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Characterization of poly (*N*-vinyl formamide) by size exclusion chromatography–multiangle light scattering and asymmetric-flow field-flow fractionation–multiangle light scattering

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ABSTRACT: The molar mass and the radius of gyration of three poly *N*-vinyl formamide (polyNVF) synthesized in aqueous solution polymerization were characterized using two different fractionation techniques: size exclusion chromatography (SEC) and asymmetric-flow field-flow fractionation (AF4) coupled with a multiangle light scattering (MALS) and a refractive index (RI) detector. For the sake of comparison, the polymers were also characterized by MALS using the Zimm plot approach (no fractionation). The dn dc^{-1} of the poly (*N*-vinyl formamide) was measured (0.1564 mL g^{-1}) and it was found to be insensitive to the molar mass (in the range 150–450 kDa) and also to the eluents used (DDI water or mixed eluent DDI water/acetonitrile (80 : 20) at pH = 5.5). Interestingly, the concentrations of the samples injected in the SEC and AF4 should be different because concentrations in the range of 20–40 mg mL^{-1} used for the AF4 caused overloading and anomalous elution in the SEC and hence misleading molar masses. At adequate concentrations in each fractionation equipment, the molar masses were in reasonable good agreement although AF4/MALS provided larger values than the other two techniques likely because samples were not filtered before injection. © 2015 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2015**, *132*, 42434.

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INTRODUCTION

Functional water-soluble polymers and their copolymers find widespread applications in pharmaceuticals, waste water treatment, consumer products, paper manufacturing, and cosmetics. These polymers are mostly produced by free-radical polymerization in aqueous phase.

N-Vinylformamide (NVF), an isomer of acrylamide, has a special interest because of its low toxicity and high reactivity in homo- and copolymerization reactions. Moreover, NVF polymers can be easily hydrolyzed to form polymers with primary amine functionality, increasing the application fields where these polymers can be implemented.

Size exclusion chromatography (SEC) coupled with multiangle light scattering (MALS) and refractive index (RI) detectors is one of the most powerful tool for the molecular characterization of the polymers due to the ability of SEC/MALS/RI to determine the absolute molar mass and root-mean square (RMS) radius for every eluting polymer fractions from the columns. In addition, the information about the polymer chain structure can also be obtained from the relationship between the molar mass and the size of the polymer chains.

However, very often an anomalous elution has been observed when size exclusion chromatography (SEC) has been used in combination with a light scattering detector; namely, the molar mass increases with elution time at longer elution times.[1–8]

This phenomenon is attributed to an artifact, which is related to the signal-to-noise ratio and data treatment[1] and/or to co-elution.[2–8] However, the origin of co-elution can be diverse. If there is an enthalpic interaction between the polymer and the stationary phase[2,3] (column), a retention of the polymer might occur and as a consequence, polymers presenting higher hydrodynamic volume will elute later resulting in higher measured average molar mass at each elution time. On the other hand, co-elution can also be related to the conformation of the polymer.[4–8] Branched polymers present a more compact structure and lower hydrodynamic volume than the linear polymers of the same molar mass and composition; for this reason, polymers presenting similar hydrodynamic volume but very different

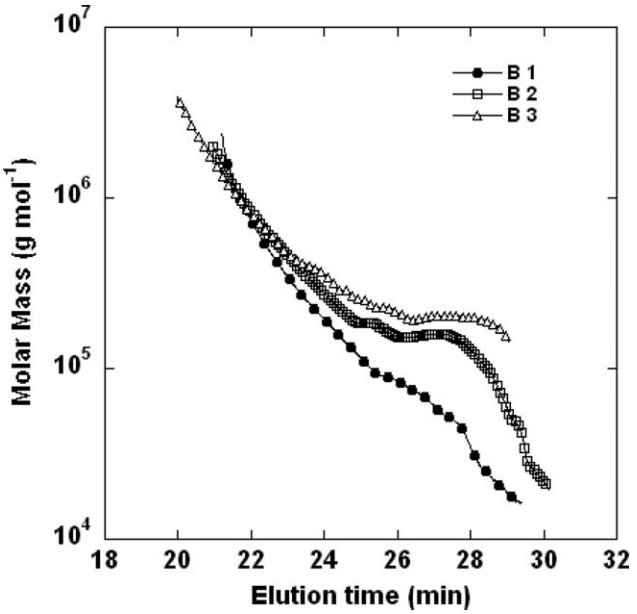

Figure 1. Evolution of the absolute molar mass for B1, B2, and B3 injected in approximately 40 mg mL^{-1} range.

molar mass will elute at the same time provoking an increase of the measured average molar mass at each elution time.

In aqueous systems, it has been reported that various polymers containing polar functional groups (poly-*N*-vinyl pyrrolidone,[9,10] poly-*N*-vinyl pyridine,[11] oxazolyne-type polymers,[12] cellulose,[13–15] and starch[16]), similar to the polymer analyzed in this work, presented a strong tendency to interact with the SEC columns commonly leading to sample adsorption or shear degradation.

Alternatively, asymmetric-flow field-flow fractionation, AF4,[17,18] has been used to avoid interactions between the polymer and the stationary phase. Although the macromolecules analyzed by AF4 are in contact with the semipermeable membrane of the accumulation wall, the total contact surface is much lower compared to the packed porous SEC columns and thus enthalpic interactions are less likely.[19] The potential of AF4/MALS for the characterization of water-soluble polymers have been demonstrated by previous works.[14,20–24] The characterization of the molar mass distribution of polyNVF has been reported in literature. In most of the works, SEC/MALS,[25,26] SEC/TD,[27] and SEC/RI[28] has been used. The Zimm plot analysis has also been reported.[29] However, the value of the dn dc^{-1}, which is essential for the accurate determination of the molar mass by static light scattering, is seldom reported.[26,30] Furthermore, to the author's knowledge, nothing related to the molecular characterization of polyNVF by AF4/MALS except for the author's previous publication[30] has been reported. In that work, the aqueous solution batch polymerization of NVF was extensively investigated and the kinetics and molar masses successfully modeled. Furthermore, extremely high molar mass polyNVF was produced by inverse microemulsion polymerization and its use as flocculants assessed. Also polyvinylamine (PVAm) was produced by basic hydrolysis of the polyNVF synthesized by batch solution polymerization and PVAm was used as polymeric stabilizer in surfactant-free emulsion polymerization to produce pH-responsive poly(MMA) latex with potential applications in biomedicine and paper industry. The values of the molar masses reported in our previous work[30] were analyzed by AF4/MALS since anomalous elution was observed when they were analyzed by SEC/MALS (Figure 1), but the details of the assessment of the different characterization techniques analyzed to determine the optimal analysis conditions were not included and are the core of this work.

The anomalous elution observed in SEC/MALS was first considered the result of the enthalpic interactions between the stationary phase and the polyNVF polymer. However, in this work, we demonstrate that polyNVF does not present any enthalpic interactions with the stationary phase of the columns and that the anomalous elution observed in the analysis of Figure 1 aroused from the column overloading due to the high concentrations employed. Column overloading led to a substantial amount of polymer retained in the column; namely, low mass recoveries. This effect was more pronounced the higher was the molar mass (see Table I). Indeed, this is an important issue when characterizing the molar mass distribution of polymers with low value of dn dc^{-1} and low molar masses. In an attempt to increase the signal of both detectors (RI and LS) to allow accurate characterization of the molar masses, one tends to increase concentration of the sample injected and hence might favor elution artifacts due to overloading of the columns.

In this work, the details of the characterization of the molar mass distribution of three polyNVF synthesized by aqueous solution polymerization are presented. SEC/MALS, AF4/MALS, and MALS using Zimm plot approach were used to determine the most accurate conditions and optimal characterization technique to analyze polyNVF in a broad range (150–450 kDa) of molar masses. In addition, the refractive index increment (dn dc^{-1}) was measured in two different carriers. The aim of analyzing the polyNVF by two different fractionation techniques (SEC and AF4) was to determine possible enthalpic interactions between the polymer and the stationary phase and its effect on the measured molar mass and root mean square radius. In addition, the nonfractionated analysis should help in elucidating anomalous effects in the separation.

EXPERIMENTAL

Materials

NVF (Aldrich, Madrid, Spain, 98%, stabilized with 25–50 ppm Tempo/Tempol) was distilled under vacuum and stored at −10°C.

Table I. Absolute Weight-Average ($\overline{M_W}$) Molar Mass Calculated from SEC/MALS/RI

	SEC/MALS		
Sample	($\overline{M_W}$) (g/mol)	[a]Recovery (%)	Concentration (mg mL^{-1})
B1	135,900	78.6	40.02
B2	199,200	80.2	41.07
B3	327,800	54.6	41.2

[a] The mass recovery was calculated using the dn dc^{-1} value and the area of the integrated RI signal.

2,2′-azobis(2-methylpropionamide)dihydrochloride (AIBA) free radical initiator was used as received. For the preparation of the polymerization solutions, distilled water was used.

Polymerization of NVF

Homopolymerization reactions were carried out in a reaction calorimeter (RC1, Mettler Toledo, Barcelona, Spain). Three reactions with different monomer concentrations (6% (B1), 9% (B2), and 15% (B3)) were carried out at the same temperature (70°C), polymerization time (120 min), and initiator concentration (1.47 mmol L^{-1}).

The aqueous monomer (NVF) solution was heated to the reaction temperature under constant stirring and nitrogen atmosphere. Once the desired temperature was reached, polymerization was started by a shot of a known amount of a solution of AIBA initiator in distilled water.

Sample Preparation

Once the polymerization reaction was finished, a certain amount of the polymer aqueous solution was added into methanol to extract the polymer. Afterward, the polymer was dissolved in water and subsequently precipitated in methanol. The last step was repeated three times in order to remove the unreacted NVF (if any), which is liquid at room temperature (boiling point of 210°C) and achieve a high-purity polymer. Then the polymer was left in the vacuum oven at 70°C during 24 h. Once the polymer was dried, the required aqueous solutions for the SEC and AF4 were prepared. In order to assure reproducible results, each sample was injected three times in both equipments.

Refractive Index Increment, dn dc^{-1}

The refractive index increment (dn dc^{-1}) was measured by means of an Optilab T-Rex differential refractometer ($\lambda = 658$ nm) (Wyatt Technology Corp., Santa Barbara, CA, USA) at 35°C. The equipment consisted of a LC20 pump (Shimadzu, Izasa S.A., Barcelona, Spain) and a Rheodyne manual injector coupled with a 2 mL sample loop.

The experiment was carried out by injecting six samples (in the 0.5–5.0 mg mL^{-1} concentration range) of the same batch of polymer. The samples were prepared as follows. First, the main solution was prepared by dissolving the dried polymer in DDI water (Milli-Q, Millipore Ibérica S.A.U, Madrid, Spain) (5.0 mg mL^{-1}). The rest five solutions (4.0, 3.0, 2.0, 1.0, and 0.5 mg mL^{-1}) were prepared by dilution of the mother solution.

The analysis was performed at 35°C in two different mobile phases at a flow rate of 1 mL min^{-1}: on one side with DDI water (stabilized with sodium azide) and on the other side with a mixed eluent water/acetonitrile (80 : 20 by weight) with 0.15 mol L^{-1} NaCl and 0.03 mol L^{-1} NaH_2PO_4 providing a pH = 5.5.

The dn dc^{-1} was calculated from the slope obtained from the plot of the refractive index against concentration.

Weight-Average Molar Mass ($\overline{M_w}$) and Root-Mean Square Radius (z-Averaged Radius, R_z)

MALS (Zimm Plot Approach). The absolute weight-average molar mass ($\overline{M_w}$) and the root-mean square radius (R_z) of the polymers (no fractionation of the sample) were characterized by a DAWN Heleos multiangle (18 angles) light scattering laser photometer equipped with an He–Ne laser ($\lambda = 658$ nm) (Wyatt Technology Corp., Santa Barbara, CA, USA) at 35°C. The setup used to measure the dn dc^{-1} of the samples was used here too.

The experiment was carried out by injecting six samples of concentrations of the polymer in the range 0.5–5.0 mg mL^{-1}. The same sample preparation procedure used to measure the dn dc^{-1} was used here.

The analysis was performed at 35°C and DDI water (stabilized with sodium azide) was used as mobile phase at a flow rate of 1 mL min^{-1}.

Before injecting the samples in the equipment, the polymer solutions were filtered by a 0.45 μm nylon filter (Scharlab, Barcelona, Spain).

The molar mass and the root-mean square radius were calculated from MALS data using the Zimm plot (with first-order Zimm formalism) from the ASTRA software v.6.0.3. of Wyatt.

SEC/MALS/RI. The molar mass and the root-mean square radius of the polymers were analyzed by SEC/MALS/RI. The equipment used for the SEC separation was that used for the AF4 separation and was already described in Ref. [30] and it will not be described here again.

Separation was carried out using three columns in series (Ultrahydrogel 120, 250, and 2000 with pore sizes of 120, 250, and 2000 Å, respectively, Waters, Barcelona, Spain).

The analyses were carried out at 35°C and a mixed eluent water/acetonitrile (80 : 20 by weight) with 0.15 mol L^{-1} NaCl and 0.03 mol L^{-1} NaH_2PO_4 providing a pH 5.5 was used as mobile phase at a flow rate of 1 mL min^{-1}.[26]

The dried polymers were dissolved in the mixed eluent at 1.0 mg mL^{-1} concentration and afterward there were filtered and injected (100 μL) into the equipment.

The SEC/MALS/RI data was analyzed by using the ASTRA software version 6.0.3 (Wyatt technology, USA). The absolute molar mass and the radius of gyration were calculated from the MALS/RI data using the Debye plot (with first-order Zimm formalism).

AF4/MALS/RI. The molar mass and root-mean square radii of the polymers were analyzed by AF4/MALS/RI. The equipment and calibration of the detectors used were already described in Ref. [30] and are not repeated here.

The separation in the AF4 equipment is carried out by a flow in an open channel where a perpendicular flow force is applied[19] (there is not a stationary phase). The channel consists of two plates joined together that are separated by a spacer. The bottom plate is permeable, made of a porous frit covered by a semipermeable membrane (permeable for the molecules of carrier, but impermeable for the polymer molecules).

The laminar flow of the carrier creates a parabolic flow profile within the channel; that is, the carrier moves more slowly closer to the channel walls compared to the channel center. The analyzed molecules and particles are driven by the cross-flow

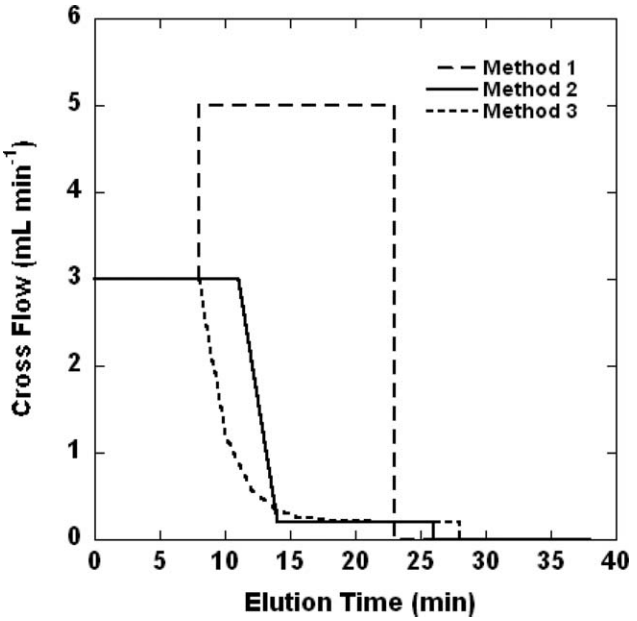

Figure 2. Evolution of the cross-flow with elution time for the three different methods.

toward the bottom wall of the channel. Diffusion creates a counteracting motion due to the channel center, where the axial flow is faster. The velocity gradient inside the channel separates the molecules and particles according to their hydrodynamic size in such a way that smaller molecules elute before than the larger ones. This means that the AF4 separation is the opposite of SEC separation, in which the large molecules elute first.[19]

In this work, separation was carried out using AF4 fractionation equipment on a 27.5 cm trapezoidal channel mounted on a PEEK (polyether ether ketone) lower block with a stainless-steel frit. The upper block was aluminum with a polycarbonate window. The channel thickness spacer was 350 μm. The accumulation wall was a Nadir regenerated cellulose membrane with a cut-off molar mass of 10,000 Da. AF4 flow control was maintained with a Wyatt Eclipse 3 AF4 Separation System controller (Wyatt Technology, Santa Barbara, CA).

The cross-flow was applied from minute 8 on, time where the fractionation takes place. The cross-flow profile employed in AF4 is the key parameter to achieve a good separation and unfortunately is system dependent. This means that the optimum cross-flow profile should be assessed for each polymer system. For the polyNVF used in this work, three cross-flow profiles were analyzed: constant (method 1), linear (method 2), and exponential (method 3). Figure 2 presents the three profiles used to find the best separation conditions and Figure 3 presents the RI raw data and molar mass as function of the elution time for a polyNVF sample B3, see below for the characteristics of the sample.

Note that the elution time in Figure 3 increases from right to left to make the chromatogram comparable with those of SEC.

In the first method, a constant cross-flow of 5 mL min^{-1} was applied for 15 min and afterward the cross-flow was abruptly decreased to 0.0 mL min^{-1}. In method 2, the cross-flow was kept constant at 3 mL min^{-1} for 3 min, then it was decreased linearly from 3 to 0.2 mL min^{-1} for 3 min, and kept constant at 0.2 mL min^{-1} for 12 min to finally being switched to 0.0 mL min^{-1}. In the last method, the cross-flow was exponentially decreased from 3 to 0.2 mL min^{-1} for 20 min. Figure 1 presents the profile of the three cross-flow methods assessed. In all the methods, the focusing was performed for 1 min at a cross-flow rate of 3 mL min^{-1} after 4 min of sample injection in focus + inject mode with an injection flow of 0.2 mL min^{-1}. Prior to the sample injection step, it was performed a 1-min focus step (cross-flow 3 mL min^{-1}) and a 2-min elution step (cross-flow 3 mL min^{-1}) for system equilibration. The detector flow during the whole experiment was set to 1 mL min^{-1}. Therefore, the injected sample elutes after the first 8 min in the cross-flow profile figure.

Although the average molar masses calculated with the three cross-flow profiles did not differ substantially, the separation in the channel did, as can be observed in Figure 3 in the RI signal and the molar masses at each elution time. Thus in method 1, most of the fractionation occurred in the last 5 min of cross-flow profile whereas in methods 2 and 3, fractionation was smoother over the whole profile. Indeed, methods 2 and 3 only differed in the first minutes of the cross-flow profile where the fast exponential decay did not fractionate well the low molar mass chains. Based on these results, it was decided to use method 2 for the rest of the samples analyzed in this work.

The calibration of the MALS equipment and the sample preparation were done as described in the section entitled "SEC/MALS/RI" for the SEC/MALS/RI, but higher concentrations were injected; about 40 mg mL^{-1}. In this equipment, samples were not filtered because separation takes place in a wide-enough (350 μm) channel as to avoid any clogging. On the other hand, it was attempted to use lower sample concentrations (similar to those used in the SEC analysis), however, due

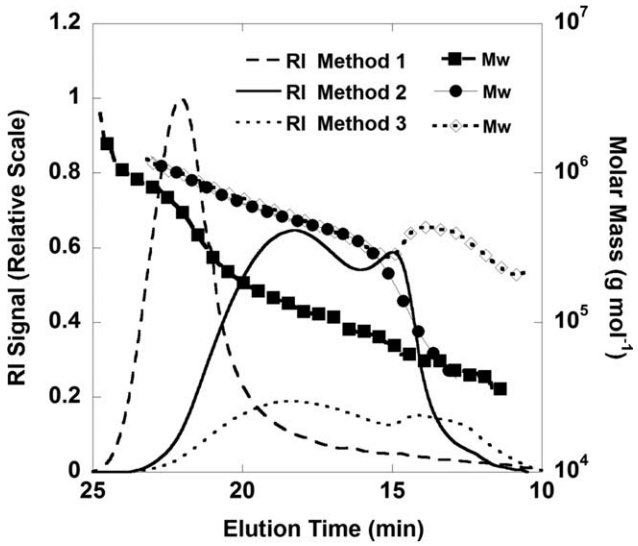

Figure 3. RI and molar mass versus elution time for AF4 analysis of sample B3 with the three cross-flow methods. The elution time axis is in the reverse order to make it similar to SEC separation.

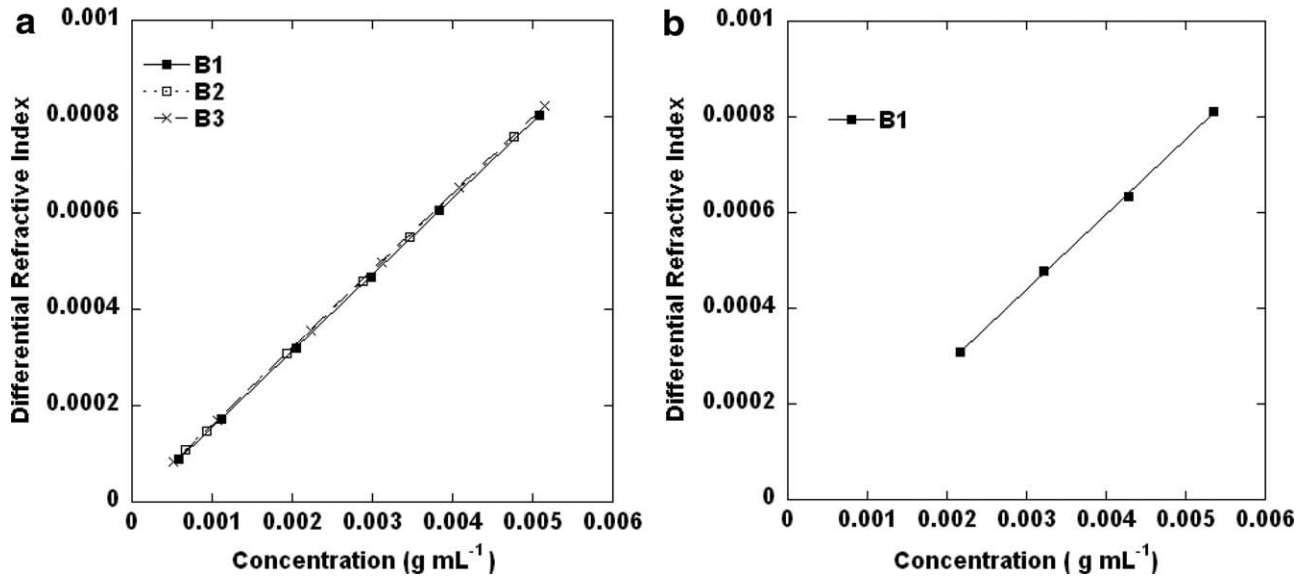

Figure 4. (a) dn dc^{-1} results for B1, B2, and B3 using DDI water stabilized with sodium azide as the mobile phase. (b) dn dc^{-1} results for B1 in the mixed eluent (DDI water/acetonitrile buffered at pH = 5.5).

to the higher sample dilution occurring in the AF4 (caused by the cross-flow), higher concentrations were required to obtain reasonable signal-to-noise ratios.

RESULTS AND DISCUSSION

dn dc^{-1}

Figure 4a presents the differential refractive index (n) vs concentration (c) in DDI water stabilized with sodium azide for the three polyNVF synthesized with different monomer concentration. From the slope of this graph, the dn dc^{-1} values were obtained for the calculation of the absolute molar mass in the MALS, SEC/MALS, and AF4/MALS analyses.

The values of dn dc^{-1} for the three polyNVF samples B1, B2, and B3 were 0.1589, 0.1587, and 0.1595, respectively.

In order to discard the mobile phase effect on the dn dc^{-1}, the dn dc^{-1} of sample B1 in the mixed eluent (DDI water/acetonitrile buffered at pH = 5.5) was measured (see Figure 4b). The dn dc^{-1} measured under these conditions (0.1564 mL g^{-1}) was very close to the average dn dc^{-1} obtained in DDI water stabilized with sodium azide (0.1590 mL g^{-1}). Therefore, the dn dc^{-1} = 0.1564 mL g^{-1} was used in the SEC/MALS, AF4/MALS, and Zimm plot.

Molar Mass and Root-Mean Square Radius Characterization

Absolute weight-average molar masses ($\overline{M_w}$) and z-average mean square radii (R_z), also known as radius of gyration, were calculated from SEC-AF4/MALS/RI data.

It is worth noting that in SEC-AF4/MALS/RI chromatography to accurately calculate the molar mass, the signal of the detectors should be sufficient in the whole elution range. This is particularly important because at small elution volumes (in SEC), MALS signal is more sensitive than RI and the opposite happens at large elution times. All the molar mass data reported in this work was computed ensuring that the signal/noise ratio was reasonably good for the analysis.

The polyNVF analyzed in this work should be rather linear. Nevertheless, some authors have claimed reaction mechanisms that might produce branches during the polymerization of *N*-vinyl formamide. Thus Gu *et al.*[28,31] have included intermolecular chain transfer to polymer in the mathematical model of the aqueous phase solution polymerization of NVF to predict the kinetic and the molar masses. Also Stach *et al.*[26] claimed that quaternary carbons detected in ^{13}C NMR spectra of polyNVF were caused by chain transfer to polymer reactions that produced branched structures (unfortunately, the branching density was not quantified). In this work, ^{13}C NMR spectra for the polyNVF analyzed was carried out and quaternary carbon peaks were not observed nor any other shift that might be related with a branched structure and these results were in agreement with the results obtained in SEC/MALS.

In contrast with the results shown in Figure 1 for higher concentrations of the samples (*ca* 40 mg mL^{-1}), Figure 5 shows that the molar mass monotonously decreases with the elution time for the three samples, namely, molar mass decreases with the elution time during the whole elution range. This behavior is a clear indication of an ideal elution that will not be observed in case the polymers were branched. In addition, the conformation plot also presents the same ideal elution with slopes in the range of 0.5–0.6 corresponding to linear polymer chains.

The same polyNVF samples were analyzed (at higher concentrations as explained in the experimental section) in the AF4/MALS and presented the same behavior (Figure 6). They present a monotonously decreasing elution curve, namely, molar mass increases with the elution time during the whole elution range (note that the slope change observed in the molar mass vs elution time at around 15–16 min is the consequence of the cross-flow variations during the experiment as described in Figure 2). Furthermore, the conformation plots present the same trend and the slopes (0.5–0.6) confirm that the three polymers present a linear conformation.

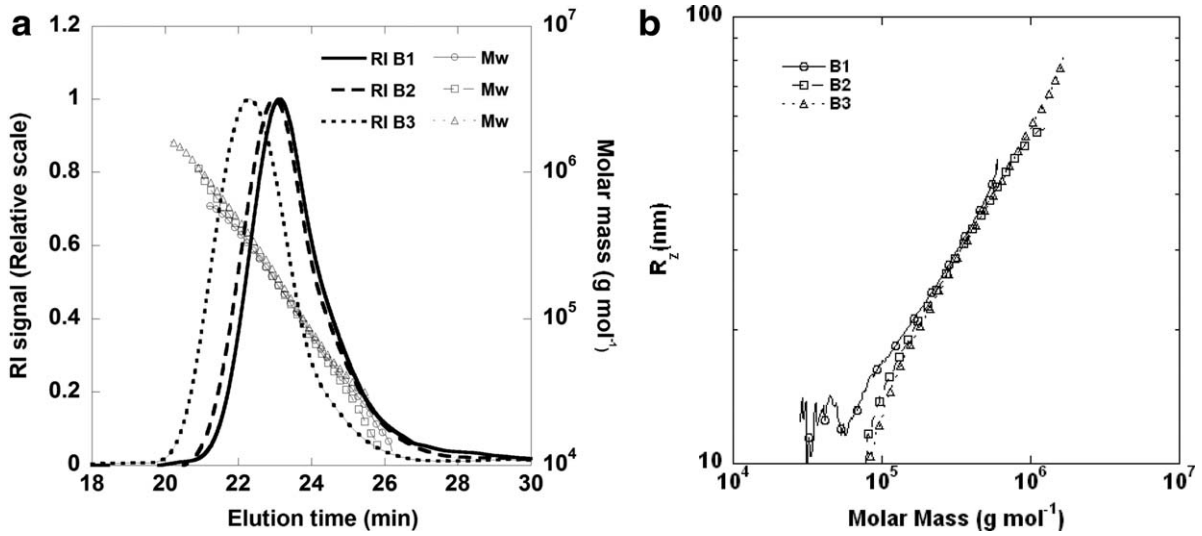

Figure 5. Comparison of the refractive index chromatogram and molar mass versus elution time (left) and conformation plot (right) for SEC analysis of samples B1, B2, and B3.

Besides the fractionated experiments (SEC/MALS and AF4/MALS), the same samples were analyzed without fractionation by MALS.

Zimm plots of good quality as those illustrated in Figures 7–9 were obtained. The obtained weight-average molar masses and radius of gyration were calculated from MALS data using the Zimm plot (with first-order Zimm formalism) and are summarized together with the weight-average molar masses obtained by SEC/MALS and AF4/MALS in Table II.

The trend in all the techniques assessed is the same; as expected in batch free-radical homogeneous polymerization, the absolute weight-average molar mass increased with monomer concentration.

Interestingly, the molar mass measured by the Zimm plot approach (no sample fractionation) and by the two fractionating techniques (SEC and AF4) were in rather good agreement, even if in Zimm plot and SEC/MALS, the samples were filtered and those injected in the AF4/MALS were not. This would explain the slightly lower values measured by the Zimm plot method, and the SEC/MALS technique. It must be said that the mass recovery in both cases, SEC and AF4, was higher than 80%. It is also worth noting that the concentration of the samples injected in the two fractionating instruments; *ca* 40 mg mL^{-1} in the AF4 and 1–3 mg mL^{-1} in the SEC were very different. The lack of stationary phase and the higher dilution of the sample due to the cross-flow employed in the AF4 allows working in a broader and higher concentration range without

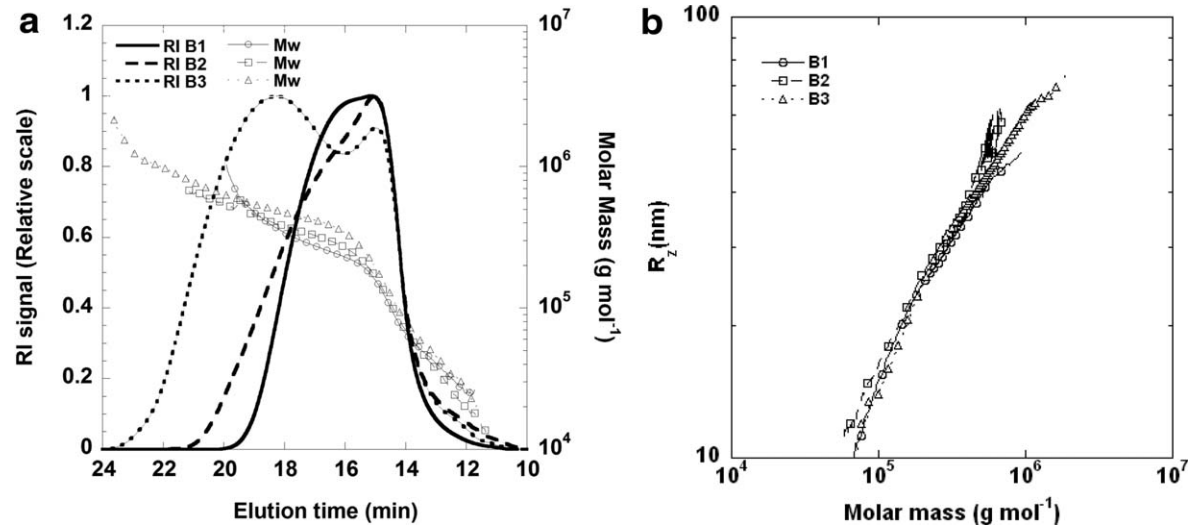

Figure 6. Comparison of the refractive index chromatogram and molar mass versus elution time (left) and conformation plot (right) for AF4 analysis of samples B1, B2, and B3. AF4 conditions: detector flow 1 mL min^{-1}, spacer 350 μm. Elution starts at 8 min. Cross-flow 3 mL min^{-1} for 3 min then linear decay to 0.2 mL min^{-1} within 3 min + 12 min at 0.2 mL min^{-1}.

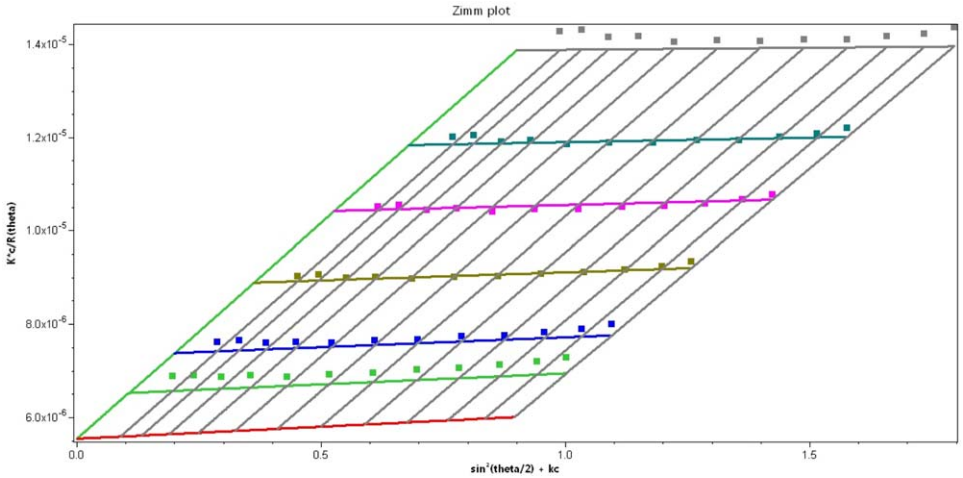

Figure 7. Zimm plot for sample B1. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

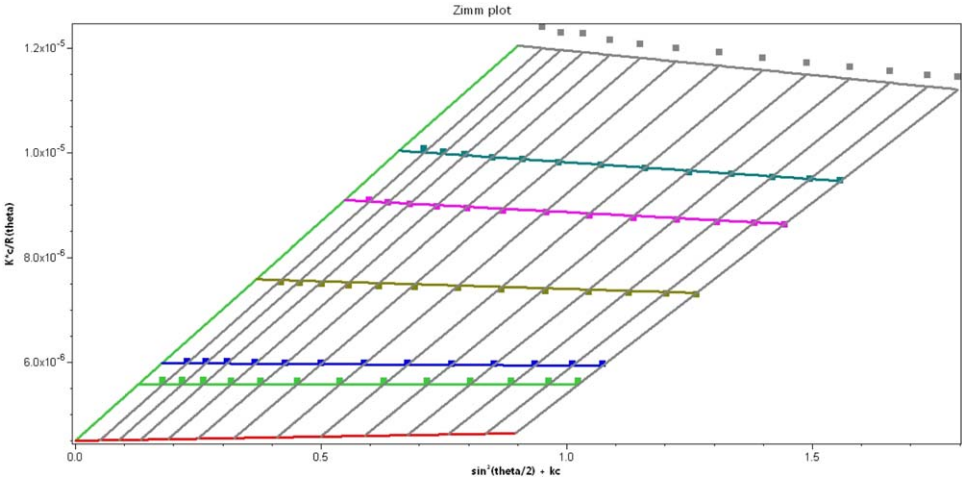

Figure 8. Zimm plot for sample B2. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

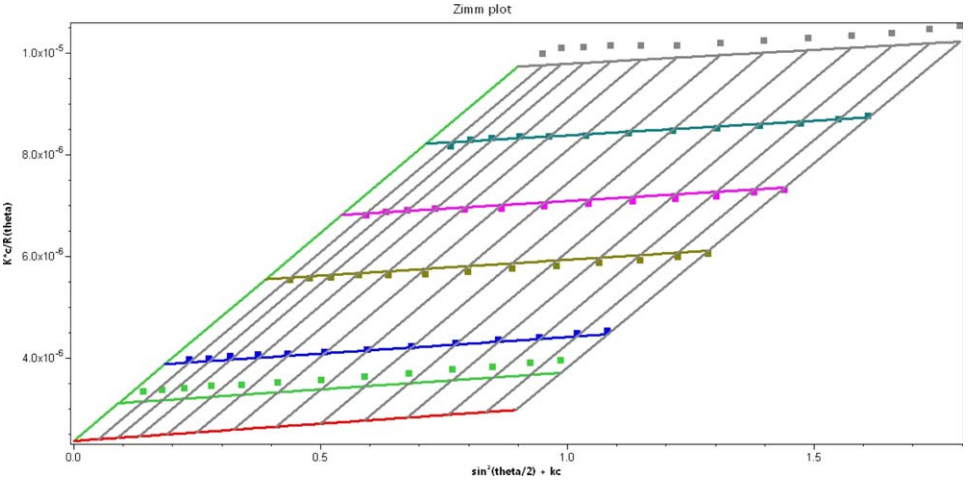

Figure 9. Zimm plot for sample B3. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Table II. Absolute Weight-Average $(\overline{M_W})$ Molar Mass and Radius of Gyration (R_Z) Calculated from Zimm Plot (MALS), SEC/MALS/RI, and AF4/MALS/RI

	MALS		SEC/MALS		AF4/MALS	
SAMPLE	$(\overline{M_W})$ g mol^{-1}	R_Z (nm)	$(\overline{M_W})$ g mol^{-1}	R_Z (nm)	$(\overline{M_W})$ g mol^{-1}	R_Z (nm)
B1 (6%)	177,500 ± 4400	20.9 ± 5.3	168,200 ± 672.8	27.1 ± 0.6	217,000 ± 870.8	28 ± 0.5
B2 (9%)	228,100 ± 4800	14.4 ± 5.7	209,900 ± 1049.5	30.6 ± 0.6	263,500 ± 2371.5	35.2 ± 1.1
B3 (15%)	415,800 ± 22,700	36.5 ± 6.1	417,800 ± 2089	44.6 ± 0.4	445,600 ± 1336.8	44.3 ± 0.3

affecting the results up to a certain extent. On the contrary, when samples with the same concentration as that used in the AF4 were injected in the SEC instrument column overloading effects were noticed leading to anomalous elution curves at high elution times as shown in Figure 1. The molar masses calculated under this condition were substantially smaller as well as the recovery calculated from the RI data (see Table I).

CONCLUSIONS

The determination of the optimal conditions for the characterization of the molar mass distribution and the radius of gyration of a water-soluble polymer, poly *N*-vinylformamide, by different fractionation techniques was pursued in this work. The fractionation techniques employed (packed columns, asymmetric flow field-flow fractionation, and no-fractionation) were coupled with refractive index and MALS for accurate determination of the molar masses.

Any possible anomalous elution arising from branching and/or stationary phase interaction has been discarded for the three polyNVF with widely different average molar masses (150–450 KDa) synthesized by aqueous solution polymerization.

The dn dc^{-1} values required to determine molar mass were determined in the two different eluents employed in this work. It turned out that neither the molar mass nor the eluents had a substantial impact in the dn dc^{-1} value calculated.

The molar mass distributions of the 3 polyNVF were assessed by using SEC/MALS, AF4/MALS, and Zimm plot MALS analysis. It was found that the SEC/MALS, AF4/MALS, and Zimm plot MALS provided very similar results in a broad range of molar masses although SEC/MALS and Zimm plot MALS provided smaller molar masses likely due to the fact that the samples were filtered before injection. Noticeable was also that high sample concentrations could be injected to the AF4 (*ca* 40 mg mL^{-1}) because of the additional dilution occurring in the system due to the cross-flow. This concentration range of the sample caused overloading and anomalous elution in the SEC equipment and hence it was not possible to compare the samples in the same concentration range although lower concentrations can be analyzed in AF4. This is important because in addition to the inherent advantages of the AF4 fractionation (reduced interaction with the polymer), this feature allows injecting higher concentrations without affecting the accuracy of the measurements, which it might be very useful in certain complex samples or for polymers with a low dn dc^{-1}.

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REFERENCES

1. Tackx, P.; Bosscher, F. *Anal. Commun.* **1997**, *34*, 295.
2. Berek, D. *Macromol Symp.* **2004**, *216*, 145.
3. Berek, D. *J. Sep. Sci.* **2010**, *33*, 315.
4. Johan, C.; Kilz, P. *J. Appl. Polym. Sci. Appl. Polym. Symp.* **1991**, *48*, 111.
5. Frater, D. I.; Mays, J. W.; Jackson, C. *J. Polym. Sci. B* **1997**, *1997*, 141.
6. Gerle, M.; Fischer, K.; Roos, S.; Mueller, A. H. E.; Sheiko, S. S.; Prokhorova, S.; Moller, M. *Macromolecules* **1999**, *32*, 2629.
7. Podzimek, S.; Vlcek, T.; Johann, C. *J. Appl. Polym. Sci.* **2001**, *81*, 1588.
8. Gaborieau, M.; Castignolles, P. *Anal. Bioanal. Chem.* **2011**, *399*, 1413.
9. Striegel, A. M.; Kirkland, J. J.; Yau, W. W.; Bly, D. D. Modern Size-Exclusion Liquid Chromatography, Willey: New York, **2009**.
10. Stach, M.; Lacìk, I.; Chorvát, D.; Buback, M.; Hesse, P.; Hutchinson, R. A.; Tang, L. *Macromolecules* **2008**, *41*, 5174.
11. Park, S.; Chang, T. *Macromolecules* **2006**, *39*, 3466.
12. Baumgaertel, A.; Weber, C.; Fritz, N.; Festag, G.; Altuntas, E.; Kempe, K.; Hoogenboom, R.; Schubert, S. *J. Chromatogr. A* **2011**, *1218*, 8370.
13. Nilsson, S.; Sundelöf, L. O.; Porsch, B. *Carbohydr. Polym.* **1995**, *28*, 265.
14. Wittgren, B.; Wahlund, K. G. *Carbohydr. Polym.* **2000**, *43*, 73.
15. Dupont, A. L.; Mortha, G. *J. Chromatogr. A* **2004**, *1026*, 129.
16. Vilaplana, F.; Gilbert, R. G. *Macromolecules* **2010**, *43*, 7321.
17. Giddings, J. C. *Science* **1993**, *260*, 1456.

18. Angoy, M.; Bartolomé, M. I.; Vispe, E.; Lebeda, P.; Jiménez, M. V.; Pérez-Torrente, J. J.; Collins, S.; Podzimek, S. *Macromolecules* **2010**, *43*, 6278.
19. Podzimek, S. Light Scattering, Size Exclusion Chromatography and Asymmetric Flow Field Fractionation: Powerful Tools for the Characterization of Polymers, Proteins and Nanoparticles; Wiley: Canada, **2011**.
20. Giddings, J. C.; Benincasa, M. A. *Anal. Chem.* **1992**, *64*, 790.
21. Kirkland, J. J.; Dilks, C. H.; Rementer, S. W. *Anal. Chem.* **1992**, *64*, 1295.
22. Roessner, D.; Kulicke, W. M. *J. Chromatogr. A* **1994**, *687*, 249.
23. Wittgren, B.; Wahlund, H. D.; Wesslén, B. *Macromolecules* **1996**, *29*, 268.
24. Jiang, X.; Chu, Y.; Liu, J.; Zhang, G.; Zhuo, R. *Chinese J. Polym. Sci.* **2011**, *29*, 421.
25. Yacoub, K. PHD Dissertation, Lehigh University, **1994**.
26. Stach, M.; Lacík, I.; Kasák, P.; Chorvát, D.; Saunders, A. J.; Santanakrishnan, S.; Hutchinson, R. A. *Macromol. Chem. Phys.* **2010**, *211*, 580.
27. Marheka, J. N.; Marascalco, P. J.; Chapman, T. M.; Rusell, A. J.; Kameneva, M. V. *Biomacromolecules* **2006**, *7*, 1597.
28. Gu, L.; Zhu, S.; Hrymak, A. N.; Pelton, R. H. *Polymer* **2001**, *42*, 3077.
29. Witek, E.; Pazdro, M.; Bortel, E. *J. Macromol. Sci. Part A: Pure Appl. Chem.* **2007**, *44*, 503.
30. Zatary, J.; Agirre, A.; de la Cal, J. C.; Leiza, J. R. *Macromol. Symp.* **2013**, *333*, 80.
31. Gu, L.; Zhu, S.; Hrymak, A. N.; Pelton, R. H. *Macromol. Rapid Commun.* **2001**, *22*, 212.