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Synthesis and characterization of water-swellable α, β-polyasparthydrazide derivatives

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Abstract: The synthesis of a new crosslinked polymer by reaction of α, β-polyasparthydrazide and glutaraldehyde is reported. Different crosslinking degrees were obtained by varying the ratio between the aldehyde and the starting polymer. The crosslinked polymer was characterized by water swelling tests and thermal analysis. In particular, the crosslinking density and its effects on the glass transition temperature of the material were studied. Finally, the microstructure of the obtained polymer was observed using scanning electron microscopy.

Key words: Crosslinked α,β-polyasparthydrazide – hydrogels – swellable micromatrices

Introduction

Hydrogels are three-dimensional networks in which individual hydrophilic polymer chains are connected by physical or chemical bonds. When these systems are in contact with an aqueous medium, a process of water penetration occurs that is associated with swelling of the networks. The presence of intermolecular bonds gives rise to the integrity and physical stability of these systems, therefore they swell without dissolving in an aqueous liquid [1]. Due to their high water affinity, hydrogels are biocompatible systems [2] and, for this reason, in recent years they have received considerable attention in biomedical and pharmaceutical fields ranging from soft contact lenses to drug-delivery systems. In particular, in addition to their inertness and good biocompatibility, due to their ability to release entrapped drugs in aqueous medium, hydrogels are suitable as carriers in the sustained and/or controlled delivery of bioactive agents. It has been shown that the release rate of entrapped drug can be regulated by controlling the crosslinking density of the polymer and its water swelling [3].

In this work, we present the synthesis and characterization of polymeric networks based on α, β-polyasparthydrazide (PAHy) chemically crosslinked by glutaraldheyde.

PAHy is a new linear water-soluble polymer which was synthesized by coupling a polysuccinimide (PSI) with hydrazine and pharmaceutically characterized [4, 5]. Tests of the polymer on laboratory animals established its potential use as a plasma substitute. In effect, solutions of PAHy are non toxic, non immunogenic, and able to improve the lowering of blood values caused by a controlled bleeding.

Hemodynamic studies have shown no significant variation in systolic and diastolic pressure or in heart beat following PAHy solution administration. PAHy has also been proposed as a drug carrier because the hydrazine groups present in the polymer allow it to be bonded with drugs bearing either carboxyl or aldehyde groups to produce water-soluble polymeric prodrugs.

Now, we wish to extend the pharmaceutical application of PAHy as hydrophylic matrix to form hydrogels systems suitable in the release of bioactive agents.

In this paper, we report the synthesis of new polymeric networks based on PAHy at various crosslinking density. We have chosen glutaraldehyde as crosslinking agent because this bifunc-

tional molecule has been utilized successfully to crosslink various enzymes, proteins, and polymers [6, 7]. The characterization of these new hydrogels was done through water swelling measurements, thermal analysis, and scanning electron microscopy.

Experimental

Chemicals

DL-aspartic acid, hydrazine hydrate, *N*,*N*-dimethylformamide (DMF), glutaraldehyde (50% aqueous solution), sulfuric acid and acetic acid were from Fluka (Switzerland).

PAHy preparation

α, β-polyasparthydrazide (PAHy) was prepared via the polysuccinimide (PSI) by polycondensation of DL-aspartic acid in the presence of H_3PO_4 at 180 °C followed by reaction with hydrazine in DMF solution. PAHy was isolated by filtration, washed several times with acetone and dried. An aqueous solution of PAHy was dialyzed for 3 days against several changes of distilled water using Visking Dialysis Tubing (18/32 inch) with a molecular weight cutoff of 12 000–14 000.

After dialysis, the PAHy was recovered by lyophilization in a yield of 96% w/w based on the starting PSI. Analytical data of PAHy were in agreement with literature values [4].

PAHy crosslinking procedure

To a PAHy aqueous solution (1 g in 35 ml of distilled water) the following reagents were added sequentially, with stirring: 5 ml 10 vol% acetic acid, 1.6 ml 1 vol% sulfuric acid (absent for the samples 2′ and 4′) and 50 vol% glutaraldehyde. The glutaraldehyde was added to the reaction mixture at regular intervals of time (15 min). The resulting reaction mixture was continuously stirred for 24 h at room temperature (about 25 °C). After this time, the obtained solid product was isolated by filtration, washed several times with ethanol and then dried in a rotary evaporator at 10^{-1} mmHg and 40 °C for 24 h.

The glutaraldehyde/PAHy ratio was indicated through [3] :

X = moles of glutaraldehyde/moles of PAHy repeating unit

In particular, the following samples were prepared:

Sample 1 ($X = 0.2$)

Quantity of glutaraldehyde used: 277μl
Yield: 96.0% w/w
Analysis: Calculated for $C_9H_{14.8}N_6O_4$: C, 39.88; H, 5.50; N, 31.01.
Found: C, 39.93; H, 5.55; N, 31.07.

Samples 2 *and* 2′ ($X = 0.4$)

Quantity of glutaraldehyde used: 555μl
Yield: 97.6% w/w
Analysis: Calculated for $C_{10}H_{15.6}N_6O_4$: C, 42.31; H, 5.54; N, 29.61.
Found: C, 42.37; H, 5.57; N, 29.70.

Sample 3 ($X = 0.8$)

Quantity of glutaraldehyde used: 1.105μl
Yield: 98.2% w/w
Analysis: Calculated for $C_{12}H_{17.2}N_6O_4$: C, 46.57; H, 5.60; N, 27.15.
Found: C, 46.63; H, 5.67; N, 27.22.

Samples 4 *and* 4′ ($X = 1.2$)

Quantity of glutaraldehyde used: 1.660μl
Yield: 98.3% w/w
Analysis: Calculated for $C_{13}H_{18}N_6O_4$: C, 48.44; H, 5.63; N, 26.07.
Found: C, 48.52; H, 5.66; N, 26.15.

Sample 5 ($X = 1.8$)

Quantity of glutaraldehyde used: 2.489μl
Yield: 97.8 w/w
Analysis: Calculated for $C_{13}H_{18}N_6O_4$: C, 48.44; H, 5.63; N, 26.07.
Found: C, 48.56; H, 5.70; N, 26.18.

Materials characterization

Elemental analyses (C, H, N) were carried out on 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.

Swelling measurements were done by keeping the crosslinked samples in contact, through a semipermeable membrane, with distilled water for 7 days at 25 °C.

Thermal analysis was performed by means of a Perkin Elmer DSC 7B calorimeter. Samples were heated from − 10 °C up to 250 °C; the heating rate was 2 °C /min. Before each test the samples were carefully dried for 72 h under vacuum in the presence of P_2O_5 and then ground in a mortar in order to ensure a good contact with the aluminum pan. The glass transition temperature, *Tg*, was determined as the temperature corresponding to a change of the slope in the specific heat-temperature plot.

Morphological investigation was made by a Philips 501 scanning electron microscope (SEM): the samples surface was made conductive by the deposition of a layer of gold in a vacuum chamber.

Results and discussion

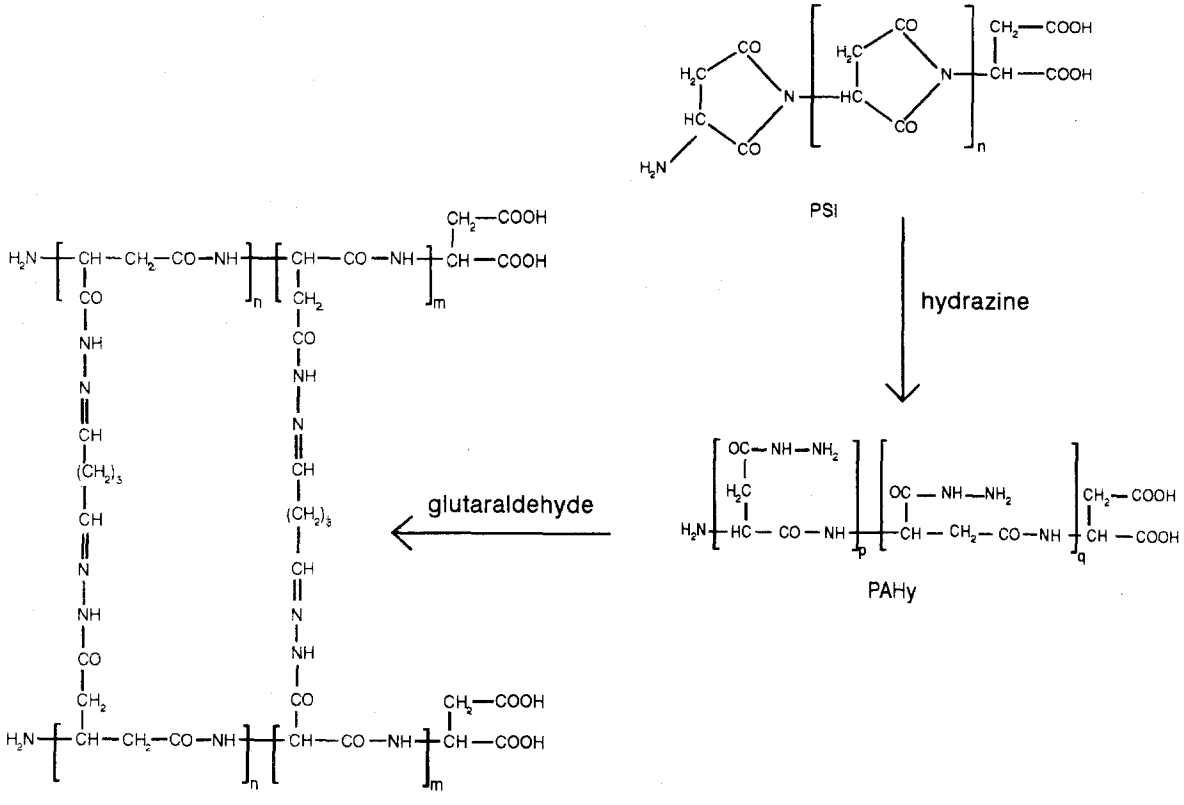
An outline of the steps involved in the synthesis of PAHy hydrogels is given in scheme 1.

The obtained networks are insoluble in water, common organic solvents (*N*,*N*-dimethylformamide, dimethylsulfoxide, methylene chloride, acetone, ethanol, methanol) and basic or acid aqueous solution. This observation confirms that in our prepared systems the individual chains of PAHy are crosslinked by chemical bonds.

In addition, since sulfuric acid is not desirable in pharmaceutical dosage forms, we prepared two batchs of network (samples 2′ and 4′) in the absence of this acid (see the Experimental Section).

The crosslinked polymers were characterized by swelling tests. Swelling effects were determined by the ratio:

$$Q = [(Ws - Wd)/Ws] \times 100,$$



scheme 1

where Ws and Wd are the weight of swollen and the unswollen samples, respectively. In Fig. 1 Q is reported as a function of X, i.e., the ratio of moles of glutaraldehyde to moles of PAHy repeating unit.

It can be observed that Q continuously decreases with X, thus indicating a continuous increase of the crosslinking degree. The effectiveness of the aldehyde as crosslinking agent becomes less marked for X values greater than 1.2. This can be related to a very high crosslinking density yet obtained for $X = 1.2$. Furthermore, it is worth nothing that no significant differences are seen between data relative to samples 2 and 2′ and between 4 and 4′. This result indicates that both acetic and sulfuric acid act in the same way as catalysis agents in the crosslinking reaction of the polymer.

For a better understanding of the structural changes in the polymer, following the crosslinking, calorimetric tests have been performed. The absence of melting peaks in the DSC thermograms indicates that the polymer is always amorphous. Significant effects, on varyng the crosslinking density, have been observed on the glass transition temperature. In Fig. 2 Tg values as a function of X are reported.

Tg decreases up to $X = 0.8$, then quickly increases until values higher than the glass transition temperature of the starting uncrosslinked

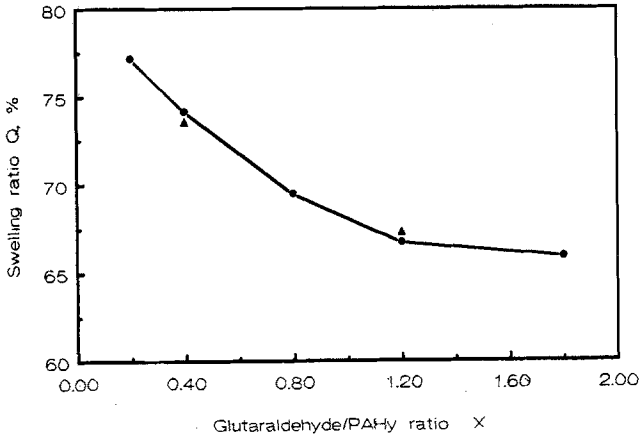


Fig. 1. Swelling ratio, Q, versus glutaraldeyde/PAHy ratio, X. (●) samples prepared in the presence of sulfuric acid (△) samples prepared in the absence of sulfuric acid

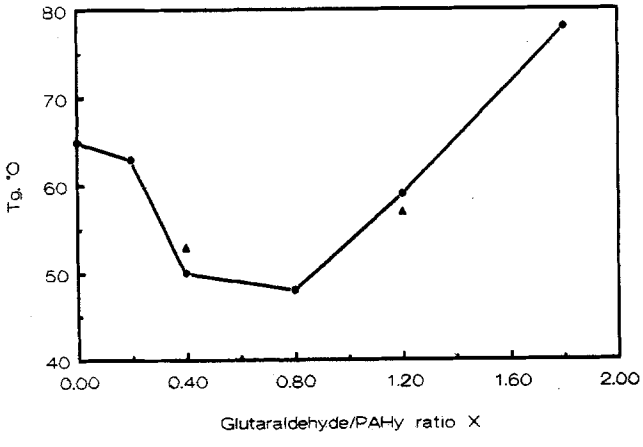


Fig. 2 Glass transition temperature, Tg, versus glutaraldeyde/PAHy ratio, X. Key for symbols as in Fig. 1

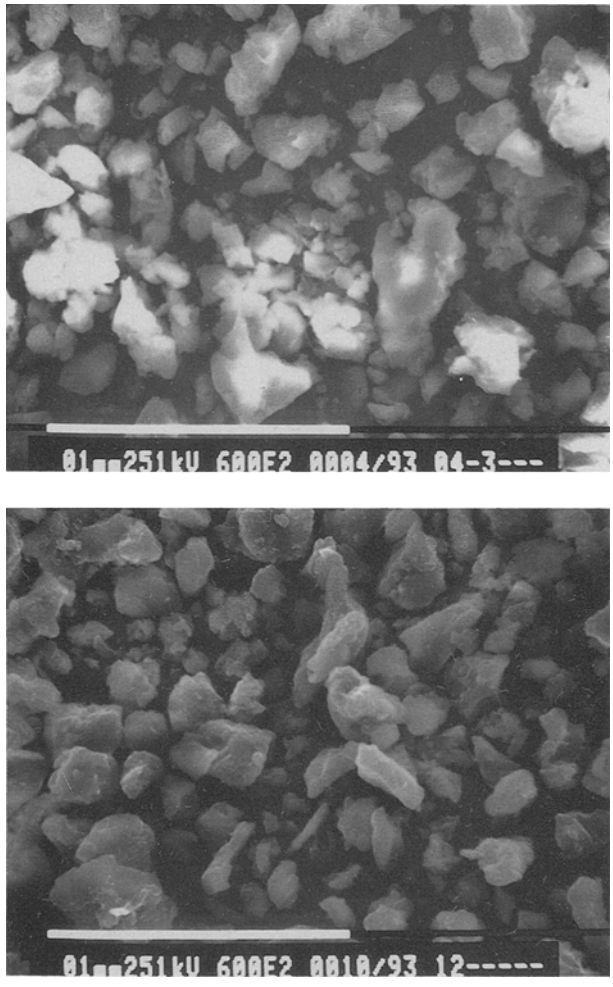


Fig. 3 Scanning electron micrographs. a) sample 2 b) sample 4

polymer are obtained. This trend can be related to two different and opposing factors. On the one hand, as was also observed from swelling data, the increase in the aldehyde percentage increased the degree of crosslinking and the rigidity of the structure. On the other hand, we have to consider that the glutaraldehyde, during its crosslinking action, acts as bridge between two different chains of α, β-polyasparthydrazide (see scheme 1), thus reducing the total polarity of the polymer. It is well known that the first effect causes an increase of the glass transition temperature, whereas the latter causes a decrease. We can conclude that up to the glutharaldehyde percentage corresponding to $X = 0.8$ the prevailing effect is the decrease in the polarity, whereas at higher values the prevailing effect is the increase in rigidity of the structure.

Finally, the crosslinked polymer was analyzed by scanning electron microscopy. No significant differences were observed on varyng the crosslinking degree. In fact, all samples showed an almost homogeneous presence of microparticles smaller than 100 μm. This can be seen in Fig. 3, where SEM micrographs of the samples 2 ($X = 0.4$) and 4 ($X = 1.2$) are reported for example.

Concluding remarks

In this work, the results of the crosslinking of α, β-polyasparthydrazide with glutaraldeyde are presented. The obtained networks have been studied as new hydrogels. For this purpose, swelling tests were performed and data indicate a good affinity of these systems towards the aqueous medium. Also, other characterizations were determined. In particular, glass transition values, determined through calorimetric analysis, indicate a peculiar effect of the glutaraldehyde grafting in the PAHy chains. Finally, SEM analysis showed the presence of an almost homogeneous distribution of microparticles in all crosslinked materials.

Acknowledgements

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