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Design of Salbutamol Sulphate Gastroretentive Nanoparticles via Surface Charge Manipulation

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***Abstract*—**In the present study, development of salbutamol sulphate nanoparticles that adhere to gastric mucus was investigated. Salbutamol sulphate has low bioavailability due to short transit time in gastric. It also has a positive surface charge that provides hurdles to be encapsulated by the positively strong mucoadhesive polymer of chitosan. To overcome the difficulties, the surface charge of active ingredient was modified using several nonionic and anionic stomach-specific polymers. The nanoparticles were prepared using ionotropic gelation technique. The evaluation involved determination of particle size, zeta potential, entrapment efficiency, in vitro drug release and in vitro mucoadhesion test. Results exhibited that the use of anionic alginate polymer was more satisfactory than that of nonionic polymer. Characteristics of the particles was nano-size, high encapsulation efficiency, fulfilled the drug release requirements and adhesive towards stomach for around 11 hours. This result shows that the salbutamol sulphate nanoparticles can be utilized for improvement its delivery.

***Keywords*—**Mucoadhesive, salbutamol sulphate, nanosize, anionic polymer.

I. Introduction

THE oral route of drug administration has been the one used most for both conventional and novel drug delivery. The reasons are obviously because of the ease of administration and widespread acceptance by patients. However, there are limitations of oral route administration. The uptake of drugs is often limited by the short contact time between the formulation and the absorption membrane and by a fast washout. This could results in a low bioavailability. Salbutamol sulphate has a specific absorption site, which is in stomach and upper part of small intestine, and would be degraded in colon. Enhancement of salbutamol sulphate absorption might minimize this drawback. It might be achieved by increasing the surface area as well as prolong the contact of salbutamol sulphate and stomach compartment. From this point, nanoparticle of salbutamol sulphate using stomach-specific mucoadhesive polymer would be advantageous. Nano-sized particles have much smaller size of particle and increased surface area, and thus, improve the absorption of salbutamol sulphate. Nanoparticles are small enough to avoid significant steric inhibition by the dense fiber mesh in the stomach, so it can penetrate mucus [1]. Mucoadhesive polymer aided nanoparticles also enhance the absorption of salbutamol sulphate by adhering longer to stomach compartment. Chitosan has a strong ability to enhance hydrophilic compounds transport across mucosal epithelial membrane [2]. Thus, it can be assumed that chitosan-based nanoparticles might increase the epithelial permeability of salbutamol sulphate. Since surface charge of chitosan and salbutamol sulphate is the same; positive charge, encapsulation of the drug in chitosan based-nanoparticles subsequently providing mucoadhesive system to the gastric would be difficult. It has been demonstrated that the use of lipid vesicles, liposomes, enhanced the encapsulation of salbutamol sulphate approximately 80% [3]. When anionic polymeric polyvinyl sulfonate was used, the salbutamol sulphate was encapsulated approximately 63%. In this study, we intended to clarify the issue by modifying the surface charge of salbutamol sulphate using nonionic and anionic of mucoadhesive polymers. It was reported that various polymers were used for gastroretentive purpose via mucoadhesive system such as polyethylene oxide, hydroxypropyl methylcellulose, carboxy methyl cellulose and sodium alginate [4-7]. These polymers could be combined with chitosan in nanoparticles preparation.

In this research, nanoparticles of salbutamol sulphate for adhering to the gastric mucus were prepared and evaluated. With this research, it is expected that salbutamol sulphate nanoparticles would have a long contact time to stomach and thus, results in greater absorption and bioavailability.

II. Materials and Methods

A. Materials

Chitosan (CS) was purchased from PT. Biotech Surindo (Cirebon, Indonesia). Salbutamol sulphate was kindly gifted from PT. Dankos Farma (Jakarta, Indonesia). Polyethylene oxide (PEO) WSR 303 was gifted from PT. Kalbe Farma (Jakarta, Indonesia). Sodium carboxymethylcellulose (CMCNa) and sodium alginate were purchased from PT. Brataco Chemica (Bandung, Indonesia). Hydroxypropyl methylcellulose (HPMC) and HPMC sustained release (HPMC SR) were gifted from PT. Jebsen & Jessen Chemicals (Jakarta, Indonesia). Calsium chloride ($CaCl_2$), sodium tripolyphosphate (TPP), pepsin, lecithin, sodium taurocholate, acetic acid and polyvinyl alcohol (PVA) were purchased from Sigma-Aldrich Pte. Ltd (Singapore). Other materials used in this study were proanalytic grade.

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B. Methods

1. Preparation of drug-loaded CS nanoparticles

The nanoparticles were formed by inducing the ionotropic gelation of a CS with TPP. Firstly, every material was dissolved first in different beakers; CS was dissolved in acetic acid 1% with concentration of 3.15 mg/mL, salbutamol sulphate was dissolved in aquadest with concentration of 0.945 mg/mL, TPP was dissolved in aquadest with concentration of 1.65 mg/mL. Then, 1 mL of the drug solution was added to 3 mL of the CS solution in a vial under magnetic stirring at room temperature. Three mL of aquadest was added to the mixture. Finally, 2 mL of the TPP solution was added into the mixture drop by drop using 1 mL syringe under magnetic stirring so that the final concentration was 0.4 mg/mL. The magnetic stirring was maintained for 10 minutes to enable complete stabilization. The nanoparticles were then transferred to macro centrifuge tube and isolated by centrifugation with speed 3220 x g for 50 minutes at room temperature. Supernatants were collected using pipette and the nanoparticles were then transferred into a vial and resuspended and sonicated for 10 minutes.

2. Preparation of drug-loaded CS nanoparticles using nonionic polymers

In the preparation of nanoparticles, nonionic mucoadhesive polymers such as HPMC, HPMC SR and PEO were used. PEO and HPMC and HPMC SR polymers were also used in combination with CS. The ratio used for the combination of PEO and HPMCs was 1:1. Firstly, those polymer were initially prepared with various concentrations of 1.575 mg/mL, 3.15 mg/mL, and 6.3 mg/mL. The concentration of CS, TPP and salbutamol sulphate were prepared as explained above. Nanoparticles were obtained spontaneously on incorporation of 2 mL of the TPP solution (using 1 mL syringe) to 7 mL of mixture containing 3 mL of the CS solution, 3 mL of the PEO solution and 1 mL of the Salbutamol sulphate solution inside a vial. For the combination between CS, HPMCs and PEO, volume of PEO and HPMC used of each was 1.5 mL with the total of 3 mL that has the same concentration. The mixing process was done under magnetic stirring at room temperature. Magnetic stirring process was maintained for 10 minutes for stabilization purpose. Next, the nanoparticles were transferred to centrifuge tube and isolated by centrifugation with speed 3220 x g for 50 minutes at room temperature. The supernatants were collected using pipette and the nanoparticles were then transferred into a vial and resuspended and sonicated for 10 minutes.

3. Preparation of drug-loaded CS nanoparticles using anionic polymers

Two types of polymers used in this study were CMC Na and sodium alginate (ALG) to prepare the nanoparticles. CMC Na polymer was used in combination with CS with initial concentrations of 0.315 mg/mL, 0.21 mg/mL, and 0.15 mg/mL.

TABLE I
FORMULA OF SALBUTAMOL SULPHATE NANOPARTICLESS

For-mula	Final concentration (mg/mL)							
	SS	CS	PEO	CMC Na	HPMC	HPMC SR	ALG	PVA
F1	0.1	1.0	-	-	-	-	-	-
F2	0.1	1.0	2.0	-	-	-	-	-
F3	0.1	1.0	1.0	-	-	-	-	-
F4	0.1	1.0	0.5	-	-	-	-	-
F5	0.1	1.0	-	0.1	-	-	-	-
F6	0.1	1.0	-	0.07	-	-	-	-
F7	0.1	1.0	-	0.05	-	-	-	-
F8	0.1	1.0	-	-	2.0	-	-	-
F9	0.1	1.0	-	-	1.0	-	-	-
F10	0.1	1.0	-	-	0.5	-	-	-
F11	0.1	1.0	-	-	-	2.0	-	-
F12	0.1	1.0	-	-	-	1.0	-	-
F13	0.1	1.0	-	-	-	0.5	-	-
F14	0.1	1.0	2.0	-	2.0	-	-	-
F15	0.1	1.0	1.0	-	1.0	-	-	-
F16	0.1	1.0	0.5	-	0.5	-	-	-
F17	0.1	1.0	2.0	-	-	2.0	-	-
F18	0.1	1.0	1.0	-	-	1.0	-	-
F19	0.1	1.0	0.5	-	-	0.5	-	-
F20	0.1	0.12	-	-	-	-	0.6	-
F21	0.1	0.12	-	-	-	-	0.6	-
F22	0.1	0.12	-	-	-	-	0.6	-
F23	0.1	0.12	-	-	-	-	0.6	-
F24	0.14	0.12	-	-	-	-	0.6	-
F25	0.12	0.12	-	-	-	-	0.6	-
F26	0.1	1.0	1.0	-	-	-	-	0.4
F27	0.1	1.0	1.0	-	-	-	-	0.8
F28	0.1	1.0	1.0	-	-	-	-	1.2
F29	0.1	1.0	1.0	-	-	-	-	1.6
F30	0.1	1.0	1.0	-	-	-	-	2.0

SS: salbutamol sulphate, CS: chitosan, ALG: sodium alginate

The preparation methods of nanoparticles were similar to the preparation methods of salbutamol sulphate-loaded CS nanoparticles using nonionic polymers. There are four methods in preparing these nanoparticles: coated (method A), coated and adjusted (method B), blended (method C), blended and adjusted (method D). Firstly in method A, sodium alginate and $CaCl_2$ were dissolved in water with concentration 0.08% and 13.55 mM respectively and CS was dissolved in 1% acetic acid with concentration 0.05%. The nanoparticles were prepared by adding 6.6 mL ALG solution into a vial containing 0.945 mg of salbutamol sulphate. After dissolved, 0.3 mL $CaCl_2$ was added drop by drop using 1 mL syringe into the solution containing ALG and salbutamol sulphate so that its final concentration was 0.1 mg/mL. The mixture was stirred for 10 minutes at room temperature and then 2.1 mL of CS was added drop by drop using 1 mL syringe into the mixture. The mixture was stirred for another 10 minutes at room temperature for complete stabilization. Next, the nanoparticles were transferred to centrifuge tube and isolated by centrifugation with speed 3220 x g for 50 minutes at room temperature. The supernatants were collected and the nanoparticles were then transferred into a vial and resuspended and sonicated for 10 minutes. In method B, the stock solution of ALG and CS were adjusted first to pH 5. The next procedures were similar to method A. In method C, the nanoparticles were prepared by adding 6.6 mL ALG solution into a vial containing 0.945 mg of salbutamol sulphate. After dissolved, 2.1 mL of CS was added into it and stirred for 10

minutes. Then, 0.3 mL $CaCl_2$ was added drop by drop using 1 mL syringe into the mixture. The mixture was stirred for another 10 minutes at room temperature for complete stabilization. Next, the nanoparticles were transferred to centrifuge tube and isolated by centrifugation with speed 3220 x g for 50 minutes at room temperature. The supernatants were collected and the nanoparticles were then resuspended and sonicated for 10 minutes. In method D, the stock solution of CS and ALG were adjusted first to pH 5. The next steps were similar to method C.

4. Preparation of drug-loaded CS nanoparticles using Combination of nonionic and anionic polymers

In this type of nanoparticles, PEO as nonionic polymer was combined with polyvinyl alcohol (PVA) to prepare the salbutamol sulphate-encapsulated CS nanoparticles. The concentration of CS, salbutamol sulphate and TPP were prepared as explained above. PVA was used in this study with various final concentrations of 0.4, 0.8, 1.2, 1.6 and 2 mg/mL. Firstly, PVA was dissolved in 90^{o}C water and it was stirred for 5 min and allow to cool down. One mL of the Salbutamol sulphate solution was then added to PVA solution. Then, 3 mL each of CS and PEO was added to the salbutamol sulphate-PVA solution inside the vial. Finally, 2 mL of the TPP solution was added to the mixture using 1 mL syringe under magnetic stirring and room temperature to make up a final volume of 9 mL. The magnetic stirring was maintained for 10 minutes to let stabilization process occur. The nanoparticles were then transferred to centrifuge tube and isolated by centrifugation with speed 3220 x g for 50 minutes at room temperature. The supernatants were collected using pipette and the nanoparticles were then transferred into a vial and resuspended and sonicated for 10 minutes.

5. Physicochemical characterization of nanoparticles

The particle size, polydispersity index and zeta potential of each sample were determined using Delsa Nano C particle analyzer (Beckman Coulter). After the nanoparticles in glass tube was sonicated for approximately 10 min in a bath-type sonicator (Branson Ultrasonics 5510) and resuspended, sample was poured into a cuvette and examined using this instrument.

6. Determination of entrapment efficiency of salbutamol sulphate in the nanoparticles

The supernatant of salbutamol sulphate-loaded nanoparticles were separated by centrifugation at 3220 x g for 50 minutes at room temperature. The amount of free salbutamol sulphate was measured in the supernatant by UV spectrophotometry at wavelength 275 nm. The salbutamol sulphate entrapment efficiency (EE) of the nanoparticles was calculated from equation:

$$EE = \frac{\text{total amount of drug} - \text{amount of unbound drug}}{\text{total amount of drug}} \text{ x } 100$$

7. Evaluation of in vitro release of salbutamol sulphate

The salbutamol sulphate-loaded nanoparticles equivalence to ~ 4.2 mg salbutamol sulphate were incubated in an orbital shaker with constant temperature, 37 ± 0.5^{o}C and constant rpm, 100 rpm in 5 mL of fasted state simulating gastric fluid (FaSSGF) medium. At appropriate intervals (0.5, 1, 2, 3, 4, 6, 9 and 12 hours), the nanoparticles were centrifuged at 3220 x g for 20 minutes at room temperature and 1 mL supernatant was withdrawn using pipette for sampling and analyzed by UV – Vis spectrophotometry at wavelength 277 nm. The 1 mL supernatant was replaced with fresh FaSSGF medium (pre-warmed to 37 ± 0.5^{o}C). All the experiments were performed in triplicate.

8. Evaluation of in vitro mucoadhesion test

In this study, male *New Zealand Albino sp* rabbit, aged 2 months old with around 2.5 kg body weight, were used. The rabbits were housed at room temperature and were maintained on a 12-h light/dark cycle. During the acclimatization the rabbits were allowed free access to food and water. The experimental protocol were followed the animal care guidance of School of Pharmacy ITB. Rabbit stomach was cleaned and cut into 2 x 4 cm size. The stomach then was mounted on an object glass using cyanoacrylate glue. A known quantity of nanoparticles (200mg) was spread randomly onto the stomach. Prior to testing, the nanoparticles were loaded with water soluble blue color substance. In the preparation of nanoparticles, the salbutamol sulphate was substituted by 20 mg of water soluble blue color substance. A beaker was filled with 100 mL Krebs buffer medium at 37 ± 0.5^{o}C and stirred using spindle at 100 rpm. The nanoparticles were observed at 3, 6, 9, 15, 30 minutes and for every 30 minutes interval until 720 minutes. The time for complete erosion of the nanoparticles from the stomach was determined visually and recorded as an indication of the ex vivo adhesion time. The evaluation was continued using Krebs buffer medium for comparison.

III. Results and Discussion

The nanoparticles preparation was developed using 30 formulas, with various ratios of polymer to co-polymers as well as ratios of drug to polymers (Table I). F20 until F23 differed in preparation method by which F20 used method A, F21 used method B, F22 used method C, and F23 used method D. F24 and F25 used method B but different in concentration of salbutamol sulphate. F26-F30 used polymers combination between fixed amount of PEO and variation of final concentration of PVA.

The CS nanoparticles were formed by inducing the gelation of a CS solution with polyanion TPP. CS is a cationic polyelectrolyte which its gelation is induced by controlling the interaction with the counterion, TPP. TPP is classified as low molecular type of counterions besides hydrophobic type and high molecular weight type of counterions [8]. The inter and intramolecular linkages created between TPP and the positively charged amino groups of CS are responsible for the gelation process. The concentration of CS and TPP must be chosen carefully. The formation of nanoparticles is only possible for some specific concentrations of CS and TPP. The final CS concentration can be approximately 4 mg/mL while the maximum TPP concentration is only 0.75 mg/mL. The

characteristics of nanoparticles including size were dependent on both CS and TPP concentration [9]. If the amount of polyanion is extremely high, the size could also become large or, in extreme cases would cause precipitation. However, if the concentration of TPP is excessively low, the nanoparticles could not be formed or the quantity of the formed nanoparticles is too low. The ratio of CS/TPP is also important in the formation of nanoparticles. Larger CS/TPP ratio yielded particles with larger size but obviously lower cross-linking density [10].

There were several polymers are used besides CS. All of the polymers are mucoadhesive polymers as the primary purpose of this research is to prolong the contact time between nanoparticles and stomach. In addition, CS and PEO has a surface charge which can be modulated from high to low positive values, nontoxic, biocompatible and has a pH-dependent dissolution behavior. The incorporation of PEO in the gel system is through intermolecular hydrogen bonding between the electropositive amino hydrogen of CS and electronegative oxygen atom of polyethers, thus forming a CS/PEO semi-interpenetrating network [9].

Besides PEO, CS also being combined with CMC Na. CS and sodium CMC also make the nanoparticles biodegradable and biocompatible, making them suitable candidates for pharmaceutical application. The nanoparticles were formed by the electrostatic interaction between negatively charged carboxylic acid groups on CMC and positively charged amino groups on CS. The concentration of CMC Na allowed to make nanoparticles is much lesser than the others as the molecular weight of the polymers is too high for nanoparticles formation [11].

ALG was also being considered to be used in the preparation of nanoparticles. ALG nanoparticles can be obtained easily by inducing gelation with calcium ion ($CaCl_2$). Such easy-gelling property can be used to produce a pre-gel consisting of very small aggregates of gel particles, followed by the addition of an aqueous polycationic solution such as CS to make polyelectrolyte complex coating. CS/ALG polyionic complexes form through ionic gelation via interaction between the carboxyl groups of ALG and the amine groups of CS [12]. The calcium ion binds preferentially with gluronic acid blocks of the ALG macromolecule. The pregel state is necessary to enable the ionic interactions between ALG and CS to form nanosphere. Formation of nanospheres required a low concentration of $CaCl_2$ (less than 0.2% mass ratio) to form the negatively charged, calcium alginate pregel that is subsequently enveloped by the positively charged CS. Cationic polymers restrict further cooperative binding between calcium and alginate ions. For the preparation of nanoparticles, polymers weight ratios are selected to ensure that all batches of CS/ALG nanospheres have submicron size with the smallest possible size [12].

The size of nanoparticles obtained in formula F1-F13 was less than 500 nm as summarized in Table II. In these formulas, CS was used alone or along with one copolymer: CMC Na, HPMC, HPMC SR, or PEO. In contrast, when more than two

TABLE II
PHYSICOCHEMICAL CHARACTERISTICS OF NANOPARTICLES

Formula	Size (nm)	PI	ζ (mV)	Entrapment (%)
F1	191	0.45	37.05	7.16
F2	223	0.43	21.15	22.02
F3	296	0.42	24.96	49.62
F4	214	0.44	23.56	35.17
F5	488	0.25	15.14	16.33
F6	374	0.27	13.01	12.86
F7	373	0.30	9.39	10.09
F8	339	0.25	30.24	23.52
F9	250	0.44	30.06	22.33
F10	214	0.30	29.19	16.21
F11	312	0.39	30.12	31.12
F12	323	0.42	30.56	37.15
F13	286	0.42	29.67	30.48
F14	810	0.33	27.19	16.78
F15	721	0.29	31.77	29.49
F16	661	0.31	28.51	21.31
F17	618	0.21	27.89	29.98
F18	558	0.29	29.21	33.73
F19	532	0.33	28.90	30.97
F20	1065	0.45	19.34	60.12
F21	299	0.34	24.34	76.46
F22	1121	0.41	15.23	31.74
F23	852	0.21	17.90	23.52
F24	288	0.39	23.12	74.21
F25	291	0.39	24.98	75.89
F26	299	0.27	28.64	85.04
F27	179	0.25	27.48	85.35
F28	184	0.26	22.90	72.24
F29	199	0.28	28.09	64.17
F30	283	0.25	26.26	68.24

polymers were used as stated in F14-F19, the size of nanoparticles became larger,being more than 500 nm. This might be due to saturation of nanoparticles with surface charge and thus the size increased [12]. Mucoadhesive nanoparticles should be small enough, preferably 200 – 500 nm in order to penetrate mucus [1]. With this understanding, F14-F19 is not considered to have good results in size. In F21, F24, and F25, where CS was combined with ALG in nanoparticles preparation, the particles size obtained was less than 500 nm. This result is consistent with other formulas using CS alone or in combination with one copolymer. Despite this fact, F20 (coated-method A) and F22 (blended-method C), which involve only two polymers (CS and ALG), resulted in relatively large particles at more than 800 nm. CS is likely to precipitate out from solution upon addition of an alginate in higher pH, resulting in less CS available for nanoparticles formation. While the pKa of CS is known to be 6.5, an alginate solution of neutral pH, upon addition, would result in the majority of amine groups of CS being unprotonated and, therefore, unable to participate in ionic interactions with alginate. A few protonated groups available for interaction would result in weaker electrostatic interaction with the alginate gel, leading to larger particle sizes to be produced [12]. For F23 (blended and adjusted pH-method D), eventhough it shows decreasing in size compared to F22 (which the pH was not “,adjusted), that size was considered as large. This was due to the method by which it mixed CS and ALG prior to $CaCl_2$ addition. The mixing between ALG and $CaCl_2$ will form pregel state. It was stated that the pregel state was necessary to enable the ionic interactions between alginate and chitosan to form nanosphere.

Formation of nanospheres required a low concentration of CaCl2 (less than 0.2% mass ratio) to form the negatively charged, calcium alginate pregel that was subsequently enveloped by the positively charged chitosan. [12]. This may be the reason why alginate should be mixed first with $CaCl_2$ before the addition of CS.

In various polymer concentrations, increasing size of nanoparticles was observed when higher polymer concentration was used. The saturated state of nanoparticles with surface charge as mentioned above was probably the cause [12]. In addition, PEO and CS interaction in nanoparticles forms CS-PEO semi-interpenetrating network. Consequently, the increase of PEO concentration lead to an increase of the particle size [9]. However, in CS-HMPC SR nanoparticles (F11-F13) this trend was absent.

The value of zeta potential in all preparations was more than + 9.0 mV. Positive zeta potential indicates that the surface of nanoparticles was preferably composed by CS [13]. The positively charge of nanoparticles would allow to interact with negatively charge of mucin, primary constituents of mucus. Thus, adhesiveness between nanoparticles and gastric mucus would be formed via ionic interaction. The measurement of zeta potential is important because zeta potential is related to the stability of colloidal systems. If the value of particle zeta potential is large, the colloidal system will be stable. Conversely, particles agglomeration is expected when the zeta potential is small [12]. Despite of no ionic interaction present between HPMC and CS due to neutral natural of the former polymer, the combination of CS/HPMC was chosen because HPMC could provide highly viscous gel barrier which is useful in drug release. Besides normal HPMC, HPMC sustained release with MW of 10,000 was also chosen as it could provide greater gel barrier [14].

When nanoparticles prepared by using CS alone, the entrapment efficiency was 7.16%. This low value was likely caused by partial repulsion of CS and SS. In CS microsphere containing gentamicin sulphate, low encapsulation efficiency was observed and this may be due to the partial repulsion of CS and gentamicin sulphate as both of them being positively charged [15]. Nonionic polymer used in this experiment: PEO, HPMC, and HPMC SR, were combined with CS to prepare salbutamol sulphate nanoparticles. Entrapment efficiency of drug was drastically changed when nonionic polymers were used. However, the entrapment efficiency in these formulas was less than 50%. The combination of three polymers, CS, PEO and HPMC or HPMC SR even resulted in entrapment efficiency at less than 35%. For polymer such as HPMC, ionic interaction was absent between HPMC and CS [14]. This could be the reason of low entrapment using nonionic polymers. The negatively charged polymer enables the positively charged SS to interact with it and thus results in increased drug entrapment [16]. In addition, it is reported that high encapsulation efficiency of gentamicin sulphate was observed in hyaluronic acid microspheres, an anionic polymer [15]. Surprisingly, it can be seen in F5-F7 that when CS and CMC Na were used, the

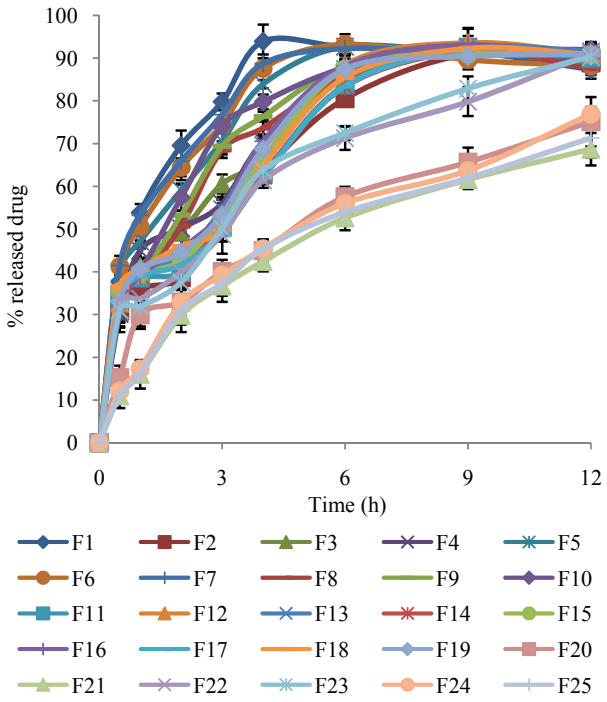

Fig. 1 Salbutamol sulphate release profiles from nanoparticles

entrapment efficiency was limited approximately 10.09-16.33%. Although CMC Na is anionic, the molecular weight of CMC Na was excessively high for nanoparticles formation [11]. When chitosan was combined with alginate, refers to F20-F25, the entrapment efficiency was within a range from 23.52-76.46%. It was obvious that adjustment of pH of 5 was critical. Failure in pH controlling (method C and D) resulted in weaker electrostatic interaction, large particles and low entrapment efficiency (F22-F23). Additionally, entrapment efficiency from method B for approximately 74-76% was higher than that from method A limited approximately 60%. These results were also in line with the previous consideration of the importance of pH adjustment of CS and ALG solution. Furthermore, combination of PVA and PEO was significantly enhanced the entrapment efficiency of SS approximately 85%. However, the higher PVA concentration, the lower entrapment efficiency. It is presumably that higher concentration of PVA provided a steric barrier for drug solution to be encapsulated into the particles.

The requirement of salbutamol sulphate release from controlled release dosage form is not set up in monography yet, thus, general requirement of controlled drug release was used. According to Banakar, in the book of Pharmaceutical Dissolution Testing [17]:

1. At the time when it is similar to 0.25 dose ($Q_{0.25}$), the percent of drug released is between 20-50%
2. At the time when it is similar to 0.5 dose ($Q_{0.5}$), the percent of drug released is between 45-75%

3. At the time when it is similar to 1 dose (Q_1), the percent of drug released is not more than 75%

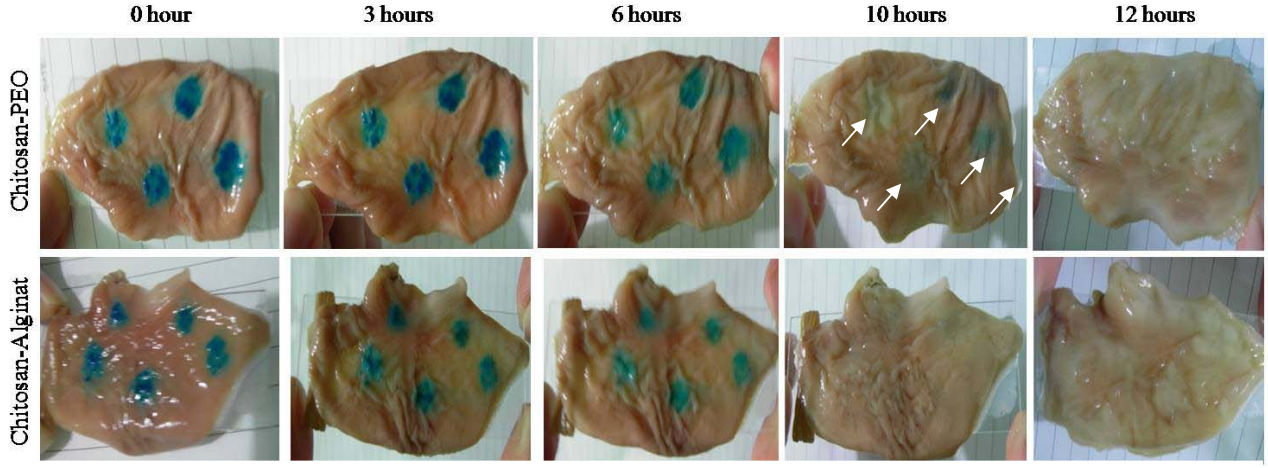

Fig. 2 Mucoadhesive testing of nanoparticles on the gastric rabbit using FaSSGF medium. Above panel: F3 PEO-chitosan based nanoparticles. Lower panel: Alginate-chitosan based nanoparticles

The dose refers to the frequency of administration drug. In this research, the frequency is 12 hours once. Thus, the requirement of salbutamol sulphate release is 20-50% after 3 hours, 45-75% after 6 hours, and ≥ 75% after 12 hours.

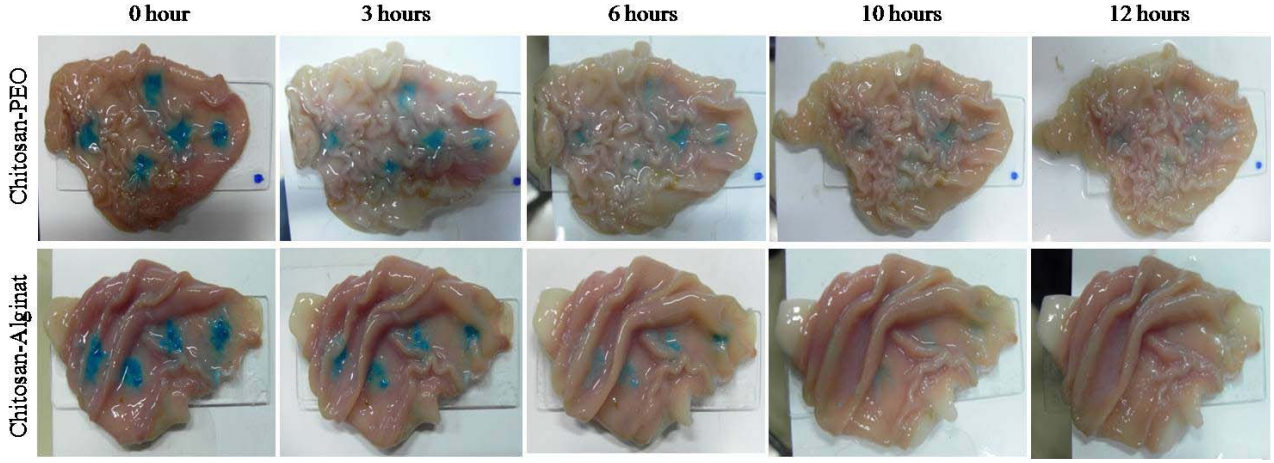

Fig. 3 Mucoadhesive testing of nanoparticles on the gastric rabbit using Krebs buffer medium. Above panel: F3 PEO-chitosan based nanoparticles. Lower panel: Alginate-chitosan based nanoparticles

For F1-F19, > 75% of salbutamol sulphate was released after 6 hours as seen in the Fig. 1. This due to CS polymer was soluble in acidic solution, while sodium CMC, HPMC, HPMC SR and PEO were hydrated in acidic solution. Meanwhile, at F20-F25, <75% of salbutamol sulphate release after 6 hours was due to the alginate polymer that is stable in acidic solution (not solubilize by acidic solution). The released drug from F26-F30 was very limited approximately 7% until 12 h. It is presumably that there was a steric hindrance derived from PVA and PEO that inhibited the release of drug. In F1-F25, they exhibited fast release at initial phase. This was consistent for all formulations, and is most likely to be due to the presence of salbutamol sulphate on the surface of the nanoparticles or defects such as pores or cracks within the matrix. The initial rapid release is common and may have a functional use in providing an initial dose during drug delivery, minimizing any lag period [15]. F24 and F25 were developed based on the result of release study of F21. The amount of salbutamol sulphate in F24 and F25 was higher than in F21 as to make the potential F21 which had small size of nanoparticle, high entrapment efficiency and good controlled release met the requirement of mucoadhesive preparation. Over the formulas, only F24 fulfilled the requirement of controlled release of salbutamol sulphate.

Next, mucoadhesion test was performed for 2 formulas (F3 & F24). These two formulas were chosen as the best representative of the anionic and nonionic co-polymers in terms of size, entrapment efficiency, and release study results. This method was chosen due to it is simple to be performed. In this test, rabbit stomach was used and the media used were Krebs buffer and FaSSGF medium. FaSSGF was used in order to simulate the condition of stomach. However, it seems that it did

not retain the cell to live longer. When chitosan-alginate nanoparticles were applied to the gastric section, the particles were likely removed after 10 hours (Fig. 2). It is probably that intimate contact between the nanoparticles and gastric mucus was eliminated due to insufficient production of mucin. Due to this limitation, Krebs buffer was used as it was more physiologic medium. After changing the medium, the chitosan-alginate nanoparticles retained until 11 h. Furthermore, combination of chitosan-PEO provides longer contact to the gastric mucus in both FaSSGF and Krebs buffer medium (Fig. 3, Table III). It is likely that binding between nonionic-mucus interactions via hydrogen bonding was stronger than that of anionic-mucus interaction through ionic interaction. Based on the results, Krebs buffer can retain the nanoparticles more longer than FaSSGF medium as the cell live more longer, thus interact with nanoparticles more longer. Although CS-PEO retained longer until 12 h, the release of drug did not meet the requirements. Therefore, F24 was the best formula with the size of 288 nm, 74, 21% of entrapment efficiency, met all the drug release requirements and approximately 11 h retained on the stomach.

TABLE III
CHARACTERISTICS OF RETENTION TIME OF NANOPARTICLES AFTER IN VITRO MUCOADHESIVE TEST

Formula	In vitro retention time (hours)	
	FaSSGF medium	Krebs buffer medium
F3	11.5	12
F24	10	11

IV. CONCLUSION

The results of the present study indicate that anionic polymer alginate and a specific preparation method determines the encapsulation ratio of salbutamol sulphate into gastroretentive mucoadhesive nanoparticles. In addition, the chitosan and alginate in the form of nanoparticle induce prolonged contact time in gastric. Collectively, the salbutamol sulphate-loaded chitosan and alginate-based nanoparticles can be utilized for providing better absorption state of the drug.

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