SOME CHARACTERISTICS OF FISH SCALE COLLAGEN/ALLOPURINOL BIOCOMPOSITE

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Abstract:

In this work, collagen extracted from fresh-water fish scales has been used as a matrix in the drug delivery polymeric system. Allopurinol has been applied to reduce the concentration of uric acid in blood and used as a model drug in collagen drug delivery system. The functional groups in collagen/allopurinol biocomposite as well as the morphology of this material were assessed by infrared (IR) spectroscopy and scanning electron microscopy (SEM). Allopurinol content released from collagen/allopurinol biocomposite in simulated body fluids was determined based on the optical absorbance of solutions containing drug by ultraviolet – visible (UV-Vis) spectra and allopurinol calibration equations. The analysis results of IR spectra of biocomposite samples indicated that the collagen/allopurinol biocomposite contains N–H, C–H, O–H linkages in collagen and C=O, C=N, N-H linkages in allopurinol. The SEM images showed that the morphology of collagen/allopurinol biocomposites differs to that of allopurinol. The allopurinol could release from the biocomposites in simulated body fluids (pH 2 and pH 7.4) according to two stages: fast release stage for 1 first hour and slow release stage in following hours. At the same time of testing, the content of allopurinol released from collagen/allopurinol biocomposite is higher than that from allopurinol free (allopurinol unloaded by collagen).

Keywords: *Allopurinol; Fish scale collagen; Characteristic; Drug release.*

1. Introduction

Fish scales, a re-product of fish processing, are one of sources for extraction of fish collagen. It is type I collagen with fibril structure formed by amino acids, especially, glycine, proline and

hydroxyproline. Fish collagen has been focused on study due to its great properties such as high absorbance, safety, less fatty, and good biocompatibility. Therefore, it is potential to apply fish collagen in polymeric drug delivery system. The aim of this study is using fish collagen which was extracted from fresh-water carp fish scales for loading allopurinol to improve the solubility of drug and control the drug release in simulated body fluids because of allopurinol's poorly water-solubility. The IR spectroscopy and SEM methods have been conducted to evaluate the functional groups as well as the morphology of the collagen/allopurinol biocomposite. The drug release content will be determined by using UV-Vis spectrometer.

2. Research overview

In the fish processing, fish scales are one of the wastes that are directly discharged into the environment, causing environmental pollution. The composition of fish scales contains hydroxyapatite minerals and collagen, which are materials applied in many different fields. Collagen extracted from fish scales is a type I collagen (Shalaby et al, 2020). In some countries, collagen extracted from animals such as pigs or cows is banned due to religious barriers. The process of extracting collagen from animals is quite complicated, time consuming and expensive. Nowadays, collagen from fish is attracting a lot of attention thanks to its high absorbance ability, biocompatibility, safety, high stability and absorption, good bonding effect (Yamada et al, 2014; Pati et al, 2012; Nagai et al, 2004). Collagen is a potential material for drug delivery and biomedical applications because it can form high-strength and stable fibers by its self-agglutination and cross-linking properties (Cherim et al, 2018; Lee et al, 2001; Quang et al, 2021; Trang et al, 2020; Ning et al, 2021; Subhan et al, 2021).

In this study, collagen was extracted from Vietnamese carp scales by chemical method. The extracted collagen used to load the allopurinol drug having poorly water solubility (Qurie et al, 2022). In some our previous studies, we mentioned a combination of collagen from fish scales with carrageenan to carry allopurinol to reduce uric acid in the blood (Chinh et al, 2020; Manh et al, 2020; Manh et al, 2019). These studies have shown that the solubility of allopurinol is improved when carried by the collagen/carrageenan biocomposites. However, the authors did not study the use of fish collagen alone to carry allopurinol in the presence of a crosslinking agent such as glucose (Simon et al, 2014) to improve the solubility of allopurinol.

Therefore, in this paper, the results of studies on the effect of glucose cross-linked collagen on the properties of allopurinol will be presented.

3. **Experimental**

3.1. Materials

Allopurinol (purity ≥ 98%, molecular weight of 136.11 g/mol, melting point > 350^{o}C, solubility in NaOH 1M (soluble 50 mg/mL)) was purchased from Sigma Aldrich (USA).

Some chemicals consist of CH_3COOH 99,5%, NaOH, NaCl, glucose, KH_2PO_4, HCl 37%, KCl made in China were used as received.

NaOH 0.1M, H_2SO_4 0.5M/HCl 0.2M and CH_3COOH 0.5M solutions were were used to remove lipids on fish scales, demineralize and separate collagen, respectively according to the procedure reported in our previous research (Chinh et al, 2019).

3.2. Preparation of collagen/allopurinol biocomposite

Collagen/allopurinol biocomposites with various content of allopurinol (5-15 wt% in comparison with collagen weight) were prepared as follows: 1 gram of collagen was dissolved in 20 mL of acid acetic 1%. The

glucose solution (2% by mass of collagen) was added slowly into the mixture and the mixture then was stirred continuously for 2 hours to obtain the solution of A.

Allopurinol was dissolved into NaOH 0.01 M to obtain the solution of B. The solution of B was dropped in the solution A and homogenized at 20,000 rpm to obtain the solution C. NaCl in solid (1 wt% by mass of solution) was added in the solution C, and cooled for 24 hours to settle the biocomposite.

The biocomposite was decanted, centrifuged, and washed with distilled water to remove excess NaCl, then freeze-dried the solid part for 48 hours. Next, the product was ground with an agate mortar to obtain a powdered collagen/allopurinol biocomposite (Figure 1). Powdered biocomposites were stored in sealed PE tubes to avoid moisture absorption.

Figure 1: Powdered collagen/allopurinol biocomposite (CA10 sample)

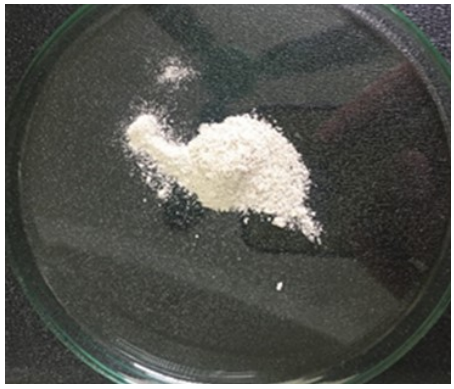


Table 1: Composition of collagen/allopurinol composites

Sample	Collagen (g)	Allopurinol (g)	Glucose (g)
CA5	1	0.05	0.02
CA10	1	0.10	0.02
CA15	1	0.15	0.02

Composition of collagen/allopurinol composites collagen/allopurinol was signed in Table 1.

3.3. Research methods

Fourier Transform Infrared (FTIR) spectra of investigated samples were recorded by using a Nicolet iS10 spectrophotometer (Thermo Scientific, USA) at Institute for Tropical Technology – Vietnam Academy of Science and Technology (VAST) at room temperature with a resolution of 8 cm^{-1} and averaging 32 scans.

Field emission scanning electron microscopy (FESEM) was carried out on a FESEM S–4800 (Hitachi, Japan) at National Institute for Hygiene and Epidemiology. Samples were coated with Pt to increase conductivity.

Ultraviolet-visible (UV-Vis) spectra of the samples conducted on a UV–Vis spectrophotometer (S80 Libra, Biochrom, Anh) at Institute for Tropical Technology (VAST) to evaluate drug concentrations released from biocomposite samples.

3.4. Drug release

The drug content of allopurinol released from collagen/allopurinol biocomposite was studied in pH 2 and pH 7.4 solutions, corresponding to the pH in the stomach and duodenum in the human body.

0.01 gram of collagen/allopurinol biocomposite was added in 200 mL of buffer solution. The solution was stirred for 12 hours at 37^{o}C and 400 rpm. After every 1 hour of stirring, 5 mL of the mixture was aspirated and filtered through filter paper, and 5 mL of fresh buffer solution was added to the testing solution. Next, the optical absorbance of the filtered solution was measured at wavelengths from 200 nm to 400 nm on a UV–Vis device. The amount of released allopurinol was calculated according to the standard curve equation of allopurinol in the buffer solutions using the excel software (Chinh et al, 2020; Manh et al, 2021). The allopurinol release percentage can be determined by the following equation:

$$\%Allopurinol = \frac{m_t}{m_0}.100 \ (1)$$

In which, m_t and m_0 represents the amount of allopurinol loaded and amount of drug released at a time t, respectively.

4. Results and discussion

4.1. The yield of the collagen/allopurinol biocomposites processing

The mass of collagen/allopurinol biocomposites was listed in Table 2. Based on the data in Table 2 and Table 1, the yield of the process of collagen/allopurinol preparation was calculated. When increasing allopurinol content in the biocomposite, the yield of the processing increased. However, the yield of the processing was quite low, indicating that the amount of allopurinol and collagen was removed quite a lot during the processing.

Table 2: The mass of collagen/allopurinol composites and the yield of the respective preparation process

Sample	CA5	CA10	CA15
Mass (g)	0.059	0.087	0.178
Yield (%)	5.51	7.77	15.21

4.2. Morphology of collagen/allopurinol biocomposites

Figure 2: SEM image of allopurinol

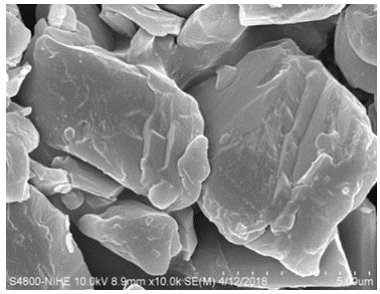
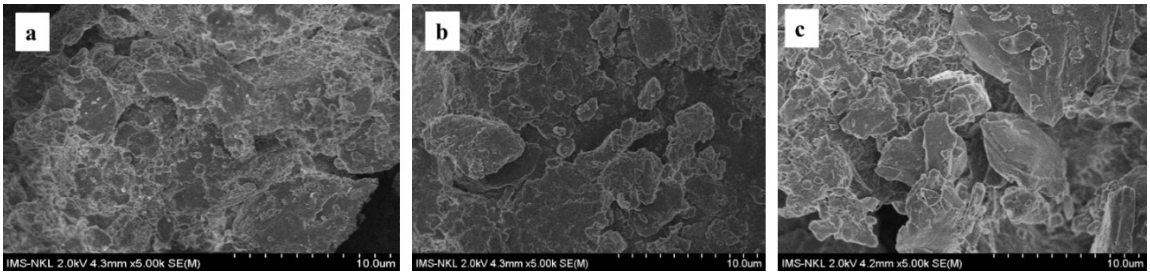


Figure 2 is the FESEM image of allopurinol at 10000X magnification. Allopurinol was of cubic shape with the diameter in the range of 2-5 µm.

The morphology of collagen/allopurinol biocomposites was also of cubic shape with the diameter in the range of 1 – 10 µm as assigned in Figure 3. On the surface of the blocks, it was appeared many nanometer-sized particles. This showed that a part of the collagen/allopurinol biocomposite material was formed in nano form.

Figure 3: FESEM images of collagen/allopurinol composites: CA5 (a), CA10 (b) and CA15 (c)



4.3. Infrared spectra of collagen/allopurinol composites

IR spectra of allopurinol and collagen/allopurinol biocomposites were presented in Figure 4. The wavenumbers characterized for some functional groups in these IR spectra were listed in Table 3. It can be seen the main functional groups of allopurinol were found at 3165 cm^{-1} (stretching vibration of amine group), 2852 cm^{-1} (stretching vibration of =C-H linkage), 1696 cm^{-1} (stretching vibration of C=O group), 1580 cm^{-1} (bending vibration of N-H group) and 1226 cm^{-1} (stretching vibration of C=N group). Fish collagen contains amide groups (amide A, B, I, II, II) as reported in the previous studies (Chinh et al, 2020, Simon et al, 2014), so we did not report the

IR spectrum of collagen here. As loaded by collagen, the positions of the peaks characterized for the amine and carbonyl groups of allopurinol were slightly shifted as seen from Table 3.

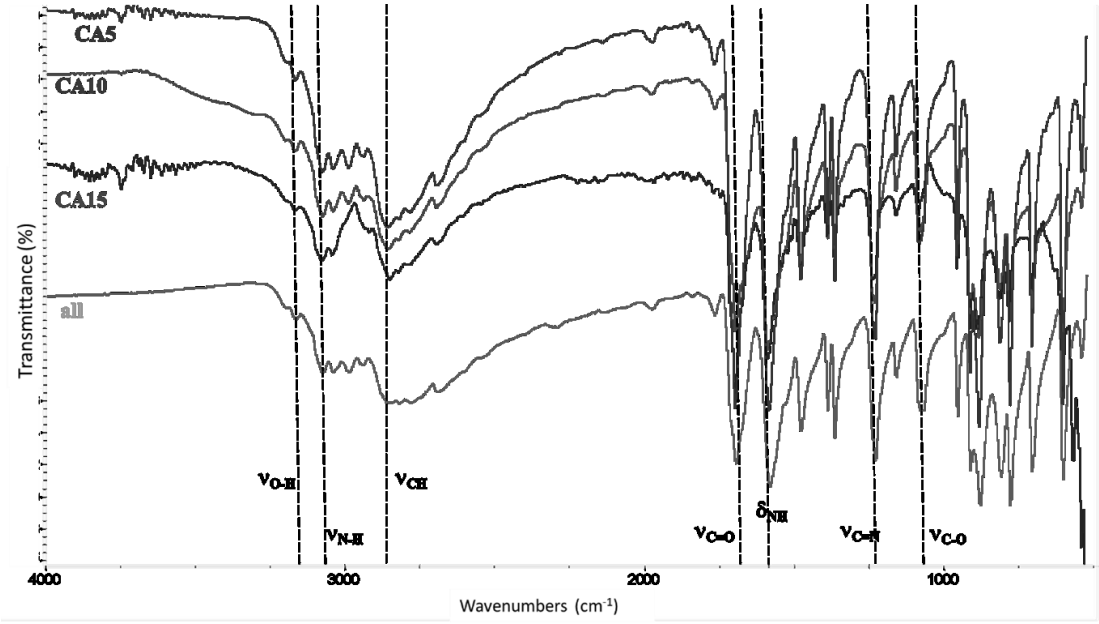
Table 3: Wavenembers (cm^{-1}) correspond to main functional groups of allopurinol and collagen/allopurinol biocomposites

Sample	$\nu_{N-H, OH}$	ν_{C-H}	$\nu_{C=O, amide I}$	$\delta_{N-H, amide II}$	$\nu_{C=N, amide III}$	ν_{C-O}
Collagen (Chinh et al, 2020, Simon et al, 2014	3294, 3076	2934	1630	1546	1238	-
Allopurinol	3161, 3070	2858	1692	1582	1228	1084
CA5	3165, 3076	2852	1694	1580	1226	1082
CA10	3164, 3078	2851	1697	1589	1227	1086
CA15	3267, 3076	2854	1695	1586	1229	1082

4.4. The drug content of allopurinol released from collagen/allopurinol biocomposites

Figure 4 is the graph of the drug content of allopurinol released from collagen/allopurinol biocomposites (CA5, CA10, CA15) and free allopurinol in pH = 2 buffer solutions.

Figure 4: IR spectrum of composites allopurinol (All), CA5, CA10 and CA15



It can be seen that, allopurinol amount released from free allopurinol for 6 hours of testing are 40.12 %. Thereafter, the drug concentration in the solution decreased slightly due to dilution. The release of allopurinol from the biocomposites occured in two stages: (1) the rapid release process took place for 1 first hour of testing, and (2) then, the slow one indicated the controlled release of drugs at the end of period. After 12 hours of testing, the drug content of allopurinol released from collagen/allopurinol biocomposites (CA) was higher than the amount of allopurinol released from the free allopurinol (all). In addition, the ratio of allopurinol and collagen significantly affected the release of allopurinol from collagen/allopurinol composites in the pH 2 buffer solution. The content of allopurinol in the biocomposite is inversely proportional to the amount of drug released.

Figure 5: *In vitro* drug release of allopurinol

from free allopurinol (all) and collagen/allopurinol biocomposites (CA) in pH = 2 buffer solutions

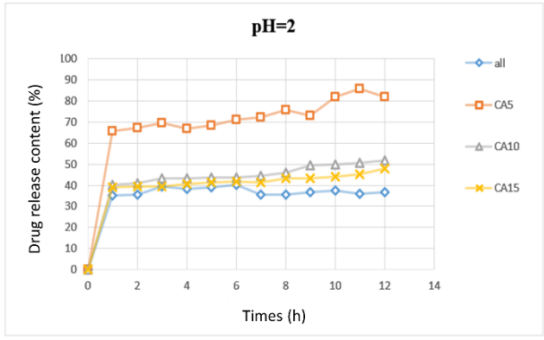
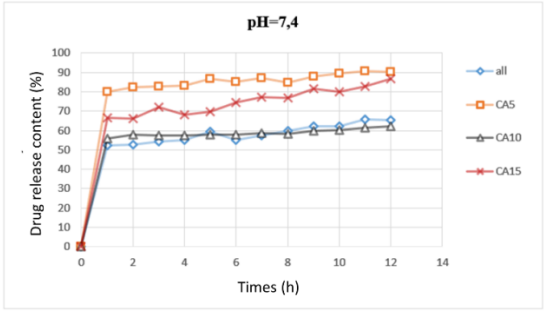


Figure 6: *In vitro* drug release of allopurinol from free allopurinol (all) and c collagen/allopurinol biocomposites (CA) in pH = 7.4 buffer solutions.



In the pH 7.4 buffer solution, the drug content of allopurinol released from collagen/allopurinol biocomposites was significantly higher than the amount of allopurinol released from free allopurinol. After 12 hours of testing, the drug content of allopurinol released from all composites in pH 7.4 buffer solution was remarkably higher than that in pH 2 buffer solution. For example, the allopurinol amount released for 12 testing hours from CA5, CA10, CA15 in pH 7.4 buffer solutions were 90.25%, 62.07%, 82.78%, respectively.

5. Discussion

From above results, it can be recorgnized that the collagen/allopurinol biocomposites were prepared successfully. The yield of the processing was increased as increasing allopurinol content due to allopurinol has a crystal structure (Chinh et al, 2020, Manh et al, 2021) which could help to form particles easier.

The collagen/allopurinol biocomposites are formed in micro-meter size particles combined with nano-meter size ones. The reduction in the size of allopurinol as loaded by collagen contributed to the improvement of the solubility of drug in aqueous solution.

The shift in the wavenumbers of main functional groups in the IR spectra of collagen/allopurinol biocomposites as compared to that of allopurinol suggested that allopurinol could interact to collagen through the dipole–dipole interactions and hydrogen bonds between the amine and carbonyl groups in allopurinol and the amide and carboxyl groups in collagen (Chinh et al, 2020). The change of allopurinol content in the biocomposites affect unsignificantly to the characteristic bands on the IR spectra of the biocomposites. A small difference in IR spectrum of CA10 was observed at above wavenumers range as compared to that of CA5 and CA15. This may be due to in this sample collagen interacted with allopurinol stronger than in others, the wavenumbers of functional groups in CA10 shifted more than that in others.

The results of drug release study showed that using collagen extracted from fish scales for loading allopurinol drug is very suitable to improve the solubility of allopurinol in buffer solutions. Thanks to the interaction between collagen and allopurinol and the loading by collagen leading to the reduction in crystal degree of allopurinol, therefore, the solubility of allopurinol loaded by collagen was improved significantly, higher from 1.5 to 2 times as compared to that of free allopurinol. This may be due to the fact that the interaction between collagen and allopurinol in the biocomposites prepared at the smaller content of drug was stronger than that of the biocomposites with higher content of

allopurinol. Therefore, at high drug contents, the role of collagen in controlling drug release with allopurinol was reduced (Cherim et al, 2018; Ning et al, 2021). Allopurinol from collagen/allopurinol biocomposites is preferable for release in the neutral solution than acid solution because allopurinol is readily soluble in alkaline solutions (Trang et al, 2020). Moreover, the most suitable content of collagen for best released drug is 95 wt.%. Therefore, 5 wt.% of allopurinol is the most suitable content for the fabrication of collagen/allopurinol composites with better solubility.

The enhancement in solubility of allopurinol is a great result, suggesting that the collagen/allopurinol biocomposites are potential for application in biomedicine. However, it is still necessary to evaluate the toxicity in animals and efficacy of the biocomposites in treatment on reduction of uric acid in blood in mice.

6. Conclusions

In this work, the allopurinol/allopurinol biocomposite powder (CA) is prepared by solution method combines centrifugation and lyophilization. The morphology of prepared biocomposites was of cubic shape with diameter in the range of 1 – 10 μm and was many nanometer-sized particles on the surface of the blocks. The release of allopurinol from the biocomposites in pH 2 and pH 7.4 buffer solutions occured in two stages: (1) the rapid release process took place for 1 first hour of testing, and (2) then, the slow and controlled release in following hours. Collagen improved the release of allopurinol in the two tested pH solutions. The allopurinol content suitable for preparation of the biocomposites is 5 wt%. The collagen/allopurinol biocomposites is a potential material for applications in biomedicine.

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CHẾ TẠO VÀ XÁC ĐỊNH CÁC ĐẶC TRƯNG CỦA VẬT LIỆU TỔ HỢP COLLAGEN TỪ VẢY CÁ MANG DƯỢC CHẤT ALLOPURINOL

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Tóm tắt:

Collagen từ vảy cá được chú trọng nghiên cứu nhờ những đặc tính tuyệt vời như độ hấp thụ cao, an toàn, ít béo, tương hợp sinh học tốt. Là collagen loại I với cấu trúc dạng sợi được hình thành bởi các axit amin, đặc biệt là glycine, proline và hydroxyproine. Vảy cá, một sản phẩm phế thải từ cá, chứa collagen loại I. Việc chiết xuất collagen từ vảy cá góp phần giảm thiểu ô nhiễm môi trường. Trong nghiên cứu này, collagen được chiết xuất từ vảy cá nước ngọt đã được sử dụng như một chất mang trong hệ thống polyme mang thuốc. Allopurinol có tác dụng giảm nồng độ axit uric trong máu và được sử dụng làm thuốc trong hệ thống mang thuốc bởi collagen. Các nhóm chức trong tổ hợp collagen/allopurinol cũng như hình thái học của nó được đánh giá bằng quang phổ hồng ngoại (IR) và kính hiển vi điện tử quét (SEM). Hàm lượng allopurinol giải phóng từ tổ hợp collagen/allopurinol được xác định dựa trên độ hấp thụ quang học trên phổ tử ngoại – khả kiến (UV-Vis) của thuốc trong dịch mô phỏng cơ thể. Kết quả phân tích phổ IR của các mẫu nghiên cứu cho thấy tổ hợp collagen/allopurinol chứa các liên kết N-H, C-H, OH trong collagen và các liên kết C=O, C=N, N-H trong allopurinol. Hình ảnh SEM cho thấy hình thái của tổ hợp collagen/allopurinol khác với hình thái của allopurinol. Allopurinol có thể giải phóng từ tổ hợp collagen/allopurinol trong dịch mô phỏng cơ thể (pH 2 và pH 7,4) theo hai giai đoạn: giai đoạn giải phóng nhanh trong 1 giờ đầu tiên và giai đoạn giải phóng chậm trong những giờ tiếp theo. Tại cùng một thời điểm thử nghiệm, hàm lượng allopurinol được giải phóng từ tổ hợp collagen/allopurinol cao hơn so với allopurinol tin khiết (allopurinol không được mang bởi collagen).

Từ khóa: *Allopurinol; Collagen từ vảy cá; Đặc trưng; Giải phóng dược chất.*