Development of keratin based hydrogels for biomedical applications

IOP Conf. Series: Materials Science and Engineering **702** (2019) 012031 doi:10.1088/1757-899X/702/1/012031

Development of keratin based hydrogels for biomedical applications

M S B Husain, A Gupta*, B Y Alashwal

Faculty of Chemical and Process Engineering Technology, College of Engineering Technology, Universiti Malaysia Pahang, 26300 Gambang, Pahang, Malaysia.

*E-mail: arungupta10@gmail.com

Abstract. Synthesis of hydrogel was developed by using keratin protein extracted from a chicken feather. Further, polyvinyl alcohol (PVA), polyvinylpyrrolidone (PVP), and starch have been commonly using for biomedical applications hydrogels due to their non-toxicity and, water solubility. The first sample, (KS-50) was mixed keratin, PVA/PVP and starch, the second sample, (K-50) was mixed the total weight of the (KS-50) without starch. KS-50 and K-50 were synthesized through cyclic freezing/thawing technique. Moreover, characteristics of the hydrogels, scanning electron microscopy (SEM) examined clearly exhibited porous structures, and the chemical structure of the compound was investigated by Fourier transform infrared spectroscopy (FTIR). The swelling degree of KS-50 exhibited slightly more pore size compared with K-50 hydrogels.

1. Introduction

The hydrogels are generally polymeric networks seen in cross-linked structures. They can be distinguished by their indistinct viscoelastic performance, hydrophilic nature. Elevation-water content facilitates the transfer the metabolic waste, nutrients, and oxygen [1]. Due to the presence of physical or chemical crosslinks like crystallites networks are collected from copolymers and homopolymers insoluble [2]. Modulating the polymer concentration and methods of synthesis allows tuning of specific hydrogel properties like biodegradability, swelling behavior, and porosity [3]. The hydrogels have been applied as scaffold materials the controlled release of drugs, proteins, and other applications [4-6]. Also, hydrogels are attractive for biomedical applications because of their diffusion properties/good water sorption biocompatibility [7].

Keratin turns into one of the most significant biomaterials through commercially available biomacromolecules due to its relatively good biocompatibility, excellent mechanical properties, high physicochemical, and good formatting-film ability [8-9]. Feather keratins from chicken feathers, that are considered to be the essential waste in the industry of poultry, because of coloring-dyeing and perming the control of the quality of the feather keratin is easier than hair keratin [10-11]. The featherweight of pure keratin is 90% feathers waste are a source of amino acids, protein, and nitrogen [12-[3]. Due to a great number of disulfide bonds, the keratin is a group of cysteine-rich structural proteins established in the cells of vertebrates that display high-rise mechanical strength [14-16]. Keratin has been utilized in several biomedical applications because of its biodegradability and biocompatibility [17-18].

Starch is one of the cheapest and most abundant polysaccharides. Also, the starch is a very cheap polysaccharide due to every starch derivatives are biodegradable and biocompatible polymers that will be utilized biomedical applications [19-21].

PVA based hydrogel is one of the famous polymer gel because of its good biocompatibility and utilized in several numbers of biomedical applications, such as, implants, dressings in wounds management, contact lenses, and drug delivery devices [22]. It has raised attention due to that it's water-soluble, non-toxic, and biocompatible properties [23-25]. PVP is one of the most common water-soluble, biodegradable, biocompatible, and low toxicity synthetic polymers [19, 24]. It is utilized in many biomedical applications and separation processes to raise the hydrophilic character of the mixed polymeric materials [26].

This paper presents a methodology for the development of hydrogels, composed of keratin protein, PVA, PVP, and Starch by using physical crosslinking by the freeze-thawing method for potential biomedical applications.

2. Materials and Methods

2.1. Materials

The chicken feathers have taken from the chicken plant at Jaya Gading, Kuantan, Malaysia. Polyvinyl alcohol (PVA), Polyvinylpyrrolidone (PVP), Sodium hydroxide (NaOH), and Hydrochloric acid (HCl) were purchased from R&M, chemicals, Kuala Lumpur, Malaysia, and, starch was purchased from Chemmart Asia Sdn. Bhd.

2.2. The keratin protein Extraction from chicken feather

Keratin protein solution was prepared in the chemical engineering lab at University Malaysia Pahang by cleaning feathers according to previously studied methods. The 100g of cleaned, dried and blended chicken feathers were added in 1L of (1N) sodium hydroxide solution in a 2L of the conical flask. The temperature of the mixture of the solution was 50°C, pH was 12 and the mixture was continuously stirred for 4h and cool to room temperature. After that, the solution was filtered through filter stainless steel then centrifuged at 10,000rpm for 10min [27], [28]. The keratin protein was collected carefully and then was filtered through filter paper and of the solution was adjusted to pH 7 with HCl (2N) and stored in the laboratory bottle for the synthesis of hydrogels.

2.3. Preparation of Polyvinyl Alcohol (PVA) Solution

A mass of 10g PVA powder was weighed by using the analytical balance. Then, the powder was added into 100ml distilled water with stirring until PVA dissolved completely. In the same time, the temperature was kept constant in the range of 70- 80°C [29]. The stirring process was done on a hot plate stirrer and continuously stirred until all the solutes dissolved thoroughly [30].

2.4. Preparation of Polyvinylpolypyrrolidone (PVP) Solution

A mass of 10g PVP powder was weighed by using the analytical balance. Then, the powder was added into 100ml distilled water with stirring until PVP dissolved completely. The stirring process was done manually by using a stirring rod for about 15 minutes [31].

2.5. Preparation of hydrogel film

The first sample, the keratin hydrogel (KS-50) was mixed with 50mL of keratin, 30mL of PVA 10mL of PVP, and 10g of starch with continuous stirring at 60 °C for 30min. The second sample, keratin hydrogel (K-50) was the total weight of the (KS-50) without starch and under stirred at 60°C for 30min. The proper amount of this mixture (15mL) were poured in plastic Petri dishes, followed by freezing at −20°C for 8h and thawing for 6h at 25°C for 3 continuous cycles to form hydrogel for further analysis [32], [33].

2.6. Scanning Electron Microscope (SEM)

Hitachi's Tabletop Electron Microscope (TM3030) was selected for carrying out the analysis related to the molecular structure of the study of the hydrogel samples [25].

2.7. Fourier Transform Infrared Spectroscopy (FTIR)

The Perkin-Elmer Model 1000 Series FTIR device was utilized to select spectra of the hydrogel. The frequency scope of the spectra was between 4000 cm^{-1} to 500 cm^{-1}. The all data obtained for hydrogel was through the utilizing of the software of FTIR [25].

2.8. Degree of the Swelling ratio

The hydrogel ability for fluid uptake was measured utilizing an electronic balance by immersion in phosphate-buffered saline (PBS). The hydrogels pieces were weighed (W_d) and put in PBS at 37 °C, and pH 7.4. The swollen hydrogel discs were measured at 10, 20, 30, 40, 50, 60, 1440 minutes, the surface was wiped gently with blotting paper to eliminate surface-adsorbed fluid, and the hydrogels weighed again (W_w). The degree of swelling defines the ability for fluid uptake by the following equation (1) [34]:

$$\text{The Swelling Degree } (\%) = \left[\frac{W_w - W_d}{W_d}\right] \times 100\% \quad (1)$$

Where W_d is the initial weight of the disc at time 0, and W_w is the weight of the disc at time t.

3. Results and Discussion

3.1. SEM analysis

SEM micrographs of the top view of the KS-50 (A) and K-50 (B) smooth with no particular structure. Figure 1, freeze-dried KS-50 and K-50 hydrogels clearly exhibited porous structures. Pore sizes can affect the physicochemical characteristics of the outcoming KS-50 and K-50 hydrogels, like the degree of swelling [35, 26].

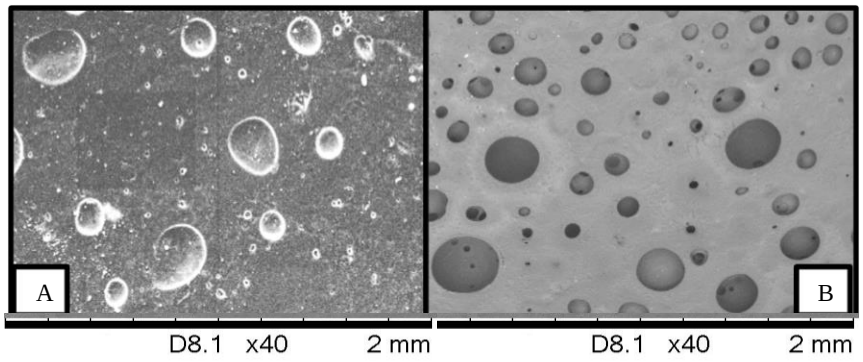


Figure 1. The SEM of KS-50 and K-50 keratin hydrogel samples.

3.2. FTIR analysis

FTIR measurement was utilized to recognize the chemical structure of the hydrogel and the results are shown in figure 2. The attained results validate the presence of keratin chemical structure when compare to the previous study in which keratin extracted from the chicken feathers [28]. The prepared hydrogels demonstrated transmission bands of the peptide bonds (-CONH) and are known as Amide A (3298cm^{-1}) ascribed to stretching vibration of -N-H and-O-H, Amide I (1651cm^{-1}) which are related to C=O stretching bonds, Amide II (1427cm^{-1}) is for N-H bending and C-H stretching, Amide III (1252cm^{-1}) derived from C-N stretching and N-H bending [36], [37]. The closeness of hydrophilic and hydrophobic moieties which was confirmed by FTIR makes them soluble in both aqueous and organic medium. This made it essential for medication framework [38]. The vibration bands from 950cm^{-1} recognized to alcohol. The presence of more -OH gatherings contributed to hydrogen bonding in the hydrogel. Thus, FTIR spectra the similar peaks demonstrated the presence of chemical groups in hydrogels that they can improve their physical and chemical properties for various for potential biomedical applications.

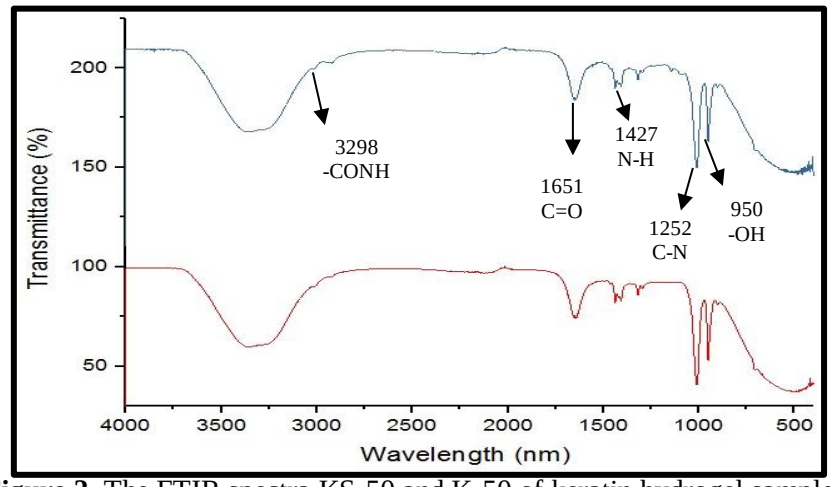
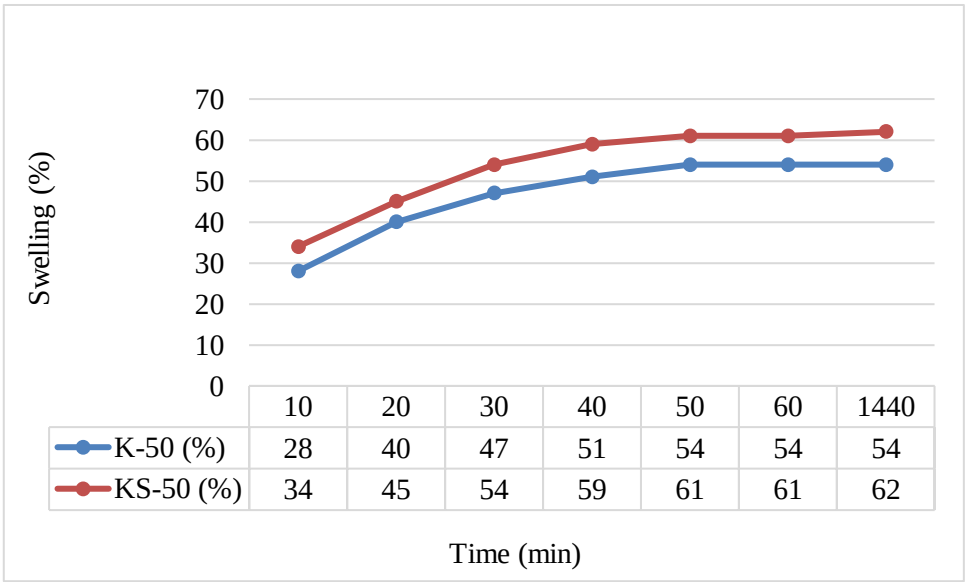


Figure 2. The FTIR spectra KS-50 and K-50 of keratin hydrogel samples.

3.3. Equilibrium degree of swelling

The swelling percentage of the KS-50 has increased only slightly with the time compared with the K-50 and the equilibrium swelling was reached at 50minutes for both KS-50 and K-50 as shown in figure 3. The size of pores is the major factor that controls the swelling degree of the hydrogel [39]. The sample could rapidly restore to the original state after being pressed, suggesting a sponge-like characteristic [40]. For most of the keratin hydrogels, the swelling properties are effective for biomedical applications.



	10	20	30	40	50	60	1440
K-50 (%)	28	40	47	51	54	54	54
KS-50 (%)	34	45	54	59	61	61	62

Figure 3.3 The swelling KS-50 and K-50 of keratin hydrogel samples.

4. Conclusion

In summary, the synthesis of keratin-based hydrogel was successfully developed using PVA/PVP/starch. Although the analysis of the results described good properties of hydrogel (K-50), the desired development was seen in the presence of starch (KS-50). FTIR spectra showed that peaks appeared in the hydrogel designating that they provide the macromolecular composition of the keratin, and SEM exhibited a large of pore sizes in the smooth surface. Moreover, the swelling percentage of the KS-50 has increased only slightly compared with the K-50 and the samples have developed like a

hydrophilic sponge. However, the developed keratin hydrogels have a future in several biomedical applications.

Acknowledgments
Authors are thankful to University Malaysia Pahang (UMP) for financial support (Financial Aid (PGRS) number: 180334) and providing facilities.

References

[1] Ågren, M ed 2016 Wound Healing Biomaterials -Volume 2: Functional Biomaterials (Woodhead Publishing)
[2] Peppas N A, Hilt J Z and Thomas JB 2007 Nanotechnology in therapeutics: Current technology and applications (Horizon Scientific Press)
[3] Hemshekhar M, Thushara R M, Chandranayaka S, Sherman L S, Kemparaju K and Girish K S 2016 Emerging roles of hyaluronic acid bioscaffolds in tissue engineering and regenerative medicine *International Journal of Biological Macromolecules* 917-928
[4] Hennink W E and van Nostrum C F 2012 Novel crosslinking methods to design hydrogels *Advanced Drug Delivery Reviews* 223-36
[5] George A, Augustine R and Sebastian M eds 2014 Diabetes Mellitus and Human Health Care: A Holistic Approach to Diagnosis and Treatment (Apple Academic Press Canada)
[6] Koehler J, Brandl F P and Goepferich A M 2018 Hydrogel wound dressings for bioactive treatment of acute and chronic wounds *European Polymer Journal* 1-11
[7] Spanoudaki A N N A, Fragiadakis D A N I E L, Vartzeli-Nikaki K A L L I O P I, Pissis P O L Y C A R P O S, Hernandez J C R and Pradas M M 2006 Nanostructured and nanocomposite hydrogels for biomedical applications (Springer Dordrecht) pp 229-40
[8] Xu S, Zhang C, Zhang A, Wang H, Rao H and Zhang Z 2016 Fabrication and biological evaluation in vivo of an injectable keratin hydrogel as filler materials Journal of Bioactive and Compatible Polymers 179-190
[9] M M S B Husain, A Gupta, and B Y Alashwal, and S Sharma 2018 Synthesis of Hydrogel Using Keratin Protein from Chicken Feather Proceedings of ICCEIB2018 **193** 16
[10] Sharma S, and Gupta A 2016 Sustainable management of keratin waste biomass: applications and future perspectives Brazilian Archives of Biology and Technology **59**
[11] Wang J, Hao S, Luo T, Cheng Z, Li W, Gao F, Guo T, Gong Y and Wang B 2017 Feather keratin hydrogel for wound repair: preparation, healing effect and biocompatibility evaluation Colloids and Surfaces B: Biointerfaces **149** 341-50
[12] Kshetri P, Roy S S, Sharma S K, Singh T S, Ansari M A, Prakash N and Ngachan S V 2019 Transforming chicken feather waste into feather protein hydrolysate using a newly isolated multifaceted keratinolytic bacterium Chryseobacterium sediminis RCM-SSR *Waste and Biomass Valorization* 1-11
[13] Alashwal B Y, Bala M S, Gupta A, Sharma S and Mishra P 2019 Improved properties of keratin-based bioplastic film blended with microcrystalline cellulose: A comparative analysis *Journal of King Saud University-Science* In Press
[14] Reichl, S, Borrelli M and Geerling G 2011 Keratin films for ocular surface reconstruction Biomaterials **32**(13) 3375-86
[15] Reichl S, 2009 Films based on human hair keratin as substrates for cell culture and tissue engineering Biomaterials **30**(36) 6854-66
[16] Yua H M, Gupta A, Guptab R and Husaina M S B 2017 Investigation of bioplastic properties developed from acrylate epoxidized soybean oil through ring opening polymerization process *Journal of Chemical Engineering and Industrial Biotechnology* 29-41
[17] Vasconcelos A and Cavaco-Paulo A 2013 The use of keratin in biomedical applications *Current Drug Targets* **14**(5) 612-9
[18] Rouse J G and Van Dyke M E 2010 A review of keratin-based biomaterials for biomedical applications *Materials* 999-1014
[19] Kamoun E A, Chen X, Eldin M S M and Kenawy E R S 2015 Crosslinked poly (vinyl alcohol)

hydrogels for wound dressing applications: A review of remarkably blended polymers *Arabian Journal of Chemistry* 1-14
[20] Sharma S Sharma D and Kanwar K 2015 Technology refinement for micropropagated Aloe vera L.: a miracle plant *Research in Plant Biology* **5**(4) 1-10
[21] Haesler E 2017 Evidence summary: Wound management low resource communities-Aloe vera for wound healing *Wound Practice & Research: Journal of the Australian Wound Management Association* 115
[22] Kokabi M, Sirousazar M and Hassan Z M 2007 PVA–clay nanocomposite hydrogels for wound dressing *European Polymer Journal* 773-81
[23] Swaroop K, Francis S and Somashekarappa H M 2016 Gamma irradiation synthesis of Ag/PVA hydrogels and its antibacterial activity *Materials Today: Proceedings* 1792-98
[24] Husain M, Gupta A, Alashwal B, and Sharma S 2018 Synthesis of PVA/PVP Based Hydrogel for Biomedical Applications: A Review *Energy Sources, Part A: Recovery, Utilization, and Environmental Effects* **40**(20) 2388-93
[25] Ramakrishnan N, Sharma S, Gupta A, and Alashwal B Y 2018 Keratin based bioplastic film from chicken feathers and its characterization *International Journal of Biological Macromolecules* **111** 352-358
[26] Abd El-Mohdy H L and Ghanem S 2009 Biodegradability, antimicrobial activity and properties of PVA/PVP hydrogels prepared by γ-irradiation *Journal of Polymer Research* **16**(1)
[27] Bao Y, Ma J and Li N 2011 Synthesis and swelling behaviors of sodium carboxymethyl cellulose-g-poly (AA-co-AM-co-AMPS)/MMT superabsorbent hydrogel *Carbohydrate Polymers* 76-82
[28] Sharma S, Gupta A, Chik S M S B T, Kee C Y G and Poddar P K 2017 Dissolution and characterization of biofunctional keratin particles extracted from chicken feathers *IOP Conference Series: Materials Science and Engineering* **191** 12013
[29] Imtiaz N, Niazi M B K, Fasim F, Khan B A, Bano S A, Shah G M, Badshah M, Menaa F and Uzair B 2019 Fabrication of an Original Transparent PVA/Gelatin Hydrogel: In Vitro Antimicrobial Activity against Skin Pathogens *International Journal of Polymer Science*
[30] Pan H, Fan D, Cao W, Zhu C, Duan Z, Fu R, Li X and Ma X 2017 Preparation and characterization of breathable hemostatic hydrogel dressings and determination of their effects on full-thickness defects Polymers **9**(12) 727
[31] Adjei F K, Osei YA, Kuntworbe N and Ofori-Kwakye K 2017 Evaluation of the Disintegrant Properties of Native Starches of Five New Cassava Varieties in Paracetamol Tablet Formulations *Journal of Pharmaceutics*
[32] Fahmy A, Kamoun E A, El-Eisawy R, El-Fakharany E M, Taha T H, El-Damhougy B K and Abdelhai F 2015 Poly (vinyl alcohol)-hyaluronic acid membranes for wound dressing applications: synthesis and in vitro bio-evaluations *Journal of the Brazilian Chemical Society* 1466-74
[33] Hassan C M and Peppas N A 2000 Cellular PVA hydrogels produced by freeze/thawing *Journal of Applied Polymer Science* 2075–79
[34] Ponrasu T, Veerasubramanian P K, Kannan R, Gopika S, Suguna L and Muthuvijayan V 2018 Morin incorporated polysaccharide–protein (psyllium–keratin) hydrogel scaffolds accelerate diabetic wound healing in Wistar rats *RSC Advances* 2305-2314
[35] Park M, Kim B S, Shin H K, Park S J and Kim H Y 2013 Preparation and characterization of keratin-based biocomposite hydrogels prepared by electron beam irradiation *Materials Science and Engineering: C* 5051-57
[36] Kamarudin N B, Sharma S, Gupta A, Kee C G, Chik S M S B T and Gupta R 2017 Statistical investigation of extraction parameters of keratin from chicken feather using Design-Expert *3 Biotech* **7**(2) 127
[37] Xu H, Cai S, Xu L and Yang Y 2014 Water-stable three-dimensional ultrafine fibrous scaffolds from keratin for cartilage tissue engineering *Langmuir* 8461-70
[38] Roberts M J, Bentley, M D and Harris J M 2012 Chemistry for peptide and protein PEGylation *Drug Delivery* 116–127
[39] Khan S, Ullah A, Ullah K and Rehman N U 2016 Insight into hydrogels Designed Monomers and

Polymers 456-478
[40] Feng R, Fu R, Duan Z, Zhu C, Ma X, Fan D and Li X 2018 Preparation of sponge-like macroporous PVA hydrogels via n-HA enhanced phase separation and their potential as wound dressing *Journal of Biomaterials Science, Polymer Edition* 1463-1481