



Synthesis of Natural Base Hydrogels by Using γ -Radiation Polymerization of Protein and Acrylic Monomers

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Several hydrogels were prepared using radiolytic polymerization of aqueous solutions of 2-acrylamido-2-methylpropane sulfonic acid containing appropriate comonomer such as acrylic acid. The hydrogels have been prepared at an irradiation dose of 30 kGy. The hydrogels obtained were characterized using swelling technique, thermal and spectroscopic analysis.

Key Words: Collagen, Irradiation, γ -Radiation polymerization, Monomers.

INTRODUCTION

Hydrogels are materials that exhibit the ability to swell in water and retain significant fractions of water within the structure. Their ability to absorb water is due to the presence of hydrophilic groups such as OH, CONH₂, CONH⁻, COOH and SO₃H^{1,2}. Applications of hydrogels have received considerable attention by many researchers in a variety of fields. These fields include enzyme immobilization, biosorbents in preparative chromatography and the development of various biomedical systems ranging from biosensors to artificial muscles³. Two other fields in which hydrogels are prominent include site-specific drug delivery systems⁴⁻¹² and super absorbent polymers. The application of radiation techniques to obtain polymeric materials for different purposes began in the last few years. Electron beam irradiation of aqueous solutions containing appropriate monomer mixtures such as acrylamide, acrylic acid and vinyl acetate were prepared and used as flocculants for waste water treatment and for agriculture and medicine¹³. The hydrogels based on polyelectrolyte structure have been prepared using γ -radiation of aqueous solutions of hydrophilic monomers. Aqueous solutions of acrylamide and N-vinyl-2-pyrrolidone with small amounts of maleic acid and itaconic acid were regarded as systems with potential immobilization, chelating and adsorptive properties for various bioapplications. Thus, bovine serum albumin was investigated by using gels with varying compositions of maleic acid¹⁴ and itaconic acid moieties¹⁵. The wound dressings manufactured by the simultaneous radiation induced crosslinking and sterilization of hydrophilic polymers were produced by Rosiak *et al*¹⁶. This indicates that the composition of hydrogels is

essential in determining its applications and its efficiency. Thus, the present work deals with the radiation-induced polymerization of acrylic monomers in the presence of, N,N'-methylene bis-allyamide as crosslinking agent.

EXPERIMENTAL

Hydrolyzed collagen (Parvar Novin-E Tehran Co.) was industrial grade which is available in market and has nearly 25 % insoluble phosphate salt. 2-Acrylamido-2-methylpropane sulfonic acid (Merck, Darmstadt, Germany) and acrylic acid (Fluka) were of analytical grade and used without further purification.

Preparation of hydrogels: A pre-weighed amount of hydrolyzed collagen (0.5-2.5 g) was dissolved in 50 mL degassed distilled water and filtered to remove its insoluble salt. The hydrogels were prepared by mixing monomer(s) with bidistilled water in a test tube and finally subjected to Co-60 γ -source at 30 kGy. The dose rate was kept constant at 3 Gy/s. The irradiation has been carried out using the γ -chamber cell facility in The Nuclear Radiation Center at the National Center for Radiation Research and Technology, Arak City, Iran. The hydrogels obtained after radiation were dried in under vacuum oven at 60 °C till constant weight. An inert gas (argon) was also gently bubbled into the reactor to remove the oxygen during the graft copolymerization reaction. The product was then worked up with methanol (200 mL) and dried in oven at 50 °C for 5 h.

Determination of water uptake

Suction filtration method: The sample (0.5 g) was immersed in an excess of deionized water or 0.9 wt % aqueous

solution of NaCl for 8 h to reach its swelling equilibrium at room temperature. The residual water was removed by suction filtration with an aspirator (250 mmHg) for 5 min. The gel was weighed and the equilibrium absorbency (Q_{eq}) was calculated by the following equation:

$$Q_{eq} = \frac{W_{wet} - W_{dry}}{W_{dry}} \quad (1)$$

where W_{dry} is the weight of the dried sample and W_{wet} is the weight of the swollen sample.

Tea-bag method: The tea bag was made of 250-mesh nylon screen. A tea bag containing the sample (0.5 g) was immersed entirely in deionized water or the saline solution until the swelling equilibrium was reached and the tea bag was then hung for 15 min to drain the excess solution and weighed. Q_{eq} was calculated by eqn. 1.

Infrared analysis: The samples were crushed with KBr to make pellets. Spectra were taken on an ABB Bomem MB-100 FTIR spectrophotometer.

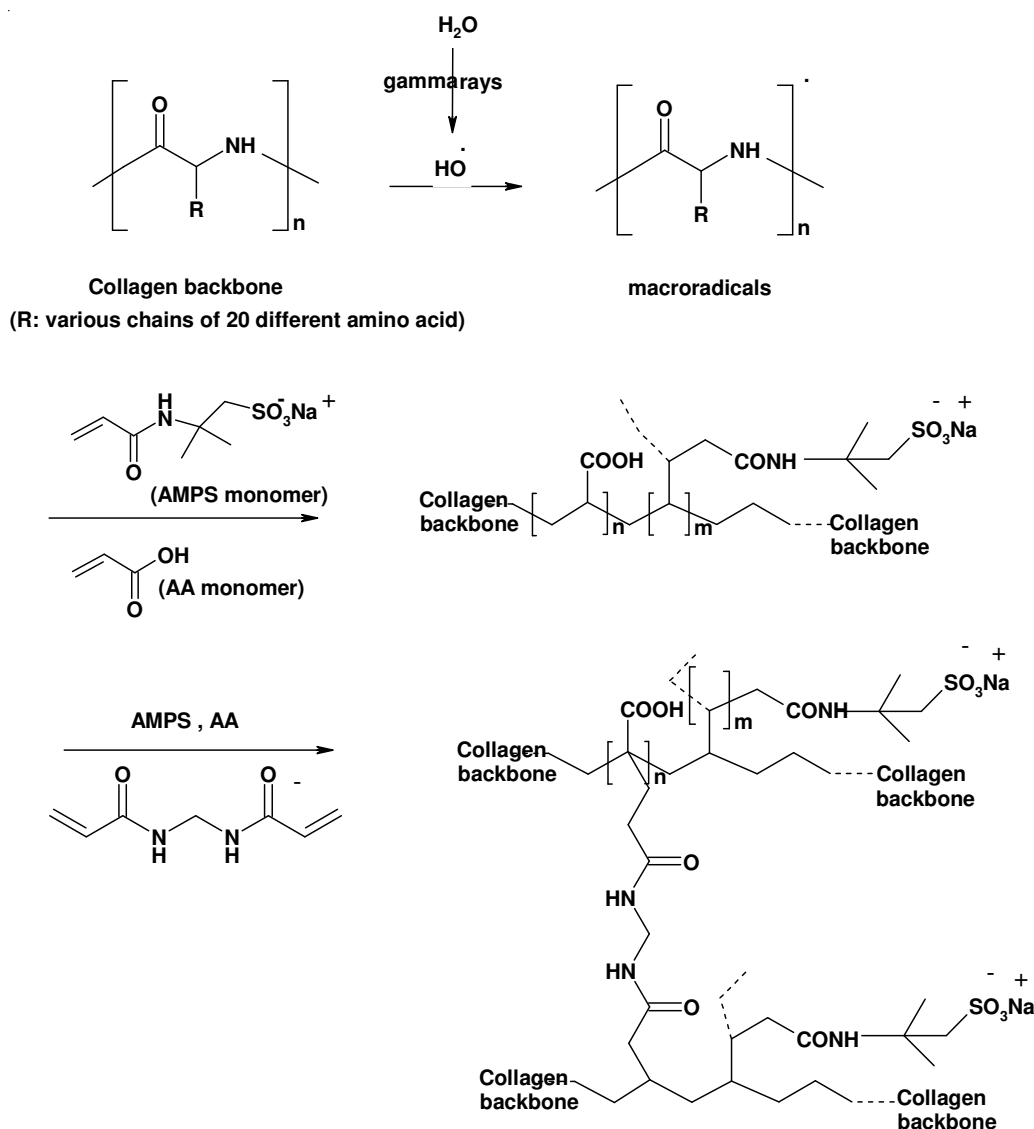
Morphological analysis: Morphology is a critical factor to analyze final result. In this work, the morphology of the

hydrogels was observed by a scanning electron microscope (SEM; Leo, 1455 VP) operating at an accelerating voltage of 20 kV. All samples were mounted on a copper stub and sputter-coated with gold to minimize charging.

Thermal analysis: Thermogravimetric analyses were performed on a Universal V4.1D TA Instruments (SDT Q600) with 8-10 mg samples on a platinum pan under nitrogen atmosphere. Experiments were performed at a heating rate of 10 °C/min until 600 °C.

RESULTS AND DISCUSSION

Grafting mechanism: A general reaction mechanism for graft copolymerization of 2-acrylamido-2-methylpropane sulfonic onto collagen backbones in the presence of γ -radiation is shown in **Scheme-I**. In the first step, the γ -radiation, with dose of 30 kGy, produce the radicals from one of the functional groups (*i.e.*, COOH, SH, OH and NH₂) in side chains of the collagen backbones. Then these macroradicals initiate grafting of monomers onto collagen backbones leading to a graft copolymer. Since a crosslinking agent, *e.g.*, N,N-methylene bis-acrylamide, is presented in the system, the copolymer



Scheme-I: Proposed mechanistic pathway for synthesis of H-collagen-g-poly(AMPS-co-AA) hydrogel

comprises a crosslinked structure that has called super absorbent hydrogel.

Evidence for grafting: Fig. 1 shows the IR spectra of the collagen and the resulted graft copolymer. The band observed at 1658 cm^{-1} can be attributed to C=O stretching in carboxamide functional groups of substrate backbone (Fig. 1a). The broad band at $3600\text{--}3200\text{ cm}^{-1}$ region is due to stretching of -OH groups of the collagen. The collagen-g-AMPS-co-AA hydrogel comprises a collagen backbone with side chains that carry sulfate and carboxylate groups that are evidenced by a new characteristic absorption band at $1710\text{--}1222\text{ cm}^{-1}$ (Fig. 1b). This peak attributed to ester sulfate stretching of 2-acrylamido-2-methylpropane sulfonic and carbonyl stretching of acrylic acid groups. The stretching band of -NH overlapped with the OH stretching band of the collagen portion of the copolymer^{17,18}.

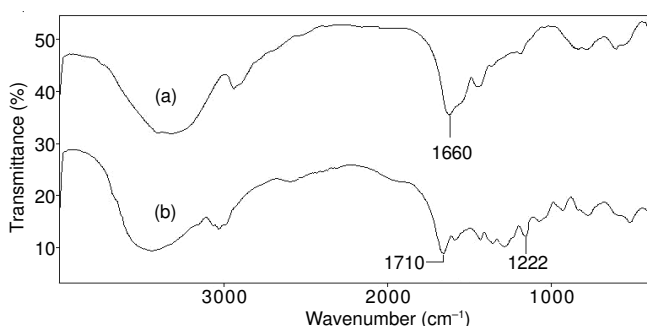


Fig. 1. FTIR spectra of collagen (a) and collagen-g-poly(AMPS-co-AA) hydrogel (b)

Scanning electron microscopy: One of the most important properties that must be considered is hydrogel microstructure morphologies. Fig. 2 shows the scanning electron microscope images of the hydrogel. This picture verifies that the synthesized polymer in this work have a porous structure. It is supposed that these pores are the regions of water permeation and interaction sites of external stimuli with the hydrophilic groups of the graft copolymers.

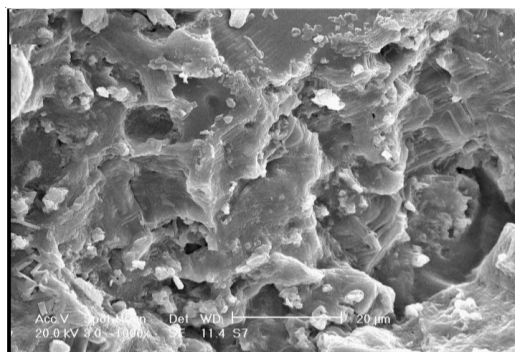


Fig. 2. SEM photographs of the hydrogel. Surfaces were taken at a magnification of 1000X and the scale bar of $20\text{ }\mu\text{m}$

Thermal gravimetric analysis (TGA): Thermogravimetric analysis (TGA) method was employed to thermally characterize the hydrogel in comparison with the intact

polysaccharide (Fig. 3). In the TGA curve of collagen-g-poly(AMPS-co-AA) *ca.* 10-12 % loss in weight is observed below $130\text{ }^{\circ}\text{C}$. This was attributed to the removal of the absorbed water. Fig. 3 shows that degradation of native collagen is faster than that of grafted collagen. *Ca.* 45 % weight loss takes place in the temperature of $280\text{ }^{\circ}\text{C}$ for collagen. A residual weight of 72 % is observed at $280\text{ }^{\circ}\text{C}$ for collagen-g-poly(AMPS-co-AA) sample.

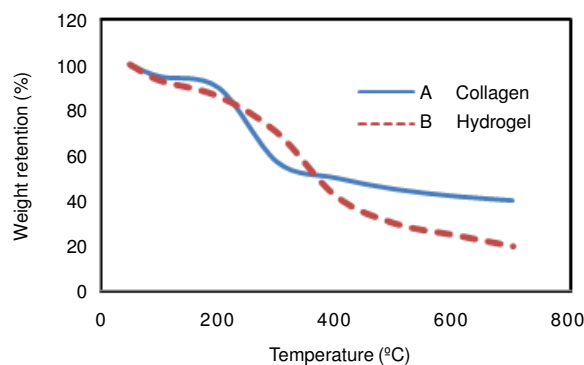


Fig. 3. TGA curves of (a) collagen and (b) collagen-g-poly(AMPS-co-AA)

Conclusion

The monomers, 2-acrylamido-2-methylpropane sulfonic acid (AMPS) and acrylic acid (AA), can be easily graft copolymerized onto collagen using γ -radiation as an initiator in aqueous medium. In order to prove that 2-acrylamido-2-methylpropanesulfonic acid molecules were grafted, FTIR, SEM spectroscopies and TGA analysis were used. The study of FTIR spectra shows that in the hydrogel spectrum a new absorption bands at $1710\text{--}1221\text{ cm}^{-1}$ was appeared that attributed to the formation sulfonate and carboxylate anions onto polysaccharide backbones, respectively.

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