The effect of variation in the amount of Hemicellulose from Corn Cobs (Zea mays L) and pH on Metronidazole Microparticle Release

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Abstract. Objective: The present study was conducted to investigate the percentage of metronidazole microparticle release in an acidic environment at pH 1.2 and an alkaline environment at pH 7.4 and pH 8 in which the highest release occurred and to investigate the microparticle release kinetics. Method: The research method used was an experimental method by making metronidazole microparticles with variations in the amount of hemicellulose from corn cobs which were combined with sodium tripolyphosphate (STPP) and propylene glycol (PG). The mixture was then put into capsules made from a combination of hemicellulose and sodium alginate (3:1). Results: Formula IV provided the smallest percentage of release in the gastric medium at pH 1.2 which was in the 240th minute with 1.80 ± 0.06 percent, followed with the formula IV in the intestinal medium at pH 7.4 in the 600th minute with (3.14 ± 0.13) percent. The largest release in formula IV was found in the colon medium at pH 8 which was in the 600th minute (66.21 ± 0.82) percent. The metronidazole microparticle formula using 200 mg of hemicellulose was not significantly different from using 250 mg of hemicellulose. The metronidazole microparticle release kinetics were formulated using 50, 100, 150, 200, and 250 mg of hemicellulose, all of which followed the zero order, first order, and the Higuchi order release kinetics. Conclusion: The release kinetics of metronidazole available on the market showed greater results than the metronidazole microparticles release which was formulated with variations in the amount of hemicellulose in various mediums.
1. Introduction
A drug release profile is an important part of the success of a preparation formulation in which the focus is the control of the drug release rate. Thus, the drug release kinetics and the duration of the drug to be released from the preparation can be obtained based on the information regarding the drug release. Drug manufacturers, however, rarely provide information about dissolution profile in the drug information sheets or in other media [1].

Microencapsulation is one of the techniques which can be used to make slow-release or controlled release preparations. Microencapsulation is a thin coating process of solid particles, liquid droplets, and dispersion of liquid by coating materials. The drug ingredients contained in a slow-release preparation will be released slowly through the mechanism of erosion and diffusion of the particles. The speed of the drug release from this preparation can be improved by reducing the molecular weight of the polymer and the particle size and controlling the polymer used as the matrix material[2].

One of the carrier materials which can be used in the formulation of the slow-release preparation so that it can be controlled and not break or dissolve in the gastric is hemicellulose. The reason is that hemicellulose is difficult to expand or dissolve in acidic pH, but it easily dissolves in alkali. Thus, hemicellulose can be used to make a drug substance into microparticles which are not easily dissolved in the gastric with acidic pH but easily dissolved in the colon which has an alkaline pH[3].

Hemicellulose is a fiber widely found in various natural materials, such as corn cobs which are frequently wasted, especially in Medan City, North Sumatra. Similarly, sodium alginate is obtained from brown algae which are widely available in Indonesian waters[4]. Previous studies have been conducted using a combination of chitosan and chondroitin sulfate and pectin and inulin as the carrier [5,6]. The results of these studies can improve the drug administration for colon in a slow-release preparation.

The researchers were interested in isolating hemicellulose from corn cobs used to produce microparticles from metronidazole material. This material was put into capsules made from a combination of hemicellulose and sodium alginate as the isolation results. In order to determine the metronidazole release profile in the drug preparation, an evaluation was conducted using a dissolution test in a gastric medium at pH 1.2, an intestinal medium at pH 7.4, and a colon medium at pH 8.

2. Methods and materials

2.1. Materials
The materials used in this research were isolation hemicellulose from of corn cob, isolation alginate from seaweed, metronidazole analytical standard (Sigma–Aldrich), sodium hydroxide (Merck), hydrochloric acid (Merck), acetic acid (Merck), sodium chloride (Merck) and potassium dihydrogen phosphate (Merck).

2.2. Preparation of the metronidazole microparticles
The preparations made into 20 capsules, the weight of materials for each formula. Hemicellulose which weighted according to each formula dissolved with 100 ml of 0.1 N sodium hydroxide. Subsequently, 100 ml of 0.1 N acetic acid was added while stirring, and its pH checked until 7.5. After it was rotated using a stirrer for 2 hours until homogeneous, STPP (sodium tripolyphosphate) added, stirred once again until homogeneous, and PG (polyethylene glycol) was also added dropwise while stirring until homogeneous. The next step was the metronidazole was added, stirred until homogeneous, and centrifuged at 1000 rpm for 3 hours so that the precipitate formed. The precipitate was separated, dried in an open room at room temperature, and protected from the sun (microparticles formed)[7].

| Table 1: Composition of metronidazole microparticle formula |
|------------------------------------------------------------|
| Material                     | Formula I | Formula II | Formula III | Formula IV | Formula V |
| Hemicellulose of corn cobs (mg) | 50        | 100        | 150         | 200        | 250        |
The weight of microparticles produced from each formula was made 10 grams with the addition of lactose and homogenized. The microparticles were divided into 20 parts in which each part was 500 mg subsequently. It was then put into capsules made from a combination of hemicellulose and sodium alginate.

2.3. Preparation of the primary standard solution
The primary standard solution of metronidazole was made with various solvents, such as the medium gastric solution at pH 1.2, the medium intestinal solution at pH 7.4, and the colon medium solution at pH 8. An amount of 50 mg of metronidazole standard was put into a 100 ml of volumetric flask. It was then dissolved with the artificial gastric medium at pH 1.2, filled to the marked line, and shaken until homogeneous so that the primary standard solution obtained with a concentration 500 µg/ml. 10 ml of the primary standard solution was put into a 100 ml of volumetric flask. Subsequently, it was diluted with the artificial gastric medium at pH 1.2, filled to the marked line, and shaken until homogeneous so that the secondary standard solution was obtained with a concentration 100 µg/ml. The same procedure was done for the metronidazole standard solution made using the intestinal medium at pH 7.4 and the colon medium at pH 8 [8].

2.4. Determination of the maximum wavelength absorbance of metronidazole
As much as 6.0 ml of the secondary standard solution of metronidazole (100 µg/ml) was put into a 50 ml of the volumetric flask, filled into the artificial gastric medium at pH 1.2 until the marked line, and shaken until homogeneous so that a solution was obtained with a concentration 12.0 µg/ml. After that, the absorption measured at a wavelength of 200-400 nm. The artificial gastric medium at pH 1.2 was used as the blank so that a maximum wavelength of metronidazole is in a solution at pH 1.2 obtained. Similarly, a solution was made using a solvent of the intestinal medium at pH 7.4 and the colon medium at pH 8. First, 4.0 ml of the secondary standard solution of metronidazole (100µg/ml) was put into a 50 ml of volumetric flask, filled into the artificial intestinal medium at pH 7.4, shaken until homogeneous so that a solution was obtained 8.0 µg/ml to the marked line. The same procedure also performed with the colon medium at pH 8 [8].

2.5. Determination of linearity of the calibration curve
3.0 ml, 4.5 ml, 6.0 ml, 7.5 ml, and 9.0 ml of the secondary standard solution put into a 50 ml of volumetric flask. The volume was adjusted with the artificial gastric medium at pH 1.2 to the marked line and homogenized. The metronidazole solution was obtained with a concentration of 6.0 µg/ml, 9.0 µg/ml, 12.0 µg/ml, 15.0 µg/ml, and 18.0 µg/ml respectively. After the absorption of the maximum wavelength measured (in point 3.5.2) using the solution blank of the artificial gastric medium at pH 1.2, the linearity of the calibration curve obtained, and the regression line equation calculated. The same procedure was also done to determine the calibration curve linearity and the regression line equation of the metronidazole solution in the medium intestinal solution at pH 7.4 and the colon medium solution at pH 8. This procedure used the metronidazole standard solution with a concentration of 4.00 µg/ml, 6.00 µg/ml, 8.00 µg/ml; 10.00 µg/ml, and 12.00 µg/ml. Subsequently, 2.0 ml, 3.0 ml, 4.0 ml, 5.0 ml, and 6.0 ml of the secondary standard solution of metronidazole (100 µg/ml) were put into a 50 ml of volumetric flask, homogenized, and the volume was adjusted in the intestinal medium at pH 7.4 to the marked line [8].
2.6. The preparation dissolution test
The dissolution test was conducted to determine the profile of the metronidazole release percentage differences from various formulas, mediums, and times using a dissolution tester tool. Sample: metronidazole microparticles with 50 mg, 100 mg, 150 mg, 200 mg, and 250 mg of hemicellulose was included in the combination of hemicellulose and sodium alginate capsules.

2.7. The dissolution test in the changing pH medium (gastric at pH 1.2, intestine at pH 7.4, and colon at pH 8)
900 ml of the artificial gastric dissolution medium at pH 1.2 put into the dissolution container, and the temperature set to 37±0.5°C. The paddle set to a height of 2 cm from the bottom of the tube and rotated with a stirring speed of 100 rpm. The tested capsules put into the container. 5 ml of aliquots were taken using a syringe at time interval starting from the 5th, 15th, 30th minute, and followed with every 30 minutes to the 600th minute. The process did in the same place which was in the middle of the surface of the dissolution medium and the upper part of the paddle. The absorbance was measured with a UV spectrophotometer at the maximum wavelength. The absorbance and the percentage of the metronidazole released at the time of aliquot collection obtained. The same procedure was also done in the artificial intestinal medium at pH 7.4 and the artificial colon medium at pH 8 [8].

3. Results and discussion

3.1. Microparticles
The formulation of metronidazole microparticles was made using various amounts of hemicellulose and combined with STPP (sodium tripolyphosphate) and PG (polyethylene glycol). The evaluation results of the particle size of the various formulas obtained see in Table 2
Table 2: The evaluation results of the microparticle formulas

| No | Formula | Hemicellulose (mg) | Particle size obtained (µm) |
|----|---------|--------------------|----------------------------|
| 1  | F1      | 50                 | 0.4536                     |
| 2  | F2      | 100                | 0.6359                     |
| 3  | F3      | 150                | 0.7173                     |
| 4  | F4      | 200                | 0.8932                     |
| 5  | F5      | 250                | 0.9185                     |

3.2. Maximum wavelength of metronidazole in the medium at pH 1.2

Based on the measurements in the gastric medium at pH 1.2, the maximum absorption of metronidazole standard at a wavelength of 276.20 nm was 0.4710. The maximum absorbance curve of metronidazole in a medium at pH 1.2 can see in Figure 1.

![Figure 1](image)

Figure 1. The maximum absorbance curve of metronidazole in the medium at pH 1.2

3.3. The calibration curve of metronidazole in the medium at pH 1.2

The measurement results can see in Table 3 and Figure 2.

Table 3: The calibration curve of metronidazole in the medium at pH 1.2

| Concentration (µg/ml) | Absorbance |
|----------------------|------------|
| 0.000                | 0.000      |
| 6.000                | 0.2017     |
| 9.000                | 0.3114     |
| 12.000               | 0.4213     |
| 15.000               | 0.5240     |
| 18.000               | 0.6390     |
The determination results of the calibration curve linearity of metronidazole standard in the gastric medium were 0.99949 which was greater than 0.995 whereas the regression equation was: $Y = 0.04107X - 0.0085$.

3.4. **Maximum wavelength of metronidazole in the medium at pH 7.4**

The maximum absorbance curve of the metronidazole standard solution in the medium at pH 7.4 in Figure 3.

3.5. **The calibration curve of metronidazole in the medium at pH 7.4**

The calibration curve of the metronidazole standard obtained by measuring the absorbance at a maximum wavelength of 320.00 nm and concentrations of 4.0 µg/ml 6.0 µg/ml, 8.0 µg/ml, 10.0 µg/ml, and 12.0 µg/ml in the artificial intestinal medium at pH 7.4. The measurement results can see in Table 4 and Figure 4.
Table 4: The determination results of the calibration curve of metronidazole in the medium at pH 7.4

| Concentration (µg/ml) | Absorbance   |
|-----------------------|--------------|
| 0.000                 | 0.0000       |
| 4.000                 | 0.2032       |
| 6.000                 | 0.3094       |
| 8.000                 | 0.4193       |
| 10.000                | 0.5310       |
| 12.000                | 0.6278       |

Figure 4. The calibration curve of metronidazole in the intestinal medium at pH 7.4

The determination results of the calibration curve linearity of the metronidazole standard in the intestinal medium at pH 7.4 showed the following regression equation: $Y = 0.06365X - 0.01158$. 

3.6. Maximum wavelength of metronidazole in the medium at pH 8

The measurements using the colon medium at pH 8 showed that the maximum absorbance found was 0.4778 in the wavelength of 319.40 nm. The maximum absorbance curve of the standard metronidazole solution in the medium at pH eight see in Figure 5

Figure 5. The maximum absorbance wavelength of metronidazole in the medium at pH 8
3.7. The calibration curve of metronidazole in a medium at pH 8
The measurement results can be seen in Table 5 and Figure 6.

Table 5: The determination results of the metronidazole calibration curve in the pH 8 medium

| Concentration (µg/ml) | Absorbance |
|-----------------------|------------|
| 0.000                 | 0.000      |
| 4.000                 | 0.2145     |
| 6.000                 | 0.3194     |
| 8.000                 | 0.4233     |
| 10.000                | 0.5207     |
| 12.000                | 0.6291     |

Figure 6. The calibration curve of metronidazole in the colon medium at pH 8

3.8. The dissolution test results of hemicellulose metronidazole microparticle capsules
The dissolution test in this study was done to describe metronidazole release from the preparations made in the stomach, intestine, and colon. By the preparation formula, there was no release in the stomach and intestine, but there was a release in the colon. Therefore, the dissolution test was performed in the artificial gastric medium at pH 1.2, artificial intestinal medium at pH 7.4, artificial colonic medium at pH 8, and the changing medium using the three mediums.

3.9. The dissolution test results of preparation in the artificial gastric medium at pH 1.2
The dissolution did by using an artificial gastric medium of pH 1.2, the retrieval time aliquat 5 ml every 30 minutes, from minutes to 5 to 360. The microparticles of metronidazole included in the combination of hemicellulose-alginate capsules (3: 1). Be able to survive can not be separated in the medium pH 1.2 because hemicellulose used as the material forming the capsule shell, is not soluble in acidic pH. It proves that the hemicellulose as the carrier can protect the active ingredients from acidifying, so it does not obtain data results percent release of the drug, whereas in a conventional capsule dosage metronidazole visible dissolution test results in minutes to 120 already perfect regardless of (86.39 ± 1, 01)%,

Thus, it fulfilled the requirements of the Indonesian Pharmacopoeia Fifth Edition (2014) in which the amount of dissolved metronidazole is not less than 85% (Q) of the amount indicated on the label with tolerance within 60 minutes [8].
3.10. The dissolution test results of preparation in the artificial intestinal medium at pH 7.4

The dissolution test was conducted using the artificial intestinal medium at pH 7.4, and 5 ml of the aliquot was collected every 30 minutes starting from the 5th minute to the 600th minute. The percentage of release calculated by the regression line equation obtained (at point 4.2.4), namely \( Y = 0.06365X - 0.01158 \). The calculation results of the dissolution test from various formulas, can see in Figure 7.

3.11. The dissolution test results of hemicellulose metronidazole microparticle capsules

The dissolution test in this study was done to describe metronidazole release from the preparations made in the stomach, intestine, and colon. Following the design of the preparation formula, there was no release in the stomach and intestine, but there was a release in the colon. Therefore, the dissolution test was performed in the artificial gastric medium at pH 1.2, artificial intestinal medium at pH 7.4, artificial colonic medium at pH 8, and the changing medium using the three mediums.

3.12. The dissolution test results of preparation in the artificial gastric medium at pH 1.2

The dissolution did by using an artificial gastric medium of pH 1.2, the retrieval time aliquot 5 ml every 30 minutes, from minutes to 5 to 360. The microparticles of metronidazole included in the combination of hemicellulose-alginate capsules (3: 1). It can not be separated in the medium pH 1.2 because hemicellulose used as the material forming the capsule shell, is not soluble in acidic pH. The proves that the hemicellulose as the carrier can protect the active ingredients from acidifying, so it does not obtain data results per cent release of the drug, whereas in a conventional capsule dosage metronidazole visible dissolution test results in minutes to 120 already perfect regardless of (86.39 ± 1.01)%.

Thus, it fulfilled the requirements of the Indonesian Pharmacopoeia Fifth Edition (2014) in which the amount of dissolved metronidazole is not less than 85% (Q) of the amount indicated on the label with tolerance within 60 minutes [8].

3.13. The dissolution test results of preparation in the artificial intestinal medium at pH 7.4

The dissolution test was conducted using the artificial intestinal medium at pH 7.4, and 5 ml of the aliquot was collected every 30 minutes starting from the 5th minute to the 600th minute. The percentage of release calculated by the regression line equation obtained (at point 4.2.4), namely \( Y = 0.06365X - 0.01158 \). The calculation results of the dissolution test from various formulas can saw in Figure 7.
Figure 7. The curve of the preparation dissolution in the medium at pH 7.4

Figure 7 showed that the average cumulative percentage of metronidazole release from formula IV using 200 mg of hemicellulose and formula V using 250 mg of hemicellulose was not significantly different. There was a small amount of metronidazole released in the medium at pH 7.4 up to the 600th minute in which formula IV was $(3.14 \pm 0.06)$ percent and formula V was $(2.98 \pm 0.13)$ percent. In contrast, the dissolution test results of the metronidazole capsule preparation available on the market showed that the preparation was completely released in the 240th minute with $85.79 \pm 0.65$ percent. This result fulfilled the requirements of the Indonesian Pharmacopoeia Fifth Edition (2014) in which the amount of metronidazole dissolved is not less than 85% (Q) of the amount stated on the label with tolerance within 60 minutes.

3.14. The dissolution test results of preparation in the artificial colon medium at pH 8

Dissolution was conducted using the artificial colon medium at pH 8 with 5 ml of the aliquot was taken every 30 minutes starting from the 5th minute to the 600th minute. The percentage of release calculated by the regression line equation (at point 4.2.6) which was $Y = 0.05914X + 0.004225$. The calculation results of dissolution test from various formulas and metronidazole capsule Figure 8.
Figure 8. The curve of the preparation dissolution test in the medium at pH 8

Figure 8 showed that the average cumulative percentage of metronidazole release in the 600th minute from formula IV using 200 mg of hemicellulose with (70.01 ± 0.46) percent was not significantly different from formula V using 250 mg of hemicellulose with (67.80 ± 0.45) percent. Metronidazole microparticles in the capsule of hemicellulose-alginate combination can be released well in the medium with pH 8. It is because hemicellulose as the form of the capsule is quickly swelling dissolved in pH 8. In contrast, it is difficult to dissolve in the acid medium at pH 1.2 and a weak base at pH 7.4. Based on the dissolution test results in the 240th minute, the metronidazole capsule preparation available on the market has completely dissolved with (86.32 ± 0.39) percent. It fulfilled the requirements of the Indonesian Pharmacopoeia Fifth Edition (2014) in which the amount of metronidazole dissolved is not less than 85% (Q) of the amount stated on the label with tolerance within 60 minutes.

3.15. Dissolution test of preparation in the changing medium pH 8

The dissolution test using changing pH mediums was done in the artificial gastric medium at pH 1.2 for 3 hours, proceed with the artificial intestinal medium at pH 7.4 for 4 hours, and continued in the artificial colon medium at pH 8 for 4 hours.

The test was conducted on formula IV using 200 mg of hemicellulose because formula IV was found to be the best formula according to the ANOVA and the Tukey test results. The aim was to see the profile and description of the metronidazole release from the microparticle formula using hemicellulose in the combination of hemicellulose and sodium alginate capsules in the gastric, intestinal, and colon medium. The results can be seen in and Figure 9.
Figure 9 shows that metronidazole formulated in the form of microparticles using 200 mg of hemicellulose for 125 mg of metronidazole has a percentage of metronidazole release that is virtually absent in the gastric media at pH 1.2 and on the intestinal media at pH 7.4. In contrast, the percentage of release is relatively good in the large intestinal medium at pH 8. This may be because hemicellulose can bind metronidazole well and cannot be easily expanded and dissolved in pH 1.2 and pH 7.4 whereas it is easy to expand and dissolve in pH 8 [9].

In terms of the chemical properties, hemicellulose can decompose with alkaline solvents, and the bond is weak, so it is easily hydrolyzed. Degradation of cellulose or hemicellulose chains easily occurs through alkalis causing hydrolytic decomposition in the glucosidic bonds. The acetyl group found in hemicellulose is highly sensitive to alkali, and glucuronic acid compounds can slow the breakdown of the carbohydrate chain through the last group. Moreover, hemicellulose is not fiber, has non-crystalline properties, natural to expand, slightly soluble in water, highly hydrophilic, and easily soluble in alkali [9].

The best formula obtained 200 mg of hemicellulose. However, but the result was not significantly different from using 250 mg of hemicellulose. It can see from the dissolution test results of the formula. It put into a combination of hemicellulose and sodium alginate capsule. It was not braking in the gastric medium at pH 1.2 and intestinal medium at pH 7.4 although there was a small release of metronidazole in the 360th minute which was (0.00) percent in the medium at pH 1.2 and (2.69 ± 0.05) percent in the medium at pH 7.4. The presence of this release is likely to occur permeation or diffusion through the pore. In the artificial colon medium at pH 8, the formula was broken, and the metronidazole was released perfectly with (74.24 ± 0.045) percent.

4. Conclusions
The smallest percentage of release was found in the gastric medium at pH 1.2 and intestinal medium at pH 7.4 whereas the largest percentage of release was found in the colon medium at pH 8. The best metronidazole microparticle formula put in a capsule combination of hemicellulose and sodium alginate (3:1) was 200 mg of hemicellulose. Microparticles of Metronidazole that formulated with various amounts of hemicellulose in the capsule combination of hemicellulose and sodium alginate (3:1) produced smaller metronidazole release than the metronidazole release on the market in the gastric medium at pH 1.2, intestinal medium at pH 7.4, and colon medium at pH 8.
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6. Conflict of interests
The authors have declared “no conflicts of interest with respect to the research, authorship, and/or publication of this article”

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