Functionalization of a Polyaspartamide with Glycidyl Methacrylate: A Useful Method to Prepare Hydrogels Through Gamma Irradiation

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SUMMARY: α,β-Poly(N-2-hydroxyethyl)-DL-aspartamide (PHEA) was derivatized with glycidyl methacrylate (GMA). Aqueous solutions of the obtained copolymer PHEA-GMA (PHG) were irradiated by gamma rays with a dose rate of 0.5 KGy/h and at zero °C in the presence or in the absence of N,N'-methylenebisacrylamide (BIS). New hydrogel systems were obtained and characterized by FT-IR analyses and swelling measurements in aqueous medium at different pH values.

Introduction

In the recent years intensive studies have been performed in order to develop new materials for biomedical applications[1)]. In this contest, α,β-poly(N-2-hydroxyethyl)-DL-aspartamide (PHEA) is a polymer which exhibits many attractive properties, such as water solubility, absence of toxicity, antigenicity and teratogenicity. Due to its physico-chemical properties and biocompatibility, PHEA has been already proposed as plasma expander and carrier for macromolecular prodrugs [2)].

Furthermore, recent studies have shown the potential application of hydrogels based on this polymer for the sustained and/or controlled release of drugs [3, 4)]. These hydrogels have been obtained through gamma irradiation of aqueous solutions of PHEA using high irradiation doses (about 550 kGy). Now, in order to improve the reactivity of PHEA towards the radical

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reactions, we have partially modified the structure of PHEA introducing groups bearing unsaturations, i. e. by functionalization of the polymer with glycidyl methacrylate (GMA).

Experimental

Materials

All reagents were of analytical grade, unless otherwise stated. Anhydrous N,N-dimethylacetamide (DMA) and N,N'-methylenebisacrylamide (BIS) were from Fluka (Switzerland). Glycidyl methacrylate (GMA), 4-dimethylaminopyridine (4-DMAP) and D_2O (isotopic purity 99.9%) were purchased from Aldrich Chemical Co. (St. Louis, MO, USA). PHEA was prepared according to a procedure elsewhere reported [5].

Apparatus

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

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

Gamma irradiation was performed by means of the IGS-3, a panoramic 3000 Ci ^{60}Co irradiator at 0°C and under nitrogen. The dose rate, measured by a PTW Universal Dosimeter, was 0.5 kGy/h and a variance of 5% in the absorbed dose was accepted.

Centrifugation was performed with an International Equipment Company Centra MP4R equipped with a 854 rotor and temperature control.

Derivatization of PHEA with glycidyl methacrylate (PHG copolymer)

The reaction between PHEA and GMA was carried out in organic phase using a procedure elsewhere reported. In particular, 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= 1 and Y = 1.5 being:

X = moles of derivatizing agent / moles of PHEA repeating unit

Y = moles of catalyst (4-DMAP) / moles of derivatizing agent (GMA)

After 48 hours, the reaction solution was precipitated in 80 ml of 1-butanol (where GMA is freely miscible) and centrifuged for 20 min at 12000 r.p.m. and 4°C. The product was isolated, washed several times with acetone (where GMA is freely miscible while PHEA is insoluble) and dried under vacuum. PHG copolymer obtained was 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 polymer solution was concentrated under vacuum and lyophilized. The product was obtained with a yield of 98% (w/w) based on the starting PHEA.

FT-IR spectra (KBr) showed a broad band centred at 3293 cm^{-1} and a band at 3078 m cm^{-1} (ν_{as} OH + ν_{as} NH + ν_s NH) and bands at 1720 m (ν_{as} CO), 1651 vs (amide I + ν C=C), 1542 s (amide II), 1405 w (scissoring RR'C=CH_2), 1180 m (ν_s COO + ether COC) and 954 m-w (wagging RR'C=CH_2) 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-], 4.55 - 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**].

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

DD = (acrylic groups / polymer repeating unit) x 100

In particular, 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 awardable to-CO-C(**CH_3**)=CH_2 as well as to protons between 5.75 and 6.15 δ awardable to -CO-C(CH_3)=**CH_2** that belong to linked GMA. The degree of derivatization was expressed as mean value and resulted to be 29 mol%. Each determination was carried out in triplicate and the maximum estimated error was 3%.

Formation of polymer networks by ^{60}Co irradiation

Aqueous solutions of PHG copolymer (40 mg/ml) with or without N,N'-methylenebisacrylamide (BIS) (25 mol% relating the moles of GMA linked to PHEA) were irradiated for different times under nitrogen at 0°C with a dose rate of 0.5 kGy/h. At suitable time intervals polymer solutions were observed and the samples producing an insoluble network were removed. Each gel was purified by several washing with distilled water, then lyophilized and weighed in order to determine the insoluble fraction.

FT-IR spectra (KBr) of all networks obtained in the presence or not of BIS showed a broad band centred at 3335 cm^{-1} and a band at 3078 m cm^{-1} (ν_{as} OH + ν_{as} NH + ν_s NH) and bands at 1730 m (ν_{as} CO), 1652 vs (amide I + ν C=C), 1542 s (amide II) and 1185 m (ν_s COO + ether COC) cm^{-1}.

Results and discussion

The reaction between glycidyl methacrylate (GMA) and α,β-poly(N-2-hydroxyethyl)-DL-aspartamide (PHEA) gave rise to the formation of PHG copolymer which was characterized by FT-IR and ^{1}H-NMR analyses. FT-IR spectroscopy of the purified copolymer evidenced the presence of the characteristic bands of GMA at 1720 (ν_{as} CO), 1176 (ν_s COO + COC ether) and 951 (wagging RR'C=CH_2) cm^{-1} which were absent in the starting polymer .

^{1}H-NMR spectrum of PHG confirmed the introduction of double bonds in the side chains of PHEA and allowed to calculate the degree of derivatization (see experimental) which resulted to be equal to about 29 mol%.

The effect of irradiation is shown in Fig.1, where gel fractions, recovered after irradiation process for both PHG alone and PHG in the presence of BIS, known crosslinking agent, are reported as a function of the absorbed dose. In both cases significant amounts of gel fractions were formed. In particular in the absence of crosslinking agent, gel fraction was observed beginning from 0.4 kGy and a plateau value of the gel fraction yield equal to about 70 % (w/w) was achieved. Crosslinking was more effective in the presence of BIS in fact gel fraction was observed beginning from 0.26 kGy and the plateau value was established at about 90%.

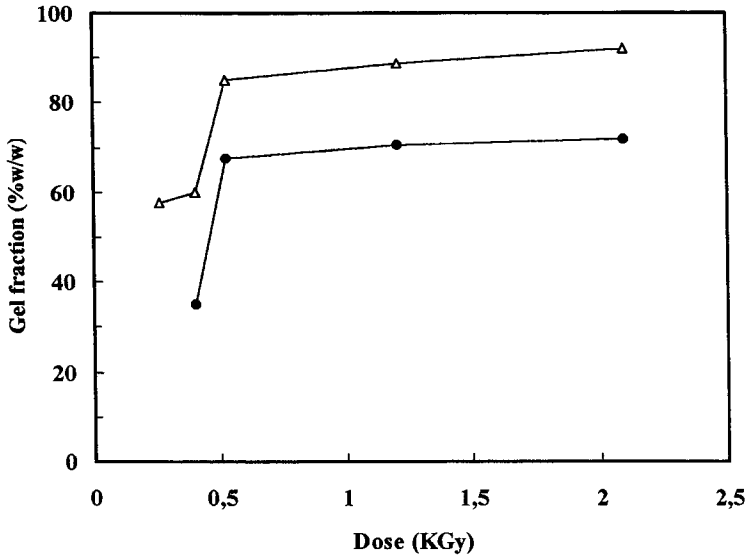

Fig. 1 - Gel fractions of PHG-γ rays (•) and (PHG + BIS)-γ rays (△) samples versus absorbed irradiation dose

All the obtained samples were insoluble in water and in the common organic solvents, such as dichloromethane, acetone, ethanol, dimethylsulfoxide, dimethylacetamide, dimethylformamide.

FT-IR spectra of PHG hydrogels, obtained in the presence or not of BIS, showed different peaks in comparison with starting PHG. In particular the band at 951 cm^{-1} disappeared and the peak of carbonilic groups shifted to 1730 cm^{-1}. This result advised that the formation of PHG networks involves the breakage of unsaturations present in the GMA residues and in BIS molecules (when present) and confirmed the presence of ester groups in the structure of obtained hydrogels.

The affinity of prepared hydrogels towards the aqueous medium at different pH value has been evaluated by swelling studies.

Water content (WC %) was calculated as follows: $WC\ \% = (Ws - Wd)/ Ws \times 100$

where Ws and Wd are the weight of swollen and dry sample respectively.

The values of WC % of the samples of PHG irradiated with and without BIS reported in Tab. 1 showed the high affinity of these hydrogels towards aqueous medium.

Tab. 1. Swelling ability of PHG -γ rays and (PHG + BIS) - γ rays samples

	Water content (WC%)			
	H_2O	pH 1	pH 6.8	pH 7.4
PHG-γ rays	91.46	85.7	91.2	92.5
(PHG + BIS)-γ rays	84	60	87.5	88.2

Acknowledgements

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