NMR Studies, Molecular Characterization, and Degradation Behavior of Poly(amido amine)s. 1. Poly(amido amine) Deriving from the Polyaddition of 2-Methylpiperazine to 1,4-Bis(acryloyl)piperazine

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ABSTRACT: Poly(amido amine)s (PAAs) are a family of water soluble polymers endowed with properties which find useful applications in the medical field. In order to perform degradation studies on PAAs by using GPC in aqueous media, a calibration curve was derived by following a purposely developed procedure. A set of PAA samples having different molecular weights were synthesized, by means of stepwise polyaddition reactions, carried out with nonstoichiometric ratios of the monomers. A careful characterization of the samples was performed by means of ^1H, ^{13}C, and bidimensional homo- and heterocorrelated NMR spectrometry. Particular attention was devoted to the quantitative determination of end functionalities, which enabled us to calculate the number-average molecular weight of PAA samples. The GPC results, obtained in buffered aqueous solution, were combined with NMR data on number-average molecular weight, and by means of a nonlinear least squares fitting procedure, following iterative routines, a linear calibration curve of log M versus retention times was obtained. Weight averages and the polydispersity index for the PAA samples were then determined. Finally, from viscometric measurements, the Mark–Houwink constants were also determined using number-average molecular weights.

Introduction

Poly(amido amine)s (PAAs) are a family of polymers characterized by the presence of amido and tertiary amino groups regularly arranged along the macromolecular chain. Linear PAAs are obtained by the polyaddition reaction of primary monoamines or bis(secondary amine)s to bis(acrylamide)s, according to Scheme 1.[1]

The polymerization reaction can be carried out in water or alcohols, at room temperature, and without any added catalyst. It takes place easily with almost every aliphatic and cycloaliphatic amine, also when side substituents such as hydroxyl, carboxylic, or additional tertiary amino groups are present.

Most PAAs are water soluble or at least water swellable. In aqueous solution they behave as bases of medium strength. The protonation and heavy-metal ion complexing behavior of a large number of PAAs have been thoroughly studied.[2–5] Some PAA–metal complexes exhibit interesting properties as oxidation catalysts.[6,7] PAAs can be easily grafted onto the surface of various polymeric and inorganic materials, thus modifying dramatically their surface properties,[8–11] or enter as constituents of block copolymers.[12,13]

The main interest in PAAs lies in their potential use in the biomedical field, being able to form stable complexes with heparin.[1] Either PAA-grafted biomaterials or PAA-based cross-linked hydrogels can in fact be stably heparinized, acquiring nonthrombogenic properties,[8–11,14] or act as selective heparin absorbers from plasma or blood.[15]

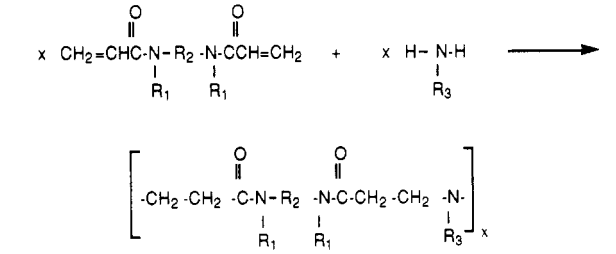
More recently, PAAs are being considered as soluble carriers for anticancer drugs. Preliminary in vitro studies have demonstrated that some PAAs are endowed with a fairly good cell compatibility.[16]

All PAAs are in principle degradable in water, since they contain hydrolyzable amidic bonds in their main chain, together with nucleophilic ter-aminic functions in the β position. This particular property of PAAs is a double-edged one: it turns out to be advantageous when PAAs are conceived as soluble carriers for preparing polymeric prodrugs but disadvantageous for those applications in which the absence of leachable products is required. This is particularly true in the case of deheparinizing resins or heparinizable biomaterials. Therefore, a study intended at determining their degradation rate is relevant to their use.

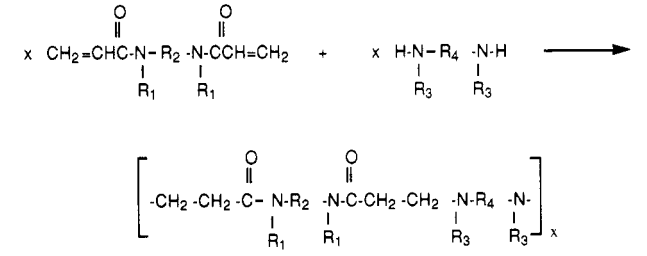
So far, degradation studies on PAAs have been carried out in our laboratory by using GPC and viscometric techniques.[17] However, in the absence of proper calibration standards, no absolute molecular weight determina-

Scheme 1. Synthetic Pathways for the Formation of PAAs

a)



b)



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tions can be performed and, therefore, no absolute degradation rate can be determined. On the other hand, such determinations are obviously necessary, especially if PAAs of different structures and molecular weights have to be compared in this respect.

The usual molecular weight measurement techniques are difficult to perform on most PAAs. In particular, VPO (vapor phase osmometry) determinations prove unreliable for these polymers, which are highly polar and, besides being poorly soluble in solvents other than water, tenaciously retain traces of moisture. As far as light scattering measurements are concerned, the rather low molecular weight values of most PAAs make this technique unsuitable for weight-average determinations. On the other hand, the moderate molecular weights involved make NMR an appropriate technique for number-average molecular weight ($\bar{M}_n$) determination of PAAs with recognizable end groups. Furthermore, no careful NMR studies have been performed till now on this class of compounds.

This paper is the first one of a series intended to determine relationships between molecular weight and GPC retention times for different PAAs, starting from their NMR characterization. The goal of the general research program is to compare the absolute degradation rates under different conditions, especially as regards temperature, pH, and ionic strength. In this paper we report on a ^1H, ^{13}C, and bidimensional NMR characterization performed on the poly(amido amine) deriving from the polyaddition of 2-methylpiperazine to 1,4-bis(acryloyl)-piperazine. The assignment of the signals due to the terminal groups and the determination of the ratio of the terminal atoms to the internal ones is given, at least for moderate molecular weights, in order to obtain average molecular weight measurements. As a second step a calibration curve relating molecular weight to GPC retention times is derived. In order to achieve this aim, a set of PAAs having different molecular weights were synthesized and characterized by GPC, their number-average molecular weights were determined *via* NMR and the calibration curve was then obtained by combining GPC and NMR data. Finally, from viscometric measurements, and using the NMR molecular weights, the Mark–Houwink constants were also determined.

Experimental Section

Measurements. Intrinsic viscosities were measured at 20 °C in Tris buffer pH 8.09 by means of Ubbelohde viscometers.

Gel permeation chromatograms were obtained at 20 °C by making use of TSK-GEL G3000 PW and TSK-GEL G4000 PW columns connected in series, using Tris buffer pH 8.09 as the mobile phase. Conditions: sample concentration, 2 mg/mL; injection volume, 25 μL; loop size, 20 μL; flow rate, 1 mL/min (Knauer model HPLC pump 64); column dimensions, 300 mm × 7.5 mm. The products were checked by a Knauer model UV detector operating at 230 nm.

Nuclear magnetic resonance spectra were obtained at frequencies of 200 and 300 MHz for the hydrogen nucleus and at 50.3 and 75.5 MHz for the ^{13}C nucleus. Varian Gemini 200 and Bruker AMX 300 instruments were used, respectively. The pulse width was in the range 5–12 μs for ^1H and 6–13 μs for ^{13}C. A total of 256 scans were acquired for ^1H, and 1000 up to 10 000 scans, for ^{13}C. The polymers were dissolved in $CDCl_3$ (2% w/w for ^1H and 8% w/w for ^{13}C) and analyzed at 30 °C.

Hydrogen homocorrelated spectra were run, acquiring 256 data points for 256 single spectra. Zero filling, sensitivity enhancement, and apodization functions were applied. A conventional COSY experiment, constituted by two 90° pulses divided by an incremental time, was adopted.[18] ^1H—^{13}C heterocorrelated spectra were carried out in the form which leads to decoupled spectra in both dimensions.[18] 1024 × 256 matrices were elaborated in this case.

The 2D spectra of the polymers were run overnight; the spectra of the monomers or of reference compounds required at least 4 h of total acquisition time.

Materials. 1,4-Bis(acryloyl)piperazine was prepared as previously described;[19] 2-methylpiperazine was purchased from Fluka and recrystallized from *n*-heptane (1 g of 2-methylpiperazine in 20 mL of solvent) before use.

Synthesis. Polymer I. 1,4-Bis(acryloyl)piperazine (0.3807 g, 1.960 mmol) was dissolved into 0.1402 g/mol 2-methylpiperazine aqueous solution (2.00 mL, 2.799 mmol); water was added (0.6 mL) in order to obtain a 0.25 g/mL monomer concentration. The reaction mixture was then allowed to react for 5 days at 25 °C and, finally, freeze-dried.

Polymer II was prepared in the same way as **I**, employing 0.4349 g of 1,4-bis(acryloyl)piperazine (2.239 mmol) and adding 0.8 mL of water.

Polymer III was prepared in the same way as **I**, employing 0.4892 g of 1,4-bis(acryloyl)piperazine (2.519 mmol) and adding 1.0 mL of water.

Polymer IV was prepared in the same way as **I**, employing 0.5167 g of 1,4-bis(acryloyl)piperazine (2.660 mmol) and adding 1.1 mL of water.

Polymer V was prepared in the same way as **I**, employing 0.5436 g of 1,4-bis(acryloyl)piperazine (2.799 mmol) and adding 1.2 mL of water.

Polymer VI was prepared in the same way as **I**, employing 2.718 g of 1,4-bis(acryloyl)piperazine (14.00 mmol) and 10.00 mL of 01402 g/mL 2-methylpiperazine aqueous solution (14.00 mmol) and adding 5.0 mL of water.

Results and Discussion

Synthesis. The PAA chosen as the model compound in this paper, besides containing no possibility of hydrogen bond along the macromolecular chain, is characterized by the asymmetric structure of its repeating unit, leading to a lack of regularity of its macromolecular configurations. Therefore, it turns out to be highly soluble both in aqueous media and in some organic solvents. This property proves particularly useful, since it makes it easier to perform analytical tests in solution.

Samples of different molecular weights were synthesized by properly selecting the molar ratio of the monomers in the reaction mixture. In fact, in a stepwise polymerization, the expected number-average degree of polymerization $\overline{DP}_{n,theor}$ is given by[20]

$$\overline{DP}_{n,theor} = \frac{1+r}{1-r} \tag{1}$$

r being the ratio of the moles of the minor component to the moles of the major one.

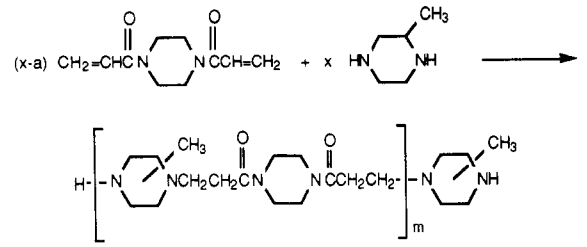
The polymerization reaction was carried out in water at 25 °C according to Scheme 2.

The values of the molar ratio selected and of the corresponding $\overline{DP}_{n,theor}$ are listed in Table 1, columns 2 and 3, respectively. Besides leading to polymer samples of different molecular weights, the selected ratios allowed us to obtain oligomers end-capped with either one of the monomeric units.

NMR spectrometry (see below) allowed us to detect, for each sample, the expected chain end group, i.e. that deriving from 2-methylpiperazine, in the same spectral region where it had been observed in the pure monomer. The presence of end-functional groups different from those predictable on the basis of the known molar ratios would have suggested side reactions, which, apparently, did not take place.

Scheme 2. Polymerization Reaction and Chemical Structure of the PAA Samples

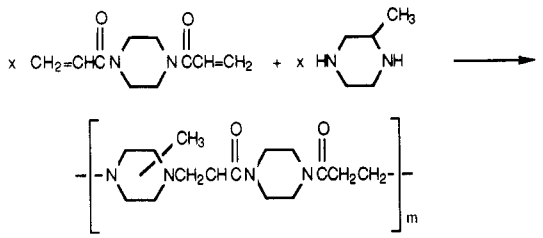
a)



I-IV

where, according to eq. (1), m=x/a - 1
Polymer I: x/a=3.33
Polymer II: x/a=5
Polymer III: x/a=10
Polymer IV: x/a=20

b)



V-VI

Table 1. Characterization and Labeling of the PAA Samples

sample	r[a]	$\overline{DP}_{n,theor}$[b]	[η] (dL/g)
I	0.70	5.7	0.046
II	0.80	9.0	0.050
III	0.90	19	0.091
IV	0.95	39	0.187
V	1.0	∞	0.369
VI	1.0	∞	0.454

[a] Molar ratio of the monomers (n_{BAP}/n_{2MP}). [b] Expected number-average degree of polymerization (from eq 1).

Owing to the difficulty of picking exact equimolarity, two samples (**V** and **VI**) were prepared by attempting to use an equimolar ratio of the monomers. In fact, small deviations from stoichiometry largely affect molecular weight, as eq 1 clearly suggests.

All samples were directly freeze-dried from the polymerization media, without any previous solvent/nonsolvent fractionation step. However, we can demonstrate, by virtue of separate freeze-drying experiments performed on 2-methylpiperazine alone, that during the drying procedure the residual aminic monomer, which should be present according to theory,[20] is quantitatively lost by evaporation.

Viscometric Characterizations. Viscometric measurements were performed in Tris buffer pH 8.09 (solution 0.1 M in Tris and 0.2 M in NaCl) in order to avoid the polyelectrolyte effect. In fact, the PAA considered is only slightly ionized at pH 8.09, since its pK_{a1} (we can recall here that PAAs, as a rule, exhibit sharp basicity constants) is 7.09.[1] In addition, the presence of NaCl in solution further lowers polyelectrolyte behavior. Actually, using this solvent, the η_{sp}/c and $\ln \eta_{rel}/c$ plots versus concentration c are linear over a wide range of concentrations. In Table 1, column 4, the intrinsic viscosity values of the samples are listed. These values increase as r approaches unity, in qualitative agreement with eq 1; **VI** shows higher intrinsic values than **V**, thus indicating higher molecular weight values. This conclusion is also supported by GPC and NMR measurements, as later discussed. The better results obtained with sample **VI** are probably due to the larger amount of monomers employed in its synthesis, which lowers the experimental error during weighing procedures.

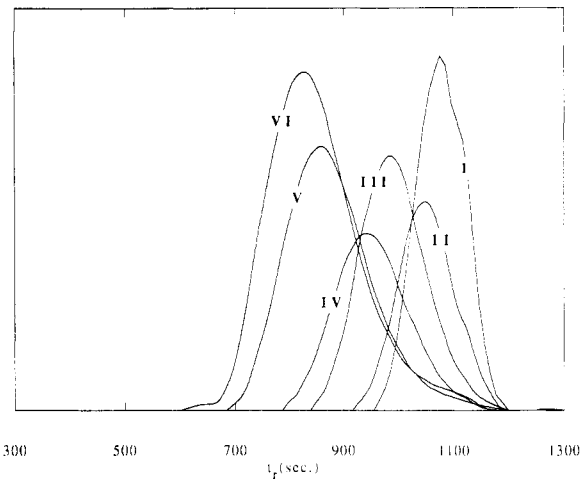


Figure 1. GPC runs of the PAA samples, following the labeling of Table 1.

GPC Characterizations. The GPC chromatograms were run in Tris buffer pH 8.09. Actually, previous studies had shown that lower pH values cause adhesion of PAAs onto the stationary phase, owing to the presence of large percentages of the macromolecules in the ionized form. On the other hand, at higher pH values, PAAs may undergo fast degradation.[16] The observed retention times turned out to be unaffected by moderate changes in the concentration of the solution injected, thus further confirming that the mobile phase chosen for this work is able to eliminate the polyelectrolyte effect in PAAs.

GPC chromatograms of PAAs from **I** to **VI** are shown in Figure 1: they move toward lower retention times, that is toward higher molecular weight regions, and become broader passing from **I** to **VI**. This is in agreement with the conventional theory of stepwise polyaddition for which, when the monomer ratio r approaches unity, the molecular weight tends toward an infinitive value and molecular weight distribution broadens.

NMR Characterizations. The NMR characterization was essentially carried out in two steps. During the first one, indicated as (*a*), the assignments of the resonances were performed by applying 1D and 2D spectroscopies to hydrogen-1 and carbon-13 nuclei. As a second step (*b*), signals and experiments were chosen in order to quantify the content of specific structures, such as chain ends, defects, or further reactions happening on the polymerized species.

As for the step *a*, the ^1H homocorrelated spectra, hereafter also indicated as COSY spectra, were performed on a high-viscosity and a low-viscosity polymer obtained by performing the polymerization with excess 2-methylpiperazine, in order to identify the connectivity of the chain signals and of the chain-end signals. The monodimensional ^1H spectrum is reported in the same scale (Figure 2). This experiment, in the version here adopted (see Experimental Section) shows cross peaks when hydrogens are directly coupled one another.

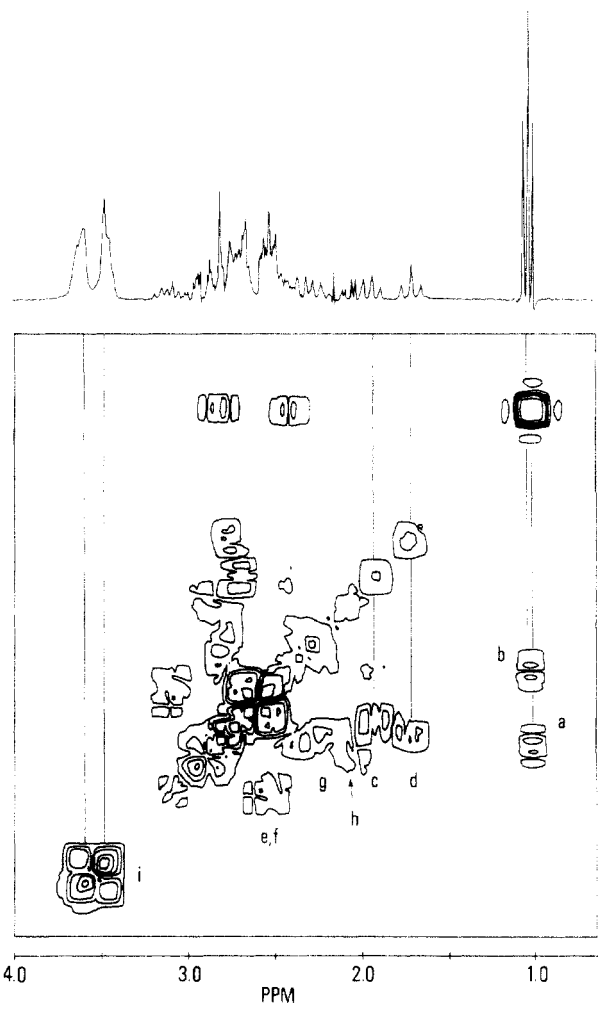


Figure 2. ^{1}H homocorrelated spectrum of the PAA samples, as recorded at 200 MHz. The 1D spectrum is reported in the same scale.

By just some basic assumptions on the chemical shifts of groups of signals and by comparison to the monomer spectra and reference spectra run in one and two dimensions (not shown), the full assignment of the ^{1}H spectra could be achieved, as reported in Table 2 and referred to in Chart 1. Therefore, the ^{1}H spectrum contains detailed information on the chemical structure, with the exception of the region at 2.5–3 ppm, where several resonances due to different groups overlap. Groups of signals, labeled by capital letters (Table 2, Chart 1), are clearly distinguished one from the other because of the interruption of the coupling between hydrogens, due to a few structural features, such as amide and amine functions.

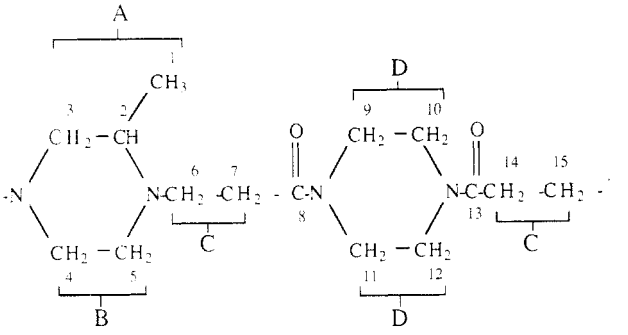
Only some of the cross peaks in the COSY spectra are present in the higher molecular weight polymers, indicating that the ones appearing in the oligomers are due to the chain-end monomer units. Particularly remarkable are the cross peaks indicating the connection between the methyl signal at about 1 ppm and methine signals at 2.40 and 2.85 ppm. The former is due to inner monomer units of the chain and the latter of the monomer units of the chain ends. Therefore, the methyl region is composed of two partly overlapped doublets, produced by the coupling to the methine carbons. They show a J coupling of 20 Hz. Thus, the shift, accidentally identical to the value of the coupling constant, gives as a result of pseudotriplet. The decreasing intensity of the high-field signal, observed in samples of increased viscosity, confirms the assignments (Figure 3).

Table 2. Hydrogen Spectrum Assignments, As Derived by 2D COSY[a]

2D COSY correlations	chemical shift	multiplicity	assignments
	0.86–0.89	doublet	$1A_m$
a	1.00–1.04	doublet	$1A_t$
b	1.04–1.07	doublet	$1A_p$
	1.57	broad singlet	NH_m
d	1.72	triplet	$6C_t$, $15C_t$
c	1.94	triplet	$6C_p$, $15C_p$
g	2.08	doublet of doublet	$4B_t$, $5B_t$
g	2.30	doublet of doublet	$4B_p$, $5B_p$
h	2.22	doublet of doublet	$4B_m$, $5B_m$
b	2.40	complex	$2A_p$
e, f	2.50–2.60	complex	$7C_p$, $14C_p$, $7C_t$, $14C_t$
c	2.65	complex	$7C_p$, $14C_p$
f	2.70	complex	$6C_p$, $15C_p$, $6C_t$, $15C_t$
d + g	2.75	complex	$6C_t$, $15C_t$, $4B_t$, $5B_t$
h + f	2.80–2.85	complex	6Cp, $15C_p$, $4B_p$, $5B_p$
a	2.85	complex	$2A_t$
a	2.95	complex	$3A_t$
g	2.90	complex	$4B_t$, $5B_t$
e	3.20	complex	$3A_p$
i	3.45	broad	$9–12D_p$
i	3.60	broad	$9–12D_p$

[a] The lower case letters in the first column indicate a correlated group of hydrogens. Capital letters indicate groups of hydrogens (see Scheme 3). Lower case letters mean, respectively, m = monomer, t = terminal group, p = polymer. Chemical shifts (CSs) are given in ppm units from TMS. The CSs of the monomer are not reported when heavily overlapped with the polymer signals.

Chart 1. Labeling of Carbon and Hydrogen Atoms of PAA[a]



[a] The groups of coupled hydrogens are labeled by capital letters. See Table 2.

Therefore, the outer signals of this region can be assigned to the polymer chain and to the chain end and they constitute a first source for quantitative measurement of the species. If the spectrum is recorded in D_2O as a solvent (not shown), the chain-end doublet shifts downfield, thus producing a complete separation of the signals in the region.

By a similar procedure, i.e. the interpretation of the COSY spectrum and signal intensity for different samples, the signals at 1.94 and 1.72 ppm were interpreted as triplets due to inner monomer units and chain ends, respectively; they are observed by methylenes 6 and 15, belonging to the group indicated as "C" (Table 3).

The ^{13}C-decoupled NMR spectrum presents a number of major signals for samples **I–VI**, consistent with the structure and symmetry of Chart 1. Minor signals appear in the low molecular weight samples and are assigned to the end monomer units.

By the ^{13}C—^{1}H correlated spectrum the assignments already discussed for the ^{1}H spectrum could be transferred to the carbon dimension (Figure 4). The chemical shifts and assignments are given in Table 3. The carbon spectrum is, however, less suitable to a quantitative

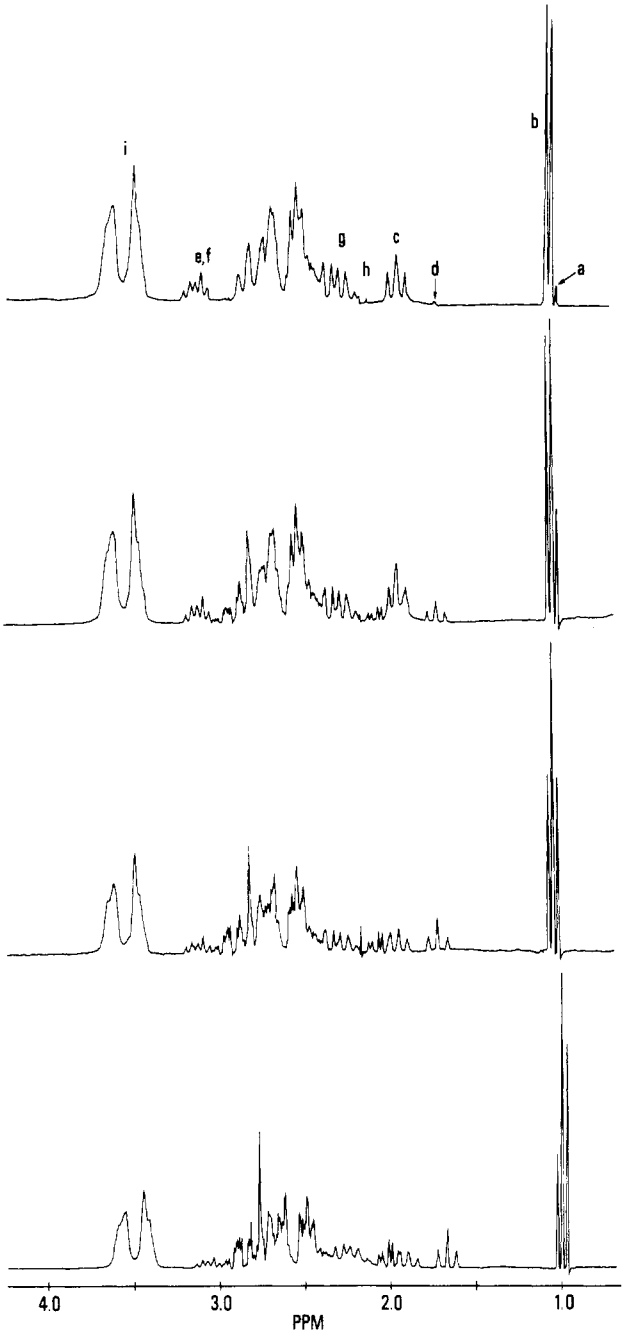


Figure 3. ^1H spectra of the PAA samples, as recorded at 200 MHz (respectively, from the bottom: I, II, III, V of Table 1).

Table 3. ^{13}C Assignments by ^1H—^{13}C Heterocorrelated Spectrum[a]

2D ^{13}C–^1H	chemical shift	assignments
a	16.9	$1A_p$
b	19.8	$1A_t$
c	30.4	$7C_p$, $7C_t$
d	30.8	$14C_p$, $14C_t$
e	41.5	$9–12D_p$
f	45.4	$9–12D_p$
g	45.8	$3A_t$
h	49.2	$3A_p$
i	50.5	$2A_t$
	51.6	$2A_p$
j	53.4	$5B_p$
k	53.7	$5B_t$
l	53.8	$4B_p$
m	54.3	$4B_t$
n	54.8	$6C_p$, $6C_t$
o	60.9	$15C_p$
p	61.5	$15C_t$
	170.5	C=O
	170.8	C=O

[a] For labeling, see Chart 1 and Table 2.

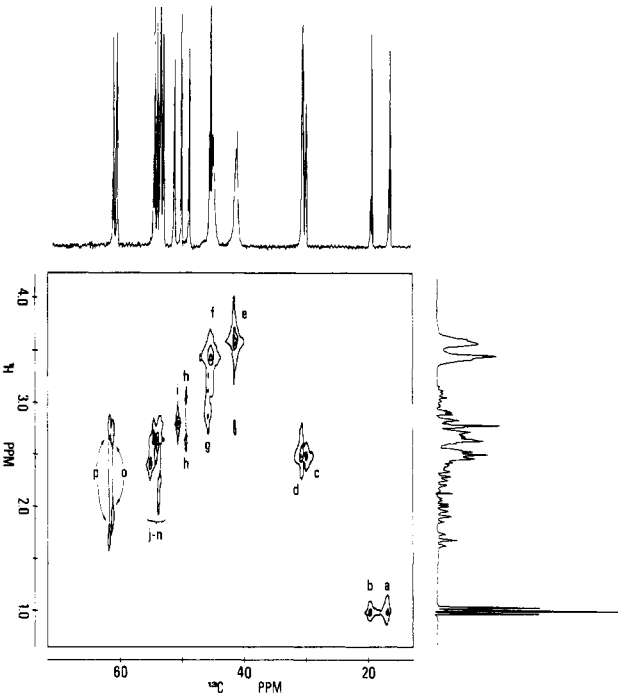


Figure 4. ^1H—^{13}C heterocorrelated spectrum of the PAA samples, as recorded at 200 MHz. The reference monodimensional spectra are reported aside for comparison.

evaluation, due to longer relaxation times of carbon atoms, as compared to hydrogen atoms.

In the carbon spectra also ^{13}C=O signals can be detected at about 170 ppm, but obviously they are not present in the heterocorrelated spectrum, because no hydrogens are directly connected to that group. The splitting can be reasonably due to the sensitivity of the C=O in the amide group to the regioisomeric approach of the 2-methylpiperazine unit.

Due to the chain continuity principle, the number of head-to-head and tail-to-tail insertions must be balanced: in fact two equally intense signals were detected. The peaks, at 41.5 and 45.4 ppm, group D, are apparently wide, being affected by nitrogen quadropolar interaction, as also occurs in the hydrogen dimension.

The quantitative estimate of the chain ends versus the inner chain signals (step *b*) was essentially based on the ^1H NMR spectra. In particular, the ratio derived from methyls at about 1 ppm (two partly overlapped doublets; see Table 2 and Figure 3) was averaged to the ratio taken by the methylene triplets at 2.45 and 2.90 ppm (not overlapped).

On the other hand a sample obtained by excess 1,4-bis(acryloyl)piperazine was characterized by hydrogen and carbon resonances in the region of the double bonds. No evidence for end groups of this kind was gathered in samples **I–VI**. Carboxylic groups, which could derive from incomplete reaction and subsequent hydrolysis in the basic medium, were not identified, as well.

These data lead to the reasonable conclusion that the polycondensation reaction occurs by the above mentioned path to high conversions, without any degradation reaction.

We cannot give any evidence for the presence of large cyclic structures, since the chemical shift remains substantially unchanged as compared to that for the infinite length polymer. The presence of short cyclic structures could, however, be detected since the average conformation is affected in this case by some strain, thus leading to a

different chemical shift. This fact was demonstrated during the NMR characterization of other polycondensation polymers.[21] No evidence for short cyclic structures was collected. On the other hand, short cycles should be improbable, due to the relative stiffness of the monomer structures. Also, no evidence for possible monomer excess left in the polymer could be obtained.

Molecular Weight Determination by NMR. In order to estimate the number-average molecular weight $\bar{M}_{n,exp}$ of samples **I–V**, which are end-capped with 2-methylpiperazine units, we first considered the ratio of methyl hydrogens of inner 2-methylpiperazine units (indicated as 1Ap in Table 2) to those of terminal units ($1A_t$). The signals attributable to either units are partially overlapped doublets, which give a pseudotriplet. Therefore, we decided to make our calculations considering the ratio R between their external peaks, which do not overlap. We expected in fact this ratio to be equal to that of the overall doublets, since $1A_p$ and $1A_t$ couple with hydrogens having a large difference in chemical shift compared to their coupling constants and, therefore, the relative height of their single peaks is the same as the overall signal. The validity of this assumption was also confirmed by recording the spectra in D_2O, where the doublets are completely separated.

Let $\bar{N}_{2MP}$ and $\bar{N}_{BAP}$ indicate the average number per chain of 2-methylpiperazine and 1,4-bis(acryloyl)piperazine units, respectively. Then

$$\bar{M}_{n,exp} = \bar{N}_{2MP}M_{2MP} + \bar{N}_{BAP}M_{BAP} \tag{2}$$

where M_{2MP} and M_{BAP} are the molecular mass of 2-methylpiperazine and 1,4-bis(acryloyl)piperazine, respectively.

Since we are dealing with samples end-capped with amine units

$$\bar{N}_{2MP} = \frac{\overline{DP}_n + 1}{2} \tag{3a}$$

$$\bar{N}_{BAP} = \frac{\overline{DP}_n - 1}{2} \tag{3b}$$

On the other hand the number of inner 2-methylpiperazine goups, $\bar{N}_{2MP,i}$, is

$$\bar{N}_{2MP,i} = \bar{N}_{2MP} - 2 = \frac{\overline{DP}_n - 3}{2} \tag{4}$$

Therefore the ratio R can be easily calculated, by taking into account that there are three hydrogens per methyl group, as

$$R = \frac{\bar{N}_{2MP,i} \times 3}{2 \times 3} = \frac{\bar{N}_{2MP,i}}{2} = \frac{\overline{DP}_n - 3}{4} \tag{5}$$

That is

$$\overline{DP}_n = 4R + 3 \tag{6}$$

Substitution of (6) into (3a) and (3b) leads to

$$\bar{N}_{2MP} = 2R + 2 \tag{7a}$$

$$\bar{N}_{BAP} = 2R + 1 \tag{7b}$$

Finally, substitution of (7a) and (7b) into (2) gives

$$\bar{M}_{n,exp} = (2R + 2)M_{2MP} + (2R + 1)M_{BAP} \tag{8}$$

Table 4. Comparison between Calculated and Experimental (NMR) Molecular Masses of the PAA Samples

sample	R^a	$\bar{M}_{n,exp}{}^b$	$\bar{M}_{n,calc}{}^c$	% δ^d
I	0.958	960	940	−1.8
II	1.41	1225	1200	−2.0
III	2.90	2100	2150	2.5
IV	4.21	2870	3090	7.8
V	10.9	6810	6280	−7.8

[a] Ratio of $1A_p$ to $1A_t$ hydrogen areas (see Scheme 3 and Table 2). [b] Experimental (NMR) number-average molecular weight, calculated using eq 8. [c] Calculated number-average molecular weight, obtained from eq 19 (a = 18.561 and b = −0.0108, as determined from the fitting procedure). [d] δ % = $[(\bar{M}_{n,calc}-\bar{M}_{n,exp})/[\bar{M}_{n,exp}] \times 100$.

The values of the experimental ratios R and of the corresponding number-average molecular weights calculated according to (8) are listed in Table 4, columns 2 and 3. The above results clearly demonstrate that the molecular mass depends on the stoichiometric ratio, roughly following the conventional theory on stepwise polymerization.

GPC Calibration Making Use of NMR Data. The following iterative procedure was developed. Since the GPC chromatograms were obtained using a UV detector operating at 230 nm, we had to take into account that the absorbance of polymer chains depends on their molecular weight. In fact, if one considers equal amounts of chains having different molecular weights, the number of carbonyl groups (the only groups absorbing at this wavelength) is lower for lower molecular weight chains, which, therefore, show a smaller absorbance. This aspect was particularly relevant in our calculations, as we considered PAA samples having moderate molecular weights; thus we did not suppose that the height $h_i^{(k)}$ of the chromatogram k at time $t_i^{(k)}$ was simply proportional to the amount $w_i^{(k)}$ of the chains with this retention time, but that $h_i^{(k)}$ was proportional to the number $N_i^{(k)}$ of carbonyl groups contained in the fraction $w_i^{(k)}$:

$$h_i^{(k)} = kN_i^{(k)} \tag{9}$$

In turn, since a chain having degree of polymerization $DP_{n,i}^{(k)}$ has $DP_{n,i}^{(k)} - 1$ carbonyl groups, $N_i^{(k)}$ reads

$$N_i^{(k)} = n_i^{(k)}(DP_{n,i}^{(k)} - 1) \tag{10}$$

$n_i^{(k)}$ being the number of chains with retention time $t_i^{(k)}$. On the other hand the number-average molecular weight of sample k, $\bar{M}_{n,calc}^{(k)}$, is defined as follows:

$$\bar{M}_{n,calc}^{(k)} = \sum_i n_i^{(k)}M_i^{(k)} \Big/ \sum_i n_i^{(k)} \tag{11}$$

Combination of (9) and (10) gives

$$n_i^{(k)} = h_i^{(k)}/k(DP_{n,i}^{(k)} - 1) \tag{12}$$

while substitution of $n_i^{(k)}$ into (11) leads to

$$\bar{M}_{n,calc}^{(k)} = \sum_i \frac{h_i^{(k)}}{DP_{n,i}^{(k)} - 1} M_i^{(k)} \Big/ \sum_i \frac{h_i^{(k)}}{DP_{n,i}^{(k)} - 1} \tag{13}$$

Since the samples involved are end-capped with 2-methylpiperazine

$$M_i^{(k)} = \frac{DP_{n,i}^{(k)} - 1}{2} M_{BAP} + \frac{DP_{n,i}^{(k)} + 1}{2} M_{2MP} \quad (14)$$

and therefore

$$DP_{n,i}^{(k)} - 1 = \frac{2(M_i^{(k)} - M_{2MP})}{M_{2MP} + M_{BAP}} \quad (15)$$

Substitution of (15) into (13) gives

$$\bar{M}_{n,calc}^{(k)} = \sum_i \frac{h_i^{(k)}}{M_i^{(k)} - M_{2MP}} M_i^{(k)} \Big/ \sum_i \frac{h_i^{(k)}}{M_i^{(k)} - M_{2MP}} \quad (16)$$

So far no mention has been made of the expression of $M_i^{(k)}$. In the following treatment the simplest and most widely used equation relating molecular weight to GPC retention time is adopted,[22] i.e.:

$$\ln M_i^{(k)} = a + bt_i^{(k)} \quad (17)$$

which is equivalent to

$$M_i^{(k)} = \exp[a + bt_i^{(k)}] \quad (18)$$

Substitution of $M_i^{(k)}$ into (16) finally leads to

$$\bar{M}_{n,calc}^{(k)} = \sum_i \frac{h_i^{(k)} \exp[a + bt_i^{(k)}]}{\exp[a + bt_i^{(k)}] - M_{2MP}} \Big/ \sum_i \frac{h_i^{(k)}}{\exp[a + bt_i^{(k)}] - M_{2MP}} \quad (19)$$

The only unknown quantities, in this relation, are the a and b parameters, which can be determined by attaining the best fit of $\bar{M}_{n,calc}^{(k)}$ with experimental values, $\bar{M}_{n,exp}^{(k)}$, obtained, in turn, by NMR data. More precisely, a and b are determined by minimizing the percentage mean square error ϵ, defined as

$$\epsilon = \left(\frac{\sum_k \left(\frac{\bar{M}_{n,calc}^{(k)} - \bar{M}_{n,exp}^{(k)}}{\bar{M}_{n,exp}^{(k)}} \right)^2}{N} \right)^{1/2} \times 100 \quad (20)$$

N being the number of samples examined.

In order to perform this procedure the following initial data are required. Firstly, $t_i^{(k)}$ and $h_i^{(k)}$ pairs for every sample k are derived by choosing a set of equally spaced retention times $t_i^{(k)}$ and reading on the corresponding chromatogram the height $h_i^{(k)}$. Secondly, initial values a_0 and b_0 of parameters a and b are needed; reasonable values are obtained by assuming that a semilogarithmic relation holds between $\bar{M}_{n,exp}^{(k)}$ and the retention times $t_{max}^{(k)}$ of the peak maximums:

$$\ln \bar{M}_{n,exp}^{(k)} = a_0 + b_0 t_{max}^{(k)} \quad (21)$$

Then a_0 and b_0 values are determined by means of the least squares method.

At this point one can calculate initial $\bar{M}_{n,calc}^{(k)}$ and ϵ values, using eqs 19 and 20, respectively. The correct values of a and b are then found iteratively so that ϵ is a minimum.

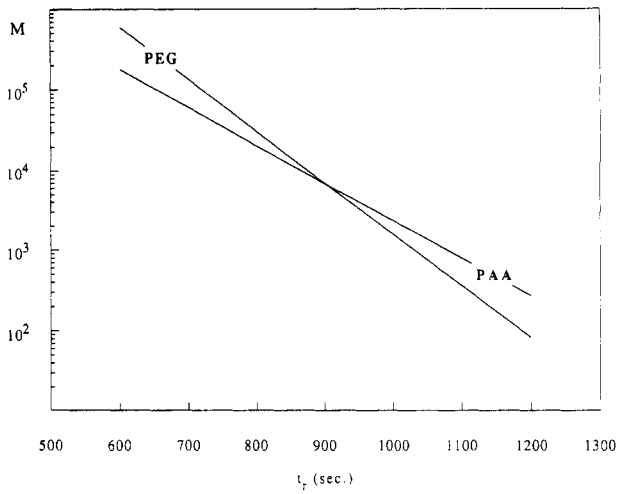


Figure 5. GPC calibration curve determined for PAA (see eq 22), in comparison with that relative to monodisperse standard PEGs.

We applied the above procedure only to samples **I**–**V**, as in the case of **VI** we could not obtain a reliable NMR molecular weight value, owing to its high molecular weight. The retention times selected were spaced by 15 s. This involved using a number of points ranging from 20 for sample **I** to 37 for **V**. Initial values of a_0 and b_0, calculated by means of eq 21, were 16.313 and 0.0088, respectively. After the minimization procedure we finally obtained the following calibration curve:

$$\ln M_i = 18.561 - 0.0108 t_i \quad (22)$$

where retention times t_i are expressed in seconds and superscript (k) has been removed since (22) is independent of the sample considered. For comparison puposes, the graph relative to this equation is shown in Figure 5, together with the calibration curve determined under the same experimental conditions from *monodisperse* standard PEGs.

The reliability of eq 22 emerges from Table 4, columns 3–5, where the calculated values of the number-average molecular weight are compared with the experimental ones, to which they are fairly close. The percentage difference % δ is in no case more than 8%, a value comparable to the sensitivity of the NMR experiment.

Thus far it was possible to calculate other parameters, such as weight-average molecular weights $\bar{M}_{w,calc}^{(k)}$, starting from the known expression

$$\bar{M}_{w,calc}^{(k)} = \sum_i n_i^{(k)} [M_i^{(k)}]^2 \Big/ \sum_i n_i^{(k)} M_i^{(k)} \quad (23)$$

where $n_i^{(k)}$ is given by eq 12. The same set of $t_i^{(k)}$, $h_i^{(k)}$ pairs as for the corresponding $\bar{M}_{n,calc}^{(k)}$ determination was employed, as well as the values of the parameters a and b obtained by the minimization procedure.

The polydispersity index $d_{calc}^{(k)}$ of each sample was also determined, according to

$$d_{calc}^{(k)} = \bar{M}_{w,calc}^{(k)} / \bar{M}_{n,calc}^{(k)} \quad (24)$$

The single calculated values are reported in Table 5, columns 2 and 3. They increase as r approaches unit, up to 2.07 for sample **V**; this involves broadening of the respective molecular weight distributions, as GPC chromatograms also clearly show (see Figure 1). This result is in agreement with the theory on stepwise polymerization,

Table 5. Molecular Masses and Polydispersity of the PAA Samples

sample	$\bar{M}_{w,calc}$[a]	d_{calc}[b]
I	1160	1.23
II	1590	1.32
III	3170	1.47
IV	5060	1.64
V	12990	2.07

[a] Weight-average molecular weight, calculated from eq 23. [b] Polydispersity (from eq 24).

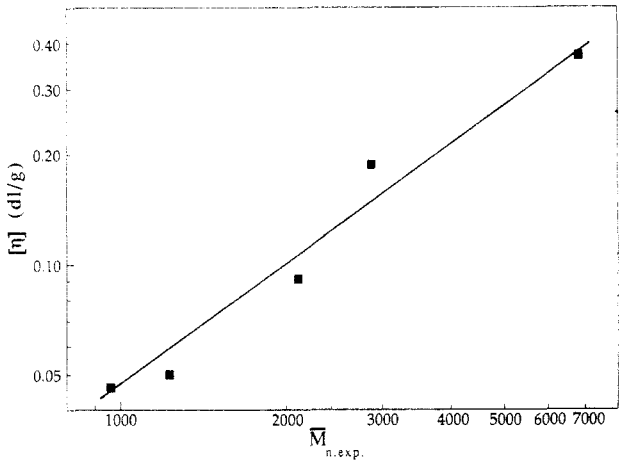


Figure 6. Intrinsic viscosity (measured at 20 °C in Tris buffer pH 8.09) versus number-average molecular weight.

which, in particular, predicts a value of 2 for the polydispersity index of polymers obtained by starting from an equimolecular ratio of monomers.

Determination of the Mark–Houwink Constants. The Mark–Houwink constants κ and α were calculated by supposing that

$$[\eta] = \kappa(\bar{M}_{n,exp})^{\alpha} \quad (25)$$

and plotting $[\eta]$ versus $\bar{M}_{n,exp}$ (Figure 6). Then, by a least squares fitting, $\kappa = 1.76 \times 10^{-5}$ and $\alpha = 1.14$ were obtained. Owing to the low intrinsic viscosity of most of our samples, the reported values may not be very precise; nevertheless, in our opinion, the high value of α is not unreasonable, as this PAA contains two rings per repeating unit in its main chain and, therefore, it is likely to assume an extended chain conformation in solution.

Conclusions

From the results reported in this paper, it is possible to obtain a careful characterization of a set of PAA samples by hydrogen and carbon-13, high-resolution NMR and bidimensional spectra. Particular attention was paid to the end group functionalities in order to perform an absolute molecular mass determination. As expected on the basis of Flory's theory on stepwise polymerization, the obtained values increase with the molar ratio r of the monomers. Furthermore, no evidence for chain end groups other than those predictable on the basis of the r values was gathered, thus suggesting that no degradative process was active to a significant extent under the adopted experimental conditions.

By combining NMR molecular mass determinations with the GPC data, a calibration curve was derived. Excellent agreement between experimental and calculated $\bar{M}_n$ values was obtained by a simple semilogarithmic function. In addition, from the calibration curve it was possible to calculate the polydispersity index for every sample. Their values increase with r up to 2.07 for the sample prepared starting from an equimolar ratio of the monomers, a value consistent with that expected from the theory.

It is worthwhile to stress that the mathematical treatment applied in this paper involves a nonlinear least squares procedure, which is generally valid in the case of two or more polydisperse polymer samples. Furthermore, the proposed procedure takes into account the length of the polymeric chains, so that it can be reasonably expected to be reliable also when low molecular weight polymers are considered.

Further work on other PAAs, as well as on the determination of the absolute degradation rate of PAAs themselves in relation with their molecular structure, is presently in progress and will be published in forthcoming papers.

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