binary mixtures, comprising the solvent and non-solvent in different ratios. It should be noted that the mixture having the lowest solvent-to-non-solvent ratio will have the least solvent power and the one having the highest ratio will have the highest solvent power. Next, the binary mixture of the lowest solvent power is added to the powdered polymer sample. The polymer is allowed to swell fully and then the entire content is stirred well to enhance the process of dissolution of the lowest molecular weight species in the solvent mixture. Depending on the quantity of the polymer taken, the stirring time should be adjusted to achieve the complete dissolution of all the soluble components. At this stage, the stirring is stopped, the solution filtered off and the filtrate collected. The first fraction is recovered by completely evaporating the solvent from the filtrate. To the swollen mass left undissolved by the first lot of the binary mixture (i.e., to the solid obtained by filtration), the binary mixture with the next higher solvent power is added and stirred and the whole process repeated till the second fraction is recovered. In this way, by successive dissolution with binary mixtures of increasing solvent powers, a series of fractions with increasing average molecular weights can be separated.

13.3.3 Gradient Elution Technique

The gradient elution technique too works on the partial dissolution principle explained above. In this technique, the polymer is precipitated on glass wool packed at the top of a column filled with silica or alumina, as shown in Fig. 13.4. The polymer is eluted successively with solvent/non-solvent mixtures of increasing solvent power. The lowest molecular weight fraction will be eluted first and the highest one will emerge last from the column. The efficiency of the method is increased by maintaining a temperature gradient in the column. The upper end of the column is kept at a slightly higher temperature than the lower end. The temperature gradient makes each molecular species undergo a series of dissolutions and precipitations at different levels of the column and, hence, improves the efficiency of fractionation.

13.3.4 Gel Permeation Chromotographic Technique

A rather quick and efficient method for polymer fractionation and simultaneous computation of the molecular weight distribution curve is provided by the gel permeation chromotography (GPC)

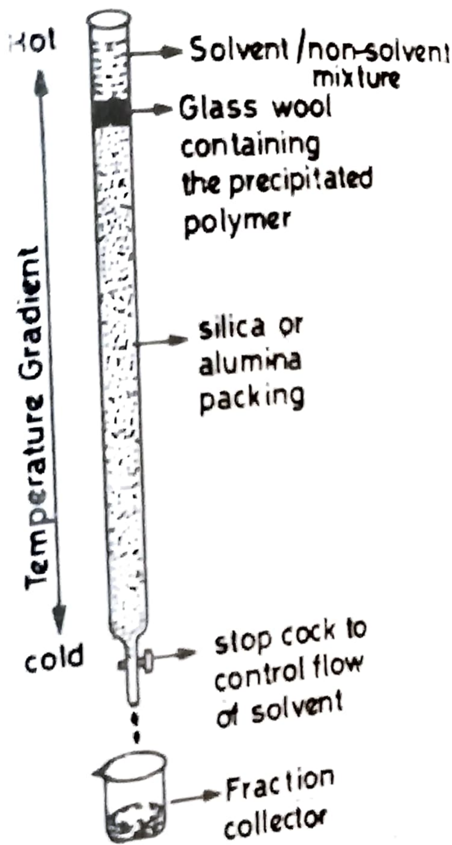


Fig. 13.4 A simple laboratory set-up used for gradient elution method of fractionation.

The principle of GPC is as follows. In any column chromatographic process, as the solute travels inside the column from one end to another, it is transferred between two phases, one remaining stationary and the other mobile. In solid-liquid chromatography, the stationary phase is a solid (such as silica, alumina or sintered glass powder packed in the column) and the mobile phase is a liquid (i.e., the solvent used to elute the solute). In liquid-liquid chromatography, both the phases are liquids, the two being immiscible with each other.

In GPC, however, a single liquid, viz., the solvent itself, acts as both stationary as well as mobile phases. But how can this be possible ?. The column in GPC is filled with a material, called 'gel'.* The latter is in the form of fine spherical beads (usually 100 μ in diameter and made of cross-linked styrene-divinyl benzene copolymer or glass) containing a large amount of micropores of uniform size. The gel beads are hard and incompressible and are prepared in

*...different from the one used earlier in Section

different porosity grades, i.e., having a pore size ranging form 50 Å to 1,000 Å. The column can be packed either with gel beads of the same pore size or with beads of different porosities. When the GPC column is filled with a suitable solvent, the solvent occupies not only the free volume between the beads (void volume) but also the volume of the pores (pore volume) inside the gel beads. The solvent phase occupying the void volume acts as the mobile phase, while that occupying the pore volume acts as the stationary phase (Fig. 13.5).

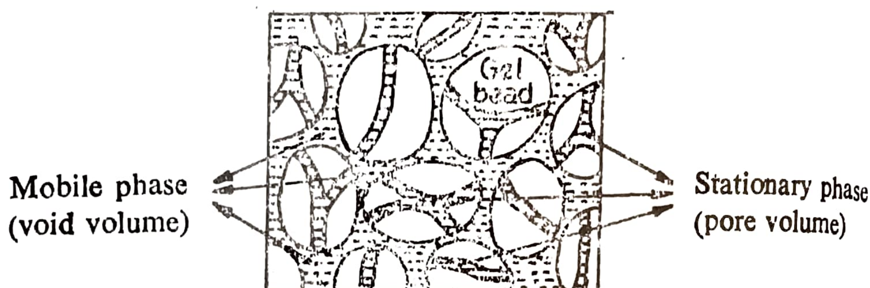


Fig. 13.5 Schematic diagram showing gel beads, the pore volume and void volume in a GPC column.

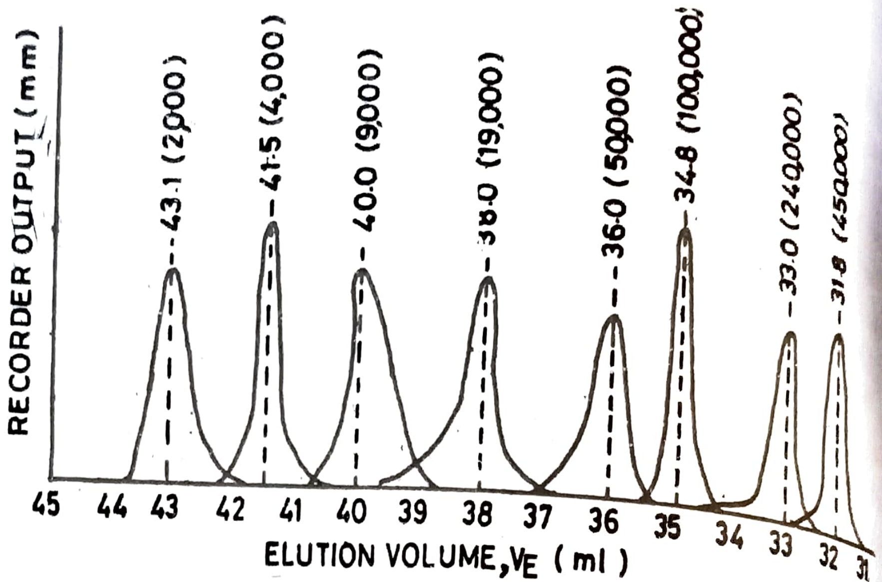
The GPC operates on the principle that polymer molecules in solution separate according to their size and not chemical interaction or chemical retention. The separation is based on the difference in the hydrodynamic volumes of polymer molecules of varying molecular weights, in their dissolved state. When a dilute solution (say, 0.5% concentration) of the polymer sample in a suitable solvent is injected at the top of the column and the column is eluted with the solvent, the polymer solution along with the solvent starts streaming down the column. The mobile phase around the gel beads now contains polymer molecules, whereas the stationary phase within the gel beads does not contain any. Due to this difference in polymer concentration between the mobile and the stationary phases, the polymer molecules start diffusing into the stationary phase so as to equalise the concentration. Athough all the polymer molecules try to enter the pore volume, the pore size restricts the entry and allows molecules up to a certain hydrodynamic size only to enter the pores. Molecules of bigger size are thus 'excluded'* from entering the pores and are washed down the column by the solvent. The

*For this reason, GPC is also termed size exclusion chromotography.

highest molecular weight fraction therefore elutes out of the column first. Among the molecules that can enter the pores the lowest molecular weight species, due to their lowest hydrodynamic size, are able to diffuse faster into the pores and, hence, occupy the maximum part of the pore volume; while those of intermediate sizes diffuse slowly and are able to occupy only a part of the pore volume. As the column is eluted further with a pure solvent, the mobile phase becomes devoid of polymer molecules, whereas the stationary phase contains a large quantity. This difference in concentration drives the polymer molecules in the opposite direction, which now start diffusing out of the pores. In this diffusion process (in and out of the pore volume), the smallest molecular weight species are retained in the column for the maximum period and, hence, elute last. The molecules of intermediate sizes elute in between.

If we denote the pore volume by V_p and the void volume by V_0, then the largest molecules, which are totally excluded from the pores, elute out of the column when a solvent volume equal to V_0 elutes out after the injection of the sample. The smallest molecules which permeate the maximum volume of the pores elute out at a volume of $(V_0 + V_p)$. The intermediate size molecules elute between V_0 and $(V_0 + V_p)$. Hence, the elution volume directly gives an estimate of the molecular size. In practice, however, no attempt is made to actually compute V_0 or V_p. Calibration curves in the form of log M versus elution volume V_E are drawn using a series of 'standards' of known molecular weights and narrow molecular weight distribution. Chromotograms of these standards are run and the elution volume corresponding to the peak position of bands obtained for each 'standard' sample (Fig 13.6) is read. From the knowledge of the molecular weight of the standards and their corresponding elution volume (see Table 13.2), a calibration curve of log M against V_E is drawn (Fig. 13.7). This peak position calibration method has been found to give reliable results, provided the standards used are of narrow molecular weight distribution (i.e. $\overline{M}_w/\overline{M}_n < 1.2$) and the chemical types of the 'standard' and the sample are the same.

The GPC apparatus (Figs. 13.8 a and b) consists of two columns: one for injecting the sample and other serving as a reference column. The solvent elutes through both the columns. A differential refractometer is used to measure the refractive index difference



Instrument: Waters ALC/GPC-244
Column: μ Styragel 10^5, 10^4, 10^3, 500 and 100 Å
Sensitivity: (RI) 2×
Pressure: 700 psi

Solvent: Toluene
Concentration: 0.5%
Injection volume: 100 μl (each)
Temperature: 25°C

Fig. 13.6 Gel permeation chromotograms of a number of 'standard' polystyrene samples showing elution volumes corresponding to different molecular weights (Figures given in brackets are molecular weights).

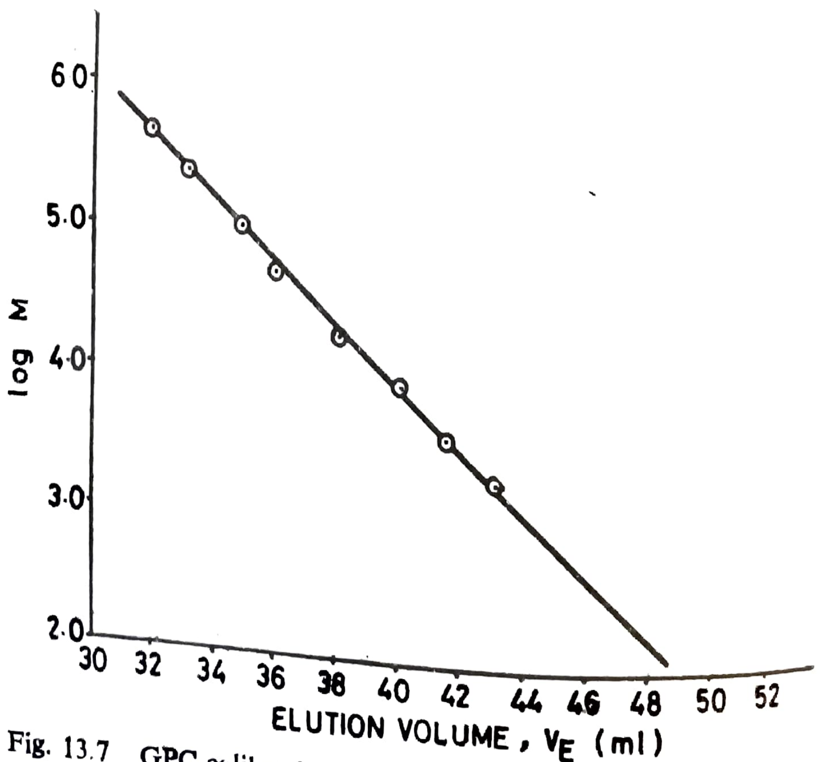
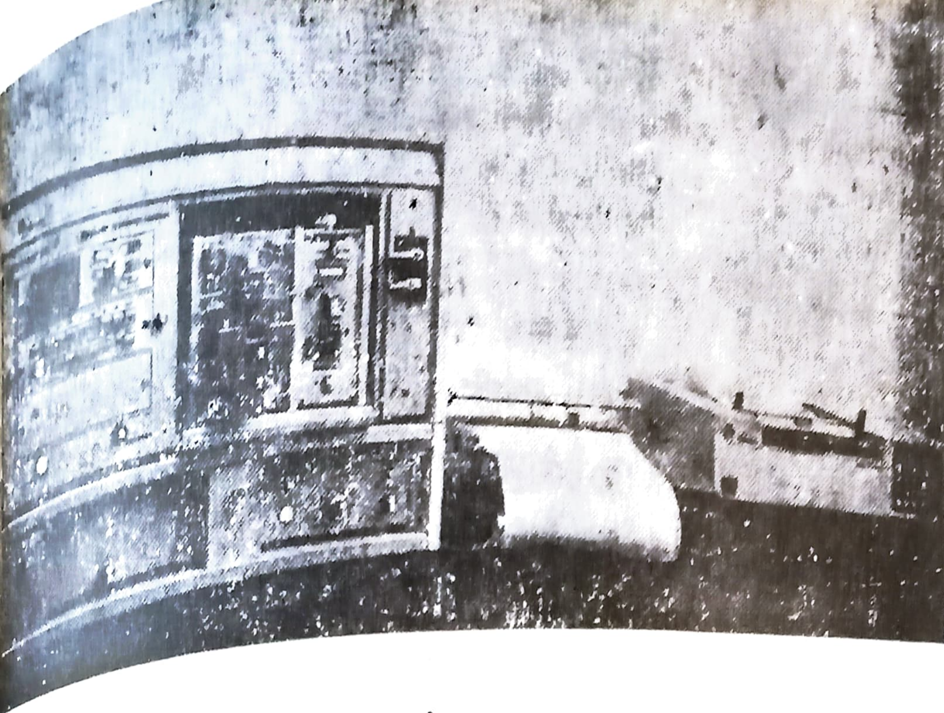
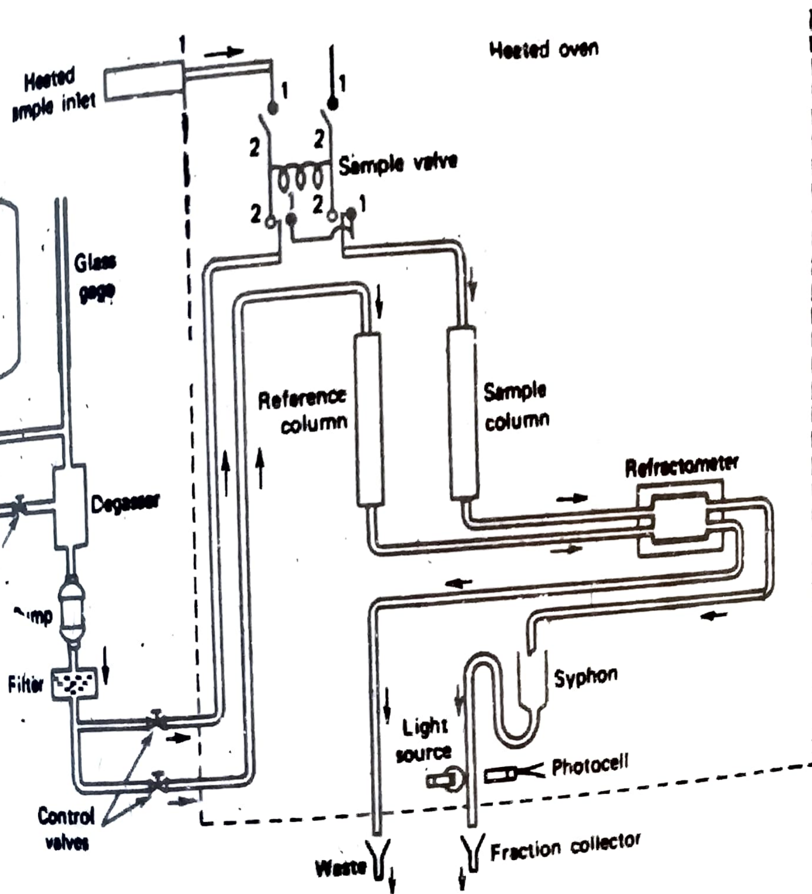


Fig. 13.7 GPC calibration curve showing the relation between log M and V_E (Data from Fig. 13.6).



(a)



(b)

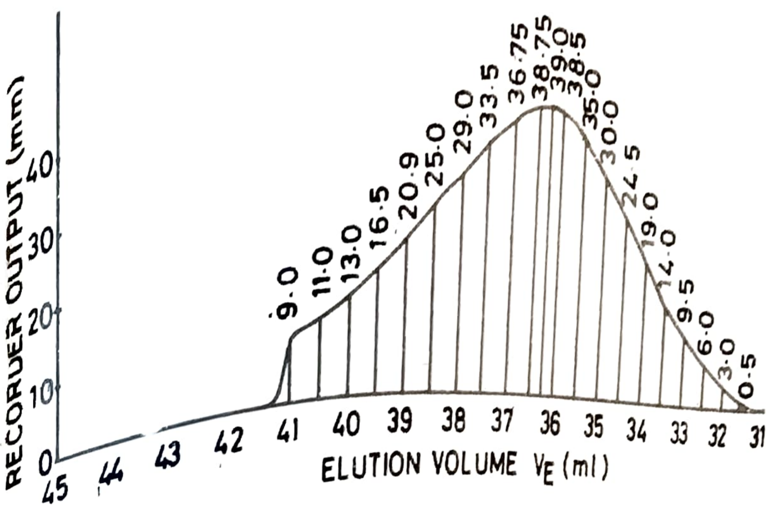
Fig. 13.8 (a) GPC apparatus. (*Courtesy*: Waters Associates Ltd., Singapore.) (b) Flow scheme of a gel permeation chromotograph.

Table 13.2 GPC data for calibration standards (polystyrene) in toluene solutions at 25°C*

Polystyrene standards, serial no.	Average molecular weight of the standard, $\bar{M}$	log $\bar{M}$	Elution volume corresponding to the standard, V_E (ml)
1	450,000	5.6532	31.80
2	240,000	5.3802	33.00
3	100,000	5.0000	34.80
4	50,000	4.6990	36.00
5	19,000	4 2788	38.00
6	9,000	3.9542	40.00
7	4,000	3.6021	41.50
8	2,000	3.3010	43.10

*Data computed from Fig. 13.6.

between the polymer solution and the pure solvent eluting out of the sample and reference columns, respectively. This refractive index difference directly gives the concentration of the polymer being eluted at different elution volumes. From a knowledge of elution volume and refractive index and by comparison with the calibration curve, we can directly get the weight and the molecular weight of different fractions eluting at different elution volumes. In the case of completely automated equipments, the data are processed and recorded in the form of a plot of elution volume versus recorder output of the differential refractometer. The elution volume corresponds to the molecular weight and the recorder output to the weight concentration of the polymer. This plot directly gives the molecular weight distribution. Such a distribution curve for a polystyrene sample is given in Fig. 13.9. This curve can also be used to find out the $\bar{M}_w$ and $\bar{M}_n$ values of the polymer sample as now explained. From the curve, the heights (h) of the recorder output axis corresponding to different elution volumes (V_E) are read and from the calibration curve, molecular weights corresponding to these elution volumes are noted. The values are tabulated as shown in Table 13.3. The height of the curve in Fig. 13.9 at any



Instrument: Waters ALC/GPC-244
Column: μ Styragel-10^5, 10^4, 10^3, 500 and 100 A

Sensitivity: (RI) 3 ×
Pressure: 700 psi

Solvent: Toluene
Concentration: 1.0%

Injection volume: 500 μl
Temperature: 25°C

Fig. 13.9 GPC chromotogram of a polystyrene sample.

particular elution volume is a measure of the concentration of molecules of the corresponding molecular weight. This height h can, therefore, be equated to $n_i M_i$ and the molecular weight M to M_i of the various equations we have come across in Chapter 3. The $\overline{M}_n$ and $\overline{M}_w$ of the sample can be calculated as follows.

From Table 13.3, we have

$\Sigma h = 452.40$, $\Sigma(h/M) \times 10^3 = 16.2107$ and $\Sigma(h \cdot M) = 29{,}552{,}925$

Now,

$$\overline{M}_n = \frac{\Sigma n_i M_i}{\Sigma n_i} = \frac{\Sigma h}{\Sigma(h/M)} = \frac{452.40 \times 10^3}{16.2107} = 27{,}907 \text{ (or, say) } 28{,}000$$

$$\overline{M}_w = \frac{\Sigma n_i M_i^2}{\Sigma n_i M_i} = \frac{\Sigma(h.M)}{\Sigma h} = \frac{29{,}552{,}925}{452.40} = 65{,}325 \text{ (or, say) } 65{,}500$$

13.4 MOLECULAR WEIGHT DETERMINATION

In Chapter 3 we have referred to various molecular weights viz., number-average ($\overline{M}_n$), weight-average ($\overline{M}_w$), viscosity-average ($\overline{M}_v$), etc. Let us now see how they are experimentally determined.

Table 13.3 GPC data for a polystyrene sample*

Elution volume, V_E (ml)	Recorder output height, h (mm)	Molecular weight, $\bar{M}$	$(h/M) \times 10^3$ (mm)	$(h \cdot M)$ (mm)
31.5	0.5	500,000	0.0010	250,000
32.0	3.0	395,000	0.0075	1,185,000
32.5	6.0	310,000	0.0193	1,860,000
33.0	9.5	240,000	0.0395	2,280,000
33.5	14.0	190,000	0.0736	2,660,000
34.0	19.0	150,000	0.1266	2,850,000
34.5	24.5	120,000	0.2041	2,940,000
35.0	30.0	94,000	0.3191	2,820,000
35.5	35.0	74,000	0.4729	2,590,000
36.0	38.5	58,000	0.6637	2,233,000
36.25	39.0	52,000	0.7500	2,028,000
36.5	38.75	45,500	0.8516	1,763,125
37.0	36.75	36,000	1.0208	1,323,000
37.5	33.5	29,000	1.1551	971,500
38.0	29.0	22,500	1.2888	652,500
38.5	25.0	17,500	1.4285	437,500
39.0	20.9	14,000	1.4928	292,600
39.5	16.5	11,000	1.5000	181,500
40.0	13.0	8,600	1.5116	111,800
40.5	11.0	6,800	1.6176	74,800
41.0	9.0	5,400	1.6666	48,600

*Data computed from Figs. 13.7 and 13.9.