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SYNTHESIS AND CHARACTERIZATION OF CHITOSAN- ALGINATE FOR CONTROLLED RELEASE OF ISONIAZID DRUG

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ABSTRACT

The aim of this research was to synthesize and characterize chitosan-calcium alginate as matrix isoniazid encapsulation to produce controlled release isoniazid drug. The microparticles were evaluated for surface morphology, functional groups, size particles, drug content and swelling index. The drug release kinetic was investigated at gastric and intestinal artificial pH. The results showed that isoniazid-calcium alginate-chitosan has majority particle diameter of 1001-1500 nm. The release mechanism of isoniazid was through combination of erosion and diffusion.

Keywords: chitosan alginate; controlled release; isoniazid

ABSTRAK

Tujuan penelitian ini adalah sintesis dan karakterisasi kitosan-kalsium alginat sebagai matrik enkapsulasi isoniazid untuk menghasilkan isoniazid sistem lepas terkontrol. Mikropartikel yang dihasilkan dianalisis morfologi permukaan, gugus fungsional, ukuran partikel, kadar obat, dan index swelling. Laju pelepasan obat dilakukan pada pH larutan lambung dan usus buatan. Hasil penelitian menunjukkan bahwa mikrokapsul isoniazid-kalsium alginat-kitosan mayoritas mempunyai diameter partikel 1001-1500 nm. Mekanisme pelepasan isoniazid adalah melalui kombinasi erosi dan difusi.

Kata Kunci: kitosan alginat; sistem lepas terkontrol; isoniazid

INTRODUCTION

During the past two decades, biodegradable polymers have attracted attention research to be developed as biomaterials, carriers of immobilized enzyme and cell, biosensor, for tissue engineering, materials for orthopedic and controlled drug delivery system [1-8]. Natural polymers have potential pharmaceutical applications because of low toxicity, biocompatibility and biodegradable characteristic [9-11]. Some materials can be used to coat drug, for examples chitosan-glutaraldehyde [12]; chitosan-gom guar [13]; alginate- chitosan [8,10,15]; gelatin [16]; chitosan-TPP [17]. In this research double coated chitosan-calcium alginate for isoniazid control release model has been development. The encapsulation of isoniazid with chitosan and alginate, was not only capable of improving the drug(s) encapsulation efficiency and bioavailability, but also reducing the dose and dosing frequency when compared to alginate alone microparticles [11-12]. The aims of this study is developing a natural polymer for

controlled released drug system to reduce the incidence and severity of side effects of the drug.

Active component met in commercial Tuberculosis drug is isoniazid. This active compound has the character of antibacterial and short bill time so that it has to be taken continuously. It causes intestine and stomach irritation. Release system in control represent method can be used to reduce the side effects. Silva et al. [20] showed that double coated system of alginate-chitosan reduce the porosity and improve the stability of the product. The negative charged carboxylic acid groups of alginate formed ionic bond with the positive charged amino groups of chitosan [21]. Moreover, chitosan has been reported of mucoadhesion, which increases the contact time of the drug with the mucosa, and the ability to induce a transient opening of epithelial cell tight junctions [11,20]. Usage of alginate-chitosan as matrix of encapsulation has low toxicity because it can be used to improve therapeutic efficacy and to reduce the adverse effects, fluctuations in plasma concentration,

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and to decrease the dose and dosing frequency [22]. The controlling preparation release of isoniazid is very important. In this study, therefore we investigated the synthesis and characterization of chitosan calcium alginate as matrix in isoniazid encapsulation. The performances of them were evaluated based on the data of surface morphology, functional groups, size particles, drug content, and swelling index. The release kinetic of drug was investigated in artificial gastric and intestinal medium.

EXPERIMENTAL SECTION

Materials

Chitosan with minimum 85% deacetylated was purchased from BrataChem Indonesia. The other materials used were sodium alginate, chloride buffer solution pH 1.2, phosphate buffer solution pH 7.4; were purchased from Dianum Indonesia, isoniazid were obtained from PT Kalbe Farma Indonesia. Acetic acid, NaOH and other chemicals were used as analytical grade and purchased from Merck.

Instrumentation

The equipments utilized in this experiment were Spectrophotometer Infra Red (FTIR Perkin Elmer Frontier-89485), SEM JEOL, JSM-1600 for morphology analysis, Particle size was analysis with Zetasizer 3000HS and Spectrophotometer UV-Vis Shimadzu-1700 was for isoniazid analysis.

Procedure

Coated of isoniazid with calcium alginate

Isoniazid weighed 0.1 g was dissolved in 100 mL distilled water containing methanol (methanol/water 1:4 v/v). The sodium alginate 2% (b/V) was added to this solution. After mixing for 20–30 min, this solution was allowed to elapse in order to make the solution bubble-free. The mixture was passed through a peristaltic pump and allowed to fall drop wise at 60 drops/min into 50 mL of 0.1 M calcium chloride. In order to get isoniazid: alginate ratio of 60:40, 6 mL of 1% isoniazid was added to 2% alginate solution (composition ratio isoniazid: calcium alginate of 90:10; 80:20; 70:30; 60:40; 50:50 and 40:60; 30:70; 20:80 and 10:90 as formula code A-I), respectively. The beads were formed immediately and were left as such for 24 h at room temperature. Subsequently, the beads were recovered by filtration, washed with distilled water and dried at room temperature.

Coated isoniazid-calcium alginate with chitosan

Isoniazid coated calcium alginate were soaked in chitosan solution. Concentration of chitosan was varied 0.1-0.5% (w/v) and time of soaked is 10 min, after filtered and dried at room temperature during 24 h, produces isoniazid microparticles. To prepare 0.1% (w/v) chitosan solutions, 100 mg of chitosan dissolved with 100 mL of acetic acid 1M solution.

IR Spectra of microparticles

The characterization of chitosan, calcium alginate, chitosan calcium alginate and chitosan calcium alginate isoniazid functional groups were performed FT-IR Perkin Elmer Frontier-89485 in range 400–4000 cm^{-1} using a resolution of 4 cm^{-1} and 10, weighed amount of drug was mixed with potassium bromide.

Estimation of drug content

Phosphate buffer (pH 7.4) was used to extract isoniazid from microparticles. As much as 50 mg microparticles of each variant was digested, and then extracted with 100 mL of phosphate buffer pH 7.4. The suspension was centrifuged at 5000 rpm for 30 min. The supernatant was assayed UV-Vis Spectrophotometer at the wavelength 262 nm for determination of isoniazid content.

In vitro dissolution test

The dissolution test was conducted using 2 types of dissolution device (paddle method). About 500 mg isoniazid encapsulated placed at dissolution chamber. The test was conducted on gastric medium pH 1.2 and intestinal medium pH 7.4 at 37 °C. About 15 mL of aliquot were sampled from gastric and intestinal medium each time for analyzing the isoniazid content at a fixed time interval. The amount of isoniazid released was analyzed using UV-Vis Spectrophotometer at 262 nm. In order to investigate model of release from the microparticles, the release data were analyzed with the following mathematical models [18]:

Zero order Kinetics: $Qt = K_0t$

First order Kinetics: $\log \left(\frac{Q_t}{Q_0} \right) = - \frac{K_1 t}{2.303}$

Korsmeyer and Peppas equation: $Qt = K_{kp} t^n$

Higuchi's equation: $Qt = K_H t^{1/2}$

Where, Qt is the percent of drug released at time 't'; K_0 , K_1 , K_{kp} , and K_H are the coefficients of Zero order, First order, Korsmeyer-Peppas and Higuchi's equations.

Measurement of swelling index

To measure the swelling index, isoniazid microparticles were immersed in various buffer solutions (pH 7.4 and 1.2) at 37 °C. After that excess

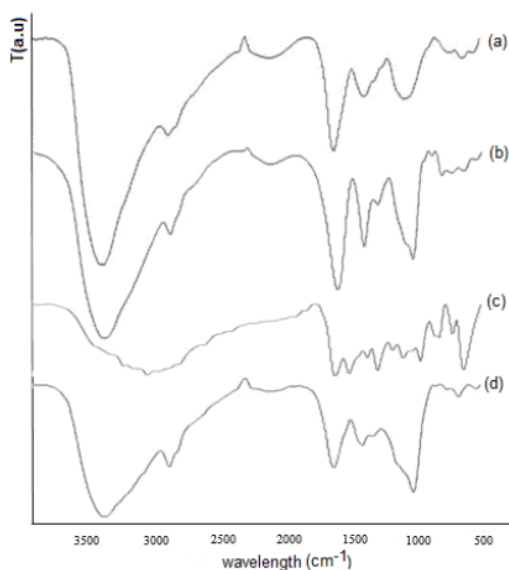


Fig 1. FTIR Spectra of (a) chitosan; (b) calcium alginate (c) isoniazid (d) isoniazid calcium alginate chitosan

water on the surface was removed by filter paper, the weight of the swollen sample was weighed at various time intervals. The procedure was repeated until there is no further weight increase. The swelling index was calculated [13]:

$$SI = \frac{W_a - W_b}{W_b}$$

Where, *SI* is swelling index; *W_a* and *W_b* represent the weight of swollen and dry samples, respectively.

RESULT AND DISCUSSION

Characteristic of Microparticles

The FTIR spectra of chitosan, calcium alginate, isoniazid and chitosan-calcium alginate-isoniazid are shown in Fig. 1. Many vibration peaks of chitosan were observed such as the characteristic broad peak at 3450 cm^{-1} indicated to either O-H or -N groups or both. Free amino group of glucosamine absorption is between 1220 and 1020 cm^{-1} . The characteristic peak at 1623 cm^{-1} assigned as -C=O group from chitosan and calcium alginate (1624 cm^{-1}), which apparently decreased after chitosan being incorporated with calcium alginate (Fig. 1d). This observation indicate that the carboxylate groups of calcium alginate were dissociated become COO⁻ groups which it complexed with protonated amino groups of chitosan through electrostatic interaction. The increase in the intensity peak at 1084 cm^{-1} after isoniazid was encapsulated in

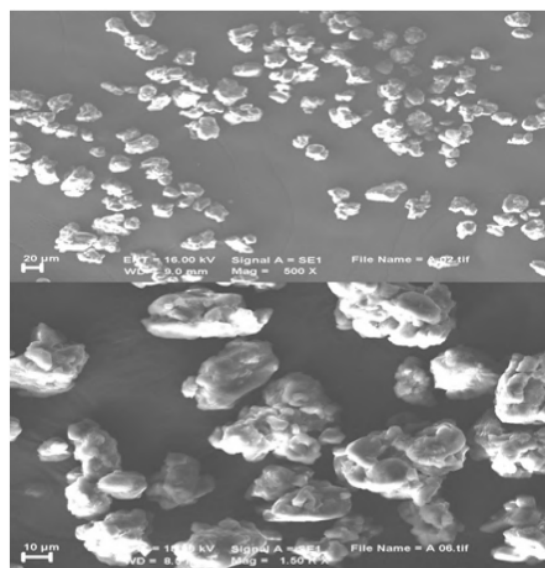


Fig 2. SEM image of chitosan-alginate-isoniazid particles

chitosan calcium alginate (Fig. 1d). In FTIR spectra of isoniazid (Fig. 1c), a peak at 1384 cm^{-1} indicated the C-O stretching and peak at 1184 cm^{-1} is due to ketone groups from isoniazid.

The analysis morphology by SEM (Fig. 2) showed that chitosan-calcium alginate isoniazid microparticles have spherical and smooth surface. Microparticles as targeted carrier for isoniazid were prepared with calcium chloride as crosslink agent using biocompatible and biodegradable polymer of chitosan. The solubility of chitosan in acetic acid is important factor for the microparticles formation.

The drop of suspension isoniazid-alginate into condensation of CaCl_2 cause to be formed crosslink of alginate intermolecular with ion of Ca^{2+} . After 10 min, gel of alginate becomes harder because crosslinked among alginate with Ca^{2+} have been perfection. After isoniazid an encapsulation at calcium alginate, this microparticle soaked at chitosan solution. During the soaking in chitosan solution process, ionic bonds between chitosan and calcium alginate were formed (Fig 3). The composition of isoniazid:alginate:chitosan has effect on the particle sizes of microparticles. Chitosan-calcium alginate-isoniazid have majority particle size diameter in 50-1000 nm with positive zeta potential at maximum and minimum chitosan concentration. Table 1 showed that particles size diameter depends upon the alginate and isoniazid concentration. The best of composition chitosan: alginate: isoniazid was 0.3:60:40. The previous research

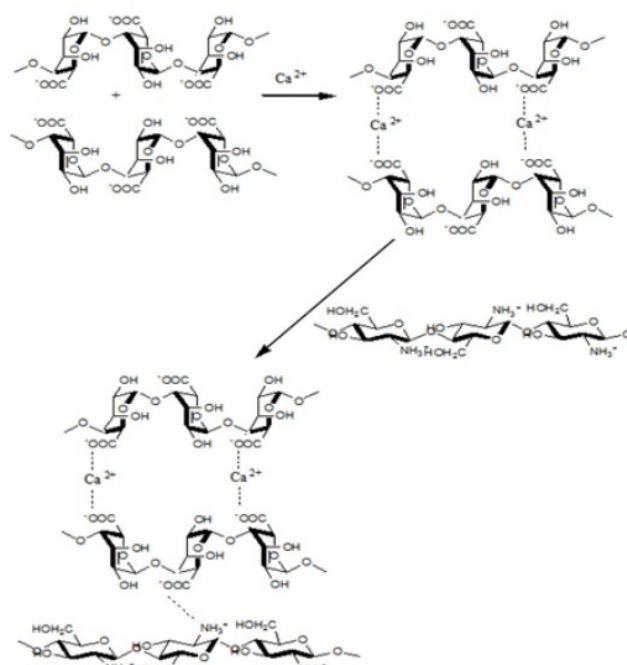


Fig 3. Mechanism interaction of alginate-calcium and chitosan

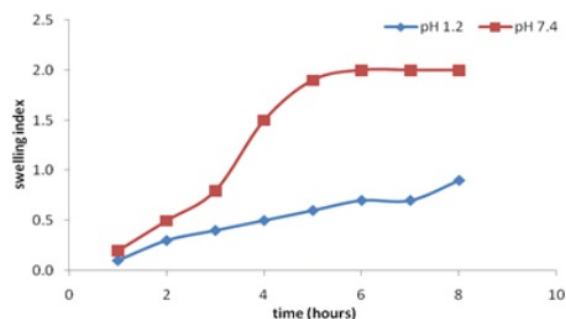


Fig 4. Swelling index of isoniazid encapsulated (Formula F) in some medium

Table 1. Particle size distribution of microparticles (%)

Formula	Particle diameter (nm)			
	50-250	251-500	501-1000	1001-1500
A	5.0	2.5	16.9%	75.6
B	6.3	10.1	10.9 %	72.7
C	10.7	17.4	6.6%	65.3
D	13.5	20.2	10.6%	55.7
E	15.4	23.4	17.5%	43.7
F	17.3	35.4	32.1%	15.2
G	13.2	25.3	31.2%	30.3
H	14.3	20.3	18.0%	47.4
I	17.3	15.9	16.3%	50.5

data indicate that the best concentration of chitosan solution was 0.3%. Increase of concentration of chitosan will decrease microparticles mass but not significant. This case is because there was osmosis processes within gel of alginate when microparticles were soaked in chitosan solution.

Drug Content

Drug content and percentage yield of all the formulations were satisfactory and each formulation demonstrated high value. The value of nanoparticles in the microparticles with composition ratio chitosan: alginate: isoniazid of 0.3:40:60 showed more than the other composition ratio. The amount of free isoniazid was measured in the clear supernatant by UV-Vis at wavelength of 262 nm. The isoniazid loading capacity of the nanoparticles and their % loading capacity were calculated. In microparticles with composition ratio of 0.3:40:60 the drug content was 90.3%. Ionic interaction between the polymer and drug may lead to increase entrapment of the drug in microparticles.

Swelling Index

Swelling index of the microparticles is given in Fig. 4. The chitosan calcium alginate isoniazid micro

Table 2. Dissolution kinetic of isoniazid in artificial gastric medium

Formula	Zero order		First order		Higuchi		Korsmeyer-peppas		
	R^2	K_0 (min^{-1})	R^2	K_1 (min^{-1})	R^2	K_H ($\text{min}^{-1/2}$)	R^2	K_{KP} (min^{-n})	N
A	0.7345	0.0124	0.7356	0.0001	0.6789	0.3345	0.9201	0.8876	0.4656
B	0.8357	0.0456	0.7689	0.0003	0.7659	0.4532	0.8976	0.9891	0.4673
C	0.7879	0.0387	0.7861	0.0007	0.7851	0.4561	0.9237	0.9679	0.4871
D	0.8323	0.0587	0.7190	0.0009	0.7563	0.3298	0.9192	1.0237	0.5671
E	0.8435	0.0591	0.8023	0.0004	0.7892	0.5348	0.9329	1.1045	0.5723
F	0.8789	0.0678	0.7845	0.0005	0.8324	0.4367	0.9187	1.051	0.5901
G	0.8801	0.0435	0.8023	0.0006	0.7934	0.5023	0.9324	1.2391	0.6234
H	0.7923	0.0465	0.7567	0.0003	0.8322	0.5109	0.9147	0.9982	0.6241
I	0.7786	0.0564	0.7645	0.0008	0.8412	0.4598	0.9524	0.9782	0.6323

Table 3. Dissolution kinetic of isoniazid in artificial intestinal medium

Formula	Zero order		First order		Higuchi		Korsmeyer-peppas		
	R^2	K_0 (min^{-1})	R^2	K_1 (min^{-1})	R^2	K_H ($\text{min}^{-1/2}$)	R^2	K_{KP} (min^{-n})	N
A	0.0734	0.0124	0.0356	0.0001	0.5789	0.6347	0.9203	0.9876	0.4561
B	0.0421	0.0351	0.0675	0.0002	0.5324	0.9876	0.8891	1.0231	0.5467
C	0.0691	0.0321	0.0561	0.0004	0.5109	0.9982	0.9109	1.0673	0.5871
D	0.0489	0.0267	0.0632	0.0001	0.4891	0.9293	0.9397	1.0562	0.5907
E	0.0532	0.0971	0.0545	0.0003	0.5089	0.9540	0.9408	1.1032	0.6782
F	0.0287	0.0659	0.0451	0.0005	0.5287	0.9918	0.9671	1.1253	0.7809
G	0.0489	0.0564	0.0582	0.0007	0.5427	0.9819	0.9108	1.0892	0.7674
H	0.0754	0.0651	0.0467	0.0006	0.5501	0.9654	0.9532	1.0765	0.7509
I	0.0681	0.0328	0.0406	0.0008	0.5602	0.9876	0.9451	1.0654	0.7543

particles showed swelling index value 0.7 at the end of 6 h in the pH 1.2 and 2.0 in the pH 7.4. The results showed that swelling index of microparticles was higher in pH 7.4 than pH 1.2. Data test swelling used to know how index of swelling isoniazid at various pH solution. At this research use of buffer solutions pH 1.2 (artificial gastric medium) and pH 7.4 (artificial intestinal medium) coming near solution in digestive system. Fig. 4 showed that in acidic medium, the calcium alginate beads were converted to the insoluble alginic acid beads which has a low swelling index. In pH 7.4, the microparticles have high swelling index. This may be due to the less solubility of chitosan (in the outer layer) at higher pH that restricted them from further expansion.

Dissolution release

Encapsulation double coating with chitosan as outer layer has to enhance microparticle's stability in gastric acidic medium, because in this medium chitosan was soluble, but calcium alginate was insoluble, so that calcium alginate is still coated with isoniazid. In intestinal medium, this medium was able to destroy crosslink bond between alginate and Ca^{2+} , thus alginate was dissolved and isoniazid was totally released in this medium.

Result of dissolution test is later the analysis into kinetics model release of order drug zero order, first order, Higuchi & Korsmeyer-Peppas. Study kinetic release isoniazid in artificial gastric medium and

intestinal medium were showed in Table 2 and 3. At these tables showed that isoniazid release were dominant the Korsmeyer-Peppas kinetics model both in artificial gastric and intestinal medium. Pursuant to price of n obtained, all formula have n between 0.45 and 0.89 indicating that release of drug follow release mechanism is non-Fickian or Fickian of anomalous diffusion. This indicated that the release mechanism of drug is through mechanism combination of erosion and diffusion.

CONCLUSION

Encapsulation of isoniazid has been prepared with double coated method, alginate as inner layer and chitosan as outer layer with crosslink agent of calcium ion. The value of nanoparticles in the microparticles with composition ratio chitosan:alginate:isoniazid of 0.3:40:60 showed more than the optimal composition. Kinetic study of the release isoniazid in artificial gastric and intestinal medium showed that the release has followed the Korsmeyer-Peppas kinetic model both in artificial gastric and intestinal medium. In all cases, the model gives n between 0.45 and 0.89 indicating that release of drug follows release mechanism is non-Fickian or Fickian of anomalous diffusion. This indicated that the release mechanism of drug is through mechanism combination of erosion and diffusion.

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