

Synthesis and Characterization of Superabsorbent Hydrogels From Waste Bovine Hair Via Keratin Grafted With Acrylic Acid (AA) and Acrylamide (AAM)

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Research Article

Keywords: Keratin, hydrogel, superabsorbent, bovine hair, leather waste

Posted Date: May 18th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-446130/v1>

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Abstract

The leather industry, due to its way of using chemicals during treatment and releasing them back to the environment makes it one of the hazardous sectors. It is not only releasing the chemicals also during the process precious biomass such as collagen and keratin discarded as waste. In this study, bovine hair which occurs during the treatments of the bovine leather process is supplied as waste and converted to superabsorbent hydrogel via grafting of keratin with monomers (Acrylic Acid (AA), Acrylamide (AAm)) in the presence of N, N'-methylene bisacrylamide (NMBA) used as a crosslinking agent and ammonium persulfate (APS) as an initiator. Keratin is selected to enhance the biocompatibility of the hydrogel. Thus, different keratin/monomer ratios, crosslinking agents, and initiator amounts were accepted as variables, and reaction conditions were optimized to achieve the highest swelling capacity. Synthesized hydrogels were chemically, morphologically, and thermally characterized via Fourier transform infrared (FTIR) spectroscopy, scanning electron microscopy (SEM), differential scanning calorimeter (DSC), and thermal gravimetric analyzer (TGA). FTIR, DSC, and TGA results confirmed the grafted structure. The maximum swelling ratio was recorded at pH 9, at the end of 48 hours as 1791%. Sponge-like hydrogels were successfully obtained, and waste keratin is successfully valorized by means of hydrogels which can be used in high-value-added areas.

Statement Of Novelty

This work contains novelty in terms of valorization of bovine hide waste which occurs during the leather process. These waste materials valorized as keratin source and can be used in several applications and in this study obtained keratin was used for synthesizing superabsorbent hydrogels.

Introduction

Hydrogels are extraordinary polymeric materials that have inherently superabsorbent properties thanks to their organized cross-linked network structure [1]. They are obtained from synthetic or natural polymers. Examples of hydrogels derived from synthetic polymers are poly (lactic-co-glycolic acid) (PLGA) [2], polyacrylamides [3], polyhydroxyethylmethacrylate (PHEMA). Natural polymers which are used to produce hydrogels are proteins (collagen and gelatin [4], soy and fish proteins [5]), carbohydrates (starch) [6], cellulose, and its derivatives [7].

Hydrogels are used in versatile applications such as drug release, wound healing, fertilizer, etc. [8]–[10]. Basically, they maintain the moisture on the surface of the wound and enhance the healing process while reducing the pain and infections [1], [11]. Also, hydrogels can be used as a solution for water scarcity by increasing crop growth and soil irrigation efficiency and which are not hazardous for the environment [12]. Moreover, they improve the root development of plants, minimize nutrient losses through leaching, and even prevent soil erosion with their additives [13], [14].

Keratin is one of the most abundant protein sources from hair, wool, feather, nails, horns, and hooves [15]. Valorization of keratin-based waste is possible in different ways according to keratin source and waste type [16], [17]. As biopolymer; keratin has remarkable properties like biocompatibility, biodegradability, and mechanical stability and can be cast as film, sponge, or hydrogel forms to serve biomedical applications [18], [19].

Keratin is a polypeptide made of different amino acids that have inter-molecular bonding of the disulfide cysteine amino acid and inter-and intra-molecular bonding of polar and nonpolar acids. Keratin proteins contains cysteine residue and small amounts of lipid (0.1%) and minerals (0.5%) [15]. Inter and intra-chain cross-links of cysteine disulfide bonds give keratin high stability together with the lower solubility. Due to the nature of keratin, it is biodegradable, biocompatible, and hydrophilic, and the properties of the keratin vary according to the extraction method [20].

Feather waste is one of the challenging issues of the poultry industry [9]. Moreover, the leather industry also gives keratin as the waste end of the dehairing process. Dehairing process of the skin can be done via acidic treatment or enzymatic treatment. If the acidic process is followed keratin molecules are damaged and recovery of keratin from wastewater stream becomes more difficult. If, the enzymatic process is applied for dehairing than, keratin recovery from the waste would be simpler.

In this study, bovine hair, which is a waste of the local leather production line is used as a keratin source. Keratin hydrogel synthesis conducted with AA, AAm, and a combination of AA/AAm monomers under changing initiator and cross-linking ratios. Hydrogels were successfully synthesized and their swelling properties in neutral, acidic, and basic mediums were recorded and the highest values exceed 1700% swelling capacity. With this proposed method, one of the leather industry waste is effectively converted to high added value material namely hydrogel.

Materials And Methods

Chemicals and Reagents

N, N'-methylene bisacrylamide (NMBA, 98%) as a crosslinking agent, ammonium persulfate (APS, 99.9%) as initiator, and the Acrylic acid (AA), Acrylamide (AAm) were supplied from Sigma Aldrich. Ethanol (EtOH, 99.8 %) was used to adjust pH. Sodium dodecyl sulfate (SDS), sodium hydroxide (NaOH), and hydrochloric acid (HCl, 37%) used for washing and alkali hydrolysis of the hair were also obtained from Sigma Aldrich. Sodium chloride (NaCl) and potassium hydroxide (KOH) were supplied from Sigma Aldrich and used as medium chemicals for calculating the swelling values. Bovine hair feather was supplied from Iskefe Leather tannery as a waste of the leather processing line and used to obtain keratin.

Preparation of Keratin Hydrolysate

Keratin-containing solution was obtained from the industrial leather tannery as a byproduct of the leather dehairing process under the tanning process. In the hair removal process, the leather is treated with

various chemicals, and anti-bacteria, and fungi agents. Meanwhile, the bovine hair on the skin is loosened from the hair follicles without damaged by the keratinase enzyme and passes into the treated solution. Hydrolyzed keratin is obtained from the keratin-derived protein in this solution. The bovine hair obtained from the skin is purified from dirt and oil with 0.5% (w/v) SDS and 95% (v/v) EtOH and made ready for extraction studies. Bovine hair was partially hydrolyzed using 1 M NaOH. 40 g of bovine hair was added to 180 mL of 1 M NaOH and kept at 95 °C for 8 h. After the hair is completely dissolved, the solution is filtered through a coarse filter and then its pH is adjusted to 6-8 with HCl. Approximately Brix value is obtained 20% at the end of the extraction processes.

Synthesis of Keratin Hydrogels

0.1 g of NMBA is dissolved in 5 mL of distilled water and 6 g of AAm/AA is added. In addition, 1.5 g of keratin hydrolysate is dissolved in 25 ml of distilled water (reaction temperature 75 ± 2 °C). Afterward, 0.75 g APS are added in keratin hydrolysate solution and mixed for 10 minutes. After mixing, NMBA and monomer (AAm and/or AA) solution were added to the polymerization medium. The reaction continues for 1 hour at 400 rpm, 75 °C. The gel structure is first washed in 100 mL EtOH for 2 hours. Afterward, the product put into fresh EtOH remains for 24 hours and then it was left to dry in the furnace. Reaction conditions given in Table 1 and for AAm/AA blend ratios (w/w) as 50/50, 70/30, and 30/70 were studied.

Table 1: Polymerization parameters for keratin hydrogels.

	Keratin (g)	AA (g)	AAM (g)	Crosslinking Agent (g)	APS Initiator (g)
Sample 1	1.5	-	6	0.01	0.75
Sample 2	1.5	-	6	0.05	0.75
Sample 3	1.5	-	6	0.10	0.75
Sample 4	1.5	-	4	0.01	0.75
Sample 5	1.5	-	8	0.01	0.75
Sample 6	1.5	6	-	0.01	0.75
Sample 7	1.5	6	-	0.05	0.75
Sample 8	1.5	6	-	0.1	0.75
Sample 9	1.5	4	-	0.05	0.75
Sample 10	1.5	8	-	0.05	0.75
Sample 11	1.5	3	3	0.05	0.75
Sample 12	1.5	4.2	1.8	0.05	0.75
Sample 13	1.5	1.8	4.2	0.05	0.75
Sample 14	3.0	-	4	0.01	0.75
Sample 15	4.5	-	4	0.01	0.75

*All other parameters were kept constant.

Results And Discussion

SDS-PAGE was performed according to the Laemmli method to detect changes in molecular weight after bovine keratin hydrolysis. Sample (4 mg) was dissolved in 1000 μ l of pure water. For Tris / Glycine / SDS Gels, the sample was diluted with Laemmli Buffer at a ratio of 1:3 (4x sample buffer). The sample protocol was prepared as a total volume of 12 μ l. The sample (5 μ l) was loaded into the individual lanes in the freshly pre-prepared 17% acrylamide gel prepared with TEMED initiator [21]. After 1 hour of electrophoresis at 200 V, the gel was fixed at 50 rpm when shaking in 10% Acetic acid, 40% Ethanol buffer for 45 minutes. The gel was then washed with distilled water and incubated with 100 mL of Coomassie brilliant blue staining solution for 16 hours at 50 rpm on a shaker. The gel was then washed with distilled water, allowed to dry under room conditions, and images of the bands were obtained. Molecular weight of keratin hydrolysate measured as 10-15 kDa according to stains, and which is accordance with the literature [22].

The chemical structure of hydrolyzed keratin hydrogels was determined by Fourier Transform Infrared spectrophotometer (FTIR) by using Agilent 600-IR Series equipment in the range of 4000 – 650 cm^{-1} .

FTIR results given in **Figure 1** for all synthesized samples and keratin hydrolysate. **Fig.1a** shows the changing amount of AAm inside the hydrogel structure whereas, Fig.1b shows the different ratios of AA and **Fig.1c** shows the blend concentrations of AAm/AA. Characteristic peaks of the keratin hydrolysate and keratin hydrogels are shown in **Fig.1d** Due to the α -helix and unordered structure of the hair keratin peak around 1650 cm^{-1} related to Amide I [1]. Around 3314 cm^{-1} Amide A band is located [20] and Amide I-II-III peaks located at around 1660 cm^{-1} , 1553 cm^{-1} due to the C=O stretching, and 1330 cm^{-1} (NH_2), respectively [23]. AA also represents characteristic peaks at 1712 cm^{-1} , 1580 and 1403 cm^{-1} which are related to $-\text{COOH}$ stretching, COO^- asymmetric stretching and COO^- stretching, respectively [22] and overlapped with the amide peaks of the biopolymer.

Measurement of Gel Content (%)

For the gel content calculation of hydrolyzed keratin hydrogels, the initial mass of the dried sample was determined as 0.1 g. The samples were swollen in distilled water for 48 hours and then filtered through coarse filter paper. The extracted gel samples were dried under vacuum at $40\text{ }^\circ\text{C}$ for 24 hours. The final product obtained was weighed for weight determination. Gel content (% gel) was calculated by Eq. (1):

$$\% \text{ Gel} = (\text{final mass}/\text{initial mass}) \times 100 \quad (1)$$

The gel contents (%) of sample 4, 14, and 15 were observed as 46.9%, 20.9%, 23.3%, respectively. Increasing the keratin concentration did not contribute to the gel content of the sample.

Swelling Measurements

The keratin hydrogel could swell in medium solution at neutral pH and room temperature. At regular intervals, the mass measurements were performed. The swelling behavior of Hydrogel was investigated for ... minutes. The Swelling ratio or swelling capacity (S%) of keratin hydrogels was calculated according to following Eq. (2):

$$\% \text{ swelling} = (W_t - W_0) / W_0 \times 100 \quad (2)$$

where W_t and W_0 specify the weight of the swollen superabsorbent collagen hydrogel at equilibrium and the weight of the initial dry superabsorbent at time 0, respectively.

Swelling in Different Media

The swelling properties of the keratin hydrogel samples at different NaCl concentrations were measured using the method applied in the "Swelling Measurements" section. At the same time, the swelling properties of keratin hydrogel samples were investigated in different pH environments and these pH values were determined as 1, 3, 5, 7, 9 and 11. pH environment was prepared using 5 M HCl and 1 M KOH.

Swelling Properties

Swelling in Distilled Water

Swelling of hydrogels relies on the anion-anion electrostatic repulsion force. The experimental method was determined by reference publications showing the highest swelling degrees for biobased AA hydrogels as; 501 g/g [22], 674 g/g [24], 920 g/g [25], 197 g/g [26] and for biobased AAm hydrogel 775 g/g [27]. The swelling ratios of this work were recorded in between 137.2% to 1430.7%. Maximum swelling ratio was obtained for 48 hours for sample 14. The swelling capacity increases with time, but with further increase in time reaches a limiting value which is the equilibrium value [24]. The increase in the swelling values of keratin hydrolysate hydrogel samples in water over time can be explained by the fact that water is a good solvent for keratin hydrolysate hydrogels. An interaction occurs between the water molecules that rapidly enter the polymer chains and the polymer chains, and due to this interaction, the groups of molecules repel each other, and the molecules move away from each other. This situation can be enhancing the swelling values. When AAm and AA monomers are compared, it is proved by high swelling capacity values that keratin hydrolysate has better molecular interaction with AAm monomer.

In addition, the effect of keratin hydrolysate weight on hydrogel swelling is shown in **Figure 2**. Swelling capacity was generally increased by increasing the keratin hydrolysate weight from 1.5 to 4.5 g from 955.5 to 1430.7%. It is seen that the active sites can easily react with the monomers as the keratin hydrolysate weight increases. However, increasing the keratin hydrolysate content by more than 3.0 g causes a decrease in diffusion of monomers to active sites to produce crosslinked hydrogels [28].

Moreover, it can be said that with the increase of NMBA, the amount of crosslinking agent increases, the polymeric structure becomes tighter. The increase in the amount of cross-linking makes it difficult for water molecules to enter the hydrogel and to move the polymer chains apart, and therefore a decrease in swelling values occurs. A slight increase in the degree of crosslinking in all hydrogels results in a significant decrease in swelling capacity values [25], [26], [28], [29], [30]. Swelling values of the samples during different time periods were given in **Table 2** and for sample 6 hydrogel formation can not obtained thus swelling performance could not recorded.

Table 2: Swelling ratio of the hydrogel samples.

	Swelling ratio (%)				
	1 hour	6 hours	12 hours	24 hours	48 hours
Sample 1	196.7	645.2	729.5	900.6	1115.7
Sample 2	284.1	654.7	800	686.2	807.2
Sample 3	508.4	603.9	666.1	635.3	702.1
Sample 4	184.6	630.6	799.8	973.3	1040.9
Sample 5	241.5	796.7	858.4	990.5	1198.1
Sample 6	N/A	N/A	N/A	N/A	N/A
Sample 7	200.0	251.0	342.6	272.0	245.5
Sample 8	137.5	285.5	293.2	306.9	291.1
Sample 9	155.3	190.4	198.2	211.2	245.5
Sample 10	149.6	217.5	233.6	115.4	170.8
Sample 11	143.3	167.6	154.3	132.2	137.2
Sample 12	106.2	286.3	213.6	356.7	473.5
Sample 13	139.7	260.8	263.7	258.2	273.7
Sample 14	139.2	328.6	874.2	1020	1430.7
Sample 15	185.2	548.1	639.2	978.6	955.5

Effect of Salinity on Swelling

The swelling capacity is mainly related to the charge number and ionic strength of the solution, as well as the flexibility of the polymer network, the presence of hydrophilic functional groups and the crosslink density [25], [30]. The addition of salt can affect hydrogel formation, stability, and properties by changing the magnitude and range of electrostatic interactions in the system. It is thought that the interactions that hold the hydrogel particles together increase with increasing NaCl concentration, which confirms the increase in swelling capacity values. At the same time, the weight of the hydrogels gradually increased with increasing salt concentration. This can be attributed to the increase in hydrogel particle size [31].

Figure 3 represents the swelling capacity of the samples during 24h and 48h for changing NaCl concentrations.

Effect of pH on Swelling

The swelling capacity of the keratin hydrogels was studied at various pH, ranging from 1.0 to 11.0 (**Fig.4**) and keratin hydrogels exhibit swelling changes at a wide range of pH values. Stock solutions 1 M KOH and 5 M HCl were diluted with distilled water to reach desired basic and acidic pH values. Ionic bonds, hydrogen bonds and hydrophobic bonds has a significance on the properties and stability of the keratin thus, ionic bonds make it pH dependent [15]. Maximum swelling ratio was obtained at pH 9 for 48 hours for sample 4 (1791%) and sample 15 (1502.8%). The hydrolysis of certain chains of the sample at pH 9 may have allowed the hydrogel to swell further. Evidence for this can be given the loss of mass between 24 and 48 hours measurements [22], [32].

It is well known that swelling of a hydrogel is induced by electrostatic repulsion of the ionic charges of its network. Under acidic pH (≤ 4) most of the carboxylate anions are protonated, thus removing the major anion-anion repulsive forces and consequently reducing swelling values [26], [25], [33]. The minimum swelling is observed at low pH due to a collapsed and disordered protein network with water tightly adsorbed to the hydrophilic sites [34]. As the pH of the swelling medium increases, the hydrogel swells due to anion-anion-repelling electrostatic forces. This results in a more hydrophilic polymer network. As the pH increases, higher water absorption occurs [26], [35].

Thermal characterization of samples was investigated via Differential scanning calorimeter (DSC), Mettler Toledo DSC-1 Star System instrument under N_2 atmosphere. DSC conditions are 25 to 300 °C, at a heating rate of 10 °C/min. The DSC was used to compare the thermal properties of grafted keratin hydrogels and keratin hydrolysate (**Fig.5a**). The denaturation temperature observed around 80 °C for keratin hydrolysate. The second endothermic peak observed at approximately 210 °C, which was reported as helix denaturation in keratin [36]. Denaturation temperatures of graft copolymers are higher than keratin hydrolysate. The second endothermic peak of the grafted copolymers was observed at about 265 °C. These results show evidence of the improved thermal properties of grafted keratin hydrogels compared to keratin hydrolysate.

Thermogravimetric Analysis (TGA) was performed via Mettler Toledo TGA 1 Star system. Samples were heated from room temperature to 600 °C under the nitrogen atmosphere with a heating rate of 10 °C/min. The TGA was used to compare the thermal stability of grafted keratin hydrogels and keratin hydrolysate (**Fig.5b**) The thermal stability of the grafted keratin hydrolysate was improved as is obvious from the TGA curve. The TGA curve shows that the main degradation stage of keratin consists of several overlapping stages. TGA of keratin hydrolysate shows a weight loss in 3 distinct stages. The first stage ranges between 25-140 °C, a loss of about 4.9% in weight was observed, which corresponds to absorbed water [16]. Degradation of keratin hydrolysate was started slowly at around 180 °C. The two main stages, partially separated at about 280 °C, follow maximum degradation rates at 222.1 and 315.6 °C, respectively. Mass loss is 20% by weight below 280 °C. If it is over 300 °C, it is a maximum of 25% by weight.

The decomposition temperatures of grafted copolymers are higher than keratin hydrolysate. The second stage of weight loss starts at about 200 °C, and continues up to 300 °C, during which time, about 50% weight loss occurs due to the degradation of keratin. Generally, keratin hydrolysate degradation is higher than grafted keratin hydrolysate. The third stage of weight loss starts at approximately 340 °C and continues up to 440 °C. Approximately 20% weight loss occurs between this temperature range. All this shows that the copolymers have better thermal stability than pure keratin hydrolysate [27]. The total weight loss of grafted keratin hydrolysate is higher than that of keratin hydrolysate.

The morphology of hydrolyzed keratin hydrogels networks was investigated by Scanning Electron Microscopy (SEM), (Quanta 400F Field Emission). The samples were coated with a thin layer of gold before analysis under high vacuum. **Figure 6** shows scanning electron microscopic images of grafted keratin hydrogels. These pictures confirm that the graft copolymers have a porous structure. Sample 4, 14, and 15 contains 1.5, 3.0, and 4.5g keratin, respectively and increased amount of keratin and pore size relationship was investigated. When sample 8 which contains AA, is compared with the others which contains AAm did not represent sponge like structure thus, swelling properties of sample 8 and AA content samples are lower. Pore diameter values for samples 4, 14, and 15 were calculated as $4.7 \pm 1.8 \mu\text{m}$, $3.7 \pm 1.1 \mu\text{m}$, $4.6 \pm 3.1 \mu\text{m}$, respectively. Increased amount of keratin reduces the pore diameter and enhance the pore amount which is an advantage for swelling behavior of the hydrogel samples. For sample 14, higher amount of keratin containing samples, represents higher swelling capacity thanks to its smaller pore diameter and expanded pore distribution.

Conclusion

In this study, waste valorization by means of recovery of bovine hair from the leather tannery waste stream was used as a keratin source, and keratin hydrolysate was successfully produced out of it. Superabsorbent hydrogels were synthesized by free radical graft copolymerization of hydrolyzed keratin with AA/AAm, and a crosslinker was selected as NMBA. FTIR spectra, thermal and morphological analysis show that graft copolymerization was successfully achieved. The swelling capacity of hydrogels was pH-dependent moreover, affected by the salt concentration. The maximum swelling ratio was recorded at pH 9, at the end of 48 hours as 1791%. The optimum formulation of synthesized hydrogel consists of 3g keratin hydrolysate, 4g AAm copolymer, and the swelling capacity was 1430.7%. With this proposed method, bovine hair, one of the leather industry wastes used as a biopolymer source and has been effectively transformed into high value-added superabsorbent hydrogels.

Declarations

Acknowledgement

This research is funded by The Scientific and Technological Research Council of Turkey under project number 180086. Also, the authors acknowledge Collagen R&D Technology (www.collagenarge.com) for their support.

Declarations

Conflict of interest: The authors declare no competing interest.

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Figures

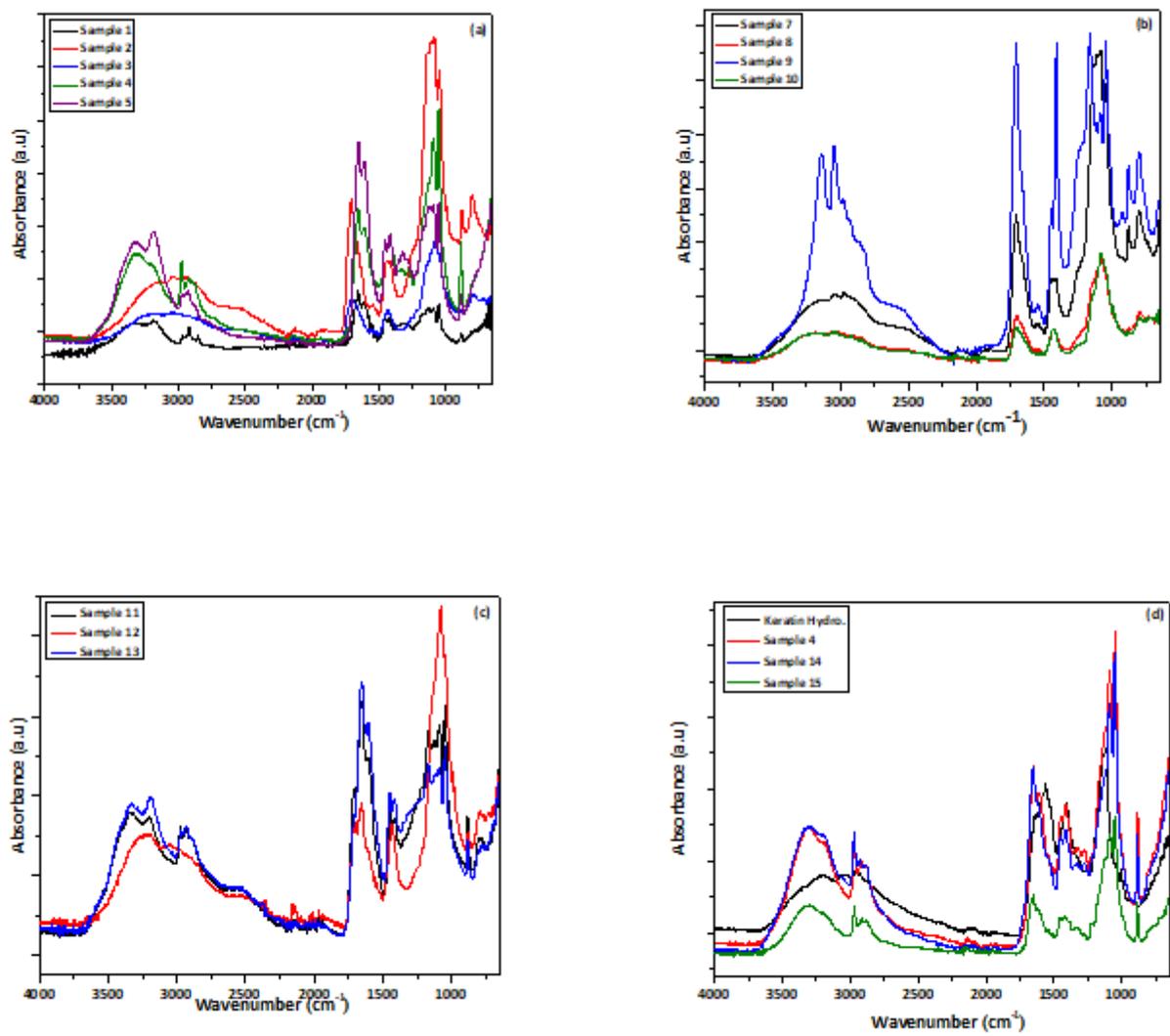


Figure 1

a) sample 1-5, b) sample 7-10, c) sample 11-13, d) keratin hydrolysate, sample 4, 14, 15.

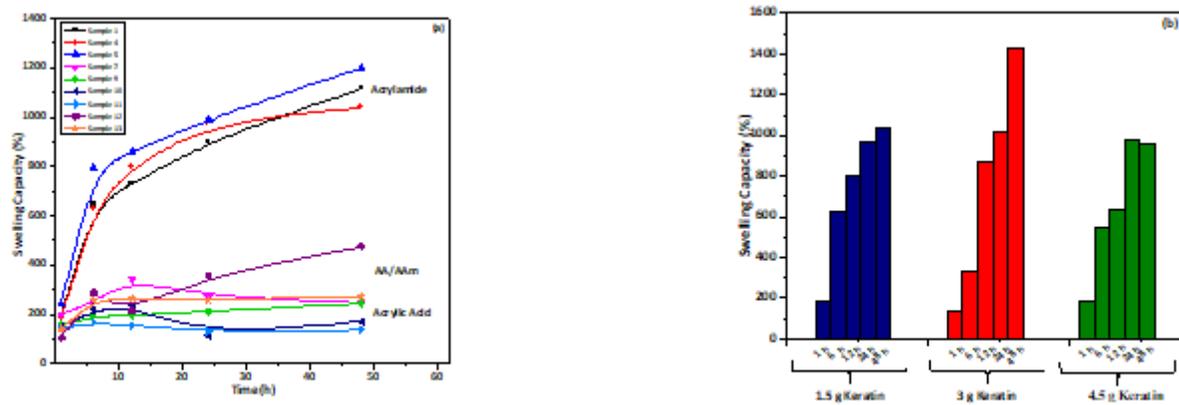


Figure 2

Effect of blend concentrations on swelling capacity, a) sample 1, 4, 5, 7, 9-13 b) sample 4, 14, and 15.

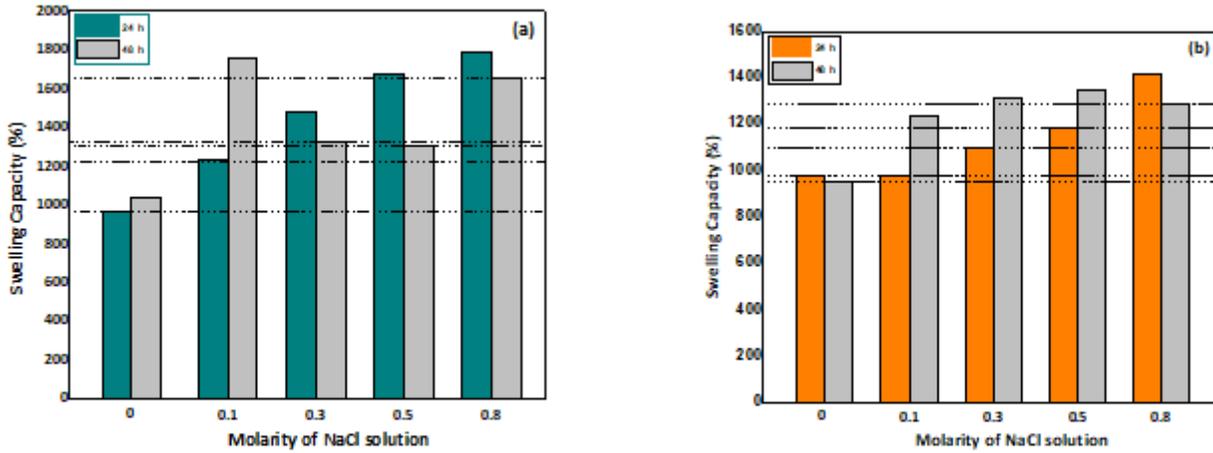


Figure 3

Effect of blends concentrations on swelling capacity for a) sample 4, and b) sample 15.

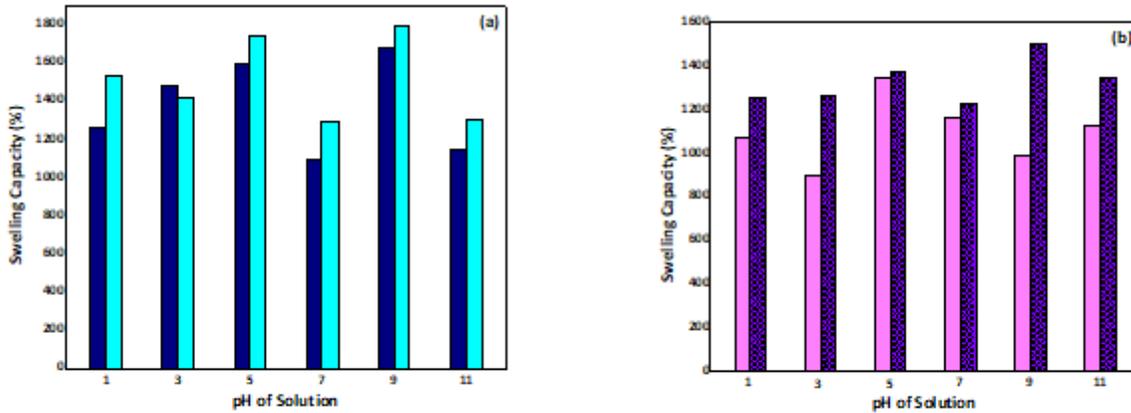


Figure 4

effect of pH on swelling capacity for a) sample 4, and b) sample 15.

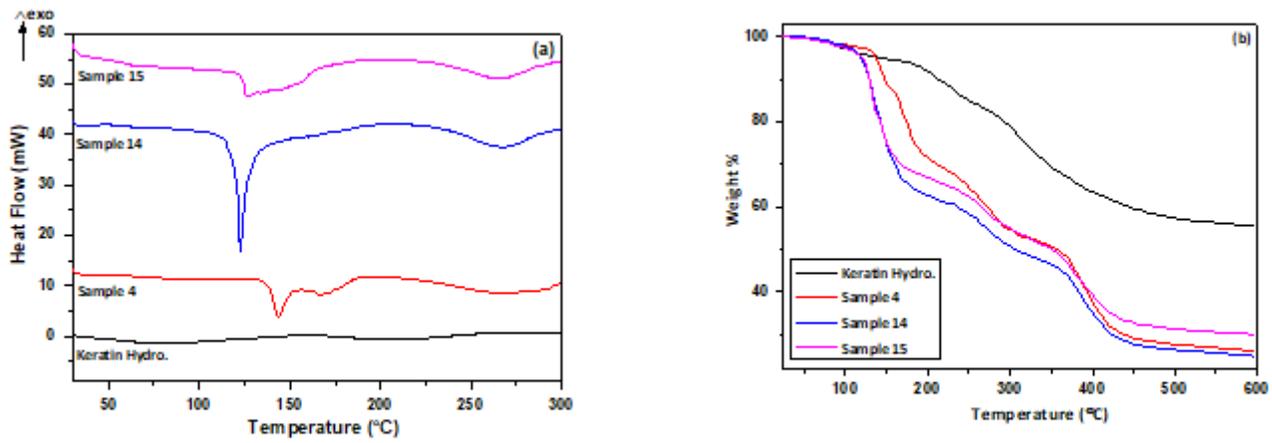


Figure 5

Effect of blends concentrations on thermal properties; a) DSC curve of keratin hydrolysate, sample 4, sample 14, and sample 15, b) TGA curve for keratin hydrolysate, Sample 4, sample 14, and sample 15.

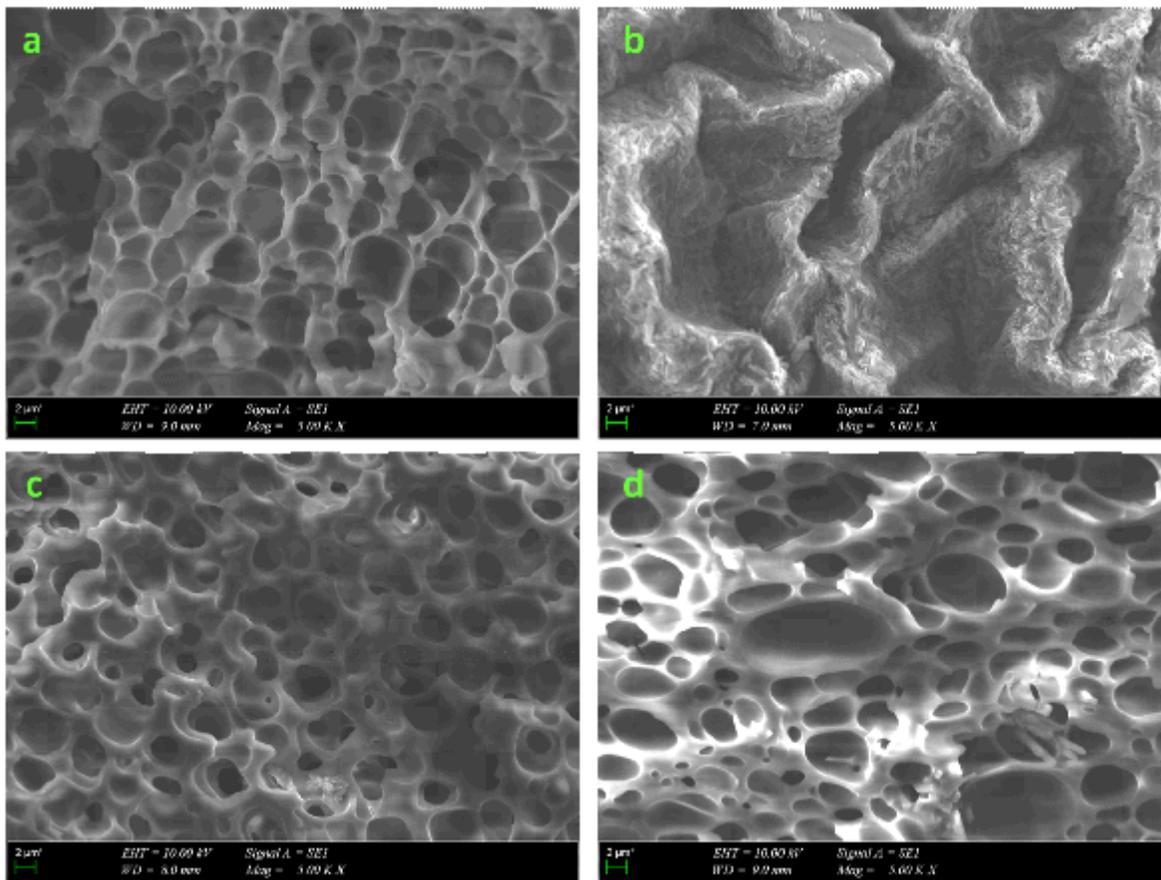


Figure 6

SEM images of a) Sample 4, b) Sample 8, c) Sample 14, and d) Sample 15, and scale is 2µm.