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Morphology, Structure and Thermal Properties of Human Hair Keratin/ Egg White Protein Blend Particles: Influence of Different Crosslinking Agents

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The effect of studied crosslinking agents *viz.* acetic acid, polyethylene glycol diglycidyl ether (PEGDE) and genipin on the morphology, structure and thermal properties of human hair keratin/egg white protein (HHK/EWP) blend particles were investigated. The HHK/EWP blend particles were prepared by the reported water-in-oil emulsification solvent diffusion method with the addition of selected crosslinking agents. Scanning electron microscopy (SEM) images revealed that the use of these agents resulted in spherical HHK/EWP particles with variable sizes, arranged in the order of increasing particle sizes as follows: acetic acid, PEGDE and genipin. The addition of the crosslinking agents enhanced the interactions between the proteins, as indicated by the shift of peak positions in FTIR spectra. Moreover, the interactions also increased the decomposition temperature of the HHK/EWP blend particles. The obtained results provided important information to further improve the properties of HHK/EWP blend particles for a wider range of applications.

Keywords: Biomaterial, Human hair keratin, Egg white protein, Crosslinking agent, Morphology, Secondary structure.

INTRODUCTION

Protein-based materials have received extensive attention due to their desirable properties including water-solubility, biocompatibility, biodegradability, and non-toxicity [1]. Many natural proteins such as collagen, elastin, keratin and silk fibroin have been extensively studied and applied in many fields [2-5]. Among them, keratin is one of the most abundant proteins found as a major component of hair, wool, feather, horns and nails of mammals, reptiles and birds [6-8]. Keratin can be categorized into α, β and γ-keratin based on its structural configuration, molecular weight and sulfur contents [9]. It is an insoluble protein due to disulfide bonds in its structure [10]. Keratin has been used in materials for scaffolds in tissue engineering and drug delivery system [11,12], where it shows a great advantage because of the presence of cell adhesion motifs such as RGD (Arg-Gly-Asp) and LDV (Leu-Asp-Val), which mimic the sites of cellular attachment [13]. The keratin-based has been performed in various forms such as sponges, patches, hydrogels, and fibrous scaffolds [14-17]. However, keratin materials have several disadvantageous properties as they are brittle and fragile with low flexibility [11]. These limit the applications of keratin products. Therefore, keratin blended with other natural polymers has attracted a lot of attention [18-20].

Egg white composed of more than 150 proteins-the majority includes ovalbumin, ovotransferrin, ovomucoid, lysozyme, ovomucin and globulin. The fresh egg weight of chicken egg white is composed of water (88%, w/w) and protein (10%, w/w) [21]. The egg white proteins showed various biological activities such as antimicrobial [22,23], antioxidant, antiviral, anticancer, immunomodulatory and protease inhibitory activities [24,25]. The egg white composed of many molecular sizes of proteins which makes it suitable for manufacture of bioplastics [26-28] and as a novel alternative material for food industry [29-31]. Moreover, egg white protein (EWP) has been blended with other polymers as well as inorganic functional materials [32]. Previously, we successfully synthesized of human hair keratin (HHK) and egg white protein (EWP) blend particles by emulsification solvent diffusion method [33]. The addition of protein crosslinking agents to the blended polymers can potentially enhance the interactions between HHK and EWP. As some crosslinkers could exhibit a certain level of physiological toxicity

[34,35], this study focused on the following biocompatible and non-toxic agents *e.g*. acetic acid, polyethylene glycol diglycidyl ether (PEGDE) and genipin.

Acetic acid is safe and has already been used by the meat products industry [36]. Moreover, it has been shown to interact with egg white proteins *via* hydrogen bonds [37,38]. Polyethylene glycol diglycidyl ether (PEGDE) is a synthetic polyether that is amphiphilic and soluble in water as well as in many organic solvents [39]. It has been used widely in biomedical and biomaterial research [40]. Genipin a natural extract from *Gardenia jasminoides* Ellis has been considered [41] due to its low cytotoxicity and ability to self-polymerize [42,43]. In this work, these three crosslinkers were separately added to the HHK and EWP mixture prior to the formation of the HHK/EWP blend particles. The effects of these crosslinking agents on the blend particle properties including morphology, secondary structure, as well as thermal stability were evaluated and compared.

EXPERIMENTAL

Egg white protein (EWP) and human hair keratin (HHK) were prepared by reported method [33].

Preparation of HHK/EWP blend particles: The HHK/EWP blend particles were prepared by the water-in-oil (W/O) emulsification-diffusion method [5]. The HHK was blended with EWP to obtain a 1:3 (v/v) ratio and used as a water (W) phase. The oil (O) phase in this work is ethyl acetate (100 mL). The stirring speed was adjusted in range 500-700 rpm and the different concentrations of the each crosslinked agents (acetic acid, PEGDE and genipin) were varied to observe their effect on the particle properties.

Morphological observation: The prepared HHK/EWP blend particles were observed morphology under a scanning electron microscope (JEOL, JSM-6460LV, Japan). The dried particles were placed on stubs, then coated with Au to induce electrons on the surfaces of particles.

Secondary structure investigation: The particle samples were prepared by blending with KBr. The secondary structure of particles was analyzed by Fourier transform infrared (FTIR) spectroscopy (Perkin Elmer-Spectrum Gx, USA) in region of 4000-400 cm^{-1}.

Thermal stability analysis: A thermogravimetric analyzer (TGA) (SDTQ600, TA-Instrument Co. Ltd., New Castle, USA) was used for thermal stability investigation of the prepared particles. The suitable weights of particle were heated from 50-800 ºC with 20 ºC/min of heating rate under N_2 atmosphere.

RESULTS AND DISCUSSION

Morphological observation: Based on this investigation, the suitable ratio of water and oil (W:O) phases used for the preparation of the particle was 1:100. The HHK/EWP blend particles with and without crosslinking agents had spherical shapes as revealed by the SEM imaging (Fig. 1). The particles were varied in sizes, listed in the increasing order as the following crosslinking agents were used: without crosslinking agents (Fig. 1a), acetic acid (Fig. 1b), PEGDE (Fig. 1c) and genipin (Fig. 1d), respectively. This suggested that the chemical structure of each agent affected the size of the particles. It is found that the suitable crosslinker concentrations and stirring rates to be 1% at 700 rpm, 1% at 500 rpm, and 5% at 700 rpm for acetic acid, PEGDE and genipin respectively. Under the mentioned conditions, the blend particles prepared using these agents all showed dense texture and similar surface morphology. The result indicated that rate of spinning as well as concentration of crosslinking agents directly affected on shape and size of particles. With all crosslinking agents, the particles have more spherical shape and smaller size than the crosslinker free particle. The acetic acid interacts with acidic and amino groups of proteins, leading to the intact texture of particle [37]. The synthetic PEGDE is one of popular crosslinker using for biomedical and biomaterial research. This was due to its wide range end-groups [40] and solubility in various solvents [39]. Among the three crosslinkers, the smallest size of particle was obtained by mixing with genepin. This might be expected that the genipin supported the rheology of polymer solution by increasing the distance between protein molecules from its ring structure. It has been employed for the crosslinking of various amino-containing polymeric molecules [37,44-46] due to its low cytotoxicity and ability [42].

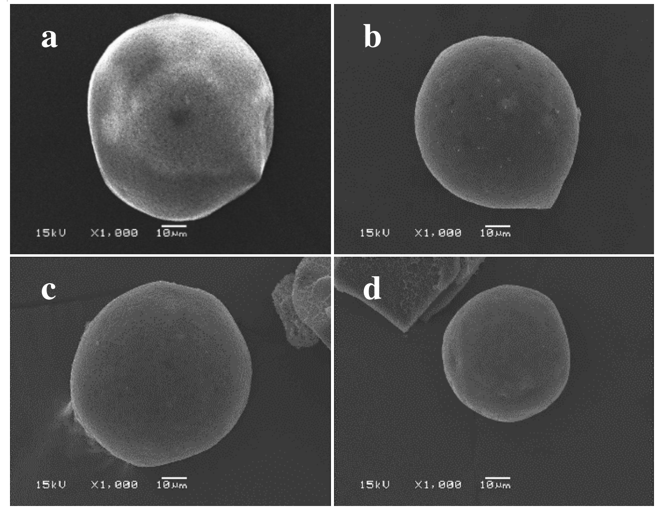

Fig. 1. SEM micrographs of HHK/EWP blend particles without cross-linked agent (a), 1% (v/v) acetic acids with stirring rate of 700 rpm (b), 1% PEGDE with stirring rate of 500 rpm (c), and 5% genipin with stirring rate of 700 rpm (d) at 1000X magnifications. Scale bars = 10 μm

Secondary structure of particles: The FTIR spectrum of HHK/EWP particles without any crosslinking agent (Fig. 2a) showed absorption peaks of amide group (R-COONH-R) in amino acids: amide I (1700-1600 cm^{-1}) accounted for the carbonyl group (-CO-), amide II (1600-1500 cm^{-1}) accounted for amine group (-NH-) and methyl group (-CH-) and amide III (1300-1150 cm^{-1}) accounted for -CN- *str.*, plane -NH-, -C-C- and -CO- *str.* [34,47]. The absorption peaks at 3275 cm^{-1} (assigned to CH_2 vibration and H-C=O amide A), 1627 cm^{-1} (assigned as C=O *str.*, amide I), 1532 cm^{-1} (O=C-NH, amide II) and 1072 cm^{-1} (O=C-SH *str.*) were observed for the KT/EW blend particles. The absorption peaks at 1627 cm^{-1} and 1532 cm^{-1} were responsible for amide I and amide II, respectively and indicated that the blend particles have the β-sheet structure.

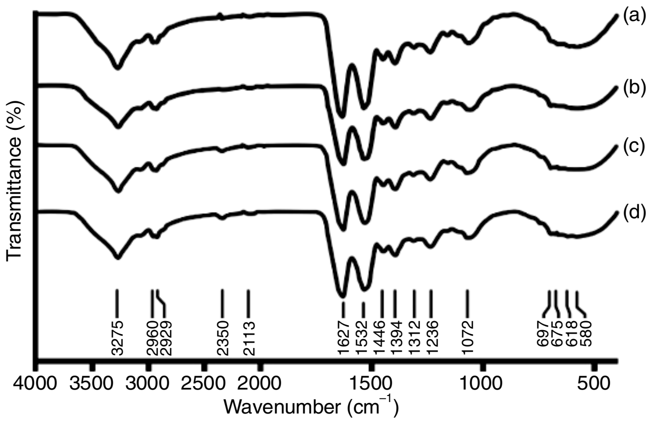

Fig. 2. FTIR spectra of HHK/EWP blend particles without cross-linked agent (a), and with 1% (v/v) acetic acids with stirring rate of 700 rpm (b), 1% PEGDE with stirring rate of 500 rpm (c), and 5% genipin with stirring rate of 700 rpm (d)

The β-sheet structure generally makes the materials brittle, fragile and very hard to use. However, in this case of HHK/EWP blend particles, the globular and hydrophilic proteins in the EWP helped increase the particle size as shown in Fig. 1. The crosslinking agents acted as strength improvement of the blend particles (Fig. 1b-d). The addition of the crosslinking agents introduced chemical interactions and resulted in slight changes of FTIR profiles and peak positions depending on the cross-linked agent used. This suggested that different structures of the blend particles were affected by the type of crosslinking agents, especially at the amide I and amide II groups of β-sheet structure.

Thermal stability of particles: The thermogravimetric analysis of HHK/EWP blend particles resulted in the DTG curves shown in Fig. 3. All the prepared particles have main maximum temperature of decomposition rate ($T_{d,max}$) higher than 300 ºC. Generally, all the particles showed decomposition peaks near 100 ºC, which was due to the evaporation of water in the materials [41]. The temperatures in the range of 220-250 ºC are the decomposition point of the egg white protein. The keratin showed a decomposition point in the range of 250-300 ºC [44]. The blend particles showed variable decomposition points depending on the crosslinking agents used. The results indicated that all crosslinking agents helped to increase the thermal property of the particles comparing to the particle without crosslinking agent (Fig. 3a). Among all the blend particles, the particles prepared using genipin as the crosslinker showed the highest $T_{d,max}$, which were at 240, 735 ºC (Fig. 3d). However, the particle blended PEGDE showed small peaks at 416 and 731 ºC (Fig. 3c). This might be caused by the interaction forming between the end groups of PEGDE and carboxylic acid or amino groups of proteins.

Conclusion

The addition of acetic acid, PEGDE and genipin as crosslinking agents in the preparation of human hair keratin/egg white protein (HHK/EWP) blend particles resulted in particles with variable sizes, secondary structures, and thermal properties. All the crosslinkers affected the particles by increasing the β-sheet structure *via* H-bond and electrochemical interactions.

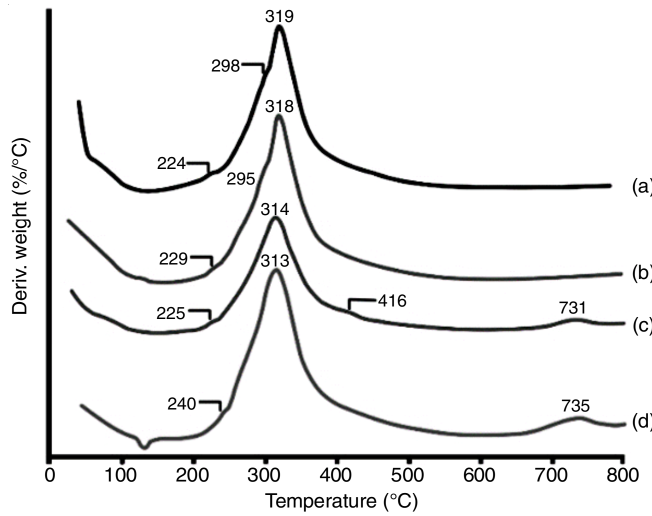

Fig. 3. DTG curves of HHK/EWP blend particles without cross-linking agent (a), and with 1% (v/v) acetic acids with a stirring rate of 700 rpm (b), 1% PEGDE with a stirring rate of 500 rpm (c), and 5% genipin with stirring rate of 700 rpm (d)

These enhanced interactions improved the thermal properties of the blend particles. However, the variable effects for each used crosslinking agents were also observed. Further studies such as the effect of these crosslinking agents on the blend particles performances such as water resistance, drug carrier and dissolution would be performed to obtain informative data for specific purposes.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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