Dilute-Solution Properties of a Polymeric Antitumor Drug Carrier by Size-Exclusion Chromatography, Viscometry, and Light Scattering

R. MENDICHI,[1] V. RIZZO,[2] M. GIGLI,[2] A. GIACOMETTI SCHIERONI[1]

[1] Istituto di Chimica delle Macromolecole (CNR), Via Bassini 15, 20133 Milan, Italy

[2] Pharmacia & Upjohn, Viale Pasteur 10, 20014 Nerviano (MI), Italy

Received 10 November 1997; accepted 20 March 1998

ABSTRACT: An investigation is reported on the dilute-solution properties of PNU166945, a conjugate between a synthetic polymeric drug-carrier poly[*N*-(2-hydroxypropyl)-methacrylamide)] (PHPMA) and the antitumor drug Paclitaxel. Thirteen fractions of the conjugate PNU and six fractions of the polymeric drug-carrier PHPMA were prepared and characterized by size-exclusion chromatography, viscometry, and light scattering. The molar mass distribution, intrinsic viscosity $[\eta]$, and dimensions $\langle s^2 \rangle^{1/2}$ of each fraction were determined. From M, $[\eta]$, and $\langle s^2 \rangle^{1/2}$, the constants of the power laws $[\eta] = f(M)$ and $\langle s^2 \rangle^{1/2} = f(M)$ were determined. A Stockmayer–Fixman plot was utilized to derive the unperturbed dimensions of the macromolecules. The presence of the drug considerably influences the conformation of the macromolecules. For PHPMA and PNU, respectively, the slopes of the power law $[\eta] = f(M)$ were 0.69 and 0.617, the slopes of the power law $\langle s^2 \rangle^{1/2} = f(M)$ were 0.55 and 0.48, and the Kuhn statistical segments were 1.7 and 2.1 nm. To our knowledge, this is the first time that an exhaustive molecular characterization of a conjugated polymeric system has been presented. © 1998 John Wiley & Sons, Inc. J Appl Polym Sci 70: 329–338, 1998

Key words: drug-carrier; size-exclusion chromatography; light scattering; intrinsic viscosity; unperturbed dimensions

INTRODUCTION

Interest in the area of polymeric drug-delivery systems for cancer chemotherapy is increasing considerably[1–3]; hence, the interest in their molecular characterization. Polymer-based drug-delivery systems are usually designed to improve the pharmacokinetic profile of an antitumor agent, by improving tumor-specific targeting and allowing long-term controlled release. In comparison to other macromolecular carriers, synthetic polymers offer the advantage of optimizable features, such as molar mass and chain-linked targeting moieties. On the other hand, synthetic polymers are inherently heterogeneous, as far as molar mass distribution (MMD) and drug loading/distribution are concerned. This poses complex problems to the characterization of any new drug-delivery system.[4] An accurate molecular characterization of the polymeric-conjugated systems includes the knowledge of the true, not relative, MMD. This feature is particularly important, because it can modulate the plasma clearance and the polymer accessibility to target cells.[5] Besides, accumulation of nondegradable polymers in the body of the patients can be avoided by keeping the molar mass lower than the renal excretion threshold. Finally, the accessibility of the drug

Correspondence to: R. Mendichi.

Journal of Applied Polymer Science, Vol. 70, 329–338 (1998)
 CCC 0021-8995/98/020329-10

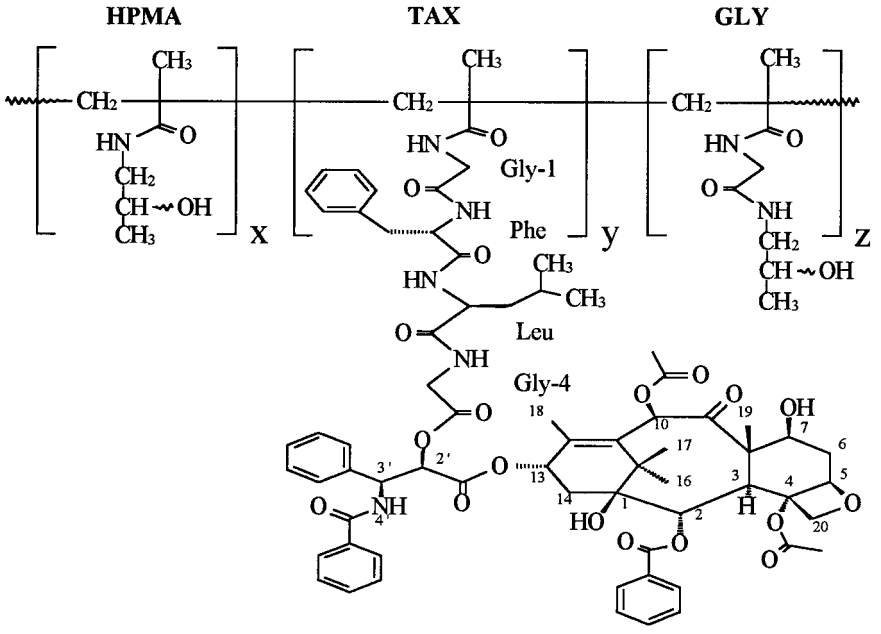

Figure 1 Structure of PNU, a random copolymer based on the HPMA monomer, a carrier containing the Paclitaxel antitumor drug connected through a tetrapeptide spacer. Composition (moles): HPMA 96.61%; TAX 0.97%; GLY 2.42%.

may be influenced in some way by the conformation of the polymeric carrier that has therefore to be deeply investigated.

PNU166945, in short PNU, is a water-soluble conjugate between a synthetic polymeric carrier poly[*N*-(2-hydroxypropyl)methacrylamide)] (PHPMA) and the antitumor drug Paclitaxel, covalently bound via a tetrapeptidyl spacer (Fig. 1). Paclitaxel possesses a well-known anticancer activity.[6] The improved antitumor activity of PNU, compared to a free (water-insoluble) drug, has been demonstrated preclinically.[7] PNU is a terpolymer with a component (HPMA) absolutely predominant with respect to the other two comonomers, which makes the study of the dilute solution properties particularly meaningful. On average, the drug content in the final PNU product is about 5% by weight, but the notable molar mass difference between the three constituent comonomers strikingly reduces this figure in a comparison of molar ratios. The molar mass of HPMA is 143 g/mol, that of the drug-carrying comonomer, denoted in Figure 1 by TAX, is 1.296 g/mol, and the third comonomer, denoted by GLY, has a molar mass of 200 g/mol. Composition analysis of a typical batch of PNU indicates that the three monomers HPMA, TAX, and GLY are in the molar ratio 1 : 0.01 : 0.025. HPMA is 96.6% in moles, and the units containing the antitumor drug are less than 1%. In this study, we investigated the MMD, intrinsic viscosity, dimensions, and conformation of the carrier and of the conjugated polymeric system. The study used a variety of techniques for the characterization of macromolecules in solution, such as multiangle light scattering (MALS) and viscometry, both off-line and on-line, to a size-exclusion chromatography (SEC) system.

EXPERIMENTAL

Materials

Unfractionated PNU samples were Pharmacia and Upjohn (Nerviano, Italy) products. Thirteen PNU fractions were obtained as described below. Six PHPMA fractions were prepared by Dr. K. Ulbrich (Institute of Macromolecular Chemistry, Czech Academy of Sciences, Prague). Seven narrow MMD poly(ethylene oxide) (PEO) standards were purchased by Toyo Soda (Tokyo, Japan). Six narrow MMD poly(ethylene glycol) (PEG) standards were purchased by Polymer Laboratories (Shropshire, UK). *N,N*-Dimethylformamide (DMF) from Aldrich (Steinheim, Germany) and methanol from Baker (Deveuter, The Netherlands) were HPLC grade. Acetic acid from Carlo Erba Analyticals (Milan, It-

aly) and LiBr from Sigma (Steinheim, Germany) were analytical grade.

PNU Fractionation

Four PNU samples with a broad molar mass distribution were prepared as starting material for the preparation of the fractions, as described elsewhere.[8] Tailoring of the polymer molar mass distribution was obtained by varying the concentration of the polymerization initiator in the synthesis of the polymeric precursor. Details of the PNU fractionation using a semi-preparative chromatographic system were described elsewhere.[9]

Chromatographic Systems

An integrated 150CV (Waters) system, composed of a liquid chromatograph equipped with an on-line single-capillary viscometer (SCV), a refractive index (DRI), and an additional MALS detector was used (SEC–MALS–SCV). The description of the SEC–MALS–SCV multidetector system and related problems were reported in detail previously.[10,11] A ternary mobile phase (DMF + 0.01M LiBr + 0.05M CH_3COOH) that proved suitable in the study of similar polymers[12] was used. The column set was composed of two Waters Styragel columns (HR4, HR3), the flow rate was 0.8 mL/min, the column and detector temperatures were 50°C, and the eluent was degassed with helium.

Light Scattering

Measurements were performed with a MALS photometer Dawn DSP-F (Wyatt). The MALS instrument, vertically polarized laser of 632.8 nm of wavelength, measures the intensity of the scattered light at 15 fixed angular locations ranging in DMF from 16.3° to 157.3°. The calibration constant was calculated using toluene as a standard (Rayleigh factor $R_\theta = 1.406 \times 10^{-5}$ cm^{-1}). The angular normalization was carried out by a concentrated solution of a narrow low molar mass PEG standard ($M = 12.6\ K$ g/mol, $D = 1.03$), assumed as an isotropic scatterer. Details of the MALS hardware and software were described elsewhere.[13]

Each PHPMA and PNU fraction was characterized both by a static off-line mode and by an on-line mode. Methanol as a solvent was preferred in the off-line MALS, because of the sizable increase of the polymer dn/dc in this solvent, when compared to that in DMF. Off-line MALS data analysis was performed using the usual Zimm double-extrapolation.[14] Unfortunately, methanol is incompatible with the used commercial SEC columns; therefore, in the SEC characterization, we used the mobile-phase DMF + 0.01M LiBr + 0.05M CH_3COOH. The refractive index increments, dn/dc, of the polymers were determined using a KMX-16 differential refractometer from LDC Milton Roy in the above-cited solvents.

Viscometry

The intrinsic viscosity $[\eta]$ of the samples was generally determined with the on-line SCV detector included in the Waters 150CV SEC system. Two PNU fraction measurements were carried out also in a static off-line mode with a micro-Ubbelohde capillary viscometer. In the SCV on-line SEC detector, a differential transducer continuously monitors the pressure drop across a stainless-steel capillary tube. From the signals of the two detectors, viscometer, and concentration, the local $[\eta]_i$ value is obtained for each slice of the chromatogram. Details of the SCV detector, hardware and software, were described elsewhere.[15] The signal of the viscometer detector depends on the intrinsic viscosity and on the concentration of the solution. Hence, to obtain a constant signal-to-noise ratio, the concentration of the samples was calculated so that $[\eta]\ c = 0.1$. On-line SCV detection is based on the concept of the universal calibration.[16] The universal calibration curve was generated using 13 narrow PEO/PEG standards with the molar mass ranging from 440 to 8.5×10^5 g/mol.

RESULTS AND DISCUSSION

Data on the PHPMA fractions have already been reported both in the SEC mobile phase[9,12,17] and in methanol. The six PHPMA fractions encompassed a broad molar mass range (from 7.8×10^3 to 3.3×10^5 g/mol) and the dispersity D varied from 1.07 to 1.15. The weight-average molar mass M_w, $[\eta]$, and z-average root mean-square radius $\langle s^2 \rangle_z^{1/2}$ were determined for each fraction. The corresponding values in the SEC mobile phase or in methanol are listed in Table I.

M_w, $[\eta]$, and $\langle s^2 \rangle_z^{1/2}$ values for the PNU fractions are listed in Table II. With respect to the PHPMA fractions, the 13 PNU fractions encompassed a broader molar mass range (from 4.8

Table I Summarized Data for Six Fractions of the Polymeric Drug-Carrier PHPMA

M_w [a] (g/mol)	$[\eta]$ [b] (dL/g)	$\langle s^2 \rangle_z^{1/2c}$ (nm)	$A_2 \times 10^4$ [a] (mol mL g^{-2})	M_w/M_n	M_z/M_w
330,000	0.814	21.7	4.51	1.15	1.15
188,000	0.559	16.0	4.65	1.11	1.12
101,500	0.354	11.2	6.21	1.07	1.10
72,600	0.264	9.7	5.85	1.08	1.10
27,760	0.135	6.0	6.84	1.15	1.18
7800	0.064	—	—	1.28	1.25

[a] Off-line MALS, methanol.
[b] On-line SEC–SCV, DMF.
[c] On-line SEC–MALS, DMF.

$\times 10^3$ to 8.3×10^5 g/mol), but the dispersity was correspondingly higher (from 1.17 to 1.70).

Viscometer and light scattering on-line detectors provide plenty molecular parameters and related different averages. Numeric, weight, and *z*-averages of the molar mass, intrinsic viscosity, and dimensions allow us to use the opportune average every time the data analysis requires congruent averages. Tables I and II summarize the results of the molecular characterization, respectively, for PHPMA and PNU and report only the M_w, $[\eta]$, $\langle s^2 \rangle_z^{1/2}$, A_2, M_w/M_n, and M_z/M_w dispersity index, which are the most significant data.

Viscometric Characterization

Intrinsic Viscosity

Off-line Ubbelohde viscometer $[\eta]$ values of two PNU fractions were used as a reference for the SCV value. The agreement between the $[\eta]$ values, as obtained by the on-line SCV detector and by the off-line Ubbelohde viscometer, was very good (see Table II). Therefore, the $[\eta]$ values as obtained by the on-line SCV detector were used in the following elaboration:

$[\eta] = f(M)$

The power laws $[\eta] = kM^a$, the Mark–Houwink–Sakurada equation, for PHPMA and PNU are shown in Figure 2. The data, from Tables I and II, are very well fitted except for the lowest molar mass PNU fraction, 4800 g/mol, which was excluded from the data analysis. Values of $[\eta]$ and of the weight-average molar mass M_w were used for the numerical evaluation of the constants k and a of the Mark–Houwink–Sakurada equation for PHPMA and PNU in the SEC mobile phase at

Table II Summarized Data for Thirteen Fractions of the Conjugate Polymeric System PNU

M_w [a] (g/mol)	$[\eta]$ [b] (dL/g)	$\langle s^2 \rangle_z^{1/2c}$ (nm)	$A_2 \times 10^4$ [a] (mol mL g^{-2})	M_w/M_n	M_z/M_w	$[\eta]$ [d] (dL/g)
830,000	1.140	31.9	7.56	1.70	1.74	1.137
322,000	0.602	17.9	7.63	1.60	1.70	
252,000	0.512	17.3	7.24	1.58	1.70	
188,500	0.441	14.0	4.65	1.57	1.66	
128,600	0.343	12.6	5.17	1.50	1.61	
77,300	0.270	8.6	5.13	1.23	1.25	
54,600	0.208	6.9	8.07	1.22	1.30	
38,500	0.171	6.1	5.51	1.28	1.41	
26,900	0.136	—	5.22	1.40	1.54	
18,600	0.109	—	9.80	1.17	1.21	0.105
8100	0.072	—	10.1	1.20	1.20	
6200	0.060	—	18.8	1.28	1.20	
4800	0.050	—	17.7	1.40	1.60	

[a] Off-line MALS, methanol.
[b] On-line SEC–SCV, DMF.
[c] On-line SEC–MALS, DMF.
[d] Off-line Ubbelohde viscometer, DMF.

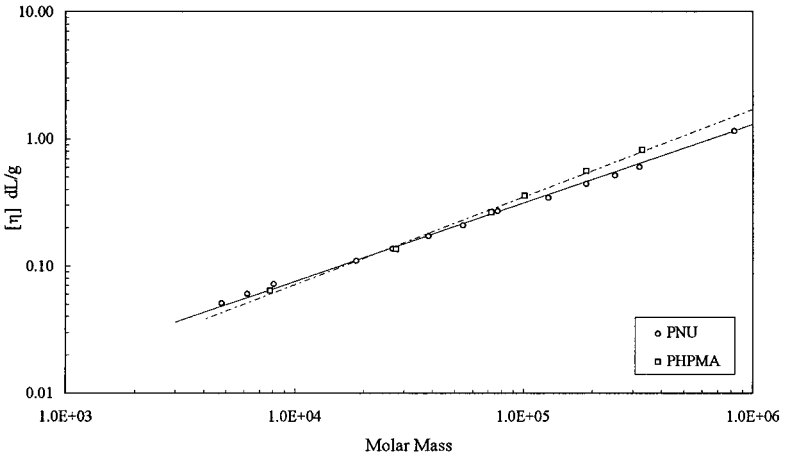

Figure 2 Comparison of the $[\eta] = f(M)$ power laws, the Mark–Houwink–Sakurada equation, for PHPMA and PNU in the SEC mobile phase.

50°C, as reported in Table III. The slope of the Mark–Houwink–Sakurada equation of 0.690 ± 0.004 for PHPMA and 0.617 ± 0.005 for PNU indicate that the SEC mobile phase is a moderately good solvent for these polymers. Besides, the meaningful difference of the two slopes demonstrates the sizable influence of the drug on the conformation of the macromolecules.

Light-scattering Characterization

The refractive index increment data for the PHPMA carrier and for the PNU conjugate are shown in Table IV. Expectedly, the dn/dc for PHPMA and PNU are quite similar.

Molar Mass and Dimensions

M_w values for PHPMA and PNU fractions, as obtained by SEC–MALS, were very close to those obtained by off-line MALS in methanol. Therefore, only the latter values are reported in the tables. Furthermore, the difference between the macromolecule dimensions, $\langle s^2 \rangle_z^{1/2}$, in methanol and in the SEC mobile phase is within the experimental uncertainty. Therefore, the tables report only the $\langle s^2 \rangle_z^{1/2}$ values in the SEC mobile phase.

Table III Constants of the Mark–Houwink–Sakurada Equation for PHPMA and PNU in the SEC Mobile Phase at 50°C

Polymer	$k \times 10^4$ (dL/g)	a
PHPMA	1.24	0.690
PNU	2.58	0.617

$\langle s^2 \rangle^{1/2} = f(M)$

At variance with the derivation of the Mark–Houwink–Sakurada equation parameters, the procedure used to obtain the constants K and α of the power law $\langle s^2 \rangle^{1/2} = KM^{\alpha}$ had to consider the following two problems: First, we have to take into account the residual dispersity of PHPMA and, to a greater extent, of PNU fractions. Second, the intrinsic limitation of the used light-scattering device prevents significant measurement of the macromolecule dimension when this is lower than 8–10 nm. As a consequence, the accessible experimental $\langle s^2 \rangle^{1/2}$ range in static off-line MALS is limited and use of the SEC–MALS data is preferable. Each SEC–MALS chromatogram produces two direct functions: $M = f(V)$ and $\langle s^2 \rangle^{1/2} = f(V)$, where V denotes the elution volume. From these two

Table IV Refractive Index Increment, *dn/dc*, for PHPMA and PNU

Polymer	Solvent	T (°C)	dn/dc (mL/g)
PHPMA	Methanol	25	0.198
PHPMA	DMF[a]	50	0.106
PNU	Methanol	25	0.200
PNU	DMF[a]	50	0.112

[a] DMF + 0.01M LiBr + 0.05M CH_3COOH.

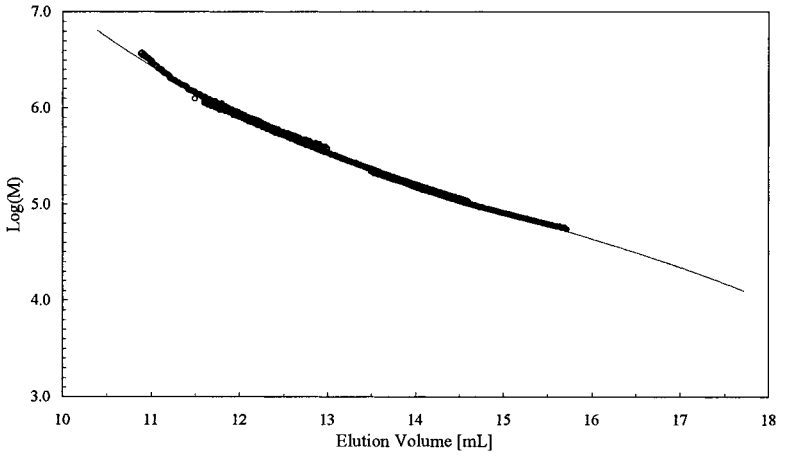

Figure 3 Experimental calibration $\mathrm{Log}(M) = f(V)$ of the SEC system, by MALS, for PNU constructed by gathering the data of seven higher molar mass PNU fractions.

experimental functions, we obtain a third derived function: $\langle s^2\rangle^{1/2} = f(M)$. For both PHPMA and PNU, three "master" functions are obtained upon gathering the "good data region" of each chromatogram, where the signal-to-noise ratio is optimal. The first experimental "master" calibration $M = f(V)$ is shown in Figure 3; the second one, $\langle s^2\rangle^{1/2} = f(V)$, is shown in Figure 4; and, finally, the third one, $\langle s^2\rangle^{1/2} = f(M)$, is shown in Figure 5. This approach overcomes the above two problems. First, assuming ideal SEC fractionation, that is, the absence of band-broadening, we have a quasiuniform composition with respect to the molar mass and dimension of each slice of the chromatograms, and for every slice of the chromatograms, we can assume that $M_z \approx M_w \approx M_n$ and $\langle s^2\rangle_z^{1/2} \approx \langle s^2\rangle_w^{1/2} \approx \langle s^2\rangle_n^{1/2}$. In addition, this procedure leads to a derivation of the power law constants from a very wide range of molar mass: 920 points for PNU (M from 1.25×10^5 to 5.0×10^6 g/mol, $\langle s^2\rangle^{1/2}$ from 12 to 70 nm) and 550 points for PHPMA (M from 5.7×10^4 to 2.0×10^6 g/mol, $\langle s^2\rangle^{1/2}$ from 8 to 60 nm).

Figure 5 shows the comparison between the power laws for PHPMA and PNU as constructed with the above-described method. The constants K and α of the equation are reported in Table V. The slope $\alpha = 0.544 \pm 0.018$ for PHPMA in the SEC mobile phase at 50°C is typical of random coils in a good solvent. The correspondent slope for PNU, $\alpha = 0.478 \pm 0.017$, is significantly different from the above one.

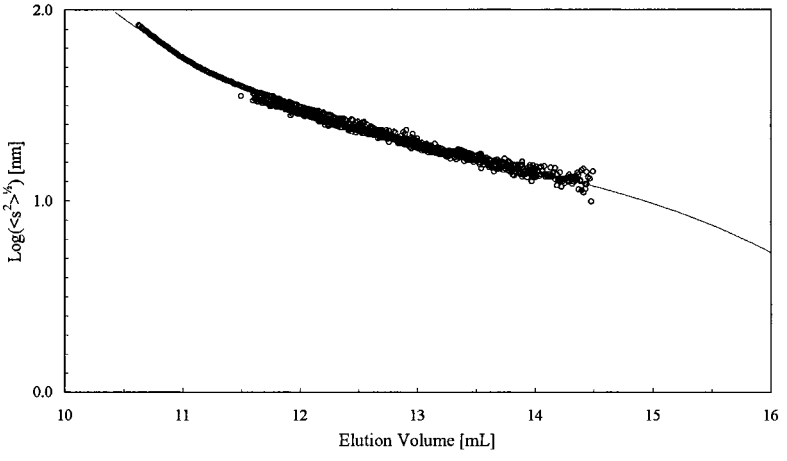

Figure 4 Experimental calibration $\mathrm{Log}(\langle s^2\rangle^{1/2}) = f(V)$ of the SEC system, by MALS, for PNU constructed by gathering the data of seven higher molar mass PNU fractions.

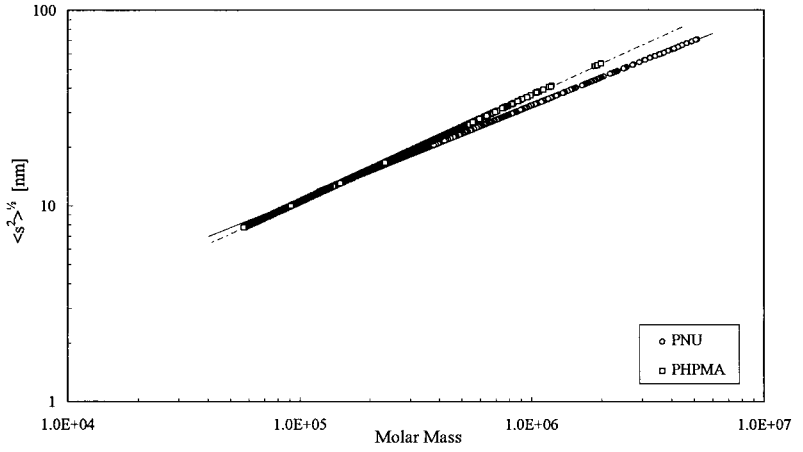

Figure 5 Comparison of the $\langle s^2 \rangle^{1/2} = f(M)$ power laws for PHPMA and PNU constructed by gathering the data of seven higher molar mass PNU fractions.

$A_2 = f(M)$

The second virial coefficient A_2 is also a power law of the molar mass: $A_2 = k \times M_w^{\nu}$. Figure 6 shows the A_2 versus M_w double logarithmic plot for PHPMA and PNU polymers in the methanol solvent. The slope "ν," calculated using the data reported in Tables I and II, was approximately -0.19 for both PHPMA and PNU. In the two-parameter theory, A_2 depends on the interpenetration function $\Psi(z)$, a function of the excluded volume z, eq. (1). It is well known that if $\Psi(z)$ asyntotically converges, for an infinite chain length, to a finite value the slope converges to -0.2 (ref. 18) in good agreement with our finding. From the PHPMA and PNU data, we obtained $\Psi(z)$ ranging from 0.15 to 0.2, slightly lower than the values found for polystyrene in a good solvent. We can conclude that the homopolymer PHPMA behaves in methanol like a flexible linear polymer in a good solvent. Besides, as is apparent in Figure 6, the A_2 values of PHPMA and PNU substantially agree. The differences are within the experimental uncertainty:

Table V Constants of the Power Law $\langle s^2 \rangle^{1/2} = f(M)$ for PHPMA and PNU in the SEC Mobile Phase at 50°C

Polymer	K (nm)	α
PHPMA	0.020	0.544
PNU	0.044	0.478

$$A_2 = \Psi(z)\frac{4\pi^{3/2}N_A\langle s^2\rangle^{3/2}}{M^2} \quad (1)$$

Unperturbed Dimensions

The unperturbed dimension represents the size of the macromolecules in an ideal θ condition. Unperturbed dimensions are normally expressed as $(\langle r^2\rangle_0/M)^{1/2}$ or K_θ or C_∞ or λ^{-1}, where $\langle r^2\rangle_0$ denotes the unperturbed mean-squared end-to-end distance; $K_\theta = \Phi_0(\langle r^2\rangle_0/M)^{3/2}$ and $\Phi_0 = 2.5 \times 10^{21}$, the Flory constants, λ^{-1}, the Kuhn statistical segment; $C_\infty = \langle r^2\rangle_0/nl_0^2$, the characteristic ratio; l_0, the length of the repeating unit, and n, the number of units. Estimation of the unperturbed dimension can be done in a number of ways[19] starting from the intrinsic viscosity or light-scattering data. Since no measurements were carried out in the θ solvent, we estimated the unperturbed dimensions by an appropriate extrapolation method, that is, the Stockmayer–Fixman plot.[20] The authors suggested using the equation

$$\frac{[\eta]}{M^{1/2}} = K_\theta + 0.51\ \Phi_0 B M^{1/2} \quad (2)$$

where B is a thermodynamic parameter that depends on the polymer partial specific volume, on the molar volume of the solvent, $\langle r^2\rangle_0/M$, and on the interaction parameter. From eq. (2), a plot of $[\eta]\ M_v^{-1/2}$ versus $M_v^{1/2}$ should yield K_θ as the intercept. Such a plot for PNU fractions is shown in Figure 7. From K_θ it is a simple matter to

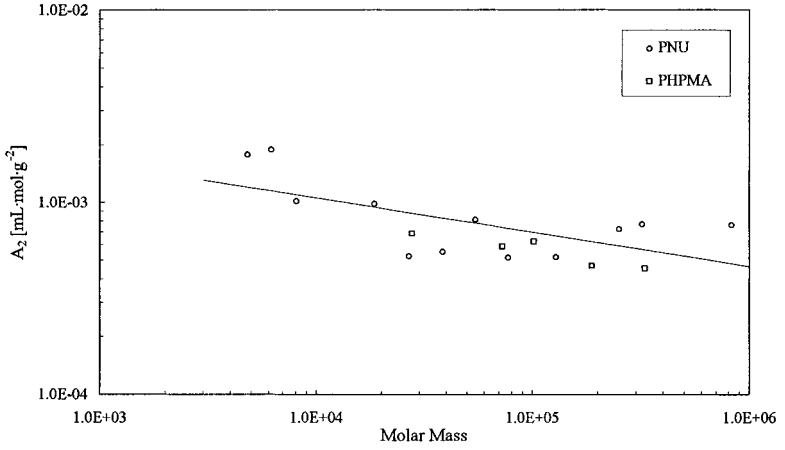

Figure 6 $A_2 = f(M)$ power law for PHPMA and PNU in methanol solvent.

calculate $(\langle r^2\rangle_0/M)^{1/2}$ and λ^{-1}. In the calculation, we used $m_0 = 71.5$ g/mol (m_0 is the molar mass of the repeating unit) and $l_0 = 0.154$ nm for the C—C bond.

The unperturbed parameters for PHPMA and PNU obtained with the intrinsic viscosity method are shown in Table VI. The results of this analysis are $\lambda^{-1} = 1.7 \pm 0.1$ nm for PHPMA and $\lambda^{-1} = 2.1 \pm 0.1$ nm for PNU. Our $(\langle r^2\rangle_0/M)^{1/2}$ result, 6.03×10^{-2} nm, is in good agreement with that obtained by Bohdanechy et al.[21] for PHPMA in pure DMF, $5.85–6.04 \times 10^{-2}$ nm. Similarly, our K_θ result for PHPMA, 5.49×10^{-4} dL/g, agrees very well with the results obtained by Bohdanechy: $5.0–5.5 \times 10^{-4}$ dL/g.

Alternatively, the unperturbed dimensions may be derived from the $\langle s^2\rangle_z^{1/2}$ and M_z light-scattering data. One method is a variation of the Stockmayer–Fixman viscosity plot.[22] In such a method, $(\langle s^2\rangle_z/M_z)^{3/2}$ is plotted as function of $M_z^{1/2}$ and extrapolated to zero molar mass. Such a plot is shown in Figure 8. For PNU, this analysis produces a result, $\lambda^{-1} = 2.0 \pm 0.2$ nm, which is very close to that of the intrinsic viscosity method. For PHPMA, this method leads to values (not reported) which are higher than those of the intrinsic viscosity data, probably because of the comparatively higher scatter of the $\langle s^2\rangle^{1/2}$ data.

As in the case of power laws, the difference between PHPMA and PNU are meaningful. In spite of the very low drug content, the stiffness of the PNU chains, as estimated by the Kuhn's statistical segment length, increases from 1.7 to 2.1 nm with respect to that of the PHPMA homopolymer.

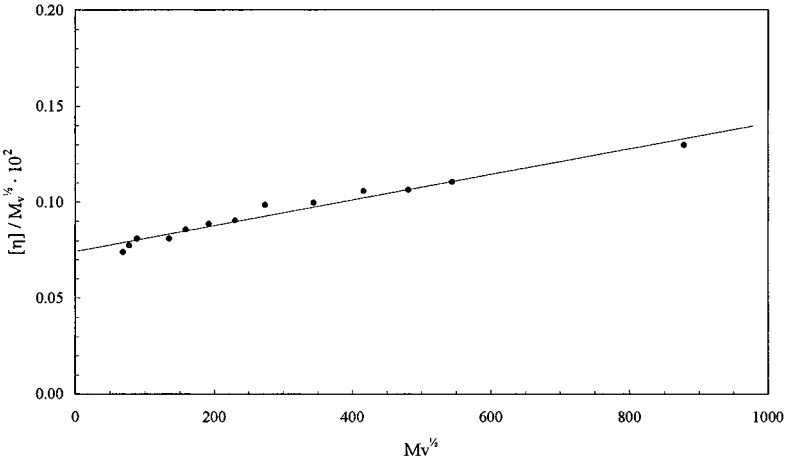

Figure 7 Stockmayer–Fixman plot, $[\eta]/M_v^{1/2}$ against $M_v^{1/2}$, from PNU intrinsic viscosity data used to calculate the unperturbed dimensions of the macromolecules.

Table VI Unperturbed Dimensions for PHPMA and PNU in the SEC Mobile Phase at 50°C

Polymer	$K_\theta \times 10^4$ (dL/g)	$(\langle r^2 \rangle_0/M)^{1/2}$ (nm)	λ^{-1} (nm)
PHPMA	5.49	6.03×10^{-2}	1.7
PNU	7.69	6.75×10^{-2}	2.1

CONCLUSIONS

Thirteen fractions of the PNU conjugate polymeric system and six fractions of the polymeric drug-carrier PHPMA having a wide range of molar mass were separated and characterized. By combining multiangle light scattering and viscometry both off-line and on-line to an SEC system, the molecular properties of these two polymers were studied. A complete molecular characterization using molar mass, intrinsic viscosity, dimensions, second virial coefficient, and related averages were obtained. To our best knowledge, this is the first exhaustive molecular characterization of a conjugated (carrier plus bound antitumor drug) polymeric system in solution.

The PHPMA homopolymer in methanol and in SEC mobile-phase solvents behaves as a flexible linear polymer in a moderately good solvent. As verified by the $[\eta] = f(M)$ and $\langle s^2 \rangle^{1/2} = f(M)$ power laws and by the unperturbed dimensions, the drug exerts a considerable influence on the macromolecules of the conjugate PNU. The slope of the power law $[\eta] = f(M)$ changes from 0.69 for PHPMA to 0.617 for PNU; the slope of the power law $\langle s^2 \rangle^{1/2} = f(M)$ changes from 0.544 for PHPMA to 0.478 for PNU; and the Kuhn's statistical segment length changes from 1.7 nm for PHPMA to 2.1 nm for PNU. These observations converge to a univocal conclusion: The presence of the drug significatively influences the conformation of the macromolecules. In view of the large predominance of the HPMA monomer in the PNU conjugate, the observed conformational difference between PNU and PHPMA demonstrates that the drug behaves as a bulky lateral group. In this conclusion, we made two assumptions: (i) the content of the drug is independent of the polymer chain length; and (ii) there is no interaction between drug molecules. Most likely, a violation of the first assumption would only affect rather than eliminate the above finding, because the optical factor dn/dc of the homopolymer and copolymer are very close (see Table IV). The second assumption requires further comments: Intermolecular interaction is taken care of by extrapolation to infinite dilution. Intramolecular interaction is more insidious, although it is made unlikely by the very low abundance of drug-carrying units: About one in every 100 monomer units. A final and decisive observation is the close correspondence of the second virial coefficient of the PNU and PHPMA samples with a similar molar mass; see A_2 values for PHPMA and PNU in Figure 6, which are in the range of flexible linear polymers in a good sol-

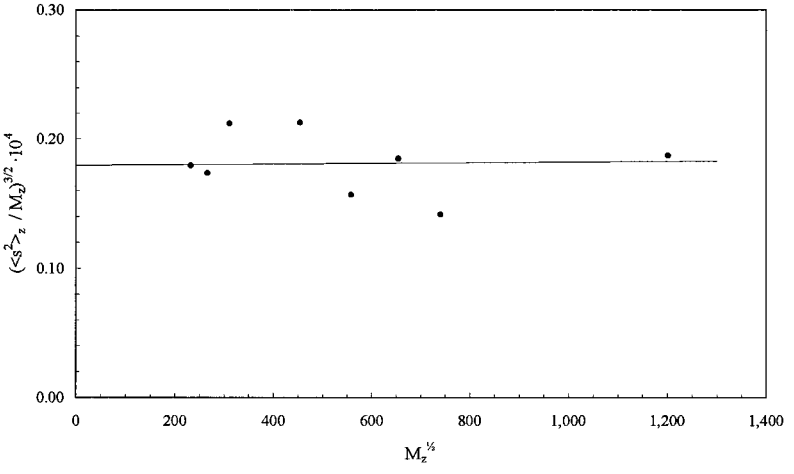

Figure 8 Modified Stockmayer–Fixman plot, $(\langle s^2 \rangle_z/M_z)^{3/2}$ against $M_z^{1/2}$, from PNU light-scattering data used to calculate the unperturbed dimensions of the macromolecules.

vent, thus excluding strong intramolecular and/or intermolecular interaction among the drug moieties in PNU.

REFERENCES

1. T. Konno and H. Maeda, *Neoplasma of the Liver,* K. Okada and K. G. Ishak, Eds., Springer-Verlag, New York, 1987, pp. 343–352.
2. J. Kurtzberg, J. O. Moore, D. Scudieri, and N. J. Franklin, *Proc. A.A.C.R.,* **29,** 213 (1988).
3. P. A. Vasev, R. Duncan, S. B. Kaye, and J. Cassidy, in *8th European Conference on Clinical Oncology Cancer Research and Cancer Nursing,* Paris, 1995.
4. U.S. Dept. of Health and Human Services, Food and Drug Administration, *Guideline on the Preparation of Investigational New Drug Products,* 1991.
5. R. Duncan, M. K. Pratten, H. C. Cable, H. Ringsdorf, and J. B. Lloyd, *Biochem. J.,* **196,** 49 (1981).
6. R. Pazdur, A. P. Kudelka, J. T. Kavanagh, P. R. Cohen, and M. N. Raber, *Cancer Treat. Rev.,* **19,** 1 (1993).
7. E. Pesenti, C. Franzetti, G. Biasioli, M. Ciomei, W. Paston, A. Marsiglio, S. Stegnjaich, N. Mongelli, and M. Grandi, *Proc. A.A.C.R.,* **36,** 307 (1995).
8. N. Mongelli, F. Angelucci, E. Pesenti, A. Suarato, and G. Biasoli, U.S. Pat. 5,473,055 (1995).
9. R. Mendichi, V. Rizzo, M. Gigli, G. Razzano, F. Angelucci, and A. Giacometti Schieroni, *J. Liq. Chrom. Rel. Technol.,* submitted.
10. R. Mendichi, G. Audisio, R. Maffei Facino, M. Carini, A. Giacometti Schieroni, and L. Saibene, *Int. J. Polym. Anal. Charact.,* **1,** 365 (1995).
11. R. Mendichi and A. Giacometti Schieroni, in *Proceedings of the 9th ISPAC,* Oxford, UK, 1996, B22.
12. R. Mendichi, V. Rizzo, M. Gigli, and A. Giacometti Schieroni, *J. Liq. Chrom. Rel. Technol.,* **19,** 1951 (1996).
13. P. J. Wyatt, *Anal. Chim. Acta,* **272,** 1 (1993).
14. B. H. Zimm, *J. Chem. Phys.,* **16,** 1099 (1948).
15. J. Lesec, M. Millequant, and T. Haward, in *Proceedings of the International GPC Symposium,* San Francisco, 1991, p. 285.
16. Z. Grubisic, P. Rempp, and H. Benoit, *J. Polym. Sci. Part B,* **5,** 753 (1967).
17. K. Ulbrich, private communication, 1993.
18. H. Yamakawa, *Modern Theories of Polymer Solutions,* Harper and Row, New York, 1971.
19. A. A. Abdel-Azim, *Makromol. Chem. Phys.,* **195,** 159 (1994).
20. W. H. Stockmayer and M. Fixman, *J. Polym. Sci. Part C,* **1,** 137 (1963).
21. M. Bohdanechy, H. Bazilova, and J. Kopecek, *Eur. Polym. J.,* **10,** 405 (1974).
22. H. Bauman, *J. Polym. Sci. Polym. Lett.,* **3,** 1069 (1965).