NOAA TECHNICAL MEMORANDUM NMFS SEFC - 234

BIOMEDICAL TEST MATERIALS PROGRAM:

PRODUCTION METHODS AND SAFETY MANUAL

Jeanne D. Joseph
Editor

October 1989

U.S. DEPARTMENT OF COMMERCE
National Oceanic and Atmospheric Administration
National Marine Fisheries Service
Southeast Fisheries Center
Charleston Laboratory
P.O. Box 12607
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FOREWORD

The Fish Oil Biomedical Test Materials Program is a cooperative effort that was established by a Memorandum of Understanding between the National Institutes of Health (NIH); the Alcohol, Drug Abuse, and Mental Health Administration (ADAMHA); and the National Oceanic and Atmospheric Administration-Department of Commerce (DOC). The program is designed to provide a long-term, consistent supply of quality assured/quality-controlled test materials to researchers in order to facilitate the evaluation of the role that n-3 fatty acids play in health and disease. The production facility is located within the National Marine Fisheries Service (NMFS), Charleston Laboratory.

The purpose of this operator's manual is to provide a set of instructions for properly and safely (1) operating the production equipment, (2) storing the test materials produced, and (3) disposing of wastes generated. Maintenance of the facility and equipment are also addressed.

Safety is of particular concern in an environment such as the production facility. Large quantities of flammable ethyl alcohol and hazardous metallic sodium are used. All employees in the plant are expected to be familiar with the safety and evacuation procedures described in this manual.

The contributions of the following Charleston Laboratory staff members to this manual are gratefully acknowledged: Thomas Brown, Donald E. Duesler, Robert C. Ernst, Jr., Robert E. Roberts, and Joe L. Wade. The contributions of William Nilsson and Erich Gauglitz of the NMFS Seattle Laboratory to the chapter on supercritical CO_2 fractionation are also very much appreciated.

With the exception of five figures in the section on supercritical fluid CO_2 fractionation that were provided by staff of the NMFS Seattle Laboratory and one HPLC chromatogram, the remainder of the illustrations were produced by the editor, using the software package, GenericCADD, Level 3, Generic Software Inc., Bothell, WA.

The manual will be revised as modifications and improvements are made in production procedures.

INTRODUCTION

Fats, oils, and lipids are terms that are often used synonymously, but there are subtle differences in their meanings. Both fats and oils consist, primarily, of triglycerides, but also may include minor amounts of hydrocarbons, wax esters, alkoxyglycerols, esterified and free sterols, non-esterified fatty acids, and partial glycerides. Fats and oils are produced from plant and animal tissues by the physical process of rendering or by chemical extraction with non-polar solvents such as hexane. The only difference between fats and oils is that at ambient temperature, fats are solid while oils are liquid. In contrast, lipids are extracted from tissues with a mixture of organic solvents, one of which must be an alcohol. In addition to the compound classes found in fats and oils, lipids may include a wide variety of polar molecular species such as phospholipids, phosphonolipids, sphingolipids, cerebrosides, and other complex organic compounds.

The nomenclature commonly used in lipid chemistry to identify component fatty acids of the triglycerides specifies first, the number of carbon atoms in the chain, followed by a colon and a second number specifying the number of double bonds in the molecule. Unsaturated fatty acids are designated by one of two symbols, "ω-" (ω = Greek "omega") or "n-", followed by the position of the first double bond relative to the non-polar end of the molecule. This nomenclature is suitable only for polyunsaturated fatty acids that have methylene-interrupted double bond systems. For polyunsaturates with non-methylene-interrupted double bonds, the number designating the number of double bonds is followed by the Greek letter "delta" (Δ) and the positions of the double bonds relative to the polar, or carboxyl, end of the molecule. Thus, 18:2n-6 identifies linoleic acid, a fatty acid with 18 carbons and two methylene-interrupted double bonds, the first of which is located between the sixth and seventh carbon atoms in the chain. The polyunsaturates of intense current interest, eicosapentaenoic and docosahexaenoic acids, are abbreviated as 20:5n-3 and 22:6n-3, respectively, and are even more familiarly known as EPA and DHA, respectively.

While "n-" is presently preferred over "ω-" as the initial double bond locator, "ω-" lives on in the commonly used collective adjective, "omega-3" as in omega-3 fatty acids, and the quarterly newsletter, Omega-3 News.

Menhaden oil serves as the raw material for production of biomedical test materials at the Charleston Laboratory. Menhaden are small oily and bony fish that yield, by far, the major portion of the industrial fish oil produced in the U.S. The fish are found in dense schools during spring, summer, and autumn months in Atlantic coastal waters of southern Canada and the Atlantic and Gulf coasts of the U.S. In the commercial fishery, the schools

are located by spotter planes and harvested by small boats that encircle and capture the schools with purse seines. The fish are then pumped aboard mother ships where they are stored in refrigerated holds. Upon return of the mother ship to the rendering plant, generally no more than 24 hours after harvest, the fish are cooked and pressed to yield oil, fish solubles and fish meal.

The company from which the Charleston Laboratory obtains its fish oil uses a proprietary process to produce a partially refined menhaden oil. The oil is first winterized to precipitate stearines, a mixture of saturated triglycerides consisting mostly of palmitic (16:0) and stearic (18:0) fatty acids. Next, the oil is treated with alkali to remove non-esterified fatty acids as soaps, a process known as alkali-refining. Finally, the oil undergoes a mild cold bleaching to eliminate peroxides that may have been formed during rendering and to reduce the color intensity of the oil.

The processed oil is stored in 10,000 gal tanks that are frequently drawn upon to supply customers and replenished by newly processed oil. Consequently, the fatty acid composition of the oil remains relatively constant during the fishing season. The processed oil is packaged in 55-gal food-grade drums, blanketed with N_2, and shipped in lots of 10, 20, or 30 drums to the Charleston Laboratory where it is stored under refrigeration until further refined by vacuum deodorization.

BIOMEDICAL TEST MATERIALS PRODUCTION OVERVIEW

The first test material agreed upon in the Memorandum of Understanding between the NIH, ADAMHA, and DOC is a fully refined fish oil. This product is prepared by vacuum deodorization (Fig. 1, I) from a partially refined menhaden oil that has been winterized, alkali-refined and bleached by the supplier. Deodorization yields an oil with little odor and flavor, decreased cholesterol levels, and organic contaminants that are below detectable limits. This refined oil is supplied in bulk and in soft-gelatin capsules to NIH-approved investigators.

Refined oil also serves as the feedstock for production of omega-3 concentrates which are produced in a series of steps (Fig. 1, II-V). The oil is first transesterified to yield ethyl esters (II), followed by urea adduction of more saturated esters (III). In the next step, alcohol is recovered for reuse and the crude polyunsaturated esters are recovered (IV). Finally, the omega-3 concentrates are purified by short-path distillation (V). The concentrates are also available in bulk and in soft gelatin capsules.

A portion of the concentrates is reserved as feedstock for the supercritical fluid (SCF) CO_2 fractionator (Fig. 1, VI) to produce fractions of 20:5n-3 (EPA) and 22:6n-3 (DHA), each in a purity of $\geq$ 75%.

High purity EPA and DHA ($\geq$ 95%) are produced from SCF fractions or from distilled concentrates, by high performance liquid chromatography (Fig. 1, VII) on a process-scale column containing C18 reverse phase packing. High purity materials are packaged in small vials for distribution.

As illustrated in Figure 1, all chemical processes required to produce the ethyl ester concentrates are carried out in a N_2 atmosphere.

Process Stages

I. Vacuum deodorization
II. Transesterification
III. Urea adduction
IV. Film evaporation
V. Short-path distillation
VI. Supercritical fluid fractionation
VII. High performance liquid chromatography

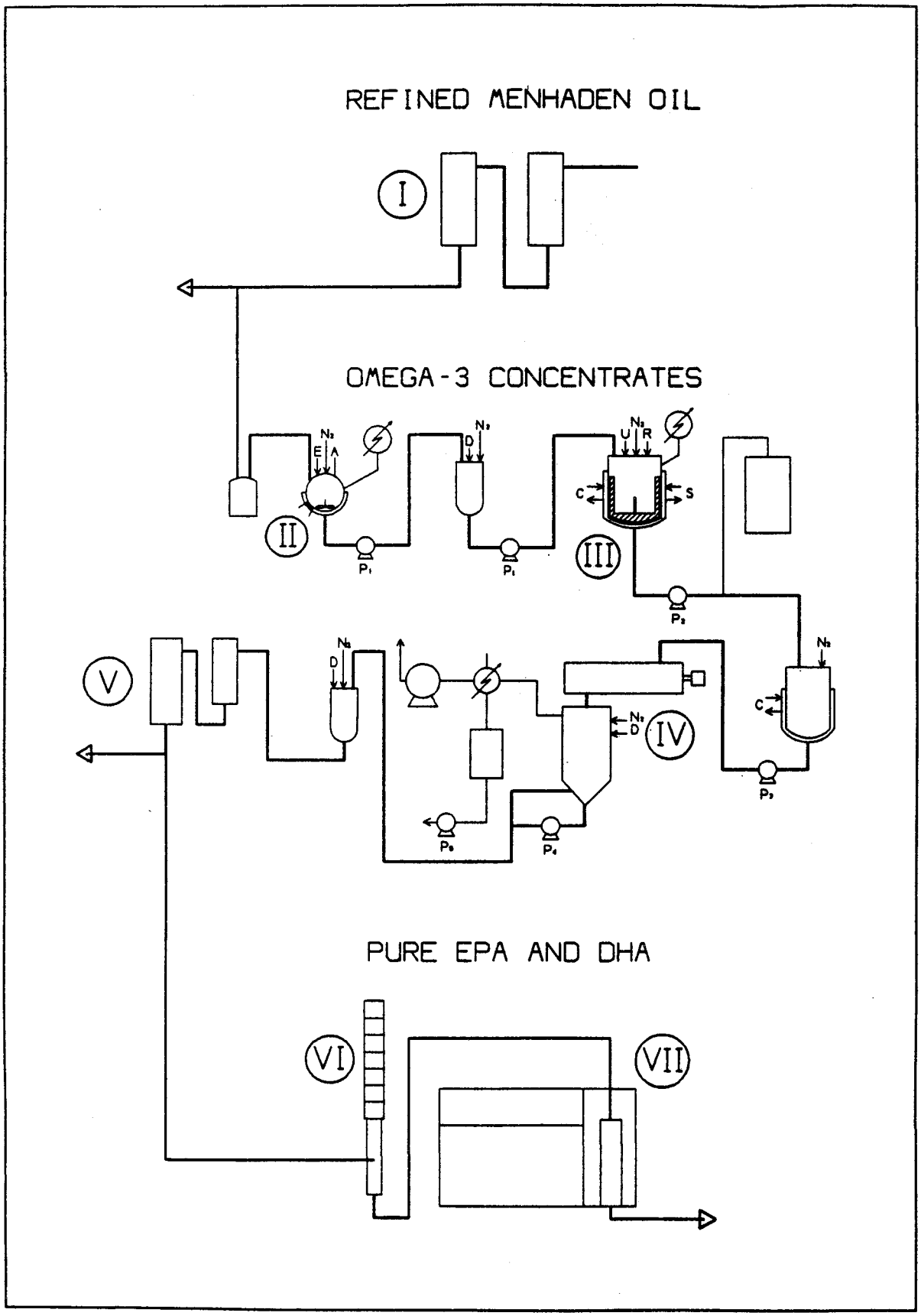


Fig. 1. Production of biomedical test materials in the Charleston Laboratory.

THE BIOMEDICAL TEST MATERIALS PRODUCTION FACILITY

Floor plans of the production facility are shown in Figures 2 and 3. As shown in Figure 2, production of test materials takes place in five laboratories, two of which are explosion proof, the reactor (Rm. 423) and evaporator (Rm. 424) laboratories. The plant has a self-contained heating and air conditioning system and a modest amount of storage space. Outside, a combination walk-in refrigerator/freezer storage unit is available. Liquid nitrogen (N_2) is the source of the gaseous N_2 which is piped to all laboratories. Also located outside are two chillers that provide refrigerated coolant for all production equipment requiring it and the liquid carbon dioxide (CO_2) tank and associated compressor needed for the supercritical fluid CO_2 fractionator. Located at the rear of the facility, outside the evaporator laboratory, are two 2200-gal fiberglass tanks and (not illustrated in Fig. 2) a 5000-gal stainless steel tank for urea waste storage. Also not illustrated are a number of 8x20 ft and 8x40 ft land-sea cargo carriers that are used for storage of fish oil (during the winter months), alcohol, sodium, urea, and wastes.

Figure 3 shows the location of the major pieces of production equipment. In addition to those items shown, there are several electronic and mechanical scales, a 20-L rotary evaporator, a drum roller, a 6-channel data logger, a drum handler, a small flammable liquids storage cabinet, and a variety of wheeled safety ladders, shelves, spare parts bins, and tool chests.

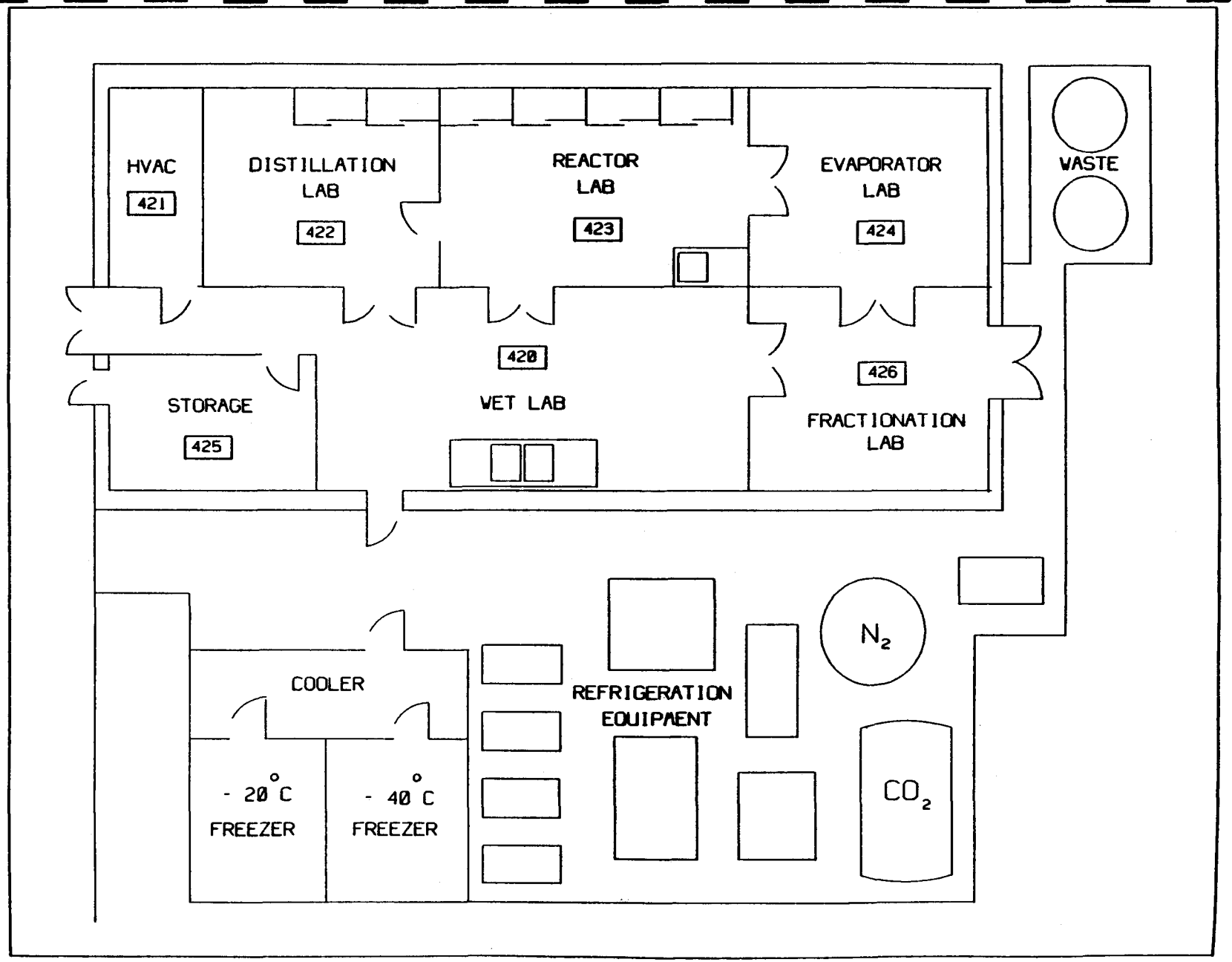


Fig. 2. Charleston Laboratory biomedical test materials production facility.

Test materials production equipment

1. Short-path still
2. Purified ester recovery still
3. Transesterification reactors, 72 L
4. Separatory funnels, 100 L
5. Scraped wall crystallizer, 300 Gal
6. Alcohol storage tanks, 250 Gal
7. Product feed tank, 250 Gal
8. Scraped wall film evaporator
9. High performance liquid chromatograph (HPLC)
10. Supercritical fluid CO_2 fractionator
11. Water deionizer
12. Low temperature (-84°C) chest freezer
13. Two-stage wiped film vacuum deodorizer
14. CO_2 compressor

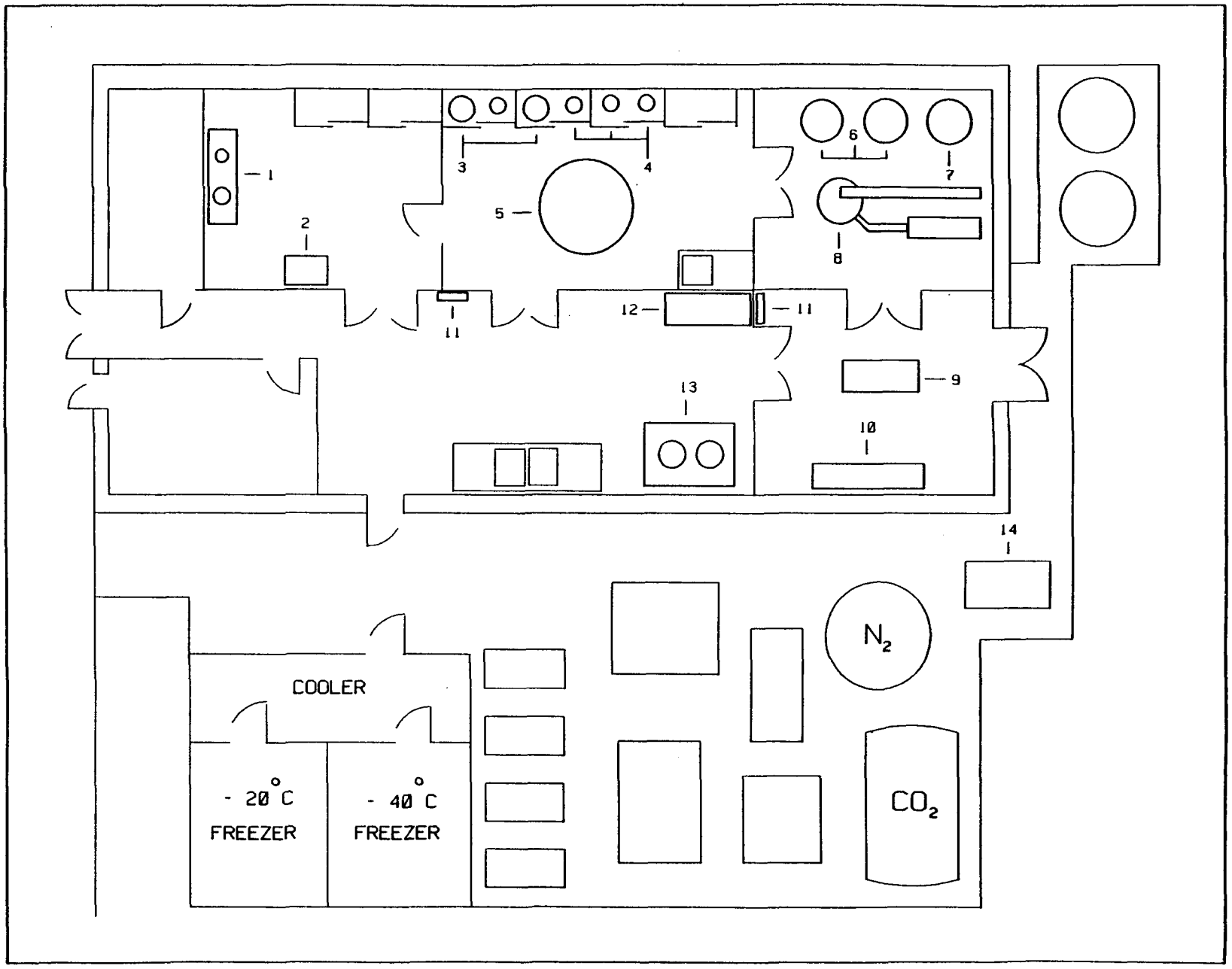


Fig. 3. Test materials production equipment.

GENERAL SAFETY AND SECURITY GUIDELINES

- Admittance is restricted to members of the Production Team, members of the QA/QC Team whose duties require that they enter the plant, and selected visitors escorted by Management.

- Production Team members will wear appropriate clothing and safety gear which are defined specifically in the sections describing production methods.

- Everyone entering the plant, including visitors, must wear disposable caps which are available in the entryway of Room 420.

- Visitors who wish to observe the vacuum stills in operation must wear safety goggles.

- Smoking and open flames are prohibited in the plant and the outside storage area.

- Two rooms in the production facility (Rms. 423 and 424) are explosion-proof laboratories. Only explosion-proof electrical equipment may be used in these areas.

- The use of personal, portable radios or tape players ("Walkmans") is not permitted in any area of the facility.

- Each Production Team member is responsible for the maintenance of order and cleanliness of his/her respective work station and for the daily proper disposal of wastes from that work station. In addition, each is to see that his/her equipment is properly secured for the night (shut down, on stand-by, or safely operating) before completing the day's shift.

- Daily plant startup/shutdown procedures are as follows:

1. The first employee to arrive on duty is to:
   a. Unlock the front gate of the utility area to allow free exit from the plant through the side door in case of fire.
   b. Unlock the chiller/freezer unit.
   c. Turn on all equipment requiring warm-up time before operating.
   d. If the electric vapor traps of the vacuum deodorizer require cleaning, the switch on the air handlers in Rm 421 (HVAC, Fig. 2) should be placed in the <UNOCCUPIED> position to evacuate odors as quickly as as possible.

2. The last employee on duty is to:
   a. Check that all trash containers have been emptied into the fireproof outdoor container near the incinerator and that all liquid and solid wastes have been

properly stored. Any failure to dispose of wastes promptly and properly is to be reported to the plant supervisor on the next working day.

b. Replace the padlock on the door of the walk-in chiller/freezer unit and make certain that it is locked.

c. Check that all gates of the utility area and all doors of the plant are locked.

Since the alcohol used in all production steps is undenatured, tax-free ethanol, accurate records of usage must be kept. One member of the Production Team is responsible for security of the alcohol supply and for maintenance of usage records. A spare key to the outside alcohol storage area is kept in a locked key cabinet in the office of the Laboratory's Administrative Assistant. Spare keys to padlocks securing alcohol supply lines to the production equipment are kept by the Production Project Leader.

EVACUATION PROCEDURES

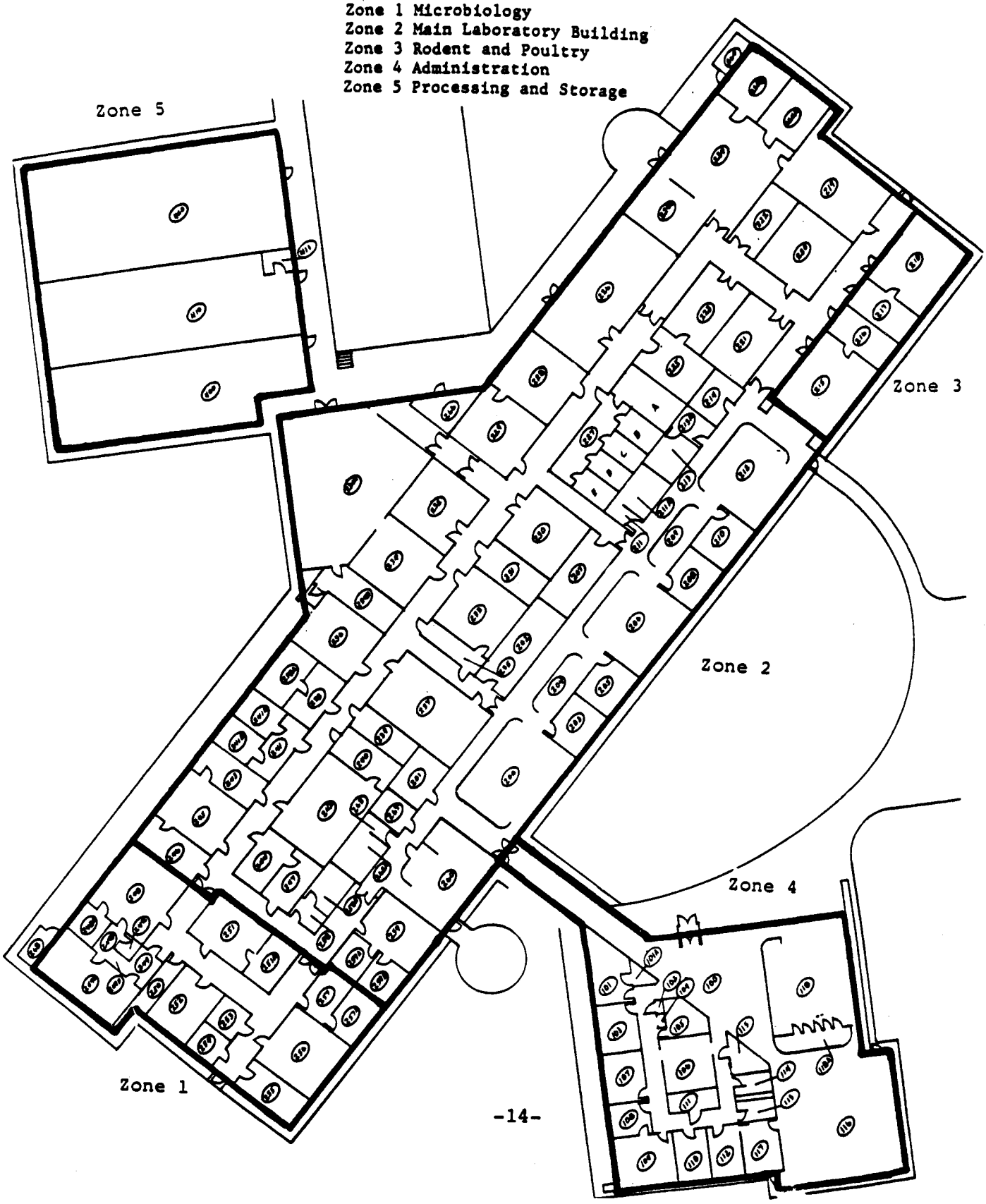
The Charleston Laboratory consists of two separate buildings joined by a breeze-way, a design which prevents or reduces the possibility of fire spreading from one section to the other. The Laboratory is divided into five Zones. Zones 1-4 include the main laboratory building, consisting of both office space and laboratories; the Production facility is located in Zone 5. When a fire alarm is activated, the location of the alarm is identified on an annunciator panel in the Laboratory's reception area. The receptionist on duty announces the Zone from which the alarm has come, but all staff, regardless of where they happen to be at the time, are to leave the Laboratory by the nearest exit as quickly as possible and assemble in the front parking lot.

The existence of the breezeway separating Zone 5 from Zones 1-4 provides an opportunity for orderly shut-down of operating equipment in the Production plant before evacuation, provided the alarm is in Zones 1-4. These procedures are described under the sections entitled Emergency Shut-down Procedure for each piece of equipment in Production Methods. In general, these procedures require that pumps transporting liquids (solvents, fish oil, and/or esters) be shut down to prevent fueling any local fire.

If the alarm is in Zone 5 which includes not only the Production facility but also a large storage area and the Experimental Processing Laboratory (EPL), an orderly shut-down is still desirable unless it is evident that a dangerous situation exists in the Production plant, itself. If there is a fire in either of the explosion-proof laboratories (Rms. 423 and 424), all personnel are to evacuate the plant immediately by the nearest exit, activating the fire alarm by the exit, if necessary.

FIRE ZONES

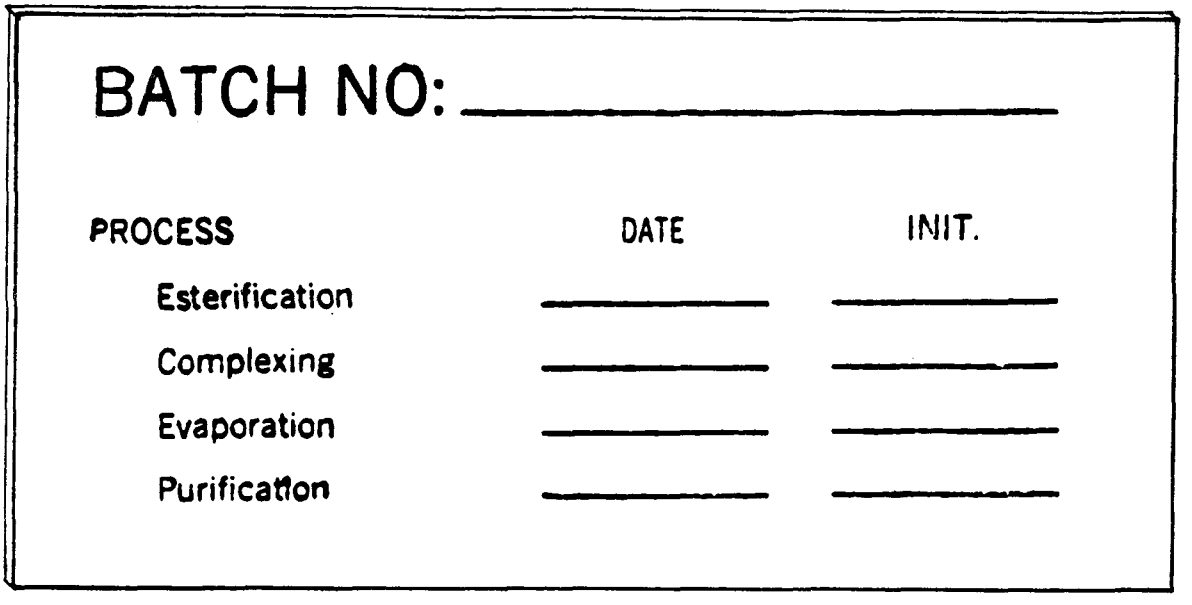
Zone 1 Microbiology
Zone 2 Main Laboratory Building
Zone 3 Rodent and Poultry
Zone 4 Administration
Zone 5 Processing and Storage



PROCESS CONTROL AND PRODUCT INVENTORY

The daily output of the vacuum deodorizer is assigned a five-digit "batch" number consisting of the last two digits of the calendar year and the three-digit day of the year. These batches are then combined at a later date to form "lots" as necessary to meet the research needs of investigators.

Individual batches of refined oil are used in the production of omega-3 concentrates. These batches are tracked through processing by information entered on 3X5 inch cards that accompany each batch. The rubber-stamped cards, illustrated below, document the batch being processed, the date each production step is carried out, and the individual responsible for each operation. These cards are placed in transparent envelopes affixed to each piece of equipment. After processing is complete, the cards are placed in transparent envelopes attached to the product containers where they remain until the product is packaged for shipment to researchers. The cards are then archived for future reference.



Test materials, and their intermediates as well, are assigned batch numbers for tracking purposes. Once a number of batches are pooled to form a lot, the numbers assigned are preceded by the letter "L" (lot). These letters are followed by the five digits discussed above. These digits are followed by the letters "B" or "A", denoting that the raw material used was vacuum or steam deodorized, respectively. Finally, another letter designates the specific process that has yielded the product or intermediate.

These designations are as follows:

- B...deodorized oil
- C...mixed ethyl esters
- D...crude concentrate of n-3 polyunsaturates
- E...short-path still first-stage distillate
- F...short-path still second-stage distillate
- G...CO_2 fractionated esters
- H...HPLC fractions (dilute)
- I...Purified neat EPA/DHA

Since more than one fraction will be produced by CO_2 fractionation, HPLC fractionation, and solvent stripping of the HPLC fractions, these fractions are further identified by a period (.) followed by the numbers 1-9. As an example of this coding system, the label "89002BH.1" would represent fraction 1 from the HPLC, produced on January 2, 1989 from an ethyl ester concentrate derived from vacuum deodorized oil. The number L89091BF would indicate a lot of ester concentrates pooled from batches on April 1, 1989.

Since HPLC peaks may be "sliced" to enhance purity, these slices are identified by lower case letters, "a" being assigned to the first slice. As an example, the code 89003BH.2a would denote the first slice of peak #2 (16:3n-4) produced on January 3, 1989 from ester concentrates of vacuum deodorized oil.

Tenox 20A containing the antioxidant tertiary butyl hydroxyquinone (TBHQ) and *alpha*- and *gamma*-tocopherols are routinely added to the deodorized oil. However, on special request, deodorized oil may be produced with only the antioxidant Tenox 20A added, or with no antioxidant at all. Codes for these oils begin with "L", the five-digit "batch" number, and the designation BT for oils with TBHQ only or BO for oils containing no added antioxidant.

Operation of the processing equipment is documented on specially designed forms that are bound into log books, 100 pages/book. Copies of these forms follow this section of the Manual.

Refrigerator or freezer storage of all materials produced, whether intermediate or finished products, is documented in a bound notebook.

VACUUM STRIPPING...OPERATING PARAMETERS

FISH OIL

Oil supplier: _____
Date received: _____
Lot number: _____
Drum number: _____

DEODORIZATION

Date: _____
Time began: _____
Time completed: _____
Operator: _____

OPERATING PARAMETERS	STAGE 1	STAGE 2
Temperatures (C°)		
Still body:	_____	_____
Film:	_____	_____
Vapor:	_____	_____
Vacuum (torr)	_____	_____
Wiper speed (rpm)	_____	_____

PRODUCT

Batch No.: _____BB
Yield (Kg): _____
Analyses requested:

POV:	_____	Cholesterol:	_____
FFA:	_____	PCBs:	_____
GLC:	_____	Pesticides:	_____

COMMENTS

_____
_____
_____

TRANSESTERIFICATION DATA SHEET

Feed: _____B	Date: _____	Operator: _____

OPERATION	REACTOR 1	REACTOR 2
Nitrogen flush (time)		
Mixer on (time)		
Oil in (time/kg)	|	|
Heat on (time/settings)	|	|
Alcohol in (time/L)	|	|
Heat adjust (time/settings)	|	|
Temperature check (time/C°)	|	|
Temperature check (time/C°)	|	|
SEOX added (time/L)	|	|
Heat off (time/C°)	|	|
Mixer off (time/C°)	|	|
Lower phase drained (time/C°)	|	|
Nitrogen flush sep funnel (time)		
Ester transfer complete (time/L)	|	|
First wash water in (time/L)	|	|
Water drained (time/ester L)	|	|
Second wash water in (time/L)	|	|
Water drained (time/ester L)	|	|
Third wash water in (time/L)	|	|
Water drained (time/ester L)	|	|
Transfer ({C}ryst. or {S}torage)		

PRODUCT

Batch No.: _____C
QC analyses: _____

COMMENTS

_____
_____

UREA ADDUCTION DATA SHEET

Feed:_____C | Run No:____ | Operator:_____ | Date:_____

SYSTEM CHECK

System clean:_____ Tank:_____ Lines:_____ Tank C:_____
Nitrogen flush:_____ Condenser on:_____ Steam (psig):_____
Valves, bottom:_____ Jackets: B:_____ M:_____ T:_____ Pump H2O:_____
Data logger on-line:_____

REACTANTS		SCHEDULED	ACTUAL
Alcohol (Lot #):	_____	_____gal	_____gal
Urea (Lot #):	_____	_____lbs	_____lbs
Esters:	_____	_____kg	_____kg

TIME	EVENT			
_____	Charge alcohol	_____gal		
_____	Mixer on			
_____	Charge urea	_____lbs		
_____	Heat on	_____F°	_____C°	Jackets _____
_____	Temp up (80 C°)	_____F°	_____C°	
_____	Add esters			
_____	Esters in	_____F°	_____C°	
_____	Heat off	_____F°	_____C°	
_____	Begin chilling	_____F°	_____C°	Jackets _____
_____	Mixer off	_____F°	_____C°	
_____	Start syphon	_____F°	_____C°	
_____	Start bottom drain			
_____	Draining complete			

UREA BY-PRODUCT

Level of drained waste, _____ in
_____ Water added to waste, _____ gal
_____ Mixer on
Waste storage tank: A __; B__; C__
_____ Begin pumping, _____ F°;_____ C°
_____ Tank emptied, _____ gal (total volume)
Current contents storage tank: ____ gal

COMMENTS

_____
_____
_____
_____
_____

FILM EVAPORATOR DATA SHEET

BATCH NO:_____D | RUN NO:_____ | SIGNED _____ | DATE _____

Item / Time					
A Tank Level					
B Tank Level					
Feed Tank Level					
Nitrogen Flush					
Feed Pump Setting					
Feed Pressure PSI					
Feed Rate GPM					
Percent Power					
High Steam PSI					
Controller Setting PSI					
Low Steam PSI					
Steam Temperature F°/C°					
Bottoms Pump Setting					
Bottoms Pressure PSI					
Bottoms Temp. F°/C°					
Water Feed On/Off					
Nitrogen Flush					
Vapor Temperature F°/C°					
Vapor Pressure PSI					
Cool Water Out F°/C°					
Cool Water In F°/C°					
EtOH Rec. Level					
EtOH Pump PSI					
EtOH Take-off On/Off					
Vac. Pump Water GPM					
Vac. Pump Pressure PSI					

NOTES: _____

RECOVERY OF CRUDE OMEGA-3 ESTERS

Feed: _____C	Operator:_____	Date:_____

Original weight (kg) of stripped oil:_____

EVENT	FUNNEL 1	FUNNEL 2
Nitrogen flush (time)		
Concentrate (L)		
Mixed (time)		
Lower phase drained (time)		
First wash (time/L)		
Water drained (time/esters left)		
Second wash (time/L)		
Water drained (time/esters left)		
Esters combined (time/L)		

PRODUCT

Batch No.: _____D Esters recovered (kg):_____ Yield (%):_____ QC analyses requested:_____

COMMENTS

_____ _____ _____ _____

SHORT PATH STILL...OPERATING PARAMETERS

Feed: _____D	Operator: _____	Date: _____

FEED

Initial weight (kg): _____
Tare weight (kg): _____
Throughput (kg): _____

DISTILLATION

Heating mantles on: _____
Distillation begins: _____
Distillation complete: _____

VACUUM (Torr)	Initial	Final
First stage	_____	_____
Second stage	_____	_____

WIPER SPEED (RPM): First stage: _____ Second stage: _____

TEMPERATURES (C°)	1st stage	2nd stage upper	2nd stage lower	Collar
Setpoint:				

THERMOCOUPLES (C°)

Time (min):						
T-1	_____	_____	_____	_____	_____	_____
T-2	_____	_____	_____	_____	_____	_____
T-3	_____	_____	_____	_____	_____	_____
T-4	_____	_____	_____	_____	_____	_____
T-5	_____	_____	_____	_____	_____	_____
T-6	_____	_____	_____	_____	_____	_____
T-7	_____	_____	_____	_____	_____	_____
T-8	_____	_____	_____	_____	_____	_____
T-9	_____	_____	_____	_____	_____	_____
T-10	_____	_____	_____	_____	_____	_____
T-11	_____	_____	_____	_____	_____	_____
T-12	_____	_____	_____	_____	_____	_____

FEED RATE

Metering valve setting: _____	Pump controller setting: _____

PRODUCT	1st stg. dist.	2nd stg. dist.	2nd stg. res.
Yield (Kg):	_____	_____	_____
Yield (%):	_____	_____	_____
Batch No.:	_____E	_____F	
QC analyses requested:	_____		

SCF FRACTIONATION...OPERATING PARAMETERS

Feed: _____F	Run # (M\D\Y\#: _____	Initials: _____

Feed Weight (g): _____

OPERATING PARAMETERS

Initial gas meter reading: _____
Flow rate (L/min): _____
Temperature profile (C°):
RT ____ (1) ____ (2) ____ (3) ____ (4) ____ (5) ____ (6) ____ (7)____
Initial pressure: _____

ANALYTIC SAMPLING

Time	Gas volumes from		to	Psig	Sample #	Gas inlet temp.
		-				
		-				
		-				
		-				
		-				
		-				
		-				
		-				
		-				
		-				
		-				
		-				
		-				
		-				
		-				

FRACTIONATION

Time	Gas volumes			Psig	Fraction #
		-			
		-			
		-			
		-			
		-			

POT WEIGHTS

Pot #	Tare	Gross	Net

COMPRESSOR RUN TIME

Time on: _____	Time off: _____	Total time: _____

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY...OPERATING PARAMETERS

Run No. (M/D/Y/#): _____ | Operator: _____

FEED

Volume (ml): _____
Sample code: _____BE
_____BF
_____BG

SOLVENT

Ethanol/water (V/V): ____ /____
Pump setting: _____
Column flow (ml/min): _____
Detector flow (ml/min): _____

PRESSURE (PSI)

Pulse dampener: _____
Radial compression: _____
Hydraulic: 350

COLUMN/COLUMN NO

Delta-pak C18
Prep scale #: _____
Process scale #: _____

PEAK SEPARATOR

Peak duration (min): _____
Peak delay (min): _____
Void timer (min): _____
Slope sensitivity: High ____/ Low ____

RECORDER

Chart speed (cm/min): _____ | Attenuation: _____

PRODUCT CODES

Esters	HPLC Fractions [#####BH.1(a-d)]	Neat Esters [#####BL.1(a-d)]
16:4	_____BH.1__	_____BI.1__
16:3	_____BH.2__	_____BI.2__
18:4	_____BH.3__	_____BI.3__
20:5	_____BH.4__	_____BI.4__
22:6	_____BH.5__	_____BI.5__

Attach photocopy (65% reduction) of chromatogram on facing page. Mark all cuts with colored ink and label cuts with proper product code.

VACUUM DEODORIZATION

I. Principle of Operation

The 55-gal drum of partially refined fish oil is pressurized with N_2 to start the oil flowing to the feed pump of the first stage of the deodorizer. The oil is pumped through the first stage where, under vacuum, it is spread in a thin layer over the inner wall of the still body by variable-speed Teflon wipers. In this stage, the oil is heated and degassed; some short-chained volatiles are removed and condensed in the first-stage electric vapor trap. The oil enters the sight-glass where a constant level indicates that the pumping rates of the two feed pumps are balanced. The second stage feed pump transfers the oil to the molecular still body where variable-speed carbon wipers spread it into a thin film on the inner wall of the still body. This stage is operated at a temperature sufficiently high and vacuum sufficiently low to distill volatile odors, flavors, cholesterol, PCBs, and pesticides from the oil. These materials condense on the internal or external condensers and are collected in the waste receivers. Volatiles that are not condensed in the external condenser pass into the second-stage electric vapor trap. The refined oil passes through the product cooler, through the second-stage sight-glass, and into the product receiver. When full, an automatic level switch in the product receiver turns on the product pump which delivers the oil to an evacuated product container through a 150 mm Teflon membrane filter (5-10 *u* pore size).

II. Equipment

The equipment, schematically illustrated in Figure 4, consists of a custom-designed stainless steel two-stage wiped-film molecular still assembled by Pope Scientific Inc., Menomonee Falls, WI. Both still bodies are heated by metal band heaters. A central control panel (Fig. 5) includes gauges, switches, and regulators to control and/or monitor temperatures, pressures, wiper speeds, and feed rates for the two feed pumps. Bleed valves for the two vacuum pumps are supplied with N_2 and the pumps are protected from volatiles condensation by electric vapor traps. In addition, 16-gal (*ca* 60-L) evacuated stainless steel pressure vessels are used to collect the deodorized oil. These vessels are equipped with stainless steel quick-disconnect fittings and a full-length dip tube to permit purging of the oil with N_2, pressurization with N_2, or application of a vacuum for product transfer in an inert atmosphere.

III. Operating Procedure

A. Procedure (numbers in brackets refer to elements in Figs. 4 and 5)

1. Turn on the circulating coolant bath and make certain that coolant is circulating through the condenser [10] and the

Components of the vacuum deodorizer

First Stage

1. Feed pump
2. Sight glass
3. Evaporator body
4. Wiper drive mechanism
5. Electric vapor trap
6. Mechanical vacuum pump

Second Stage

7. Feed pump
8. Wiper drive mechanism
9. Molecular still body
10. External condenser
11. Heated circulating bath
12. Product cooler
13. Electric vapor trap
14. Wastes receiver
15. Sight glass
16. Product receiver
17. Mechanical vacuum pump
18. Product pump
19. In-line Teflon filter
20. Oil re-circulation valve
21. Oil re-circulation valve

Note: For the sake of clarity, only the piping associated with the fish oil feed has been shown. For the same reason, the position of some of the components shown in the diagram differs slightly from their actual position on the support. The second-stage diffusion pump and the coolant bath are not shown.

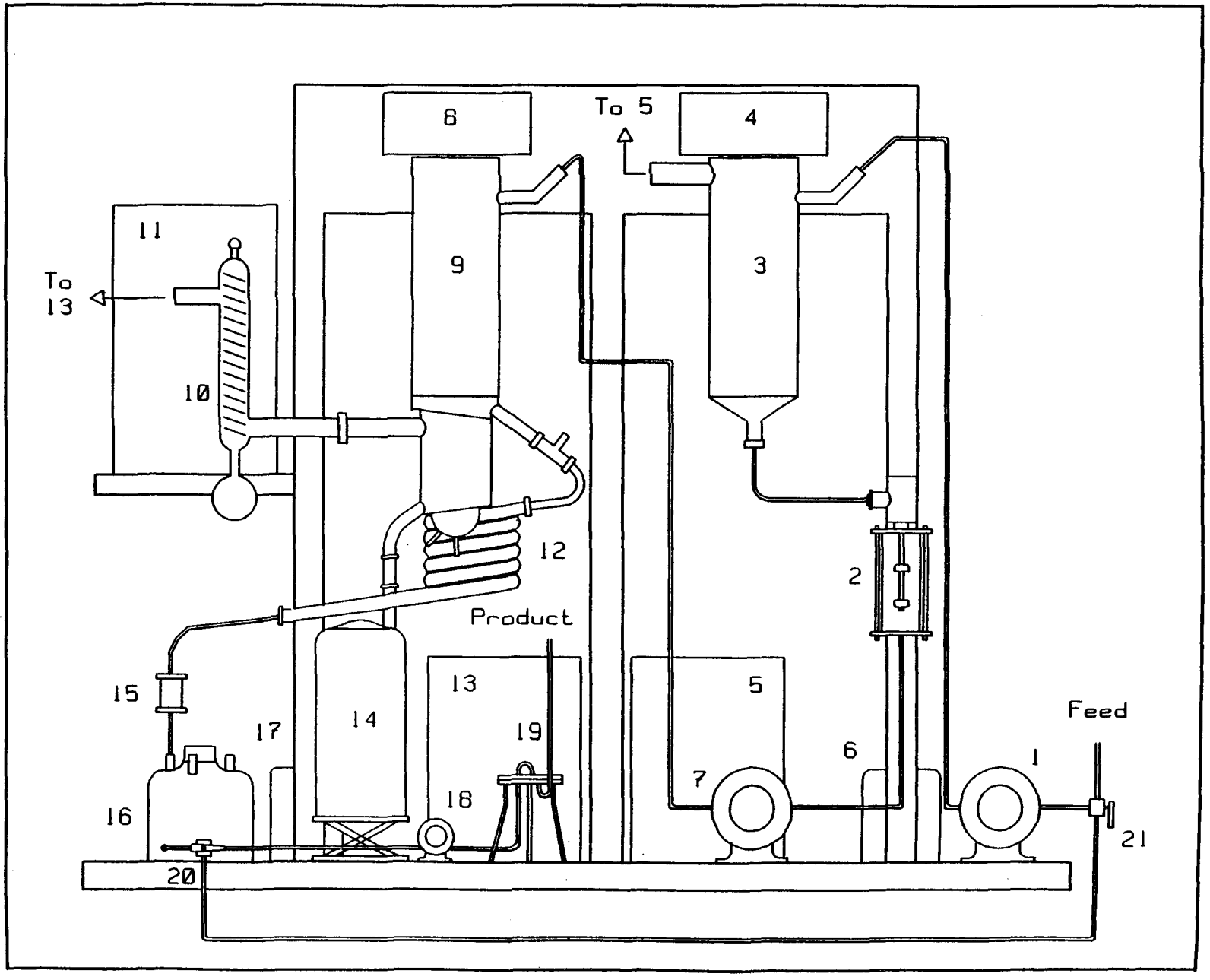


Fig. 4. Two-stage wiped film vacuum deodorizer.

Control panel toggle (power) switches:

S1 - Diffusion pump
S2 - Second stage vacuum pump
S3 - First stage vapor trap
S4 - First stage vacuum pump
S5 - Heated bath
S6 - Product pump
S7 - Temperature indicator
S8 - RPM indicator
S9 - Second stage still body heater
S10 - First stage still body heater

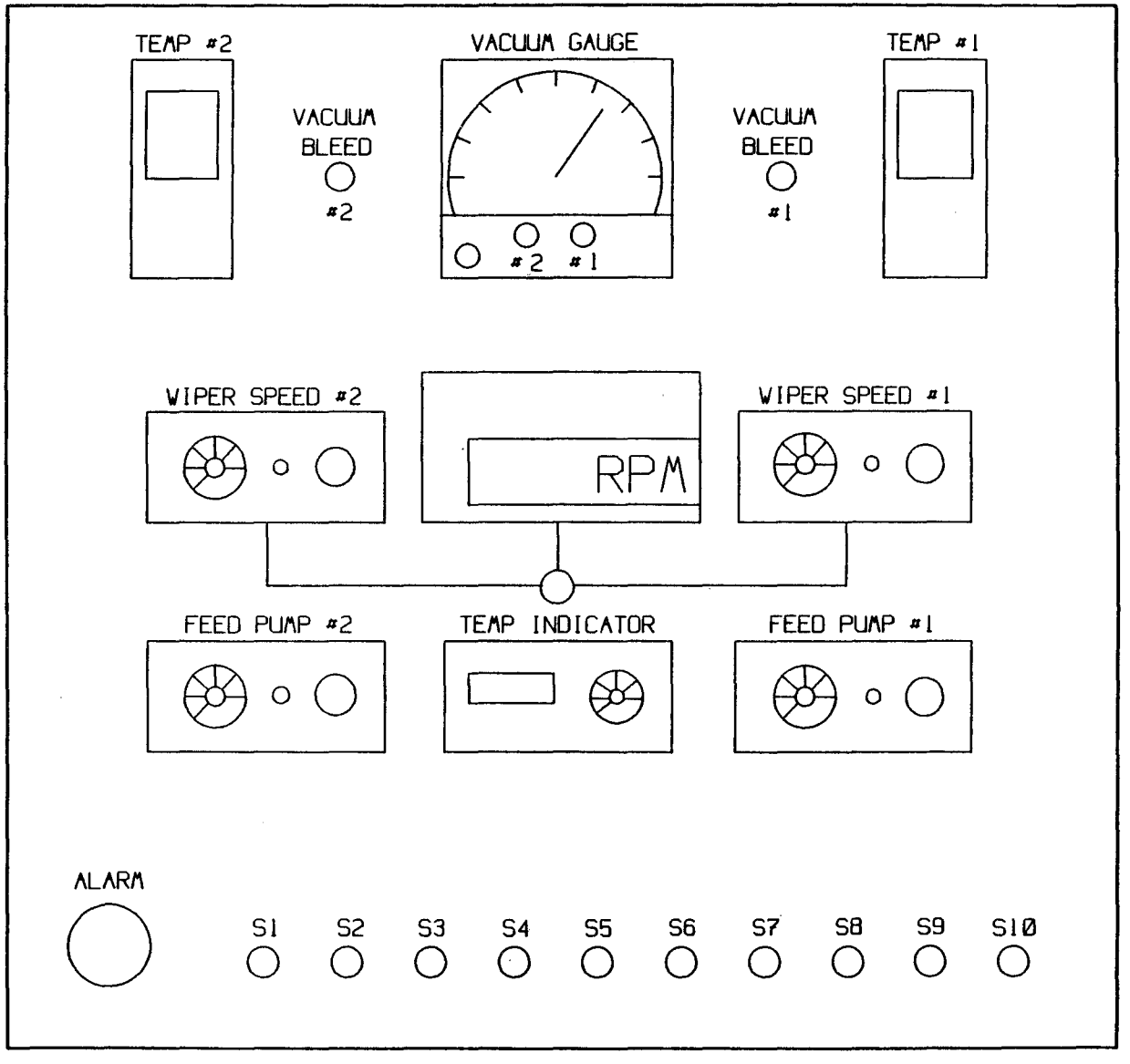


Fig. 5. Control panel of the vacuum deodorizer.

product cooler [12].

2. Turn on both electric vapor traps [5 and 13] and the heated circulating bath [11].
3. Make sure the selector switch is in the <O> position when starting the heated circulating bath. When the temperature reaches 80°C, place the switch in the <I> position. These are international symbols representing the binary numbers, 1 (on) and 0 (off).
4. For each kg of oil to be deodorized, weigh out into separate 250 ml beakers, 1.0 g Tenox-20A (20% TBHQ - tertiary butyl hydroquinone), 1.15 g Tenox 5-67 (*alpha*-tocopherol), and 3.1 g Tenox GT-1 (*gamma*-tocopherol). Transfer as completely as possible to a 16-gal product receiver. Attach the receiver to a vacuum pump and begin evacuation.
5. Evacuate a 5-gal product receiver.
6. When the vapor traps reach operating temperature, (-50°C), turn on both vacuum pumps [6 and 17]. Check the vacuum gauge on the control panel to be sure a vacuum is being pulled.
7. Turn on the diffusion pump switch (S1 on control panel) and allow pump to heat 30 min before turning on the pump itself.
8. When the vacuum of the first stage approaches 400*u* and that of the second stage, 300*u*, attach the 5-gal evacuated product receiver.
9. Set the product pump switch (S6 on control panel) to <AUTOMATIC>.
10. Turn on both wiper drives to 50 rpm, pressurize oil supply drum with N_2 and begin pumping oil.
11. Bring pumping rate of both feed pumps [1 and 7] up to a dial setting of '4' and '2.8', respectively.
12. Turn on both still body heaters [S9 and S10]. Set the first stage to 150°C, the second to 260°C.
13. Increase the speed of the first stage wiper from 50 to 150 rpm.
14. Set the speed of the second stage wiper at 150 rpm and increase to 250 rpm when it is operating smoothly.
15. After 30 min replace the 5-gal product receiver with the 16-gal evacuated receiver, supported on a mechanical scale. Record the weight and begin to collect product.
16. Monitor the product cooler to be sure the bottom is cool to the touch. If it is not, check that coolant is circulating through the cooler and consult the plant supervisor.
17. Monitor and empty the waste receiver [14] as necessary.
18. When the target weight of the product receiver is reached, replace the filled container with another evacuated receiver containing the proper amount of antioxidants and continue to collect product.

B. Shut-down

1. When the last run of the day is finished, open the two valves [20, 21] that allow recirculation of feed through the system.
2. Close the diffusion pump high vacuum valve and low vacuum valve. Shut off the pump.
3. Close the valve of the first stage vacuum pump. Let the pump continue to operate.
4. Turn off heaters to both still bodies.
5. Reduce wiper speed to 50-70 rpm.
6. Turn off the heated bath [11].
7. Turn off the vapor traps [5, 13].
8. Turn off the vacuum pump used to evacuate the product tank.
9. Cool both still bodies to 50°C.
10. Turn off first and second stage feed pumps [1, 7].
11. Turn off first and second stage wipers [4, 8].
12. Turn off the second stage vacuum pump [17].
13. Turn off the first stage vacuum pump [6].
14. Turn off the coolant bath.

IV. Product Storage

The deodorized oil is collected in 16-gal stainless steel pressure vessels, each constituting one "batch" and stored at 5°C. For future shipment as bulk oils, two batches (56 kg each) may be bulked into one "lot" in a 37-gal stainless pressure vessel for freezer storage until packaged for shipment. If the oil is to be used in the production of omega-3 concentrates, 40 kg will constitute one batch and be stored in the pressure vessel itself. In either event, before storage, the container is to be placed on the drum roller to thoroughly mix the oil and antioxidants. Transparent envelopes large enough to accept a 3"x5" information card are attached to each storage container to identify the contents by batch or lot number. Using the rubber stamp, stamp a card, fill in the batch number and place in the envelope before storing the container in the refrigerator or freezer.

V. Equipment Cleaning and Maintenance

Before daily use, the oil of all vacuum pumps is inspected and changed, as necessary. Following daily operation, the waste receivers are emptied and washed with hot detergent solution; the vapor traps must thaw overnight before they can be cleaned. After thawing, the traps are first drained and then wiped with paper toweling. The air handler switch in Rm. 241 should the be placed in the <OCCUPIED> position.

After every 13 weeks of operation, the first still body is cleaned by flushing with alcoholic alkali, followed by copious volumes of deionized water and absolute alcohol. At this time, the second still body is disassembled and cleaned by hand using an oven cleaner and steel wool. The carbon blades are replaced and all

gaskets examined and replaced if necessary. Every 26 weeks, both still bodies are disassembled, examined, and hand cleaned.

All pressure vessels and storage containers are filled with hot FDA-approved detergent solution as soon as they are emptied, and allowed to stand overnight. The next day they are scrubbed with fresh detergent, rinsed with tap water, deionized water, and absolute ethanol.

VI. Waste Disposal

Wastes consist of (1) PCBs and pesticides that collect in the receiver of the second-stage external condenser, (2) vapor trap condensables (3) semi-solid wastes consisting of cholesterol, free fatty acids, etc. that collect in the second-stage waste receiver, (4) used vacuum pump oil, (5) alcoholic alkali used to clean the deodorizer, and (6) oily toweling (see below). Each type of waste must be placed in a separate container, clearly and prominently labeled, and stored properly for disposal.

VII. Safety

* Because the deodorizer is operated at low pressure and contains glass components, the operator must wear safety goggles while operating the equipment.
* The wearing of safety shoes is imperative since a full 55-gal drum of fish oil weighs 193 kg and a filled 16-gal pressure vessel, about 75 kg.
* The exposed metal of both of the still bodies is very hot during operation, and the operator must exercise caution when working in these areas.
* Gloves, safety goggles, and a respirator with an organic vapor cartridge are to be worn when cleaning the vapor traps.
* All oil spills on the platform and floor are to be cleaned up immediately and the used toweling placed in the covered safety can. This can must be emptied into the covered safety can located near the incinerator before the operator completes the shift.

VIII. Emergency Shut-down Procedure

When the fire alarm sounds, the deodorizer should continue to operate, but unattended, since proper shutdown requires too much time in an emergency situation. To avoid the risk of filling the product receiver and flooding the vacuum pump with fish oil, an empty evacuated product receiver must be available at all times the deodorizer is in operation. The operator can then switch to the empty receiver before leaving the plant.

In case of a fire in the plant (other than in the Reactor Lab or the Evaporator Lab, Rms. 423 and 424, respectively):

1. First, activate the fire alarm.

2. Throw the main circuit breaker of the deodorizer, located in the compartment below the control panel.
3. Open both vacuum pump bleed valves.
4. Evacuate the plant.

If the fire is located in Rooms 423 or 424, leave the plant immediately by the nearest exit, activating the fire alarm beside the exit if necessary.

IX. Appendix

Equipment and Materials Suppliers:

Vacuum deodorizer
Pope Scientific, Inc.
(414) 251-9300
P.O. Box 495
N90 W14337 Commerce Dr., Menomonee Falls, WI 53051
Contact: Dean Segal (engineering)
John Oxendorf (price information)

Vacuum pumps and gauge
Edwards High Vacuum, Inc.
(800) 828-6691 or (615) 691-3850
P.O. Box 30381
Knoxville, TN 37930-0381
Contact: Kevin Bloomer, sales engineer

Vacuum fittings
Cooper River Valve and Fitting
552-5545
4669 Franchise Street
Charleston Heights, S.C.
Contact: John Chambliss

Electric vapor trap
Southeastern Scientific Corp.
(704) 542-3508
P.O. Box 2093
Charlotte, N.C. 28211
Contact: John Hardin

Pressure vessels
Alloy Products Corp.
(414) 541-0958
1045 Perkins Ave.
Waukesha, WI 53186
Contact: Craig Bear

Wiper drive speed controller/feed pump controller
Graham Co.
(414) 355-8800
Box 23880
Milwaukee, WI 53223

Temperature controller
Shealy Electric Wholesalers (Watlow area representative)
(803) 252-5668
Columbia, SC

Antioxidants
Eastman Chemical Products, Inc.
(800) 327-8626
P.O. Box 431
Kingsport, TN 37662
Contact: Rama Wineman

Nitrogen supply
National Welders
(Laboratory Purchasing Agent places blanket purchase orders)

PREPARATION OF SODIUM ETHOXIDE

I. Principle of Operation

Sodium ethoxide is synthesized in the production plant, rather than purchased, for reasons of both economy and purity. Metallic sodium and absolute ethyl alcohol are reacted under a nitrogen atmosphere to exclude moist air, with constant stirring. The sodium is cut into small pieces and added to the alcohol very slowly to avoid generating appreciable heat, thus eliminating the need for a cooling bath.

II. Equipment

Supplies and materials needed include two 10 L-Schott bottles with caps, two magnetic mixers with stirring bars, a sharp knife, absorbent paper towels, disposable plastic gloves, two 8-oz jars with lids, each containing 170 g pre-weighed metallic sodium under mineral oil, a screw-capped plastic jar, two plastic buckets large enough to accommodate the Schott bottles, absolute ethanol, and a N_2 supply.

III. Operating Procedure

Preparation of the sodium ethoxide must be carried out in hood #2 (Rm. 422) with the fan on. To prepare sufficient sodium ethoxide for <u>one</u> 40 kg batch of oil, place 7 L absolute ethanol in a 10-L Schott bottle flushed with N_2 and place the bottle in the plastic bucket. Place the bucket on a magnetic stirrer and add the stirring bar. Open a container of previously weighed sodium, remove a few pieces and wipe off the mineral oil with absorbent towels. Turn on the stirrer to moderate speed, then add 4-5 pieces of sodium (approximately 1 cm^3 in size) to the ethanol. Continue until all sodium has been added and dissolved. <u>No more than 4-5 pieces should be added within a 15-20 min period to avoid overheating the container</u>. It is necessary to keep a constant flow of N_2 over the solution until all sodium has dissolved. Therefore, if at the end of the day there are still small flakes of sodium present, reduce N_2 flow and continue to stir the solution overnight. Otherwise, the bottle can be tightly capped.

IV. Product Storage

The sodium ethoxide may be stored in the hood overnight in the tightly capped Schott bottle.

VI. Waste Disposal

The waste generated during the preparation of sodium ethoxide consists of used plastic disposable gloves and paper toweling contaminated with mineral oil and small flakes of sodium. These

wastes must be placed in a plastic jar with a tightly fitting cover containing a small volume of alcohol, removed from the plant and the contents burned in the incinerator as soon as possible.

VI. Equipment Cleaning and Maintenance

The only cleaning required is routine washing of the Schott bottles, the caps, the stirring bar, and the knife in detergent solution followed by thorough rinsing with water. The bottles and caps should also be rinsed with a small quantity of absolute alcohol to speed drying.

VII. Safety

* Extreme caution is required in working with metallic sodium because of its violent reaction with water and the accompanying evolution of H_2 gas. Therefore there must be NO contact of the sodium with water and even though the sodium is shipped in sealed cans, supplies must be stored in a container under mineral oil in the flammables cabinet in Storage Container E. To avoid even a remote chance of water leaks in the vicinity of exposed sodium, all valves in the water supply to hood #2 have been shut off above the ceiling.
* In preparing the sodium for reaction with ethanol, the worker must wear disposable plastic gloves and safety goggles. As soon as possible, the knife used to cut the sodium must be placed in a container of absolute ethanol with the blade submerged. At the end of the day, the knife may be safely washed with water and dried carefully. Periodically, this ethanol should be added to the drum containing waste alcohol and fish oil derived from cleaning of the vacuum deodorizer. As much as possible, the reaction should be carried out behind the closed doors of the hood.
* In case of a major spill of dissolved Na in alcohol, large spill pillows (4 L-capacity) are located in Room 420, over the chest freezer. Small spill pillows (1-L capacity) are located in Room 423 near the safety shower. If the spill pillows are needed, they should be placed, after use, in the individual plastic bags they were packaged in and placed in the solid waste can located outside near the incinerator. In case of breakage of the Schott bottle during preparation, the plastic bucket will retain the sodium and ethanol. If this occurs, allow all sodium to react before diluting the sodium ethoxide copiously with tap water and disposing down the drain.

VIII. Emergency Shut-down Procedure

If the fire alarm sounds while the sodium is being cut up for reaction, return all sodium to the container of mineral oil and

cap tightly, place the knife in the container of alcohol, place paper toweling and gloves in the plastic waste jar and cap tightly. Evacuate the plant, taking along the jar of sodium and the waste jar. Leave the waste jar beside the incinerator but bring the jar of sodium and report to the front parking lot. If the alarm sounds during the reaction, return any exposed sodium to the jar of mineral oil, leave the stirrer and N_2 flow on, and leave the plant, removing waste jar and unreacted sodium, as described above. If the fire is in the facility itself, activate the fire alarm at the nearest exit if necessary, and evacuate the plant immediately, removing any unreacted sodium.

IX. Appendix

Equipment and Materials Suppliers:

Schott bottles
Corning
Sales/Service
(607) 737-1672
P.O. Box 5000
Corning, NY 14830
Contact: Barbara Harr

Magnetic stirrer
Cole-Parmer Instrument Co.
(800) 323-4340
7425 North Oak Park Ave.
Chicago, IL 60648

Sodium (1 lb bars in evacuated cans)
Baxter
(800) 438-1234
8350 Arrowridge Blvd.
Charlotte, NC 28217
Contact: Glenna

Absolute ethanol
Aaper Alcohol
(800) 626-5281
P.O. Box 339
Shelbyville, KY 40065
Contact: Harold
or
Quantum
(800) 543-5900

TRANSESTERIFICATION

I. Principle of Operation

Since the long chained polyunsaturated fatty acids of fish oils cannot be substantially concentrated in the form of triglycerides, it is necessary to hydrolyze the triglycerides and convert their constituent fatty acids to esters. Vacuum deodorized fish oil is reacted with a catalytic amount of sodium ethoxide and absolute ethanol at 60°C, under N_2, to simultaneously hydrolyze the oil and convert the released fatty acids to ethyl esters. After 60 min, the reaction mixture is cooled and allowed to stand until the lower glycerol/alcohol phase separates from the esters. The lower phase is discarded and the esters are pumped to 100-L separatory funnels where they are blanketed with N_2 and washed several times with deionized water.

II. Equipment

The equipment consists of two 72-L all glass/Teflon reaction flasks and two 100-L separatory funnels. Each reaction flask has a N_2 sparge tube, a thermowell and thermometer, a side arm with Teflon stopper for addition of materials, an air-driven stirrer, an internal glass cooling coil, a heating mantle, and an external condenser. Each separatory funnel has a polycarbonate lid that is fitted with a N_2 supply and a deionized water supply. Air-driven pumps are used to transfer esters from the reactors to the separatory funnels and from the funnels to the crystallizer. Nitrogen, air, and chilled water are supplied to the hoods where the reaction is carried out. The relationship of this equipment to the rest of the equipment needed to produce omega-3 concentrates is shown schematically in Figure 6.

III. Operating Procedure

1. Using N_2 pressure available at the hoods where the reactors are located, transfer 40 kg of refined oil from the 16-gal pressure vessel in which it was stored, to each of two 72-L reactors being purged with N_2. Nitrogen pressure should be sufficient to complete the transfer in about 20 min.
2. Turn on the stirrer and set the temperature controllers for the heating mantles at '6'.
3. When oil transfer is complete, add 9 L absolute ethanol to each reactor.
4. Set the temperature controllers to 'High'
5. When the temperature of the solutions is 55°C (about 45 min required), add 7 L of sodium ethoxide to each reactor.
6. Turn off the temperature controllers.
7. After 60 min, turn off the stirrers, and allow the

Elements in the production of ester concentrates

Equipment

1 - Deodorized triglycerides
2 - Transesterification reactor
3 - Separatory funnel
4 - Adduct crystallizer
5 - Solid waste receiver
6 - Evaporator feed tank
7 - Scraped film vacuum evaporator
8 - Vapor separator
9 - Alcohol receiver
10 - Vacuum pump
11 - Separatory funnel
12 - Product (crude ester) receiver

Supplies/Services

E - Sodium ethoxide
D - Deionized water
A - Anhydrous alcohol
R - Reclaimed alcohol (95%)
U - Urea
C - Jacket coolant
S - Steam

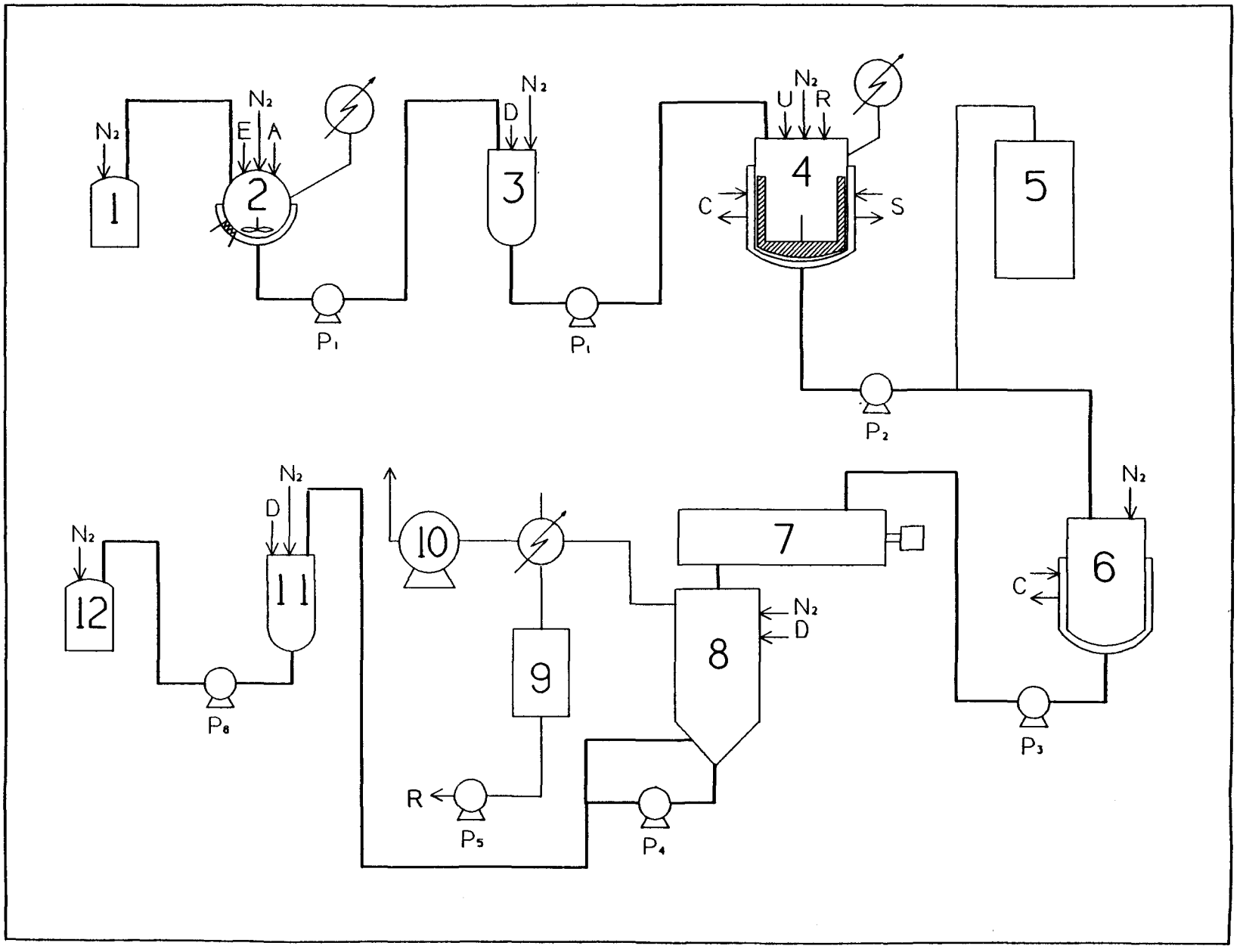


Fig. 6. Esterification-adduction-concentration flow sheet.

solutions to stand for an additional 60 min.

8. Drain the glycerol/alcohol lower phase as completely as possible.
9. Pump the esters to the separatory funnels that have been purged with N_2. Maintain N_2 flow.
10. Wash the esters first with 10 L of deionized water, then three times more with 5-L aliquots of deionized water, draining water after each wash.
11. Warning! Do not leave the funnels unattended while draining is in progress.

IV. Product Storage

Under ordinary operating procedure, the esters will be pumped to the crystallizer for urea adduction. However, if the esters must be stored, they may be transferred to evacuated 16-gal product receivers and stored in the cooler or freezer, depending upon expected storage time (walk-in cooler for <24 hours, -10°freezer for >24 hours).

V. Equipment Cleaning and Maintenance

Thorough washing of all glassware with detergent solution followed by rinsing with tap water, deionized water, and finally, absolute ethanol, is the only cleaning required unless there are spills. In that event, the pans in the floor of the hoods will also need to be cleaned with detergent.

VI. Waste Disposal

The wastes generated by this procedure consist of glycerol and water used to wash the esters, both of which may be drained from the bottom of the reactor into the cup sink in the hood. Tap water should be used to dilute the glycerol flowing through the cup sink as a routine practice.

In case of a major spill, large spill pillows (4-L capacity) are located in Room 420, over the chest freezer. Small spill pillows (1-L capacity) are located within the reactor lab (Rm. 423) near the safety shower. If the spill pillows are needed, they should be placed, after use, in the individual plastic bags they were packaged in and placed in the solid waste can located outside near the incinerator.

VII. Safety

* Since there is always some hazard in working with glass equipment at elevated temperatures, safety goggles should be worn and the reaction should be carried out behind the closed door of the hood.
* The safety trays in the floors of the hoods will contain the contents of a broken reactor or separatory funnel and

can be cleaned out using 4-L spill pillows but only when the spill is cold.

* Broken glassware should be disposed of in a 55-gal drum labeled "Broken Glass". Very cautiously, recover reusable ground glass joints, Teflon stopcocks, etc., before disposal.

VIII. Emergency Shut-down Procedure

If the fire alarm sounds during the reaction, close the door to the hood but leave the air-driven motors on. Shut off the heating mantles and evacuate the plant.

If a fire occurs in Rms. 423 or 424, close the door to the hood and leave the plant immediately by the nearest exit, activating the fire alarm by the exit, if necessary.

IX. Appendix

Equipment and Materials Suppliers:

Glassware
Tudor Scientific Glass, Inc.
(803) 279-4666
555 Edgefield Road
Belvedere, SC 29841
Contact: Chris Schute
Tom Tudor

Transfer pump (explosion-proof)
Cole-Parmer Instrument Co.
(800) 323-4340
7425 North Oak Park Ave.
Chicago, IL 60648

Absolute ethanol
Aaper Alcohol
(800) 626-5281
P.O. Box 339
Shelbyville, KY 40065
Contact: Harold

UREA ADDUCTION

I. Principle of Operation

Urea adduction of more saturated fatty acids and esters is a classical analytical technique for concentrating polyunsaturated acids and esters. When a hot solution of urea and alcohol is allowed to cool, the urea precipitates in the form of hexagonal crystals with a central channel about 6.5 A in diameter. When this occurs in the presence of straight chained organic molecules having a chain length greater than six carbons, adducts are formed between the organic molecules and the urea crystals. Saturated compounds readily form adducts, monoenes less so, and dienes even less. Polyunsaturates do not form adducts because their molecules are too bulky to fit within the channel of the urea crystal. In production, this small-scale analytic procedure has been scaled up to produce concentrates of omega-3 esters in quantity. In production scale, urea is dissolved in hot (80°C) 95 wt.% ethyl alcohol under a N_2 blanket. The esters (80 kg) are added and dissolved in the solution. Upon cooling overnight, the more saturated esters complex with urea and precipitate from solution while the polyunsaturates remain in the alcoholic liquor. The crystals are allowed to settle and the alcoholic solution of unsaturated esters is siphoned and drained from the crystals. This liquor is then concentrated in a film evaporator and washed with water to recover the unsaturates, as described in the next section of this Manual.

II. Equipment

The crystallizer system consists of: a 300-gal, jacketed, stainless steel vessel with a two-speed, scraped surface mixer; a variable speed, rotary pump; a reflux/take-off condenser; a data-logger; and associated valves and piping. The crystallizer is designed with three jackets (upper, middle, and lower) any or all of which may be heated by steam and cooled with tapwater and chilled coolant. Automatic switches of the data logger turn off the mixer and coolant supply when the internal temperature reaches the set-point (5°C). If the temperature subsequently rises, the coolant supply is restored until the temperature reaches the setpoint again. Nitrogen is supplied to the crystallizer throughout the entire process. The equipment is illustrated schematically in Figure 6 and, in greater detail, in Figure 7.

III. Operating Procedure

A. To prepare for operation, check alcohol, vessel, piping, valve settings, cooling water, steam, pumps, meters, temperature recorder, data logger and N_2 purge. Numbers in brackets, below, generally refer to valves and equipment located in Rm. 423 and are illustrated in Figure 7. In a few instances, the valves referred to are located in Rm. 424 and

Room 423 Valve List

1. Crystallizer bottom drain
2. Adduct liquor take-off
3. Adduct liquor recycle
4. Adduct liquor syphon
5. Alcohol line flush
6. Alcohol charge
7. Nitrogen supply
8. Nitrogen pressure regulator
9. Potable water hose bibb
10. Potable water to jackets
11. Condenser coolant supply
12. Condenser coolant return
13. Low temperature coolant supply
14. Low temperature coolant return
15. Coolant supply cross-over
16. Coolant return cross-over
17. Condenser pressure relief
18. Condenser air vent valve
19. Coolant supply drain
20. Top jacket pressure relief
21. Top jacket steam inlet
22. Top jacket coolant outlet
23. Mid jacket steam inlet
24. Mid jacket coolant outlet
25. Mid jacket pressure relief
26. Top jacket coolant inlet
27. Top jacket steam condensate outlet
28. Top jacket drain
29. Mid jacket coolant inlet
30. Mid jacket steam condensate outlet
31. Mid jacket drain
32. Bottom jacket coolant outlet
33. Bottom jacket steam inlet
34. Bottom jacket pressure relief
35. Bottom jacket coolant inlet
36. Bottom jacket steam condensate outlet
37. Bottom jacket drain
38. Steam drip leg condensate outlet
39. Steam drip leg drain
40. Condenser alcohol recycle
41. Condenser alcohol drain
42. Condenser alcohol take-off

Room 423 Equipment List

N_2	Nitrogen supply
CS	Coolant supply
CR	Coolant return
PCW	Potable cold water
LCS	Low temperature coolant supply
LCR	Low temperature coolant return
LSS	Low pressure steam supply
SCR	Steam condensate return
C	Condenser
A	Alcohol charge
E	Ester charge
D	Drain
S	Syphon
P	Transfer pump
G	Sight-glass
UJ	Upper jacket
MJ	Mid jacket
LJ	Lower jacket
TM	Totalizing meter

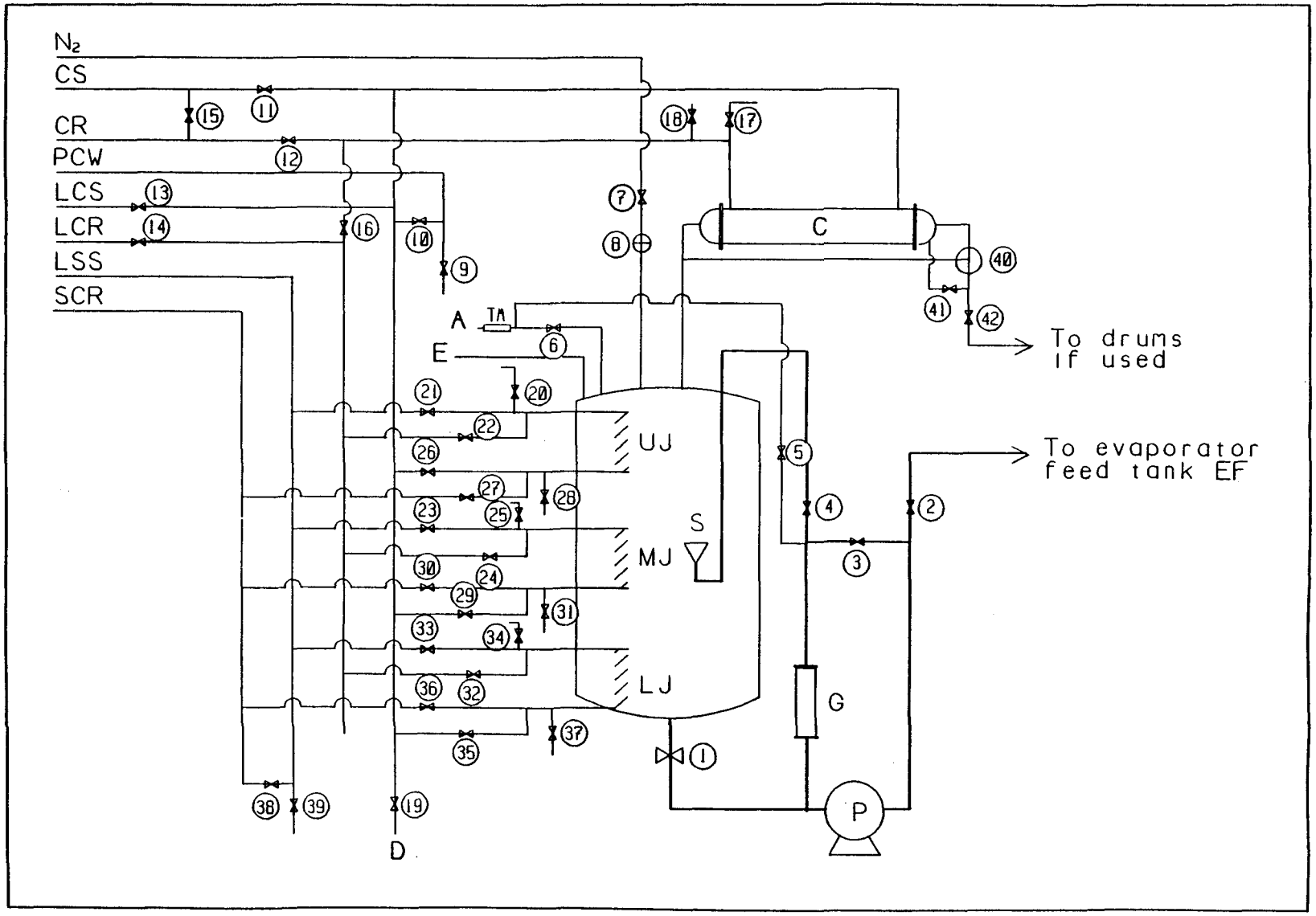


Fig. 7. Crystallizer valve and equipment diagram (Room 423).

illustrated in Figure 8 (see next section on film evaporation). In these cases, the room number is included in the brackets.

Check all valves for proper position before operating the chiller or crystallizer. Specifically, valves [1, 2, 4, 5, 6, 7, 8, 9, 10, 11, 12, 15, 16, 19, 20, 21, 22, 24, 25, 26, 27, 28, 29, 30, 31, 32, 34, 35, 36, 37, 38, and 40] must be closed. Valves [3, 13, 14, 23, 33, 39, and 40] must be open (close valve [40] when it is steaming).

B. Charge crystallizer with alcohol and urea. Heat to 80°C with mixer on high. Cut heat, charge esters, start cooling.

1. Turn on chiller (see Appendix to this section, p. 49).
2. Record alcohol level in storage tank [A or B] and time of start, making sure alcohol is of correct strength and purity.
3. Flush crystallizer with N_2 [7, 8], then adjust flow to blanket the solution.
4. Set totalizing meters at pump and at crystallizer to zero.
5. Open alcohol valve at bottom of alcohol tank [424; 11 or 13].
6. Turn on transfer pump.
7. Open transfer valve near pump [424; 7].
8. Charge required gallons of alcohol to crystallizer (209 gal) by opening the alcohol valve at the crystallizer [6].
9. Turn off transfer pump, close valves [424; 11 or 13: 424; 7: 423; 6]. Record time and volume (gal).
10. Turn mixer on high. Set mixer switch (above data logger) to 'AUTO'.
11. Charge required weight (220 Kg = 484 lb) of urea to crystallizer. Record.
12. Open main steam valve [424; 4]. Turn on steam to mid jacket [24, 31]. Open valves [23] and [33].
13. Heat to required temperature (80°C). Record time and temperature.
14. Reduce steam to mid jacket [24].
15. Pump required esters (80 Kg) to crystallizer. Record.
16. Shut off steam [24] when temperature returns to 80°C.
17. Shut off steam condensate [31]. Vent jacket [32].
18. Turn on cooling water to top [27, 23] and bottom [36, 33] jackets. Record time and temperature.
19. Set mixer to low speed.
20. When the contents of the crystallizer reaches 5°C the mixer will shut off.
21. Cool overnight.

C. Transfer of liquor from crystallizer

1. Record temperature and time.

2. Prepare siphon and pump. Turn on distilled water flow to pump seal.
3. Check evaporator feed tank (Rm. 424). Flush with N_2 and adjust flow to blanket the liquid. Check that bottom valve [17] is closed
4. Open by-pass valve [423; 3]. Check distilled water flow to seal on transfer pump.
5. Turn on pump. Set speed to '5'.
6. At crystallizer, open transfer valve [2] to feed tank.
7. Open siphon valve [4].
8. Close by-pass valve [3].
9. Pump top liquor to feed tank.
10. When the top liquor is removed, insert the siphon line into the crystal mass as far down as possible, near the center of the tank.
12. With screen in line, open bottom valve [1] of crystallizer.
13. Almost close by-pass valve [3]. Check at evaporator feed tank that liquid is pumping over.
14. Continue pumping bottoms until flow is very low (this will take 3.5-4 hours). Check level of liquor in feed tank.
15. When liquor flow is very low, shut off pump and distilled water to the pump seal.
16. Remove siphon line and close lid.
17. Turn off nitrogen.

IV. Product Storage

Since urea adduction is only the first of three interrelated steps in concentrating polyunsaturated ethyl esters, no finished product results from this step. While chilling for crystallization, the system may be held in stand-by condition by continued chilling and blanketing with N_2. Liquor that has been transferred to the evaporator feed tank may be held under N_2 and kept cold (0-5°C) by circulating coolant in the jacket.

V. Equipment Cleaning and Maintenance

Rinse crystallizer well with 8 to 10 gallons of hot tap water (use hose) immediately after use and discard into floor drain. Follow this rinse with hot detergent solution and scrub the interior of the crystallizer with a long handled brush. Rinse thoroughly with hot tap water, deionized water, and absolute ethanol.

VI. Waste Disposal

The adduct crystals are slurried with water to store as by-product for disposal. Rinse tank and pipe lines.

1. Add required gallons of tap water with hose (30 gal).
2. Turn mixer on low, then high.

3. Mix thoroughly.
4. Remove in-line screen at bottom valve [1].
5. Connect transfer pipeline to urea by-product storage tank.
6. Open valves [1, 2] and pump urea solution to storage.
7. Disconnect pipeline to storage and re-connect to feed tank line.

VII. Safety

* Hoods #3 and #4 must be operating (#4 on HIGH) when the crystallizer is in use.
* Ethyl alcohol is potentially toxic. Avoid breathing vapors by maintaining proper ventilation, avoiding spills and leaks, keeping operating and storage vessels closed, maintaining constant vigilance and care. Toxicity level is highly subject to individual tolerances but can impair judgement at relatively low levels of exposure. Lethal dosage levels are relatively high and, at low rates of exposure, are preceded by intoxication. Respirators with organic vapor cartridges have been provided to all personnel for use if ethanol vapors become objectionable.
* Ethyl alcohol is a flammable solvent, will easily ignite and burn unless highly diluted with water. A fire can be especially dangerous because the flames may be almost invisible. It will form explosive mixtures with the proper amount of air. Water dilution is the most effective countermeasure for minor spills, etc.
* Major spills (*ca* 20 gallons or more) should receive special, supervised attention. Provisions are made to handle large spills by entrapment in the outside 1000 gal sump. In this event, make certain immediately that the sump pump is turned off so alcohol will not be pumped to the sewerage system The pump switch is outside near the sump pump. Normally, the explosive vapor detection system will sound an alarm and disconnect the pump automatically. Further precautions should be taken by disconnecting the pump with the outdoor local switch. Follow up any spill with a water hose flush of the floor and drains since vapors can possibly leak into other areas of the building (although this is not likely). Also turn local hood fans on high and open hood doors partly if conditions permit.
* Care must be maintained in handling alcohol and oil-contaminated wastes and materials (towels or other clean-up materials.)
* Make certain all valves to large vessels containing alcohol are kept closed except when necessary during immediate operation.
* Open all disconnect switches at shut-down and close all valves as necessary.

* Keep floors rinsed down and obstacles out of the way, especially during operation.
* Neoprene gloves are recommended because of the dehydrating effect of ethanol on exposed skin.
* Safety shoes should be worn necause of the necessity for handling 55 gal drums of ethanol.

VIII. Emergency Shut-down Procedure

If a fire occurs in the immediate vicinity (Rms. 423 and 424), all personnel are to evacuate the plant immediately. Activate the fire alarm at the nearest exit on leaving. Fire protection devices (heat and smoke detectors and sprinkling system) will take over. The main circuit breaker in panel E2 (Rm. 426) may be turned off to shut off all power to Rms. 424 and 426 if this can be done safely.

If fire alarms indicate possible fire in other parts of the main laboratory or office spaces (i.e., Zones 1-4), make a careful, safe shut-down of the operation so as not to damage the equipment or material being processed and evacuate the plant. For example, if charging the crystallizer or heating its contents, close all transfer valves, turn off steam and pumps, leave the mixer on, and vacate the facility, assisting others as necessary.

IX. Appendix

A. Water chiller start-up procedures

The Capitol water chiller is used with the crystallizer.

1. Check coolant level in tank. Add coolant (food-grade 50% propylene glycol) when level is below mark.
2. Open valve [A] and close valve [B]. These valves are located on the heat exchanger. One of the valves must be open during chiller operation.
3. Depress the <START> button on the control panel.
4. Check pressures and temperatures for operation.
   - Suction pressure: 30-50 psi
   - High pressure: 250-275 psi
   - Coolant supply pressure: 20-30 psi
   - Coolant return pressure: 5-15 psi
   - Coolant temperature when stabilized: 10-13°F, Maximum, 30°F
5. Place the control switch (located in Rm. 420, above the data logger) in the <AUTO> position.

B. Preparation of 95 wt.% Ethanol

In order to reuse ethanol recovered during film evaporation of the concentrates, it is necessary to add absolute ethanol to bring the strength of recovered ethanol (generally 90-94 wt.%) to 95% for reuse in subsequent urea adductions. The following program was written in BasicA by R.C. Ernst, Jr. to simplify the necessary calculations. The program has been edited, removing spaces needed for proper display on a computer monitor, to improve legibility.

```
900 '            ************* ALCALC ******************
940   KEY OFF
950   KEY (1) ON
970  GOSUB 3000: GOTO 4280     ' Initialize and start program
1000 '           ******** ALCOHOL INTERPOLATION*********
1010 '                        TEMP,CONC,SPGR
1050 '                 X(N) VS Y1(N) @ 50 F, X-->SP(1)
1060 N=3
1070   A=X
1080     B=0
1090   FOR J=1 TO N
1100       T=1
1110      FOR I=1 TO N
1120         IF I=J THEN 1140
1130            T=T*(A-X(I))/(X(J)-X(I))
1140      NEXT I
1150          B=B+T*Y1(J)
1160   NEXT J
1170     SP(1)=B
1180 '                  *************************
1190 '                 X(N) VS Y2(N) @ 77 F, X-->SP(2)
1200   A=X
1210     B=0
1220   FOR J=1 TO N
1230       T=1
1240      FOR I=1 TO N
1250         IF I=J THEN 1270
1260            T=T*(A-X(I))/(X(J)-X(I))
1270      NEXT I
1280          B=B+T*Y2(J)
1290   NEXT J
1300     SP(2)=B
1310 '                  *************************
1320 '                 X(N) VS Y3(N) @ 104 F, X-->SP(3)
1330   A=X
1340     B=0
1350   FOR J=1 TO N
1360       T=1
1370      FOR I=1 TO N
1380         IF I=J THEN 1400
1390            T=T*(A-X(I))/(X(J)-X(I))
1400      NEXT I
```

```
1410          B=B+T*Y3(J)
1420    NEXT J
1430      SP(3)=B
1440 '                  *************************
1450 '              TT(N) VS SP(N) @ CONC. X, TEMP-->SPGR
1460 N=3
1470 A=TEMP
1480      B=0
1490    FOR J=1 TO N
1500        T=1
1510       FOR I=1 TO N
1520          IF I=J THEN 1540
1530             T=T*(A-TT(I))/(TT(J)-TT(I))
1540       NEXT I
1550           B=B+T*SP(J)
1560    NEXT J
1570      SPGR=B
1580 RETURN
1600 '           *************** TABLE *****************
1610 CLS:GOSUB 4260:PRINT" POUNDS TO GALLONS VS TEMPERATURE
     ("CHR$(248) "F)"
1620 PRINT  "-------------------------------------------------"
1630 PRINT "TEMP.("CHR$(248)"F)",:PRINT USING "####.# _LB."
     ;W3;W4;W2;W1
1640 PRINT ,:PRINT USING "_OF #### _%       ";95,95,100;
     :PRINT USING "_OF ####.# _%     ";C1
1650 PRINT "-------","-----------","-----------","-----------",
     "-----------"
1660       FOR TEMP=60 TO 94 STEP 2
1670 X=95:GOSUB 1060:G95=W3/(8.33 *SPGR)
1680 V4=G95/W3*W4
1690 X=100:GOSUB 1060:G100=W2/(8.33 *SPGR)
1695 IF C1=0 THEN GR=W1/8.33:GOTO 1710
1700 X=C1:GOSUB 1060
1705 GR=W1/(8.33*SPGR)
1710 PRINT TEMP,:PRINT USING "####.# ";G95,V4,G100,GR
1720       NEXT
1750 RETURN
1770 GOSUB 2130
1910 '           ********** STANDARD CHARGE ************
1930 PRINT           "A standard charge consists of the following."
1940 PRINT:PRINT     "Alcohol (95 w%).....632 Kg  (1390.4 lbs)"
1950 PRINT           "Urea.....................220 Kg  ( 484 bs)"
1960 PRINT           "Esters...................80 Kg  ( 176 bs)"
1970 RETURN
2000 '           ****** NEW CHARGE CALCULATIONS ********
2010 UREAK=ESTERK*220/80:UREAP=UREAK*2.2:ALCK=ESTERK*632/80
     :ALCP=ALCK*2.2
```

```
2020 ESTERP=ESTERK*2.2
2030 ALCK=FN B(ALCK):ALCP=FN B(ALCP):UREAK=FN B(UREAK):UREAP=FN
     B(UREAP):ESTERK=FN B(ESTERK):ESTERP=FN B(ESTERP)
2040 PRINT:PRINT "Your new charge consists of the following."
2050 PRINT:PRINT "Alcohol (95%)...";ALCK;" Kg (";ALCP;" Lbs)"
2060 PRINT       "Urea.....";UREAK;" Kg (";UREAP;" Lbs)"
2070 PRINT       "Esters.....";ESTERK;" Kg (";ESTERP;" Lbs)"
2080 RETURN
2240 '          ********** CALCULATIONS **********
2250 CLS
2260 COLOR 15,0:PRINT TAB(18)"UREA ADDUCTION CHARGE AND ALCOHOL
     MIX":COLOR 7,0
2270 PRINT
2275 GOSUB 1930
2280 ON KEY (1) GOSUB 4100:GOSUB 4220:PRINT
     :INPUT "Kg of esters to run = ";ESTERK:P=CSRLIN-1
2285      IF ESTERK=0 THEN ESTERK=80:LOCATE P,23:PRINT ESTERK
          :GOTO 2300
2290      IF ESTERK >85 THEN :COLOR 15,0:PRINT"OUT OF RANGE
          (40-85)  You will exceed the capacity of the
          crystallizer.":COLOR 7,0:GOTO 2280
2295      IF ESTERK <40 THEN GOSUB 4130
2300 ON KEY (1) GOSUB 4070:GOSUB 4190:PRINT "(Enter 100% water if
     you want to make new 95% alcohol.)":INPUT "Wt% water in
     reclaimed alcohol = ";WR:P=CSRLIN-1
2305      IF WR=100 THEN C1=0:PRINT "You are going to add water
          to new, 100% alcohol to make 95 Wt% alcohol."
          :T1=70:SPGR1=1:GOTO 2340
2307      IF WR=0 THEN WR=5:LOCATE P,32:PRINT WR
2310      IF WR >20 OR WR <5 THEN COLOR 15,0:PRINT "OUT OF RANGE
          (5-20 OR 100) ":COLOR 7,0:GOTO 2300
2315 C1=100-WR
2320 PRINT "Temperature of reclaimed alcohol (" CHR$(248)
     "F) = ";:INPUT ;T1:P=CSRLIN
2325      IF T1=0 THEN T1=78:LOCATE P,40:PRINT T1
2330      IF T1 >104 OR T1 <50 THEN COLOR 15,0:PRINT" OUT OF
          RANGE (50-104 F) ":COLOR 7,0:GOTO 2320
2340 GOSUB 2000:W4=FN B(ALCP)
2350 W3=ALCP+12.6
2355      IF C1=0 THEN GOTO 2380
2360 TEMP=T1:A=C1:X=C1
2370 GOSUB 1050:SPGR1=SPGR
2380 C3=95
2390 WW3=W3*(100-C3)/100
2400 W1=WW3*100/(100-C1)
2410 V1=W1/(8.33*SPGR1):V1=FN B(V1)
2420 W2=W3-W1
2430 PRINT
2440 W1=FN D(W1):C1=FN D(C1):W2=FN D(W2):W3=FN D(W3):W4=FN D(W4)
2450 PRINT "Mix ";W1;"pounds (";V1;"gallons @";T1;CHR$(248);"F)
     of";
```

```
2455      IF C1=0 THEN PRINT " water ":GOTO 2460
2457 PRINT ;C1;"% alcohol "
2460 PRINT "with";W2;"pounds of 100% alcohol to make ";W3;
     "pounds of 95 Wt% alcohol,"
2470 COLOR 15,0:PRINT "but you will only use ";W4;"pounds of your
     95% to process esters.":COLOR 7,0
2480 PRINT
2490 INPUT "Press <Return> to create table ",R$
2500 GOSUB 1600
2530 INPUT "Do you want another calculation (Y,N) "; R$
2540      IF R$="Y" OR R$="y" THEN CLS:GOTO 2270
2545 GOSUB 4240
2550 GOTO 4900
3000 '          ********** INITIALIZATION *************
3010 DIM X(10),Y1(10),Y2(10),Y3(10)
3020 '                  *** DEFINE FUNCTIONS ***
3030 DEF FN A(X)=INT(X*10000+.5)/10000
3040 DEF FN B(X)=INT(X*100+.5)/100
3050 DEF FN C(X)=INT(X*1000+.5)/1000
3060 DEF FN D(X)=INT(X*10+.5)/10
3070 '                  **** SET VARIABLES *****
3080 A=0:B=0:C1=0:C3=0:G100=0:G95=0:GR=0:I=0:J=0:N=0:SPGR=0
     :T=0:TEMP=0
3090 V4=0:W1=0:W2=0:W3=0:W4=0:X=0:SPGR1=0:T1=0:V1=0:WR=0
     :WW3=0:LL=0:P=0
3120 '             **** WT%, TEMPERATURE, SPGR DATA ****
3130    N=3       :TT(1)=50        :TT(2)=77        :TT(3)=104
3140 X(1)=100     :Y1(1)=.79784    :Y2(1)=.78506    :Y3(1)=.77203
3150 X(2)=90      :Y1(2)=.82654    :Y2(2)=.81362    :Y3(2)=.80028
3160 X(3)=80      :Y1(3)=.85197    :Y2(3)=.83911    :Y3(3)=.82578
3200 '                    **** KEY SUB LABELS ****
3240 HA$="Press <F1> then <Return> to skip instructions "
3250 HB$="Press <F1> then <Return> to back up"
3260 HH$="Press <F1> then <Return> to re-enter last entry"
3270 HP$="Press <F1> to stop table and re-enter last entry"
3280 EE$="                                                "
3290 RETURN  '        ** END OF INITIALIZATION **
4060 REM         ************* KEY SUBS ***************
4070 RETURN 2250
4080 RETURN 2300
4090 RETURN 2320
4100 RETURN 4340
4110 INPUT "Press <Return> to continue: ",R$:P=CSRLIN-1:LOCATE P
     :PRINT "                                          ": RETURN
4120 RETURN 4280
4130 REM         ************* CAUTION ****************
4140 PRINT
4150 COLOR 15,0:PRINT "CAUTION: IT IS NOT ADVISABLE TO PROCESS
     LESS THAN A 40 KG ESTER CHARGE IN THE CRYSTALLIZER.  DO SO,
     ONLY WITH PROPER ADJUSTMENTS OF TEMPERATURE PROBE, JACKETS,
     AND DATA LOGGER.  KNOW YOUR SYSTEM WELL. "
4160 PRINT "Press <F1> to enter another charge."
```

```
4170 COLOR 7,0
4180 RETURN
4190 REM         ********* PRINT KEY LABEL *************
4200 LL=CSRLIN:LOCATE 25:COLOR 15,0:PRINT HH$:COLOR 7,0:LOCATE LL
4205 RETURN
4210 LL=CSRLIN:LOCATE 25:COLOR 15,0:PRINT HA$:COLOR 7,0:LOCATE LL
4215 RETURN
4220 LL=CSRLIN:LOCATE 25:COLOR 15,0:PRINT HB$:COLOR 7,0:LOCATE LL
4230 RETURN
4240 LL=CSRLIN:LOCATE 25:PRINT EE$:LOCATE LL-2
4250 RETURN
4252 LL=CSRLIN:LOCATE LL-1:PRINT EE$:LOCATE 25 :PRINT EE$
     :LOCATE LL-2
4260 LL=CSRLIN:LOCATE 25:COLOR 15,0: PRINT HP$:COLOR 7,0:LOCATE LL
4270 RETURN
4280 '           ***** PRINT INSTRUCTIONS **************
4290 GOSUB 4600:ON KEY (1) GOSUB 4070: GOSUB 4210
4300 COLOR 15,0:PRINT SPC(35)"ALCALC":COLOR 7,0
4310 PRINT:PRINT "This program calculates the components and
     alcohol mixes used in our urea adduction operation."
4320 PRINT:COLOR 15,0:PRINT "If you want a print-out of the
     instructions, get your printer ready and press <Ctrl><PrtSc>
     once then <Return>.":COLOR 7,0
4330 LOCATE 23:INPUT "Press <Return> to continue.",R$
4340 '           ********** INSTRUCTIONS *************
4350 CLS:COLOR 15,0:PRINT SPC(28)"ALCALC INSTRUCTIONS":COLOR 7,0
4360 GOSUB 4220
4370 PRINT:PRINT "This program calculates the components used
     in our urea adduction operation."
4380 PRINT:PRINT "It also calculates the weight and gallons of
     alcohol to make 95 Wt% from a weaker, reclaimed alcohol
     or from 100% alcohol, for use in our formulation."
4390 PRINT:PRINT "You must know the kg of esters you will use
     to make a batch.  However, 80 kg is  the normal default
     (just press <Return>)."
4400 PRINT:PRINT "Because the volume of alcohol varies with
     composition and temperatures it is necessary to know the
     weight percent water and the temperature of the alcohol."
4410 PRINT:PRINT "Since you can not know the temperature of
     your strengthened, reclaimed alcohol until you have made up
     the alcohol mix, this program will calculate a table of
     volumes at different temperatures for you to use."
4420 ON KEY (1) GOSUB 4120:LOCATE 23: INPUT "Press <Return> to
     continue ",R$
4430 CLS: PRINT "If you wish to make all new 95% alcohol,
     enter 100 for the % water in reclaimed alcohol.  Use
     deionized water and weigh it out."
4440 PRINT:PRINT "If you wish to stop this program at any time,
     you can use <Ctrl><Break>."
4450 PRINT:PRINT "If you make a wrong entry before hitting
     <Return>, then <Back Space> and correct."
4460 PRINT:PRINT "If you wish to change an entry after <Return>,
     then press <F1> and then <Return>."
```

```
4470 PRINT:PRINT "Press <F1> then press <Return> several times to
     step back several steps."
4480 PRINT:COLOR 15,0:PRINT "If you wish, you can print the data
     out as you run this program.  Get your printer ready,
     press <Ctrl><PrtSc> and then <Return>."
4490 PRINT:PRINT "If you are already printing do not press
     <Ctrl><PrtSc> unless you want to stop printing now and
     use <Shift><PrtSc> to print out any particular screen
     showing."
4500 PRINT:PRINT "To stop your printer from printing every-
     thing after you have activated <Ctrl><PrtSc>, again press
     <Ctrl><PrtSc>.":COLOR 7,0
4510 ON KEY (1) GOSUB 4100:GOSUB 4220:LOCATE 23:INPUT "<Return>
     to continue. ",R$::GOTO 2240
4600 '            ************* CREDITS *****************
4620 CLS:B=25
4630 LOCATE 5,B:PRINT"*****************************"
4640 LOCATE  ,B:PRINT"*          ALCALC           *"
4650 LOCATE  ,B:PRINT"*       R.C.ERNST,JR.       *"
4660 LOCATE  ,B:PRINT"*   11-4-88.....7-16-89    *"
4670 LOCATE  ,B:PRINT"*****************************"
4680 LOCATE 12,1
4690 RETURN
4900 '           *************** END ******************
4920 LOCATE LL-1:PRINT EE$:LOCATE LL-1:PRINT "This is the END.
     GOOD-BYE.":COLOR 7,0
4940 END
```

C. Equipment and Materials Suppliers:

Crystallizer
Lee Process Systems Co.
(814) 342-0470
P.O. Box687
Philipsburg, PA
Contact: Jack Speece

Crystallizer condenser
ITT Standard (American Standard Heat Transfer Div.)
(716) 897-2800
Buffalo, NY 14240

Sanitary valves and fittings
Carolina Stainless Products
(919) 886-4477
611 Colonial Dr.
High Point, NC 27262
Contact: Bob Steel

Transfer (Triclover) pump
OEC Fluid Handling, Inc.
(803) 573-9311
Spartanburg, SC 29304

Alcohol holding tanks and evaporator feed tank
Custom Metalcraft, Inc.
(417) 862-0707
P.O. Box 10687
Springfield, MO 65808

Alcohol transfer pump
Cole-Parmer Instrument Co.
(312) 647-9660
7425 North Oak Park Ave.
Chicago, IL 60648

Pressure filter
Alsop Engineering Co.
(914) 338-0466
P.O. Box 3427
Kingston, NY 12401
Contact: Greg Haver

Urea
Columbia Nitrogen Corp.
(800) 241-4630
P.O. Box 1483
Augusta, GA 30906
Contact: Susan Hyer

Absolute ethanol
Aaper Alcohol
(800) 626-5281
P.O. Box 339
Shelbyville, KY 40065
Contact: Harold

FILM EVAPORATION

I. Principle of Operation

The solution of polyunsaturated fatty acid ethyl esters resulting from urea adduction is concentrated in a mechanically-aided film evaporator under vacuum. The alcohol is recovered for reuse. Since residual urea precipitates from solution as the alcohol is evaporated, water is added to the concentrate to redissolve the urea and dilute residual alcohol. This intermediate evaporator product is continuously pumped to separatory funnels for phase separation. After the mixture separates into organic and aqueous phases, the aqueous lower phase is drained and the esters are recovered. Residual alcohol and urea are further removed by several water washes of the esters to yield the crude concentrates as described in the next section of this Manual.

II. Equipment

The evaporator system consists of a 250-gal, jacketed feed tank; a variable-speed, rotary feed pump; a scraped surface evaporator; a vapor separator with an associated variable-speed rotary pump for circulating and removing bottoms product; a single pass condenser; a condensate receiver; a centrifugal condensate pump; a water-seal vacuum pump; two 250-gal alcohol holding tanks; steam controller; source of deionized water; N_2 supply; a leaf filter; two 100-L separatory funnels; and associated valves, piping, gauges, and level controls. A schematic diagram of the equipment and its relationship to the crystallizer is shown in Figure 6. Figure 8 is a more detailed diagram of the piping and valving of the evaporator and ancillary equipment. All valves specified in the next section are illustrated in Figure 8 and are located in Rm. 424.

III. Operating Procedure

A. Check evaporator system and ready for operation.
   1. Close all valves.
   2. Turn on both Capitol and Custom water chillers (see Appendix to this section, p 63).
   3. Turn on cooling water to condenser [45] and open the outlet valve [40].
   3. Open alcohol condensate valve [42] below the condenser.
   4. Turn on air to steam pressure controller. Bleed off water through valve [24].
   5. Turn on main steam valve [22] and clear drip leg [23].
   6. Open drain valve [31] and allow evaporator condensate to drain.
   7. Attach product transfer line to leaf filter and to the separatory funnels. Purge separatory funnels with N_2.

Room 424 Valve List

1. Crystallizer low pressure steam supply
2. Crystallizer steam supply reducing
3. Crystallizer steam supply by-pass
4. Crystallizer main steam supply
5. Tank A main inlet
6. Tank A recovered alcohol inlet
7. Alcohol to crystallizer
8. Tank B main inlet
9. Tank B recovered alcohol inlet
10. Tank drum-off
11. Tank A bottom
12. Drum unloading
13. Tank B bottom
14. Alcohol storage to feed tank
15. (future use)
16. Evaporator feed tank nitrogen flush
17. Evaporator feed tank outlet
18. Evaporator feed control
19. Cold water hose
20. Hot water hose
21. Compressed air outlet 2
22. Evaporator main steam
23. Evaporator main steam drip
24. Instrument air inlet
25. Evaporator high pressure steam
26. Evaporator steam pressure controller
27. Evaporator steam controller by-pass
28. Evaporator low pressure steam
29. Evaporator pressure relief
30. Evaporator steam condensate return
31. Evaporator steam condensate drain
32. Evaporator produce take-off control
33. Evaporator product take-off to separator funnel
34. Evaporator product to feed tank
35. Evaporator product pump inlet
36. Evaporator product recycle
37. Evaporator bottoms drain
38. Deionized water control
39. Evaporator nitrogen flush & bleed
40. Condenser coolant supply
41. Condenser coolant drain
42. Alcohol condensate
43. Alcohol condensate recycle
44. Alcohol condensate take-off control
45. Condenser coolant return
46. Condenser coolant relief
47. Vacuum control bleed
48. Vacuum shut off
49. Vacuum pump
50. Vacuum pump water supply
51. Vacuum pump water recycle
52. Vacuum pump water drain
53. Evaporator feed tank coolant supply
54. Evaporator feed tank coolant return
55. Coolant supply take-off
56. Coolant return take-off
57. Coolant supply room cut-off
58. Coolant return room cut-off
59. Deionized water supply tank outlet
60. Deionized water supply
61. Deionized water supply take-off
62. Evaporator deionized water feed tank
63. Compressed air outlet 1
64. Deionized water in-line valve

Room 424 Equipment List

A	Alcohol tank A
B	Alcohol tank B
EF	Evaporator feed tank
FE	Film evaporator
C	Condenser
AR	Alcohol condensate receiver
EPR	Evaporator product receiver & vapor separator
DWS	Deionized water supply
DWF	Deionized water feed tank
VP	Vacuum pump
WS	Vacuum pump water separator
SPC	Steam pressure controller
FM	Flow meter
TM	Totalizing meter
P1	Alcohol transfer pump
P2	Evaporator feed pump
P3	Product circulating pump
P4	Alcohol condensate pump
N_2	Nitrogen supply
CS	Coolant supply
CR	Coolant return
SCR	Steam condensate return
PCW	Potable cold water supply
PHW	Potable hot water supply
CA	Compressed air supply
SS	Steam supply
D	To drain

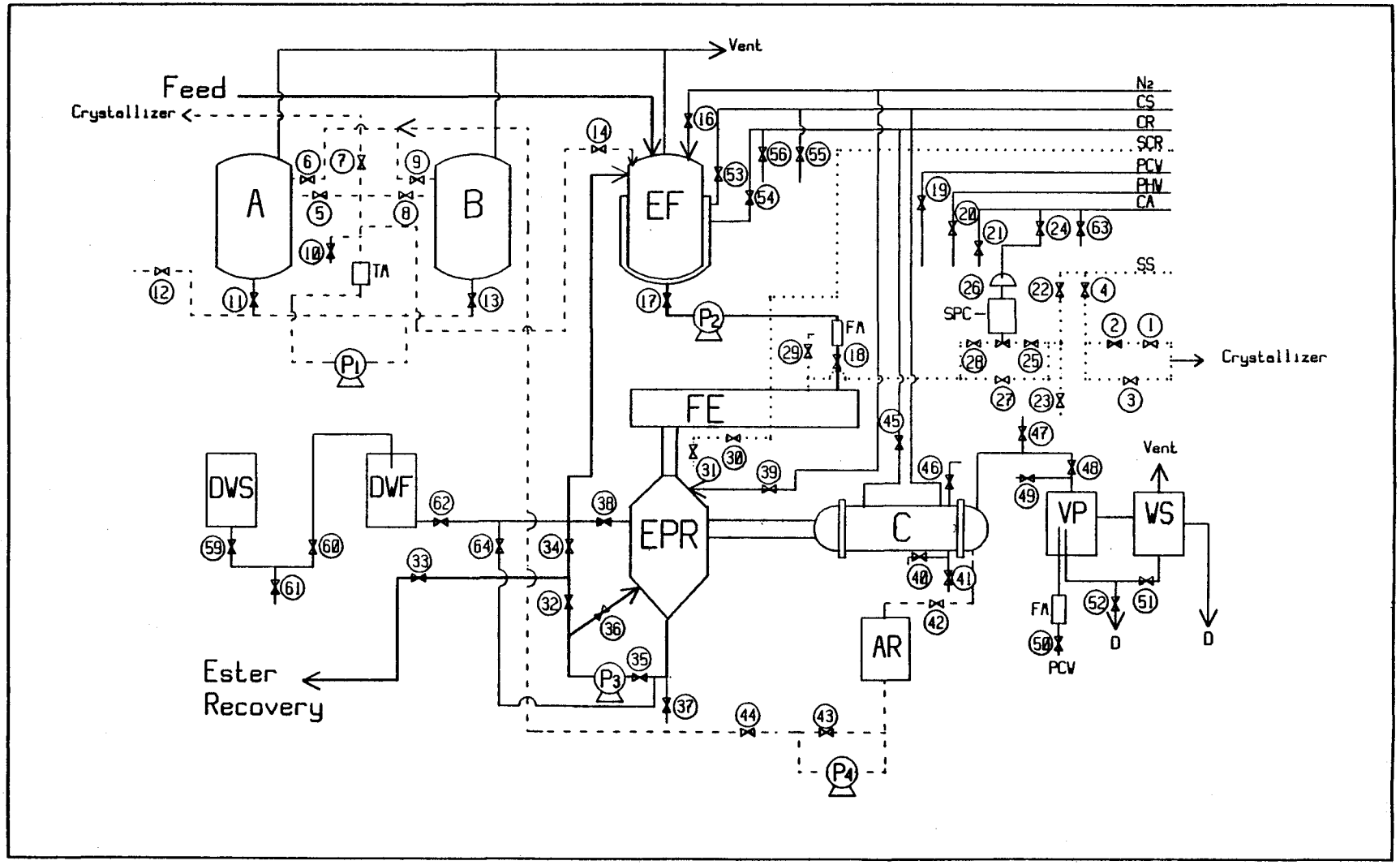


Fig. 8. Film evaporator valve and equipment diagram (Room 424).

B. Start up evaporator system. Note start-up time.
   1. Open nitrogen bleed valve [39] at separator, slightly.
   2. Open N_2 valve [16] at feed tank one-quarter turn. Adjust flow if necessary.
   3. Open feed tank bottom valve [17].
   4. Turn on feed tank pump. Set speed to '4.5' to give about 10 psig.
   5. Set feed flow with back pressure valve [18] for 1.0 gpm.
   6. Start evaporator rotor.
   7. Open the cold water valve [50] to the vacuum pump and set for 3.0 gpm.
   8. Turn on vacuum pump.
   9. Open main vacuum valve [48]. Adjust to 5.2 inches of vacuum.
   10. Open recycle valves [35, 36] at vapor separator.
   11. When liquid is visible in sight-glass, turn on bottoms pump. Set speed to '6'.
   12. Throttle vapor separator recycle valve to about 10 psig.
   13. Open take-off control valve [32] and recycle valve [34] to feed tank.
   14. Open low pressure steam valve [28] to evaporator.
   15. Set controller to 4 psig (auto).
   16. Turn off steam drip leg [23], then fully open steam valve [25] to controller.
   17. Watch fluid level in vapor separator sight-glass. Keep level low. When the level in the alcohol condensate tank reaches '4', or when urea solids can be seen in sight-glass, shut off take-off valve [34] to feed tank. Open product take-off valve [33] to separatory funnel. Close recycle valve [36].
   18. Open deionized water in-line valve [64] 1/8 turn for a continuous feed of water. To prevent solid urea plugging the pipes, open recycle valve [36] and close the take-off valve [33] for 30-45 sec. Monitor the sight-glass and repeat as necessary.
   19. With the level in the alcohol condensate tank at '4' open recycle valve [43] and turn on condensate pump. Record level in tank and the time. Close recycle valve [43] to create positive pressure on pump discharge (+10 psig). Open condensate valve to tank B [9]. Go back and adjust take-off valve [44] periodically to maintain constant level in receiver tank.
   20. Start-up is now complete. Record time and start taking data readings. Watch and adjust conditions to maintain steady-state conditions throughout run.

C. Maintain operating conditions by appropriate adjustments during run. Take data at required intervals (every hour). Once the first separatory funnel is full (90-100 L), move the transfer line to the second funnel.

D. Prepare for shut-down.

1. Ready 2 gal of deionized water to flush through feed tank when empty.
2. Open alcohol condensate take-off valve [44] fully.
3. Check volume in second separatory funnel.

E. Shut-down procedure.

1. When feed runs out, follow with 2 gal of deionized water.
2. The following steps must be taken as soon as possible and in the exact order listed:
   - Turn off steam valves [22, 25] to evaporator.
   - Shut off instrument air inlet.
   - Close main vacuum valve [48] and cold water valve [50].
   - Shut off vacuum pump.
3. Pour an additional 2 gal deionized water into feed tank.
4. Turn off alcohol condensate pump.
5. Turn off evaporator rotors.
6. Close water in-line inlet valve [64] and open water feed valve [38] to evaporator tank.
7. Turn off feed pump.
8. Close water feed valve [38] and product take-off valve [33] and open evaporator product recycle valve [36].
9. After 1-2 min, pump out bottoms to separatory funnels.
10. Turn off evaporator bottoms pump.
11. Close nitrogen valves [16, 39].
12. Turn off chillers.
13. Record final volume of recovered alcohol in tank B.

IV. Product Storage

The concentrate from the evaporator system is held under N_2 overnight in the 100-L separatory funnels prior to final recovery of the polyunsaturated esters (described in the next section, Recovery of Omega-3 Concentrates).

V. Equipment Cleaning and Maintenance

After use, flush the feed tank and film evaporator thoroughly for at least 15 min with hot tap water followed by a deionized water rinse. Place about 2 gal of absolute ethanol in the crystallizer and pump it into the feed tank and through the film evaporator, flushing all transfer lines in the process.

VI. Waste Disposal

Only small volumes of very dilute alcohol generated in cleaning the equipment are produced in this process. This can be allowed to enter the floor drains followed by a water flush.

VII. Safety

* Ethyl alcohol is potentially toxic. Avoid breathing vapors by maintaining proper ventilation, avoiding spills and leaks, keeping operating and storage vessels closed, maintaining constant vigilance and care. Toxicity level is highly subject to individual tolerances but can impair judgement at relatively low levels of exposure. Lethal dosage levels are relatively high and, at low rates of exposure, are preceded by intoxication. Respirators with organic vapor cartridges have been provided to all personnel for use if ethanol vapors become objectionable.
* Ethyl alcohol is a flammable solvent, will easily ignite and burn unless highly diluted with water. A fire can be especially dangerous because the flames may be almost invisible. It will form explosive mixtures with the proper amount of air. Water dilution is the most effective countermeasure for minor spills, etc.
* Major spills (*ca* 20 gallons or more) should receive special, supervised attention. Provisions are made to handle large spills by entrapment in the outside 1000 gal sump. In this event, make certain immediately that the sump pump is turned off so alcohol will not be pumped to the sewerage system. The pump switch is outside near the sump pump. Normally, the explosive vapor detection system will sound an alarm and disconnect the pump automatically. Further precautions should be taken by disconnecting the pump with the outdoor local switch. Follow up any spill with a water hose flush of the floor and drains since vapors can possibly leak into other areas of the building (although this is not likely). Also turn local hood fans on high and open hood doors partly if conditions permit.
* Care must be maintained in handling alcohol and oil contaminated wastes and materials (towels or other clean-up materials.)
* Make certain all valves to large vessels containing alcohol are kept closed except when necessary during immediate operation.
* Open all disconnect switches at shut-down and close all valves as necessary.
* Keep floors rinsed and obstacles out of the way, especially during operation.
* Neoprene gloves are recommended because of the dehydrating effect of ethanol on exposed skin.

VIII. Emergency Shut-down Procedure

If a fire occurs in the immediate vicinity (Rms. 423 and 424), all personnel are to evacuate the plant immediately. Activate the

fire alarm at the nearest exit on leaving. Fire protection devices (heat and smoke detectors and sprinkling system) will take over. The main circuit breaker in panel E2 (Rm. 426) may be turned off to shut off all power to Rms. 424 and 426 if this can be done safely.

If fire alarms indicate possible fire in other parts of the main laboratory or office spaces (i.e., zones 1-4), make a careful, safe shut-down of the operation so as not to damage the equipment or material being processed before evacuating the plant. Follow the steps listed below.

- Close evaporator main steam valve [22].
- Close tank bottom valves [11, 13, and 17] and condensate valves [6, 9].
- Shut off evaporator feed pump [P2] and alcohol transfer pump [P1].
- Close deionized water valve [38].
- Close N_2 valve [39]*.
- Shut off the product pump [P3] and the alcohol condensate pump [P4].
- Close main vacuum valve [48] and cold water valve [50].
- Shut off the vacuum pump and the evaporator rotor.
- Shut off evaporator bottoms pump.

---

* This step may be omitted if/when a pressure relief has been installed. Otherwise the valve must be closed to prevent an increase in the pressure.

IX. Appendix

A. Water chiller startup procedures

The Custom Research water chiller is normally used with the film evaporator. However, if additional cooling capacity is required, the Capitol water chiller can be added as described in steps 7-11, below.

1. Check coolant level in tank. Add coolant (food-grade 50% propylene glycol) when level is below mark.
2. Valves [C] and [D] are set for manual operation. Do not change settings.
3. Place the control switch (on the panel) in the <ON> position.
4. Place circuit breaker [1] (pump) in the <ON> position.
5. Place circuit breaker [2] (condensing unit) in the <ON> position.
6. Check pressures and temperatures for operation.
   - Coolant supply pressure: 40-45 psi
   - Coolant return pressure: 8-10 psi
   - Coolant temperature (condensing unit): -5° to +5°F,
   - Coolant temperature (tank): 40°F
7. To add the Capitol water chiller, place the control switch (in Rm. 420, above the data logger) in the <MAN> (manual) position.
8. Check coolant level in the tank. Add coolant when level is below mark.
9. Open valve <B> and close valve <A>. One of these valves must be open during chiller operation.
10. Depress <START> button on control panel.
11. Check pressures and temperatures for operation.
    - Suction pressure: 30-50 psi
    - High pressure: 250-275 psi
    - Coolant supply pressure: 20-30 psi
    - Coolant return pressure: 5-15 psi
    - Coolant temperature when stabilized: 10-13°F, Maximum, 30°F

B. Equipment and Materials Suppliers:

Votator, the manufacturer of the film evaporator, and supplier of ancillary equipment, has provided duplicate copies of an operating and maintenance instruction manual for the equipment. This manual includes sources of spare parts. One copy is located in R.C. Ernst's office area and the second copy is in the Project Leader's office.

RECOVERY OF CRUDE OMEGA-3 CONCENTRATES

I. Principle of Operation

The ester concentrate is pumped from the film evaporator into separatory funnels and a dilute solution of HCl added. When the mixture separates into two phases, the lower aqueous phase is drained and the esters are washed six times with hot deionized water. The esters are filtered through an in-line filter to remove any particulate material before they are placed in 10-L glass Schott bottles and blanketed with N_2. After storage for 24 hours at -40°C to precipitate non-ester impurities, the esters are filtered before distillation.

II. Equipment

The concentrates are recovered in two 100-L separatory funnels with polypropylene lids fitted with quick-connect fittings for the esters, deionized water and N_2 supply. An air-driven pump transfers the concentrates, through a 7u in-line filter, to 10-L glass bottles. Concentrated HCl, a 1-L graduated cylinder, a container for dilute HCl solution, a Teflon stirring rod, plastic gloves, glass vessel for heating deionized water, a filter stick, source of vacuum, and safety goggles are also needed.

III. Operating Procedure

1. Prepare separatory funnels to receive the concentrates by closing bottom valves and attaching quick-connect fittings for the esters, deionized water and N_2.
2. Purge both funnels with N_2. Transfer 90 L of evaporator product into one funnel, the remaining product into the second.
3. Slowly add aqueous HCl (10:90, HCl:H_2O), 100 ml/L of product.
4. With a continuous N_2 flow, stir the mixture with a Teflon stirring rod.
5. Cover the funnels with lids and let stand overnight.
6. The next day, drain the lower phase from each funnel.
7. Combine the upper phases into one funnel.
8. Record the volumes of the concentrates and begin washings. Heat deionized water in one of the trans-esterification reactors. Use 0.7 L hot deionized H_2O/L concentrates for each washing.
9. For the first three washes, gently stir the mixture of esters and water then allow to stand until two distinct phases are formed (one-half hour). Drain the lower phases from the funnel. Allow each of the last three washes to stand at least one hour.
10. After the last wash, the concentrates are passed through the in-line filter into the storage bottles that have been thoroughly flushed with N_2.

11. Record the weights and place bottles in the -40°C chest freezer in Room 420 for at least 24 hours.
12. Filter the crude concentrates, using a filter-stick and moderate vacuum (Fig. 9), into a clean dry bottle and store in the walk-in cooler overnight, if necessary.

IV. Product Storage

For storage of more than 24 hours, the esters should be placed in the freezer at -40°C.

V. Equipment Cleaning and Maintenance

Wash the separatory funnels and lids carefully in hot detergent solution. Pump the wash water through the transfer lines and air driven pump. Rinse copiously with tap water and deionized water and allow to drain. Finally, rinse with absolute ethanol.

VI. Waste Disposal

The waste generated by this procedure consists of dilute solutions of ethanol, HCl, and urea which can be drained into the cup sink inside the hood followed by a water flush.

VII. Safety

* Plastic gloves and safety goggles must be used for personal protection.
* Spills resulting from funnel breakage will be contained in the catch basin in the floor of the hood.
* Do not leave the funnels unattended while draining.

VIII. Emergency Shut-down Procedure

If it is necessary to evacuate the facility, shut off the flow of any and all liquids, leave the N_2 flow on, close hood doors, and leave the building.

IX. Appendix

Equipment and Materials Suppliers:

Glass reactor/separatory funnels
Tudor Scientific Glass, Inc.
(803) 279-4666
555 Edgefield Road
Belvedere, SC 29841
Contact: Chris Schute
Tom Tudor

Hydrochloric acid
Baxter
(800) 438-1234
8350 Arrowridge Blvd.
Charlotte, NC 28217
Contact: Glenna

Transfer pump
Cole-Parmer Instrument Co.
(800) 323-4340
7425 North Oak Park Ave.
Chicago, IL 60648

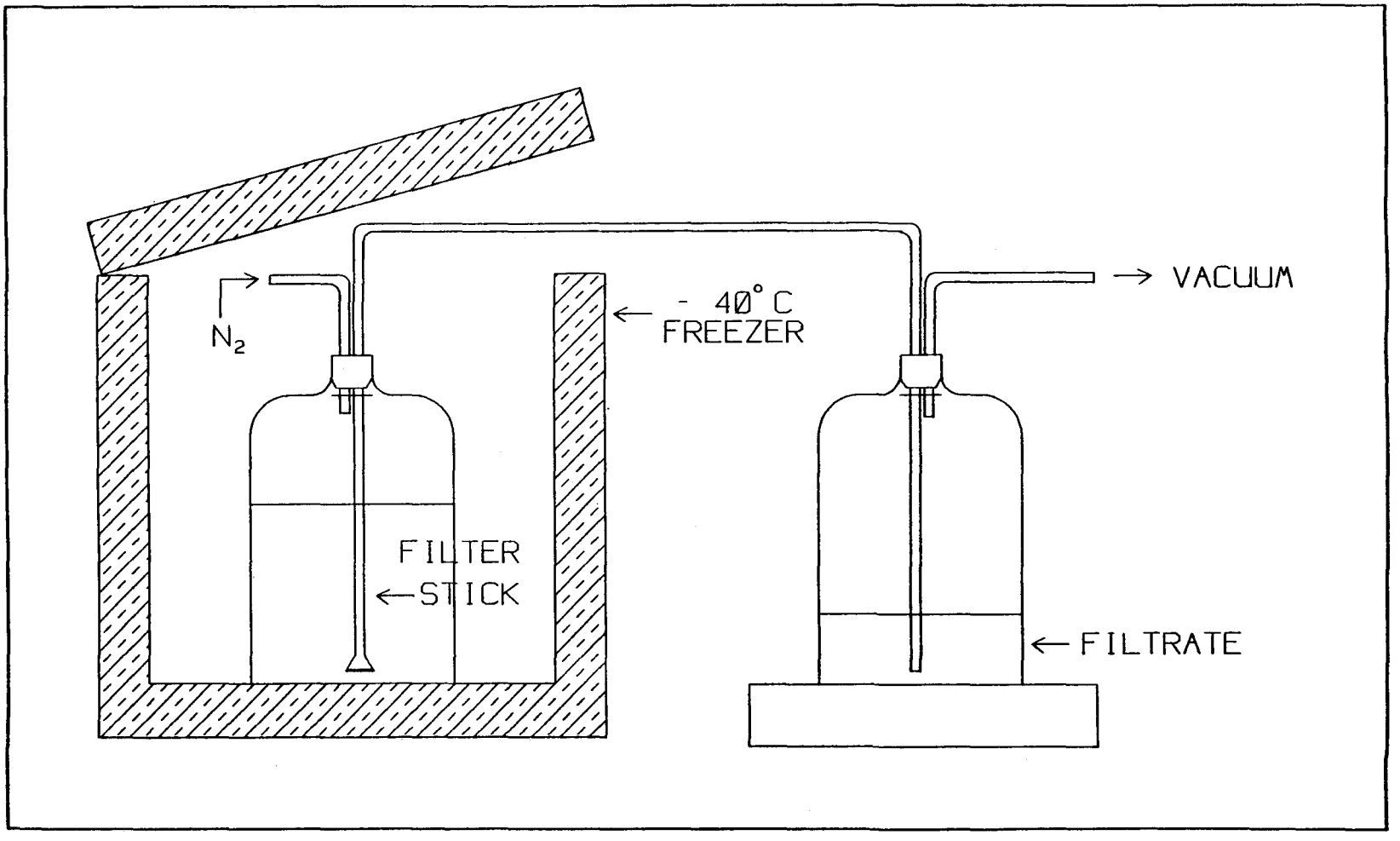


Fig. 9. Filtration of crude concentrates.

SHORT-PATH DISTILLATION

I. Principle of operation.

Final purification of the crude concentrates is carried out in a two-stage wiped film molecular still. The 10-L Schott bottle containing the crude ester concentrates is pressurized with N_2 to transfer them into the first stage of the still. In this stage, the concentrates are degassed and heated. Any hydroperoxides that may have developed during earlier production steps are decomposed and the resulting short-chained volatile decomposition products are distilled off. In addition, a substantial portion of two 16 carbon polyunsaturates that are not omega-3 esters (16:3n-4 and 16:4n-1) are distilled off. The residue of this first stage which contains the omega-3 esters serves as the feed for the second stage. In the second stage, operated at a higher temperature and lower pressure than the first stage, the omega-3 esters, 18:4, 20:5, and 22:6, are distilled, leaving a residue containing polymers, color bodies, and cholesterol.

II. Equipment

The molecular still was constructed from off-the-shelf items and electronic components purchased from Pope Scientific, Inc., Menomonee Falls, WI. The first stage is a four inch (diameter) glass still containing a stainless steel wiper drive assembly and Teflon blades. The second stage is similar but is a six inch still. Both still bodies are heated by Glas-Col heating mantles. The vacuum pumps of both stages are protected by glass condensers and electric vapor traps. Feed for the still is contained in 10-L Schott bottles. The products are stored in 5-gal stainless steel pressure vessels. Ancillary equipment includes an in-line 7 u filter and a metering feed valve, both in the feed line, and a feed pump for the second stage. A dedicated water chiller is used to provide coolant for the condensers. Electronics include a two-stage Pirani pressure gauge, two wiper drive controllers, three temperature controllers, a multi-channel temperature monitor, and a flow controller for the second-stage feed pump. A diagram of the equipment is shown in Figure 10.

III. Operating Procedure

A. Initial preparation
   1. Remove 10-L bottle of esters from the chiller.
   2. Prepare a 12-L round-bottom flask for distillate collection. Record weight of flask with stopper inserted.
   3. Prepare two clean 2-L round-bottom flasks. Record the weight of one with a stopper (for collection of first-stage distillate). Record the weight of the second flask without stopper (for collection of second-stage residue).

Components of the short-path still

1 - Still body temperature controllers
2 - RPM indicators
3 - Two-stage Pirani gauge
4 - Feed pump controller
5 - In-line 7u filter
6 - 1st stage wiper drive controller
7 - Sight-glass
8 - Pressure equilization valve
9 - Master feed valve
10 - Metering valve
11 - Temperature monitor
12 - 1st stage wiper drive
13 - 1st stage internal condenser
14 - 1st stage external condenser
15 - 1st stage still body
16 - 1st stage distillate receiver
17 - Condenser coolant
18 - 1st stage residue flask
19 - Feed pump
20 - 2nd stage mantle temperature controllers
21 - 2nd stage drive controller
22 - 2nd stage wiper drive
23 - Heating collar
24 - Upper mantle
25 - 2nd stage still body
26 - Lower mantle
27 - 2nd stage internal condenser
28 - 2nd stage residue flask
29 - Condenser coolant
30 - 2nd stage distillate receiver
31 - 2nd stage external condenser

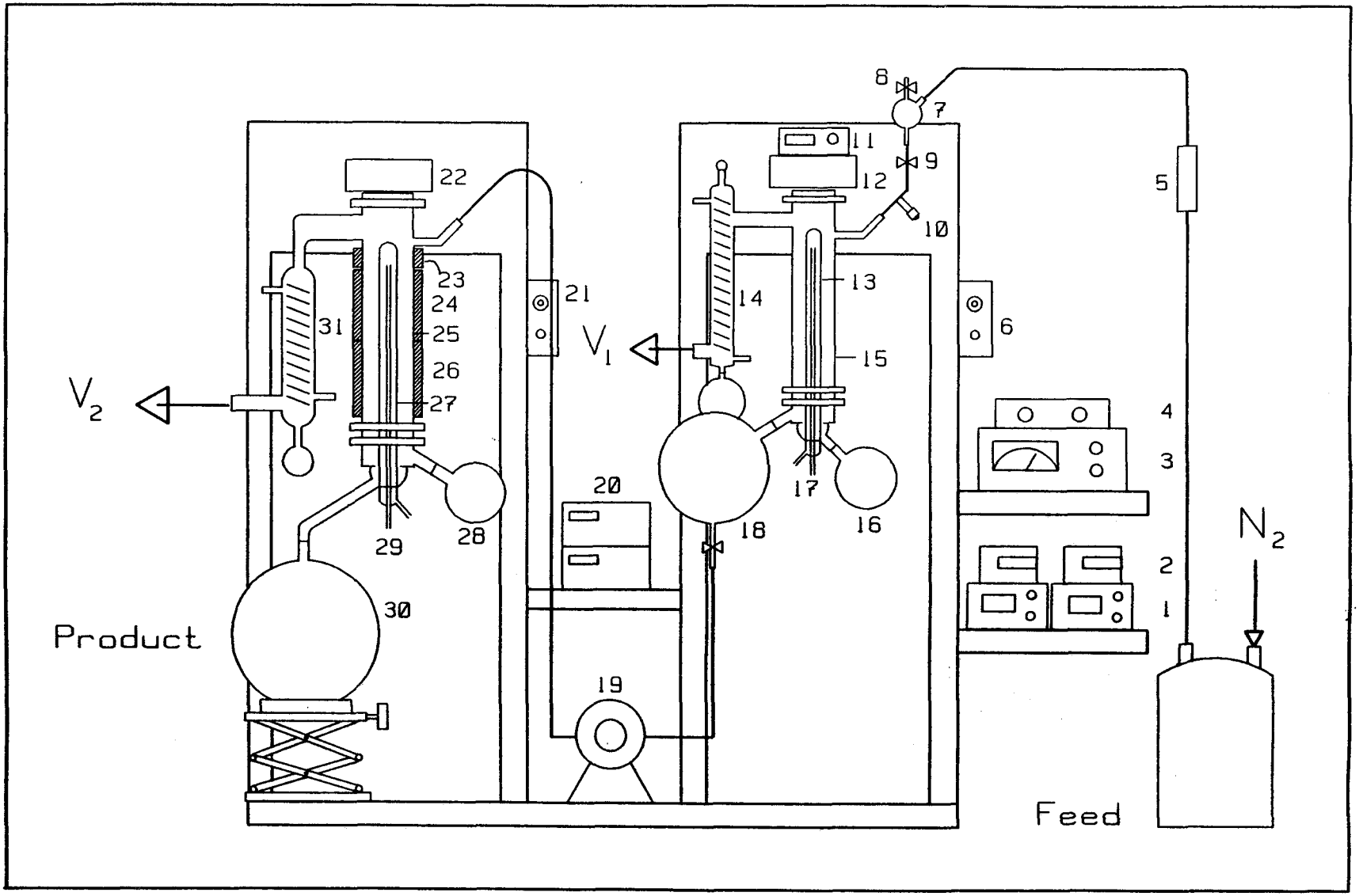


Fig. 10. Two-stage wiped film short-path still.

4. Clean, dry, and grease (vacuum grease) both electric vapor traps. Also grease glass connections on the still bodies for the 12-L and 2-L receivers.

B. Procedure

1. Turn on the chilled water system.
2. Power up the electric vapor traps.
3. Turn on power to vacuum pumps (speedi-valve on top of pump must be closed).
4. Place the 2-L round bottom flasks on both stills at [16] and [28] (Fig. 10).
5. Place the 12-L flask at [30].
6. Check to ensure that N_2 bleed is turned off to both stills. Close valve [9].
7. Open speedi-valve on V_1 pump when cold trap reaches -50°C. Vacuum should pull to *ca* 0.2 torr as read on the vacuum gauge [3]. Set the gauge [3] to read in torr and selector switch at <1>.
8. Open speedi-valve on V_2 vacuum pump to pull vacuum when the cold trap reaches -50°C. V_2 should be *ca* 100*u* with the vacuum gauge [3] selector switch at <2>.
9. Place the 10-L bottle containing esters in-line as shown in Figure 10. Connect N_2 line to pressurize bottle. When flask [7] is half full, close valve [8].
10. Turn on the temperature controller for stage 1 (1, right]. Set at 141°C by depressing knob and turning appropriately. At this time, turn on the first-stage wiper [6] and set rpm to 50. The speed is read at [2, right].
11. Turn on the temperature controller [1, left] for the stage 2 upper mantle [24] and set at 128°C. Set the temperature controller [20, upper] for the second-stage bottom mantle [26] at 163°C. Set the temperature controller [20, bottom] for the collar [23] at 66°C. Turn on the second-stage wiper [21] and set at 50 rpm and read at [2, left].
12. Once operating temperatures have been reached, distillation can begin. It is not necessary to wait for stage 2 to reach the temperature setpoint before starting stage 1.
13. Set wiper speed of stage 1 to 100 rpm.
14. Set metering valve [10] to '25' (*ca* 1 L/hr).
15. Open stopcock [9] on flask [7] to establish flow.
16. Turn on the second-stage feed pump [19] and use the control [4] to remove the first-stage residue from flask [18] slightly faster than it enters the flask. Stage 1 distillate collects in flask [16].
17. When the residue begins to enter the second stage, increase second-stage wiper speed to 200 rpm.
18. The second-stage distillate collects in flask [30]. Second-stage residue collects in flask [28].

C. Shut-down

1. When flow from the ester supply bottle ceases, disconnect the bottle.
2. Allow flow to continue through stopcock [9]. Watch pressure. When the last of the feed enters the metering valve [10] (the pressure will increase), close stopcock [9]. Do not use the metering valve to close the system.
3. Reduce heat to the first-stage still body to 90°C when flow reaches the bottom of the body. Reduce wiper speed to 50 rpm.
4. Continue operation of the feed pump [19].
5. When the liquid level falls below the stopcock of receiver [18], close the stopcock.
6. Close down the feed pump [19] when no more material enters the top of the second-stage still body.
7. Reduce heat to the second stage still to 90°C after the material has drained to the bottom of the still body. Set wiper speed to 50 rpm.
8. Close speedi-valve on V_1; open N_2 bleed.
9. When the pressure falls, remove the first-stage distillate [16] for storage, replace the flask with another 2-L round bottom flask, and close the N_2 bleed.
10. Close the speedi-valve on V_2, open the N_2 bleed, and remove the second-stage residue [28] and distillate [30]. Replace both flasks with clean 2 L round-bottom flasks, and close the N_2 bleed.
11. Turn off the vacuum pumps
12. Clean the still as described below.

IV. Product Storage

Both first- and second-stage distillates should be stored in 5-gal stainless steel pressure vessels under N_2 at -40°C.

V. Equipment Cleaning and Maintenance

A. Short-path still

1. After each use, replace the empty ester supply bottle with a 5-gal pressure vessel filled with absolute ethanol. Pressurize the vessel with N_2 and establish a flow to flask [7].
2. Open the metering valve [10] fully.
3. Open the stopcock of flask [18] and increase the first-stage wiper speed to 100 rpm.
4. Increase the speed of the second-stage wiper to 100 rpm.
5. Wash the entire system with *ca* 1.5 L of alcohol or until clean.
6. Turn off heating mantles to both stages. Open the N_2 bleed valves and purge the system of alcohol.

7. Dispose of waste alcohol in properly-labeled alcohol waste drums.

B. Glassware

1. Soak all used glassware in hot detergent solution overnight.
2. Wash soaked glassware in fresh, hot detergent solution, then rinse well with tap water, deionized water and, finally, absolute ethanol.
3. Invert and allow to air dry.

VI. Waste Disposal

Liquid wastes will include (1) pot residue, (2) condensed materials in the electric vapor traps, (3) used vacuum pump oil, and (4) alcohol used to clean the still. These wastes can be combined with those of the vacuum deodorizer for disposal in individual, well-labeled containers. Oily toweling is to be placed in the covered safety cans during the day and removed from the plant at the end of the day.

VII. Safety

* Since the short-path still operates at low pressure and high temperature, safety goggles must be worn at all times when operating the still. A full-face shield, provided to all workers, is highly recommended.

VIII. Emergency Shut-down Procedure

When the fire alarm sounds, turn off the Teflon valve [9], the second stage feed pump, the wipers, and the heating mantles. Leave the plant if the alarm is from Zones 1-4. In case of a fire in the production plant itself, activate the nearest fire alarm and evacuate the plant immediately.

IX. Appendix

Equipment and Materials Suppliers:

Glassware
Tudor Scientific Glassware
(803) 279-4666
555 Edgefield Road
Belvedere, SC 29841
Contact: Chris Schute or Tom Tudor

Heating mantle
Glas Col
(812) 235-6167
711 Hulman Street

P.O. Box 2128
Terre Haute, IN 47802
Contact:

Temperature monitor/controllers
Pump
Cole-Parmer Instrument Co.
(800) 323-4340
7425 North Oak Avenue
Chicago, IL 60648

Vacuum pumps and gauge
Edwards High Vacuum, Inc.
(800) 828-6691 or (615) 691-3850
P.O. Box 30381
Knoxville, TN 37930-0381
Contact: Kevin Bloomer, sales engineer

Vacuum fittings
Cooper River Valve and Fitting
552-5545
4669 Franchise Street
Charleston Heights, S.C.
Contact: John Chambliss

Electric vapor trap
Southeastern Scientific Corp.
(704) 542-3508
P.O. Box 2093
Charlotte, N.C. 28211
Contact: John Hardin

SUPERCRITICAL FLUID GASEOUS EXTRACTION

I. Principle of Operation

Carbon dioxide, above its critical temperature (31.1°C) and pressure (1070 psig) has a liquid-like density and behaves like liquid with substantial solvent power. A stainless steel column, packed with Propak (a patented stainless steel column packing material) and heated by a series of silicone heaters, is charged with distilled omega-3 concentrate. Carbon dioxide under pressure percolates through the esters which differentially dissolve in the CO_2. As the mixture of CO_2 and esters rises through the column, it enters the heated zone where the CO_2 becomes less dense and the longer chained esters become less soluble. These materials tend to come out of solution and fall back down the column while the shorter chained esters are carried out of the column.

II. Equipment

The equipment is illustrated schematically in Figure 11 and the column in Figure 12. Compressed CO_2 enters the system through the back pressure regulator [BPR] and the stem valve [SV1]. The supercritical fluid passes up through the column with its seven heated zones which are regulated by temperature controllers mounted in the control box. Fractions are collected into stainless steel 1-gal pressure vessels by opening the stem valve [SV2]. In the collection vessels, the gas expands to atmospheric pressure and the esters are deposited in the vessels. The gas passes from the collection vessels through a trap containing glass wool to the rotameter and the dry test meter which measures the volume of gas used. Small analytical samples may be taken at intervals by attaching a glass U-tube to the third stem valve [SV3]. The compressor, along with its ancillary circulating coolant system, and the liquid CO_2 supply tank are located outside the plant on a concrete pad.

III. Operating Procedure

A. Packing the column

1. Insert the Viton O-rings, top and bottom.
2. Remove the thermocouple probes. Otherwise the column will not pack properly.
3. Take a piece of Pyrex wool that will fit snugly and insert it in the bottom of the column. This will retain the packing when replacing the O-ring. Screw on the bottom endcap and tighten with the spanner wrench. To ensure that the Pyrex is firmly packed on the bottom, a long piece of rigid 1/2" polyethylene tubing can be brought in from the top to use as a tamping device.
4. Pack the region of the column below the feedport with Propak by pouring it in from the top. This will require approximately 205 g.

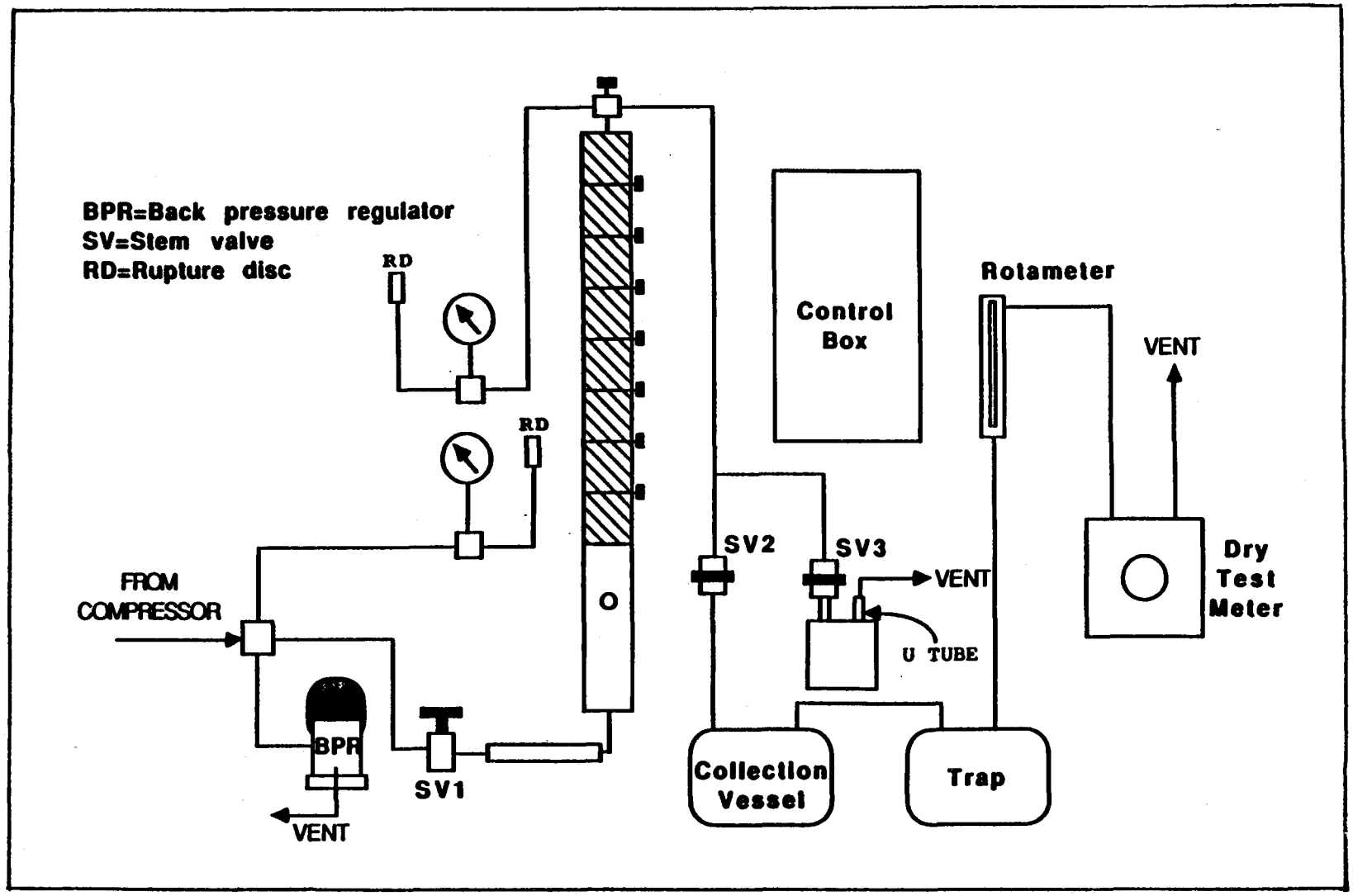


Fig. 11. Simple block diagram of the SCF fractionation unit.

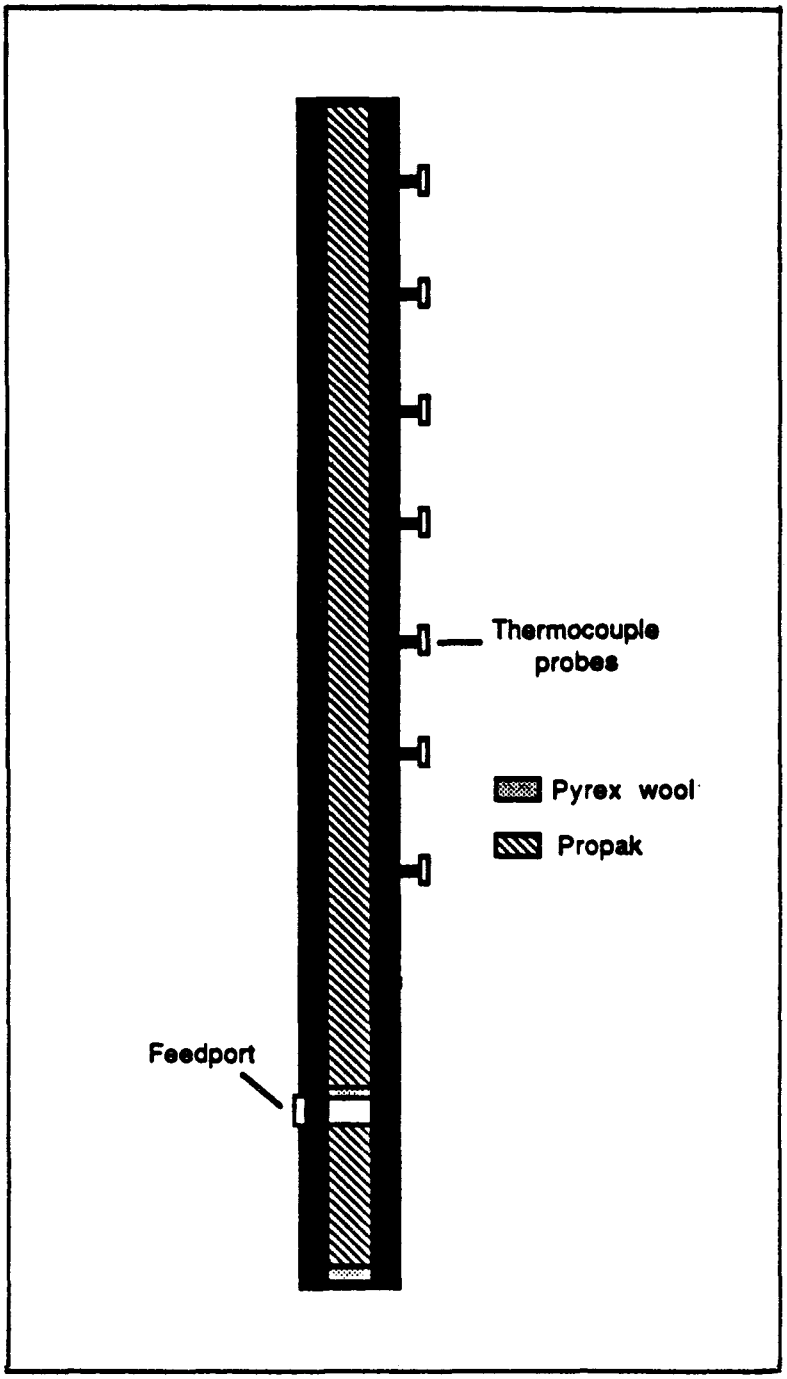


Fig. 12. The SCF fractionation column.

5. Using the polyethylene tubing, insert another piece of Pyrex wool from the top and force it down the column until it is just above the feedport (see diagram). A piece of 1/4" stainless steel tubing inserted in the feed port will insure that the Pyrex wool is positioned properly.
6. Pack the remainder of the column with Propak to just below the top (this should require *ca* 910 g). Inspect the O-ring to be sure that no Propak will interfere with the seal. Screw on and tighten the top endcap.
7. Attach and tighten the connecting fittings and the thermocouple probes.

B. Loading the feed stock

1. The esters are loaded using a separatory funnel with a tip altered to fit into the feedport. After weighing the feedstock in the funnel (200 g/charge), introduce the feedstock at a moderate rate such that the feedstock does not dribble out of the feedport.
2. Once loaded, close up the feedport. The system is now ready to be pressurized.

C. Operation of the CO_2 compressor

1. Uncover the water chiller.
2. Start water chiller (check setpoint, +5°C).
3. Close disconnect switch [switch B] (Fig. 13) for compressor
4. When coolant reaches setpoint, check return for proper flow.
5. Check level of oil in compressor crankcase sight-glass.
6. Check open position of pressure relief bypass valves [1 and 2] (Fig. 13).
7. Open CO_2 tank valve [1] (Fig. 14).
8. Set CO_2 supply regulator [3] (Fig. 14) to 30 psi. Check pressure gauge at compressor for 30 psi.
9. The compressor utilizes an oil pressure safety switch which requires the following procedure to be followed exactly to start the compressor. Depress the <START> push button on the magnetic starter and the <START> push button located in the front center area of the compressor, simultaneously. Hold the push button located front center for approximately 10 sec before releasing. If the compressor does not continue to operate, restart using the above procedures, and hold the front center push button for 20 sec (this should provide adequate operating time for the oil pressure safety switch to close).
11. Check oil flow in both sight-glasses (next to valves [1] and [2], Figure 13).
12. With the compressor operating, the oil level in the crankcase should be at the mid-point of the sight-glass.

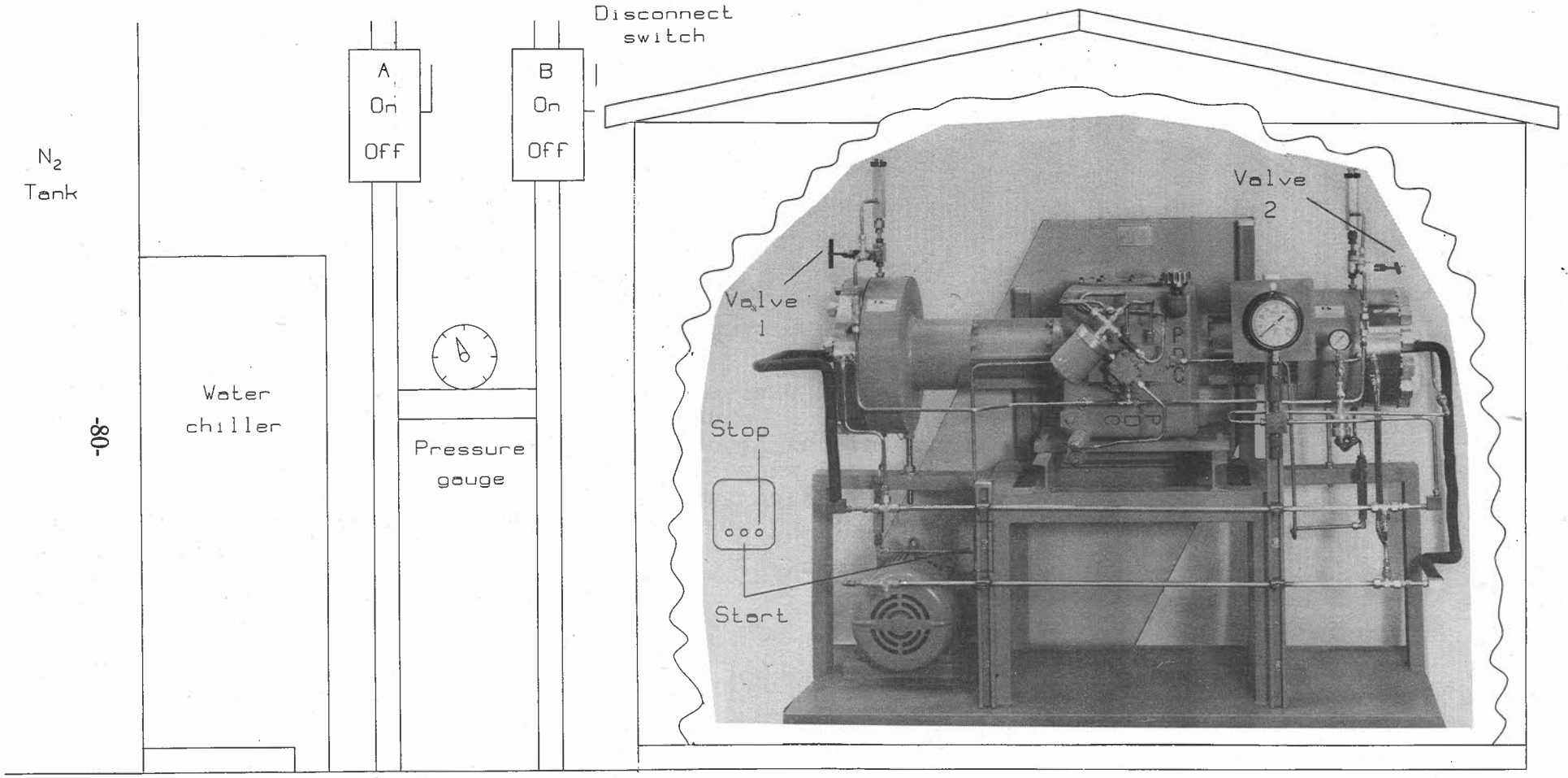


Fig. 13. Carbon dioxide compressor

Components of the CO_2 supply tank

1 - Tank valve
2 - Pressure gauge
3 - Supply regulator
4 - Contents gauge
5 - Pressure gauge

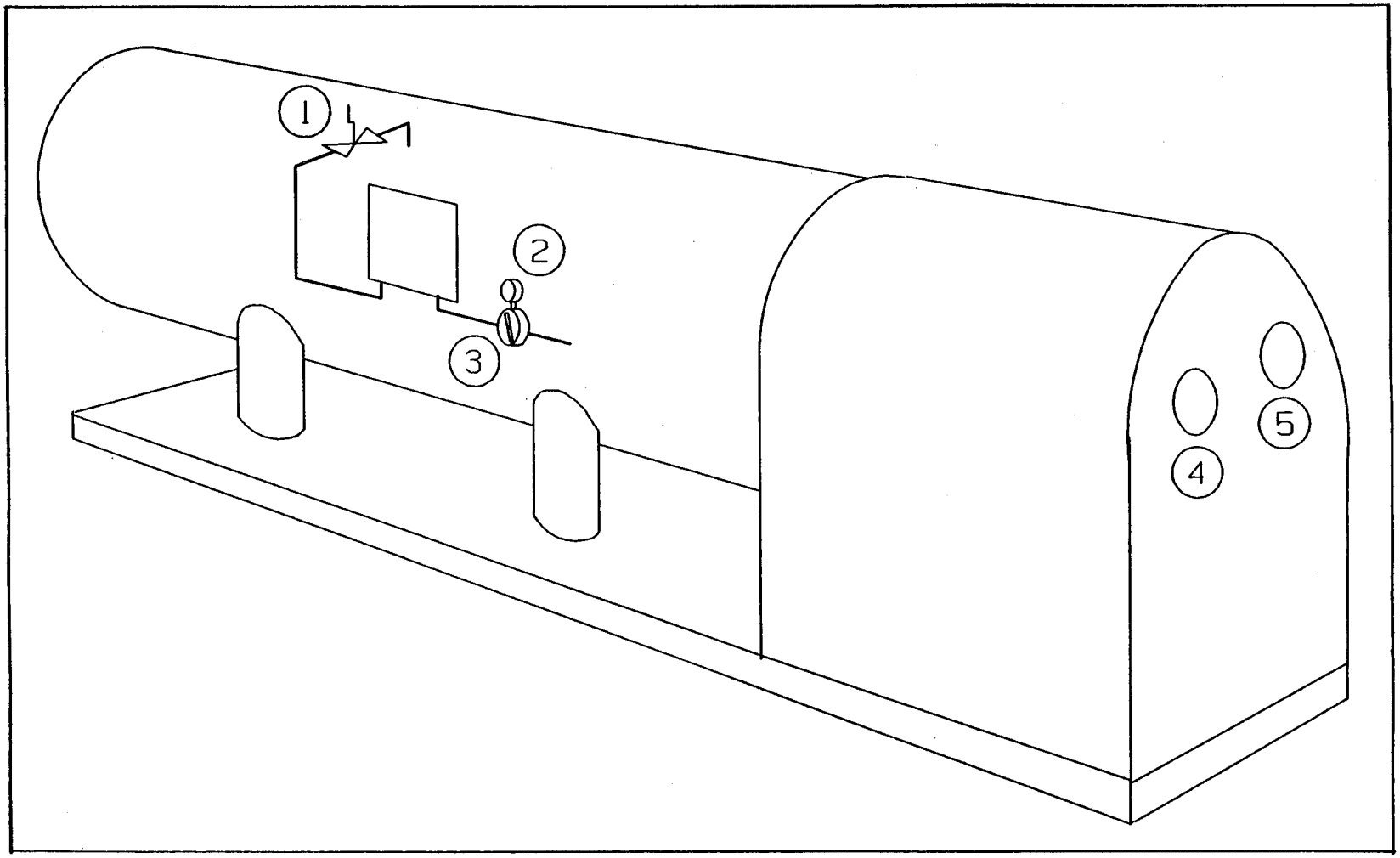


Fig. 14. Supercritical fluid CO_2 supply tank.

13. When the initial priming is complete, foamy oil will appear in the bypass sight-glasses, indicating displacement of air mixed with oil inside the cavity.
14. After approximately a minute, close the oil relief valves, [1] and [2], simultaneously. Oil flow in the sight-glasses will stop momentarily and then reappear
15. Operate in this mode until clear oil is visible in the sight-glasses. Once this is achieved, a regular tapping sound will be audible. This indicates the proper opening and closing of the check and relief valves.
16. Reset the CO_2 supply regulator [3] (Fig. 14) to the required operating pressure, not to exceed 150 psi.
17. Foamy oil may reappear momentarily in the sight-glasses, but this is normal.
18. The compressor will now start to increase discharge pressure as the back pressure regulator of the SCF fractionator is adjusted.

D. Pressurizing the system

Refer to Figure 11 for the following instructions.

1. Turn on the compressor chiller and compressor.
2. Open the expansion valve [SV3] 1/2 turn and slowly crack the stem valve [SV1] to begin passing CO_2 through the feedstock. Purge the system in this manner for 5 minutes, then close [SV3] (no U-tube should be attached to [SV3] during purging).
3. Turn on power regulator for the SV2 and back pressure heating tapes to '8'.
4. Next, set the temperature controllers to the desired temperatures. Information on the temperature controllers can be found in Section IX, Appendix. Briefly, each controller has 8 functions, only one of which the operator will deal with routinely (the set value). Initially, the controller will display both the measured (PV) and the set-values (SV), simultaneously. To change the set value:
   a. Press the [PARA] key once which will display the set value symbol (S8) on the measured value display. Additional pressing will step through the parameters and return to the beginning.
   b. Press the [◂] key while pressing the hidden key which is located to the right of the [PARA] key. The least significant digit on the SV display will light brightly. To adjust the display value for this digit, press the "up" [▲] or "down" [▼] key. Either key can be held down continuously until the desired value has been reached.
   c. To set the next most insignificant digit, press the [◂] key. The digit to be set will light brightly and is set in the same manner as above. Repeated pressing will cycle through the three digits. The controllers may be set while the feedstock is being loaded.

5. The controllers will begin to supply current to the silicon rubber heaters. Temperatures in all zones should reach set values in about 20 min. Once equilibration has occurred, begin to pressurize the column by slowly cracking the stem valve [SV1] between the column and back pressure regulator.
6. Attach the pressure vessels (tared receiver and trap).
7. Continue to pressurize the system by rotating the handle on the back pressure regulator in a clockwise direction as indicated on the regulator. Increase the pressure to 1500 psig. Be careful not to overshoot by rotating the back back pressure regulator too rapidly. As the system is pressurized, it is normal for the display values on the temperature controllers to jump several degrees.

E. Collection of fractions

1. Record the reading of the dry gas meter (DGM).
2. Open [SV2] to obtain a CO_2 flow rate of about 50 L/min (or about 20% on the rotameter). Then increase the pressure to 1900 psig at a rate of about 50 psig/min (8 min).
3. If the column temperatures are close to setpoint (within 2°C), increase the flow rate to 100 L/min (about 40% on the rotameter). Collect the first fraction eluted with the first 9500 L CO_2 (i.e., the reading obtained at time 0 [# 1, above] plus 9500). Close [SV2].
4. Increase the pressure to 2000 psig and as quickly as possible, replace the first receiver with another tared receiver, open [SV2], and collect the second fraction over the next 6000 L CO_2 (i.e., a total of 15,500 L.)
5. Repeat three times more, increasing the pressure by 1000 psig increments, beginning the third collection at 15,500 L CO_2, the fourth at 30,500 L CO_2, and the final collection at 39,000 L CO_2.
6. At 42,000 L CO_2, substitute a 1-L glass filter funnel for the stainless steel receiver and check for any residual fifth fraction by visual observation.
7. Throughout the fractionation, care should be taken to keep the flow rate constant (setpoint, $\pm$5%). The flow rate usually is observed to fall somewhat and it is often necessary to adjust it a bit. If the flow rate falls a bit and stays there, the separation and therefore product yield will not suffer, but it will increase the length of time of a batch cycle.

F. System shut-down

1. Column

   a. Isolate the column by closing the stem valve [SV1] between the column and the back pressure regulator. THIS IS IMPORTANT because rapid decompression may damage the seat in the back pressure regulator.

b. Reset all controllers to 0°C, as described above.
c. Reduce the system pressure by rotating the handle on the back pressure regulator slowly counter-clockwise.
d. Vent the column into the last collection vessel. For overnight shut-down, maintain the pressure at 100 psi. Complete venting for overnight shutdown would expose any trace of esters which might still remain in the unit, as well as non-ester impurities, to oxygen.
e. Make certain all heaters, including the heater at the expansion valve, are off.

2. Compressor

a. Ascertain that the back pressure regulator is in the fully open position.
b. Reset the CO_2 supply regulator [3] (Fig. 14) to 30 psi.
c. Open the oil relief valves, [1] and [2] simultaneously.
d. Shut down the compressor by pushing the <STOP> button on the magnetic starter.
e. Open the disconnect switch [B].
f. Close the CO_2 tank valve [1] (Fig. 14).
g. When CO_2 tank pressure reaches 0 psi at the compressor, reset the CO_2 supply regulator [3] (Fig. 14) to the fully open position.
h. Continue to operate the water chiller for 15 min, then shut down.
i. Cover the water chiller.

IV. Product Storage

During the four days of operation in each week, the products may be stored in the individual fraction receivers in the cold room. On Fridays, however, or when interrupting operations for more than 24 hrs, the fractions should be stored in brown glass (plastic coated) bottles of appropriate size, under N_2, at -40°C.

V. Equipment Cleaning and Maintenance

The column was originally supplied with stainless steel "C" rings because of a desire to have all-stainless steel internals. However, it was found that the C-rings generally could not be reused. Due to their expense (about $50 each), O-rings made of a Viton which is FDA-approved for food applications were chosen. In general, these O-rings also cannot be reused because they tend to crack upon decompression. However, they are much less expensive (about $0.90) than the C-rings.

The frequency at which the system must be cleaned will depend on the quality of the ester feedstock. If the feedstock contains only ethyl esters, essentially no residual material will remain in

the system after a batch cycle as described above. Thus, in theory, only occasional cleaning would be necessary. In the past, however, non-ester impurities ranging from *ca* 7-15 wt.% have been found in the ethyl ester n-3 concentrate. These impurities (probably primarily glycerides, sterols, and polymeric materials) must be removed weekly from the system. To do so, ethanol is used as a 'cosolvent' along with SCF CO_2 in the following procedure:

A. Cleaning the column

1. Remove the bottom endcap. Put a tray underneath the column to catch packing and pull out the bottom Pyrex wool plug. Packing from the section of the column below the feedport should, at least in part, come down spontaneously. Any remaining packing may need to be coaxed out using a semi-flexible probe such as a small "snake".
2. Using this flexible probe, pull down the Pyrex wool plug above the feedport. The remainder of the packing will usually fall down the column. If not, pull the top endcap and pour *ca* 1/2 liter of ethanol down the column to loosen the packing. If packing still will not fall, pull the thermocouple probes, starting from the bottom probe mounted in the body of the column, and use the flexible probe to free the packing.
3. After all the packing has been removed, replace the Viton O-rings with the ethylene-propylene O-rings. (The latter will withstand the CO_2-ethanol environment better and can be reused in subsequent cleanups). Insert the thermocouple probes and tighten the bottom endcap and fittings. From the top, pour 1 liter of ethanol into the column and tighten the top endcap and fittings.
4. Turn on the heating tapes to the back pressure regulator and [SV2]. Turn on the compressor and set all controllers at 60°C. Pressurize the system to 100 psi after purging the column for 30 min. After equilibration, pressurize the system to 4000 psi and allow it to sit for *ca* 1 hour. Draw off the solvent through the expansion valve at a flow rate of *ca* 15% on the flowmeter. When the solvent is depleted, set the controllers to 0°C, shut down the compressor, and open the system to allow any residual solvent to evaporate overnight.
5. Flush the rotameter with solvent from a squirt bottle to keep the float from seizing due to polymerized entrained esters.

B. Cleaning the Propak

1. An ultrasonic cleaning bath is used to clean the packing. The unit has automatic controls with a timer for heating and washing for pre-set time and temperature. Proper setting will prevent possible damage to the heating elements due to evaporation of the tank water.

2. Mix about 1 part liquid detergent with 4 parts hot water to give sufficient solution to fill the insert tray to a depth of about 2.5 inches.
3. Fill the main tank with hot water about 1/3 full and add about 1 tsp of FDA-approved powdered detergent.
4. Pour the Propak from one fractionation, pre-cleaned with ethanol, into the insert tray. It should be completely covered by the detergent solution.
5. Put the insert tray into the main tank and check to see that the water level inside the tank is within 1" of the top. Adjust the water level if necessary. (Note: this level must be maintained or the heating elements may burn out).
6. Push <MODE> until the display reads 'SET TEMP'.
7. Push <SET> until the display reads '60'.
8. Push <START> ('HEAT ON' should light up).
9. Push <MODE> until the display reads 'SET TIME'.
10. Push <SET> until display reads '60'.
11. Push <START>. The sonicator should emit a soft "buzz".
12. When the sonicator stops (after 60 min) rinse with tap water and deionized water, then oven-dry the Propak for the next use.

CAUTION: Do not leave packing in the solution for long periods of time. Use fresh cleaning solution for each batch and change the tank water with each use. NEVER stick any metal objects in the tank or insert tray while the sonicator is operating.

VI. Waste Disposal

The only solid waste generated by the system is the glass wool packed into the column and the trap. This can be disposed of along with other oil- or solvent-contaminated solid wastes. The liquid waste consists of ethyl alcohol used to clean the column and to pre-clean the Propak. This can be added to that generated in cleaning the molecular stills.

VII. Safety

* High pressure equipment can be dangerous if not maintained and operated properly.
* CO_2 can be extremely cold and can cause frost-bite.
* All connections must be clean and tight before pressurizing the system.
* CO_2 is not toxic but can cause suffocation unless adequate ventilation is maintained at all times.
* Rupture disks should prevent over-pressurizing the system but they must be kept open and free of dirt or other obstructions. Care must be taken to avoid damage to the disks. Since the tubing to the disks is small in diameter, the tubing should be checked for obstructions several times a year.

* Keep all vent lines beyond the high pressure valves open and clear of obstructions to prevent excessive pressure on these lines. Never install valves or closed containers on the vent line.
* Heating tapes will be very hot. CAUTION!
* Proper procedures must be followed to prevent danger to personnel or damage to equipment.

VIII. Emergency Shut-down Procedures

A. Compressor shut-down due to power interruption

1. Close valves [SV1], [SV2], and [SV3].
2. Restart compressor immediately.
3. Slowly reopen [SV1] after line pressure returns to the set pressure.
4. Continue operation.

B. Electric power failure

1. Close valve [SV1] between the column and the BPR.
2. Close valve [SV2].
3. Slowly open the BPR while turning off all heaters.
4. Shut off the flow of CO_2 at the CO_2 tank ([1], Fig. 14).
5. Back off on the CO_2 pressure regulator ([3], Fig.14).
6. Open the by-pass valves ([1, 3], Fig. 13) on the compressor.
7. Open the disconnect switch ([B], Fig.13) to the compressor.

C. Fire alarm

1. If the alarm originates in Zones 1-4, there is no immediate danger in the production plant (zone 5). The system can be left in stable operation, unattended, for 10 to 15 min (provided there is no power interruption) if the collection vessel will not need to be changed within this period. The CO_2 flow rate may be decreased if desired to prolong the operating time.
2. Operation may be temporarily suspended by closing valves [SV1], [SV2], and [SV3] <u>only</u>. The system will hold on stand-by for 30-60 min.
3. If the alarm originates in Zone 5 but there is no fire in the production plant, follow step C1, above. In case of a fire in the plant, <u>activate the nearest fire alarm</u> and leave the plant immediately. If safety permits, set the compressor disconnect switch to off and close the valve on the CO_2 tank.

IX. Appendix

A. Sample Size

This unit is designed to fractionate 200 g of fatty acid ethyl ester concentrates in a single batch cycle, assuming that the feedstock is pure esters. If it is found that a feedstock contains 'non-ester' impurities, the initial charge will have to be adjusted. For example, if it is found that the feedstock contains 10 wt.% of an uncharacterized material that will not dissolve in CO_2, the proper charge would be 220 g. It is not necessary to process the maximum charge. However, there is a minimum charge below which the yield of 90% pure products begins to suffer. For this system, the minimum is about 150 g.

B. Fractionation Curves

Figure 15 is a plot of the weight % collected vs. the composition of the fractions in units of peak area %. This run was performed on 100 g of n-3 ester concentrate. Also shown is the pressure programming used to perform this fractionation, (see section C of this Appendix). Pressure programming, as opposed to isobaric fractionation, offers improved separation although at the cost of a higher solvent-to-feed ratio.

Figure 16 is a plot from the same fractionation, but the abscissa is liters of CO_2, i.e. the total number of liters of CO_2 passed through the dry gas meter to that point in the fractionation. As indicated, EPA of 90% purity was collected in this fractionation. This plot is of more practical value to the operator since the flow of CO_2 through the dry gas meter can be monitored continually, whereas the wt.% eluted at any time can be determined only after the fractionation is completed.

Figure 17 illustrates the fractionation of 200 g of n-3 ethyl ester concentrate, following the procedures detailed in section D (p. 84), to produce five fractions. The first fraction, primarily 16:3w4, 16:4w1, 18:4w3, and some EPA was eluted with 9500 L CO_2. The next fraction (elution volume 15,500 L), considerably smaller, contains EPA of about 70-80% purity. This second fraction should not be discarded for, as soon as 200 g of this fraction has been accumulated, it can be used as a feedstock for obtaining 90% EPA in much better yield than is possible with the usual feedstock. Not only is it higher in EPA, it also should contain an insignificant amount of DHA (<2%). Thus, with this feedstock, one need only 'strip out' the lower molecular weight components; once these components are removed, the esters remaining in the column should contain EPA of 90% purity or better, which can be collected at 2500 psi (at this pressure, EPA will come over quite rapidly). The third fraction (elution volume 30,500 L) contains primarily EPA of better than 90% purity. Fraction 4 (elution volume 39,000 L) is about a 40%:40% mixture of EPA:DHA. This material might also be desirable for isolating EPA and particularly DHA of 90%

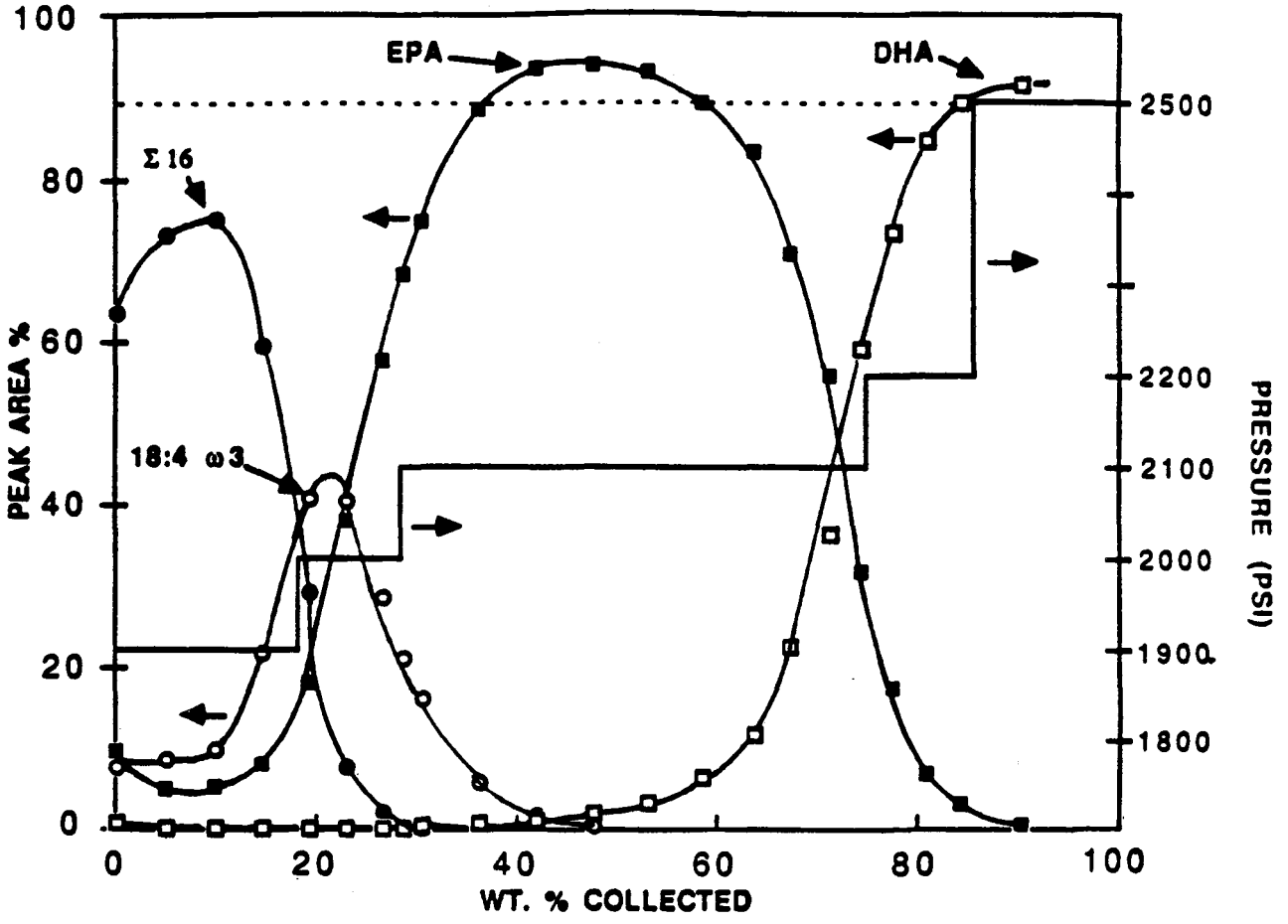


Fig. 15. Fractionation curves for the SCF fractionation of Charleston Laboratory crude omega-3 concentrates. Column temperatures were (from the top of the column) 80, 72, 65, 59, 54, and 50°C, except at 2500 psi where all zones were set at 60°C. The pressure profile is overlayed with the scale on the right vertical axis. The left hand curve, designated 16, is the sum of 16:3w4 and 16:4w1.

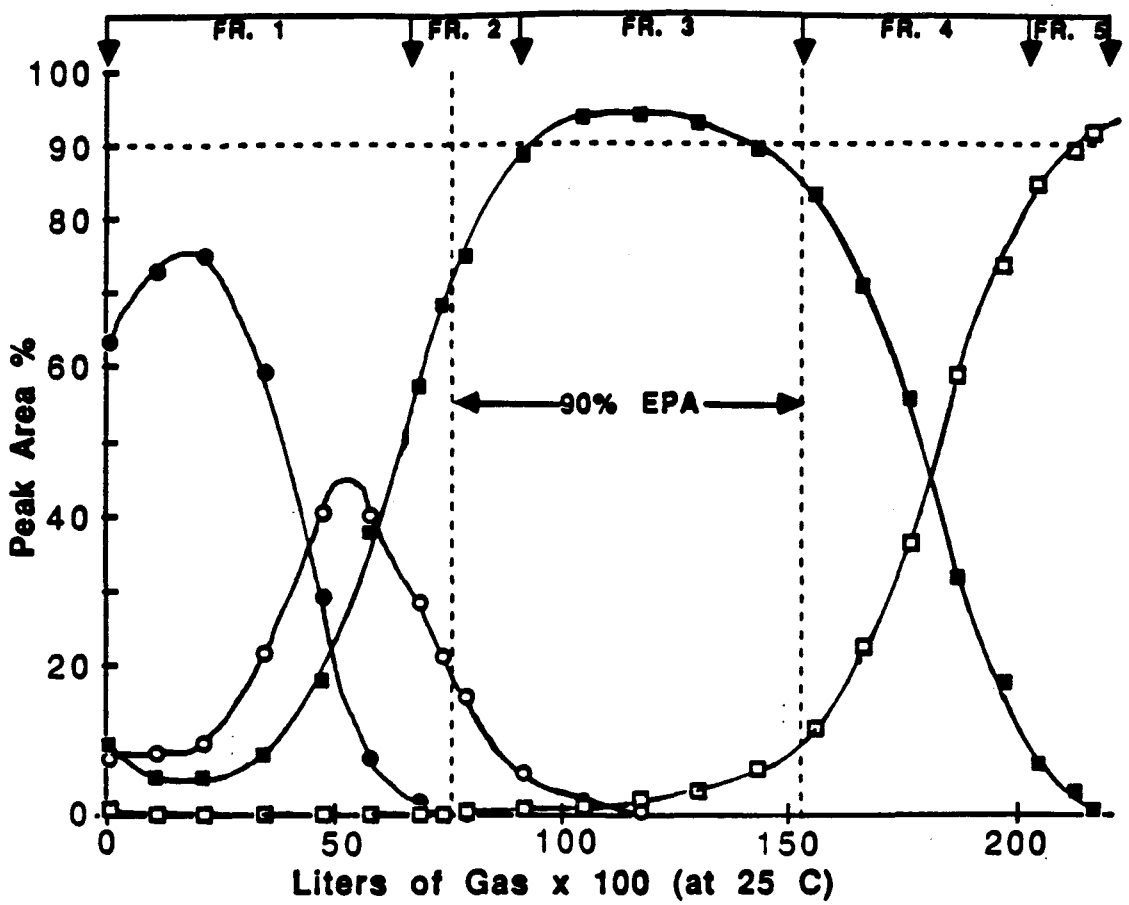


Fig. 16. A plot of liters of CO_2 used vs composition of fractions in units of GC peak area percent for the fractionation of Charleston Laboratory crude omega-3 concentrates. Conditions are identical to those of Fig. 11. Suggested points at which fractions should be cut are indicated at the top of the plot.

CO_2 FRACTIONATION DATE: 5/12/88

FR. #	1	2	3	4	5
WT.(g)					
C 16	21.2	0.7	--	--	--
18:4	6.5	5.2	1.7	--	--
20:5	4.0	12.5	56.9	16.7	0.2
22:6	--	0.2	5.4	25.6	14.6
WT. (g)	35.5	21.6	69.6	46.7	16.3

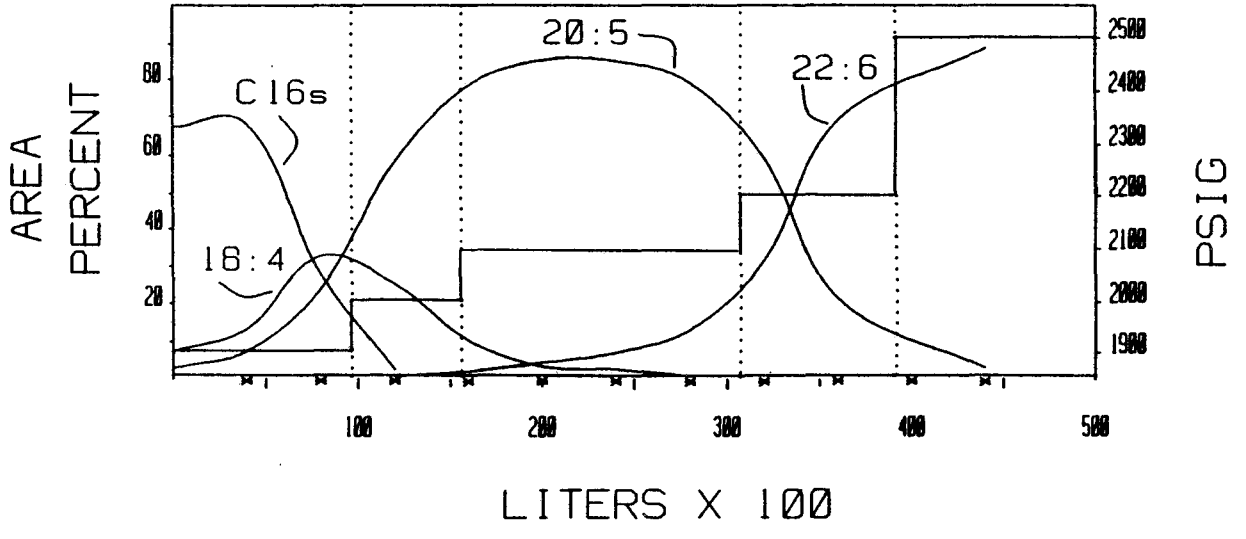


Fig. 17. Fractionation curve and fraction composition of Charleston Laboratory crude omega-3 concentrates processed by SCF Technology as described in the text.

purity. Finally, the last fraction is DHA of high purity.

C. Pressure Programming

The lower the pressure of the SCF CO_2, the higher the selectivity and the better the separating power of the fluid. The selectivity is always observed to increase as the pressure is decreased. However, with a decrease in pressure, a decrease in ester solubility is also observed. Therefore, while the fluid is observed to become much more selective at lower pressures, the time necessary to perform the fractionation eventually becomes prohibitive. Pressure programming is a means to take advantage of the higher selectivity of CO_2 at lower pressures without incurring a large increase in the time necessary to perform the fractionation.

The theory behind pressure programming is as follows. The n-3 concentrate used as feedstock contains esters with 16-, 18-, 20-, and 22-carbons. To a first approximation, esters of the same chain length are equally soluble in CO_2. Therefore, the mixture may be considered to be a 4-component mixture. Initially, the pressure is chosen to be low enough to take advantage of high selectivity, but not so low that the most soluble components (initially the 16-carbon esters) are so insoluble that the time necessary to elute them is unreasonably long. Once the 16-carbon esters are depleted, the pressure is raised to a higher value at which time the next most soluble of the components is stripped out of the mixture, (in this case, the 18-carbon esters), etc. There are, of course, quite a number of possible programming schemes. For simplicity and yield of product per unit time, the scheme shown in Figure 17 is recommended.

D. Temperature Controllers

The Syscom REX C4 controllers are mounted in a control box. A wiring diagram for a single controller and relay is shown in Figure 18. These controllers have the feature of 'PID Control'. 'P' refers to 'time Proportioning control', 'I' refers to automatic reset or 'Integral', while 'D' refers to rate or 'Derivative.' Each of these must be set to values which optimize temperature control. The appropriate combination of values depends upon the 'load,' which in the present case is the column and its contents at the process CO_2 flowrate. Finding the optimum values would be a time-consuming and tedious exercise if one were to do so manually. However, these controllers have an auto-tuning feature. Once this feature is activated, the controller will locate optimum values on its own, usually in about 15-20 min. The optimum values have been set and have been locked in to prevent inadvertent alteration.

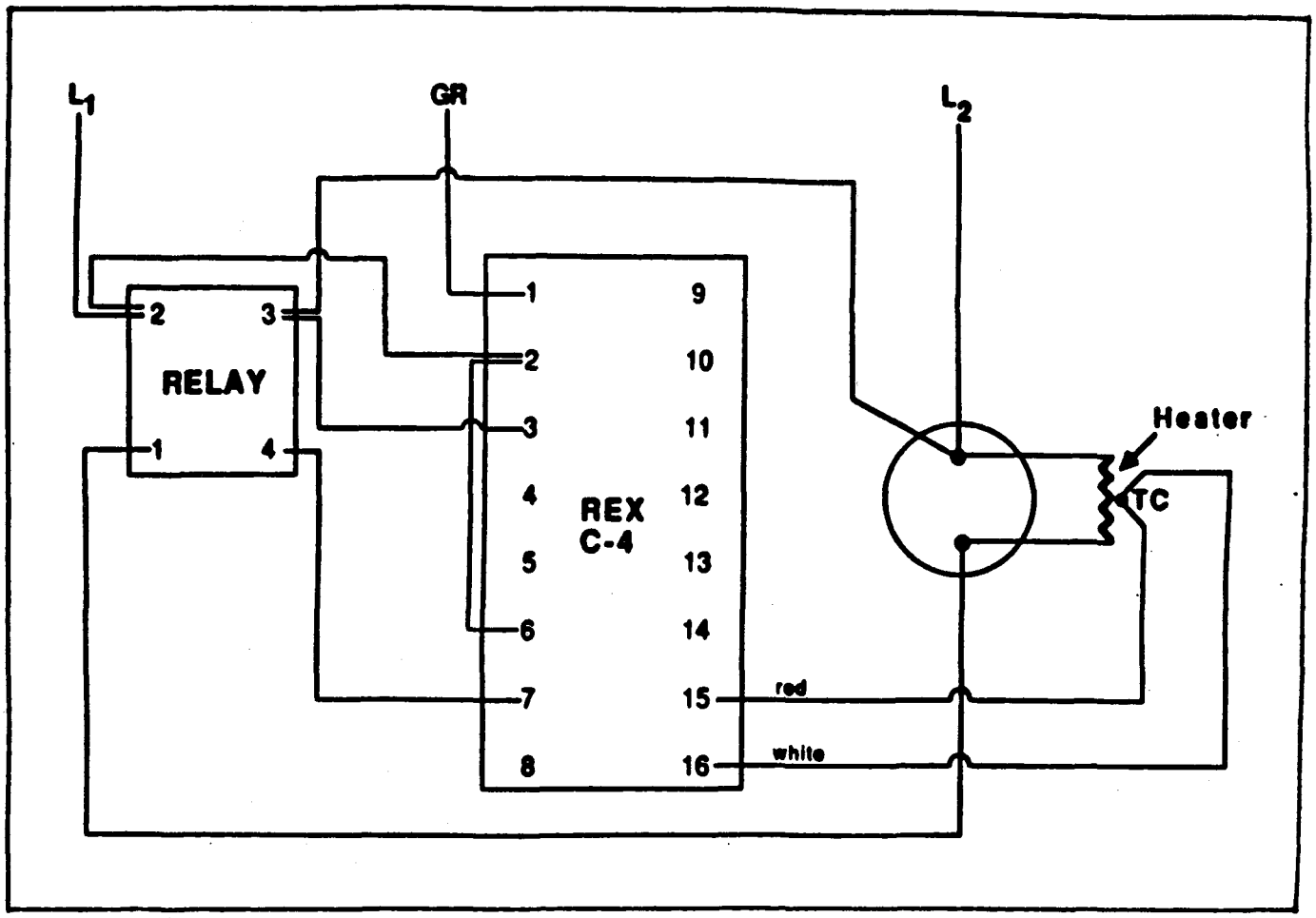


Fig. 18. Wiring diagram for a Syscon REX C-4 temperature controller.

E. Equipment and Materials Suppliers:

Addresses and phone numbers of the nearest suppliers or representatives for most of the major components in the unit are listed below:

High pressure fittings, gauges
Newport Scientific
(301) 498-6725
8246 E. Sandy Court
Jessup, MD 20794-0189
Contact: Phil Davidson

Column, endcaps
Temco Inc.
(918) 834-2337
P.O. Box 582550
Tulsa, OK 74158
Contact: Paul Brauer

Compressor and compressor accessories
PDC Inc.
(215) 443-9442
1875 Stout Dr.
Warminster, PA 18974
Contact: Ed Lamm

Propak
Scientific Development Company
(814) 237-4132
State College, PA 16801

Syscon Controllers
Sentry Power
(704) 847-5861
PO Box 2629
Matthews, NC 28106

Gas meter
Energy Control Inc.
(704) 375-1701
2150 Hawkins Rd.
Charlotte, NC 28203

Back Pressure Regulator
Tescom
(612) 441-6330
12616 Industrial Blvd.
Elk River, MN 55330
Contact: Rick Lindbloom

Silicone rubber heaters
Shealy Electrical Wholesalers
(803) 252-5668
(Watlow area representative)
Columbia, SC

Carbon dioxide
Sunox, Inc.
Laboratory Purchasing Agent deals with blanket purchase orders

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

I. Principle of Operation

High performance liquid chromatography (HPLC) is a separation process that is similar to packed column gas-liquid chromatography except that the mobile phase of the system is a liquid, rather than a gas, under pressure. The stationary phase contained within the column is an inert particulate support, coated with a liquid phase selected to differentially retard the elution of the components of the feed stock. In this way, the feed is fractionated into its individual components. Depending upon the dimensions of the column and the weight of feed to be fractionated, the process is known as analytical-, preparative-, or process-scale HPLC. A process-scale HPLC is used at the Charleston Laboratory to further purify EPA and DHA fractions obtained by supercritical fluid CO_2 fractionation.

II. Equipment

The HPLC used for isolation of pure 20:5n3 (EPA) and 22:6n3 (DHA) is a Waters Kiloprep 250 Preparative HPLC System that has been used to produce 98% pure EPA and 92% pure DHA, a method developed by Judith Krzynowek of the NMFS Gloucester Laboratory. This system employs a polyethylene column, 10 cm x 60 cm, containing a non-polar reverse phase packing (Deltapak C18, 15 u, 100 A). The column is radially compressed, hydraulically, to reduce dead volume in the column and provide a more uniform column bed and, therefore, more efficient separations. A chromatogram of the separation of 30 g of distilled n-3 concentrates is illustrated in Figure 19.

The system has been modified to include a smaller column (5.7 cm x 30 cm) containing the same column packing as the process-scale column. This smaller column permits continued methods development while requiring less solvent and sample. The Kiloprep 250 is capable of gradient elution, although this procedure cannot be used with the differential refractometer that detects the elution of sample components. Instead, the gradient elution module is used to prepare the ethanol/water solvent. The equipment is illustrated schematically in Figures 20 and 21. A separate pump has been added to deliver the sample directly to the head of the column, thereby greatly reducing the dead volume of the system and improving separation of the eluted fractions.

III. Operating Procedure

A. Start-up

1. Turn on the circulating water bath ([10], Fig. 20) and set at 35°C if necessary.
2. Switch on the multisolvent delivery system [11].

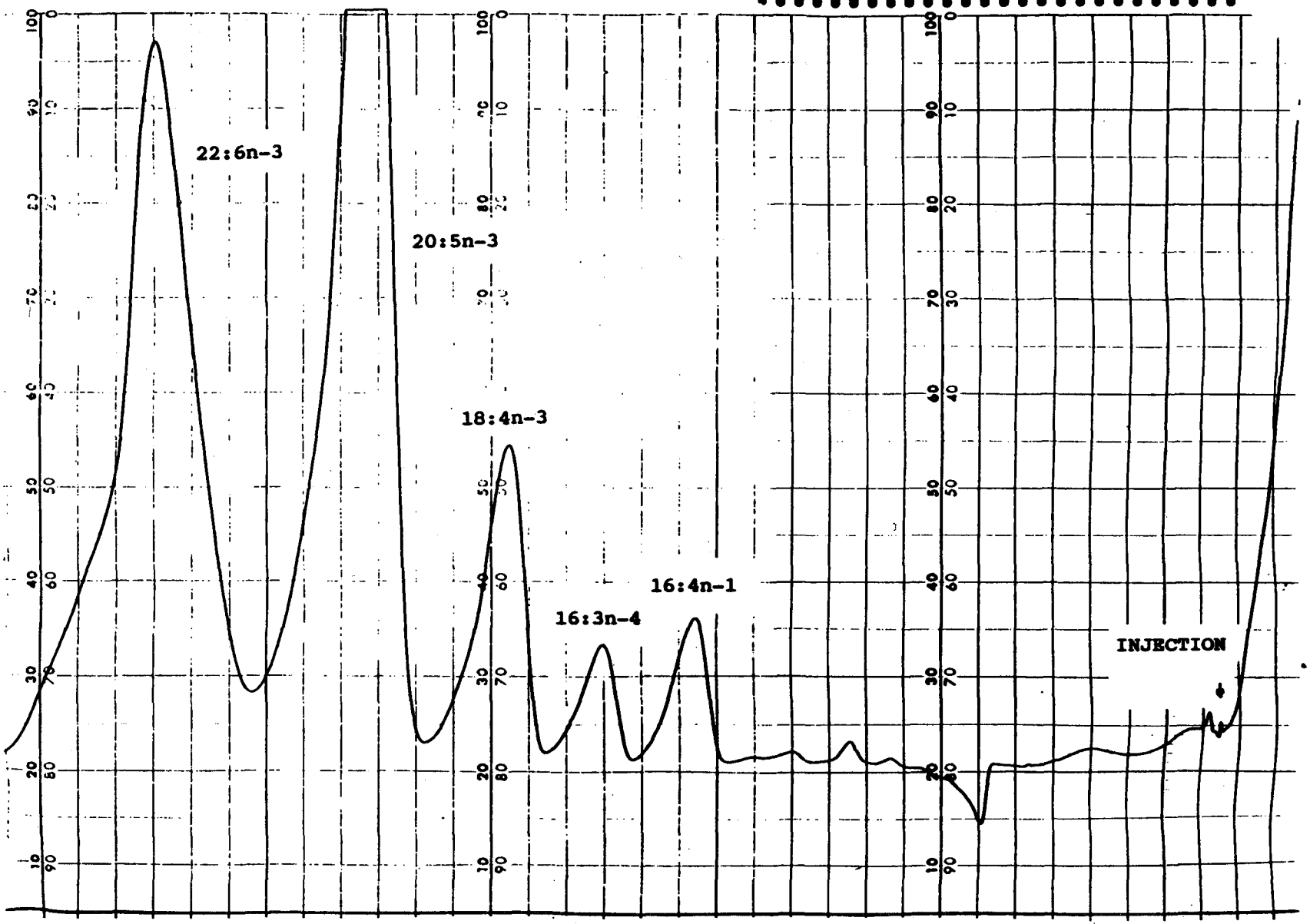


Fig. 19. Separation of distilled omega-3 ester concentrates (30 g) on a 10 cm x 60 cm Deltapak reverse phase C18 column installed in a Waters Kiloprep 250 HPLC, using 80/20 ethanol/water as eluent. Chart speed: 0.5 cm/min.

Components of the high performance liquid chromatograph

1 - Fraction collect timer
2 - Fraction collector ports
3 - Detector
4 - Column
5 - Sample feed pump
6 - Sample inlet line
7 - 3-Way column valve
8 - Head crank
9 - Prep-scale column
10 - Constant temperature bath
11 - Multisolvent delivery system
12 - Solvent delivery line
13 - Solvent reservoir
14 - Recorder
15 - Peak separator
16 - Fraction collector control panel
17 - HPLC status panel
18 - Fraction collection valve
19 - Inlet C

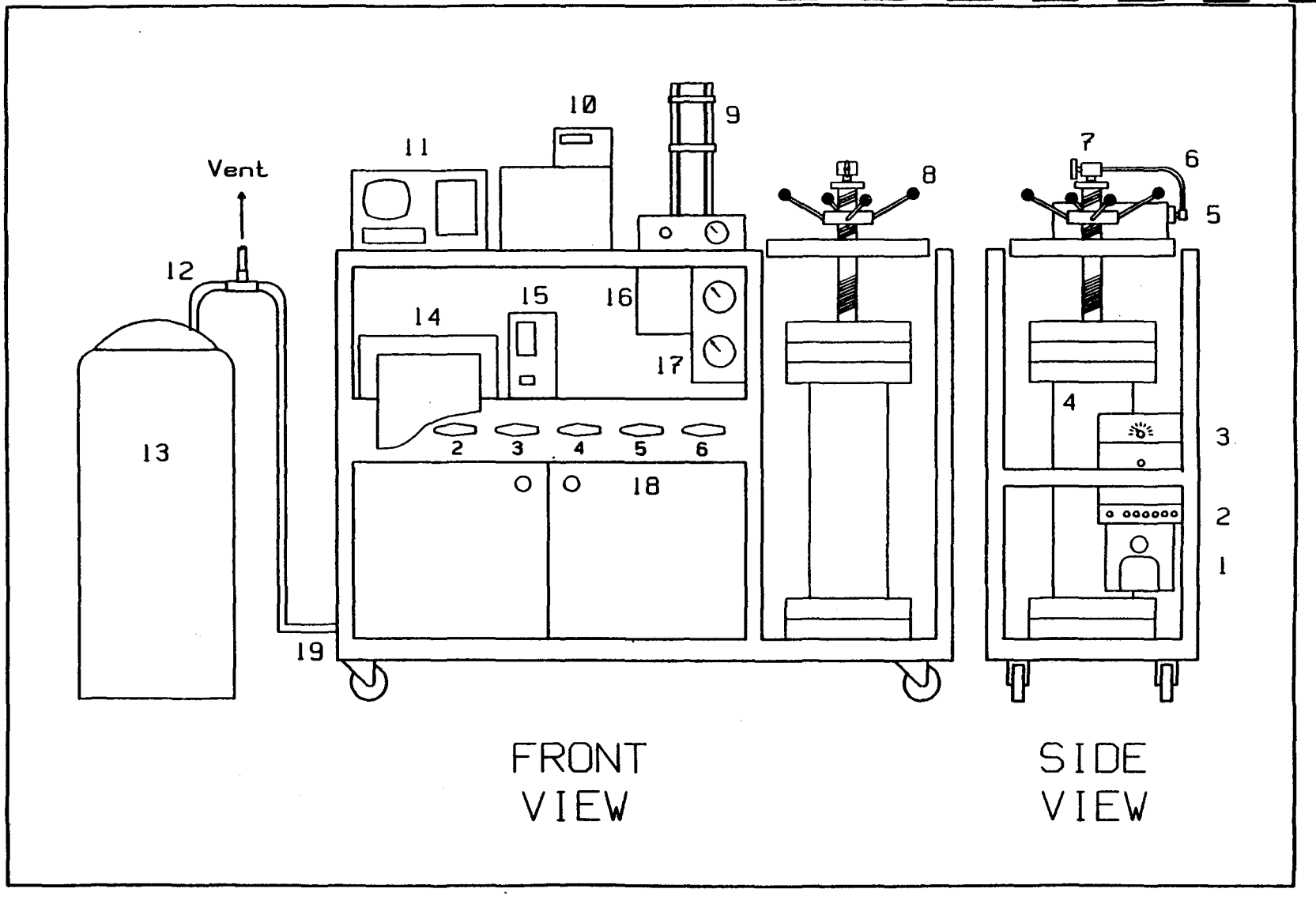


Fig. 20. Schematic diagram of the high performance liquid chromatograph.

Components of the HPLC Control Panels

The smaller panel on the left contains switches for manual and automatic operation of the 6-port fraction collect valve. For automatic operation, both toggle switches are placed in the <AUTO> position and the <HOME> rocker switch is pressed to select the first port of the fraction collect valve. The ports can be advanced or the run aborted, manually.

The larger panel has switches for the solvent pumps, the gradient pumps, N_2 valves to control the pressure of the pulse dampener, and four panel lights indicating operation. Radial compression of the column is controlled by a manual hydraulic pump located inside the cabinet of the HPLC.

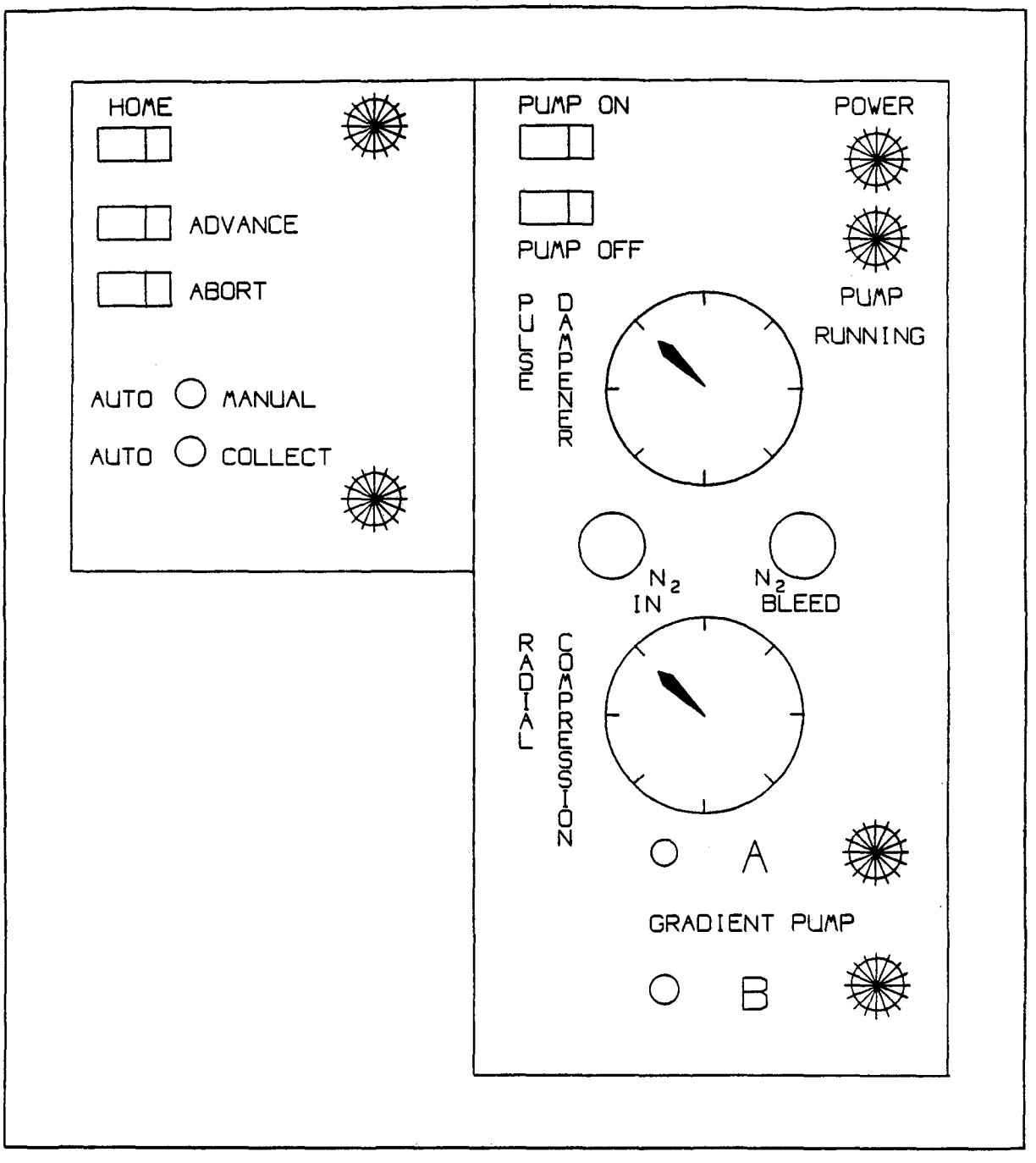


Fig. 21. Control panel of the high performance liquid chromatograph.

3. If it is necessary to make up solvent, carry out the following steps. If not, go to step 4.
    a. Press the <OPERerate GRADient> key [11].
    b. Enter Table #8 by pressing <8><ENTER> [11].
    c. Insure that there is sufficient alcohol and water to make up the desired amount of solvent.
    d. Start solvent feed tank stirrer.
    e. Turn on gradient pumps (inside instrument cabinet) using the toggle switches on the control panel (Fig. 21). The pumps are set to deliver ethanol and water in a ratio of 80 to 20 (v/v).
    f. At this time, press <OPER GRAD> [11].
    g. Connect Teflon tube, the outlet of the gradient mixer, to solvent feed tank [13]. Make sure the tank is vented.
    h. When tank contains desired amount of solvent, press <6> <ENTER> [11]. Then press <OPER GRAD> [11].
    i. Turn off gradient pumps and disconnect Teflon tube.
    j. Evacuate the solvent feed tank for 5 min, using a vacuum pump. Then vent the tank.
    k. Attach braided stainless steel line from inlet C [19] to the solvent feed tank. The system is now prepared for solvent delivery.
4. Adjust the pressure on the pulse dampener to 550 psig by admitting or bleeding N_2, using the valves on the control panel (Fig. 21).
5. Radial cartridge pressure (Fig. 21) should be 350 psig.
6. Turn valve 5 [18] (Fig. 20) on the front panel to the 'COLLECT' position.
7. Set both toggle switches on the smaller control panel to <AUTO>, press the <HOME> toggle switch and start the solvent feed pumps (Fig. 21).
8. Vent the pump heads as follows: While the pumps are running, reduce the setting on the left pump gauge (inside the cabinet) to 5 mm (red hand on gauge). Turn the valve on the pump head to vent the line. Then adjust gauge to 30 mm (red hand) and allow the pump to run until no air is evident. Re-adjust gauge to 5 mm (red hand) and turn the valve to the online position. Finally, adjust the gauge to 10 mm (red hand)/20 mm (black hand). Repeat these operations for the right pump head.
9. Flush the column for 3 min, then flush reference cell of the RI detector for 1 min, using the flush valve located on the panel below the detector ([3], Fig. 20). However, if the column contains 100% ethanol (due to washing the column after the previous day's work), flush the column with ethanol/water (80/20, v/v) until the pulse dampener pressure gauge (Fig. 21) reads 900-1,000 psi. Now, the reference cell can be flushed.
10. To load the sample onto the column, turn on the sample pump. Place the sample uptake line in the sample

container and flush the line with sample. The valve ([7], Fig. 20) on top of the column should be in the vertical position.

11. Turn off the solvent pumps and allow the pressure in the system to drop for 30 sec.
12. To inject the sample, turn the column valve ([7], Fig. 20) to the horizontal position and set the sample pump switch to the <RUN> position. For each 10 g of sample injected, operate the sample pump for 1 min and 6 sec. Once the amount of sample desired has been injected, turn the sample pump switch to <STANDBY>.
13. Turn the valve at the head of the column to the vertical position.
14. Start the solvent pumps and strip-chart recorder. Press the unlabeled key that lies immediately below the 'START RUN' command on the monitor of the Waters 600 module (multi-solvent delivery system [11], Fig. 20).
15. After 2 min, turn on the peak separator [15] and re-set the void timer. Set the timer appropriately to collect the peaks of interest. The void time is equal to the retention time of the first peak to be collected, minus three min.
16. Attach labeled receivers to the proper outlets ([2], Fig. 20) of the 6-port fraction collector valve.
17. Set the sample pump switch in the <RUN> position and rinse the line with about 20 ml of absolute ethanol. Return the pump switch to <STANDBY>.

B. Shut-down

1. After the DHA peak has eluted from the column, switch the solvent pump off. Turn valve 5 [18] on the front panel to the vertical position to seal the system.
2. Turn off the sample delivery pump.
3. Turn off the stirrer beneath the solvent feed tank.
4. Turn off the circulating water bath.

C. Product recovery

The purified esters are recovered from the solvent in an all-glass/Teflon wiped film still, illustrated in Figure 22. This still is operated as follows:

1. Turn on the chiller (35°C).
2. Turn on the vapor trap.
3. Attach the solvent receiver and product receiver [11].
4. When the vapor trap reaches -40°C, start the vacuum pump.
5. Turn on the heating mantles [10] (setpoint, 90°C), and the feed line heater tape [6] (setpoint, 100°C). Turn on the still body heater tapes [12] (setpoint, 35°C).
6. Set wiper drive speed at '6' on the speed control [4].
7. Set the metering valve [5] at '10'.
8. When the vacuum reaches 100 torr, connect N_2 line to feed flask and the feed line to the still body.

Components of the EPA/DHA recovery still

1 - Temperature controllers
2 - Pirani vacuum gauge
3 - Temperature monitor
4 - Wiper drive controller
5 - Metering valve
6 - Heated inlet line
7 - Wiper drive
8 - Vacuum sensor
9 - Still body
10 - Heating mantles
11 - product receiver
12 - Heating tapes
13 - Entrained ester receiver
14 - Primary condenser
15 - Glass bead packing
16 - Solvent recovery line
17 - Secondary condenser
18 - Secondary condenser

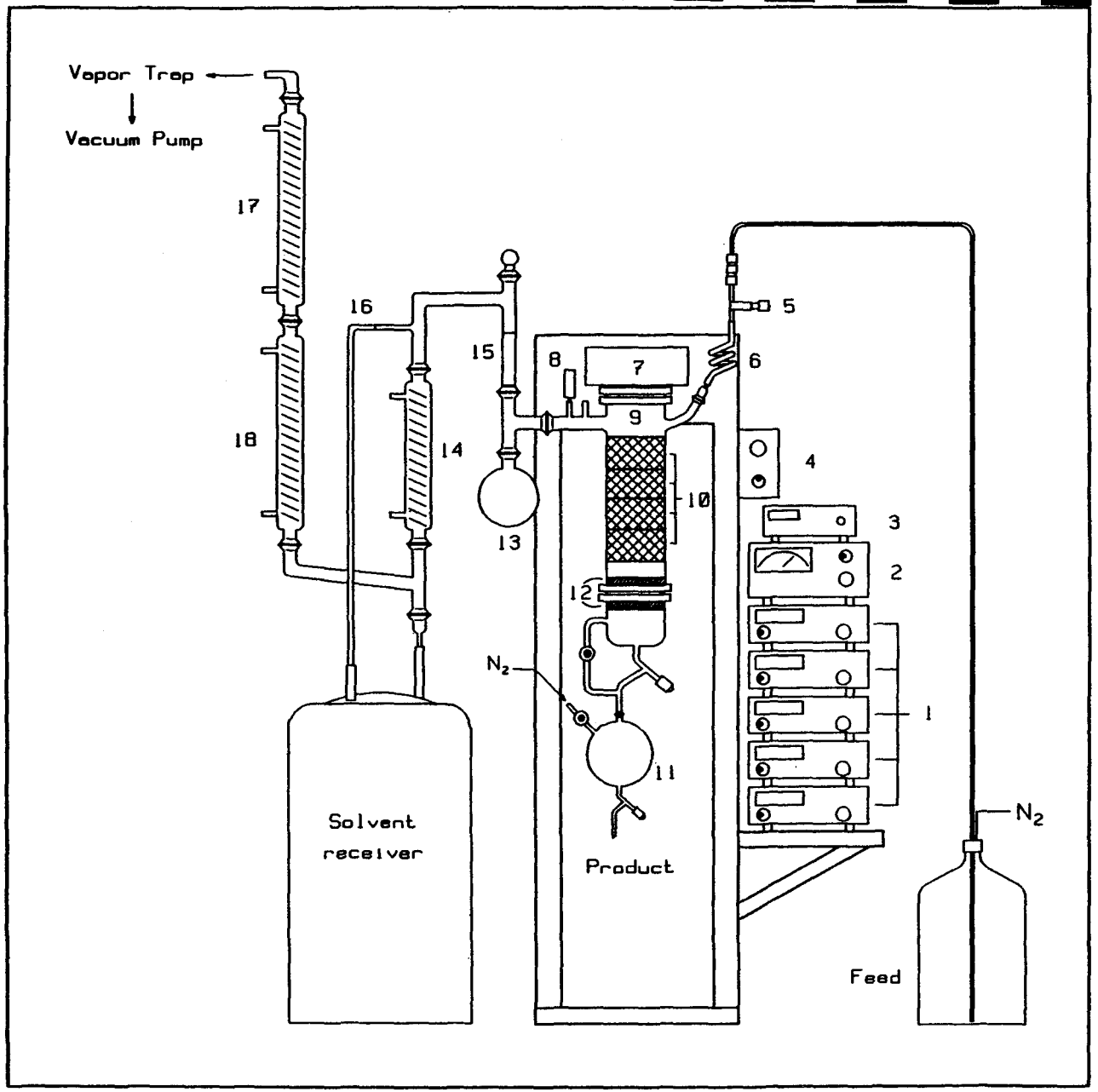


Fig. 22. EPA/DHA recovery still.

8. When the vacuum reaches 100 torr, connect N_2 line to feed flask and the feed line to the still body.
9. Adjust the metering valve [5] to give a feed rate of about 60 ml/min.
10. When distillation is complete, isolate the vacuum pump using the speedi-valve mounted on the pump.
11. Introduce N_2 into the product receiver [11] *via* the receiver sidearm and remove the receiver.
12. Immediately drain the contents of the receiver into an appropriately-sized brown glass bottle that has been thoroughly flushed with N_2, blanket well with N_2, cap tightly, and place in the -40°C chest freezer.
13. Turn off power to all heating tapes and the two upper mantles.
14. Clean the still immediately after use, as described in Section V, below.

IV. Product Storage

All products are currently stored in brown glass bottles with Teflon-lined caps in the -40°C chest freezer. Storage studies are underway to evaluate the use of cryovials at -40°C and -70°C as well as evacuated amber glass ampules.

V. Equipment Cleaning and Maintenance

The recovery still and all electronic components of the HPLC must be kept free of alcohol and organic materials. The HPLC column requires routine cleaning every other day of use. Beyond this, the HPLC requires little cleaning. The recovery still must be cleaned after each use.

A. HPLC Column
1. After the last component has eluted from the column, shut off the solvent feed pumps.
2. Switch the braided stainless steel solvent feed line to a drum of absolute ethanol. Start the solvent feed pump and flush the column for 10 min. Ethanol remains in the column overnight.
3. After washing the column, turn off the solvent pumps and all other devices as indicated in normal shut-down mode.

B. Recovery still
1. Shut off the vacuum pump and electric vapor trap.
2. Replace the product receiver (removed to recover the product) with a clean flask and open the valve to the still body. Check that the flask's side-arm and bottom stopcocks are closed.
3. Introduce absolute ethanol through the feed line into the system at a very low flow-rate (*ca* 25-30 ml/min).
4. After all surfaces of the still have been wetted, open the N_2 valve to the system to eliminate any remaining vacuum. This can be done before all of the ethanol enters the still.

5. Distill approximately 2 gal of absolute alcohol through the still.
6. Turn off the heating mantles.
7. Allow all alcohol to drain from the still and discard into properly labeled containers.
8. Remove the product receiver from the still. Remove stopcocks and wash both flask and stopcocks in hot detergent solution. Rinse both copiously with tap water, deionized water and, finally, absolute ethanol.

VI. Waste Disposal

Used 80% ethanol, the only waste product from the HPLC, is stored in 55 gal drums in an outdoor facility. Quarterly, it is disposed of by incineration by a licensed waste handler.

VII. Safety

* Ethyl alcohol is potentially toxic. Avoid breathing vapors by maintaining proper ventilation, avoiding spills and leaks, keeping operating and storage vessels closed, maintaining constant vigilance and care. Toxicity level is highly subject to individual tolerances but can impair judgement at relatively low levels of exposure. Lethal dosage levels are relatively high and, at low rates of exposure, are preceded by intoxication. Respirators with organic vapor cartridges have been provided to all personnel for use if ethanol vapors become objectionable.
* Ethyl alcohol is a flammable solvent, will easily ignite and burn unless highly diluted with water. A fire can be especially dangerous because the flames may be almost invisible. It will form explosive mixtures with the proper amount of air. Water dilution is the most effective countermeasure for minor spills, etc.
* Major spills (ca 20 gallons or more) should receive special, supervised attention. Provisions are made to handle large spills by entrapment in the outside 1000 gal sump. In this event, make certain immediately that the sump pump is turned off so alcohol will not be pumped to the sewerage system The pump switch is outside near the sump pump. Normally, the explosive vapor detection system will sound an alarm and disconnect the pump automatically. Disconnect the pump with the outdoor local switch in any event. Follow up any spill with a water hose flush of the floor and drains since vapors can possibly leak into other areas of the building (but not likely). Turn local hood fans on high and open hood doors partly if conditions permit.
* Care must be maintained in handling alcohol and oil contaminated wastes and materials (towels or other clean-up materials.)
* Keep floors rinsed and obstacles out of the way, especially during operation.

* Safety shoes should be worn necause of the necessity for handling 55 gal drums of ethanol.

VIII. Emergency Shut-down Procedure

If a fire occurs in the plant and the facility must be evacuated, activate the nearest fire alarm before shutting off the solvent delivery pump and the main power switch and exiting the building.

IX. Appendix

Equipment and Materials Suppliers:

HPLC
Waters Chromatography
(508)478-2000
34 Maple Street
Milford, MA
Contact: Jim Landers, service engineer
Hal Crow, area representative (1-800-334-5167)

Glassware
Tudor Scientific Glassware
555 Edgefield Road
Belvedere, SC 29841
(803) 279-4666
Contact: Chris Schute or Tom Tudor

Silicone rubber heaters
Shealy Electrical Wholesalers
(803) 252-5668
(Watlow area representative)
Columbia, SC

Temperature monitor/controllers
Vacuum pump
Cole Parmer
7425 North Oak Avenue
Chicago, IL 60648
(800) 323-4340

Vacuum pumps and gauge
Edwards High Vacuum, Inc.
(800) 828-6691 or (615) 691-3850
P.O. Box 30381
Knoxville, TN 37930-0381
Contact: Kevin Bloomer, sales engineer

Vacuum fittings
Cooper River Valve and Fitting
552-5545
4669 Franchise Street
Charleston Heights, S.C.

Electric vapor trap
Southeastern Scientific Corp.
(704) 542-3508
P.O. Box 2093
Charlotte, N.C. 28211
Contact: John Hardin

Routine Maintenance

A. Pumps

1. Direct-drive pumps.
To attain long-term reliable operation, maintenance is required at regular intervals. Normally, direct-drive pumps should require no servicing other than replacement of the ball bearings and the mechanical seal.

- Initial startup - with the pump operating:
  * Take and record amperage readings of the pump motor.
  * Take and record discharge pressure readings.
  * Take meter readings and record flow rate/min.

- Daily:
  * Visually inspect for liquid leaks at the mechanical seal, gasket, and pipe fittings.
  * Check any unusual noise or erratic operation.
  * Vent air from pump when cavitation is apparent.

- Semi-annually - with pump operating:
  * Take amperage readings of the pump motor and compare with initial value.
  * Take meter readings and compare with initial value.
  * Disassemble and inspect pump impeller for wear.
  * Inspect impeller waterway passages for restrictions or debris.
  * Inspect mechanical seal for wear, scoring, chips or broken surfaces.
  * Inspect spring for rust and mechanical damage.
  * Replace seal if any of the above physical defects is present.
  * Disassemble and inspect the bearings to determine the condition of the grease and replace the bearings, if necessary.
  * Reassemble in reverse order.

2. Variable-drive pumps.
These pumps also require regular maintenance. In addition to the maintenance instructions outlined for direct-drive pumps, variable-drive pumps require additional maintenance.

- Daily:
  * Visually inspect for grease leaking from bearing seals or bearing housing.
  * Visually inspect for belt particles.
  * Check any unusual noise or erratic operation.

- Semi-annually:
  * Perform daily maintenance.

* Remove side plates and visually inspect the condition of the belt. Replace if worn or cracked.
* With the pump operating, visually monitor the performance of the variable drive as the control is adjusted from the set point to high and low rpm. Before turning pump off, return the control to the set point position.
* Lubricate bearings and movable sheave in accordance with manufacturer's instructions attached to the drive.

3. Gear-drive pumps

- Daily:
  * Visually inspect oil level.
  * Visually inspect for leaking seals and gaskets.
  * Visually inspect coupling for alignment and wear.
  * Check any unusual noise or erratic operation.

- Semi-annually:
  * Perform daily maintenance.
  * Remove coupling and visually inspect the condition of the rubber insert. Replace if worn or cracked.
  * Change oil in the gear housing in accordance with manufacturer's instructions attached to the drive.
  * With the pump operating, monitor the performance of the variable drive as the control is adjusted from low to high rpm. Before turning pump off, return the control to the low rpm position.

B. Gear reduction drives (crystallizer and film evaporator)
To attain long-term, reliable performance, carry out the following steps at the stated intervals.

- Daily:
  * Visually inspect oil level.
  * Visually inspect for leaking seals and gaskets.
  * Visually inspect coupling for alignment and wear.
  * Check any unusual noise or erratic operation.

- Semi-annually:
  * Perform daily maintenance.
  * Re-position coupling, if loose, and tighten.
  * Change oil in the gear housing in accordance with manufacturer's instructions.
  * When drive is installed overhead, tighten all bolts and nuts securing drive to motor and frame or base.

C. Water chillers
For reliable, long-term operation, the following maintenance must be carried out at the recommended intervals.

- Daily:
  * Record the pressure readings of the refrigerant gauges.
  * Record the coolant and ambient temperatures, and check the coolant level.
  * Visually inspect for water or oil leaks.
  * Check condenser for restrictions.
  * Check refrigerant sight-glass for bubbles.
  * Review equipment performance and check any unusual noise or erratic operation.

- Semi-annually:
  * Clean condenser coil (air-cooled condensing units).
  * Check compressor oil level.
  * Activate safety controls to insure they are operational.
  * Leak-test refrigeration circuit.
  * Inspect all electrical components and insure all connections are tight.
  * Take amperage readings on compressor and sump.
  * Check specific gravity of coolant.
  * Clean storage tank as required.
  * Check calibration of temperature controller.

TROUBLE-SHOOTING ANCILLARY EQUIPMENT

WATER CHILLER (CAPITOL)

Problem	Corrective Action
Water chiller not operating.	Check disconnect switch on unit. Reset circuit breaker #C-1, panel MCC-4, Room 410. Place control switch above recorder in Room 420 to manual position. Check temperature set point (on chiller unit). Check coolant level. Reset high-pressure limit switch (in control panel). Press start button on control panel.
Circulating pump is operating, chiller will not start.	Check temperature set point. Check coolant flow switch for closed position (next to pump).
Chiller and circulating pump are operating, coolant is not cooling crystallizer.	Open flow control valve "A" and close valve "B".*
Chiller and circulating pump are operating, coolant is not cooling heat exchanger.	Open flow control valve "B" and close valve "A".*
Coolant slow in reaching temperature set point.	Check sight glass, add refrigerant (R-502).

* One of these valves must be open any time pump is operating.

WATER CHILLER (CUSTOM RESEARCH)

Problem	Corrective Action
Water chiller not operating.	Reset circuit breaker #7, panel MCC-4/B, Room 410. Check circuit breakers (on chilling unit). Check electrical power switch (on temperature control panel). Check coolant flow switch for closed position (next to heat exchanger). Reset oil pressure switch (on compressor). Reset high-pressure limit switch, (on compressor). Check flow control valves "C" and "D" for open position. Check circulating pump for flow.
Coolant slow in reaching temperature set point.	Check sight glass, add refrigerant (R-22).

COOLING TOWER (FAN)

Problem	Corrective Action
Fan not operating.	Check disconnect switch (on unit). Check circuit breaker #B-3, panel EMCC-4, Room 410. Check fan operation with test switch. If fan does not operate, reset thermal overload (push red bar on magnetic motor control, inside panel). If fan operates, cooling water temperature is lower than thermostat set point.

COOLING TOWER (PUMP)

Problem	Corrective Action
Pump not operating.	Check disconnect switch (on unit). Check circuit breaker #B-4, panel EMCC-4, Room 410.
Pump is operating but not pumping sufficient cooling water.	Open disconnect switch for fan and pump. Remove inspection panel (pump end). Check water level in cooling tower tank (water should cover pump inlet pipe). Remove screen on pump inlet and clean. Replace screen. Restart fan and pump. Reset high pressure switches on freezer and water chiller compressors, if necessary.

WALK-IN FREEZER (ROOM "A")

Problem	Corrective Action
Condensing unit not operating.	Check disconnect switch (at condensing unit). Check circuit breaker #C-3, panel EMCC-4, and circuit breaker #11, panel E-1, Room 410.
Unit has electrical power, compressor will not start.	Reset high-pressure limit switch (on compressor).
Unit starts, but will not continue to operate.	Check cooling tower fan or pump operation. Reset high-pressure limit switch.
Room slow in reaching temperature set point.	Check time clock for defrost schedule. Check evaporator fan operation. Check cooling tower fan operation. Check sight glass, add refrigerant (R-12).
Room not maintaining temperature set point.	Check cooling tower fan operation. Check sight glass, add refrigerant (R-12).

WALK-IN FREEZER (ROOM "B" PRIMARY/SECONDARY)

Problem	Corrective Action
Condensing unit not operating.	Check disconnect switch (at condensing unit). Check circuit breaker #C-5, panel EMCC-4, and circuit breaker #12, panel E-1, Room 410.
Unit has electrical power, compressor will not start.	Reset oil pressure switch* (on compressor). Reset high-pressure limit switch (on compressor). Check position of transfer switch on control panel.
Unit starts but will not continue to operate.	Check cooling tower fan or pump operation. Reset high-pressure limit switch.
Room slow in reaching temperature set point.	Check time clock for defrost schedule. Check evaporator fan operation. Check cooling tower fan operation. Check sight glass, add refrigerant (R-502).
Room not maintaining temperature set point.	Check cooling tower fan operation. Check sight glass, add refrigerant (R-502).

* Oil pressure switch will open after a 2-minute power interruption. Restart could be delayed due to a time delay switch in the electrical system.

WALK-IN FREEZER (ROOM "C")

Problem	Corrective Action
Condensing unit not operating.	Check disconnect switch (at condensing unit). Check circuit breaker #C-4, panel EMCC-4, and circuit breaker #13, panel E-1, Room 410.
Unit has electrical power, compressor will not start.	Reset oil pressure switch* (on compressor). Reset high-presure limit switch (on compressor).
Unit starts, but will not continue to operate.	Check cooling tower fan or pump operation. Reset high-pressure limit switch.
Room slow in reaching temperature set point.	Check time clock for defrost schedule. Check evaporator fan operation. Check cooling tower fan operation. Check sight glass, add refrigerant (R-502).
Room not maintaining temperature set point.	Check cooling tower fan operation. Check sight glass, add refrigerant (R-502).

* Oil pressure switch will open after a 2-minute power interruption. Restart could be delayed due to a time delay switch in the electrical system.

AIR CONDITIONING AND HEATING

Problem	Corrective Action
Air conditioning system not operating.	Check disconnect switch (at unit). Check circuit breaker #B-4, panel MCC-4 and circuit breaker #9, panel E-1, Room 410.
Unit has electrical power, blower will not start.	Check hoods 3 and 4 exhaust blowers for operation. Both blowers must be operating to close inter-lock switches. Check for air movement in hoods 3 and 4. When no air movement is detectable, check the operation of the motor and belt (on roof).
Unit is operational, but not cooling rooms.	Place control switch (at control panel) in "Unoccupied" position. Check setting on thermostat (Room 420).

SUPERCRITICAL FLUID CO2 EQUIPMENT

Problem	Corrective Action
Column not heating.	Check fused disconnect switch for closed position (at column). Check fuses in disconnect switch. Check circuit breaker #29, panel A, section B, Room 410. Check heater controls.
CO_2 compressor unit not operating.	Check fused disconnect switch for closed position (at compressor). Check fuses in disconnect switch. Check circuit breaker #30, panel A, section B, Room 410. Check cooling water flow. Check diaphram leak gauges (on compressor).
Cooling unit not operating.	Check fused disconnect switch for closed position (at compressor). Check fuses in disconnect switch. Check circuit breaker #32, panel A, section B, Room 410. Check that cable is plugged into receptacle.
CO_2 tank unit not operating.	Check fluid and pressure levels for operation. Check fused disconnect switch for closed position (at tank). Check fuses in disconnect switch. Check circuit breaker #10, panel MCC-4/B, Room 410. Check disconnect switches (in tank compartment). Check valves for correct position for normal operation (in tank compartment). Check header valve (on top of tank). Check pressure regulating valve (on side of tank).

Units and conversion factors

Temperature

°F = (1.8 x °C) + 32 °C = 0.555 (°F −32) °K = °C + 273.2

Fahrenheit	Celsius	Rankine	Kelvin
602	316.7	1061.7	589.9
572	300.0	1031.7	573.2
542	283.3	1001.7	556.5
512	266.7	971.7	539.9
482	250.0	941.7	523.2
452	233.3	911.7	506.5
422	216.7	881.7	489.9
392	200.0	851.7	473.2
362	183.3	821.7	456.5
332	166.7	791.7	439.9
302	150.0	761.7	423.2
272	133.3	731.7	406.5
242	116.7	701.7	389.9
212	100.0	671.7	373.2
182	83.3	641.7	356.5
152	66.7	611.7	339.9
122	50.0	581.7	323.2
92	33.3	551.7	306.5
62	16.7	521.7	289.9
32	0.0	491.7	273.2
2	− 16.7	461.7	256.5
−28	− 33.3	431.7	239.9
−58	− 50.0	401.7	223.2
−88	− 66.7	371.7	206.5
−118	− 83.3	341.7	189.9
−148	−100.0	311.7	173.2
−178	−116.7	281.7	156.5
−208	−133.3	251.7	139.9
−238	−150.0	221.7	123.2
−268	−166.7	191.7	106.5
−298	−183.3	161.7	89.9
−328	−200.0	131.7	73.2
−358	−216.7	101.7	56.5
−388	−233.3	71.7	39.9
−418	−250.0	41.7	23.2
−459.7	−273.2	0.0	0.0

Length

1 angstrom (Å) = 10^{-10} m = 10^{-8} cm
1 fermi = 1 femtometer (fm) = 10^{-15} m = 10^{-13} cm
1 micron (μ) = 1 μm = 10^{-6} m = 10^{-4} cm = 10^{4} Å
1 light-year = 5.88 x 10^{12} mi = 9.461 x 10^{12} km

Units of length

Unit	Inches	Feet	Yards	Centimeters	Meters
Inch	1	0.08333	0.02777	2.54	0.0254
Foot	12	1	0.3333	30.48	0.3048
Yard	36	3	1	91.44	0.9144
Mile	63,360	5280	1760	160,934.4	1609.34
cm	0.3937	0.03281	0.01094	1	0.01
Meter	39.37	3.2808	1.09361	100	1

Units of area

Unit	Square inches	Square feet	Square yards	Square centimeters	Square meters
Sq inch	1	0.006944	0.000772	6.45162	0.000645
Sq foot	144	1	0.1111	929.034	0.092903
Sq yard	1296	9	1	8361.31	0.836131
Sq cm	0.155	0.001076	0.00012	1	0.0001
Sq meter	1550	10.7639	1.19599	10,000	1

If you need additional technical information or data on pressure and flow systems, refer to the Pump section on pages 724-726.

Units of capacity and liquid measure

Unit	Fluid drams	Fluid ounces	Quarts	Gallons	Liters
Fl Dram	1	0.125	0.003906	0.000976	0.003697
Fl Ounce	8	1	0.03125	0.007812	0.028413
Quart	256	32	1	0.25	0.94635
Gallon	1024	128	4	1	3.78541
Milliliter	0.2705	0.03381	0.001057	0.000264	0.001
Liter	270.512	33.8140	1.05669	0.264172	1
Cubic inch	4.432	0.5541	0.017316	0.004329	0.016387

Volume

lbs water x 0.119 = gallons
gal (Brit) x 1.2 = gallons (US)
gal x 128 = fluid oz
cubic ft x 7.48 = gallons
cubic in x 0.00433 = gallons
gal x 3.785 = liters

liter x 0.264 = gallons
cubic meters x 264.2 = gallons
cubic meter x 1000 = liters
liters x 1000 = cubic cm
cubic cm x 0.0338 = fluid oz
fluid oz x 29.57 = cubic cm

Units of volume

Unit	Cubic inches	Cubic feet	Cubic yards	Cubic centimeters
Cubic inch	1	—	—	16.3872
Cubic foot	1728	1	0.03704	28.317
Cubic yard	46,656	27	1	764,561
Cubic cm	0.06102	—	—	1
Cubic meter	61,023	35.314	1.3079	1,000,000

Velocity

60 mph = 88 fps = 26.82 m/s
1 mph = 0.4470 m/s = 1.609 km/h = 1.47 fps

Force

1 newton (N) = 10^{5} dynes = 0.2248 lb
1 lb = 4.448 N
1 ton = 2000 lbs

Weight and mass

Weight = mass x acceleration due to gravity
An object of weight 1 lb has mass 453.6 g = 0.4536 kg
An object of mass 1 kg has weight 9.807 N = 2.205 lbs
1 metric ton = 1000 kg = 10^{6} g

Units of mass

Unit	Grains	Avdp oz	Avdp lbs	Grams
Grain	1	0.00229	0.000143	0.064799
Apoth scruple	20	0.04571	0.002857	1.29598
Pennyweight	24	0.05486	0.00343	1.55517
Avdp dram	27.344	0.0625	0.003906	1.77185
Apoth dram	60	0.1371	0.008571	3.88794
Avdp ounce	437.5	1	0.0625	28.3495
Apoth ounce	480	1.09714	0.068571	31.1035
Apoth pound	5760	13.1657	0.822857	373.242
Avdp pound	7000	16	1	453.592
Gram	15.432	0.03527	0.002205	1
Kilogram	15,432	35.2739	2.20462	1000
Short ton	—	—	2000	—
Long ton	—	—	2240	—
Metric ton	—	—	2204.6	—

Flow

C_v = flow coefficient factor = flowrate (US gpm) across a restriction (e.g. valves, fittings, etc.) at a 1 psig pressure drop
Q = flowrate (gpm)
Δp = pressure drop (psig) across restriction
= inlet pressure − outlet pressure
$C_v = Q/\Delta p$

Units and conversion factors

Specific heat

1 cal/g·C° = 1 Btu/lb·F°
= 4.184 J/g·C°
= 4184 J/kg·C°

Latent heat

1 cal/g = 1.80 Btu/lb
= 4.184 J/g
= 4184 J/kg

mm vs inches

mm	Inches
1	0.03937
2	0.07874
3	0.11811
4	0.15748
5	0.19685
6	0.23622
7	0.27559
8	0.31496
9	0.35433
10	0.39370
11	0.43307
12	0.47244
13	0.51181
14	0.55118
15	0.59055
16	0.62992
17	0.66929
18	0.70866
19	0.74803
20	0.78740
21	0.82677
22	0.86614
23	0.90551
24	0.94488
25	0.98425
25.4	1.0
38.1	1.5
50.0	1.968
50.8	2.0
75.0	2.953
76.2	3.0
100.0	3.937
101.6	4.0
127.0	5.0
152.4	6.0

Work and energy

1 joule (J) = 10^7 ergs = 0.239 cal
= 0.7376 ft·lb
1 cal = 4.184 J = 3.086 ft·lb
1 ft·lb = 1.356 J
1 Btu = 1054 J = 252 cal = 778 ft·lb
1 electron volt (eV) = 1.60×10^{-19} J
1 kilowatt hour (kWh) = 3.60×10^6 J

Power

1 watt (W) = 1 J/s = 0.738 ft·lb/s
1 horsepower = 0.746 kilowatt (kW)
= 550 ft·lb/s
= 3.30×10^4 ft·lb/min
1 Btu/hr = 2.93×10^{-4} kW

Inch equivalents

Fraction inches	Decimal inches	Millimeters
1/64	0.015625	0.397
1/32	0.03125	0.794
3/64	0.046875	1.191
1/16	0.0625	1.588
5/64	0.078125	1.984
3/32	0.09375	2.381
1/8	0.125	3.175
3/16	0.1875	4.763
1/4	0.25	6.35
5/16	0.3125	7.938
3/8	0.375	9.525
7/16	0.4375	11.113
1/2	0.50	12.70
9/16	0.5625	14.288
5/8	0.625	15.875
11/16	0.6875	17.463
3/4	0.75	19.05
13/16	0.8125	20.638
7/8	0.875	22.225
15/16	0.9375	23.813
1	1.0	25.40

Important metric prefixes

Prefix	Abbreviation	Meaning	Typical examples
peta-	P	$\times 10^{15}$	1 petayear = 10^{15} years
tera-	T	$\times 10^{12}$	1 terayear = 10^{12} years
giga-*	G	$\times 10^9$	1 gigahertz (radar frequency) = 10^9 Hz
mega-	M	$\times 10^6$	1 megaton (equivalent TNT strength of nuclear weapon) = 10^6 tons
kilo-	k	$\times 10^3$	1 kilogram = 1000 g
deci-	d	$\times 10^{-1}$	1 decimeter = 0.1 m
centi-	c	$\times 10^{-2}$	1 centimeter = 0.01 m
milli-	m	$\times 10^{-3}$	1 milliampere = 0.001 A
micro-	μ	$\times 10^{-6}$	1 microvolt = 10^{-6} V
nano-*	n	$\times 10^{-9}$	1 nanosecond = 10^{-9} second
pico-*	p	$\times 10^{-12}$	1 picofarad = 10^{-12} F
femto-	f	$\times 10^{-15}$	1 femtometer (approximate size of a proton) = 10^{-15} m

*In older literature, certain double prefixes are used: kilomega (kM) for 10^9; millimicro (mμ) for 10^{-9}; micromicro (μμ) for 10^{-12}.

If you need additional technical data or information on pressure and flow systems, refer to the Pump section on pages 724-726.

Glossary of terms for various types of instrumentation and measurements

Conductivity

Cell constant—The ratio of distance between conductance electrodes to the area of the electrode surface (l/A), expressed as K_c/cm.

Conductance—Measure of the ability of a solution to conduct electricity; thus it is the reciprocal of the resistance.

Mho—(micro- or milli-)—same as the siemens, S. See definition below.

Siemens—(micro- or milli-)—Unit of conductance of a solution. This value is the product of solution conductivity (L) in siemens/cm and cell constant K_c/cm. The conductance of a solution in siemens (S) is the reciprocal of its resistance in ohms. Formerly known as the mho (℧); reciprocal of Ω.

Temperature compensation—A correction for solution temperature to equal a conductivity value which would be measured at 25°C. Usually done electronically within an instrument, using an external detector to measure solution temperature.

Control definitions

On/off control—Control response in which function is either fully on or fully off.

Proportional band—Range of values above and below the set point; where control action is full on below the band, full off above the band and proportional in between.

Proportional control (P)—Control response proportional to deviation from set point within proportional band.

PID control—Control algorithm that combines Proportional, Integral (reset) and Derivative (rate) controls providing a more responsive control system.

Rate or Derivative (D)—Control action that speeds a correction based upon the deviation rate of change from the set point.

Reset or Integral (I)—Control action that changes proportional control depending upon time deviation from the set point.

Time proportional (P)—Control response is full on or full off but on and off duration is proportional to deviation from set point.

pH

pH—A term used to describe the hydrogen ion activity of a system. Equal to the $-\log a_{H^+}$; where a_{H^+} is the activity of the hydrogen ion. In dilute solutions the activity is essentially equal to the concentration, and pH is defined as the $-\log [H^+]$, where $[H^+]$ is the hydrogen ion concentration in moles per liter. A solution of 0 to 7 pH is "acidic", pH of 7 is neutral, and pH over 7 is "alkaline" or "basic."

pH temperature compensation—Correction for the influence of temperature on the pH sensing electrode. Instruments with temperature compensation indicate theoretical 25°C pH value, regardless of actual solution temperature.

Slope or slope adjustment—Correction (within a pH meter) for changes in an electrode's response from an ideal response.

Standardization—A method of compensating for the inaccuracy of a pH electrode. The meter is "standardized" (adjusted) to give an ideal response to a standardizing solution (buffer). The pH of other solutions can then be more accurately measured.

Thermometry

Bimetal thermometer—A temperature-sensing instrument where two dissimilar metals are bonded together in a narrow strip and coiled into the shape of a helix or spiral. The differential expansion of the dissimilar metals actuates a pointer, indicating temperature.

Cold junction—The reference junction of thermocouple wires leading to the measuring instrument, normally at room temperature.

Infrared thermometer—A thermometer that measures emitted infrared radiation (heat) to determine the temperature of an object.

Resistance Temperature Device (RTD; platinum)—A temperature-measuring device in which the resistance of the sensing element is an accurately known function of temperature.

Thermistor—A resistive circuit component having a high negative temperature coefficient of resistance, so that its resistance decreases as temperature increases. Made of a rugged two-terminal ceramic-like semiconductor bead, rod, or disc. The term is derived from "thermal resistor".

Thermocouple—A device consisting of two dissimilar metals joined together at both ends. The thermoelectric voltage developed between the two junctions is proportional to the temperature difference between the junctions. Thus the device is used to measure the temperature at one of the junctions when the other is held at a fixed, known temperature.

Solid-state temperature sensor—A temperature sensor which uses no mechanical parts. A temperature change produces a linear current or voltage response which is transferred or amplified by a silicon device.

WEIGHT CONVERSION TABLE FOR FISH OILS

Lbs = Kg * 2.2
L = Kg/0.95
Gal = L/3.785

The column headed "Lbs/Kg" represents the number to be converted.
Read the equivalent in "Lbs" (left) or "Kg" (right).

Lbs	Lbs/Kg	Kg	Liters	Gallons
0.22	0.1	0.045	0.05	0.01
0.44	0.2	0.091	0.10	0.03
0.66	0.3	0.136	0.14	0.04
0.88	0.4	0.182	0.19	0.05
1.10	0.5	0.227	0.24	0.06
1.32	0.6	0.273	0.29	0.08
1.54	0.7	0.318	0.33	0.09
1.76	0.8	0.364	0.38	0.10
1.98	0.9	0.409	0.43	0.11
2.20	1.0	0.455	0.48	0.13
4.40	2.0	0.909	0.96	0.25
6.60	3.0	1.36	1.44	0.38
8.80	4.0	1.82	1.91	0.51
11.0	5.0	2.27	2.39	0.63
13.2	6.0	2.73	2.87	0.76
15.4	7.0	3.18	3.35	0.88
17.6	8.0	3.64	3.83	1.01
19.8	9.0	4.09	4.31	1.14
22.0	10.0	4.55	4.78	1.26
44.0	20.0	9.09	9.57	2.53
66.0	30.0	13.64	14.35	3.79
88.0	40.0	18.18	19.14	5.06
110.0	50.0	22.73	23.92	6.32
132.0	60.0	27.27	28.71	7.58
154.0	70.0	31.82	33.49	8.85
176.0	80.0	36.36	38.28	10.11
198.0	90.0	40.91	43.06	11.38

TEMPERATURE CONVERSION TABLE (SAUVEUR)

°F = (9/5 * C) + 32
°C = (F - 32) * 5/9

The center column represents the number to be converted.
Read the equivalent in °C (left) or °F (right).

°C	°C/F	°F	°C	°C/F	°F	°C	°C/F	°F
-40.0	-40	-40.0	2.8	37	98.6	26.7	80	176.0
-34.4	-30	-22.0	4.4	40	104.0	28.3	83	181.4
-28.9	-20	-4.0	5.0	41	105.8	28.9	84	183.2
-23.3	-10	14.0	5.6	42	107.6	29.4	85	185.0
-17.8	0	32.0	6.1	43	109.4	30.0	86	186.8
-17.2	1	33.8	6.7	44	111.2	30.6	87	188.6
-16.7	2	35.6	7.2	45	113.0	31.1	88	190.4
-16.1	3	37.4	7.8	46	114.8	31.7	89	192.2
-15.6	4	39.2	8.3	47	116.6	32.2	90	194.0
-15.0	5	41.0	8.9	48	118.4	32.8	91	195.8
-14.4	6	42.8	9.4	49	120.2	33.3	92	197.6
-13.9	7	44.6	10.0	50	122.0	33.9	93	199.4
-13.3	8	46.4	10.6	51	123.8	34.4	94	201.2
-12.8	9	48.2	11.1	52	125.6	35.0	95	203.0
-12.2	10	50.0	11.7	53	127.4	35.6	96	204.8
-11.7	11	51.8	12.2	54	129.2	36.1	97	206.6
-11.1	12	53.6	12.8	55	131.0	36.7	98	208.4
-10.6	13	55.4	13.3	56	132.8	37.2	99	210.2
-10.0	14	57.2	13.9	57	134.6	37.8	100	212.0
-9.4	15	59.0	14.4	58	136.4	43.3	110	230.0
-8.9	16	60.8	15.0	59	138.2	48.9	120	248.0
-8.3	17	62.6	15.6	60	140.0	54.4	130	266.0
-7.8	18	64.4	16.1	61	141.8	60.0	140	284.0
-7.2	19	66.2	16.7	62	143.6	65.6	150	302.0
-6.7	20	68.0	17.2	63	145.4	71.1	160	320.0
-6.1	21	69.8	17.8	64	147.2	76.7	170	338.0
-5.6	22	71.6	18.3	65	149.0	82.2	180	356.0
-5.0	23	73.4	18.9	66	150.8	87.8	190	374.0
-4.4	24	75.2	19.4	67	152.6	93.3	200	392.0
-3.9	25	77.0	20.0	68	154.4	98.9	210	410.0
-3.3	26	78.8	20.6	69	156.2	100.0	212	413.6
-2.8	27	80.6	21.1	70	158.0	104.4	220	428.0
-2.2	28	82.4	21.7	71	159.8	110.0	230	446.0
-1.7	29	84.2	22.2	72	161.6	115.6	240	464.0
-1.1	30	86.0	22.8	73	163.4	121.1	250	482.0
-0.6	31	87.8	23.3	74	165.2	126.7	260	500.0
0.0	32	89.6	23.9	75	167.0	132.2	270	518.0
0.6	33	91.4	24.4	76	168.8	137.8	280	536.0
1.1	34	93.2	25.0	77	170.6	143.3	290	554.0
1.7	35	95.0	25.6	78	172.4	148.9	300	572.0

thompson chemtrex™

Chemical Resistance Guide

Corrosion Resistance Rating
A — Excellent
B — Good
C — Fair
X — Not Recommended

Maximum Satisfactory Temperature
1 — 68°F (20°C)
2 — 104°F (40°C)
3 — 140°F (60°C)
4 — 176°F (80°C)
5 — 212°F (100°C)
6 — 248°F (120°C)

CHEMICAL	CPVC	POLYPROPYLENE	PURE PVDF FLUOROPOLYMER	TEFLON®	VITON	ETHYLENE PROPYLENE	316SS	HASTELLOY C	CARBON / GRAPHITE	CERAMIC
Acetaldehyde	X	B[3]	X	A[6]	X	B[3]	A[6]	A[5]	A[6]	A[6]
Acetic Acid, 20%	A[3]	A[2]	A[3]	A[6]	B[1]	A[2]	A[6]	A[6]	A[4]	A[4]
Acetic Acid, 80%	B[1]	B[1]	B[1]	A[6]	B[1]	—	A[6]	A[6]	A[4]	A[4]
Acetic Acid, Glacial	X	C[2]	B[4]	A[6]	B[1]	X	A[6]	A[5]	A[5]	A[5]
Acetic Anhydride	X	C[2]	C[2]	A[6]	X	C[1]	A[6]	A[5]	A[6]	A[6]
Acetone	X	A[2]	X	A[6]	X	B[2]	A[6]	A[5]	A[5]	A[5]
Aluminum Chloride	A[2]	A[4]	A[2]	A[6]	A[5]	A[4]	X	A[5]	A[5]	A[5]
Aluminum Fluoride	A[4]	A[4]	A[6]	A[5]	A[5]	A[4]	X	B[4]	A[5]	—
Aluminum Hydroxide	A[4]	A[4]	A[6]	A[5]	A[4]	A[3]	A[4]	B[4]	A[6]	A[6]
Aluminum Sulfate	A[4]	A[4]	A[5]	A[5]	A[3]	A[3]	X	B[4]	A[5]	A[5]
Ammonia Liquid	A[3]	A[1]	A[5]	A[6]	C[1]	B[3]	A[5]	A[5]	A[5]	A[5]
Ammonium Bifluoride	A[4]	A[4]	A[6]	A[6]	A[3]	A[3]	A[5]	B[4]	A[6]	A[6]
Ammonium Carbonate	A[4]	A[4]	A[6]	A[6]	A[5]	A[4]	A[6]	B[4]	A[6]	A[6]
Ammonium Chloride	A[3]	A[3]	A[6]	A[6]	A[5]	A[4]	A[5]	A[5]	A[6]	A[6]
Ammonium Hydroxide	A[3]	A[4]	A[6]	A[6]	B[2]	A[3]	A[6]	B[4]	A[5]	A[5]
Ammonium Nitrate	B[3]	A[4]	A[6]	A[6]	A[4]	A[4]	A[6]	B[4]	A[5]	A[5]
Ammonium Persulfate	A[3]	A[3]	A[6]	A[6]	A[4]	A[4]	A[6]	B[4]	A[6]	A[6]
Ammonium Phosphate	A[4]	A[4]	A[6]	A[6]	A[4]	A[4]	A[6]	B[4]	A[5]	A[5]
Ammonium Sulfate	A[4]	A[4]	A[6]	A[6]	A[4]	A[4]	A[2]	B[4]	A[6]	A[6]
Amyl Acetate	X	X	A[1]	A[2]	X	B[1]	A[2]	A[2]	A[2]	A[2]
Amyl Alcohol	A[3]	A[3]	A[5]	A[6]	A[3]	A[4]	A[6]	A[5]	A[6]	A[5]
Amyl Chloride	C[2]	X	A[3]	A[6]	B[1]	X	A[6]	A[1]	A[5]	A[5]
Aniline	C[1]	B[2]	A[1]	A[6]	B[3]	B[1]	A[6]	B[1]	A[6]	A[6]
Aqua Regia	C[2]	C[2]	A[4]	A[5]	C[1]	B[2]	X	C[2]	X	X
Arsenic Acid	A[1]	A[2]	A[4]	A[6]	A[3]	A[2]	A[6]	B[4]	A[6]	A[6]
Barium Carbonate	A[4]	A[4]	A[6]	A[6]	A[6]	A[4]	A[6]	B[3]	A[6]	A[6]
Barium Chloride	A[4]	A[4]	A[5]	A[6]	A[6]	A[4]	A[6]	B[3]	A[6]	A[6]
Barium Hydroxide	A[4]	A[4]	A[6]	A[6]	A[6]	A[4]	A[6]	B[3]	A[6]	A[6]
Barium Sulfate	A[4]	A[4]	A[6]	A[6]	A[6]	A[4]	A[6]	A[5]	A[6]	B[4]
Barium Sulfide	A[4]	A[4]	A[6]	A[6]	A[6]	A[3]	A[6]	A[6]	A[6]	A[6]
Beer	A[4]	A[4]	A[6]	A[6]	A[4]	A[4]	A[6]	A[1]	A[6]	A[6]
Benzaldehyde	X	A[1]	A[3]	A[2]	X	A[1]	A[3]	A[3]	A[3]	A[3]
Benzene (Benzol)	C[1]	B[1]	A[1]	A[6]	B[4]	X	A[6]	B[3]	A[6]	A[6]
Bensoic Acid	X	X	X	A[5]	A[3]	—	A[5]	A[6]	—	—
Benzoic Acid	A[2]	A[1]	A[5]	A[5]	A[4]	A[3]	A[5]	B[1]	A[3]	B[2]
Borax (Sodium Borate)	A[4]	A[4]	A[6]	A[6]	A[4]	A[3]	A[6]	B[4]	A[6]	A[6]
Boric Acid	A[3]	A[4]	A[6]	A[6]	A[4]	A[3]	C[4]	A[6]	A[4]	B[3]
Brine	A[4]	A[4]	A[6]	A[6]	A[4]	A[3]	—	—	—	—
Bromine Water	C[1]	C[1]	A[3]	A[3]	A[2]	X	C[4]	A[6]	—	—
Butyl Acetate	C[1]	C[1]	A[1]	A[3]	X	B[1]	C[4]	A[3]	A[3]	A[3]

CHEMICAL	CPVC	POLYPROPYLENE	PURE PVDF FLUOROPOLYMER	TEFLON®	VITON	ETHYLENE PROPYLENE	316SS	HASTELLOY C	CARBON / GRAPHITE	CERAMIC
Butyric Acid	X	A[4]	A[6]	A[6]	B[1]	B[1]	A[6]	A[1]	A[6]	—
Calcium Bisulfide	A[2]	A[3]	A[5]	A[3]	A[3]	X	A[5]	A[6]	—	A[3]
Calcium Carbonate	A[4]	A[6]	A[6]	A[6]	A[6]	A[3]	A[6]	B[4]	A[6]	A[6]
Calcium Chloride	A[4]	A[4]	A[6]	A[6]	A[3]	A[4]	A[6]	A[6]	A[6]	A[6]
Calcium Hydroxide	A[3]	A[4]	A[6]	A[3]	A[3]	A[4]	A[6]	A[6]	A[6]	A[6]
Calcium Hypochlorite	A[2]	A[2]	A[4]	A[3]	A[3]	B[2]	C[4]	B[2]	A[4]	A[4]
Calcium Sulfate	A[4]	A[4]	A[5]	A[3]	A[3]	A[4]	B[4]	B[4]	A[3]	A[3]
Carbon Bisulfide	A[3]	A[4]	A[4]	A[3]	A[3]	A[4]	A[3]	A[6]	—	A[3]
Carbon Tetrachloride	C[1]	X	A[3]	A[6]	B[1]	X	B[5]	A[1]	A[6]	A[6]
Carbonic Acid	A[2]	A[3]	A[6]	A[6]	A[4]	A[4]	A[6]	A[1]	A[6]	A[6]
Chloracetic Acid	C[3]	C[1]	A[1]	A[4]	X	B[3]	C[3]	A[4]	A[3]	—
Chlorine Water	A[2]	C[1]	A[6]	A[6]	C[1]	B[1]	X	A[4]	A[5]	—
Chlorobenzene	X	B[1]	A[2]	A[3]	A[1]	X	A[3]	B[3]	A[3]	A[3]
Chloroform	X	B[1]	A[2]	A[3]	B[1]	X	A[3]	A[1]	A[3]	A[3]
Chlorosulfonic Acid	X	X	C[1]	A[4]	X	X	C[3]	A[1]	A[4]	C[3]
Chromic Acid, 10%	A[3]	X	A[6]	A[6]	A[1]	B[1]	A[6]	B[2]	A[6]	C[3]
Chromic Acid, 50%	C[1]	X	X	A[2]	A[3]	X	B[3]	B[1]	A[1]	X
Citric Acid	A[2]	A[2]	A[3]	A[3]	A[3]	A[4]	A[3]	A[4]	A[3]	A[3]
Copper Chloride	A[4]	A[4]	A[6]	A[6]	A[3]	A[4]	X	—	—	A[6]
Copper Cyanide	A[1]	A[3]	A[4]	A[3]	A[3]	A[3]	A[3]	B[2]	A[3]	A[3]
Copper Nitrate	A[2]	A[3]	A[3]	A[3]	A[3]	A[4]	A[3]	A[1]	A[3]	A[3]
Copper Sulfate	A[4]	A[4]	A[3]	A[3]	A[3]	A[4]	A[3]	A[4]	A[3]	A[3]
Cresylic Acid	A[3]	X	B[1]	A[4]	A[4]	X	A[4]	B[1]	A[3]	A[3]
Ethyl Acetate	X	B[2]	A[1]	A[4]	X	B[1]	A[4]	A[4]	A[4]	A[4]
Ethyl Chloride	X	C[1]	A[3]	A[4]	B[3]	A[3]	A[4]	B[3]	A[4]	A[4]
Ethylene Dichloride	X	C[1]	A[4]	A[6]	A[1]	X	A[6]	B[3]	A[6]	A[6]
Ethylene Glycol	A[3]	A[3]	A[3]	A[6]	A[3]	A[4]	A[6]	B[1]	A[3]	A[3]
Fatty Acids	B[3]	A[1]	A[6]	A[6]	A[1]	X	A[6]	A[6]	A[6]	A[6]
Ferric Chloride	A[3]	B[1]	A[3]	A[4]	A[3]	A[3]	X	B[2]	A[3]	A[3]
Ferric Nitrate	A[4]	A[3]	A[6]	A[6]	A[6]	A[4]	A[6]	B[1]	A[3]	A[3]
Ferric Sulfate	A[3]	B[3]	A[3]	A[4]	A[3]	A[3]	C[3]	A[1]	A[4]	A[4]
Ferrous Chloride	A[4]	A[4]	A[6]	A[6]	A[4]	A[4]	X	B[1]	A[4]	A[4]
Ferrous Sulfate	A[4]	A[4]	A[6]	A[6]	A[4]	A[4]	A[6]	B[3]	A[4]	A[4]
Fluoboric Acid	A[3]	A[3]	A[6]	A[6]	A[4]	A[3]	C[3]	A[1]	A[3]	X
Fluosilicic Acid	A[3]	A[3]	A[6]	A[6]	A[5]	A[3]	A[6]	B[3]	A[5]	X
Formaldehyde	A[4]	A[4]	A[2]	A[6]	A[4]	A[4]	A[6]	A[6]	—	A[6]
Formic Acid	A[1]	A[1]	A[4]	A[6]	X	A[4]	B[5]	A[5]	A[3]	A[5]
Freon 12 (wet)	A[3]	X	A[6]	A[6]	B[1]	B[1]	X	A[3]	—	A[4]
Fruit Juice	A[4]	A[4]	A[6]	A[6]	A[6]	—	A[6]	A[6]	—	A[6]
Fuel Oils	—	C[1]	B[3]	A[4]	A[4]	X	A[4]	A[1]	A[4]	A[4]

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Furfural	X	C[1]	A[1]	A[4]	B[2]	A[3]	A[4]	B[3]	A[4]	A[4]
Gasoline	C[1]	X	A[4]	A[4]	B[2]	X	A[4]	A[4]	A[4]	A[4]
Glycerine (Glycerol)	A[3]	A[4]	A[4]	A[4]	A[4]	A[4]	A[4]	A[4]	A[4]	A[4]
Heptane	A[3]	B[2]	A[4]	A[4]	A[3]	X	A[4]	A[4]	B[3]	A[4]
Hexane	B[1]	B[2]	A[4]	A[4]	A[1]	X	A[4]	A[4]	A[4]	A[4]
Hydrobromic Acid, 20%	A[4]	A[4]	A[4]	A[4]	A[3]	A[3]	X	A[1]	A[4]	A[4]
Hydrochloric Acid, 0-25%	A[4]	A[4]	A[4]	A[4]	A[3]	A[3]	X	B[2]	A[4]	A[4]
Hydrochloric Acid, 25-40%	A[3]	A[4]	A[4]	A[4]	B[2]	B[2]	X	C[3]	A[4]	A[4]
Hydrocyanic Acid	A[3]	A[3]	A[4]	A[4]	B[1]	B[1]	A[4]	—	A[4]	A[4]
Hydrofluoric Acid, 10%	A[1]	A[1]	A[4]	A[4]	A[3]	A[3]	X	A[3]	A[3]	C[2]
Hydrofluoric Acid, 30%	A[1]	A[1]	A[4]	A[4]	A[4]	A[3]	X	A[1]	A[3]	X
Hydrofluoric Acid, 60%	B[1]	A[1]	A[3]	A[4]	A[4]	A[2]	X	A[1]	A[2]	X
Hydrofluorsilicic Acid	—	A[3]	A[1]	A[4]	A[4]	A[4]	X	B[2]	—	X
Hydrogen Peroxide, 50%	X	C[1]	A[3]	A[4]	A[1]	C[1]	C[4]	A[3]	C[3]	—
Hydrogen Peroxide, 30%	X	C[1]	A[4]	A[4]	A[2]	B[1]	C[4]	A[3]	C[3]	—
Hydrogen Peroxide, 90%	A[4]	A[4]	A[1]	A[4]	A[1]	X	C[4]	C[3]	C[3]	—
Hydrogen Sulfide (aq. sol.)	A[4]	A[3]	A[4]	A[4]	A[4]	A[3]	C[4]	A[1]	A[4]	A[4]
Iodine (in alcohol)	B[2]	A[4]	A[4]	A[4]	B[1]	X	X	X	—	—
Kerosene	A[3]	A[1]	A[4]	A[4]	A[1]	X	A[4]	A[4]	A[4]	A[4]
Ketones	—	C[3]	C[1]	A[4]	X	X	A[4]	A[4]	A[4]	C[3]
Lacquer Thinners	—	C[3]	—	A[3]	X	X	A[3]	C[3]	A[4]	—
Lactic Acid	A[2]	A[3]	A[4]	A[4]	A[3]	A[3]	X	B[1]	A[4]	A[4]
Lead Acetate	A[4]	A[4]	A[4]	A[4]	A[2]	A[4]	B[3]	B[1]	A[4]	A[4]
Liquors	—	—	—	—	—	—	A[4]	A[4]	—	—
Lubricants	—	A[1]	A[3]	A[4]	A[4]	A[4]	A[4]	A[4]	A[4]	A[4]
Magnesium Chloride	A[4]	A[4]	A[4]	A[4]	A[4]	A[4]	B[4]	A[2]	A[4]	—
Magnesium Hydroxide	A[4]	A[4]	A[4]	A[4]	A[3]	A[4]	A[4]	B[1]	A[4]	A[4]
Magnesium Nitrate	A[3]	A[3]	A[4]	A[4]	A[3]	A[3]	A[4]	A[3]	—	—
Magnesium Sulfate	A[4]	A[4]	A[4]	A[4]	A[3]	A[4]	B[4]	A[2]	A[4]	A[4]
Maleic Acid	A[4]	A[4]	A[4]	A[4]	A[2]	A[1]	A[4]	B[3]	A[4]	A[4]
Mercuric Chloride	A[3]	A[4]	A[4]	A[4]	A[3]	A[3]	X	C[1]	X	B[2]
Mercuric Cyanide	A[4]	A[4]	A[4]	A[4]	A[1]	A[1]	A[4]	—	A[4]	A[4]
Mercury	A[4]	A[4]	A[4]	A[4]	A[1]	A[1]	A[4]	A[4]	C[2]	A[4]
Methyl Alcohol	A[1]	A[3]	A[4]	A[4]	B[2]	A[3]	A[4]	A[4]	A[4]	A[4]
Methyl Chloride	X	B[1]	A[4]	A[3]	X	B[1]	A[4]	A[3]	A[4]	A[4]
Methyl Ethyl Ketone	X	B[1]	X	A[4]	X	B[1]	A[4]	A[4]	A[4]	A[4]
Methyl Isobutyl Ketone	X	B[1]	A[1]	A[3]	X	B[1]	A[3]	A[3]	A[4]	A[4]
Methylene Chloride	X	X	B[1]	A[3]	C[1]	X	A[4]	B[3]	A[4]	A[4]
Naphtha	A[3]	A[1]	A[3]	A[3]	A[1]	X	A[3]	B[3]	A[4]	A[4]
Naphthalene	X	B[1]	A[4]	A[3]	A[1]	X	A[3]	A[3]	A[3]	A[3]
Nickel Chloride	A[4]	A[4]	A[4]	A[4]	A[3]	A[4]	C[3]	B[3]	A[3]	A[3]
Nickel Sulfate	A[3]	A[3]	A[4]	A[4]	A[4]	A[4]	B[3]	B[2]	A[4]	A[4]
Nitric Acid, 10%	A[4]	A[3]	A[4]	A[3]	A[3]	A[2]	A[3]	A[1]	A[3]	C[2]
Nitric Acid, 30%	A[2]	A[2]	A[3]	A[4]	A[4]	A[2]	A[3]	A[1]	A[4]	X
Nitric Acid, 50%	A[1]	A[1]	A[3]	A[3]	A[3]	X	B[3]	A[1]	X	X
Nitric Acid (anhydrous)	X	C[3]	A[3]	A[3]	A[3]	X	X	B[1]	X	X
Nitrobenzene	X	B[1]	A[1]	A[4]	A[1]	B[1]	A[4]	X	B[3]	A[4]
Oils and Fats	B[3]	A[1]	A[4]	A[4]	A[1]	X	A[4]	A[4]	A[4]	A[4]
Oleic Acid	C[3]	C[3]	A[3]	A[4]	B[3]	B[1]	A[4]	A[2]	A[4]	A[4]
Oleum	X	X	C[1]	A[4]	A[4]	X	A[4]	B[3]	X	—

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Oxalic Acid	A[1]	A[2]	A[3]	A[1]	A[3]	A[3]	A[1]	B[3]	A[1]	A[1]
Phenol	A[1]	A[2]	A[2]	A[2]	A[4]	B[1]	A[4]	A[4]	A[4]	A[4]
Phosphoric Acid, 50-100%	A[2]	A[1]	A[4]	A[4]	A[4]	A[4]	B[3]	A[2]	A[4]	B[4]
Phosphoric Acid, 0-50%	A[3]	A[4]	A[4]	A[4]	A[4]	A[4]	B[4]	A[2]	A[4]	B[4]
Pickling Solutions	A[3]	A[4]	A[4]	A[4]	—	—	—	—	—	—
Potassium Bicarbonate	A[4]	A[4]	A[4]	A[4]	A[3]	A[4]	A[4]	B[3]	A[3]	A[3]
Potassium Bromide	A[4]	A[4]	A[3]	A[4]	A[3]	A[4]	A[4]	B[3]	A[4]	A[4]
Potassium Carbonate	A[4]	A[4]	A[4]	A[4]	A[3]	A[4]	A[4]	B[3]	A[4]	A[4]
Potassium Chlorate	A[3]	A[3]	A[4]	A[4]	A[3]	A[2]	A[4]	C[2]	C[3]	A[4]
Potassium Chloride	A[4]	A[4]	A[4]	A[4]	A[3]	A[4]	A[4]	B[3]	A[4]	A[4]
Potassium Cyanide	A[3]	A[3]	A[4]	A[4]	A[1]	A[1]	A[4]	B[2]	A[4]	A[4]
Potassium Dichromate	A[3]	A[3]	A[4]	A[4]	A[3]	A[4]	A[4]	B[3]	A[3]	A[3]
Potassium Hydroxide	A[3]	A[4]	A[2]	A[4]	X	A[3]	B[4]	B[1]	C[2]	X
Potassium Nitrate	A[3]	A[3]	A[4]	A[4]	A[3]	A[4]	A[4]	B[1]	A[3]	A[3]
Potassium Permanganate	A[1]	A[2]	A[3]	A[3]	A[2]	A[3]	B[4]	A[1]	A[3]	A[3]
Potassium Sulfate	A[4]	A[4]	A[4]	A[4]	A[3]	A[3]	B[4]	B[1]	A[4]	A[4]
Propyl Alcohol	A[3]	A[3]	A[4]	A[4]	A[3]	A[4]	A[4]	A[4]	A[4]	A[4]
Soaps	A[4]	A[4]	A[4]	A[4]	A[3]	A[4]	A[4]	A[4]	A[4]	A[4]
Sodium Acetate	A[4]	A[4]	A[4]	A[4]	B[1]	A[4]	A[4]	A[4]	A[4]	A[4]
Sodium Bicarbonate	A[4]	A[4]	A[4]	A[4]	A[3]	A[4]	A[4]	B[1]	A[4]	A[4]
Sodium Bisulfate	A[3]	A[3]	A[4]	A[4]	A[3]	A[4]	A[4]	B[2]	A[4]	A[4]
Sodium Bisulfite	A[3]	A[3]	A[4]	A[4]	A[3]	A[4]	A[4]	B[3]	A[4]	A[4]
Sodium Carbonate	A[4]	A[4]	A[4]	A[4]	A[3]	A[4]	B[4]	A[4]	A[4]	A[4]
Sodium Chlorate	A[3]	A[3]	A[4]	A[4]	A[3]	A[3]	A[4]	B[1]	C[3]	A[4]
Sodium Chloride	A[4]	A[4]	A[4]	A[4]	A[4]	A[4]	A[4]	A[4]	A[4]	A[4]
Sodium Cyanide	A[3]	A[3]	A[4]	A[4]	A[4]	A[4]	A[4]	A[4]	A[4]	A[4]
Sodium Hydroxide, 20%	A[3]	A[3]	A[2]	A[4]	C[2]	A[3]	B[3]	B[3]	A[2]	X
Sodium Hydroxide, 50%	A[3]	A[3]	A[1]	A[4]	X	A[4]	X	B[3]	A[2]	X
Sodium Nitrate	A[4]	A[4]	A[4]	A[4]	A[3]	A[4]	A[4]	B[3]	C[3]	A[3]
Sodium Silicate	A[2]	A[3]	A[4]	A[4]	A[4]	A[4]	A[4]	B[3]	A[4]	A[4]
Sodium Sulfate	A[4]	A[4]	A[4]	A[4]	A[3]	A[4]	A[4]	B[3]	A[4]	A[4]
Sodium Sulfide	A[4]	A[4]	A[4]	A[4]	A[4]	A[4]	A[4]	B[1]	A[4]	A[4]
Stannic Chloride	A[3]	A[3]	A[4]	A[4]	A[4]	A[3]	X	B[3]	A[4]	A[4]
Stearic Acid	A[3]	B[3]	A[4]	A[4]	A[2]	X	A[4]	B[3]	A[4]	A[4]
Stoddard's Sulfuric	C[1]	C[2]	A[4]	A[4]	A[3]	X	A[4]	A[4]	A[1]	A[1]
Sulfuric Solvent	—	—	—	—	—	—	C[3]	B[2]	—	—
Sulfuric Acid, 0-10%	A[4]	A[4]	A[4]	A[4]	A[4]	A[4]	X	B[1]	A[1]	A[3]
Sulfuric Acid, 10-75%	A[4]	A[3]	A[3]	A[4]	A[4]	A[2]	X	B[1]	A[1]	X
Sulfuric Acid, 75-100%	A[2]	A[2]	A[4]	A[4]	A[4]	A[1]	X	X	C[1]	X
Tannic Acid	A[1]	A[3]	A[4]	A[4]	A[3]	B[1]	C[2]	B[1]	A[4]	A[4]
Tanning Liquors	A[1]	A[1]	A[1]	A[4]	A[3]	B[1]	A[4]	B[3]	A[4]	A[4]
Tartaric Acid	A[2]	A[2]	A[4]	A[4]	A[1]	A[4]	B[4]	B[3]	A[4]	A[4]
Tetrahydrofurane	X	B[1]	B[1]	A[3]	X	X	A[3]	A[3]	A[4]	A[4]
Toluene (Toluol)	X	B[1]	A[2]	A[4]	B[1]	X	A[4]	A[4]	A[4]	A[4]
Trichloroethylene	X	B[1]	A[3]	A[4]	A[1]	X	A[4]	A[4]	A[4]	A[4]
Tricresylphosphate	X	A[1]	X	A[4]	B[2]	A[3]	A[4]	A[4]	A[4]	A[4]
Turpentine	A[4]	B[1]	A[4]	A[4]	A[1]	B[1]	A[4]	B[3]	A[4]	A[4]
Urea	A[4]	A[4]	A[4]	A[4]	A[1]	A[1]	A[4]	A[4]	—	—
Vinegar	A[3]	A[3]	A[3]	A[4]	A[3]	A[3]	A[4]	A[4]	A[4]	A[4]
White Liquor	A[2]	A[2]	A[3]	A[4]	A[2]	A[4]	A[4]	A[4]	A[4]	A[4]
Xylene (Xylol)	X	X	A[4]	A[4]	B[1]	X	A[4]	A[1]	A[3]	A[4]
Zinc Chloride	A[4]	A[4]	A[4]	A[4]	A[3]	A[4]	B[4]	X	A[3]	A[3]
Zinc Sulfate	A[4]	A[4]	A[4]	A[4]	A[3]	A[4]	B[3]	X	A[3]	A[3]

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