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EFFECT OF CROSS-LINKED AGENTS ON KERATIN FILMS PROPERTY

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ABSTRACT: This research was aimed to investigate the effect of cross-linked agents on the properties of keratin films. The films were prepared by mixing all solutions until homogeneous before pouring into polystyrene plates and dried in an oven at 40 ºC for 3 days. The obtained films were characterized for their morphology and secondary structure using scanning electron microscope (SEM) and Fourier transform-infrared (FT-IR) spectrophotometer. The native keratin film had smooth surfaces with slight fragile throughout the film area. Considering among the cross-linked agent, the keratin film cross-linked with glutaraldehyde had the smoothest surfaces than others. In addition, the differences of cross-linked agents were affected on different morphology of the films. The secondary structure analysis found that random coil structures of films had been changed into β-sheet after blending with cross-linked agent. This reflected to increase of dense texture, strength and thermal stability of the films. The results could be suggested that the morphology, secondary structure and thermal stability of the keratin films were depended on content and type of cross-linked agents used.

Keywords: Film, Keratin, Morphology, Secondary structure

1. INTRODUCTION

Keratin is a structural protein which composed unique biological and chemical properties [1], [2]. It can be extracted from several original sources. Recently, the keratin has been used in many applications [3], [4]. It can be prepared in many forms, especially film. It has also exhibiting as a high potential scaffold material for several tissue engineering applications. Previous reports have been developed novel keratin by cross-linking of disulfide bonds. The recycled keratin biomaterials could be fabricated into films to be used as drug release carriers. However, keratin film composed of the limited applications which are high fragile, low strength and flexibility [5]. Moreover, polymers blend have been used to improve some limitations of the keratin material include cross-linked agents [6], [7]. However, the effect of cross-linked agents on keratin property is a little information in Thailand. Therefore, the objective of this work is to study the effect of different cross-linked agents on keratin films properties, especially morphology, secondary structure and thermal stability.

2. METHODOLOGY

Human hair was gathered from local hair salon in Maha Sarakham Province, Thailand. The hair was wormed at 40 °C before immersing in n-hexane for 12h to remove external lipid. Other chemical reagents used were analytical grade.

2.1 Preparation of Keratin Solution

Human keratin was extracted using modification of Shindai method [26]. Briefly, 10g of hair sample was dissolved using mixture solution of 7g urea, 2g sodium dodecyl sulfate (SDS), 0.8g sodium hydroxide (NaOH) in 100 mL distilled water at 70 °C with stirrer until homogeneous dissolution. The obtained solution was then dialysis against distilled water for 3 days.

2.2 Preparation of Keratin Blend Films

A 1% wt keratin solution was firstly mixed with different ratios of cross-linked agents in beakers. The mixture solutions were then poured in polystyrene plates with diameter of 9 cm. The plates were then dried in an oven at 40 °C for 3 days to obtain the blend films.

2.3 Characterizations

2.3.1 Morphological observation

All of films were dehydrated and cut (~ 1cm in length) before observing their morphology under the scanning electron microscope (SEM) (JEOL, JSM-6460LV, Tokyo, Japan). The samples were sputter coated with gold by double sides of carbon for enhancing surface conductivity. Current and voltage were adjusted to give power of 2W (3mA, 15 kV) for 3 min.

2.3.2 Secondary structure analysis

All of films were investigated their secondary structures using attenuated total reflection infrared

(ATR-IR) spectroscopy (Perkin Elmer-Spectrum Gx, USA) in the spectral region of 4000-400 cm^{-1} at 4 cm^{-1} spectral resolution and 32 scans.

2.3.3. Thermal properties study

The keratin/sericin blend films with 8-10 mg were loaded in a platinum crucible. The thermogravimetric analysis (TGA) was then performed using TA instruments, SDT Q600 (Luken's drive, New Castle, DE). The samples were non-isothermal heated from 50 °C to 800 °C at a heating rate of 20 °C/min. The TGA was carried out in nitrogen with the flow rate of 100 mL/min.

3. RESULTS

3.2 Morphological study

The morphology of films was investigated using scanning electron microscope (SEM). The results found that keratin (Fig. 1a) had smooth surface with homogeneous texture of cross-section without phase separation. Keratin blended PEGDE resulted to increase rough surface as well as cross-section (Fig. 1b) comparison to native keratin film. In contrast the keratin blended with glutaraldehyde (Fig. 1c) and genipin (Fig. 1d) had smoother surface than the native keratin film. Considering at the edge of film, the keratin blended genipin indicated some small phase separation which does not find in other films.

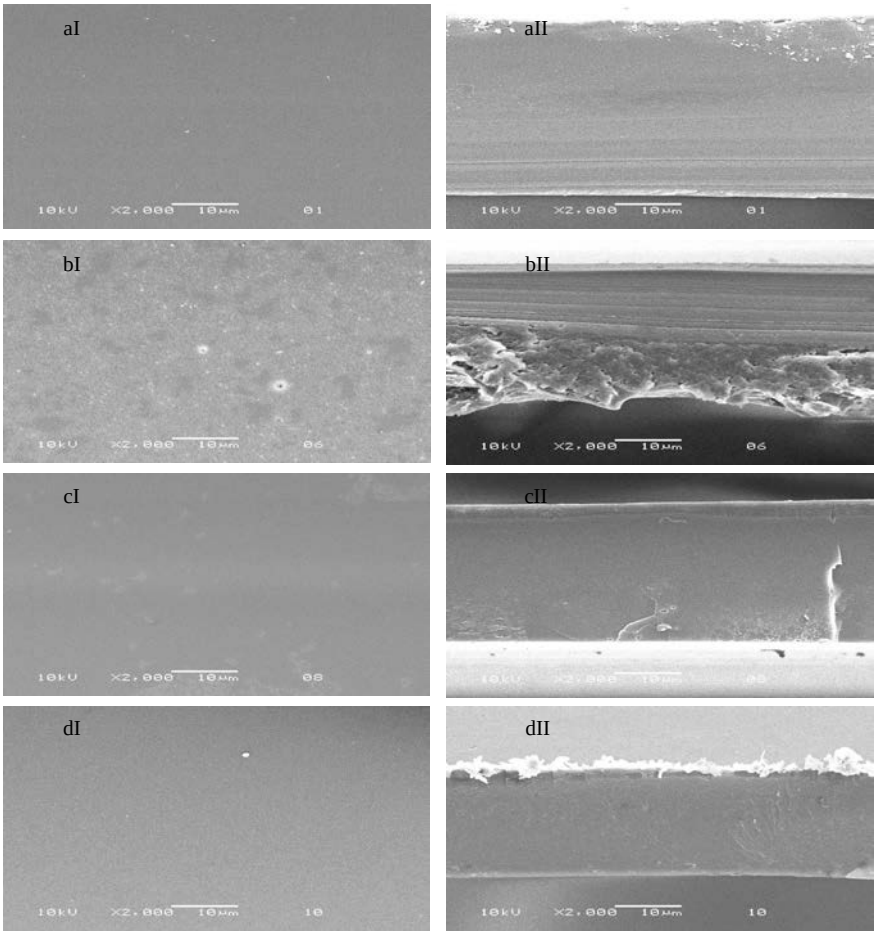


Fig. 1 SEM micrographs native keratin film (a), keratin blended with PEGDE (b), glutaraldehyde (c) and ginipin (d). Column I present as surface and column II as cross-section of films. (All bars =10µm).

3.2 Secondary structure

The results found that the native keratin film appeared the absorption peaks at 1671, 1539 and 1232 cm^{-1} [Fig. 2a]. The keratin film blended with PEGDE showed the absorption peaks at 1664 and 1234 cm^{-1} [Fig. 2b], glutaraldehyde showed at 1622, 1571 and 1236 cm^{-1} [Fig. 2c]. In addition, the keratin blended genipin showed the absorption peaks at 1628, 1553 and 1236 cm^{-1} [Fig. 2d].

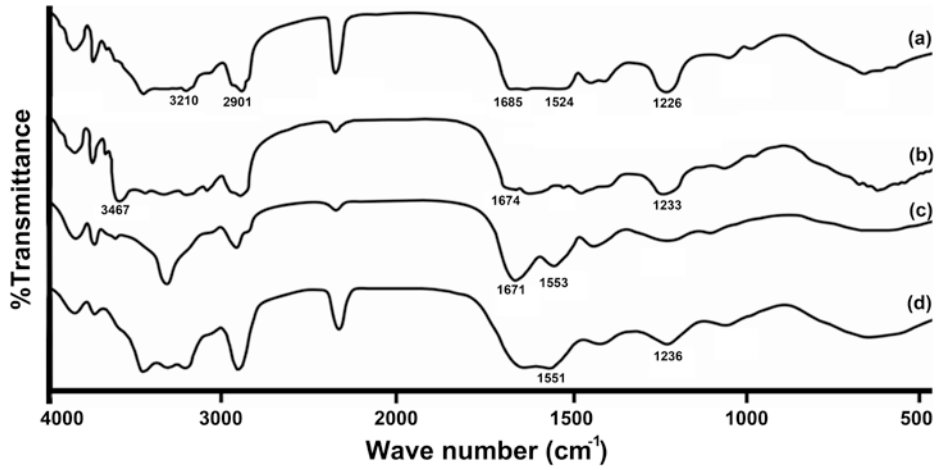


Fig. 2 FTIR spectra of native keratin (a), keratin blended with 0.1% PEGDE 0.01 g (b), keratin blended with genipin (c) and keratin with glutaraldehyde (d).

3.3 Thermal stability

The thermal stability of the films indicated by DTG curves as shown in Fig. 3 which showed the maximum temperature of decomposition ($T_{d,max}$) summarized in Table 1. The native keratin started weight lose at about 105 °C and had $T_{d,max}$ at 311 °C (Fig. 3a). Among the cross-linked agents, keratin blended genipin showed the highest of $T_{d,max}$ at 316 °C (Fig. 3f,g), then PEGDE at 314 °C (Fig. 3b,c) and glutaraldehyde had the lowest of $T_{d,max}$ at 304 °C (Fig. 3d,e).

4. DISCUSSION

SEM micrographs showed different surfaces and texture of cross-section according the unique characteristic of each cross-linked agent. Generally, keratin is varied in size, molecular weight and amino acid components. The cross-linked agents influenced on the dense texture of the film. This was due to their acting as binder between keratin molecules to more close together. The secondary structure of protein was mainly observed in the region of amide I, II and III regions [8].

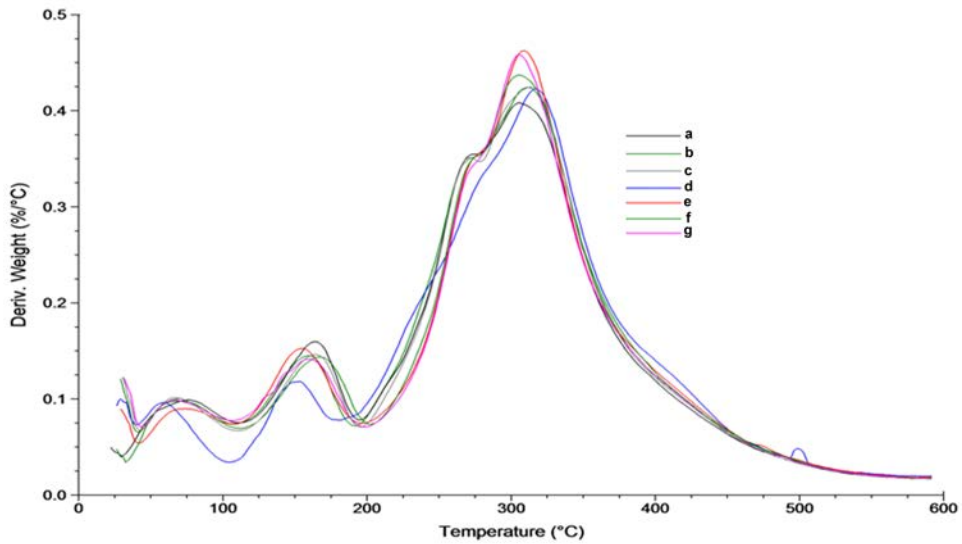


Fig.3 DTG curve of native keratin (a), keratin blended with PEGDE of 0.01 g (b) and 0.02 g (c), glutaraldehyde of 0.01 g (d) and 0.02 g (e), and genipin of 0.01 g (f) and 0.02 g (g).

Table 1 Temperature of maximum decomposition of keratin films blended with different cross-linked agents.

Type of films	$T_{d,max}$ (°C)
Keratin	311
Keratin/PEGDE	314
Keratin/glutaraldehyde	304
Keratin/genipin	316

Amide i was the stretching of carbonyl group (-c=o) while amide ii was blending of –nh group and stretching of –ch. On the other hand, amide iii was stretching of –cn and the plane of –nh as well as stretching of –cc and –c=o [9]. Ftir spectra suggested that the native keratin composed of α-helix and random coil structures. Glutaraldehyde, the smallest chemical structure, helped to decrease the crystalline of the keratin film and resulted to decrease thermal stability of keratin film. However, the keratin films blended pegde and genipin increased thermal stability. This might be suggested that pegde and genipin are complex chemical structures which are interacted with more keratin molecules than glutaraldehyde and affected to increase α-helix and β-sheet structures. The decomposition of all films indicated at least 2 steps with the td,max higher than 300 ±30 (300 □c). This temperature characterize decomposition of amino acid side chain and peptide bonds between amino acids [10].

CONCLUSION

The native keratin had smooth surfaces with some particles with homogeneous texture without phase separation. The keratin film blended with cross-linked agents affected to impact texture of the films and increased thermal stability according to change their secondary structure, except glutaraldehyde. The finding results may use for preparation keratin film for several applications, especially biomedical and drug controlled release.

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