

AUTOMATION IN FUNCTIONAL GROUP ANALYSIS

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INTRODUCTION

Reliable analytical data are becoming increasingly important for an understanding of behaviour in all branches of science. Much that has been done in the past has been empirical and, therefore, useful only on a relative basis. Under such conditions, when the system or process is changed, direct comparisons of data often are meaningless. Analysis encompasses all types of measurement. The broadly capable analytical chemist, therefore, is skilled in a variety of techniques and knowledgeable in such branches of science as physical chemistry, physics, mathematics, chemistry, and engineering. He is concerned with reliability of analysis and is interested in accuracy and, thus, in quantitative measurements. He develops mechanical aptitude in adapting basic analytical tools to a variety of applied problems. Solutions to many of these problems require lengthy separations and multistep analyses. When performed manually, there are numerous difficulties which can lead to significant errors affecting both precision and accuracy.

Most of the tools used for analysis are now automated to some degree. Usually, this involves a means for recording results and, thus, eliminates the need for the individual to record data by hand. Even the analytical balance and the desk calculator may now be provided with a print-out system. In fact, tie-in of desk calculator with time-sharing computer is relatively straightforward. Most spectrometers can be equipped with digital readout. Some, such as the mass spectrometer, can be designed to use direct digital recorders. Others, such as absorption spectrometers, may require analogue-to-digital converters.

Much of the justification for automated systems is associated with labour or time saving in routine analyses. Where there is a high degree of activity in this area, quite expensive automation can be shown to result in considerable cost savings. In the research and development laboratory, we have found great improvements in reliability of analysis and often in lowered limits of detection.

Some of our experiences in automating techniques for functional group analysis are reported in this communication. These include systems for use with a colorimeter, infrared spectrophotometer, peak-resolving analogue computer, and thermal analysis instruments.

COLORIMETRY

Automated instrumentation for colorimetric analysis has made feasible considerably lowered limits of detection for many functional groups, anions,

and cations. In addition, with this equipment, rate studies can be made with better control than is achieved normally by conventional manual procedures.

Applications have been described for use in agricultural and food chemistry, pharmaceutical and clinical chemistry, metallurgy, water and air pollution studies, etc. In the preface to a bound copy of papers presented at a symposium on automation in analytical chemistry¹, Dr. L. T. Skeggs, Jr., clearly described the need for automation, and subsequent developments, in the clinical laboratory. Quotes from the preface follow.

"Ever since the close of World War II, clinical laboratories have been overwhelmed with requests for an ever-increasing number of determinations. The introduction of semi-automatic pipettes, photoelectric colorimeters, and flame photometers provided only minor relief, and, at times, it appeared that the insistent demand for analyses would quickly outstrip the availability of trained technologists to perform them.

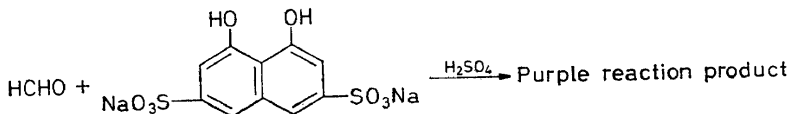
"As a biochemist in an overburdened clinical laboratory, I was acutely aware of this problem and conceived of a completely automatic method of continuous analysis. The idea was quickly put to the test. A fully functional model was constructed which determined urea and glucose in blood accurately and automatically . . ."

"The success of the method is not due solely to the fact that it is an easier, quicker, and more economical way to conduct an analysis. . . . The automatic, continuous flow method of analysis has certain inherent advantages which permit results to be obtained and experiments to be conducted that are either very difficult or simply cannot be performed by other methods."

"Perhaps the greatest advantage of the automatic, continuous method is that it permits not only analyses of the same sort to be performed on a large number of samples but that it also makes it possible to conduct many different kinds of determinations on a single sample. This new concept was first introduced when it was discovered that it was easier and better to do both urea and glucose on every blood sample on which either determination had been ordered."

We have automated many batch methods of analysis for both functional groups and elements². In general, previously published colorimetric procedures were used with resultant marked improvements in precision and accuracy as well as considerable saving in time per analysis.

Modification of the chromotropic acid (CTA) procedure for formaldehyde provides a good example,



This procedure was adapted to the "AutoAnalyzer" (Technicon Instruments Corp., Chauncey, New York). The flow diagram, *Figure 1*, shows schematically the feed system, time-delay mixing coils, heating bath, and colorimeter. Absorbance is measured with a filter photometer at 588 $m\mu$. The absorption maximum is actually at 570 $m\mu$, but there is essentially no loss of sensitivity at 588 $m\mu$. Without scale expansion, as little as 0.05 ppm formaldehyde can be detected readily. Optimum range is from 0 to 10 ppm. The system provides for automatic dilution for higher concentrations in the original sample.

Determination of small amounts of methanol via oxidation to formaldehyde illustrates the versatility of the automated system. Earlier work on the batch method in our laboratory has indicated about 12 per cent conversion to formaldehyde by permanganate oxidation in aqueous phosphoric acid solution. Conversion to formaldehyde in the batch method was raised to

20 samples/hour

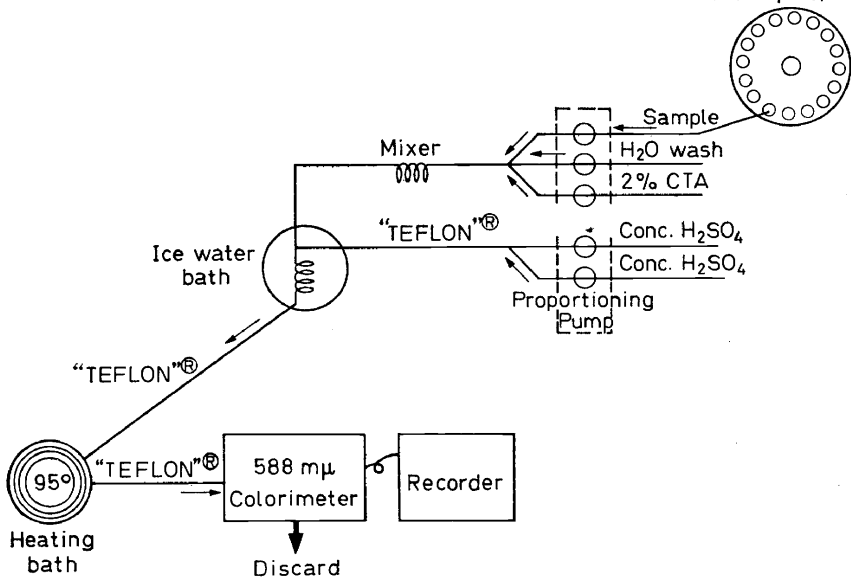


Figure 1. Flow diagram for determination of formaldehyde ("TEFLON"® is the trademark of E. I. du Pont de Nemours and Co., for its fluorocarbon resins)

about 20 per cent when a low concentration (*c.* 0.4%) of ethanol also was present. The automated procedure provides a convenient means for studying kinetics of reaction, using the flow system shown in Figure 2. Initial oxidation is made in the upper half of the manifold. Following this reaction, sodium bisulphite is added to decolorize excess permanganate. Then, CTA

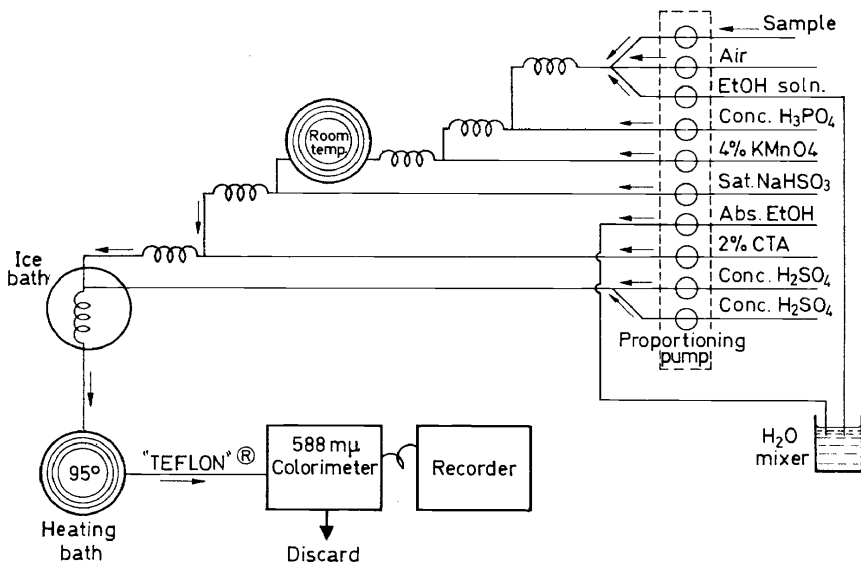
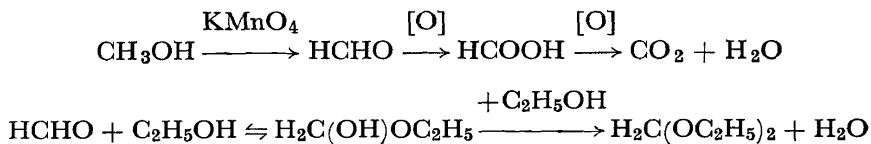


Figure 2. Flow diagram for determination of methanol

reagent and sulphuric acid are added for the colorimetric formaldehyde reaction. In establishing optimum ethanol level, its concentration was varied from about 0 to about 16 vol. per cent equivalent to 0.47 per cent in the reaction mixture.

The data indicate an optimum concentration of ethanol at 0.09 per cent in the reaction mixture. It is likely that ethanol serves to reduce the amount of formaldehyde oxidized by permanganate to carbon dioxide and water. This might well result from combinations of ethanol with formaldehyde to form hemiacetal or acetal which is more stable toward oxidation than is formaldehyde,



Following addition of concentrated sulphuric acid, the acetal is hydrolyzed, making the total formaldehyde available for the CTA reaction.

Measurement of loss of carbon dioxide from a carbonated liquid is a further interesting example of the advantages of automation in a kinetic study. Batch methods are quite unreliable since periodic contact of the sample with a sampling device, such as a pipette, is likely to result in sudden increased evolution of carbon dioxide. Adaptation to the "AutoAnalyzer" was straightforward. The arrangement is shown in *Figure 3*. The carbonated

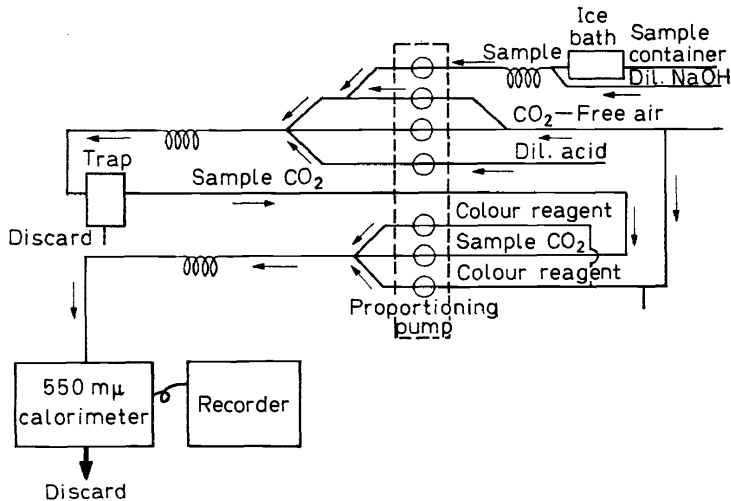


Figure 3. Flow diagram for evolution and determination of carbon dioxide

liquid is sampled continuously and the chilled sample stream mixed as quickly as possible with a stream of dilute sodium hydroxide. This treatment minimizes any loss of carbon dioxide as it is carried through the proportioning pump. The stream is then mixed with CO_2 -free air and dilute sulphuric acid to reliberate the carbon dioxide and fed to a small bubble

trap from which a portion of the CO₂-air mixture is taken off overhead. This gas is carried into a carbonate buffer solution containing phenolphthalein. The carbon dioxide content, measured continuously, is proportional to the degree of bleaching of the indicator colour.

Figure 4 shows results obtained from sampling a carbonated beverage at room temperature. Results are plotted as per cent transmittance vs. time. Maximum in carbon dioxide content (about 0.7 per cent) was observed in 2-3 min after introduction of the sample, after which the rate of loss was fairly rapid. Half of the gas was evolved in about 30 min. Then, the rate of loss decreased, as indicated by the fraction remaining.

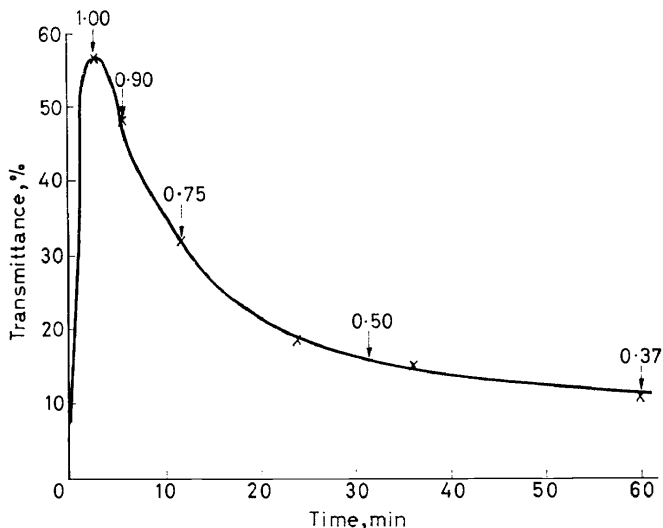


Figure 4. Rate of decarbonation of soda water

INFRARED SPECTROMETRY

Infrared spectrophotometry provides the basis for probably the most versatile technique for functional group analysis. It combines the desirable features of (a) small sample size; (b) use of solid, liquid, or gaseous sample; (c) being usually non-destructive; and (d) a means for detection of a variety of groups in a single scan. Like all so-called general techniques, it is subject to interferences. There is a considerable variation in intensity of absorption for different types of groups. Consequently, weakly absorbing species may not be resolved from broad, strongly absorbing functional groups. For example, the oxidation of polyolefins leads to a variety of oxygen-containing groups. The reactions appear to proceed by a free radical mechanism³ with a hydroperoxide group as the intermediate. The infrared spectrum from 2 to 15 microns of a film of polyethylene after heating for 2.5 hours at 150°C is shown in Figure 5. Accurate determination of low levels of the hydroperoxide function is not feasible because of its weak absorption and its equilibrium between bonded and unbonded species.

The selective reaction of hydroperoxides with sulphur dioxide⁴ was used effectively to permit determination of parts-per-million concentrations in polyethylene⁵. After exposure of film to sulphur dioxide, the —OOH band had disappeared, and the C=O absorption was broadened. As shown in *Figure 5*, new moderate-to-strong bands appeared at about 7.1, 8.3, and 10.7 microns. These have been assigned to sulphate groups. That at 8.3 μ was quite free of other group absorptions, permitting determination of less than 1 ppm of hydroperoxide⁵.

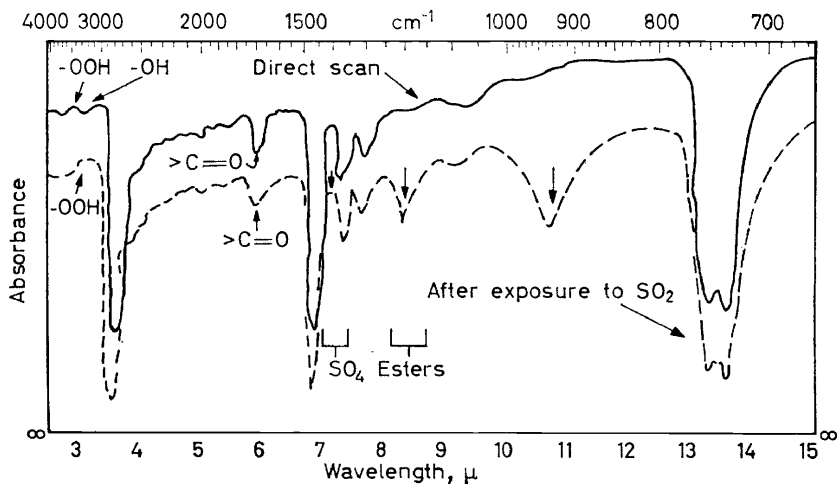


Figure 5. Infrared spectrum of partially oxidized polyethylene (Direct scan: solid line. After SO₂ treatment: broken line)

Much of our work is associated with analysis of polymer films. For routine determinations, a few analytical wavelengths can be chosen, the intensity of which are related to concentration. We had a need for a versatile infrared analyzer capable of performing a variety of analyses and working independently; i.e., without the need for operators. An automated infrared analyzer was assembled, *Figure 6*, consisting of a control programme, a double-beam infrared spectrophotometer, and a dual readout system⁶. This basic system has been retained in our updated equipment. However, numerous improvements have been made in controls and in design. *Figure 7* shows schematically our current arrangement employing a Perkin-Elmer Model 221 infrared spectrophotometer. *Figure 8*† shows a photograph of the system for handling films: (1) sample feed to the spectrometer, (2) programmer, (3) analogue-to-digital converter, and (4) teletype punch. *Figure 9*† shows a close-up of the sample system. The film samples are mounted on 5 cm × 5 cm cards and placed in a standard slide projector carriage. The sample is pushed automatically into position in the infrared beam. Upon completion of the scan, as established by the programmer, the sample is returned to the carriage and the next sample pushed into place. The arrangement shown permits successive, automated analyses of as many as 72 samples.

† Figures 8 and 9 are given in the section printed on art paper

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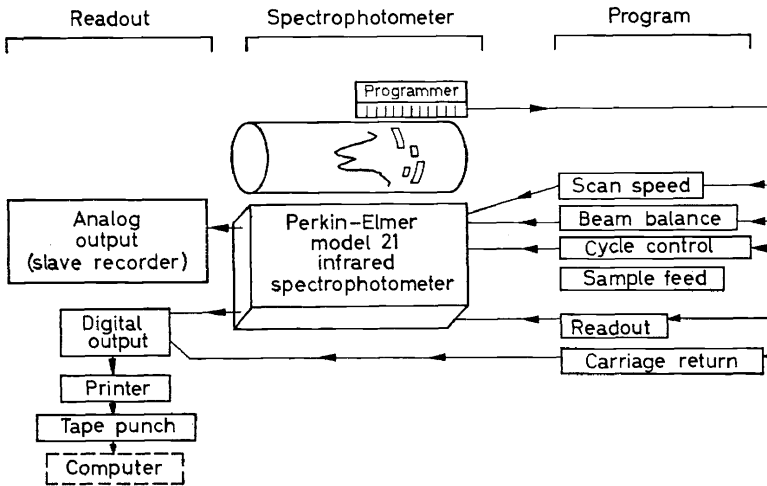


Figure 6. Block diagram of automated infrared spectrophotometer

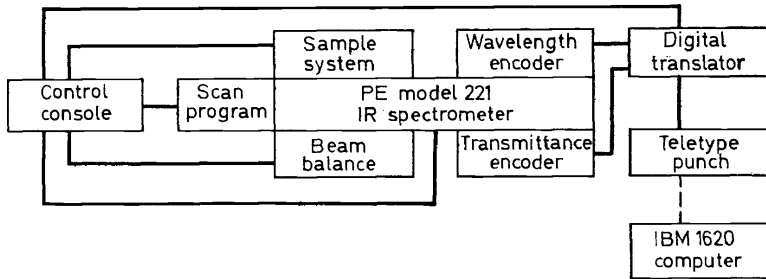


Figure 7. Automated infrared spectrophotometer

The system can be applied to most infrared analyses which can be made manually. Three principal steps are programming, scanning, and computing. A generalized example for the determination of a minor component, X , in a matrix, M , illustrates the principle. Figure 10 shows the sequence followed. The ratio of the absorbance of band X to that of the internal standard band M serves as a measure of the concentration of X ; this relation is assumed to be linear over the range of concentration of X .

The automated system has been used on a variety of routine analyses, principally end groups and crystallinity in polymers. In all cases, analytical results have been at least as good as those by the corresponding manual procedure. In a check of instrumental reproducibility, a standard deviation of 0.045 per cent was found from 65 determinations on a single film sample over a 24-hour period. Over a period of three months, a standard deviation of 0.1 per cent was observed, about the same as that found by the manual method.

In nearly all cases, use of the automatic infrared analyzer has permitted analyses of replicate samples to improve precision at essentially no increase in cost. The analyzer also has provided for inclusion of standard samples at

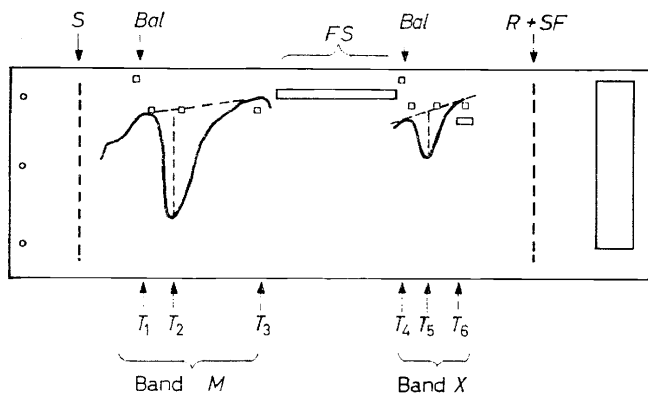


Figure 10. Typical programme sheet and operations

S, Start	SF, Sample feed
Bal, Beam balance	T, Readout, peak transmittance
FS, Fast scan	M, Matrix component
R, Recycle	X, Determined component

more frequent intervals than would have been feasible by the manual method. In this way, accuracy of analysis has been improved significantly at very low cost. Finally, the automatic analyzer has freed skilled personnel and valuable equipment for non-routine operations during the regular working day, since the routine, automated analyses could be performed primarily during off-shift hours.

ANALOGUE COMPUTER

Automation of conventional manually operated laboratory instruments may be relatively straightforward. Automation can be achieved in varying degree from a means for handling results to eliminate hand calculation or manipulation to a fully automated system providing for direct sampling, unattended instrument operation with on-line computer, or direct access to a time-sharing computer. The type and degree of automation obviously depends on the need.

Where resolution is desired of overlapping peaks in spectra or other curves representing sums of peaks or distribution functions, an analogue computer is quite convenient. Such equipment was devised in the United States by scientists at the National Institutes of Health⁷. A prototype of a modified unit constructed in our laboratories is shown in *Figure 11* (given in the section printed on art paper). This equipment provides a means for producing the individual component parts through function generator channels. That shown in the figure provides nine channels, permitting direct separation of as many as nine peaks which contributed to the original curve. Direct matching with the original is made through a modified photographic enlarger directly on a copy of the original curve. We have used the computer to resolve complex curves from a variety of instruments. *Figure 12* (given in the section printed on art paper) illustrates application to (a) an x-ray diffraction pattern of nylon, 2θ vs. x-ray intensity, showing resolution into two crystalline peaks over the broad peak resulting from

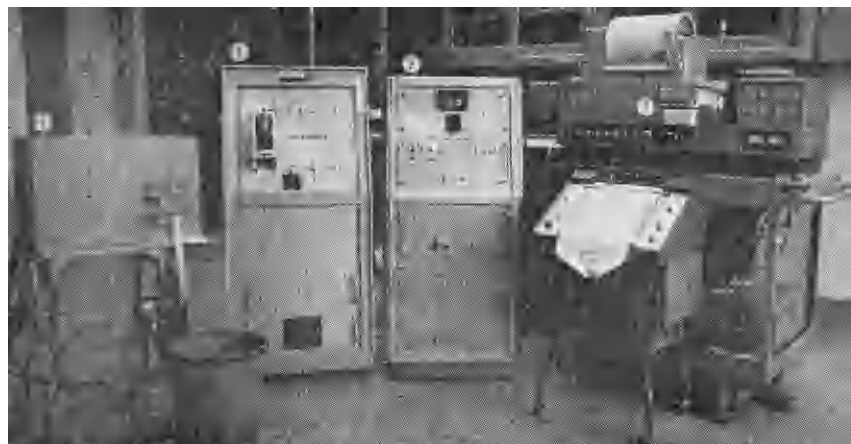


Figure 8. Automated infrared spectrophotometer

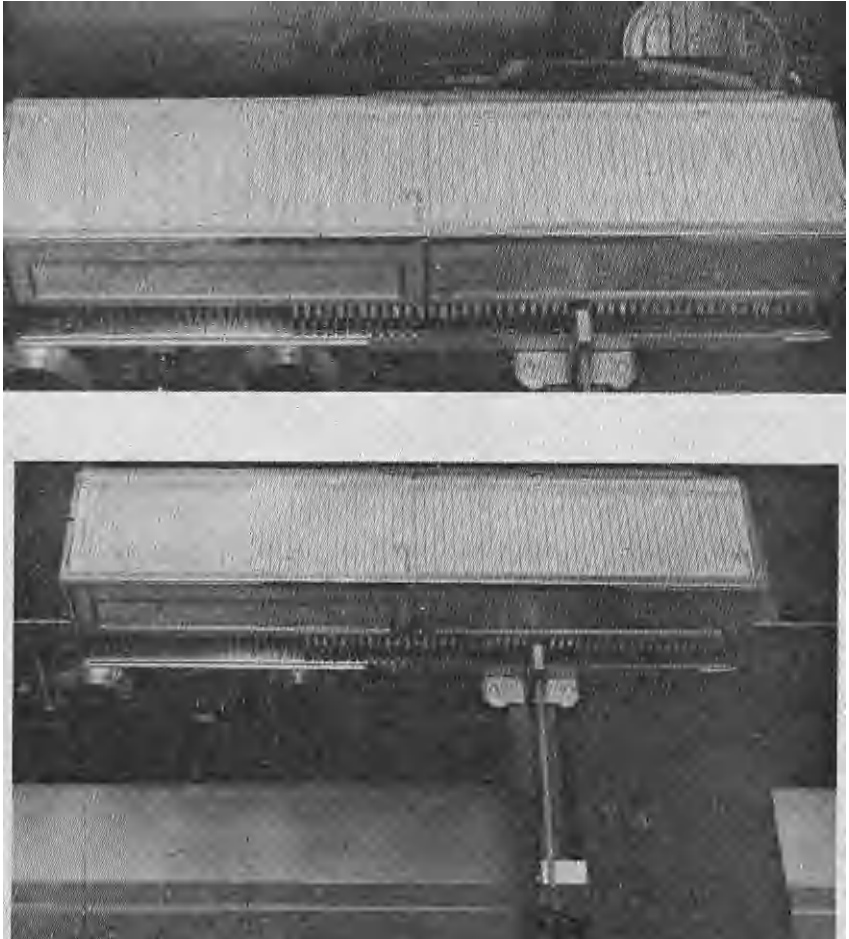


Figure 9. Automated film sample feed system

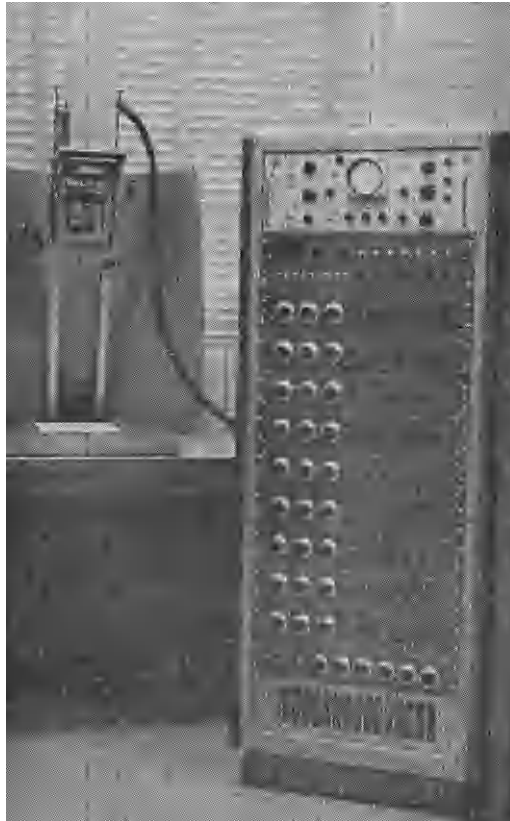


Figure 11. Peak resolving analogue computer

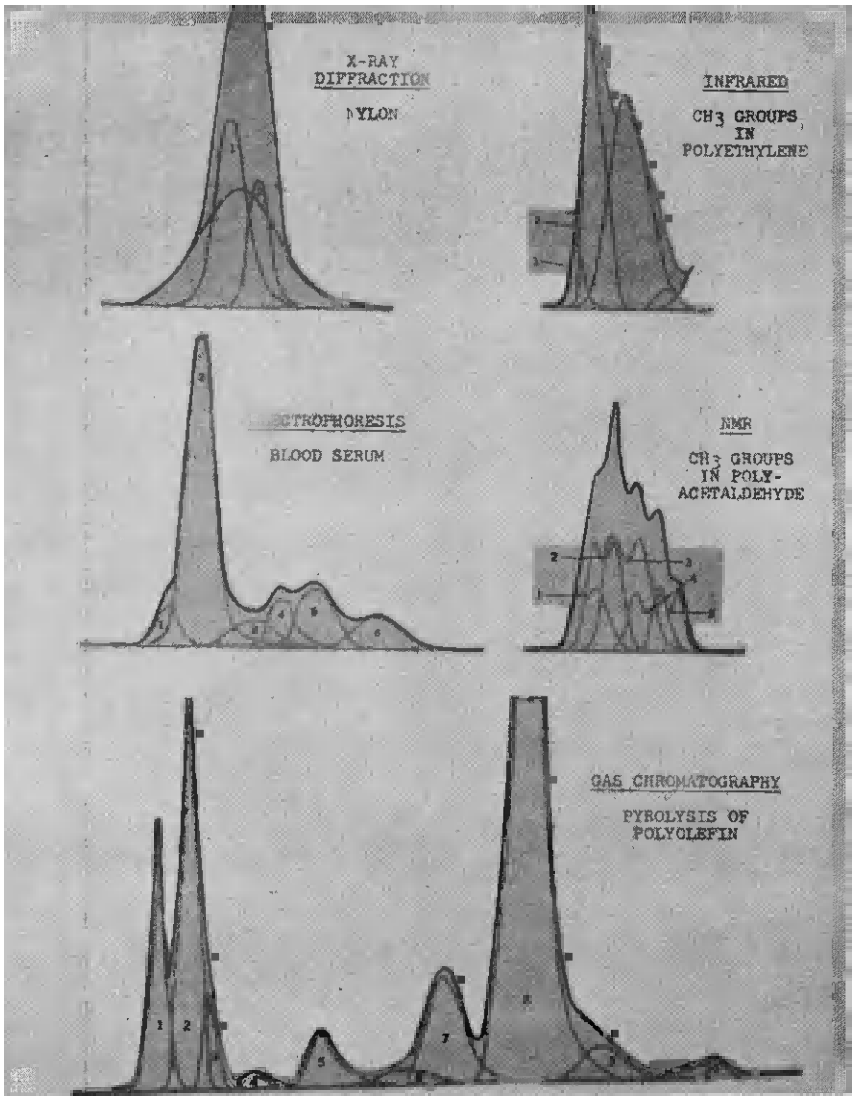


Figure 12. (a) X-ray diffraction pattern of nylon, 2θ vs. x-ray intensity. (b) Infrared absorption of polyethylene, frequency vs. absorbance. (c) Electrophoresis curve from normal blood serum. (d) The high-resolution n.m.r. spectrum of polyacetaldehyde. (e) A gas chromatogram of the hydrocarbon pyrolysis products

scatter by the amorphous regions; (b) infrared absorption of polyethylene, frequency vs. absorbance, in the 7 to 8 μ region—resolution is shown of the CH_3 —group symmetrical deformation band, 1, at 7.255 μ from the intense $-\text{CH}_2-$ deformation bands, 2; (c) the electrophoresis curve from normal blood serum showing resolution into six components: prealbumin, albumin, α_1 -globulin, α_2 -globulin, β -globulin, and γ -globulin; (d) the high-resolution nuclear magnetic resonance spectrum of polyacetaldehyde showing resolution into five doublets resulting from spin-spin interactions of CH_3 —protons with CH —protons; and (e) a gas chromatogram of the hydrocarbon pyrolysis products from a polyolefin. A suitable peak-resolving analogue computer is now available commercially.

Application of the analogue method to determination of methyl groups in polyethylene represents a particularly interesting technique. Current methods being evaluated by the American Society for Testing and Materials⁸ require compensation during the actual infrared measurement. One method uses compensation with a standard sample film or wedge of known methyl content. The other method uses compensation with a wedge of polymethylene or a polyethylene of known low-methyl content. In the more rapid analogue method, direct resolution is made of four Lorentzian-shaped bands at 7.255 μ from CH_3 groups and at 7.31, 7.39, and 7.47 μ from CH_2 groups and a Gaussian-shaped band at 7.67 μ from CH_2 group vibrations in the amorphous phase. A straight-line relationship has been observed between methyl groups as determined via the analogue computer vs. methylene groups as calculated from compensation.

THERMAL TECHNIQUES

Automation has played a prominent role in design and use of equipment for measurements of thermally induced transitions. A few years ago each technique, such as differential thermal analysis (DTA) or thermogravimetric analysis (TGA), was handled as a separate measurement. Often, objective correlations of relative behaviour between DTA, TGA, etc., were not feasible because of difficulties in exactly reproducing the environment. This problem has been overcome by combining techniques into a common system which provides for simultaneous measurement.

Paulik, Paulik, and Erdey⁹ have devised an automated instrument for DTA, TGA, and DTG (first derivative of TGA) with photographic recording. The three curves are recorded simultaneously vs. time. A schematic diagram of their instrument is shown in *Figure 13*, consisting of the furnace in which sample and reference are placed; the balance; and the circuitry for DTA, TGA, and DTG measurements.

Berg and Burmistrova¹⁰ devised apparatus for simultaneous recording of DTA and electrical conductivity. A schematic diagram is shown in *Figure 14*. Sample and reference are placed in the cells. Electrical conductivity is measured between two platinum electrodes and DTA from a thermocouple placed in the sample. Photographic recording is used. Four rectifiers are shown in *Figure 14*. Alternative equipment having a single rectifier also was used in analyses of inorganic substances.

In our laboratory, a direct recording system has been devised for DTA

and electrical conductivity. The latter has been termed dynamic electrothermal analysis (ETA)¹¹. The original DTA circuitry, shown as a solid line in *Figure 15*, employed an X-Y recorder to plot ΔT vs. T . Simultaneous ETA requires addition of a separate link, dashed line, connection from the cell with d.c. source, electrometer, and an X-Y-Y recorder.

The apparatus now has been modified further to provide for simultaneous TGA-DTG-DTA-ETA¹². Basic components are TGA coupled

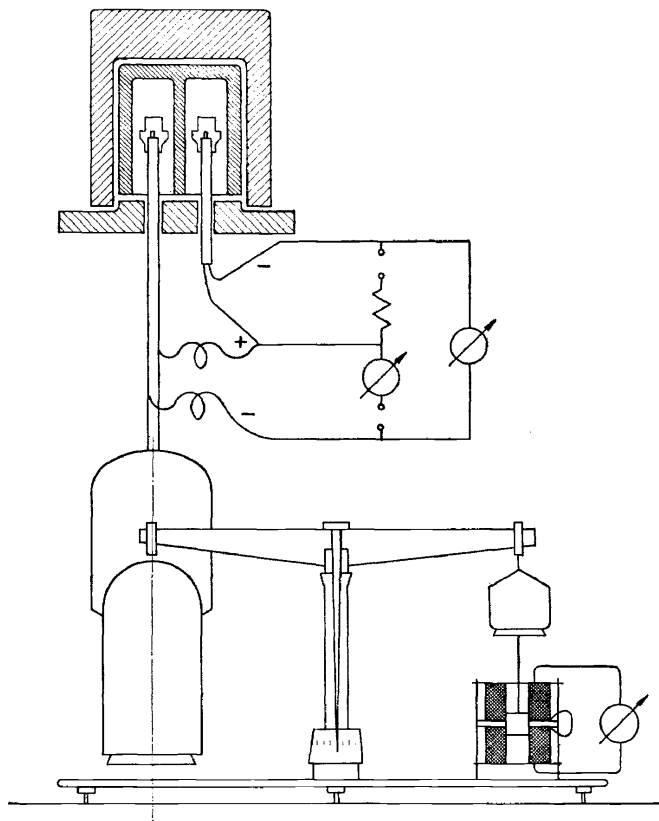


Figure 13. The derivatograph

to DTA (*Figure 16*). The key to this equipment is in modification of the TGA accessory. *Figure 16* shows how the sample is placed within the furnace tube, together with the leads for the several measurements.

Advantages of the simultaneous measurement are indicated in studies of urea and pyromellitic acid dihydrate. *Figure 17* shows the behaviour of urea from room temperature to about 450° in a nitrogen atmosphere. Note that, although no weight loss is observed prior to the DTA melting endotherm at about 133° , there is a significant change in electrical conductivity. Decomposition is evident from about 190° , as shown in the TGA and DTG curves. A sharp change in the ETA curve, on the other hand, occurs at the melting point, quickly reaching a minimum value. No further

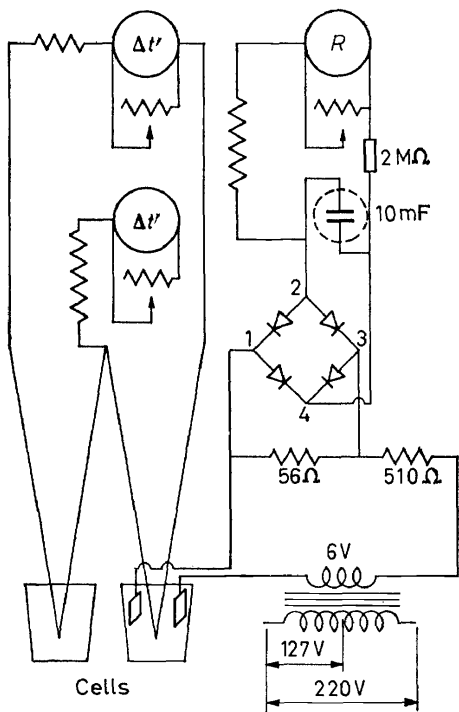


Figure 14. Schematic diagram of simultaneous recording of DTA-electrical conductivity of a sample with four rectifiers

marked change in the ETA curve occurs until a temperature of about 250° is reached which coincides with a DTA endotherm and a change in rate of weight loss. Doubtless, these and transitions up to about 400° are associated with thermal decompositions and recombinations to form such

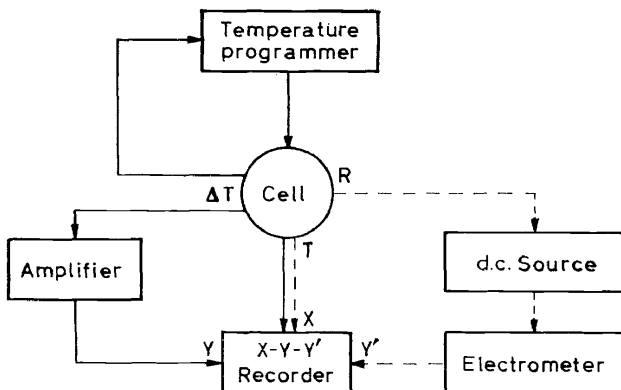


Figure 15. Schematic diagram of DTA-ETA apparatus

products as biuret, cyanuric acid, amides of cyanuric acid, and cyanuric triurea. We have not isolated products from various stages of the decomposition. However, the technique is well suited for such study.

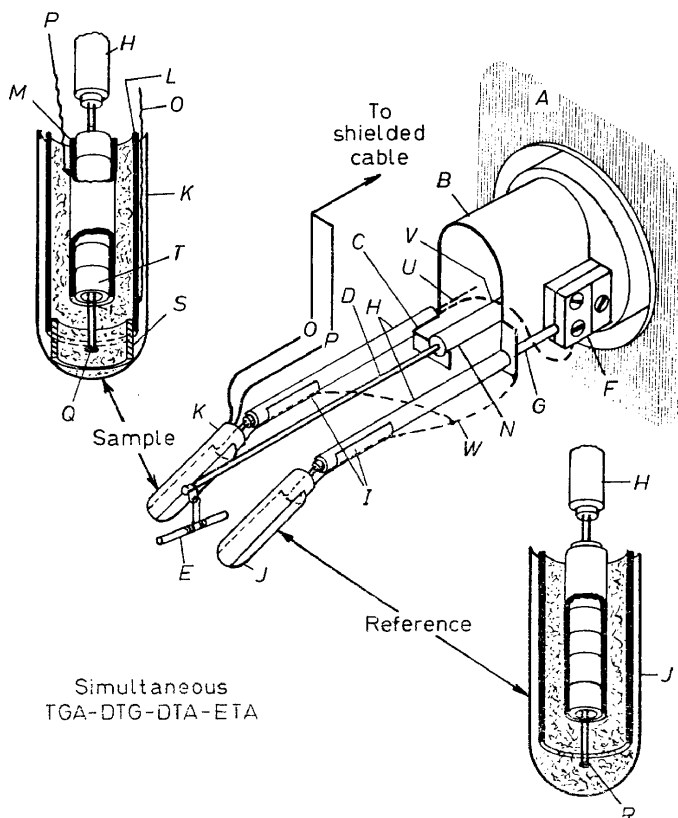


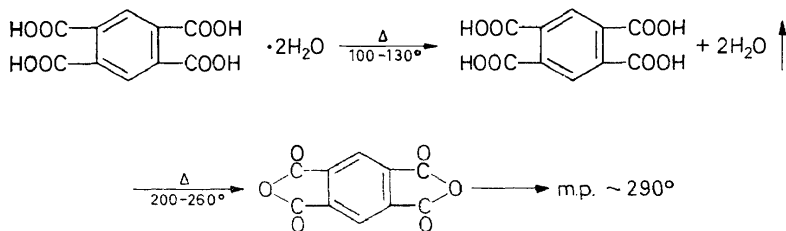
Figure 16. Sample handling system for simultaneous TGA-DTG-DTA-ETA

- | | |
|----------------------------------|------------------------------------|
| A, Balance Housing | K, Sample Quartz Tube |
| B, Balance Beam Sheath | L, Outer Platinum Electrode |
| C, Beam Stop | M, Centre Platinum Electrode |
| D, Quartz Beam | N, Cold Beam Member |
| E, Sample Container | O, P, Platinum Lead Wires |
| F, Thermocouple Block | Q, Sample Thermocouple Junction |
| G, Sample Measuring Thermocouple | R, Reference Thermocouple Junction |
| H, Ceramic Tubing | S, Spacer |
| I, Platinum Jacket | T, Ceramic Insulation |
| J, Reference Quartz Tube | U, V, Sample Thermocouple Wires |
| | W, Platinum Grounding Wire |

Further application of the technique is illustrated by the thermal behaviour of pyromellitic acid dihydrate. Figure 18 provides TGA, DTG, DTA, and ETA data for (1), the original sample heated from room temperature

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to 300°. After cooling, (2), the residue was reheated to about 500°¹¹. The transitions are consistent with the following sequence:



On recycling the residue, now anhydride, the dehydration steps are eliminated. A new small DTA endotherm appears at about 225°, indicating a crystalline transition in the dianhydride. Following melting at about 290°,

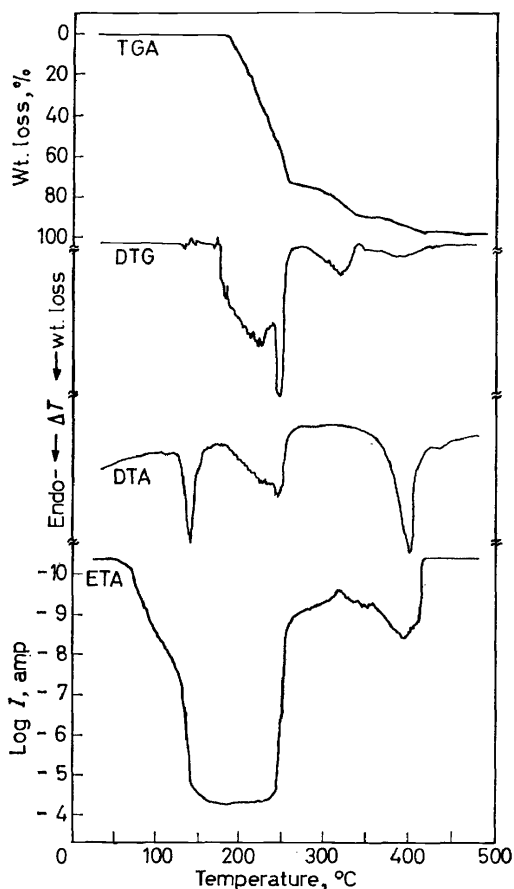


Figure 17. TGA-DTG-DTA-ETA of urea

the anhydride is almost completely volatilized near 400°, as shown by the final step of the weight loss curve, the sharp endotherm, and the rapid decrease in electrical conductivity.

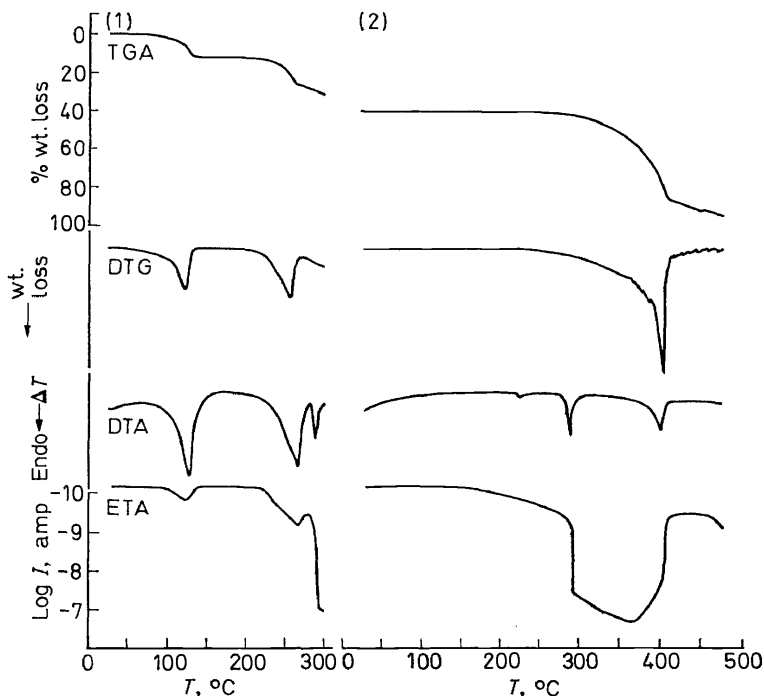


Figure 18. TGA-DTG-DTA-ETA of pyromellitic acid

SUMMARY, CONCLUSIONS

The extent to which automation should be carried in the laboratory obviously depends on the need. There is little doubt that automated readout is highly desirable. Where extensive routine work is done, a considerable investment in equipment can be justified.

The system described for automated infrared analysis is an example of control of programming as well as readout. Currently, for x-ray diffraction experiments, we are providing a cybernetic system with on-line computer, as shown in the schematic diagram in *Figure 19*. This same approach can be used for other instrumentation for functional group analysis, employing on-line or time-sharing computer.

My discussion here has indicated the varying degrees of automation which we employ in the analytical laboratory associated with a research and development effort. Stress has been placed on those techniques used broadly for functional group analysis at concentrations ranging from ppm to several per cent. Included are colorimetry for selective trace analyses, infrared for a range of concentrations, analogue computer for resolving overlapping bands, and thermal techniques for transitions.

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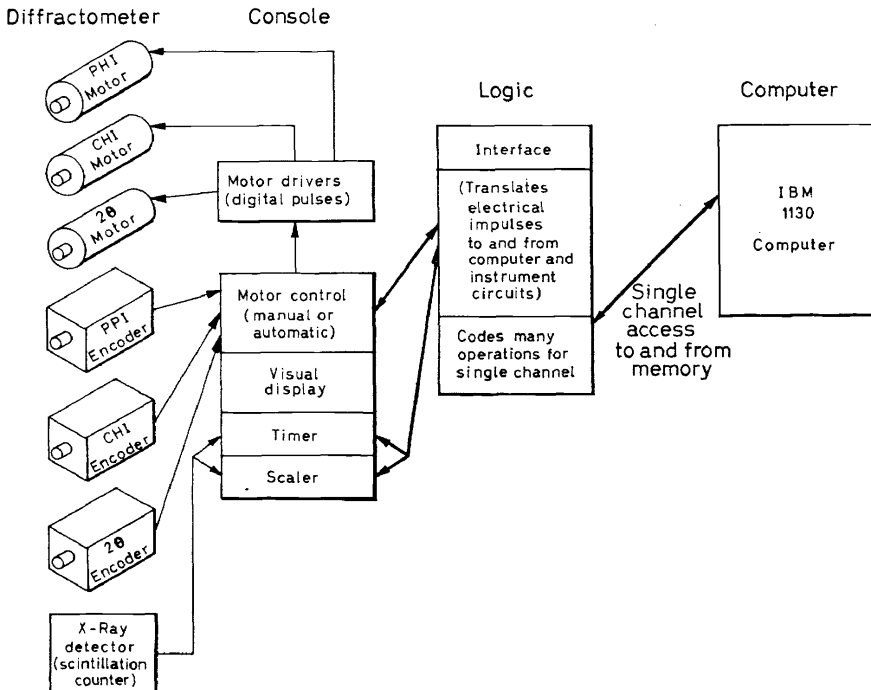


Figure 19. Cybernetic system for x-ray diffraction

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