

APPLICATION OF GEL CHROMATOGRAPHY*

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INTRODUCTION

The term Gel Chromatography refers to the process of separation of solutes caused by the passage of a solution through a column filled with a porous gel. The dissolved particles may enter the pores fully or partially or they may remain completely excluded. This results in a sorting out of the dissolved particles according to their size, so that the largest particles migrate with the highest rate.

The procedure was applied in about 1960 to aqueous solvents¹ with great success and to organic solvents² with limited success for the separation of polymers and was named gel filtration. Crosslinked dextran proved to be a very good porous gel. After a few years a highly cross-linked polystyrene³ became available as a gel for organic solvents, which resulted in a breakthrough of this separation technique, here named Gel Permeation Chromatography, as regards its application to synthetic polymers.

As in the title of Determann's book⁴, the term Gel Chromatography describes a separation process on porous gels. The term Chromatography refers to the experimental procedure and not to the micro mechanism. As with most of the chromatographic techniques applied to polymers, the classical chromatographic effect due to interactions with the surface of the bed material is quite undesirable. Every adsorption-desorption step complicates the chromatographic separation of polymers.

The numerous review articles on gel chromatography published so far can be divided roughly according to the solvents used⁵⁻¹³. Those for aqueous solvents deal mainly with biopolymers, whereas those for organic solvents are concerned mainly with synthetic polymers¹⁴⁻¹⁶. The recent monograph of Determann⁴ covers both types of solvent and polymer and gives an excellent description of the situation in gel chromatography at the end of 1966.

This presentation is likewise a review and covers also the possibilities of gel chromatography in analytical and preparative procedures. It will concentrate on synthetic polymers in organic solvents, since there are certainly more unsolved problems with polydisperse threadlike polymer compounds than with monomolecular biopolymers of globular form.

This paper is divided into two sections. The first treats the experimental details, while the second handles particularly the evaluation of the experiments. Finally, the preparative possibilities of this method are briefly discussed.

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EXPERIMENTAL TECHNIQUE

General arrangement of gel chromatography apparatus

In gel chromatography columns are nearly always filled with gel particles of small diameters. The solute is separated by transporting it with an eluent through the gel bed.

In the simplest case, as demonstrated in *Figure 1*, a vertical column with a fraction collector below the outlet is sufficient. The solvent flows from a reservoir by its own hydrostatic pressure through the column. The individual fractions in the collector are analysed separately.

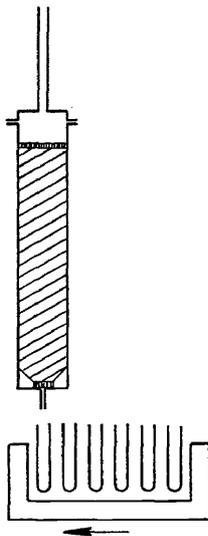


Figure 1. Simple column with fraction collector.

A nearly automatic apparatus with a pump for the solvent flow is shown schematically in *Figure 2*. From the reservoir (V) the solvent passes through

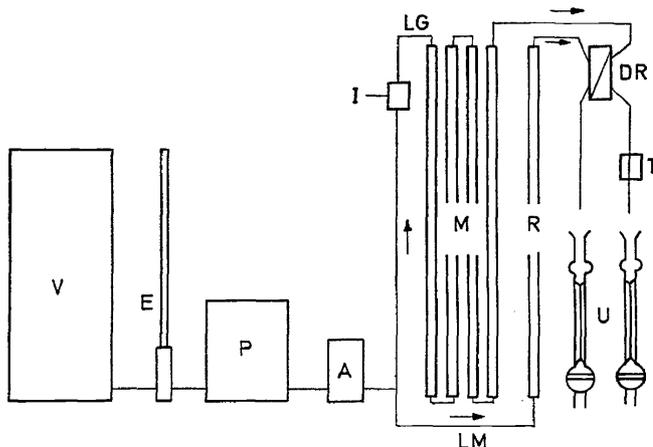


Figure 2. Integrated gel chromatography system with a pump and measuring devices for concentration (DR), volume (T), and molecular size (U).

the degasser (E), the pump (P) and the pulsation damper (A), if no pulseless pump is applied. If a differential detector is used, the solvent flow is divided into the measuring (M) and reference (R) columns and combined in the detector (DR). At (I) is mounted an injection valve for the loading of the samples. Behind the device (DR) detecting the concentration of the solute in the elution volume is placed a volume counter (T), and possibly an apparatus for measuring the molecular weight in the eluted volume, e.g. in *Figure 2* a viscometer (U). The outputs of (DR), (T) and (U) are recorded graphically or digitally.

Injector system

It is essential, in order to obtain a good resolution, to achieve an exact sample loading. With a column, open at the upper end and of not too small a diameter, the sample solution may be carefully poured onto the gel surface or a porous plate covering the gel. However, when working with higher pressure the pump-column system has to be closed. The loading in this case can be done by a three-way glass cock. A six-port valve, as installed in the Waters GPC, works better. Such a valve, however, exhibits sharp bends at the interface between solvent and sample and so contributes somewhat to the undesirable axial dispersion of the elution diagram. In the flow system outside the columns from the injector to the detector an appreciable spreading occurs, which results in an axial dispersion comparable with that of a well-packed column¹⁷. For this reason since 1966 a simplified injection valve has been used, which was developed for chromatography with porous membranes¹⁸, where a very exact loading is required on account of the small sample size.

This valve is shown schematically in *Figure 3* and consists of two fixed cylinders of Teflon (T) with a rotating cylinder of stainless steel (S) between

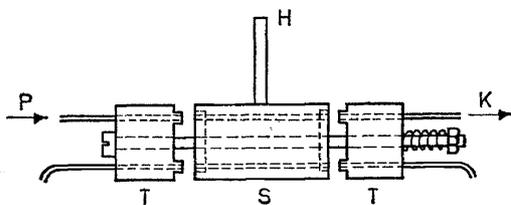


Figure 3. Injection valve with straight flow of the sample solution.

them. The three parts are mounted on an axis and pressed together by a spring (right side of *Figure 3*), so that even at high pressures (15 atm) no leakage can take place. The solvent from the pump passes the upper channels in the direction to the columns (P → K). The lower channels are filled with the sample. Turning the lever (H) by 90°, to the back in *Figure 3*, brings the sample into the liquid flowing to the columns, which can always move straight ahead. The flux is less disturbed as compared with the three-fold sharp bend occurring in other valves. For larger sample volumes an additional loop is installed in the lower channel of the middle piece.

Columns

The column fillings should be inert towards solvent and solute and capable of withstanding the high pressures necessary for the desired flow rate. For all rigid gels stainless steel tubing is a suitable material. For low pressure as used with swelling soft gels, glass tubes may be employed. The lengths and diameters of the columns vary appreciably. For swelling gels larger diameters are mostly used. It is convenient to use sections of columns, of e.g. not more than 150 cm length, connected in series, and to avoid extremely long straight or bent columns.

The technique of packing a column has a great influence on the resolution power. It is generally simpler to pack a soft gel, since small irregularities in the geometric arrangement are to a great extent adjusted after some time. Two methods have been reported for packing the quite rigid highly cross-linked styrene gels of Moore³. One can use a 2 per cent emulsion of the gel¹⁹, which is heated for degassing, then cooled down and brought as a slurry into the column. Column rotation during packing at about 1 r.p.m. enhances even spreading of the gel. The Waters Association apply and recommend a packing of the gel dissolved in a solvent mixture with the same density as the gel particles. A pump with greater flow rate is needed for this procedure. Both techniques allow the rigid styrene gels to be packed into columns of about 0.8 cm diameter, so as to produce a high plate count of 2000–6000 per metre column length with trichlorobenzene in tetrahydrofuran. One needs essentially a good gel consisting of uniform small spheres.

Polymer based gels

The separating gels used for gel chromatography can be divided into swelling and non-swelling materials. Additional cross-linking of linear polymers or a weak cross-linking during polymerization produce gels, which take up great amounts of solvent and swell appreciably. To this type belong the dextran gels, Sephadex^a, which proved to be very valuable for measuring and separating proteins, and other water-soluble polymers. Similar properties are shown by the Bio-Gels^b based on polyacrylamide^{20, 21} and agarose-gels^{22, 23, a, d}. It is possible to solubilize the Sephadex gels by introducing organophilic side groups. Similar swelling gels result from the copolymerization of methylmethacrylate^{24, 25}, and styrene with divinylbenzene²⁶, or of vinylacetate with divinyl compounds²⁷. Gels of larger pore size, which are needed for the separation of higher molecular weight compounds if produced in this manner, become mechanically less stable. They tend to condense slowly under their own weight and can be used only with a low external pressure.

Very rigid polymer gels of large pore size were produced by Moore³, who copolymerized styrene with divinylbenzene in the presence of different diluents, e.g. in a mixture of toluene and *n*-dodecane. The pore size of this Styragel^c depends on the composition of the reaction mixture, but all gels are rigid and swell only very little. Very recently, similar gels with large pores and great rigidity²⁸ were also prepared from vinyl acetate and divinyladipate in suitable diluents.

Inorganic gels

Silica gels are principally applicable to gel chromatography. Recently it became possible to produce this material as beads with well-defined diameters and pore sizes³¹. It is commercially available as Spherosil[®] or Porasil[®] and has already been used extensively in gel chromatography.

A glass powder of uniform pore size was developed by Haller³². This involves the use of sintered borosilicate glass, which is thermally treated to produce heterogeneous regions. The glass is crushed and the silica-poor phase is leached out leaving the desired pore system. The powder is well suited as a packing for polymers^{32, 33}. A similar glass, but with a much broader pore size distribution³⁴ can be purchased as Bio Glas^b.

At Mainz some compounds based on silica and aluminium oxide³⁵ were tested, because some of them possessed a favourable pore size distribution. They proved to be applicable as a separating gel for different polymers. All these inorganic gels are rigid, and the pore sizes do not vary in different solvents. Their fundamental advantage is that they can be used in nearly all solvents. The packing into the columns is very easy, involving essentially a slow rinsing of the dry gel into the column, which is gently vibrated.

A disadvantage is that the powder results from a crushing process, and therefore the particles are usually of irregular shape. This makes it more difficult to obtain tight packing.

Another disadvantage of inorganic gels is based on the interactions, which are possible with the polymer and evidently occur more often than with polymer gels. The gel chromatographic separation can be counterbalanced by an adsorption chromatographic process, diminishing and/or retarding the elution peaks. Under extreme circumstances it is possible to elute a low molecular weight before a higher one³⁶.

Concentration measurement

One of the most essential improvements in gel chromatography has been the use of a detector for continuous measurements of the concentration. This means that no isolation is necessary for the determination of the mass of the polymer in the eluted volume. There are various possibilities for measuring the concentration. Basically any property proportional to the concentration can be used.

For proteins in aqueous solvents the absorption of ultraviolet light is frequently applied. For this purpose flow photometers with small cells are needed, which show a low turbulence. Infrared absorption can also be used. Spectroscopic detectors have the advantage of high sensitivity in some cases and are able to detect specific chemical groups. The number of synthetic polymers to which they are applicable has hitherto been somewhat limited.

For polymers in organic solvents measurements of the difference in refractive index have a much wider range of applicability. For this reason such polymers are nearly always recorded by a differential refractometer. This records with high sensitivity and good reproducibility the refractive index differences, which are, for molecular weights over 3000, proportional to the concentration of the solute. For oligomers the decrease of dn/dc with decreasing molecular weight has to be considered, when the mass of single components is evaluated.

A further possibility exists in measuring the density of solutions, which can now be done rapidly and with great accuracy. Recently, a technique was devised involving working with small volumes of liquid and suited for operating continuously on flowing liquids³⁷. The inherent frequency of a bending oscillator filled with the solution is measured. In practice this is a hollow glass body excited to produce undamped oscillations. The frequency depends on the mass of the oscillator, and hence on the density of the liquid contained in it. Thus, a frequency meter serves to calculate the density of a solution, which is, for polymers above the oligomer region, likewise proportional to the concentration.

Volume measurement

For measuring the effluent volume, a siphon is often used. This is emptied e.g. after every 5 ml and then sends a pulse to the recorder, which produces a sharp vertical line. The siphon should be placed in an atmosphere saturated with solvent vapour, especially if low elution rates are used. The actual volume becomes greater for higher flow rates on account of the time needed for discharging the siphon³⁸. According to our experience, a drop counter using a light barrier and a photoelectric cell are preferable. The drop size depends only very little on the dissolved molecules in the eluate. Further, it is possible to divide the volume into smaller units, e.g. 1 or 2 ml. A siphon of so small a volume does not work very well. An electronic counter with preselectable count numbers and automatic zero positioning for small drops allows more precise volume measurements.

Molecular weight determination

For the evaluation of gel chromatography diagrams, representing the relation between elution volume V_e and the corresponding mass of the solute w_{Ve} , separate calibrations with narrow fractions are needed to obtain a relation between V_e and the molecular weight M . The possibilities of gel chromatography could be applied much more widely if separate calibration could be avoided. A way of doing this is to incorporate a method for molecular weight determination into the gel chromatography system. For example, a light scattering apparatus recently described³⁹ can be placed behind the differential refractometer. This apparatus allows continuous intensity measurements on a flowing liquid. For polymers in a θ -solvent the quantity c/M is recorded at a fixed observation angle. M can be then calculated from c/M and c which has been obtained using the differential refractometer. It is possible that the continuous light scattering measurements and their evaluation will be somewhat more difficult than automatic viscosity measurements. It was therefore decided to develop a continuous viscometric determination of molecular weights and to measure continuously the viscosity η_e of the eluent volumes. From η_e together with η_0 of the pure solvent $\eta_{sp} = (\eta_e - \eta_0)/\eta_0$ can be determined and the molecular weight M can then be calculated from one of the usual formulae

$$\eta_{sp}/c(1 + k \times \eta_{sp}) = K \times M^a. \quad (1)$$

The concentration c is determined by an integration of w_{Ve} over the volume loaded into the viscometer.

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For the measurement of η_e a new type of automatic viscometer is needed, allowing not only the timing but also the filling and emptying to be done automatically³⁹. *Figure 4* shows a viscometer out of a system consisting of six viscometers arranged circularly; these are alternately filled with pure solvent and with eluted solution. A rotary electromagnet (M) closes the valve (H)

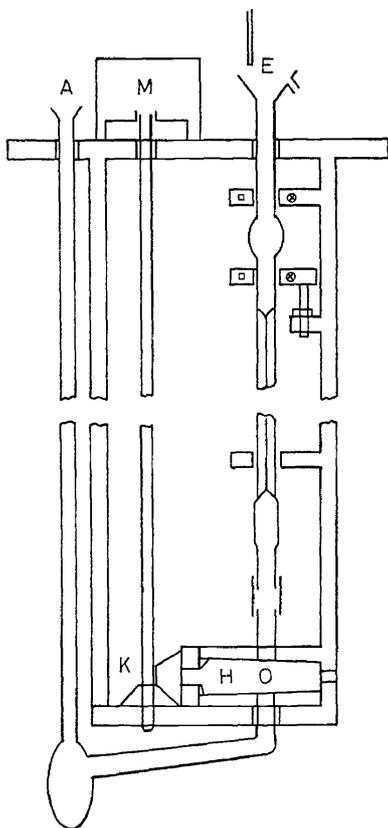


Figure 4. Viscometer for automatic loading, emptying, and timing.

and the liquid enters the viscometer at (E). Two light barriers together with two photo-diodes operate the magnet and an electrical time counter. When the valve (H) is open, the liquid flows freely through the viscometer and is finally withdrawn by suction through a narrow tube at the opening (A). The effluent times of the counter can be stored on a punch tape. As indicated in *Figure 2* one viscometer is filled with solvent, and another viscometer is at the same time filled with solution. The viscometers start the measuring process one after the other with a 60 sec time interval. In this way, out of each 2 ml of the effluent, 0.8 ml (the viscometer volume) is measured at a standard flow rate of 1 ml/min. This results in the mean average molecular weight of 0.8 ml eluate at 2 ml intervals. It is easy to change this to 1 ml

intervals if all viscometers are filled with solution. Spare viscometers allow rapid replacement of a viscometer with an unclean capillary.

Resolving power

The solution of a column or a set of columns, always with the injection and detection system and the connecting tubes included may be characterized by the number of theoretical plates according to the usual chromatographic relation

$$n = \left(\frac{V_e}{\sigma}\right)^2 = \left(\frac{4V_e}{b}\right)^2; \quad (2)$$

V_e is the elution volume at the peak of a monodisperse substance, σ is the standard deviation and b is the width in volume units at the peak base, between the tangents drawn through both inflection points of the elution peak. Dividing n by the column length L results in $N = n/L$, i.e. the number of plates per metre or per foot. $H = N^{-1}$ is the height equivalent to a theoretical plate H.E.T.P. The plate count may be influenced by the diameter of the column, although the diameter does not enter explicitly into the formula of classic chromatography.

The dependence of the elution peak on the molecular weight is often given by a logarithmic relation

$$\log M = A - B.V_e \quad (3)$$

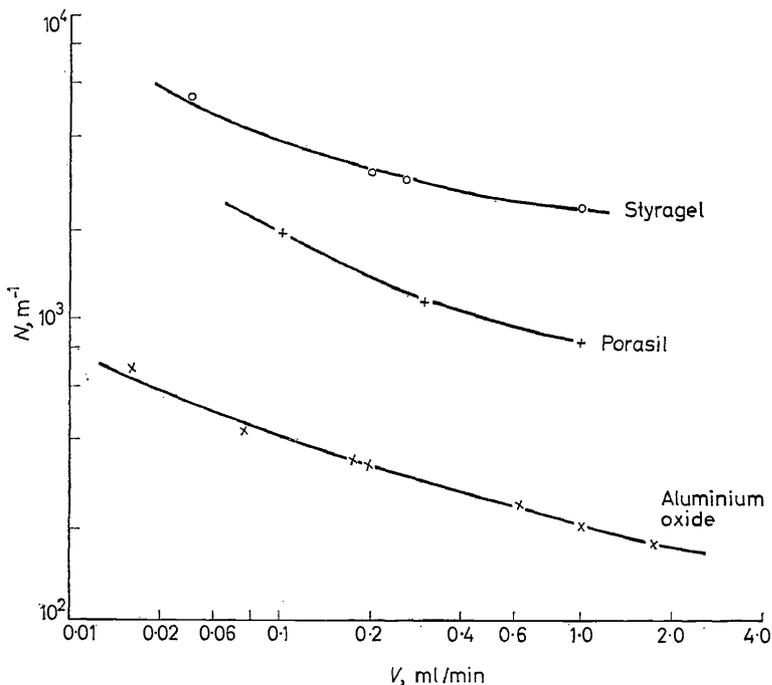


Figure 5. Plate count (N) of trichlorobenzene in THF in relation to the flow rate V (ref. 35).

Hence B , or more generally $d(\log M)/dV_e$, is a measure of the resolving power of a column. To get a good resolution of the product B , σ has to be small. Because $V_e \propto L$, σ rises as $L^{1/2}$, according to Eq. (2). Since at the same time B decreases as L^{-1} , the resolving power increases roughly with the square root of the column length. If N is determined for a low molecular weight substance, it reflects the quality of the column packing rather than the resolution expected for high polymers. Besides an increase of the column length, a decrease in the flow rate effectively improves the column resolution^{33, 40, 41}. *Figure 5* shows (for different gels in columns of 0.8 cm diameter and with tetrahydrofuran as solvent) how N for trichlorobenzene depends on the elution rate v . A steady increase of N is observed when v is decreased down to 0.015 ml/min. A decrease of N for low values of v cannot, as would be expected from axial dispersion, be observed in this range of column diameter. The relation between N and v is a complex one. In *Figure 5* an acceptable representation is given by $N \propto v^{-1/3}$ or $N \propto v^{-1/4}$ for various gels. The standard deviation σ and with it the undesirable axial dispersion decreases slowly but distinctly with diminished flow rate. For polymers, the effect on the resolution is often higher than for low molecular weight compounds^{40, 42, 43}.

POSSIBILITIES OF THE APPLICATION OF GEL CHROMATOGRAPHY

Calibration and molecular weight determination

It is common to establish the relation

$$\log M = f(V_e) \quad (4)$$

for the calibration of a gel chromatography column or a column set. One has to use the same solvent, temperature, elution velocity and the same polymer, which is to be studied later. The injection volume and the concentration of the calibrating samples of known molecular weight and of narrow distribution have to be kept constant and as low as possible. Obviously a detector of sufficient sensitivity must be used.

For calibration with samples of broad molecular weight distribution special techniques are available^{63, 64}. The first moment of the elution curve may be used.

A plot of $\log M$ versus the maximum of the elution peak (cf. *Figures 6 and 7*) corresponds to Eq. (4) and often shows the linear relationship of Eq. (3). Yet the calibration curves depend on the injected volume and the concentration⁴⁰. A fixed volume value, e.g. 0.5 or 1.0 ml, is required. For a constant injection volume the elution peak of high molecular weight samples tends to move to lower values of V_e if the concentration is decreased. For an exact evaluation the calibration curves as well as the recorder curves for the desired molecular weight distribution should be extrapolated to $c = 0$ if the molecular weights are high. This is valid especially if the distributions are broad, for in this case the individual species pass the detector at much lower concentration than the narrow calibrating polymers⁶⁴.

With proteins this problem is less pronounced. Thus, calibration curves could often be used directly to determine the molecular weight of other compounds by gel chromatography. This is also a very useful method for the

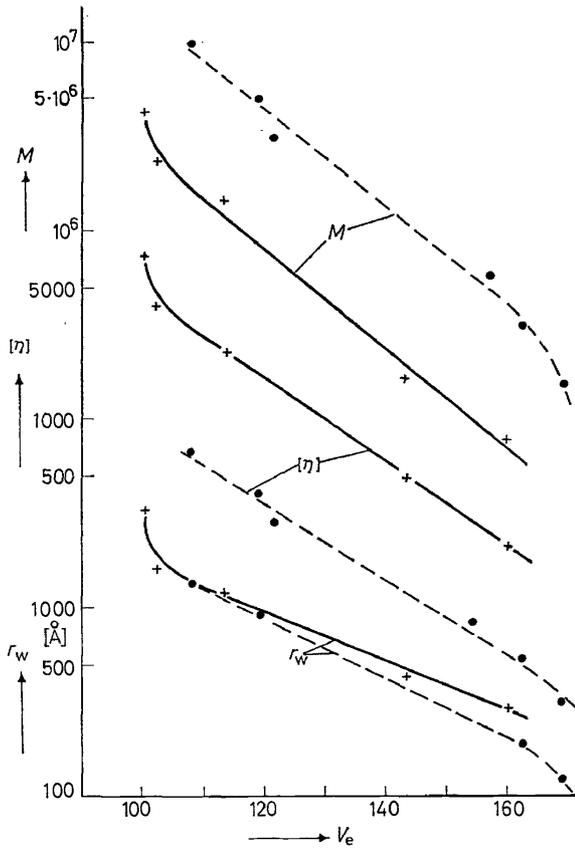


Figure 6. Calibration of Styragel columns (10^7 10^6 10^5) for acetone and for $v = 1$ ml/min. Molecular weight M , intrinsic viscosity $[\eta]$, and light scattering radius r_w (\AA) are plotted for (●) Polymethacrylate, and (+) cellulose trinitrate as a function of the elution volume.

determination of molecular weights of non-uniform synthetic polymers. The accuracy is nearly comparable with that obtained by viscometric determination of M_η . M_η is, however, calculated from the general relation

$$[\eta] = K \cdot M^a \quad (5)$$

in contrast to M_{GC} resulting from Eq. (3) or (4), which are valid only for a special column and a special system. Moreover, the gel chromatograph is much more expensive than a viscometer. An advantage of gel chromatography is that it is not necessary to know the exact concentration. Low molecular weight contaminations, including humidity, are harmless in contrast to their larger effect on $[\eta]$. The danger of an experimental failure is also less pronounced than with viscometry.

If by a fractionation technique such as pure elution or the Baker-Williams method a great number of fractions are separated, it is very convenient

to measure M_{GC} . Apart from the molecular weight itself, information can be obtained regarding the width of the fractions. The molecular weight average is

$$M_n \leq M_{GC} \leq M_w \quad (6)$$

if M_{GC} is calculated from the elution maximum. The equals sign is valid for monomolecular polymers. But for all polymers having a Schulz distribution⁴⁴ it can be shown⁴⁵ that $M_{GC} = M_w$, whilst for other non-uniform polymers $M_n < M_{GC}$.

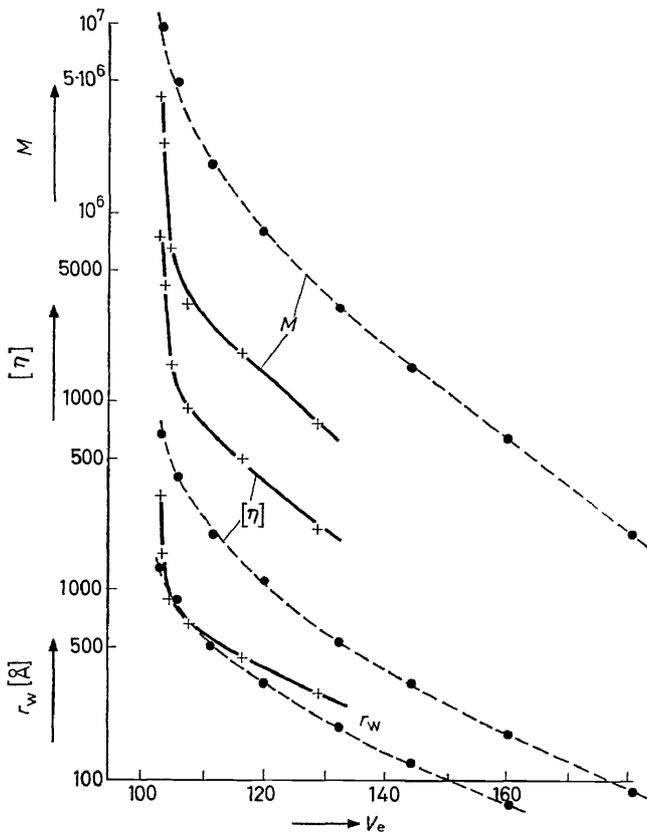


Figure 7. Calibration of Porasil columns (F, E, D, C, B) for acetone and for $v = 1$ ml/min. Molecular weight M , intrinsic viscosity $[\eta]$ and light scattering radius r_w (Å) are plotted for (●) Polymethacrylate and (+) cellulose trinitrate as a function of the elution volume.

General remarks concerning calibration

In connection with calibration, it is of interest to test whether the elution volume is a function of a universal parameter for all polymer solvent systems. Sometimes the chain length L of the fully stretched polymer is used. This is of course only a rough approximation. It surely is not permissible to take a vinyl polymer calibration to calculate a reasonable value of M_{GC} for a chain

with a different backbone, such as polysaccharides (*see Figure 8*). Different curves for molecular weight, intrinsic viscosity and radius of gyration (determined by light scattering) result for different polymers in the same column. It was found empirically, that a reasonable agreement for cellulose trinitrate and vinyl polymers in Styragel with THF could be obtained by plotting the product $M^{1/2} [\eta]^{1/3}$ versus V_e ⁴⁰. It can also be shown^{47, 48}, that another combination of $[\eta]$ and M , i.e. the product $M \cdot [\eta]$, generalizes the elution behaviour of many polymers. This is valid especially for linear and branched molecules of the same polymer.

The product $M \cdot [\eta] = \Phi \cdot r^3$ is also called the hydrodynamic volume⁴⁷. In most cases it was tested with Styragel and THF as solvents. For other gels the agreement is sometimes not quite as good. In the case of Porasil the elution volume for polystyrene in THF and dextran in water are only approximately the same for the same $M \cdot [\eta]$ value¹⁷. In particular, values of V_e the upper separation limits differ in both systems. This is in contrast with the postulate that the hydrodynamic volume is the parameter governing the separation mechanism of gel chromatography.

Since fractions of cellulose trinitrate were available, the molecular weight and the light scattering radius of gyration r_w of which had been measured in acetone⁴⁹, these fractions were used to calibrate Styragel^c and Porasil^c columns for acetone. The Styragel should be packed for acetone in a special way, because the volume of the gel in the non-solvent is somewhat smaller than in solvents for the material of the gel matrix. For comparison, atactic polymethylmethacrylate was used with radii of gyration likewise known from light scattering^{50, 51}.

Figures 6 and 7 show the logarithms of M , $[\eta]$ and r_w plotted against the elution volumes for the two gels. Both sets of results show that the radii of gyration in these cases give an acceptable agreement. For smaller cellulose molecules the deviation from the behaviour derived with the vinyl polymer is likely to become greater, since the slopes of the two polymer lines are different. Unfortunately, it is not easy to prepare fractions of CTN of low molecular weight and for lower polymethylmethacrylate molecules r_w cannot be measured by light scattering.

The parameters containing the combination of $[\eta]$ and M describe quite well the behaviour of the two polymers in Styragel; but two parallel lines remain. For Porasil the curves likewise run nearly parallel, but are more shifted, so that the values of both parameters for PMMA are about 1.5 times greater than those for CTN, as can be seen in *Figure 8*. This figure includes the straight chain lengths, L , of the two different polymers, which differ by a factor of 7 or more.

Polymers of the same type differing in the degree of branching only can usually⁶⁸ be represented by a unique $M \cdot [\eta] = f(V_e)$ relation. An attempt was made to determine the branching density by gel chromatography⁵². The measured $[\eta]_{br}$ and the elution volume allow the evaluation of the molecular weight M_{br} . This M_{br} is used to obtain $[\eta]_{lin}$ from the relation $[\eta]_{lin} = K \cdot M^a$ which must be known. From the statistics of branched polymers the ratio $g = \langle r^2 \rangle_{br} / \langle r^2 \rangle_{lin}$ can be calculated⁵³. A unique dependence between g and the viscosity ratio $[\eta]_{br} / [\eta]_{lin}$ is not available. Relations of the form $[\eta]_{br} / [\eta]_{lin} = g^a$ (exponent a varying between 3/2 and 1/2)

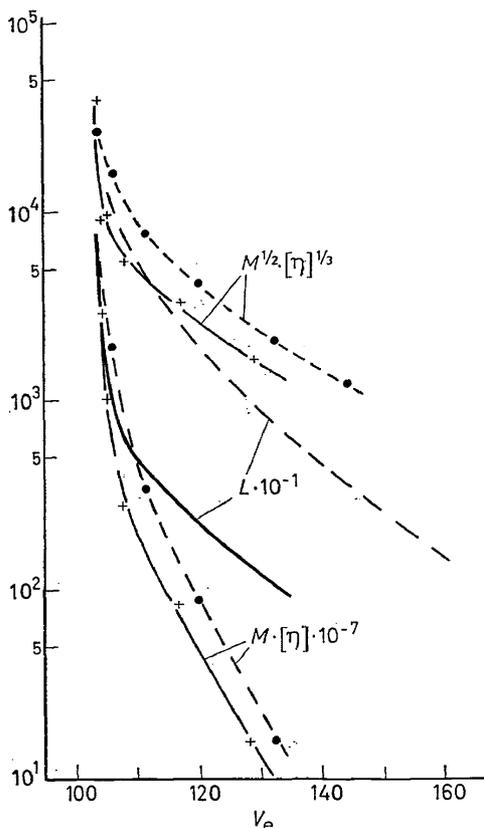


Figure 8. Behaviour of the parameters M , $[\eta]$, $M^{1/2} \cdot [\eta]^{1/3}$ and the straight chain length L in relation to the effluent volume for (●) Polymethylmethacrylate and (+) Cellulose trinitrate in Porasil columns (F, E, D, C, B) in acetone.

do however exist^{53, 54}. Experiments are best represented by low exponents from 1/2 to 1. For the exponent 1/2 a detailed theoretical analysis of gel chromatography diagrams of branched and unbranched polymers has been given⁶⁹.

Molecular weight distributions from gel chromatography

Gel chromatography separates polymer mixtures according to the size of the dissolved molecules. As a consequence it must in principle be possible to calculate the molecular weight distribution from the elution diagram. It has been seen that each molecular species travels with a distinct velocity through the column. Its boundary, quite sharp at the start, is spread out by axial dispersion. The height of the elution curve at any point does not represent the quantity of one species but includes the contributions of many neighbouring sizes. In most cases the molecular weight distribution calculated directly from the chromatogram becomes too broad.

With polymers of very narrow distribution the elution curve is always too broad. For broader distributions it is sometimes found, especially for low flow rates, that the elution curve is too narrow^{42, 70}. This means for instance that a polymer with $M_w/M_n = 1.5$ shows an elution diagram which uncorrected for axial dispersion gives $M_w/M_n < 1.5$. It is of course quite easy to increase the axial dispersion in any way, e.g. by increasing the flow rate, using a column with low plate count or to perform an unfavourable sample loading, etc. and to reach a value of M_w/M_n greater than 1.5. But frequently the value of M_w/M_n from gel chromatography is too high, and a correction for axial dispersion becomes necessary. Nevertheless, in practice there are many cases in which the uncorrected diagram is very helpful. *Figure 9* represents such a diagram for an anionically prepared

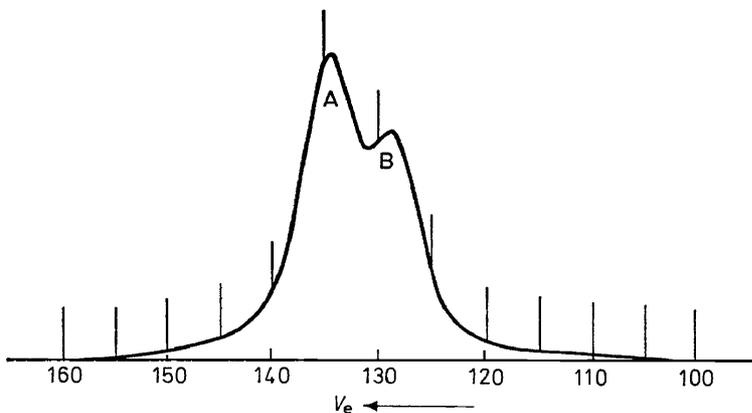


Figure 9. Elution diagram of an anionic polystyrene in Styragel (10^6 , 10^5 , $2 \cdot 10^4$, 10^3) with THF and $v = 1$ ml/min. The peaks P = 14 000 (B) and P = 7000 (A) result from chains growing on both sides or on one side only.

polystyrene. The areas below the peaks reflect directly the ratio of two components, whose chain lengths differ by a factor of 2. The two kinetic lengths result from partial termination of one side of the bianion at the start. This is only one example of how to control a polymerization process in the laboratory or in the industrial plant directly by means of the recorder trace and of how to obtain immediately valuable information.

For the correction of the elution diagram with regard to the axial dispersion, several methods are available. Tung⁵⁶ uses an analytical technique and makes the assumption that each molecular species passes the detector with a Gaussian distribution. A constant, molecular weight-independent resolution factor, e.g. the σ corresponding to the molecular weight of the elution peak, permits a polynomial evaluation. For $\sigma = f(M)$ a calculation by Gaussian mechanical quadrature is possible. Hess and Kratz⁵⁷ and Smith⁵⁸ apply a numerical technique and also assume the Gaussian type elution of monomolecular compounds. Their resolution factor may depend on M . For higher molecular weights a log-normal shape or a shape consisting

of two Gaussian halves, each with its own σ -value improves the result. For one method⁵⁷ a sometimes unfavourable matrix inversion impedes the application⁶².

It is necessary to know the dependence of the resolution on the molecular weight on the elution volume. The function $\sigma = f(V_e)$ is determined, since monomolecular compounds of high molecular weight are not available, from polymers with narrow distributions or by a flow reversal technique⁵⁶.

The separate determination can be avoided by a correction method of Pickett *et al.*⁵⁹, whose computer program involves the peak volume and the standard deviation of narrow fractions. From the known values of M_w and M_n , the contributions of uniform polymers are calculated, including those of interpolated M values, and stored in the computer. A multivariable search technique is used to make selections from a number of constructed chromatograms with different compositions in species and this yields a chromatogram which has a minimum deviation from the actual one.

For all methods cited a computer evaluation is required. For Tung's equation of axial diffusion solutions were also reported^{60, 61}, which do not need this auxiliary analysis.

The basic assumptions for all correction techniques are reasonable. The fewest presuppositions are required with the Pickett method⁵⁹. It is very interesting to see how the different corrections work on the same elution diagram and on diagrams of the same polymer in different columns. This behaviour was tested by Duerksen and Hamielec⁶² using polystyrenes prepared by free radical polymerization with AIBN. We do not consider the polymer except the one with a medium molecular weight ($M_n \sim 75\ 000$). In *Figure 10* chromatograms of three column sets and varied flow rates are

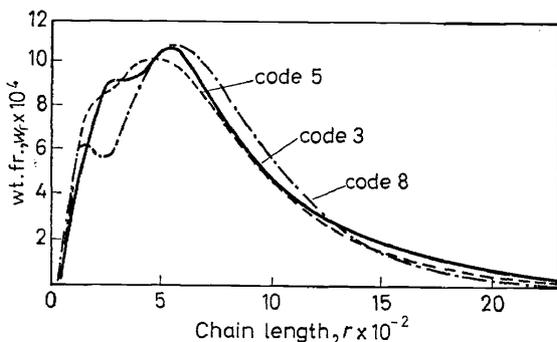


Figure 10. Molecular weight distributions calculated by the Tung⁵⁶ method for a polystyrene in THF on Styragel⁶² [code 3: columns 10^4 , 10^4 , 900, 800, 800; $v = 1$ ml/min. code 5: columns 10^5 , 10^4 , 800; $v = 1$ ml/min. code 8: columns 10^5 , 800; $v = 2$ ml/min.]

used to generate by Tung's⁵⁶ polynomial method the molecular weight distribution. The agreement between the three sets is satisfactory, and two distinct peaks are always obtained or at least some indication of them is observed. Most pronounced is the bimodal distribution at the two column set with high elution rate, which in reality should show a less good resolution. *Figure 11* compares for elution rates for 10^5 , 10^4 , 800 and the standard value

of 1 ml/min the distributions as calculated by the techniques of Tung⁵⁶, Smith⁵⁸ and Pickett⁵⁹. The Smith method results in lower oscillations in the molecular weight distribution, whilst from the Pickett method a multi maximum molecular weight distribution is obtained. If the oscillations represent real peaks in the polymer, they should be the same for different columns. This is not the case.

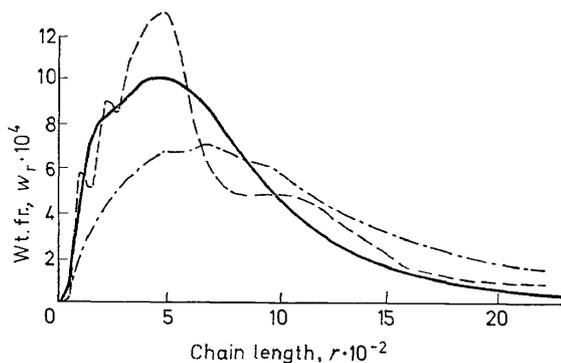


Figure 11. Molecular weight distributions calculated⁶² for the polystyrene of Figure 10 and code 5 according to (—) Tung⁵⁶; (---) Smith⁵⁸; (- - -) Pickett, Cantow, and Johnson⁵⁹.

According to the polymerization technique⁶² and the knowledge of the molecular weight distribution of such polymers, a bimodal distribution—let alone a multimodal one—is not very likely. It is possible that the oscillations are mathematically generated or that they result from experimental inaccuracies. Minimum deviations in the chromatogram baseline exhibit large effects on the sensitive correction methods. They can easily result from small changes in temperature and pressure. It is common to use a fictitious ideal straight baseline, so that the oscillations which occur even for differential methods, are not eliminated. Until this difficulty is overcome, repeated runs under constant conditions have to be evaluated.

It must be appreciated that all correction methods are able to depress the excessive measured value of M_w/M_n to the expected value. In order to represent the details of the molecular weight distribution in a reliable manner some further efforts in improving the experimental and evaluation techniques are needed in order to obtain more and more precise information from gel chromatography.

Preparative gel chromatography

Separations of molecules of distinct size were performed by gel chromatography with great success, such as separations of low molecular weight compounds from proteins, and of different proteins from one another, etc.

Oligomers in organic solvents are separable. An example⁶⁷ is presented in Figure 12. A column of 200 cm length and 5 cm diameter is filled with a

cross-linked styrene gel, which swells appreciably. For a standard oligostyrene and an elution rate of 3.3 ml/min a complete isolation of the individual degrees of polymerization up to $P \sim 15$ is obtained. This is done in a relatively short time, producing the dimer after about 12 h at the column end. Such diagrams can be used to determine the different molecular weight averages of oligomers with an accuracy impossible for other techniques. From *Figure 12* it can be shown that $M_n = 580$ and $M_w = 675^{67}$.

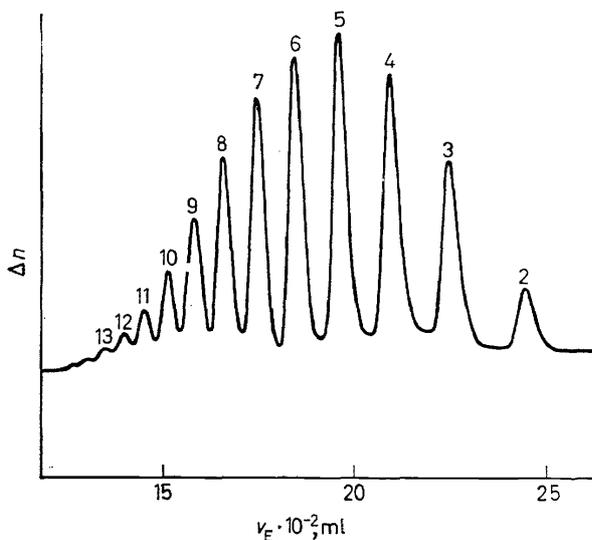


Figure 12. Complete separation of an oligostyrene into individual degrees of polymerization⁶⁷

A comparable resolution could be obtained with Styragel in a set of columns of 0.8 cm diameter and an overall length of 4900 cm and with an elution rate of 0.4 ml/min⁶⁵. The possibility of a complete separation gradually disappears with the ratios between two succeeding species becoming too low.

For higher molecular weights, swelling gels are not so well suited and one has to use the rigid or almost non-swelling gels with columns of larger diameter. Fractionation of greater sample amounts then becomes possible. But the packing technique seems to be more difficult than with columns of smaller cross-sections.

There are not many papers dealing with preparative gel chromatography on synthetic polymers. It is likely that the situation will soon be changed since a commercial apparatus is now available by means of which effective fractionations on high molecular weight polystyrene and polyethylene have been performed⁶⁶.

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