



## Synthesis and Characterization of Karaya Gum/Chitosan Composite Microspheres

Ji Zhang<sup>1,2,3\*</sup>, Zhaoli Du<sup>1,2</sup>, Shengjun Xu<sup>1</sup>, and Shengtang Zhang<sup>1</sup>

(1) College of Life Science, Northwest Normal University,  
Lanzhou-730070, PR China

(2) Engineering Technology Research Center of the Effective Components of  
Characteristic Plants of Gansu, Lanzhou-730070, PR China

(3) School of Life Science and Chemistry, Tianshui Normal University,  
Tianshui-741001, PR China

Received 3 August 2008; accepted 16 March 2009

### ABSTRACT

Composite microspheres were prepared by suspension polymerization of karaya gum and chitosan in oil-water emulsion using glutaraldehyde as a cross-linker. The composite microspheres morphology was investigated by scanning electron microscopy. It was found that the products were composed of a large quantity of microparticles with average diameters of 250-350  $\mu\text{m}$  and a few microspheres with diameters of several micrometers. The composite microspheres are spherical particles with asperous surfaces. The thermal behaviour of composite microspheres was studied using both methods of thermogravimetric analysis and differential thermal analysis, further confirming the cross-linking reaction. The results suggest that the composite microspheres show good heat resistance after cross-linking reaction. In order to obtain an insight into the karaya gum/chitosan interactions, the composite microspheres were characterized by Fourier transform infrared spectroscopy (FTIR) to confirm the formation of Schiff's. At the same time, the infrared spectrum indicates the presence of special functional groups on the surface of the microspheres, which involve ion-carboxyl, aldehyde, amino, and hydroxyl groups. The swelling kinetics of the composite microspheres at different pH values has been investigated. The composite microspheres swell at lower pH (<6) as well as higher pH range (pH>9), and the deswelling occurs in the pH range from 6 to 9.

### Key Words:

composite microspheres;  
karaya gum;  
chitosan;  
properties.

### INTRODUCTION

In recent years, the development of appropriate carriers for the immobilization of biomolecules has been a challenging effort for researchers. Therefore, different types of natural and synthetic polymers have received much attention in chemical and biomedical applications. A special interest in developing these polymer-based materials is oriented to prepare microspheres and nanoparticles. So far, several studies have been reported on the development of these carriers (i.e., calcium

alginate, polystyrene, polyacrylamide, polyvinyl alcohol, nitrocellulose, etc.) which have been used in the preparation of microspheres [1-4]. These microspheres can be used as support materials and stabilized in a fluidized bed reactor. The most popular applications of microspheres are wastewater treatment, immobilization of enzymes or other biomolecules, and the preparation alginate, polystyrene, polyacrylamide, polyvinyl alcohol, nitrocellulose, etc.), which have been used

(\* ) To whom correspondence to be addressed.  
E-mail: [duzhaoli@126.com](mailto:duzhaoli@126.com)

in the preparation of microspheres [1-4]. These microspheres can be used as support materials and stabilized in a fluidized bed reactor. The most popular applications of microspheres are wastewater treatment, immobilization of enzymes or other biomolecules, and the preparation of immunological assays [5-7]. In a word, the composite microspheres have potential extensive applications in the fields of medical science, biology, catalysis, and many other areas.

Karaya gum is a complex polysaccharide. The primary structure is shown to be composed of *D*-glucuronic acid, *D*-galacturonic acid, *D*-galactose, *L*-rhamnose and acetyl groups, in different proportions according to the quality, type, and origin of the polysaccharide [8]. The average composition of acetyl free samples is 60% of neutral sugar (rhamnose and galactose) and 40% of acidic sugar residues (galacturonic and glucuronic acid) [9]. Native polysaccharide contains approximately 8% acetyl groups [10].

In this study a novel composite microsphere was prepared and characterized using deacetylated karaya gum and chitosan. The polymeric microspheres were prepared by suspension polymerization [11]. The composite colloid solution of deacetylated karaya gum and chitosan was dispersed as droplets in the dispersion medium and glutaraldehyde was used as the cross-linker. The properties of the composite microspheres, such as morphology, thermal stability, FTIR spectra and pH sensitive behaviour were evaluated.

## EXPERIMENTAL

### Materials

Karaya gum was purchased from the technology company of Anwen hydrosol (Tianjing, China) which was extracted from *Sterculia* tree. The molecular weight was  $9 \times 10^6$  D and the acetyl group's concentration was 8% in mol. Chitosan ( $M_w = 5 \times 10^4$  D, DD  $\geq$  85%) was obtained from Yuhuan Company (Zhejiang, China). The molecular weight of chitosan was determined by the capillary viscometry method using an Ubbelohde viscometer at 25°C. The sodium hydroxide was used to remove acetyl groups of karaya gum and glutaraldehyde (Tianjing, China). The suspension medium was composed of a mixture

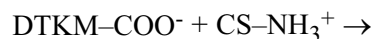
of mineral oil and petroleum ether, and Tween-80 was added as an emulsifier. All chemicals were of analytical grade.

### Preparation of Deacetylated Karaya Gum

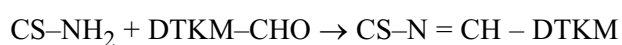
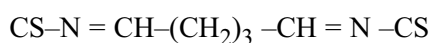
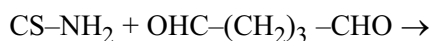
Deacetylated karaya gum was prepared by alkali treatment as follows: karaya gum (100 g) was added into aqueous solutions of sodium hydroxide (1000 mL, 4 w/w%) at 60°C for 24 h with gentle shaking [12]. During the process, sodium tetraborate (2 g) was added to prevent polymer degradation [13]. Then karaya gum polymers were dialyzed by bag filter (molecular cut off: 8000-10000) to form a colloid solution. The colloid concentration was more than 1%. In this experiment, we measured the solution's concentration and prepared a 1% deacetylated karaya gum solution. Karaya gum in the concentration used in this work was quite insoluble, but after deacetylation the solubility increases. The colloid solution of deacetylated karaya gum was used to prepare microspheres without further purification.

### Preparation of Composite Microspheres

The composite microspheres were prepared using the suspension polymerization method: to 1% (mass fraction) deacetylated karaya gum solution, an equal quantity of chitosan powder was added, which was followed by dropwise addition of 1% glacial acetic acid solution to form a complex. The polyelectrolyte complex reaction at 25°C in the acidic condition between deacetylated karaya gum (DTKM) and chitosan (CS) can be represented by:



Then the mixture (40 mL) was added to a flask containing the dispersion medium (80 mL) which was composed of mineral oil, petroleum ether (1:1, v/v) and an emulsifier (Tween-80, 3 mL). During this process, the dispersion medium was stirred at 400-800 rpm at 40°C. After 10 min, 3 mL glutaraldehyde (55%) was added and the temperature was raised to 60°C. The temperature was maintained for about 1 h to carry out the cross-linking reaction. The cross-linking reaction can be represented by:



Later, the composite microspheres were collected and washed consecutively with acetone and ethanol. Then they were placed into hydrochloric solution (1 mol/L) for one day and washed with distilled water. Finally, the composite microspheres were dried at 60°C in a vacuum oven for two days.

## Characterization

### Morphology

Scanning electron microscopy (SEM) was performed with a Philips HL-20 microscope. Samples of the composite microspheres were dropped onto a sample holder and placed in a vacuum oven at room temperature to dry. The samples were coated with gold and then SEM micrographs were obtained.

### Thermal Stability

The thermal stability of the composite microspheres was measured by a Perkin-Elmer instrument. Samples were accurately weighed to 4.0-5.0 mg and were purged with air (heating range: 0-600°C; heating rate: 10°C/min). Indium (mp = 156.8°C) was used as standard reference to calibrate the temperature and energy scales of the instrument [14-15].

### Measurement of Apparent Density

The apparent density of the composite microspheres was determined by graduated cylinder method. The measurements were conducted in five replicates, and average apparent density of the composite microspheres was found to be 0.78 g/mL.

### Measurement of pH Sensitivity and Swelling Ratio

The pH-sensitivity of microspheres was investigated in the range of 1-14. A certain amount of dry microspheres was immersed in separate buffer solutions with pH 1 to 14 at room temperature. Then the weight of the swollen microspheres was frequently measured after wiping the excess solution off the surface with filter paper and calculated the swelling ratio (SR) [16-17].

The swelling ratio (SR) of microspheres was deter-

mined by immersion in buffer solutions at each predetermined time at room temperature with gentle shaking as the following procedures: first, the dried microspheres were immersed in buffer solutions; followed by removal of the samples from the buffer solutions and wiping with a filter paper to remove excess solutions from their surfaces. Finally the microspheres were immediately weighed on an electronic balance. The swelling ratio (SR) of microspheres was calculated by the equation:

$$\text{SR} = (\text{W}_s - \text{W}_d) / \text{W}_d$$

where,

$\text{W}_s$  : weight of swollen microspheres;

$\text{W}_d$  : initial dried weight of microspheres

Each swelling experiment was repeated six times and the average value was recorded.

### FTIR Spectroscopy

The functional groups of microspheres were identified by the basic Fourier transform infrared spectroscopy (FTIR, Alpha-centauri). In a typical procedure, approximately 0.2 mg of microspheres was crushed to make potassium bromide pellets under a hydraulic pressure of 500 kg/cm<sup>2</sup>. The spectra were taken in the wavelength region between 400 and 4000 cm<sup>-1</sup> [18].

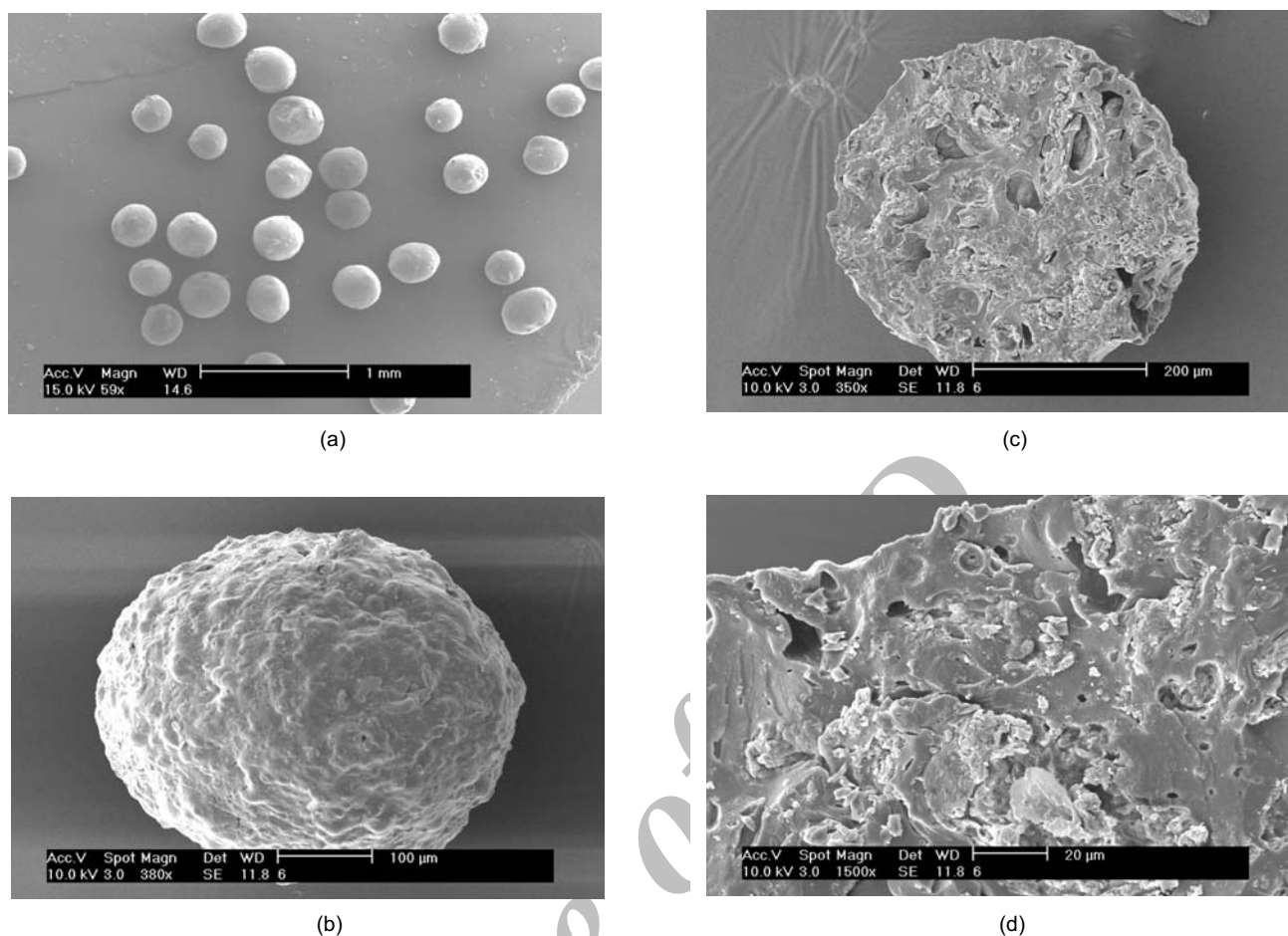
## RESULTS AND DISCUSSION

### Morphology of the Composite Microspheres

Figure 1 shows the SEM micrographs of the composite microspheres. It may be observed in Figures 1a and 1b that the prepared products had a rather rough surface with average diameter of about 250-350 μm; Figures 1c and 1d show the microstructure of the section cut straight through the composite microspheres, and it can be clearly seen that they are porous.

### Dynamic Thermogravimetric Analysis

The thermogravimetric curves of chitosan, deacetylated karaya gum and microspheres show three stages in Figure 2a. The first stage is attributed to desorption of moisture as hydrogen bound water to the polysaccharide structure and the proportion of weight loss was



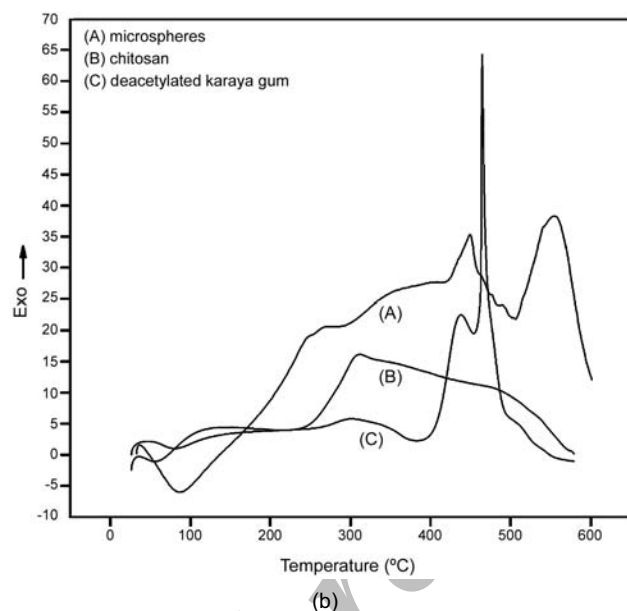
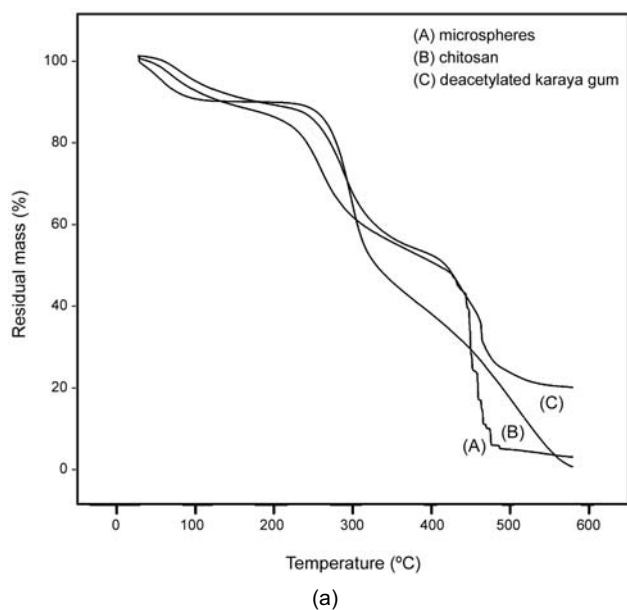
**Figure 1.** Scanning electron microscopy of composite microspheres at different magnifications: (a) 60 $\times$ , (b) 380 $\times$ , (c) 350 $\times$  and (d) 1500 $\times$ .

about 17% for these three materials, which are correlated to three endothermic peaks in the DTA curve in Figure 2b. The endothermic peak strength of microspheres has increased, as shown in Figure 2b, which is due to the volatilization of the solvent. The second and third stages of TG curve are probably due to molecular degradation and decomposition reactions of polysaccharide. The initial decomposition temperature of chitosan is observed at 150 $^{\circ}\text{C}$  whereas final decomposition temperature is observed at 568 $^{\circ}\text{C}$ , which correlates with the main exothermal peaks at 290 $^{\circ}\text{C}$ -320 $^{\circ}\text{C}$ . The weight loss is 80.4%, indicating energy release through the weight loss. The thermal degradation temperatures of deacetylated karaya gum are 190 $^{\circ}\text{C}$ -384 $^{\circ}\text{C}$  and 384 $^{\circ}\text{C}$ -570 $^{\circ}\text{C}$  where the weight losses are 35.5% and 33.5%, respectively. They correspond to two of DTA exothermal peaks at 280 $^{\circ}\text{C}$  and 469 $^{\circ}\text{C}$ , respectively.

Compared with chitosan and deacetylated karaya gum, the microspheres are observed to show a shift towards high temperature regions for the difference in decomposition temperature and the exothermic peaks. These are two exothermic peaks at 449 $^{\circ}\text{C}$  and 555 $^{\circ}\text{C}$ , respectively. This finding has confirmed the cross-linking between the chitosan and deacetylated karaya gum. The cross-linker has led to the formation of a composite of karaya gum and chitosan. Therefore, it may be concluded that the modification has induced thermal stability in the cross-linked networks. This observation is further supported by the decomposition temperature [14-15].

#### **pH Sensitive Behaviour and Swelling Kinetics**

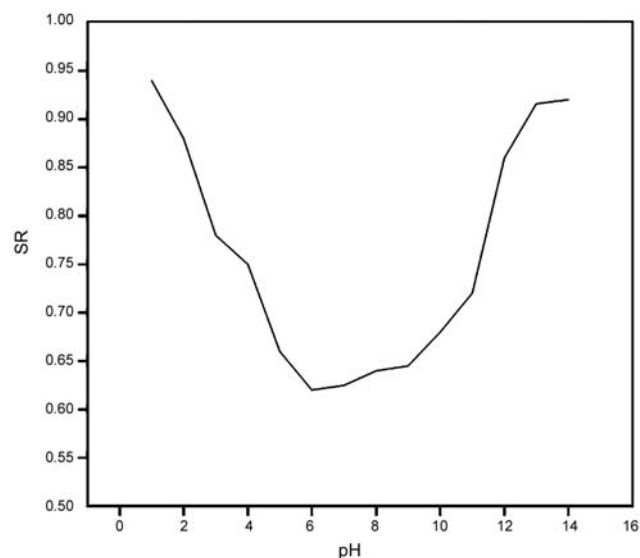
In the experiment, chitosan can absorb six times as much water as its own weight and karaya gum can absorb one hundred times as much water as its own



**Figure 2.** TG curves (a) and DTA curves (b) of (A) microspheres, (B) chitosan, and (C) deacetylated karaya gum samples.

volume at pH = 7. After cross-linking reaction, the swelling capacity of composite microspheres has been decreased significantly and it is affected by different pH values.

As it is shown in Figure 3, pH sensitive characteristics of the composite microspheres were investigated by swelling behaviours. The composite microspheres swelled at lower pH (<6) and also at higher pH range (pH>9). However, deswelling has occurred in



**Figure 3.** Swelling of microspheres at different pH values.

the range of pH 6-9. The swelling capacity is being dependent on the effect of pH on the charged groups introduced on the composite microspheres, because the composite microspheres possess both carboxyl and amino groups. The surplus of amino groups forms ionic bonds and hydrogen bonds with the carboxyl groups of deacetylated karaya gum, which has consumed most of ionic groups. As a result a network is formed with oppositely charged structures which can change the charge state of the ionic groups with varying pH [16].

Under low pH conditions, most of the carboxyl groups are in  $-\text{COOH}$  form, while most of the amine groups of the composite microspheres are in  $-\text{NH}_3^+$  form. Conversely, under high pH conditions, most of the amine groups of the composite microspheres are in  $-\text{NH}_2$  form, while most of the carboxyl groups of the composite microspheres are in  $-\text{COO}^-$  form. In these pH regions, the composite microspheres were swelled due to the increase of ionic repulsion. If we could assume the composite microspheres having an isoelectric point (pI) like protein molecules, the pI should be in the pH region 6-9. Most of the ionic groups are absent due to protonation of the carboxyl group and deprotonation of the amino group at pH 6-9, which have led to a more compact structure and therefore, less water absorption. Thus, the composite microspheres have deswelled in this pH region [17].

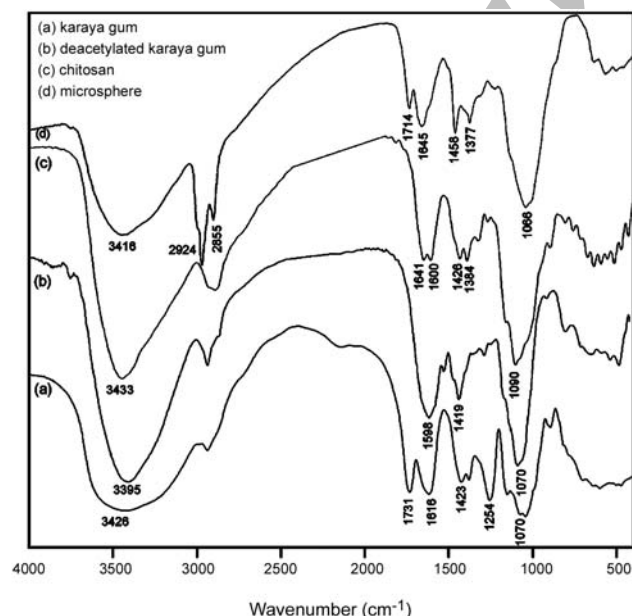
### FTIR Spectroscopy Analysis

The FTIR spectra of karaya gum, deacetylated karaya gum, chitosan, and microspheres films are shown in Figure 4 for comparative purposes. The characteristic peaks at  $1731\text{ cm}^{-1}$  and  $1254\text{ cm}^{-1}$  are attributed to acetyl groups of karaya polysaccharide in Figure 4a. The absorptions at  $1616\text{ cm}^{-1}$  and  $1423\text{ cm}^{-1}$  are due to carboxylate groups of the uronic acid residues. The absence of peaks at  $1731\text{ cm}^{-1}$  and  $1254\text{ cm}^{-1}$  in Figure 4b indicates that the sample is a deacetylated form of karaya polysaccharide [10]. Chitosan in Figure 4c shows its characteristic peaks as follows: the peaks at  $1426$  and  $1384\text{ cm}^{-1}$  belong to the N–H stretching of the amide and C–N stretching vibration, respectively; the peak at  $1641\text{ cm}^{-1}$  is assigned to the stretching of C=O and the peak at  $1600\text{ cm}^{-1}$  is assigned to the bending vibrations of  $-\text{NH}_2$  groups [18].

In Figure 4d the FTIR analysis is based on the identification of absorption bands concerned with the functional groups present in the composite microspheres. The bands wavenumber ( $\text{cm}^{-1}$ ) are as follows:

- CO stretching vibration: the new absorption peak appears at  $1714\text{ cm}^{-1}$  indicates that there are aldehyde groups in the composite microspheres.

- C=N stretching vibrations: the absorbance peak at  $1645\text{ cm}^{-1}$  is wider and stronger than that of pure



**Figure 4.** Infrared spectra of: (a) karaya gum, (b) deacetylated karaya gum, (c) chitosan, and (d) microspheres samples.

chitosan, thus the peak indicates the formation of Schiff's base as a result of the reaction between carbonyl groups of glutaraldehyde and amine groups of chitosan chains.

- C–OH bending vibration: this peak indicates the existence of ion-carboxyl in the composite microspheres.

- C–N stretching of the amino groups.

In addition, these results suggest that the composite microspheres are composed of carbohydrate polymers with functional groups [19-20].

### CONCLUSION

A newly designed microsphere was successfully prepared. The glutaraldehyde has been used successfully to prepare physically cross-linked microspheres using deacetylated karaya gum and chitosan. The optimal conditions were obtained for preparation of microspheres for the various areas of biochemical and biotechnological applications: such as immobilization of biomolecules, wastewater treatment, and drug delivery systems. The composite microspheres were characterized based on morphology, dynamic thermogravimetric analysis, FTIR spectroscopy analysis, swelling kinetics and pH responsive behaviours.

### ACKNOWLEDGEMENTS

The research works in this dissertation are supported by the National Nature Science Foundation (Project Number: 50773064) and the Open Fund of Institute of Polymers of Northwest Normal University.

### REFERENCES

1. Fundueanu G, Esposito E, Mihai D, Carpov A, Desbrieres J, Rinaudo M, Nastruzzi C, Preparation and characterization of Ca-alginate microspheres by a new emulsification method, *Int J Pharm*, **170**, 11-21, 1998.
2. Yap FL, Zhang Y, Assembly of polystyrene microspheres and its application in cell micropat-

- terning, *Biomaterials*, **28**, 2328-2338, 2007.
3. Zhang Y, Fang Y, Xia HY, Xie YX, Wang RF, Li XJ, Preparation of AgCl-polyacrylamide composite microspheres via combination of a polymer microgel template method and a reverse micelle technique, *J Colloid Interface Sci*, **300**, 210-218, 2006.
  4. Oliveira HP, Tenório AC, Lima EG, Melo CP, Dielectric characterization of colloidal solutions of retinoic acid embedded in microspheres of polyvinyl alcohol, *Colloids Surf A*, **257-258**, 3-7, 2005.
  5. Denkba EB, Kiliçay E, Birlikseven C, Öztürk E, Magnetic chitosan microspheres: preparation and characterization, *Funct Polym*, **50**, 225-232, 2002.
  6. Govender S, Pillay V, Chetty DJ, Essack SY, Dangor CM, Govender T, Optimisation and characterisation of bioadhesive controlled release tetracycline microspheres, *Int J Pharm*, **306**, 24-40, 2005.
  7. Kosaraju SL, D'ath L, Lawrence A, Preparation and characterisation of chitosan microspheres for antioxidant delivery, *Carbohydr Polym*, **64**, 163-167, 2006.
  8. Le CD, Irinei F, Muller G, Solution properties of gum exudates from *Sterculia urens* (Karaya gum), *Carbohydr Polym*, **13**, 375-386, 1990.
  9. Stephen AM, Churms SC, Polysaccharides and mucilages. In: *Food Polysaccharides and Their Applications*, Stephen AM (Ed), Marcel & Dekker, New York, 377-440, 1995.
  10. Meer W, Gum arabic. In: *Handbook of Water-soluble Polysaccharides and Resins*, Davidson RL (Ed), McGraw-Hill, New York, Ch 8, 3-4, 1980.
  11. Zhu A, Shi Z, Cai A, Zhao F, Liao T, Synthesis of core-shell PMMA-SiO<sub>2</sub> nanoparticles with suspension-dispersion-polymerization in an aqueous system and its effect on mechanical properties of PVC composites, *Polym Test*, **27**, 540-547, 2007.
  12. Brito ACF, Silva DA, Paula RCM, Feitosa JPA, *Sterculia striata* exudate polysaccharide: characterization, rheological properties and comparison with *Sterculia urens* (karaya) polysaccharide, *Polym Int*, **53**, 1025-1032, 2004.
  13. Paulino AT, Simionato JI, Garcia JC, Nozaki J, Characterization of chitosan and chitin produced from silkworm chrysalides, *Carbohydr Polym*, **64**, 98-102, 2006.
  14. Maciel JS, Silva DA, Paula HCB, Paula RCM, Chitosan/carboxymethyl cashew gum polyelectrolyte complex: synthesis and thermal stability, *Eur Polym J*, **41**, 2726-2733, 2005.
  15. Yuan Y, Chesnutt BM, Utturkar G, Haggard WO, Yang Y, Ong JL, Bumgardner JD, The effect of cross-linking of chitosan microspheres with genipin on protein release, *Carbohydr Polym*, **68**, 561-567, 2007.
  16. Du J, Dai J, Liu JL, Dankovich T, Novel pH-sensitive polyelectrolyte carboxymethyl Konjac glucomannan-chitosan beads as drug carriers, *Funct Polym*, **66**, 1055-1061, 2006.
  17. Paula HCB, Gomes FJS, Paula RCM, Swelling studies chitosan/cashew nut gum physical gels, *Carbohydr Polym*, **48**, 313-318, 2002.
  18. Pawlak A, Mucha M, Thermogravimetric and FTIR studies of chitosan blends, *Thermochim Acta*, **396**, 153-166, 2003.
  19. Xiao Y, Zhou XH, Synthesis and properties of a novel crosslinked chitosan resin modified by L-lysine, *Funct Polym*, **68**, 1281-1289, 2008.
  20. Dai YN, Li P, Zhang JP, Wang AQ, Wei Q, Swelling characteristics and drug delivery properties of nifedipine-loaded pH sensitive alginate-chitosan hydrogel beads, *J Biomed Mater Res B*, **86**, 493-500, 2008.