CHROM. 4648

Two multi-temperature bath control units for single-column amino acid analyzers

Ion-exchange chromatography of the eighteen amino acids commonly found in protein hydrolysates is relatively simple, and is easily accomplished on most commercial amino acid analyzers; resolving the many ninhydrin-positive compounds present in physiological fluids[1-3] is, however, considerably more difficult. Most amino acid analyzers currently being manufactured use dual-column discontinuous buffer systems to improve resolution and decrease the length of time required to complete the assay. The older, single-column analyzer, however, can compete favorably with the dual-column systems in resolution if utilized with newly developed sodium citrate[4,5] or lithium citrate[6] buffer systems which allow the separation of ninhydrin-positive compounds unresolved or destroyed with the older buffer systems. These new systems require changing the column temperature at specific points during the analysis (*e.g.*, 37° to 60° at 2½ h, or 35° to 70° at 6 h), and the ability to change column temperature automatically at a fixed point during the analysis is a distinct advantage from the point of view both of personnel time and reproducibility of resolution. Some laboratories may have neglected to use these superior buffer systems because of the lack of an inexpensive automatic method of changing column temperature. This laborabory currently uses two different automatic multi-temperature bath control units with our three Technicon NC-1 amino acid analyzers, and both units are described in this paper.

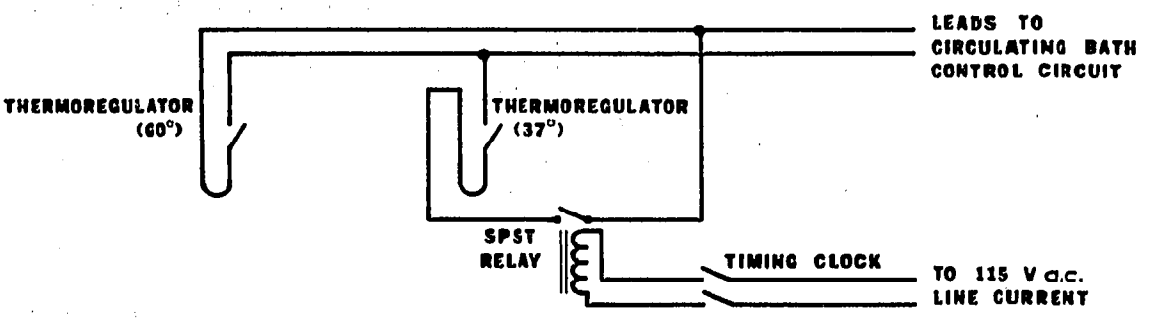

Fig. 1. Circuit diagram for two-temperature operation with control unit No. 1.

Materials and methods

Control unit No. 1. The temperature control mechanism of many common circulating baths is actuated by a modified mercury thermometer-thermoregulator, the mercury closing a circuit when in contact with an adjustable wire. When this control circuit is closed, current ceases to flow to the heating element; when the mercury breaks contact (reflecting a lowered bath temperature) current flows to the heating element. A simple method of automatically changing the operating temperature of the bath is to introduce two, three (or more) identical mercury thermometer-thermoregulators into the system, wired through timed relays to the electronic control of the bath. This laboratory uses a Haake bath fitted for a second thermometer-thermoregulator (a 7/8 in. hole must be drilled into the top plate of the bath) which is connected to the electronic control of the bath in the manner indicated in Fig. 1. The

parts and labor required, excepting the circulating bath, are: one timing clock (Fisher Scientific No. 6-663-50VI); one single-pole, single-throw relay, 115 V a.c. (Allied Radio No. 4657); one thermometer-thermoregulator (Technicon Corp. DW-17790, Scientific Products W 3097-2); one dual Banana Plug (Allied Radio, No. 26 C 1792); two Type 224BB Dual Binding Posts (Allied Radio No. 47 C 1328); one hole drilled to slightly exceed the outer diameter of the thermoregulator in the top plate of the heating bath. The use of the dual binding posts and banana plug may be omitted and splicing used instead.

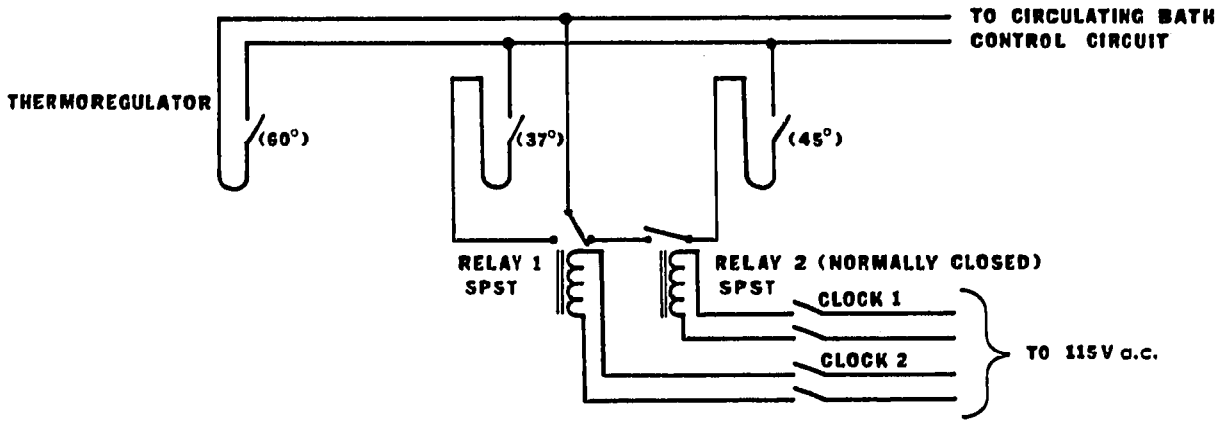

Fig. 2. Circuit diagram for three-temperature operation with control unit No. 1.

In two-temperature operation, the appliance clock holds the relay closed during the initial period of operation at the lower temperature, and opens it to bring the column up to higher temperature operation. During the regeneration cycle, the appliance clock closes the relay allowing the column temperature to fall to starting temperature, *e.g.*, if an analysis with the EFRON buffer[4] is begun at an arbitrary time setting of 12:00 p.m. (on a 24-h clock), the clock should be set to turn "on" at 10:30 a.m. and "off" at 2:30 p.m. In some instances we have found it useful to have a three-temperature control system for certain separations; the circuitry for such a system is illustrated in Fig. 2. In this system, clock No. 1 activates the single-pole single-throw (SPST) relay to place the 35° thermoregulator in the circuit, and deactivates it to place the 45° thermoregulator in the circuit. The second single-pole single-throw relay, wired to clock No. 2, should be wired normally closed, opening when the relay is activated by the clock. Clock No. 2 activates the relay to increase the bath temperature to the 60° level and holds it open during the desired time interval. The temperatures mentioned are only representative, but their relationship must be preserved since the thermoregulator with the lowest setting actually connected to the heating bath electronics will determine the bath temperature. For example, when the relay in Fig. 1 is closed, both thermoregulators are connected to the heating bath electronics; but the operating temperature is controlled by the thermoregulator with the lowest setting (in this case 37°).

This system has proven reliable, reproducible and flexible with respect to both temperature and time-interval modifications. Moreover, it can be used with any other constant-temperature control mechanism (*e.g.*, Fisher No. 15-445-47V2) employing the mercury-type thermoregulator, and may be added to the constant-temperature bath for less than fifty dollars.

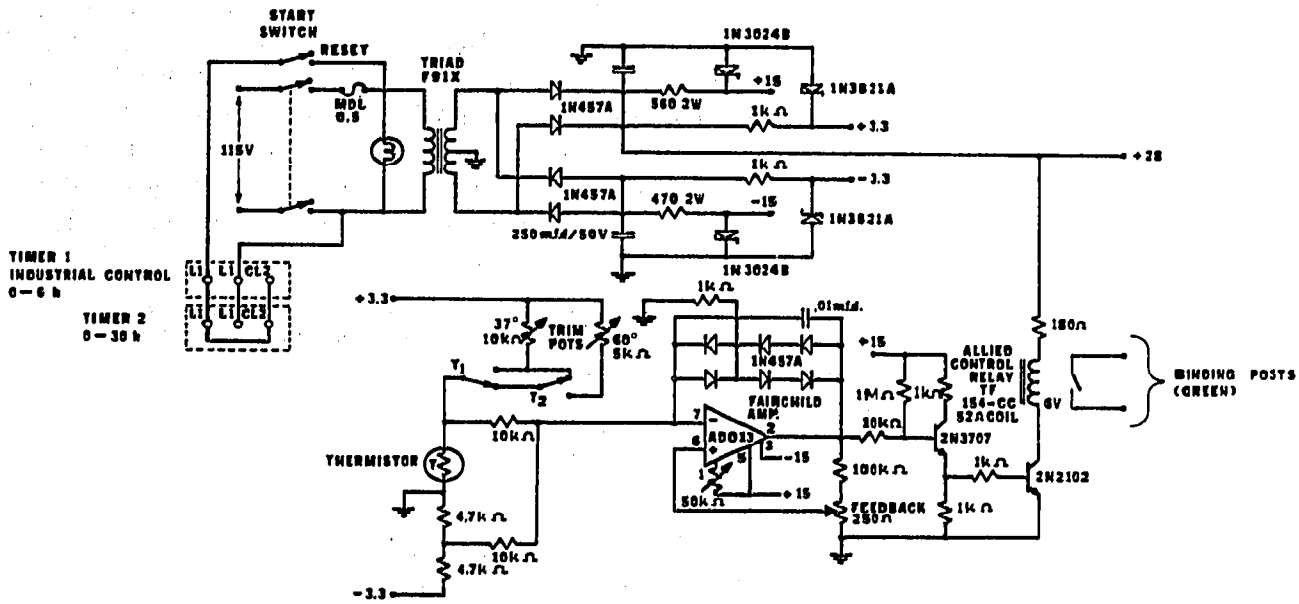

Fig. 3. Circuit diagram for control unit No. 2.

Control unit No. 2. This unit (Fig. 3) consists of a multi-tap constant-voltage supply and a variable feedback amplifier circuit; the timing control is achieved by industrial timers regulating which variable resistors (trimpots) are placed in the half-bridge configuration to balance the thermistor (Veeco T41A11) located in the heating bath. Fig. 4 shows the timing switch configuration during each of the three phases of the analysis, 37°, 60°, and regeneration cycle. The degree of imbalance between trimpot and thermistor is sensed by the operational amplifier which has a controllable degree of hysteresis through variable positive feedback. The diodes in the negative feedback loop in this amplifier provide a high overall amplification when the imbalance between trimpot and thermistor is small, *i.e.* when the signal level is low and the diodes thus non-conducting. When the imbalance is large, the amplifier output is limited by these diodes which now present a low value feedback path ensuring that the amplifier remains within its operating range.

The heating element is controlled by a relay (TF154-C-C) which substitutes for the mercury thermoregulators of control unit No. 1. Transistors 2N2101 and 2N3707 provide a power stage for the operational amplifier since it does not have sufficient

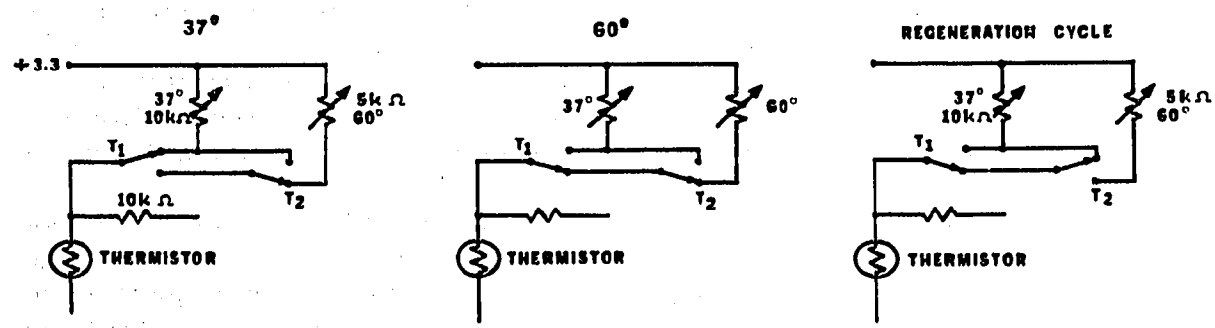

Fig. 4. Timing switch configuration for control unit No. 2 during the various phases of a typical two-temperature analysis (37°, 60° and regeneration cycle).

output drive to control the relay directly. Trimpots are used so that the temperatures may be preset to any desired value. The electronic system is conventional and a variety of alternatives is possible.

Discussion

The analysis of physiological fluids for ninhydrin-positive materials remains considerably more complex than that of simple protein hydrolysates. Urine samples present a particular problem, since a single broad peak may represent several components[1–3]. In the past, numerous laboratories have used such single-column analyzers for the analysis of the amino acid composition of physiological fluids. The major criticism of such systems has been the destruction of glutamine and asparagine during the analysis, and the lack of resolution of certain components. Both EFRON and her collaborators[4,5] and PERRY *et al.*[6] have pointed out the disadvantages of the usual buffer systems used with single-column analyzers, and have devised new sodium[4,5] and lithium[6] citrate buffer gradients. With the use of these buffer systems, the resolution of the single-column analyzer is equal to that of the dual-column systems. Both buffer systems require a change in column temperature at specific points during the analysis. This requirement is inconvenient since it may require the presence of laboratory personnel in the evening, and the temperature change must be made at a precise and reproducible interval, a condition difficult to fullfil in a busy overworked laboratory.

In the course of our experiments, we have used six different Technicon NC-1 amino acid analyzers with ten different batches of Technicon Chromobeads Type B resin. Each batch of resin has unique capabilities of resolution, even when buffer composition, column temperature and flow rate are accurately controlled. Experimentation with interval of operation at the starting temperature is required to achieve maximum resolution from each batch of resin. The two systems described above were designed to solve the above problems, and give accuracy and flexibility of both temperature and time control to obtain maximum resolution with each resin batch. These control units have been tested in our laboratory for the past two years using various buffer systems with no component failure.

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