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Standard Practice for Use of Liquid Exclusion Chromatography Terms and Relationships¹

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INTRODUCTION

Liquid exclusion chromatography (LEC) began as “gel filtration chromatography” which is attributed to Porath and Flodin.² With the invention of new column packings by Moore³ for organic-phase work a new form of LEC developed which commonly became known as gel permeation chromatography or GPC. Liquid exclusion chromatography is a form of liquid chromatography (some other forms being partition, ion-exchange, and adsorption) and as such is the preferred name for the technique; however, the reader must be aware that other names are common in the literature, the most prevalent being those cited above. LEC differs from all other chromatographic techniques in that only the exclusion mechanism may be operative if meaningful data are to result. Most other chromatographic mechanisms operate in essentially the opposite way, that is, with small molecules exiting first. Any combination of mechanisms causes confusion and is misleading.

Liquid exclusion chromatography as used for the analysis of polymers has grown and matured since the first issuance of this practice in 1972. Therefore, some infrequently used or “outdated” terms have been deleted and some modern practices (or terms) have been included. Modern developments include the use of constant-volume pumps, use of “microparticle” column packings and much smaller columns, and automated data-handling procedures. In addition, SI units as recommended in ASTM Standard E 380 for Metric Practice⁴ are now used.

1. Scope *

1.1 This practice covers the definitions of terms and symbols most often used in liquid exclusion chromatography. Wherever possible, these terms and symbols are consistent with those used in other chromatographic techniques.⁵ As additional terms and relationships are developed, they will be incorporated.

NOTE 1—There is no similar or equivalent ISO standard.

2. Terminology Apparatus Definitions

2.1 *sample inlet system*—a means of introducing samples onto the column.

2.2 *direct injector*—a device for introducing a sample from a source external to the column directly onto the column (for example, septum-syringe injector).

2.3 *by-pass or loop injector*—the injector most common in liquid exclusion chromatography and which utilizes a sample chamber that can be filled with sample while it is temporarily external to the flowing liquid stream. It can be manipulated by means of a valving device to sweep the sample with eluent into the column.

2.4 *columns*—tubes that contain the column packing.

2.5 *column end-fittings*—devices that prevent the column packing from passing through them but which are permeable to the eluent (solvent or solution).

2.6 *detectors*—devices that sense and measure the concentration or other physical property of solute components in the solution (eluate) passing through.

2.7 *differential detectors*—devices that sense and measure the difference in a physical or chemical property between a solution (solvent containing solute components) and a reference liquid (for example, solvent alone).

2.8 *absolute detectors*—devices that sense and measure the absolute concentration or other physical property of solute components contained in the eluate.

2.9 *collection devices*—devices used to collect discrete portions of an eluate according to a preset cycle (for example, times, volume, etc.).

¹ This practice is under the jurisdiction of the ASTM Committee D-20 on Plastics and is the direct responsibility of Subcommittee D 20.70 on Analytical Methods (Section D20.70.02).

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² Porath, J., and Flodin, P., *Nature*, NTRWA, Vol 183, 1959, p. 1657.

³ Moore, J. C., *Journal of Polymer Science*, JPYAA, Part A, Vol 2, 1964, p. 835.

⁴ *Annual Book of ASTM Standards*, Vol 14.02; excerpts in all volumes.

⁵ *Journal of Gas Chromatography*, JCHSB, Vol 66, 1968.

***A Summary of Changes section appears at the end of this standard.**

2.10 *pump*—any device that causes mobile phase to flow through the columns.

3. Reagent Definitions

3.1 *eluent*—the mobile phase or solvent used to sweep or elute the sample (solute) components into, through, and out of the column. Its composition is the same as the stationary liquid phase.

3.2 *eluate*—the liquid emerging from the column.

3.3 *solutes*—dissolved substances that, in LEC, are caused to pass through the column and to influence the detector response.

3.4 *column packing*—the stationary phase which consists of microporous material and the stationary liquid phase contained in the pores.

4. Performance in Terms of Resolution, Column Efficiency, and Precision

4.1 Resolution is the resultant of two effects, the separating power of the column packing and the efficiency or peak broadening. The separating power of the column packing is dependent on pore size and pore volume. Peak broadening depends on the nature of the column packings, on how well the columns are packed, and on instrumental components external to the columns. The equations used in LEC (GPC) are similar to those used in other chromatographic techniques. Reference may be made to any standard chromatography text. Resolution for any two samples is defined by the following equation:

$$R_{1,2} = 2[(V_{R_1} - V_{R_2})/(W_1 + W_2)] \quad (1)$$

where subscripts 1 and 2 refer to samples 1 and 2. (See tables for symbols used in this equation.) For complete separation, $R_{1,2}$ must be 1.25 or greater.

4.2 Column efficiency is a measure of peak spreading or the rate of generation of variance with column length. For a monodisperse material, efficiency is the number of theoretical plates, N , for the entire system defined as follows: (See tables for symbols used in this equation.)

$$N = 16(V_R/W)^2 \text{ or } \sigma = N/V_R \quad (2)$$

This expression includes all contributions to peak broadening.

4.3 Precision and accuracy are used according to their accepted definitions. Precision is inherent to the system. Both precision and accuracy are dependent on the method of calibration and treatment of the data as well as on the resolving power of the columns. The accuracy must be determined by comparison with other methods. For example, the molecular-weight distribution can be compared with that obtained from equilibrium ultracentrifugation. More commonly the weight- and number-average molecular weights computed from the LEC (GPC) trace are compared with those measured by light scattering, (both static and on-line), osmometry, and on-line viscometry.

5. Readout Definitions

5.1 *chromatogram*—a plot of detector response against volume of eluate emerging from the system. An idealized chromatogram obtained with a detector providing differential response is shown in Fig. 1.

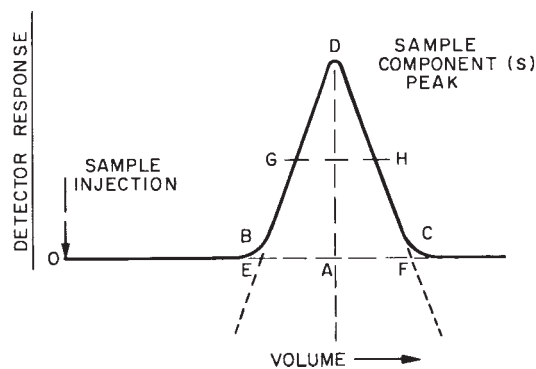


FIG. 1 Typical Chromatogram

5.2 The definitions that follow apply to chromatograms obtained directly by means of differential detectors:

5.2.1 *baseline*—the portion of the chromatogram recording the detector response when only eluent emerges from the column.

5.2.2 *peak*—the portion of the chromatogram recording the detector response while a single component or a single distribution (two or more sample components that emerge together as a single peak) is eluted from the column.

5.2.3 *peak base, BC*—an interpolation of the baseline between extremities of the peak.

5.2.4 *peak area, BGDHCAB*—the area enclosed between the peak and the peak base.

5.2.5 *peak height, AD*—the dimension from the peak maximum to the base measured in the direction of detector response.

5.2.6 *peak width, EF*—the retention volume interval of the segment of peak base intercepted by tangents to the inflection points on either side of the peak.

5.2.7 *half width, GH*—the retention volume interval of a line parallel to the peak base, bisecting the peak height, and terminating at the sides of the peak.

6. Volume Parameters

6.1 Volume parameters expected to be involved in LEC are listed in the glossary of terms found in Tables 1 and 2. These terms are intended for use where the primary mechanism of separation depends on the size of the solute molecules. The hydrodynamic volume of polymers usually may be related to V_R independent of molecular weight and structure, for example, in describing the exclusion limit. Hydrodynamic volume is related to the product of intrinsic viscosity and molecular weight. For small molecules, molar volume is a generally applicable parameter for correlation with retention volume. Molar volumes can be readily calculated from experimentally measured densities or estimated at any temperature of interest by use of critical properties. Of course, determination of the molecular weight distribution of a polymer by LEC requires a known relationship between molecular weight of the polymer and retention volume.

7. Presentation of Data

7.1 Calibration procedures and data should be included in all LEC publications as follows:

TABLE 1 Recommended List of Terms and Symbols for Use in LEC—Part I

PART I—LEC PARAMETERS					
No.	Parameter	Symbol	Units ^A		Definitions
			SI	Common	
1	Peak width	W	cm ³	ml	The distance between the baseline intercepts of lines drawn tangent to the points of inflection of the trace
2	Peak width at half height	$W_{1/2}$	cm ³	ml	Peak width measured parallel to baseline at 50 % of its height
3	Peak area	A	cm ²	cm ²	The area enclosed between the peak and its base, $ABGDHCA$ in Fig. 1
4	Retention volume	V_R	cm ³	ml	Volume of liquid that has passed through the system from middle of sample injection period to peak maximum
5	Volume of mobile phase in interstitial volume or void volume	V_o	cm ³	ml	Volume of mobile phase in the interstices between the gel particles
6	External volume	V_{ext}	cm ³	ml	Contributions to V by all system components external to column
7	Stationary liquid volume	V_i	cm ³	ml	Total carrier volume contained within the porous support
8	Total liquid volume	V_t	cm ³	ml	$V_i + V_o + V_{ext}$
9	Hydrodynamic volume	V_h	cm ³ ·mol ⁻¹	ml/mol	A polymer molecular property proportional to $\{\eta\}M$. See Part II.
10	Exclusion limit	$V_{h,max}$	cm ³ ·mol ⁻¹	ml/mol	Max V_h that entered into pore
11	Column plate count or number of theoretical plates for system	N	—	—	$16(V_R/W)^2$ for Gaussian peak
12	Effective plates	$N_{(eff)}$	—	—	$16[(V_R - V_o)/W]^2$
13	Reduced H	h	—	—	H/D_w
14	Height equivalent to theoretical plate for system	H	mm	mm	L/N where L is the column length
15	Resolution	$R_{1,2}$	—	—	$2[V_{R1} - V_{R2}]/W_1 + W_2]$
16	Specific resolution	R_s	—	—	$R_{1,2}/[\log_{10}(M_2/M_1)]$
17	Mobile phase velocity	μ	cm·s ⁻¹	cm/s	F divided by internal cross-sectional area of the column
18	Eluent flowrate	F	cm ³ ·s ⁻¹	ml/min	Eluent volume flow at column temperature
19	Distribution coefficient	K	—	—	$(V_R - V_o)/V_i$
20	Solute capacity factor	K'	—	—	$(V_R - V_o)/V_o = KV_i/V_o$
21	Diffusion coefficient of solute	D_s	cm ² ·s ⁻¹	cm ² /s	
22	Molar volume	V_m	cm ³ ·mol ⁻¹	cm ³ /mol	A basic molecular property
23	Calibration curve	—	—	—	Relationship between a molecular parameter (such as M , V_m , or V_h) and V_R
24	Standard deviation for a Gaussian curve	σ	cm ³	ml	$W/4 = (1/2)W_{1/2} \times (2 \times \ln 2)^{-1/2} = (N/V_R)^{1/2}$
25	Pore diameter		mm	mm	(determined by physical measurement)
26	Time	t	s	min	
27	Temperature	T	K	°C	For fundamental calculations
28	Column inlet pressure	P	Pa	psi	
29	Pressure drop	Δp	Pa	psi	
30	Weight	w	kg	g	
31	Density	ρ	kg·m ⁻³	g/cm ³	

^A It is recommended that reported values first be stated in the units as actually measured followed by SI units in parentheses if SI is not the unit of measurement.

7.1.1 Number, length, inner diameter, exclusion limit, and theoretical plate numbers of the columns.

7.1.2 Type of column packing, solvent, temperature, solute concentration, and flowrate.

7.1.3 Type and average molecular weights of the calibration polymers, together with the limiting viscosity number (intrinsic viscosity) of these polymers in the above solvent at operating temperature. In case of small molecules, the molar volumes of the calibration samples should be given.

7.1.4 Concentration of calibration solution and injection volume.

7.1.5 Retention volume or time for each calibration sample.

7.2 Sample procedures and data for unknown polymer samples should be included as in 7.1.1-7.1.5, except any unknown data in 7.1.3.

7.3 Calculation procedures should be described. If the calculations are carried out by computer, the origin of the program should be reported. The same applies to any curve shaping that may have been done. (Often further corrections are applied in order to obtain more accurate quantitative information.)

8. Keywords

8.1 chromatogram; chromatography; gel permeation chromatography; liquid chromatography; liquid exclusion chromatography; molecular weight; molecular weight distribution; size exclusion chromatography

TABLE 2 Recommended List of Terms and Symbols for Use in LEC—Part II

PART II—PARTICLE AND MOLECULAR PARAMETERS

No.	Parameter	Symbol	Units ^A		Definitions
			SI	Common	
1	Molecular weight	M	$\text{g}\cdot\text{mol}^{-1}$	g/mol	The sums of all the atomic weights of the atoms in a molecule
2	Weight differential distribution of molecular weights	$f_w(M)$	—	—	Plot of weight population density as a function of M or the frequency distribution of M
3	Number-average molecular weight	\bar{M}_n	$\text{g}\cdot\text{mol}^{-1}$	g/mol	First moment or mean of the number distribution of molecular weights can be expressed in terms of $f_w(M)$: $\int f_w(M)dM/\int (1/M)f_w(M)dM$
4	Weight-average molecular weight	\bar{M}_w	$\text{g}\cdot\text{mol}^{-1}$	g/mol	First moment or mean of the weight distribution of molecular weight: $\int Mf_w(M)dM/\int f_w(M)dM$
5	Z-average molecular weight	\bar{M}_z	$\text{g}\cdot\text{mol}^{-1}$	g/mol	First moment or mean of the Z-distribution of molecular weights which can be expressed in terms of $f_w(M)$: $\int M^2f_w(M)/\int Mf_w(M) dM$
6	Z + 1-average molecular weight	\bar{M}_{z+1}	$\text{g}\cdot\text{mol}^{-1}$	g/mol	First moment or mean of the Z + 1 distribution of molecular weights which can be expressed in terms of $f_w(M)$: $\int M^3f_w(M)dM/\int M^2f_w(M)dM$
7	Polydispersity factor	P or d	—	—	\bar{M}_w/\bar{M}_n
8	Intrinsic viscosity	$[\eta]$	$\text{m}^3\cdot\text{kg}^{-1}$	dl/g	See ASTM Method D 2857, Test for Dilute Solution Viscosity of Polymers, <i>Annual Book of ASTM Standards</i> , Vol 08.02.
9	Mark-Houwink intrinsic viscosity—molecular weight relation	—	—	—	$[\eta] = KM^a$ where K and a are empirically determined parameters for a given polymer solvent system at a specific temperature
10	Viscosity average molecular weight	\bar{M}_v	$\text{g}\cdot\text{mol}^{-1}$	g/mol	$([\eta]/K)^{1/a} = [\int M^a f_w(M)dM/\int f_w(M)dM]^{1/a}$
11	Instrument symmetrical spreading parameter	X_1	—	—	Used to correct for symmetric band broadening. See Method D 3536, Test for Molecular Weight Averages and Molecular Weight Distribution of Polystyrene by Liquid Exclusion Chromatography (Gel Permeation Chromatography—GPC), <i>Annual Book of ASTM Standards</i> , Vol 08.03. $(1/2) \left[\frac{\bar{M}_n(t)}{\bar{M}_n(u)} + \frac{\bar{M}_w(u)}{\bar{M}_w(t)} \right]$
12	Instrument skewing parameter	X_2	—	—	Used to correct for nonsymmetric band broadening. See Method D 3536. $\frac{[\bar{M}_n(t)/\bar{M}_n(u)][\bar{M}_w(t)/\bar{M}_w(u)] - 1}{[\bar{M}_n(t)/\bar{M}_n(u)][\bar{M}_w(t)/\bar{M}_w(u)] + 1}$
13	Uncorrected molecular weight averages	$\bar{M}_n(u)$ $\bar{M}_w(u)$	$\text{g}\cdot\text{mol}^{-1}$	g/mol	Number and weight average molecular weights uncorrected for instrument spreading
14	Corrected molecular weight averages	$\bar{M}_n(c)$ $\bar{M}_w(c)$	$\text{g}\cdot\text{mol}^{-1}$	g/mol	Number and weight average molecular weights corrected for instrument broadening
15	Observable or “true” molecular weight averages	$\bar{M}_n(t)$ $\bar{M}_w(t)$	$\text{g}\cdot\text{mol}^{-1}$	g/mol	Number and weight average molecular weights obtained by classical methods such as osmometry and light scattering
16	Weight differential distribution of particle diameter	$f_w(D)$	—	—	Plot of weight population density as a function of D or the frequency distribution of D
17	Weight-average particle diameter	\bar{D}_w	m	μm	First moment or mean of the weight distribution of particle diameters: $\int Df_w(D)dD/\int f_w(D)dD$
18	Number-average particle diameter	\bar{D}_n	m	μm	First moment of the number distribution of particle diameters can be expressed in terms of $f_w(D)$: $\int (1/D^2)f_w(D)dD/\int (1/D^3)f_w(D)dD$
19	Lowest molecular weight	M_L	$\text{g}\cdot\text{mol}^{-1}$	g/mol	Lowest value of molecular weight in the molecular weight distribution
20	Highest molecular weight	M_H	$\text{g}\cdot\text{mol}^{-1}$	g/mol	Highest value of molecular weight in the molecular weight distribution
21	Weight cumulative (or integral) distribution of molecular weights	$I_w(M)$	—	—	Sum of weight fractions as a function of molecular weight;
22	Variance of molecular weight distribution	σ_j^2	$(\text{g}\cdot\text{mol}^{-1})^2$	$(\text{g/mol})^2$	$\int_{M_L}^M f_w(M)dM/\int_{M_L}^{M_H} f_w(M)dM$ Second moment about the mean of a specific type of molecular weight distribution ($j = n, w, z, z + 1$); a measure of the breadth of the distribution

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SUMMARY OF CHANGES

Committee D-20 has identified the location of selected changes to this practice since the last issue that may impact the use of this practice.

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| (1) Added ISO equivalency statement. | and other techniques used for comparison. |
| (2) Added “or other physical property” to 2.6 to reflect use of light scattering detectors. | (5) Deleted part of 6.1 referring to retention volumes since most modern data systems are based on time. |
| (3) Added “or solvent” to reagent definition in 3.1. | (6) Added “or time” to 7.1.5. |
| (4) Last statement of 4.3 was modified showing osmometry | (7) Added “molecular weight distribution” to Keywords. |

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