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SOME CHARACTERISTICS AND ALLOPURINOL RELEASE OF CARRAGEENAN/ ALLOPURINOL FILMS USING POLYETHYLENE OXIDE AS A DISPERSION AID AGENT

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Abstract. This paper presents the effect of carrageenan (CG) on some characteristics and drug release of allopurinol in the presence of polyethylene oxide (PEO) as a dispersion aid agent. The samples were prepared in film shape by solution method, in which, the content of PEO was changed from 1 wt.% to 5 wt.% and the content of allopurinol was fixed at 10 wt.% in comparison with carrageenan weight. Fourier transforms infrared (FTIR) spectroscopy and Field emission scanning electron microscopy (FESEM) methods were used to evaluate interactions and morphology of CG/PEO/allopurinol films. Ultraviolet-visible spectroscopy (UV-Vis) analysis was used to determine drug loading capacity and drug release of CG/PEO/allopurinol films. The IR spectra of CG/PEO/allopurinol films showed that the sulfate groups in CG interacted with C-O and OH groups in PEO and C=O, N-H groups in allopurinol. The SEM images of films indicated that allopurinol could disperse regularly in CG matrix as using 2 wt.% PEO. The drug loading capacity of CG films was reached from 49.33 to 92.32 %, depending on the PEO content. From the data of drug release of CG/PEO/allopurinol films in pH 7.4 and pH 2 buffer solutions, the CG/PEO/allopurinol film prepared at 2 wt.% of PEO had best drug release control ability.

Keywords: carrageenan, drug release, drug loading capacity, allopurinol, morphology.

Classification numbers: 2.7.1, 2.9.4.

1. INTRODUCTION

Carrageenan (CG) is a polysaccharide extracted from certain species of red seaweeds with galactose and anhydrogalactose units linked by glycosidic units [1, 2]. In recent years, CG is

increasingly used in various nonfood products industry, especially in pharmaceutical industry because of its good compatibility, nontoxic, bioactivity, persistent viscoelasticity, high robustness, and nonirritating [3-6]. CG is a promising carrier for both conventional and advanced drug delivery systems, for example, anti-inflammatory drugs, ibuprofen [5, 7-8]. Solubility and viscosity of CG in solutions depends on the types, molecular weight, content, presence of cations in solution. Interestingly, the nature of CG is hydrophilic; therefore, CG could be used to enhance the dissolution rate of poorly water soluble drugs, leading to increase in their oral bioavailability [9]. Ghanam *et al*. fabricated microcrystalline cellulose pellets coated with κ-CG for loading bisacodyl [10]. In neutral condition, there is about 20-24 % of the drug released from cellulose pellets but there is over 80 % of the drug released from κ-CG coated cellulose pellets. As only CG used as a drug carrier, CG is usually converted to gel form by heat and cations (K^+, Ca^{2+}, Na^+, etc.) because in gel form, the helices chains in CG structure are agglomerated together, leading to a tighter structure of gel CG. Varghese *et al*. studied the release of quercetin from gelatin-CG hydrogels (the ratio of gelatin/CG =1/2) and confirmed that CG contributed in improvement of quercetin release form hydrogels by an increase in hydrogel porosity [11]. CG contributed in the enhancement of the solubility, dissolution rate as well as bioavailability of poorly soluble drugs and anticancer drugs [12-18].

This report related to study on drug loading ability of CG films. Allopurinol – a potent inhibitor of xanthin oxydase, which reduces the concentration of uric acid in blood and urine – is chosen as a model drug for loading by carrageenan. However, the nature of allopurinol is a poorly water soluble drug, it is difficult to disperse regularly in CG film. Therefore, polyethylene oxide (PEO) was used as a dispersion aid agent for allopurinol in CG film because PEO is known as a stabilizer or an emulsifier and a compatibilizer shown in our previous reports [19-21]. The solution method is one of popular methods for preparation polymer loading drug, especially, carrageenan film loading drug (curcumin, ibuprofen, anti-microbial and anti-inflammatory drugs, etc.) because solvent cast films are used as oral strips with potential to adhere to the mucosal surface, hydrate and deliver drugs across the buccal membrane [22-24]. The dispersion and release ability of allopurinol from CG film were investigated and discussed.

2. MATERIALS AND METHOD

2.1. Materials and devices

Some chemicals consist of carrageenan (in powder with content of potassium ≤ 11 %, calcium ≤ 3.5 %, and sodium ≤ 2 %, moisture content ≤ 12 %), poly(ethylene oxide) (PEO) (*Mv* 100000, glass temperature -67.0 ^oC, polydispersity index PDI = 1.02–1.12), allopurinol (purity ≥ 98 %, molecular weight of 136.11 g/mol, melting point > 300 ^oC, solubility in NaOH 1M (soluble 50 mg/ml)) were purchased from Sigma Aldrich (USA). Other chemicals: HCl 36.5 %, NaOH, KH_2PO_4, CH_3COOH 99 %, NaCl made in China were used as received.

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 in the wavenumbers range from 400 to 4000 cm^{-1} 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 device (Hitachi – Japan) at National Institute for Hygiene and Epidemiology. Ultraviolet - visible (UV-Vis) spectra of the samples conducted on spectrophotometer (CINTRA 40, GBC,

USA) at Institute for Tropical Technology (VAST) was used to determine optical density, as a basis on calculation of drug release content of CG/allopurinol films in different pH solutions.

2.2. Preparation of carrageenan/allopurinol film

Carrageenan/allopurinol films were prepared as follows: 0.1 gram of carrageenan and various content of PEO (0-5 wt%/wt.% in comparison with carrageenan weight) was dissolved in 15 mL of distilled water. This solution was heated to 80 ^{o}C and kept at this status for 30 minutes. Then, this solution was cooled to 50 ^{o}C and added KCl (1 wt.% in comparison with carrageenan weight). Next, the solution was cooled to room temperature before dropping 0.01 gram of allopurinol in 10 mL of NH_4OH 1M solution and combining with high speed stirring at a speed of 20000 rpm. The obtained mixture was stirred at a speed of 20000 rpm for 15 minutes and poured into a petri dish for natural dry. The CG/allopurinol films with other content of PEO (FCPA) were signed in Table 1.

Table 1. Composition and abbreviation of carrageenan/allopurinol films with different content of PEO.

Weight ratio of G/allopurinol/PEO (wt.%/wt.%/wt.%)	**Weight of CG/allopurinol/PEO (gram:gram:gram)**	**Abbreviation**
10/1/0	0.1 : 0.01 : 0.0	FCPA.0
10/1/1	0.1 : 0.01 : 0.001	FCPA.1
10/1/2	0.1 : 0.01 : 0.002	FCPA.2
10/1/3	0.1 : 0.01 : 0.003	FCPA.3
10/1/4	0.1 : 0.01 : 0.004	FCPA.4
10/1/5	0.1 : 0.01 : 0.005	FCPA.5

2.3. *In-vitro* drug release study

The *in-vitro* allopurinol release test from the CG/allopurinol films was carried out as follows: 150 mg of each sample was immersed in 200 mL of pH 7.4 phosphate buffer solution (corresponds to the environment of simulated intestinal fluid) or pH 2 buffer solution (correspond to the environment of simulated gastric fluid) at 37 ^{o}C and placed in a incubated shaker at 120 rpm. At predetermined time intervals, 5 mL of aliquots was withdrawn while 5 mL of fresh buffer solution was added contemporaneously into the solution to maintain the total volume. The concentration of released allopurinol was calculated by UV - Vis method, based on the calibration equations of allopurinol in pH 7.4 ($y = 1898x + 0.0047$, $R^2 = 0.9998$, λ_{max} = 249.81 nm) and pH 2 ($y = 8225.3x + 0.0272$, $R^2 = 0.9997$, λ_{max} = 257.06 nm) solutions, in which x is the concentration of allopurinol (mol.L^{-1}) and y is the absorbance of solution. The allopurinol release percentage can be determined by the following equation:

$$\text{Drug release [\%]} = C_{(t)}.100/C_{(0)} \quad (1)$$

where $C_{(0)}$ and $C_{(t)}$ represents the amount of allopurinol loaded and amount of drug released at a time t, respectively. All studies were done in triplicate.

3. RESULTS AND DISCUSSION

3.1. Infrared spectra

IR spectra of carrageenan, allopurinol and the CG/allopurinol films were presented in Fig. 1. It can be observed that some characteristic absorbance peaks of carrageenan coressponding to the vibration of O-H (stretching), S=O (stretching), O-H (bending), C-O, and S=O (bending) groups at 3392; 2360; 1636; 1154 and 842 cm^{-1}, respectively [15].

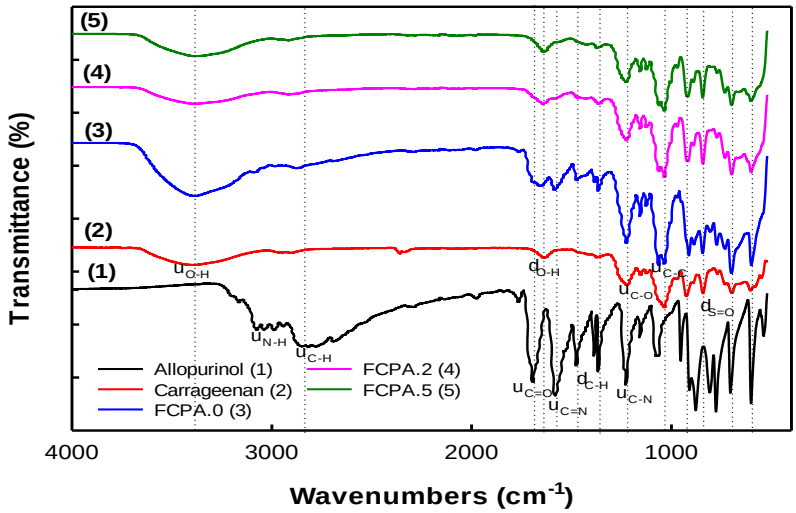

Figure 1. IR spectrum of allopurinol, carrageenan and the CG/allopurinol films.

In the IR spectrum of allopurinol, the vibration of secondary amine, tertiary amine, C=O, C=N and C-N was also found at 3169; 2817; 1694; 1580 and 1226 cm^{-1}, respectivelly [25]. In comparison of the IR spectrum of carrageenan in the CG/allopurinol film prepared without PEO (FCPA.0), it was seen that the appearance of some new peaks characterized for stretching vibration of secondary amine (3084 cm^{-1}), tertiary amine (2864 cm^{-1}), C=N (1584 cm^{-1}) groups in the allopurinol molecular. This can confirm that the allopurinol was loaded by carrageenan. Moreover, the slight shift in wavenumbers of stretching vibration of amine groups and the broaden sulfate group peak could be also attributed by the hydrogen bonds and strong electrostatic binding between the amine group of allopurinol with the hydroxyl and sulfate groups of carrageenan. Matej *et al.* also showed that the formation of strong electrostatic binding between doxazosin drug and carrageenan [26]. When using PEO as a dispersion aid agent, the position of characteristic peaks in IR spectra of CG/allopurinol films has not changed. This exhibits that the presence of PEO does not influence on the interaction between CG and allopurinol.

3.2. Morphology

The morphology of allopurinol was of bar and cubic shape with diameter in the range of 200 nm – 10 µm as assigned in Fig. 2. As loaded by carrageenan, the size of allopurinol was extremely decreased to 50-250 nm (Fig. 3 A). This can be explained by the dissolution of allopurinol in NaOH solution and the physical interactions between allopurinol and carrageenan leading to the reduction in the size of allopurinol dispersed phase. However, it can be seen the

agglomeration of allopurinol in the CG matrix. Observation from Fig. 3 B and Fig. 3 C, it was clear that allopurinol was dispersed more regularly in CG matrix by presence of PEO. At 2 wt.% of PEO, the film had a uniform and tight structure. Allopurinol particles were covered by the polymer. At 5 wt.% of PEO, some allopurinol particles still appeared on the surface of the films but the agglomeration of these particles was decreased. The dispersion of allopurinol in the carrageenan matrix can influence on the drug release from the carrageenan films as investigated below.

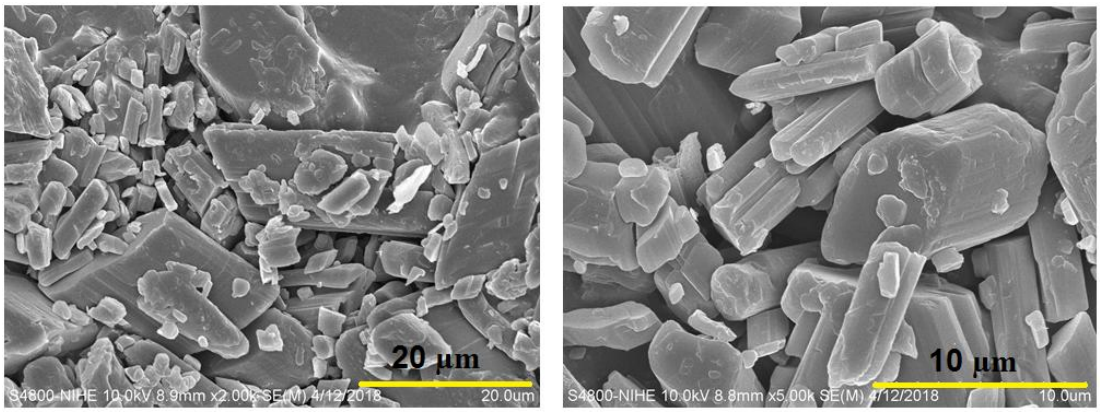

Figure 2. SEM images of allopurinol at magnification of 2000 times (left) and 5000 times (right).

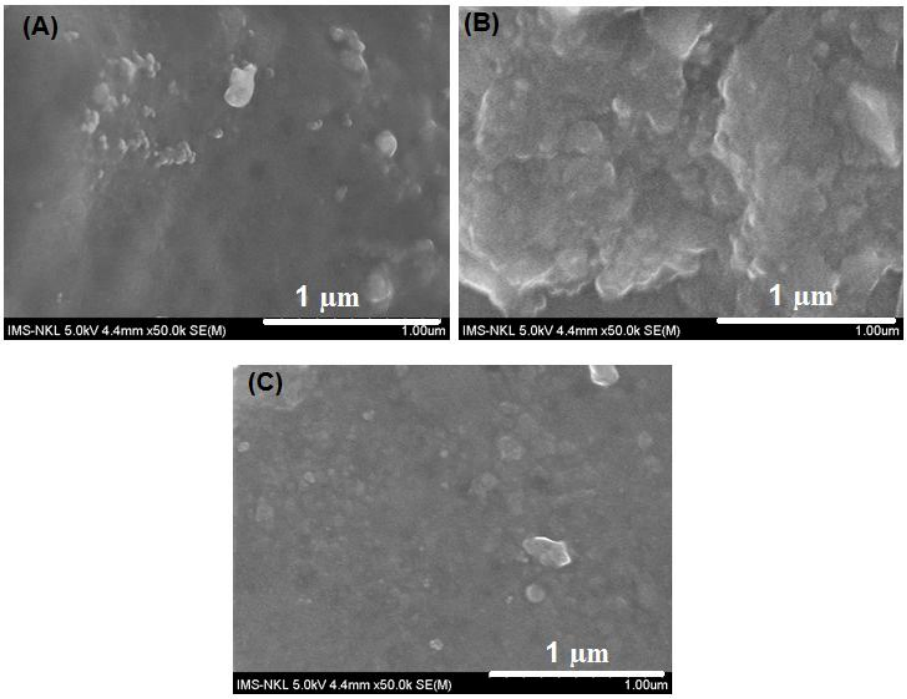

Figure 3. SEM images of the CG/allopurinol films with and without PEO:
FCPA.0 (A), FCPA.2 (B), FCPA.5 (C).

3.3. Drug release study

Drug release study in simulated body fluids plays an important role in the evaluation of bioavailability of drug. In this study, the allopurinol release content from the carrageenan/allopurinol films with and without PEO in different simulated body fluids was investigated and compared with the control sample (crystalline allopurinol) as performed in Fig. 4.

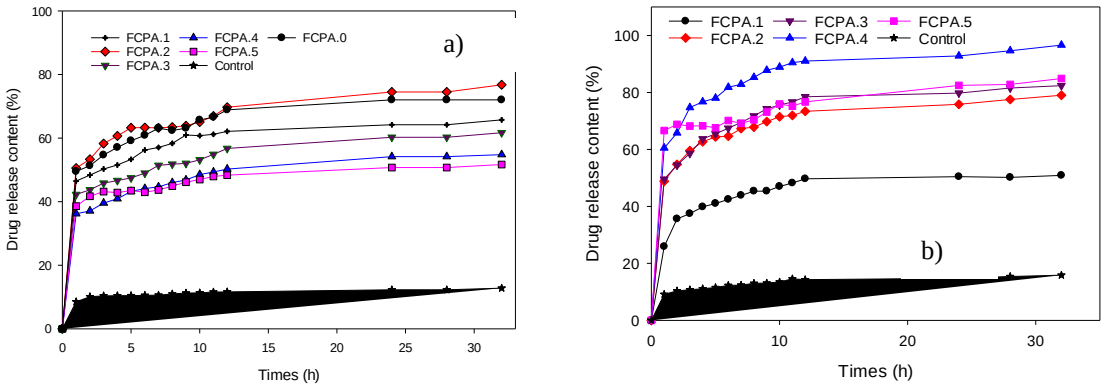

Figure 4. Drug release content from the CG/allopurinol films with and without PEO in pH 7.4 solution (a) and pH 2.0 solution (b), control: crystalline allopurinol.

Table 2. Regression coefficient (R^2) obtained from kinetic equations reflecting allopurinol release from the CG/allopurinol films in pH 7.4 and pH 2 solutions. ZO: zero order kinetic, FO: first order kinetic, HG: Higuchi model, HC: Hixson Croswell model, KMP: Korsmeyer–Peppas model.

Sample	ZO	FO	HG	HC	KMP
pH 7.4					
FCPA.1	0.7037	0.6703	0.8578	0.7037	0.9447
FCPA.2	0.8306	0.7792	0.9364	0.8306	0.9734
FCPA.3	0.8564	0.8216	0.9555	0.8564	0.9664
FCPA.4	0.8163	0.7716	0.9376	0.8163	0.9745
FCPA.5	0.8566	0.8282	0.9417	0.8566	0.9433
pH 2					
FCPA.1	0.5696	0.4883	0.7519	0.5696	0.8787
FCPA.2	0.7059	0.6443	0.8649	0.7059	0.9655
FCPA.3	0.6621	0.6084	0.8323	0.6621	0.9468
FCPA.4	0.6411	0.5872	0.8139	0.6411	0.9376
FCPA.5	0.9294	0.9168	0.9324	0.9294	0.8182
FCPA.0	0.7694	0.7278	0.9152	0.7694	0.9722

It can be seen that allopurinol was released slowly with a low content from the control sample in both pH 7.4 and 2 solutions due to the nature of allopurinol which is poorly dissolved in the acidic and aqueous solutions. In contrast, allopurinol released from the carrageenan/allopurinol with and without PEO was much faster than that from control and according to two stages (burned or fast release during 1 first testing hour and then slow and continuous release) with the drug release content was higher many times than that from the control sample in the same pH solutions. This phenomenon is similar to the release of Ibuprofen from carrageenan film [22]. The significant increase in content of allopurinol released from the carrageenan/allopurinol films could be explained by the hydrophilic nature and swellability of carrageenan and interaction between carrageenan and drug [7]. According to Nerurkar *et al*., the rate at which the drug is released from the swellable hydrophilic matrices is determined by numerous processes such as hydration of the polymer that leads to swelling, diffusion of the drug through the hydrated polymer, drug dissolution and polymer erosion. Many of these processes occur simultaneously to release the drug. The variation in PEO content had an unlike effect on the allopurinol release content from these films in the pH 7.4 and pH 2 solutions.

This can be affected by the interaction ability of PEO with carrageenan and allopurinol to be different at various content of PEO, therefore, dispersion of allopurinol in the carrageenan matrix using PEO was distinguished. Among investigated samples, the CG/allopurinol films containing 1 – 2 wt.% of PEO exhibited higher release in pH 7.4 solution and slower release in pH 2.0 solution.

In vitro drug release data were fitted to Zero order, First order, Higuchi, Hixson-Crowell and Korsmeyer-Peppas models for understanding the mechanism of drug release from the film formulations [22]. The regression coefficients (R^2) obtained from kinetic equations reflecting allopurinol release from the CG/allopurinol films with and without PEO in pH 7.4 and pH 2 buffer solutions are listed in Table 2. It can be seen that the regression coefficient obtained from Korsmeyer–Peppas model are highest. This evidence showed that the release of allopurinol from the CG/allopurinol films is followed a complex mechanism, including to the swelling of polymer, the dissolution of polymer, the diffusion of drug, the erosion film, etc. [22, 24]. The regression coefficient obtained from Higuchi model is higher than that from Hixson-Crowell model corresponding to the diffusion mechanism has more advantage than the erosion mechanism.

4. CONCLUSIONS

In this paper, the influence of polyethylene oxide (PEO) as a dispersion aid agent on IR spectra, morphology and drug release of carrageenan film loading allopurinol was investigated. The IR spectra showed the existence of hydrogen bonding between PEO, carrageenan and allopurinol. The SEM images indicated that the presence of PEO helps to improve the dispersion ability of allopurinol in the carrageenan matrix. The results of drug release proved that allopurinol loaded by carrageenan with and without PEO had release ability in simulated body fluids better than that of the control sample. The PEO content of 1-2 wt.% was suitable for improvement in the interaction, dispersion and drug release of the carrageenan/allopurinol film. The Korsmeyer–Peppas model was suitable for the release mechanism of allopurinol from the carrageenan/allopurinol films.

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