AN AUTOMATED FLUORESCENCE-MONITORED SYSTEM FOR THE SEPARATION OF SERUM PROTEINS ON SEPHADEX*

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The system described here resulted from the need for a rapid, reproducible, relatively inexpensive automatic system for providing clean separations of serum proteins on a routine basis. The separation is based on the use of a column of DEAE-Sephadex A-50, Medium (Pharmacia Fine Chemicals, Inc., Uppsala, Sweden) which allows for the elution of four major fractions by the sequential use of appropriate eluents[1]. Automation is accomplished by putting the column effluent through a continuous flow Turner Fluorometer, Model 110 (G. K. Turner Associates, Palo Alto, Calif.), which has been made to drive a meter, the needle of which comes between a light source and a photoelectric cell as the proteins come off the column. The photoelectric cell, in turn, controls the mechanism for changing the eluent flowing on to the column and for advancing the fraction collector to the next position for collection of the effluent. Excluding the fluorometer (cost approximately $1,100), the cost of the remaining components is estimated at less than $200. A preliminary version of this apparatus was described recently[2].

MAISEL[3] has described a system for the preparative electrophoresis of proteins using acrylamide gels and ROUBAL AND TAPPEL[4] have reported the use of an automated Sephadex column for the separation of proteins, which depends on the blue color of the Lowry method for proteins as a means of monitoring the effluent from the column.

DESCRIPTION OF APPARATUS

Fig. 1 is a block diagram of the components of the system and their relationship to each other. The eluent inlet valves (V) are composed of solenoid-operated clamps, through which pass the tubes from the eluent reservoirs to the Sephadex column. A short length of rubber tubing through the clamp allows the valve to start and stop the flow of eluent at the proper time. Only one valve is open at one time.

A jack was added to the fluorometer to allow for by-passing the fluorometer meter when an external meter was plugged in. For greatest reliability, a voltage regulator (not shown) should be included in the fluorometer power line.

The external meter (M) was altered as follows: A photoelectric cell (PC) was mounted flush with the face of the meter and a light source was mounted on the outside of the meter facing the PC. A piece of light-weight carbon paper, large enough

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to cover the face of the PC, was mounted on the meter needle which moves between the PC and its light source.

The fraction collector consists of a brass arm, carrying the effluent tube from the fluorometer, which is rotated to its proper positions by a slow speed motor controlled by micro switches.

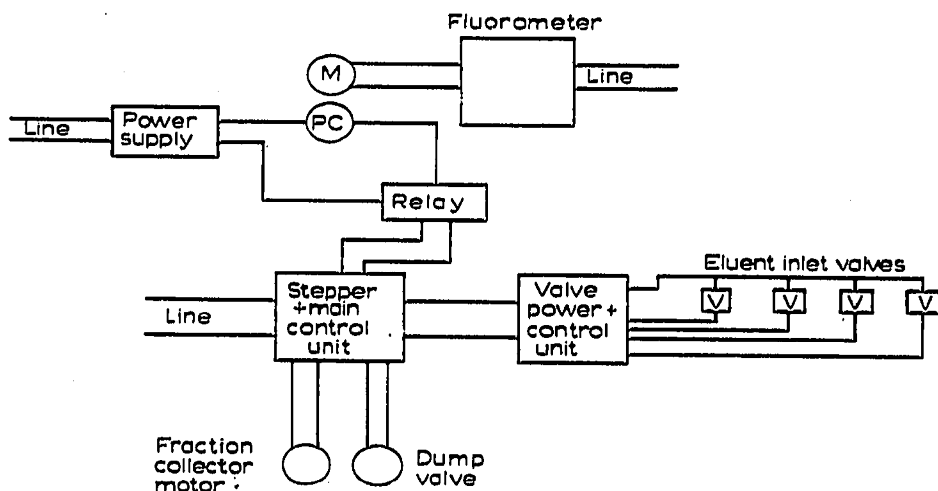

Fig. 1. Block diagram of automated serum protein fractionator system.

The dump valve is a solenoid-operated clamp situated on the effluent line between the Sephadex column and the fraction collector. It remains closed as long as a sample is being collected and opens as soon as the meter needle drops as a result of a decrease in protein concentration in the effluent. In this way only the major parts of the peak concentrations of the protein fractions are collected and the dilute valley portions are discarded.

OPERATION OF APPARATUS

A typical run is performed as follows: The day before, 3 g of DEAE-Sephadex A-50, Medium, is conditioned as directed[1], using 0.015 M (instead of 0.02 M) phosphate buffer, pH 6.6 for the final wash. Also before use, the serum protein fraction is dialyzed for 24 h in the refrigerator against 0.015 M phosphate buffer, pH 6.6, and then centrifuged immediately before use. The column is poured into a 16 mm (i.d.) glass tube to a height of approximately 100 mm. Five ml of rat serum is carefully added to the top of the column after turning on the system and adjusting the fluorometer. (The fluorometer is set as follows: Primary filter, 7–54; secondary filters, 7–60 and 50% N.D.; opening size 3 ×. With these settings, solutions containing protein at a concentration of approximately 50 μg/ml are rejected and those above 50 μg/ml are collected. For the large range of other possible sensitivity settings, see Turner advertising literature.) The first eluent is 0.015 M phosphate buffer, pH 6.6, which washes off the γ-globulin fraction. As this fraction starts coming off the column, the meter needle rises until it comes between the light source and the photoelectric cell (PC). This automatically causes the dump valve to close and start the collection of the fraction.

When the γ-globulin fraction is almost finished, the concentration of protein in the effluent decreases and the needle drops away from the PC. This shuts off the first eluent, the phosphate buffer, opens the clamp on the second eluent, 0.07 *M* NaCl, opens the dump valve and rotates the fraction collector arm to the next position. When the β-globulin fraction starts coming off the column, the meter needle covers the face of the PC, the dump valve closes and the fraction is collected. A similar sequence of events takes place for the albumin fraction, eluted with 0.21 *M* NaCl instead of 0.17 *M* NaCl as in the original directions[1], and for the α-globulin fraction, eluted with 0.38 *M* NaCl.

When the meter needle drops away from the PC after the last fraction is collected, the system turns itself off. The usual run using 5 ml rat serum takes about six hours.

RESULTS

In order to determine the quality of the separation, one-tenth, or less, of each of the four fractions collected is highly concentrated by an ultrafiltration technic which is a modification of the LKB-Produkter AB system (LKB-Produkter AB, Stockholm, Sweden). Instead of the relatively large LKB filter frame, a small, plastic, medium-pore, gas dispersion tube or filter candle, 12 × 40 mm (Bel-Art Products, Pequannock, N.J.) is used. Over this is pulled the closest-fitting dialysis tubing, tied at the bottom end. The open end of the dialysis tubing is then fixed tight to the stem of the filter candle by putting one band of rubber tubing over the stem inside the end of dialysis tubing and then stretching another band of rubber tubing over the outside of the dialysis tubing, thus sandwiching the dialysis tubing between bands of rubber tubing. Vacuum can then be applied at the stem end of the filter candle as the dialysis end dips into the solution to be concentrated. For samples suitable for electrophoresis, the ultrafiltration is carried out almost to dryness. Results of a separation (electrophoresis on Whatman No. 3 MM paper strips, barbital buffer, pH 8.6, approximately 18 h at 175 V, stained with bromphenol blue), accomplished with this apparatus, are shown in Fig. 2. Except for the trailing commonly seen in paper electrophoresis of albumin, the efficiency of separation of the remaining fractions is obvious. The remainder of each fraction, not used for electrophoresis, can be used for whatever purpose required.

The entire process can be carried out at room temperature between 21 and 24°.

DISCUSSION

Although a typical run is described only for a 5 ml serum sample, larger columns for larger samples can easily be accommodated. Other adaptations related to sensitivity and number of fractions to be collected can also be made quite readily. The automation, reproducibility, and size of sample which can be handled by this system make it extremely useful in all applications requiring routine handling of relatively large numbers of samples.

Since the system depends on the fluorescence of proteins for its monitoring capacity, substances which tend to quench such fluorescence should be avoided, if possible.

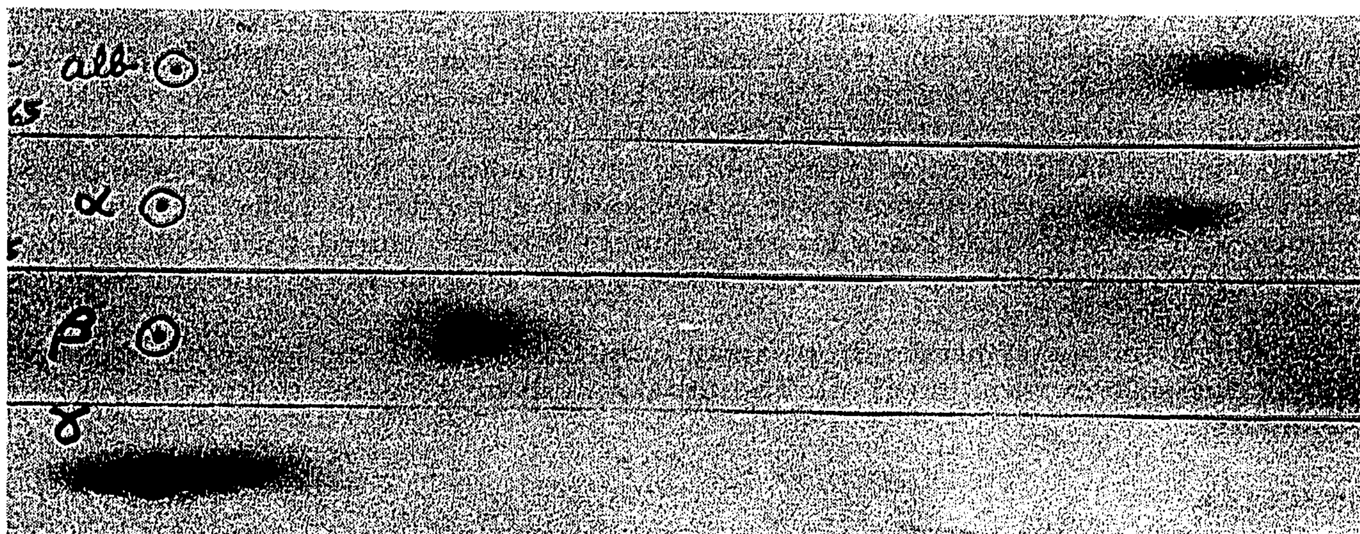

Fig. 2. Electropherograms of separated fractions of rat serum on 1 in. wide strips of Whatman 3 MM paper.

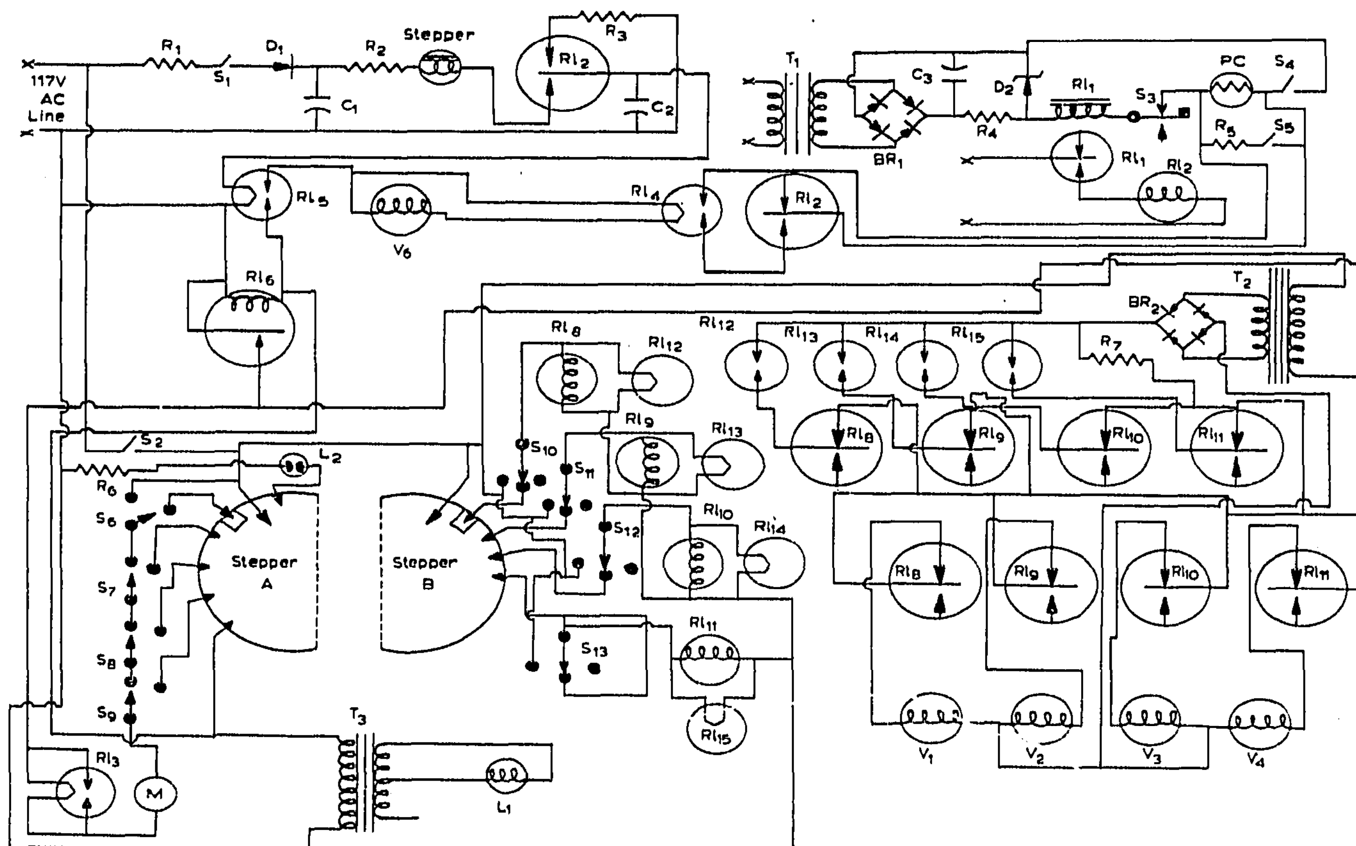

Fig. 3. Schematic wiring diagram of automated serum protein fractionator system. See List of Parts for identification of components.

LIST OF PARTS AND SPECIFICATIONS

Fig. 3 is the schematic wiring diagram for the system. The component parts are listed below:

RL_1 = Sigma 9000 Ω 11 F relay
RL_2 = DPDT 117 V AC relay
RL_3 = Amperite delay relay, 115NO60T
RL_4 = Amperite delay relay 115NC120T
RL_5 = Amperite delay relay, 115NO2T
RL_6 = DPDT 117 V AC relay
RL_8, RL_9, RL_{10}, RL_{11} = DPDT 117 V AC relay
RL_{12}, RL_{13}, RL_{14}, RL_{15} = Amperite delay relay, 115NO5T
T_1 = Transformer 117 V/24 V, 0.3 A
T_2 = Transformer 117 V/24 V, 5 A
T_3 = Transformer 117 V/2.5 V center tap, 1 A
L_1 = No. 222 lamp
L_2 = NE2 neon lamp

Stepper = 6 position 24 V stepping switch

M = 6 RPM slow speed motor
S_1 = SPST toggle switch
S_2 = SPST toggle switch
S_3 = SPDT push button switch
S_4, S_5 = SPST toggle switch
S_6, S_7, S_8, S_9 = SPDT micro switch
S_{10}, S_{11}, S_{12}, S_{13} = SPDT toggle switch
D_1 = Silicon diode 500 mA, 400 PIV
D_2 = Zener diode, 27 V, 1 W
BR_1 = Silicon bridge rectifier, 500 mA, 100 PIV
BR_2 = Selenium bridge rectifier, 5 A, 26 V AC
PC = Clarex CL-4 CdS photocell
V_1, V_2, V_3, V_4 = Spring loaded solenoids, 10 V DC
V_6 = Spring loaded solenoid, 115 V AC
C_1, C_2 = 200 μF, 250 V capacitor
C_3 = 250 μF, 50 V capacitor

Resistors:

R_1 = 100 Ω 1 W carbon
R_2 = 47 Ω 1 W carbon
R_3 = 1500 Ω 1 W carbon
R_4 = 2700 Ω 1 W carbon
R_5 = 180 Ω $^1/_2$ W carbon
R_6 = 220 kΩ $^1/_2$ W carbon
R_7 = 10 Ω 100 W wire wound.

SUMMARY

A system for automatically separating serum into its major protein fractions is described. This system is based on the use of a DEAE-Sephadex column and a flow-through fluorometer, which monitors the fractions as they come off the column and drives the automatic mechanisms which change eluents and fraction collector positions at the appropriate times. This apparatus, completely automated at low expense, can provide rapid, reproducible, preparative scale serum protein fractionations on a routine basis. A description of the apparatus and a schematic wiring diagram are included.

REFERENCES

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