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A Punched Tape Controlled Peptide Synthesizer

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A technical description of a full-automatic system for solid-phase peptide synthesis is presented. The information to the synthesizer is given *via* punched tape. The use of liquid valves is almost avoided by using gas pressure for transferring reagents and solvents. The system allows an easy exchange of programs between different laboratories.

With the introduction of the solid-phase method of peptide synthesis by Merrifield [1,2] automation of the peptide synthesis procedure was made possible. As the increasing peptide chain is made insoluble in the applied solvents by covalent binding to a styrene-divinylbenzene copolymer, all separations during the synthesis can be carried out by filtration. Merrifield *et al.*[3,5] and Merrifield [4] have published data on an automatic system based on the employment of liquid selector valves controlled by a stepping drum programmer. The system presented here is controlled by punched tape, and avoidance of liquid valves has been made possible by the employment of gas pressure for pipetting. A preliminary account has been given.[6]

GENERAL DESCRIPTION OF THE SYSTEM

An example of a cycle with dicyclohexylcarbodiimide (DCC) coupling, resulting in the elongation of the peptide chain by one amino acid residue, is presented in Table 1. The cycle comprises a total of 26 treatments. Each treatment in the automation described here involves the following operations: a) measuring-off, b) transfer to the reactor, c) reaction or washing, d) emptying of the reactor.

The reactor is not emptied between the sixteenth and seventeenth treatment resulting in a total number of 103 operations in each cycle. A schematic drawing of the synthesizer is given in Fig. 1.

The necessary information is transferred from the punched tape to the control unit by means of a tape reader, and the read code is displayed on the control unit. The tape is punched on an ordinary 8-channel Teletype printer as it is possible to assign the various letter and digit symbols to different opera-

Table 1. One cycle in the solid-phase synthesis with DCC-coupling.

Treatments	Reagents and solvents	ml	Duration (min)
1	N HCl in AcOH	50	30
2–4	3×wash with AcOH	3×50	3×5
5–7	3×wash with EtOH	3×50	3×5
8–10	3×wash with CH_2Cl_2	3×50	3×5
11	triethylamine in CH_2Cl_2	50	10
12–15	4×wash with CH_2Cl_2	4×50	4×5
16	t-BOC-amino acid in CH_2Cl_2	30	10
17	Dicyclohexylcarbodiimide in CH_2Cl_2	30	120
18–20	3×wash with CH_2Cl_2	3×50	3×5
21–23	3×wash with EtOH	3×50	3×5
24–26	3×wash with AcOH	3×50	3×5

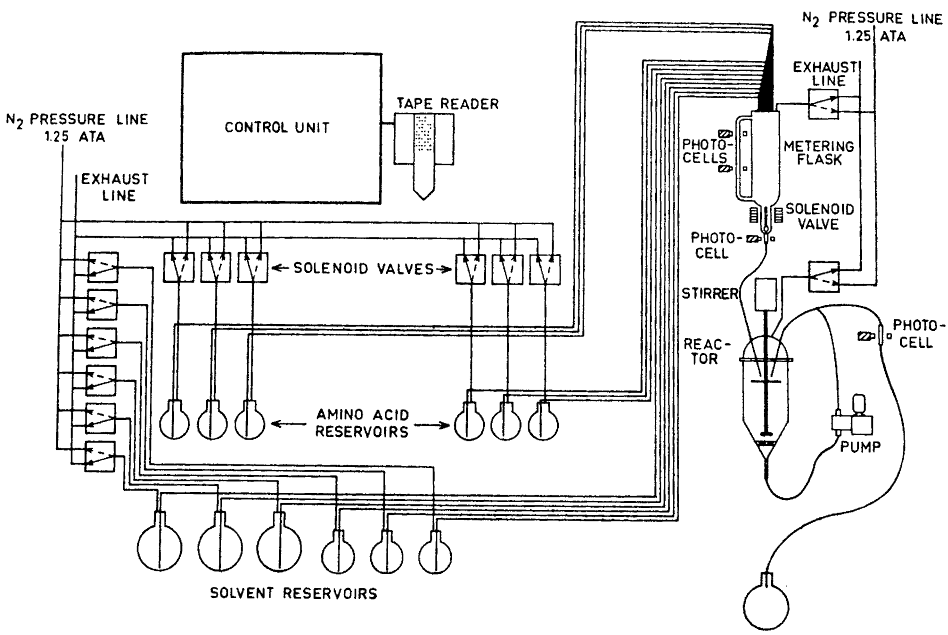


Fig. 1. Schematic drawing of the solid-phase peptide synthesizer. For the sake of clarity only a few of the reservoirs are shown in the figure. All reservoirs are connected to the metering flask by separate tubings. In the figure, however, it has not been possible to demonstrate this at the inlet of the metering flask due to fusion of the lines.

tions (Table 2). On coding for a specific peptide the punched tapes for the individual amino acids are copied on one long tape in the sequence desired.

The various operation units are energized by the control unit. When a function has been properly executed, the tape reader is signalled to advance one step.

Table 2. "Code" represents the symbols on the keyboard of the Teletype printer and "Indication" the read-out of the control unit display tubes.

Code	Indication	Amino acid
A	0.1	Alanine
B	0.2	Arginine
C	0.3	Asparagine
D	0.4	Aspartic acid
E	0.5	Cysteine
F	0.6	Glutamine
G	0.7	Glutamic acid
H	1.0	Glycine
I	1.1	Histidine
J	1.2	Isoleucine
K	1.3	Leucine
L	1.4	Lysine
M	1.5	Phenylalanine
N	1.6	Proline
O	1.7	Serine
P	2.0	Threonine
Q	2.1	Tyrosine
R	2.2	Valine
Y	3.1	Methionine
Z	3.2	Tryptophan

Code	Indication	Solvent/reagent
S	2.3	Methylene chloride
T	2.4	Ethanol
U	2.5	Acetic acid
V	2.6	Triethylamine in methylene chloride
W	2.7	DCC in methylene chloride
X	3.0	N HCl in acetic acid

Code	Indication	Function
1	6.1	Draining metering flask
2	6.2	Draining reactor
3	6.3	Stirring 5 min
4	6.4	Stirring 10 min
5	6.5	Stirring 30 min
6	6.6	Stirring 1 h
7	6.7	Stirring 2 h

Application of nitrogen pressure to the desired reservoir through a three-way valve serves to transfer the amino acid derivatives, reagents, and solvents. The liquid is thus through separate tubings forced up into the metering flask where measuring takes place by a photo-cell positioned according to the volume desired. When the proper quantity of liquid has been measured, the three-way valve is changed over to exhaust at the same time as the pressure line is closed. As the metering flask is located at a higher level than the reservoir, the liquid remaining in the tubing flows back to the reservoir. As all tubings except the one in use are empty contamination is avoided.

The liquid is transferred to the reactor on activation of a glass solenoid valve. The emptying is supervised by a photo-cell. On complete emptying the reactor agitation is started. After the preset time the mixture is filtered through a sintered glass disk by applying a pressure. The spent liquids are collected in a waste flask, while the polymer with the attached peptide remains in the reactor. The emptying is also here supervised by a photo-cell.

In case of malfunction of an operation an alarm system described below is activated, and the system is paralyzed with the display tubes indicating the faulty operation.

The system has been tested by the synthesis of bradykinin, angiotensin I and II, and kallidin.

As a number of 64 codes exceeds what usually is necessary for the synthetic procedure, the additional codes may be used for temperature control of the reactor and for an arrangement for collecting the spent solvents.

The use of punched tape allows an easy exchange of programs between different laboratories.

EXPERIMENTAL

Reservoirs. The control unit allows the use of 46 reservoirs. For amino acid derivatives 500 ml round bottom flasks are used, and for reagents and solvents flasks with a volume of 1 and 5 l, respectively, are used. The flasks are all provided with one *B* 29 ground joint and a corresponding *B* 29 dome with inlet tube for the nitrogen pressure (1/4″ O.D.) and liquid outlet tube reaching the bottom of the flask. The outlet tubes are provided with a tube nipple (approximately 4 mm O.D.).

Metering flask. The metering flask is made from a 30 mm O.D. glass cylinder, length 400 mm, volume approximately 120 ml. The cylinder is provided with a side arm (6 mm O.D. glass tube) upon which the liquid detection is carried out. The outlet from the metering flask consists of a glass solenoid valve (Scaniameter A/S, Copenhagen, Denmark).

Reaction system. Two types of reaction systems have been developed. One with agitation effected by stirring (A) and one by shaking (B). The reaction system (A) consists of a glass reaction vessel shown on Fig. 2, a circulation pump and a stirring

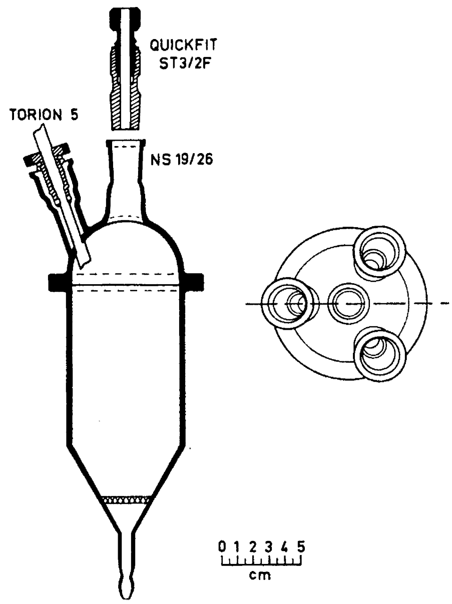


Fig. 2. Reactor. In the sectional drawing only one of the three Torions is shown.

device. The reactor body is a 500 ml filter jar (porosity 1) with a ground flange joint at the top. The bottom outlet is connected to a teflon membrane pump (Seybert Rahier model R 408). The function of the pump is to establish a continuous washing of the reactor wall during the reactions. The cover for the reactor body provided with a ground flange joint contains 3 Torion 5 joints (Sovirel France) as lead-in for the 5 mm O.D. teflon tubes from the metering flask, the circulation pump and the nitrogen pressure valve. In the centre of the cover a *B* 19 ground joint and a corresponding Quickfit ST 3/2F stirring head guides the propeller stirrer with which the agitation of the reaction mixture is performed. Above the highest liquid level in the reactor a teflon disk of 50 mm diameter is attached to the stirrer. The purpose of the disk is to eject the circulating liquid on the reactor wall. (B). Reactor agitation can also be effected by a shaking device giving approximately 400 strokes per minute with an amplitude continuously variable from 0 to 50 mm. The reactor consists of a 300 ml cylindrical filter jar (porosity 1) provided with an outlet below the filter and a flange joint at the top. The corresponding cover contains inlet tubes for liquid and nitrogen provided with plash heads.

Waste flask. As waste flask serves a 20 l round bottom flask provided with a teflon stopcock (4 mm bore) at the bottom and a *B* 29 ground joint at the top. The corresponding dome contains an inlet tube with tube nipple for connection with the teflon tube from the reactor outlet, and an outlet tube (1/4″ O.D.), which can be connected to a vacuum system or to exhaust.

Acid traps. Acid traps filled with sodium hydroxide pellets are used in order to protect the solenoid valves from HCl vapours. They are placed in the pressure line for the reservoir containing HCl in acetic acid, in the reactor flush line, and in the vacuum line. The traps consist of 500 ml Erlenmeyer flasks with *B* 29 ground joint and corresponding dome with in- and outlet of 1/4″ O.D. glass tubing.

Nitrogen pressure system. The solenoid valves for the nitrogen pressure system are stainless steel three-way solenoid valves (Lucifer Ltd., Geneva, Switzerland, model 131A-53 with 24 volt D.C. minicoils for AMP plugs). The valves are mounted on a panel with common pressure and exhaust manifolds. The manifolds are built up of 1/4″ O.D. nylon tubing and nylon fittings (Imperial Eastman Corporation, Chicago, U.S.A., models 264-N, 265-N, 269-N, 270-N and 272-N). The valves are connected to the appropriate reservoirs with 1/4″ O.D. nylon tubing and nylon fittings model 268-N and 262-N, the latter being a 1/4″ – 1/4″ union which establishes the connection between the nylon tube and the 1/4″ glass tube on the flask dome. Between the acid trap and the reservoir containing HCl in acetic acid PVC tubing is used instead of nylon tubing.

Liquid tubing. Each reservoir is connected to the top of the metering flask with a 2 mm I.D. teflon tube. The tube is expanded somewhat and pressed upon the nipple of the reservoir and then locked with a small piece of PVC tubing. The free ends of the tubes are assembled in a bunch in the top of the metering flask. The connections between the metering flask, pump and waste flask, and the reactor are made of 4 mm I.D. teflon tubing and locked with 5 mm I.D. PVC tubing.

Frame. The system has been built up on a rack 70 cm broad × 200 cm high. The reservoirs and the valve panel are placed on one side of the rack, and the metering flask, the reaction system and the waste flask on the other side.

Control unit. The control unit, Figs. 3 and 4, consists of two main parts: a functional part and a control part. The functional part includes a tape reader, code converter, 64-code panel, solenoid drivers, timer for stirring, and a relay driver for the stirrer and pump motors.

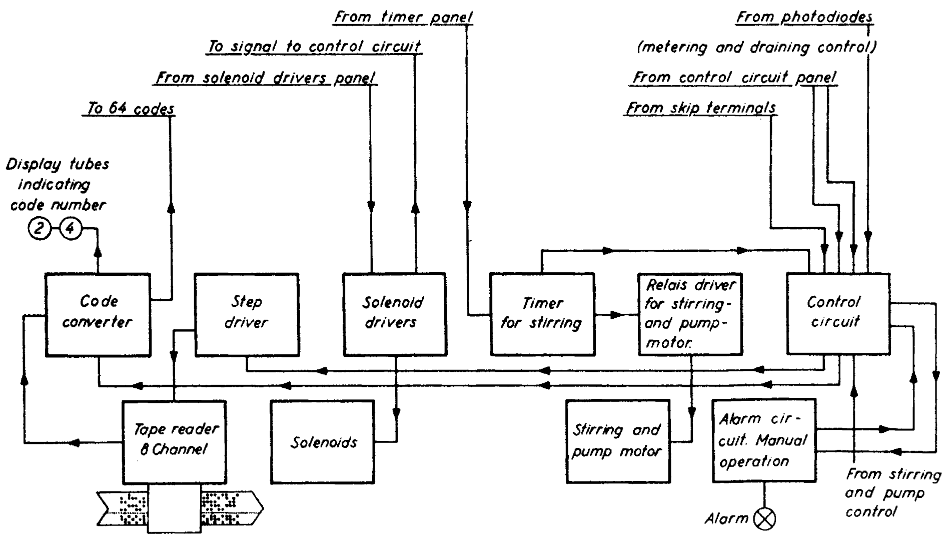


Fig. 3. Block diagram of the control unit.

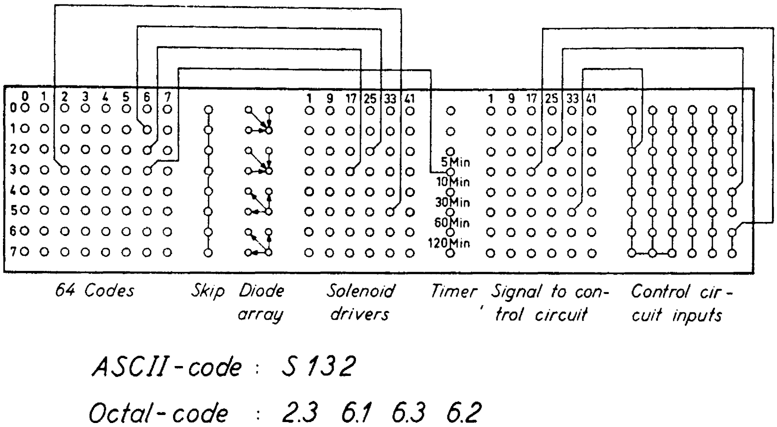


Fig. 4. Crossconnecting panel showing the connections mentioned in the text.

The control part includes "signal to control circuit" panel, "control circuit input" panel, control circuit, alarm circuit, and also the manual operation circuits.

The tape reader (Great Northern Telegraph Works model 24) is capable of reading 5- to 8-unit punched tape or edge-punched cards of up to 5 inches' width.

The code converter changes the used ASCII code (The American Standard Code for Information Interchange) to an octal code with two digits resulting in 64 different combinations. Furthermore, the code converter is so constructed that unwanted code combinations, *e.g.* "car return" and "line feed", do not reach the 64-code panel, but will be skipped. The output of the code converter is shown on two display tubes for indication of the last read code. The 64-code panel contains the output terminals of the code converter and from here the signals are coupled to the desired function by means of plug-in wires.

To locate the terminals on the 64-code panel the letter S may be taken as an example. It will be read by the tape reader as 110 01011 and converted to 2.3 in the octal code. The terminal is seen on Fig. 4 as the one connected to solenoid driver 38.

The skip terminals, causing the tape reader to step, are to be used if the punched tape contains undesired codes from the 64-code panel.

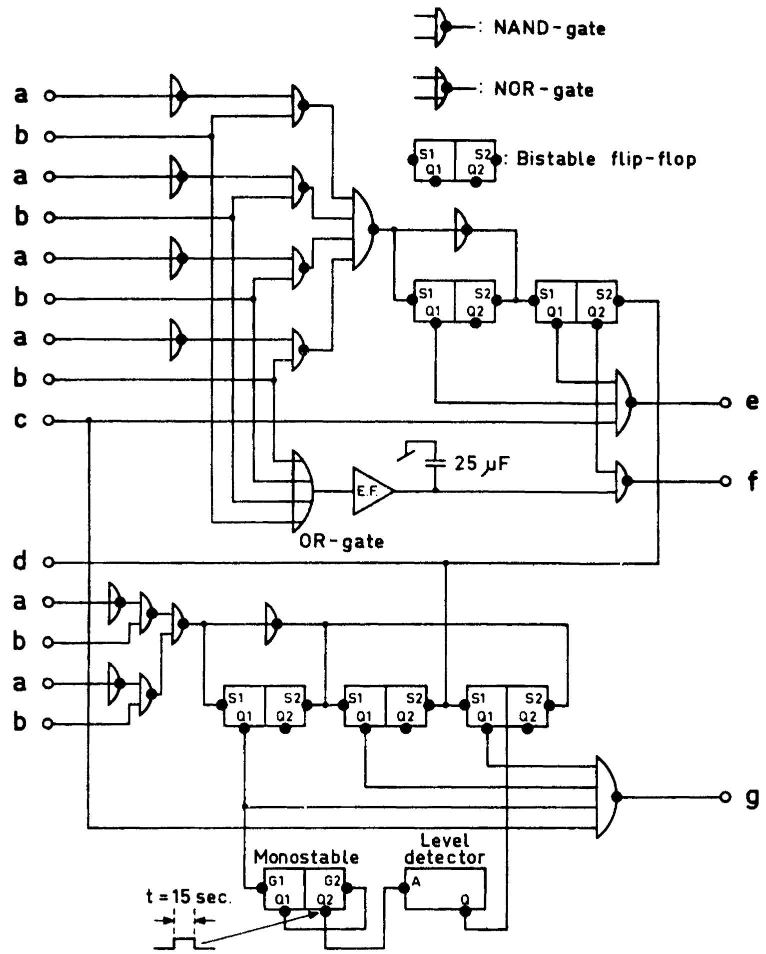
The diode array includes two types (Fig. 4). The upper two connect more codes to the same function as the signal follows the direction of the arrows. The lower two are used when a code must be connected to several functions as the signal again follows the direction of the arrows.

The "solenoid drivers" panel contains input terminals for each solenoid driver, and the solenoid can be chosen at will. The "solenoid drivers" unit consists of 48 power amplifiers. Each amplifier drives a solenoid valve and at the same time the signal passes to the "signal to control unit" panel *via* a diode arrangement, so that 1 on the solenoid panel corresponds to 1 on the "signal to control unit" panel *etc.*

Stirring times are obtained by precoding the crossconnection panel by connecting the time panel to the 64-code panel.

In the equipment used in our laboratory stirring times of 5, 10, 30, 60, and 120 min are available. If a multiple or a combination of these times is wanted, this is brought about by punching, repeating or combining the proper codes on the tape.

The "timer for stirring" in system A consists of an oscillator and a frequency divider. The frequency is divided until the period time has reached the desired value. In the system B with reactor agitation by shaking the reaction time is determined by a preset number of strokes counted by a photocell system. Control of the timing and of the shaking device is achieved by comparing the number of strokes with the number of pulses from



a: From control ciruit input panel
b: From photodiodes
c: Blocking signal from alarm circuit
d: Reset signal from alarm circuit
e: Step signal
f: Alarm signal
g: Step signal

Fig. 5. Electrical diagram of control circuit.

the oscillator, which in the previous mentioned arrangement is directly used for timing of the stirring.

The "control circuit input" panel consists of the input terminals to the control circuit connected in groups with 24, 8, 8, and 4 terminals, respectively, to determine the liquid level in the metering flask, and 4 terminals connected together two and two to control the draining of the metering flask and reactor.

The control circuit is so constructed that a double control is achieved of filling and draining the metering flask as well as draining the reactor. Thus not only a signal from the "control circuit input", which is precoded, but also a signal from the photodiodes is necessary to prevent alarm. The same conditions are valid for stirring and pumping, as a signal is needed from the timer for stirring as well as from the stirrer and pump control. The draining signal has a delay of about 15 sec to allow for the last drops, a delay which has proved sufficient with the solvents used. Furthermore, the timer unit is connected to the control circuit in such a manner that a function cannot last for more than 5 min. If this time is exceeded, except for the stirring times, the control unit alarm is activated, for example if a reservoir is empty.

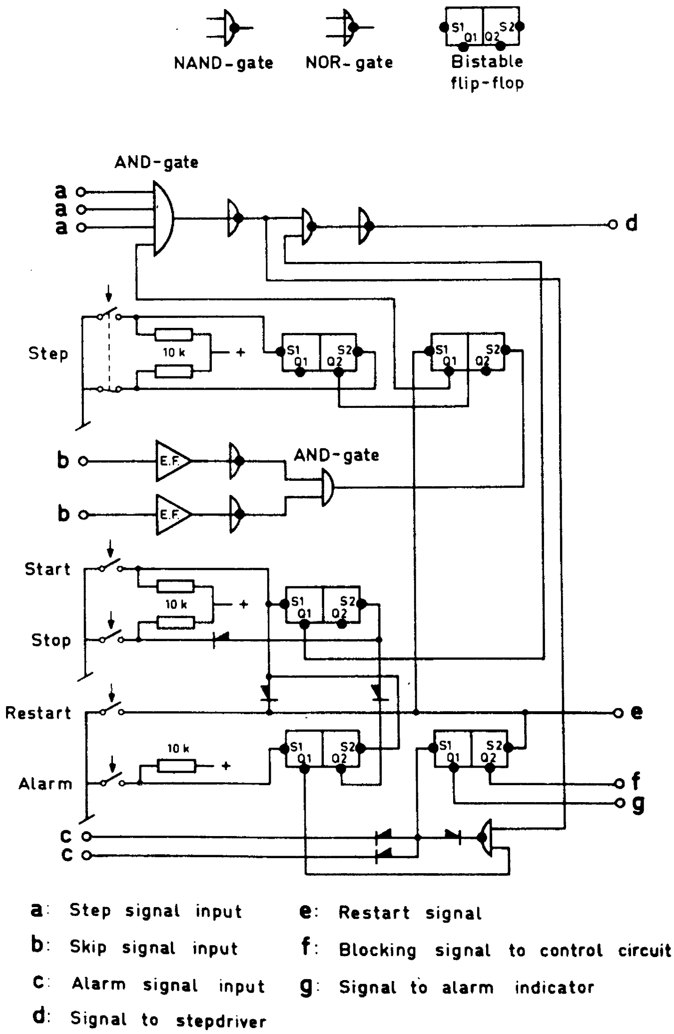


Fig. 6. Electrical diagram of alarm circuit and manual operation circuit.

The alarm circuit consists of a blocking circuit for the step driver prohibiting the tape reader to step if any of the signals to the control circuit are incorrect. Moreover, on activation of the alarm system all functions are interrupted. The manual operation circuits for start, stop, restart, alarm, and step are mainly included in the alarm circuit.

Operation knobs. The start and stop knobs, respectively, block and deblock the step driver in such a way that the unit is locked in the function being executed. The alarm knob makes the unit enter into alarm, when it has finished the function which has commenced, or when the step knob is switched. The alarm is indicated by a lamp.

If the step knob is switched the tape reader will move one step unless the alarm system has been activated or the stop knob has been switched immediately before.

The restart knob makes the unit start from the beginning of a function regardless of whether a function is being executed or inactivated by alarm. If the unit, due to a defect, goes into alarm when the tape reader steps, it is not possible to make it operate by means of the restart knob before the defect has been corrected, for example if one of the light sources in the photo-cell system has burnt out.

The level of the liquid in the metering flask, and the draining of both the metering flask and the reactor as well as stirring and pumping are controlled by a lamp and a photo-cell. To obtain a well defined signal from the photo-cells, these are followed by an amplifier. The incident light is a parallel beam of light rays passing a diaphragm measuring 2×14 mm, the longer side of which is placed parallel to the tube. Due to the deflection when the tube is filled with liquid a precise indication is obtained.

The electronic circuits are made of DTL (Diode Transistor Logic) silicon monolithic integrated circuits as far as this was possible at the moment of developing. The complete schematical diagram of the control unit is shown on Fig. 3, and the control circuit and the alarm circuit and circuit for the manual operation on Figs. 5 and 6. The entire system is built up on 16 printed wire circuits which together constitute a plug-in system.

The coding is effected by means of a tape containing the desired functions, for instance:

1) measuring methylene chloride into metering flask, code: S.
2) emptying the measured methylene chloride into the reactor, code: 1.
3) stirring the reactor contents for 5 min, code: 3.
4) draining the reactor, code: 2.

The codes are presented on the display tubes as: 2.3; 6.1; 6.3; 6.2; and are shown with the necessary connections in Fig. 4. Thus, with the crossconnecting panel properly precoded, all commands to the system are given *via* punched tape, allowing the synthetic procedure to be carried out full-automatically.

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