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# Preparation, Characterization and Properties of Protein Nanoparticles from Feather Waste

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THE PURPOSE of this paper was to investigate the feasibility of a newly developed method for producing protein nanoparticles (PNP) from chicken feather, prepared by the reduction technique with plant extracts followed by ultrasonic treatment. Characterization of the prepared sample was confirmed using particle size distribution, zeta potential, X-ray diffraction, Fourier-transform infrared, and scanning electron microscope. The particle size distribution results of the sample showed that the average diameter was 69 nm width by 60 nm length. Scanning electron microscope study showed that the diameter of the sample was about 106.8–215.7 nm. Radiographic analysis shows obviously that the crystallinity of PNP decreases with their particle size. The results indicated the positive effect of sonication on reducing particles size after reduction process while maintaining the basic chemistry and structural integrity of the native keratin protein. For instance, the authors have shown that preparation of PNP could be produced from feather waste, which is believed to be the most promising approach because of the milder process conditions, leaving no harmful byproducts. These results are very important for industrial application with the production of PNP as an inexpensive source from feather waste.

**Keywords:** Chicken feather, Nanoparticles, Particle size, Reduction, Sonication, Zeta potential.

## Introduction

Nanotechnology is an emerging interdisciplinary field that is expected to have wide ranging implications in all fields of science and technology such as material science, mechanics, electronics, optics, medicine, plastics, energy, and aerospace [1]. Nanotechnology is the technology of the extremely small; one nm was defined as one billionth of a meter. In comparison, 1 nm is one fifty-thousandth of the diameter of a human hair, or, if a nanometer is scaled to the diameter of a child's marble, then a meter would have to be scaled to the diameter of the Earth [2]. Nano-biotechnology have wide range of application, one of them is application of nanoparticles for delivery system [3]. The processing requirement for creative finishing technologies e.g. nanotechnology, to give the request useful properties in various textile sector applications, without unfavorably influencing the earth has become in link manner [4]. Another application of nanotechnology is to impart multi-functional properties for cotton fabric such as flame retardance, self-cleaning and anti-crease using environmentally friendly finishing agents including citric acid, $TiO_2$ nanoparticles and flame retarding agent based on phosphate salts [6]. Over the past few decades, there has been considerable interest in developing biodegradable nanoparticles (liposome, virus like particle (VLP), protein, etc.) as effective food and drug delivery device [7]. Among these colloidal system those based on protein may be very promising since they are biodegradable and non-antigenic relatively easy to prepare and their size distribution can be monitored easily [8]. Feather is a waste product generated in large quantities from industrial poultry processing. Recycling of this renewable source of biopolymers has been the objective of many researches due to its high protein content,

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biodegradability, and biocompatibility [8, 9]. An estimated 15 million tons of chicken feathers are available globally each year as a by-product of meat manufacture. The raw material is tough and chemically resistant [10]. Currently the feathers are disposed of in landfill, burned or processed to make a low-grade animal feedstock. In recent years, however, some innovative uses of waste feather have been proposed [11]. Chicken feathers contain 91% protein (keratin), 1% lipids, and 8% water [12]. Our previous work indicated that the feather total amino acids were of 334.1 mg/l at the optimum condition of hydrolysis [13]. Various potential applications have emerged due to recent interest in transforming the chicken feathers into useful products. The native or processed fibers from feathers have also been tested for composite applications [14]. Keratin protein has fibrous matrix which act as nano-filtering sponge can be used for cleaning industrial effluents by the process of adsorption [15]. Our previous work on the removal of some polluting metals from industrial water using chicken feathers was carried out [13]. Another application which attracted attention is use in computer chips [16]. Several studies were also carried out to investigate chicken feather fiber based composites [17]. Another application of the utilization of feather waste to improve the properties of the Egyptian cotton fabrics was carried out [18]. Suspended keratin-based binder (Su-KBB) was prepared from renewable cheap natural resources; namely, coarse Egyptian wool fleece or feather to replace the commercially used synthetic binders required for pigment printing technique [19]. The purpose of this paper investigates the feasibility newly developed method of producing protein nanoparticles from chicken feather prepared by the reduction technique with plant extracts followed by ultrasonic treatment.

## Materials and Methods

*Materials*

Chicken feather was selected as the functional for PNP. All chemicals used were of analytical grade using double distilled water (18.5 MΩ.cm$^{-1}$).

*Methods*

*PNP preparation technique*

1- Purification and treatment of chicken feather was prepared according to our previous work [13].

2- 100 g of barely was boiled in $H_2O$ for 1 h, then filtered, and the aqueous extract was added to the portentous solution in the ratio of 2:1 respectively and boiled for 1h to complete the reduction reaction .

3- The precipitant was separated using centrifugation at 12000 rpm, and it was used for the characterization of portentous nanoparticles.

4- Ultrasonic process technique was carried out at the optimum condition as described in our previous work [20].

5- The produced sample allowed cooling down and the produced nano suspension was centrifuged for 15 min at 10,000 rpm. The precipitate was re-suspended in distilled water and dialyzed with tap water. The dried sample was used for the evaluation tests.

*Characterization of the prepared PNP*

*Size and zeta potential analysis:* Particle size distribution analysis was conducted by Dynamic Light Scattering (DLS) using a Zetasizer NanoZS instrument (Malvern, UK), under the following conditions: dispersant water, material refractive index 1.47, dispersion refractive index 1.33, viscosity 0.8872 cP, temperature 25°C and general calculation model for irregular particles. Three measurements of 10s each were taken and the averaging was done. The zeta-potential (estimated as surface charge) tests of the PNP were conducted with the Zetasizer NanoZS Instrument (Malvern, UK). Experiments were performed in a cuvette consisting of 4 ml 0.1 wt% PNN, solutions were all adjusted at pH values of 7, under the following condition:. temperature 25°C, zeta runs 12, count rate (kcps) 80, measurement position( mm 2.0), cell descreption (clear dispospal zeta c, and attenuator 5.

*X-Ray diffraction analysis (XRD):* The crystallinity phase present in the samples were determined using an X-ray diffraction (Xpert MDP diffractometer with Co tube) at 40 kV and 30 mA. The fabric sample was gold-coated for 15 min in Emitech K550 metalizer with argon as a carrier gas.

*FTIR analysis:* The Fourier Transform Infrared (FTIR) Analysis were carried out for samples Using FTIR Model Cary 630 FTIR spectrometer produced by Agilent technologies Company, for both Qualitative and Quantitative(for liquid samples) analysis, in spectral range (wavenumbers cm$^{-1}$) from 4000cm$^{-1}$ to 400cm$^{-1}$ without any treatment.

*Scanning electro microscopy (SEM)*

The metalized sample was scanned in a Zeiss DSM 940A SEM under accelerated electrons with 15 kV of energy. The width of 31 individual fibers was measured, and the mean, standard deviation, and confidence interval were calculated**.**

*Theory /calculation*

PNP was synthesized from feather waste solution after reduction reaction with barely extract solution in the ration of 2:1 respectively followed by ultrasonic technique. The results are very important for industrial application with the production of PNP as an inexpensive source from feather waste as a by-product. Our future research will focus on the fact that PNP will apply in different industrial application especially in textile finishing.

## Results and Discussions

*Characterization and evaluation of the PNP*

*Size and zeta potential*

The results obtained indicated a notable distribution in the paricle size values for the PNP. According to our observed results, the particle size was relatively non-uniform. Figure 1 showed that the particle size (d. nm) were 58, 68, 78 and 105 in the number % of 6, 15, 21, 18, and 13 respectively. The results obtained from Fig.1 showed that the PNP width (d.nm) was 54.67. Kumaresh et al. [20] poimted out that It is difficult to obtain protein nanoparticle less than 500 nm by emulsification crosslinking method [21]. Our results showed that the method of PNP perpearation is more effective compared with the other method of preparations. The results obtained is agreement with the results of Hans and Lowman results which indicated that the size and surface charge of the nanoparticles are crucial for their uptake [22].

Zeta potential (estimated as surface charge) can be measured by tracking the moving rate of negatively or positively charged particles across an electric field [23]. Usually a value less than –15mV represents the onset of agglomeration. Values greater than –30 mV generally signifies that there is sufficient mutual repulsion which results in colloidal stability [24]. As shown in Fig. 2, the zeta potential value of the PNP suspension was -15.7 mV attributed to that the ionic bond formed by electrostatic attraction of the NHs+ group of the diamino acids and the COO- group of the dicarboxylic acids could be broken. The highest surface charge imparts electrostatic repulsive forces to the system, preventing the binding between PNP-PNP, and thus homogeneous suspension is obtained. The uniform dispersion of PNP is critical to improve the mechanical properties of the final PNP products promoting the actual formation of hydrogen bonding as cationized agents.

*FTIR analysis*

Our previous work pointed out that sodium hydroxide plays an important role in dissolving feathers, .the dissolving rate of feathers is highly in alkaline solution of 0.9 M NaOH [13]. This is because in the alkaline state the proton will be removed from the amino group and the ionic bond formed by electrostatic attraction of the NHs+ group of the diamino acids and the COO- group of the dicarboxylic acids can be broken [25]. Due to some reasons these ionic bond must be broken first to reduce the disulfide bonds of the keratin and dissolve the feathers. It has been noted that Carbon, nitrogen and oxygen are major elements in neat keratin (CFK). FT-IR spectroscopic analysis is used to determine characteristic peaks in functional group (4,000–1,400 $cm^{-1}$) and finger print(400–1,400 $cm^{-1}$) region [26]. From Fig. 3 the interval from 3600 to 3100 $cm^{-1}$ neat showed stretching vibration related to N-H of keratin. The amide I region lies in the range between 1600 and 1700 $cm^{-1}$, while amide II and amide III absorption bands comes at around 1530 and 1220 $cm^{-1}$. Amide I refers to the secondary structure of protein and arises mainly from C=O stretching, with a minor contribution from C-N stretching while the amide II band originates from N-H bending and C-H stretching vibrations. Overall, there are no significant changes in the spectra of neat and modified keratin fibers, but a slight difference in the peak intensities of neat and modified keratin fibers can be observed.

*X-Ray diffraction analysis*

The results obtained in Fig. 4 (A, and B) exhibits three narrow peaks around 2θ = 38°, 64° and 77°, which corresponds to the inter plenary spacing of 2.38231 Å, 1.44719 Å and 1.23161 Å, and relative intensity (%) of 9.97%, 43.12% and 50.54% respectively, which indicated the strong crystalline characteristics related to 100% of crystallinity to peak 4°, that besides being narrow, it is the most intense, around 44°, which corresponds to the inter plenary spacing of 2.05916. These effects are caused by the presence of crystalline regions within the sample. However, the existence of the reflection band around 2θ =10° and 20°, clearly shows that the material may have an amorphous region.

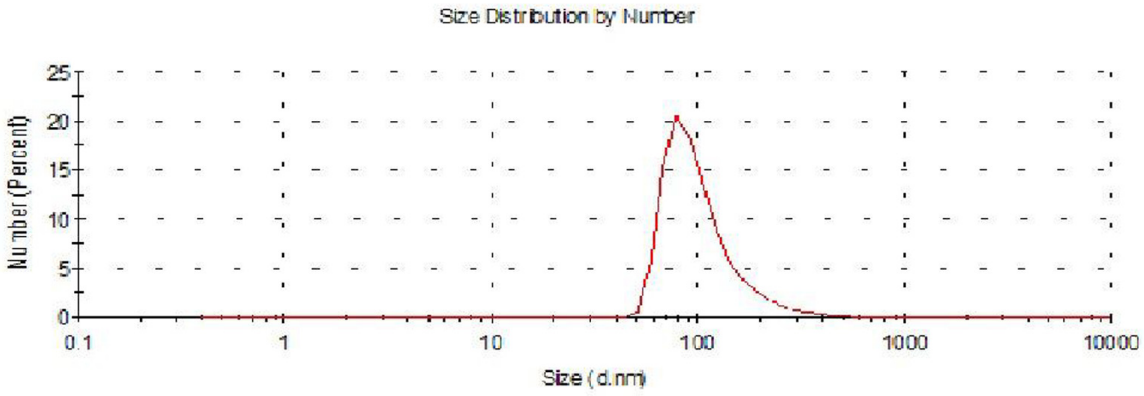


**Fig. 1. Partizle size distribution of protein nanoparticle.**

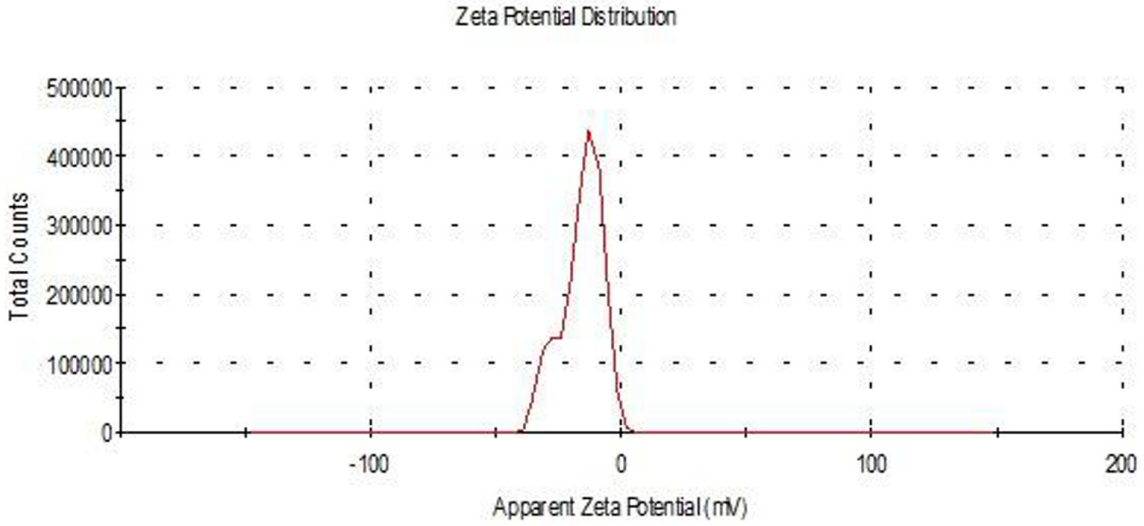


**Fig. 2. Zeta potential of protein nanoparticle.**

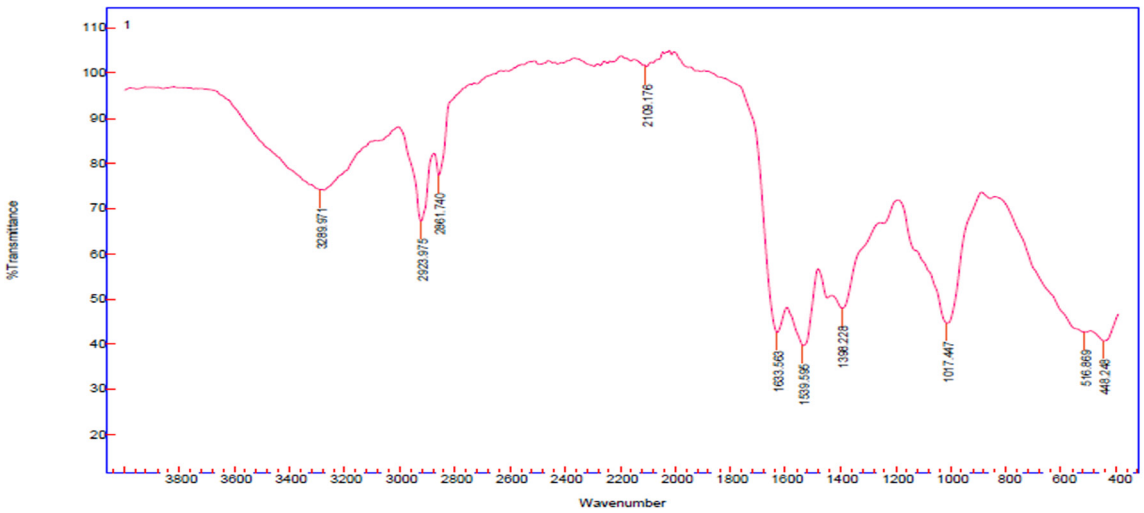


**Fig. 3. Fourier-transform infrared analysis of protein nanoparticle.**

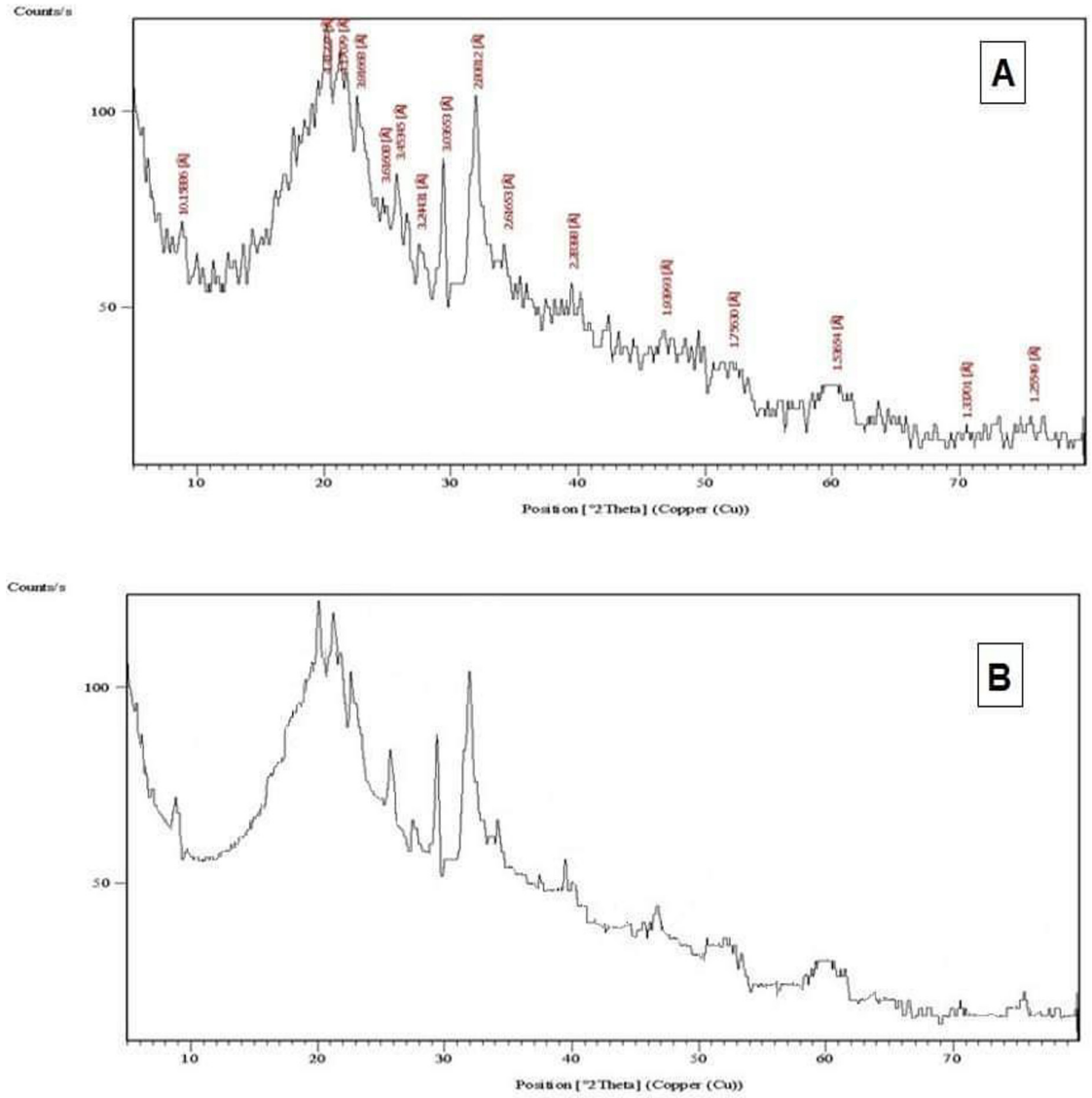


**Fig. 4 (a and b) X-ray diffraction of protein nanoparticle.**

*Scanning electron microscopy*

Scanning electron microscopy (SEM) is useful for detailed study of a specimen's surface. A high-energy electron beam scans across the surface of a specimen, usually coated with a thin film of gold or platinum to improve contrast and the signal-to-noise ratio. As the beam scans across the sample's surface, interactions between the sample and the electron beam result in different types of electron signals emitted at or near the specimen surface. These electronic signals are collected, processed, and eventually translated as pixels on a monitor to form an image of the specimen's surface topography that appears three dimensional. Low-energy secondary electrons excited on the sample's surface are the most common signal detected. High-energy backscattered electrons and X-rays are emitted from below the specimen surface, providing information on specimen composition. From Fig. 5, it has been noted that the PNP particle size was relatively non-uniform. The particle size (d. nm) were ranged from 106.8 to 215.7 which was slightely different with the particle size distripution measurements. These measurements confirmed through SEM analysis might suffer from the severe agglomeration of powder resulting in the inaccurate size measurements through SEM. In order to prevent agglomeration, the powder was put in a dynamic chamber, which induced water current to prevent agglomeration of the powder. The SEM analysis of the sample under investigation in Fig. 5 provides evidence that the PNP could be obtained.

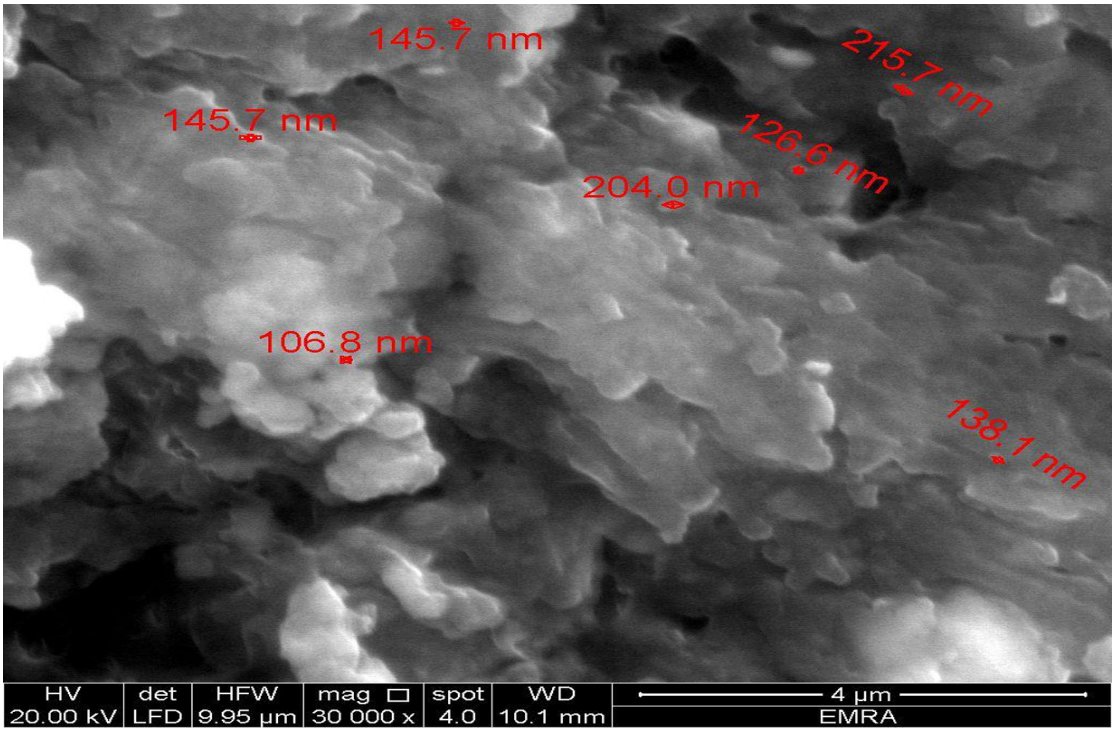


**Fig. 5. Scanning electron microscope of protein nanoparticle.**

*Ultrasonic technique treatment*

Our previous results revealed that the particles size became smaller with increasing the number of the ultrasonic doses. The properties of particle size would be affected by the number of ultrasonic doses [20]. The results indicated the positive effect of sonication on reducing particles size after reduction process, while maintaining the basic chemistry and structural integrity of the native keratin protein. The results were in accordance with the results obtained by Eslahi et al. [27].

## Conclusions

Protein, including variety of natural proteins and engineered synthetic polypeptides, based platforms of biomaterials has very promising potential for different industrial applications. . Many synthesis methods for protein nanoparticles were developed and the protein characters of biodegradability, biocompatibility, and even the effect of particles sizes act significant roles on the application of protein nanoparticles in industrial application specially for textile finishing. Nanocomposite of Protein nanoparicles have great potential application to the future development textile finishing due to its high loading efficiency and targeting effect. PNP was synthesized from feather waste solution after reduction reaction with barely extract solution in the ration of 2:1 respectively followed by ultrasonic technique. Preparation, characterization and properties of PNP was confirmed using Particle size distribution, Zeta potential, X-Ray diffraction (XRD), Fourier transfer infrared (FTIR), and Scanning Electron Microscope (SEM) analysis. The results are very important for industrial application with the production of PNP as an inexpensive source from feather waste as a by-product.

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