Research Article

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Disintegration, *In vitro* Dissolution, and Drug Release Kinetics Profiles of κ-Carrageenan-based Nutraceutical Hard-shell Capsules Containing Salicylamide

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Abstract: The release of drugs from solid drug delivery materials has been studied intently in recent years. Quantitative analyses achieved from *in vitro* dissolution becomes easier if a zero-order mathematical model is used. Non-gelatin nutraceutical hard-shell capsules of zero size (approximately 0.7-0.8 cm) were produced from carrageenan-based natural polymers, namely carrageenan-alginate (CA) and carrageenan-starch (CS). Disintegration, dissolution and zero-order drug release kinetics of hard-shell capsules containing 100 mg of salicylamide were studied. The disintegration time of CA and CS were observed to be less than 30 min for both CA and CS. *In vitro* dissolution profile showed that the percentage dissolution of CA capsules was better at pH 4.5, while that of CS was poor at pH 1.2, 4.5 and 6.8. Determination of drug release kinetics profiles of carrageenan-based hard-shell capsules utilized the Noyes-Whitney and Peppas-Sahlin modification rules for zero-order. The drug release from carrageenan-based capsules followed zero-order kinetics, especially at pH 6.8, and was compared to the Higuchi model. Salicylamide in CA hard-shell capsules at a pH 6.8 had a release rate constant (k_H) of 2.91 %(ppm/ppm) $min^{-1/2}$, while the release rate constant of CS was 0.36 %(ppm/ppm) min^{-1}.

Keywords: Dissolution; Drug release kinetics; Salicylamide; k-Carrageenan; Capsule.

1 Introduction

Commercially available hard-shell capsules are generally made from gelatin, which is produced from bone and skin of cows, pigs or buffalos. Gelatin hard-shell capsules were introduced in 1931 by Arthur Cotton [1]. The first non-gelatin hard-shell capsule was produced in 1989 with the trademark of Vegicaps, and it was made from a material, hydroxypropyl methylcellulose (HPMC), that is appropriate for vegetarians or vegans. As an addition, carrageenan-based non-gelatin hard-shell capsules have been patented and produced, i.e. Quali-V [2].

Carrageenan is one of the most important commercial marine products, along with alginates and agars. Indonesia and the Philippines are two countries that produce much of the carrageenan for the rest world [3]. It is extracted from red seaweeds, especially from the Rodhophyceae family, including *Chondruscripus*, *C. ocellatus*, *Eucheuma cottonii*, *E. spinosum*, *E. gelatinae*, *Furcellaria fatigiata*, *Gigartina stellata*, *G. acicularis*, *G. pistillata*, *G. canaliculata*, *G. chamissol*, *G. radula*, *G. skottsbergii*, *Gymnogongrus furcellatus*, *Hypneamusciformis* and *H. spicifera* [4].

Carrageenan is a sulfated polysaccharide that is classified based on the position of the link of anhydrogalactose bond and the number and position of ester sulfate. Subclasses of carrageenans include α, β, k, i, and q [5] as well as m and ν [6]. k-Carrageenan is mainly obtained from tropical seaweed of the *Eucheuma*

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cottonii or *Kappaphycus alvarezii* species. Commercial k-carrageenan contains an ester sulfate and D-galactose-4-sulfate 3,6-anhydro-D-galactose at levels of approximately 25 to 30% and 28-35%, respectively [5,6]. The molecular weight of carrageenan is in the range of 100 to 1000 kDa. It is a strong anionic polymer possessing excellent gelling, thickening, stabilizing [5,7] and emulsifying [6] properties.

Carrageenan is also used for non-foods industries, including as a pharmaceutical excipient and as a medicine [6]. Carrageenan has some bioactivities, such as antioxidant, antimicrobial towards *Salmonella typhimurium* and *E. coli O157:H7* strains, immunomodulatory, antiviral, antitumor, anticoagulant and antithrombotic properties [8]. It is regarded as a nontoxic and nonirritating agent for use in nonparenteral formulations, and it is well known as an anti-inflammatory agent [9]. Alginate is used as a dietary fiber, and it can stimulate the immune system, reduce intestinal absorption, increase satiety, reduce glycemic index and modulate colonic microflora [8]. Alginate can be consumed as a beverage to reduce the blood sugar level [10].

Due to these important consumer-targeted properties, combinations of carrageenan-alginate and carrageenan-starch are being developed as new products as non-gelatin nutraceutical (nutrition and pharmaceutical) hard-shell capsules. This nutraceutical capsule offers unique inherent acid-resistant natural polymer properties. Thus, the capsule resists disintegration in the stomach but opens immediately once the pH rises above 4.5, out of the acidic environment of the stomach. Disintegration starts approximately 25 minutes after the ingestion of the capsule, and the dissolution of drugs begin after 50 minutes. The complete release of the gelatin hard-shell capsule takes place in the small intestine around 20 min after the onset of release when the pH shifts more to a neutral pH of 6.8. Therefore, the nutraceutical capsule is acid-resistant and delivers to the small intestine [11].

In vitro dissolution is an important factor in drug absorption, distribution, metabolism and excretion (ADME). Dissolution is defined as the rate of transfer of mass from the dosage form into a liquid medium at a suitable temperature for standardization [12]. Many kinetics models have been used to describe drug dissolution and release from solid dosage forms [13]. Drug release can be divided into several modes, including immediate release, modified-release, extended-release, controlled-release and pulsatile-release [12]. Salicylamide is a non-steroidal and anti-inflammatory agent; it has analgesic, antipyretic and platelet inhibitory actions. Salicylamide is stable in acid and weak base, so it can be used as a model of drug release kinetics. Carrageenan can be used to control the release of drugs, improve the dissolution of drugs and has been tested for its potential use in broader biomedical application [6,14].

Information about drug release kinetics of carrageenan-based hard-shell capsules is still limited. There are reports on drug release properties during diffusion from layer-by-layer self-assembled κ- carrageenan-chitosan nanocapsules [15], application of κ-carrageenan as a sustained-release matrix in floating tablets containing sodium salicylate [16] and drug release kinetics and front movement in matrix tablets containing diltiazem or metoprolol/λ-carrageenan complexes [17]. However, none of these reports investigated drug release kinetics from nutraceutical hard-shell capsules. This paper reports the study of disintegration, dissolution and drug release kinetic profiles of k-carrageenan-based nutraceutical hard-shell capsules containing salicylamide.

2 Experimental Method

2.1 Materials

Food grade k-carrageenan and sodium alginate were purchased from Kappa Carrageenan Nusantara, Inc., Pasuruan, Indonesia. Carrageenan-alginate (CA) and carrageenan-starch (CS) hard-shell capsules were prepared in Kapsulindo Nusantara, Inc., Bogor, Indonesia. All water used was deionized.

2.2 Preparation of Hard-Shell Capsules

Carrageenan and alginate were combined and mixed into the water gently at room temperature. Then, the temperature was raised to 70°C to create a homogeneous solution. The solution was poured into a bath and printed using zero size dipping bars and dried at room temperature for 4 h to produce capsules. The same procedure was conducted to create CS capsules.

2.3 Disintegration Test

The disintegration of capsules was tested using a disintegration tester type Veego-202397. Six chambers were filled with 900 mL of water. Six carrageenan-based (CA and CS) capsules were put in each basket and heated at 37±0.5°C. The apparatuses were slowly rotated until hard-shell capsules were disintegrated completely.

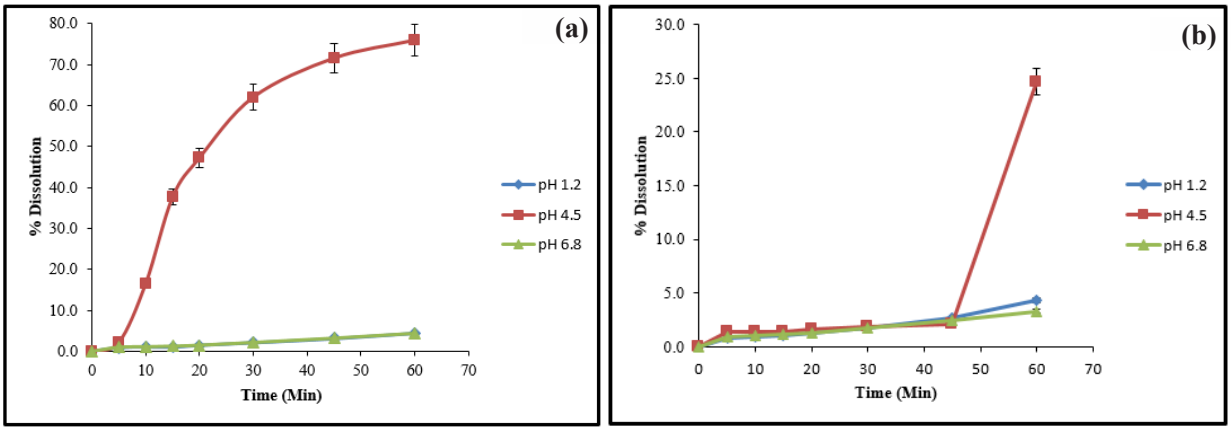

Figure 1: Dissolution profile of salicylamide released from (a) CA and (b) CS capsules at pH 1.2, 4.5 and 6.8. The dissolution of the drug capsules was carried out in triplicate for each formulation, and results are reported as averages +/- standard deviation.

2.4 Dissolution Test and Release Kinetics Analyses

An Erweka Dissolution Tester type DT 820 was adjusted to 37°C and rotated at 100 rpm. Three of each of the carrageenan-based (including CA and CS) zero size capsules were filled with 100 mg of salicylamide and put in the basket holder, which contained 900 mL of medium. The dissolution media was created as described by the United States Pharmacopeia (USP) at pH 1.2 (0.1 mol/L HCl), pH 4.5 (citrate buffer) and pH 6.8 (phosphate buffer). All media were calibrated with a pH meter prior to each assay. Samples (5 mL) were taken after 5, 10, 15, 20, 30, 45 and 60 min, and volumes were maintained by refilling with 5 mL of media. All samples were analyzed using a UV-Vis spectrometer at 298 and 299 nm for pH 4.5 and 1.2 / 6.8, respectively. The data were analyzed statistically to obtain dissolution and kinetics profiles in all media. Drug release kinetics profiles utilized the Noyes-Whitney rules for zero-order calculation. The best profile of the graphs from the Noyes-Whitney and Peppas-Sahlin equations were used to determine k_H using the Higuchi model.

Ethical approval: The conducted research is not related to either human or animal use.

3 Results and Discussion

3.1 Disintegration of CA and CS

The CA and CS hard-shell capsules disintegrated completely by 12.80 and 25.79 min, respectively as shown in **Table 1**. All of the capsules ruptured within 30 min, which meets the USP recommendation for the disintegration of dietary supplements [19].

Table 1: Disintegration times of capsules.

Capsules	CA (min)	CS (min)
1	11.28	20.04
2	11.28	25.52
3	12.13	27.02
4	13.47	27.02
5	14.33	27.58
6	14.33	27.58
Mean	**12.80**	**25.79**
Standard Deviation	**1.43**	**2.92**

Starch is a semicrystalline material that contains the crystalline form of a linear structure of amylose and the amorphous form of branched amylopectin [20], while sodium alginate is a linear unbranched copolymer composed of β-D-mannuronic acid (M) and α-L-guluronic acid (G) linked by 1→4 glycosidic bonds and appears as an amorf [21]. Therefore, the structure of CS is more rigid than CA. This rigid structure likely prevents CS from being ruptured by the medium better than CA; this model correlates with the higher disintegration time of CS relative to CA. As an addition, Glube *et al.* reported that hard-shell capsules made of hypromellose (HPMC)–carrageenan that contained green tea extracts disintegrated within 20 min [19]. It is seen that the order of disintegration time from the slowest to the fastest time would be CS > HPMC-carrageenan > CA > gelatin. Therefore, relative to gelatin, the disintegration time of CA was better than other materials, which was HPMC in this case.

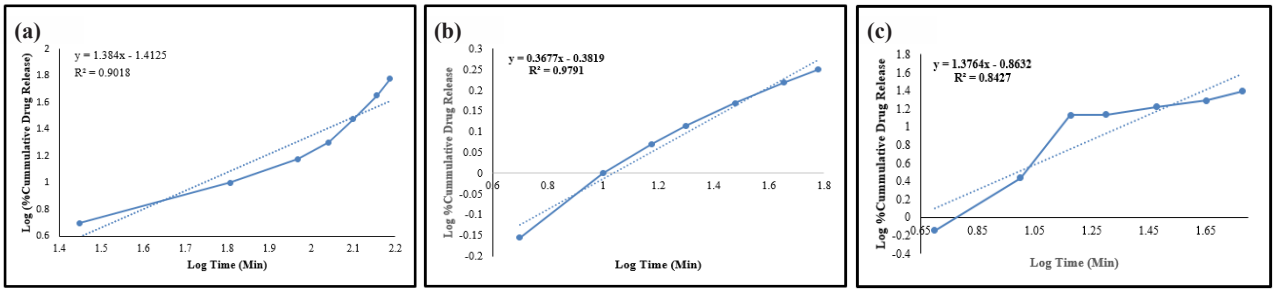

Figure 2: The zero-order drug release profile of salicylamide from CA capsules at pH (a) 1.2, (b) 4.5, and (c) 6.8.

3.2 Dissolution and release kinetics analyses of salicylamide released from CA and CS

The dissolution of salicylamide from hard-shell capsules of CA reached approximately 70% at pH 4.5 within one hour **(Figure 1a)**, while in CS **(Figure 1b)**, it was poorly dissolved in all of the media conditions compared to CA hard-shell capsules. The disintegration test does not require the drug to be inside the capsules (USP NF-32), while the dissolution test requires it (USP 711). The salicylamide substance enters into the solvent to produce a solution. Interactions (especially hydrogen bonds) between salicylamide and CA and CS might slow the solvation process. The percentage of dissolution of salicylamide from the hard-shell capsules of CA and CS at pH 1.2, 4.5 and 6.8 is shown in **Figure 1**. In general, salicylamide dissolution increased over time in all three media.

The differences of dissolution may be due to a variety of characteristics of the matrix material, such as composition, structure, gelling, pH, temperature and ionic strength [22]. Chiwele *et al.* reported that the empty hard-shell capsules of gelatin, gelatin-PEG (polyethylene glycol) and HPMC at pH under 5.8 and at 10°C to 55°C dissolved rapidly, while gelatin and gelatin-PEG did not dissolve at a temperature below 30°C [23]. There was a significant increase in dissolution for capsules at pH 4.5 shown in **Figure 1b**. One possible explanation could be that the capsules were significantly dissolved at 50 min, which increased the dissolution of salicylamide into the medium exponentially.

Many mathematical models for determining drug release from solid dosage forms are available: the Noyes-Whitney rule, Higuchi, Hixson-Crowell, Korsmeyer-Peppas, Baker-Romdale, Weibull, Hopenberg and Gompertz model are used routinely. In this experiment, the mathematical model of drug release used is shown in equation (1), a modification of the Noyes-Whitney equation, for zero-order release kinetics calculations [13,18].

Table 2. The ko and R^2 value of zero-order kinetics of carrageenan-based hard-shell capsules

pH	k_0 CA ppm/min	R^2	k_0 CS ppm/min	R^2
1.2	3.28	0.90	0.058	0.87
4.5	4.14	0.98	0.23	0.92
6.8	0.46	0.84	0.067	0.82

$$Q_t = Q_0 + K_0\, t \quad (1)$$

In equation (1), Q_t is the amount of drug dissolved in solution at time t, Q_0 is the initial amount of drug in the solution (typically, $Q_0 = 0$), and k_0 is the zero-order release constant. Costa *et al.* reported that the dosage forms of drugs following zero-order release are the ideal model of drug release [13]. The logarithmic form of equation (1) is shown in equation (2):

$$\log Q_t = \log K_0 + \log t \quad (2)$$

The kinetics of the release of the drug can be classified as zero or first-order. The drug release in this experiment used the Noyes-Whitney equation model**. Figure 2** compares a zero-order kinetic model to the drug release profile of salicylamide from CA hard-shell capsules. Since the only pH where salicylamide dissolved up to 80% was 4.5 **(Figure 2b)**, there was only one kinetic profile that could be calculated. The salicylamide was released in the CA capsules more quickly at pH 4.5, and **Figure 2b** also shows linearity. The zero-order release constants of salicylamide in CA at pH 1.2 **(Figure 2a)** and 4.5 were 3.28 and 4.14 ppm/min **(Table 2)**. The zero-order release constant of salicylamide in CA at pH 4.5 **(Figure 2b)** was 4.14 ppm/min **(Table 2)**. Therefore, we suggest further study of CA capsules at pH 4.5. Even though CA capsules had poor solubility at pH 1.2 and 6.8 **(Figure 2c)**, we also suggest further study for medicines with long release time that would be suitable for these features.

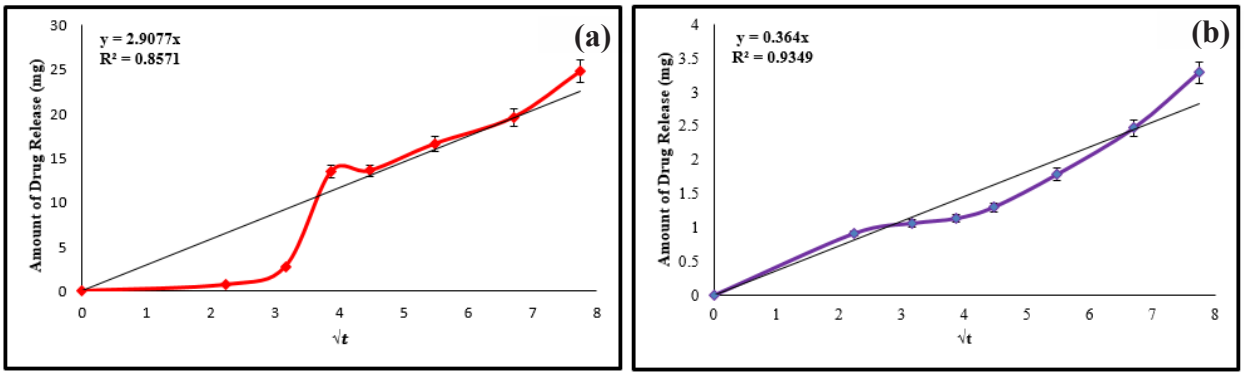

Figure 3: The Higuchi model of drug release of salicylamide in hard-shell (a) CA and (b) CS capsules at pH 6.8

What seems interesting is that the capsules exhibit good solubility in a weakly acidic environment but not in either strongly acidic or more basic environments. No research to support or explain these results could be found. However, one possible explanation could be that the solubility of carrageenan might be affected by its sulfate groups. A citrate buffer solution that created a weakly acidic environment might increase its solubility by decreasing intramolecular hydrogen bonding in carrageenan's secondary structure, as this structure is the main feature of carrageenan's gelling ability [23]. Thus, when intramolecular interactions are broken, carrageenan would be expected to dissolve easily. This might not happen at pH 1.2 and pH 6.8, where HCl and phosphate buffers were used, because the buffer molecule itself could not form hydrogen bonds. Finally, since CS capsules have poor solubility at all pH values examined, release kinetic profiles could not be developed for the material due to Peppas-Sahlin's rules [25]. Thus, we suggest that further research related to the dissolution time of CS capsules for more than 1 h should be conducted. The zero-order kinetic constants for CA capsules were 3.27 and 4.14 ppm/min **(Table 2)** for pH 1.2 and 4.5, respectively.

Analysis of drug release from carrageenan-based hard-shell capsules resulted in linear graphs that followed zero-order kinetics in the Noyes-Whitney model and were good mathematically and kinetically modeled at pH 6.8. This pH was applied to investigate the diffusion coefficient using the Higuchi model. **Figure 3** represents the drug release of salicylamide in CA and CS hard capsules at pH 6.8 using the Higuchi model [12]. The graphs resulted from the Higuchi model calculation **(Figure 3)** represented the release kinetics process of drugs that were released from a swellable polymer, where the graph was plotted from the amount of drug released against the square-root of time. The simplified mathematical form of the Higuchi model is shown by equation (3).

$$Q_t = k_H \sqrt{t} \quad (3)$$

In this equation, Q_t is the amount of drug released at time t, and k_H is the release rate constant for the Higuchi model. This model features the release from both planar and spherical surfaces, where two geometric systems have been considered: the undirectional leaching of a simple planar surface and three-dimensional leaching from a spherical pellet [26].

We chose to examine the data at pH 6.8 because of its good linearity in **Figure 3b** compared to the other results, even though the capsules were less soluble in this environment. Another reason to be highlighted was that the only parameter affected by the acidity was the rate of release, but all capsules dissolved eventually. Therefore, capsules could still be used at every pH for any drug that meets compatible properties.

The graph shows that salicylamide in CA is released with a rate constant (k_H) of 2.91 %(ppm/ppm) $min^{-1/2}$ **(Figure 3a)**, while CS was 0.36 %(ppm/ppm) $min^{-1/2}$ **(Figure 3b)** at pH 6.8. At pH 6.8, the hard-shell capsule of CA has a bigger k_H than does CS. They had diffusion coefficients that were large, and CA releases more under that condition. Finally, developments are needed for this study to be applied in the human body. *In vivo* studies must be performed to support this information with respect to toxic dosages. One example might be work similar to that carried out by Zhang *et al.* in which they studied nanoparticular melamine in a mouse model [27].

4 Conclusions

CA had lower stability or was more readily dissolved in a weaker acid medium (pH 4.5) than other natural, carbohydrate-based polymers. Salicylamide in CA was released into the acidic medium more readily than CS.

One reason to explain the phenomenon is that starch has smaller membrane pores and is more stable in a strongly acidic medium (pH 1.2). Salicylamide, in CS, results in a smaller percentage of drug release than that of CA. Dosage forms that prolong release can maintain drug concentrations for longer periods in a more optimal therapeutic range while minimizing toxicity.

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Conflicts of interest: The authors declare that there is no conflict of interest.

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