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RESEARCH FOR PRODUCTION AND CHARACTERISTICS OF CARRAGEENAN/COLLAGEN COMBINED (FROM FISH SCALES) WITH PHARMACEUTICAL ALLOPURINOL

SUMMARY OF THESIS DOCTOR OF CHEMISTRY

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LIST OF THE PUBLICATIONS RELATED TO THE DISSERTATION

1. **Quoc Manh Vu**, Thuy Chinh Nguyen, Duong My Ngoc Dam, Quoc Trung Vu, Trong Lu Le, Tran Dung Hoang, Thi Kim Ngan Tran, Tuan Anh Nguyen, Phi Hung Nguyen, and Hoang Thai, *A Novel Method for Preparation of Carrageenan/Fish Scale Collagen/Allopurinol Biocomposite Film*, International Journal of Polyme Science, **2021**, Article ID 9960233, 10 pages (Q2-SCIE).
2. Nguyen Thuy Chinh, **Vu Quoc Manh**, Thai Hoang, Kavitha Ramadass, C.I. Sathish, Vu Quoc Trung, Tran Thi Kim Ngan, Ajayan Vinu, *Optimizing the component ratio to develop the biocomposites with carrageenan/collagen/allopurinol for the controlled drug release*, Journal of Drug Delivery Science and Technology, **2022,** *68*, 102697, (Q1-SCIE)
3. Chinh Thuy Nguyen, **Manh Quoc Vu**, Thuy Thi Phan, Trung Quoc Vu, Quan An Vo, Giang Long Bach, Hoang Thai, *Novel pH-sensitive hydrogel beads based on carrageenan and fish scale collagen for allopurinol drug delivery*, Journal of Polymes and the Environment, **2020,** *28*, 1795–1810 (Q1-SCIE).
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INTRODUCTION

1. The Importance of the Research

Polymer hydrogel systems, particularly those made of carrageenan (CAR) and collagen, offer numerous benefits for drug delivery such as biocompatibility, controlled drug release, and easy binding to medicinal plants. These properties are crucial for developing new drug forms for treating various diseases. Carrageenan, particularly in composite nano-systems with a nano-size, possesses unique properties that are widely used in the pharmaceutical industry for producing therapeutic drugs like anticoagulants and anti-cancer drugs. Collagen is also utilized in drug manufacturing, hemostasis, wound healing, tissue, and cell culture. However, obtaining high-quality collagen from animal skin poses a risk of contamination from diseases like mad cow and foot-and-mouth disease in pigs, making the obtained collagen's quality uncertain. Allopurinol (ALP) is a potent substance used in treating gout and hyperuricemia by significantly reducing uric acid levels in the blood.

Thus, the objective of this PhD thesis is to investigate the "Research for production and characteristics of carrageenan/collagen combined (from fish scales) with pharmaceutical allopurinol". The thesis aims to extract collagen from freshwater fish scales in Vietnam and use this collagen in combination with carrageenan to develop materials that can carry the ALP drug to help treat gout.

2. Thesis Objectives

+ Setting up a procedure for extraction of collagen from freshwater fish scales including carp (*Cyprinus carpio*), rohu (*Labeo rohita*), grass carp (*Ctenopharyngodon idella*), and tilapia (*Oreochromis niloticus*) fish scales.

+ Preparing carrageenan/collagen (from fish scales) composite for loading allopurinol using ionic gelation method.

+ Evaluating the potential of carrageenan/collagen/allopurinol composites in reducing uric acid concentration in blood.

3. The main research contents of the thesis

- Research on collagen extraction conditions from Vietnamese freshwater fish scales (carp - *Cyprinus carpio*, rohu - *Labeo rohita*, grass carp - *Ctenopharyngodon idella*, tilapia - *Oreochromis niloticus*).

- Research on the manufacturing conditions and composition ratio between carrageenan and collagen polymers (from fish scales) bearing ALP to obtain a polymer combination carrying the drug in film and granular form by solution and gel methods - ionize.

- Research on the release of ALP drug from the Car/C/ALP polymer combination.

- In vivo experimental research on mice using the natural polymer combination Car/C (from fish scales) containing ALP to reduce uric acid in the blood.

CHAPTER 1. OVERVIEW

1.1. Collagen overview

The amino acid content in fish-derived collagen is lower than in animal-derived collagen. Based on the structure and origin of collagen, there are the following types: Fibrous collagen, membrane collagen, seed chains, and short chains.

Collagen is a type of protein so it has the following basic properties: water absorption, acid-base hydrolysis, high-temperature denaturation, biocompatibility...

With many good properties, collagen has been researched and extracted from many sources such as: animal skin, fish skin, bones and fish scales.

Collagen is used in many different industries such as: In food production, medicine, pharmaceuticals, and cosmetics.

1.2. Carrageenan overview

Carrageenan is a polysaccharide of galactose - galactan extracted from *Chondrus crispus*, a red seaweed. It contains D-galactose and 3,6-anhydro D-galactose moieties that are linked by alternating β-D (1-4) and α-D (1-3) galactoside bonds. There are three main types of carrageenan, which are κ-carrageenan, ι-carrageenan, and λ-carrageenan, based on their structure. Researchers and producers extract carrageenan from seaweed using various methods, such as chemical, enzyme, and microwave techniques.

1.3. Overview of natural polymers for drug delivery

CAR is preferred in drug delivery systems due to its biological and chemical properties. CAR has a versatile chemical structure that makes it useful in drug preparation and manufacturing, and it is used in various forms such as tablets, suppositories, films, and fast-dissolving inserts. The study also explored the potential of ι-CAR and λ-CAR in carrying and regulating the release of drugs in a controlled-release tablet formulation. The CAR matrix was infused with theophylline, sodium salicylate, and chlorpheniramine maleate at a concentration of 500 mg.

In recent years, scientific research has focused on studying collagen and its potential in drug delivery. Different drugs such as doxycycline, triphala, tobramycin or ciprofloxacin, gentamicin have been investigated in conjunction with collagen. Researchers have looked at the release of various drugs from composite materials that involve collagen, such as poly(N-isopropylacrylamide) nanoparticles, hydrogels made of collagen/poly(vinyl alcohol), and nanoparticles with an interwoven polymeric network structure. Furthermore, collagen-based nanogel systems have been developed for wound healing, and hydrogel systems consisting of carrageenan and collagen have been used to research and treat bone tissue growth.

In the field of pharmaceuticals, there has been a focus on developing extended-release formulations for allopurinol, a xanthine oxidase inhibitor used to treat gout and hyperuricemia. Conjugate systems such as polyvinylpyrrolidone (PVPk30), polyethylene glycol 6000 (PEG 6000), and chitosan superparamagnetic nanoparticles have been developed to control the delivery and release of allopurinol. However, there have been very few studies using natural polymers such as chitosan, carrageenan, and collagen as carriers of the drug. The combination of carrageenan and collagen, known as CAR/C, has not been well-researched as an allopurinol carrier. A hydrogel made of CAR/C can provide physical interactions with allopurinol, improving the solubility of the drug and increasing its bioavailability when administered. As such, the topic has both scientific and practical significance.

1.4. Overview of allopurinol and allopurinol carrier polymers

ALP is a xanthine oxidase inhibitor, commonly used in the treatment of gout and hyperuricemia. The biological half-life is 1–3h, which clearly indicates the need to develop extended-release formulations.

Pharmacodynamics: Reduces uric acid levels in the blood. Uric acid forms crystals in body tissues that cause gout. Increased uric acid also causes kidney

stones. If ALPopurinol is used directly, it can cause skin side effects (rash) or cause fever, tremors and vasculitis leading to kidney and liver damage.

ALP is excreted in the urine as metabolites, with a half-life of about 1h (oxypurinol 18 - 20h). Approximately 20% of ALP taken orally is eliminated in the faeces.

To date, various conjugate systems have been developed for the controlled delivery and release of allopurinol. Hydrophilic drug carriers such as polyvinylpyrrolidone (PVPk30), polyethylene glycol 6000 (PEG 6000), polyoxyethylene (PEO) tablet formulation, improve the bioavailability of allopurinol.

Chitosan superparamagnetic nanoparticles carrying allopurinol (A-CNPs) in the treatment of hyperuricemia have been studied and fabricated. The grain sizes obtained range from 46.40 to 91.65 nm. *In vivo* studies of A-CNPs showed a 19.07-fold increase in renal absorption of allopurinol compared with purified allopurinol after 2 h of dosing in mice.

From the above studies and presentations, it can be seen that the solution to systematically treat fish scales to limit environmental pollution and collect collagen has not been given adequate attention. Studies on polymers carrying allopurinol are mainly semi-synthetic polymers such as PEG 6000, PVPk30, poly(ethyl-cyanoacrylate), polymethylmetacrylate... There are very few studies using natural polymers (such as chitosan, carrageenan, collagen) as a carrier of the drug allopurinol. Especially, so far, making carrageenan/collagen (CAR/C) combination as an allopurinol carrier has not been focused on research. The combination of 2 polymers CAR/C will be effective in the treatment of gout thanks to the advantages of these polymers. Hydrogel CAR/C makes it easier to control and release allopurinol thanks to physical interactions between allopurinol with carrageenan and collagen, while improving the solubility of allopurinol, increasing the bioavailability of the drug when administered. Therefore, the topic has scientific and practical significance.

CHAPTER 2. EXPERIENCE

2.1. Chemicals and tools

$Ca(OH)_2$, KCl, citric acid, KH_2PO_4, HCl 37%, concentrated H_2SO_4, H_3PO_4, solid NaOH, concentrated CH_3COOH, solid NaCl: a commercial product of China.

Carrageenan, allopurinol, polyethylene oxide (PEO): Product of Sigma-Aldrich (USA).

2.2. Extraction of collagen from fish scales (carp family in the north of Vietnam)

Step 1 – Mixed fish scales including: scales of carp, drifting fish, carp, and tilapia are washed and dried. In the next processing steps, the flakes soaked in the survey solutions were carried out at 4 ^oC.

Step 2 – Soak fish scales in NaOH solution.

Step 3 – Soak fish scales washed in step 2 in mixed solutions of H_2SO_4 and HCl acids at different concentrations

Step 4 – Soak fish scales in 0.5 M CH_3COOH solution for 24h.

The collagen was precipitated with NaCl, filtered and dialyzed for 48h to obtain pure collagen.

2.3. Production of carragennan/collagen/allopurinol combination

Dissolve CAR in 40 mm water at 80 °C for 15 min, cool further with KCl solution and magnetic stir for 15 min (beaker A). Dissolve collagen in 0.5 M CH3COOH solution (beaker B). Dissolve allopurinol in 0.5 M NaOH solution (C cup) Slowly pour beaker (C) into beaker (A) to obtain solution (D). Stir beaker D on a magnetic stirrer for 30 min. Add slowly to the end of solution (B) into solution (D), combined with ultrasonic stirring at a speed of 20,000 rpm. Continue stirring the mixture for 60 minutes. Then, it was ultrasonically stirred at 20,000 rpm (3 times, 10 minutes/time). The mixture is fine if poured into a petri

dish and allowed to evaporate naturally to obtain composite films. If centrifuged, freeze-dried to obtain granules.

2.5. Research Methods

Scanning electron microscope (SEM): measured on FESEM S-4800, Hitachi, Japan.

Dynamic light scattering (DLS): measured on a Zetasizer Ver 620 instrument.

Fourier Transformation Infrared (FTIR) spectra: recorded with a Fourier Nexus 670 instrument (USA).

X-ray diffraction (XRD): recorded on a D8 ADVANCE, Brucker.

Differential scanning calorimetry (DSC): recorded on a DSC131 instrument (Setaram, France).

Thermal gravimetric analysis (TGA): recorded on a DTG 60H instrument.

UV-Vis absorption spectroscopy method: Using UV-Vis spectrophotometer (Cintra 40, GBC, USA).

High-performance liquid chromatography (HPLC) method: performed at the National Institute of Food Safety and Hygiene (test methods H.HD.QT.046 and H.HD.QT.112).

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE)

Two-dimensional nano-liquid chromatography-mass spectrometry (nanoLC-MS/MS).

In-vivo test on mice.

2.6. Research on allopurinol release from CAR/C composites carrying ALP in different pH environments

2.7. In vivo testing using CAR/C/ALP composite beads on animals (normal mice and mice injected peritoneally with potassium oxalate)

CHAPTER 3: RESULTS AND DISCUSSION

3.1. Extract collagen from freshwater fish scales

3.1.1. *Effect of lye solution on collagen mackerel scale treatment*

The results of fish scale treatment by time and concentration of NaOH solution. We found that 0.5 M NaOH solution, soaking time of 8 h is the most appropriate, the scales are clean of dirt and protein.

3.1.2. Effect of acid mixture on scab treatment of collagen collectors

Fish scales after being pre-treated and alkaline treatment are carried out in the next steps with the conditions for extracting collagen as shown in Table 3.3. The volume of collagen obtained when treating fish scales with a mixed solution of 0.5 M H_2SO_4 + 0.2 M HCl and 0.5 M CH_3COOH solution was larger than that of other acidic solutions. Hemodialysis for 48 h obtained pure collagen.

3.1.3. Determination of purity and content of amino acids in collagen obtained from a mixture of fish scales

Figure 3.1 is the EDX spectrum of the collagen sample. With undiluted collagen containing elements C, O, N, S, Na and Cl. After 24 h of dialysis, the content of elements Na and Cl and S decreased. After 48h of dialysis, all NaCl and most of S.

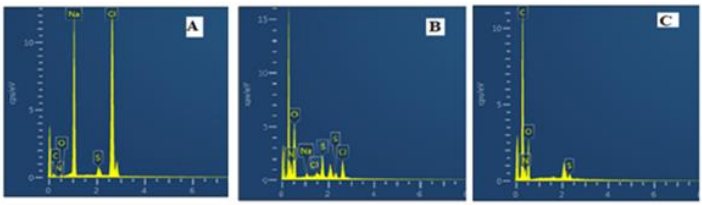


Figure 3.1. EDX spectrum of crude collagen (A); collagen dialysate after 24 h (B) and collagen dialysate after 48 h (C)

Table 3.5 presents the results of research on the amino acid composition of collagen extracted from Vietnamese fish scales containing 18 amino acids.

Table 3.5. The content of amino acids in the pure collagen solution obtained from fish scales

		Collagen from carp scales		Acid-soluble Carp Scale collagen		Carp Scale collagen	
TT	Amine acid	mg/ml	%	Coarse scum/10^3	%	Coarse scum/10^3	%
1	Threonine	1.704	39.79	21	2.05	23	2.25
2	Proline	0.487	11.37	110	10.74	115	11.23
3	Glutamic acid	0.550	12.84	77	7.52	76	7.42
4	Arginine	0.425	9.92	50	4.88	55	5.37
5	Serine	0.357	8.34	38	3.71	35	3.42
6	Alanine	0.218	5.09	117	11.43	119	11.62
7	Glycine	0.140	3.27	306	29.88	336	32.81
8	Aspatic acid	0.099	2.31	49	4.79	48	4.69
9	Cysteine	0.059	1.38	32	3.13	-	-
10	Histidine	0.047	1.10	6	0.59	5	0.49
11	Lysine	0.047	1.00	26	2.54	26	2.54
12	Leucine	0.039	0.91	24	2.34	21	2.05
13	Phenylalanine	0.028	0.65	15	1.46	12	1.17
14	Valine	0.026	0.61	19	1.86	19	1.86
15	Isoleuocine	0.022	0.51	12	1.17	9	0.88
16	Methyoline	0.020	0.47	12	1.17	14	1.37
17	Tryptophan	0.012	0.28	-	-	-	-
18	Tyrosine	0.007	0.16	21	2.05	3	0.20
19	Hydroxyprolin	-	-	89	8.69	77	7.52
20	Hydroxylysin	-	-	-	-	8	0.87
21	Collagen	0,29	6,77				

3.1.4. Characterization, properties and structural morphology of collagen obtained from a mixture of fish scales

Figure 3.2 is the FT-IR spectrum of purified collagen obtained from a mixture of fish scales. On the FTIR spectrum, there is a full spectrum pattern characteristic for the valence oscillations of the N-H group at wave number 3305.19 cm^{-1}, the deformation vibrations of the amide order 1, 2,

and 3, respectively: 1650.3 cm^{-1}; 1547.91 cm^{-1} and 1236.59 cm^{-1}.

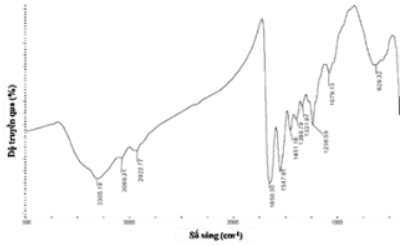


Figure 3.2. FTIR spectrum of collagen after dialysis 48 h

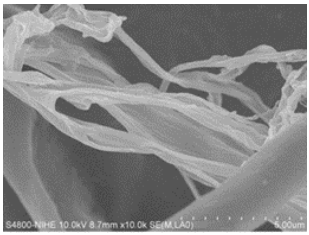


Figure 3.4. SEM image of fine collagen after 48 h of dialysis

Figure 3.2. FTIR spectrum of collagen after dialysis for 48 h Figure 3.4. SEM image of fine collagen after 48 h of dialysis.

Thermal characterization of collagen with an exothermic peak at 116 ^oC (ΔH = 3.7 J/g). Collagen loses mass in 3 steps: Step 1 - corresponds to the loss of water in collagen; Step 2 - corresponds to the breakdown of proteins in collagen; Step 3 - corresponds to the decomposition of aromatic rings in collagen, the combustion of C to CO_2.

The denaturation temperature of the obtained collagen was at 32.2 °C. The results of electrophoresis (SDS-PAGE) showed that the collagen obtained was type I with α1 and α2 chains of 139 and 129 kDa respectively and a β chain.

The SDS-Page electrophoresis method identified 5 proteins belonging to the group of skin-scale proteins (collagen) of the fish family with a large pairing coefficient, of which the hypothetical protein cypCar_00045321 [Cyprinus carpio] had the highest pairing score (318).

3.2. Carragennan/collagen/allopurinol complex membrane

3.2.1. Allopurinol - carrying efficiency of CAR/C composite membranes

ALP in 1 M NaOH solution: λ_{max} = 277.98 nm, standard curve equation: y = 8621.6x + 0.2358, R^2 = 0.9962.

The results determined the ALP-carrying efficiency of the CAR/C composite film samples with 5% of ALP mass (by total polymer mass) fabricated at the KCl gel-forming agent contents (0.5%; 1%; 2% and 5 %) differ by 28.35%, respectively; 74.31%; 46.3% and 47.42 %.

3.2.2. FTIR spectrum of CAR/C/ALP composite films

Figure 3.10 is the FTIR spectrum of ALP, CAR and collagen. Spectral ridges characteristic for amide group oscillations of collagen appear at 3294 cm^{-1} (amide A), 3076 cm^{-1} (amide B), 1630 cm^{-1} (amide I), 1546 cm^{-1} (amide II).) and 1236 cm^{-1} (amide III). CAR's spectral fringes: within 3000–3600 cm^{-1} and at 1636 cm^{-1}. Cloud spectrum at 1223 cm^{-1} of the S=O bond. of ALP: spectral fringes at 3165 and 3074 cm^{-1} of NH, spectral fringes at 3030 cm^{-1} of CH, spectral fringes at 1765 and 1694 cm^{-1} of the C=O bond.

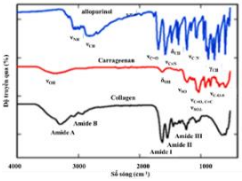


Figure 3.10. FTIR spectrum of ALP, CAR and collagen

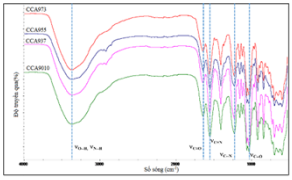


Figure 3.11. FTIR spectra of the CCA . composite film

When the CAR/C combination carries ALP, the positions of the spectral fringes of the feature groups in each part are changed. For CAR, the spectral pattern of the O–H group is 3371 cm^{-1}, the magnetic C=O group is 1642 cm^{-1}, and the C–O group is 1065 cm-1. With collagen: O–H group fluctuation is 3371 cm^{-1}, primary amide is 1642 cm^{-1}. With allopurinol: the N–H group is 3371 cm^{-1}, the C=N group is 1564 cm^{-1}, the C–N group is 1229 cm^{-1}. This suggests that carrageenan, collagen and allopurinol can interact with each other through hydrogen bonding, this hypothesis is depicted in Figure 3.13

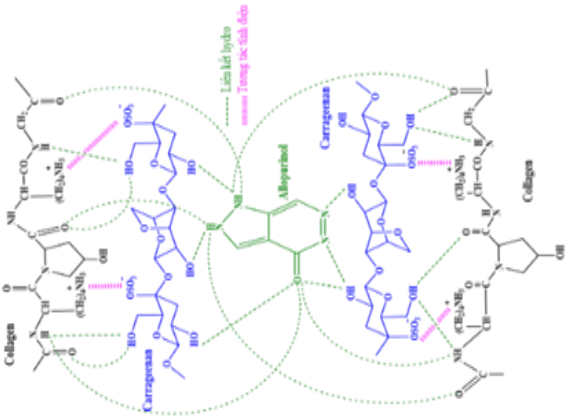


Figure 3.13. Some interaction hypotheses in the CAR/C/ALP composite membrane

3.2.3. X-ray diffraction (XRD)

X-ray diffraction (XRD) pattern of ALP. It can be seen that ALP has a crystalline structure with the appearance of a diffraction angle at 10.66º; 12.16º; 14.96º; 17.46º; 20.26º; 21.26º. When carried by the combination CARr/C (95/5) and 1% KCl used, the peaks and diffraction angles of ALP have a significant shift.

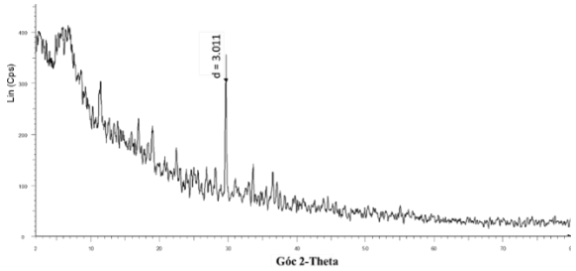


Figure 3.15. XRD pattern of CAR/C/ALP composite membrane at carrageenan/collagen ratio 95/5, 1% KCl gelling agent

3.2.4. SEM image of CAR/C/ALP composite film

It can be seen that ALP is dispersed in the CAR/C/ALP composite film with a size of about 50 nm – 100 nm. With CAR/C ratios of 99/1 and 90/10, the composite membrane has an uneven structure. The

composite membrane sample with the CAR/C ratios of 95/5 has the most uniform structure. Samples containing different amounts of collagen had a particle size of ALP about 50 – 350 nm.

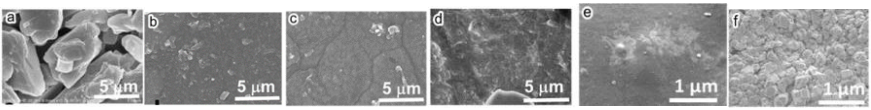


Figure 3.16. SEM images of CAR/C/ALP composite membranes, 1% KCl gelling agent: allopurinol (a), CCA991-5 (b), CCA955-5 (c), CCA9010-5 (d), CCA955-3 (e) and CCA955-10(f)

3.2.6. Research on ALP release from CAR/C/ALP (CCA) composite membranes in pH 2 and pH 7.4 buffer solutions

ALP in pH 2 buffer solution has: λmax = 274.98 nm, the standard curve equation is y = 9756x – 0.326 (R^2 = 0.991).

ALP in pH 7.4 buffer has λmax = 276.69 nm, the standard curve is y = 9945.3x – 0.4437 (R^2 = 0.9901).

Most ALP are released within the first hour. In the control sample, after 1 hour of testing, there was 9.14% ALP release in pH 2 buffer solution and increased to 15.87% after 32 h. In pH 7.4 buffer solution, 8.49 % ALP was released after 1 hour and after 32 h it was 12.79%. The content of ALP released from the CCA composite membranes in contrast, released in the pH 7.4 buffer solution was greater than that in the pH 2 buffer solution. Thus, the CAR/C/ALP combination helps control the release of ALP in acidic environment.

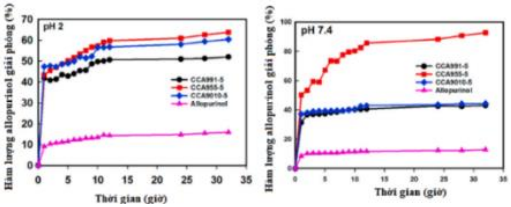


Figure 3.20. Graph of ALP release from CCA composite membranes in pH 2 and pH 7.4 buffers

Although the CCA composite membrane was fabricated at the same

ALP content, ALP was released faster from the CCA955-5 sample than from the CCA9010-5 and CCA991-5 samples in both pH 7.4 buffers and pH 2. The CAR/C ratio of 95/5 is suitable to fabricate CCA composite membrane which helps to control ALP release well.

As Figure 3.22 shows, most of the ALP crystals were dissolved in the first hour with the amount of ALP liberated 8.488 % and 9.135 %, respectively, in pH 7.4 and pH 2.0 buffer solutions, respectively. The liberated ALP content was increased to 2 to 3 %, depending on the pH of the solution in the following hours.

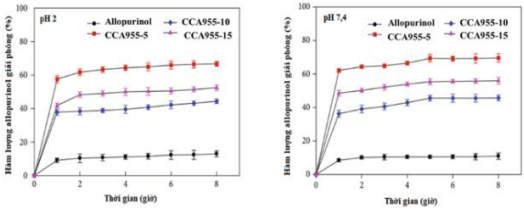


Figure 3.22. Graph of the release of pure ALP and from CCA membranes with varying ALP content in pH 2 and pH 7.4 buffers

3.3. Carragennan/collagen/allopurinol (ACC) granules

3.3.1. FTIR spectrum of CAR/C/ALP (ACC) particles

Figure 3.23 is the FTIR spectrum of CAR, ALP, C and ACC composite particles fabricated at different CAR/C ratios. On the FTIR spectrum of CAR, a full spectrum of characteristic fringes appears: valence oscillations of –OH groups at 3450 cm^{-1}, C=O groups at 1600 cm^{-1}, C–O groups at 1200 cm^{-1} and groups S=O (in the sulfate ester group) at 1100 cm^{-1}. In the FTIR spectrum of ACC, there is a characteristic absorption pattern for the valence vibration of the –NH group at 3305.19 cm^{-1}. In addition, the spectral pattern is typical for deformation fluctuations of primary amide at 1650.3 cm^{-1}, secondary amide at 1547.91 cm^{-1}, tertiary amide at 1236.59 cm^{-1}.

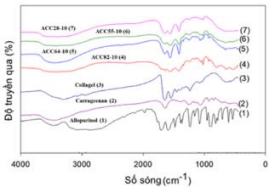


Figure 3.23. FTIR spectra of CAR, C, ALP and ACC composite particles at different CAR/C ratios

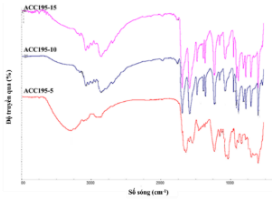


Figure 3.24. IR spectra of ACC19-5, ACC19-10 and ACC19-15 composite particles

3.3.2. The particle size distribution of the ACC composite particles

Table 3.12 and Table 3.13 can be seen that the ACC composite particles with different CAR/C ratios have nano size, of which the sample ACC28 has the smallest average size of 200.80 nm and the ACC19-10 particle has the smallest size. 170.92 nm average.

Table 3.12. Average particle size of ACC28-10, ACC55-10 and ACC64-10 nanoparticles

Sample	particle size (nm)	Average particle size (nm)
ACC28-10	150 – 300	200.80 ± 7.19
ACC55-10	225 – 400	309.10 ± 14.76
ACC64-10	400 – 800	547.00 ± 31.74

Table 3.13. Average particle size of ACC19-5, ACC19-10 and ACC19-15 nanoparticles

Sample	Average particle size (nm)
ACC19-5	180.55 ± 17.30
ACC19-10	170.92 ± 21.80
ACC19-15	340.00 ± 28.54

3.3.3. SEM image of ACC nanocomposite particles

SEM images of samples ACC28, ACC55, ACC64 are presented in Figure 3.26. It can be seen that the particles have unequal sizes from 2 to 50 µm.

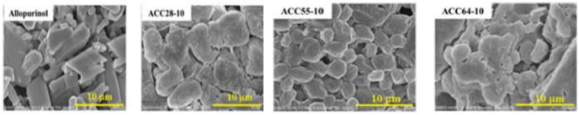


Figure 3.26. SEM images of ALP and ACC nanoparticles with different CAR/C ration

Figure 3.27 is the SEM image of the ACC composite particle samples fabricated at ALP concentrations, the particle size is quite uniform in the range of 50 - 250 nm, smaller than allopurinol.

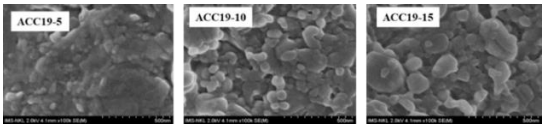


Figure 3.27. SEM image of ACC composite particles with different ALP content

3.3.5. Allopurinol carrying efficiency of ACC nanocomposites

Table 3.15 presents the results of determining the allopurinol carrying efficiency of the ACC nanocomposite samples fabricated at different CAR/C ratios and the fabricated ACC nanoparticles varied with allopurinol content.

Table 3.15. ALP-carrying efficiency of ACC and CCA composite particles

No	Sample	Allopurinol carrying efficiency (%)
1	ACC28-10	70.92
2	ACC55-10	66.79
3	ACC64-10	68.10
4	ACC19-5	70.30
5	ACC19-10	80.79
6	ACC19-15	60.55

3.3.6. Research on ALP release from CAR/C/ALP nanocomposite particles in buffer solution pH 2 and pH 7.4

It can be seen that ALP released from ACC nanocomposites occurs in 2 phases: rapid release in the first 11 h and then a slow, controlled release phase. The content of ALP released from the ACC28-10 nanocomposite particles after 32 h of testing was higher than that of the pure composite and ALP particles.

The content of ALP released from the ACCA19-15 nanocomposite particles after 32 h of testing was larger than that of the pure ACC (variable ALP content) and pure ALP nanoparticles.

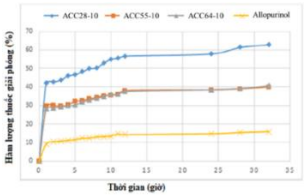
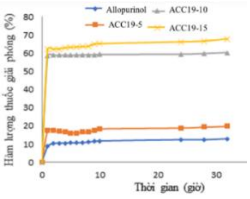


Figure 3.29. Graph of ALP release from pure ALP and ACC nanoparticles in pH 2 buffer



Hình 3.30. Graph of ALP release from pure ALP sample and CCA nanoparticles in pH 2 buffer

It can be seen that the release of allopurinol from the ACC nanocomposites occurs in 2 phases: rapid release during the first 11 h and then a controlled slow release phase. The content of ALP released from the ACCA28-10 nanocomposite particles after 32 h of testing was larger than that of the pure ACC (variable ALP content) and pure ALP nanoparticles.

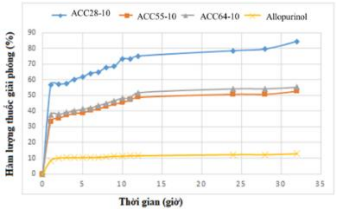


Figure 3.31. Graph of ALP release from ACC nanocomposites (different CAR/C ratio) in pH 7.4 buffer solution

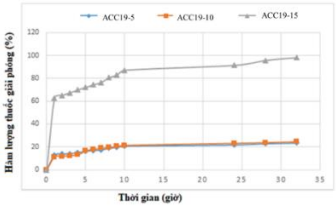


Figure 3.32. Graph of ALP release from ACC nanoparticles (different ALP content) in pH 7.4 buffer solution

Figure 3.32 shows ALP content released from ACC nanoparticles with ALP content (5 %, 10 % and 15%) and pure ALP sample in pH 2 buffer solution. ALP content released from nest particles ACCA19-15 nanocomposite after 32 h of testing is larger than pure ACC (variable ALP content) and pure ALP nanocomposite particles.

3.4. In vivo testing using CAR/C/ALP nanocomposite particles on animals

3.4.1. Determination of acute toxicity of CAR/C/ALP nanocomposite particles on tested mice

The results of the acute toxicity test showed that the ACC19-10 nanocomposite phase probe dose showed that the ACC19-10

nanocomposite at the test doses did not cause acute toxicity in mice.

Image results of normal liver, kidney and spleen parenchyma showed that when taking ACC19-10 nanoparticles, there was no damage to the morphology and structure of liver, kidney and spleen.

3.4.2. Determination of semi-permanent toxicity of CAR/C/ALP nanocomposite particles on tested mice

The increased weight of mice showed that oral administration of ACC19-10 nanocomposite particles did not affect the weight development of mice.

Hematological indicators showed that ACC19-10 nanoparticles did not affect hematopoietic function in experimental animals (Table 3.21).

Table 3.21. Haematological indexes of groups of mice before and after drinking ACC19-10 nanocomposite beads after 14 and 28 days

Group	Before (1)	After 14 day (2)	After 28 days(3)	P
Red blood cell count (T/L)				
Control group	7.18±0.76	7.05±1.18	7.04±0.40	p>0.05
Value group 1	7.12±0.87	7.05±0.63	7.10±0.69	p>0.05
Value group 2	6.93±1.39	6.84±1.27	7.02±0.78	p>0.05
P	p>0.05	p>0.05	p>0.05	
Hg concentration (g/dl)				
Control group	132.50±10.5	130.40±12.57	132.50±9.64	p>0.05
Value group 1	129.50±8.11	130.30±11.14	130.50±10.22	p>0.05
Value group 2	129.60±6.40	129.40±6.74	129.80±4.64	p>0.05
P	p>0.05	p>0.05	p>0.05	
WBC count (G/L)				
Control group	7.87±1.89	7.74±1.16	7.88±1.95	p>0.05
Value group 1	7.87±2.08	7.88±2.29	7.83±1.28	p>0.05
Value group 2	7.71±1.92	7.76±1.47	7.83±2.09	p>0.05
P	p>0.05	p>0.05	p>0.05	
Platelet count (TC/ml)				
Control group	764.40±244.89	785.80±247.02	788.60±261.29	p>0.05
Value group 1	768.20±209.37	790.80±233.30	766.20±201.79	p>0.05
Value group 2	779.70±284.57	762.60±262.10	767.10±264.71	p>0.05
P	p>0.05	p>0.05	p>0.05	

Table 3.22 shows the biochemical indexes of mice before and after

drinking ACC19-10 nanoparticles for 14 and 28 days. The results showed that ACC19-10 nanoparticles did not affect liver and kidney function in experimental animals.

Table 3.22. Biochemical indicators of mice before and after drinking ACC19-10 nanocomposite beads after 14 and 28 days

Group	Before (1)	After 14 days	After 28 days	P
AST Concentration (UI/L)				
Control group	69.90±7.50	70.60±12.47	71.10±11.64	p>0.05
Value group 1	69.50±14.37	69.60±11.25	69.10±12.07	p>0.05
Value group 2	69.70±7.78	69.30±8.31	69.10±10.63	p>0.05
P	p>0.05	p>0.05	p>0.05	
ALT concentration (UI/L)				
Control group	105.00±6.25	104.10±6.94	103.60±3.34	p>0.05
Value group 1	107.50±8.96	107.90±8.09	107.60±7.53	p>0.05
Value group 2	106.90±9.97	108.10±7.23	107.40±10.39	p>0.05
P	p>0.05	p>0.05	p>0.05	
Ure oncentration (mmol/l)				
Control group	5.53±0.62	5.76±0.61	5.83±0.60	p>0.05
Value group 1	5.63±1.30	5.77±0.72	5.85±0.96	p>0.05
Value group 2	5.84±0.57	5.87±0.67	5.69±0.68	p>0.05
P	p>0.05	p>0.05	p>0.05	
Creatinin concentration (µmol/l)				
Control group	63.80±5.37	63.30±6.55	62.90±4.28	p>0.05
Value group 1	62.70±6.82	63.50±5.70	62.50±8.25	p>0.05
Value group 2	62.90±8.52	63.40±6.47	63.60±6.52	p>0.05
Control group	p>0.05	p>0.05	p>0.05	

3.4.3. Investigate the effect of CAR/C/Al nanoparticles on the reduction of urate/uric acid levels in the blood of tested groups of mice

During the test, no mice died and the weight of mice increased normally, only causing hyperuricemia model as well as evaluating the effect of ACC19-10 nanocomposite particles. Thus, the model of hyperuricemia in rats using potassium oxalate is safe.

Table 3.25 shows the blood uric acid levels of rats in the study groups. The white control group had a concentration in the range of 40 ÷ 77 µmol/l.

Table 3.25. Uric acid concentration in the white control group; disease; drink ALP and nano-ALP

Times	Control group		Disease group		Drink ALP		Drink nano-ALP	
	Oscillate (μmol/l)	TB (μmol/l)	Oscillate (μmol/l)	TB (μmol/l)	Oscillate (μmol/l)	TB (μmol/l)	Oscillate (μmol/l)	TB (μmol/l)
fter 1h	49 ÷ 76	66.90 ± 9.82	88 ÷ 133	97.6 ± 15.90	50 ÷ 88	71.60 ± 12.64	50 ÷ 81	66.80 ± 9.92
After 2h	50 ÷ 77	66.80 ± 8.53	86 ÷ 133	105.70 ± 19.35	55 ÷ 88	74.80 ± 10.77	50 ÷ 83	69.90 ± 8.49
After 4h	58 ÷ 77	68.60 ± 5.15	88 ÷ 170	123.60 ± 28.45	50 ÷ 97	76.70 ± 17.02	58 ÷ 75	68.50 ± 4.95
After 8h	40 ÷ 75	67.30 ± 10.45	88 ÷ 160	119.50 ± 27.80	59 ÷ 108	86.60 ± 15.57	59 ÷ 85	73.70 ± 8.27
After 16h	45 ÷ 75	66.90 ± 9.82	88 ÷ 146	112.20 ± 18.18	89 ÷ 165	115.70 ± 26.27	55 ÷ 110	83.40 ± 16.17
After 24h	55 ÷ 76	67.4 ± 6.40	82 ÷ 147	106.9 ± 25.20	81 ÷ 145	112.20 ± 21.36	88 ÷ 132	101.1 ± 13.20
Synthetic	40 ÷ 77	67.32 ± 8.22	82 ÷ 170	110.92 ± 23.70	50 ÷ 165	89.60 ± 24.94	50 ÷ 132	77.23 ± 15.88

The level of reduction in blood uric acid levels in the group of rats drinking ALP after 1 hour to 8 h of drinking ranged from 26.64% to 37.94%, after 16 h and 24 h, there was no reduction. Meanwhile, the group of mice that drank ACC19-10 nanoparticles reduced by 29.90 ± 13.58% (Table 3.27). Thus, the ACC19-10 nanocomposite particles at the same therapeutic concentration had a stronger effect on reducing uric acid levels in the blood of rats than in the group of mice that drank ALP.

Table 3.27. Rate of reduction in blood uric acid in groups of mice

Time	Allopurinol group	nano-allopurinol group
After 1 h	26.64 %	31.56 %
After 2 h	29.23 %	33.87 %
After 4 h	37.94 %	44.58 %
After 8 h	27.53 %	38.33 %
After 16 h	-3.11 %	25.67 %
After 24 h	-4.95 %	5.43 %
Average	18.88 ± 18.21 %	29.90 ± 13.58 %

From these results, researchers need to continue to study to apply the combination CAR/C/ALP in clinical to increase the uric acid lowering effect of ALP in the near future.

CONCLUSION

1. The appropriate conditions have been selected to extract collagen from the mixture of Vietnamese freshwater fish scales. By using suitable acid and alkali agents, collagen was separated with 0.5 M CH_3COOH solution, then solid NaCl was used to precipitate the collagen. Collagen dialysis for 48 h yielded pure collagen type I, containing α1, α2 and β chains, purity > 99%, fibrous with fiber diameter 0.5-1 μm, denaturation temperature of 32.3 ^oC, contains 20 amino acids.

2. The CAR/C/ALP composite membrane has been prepared with the appropriate gelation content of KCl 1% and used this gelation concentration for further studies. The proper CAR/C ratio is 95/5. ALP is dispersed in the CAR/C mixture with the size of 50-100 nm, interacting with carrageenan and collagen by hydrogen bonds, dipole-dipole interactions and electrostatic interactions.

3. CAR/C/ALP nanocomposite particles have been fabricated at different CAR/C ratios and ALP contents by ionizing gel method. Samples were dried by lyophilization. The appropriate ratio of CAR/C for nanocomposites CAR/C/ALP, CAR/C ratio for the smallest particle size is 10/90.

4. The results of the determination of content released from CAR/C/ALP membrane and particle samples in solutions simulating gastric juice (pH 2.0) and intestinal fluid (pH 7.4) showed that during the period, the concentration of ALP released from the nanoparticles was larger than that from the pure ALP sample (3.27-7.25 times higher). The process of dissolving ALP from the compound seeds follows two stages: a rapid release phase and a slow, controlled release phase. After 32h of testing, the concentration of ALP drug released from the composite particle samples prepared under different conditions in the pH 2 buffer solution reached from 19,647 to 67,650 %, in the pH 7.4 buffer solution reached from 23 .33 – 98.082%.

5. The process of ALP release from CAR/C/ALP composite

membranes in pH 2 and pH 7.4 buffer solutions mainly follows the KP model with diffusion coefficient of n = 0.361. ALP release from nanocomposites mainly follows the KP model with diffusion coefficient n = 0.1863 (in pH 2) and Higuchi model (in pH 7.4).

6. CAR/C/ALP nanocomposite particles at the maximum test phase dose of 1500 mg/kg did not cause acute toxicity in test rats. CAR/C/ALP nanoparticles with 2 doses of 200 mg/kg and 1000 mg/kg, tested for 28 days in white rats, did not affect hematopoietic function, structure and function of liver, kidney and liver. spleen. CAR/C/ALP nanocomposites are safe at the tested doses. Nanoparticles carrying ALP reduced uric acid levels in the blood of mice more strongly than those with increased uric acid.

REQUEST

1. Research and build a model for extracting collagen from freshwater fish scales in Northern Vietnam according to pilot scale and orientation to industrial scale production.

2. Research on preparation and clinical trials towards drug application based on CAR/C/ALP nanocomposite particles = 1/9/10 in the treatment of reducing blood uric acid in the treatment of gout.

NEW CONTRIBUTIONS OF THE THESIS

1. The process of extracting collagen from freshwater fish scales has been developed in Vietnam. The obtained collagen is pure collagen type I, containing α1, α2 and β chains, fibrous with a fiber diameter of 0.5-1 μm, denaturation temperature of 32.3 oC.

2. Successfully fabricated CAR/C/ALP composite membranes and particles. The composite films have a 28.35-78.03 % ALP carrying efficiency, depending on the fabrication conditions, while the composite particles have a higher allopurinol carrier efficiency of 27.11-80.79%.

3. Find the most suitable KP model to reflect the release of ALP from the composite membrane and the CAR/C/ALP nanocomposites at pH 2 and pH 7.4.

4. From the results of in vivo testing on mice using CAR/C/ALP nanocomposite particles, it is confirmed that the composite particles have the effect of lowering uric acid in the blood and do not cause toxicity and side effects at the test doses (200 mg/kg, 1000 mg/kg and 1500 mg/kg).