

FABRICATION OF NANOFIBRES BY ELECTROSPINNING USING KERATIN FROM WASTE CHICKEN FEATHERS, PVA AND AgNPs

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ABSTRACT

Objective: To prepare and characterise keratin from chicken feathers (CF), collected from the slaughter house, and to blend with poly vinyl alcohol (PVA) and biosynthesised silver nanoparticles (AgNPs) and to convert into nanofibers by an electrospinning process.

Methods: The extraction of keratin from chicken feathers was done by sodium m-bisulphite. The solution was subjected to ammonium sulphate precipitation to separate keratin. The nanoparticles were synthesised using *tridax procumbens*. The isolated keratin and PVA was mixed in the ratio of 50:50 with 1 ml of biosynthesised nanoparticles was blended and made into nanofibers by electrospinning technique.

Results: The precipitated protein was analysed using FT-IR analysis confirming the presence of β -keratin in the sample isolated from chicken feathers and the concentration of keratin was estimated to be 1.85 g/ml. PVA solution with 4% w/v had the best film forming ability. The solution containing keratin, PVA and silver nanoparticles was prepared in various proportions. These solutions when subjected to electrospinning, fibrous network was observed in 50:50 (PVA: Keratin) ratio with 1 ml of synthesised silver nanoparticle solution. Hydrogen bonding between keratin and PVA indicated in the XRD analysis showed successful film forming of the nanofiber, the DSC analysis also showed similar results as the obtained peak was at 214 °C which is in between the characteristic heat degradation temperature of both the keratin and PVA. The thermogravimetric analysis (TGA) showed high thermal stability as the complete degradation of the nanofiber was observed at 420 °C. Incorporation of metal nanoparticles by herbal approach using *tridax procumbens* in the nanofibers provided the antimicrobial properties. The nanofibers obtained by electrospinning process appeared stable and continuous for solutions containing no more than 50% wt of CF. The average diameter of the nanofibers increased as the CF content increased.

Conclusion: Keratin isolated from the waste chicken feathers impregnated with biosynthesised silver nanoparticles using *tridax procumbens* and PVA can be converted into nanofibers by electrospinning process. Thus, the biocomposite nano fibers are shown as a novel eco-friendly material that must be adequately applied in the development of green composites for the biomedical applications such as wound dressings.

Keywords: Keratin, Chicken feathers, PVA, *Tridax procumbens*, Nanofibres, Electrospinning

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INTRODUCTION

Wound healing is a biological process, where the tissue regeneration of the damaged tissue takes place. Wound healing products are highly in demand due to the increase in population, the patients should be able to recover faster with less trauma. The important factor for the increase in bio-based wound healing products are because of the increasing waste in the environment such as medical waste in the form of gauze, cotton and wound dressings stained with blood, which possess hazardous to the surroundings. This can be avoided using bio-based wound healing products as the products are absorbable and eliminate the need for waste management [1]. Chicken feathers contain over 90% of protein, mainly β -keratin, fats and water, which can be utilized for industrial applications [2]. The chicken feathers leads to accumulation, and imparts an important environmental risk to the poultry farming industry and landfills. Keratin is an insoluble protein matrix which forms the feathers, hair, nails, beaks, horn and hooves [3, 4]. They are known to have a high tensile strength in comparison to all other biological materials and are considered as dead tissues [5]. Keratinous materials exhibit various characteristics such as high stress, water resistant, therefore acts as a diffusion barrier and resists penetration based on their mechanical properties, which are influenced by the disulphide bonds present between two cysteine molecules [6]. It was reported that the feather keratin is a potential source of inexpensive, ecofriendly and commercial biomaterial [7, 8]. Shindai method to extract the keratin from poultry feathers under reducing conditions were followed by many researchers [9-11].

The soluble keratin have applications in wound healing, healing of burns, cell seeding, cell proliferation, diffusion and in drug. Keratin

is extensively used for wound healing as it helps for the increased proliferation of keratinocytes which helps with faster healing of the wounds [12, 13]. Studies showed that wounds treated with keratin-based product healed 21% more in 4 d, than wounds treated with the keratin-free product [14]. Developing regenerated keratin fibers could not only provide new sources for biomedical industry to alleviate the fiber shortage, but also add value to poultry industry and address related environmental concerns.

Poly Vinyl Alcohol (PVA) is a water soluble, flexible, non toxic and biodegradable synthetic polymer with film forming properties. It does not impart any harmful side effects and has a high tensile strength, retains water which allows it to form films with embedded substances in its matrix. It easily adheres to surfaces, even in the nano range [15]. Thus, natural polymers are blended into synthetic polymers, the favorable biological functionality is contributed by natural polymers, and the mechanical stiffness is provided by their synthetic counterparts. Such type of hybrid system is expected to significantly improve material properties, by providing a stable, nurturing environment for a broad array of biomedical applications [16]. *Tridax Procumbens*, is a weed widely available is tropical and subtropical regions. It has pharmacological properties which include antioxidant and anti-inflammatory properties [17], enhanced blood clotting [18], hepatoprotective, wound healing, immunomodulatory, antimicrobial, antiseptic, hypotensive and bradycardiac effect [19-22]. Nanoparticles synthesised using chitosan, gelatin beads, conjugated linoleic acid, ricinoleic acid and silver nanoparticle impregnated cellulose showed better antimicrobial properties [23]. Electrospinning is a simple and versatile technique to produce micro and nanofibers of polymers, because it provides a potential way to fabricate continuous nanofibers for structural designs [24-26]. The

nanofibres formed using electrospinning are also highly comfortable due to their ultrafine fibres and used for the wound healing gives a major advantage of matrix structure, which gives an option of incorporating drugs, enzymes and other antimicrobial agents that can help with healing the wound [27].

In the present study, chicken feather (CF) was extracted using sodium m-bisulphite and blended with PVA. And biosynthesised AgNPs from *tridax procumbens* are incorporated during electrospinning process. It was expected that the affinity between CF and PVA would facilitate the fibre formation during the spinning process and the AgNPs would facilitate the antimicrobial properties to the nanofibres.

Experimental methods

MATERIALS AND METHODS

The chicken feathers were obtained from the local chicken shop in Chennai. Ethanol, sodium m-bisulphite (40 % solution), and sodium hydroxide were bought from Himedia and the PVA was bought from Sigma Aldrich. All the chemicals used were of the analytical grade. The plant *Tridax Procumbens* was collected near SRM Lake and was certified for species identification at Plant anatomy research centre.

Keratin Extraction from chicken feathers

Pretreatment of chicken feather

The chicken feather were freshly collected from the market and it was washed repeatedly with water to remove the dirt, blood and dung. The chicken feathers were again soaked in detergent for 1 hour to remove the bad odour, drained and washed thoroughly. The feathers were then soaked in ethanol for 24 h, drained and washed with distilled water. Once the chicken feathers were thoroughly washed, the feathers were spread in the fume hood for about three days and thereafter they were kept in a ventilated oven for 24 h at 45 °C to completely remove the moisture. The dried CF were stored in a desiccator at room temperature, until they were used for the experimental work.

Extraction of keratin from chicken feathers

In the reduction process, the quill was removed from the feathers and the defatted feathers (10 g) were put in 250 ml of aqueous solutions containing sodium m-bisulphite and the mixture was shaken at 50 °C for 2 h. The mixed solution was left in a temperature controlled shaker for 2 d at 50 °C. The solubilized feather solution is centrifuged at 10,000 rpm for 30 min. The supernatant was used as hair protein extraction and the pellet was recovered, washed with double distilled water and used as an extracted sample [28]. Ammonium sulphate precipitation was carried out to precipitate the crude protein extract. Three concentrations of ammonium sulphate solution such as 80%, 90% and 100% were prepared using the ammonium precipitation standard chart. For each concentration of ammonium sulphate salt, 30 ml of ice cold distilled water was used with constant stirring at 4 °C. The dissolved ammonium sulphate solution is then added to equal volume of solubilized feather solution in the falcon tubes. The falcon tubes were incubated at 4 °C overnight and the solution was centrifuged at 10,000 rpm for 20 min. The supernatant was discarded and the pellet was re-suspended in 1 ml of distilled water and stored at 4 °C for further use. The precipitated protein is washed with water several times and then, 2M sodium hydroxide solution is used to obtain protein back in the solution form. Then it was lyophilized and stored for further use. The protein concentrations were determined by the Bradford assay using bovine serum albumin as the standard. The physical properties of chicken feathers like length, moisture content, density, fineness, fibre strength, elongation at break and moisture regain were studied. The chemical action of acid, alkali, solvent, hot and cold water were analysed.

Preparation of AgNPs

The leaves and stems of *tridax procumbens* were thoroughly washed with tap water to remove soil, dirt and finally washed with double distilled water. To prepare the extract, 25 grams of leaves and stems were finely cut and were boiled in 100 ml of deionised water for 15-

20 min in a 250 ml of Erlenmeyer flask and the solution were decanted, then filtered using whatmann filter paper No.1 to obtain the extract of definite concentration. 10 ml of extract, which was in yellowish color was added to 100 ml of 5 mmol AgNO₃ at room temperature. The formation of silver nanoparticles (AgNPs) was indicated by the development of brown color. The reduction of silver was analysed by UV spectrophotometer within the range of 300-600 nm [29].

Preparation of polymer solutions for electrospinning

Polymer solution for electrospinning were prepared by dissolving CF, PVA and AgNPs at different weight ratios in distilled water. CF powders were dispersed in water and completely dissolved by dropping a 1 mmol NaOH solution under continuous stirring at 45 °C. Different weight ratios of PVA was added to the resulting CF solution and pH value of mixture was adjusted to 8.5. The mixtures were mixed vigorously both by manual agitation and vortexing to achieve homogeneous distributions. A series of CF/PVA/AgNPs solutions labelled CF₁₀₀/PVA₀/AgNPs, CF₈₀/PVA₃₀/AgNPs, CF₆₀/PVA₄₀/AgNPs, CF₅₀/PVA₅₀/AgNPs, CF₄₀/PVA₆₀/AgNPs, CF₂₀/PVA₈₀/AgNPs and CF₀/PVA₁₀₀/AgNPs. In all the ratios, 1 ml of AgNPs are maintained throughout.

Electrospinning and stabilizing of CF/PVA/AgNPs

Keratin/PVA/AgNPs biocomposite film were prepared using different ratios of keratin, PVA and AgNPs. The electrospinning experiments were performed at room temperature. The polymer solution was placed into a 2 ml syringe with a needle having an inner diameter of 0.4 mm. A clamp connected with high voltage power supplier, which can supply positive voltage from 0 to 240 kV, was attached to the needle. A rotary collector drum with aluminium foil wound on it was placed in front of the needle at the distance of 10 cm from the tip of the needle. The polymer jets were generated from the needle by high voltage field to the collector and formed the nanofiber mesh on the rotating drum. A grounded drum with aluminium foil was placed at a distance of 12 cm from the capillary tip. The applied voltage and flow rate of the solution were fixed at 20kV and 0.6 ml/h flow rate, and 400 rpm drum speed with distance of 12 cm respectively.

Characterisation of keratin./PVA/AgNPs nanofibres

Scanning electron microscope (SEM)

Surface morphology of the bio composite film was visualised by scanning electron microscope. Gold coating was done on bio composites using ion coater 0.1Torr pressure, 20mA current, and 70s coating time using a 15kV accelerating voltage.

Fourier transform infra red (FTIR)

The FTIR spectra of the nanofiber samples were recorded with an infrared spectrometer using Agilent Technologies 6000 series for a wavenumber from 4000 to 650 cm⁻¹ (Ming He et al.,2017).

XRD analysis

The prepared nanofibers were scraped out of the metal sheet resulting in a powder and given for XRD analysis at SRM University using Thermo Scientific ARL 9900 Pot Flux X-Ray Analyzer.

Differential scanning calorimetry

To analysis the thermal properties of the prepared nanofiber was performed using differential scanning calorimeter (DSC 4000 System, 100-240V/50-60Hz) at CATERS, CLRI. The calorimeter cell was flushed with 100 ml/min nitrogen. The runs were performed on the conditioned samples (24 °C, 65% R. H.) from 30 to 400 °C, at the heating rate of 10 °C/min.

Thermogravimetric analysis

TGA analysis using LABSYS evo TGA 1150 at CATERS, CLRI were performed for the prepared nanofibers. The sample was subjected temperature ranging from 25 °C to 600 °C at a heating rate of 10 °C per minute in nitrogen atmosphere. About 3 mg of sample were used in each test using Al₂O₃ crucibles.

Statistical analysis

The data are shown as mean±SEM. Differences between experimental results were evaluated according to a one-way analysis of variance (ANOVA), with considered statistically significant. All experiments were performed in triplicate.

RESULTS AND DISCUSSION

Physico chemical properties of the CF/PVA/AgNps

The physical properties of the CF was studied. It was found that the length of the feather was 23-37 mm, the moisture content is about 12-13.6%, density is 1.03-1.12 g/cc, fineness is 3.98 micron, elongation at break 1-5%, moisture gain is 13-13.95%. The chemical

properties of the CF were studied. It was found that the feathers are completely damaged and the weight loss was observed with 1N HCl acid and with 5% NaOH, the feathers were completely dissolved. The CF was sensitive to solvent and there was a weight loss. In the action of hot water on CF showed less absorbance and there was also loss in weight.

Keratin from chicken feathers

The protein content was calculated from the precipitated protein extract and it was found to be 1.64 mg/ml for 80%, 1.41 mg/ml for 90% and 1.85 mg/ml for 100% ammonium sulphate. The protein content was found to be highest in the case of 100% ammonium sulphate. The precipitated protein was characterized by FTIR [30].

Agilent Resolutions Pro

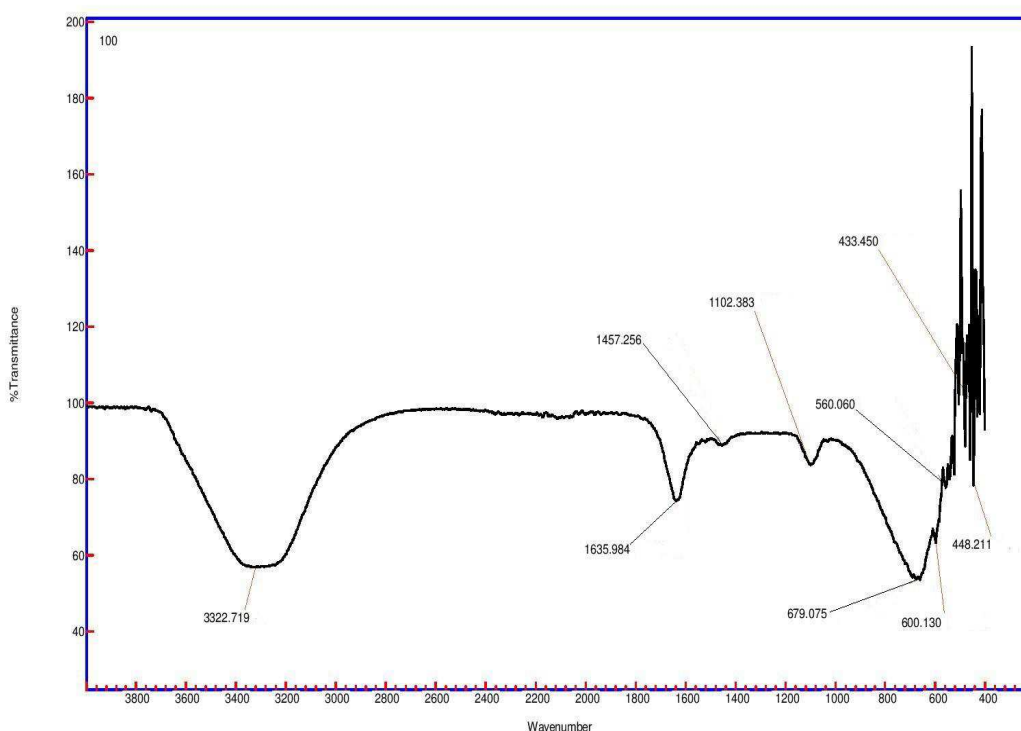


Fig. 1: FTIR analysis of the precipitated protein solution using 100% saturated solution of ammonium sulphate

The solubilized feather solution precipitated with the 100% ammonium sulphate concentrations were characterized by FTIR. The FTIR spectra of extracted keratin in the region 400–4000 cm^{-1} are given in fig. 1, where the characteristic absorption bands are mainly assigned to the peptide bonds (CONH). A broad absorption band of CF appearing at 3322 cm^{-1} is mainly due to hydrogen bonded N-H stretching vibrations, as the peptide N-H groups form hydrogen bonds with amide C=O in the native secondary structure [31]. Amide I band is the most intensely and widely used, among all the amide bands of the backbone peptide groups in the proteins. The amide I band is connected mainly with the C=O stretching vibration and it occurs in the range of 1700–1600 cm^{-1} . This band arises mainly from the C=O stretching vibration of the amide carbonyl group, and it is weakly coupled with the in-plane N-H bending and the C-N stretching vibration, and it appears in the region between 1700 and 1600 cm^{-1} . It is commonly found in keratin, and the amide showed C=O stretching vibration within range of 1700-1600 cm^{-1} , C-H stretching vibration at 1520 cm^{-1} and 1220-1300 cm^{-1} shows C-N

stretching [32-34]. It has been studied previously that the peaks between 1610-1630 cm^{-1} , which is observed in the FTIR of the protein samples proving the presence of keratin retaining its native structure [35]. It can be seen from fig. 1 that these bands exist in the extracted keratin from the chicken feathers. Thus the product obtained at the end of the extraction confirmed true keratin protein from chicken feathers without the presence of any foreign materials.

XRD analysis of nanofiber

The X-ray diffraction (XRD) is the most widely used technique for general crystalline material characterization. It is used to measure the average spacing's between layers or rows of atoms, determine the orientation of a single crystal or grain. The % crystallinity of CF was found to be 24. This fact is supported by earlier research [36]. From the fig. 2, the appearance of new crystallinity peaks suggesting the formation of other crystalline patterns at a greater angle, e. g. at 2θ is 24 in the sample that was chemically treated with sodium bisulphite, is indexed to its strand secondary structure.

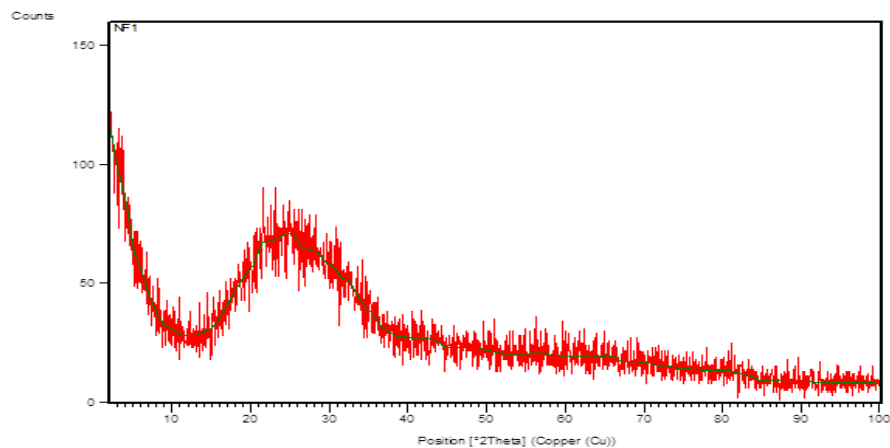


Fig. 2: XRD analysis of electrospun nanofiber containing keratin, PVA, AgNps (50:50)

The nanofiber obtained showed a peak at about 23 °(2θ) observed in fig. 2. The crystallinity of the keratin composite nanofibers lies

between 22.4% and 23.5%. The XRD result showed the presence of hydrogen bonding between the PVA and keratin [37].

SEM analysis

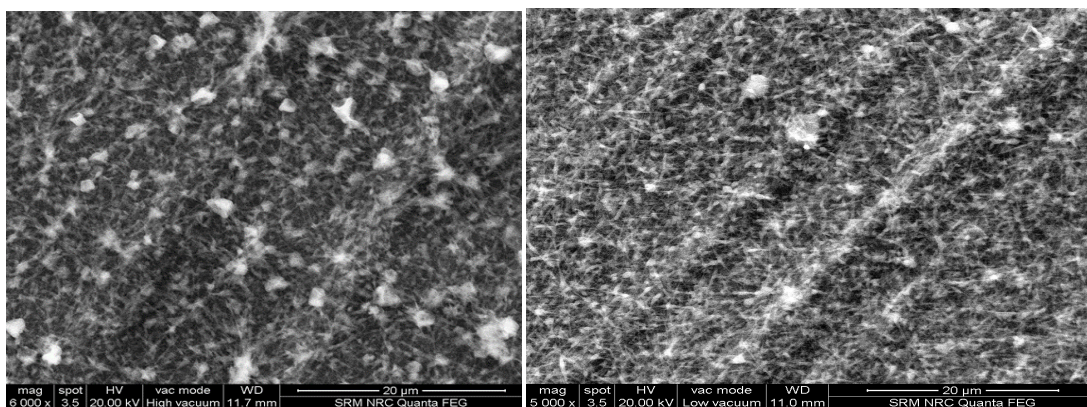


Fig. 3a): SEM image of electrospun nanofiber at the blending ratio of 60:40 of PVA to keratin with silver nanoparticles at magnification of 6000 x. 3b) SEM image of electrospun nanofiber at the blending ratio of 40:60 of PVA to keratin with silver nanoparticles at magnification of 6000 x

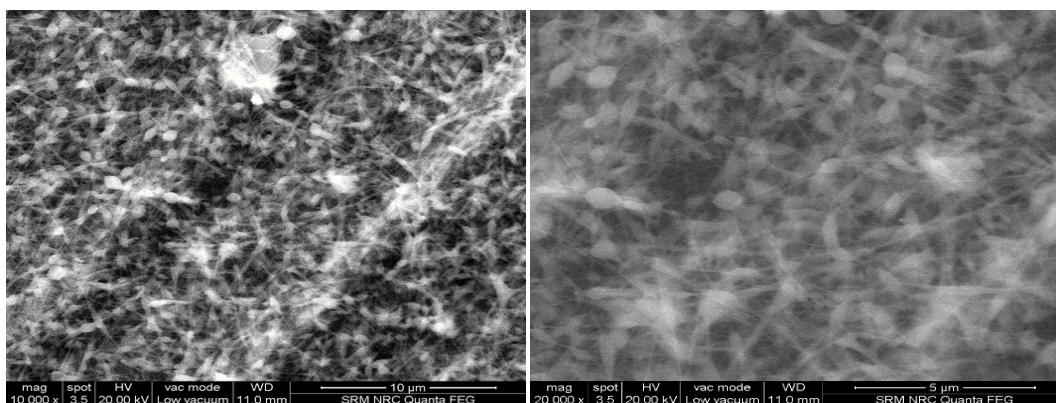


Fig. 3b) and 3 c): SEM image of obtained electrospun nanofiber at the ratio of 50:50 of PVA to keratin with silver nanoparticles (b) at magnification 20 000 x (c) at magnification 10 000 x

Fig. 3a) and fig. 3b) are the SEM images of the electrospun nanofibers in the ratio of 60:40 and 40:60 of keratin and PVA. The nanofibers are obtained were discontinuous film structure and it

was clearly seen in the SEM images. The SEM images of electrospun nanofibers were performed on 50:50 ratio blend, because the rest of the ratios experimented appeared unstable. In the ratio of 90:10 of

keratin to PVA with 1 ml of silver nanoparticle solution, the nanofibres formation does not take place, because the keratin does not possess film forming properties. It was evident that the increase in the ratio of keratin does not form any fibre structure on the electrospinning drum [38]. In the case of increase in the PVA concentration, the nanofibres were straight and uniform. This is mainly due to the film forming properties of PVA. When the CF content was increased further, the electrospinning was not successful [35]. In the case of other ratios, poor morphologies of nanofibres exhibiting many large beads were obtained and the fibres were difficult to handle (data not presented). In a study, it was reported that the fibre structure was not constructed by electrospinning keratin/fibroin blend solutions when the keratin content exceeded 35% [39]. Similarly, polymer solutions containing no more than 40wt% of feather keratin can be electrospun [35]. The presence of silver nanoparticles are also clearly visible in the figures.

The SEM image of the 50:50 ratio seen in fig. 3a, showed a distinct fibrous matrix structure, stable, smooth bead free, randomly oriented and formed a continuous fiber mat at 20 kV during the electrospinning process. The SEM image of the 50:50 ratio is seen in fig. 3a and 3b, which shows distinct fibrous structure with the silver nanoparticle observed in spherical particles embedded in the fiber matrix. Therefore, the optimum ratio for the nanofiber synthesis is 50:50 of keratin solution to PVA and 1 ml of silver nanoparticle solution [40]. Moreover, the surface of the nanofibers shows heterogeneous microstructures, the surface becomes brighter and also causes roughness, which is the characteristic of increased surface activity of CF. It should be noted that to obtain satisfactory fibres, the ratio of CF in the blend solution had to be less than or equal to 50wt %. (CF₅₀/PVA₅₀). Therefore, in the present study, only electrospun fibres from solution containing no more than 50wt% of CF were considered.

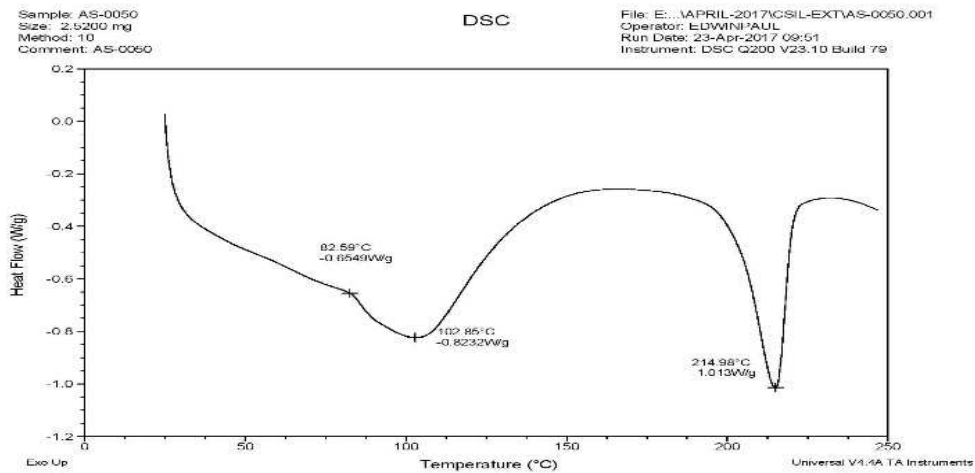


Fig. 4: DSC analysis of electro spun nanofiber containing Keratin, PVA, AgNps (50:50:1)

Differential scanning calorimetry of nanofiber

The DSC analysis result shown in fig. 4 was obtained and showed a characteristic peak at a temperature of 214 °C. This indicates the formation of hydrogen bonds between keratin and PVA, confirming that single complex was formed correlating to the results obtained by Yao Dou *et al.*,2015. A low temperature broad peaks below 100 °C is indicative for the evaporation of residual moisture and denaturation of the protein. It is a distinctively

different behavior with broader denaturation. The DSC of CF shows an endothermic peak at <215 °C, which is usually assigned to α-helix disordering and decomposition [41]. The endothermic peak observed at about 215 °C for the CF was attributed to the crystalline melting (T_m) of the nanofiber, and the peak area represented the crystallinity of the nanofibers [42]. These observations suggest the loss of α-helix structures and gain of amorphous behavior, especially marked with a broadened melting curve trend [43].

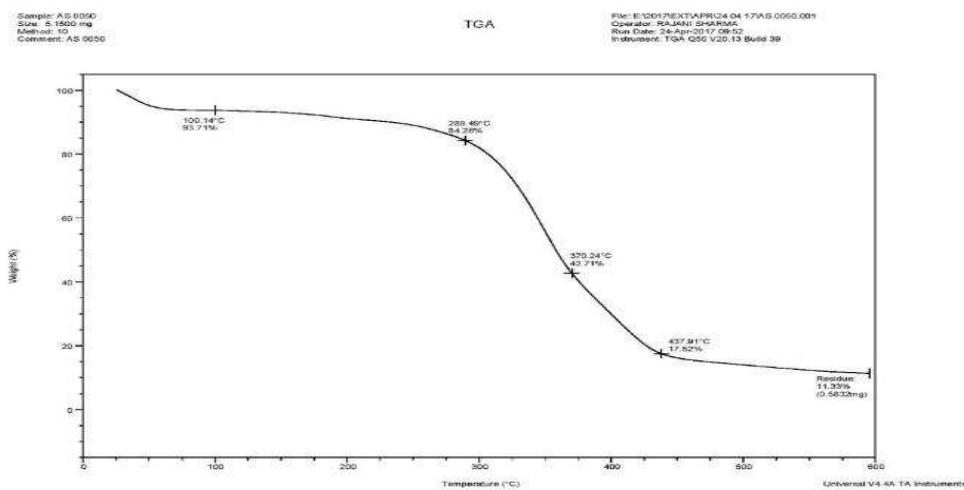


Fig. 5:TGA analysis of electrospun nanofiber containing keratin, PVA, AgNps (50:50:1)

Thermogravimetric analysis of nanofibre

The TGA result shown in fig. 5, shows that the initial weight loss at 100-280°C is due to moisture loss and weight percentage of the nanofibre reaches its lowest point at 280-420 °C, indicating that it has a high heat resistance capacity in comparison to the nanofiber formed in earlier studies which had a decreased thermostability [44, 45]. The TGA curves as a function of temperature of the keratin nanofibres showed the decomposition in the temperature range of 280–412 °C. At this juncture, a total weight loss of ca. 90% was observed. Finally, thermogravimetric analysis showed the higher thermal stability of keratin/PVA nanofibers β -sheet crystalline structure formed during cast solidification.

CONCLUSION

In summary, a simple and effective method was developed to obtain novel keratin biomaterials from disused feathers. The antimicrobial properties of synthesised silver nanoparticles provides effective against human pathogens. Incorporation of metal nanoparticles by herbal approach using *tridax procumbens* in the nanofibers may have persuasive applications in medical therapeutics. Thus, we can conclude that keratin isolated from the waste chicken feathers impregnated with silver nanoparticles and PVA can be converted into nanofibers by electrospinning process. Thus, keratin fibers from chicken feathers are shown as a novel eco-friendly material that must be adequately applied in the development of green composites.

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AUTHORS CONTRIBUTIONS

All the author have contributed equally

CONFLICT OF INTERESTS

The authors declared that they have no conflict of interests in this work

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