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Note

Characterization and molecular weight determination of water-soluble polyethylene glycol oligomers using open-tubing liquid chromatography-mass spectrometry

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Characterization of water-soluble polyethylene glycol (PEG) oligomers by liquid chromatography (LC) has been previously reported¹⁻³. Either refractive index detection¹ or low-wavelength UV detection (180-220 nm)^{2,3} was used. The mobile phases used in the separations were methanol-water¹ and acetonitrile-water^{2,3} while the stationary phases were octyl-bonded^{1,2} or phenyl-bonded silica³.

The molecular weight of polymers is commonly determined by size-exclusion chromatography⁴. A previous study⁵ demonstrated that the liquid chromatogram of oligomers can be used to determine the molecular weight. Polyethylene glycol was derivatized and its gas chromatogram was used to determine the molecular weight⁶⁻⁸.

This report describes an open-tubing LC-mass spectrometry (MS) method to characterize PEG oligomers. The mass spectrum of PEG 400 showed major abundances of the $M + 1$, $M + 2$ and $M + 3$ mass ions; while the abundances of other mass fragments were minute. The relative abundances of the molecular ions were used to calculate averaged molecular weights and the polydispersity.

EXPERIMENTAL

Mass spectrometer

An HP-5987A quadrupole mass spectrometer system was used with a high-performance liquid chromatography (HPLC) interface. The HPLC interface included a Beckman 112 solvent delivery module, a cryopump and a sample introduction probe. The cryopump consisted of a liquid nitrogen transfer line and an automatic liquid level monitoring and filling station. In this experiment, an open stainless-steel tubing (1 ft. \times 1/16 in. I.D.) was used to connect the sample introduction probe and the liquid chromatograph, and no HPLC column was used. The HP-5987A LC-MS interface operates on Baldwin and McLafferty's split chemical-ionization (CI) principle⁹. A portion of the eluate from the LC instrument (1-4%, depending upon flow-rate and source conditions¹⁰) is injected into the MS source, the solvent vapors acting as the ionization gas in the CI mode of operation. The function of the cryopump is to increase the pumping speed (*ca.* ten-fold) for the most common HPLC solvents over a similar operation by means of a diffusion pump alone¹¹; consequently more sample can be introduced into the MS source.

The mobile phase used was methanol-water (80:20) at a flow-rate of 0.5 ml/min. A high abundance of solvent ion at m/z 65, $[(\text{CH}_3\text{OH})_2 + \text{H}]^+$, was observed; thus the mass linear scan range was set beyond m/z 100 to enhance the visibility of the molecular ion distribution of PEG 400 oligomers, and the ethylene glycol monomer (MW = 62) was not included in the mass spectrum. The ratio of ethylene glycol monomer to the total PEG 400 oligomer was less than 0.1%, which was calculated from a separate MS scan (40–800 a.m.u.) and was confirmed by the HPLC chromatogram of a previous study of PEG 400 oligomers³.

Sample

The PEG 400 sample has been described previously³. The sample concentration was 0.1% (w/w) in a solution of methanol-water (80:20).

RESULTS AND DISCUSSION

Fig. 1 shows the elution peak of the PEG oligomers (molecular weight 400, PEG 400) in an open stainless-steel tubing. The mass spectrum of this peak (Fig. 2) shows the distribution of the molecular ions ($M + 1$, $M + 2$ and $M + 3$) of PEG 400 oligomers of up to 15 repeating units. As illustrated in Fig. 2, the $M + 1$ molecular ions were the most abundant. Table I lists the relative abundance of these molecular ions, which can be used to calculate the averaged molecular weights. Among the $M + 1$ mass ions, the m/z 371 (degree of polymerization = 8) mass ion had the highest abundance, while among the $M + 2$ and $M + 3$ mass ions, the m/z 415 and 416 mass ions were the most abundant (degree of polymerization = 9). The number-averaged molecular weight (M_n), weight-averaged molecular weight (M_w) and z -averaged molecular weight (M_z) can be expressed as

$$M_n = (\sum M_i P_i) / (\sum P_i) \quad (1)$$

$$M_w = (\sum M_i^2 P_i) / (\sum M_i P_i) \quad (2)$$

$$M_z = (\sum M_i^3 P_i) / (\sum M_i^2 P_i) \quad (3)$$

where P_i and M_i are the increments of relative abundance and the molecular weight, respectively. The polydispersity is defined as $P = M_w/M_n$.

Table II lists the values calculated for M_n , M_w , M_z and the polydispersity using $M + 1$ molecular ions alone, as well as the corrected values using the relative abundances of $M + 2$ and $M + 3$ molecular ions as correction factors. As illustrated in

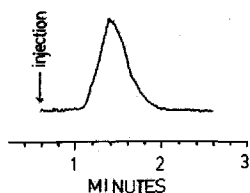


Fig. 1. Total ion current plot of the elution of PEG 400.

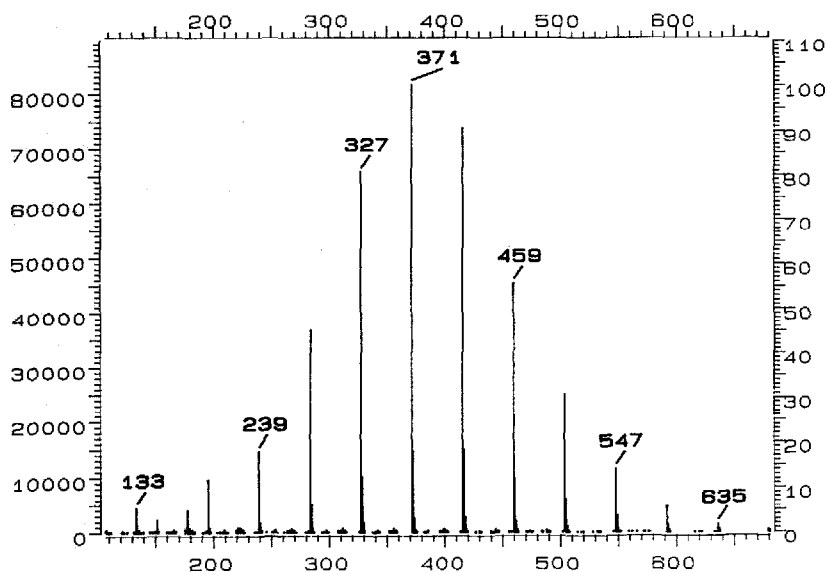


Fig. 2. Mass spectrum of PEG 400 (open-tubing LC-MS).

this table, the deviations between the uncorrected and corrected values decreased in the order M_n , M_w , M_z and polydispersity. The largest deviation was 1.03%. This illustrates that mass ions of $M + 1$ were the major ions formed in the chemical ionization environment of methanol and water. Two mass ions of impurities were observed, viz. m/z 133 and 177; both are unidentified presently.

TABLE I

RELATIVE ABUNDANCE OF MOLECULAR IONS

n = Degree of polymerization, M = molecular weight.

n	M	Relative abundance (%)		
		$M + 1$	$M + 2$	$M + 3$
2	106	3.3142	—	—
3	150	2.9567	—	—
4	194	11.7109	1.1095	0.1674
5	238	17.7905	2.0313	0.3539
6	282	44.4668	6.0186	0.9457
7	326	80.1530	12.1927	2.0708
8	370	100.0000	18.0093	3.1600
9	414	90.6647	18.3515	3.3429
10	458	56.2123	12.1150	2.4175
11	502	31.4472	7.5634	1.4849
12	546	14.3376	3.8164	0.7209
13	590	5.7831	1.5088	0.3587
14	634	1.6296	0.5679	0.1016
15	680	0.4627	0.1351	—

TABLE II
MOLECULAR WEIGHT AND POLYDISPERSITY

Numbers in parentheses are the differences between uncorrected and corrected values.

Parameter	Mass ions used in the calculation		
	$M + 1$	$M + 1$ and $M + 2$	$M + 1$, $M + 2$ and $M + 3$
P_i	460.9299	544.3476	559.4719
$P_i M_i$	174625.454	208027.9048	214161.8310
$P_i M_i^2$	69708807.700	83651536.9600	86240648.6300
$P_i M_i^3$	$2.907264 \cdot 10^{10}$	$3.1511864 \cdot 10^{10}$	$3625186 \cdot 10^{10}$
M_n	378.8547 (1.03%)	382.1600 (0.17%)	382.7928
M_w	399.1904 (0.86%)	402.1169 (0.14%)	402.6892
M_z	417.0583 (0.78%)	419.8206 (0.13%)	420.3570
$P = M_w/M_n$	1.0538 (0.17%)	1.0522 (0.02%)	1.0520

In summary, open-tubing LC-MS provides a rapid method for the characterization of PEG oligomers. Its applicability to other types of polymers is currently under investigation. In general, it is limited by both the mass fragmentation of the samples and the available linear mass scan range of the mass spectrometer.

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