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Development of Alginate – Gum Arabic Beads for Targeted Delivery of Protein

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Abstract

Controlled release beads were prepared by using the combination of alginate and gum Arabic through ionotropic gelation method. Bovine serum albumin was used as model protein for in vitro assessments. The effect of amount of sodium alginate and gum Arabic as the factor affecting protein encapsulation efficiency and protein release were optimized and analyzed by using RSM-FCCD. It was observed that protein encapsulation efficiency was increased and protein release was decreased with the increase of both of the amount of sodium alginate and gum Arabic, used as polymer blend. The optimized beads showed high encapsulation efficiency (87.5 ± 3.65%) with suitable protein release (100% protein release after almost 4 hrs). The swelling of beads were highly influenced by pH of dissolution medium. These beads were also characterized by FT-IR spectroscopy, SEM and TA for protein-excipients interaction, beads surface morphology and beads strength, respectively. These calcium alginate/gum Arabic beads have good potential to be used as delivery vehicle for protein drugs.

Keywords: Alginate; Gum Arabic; Controlled release; Encapsulation; Targeted delivery

Introduction

Advances in biotechnology over the past few years have driven the production of various clinically useful protein and peptides. However, administration of protein and peptide drug is quite challenging in terms of sustained delivery, targeting formulations and controlled manner. Unlike synthetic pharmaceutical, protein is more sensitive because of their diffusivity and low partition coefficient [1]. Due to these features, proteins may undergo chemical changes, proteolysis and denaturation during passing through the human gut [2,3]. Many attempts have been carried out in order to improve stability of protein in human body. The encapsulation of protein drugs using different biodegradable and biocompatible polymers have been paid much attention in recent years [4,5]. Encapsulation of protein by means is to incorporate a protein drug into a suitable matrix that can provide protection during exposure to the harsh condition of the human gastrointestinal tract. In addition, encapsulation also will help in attaining the sustained release of the drug at the targeted site over a long period of time.

Development of ionotropic gelation technique in producing biopolymeric beads as carrier in drug delivery system has gain great attention recently [6,7]. Through this technique, beads are formed when ionic polymer such as alginate and gum Arabic undergo ionotropic gelation and precipitate due to electrostatic interaction between oppositely charged species. This is a very simple technique, non-toxic and easy to control in term of production [6].

Alginates (ALG) are natural occurring polysaccharides extracted from brown algae that have been investigated since decades ago for its unique characteristics. These characteristics have enabled alginate to be used as matrix for protein delivery. Alginate is a polyanionic copolymer of guluronic and manuronic acids that can form hydrogel beads through ionotropic gelation by the addition of divalent cations in aqueous liquid [8]. Besides proteins [9,10], the other bioactive agents that can be entrapped into alginate matrices are including cells and DNA [11,12]. This is due to the relatively mild gelation process.

Even though alginate beads are easy to be prepared through ionotropic gelation method, there is a major problem regarding drug loss during beads preparation due to the porosity of alginate. In addition, alginate is unstable in acidic environment which can cause the decarboxylation of alginate. Therefore, many modifications based on the combination of alginate with other polymers as drug carrier have been investigated [10,13,14].

Gum Arabic (GA) is a biodegradable and biocompatible natural gum, exudate from Acacia Senegal tree. It has been used widely as stabilizer, thickening agent and emulsifier in the food industry. Gum Arabic is also gaining popularity in pharmaceutical sector due to its physical properties such as non-toxic, highly soluble, pH stable and gelling characteristics [15]. Gum Arabic is complex polysaccharide with highly branched, consist of mixed calcium, magnesium and potassium salt of polysaccharidic acid. Its main chain is composed of 1,3-linked β-D-galactopyranosyl units and the side chain (2 to 5 1,3-linked β-D-galactopyranosyl units) are joined to the main chain by 1,6-linkages. Both the main and side chains comprise of α-L-arabinofuranosyl, α-L-rhamnopyronosyl, β-D-glucuronopyranosyl and 4-O-methyl-β-D-glucuronopyranosyl [15,16]. It has been recently reported that gum Arabic has been combined with alginate as drug delivery system to release glibenclamide [13].

Optimization is defined as statistical experimental design methodologies and has been used widely to produce optimum

response. Central composite design, which is a response surface design, is one of the most reliable statistical optimization designs [17,18]. It is very flexible and efficient, offering much info regarding experiment variable effects and overall experimental error in a minimal number of required runs [19]. In this study, an optimized formulation of alginate and gum Arabic as encapsulating matrices using Response Surface Methodology (RSM) is determined. It is our aim to utilize optimized alginate-gum Arabic beads to deliver BSA orally in the small intestine. Infrared (IR) spectra and Scanning Electron Microscopy (SEM) have been employed to investigate the protein-excipient interaction and the beads surface morphology. We also used Texture Analyzer (TA) to measure the mechanical strength of the beads. The main site for the drug absorption in man is considered to be small intestine due to its high effective surface area. If any, only little drug absorption will occur in stomach and large intestine. Therefore, the bead was aimed to protect the BSA during exposure to acidic condition in stomach and once reach the targeted site (small intestine) the beads will slowly disintegrate and release the BSA. It seems that alginate-gum Arabic beads have potential use as a vehicle for targeted drug delivery system.

Materials and Methods

Materials

Sodium alginate (ALG) and bovine serum albumin (≥98%) powder were obtained from Sigma-Aldrich Co, St. Louis, MO, USA. Gum Arabic was given by Natural Prebiotic Sdn. Bhd. (Selangor, Malaysia) as a gift. All other chemicals are of Analytical Reagent (AR) grade.

Beads preparation

The calcium alginate-gum Arabic beads containing bovine serum albumin were prepared by using ionotropic gelation method where calcium chloride ($CaCl_2$) was used as cross-linker in ionotropic gelation. Sodium alginate and gum Arabic were allowed to dissolve in deionized water containing bovine serum albumin (BSA) (3 mg/ml). The formulation was as follow: sodium alginate (2–3% w/v) and gum Arabic (1–2% w/v). The final calcium alginate-gum Arabic solution containing BSA were ultrasonicated for 15 min for debubbling. Approximately 1 ml of the resulting solution which contained 3 mg of BSA was injected through a syringe needle (23G) into 50 ml of 0.2 M $CaCl_2$ solution for hardening process. The beads were formed instantly and were retained in $CaCl_2$ solution for 30 min in order to form rigid beads. Then, the wet beads were filtered and washed at least two times with distilled water. The rinsed beads were allowed to dry at room temperature for overnight. The dried calcium alginate–gum Arabic beads containing BSA were stored in a refrigerator until used.

Experimental design

Response Surface Methodology-Face Centered Composite Design (RSM–FCCD) has been applied to design the experiments, model and optimize three response variables including protein encapsulation efficiency (PEE,%), protein release at 2 h in SGF (%) and time for 100% release (min) in SIF. The amounts of sodium alginate and the gum Arabic were defined as the selected independent formulation variables (factors). Each factor was coded at three levels between 1 and +1, where the factor alginate and gum Arabic were changed in the ranges. Nine experiments were augmented with three replications at the center points to evaluate the pure error and to fit a quadratic model. Minitab^{TM} version 14.0 software (Minitab Inc., PA, USA) was used for regression analysis of experimental data and to plot response surface. The 'response optimizer' in Minitab^{TM} was employed to determine the optimum setting for each independent variable that contributed to the optimum predicted responses. In addition, the interaction effect between significant variables were also can be analyzed by using response surface plots. The polynomial mathematical model generated by central composite design was expressed as follow:

$$y=b + bx1 + bx2+ bx1x2 + b4x12+ b5x22 + \varepsilon$$

Where y is the response, b is the intercept and b^1, b^2, b^3, b^4, b^5 are regression coefficients. $x^1$and x^2 and individual effects; x^{12} and x^{22} are quadratic effects; x^1x^2 is the interaction effect, and ε is the residual.

Determination of protein encapsulation efficiency, PEE (%)

The amount protein (BSA) loaded in the beads was estimated by using digestion method. Beads containing 3 mg BSA was dissolved in 20 ml of 0.1 M Phosphate Buffer Saline (PBS), pH 7.4 for at least 18 hrs at 25°C ± 0.5°C. The experiment was done in triplicate. BSA content was spectrophotometrically assayed using UV-Vis spectrophotometer at 595 nm (Bradford, 1976). The percentage of loading efficiency was calculated by expressing the actual amount of protein loaded (L) divided by the theoretical amount of protein loaded (L), as a percentage.

$$\text{Loading efficiency}=(L/L) \times 100$$

In vitro protein release studies

The release of BSA from various ionotropically gelled calcium alginate-gum Arabic was tested in two different pH solutions which mimicking mouth to small intestine transit. The studies were carried out in glass bottle in shaking water bath. The dissolution rates were measured at 37 ± 1°C fewer than 50 rpm speed. Beads containing 3 mg BSA were tested for protein release in 5 ml of simulated gastric fluids, SGF (0.1 M HCl, pH 1.2) for 2 h as the average gastric emptying time is about 2 h. Then, the dissolution medium was replaced by simulated intestinal fluid, SIF (phosphate buffer, pH 7.4) for next hours until the beads were disintegrate. At regular time intervals, 0.5 ml of aliquots was collected and analyzed to determine the protein release from the beads by using Bradford's method. An equal volume of same dissolution medium was replaced to maintain a constant volume. The cumulative percentage of BSA release from the beads in dissolution medium was calculated.

Beads stability and swelling behavior

The swelling behavior of beads was evaluated in two different aqueous media: 0.1 N HCl, pH 1.2 (SGF) and phosphate buffer, pH 7.4 (SIF). Briefly, 100 mg of dried beans were exposed to 20 mL of SGF for 120 min and SIF for 180 min. The condition during incubation period was maintained at 37 ± 1°C with 110 rpm shaking. The swelled beads were removed at predetermined time interval and weighed after blotting the surface with filter paper to remove the excess moisture. All experiments were done triplicate. Swelling index was calculated using the following formula:

Fourier transform-infrared (FT-IR) spectroscopy

FT-IR was conducted with a Thermo Scientific Nicolet 6700 spectrophotometer (USA). The control of instruments, data collection

and primary analysis were processed by OMINIC software. The sample of pure BSA, polymers and dried BSA loaded beads were crushed into powder and 5 mg of the sample was ground completely with 950 mg of spectroscopy grade KBr powder. Then, the mixture was pressed into a pellet with a die press and placed in the sample holder. Spectral scanning was done in the wavelength region between 400 and 4000 cm^{-1} at a resolution of 4 cm^{-1} with scan speed of 1 cm^{-1}.

Surface morphology analysis

The surface morphology of the dried formulated beads was analyzed using Scanning electron microscopy (SEM) (LEO 1455 VP SEM, Oberkochen, Germany). Beads were mounted on aluminum stub using a double-sided adhesive tape. Subsequently, the beads were gold coated with a sputtering coater (Bal-TEC SCD 005, Spulter Coater, Principality of Leichtenstein, and Switzerland) to make them electrically conductive and their morphology was examined. Samples were viewed at an accelerating voltage of 20kV, using a second detector at high vacuum mode.

Beads' strength determination

The method for determining the strength (g) of the beads was modified from a previous study reported by Edward-Lévy and Lévy (1999). The analysis of mechanical behavior of beads was carried out by using texture analyzer (T.A.HD plus, Stable Micro System, UK) with a 5 kg load cell equipped and a delrin cylindrical probe of 5 mm in diameter (Plate 4.1). The probe was positioned to touch the beads, recorded as initial position and the probe flattened the beads. The compression of the beads was measured using following conditions: Test mode: Compression (g), Pretest speed: 2 mms^{-1}, Test speed: 2 mms^{-1}, Post-test speed: 2 mms^{-1}, Target mode: strain, Distance: 5 mm, Strain: 50%, Trigger type: Auto (force), Trigger force: 5 g. The probe was removed when the beads was compressed to 50% of its original height .The maximum force (g) at 50% displacement represent the strength of the beads was recorded and analyzed by Texture Exponent 32 software program (version 3.0). The bead strength was examined before and after being exposed to simulated gastric fluid (SGF) and intestinal fluid (SIF). Wet beads were exposed to 20 mL of SGF for 120 min and 60 min in SIF. A single wet bead was tested each time and 5 replications were performed on each sample.

Statistical analyses

One-way ANOVA was performed to examine significant differences between normally distribution data. Tukey's test was applied to perform multiple comparisons between means within each analysis. Probability level of less than 0.05 was considering ($p<0.05$). All data was analyzed using MINITAB version 16 (Minitab Inc., PA, USA).

Results and Discussion

Formulation optimization by central composite design

Planning pharmaceutical formulation with the least of trial is very challenging for pharmaceutical researchers [20]. In conventional optimization method, a single factor is varied meanwhile the other factors are fixed at a particular set of settings. However, this method is time consuming and less effective as it does not consider the interactive effect of all the main factors. If several factors are to be considered at the same time, their interactions are not noticeable even for the dominant ones. Therefore, using statistical tool such as central composite design is very useful because it helps to study the effect of independent variables influencing the responses by changing them simultaneously. This approach provides statistically reliable results with fewer numbers of experiments and can be applied for the development, improvement and optimization of the biomanufacturing process [21-23]. Conventional screening has been applied to estimate the range of encapsulating matrices compositions using alginate and gum Arabic to encapsulate BSA. Based on the screening process, a central composite design with total 9 experimental formulations of calcium alginate/gum Arabic containing BSA was proposed for two factors: amount of alginate, x^1 and amount of gum Arabic, x^2 used in polymer-blend. The effects of these factors on protein encapsulation efficiency (y^1), the amount of protein release in SGF (y^2) as well as time taken for 100% release of protein in SIF (y^3).

Based on the estimated coefficient of the experimental result, the estimated polynomial regression model relating the PEE (%) as response became:

$y1=13.99 + 22.57\ x1 + 9.28\ x2–0.01x12 + 4.59\ x22–5.30\ x1x2$ [$R2=0.964$; $p<0.1$]

The regression model equation for R2h (%) can be predicted as follows:

$y2=81.60–42.99\ x1–15.57\ x2 + 5.66\ x12 + 0.64\ x22 + 4.32\ x1x2$ [$R2=0.972$; $p<0.1$]

The regression model equation relating Tr (min) as response became:

$y3=41.47–23.25\ x1–48.95\ x2 + 11.32\ x12 + 11.32\ x22 + 15.00\ x1x2$ [$R2=0.976$; $p<0.1$]

Model building steps has been carried out by excluding non-significant terms ($p>0.1$). Starting with full quadratic terms, the most non-significant terms will be eliminated first. The same procedure is applied for the next step until all the non-significant terms are eliminated. After eliminating non-significant terms ($p>0.05$), the regression model equation for y2 and y3 responses became:

$y1=4.35 + 22.52\ x1 + 23.10\ x2–5.30\ x1x2$

$y2=81.29–43.84\ x1–13.65\ x2 + 5.83\ x12 + 4.32\ x1x2$

$y3=-51.63 + 33.33\ x1–15.00\ x2 + 15.00\ x1x2$

These models were evaluated statistically by applying one-way ANOVA ($p<0.05$). Response Surface Methodology (RSM) software generated three-dimensional (3D) response surface plot and contour plot relating investigated response, PEE (%). The 3D response surface plot is very helpful in understanding about the main and interaction effects on the factors; meanwhile two-dimensional (2D) contour plot provides a visual illustration of values of the response [17,24].

The 3D response surface plot relating PEE (%), R2h (%) and Tr (min). Meanwhile, the 2D contour graph relating PEE (%), R2h (%) and Tr (min) are also presented. Based on 3D response surface plot, it can be noted that PEE (%) increases with the increase of both the amount of both sodium alginate (x^1) and gum Arabic (x^2). On the other hand, the response surface plot relating R2h (%) depicted the decrease in R2h (%) with the increase of both the amount of sodium alginate and gum Arabic. In the meantime, the increase in Tr (min) was observed from 3D response surface plot as both the amount of sodium alginate and gum Arabic increased.

A numerical optimization method using the desirability approach was used to develop a new formulation of sodium alginate and gum Arabic with the desired responses. Constraints like maximizing protein encapsulation efficiency (PEE), minimizing the protein release in SGF (R2h) and maximizing the time taken for protein release in SIF (Tr) were set as the goals to locate the optimum settings of factors in the new formulations. 'Response optimization' in Minitab^{TM} version 14.0 software (Minitab Inc., PA, USA) was employed for the optimization process. In order to obtain the desired optimum responses, the factors were restricted to 2% (w/v) ≤ x1 ≤ 3% (w/v) and 1% (w/v) ≤ x2 ≤ 2% (w/v); while the responses were restricted to 70% ≤ PEE ≤ 100%, 0% ≤ R2h ≤ 4% and 90 min ≤ Tr ≤ 120 min.

The optimized formulation was developed using 3% (w/v) sodium alginate and 2% (w/v) gum Arabic. The optimized beads containing BSA were evaluated for PEE (%), R2h (%) and Tr (min) for verification. The optimized beads which contain BSA showed PEE value of 87.5 ± 3.65%, R2h (%) value of 3.12 ± 4.14% and Tr (min) value of 110 ± 0 min. It can be observed that the experimental and predicted values for all the responses were significantly different ($p>0.05$) with the prediction error ranged between 0.92 and 2.30%. This result suggests that mathematical models obtained from central composite design were well fitted.

Protein encapsulation efficiency, PEE (%)

The PEE (%) of all these calcium alginate/gum Arabic beads containing BSA was within the range 62.3 ± 3.87 to 87.5 ± 3.65%. It can be noted that PEE (%) increases with the increase of both the amount of both sodium alginate (x^1) and gum Arabic (x^2). The increased of PEE (%) with the increasing amount of sodium alginate and gum Arabic probably due to the high viscosity of polymer solution. High viscosity of solution can be obtained when the amount of polymer addition increases and through this method, the drug leaching during beads preparation might be prevented and result in high encapsulation efficiency [13]. In this study, high protein encapsulation efficiency, PEE (%) was desired in order to make sure that optimum protein densities were able to reach the target area.

In vitro protein release

Initially, the release amount of BSA was minor in SGF throughout the 2 h incubation (pH 1.2) and this is due to unique characteristics of alginate. Alginate is a hydrophilic polysaccharide and water soluble. However, alginate is insoluble under acidic condition. At low p^H, the quantity of positively charged ions is high and they decrease the electrical repulsion between negatively charged alginate molecules [25]. This results to protonation of alginate into insoluble form of alginic acid. Therefore, at acidic pH, penetration of dissolution fluid through the polymer is slowed down and the amount of protein release is minimal. Moreover, the introduction of other polysaccharides into alginate matrices increases the beads viscosity and allows the synergistic interaction which enhances the stability of beads in low pH solution.

Once pH was increased to 7.4, the protein release gradually increases up to 100%. This behavior is due to the deprotonation of alginic acid that occurs at higher pH [26]. It will draw fluid into the beads which led to swelling and disintegration. Consequently, the protein release from the beads occurred rapidly. The pattern of protein release in this study indicates that protein can be continuously released from the acidic (pH 1.2) to nearly neutral condition (pH 7.4) in hich the release amount and speed of release were much higher and faster than those in acidic medium.

The protein release pattern obtained in this study was relevant with the transit time of pharmaceutical dosage in human body. The previous study has reported that the average gastric emptying for pharmaceutical dosage was 2 hrs while the small intestine transit time was 3 hrs [27]. The targeted site which is the small intestine is quite long and nearly all drugs or vitamin and minerals can be absorbed from different area along its surface. However, the absorption of most drugs and minerals is occurs at the part of intestine closest to the stomach (duodenum) and the middle part of the small intestine (jejenum).Since the transit time of pharmaceutical drugs in small intestine was about 3 hrs, thus we can assume that most of the proteins released during the 2 hrs in SIF can be successfully absorbed into the small intestine region.

Beads stability and swelling behavior

The stability of the beads during SGF and SIF treatment is signified through the swelling behavior. The swelling index of the beads was initially lower in acidic pH (0.1 N HCl pH 1.2) compare to that of in alkaline phosphate buffer (pH 7.4), indicating a pH-sensitive swelling behavior. Low swelling index in acidic environment was probably due to the shrinkage of alginate. On the other hand, the swelling behavior of beads in SIF could be explained by the ion exchange phenomenon occurs between calcium ions in the beads and sodium ions in phosphate buffer. Sodium alginate is a polysaccharide with highly hydrophilic properties due to the –OH and –COOH groups present in its chain [28]. This characteristic enables alginate to cross-link with the positive charge ions, Ca^{2+} in $CaCl_2$ during hardening process of the beads. In acidic environment, the ionic strength was stronger due to the stability of negative and positive charges. However, at pH 7.4 (near to neutral), water tends to penetrate into the chain to form hydrogen bond through –OH and –COOH groups and fills up the space along the chain [29]. Finally, calcium ions in egg-box buckle structure diffuse into dissolution medium and alginate beads begin to swell substantially, which cause the disintegration of the beads at higher speed. This phenomenon may also occur in case of gum Arabic beads due to the present of –COOH group in its chain [17]. In conclusion; the beads are very stable in acidic medium, pH 1.2 and started to lose their stability at higher pH, 7.4.

FT-IR spectroscopy

The natural polymers (alginate and gum Arabic) that have been used in this study as microsphere matrices for drug delivery system are referred as the excipients. The interaction between protein and excipients is important to be studied as the polymers might be not compatible and affect the stability of the encapsulated protein. Besides that, we also interested in the interaction between various functional groups present in the polymers which contributes to the stability of the encapsulating matrices. FT-IR spectrum for sodium alginate, gum Arabic, alginate/gum Arabic beads without BSA, alginate/gum Arabic beads containing BSA and pure BSA. The spectra of sodium alginate and pure BSA have been discussed in the previous sub topic. In the FT-IR spectrum gum Arabic, a broad absorption band was observed near 3400 cm^{-1} which was the characteristic peak of the glucosidic ring and also might due to the stretching of –OH hydroxyl group. The peak at 2933 cm^{-1} was attributed to the C–H stretching vibration. The strong peak at 1610 cm^{-1} was indicated the presence of –COO (asymmetric) vibration meanwhile the weak absorption around 1420 cm^{-1} was due to

the –COO (symmetric) stretching. Around 1200–900 cm^{-1}, strong peaks were detected and they were finger print of carbohydrates [30].

The FT-IR spectra of alginate/gum Arabic beads without BSA showed the characteristics peaks of both alginate and gum Arabic without any major shifting or deviation. However, the peak of C–H stretching in gum Arabic at 2933 cm^{-1} disappeared and it might attribute to the interaction with alginate. The peaks of –COO (asymmetric) and –COO (symmetric) stretching from both polymers near 1600 and 1400 cm^{-1} respectively were combined and formed new stronger peaks at the same region.

On the other hand, in the FT-IR spectrum of alginate/gum Arabic beads containing BSA, numerous characteristic peaks of alginate, gum Arabic and BSA were shown, suggesting there is no interaction between protein and polymers. Therefore, alginate and gum Arabic are compatible with BSA and suitable to be used as encapsulating matrices.

Beads morphology

The shape and morphological analysis of optimized alginate-gum Arabic beads loaded with BSA before and after SGF and SIF exposures were visualized using SEM at different magnification. It was observed that these beads possessed a homogenous and compact structure with spherical shape. Detailed examination of alginate-gum Arabic beads showed a denser surface and polymeric debris was seen on the beads surface which probably due to the procedure of beads preparation such as instantaneous gel beads preparation and development of the polymer blend matrix [17].

After 120 min (2 hrs) of SGF incubation, beads were still intact with slightly differences in their morphology. The size beads were slightly increased which indicated the beads swelling behavior in SGF. Beads showed swollen behavior with small erosion on the surface after SGF treatment indicating the beads experience the early sign of disintegration activity. The incorporation of gum Arabic into alginate matrices might not give much difference under SGF exposure. This is because the main component of the matrices is alginate which is very stable in acidic condition. Nevertheless, during incubation in SIF, there was large difference in bead morphology which explained the release profile of BSA from the beads. After 120 min of exposure in SIF (pH 7.4), the beads obviously lost their shape due to the erosion and swelling activities.

Beads strength analysis

The mechanical properties of beads like alginate are not easy to be measured due to the large amount of water that contained inside the beads. In this study, the high compression speed that has been used in this analysis was able to minimize the time-dependent behavior [31].

The mechanical strengths (g) of optimized alginate/gum Arabic beads before and after being exposed to SGF and SIF. The initial bead strength was relatively high (178.25 ± 10.03 g) and it could due to the high concentration of sodium alginate in the polymer blend. According to the study carried out by Ouwerx et al. [32], the gel strength of alginate beads was highly influenced by the concentration of alginate used in the polymer solution. High concentration of alginate results in high viscosity of solution; hence produce more rigid and strong beads. After being exposed to simulated gastric fluid, SGF (pH 1.2) for 2 h, the beads strength has been reduced (111.78 ± 6.79 g). These results probably related to the decarboxylation of alginate at low p^H (10). Sodium alginate contains carboxylic acid groups (RCOOH) that are protonated in acidic environment as following:

$$[RCOO^-]gel + [H^+]aq \rightarrow [RCOOH]gel$$

This results in low charge density and the content of mobile counter ions within the beads which leading to gel shrinking [33]. Even though the beads strength was decreased after SGF exposure, the beads were still intact and the values of beads strength are comparatively high to protect the protein from being released into the gastric region. These results are parallel with the findings obtained in previous chapter which showed that only a trace amount of protein was released during 2 hrs exposure to SGF.

The results show a drastic reduction of beads strength after being introduced to SIF (46.87 ± 2.30 g). In basic surrounding conditions, the carboxylic acid groups (RCOOH) in alginate are deprotonated and produce carboxylate ion with negative charge (RCOOH-) as following:

$$[RCOOH]gel + [OH^-]aq \rightarrow [RCOO^-]gel + H_2O$$

When carboxylic acid groups exposed to basic solution, the hydrogen is dissociate and cause the formation of lots negative charges along the backbone of polymer chain. These negative charges repel each other and force the polymer to uncoil. This process also called as chain relaxation. In addition, the negative charges also cause the attraction of polymer to water increases [34]. When the structure began to loosen, and water molecule penetration is enhanced, the swelling process occurs and subsequently led to mechanical and physical instability of beads [35-37]. In this study, the beads strength is targeted to be low after SIF exposure in order to give indication that the beads are ready to release the protein into the intestinal region.

Conclusion

As conclusion, calcium alginate/gum Arabic beads containing BSA were successfully prepared by ionotropic gelation method and optimized using central composite design. The optimized beads showed encapsulation efficiency of 87.5 ± 3.65% with suitable sustained protein release pattern (100% protein release after almost 4 hrs) which could possibly be advantageous for targeted protein drug delivery in small intestine. The encapsulation technique in preparing calcium alginate/gum Arabic beads was found to be simple, mild, easily controllable, low cost and reproducible. In addition, the natural polymers used for the formulation such as sodium alginate and gum Arabic are also cheap and abundant. The pH values of dissolution medium were highly affected the protein release profile, swelling behavior and beads strength of calcium alginate/gum Arabic beads. At acidic pH, a trace amount of protein was released as the beads undergo a low degree of swelling. In contrast, the beads started to swelled rapidly and released protein from the beads when exposed to basic medium. However, more in vivo studies were required in order to confirm these observations.

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Declaration of Interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of paper.

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