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Acryloylated α,β-poly (N-hydroxyethyl)-DL-aspartamide and α,β-polyasparthydrazide. Synthesis, characterization and radiation formation of polymeric networks

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Abstract In the present study the derivatization of two water-soluble and synthetic polymers, such as α,β-poly(N-hydroxyethyl)-DL-aspartamide (PHEA) and α,β-polyasparthydrazide (PAHy), with glycidylmethacrylate (GMA) is described. This reaction allowed the introduction of double bonds in the macromolecular chains of PHEA and PAHy in order to make easier the crosslinking by a radical mechanism. Different parameters affected the reaction of derivatization, such as reaction pH, GMA concentration and reaction time. As far as PHEA is concerned the amount of GMA linked to the polymer increased until reaching a plateau. On the contrary, the reaction of PAHy with GMA proceeded with a zero order kinetics and the GMA amount in the polymer increased regularly. Some aqueous solutions of PHEA-GMA and PAHy-GMA copolymers at various GMA content were submitted to gamma radiation processing, thus obtaining crosslinked structures. The derivatization of PHEA and PAHy with GMA was a convenient method to introduce insaturations in their chains and it allowed to obtain gels at lower doses with respect to the starting polymers.

Key words Acryloylated polymers – α,β-poly(N-hydroxyethyl)-DL-aspartamide – α,β-polyasparthydrazide – glycidylmethacrylate – crosslinking – hydrogels

Introduction

Biocompatible polymers are increasingly used in the field of pharmaceutical technology. In particular, one of the main areas in which biopolymers have been more exploited is that of controlled drug delivery [1, 2]. Several hydrophilic macromolecules, both natural and synthetic, have been successfully utilized to prepare hydrogels for biomedical purposes and as systems able to release bioactive molecules [3–6]. Among the network producing methods, radical polymerization and crosslinking seem to be very profitable, because of their ease and speed of carrying it out and, moreover, because systems of different shape and size can be easily obtained [7]. Polymer crosslinking by a radical mechanism can be basically performed by two methods. The first one is a chemical method, based on the use of radical initiators and catalysts [8, 9]; the second one is the high-energy radiation technique. Several advantages in the use of radiation technology in the hydrogel preparation have been described, such as product sterilization and lack of toxic chemical residuals [10, 11]. It is well known that with exposing a macromolecule to ionizing radiation, several phenomena can occur. Network formation depends mainly on the polymer structure and on radiation processing conditions, such as total dose, temperature, presence and type of solvent. A possible way to promote the polymer reactivity towards reactions based

on radical mechanisms is the introduction of double bonds in the macromolecular chains [12, 13].

The present work describes the derivatization of two polymers, such as α,β-poly(N-hydroxyethyl)-DL-aspartamide (PHEA) and α,β-polyasparthydrazide (PAHy), with glycidylmethacrylate (GMA). Some derivatizing acryloyl agents have already successfully used for other hydrophilic macromolecules [14]. PHEA and PAHy are two water-soluble and synthetic polymers derived from the polysuccinimide [15, 16]. Biological studies have shown that these macromolecules are highly biocompatible and for this reason have been proposed as plasma expanders and carriers for macromolecular prodrugs [17–19]. In addition, PHEA and PAHy have been already crosslinked, thus providing matrices able to release small molecules in physiological-like media [20, 21]. Acryloylated polymers, PHEA-GMA and PAHy-GMA, originating from the derivatization of these two macromolecules with GMA, have been characterized by elemental analysis, Fourier transform infrared spectroscopy (FT-IR) and ^{1}H nuclear magnetic resonance (^{1}H-NMR). Finally, the ability of PHEA-GMA and PAHy-GMA at various insaturation content to give crosslinked structures by gamma radiations has been studied. The chemical modification extent of PHEA and PAHy has been an important variable determining the formation of crosslinked matrices.

Materials and methods

Chemicals

All the reagents used were of analytical grade, otherwise stated. DL-aspartic acid, ethanolamine, hydrazine hydrate, N,N-dimethylformamide (DMF), were from Fluka. Glycidyl methacrylate (GMA), D_2O (isotopic purity 99.9%), and dimethyl-d_6 sulfoxide (isotopic purity 99.9%) were purchased from Aldrich Chemical Co.

PHEA synthesis

α,β-poly(N-hydroxyethyl)-DL-aspartamide (PHEA) was prepared by reaction of a polysuccinimide (PSI), obtained by thermal polycondensation of DL-aspartic acid, with ethanolamine in DMF solution and purified according to a procedure reported elsewhere [15]. Spectroscopic data (FT-IR and NMR) were in agreement with the values reported in the literature [20]. The batch of PHEA used in the present study had a weight-average molecular weight of 56900 ($M_w/M_n = 1.89$) determined by light scattering measurements, using a Dawn DSP-F Laser Spectra Physics Spectrometer.

PAHy synthesis

α,β-polyasparthydrazide (PAHy) was prepared by reaction of PSI with hydrazine in DMF solution and purified according to a procedure already reported [16]. Spectroscopic data (FT-IR and NMR) were in agreement with the literature values [18]. PAHy weight-average molecular weight determined by light-scattering, was 41300 ($M_w/M_n = 1.88$).

Analytical methods

Elemental analyses (C, H, N) were carried out using a Carlo Erba model 1106 analyzer; compounds were quantitatively dried before analysis under reduced pressure (10^{-3} mmHg) at room temperature for 48 h on P_2O_5.

FT-IR spectra were recorded in Nujol using a Perkin–Elmer 1720 Fourier Transform Spectrophotometer.

The ^{1}H-NMR spectra were obtained with a Bruker AC-250 instrument operating at 250.13 MHz.

PHEA derivatization with GMA (samples a_1–a_5 and b_1–b_5)

PHEA was dissolved in a sodium carbonate aqueous solution (0.1 M, pH 11). Glycidyl methacrylate was added at the suitable quantity according to X defined as:

X = moles of GMA/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, 21 days were carried out for each X value. In particular, the following samples were prepared:

Sample a_1:
1 g of PHEA in 15 ml of Na_2CO_3 0.1 M (pH 11)
1.14 ml of glycidyl methacrylate ($X = 1.35$)
Reaction time: 24 h
Yield: 95% (w/w) based on starting PHEA
Analysis: Calculated for $C_{6.37}H_{10.54}N_2O_{3.16}$: C, 46.17; H, 6.41; N, 16.91
Found: C, 46.41; H, 6.71; N, 16.64

Sample a_2:
1 g of PHEA in 15 ml of Na_2CO_3 0.1 M (pH 11)
1.14 ml of glycidyl methacrylate ($X = 1.35$)
Reaction time: 5 days
Yield: 97% (w/w) based on starting PHEA
Analysis: Calculated for $C_{6.54}H_{10.78}N_2O_{3.23}$: C, 46.45; H, 6.43; N, 16.57
Found: C, 46.01; H, 6.66; N, 16.33

Sample a_3:
1 g of PHEA in 15 ml of Na_2CO_3 0.1 M (pH 11)
1.14 ml of glycidyl methacrylate ($X = 1.35$)
Reaction time: 7 days
Yield: 97% (w/w) based on starting PHEA
Analysis: Calculated for $C_{6.56}H_{10.81}N_2O_{3.24}$: C, 46.47; H, 6.43; N, 16.52
Found: C, 46.03; H, 6.58; N, 16.21

Sample a_4:
1 g of PHEA in 15 ml of Na_2CO_3 0.1 M (pH 11)
1.14 ml of glycidyl methacrylate ($X = 1.35$)
Reaction time: 10 days
Yield: 96% (w/w) based on starting PHEA
Analysis: Calculated for $C_{6.59}H_{10.84}N_2O_{3.25}$: C, 46.54; H, 6.42; N, 16.47
Found: C, 46.78; H, 6.22; N, 16.53

Sample a_5:
1 g of PHEA in 15 ml of Na_2CO_3 0.1 M (pH 11)
1.14 ml of glycidyl methacrylate ($X = 1.35$)
Reaction time: 21 days
Yield: 95% (w/w) based on starting PHEA
Analysis: Calculated for $C_{6.61}H_{10.88}N_2O_{3.26}$: C, 46.56; H, 6.43; N, 16.43
Found: C, 46.40; H, 6.68; N, 16.13

Sample b_1:
1 g of PHEA in 15 ml of Na_2CO_3 0.1 M (pH 11)
1.69 ml of glycidyl methacrylate ($X = 2$)
Reaction time: 24 h
Yield: 96% (w/w) based on starting PHEA
Analysis: Calculated for $C_{6.41}H_{10.59}N_2O_{3.18}$: C, 46.22; H, 6.41; N, 16.82
Found: C, 46.56; H, 6.60; N, 16.71

Sample b_2:
1 g of PHEA in 15 ml of Na_2CO_3 0.1 M (pH 11)
1.69 ml of glycidyl methacrylate ($X = 2$)
Reaction time: 5 days
Yield: 98% (w/w) based on starting PHEA
Analysis: Calculated for $C_{6.58}H_{10.82}N_2O_{3.25}$: C, 46.50; H, 6.42; N, 16.48
Found: C, 46.81; H, 6.57; N, 16.33

Sample b_3:
1 g of PHEA in 15 ml of Na_2CO_3 0.1 M (pH 11)
1.69 ml of glycidyl methacrylate ($X = 2$)
Reaction time: 7 days
Yield: 97% (w/w) based on starting PHEA
Analysis: Calculated for $C_{6.62}H_{10.88}N_2O_{3.26}$: C, 46.59; H, 6.43; N, 16.42
Found: C, 46.28; H, 6.31; N, 16.12

Sample b_4:
1 g of PHEA in 15 ml of Na_2CO_3 0.1 M (pH 11)
1.69 ml of glycidyl methacrylate ($X = 2$)
Reaction time: 10 days
Yield: 96% (w/w) based on starting PHEA
Analysis: Calculated for $C_{6.67}H_{10.95}N_2O_{3.29}$: C, 46.63; H, 6.42; N, 16.31
Found: C, 46.88; H, 6.70; N, 16.17

Sample b_5:
1 g of PHEA in 15 ml of Na_2CO_3 0.1 M (pH 11)
1.69 ml of glycidyl methacrylate ($X = 2$)
Reaction time: 21 days
Yield: 98% (w/w) based on starting PHEA
Analysis: Calculated for $C_{6.71}H_{11.02}N_2O_{3.31}$: C, 46.67; H, 6.43; N, 16.22
Found: C, 46.75; H, 6.56; N, 16.04

After the established time, the reaction mixture was precipitated in 150 ml of acetone and centrifuged for 20 min at 5000 r.p.m. The product was isolated, washed several times with acetone (where GMA is freely miscible while the polymer is unsoluble) and dried under vacuum. Then the PHEA-GMA copolymer was dissolved in 20 ml of distilled water and extensively dialyzed using Visking Dialysis Tubing (18/32 inch) with a molecular weight cutoff of 12 000–14 000. After dialysis, the solution was concentrated under vacuum and *lyophilized.*

FT-IR spectra (nujol) showed a broad band centered 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}.

^{1}H-NMR (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–], 4.65 [m, 2H, –CH(OH)–**CH_2**–O–CO–], 4,76 [m, broad, 1H, –NH–**CH**(CO)–CH_2–], 5.75 and 6.15 [2s, 2H, –CO–C(CH_3) = **CH_2**].

PAHy derivatization with GMA (samples c_1–c_5)

PAHy was dissolved in phosphate buffer solution (PBS) (KH_2PO_4, K_2HPO_4, pH 8.5). Glycidyl methacrylate was slowly added in order to have a X of 1 (defined as moles of GMA/moles of PAHy repeating unit). The two-phase system was kept at room temperature under continuous stirring. Reactions at 2, 4, 8, 16 and 24 h were done. In particular the following samples were prepared:

Sample c_1:
500 mg of PAHy in 10 ml of PBS (pH 8.5)
520 μl of glycidyl methacrylate ($X = 1$)

Reaction time: 2 h
Yield: 96% (w/w) based on starting PAHy
Analysis: Calculated for $C_{4.35}H_{7.5}N_3O_{2.15}$: C, 38.35; H, 5.55; N, 30.85
Found: C, 38.48; H, 5.71; N, 30.58

Sample c_2:
500 mg of PAHy in 10 ml of PBS (pH 8.5)
520 μl of glycidyl methacrylate ($X = 1$)
Reaction time: 4 h
Yield: 97% (w/w) based on starting PAHy
Analysis: Calculated for $C_{4.57}H_{7.82}N_3O_{2.24}$: C, 39.03; H, 5.60; N, 29.88
Found: C, 38.89; H, 5.88; N, 30.04

Sample c_3:
500 mg of PAHy in 10 ml of PBS (pH 8.5)
520 μl of glycidyl methacrylate ($X = 1$)
Reaction time: 8 h
Yield: 97% (w/w) based on starting PAHy
Analysis: Calculated for $C_{5.2}H_{8.72}N_3O_{2.51}$: C, 40.71; H, 5.72; N, 27.39
Found: C, 40.99; H, 5.93; N, 27.06

Sample c_4:
500 mg of PAHy in 10 ml of PBS (pH 8.5)
520 μl of glycidyl methacrylate ($X = 1$)
Reaction time: 16 h
Yield: 98% (w/w) based on starting PAHy
Analysis: Calculated for $C_{6.68}H_{10.83}N_3O_{3.15}$: C, 43.71; H, 5.95; N, 22.89
Found: C, 43.54; H, 5.89; N, 22.77

Sample c_5:
500 mg of PAHy in 10 ml of PBS (pH 8.5)
520 μl of glycidyl methacrylate ($X = 1$)
Reaction time: 24 h
Yield: 98% (w/w) based on starting PAHy
Analysis: Calculated for $C_{7.82}H_{12.46}N_3O_{3.64}$: C, 45.43; H, 6.07; N, 20.32
Found: C, 45.64; H, 6.37; N, 20.41

After the established time, the reaction mixture was precipitated in 200 ml of acetone and centrifuged for 20 min at 5000 r.p.m. The product was isolated, washed several times with acetone (where PAHy is insoluble) and dried under vacuum. Then the PAHy-GMA adduct was dissolved in 10 ml of distilled water and extensively dialyzed using Visking Dialysis Tubing (18/32 inch) with a molecular weight cutoff of 12 000–14 000. After dialysis, the solution was concentrated under vacuum and lyophilized.

FT-IR spectra (nujol) showed a broad band centered 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}.

^{1}H-NMR (DMSO-d_6): δ 1.88 [s, 3H, –CO–C(**CH_3**) $=CH_2$], 2.4–3.1 [m, broad, 4H, –CH–**CH_2**–CO–NH–, –NH–**CH_2**–CH(OH)–CH_2–], 3.6–4.15 [m, broad, 3H, –O–CH_2–**CH**(OH)–CH_2–, –CH(OH)–**CH_2**–O–CO–], 4.55 [m, 1H, –NH–**CH**(CO)–CH_2–] 5.67 and 6.06 [2s, 2H, –CO–C(CH_3) = **CH_2**].

Radiation processing

Aqueous solutions (40 mg/ml) of PHEA, PAHy, PHEA-GMA and PAHy-GMA 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 6.30 kGy/h 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 exposure of solutions was continued until 600 kGy even if some samples did not give rise to gels or insoluble products.

Results and discussion

The reaction between α,β-poly(N-hydroxyethyl)-DL-aspartamide (PHEA) and α,β-polyasparthydrazide (PAHy) with the glycidyl ester of metacrylic acid produces PHEA-GMA and PAHy-GMA conjugates containing pendant unsaturations. The chemical structure of the modified PHEA and PAHy is shown in Fig. 1.

FT-IR analysis of PHEA-GMA and PAHy-GMA adducts evidenced the presence of a characteristic band of the derivatizing agent at 1171 cm^{-1}, which is absent in the starting PHEA and PAHy (see Fig. 2A and B respectively).

The degree of derivatization (*DD*) was determined by ^{1}H-NMR and was calculated by the following ratio:

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

PHEA-GMA conjugates spectra were recorded in D_2O. *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 and 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

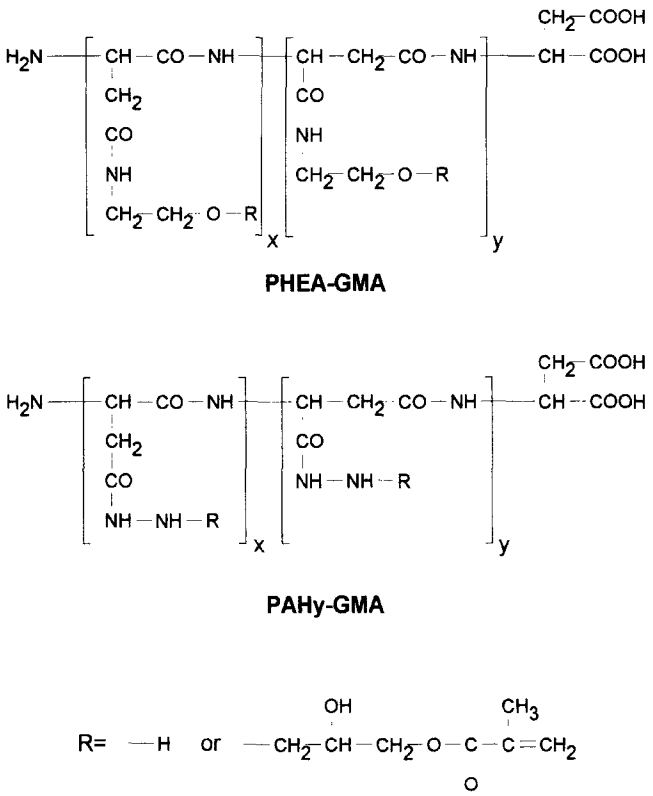

Fig. 1 Chemical structure of glycidyl methacrylate derivatized PHEA and PAHy

3%. The derivatization kinetics of PHEA, relative to $X = 1.35$ and $X = 2$, are reported in Fig. 3.

It was found that the amount of unsaturation in PHEA-GMA depended on X and on the time of reaction. A plateau was reached after 10 days, regardless of X.

The maximum quantity of GMA inserted in PHEA chains was 10.19%.

Referring to PAHy-GMA conjugates, spectra were performed in DMSO-d_6 and the *DD* was calculated by 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 and 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 degree of derivatization was expressed as a mean value. Each determination was carried out in triplicate and the maximum estimated error was 5%. Figure 4 shows the kinetics of the reaction between PAHy and GMA.

PAHy reacted more rapidly and more extensively than PHEA. The higher reaction rate with PAHy can reasonably be ascribed to the presence of hydrazine functions. Besides, in the experimental conditions used, the reaction between PAHy and GMA followed a zero order kinetics.

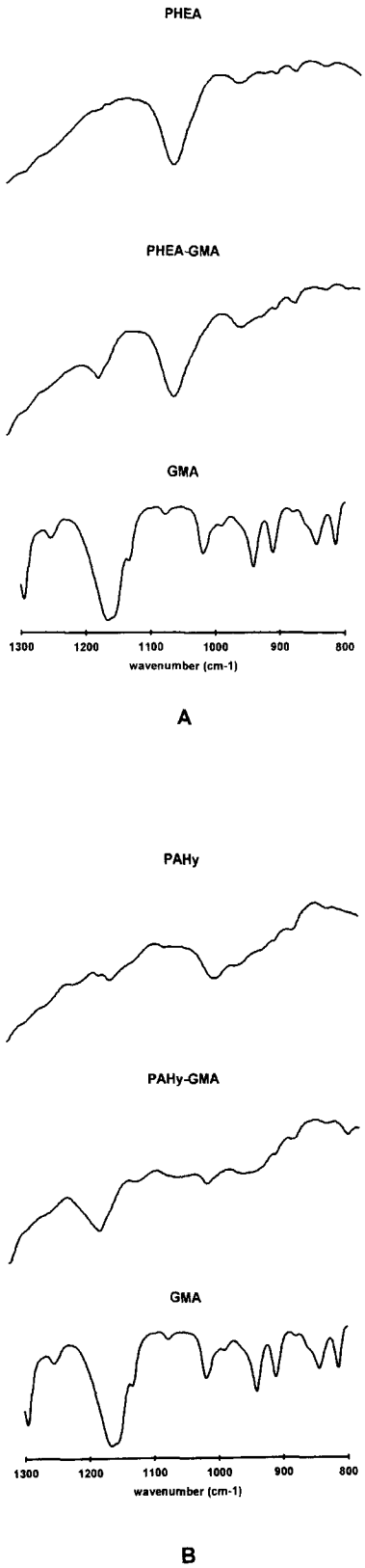

Fig. 2 FT-IR spectra from 800 to 1300 cm^{-1}. A: spectra of PHEA, PHEA-GMA (sample a_5) and GMA; B: spectra of PAHy, PAHy-GMA (sample c_3) and GMA

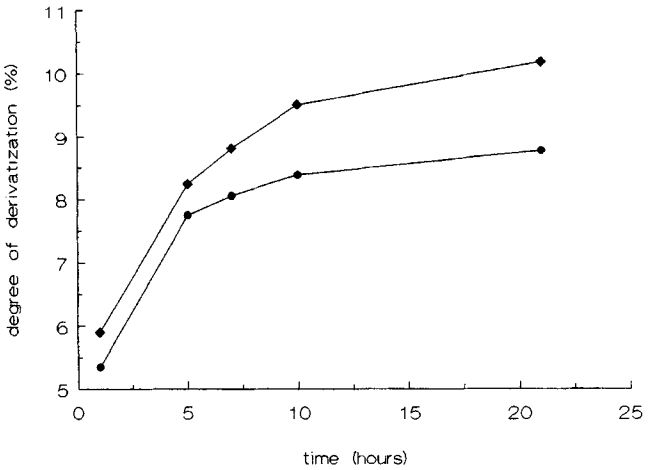

Fig. 3 Degree of PHEA derivatization by glycidyl methacrylate as a function of time (●) samples a_1–a_5 ($X = 1.35$); (◆) samples b_1–b_5 ($X = 2$)

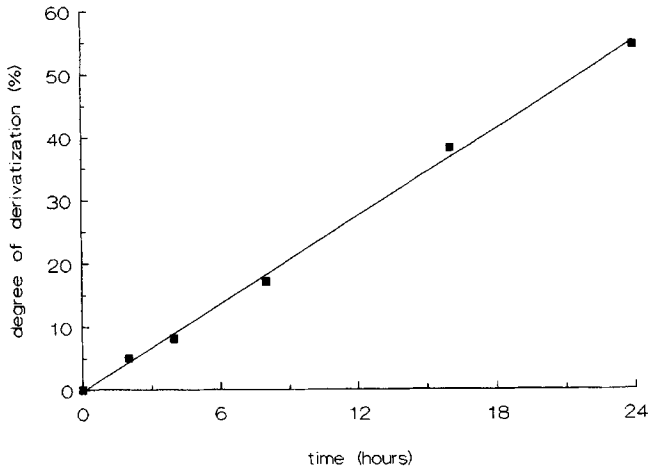

Fig. 4 Degree of PAHy derivatization by glycidyl methacrylate as a function of time (samples c_1–c_5)

The following equation was obtained by a linear regression of *DD* data versus time:

$$DD = 2.31 \times h - 0.27;\ r = 0.999\ .$$

The *DD* ranged from 5.02% to 54.60% of acrylic groups.

Finally, PAHy-GMA and PHEA-GMA conjugates at various unsaturation content were irradiated and the dose gel was determined. As far as PAHy-GMA polymers are concerned, only samples c_4 and c_5 (containing the 38.35% and 54.60% of acrylic groups, respectively) gave rise to an insoluble network. The dose gel was 18 kGy for the sample c_4 and 6 kGy for the sample c_5. All the other PAHy-GMA conjugates (c_1, c_2 and c_3) and the starting PAHy did not produce gel until 600 kGy.

With reference to the PHEA-GMA conjugates, we irradiated only the sample b_5 (having the highest *DD*) and the starting PHEA. The dose gel was 450 kGy for sample b_5 and 600 kGy for PHEA. It can be deduced that the gel dose decreases, but it remains quite high. These results can be explained by considering the low content of unsaturation in the structure of the sample b_5.

In conclusion, the derivatization of PHEA and PAHy with glycidyl methacrylate seems to be a profitable method to introduce pendant double bonds in their chains and, at least for PAHy, it allows hydrogels to be obtained by γ-radiations at quite low doses.

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