Effect of Preparation Method on the Capture and Release of Biologically Active Molecules in Chitosan Gel Beads

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ABSTRACT: Model bioactive compounds with different molecular and functional characteristics were entrapped in gel beads prepared by polyelectrolyte complexation of chitosan and pentasodium tripolyphosphate (TPP). Three compounds of interest to the food and biomedical industries were tested: (1) lysine, (2) bovine serum albumin (BSA), and (3) β-galactosidase. Effects of the compound concentration in the initial chitosan solution, pH of the curing solution, and length of the curing phase on the capture efficiency were evaluated. Release rates for lysine and BSA into a phosphate buffer, distilled water, and a synthetic ocean solution were observed, and the activity of the entrapped β-galactosidase was determined. The capture efficiencies for lysine and BSA decreased as the concentration increased. The capture efficiency for lysine ranged from 90% at pH 5 to 20% at pH 8.6. There was no significant effect of the release media on the rate of release. BSA and lysine release reached 90% of the maximum after 100 and 50 min, respectively. The capture efficiency of β-galactosidase was not affected by the pH of TPP; however, enzyme activity in the beads decreased as the pH increased. Beads prepared in the pH 8.6 TPP solution had significantly higher rates of enzyme release over time. © 2002 John Wiley & Sons, Inc. J Appl Polym Sci 84: 405–413, 2002; DOI 10.1002/app.10393

Key words: biopolymer; microencapsulation; enzymes; gels; polyamines

INTRODUCTION

Entrapment of bioactive molecules, such as enzymes, drugs, vitamins, and peptides, in stabilizing matrices has been practiced by the pharmaceutical, food, biomedical, chemical, and waste-treatment industries for many years. Although specific requirements are industry- and application-dependent, general desirable attributes of an entrapment matrix include low cost, ease in handling, abundance, and versatility. Over the past decade, the biopolymer chitosan has attracted interest as a potential matrix for the immobilization or controlled release of cells and numerous bioactive compounds.[1,2]

Chitosan, a polyamine produced primarily from shellfish chitin, is inexpensive, nontoxic, and forms gels readily.[3–5] Recent research has focused primarily on its use in microbead drug-delivery and enzyme-immobilization systems. Chitosan microspheres crosslinked with glutaraldehyde have been successfully prepared for the immobilization of urease, tannase, and peroxidase,[6–8] and the controlled release of progesterone, mitoxantrone, 5-fluorouracil, and methotrexate.[9–12]

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However, due to its toxicity, glutaraldehyde is not appropriate for some chitosan microsphere applications.[13] Another method of microsphere preparation relies on the electrostatic attraction between polyelectrolytes in an aqueous solution. Once dissolved in a weakly acidic solution, the polycation chitosan forms ionic crosslinks with polyanions such as sodium tripolyphosphate (TPP), xanthan, and alginates. This ionotropic gelation of chitosan has been used in the immobilization of *Amaranthus tricolor* and *Corynebacterium glutamicum* cells and in the controlled release of indomethacin and 6-mercaptopurine.[14–17]

Various studies have been undertaken to describe the effects of several process variables on the controlled release of single compounds, usually drugs. Drug capture and release characteristics of chitosan beads prepared using the ionotropic gelation method were shown to be influenced by the concentration and molecular weight of chitosan, pH, and the ionic strength of the polyanionic solution.[16,17] The first objective of the study reported here was to evaluate the effects of several processing variables on the entrapment capacity of chitosan gel beads produced by ionotropic gelation. This method was chosen because the mild processing conditions of ionotropic gelation were the least damaging to the biological molecules encapsulated. The second objective was to observe the effects of the release media properties on the release rate of three model compounds with different characteristics. A third objective was to determine the effect of the entrapment process on the function of the entrapped molecule. Entrapment or immobilization of an enzyme within a matrix is a useful system for reuse or protection of an enzyme; however, the entrapment process can result in the loss of enzyme activity or function. The functionality of one of the entrapped molecules, an enzyme, was quantified. Three compounds of interest to the food and biomedical industries were tested: (1) the basic amino acid lysine; (2) bovine serum albumin (BSA), a medium molecular weight protein commonly used as a model protein; and (3) β-galactosidase, an enzyme which cleaves lactose into glucose and galactose.

The long-term goal of this research is to understand the physical factors that affect release and activity of a variety of biological compounds from chitosan beads and to use this information to design chitosan bead controlled-release systems. Previous reports demonstrate the effect of processing conditions on entrapment and release of a single compound from chitosan matrices, but this is the first study to investigate a variety of types of biological molecules under the same system.

EXPERIMENTAL

Materials

High molecular weight chitosan (average molecular weight 1,000,000) prepared from crab or shrimp shells was obtained from the Aldrich Chemical Co. (Milwaukee, WI). BSA, L-lysine monohydrochloride, methylamine hydrochloride, dansylchloride, and β-galactosidase (EC 3.2.1.23; from *Aspergillus oryzae*, activity of 9.6 units/mg) were purchased from the Sigma Chemical Co. (St. Louis, MO). HPLC-grade acetonitrile and water were purchased from EM Science (Gibbstown, NJ). Food-grade TPP was donated by the FMC Corp. (Lawrence, KS). All other chemicals were of reagent grade or higher and were purchased from EM Science or Fisher Scientific (Fair Lawn, NJ).

Preparation of Chitosan Microspheres

The chitosan gel beads were made using the ionotropic gelation method.[18] Chitosan (1.5 g) was dissolved in 100 mL of 3% (v/v) acetic acid and extruded dropwise through a syringe equipped with a 21-gauge hypodermic needle into 30 mL of a 15% (w/v) TPP solution (Fig. 1). Beads were extruded at a rate of 60 beads/min from a height of 7 cm. The pH of the TPP solution was adjusted with dilute hydrochloric acid to produce three curing solutions of pH of 5, 7, and 8.6. Gel beads were formed immediately upon contact with the TPP curing solution. After a specified time period, the beads were removed from the TPP and rinsed in distilled water. The size distribution of the microspheres was determined by manual measurement with a vernier caliper. The viscosity of the chitosan solutions prior to microsphere preparation was determined using a rheometer (Bohlin CS 50 CVO).

BSA and Lysine Entrapment

The compound of interest (BSA or lysine) was dissolved in 3% acetic acid under gentle stirring at three concentrations: 0.05, 0.5, and 5.0% (w/v). Finely ground chitosan was added to the solution at a concentration of 1.5%, and the solution was stirred with a magnetic stirrer until the chitosan

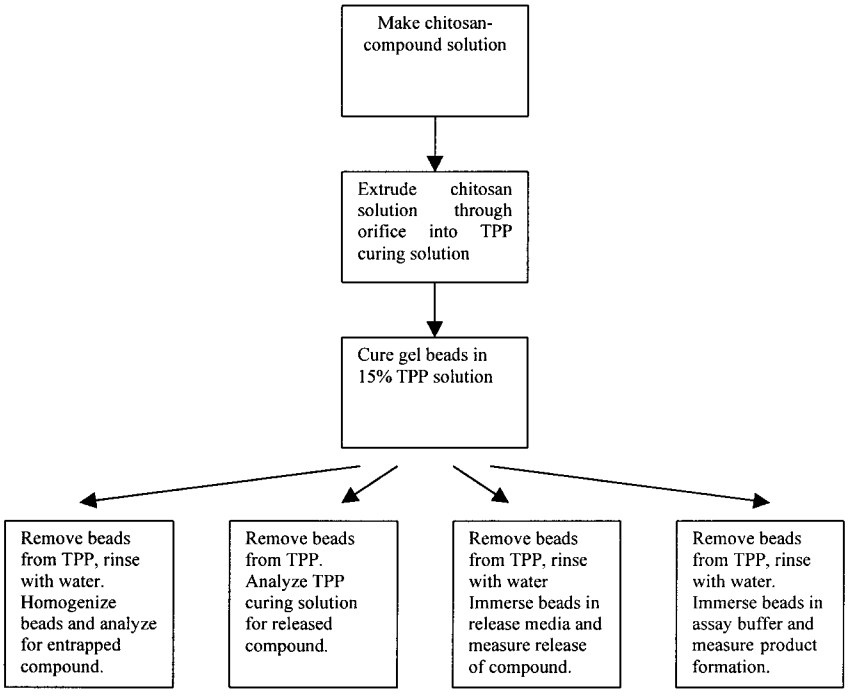

Figure 1 Flow diagram of experimental design.

was dissolved, approximately 20 min. Beads were made in the manner described above, using a controlled volume of the chitosan–BSA or chitosan–lysine solution. Each capsule was counted as it was formed. After gelation in the TPP curing solution (pH 5, 7, or 8.6), the gel beads were removed, rinsed briefly with distilled water, and then homogenized in 5 mL of 2*N* HCl. The homogenate was analyzed for the entrapped compound and the entrapment capacity determined. The capture efficiency (% CE) of each compound was determined by dividing the actual amount of the compound analyzed in the bead by the amount of the compound in the chitosan solution prior to bead formation. Each experiment was conducted in triplicate. One-way analysis of variance ($P < 0.05$) was used to detect differences in the bead size among treatments.

Release During Curing

The release rate of the encapsulated compound from the gel matrix into the TPP solution during curing of the microspheres was determined. The compound release was measured after 0.5, 1, 5, 10, 20, 60, and 120 min of curing in the TPP solution. For the chitosan–BSA microspheres, the beads were sampled at the specified time periods and analyzed for the BSA remaining in the microsphere. Lysine release was determined by sampling and analyzing the TPP curing solution. The microspheres were made using the same method as described above.

Release into Selected Media

Release rates were determined for BSA and lysine into three different media with diverse potential applications: sodium phosphate buffer (0.2*M*, pH 7.8), distilled water (pH 5.8), and a synthetic ocean solution (29.5 g/L, pH 8.8; CrystalSea®; Marine Enterprises International, Baltimore, MD). Based on the results of the previous entrapment studies, it was decided to prepare microspheres at pH 5, using 1% of BSA or lysine. Five-milliliter aliquots of the chitosan–BSA or chitosan–lysine solution were made into microspheres, removed from TPP after a 10-min curing phase, and then resuspended into 100 mL of the selected release medium. The beads and release media were stirred at a constant rate of 100 rpm and maintained at room temperature (~25°C). Samples of the release medium were removed and analyzed at time intervals of 0.25, 0.5, 1, 2, 4, 8, and 12 h until the maximum release was reached.

BSA and Lysine Analyses

The concentration of BSA in the microspheres was determined by the Lowry protein assay.[19]

Table I Viscosity and pH of a 1.5% Chitosan Solution, with Various Concentrations of BSA or Lysine Added

Measurement	Chitosan Control	BSA Concentration			Lysine Concentration			
		0.05%	0.5%	5.0%	0.05%	0.5%	1%	5%
Viscosity (cP)	3250	550	250	110	2800	2300	1600	300
pH	3.55	3.49	3.55	3.98	3.69	3.63	3.75	3.61

Lysine concentration in the TPP and different media was determined by reversed-phase high-performance liquid chromatography (HPLC) following precolumn derivitization with 5-dimethylaminonaphthalene-1-sulfonyl chloride.[20] The derivitized samples and standards were filtered (0.45 μm) and then injected (10 μL) with an autosampler into a Hewlett–Packard (Burlington, MA) Model 1050 HPLC, using a Spherisorb ODS column (Phenomenex; Torrance, CA) at ambient temperature. The mobile phase (pH 7) was acetonitrile/0.01*M* potassium phosphate buffer (50:50). Absorbance was monitored at 254 nm with a photodiode array detector (HP Model 1050). Quantitative determination of lysine in the samples was evaluated by comparing the peak area of the samples with dansylated lysine standards having concentrations of 1, 2, 3, and 6 μg lysine/mL.

β-Galactosidase Entrapment

The process of immobilizing or entrapping an enzyme within a support matrix can destroy the enzyme or reduce its activity or function. Additionally, a successfully entrapped enzyme may leak out of the matrix. A study of the immobilization and the resulting activity of the enzyme β-galactosidase was undertaken to determine if the encapsulation process and conditions affect the activity of the encapsulated molecule. Beads were made as previously described and extruded into a TPP solution of pH 5, 7, and 8.6. Beads were cured in TPP for 10 min as determined by the release-during-curing studies. One initial enzyme concentration, 200 units of β-galactosidase per milliliter of the chitosan solution, was used. After extrusion and curing, the amount of the enzyme remaining in the bead was measured as total protein, and the capture efficiency of β-galactosidase was determined. The enzyme powder initially added to the chitosan solution was found to be approximately 13% protein, based on the Lowry protein assay. The enzyme activity of the β-galactosidase–chitosan beads was assayed immediately after bead formation, using *O*-nitrophenyl β-D-galactopyranoside (ONPG) as the substrate.[21] The beads were immersed in the 25°C, pH 7.3, assay buffer and subjected to gentle stirring at 100 rpm, and the hydrolytic formation of free nitrophenol was measured spectrophotometrically at 405 nm. After measurement of the enzyme activity was completed, the beads were removed from the assay buffer and reanalyzed for total protein content to determine the extent of leakage of the enzyme from the beads into the assay buffer.

RESULTS AND DISCUSSION

This method of microsphere preparation resulted in uniformly spherical beads with an average diameter of 3.04 mm with a 0.13 mm standard deviation (n = 12). The beads were stable in water for at least 1 week and stable for at least 3 days in pH 5 and 7 TPP. In pH 9 TPP, the beads were observed to degrade rapidly over a period of several hours.

Chitosan Solution Properties

It was expected that the viscosity and pH of the chitosan solutions would affect the capture efficiency and formation of the gel beads. Thus, the effects of BSA and the lysine concentration on the viscosity and pH of the resultant chitosan solutions were measured (Table I). As expected, the viscosity of the chitosan solutions decreased with increasing concentrations of BSA and lysine, with BSA having a stronger effect on the solution viscosity than had lysine.

Capture Efficiency

During the chitosan bead formation process, some of the entrapped compound may be destroyed or

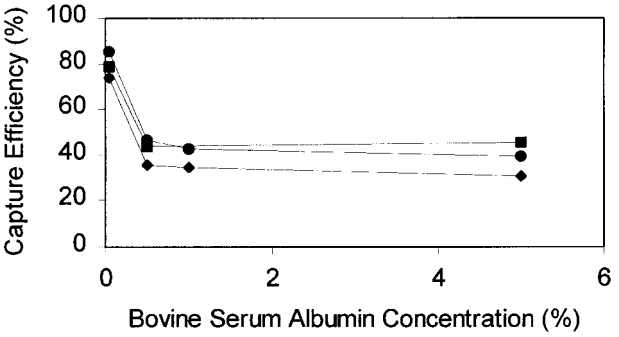

Figure 2 BSA capture efficiency as a function of solution concentration (0.05, 0.5, 1.0, and 5% BSA) and pH of the TPP curing phase (average of three experiments): (♦) pH 8.6; (■) pH 7; (●) pH 5. Standard error ranged from 1.60 to 6.65, 0.46 to 10.1, and 0 to 3.8 for pH 8.6, 7, and 5, respectively.

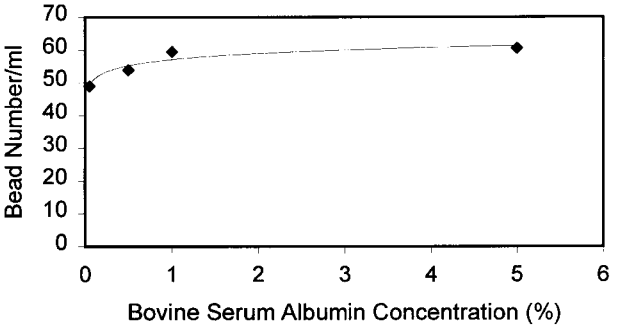

Figure 4 Number of beads formed per milliliter of chitosan–BSA solution with initial concentrations of 0.05, 0.5, 1.0, and 5.0% BSA. Each value is the average of three experiments and the standard error ranged from 0.82 to 1.42. One-way analysis of variance indicated a significant ($P < 0.05$) effect of the BSA concentration on the bead number.

lost. As it is desirable to minimize this loss, this project was undertaken to determine the optimal conditions for making chitosan gel beads with the highest capture efficiency of the desired compound. The capture efficiency of the desired compound was measured using the following equation:

$$\text{Capture efficiency (\%)} = (AQ/TQ) \times 100 \quad (1)$$

where AQ is the actual quantity of amino acid or protein in the bead, and TQ, the theoretical quantity (i.e., the initial loading concentration).

The capture efficiencies for BSA and lysine determined for varying the initial concentrations and pH of TPP are shown in Figures 2 and 3. The

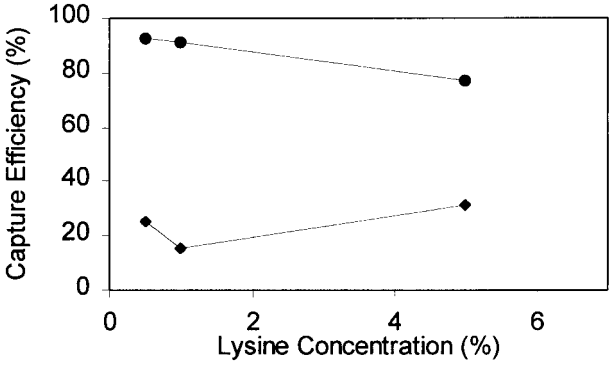

Figure 3 Lysine capture efficiency as a function of solution concentration (0.5, 1.0, and 5% lysine) and pH of the TPP curing phase (average of three experiments): (♦) pH 8.6; (●) pH 5. Standard error ranged from 1.80 to 4.40, and 0.7 to 1.85 for pH 8.6 and 5, respectively.

capture efficiencies for both compounds were found to be higher at lower initial concentrations and decreased as the initial concentration increased. This trend was more apparent for BSA than for lysine. The average capture efficiency for a 0.05% BSA solution was 80%, compared to 40–50% for a 5% solution. The lysine capture efficiency in pH 5 TPP was 95% for the 0.5% initial concentration and decreased to 80% at a 5% lysine concentration. The lower capture efficiency of BSA at high initial concentrations may be related to the fluid properties of the chitosan solution, which are known to affect the size and, thus, the surface area of a droplet extruded from an orifice. Lower solution viscosities resulted in smaller and more numerous beads formed from the same initial volume of the chitosan solution in comparison to a higher solution viscosity. The number of beads formed from the chitosan–BSA solution is shown in Figure 4. A larger number of beads were formed per unit volume at the higher BSA concentrations, giving a higher overall surface area available for the release of the dissolved compound during curing in TPP.

Capture efficiencies were also significantly affected by the pH of the TPP curing solution. The capture efficiencies of both compounds were lower when the beads were cured in TPP of higher pH. Bead formation at pH 8.6 resulted in the lowest capture efficiency of BSA and lysine. The effect of pH on the capture efficiency was stronger for lysine entrapment than for BSA. BSA and lysine are both positively charged in the acidic chitosan solution. During extrusion of chitosan into TPP, the positively charged lysine moved more readily

into the basic TPP than into the acidic TPP. Because of size effects, the lysine was able to move more freely into the basic medium in contrast to the slower diffusion observed with the larger BSA molecule.

Overall, very high capture efficiencies were obtained for lysine, reaching 90% at a pH of 5. The capture efficiencies of BSA were in the range of 40–50%. The exception was observed when beads were made with a low initial concentration (0.05% BSA in chitosan). At this initial BSA concentration, a high capture efficiency, 80%, was achieved. Other researchers observed similar capture efficiencies with other compounds. Sezer and Akbuga[22] were able to achieve very high capture efficiencies (90%) of piroxicam in chitosan matrices and observed an increase in drug loading with decreasing TPP pH. Another study[18] showed that the capture efficiency depended on the nature of the compound and the initial concentration in chitosan: Higher entrapment was achieved with water-insoluble compounds, and entrapment of the water-insoluble drug sulfadiazine increased with increasing initial concentration.

Release During Bead Production

As it was anticipated that the loss of an encapsulated compound occurred during the curing phase, capture efficiency was measured as a function of time in the curing solution. The capture efficiencies of BSA and lysine as a function of time in the curing solution are shown in Figures 5 and 6, respectively. In both cases, the maximum capture efficiency occurred at the shortest time spent in TPP, indicating that loss was rapid and the

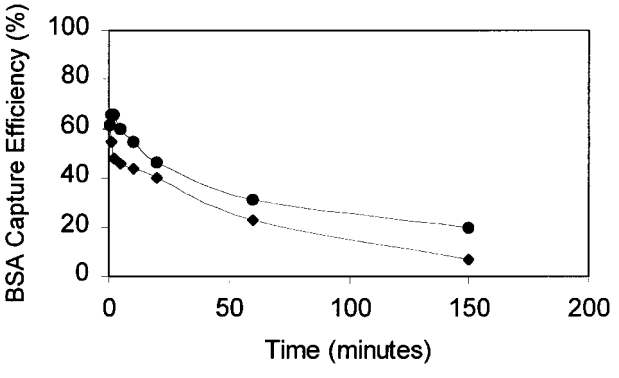

Figure 5 Effect of time in TPP on the capture efficiency of 0.5% BSA: (♦) pH 8.6; (●) pH 5. Standard error ranged from 0.94 to 6.7 and 0.23 to 2.46 for pH 8.6 and 5, respectively.

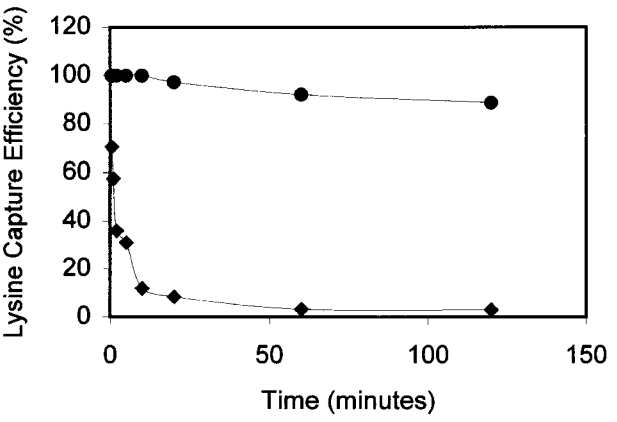

Figure 6 Effect of time in TPP on the capture efficiency of 0.5% lysine: (♦) pH 8.6; (●) pH 5. Standard error ranged from 1.18 to 6.79 and 1.58 to 6.2 for pH 8.6 and 5, respectively.

importance of minimizing the time spent in TPP. However, because the interaction of the negatively charged TPP with the positively charged amine group in the chitosan acted to crosslink the chitosan chains and form the matrix, insufficient contact with TPP resulted in a weakly formed bead. As seen during the capture efficiency studies, the pH of the TPP affected the release of both BSA and lysine. At pH 5, most of the lysine remained entrapped over a 2-h period, whereas at pH 8.6, the capture efficiency decreased rapidly from 80% to less than 20% in the first 20 min. After 2 h, over 95% of the lysine was released into the basic TPP. The release of BSA into the TPP was less dependent on the pH. After 2 h, 95% of the BSA was released into the pH 8.6 TPP and 80% of the BSA was released into the pH 5 TPP.

The amine groups of the chitosan are positively charged at pH 5, leading to the formation of ionic linkages with the TPP. At pH 8.6, the positively charged amine groups are converted to the unionized state, resulting in reduced ionic interactions with the TPP counterion. This may account for the weaker beads and higher release of entrapped compounds observed when curing in TPP of differing pHs.

The majority of chitosan-release studies reported in the literature utilized dried chitosan beads or glutaraldehyde crosslinked chitosan matrices, which differ from the gel beads in this study. Several of these studies also report a pH-dependent release. One study reported that the pH of the environment affected the release of thiamine hydrochloride from chitosan matrices, with the drug being released at a higher rate into

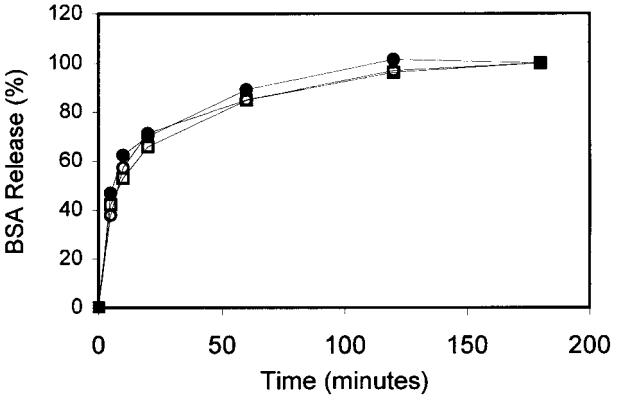

Figure 7 Release of 1% BSA from chitosan beads into various media: (○) water; (□) phosphate buffer; (●) simulated ocean solution. Standard error ranged from 0.07 to 2.49, 1.51 to 4.36, and 3.84 to 6.96 for water, the phosphate buffer, and the simulated ocean solution, respectively.

acidic medium than into basic medium.[23] Other researchers reported that water-soluble drugs (caffeine, salicylic acid, and quinidine) entrapped in chitosan by polyelectrolyte complexation were lost rapidly to the TPP phase, within a period of 15 min.[18] They also determined that by adjusting the pH of the TPP water-soluble compounds could be entrapped into chitosan beads at high capture efficiencies.

Release into Different Media

The release profiles for BSA and lysine from the chitosan beads are shown in Figures 7 and 8, respectively. Initial loading of BSA and lysine was determined to be 5 and 9 mg/mL, respectively, based on the capture efficiency of a 1% solution during a 10-min curing time in pH 5 TPP. There was no difference in the rate of release of BSA into water (pH 5.8), phosphate buffer (pH 7.8), or artificial ocean (pH 8.8). The BSA released reached 90% of the maximum after a period of 100 min. The release profile for lysine was steeper than that for BSA. The rate of release of lysine was higher, with 90% of the maximum released occurring in less than 50 min. Possible explanations are the higher concentration gradient driving the diffusion of lysine (as lysine entrapment efficiency was much higher, the lysine beads had a higher actual lysine concentration) or the increased mobility of lysine in the chitosan matrix as compared to BSA. For both BSA and lysine, there did not appear to be a significant dependence of the release rate on the release medium. This was somewhat unexpected, as it was observed that the release into TPP was pH-dependent and the release media differed in pH. However, other factors such as degradation or swelling of the chitosan matrix may have also affected the release. Although it was expected that the release into the more acidic medium (water) would be slower, the osmotic pressure in the pure water was greater than that of the phosphate buffer and may have driven the BSA or lysine out of the bead, offsetting the pH effects. The pH and ionic strength-controlled swelling of dried chitosan beads was shown to affect the release of entrapped compounds.[24] Swelling effects have not been studied with gel beads and may not be as significant as in the dried bead research studies, but still warrant further investigation. Overall, the complete release of BSA and lysine in less than 2 h was extremely rapid and may limit potential applications unless further optimization is undertaken.

The results reported in the literature for the release of compounds from chitosan matrices vary widely in the method of making the bead and the nature of the compound. These factors are relevant in evaluating the release kinetics from the chitosan beads. Gupta and Ravi Kumar[23] observed a slow release of thiamine hydrochloride from glutaraldehyde crosslinked, dried chitosan beads, less than a 20% release over a 72-h period in the maximum case, under unstirred conditions. Bodmeier and coworkers[18] found that for the water-insoluble drug, sulfadiazine, encapsulated in freeze-dried chitosan beads, 70–100% of the drug

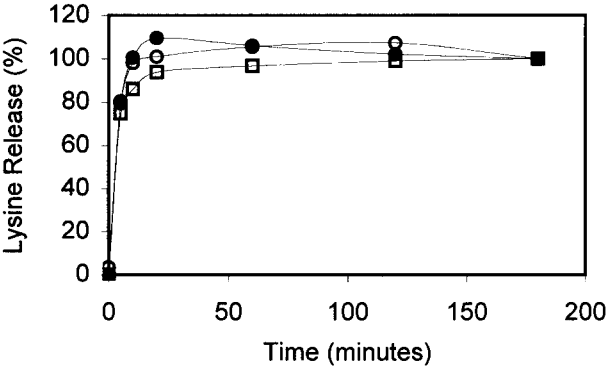

Figure 8 Release of 1% lysine from chitosan beads into various media: (○) water; (□) phosphate buffer; (●) simulated ocean solution. Standard error ranged from 2.04 to 5.41, 2.51 to 6.6, and 0.38 to 2.91 for water, phosphate buffer, and simulated ocean solution, respectively.

Table II Capture and Activity of β-Galactosidase in Chitosan Gel Beads

pH of TPP Curing Solution	Capture Efficiency (%)	Enzyme Activity in Beads (U/mL Original Chitosan)	Enzyme Activity in Beads (U/mg Protein Entrapped)	Protein Remaining in Bead (mg)
5	55.7	10.54	7.56	1.44
7	—	9.38	—	1.65
8.6	47.4	8.33	6.81	0.38

Each value is the average of duplicate experiments. Standard deviations were less than 5%. Initial enzyme loading of 200 U/mL chitosan, 77.5 U/mg protein, and 2.58 mg protein.

was released within 12 h depending on the initial content of the compound and the pH and concentration of the release medium. Mi and coworkers[16] investigated the release of 6-mercaptopurine from chitosan beads prepared in a manner similar to the methods presented in this article and found that maximum release was attained in as little as 2 h and as long as 20 h, depending on the molecular weight of the enzymatically hydrolyzed chitosan used and the anionic curing agent used.

Theoretical Treatment of Release

Modeling the controlled release of compounds from polymeric matrices can be employed to identify the nature of the release from the matrix. The power-law expression was developed by Higuchi[25] to describe dissolution and diffusion of a drug from a matrix and was extended by Ritger and Peppas[26] to the following form:

$$\frac{M_t}{M_\infty} = kt^n \tag{2}$$

where M_t and M_∞ represent the amount of the compound released at time t and the overall amount released, respectively. The term k is a release constant and n is an exponent indicative of the release mechanism. Information about the release mechanism can be gained by fitting the release data shown in Figures 7 and 8 (up to 60% release) to the above equation and determining the value of n. Different values of n can be compared to the values for various geometries reported by Ritger and Peppas.[26] For spheres, a value of n larger than 1.0 indicates zero-order release, a value of n between 0.43 and 1.0 indicates anomalous (non-Fickian) release, and a value of n less than 0.43 indicates a compound release due to Fickian diffusion. The release data shown in Figures 7 and 8 were fit to eq. (2), and the constants were determined for the release of BSA and lysine from chitosan gel matrices into the three different media. In all instances, the value of n determined from the data was less than 0.43, indicating the primary mechanism of release for all systems studied was through Fickian diffusion, suggesting that release of the molecules occurred without alteration in the structural properties of the matrix.

Enzyme Immobilization

The results of the β-galactosidase experiments are presented in Table II. The capture efficiency was found to be approximately 50% for both pH 5 and 8.6, a result that was not strongly affected by the pH of the TPP. These results were very similar to the capture efficiency trends observed for BSA under similar conditions. The activity of the bead-immobilized enzyme was related to the initial volume of the solution used to make the beads, shown in column 3 of Table II. The enzyme activity per initial solution volume was highest in the beads made at pH 5, reaching 10.54 U/mL, and decreased to 8.33 U/mL in beads made at a pH of 8.6. The total activity was normalized to the protein content in the beads and compared to the initial activity per protein in the chitosan solution. Data available for the beads made in pH 5 and 7 TPP indicate that the enzyme activity per milligram protein remaining was approximately 10% of the initial activity. The results of the previous experiments indicated that protein is released from the bead when placed in an aqueous solution. This represents a potential problem in immobilized enzyme applications where it is desirable for the enzyme to remain in the bead. For the results shown in Table II, it is possible that the enzyme leaked into the buffer solution and catalyzed the conversion of ONPG in the buffer. To determine if the enzyme leaked from the

beads, the protein remaining in the bead was measured after the time spent in the assay buffer (20 min). For beads made at a pH of 5 and 7, there appeared to be minimal leakage from the bead into the enzyme buffer. This was not the case for the beads made at pH 8.6, for which the capture efficiency decreased from 47.4% immediately after the beads were made to 14.7% after the 20 min spent in the assay buffer.

In comparing the remaining enzyme activity on a volume chitosan solution basis to a protein basis, the enzyme activity per milliliter chitosan solution was 5% of the initial solution, while the enzyme activity per milligram protein was 10% of the initial solution. This indicates that there was some loss of volume during the bead-making process, most likely due to release of acetic acid from the chitosan solution into the TPP.

CONCLUSIONS

In conclusion, gel chitosan beads were determined to represent a potential matrix for the immobilization and controlled release of a variety of biological molecules that had not previously been studied together in the same system. Processing conditions were mild so as not to destroy these biological molecules and were found to affect the final characteristics of the matrix-entrapped compound complex, as summarized here:

- The pH of the TPP curing solution affected the capture efficiency of the compound. pH may influence the charge of chitosan and subsequently its interaction with other molecules. The pH 5 TPP resulted in the highest capture efficiencies of lysine and BSA. More lysine and BSA were released into the pH 8.6 TPP over time than was released into the pH 5 TPP over time.
- The different release media did not affect the rate of release of entrapped BSA or lysine from chitosan beads. Lysine was released into all media at a faster rate than BSA was released.
- Release of lysine and BSA into the release media followed the Fickian diffusion model.
- β-Galactosidase was successfully entrapped in chitosan beads and remained active in this state, as was demonstrated by conversion of the ONPG substrate. The pH of the curing solution did not have a strong effect on the capture efficiency of β-galactosidase.
- The pH of the TPP curing solution affected the retention of the β-galactosidase enzyme within the beads when placed in the ONPG assay buffer. Beads made at pH 8.6 showed a loss of β-galactosidase during the activity assay, as determined by protein measurements immediately following bead making and immersion in the assay buffer.

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