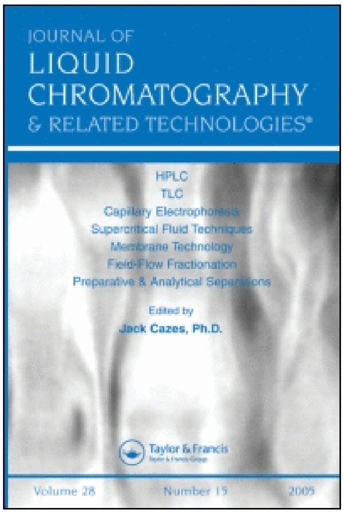

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Size Exclusion Chromatography of Quaternized Poly(vinylpyrrolidone-co-dimethyl Amino Ethyl Methacrylate) Copolymer (PVPDMAEMA): Absolute Molecular Weights and Molecular Weight Distributions by Low Angle Laser Light Scattering and Universal Calibration

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SIZE EXCLUSION CHROMATOGRAPHY OF QUATERNIZED POLY(VINYL-PYRROLIDONE-CO-DIMETHYL AMINO ETHYL METHACRYLATE) COPOLYMER (PVPDMAEMA): ABSOLUTE MOLECULAR WEIGHTS AND MOLECULAR WEIGHT DISTRIBUTIONS BY LOW ANGLE LASER LIGHT SCATTERING AND UNIVERSAL CALIBRATION

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ABSTRACT

In 0.1M tris. pH 7 buffer containing 0.5M $LiNO_3$, PVPDMAEMA (which exists as a cationic polyelectrolyte) was found to elute based on hydrodynamic volume on Waters Ultrahydrogel columns. Within the exclusion limit of the column set an accurate weight average molecular weight of PVPDMAEMA can be obtained by universal calibration which is in very good agreement with the SEC/LALLS result.

INTRODUCTION

In a recent work (1) Nagy and Terwilliger showed that universal calibration can be used successfully for a cationic polymer, poly(2-vinyl pyridine), on Synchrom Catset columns which are specially treated silica gels in an aqueous mobile phase of 0.1% trifluoroacetic acid/0.2M $NaNO_3$ at 35°C. Kato and Hashimoto (2) showed that TSK-PW columns are suitable for size exclusion chromatography (SEC) of cationic polymers in a mobile phase of 0.5M aqueous acetic acid with 0.3M sodium sulfate (pH = 2.9). Our laboratory has previously demonstrated that universal calibration can be used with the TSK-PW columns for a water soluble polymer, polyvinylpyrrolidone, in a mobile phase of water/methanol (50:50) with 0.1M lithium nitrate (3). We also showed that universal calibration can be used with Waters Ultrahydrogel columns (which are equivalent to the TSK-PW columns) for an anionic polymer, poly(methyl vinyl ether-co-maleic anhydride) copolymer, in a mobile phase of 0.1M tris. pH 9 buffer with 0.2M lithium nitrate (4).

We would like to report the results of recent work on the SEC/LALLS (size exclusion chromatography with low angle laser light scattering detector) and universal calibration behavior of a cationic polymer using Waters Ultrahydrogel columns. The cationic polymers used in this study were quaternized poly(vinyl pyrrolidone-co-dimethyl amino ethyl methacrylate) copolymers (PVPDMAEMA). The composition and synthesis of these copolymers have been discussed in detail in two earlier GAF patents (5,6). The determination of absolute molecular weights and molecular weight distributions of PVPDMAEMA polymers by SEC/LALLS and universal calibration methods will be presented.

EXPERIMENTAL

1. Polymers

The PVPDMAEMA samples employed were the Gafquat® 734 (low molecular weight grade), 755 (high molecular weight grade), and 755N (neutralized high molecular weight grade) samples obtained from GAF Chemicals Corporation. Seven Toyo Soda polyethylene oxide (PEO) standards were used in the universal calibration portion of this work whose MW's ranged from 860000 to 12600 amu with polydispersities ranging from 1.02 to 1.10. The concentrations of the PEO standard solutions used in SEC calibration were 0.05% (w/v).

2. Chromatograph

The chromatograph employed in the SEC/LALLS work was a Waters Model GPC-1 with a Rheodyne model 7125 injector (100 μl loop). The RI detector used was a Waters model 410 RI detector with a 4X detector setting. The mobile phase used in the SEC/LALLS work is 0.1M tris. pH 7 buffer with 0.3M $LiNO_3$.

The chromatograph used in the universal calibration work was a Waters Model 150C GPC. The injection volume was 100 μl. The mobile phase used in universal calibration work was identical to that used for SEC/LALLS.

3. LALLS

The LALLS detector was an LDC/Milton Roy (Chromatix) Model CMX-100 whose He-Ne Laser operates at a wavelength of 632.8 nm. The LALLS incident power settings were 150 mV and 100 mV for the Gafquat 734 and 755 samples respectively. A 0.45 μm Millipore filter was installed before the LALLS detector. Very little or no spiking was noticed in any of the LALLS curves.

The laser differential refractometer, an LDC/Milton Roy (Chromatix) Model KMX-16 (used to determine dn/dc) also operated at 632.8 nm and was thermostatted at 25°C.

4. Viscosity

Viscosities were determined at 25°C using a 0.64 mm i.d. Ubbelohde viscometer and a Schott model AVS automated viscometer. Four dilutions were made for each sample in the intrinsic viscosity determination.

5. Data Acquisition

The data acquisition system consisted of a Digital Equipment Corporation MINC-11 computer and an LDC/Milton Roy (Chromatix) Model CMX-10 interface box. The Chromatix MOLWT3 and GPC3 programs were used to perform SEC/LALLS and SEC/universal calibration calculations respectively. For universal calibration work, the data collection window was 31 minutes and 600 data points were recorded per chromatogram for universal calibration.

6. Chemicals

Tris(hydroxy methyl) aminomethane, HNO_3, and $LiNO_3$ used were reagent grade from Aldrich.

7. Sample Preparation

The concentrations of the sample used in this work were listed in Table 1.

The tris-buffer solutions were prepared from 0.1M tris(hydroxymethyl) aminomethane with 0.3 or 0.5M $LiNO_3$ and adjusted to pH 7 with HNO_3. The various PVPDMAEMA sample

Table 1

Sample Concentrations in the Experiments

sample	intrinsic viscosity	SEC	LALLS	$\frac{dn}{dc}$
734	1%	0.25 %	0.5 %	-----
755 & 755N	0.5 %	0.25 %	0.1 %	0.319% 0.408% 0.474% 0.618% 0.845%

solutions were placed on a slowly rotating wheel until the solutions were homogeneous (about 4 hours).

8. Calculation of Molecular Weights

For the SEC/LALLS experiment, MOLWT3 calculates the instantaneous timeslices of molecular weight, M_i, from the corresponding instantaneous concentration, c_i and the excess Rayleigh scattering factor, $R_{\theta,i}$, according to a modified form of the Debye equation:

$$\frac{Kc}{R_{\theta,i}} = \frac{1}{M_i} + 2A_{2,i}C_i$$

where, A_2 equals the second virial coefficient and the optical constant, K, is proportional to the squares of both the refractive index, n, and (dn/dc). The $A_{2,i}$ term is neglected in this work. This introduces only about 2% error to the final $\bar{M}_w$ because the C_i term is extremely small in a SEC/LALLS experiment (about 5×10^{-4}%). For universal calibration, GPC3 calculates M_i according to

$$M_i = \left\{ \frac{[\eta_{s,i}]M_{s,i}}{K} \right\}^{1/(a+1)}$$

where K and a are the Mark-Houwink constants for PVPDMAEMA in the SEC mobile phase and $[\eta_{s,i}]$ and $M_{s,i}$ are the instantaneous values of the PEO standards' intrinsic viscosities and molecular weights respectively. The latter are taken from a previously computed calibration curve for $[\eta_{s,i}]M_{s,i}$ versus $V_{e,i}$ (the elution volume) constructed from the individual PEO standards' $[\eta_s]$ and M_s values.

RESULTS AND DISCUSSION

A. Refractive Index Increments

The refractive index increment (dn/dc) of the 755 sample in the pH 7 mobile phase with 0.3M $LiNO_3$ at 25°C, which is needed for the calculation of the Raleigh optical constants (K), was found to be 0.151 ml/g. The same value was used for the 734 sample due to the fact that no molecular weight dependence was noticed for polymers with molecular weight higher than 50,000 in this laboratory in two previous studies (3,4). The 734 sample is a 50% solution in anhydrous ethanol (SDA-40). It is extremely difficult, if not impossible, to completely remove SDA-40 from the very polar PVPDMAEMA polymer and to determine dn/dc accurately.

B. Viscosity Data

The intrinsic viscosity data of the PEO standards and the PVPDMAEMA samples are compiled in Table 2.

Table 2

Intrinsic Viscosity Data of the PVPDMAEMA
Samples and PEO Standards in pH 7 Buffer
at Different Salt Concentrations

Sample	[η] in 0.3M $LiNO_3$	[η] in 0.5M $LiNO_3$
734	0.646	0.647
755	2.14	2.15
755N	2.22	2.22
PEO-860000	5.280	5.353
570000	4.001	-----
270000	2.341	2.418
160000	1.603	1.568
85000	0.957	0.970
45000	0.595	0.592
21000	-----	0.374
12600	-----	0.265

Table 3

Mark-Houwink Constants and Coefficients of Linear Regression of PEO Standard and PVPDMAEMA in pH 7 Buffer 0.5M $LiNO_3$

Sample	K	a	r
PEO	2.8×10^{-4}	0.72	0.9994
PVPDMAEMA	1.42×10^{-4}	0.67	0.9975

It should be noticed that for the nonionic polyethylene oxide samples and the cationic PVPDMAEMA samples the intrinsic viscosities are virtually the same in both 0.3M $LiNO_3$ and 0.5M $LiNO_3$ pH 7 buffer solutions. This indicates that the 0.3M $LiNO_3$ concentration is adequate to neutralize the electric repulsion forces between the cationic monomer units in the polymer chain.

From the data in Table 2 and Table 4, the Mark-Houwink constants, K and a, for PEO and PVPDMAEMA in the pH 7 buffer at 25°C can be obtained by linear regression analysis of Log[η] vs LogM. The results along with the coefficients of linear regression (r) are shown in Table 3.

The values of the exponent of the Mark-Houwink constants for PVPDMAEMA and PEO in the pH 7 buffer are typical of polymers in a good solvent. This indicates that the ionic strength of the 0.1M tris. pH 7 buffer with 0.3M $LiNO_3$ is strong enough to neutralize the electrostatic repulsion of the cationic units of PVPDMAEMA in this buffer. In other words both the PEO and PVPDMAEMA behave like random coils in the pH 7 buffer solution.

C. Light Scattering Data

Virtually no spiking and very stable RI and LALLS baselines were obtained in SEC/LALLS experiments. No indication of a highly branched high molecular weight component was noticed for

Table 4

Absolute Molecular Weights and Molecular Weight Distributions of PMVEMA Samples from SEC/LALLS

Sample	$\bar{M}_w$	$\bar{M}_n$	$\bar{M}_w/\bar{M}_n$
734	3.00×10^5	1.15×10^5	2.61
755	1.63×10^6	7.04×10^5	2.32
755N	2.02×10^6	8.89×10^5	2.27

this sample from the SEC/LALLS chromatogram. The absolute molecular weights and molecular weight distributions of the PVPDMAEMA samples obtained from SEC/LALLS are shown in Table 4. As indicated in an earlier paper (1) the number average molecular weight may be over estimated and the polydispersity may be under estimated by SEC/LALLS.

D. Recovery of PVPDMAEMA from Ultrahydrogel Columns in pH 7 Buffer with 0.5M $LiNO_3$

PVPDMAEMA samples were found to elute from the Ultrahydrogel columns with 100% recovery in the pH 7 buffer with 0.5M $LiNO_3$. The peak areas (determined by the cut and weigh technique) of a 755 sample SEC peak (0.25%) were found to increase linearly with injection volume (Table 5) and can be linearly extrapolated to an intercept coincident with the origin and possesses a linear regression coefficient of 0.9999. If the PVPDMAEMA samples were partially retained by adsorption on the columns, the relationship would not be linear and there would be a positive intercept on the ordinate.

E. Universal Calibration

In the early stage of this study the PVPDMAEMA samples were found to elute quantitatively from Ultrahydrogel columns in the

Table 5

SEC Peak Areas of a 0.25% 755 Sample Solution at Various Injection Volumes

Injection Volumes (ml)	Peak Areas
40	0.0647
80	0.1304
120	0.1928

Table 6

Absolute Molecular Weights and Molecular Weight Distributions of the PVPDMAEMA Samples by Universal Calibration

Sample	$\bar{M}_w$	$\bar{M}_n$	$\bar{M}_w/\bar{M}_n$
734	$3.31x10^5$	$1.10x10^5$	3.01
755	$1.72x10^6$	$4.83x10^5$	3.55
755N	$2.02x10^6$	$5.23x10^5$	3.51

pH 7 buffer with 0.3M $LiNO_3$ as in the SEC/LALLS work. However it was found later the PVPDMAEMA samples were partially retained by the columns in this mobile phase, probably due to the aging of the columns and to the fact that these columns were used for other water soluble polymers and polyelectrolytes during this period. Therefore the salt content was raised to 0.5M $LiNO_3$ to ensure 100% recovery and that size exclusion mechanism remained the only separation mode in this phase of the work.

The absolute molecular weights and molecular weight distributions of the PVPDMAEMA samples in pH 7 buffer with 0.5M $LiNO_3$ by universal calibration using PEO standards are shown in Table 6. A least squares linear fit was used in calibration since the column set was found to be linear ($\text{Log } M = 10.77 - 0.17\ V$) with a coefficient of linear regression equal to 0.9990 for the PEO standards (where M and V are the molecular weight and retention volume of each standard).

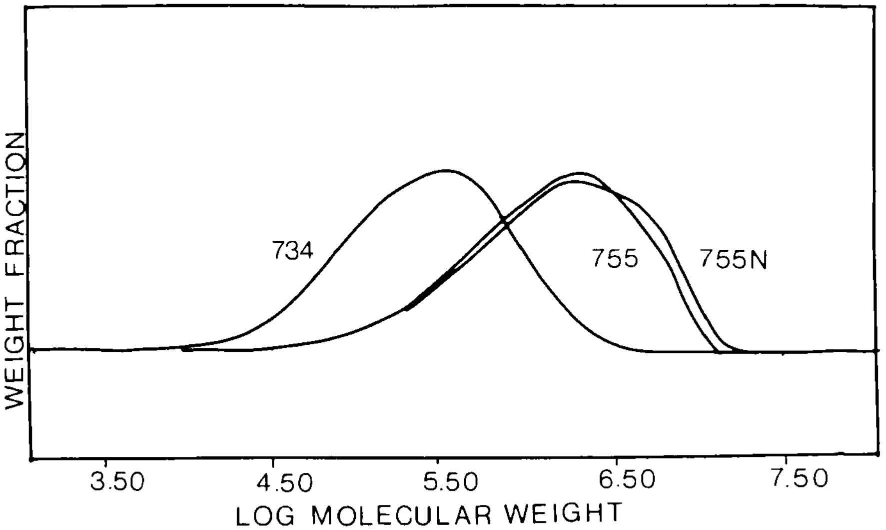

FIGURE 1 Overlay of Weight Fraction Distributions of 734, 755, and 755N

The agreement in $\bar{M}_w$ data between SEC/LALLS and SEC/universal calibration is very good for the Gafquat® polymers. This indicates that the separation mechanism of the nonionic polymer PEO and the PVPDMAEMA polymer (as a cationic polyelectrolyte) in the pH 7 buffer with 0.5M $LiNO_3$ is based on hydrodynamic volume $[\eta]$ M, where $[\eta]$ is the intrinsic viscosity and M is the molecular weight. The results also confirms an earlier finding (3) that the Mark-Houwink constants may be obtained from broad distribution polymers without fractionation for the purpose of calculating molecular weights by the universal calibration method. The overlay of the chromatograms of the PVPDMAEMA polymers is shown in Fig. 1. As indicated in an earlier paper (1) the number average molecular weight may be under estimated and the polydispersity may be over estimated by SEC with universal calibration.

CONCLUSIONS

In the 0.1M tris. pH 7 buffer containing 0.5M $LiNO_3$ the nonionic PEO and the PVPDMAEMA (which exists as cationic polyelectrolyte) were found to elute based on hydrodynamic volume on Waters Ultrahydrogel column. Within the exclusion limit of the column set absolute weight average molecular weights of PVPDMAEMA can be obtained by universal calibration which are in very good agreement with the SEC/LALLS result. The refractive index increment of the PVPDMAEMA in the pH 7 tris. buffer 0.3M $LiNO_3$ was found to be 0.151 ml/g. The Mark-Houwink constants of the PVPDMAEMA in the pH 7 tris. buffers with 0.3 or 0.5M $LiNO_3$ were found to be 1.42×10^{-4} and 0.67 for K and a respectively, which are typical of a polymer in a good solvent.

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