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Research Article

Study of Alginate-Glycerin Microspheres for Release Profile of Human Serum Albumin at Different pH Values

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Abstract — In this study, alginate-glycerin microspheres encapsulated with human serum albumin (HSA) as a model protein were synthesized utilizing the ionotropic gelation process with calcium ions acting as a cross-linker. The encapsulated microspheres were then tested for release profile using the Bradford method in simulated gastric and intestinal fluids (SGF and SIF). The native alginate and the encapsulated microspheres were characterized by FTIR analysis. The results demonstrated that the encapsulated microspheres displayed the gradual release profile of HSA in the simulated gastric fluid (SGF) compared to release profile obtained in simulated intestinal fluid (SIF), that revealed cumulative release of 46 µg/ml of the model protein over a period of 2 hours' incubation time. Furthermore, the in cooperated glycerin molecules improved the stability and sustained the released of the HSA in simulated intestinal fluid. In conclusion, the encapsulated microspheres demonstrated promising applications as intestinal-targeted protein vehicles.

Keywords — Alginate, Protein, Delivery, Glycerin, microspheres, pH-values

1. Introduction

Sodium alginate, a linear polysaccharide derivative of alginic acid, has two residues of 1,4-β-D-mannuronic (M) and 1,4-α-L-guluronic (G) acids [1] as shown in Fig. 1, is widely used in pharmaceuticals and biotechnology industry. Because alginates have interesting properties which include biocompatibility, ability to generate hydrogel microspheres at room temperature and the system's biodegradation under normal physiological settings. In addition to its thickening and stabilizing properties which are essential in textile, food and beverages industries [2]. These properties possess by alginate makes it a fascinating biopolymer for the immobilization of proteins and enzymes as well as development of controlled-release systems [3, 4].

Alginates are primarily derived from three species of brown algae (kelp), namely Laminaria hyperborean, Ascophyllum nodosum, and Macrocystis pyrifera [5]. Other sources include Sargassum species [6]. Alginate has also been extracted from bacteria, including Azotobacter vinelandii and numerous Pseudomonas species [7, 8]. Proteins are more responsive than synthesized pharmaceuticals due to their diffusion and low partition coefficient [9]. Because of these characteristics, proteins are susceptible to some physical and chemical changes such as denaturation and proteolysis during passing through the human gut [10]. Several studies have been conducted to increase the stability of proteins under various physiological situations. The encapsulation of protein medicines with various biodegradable and biocompatible polymers has received a lot of attention in recent years [11, 12 & 13].

2. Related Work

Many experiments have been undertaken using alginate microspheres in controlled-delivery systems for various proteins. Recently, Alginate and inulin hydrogels encapsulated with bovine serum albumin (BSA) as a model protein were examined for targeted release [14]. The developed hydrogels fairly demonstrated good stability and release efficiency at the target area. In another study conducted by [15], it was also reported that pea protein was encapsulated in an alginate matrix and its release behavior was evaluated in various fruit juices.

In this study, human serum albumin (HSA) was encapsulated with glycerine in alginate hydrogels in the form of microspheres, and the model protein's release behaviors were examined in two pH solutions that mimicked stomach and small intestine environments. HSA, the most prevalent protein in the plasma, has various physiological functions, including

binding and transport of many materials such as fatty acids, hormone, amino acids and toxic metabolites [16-20]. In addition, it has antioxidant and anti-inflammatory actions [21].

3. Experimental Method

3.1 Materials and Reagents

Merck (South Africa) supplied sodium alginate and human serum albumin (≥98%) powder. Glycerol (99%) was acquired from JHD Shantou in China. All other compounds are Analytical Reagent (AR) grade. The equipment used in the study include; Electric Weighing balance (AR2140), FT-IR Spectrophotometer (630FTIR, Shimadzu Scientific instrument), UV-Vis Spectrophotometer (T60 UV-Vis spectrophotometer).

3.2 Preparation of Encapsulated Microspheres

Calcium alginate-glycerol microspheres containing HSA were made utilizing the ionotropic gelation process using calcium chloride ($CaCl_2$) as a cross-linker (scheme 1). An alginate solution of 2.0% (w/v) was produced, and HSA was added at a concentration of 3 mg/ml. The resultant solution was then dropped (injected) into 50 ml of $CaCl_2$ solution (0.2 M) using a syringe needle and the microspheres were left in the $CaCl_2$ solution for 30 minutes to stabilize. The hydrogel microspheres were then filtered and rinsed twice with extra distilled water. The rinsed microspheres were left to dry at room temperature for four days. The dried encapsulated microspheres containing HSA were refrigerated until needed. [13].

3.3 Fourier Transform-Infrared (FT-IR) spectroscopy analysis

FT-IR analysis was performed with a spectrophotometer (FTIR-630, Shimadzu Scientific equipment). A pure sample of HSA, sodium alginate, and dry HSA loaded microspheres were ground separately into powder, and 5 mg of the sample was combined with 950 mg of spectroscopic grade KBr powder. The mixture was then crushed using a die to produce a pellet, which was placed in the sample holder. The samples were then scanned between 400 and 4000 cm-1 (wavenumber) at a resolution of 4 cm-1 and a scan speed of 1 cm-1.

3.4 In vitro Protein Release Studies

The method reported by Bradford [22] was adopted with little modifications, two different *pH* solutions mimicking stomach and small intestine transit were used to investigate the release of HSA loaded microspheres at 50 rpm speed and 37^{o}C. The release rates were studied in 10 ml of stimulated gastric fluid, SGF (0.1M. *pH* 1.2) for 2 hours at 30 minutes' time interval, 0.5 ml of aliquots were collected and analyzed for the protein release from the microspheres by T60 UV-Vis spectrophotometer.

3.5 Swelling Behaviours Test of the Encapsulated Microspheres

The dried encapsulated microspheres (100 mg) were immersed in 20 mL of simulated gastric fluid SGF (0.1M HCl, pH 1.2) for 2 hours and simulated intestinal fluids SIF (Phosphate buffer, pH 7.4) for 1.5 hours at 37°C and shaken at 50 rpm. The swollen microspheres were removed every 30 minutes, and the sample was wiped with Whatman filter paper, which absorbed excess water from the microsphere's surface [18].

The swelling percent was calculated using the formula shown in equation 1;

$$S\% = \frac{Wt-Wo}{Wo} \times 100$$

Equation 1. Where S% is the swelling index, Wt is the weight of the microsphere after swelling, and Wo is the dried weight of microspheres.

4. Results and Discussion

Characterization (FTIR Analysis)

FTIR analysis was carried out to ascertain the functional groups present in both native sodium alginate and encapsulated microspheres.

The FT-IR spectra of native alginate and encapsulated microspheres were presented in figure 2 with distinct absorption peaks. At 3242 cm^{-1}, a broad absorption peak was caused by O-H stretching vibration, indicating the existence of hydroxyl groups. The absorption peak at 2922 cm^{-1} was assigned to the C-H stretching vibration. The significant absorption peaks at 1595 cm^{-1} and 1401 cm^{-1} in Fig. 2(a) revealed the presence of -COO (asymmetric) vibration and -COO (symmetric) stretching, respectively (characteristics of carbonyl group resonance to carboxylic salt) [23, 24]. The absorption peak at 1021 cm^{-1} was attributed to the stretching vibration of C-O [25].

The encapsulation of protein polymer into alginate matrix revealed some additional characteristic peaks in the spectrum of encapsulated microspheres (Fig. 2(b)) and the significant changes in the spectrum were seen at 1587 cm^{-1} (which overlaps with –COO (asymmetric) and 1028 cm^{-1} due to the stretching of C=N and C-N, respectively. These peaks confirmed the presence of the model protein (HSA) in the encapsulated microspheres.

In vitro protein release

In-vitro protein release behaviours of the encapsulated microspheres were measured using a Bradford method [22] and the Wavelength throughout the study was 595nm. It was conducted for 2 hours (at interval of 30 minutes) and the results obtained are presented in Figure 3. The release behaviour of the model protein in simulating SGF was low over the period of the study (2 hours), starting at an initial rate of 32 μg/ml with cumulative release increasing slowly up to 34 μg/ml. This is because at low pH, protonation of alginate occurred and greatly affects the movement of the fluid into the shrunken polymer matrix as depicted in Figure 3. Many studies have been reported on the stability of alginate in acidic environment that indicated the shrinking and strongly holding the bioactive materials in the matrix [26].

Furthermore, addition of glycerine in the alginate matrix fairly improved the release of the protein in the medium. On the other hand, at high pH (phosphate buffer saline pH 7.4) the protein release behaviours was significantly increased. This may be attributed to the increased in fluid uptake which usually occurs at alkaline pH [27]. This enables fluid to penetrate into the microspheres which leads to swelling and little disintegration over a period of incubation time, with cumulative release of 46 µg/ml. The release behaviours observed in this study was similar to release pattern reported by [13] using bovine serum albumin as a model protein. Because of the stability of alginate in gastric environment, thus, sodium alginate is widely employed to prepare intestinal-target drug delivery vehicles [28].

Microspheres Stability and Swelling Behaviours

The stability and swelling behaviours are important factors that influence the release of bioactive substances. The encapsulated microspheres (100 mg) were placed in SGF at 37^{o}C and stirred at 50 rpm. The swelled microspheres were removed at every interval of 30 minutes as depicted in Figure 4. The results showed that swelling rate was slow at lower pH over the study period (2 hours), the highest swelling index obtained was 194%. This is due to protonation of alginate that prevents the fluid to get through the alginate matrix. This behaviour may be useful in oral delivery systems when exposed in acidic medium. Alginate can cross-link with positive charge ions, Ca^{2+} in $CaCl_2$, during the hardening phase of microsphere formation. In acidic environments, the ionic strength is greater due to the stability of negative and positive charges.

However, the swelling index in phosphate buffer solution (pH 7.4) was significantly increased as shown in Figure 5., it was observed that swelling rate as well as size were higher than in simulated stomach gastric fluid (SGF).

The swelling behaviour of alginate beads in gastric and intestinal fluids was investigated by [29], and it was discovered that the beads tended to shrink in the gastric environment (pH 2) but expanded in the intestinal environment (pH 8). They also explain that in the phosphate buffer solution, an increased ion exchange between Ca^{2+} ions found in alginate beads and Na^{+} ions occurred, resulting in beads disintegration. The ionic strength was greater in acidic environments due to the stability of negative and positive charges than in pH 7.4 (near neutral), where water tends to penetrate the chain to form hydrogen bonds via -OH and -COOH groups and fills the space along the chain. These literature findings are consistent with those reported in this study.

Figures and Tables

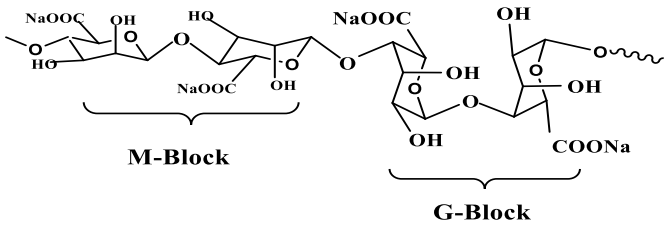


Figure 1: Molecular Structure of Alginate

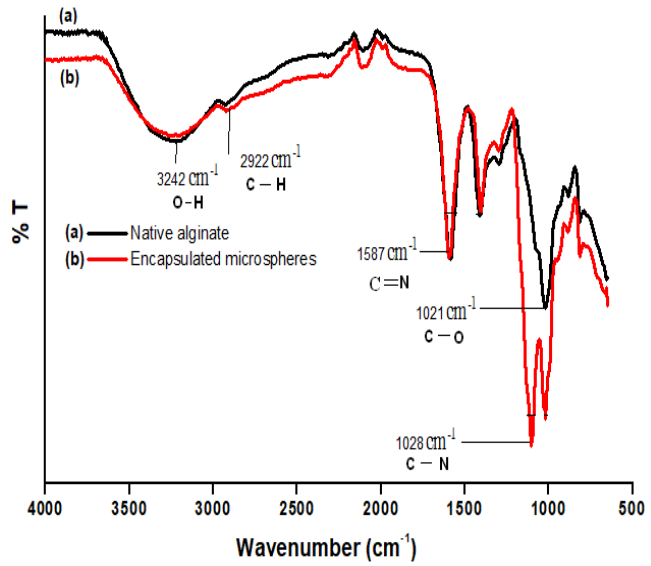


Figure 2: FTIR Spectra of (a) Native alginate (b) Encapsulated microspheres

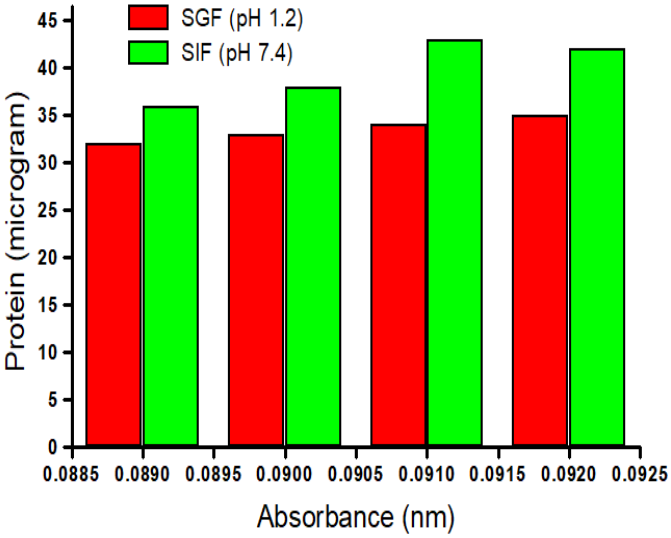


Figure 3: In-vitro Protein Release Results in SGF and SIF at Different pH Values

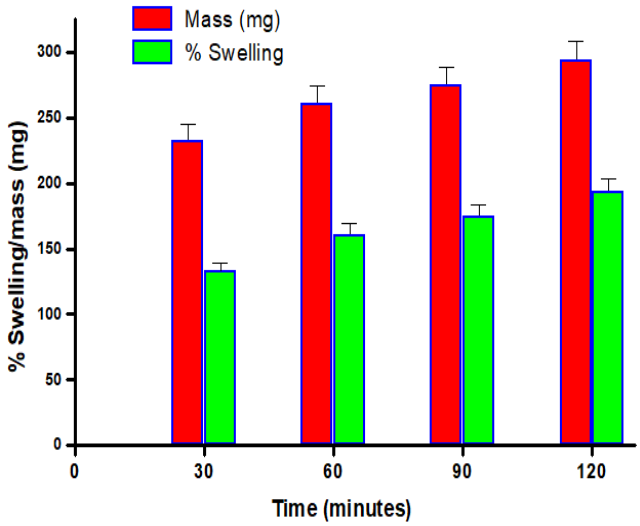


Figure 4: Swelling index and mass of the encapsulated microspheres at *pH* 1.2 (SGF)

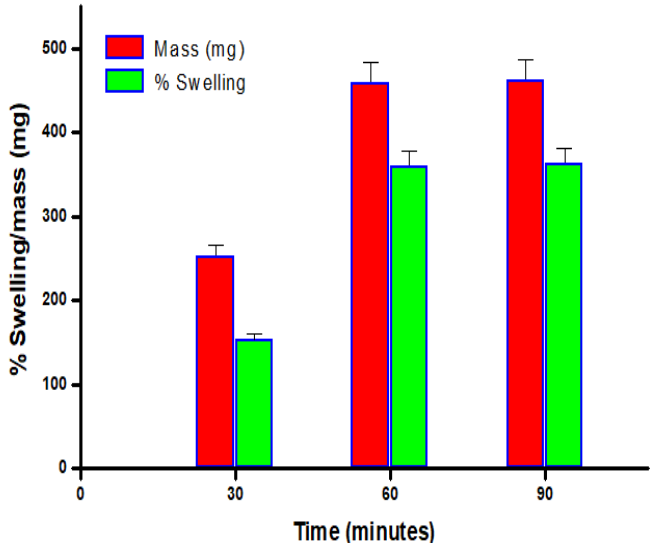
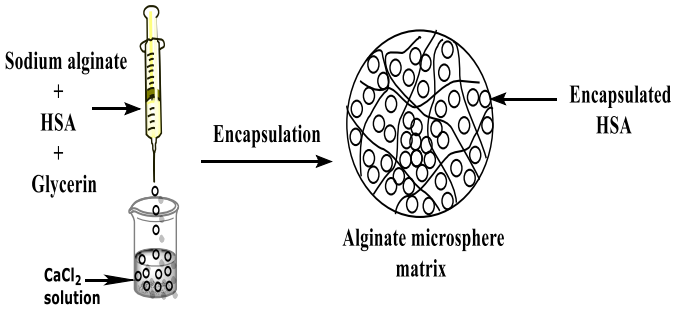


Figure 5: Swelling index and mass of the encapsulated microspheres at *pH* 7.4 (SIF)



Scheme 1: Schematic illustration for the preparation of HSA cross-linked calcium alginate microspheres.

5. Conclusion and Future Scope

It can be concluded that encapsulated alginate-glycerin microspheres containing HSA were successfully prepared by ionotropic gelation method. The encapsulated alginate-glycerin microspheres containing HSA were used to study the release profile using UV-visible spectrophotometer, and monitored the swelling behaviours and microsphere stability. The encapsulated microspheres have shown *pH* sensitive swelling behaviours where slow rate was observed acidic environment. However, the release of the protein from microspheres in simulated intestinal fluid was greatly improved by synergy effect of glycerin in the microspheres. The FT-IR spectrum of the encapsulated microspheres showed that the addition of the protein polymer and glycerin to alginate resulted in additional characteristics confirming the encapsulation has taken place. Based on the good release profile in SIF, the encapsulated microspheres demonstrated promising applications in targeted release of proteins in intestinal environment.

In the future research, other therapeutic agents would be study for controlled release in different media. In Vitro-In Vivo Correlation (IVIVC) should also be performed to establish a link between in vitro HSA release profiles from alginate-glycerin microspheres and in vivo pharmacokinetic behavior in animal models or human subjects; this would validate the relevance of in vitro dissolution studies for predicting the performance of microsphere formulations in vivo. Multifunctional Microspheres should be identified, which means investigating the possibility of incorporating additional functionalities into alginate-glycerin microspheres, such as imaging agents or stimuli-responsive components, to allow real-time monitoring of drug release or triggered release in response to specific physiological cues. Alternative polymers, such as chitosan, poly(lactic-co-glycolic acid) (PLGA), or polyethylene glycol (PEG), should also be employed and analyzed for their potential in controlling release profiles at various pH values, in addition to alginate.

Data Availability

Because of the technical and time limitations, the raw data required as part of an ongoing study cannot be shared.

Conflict of Interest

The authors have no any potential conflicts of interest to declare.

Funding Source

The authors do not receive any financial assist from any agency or institution for the research.

Authors' Contributions

The author Zuhra Muhammad Dikko[2] and Ibrahim Usman Gafai[3]* carried out the research and Dr. Ahmed Salisu[1] supervised the research and drafted the manuscript. All authors reviewed and edited the manuscript and approved the final version of the manuscript.

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