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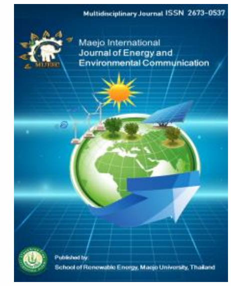

ARTICLE

Synthesis of keratin spray from chicken feathers for biomedical applications

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A B S T R A C T

There is a pressing need to develop wound healing spray that can utilize it for the treatment of skin rebuilding. Wound healing involves the regeneration and tissue repair process with the sequence of molecular and cellular measures that ensue the onset of a tissue lesion to reestablish the damaged tissue. This study aimed to produce the best formulation of keratin-based wound spray which was prepared by varying of the keratin concentration to suit for human nature skin and can be promoted for the wound healing process. A wound-healing bouquet was prepared with keratin as the primary substituent mixed with 2-phenyl ethanol, methyl lactate, and methyl propanediol to rejuvenate the skin effectively. The keratin has been extracted from chicken feathers which have high levels of protein source. Keratinocytes containing keratin migrate from the wound edges to cover the wound during the remedial process. The samples are characterized by Fourier Transform Infrared Spectroscopy (FTIR) to determine the functional groups and Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) for heavy metal analysis. The characteristics of keratin-based wound spray from chicken feather made it an effective wound care product. The pH value of the formulation possesses a little acidic nature (pH 5.56) where is considered an appropriate nature to prevent the growth of bacteria. This wound healing spray gives a suitable domain of cure efficiency to the injured region as they have ideal levels of pH.

1. Introduction

Keratin is protein building blocks that have amino acid chains, coiled (Saron et al. 2011), cross-linked and are the most abundant proteins found in the body of humans, mammals, birds, and reptiles (Soubam and Gupta, 2021; Reddy et al., 2021). As part of the epithelial cytoskeleton, keratins are essential for the mechanical stability and integrity of epithelial cells and tissues (Alashwal et al., 2020). Some keratins also have regulatory functions and are involved in intracellular signalling pathways, e.g. protection from stress, wound healing, and apoptosis (Moll et al., 2008) Keratin represents the most abundant structural proteins in epithelial cells, and together with collagen, is the most important biopolymer in animals. According to the Ashby map (Ashby et al., 1995), keratin

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is among the toughest biological materials, possessing both high toughness and high modulus, although it is solely composed of polymeric constituents, and seldom contains minerals Keratinous materials, formed by specifically organized keratinized cells filled with mainly fibrous proteins (keratins) Experimental study on the mechanical properties of the horn sheaths from cattle (Bala et al., 2020, Yua et al., 2017). Chicken feathers are composed of over 90% keratin protein. The feathers constitute up to 10% of the total chicken weight. Worldwide, millions of tons of feathers are generated annually as waste by poultry-processing industries (Taskin et al., 2011). Feather keratin has a high content of cysteine in the amino acid sequence and cysteine has -SH groups that cause the sulfur–sulfur (disulfide) bonding. This high content of cysteine makes the keratin stable by forming a network structure by joining adjacent polypeptides by disulfide cross-links (Thornton, 2018). One of the main sources of keratin waste is the poultry industry (Bala et al., 2018). The USDA Foreign Agricultural Services (2015) estimates that Malaysian broiler (chickens that are raised for meat) chicken production has increased throughout 10 years. Considering feathers are made in 90% of keratin and constitute 5-7% of the total weight of an adult chicken, the poultry industry generates thousands of kilograms of waste feathers as a byproduct when the birds are processed in commercial dressing plants (Nagal et al., 2010; Cheong et al., 2017)

The humongous volume of waste feather creates a serious solid waste problem in many countries. Waste feathers are often buried in landfills or piled in dumpsites since the traditional method of waste are expensive and difficult (Alashwal et al., 2020). However, feathers are naturally resistant to deterioration and persist in the environment for decades. Thus, it pollutes the environment as they take up large space in landfills and have a bad odour from residual manure, as well as their blood (Acda, 2010). Furthermore, up until now, there are nobody has ever done wound healing spray using keratin protein specifically from chicken feathers (Husain et al., 2018b; Alashwal et al., 2019). The literature on the development of wound healing spray using keratin protein from chicken feathers not available yet. There are lots of wound healing products sold in the market but not all products can heal the wound perfectly. This study intends to produce an empirical formula of wound healing spray, which is suit to the human skin nature and can be used efficiently and fast for healing the wound (Kong et al., 2007). The skin can be damaged in a variety of ways depending upon the mechanism of injury for example of inflammation, deep abrasions, lacerations, rupture injuries, and penetrating wounds (Belarmino et al., 2012). A wound is a breakdown in the protective function of the skin where the loss of continuity of epithelium, with or without loss of underlying connective tissue for example muscle, bone, or nerves that are caused by an injury (Li et al., 2017). The skin can be damaged in a variety of ways depending upon the mechanism of injury for example of inflammation, deep abrasions, lacerations, rupture injuries, and penetrating wounds (Wedro, 2017). Thus, due to the function of 2-phenylethanol, methyl lactate, methylpropanediol, inside the keratin-based wound healing spray promotes the healing process. In previous studies, the most commonly used topical antibiotics include neomycin, bacitracin, silver sulfadiazine, and mupirocin (Husain et al., 2018a, 2019). However, the usage of silver nitrate is limited in burn wounds because the application of silver over a large body surface area may lead to electrolyte imbalance. Neomycin has been reported to cause allergic contact dermatitis when applied to intact skin (Bhuyar et al., 2018; Abayomi et al., 2016). There are varieties of wound dressings in the market, which are functioned to preserve hydration within the wound in order to optimize such as the regeneration, the protection against infection, and avoiding the disruption of the wound base (Flanagan et al., 2000). Therefore, there are numbers of biomaterials that are currently being investigated for wound dressings consist of alginates, hydrocolloid (Dreifke et al., 2014; Boateng et al., 2007). Generally, the products are pharmaceutically available on the form of gels, thin films and foam sheets Development of keratin-based hydrogels for biomedical applications (wang et al., 2015; Gupta et al., 2020).

To check the properties of made wound healing sprays, they were characterized by using Fourier Transform Infrared Spectroscopy (FTIR), Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES), pH and density (Fabian et al., 2002). This study provides a remarkable platform to produce the value-added products from the waste biomass of chicken feathers in an eco-friendly manner. It also directed the bioremediations of huge poultry waste.

2. Materials and Methods

2.1. Materials

Chemicals were obtained from one source, namely Gardner Global Enterprise, Kuantan, Pahang, Malaysia, 2-phenylethanol (purity ≥ 99% (GC)), methyl lactate (purity ≥ 98% (GC)), and methylpropanediol (purity 98%). The keratin protein extracted from chicken feathers was prepared inside the Faculty of Chemical and Natural Resources Engineering laboratory at Universiti Malaysia Pahang.

2.2. Keratin protein preparation

Chicken feathers were collected from chicken processing plants. After feather treatment process Characterization of dehydrated keratin protein extracted from chicken feather , 160 g of sodium hydroxide were dissolved in 4 L of distilled water. 200 g of chicken feathers were added into the mixture solution. The mixture was stirred for 6 hours and was heated at 60°C Improved properties of keratin-based bioplastic film blended with microcrystalline cellulose: A comparative analysis . The solution was filtered and centrifuged at 10000 rpm for 15 min. The supernatant liquid will be collected and filtered to make it particle free. The pH of the resulting keratin protein was maintained in the range of 7.0-8.0 (Gupta et. al., 2012). The keratin protein solution were then analysed using Fourier Transform Infrared Spectroscopy (FTIR) equipment Characterization of dehydrated keratin protein extracted from chicken.

2.3. Wound healing spray production

2-phenylethanol was mixed with water until it was homogenous. Methyl lactate and methylpropanediol were added into the mixture. The mixture solution was stirred for 20 minutes. The keratin solution was then added to the mixture and stirred for another 10 minutes. The pH value and density of the formulations were measured. Four formulations were made with four different ratios of keratin solution and chemical mixture solution. The ratio used were (keratin : chemical = 10:50-40:20 (ml)) to obtain the best formulation of wound healing spray. pH test and density were performed for every formulations. The wound healing solution then proceeds with FTIR analysis, and Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) analysis.

2.4. FTIR analysis

FTIR was utilized to examine the nearness of particular chemical functional groups in the samples. Each of the sample solutions were collected and analyzed using FTIR and its wavelength graph obtained will be compared with the keratin protein solution graph. FTIR spectra were obtained in the range of wave number 4000 to 400 cm-1 (Paragon 1000, Perkin-Elmer, USA). The FTIR spectra were normalized and major vibration bands were associated with chemical groups.

2.5. Heavy Metal test

Heavy metals such as arsenic (As), cadmium (Cd), copper (Cu), iron (Fe), lead (Pb), and zinc (Zn) are dangerous if it were consumed by human as by accident or not. Thus, heavy metal test were conducted in order to detect, measure or monitor the quantity of specific potentially toxic metals in each of the formulations. This test was conducted by Lab Technician in CARIFF at Universiti Malaysia Pahang.

3. Results and Discussion

3.1. pH test

The pH value result of each spray formulation was stated in Table 2. The pH of each of formulation was measured over the course of 7 days. After the production, the average pH values ranged from 7.24 to 7.46 for all of the formulation. After 7 days, the pH was reduced in the range of 5.15 and 5.56 for F1 to F4, respectively. It may indicate that some form of chemical reaction may be taking place (Lambers et al., 2006). As indicated by (Parsons et al., 2005; Slone et al., 2010), it has been suggested that dressings with a slightly acidic pH (similar to that of healthy skin; pH of 5.5) may be most comfortable to wear.

3.2 Density

The density of each formulation is stated in Table 3. The densities of the liquid spray formulations were confined to be in the range of 1.0064 g/mL to 1.0234 g/mL. Since density is slightly equal to that of water (1.000 g/mL), they are largely unaffected by the spray composition.

Table 1 Formulation of Wound Healing Spray using keratin from chicken feathers.

Compound	Formulation, % w/w			
	F1 (10:50)	**F2 (20:40)**	**F3 (30:30)**	**F4 (40:20)**
Keratin protein solution	16.67	33.33	50.00	66.67
2-phenylethanol	1.00	1.00	1.00	1.00
Methyl lactate	8.33	8.33	8.33	8.33
Methylpropane-diol	13.33	13.33	13.33	13.33
Water	60.67	44.00	27.33	10.67

Table 2 pH value for each wound healing.

Spray Formulation	pH value							
	pH after production				**pH after 168 hours**			
	1	2	3	Average	1	2	3	Average
Pure keratin	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0
F1 (10:50)	7.25	7.1	7.36	7.24	5.15	5.18	5.13	5.15
F2 (20:40)	7.28	7.15	7.56	7.33	5.34	5.33	5.35	5.34
F3 (30:30)	7.25	7.33	7.56	7.38	5.48	5.44	5.47	5.46
F4 (40:20)	7.28	7.47	7.63	7.46	5.55	5.56	5.58	5.56

Table 3 Density value for each wound healing spray.

Spray Formulation	Density (g/ml)			
	1	**2**	**3**	**Average**
F1 (10:50)	1.0048	1.0156	1.0102	1.0102
F2 (20:40)	1.0201	1.0032	0.9959	1.0064
F3 (30:30)	1.0199	1.0212	1.0207	1.0206
F4 (40:20)	1.0234	1.0190	1.0278	1.0234

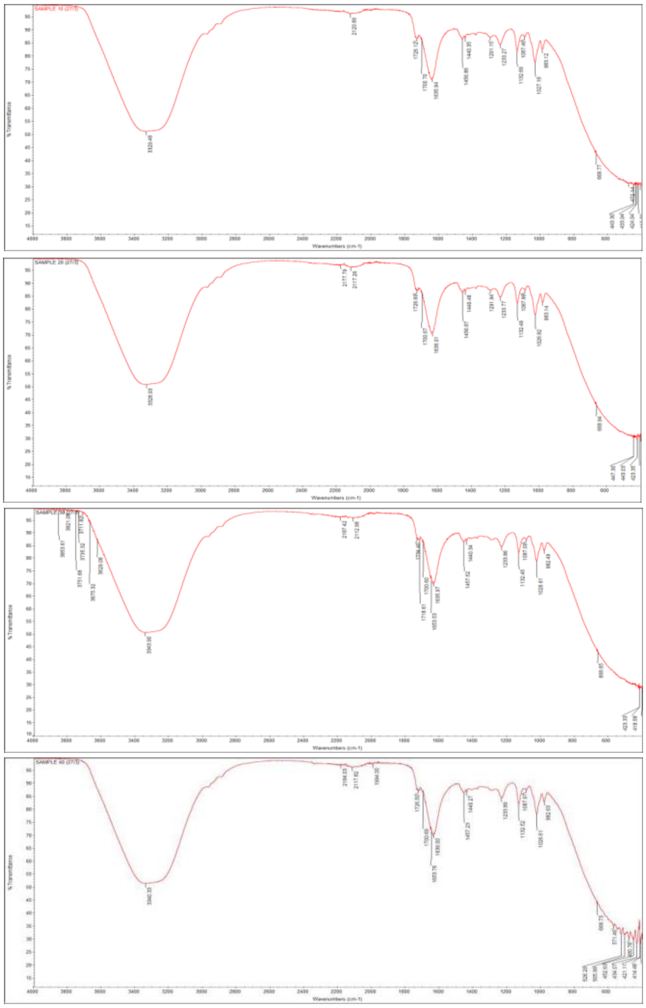

Fig. 1. FTIR result for every formulation.

3.3 Fourier Transform Infrared Spectroscopy (FTIR) analysis

FTIR was utilized to examine the nearness of particular chemical functional groups in the samples. From the result of FTIR spectroscopy obtained, all spray formulations demonstrated the presence of water OH stretch, alcohol OH stretch, C=O, –NH, and –COOH functional. It demonstrated the stretch of water –OH groups from 3700-3100 cm^{-1}. The presence of 2-phenylethanol shows the alcohol –OH stretch from 3600-3200 cm^{-1}. –COOH stretch range from 1200-1020 cm^{-1} was seen as the presence of methylpropanediol. The stretch of C=O ester in the range of 1750-1720 cm^{-1} was shown as the presence of methyl lactate in the formulations.

3.4 Heavy metal test

The concentration of heavy metal namely Arsenic (As), Cadmium (Cd), Copper (Cu), Iron (Fe), Lead (Pb), and Zinc (Zn) was measured for each formulations. The results are shown in Table 4. From the results it can be seen that the concentration of each elements inside each formulation is in negative value. Based on the calibration curve obtained from the lab, the error is very high (> 0.00001). The calibration curve for Cadmium, copper, lead, zinc, iron, and arsenic element is 0.027, 0.023, 0.026, 0.027, 0.025, and 0.00063, respectively. Since the results obtained follows the calibration curves, the concentration of the elements inside each formulations is inaccurate.

Table 4 Heavy metal concentrations (mg/L) for each wound healing spray.

Spray formulations	As	Cd	Cu	Fe	Pb	Zn
F1 (10:50)	-0.549	-1.474	-1.376	0.633	-1.550	-0.356
F2 (20:40)	-0.552	-1.481	-1.371	0.099	-1.547	-0.432
F3 (30:30)	-0.546	-1.482	-1.358	-0.274	-1.550	-0.297
F4 (40:20)	-0.547	-1.478	-1.336	-0.096	-1.553	-0.201

4. Conclusion

Keratin biomaterials have been in collective conscience by reserachers since many years, yet there is no much evidence of current use for clinic for keratin application. The main challenge is to develop wound healing spray formulation from natural biomateriak that have the capacity to positively influence the wound heal. In the present article, the keratin protein still exists inside all of the formulations. Thus, the liquid formulations can be used as a wound healing spray to promote wound healing process. The pH of slightly acidic (pH 5.56) also shows that the spray is suitable to be applied on human skin.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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