Gel permeation chromatography separates sample molecules by differences in effective molecular size in solution. Separation is accomplished as a result of the pore size distribution in the packing material. Molecules too large to penetrate any of the pores are unretained by the column packing (totally excluded) and elute first. Slightly smaller molecules penetrate some of the pores, are retained on the column, and elute somewhat later. Molecules small enough to penetrate all of the pores are retained on the column longest and elute last. The consistency of this technique allows determinations of weight-average molecular weight ($\langle M_w \rangle$), number-average molecular weight ($\langle M_n \rangle$), and molecular weight distribution, and dispersity for polymeric materials.

In many instances, it is not necessary to obtain numerical values for these parameters. Differences in molecular weight distributions are obvious if GPC curves are compared. Curve overlays are sufficient for most quality control uses.

If it is deemed necessary to obtain numerical data, attention must be paid to:

- Sample preparation,
- Sample injection,
- Column selection,
  - Calibration method,
  - Baseline determination, and
  - Computation.

**SAMPLE PREPARATION**

Sample preparation for GPC, as for any mode of liquid chromatography, is usually quite easy. The polymer to be analyzed is dissolved in the mobile phase and chromatographed within the next 24 hours. Agitation (with a magnetic stirrer or laboratory shaker) aids in dissolving the sample. ULTRASONIC DEVICES MUST NOT BE USED BECAUSE THEY MAY CAUSE POLYMER DEGRADATION.

After the sample is dissolved, it should be filtered to remove any material likely to clog the columns. Filters with pore sizes between 0.4 µm and 0.5 µm should be used. Waters Associates' Sample Clarification Kit for organic solutions provides a convenient means of filtering samples for the ALC/GPC 200 series.
of instruments. For filtering aqueous solutions for use with the water-wettable GPC packing materials use Waters' Sample Clarification Kit for aqueous solutions. Both kits are described in DS-043.

The presence of a cross-linked microgel in a sample that appears clear to the eye may be revealed by filtration. If a microgel is present, it can clog the filter and prevent the passage of smaller molecules. If the filter clogs, excessive and increasing pressure will be needed for filtration. Should this occur, decrease the sample concentration and/or use a prefilter.

For most polymers a sample concentration of 0.25% will minimize the possibility of viscous fingering caused by high concentrations of polymer, particularly high molecular weight polymers. Viscous fingering can result in changes in peak retention volumes and band shapes.

An unknown sample should be chromatographed at successively decreasing concentrations until constant retention volume and chromatographic band shape are observed. However, a balance must be made between a concentration that avoids viscous fingering and one that provides adequate detector response. A response that approximates that obtained with the calibration standard is ideal.

Along with sample concentration, mobile phase consistency is an important consideration in sample preparation. All solvents require agitation. This may be supplied by a magnetic stirrer or by a large mixing chamber, depending upon the GPC instrument used. A list of the most commonly used GPC solvents is given in the table below.

**SAMPLE INJECTION**

Samples can be injected through a fixed volume valve and loop as in traditional GPC or the Model U6K Universal Injector may be used. For high-speed/high-resolution GPC, on µSTYRAGEL® the Model U6K must be used. For a complete description of this injector, refer to DS 036.

Before injecting the sample, however, a stable baseline must be obtained.

**COLUMN SELECTION**

Use of the proper columns is essential in obtaining optimum separations. Columns should be chosen so that their calibration curves are linear over the polymer distribution range. Samples may have to be chromatographed initially on a very broad range column set in order to estimate the range of interest.

---

### MOST COMMONLY USED GPC SOLVENTS

<table>
<thead>
<tr>
<th>SOLVENT</th>
<th>BOILING POINT (°C)</th>
<th>DENSITY</th>
<th>VISCOSITY</th>
<th>REFRACTIVE INDEX</th>
</tr>
</thead>
<tbody>
<tr>
<td>TETRAHYDROFURAN</td>
<td>66</td>
<td>0.8892</td>
<td>0.51 @ 25°</td>
<td>1.4070</td>
</tr>
<tr>
<td>1,2,4−TRICHLOROBENZENE</td>
<td>213</td>
<td>1.4623</td>
<td>0.50 @ 135°</td>
<td>1.5524</td>
</tr>
<tr>
<td>TOLUENE</td>
<td>110.6</td>
<td>0.866</td>
<td>0.52 @ 25°</td>
<td>1.4893</td>
</tr>
<tr>
<td>meta−CRESOL</td>
<td>202</td>
<td>1.034</td>
<td>16.9 @ 20°</td>
<td>1.5348</td>
</tr>
<tr>
<td>N,N−DIMETHYL FORMAMIDE*</td>
<td>153</td>
<td>0.9445</td>
<td>0.90 @ 25°</td>
<td>1.4280</td>
</tr>
<tr>
<td>WATER†</td>
<td>100</td>
<td>0.9999</td>
<td>1.0 @ 20°</td>
<td>1.3330</td>
</tr>
<tr>
<td>CHLOROFORM</td>
<td>61.2</td>
<td>1.489</td>
<td></td>
<td>1.4476</td>
</tr>
<tr>
<td>1,1,2,2 TETRACHLOROETHANE</td>
<td>146.5</td>
<td>1.58658</td>
<td></td>
<td>1.4941</td>
</tr>
<tr>
<td>TRIFLUOROETHANOL</td>
<td>73.6</td>
<td>1.3823</td>
<td>0.9 @ 38°</td>
<td>1.2907</td>
</tr>
</tbody>
</table>

*DMF should not be used with packings with pore sizes less than 10⁴ Å.
†Water should not be used across STYRAGEL or µSTYRAGEL.
Such a broad range column set is fashioned by joining several columns of different exclusion limits and different linear ranges. However, for optimum resolution, a column set should be chosen so that each column plays an important part in the separation. The range of the column set should only be broad enough to cover the range of interest.

CALIBRATION METHOD

Both direct and indirect methods of calibrating GPC columns have been used to obtain molecular weight averages. With polymers for which narrow molecular weight distribution standards are commercially available (e.g., polystyrene), a direct calibration on the basis of molecular weight can be made. If standards are not commercially available, they must either be prepared or an indirect method used.

Direct Calibration

Prepare fresh calibration standards in the same manner as samples, but use 0.1% concentrations. (Standards available from Waters Associates are listed in the table on the following page.) As many standards as possible should be run in the range of interest. If the sample is totally unknown, it may be necessary to chromatograph it first to determine the estimated range.

Be sure each injection point is clearly marked. With the Model U6K Universal Injector this is done automatically.

Measure the retention volume or time of each standard from the start of injection to the maximum of the chromatographic peak. Plot this value (on the linear x-axis) on semilog paper vs. the corresponding value (on the logarithmic y-axis) of the peak molecular weight, peak M (listed on the standard), for each standard. Draw a smooth curve through the points plotted to obtain the calibration curve.

Indirect Calibration

If narrow molecular weight distribution standards of the polymer of interest do not exist, such standards can be prepared by GPC fractionation of a broad molecular weight distribution sample or an indirect method of calibration can be applied.

Stylized and actual GPC calibration curves are shown above. It should be emphasized that each calibration curve is related to a unique set of columns. If even one column of that set is replaced—even by another with the same exclusion limit—the whole set must be recalibrated.
POLYSTYRENE

<table>
<thead>
<tr>
<th>Catalog Number</th>
<th>Nominal Weight Average Molecular Weight $M_w$</th>
<th>Suggested Concentration %</th>
</tr>
</thead>
<tbody>
<tr>
<td>26971</td>
<td>2,000</td>
<td>0.25</td>
</tr>
<tr>
<td>25169</td>
<td>4,000</td>
<td>0.25</td>
</tr>
<tr>
<td>25171</td>
<td>10,000</td>
<td>0.25</td>
</tr>
<tr>
<td>25168</td>
<td>20,000</td>
<td>0.25</td>
</tr>
<tr>
<td>25170</td>
<td>80,000</td>
<td>0.10</td>
</tr>
<tr>
<td>41996</td>
<td>100,000</td>
<td>0.10</td>
</tr>
<tr>
<td>41984</td>
<td>200,000</td>
<td>0.10</td>
</tr>
<tr>
<td>25166</td>
<td>400,000</td>
<td>0.05</td>
</tr>
<tr>
<td>25167</td>
<td>700,000</td>
<td>0.05</td>
</tr>
<tr>
<td>61970</td>
<td>2,500,000</td>
<td>0.05</td>
</tr>
<tr>
<td>41746</td>
<td>4,000,000</td>
<td>0.05</td>
</tr>
</tbody>
</table>

POLYPROPYLENE (GLYCOL)

<table>
<thead>
<tr>
<th>Catalog Number</th>
<th>Nominal Weight Average Molecular Weight $M_w$</th>
<th>Suggested Concentration %</th>
</tr>
</thead>
<tbody>
<tr>
<td>41993</td>
<td>800</td>
<td>0.25</td>
</tr>
<tr>
<td>41994</td>
<td>1,200</td>
<td>0.25</td>
</tr>
<tr>
<td>41985</td>
<td>2,000</td>
<td>0.25</td>
</tr>
<tr>
<td>41983</td>
<td>4,000</td>
<td>0.25</td>
</tr>
</tbody>
</table>

A GPC Calibration Kit (request DS 045) is also available from Waters Associates. The kit contains standards, syringes, and semilog graph paper.

One of these indirect methods which can be applied successfully is the "Q-Factor Method". Briefly, it consists of estimating the molecular size averages of the polymer from its GPC curve and a molecular size calibration curve for polystyrene. The polystyrene calibration curve is prepared as described in the section on direct calibration except that the MOLECULAR SIZE (extended chain length in ångström) is plotted instead of molecular weight—molecular size is given on the standard container. Molecular size is then calculated for the polymer according to the equations given in the section on computation. These molecular size averages are then converted to molecular weight averages by a $Q$ factor, i.e., a conversion factor determined for the polymer of interest.

$$
\bar{M}_w = Q\bar{A}_w, \bar{M}_N = Q\bar{A}_N
$$

$Q$ is experimentally determined by obtaining GPC curves for a number of samples of the polymer of interest whose molecular weight averages are known (via light scattering, osmometry, viscometry, etc.) $Q$, the number of molecular weight units per ångström of molecular size, can then be calculated by the following equations:

$$
Q = \frac{\bar{M}_w}{\bar{A}_w} = \frac{\bar{M}_N}{\bar{A}_N}
$$

Similar $Q$ values should be obtained from both equations. If not, the $Q$-factor method is not applicable, and another calibration method must be chosen.

When using the $Q$-factor method, the analyst should realize that wide deviations in results will result if his samples are of copolymers which vary in composition, blends of two polymers where the blend ratios differ, or polymers where the degree of branch- ing or cross-linking varies from sample to sample.

**BASELINE DETERMINATION**

On the chromatogram, mark the retention volumes for the start ($V_i$) and finish ($V_t$) of the polymer chromatogram. Draw a linear baseline from before $V_i$ to after $V_t$. If the baseline is drawn from $V_i$ to $V_t$, small rises may be overlooked.
Once the baseline has been determined, measure peak heights to three significant figures for about 15 equally spaced points along the GPC curve. Tabulate these data under the headings shown below.

<table>
<thead>
<tr>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>RETENTION VOLUME OR COUNTS</td>
<td>HEIGHT (mm)</td>
<td>CHAIN LENGTH OR MOL WT</td>
<td>Col 2/Col 3</td>
<td>Col 2 X Col 3</td>
</tr>
</tbody>
</table>

If calibrating directly, list molecular weights in column three and determine molecular weight averages by the following equations:

\[
\bar{M}_N = \frac{\sum \text{Column } 2}{\sum \text{Column } 4}
\]

\[
\bar{M}_W = \frac{\sum \text{Column } 5}{\sum \text{Column } 2}
\]

These equations are based on the theoretical equation:

\[
\bar{M}^* = \frac{\sum_i N_i M_i^b}{\sum_i N_i M_i^b - 1}
\]

where \(b\) is a constant, any whole integer, and \(\bar{M}^* = M_N\) when \(b = 1\), \(M_W\) when \(b = 2\), and \(M_Z\) when \(b = 3\).

The elution volume is directly relatable to \(M_i\), the molecular weight, and the amplitude of the curve is directly relatable to \(N_i\), the number of molecules present at that molecular weight.

If using the Q-factor for indirect calibration, list chain length in angstroms in column three and determine molecular size averages by replacing \(M\) in the preceding equations with \(A\).

\[
\bar{A}_N = \frac{\sum \text{Column } 2}{\sum \text{Column } 4}
\]

\[
\bar{A}_W = \frac{\sum \text{Column } 5}{\sum \text{Column } 2}
\]

\[
\bar{A}^* = \frac{\sum_i N_i A_i^b}{\sum_i N_i A_i^b - 1}
\]

then \(\bar{A}^* = \bar{A}_N\) when \(b = 1\), \(\bar{A}_W\) when \(b = 2\), and \(\bar{A}_Z\) when \(b = 3\).

**Example**

The chromatogram on the preceding page is of a polystyrene sample. Since narrow molecular weight distribution standards are available for this material, a direct calibration method was chosen. Eight polystyrene standards were run and the calibration curve shown below obtained.
Values obtained from the chromatogram and from the calibration curve were then entered on the sheet shown here. $\bar{M}_N$ was calculated to be 13,000 and $\bar{M}_W$ 2,068; $D = 2.5$.

<table>
<thead>
<tr>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RETENTION VOLUME OR COUNTS</td>
<td>HEIGHT (mm)</td>
<td>CHAIN LENGTH OR MOL WT</td>
<td>Col 2/Col 3</td>
</tr>
<tr>
<td>30</td>
<td>1.0</td>
<td>340K</td>
<td>0.000029</td>
<td>340,000</td>
</tr>
<tr>
<td>31</td>
<td>17</td>
<td>162K</td>
<td>0.000105</td>
<td>2,754,000</td>
</tr>
<tr>
<td>32</td>
<td>82</td>
<td>77K</td>
<td>0.001065</td>
<td>6,314,000</td>
</tr>
<tr>
<td>33</td>
<td>194</td>
<td>35K</td>
<td>0.005543</td>
<td>6,790,000</td>
</tr>
<tr>
<td>34</td>
<td>180</td>
<td>19K</td>
<td>0.009474</td>
<td>3,420,000</td>
</tr>
<tr>
<td>35</td>
<td>90</td>
<td>12K</td>
<td>0.007500</td>
<td>1,080,000</td>
</tr>
<tr>
<td>36</td>
<td>41</td>
<td>7.8K</td>
<td>0.006256</td>
<td>319,800</td>
</tr>
<tr>
<td>37</td>
<td>26</td>
<td>5.2K</td>
<td>0.00500</td>
<td>135,200</td>
</tr>
<tr>
<td>38</td>
<td>13.5</td>
<td>3.6K</td>
<td>0.003750</td>
<td>48,600</td>
</tr>
<tr>
<td>39</td>
<td>8.5</td>
<td>2.0K</td>
<td>0.004250</td>
<td>17,000</td>
</tr>
<tr>
<td>40</td>
<td>6</td>
<td>1.3K</td>
<td>0.004615</td>
<td>7,800</td>
</tr>
<tr>
<td>41</td>
<td>2.5</td>
<td>820</td>
<td>0.003049</td>
<td>2,050</td>
</tr>
<tr>
<td>42</td>
<td>0.5</td>
<td>510</td>
<td>0.000980</td>
<td>255</td>
</tr>
<tr>
<td>662</td>
<td></td>
<td></td>
<td>0.050616</td>
<td>21,228,705</td>
</tr>
</tbody>
</table>

$\bar{M}_N = \sum \text{Col 2/Col 4}$

$\bar{M}_W = \sum \text{Col 5/Col 2}$

$\bar{M}_N = 662/0.05062 = 13,000$

$\bar{M}_W = 21,228,705/662 = 32,067.53$

The calibration techniques described here are general approaches. The following list of references will be useful if more sophisticated calibration techniques are required.
REFERENCES

(1) ALLIET, D.F., and Paccio, J.M., “Calibration of Gel Permeation Chromatography Based Upon Polymer Coil Size”, Reprints, Sixth International Seminar on Gel Permeation Chromatography, Miami Beach, Fla., 1968.

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(10) BLY, D.D., “Unique Properties and Uses of the Linear Log of Calibration in Gel Permeation Chromatography”, Reprints, Sixth International Seminar on Gel Permeation Chromatography, Miami Beach, Fla., 1968.


(32) GULIANA, R., and Wild, L., "Molecular Weight Distribution of Branched Polyethylene by Gel Permeation Chromatography", Reprints, Sixth International Seminar on Gel Permeation Chromatography, Miami Beach, Fla., 1968.


