Separation Science and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713708471

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To cite this Article Barrall II, Edward M. and Johnson, Julian F.(1970) 'Chromatographic Instrumentation and Detection of Gel Permeation Effluents', Separation Science and Technology, 5: 4, 415 — 427
To link to this Article: DOI: 10.1080/00372367008068440
URL: http://dx.doi.org/10.1080/00372367008068440

SEPARATION SCIENCE, 5(4), pp. 415–427, August, 1970

Chromatographic Instrumentation and Detection of Gel Permeation Effluents*

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Summary

The basic instrumentation required for gel permeation chromatography are described. The various choices for sample injectors, pumping systems, detectors, and recording techniques are discussed and critically evaluated. Column choice, construction, etc., are omitted since these items are very well covered by other Symposium papers.

INTRODUCTION

The instrumentation for gel permeation chromatography (GPC) is relatively simple in concept but somewhat involved in execution. All functional instruments contain the following systems: (a) sample injection, (b) pumping, (c) chromatographic column, (d) sample detection, (e) eluent volume detection, and (f) data recording. External to the apparatus flow system are the sample preparation apparatus and column temperature regulation control. A detailed discussion of each of the above items with the exception of the chromatographic column will be the subject matter of this paper. It is not intended that this discussion be an exhaustive survey of the very large literature in this field. However, examples will be cited to illustrate particular points when necessary for clarity.

* Presented at the ACS Symposium on Gel Permeation Chromatography, sponsored by the Division of Petroleum Chemistry at the 159th National Meeting of the American Chemical Society, Houston, Texas, February, 1970.

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SAMPLE INJECTION

Some means must be provided for the introduction of the sample in solution into the chromatograph. This problem is complicated by three factors: (a) relatively high hydrostatic pressure in the GPC system before the columns and after the pump, (b) flow properties of two liquids of different densities, and (c) danger of introducing gas bubbles. The objective of the instrumentation for sample addition is to introduce a known amount of sample onto the column with a minimum loss of resolution.

Systems for GPC sample addition fall into two main types: multiport valves and hypodermic injection systems. Figure 1 shows an injection system based on a multiport valve described by Bombaugh, Dark, and King (*1*). The sample loop is filled with a polymer solution of the desired concentration while solvent is flowing through the column. Interchangeable loops of different volumes are available. To inject the sample the valve is turned 90°, causing solvent flow to be directed through the sample loop. Valving of the sample injection system has been a problem in the past. The most common difficulty reported is solvation of the valve gasketing by the GPC solvent. Leak-

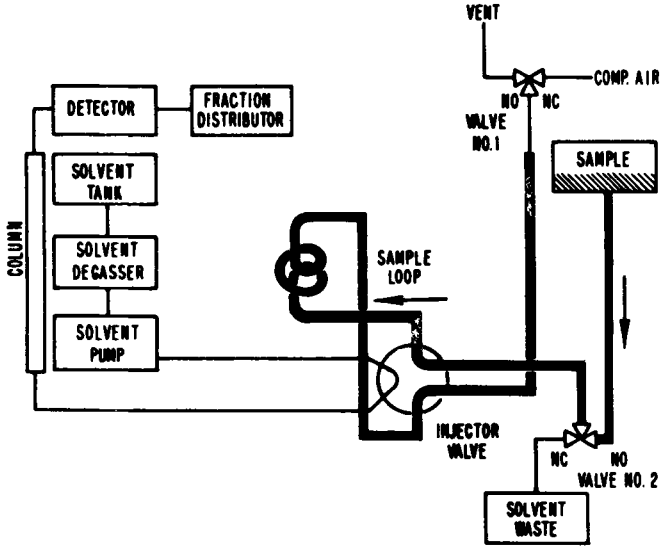

FIG. 1. Loop injection system. Sample filling loop is shown in bold lines (*1*).

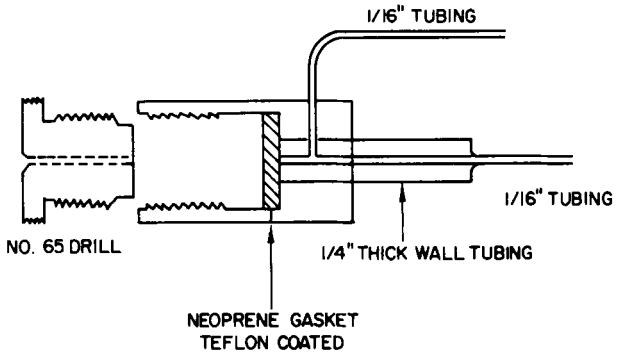

FIG. 2. Injection assembly (*5*).

ing valves and plugged lines are the general result. It is now possible to obtain reasonably long lasting sliding plate and O ring valves with insoluble Teflon surfaces. This type of sample injection system has the ability to introduce reproducible amounts of sample and is readily automated. Improvements in valve packing and construction have made possible the introduction of automatic injection systems. A commercially available system, Waters Associates, has six sample loops and will automatically inject six previously loaded samples at any selected fixed interval.

In general, for a given amount of polymer, reducing the volume of the sample loop reduces the peak broadening, see for example the work of Billmeyer and Kelley (*2*). However, since this necessarily causes an increase in the concentration of the polymer in solution, there is a point where the increase in resolution due to decreasing solution injected is counteracted by the irregular shape of injection of a very viscous sample. This "viscous fingering" effect has been discussed by Little et al. (*3*). The balance between sample size and concentration for optimum resolution is a complex function depending at least on flow rate and molecular weight and probably on other factors (*4*).

A typical hypodermic injection system developed by Gamble, McCracken, and Wade is shown in Fig. 2 (*5*). The polymer solution is added from the syringe which is inserted through the Neoprene gasket positioned by the guide hole. The solution is added directly into the flowing solvent at the head of the column. Sample volumes are less reproducible than with the multiport valve but the apparatus is compact, inexpensive, and easy to use. Jentoft and Gouw (*6*) have described modifications of the septum-hypodermic sample system to allow injection onto columns operating at pressures up to 1000 lb/in.^{2}

PUMPING SYSTEM

The pumping system is required to produce a constant but readily variable flow rate typically in the range of 0.1 to 5 ml/min, at back pressures up to 250 lb/in.^{2} These limits are, of course, a rather arbitrary selection, for higher and lower flow rates have been used and higher back pressures are occasionally encountered. Pressure fluctuations must be minimized as these are frequently a source of noise in the detector. The more common pumping systems are based on commercially available reciprocating or pulsing positive displacement pumps with an adjustable delivery volume. These pumps are used in conjunction with a damping system to reduce pressure pulses. A particularly effective small pulsation damper has been described by Ross and Castro and is shown in Fig. 3 (*7*).

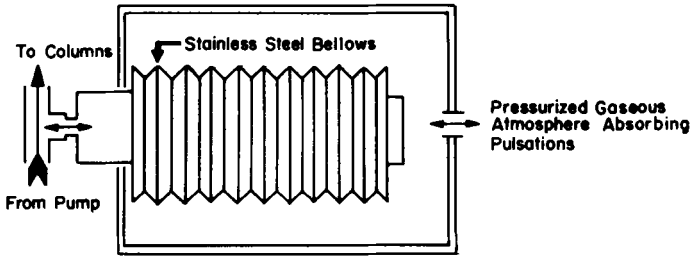

FIG. 3. Pulsation damper (*7*).

Jentoft and Gouw (*8*) and others (*9*) have reported on pulseless pump designs. Figures 4a and 4b illustrate typical designs. In the system shown in Fig. 4b constant volume flow is obtained using commercially available constant volume pumps (Zenith Products Co., West Newton, Mass., No. 1/2B-4391 with Teflon U-seals). The necessary low flow rates are obtained by operating two pumps differentially. That is, one pump delivers a volume V-1 as the source for the second pump which delivers a slightly smaller volume V-2. The excess volume, V-1 minus V-2, from a T-connection between the two pumps is the flow delivered to the chromatograph. Since these are gear-type pumps, the pumped fluid must have a considerable viscosity to achieve constant volume flow. High viscosity for constant volume requires an intermediate pumping fluid. Mineral oil of about 200 cSt was used to displace mercury which subsequently displaces the chromatographic solvent. The pump delivery rate is very constant against variable pressure heads,

easily variable by the adjustment of system parameters and gear ratios, and essentially pulse free. The pump has one small disadvantage, the solvent reservoir must remain sealed during pump operation.

A particularly simple and quite satisfactory pump consists of a pneumatic pump (Waters Associates, Framingham, Mass.) discharging through a capillary restrictor into the gel permeation column (*10*). The pump is simply a polyethylene wash bottle in a metal container to which air pressure can be applied. While not a continuous pump, the flow is constant for a volume of about 300 ml which is sufficient for most analytical GPC measurements.

SAMPLE DETECTORS

It is possible to characterize fractions collected by gel permeation in the classical manner, that is by collection, evaporation of stabilizer-free solvent, weighing, and determination of molecular weight. However, one of the major advantages of GPC is that continuous detectors may be used to monitor and record the polymer concentration in the eluent.

Differential refractometers to detect polymers in organic solvents were first introduced by Moore (*11*) and are the most widely used detectors. A differential detector is required because of the low concentrations involved. The usual sample of polymer contains 2–10 mg of polymer which elutes from the column in 25–75 ml of solvent. With the refractometer detector the solvent-polymer system is chosen to give a maximum difference in the refractive index. Quantitatively, the detector response depends on the relationship that Δn is proportional to Δc where Δc is the concentration of the polymer. In turn this implies that the refractive index of the polymer is independent of molecular weight. While this is generally true for homologous series with molecular weights above a few thousand, some error may be introduced at lower molecular weights unless a correction factor is introduced to account for the small dependence on molecular weight (*12*).

Differential refractometry is not satisfactory for monitoring the fractionation of a mixture unless response factors are known for each component. There is no *ab initio* rule for determining if a small peak in a mixture represents a trace component with a large refractive index difference from the solvent or a major component with a refractive index very similar to the solvent. Also, low liquid holdup in the cell is necessary to prevent excessive loss of resolution. For example, when the volume of the detector cell was decreased from 0.070

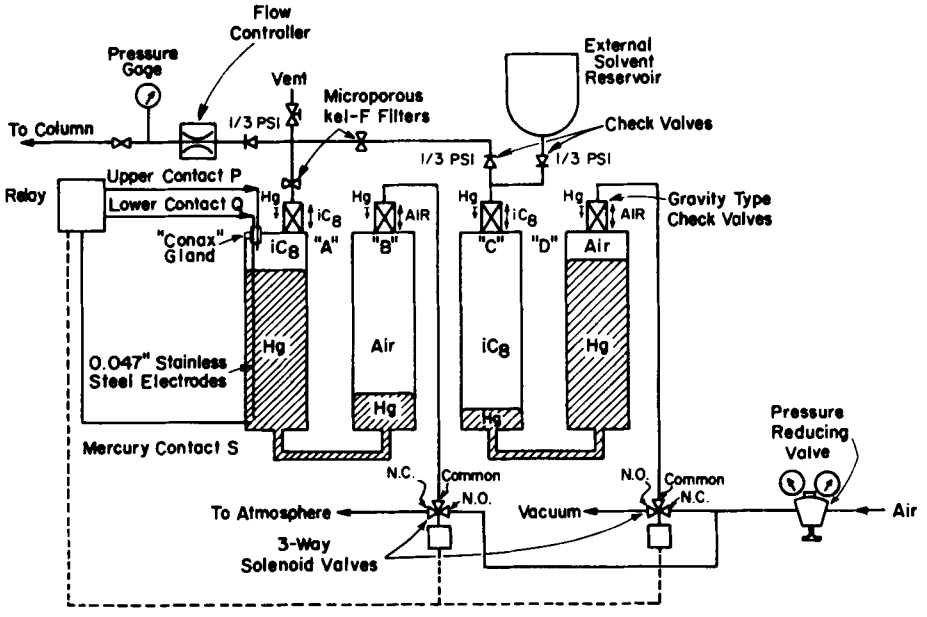

FIG. 4a. Pulseless high pressure pump (*8*).

to 0.010 ml, Billmeyer and Kelley observed a threefold gain in sensitivity and a reduction of one-half in the zone broadening due to the cell (*2*). Johnson, Campanile, and Lefebre (*12a*) reported on the design of a low holdup reflection type refractometer with a limit of detection for Δn of 1×10^{-6} and a noise and drift for 1 hr periods of only 5×10^{-7}.

Since the refractometer is a differential detector, a reference, flowing stream of solvent must be supplied to one side of the refractometer cell. The reference stream is usually provided by the eluent of a relatively short section of packed column. Since the differential refractometer is measuring differences between the sixth and seventh decimal of refractive index during a chromatogram, it is very sensitive to minor solvent and flow variations (surges). Care must be used to balance the flow rate and pressure drop across the reference and working columns if a smooth base line is to be obtained.

In some cases infrared spectrometers may also be used as detectors with certain advantages over the refractometer type. An example is given by Ross and Castro (*7*), who found that very satisfactory

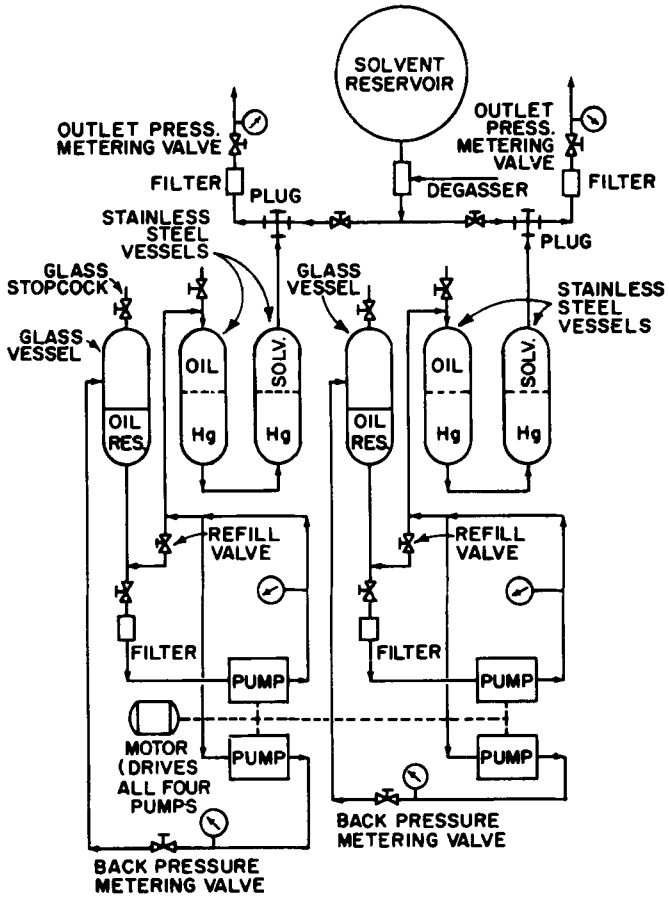

FIG. 4b. Dual constant volume pumping unit (*9*).

results could be obtained by monitoring the 3.4 μ band for polyethylene molecular weight distributions using perchloroethylene at 110°C as a solvent. Sensitivity was stated to be better and noise due to temperature fluctuations less than with a differential refractometer at high temperatures. It was necessary to add a zero suppressor circuit so that the 80–100% transmission range appeared as full scale on the recorder. In this region concentration was found to be a linear function of optical density, see Fig. 5.

Rodriguez, Kulakowski, and Clark (*13*) have utilized infrared detection to determine relative concentrations of functional groups as

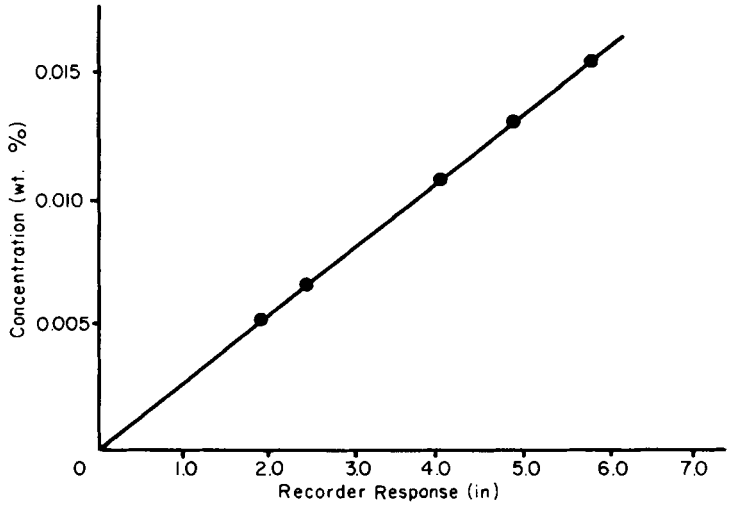

FIG. 5. Response of recorder with polyethylene concentration at 3.4 μ (2940 cm^{-1}) (7).

well as total polymer concentration. The importance of such measurements in copolymer analysis is apparent. For example, as shown in Fig. 6, Terry and Rodriguez determined methyl methacrylate from the 1731 cm^{-1} band and styrene from the 698 cm^{-1} band (*14*). Phenyl

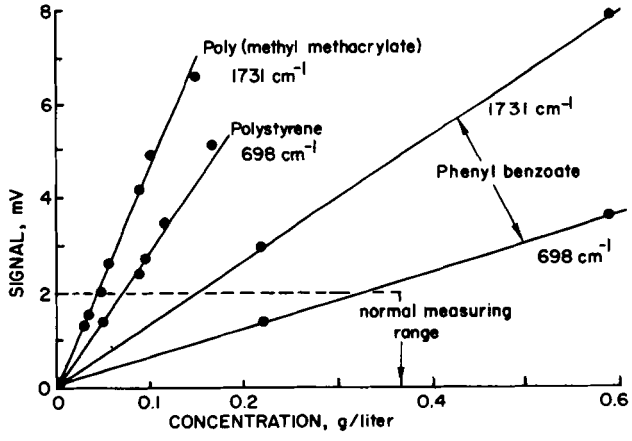

FIG. 6. Calibration curves for infrared detector. Solvent: trichloroethylene (*14*).

benzoate, which elutes separately from the polymer, was measured at both frequencies to provide an internal standard. In addition, the flow may be stopped when a peak appears and a complete spectrum recorded. This is particularly useful for low molecular weight compounds and in identification of additives in polymers. The spectrometer need not be a rapid scan type as essentially no column resolution is lost when flow is interrupted for periods of several hours (*15*).

The search for more sensitive detectors to permit smaller sample sizes and higher resolution led to the development by several research groups of a detector based on flash evaporation of the eluent. This deposits the nonvolatile solute on a moving chain or cord which carries it into a pyrolyzer after which the amount of pyrolysis products is determined by a very sensitive detector. In the above case this was the well-known flame ionization detector used in gas chromatography (*16*, *17*). A very sensitive detector of this type developed by Johnson, Seibert, and Stross (*18*) is shown in Fig. 7. While the construction and operation is much more complex than previous detectors, the sensitivity is much higher. As Fig. 8 shows, 0.1 μg amounts are detectable. The

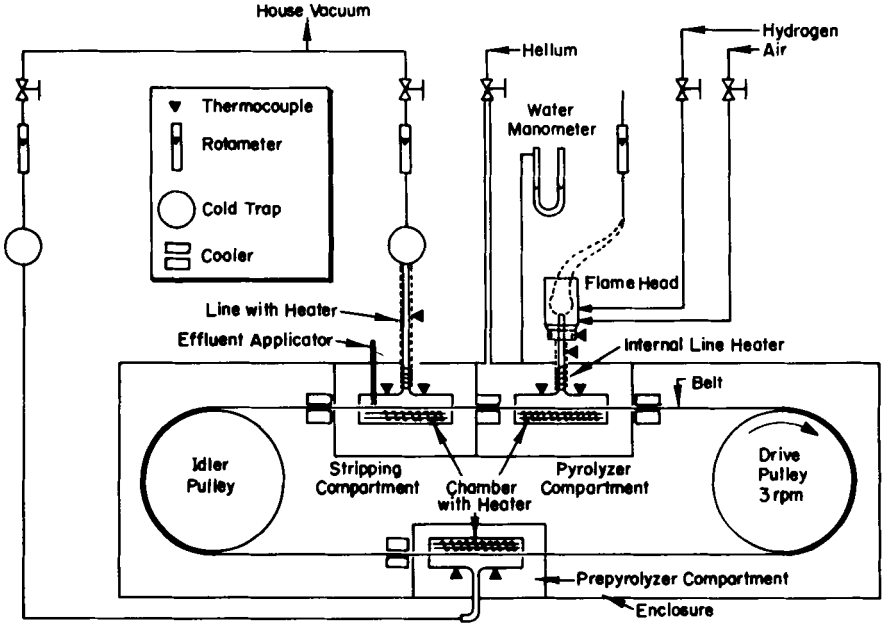

FIG. 7. Schematic of belt detector (*18*).

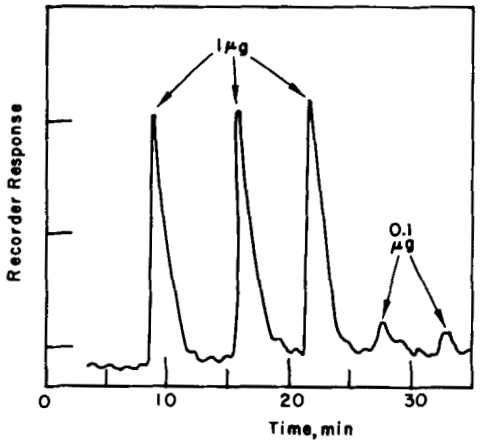

FIG. 8. Belt detector response, polystyrene (*18*).

nickel chromium alloy belt moves at a speed of about 2.5 cm/sec. A temperature gradient is used to remove the solvent in the stripper section. The gradient is usually somewhat below the solvent boiling point at the entrance to 100°C above the boiling point at the exit. The optimum temperature for the pyrolysis chamber depends on the specific polymer, ranging from 450–550°C. A flame ionization detector and electrometer comprise the detector.

Polyether polyols have been separated by GPC and the concen-

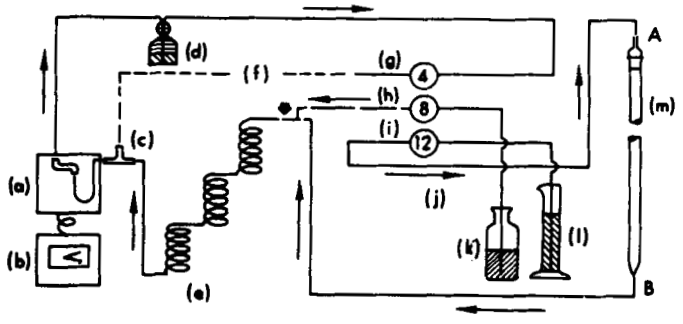

FIG. 9. Flow diagram of gel permeation chromatograph using a recording colorimeter detector (*19*): (a) colorimeter, (b) recorder, (c) separator, (d) displacement bottle, (e) mixing coil, (f) waste, (g) Tygon tube, (h) Tygon tube, (i) Acidflex, (j) proportioning pump, (k) cobalt thiocyanate reagent, (l) EDC reservoir, (m) column.

tration determined from the cobalt thiocyanate complex using a recording colorimeter by Kondo, Hori, and Hattori (*19*). The apparatus shown in Fig. 9 was sensitive with a linear response versus concentration. Its applicability is limited to systems where a suitable reagent exists.

For solvent-polymer systems where they are applicable, conductivity detectors (*20*) and ultraviolet spectrometers (*21*, *22*) are sensitive, inexpensive instruments.

ELUENT VOLUME DETECTORS

To record the volume of eluent through the column as a function of time, a siphon that discharges when full and at each discharge sends a signal to the recorder where a blip is produced is most frequently used. This is a convenient arrangement, but is subject to error. Yau, Suchan, and Malone (*23*) in studies of the volume delivered by the siphon as a function of flow rate identified two sources of error. One was due to evaporation from the siphon and the other due to eluent continuing to flow into the siphon while it is discharging. Similar results were reported by Little et al. (*3*). Various methods of saturating the air in the siphon chamber with solvent vapor have been used to minimize evaporation. Cooper has found (*10*) that a siphon designed by Gray (*24*) gives more reproducible results. It would seem that developing a more accurate method for measuring the eluent volume would be a rewarding research area.

COMMERCIAL INSTRUMENTS

Recently, the number of instrument manufacturers interested in the liquid–solid and gel permeation market has increased. Older manufacturers have diversified their lines and now offer a large number of instruments in various price ranges for specific applications. Instruments satisfactory for GPC are currently being produced by Waters Associates, 16 Fountain St., Framingham, Mass.; du Pont Instrument Products Division, Wilmington, Delaware; Varian-Aerograph, Walnut Creek, Calif.; and Nester-Faust, 2401 Ogletown Rd., Newark, Del. Most of these companies also sell components as well as complete chromatographs. The cost of many commercial instruments is below the cost of custom construction in industry. Academic research workers can probably construct chromatographs more cheaply from components in highly specialized situations. However, the instrumentation scene

is rapidly changing and should be reviewed in detail prior to construction or purchase of GPC equipment.

DATA RECORDING

Conventionally, the signal from the polymer concentration detector and the eluent volume detector are recorded on standard potentiometer recorders. Other more sophisticated systems may be used. For example, one commercially available system (Waters Associates, Framingham, Mass.) uses a digital curve translator to monitor the signal from three gel permeation chromatographs simultaneously and provides both a printed and punched paper tape record of the output. Gregges, Dowden, Barrall, and Horikawa (*25*) have reported a system for direct reading of GPC data into the analog/digital interface of an IBM 1800 computer. The system was designed to work with the Waters Associates automatic sample injection system or manual injection. The final read out is both graphical and punched cards. The cards are in a form suitable for most of the currently employed data reduction programs.

Acknowledgments

The authors are pleased to acknowledge the courtesy of the editors and publishers of the following journals for permission to reproduce the figures cited: *Analytical Chemistry,* Figs. 4a, 7, and 8; *Bunseki Kagaku,* Fig. 9; *Journal of Polymer Science,* Figs. 1, 3, 5, and 6; *Journal of Chromatography,* Fig. 4b; and Waters Associates, Inc., Fig. 2.

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Received by editor March 17, 1970