT ANALYZING DEVICES

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(Received October 19th, 1961)

The use of instruments to continuously measure and record the light absorption of eluted materials has greatly facilitated the application of ion-exchange separations. When these devices are used with a fraction collector, it is convenient to use them in conjunction with an event marker in order to locate the fractions corresponding to the absorption areas shown on the strip chart recording.

There are at least three commercial firms which market continuous flow photometer systems with strip chart recorders and event markers*. From readily available components, we have assembled an effluent monitoring system which is more versatile than the commercial devices. The event marking unit used in this system is mechanically independent of the other components, and thus it can be used in conjunction with most fraction collectors and strip chart recorders. The event mark is made by the same pen that indicates the transmission; therefore no extra pen is required.

One of the problems with a continuous flow system is that a slow rate of elution yields a very long recording. This can be compensated for by the use of a recorder with a slow chart speed. However, if a slow chart speed is employed and small fractions are collected, or if the elution flow rate is fast, poor resolution is obtained. In the system described here, we have used a strip chart recorder which is available with 16 chart speeds ranging from 1 in./h to 16 in./min. In addition the instrument can be supplied equipped with two chart speeds.

An event marker which marks every fraction, when used with very slow chart speeds and fast elution, can yield a recording where the event indications are so close together that they are difficult to distinguish. The equipment described here overcomes that problem in that it can be set to mark the collection of a group of fractions as well as individual fractions. Through the use of a rotary switch and latching relay, the event mark can be adjusted to indicate the collection of from 1 to 20 fractions. That is, if the rotary switch is set on position 5, then every 5th fraction will result in an event mark on the strip chart recording.

The system described here operates as follows (see Fig. 1): When the fraction collector turntable motor operates, leads across this motor also actuate the latching coil

* Canal Industrial Corp., 4940 St. Elmo Avenue, Bethesda 14, Md. Gilson Medical Electronics, Middleton, Wisc. LKB Instruments, Inc., 4840 Rugby Avenue, Washington 14, D.C.

(LC) of the latching relay. When the latching relay advances to the setting of the rotary switch (S_4), the reset coil (RC) is actuated to reset the latching relay. At the same time, current flows through the switching relay (R_2), to open the circuit to the time delay relay (TDR). The time delay relay then opens the positive lead from the photometer unit to the strip chart recorder, for a preset length of time. The recorder

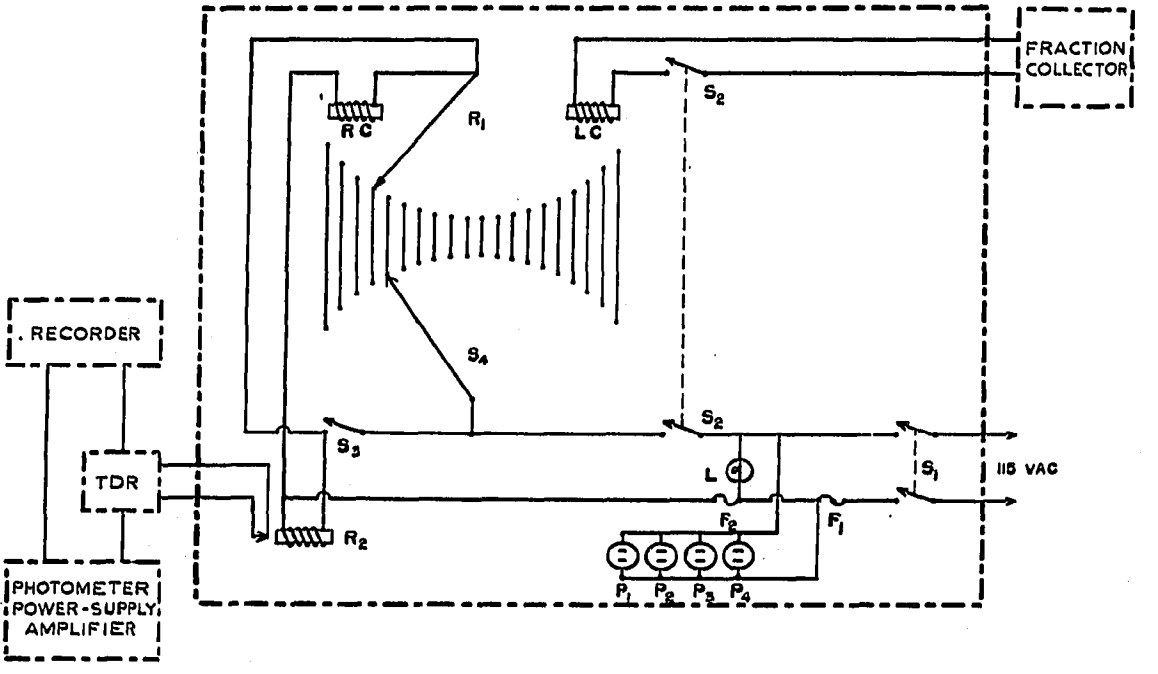

Fig. 1. Schematic diagram of event marking circuit. S_1 = DPST; S_2 = DPST; S_3 = SPST momentary; S_4 = SP20T rotary; R_1 = Latching relay, 21 position 115 VAC, electrical resetting; R_2 = SPST relay, 115 VAC normally closed; TDR = SPST variable time delay relay; L = Pilot light, 115 VAC neon with self contained resistor; F_1 = 5 A fuse; F_2 = 0.5 A fuse; P_1, P_2, P_3 and P_4 = Standard 115 VAC outlets.

responds by an excursion of the pen from the transmission tracing to a point beyond the zero percent transmission setting. When the time delay relay closes the recorder lead the pen then returns to the transmission tracing. The event mark is then a vertical straight line extending from the absorption curve to a point off the recording area (see Fig. 2). The momentary switch (S_3) is used to manually reset the latching relay, and it can also be used to indicate the start or finish of a particular eluant, change in flow rate or other change in conditions. When switch S_2 is open, the other components and the outlets (P_1–P_4) can be used without the event marking system. This is especially convenient for resetting the fraction collector.

The fraction collector used is a Model 1205 sold by Research Specialties Company, 200 South Garrard Blvd., Richmond, Calif. This fraction collector is easily connected to the event marking system through an accessory outlet plug supplied as part of the instrument. By wiring the latching coil across the turntable motor leads (pins A and B), the event marking process can be initiated when the fraction collector is operating with volumetric siphoning, drop counting or constant timing.

The latching relay is a Guardian Mer-115, 21 point electrical reset stepper manufactured by Guardian Electric Mfg. Co., 1621 W. Walnut Street, Chicago 12, Ill.

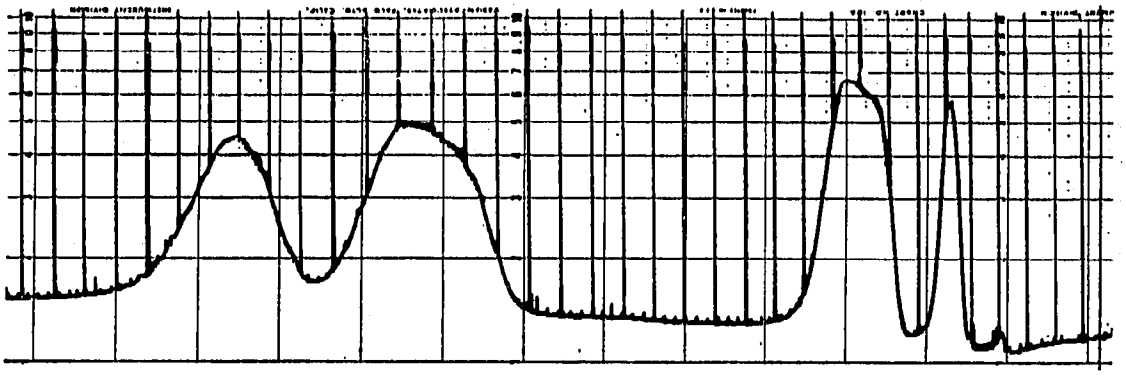

Fig. 2. An illustration of the use of the event marking system. This shows the ion-exchange separation of ribonucleotides from the alkaline hydrolysis of ribonucleic acid, using gradient elution with 4 *N* formic acid, according to the procedure of HURLBERT *et al.*[1]. The absorption peaks of the 2′-, 3′- nucleotides, in the order of their emergence from right to left, correspond to cytidylic, adenylic, guanylic and uridylic acids respectively. The event marker was set to mark every second 10-ml fraction. The chart speed was 1 in./h.

Only the first 20 of the 21 contacts were used. The 20 position switch is a Model MS-20-1 from J-B-T Instruments, Inc., New Haven, Connecticut. The time delay relay used here is a Model CR 7504-A3B from General Electric, Schenectady, New York. This is a normally open switch and thus requires another relay switch (R_2) to actuate it. It would be more convenient to use a normally closed unit. The time delay relay should have a closing delay of at least twice the full scale response time. A delay of approximately 3 sec was found adequate for the recorder used here. If the motor actuation time of the fraction collector is equal to or greater than the response time of the recorder used, then the necessity of a time delay relay would be eliminated.

The strip chart recorder is a Varian Model G-10, 100 mA input, with a full scale response time of 1 sec. This instrument is obtainable from Varian Associates, 611 Hansen Way, Palo Alto, Calif. Chart speeds of 1 in./h and 6 in./h appear to be suitable for most ion-exchange procedures.

The distance the pen travels beyond the zero percent transmission point during an event marking excursion is regulated by adjustment of a stop ring on the pen slide. This stop ring can be so adjusted that the event mark is always discernible even when the transmission is zero percent.

The photometric device is an LKB 4701 Uvicord Ultraviolet Absorptiometer distributed by LKB Instruments, Inc., 4840 Rugby Avenue, Washington 14, D.C. This particular instrument has terminals for 10 or 100 mA outputs. This instrument measures light absorption primarily at 254 mμ.

The 115 VAC outlets, P_1, P_2, P_3 and P_4 are used to power the recorder, photometer power supply-amplifier, time delay relay and a magnetic stirrer for gradient elution. The fraction collector is connected to a separate electrical source so that it can be used independently of the other devices.

ACKNOWLEDGEMENT

This investigation was supported in part by the U.S. Army Medical Research and Development Command, Department of the Army under Research Contract No. DA-49-193-MD-2129.

SUMMARY

A simple event marking device is described for use with fraction collectors and eluant analyzing instruments. The device is mechanically independent of the other units in the chromatographic system and can be adjusted to mark the collection of single or multiple (up to 20) fractions.

REFERENCE

[1] R. B. HURLBERT, H. SCHMITZ, A. F. BRUMM AND V. R. POTTER, *J. Biol. Chem.*, 209 (1954) 23.