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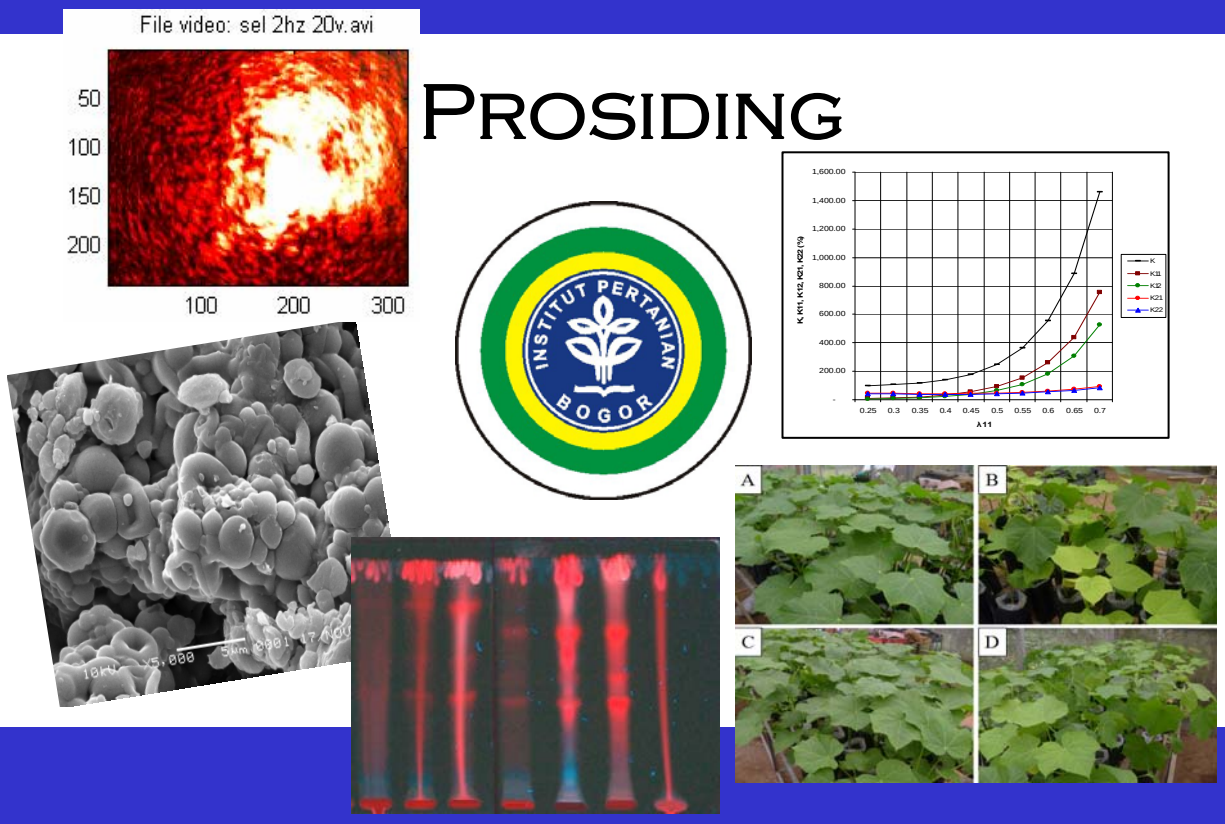
SEMINAR NASIONAL SAINS III
13 NOVEMBER 2010

Sains Sebagai Landasan Inovasi Teknologi dalam Pertanian dan Industri



PROSIDING

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PROSIDING

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KATA PENGANTAR

Ketahanan pangan dan kemandirian energi merupakan isu sentral nasional dan dunia untuk mengimbangi terus bertambahnya jumlah penduduk, semakin menyempitnya lahan yang disertai tidak terlalu signifikannya peningkatan produktivitas pertanian, ditambah lagi dengan masalah global menurunnya kualitas lingkungan. Untuk mengatasi permasalahan-permasalahan ini tentunya dibutuhkan inovasi-inovasi. Inovasi akan menjadi lebih bermakna dan berhasil guna bila berlandaskan kepada sains dan teknologi.

Banyak perguruan tinggi dan lembaga litbang departemen atau bahkan divisi litbang di perusahaan terus melakukan penelitian dan pengembangan yang didasarkan pada pemanfaatan dan pengembangan sains dan teknologi untuk mengembangkan dan menghasilkan inovasi-inovasi dalam upaya untuk meningkatkan produktivitas serta meningkatkan nilai tambah. Seminar Nasional Sains III (2010) yang diselenggarakan atas kerjasama FMIPA-IPB dan MIPA*net*, diharapkan menjadi sarana dan upaya untuk menjalin komunikasi antar pelaku dan institusi yang terlibat untuk mengoptimumkan pemanfaatan sains sebagai landasan dalam mengembangkan dan menghasilkan inovasi-inovasi dalam upaya menjawab tantangan ketahanan pangan dan kemandirian energi. MIPA*net* adalah Jaringan Kerjasama Nasional Lembaga Pendidikan Tinggi Bidang MIPA yang didirikan pada tanggal 23 Oktober 2000.

Makalah-makalah hasil penelitian dipresentasikan pada empat kelas paralel yaitu *Biological Science*, *Biochemistry*, *Chemistry*, serta *Physics & Mathematical Science*. Selain itu beberapa makalah juga ditampilkan pada sesi Poster. Makalah-makalah tersebut sebagian besar merupakan isi dari prosiding ini. Seminar dihadiri oleh peneliti dari balitbang-balitbang terkait dan dosen-dosen perguruan tinggi, mahasiswa pascasarjana serta guru-guru SMA.

Ucapan terima kasih disampaikan kepada FMIPA-IPB dan MIPA*net* yang telah mendukung penuh kegiatan Seminar Nasional Sains III ini. Juga kepada Panitia Seminar, para mahasiswa, dan semua pihak yang telah mensukseskan acara seminar ini. Kami juga sangat berterima kasih kepada semua pemakalah atas kerjasamanya, sehingga memungkinkan prosiding ini terbit. Semoga prosiding ini bermanfaat bagi semua pihak.

Bogor, Desember 2010

Dekan FMIPA-IPB,

Dr. drh Hasim, DEA

DAFTAR ISI

CHEMISTRY

DISSOLUTION BEHAVIOR OF KETOPROFEN DOUBLE COTED BY CHITOSAN-GUM GUAR WITH ALGINAT-$CaCl_2$

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Abstract

High dose ketoprofen administration as anti-inflammatory drug can cause gastrointestinal bleeding. To minimize this drawback, we have successfully double coated guar gum-modified chitosan microcapsules containing ketoprofen with alginate. The results showed that this double coating had improved the microcapsule's stability in the gastric acid medium. The ketoprofen releasing kinetic models, both at gastric and intestinal pH, are dominated by the Korsmeyer-Peppas model suggesting that this action is following the diffusion mechanism. This kinetic model is the closest approximation to the real releasing condition.

Keywords: chitosan, ketoprofen, microcapsule, dissolution

1. INTRODUCTION

Ketoprofen is one of the non-steroidal anti-inflammatory drugs (NSAID) which is able to suppress pain by inhibiting the prostaglandin synthesis. Its solubility in water is low and high dose administration (> 300 mg) can cause gastrointestinal bleeding [1]. Thus a specific drug delivery system, which is able to minimize those drawbacks, is highly needed.

Controlled release system method is able to minimize the negative side effect to the digestive system and overcome the short elimination time [2]. One of this is the microencapsulation. This method is able to maintain the therapeutic dose and activity of a continually administered drug. Some coating materials have already been studied for this application, alginate, chitosan [3], pectin [4], gelatine [5], polyacrylate, hydroxypropylmethylcellulose, and ethylcellulose [6] are just a few examples. Besides that, chitosan-carboxymethyl cellulose (CMC) has already been developed [7]. The difference between chitosan and CMC solubility is the main problem of this system. This difference made the resulted gel not homogenous. Sugita *et al.* [8] used chitosan-guar gum to coat ketoprofen because this modified chitosan is able to form homogenous gel. However, guar gum was not strong enough to protect the microcapsules in the gastric environment and destroyed at the 90th minute [8]. Thus another kind of polymer which is

strong enough to protect the microcapsules from the gastric acid is needed. In this study, alginate was applied as the external coating material for the chitosan-guar gum microcapsule. This choice is made based on alginate's properties that is indestructible and able to form gel at gastric pH (< 3) [9], and also can undergo a spontaneous reaction with chitosan [10].

Chitosan application as drug coating material has been extensively studied, for example as ketoprofen [11] and propanolol hydrochloride [6] coating material. Beside its application as single material, chitosan has also been combined with other polymers in its application, for example chitosan-CMC for indometasin coating material and alginate-chitosan for insulin hormone [12]. Meanwhile study regarding combination between chitosan and guar gum has been conducted by Sugita *et al*. (2007) which resulting the uniform sized microcapsules (0.4-5 μm). However, double coating of chitosan-guar gum microcapsule with alginate has never been done before.

2. MATERIAL AND METHODS

2.1. Material and Instrument

Chitosan used in this study was obtained from Bratachem with moisture content, ash content, deacetylation degree, and molecular weight specifications of 7.5%, 0.11%, 74.41%, and $(2.43894–6.31553)10^6$ g/mol respectively. The other materials used were glutaraldehyde, guar gum, chloride buffer solution (KCl-HCl)-water pH 1.2, phosphate buffer solution (KH_2PO_4-NaOH)-water pH 7.4, Tween-80, $CaCl_2$, alginate, and ketoprofen active compound which was obtained from PT Kalbe Farma.

Instruments used in this study were glass wares, hot plate, J.P. SELECTA oven, magnetic stirrer, Bruker Tensor 37 FTIR spectrophotometer, Ostwald-Cannon-Fenske viscometer, diffusion cell instrument with water bath, aerator, UV-1700 PharmaSpec spectrophotometer, SEM JEOL JSM-5310LV, sieve shaker, Hansen paddle dissolution assay, and Minitab Release 14 software.

2.2. Method

2.2.1. Microcapsule Preparation [10,13]

As much as 228.6 ml of 2.5% (w/v) chitosan solution in 1% (v/v) acetic acid solution mixed with 38.1 ml of 0.35% guar gum solution with stirring. After that, 7.62 ml of 3.75% glutaraldehyde was added to the mixture and stirred until homogenous.

As much as 250 ml 0.8% (w/v) ketoprofen solution in 96% ethanol was mixed with chitosan-guar gum so the weight ratio of chitosan-ketoprofen becomes 2:1. After that, 5 ml of 2% Tween-80 was added to the mixture and stirred at room temperature (Sugita *et*

al., 2006). The microcapsule was made by adding the chitosan-guar gum mixture to alginate solutions of different concentration (1, 2, and 3% (w/v)) dropwise via a syringe. Then the microcapsules was filtered and washed with $CaCl_2$ solutions with different concentration of 0.05, 0.10, and 0.15 M. The chitosan-guar gum and alginate coated ketoprofen microcapsules then dried by oven at 40 °C for 3 hours.

2.2.2. Encapsulation efficiency [14]

NaOH was used to extract ketoprofen from the microcapsule. As much as 50 mg microcapsules of each variant was digested, then extracted with 80 ml of 0.1 M NaOH for 5 minutes. The extract was filtered and diluted by NaOH to 100 ml volume. The extracted ketoprofen concentration was measured by UV spectrophotometer at 257 nm. The obtained absorbance were used to calculate the ketopfofen concentration via a standard curve.

2.2.3. *In vitro* dissolution test [15]

The dissolution test was conducted using the type 2 dissolution device (paddle method). The microcapsule was weighted (500 mg) and placed at the dissolution chamber. The test was conducted on gastric medium (pH 1.2) for 3 hours and intestinal medium (pH 7.4) for 6 hours at 37 ± 0.5 °C with paddling speed 150 rpm. Fifteen milliliters of aliquots were sampled every 15 minutes from the gastric and intestinal medium. After each time an aliquot was taken, the removed volume was replaced with the new medium solution with the same volume and temperature. The dissolution medium volume was 500 ml. Aliquot's ketoprofen concentration was measured at 258.6 nm (for dissolution at pH 1.2) and 260 nm (for dissolution at pH 7.4). The dissolution kinetic was studied by plotting the ketoprofen release percentage versus dissolution time and then determines the reaction order and the ketoprofen release model.

2.2.4. Microcapsule characterization

The microcapsule's morphology characterization was conducted to the empty, filled, and acid and base dissolute ketoprofen microcapsule using scanning electron microscope (SEM). While the particle size measurement was conducted using sieve shaker and photo-stereo microscope.

3. RESULTS

Alginate concentration variation effect the resulting gel strength. Microcapsule gel made using 1% (w/v) alginate is the most fragile. Besides that, this microcapsule gel also has the biggest size compared to the gel made using 2 and 3% (w/v) alginate. After

washed by $CaCl_2$, the gel becomes harder and clustered during drying. The dried microcapsules containing ketoprofen are yellow while the empty ones are paler.

Microcapsules resulted from all formula could not through the 35 mesh sieve, in other words the size are $\geq$ 500 μm and they looks more bulky and filled than the blank (microcapsules without ketoprofen). These results are in agreement with the photo-stereo microscope observation of which results are listed in Table 1.

Table 1 Microcapsule's sizes

Variation	Microcapsule's Sizes (μm)
Alginate 1%, $CaCl_2$ 0.05M	1075–2000
Alginate 1%, $CaCl_2$ 0.10M	1025–1975
Alginate 1%, $CaCl_2$ 0.15M	1125–2000
Alginate 2%, $CaCl_2$ 0.05M	775–1200
Alginate 2%, $CaCl_2$ 0.10M	700–1335
Alginate 2%, $CaCl_2$ 0.15M	625–1475
Alginate 3%, $CaCl_2$ 0.05M	725–1225
Alginate 3%, $CaCl_2$ 0.10M	725–1400
Alginate 3%, $CaCl_2$ 0.15M	750–1125

3.1. Microcapsule Moisture Content

The obtained microcapsules had different moisture contents ranging from 13.91 to 25.84% (Table 2). Table 2 shows that the increasing $CaCl_2$ concentration will increase the moisture content of the microcapsules made using 1 and 2% (w/v) alginate. Besides that, the increase in alginate concentration is also tending to increase moisture content.

Table 2 Chitosan-alginate ketoprofen microcapsule's moisture contents

Treatment		Moisture Content
Alginate (% [w/v])	$CaCl_2$ (M)	(%)
1	0.05	14.48
1	0.10	14.73
1	0.15	23.36
2	0.05	16.24
2	0.10	18.14
2	0.15	25.84
3	0.05	24.79
3	0.10	17.43
3	0.15	13.91

3.2. Ketoprofen Release

The maximum ketoprofen release percentage in gastric medium was very low, ranging from 3.18-12.97%. It was also not released completely in intestinal medium until the 360th minute. However, the maximum ketoprofen release percentages in intestinal medium were still higher compared to the gastric medium release, i.e. 18.34-54.97%.

Fehling tests to the 360th minute's aliquots taken from the intestinal medium dissolution test showed negative results.

The best microcapsules were obtained from the H formula with 3% (w/v) alginate and 0.15 M $CaCl_2$. Figure 1 shows that ketoprofen releases at gastric medium were controlled. While at the intestinal medium, ketoprofen concentration reached its maximum value at the 90th minutes and became relatively steady afterwards. Ketoprofen concentrations at the equilibrium condition were 110-120 mg/l.

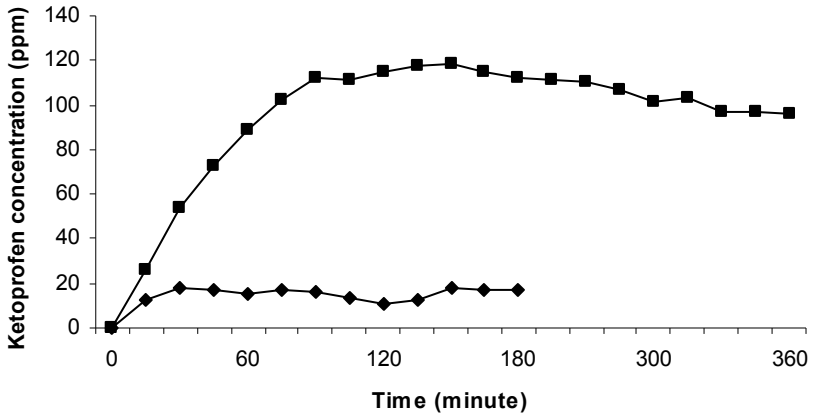


Figure 1 Acidic (♦) and basic (■) dissolution behavior of the best produced microcapsules (3% (w/v) alginate and 0.15 M $CaCl_2$).

According to Table 3, ketoprofen releases were dominated by the Korsmeyer-Peppas kinetic model both in artificial gastric and intestinal medium. However, the F, G, and H formula were following the Higuchi kinetic model in the gastric medium dissolution. These models were assigned by calculating their determination coefficient towards each formula. Comparison between dissolutions in artificial gastric and intestinal medium shows that the rate constant (*k*) in gastric medium is tends to be lower than in base medium. The same thing was also happening to the maximum rate of release calculated by the Korsmeyer-Peppas and Higuchi kinetic models (Figure 2). Figure 2 also implied that the kinetic models had provided the best approximation to the real value.

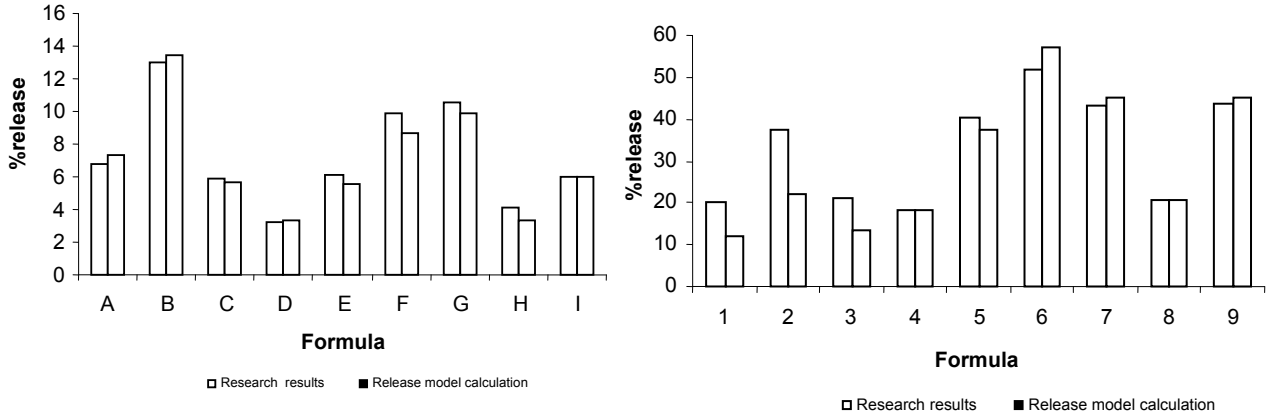


Figure 2 Maximum ketoprofen release percentages in acidic (a) and basic (b) medium based on model calculations and experimental results.

Table 3 Dissolution kinetic determination in artificial gastric and intestinal medium

Artificial gastric medium

Formula	Zero order		First order		Higuchi		Hixson-Crowell		Korsmeyer-Peppas		
	R^2	k (min)$^{-1}$	R^2	k (min)$^{-1}$	R^2	k (min)$^{-1/2}$	R^2	k (min)$^{-1/3}$	R^2	k (min)$^{-n}$	n
A	0.8138	0.0242	0.8139	0.0003	0.8399	0.4388	0.8139	0.0004	**0.886**[a]	0.889	0.3921
B	0.8896	0.0441	0.896	0.0005	0.9615	0.8189	0.8939	0.0007	**0.978**[a]	1.845	0.3832
C	0.3359	0.0098	0.2755	0.0010	0.4367	0.1995	0.2783	0.0015	**0.5639**[a]	1.744	0.2262
D	0.8383	0.0096	0.8395	0.0001	0.9030	0.1774	0.8391	0.0002	**0.9261**[a]	0.633	0.3173
E	0.8292	0.0218	0.8251	0.0005	0.8381	0.3917	0.8265	0.0007	**0.8556**[a]	0.699	0.4001
F	0.8551	0.0430	0.8948	0.0004	**0.902**[a]	0.6424	0.8944	0.0006	0.8702	1.044	0.4173
G	0.9125	0.0403	0.9154	0.0004	**0.9458**[a]	0.7334	0.9145	0.0007	0.1710	6.674	0.0200
H	0.878	0.0145	0.8491	0.0002	**0.8873**[a]	0.2608	0.8777	0.0002	0.8760	0.437	0.4111
I	7×10^{-7}	16.050	0.1649	0.0001	5×10^{-7}	0.0002	4×10^{-7}	1×10^{-5}	**0.6603**[a]	1.473	0.2745

Artificial intestinal medium

Formula	Zero order		First order		Higuchi		Hixson-Crowell		Korsmeyer-Peppas		
	R^2	k (min)$^{-1}$	R^2	k (min)$^{-1}$	R^2	k (min)$^{-1/2}$	R^2	k (min)$^{-1/3}$	R^2	k (min)$^{-n}$	N
A	0.0781	0.0148	0.0527	0.0001	0.2938	0.5697	0.0676	0.0002	**0.8184**[a]	1.1393	0.4726
B	0.0991	0.0295	0.0676	0.0003	0.3347	1.0785	0.0777	0.0005	**0.8504**[a]	1.1534	0.5796
C	0.0693	0.0145	0.0527	0.0001	0.0693	0.8718	0.058	0.0002	**0.8173**[a]	1.1429	0.4788
D	0.1918	0.0211	0.1784	0.0002	0.3042	0.0044	0.1829	0.0003	**0.8742**[a]	1.1048	0.4744
E	0.0923	0.0309	0.0584	0.0003	0.3225	1.1485	0.0691	0.0005	**0.8500**[a]	1.1553	0.5908
F	0.2798	0.0713	0.1632	0.0008	0.4358	1.4738	0.1796	0.0011	**0.8980**[a]	1.1397	0.6644
G	0.1692	0.0461	0.1283	0.0005	0.4509	0.7057	0.1419	0.0008	**0.8768**[a]	1.1475	0.6237
H	0.1791	0.0223	0.2202	0.0003	0.6424	1.6924	0.1587	0.0003	**0.9198**[a]	1.1153	0.4971
I	0.3512	0.0628	0.3353	0.0008	0.0178	0.1345	0.0002	1×10^{-5}	**0.9198**[a]	1.1120	0.6284

[a] The highest determination coefficient

Captions: [a] A = alginate 1%, $CaCl_2$ 0.05 M
B = alginate 1%, $CaCl_2$ 0.1 M
C = alginate 1%, $CaCl_2$ 0.15 M
D = alginate 2%, $CaCl_2$ 0.05 M
E = alginate 2%, $CaCl_2$ 0.10 M
F = alginate 2%, $CaCl_2$ 0.15 M
G = alginate 3%, $CaCl_2$ 0.05 M
H = alginate 3%, $CaCl_2$ 0.1 M
I = alginate 3%, $CaCl_2$ 0.15 M

Equation for zero order $Q = kt$; first order $\ln [A]_t = \ln [A]_o - kt$; Higuchi $Q = kt^{1/2}$; Hixson-Crowell $Q = kt^n$; Korsmeyer-Peppas $Qo^{1/3} - Qt^{1/3} = kt$. (Q = persen release, k = rate constant, and t = time of release)

3.3. Microcapsule Morphology

SEM image of microcapsules obtained from the best formula, 3% (w/v) alginate and 0.10 M $CaCl_2$, clearly shows that the surfaces were very tight with a few shallow cracks (Figure 3). Besides that, there were also fragments of crushed layer in the surface.

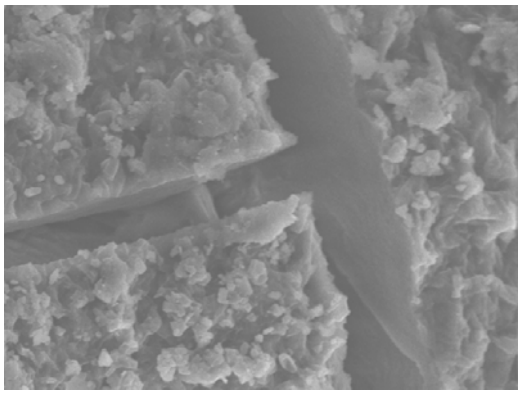
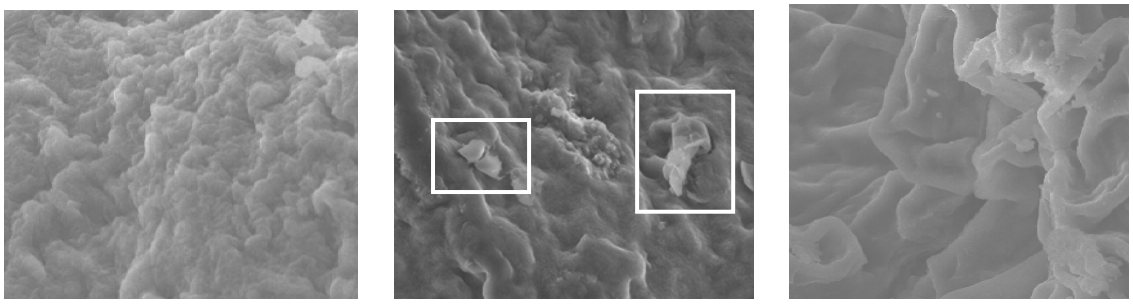


Figure 3 Ketoprofen microcapsule's surface SEM image (3% alginate and 0.15 M $CaCl_2$) at 1500 times magnification.

After gastric and intestinal dissolution, the microcapsule's surface looks remain unchanged but with bigger size. However, the surface swelling in gastric medium is bigger than in intestinal medium. During the dissolution test, the gastric medium color turned to yellowish. But none of these were happened in the intestinal medium.

Observation to the SEM images taken from the microcapsule surface that had been spent 180 minutes in the gastric dissolution test shows that the surface had not significantly changed compared to the initial image (Figure 4a and b). Beside that, the surface contains fragments after gastric dissolution. Figure 4c has a different morphology compared to the initial image.



(a) (b) (c)

Figure 4 SEM images taken from ketoprofen microcapsule before dissolution (a), after acidic dissolution (b), and after basic dissolution (c) at 1000 times magnification (inset: crushed alginate layer fragments).

4. DISCUSSION

Ketoprofen microencapsulation with guar gum-modified chitosan and alginate double coating has been successfully conducted by means of a chemical method. The first layer coated the ketoprofen is the glutaraldehyde linked chitosan with guar gum as the interpenetrating agent. Due to its low solubility in chitosan solution, the ketoprofen was dissolved in ethanol prior to mixing with chitosan solution.

The second coating layer was introduced via soaking in alginate solution. Swelling occurred to microcapsule gel due to high free water content around the gel in 1% alginate solution. While in 2 and 3 % alginate solution, the free water contents around the gel were lower. The smaller gel size in the 1% alginate solution was also due to the stronger cross-linking between the negative charged alginate and the positive charged chitosan [10].

The washing step with $CaCl_2$ solution was intended to strengthen the outmost layer of alginate [16] by means of cross-linking formation at the alginate's guluronate residue [17] thus made the dried microcapsule tend to clustered. Besides that, during the drying process, intermolecular hydrogen bonds between alginates from different microcapsules were formed [16]. Alginate at the external surface of the microcapsules also made the surface more hygroscopic. Alginate is a hydrocolloid which contain large amount of hydroxyl groups.

4.1. Ketoprofen Release from the Microcapsule

Compared to Sugita *et al*. [8], double coating with alginate as the additional layer has been proven here to be able to minimize ketoprofen release in gastric acidic environment. This fact reinforce Silva *et al*. [16] who stated that double coating is able to enhance microcapsule's stability in gastric environment. Tan *et al*. [18] stated that the increasing alginate concentration caused the resulted microcapsule's surface to have too

few pores thus made ketoprofen difficult to get through it. This statement was proved by the fact that the *k* value we obtained from the dissolution at the intestinal medium is higher than the value obtained from the gastric dissolution. However, the strong cross-linking between guar gum-modified chitosan and glutaraldehyde also caused the ketoprofen won't be released completely before the 360^{th} minute.

Because ketoprofen can initiate gastric bleeding and its absorption process is happening in the intestine, the microcapsules are considered good if only a little ketoprofen released in the gastric and more in the intestine. Besides that, another parameter which needs to be met by a good microcapsule is the low moisture content and high encapsulation efficiency. According to the scoring result, the best microcapsule was obtained from the H formula with 3% alginate and 0.15 M $CaCl_2$.

4.2. Microcapsule Morphology

In gastric medium, the medium solution will penetrate the microcapsule's surfaces thus swelled and even dissolved the chitosan. This also observed by the color change of the medium solution to yellowish after the gastric dissolution. However, this could not be happened in the intestinal medium due to the characteristic of chitosan which is insoluble in alkali environment.

The resemblances between SEM images of the microcapsule's surface before and after gastric dissolution test occurred because the outermost microcapsule's layer, i.e. Ca^{2+} cross linked alginate, was not totally affected by the gastric environment. Alginate layer was swelled because it was pushed by the swelled inner chitosan-guar gum matrix. However, some alginate layer, which is too thin, was unable to hold this force and then crushed. The fragments of this destroyed alginate layer was scattered around the microcapsule's external surface.

The differences between the SEM images taken before and after the intestinal dissolution reinforced Ivanova *et al*. (2000) [19], who stated that intestinal buffer solution is able to destroy cross-linking bonds between alginate and Ca^{2+}, thus alginate will be dissolved. This means that the SEM image after intestinal dissolution is showing the surface of chitosan-guar gum matrix. Because alginate layer has been destroyed, ketoprofen will be released more easily from the matrix when it made contact with the intestinal medium. This release is also accommodated by large amount of channels which enlarged the microcapsule's surface area that in contact with the medium.

5. CONCLUSIONS

Alginate double coating application to chitosan-guar gum microcapsule has been proved able to enhance microcapsule's stability in gastric acidic medium. The best

produced microcapsule was made from 3% (w/v) alginate and 0.15 M $CaCl_2$. Ketoprofen releases both in acidic gastric pH and basic intestine pH were dominated by Korsmeyer-Peppas kinetic model. This assigned model is the best approximation to the real condition in this study.

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