High Molecular Weight Hydrophilic Functional Copolymers by Free-Radical Copolymerization of Acrylamide and of *N*-Acryloylmorpholine with *N*-Acryloxysuccinimide: Application to the Synthesis of a Graft Copolymer

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ABSTRACT: Free-radical solution copolymerization of acrylamide (AAm) and of a disubstituted acrylamide derivative, *N*-acryloylmorpholine (NAM), with N-acryloxysuccinimide (NAS) was investigated with the aim to obtain a copolymer of at least 100,000 g mol^{-1}. Different polymerization conditions likely to increase the molecular weight were studied such as monomer and initiator concentrations, temperature, and nature of the solvent. The molecular weights were determined by SEC using a light-scattering detector. The grafting of end-functionalized polysaccharide chains onto such high molecular weight poly(NAM-*co*-NAS) was performed and a graft copolymer bearing a high number of saccharidic branches was obtained. © 2003 Wiley Periodicals, Inc. J Appl Polym Sci 88: 1808–1816, 2003

Key words: hydrophilic polymers; radical polymerization; polysaccharides; graft copolymers; gel permeation chromatography (GPC)

INTRODUCTION

Water-soluble acrylamide-based copolymers proved to be very useful in a wide range of applications, from industrial and environmental fields (as flocculating agents)[1] to biological and pharmaceutical fields (as gel or membrane).[2] The nature of the comonomer usually determines the type of application. For instance, copolymers of acrylamide (AAm) with ionizable comonomers either anionic (like acrylic acid[3,4]) or cationic [like (dimethylamino)ethyl methacrylate[5]] provide material which may act as efficient water-purification agents. Moreover, copolymers of AAm with reactive monomers (like activated ester derivatives) may serve as convenient supports for immobilization of various specific side groups.[6]

Among the monomers bearing an activated ester function, *N*-acryloxysuccinimide (NAS) is often used due to its high reactivity and specificity to covalently bind biomolecules like proteins[7] or DNA fragments.[8] It has been copolymerized with AAm to produce a gel suitable for enzyme immobilization.[9] Alternatively, monosubstituted AAm derivatives, such as *N*-isopropylacrylamide (NIPAM), have also been copolymerized with NAS to synthesize thermosensitive copolymers for the immobilization of fluorescent compounds,[10] monoclonal antibodies,[11,12] and immunoglobulins.[13] Moreover, hydrophilic disubstituted AAm derivatives, such as *N*-acryloylmorpholine (NAM), have been copolymerized with NAS, leading to copolymers which, for instance, provide steric protection to enzymes.[14]

The latter monomer, NAM, presents several outstanding features[15–19]: a solubility in a wide range of solvents, from aqueous solutions to organic ones (chloroform, tetrahydrofuran, dioxane), an ability to give high molecular weight polymers with a virtual lack of toxicity, and being themselves soluble both in polar or low-polar solvents. NAM was used for many years to synthesize crosslinked networks for gel-phase synthesis of peptides,[20–23] semipermeable membrane for plasma separation,[24] and polymeric supports for gel chromatography[25] and capillary electrophoresis.[2] Linear poly(NAM) was also used for drug-delivery applications, protecting liposomes from blood clearance, or increasing the lifetime of enzymes both *in vitro* and *in vivo*.[14]

As part of a program to obtain water-soluble multifunctional polymers for the immobilization of single-stranded DNA probes, this work aimed at reporting the synthesis of copolymers of AAm or NAM with NAS, poly(AAm-*co*-NAS) and poly(NAM-*co*-NAS), respectively. Most of the studies previously related to these comonomer pairs were concerned with relatively short copolymer chains, that is, ranging from

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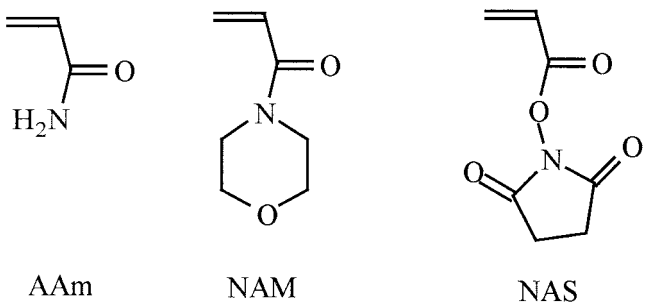


AAm NAM NAS

Scheme 1

6000 to 30,000 g mol^{-1}.[9,14,26–29] An article by Ferutti et al.[30] claimed the preparation of high molecular weight polymers of NAM with acrylic and methacrylic esters of *N*-hydroxysuccimide but without precise molecular weight data. In view of obtaining polymer chains offering many potential immobilization sites while remaining highly water-soluble (i.e., consisting of a low percentage of reactive comonomer), the synthesis of high molecular weight copolymers was a prerequisite. For that purpose, the influence of various polymerization conditions was investigated so as to obtain polymer chains of at least 100,000 g mol^{-1}.

As an illustration, such a reactive copolymer was further used for the grafting of short saccharidic chains [a galactose-substituted vinyl ether homopolymer,[31] poly(GVE)–NH_2)]. In fact, these chains had previously been end-functionalized by an amino group, allowing their covalent binding onto the NAS residues along the poly(NAM-*co*-NAS) reactive backbone. The efficiency of the binding reaction was determined through the characterization of the obtained graft copolymer by SEC analysis.

EXPERIMENTAL

Materials

AAm (99% from Merck) was purified by recrystallization in chloroform and stored at −20°C without an inhibitor. NAM (99% from Polysciences, Inc.) was purified by distillation under reduced pressure. NAS was synthesized via straightforward chemistry[9] from distilled acryloyl chloride (97% from Aldrich) and *N*-hydroxysuccinimide (NHS, 97% from Aldrich). Azobisisobutyronitrile (AIBN, 98% from Merck), used as a polymerization initiator, was purified by recrystallization in ethanol. Dimethylformamide (DMF, 99.8% from Aldrich), toluene (99.5% from Merck), and *N*-methylpyrrolidone (99.5% from SDS) were used as received. Dioxane (99.8% from Aldrich) and acetonitrile (99.0% from Acros) were distilled. The chemical structure of the three monomers is described in Scheme 1.

Polymerization procedure

Polymerization experiments were performed in a three-necked round-bottomed flask equipped with a condenser, a magnetic stirrer, and a nitrogen inlet. The reaction vessel was loaded with the solvent and the comonomer mixture (Table I) and purged with nitrogen for 1 h at 20°C. Then, the temperature was increased to 60°C using a thermostated oil bath. Finally, the initiator, preliminary dissolved in the purged solvent, was added to the reaction mixture (zero time), and the copolymerization was carried out under a nitrogen atmosphere. After 2–4 h, the polymerization mixture was placed at −20°C in the presence of hydroquinone traces to stop the polymerization.

Characterization of the copolymers

Copolymers were precipitated in a large volume of ethylic ether, recovered by filtration, washed several times with the same solvent, and finally dried under vacuum to a constant weight. The complete elimination of residual monomers was checked by ^{1}H-NMR analysis.

TABLE I
Free-radical Copolymerization of AAm or NAM with NAS

REF	[*M*] (mol L^{-1})	[*I*]/[*M*] (%)	*T* (°C)	Solvent	Polymerization period (h)	NAS comonomer
FD2	1.00	1	60	DMF	4	AAm
FD3	0.80	1	60	DMF	4	AAm
FD20	0.65	1	60	DMF	4	AAm
FD5	1.00	0.5	60	DMF	2	AAm
FD6	1.00	0.5	60	Acetonitrile	2	AAm
FD7	1.00	0.5	60	*N*-methylpyrrolidone	2	AAm
FD19	1.00	1	55	DMF	4	AAm
FD21	1.00	1	50	DMF	4	AAm
FD14	1.00	0.5	60	DMF	2	NAM
FD15	1.00	0.5	60	Toluene	4	NAM
FD16	1.00	0.5	60	Dioxane	2	NAM

V = 5 mL, AAm /NAS and NAM/NAS molar ratio in the comonomer feed = 80/20.

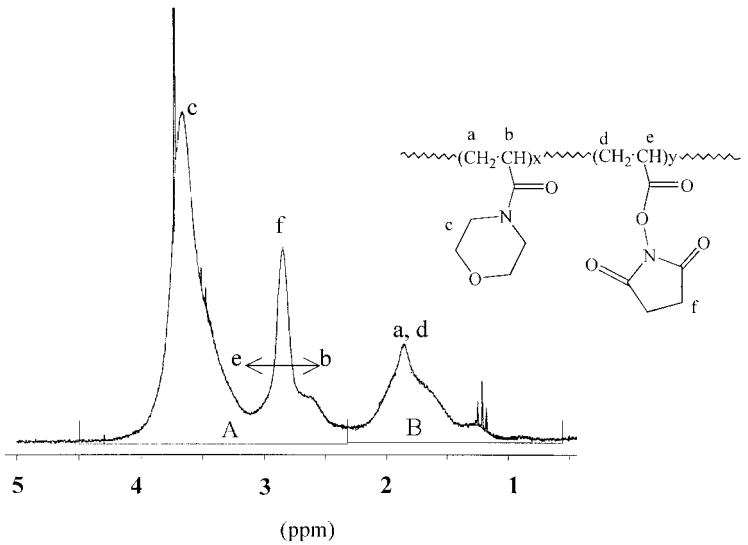


Figure 1 ^{1}H-NMR spectrum (200 MHz) of poly(NAM-*co*-NAS) in $CDCl_3$.

Molecular weights and molecular weight distributions (MWDs)

Molecular weights and MWDs were determined with a light-scattering apparatus [three-angle laser light-scattering photometer (TALLS) from Wyatt Technologies] associated with a differential refractometer (DRI Waters 410), as an on-line double detection for size-exclusion chromatography (SEC) analysis. Analyzes were performed by injection of 100 μL of the polymer solution ($C = 5 \times 10^{-3}$ g mL^{-1}) in an injection loop connected to a Waters 510 pump and Waters Ultrahydrogel 2000 and 500 columns. The eluent used was a borate buffer, 0.05 mol L^{-1} (pH 9.3), at a flow rate of 0.5 mL min^{-1}.

The specific refractive index increments (*dn/dC*) of poly(AAm-*co*-NAS) and poly(NAM-*co*-NAS) were determined with the same eluent, using a Brice Phoenix differential refractometer equipped with a filtered white-light source at 530 nm. In the case of poly(NAM-*co*-NAS) (FD16), the molecular weight was also determined by static light-scattering measurements in DMF with a Brookhaven instrument equipped with a 2-W laser Model 2560 from Spectra Physics (ionized argon) and using a cell dipped in a decaline bath. The specific refractive index increment (*dn/dC*) was determined in DMF using the differential refractometer. Light-scattering data were exploited so as to calculate the weight-average molecular weight, M_w, and the second virial coefficient, A_2.

Average copolymer composition (^{1}H-NMR)

The average copolymer composition was determined from ^{1}H-NMR spectra of the copolymers (Brücker AM 200, 200 MHz). In the case of the poly(NAM-*co*-NAS) copolymer (Fig. 1), the *B* region could be assigned to the superposition of the methylene protons of the main chain, whereas the *A* region included all the other protons, according to the following equations:

$$A = 9\ H_{\mathrm{PNAM}} + 5H_{\mathrm{PNAS}}$$

$$B = 2\ H_{\mathrm{PNAM}} + 2H_{\mathrm{PNAS}}$$

where H_{PNAM} and H_{PNAS} represent one proton stemming, respectively, from the NAM and the NAS units in the copolymer. *A* and *B* values were determined by planimetry of the corresponding areas.

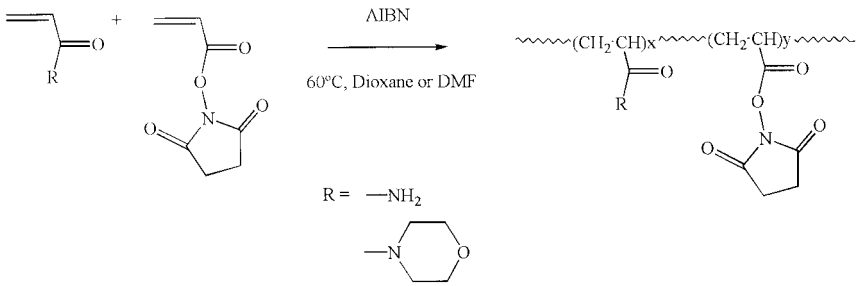
Grafting of side chains onto a poly(NAM-*co*-NAS) copolymer backbone

Saccharidic side chains, poly(GVE)–NH_2 (6000 g mol^{-1}, 120 mg, 2×10^{-5} mol), and backbone chains, poly(NAM-*co*-NAS) (60/40 NAM/NAS molar ratio, 121,000 g mol^{-1}, 23 mg, 1.9×10^{-7} mol), were dissolved in DMF (2 mL). The mixture was maintained under vigorous stirring at 40°C (thermomixer) for 5 days. The raw product, isolated after evaporation of the solvent, was analyzed by SEC with DMF as the eluent (Polymer Laboratories PLgel Mixed-D column) and with PS standards for the calibration (Waters 410 refractometric detector).

RESULTS AND DISCUSSION

Synthesis of the copolymers

Free-radical copolymerization of AAm and of the disubstituted AAm derivative, NAM, with NAS was performed in a homogeneous solution using AIBN as the initiator (Scheme 2). As explained in the Introduction,



Scheme 2

one of our main goals was to synthesize a long-chain copolymer of at least 100,000 g mol^{-1}. Moreover, taking into account that the AAm/NAS and NAM/NAS comonomer pairs copolymerize in a statistical way[9,32] and to favor the spacing out of the activated ester functions along the chain, the initial comonomer mixture was chosen equal to an 80/20 molar ratio.

According to the definition of the kinetic chain length, ν, many parameters would *a priori* play a role for controlling the molecular weight. In fact, the kinetic chain length can be expressed as follows[33]:

$$\nu = \frac{k_p[M]_T}{(2k_d f k_t[I])^{1/2}} \quad (1)$$

with $[M]_T$ the total monomer concentration, $[I]$ the initiator concentration, k_t the termination rate constant, k_d the initiator decomposition rate constant, k_p the propagation rate constant, and f the initiator efficiency.

To ensure increase of the molecular weights, the influence of the following parameters was studied: (i) the total monomer concentration $[M]_T$, (ii) the initiator concentration $[I]$, (iii) the temperature T, (iv) the nature of the solvent, and (v) the nature of the hydrophilic comonomer. The experimental conditions used for the various copolymerization assays are reported in Table I and the copolymer molecular weights determined by light scattering are compared in Table II.

Influence of $[M]_T$, $[I]$, and T

First, three copolymerization experiments (FD2, FD3, FD20) were carried out with AAm and NAS, solubilized in DMF at 60°C, with a 1.00, 0.80, and 0.65 mol L^{-1} total monomer concentration, respectively (Table I). The initiator concentration was 1% of the total monomer concentration. After 4 h of polymerization, the copolymers were precipitated and analyzed in terms of the number-average molecular weight (M_n), peak molecular weight (M_{peak}), and polydispersity index (I_p). According to Table II, the obtained molecular weights exhibited a trend in agreement with eq. (1), that is, an increase of M_n with increasing $[M]_T$. However, for an $[M]_T$ value equal to 1.00 mol L^{-1}, the M_n value was only 33,100 g mol^{-1}.

Concerning the influence of the initiator concentration, a polymerization assay, FD5, was carried out in the same conditions as was FD2 except that the initiator concentration was divided by two ($[I]$ = 0.5% $[M]_T$). As expected, the molecular weight of the obtained copolymer increased, from 33,100 g mol^{-1} (FD2) to 45,200 g mol^{-1} (FD5) (Table II).

Then, the influence of T was investigated, knowing that a decrease of the temperature should induce a diminution of k_d and, thus, an increase of ν. However, the influence of the temperature on the molecular weights was difficult to prove since the molecular weights corresponding to the polymerization experiments carried out at 55°C (FD19) and at 60°C (FD2) were quite similar (Table II) and since no polymer was recovered at 50°C along with the usual polymerization time (4 h) (FD21).

Considering these first three parameters, the polymerization conditions providing the highest molecular weight copolymers were those corresponding to the FD5 experiment ($[M]_T$ = 1 mol L^{-1}, $[I]$ = 0.5% $[M]_T$, T = 60°C). However, the obtained value (45,200 g mol^{-1}) was still far from the desired 100,000 g

TABLE II
Molecular Weight Characteristics of the Obtained Copolymers

REF	M_n (g mol^{-1})	M_{peak} (g mol^{-1})	I_p
FD2	33,100	37,700	1.5
FD3	27,300	25,500	1.7
FD20	12,090	nd	1.7
FD5	45,200	55,500	1.4
FD6		No polymerization	
FD7	7000	nd	nd
FD19	27,600	32,400	1.8
FD21		No polymerization	
FD14	79,400	89,600	1.6
FD15		No polymerization	
FD16	107,400	133,500	1.6

nd: not determined. SEC–TALLS analyses in borate buffer.

mol^{-1}. Consequently, the influence of both the solvent and the comonomer was then examined.

Influence of the solvent and the comonomer

The relatively low molecular weights could probably be explained by the transfer activity of the solvent, DMF. The range of solvents which could be used was very limited (the solvent should, indeed, solubilize the two monomers, AAm, hydrophilic, and NAS, hydrophobic, as well as the formed copolymer. In addition, it should be inert toward the NAS activated ester function). Other chosen solvents were acetonitrile and *N*-methylpyrrolidone. No polymer was obtained in acetonitrile, and in the case of *N*-methylpyrrolidone, a copolymer with an M_n value of 7000 g mol^{-1} only was formed (FD6 and FD7 experiments, respectively, carried out in the same conditions as FD5).

Then, the influence of the nature of the comonomer was evaluated by substituting AAm by one of its disubstituted derivatives, NAM, in the FD14 assay where all the parameters were kept identical to that of the FD5 experiment. An important increase of the number-average molecular weight (M_n) was observed, from 45,200 g mol^{-1} for poly(AAm-*co*-NAS) (FD5) to 79,400 g mol^{-1} for poly(NAM-*co*-NAS) (FD14), as shown in Table II. If we consider that transfer is only due to the solvent, this result would indicate that a NAM-ended macroradical is less reactive toward the DMF protons than is an AAm-ended macroradical. This molecular weight increase really corresponds to an increase of the average polymerization degree (X_n) and not only to the fact that NAM monomer has a higher molar mass than that of AAm. Considering that the NAS molar content in each copolymer is equal to 20% (quantitative conversion of the comonomers in both cases), such an M_n increase would correspond to an X_n increase from 498 (FD5) to 540 (FD14).

As the NAM/NAS copolymerization system led to a significant M_n increase in comparison with the AAm/NAS system, further study was performed, allowing examination of the influence of various solvents; FD14 was compared to FD15 and FD16 assays, carried out in toluene and dioxane, respectively, instead of DMF. No polymerization was observed in toluene; on the contrary, copolymerization proceeded smoothly in dioxane and the desired molecular weight could be obtained. The jump from 79,400 g mol^{-1} (FD14) to 107,400 g mol^{-1} (FD16) can probably be assigned to a weaker transfer activity of dioxane compared to DMF.

All the obtained copolymers exhibited a polydispersity index ranging between 1.5 and 1.8 (Table II), indicating that, in this system, termination reactions predominantly occurred by combination as generally reported for polyacrylates and polyacrylamides.

Several points were noticed about the SEC analysis:

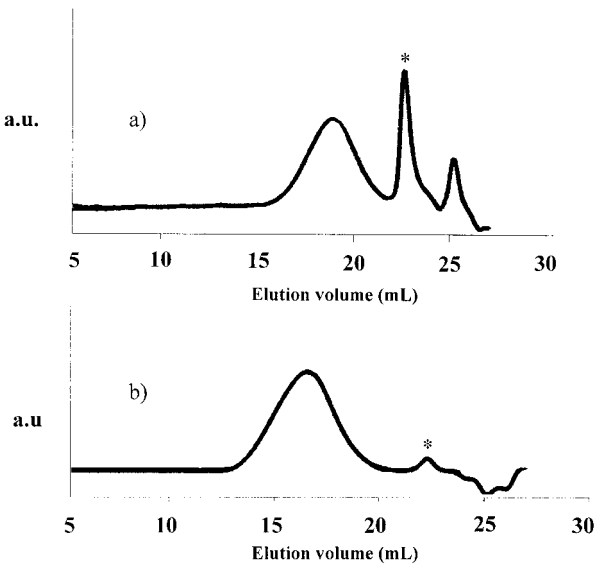


Figure 2 (a) Poly(AAm-*co*-NAS) and (b) poly(NAM-*co*-NAS) refractometric traces by SEC–TALLS.

(i) The molecular weight determination by SEC–TALLS with borate buffer as the eluent previously required the determination of the polymer refractive index increment (*dn/dC*) with the same eluent. The *dn/dC* values for poly(AAm-*co*-NAS) and for poly(NAM-*co*-NAS) were found to be 0.141 and 0.162 mL g^{-1}, respectively.

(ii) NHS is released when the activated ester function is hydrolyzed, leading to an acrylic acid moiety. Such hydrolysis occurred when the polymer was dissolved in the aqueous buffer. To limit this phenomenon as much as possible, all the SEC analyzes were performed immediately after dissolution. Nevertheless, the use of a refractometric detector allowed us to evidence the presence of NHS in some cases.

For poly(AAm-*co*-NAS) copolymers, a NHS peak systematically appeared on the refractometric trace [Fig. 2(a), starred peak], which was indicative of the hydrolysis of some activated ester functions. However, the quantitation of the hydrolysis extent was difficult. In the case of poly(NAM-*co*-NAS) copolymers, the hydrolysis seemed to be very limited compared with the previous case [Fig. 2(b), starred peak], a phenomenon which might be explained by the presence of the morpholine cycles ensuring some steric protection of the activated esters against hydrolysis.

Complementary characterization of the copolymers

With a view to obtain a more complete description of the final materials before performing further grafting

reactions, some of the copolymers were characterized using both static light scattering and ^{1}H-NMR.

Static light-scattering analysis of poly(NAM-*co*-NAS) copolymer

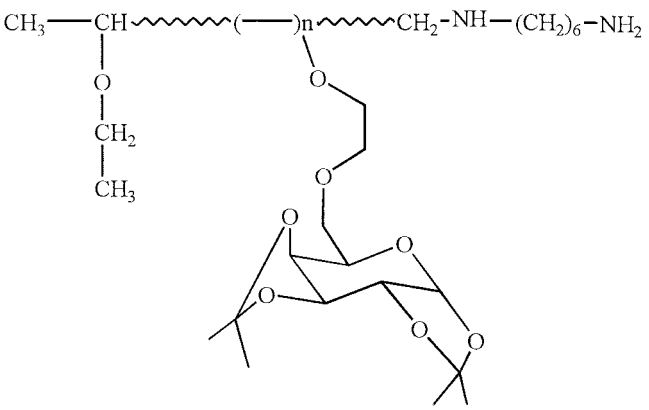
Static light-scattering measurements were carried out for copolymer FD16 in DMF after determination of the corresponding *dn/dC* value (0.076 mL g^{-1}). From the corresponding Zimm plot, a weight-average molecular weight of 215,000 (±2.6 %) g mol^{-1} was determined, a value consistent with that obtained from SEC–TALLS measurements in borate buffer (172,000 g mol^{-1}). The second virial coefficient, A_2, was found equal to 1.46 cm^3 mol g^{-2}, confirming that DMF is a good solvent for poly(NAM-*co*-NAS).

^{1}H-NMR analysis of poly(AAm-*co*-NAS) and poly(NAM-*co*-NAS) copolymers

In the case of poly(AAm-*co*-NAS), the determination of the average copolymer composition could not be performed by ^{1}H-NMR for several reasons: (i) The copolymer was not soluble in $CDCl_3$, which implied the use of DMSO-d_6 to carry out the analysis; then, contributions of water and DMSO peaks could be only roughly quantified; (ii) the copolymer peaks were not well resolved and it was necessary to perform a deconvolution.

On the contrary, the average composition of the poly(NAM-*co*-NAS) synthesized in dioxane could be estimated by ^{1}H-NMR analysis in $CDCl_3$, as explained in the Experimental section (Fig. 1). However, the obtained value, 67/33 NAM/NAS molar ratio, was significantly different from the initial comonomer ratio (where NAM/NAS = 80/20), a discrepancy again due to the peak overlapping and to the contribution of the water peak. Note that the methylene groups of the activated ester residues corresponded to the sharp peak at 2.8–2.9 ppm (f in Fig. 1).

Finally, the same optimized conditions as for FD16 were applied to different NAM/NAS comonomer mixtures[32] with various molar ratios (80/20, 70/30, 60/40, 50/50, 20/80) and it was found that whatever the molar ratio the number-average molecular weight of the obtained copolymers was around 100,000 g mol^{-1}. A detailed kinetic study of the copolymerization of this binary system has already been published[32]; from the conversion versus time curves (obtained by ^{1}H-NMR analysis of the polymerization media), the so-called reactivity ratios of the NAM/NAS comonomer system were determined, such as r_{NAM} = 0.63 and r_{NAS} = 0.75. These values evidenced an azeotropic composition for the 60/40 molar ratio which was experimentally corroborated. At this ratio, the conversion-versus-time curves of both monomers were identical,[32] and, as a consequence, the composition of the copolymer chains was constant whatever the conversion. These very homogeneous copolymer chains provided a particularly interesting material for biological applications.



Scheme 3

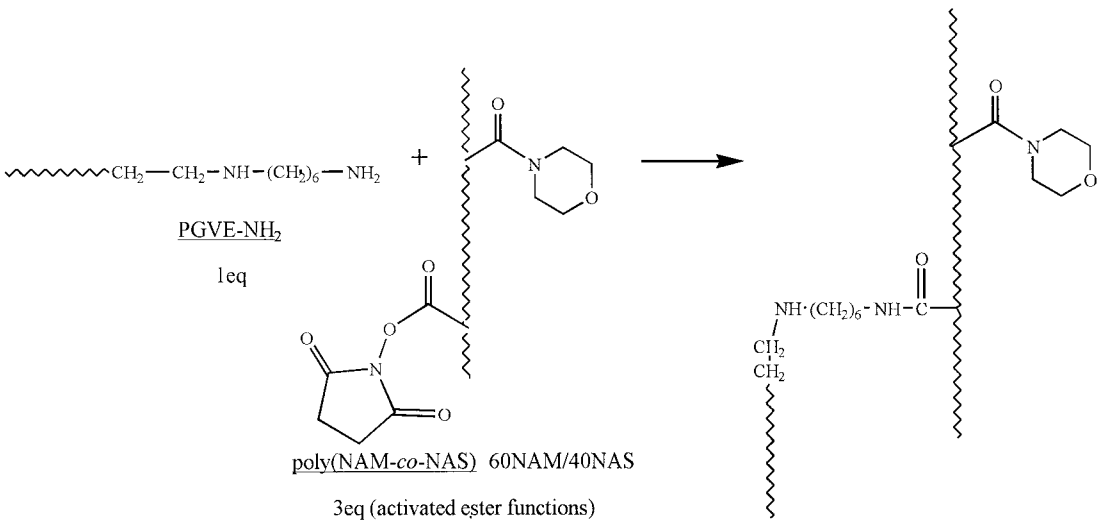
Grafting of polysaccharidic side chains onto a poly(NAM-*co*-NAS) backbone

Then, as an illustration of NAS function reactivity, and to obtain a graft copolymer potentially useful for DNA probe binding, some poly(NAM-*co*-NAS) synthesized at the azeotropic composition was used as a reactive backbone for the grafting of saccharidic polymer chains bearing a primary amino chain end (poly(GVE)–NH_2, Scheme 3), via the "grafting onto" technique (Scheme 4). The synthesis of the corresponding saccharidic monomer (GVE) and its polymerization via a "living" cationic process as well as the end-functionalization reaction were described in a previous article.[31]

The grafting reaction was performed in a DMF solution at 40°C, with 3 equivalents of the activated ester function per saccharidic chain. Since DMF proved to be a good solvent for the backbone, it should favor expansion of the polymer chains and then accessibility of the NAS reactive functions toward the amino chain ends. Considering that the M_n of the backbone was 121,000 g mol^{-1}, corresponding to 318 NAS functions per copolymer chain, a quantitative binding yield should lead to a graft copolymer bearing an average of 106 saccharidic side chains.

After 5 days of reaction, a precipitation test in diethylic ether (solvent of the saccharidic grafts but nonsolvent of the backbone) showed that no polymer chain could be recovered. It was a preliminary indication of a successful grafting if considering that the grafted saccharidic chains could disfavor the precipitation of the graft copolymer.

An SEC study was performed to evidence the occurrence of the grafting and to determine the grafting yield. First, the grafts and backbone were analyzed



Scheme 4

independently and then the raw product was obtained after 5 days of reaction [Fig. 3(a,b,d)]. In all the analyzes, toluene was used as the internal elution reference (elution volume: 12.2 mL). Comparison of the chromatograms allows one to elucidate the different peaks of the raw product chromatogram [Fig. 3(d)]:

- A peak of very weak intensity at a 10.2-mL elution volume, absent in the chromatogram of the backbone [Fig. 3(b)] but present in the chromatogram of the grafts [Fig. 3(a)]. It corresponded to saccharidic moieties released during the synthesis of the saccharidic chains as previously explained.[31]

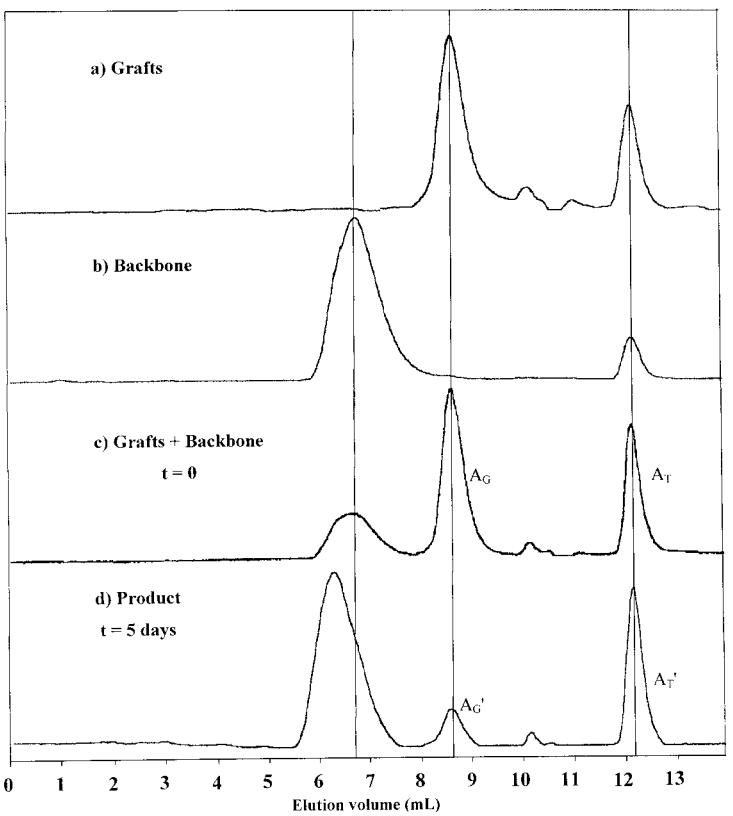


Figure 3 SEC chromatograms (eluent DMF, polystyrene standards) of (a) the grafts, (b) the backbone, (c) the initial mixture: grafts + backbone, and (d) the product after 5 days of reaction.

- A peak at a 8.6-mL elution volume, corresponding to the residual saccharidic grafts, poly(GVE)–NH_2.
- A peak at a 6.4-mL elution volume, which did not correspond to the backbone [elution volume 6.7 mL, Fig. 3(b)]. According to this value, the molecular weight of the corresponding population would reach 360,000 g mol^{-1}. Considering the molecular weight of the backbone alone, this result is another argument confirming that the grafting took place.

Nevertheless, to obtain more evidence, two complementary experiments were performed. First, grafts and the backbone were separately dissolved in DMF and both samples were placed under stirring for 5 days at 40°C. After evaporation of the DMF, the SEC analyzes of the isolated products exactly corresponded to Figure 3(a,b), an indication that both grafts and the backbone were stable under these conditions. In a second test experiment, a mixture of grafts and the backbone with the same proportion as for the grafting experiment, was analyzed by SEC immediately after preparation [Fig. 3(c)]. The corresponding chromatogram clearly confirmed that (i) the peak corresponding to the backbone was significantly different from the peak at a 6.4-mL elution volume and (ii) the peak area corresponding to the grafts significantly decreased between the initial and final mixture [see Fig. 3(c,d)]. In conclusion, the population of chains eluted at 6.4 mL corresponded to the expected graft copolymer.

The grafting efficiency, R (number of grafted saccharidic chains/number of introduced saccharidic chains), can be evaluated by the following equation [Fig. 3(c,d)]:

$$R = 1 - \frac{A'_G/A'_T}{A_G/A_T} \quad (2)$$

where A_G is the peak area of the introduced saccharidic chains; A_T, the peak area of toluene in the initial mixture; A'_G, the peak area of the residual saccharidic chains; and A'_T, the peak area of toluene in the final product. It is worthy to note that such calculation was truly appropriate since only peaks corresponding to chains of the same nature were compared (saccharidic chains) and since analyses were normalized to the toluene peak area. Then, a grafting efficiency of 92% was obtained, indicating that the graft copolymer consisted of an average of 97 saccharidic grafts per backbone chain.

CONCLUSIONS

The purpose of this study was to investigate the influence of various parameters on the molecular weight of copolymers of AAm and of NAM with an activated ester-type monomer, NAS, to prepare polymer chains of at least 100,000 g mol^{-1}. Different polymerization conditions liable to increase the molecular weight such as the total monomer concentration $[M]_T$, the initiator concentration $[I]$, the temperature T, and the nature of the solvent were examined. The following conditions allowed us to isolate a poly(NAM-*co*-NAS) copolymer of 107,000 g mol^{-1}: $[M]$ = 1 mol L^{-1}, $[I]$ = 0.5% $[M]_T$, T = 60°C, and solvent = dioxane.

Poly(NAM-*co*-NAS) was then used as a reactive backbone for the grafting of saccharidic side chains bearing a primary amino chain end, [poly(GVE)–NH_2], via the "grafing onto" technique. The grafted structure was evidenced by SEC. A grafting efficiency of 92% was calculated, corresponding to a graft copolymer bearing 97 saccharidic grafts per backbone chain, that is, an average of 1700 saccharidic residues.

Since saccharidic moieties are potential immobilization sites for biomolecules, the obtained structure is a very attractive material. For instance, DNA probes could be covalently bound to similar galactose moieties (recovered after a deprotection step), as was demonstrated in a recent study carried out with poly(GVE) linear chains.[31] The successful binding of these DNA probes on the recovered hydrophilic polymer is very promising regarding the saccharidic graft copolymer. Moreover, a better control of the graft copolymer architecture can be considered via the synthesis of the backbone by controlled radical polymerization. This strategy has been recently developed together with the synthesis of the grafts by "living" cationic polymerization, and the results will be published in a future article.[34]

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