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Physicochemical Properties of Glucomannan-Alginate as Vitamin C Excipient

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This work investigated the physicochemical properties of glucomannan-alginate combination as an excipient of vitamin C encapsulation. The effects of glucomannan-alginate ratio, concentration of vitamin C and preparation method on the encapsulation physicochemical properties were studied. Swelling and solubility of the vitamin C encapsulation were determined in 2 pH conditions which mimic the environment pH of the stomach and the lower gastrointestinal tract. The ratio of glucomannan-alginate on swelling at both pHs had a similar impact on the solubility. Higher values of swelling and solubility were observed at pH 6.8 than those at pH 1.2. The second method preparation of 1:1 (w/w) ratio of glucomannan-alginate showed the most soluble excipient in pH 6.8. The highest viscosity was observed at encapsulation of 3% vitamin C prepared using the second method of 3:1 (w/w) of glucomannan-alginate.

Keywords: alginate, glucomannan, solubility, swelling, viscosity.

1. Introduction

Humans rely on an exogenous source of vitamin C due to the lack of enzyme L-gulonolactone oxidase [1]. This vitamin is sensitive to be degraded due to the environment changing. The vitamin C absorption occurs in the small intestine. This absorption depends on the dose intake. Only 80-90% of 30-180 mg/day vitamin C intake is absorbed while the rest is discharged in urine. When the intake dose increases over 1,000 mg/day, the absorption drops to less than 50% [2]. Hence, it is important to retain a continuous low-dose supply of vitamin C to maximise the absorption process.

To provide the continuous supply of vitamin C, it is important to create a mode which controls the release as well as to deliver the vitamin to the right site. Encapsulation using an appropriate excipient could help in achieving these intentions. The excipient should be a pH-sensitive, which passes pH 1-2.5 of the stomach and delivers to the small intestine (pH 6.6-7.5) where the absorption is taken place [3]. Considering the dose-dependent absorption, the excipient should allow releasing the vitamin in specific rate solubility [4]. Hence, finding the excipient with those proper characteristics is important.

Glucomannan has a potency as vitamin C excipient. This polysaccharide consists of D-mannose and D-glucose with β-(1→4) linkage. Its unhydrolysed property during enzyme digestion in the human stomach [5] allows this copolymer to deliver the vitamin pass through the stomach. However, glucomannan high solubility needs to be modified to increase the encapsulation efficiency.

Alginate is a polysaccharide extracted from brown algae. This polyanionic saccharides consists of two acids i.e. guluronic and manuronic acids. It forms beads through ionotropic gelation in divalent cation solution. This unique property has attracted researchers to use it as drug carrier [6]. However, its instability in the acidic environment [7] and its porosity [8] limit alginate application as a drug matrix.

Fan et al. [9] had been studied the structure and properties of blend fiber prepared from glucomannan-alginate. They found the strong interaction of both polysaccharides depends on the glucomannan concentration. Introducing glucomannan in the blend fiber significantly improves water-retention property of the blend fiber compared to that of lone alginate fiber.

Synergistic polysaccharides are often encountered in drug excipient [10]. Wang and He [7] reported introducing glucomannan into alginate excipient lead to form stronger and more stable gels than those that composed of lone alginate. They reported a combination of native glucomannan and alginate as an excipient of water-soluble

insulin increases loading efficiency but reduces the release rate at pH 7.4. This combination also shows higher water hydration at pH 7.4 than that at 0.1 N HCl. This condition fits to support glucomannan-alginate combination as an excipient for vitamin C controlled release. However, application of this combination as vitamin C excipient has not been reported.

To extend the applications of this combination matrix on encapsulating vitamin C, the properties of this matrix were crucial to be understood. Hence, this work investigated the physicochemical properties of the combination of glucomannan-alginate for vitamin C excipient.

2. Materials and Method

2.1 Materials

Glucomannan of A. oncophyllus flour was extracted using the method of Wardhani et al. [11]. Alginate was purchased from PT. Multi Kimia Raya, Semarang. Distilled water was provided by Laboratorium Terpadu, Diponegoro University-Semarang, Indonesia. Other chemicals were obtained from Merck, Indonesia.

2.1 Bead preparation method

The bead was prepared based on the method of Muhammed et al. [6]. Vitamin C (1, 3 and 5% total excipient) was mixed with a combined solution of glucomannan and alginate in certain ratio (100 ml, 3%) was prepared. The mixture was dropped into a $CaCl_2$ solution (0.2 N, 200 ml) using syringe needle. After 1h, the beads were collected and freeze-dried. The bead was subject to physicochemical determination.

Three encapsulation methods were applied to study the effect of bead preparation method. Firstly, both excipients were mixed with the vitamin C. Secondly, vitamin C was encapsulated using glucomannan before coated with alginate. In the last method, vitamin C was encapsulated with alginate followed by coating with glucomannan.

2.3 Swelling and solubility

Bead swelling and solubility were determined based on the method of Muhammed et al. [6] in two solutions i.e. 0.1M HCl (pH 1.2) and pH 6.8 of phosphate buffer (0.1M). Briefly, 100 mg of the dried bead was dissolved in 100 ml of each solution at 37^{o}C and stirred at 100 rpm for 1h. The swollen beads were collected and weighted after blotting the bead surface with filter paper to remove excess moisture.

Meanwhile, the filtrate was centrifuged at 4,000 rpm for 20 min. The supernatant was collected and weighed afterwards. The collected supernatant was oven-dried and reweighted. The swelling index and solubility of the sample are calculated following equation [1] and [2].

$$\text{Swelling index (\%)} = ((m_{sb}-m_{db})/ m_{db}) \times 100\% \quad [1]$$

$$\text{Solubility (\%)} = (m_{ds}/m_{ws}) \times 100\% \quad [2]$$

where m_{sb}, m_{db}, m_{ds}, and m_{ws} are the weight of swollen bead, dried bead, dried supernatant, and the wet supernatant, respectively.

2.4 Intrinsic Viscosity

A series of the matrix solution concentration was prepared at 0.01-0.1% which then centrifuged for 20 min at 4,000 rpm. The specific viscosity of the supernatant was measured with Cannon Fenske viscometer against distilled water as a blank. The intrinsic viscosity was found by plotting the reduced viscosity against concentration and extrapolating to zero concentration.

3. Results and discussion

In this study, glucomannan and alginate were combined for excipient of vitamin C by varying three variables i.e. ratio of glucomannan-alginate, vitamin C concentrations and encapsulation preparation method. All samples were subject to the physicochemical determinations. This determination refers to a property which depends on joining action of physical and chemical properties. Swelling and solubility were observed at two pH solutions which represented the environment of gastric fluid (pH 1.2) and intestinal fluid (pH 6.8).

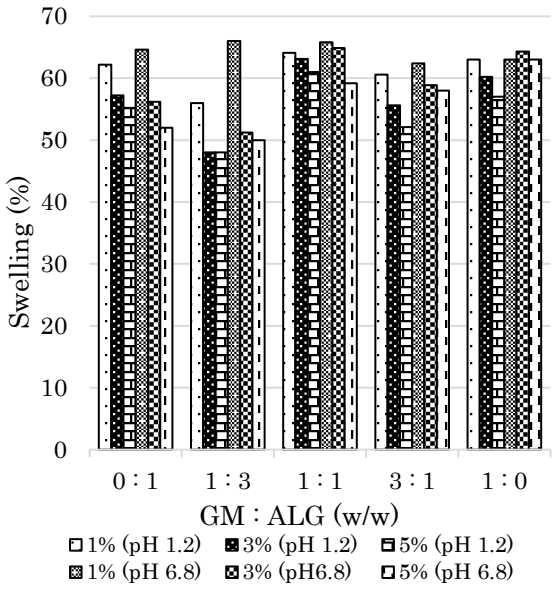


Fig. 1: Effect of ratio glucomannan-alginate and vitamin C concentration on swelling at HCl solution (pH1.2) and phosphate buffer solution (pH 6.8).

3.1 Swelling

Swelling refers to the ability of a polymer in absorbing solvent and expanding their volume. Figure 1 shows the

swelling of the sample at various ratios of glucomannan-alginate and vitamin C concentrations. In general, effect of ratio of glucomannan-alginate and vitamin C concentration on the swelling values in pH 1.2 was lower than those in pH 6.8. In this study, the pH 6.8 of phosphate buffer solution was prepared using monosodium phosphate and disodium phosphate. These sodiums could interact with the Ca^{2+} of $CaCl_2$ which linked to carboxylic groups of alginate during cross-link process. This replacement process of a bivalent ion with monovalent caused the breakup of the "egg-box" structure [12]. The egg box structure is described as the structure of calcium ions which bound in the periodic chelation sites located between two polyuronate chains [13]. The breakup increased the distance between the polymeric chains and favoured the fluid absorption. As a result, the swelling of the matrix at pH 6.8 was higher than that at an acidic solution. Using a combination of alginate-chitosan excipient, Segale et al. found higher swelling was also observed in pH intestinal [12].

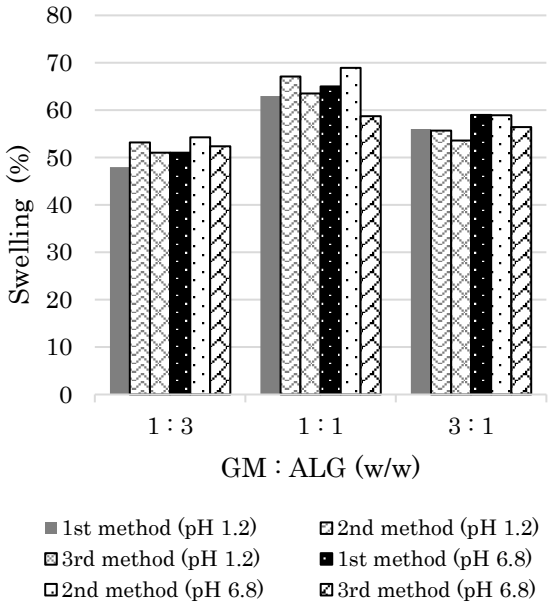


Fig. 2: Effect of preparation method on beads swelling at HCl solution (pH 1.2) and phosphate buffer solution (pH 6.8)

Figure 1 also shows the effect of ratio glucomannan-alginate on the swelling, in which the swelling was higher in the combined matrix than that of the lone glucomannan. Wang et al. [7] reported lower swelling index of lone glucomannan was due to the irreversibility of the glucomannan bead to swell after bead-drying process. Blending both compounds allowed more surface gap of these mixed hydrogels and provided more space to store more water. Increasing vitamin C concentration led to decrease the swelling in both pHs. Higher concentration of vitamin C to be encapsulated resulted in more vitamin C filled the excipient gap since molecule size of vitamin C was smaller than that of both excipients. This occupation reduced the free space which was available for storing the water. As a result, increasing vitamin C reduced the swelling index.

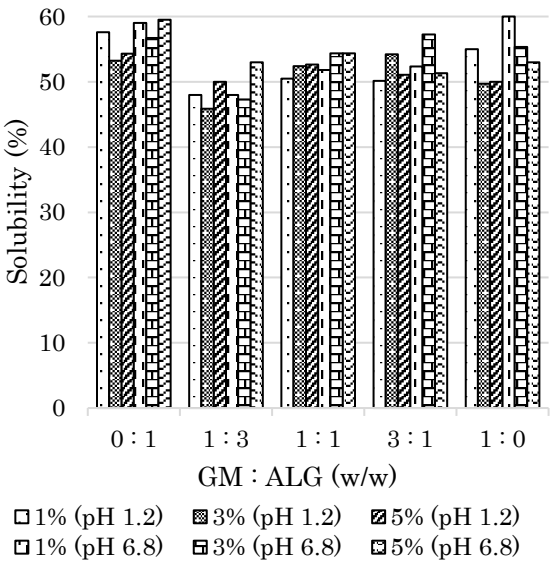


Fig. 3: Effect of ratio glucomannan–alginate and concentration of vitamin C on solubility at HCl solution (pH 1.2) and phosphate buffer solution (pH 6.8)

Furthermore, reducing ratio of glucomannan-alginate also reduced the swelling value. This swelling reduction could be due to the increasing number of carboxylic group of alginate. This functional group allowed to form strong hydrogen bond and resisted to water penetration [14].

Figure 1 also depicts the excipient of 1:1 (w/w) of glucomannan-alginate ratio had the highest swelling value. This swelling value could be due to the balance molecule interaction between glucomannan and alginate and hence maximise the water absorption. This result was supported by Zhen et al. [15]. They simulated energy variety in the glucomannan-alginate composite and found a large number of hydrogen bonds was formed not only between each glucomannan and alginate molecule but also existed among respective molecule of each polymer. These bonds led to better synergistic effect because the chain segments of glucomannan surrounded the alginate molecules spirally and irregularly, leading to the strong nonbonding intermolecular forces [15].

Regardless the encapsulation method, lower swelling index was observed at acidic solution than that of neutral one (Figure 2). The second encapsulation method showed higher swelling than other methods. In this method, vitamin C was firstly encapsulated with glucomannan before coating with alginate. This outer layer was cross-linked with $CaCl_2$ during bead formation. This divalent

cation helped in forming hydrogen bond with water during swelling determination and resulted in higher swelling index. The highest swelling was achieved in the second method preparation with similar amount of each polysaccharides (1:1, w/w). This similar ratio allowed to maximize the interaction of hydrogen bond and the water absorption.

3.2 Solubility

Effect of glucomannan-alginate ratio and vitamin C concentration on the solubility of the encapsulation in 2 pHs is presented in Figure 3. Glucomannan and alginate are hydrophilic polysaccharides and water soluble. Solubility of alginates is influenced by the total ionic strength of the solution, the free calcium concentration, and pH of the solvent [16]. Fan et al. [9] reported a strong interaction of glucomannan-alginate combination affects on their miscibility. The presence of acetyl groups was responsible for glucomannan solubility [17]. Moreover, the quantity of hydrogen bonding was also affected by the number of hydroxyl groups in the glucomannan [18].

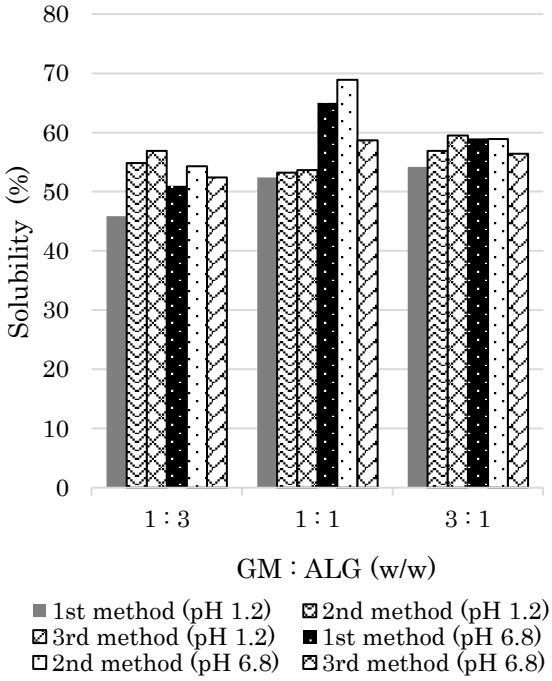
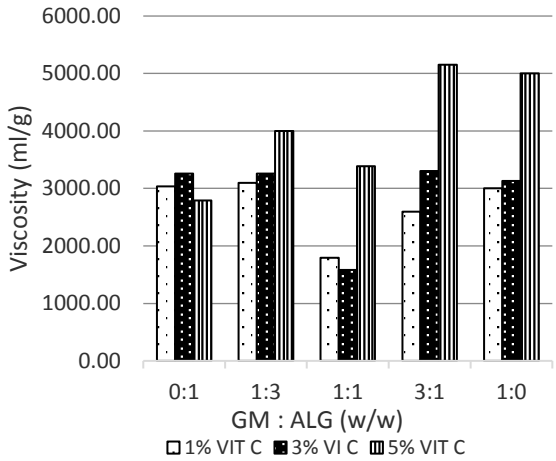


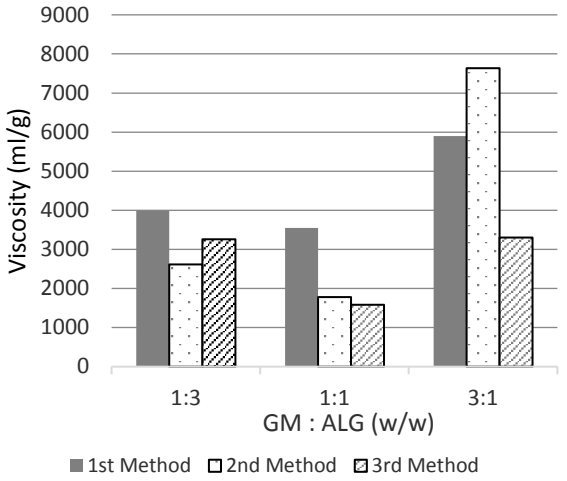
Fig. 4: Effect of encapsulation preparation on solubility at HCl solution (pH 1.2) and phosphate buffer solution (pH 6.8).

The solubility of the mixed matrix was lower in acidic solution than that of pH 6.8 (Figure 3). Alginate has carboxylic acid groups (RCOOH) which are protonated in alkaline conditions. It produced negative charges which attract more water to penetrate and result in instability of bead. As a consequence, the solubility of alginate increased in higher pH solution.

However, alginate was insoluble under acidic condition. The number of ions positive in acidic solution was higher than in higher pH solution. This higher positive charged decreased the electrical repulsion between negatively charged alginate molecules and resulted transformation of alginate into an insoluble form of alginic acid. This condition reduced penetration of solvent during solubility determination [6]. Moreover, combination of glucomannan and alginate decreased solubility. Mohamed et al. [6] reported this combination led to synergistic interaction between these polymers hence enhance the stability of the bead. As a result, lower solubility was observed in the mixed excipient.



(a)



(b)

Fig. 5: Effect of glucomanna-alginate ratio, concentration of vitamin C (a) and encapsulation method (b) on viscosity of combined excipient.

Figure 4 describes the solubility of vitamin C encapsulation which prepared by various preparation methods. In general, the solubility of the beads was lower in acid solution. The combination matrix which mixed with the vitamin C (method 1) had lower solubility than other methods. This could be due to hydrogen bonding which formed not only intern of the glucomannan or alginate molecules but also extern between glucomannan and alginate molecule. This result showed that combination matrix allows the synergistic effect to form the hydrogen bonding and reduced the solubility. Similar result was reported by Fan et al. [9]. On the other side, vitamin C encapsulated with alginate followed by glucomannan as the outer layer (method 3) had higher solubility. The solubility of glucomannan relies on the acetyl groups which found randomly in low degree at C6 position [4]. Since glucomannan is neutral soluble material, the effect of bead-forming through ionotropic gelation using $CaCl_2$ could not as strong as that on alginate. Alginate is a polyanionic copolymer which forms rigid bead through the addition of divalent cations in aqueous liquid [6].

3.3 Viscosity

Viscosity represents a resistance of a fluid to flow. Figure 5 describes the effect of the combined matrix and encapsulation method on viscosity. In general, 5% of vitamin C in various matrix ratio depicts the higher viscosities. It seemed that the viscosity was not only affected by the total amount of the matrix. High concentration of vitamin C also improved the viscosity. Moreover, the synergistic effect of the matrix, as well as the interaction between the matrix with the active compounds, could contribute to the final viscosity of the mixtures.

The highest viscosity was achieved by second preparation method of 3:1 (w/w) of glucomannan-alginate ratio (Figure 5-bottom). This could be because in preparation of viscosity determination, the beads swelled as explain in 3.1 section. This swelling was followed with broken off the outer layer and resulted in dissolving the glucomannan matrix. Glucomannan is knows as one of the high viscosity compound. Since the concentration of the glucomannan was three times higher than other ratios, hence the viscosity of 3:1 glucomannan-alginate ratio increased significantly.

4. Conclusion

The impact of ratio of glucomannan-alginate on swelling at both pHs was similar with that on solubility. The higher swelling index was observed at 1:1 (w/w) glucomannan-alginate of all preparation methods in both pH conditions. This ratio of the second method preparation had the highest solubility in pH 6.8. The highest viscosity was observed in the beads which prepared with the second method of higher glucomannan ratio.

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