Journal of Applied Polymer Science

# *In Vitro* Evaluation of Spray-Dried Chitosan Microspheres Crosslinked with Pyromellitic Dianhydride for Oral Colon-Specific Delivery of Protein Drugs

**Iman Kavianinia,[1] Paul G. Plieger,[1] Nadia G. Kandile,[2] David R. K. Harding[1]**

[1]Department of Chemistry, Institute of Fundamental Sciences, Massey University, Palmerston North, New Zealand

[2]Department of Chemistry, Faculty for Women, Ain Shams University, Heliopolis, Cairo, Egypt

Correspondence to: I. Kavianinia (E-mail: i.kavianinia@massey.ac.nz)

**ABSTRACT:** Chitosan microspheres have been prepared using a spray-drying method, and crosslinked with pyromellitic dianhydride. The chemical structure of the modified chitosan was characterized by FTIR spectroscopy and solid state 13C NMR analysis. The particle size and morphology of the crosslinked chitosan were investigated. These microspheres were evaluated for colon-specific delivery of bovine serum albumin (BSA) as a model protein drug. The results indicate that the drug was released as follows: 37.1 ± 2.8% after 2 h in SGF, 73.1 ± 4.8% after 8 h (2 h in SGF+ 6 h in SIF), and 80.9 ± 4.1% after 12 h in SCF. The effect of $\beta$-glucosidase on the drug release was also examined. The encapsulation efficiency was decreased from 88.4 ± 3.1% to 62.8 ± 2.9%, with increasing BSA concentration. Loading capacity was significantly increased from 6.3 ± 0.3% to 41.8 ± 4.1% by increasing the initial BSA concentration. © 2014 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2014**, *131*, 40514.

**KEYWORDS:** drug delivery systems; polysaccharides; swelling

Received 21 November 2013; accepted 27 January 2014

**DOI: 10.1002/app.40514**

## INTRODUCTION

Therapeutic peptides and proteins have attracted the attention of scientists because of their great potential for treatment of numerous diseases as a result of their exquisite selectivity and their ability to provide effective and potent action and fewer side effects.[1] Oral delivery is by far the most widely used route of administration because of its simplicity and convenience, although injection remains the most common means for administering therapeutic protein and peptide drugs.[2–4] Overcoming obstacles such as acid-catalyzed degradation and extensive hydrolysis of protein and peptide drugs in the gastrointestinal tract requires the efficient delivery of these drugs into the systemic circulation.[5] Pharmaceutical researchers have tried various strategies to overcome such obstacles and to develop effective peptide and protein drug delivery systems. In recent years, delivery of therapeutic peptides and proteins into systemic circulation through colonic absorption has attracted great interest because of the relatively low activity of proteolytic enzymes in the colon.[6,7] Among the various approaches for colon-targeted drug delivery, using biodegradable polymers such as chitosan, which is degraded by the colonic microflora, holds great promise.[8,9] Chitosan microspheres as drug carriers for site specific delivery of drugs have been prepared using different techniques such as spray-drying, solvent evaporation, and ionic/covalent crosslinking.[10,11] Lorenzo et al. prepared chitosan microspheres for delivery of sodium diclofenac using a spray drying technique. These microspheres were enteric coated with Eudragit L-100 or Eudragit S-100. No release was seen in acidic pH for 3 h, but at higher pH Eudragit dissolved and the swelling of chitosan led to continuous drug diffusion which was complete in 4 h.[12] Chitosan crosslinked with sodium cellulose sulfate and sodium polyphosphate have also been prepared and loaded with 5-aminosalicylic acid (5-ASA) as a model drug.[13] Mura et al. designed *N*-succinyl-chitosan microparticles using spray drying for colon-specific delivery. In their fluids simulating gastric content (pH 2.0), the 5-ASA released was less than 10% during the first 2 h, whereas at pH 7.4 the drug release after 4 h reached to 51%.[14]

Modification of chitosan is a common method to prevent rapid release of drugs. Drug release from unmodified chitosan is compromised due to the swelling and subsequent rapid dissolution of chitosan.[15] Therefore, many investigations have been pursued to develop new reagents to crosslink chitosan.[8]

Our group has recently developed a new generation of crosslinked chitosans (Cts) using dianhydrides as the crosslinking agent.[20] Herein, we examined for the first time the ability of a chitosan microsphere crosslinked with pyromellitic dianhydride

(PMDA) for controlled drug release. In this study, bovine serum albumin (BSA) as a model protein drug was used to investigate the drug release behavior of spray-dried Cts-PMDA microspheres. The effect of initial BSA concentration in encapsulation efficiency (EE) and loading capacity (LC) was also investigated.

## MATERIAL AND METHODS

### Materials

Chitosan (Cts) and bovine serum albumin (BSA) were purchased from Acros Organics. Pyromellitic dianhydride (PMDA) and $\beta$-glucosidase from almonds (8.1 U/mg) were purchased from Sigma–Aldrich.

### Preparation of the Spray-Dried Chitosan Microspheres

Chitosan solution (1.5%, w/w) was prepared by dissolving 10 g of chitosan in an aqueous solution of acetic acid (2%, v/v) at room temperature. Subsequently, the completely dissolved chitosan solution and air were passed separately to the nozzle of a spray dryer at a feed rate of 9 mL/min in a Büchi-B190 spray dryer (Büchi, Switzerland). The inlet temperature was controlled at 140°C. The chitosan microspheres were collected from the cyclone of the spray dryer.

### Crosslinking of the Spray-Dried Chitosan Microspheres

A total of 1.5 g of the spray dried chitosan microspheres (0.0093 mol of glucosamine residues) were dispersed into 40 mL of acetic acid for 10 min then dimethylformamide containing 4 eq PMDA (8.12 g) was added and heated at 130°C. Crosslinked chitosan microspheres were washed with methanol and 0.1*N* NaOH followed by rinsing with ethanol to remove NaOH and vacuum dried to evaporate ethanol.

### Characterization

The average molecular weight ($M$) of the chitosan was determined by the viscometric method. Chitosan samples were prepared in 0.1*M* acetic acid/0.2*M* sodium chloride aqueous solutions. The relative viscosity ($\eta$) of chitosan solutions were measured using a U Cannon-Fenske routine viscometer (Cannon Instrument Co., State College, PA) at 25°C.

The average molecular weight ($M$) of chitosan was calculated from the intrinsic viscosity by Mark–Houwink–Sakurada's empirical equation[21]:

$$[\eta] = kM^a$$

where $k = 1.81 \times 10^{-3}$ and $a = 0.93$ in the prepared solution at 25°C.

Elemental analysis was performed using a Carlo Erba Elemental Analyser EA 1108 using a flash combustion technique. The analyses were carried out at the Campbell Microanalytical Laboratory, Otago University, Dunedin, New Zealand.

Crosslinking degree was determined by elemental analysis on the basis of the percentage of nitrogen in the product by the following equation[22]:

$$\frac{(\mathrm{N}-7.7)}{\left(\frac{938}{143+\mathrm{M}}+\frac{462}{185+\mathrm{M}-7.7}\right)}\times 100$$

where $N$ is the percentage of nitrogen content and $M$ is the molecular weight of crosslinking agents.

FTIR spectra were recorded using a Nicolet 5700 FTIR Spectrophotometer. Cts and Cts-PMDA microsphere samples were prepared by processing compressed KBr disks.

Solid-state carbon-13 ($^{13}C$) magic angle spinning (MAS) NMR spectra were obtained at a $^{13}C$ frequency of 50.3 MHz on a Bruker (Rheinstetten, Germany) DRX 200 Mhz spectrometer. Crosslinked chitosan microspheres were subjected to particle-size distribution analysis using a particle size analyzer (Mastersizer 2000, Malvern Instruments Ltd, Malvern, Worcestershire, UK).

The morphological features of the microspheres were examined by an FEI Quanta 200 Scanning Electron Microscope (Eindhoven, The Netherlands) at an accelerating voltage of 20 kV. The samples were mounted onto stubs using a double sided adhesive tape and sputter coated with gold.

### Determination of the Swelling Behavior of the Microparticles

The dry microspheres (50 mg) were immersed in both simulated gastric fluid (SGF, pH 1.2) and simulated intestinal fluid (SIF, pH 7.4). In addition a test was carried out for 2 h in SGF, followed by 6 h in SIF and then 12 h in simulated colonic fluid (SCF, pH 7) to simulate the swelling behavior of microspheres in the gastrointestinal tract. At predetermined time, the swollen samples were collected with a centrifuge, blotted with filter paper for the removal of the absorbed water on the surface, and then weighed immediately. The percentage of swelling $S$ (%) of microspheres in the media was then calculated from the formula[23]:

$$\mathrm{S}(\%) = \left[\frac{\mathrm{W_s}-\mathrm{W_d}}{\mathrm{W_d}}\right]\times 100$$

where $W_s$ and $W_d$ are the weights of swollen and dried microsphere.

### BSA Loading and Release Experiments

A total of 500 mg of dried particles was soaked for 6 h in 5 mL of phosphate-buffer solution (PBS) at pH 7.4 containing various concentrations of BSA (0.1, 0.2, 0.4, 0.6, 0.8, and 1 mg/mL) for remote loading. Subsequently, excess surface BSA solution, if any, was removed using filter paper and the protein-loaded particles were dried at 4°C.

**Protein EE and LC.** Protein content inside the microparticles was determined by suspending 50 mg of particles in 5 mL phosphate saline buffer (pH 7.4) for 24 h. BSA-loaded microspheres were separated from the solution by centrifugation at 4000 rpm for 10 min. Then, the supernatant from the centrifugation was decanted carefully, and the protein content in the supernatant was analyzed by HPLC. All samples were analyzed in triplicate. The BSA LC and EE of Cts-PMDA microsphere were calculated according to the following formula:[24]

$$\mathrm{LC} = \left[\frac{\text{Total amount BSA} - \text{Free amount BSA}}{\text{Microparticle weight}}\right]\times 100$$

$$\mathrm{EE} = \left[\frac{\text{Total amount BSA} - \text{Free amount BSA}}{\text{Total amount BSA}}\right]\times 100$$

***In Vitro* Drug-Release Study.** For the BSA release experiments, 50 mg of drug-loaded microparticles were immersed in 5 mL of simulated gastrointestinal fluid (SGF, pH 1.2) and simulated intestinal fluid (SIF, pH 7.4) at 37°C. In addition, a test was carried out to

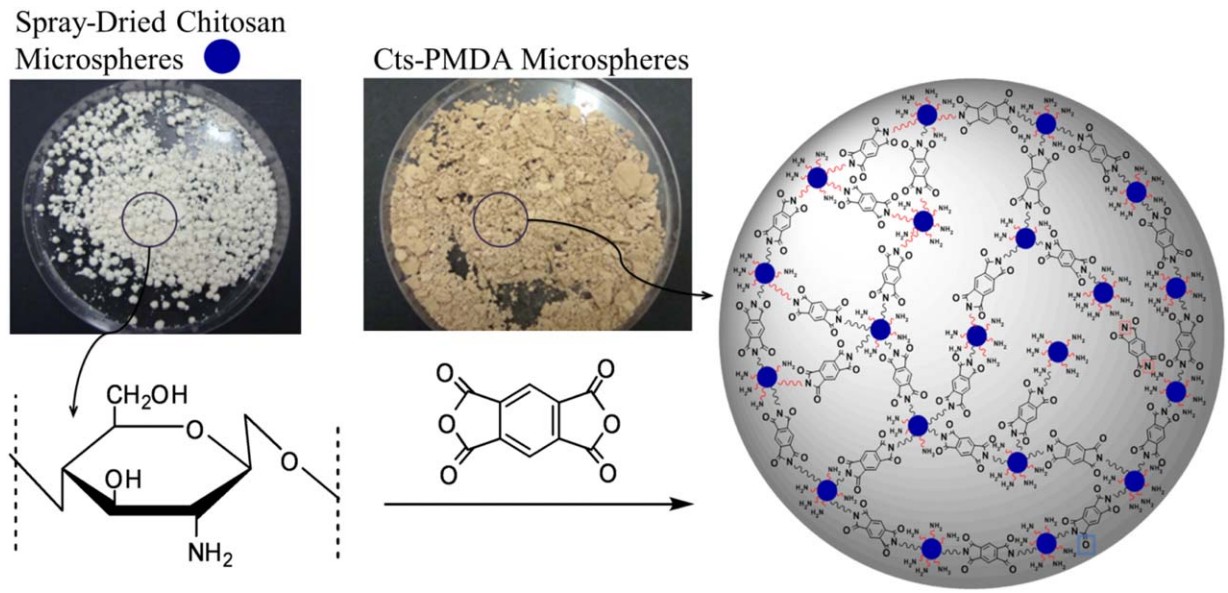


**Figure 1.** The schematic representation of Cts-PMDA microsphere. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

simulate passage through the GI tract. At first, the drug release was determined in SGF for 2 h, followed by 6 h in SIF, and then the matrix was transferred to simulated colonic fluid (SCF, pH 7) with and without $\beta$-glucosidase (0.02% w/v) for 12 h. At the desired times, an aliquot of sample (100 $\mu$L) was withdrawn and protein content was estimated by HPLC method. The dissolution medium was replaced with fresh buffer to maintain total volume after each withdrawal.

### HPLC Protein Analysis

The quantitative determination of BSA was determined using a high-performance liquid chromatography (HPLC) system

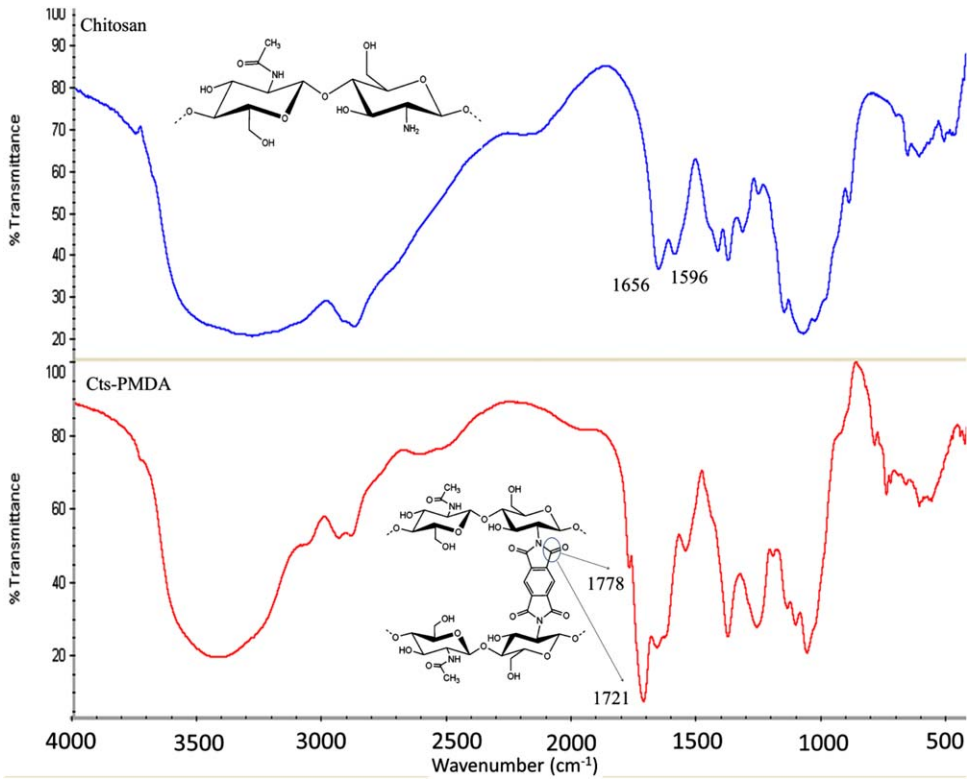


**Figure 2.** Fourier transform spectroscopy spectra of Cts and Cts-PMDA microsphere. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

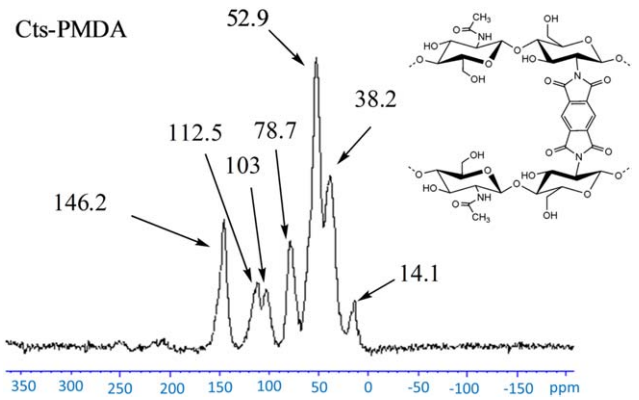


**Figure 3.** 13C DP-MAS spectra of chitosan-PMDA. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

consisting of a Waters 2690 Separation module and a Waters 996 photodiode Array detector, equipped with a Phenomenex® C18 reverse-phase column (250 × 4.6 $mm^2$, 5 $\mu$m). The separation was performed using a gradient between mobile phase A (water/acetonitrile, 90/10 v/v and 0.05% TFA) and mobile phase B (100% acetonitrile, 0.04% TFA).[25] The injection volume was 50 µL and elution was performed at a flow rate of 1 mL/min. The wavelength of the detector set at 280 nm. The peak corresponding to BSA has the retention time of 9.62 min. We observed linear calibration curves with correlation coefficients of 0.999.

### Statistics

All analyses were carried out in triplicate ($n = 3$) and were expressed as means ± SD. Analysis of variance was used to determine statistical significance. A difference was considered statistically significant if the $P$ value is less than 0.05.

## RESULTS AND DISCUSSION

### Preparation of the Spray-Dried Chitosan Microspheres

The degree of deacetylation of chitosan used in this study was calculated at 70% by elemental analysis (C, 43.8; N, 8.02; and H, 6.98). The approximate molecular weight of chitosan was also determined to be 162 kDa by the Mark–Houwink viscometry method.

The chitosan microspheres were crosslinked with PMDA to improve the stability of chitosan in the acidic medium of the stomach. Addition of dianhydride to chitosan led to the formation of the cyclic imide (Figure 1). The heterocyclic imide linkage offers excellent thermal, mechanical, and chemical stability to the chitosan.[22] The degrees of substitution (DS) was calculated 73% from elemental analysis (C, 46.1; N, 4.81; and H, 4.61). This high DS is related to the electron affinity of PMDA (Ea = 1.90 eV) which governs the reactivity of the dianhydride.[26]

The IR spectra for Cts and Cts-PMDA are compared in Figure 2. After crosslinking chitosan with PMDA, two new peaks appear at 1778 and 1721 $cm^{-1}$ which correspond to the characteristic symmetrical and unsymmetrical C═O stretching bands of the cyclic imide, respectively. The disappearance of the vibrational band corresponding to the primary amino group at 1596 $cm^{-1}$ further confirms that the chitosan had been modified.

To further validate the structure, the 13C DP-MAS spectrum of dianhydride crosslinked chitosan microsphere was obtained (Figure 3). Chitosan backbone related signals appear at 78.7(C4), 52.9 (C3/C5), and 38.2 ppm (C2/C6). The signal at 146.2 ppm is attributed to the carbonyl group carbon of the cyclic imide and signals at 112.5 and 103 ppm are attributed to the phenyl ring.

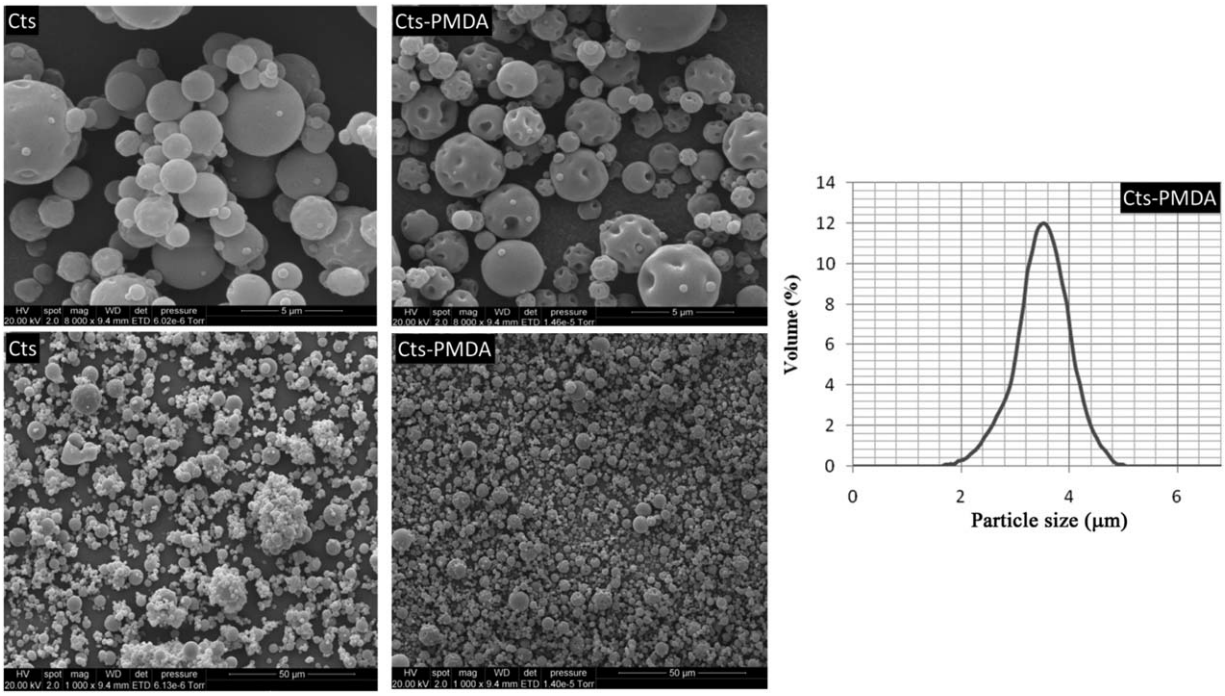


**Figure 4.** SEM images and size distribution of chitosan and Cts-PMDA microparticles.

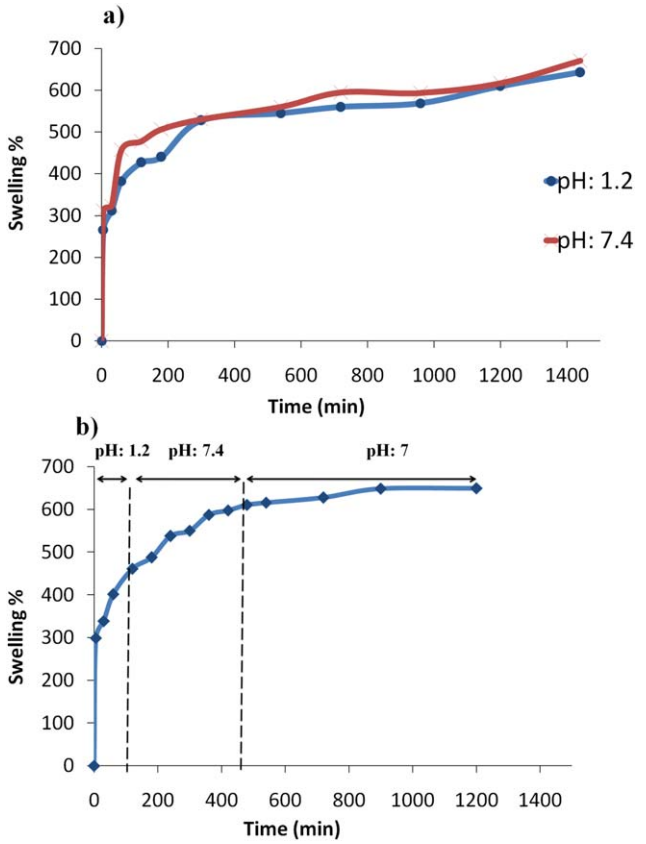


**Figure 5.** Swelling behavior of Cts-PMDA microspheres in (a) SGF and SIF (b) simulated gastrointestinal tract (2 h in pH 1.2, 6 h in pH 7.4, 12 h in pH 7). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Scanning electron microscopy (SEM) study by Wei et al. indicated that microspheres with a size of below 7.2 μm can be adsorbed in the gastrointestinal tract.[27] The particle-size distribution analysis of Cts-PMDA microspheres prepared in this study showed the average size of 3.55 μm, Figure 4.

SEM was used to visualize the surface morphology of the spray-dried microspheres as shown in Figure 4. The noncrosslinked microspheres were almost spherical in shape with a smooth surface. After crosslinking chitosan with PMDA, a well-defined change in the surface morphology of the spray dried chitosan microspheres was observed. The SEM photographs show that the surface roughness increased after crosslinking; however, the particles were still nearly spherical in shape.

### Swelling Studies

The swelling behavior of a hydrogel plays a crucial rule in controlling the rate of drug release from hydrogel networks. The swelling behavior of the Cts-PMDA microspheres was analyzed at pH 1.2 and 7.4. As shown in Figure 5(a), the crosslinked chitosan matrix produced a higher degree of swelling at pH 7.4 ($671.7 \pm 2.9\%$) than at pH 1.2 ($643.5 \pm 3.2\%$). This can be explained by the fact that at pH 1.2, the swelling is controlled mainly by repulsion of the protonated amino group on the C-2 carbon of the chitosan component.[28] Therefore, reducing the number of amino groups after crosslinking has led to a reduction in the swelling percentage of the matrix. Partial dissolution of the matrix at pH 1.2 also has an effect on this trend. For further simulating gastrointestinal tract conditions, the swelling characteristics of the Cts-PMDA microsphere were determined by immersing dried test samples to swell in a solution at pH 1.2 for 2 h, and subsequently in another solution at pH 7.4 for 6 h then 12 h in pH 7, Figure 5(b). Results also indicated that matrix swelled $650 \pm 4.2\%$ at the end of third step, Figure 5(a).

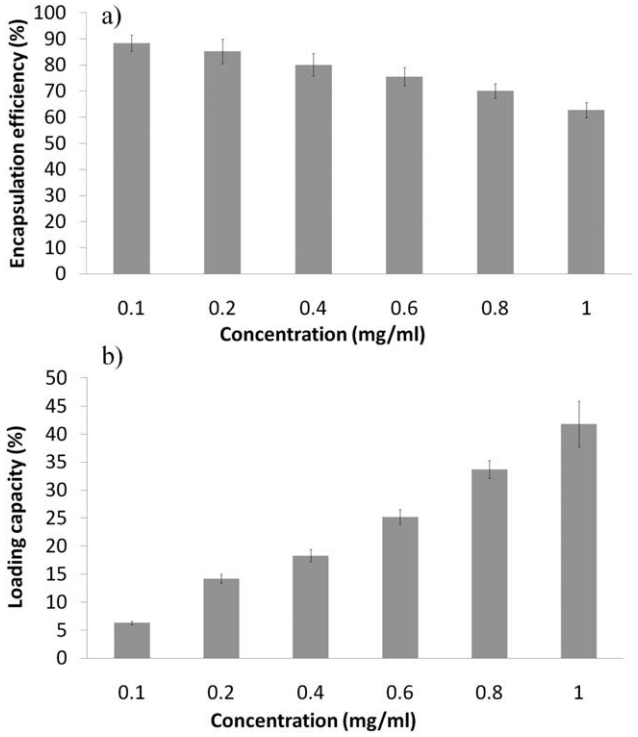


**Figure 6.** The influence of BSA initial concentration on (a) encapsulation efficiency and (b) loading capacity of Cts-PMDA microspheres.

### Microsphere EE and LC Study

The drug EE and LC was found to be dependent upon the initial BSA concentration. Cts-PMDA microspheres were loaded

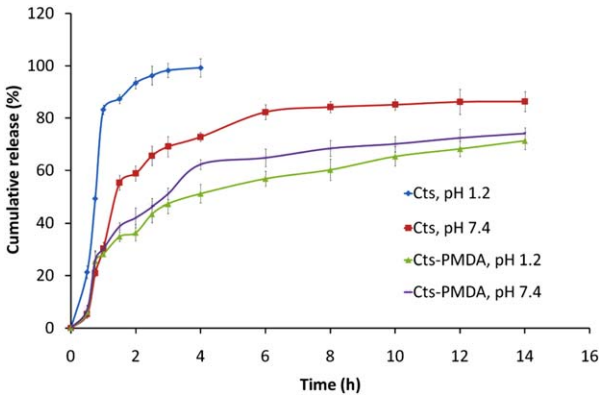


**Figure 7.** Effect of pH on cumulative release of BSA from Cts and Cts-PMDA microspheres. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

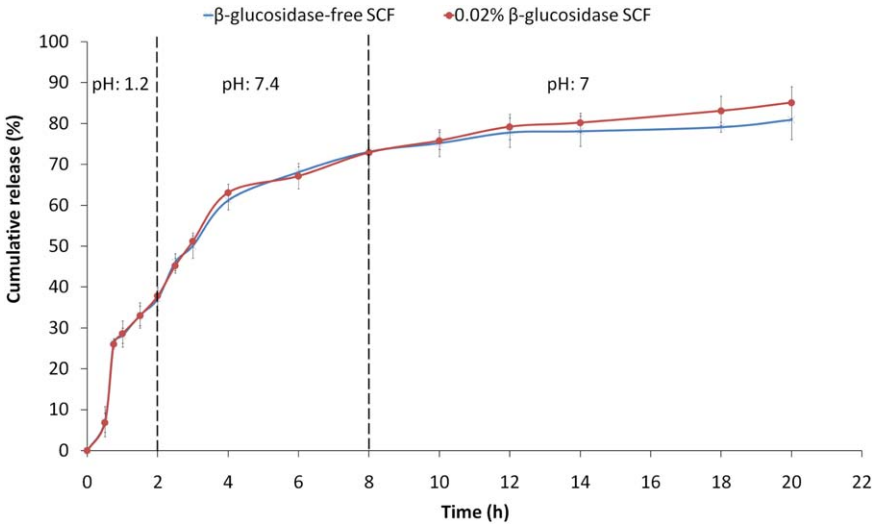


**Figure 8.** The BSA release profile in simulated gastrointestinal fluid for 2 h, followed by 6 h in simulated intestinal fluid and then 12 h in simulated colonic fluid. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

with different concentrations of BSA. It was observed that a higher initial concentration of BSA led to lower EE. Results showed that the protein EE decreased slightly from 88.4 ± 3.1% to 62.8 ± 2.9%, Figure 6(a), when the initial BSA concentration was increased. Similarly, the LC was also affected by the initial BSA concentration. However, the results indicate that the protein LC was enhanced dramatically from 6.3 ± 0.3% to 41.8 ± 4.1% by increasing the initial BSA concentration from 0.1 to 1 mg/mL [Figure 6(b)].

### *In Vitro* Release Study

*In vitro* BSA release tests in SGF and SIF were performed to provide a comparison between the release profile of the spray-dried microsphere with and without PMDA crosslinking. As shown in Figure 7, within the first 2 h, 93.4 ± 3.2% of loaded BSA was released at SGF (pH 1.2) from the unmodified chitosan microsphere. This observation can be attributed to the dissolution of the chitosan in the acidic media. These results indicate that the uncrosslinked chitosan fails to provide adequate retention of encapsulated proteins at stomach pH. On the other hand, the amount of BSA released from the crosslinked microspheres at SGF was relatively low; 36.4 ± 3.1% was released after 2 h. This delayed release characteristic could be explained as being due to the network of the crosslinked structures of Cts-PMDA controlling the release of BSA from the chitosan microspheres.

At SIF (pH 7.4), the amount of BSA released from Cts and Cts-PMDA was 82.3 ± 2.1% and 64.8 ± 3.4% after 6 h, respectively. These results also indicated that during the same length of time (2 h), 36.4 ± 3.1% of BSA was released from Cts-PMDA in SGF (pH 1.2), whereas 42.1 ± 3.8% of BSA was released in SIF (pH 7.4). This result can be attributed to the higher swelling percentage of the Cts-PMDA microspheres in pH 7.4 than at pH 1.2, which leads to the opening up of the pores and channels of the matrix. Within 1 h ~30% of the protein is released from the crosslinked chitosan microspheres at either pH 1.2 or 7.4. This initial burst is attributed to the release of BSA on or near the surface of the microspheres.

It is clear from Figure 7 that after 14 h less than 80% of BSA has been released in either acidic or basic media from the Cts-PMDA microspheres. This observation can be related to the entanglement of the BSA molecules within the hydrogel network which prevent release until the polymer matrixes are degraded.

To further simulate gastrointestinal tract conditions, the Cts-PMDA microspheres were incubated in a acidic pH (1.2) environment to simulate the retention time in the stomach, then subsequently in another solution at pH (7.4) to simulate the retention time in the small intestine for additional 6 h, followed finally by 12 h in simulated colonic fluid (pH 7). The results of this study are shown in Figure 8 which indicates that cumulative release from the Cts-PMDA microspheres was found to be 37.1 ± 2.8% after 2 h in SGF and 73.1 ± 4.8% at the end of 8 h (2 h in SGF+ 6 h in SIF). The results reveal that after 12 h in SCF 80.9 ± 4.1% of the drug was released.

The drug release in SCF was also evaluated in the presence of an enzyme. Because a similar degradation function of commercially available almond $\beta$-glucosidase occurs with chitosan as that of colonic enzymes, $\beta$-glucosidase was used to simulate the colonic medium.[29] When compared with the results where there was an absence of the enzyme at SCF (pH 7), after 12 h, the release of BSA was increased by almost 6% in the presence of the $\beta$-glucosidase, indicating that the enzyme had catalyzed the hydrolysis of the crosslinked chitosan.

## CONCLUSIONS

Chitosan microspheres crosslinked with the pyromellitic dianhydride intended for delivery of protein to the colon were successfully prepared. BSA as a model protein was then loaded into the microsphere by the diffusion filling method (remote loading). Altering the concentration of BSA from 0.1 to 1 mg/mL enhanced significantly the LC from 6.3 ± 0.3% to 41.8 ± 4.1% and decreased the EE from 88.4 ± 3.1% to 62.8 ± 2.9%. A number of factors such as time, pH, and enzyme availability were

investigated for their influence on the release of the BSA from the microspheres. The results from this study clearly show the potential of Cts-PMDA microspheres for drug delivery purposes.

## ACKNOWLEDGMENTS

The authors thank Dr. Jason Hindmarsh and Mr. David Lun (Massey University) for assistance and providing access to the solid-state NMR spectrometer and HPLC analysis, respectively.

## REFERENCES

1. Frokjaer, S.; Otzen, D. E. *Nat. Rev. Drug Discov.* **2005**, *4*, 298.
2. Morishita, M.; Peppas, N. A. *Drug Discov. Today* **2006**, *11*, 905.
3. Goldberg, M.; Gomez-Orellana, I. *Nat. Rev. Drug Discov.* **2003**, *2*, 289.
4. Zhang, Y.; Wei, W.; Lv, P.; Wang, L.; Ma, G. *Eur. J. Pharm. Biopharm.* **2011**, *77*, 11.
5. Markovsky, E.; Baabur-Cohen, H.; Eldar-Boock, A.; Omer, L.; Tiram, G.; Ferber, S.; Ofek, P.; Polyak, D.; Scomparin, A.; Satchi-Fainaro, R. *J. Control. Release* **2012**, *161*, 446.
6. Watts, P. J.; Lllum, L. *Drug Dev. Ind. Pharm.* **1997**, *23*, 893.
7. Renukuntla, J.; Vadlapudi, A. D.; Patel, A.; Boddu, S. H.; Mitra, A. K. *Int. J. Pharm.* **2013**, *447*, 75.
8. Bhattarai, N.; Gunn, J.; Zhang, M. *Adv. Drug Deliv. Rev.* **2010**, *62*, 83.
9. Chourasia, M.; Jain, S. *Drug Deliv.* **2004**, *11*, 129.
10. Yeo, Y.; Baek, N.; Park, K. *Biotechnol. Bioproc. E.* **2001**, *6*, 213.
11. Han, D.; Lei, H.; Zhao, K.; Zhu, L.; Li, X. *J. Wuhan Univ. Technol. Mater. Sci. Ed.* **2012**, *3*, 459.
12. Lorenzo-Lamosa, M.; Remunan-Lopez, C.; Vila-Jato, J.; Alonso, M. *J. Control. Release* **1998**, *52*, 109.
13. Wu, Q. X.; Yao, S. *J. Colloid Surf. B* **2013**, *109*, 147.
14. Mura, C.; Nacher, A.; Merino, V.; Merino-Sanjuan, M.; Carda, C.; Ruiz, A.; Manconi, M.; Loy, G.; Fadda, A.; Diez-Sales, O. *In. J. Pharm.* **2011**, *416*, 145.
15. Lee, R. J.; Tamm, T.; Temmer, R.; Aabloo, A.; Kiefer, R. *Synth. Met.* **2013**, *164*, 6.
16. Termsarasab, U.; Cho, H. J.; Moon, H. T.; Park, J. H.; Yoon, I. S.; Kim, D. D. *Colloid Surf. B.* **2013**,*109*, 280.
17. Mirzaei, B. E.; Ramazani, S. A. A.; Shafiee, M.; Danaei, M. *Int. J. Polym. Mater. Polym. Biomater.* **2013**, *62*, 605.
18. Dünnhaupt, S.; Barthelmes, J.; Iqbal, J.; Perera, G.; Thurner, C. C.; Friedl, H.; Bernkop-Schnürch, A. *J. Control. Release* **2012**, *160*, 477.
19. Rekha, M.; Sharma, C. P. *J. Control Release* **2009**, *135*, 144.
20. Kavianinia, I.; Plieger, P. G.; Kandile, N. G.; Harding, D. R. *Carbohydr. Polym.* **2012**, *87*, 881.
21. Rashid, T. U.; Rahman, M. M.; Kabir, S.; Shamsuddin, S. M.; Khan, M. A. *Polym. Int.* **2012**, *61*, 1302.
22. Kavianinia, I.; Plieger, P. G.; Kandile, N. G.; Harding, D. R. *Carbohydr. Polym.* **2012**, *90*, 875.
23. Shi, X. Y.; Tan, T. W. *Biomaterials* **2002**, *23*, 4469.
24. Zhang, H.; Oh, M.; Allen, C.; Kumacheva, E. *Biomacromolecules* **2004**, *5*, 2461.
25. Nicolas, J.; San Miguel, V.; Mantovani, G.; Haddleton, D. M. *Chem Commun.* **2006**, 4697.
26. Kim, B. S.; Bae, S. H.; Park, Y. H.; Kim, J. H. *Macromol. Res.* **2007**, *15*, 357.
27. Wei, W.; Wang, L. Y.; Yuan, L.; Yang, X. D.; Su, Z. G.; Ma, G. H. E. *Eur. J. Pharm. Biopharm.* **2008**, *69*, 878.
28. Berger, J.; Reist, M.; Mayer, J.; Felt, O.; Peppas, N.; Gurny, R. *Eur. J. Pharm. Biopharm.* **2004**, *57*, 19.
29. Zhang, H.; Alsarra, I. A.; Neau, S. H. *In. J. Pharm.* **2002**, *239*, 197.