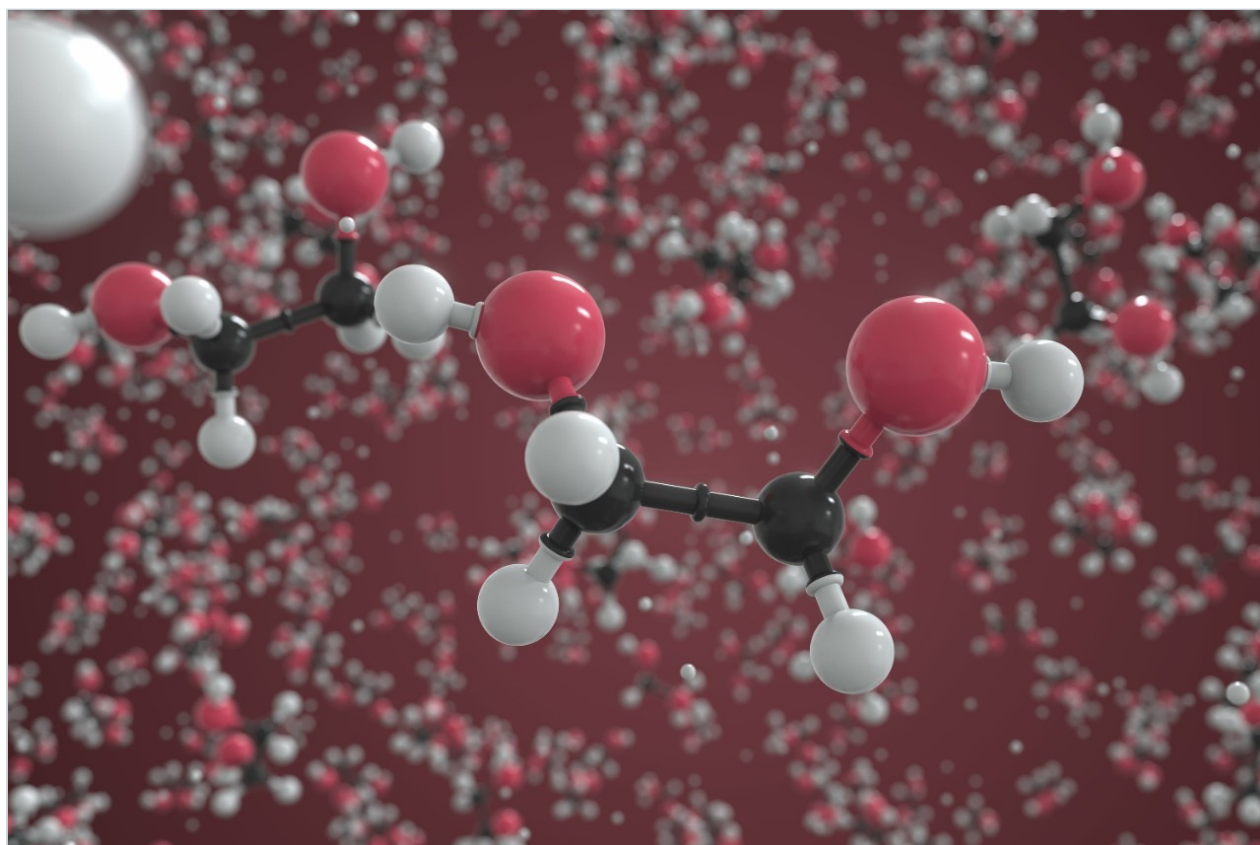


Application Note

Arc HPLC Aqueous SEC/GPC Separation of PEO/PEG

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This is an Application Brief and does not contain a detailed Experimental section.

Abstract

This application brief assesses the performance of the Waters Arc HPLC System for Gel Permeation Chromatography (GPC), also known as Size Exclusion Chromatography (SEC) of polyethylene oxide/polyethylene glycol (PEO/PEG).

Benefits

- The Arc HPLC System, running Empower 3 Software, delivers precise flow rate to obtain accurate, reproducible peaks in GPC analysis
- The Ultrahydrogel Linear Column provides polymer size exclusion separation nominal 7 million Daltons



**Ultrahydrogel Linear
(WAT011545)**



**Polyethylene glycol
(WAT035711) and
polyethylene oxide
(WAT011572) standards kits**



Introduction

Gel permeation chromatography requires a high-performance liquid chromatography (HPLC) system that delivers precise flow and reproducible results. The GPC technique plots retention time against the log of the molecular weight, thus, the pump must produce a consistent isocratic flow to provide reproducible chromatographic peaks.¹ The most often used method for determining the molecular weight of an unknown sample is by comparing the sample against a relative calibration curve of narrow molecular weight polymer standards.²

Experimental

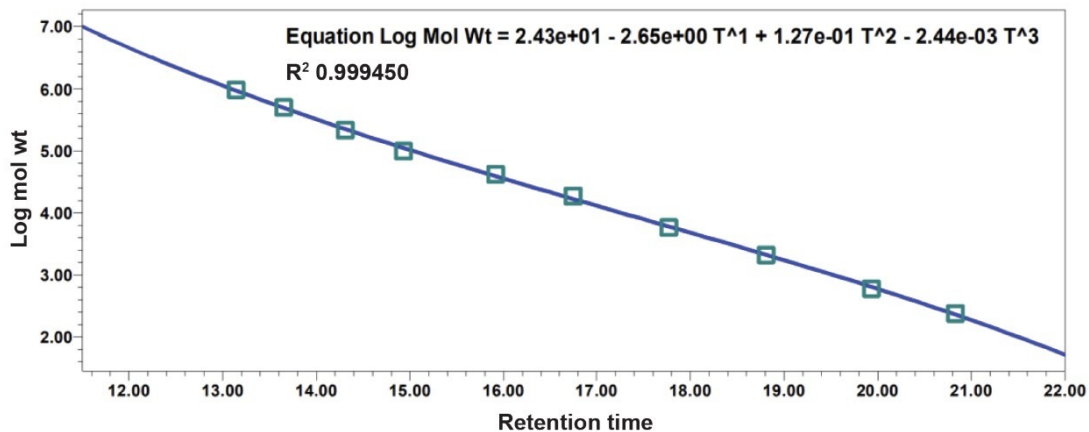
The flow rate is one of many listed Arc HPLC GPC method parameters in Table 1. This application uses polyethylene glycol and polyethylene oxide standards (PEG/PEO) for the calibration curve (Figure 1).

Method parameters	Column 1
System:	Arc HPLC
Mobile phase:	0.1 M Sodium nitrate in DI water (isocratic flow)
Seal wash:	50/50 Water/methanol
Flow rate (mL/Min.):	1
Run time (min.)	30
SM wash & purge:	DI Water
Sample conc. (mg/mL):	1.5
Sample temp. (°C):	25
Injection volume (µL):	50
Column temp. (°C):	30
Particle chemistry:	Hydroxylated polymethacrylate-based gel
Column dia (mm):	7.8
Column length (mm):	300
Particle size (mm):	10 µm
Pore size (Å):	Pore size blend, exclusion limit of 7M Daltons
Detector:	W2414 RI
Detector temp (°C):	30
Sample information	
	Polyethylene glycol/polyethylene oxide (PEG/PEO)
Molecular weight:	Mp = 238 - 969 k Daltons
Software	
Empower 3, FR5	with GPC option

Table 1. Method parameters table.

GPC calibration curve information

Sample Name:	****	A:	2.432678e+01	D:	-2.439494e-03
Processing Method:	ArchHPLC PEO PEG 1ml Lin 30min	B:	-2.648674e+00	E:	0.000000e+00
Fit Order:	3	C:	1.273373e-01	F:	0.000000e+00
Cal Curve ID:	1718	R:	0.999725	R ² :	0.999450
Date Calibrated:	7/15/2021 9:47:03 AM EDT	v0:	11.500000	vt:	22.000000



GPC Calibration Table

	Retention time (min)	MOL wt. (Daltons)	Log mol wt.	Calculated weight (Daltons)	% Residual
1	13.141	969000	5.9863	941646	2.905
2	14.931	99000	4.9956	111481	-11.196
3	17.770	5800	3.7634	6039	-3.966
4	13.651	504000	5.7024	493664	2.094
5	15.914	42000	4.6232	39162	7.246
6	19.932	599	2.7774	638	-6.180
7	13.651	217000	5.3365	225328	-3.696
8	16.748	18600	4.2695	16761	10.971
9	18.808	2100	3.3222	2111	-0.525
10	20.828	238	2.3766	228	4.342

Figure 1 and Table 2. Relative calibration curve using PEG/PEO narrow molecular weight calibration standards with the resulting table of retention times, calculated weight, and % residual values.

The excellent flow rate precision of the Arc HPLC Pump is demonstrated in an overlay of six injections of a PEO/PEG mixed molecular weight standard as shown in Figure 2. Relative standard deviations of the peak retention time and peak molecular weight (Mp) at <0.3% are obtained on a Waters Arc HPLC System using two linear GPC columns and a 30-minute run time (Table 3 and 4).

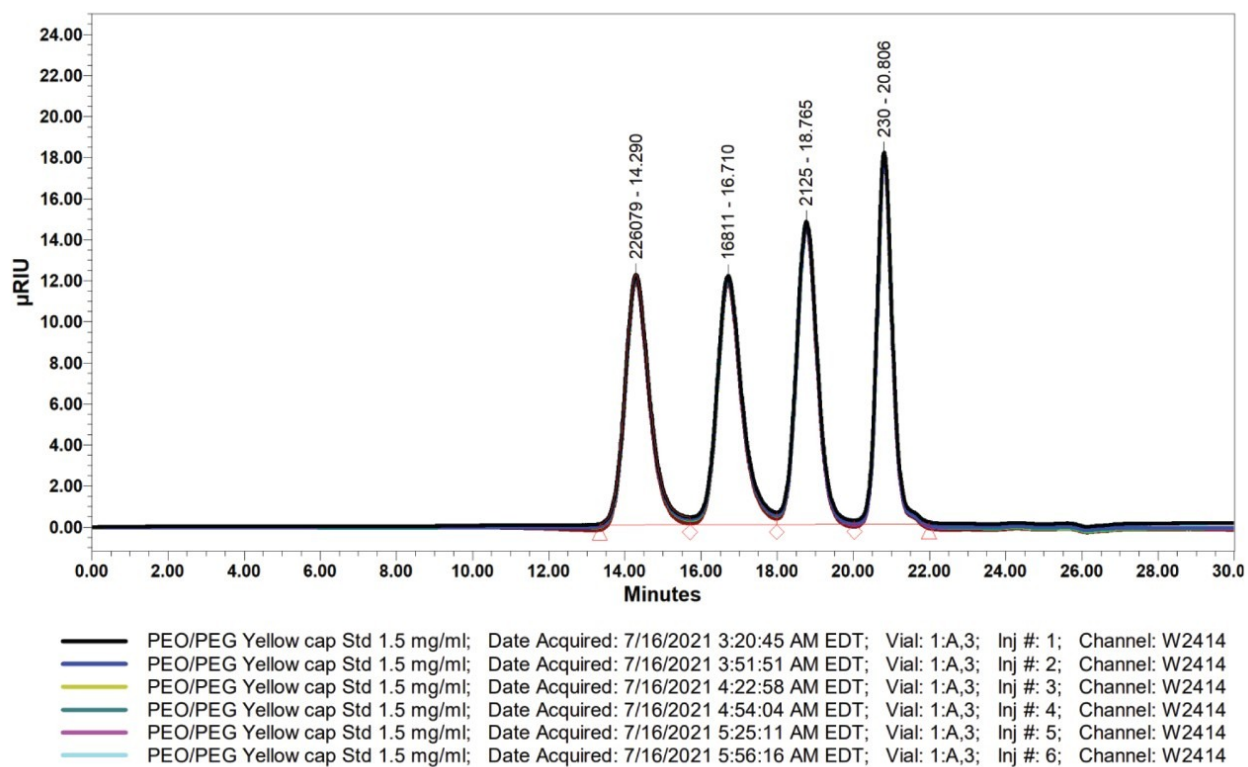


Figure 2. Six injections of the PEO/PEG calibration standard.

Peak results
retention time summarized by name

	Y1 (min)	Y2 (min)	Y3 (min)	Y4 (min)
1	14.289	16.712	18.767	20.808
2	14.289	16.708	18.764	20.805
3	14.290	16.708	18.764	20.808
4	14.290	16.708	18.765	20.807
5	14.290	16.710	18.765	20.806
6	14.292	16.714	18.769	20.811
Mean	14.2902	16.7100	18.7655	20.8074
Std. dev.	0.0012	0.0025	0.0019	0.0020
% RSD	0.0081	0.0151	0.0101	0.0096

Peak results MP summarized by name

	Y1 (Daltons)	Y2 (Daltons)	Y3 (Daltons)	Y4 (Daltons)
1	226523	16765	2121	229
2	226427	16843	2128	230
3	226130	16836	2126	229
4	226104	16837	2126	229
5	226079	16811	2125	230
6	225653	16742	2117	228
Mean	226153	16806	2124	229
Std. dev.	307	43	4	1
% RSD	0.14	0.25	0.19	0.24

Table 3 and 4. Empower 3 data report from six injections of the yellow cap PEO/PEG calibration standard peak retention time and peak molecular weight (Mp).

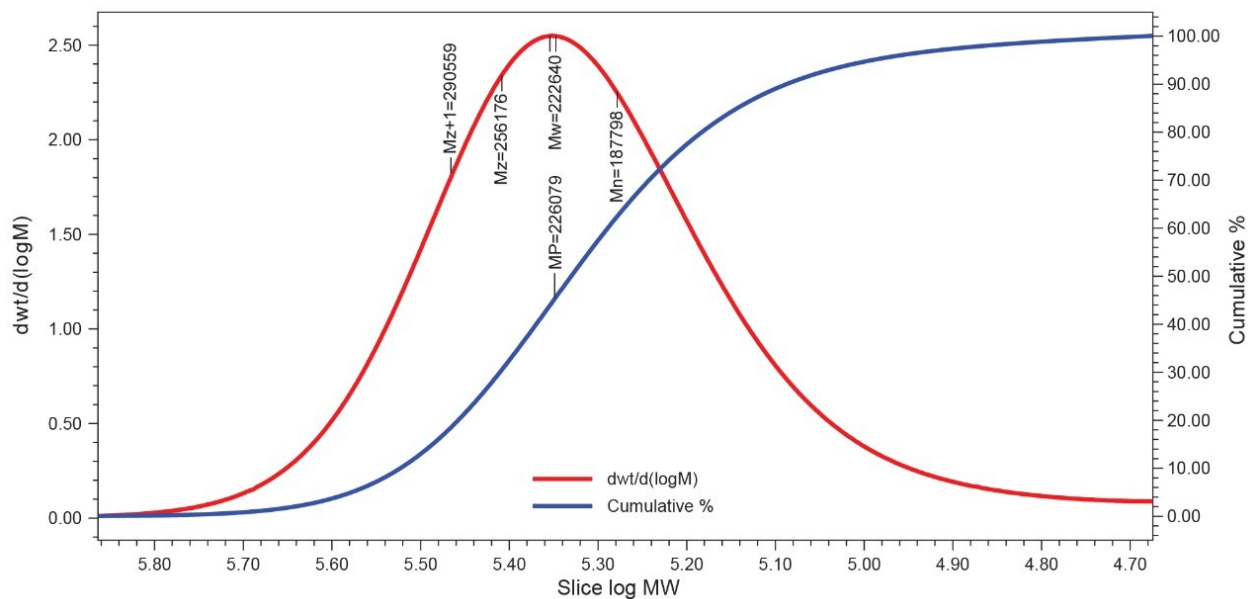


Figure 3. Molecular weight distribution and %Cumulative of the 227 kDalton PEO/PEG calibration standard are depicted as an example of one of the automatically calculated features of the Empower 3 GPC option.

Empower 3 has many built-in features that enable sophisticated reporting of data usually done in spreadsheets. A popular reporting area is column plate count, which is included with the system suitability feature. By reporting the column plate counts, the column efficiency can be charted and observed for any changes in performance (Table 5).

	Name	Mn (Daltons)	Mw (Daltons)	MP (Daltons)	Mz (Daltons)	Mz+1 (Daltons)	Ploydisperity	Mz/Mw	Mz+1/Mw	Area ($\mu\text{V}\cdot\text{sec}$)	% Area	Height (μV)	USP plate count
1	Y1	187798	222640	226079	256176	290559	1.186	1.151	1.305	565016	24.99	12190	2328
2	Y2	14435	16580	16811	18766	21167	1.149	1.132	1.277	575722	25.47	12125	3174
3	Y3	1951	2139	2125	2321	2507	1.096	1.085	1.172	586796	25.96	14757	5396
4	Y4	209	227	230	244	260	1.087	1.071	1.143	532986	23.58	18102	12578

Table 5. Empower 3 calculated peak fields.

Results and Discussion

The Arc HPLC with Empower 3 Software is a very capable GPC data processing tool for fundamental and complex chromatograms and calculations. In this experiment, the fundamental GPC calculations are demonstrated. The narrow PEO/PEG standards are used as calibration standards, and as broad unknowns for this experiment. The

choice of method and standards used in this application are common for aqueous GPC of polymers like carbohydrates.³ The linear Ultrahydrogel Column is chosen for a wide molecular range nominal to seven million Daltons, and two of the same column are used for higher resolution of polymer peaks.⁴ The Empower 3 Software's partial list of reportable peak result fields includes Mn, Mw, Mp, Mz, Mz+1, and polydispersity (Table 5). Relative and modified universal calibration types employing point-to-point and first- through fifth-order fits are available, and an example of relative calibration is shown in Figure 1.

Conclusion

The Arc HPLC Pumps in isocratic mode provide the excellent flow precision necessary for high-quality gel permeation chromatography.

All basic GPC calculations can be calculated using the Empower 3 Software with the GPC option, and numerous complex GPC calculations are accessible.

Short analysis time of 30 minutes was demonstrated using two linear columns.

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