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Application of CornCobs Hemcellulose Microparticle Carrier With Insulin as a Model

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ABSTRACT

Hemicellulose is a biodegradable polymer in the colon because bacteria of human colon degrade them and thus make potentially useful in colon targeted drug delivery systems, these polymers are hydrophilic and hydrogel in nature and have limited swelling characteristics in acidic pH. The aim of this study is to experiment with corn cobs hemicelluloses to be used for microparticle carrier of solid oral dosage forms for colonic delivery of insulin. This research was conducted by preparation of insulin hemicellulose microparticles with varying concentrations of 0.5%, 0.75% and 1% hemicellulose and 0.1% TPP and 30 IU insulin. Insulin hemicellulose microparticles testing performed of the solubility in pH 2; pH 5; pH 7; pH 7.5 and pH 8, as well as SEM and PSA testing. Results showed that insulin hemicellulose microparticles having a particle size with an average of 12-17 μm and solubility obtained that these microparticles insoluble in pH 2; pH 5; slightly soluble in pH 7 and soluble in pH 7.5 and pH 8. It can be concluded that the corn cob hemicellulose can be developed for raw material of microparticles drug that are absorbed in the colon.

Keyword: application, hemicelluloses, carrier, microparticles, insulin

INTRODUCTION

Drug dosage forms contain many components in addition to the active pharmaceutical ingredient(s) to assist in the manufacturing process as well as to optimize drug delivery. Due to advances in drug delivery technology, excipients are currently included in novel dosage forms to fulfil specific functions and in some cases they directly or indirectly influence the extent and rate of drug release and absorption. Hemicellulose has many requirements expected of pharmaceutical excipients such as non-toxicity, stability, availability and renewability they are extensively investigated for use in the development of solid oral dosage forms. Hemicelluloses are structural polysaccharides which are the second most abundant heteropolymers present in nature accounting for one third of total components available in the plants [1,2].

Hemicellulose is a natural polymer that is nontoxic, mucoadhesive, biodegradable, biocompatible. These components consist of a group of complex polysaccharides that are bound to the surface of cellulose microfibrils but their structure prevents them from forming microfibrils by themselves. These polysaccharides consist of xyloglucans, xylans and mannans that can be extracted from the plant cell wall with a strong alkali. They have backbones made up of β -1,4-linked D-glycans. Xyloglucan has a similar backbone as cellulose, but contains xylose branches on 3 out of every 4 glucose monomers. The β -1,4-linked D-hemicellulose backbone of arabinose hemicelluloses contains arabinose branches [3,4-6].

Mannan is one of the important member of the hemicellulose family and can be divided to four subfamilies: linear mannan, glucomannan, galactomannan, and galactoglucomannan [7].

Mannan is present in different forms, each having a β -1,4-linked backbone containing mannose (linear mannan) or a combination of glucose and mannose residues (glucomannan) and occasional side chains of α -1,6-linked galactose residues. In the backbone, mannose and glucose units can also be acetylated at C-2 or C-3 [8,9].

Glucomannan is a hydrocolloidal polysaccharide of the mannan family consisting of β -1,4 linked D-mannose and D-glucose monomers with acetyl side branches on some of the backbone units. The acetyl groups contribute to its solubility and swelling capacity and assist in making it a soluble natural polysaccharide with the highest viscosity and water-holding capacity. It has been investigated as an effective excipient in controlled release drug delivery devices in combination with other polymers or by modifying its chemical structure. Konjac glucomannan cross-linked with sodium tri polyphosphate formed hydrogel systems that could sustain hydrocortisone release dependent on cross-linking density and enzymatic degradation [8,9].

Targeting pharmaceutical drugs to the colon makes it possible to achieve local or systemic drug delivery to this site. To deliver the compounds in a non-degraded form to the lower part of the gastrointestinal tract, they must first of all pass through the stomach, the upper part of the intestine and must use the characteristics of the colon to specifically release the drugs in this part of the digestive tract. Various bacteria present in the colon secrete many enzymes which can cause hydrolytic cleavage of glycosidic bonds e.g. β -D-galactosidase, amylase, pectinase, β -D-glucosidase, dextranase, α -D-xylosidase [8,9].

Colon is used for systemic absorption of proteins and peptides also proteolytic activity of colon mucosa is much less than that observed in small intestine. Drug targeting to specific sites of action offers several advantages over non targeted drugs such as prevention of side effects and reduction of doses [8,9].

Insulin, 51 amino acid protein, can get deteriorated by gastric pH and intestinal enzymes, and even intestinal epithelial cell membranes serve as absorption barrier for intact peptide structure resulting in less than 1 % bioavailability of total insulin taken orally [10,11].

Several methods of isolations have been developed in order to isolate hemicellulose based on solubility. Isolation of hemicellulose process must conform with the principles of green chemistry. The hemicellulose can be utilized as a new alternative polymer for various applications especially in pharmaceutical preparations [12].

Polymeric microparticles have turned out to be a promising approach as a targeted drug delivery system for the treatment at colon. Egito and colleagues have been working for over a decade on the extraction of hemicellulose from corn cobs and its use for the development of microparticles as drug carriers for colon specific delivery of anti-inflammatory and toxic drugs, such as sodium diclofenac (SD), 5-aminosalicylic acid (5-ASA), and usnic acid (UA). Hemicellulose-coated microparticles have also been developed by Egito and co-workers in order to deliver magnetite particles [13]. Different microencapsulation techniques have been used for the production of hemicelluloses based microparticles. Coacervation, interfacial cross-linking polymerization, and spray drying have been shown to be the most successful methodologies for that purpose [13,14].

Simple isolation technology of hemicelluloses and manufacture of microparticles does not require equipment and not difficult that will be developed according to the needs of the pharmaceutical industry, economic terms, this study is very promising, because it is in line with the increase in corn production, the availability of corn cobs. Hemicellulose has the properties of the acid-insoluble and soluble in alkaline as well as the most important that these polymers will biodegrade in the colon so that it will release a drug that is acid resistant compound especially peptides and proteins, including insulin, therefore corn cob hemicellulose can be developed for drug release in the colon

The aim of this research to produce and stabilize corn cobs hemicelluloses based drug microparticles produced with ionic gelatin method using tripolyphosphate as crosslinker and insulin is a model. The performance of the particles was evaluated utilizing different solid state characterization methods, solubility in acid and alkaline, SEM and PSA.

MATERIALS AND METHODS

Materials and Reagent

Corn Cobs were obtained from local Corn in Medan, Indonesia. Insulin (Sigma) NaOH (E.Merck), 35% H₂O₂ (E.Merck), 96% ethanol (E.Merck), CH₃COOH (E.Merck), Sodium Tri polyphosphate (E.merck), Propylene Glycol (E.merck), 0.1N HCl (EMerck), pH 7.5 Buffer, All other chemicals used were of analytical grade.

Apparatus

Magnetic Stirrer, hot plate, universal indikator, Filter membran PTFE @100, Centrifuge, pH meter, Homogenizer, Particle size analyzer, Viscometer, thermostat, microscope, scanning electron microscope, FTIR (Hitachi) HPLC (Shimadzu) Spektrofotometri UV (Hitachi),

Preparation and isolation of corn cobs hemicelluloses

Preparation begins with drying the corn cobs, then cut them small with the size of 1 cm per side, crushed and sieved. This fine powder is used to identify the corn cobs, determination the Kappa Numbers for delignification and isolation of corn cobs hemicelluloses [12].

Preparation of insulin –corn cobs hemicelluloses microparticles.

Hemicellulose weighed 500 mg, 750 mg and 1 gram, then each dissolved in 100 ml of 0.1 N NaOH and 100 ml of 0.1 N acetic acid, the obtained solution containing hemicellulose concentration of 0.5%, 0.75% and 1%. Hemicellulose in acetic acid is added with hemicelluloses in 0.1 N NaOH dropwise, while stirring with a magnetic stirrer at 500 rpm and pH checked with a pH meter to obtain a pH of 7.5. After that 0.1% STPP was added to hemicelluloses solution in pH 7.5 and stirred the solution with a magnetic stirrer at a speed of 3000 rpm for 30 minutes to obtain a homogeneous turbid solution. To the hemicelluloses suspension gives 0.5 ml propylene glycol and then stirred with a speed of 3000 rpm for 15 minutes, then the hemicellulose suspension allowed to stand for 24 hours. To the hemicelluloses suspension respectively added 30 iu insulin in buffer solution pH 7.5 and stirred with a magnetic stirrer at 500 rpm for 15 minutes, then centrifuged for 10 minutes at a speed of 5000 rpm, and the solution was separated. The residue was dry by putting in a glass plate and dry with air dryer for 48 hours and then crushed particles obtained by the mortar until smooth [15].

Solubility test of insulin corn cobs hemicellulose microparticles

Insulin corn cobs hemicelluloses microparticles as much as 100 mg suspended in a buffer solution of pH 2, pH 5, pH 7, pH 7.5 and pH 8 and then in the stirrer with a magnetic stirrer at 500 rpm for 10 minutes and then then allowed to stand for 90 minutes and observed the solubility of the microparticles [12].

Characterization of Corn Cobs Hemicellulose Using Infrared Spectrophotometry (FTIR)

Insulin corn cob hemicellulose micro particles weighed 1 mg and 200 mg of potassium bromide. Then put into a mortar, crushed until homogeneous, and then put into an FTIR instrument is further analyzed in the wave number range 4000 - 500 cm⁻¹ and recorded vibration spectrum Infrared [12].

Characterization of Corn Cobs Hemicellulose Using High Performance Liquid Chromatographi (HPLC)

Method of characterization by High Performance Liquid Chromatographi as follows: 25 mg of insulin-corn cobs hemicellulose weighed, and then put into 50 ml volumetric flask and added pure water to mark. Shaken, then filtered (first few ml of filtrate was discarded). Solution was then filtered through a membrane filter of 0.2 µm Cellulose Nitrate. Then about 50 ml solution was injected into the HPLC system via a loop injector with a 20 ml, using an isocratic elution system with distilled water with a mobile phase, flow rate 0.8 ml / min. The detection was made using a UV detector at a wavelength of 280 nm. The chromatogram recorded and performed a qualitative analysis by the area, peak height and retention time insulin hemicelluloses microparticles [16].

Particle size evaluation

Particle size evaluation was done using particle size analyzer [15].

Particle morphology evaluation

Particles surface and morphology of particles were observed by scanning electron microscope (SEM) in different magnification [15].

RESULTS AND DISCUSSION

Insulin –corn cobs hemicelluloses microparticles were prepared with ionic gelation using tri polyphosphatas crosslinker, and propylene glycol, then spray dried in room temperature for 5 hour. Ionic gelation is based on the ability of polyelectrolytes (polymer) to crosslink in the presence of counter ions (crosslinker) to form hydrogel particles. Microparticles obtained by testing the solubility in a wide range of pH, FTIR, HPLC, SEM and PSA results are as follows

Characterization solubility of Insulin hemicelluloses microparticles

The test results on the solubility in a wide range of pH can be seen in the table 1 below:

Table 1. Characterization of corn cob hemicelluloses microparticles with solubility

Characterization	Insulin hemicelluloses microparticle
Solubility	Aquadest
	Hot Aquadest
	pH 2
	pH 5
	pH 6
	pH 7
	pH 7.5
	pH 8

Based on the Table 1 above, it can be seen that the solubility of microparticles hemicellulose insulin that microparticles is not soluble in pH 2, pH 5 and pH 6, and this means that the STPP in the bond links this cross is not at all affect the solubility of particles hemicellulose, as well as to the entrapment of insulin in nanoparticles hemicellulose STPP. Solubility occurs at pH 7 but takes 90 minutes and pH 7.5 solubility occurred at a time of 60 minutes means there have been splits and insulin particles contained inside the particles had dissolved, while at pH 8, after the microparticles allowed to stand 30 minutes the amount of soluble grow this large, indicating that the majority of microparticles has been dissolved. But unfavorable pH 8 for release in the colon where insulin is degraded in the pH 8, so no longer efficacious as oral anti-diabetes. Therefore, the best pH is pH 7.5 as well as microparticles has been broken, but the presence of bacteria in the colon which will help release the xylanase enzyme degradation of hemicellulose, which will accelerate the entrapment of insulin into the blood.

Characterization of Insulin hemicelluloses microparticles by FTIR

The results of FTIR measurements of insulin hemicellulose microparticles can be seen in the Figure 1 and Figure 2 below

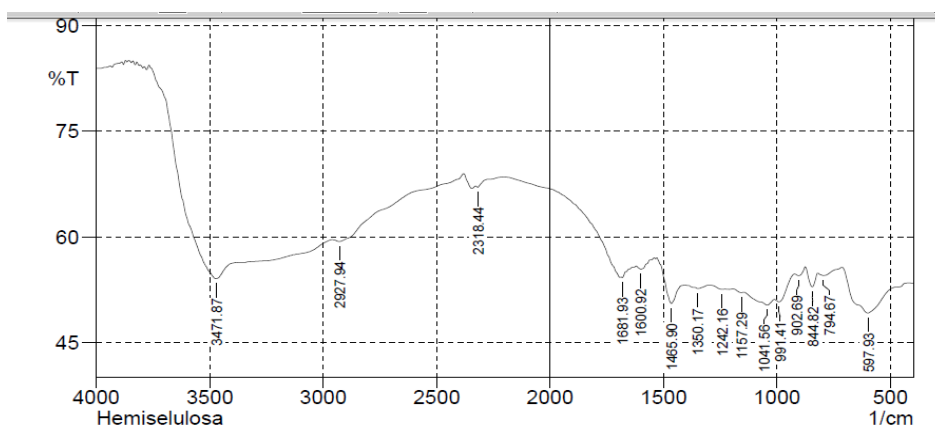


Figure 2. FTIR image corncobs hemicellulose [15]

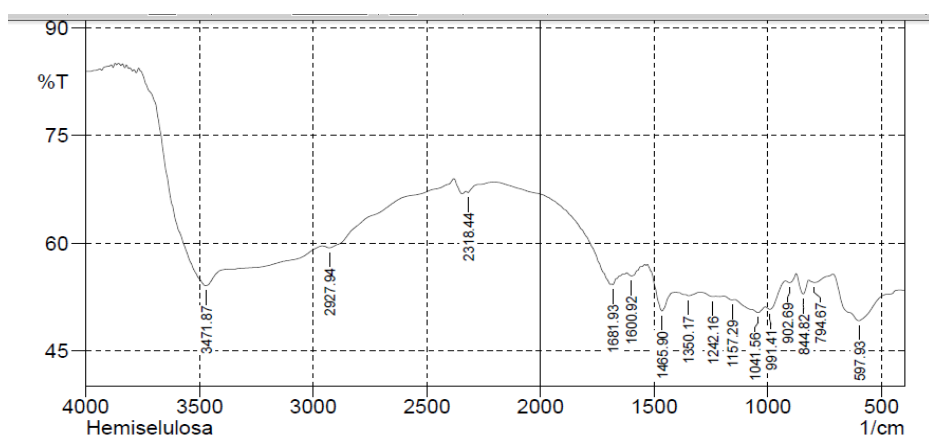


Figure 2. FTIR image of insulin hemicelluloses microparticles

Based on Figure 1 and Figure 2 above, it can be seen that the vibration infrared image of both compounds, in the region of 1820-1600 cm^{-1} adalah vibration area group carbonyl and vibration widened near 3400-2400 cm^{-1} is the vibration area group hydroxyl, this means that the samples have a group carbonyl and hydroxyl groups, but the hemicellulose STPP occurred accretion vibration of 3 pieces in the region hydroxyl this shows the change of vibration due to the influx of STPP also containing groups hydroxyl and provide vibrational fingerprint same area 1500 - 500 cm^{-1} . While the image keiga with the entry of insulin is a protein compound that has a group of the amino acid carboxylic and amine then an increase of vibration from the group of amines, this increase looks very little, this is due to the concentration of insulin in the microparticles are so few that the vibrations that occur very small. as it is known that all three samples FTIR fingerprint samples have different forms is one of the identification of organic compounds, the same as any compound having the same fingerprint. This means that all three samples having functional groups and different fingerprints.

Characterization of insulin hemicelluloses microparticles by HPLC

Identify chromatogram of corn cobs' hemicellulose with HPLC by using a C18 column with aquabidestilata with flow rate 0.8 mL/min and the ultra violet detector at a wavelength of 280 nm can be seen at Figure 4 and Figure 5 below.

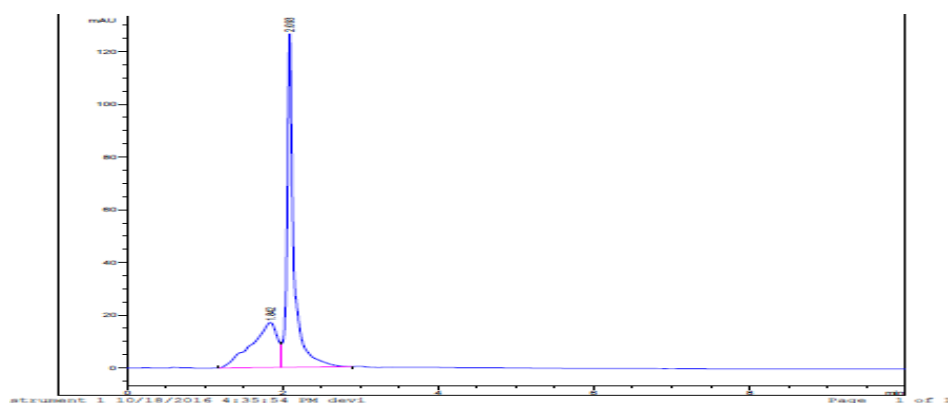


Figure 3. Chromatogram of corn cob hemicellulose by HPLC [15].

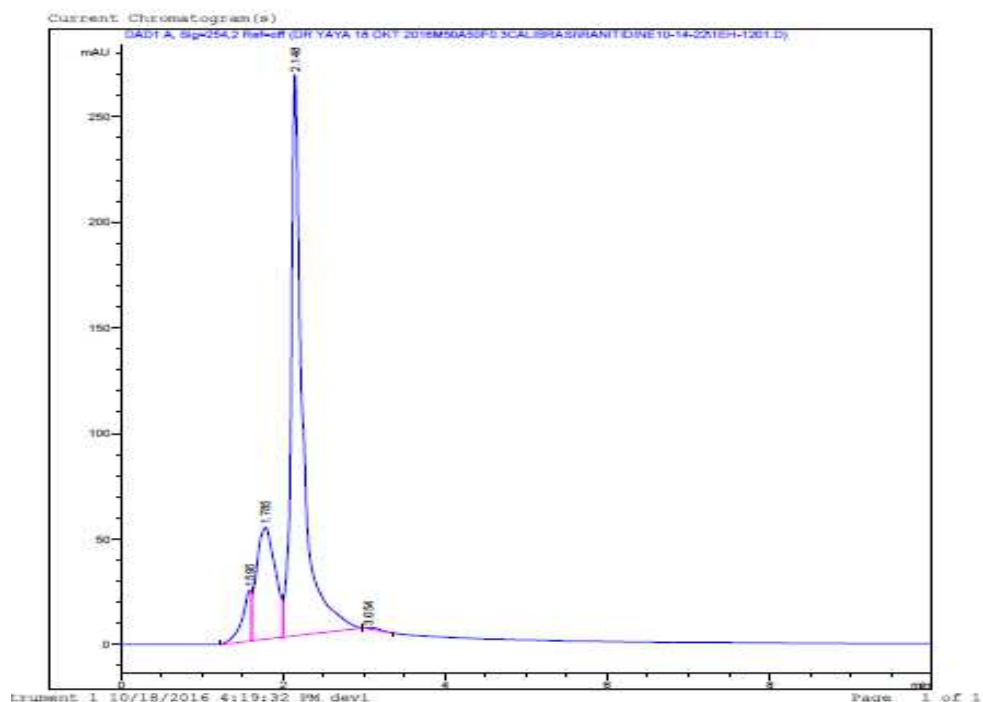


Figure 4. Chromatogram of Insulin hemicelluloses microparticle by HPLC

Based of Figure 3 and Figure 4 below it can be seen that the results of HPLC measurements at hemicellulose, there are two peaks that have retention time are 1.84 minutes and 2.09 minutes. In the measurement of insulin hemicellulose there are four peaks with each retention time are 1:59 min., 1, 79 min., 2.15 minutes and 3.05 minutes. Chromatogram contained in hemicellulose and insulin hemicellulose are two peaks adjacent retention time is 1.79 minutes and 1.84 minutes and 2.09 and 2.15 minutes, this shows that the two samples of the particles have the same peak with research conducted muchlisyam (2013) on the identification of a corn cob hemicellulose which has a peak hemicellulose one with retention time is 1.8 minutes. The existence of these differences were not significant, it's indicate factors that influence the process of making the formulations.

Based on the above it can be said that STPP ties with hemicellulose based on the structure of hemicellulose-containing galaktomanan to bind STPP form ionic bonds. Based on these bonds, the STPP can be made into microparticles with hemicellulose, and it's depends on the hemicelluloses concentration. The microparticles will be formed properly if the concentration of hemicellulose in pH 7.5 at a low concentration. While there is a change of insulin hemicellulose microparticles peak with the peak gain at the retention time.

Characterization morphology surface of Insulin hemicelluloses microparticles using scanning electron microscopy(SEM).

The surface characteristics of the sample are obtained from the secondary electrons emitted from the sample surface. The microparticles must be able to with stand vacuum, and the electron beam can damage the polymer. The mean size obtained by SEM is comparable with results obtained by particle size analyzer. Moreover, these techniques are time consuming, costly and frequently need complementary information about sizing distribution.

Measurement results of insulin hemicelluloses microparticles can be seen in the Figure 5 Figure 6 dan Figure 7 below.

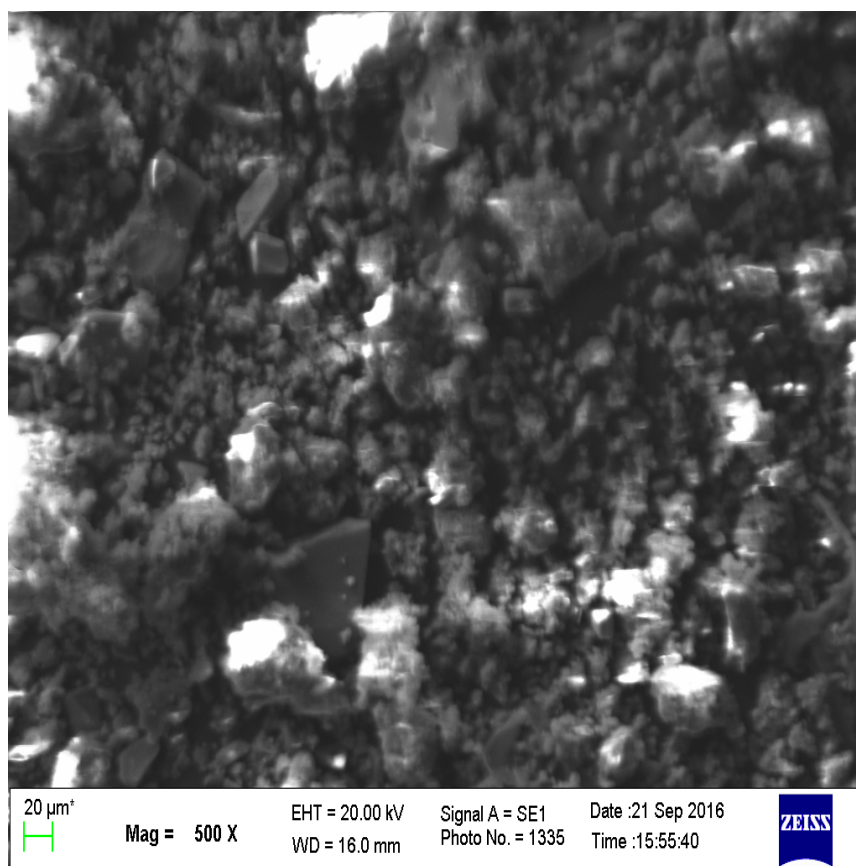


Figure 5. SEM image of hemicellulose particles[15].

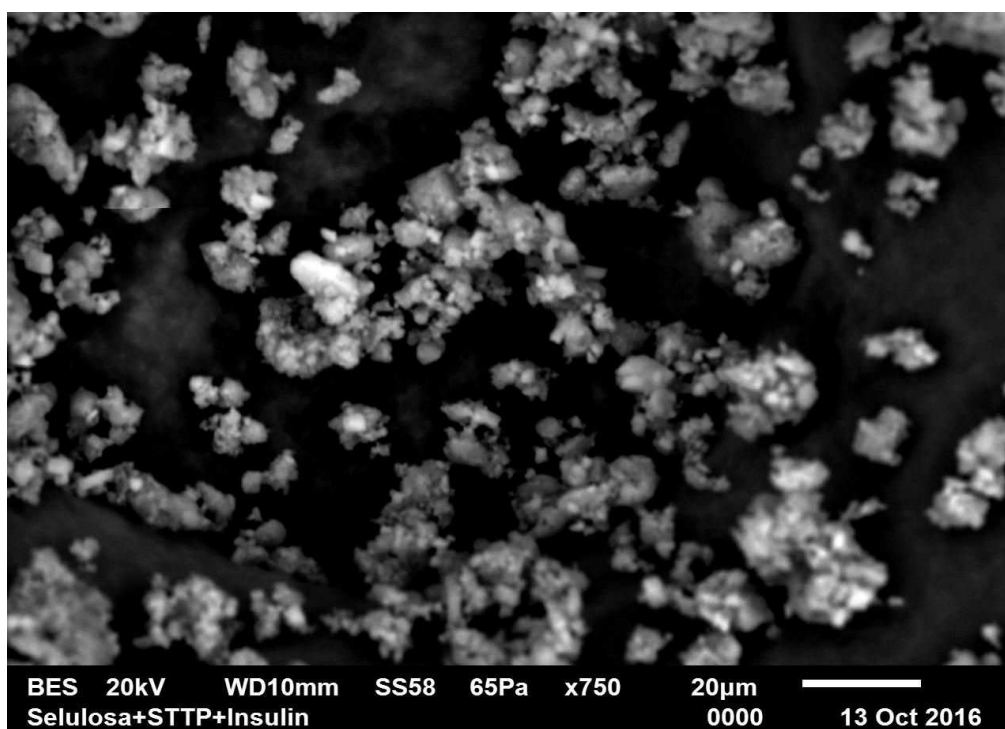


Figure 6. SEM image of 0.75% hemicelluloses with insulin microparticles

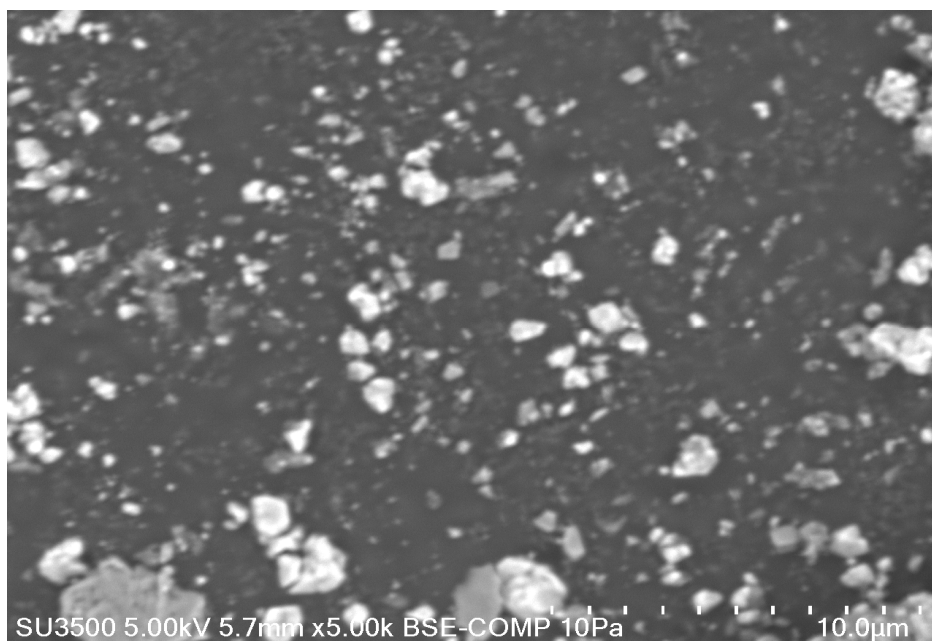


Figure 7. SEM image of 0.5% hemicelluloses and insulin microparticles

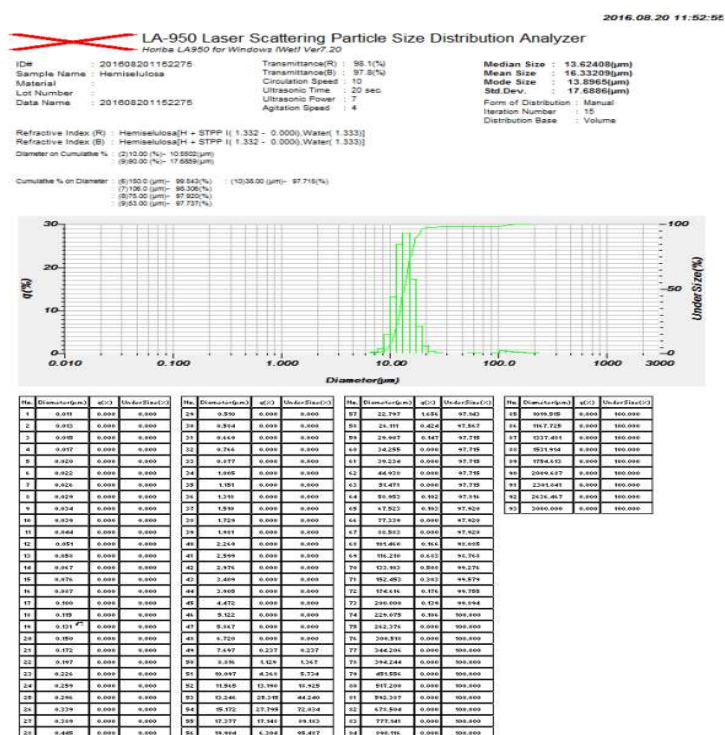


Figure 8. Particle size distribution of insulin with 0.75% hemicellulose microparticles

Based on Figure 5, Figure 6 and Figure 7 above, it can be seen the test results morphology using scanning electron microscopy (SEM) is giving morphological examination with direct visualization. In figure 6 still plenty clot in a

larger size of 50-200 μm , whereas in microparticles with a basis of 0.75% hemicellulose can be seen that the particle distribution is already in the magnitude of 13-45 μm and uneven, and there were clumps. At the microparticles with a basis of hemicellulose concentration of 0.5% is still in the form of particles 13 to 17 μm region and its distribution were almost evenly, and this can be shown in the particle size with the PSA test.

Identification Insulin Hemicellulose Microparticle With PSA

Identification of hemicellulose microparticles can be seen at figure 8 and 9.

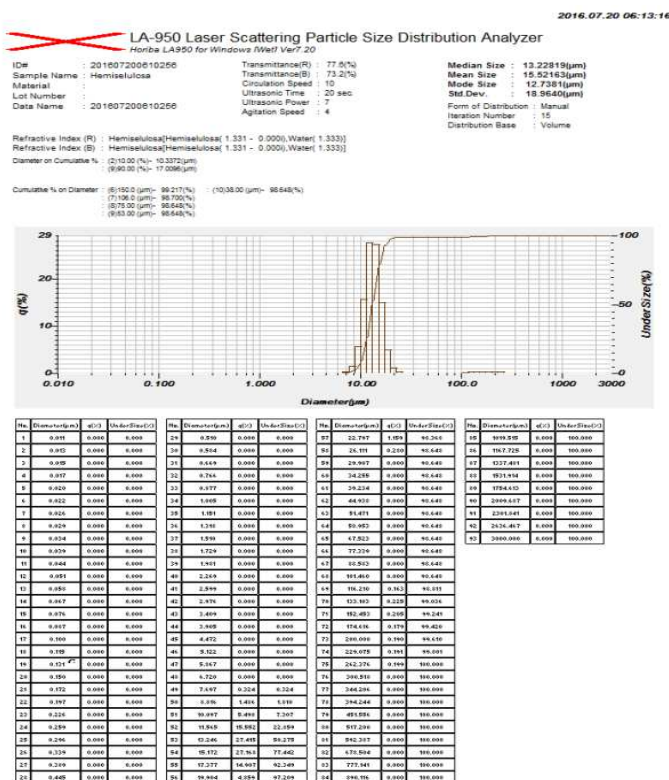


Figure 9. Particle size distribution of insulin with 0.5% hemicellulose microparticles

Based on Figure 8 and Figure 9 above, it can be seen that the results hemicellulose with insulin microparticles at concentrations of 0.75% hemicellulose obtained particle size standard deviation of 13.22 to 18.96 μm and a concentration of 0.5% was obtained particle size of 13.62 μm with a standard deviation of 17.68 μm . This shows that with a concentration of 0.5% was not achieved just the size of nanoparticles derived size. Therefore, to obtain particle sizes in the nanoparticle form of the concentration of hemicellulose must be in a very small, however hemicellulose can be used as carrier for the manufacture of microparticle for colon region.

CONCLUSION

Insulin hemicellulose microparticles can be made by using corn cob hemicellulose as a base material with a concentration of 0.5%. These microparticles can be dissolved within 60 min at pH 7.5, and has a particle size of 12-17 μm and can be developed for microparticles to drug release in the colon

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