COMPUTER AUTOMATION OF A NOVEL ION-EXCHANGE PROCESS FOR THE SIMULTANEOUS RECOVERY OF LYSOZYME AND AVIDIN FROM CHICKEN EGG ALBUMEN

by

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ABSTRACT

A three-column ion-exchange system was designed, fabricated and computer-automated to accommodate a novel 'elution looping' process developed by Dr. Tim Durance (U.B.C. Department of Food Science) during his doctoral studies on the recovery of lysozyme and avidin. This processing technique enhances the simultaneous recovery of these two pharmaceutically important proteins from chicken egg albumen. The processing system prototype was sized to handle throughput rates between approximately five and 300 liters per day of albumen to facilitate both laboratory and small commercial scale work. Very efficient use is made of the ion-exchange resin due to a two-column cascaded feed arrangement.

The processing control software was designed to provide flexibility and ease of operation in setting up new and existing method files, allowing for the selection of any column or group of columns to use and providing a 'staged-shutdown' approach toward handling columns fouled with congealed albumen during unattended operation. This approach attempts to maximize the productivity of the system even when one or two of the columns has become fouled with congealed albumen.

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INTRODUCTION

The primary goal in producing this work has been to take a novel ion-exchange method, developed in the Department of Food Science at the University of British Columbia (Durance, 1987) for the simultaneous recovery of lysozyme and avidin from chicken egg albumen, and to develop a computer-automated continuous-production system to accommodate this process.

Sub-goals supporting this main goal make up the basic design specifications for the system and include the following:

1. The system must be capable of operating continuously according to the novel operating sequence established by Durance (1987).
2. The system should provide for continuous feeding of albumen to ion-exchange columns.
3. The column arrangement should efficiently use the resin beds in order to minimize the quantity of resin required for a given production rate.
4. The physical structure of the recovery system must promote hygienic operation and ease of maintenance.
5. The physical plant should be compact and portable for use in research.
6. The operating system must be "user friendly", enabling it to be invoked easily by an operator with a basic knowledge of the process.
7. File handling routines should allow for the reading/writing of method files to/from storage media

(either floppy or hard disk) as well as providing printed output for record-keeping.

8. There should be some degree of flexibility in selecting which and how many columns are to be used in order to accommodate both production and research needs.
9. Columns should be easily and quickly replaced to accommodate the use of columns of different sizes and to allow for rapid in-process cleaning of the columns should congealed albumen be filtered out of the feed flow stream by the resin, restricting the flow rate.
10. The pump capacities should cover a broad range of flow rates to accommodate the various sizes of columns that may be used to allow for the variation of contact times between the process liquids and the ion-exchange resin.

1. BACKGROUND

Ion-exchange Chromatography

The theory of ion-exchange was initially studied during efforts to understand the mechanism of nutrient transport in soils . Clay particles were observed to reversibly bind inorganic nutrient cations such as Na^+, K^+, Ca^{+2} and NH_4^+ as illustrated in Equation 1.1 (Salisbury and Ross, 1978):

$$2NH_4^+ + clay{\cdot}Ca \quad Ca^{+2} + clay{\cdot}(NH_4)_2 \qquad 1.1$$

It was discovered that different cations could be classified according the relative strengths of their attractions for the negatively charged bonding sites. The inequality which describes this pattern is called the Hofmeister or lyotropic series:

$$Al^{+3}>H^+>Ba^{+2}>Mn^{+2}>Ca^{+2}>Mg^{+2}>(K^+=NH_4^+)>Na^+>Li^+ \qquad 1.2$$

The strongest to weakest attractions are shown from left to right, with K^+ and NH_4^+ exhibiting roughly equal attractions to negatively charged sites. A cation residing at a negatively charged site will generally relinquish its position to another ion having a stronger electrostatic attraction as indicated by its position in the series. This is an equilibrium reaction which can be reversed by increasing the concentration of the less strongly attracted ion. It is due to this fact that an ion-

exchange material can be stripped of strongly held ions and "regenerated" by their replacement with less strongly held ions so that the exchange process can begin again.

Commercial ion-exchange processes involve the cyclic reversal of equilibrium equations such as Equation 1.1. The exchange of sodium ions for calcium and magnesium ions in water softening systems is one of the most wide spread. Of greater importance to the bio-processing industries are exchanges involving proteins in order to effect their recovery.

Ionic proteins often exist as sols or colloidal dispersions which rely upon the affinity of the protein for water, rather than upon the electrostatic repulsion of like charges, for stability. For this reason they are called hydrophilic colloids and include such materials as soaps, soluble starch, soluble proteins and synthetic detergents (Clark et al., 1977). Hydrophobic colloids, on the other hand, rely upon the dielectric property of water to prevent flocculation and settling of these ions. Due to the polar nature of water molecules, colloids with a negative surface charge become surrounded with a layer of water molecules oriented with the positive pole toward the surface. The negative pole of each water molecule points away from the particle resulting in a negatively charged "shell" some distance removed from the actual particle. Other similar particles are repelled by the like charges before the particles are in close enough proximity for the attractive Van der Waals forces to bind them together. Metal oxides, usually positively charged, form hydrophobic sols (Clark et al., 1977).

With a size range of roughly 1-200 nm, these particles are subject to Brownian motion caused by the uneven distribution of collisions with molecules of the continuous phase. These random collisions tend to destabilize sols by forcing particles into sufficiently close contact that Van der Waals forces dominate over the electrostatic repulsion . Destabilization also occurs when the surface charge on a particle is neutralized by altering the pH of the aqueous system. Repulsive forces are reduced by coagulation (charge neutralizaton by the attachment of electrolyte counter-ions) allowing flocculation (chemical bridging between particles) to occur followed by settling (Clark et al., 1977).

The pH at which the electrostatic charge is neutralized is called the isoelectric pH or the isoelectric point, pI, of the colloid, at which point its tendency to remain dispersed is at a minimum. Lysozyme exibits a pI of 11.0 while avidin has a pI of 10.0. While many proteins including lysozyme have been recovered by the method of isoelectric precipitation, the major problem encountered with this method is that the feed stock from which the protein was recovered becomes contaminated with the neutralizing electrolytes, often making it unfit for subsequent use or processing (Durance, 1987). In order to overcome such problems, ion-exchange resins were devloped with charged chemical groups covalently bound to an insoluble porous matrix. Counter-ions could then be attached and reversibly exchanged with other ions of the same charge without altering the matrix (Pharmacia, 1980). The major benefit of this is that specific

molecules, including many pharmaceutically important proteins, can be specifically targetted and very effectively and efficiently secured onto the charged sites of the matrix without the need to add chemicals to the feed stock. In the instance of lysozyme and avidin recovery, once these have been secured onto the matrix, displacing the initial counter-ions, the spent feed stock can be processed exactly as though the protein extraction had not taken place and with no loss of functionality in terms of gel strength, whipability or nutritional value (Li-Chan et al., 1986).

A typical set of steps in the general operation of an ion-exchange column includes:

1. If the resin is being used for the first time, it should be prepared for use following the manufacturer's directions. This involves, for the Duolite C-464, washing the resin with acid, with deionized water, with alkali and then water again followed by equilibration buffer (Li-Chan et al., 1986).

2. The feed stock is then fed in downflow through the packed bed until the predetermined percentage of product is observed to be passing through the column outlet. At this point, the resin bonding sites are essentially occupied, except in the lower portion of the bed. The proportion of unexchanged sites remaining at breakthrough depends largely upon the feed flow rate and the diffusion rates for the desired product in both the liquid and solid phases. A high feed flow rate gives a low contact time

which reduces the probability that a particular molecule within the feed liquid will find an exchange site prior to exiting the bed. The contact time should be chosen with care to optimize the balance between rate of production and efficiency of recovery.

3. Backwash the column with deionized water or a suitable buffer in order to remove non-adsorbed proteins from the resin.

4. Pass a solution of counter-ions down through the column to elute the ionically bonded proteins. As the product elution step proceeds, an ultra-violet spectrophotometer is frequently used to monitor the concentration of protein in eluting buffers by comparing the absorbance of light at 280 nm wavelength between the protein-free eluting buffer and the process eluant.

5. Re-equilibrate the column for the next feed cycle by passing equilibration buffer through the column to replace the eluting counter-ions with the ion most appropriate for the capture of product ions. The process is now ready to return to the application of feed (Step 2).

This cycle consisting of steps 2 to 5 continues until the feedstock is exhausted, or a planned shut-down occurs, or in the long run until the resin is no longer able to carry out its function due to deterioration. Figure 1.1 shows schematically in flow chart form the sequence of procedures for the initial preparation and cyclic operation.

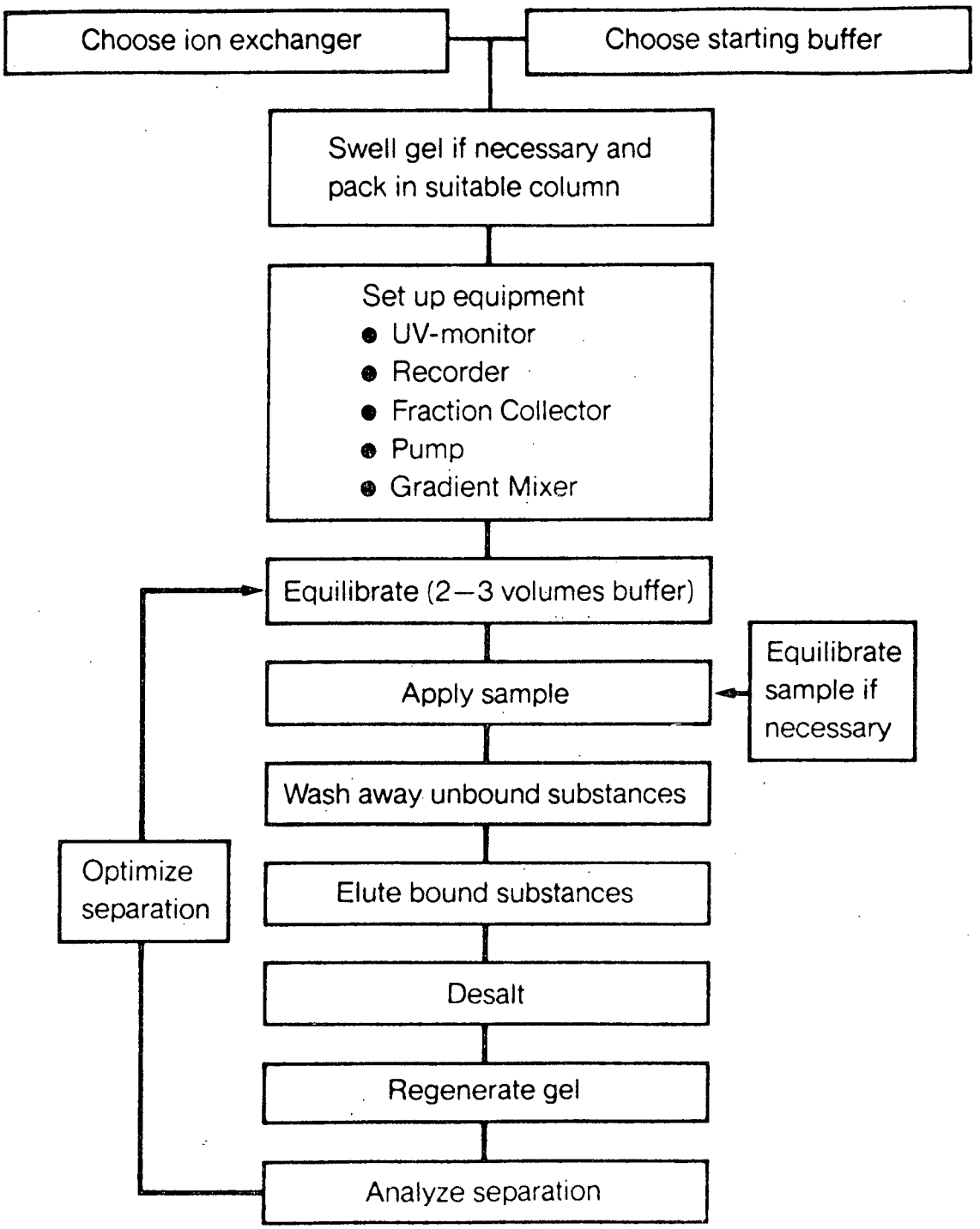


FIGURE 1.1: Flowchart for a typical ion-exchange procedure

Ion-Exchange Resins

The most basic distinction between resin types is whether the counter-ions (i.e. exchangeable ions) are positively or negatively charged. Negatively charged resins having positively charged counter-ions and are called cation exchangers while positively charged resins with negatively charged counter-ions are termed anion exchangers. Within each type of exchanger, the various covalently bound ions can be classified according to the strength with which they bind counter-ions. Strong ion exchangers maintain a state of complete ionization over a wide range of pH while weak exchangers are much more influenced by the effect of pH on the degree of ionic dissociation (Pharmacia, 1980).

Table 1.1, below shows a sampling of covalently bonded ionic groups used in modern resins to produce strong, moderate and weak exchangers.

Table 1.1 Functional groups used for ion exchangers (Pharmacia, 1980)

ANION EXCHANGERS	FUNCTIONAL GROUP
Aminoethyl (AE-)	$-OCH_2CH_2NH_3^+$
Diethylaminoethyl (DEAE-)	$-OCH_2CH_2N^+H(CH_2CH_3)_2$
Quaternary aminoethyl (QAE-)	$-OCH_2CH_2N^+(C_2H_5)_2CH_2CH(OH)CH_3$
CATION EXCHANGERS	
Carboxymethyl (CM-)	$-OCH_2COO^-$
Phospho	$-PO_4H_2^-$
Sulphopropyl (SP-)	$-CH_2CH_2CH_2SO_3^-$

Strong exchangers are formed using sulphonic and quaternary amino groups while the phospho group is of intermediate strength and the others are considered weak exchangers (Pharmacia, 1980).

Other factors to be considered when selecting a resin type include the porosity of the matrix as determined by the degree of polymer cross-linking, the change in resin volume during changes in pH, ionic strength and counter-ion, the chemical stability of the resin in the solvent used, the physical durability of the resin, and the mesh size and size distribution of the resin.

Factors Affecting Exchange Dynamics

The degree of divinylbenzene cross-linkage in a sulphonated polystyrene resin determines the pore size within. A highly cross-linked matrix provides a large number of charged sites while the small pore diameters prevent the entry of large molecules including most proteins. In order to allow proteins access to internal exchange sites, and thus increase the exchange capacity, resins were developed with an open macroporous structure. These resins are generally produced in the form of small spherical beads which are available in a variety of sizes usually between about 16 and 400 mesh (U.S. standard sieve).

The size and shape of the molecule as well as its surface charge distribution are of great importance in biological ion-exchange processes. The resin pore diameter can be chosen so that only particular molecules which are smaller than the pore diameter can enter the resin matrix. This allows for separation

in part by size exclusion as well as by ion-exchange since large ionic species can access only superficial bonding sites on the resin, while the myriad internal sites are reserved for smaller ions.

Liquid-phase diffusion coefficients for feedstock constituents can be increased by decreasing the liquid viscosity, which is in turn lowered by raising the temperature. Higher diffusion rates promote more rapid exchange kinetics and therefore allow for higher flow rates. Homogenization of biological liquids can reduce the viscosity by exposing congealed or coagulated protein to high shear forces which break them apart to make them more free-flowing. Durance (1987) used a Manton-Gaulin high pressure (6.9 MPa), small orifice homogenizer to reduce the albumen viscosity prior to feeding to the column. The major reason for homogenizing the egg albumen was to break up congealed lumps of protein in order to prevent plugging of the packed resin bed.

The contact time between the liquid and resin greatly influences the product recovery efficiency. Since the attachment of any particular ion to an exchange site is a stochastic process, some minimum contact time is required in order to ensure a given level of product recovery.

The presence of extraneous ions competing for bonding sites hinders the efficiency of product recovery in many ion-exchange processes. The recovery of lysozyme and avidin is not particularly hindered in this way since their isoelectric points are markedly higher than those of most other proteins.

Recovery of Lysozyme and Avidin

Lysozymes, basic proteins having an average size of approximately 15,000 daltons, are produced in animals, plants, and insect and fungal cells (Durance, 1987). They exhibit bacteriocidal characteristics since they can lyse certain bacterial cell walls including those of Micrococcus lysodeikticus upon which a popular assay for lysozymes has been developed. In this assay, a liquid suspected of containing lysozyme is mixed with a slurry of M. lysodeikticus and then observed turbidimetrically. If mixing the feed liquid with the dead cell slurry causes an increase in the transmission of visible light compared with a feed containing no lysozyme, then the percent increase in transmission gives a quantitative measure of lysozyme concentration (Jollès et al., 1965).

The dominant commercial source of lysozyme is chicken egg albumen which contains approximately 88% water and 10.2% protein (Wilkinson and Dorrington, 1975). Lysozyme accounts for about 3.5%, while avidin makes up only 0.05% of this total protein (Li-Chan et al., 1986).

The recovery of lysozyme has been shown to be between 90 and 95% using Duolite C-464 cation exchange resin in a packed bed (Li-Chan et al., 1986). This high recovery along with desirable physical and chemical qualities including a low degree of swelling/shrinking during cyclic changes in the liquid phase ionic strength, good chemical inertness and physical stability make this resin a good choice.

Elution-Looping Technique

The novelty of the process for which this system was designed and built is the way in which both lysozyme and avidin can be recovered simultaneously. As mentioned earlier in this section, the concentration of lysozyme is 70 times greater than that of avidin. Thus, if both proteins were eluted after each column feeding, the presence of avidin is almost totally masked by the large lysozyme peak. In order to better separate the two peaks, the fact that lysozyme can be eluted using a lower ionic strength saline solution than that required for avidin elution (although the opposite case would be expected from their relative pI's (Durance, 1987)) is used to advantage.

The avidin is allowed to remain on the column for a specified number of feed/regeneration cycles while lysozyme is removed each time. As the quantity of avidin bound to the resin increases with each cycle, its delayed removal produces a peak which is larger and more pure than that from a single cycle. While the clarity and purity of this delayed peak increases with the number of cycles between avidin elutions, the optimum has not yet been determined. This modified recovery process which Durance has termed "elution-looping" promotes the simultaneous recovery of two commercialy important proteins on a single column with high initial purities and recoveries.

2. AUTOMATION OF LYSOZYME/AVIDIN RECOVERY

Prior to discussing the particular approach taken to automating the recovery of these two well known proteins, it should be noted that the intent of this paper is to provide a total-system prototype, both hardware and software, for the recovery of lysozyme and avidin using the elution-looping technique. It does not attempt to introduce any radically new concepts in the general process of ion-exchange or in the equipment that is used. It does, however, attempt to present a system package that is particularly suited for both laboratory experimentation and commercial production. The complete flexibility regarding which and how many of the three columns are to be used enhance the experimental capabilities of the system. The "staged shutdown" capability allows for the unattended operation of the system during which time the software will modify the operating mode to exclude any column(s) which become clogged with congealed egg white and continue processing with whatever column(s) remain.

The operating logic of the system will be explained in more detail in the following sections describing the physical plant and the electronic control circuits.

Physical Plant

Lysozyme and avidin have been recovered in both batch and continuous ion-exchange arrangements. While batch methods exploit

the least expensive technology in their operation, they tend to be relatively labour intensive and inefficient in terms of the optimum use of resin. If sufficient resin is used to ensure an accurate contact time between the resin and the feed liquid, the result is a packed bed. If less resin is used, then some form of agitation is required in order to insure a uniform contact time over all of the feed stock. Ion-exchange resins tend to be easily damaged during agitation with an impeller, either open, or enclosed as in a pump. Packed column continuous feed systems, on the other hand, are better suited to optimizing operating conditions including contact times. The number of columns used dictates whether the process is continuous or not on the basis of feed input. Multiple-column systems are generally operated as multiple individual columns which are fed in sequence.

The feeding of a given column continues until product molecules begin to break through into the column effluent. At this point, since the adsorption of ions is a stochastic process, a significant portion of the resin bed at the bottom of the column is unsaturated. The exact proportion of under-used resin varies directly as the flow rate, or inversely as the contact time. If all of the resin could be fully loaded, then the capacity of the column would be increased to accommodate more feed, or the volume of the column could be reduced while treating the same quantity of feed.

This end was accomplished by operating two columns in a cascade arrangement wherein the feed is introduced into the top of the first column, and its effluent feeds into the top of the

second column. The first column can then be allowed to approach saturation while the product ions which break through are captured in the second column. This is the same effect as sampling a single tall column at its midpoint for saturation while not having to worry about losing product due to breakthrough. The benefit of the having the columns separated is that upon saturation of the first column, the feed inlet can be directed to the top of the second column while the first one is regenerated (eluted and re-equilibrated with the desired form of ion). In order for the this sequence to continue, a third column is required. Provided that the time required to load a column with the product ions is greater than the time required for regeneration, only three columns are necessary. If the feed time is less than that required for regeneration, then either the feed step would have to be interrupted or an additional column would be needed.

Two sets of names are used in referring to the columns. In order to refer to a particular column regardless of its current function, each is given an absolute name: "A", "B" and "C". In order to describe a given column with respect to its current function, relative names are also used: "Primary" (1°), "Secondary" (2°) and "Regenerating" (R).

Figure 2.1 illustrates the use of three columns which operate in this cascade sequence. The three columns exchange operating duties in a continuous cycle such that the most recently regenerated column becomes the secondary one in the two-columns series, the column that was the secondary becomes the

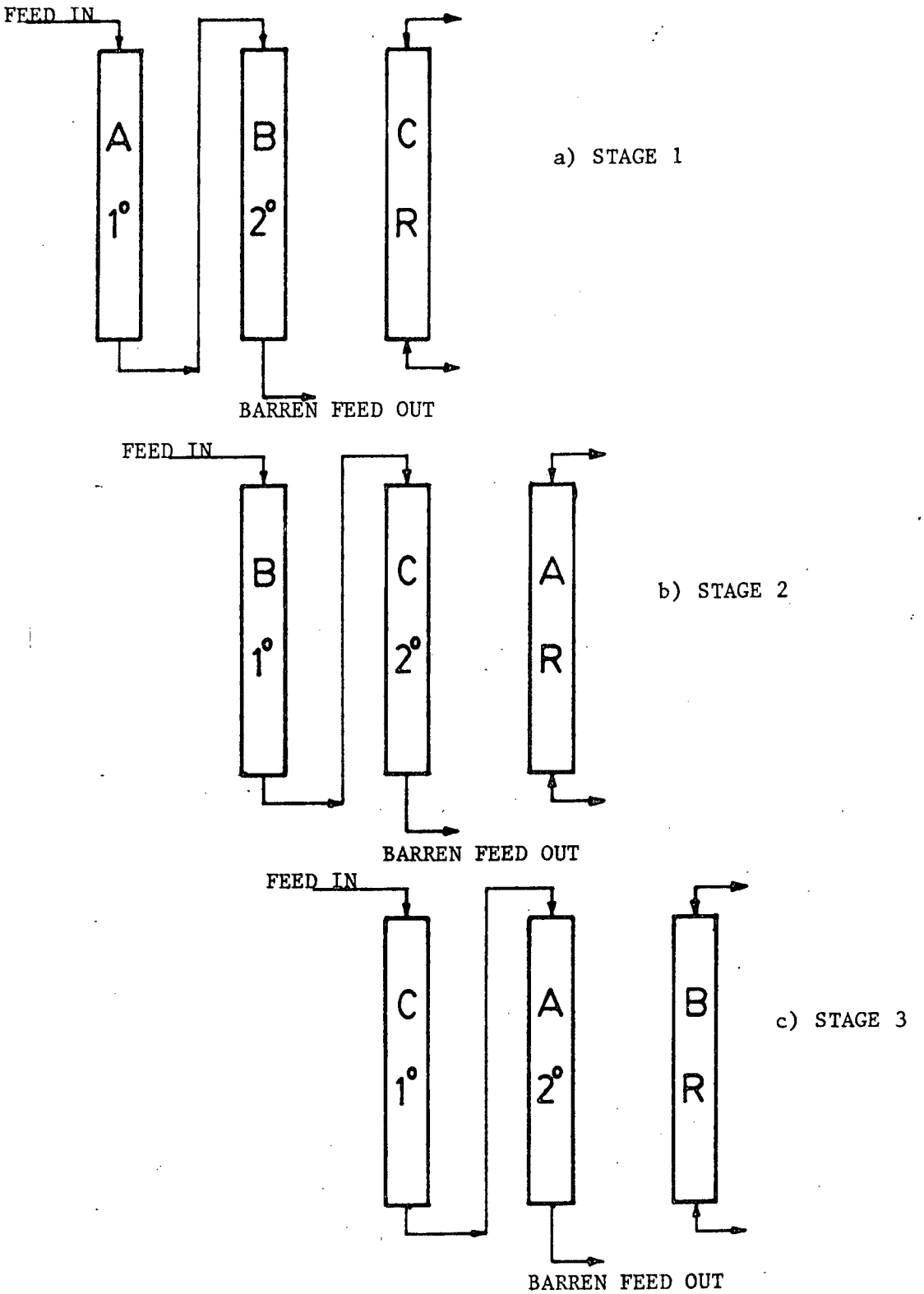


FIGURE 2.1: Cascade sequence for columns A, B and C as they operate in the capacity of primary and secondary feed, and regeneration

primary, while the primary column begins the regeneration stage.

During the cascade feeding of the primary and secondary columns, the remaining exhausted (fully loaded) column is exposed to the following regeneration steps:

1. The egg white in the column is removed by backwashing with starting buffer in order to remove any unadsorbed protein prior to stripping off the product ions.
2. A weak saline solution eluant is passed downward through the column to remove the lysozyme while leaving the avidin bound to the resin as per the elution-looping technique mentioned earlier.
3. If the specified number of loops have been performed, removing only the lysozyme, then a strong saline eluant is passed through the bed to remove the enhanced avidin peak.
4. If just the weak saline eluant has been used, then no further equilibration is necessary, however, if the strong saline was used, then the resin must be equilibrated prior to returning the column to feed duty.

This sequence of regeneration steps is thus applied to the column labelled "R" in each of the three STAGEs shown in Figure 2.1.

Operating Sequence Nomenclature

The various valve operating sequences must be labelled unambiguously in order to prevent errors in control programming. The following terms describe these sequences, beginning with the most fundamental division and progressing to the most comprehensive. These terms will be presented in capital letters wherever they are used to indicate these specific logical divisions as defined below:

STEP: The term STEP indicates a particular control setting for the 23 flow control valves, 3 pumps and the homogenizer. Each of these units can be either ON or OFF, that is, energized or not. The ON/OFF patterns for the various STEPs are shown in Appendix A. STEP time durations are assigned by the operator either through the uploading of an existing method from diskette or through the direct interactive method.

STAGE: For the column being regenerated, the term STAGE represents the total number of STEPS involved in the regeneration process. The number of STEPS in a STAGE varies dependent upon whether or not the avidin as well as the lysozyme is to be stripped from the column. For the column(s) being fed, the STAGE is equivalent to the single feed control setting of the valves, feed pump and homogenizer. The actual duration of the STAGE is the greater of the length of time required for the sequence of

regeneration STEPS or the specified feeding time.

CYCLE: If three columns are being used, then in one CYCLE each of the columns has occupied the position of "primary", "secondary" and "regeneration". CYCLE and STAGE are synonymous when running fewer than three columns since the control settings are no longer completely independent for feeding and regeneration. In the case of two active columns, the cascade feed can only take place if the regeneration procedure is completed for the second column. Since non-cascade feeding can progress while regeneration is carried out in the second column, the feeding and regeneration can be carried out in parallel but only in the forward direction, as defined by the normal operation of three columns. For a single column, it is obvious that only one operation can be performed at a time, and that feeding and regeneration must take place in series rather than in parallel. For one or two columns, then, the combined set of STEPs for feeding and regeneration is considered as a single sequence, making redundant the use of both of the terms, STAGE and CYCLE. Since CYCLE appears to better describe the completion of all required STEPs for each column, the term STAGE was dropped in the operation of just one or two columns.

The incorporation of the elution-looping technique requires that a count be kept of the number of CYCLEs run so that the avidin can be stripped from the column(s)

after the appropriate CYCLE number.

BLOCK: This counter simply indicates the number of times that avidin has been removed from the column. It increments each time the CYCLE counter reaches the predetermined value and is not trivial since the number of CYCLEs between subsequent avidin removals can be varied at any time during the process via the routine, MPC (manual process control).

Flow Path Design

The cascade approach used to recover the target ions was incorporated into the system plumbing arrangement, with an attempt made to minimize the internal volume of the system while accomodating a wide range of flow rates for different size columns. Figure 2.2 illustrates the liquid flow path and valve arrangement. The arrangement of these valves with respect to their ON/OFF states was of critical importance in minimizing the number of valves required. Of the 23 electric solenoid valves used, six are simple two-way valves which either permit liquid to flow through them or block its path completely (valves 9-14). The other 17 are three-way valves which can select either of two input streams and allow one of them to exit the valve (mixing valves) or can direct one input line into either of two output lines (diverting valves). These three-way vlaves cannot prevent liquids from flowing through them, but can only select the

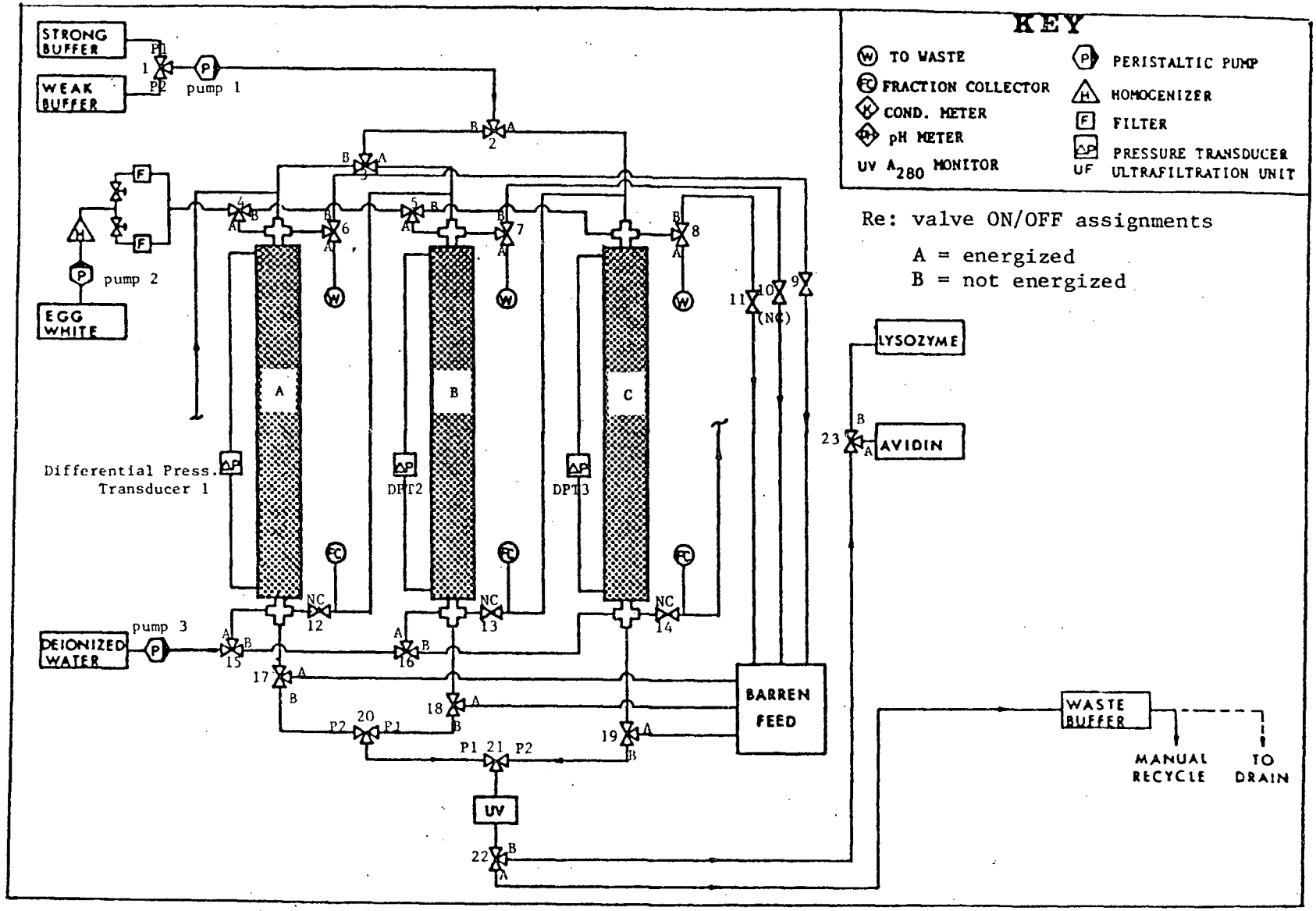


FIGURE 2.2: Liquid flow path, valve and ancillary equipment arrangement

appropriate flow path. If one of these valves is operated to permit flow through one of the two possible pathways, then the alternate path is blocked off. In this way each valve can provide dual functions at any given time.

Great care had to be taken to assure that a given physical arrangement of valves and possible flow paths would be able to accommodate every required flow arrangement, allowing for the cascade feed approach and the isolation of the regenerating column from the others through its various steps. This flow path integrity had to be maintained throughout the rotation of column duties from STAGE to STAGE.

In tracing through the flow system, it might appear that certain flow paths cannot be blocked off as required for a given sequence, however, the use of positive displacement pumps effectively provides the system with three more two-way valves as they are turned on or off.

Once the valve placements within the system satisfied the flow path criteria, the matching of inlet lines with particular input ports on the mixing valves, or outlet lines with output ports on diverting valves was done to minimize the amount of time that each valve was required to be energized. This in turn minimized the heat loading from the solenoids which would be transferred to the product or to the surrounding environment. This might be a significant heat source to be considered if the system were to be operated in a refrigerated room.

Flow Handling Equipment

Valves: The valves used in the prototype system are Burkert (West Germany) 24v D.C. electric solenoid valves designed for low pressure operation. Six two-way valves (Model 121-A), two three-way mixing valves (121-E) and 15 diverting valves (121-F) were used in the system with fittings sized to accommodate the 1/4 inch (6.4mm) outside diameter, 1/8 inch (3.2mm) inside diameter plastic tubing used.

Pumps: Three peristaltic, positive displacement pumps were used to circulate the liquids through the system. These are especially suited for low pressure systems and allow infinite adjustment of rotor speed over a wide range of liquid flow rates. Since the rollers on the pump rotor squeeze the liquid through the flow line, no direct contact with the liquid is ever made, enhancing the level of hygiene of the system. One pump moves feed liquid (egg albumen) through the system; another provides backwashing buffer, while the third one does double duty by providing either strong or weak eluent as required with the aid of a diverting valve. The pump speeds must be set manually on the controller for each pump, while the ON/OFF setting for each was made controllable by the software.

Columns: Quick disconnect fitting were used on either end of the columns to allow for the rapid replacement of columns of various sizes and aspect ratios (ratio of height to diameter) during process optimization steps (not part of this study). The columns fabricated for this system were 12" (305mm) long, made from 1"i.d.(25.4mm) clear acrylic tube, selected for its transparency and chemical inertness and sized roughly for the production of 25 liters per day of albumen. This processing rate was selected as a compromise between the minimum scale of operations which could provide for both laboratory scale research runs and small industrial scale processing of up to approximately 250-300 liters per day at the limit of the pumps. Figure 2.3 shows the construction of a typical commercial column. Screens provide the primary support for the resin column in both upflow and downflow operation. Filters at either end serve to prevent resin fines from escaping into the flow lines and to help prevent the resin bed from being blocked with congealed albumen.

Homogenizer: A laboratory size Manton-Gaulin high pressure orifice homogenizer used by Durance (1987) was effective in breaking up congealed egg white and resuspending the protein, however, its substantial size and weight made it unsuitable for use in the portable style system that was developed. The major criteria, besides portability, for

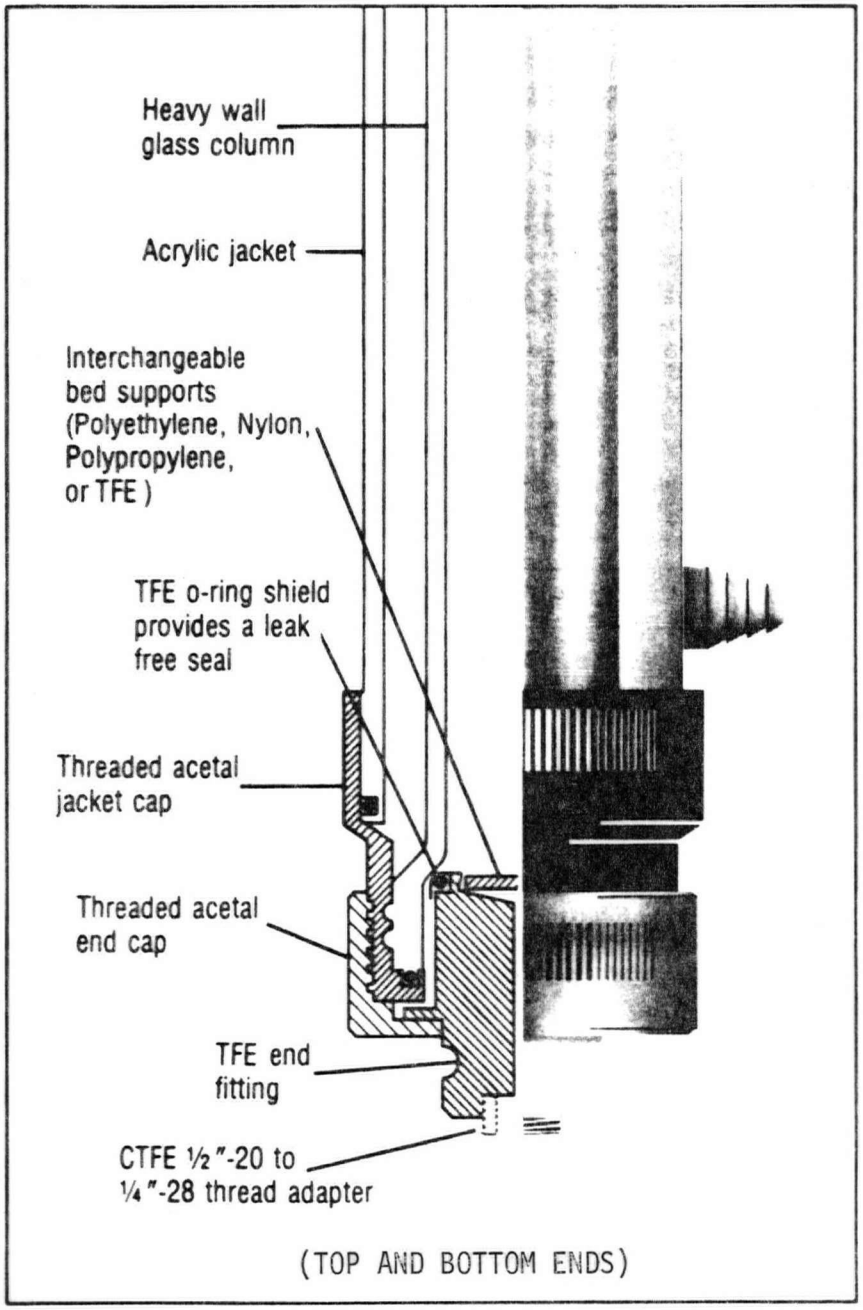


FIGURE 2.3: A typical laboratory style chromatography column with temperature control jacket (Kontes Scientific Glassware/Instruments)

the selection of a homogenizer were the possible range of flow rates, continuous processing capability, and the exclusion of air from the product while processing. While the range of flow rates needed to be matched to the desired range of processing rates as defined by the pump capacities, the prevention of air bubble entrainment in the feed stream was critical to avoid protein denaturation resulting from the high surface free energy at the gas/liquid interface. Equipment using blendor-style rotating blades were thus unsuitable due to the large-scale entrainment of air and the batch operation style, since no continuous-flow sealed processing vessel was readily available. The extremely high shear forces encountered in this type of equipment might well be much greater than is required to liquify the congealed protein, even to the point of denaturing at least the larger individual protein molecules.

The most suitable type of homogenizer found, within the above constraints, was a mortar and pestle style unit in which a Teflon (Reg.TM E.I. duPont de Nemours & Co.) rod rotates within a precision bore borosilicate glass tube. The albumen can be continuously fed through the narrow gap between the rotor (pestle) and stator (mortar). The amount of shear to which the product is subjected can be precisely controlled over a wide range by varying the speed of rotation and diameter of the pestle with respect to the fixed tube, and the feed rate,

which governs the length of time which the liquid takes to pass through the shear zone. No air is entrained in this type of unit and the shear forces can be maintained at a low enough level that bubbles are not created due to cavitation. Although this was the unit of choice, its high cost (approx. U.S. $3000) precluded its use in this prototype system.

The unit chosen was an ultrasonic horn with a totally enclosed through-flow processing cell with cooling jacket. Although microscopic bubbles are formed and collapse during excursions of the vibrating horn tip, the intensity of the vibration can be controlled, and although the vigorous tip oscillations transmit a considerable amount of heat to the liquid, the cooling jacket can be used to maintain temperatures below that which will cause the protein to denature. Again, the flow rate of liquid through the shear zone dictates, in large part, the degree of heat buildup in a given volume of liquid flowing through the unit.

Durance (1987) found, when using batch homogenized feed, that the albumen tended to recoagulate over a period of time while waiting to be processed. It was to alleviate this problem that a continuous-flow homogenizer was placed in-line between the refrigerated feed storage and the dual inlet feed filters.

Tubing: The flow tubing used in the prototype is Tygon (Reg. TM Norton Company) R-3603 formulation, a clear, flexible plastic tubing with a 1/4 inch (6.4mm) outside diameter and a 1/16 inch (1.6mm) wall thickness. This tubing is excellent for its smooth bore, clarity and ability to bend sharply without collapsing, for quick and easy setups. It is autoclavable at 121 degrees centigrade for 30 minutes at 15 pounds per square inch (psi) (103.4 kPa) pressure, has a useful operating temperature range of -45 C to +74 C, and it will not age or oxidize. The maximum working pressure for this tubing is 44 psi (303.4 kPa) at an ambient temperature of 21 C (Cole-Parmer Instrument Company 1987-88 catalogue, p558).

The length of tubing in the system was kept short by expedient placement of valves on the mounting frame, although an optimization routine could be used to determine the very best possible arrangement. For the purposes of this study, the time required to set up such a routine for computer aided optimization, in light of the relatively insignificant reduction in system internal volume, was deemed excessive.

Filters: Since chicken egg albumen has a slight tendency to recongeal, even at room temperature after homogenization, two filters were placed in parallel between the homogenizer and the columns. The filter holders (Sartorius D-3400, SM16508B), are constructed of clear

polycarbonate with a small internal volume. 50mm diameter Whatman GF/D filters (Whatman Ltd.) with a particle retention of 2.7 µm were used because of their high flow capacity, adequate filtration capacity (generally used as a prefilter for membrane filtration), and low cost.

Either filter can be clamped off from the system and changed independently while the process continues with the remaining filter. Secondary filters are located at either end of each resin column. These play a dual role in preventing the resin bed from becoming fouled should one of the prefilters become ruptured and in preventing the resin fines from escaping the column into the flow lines. The main filter supports within the columns are discs of 40x60 mesh stainless steel screen next to the resin bed. The filter material used in the columns was a synthetic non-woven cloth of fine but undetermined particle size retention held in place from the other side by a disc of coarse synthetic sponge-like material used in domestic scouring pads for dish washing. The spongy pads held the filter cloth tightly against the screen to prevent leakage of resin and/or congealed albumen around the sides of the filter. Support from both sides of the filters was required since fluid flows through the columns in both upflow and downflow as required by the control program.

Pressure Transducers: Due to the tendency of the egg white to congeal and foul the filters and resin, the operating condition of the filters is monitored to alert the control program and the operator when a flow blockage is imminent. The differential pressure is monitored across each of the three columns as well as across the inlet filters. The transducers (Honeywell) have a nominal capacity of measuring ± 5 psi (34.5 kPa) according to the unit specifications, however, all four of the transducers were tested and found to provide a useful signal output up to about +7.3 psi (50.3 kPa) and -5.0 psi (34.5 kPa). The requisite DC power source was provided from the computer interface while the output voltage from each pressure sensor was monitored by a separate channel of the analog to digital (A/D) converter board (described in a following section) via the screw terminal connector. These signals, once processed, provide the control program with information which allows it to modify or terminate the operating routine to suit the physical situation within the chromatography system.

Ultraviolet Monitor: Ultraviolet (UV) light at a wavelength of 280nm is absorbed by certain amino acid groups. The degree of light absorbance associated with these groups can be used to indicate the concentration of proteins which contain them. For the purpose of observing the concentration of eluting proteins, an in-line UV monitor

unit (UV-1 Single Path Monitor Optical Unit and UV-1 Single Path Monitor Control Unit by Pharmacia Fine Chemicals) was placed between the processing columns and the two solenoid valves which control the flow of eluting liquid to waste or to the appropriate product reservoir, depending upon whether or not the eluant contains protein at a concentration higher than some specified threshold value. The analog signal coming from the UV monitor is fed to the A/D board as an input signal for the control software. The control program monitors this signal in order to route the eluting liquid to the appropriate vessel.

The UV-1 output signal is in the range 0-10 mv DC.

Electronic Flow Control Hardware

Figure 2.4 shows the general arrangement of components which perform the electric and electronic control functions required to translate the computer output into a working flow control system.

DC Power Source: A 24 volt (v) DC power source is required to operate the flow control solenoid valves. Each valve takes an inrush current of approximately 1.1 amperes (A) and a holding current of 0.4 A. The power source used is one that was available from a previous project, a Power-One (Power-One Inc.) 7.2 A unit which proved adequate for the purpose.

The output of this unit was used as an input to the

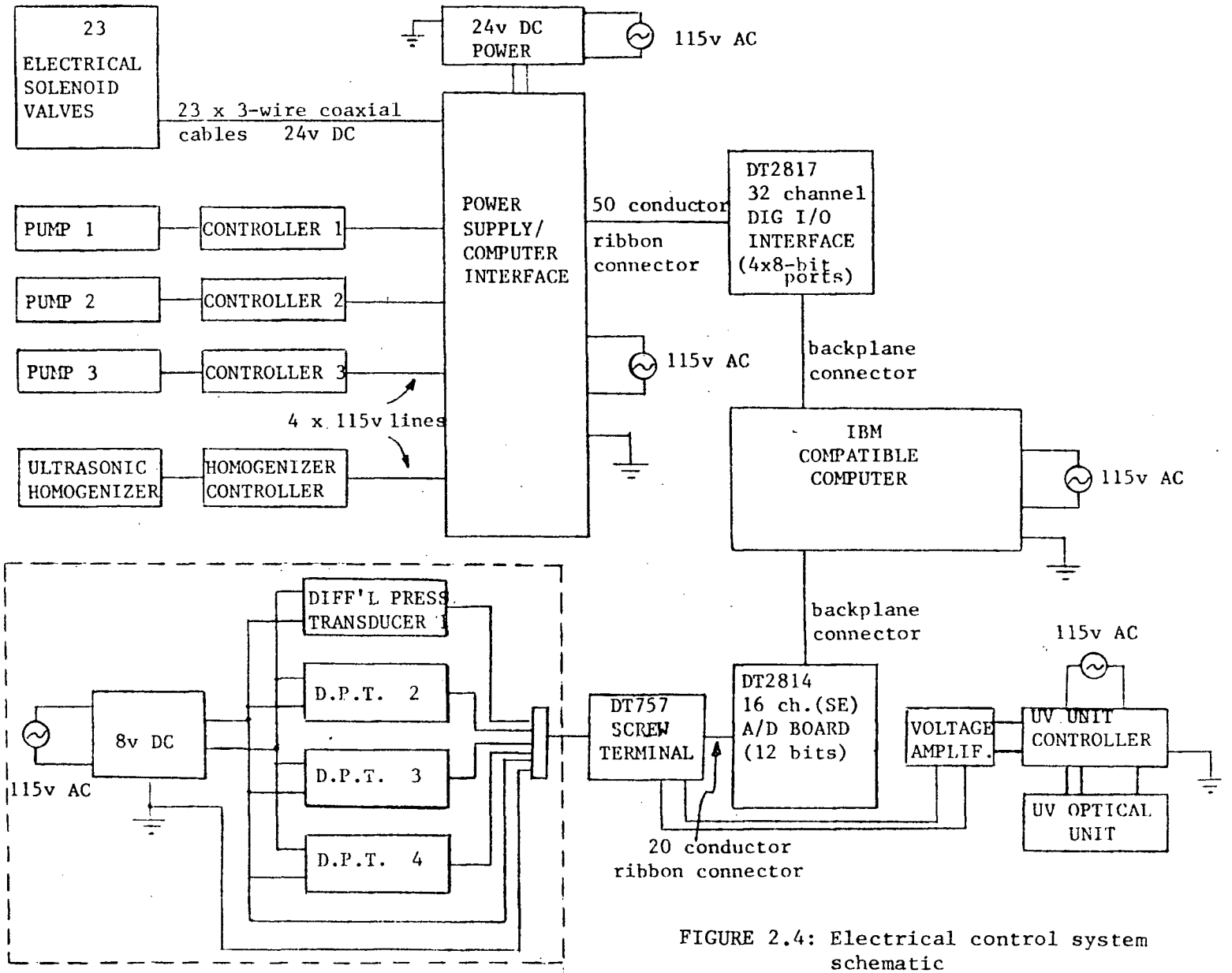


FIGURE 2.4: Electrical control system schematic

computer/system interface wherein the control signals from the computer digital output lines were used to help switch individual valves on and off according to the control program.

System Control Interface: The interface is the power dispatching center of the system. Its inputs consist of the 24v DC output from the power source mentioned above, 115v AC power used to drive the homogenizer and three peristaltic pumps, and the LSTTL (large scale transistor-transistor logic) signals from the computer.

The 2.4v (minimum) signal supplied by the digital input/output (Dig I/O) board mounted in one of the computer card slots arrives at the interface with a maximum 15.0 mA. This miniscule current must be amplified in order to switch on the flow control solenoids. A Darlington transistor arrangement, as shown in Figure 2.5 for a single channel, is used to achieve the desired electrical output. The digital output signal from the I/O board supplies the initial base current for the TIP120 Darlington-connected silicon power transistors (Texas Instruments, Inc) which then passes 24v DC current at the required amperage through the 4N32 opto-isolator (Motorola) to one of the 23 solenoids.

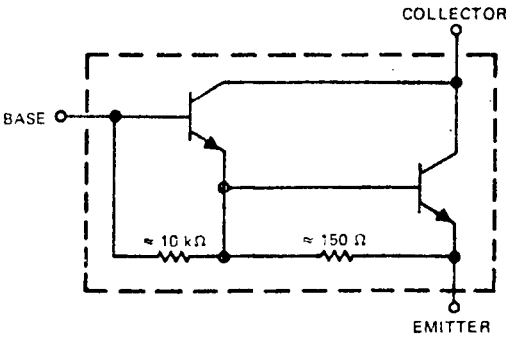
The four 115v AC outputs are triggered in a similar manner, but with the addition of a moving contact relay for each of the four channels.

TYPES TIP120, TIP121, TIP122
N-P-N DARLINGTON-CONNECTED SILICON POWER TRANSISTORS

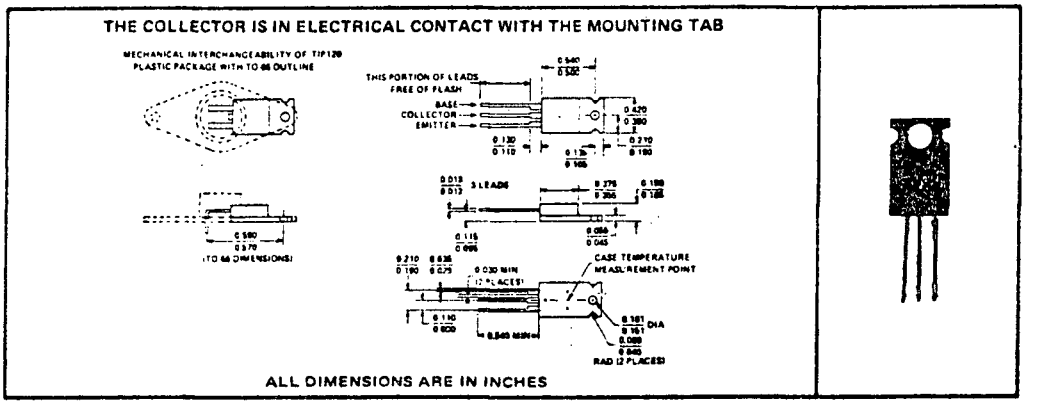
DESIGNED FOR COMPLEMENTARY USE WITH TIP125, TIP126, TIP127

- 65 W at 25°C Case Temperature
- 5 A Rated Collector Current
- Min h_{FE} of 1000 at 3 V, 3 A
- 50 mJ Reverse Energy Rating

device schematic



mechanical data



absolute maximum ratings at 25°C case temperature (unless otherwise noted)

	TIP120	TIP121	TIP122
Collector-Base Voltage	60 V	80 V	100 V
Collector-Emitter Voltage (See Note 1)	60 V	80 V	100 V
Emitter-Base Voltage	5 V	5 V	5 V
Continuous Collector Current	5 A		
Peak Collector Current (See Note 2)	8 A		
Continuous Base Current	0.1 A		
Safe Operating Areas at (or below) 25°C Case Temperature	See Figures 7 and 8		
Continuous Device Dissipation at (or below) 25°C Case Temperature (See Note 3)	65 W		
Continuous Device Dissipation at (or below) 25°C Free-Air Temperature (See Note 4)	2 W		
Unclamped Inductive Load Energy (See Note 5)	50 mJ		
Operating Collector Junction Temperature Range	−65°C to 150°C		
Storage Temperature Range	−65°C to 150°C		
Lead Temperature 1/8 Inch from Case for 10 Seconds	260°C		

NOTES: 1. These values apply when the base-emitter diode is open-circuited.
2. This value applies for $t_w \leq 0.3$ ms, duty cycle ≤ 10%.
3. Derate linearly to 150°C case temperature at the rate of 0.52 W/°C or refer to Dissipation Derating Curve, Figure 9.
4. Derate linearly to 150°C free-air temperature at the rate of 16 mW/°C or refer to Dissipation Derating Curve, Figure 10.
5. This rating is based on the capability of the transistors to operate safely in the circuit of Figure 2. L = 100 mH, R_{BB2} = 100 Ω, V_{BB2} = 0 V, R_S = 0.1 Ω, V_{CC} = 20 V. Energy = $I_C^2 L/2$.

FIGURE 2.5: Darlington-connected N-P-N silicon power transistors

Analog to Digital (A/D) Board: Most system monitoring devices produce an analog signal based on either voltage or current levels. In order for digital computers to perform any operations using this data, it must be converted to binary format. The A/D board takes a 'snapshot' of the measured parameter and then produces a digital representation of its value.

The A/D board selected for use in this system is the DT2814 by Data Translation (Marlboro, MA). It is a half-size board including 16 single-ended (SE) analog input channels, a 12-bit A/D converter and a combination hardware/software programmable pacer clock to set the sampling frequency. The maximum sample throughput for the board is 25 kHz.

Figure 2.6 gives the general layout of the card and its connection to the IBM PC style Bus (PC/XT/AT), while Figure 2.7 shows the user pin assignments.

The general operation of the board is carried out by writing to and reading from two registers located in computer memory at the Base address 220H (i.e. Hexadecimal 220) and Base+1 (221H). A WRITE command to the Base, or control/status register is used to set the control bits. Bits 0-3 are used to inform the multiplexer which one of the 16 input channels is to be sampled. Bit 4 (ENB) enables or disables the on-board pacer clock for continuous sampling, while bits 5-7 specify the decade divisor for the hardware-jumpered base frequency for

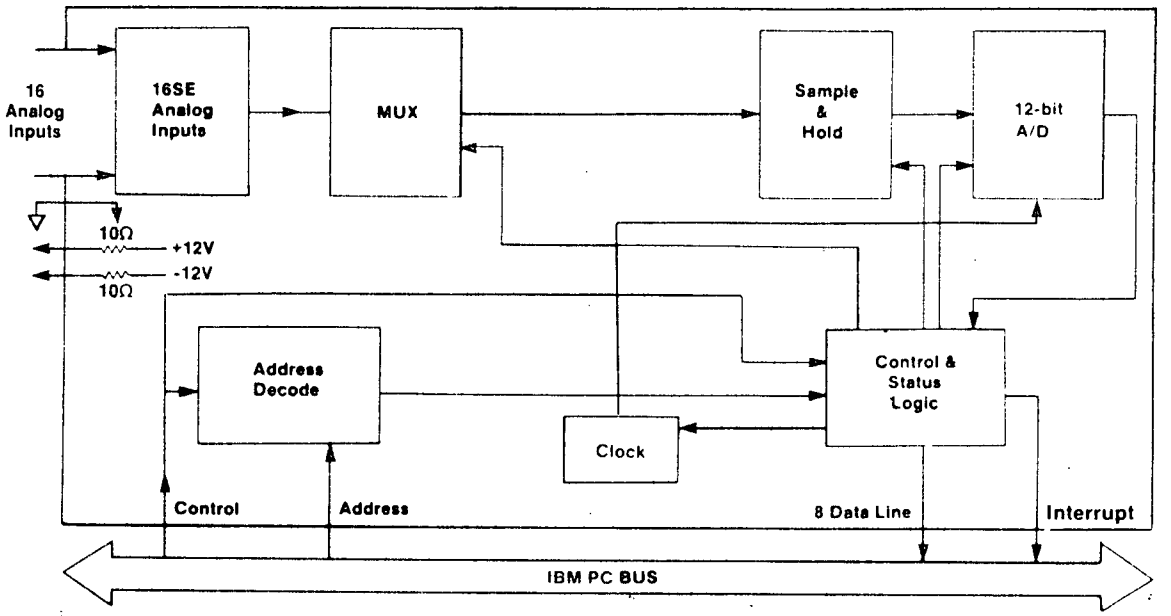


FIGURE 2.6: Card layout for DATA Translation DT2814 A/D card

PIN FUNCTION	PIN		PIN FUNCTION
Channel 0	1	2	Channel 8
Channel 1	3	4	Channel 9
Channel 2	5	6	Channel 10
Channel 3	7	8	Channel 11
Channel 4	9	10	Channel 12
Channel 5	11	12	Channel 13
Channel 6	13	14	Channel 14
Channel 7	15	16	Channel 15
Power Ground	17	18	Analog Ground
+12V Out	19	20	-12V Out

FIGURE 2.7: DATA Translation DT2814 A/D card user connections

sampling rate.

The useable sampling frequency range is 0.005 Hz to 20 kHz, however, for the purpose of the control software, continuous sampling is not required but rather single values are requested periodically by the control program. Within the program, a channel is selected and that number along with a zero value for Bit 4 (ENB) are written to the Base address. When this register receives the value sent, the on-board multiplexer (MUX) selects the desired channel and the sampling sequence is triggered.

To read the digital value, a READ command should be sent to the Base address which now returns status information regarding the data conversion. Bits 0-3 still contain the selected channel number, for verification, and Bit 4 is still the enable/disable flag. Bit 5, however, returns a value of 1 if the A/D conversion is in progress or 0 if it is complete. Bit 6 (ERR) returns a 1 if an error is encountered during a conversion as a result of too fast a clock speed or a failure to clear the data register prior to the next conversion. Too high a clock speed results in a second conversion being initiated before the first one is complete. Bit 7 (FINISH) is set (1) if the conversion is complete and the data register value is valid, or is cleared (0) if the conversion is incomplete and the data register value is invalid.

Where samples are taken in the control program, the

selected channel is written to Base and then the FINISH bit is monitored until set, at which time the data register (Base+1) double byte is READ. In reading the data, two separate READ statements to BASE+1 are required: the first yields the most significant 8 bits while the second retrieves the least significant 4 bits. The 4 bits occupy the most significant positions of the second byte read at Base+1. In order to arrive at a single meaningful value from the combination of these two values, the first one must be multiplied by 16 (i.e. shifted four binary columns to the left) while the second must be divided by 16 in order to compensate for the fact that it occupies the left (most significant) half of the second word read.

Signals get from the sampling instruments to the A/D board via a screw terminal (DT757) and 20 conductor ribbon cable.

Digital Input/Output (I/O) Board

This Data Translation (Marlboro, MA) board, DT2817, is a half-size card containing 32 channels. These are programmable for either input or output in groups of 8 channels per port for each of 4 ports. The Base address is factory set at 228H, although it can be changed via on-board jumpers, and contains the control register. Addresses Base+1 to Base+4 contain the ON/OFF status for

each of the 4 ports.

A WRITE statement containing the I/O status of the 4 ports must first be sent to the Base address. Only the four least significant bits are used in this statement: a BIT 0 value of 0 sets port 0 (channels 0-7) as input, while a value of 1 sets them for output. The same pattern holds for BITS 1-3 for ports 1-3 (channels 8-32).

For input signals to the card, a BIT value of 0 (logical low) corresponds to a maximum voltage of 0.8v at -0.2mA, while a value of 1 (logical high) corresponds to a minimum 2.0v at 20.0μA. Output signals must a maximum 0.4v at 24.0mA for a logical 0, while a high signal must be a minimum of 2.4v at -15.0 mA.

The DT2817 is connected to the IBM-PC/XT bus with pin assignments as shown in Figure 2.8. The 32 I/O lines are connected to the system interface by a 50 conductor ribbon cable with pin assignments as shown in Figure 2.9.

Process Control Computer

The computer used to monitor and control the ion-exchange system is an IBM PC/XT compatible unit. It contains a multi-purpose card from which the clock is extensively used in the control routines. The on-board memory contains 640 kbytes of random access memory (RAM). The computer clock operates at the standard PC/XT

PIN	MNEMONIC	SIGNAL DESCRIPTION
A1	-I/O CH CK	No Connection
A2	+D7	Data Bit 7 (MSB)
A3	+D6	Data Bit 6
A4	+D5	Data Bit 5
A5	+D4	Data Bit 4
A6	+D3	Data Bit 3
A7	+D2	Data Bit 2
A8	+D1	Data Bit 1
A9	+D0	Data Bit 0 (LSB)
A10	-I/O CH RDY	No Connection
A11	+AEN	Address Enable
A12	+A19	No Connection (MSB)
A13	+A18	No Connection
A14	+A17	No Connection
A15	+A16	No Connection
A16	+A15	No Connection
A17	+A14	No Connection
A18	+A13	No Connection
A19	+A12	No Connection
A20	+A11	No Connection
A21	+A10	No Connection
A22	+A9	Address Bit 9
A23	+A8	Address Bit 8
A24	+A7	Address Bit 7
A25	+A6	Address Bit 6
A26	+A5	Address Bit 5
A27	+A4	Address Bit 4
A28	+A3	Address Bit 3
A29	+A2	Address Bit 2
A30	+A1	Address Bit 1
A31	+A0	Address Bit 0 (LSB)
B1	GND	Ground
B2	+RESET DRV	Reset Driver
B3	+5V	+5 Volt Power
B4	+IRQ2	No Connection
B5	-5VDC	No Connection
B6	+DRQ2	No Connection
B7	-12V	No Connection
B8	RESERVED	No Connection
B9	+12V	No Connection
B10	GND	Ground
B11	-MEMW	No Connection
B12	-MEMR	No Connection
B13	-IOW	I/O Write Command
B14	-IOR	I/O Read Command
B15	-DACK3	No Connection
B16	+DRQ3	No Connection
B17	-DACK1	No Connection
B18	+DRQ1	No Connection
B19	-DACK0	No Connection
B20	CLOCK	6MHz
B21	+IRQ7	No Connection
B22	+IRQ6	No Connection
B23	+IRQ5	No Connection
B24	+IRQ4	No Connection
B25	+IRQ3	No Connection
B26	-DACK2	No Connection
B27	+T/C	No Connection
B28	+ALE	No Connection
B29	+5V	+5 Volt Power
B30	+OSC	14.318MHz
B31	+GND	Ground

FIGURE 2.8: Digital I/O card backplane connections for DATA Translation DT2817

SIGNAL NAME	PIN NO.		SIGNAL NAME
Digital Ground	1	2	Digital Ground
Port 0, bit 0	3	4	Port 1, bit 0
Port 0, bit 1	5	6	Port 1, bit 1
Port 0, bit 2	7	8	Port 1, bit 2
Port 0, bit 3	9	10	Port 1, bit 3
Port 0, bit 4	11	12	Port 1, bit 4
Port 0, bit 5	13	14	Port 1, bit 5
Port 0, bit 6	15	16	Port 1, bit 6
Port 0, bit 7	17	18	Port 1, bit 7
+5V Out(1A max)	19	20	+5V Out(1A max)
Digital Ground	21	22	Digital Ground
Digital Ground	23	24	Digital Ground
Digital Ground	25	26	Digital Ground
Digital Ground	27	28	Digital Ground
Digital Ground	29	30	Digital Ground
+5V Out(1A max)	31	32	+5V Out(1A max)
Port 2, bit 0	33	34	Port 3, bit 0
Port 2, bit 1	35	36	Port 3, bit 1
Port 2, bit 2	37	38	Port 3, bit 2
Port 2, bit 3	39	40	Port 3, bit 3
Port 2, bit 4	41	42	Port 3, bit 4
Port 2, bit 5	43	44	Port 3, bit 5
Port 2, bit 6	45	46	Port 3, bit 6
Port 2, bit 7	47	48	Port 3, bit 7
Digital Ground	49	50	Digital Ground

FIGURE 2.9: Digital I/O card user connections for DATA Translation DT2817

frequency of 4.77 MHz. The only additions to the system were the two I/O cards used to interface with the process monitoring and control equipment.

The control system was purposely kept as simple as possible so that the functionality of the software was not overcome by requirements for expensive graphics cards and massive memory upgrades. While a real-time color graphic display of the physical system is often more desirable than text only, the costs involved in developing and accomodating such a display are high and unwarranted at this time.

3. PROCESS CONTROL SOFTWARE

Mandatory Control Functions

The most fundamental requirements of the control software include monitoring the condition of the system via electronic sensors, accounting for the elapsed time of various sequence steps and providing real time control output signals to the physical flow control system.

As in any decision-making process, it helps to have an up-to-the-minute account of pertinent facts. Those most indicative of the state of the current ion-exchange process include the pressure drop across each of the columns and across the inlet albumin filters, the presence or absence of proteins in the eluted fraction of the column undergoing the regeneration procedure, and the temperature and flow rates of the system liquids. Due in part to the costs involved in securing extra sensors for thermal and volumetric or mass flow measurement and in part to the relative ease with which both the temperature and flow rates can be controlled, only the pressure sensors and ultraviolet monitor were used.

Elapsed times for the various STEPs involved in the process were used as the basic criteria for triggering STEP changes.Since during the course of a run, certain parameters are subject to some degree of variation due to changes in physical and/or chemical properties of the organic feed liquid and the resin, an event-controlled sequence is in theory preferrable to a rigid, timed one. Event control allows for optimal use of the system

while timed control has the benefit of simplicity in terms of the sensing equipment requirements and software structure. Since no suitable on-line assay has been perfected for the detection of lysozyme in the albumin leaving the primary feed column, a timed procedure becomes the default method of choice.

Output control signals are stored in array form on the program diskette and are uploaded as arrays into computer memory during the initialization procedure prior to the operator's interactive participation in beginning a run. The control values for each STEP are stored as four 8-bit words with each bit contributing the ON/OFF status of one output line from the digital I/O card to the control interface. These array values are fixed according to the physical structure of the flow control system and cannot be altered from within the program.

Control Enhancements

The basic control of the ion-exchange system presented herein is relatively straight forward and simple as is indicated by the modest length of the previous section. At this point, the control program would operate in much the same manner as a mechanical clock used to trigger a signal that would increment the STEP number with its corresponding control output values. No information would be passed along to the operator, and all control values would have to be assigned to fixed arrays by editing the program between runs. However, since few operators want to be bothered with having to modify the software for each change of conditions, an interactive program which provides

menus, prompts and validity checks for required input data is a far more useful vehicle through which to accomodate rapid set-up procedures.

The following sections outline these and other enhancements which add to the flexibility and user-friendliness of the control environment.

Interactive input:

Interactive data entry provides a fast and safe method of allowing operating parameters to be set and modified by the operator without his/her having to modify the control program. It is a quick data entry method since all potential changes are presented on the monitor in a logical progression and important value assignments are not easily overlooked. Information is prompted for as required. The safety of data entry is augmented since the program can screen the input data against pre-set limits to prevent untenable situations.

The use of menu structures allows the operator to select particular parameters for which to assign values or simply to view existing ones without having to progress through the entire parameter list each time. Once presented with a menu of options, a selection is generally made by entering the line number to its left. This allows the operator great flexibility and speed in adressing particular parameters via single key stroke selections. If data input is called for once the selection has been made, values are prompted for on

the screen, and once entered, they are tested to ensure program integrity. Appropriate error messages are displayed in the event of inappropriate entries.

Parameters which can be assigned values prior to or during a run are described in the following sections.

Visual display:

Just as the software requires certain information in order to provide appropriate control sequences for the system, the operator also must remain informed as to its state so that he/she can make knowledgeable adjustments to the control parameters as they are needed.

System information is displayed in text format as the simplest case, although a graphical display of the physical system would be more aesthetically pleasing. The monitored and calculated parameter values are updated several times per second. Figure 3.1 shows a typical screen during automatic process control (APC).

At the top of the screen, the title indicates that the system is being actively controlled by the program; the alternative to automatic control is manual process control (MPC) which can be accessed by pressing the "M" key at any time during the course of the run as noted in the bottom line of the screen.

The second line provides information on the number of columns operating and whether or not a staged shut-down (described in a following section) is in progress, as well as

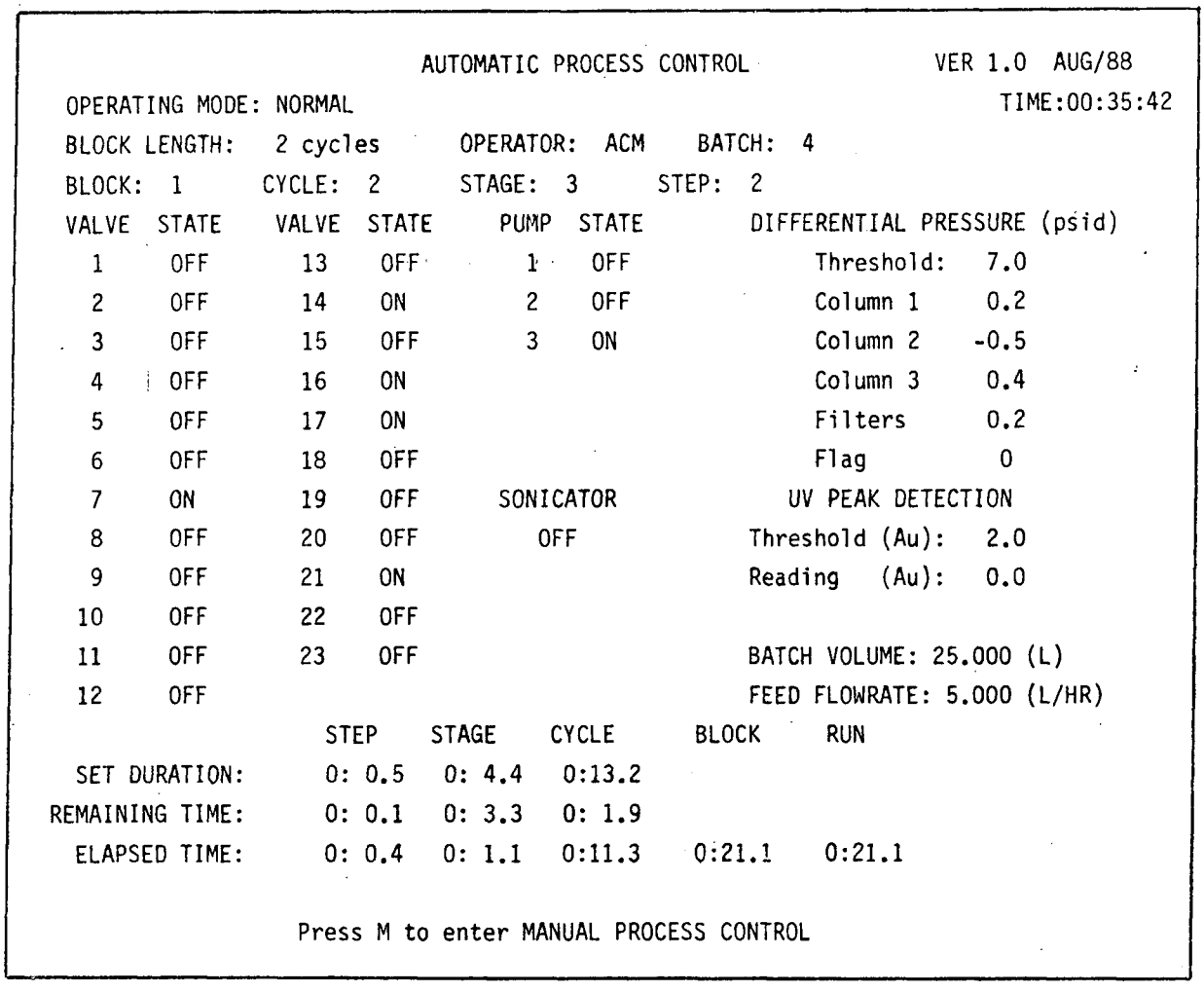


FIGURE 3.1: Typical APC (Automatic Process Control) screen

the current time.

Line 3 shows the values of BLOCK length, which is the number of elution cycles per column between removals of avidin (as per the elution-looping concept discussed in section 2 of this paper), the Operator Identification label, and the Batch Number.

Line 4 shows the current values of the sequence indicies, BLOCK, CYCLE, STAGE and STEP.

The left-most two thirds of the screen from line 5 to line 17 are the state indicators for all controlled equipment. The accuracy of the displayed values, ON/OFF, is ensured by having them read from the digital I/O card output latches rather than simply assigning them the intended values harboured by the software. This feature is invisible to the operator, although it is a useful trouble-shooting tool when comparing the actual outputs to the intended ones as shown in Appendix A.

The right third of the screen displays the current values of all monitored variables as well as the threshold values which when exceeded are used to trigger some form of control event to override the normal timed sequences. Below these are listed the Batch Volume and Feed Flowrate for general information only since they are neither monitored nor controlled by the program. In a commercial operation it may well be expedient to monitor the various liquid flow rates for both system logging and control purposes.

The next four lines of the display show the allotted,

remaining and elapsed time for each of the control sequence times.

While this screen arrangement is not necessarily optimized for the usefulness of all data displayed or the speed with which it can be digested by the operator, it is functional and adequate for this version of the program.

Default drive path:

Menu 1 asks the operator to specify what type of disk drive the computer uses, and from this the default drive path is assigned Drive A for single floppy drives, Drive B for dual floppy drives, and Drive C for hard disk systems. This path can be changed at any time during the pre-run set up procedure or during the program via Menu 2. Even when the default path is specified, it can be overridden during storage and retrieval operations on method files by specifying the method file path in response to the prompt. If no path is specified, then the default path is used.

File handling routines:

In most chromatographic work, some attempt is made to optimize process parameters for the recovery of specific products from a given feed stock. A listing of these run parameter values is called the "method". When a method is to be used more than once, it is very desirable to store it in a method file on a computer diskette for subsequent retrieval. This not only speeds the set-up procedure greatly, but also

prevents the incorporation of typographical errors into the method.

Three routines have been written for the storage, retrieval and output to printer of method files. These routines are accessed from the third menu in the initialization section of the program.

Upon invoking either the storage or retrieval routines, the option exists to view the method file names prior to entering the name of the selected file. The operator may also decline this option and enter the drive path and file name at the prompt. If the default drive path (as established at the outset of the program) is to be used, then only the file name needs to be entered. If the view-files option is selected, then a search is made of the method file disk in the specified drive path. Only files having the extension, ".SET" (indicating "set-up" data) are selected in a directory search. The operator does not have to concern him/herself about the use of the extension since the method storage routine automatically attaches it to any files to be stored. When the directory search produces file names ending with ".SET", they are piped to a DOS SORT routine for alphabetizing and are then redirected into a temporary file from which they are recalled for display in blocks of 25 names. Once the desired file has been identified, its name is keyed in by the operator. If this routine was accessed through the method storage routine, then it may be that the operator simply wants to avoid overwriting an existing file

or to ensure overwriting the correct one. If the method files are viewed via the retrieval routine, then once the file name has been entered, the contents of the file are up-loaded and assigned to the appropriate parameter labels.

In order to prevent accidentally overwriting method files and/or "crashing" the program , a wide variety of DOS file error handling interrupts have been accomodated in the program's control structure. Thus, even if an operator keys in an existing file name when down-loading a new method, the program will alert him/her to the fact and offer the alternatives of confirming the action, assigning a new file name, or aborting the procedure.

If an attempt is made to up-load a method from a non-existent file, the operator is alerted to the fact, and the options of re-entering the file name or aborting the procedure are presented for alternate action.

The data stored in these method files includes the STEP time values for all system operating modes, and the values of the process constants presented in Menu 4.

Staged shutdown:

The concept of a system which can continue to operate in the event that one or two of the three columns becomes fouled with congealed albumin was incorporated into the program to promote the highest possible production from an unattended system. Such a system could be left to operate 24 hours per day with only a single work shift to maintain it.

Rather than providing a system with a simple alarm and a total shutdown when a blockage occurs, the software brings the impending blockage to the attention of the operator when the pressure drop across one of the columns or the inlet filters exceeds a lower overpressure threshold. If the partial blockage is not removed and the high overpressure threshold is reached, then the feed pump is stopped until the regenerating column has completed its regeneration sequence and is ready to have albumen passed through it. Once the regeneration is complete, process control is passed from normal 3-column operation to the routine APC which causes the fouled column to be backwashed with phosphate buffer to remove the feed liquid from the resin bed.

Process control is then passed to a routine which uses a modified control sequence to permit operation of the two remaining columns while keeping track of the elapsed number of CYCLEs required for the elution-looping procedure. The process continues with these two columns until the offending column is cleaned and returned to service, or one of the remaining two becomes fouled or a total shutdown is effected by a programmed endpoint or a manual intervention. If one of the two columns is badly fouled, then a sequence of events occurs which is similar to that for the transition from three columns to two, leaving one column still active.

The staged shutdown method of operation thus provides for a well controlled system response in the event of a column or filter failure due to fouling. This control strategy is

optimized for continuous production work with a minimum of operator intervention.

Column selection:

At the beginning of the program, Menus 2 and 2.1 provide for the selection of the initially active column(s). All three columns, any combination of two, or any single column can be selected. This flexibility facilitates both commercial and research work.

Manual process control:

This mode is entered from any of the automatic control routines by depressing the "M" key. Several options are available while the status of the flow control hardware remains fixed as it was when the key was pressed. From the main MPC menu, STEP time durations and process constants can be viewed and/or changed, equipment ON/OFF status can be toggled for any valve, pump or the homogenizer, the UV monitor baseline can be set, and a DOS shell can be set up using the SYSTEM command to allow other DOS activities to be run without exiting the control program. The final selection permits the return of control to the automatic routine from which the MPC call was made.

Alarms:

Both audible and visual alarms are provided to alert the operator to overpressure conditions within the system in

order to allow time for correction of the problem before a staged shut down takes place. A warning message is also presented at the bottom of the screen.

The audible alarm can be shut off by a keystroke, while the message remains until the situation is remedied. Audible "beeps" are also used to indicate incorrect or out of range data entries prior to reprompting for correct data.

Start time options:

This feature allows the process to be initiated either immediately or at some specified date and time. The delayed start option is likely to be most beneficial to researchers who often find it difficult to conduct chromatographic runs within the bounds of normal working hours. With this option, a run can begin unattended at any time of day or night and on any date so that the products of the run can be ready for analysis as soon as the staff arrives in the morning. This capability could speed up research involving long run times.

Step time assignments:

As selected from Menu 2, option 3 allows process STEP durations to be assigned to all STEPs having the same function in each of the three operating modes (one, two or three columns active) simultaneously. When this selection is made, Menu 2.3, STEP descriptions, is presented without reference to any particular operating mode. Once entered, these durations are then written to the STEP time array

variables for each of the modes. STEP times for individual modes can be altered without affecting the time duration of similar STEPs in any other mode. The current STEP time durations can also simply be viewed for all or any single operating mode without being queued for changes.

Run completion setpoint:

The endpoint for a run can be prior to beginning the run or at any time within the run by specifying the BLOCK, CYCLE, STAGE and STEP number at which termination should occur. When these values match the corresponding ones from the screen display the process is halted and a message is displayed to show whether the shutdown was planned or occurred due to malfunction. By setting the endpoint prior to beginning a delayed start run, the process can be made, for instance, to start a run during the night and stop after one CYCLE or one BLOCK, whether or not the apparatus is attended by an operator, so that analyses can be undertaken immediately as personnel are available.

Automatic column cleaning:

When any column(s) is (are) retired from the process due to fouling, it (they) is (are) backwashed with phosphate buffer to remove any albumen and thus prevent it from being stored at room temperature in the resin bed for prolonged periods of time. The same treatment is accorded all active columns at the normal termination of a run.

Program Structure and Operation

The physical operation of the system involves five basic steps:

1. Turn on power to all system components including:
   - computer
   - printer
   - DC power source
   - control interface
   - pumps (ensure correct pumping speed)
   - homogenizer (ensure correct intensity)
   - differential pressure transducer module
   - UV monitor detector and controller

2. Place the program diskette in drive A for single and dual floppy drives.

3. (a) For floppy drives, simply type in "IX" and then press "ENTER" (or "RETURN" depending upon the keyboard), and the program, which resides in the diskette file "IX.EXE", will begin as soon as it is read into the computer memory.
   (b) For hard disk drives having the program in storage, prefix the program name with the appropriate drive path (see DOS manual for details).

4. Enter the run data required via the interactive menus

5. Start the automatic control portion of the program via the "Start time options" selection from the Main Menu.

The general control structure of the computer program is shown in Figure 3.2 with arrow heads representing the direction of control flow. Lines having arrows on both ends indicate subroutine CALL and return sequences. Included in the figure are the most prominent menu- and non-menu subroutines. The menu-, or interactive portions of the program are shown with the available selections as found in the program. The non-menu routines are comprised of the initial declaration statements and the assigning of default values to control, STEP duration and process constants arrays.

At the start of a new run, the Main Menu is presented to the operator. The seven options are presented in the order in which they will generally be used in the course of a run.

The first option, "System Configuration", allows the user to specify what type of disk drive arrangement is being used, which and how many columns are to be used, whether or not the columns are to be equilibrated after the stripping of lysozyme with weak saline solution, and how many CYCLEs are to be run prior to the removal of avidin from the column(s). The default values provided are: the previous entry for drive type (stored after each run), all three columns, no equilibration, and the previous entry for the elution-looping CYCLE number, respectively.

Option 2, "Files (Recall;Save;List)", provides access to the file handling routines through which method files can be up-

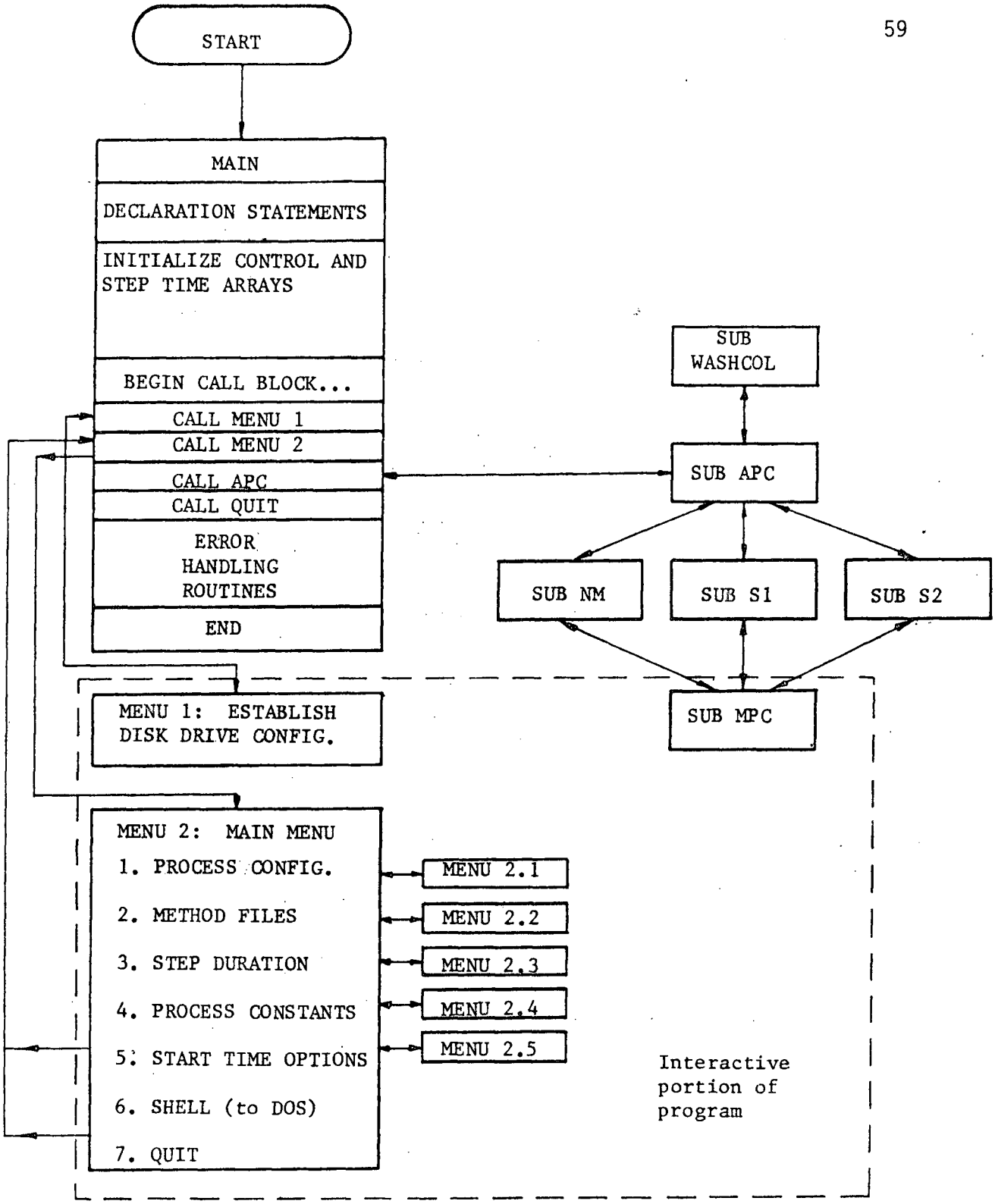


FIGURE 3.2: General control structure of process software

loaded to the program arrays for use in the current run, down-loaded from the run arrays to disk file, or listed to the printer (LPT1).

Option 3, "STEP durations", allows the operator to view the current length of STEP times in any or all of the operating modes, from normal operation and staged shutdown modes. Values can be changed by direct keyboard input at this time. The new values will be used in the automatic control portion of the program, but in order to save them for future use they must be stored on disk using option 2.

Option 4, "Process Constants", shows a list of parameters and their current values. Some of these, such as 'Operator ID' and 'Run number', are provided strictly for the sake of run documentation while others such as the pressure and UV threshold values allow for the sensitivity of the control system to be varied. The A/D and Dig I/O card base addresses can be changed from the factory-set values should it become necessary. Most users will never change the default settings supplied with the program, however, when the method file is stored via option 2, the current address values are included.

Option 5, "Start Time Options", provides entry to the automatic control routines. The run can be started immediately or at any time specified using the delayed-start option.

Option 6, "SHELL", is not required to be used, however, it allows the operator to temporarily install a second copy of COMMAND.COM with which to run other DOS software while keeping the control program resident and ready to go. As outlined in the

DOS documentation, the resident program can be reentered by typing "EXIT" at the DOS prompt for the second level COMMAND.COM.

Option 7, "Quit", brings up another menu which prompts for confirmaton of this action before allowing the program to end, and allows for the last minute saving of the method file before this data is lost.

When all of the run parameters have been set and the run has been initiated, control passes to the subroutine, APC (automatic process control), which selects the proper operating mode based upon which column(s) are to be used. For the sake of this process description, the normal three-column configuration will be considered. The other operating modes, for two or one column(s), follow a similar procedure.

For the operation of three columns, then, control would be passed to the subroutine, "NM" (normal mode), which would then provide the following general sequence of events for control:

- Elapsed times are initialized for RUN, BLOCK, CYCLE, STAGE and STEP variables as a "DO LOOP" is entered for each of the latter four parameters (recall that STAGE is eliminated if fewer than three columns are used). The STEP loop is the inner-most, while it contains a STEP time loop in which the basic control of the system is located.
- Within the STEP number loop, but prior to the start of the time loop, the STEP number is monitored to ensure that the avidin removal STEP is not executed unless the current CYCLE number matches the number of CYCLEs specified for elution-looping. As well, the total time for the

regeneration STEPs is compared with the cascade feed duration (cascade or non-cascade time, depending upon which column is being fed, for two columns; non-cascade time for one column) to determine the total STAGE duration (or CYCLE for fewer than three columns).

- The flow control output array values are then sent to the Dig I/O board to establish the correct liquid flow paths and pump/homogenizer assignments for the first STEP of the first STAGE (column A is primary, B is secondary, C is regenerating).
- The static portion of the run time visual display is established to which the values to be updated with each pass of the control loop will be superimposed. As well, the column/feed-filters overpressure alarms are initialized.
- The STEP time loop is entered within which all of the automatic control functions are executed for the current STEP. The loop is exited when the STEP time is complete or when the regenerating column is ready to become the secondary column for the next STEP, which ever requires the most time.
- Within this STEP time loop the visual display of the processing system parameter values is updated with each pass through the loop.
- The ON/OFF state of the feed pump is monitored as the routine controls it so that the elapsed feed time, rather than the elapsed clock time, signals the end of the loop

and the beginning of the next STEP and its corresponding system control output.

- The pressure transducers are next tested against the set limits to check to overpressure conditions. If an error condition exists, then an audible as well as a visual alarm is turned on. While the audible alarm can be turned off with a keystroke as per the prompt, the visual message remains on the screen until the condition is corrected or the end of the STAGE at which time program control would be transferred back to APC from which an alternate operating mode would be selected.
- If the current STEP requires a column to be eluted, then the UV monitor output is tested against the appropriate threshold value to determine the presence or absence of a protein peak. If a peak is present then the liquid containing the protein fraction is routed to the appropriate storage container for either lysozyme or avidin.
- The presence of an eluting peak will override the STEP time as the controlling factor in the event that the STEP time has elapsed prior to the end of a peak to prevent the loss of product. If an extended STEP time is required, both audible and visible alarms are invoked.
- Keyboard input is tested to see if "M" has been pressed, transferring control to MPC (Manual Process Control), or in the event of an extended STEP duration due to peak elution, if any key has been depressed to stop the audible

alarm.

- A test is made to see, when the current STAGE time is complete, if the elapsed feed time equals the cascade feed time or if feed time was lost due to filter maintenance down time.
- The STEP time loop is tested for completion. Upon completion, the time loop is exited and the STEP number loop increments by one and restarts the STEP time loop. When the STEP number loop is exited, the STAGE number loop is incremented by one. The completion of the STAGE increments the CYCLE loop, which when completed increments the BLOCK loop which is essentially an infinite loop unless an endpoint has been specified via MPC.
- At any point within the process, the run is terminated when a specified end point has been reached or when overpressure conditions force a shutdown, either staged or immediate depending upon whether the source of the problem was the columns or the inlet feed filters.

The operation of modes S1 and S2 for one and two columns shut down, respectively, is very similar to that described above for the NM routine. As each fouled column is removed from operation during a staged shutdown, it is backwashed with equilibrating buffer to remove the egg white from the resin bed. If three columns are being used (NM routine) and an immediate shutdown is forced due to the feed filters plugging, then all three would be cleaned out in sequence prior to the system

shutting down. This gives added protection against the growth of microorganisms even though the lysozyme is an anti-bacterial agent.

Ending a run:

When a run is completed or has been terminated prematurely, the APC routine passes control back to the MAIN program segment after clearing the screen and displaying an end-of-run message indicating briefly the cause of the termination. At this point, the next keystroke produces a small end-of-run menu which allows the operator to start a new run, to save the current method to a disk file, and/or to exit the program.

4. DISCUSSION AND FUTURE CONSIDERATIONS

While no albumen has been run through the system thus far, the various operating modes, alarms and control transfer conditions have been tested using dyed water and found to perform adequately. As in any project of this kind, approaching a solution to the stated objectives often raises the lid on Pandora's Box of program revisions and "new and useful" routines to add to the existing framework. The addition of more and more subroutines that were not considered wnen the program architecture was laid out usually leads to a patchwork quilt of code. Such programs may fulfill the immediate processing requirements, but lack the grace and simplicity of a mature program which has been carefully reduced to the kernel of usefullness. Parts of this program have been reworked several times while others have not been so colsely scrutinized. When working with a moderately long program such as this, the constant threat to ever completing the initial version is the wellspring of ideas for improvements to what has already been committed to code.

The physical plant and control software designs together meet and exceed the basic design specifications listed in the introduction of this paper. Not only does the software promote an orderly shutdown in the case of column fouling, but it also allows for the removal of the offending column while continuing to run what is left of the system. This approach attempts to maximize production with a minimum of supervision.

The delayed run/start feature provides a great deal of operating flexibility, which is especially desirable for use in reasearch. The MPC routine gives great flexibility in allowing the operator to modify the values of most parameters which materially affect the course of events within the process.

While a host of smaller operating enhancements have been included in the software, there are always new functions and refinements to be considered. One such addition that would be useful in tracking the behavior of the system over a long unattended period is a logging routine that notes and time stamps every change of operating parameter during the run and makes is easy to dump to the printer as a run report.

While the program provides adequate control in its current form, the marketing of such a product for commercial use would necessitate a complete rewrite, taking into account all of the enhancements which have been add-ons to this version. The run-time display should be redesigned to provide only the most essential information, with the less important data being relegated to a secondary screen which could be called up with a single keystroke. The aesthetics and uniformity of presentation of the interactive and data display screens would also need to be improved.

Other future considerations in terms of the enhancement of the process itself might include the addition of a secondary purification step for the proteins and an ultrafiltration (UF) module to concentrate the protein fractions without concentrating the accompanying salts. The salt solutions recovered using UF

could be recycled as shown in Figure 4.1. Two more pumps would be required to recirculate the liquids, and the conductivity meter and pH meter shown would allow the operator to ensure that the proper levels of salt concentration and pH were maintained. A more sophisticated sytem again would have automatic control over concentration and pH by incorporating computer-controlled dispensing pumps for concentrated eluant and acid and base solutions.

Since the physical plant and software have been developed expressly to accomodate the recovery of avidin and lysozyme using the elution-looping technique, the general market appeal for such a system is likely to be quite limited. The physical plant itself, however, can be used with virtually any chromatographic procedure if accompanied by the appropriately modified software. For example, with a very minor modification to the flow control hardware and a moderate restructuring of the software,the system could be performing Immobilized Metal Affinity Chromatography (IMAC) for the recovery of immunoglobulins.

The use of automatic control in the field of food processing, and bio-processing in general, has the potential to greatly benefit both the producer and the consumer through the enhancement of production process efficiency, cost effectiveness and quality control.

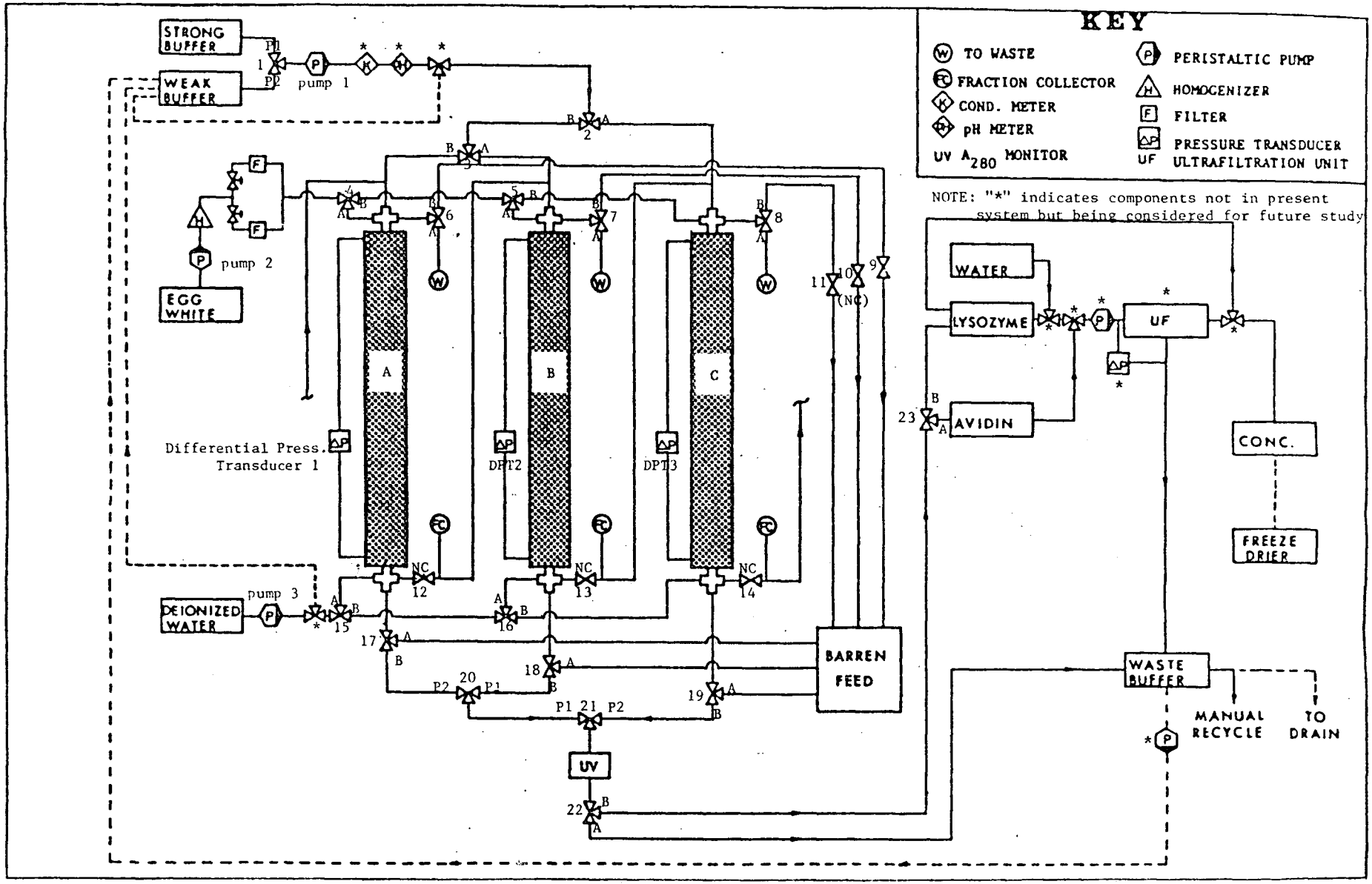


FIGURE 4.1: General system layout including potential future additions for nearly total automation of control

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APPENDIX

OPERATIONAL STATES FOR NORMAL AND DISABLED SYSTEM MODES

SEQUENCE No.	column A	B	C	DESCRIPTION	VALVE NUMBER 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	PUMPS 1	2	3	HOMOG-ENIZER
NORMAL MODE (3-column cycle: 2-column cascade)																															
a	1	2	R	back-rinse C to barren	0	0	0	1	0	0	0	0	0	0	1	1	0	0	0	0	0	1	0	1	1	0	0	0	1	1	1
b				back-rinse C to waste	0	0	0	1	0	0	0	1	0	0	0	1	0	0	0	0	0	1	0	1	1	0	0	0	1	1	1
c				apply weak saline to C	0	1	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	1	0	1	0	1	1	0	1
d				apply strong saline to C	1	1	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	1	0	1	1	1	1	0	1
e				equilibrate C	0	0	0	1	0	0	0	1	0	0	0	1	0	0	0	0	0	1	0	1	1	1	0	0	1	1	1
f				C is idle	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	1	0	1
a	R	1	2	back-rinse A to barren	0	0	0	0	1	0	0	0	1	0	0	0	1	0	1	0	0	0	1	1	0	0	0	0	1	1	1
b				back-rinse A to waste	0	0	0	0	1	1	0	0	0	0	0	0	1	0	1	0	0	0	1	1	0	0	0	0	1	1	1
c				apply weak saline to A	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	1	1	0	0	1	1	0
d				apply strong saline to A	1	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	1	1	1	0	1	1	0
e				equilibrate A	0	0	0	0	1	1	0	0	0	0	0	0	1	0	1	0	0	0	1	1	0	1	0	0	1	1	1
f				A is idle	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	1	0	1
a	2	R	1	back-rinse B to barren	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	1	1	0	0	0	1	0	0	0	1	1	1
b				back-rinse B to waste	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	1	1	0	0	0	1	0	0	0	1	1	1
c				apply weak saline to B	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	1	1	1	0	1	1	0	1
d				apply strong saline to B	1	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	1	1	1	1	1	1	0	1
e				equilibrate B	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	1	1	0	0	0	1	1	0	0	1	1	1
f				B is idle	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	1	0	0	0	1	0	1
SHUTDOWN MODE: S1(a) (Column A is down; 2-col. intermittent cascade)																															
a	*	1	2	cascade B-C	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	1	0	1
b	*	R	1	backwash B to barren	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	1	1	1
c	*	R	1	backwash B to waste	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	1	1	1
d	*	R	1	apply weak saline to B	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	0	1	1	0	1
e	*	R	1	apply strong saline to B	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	0	1
f	*	R	1	equilibrate B	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	0	0	1	1	1
g	*	1	R	backwash C to barren	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	1	0	0	0	1	1	1
h	*	1	R	backwash C to waste	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	1	1	1
i	*	1	R	apply weak saline to C	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	1	1	1
j	*	1	R	apply strong saline to C	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	1	1	1	0	1
k	*	1	R	equilibrate C	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	1	1	1

OPERATIONAL STATES FOR NORMAL AND DISABLED SYSTEM MODES (cont'd)

SEQUENCE No.	column A	B	C	DESCRIPTION	VALVE NUMBER 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	PUMPS 1	2	3	HOMOG-ENIZER
SHUTDOWN MODE: S1(b) (Column B is down; 2-col. intermittent cascade)																															
a	2	*	1	cascade C-A	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	1	0	0	1	0	0	0	0	0	1	0	1
b	1	*	R	backwash C to barren	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	1	1	1
c	1	*	R	backwash C to waste	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	1	1
d	1	*	R	apply weak saline to C	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	0
e	1	*	R	apply strong saline to C	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	1	0
f	1	*	R	equilibrate C	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	1	1
g	R	*	1	backwash A to barren	0	0	0	0	1	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	1	1	1
h	R	*	1	backwash A to waste	0	0	0	0	1	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	1	1	1
i	R	*	1	apply weak saline to A	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	1	0	1
j	R	*	1	apply strong saline to A	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	0	1
k	R	*	1	equilibrate A	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	1	1
SHUTDOWN MODE: S1(c) (Column C is down; 2-col. intermittent cascade)																															
a	1	2	*	cascade A-B	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	1	0	1
b	R	1	*	backwash A to barren	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	1	1	1
c	R	1	*	backwash A to waste	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	1	1	1
d	R	1	*	apply weak saline to A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	1	0	1	1	0	1
e	R	1	*	apply strong saline to A	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	1	1	1	1	0	1
f	R	1	*	equilibrate A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	1	0	0	1	1	1
g	1	R	*	backwash B to barren	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0	1	1	1
h	1	R	*	backwash B to waste	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	1	1	1
i	1	R	*	apply weak saline to B	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	1	1	0	1	1	0	1
j	1	R	*	apply strong saline to B	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	1	1	1	1	1	0	1
k	1	R	*	equilibrate B	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	1	1	0	0	1	1	1

OPERATIONAL STATES FOR NORMAL AND DISABLED SYSTEM MODES (cont'd)

SEQUENCE No.	column A	B	C	DESCRIPTION	VALVE NUMBER 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	PUMPS 1	2	3	HOMOG-ENIZER
SHUTDOWN MODE: S2(a-b) (A & B down; C operating alone)																															
a	*	*	1	feed C	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	1
b	*	*	R	backwash C to barren	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0
c	*	*	R	backwash C to waste	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0
d	*	*	R	apply weak saline to C	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0
e	*	*	R	apply strong saline to C	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0
f	*	*	R	equilibrate C	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0
SHUTDOWN MODE: S2(b-c) (B & C down; A operating alone)																															
8a	1	*	*	feed A	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	1
8b	R	*	*	backwash A to barren	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	1	0
8c	R	*	*	backwash A to waste	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	1	0
8d	R	*	*	apply weak saline to A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0	0
8e	R	*	*	apply strong saline to A	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	0	0
8f	R	*	*	equilibrate A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	0
SHUTDOWN MODE: S2(c-a) (C & A down; B operating alone)																															
a	*	1	*	feed B	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	1
b	*	R	*	backwash B to barren	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0
c	*	R	*	backwash B to waste	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0
d	*	R	*	apply weak saline to B	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	1	0	0
e	*	R	*	apply strong saline to B	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	0	0
f	*	R	*	equilibrate B	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	1	0
TOTAL SHUTDOWN MODE: S3 (all columns down)																															
9a	*	*	*	backwash A to barren	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0
9b	*	*	*	backwash A to waste	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0
9c	*	*	*	equilibrate A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0	0
9d	*	*	*	backwash B to barren	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0
9e	*	*	*	backwash B to waste	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0
9f	*	*	*	equilibrate B	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	1	0	0
9g	*	*	*	backwash C to barren	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0
9h	*	*	*	backwash C to waste	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0
9i	*	*	*	equilibrate C	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0
9j	*	*	*	all power off	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0