# สมบัติและรูปแบบการปลดปล่อยยาของฟิล์มผสมระหว่างเคราตินและแป้ง

# Properties and Drug Release Profile of Keratin/Starch Blend Films

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## บทคัดย่อ

งานวิจัยนี้มีวัตถุประสงค์เพื่อศึกษาสมบัติและรูปแบบการปลดปล่อยยาของฟิล์มผสมระหว่างเคราตินและแป้ง โดยทำการเตรียมฟิล์มด้วยการผสมสารละลายเคราตินที่สกัดจากเส้นผมมนุษย์และแป้งมันสำปะหลังก่อนนำไปเทบนจานเพาะเชื้อสไตรีนแล้วทำการระเหยตัวทำละลายโดยการอบที่อุณหภูมิ 40 องศาเซลเซียส เป็นเวลา 3 วัน นอกจากนี้ ยังทำการเตรียมฟิล์มที่ผสมยาคลอเฮกซิดีนสำหรับศึกษารูปแบบการปลดปล่อยโดยใช้วิธีการเดียวกับที่กล่าวมา นำฟิล์มที่เตรียมได้ไปตรวจสอบสัณฐานวิทยาโครงสร้างทุติยภูมิและสมบัติเชิงความร้อนด้วยกล้องจุลทรรศน์อิเล็กตรอนแบบส่องกราด (SEM), Fourier transform infrared (FTIR) สเปกโทรโฟโตมิเตอร์และ thermogravimetric analyzer (TGA) ตามลำดับ รวมทั้งตรวจสอบการละลายและพฤติกรรมการปลดปล่อยยาของฟิล์มผสมอีกด้วย ผลการทดลองแสดงให้เห็นว่าสมบัติของฟิล์มเคราตินแตกต่างจากฟิล์มผสมระหว่างเคราตินและแป้ง การผสมแป้งกับเคราตินทำให้การละลายเพิ่มขึ้น โดยเฉพาะอย่างยิ่งในอัตราส่วนเคราตินต่อแป้งเป็น 1 ต่อ 2 ที่พบว่าละลายมากที่สุด ฟิล์มผสมระหว่างเคราตินและแป้งมีอัตราการปลดปล่อยยาต่ำและแตกต่างกันตามอัตราส่วนที่ใช้ ผลการทดลองที่พบในการศึกษาครั้งนี้แสดงให้เห็นว่าสามารถเตรียมฟิล์มให้มีสมบัติและรูปแบบการปลดปล่อยยาแตกต่างกันเมื่อผสมกับแป้งได้

**คำสำคัญ:** ฟิล์ม เคราติน สมบัติ

## Abstract

This work aimed to study keratin/starch blend film properties and their drug release profiles. Films were prepared by mixing keratin solution, extracted from human hairs and cassava starch solution, poured in polystyrene culture plates, and evaporated in an oven at 40 °C for 3 days. The keratin blended films containing chlorhexidine, for studying the drug release pattern, were also prepared by the same procedure. The obtained films were determined for their morphology, secondary structure and thermal properties using scanning electron microscope (SEM), Fourier transform infrared (FTIR) spectrophotometer and thermogravimetric analyzer (TGA), respectively. In addition, dissolution and drug release patterns from the blended films were also determined. The results showed that the native keratin film properties were different from the blended films. Blending keratin with starch led to an increase in their dissolution, especially the keratin/starch ratio at 1:2, which showed the highest dissolution. The keratin/starch blend films showed a slow rate of drug release which varied following the ratio used. The obtained results from this work indicated that keratin film can be prepared in various properties and drug release patterns by blending with starch.

**Keywords:** Film, Keratin, Properties

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## Introduction

Keratin is the structural fibrous protein providing an outer covering for things such as wool, hair, feathers, nail, and horns of mammals, reptiles and birds[1]. Keratin is chara cteristically abundant in cysteine residues (7–20 number% of the total amino acid residues)[2] which are oxidized to give inter- and intra-molecular disulfide bonds, which may result in the mechanically strong three-dimensional linked network of keratin fiber[3].

The keratin-based biomaterials for many applications have been developed and prepared in various forms such as films, sponges, porous membrane, fibers and scaffolds[4]. Keratin has a wide range of advantages such as environmental stability, biodegradability, and biocompatibility. Thus, it is possible to develop biodegradable materials based on keratin, such as films for compostable packaging or paper coatings and drug delivery system[5]. It has exhibited high potential as a scaffold material for several tissue engineering applications such as support fibroblast cell growth, support cultivation of osteoblast cell and an important role in the protective function of the skin[6]. In addition, extracted keratins are capable of forming self-assembled structures that regulate cellular recognition and behavior. These qualities have led to the applications of keratin biomaterials in wound healing, drug delivery, trauma and medical devices[7]. Keratin biomaterial is an appealing choice for therapeutic development as it can be easily manufactured, whether as an allogeneic or autologous product[8]. Previous literatures have been developed to obtain novel keratin biomaterials from disused feathers. By cross-linking of disulfide bonds, recycled keratin biomaterials could be fabricated into films to be used as drug release carriers. In this work, keratin films were mixed with starch, polysaccharide polymer, to study their properties and drug release profile using chlorhexidine diacetate (CHX diacetate) as a model drug. Furthermore, dissolution of films was also determined.

## Materials and methods

### Extraction of keratin from human hair

Keratin was extracted from human hair collected from a hair salon in Maha Sarakham Province, Thailand. The procedure used to extract keratin followed the Shindai method with some modifications[10-11]. The hair was firstly washed twice with distilled water and then immersed in hexane for 12 h to remove some lipid components. The 1 g of dried hair was put in 20 mL of the mixture solution of 0.02 M NaOH, 0.5 M urea, 0.26 M SDS and distilled water at 70 °C with stirring until the hair was completely dissolved. The solution was then dialyzed against distilled water for 3 days with dialysis tube (MW. cut off = 3,500 Da). The 1 mL of keratin solution was pipetted and placed into a beaker of known weight then evaporated in an oven to find the concentration (%w/v).

### Preparation of starch solution

Starch solution with concentration of 2% (w/v) was prepared by weighing 2g of cassava starch powder and then adding into 100 mL of distilled water, heated on hotplate at 100 °C for 5 min with stirring until it gelatinized, and allowed to cool at room temperature

### Preparation of keratin/starch blend films

The keratin/starch (K/St) blended films were prepared by mixing the diluted keratin solution of 2% (w/v) and 2% (w/v) starch solution with different ratios (2:1, 1:1, 1:2). The mixture solution of each ratio with total volume of 21 mL and 0.04 g of CHX diacetate was added before pouring onto 9 cm polystyrene plates. They were dried in an oven at 40 °C for 3 days to obtain the blend films.

### Morphology study of keratin/starch blend films

Samples were dehydrated and then mounted on the stub with double-sided carbon tapes. They were sput tercoated with gold for enhancing surface conductivity. Current and voltage were adjusted to give power of 2 W (3 mA, 15 kV) for 3 min. The samples were examined using scanning electron microscope (SEM) (JEOL, 6460LV).

### Secondary structure analysis

The samples were dehydrated and analyzed for their secondary structures using Fourier transformed infrared (FTIR) spectrophotometer (Perkin Elmer- Spectrum GX). A region from 400 to 4000 $cm^{-1}$ was used for scanning at 4 $cm^{-1}$ resolution with 32 scan.

**Thermal behavior analysis**

A thermogravimetric analyzer was used to determine the thermal decomposition patterns of the films. Samples were loaded in platinum crucible. The analysis condition was 50-800 °C at heating rate of 20 °C/min under nitrogen atmosphere. The analyses of the TG data were done using TA instrument's Universal Analysis 2000 software (version 3.3B).

**Dissolution study**

Sample films without a model drug were tested by dissolving in phosphate buffer saline (PBS, pH 7.4) and stand at 37 °C for 7 days. The buffer solution was changed every day and replaced with fresh solution at the same volume. The drying film was rinsed twice with distilled water before submersing in the PBS.

**Drug release study**

The blended films were immersed in a 10 mL of PBS, pH 7.4 solution at 37 °C for 48 h with continuously shaken at 150 rpm. The supernatant of 5 mL was collected at a designation time interval for examining. The absorbance at 254 nm of the collected PBS solution was measured to find amount of released CHX diacetate according to the standard curve. Finally, supernatant (collected PBS solution) was replaced by fresh PBS solution.

## Results and discussion

**Morphology observation**

Figure 1 shows the morphological study of the keratin/ starch blend films. In the left column, the keratin/starch (KSt) blend films indicated rough texture with different patterns. The porous like sponge texture of the film was found at the ratio of 1:2 (KSt2). This ratio obtained the film with many porous and rough surfaces covering the area. The films with a half sponge and smooth texture were found in the blend film at 1:1 ratio (KSt1) while the lower sponge was observed in the blend film at 2:1 (KSt3).

In the right column, the smoothest film surfaces were found in 1:1 ratio (KSt1). At 2:1 ratio (KSt3), the film has a smooth surface but a non-homogeneous region was also observed. Starch is a type of polysaccharide and non-soluble in water. The hydroxyl group (OH) in starch molecules can interact with water via H-bonds and the result to appear sponged like in the film. This might be caused from the evaporation of water molecules.

**Secondary structure**

Generally, the absorption peaks of amide regions including amide I (1700-1600 $cm^{-1}$), amide II (1600-1500 $cm^{-1}$), amide III (1300-1200 $cm^{-1}$), amide IV (767-625 $cm^{-1}$) and amide V (800-640 $cm^{-1}$) were used to investigate the secondary structure of protein[9,12]. As shown in Figure 2, the native keratin film showed strong absorption peaks at about 1678, 1561 and 1221 $cm^{-1}$ for amide I, II and III, respectively. These peaks were assigned as co-existed structure between $\alpha$-helix and disordered structure[13]. The keratin/starch blend films showed strong absorption peaks that were similar to a native keratin film in all of amide regions. All of absorption peaks indicated the $\alpha$-helix co-existed with random coil structures[14]. However, it should be thought that keratin and starch may form a homogeneous texture since the hydroxyl groups (-OH) in the glucose units can be interacted with the amino groups ($-NH_2$) or carbonyl group (C=O) of the keratin protein. This was according to the absorption peak of about 3200-2900 $cm^{-1}$ (amide A) disappearing at 1:1 and 2:1 ratios. These peaks are connected with the stretching vibration of N-H bonds[9]. The results indicated that this region might be changed after bonding formation between internal molecules of keratin and starch.

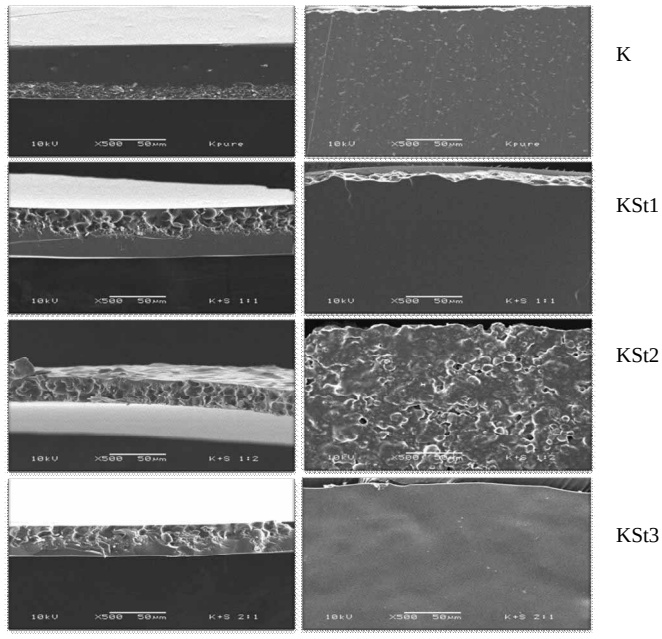


**Figure 1** SEM micrographs of different films; native keratin (K), keratin/starch blend at 1:1 (KSt1), 1:2 (KSt2) and 2:1 (KSt3) ratios. Left column presented as cross section and right column presented as surface of each films

## Thermal properties

As shown in Figure 3 and Table 1, the native keratin film showed the highest $T_{d, max}$ at 319 °C. The keratin/starch blend film showed the highest of $T_{d, max}$ at 1:2 ratio but the other ratios have similar of the $T_{d, max}$. The DTG curves presented the $T_{d, max}$ of all films are higher than 250 °C, responsibility to denaturation of $\alpha$-helix structure[15]. In addition, thermal properties were affected strongly by blending ratio which was found by the different of $T_{d, max}$ of each blending ratio. This means the component in each film was affected on the bonding formation, especially inter- and intra-molecular hydrogen bonds. At 1:1 and 2:1 ratios, $T_{d, max}$ of films slightly lower than the native keratin which indicated the lower amount of $\beta$-sheet formation.[12]

## Dissolution

As shown in Figure 4, the native keratin film has the lowest dissolution, about 1% of initial weight, but the highest dissolution about 6% was found in the blend film of 1:2 ratios (c). The keratin/starch blend films at 1:1 (b) and 2:1 (d) ratio showed similar %dissolution. However, the %dissolution of the blend film at 1:1 ration slightly dropped after 1 days of experiment. This might be affected by interaction force between keratin and starch. Moreover, the broad distribution of molecular sizes and molecular weights of starch and keratin should be affected on the bonding formation between two components and reflected on degradation pattern of the blended film.[9]

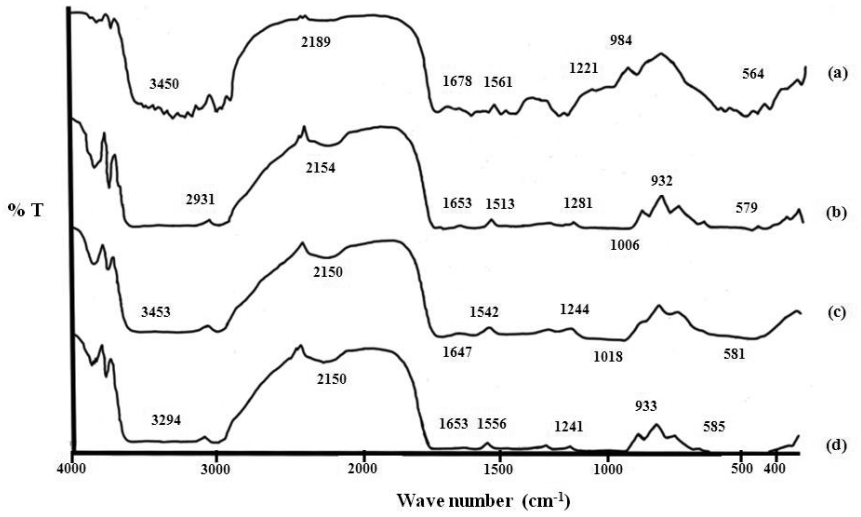


**Figure 2** FTIR spectra of native keratin (a), keratin/starch blend films at 1:1 (b), 1:2 (c) and 2:1 (d) ratios

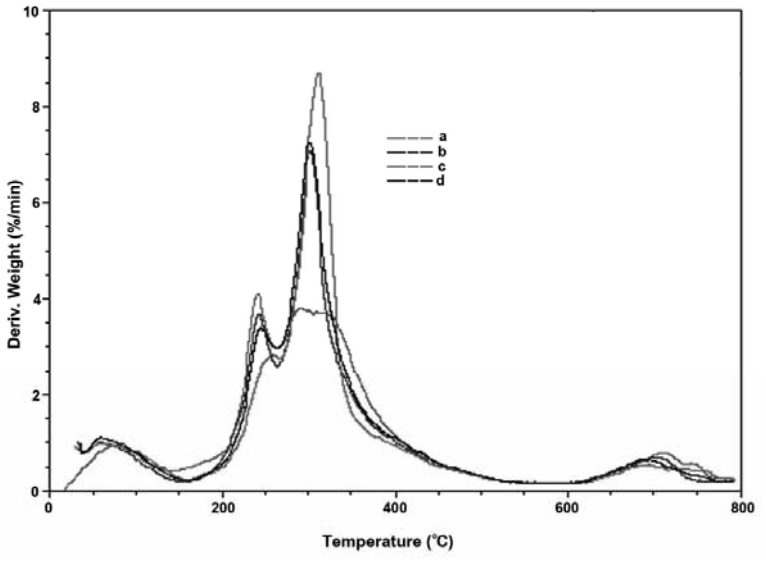


**Figure 3** DTG curves of native keratin (a) and keratin/starch blend films at 2:1 (b), 1:2 (c) and 1:1 (d) ratios

**Table 1 $T_{d, max}$ of different films.**

| Type of Films | $T_{d, max}$ (°C) |
|---|---|
| Keratin | 240 319 |
| Keratin/Starch (1:2) | 242 322 |
| Keratin/Starch (1:1) | 244 300 |
| Keratin/Starch (2:1) | 256 311 |

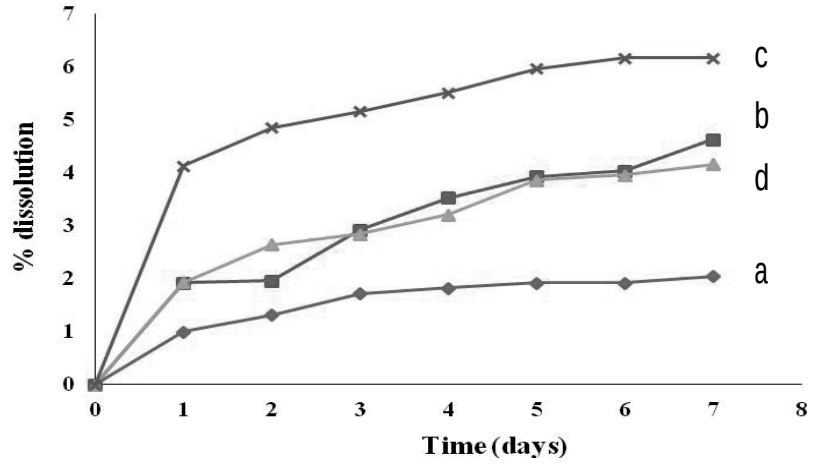


**Figure 4** Percentage of dissolution of keratin/starch blend films; native keratin (a), 1:1 ratio (b), 1:2 ratio (c) and 2:1 ratio (d)

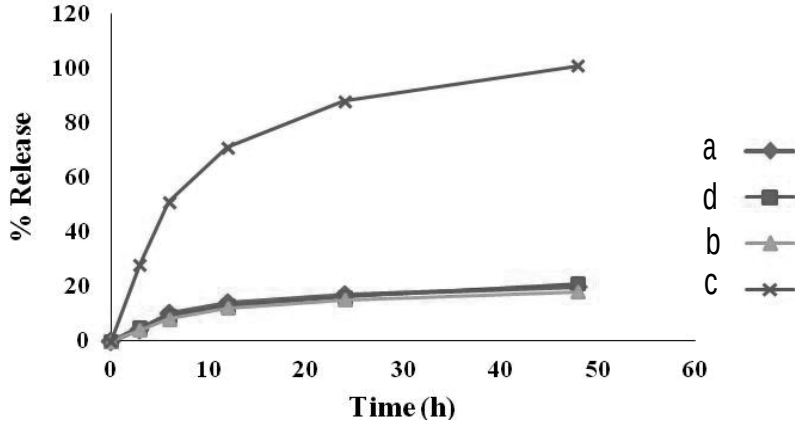


**Figure 5** Percentage CHX diacetate release from keratin/starch blend films; native keratin (a), 1:1 ratio (b), 1:2 ratio (c) and 2:1 ratio (d)

## Drug release profile

As shown in Figure 5, the keratin/starch blend film at 1:2 ratio (c) shows different pattern of drug release comparison to other films. This blended film gradually increased drug release until 100% at the end of experiment. The release pattern of CHX diacetate might be affected from the texture and dissolution of the film as well as the interaction between drug and film. Moreover, the position of the drug on the film also concerned the release rate since the drug on the film surface should be firstly released before the drug inside the film.

## Conclusions

The native keratin and keratin blended films showed smooth surfaces, except keratin/starch blend film. The native keratin film showed strong absorption peaks with $\alpha$-helix structure while the structure of the keratin blended film changed its structure after mixing with starch. Both native keratin and blended films have various decomposition peaks indicated many components composed in their structures. Thermal properties were affected strongly by blending ratio as well as types of polymer used. The keratin/starch blend film at 1:2 ratio has the highest dissolution. The drug release profiles of the blended films were depended on both ratio and type of polymer used.

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