Isoniazid Microencapsulation With HPMCP HP-50 and HPMCP HP-55 (2:3) Coating Using Solvent Evaporation Method

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Abstract
The combination formulation of tuberculosis drugs may cause interactions if these drugs are given simultaneously. Rifampin (RIF) decomposes in the stomach when given concurrently with isoniazid (INH), which results in a decrease in the bioavailability of RIF. The purpose of this study is to make INH microcapsules using HPMCP HP-50 and HP-55 coatings to prevent these interactions. The process of making INH: HPMCP HP-50 and HP-55 (2:3) microcapsules was done by using solvent evaporation method. The entrapment efficiency of INH: HPMCP HP-50 and HP-55 (2:3) were 83.21% and 91.57%, respectively. The dissolution test of INH: HPMCP HP-50 and HP-55 microcapsules met the requirements of the Indonesian Pharmacopoeia Edition V. The FTIR results showed that there was no change either in the chemical composition of isoniazid or in the coating of the microencapsulation. Scanning Electron Microscopy (SEM) showed the active substance was well coated. This study produces microcapsules that can provide a delayed release effect, so it is expected that INH: HPMCP HP-50 and HP-55 (2:3) microcapsules can be released in the intestines without interacting with RIF.

Keywords: HPMCP HP-50, HPMCP HP-55, isoniazid, microcapsules, solvent evaporation method

Mikroenkapsulasi Isoniazid - HPMCP HP-50 dan HPMCP HP-55 dengan Perbandingan (2:3) Menggunakan Metode Penguapan Pelarut

Abstrak

Kata Kunci: HPMCP HP-50, HPMCP HP-55, isoniazid, mikrokapsul, metode penguapan pelarut.
1. Introduction

Single drug therapy with isoniazid (INH) is not effective in treating tuberculosis due to the risk of developing resistance in a short time. Currently, the administration of drugs recommended by WHO for the new treatment of TB cases is a combination of three or four different first-line drugs, namely rifampin (RIF), isoniazid (INH), ethambutol (ETB) and pyrazinamide (PYR). Combination formulations of tuberculosis (TB) drugs may increase the risk of drug interactions given concurrently which can affect the bioavailability of the drugs. The TB drug combination of rifampin (RIF) taken on an empty stomach at a pH of around 1.4–2.1 will be easily decomposition into isonicotinyl hydrazone (HYD) due to the presence of INH. This reaction is thought to be the cause of the lower RIF in vivo bioavailability of the TB drug combination. RIF is converted to 3-formylrifamycin below pH 2, which then reacts with INH to form HYD. Isoniazid (INH) is soluble at pH 1.2 but has poor gastric permeability and is very well absorbed in the intestine.

Microcapsules contain solid or liquid active ingredients which are dispersed or dissolved in the matrix. Microcapsules are microscopic-sized reservoirs surrounded by walls that are capable of controlling the release of the active substances. Microcapsules have many advantages based on their structural and functional capabilities among others; they can be applied conveniently and administered via several routes. Depending on the formulation, microcapsules can be incorporated into different pharmaceutical dosage forms such as solids (capsules, tablets, sachets), semi-solids (gels, creams, pastes), or liquids (solutions, suspensions, and even parenterals). Another advantage of microcapsules preparations is that when compared to nanoparticles, they do not cross the interstitium as they are larger than 100 nm in size and transport does not go through lymph vessels, so that it can work locally.

Hydroxy Propyl Methyl Cellulose Phthalate (HPMCP HP-50 and HP 55) is a cellulose-derived enteric-coated polymer which is insoluble in gastric juice, but expands and dissolves in intestinal fluid. HPMC HP-50 and 55 are commonly used as soluble coatings, respectively at pH above 5.0 and 5.5, and can withstand the release of drugs in the stomach, so it can be used as a polymer for the purpose of delayed release. Microencapsulation can be made by several methods. One of the methods that are popular and often used is the solvent evaporation method. This method can be done by dissolving the coating in a volatile solvent and the active substance is dissolved or dispersed in the coating solution and then the solvent evaporation is carried out. This method has a short processing time, can be used for various core materials such as water-soluble or water-insoluble materials. The purpose of this study is to make INH microcapsules using HPMCP HP-50 and HP-55 (2:3) coatings to prevent interactions between INH and RIF. It is hoped that INH will not be degraded in the stomach and absorbed in the intestine after the coating, preventing interaction with RIF, which can result in a decrease in the bioavailability of RIF. Previously, similar research has been conducted on the manufacture of INH microcapsules by using HPMCP HP-50 and HP-55 (1:1) coatings but the results are not optimal.

2. Materials and Methods

2.1. Materials

Isoniazid (Amsal Chem, India), HPMC HP 50 and 55 (Shin Etsu Japan), magnesium stearate, span 80, ethanol, liquid paraffin, n-hexane, hydrochloric acid, phosphoric buffer and other materials used in the analysis.

2.2. Tools

Fourier Transform Infrared Spectroscopy (FTIR), SEM (Jeol), UV-visible Spectrophotometry (Shimadzu PC-1601), orbital shaker (IKA), dissolution test equipment (Vanguard RC-6), ultrasonic/sonifier (Branson, Model 3510E-DTH, Danbury, USA), orbital shaker graded sieve (IKA, Germany), pH meter (Boeco), analytical balance and other tools commonly used in laboratories.
2.3. Methods
2.3.1. Manufacturing of Microcapsules with HPMCP HP-50 and HPMCP HP-55 Polymers
HPMCP HP-50 and 55 were dissolved in acetone, then stirred until dissolved (polymer solution). Isoniazid was dispersed in a polymer solution and stirred until homogeneous using a homogenizer. The isoniazid dispersion and polymer solution were then emulsified into liquid paraffin containing 1 g of span 80. The emulsion solution was stirred at 900 rpm until the solvent completely evaporated and microcapsules were formed. The formed microcapsules were then collected through the process of decantation and washed twice with n-hexane to remove the attached liquid paraffin, then dried in an oven for 2 hours. Furthermore, microcapsule evaluation was carried out.16

2.3.2. Microcapsule Evaluation and Characterization17

Microcapsule Particle Size Distribution
The size distribution of the microcapsules was evaluated using a sieve shaker. A series of two sieves with sieve numbers 35 and 40 was arranged in succession from the size of the largest sieve hole. A total of 5 grams of microcapsules was placed in the top sieve, and then the sieving machine was run for 10 minutes. Each fraction in the sieve was weighed, and carried out 3 times for each formula.

Entrapment Efficiency
Weighed the equivalent of 500 mg microcapsules, then dissolved in ethanol up to 100 ml. Absorption was measured at a maximum wavelength of 266 nm by UV spectrophotometry. The test was carried out 3 times for each formula.

Isoniazid Dissolution Test
The dissolution profile of isoniazid powder and microcapsules was determined using a type 2 dissolution apparatus (paddle) at a speed of 50 rpm at 37±0.5 ºC in a solution of hydrochloric acid pH 1.2 and phosphate buffer pH 6.8 with a medium volume of 900 ml for 12 hours. Sampling of 5 ml was carried out at the 1st and 2nd hours. The sampling was continued in phosphate buffer medium pH 6.8 at 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12 hours. The samples obtained were analyzed and their absorption was measured at a wavelength of 266 nm using UV spectrophotometry. Each time a sample was taken, the volume of the medium was replaced with a new medium solution with the same volume and temperature.

<table>
<thead>
<tr>
<th>Substance</th>
<th>HPMCP HP-50</th>
<th>HPMCP HP-55</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoniazid</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Coating</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>Acetone</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Magnesium stearate</td>
<td>0.75</td>
<td>0.75</td>
</tr>
<tr>
<td>Span 80</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Liquid paraffin up to 100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 1. INH Microcapsule Formulation: HPMCP HP-50 and HP-55 (2:3)

<table>
<thead>
<tr>
<th>Size (µm)</th>
<th>Microcapsule Size (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥500</td>
<td>21,11±1,13</td>
</tr>
<tr>
<td>250-500</td>
<td>79,24±0,47</td>
</tr>
<tr>
<td>≤250</td>
<td>0,01±0,01</td>
</tr>
</tbody>
</table>

Table 2. Microcapsule Particle Size Distribution for INH-HPMCP HP-50 (2:3) and INH-HPMCP HP-55 (2:3)
Fourier Transform Infrared Spectroscopy (FTIR)
A total of 3 mg of the sample was put into a clean and dry sample holder, then sample analysis was conducted on a programmed FTIR device and the spectrum results would come out.

Microcapsule Shape and Surface Morphology
The shape and surface morphology of the microcapsules were observed using Scanning Electron Microscopy (SEM).

3. Results
Microcapsule particles with a stirring speed of 900 rpm produced the most microcapsule size of INH: HPMCP HP-50 at a size of 250-500 μm with an average percentage of 79.24%, and in INH: HPMCP HP-55 produced a slightly higher percentage of microcapsules at a size above 500 μm with an average of 61.53% (Table 2).

The entrapment efficiency of microcapsules was carried out to determine the level of active substances coated in the microcapsules. In this study, the entrapment efficiency results of INH: HPMCP HP-50 and 55 (2:3) microcapsules were 83.21% and 91.57%, respectively (Table 3).

In this study, the results of the in vitro dissolution test of pure INH in hydrochloric acid buffer medium pH 1.2 obtained solute level after 2 hours was 111.42%. Meanwhile, in INH-HPMCP (2:3) HP-50 and HP-55 microcapsules, the solute levels were 7.64% and 5.74%, respectively (Fig. 1). In vitro dissolution test results of pure INH in phosphate buffer medium pH 6.8 obtained the solute level after 2 hours was 104.62%. Meanwhile, in INH-HPMCP (1:1) HP-50 and HP-55 microcapsules, the solute levels were 95.60 and 99.62%, respectively (Fig. 2).

Table 3. INH Microcapsule Formulation: HPMCP HP-50 and HP-55 (2:3)

<table>
<thead>
<tr>
<th>No</th>
<th>Amount of Material (%) w/v</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HPMCP HP-50</td>
</tr>
<tr>
<td>1</td>
<td>87,09</td>
</tr>
<tr>
<td>2</td>
<td>82,47</td>
</tr>
<tr>
<td>3</td>
<td>80,08</td>
</tr>
<tr>
<td>Average ±SD</td>
<td>83,21%±3,56</td>
</tr>
</tbody>
</table>

4. Discussion
The process of forming microcapsules using the solvent evaporation method requires optimization of time and stirring speed. Optimization of time was carried out to find out how long it would take to form microcapsules. Microcapsules are formed when the acetone has evaporated completely. In addition, the speed of stirring (agitation) is one of the most important parameters to control the size of microspheres after the physico-chemical properties of the active substance. At the time of formation of microcapsules, the type of stirring (impeller or baffle) used determines the size of the microcapsules. If the mixing speed increases, it generally results in a decrease in particles. The optimum stirring speed in this study was 900 rpm as the size of...
the microcapsules produced was smaller than the speed of 800 rpm. Meanwhile, at 1000 rpm, no microcapsules were formed. INH microcapsules were made with a ratio of (INH: HPMC HP-50/55) 2:3 using the solvent evaporation method. The principle of making microcapsules using the solvent evaporation method is to use a volatile solvent that will dissolve the microcapsule or polymer layer and not mix with other liquid carrier phases.19

The formed microcapsules were then washed with n-hexane to remove the remaining paraffin that was still attached to the surface of the microcapsules. N-hexane has volatile criteria and cannot dissolve microcapsules that have been coated with HPMC HP-50, n-hexane will only dissolve paraffin and the remnants of magnesium stearate that are still attached to the microcapsules. In the last step, the microcapsules were vacuumed and dried at room temperature to remove the remaining n-hexan.16

The entrapment efficiency of microcapsules was carried out to determine the level of active substances coated in the microcapsules. In this study, the entrapment efficiency results of INH: HPMC HP-50 and 55 (2:3) microcapsules were 83.21% and 91.57%, respectively (Table 3.2). Various factors may affect the recovery results of microcapsules. HPMC HP-50 and 55 coatings have differences in phthalate

Fig.1. Dissolution profile of INH, INH-HPMCP HP-50 (2:2), INH-HPMCP HP-55 (2:3) in hydrochloric acid buffer solution pH 1.2

Fig.2. Dissolution profile of INH, INH-HPMCP HP-50 (1:1), INH-HPMCP HP-55 (2:3) in phosphate buffer solution pH 6.8
content, pH solubility and viscosity. HPMC
HP-50 and 55 viscosities were 55 and 40 cps,
respectively. This indicates that the viscosity
affects the adsorption of the isoniazid active
substances. High viscosity which causes an
increase in viscosity of the preparations will
inhibit the release of isoniazid drug, so that
microcapsules with HPMC HP-50 coating
will provide a lower percentage of recovery
than microcapsules with HPMC HP-55
coating. In addition to the above mentioned
factors, the amount of polymer used will
also affect the percentage of entrapment
efficiency. The less polymer used, the more
active substance will be adsorbed during
stirring. Conversely, the more polymer used,
the less active substance will be adsorbed
during stirring, this occurs because a strong
polymer wall is formed so that it will hinder
the diffusion of isoniazid.20

The dissolution test was carried out in
vitro to determine the dissolution profile of
pure INH and INH microcapsules. The test
was carried out in hydrochloric acid buffer
medium pH 1.2 for 2 hours and in phosphate
buffer medium pH 6.8 for 2 hours. Testing
on these two buffer mediums carried out so
that the conditions for the release of the active
substance are close to in vitro conditions in
the gastrointestinal tract. In this study, the
results of the in vitro dissolution test of pure
INH in hydrochloric acid buffer medium pH
1.2 obtained solute level after 2 hours was
111.42%. Meanwhile, in INH-HPMCP (2:3)
HP-50 and HP-55 microcapsules, the solute
levels were 7.64% and 5.74%, respectively.
These results have met the requirements of
the Indonesian Pharmacopoeia edition V, in
which for delayed release preparation, the percentage of solute is
not less than 80% after 2 hours (Fig. 2). These
results indicate that the INH-HPMCP HP 50
and INH-HPMCP HP-55 microcapsules met
the expected goals of producing a low level
of percent dissolution in hydrochloric acid
medium pH 1.2, which implies that after
the microcapsules were made, the release of
INH was effectively retained in the stomach
compared to the pure INH, which was
released 111.42% in the stomach. Based on
the literature, it was found that if INH is
released in the stomach, RIF will be degraded
into isonicotinyl hydrazine (HYD) due to
the presence of INH. This reaction is thought
to be the cause of the lower RIF in vivo
bioavailability of the TB drug combination.
The results of the microcapsule dissolution
test in phosphate buffer medium pH 6.8
showed that after microcapsules were made,
INH could be released and well absorbed in
the intestine. The results of dissolution test
also showed that INH does not interact with
RIF in the stomach but is released in the
intestine. For the dissolution test of RIF, it has
not been carried out. It is only based on the
literature that RIF is soluble and released in
the stomach.4,5,21

FTIR (Fourier Transform Infrared
Spectroscopy) is a spectroscopic technique
used to detect the formation of microcapsules
by looking at the spectral wave numbers in
certain groups. In the isoniazid spectrum,
there is absorption of the carbonyl group at
a wave number of 1664 cm-1. N-H strain
occurs at a wave number of 1552.32 cm -1.
In
addition, there are many tenuous vibrational
regions between 1407 and 668.53 cm-1 in
the isoniazid spectrum. The carbonyl group
was detected at 1716 cm-1 for HPMCP (19).
Several characteristics of isoniazid tenuous
vibrations were seen in the INH-HPMCP
HP-50 and HP-55 microcapsules (Fig. 3).
The FTIR spectra of isoniazid microcapsules showed the same combined peaks as isoniazid and HPMCP. The FTIR results showed that there was no change either in the chemical composition of isoniazid or in the coating of the microencapsulation. Therefore, it was concluded that no chemical reaction or decomposition occurred before or after the microcapsules were formed. 22,23,24,25

5. Conclusion

The particle size distribution test for INH: HPMCP HP-50 microcapsules gave the most particle size at > 500 µm, while for INH: HPMCP HP-55 microcapsules at a size of 250-500 µm. The entrapment efficiency of INH: HPMCP HP-50 and HP-55 (2:3) were 83.21% and 91.57%, respectively. The dissolution test of INH:HPMCP HP-50 and HP-55 microcapsules has met the requirements of the Indonesian Pharmacopoeia edition V. The FTIR test showed that the microcapsules didn’t change the chemical composition of isoniazid or the coating on the microencapsulation so that it was concluded that no chemical reaction or decomposition occurred before and after the formation of the microcapsules. Scanning Electron Microscopy (SEM) showed a spherical microcapsule surface morphology and the active substance was well coated for INH: HPMCP HP-50 (2:3), while for INH: HPMCP HP-55 (2:3) the surface of the microcapsules was round but hollow. This study produces microcapsules
that can provide a delayed release effect, so it is expected that INH: HPMCP HP-50 and HP-55 (2:3) microcapsules can be released in the intestines without interacting with RIF.

6. Acknowledgements
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