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RECENT DEVELOPMENTS IN GEL PERMEATION CHROMATOGRAPHY:
HIGH SPEED AND HIGH RESOLUTION

INTRODUCTORY LECTURE

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SUMMARY

High resolution, high speed and heavy load are each attainable by gel permeation chromatography each at the expense of the other two. However, by use of appropriate compromises any two may be increased without significant loss. For example, flow rate and load may be increased to gain throughput by recycling to increase column length. Further flow rate and column length or flow rate and temperature may be increased to increase speed. However, when highest resolution is desired, load, flow rate, and temperature must all be optimized.

INTRODUCTION

The chromatographer is confronted with a choice between three interdependent factors, *i.e.*, resolution, speed and load, which can be represented by an equilateral triangle with one factor at each apex¹. In this triangular system an increase in one factor is gained at the expense of the other two. It is the purpose of this presentation to summarize recent development in gel permeation chromatography (GPC) in terms of this interdependence and to demonstrate some useful compromises.

During the past two years several major accomplishments have occurred in gel permeation chromatography which have resulted in new developments in techniques and equipment. Increased understanding of chromatographic principles led to increased speed through increased flow rates and increased temperature. Simultaneously, developments in instrument technology led to increased resolution at increased load through the use of recycle.

RESOLUTION IN GPC

First consideration is given to resolution since GPC is primarily a separation process. Numerous workers have contributed to the advances which led to high-resolution GPC. Particular attention is given to the work of ČOUPEK AND HEITZ², who clearly showed the value of both increased capacity ratio (KV_s/V_0) and optimized

solvent velocity in obtaining high resolution. The high capacity ratio was obtained by use of highly swollen, lightly cross-linked gels. The benefit of increasing capacity ratio (K') through increased pore volume is discernible in the expanded resolution equation³, which describes resolution in terms of selectivity, capacity and efficiency,

$$R = \frac{1}{4} \left(\frac{K_2}{K_1} - 1 \right) \left(\frac{K'}{1 + K'} \right) \sqrt{n} = \frac{1}{4} \left(\frac{K_2}{K_1} - 1 \right) \sqrt{N} \quad (1)$$

where $N = 16\{(V_e - V_0)/W\}^2$ represents the number of effective plates, n the number of theoretical plates, K the distribution coefficient, $K' = KV_s/V_0$ the capacity ratio, and W the peak width.

Plate number n , widely used to evaluate the resolving power of a GPC column, can be misleading since resolution is related to the number of effective plates, N , where correction is made for column void volume. Since K_2/K_1 is related to molecular size, the selectivity term is generally fixed in GPC. The capacity term, however, must be considered when choosing a GPC column packing since the phase ratios (V_s/V_0) of commercial gels range from 0.5 to 2. (A column packed with a $V_s/V_0 = 0.5$ gel would require four times the plate number, *i.e.* four times the length, to provide equal resolution equal to a column with $V_s/V_0 = 1$.)

While highly swollen gels offer the advantage of high phase ratio they are subject to compaction when exposed to high solvent velocities. This is particularly true with macroporous gels. Consequently rigid gels⁴⁻⁷ have found virtually universal acceptance in instrumented GPC. The phase ratios of most commonly used rigid gels range between 0.8 and 1.2, with a nominal value of 1.

With the capacity term of the resolution equation limited by the phase ratio of rigid gels to values between 1 and 2 increased resolution must be attained by an increase in the number of effective plates of the column, N (*cf.* ref. 8), which is accomplished by increasing column length⁸. Increased column length is accompanied by a proportional increase in both pressure drop and cost.

An alternative to long columns as a means of increasing N is to recycle the solute through the columns^{9,10}. The approach is particularly reasonable in GPC since K values do not exceed unity and maximum elution volume is fixed by the volume of the columns in the systems. Late peaks common to sorption chromatography need not be considered.

Peak width in recycle chromatography

Peak width (W) of a single species in recycle chromatography relates to cycle number (ν) by the relationship

$$W_\nu = V_\nu \left(\frac{16H}{L_\nu} \right)^{1/2} \quad (2)$$

Since $V_\nu = \nu V_0$ and $L_\nu = \nu L_0$ peak width of the ν -th cycle is

$$W_\nu = W_0 \sqrt{\nu}.$$

For W_0 to be valid it must include the contribution to spreading of the entire system,

i.e. postdetector and pump. When injection is by means of a valve after the pump $\Delta W/\text{cycle}$ must be determined and W_0 obtained by extrapolation.

High resolution with recycle

The distance between peaks in recycle chromatography increases as a function of the number of cycles. Resolution with recycle is therefore described by the expression

$$R_v = \frac{vV_2 - vV_1}{W_v} = \frac{v(V_2 - V_1)}{W_0\sqrt{v}} = \frac{\sqrt{v}(V_2 - V_1)}{W_0} = \sqrt{v}R_0. \quad (3)$$

Actual distribution of a polydispersed solute at maximum resolution may be defined¹¹ by eqn. 4

$$(W_T/v)^2 = W_c^2/v + W_s^2 \quad (4)$$

where W_T is the total width of a distribution, W_c = width due to random dispersion, and W_s = width due to separation. A plot of $(W_T/v)^2$ versus $1/v$ yields a straight line which, when extrapolated to $1/v = 0$, defines W_s^2 at infinite cycles. By this technique WATERS¹¹ extrapolated a 16-cycle separation of a narrow fraction polystyrene with a molecular weight of 173000 to $D = 1.0025$.

THE PRACTICE OF RECYCLE CHROMATOGRAPHY

Recycle chromatography, first considered in gas chromatography¹², was introduced in gel filtration by PORATH AND BENNICH¹³ using a peristaltic pump at low pressure and low velocity. In our work¹⁰ a small volume reciprocating pump (Milton Roy) used in commercially available GPC equipment was shown to be capable of recycle operation with minimal band spreading^{10,14}. In our original work the outlet to the detector was connected to the suction of the pump with a minimum amount of small-diameter tubing. During recycle the inlet from the solvent tank was left

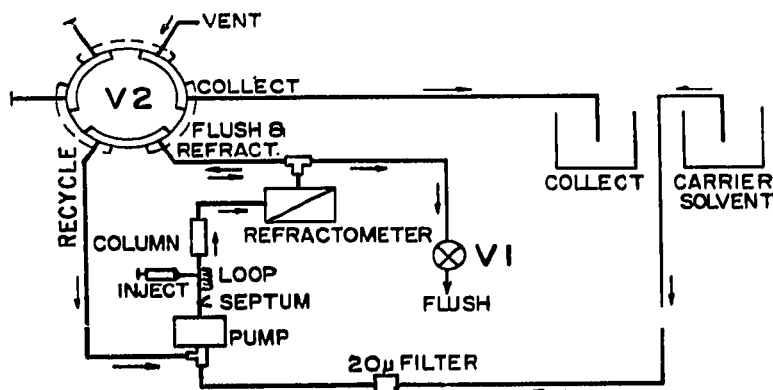


Fig. 1. Schematic diagram of recycle operation. Method of operation: (a) Recycle to collect sequence (V2 in recycle position). (1) Turn pump and recorder off. (2) Flush with approximately 15 ml of solvent by opening and closing V1. (3) Turn V2 to "collect" position. (4) Turn pump and recorder on. (b) Collect to recycle sequence (V2 in collect position). (1) Turn pump and recorder off. (2) Turn V2 to "recycle" position. (3) Flush as "recycle to collect" sequence. (4) Turn pump and recorder on.

open to prevent cavitation. The system was changed from recycle to collect modes by means of needle valve.

High-performance recycle equipment

For simplified operation a system was designed around a 6-port valve as shown in Fig. 1. The valve was designed into a Waters Associates GPC/ALC Model 301 using minimal lengths of small-diameter transport tubing. The system operates at flow rates up to 3 ml/min and pressures up to 1000 p.s.i.

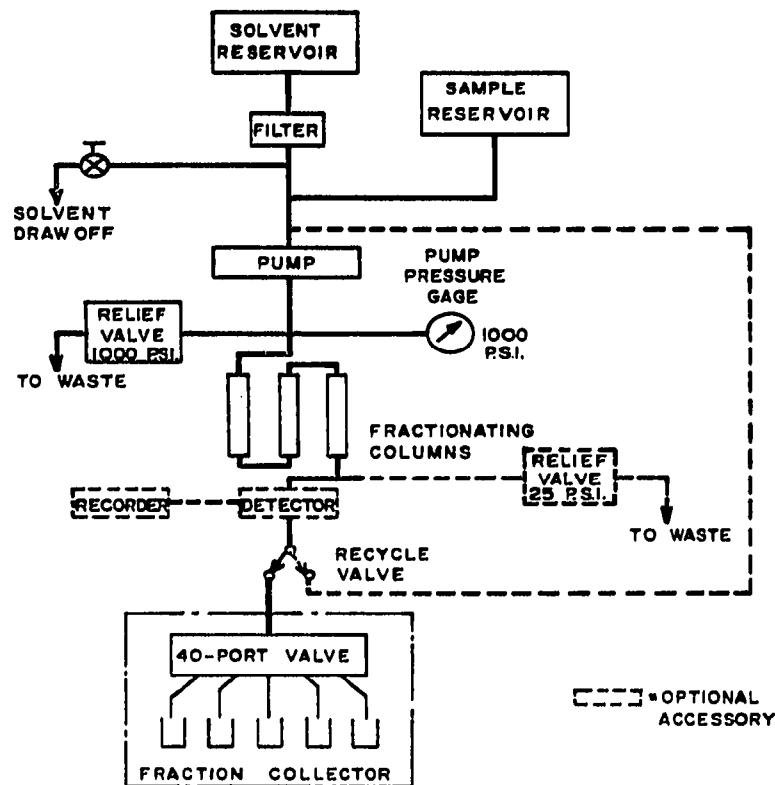


Fig. 2. Schematic diagram of Chromato-Prep.

The method of operation shown in the caption is vital to successful use of recycle since it includes a procedure to draw off the sides of the distribution to prevent peak overlap¹⁴ and also provides a method of flushing the solvent inlet Tee to prevent spurious peaks caused by reinjection of any solute which may have migrated into the inlet line during recycle operation. The recycle system shown is usable with columns ranging between 0.303 in. and 1 in. in I.D.

Large-scale GPC, using recycle and heavy load

A large-scale preparative apparatus, the "Chromato-Prep", shown schematically in Fig. 2, was developed to fractionate, in the recycle mode, gram quantities of material using columns up to 2.24 in. I.D. by 4 ft. in length. The "Chromato-Prep" is equipped with a constant displacement piston-type pump operable to 1000 p.s.i. at flow rates between 10 and 100 ml/min. Sample is injected through the pump by

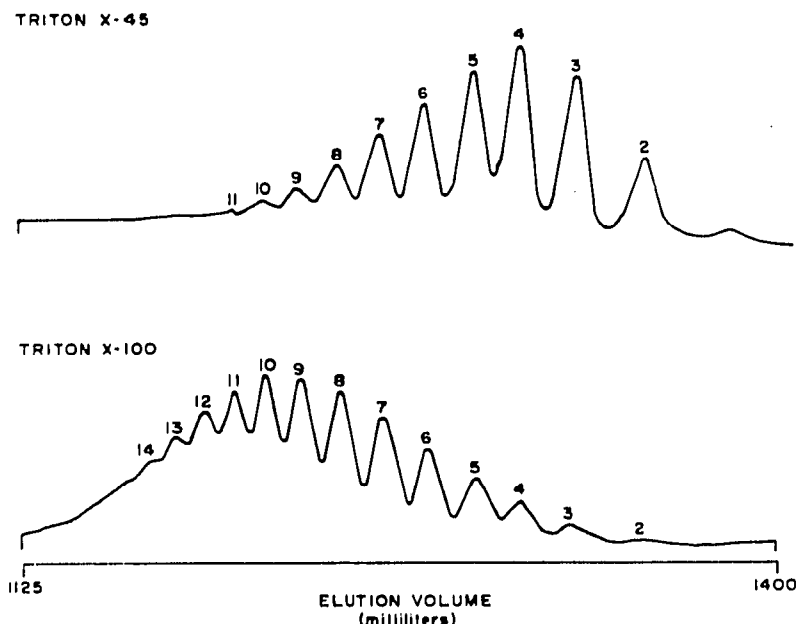


Fig. 3. High-resolution GPC separation of surfactants (nonylphenol-ethylene oxide adducts). Analytical/operating conditions: columns, 160 ft. \times 3/8 in. O.D. 500 Å gel; flow rate, 0.4 ml/min. The numbers 2 through 14 represent moles of ethylene oxide per molecule of nonylphenol.

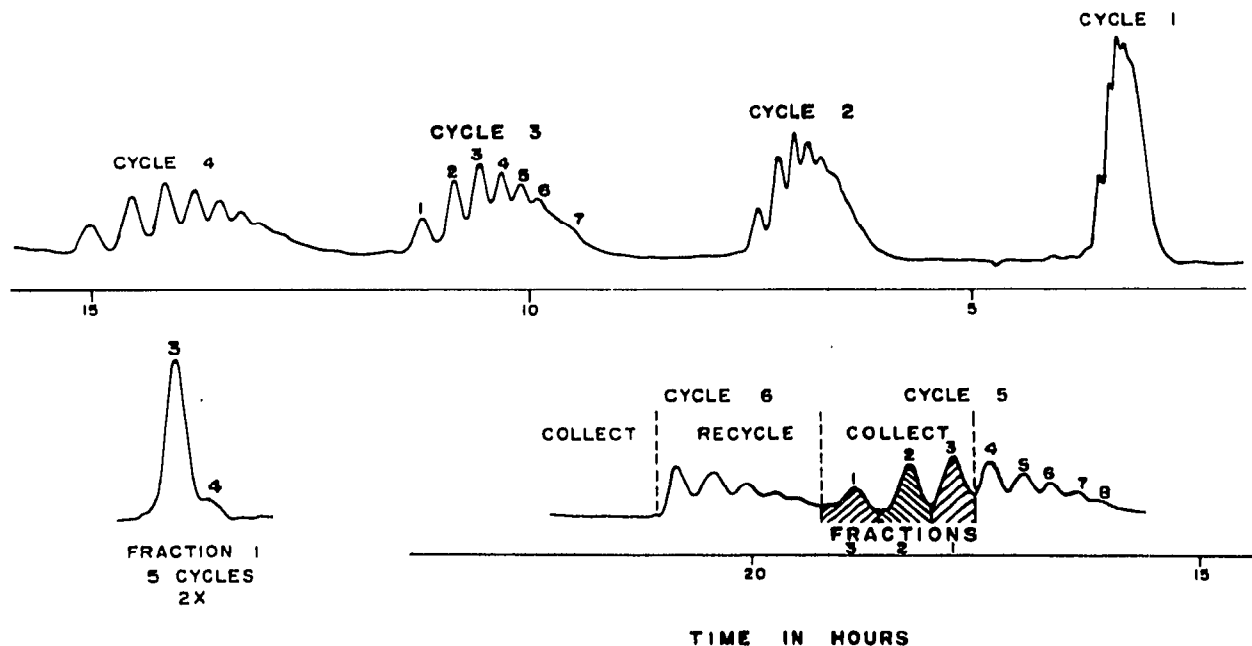


Fig. 4. Effect of cycle number on resolution. Sample, Triton X-45; concentration, 50%; injection volume, 30 μ l; solvent, THF; flow rate, 0.48 ml/min; columns, Styragel 60 Å (15 ft.).

means of remotely operated electrical controls mounted on a graphic panel on the front of the instrument. The instrument can accommodate up to four columns 4 ft. in length by 2.24 in. I.D. The columns are mounted vertically in a recess in the sides of the enclosure. Columns are of stainless steel construction designed to withstand

pressures up to 1500 p.s.i. Nominal pressure drop for 2.24 in. columns, packed with Styragel is 200 p.s.i. for toluene at a flow rate of 100 ml/min at room temperature. Provision is made for both refractive index and UV detector. The refractive index detector is equipped with a 28° cell to provide an increase in linear range at heavy sample load. A detector is essential to recycle operation since the operator must see when resolution is adequate to collect fractions or when peak sides must be drawn off to prevent overlap.

EVALUATION OF RECYCLE OPERATION

Recycle operation may be compared with single-pass chromatography by use of Figs. 3 and 4. The Triton X-45 resolved by a 160-ft. column on a single pass was also resolved by a column 15 ft. in length after six cycles. Peaks 1, 2, and 3, removed after the fifth cycle to prevent overlap, were collected as individual fractions. Peak 3 evaluated separately shows only minor contamination by peak 4. To resolve oligomers it is desirable to provide enough system capacity to obtain the desired resolution before peak overlap occurs. This is illustrated in Fig. 5. At the high flow rate six cycles were completed in 4 h, but peak overlap resulted before discrete peaks were observed. At the lower flow rate individual peaks are resolved after the second cycle and four cycles (19 h) are completed before overlap occurs. By increasing column length from 20 to 32 ft. and operating at an intermediate flow rate discrete peaks were resolved before overlap occurred¹⁴.

Load versus resolution

The deleterious effect of increased load on resolution is evident from Tables I and II (*cf.* ref. 14). Also evident is the effect of increased column capacity gained by recycle; Fig. 6 shows chromatograms of a 3.5-g load after three cycles.

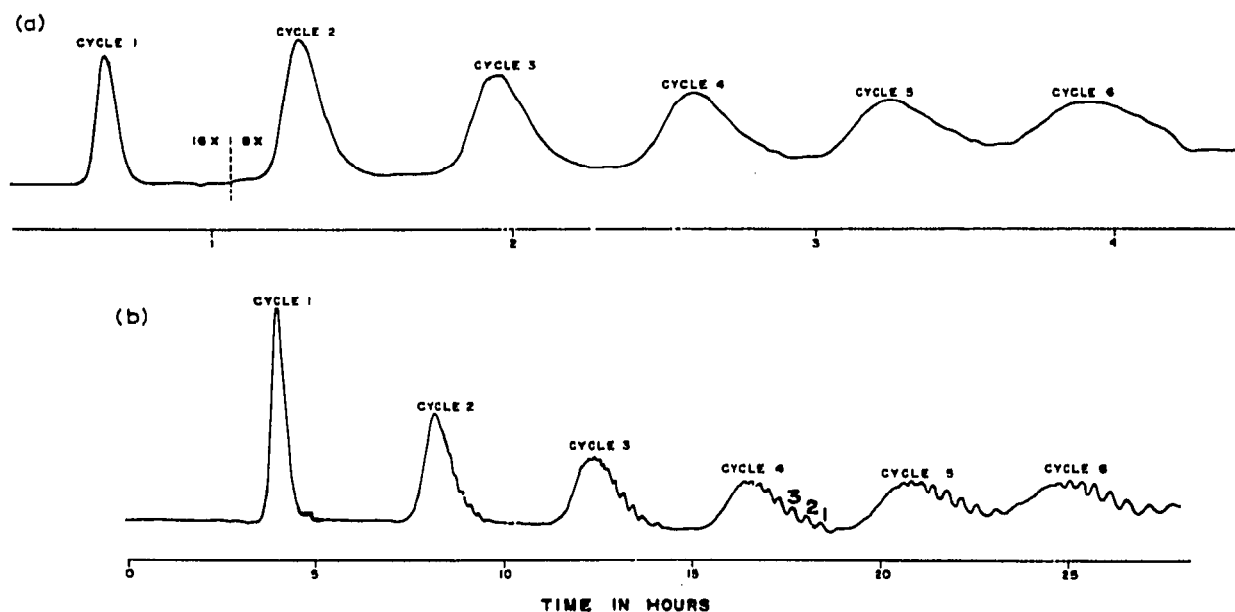


Fig. 5. Effect of flow rate on capacity requirement. Sample, Triton X-100; concentration, 50%; injection volumes, (a) 50 μ l and (b) 70 μ l; solvent, THF; flow rates, (a) 3.1 ml/min and (b) 0.5 ml/min; columns, Styragel 100 Å (8 ft.) and 60 Å (12 ft.).

TABLE I

RESOLUTION AT VARIOUS SAMPLE LOADS USING CONSTANT VOLUME (100 ml)

Sample load (g)	Concentration (mg/ml)	Resolution		
		Cycle 1	Cycle 2	Cycle 3
1.0	10	1.06	1.31	1.47
2.0	20	0.59	1.13	1.29
3.5	35	0.34	0.77	1.14
5.0	50	0.25	0.54	0.92

TABLE II

RESOLUTION AT VARIOUS SAMPLE LOADS USING CONSTANT CONCENTRATION (10 mg/ml)

Sample load (g)	Volume (ml)	Resolution		
		Cycle 1	Cycle 2	Cycle 3
1.0	100	1.06	1.31	1.47
2.2	220	0.70	1.20	1.34
3.5	350	0.39	0.91	1.14

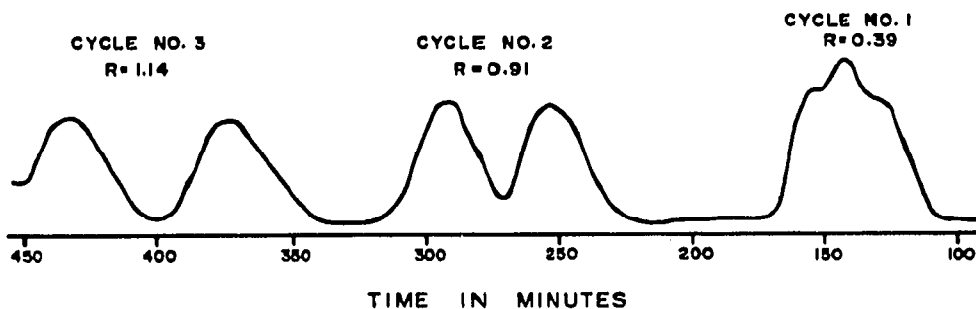


Fig. 6. Effect of recycle on resolution at heavy load. Sample, polystyrene mixture 51 K + 10.3 K (1-1); load, 3.5 g; concentration, 10 mg/ml; injection volume, 350 ml; solvent, toluene; flow rate, 14.4 ml/min; column, 2.5×10^4 Å Styragel (4 ft.).

Resolution is greatest at the low molecular weight (high K') end of the distribution. When a heavy load is applied to the column with recycle, the resolved end may be drawn off first, thereby increasing column efficiency for the more difficult end of the distribution. In this way column length is programmed according to the need of the problem and throughput is optimized.

Flow rate versus resolution at high speed

In GPC as in other forms of liquid chromatography retention time t_r may be described by equation

$$t_r = \frac{V_r}{F} = \frac{V_0 + KV_s}{F} = \frac{KV_s}{F} \left(\frac{1 + K'}{K'} \right) \quad (5)$$

In GPC as discussed earlier K , K' , and V_s are limited. Increased speed demands an increase in flow rate F . Increased flow rate increases peak widths (W). The relationship between F and W is best described in terms of solvent velocity (U) and plate height (H) by the empirical expression¹⁵:

$$H = aU^n$$

At velocities up to 2 cm/sec n values were found to be 0.3 for Styragel^{16,17} and 0.6 for Porasil¹⁵. Elution volume was shown to be invariant with flow velocity up to 2 cm/sec (*cf.* refs. 16–18). Flow rate dependence reported by some workers was seen only when large particles were used¹⁹. The large voids between the particles introduced large distances to be traversed by macromolecules, introducing non-equilibrium. With well packed columns of small uniform spherical particles steric exclusion prevails. Further, equal peak widths for all values of K' show virtually no resistance to mass transfer in the stationary phase³. Consequently increased speed is obtained by increased flow rate provided a moderate increase is made in column length to offset the efficiency loss accompanying the increase in velocity.

Resolution per unit time may therefore be increased by increasing both column length and flow rate simultaneously. This may be accomplished by recycle without increasing column head pressure. Fig. 6 shows the effect of flow rate on resolution at one, two, and three cycles. The resolution obtained in the third cycle at 121 ml/min was superior to that of the first cycle at 14.4 ml/min.

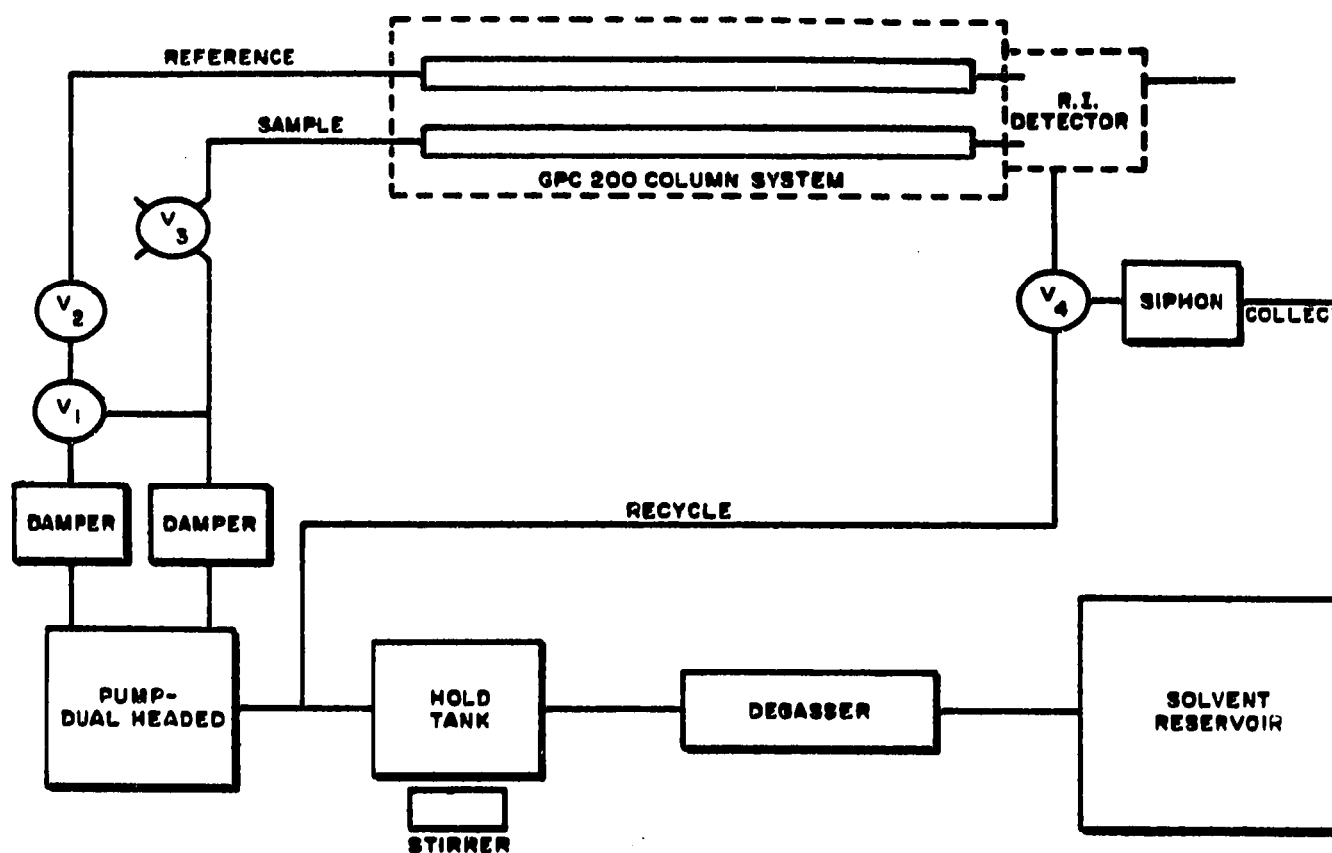


Fig. 7. High flow solvent supply system for GPC.

EQUIPMENT FOR HIGH-SPEED GPC

High-speed GPC requires equipment which affords the high pressures needed to produce high flow velocity as well as elevated column temperature to reduce solvent viscosity and increase diffusion. Two systems are described, *viz.* high-pressure and high-temperature.

High-pressure system

A high-pressure auxiliary pumping system is used with the Waters Associates GPC Model 200. A schematic diagram of the system is shown in Fig. 7. The unitized system may be used externally in place of the original pumping system. The dual-piston system affords pressures up to 1000 p.s.i. at flow rates between 0.4 and 7.0 ml/min as a dual-column system or up to 14 ml/min when flows are combined. The system injection valve is mounted outside the oven for high-pressure operation.

High-temperature system

The second system is a high-temperature oven for use with the Waters Associates GPC/ALC Model 301, which already has the high-pressure capability. The oven, designed to fit into the present column compartment, is temperature controlled to $\pm 0.1^\circ$ over a range of 35° to 200° . The oven accepts columns 2 ft. in length. Our work has shown that two such columns coupled by a short U-tube of 0.009 I.D. affords efficiencies equal to a single 4-ft. length of column.

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