Carbohydrate Polymers 98 (2013) 1366–1370

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Fabrication of distilled water-soluble chitosan/alginate functional multilayer composite microspheres

Congming Xiao*, Fei Sun

College of Material Science and Engineering of Huaqiao University, Quanzhou 362021, China

ARTICLE INFO

Article history:
Received 1 August 2012
Received in revised form 17 July 2013
Accepted 24 July 2013
Available online 6 August 2013

Keywords:
Water-soluble chitosan
Alginate
Polyelectrolyte complex
Microspheres
Layer-by-layer

ABSTRACT

Polysaccharides-based functional microspheres were fabricated under mild conditions. Firstly, magnetic alginate microspheres were prepared by emulsification/internal gelation and acted as substrates. Then the multilayer composite microspheres (MCM) were obtained through the layer-by-layer assembly of the distilled water-soluble chitosan and alginate. The components, morphology, and size distribution of the microspheres were characterized by element analysis (EA), X-ray photoelectron spectroscopy (XPS), scanning electron microscope (SEM) and laser particle size analyzer (LPSA). Both EA and XPS analysis results indicated that alternate immersion was an effective method for preparing MCM. Vibrating sample magnetometer, SEM and LPSA results showed that the microspheres had good dispersion, uniform particle size and were superparamagnetic. In addition, in vitro drug release behaviors of the microspheres were investigated by using hemoglobin (HB) and Coomassie brilliant blue G250 (CBB) as model drugs. It was found that the release rates of both HB and CBB from the composite microspheres were slower than those from the substrates.

1. Introduction

Both chitosan and alginate are naturally occurring linear polysaccharides. They are non-toxic, biocompatible, biodegradable and renewable. They are able to form physically cross-linked hydrogel via ionic cross-linking or self-assembly. The difference is that alginate is an anionic polysaccharide while chitosan is a cationic one. These unique properties make them suitable for biomedical, pharmaceutical and other applications (Balakrishnan & Jayakrishnan, 2005; Berger et al., 2004; Gu, Amsden, & Neufeld, 2004; Lee & Mooney, 2012; Pillai, Paul, & Sharma, 2009; Prashanth & Tharanathan, 2007).

Polymeric microparticles are regarded as a promising candidate to carry out the protection and controlled release of bioactive substances. Natural polymers such as alginate and chitosan are widely adopted to prepare microspheres (Tran, Benoît, & Venier-Julienne, 2011). Polyelectrolyte complexes (PEC) that achieved from water-soluble polymers are attractive alternates for producing nanoscale and microscale therapeutic vehicles (Hartig, Carlesso, Davidson, & Prokop, 2007). Chitosan and its derivatives have been applied to form polyelectrolyte complex microspheres with alginate (Lin, Liang, Chung, Chen, & Sung, 2005; Wong, Yuan, & Choong, 2011; Wen et al., 2012). However, chitosan only dissolves in dilute acid at pH below its PKa (ca. 6.3). It is not soluble in aqueous medium of higher pH value. As the bioactive substances may become invalid in acidic media, PECs formed from water-soluble chitosan (WSC) derivatives are superior to those generated directly from chitosan in terms of biomedical or pharmaceutical applications. As it is known, the reported chitosan–alginate PEC systems are seldom obtained from pure water media.

Magnetic particles respond instantaneously to external magnetic field by non-contact trigger and have been widely investigated for biomedical, catalysis and other applications (Deng, Yang, Wang, & Fu, 2003; Liu, Wang, & Yang, 2009; Liu et al., 2008). Layer-by-layer (LbL) assembly is an effective approach to design functional materials through alternate depositing charged polymer on a substrate (Hammond, 2011). In view of what have been mentioned above, we intend to form a kind of novel functional multilayer microspheres through LbL technique by using alginate and chitosan derivative, which is soluble in distilled water, as starting materials. As chitosan can be dissolved in the acid-free condition, the fabrication process of the chitosan–alginate microspheres will be optimized and become easier to be controlled. It is anticipated that the novel polysaccharide pair will be probably suitable for biomedical or pharmaceutical applications and other fields as well.

2. Experimental part

2.1. Materials

Distilled water-soluble chitosan (DWSC, the percentage of —CO—CH=CH—COOH was 36.9%) was prepared via the

* Corresponding author. Tel.: +86 0595 22691357; fax: +86 0595 22693999.
E-mail address: congmingxiao@hqu.edu.cn (C. Xiao).

0144-8617/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.carbpol.2013.07.068

esterification reaction between chitosan (minimum 90% deacetylation, viscosity average molecular weight was 2.07×10^6) and maleic anhydride according to the literature (Zhu, Pan, Liao, Zhao, and Chen, 2008). Ferrofferic oxide (Fe_3O_4) particles were prepared by chemical co-precipitation (Ménager, Sandre, Mangili, & Cabuil, 2004). Sodium alginate (SA, its weight average molecular weight was ca. 33,000), ferrous chloride ($FeCl_2{\cdot}4H_2O$), ferric chloride ($FeCl_3{\cdot}6H_2O$), sodium hydroxide, hemoglobin (HB), Coomassie brilliant blue G250 (CBB), acetic acid, Span 60, liquid paraffine, calcium carbonate, boric acid and sodium borate were all analytical grade reagents, purchased domestically and used directly.

2.2. Preparation of magnetic alginate microspheres

The emulsification/internal gelation technique (Chen & Subirade, 2006) was used to prepare magnetic calcium alginate microparticles. Briefly, 0.5 g SA was dissolved in 30 mL distilled water. A mixture of 0.2 g Fe_3O_4 and 0.3 g $CaCO_3$ was added and subjected to ultrasonic treatment for 30 min. The suspension was well mixed with 70 mL liquid paraffine containing 1% Span 60 under stirring. Subsequently, 20 mL liquid paraffine and 0.5 mL acetic acid were supplemented, and the mixture was agitated for 1 h. The formed microspheres were washed with 200 mL boric acid–sodium borate buffer saline (pH 5.4) and separated by the aid of a magnet. Such a treatment cycle was repeated until no oil residue was found on the surface of the microparticles.

2.3. Fabrication of multilayer composite microspheres

The multilayer composite microspheres (MCM) were fabricated through alternating LbL method. Typically, 1 g magnetic alginate (0-layer) microspheres were mixed with 5 mL 0.1% DWSC aqueous solution under stirring for 30 min. The formed alginate–DWSC (1-layer) microspheres were separated by the aid of a magnet and washed with distilled water. Then, 1-layer microspheres were mixed with 5 mL 1% SA aqueous solution, separated and washed in the same way to generate alginate–DWSC–alginate (2-layer) microspheres. The multilayer composite microspheres of three or more layers were obtained by repeating this process.

2.4. Characterizations of multilayer composite microspheres

Magnetic alginate microspheres suspension was sprayed on glass plate and observed with an Omec PIP9.1 particles image analyzer. The Au-coated morphology of magnetic alginate microsphere was examined with a Hitachi JSM-6700F scanning electron microscope (SEM). A predetermined amount of DWSC was dissolved in distilled water. The solution was cast onto glass, dried, and formed multilayer composite membranes with alginate by alternating LbL method. Then the percentages of nitrogen element of the membranes were analyzed with an EA3000CHNS element analyzer. X-ray photoelectron spectrometer (XPS) spectra of MCM surface were collected on a PHI Quantum 2000 scanning ESCA microprobe with Al Kα X-ray source (1486.6 eV, operated at 15 kV and 35 W). MCM was dispersed in distilled water, and its distribution was determined by using a Matersizer laser particle size analyzer. A Lake Shore 7410 vibrating sample magnetometer (VSM) was used to examine the magnetic character of the dried MCM samples.

2.5. In vitro release of model drug-loaded multilayer composite microspheres

One gram of HB or CBB was mixed with 5 mL 1% SA solution respectively, and then drug-loaded MCM was generated through alternating LbL method as described above.

One gram of drug-loaded MCM was immersed in the boric acid–sodium borate buffer saline (0.2 M, pH 7.4) until the drug was completely dissolved out, which was monitored with UV spectrometry. The solution was separated from the mixture by centrifugation. The solid particles were washed with distilled water for three times. Two solutions were merged and supplemented with some distilled water till the volume reached an exact value. The solution was analyzed with a Shimadzu UV2450 UV-visible spectrophotometer. Then, the encapsulation efficiencies (EE) were calculated according to the formula: EE (%) = drug content in MCM/initial feeding drug amount × 100.

In vitro release of drug from MCM was analyzed as followed. Thirty-milligram samples were placed in vials that contained 20 mL boric acid–sodium borate buffer saline (0.2 M, pH 7.4) and maintained at 37 °C. At timed intervals, 5 mL solution was removed and analyzed with a Shimadzu UV2450 UV-visible spectrophotometer at 406 nm (for HB) or 587 nm (for CBB), and 5 mL fresh buffer saline was added in the meantime. The release amount of HB or CBB was calculated according to the linear absorbency (A) and concentration (C) relationship (HB: $A = 8.53682C - 0.00868$, $R^2 = 0.9998$; CBB: $A = 38.76582C - 0.01323$, $R^2 = 0.99928$), and its cumulative release percentage (%) equaled to Acr/Ae × 100, where Acr and Ae were the cumulative release amount and the encapsulated one of the model drug.

3. Results and discussion

LbL assembly is predominantly driven by the electrostatic interaction. Usually, it involves adsorption and deposition of oppositely charged constituents as well as washing step to remove the unbound specimens (de Villiers, Ptto, Strydom, & Lvov, 2011). Therefore, water-soluble polyelectrolyte is preferable. In other words, DWSC is prior to chitosan for LbL assembling with SA. The water-solubility of chitosan is greatly improved by functionalizing with maleic anhydride, which is utilized to prepare DWSC in this article. The chitosan derivative is able to be dissolved in distilled water without adding acid. This is quite important for LbL assembly of DWSC with SA, as SA will gelate in the medium of low pH value.

LbL technique itself is an effective way to produce functional material. Actually, both DWSC and SA are pH-sensitive. It can be anticipated that the multilayer aggregate of DWSC–alginate is also responsive to the change of environmental pH. Magnetic alginate-based composites are a class of robust functional materials (Srivastava et al., 2012). In view of these, we adopt LbL method to prepare magnetic DWSC–alginate MCM so as to explore its functions and applications.

In order to avoid introducing other components, magnetic alginate microspheres are used as the substrates of LbL assembly. The emulsification/internal gelation technique is chosen to obtain Fe_3O_4 homogeneously dispersed alginate microsphere. As expected, Fe_3O_4 are well encapsulated in the regular spheres (Fig. 1). The slow release of calcium ions leads to slow cross-linking of SA and efficient encapsulation of magnetic particles. As shown in Fig. 2, the size of magnetic alginate microspheres is around 150 μm, which is consistent with what was observed by photomicroscope. Actually, calcium alginate microspheres are soft hydrogel beads. No obvious deformation of the microspheres is found, which suggests the amount of encapsulated Fe_3O_4 particles is enough to maintain the global shape. It is observed that the microspheres move toward an external magnet readily. In addition, the surface of MCM is rough. Obviously, this is available for adsorption.

Before we fabricate MCM through LbL assembly, the ability of alternating adsorption and combination between DWSC and alginate is evaluated. DWSC film obtained by casting is used as the

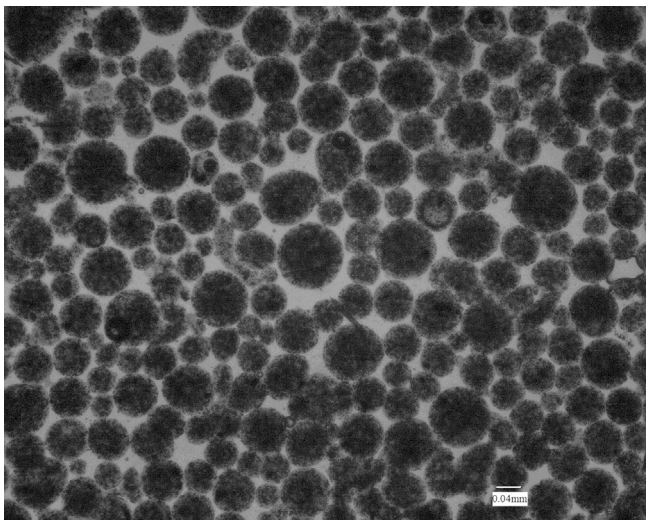

Fig. 1. Photomicrograph of magnetic alginate microspheres (magnification: 40×).

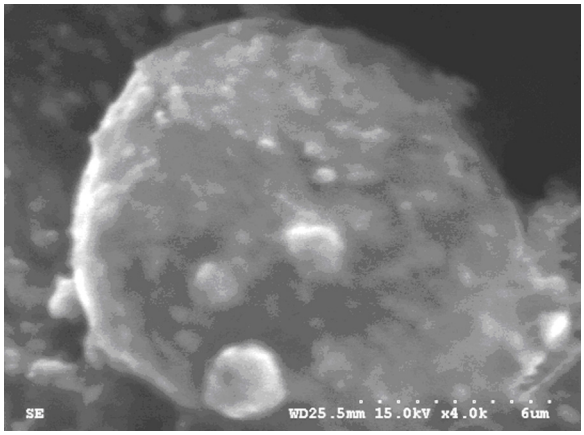

Fig. 2. Scanning electron micrograph of the morphology of magnetic alginate microspheres.

substrate, and is allowed to be immersed in SA and DWSC solution alternately. Nitrogen content of each layer is analyzed. It is found that the nitrogen percentage (N%) of DWSC–SA membrane is changed with the number of layers. N% of DWSC–SA membrane is 5.15, 5.13, 5.31, 5.26, and 5.35% when the layer number is 0, 1, 2, 3, and 4 respectively. There is no nitrogen element in SA. Thus, the total N% is reduced when the outer layer is SA. This phenomenon indicates that it is feasible to conduct LbL assembly between DWSC and SA.

Magnetic alginate microspheres are acted as the substrates (0-layer) for binding DWSC to obtain 1-layer microspheres. As DWSC is soluble in water, the excess or unbound DWSC is remained in solution or easily to be washed away. Subsequently, 1-layer

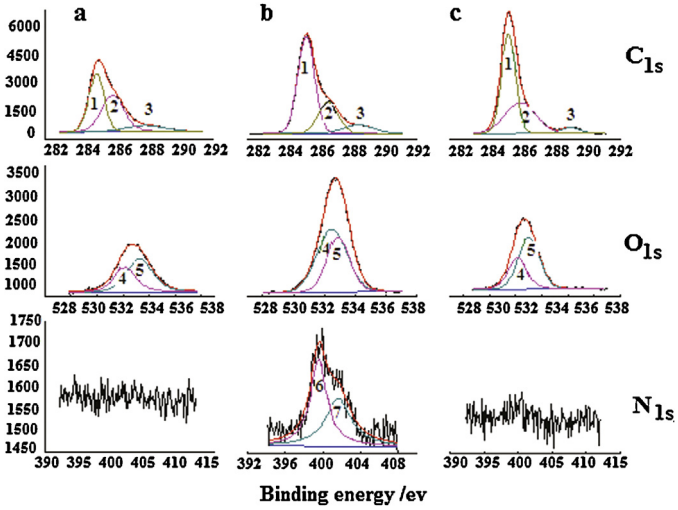

Fig. 3. X-ray photoelectron spectroscopy spectra of distilled water-soluble chitosan/alginate multilayer microspheres (a: 0-layer, b: 1-layer, c: 2-layer).

microspheres that contain amine groups capture the anionic SA molecules in the solution via the electrostatic interaction. As a consequence, 2-layer microspheres are created. Just repeat the cycles of LbL assembly of two oppositely-charged polysaccharides, MCM of three or more layers is obtained. The encapsulated magnetic substance makes MCM of different layers easy to be separated from the liquid and easy to be collected. Evidently, the alternating LbL process is facilely carried out.

The element contents on the surface of MCM are analyzed by using XPS. Fig. 3 shows the XPS spectra of 0-, 1-, and 2-layer MCM. Their detailed information about binding energies and peak areas of C, O and N element is shown in Table 1. Because there is no element in SA, no N_{1s} peak is found on the XPS spectra of 0- and 2-layer MCM, whereas two peaks of N element are found at 399.6 and 401.6 eV. These results indicate that MCM is obtained as expected. The sizes of MCM of different layers also reflect the LbL assembly between DWSC and SA is successfully carried out. The average diameter of MCM increases as the layer number of microspheres increases and the increase rates slow down as the layer number is greater than 2 (Fig. 4). DWSC is an ampholyte. The carboxylic groups on the chains may hinder the LbL assembly between DWSC and SA. Consequently, the size increment of 3- and 4-layer MCM is not high. Fig. 5 shows the magnetization profiles of 0- and 4-layer MCM. Inset one is the low-field magnetization behavior. Both coercivity and remanence are not found on profiles, which indicate MCM is superparamagnetic (Zhou, He, & Zhang, 2012). The saturation magnetic strengths of 0- and 1-layer MCM are 17.46 and 15.64 emu/g respectively, which means the effect of the layers on magnetic strength of the multilayer microspheres is limited.

HB and CBB are chosen as model drugs to represent hydrophilic macromolecule and hydrophobic low molecular weight compound respectively. Figs. 6 and 7 show the encapsulation efficiency and

Table 1
XPS analysis results of C_{1s}, O_{1s} and N_{1s} on the surface of distilled water-soluble chitosan/alginate multilayer microspheres.

MCM samples	Analysis results	1 C*—C/C*—H	2 C*—O	3 C*=O	4 C=O*/O*—H	5 C—O*	6 C—N*	7 N*—H
0-Layer	Binding energy	284.67	285.82	288.0	532.16	533.28	–	–
	Peak area	4310.3	4245.2	1095.2	1646.5	2444.7	–	–
1-Layer	Binding energy	284.82	286.24	288.21	532.48	532.89	399.57	401.66
	Peak area	7352.3	2770.2	1164.7	3791.2	2628.2	702.3	597.9
2-Layer	Binding energy	284.78	285.56	288.82	532.16	533.0	–	–
	Peak area	6205.6	4191.8	461.8	1569.6	2696.4	–	–

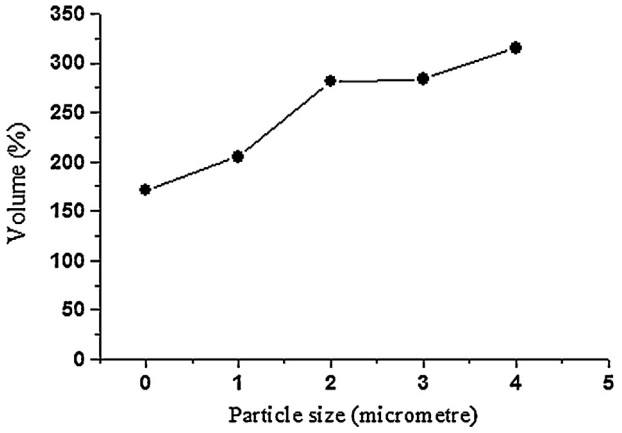

Fig. 4. Average diameters of distilled water-soluble chitosan/alginate multilayer microspheres.

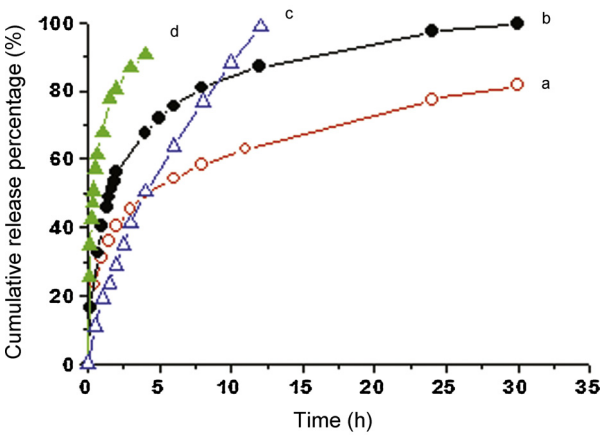

Fig. 7. Release behavior of hemoglobin-loaded (a: 1-layer, b: 0-layer) or Coomassie brilliant blue G250-loaded (c: 1-layer, d: 0-layer) distilled water-soluble chitosan/alginate multilayer microspheres.

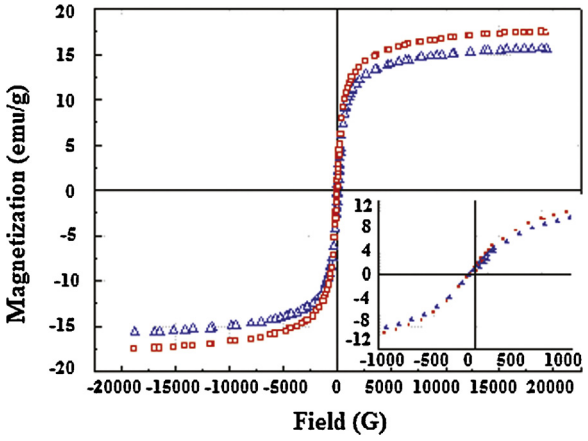

Fig. 5. Magnetization curves of distilled water-soluble chitosan/alginate multilayer microspheres (a: 0-layer, b: 4-layer).

release behavior of the drug-loaded MCM. The differences in structure and molecular weight are considered as the major factors that affect the encapsulation and release of the two model drugs. The interaction between HB and the matrix is higher than that between CBB and MCM. Moreover, the molecular weight of CBB is much lower than that of HB. Therefore, the encapsulation efficiency of MCM for CBB is much lower than that for HB, and the release rate of CBB from the matrices is higher than that of HB. The structure and size characters of CBB also result in the difference of encapsulation efficiency between 0- and 1-layer MCM for the same drug. There are evident differences of release rates between 0- and 1-layer MCM loaded with drugs. Binding another layer decreases the penetrating ability of drugs from MCM, which slows down the release rate. It is noted that the encapsulation efficiencies of 0- and 1-layer MCM for HB are 27.9 and 27.1% respectively, which suggests the effect of layer number on the encapsulation efficiency for HB is neglectable. These preliminary results indicate that alternating LbL assembly of DWSC and SA is suitable for encapsulating and release of hydrophilic macromolecule. Undoubtedly, it is meaningful to well investigate the effect of layer number on the release rate of HB from MCM. On the other hand, the encapsulation efficiency should be improved. Ribeiro and his cooperators obtained chitosan-coated alginate microspheres through the emulsification/internal gelation technique and used them as carriers for encapsulating HB. The encapsulation efficiency was as high as 89% (Ribeiro, Silva, Ferreira, & Veiga, 2005). Indeed, MCM that obtained from LbL assembly of DWSC and SA in absence of acid is advantageous for encapsulating bioactive substance. The magnetic substance embedded is propitious to LbL process. However, the presence of the magnetic particles maybe one reason for the low encapsulation efficiency. Thus, how to balance the advantages and disadvantages of layer number and magnetic substance needs to be further researched.

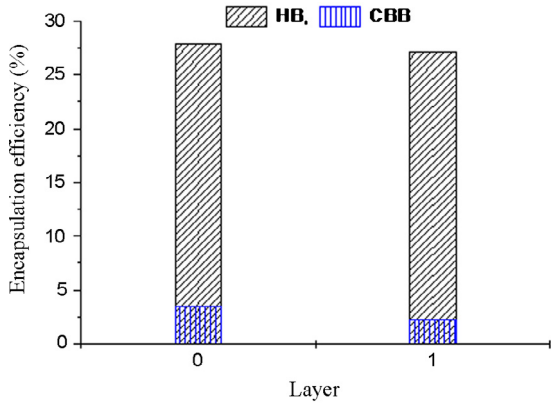

Fig. 6. Effect of layers on the encapsulation efficiencies of distilled water-soluble chitosan/alginate multilayer microspheres for hemoglobin and Coomassie brilliant blue G250.

4. Conclusions

Distilled water-soluble chitosan is successfully applied to conduct alternating LbL assembly with alginate to fabricate functional multilayer microspheres. Magnetic alginate microspheres that prepared via the emulsification/internal gelation technique are utilized as substrates. Such a strategy not only avoids the introduction of other components, but also ensures the LbL assembly is easy to be performed. The formation of multilayer microspheres is verified with several characterization methods. Two compounds of different structures and molecular sizes are assumed as model drugs to evaluate the encapsulation and release behaviors. The alternating LbL assembly of DWSC and SA is a mild and facile process, which suggests our idea may be useful and practical for encapsulating bioactive hydrophilic macromolecules if further improvement is made. It might also be considered as an effective approach in other applications.

Acknowledgements

The authors are grateful for the aids by the Natural Science Foundation of Fujian Province of China (No. 2010J01291 or E1010026) and the Basic Research Item of Central Universities (JB-JD1001).

References

Balakrishnan, B., & Jayakrishnan, A. (2005). Self-cross-linking biopolymers as injectable in situ forming biodegradable scaffolds. *Biomaterials*, *26*, 3941–3951.

Berger, J., Reist, M., Mayer, J. M., Felt, O., Peppas, N. A., & Gurny, R. (2004). Structure and interactions in covalently and ionically crosslinked chitosan hydrogels for biomedical applications. *European Journal of Pharmaceutics and Biopharmaceutics*, *57*, 19–34.

Chen, L. Y., & Subirade, M. (2006). Alginate–whey protein granular microspheres as oral delivery vehicles for bioactive compounds. *Biomaterials*, *27*, 4646–4654.

de Villiers, M. M., Ptto, D. P., Strydom, S. J., & Lvov, Y. M. (2011). Introduction to nanocoatings produced by layer-by-layer (LbL) self-assembly. *Advanced Drug Delivery Reviews*, *63*, 701–705.

Deng, Y. H., Yang, W. L., Wang, C. C., & Fu, S. K. (2003). A novel approach for preparation of thermoresponsive polymer magnetic microspheres with core–shell structure. *Advanced Materials*, *15*, 1729–1732.

Gu, F., Amsden, B., & Neufeld, R. (2004). Sustained delivery of vascular endothelical growth factor with alginate beads. *Journal of Controlled Release*, *96*, 463–472.

Hammond, P. T. (2011). Engineering materials Layer-by-Layer: Challenges and opportunities in multilayer assembly. *AIChE Journal*, *57*, 2928–2940.

Hartig, S. M., Carlesso, G., Davidson, J. M., & Prokop, A. (2007). Development of improved nanoparticulate polyelectrolyte complex physicochemistry by nonstoichiometric mixing of polyions with similar molecular weights. *Biomacromolecules*, *8*, 265–272.

Lee, K. Y., & Mooney, D. J. (2012). Alginate: Properties and biomedical applications. *Progress in Polymer Science*, *37*, 106–126.

Lin, Y. H., Liang, H. F., Chung, C. K., Chen, M. C., & Sung, H. W. (2005). Physically crosslinked alginate/N,O-carboxymethyl chitosan hydrogels with calcium for oraldelivert of protein drugs. *Biomaterials*, *26*, 2105–2113.

Liu, G. Y., Wang, H., & Yang, X. L. (2009). Synthesis of pH-sensitive hollow polymer microspheres with movable magnetic core. *Polymer*, *50*, 2578–2586.

Liu, T.-Y., Hu, S.-H., Liu, K.-H., Shaiu, R.-S., Liu, D.-M., & Chen, S.-Y. (2008). Instantaneous drug delivery of magnetic/thermally sensitive nanospheres by a high-frequency magnetic field. *Langmuir*, *24*, 13306–13311.

Ménager, C., Sandre, O., Mangili, J., & Cabuil, V. (2004). Preparation and swelling of hydrophilic magnetic microgels. *Polymer*, *45*, 2475–2481.

Pillai, C. K. S., Paul, W., & Sharma, C. P. (2009). Chitin and chitosan polymers: Chemistry, solubility and fiber formation. *Progress in Polymer Science*, *34*, 641–678.

Prashanth, K. V. H., & Tharanathan, R. N. (2007). Chitin/chitosan: Modifications and their unlimited application potential—An overview. *Trends in Food Science & Technology*, *18*, 117–131.

Ribeiro, A. J., Silva, C., Ferreira, D., & Veiga, F. (2005). Chitosan-reinforced alginate microspheres obtained through the emulsification/internal gelation technique. *European Journal of Pharmaceutical Sciences*, *25*, 31–40.

Srivastava, M., Singh, J., Yashpal, M., Gupta, D. K., Mishra, R. K., Tripathi, S., & Ojha, A. K. (2012). Synthesis of superparamagnetic bare Fe_3O_4 nanostructure and core/shell (Fe_3O_4/alginate) nanocomposites. *Carbohydrate Polymers*, *89*, 821–829.

Tran, V.-T., Benoît, J.-P., & Venier-Julienne, M.-C. (2011). Why and how to prepare biodegradable monodispersed polymeric microparticles in the field of pharmacy? *International Journal of Pharmaceutics*, *407*, 1–11.

Wen, Y. H., Grøndahl, L., Gallego, M. R., Jorgensen, L., Møller, E. H., & Nielsen, H. M. (2012). Delivery of dermatan sulfate from polyelectrolyte complex-containing alginate composite microspheres for tissue regeneration. *Biomacromolecules*, *13*, 905–917.

Wong, Y. Y., Yuan, S. J., & Choong, C. (2011). Degradation of PEG and non-PEG alginate–chitosan microcapsules in different pH environments. *Polymer Degradability and Stability*, *96*, 2189–2197.

Zhou, L., He, B. Z., & Zhang, F. (2012). Facile one-pot synthesis of iron oxide nanoparticles cross-linked magnetic poly(vinyl alcohol) gel beads for drug delivery. *ACS Applied Materials & Interfaces*, *4*, 192–199.

Zhu, A. P., Pan, Y. N., Liao, T. Q., Zhao, F., & Chen, T. (2008). The synthesis and characterization of polymerizable and biocompatible N-maleic acyl-chitosan. *Journal of Biomedical Materials Research Part B: Applied Biomaterials*, *85B*, 489–495.