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Research Article

ENZYMATIC DEGRADATION OF CROSS-LINKED EXCIPIENT MATRIX OF CO-PROCESSED XANTHAN GUM-AMYLOSE AND DISSOLUTION PROFILE OF DICLOFENAC SODIUM TABLET

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ABSTRACT

Objectives: This study aims to determine the amount of excipient that is degraded by alpha-amylase and the influence of alpha-amylase to the dissolution profile of sustained-release tablets that use matrix CL-Co-A-XG.

Methods: Excipient is cross-linked with two concentrations of sodium trimetaphospate, which are 6% (CL6-Co-A-XG) and 12% (CL12-Co-A-XG). Each excipient is made with the ratios 1:1, 1:2, and 2:1 amylose-xanthan gum. Enzymatic degradation tests are performed on excipient powders for 60 minutes. Sustained-release tablet with CL-Co-A-XG excipient as a matrix is formulated through direct compression method. Then, drug dissolution tests are performed in a phosphate buffer with a pH of 7.4 both using and without using alpha-amylase as a medium for 8 hrs.

Results: The results of this study show that CL6-Co-A-XG and CL12-Co-A-XG degraded 20% at 10 and 30 minutes, respectively. In addition, the release profile of F1-F6 tablets show the sustained-release profile that follows zero-order and Korsmeyer–Peppas kinetics and is unaffected by the presence of alpha-amylase.

Conclusions: From this study, it can be concluded that the CL-Ko-A-XG excipients are more resistant to enzymatic degradation than amylose. Therefore, this excipient shows potential as a single matrix sustained-release tablet.

Keywords: Cross-linked of excipient co-processed xanthan gum-amylose, Alpha-amylase, Dissolution profile, Enzymatic degradation, Sustained-release tablet.

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INTRODUCTION

Oral administration of drugs is a frequently used route of administration. Various forms of pharmaceutical preparations are developed around it. One of the fastest growing oral preparations today is the sustained-release tablet. Sustained-release tablet preparations contain two or more doses of a drug, which are then released over time. The sustained-release preparation aims to decrease the frequency of drug administration for drugs with short half-lives. Reduced frequency of drug delivery will improve patient compliance and reduce the risk of fluctuations in blood levels when using the drug. The popular system used in sustained -release drugs is the matrix system. The polymers used in the preparation of the sustained-release preparation matrix should have characteristics that allow it to regulate its release of the drug and keep the form of the matrix during the drug's release [1].

Polysaccharides have many hydroxyl groups, which cause the polysaccharides to have a high affinity for water. The hydroxyl groups in the polysaccharides make it possible to be modified both physically and chemically. Various polysaccharides have been modified and can be used as sustained-release tablet excipients; one of which is amylose. In his research, Ariani and Surini made such a modification by performing an amylose coupling cross-linked with xanthan gum to improve the ability of excipients in maintaining their shape [2]. Co-processes are physical modifications of the excipient by combining two or more excipients, thereby producing excipients having better physical properties. Xanthan gum is a polysaccharide that is resistant to enzymatic degradation and has a good expanding index. Based on these studies, drug release tests from tablets with a xanthan gum-amylose cross-linked formula showed a slowdown in drug release [2].

Xanthan gum-amylose crosscut exchanger excipients have many functional advantages, but the amylose contained in cross-linked xanthan gum-amylose cross-linking excipients is a substrate of alpha-amylase. Alpha-amylase is an enzyme produced by the pancreas and is present in the human digestive tract, especially the small intestine. Alpha-amylase will hydrolyze the α-D-bond (1→4) straight chain amylose glycosidic. Amylose degradation occurring in the presence of alpha-amylase within the human body may affect the dissolution of sustained-release tablets by accelerating drug release from the preparation. In this research, dissolution of sustained-release tablets in a medium containing alpha-amylase, with diclofenac sodium as the drug model, will be tested. The dissolution test was carried out in accordance with those listed in the USP 32 monographs of diclofenac sodium sustained release tablets, which was added α-amylase. In addition, an enzymatic degradation test of cross-linked xanthan gum-amylose crosscurrent excipients is performed at pH and at similar conditions with the dissolution medium. By doing this research, we hope to know the amount of enzyme-degradable excipients as well as the effect of alpha-amylase on the tablet dissolution profile.

METHODS

Tools

Following tools that were used for this study: Drum dryer (R. Simon Dryers, Nottingham, England); vibrator mill (Retsch, Haan, Germany); a sifter set (Retsch, Haan, Germany); ultraviolet (UV)-1800 spectrophotometer (Shimadzu, Kyoto, Japan); pH meters Eutech pH 510 (Eutech Instruments, Singapore, Singapore); analytical balance of Adam AFA - 210 LC (Adam Equipment, Connecticut, United States); thermal analyzer DSC 6 (Perkin Elmer, Ohio, United States); oven (Memmert, Schwabach, Germany); tablets (Korsch, Berlin, Germany); TAR-type friability tester (Erweka, Heusenstamm, Germany); TDT-08L type dissolution equipment (Electrolab, Mumbai, India); TBH 28 type hardness tester (Erweka, Heusenstamm, Germany); slurry term (Venier Caliper, Shandong, China); homogenizer EH 20112 (CKL Machinery,

Puchong, Malaysia); and moisture balance analyzer (Mettler Toledo, Giesen, Germany).

Materials

The materials used for this study were: Diclofenac sodium (Yung Zip Chemical, Taiwan); Amylose (Shangqiu Kangmedia Bio Tech, China); Xanthan gum (CV, Tristars Chemicals, Indonesia); sodium trimetaphosphate (STMP) (Shangqiu Kangmedia Bio-Tech, China); Alpha-amylase porcine (Sigma, Missouri, United States); Avicel PH 102, sodium hydroxide, hydrochloric acid, sulfuric acid, nitric acid, ammonium molybdate tetrahydrate, sodium hydroxide, sodium chloride, sodium bicarbonate, aqua destillata (Merck, Darmstadt, Germany); potassium dihydrogen phosphate, 96% ethanol (Brataco, Jakarta, Indonesia); ascorbic acid (Takeda, Tokyo, Japan); and iodine and potassium iodide (Mallinckrodt, Surrey, London).

Making excipients cross-linked co-process amylose-xanthan gum

Amylose and xanthan gum, each with a concentration of 3%, are dissolved in distilled water. Amylose and xanthan gum are mixed at predetermined ratios (1:1, 1:2, and 2:1) and homogenized at 3000 rpm for 30 minutes. The homogeneous mass is then dried with a drum drier at a temperature of 80±5°C. The obtained layer or flakes are mashed and sieved with 35 mesh sieves.

A total of 1800 mL of aquadest is heated to a temperature of 30°C, and then amylose-xanthan gum processed excipients, as much as 140 g, is added in by stirring with a stirring magnetic until completely dispersed. A 10 N sodium hydroxide solution is used to maintain the pH during the reaction; pH 11-12. To each batch added 8.4 g of STMP dissolved in 132 mL (6.6% of total aquadest) as a cross-linking agent, with continuous stirring using a homogenizer at a rate of 3000 rpm. The reaction continues for 1-4 hrs, and then stands for 12 hrs until the pH levels out. Then, the suspension is neutralized by adding a solution of 7 N hydrochloric acid to pH 6. The solid is decanted and washed with ethanol; the washing continues until the filtrate gives negative results with the ammonium molybdate reagent (non-intensive yellow). The resulting powder is dried at room temperature for 48 hrs. The result produces granules and is sieved with 35 mesh sieves. The experiment is repeated, but with using 16.8 g of STMP.

Degree of substitution

The determination of degrees of substitution is carried out chemically at CL-Ko-A-XG excipients with concentrations of STMP 6% and 12% dysfunction agents. The amount of phosphate substitution in STMP as a cross-linking agent is determined through colorimetric method. The reagents used are reagent A (10% ascorbic acid solution), reagent B (0.42% ammonium molybdate tetrahydrate solution in 1 N sulfuric acid), and mixed reactants (mixture of 1.0 mL of reactant A and 6.0 mL of reactant B).

A total of 100 mg of the sample is dried in a furnace at 600°C to ash. The ash is cooled and added into 8.0 mL of 1N sulfuric acid. Then, the sample ash is heated to a boiling water bath for 10 minutes to ensure solubility and also to ensure the phosphate conversion formed during the inbreeding into inorganic phosphate. Filtrate is filtered using Whatman 40 and transferred to a 10.0 mL measuring flask, to which aquadest and 0.1N sulfuric acid (1: 1) are then added. The 1.0 mL filtrate was pipetted and combined with aquadest and 0.1 N sulfuric acid (1: 1) to 10.0 mL. The sample is piped in at 3.0 mL and added 7.0 mL of the mixed reagent. The sample in the test tube is then shaken and incubated in a water bath at 45°C for 20 minutes and then cooled.

The blank is prepared by taking as much as 3.0 mL of aquadest mixture and 0.1N sulfuric acid (1:1) and combining 7.0 mL of the mixed reagent, and is then treated equally from the shaking stage. The absorption of the sample, blank, or standard, is measured by a viscous spectrophotometer at a wavelength of 820 nm because the sample has a blue color. Interpretation of phosphate concentration is done by extrapolating the uptake on a standard calibration curve made from the standard solution of potassium dihydrogen phosphate (100 µg P in 1 mL). Calibration curves are made at concentrations of 2.0 ppm, 5.0 ppm, 10.0 ppm, 15.0 ppm, 20.0 ppm, and 25.0 ppm. The standard solution is piped at 3.0 mL and combined with 7.0 mL of the mixed reagent. The solution is shaken and incubated in a water bath at 45°C for 20 minutes, and then cooled.

The amount of substitution is determined using the following formula, where P represents% phosphorus (% P) of phosphorylated amylose [3]. The number 162 is the molecular weight of one glucose unit, the number 3100 is the molecular weight of phosphor (P) multiplied by 100, and 102 is the molecular weight of the linked phosphate calculated as the $-PO_3Na$ group.

$$DS=\frac{162P}{3100-102P}$$

Enzymatic degradation test of cross-connection matrix xanthan gum-amylose processing

Equivalent to 50 mg of amylose from each excipient, CL6-Ko-A-XG and CL12-Ko-A-XG are dispersed in ratios of 1:1 (100 mg); 1:2 (150 mg), and 2:1 (75 mg) in a 100 mL 0.2 M phosphate buffer of 6.7 mM with a pH of 7.4 at 37°C for 10 minutes with constant stirring at 50 rpm. The alpha-amylase from pig pancreases is 58.33 mg dissolved in 10 mL of NaCl phosphate buffer with a pH of 7.4; taken 1 mL then added to medium (17.5 UI/100 mL medium). The sampling takes 1 mL at 15, 30, 60, 90, 120, 180, 240, 360, and 480 minutes after incubation. Samples are mixed with 3 mL of HCl and 5 mL of 0.1 N of iodine, and then the sufficient volume went up to 10 mL the uptake is immediately measured at a wavelength of 580 nm.

$$\%\text{Residul Amylose}=\frac{\text{Residul Amylose}}{\text{Baseline Amylose}}\times 100$$

Formulation of sustained-release tablet diclofenac sodium

The mass of the tablets is 500 mg and is made through direct forging method. Diclofenac sodium tablet formula is described in Table 1.

Evaluation of sustained-release diclofenac sodium tablets

Physical appearance

Aspects considered in a tablet's physical appearance include its size, shape, color, and surface.

Uniformity of weight

A total of 20 tablets are weighed and then the average weight of each tablet is calculated. If weighed one by one, only two tablets will have a weight that deviates from the average weight more than what is specified by column A, and none weigh more than the average as denoted by column B [4] (Table 2).

Uniformity of size

A total of 20 tablets are chosen at random and then measured in diameter and thickness using a sliding range. Unless otherwise stated, the diameter is no more than 3 times and no <1 tablet thick [4].

Tablet firmness

Tablet firmness is determined using the friability tester tool. 20 tablets are cleaned of dust, weighed, and then fed into the friability tester. The friability tester tool runs at 25 rpm for 4 minutes (100 lap times). Tablets are cleaned of dust and then weighed again. The weight difference before and after the treatment is calculated, where the tablet weight loss cannot exceed 1% [5]. Tablet firmness can be calculated using the following equation:

$$\text{Rigidity}(\%)=\frac{W1-W2}{W1}\times 100\%$$

Test index expands slow tablet formula slow

The expanding index is tested using a tablet formula F1-F6 that has been printed into tablets, weighing ± 500 mg each. Each tablet is inserted into a Petri dish containing 10 mL of phosphate buffer medium with a pH of 7.4. The expanding index is measured through the increase in tablet weight, up to the 8th hr. Tablets are weighed at 15, 30, 60, 90, 120, 180, 240, 360 and 480 minutes.

Dissolution test of diclofenac sodium tablet with and without addition of alpha-amylase

Quantitation of diclofenac sodium content in tablets

Quantitation of the diclofenac sodium content in tablets is done through UV-visible spectrophotometry method. Tablet powder equivalent to 50 mg diclofenac sodium is weighed, incorporated into a 100.0 mL measuring flask, and then dissolved with 0.1 N sodium hydroxide up to the limit. It is then filtered and 10 mL of the first filtrate is discarded. After that the filtrate is piped in 2 mL and then put into a 100 mL measuring flask, with 0.1N sodium hydroxide added to the limit. The absorption is measured at its maximum wavelength. Meanwhile, to interpret the results, a calibration curve of standard BPFI diclofenac sodium is prepared with concentrations of 4 ppm, 6 ppm, 8 ppm, 10 ppm, 12 ppm, 14 ppm, and 16 ppm in 0.1 N sodium hydroxide. Tablets are said to qualify if they contain diclofenac sodium 90.0-110.0% of the value listed on the label.

Drug release (dissolution) test

The diclofenac sodium dissolution test of a sustained-release tablet of diclofenac sodium is carried out using a type-2 dissolution device that is a type of paddle and conducted in two mediums, that is, in a 900 mL medium of 0.2 M phosphate buffer pH 7.4 and 900 mL of 6.7 mM phosphate buffer medium at pH 7.4 containing alpha-amylase. The dissolution medium is maintained at 37±0.5°C with a 50 rpm stirring speed.

Samples are taken at minute 15, 30, 60, 90, 120, 180, 240, 360, and 480. Then, the amount of diclofenac sodium dissolved is measured using a UV-visible spectrophotometer at maximum wavelength (276 nm), using the phosphate buffer 0.2 M pH 7.4 solution as the blank control. If necessary, dilution of the filtrate has been taken [6].

Table 1: Diclofenac sodium tablet formula

Materials	F1	F2	F3	F4	F5	F6
Sodium diclofenac	75	75	75	75	75	75
CL6-Ko-A-XG (1:1)	300	-	-	-	-	-
CL6-Ko-A-XG (1:2)	-	300	-	-	-	-
CL6-Ko-A-XG (2:1)	-	-	300	-	-	-
CL12-Ko-A-XG (1:1)	-	-	-	300	-	-
CL12-Ko-A-XG (1:2)	-	-	-	-	300	-
CL12-Ko-A-XG (2:1)	-	-	-	-	-	300
Avicel PH 102	125	125	125	125	125	125
Total (mg)	500	500	500	500	500	500

CL6-Ko-A-XG: Cross-linked excipient with 6% STMP amylose-xanthan gum process. CL12-Ko-A-XG: Cross-linked excipient with 12% STMP amylose-xanthan gum process

Table 2: Weight uniformity requirements

Average weight	Average weight aberration in%	
	A	B
25 mg or less	15	30
26-150 mg	10	20
150-300 mg	7.5	15
More than 300 mg	5	10

Source: Ministry of Health Republic of Indonesia, 1979

The calculation of the cumulative amount of diclofenac sodium dissolved at a given time is:

$$W_t = V_1.C + V_2 \sum_{t_0}^{t(n-1)} .C$$

$$\%\,\text{Dissolved diclofenac sodium} = \frac{W_t}{W_0} \times 100\%$$

Information:

W_t = The cumulative amount of diclofenac sodium is de-dissolved at time t

W_0 = The amount of sodium diclofenac present in the matrix

C = Concentration of diclofenac sodium which is dissolved at time t

V_1 = Dissolution test fluid volume

V_2 = Volume of pipetted fluid.

Dissolution data are calculated using the drug release kinetics model. The test is also conducted to determine the dissolution mechanism that occurs.

RESULTS AND DISCUSSION

The synthesis of excipients of cross-connection xanthan gum-amylose processing is shown in Table 3. In addition, Table 4 shows the substitution degrees.

Enzymatic degradation test of excipients

Evaluation of sustained-release diclofenac sodium tablets, according to the mass evaluation of tablets, is shown in Table 5. Further, tablet weight uniformity, size uniformity, and tablet hardness are described in Tables 6-8, respectively.

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Dissolution test of diclofenac sodium tablet with and without addition of alpha-amylase, and the quantitation of diclofenac sodium content in tablets are shown in Table 9.

Release (dissolution) drug test

Fig. 1 shows that the drug release profile of F2 in the medium containing the enzyme is not affected by alpha-amylase. Formulas 1 and 3 have a slower drug release than those in a non-enzyme medium.

Table 3: The percentage yield of excipients synthesis of the amylose-xanthan gum process and xanthan gum-amylose cross-linked excipient exchanges

Excipients	Yield (%)
Ko-A-XG 1:1	70.89
Ko-A-XG 1:2	66.42
Ko-A-XG 2:1	69.83
CL6-Ko-A-XG 1:1	99.29
CL6-Ko-A-XG 1:2	98.60
CL6-Ko-A-XG 2:1	96.82
CL12-Ko-A-XG 1:1	96.14
CL12-Ko-A-XG 1:2	97.50
CL12-Ko-A-XG 2:1	96.03

Table 4: Data of % P and degree of substitution excipients CL6-Ko-A-XG and CL12-Ko-A-XG

Excipients	% p	DS
CL6-Ko-A-XG (1:1)	1.91±0.095	0.099±0.006
CL6-Ko-A-XG (1:2)	1.77±0.095	0.090±0.006
CL6-Ko-A-XG (2:1)	1.77±0.072	0.090±0.004
CL12-Ko-A-XG (1:1)	4.78±0.176	0.289±0.013
CL12-Ko-A-XG (1:2)	4.55±0.147	0.272±0.011
CL12-Ko-A-XG (2:1)	4.42±0.152	0.262±0.011

Table 5: Mass evaluation data of F1-F6 tablets

Formula	Flow rate (gram/second)	Repose angle (0)	Hausner ratio	Compressibility index (%)	Category
1	5.05±0.13	34.63±1.14	1.15±0.01	12.78±0.96	Good
2	5.59±0.11	33.96±1.14	1.15±0.01	12.78±0.96	Good
3	5.39±0.03	35.61±0.56	1.13±0.02	11.11±1.92	Good
4	5.97±0.12	29.74±0.00	1.17±0.01	16.89±1.31	Special
5	4.33±0.16	33.97±1.14	1.23±0.02	12.54±2.47	Good
6	4.94±0.32	31.98±0.20	1.16±0.01	13.89±0.96	Good

Table 6: The result of uniform weight evaluation of F1-F6 tablets

Formula	Weight (mg)	% Deviation
1	502.15±1.87	1.00±0.00
2	501.80±2.26	1.00±0.00
3	502.19±0.76	1.00±0.00
4	502.50±2.26	1.00±0.00
5	500.30±1.56	1.00±0.00
6	503.05±1.64	1.00±0.00

Table 7: Results of uniform size evaluation of sustained-release tablets F1-F6

Formula	Diameter (cm)	Thickness (cm)	Diameter/ thickness (cm)
1	1.22±0.00	0.33±0.04	3.68±0.05
2	1.22±0.00	0.33±0.00	3.66±0.05
3	1.22±0.00	0.33±0.00	3.67±0.05
4	1.22±0.00	0.33±0.00	3.67±0.05
5	1.22±0.00	0.33±0.00	3.67±0.05
6	1.22±0.00	0.33±0.01	3.66±0.05

Table 8: Results of evaluation of hardness and firmness of sustained-release tablets F1-F6

Formula	Roughness (kP)	Rigidity (%)
1	12.58±0.38	0.59±0.02
2	12.56±0.37	0.57±0.01
3	15.23±0.55	0.59±0.01
4	12.37±0.47	0.62±0.02
5	12.04±0.49	0.62±0.02
6	15.28±0.49	0.56±0.00

Table 9: Evaluation results of sustained-release tablets

Formula	Levels (%)
1	100.83±1.29
2	99.86±1.24
3	99.06±0.85
4	99.67±0.42
5	99.35±1.6
6	100.8±1.62

Table 10: Data of kinetic calculation of diclofenac sodium dissolution from F1-F6 matrix

Formula	Parameters	Zero order	Korsmeyer–Peppas
1	r	0.987±0.002	0.990±0.004
	k	0.182±0.002	0.873±0.083
	n		0.741±0.012
2	r	0.988±0.007	0.991±0.007
	k	0.103±0.006	0.765±0.231
	n		0.639±0.016
3	r	0.991±0.001	0.986±0.004
	k	0.165±0.004	0.746±0.065
	n		0.712±0.014
4	r	0.998±0	0.979±0.004
	k	0.097±0.003	0.688±0.006
	n		0.643±0.001
5	r	0.995±0.001	0.984±0.001
	k	0.095±0.006	0.705±0.016
	n		0.645±0.012
6	r	0.979±0.003	0.971±0.001
	k	0.435±0.016	0.677±0.016
	n		0.840±0.009

K: Drug dissolution constant for each equation, N: Peppas diffusion exponent

According to Fig. 2, the drug release profiles of the F4, F5, and F6 matrix tablets are not affected by the presence of enzymes. This is due to the use of high concentration cross-linked agents, which cause the tablet to be more resistant to enzymatic degradation. Data of kinetic calculation of diclofenac sodium dissolution are shown in Table 10.

DISCUSSION

In preparing this excipient, xanthan gum-amylose is processed first. It is cross-linked using STMP at a temperature of 300°C. Based on Cury *et al*., a temperature of 300°C is the optimum temperature for the cross-linking reaction [7]. The 3% w/v dispersion of Co-A-XG is then added, with 10 N NaOH solution used to maintain the pH during the reaction of 11-12. Then, the reaction is left for the next 12 hrs without stirring to ensure the reaction is complete. The reaction is declared complete if the pH has not decreased due to the presence of H^+ released from the phosphate substitution.

The alkaline pH conditions at the time of cross-linking are required to open the STMP ring into sodium tripolyphosphate. In addition, the amylose and/or xanthan gum hydroxyl groups are ionized and may attack the phosphate in sodium tripolyphosphate under basic pH conditions. The reaction produces amylose and/or xanthan gum pyrophosphate. Amylose and/or xanthan gum pyrophosphate may be attacked by an amylose hydroxyl group and/or xanthan gum, further resulting in amylose or di-xanthan gum phosphates [8].

After the reaction is complete, the mixture is neutralized using a 7 N HCl solution until it reaches pH 6. Neutralization with the addition of HCl aims to replace the previously lost H^+ amylose proton and does not react with STMP [7]. The neutralized solution mixture is washed using 96% ethanol through the decantation principle to dissolve short-chain polysaccharides. The CL6-Ko-A-XG and CL12-Ko-A-XG excipients of 1:1, 1:2, and 2:1 ratios are produced as fine, odorless, and slightly yellowish-white granules.

Substitution degrees

In this study, the degree of excipient substitution is calculated against the number of hydroxy groups of amylose and possibly xanthan gum substituted by the phosphate group. Amylose has 3 hydroxy groups in each glucose unit at positions C-2, C-3, and C-6. The most reactive and easily substituted hydroxy group is that at position C-6 since it is the primary alcohol [9].

The determination of degrees of substitution is performed through colorimetric method [10]. Inorganic phosphates resulting from the decomposition of organic substances in excipients with annealing react

Table 11. Data of kinetic calculation of diclofenac sodium release from F1-F6 matrix

Formula	Parameters	Zero order	Korsmeyer-Peppas
1	r	0.987±0.002	0.990±0.004
	k	0.182±0.002	0.873±0.083
	n		0.741±0.012
2	r	0.988±0.007	0.991±0.007
	k	0.103±0.006	0.765±0.231
	n		0.639±0.016
3	r	0.991±0.001	0.986±0.004
	k	0.165±0.004	0.746±0.065
	n		0.712±0.014
4	r	0.998±0	0.979±0.004
	k	0.097±0.003	0.688±0.006
	n		0.643±0.001
5	r	0.995±0.001	0.984±0.001
	k	0.095±0.006	0.705±0.016
	n		0.645±0.012
6	r	0.979±0.003	0.971±0.001
	k	0.435±0.016	0.677±0.016
	n		0.840±0.009

with ammonium molybdate to form the phosphomolybdate complex. The phosphomolybdate complex is reduced by ascorbic acid to produce a blue complex.

The degree of excipient substitution does not affect the physical characteristics of excipients, but it affects the functional characteristics of the excipients, namely, the expanding index, gel strength, and viscosity which will affect the ability to release the drug from the excipient matrix.

Enzymatic excipient degradation test

The degradation tests on CL6-Ko-A-XG and CL12-Ko-A-XG are each performed for 60 minutes because the absorption after that time frame did not fall within the 0.2-0.8 range, so the test was not sensitive and accurate. In amylose, the degradation test is also performed, but during minute 15 of the test, no amylose was discontinued because it had degraded; no calculations could be performed.

Fig. 3 shows the enzymatic degradation of 1:1, 1:2, and 2:1 CL6-Ko-A-XG as well as 1:1, 1:2, and 2:1 CL12-Co-A-XG excipients. It showed that CL12-K-A-XG degraded less than CL6-Ko-A-XG. This is because the amount of xanthan gum in the excipient is greater, where xanthan is resistant to enzymatic erosion [11]. In addition, not only does amylose undergo cross-linking but xanthan gum also does. The amount of xanthan gum needed to mask the amylose, thus inhibiting enzymatic degradation, is double.

The CL12-Ko-A-XG 1:2 excipient was degraded less than CL6-Ko-A-XG 1:2. In CL12-Ko-A-XG 1:2, the amylose remained at 66.80%, whereas CL6-Ko-A-XG 1:2 was 37.48% within 1 hr. Larger STMP concentrations lead to more cross-linking in amylose and possibly xanthan gum. Cross-linking decreases the enzyme's ability to enter into the excipient, so the higher the concentration of the shelling agent, the more resistant it is to enzymatic degradation. The bond strength of amylose provides a stable and acid-resistant structure, enzymatic hydrolysis, and heat and shear stress. Overall, the CL12-Ko-A-XG excipient degraded less than CL6-Ko-A-XG with the remaining amylose counts of 37.48-44.9% and 66.80-75.29% for 1 hr of testing. 20% of the CL6-Ko-A-XG and CL12-Ko-A-XG excipients had degraded at the 10 and 30 minutes marks, which in the experiments showed an 80% amylose residue. Thus, the concentration of STMP and the amylose-xanthan gum concentration ratio used affects the excipient enzyme degradation.

Making sustained-release diclofenac sodium tablets

The addition of Avicel PH 102 is used as a binder. This is because at the time of the tablet printing process using a tablet machine, tablets

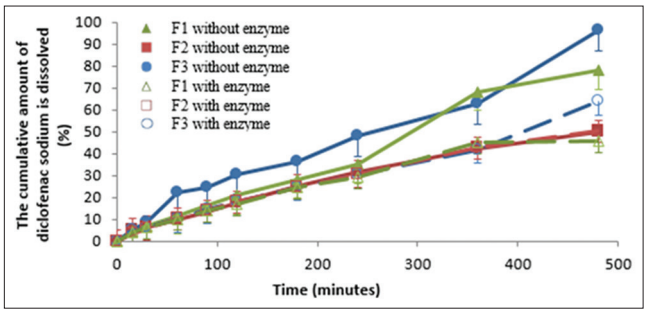

Fig. 1: Drug release profile tablets F1, F2, and F3. In phosphate buffer medium pH 7.4 without enzyme and with enzyme, each point illustrates the mean ± SD (n=3)

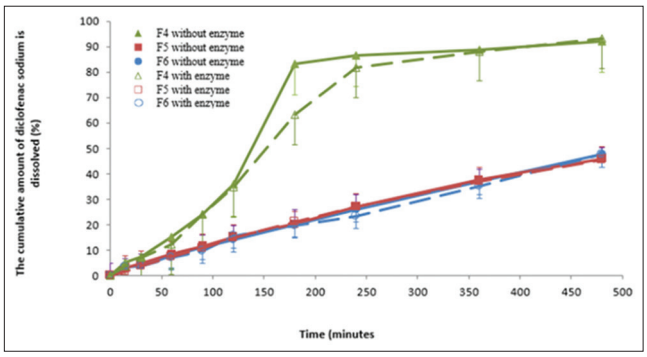

Fig. 2: Drug release profile tablets F4, F5, and F6. In phosphate buffer medium pH 7.4 without enzyme and with enzyme, each point illustrates the mean ± SD (n=3)

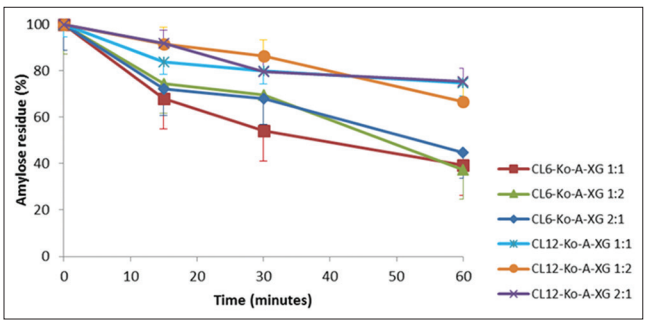

Fig. 3: Enzymatic degradation of excipients for 60 minutes. Each dots shows the average value (mean) ± SD (n=3)

that were not produced to meet the requirements of 10-20 kP hardness, so the tablet becomes fragile and cracked. In this sustained-release formulation, instead of being used solely as a binder, Avicel PH 102 also serves as a lubricant [11] that can prevent the tablets from sticking with punch and dye when printed using a tablet press. Powder evaluation occurs before the printing of tablets.

Mass evaluation of tablets

Mass evaluation is performed before tablet preparation. Evaluations performed include flow rate test, compressibility index test, repose angle test, and Hausner ratio test to assess the flow properties of the powder. Good flow properties will ensure weight uniformity. The better flow properties will ensure a full fill that is evenly distributed so that the weights are all uniform [12].

In addition to the flow rate, flow properties are also influenced by the angle of repose. The repose angle is the maximum angle that exists

between the surface of a powder stack and the horizontal plane, indicating the frictional force between the powder particles. A non-cohesive powder can flow well, spread, and form a low heap [12]. The smaller (sloping) the angle of repose, the better the flowing properties of a powder and vice versa [13].

Evaluation of sustained-released diclofenac sodium tablet

Physical appearance

Tablets are formulated as round, flat, brownish-white, and odorless. The physical appearance of tablets can be seen in Fig. 4.

Tablet weight uniformity

Based on Table 7, F1-F6 tablet evaluation results are eligible, thus there is no deviation from Column A (5%) and Column B (10%) from the average weight of each tablet formula, according to Pharmacopoeia Indonesia edition III.

Size uniformity

Based on Table 8, No formula meets the requirements of Pharmacopoeia Indonesia III edition because the tablet diameter is more than three times the thickness of the tablet. However, these requirements have been excluded on the IV Pharmacopoeia.

Tablet hardness

Hardness is useful as a physical control during the manufacturing process. Tablet rigidity is useful for knowing how resistant tablets are to shocks that occur during manufacturing, packaging, and distribution [14]. The hardness value of the tablet will generally be correlated with the randomness value of the tablet (Table 9). The harder a tablet is, the smaller the value of its rigidity, and *vice versa*. The hardness of the tablet is influenced by the amount of pressure exerted during production as well as the mass particle shape of the tablet. According to Table 9, these six formulas qualify where the weight loss in the assay test is no more than 1%, according to USP 30.

Index expands tablet formula

An index that expands a tablet formula in a medium may affect the release of the drug from the excipient to a particular medium. Testing of the expanding index is carried out on the formula of diclofenac sodium F1-F6 tablets on a phosphate buffer base with a medium pH of 7.4 for 480 minutes. This condition will show the correlation between index-expanded formulas of F1-F6 tablets with the tablets' drug release.

Fig. 5 shows the different indexes inflate each formula's tablet. This is related to the difference in tablet speed for hydrated and hydrogel strength of tablet formulations in the phosphate buffer with a medium pH of 7.4. In the tablet formulation using CL6-Ko-A-XG excipients, CL6-Ko-A-XG 1:2 (F2) has the smallest expanding index compared to CL6-Ko-A-XG 1:1 (F1) and CL6-Ko -A-XG 2:1 (F3). Tablet F2 has an expanded index value of 265.71%, whereas F1 and F3 have values of 407.20% and 451.98%, respectively. The formulation of tablets with CL12-Ko-A-XG 1:2 (F5) excipients show the smallest expansion index value of 238.88%, whereas CL12-Ko-A-XG 1:1 (F4) and CL12-Ko-A-XG 2:1 (F6) have index values of 281.41% and 803.95%, respectively. This is due to the greater amount of xanthan gum, where xanthan gum has a good expanding index in the medium [8].

The index inflated F4 tablet is smaller than F1 and F5 expands less than F2. The concentration of dysfunctional agents affects the index and expands the tablets. In the CL-Ko-A-XG excipient, the excipients are not only amylose but also cross-linked xanthan gum. In F4 and F5, the STMP concentrations used are greater than F1 and F2, so more hydrophilic groups are substituted by phosphates. Xanthan gum has gel strength and a good expanding index [11]. If the xanthan gum is dysfunctional, the xanthan gum chain bond will become stronger. Cross-linking xanthan gum will make the macromolecule matrix stiff so that the index expands the declining tablets [8].

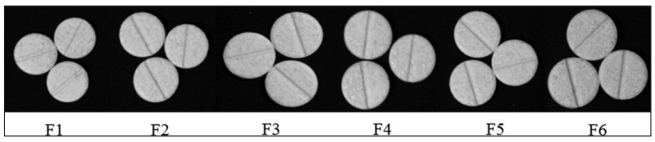

Fig. 4: Physical appearance of F1 tablets; F2; F3; F4; F5; and F6

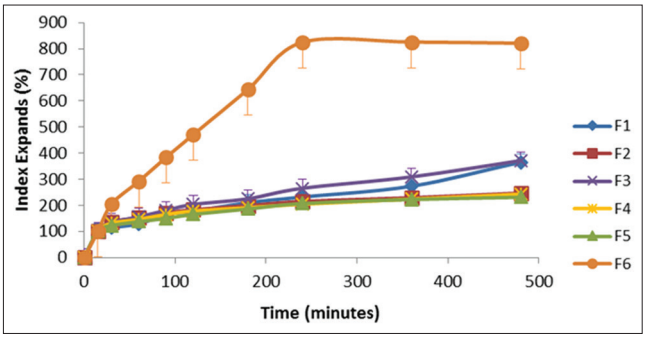

Fig. 5: Index inflate tablet F1-F6. Each point illustrates the mean ± SD (n=3)

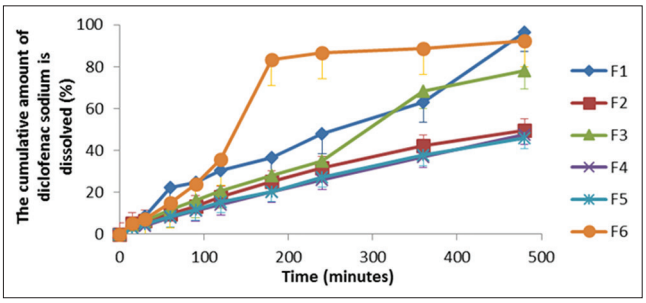

Fig. 6: Drug release profile of the formulation tablet with CL6-Ko-A-XG excipient (F1-F3) and with CL6-Ko-A-XG excipient (F4-F6) in a phosphate buffer buffer pH 7.4 without enzyme. Each point represents the mean ± SD (n=3)

Formula 6 has an expanded index larger than F3 and other formulas. This relates to the amount of amylose and STMP in the excipient. A greater the amount of amylose than the amount of xanthan gum (2:1) causes the excipient to be more easily hydrated, resulting in a larger expanded index. In addition, the STMP concentration used is greater than F3. Administrated amylose with low-to-moderate STMP concentrations will result in a closer chain linkage by strengthening hydrogen bonds with strong covalent bonds. At high concentrations of STMP, many glycemic bridges are found in chains, hydrogen bonds are blocked, and chain links are more loosely coupled with intermediate chain regulation, so the larger the index expands [15].

Determination of tablet content

This evaluation is performed to determine the dosage of the active substance in a precise pharmaceutical preparation. The pharmaceutical preparation will not cause the expected therapeutic effect if used not according to the range of active substance, or if the active ingredient is less than the dosage. If the content of an active substance is excessive, it can cause toxic effects.

The assay results in Table 10 show that the active ingredient is in the range 90.0-110.0%, thus meeting the requirements of USP 30.

Drug release (dissolution) test

In the preparation of sustained-release tablets, the dissolution test is crucial for understanding the drug's release from the tablet matrix. In this case, diclofenac sodium is used as a drug model. The sustained-release tablets are expected to retain the diclofenac sodium acid release in the phosphate buffering medium at a pH

of 7.4. The medium is used in accordance with the United States Phamacopoeia 32nd ed. [6]. The diclofenac sodium has pKa = 4, so the diclofenac sodium is soluble in alkaline pH and insoluble in acid solution. When the drug is soluble, the preparation will release the drug into the medium. The amount of potassium dihydrogen phosphate used in accordance with the literature will simulate conditions in the small intestine [16].

The drug release profile of diclofenac sodium from the matrix can be seen in Fig. 6. Formulas 1 to 3 are tablet formulas using the CL6-Ko-A-XG matrix with ratios of 1:1, 1:2, and 2:1. The diclofenac sodium release profile shows that F2 can withstand drug release for 8 hrs with a cumulative amount of 49.76%, whereas F1 and F3 indicate drug releases greater than 75-77.99% and 96.53%, respectively. This result is consistent with the inflate index of tablets showing F2 having the smallest expanding index, whereas F3 has the largest of the tablets with the CL6-Ko-A-XG excipient.

In tablets with CL12-Ko-A-XG excipients, F5 showed the smallest drug release compared to F4 and F6, at 45.90%. Formula 4 releases the drug by 47.66% after 8 hrs while F6 releases the drug as much as 92.18% after 8 hrs. This is consistent with the inflate index of tablets (Fig. 5), where tablets with excipient formulations having larger xanthan gum ratios have smaller expanding indices. Xanthan gum has a good expanding index in the medium so it can withstand a longer drug release [8].

A comparison of dissolution profiles between tablets with CL6-Ko-A-XG and CL12-Ko-A-XG excipients in Fig. 6 shows that Formula 4 releases the drug more slowly than F1. Similarly, F5 releases the drug more slowly than F2. As the index profile expands (Fig. 5), the index inflate F4 tablet is smaller than F1 and F5 is smaller than F2. This may be because not only amylose is found in the crosslinked xanthan gum-amylose xanthan excipients, but also xanthan gum. Thus, the higher the concentration of STMP in the matrix, the more the hydrophilic amylose and xanthan gum groups are substituted by the phosphate, the smaller the index expands the tablet, and the less the amount of the drug dissolved. Formula 6 shows a drug release profile faster than F3 and other forms. This is consistent with the rapid expansion index of tablets (Fig. 5).

Formula 6 releases the active substance quickly. This corresponds to the F6 index inflate rapidly expanding up to 240 minutes (Fig. 5). In addition, the F6 contains a 2:1 amylose-xanthan gum ratio with a 12% STMP concentration. The number of cross-linked amylose with higher STMP concentrations is greater than xanthan gum. Amylose with a dysregulatory concentration of more than 10 results in cross-linked amylose showing a decrease in drug release time. The three-dimensional structure of amylose hydrogel with high STMP concentrations resulted in a more split structure when compared to amylose with low-to-moderate STMP concentrations [15]. The structure causes water to penetrate more easily so the tablet expands faster.

Furthermore, the drug release test is carried out in an enzyme-containing medium. The enzyme used is alpha-amylase because the amylose present in the excipient CL-Ko-A-XG is a substrate of alpha-amylase. The medium used is a phosphate buffer at a pH of 7.4 containing 6.7 mM NaCl. The amount of NaCl added is in accordance with the Sigma-Aldrich protocol, and is intended to keep the enzyme activity in the medium.

Fig. 1 shows that the drug release profile of F2 in the medium containing the enzyme is not affected by alpha-amylase. Formulas 1 and 3 have a slower drug release than those in a non-enzyme medium. This is due to the presence of electrolytes in the dissolution medium. Sodium chloride added to the dissolution medium will form Na^+ and Cl^- ions, which will decrease the expanding ability of cross-linked amylose. The presence of electrostatic interactions between amylose and sodium chloride and the competition between amylose and sodium chloride in binding water molecules causes a decrease in the expanding ability of cross-linked amylose [17].

According to Fig. 2, the drug release profiles of the F4, F5, and F6 matrix tablets are not affected by the presence of enzymes. This is due to the use of high concentration cross-linked agents, which cause the tablet to be more resistant to enzymatic degradation. The higher the concentration of dysfunctional agents, the lower the enzyme's ability to enter the excipient. The bond strength of the amylose provides a stable and acid-resistant structure, enzymatic hydrolysis, and heat and shear stress. In the enzymatic degradation test of excipients in powder form, the presence of alpha-amylase was to ensure ease of degradation, but after being formulated and forged into tablets, the drug release is not affected by its presence.

To relate the frequency of drug administration in daily use to the amount of soluble drugs, the Banakar rules are applied. If the percentage of the solution is about 20-45%, then a sustained-release preparation can be used for 32 hrs. If the percentage of drug that dissolves is around 45-75%, it can be used for 16 hrs. If the percentage of the drug that dissolves is over 75%, then the sustained-release preparation can only be used for 8 hrs. According to Fig. 6, the percentage data relating to the amount of drug released in a phosphate buffer medium with a pH of 7.4 without enzymes for 8 hrs, Formulas 2, 4, and 5 fell between 45% and 75%. According to Banakar rules, they can be used as sustained-release tablets for 16 hrs. In a matrix of tablets with 1:1 (F4) and 1:2 (F5), CL6-Ko-A-XG 1:2 (F2) and CL12-Ko-A-XG matrices having slow-expanding indices. Thus, the amount of diclofenac sodium released by them is less. In F1, F3, and F6, the percentage of drug quantity released is over 75%, so according to the Banakar rule, they can be used as a sustained-release tablet formula for 8 hrs. However, there is a slowing of the drug release in F1 and F3 when in phosphate buffer medium with a pH of 7.4 and alpha-amylase. According to Fig. 1, the percentage of the released drug is in the range of 45-75%, which according to the Banakar rule, means it can be used as a 16-hrs sustained-release tablet. This is due to the influence of electrolytes in the medium, which leads to a decrease in its ability to inflate amylose cross-link excipients [17]. Figs. 1 and 2 show no change in the drug release profile of F1-F6 in a phosphate buffer medium with a pH of 7.4 and alpha-amylase.

Furthermore, the drug release profile obtained from the six formulas is matched by analyzing them against some drug release kinetics, such as kinetics of zero-order release, first order, Higuchi, and Korsmeyer–Peppas. In each kinetic equation matched with a prepared drug release, the value of the drug release constants (k) and correlation coefficient (r) will be obtained. If a preparation follows zero-order kinetics, then the rate of drug release is constant over time without being affected by the concentration of the drug [18].

Based on Table 11, it is shown that drug releases F1-F6 follow the kinetics of drug release of zero order and Korsmeyer–Peppas. This can be seen by the value of r at the zero order being near zero, and the value of n at Korsmeyer–Peppas being between 0.60 and 0.87. If a preparation follows zero-order kinetics, then the rate of drug release is constant over time without being affected by the concentration of the drug. The Korsmeyer–Peppas equation describes the drug release mechanism of a preparation based on a Fickian diffusion mechanism, a non-Fickian transport, or a super case-II transport mechanism based on the Peppas diffusion exponent value (n) [18].

CONCLUSION

The excipients CL6-Ko-A-XG and CL12-Ko-A-XG degraded by 20% (indicated by the remaining 80% amylose) at 10 minutes and 30 minutes degradation test with alpha-amylase, respectively. The dissolution profiles of tablet formulations with CL6-Ko-A-XG 1:2, CL12-Ko-A-XG 1:1, and 1:2 excipients were not affected by enzymatic degradation and can be used for 16 hrs, whereas the tablet formulation with excipients CL6-Ko-A-XG 2:1 and CL12-Ko-A-XG 2:1 underwent a change in dissolution profile to be slower and can be used for a sustained-release tablet of 8 hrs.

The xanthan gum-amylose cross-linking process can be an alternative to producing more functional excipients as sustained-release tablets

without the need for additional excipients in the formula. In addition, further research on the cross-linking of xanthan gum in CL-Ko-A-XG excipients is required.

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