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Graft copolymerization of Gelatin-g-poly (Acrylic acid-co-Acrylamide) and calculation of grafting parameters

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Abstract

In this research, we synthesized a novel graft copolymer of gelatin-based via radical polymerization mixtures Acrylic acid (AcA) and Acrylamide (AAm) onto gelatin backbones. The polymerization reaction was carried out in an aqueous medium and in the presence of ammonium persulfate (APS) as an initiator. Evidence of grafting was obtained by comparing FTIR and TGA analysis of CMC and the graft copolymer as well as solubility characteristics of the products. The effect of grafting variables, i.e. concentration of AAm, AcA, APS and Gelatin, and temperature was systematically optimized to achieve a highest percent grafting possible. The overall activation energy for the grafting was also estimated to be 23.30kJ/mole.

Keywords: Gelatin, Graft copolymerization, Acrylamide, Acrylic acid, Optimization.

Introduction

Free radical vinyl graft copolymerization onto natural polymers is a well-known method for synthesis of natural-based superabsorbent hydrogels. In fact, an efficient approach to modify of natural polymers in order to synthesis of natural-based SAPs is graft polymerization of vinylic monomers onto their backbones in the presence of cross-linkers. Free radical graft copolymerization with various monomers can carried out with different initiator systems (Sandle *et al.,* 1987; Athawale & Rathi, 1997; Heinze & Liebert, 2001). Although hydrogels made from synthetic polymers, such as polyacrylate posses excellent water-absorbing properties, their toxicity and non-bio-degradability might pose long-time environmental problems and limit their use in drug delivery systems and consumer products. Natural-based SAPs have attracted much attention in medical and pharmaceutical fields because of their non-toxicity, biocompatibility and biodegradability (Athawale & Rathi, 1999).

As a protein, gelatin is a biomaterial with the above mentioned essential properties. Generally, cross-linking in gelatin is used in various purposes such as gelatin swelling and gelatin hydrogels as biodegradable implants to deliver small and macromolecular drugs. Recently, attention has been focused on employing gelatin substrate to produce hydrogels with a specific response to a biological environment (Hebeish & Guthrie, 1981; Okieimen & Ogbeifun, 1996; Mohammad Sadeghi & Nahid Ghasemi, 2012).

Although, APS-initiated grafting of vinyl monomers such as methyl acrylate, ethyl acrylate and ethyl methacrylate (Okieimen, 1998), AN/methyl methacrylate mixture (Okieimen & Ogbeifun, 1996), acrylamide (AAm) (Okieimen, 2003), and 4-vinylpyridine (Leza *et al*., 1989) onto gelatin has been reported. However, to the best of our knowledge, no report has been published on the optimization graft polymerization of Acrylic acid (AcA) and Acrylamide (AAm) together onto Gelatin chains using APS-Protein initiating system. In the present study, to modify the Gelatin, the grafting of Acrylic acid and Acrylamide onto Gelatin chains in the presence of ammonium persulfate (APS) as an initiator was performed in a homogeneous system. The effect of reaction variables affecting on percent grafting was investigated.

Experimental

Materials

Gelatin (from Parvar Novin-E Tehran Co.), potassium persulfate (APS, from Fluka), Acrylamide (Merck) and Acrylic acid (Merck) were used without further purification. All other chemicals were also analytical grade. Double distilled water was used for graft copolymer preparation.

Preparation of Graft Copolymer

A general procedure for chemically graft copolymerization of Acrylamide (AAm) and Acrylic acid (AcA) onto gelatin backbones was conducted as follows: Gelatin (1.0 g) was added to a three-neck reactor equipped with a mechanical stirrer (Heidolph RZR 2021, three blade propeller type, 300 rpm), including 35 mL double distilled water. The reactor was immersed in a thermo-stated water bath preset at a desired temperature (70^{o}C). Then 0.10 g of APS as an initiator was added to gelatin solution and was allowed to stir for 10 min. After adding APS, variable amounts of AAm and AcA (AAm 0.50-2.50 g, AcA 0.50-2.50 g) were added simultaneously to the gelatin solution. After 90 min, the reaction product was allowed to cool to ambient temperature. The graft copolymer was poured to excess non solvent ethanol (200 mL) and remained for 3 h to dewater. Then ethanol was decanted and the product slices to small pieces (diameter ≈ 5 mm). Again, 100 mL fresh ethanol was added and the graft copolymer was remained for 24 h (Pourjavadi & Zohuriaan-Mehr, 2002).

Homopolymer extraction

The graft copolymer, namely gelatin-g-Poly (AAm-co-AcA), was freed from homo-polymers, by pouring 1.00 g of the product in 75 mL of DMF solution. The mixture was stirred gently at room temperature for 48 h. After

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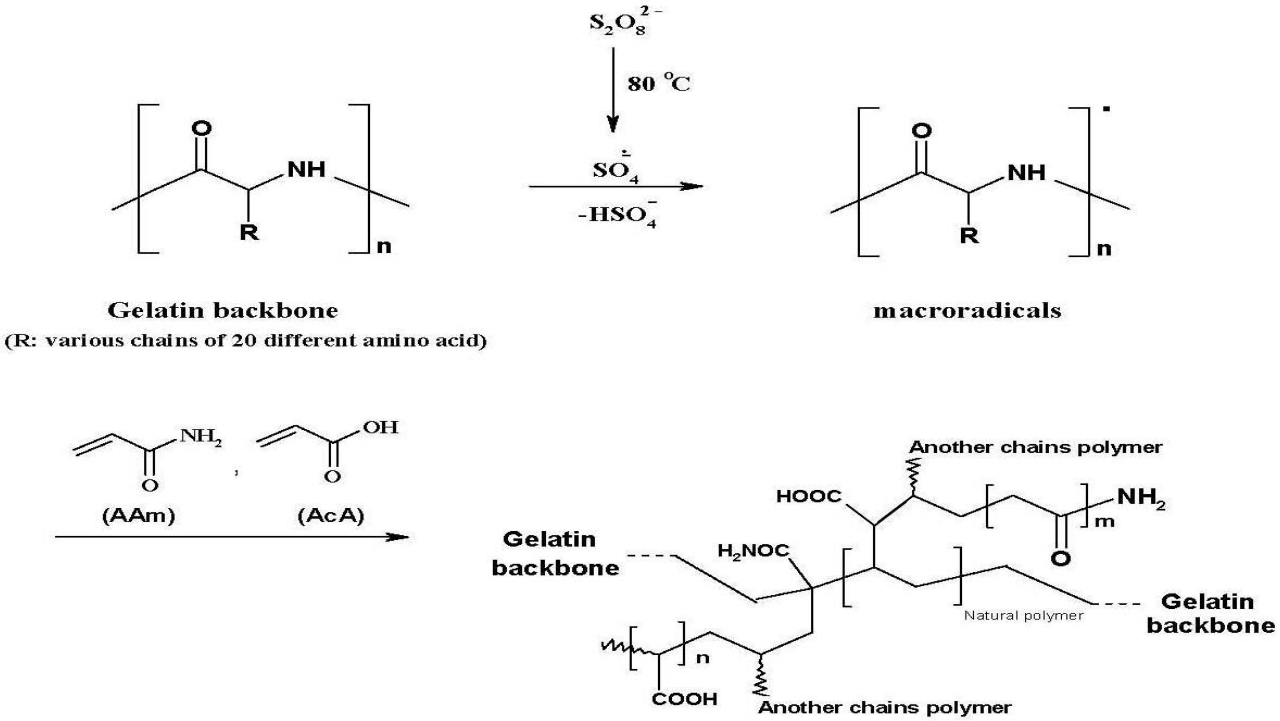
complete removal of the homo-polymers by centrifugation, the gelatin-g-Poly(AAm-co-AcA), was filtered and washed with methanol and dried in oven at 50˚C to reach a constant weight (Pourjavadi & Zohuriaan-Mehr, 2002; Zohuriaan *et al*., 2005) Although, dimethylformamide is a good solvent for PAAm and PAcA as well as a precipitant for pure gelatin or grafted gelatin, so the homo-polymers could be easily separated from the rough products. However, it seemed to be difficult to further separate the unreacted gelatin from the products and the right separation methods are still in progress in my research. In view of the modification intention, the unreacted gelatin is not very necessary to be separated from the products. So the blends of unreacted gelatin and the graft copolymer CMC-*g*-Poly (AAm-co-PAcA) were actually obtained in this research and their compositions were unknown. In several studies on the grafting modification of polymer, the unreacted substrate polymer and graft copolymer were also not separated from the products (Zhang *et al*, 2000; Zhang, 2000; Ibrahim *et al*., 2002). If the unreacted gelatin could be separated from the products, the graft copolymer CMC-*g*-Poly(AAm-co-PAcA) with higher G% could be obtained, but the values of PC% and GE% would not be affected.

Instrumental analysis

The gelatin-g-Poly(AAm-co-AcA) samples were characterized as KBr pellets using a Mattson-1000 FTIR spectrophotometer. Thermo-gravimetric analyses were also 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 700°C.

Scheme1. Proposed mechanistic pathway for synthesis of gelatin-g-poly(AAm-co-AcA) copolymer.



Grafting parameters

The grafting parameters, i.e. grafting-rati (Gr %), grafting-efficiency (Ge %), add-on value (Ad %), and homopolymers content (Hp %) used to characterize the nature of the copolymer are defined and calculated using the following equations (Fanta & Doane,1986):

$Gr\ \% = 100\ (W_2 - W_0) / W_0$ (1)

$Ad\ \% = 100\ (W_2 - W_0) / W_2$ (2)

$Hp\ \% = 100\ (W_1 - W_2) / W_1$ (3)

$Ge\ \% = 100\ (W_2) / W_1$ (4)

where W_0, W_1, and W_2 are the weight of the initial substrate, total product (copolymer and homopolymers) and pure graft copolymer (after DMF extraction) respectively.

Results and discussion

Synthesis and spectral characterization

The mixture of monomers, acryl-amide and acrylic acid was simultaneously grafted onto gelatin backbones in a homogeneous medium using APS as a radical initiator. A general reaction mechanism for gelatin-*g*- poly(AcA-co-AAm) copolymer formation is shown in Scheme 1. At the first step, the thermally dissociating initiator, i.e. APS, is decomposed under heating to produce sulfate anion-radical. Then, the anion-radical abstracts hydrogen from one of the functional groups in side chains (i.e. COOH, SH, OH, and NH_2) of the substrate to form corresponding radical. So, these macroradicals initiated monomers

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grafting onto gelatin backbones led to a graft copolymer (Mohammad & Hossein, 2010).

Infrared spectroscopy was carried out to confirm the chemical structure of the copolymer. Fig.1 shows the FTIR spectra of the gelatin substrate and the synthesized copolymer. The band observed at 1634 cm^{-1} can be attributed to C=O stretching in carboxamide functional groups of substrate backbone (Fig.1a). The graft copolymer product comprises a gelatin backbone with side chains that carry carboxylate and carboxamide functional groups that are evidenced by peaks at 1558 and 1637 cm^{-1} respectively (Fig. 1b). The characteristic band at 1558 cm^{-1} is due to asymmetric stretching in carboxylate anion that is reconfirmed by another peak at 1411 cm^{-1} which is related to the symmetric stretching mode of the carboxylate anion (Zhang *et al.,* 2000; Ibrahim *et al.,* 2002). The stretching band of the grafted carboxamide groups overlapped with that of the gelatin portion of the copolymer.

Thermogravimetric behavior

The grafting was also supported by thermo-gravimetric analysis (Fig. 2). TGA of gelatin (Fig. 2a) shows a weight loss in two distinct stages. In the TGA curve gelatin-*g*-poly (AAm-co-NaAcA) copolymer about 10-12% loss in weight is observed below 130^{o}C. This was attributed to the removal of the absorbed water. Also the Fig.2 shows that the degradation of native gelatin is faster than that of grafted gelatin. About 45% weight loss takes place in the temperature of 280^{o}C for gelatin. A residual weight of 72% is observed at 280^{o}C for gelatin-*g*-poly (AAm-co-NaAcA) copolymer. In general, the copolymer had lower weight loss than Gelatin. This means that the grafting of Gelatin increases the thermal stability of Gelatin in some extent (Doyle, 1961).

Solubility test

To obtain an additional evidence of grafting, solubility difference between the grafted and the non-grafted polymer was used. Gelatin and poly (AAm-co-AcA) are soluble in water and DMF respectively. When a reaction product was extracted with DMF and alternatively with water for 48 h, an insoluble solid still remained. A physical mixture of gelatin and poly (AAm-co-AcA) was treated in the same way and was found to dissolve completely. Therefore, it is obvious that the resulted graft copolymer was not a simple physical mixture, but some chemical bonds must exist between the gelatin substrate and poly (AAm-co-AcA) macromolecules (Mohammad & Hossein, 2010).

Optimization of polymerization

In the present investigation, the effect of concentration of gelatin, APS, AcA and AAm, along with reaction temperature was studied to optimize the reaction conditions. It may be found from the related curves (next figures) that the trends of the "changes" are similar for grafting parameters Gr, Ge, Hp, and Ad. The reason is the similar concepts applied for defining the grafting parameters (Eqs. 1-4).

Effect of initiator concentration

The effect of concentration APS on graft polymerization was studied by changing its concentration from 0.0003 to 0.0008mol/L (Fig. 3). It was observed that the grafting percent is increased versus increasing the APS concentration from 0.01 up to 0.00055 mol/L and then, it is decreased considerably with a further increase in the amount of APS. The maximum grafting percent (160%) is obtained at APS 0.00055 mol/L. The number of active free radicals on the gelatin backbone is increased in terms of the initiator levels lower than 0.00055 mol/L. This accounts for the initial increment in grafting percent up to a certain amount of APS. The grafting percent decrease after the maximum may be attributed to increased number of produced radicals led to terminating step via bimolecular collision resulting in enhanced crosslink density (Pourjavadi & Zohuriaan-Mehr, 2002).

An additional reason for decreasing the grafting percent can be related to decreasing molecular weight (MW) of the grafted polyacrylamide and polyacrylic acid at high levels of APS concentration. Since MW inversely depends on initiator concentration (I), and the higher I results in lower MW in turns to be a lower grafting percent of the copolymer (Zohuriaan *et al.,* 2005). On the other hand, free radical degradation of gelatin substrate is also possible at high APS levels. A similar observation is recently reported in the case of degradation of chitosan with potassium persulfate (Hsu & Don, 2002).

Effect of reaction temperature

To study the influence of the reaction bath temperature on the grafting parameters, the grafting of AAm, AcA onto gelatin was carried out at temperature ranging from 35-70^{o}C. As shown in Fig. 4, higher temperatures favor the rate of diffusion of monomers to the gelatin macroradicals as well as increase the kinetic energy of radical centers. In addition, higher temperatures increase the rate of decomposition of the thermally dissociating initiator APS (Zohuriaan *et al.,* 2005). The temperatures higher than the optimum value (55^{o}C), however, lead to low-graft copolymer. This percent-loss may be attributed to (a) oxidative degradation of gelatin chains by sulfate radical-anions, (b) increasing the rate of termination and chain transfer reactions, and (c) decomposition of APS to give O_2 (a radical scavenger), which reacts with primary free radicals (Eqs. 5 and 6), (Hsu *et al.,* 2002), resulting in decreased molecular weight and decreased grafting (the sulfate radical anions may react with water to produce hydroxyl radicals (Eq. 5) and finally oxygen (Eq. 6)).

$$\dot{SO}_4^- + H_2O \longrightarrow HSO_4^- + \dot{HO} \quad (5)$$

$$2\dot{HO} \longrightarrow H_2O_2 \longrightarrow H_2O + 1/2\,O_2 \quad (6)$$

The rates graft copolymerization (R_g) may be evaluated as measures of the rate of monomer disappearance by using the following equations (Fanta & Doane, 1986):

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Fig.1. FTIR spectra of pure gelatin (a) and gelatin-g-poly (AAm-co-NaAcA) copolymer(b).

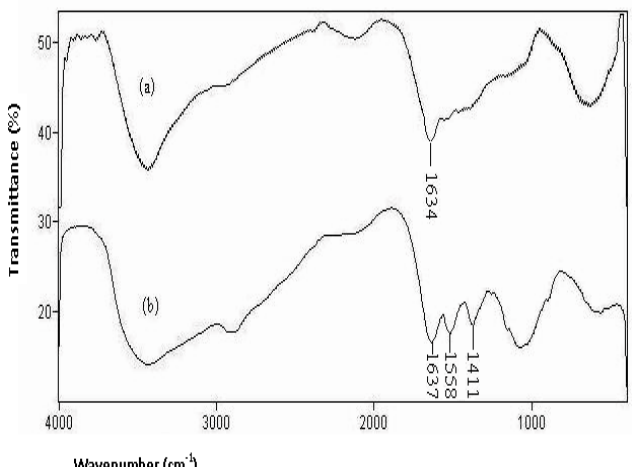


Fig. 2. TGA curves of (a) gelatin and (b) gelatin-g-Poly (AAm-co-AcA).

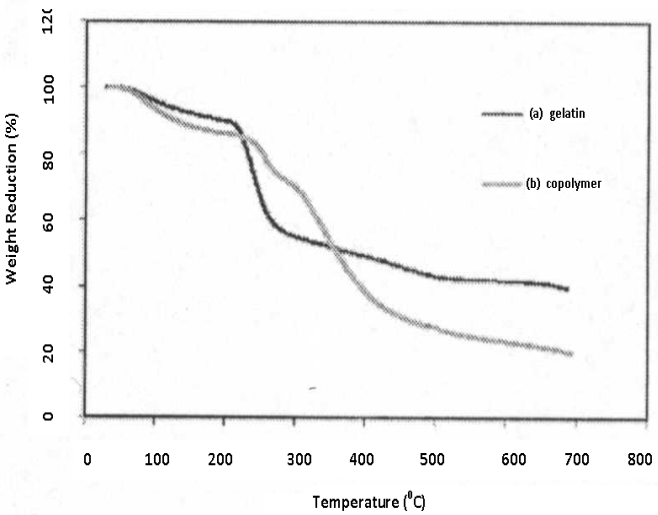


Fig.3. Grafting percent variances with concentration of ammonium persulfate variance

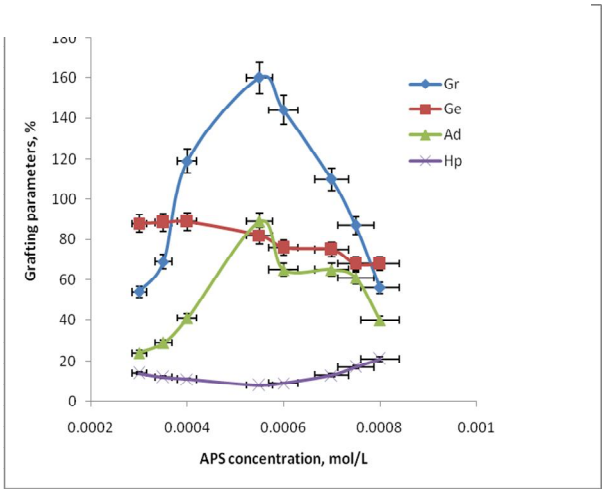


Fig. 4. Grafting percent variances with temperature variance

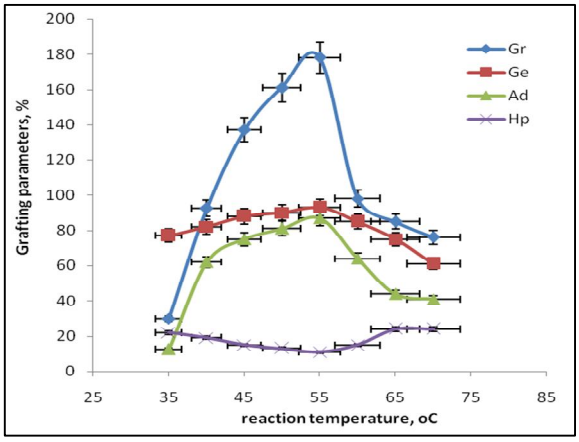


Fig. 5. Plot of lnR_g-1/T for estimating the activation energy of the graft polymerization reaction.

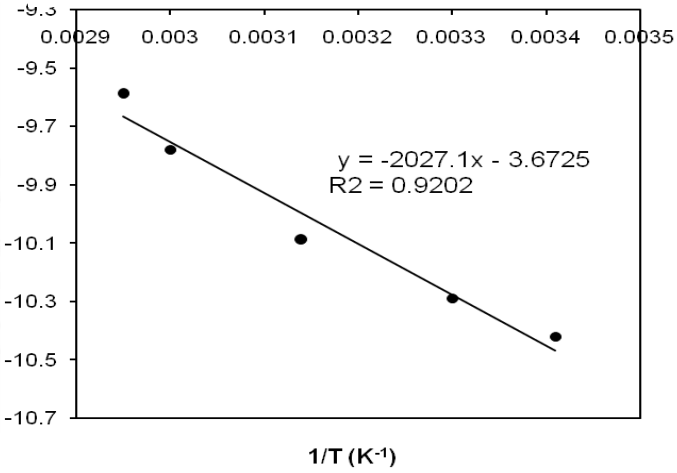
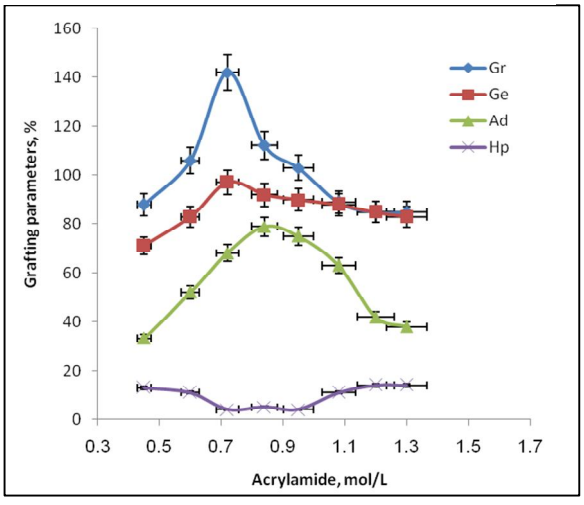


Fig. 6. Grafting percent variances with amount of acrylamide monomer variance



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$$Rg\left(mol.\ s^{-1}.m^{-3}\right) = \frac{Weight\ of\ grafted\ polymer}{Molecular weight\ of\ monomer \times [rection\ time\ (s)] \times volume\ (m^3)} \quad (7)$$

Overall activation energy of grafting (E_a) may also be estimated from the temperature data through plotting $\ln R_g$ versus 1/T ($^oK^{-1}$) for the initial portion of the data of the temperature series given in above text**.** The slope of this Arrhenius plot (Fig. 5) resulted in a rough estimation of Ea of grafting using the relationship slope = $-E_a/R$; where R is the universal gas constant. Therefore, Ea for the graft copolymerization was found to be 23.30 kJ/mole (5.57 kcal/mole).

Effect of AAm concentration

The effect of monomer amount on the grafting reaction was studied at various concentrations of AAm while other influential factors were unchanged. The grafting parameter variations are changed by the amount of charged monomer (Pourjavadi & Zohuriaan-Mehr, 2002). The results are given in Fig. 6. The grafting extent is significantly increased due to more availability of monomer for grafting. However, beyond a certain Gr value, *i.e*., 142% at AAm 3.5 mL, the trend is inversed. The conversion and the grafting efficiency (Ge) are decreased, and homopolymer content is increased noticeably from 4 to 14 percent. Thus, acrylamid in an amount of AAm 3.5 mL was recognized as an optimum monomer concentration. Once the monomer units are added, an excess of monomer can only increase the optimum volume of the reaction mixture (Pourjavadi & Zohuriaan-Mehr, 2002; Zohuriaan, 2005).

Fig. 7. Grafting percent variances with amount of AMPS monomer variance

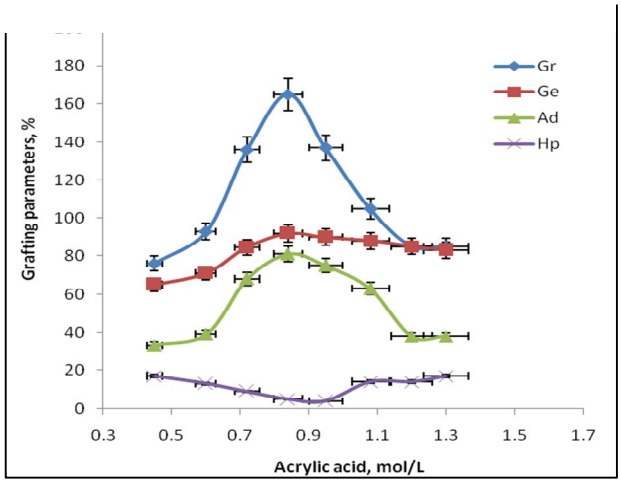
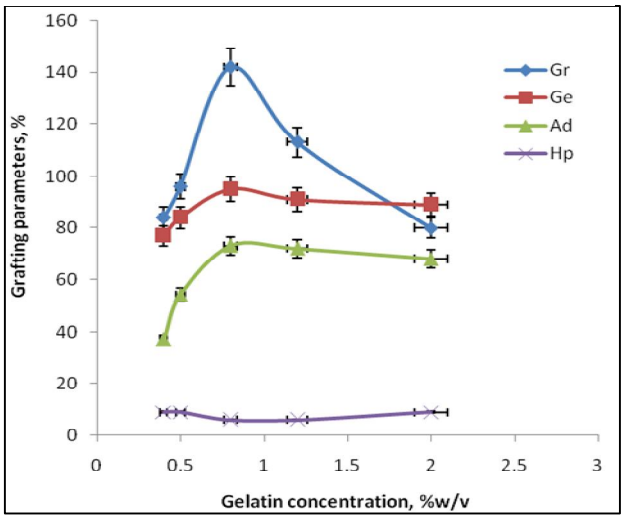


Fig. 8. Grafting percent variances with amount of gelatin variance.



Effect of AcA concentration

The Acrylic acid concentration was varied from 0.45 to 1.30 mol/L equal (1.5-4.7 mL) to study its effects on grafting parameters (Fig. 7). These parameters were found to be increased by enhancement of AcA concentration from 0.45 up to 0.84 mol/L. This behavior can be attributed to the increase of monomer concentration in the vicinity of the gelatin backbone and consequent greater availability and enhancement chances for molecular collisions of the reactants. The decrease in %Gr and %Ad after a certain level of AcA (0.84 mol/L) is probably due to preferential homopolymerization over graft copolymerization as well as increasing the viscosity of reaction medium, which hinders the movement of free radicals(Mohammad & Hossein, 2010). Needless to say, the increase in the chain transfer to monomer molecules may be other possible reason for the diminished grafting at higher AcA concentrations. Similar observations have been reported for the grafting of ethyl acrylate onto cellulose (Okieimen & Ogbeifun, 1996; Ibrahim *et al.,* 2002) and methyl acrylate onto starch (Athawale & Lele, 2002).

Effect of gelatin concentration

The related to the grafting dependence on Gelatin amount is summarized in Fig.8. Maximum grafting percent and the lowest homopolymers formation was observed at 0.80 g Gelatin, while others reactants including, monomers, initiator, and temperature were kept constant. Beyond this value, both grafting percent and add-on values are considerably reduced. This behavior is attributed to the availability of more grafting sites for initiation of graft copolymerization at higher concentration of the substrate (until 0.80 g Gelatin). However, upon further increase in the substrate concentration, increase in the reaction medium viscosity restricts the movements of macroradicals leading to decreased grafting percent and add-on values (Hsu *et al.,* 2002). It also may be attributed to deactivation of the macroradical growing chains (*e.g*., by transfer reactions, combination and/or interaction with the primary radicals) soon after their formation (Mohammad & Hossein, 2010).

Conclusion

The monomers, Acrylamide (AAm) and Acrylic acid (AcA) can be easily graft copolymerized onto gelatin

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using APS as an initiator in aqueous medium under an inert atmosphere. In order to prove that monomers were grafted, solubility test, FTIR spectroscopy, TGA analysis were used. The synthetic conditions were systematically optimized through studying the influential factors including temperature, concentration of the initiator, the monomers AAm,AcA and the substrate Gelatin. The effect of the individual factors was investigated by calculating the grafting parameters, *i.e.*, grafting-percent (Gr), grafting-efficiency (Ge), add-on value and homopolymer content (Hp). Under optimum conditions (Gelatin 0.80g, AAm 0.72 $molL^{-1}$, AcA 0.84 $molL^{-1}$, APS 0.00055 mol/L, reaction temperature 55^{o}C), the grafting parameters were achieved as 178%, 97 %, 89% and 11% respectively. Empirical polymerization rate also showed a first-order dependence on the monomer concentration and a half-order dependence on the initiator concentration. According to the slope of LnRg versus 1/T, the overall activation energy for graft copolymerization reaction was estimated to be 23.30 kJ/mole (5.57 kcal/mole).

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