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Glycidyl methacrylate derivatization of α, β-poly(N-hydroxyethyl)-DL-aspartamide and α, β-polyasparthydrazide

Gaetano Giammona*, Vincenzo Tomarchio, Giovanna Pitarresi and Gennara Cavallaro
Dipartimento di Chimica e Tecnologie Farmaceutiche, Università degli Studi di Palermo, Via Archirafi 32-90123 Palermo, Italy

α, β-Poly(N-hydroxyethyl)-DL-aspartamide (PHEA) and α, β-polyasparthydrazide (PAHy) are two synthetic macromolecules having many potential applications in the field of biomedical sciences. This paper describes the functionalization of PHEA and PAHy with glycidyl methacrylate (GMA), in order to introduce pendant double bonds in their chains. Derivatized PHEA and PAHy (samples PHG and PAG, respectively) at various GMA content have been obtained and characterized. It has been shown that the derivatization reaction can be controlled by varying some parameters as solvent, catalyst, pH, GMA concentration and reaction time. As expected, PAHy reacted more rapidly and more extensively than PHEA, reasonably because of the higher nucleophilicity of hydrazine groups. Besides, it has been observed that PHG and PAG aqueous solutions easily crosslink by gamma radiation processing and a correlation has been found between gel doses and derivatization degrees of the copolymers. The results of this study demonstrate that introduction of pendant double bonds in side chains of PHEA and PAHy is a suitable method to prepare macromolecular networks. © 1997 Elsevier Science Ltd.

(Keywords: α, β-poly(N-hydroxyethyl)-DL-aspartamide; α, β-polyasparthydrazide; derivatization)

INTRODUCTION

In recent years intensive studies have been performed in order to develop new materials for biomedical applications[1]. In this context, α, β-poly(N-hydroxyethyl)-DL-aspartamide (PHEA) and α, β-polyasparthydrazide (PAHy) (*Figure 1*) are two polymers exhibiting many attractive properties, such as water solubility, absence of toxicity, antigenicity and teratogenicity. Due to their physico-chemical characteristics and biocompatibility, PHEA and PAHy have been already proposed as plasma expanders and carriers for macromolecular prodrugs[2–4]. Furthermore, recent studies have shown the potential application of hydrogels based on these two macromolecules for the sustained and/or controlled release of antiviral and anticancer drugs[5,6].

Partial chemical modification of a macromolecule is a strategy currently pursued in order to improve its reactivity towards a particular reaction, such as crosslinking, grafting or the linkage with biologically active agent. Structure modification can be carried out by introducing suitable reactive groups in the backbone or in the side chains of the polymer. It is known, for instance, that albumin bearing acrylic residues readily gives bioerodible microspheres by emulsion polymerization[7]. A similar approach has been followed for polyesters containing backbone or pendant double bonds[8]. Among the crosslinking methods based on

radical mechanisms, gamma irradiation processing is a very effective procedure. Gamma rays are highly energetic radiations which cause ionization and formation of radicals in the starting material and the subsequent evolution of the radical species to form crosslinked structures[9]. There are in the recent literature many papers concerning the achievement of swellable networks by radical crosslinking using gamma irradiation from ^{60}Co sources[10–13]. Depending on the starting material structure, crosslinking can occur without any initiator or catalyst, thereby obtaining pure and sterile materials[14]. Previous articles have demonstrated the possibility to crosslink aqueous solutions of PHEA by gamma irradiation[15,16]. It has been found that gel dose is dependent on molecular weight and concentration of the starting polymer. However, it has been also established that high irradiation doses (about 550 kGy) are necessary to obtain micromatrices able to incorporate small bioactive molecules and to release them in a controlled way[16]. Contrary to PHEA, aqueous solutions of PAHy do not give rise to networks, whatever the polymer concentration and irradiation dose are. These problems can limit the application of PHEA and PAHy in the area of pharmaceutical technology. Our objective is to modify partially the structure of these two macromolecules by introducing groups bearing unsaturations. The present paper focuses on the functionalization of PHEA and PAHy with glycidyl methacrylate (GMA), already successfully exploited for polysaccharides[17]. The influence of reaction conditions on the derivatization

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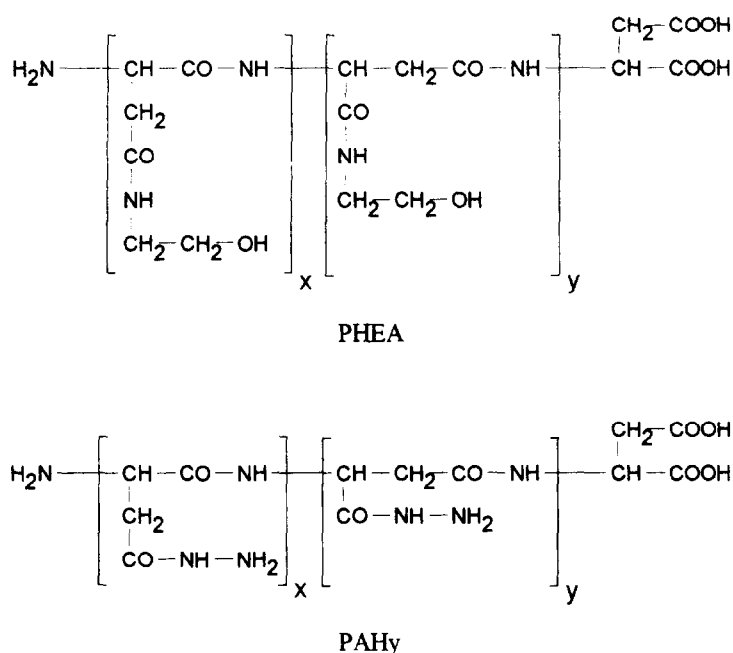* To whom correspondence should be addressed

Figure 1 Chemical structures of PHEA and PAHy

degrees of the two macromolecules has been studied. PHG and PAG copolymers differing in the content of pendant double bonds have been synthesized and characterized by elemental analysis, light scattering measurements, and Fourier transform infra-red (FTi.r.) and ^{1}H nuclear magnetic resonance (^{1}H n.m.r.) spectroscopy. Finally, some highly functionalized copolymers have been subjected to γ-irradiation processing, chosen as a model method of radical crosslinking, in order to verify the feasibility of our approach. Modified PHEA and PAHy have given rise to macromolecular networks at quite low γ-ray doses and gel dose has been found to be dependent on the degree of GMA substitution.

EXPERIMENTAL

Chemicals

All the used reagents were of analytical grade, unless otherwise stated. D,L-aspartic acid, ethanolamine, hydrazine hydrate, N,N-dimethylformamide (DMF), and anhydrous N,N-dimethylacetamide (DMA) were from Fluka (Switzerland). Glycidyl methacrylate (GMA), 4-dimethylaminopyridine (4-DMAP), D_2O (isotopic purity 99.9%) and dimethyl-d_6 sulfoxide (DMSO-d_6, isotopic purity 99.9%) were purchased from Aldrich Chemical Co. (St Louis, MO, USA).

Apparatus

Molecular weights and polydispersity indices of both starting and functionalized polymers were determined by light scattering measurements, using a Dawn DSP-F Laser Spectra Physics Spectrometer. Elemental analyses (C, H, N) were carried out on a Carlo Erba model 1106 analyser; compounds were quantitatively dried before analysis under reduced pressure (10^{-3} mmHg) at room temperature for 48 h on P_2O_5. FTi.r. spectra were recorded using a Perkin-Elmer 1720 FT spectrophotometer. The ^{1}H n.m.r. spectra were obtained with a Bruker AC-250 instrument operating at 250.13 MHz.

PHEA synthesis

α,β-Poly(N-hydroxyethyl)-DL-aspartamide (PHEA) was prepared by reaction of a polysuccinimide (PSI), obtained by thermal polycondensation of D,L-aspartic acid, with ethanolamine in DMF solution and purified according to a procedure reported elsewhere[18]. Spectroscopic data (FTi.r. and n.m.r.) were in agreement with the values reported in the literature[4]. The batch of PHEA used in the present study had a weight-average molecular weight of 56 900 ($M_w/M_n = 1.79$).

PAHy synthesis

α,β-Polyasparthydrazide (PAHy) was prepared by reaction of PSI with hydrazine in DMF solution and purified according to a procedure already described[19]. Spectroscopic data (FTi.r. and n.m.r.) were in agreement with the literature values[3]. PAHy weight-average molecular weight was 41 300 ($M_w/M_n = 1.88$).

Glycidyl methacrylate derivatization of PHEA (samples PHG_1–PHG_{29})

A first set of reactions was performed in alkaline aqueous environment according to the following procedure[20]: 1 g of PHEA was dissolved in 15 ml of sodium carbonate aqueous solution (0.1 M, pH 11), then a suitable amount of glycidyl methacrylate was added, according to X defined as:

$$X = \text{moles of derivatizing agent}/$$
$$\text{moles of PHEA repeating unit}$$

In particular, reactions with $X = 1.35$ and $X = 2$ were performed. The two-phase system was kept at room temperature under continuous stirring. Reactions at 1, 5, 7, 10 and 21 days were done for each X value (*Table 1*, samples PHG_1–PHG_{10}).

After the established time, the reaction mixture was precipitated in 150 ml of acetone and centrifuged for 20 min at 5000 rpm. The product was isolated, washed several times with acetone (where GMA is freely miscible while PHEA is insoluble) and dried under vacuum.

The above mentioned reactions gave rise to low degrees of GMA substitution (see *Figure 2*), hence a second set of reactions was carried out in organic phase using the following procedure (*Table 1*, samples PHG_{11}–PHG_{29}): 500 mg of PHEA were dissolved in 10 ml of anhydrous DMA, then 4-DMAP and GMA were added in a suitable amount according to X (see above) and Y. Y value is defined as:

$$Y = \text{moles of catalyst (4-DMAP)}/$$

$$\text{moles of derivatizing agent (GMA)}$$

Several reactions at various X, Y, and reaction times

were done in order to investigate the influence of derivatizing agent and catalyst concentrations and to study the reaction kinetics. After the established time, the reaction solution was precipitated in 80 ml of 1-butanol (where GMA is freely miscible) and centrifuged for 20 min at 5000 rpm. The product was isolated, washed several times with acetone (4 × 20 ml) and dried under vacuum.

All the PHG copolymers obtained from the two sets of described reactions were dissolved in 20 ml of distilled water and subjected to extensive dialysis utilizing Visking Dialysis Tubing (18/32 inch) with a molecular weight cut-off of 12 000–14 000. After dialysis, the solutions were concentrated under vacuum and lyophilized. Yield varied from 97 to 99% (w/w), based on the starting PHEA.

Table 1 Reaction conditions in the synthesis of PHEA-GMA derivatives (samples PHG_1–PHG_{29}).

Sample	Solvent	X	Y	Reaction time
PHG_1	Na_2CO_3, 0.1 M	1.35	0	24 h
PHG_2	Na_2CO_3, 0.1 M	1.35	0	5 days
PHG_3	Na_2CO_3, 0.1 M	1.35	0	7 days
PHG_4	Na_2CO_3, 0.1 M	1.35	0	10 days
PHG_5	Na_2CO_3, 0.1 M	1.35	0	21 days
PHG_6	Na_2CO_3, 0.1 M	2	0	24 h
PHG_7	Na_2CO_3, 0.1 M	2	0	5 days
PHG_8	Na_2CO_3, 0.1 M	2	0	7 days
PHG_9	Na_2CO_3, 0.1 M	2	0	10 days
PHG_{10}	Na_2CO_3, 0.1 M	2	0	21 days
PHG_{11}	DMA	1	1.5	2 h
PHG_{12}	DMA	1	1.5	6 h
PHG_{13}	DMA	1	1.5	12 h
PHG_{14}	DMA	1	1.5	20 h
PHG_{15}	DMA	1	1.5	24 h
PHG_{16}	DMA	1	1.5	30 h
PHG_{17}	DMA	1	1.5	36 h
PHG_{18}	DMA	1	1.5	42 h
PHG_{19}	DMA	1	1.5	48 h
PHG_{20}	DMA	1	1.5	60 h
PHG_{21}	DMA	0.5	1.5	30 h
PHG_{22}	DMA	1.5	1.5	30 h
PHG_{23}	DMA	2	1.5	30 h
PHG_{24}	DMA	1	0	48 h
PHG_{25}	DMA	1	0.5	48 h
PHG_{26}	DMA	1	1	48 h
PHG_{27}	DMA	1	2	48 h
PHG_{28}	DMA	1	2.25	48 h
PHG_{29}	DMA	1	2.5	48 h

Characterization of PHG copolymers

The results of elemental analysis of samples PHG_1–PHG_{29} are shown in *Table 2*. Elemental analysis was not calculated in samples PHG_{11}, PHG_{12}, PHG_{13} and PHG_{24} because they did not contain acrylic groups (see below).

*FT*i.r. spectra (nujol) showed a broad band centred at $3293 \, cm^{-1}$ ($-OH$, $-NH-$, $-NH_2$) and bands at 1651 (broad, amide I), 1542 (amide II) and 1171 ($C-O$ asymmetric stretching of ester group of GMA) cm^{-1}.

^1H n.m.r. (D_2O): δ 1.94 (s, 3H, $-CO-C(CH_3)=CH_2$), 2.85 (m, 2H, $-CH-CH_2-CO-NH-$), 3.39 (t, 2H, $-NH-CH_2-CH_2-O-$), 3.57 (m, 2H, $-O-CH_2-CH(OH)-CH_2-O-$), 3.68 (t, 2H, $-NH-CH_2-CH_2-O-$), 4.28 (m, 1H, $-O-CH_2-CH(OH)-CH_2-$), 4.55–4.8 (m, 3H, $-CH(OH)-CH_2-O-CO-$, $-NH-CH(CO)-CH_2-$), 5.75 and 6.15 (2s, 2H, $-CO-C(CH_3)=CH_2$).

Sample PHG_{26} was further characterized by light scattering measurements. The weight-average molecular weight was 65 300 ($M_w/M_n = 1.91$).

Glycidyl methacrylate derivatization of PAHy (samples PAG_1–PAG_6)

500 mg of PAHy were dissolved in phosphate buffer solution (PBS) (KH_2PO_4, K_2HPO_4, pH 8.5). Glycidyl methacrylate (520 μl) was slowly added in order to have
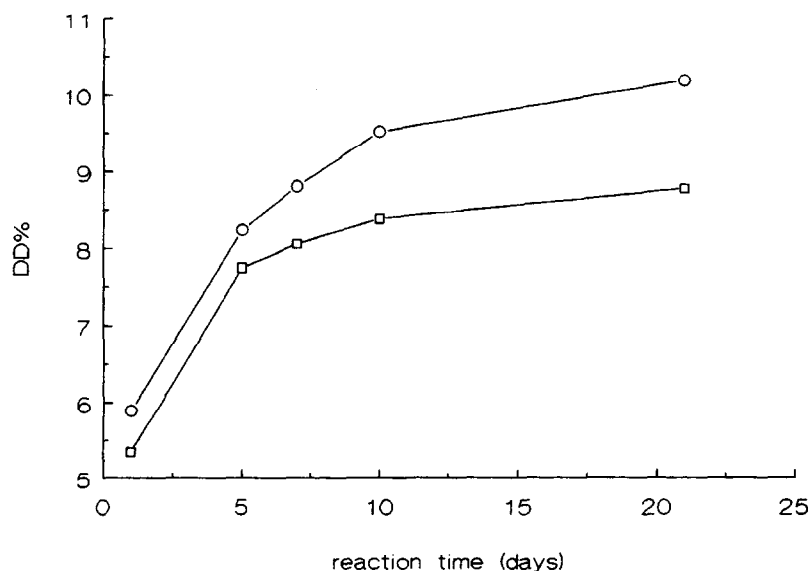
Figure 2 Degree of derivatization of PHEA in aqueous solution at pH 11 as a function of time ($\square$, $X = 1.35$; $\bigcirc$, $X = 2$)

Table 2 Elemental analysis of PHEA and PHG copolymers

Sample	Calculated for	Theoretical			Found		
		C	H	N	C	H	N
PHEA	$C_6H_{10}N_2O_3$	45.57	6.37	17.71	45.44	6.51	17.63
PHG$_1$	$C_{6.37}H_{10.54}N_2O_{3.16}$	46.17	6.41	16.91	46.41	6.71	16.64
PHG$_2$	$C_{6.54}H_{10.78}N_2O_{3.23}$	46.45	6.43	16.57	46.01	6.66	16.33
PHG$_3$	$C_{6.56}H_{10.81}N_2O_{3.24}$	46.47	6.43	16.52	46.03	6.58	16.21
PHG$_4$	$C_{6.59}H_{10.84}N_2O_{3.25}$	46.54	6.42	16.47	46.78	6.22	16.53
PHG$_5$	$C_{6.61}H_{10.88}N_2O_{3.26}$	46.56	6.43	16.43	46.40	6.68	16.13
PHG$_6$	$C_{6.41}H_{10.59}N_2O_{3.18}$	46.22	6.41	16.82	46.56	6.60	16.71
PHG$_7$	$C_{6.58}H_{10.82}N_2O_{3.25}$	46.50	6.42	16.48	46.81	6.57	16.33
PHG$_8$	$C_{6.62}H_{10.88}N_2O_{3.26}$	46.59	6.43	16.42	46.28	6.31	16.12
PHG$_9$	$C_{6.67}H_{10.95}N_2O_{3.29}$	46.63	6.42	16.31	46.88	6.70	16.17
PHG$_{10}$	$C_{6.71}H_{11.02}N_2O_{3.31}$	46.67	6.43	16.22	46.75	6.56	16.04
PHG$_{14}$	$C_{6.38}H_{10.54}N_2O_{3.16}$	46.21	6.41	16.89	46.44	6.70	17.01
PHG$_{15}$	$C_{6.57}H_{10.82}N_2O_{3.25}$	46.47	6.42	16.50	46.68	6.66	16.38
PHG$_{16}$	$C_{7.17}H_{11.67}N_2O_{3.50}$	47.35	6.47	15.40	47.41	6.61	15.32
PHG$_{17}$	$C_{7.40}H_{12.00}N_2O_{3.60}$	47.64	6.48	15.01	47.88	6.70	15.36
PHG$_{18}$	$C_{7.80}H_{12.17}N_2O_{3.77}$	48.12	6.51	14.39	48.33	6.82	14.18
PHG$_{19}$	$C_{8.19}H_{13.13}N_2O_{3.94}$	48.54	6.53	13.82	48.75	6.84	13.66
PHG$_{20}$	$C_{8.55}H_{13.64}N_2O_{4.09}$	48.93	6.55	13.35	49.08	6.48	13.21
PHG$_{21}$	$C_{6.02}H_{10.03}N_2O_{3.01}$	45.59	6.37	17.66	45.21	6.27	17.42
PHG$_{22}$	$C_{8.84}H_{14.06}N_2O_{4.22}$	49.18	6.56	12.98	49.46	6.78	12.78
PHG$_{23}$	$C_{10.27}H_{16.11}N_2O_{4.83}$	50.37	6.63	11.44	50.58	6.83	11.11
PHG$_{25}$	$C_{6.36}H_{10.51}N_2O_{3.15}$	46.19	6.40	16.94	46.31	6.53	16.84
PHG$_{26}$	$C_{7.26}H_{11.81}N_2O_{3.54}$	47.45	6.48	15.24	47.65	6.41	15.33
PHG$_{27}$	$C_{8.63}H_{13.75}N_2O_{4.13}$	48.99	6.55	13.24	49.08	6.44	13.11
PHG$_{28}$	$C_{8.67}H_{13.81}N_2O_{4.14}$	49.05	6.56	13.19	49.25	6.71	13.24
PHG$_{29}$	$C_{8.66}H_{13.81}N_2O_{4.14}$	49.00	6.56	13.21	49.12	6.69	13.25

an X of 1 (defined as moles of derivatizing agent/moles of PAHy repeating unit). The two-phase system was kept at room temperature under continuous stirring. Reactions at 2, 4, 8, 16, 19 and 24 h were done, thereby obtaining the samples PAG$_1$–PAG$_6$, respectively. After the established time, the reaction mixture was shaken with 40 ml of dichloromethane (in which GMA is freely miscible, while PAHy is insoluble) in order to extract the unreacted derivatizing agent. Then the two layers were separated and the aqueous phase was extracted twice again. The collected organic phases were dried and analysed by i.r. spectroscopy which showed the characteristic peaks of GMA, while no peak of both starting PAHy or PHG copolymer was found. Finally, the aqueous phase containing the derivatized polymer was concentrated in a rotovapour to remove the residual organic solvent and dialysed using Visking Dialysis Tubing (18/32 inch) with a molecular weight cut-off of 12 000–14 000. After dialysis, the solution was concentrated under vacuum and lyophilized. PAG derivatives were obtained with a yield of 96–98% (w/w), based on the starting PAHy.

Characterization of PAG copolymers

Table 3 shows elemental analyses of samples PAG$_1$–PAG$_6$.

*FT*i.r. spectra (nujol) showed a broad band centred at 3294 cm^{-1} (–NH–, –NH$_2$) and bands at 1657 (broad, amide I), 1526 (amide II) and 1171 (C–O asymmetric stretching of ester group of GMA) cm^{-1}. *FT*i.r. spectra

of PAG adducts made in dimethylsulfoxide solution showed a band at 1715 cm^{-1} (C=O stretching vibration of ester group of GMA).

^{1}H n.m.r. (DMSO-d_6): δ 1.88 (s, 3H, –CO–C(CH$_3$) = CH$_2$), 2.3–3.8 (m, broad, 5H, –CH–CH$_2$–CO– NH–, –NH–CH$_2$–CH(OH)–CH$_2$–, –O–CH$_2$–CH(OH)– CH$_2$–), 4.06 (m, 2H, –CH(OH)–CH$_2$–O–CO–), 4.55 (m, 1H, –NH–CH(CO)–CH$_2$–), 4.8–5.15 (m, broad, –OH), 5.66 and 6.06 (2s, 2H, –CO–C(CH$_3$) = CH$_2$), 7.9–9.15 (m, broad –NH–, –NH$_2$).

^{1}H n.m.r. (DMSO-d_6)/D$_2$O): δ 1.88 (s, 3H, –CO–C(CH$_3$) = CH$_2$), 2.3–2.9 (m, broad, 4H, –CH–CH$_2$– CO–NH–, –NH–CH$_2$–CH(OH)–CH$_2$–), 3.38 (m, 1H, –O–CH$_2$–CH(OH)–CH$_2$–), 4.08 (m, 2H, –CH(OH)– CH$_2$–O–CO–), 4.55 (m, 1H, –NH–CH(CO)–CH$_2$–), 5.66 and 6.06 (2s, 2H, –CO–C(CH$_3$) = CH$_2$.

Finally, sample PAG$_3$ was subjected to light scattering measurements which evidenced a weight-average molecular weight of 49 800 (M_w/M_n = 2.02).

Radiation processing

Aqueous solutions (40 mg ml^{-1}) of PHEA, PAHy, PHG and PAG at various GMA content were irradiated by the IGS-3, a panoramic 3000 Ci ^{60}Co irradiator at room temperature[21]. The dose rate, measured by a Fricke Dosimeter, was 6.25 kGy h^{-1} and a variance of 5% in the absorbed dose was accepted. At regular intervals of time the polymer solutions were observed and the samples producing an insoluble network were removed. The exposition of the solutions which did not

Table 3 Elemental analysis of PAHy and PAG copolymers

Sample	Calculated for	Theoretical			Found		
		C	H	N	C	H	N
PAHy	$C_4H_7N_3O_2$	37.21	5.46	32.54	37.43	5.22	32.67
PAG$_1$	$C_{4.35}H_{7.5}N_3O_{2.15}$	38.35	5.55	30.85	38.48	5.71	30.58
PAG$_2$	$C_{4.57}H_{7.82}N_3O_{2.24}$	39.03	5.60	29.88	38.89	5.88	30.04
PAG$_3$	$C_{5.2}H_{8.72}N_3O_{2.51}$	40.71	5.72	27.39	40.99	5.93	27.06
PAG$_4$	$C_{6.55}H_{10.64}N_3O_{3.09}$	43.50	5.93	23.23	43.54	5.95	23.23
PAG$_5$	$C_{7.82}H_{12.46}N_3O_{3.64}$	43.94	5.97	22.43	44.02	5.98	22.36
PAG$_6$	$C_{7.82}H_{12.46}N_3O_{3.64}$	45.43	6.07	20.32	45.64	6.37	20.41
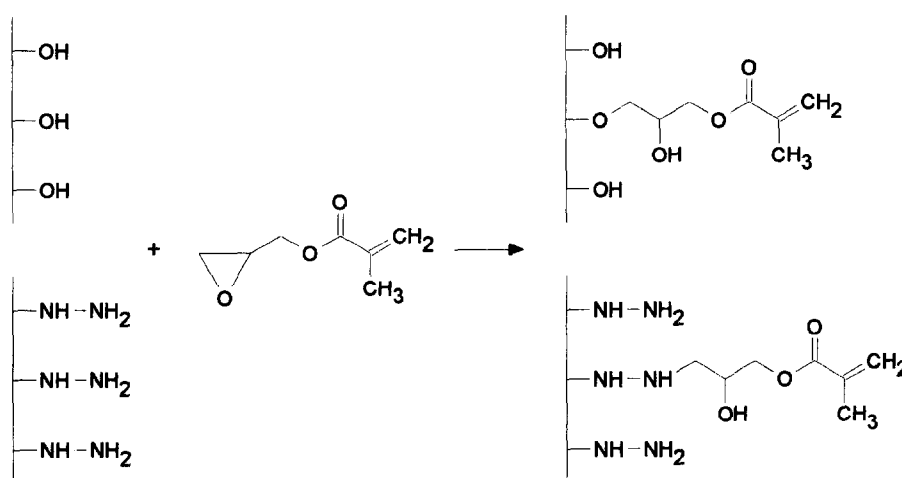
Figure 3 Reaction of PHEA (P–OH) and PAHy (P–NH–NH$_2$) with glycidyl methacrylate

produce gel structures was continued until 600 kGy. All the obtained gels obtained from PHG and PAG copolymers were lyophilized and treated with H$_2$O under continuous stirring at 40°C for 24 h. After filtration, the extraction liquid was dried under vacuum and the residue was weighed. In all samples, the amount of water soluble material resulted less than 1.5% (w/w) based on the gel weight.

RESULTS AND DISCUSSION

The present study deals with the synthesis and characterization of poly(amino acid)s containing pendant unsaturations which can be used as starting materials in the preparation of systems for drug release by radical copolymerization/crosslinking or grafting. The reaction between glycidyl methacrylate and PHEA or PAHy is schematically reported (*Figure 3*).

The functionalized polymers were characterized by *FT*i.r. and ^{1}H n.m.r. analysis. *FT*i.r. spectroscopy of the purified copolymers evidenced the presence of the characteristic band of GMA at 1171 cm^{-1} in PHG and PAG derivatives which is absent in the starting polymers (*Figure 4*).

^{1}H n.m.r. spectra of PHG and PAG confirmed the introduction of double bonds in the side chains of PHEA and PAHy (*Figure 5*).

One sample for each series of functionalized polymers (PHG$_{26}$ and PAG$_3$, respectively) were further

characterized by light scattering measurements. The determined data confirmed an increase of molecular weight of both samples compared to the starting macromolecules.

Determination of the degree of derivatization in PHG derivatives

The degree of derivatization (DD) was determined by ^{1}H n.m.r. and was calculated by the following ratio:

$$DD = (\text{acrylic groups/polymer repeating unit}) \times 100$$

PHG conjugates spectra were recorded in D$_2$O. DD was calculated comparing the integral of the peak related to protons at 2.85 δ awardable to $-CH-CH_2-CO-NH-$ (belonging to PHEA), with the integral related to protons at 1.94 δ as well as to protons between 5.75 and 6.15 δ respectively awardable to $-CO-C(CH_3)=CH_2$ and $-CO-C(CH_3)=CH_2$ that belong to linked GMA. The degrees of derivatization were expressed as mean values. Each determination was carried out in triplicate and the maximum estimated error was 3%.

Determination of the degree of derivatization in PAG derivatives

Referring to PAG conjugates, spectra were performed in DMSO-d_6 and the DD was calculated comparing the integral of the peak related to protons at 4.55 δ awardable to $-NH-CH(CO)-CH_2-$ (belonging to PAHy), with the integral related to protons at 1.88 δ as
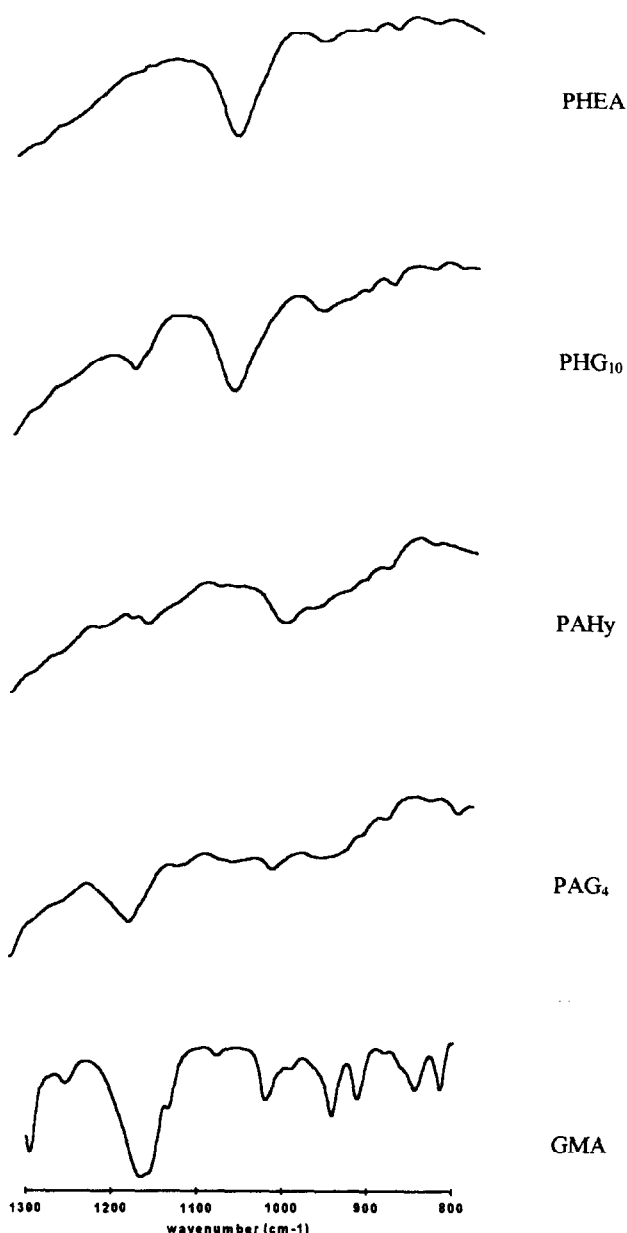
Figure 4 *FT*i.r. spectra (nujol) in the range 800–1300 cm^{-1} of PHG and PAG copolymers compared to the starting macromolecules and GMA

Figure 5 ^{1}H n.m.r. spectra of PHG$_{10}$ (A, recorded in D$_2$O) and PAG$_5$ (B, recorded in DMSO-d_6/D$_2$O)

well as to protons between 5.67 and 6.06 δ respectively awardable to $-CO-C(CH_3) = CH_2$ and $-CO-C(CH_3) = CH_2$ that belong to linked GMA. The degrees of derivatization were expressed as mean values. Each determination was carried out in triplicate and the maximum estimated error was 5%.

Derivatization of PHEA in aqueous environment

Glycidyl methacrylate derivatized PHEA was initially prepared in aqueous solution at pH 11. The derivatization kinetics of PHEA, relative to $X = 1.35$ and $X = 2$, showed that the amount of unsaturations in PHG copolymers was dependent on X and reaction time (*Figure 2*). A trend towards a plateau was reached after 10 days, regardless of X. The efficiency of this set of reactions was quite low and the maximum quantity of GMA inserted in the PHEA chains was 10.2% (sample PHG$_{10}$).
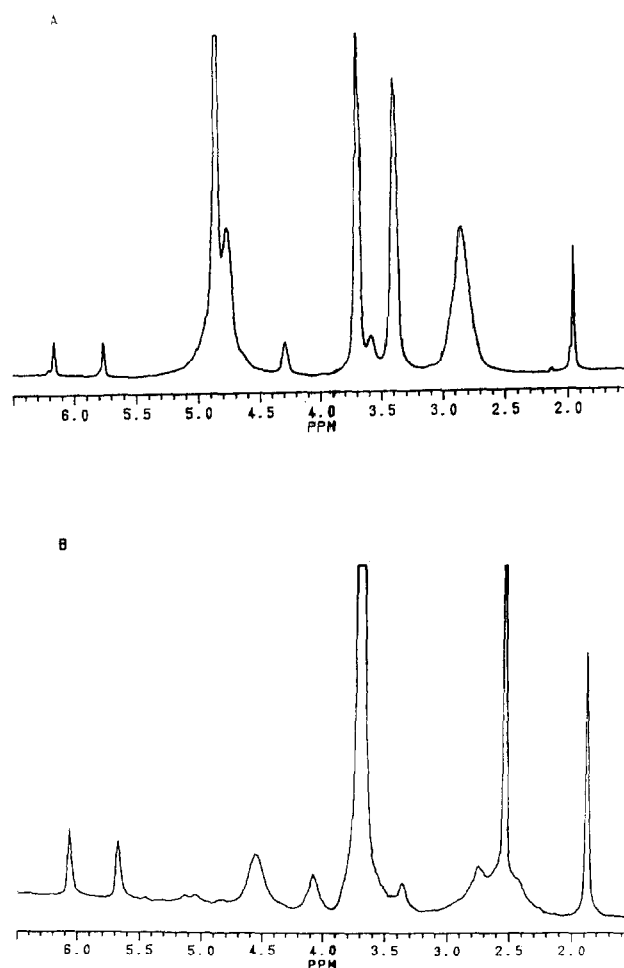
Derivatization of PHEA in DMA in the presence of 4-DMAP as a catalyst

Based on the results obtained, the reaction between GMA and PHEA was studied in anhydrous dimethyl acetamide (DMA) using 4-dimethylaminopyridine (4-DMAP) as catalyst. An X value of 1 was chosen and the effect of the reaction time on the degree of derivatization of the polymer side chains was investigated. In this environment PHEA reacted more quickly and extensively than in an alkaline aqueous environment, obtaining degrees of derivatization up to 36.4% (*Figure 6*).

A possible explanation about the difference of PHEA reactivity between aqueous environment and organic solvent is that the last set of reactions was conducted in a homogeneous phase (both PHEA and GMA are soluble in DMA) and, moreover, because of the presence of 4-DMAP as catalyst. This acid acceptor was already successfully used as catalyst in the activation of PHEA with 4-nitrophenyl chloroformate and in the derivatization of dextran with epoxide functions[22,23]. Data reported in *Figure 6* indicate that the amount of pendant unsaturations is very low until 12 h, and it quickly increases for longer reaction times. This mechanism is anything but rare in the reactivity of poly(amino acid)s. A similar profile of derivatization has been observed with alumin[7]. This trend can probably be explained by supposing that the introduction of the first GMA molecules in PHEA side chains causes a rearrangement of the conformation of the macromolecule which evolves
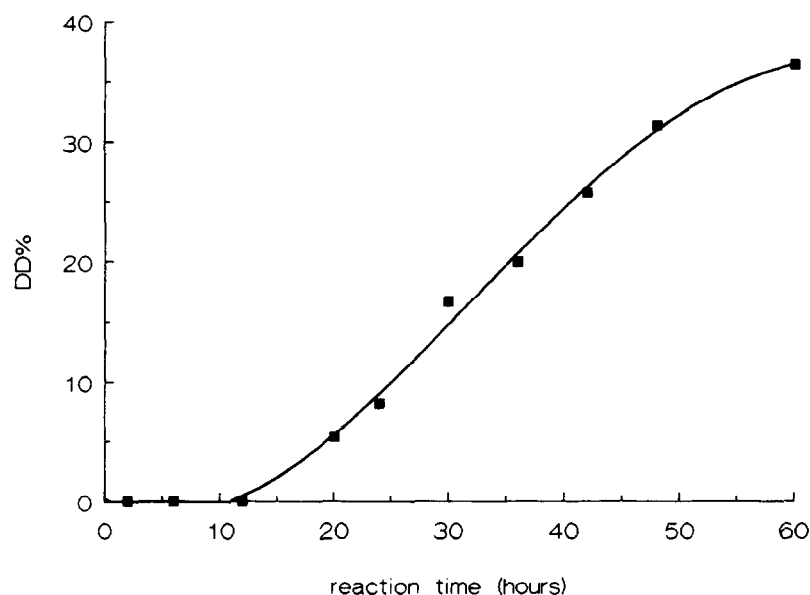
Figure 6 Degree of derivatization of PHEA in DMA in the presence of 4-DMAP as a function of time ($X = 1$, $Y = 1.5$)
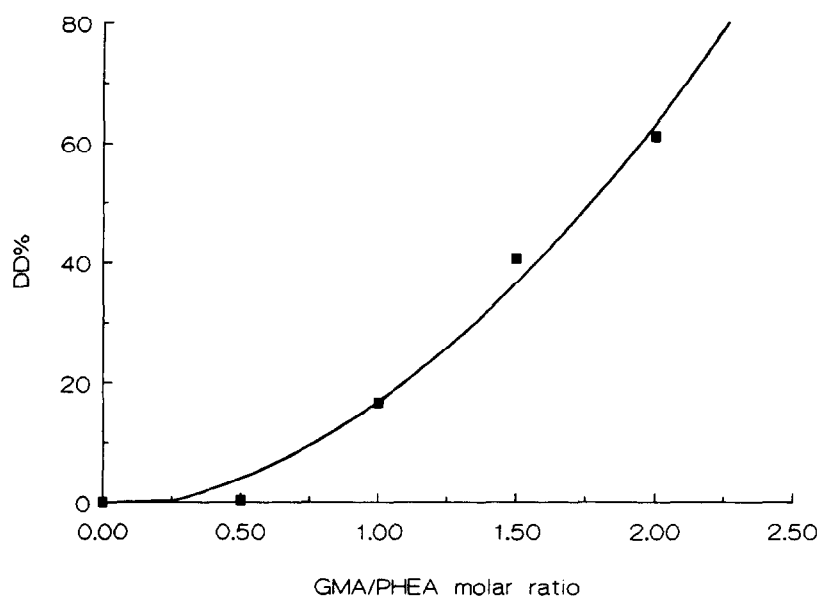

Figure 7 Degree of derivatization of PHEA in DMA in the presence of 4-DMAP as a function of X (time = 30 h, $Y = 1.5$)

in a form able to react better with further molecules of derivatizing agent.

Furthermore a subsequent set of reactions was carried out maintaining constant the reaction time as well as Y (time = 30 h and $Y = 1.5$, respectively) in order to investigate the effect of X (ratio between moles of GMA and moles of PHEA repeating unit) on DD. *Figure 7* shows that the derivatization degree was hardly dependent on X, and DD values higher than 60% were obtained with an X value of 2.

Finally, another set of reactions was performed maintaining constant the time reaction as well as X (time = 48 h and $X = 1$, respectively) in order to investigate the correlation between Y and DD. The data obtained suggest that the reactivity of PHEA is increased by increasing the DMAP/GMA molar ratio (*Figure 8*). DD values up to about 38% were obtained and a plateau was observed for Y values higher than 2.

Derivatization of PAHy in aqueous environment

The glycidyl methacrylate derivatization of PAHy was

investigated in PBS solution at pH 8.5 with an X value of 1. The degrees of derivatization vs reaction time are reported in *Figure 9*.

PAHy reacted more rapidly and more extensively than PHEA and DD ranged from 5.0% to 54.6% (samples PAG_1-PAG_6). The higher reaction rate of GMA with PAHy can reasonably be attributed to the presence of hydrazine functions. Besides, in the experimental conditions used, the reaction between PAHy and GMA followed a zero-order kinetics. The following equation was obtained by a linear regression of DD data *versus* time:

$$DD = 2.2446 \times h - 0.2381; \qquad r = 0.999$$

Formation of macromolecular networks by ^{60}Co irradiation

Some PHG and PAG conjugates at various unsaturation content was irradiated in aqueuous solution at room temperature and the dose gel (D_g) was determined. The results are reported in *Table 4*.
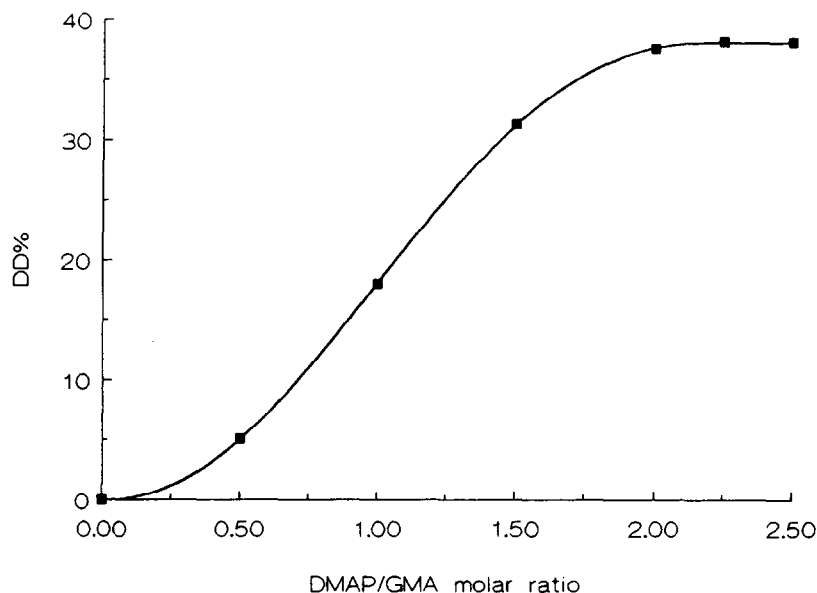
Figure 8 Degree of derivatization of PHEA in DMA in the presence of 4-DMAP as a function of Y (time = 48 h, $X = 1$)
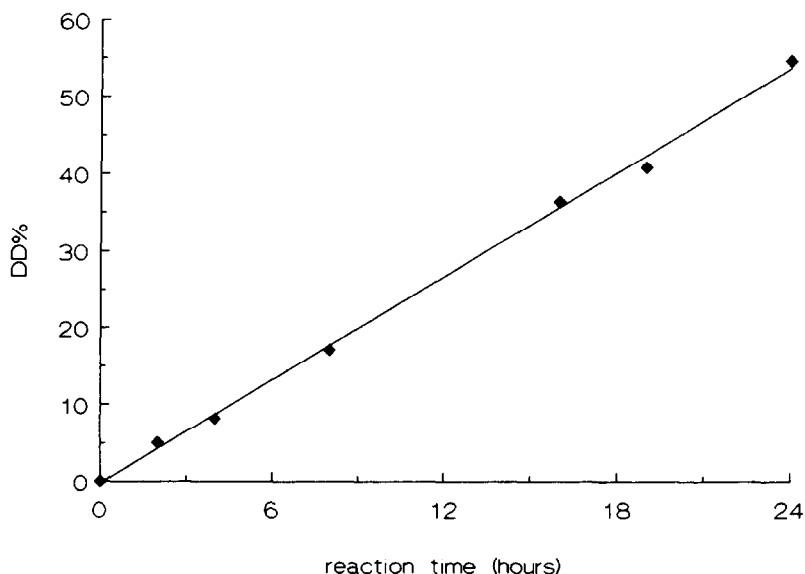

Figure 9 Degree of derivatization of PAHy in PBS pH 8.5 as a function of time ($X = 1$)

Table 4 Gamma irradiation of PHEA, PAHy and some PHG and PAG copolymers

Sample	DD%	D_g (kGy)
PHEA	0	550
PHG$_{19}$	31.3	2.5
PHG$_{22}$	40.6	1.5
PHG$_{26}$	18.1	6
PAHy	0	_^{a}
PAG$_4$	36.4	18
PAG$_5$	40.9	12
PAG$_6$	54.6	6

^{a} γ-irradiation was stopped at 600 kGy

With reference to PHG copolymers, we irradiated the samples PHG$_{19}$, PHG$_{22}$, PHG$_{26}$ (containing 31.3%, 40.6% and 18.1% of acrylic groups, respectively) and the starting PHEA. The dose gel was 2.5, 1.5 and 6 kGy for samples PHG$_{19}$, PHG$_{22}$ and PHG$_{26}$ respectively, while in the same conditions the starting polymer gave

rise to a gel structure at 550 kGy. It can be deducted that gel dose for PHG adducts is much lower than starting PHEA and, moreover, it decreases when DD increases. This result can be explained considering the varying content of unsaturations in the side chains of PHEA-GMA samples.

As far as PAG copolymers are concerned, only samples PAG$_4$, PAG$_5$ and PAG$_6$ (containing 36.41, 40.85 and 54.60% of acrylic groups, respectively) were irradiated and gave rise to an insoluble network. The D_g was 18, 12 and 6 kGy for the samples PAG$_4$, PAG$_5$ and PAG$_6$, respectively, while the starting macromolecule did not produce gel until 600 kGy (see *Table 4*).

All the obtained networks based on PHG and PAG copolymers were insoluble in water, acid or alkaline aqueous solutions and in the common organic solvents, such as dichloromethane, acetone, ethanol, dimethylsulfoxide, dimethylacetamide, dimethylformamide. Further studies are in progress to evaluate the effect of some

important parameters, such as temperature, dose rate, copolymer concentration and presence of difunctional crosslinking agents on gel doses and to characterize more extensively the obtained networks.

CONCLUSIONS

The results of this study indicate that the derivatization of PHEA and PAHy with glycidyl methacrylate is a profitable method to introduce pendant double bonds in their chains. It is possible to obtain PHG and PAG derivatives containing the expected amount of pendant unsaturations by choosing the suitable amount of derivatizing agent, catalyst and reaction time. The functionalized polymers give rise to crosslinked structures by γ irradiation, and gel dose depends hardly on the amount of acrylic groups present in the polymer chains. These findings demonstrate the effectiveness of our rationale for the production of new macromolecular networks based on PHEA and PAHy.

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