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Effect of Cross-Linked Agents on the Morphology of Keratin/Egg White Blend Particles

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The effect of cross-linked agents *e.g.* acetic acid, polyethylene glycol diglycidyl ether (PEGDE) and genipin on the morphology of keratin/egg white (KT/EW) blend particle formation by emulsification solvent diffusion method was observed. The concentration of blending solution and spinning rate (rpm) for obtaining desired particles were also studied. SEM images indicated that the shapes and sizes of KT/EW blend particles varied by the types of cross-linked agents and conditions used. A high concentration of acetic acid and PEGDE interrupted the rheology of blend solution, which interfered the particle formation as well as enhancing the protein denatured. In contrast, the high content of genipin did not affect the morphological shape and size of the particles. A high rotation per minute (rpm) rate was suitable for blend particle formation mixed acetic acid and PEGDE whilst suitable rpm rate for genipin was slower than 2 types of cross-linker. The morphological shape and surfaces of the KT/EW blend particles affected by the types and conditions used. These results obtained the informative data for the development of keratin/egg white blend particles to apply in specific fields such as drug-delivery system.

Keywords: Acetic acid, Polyethylene glycol diglycidyl ether, Genipin, Morphology, Cross-linked agents, Egg white, Keratin.

INTRODUCTION

In recent, many researchers are increasingly interested in biodegradable polymers according to their biodegradability [1]. The biodegradable polymers are generally divided into 2 groups; synthetic polymers such as polyester (polyglycolic acid (PGA), polylactic acid (PLA), polycaprolactone (PCL), polylactides-*co*-polyglycolide (PLGA)) and natural polymers (carbohydrates and proteins). Among the natural polymers, carbohydrates such as chitin-chitosan, alginate, starch, and cellulose are major natural polymers [2-4]. In addition, natural structural proteins like collagen, gelatin, elastin, silk-fibroin, and keratin are attention focused for applications [5-8]. Previous works [1,8,9] reported that the biodegradable polymers have been used in various fields, especially in biomedical applications for tissue engineering and drug delivery systems.

Keratin, a fibrous protein derived from several parts of animals including wool, nail, horn, feather and hair [10-12]. It is an insoluble protein according to disulfide bonds in its structure [13]. Recently, keratin has been used in tissue engineering and drug delivery systems [6,14]. Keratin with various forms such as films [10,15], microcapsules [16], sponges [10], hydrogel [17] and fibers [18] were reported. However, keratin material has some drawbacks with properties such as brittle, fragile and low flexibility [6]. This is the main problem for the application of keratin products. Therefore, keratin blended with high flexibility materials might be solved this problem [19].

Previous studies [20-22] have been shown that the egg white protein composed the suitability for the manufacture of bioplastics. Many studies [23-25] have revealed the feasibility of producing highly transparent bioplastics from egg white albumen as a novel alternative to the food industry. Egg white proteins have been applied in the food and cosmetics industries [26], in packaging [16], as an emulsifier and thickening agent [27]. Moreover, egg white protein has been blended with polymers such as polyvinyl alcohol, starch, cellulose acetate, and polyethylene oxide to improve its mechanical properties [28-30]. The egg white showed properly as a blending material since it is a hydrophilic protein as well as its biological properties. Sometimes, the proper properties for the application would not be obtained from only one type of polymer, especially natural polymers. The blending or composite polymers are another way

to solve this problem. Moreover, the polymer blended or composite are simple to construct without chemical treatment [31,32]. However, some factors like the compatibility of each polymer should be considered as well. To achieve this point, adding some agents including additives, plasticizer, compatibilizer, filler, reinforcing agent, nucleating agent or crosslinked agent were performed to enhance the compatibility of the blend polymers.

In this work, keratin from human hair and egg white was blended. Different crosslinked agents *e.g*., acetic acid, polyethylene glycol diglycidyl ether (PEGDE) and genipin were chosen and mixed to the blended polymers for particles preparation by a water-in-oil emulsification solvent diffusion method. The effect of the cross-linked agents on the morphology of the blend particles was observed and discussed.

EXPERIMENTAL

Egg white (EW) was derived from hen chicken. The egg solution has manually separated the yolk to obtain only egg white. The egg white concentration was prepared as 1% (w/v) by mixing with distilled water.

Human hair was warmed at 40 ºC before washing twice with distilled water. The hair sample was then immersed in *n*-hexane for 12 h to remove some lipids and waxes. The extraction procedure was then performed using a modification method [33]. Briefly, 10 g of hair sample was dissolved using a mixture solution of 0.8 M urea, 0.26 M SDS, and 0.4 M NaOH in 100 mL distilled water at 70 ºC with stirring until homogeneous dissolution. The obtained solution was then dialyzed against distilled water for three days. The %wt. of keratin was found by evaporation technique. The prepared keratin solution was added to a known weight of 10 mL beaker, then dried in an oven until the solution was fully evaporated. After cooling, the beaker was weighed to find remained keratin in the beaker. The increased weight was expressed as % wt of keratin by multiply by 100. The keratin (KT) concentration was prepared as 1% (w/v) by mixing with distilled water.

Preparation of KT/EW blend particles: The KT/EW blend particles were prepared by water-in-oil (w/o) emulsification diffusion method [34]. The keratin was blended with egg white 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 concentrations of the cross-linked agents of 1, 5, and 10% (v/v) of the blended solution were varied to observe their effect on the particle morphology. In the brief of particle preparation, ethyl acetate contained a beaker was stirred on the magnetic stirrer apparatus, then a 1.0 mL of the blended polymer solution with different amount of cross-linked agents was slowly added dropwise into the solvent with stirring continued for 30 min. During the emulsification and diffusion processes, the beaker was covered with aluminum foil to prevent the evaporation of the solvent. The particles were collected by centrifugation and then dried in a vacuum oven at room temperature until the solvent was entirely evaporated.

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

RESULTS AND DISCUSSION

Effect of acetic acid: With our experience, the suitable ratio using for the particle formation 1:100 (W:O). Fig. 1 showed

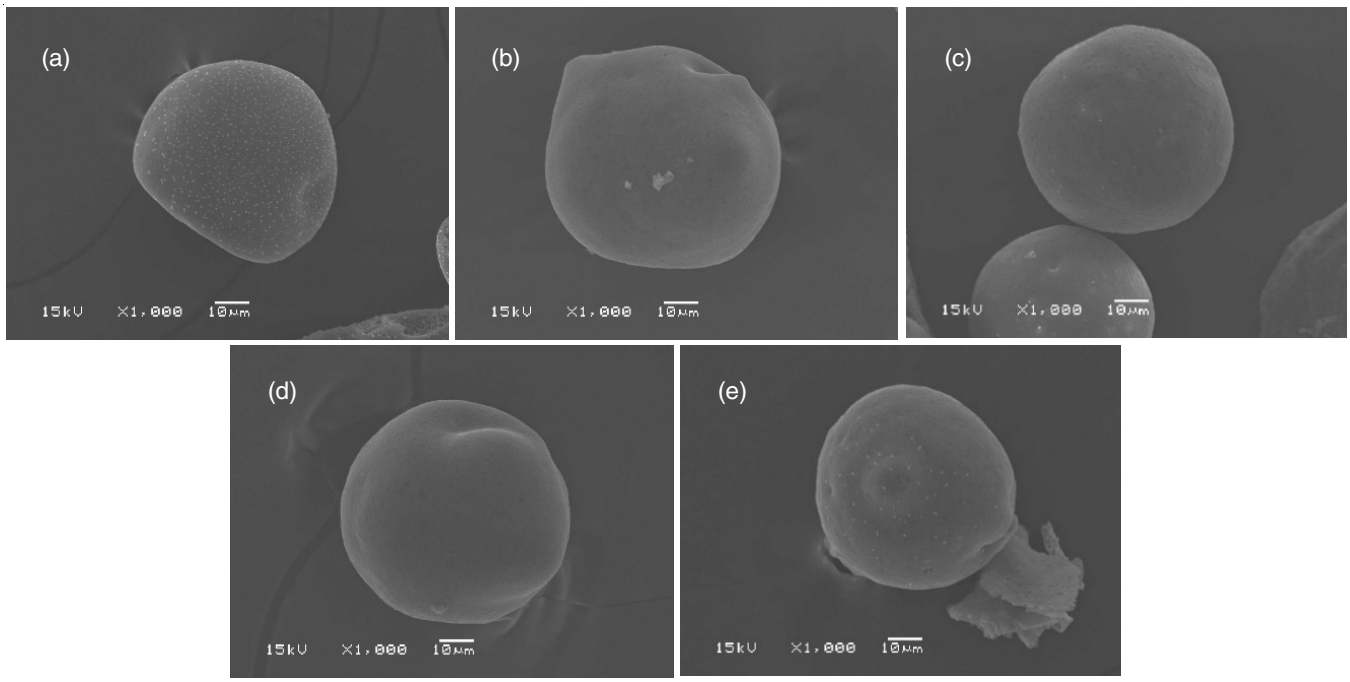

Fig. 1. SEM micrographs of KT/EW blend particles prepared by different concentrations of acetic acids; 10% (a), 5% (b) and 1% (c) (% v/v) in the blend solution using with stirring rate of 700 rpm and 1% acetic acid with stirring rate of 600 rpm (d), 500 rpm (e) at 1000X magnifications. Scale bars = 10 µm

the effect of acetic acid concentrations on the morphology of KT/EW blend particles prepared by the water-in-oil emulsification diffusion method. In general, the obtained particles had variable shapes and sizes. Most of the particles were slight spherical shapes with varied slight appearances. Comparison among the concentration of acetic acid, the lowest concentration resulted to obtain the spherical shape (Fig. 1c-e). At the highest concentration of acetic acid, the full spherical shape of the blend particles could not be conducted (Fig. 1a). This might be expected that the high concentration of acetic acid enhanced protein denatures and formed dense texture. This dense texture interfered the rheology of the solution as well as the interaction between water and protein. Moreover, the particle has a white droplet dispersed on its surface in a higher amount than other concentrations. These expected droplets were denaturing proteins of egg white by acetic acid since the isoelectric point of the egg white proteins is equal to pK_a of acetic acid (4.76). At 5% acetic acid (Fig. 1b), the blend particle could be formed spherically than 10% acetic acid used, but in lower than 1% acetic acid. With the same concentration of acetic acid, the highest rpm (Fig. 1c) showed a more spherical shape than the low stirring rate (Fig. 1 d-e). However, the smooth surface of the particle also found for the particle prepared by the stirring rate at 600 rpm (Fig. 1d). Considering particle sizes, the blend particle constructed from 10% acetic acid solution (Fig. 1a) showed the smallest size which was equal to 1% acetic acid stirring rate at 500 rpm (Fig. 1e). It does not a surprise for 10% acetic acid since this condition might be affected by proteins denature, dense texture and packed tightly particle. As a comparison to another protein like gelatin, the egg white protein could have interacted with acetic acid *via* hydrogen bonds [35]. The reason to describe for 1% acetic acid on the small particle should be affected by the interaction of acetic acid and protein *via* bond interaction. These force might be closed both keratin and egg white together and good compatibility when stirred in the oil phase. Therefore, it is concluded that the most properly prepared blend particles were 1% acetic acid with a stirring rate of 700 rpm as shown in Fig. 1c. Natural candidates such as acetic acid and lactic acid is already used by the food industry and designated by the Food and Drug Administration (FDA) as Generally Regarded as Safe (GRAS) for meat products [36,37].

Effect of polyethylene glycol diglycidyl ether (PEGDE): Ethylene glycol diglycidyl ether (EGDE) as a type of crosslinker of polyethylene glycol (PEGs) [38,39]. This polymer is amphiphilic and soluble in water as well as in many organic solvents [40]. PEGDE should be able to play the dual function of water-retaining polymer and crosslinker giving rise to particle texture with different water affinity depending on the amount of PEGDE employed [41]. Fig. 2 showed the effect of PEGDE concentrations on the morphology of KT/EW blend particles. The results indicated that all tested concentrations can be conducted the particles in spherical shapes. However, the variable morphology of the particles was observed. At 10% (Fig. 2a) and 5% (Fig. 2b) PEGDE concentrations, the surfaces of particles were rough and different patterns. The lowest concentration of used PEGDE resulted to obtain the particles with spherical shape (Fig. 2c-e). This might be expected that the high concentration of PEGDE interfered the rheology of polymer solution by bonding formation, then interrupted the particle formation. Moreover, small white droplets dispersed on the surface of particles in high PEGDE concentrations were observed. These expected droplets were aggregate proteins of egg white. At 1% PEGDE (Fig. 2c-e), the blend particles could be formed spherically.

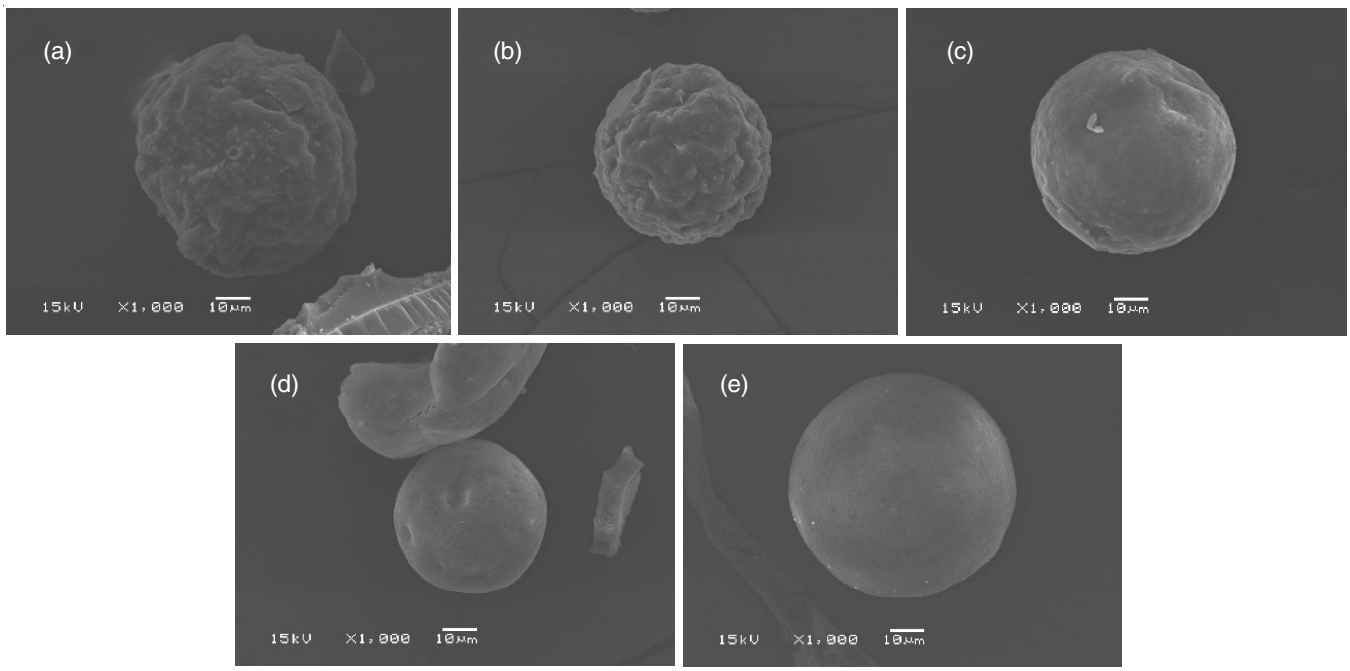

Fig. 2. SEM micrographs of KT/EW blend particles prepared by different concentrations of polyethylene glycol diglycidyl ether; 10% (a), 5% (b) and 1% (c) (% v/v) in the blend solution using with stirring rate of 700 rpm, and 1% polyethylene glycol diglycidyl ether with stirring rate of 600 rpm (d), 500 rpm (e) at 1000X magnifications. Scale bars = 10 μm

However, variable surface morphology was observed. With the same concentration, the lowest rpm (Fig. 2e) showed a more spherical shape than a high stirring rate (Fig. 2d-e). The smoothest surface of particle found in the particle prepared by the stirring rate at 500 rpm (Fig. 2e). This means that the rpm and PEGDE concentrations are the main factor on the spherical shape and smooth surface of the particle. Therefore, it is concluded that the most properly prepared blend particles were 1% PEGDE with a stirring rate of 500 rpm as shown in Fig. 2e.

Effect of genipin: Genipin, a natural extract from *Gardenia jasminoides* Ellis has been effectively and extensively employed for the crosslinking of various amino-containing polymeric molecules [42,43]. This plant extract has shown low cytotoxicity and ability to self-polymerization [9,44]. Genipin can function well in protein-protein crosslinking including egg white [45-47]. All concentrations of genipin mixed KT/EW blend solution could be constructed as spherical particles as shown in Fig. 3. The results indicated that the high concentration and rotation per minute (rpm) more effect on the particle shape than the low condition. Comparison of the concentration used, all did not be different in spherical shape and size of particles (Fig. 3a-c). However, the smoothest of particle surfaces was found by using 5% genipin (Fig. 3b). The variable in shape and size were obtained comparison between 600 (Fig. 3e) and 500 rpm (Fig. 3f). The result indicated that rpm used directly affected on shape and size of particles. The low rpm resulted to obtain variables in particle size and shape (Fig. 2d-f). This might be expected that the genipin supported the rheology of polymer solution by increasing the distance between protein molecules according to its ring structure. This means that the rpm is the main factor in the spherical shape and size of particles. By this work, high genipin concentration (5 and 10%) was suitable for crosslinked polymer blend. However, the most properly prepared blend particles were 5% genipin with a stirring rate of 700 rpm (Fig. 3b), due to cost safe. The suitable conditions that affected each cross-linked agent is shown in Fig. 4.

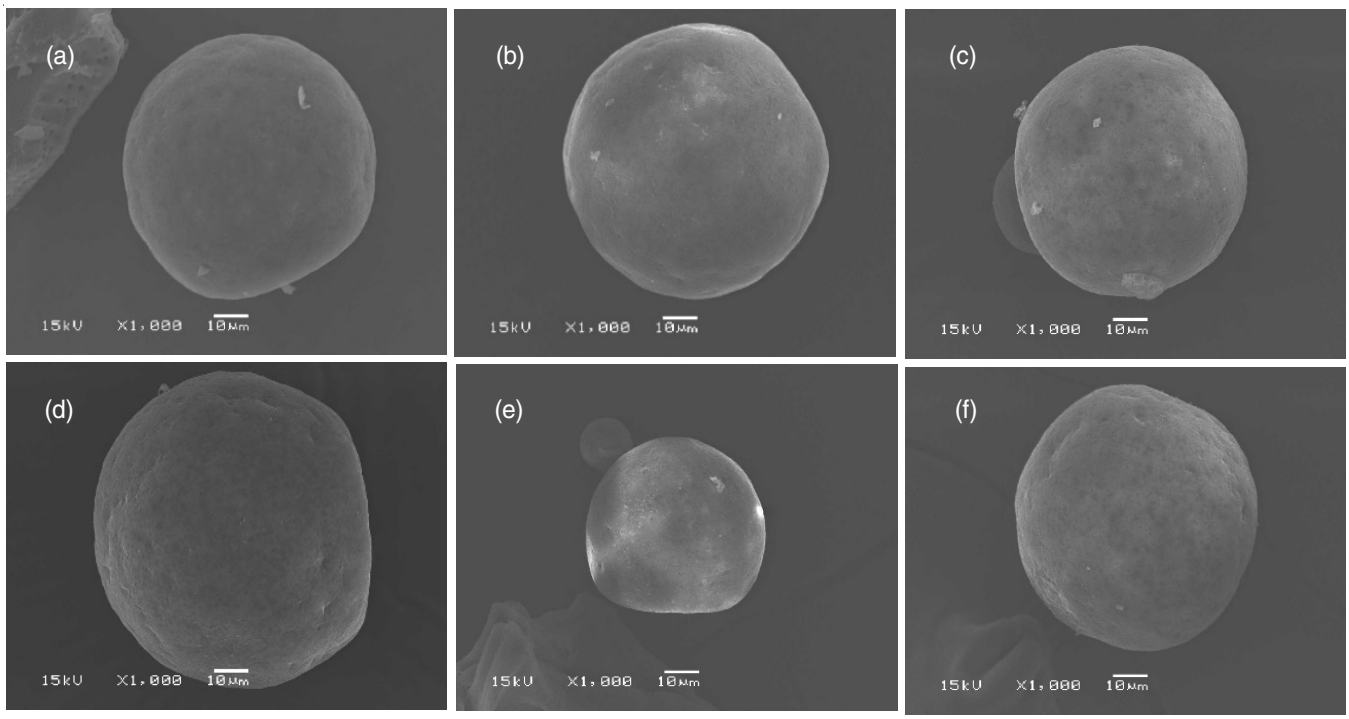

Fig. 3. SEM micrographs of KT/EW blend particles prepared by different concentrations of genipin; 10% (a), 5% (b) and 1% (c) (% v/v) in the blend solution using with stirring rate of 700 rpm, 5% (d), 1% (e) genipin with stirring rate of 600 rpm, and 1% genipin with stirring rate of 500 rpm (f) at 1000X magnifications. Scale bars = 10 μm

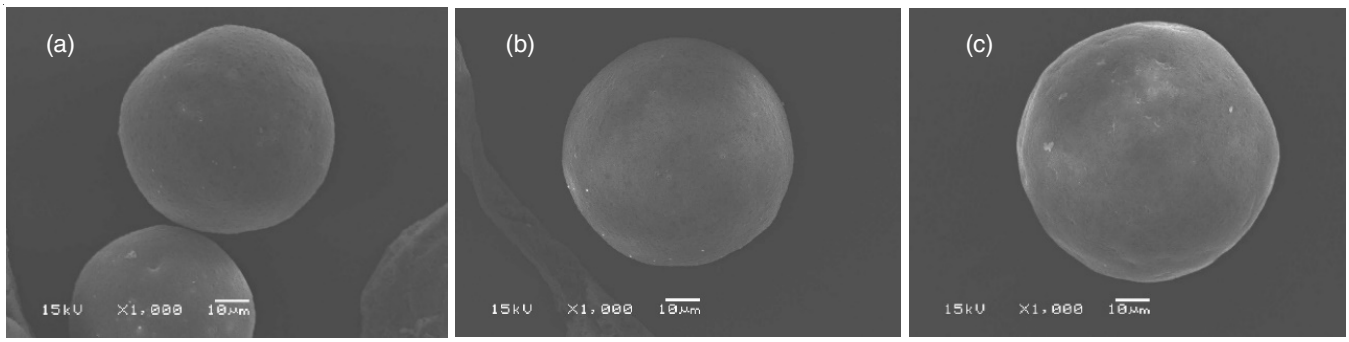

Fig. 4. SEM images of the KT/EW blend particles under the suitable conditions and mixed with acetic acid (a), PEGDE (b) and genipin (c)

Conclusion

The effect of cross-linked agents on the keratin/egg white (KT/EW) blend particles was studied and found that studied crosslinked agents [acetic acid, polyethylene glycol diglycidyl ether (PEGDE) and genipin] showed a variable effect on the shape, size and surface of the blend particles. Acetic acid at the lowest concentration (1%) and spinning rate with the highest (700 rpm) showed properly for particle blend formation while the suitable condition for PEGDE was 500 rpm with the lowest concentration used. Genipin showed different characteristics on the blend particle since the concentration did not affect the particle formation even at 10%. The spherical and consistent size obtained by using a high spinning rate. Further studies such as the effect of these cross-linked agents on other properties of the blend particles such as secondary structure, thermal behaviour or water resistance would be performed to obtain informative data for using the KT/EW blend particles in various applications.

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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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