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Extension of GPC Techniques*

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Summary

Most of the common GPC gels exhibit pore sizes which are too small to separate very extended molecules, e.g., native cellulose in solution. Several gel types were tested with vinyl polymers of molecular weights up to 10^7 and with cellulose nitrate. Large pore size Styragel with acetone as solvent proved to be the most favorable gel system yielding effective separations for polymers with coil diameters in solution up to 4000 Å.

The evaluation of GPC runs usually requires a separate calibration procedure. We attempted to determine the molecular weight of the eluate directly as it leaves the column. This is done by a special automatic viscometer that allows measurement of the viscosity of small cuts (e.g., 0.5–1 ml) of the effluent volume. A set of six capillary viscometers are loaded and unloaded continuously. The details of the apparatus are described and examples of the performance reported.

INTRODUCTION

The lecture presented at the GPC Symposium in Houston, February 24, 1970, covered two areas, the search for a gel material of large pore sizes and the development of a viscometric technique allowing continuous determination of the eluate leaving a GPC column.

For the first purpose different gel types were tested with very extended molecules in solution, e.g., native cellulose nitrate and poly-

* Based on a lecture 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.

methyl methacrylate. The best resolution was obtained with large pore size Styragels of $>5 \times 10^6$ Å using acetone as solvent. Acetone is a nonsolvent for polystyrene. It slightly shrinks the volume of the gel particles compared with tetrahydrofuran. Measurements in acetone therefore require specially packed columns, which can only be used with nonsolvents for polystyrene, the base of Styragels. As a detailed description of the packing procedure and the resolution power of this and other column fillings appeared elsewhere (*1*), we restrict this paper to a description of the new viscometer system.

Gel permeation chromatography hitherto measured only the amount of polymer contained in the effluent volume and the chemical composition of the polymer. Both variables can be reported continuously by refraction and absorption measurements. For instance, a differential refractometer allows recording of the difference of the refraction indexes of sample and reference fluid as a function of time

$$n = f(t) \tag{1}$$

with additional markings of volumes counts. Since $n \propto c \propto w_{V_e}$ and, for constant flow rate, also $t \propto V_e$, the function

$$w_{V_e} = f(V_e) \tag{2}$$

is experimentally accessible. The term w_{V_e} is the normalized weight fraction of the polymer within the eluted volume V_e.

Since the desired molecular weight distribution is

$$w_M = f(M) \tag{3}$$

one has to establish a special calibration function with narrow samples of known molecular weight of the same polymer type

$$M = f(V_e) \tag{4}$$

which allows to transform w_{V_e} to w_M and V_e to M. Likewise, each single GPC elution curve contains a relation (4). Therefore, the potential of the GPC technique will not be fully realized until one can omit the separate calibration and determine the molecular weight of the polymer directly as a function of the eluted volume.

Recently a continuous method of molecular weight determinations on a flowing solution was given by Cantow (*2*), who used a light-scattering cell with two distinct angles of observation. This method

implies the use of a θ-solvent to avoid extrapolation to zero concentration. The light-scattering apparatus together with a differential refractometer basically allows determination of w and M of solutes in column effluents.

There are now many techniques available which allow estimation of M_n by one of the colligative properties in a matter of minutes. In all of these cases equilibrium must be established, which is scarcely possible with a flowing liquid of changing composition.

A way to determine M by continuous viscometric measurements has been reported by the author (*3*). The situation seems to be somewhat more favorable than with the light-scattering technique. If η is measured continuously for small cuts $\triangle V_e$, the ratio of the viscosities of the polymer-containing effluent to that of the pure solvent, $\eta_r(V_e)$, can be calculated. The intrinsic viscosity

$$\lim_{c \to 0} \frac{\eta_r - 1}{c} = [\eta] \tag{5}$$

may be obtained by any of the numerous extrapolation methods from η_r and c, the concentration measured with a differential refractometer. $[\eta]$ is converted to the molecular weight by the relation of Kuhn-Mark-Houwink-Sakurada:

$$[\eta] = KM^a \tag{6}$$

This is a universal relation and has to be established only once for the elution solvent. It is not restricted to a special apparatus as are Eqs. (1) and (4).

DESCRIPTION OF THE GPC RECORDING UNIT

Usually the effluent from a GPC sample column passes a refraction cell and/or an absorption cell and then enters a siphon. When the siphon is emptied, a mark is produced on the chart recording the refraction difference and/or the absorbance. Since normally the flow rate is not completely constant, the distance from count to count varies slightly. The recorder trace often has to be corrected to obtain precise effluent volumes. Of course the correction can be avoided by applying a constant volume flow, which is possible by special regulating devices. It is more economical to use a drop counter such as that shown in Fig. 1. This schematic shows how the eluate passes the detectors for concentration and chemical composition before it

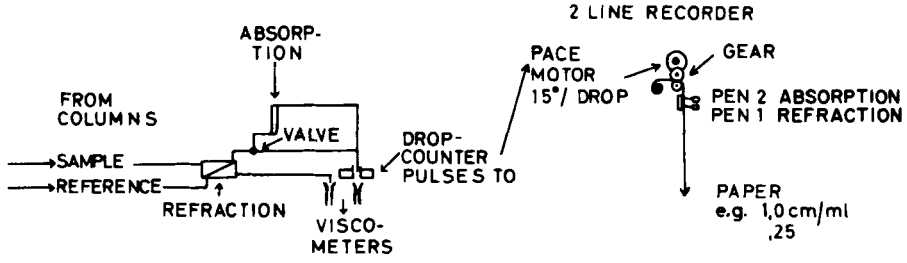

FIG. 1. Schematic diagram of detectors attached to GPC columns. Concentration is measured by a differential refractometer, chemical composition by an additional absorption spectrometer, and volume by a drop counter, which moves the chart drive of the recorder. The details of the viscometer are given in Fig. 2.

leaves the tubing as single drops which interrupt the light barrier in the drop counter and then enter a viscometer. Each drop renders a pulse, which is intensified to activate a pace motor. With our special setup, the motor axis is rotated 15 angular degrees per drop. A gear box slows down the rotary motion, so that the chart paper of the recorder moves, e.g., at a rate of 1.0 or 0.25 cm/ml, as a function of volume instead of time in any case.

The drop size depends on the surface tension, which varies slightly with polymer and polymer concentration. Since the concentration of the polymer in the effluent volume is low, the effect on the drop size and on the recorded volume is smaller than the error resulting from a very good siphon. Aside from this, it is practically impossible to use a siphon for volume counts appreciably smaller than the usual 5 ml, while a drop counter, if operated electronically, allows any reasonable volume counts to be recorded, e.g., 1 ml, 2 ml. A further advantage of a base line divided into volume units is that the evaluation and comparison of GPC runs with different flow rates are easily made.

Additional time counts are marked on the chart edge for control.

DESCRIPTION OF THE AUTOMATIC VISCOMETER

A special viscometer is needed to measure the viscosity of the effluent continuously. Many automatic viscometers which repeatedly measure the flow time of a liquid have been described and are commercially available, but they must be loaded and emptied by hand. Our viscometer has the advantage of automatic filling and unloading besides having automatic timing.

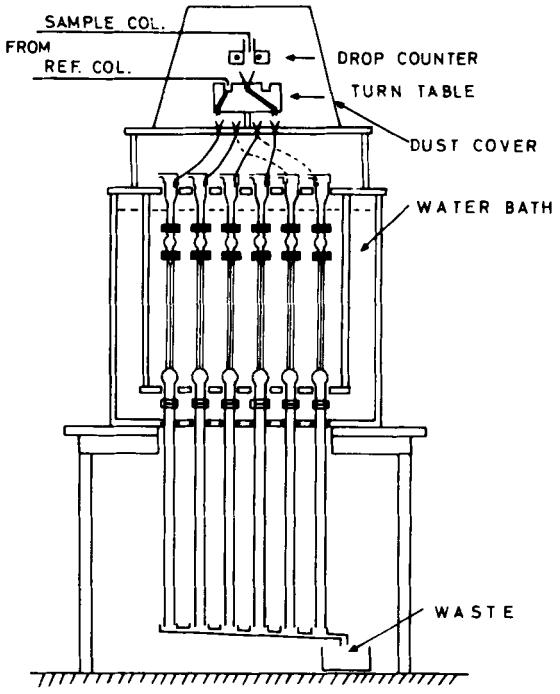

FIG. 2. Automatic viscometer with rotating turn table for consecutive filling of six viscometers. For details see text.

This is done with the arrangement demonstrated in Fig. 2. Six viscometers of free-flowing type are placed side by side in a thermostated water bath ($\Delta T < 0.003°C$). The volume of the viscometers is 0.5 ml; the capillary has a diameter of 0.03 cm and is bent backwards (below the plane of the drawing), thus accommodating a length of 17 cm and a medium driving height of 14 cm. The flow time for THF is >135 sec, while the filling time at a flow rate of 1 ml/min is less than 45 sec. This permitted omission of the valves at the outlet of each viscometer, which were previously described (*1*). The Teflon valves had been operated by two rotary magnets. They had been closed for filling only, but they had exhibited mechanical shock and leakage problems.

The timing of the six viscometers is performed by light barriers measuring the decrease of the intensity due to the falling meniscus. Two phototransistors start and stop an electronic counter for each

viscometer. The counters' digits are printed out together with the number of the viscometer being timed and the hours and minutes of daytime. If the flow exceeds 200 sec, the capillary of the viscometer is likely to be partially blocked. Then an alarm is given and the viscometer must be replaced by a spare viscometer.

The liquid which has passed the capillary leaves the viscometer via a stainless steel tube connected to its glass outlet. The stainless steel tube is placed in a boring of the thermostat bottom and tightened by a rubber bearing, which allows the raising the viscometer until the glass steel connection is above the meniscus of the water bath. In this manner each viscometer can be separatively exchanged.

For the consecutive filling of the six viscometers, a rotating turntable is used. It is moved from 30° to 30° by a pace motor not shown in Fig. 2. The sample fluid, after passing a drop counter, enters a smaller funnel from which it runs down a stainless steel tube, while the reference fluid enters a circular groove of the turntable and flows down another tube. The exits of these tubings let the liquids enter two funnels from which they are led to the viscometer entrances through a small cap. Six of these funnels are arranged on a circle at 60° intervals. By this method two measuring rhythms are possible. If the viscometers are filled alternately with reference and sample fluid, η_r is measured with 2 ml intervals. If the reference line is not used, the viscometers are filled with the sample fluid only; and η_r can be determined in 1 ml intervals as demonstrated in Figs. 3 through 5.

The turntable is triggered by the meniscus of the liquid when it passes the upper light barrier of a viscometer. It moves with a time lag of 5 sec by 30°, placing the ends of the fluid lines between two funnels. About 5 sec after that, the counter is started. The rotary movement to the next full 60° position of the turntable, regulated by a clock, always brings the ending of the central funnel to the next viscometer at each full minute. Thus, the stepwise rotation is alternately controlled by the upper light barrier and an electronic clock. The 30° positions are necessary to avoid any overfilling of the viscometers. The 60° positions are enforced by a safeguard, so that an incomplete filling of one viscometer will not ruin the correct rhythm.

The drop counter, the turntable, and the viscometer entrances are placed below a dust cover, which also maintains a solvent saturated vapor around the drops.

TEST RUNS WITH POLYSTYRENE

For our test runs, 3 Waters Styragel columns $>5 \times 10^6$, 7×10^5, and 10^4 were used with tetrahydrofuran as a solvent. The filling loop contained 1.96 ml. Standard Polystyrene 1,800,000 from Pressure Chemical Company, Pittsburgh, was used with concentrations ranging from 1 to 4 mg/ml. In Fig. 3 both the concentration and η_r, the

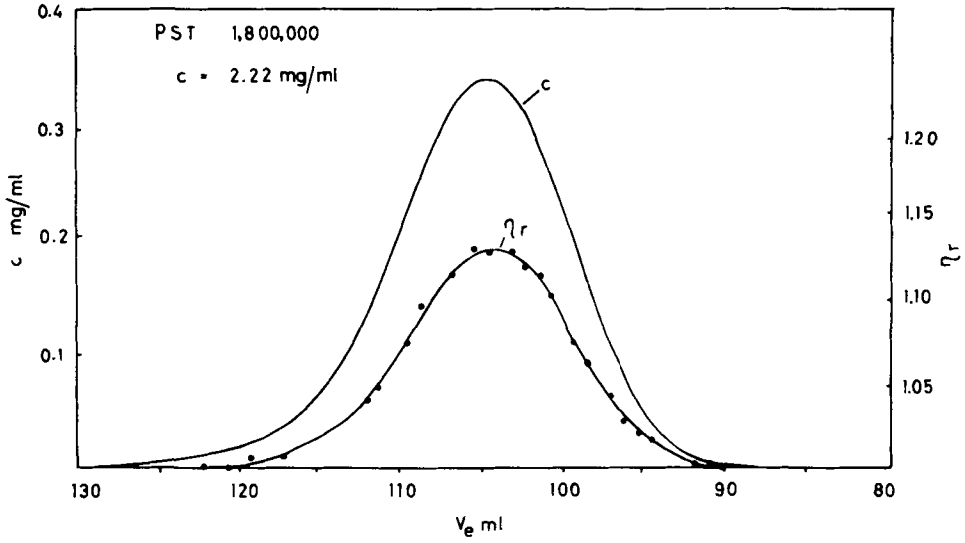

FIG. 3. Concentration and relative viscosity of a polystyrene with M = 1,800.000 after passing three Styragel columns with THF as produced by a differential refractometer and an automatic viscometer as a function of the effluent volume.

latter indicated by small open circles, are shown as a function of the effluent volume for a solution of $c = 2.22$ mg/ml. The η_r scale given at the right ordinate follows directly from the printed times of our tape. For the determination of the correct scale of the concentration, some precaution is necessary.

DETERMINATION OF CONCENTRATION

Usually it is sufficient to plot the difference of the refraction index and, hence, the concentration in arbitrary units. But for the calculation of $(\eta_r-1)/c$, it is essential to know the correct absolute value

of the concentration. In this case it is not permissible to calibrate the differential refractometer in the usual way with static fillings of polymer solutions of known concentration. After passing a GPC column, the solvent contains small amounts of water and other low molecular weight impurities which exhibit a pronounced effect on *dn/dc*. This effect is as large as that due to negative and positive tails of a GPC run. Therefore, we measured the total area below the concentration line, which corresponds to (2.22 mg/ml) (1.96 ml) = 4.34 mg of polymer. This, together with the total volume over which the polymer is distributed, allows us to calculate the concentration scale given at the left ordinate. For this method it is essential to have a correct volume base line as produced by our drop counter-step motor combination.

The same column set and polymer were tested at three more concentrations. Figure 4 shows the recorder traces from the differential refractometer for $c = 4$, 3, 2, and 1 mg/ml. The concentrations in the eluates ranged up to 0.47 mg/ml for the highest and up to 0.17 mg/ml for the lowest injected sample concentration. Concentrations >4 mg/ml exhibited an overload effect, characterized by a delayed second peak, viz., near $V_e = 117$ ml. The second peak starts to show up slightly at $c = 4$ mg/ml.

The areas under the peaks of Fig. 3 proved to be proportional to the concentration. This was not exactly the case when the usual timing method for the injection of different volumes was applied. Very

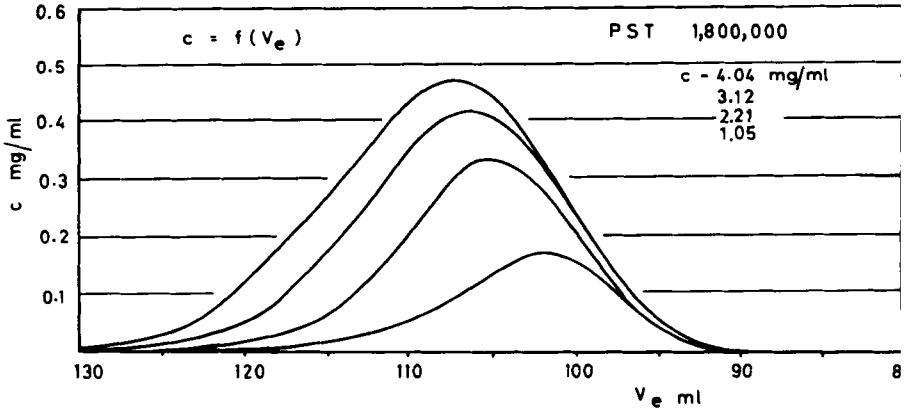

FIG. 4. Recorder traces of the differential refractometer for four different concentrations of polystyrene as a function of the effluent volume.

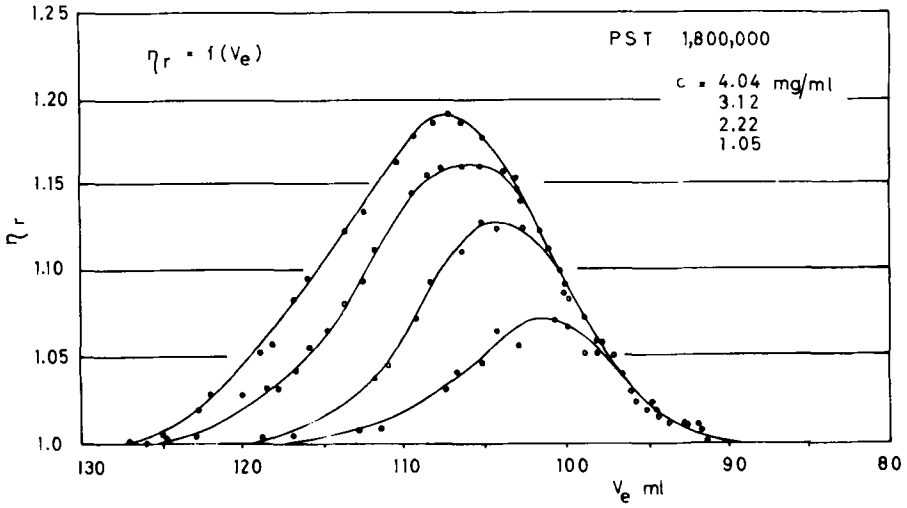

FIG. 5. Relative viscosities as a function of the effluent volume corresponding to the concentration curves given in Fig. 4.

likely some part of the polymer remains at the walls of the filling loop. It is therefore preferable to use separate filling loops of the proper volume to inject smaller volumes of solution (at Mainz 0.25, 0.5, 1.0, and 2.0 ml). The injection valve stays open for at least the threefold loop volume.

In Figure 5 the relative viscosities of the runs of Figure 4 are represented by open circles and connected by smoothed curves. Naturally the deviations are more pronounced for the lowest concentrations.

CONCLUSIONS

Our measurements reveal the feasibility of direct determinations of molecular weight in the eluates of a GPC apparatus by consecutive viscosity measurements. Since the viscosity of a solution increases with molecular weight and concentration, higher concentrations are needed for polymers of lower molecular weight. As a rough measure, the concentration may be increased inversely to the intrinsic viscosity if two samples of similar distribution but different molecular weight are tested.

The technique described here looks quite promising for further automation of GPC and its evaluation procedures.

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