Calcium modified edible Canna (*Canna edulis* L) starch for controlled released matrix

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Abstract Canna edulis L starch was modified with calcium chloride in order to form controlled released matrix. Present study aim to analyze modified starch characteristic. Four different formulation of ondansetron granules was used to provide dissolution profile of controlled released, two formula consisted of 15% and 30% modified starch, one formula utilized matrix reference standards and the last granules was negative control. Methocel-hydroxypropyl methyl cellulose was used as controlled released matrix reference standards in the third formula. Calcium starch was synthesized in the presence of sodium hydroxide to form gelatinized mass and calcium chloride as the cross linking agent. Physicochemical and dissolution properties of modified starch for controlled released application were investigated. Modified starch has higher swelling index, water solubility and compressibility index. Three of four different formulation of granules provide dissolution profile of controlled released. The profiles indicate granules which employed calcium Canna edulis L starch as matrix are able to resemble controlled drug released profile of matrix reference, however their bigger detain ability lead to lower bioavailability.

1. Introduction

Canna indica L or known as achira is a perennial plant from South America, which it cultivation has expanded to Asia. Apart from being known as top 25 source of starch, considerable studies have suggested that achira rhizomes have immense potential not only for functional ingredient in food industry but also source of biopolymer for pharmaceutical industry [1]. Canna have been proven as promising biomaterial for industrial application based on their characteristic similarities with cassava starch that common for this utilization [2]. Starch is a familiar term for complex carbohydrate, $(C_6H_{10}O_5)_x$, that can be found in the seed, bulbs and tuber of plants, composed of plentiful of atoms which corresponding to value of x, as given formula [3]. Native starch denotes for untreated starch, that can be customize through physical, chemical and enzymatic methods to enhance their functional properties as called modified starch.

Starch polymer chain are commonly cross-linked with two- or poly-function compounds such as adipic acid, citric acid or phosphoric acid, to increase polymer molecular weight, stabilized granule structure, high temperature, acidity or shear resistant paste, whereas the result depend on material and method [4,5]. Calcium chloride are also a cross-linked agent, that usually use to modified alginate or pectin, adopt to bring the same effect for *Canna edulis* L starch and studies their properties and function

as granule matrix utilize it gelling properties. Studies about utilization of calcium chloride as starch cross linking agent and it influence to starch physicochemical properties are still limited. Chowdary and Sundari (2008) was studied the utilization of calcium chloride as cross linking agent for potato starch and using the modified as matrix for diclofenac granules, but the information about characteristic of modified starch are still limited [6]. Ondansetron, for preventing postoperative nausea and vomiting and also prescribe for preventing nausea caused by pregnancy [7]. Controlled released formulation of ondansetron, aimed to tailoring drug released rate for increase patient compliance by reducing drug bioavailability and intake frequency [8].

In this study three of four formulation of ondansetron granules containing matrix granules were prepared, two formulas employing two different concentration of calcium *Canna edulis* L starch, and methocel-hydroxypropyl methyl cellulose at the third as controlled released matrix reference standards. The fourth formula set as negative controlled which only consisted drug and diluents. All formulas were evaluated for controlled release ability by analyzed drug dissolution profiles. The influence of calcium chloride as cross linking agent for *Canna edulis* L starch will be observed by physicochemical properties determination, included amylose content analysis, swelling index, water solubility, compressibility index, Hausner ratio and powder flow rate. The results shown that calcium modification on *Canna edulis* L starch influence it characteristic and dissolution profiles indicate that the modified starch regulated drug release in dissolution media.

2. Materials and Methods

2.1. Materials

The initial raw material, native *Canna edulis* L starch was isolated from achira rhizomes from Cibogo, West Java, Indonesia. Sodium hydroxide, calcium chloride, ethanol, acetic acid, iodine, hydrochloric acid, lactose monohydrate and amylum (soluble starch) were analytical grade reagents from Merck®. Methocel-hydroxypropyl methyl cellulose (Methocel K15M) also pure chemical from Sigma-Aldrich, Ondansetron hydrochloride di-hydrate was a give from PT. Combiphar Indonesia. Distilled water was used throughout the work.

2.2. Preparation of Cross-linked Starch

Cross-linked starch was prepared according to the method of Chowdary and Sundari (2008) [6]. Starch slurry from 5 part of *Canna edulis* L starch was dispersed in 50 part of distilled water, added with 10 % w/v NaOH. Solution was mixing for 30 min or continued until thick gelatinized mass was formed. A 300 mL $CaCl_2$ (20 % w/v) was added vessel contained with gelatinized mass while stirring at 1000 rpm stirrer. The stirring was remained for 1 h to precipitate calcium starch. The modified starch was collected

by vacuum filtration, washed repeatedly with distilled water and dried at 80ºC. Dried starch samples were then grounded in a mortar and sieved (mesh No. 80).

2.3. Physicochemical Analysis

2.3.1. Starch Amylose Content *Amylose Content Determination.*

was determined using calorimetric method [9]. Complex solution was obtained from 100 mg sample added with 1 mL 95 % ethanol and 9 mL 1 N NaOH, placed in 200 mL volumetric flask. The sample was heated for 10 min in water bath to gelatinized the starch, cooled and add with distilled water to up volume. A 5 mL aliquot was transferred to 100 mL volumetric flask, to which was added 1 mL 1 N CH_3COOH and 2 mL iodine solution, and the volume was made up to 100 mL with distilled water. Solution was homogenized by shaker and color intensity was determined by spectrophotometric method, that based on Beer's law, correspond to amylose content of samples, using a UV/VIS spectrometer (Shimadzu UVmini-1240).

2.3.2. Swelling Index and Water Solubility.

An aqueous solution of sample starch was made from 1 g of starch per 100 g of solution. The suspension was steady shaken for 30 min at 90ºC in thermostatic water bath (Memmert). Subsequently it was centrifuged for 10 min using 80-2 Centrifuge at 3000 rpm. In separated supernatant, dry powder was determined gravimetrically, and the precipitate remaining in the centrifuge tubes was weighed [10].

2.3.3. Compressibility Index and Hausner Ratio.

Bulk and tapped density were resolute using 100 g sample. The bulk volume was measured after manually tapping the cylinder two times on flat table top surface. The tapped volume was amount with the Electrolab ETD-1020 Tapped Density Tester after tapping in increments of 10 and 500 taps. The bulk and tapped densities were used to calculated the Carr's compressibility index (Eq. 1) and the Hausner ratio (Eq. 2) to provide a measure of the flow properties and compressibility [11].

$$\mathrm{CI} = \frac{\rho_{tap} - \rho_{bulk}}{\rho_{tap}} \, x \, 100 \qquad (1)$$

$$\mathrm{HR} = \frac{\rho_{tap}}{\rho_{bulk}} \qquad (2)$$

Where ρ_{tab} is the tab density and ρ_{bulk} is the bulk density.

2.3.4. Flow Rate

Sample flow ability was defined by angle formed by the horizontal base of the bench surface and the edge of a cone-like pile of granules known as angle of repose. Stainless steel funnel with 15 mm orifice and 120 mm tall was used, placed 16 cm above the surface. After a cone from 100 g of samples was

build, height (h) and radius (r) of the granules forming of cone of the base were measured. The angle of repose (θ) was calculated as follows:

$$\theta = \tan^{-1}\left(\frac{h}{r}\right) \quad (3)$$

2.4. Granule Preparation

Each pack of 600 mg granules contains of 16 mg of ondansetron hydrochloride di-hydrate were prepared using calcium starch and methocel K15M as matrix in different proportion, accept for control formula. The required quantities of drug matrix materials were mixed thoroughly in a mortar by following geometric dilution technique. Mucilago amylum was employing as binder and lactose as filler to form a dough mass and dried until mass water content less than 3 % afterward it passed through sieve. The mass was dried at 50^{o}C and later it passed to mesh No. 16 to obtain granules.

2.5. Ondansetron Determination

Ondansetron was estimated by spectrophotometric (Shimadzu UV mini 1240) method based on measurement absorbance at 310 nm. The method obeyed Beer's law in the concentration 6-18 μg/mL.

2.6. Drug Release Study

Drug release from matrix granules was studied using 6 station dissolution tester (LID-6, Vanguard) paddle type. A 500 mL HCl 1 N was use as dissolution media, employing a paddle stirrer at 50 rpm and temperature set at 37±0.5^{o}C. For different time intervals over period of 8 h, filtered samples of 5 mL of each station were withdrawn. For comparison, ondansetron release from methocel K15M and control, granules without matrix, are also studied.

2.7. Statistical Analysis

All experiments were performed in triplicate and Statistical Package for the Social Science (SPSS, Version 20.0) was used to statistically analyzed the result. The results were express as the mean ± SD and Paired T-test analysis express with alphabet notation (a and b) to indicate data significant differences ($P>0.05$).

3. Results and Discussion

3.1. Calcium Starch Characteristic

The modified *Canna edulis* L starch was successfully synthesize resulting off white odorless powder, which when dissolved in water giving alkaline pH. Physicochemical determination of modified starch was conduct to learn starch characteristic change that caused by forming of cross liked in starch molecules.

3.1.1. Amylose Content.

Blue color solution is formed form long branch chains amylopectin, like amylose, that bind with iodine configure a single helical complex during titration. The amount of amylose founded in calcium starch was about 26 percent lower than native (Table 1). Richard and William study found that the swelling behavior of starch is base on the property of its amylopectin content, and amylose acts as both a diluents and an inhibitor of swelling [12]. The maximal swelling are also related to the molecular weight and the shape of amylopectin [13]. The data shown in Table 1 indicate that most of swelling and water solubility of *Canna indica* L starch mostly influent by the shape of amylopectin, since higher amylose and lipid content can strongly inhibited swelling at all temperatures.

Table 1. Starch physicochemical characteristic before and after modification.

Physicochemical	Native starch	Calcium starch
Amylose content (%)	57.28±1.40[a]	31.04±0.20[b]
Swelling index (w/w)	2.13±0.10[a]	3.44±0.11[b]
Water solubility (%)	2.44±0.84[a]	17.47±0.60[b]
Compressibility index (%)	16.89±0.83[a]	23.81±0.61[b]
Hausner ratio	1.20±0.01[a]	1.29±0.03[b]
Angle of repose (θ)	Do not flow[a]	Do not flow[a]

All values are means of triplicate determination ± standard deviation

3.1.2. Swelling Index and Water Solubility.

Cross linking modification starch characteristic will be effected by chemicals and raw material [5]. Cross linked starch using different chemical and native starch tends to increasing starch structure crystallinity [14], lowering solubility [15] likewise the swelling index [16]. Other studies generate water insoluble characteristic [3] and higher degree of substitution will increased the effect [17]. Table 1 shown both swelling index and water solubility of native to modified starch have increasing value. Results presented the impact of cross linked agent gives unlikely outcome, the lower amylose content of calcium starch exhibit higher swelling index as well as water solubility. This findings can be affected by branch chain lengths distribution of amylopectin and X-ray type of starch which contribute to heterogeneous effect [22]. Characteristic of *Canna indica* L amylopectin allows starch to interact more with water molecules. Modification of calcium chloride provide the change of amylose and lipid solubility behavior in the granule, which allow the amylopectin to stretch and start starch gelatinization led to increase swelling volume and higher water intake. Starch swelling index can be used to foreseen gelatinization capability that will be exploited at controlled release mechanism. Increasing of molecular structure enlargement proficiency in water environment expected to prolong the drug-matrix binding, consequently time of release.

3.1.3. Flow Rate

Powder flow is a key requirement for pharmaseutical manufacturing process to understanding mixing, packaging and transportation. The compendium methods include several measurements among others are angle of repose, compressibility index and Hausner ratio [11]. The cross linked produced by calcium chloride upsurge starch compressibility index and Hausner ratio. Increasing value of both characteristics will aggravated powder flow rate. Both native and modified starch angle of repose cannot be identified because they were not able to form the cone in the base surface, both were not surge through the V-funnel. All three test indicate that modification enlarge starch particles size so it will takes higher force when compressing granules into tablet, raise the Hausner ratio and lowering the powder flow ability. This inability indicate that both starch will be suitable for wet granulation method in tabletization [18].

3.2. Drug Analysis

3.2.1. Ondansetron Determination

Amount of ondansetron in granules was estimated by UV spectrometric method based on the measurement of absorbance at 310 nm [19]. Series of concentration of reference ondansetron hydrochloride di-hydrate solution obtained valid linearity, $y=0.0407x + 0.0138$.

3.2.2. Release Profile

Dissolution profile of a granule or tablet depends on dissolution condition, composition, surface and hardness, among other factors [21]. Concentration of ondansetron that release from granules studied using dissolution apparatus with hydrochloric acid as dissolution liquid to mimicking digestive system. All four formulations of ondansetron, diluents and matrix was shown in Figure 1. Total drug that release from F1 was peaking immediately after 5 min and descent in line with increasing of time. Other formulation produces different outline where after the same onset drug release in constant rate.

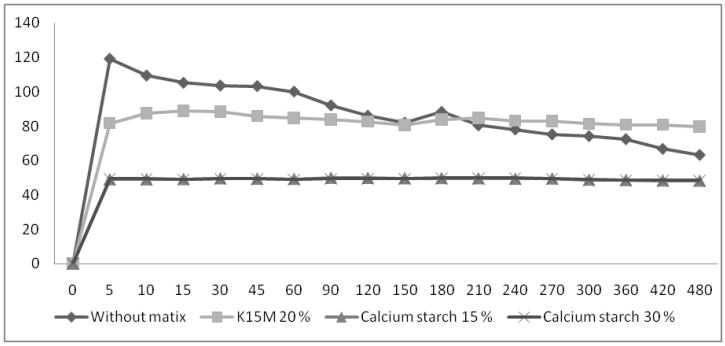

Figure 1. Ondansetron release from granule from four different formula shown that modified starch have controlled release profile.

The second formulation where metochel K15M employing as matrix give the appropriate profile as controlled released, more that 80 % of drug was in dissolution fluid. Although the profile of modified starch shown linier controlled profile but since the proportion of drug in liquid was less that level of requirement the formula optimization is still needed.

4. Conclusion

Calcium starch was synthesized from achira rhizome employing new cross linking agent produced off white and odorless powder with homogeneous particle size obtain by sieve with mess No. 80. The cross linked starch possesses a lower portion of amylose than native. Physicochemical study of the modified starch indicates that it suitable for controlled release matrix, with higher swelling index and water solubility. Based on the contradiction between amylose content and swelling behavior, indicate that in *Canna indica* L starch characteristic more affected by the shape of amylopectin.

Ondansetron granules are made with four different formulations, where two of it utilizing calcium starch as controlled released matrix. Drug release study was obtaining by observe granules dissolution profiles. Most of the water molecules interact with amylopectin structure, the entrapping mechanism inhibit by the strength of cross linked effect at the granules. The dissolution profiles indicate granules which employed calcium Canna edulis L starch as matrix are able to resemble controlled drug released profile of matrix reference, however their bigger detain ability lead to lower bioavailability.

References

[1] Andrade-Mahecha M M *et al* 2015 *Carborhydates* **123** 406–15.
[2] Valencia G A *et al* 2012 *J. Polym. Eng.* **32** 531–37.
[3] Jubril I, Muazu J and Mohammed G T 2012 *J. of App. Pharm. Sci.* **2**(2) 32–6.
[4] Wattanachant S *et al* 2003 *Food Chem.* **80**(4) 463–71.
[5] Kapelko M *et al* 2015 *Food Chem.* **167** 124–30.
[6] Chowdary K P R and Sundari P T 2008 *Int. J. Chem. Sci.* **6**(3) 1189–95.
[7] Tramèr M R *et al* 1997 *Anesthesiology* **87** 1227–89.
[8] Bhowmik D *et al* 2012 *The Pharma Innovation* **1** 24–32.
[9] William P C *et al* 1970 *Cereal Chem.* **47** 411–21.
[10] Kapelko M *et al* 2012 *Food Chem.* **135** 1494–1504.
[11] Rakhi B S *et al* 2008 *AAPS Pharm. Sci. Tech.* **9**(1) 250–8.
[12] Tester R F and Morrison W R 1990 *Cereal Chem.* **67(6)** 551–7.
[13] Alcázar-Alay S C, Meireles M A A *Food Sci. Technol.* **35(2)** 215-36.
[14] Atichokudomchai N and Varavinit S 2003 *Carbohydrate Polymers* **53(3)** 263–70.
[15] Yeh A I and Yeh S L 1993 *Cereal Chem.* **70(5)** 596–601.
[16] Hoover R and Sosulski F 1986 *Starch* **38(5)** 149–55.
[17] Koo S H *et al* 2010 *Food Hydrocolloids* **24** 619–25.
[18] Visavarungroj N *et al* 1990 *Int. J. of Pharmaceutics* **59(1)** 73–8.
[19] Dibbern H W 2002 UV and IR spectra: pharmaceutical substances (UV and IR) and pharmaceutical and cosmetic excipients (IR) (Aulendorf Germany).

[20] Smith A A *et al* 2009 *J. of Pharm. Sci. and Research* **1(4)** 48–54.
[21] Blanco M *et al* 2006 *J. Pharm. Sci.* **95(10)** 2137–44.
[22] Jane J *et al* 1999 *Cereal Chem.* **76(5)** 629–37.