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## Polymer characterization by size-exclusion chromatography with multiple detection<sup>☆</sup>

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### Abstract

Size-exclusion chromatography with coupled multiangle light scattering and differential refractometry detectors has been used to obtain molecular mass and radius of gyration distributions of polydisperse polymer samples. From these data the scaling law between dimensions and the absolute molecular mass is obtained with just one sample of each polymer. Three different kinds of polymers are presented: polystyrene which serves as reference polymer, polyphosphazenes which behave abnormally in solution and poly(ethylene oxide) which is soluble in water. Since the relationship between dimensions and molecular mass depends on the extent of interactions between chain segments and solvent molecules, the scaling law provides information about the solution properties of the polymer. © 2001 Elsevier Science B.V. All rights reserved.

*Keywords:* Multiangle light scattering detection; Detection, LC; Polymers; Polystyrene; Polyphosphazenes; Poly(ethylene oxide)

### 1. Introduction

Size-exclusion chromatography (SEC) is the most common way to obtain information about the molecular mass distribution of polymers. In this technique, it is often supposed that there is an absence of interaction between the macromolecules and the gel contained in the columns, and consequently, molecules are separated according to their size, yielding a

molar size distribution. However, the size of the polymer depends not only on its molecular mass but also on its physical architecture distribution, thus a calibration with narrow polydispersity index standards of the same polymer is required in order to obtain true molecular mass distributions. Unfortunately, suitable monodisperse standards are not always available so that other approaches like universal calibration or numerical procedures using broad polydisperse samples, must be used [1,2].

The use of a molecular mass sensitive detection method, such as multiangle light scattering (MALS) detection, coupled to a customary concentration detection method, such as the conventional refractive index (RI) detection, allows absolute molecular masses determination for each slice across a sample peak. Thus, it offers the possibility of analyzing a

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polymer sample in terms of the absolute distribution of molecular mass [3–8].

Furthermore, the angular dependence of the scattered intensity may be determined at each elution volume. Thus, the light scattering technique also provides a direct measurement of the polymer dimensions, the mean square radius of gyration,  $\langle s^2 \rangle$ . The on-line MALS detection method presents the appealing possibility of being able to obtain the relationship between molecular mass and dimensions, i.e. the scaling law, with just one sample for broad molecular mass distribution polymers. Since the polymer dimensions depend on the polymer–solvent interactions, the scaling law provides information about the solution properties of the polymer [9–12]. This technique can be carried out with minimal sample preparation, thus avoiding tedious and time consuming polymer fractionation, and can greatly speed up the elucidation of polymer solution properties.

This work presents the results obtained for different polymers using SEC with a MALS photometer and a differential refractometer (RI) as detectors. Two kinds of polymers, lipophilic polymers: polystyrene (PS), poly[(diphenoxy)phosphazene] (PDPP) and poly[di( $\beta$ -naftoxy)phosphazene] (PDNP) and a hydrophilic polymer, poly(ethylene oxide) (PEO) were studied by means of organic and aqueous SEC, respectively (Fig. 1). PS was chosen since it is a well-behaving polymer commonly used as a reference polymer [13]. On the contrary, the solution behavior of polyphosphazenes is anomalous, they do not always follow the universal calibration approach in SEC and its fractionation is very difficult [11,14,15]. PEO is a water-soluble polymer whose conformational properties in water depend on the presence of salts [16,17].

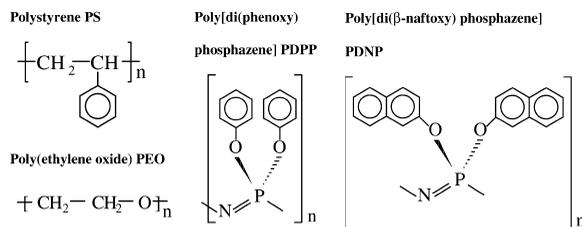


Fig. 1. Polymer structures.

## 2. Experimental

SEC measurements were carried out using Waters Associate equipment with a model 510 pump, a U6K injector, a differential RI detector model 410. A Dawn-DSP-F MALS photometer operating at 632.8 nm from Wyatt Technology was the molecular mass and size detector used. The measurements were carried out at 25°C. Calibration of the Dawn equipment was done using toluene and the normalization of the detectors was performed with standard monodisperse PS and PEO of low molecular mass that did not show angular dependence on the light scattering signal. The above standard polymers were also used to determine the interdetector volume [5]. For organic SEC, two columns PLgel mixed B (Polymer Labs.) in series were used, whereas two Ultrahydrogel Linear 6–13  $\mu$ m were used for aqueous SEC. The eluent was tetrahydrofuran (THF) freshly distilled from sodium and benzophenone for PS; THF with a 0.1% tetra-*n*-butylammonium bromide for PDPP and PDNP; water and a 0.1 M NaNO<sub>3</sub> aqueous solution for PEO. The eluents were filtered through a 0.2- $\mu$ m filter and degassed. The flow-rates were 1.0 and 0.5 ml/min for organic and aqueous SEC, respectively.

PDPP and PEO were commercial samples (Aldrich). PS and PDNP were synthesized by bulk polymerization of styrene and hexachlorocyclotriphosphazene, respectively, as explained elsewhere [15,18].

The software used, ASTRA 4.73 from Wyatt Technology, allowed on-line data of molecular mass and radius of gyration collection as well as calculation of the distributions and averages.

The differential refractive index increments for the polymers in the solvents were taken from literature [13] or measured with a Brice-Phoenix differential refractometer or an Optilab-DSP interferometric refractometer.

## 3. Theory

The basic equation when using a light scattering detector [5,19] is:

$$\frac{Kc}{\Delta R_\theta} = \frac{1}{M_w} \left( 1 + \frac{16\pi^2}{3\lambda^2} \langle s^2 \rangle \sin^2 \left( \frac{\theta}{2} \right) + \dots \right) \quad (1)$$

where  $\Delta R_\theta$  is the excess Rayleigh ratio, related to the scattered light intensity of the polymer at angle of observation  $\theta$ ,  $c$  is the concentration of the polymer solution,  $\lambda$  the light wavelength,  $\langle s^2 \rangle$  the mean square radius of gyration,  $M_w$  the weight-average molecular mass, and  $K$  is the optical constant.  $K$  is related to the wavelength in vacuum,  $\lambda_0$ , the solvent refraction index,  $n$ , Avogadro's number,  $N_A$ , and refraction index increment,  $dn/dc$  by:

$$K = \frac{4\pi^2 n^2}{\lambda_0^4 N_A} \left( \frac{dn}{dc} \right)^2 \quad (2)$$

The MALS detector measures simultaneously the excess Rayleigh ratio for each slice  $i$  at different  $\theta$  angles and the slices are assumed to be monodisperse both in composition and molecular mass [5,6]. Thus, a plot of  $Kc/\Delta R_\theta$  versus  $\sin^2(\theta/2)$ , affords to calculate the molecular mass  $M_i$  from the intercept and the mean square radius of gyration,  $\langle s^2 \rangle_i$ , from the slope for each slice of the chromatogram.

The signal of the RI detector is proportional to the polymer concentration and to the refractive index increment [8]. Thus, for each slice:

$$S^{\text{RI}} = k^{\text{RI}} c \left( \frac{dn}{dc} \right) \quad (3)$$

where  $k^{\text{RI}}$  is the absolute response factor of the detector that does not depend on the kind of polymer and can be assumed to be constant and  $c$  is the polymer concentration in the slice. When using the RI detector the concentration  $c$  will be the true concentration if the  $dn/dc$  of the slice coincides with the  $dn/dc$  of the whole sample.

## 4. Results and discussion

### 4.1. Organic size-exclusion chromatography

Fig. 2 shows the molecular mass and the root mean squared radius of gyration for PS, PDPP and PDNP, versus the elution volume,  $v_e$ , i.e. the absolute calibration curves. The corresponding signals of the RI detector for the chromatograms are superimposed. At the edges of the peaks, the signal-to-noise ratio is low and there is a larger uncertainty, especially for high elution volumes. PS shows linear calibration curves of  $M$  and  $\langle s^2 \rangle^{1/2}$  over the entire

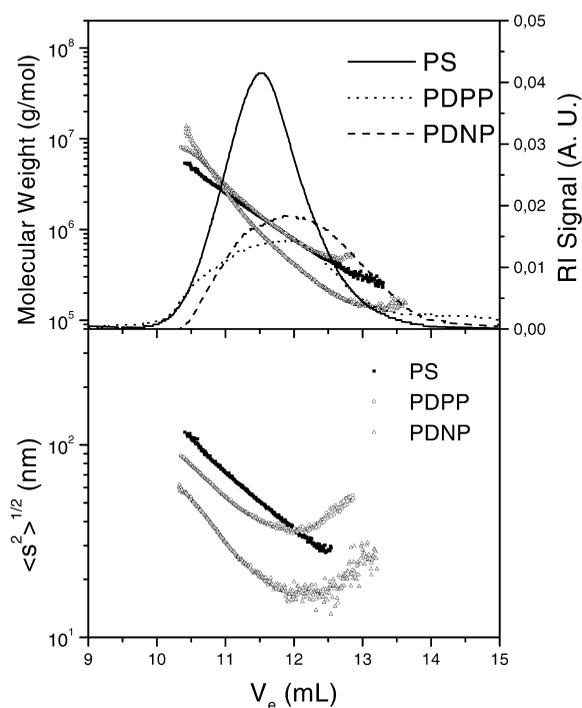


Fig. 2. Logarithm of molecular mass and root mean squared radius of gyration versus elution volume for polystyrene and polyphosphazenes. The corresponding RI signals are also shown.

range of molecular mass, whereas upward curvatures are observed for polyphosphazenes at high elution volumes. This curvature, has been found in polyphosphazenes [14,20] and other types of polymers [21,22] and indicates the existence of a small fraction of molecules, which are retarded in the columns. This fact may lead to errors if universal calibration [23] is used to obtain molecular masses, whereas using a light scattering detector it is easily visualized. In spite of being similar polymers studied under the same conditions, PDPP and PDNP have very different calibration curves.

At the very low concentrations used in SEC, the calculation of the values for the  $z$  average root mean square radius of gyration  $\langle s^2 \rangle^{1/2}$  is independent [24] of both  $dn/dc$  and  $M$  and therefore, it is an excellent parameter to study the dimensions of the polymer. However, the size of the polymer must be larger than  $\sim \lambda/20$  in order to observe the angular dependence of the scattered light intensity [19]. Thus the accuracy of the radius of gyration, obtained in the region of

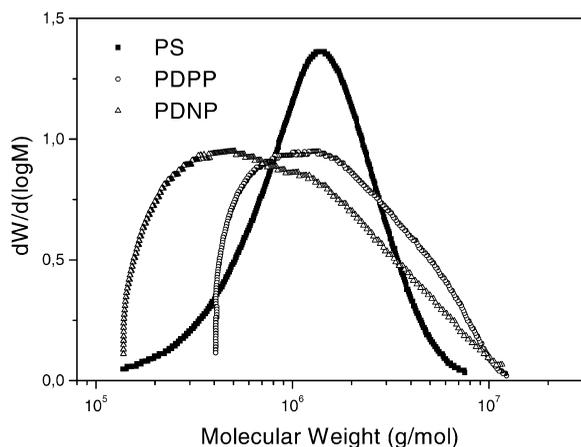


Fig. 3. Molecular mass distributions for polystyrene and polyphosphazenes.

low molecular masses, begins to deteriorate rapidly for PDNP, the sample with lower-molecular-mass chains (Fig. 2).

Fig. 3 presents the absolute molecular mass distributions (MMDs) of the polymers calculated from the combined measurements of molecular mass (MALS detector) and concentration (RI detector). Different molecular mass averages and the polydispersity index ( $M_w/M_n$ , where  $M_n$  is the number-average molecular mass), calculated from the distributions, are presented in Table 1. The three samples are polydisperse covering a broad range of molecular masses. The MMD of PDNP is more displaced to low molecular masses, as explained above.

Since the three samples are polydisperse and cover an ample range of molecular masses, the relationship between the polymer dimensions and the molecular mass can be obtained for each sample. This relationship is usually described as a scaling law of the form [9]:

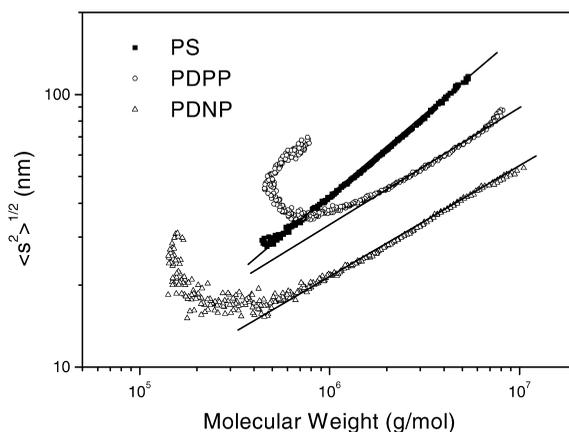


Fig. 4. Log–log plot of root mean squared radius of gyration versus molecular mass, i.e. scaling laws, for the polymers in the whole range of molecular masses. The linear fits corresponding to the scaling parameters of Table 2 are also shown.

$$\langle s^2 \rangle^{1/2} = QM^q \quad (4)$$

and yields information about the solution properties of the polymer. The power law exponent  $q$  is related to the shape of the chain and yields information about the polymer–solvent interactions and macromolecular conformation of the polymer. Values of  $0.5 \leq q \leq 0.6$  are predicted for random-coil chains, from polymers in theta conditions (0.5) to polymers in very good solvents (0.6). Rigid rod polymers present values of  $q$  as high as 1 whereas spherical particles have a  $q$  value equal to  $1/3$ .

Fig. 4 shows the log–log plot of the scaling law (Eq. (4)) obtained for the polymer samples and the values of the  $q$  and  $Q$  parameters are shown in Table 2. For PS the plot is linear and the value of  $q \approx 0.6$  indicates that, as expected, PS chains are in random coil conformation and expanded due to the excluded volume effect since THF is a very good solvent for this polymer. For the polyphosphazenes, PDPP and

Table 1  
Molecular mass averages and polydispersities for the polymer samples

	$10^{-5} M_n$	$10^{-6} M_w$	$10^{-6} M_z$	$M_w/M_n$
PS	$9.8 \pm 0.1$	$1.67 \pm 0.03$	$4.5 \pm 0.7$	$1.70 \pm 0.04$
PDPP	$9.41 \pm 0.01$	$1.89 \pm 0.01$	$3.94 \pm 0.01$	$2.01 \pm 0.01$
PDNP	$3.64 \pm 0.01$	$0.976 \pm 0.001$	$2.89 \pm 0.01$	$2.68 \pm 0.01$
PEO (water)	$9.6 \pm 2.0$	$1.8 \pm 0.3$	$3.5 \pm 2.0$	$1.9 \pm 0.5$
PEO (0.1 M NaNO <sub>3</sub> )	$10 \pm 3$	$1.7 \pm 0.4$	$5.0 \pm 6.0$	$1.6 \pm 0.6$

Table 2  
Scaling law parameters

	$10^3 Q$	$q$
PS	$12.4 \pm 0.3$	$0.59 \pm 0.01$
PDPP	$81 \pm 3$	$0.43 \pm 0.01$
PDNP	$85 \pm 1$	$0.40 \pm 0.01$
PEO (water)	$1.15 \pm 0.09$	$0.75 \pm 0.01$
PEO (0.1 M NaNO <sub>3</sub> )	$177 \pm 14$	$0.42 \pm 0.01$

PDNP, the plots show a pronounced curvature at low molecular masses as a consequence of the curvatures showed in the calibration curves (Fig. 2) explained above [14]. The values of  $q$  obtained for both polyphosphazenes are lower than 0.5 thus, THF is a bad solvent, the polymers are under  $\theta$  conditions and adopt more compact conformations. The results obtained for PDNP presented in this work are in good agreement with our previous results obtained through a much more laborious procedure [15]. The method consisted firstly in fractionation of the polymer, secondly in SEC, light scattering and viscosity measurements of the samples and thirdly since the fractionation yielded broad samples, in the use of numerical methods of adjustment [15]. The coupled SEC–MALS technique used in this work, is not only much simpler but also explains, from the plots of Figs. 2 and 4, why the fractionation was not effective.

#### 4.2. Aqueous size-exclusion chromatography

Aqueous SEC permits the determination of the molecular mass distribution of water-soluble polymers. The principal separation mechanism is similar to conventional SEC; thus, smaller polymers have more pore volume available and elute later than larger polymers. The solvents used as eluents are water, polar solvents or ionic solutions and since the polar interactions between polymers, eluent and support can be significant, additional separation mechanisms, besides the steric exclusion one, can influence the separation process. A lot of effort is being made towards the understanding and implementation of the technique [4,25] and, since the ionic strength of the solvent can affect the polymer conformation, the use of the light scattering detector can provide information about this conformational change.

Fig. 5 presents plots similar to Fig. 2, i.e. calibration curves for the PEO using the same columns, temperature and rate of elution, but two different eluents, water and a 0.1 M aqueous solution of sodium nitrate. The shapes of the SEC chromatograms and the slope of the molecular mass calibration curves in the two eluents are similar. However, the elution volumes increase markedly if salt is present, indicating a decrease of the hydrodynamic volume of the polymer when salt is added. In the lower part of Fig. 5, the calibration curves of the radius of gyration show a different slope indicating a change in the conformation of the polymer which can be the reason for the decrease of the hydrodynamic volume. This change will be more easily observed in the scaling law that will be explained below.

Fig. 6 shows the absolute molecular mass distributions obtained with the two solvents and the averaged molecular masses are presented in the last

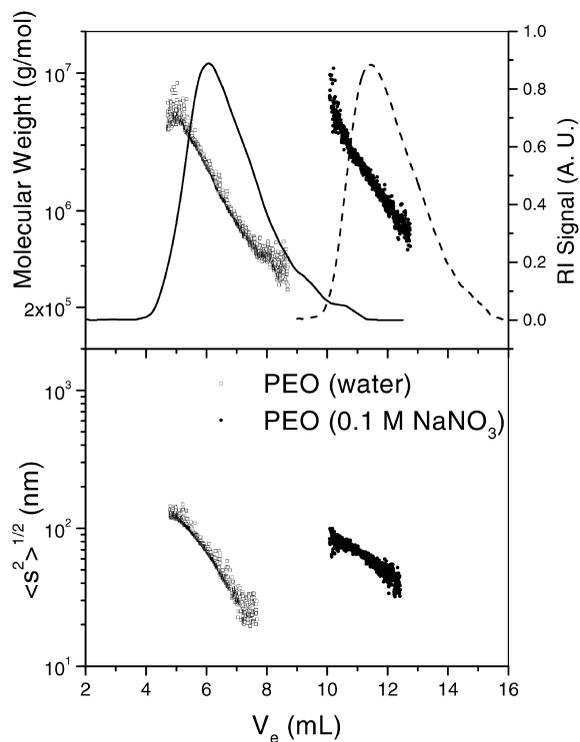


Fig. 5. Logarithm of molecular mass and root mean squared radius of gyration versus elution volume for PEO in the two eluents used. The corresponding RI signals are also shown.

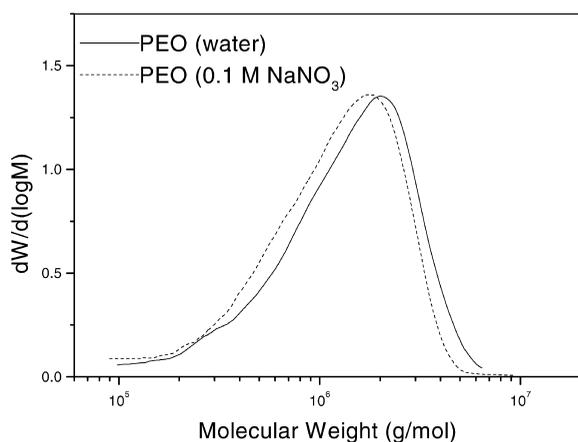


Fig. 6. Molecular mass distributions for PEO.

two rows of Table 1. They are similar within experimental error. Comparing the Figs. 2 and 5, the uncertainty in the calibration curves is larger for aqueous SEC due to the fact that the signal-to-noise ratio is lower in aqueous SEC than in organic SEC.

The scaling laws for PEO in the two solvents are presented in Fig. 7 and the scaling parameters are shown in Table 2. Although the uncertainty in the measurements is large, it is clear that the scaling law and thus the polymer conformation strongly depends on the addition of  $\text{NaNO}_3$ . The value of the  $q$

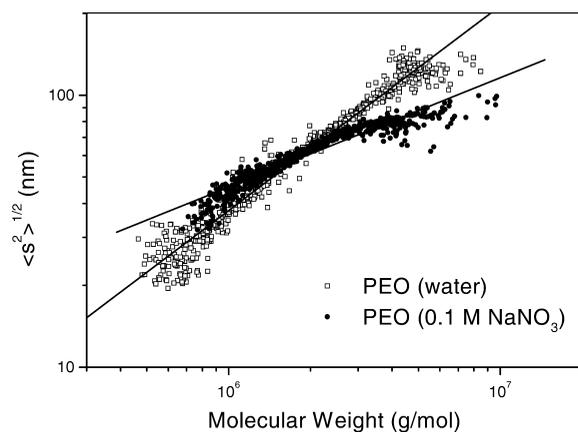


Fig. 7. Log–log plot of root mean squared radius of gyration versus molecular mass, i.e. scaling laws, for PEO in the two eluents. The linear fits corresponding to the scaling parameters of Table 2 are also shown.

parameter obtained for PEO in water, 0.75, is considerably higher than the value corresponding to a random coil polymer in a good solvent, 0.6. Thus the chain is further extended than a random coil polymer and there is a tendency of the chains to adopt rod type conformations. However, if  $\text{NaNO}_3$  is added, the polymer chain contracts up to values well below  $\theta$  conditions, and close to a spherical shape. These results agree with viscosity measurements and theoretical calculations. It has been reported that the addition of sodium or potassium salts to water reduces the viscosity of PEO of high molecular mass [26,27] and also lowers the  $\theta$  temperature [13]. Tasaki performed molecular dynamic simulations of PEO chains in water; the chain showed a preference for helical conformations stabilized by hydrogen bonds with water molecules [28]. Moreover the addition of  $\text{K}^+$  disrupted the hydrogen bonds and considerably distorted the helical conformation of the chain [16].

## 5. Conclusions

The use of a SEC–MALS–RI chromatographic system gives information about conformational properties of polymers in solution using just one broad polydisperse sample of the polymer. The technique can be applied to both lipophilic and hydrophilic polymers using organic and aqueous SEC, respectively. Moreover, the method is fast and avoids tedious and time consuming polymer fractionation.

The results obtained for a standard polymer like PS are similar to those expected whereas the anomalous behavior of polyphosphazenes PDPP is observed. In aqueous SEC, although the accuracy of the measurements is lower, the differences in the PEO conformations when a salt is added can be determined through the two different scaling law exponents obtained.

## Acknowledgements

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## References

- [1] E.V. Patton, in: B.W. Rossiter, J.E. Hamilton (Eds.), *Physical Methods of Chemistry*, 2nd ed., Wiley, New York, 1989, Ch. 7.
- [2] L.H. GarciaRubio, J.F. McGregor, A.E. Hamielec, in: C.D. Craver (Ed.), *Polymer Characterization (Advances in Chemistry Series)*, American Chemical Society, Washington, DC, 1983.
- [3] B. Trathnigg, *Prog. Polymer Sci.* 20 (1995) 615.
- [4] M. Potschka, P.L. Dubin (Eds.), *Strategies in Size-Exclusion Chromatography*, American Chemical Society, Washington, DC, 1996.
- [5] P.J. Wyatt, *Anal. Chim. Acta* 272 (1993) 1.
- [6] D.W. Shortt, *J. Chromatogr. A* 686 (1994) 11.
- [7] U. Dayal, S.K. Mehta, *J. Liq. Chromatogr.* 17 (1994) 303.
- [8] M.D. Zammit, T.P. Davis, *Polymer* 38 (1997) 4455.
- [9] P.G. De Gennes, *Scaling Concepts in Polymer Physics*, Cornell University Press, Ithaca, NY, 1979.
- [10] W.L. Mattice, U.W. Suter, *Conformational Theory of Large Molecules*, Wiley, New York, 1994.
- [11] M.P. Tarazona, *Polymer* 35 (1994) 819.
- [12] J. Búrdalo, R. Medrano, E. Saiz, M.P. Tarazona, *Polymer* 41 (2000) 1615.
- [13] J. Brandrup, E.H. Immergut, E.A. Grulke (Eds.), *Polymer Handbook*, 4th ed., Wiley, New York, 1999.
- [14] M.T.R. Laguna, E. Saiz, M.P. Tarazona, *Polymer* 41 (2000) 7993.
- [15] J. Bravo, M.P. Tarazona, E. Saiz, *Macromolecules* 25 (1992) 5625.
- [16] K. Tasaki, *Comp. Theor. Polym. Sci.* 9 (1999) 271.
- [17] M.P. Plana, E. Saiz, M.P. Tarazona, in preparation.
- [18] M.T.R. Laguna, Tesis de Licenciatura. UAH, 1998.
- [19] M.B. Huglin (Ed.), *Light Scattering From Polymer Solutions*, Academic Press, London, 1972.
- [20] J. Búrdalo, M.P. Tarazona, G.A. Carriedo, F.J. García Alonso, P.A. González, *Polymer* 40 (1999) 4251.
- [21] V. Percec, C.H. Ahn, W.D. Cho, A.M. Jamieson, J. Kim, T. Leman, M. Schmidt, M. Gerle, M. Möller, S.A. Prokhorova, S.S. Sheiko, S.Z.D. Cheng, A. Zhang, G. Ungar, D.J.P. Yeardley, *J. Am. Chem. Soc.* 120 (1998) 8619.
- [22] M. Gerle, K. Fischer, S. Roos, A.H.E. Müller, M. Schmidt, *Macromolecules* 32 (1999) 2629.
- [23] Z. Grubisic, P. Rempp, H.J. Benoit, *Polym. Sci. B* 5 (1967) 753.
- [24] P.J. Wyatt, *J. Chromatogr.* 648 (1993) 27.
- [25] J.E. Rollings, A. Bose, J.M. Caruthers, G.T. Tsao, M.R. Okos, in: C.D. Craver (Ed.), *Polymer Characterization (Advances in Chemistry Series)*, American Chemical Society, Washington, DC, 1983.
- [26] K.J. Liu, K.E. Anderson, *Macromolecules* 2 (1969) 235.
- [27] B. Briscoe, P. Luckham, S. Zhu, *Macromolecules* 29 (1996) 6208.
- [28] K. Tasaki, *J. Am. Chem. Soc.* 118 (1996) 8459.