

## Research Article

# Preparation and Characterization of Protein-Based Hydrogel: Cottonseed Protein Grafted with 2-Acrylamido-2-Methylpropanesulfonic Acid (AMPS)

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Using synthetic polymers in the production of superabsorbent polymers offers significant advantages such as low cost, extended service life, and a high water absorption rate. However, concerns about the environmental impact and potential adverse effects on plant growth arise from the degradation products of these polymers after disposal. In addition, handling these polymers can cause rashes, irritations, and even toxic shock syndrome. To overcome these issues, researchers are exploring the synthesis of superabsorbent polymers from natural sources. Cottonseed protein is identified as a potential natural polymer for the synthesis of natural superabsorbent polymers. Notably, there is no existing research on hydrogel synthesis using cottonseed protein and 2-acrylamido-2-methylpropanesulfonic acid (AMPS). This study addresses this gap by focusing on modifying cottonseed protein (CSP) through graft copolymerization, utilizing the partially neutralized form of 2-acrylamido-2-methylpropanesulfonic acid (AMPS) in a water-based solution. N,N-methylene bisacrylamide serves as the crosslinking agent, while potassium persulfate (PPS) and sodium bisulfite (SBS) function as redox initiators. The optimization of hydrogel synthesis conditions was achieved using Design Expert-11 software, adjusting the AMPS to CSP ratio. The research reveals that the hydrogel reaches its maximum swelling capacity (195.7 g/g) with 0.03 g of MBA, 0.01 g of PPS, 0.01 g of SBS, and a 1wt% AMPS to CSP ratio. Swelling properties were assessed under diverse pH conditions, and the study delved into swelling kinetics (both pseudo-first-order model and pseudo-second-order model) and performance under different loads. Grafting evidence was validated through FTIR analysis. The maximum water uptake was obtained when there was no load, and the pH value was around neutral (7). In conclusion, the results indicate that the developed hydrogel holds a promise for applications in water retention, reducing water loss, and serving as an environment-friendly, biocompatible superabsorbent polymer so we can use such hydrogel in biomedical applications.

## 1. Introduction

A hydrogel is a three-dimensional network of water-loving polymers that can expand in water, holding a significant amount of water while keeping its structure intact [1]. This is achieved through the chemical or physical linking of individual polymer chains. Hydrogels were first reported by the authors in [2]. The hydrophilicity of the network is due to the presence of hydrophilic groups such as  $-\text{NH}_2$ ,  $-\text{COOH}$ ,  $-\text{OH}$ ,  $-\text{CONH}_2$ ,  $-\text{CONH}$ , and  $-\text{SO}_3\text{H}$  [3]. Hydrogels or

absorbent polymers can be either natural or synthetic. Proteins [4, 5], chitosan [6–8], polylactic acid (PLA) [9, 10], polyglutamic acid [11], hyaluronic acid [12], and some cellulose derivatives [13, 14] are some natural sources, whereas acrylic acid polymers [15, 16], polyvinyl alcohol [17], polymethyl methacrylates [18], polyvinylpyrrolidone [18], polyacryl amide [19], and polyvinyl acetate [20] are synthetic sources [21].

Cotton, basically (*Gossypium hirsutum* L.), is the third-largest oilseed crop next to soybean and rapeseed in the

world and also a source of relatively high-quality protein [22]. Its protein is under a class of globulins (salt soluble, vicilin, and legumin families) that are the major dominant storage proteins and account for 60%–70% of seed proteins. Albumins (water-soluble) and gliadins (alkali-soluble) are in low concentrations [23]. The most abundant amino acid in cottonseed meal is arginine (15%–34% of total protein) while methionine and cysteine are the least abundant (1%–2%) [20]. But cottonseed protein utilization in food as a protein source has been limited due to the presence of the toxic substance, gossypol, the low lysine level, and high fiber content [24].

Proteins are long-chain polyamino acids arranged in a linear chain and joined together by peptide bonds ( $-\text{CO}-\text{NH}-$ ) between the carboxyl and amino groups of next amino acid residues. The presence of amino and carboxylic acid gives to protein hydrophilicity, which is recognized as surface-active components. Recently, proteins have been increasingly focused on as nonpetroleum, safe, and biodegradable and renewable resource by many researchers. Carboxyl, amido, amide, hydroxy, sulfhydryl groups exist in the main chain of the protein molecule. Chemical modification such as grafting [25], copolymerization [26], and crosslinking could result in the production of desirable protein-based materials [27].

Nontoxic biodegradable hydrogels have been studied extensively for biomedical applications such as bioadhesive, wound dressings for medical applications, biomatrix for drug delivery systems, food packaging, soil additives, industrial dewatering, and also other consumer products such as diapers and feminine sanitary napkins [28]. Proteins and polysaccharides are commonly used to form biopolymer-based hydrogel networks [29]. Protein-based hydrogels are mainly formed by noncovalent interactions between the proteins themselves, such as hydrogen bonds, hydrophobic, or electrostatic interactions, and less frequently, by covalent interactions such as disulfide bonds [30]. The properties of protein hydrogels depend on the protein's intrinsic characteristics as well as the processing conditions used to induce gelation [29]. Protein-based hydrogels have found versatile applications across different fields, including but not limited to tissue engineering [31–33], drug delivery systems [34], wound dressing [13, 35, 36], and heavy metal removal [37–39]. Based on the above considerations, an attempt has been made in this research mainly to prepare hydrogel from the CSP-co-AMPS, and with further tests and parameter consideration, the synthesized hydrogels will be applied for drug delivery purposes but the detail will be the future work of this research.

## 2. Materials and Methods

**2.1. Materials.** Cottonseed protein (CP), obtained according to the classical procedure by authors in [40], 2-acrylamido-2-methylpropane sulfonic acid (AMPS) 98.00% from Songchuan Industrial Additives Co., Ltd., Shandong, China. Potassium persulfate (KPS) 98.50% from Hebei Feisha Technology Co., Ltd., Shijiazhuang China, sodium bisulfite (SBS) 96% from Lianyungang HuaiHua International Trade

Co., Ltd., Jiangsu, China, and N,N'-methylene bisacrylamide (MBA) 99.0% Sancai Industry Co., Ltd., Shandong, China. All chemicals were analytical grade and the laboratories were from Ethiopian Institute of Textile and Fashion Technology (EiTEX), Bahir Dar University.

**2.2. Methods.** The general methods of protein-co-AMPS hydrogel preparation and the mechanistic pathway of synthesized hydrogel are shown in Figures 1 and 2 below, respectively.

**2.2.1. Graft Polymerization of AMPS to CSP.** First, the known weight of CSP (2 g) was dissolved thoroughly in 10 mL distilled water of 8–10 pH value of 0.1 M NaOH and agitated for 60 minutes using a magnetic stirrer for uniform dissolution. The second known weight of AMPS (2 g) was dissolved in 10 ml distilled water and neutralized up to 65% using 0.1 M NaOH. The initiator (0.01 g PPS) and a redox catalyst (0.01 g SBS) and crosslinker N-methylene bisacrylamide (0.03 g) were added to the measured CSP solution at an ambient temperature then. After stirring, the measured AMPS based on the ratio difference was added to the prepared CSP solution and the two solutions are mixed and immersed in a thermostated water bath which has preset at a desired temperature (35–70°C) and the mixture was kept in a thermostat water bath for 120 m.

**2.2.2. Protein-Co-AMPS Hydrogel Preparation.** Hydrogel was synthesized using solution polymerization of CSP copolymerized with 2-acrylamido-2-methylpropanesulfonic acid (AMPS) with the presence of initiators' potassium persulfate and redox catalyst sodium bisulfite and N, N-methylene bisacrylamide (NMBA) as crosslinking agent as it is explained before. The experimental design (central composite) used to determine the number of runs and the combinations were by varying the ratio of the backbone protein and the copolymer AMPS and by taking the amount of crosslinker and imitator as constant as the table below shows. The swelling capacity of hydrogel is also taken as a response. The resulting hydrogel product is scissored and put into excess absolute ethanol (200 mL) and remains until dewatered. Then, ethanol is decanted, and the product hydrogel is dried in the oven at 40°C to a constant weight. The dried product was then milled and screened to collect the samples with a particle size in the range of 40–60 mesh (Table 1).

The water uptake of protein-based hydrogel was determined using the teabag method in the following procedure [41]; for determination of the water absorbency of the hydrogels, the particles are used with 40–60 mesh sizes. The first step was to weigh 100 mg (0.1 g) of a dried sample in a nonwoven heat-sealable pouch (such as a tea bag of 200 mesh screen). The sealed pouch was then immersed in 200 ml deionized water for 24 h at room temperature ( $25 \pm 2^\circ\text{C}$ ). Then, the nonwoven bag was hung in the air for 15 min to remove the excess solution. The sample was weighted instantly and the wet weight of the swollen gel was

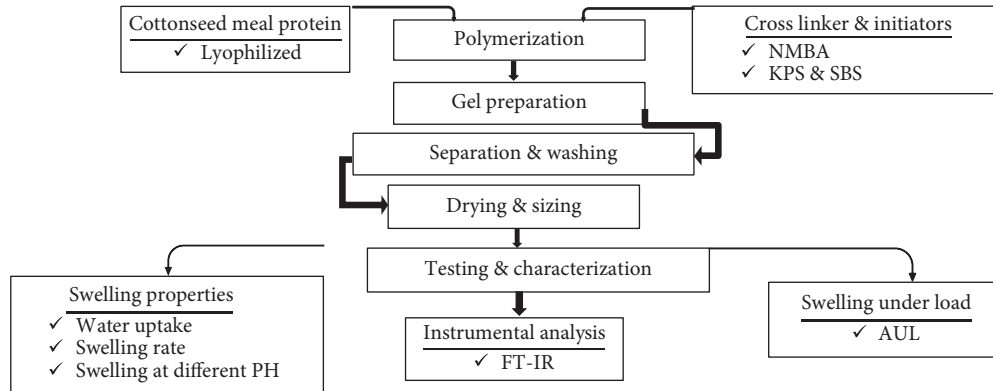


FIGURE 1: General methodology of protein-co-AMPS hydrogel preparation.

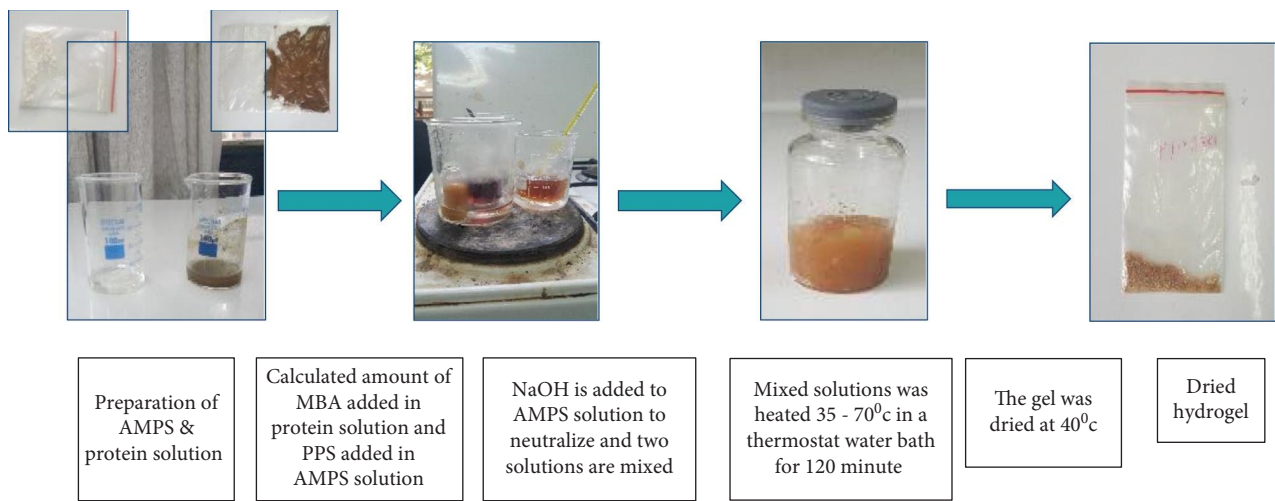


FIGURE 2: Mechanistic pathway of synthesized hydrogel.

TABLE 1: Ratio of the backbone protein and the copolymer AMPS.

|   | AMPS (%) | CSP (%) | MBA (g) | PPS (g) |
|---|----------|---------|---------|---------|
| 1 | 25       | 75      | 0.03    | 0.01    |
| 2 | 50       | 50      | 0.03    | 0.01    |
| 3 | 75       | 25      | 0.03    | 0.01    |

determined. Finally, the water uptake of the gel is measured by the weight of the wet gel divided by the weight of the dried gel according to the authors in (1).

### 2.2.3. Characterization of Hydrogel

(1) *Water Uptake/Swelling Capacity.* Of protein-based hydrogel was determined following a modified procedure [28]. A 100 mg (0.1 g) dried sample was weighed and placed in a nonwoven heat-sealable pouch (similar to a 200 mesh screen tea bag). The sealed pouch was immersed in 200 ml deionized water for 24 h at room temperature (25 ± 2°C). Afterward, the pouch was hung for 15 min to remove excess solution. The wet weight of the swollen gel was immediately measured, and the water uptake was calculated by dividing

the wet weight by the weight of the dried gel using the following equation:

$$Water\ uptake\left(\frac{g}{g}\right) = \frac{W - W_0}{W_0} \quad (1)$$

where  $W$  is the weight of swollen gel and  $W_0$  is the weight of the dried matrix.

(2) *Swelling Kinetics/Absorption Rate.* Indicating the amount of water absorbed per unit of time and reflecting liquid diffusion into polymer networks, the authors studied crosslinked protein hydrogels. Samples of SAP hydrogel (0.1 g) were placed in weighed nylon bags (200 mesh screen) and fully immersed in 200 mL distilled water. After 15 minutes of air hanging to remove excess solution, water absorbency was measured at various time points: 0, 2, 4, 6, and 8 hours. Swelling rates were calculated using (2), and initial and final swelling (swelling equilibrium) was determined after 2 and 8 hours, respectively [42].

$$S_R = \frac{S_{t+\Delta t} - S_t}{\Delta t} \quad (2)$$

where  $S_t$  is the swelling content at any time (g/g) and  $S_{t+\Delta t}$  represents the swelling content based on the dry content at “ $t + \Delta t$ .”

(3) *Swelling at Various pH Values.* Different buffer solutions with pH values ranging from 3, 5, 7, 9, and 11 were used to determine the effect of pH value on the water uptake of the hydrogel. The swelling capacity of the hydrogels at each pH value was measured using water uptake (1), at a period of constant time of 24 h.

(4) *Absorbency under Load (AUL).* The test system comprises a porous filter plate positioned in a Petri dish, with a load applied to the top of the filter plate. A filter paper is then saturated with a solution on the filter plate. A quantity of dried superabsorbent polymer (0.1 g) is placed in contact with the solution on the filter screen of the test device (a cylinder with a diameter of 2.5 inches). A standard weight (0–240 g) is applied to the top of the superabsorbent polymer to achieve a load. After 24 hours, the swollen hydrogel is weighed, and absorbency under load is calculated using the following equation:

$$AUL = \frac{\text{Weght of swollen gel} - \text{Weght of dried gel}}{\text{Weght of initial hydrogel}} \quad (3)$$

(5) *Infrared Analysis (FTIR).* The molecular structure or functional group of sample hydrogel was characterized in KBr pellets using an FTIR spectrophotometer (JASCO FT/IR-6600). 1–2 mg of protein powder was put on the disc for inspection. Transmittances are then recorded at wave numbers between 4,000 and 400  $\text{cm}^{-1}$ .

### 3. Results and Discussion

*3.1. Graft Copolymerization and Hydrogel Preparation.* Cross-linked protein-based hydrogel was prepared by redox-initiated polymerization of AMPS- g-CSP in the presence of N, N-methylene bisacrylamide as the cross-linker. The mechanism of redox initiation due to the combined action of persulfate and bisulfite has been well-reported in the literature [43]. Figure 3 below shows the reaction mechanisms of redox initiators. In the first step, it is expected to be the most predominant radical-producing reaction of persulfate ion initiator PPS and redox catalyst SBS; however, at the higher thermal dissociating condition, persulfate ion is decomposed under heating (65°C) to produce sulfate anion-radicals in reaction (3) (where R represents any of the radicals produced in reaction (1)) is again a likely contributor of radicals due to the presence of a large quantity of water present in the system [44].

Then, the anion radicals abstract hydrogen from one of the functional groups (i.e., COOH, SH, OH, and  $\text{NH}_2$ ) in side chains of the protein backbones to form corresponding macroinitiators. These macroradicals initiate the grafting of AMPS onto protein backbones leading to a graft copolymer [45]. Figure 4 shows the grafting of AMPS onto protein backbones.

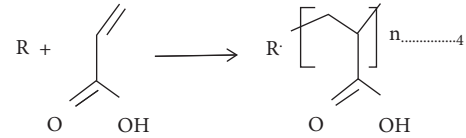
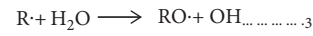
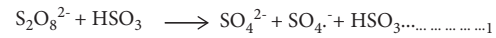


FIGURE 3: Reaction mechanism of persulfate-bisulphite redox initiation.

The effectiveness of graft polymerization is shown in the FTIR graph of Figure 5(b). The grafted polymer is then crosslinked with N, N-MBA. Figure 4 shows the graft copolymerization of AMPS monomer with protein backbone in the presence of the PPS initiator and the formation of hydrogel using MBA as crosslinker. Grafting of AMPS using PPS and SBS initiators in solution polymerization will reduce the average molecular weight of the final polymer by chain transfer to the solvent. Several methods are there to control this chain transfer such as controlling the number of monomers and initiators and selecting the proper solvent but in this case control this chain transfer; surface radical generation was done in separate steps followed by redox initiation in a solution which helps to control the degree of dilution, and therefore, the efficiency of the initiator can be controlled to regulate the viscosity.

*3.2. Effects of AMPS: CSP Concentration on Swelling.* The results obtained by changing the AMPS and CSP concentration for the graft polymerization and its swelling capacity in gram of water per gram of hydrogel are presented in Figure 6.

It is evident from Figure 6 that as the ratio of AMPS to CSP is increased from 0.33 to 1%, the swelling percentage was found to increase. This indicates enhancement of AMPS can be attributed to the increase of hydrophilic vinyl monomer concentration in the vicinity of the protein backbone due to the availability of more grafting sites that protein can be grafted and consequently greater availability and enhancement chances for molecular collisions (cross-linking) of the reactants. This observation is in close agreement with the results obtained by authors in [2]. But further increasing the concentration of AMPS to CSP above 1 wt% is probably due to preferential homopolymerization over graft copolymerization as well as increasing the viscosity of the reaction medium, which hinders the movement of free radicals [46].

*3.3. Swelling Kinetics.* Also the swelling kinetics was assessed employing pseudo-first-order kinetic models. The mathematical formulations of these models are depicted in (4) and (5). This swelling kinetic model was adopted from earlier studies conducted by Lestari et al. [46] and Chen et al. [47].

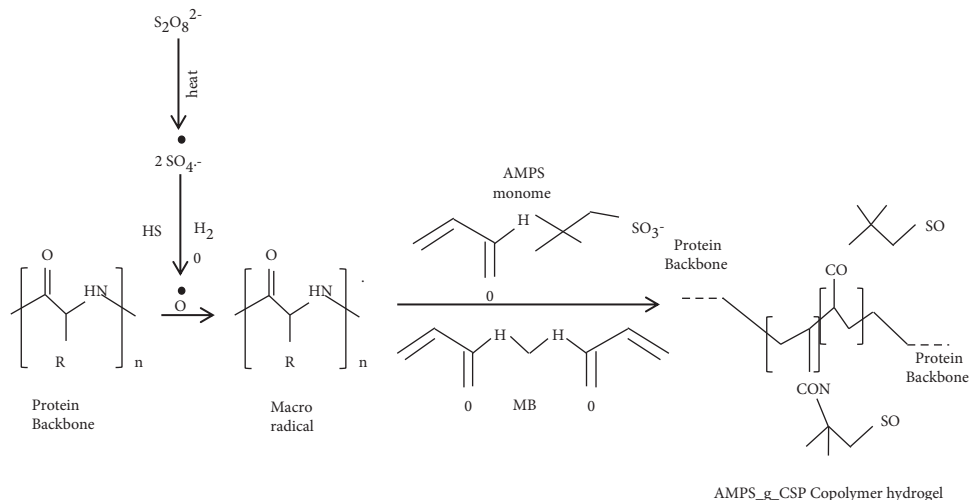
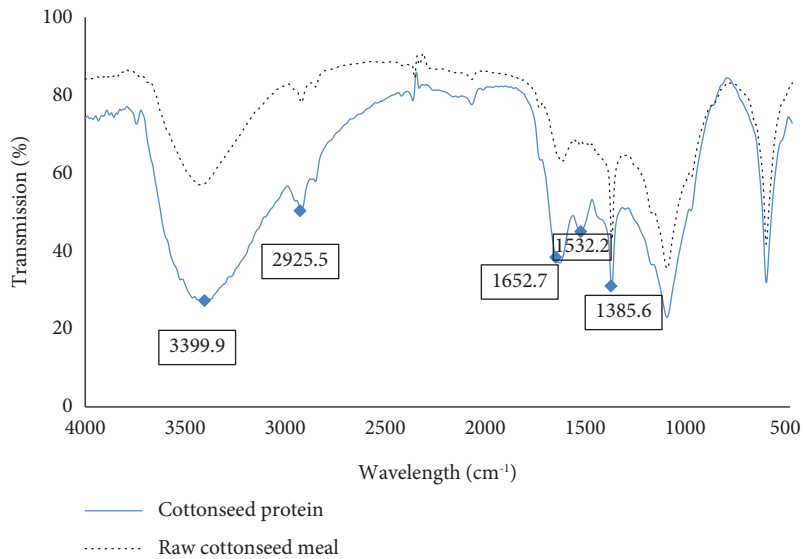
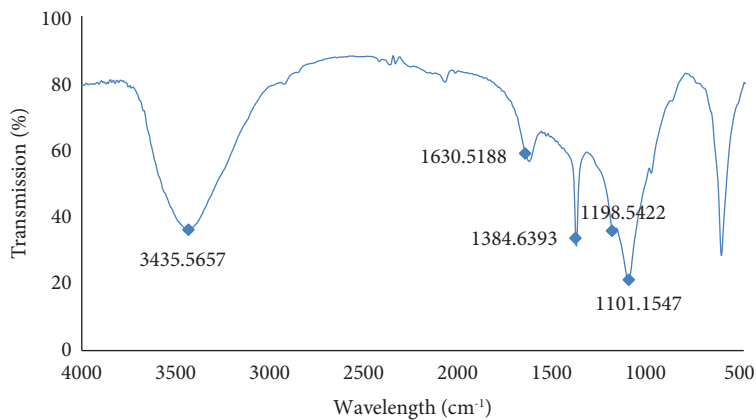


FIGURE 4: Grafting of AMPS onto protein backbones.



(a)



(b)

FIGURE 5: FTIR spectroscopy of cottonseed protein (a) and FTIR spectroscopy of AMPS-grafted CSP hydrogel (b).

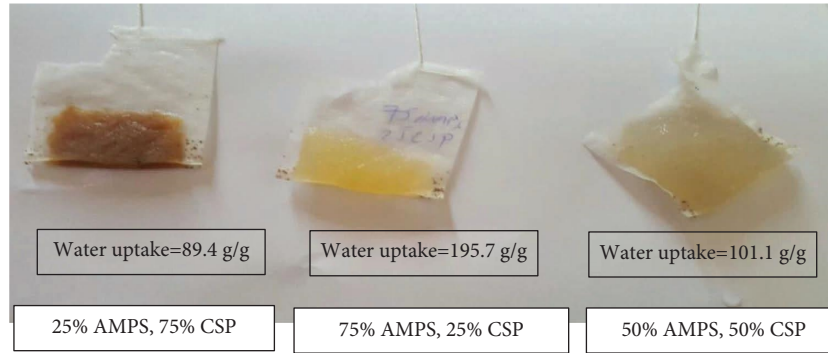


FIGURE 6: Swelling of protein-co-AMPS hydrogel by different concentration of CSP and AMPS.

$$\ln(Q_t) = \ln(Q_e) - k_1 t, \quad (4)$$

$$\frac{1}{Q_t} = \frac{1}{Q_e} + k_2 t, \quad (5)$$

where  $Q_e$  and  $Q_t$  are the absorption capacity at equilibrium and at time  $t$ ,  $k_1(\text{h}^{-1})$  and  $k_2(\text{g.g}^{-1} \text{h}^{-1})$  are swelling rate constants for the two models, respectively. But in this case, the absorption capacity at equilibrium becomes 195.7 g/g and the absorption capacity at time 0, 2, 4, 6, and 8 hours are described in Table 2.

Swelling behavior of the protein-based hydrogel, as shown in Figure 7(a), showed a water uptake of 195.7 g water/g dry gel after 24 h at room temperature. But the maximum rate of swelling, i.e., about half of the swelling capacity of hydrogel occurred in the first two hours after-ward slope increase very slowly up to 8 hours and the pattern shows the slope increased very slowly up to 24 hours.

Several factors such as the type of polymerization, monomer composition, swelling capacity, particle size and distribution, and so forth can affect the swelling rate of the superabsorbent polymer greatly [48]. The rapid increase of water absorbency of hydrogel increases instantly due to the higher surface area and contact area with water of fine powder hydrogel and the presence of more unsaturated hydrophilic groups, but the gradual increment in the swelling rate is due to the saturation of the hydrophilic group with the water [49].

The plot based on the pseudo-first-order model is shown in Figure 7(b). As expected, the plot is linear with a negative slope ( $K_1 = 0.0766$ ). However, the linear regression coefficient ( $R^2$ ) is 0.9367 which indicates that the absorption of water by the hydrogel does not follow a first-order kinetic process, where as it was also found that the relationship curve between  $t$  and  $t/q_t$  was linear with  $R^2$  value (0.9969) close to 1; Figure 7(c) indicates that the swelling behavior of hydrogel could be fitted by the pseudo-second-order kinetics model.

**3.4. Swelling at Different pH Values.** Ionic superabsorbent contains some functional groups that can be ionized at a suitable pH value; the hydrogel can exhibit swelling change at various range of pH values. The produced hydrogel in this study is pH-sensitive due to the polymer structurally

TABLE 2: The values for the finding of kinetic constants by using the pseudo-first and second-order models.

| Time (Hr) | $Q_t$ (g/g) | $Q_e$ | $Q_t - Q_e$ | $\ln(Q_t - Q_e)$ | $t/Q_t$     |
|-----------|-------------|-------|-------------|------------------|-------------|
| 0         | 0           | 195.7 | 195.7       | 2.2915908        | —           |
| 2         | 98.28       | 195.7 | 97.42       | 1.9886481        | 0.02035002  |
| 4         | 124.64      | 195.7 | 71.06       | 1.8516252        | 0.032092426 |
| 6         | 138.1       | 195.7 | 57.6        | 1.7604225        | 0.043446778 |
| 8         | 152.09      | 195.7 | 43.61       | 1.6395861        | 0.052600434 |

containing hanging acidic (carboxylic and sulfonic acids) groups. In the present study, the effect of the pH value of the swelling media on the equilibrium water absorbency of the hydrogel was investigated in swelling media of pH values 3, 5, 7, 9, and 11, and the results are depicted in Figure 8. It clearly indicates that the water absorbency increases with the pH value of the swelling media from 3 to 7 and decreases with the pH of the swelling media from 7 to 11.

The reason for this behavior may be due to the ionic strength change at different pH values. The maximum water absorbency (186.21 g/g) of the current hydrogel was obtained at a pH of around 7. This is due to the higher osmotic pressure between the hydrogel network and the external solution [50]. Anionic-type superabsorbent hydrogel is protonated in the solution at a low pH value (less than 7) but ionized at a high pH value; under low pH values, most of the carboxylate and sulfate anions are protonated, the anion-anion repulsive forces become eliminated and the network cannot expand; therefore, the osmotic pressure between the network and external solution reduces and the water absorbency decreases. On the other hand at a higher pH value (greater than 7), the ionization of the carboxylic groups in the network increased and the concentration of anionic groups in the polymer network increased, which resulted higher anion-anion repulsive forces and the network can expand, osmotic pressure between the network and external solution increases, and the water absorbency also increase. However, the reason for the swelling loss in the basic solutions is due to the “charge screening effect” of excess  $\text{Na}^+$  in the swelling media, which shields the carboxylate and sulfate anions and prevents effective electrostatic repulsion. Similar swelling behaviors have been reported in the case of such types of hydrogel systems [48].

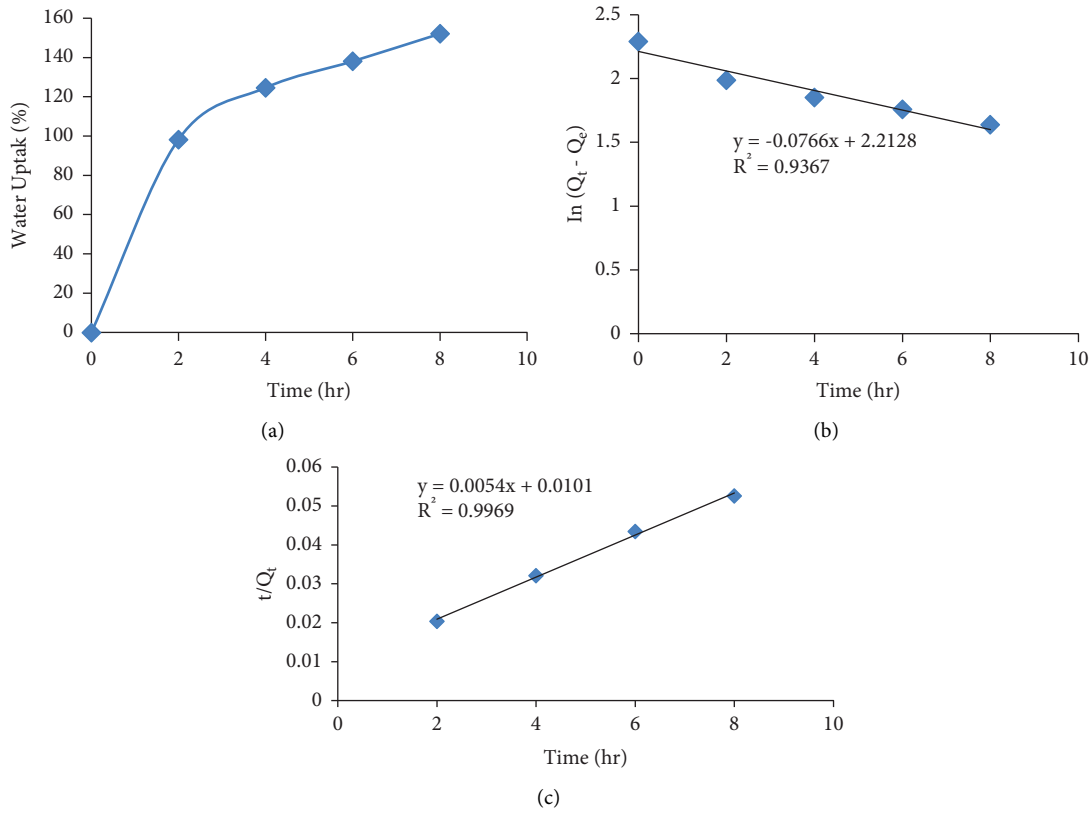


FIGURE 7: The swelling kinetics of the synthesized protein-co-AMPS hydrogel (a) experimental swelling kinetics curve, (b) pseudo-first-order kinetics model, and (c) pseudo-second-order kinetics model.

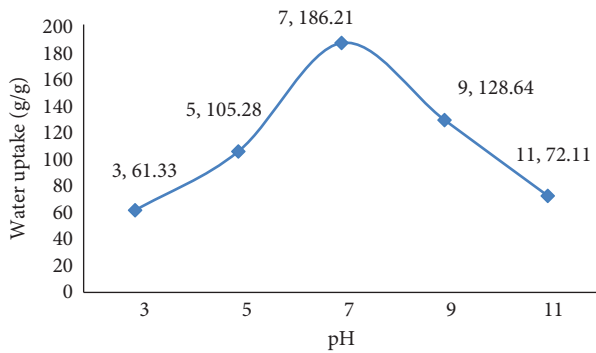


FIGURE 8: The swelling of synthesized protein-co-AMPS hydrogel at different pH.

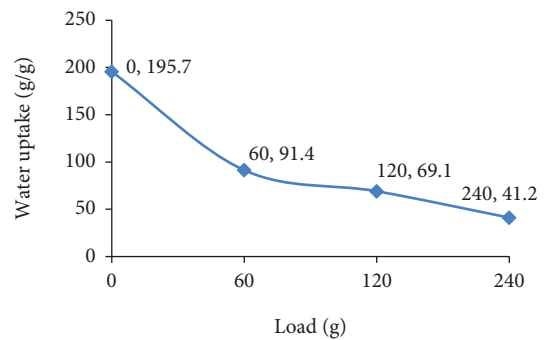


FIGURE 9: Absorbency under load of protein-co-AMPS hydrogel.

3.5. *Absorbency under Load (AUL)*. A standard weight of 60 g, 120 g, and 240 g weight was used for a given 2.5 in a diameter filter plate. Figure 9 shows the absorbency for each weight after 24 hours. The applied load has a negative effect on the water uptake of the hydrogel and it is due to the compression effect of the load which hinders the chain expansion of the hydrogel network.

The differences in water absorption are significant. It turns out to be essential for the efficiency and reasonableness of the application of superabsorbent polymers in a scattered form using mixing them with the particle in the intended application area. The maximum load (240 g) results in the

lowest water uptake (41.2 g/g) of the hydrogel. The most important property of hydrogel absorption capacity is directly linked to particle size and degree of crosslinking [50].

3.6. *Fourier-Transform Infrared (FTIR) Spectrum Analysis*. FTIR spectroscopy was used for the identification of the graft copolymer. From the representative of FTIR spectra of AMPS-g-CSP, the peaks at  $1630\text{ cm}^{-1}$  were attributed to amide I to C=O stretching in carboxamide functional groups of the protein backbone. AMPS-g-CSP shows absorption peaks at  $3435\text{ cm}^{-1}$  due to the collapse of -NH and -OH ( $-\text{SO}^3\text{H}$ ) stretching frequency of AMPS and due to overlapping contributions of the stretching vibrations of both O-H and N-H

groups of the protein backbone [45]. The protein-g-AMPS hydrogel comprises a protein backbone with side chains that carry sulfate groups that are evidenced by a new characteristic absorption band around  $1200\text{ cm}^{-1}$ . The strong stretching absorption in the region of  $1100\text{ cm}^{-1}$  represents S–O–C group; this peak is attributed to the ester sulfate stretching of AMPS. The stretching band of –NH overlapped with the OH stretching band of the protein portion of the copolymer [51, 52].

#### 4. Conclusion

A crosslinked network hydrogel composed of cottonseed protein and 2-acrylamido-2-methylpropanesulfonic acid (AMPS) has been successfully synthesized by solution polymerization using N, N-MBA as a crosslinker and potassium persulfate PPS and sodium bisulfite SBS as a redox initiator system and optimized by the response surface method. Various characterization techniques confirm the formation of hydrogel such as water uptake and FTIR spectroscopy. The effects of the ratio of cottonseed protein to AMPS, swelling kinetics, absorbency under load, and swelling at different pH values were measured. The reaction conditions were attempted to optimize for obtaining graft copolymers with higher grafting parameters. So, the reaction conditions for achieving the maximum swelling capacity ( $195.7\text{ g/g}$ ) of hydrogel were in a condition 0.03 N, N-MBA, 0.01 PPS, and 0.01 SBS with a 1wt% ratio of AMPS to CSP. The absorbency under load was reduced as the load increased and the maximum swelling rate was obtained in the first two hours. The higher swelling capacity of the hydrogel is in a neutral aqueous media. The FTIR spectroscopy clearly shows the grafting is done well. The absorption kinetics model shows the experimental data best fit with pseudo-second-order kinetics model. This observation indicates that the presence of hydrophilic, hydrophobic segments, and crosslinker moiety in the network structure control the swelling behavior of the hydrogel. This new approach showed a promising method for utilizing natural cottonseed protein in the production of the superabsorbent polymer, and the resulting hydrogel has excellent water absorbency. In addition, because of the use of protein as a natural backbone and the synthesis hydrogel has superior pH-resisting properties, it is expected that the resulting gels show more compatibility with the human body and can be used in biomedical applications as a drug delivery system with further test and conditions.

#### Data Availability

The graphical data and experimental results data used to support the findings of this study are included within the article.

#### Conflicts of Interest

The authors declare that they have no conflicts of interest.

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