

THE KINETIC RELEASE AND *In-vivo* STUDY OF ALGINATE-CHITOSAN ENCAPSULATED METFORMIN AGAINST TYPE II DIABETES MELLITUS

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ABSTRACT

This study was a purpose to determine the kinetic release of metformin encapsulated in chitosan alginate and to determine the antidiabetic effectiveness by *in vivo* study. Encapsulation of metformin was carried out by using alginate and chitosan polymers with CaCl₂ as a crosslinking agent and Tween 80 as a surfactant. A total of 30 mice were divided into 6 groups, namely the normal group with distilled water, the positive control group with metformin without encapsulation, the negative control group with Na-CMC (without drug treatment), and the treatment group (encapsulated drug with a composition of 1:1; 1:2; and 1:3). Substances used for diabetes include intraperitoneally induced alloxan monohydrate. The results showed the release of metformin in pH 1.2 of the gastric physiological solution. The mechanism is followed the Korsmeyer Peppas equation. In the intestinal physiological solution pH, 7.4 followed the Higuchi equation with the mechanism was an erosion. The analysis of surface morphology of metformin encapsulated showed agglomeration in several parts on the surface of the encapsulated metformin matrix without the addition of Tween 80 surfactant. Meanwhile, the SEM results of encapsulated metformin with the addition of surfactant Tween 80 have a smooth matrix surface and there is no agglomeration. *In vivo* test showed there was a decrease in blood sugar in the optimum composition of metformin encapsulation with a 1:2 ratio of 37.9 mg/dl.

Keywords: Metformine, Encapsulation, Kinetic Release, *In vivo*

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INTRODUCTION

Diabetes mellitus is a disease caused by the pancreas not being able to produce enough insulin and ineffective use of insulin in the body.^{1,2} Diabetes Mellitus requires treatment for a relatively long time. In type II diabetes mellitus, it is necessary to take medication regularly throughout life, while for type I diabetes mellitus patients not only take medication regularly in their lives but also must receive regular insulin injections.³ The types of drugs that are often consumed to treat diabetes are glibenclamide, Glucophage, and metformin. Metformin hydrochloride is one of the drugs recommended as a first-line therapy for type 2 diabetes mellitus⁴. Metformin can lower glucose levels by reducing hepatic gluconeogenesis.⁵ Usually, the dose of metformin HCl used in conventional preparations is 500-850 mg for use 1-3 times a day.⁶ The drug carrier control system can be synthesized using natural polymers by encapsulation.⁷ Some of the polymers commonly used for encapsulation in biomedical applications are polyglycolide copolymers, lecithin, chitosan, and alginate.⁸ Polymers that are often used in drug release control systems are chitosan and alginate polymers because both polymers have biocompatible, non-toxic, and biodegradable properties.^{9,10} The combination of the two polymers alginate and chitosan will have opposite charges so that metformin can be completely absorbed in the digestive system and can absorb the active substance of metformin more effectively.¹⁰ Chitosan still forms agglomerations on the surface of the alginate matrix which causes the swelling process of the alginate matrix to be difficult¹¹. Therefore, it is necessary to add surfactant to reduce the surface tension between alginate and chitosan.¹² The surfactant that is often used is tween 80 because it is non-toxic and biodegradable which is safe to use in drug coating. Tween 80 is surfactant non-ionic with a hydrophilic-lipophilic balance value is 15 so it tends to be polar and can be used as an emulsifier in water solvent systems.¹³ In this study, the metformin encapsulated was

evaluated in surface morphology, The release kinetics of the drug, and *in vivo* test with mice (*Mus musculus*) was conducted. In this study mice were induced by alloxan monohydrate. Alloxan is a toxic glucose analog that produces superoxide radicals in pancreatic cells, and hydroxyl radicals.¹⁴ Alloxan has a selective cytotoxic effect on pancreatic beta cells, therefore it is used as an inducer of diabetes. The test animals used in mice must have normal sugar levels in the range of 62-175 mg/dl¹⁴. In addition, mice must have blood sugar levels above normal to get diabetes. The test on mice was carried out to compare the effectiveness of existing drugs without encapsulation with those that had been encapsulated using alginate-chitosan.

EXPERIMENTAL

The materials used in this study were metformin from PT. Hex pharm Jaya, alloxan monohydrate from Nitra Kimia Yogyakarta, alginate obtained from PT. MKR Chemical Ltd Semarang, chitosan with a deacetylation degree of 85% commercially obtained from PT. Chatty Indo Chitin, SPME material with qualification pro analyst. The instruments for monitoring the blood glucose system (GlucodrTM from PT. Medisindo Bahana). The experimental animals that were used in this research are male, mice (*Mus Musculus*) weighing 25-30 grams, and their ages are 2-3 months (Following acceptance from the ethical clearance Airlangga University Indonesia). The metformin was ground with a mortar, then weighed as much as 25 grams put into a 250 mL beaker, and alginate solution was added to 25 mL of 2%. The solution was stirred for 10 minutes. Then the solution is dropped into a solution of 0.5 M CaCl₂ to form granules, and then filtered and washed with demineralized water. The granules were immersed in 3% Tween 80 solution for 30 minutes. After that was immersed for 30 minutes in chitosan 0.1%. The sample was filtered and then dried at room temperature.

Kinetic Release Controlled

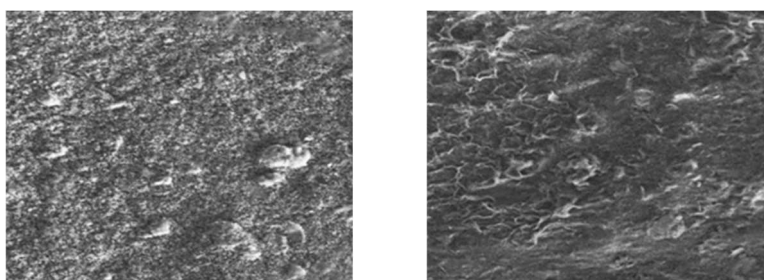
The metformin encapsulated was analyzed and controlled on the gastric medium with a pH is 1.2 and the intestinal medium with a pH is 7.4 for 60 minutes. The encapsulated metformin was placed in 2 containers containing 100 ml of the mixture of gastric and intestinal solutions for 10-60 minutes. Every 10 mL sample was taken, it must replace in a container and add to the same volume from the new solution. Dissolution of metformin in solution is measured by using a UV-Vis spectrophotometer on maximum wavelength.

In Vivo Evaluation of Metformin Encapsulated in a Diabetic Animal Model

The dose of metformin in mice was calculated using the conversion table from humans to mice weighing 20 g, the conversion value from humans to mice was 0.0026. Therefore, the dose of metformin in mice is 20 g = 2.6 mg. The dose of 1 Kg / BW for mice = $1000/20 \times 1.3 \text{ mg} = 65 \text{ mg/kg BW}$. Mice became diabetic by injecting alloxan monohydrate intraperitoneally.¹⁵

RESULTS AND DISCUSSION

This encapsulation process aims to encapsulate metformin particles on the alginate-chitosan matrix. In this study, 0.15 M CaCl₂ solution was used as a crosslinking agent. The presence of Cl⁻ ions can be identified qualitatively by dropping infiltrate with 0.1 M AgNO₃ solution^{17,18}. The addition of tween 80 in this research aims to reduce the occurrence of alginate-chitosan agglomeration and to maintain the grain size which usually becomes smaller after soaking in chitosan and drying at room temperature.^{19,20}



(a)

(b)

Fig.-1: Metformin Encapsulated without Tween 80 (A) and Metformin Encapsulated with Tween 80. 3% Magnification 500X

This can be observed in the results of the alginate-chitosan-encapsulated metformin SEM test in Fig.-1 which was carried out and showed agglomeration in several parts on the surface of the encapsulated metformin matrix without the addition of Tween 80 surfactant. Meanwhile, the SEM results of metformin encapsulated with the addition of surfactant Tween 80 have a smooth matrix surface and there is no agglomeration.

Kinetic Release Controlled of Metformin Encapsulated

The pattern of the release of the active substance metformin following the zero-order equation can be observed in Fig.-2. In gastric physiological solution pH 1.2, chitosan which is easily soluble in an acidic medium will dissolve along with the release of the metformin substance from matrix pores with diffusion, while in intestinal physiological solution pH 7.4 chitosan will be hydrated and saturated with the media so that metformin will experience erosion on the outer surface of the matrix.²¹

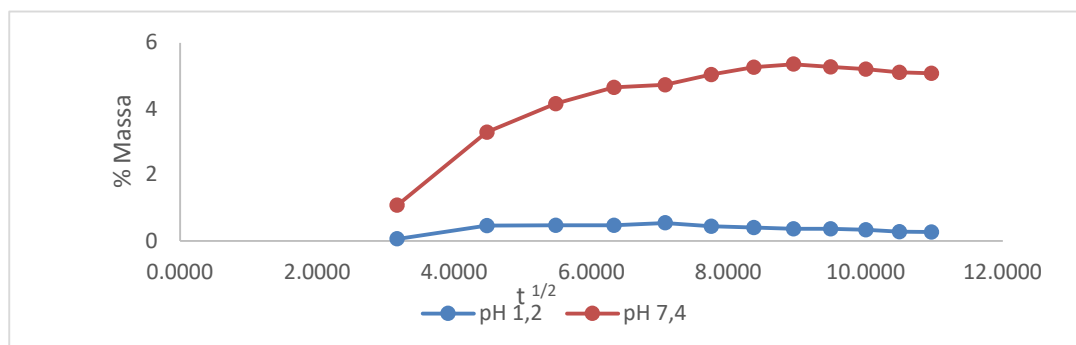


Fig.-2: The Dissolution of Metformin Encapsulation with Kinetic Order One

The Higuchi equation is used to explain the mechanism of the erosional release of the metformin in a pH 7.4 and a pH 1.2 buffer solution (Fig.-3).

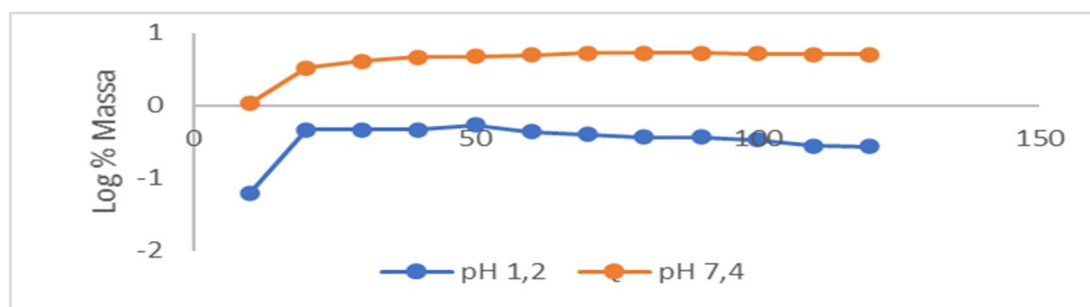


Fig.-3: The Dissolution of Metformin Encapsulation with Kinetic Higuchi

The pattern in Fig.-3 the Korsmeyer-Peppas equation is used to explain the release mechanism of metformin is diffusion in the physiological gastric solution and erosion in the intestinal physiological solution which can be indicated by the value of the diffusion exponent (n).

There can be shown that the release of metformin encapsulated by alginate-chitosan-Tween 80 at a concentration of 4% in gastric physiological solution pH 1.2 tends to in the Korsmeyer Peppas equation with a diffusion mechanism and in the intestinal physiological solution pH, 7.4 is the Higuchi equation and erosion mechanism.

Antidiabetic Test

This test was carried out by preclinical testing using experimental mice. This study begins with measuring blood sugar levels, namely on day 0 to determine the blood normal sugar levels of mice before and after mice are given treatment. The results of measuring the mean blood sugar levels of mice before the experiment (initial), after alloxan induction (pretest), and after (posttest) are in Figure-5. The graph above

shows a significant decrease in blood sugar levels of mice after treatment in group II (administration of metformin without encapsulation), followed by groups IV, V, and VI (administration of alginate-chitosan encapsulated drug metformin) compared to group III which given positive treatment with diabetes only given Na-CMC experienced a decrease in blood sugar levels which was flatter and almost no decrease was seen, while group 1 was much flatter because it was used as a normal control or only as a reference that blood sugar levels being treated had normal conditions.

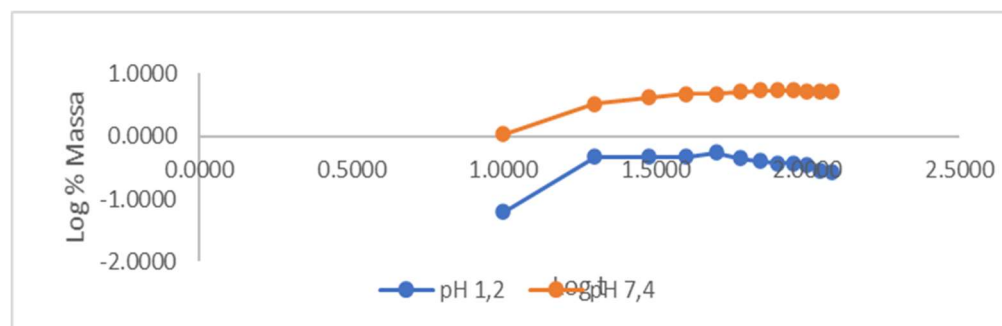


Fig.-4: The Dissolution of Metformin Encapsulation with Korsmeyer Peppas

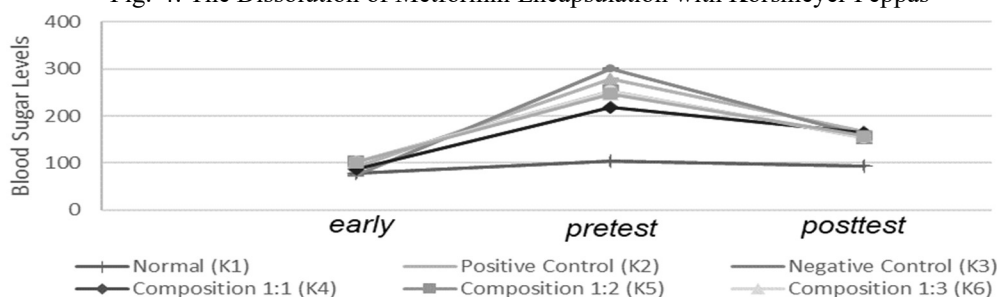


Fig.-5: Mean Blood Sugar Levels of Mice (mg/dl)

To determine whether there is a decrease in blood sugar levels, the KGD calculation is calculated from blood sugar levels after alloxan-induced (pretest) reduced by blood sugar levels after treatment (posttest). The decrease in blood sugar levels was then averaged and classified by treatment group. The mean reduction obtained from the six treatment groups is as follows.

Table-1: The Average Reduction of Blood Sugar Levels of Mice from the Six Treatment Groups

No	Treatment Group	Mean of Blood Sugar Levels of Mice (mg/ dl)
1	Normal (K1)	9.3
2	Positive Control (K2)	35.4
3	Negative Control (K3)	48.5
4	Composition 1:1 (K4)	25.5
5	Composition 1:2 (K5)	37.9
6	Composition 1:3 (K6)	32.4

No Treatment Group Mean of Blood Sugar Levels of Mice (mg/ dl)

Data measurement of blood sugar levels used is the average difference in the decrease in blood sugar levels (Table-1). The data obtained showed the average blood sugar levels of mice after alloxan administration exceeded the normal limits of blood sugar levels of mice. The average blood sugar level of mice is 73 – 96.6 mg/dl. After being treated for 25 days, it was found that the average blood sugar levels of the six treatment groups decreased (Table-1).

CONCLUSION

The mechanism release of metformin encapsulated in alginate-chitosan in the gastric pH 1.2 physiological solution followed the Korsmeyer Peppas equation with a diffusion mechanism. In the intestinal physiological solution pH 7.4 followed the Higuchi equation with an erosion mechanism. The metformin encapsulated can reduce blood sugar levels in experimental animals that are induced with alloxan. The results of encapsulation with 1:2 composition was more optimal than 1:1 and 1:3 compositions which could

have an effect on lowering blood sugar levels compared to metformin drugs without alginate-chitosan encapsulation.

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REFERENCES

1. C. L. Broadhurst and P. Domenico, *Diabetes Technology & Therapeutics*, **8(6)**, 677(2006), <https://doi.org/10.1089/dia.2006.8.677>
2. Z. Norouzi, M.Kaviani, M.Tarrahi, M.Jariani, M.Abdollahian M, et al, *Epidemiology (Sunnyvale)* **6**, 249(2016), <https://doi.org/10.4172/2161-1165.1000249>
3. M.E, Okur, I.D, Karantas, & , P.I, Siafaka, *ACTA Pharmaceutica Scientia*, **55(1)**, 61(2017), <https://doi.org/10.23893/1307-2080.aps.0555>
4. B. E. Wilson and A. Gondy, *Diabetes Research and Clinical Practice*, **28(3)**, 179(1995), [https://doi.org/10.1016/0168-8227\(95\)01097-W](https://doi.org/10.1016/0168-8227(95)01097-W)
5. A. Holstein, A. Plaschke and E.H. Egberts, *Diabetes/Metabolism Research and Reviews*, **17(6)**, 467(2001), <http://dx.doi.org/10.1002/dmrr.235>
6. A. V. Abiazem, A. B. Williams, A. I. Inegbenebor, C. T. Onwordi, C. O. Ehi-Eromosele and L. F. Petrik, *Rasayan Journal of Chemistry*, **13(1)**, 177(2020), <http://dx.doi.org/10.31788/RJC.2020.1315328>
7. G. M. Srirangam and K.P. Rao, *Rasayan Journal of Chemistry*, **10**, 46(2017), <http://doi.org/10.31788/RJC.2021.1436332>
8. S. Sahoo, A. Sasmal, R. Nanda, A. R. Phani, and P. L. Nayak, *Carbohydrate Polymer*, **79(1)**, 106(2010), <https://doi.org/10.1016/j.carbpol.2009.07.042>
9. A. A. Umaredkar, P. V. Dangre, D. K. Mahapatra, and D. M. Dhabarde, *Material Technology*, **35(11-12)**, 697 (2020), <https://doi.org/10.1080/10667857.2018.1456617>
10. A. Riefflin, U. Ayyagari, S.E. Manley, R.R. Holman and J.C. Levy, *Diabetologia*, **58(1)**, 43(2015), <https://doi.org/10.1007/s00125-014-3399-1>
11. E. Ferrannini, *The New England Journal of Medicine*, **371(16)**, 1547(2014), <https://doi.org/10.1056/NEJMcibr1409796>
12. S. E. Cahyaningrum, N. Herdyastuti, and N. Qomariah, *Indonesian Journal of Chemistry*, **15(1)**, 16(2015), <https://doi.org/10.22146/IJC.21218>
13. M, S. M., Shankrayya, J S Venkatesh, V. J., Patil, S., R. M Santhosh, & J. Rabadia, *International Journal of Scientific Research*, **2(7)**, 436(2012), <https://doi.org/10.15373/22778179/july2013/147>
14. A. Holstein, A. Plaschke and E.H. Egberts, *Diabetes/Metabolism Research and Reviews*, **17(6)**, 467(2001), <https://doi.org/10.1002/dmrr.235>
15. G. M. Karau, E. N. M. Njagi, A. K. Machocho, L. N. Wangai, and P. N. Kamau, *British Journal of Pharmacology and Toxicolog*, **3(5)**, 251(2012)
16. D. Sawe, E. Njagi, A. Muchugi and M. Ndiema, *Open Journal of Applied Sciences*, **11**, 832(2021), <https://doi.org/10.4236/ojapps.2021.117061>
17. A.C. Friedli, I. R. Schlager, and S. W. Wright, *Journal of Chemical Education*, **82(7)**, 1017(2005), <https://doi.org/10.1021/ed082p1017>
18. L. Verma, A. Khatri, B.Kaushik, U. Patil, & R. Pawar, *Indian Journal of Pharmacology*, **42**, 224(2010), <http://doi.org/10.4103/0253-7613.68422>
19. I.A Abdel-Hassan, J/A, Abdel-Barry, & S.T Mohammeda, *Journal of ethnopharmacology*, **71(1)**, 325(2000), [https://doi.org/10.1016/S0378-8741\(99\)00215-9](https://doi.org/10.1016/S0378-8741(99)00215-9)
20. S. E. Cahyaningrum, Amaria, and A. M. Sholikhah, *Rasayan Journal of Chemistry*, **14(2)**, 1273(2021), <https://doi.org/10.31788/RJC.2021.1426218>
21. Y.A Abdirahman, K.K Juma, W.A Makori, D.S Agyirifo, M.P Ngugi, et al. *Pharmaceutica Analytica Acta*, **6**, 422(2015), <http://dx.doi.org/10.4172/2153-2435.1000422>

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