Formulation And Evaluation Of Sustained Release Microcapsule Of Metronidazole

Natassya Manda Leo[1], Hakim Bangun[2,4*], Urip Harahap[3]

[1] Postgraduate Programs, Faculty of Pharmacy, Universitas Sumatera Utara, Jl. Tri Dharma No. 5, Kampus USU, Medan. Sumatera Utara, Indonesia 20155.
[2] Department of Pharmaceutical Technology, Faculty of Pharmacy, Universitas Sumatera Utara, Jl. Tri Dharma No. 5, Kampus USU, Medan. Sumatera Utara, Indonesia 20155.
[3] Nanomedicine Centre of Innovation Universitas Sumatera Utara, Jl. Tri Dharma No. 5, Kampus USU, Medan. Sumatera Utara, Indonesia 20155.
[4] Department of Pharmacology, Faculty of Pharmacy, Universitas Sumatera Utara, Jl. Tri Dharma No. 5, Kampus USU, Medan. Sumatera Utara, Indonesia 20155.
*Corresponding Author:
Email: hakim@usu.ac.id

Abstract
The oral conventional metronidazole dosage forms have a short duration of action due to gastric emptying process, hence the treatment of ulcus pepticum using metronidazole become less effective. Due to retaining the dosage form in the stomach, a preparation form of sustained released drug delivery systems has been developed. The aim of this study was to developed a sustained released drug delivery systems of metronidazole using alginate and chitosan as polymers that could last longer in stomach. The formulation consisted of variatons in chitosan 0.5%, 0.75% and 1%. The drug released test was carried out using paddle method in simulated gastric medium pH 1.2 at 37°C. The dissolution test results showed that formula with 0.75% of chitosan giving the best sustained released effect and the kinetics drug realeased of microcapsule followed Higuchi order. Based on the results of the study, it can be concluded that microcapsules of metronidazole with a combination of alginate and chitosan can be prepared as sustained released formulation.

Keywords*: Microcapsule, metronidazole, alginate, chitosan, and sustained released.*

I. INTRODUCTION

Peptic ulcer is a disease in digestive tract that is still found around the world. The prevalence of peptic ulcers in developing countries are around 80-90%, while in developed countries it is 30-40%. Ulcers caused by Helicobacter pylori are 48% and NSAID drugs are 24%. [1] Clinical research data in Indonesia also shows that out of 131 patients who were treated endoscopically, 44 people (33.6%) were infected with Helicobacter pylori [2].The pathogenesis of gastric ulcers is due to an imbalance between aggressive factors such as gastric acid, nonsteroidal anti-inflammatory drugs, alcohol, and Helicobacter pylori bacteria that can damage the mucosa and defensive factors that maintain the integrity of the gastric mucosa such as bicarbonate and prostaglandins, resulting in the disruption of mucosal tissue. The symptoms of gastric ulcers are pain and discomfort in the stomach such as nausea and vomiting [3]Helicobacter pylori can be treated using antibiotics that have local action on the stomach. Antibiotics commonly used to eradicate Helicobacter pylori are metronidazole, clarithromycin, amoxicillin, and tetracycline. The use of metronidazole as an antibiotic in the treatment of gastric ulcers is an option because metronidazole has excellent activity against anaerobic bacteria, but conventional metronidazole preparations have a short duration of action due to the gastric emptying process [4] The antibiotic metronidazole is a derivative of azomycin, a nitroimidazole generated by Actinobacteria and Proteobacteria. This chemical was utilized to treat trichomoniasis, an ailment caused by the protozoan Trichomonas vaginalis, in 1959. In addition, metronidazole has been shown to be beneficial against dysentery and liver abscess caused by the protozoan parasite Entamoeba histolytica. [5] Infections caused by anaerobic or microaerophilic microbes, such as Trichomonas vaginalis, Giardia lamblia, Entamoeba histolytica, Clostridium difficile, and Helicobacter pylori, are typically well treated with metronidazole. Metronidazole is an effective treatment for certain bacterial illnesses.

500 mg are taken orally twice daily for one week [6] .Especially for medications with a short half-life, conventional drug delivery devices cannot maintain an effective drug concentration for the needed duration. To maintain an effective drug concentration in plasma, the drug must be administered often. This can reduce patient compliance and effect of the treatment becomes less successful [5] In addition, conventional drug delivery systems have drawbacks especially for drugs that act locally in the stomach (for the treatment of ulcers), having a narrow absorption range in the gastric region. The conventional system is not able to hold conventional preparations in the stomach because of the relatively short transit time and residence time of the preparation in the digestive tract, which is 2-3 hours. This causes incomplete drug absorption from the preparation in the stomach so that the efficacy of the dose given will be reduced. [7]Microcapsules are small particles containing a core substance in the form of solids, liquids and gases which are covered by a wall of polymer or coating. Large particle size between 1-5000 m. The small particles cause the drug portion to be widely dispersed through the gastrointestinal tract so that it can increase the absorption potential of the drug [8]. Alginate and chitosan have several advantages including biodegradability, biocompatible and non-toxic. Alginate and chitosan will form an electrostatic interaction between the -COOH group of alginate and the -NH2 group of chitosan [9] Based on the introduction, researchers are interested in formulating a sustained released microcapsule with combination of alginate and chitosan.

II. METHODS

2.1 Apparatus

The apparatus used in this study were dissolution tester, spectrophotometry, hotplate, beaker glass, analytical balance and laboratory glassware.

2.2 Meterials

Alginate, calcium chloride, chitosan, distilled water, simulated gastric medium, metronidazole.

2.3 Preparation of simulated gastric fluid pH 1.2 (without enzyme)

2 g of sodium chloride was dissolved into 7 mL hydrochloric acid 37% and distilled water. Distilled water was added to adjust the volume to 1000 mL.

2.4 Preparation of metronidazole standard stock solution

20 mg of metronidazole was dissolved into simulated gastric fluid pH 1.2 (without enzyme) in a 100 mL volumetric flask and made up the volume to the mark (concentration of ibuprofen is 200 ppm).

2.5 Preparation of metronidazole absorption curve

1.5 mL of metronidazole standard stock solution is transferred and adjusted the volume to the mark of 25 mL volumetric flask with simulated gastric fluid pH 1.2 (without enzyme). The absorbance was measured with spectrophotometer UV (Shimadzu UV-1800) and scanned from 200 to 400 nm wavelengths.

2.6 Preparation of metronidazol calibration curve

Metronidazole solution was prepared un der various concentrations by transferring 0; 0,125; 0,25; 0,5; 0,625; 0,75 and 1 ml of metronidazole standard stock solution and adjusted the volume to the mark of 20 mL volumetric flasks with simulated gastric fluid pH 1,2 (withouth enzyme) (concentration of metronidazole are 0; 1; 2; 4; 5; 6; and 8 ppm). The absorbances were measured with spectrophotometer UV (Shimadzu UV-1800) under the maxium wavelength at 277 nm.

2.7 Preparation of Metronidazole Microcapsule Formulation

Dissolve 2 g of sodium alginate into distilled water. Add in 0.2 g of metronidazole and various concentration of chitosan (0.5, 0.75, and 1 g) into the alginate solution till 100 ml and stirred until a homogeneous solution was formed. The solution was dropped into 50 ml of 0.15 M CaCl2 solution using a syringe with diameter of 0.3 mm. The microcapsule were allowed to submerge in CaCl2 for 5 minutes, then filtered using filter paper. After done, dried inside an oven at 450C for 12 hours. [10]

2.8 Particle Size

The diameter measurement of the microcapsules was carried out using a caliper. Measurements were made using 10 microcapsule.

2.9 Water Content of Microcapsule

Water content is done by using a tool called moisture balance. Determination of water content is carried out by inserting microcapsules from each formula and placing them in an aluminum container [11]. The moisture content of the resulting microcapsules is important to know because it can affect the stability of a product. Requirements for a good moisture content are <5%. [12]

2.10 Dissolution studies

Dissolution of products were determined using paddle type dissolution tester under the condition of 900 mL simulated gastric fluid pH 1.2 (without enzyme), temperature of 37 ± 0.5◦C, and 100 rpm. 2 mL of aliquot was taken under the interval time of 0, 5, 10, 15, 30, 45, 60, 90, 120, 150, 180, 240, 300, 360, 420, 480, 540, 600, 660, 720 minutes and the volume was kept 900 mL by adding the same volume of simulated gastric fluid pH 1.2. Aliquot was transferred to 20 mL volumetric flask and adjusted the volume till the mark by using simulated gastric fluid pH 1.2 (withouth enzyme). [13] The absorbances were measured with spectrophotometer UV (Shimadzu UV-1800) under the maximum wavelength at 277 nm.

III. RESULT AND DISCUSSION

3.1 Formulation of Metronidazole Microcapsule

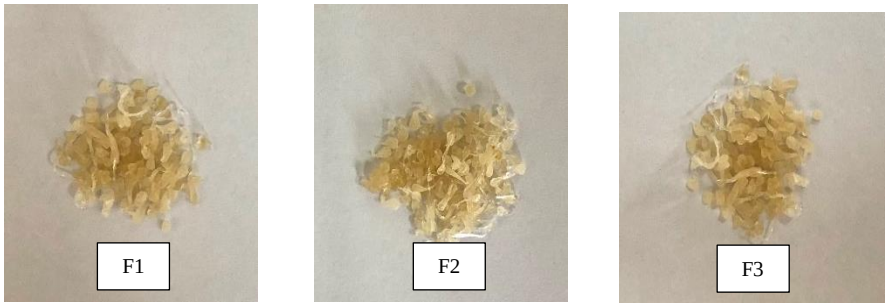

Fig 1. Metronidazole Microcapsule With Alginate and Chitosan

Information :

F1 : Microcapsule metronidazole with alginate 2% and chitosan 0.5%

F2 : Microcapsule metronidazole with alginate 2% and chitosan 0.75%

F3 : Microcapsule metronidazole with alginate 2% and chitosan 1%

3.2 Particle Size

Measurement of microcapsule preparataion was carried out using a caliper at room temperature when the preparation was finished. Particle size evaluation results of metronidazole microcapsule can be seen on Table 1

Table 1.Particle Size of Microcapsule

Formula	Diameter ± SD (mm)
F1	0,354 ± 0,008
F2	0,355 ± 0,085
F3	0,355 ± 0,085

The measurement result of metronidazole microcapsule preparation meet the requirements of microcapsule particle size, which is not more than 5 mm [14]

3.3 Water Content of Microcapsule

The water content of microcapsule can be shown in Table 2

Table 2.Water Content of Microcapsule

Formula	Water Contet (%)
F1	1,67±0,026
F2	2,08±0,083
F3	2,44±0,036

Based on Table 2 it can be seen that every formula of microcapsule is fulfill the requirement of water contet of microcapsule which is 5% [15]. The lower the water content contained in microcapsule, the more durable microcapsule will be because microbial growth will be inhibited [16]. Formula 4 has the highest water content which is 2,44±0,036%, this is because F3 has the highest chitosan concentration among the

others. The higher the concentration of chitosan, the greater water content in the microcapsules. This is because the process of losing water during the evaporation process will be increasingly difficult. A higher concentration of chitosan will minimize water loss due to transpiration so that the water content with a high concentration of chitosan will be higher than that of a lower concentration of chitosan. [17] .

3.4 In-vitro drug released studies

Metronidazole released from microcapsule can be seen on Figure 2 and Table 3

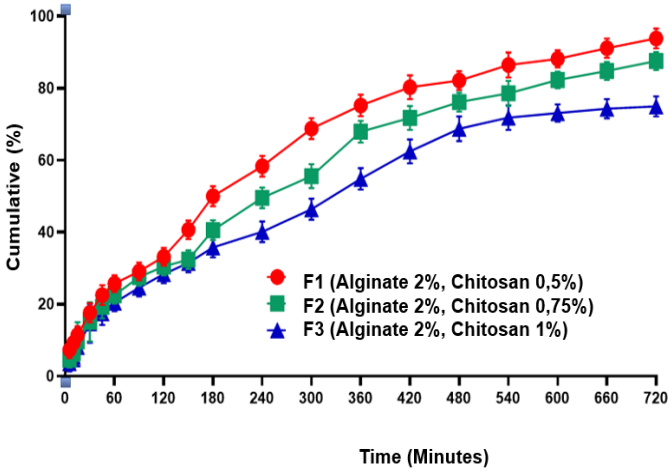

Fig 2. Dissolution curve of metronidazole microcapsule

Table 3. Cumulative percentage of Metronidazole Microcapsule

Formula	Time (Minutes)			Sustained Released Requirement [11]
	180	360	720	
F1	49.98±0.39%	75.21±0.33%	93.84±0.67%	180 minutes: 20-50%
F2	40.54±0.44%	67.93±0.31%	87.56±0.53%	360 minutes: 45-75%
F3	35.77±0.34%	54.82±0.22%	74.94±0.34%	720 minutes: >75%

In Figure 2 it can be seen that F3 has the slowest release rate due to the highest chitosan concentration among other formulas. The higher amount of chitosan causes difficulty for drug to dissolve because chitosan is weak base and insoluble in water. [18] The higher amount of chitosan causing the release of metronidazole decrease. Alginate is polyanionic and chitosan is polycationic. The carboxylic acid group of alginate interact with the amino group of chitosan and form a polyelectrolyte complex which causes a reduction in the release rate of metronidazole from microcapsules. [19] As shown in Table 1, F2 shows the best result as sustained released properties due to the percentage of the released drug is closest to the requirement of sustained released properties and the released of metronidazole from microcapsule followed Higuchi model kinetic released as shown in Table 3 and Figure 3.

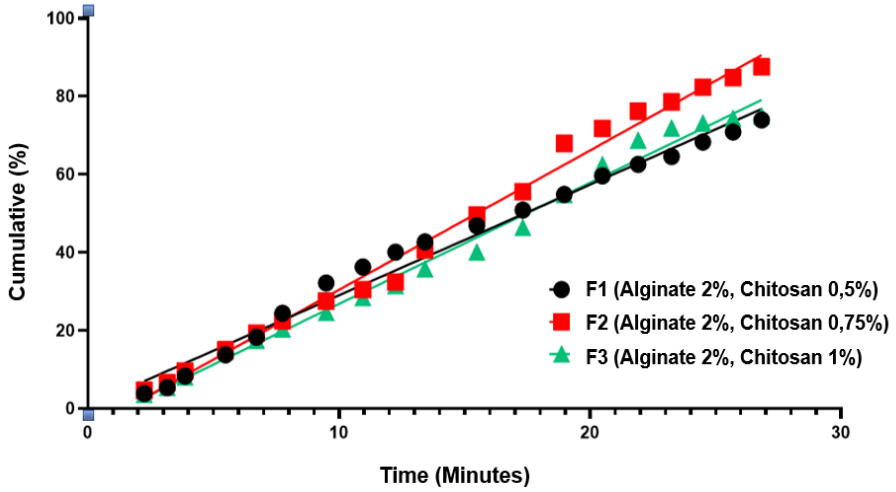

Fig 3. Higuchi plot of metronidazole released from microcapsule

Table 3. Kinetic drug relased of microcapsule

Formula	Zero-order	First-order	Higuchi	Korsmeyer-Peppas	
	R^2	R^2	R^2	R^2	n
F1	0,9276	0,7197	**0,9827**	0,9863	0,5387
F2	0,9501	0,7384	**0,9916**	0,9875	0,6000
F3	0,9507	0,7153	**0,9906**	0,9914	0,6119

Korsmeyer et al. (1983) derived a simple relationship which described drug release from a polymeric system equation [20]. To find out the mechanism of drug release, first 60% drug release data were fitted in KorsmeyerPeppas model [21]. In this model, the value of n describes the drug release mechanism; 0.45 n 0.89 corresponds to non-Fickian transport, n = 0.89 to Case II (relaxational) transport, and n > 0.89 to super case II transport [22][23]. order to examine the release kinetics, data from in vitro drug release trials were plotted as log cumulative percentage drug release vs log time. Figure 4 depicts how the microcapsule medication release followed the Korsmeyer-Peppas protocol.

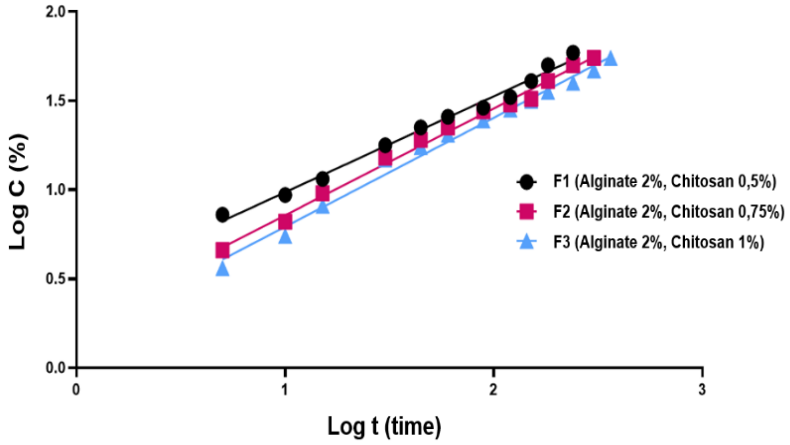

Fig 4. Korsmeyer-Peppas plot of metronidazole released from microcapsule

Based on the result from Table 2, the n values from all formulas are greater than 0.5 but smaller dan 0.89 which characterized as non-Fickian transport.

IV. CONCLUSION

From the research, it can be concluded that metronidazole microcapsule with combination of alginate and chitosan can be prepared as sustained released forms. The greatest effect is the one prepared by using alginate 2% and chitosan 0,75%

REFERENCE

[1] P. Tarigan. Tukak Gaster dan Tukak Duodenum. Dalam: Buku Ajar Ilmu Penyakit Dalam Edisi Keempat - Jilid I. Jakarta: Pusat Penerbitan Departemen Ilmu Penyakit Dalam Fakultas Kedokteran Universitas Indonesia, 2016.

[2] M. Miftahussurur, A. Syam, D. Makmun, Nusi, I.A., Zein, L.H., Akil, F. Helicobacter pylori virulence genes in the five largest islands of Indonesia. The Creative Commons Public Domain Dedication waiver. 7(26): 1-10, 2015.

[3] K. Sunil, K. Amandeep, S. Robin, S. Ramica. Peptic Ulcer: A Review on Etiology and Pathogenesis. ***International Research Journal of Pharmacy***. 3(6): 34-38, 2012

[4] E. Hajiani. Treatment for Helicobacter Pylori Infection, an overview. ***Jundishapur Journal of Microbiology. 2***(2): 41-46, 2009.

[5] A. Hoffman. Pharmacodynamic Aspects of Sustained Release Preparations. Adv Drug Del Rev. 33(3): 186-199, 1998.

[6] G. C. Rajput, Dr. Majmudar, F.D., Dr. J.K. Patel, K.N. Patel, R.S Thakor. Stomach Specific Mukoadhesif Tablets as Controlled Drug Delivery System. ***International Journal on Pharmaceutical and Biological Research***. 1(1): 30-41, 2010.

[7] L. Lachman, H.A. Lieberman, J.L. Kanig. Teori dan Praktek Farmasi Industri II. Edisi III. Jakarta: Universitas Indonesia Press, 1994.
[8] N. Sachan, et al. Biopharmaceutical classification system: A strategic tool for oral drug delivery technology, ***Asian Journal of Pharmaceutics***, 3(2), p. 76. 2009.
[10] T.M. Rasala, V.V. Kale, M.V. Bhalekar, J.G. Avari. Formulation and Evaluation of Mukoadhesif Microcapsules of Diltiazem HCl and Diclofenac Sodium by Orifice-Ionic Gelation Method. ***Journal of Pharmaceutics and Cosmetology.*** 1(1), 2014.
[11] Y. Srifiana, W. Ari, Nopriadi. Natrium Alginat Sebagai Polimer Mukoadhesif Terhadap Daya Lekat Serta Pelepasan Granul Mukoadhesif Amoksisilin. ***Jurnal Ilmu Kefarmasian Indonesia***. 17(1). 2019
[12] R. Voight. Pharmazeutische Technologie Deutscher Apotheker Verlag. 2010.
[13] A. Arianto, H. Bangun, U. Harahap, S. Ilyas. Effect of Alginate Chitosan Ratio on the Swelling, Mucoadhesive, and Release of Ranitidine from Spherical Matrices of Alginate-Chitosan. ***International Journal of PharmTech Research.*** 8(4). 2015.
[14] D.J McHugh. Production, Properties and Uses of Alginates. 1987.
[15] R. Voight. Pharmazeutische Technologie Deutscher Apotheker Verlag. 2010.
[16] B. Purnomo. Materi Kuliah Mikrobiologi. Universitas Bengkulu. Bengkulu. 2004.
[17] R. F. Sitorus, T. Karo-karo., Z. Lubis. Pengaruh Konsentrasi Kitosan Sebagai Edible Coating dan Lama Penyimpanan Terhadap Mutu Buah Jambu Biji Merah. ***Jurnal Rekayasa Pangan***. 2(1): 37-46, 2014.
[18] C. Qin, H. Li, Q. Xiao, Y. Liu, J. Zhu, Y. Du. Water-solubility of Chitosan and Its Antimicrobial Activity. Carbohydrate Polymers. 63(3). 2006.
[19] D. Kulig, Z. Anna, J. Andrzej, M. Krzysztof. Study on Alginate – Chitosan Complex Formed with Different Polymers Ratio. Polymers. 8 (167). 2016.
[20] E. Brunner: Z. Physik. Chem, 47, 56, 1904.
[21] R. W. Korsmeyer. R. Gurny. E. Doelker. P. Buri, Peppas N.A.: ***Int. J. Pharm.*** 15, 25 1983).
[22] Riger P.L, Peppas N.A.: ***J. Control.*** Rel. 5, 37. (1987).
[23] Siepmann J., Peppas N.A.: Adv. Drug Deliv. Rev. 48, 139 (2001).