A Novel Microencapsulation of Neem (*Azadirachta indica A. Juss.*) Seed Oil (NSO) in Polyelectrolyte Complex of κ-Carrageenan and Chitosan

Nirmala Devi, T. K. Maji

Department of Chemical Sciences, Tezpur University, Napaam-784028, India

Received 15 February 2008; accepted 12 January 2009
DOI 10.1002/app.30038
Published online 14 April 2009 in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: Microcapsules containing neem (*Azadirachta Indica A. Juss.*) seed oil (NSO) were prepared by encapsulation of natural liquid pesticide NSO in a polyelectrolyte complex of κ-carrageenan and chitosan. The optimum ratio between carrageenan and chitosan to form a stable polyelectrolyte complex was found as 1 : 0.36. The microencapsulation method for NSO loading was also optimized. SEM study demonstrated that the surface of the microcapsules became more irregular as oil loading increased. The release rates of NSO were studied by varying the percentage of oil loading, concentration of cross-linking agent, and polymer concentration. Fourier transform infrared spectroscopy (FTIR) study confirmed the complex formation between κ-carrageenan and chitosan. Differential scanning calorimetry (DSC) and FTIR study indicated the absence of any significant interaction between polyelectrolyte complex of κ-carrageenan -chitosan and NSO. © 2009 Wiley Periodicals, Inc. J Appl Polym Sci 113: 1576–1583, 2009

Key words: microencapsulation; neem seed oil; polyelectrolyte complex; cross-linking agent; characterization

INTRODUCTION

Botanical insecticides have long been touted as attractive alternatives to synthetic chemical insecticides for pest management because botanicals reputedly pose little threat to the environment or human health.[1] *Azadirachta Indica A. Juss.*, commonly known as the "neem" tree, produces seeds which can be extracted to get neem seed oil (NSO), that has proven its advantages over many synthetic pesticides.[2,3] But, NSO, as it is a liquid, cannot be used in soil. Microencapsulation technique seems to be the best technique to design the liquid pesticide to a solid form and for controlled delivery of the oil in the soil for long duration efficiently.

Natural polymers, due to their eco-friendly nature, cost effectiveness, free availability, and most important—their biodegradability nature, are undoubtedly the best choice for soil applications. Chitosan has attracted much attention because of its biocompatibility, antibacterial fungal, and antimicrobial properties.[4,5] Moreover chitosan on degradation, when used for controlled release formulation for delivery of NSO, may produce nitrogen which can enhance the quality of soil.

Correspondence to: T. K. Maji (tkm@tezu.ernet.in).
Contract grant sponsors: Tezpur University.

Journal of Applied Polymer Science, Vol. 113, 1576–1583 (2009)

Carrageenans, a naturally occurring high-molecular-weight polysaccharides, are made up of repeating units of galactose and 3,6 anhydrogalactose. They cosists of sulpfate esters of galactose and 3,6 anhydrogalactose joined by alternating α-1,3 and β-1,4 glycosidic linkages. Three types of carrageenans namely iota (ι), kappa (κ), and lambda (λ) with one, two, and three sulpfate groups are available. Both iota and kappa carrageenans are able to produce gels which can govern the release behavior of mixtures. Carrageenas have been investigated for use in controlled release tablets.[6] Both carrageenans and chitosan are little bit expensive but considering their multiple advantages, they can be used as an matrix for controlled release of NSO.

When two oppositely charged polyelectrolytes are mixed in an aqueous solution, a complex is formed by the electrostatic attraction between polyelectrolytes. Both chitosan and carrageenan can react to form polyelectrolyte complex. Various neutral polymers (e.g. hydroxypropylmethylcellulose [HPMC]), cationic polymer (e.g.,chitosan), and anionic polymers (κ-carrageenan, sodium alginate) have been used in the form of polyelectrolyte complexes[7] such as sodium alginate-chitosan,[8–12] polyacrylic acid-chitosan,[13] and chitosan-carrageenan[14,15] for the design of controlled release formulations. Several reports addressing the use of polyelectrolyte complex of chitosan and κ-carrageenan for controlled release of drugs have been cited.[12,14] Sakiyama et al.[15] studied the swelling behavior and other properties of this

polyelectrolyte complex. But there is little information regarding the use of this polyelectrolyte complex for encapsulation of agrochemicals. To improve the controlled release behavior, glutaraldehyde has been reported to be used as a cross-linker.[16,17]

The aim of this work is to evaluate the possibility of using chitosan κ-carrageenan complex for encapsulation of NSO and to study the release characteristic of NSO from glutaraldehyde cross-linked microcapsules prepared under various conditions.

EXPERIMENTAL

Materials

Carrageenan Type I, containing predominantly κ-and lesser amount of λ-carrageenan was purchased from Sigma-Aldrich Inc. (USA). Chitosan, medium molecular weight with brookfield viscosity ~ 200 cps was purchased from Sigma-Aldrich Inc. (USA). Glacial acetic acid (E.Merck, India), Tween 80 (E.Merck, India), glutaraldehyde 25% w/v (E. Merck, Germany) were used without further purification. The core material, cold pressed NSO was a gift sample of Ozone Biotech., Faridabad, India. Double-distilled deionised (DDI) water was used throughout the study. Other reagents used were of analytical grade.

Optimisation of chitosan and carrageenan ratio

The optimum ratio between chitosan and carrageenan was judged from the measurement of turbidity and viscosity of the supernatant solution. The complex formation between polyelectrolytes is very much dependent on pH.[18] Mixing of both chitosan and carrageenan solution at different ratio would change the pH which might affect the reaction between polyelectrolytes. For that buffer solution was used to prepare both the solution of chitosan and carrageenan.

Solutions of chitosan (0.5% w/v) and carrageena (0.5% w/v) were prepared in 0.3% acetic acid/sodium acetate buffer at two different pH namely 4 and 5. This range (4.0–5.0) was below the pH at which precipitation of chitosan occurred. The stability of carrageenan would also not be much affected at these pH. Both solutions were mixed in different proportions to make 30 mL. The mixtures were incubated at 40°C for 24 h. It was then centrifuged at 2500 rpm for 1 h. The supernatant solution was separated, viscosity and turbidity were measured. The supernatant solution did not exhibit any significant difference in both viscosity and turbidity at pH 4 and 5.This indicated that the interaction between chitosan and carrageenan would remain similar within the pH range 4.0–5.0. The determination of optimum ratio between chitosan and carrageenan was, therefore, done at pH 4.5. This pH was maintained for preparing of chitosan and carrageenan solution used for subsequent experiments.

Turbidity measurements were done to confirm the optimum ratio between chitosan and carrageenan to form an insoluble polyelectrolyte complex. Solutions of pure chitosan (0.5% w/v), carrageenan (0.5% w/v) and the supernatants from the mixture of both at different ratios were scanned in the range 200–400 nm employing UV spectrophotometer. Both the solution of pure chitosan and carrageenan showed no peak within the scanned range. But the supernatant showed a peak at 341 nm. Therefore, all the turbidity measurements reported were done at 341 nm.

Microencapsulation procedure

In order to optimize the encapsulation process, a series of experiments were conducted by varying the parameters like temperature during formation and hardening of microcapsules, time and temperature for completion of cross linking reaction. The optimized process are described as follows:

In a beaker, known amount of (100 mL) 0.3–0.85% (w/v) of carrageenan solution was taken. This polymer solution was stirred by mechanical stirrer under high agitation at (70 ± 1)°C. This temperature was maintained throughout the experiment. To this, NSO (0.68–2.04 g) was added under high agitation to form an emulsion. A known amount of (36 mL) chitosan solution of 0.3–0.85% (w/v) was added to the beaker drop wise to attain complete phase separation. However, the weight ratio of carrageenan to chitosan was maintained at 1 : 0.36 during all the experiments. At this ratio, interaction between chitosan and carrageenan took place completely as judged by the viscosity and turbidity measurements. The beaker containing the microcapsules was left to rest at this temperature for approximately 15 min. The system was then brought to 5–10°C to harden the microcapsules. The cross linking of the polymer capsule was achieved by slow addition of certain amount of glutaraldehyde (2.5–12.5 mmol/g of polymer). The temperature of the beaker was then raised to 45°C and stirring was continued for another 3–4 h to complete the cross-linking reaction. The beaker was then cooled to room temperature. The microcapsules were filtered through 300-mesh nylon cloth, washed with 0.1% Tween 80 surfactant solution to remove any oil adhered to the surface of microcapsules followed by distilled water, dried and stored inside a refrigerator in a glass ampule.

Measurements

Calibration curve of oil

A calibration curve is required for the determination of release rate of oil from the microcapsules. It was

found that 0.1 g of oil could be easily dissolved in 100 mL of water containing 0.1 g Tween 80. A known concentration of NSO in DDI water containing 0.1% Tween 80 was scanned in the range of 200–400 nm by using UV–visible spectrophotometer. For NSO having concentration in the range 0.001–0.08 g/100 mL, a prominent peak at 254 nm was noticed. The absorbance values at 254 nm obtained with the respective concentrations were recorded and plotted. From the calibration curve, the unknown concentration of NSO was obtained by knowing the absorbance value.

Viscosity and turbidity measurement

The viscosity of the supernatant solution arising from the mixing of chitosan and carrageenan was measured by using an Ubbelohde viscometer at 30°C. The optimal ratio between chitosan and carrageenan was obtained when both polymers would react completely to form an insoluble complex. At this stage, polymer in the supernatant solution would be either absent or negligible. This would make the supernatant viscosity similar or close to that of solvent viscosity.

Because of the mixing of chitosan and carrageenan solution in different ratios, turbidity appeared was different in different solutions. The optimal ratio at which complete phase separation occurred between chitosan and carrageenan solution was the point where the supernatant would have the maximum turbidity. The change in transmittance due to turbidity was monitored continuously at 341 nm wavelength using UV spectrophotometer.

Encapsulation efficiency, oil content, and oil load

A known amount of accurately weighed microcapsules was grounded in a mortar, transferred with precaution to a volumetric flask containing a known amount of 0.1% aqueous Tween 80 solution and kept for overnight with continuous stirring. The encapsulation efficiency (%), oil content (%), and oil loading (%) were calculated by using the calibration curve and the following formulae[17]

$$\text{Encapsulation efficiency } (\%) = w_1/w_2 \times 100$$

$$\text{Oil content } (\%) = w_1/w \times 100$$

$$\text{Oil load } (\%) = w_2/w_3 \times 100$$

where w, weight of microcapsules; w_1, actual amount of oil encapsulated in a known amount of microcapsules; w_2, amount of oil introduced in the same amount of microcapsules; w_3, total amount of polymer used including cross-linker.

Scanning electron microscopy study

The samples were deposited on a brass holder and sputtered with gold. Surface characteristics of the microcapsules were studied at room temperature using scanning electron microscope (model JEOL, JSM-6360) at an accelerated voltage of 10 kV.

Oil release studies

Oil release studies of encapsulated oil were done by using UV–visible spectrophotometer (UV-2001 Hitachi). A known quantity of microcapsules (0.2–0.3 g) was placed into a known volume of 0.1% Tween 80 surfactant solution. The microcapsule-Tween 80 mixture was shaken from time to time and the temperature throughout was maintained at 30°C (room temperature). An aliquot sample of known volume (5 mL) was removed at appropriate time intervals, filtered, and assayed spectrophotometrically at 254 nm for the determination of cumulative amount of oil release up to a time t. Each determination was carried out in triplicate. To maintain a constant volume, 5 mL of 0.1% Tween 80 solution was returned to the container.

Fourier transform infrared (FTIR) study

FTIR spectra were recorded using KBr pellet in a Nicolet (model Impact-410) spectrophotometer. Chitosan, carrageenan, polyelectrolyte complex of chitosan–carrageenan, NSO, NSO loaded microcapsules, and physical mixture of (NSO+ polyelectrolyte complex of chitosan–carrageenan) were each separately finely grounded with KBr and FTIR spectra were recorded in the range of 4000–400 cm^{-1}. The scanning was done thirty times before taking the final spectra.

Thermal property study

Thermal properties of chitosan–carrageenan polyelectrolyte complex, NSO, NSO loaded microcapsules and physical mixtures of (NSO+ chitosan-carrageenan polyelectrolyte complex) were evaluated using differential scanning calorimeter (DSC). The sample (6.0 mg approx.) was taken in an aluminium pan and sealed. The study was done in a differential scanning calorimeter (model DSC-60, shimadzu) at a heating rate of 10°C/min up to 400°C under nitrogen atmosphere.

RESULTS AND DISCUSSION

Viscosity and turbidity measurement

Figure 1(a) shows the change in supernatant viscosity with variation in percentage of chitosan in

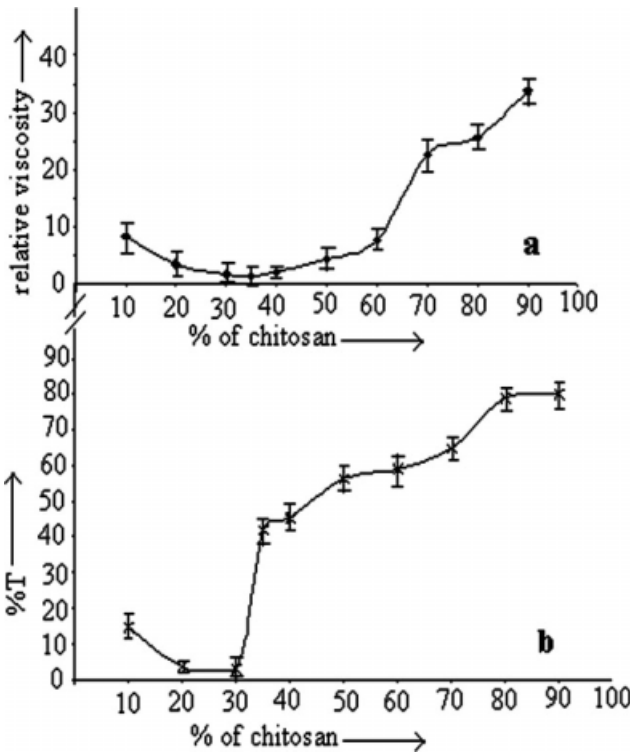

Figure 1 (a) Effect of variation of chitosan concentration in chitosan–carrageenan mixture on relative viscosity of supernatant. (b) Effect of variation of chitosan concentration in chitosan carrageenan mixture on (%) transmittance of supernatant.

chitosan–carrageenan mixture. Each value represents the mean of three values. Viscosity was found to decrease initially, reaching a minimum value, and after that it increased with the increase in the percentage of chitosan. The minimum viscosity observed when the percentage of chitosan in the mixture was in between 30 and 40. Similar type of observation was reported by Tapia et al.[12] At this percentage of chitosan (36%), both the polymers probably reacted completely to form an insoluble complex. The percentage of polymer at this stage in the supernatant would be minimum, which in turn would develop lowest viscosity. The observed higher viscosity at the latter stage might be due to the presence of unreacted chitosan in the supernatant.

The plot of % transmittance against % of chitosan is presented in Figure 1(b). Each value represents the mean of three values. The % transmittance showed a decreasing trend initially followed by an increasing trend latter. The minimum % transmittance occurred when the % of chitosan in the mixture was in between 30 and 40 and also in between these points the transmittance increased sharply. The reason for this could be explained as before. The maximum turbidity developed when the interaction between chitosan and carrageenan was maximum. The higher the turbidity, the lower was the transmittance. The % of increased chitosan latter would decrease the turbidity and hence transmittance increased.

Scanning electron microscopy study

SEM photographs of neat carrageenan + chitosan complex and NSO loaded microcapsules are presented in Figure 2. Photographs of neat carrageenan + chitosan complex [Fig. 2(a)] appeared more regular and free flowing compared to NSO loaded microcapsules[Fig. 2 (b,c)]. The surface of high NSO loaded microcapsules [Fig. 2(c)] appeared more irregular and bursting in comparison with that of low NSO loaded microcapsules [Fig. 2(b)] Similar observation was reported in the literature.[18] Figure 2(d) represents the photograph of the sample after release of NSO. Levels of NSO loading in both the samples (c and d) were almost similar. Bursting look observed in loaded samples was found to decrease after releasing of NSO.

Effect of variation of oil loading

The effect of variation of oil loading on oil content, encapsulation efficiency, and release rate is shown in the Table I and Figure 3. All experiments were carried out in triplicate and the results presented were the average value. With the increase in oil loading, the encapsulation efficiency, the release rate, and % oil content were found to increase throughout the range of oil concentration studied. At low oil load, the dispersion force of the stirrer was more efficient resulting in the generation of smaller oil vesicles. The polymer present in the mixture was enough to encapsulate these vesicles. The dispersion force became progressively difficult as the oil load increased. This would develop large oil vesicles and as a result encapsulation efficiency would increase. As the amount of polymer was fixed, therefore, the polymers would encapsulate all the large oil vesicles at the expense of decrease of thickness of microcapsule wall. The faster release rate might be due to the decrease of thickness of the capsule wall. With the decrease in wall thickness, diffusional path for the oil release became short,[19] which resulted in an increase in release rate. With increase in percent oil load, the oil content (%) increased. At very low oil load, many of the microcapsule probably contained few oil vesicles indicating that there was an abundance of the encapsulating polymer for the oil present. With the increase in oil load (%), the number of oil vesicles in the microcapsule increased which resulted in an increase in oil content. An increase in oil content (%) of microcapsules due to increase in oil loading was supported by SEM study. The

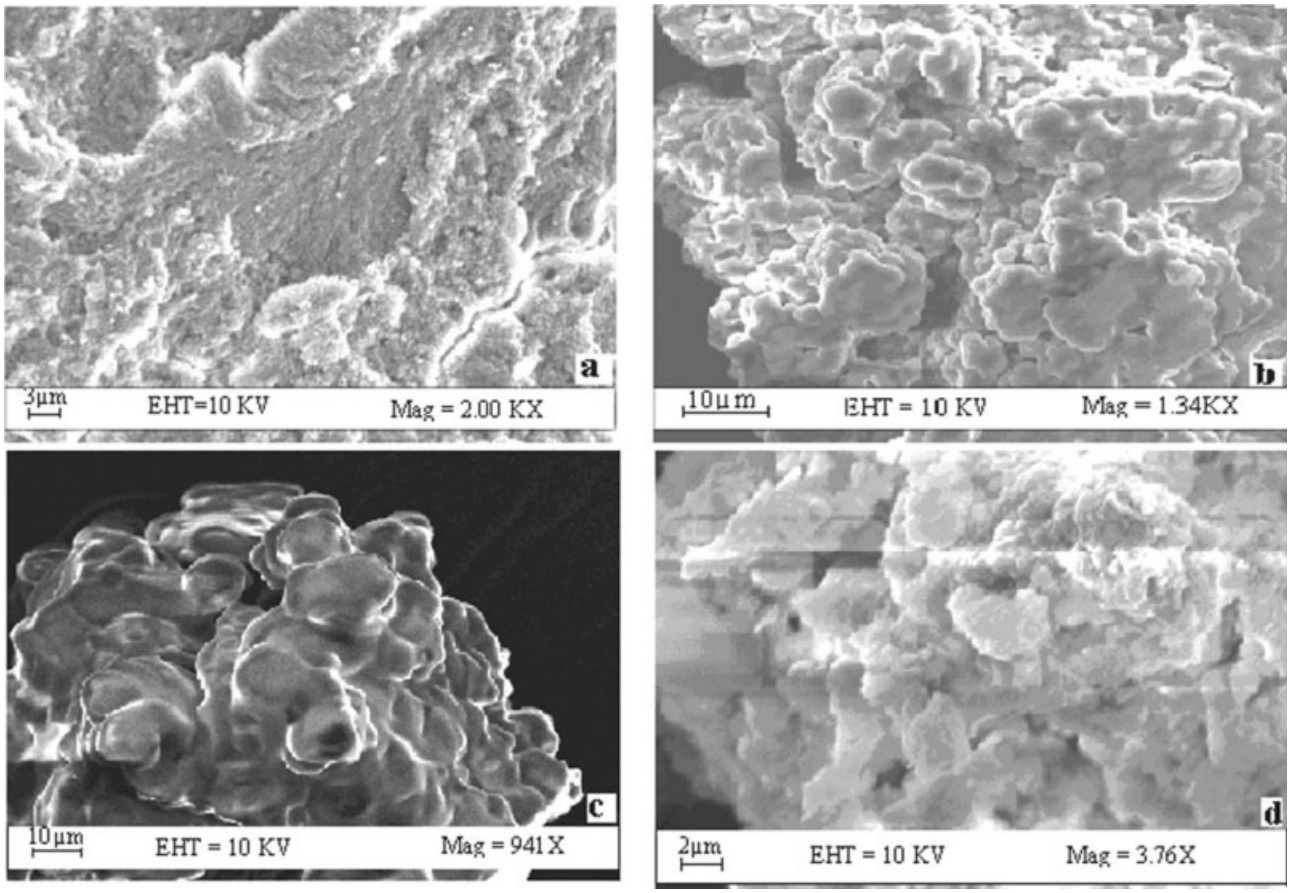

Figure 2 Scanning electron micrographs (a) neat carrageenan + chitosan complex, (b) microcapsules loaded with low percentage of NSO, (c) microcapsules loaded with high percentage of NSO, (d) microcapsules loaded with high percentage of NSO (after release of NSO).

surface characteristics of the microcapsules were found to change as oil content (%) varies.

Effect of variation of cross-linker concentration

The effect of variation of cross-linker concentration on oil loading (%), oil content (%), encapsulation efficiency (%), and release rate is shown in the Table I and Figure 4. The trends of oil loading (%) and oil content (%) shown in the table were as per expectation. With the increase in glutaraldehyde concentration, oil loading decreased for all as expected. But oil content and encapsulation efficiency increased. A decrease in trend in oil content

TABLE I
Effect of Variation of Oil Loading, Polymer and Glutaraldehyde Concentration on the Behavior of Microcapsules

Sample formulations					
Total polymer (g)	Glutaraldehyde (mmol)	NSO (g)	Oil load (%)	Oil content (%)	Encapsulation efficiency (%)
0.68	2.5	0.68	73.12	31 ± 1.0	73.39 ± 2.37
0.68	2.5	1.36	146.23	48 ± 1.3	80.82 ± 2.18
0.68	2.5	2.04	219.35	59 ± 0.5	85.897 ± 0.728
0.68	1.5	1.36	163.85	41 ± 2.0	66.02 ± 3.22
0.68	7.5	1.36	95.10	48 ± 0.74	98.47 ± 1.51
0.68	12.5	1.36	70.4	40 ± 1.0	96.78 ± 2.40
0.408	1.875	2.04	342.56	61 ± 0.9	78.80 ± 1.169
1.156	4.625	2.04	126.04	46 ± 2.3	82.49 ± 4.13

Chitosan: 0.018–0.306 g; carrageenan: 0.30–0.85 g; water: 136 mL; NSO: 0.68–2.04 g; glutaraldedyde: 1.5–12.5 mmol/g of polymer; temperature: (70 ± 1)°C.

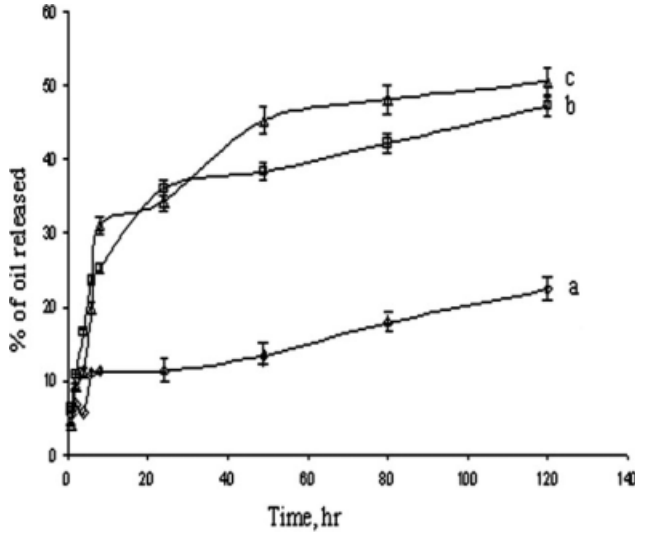

Figure 3 Effect of variation of oil loading on release profile [a: polymer 0.68 g; cross-linker 2.5 mmol; NSO 0.68 g, b: polymer 0.68 g; cross-linker 2.5 mmol; NSO 1.36 g, c: polymer 0.68 g; cross-linker 2.5 mmol; NSO 2.04 g].

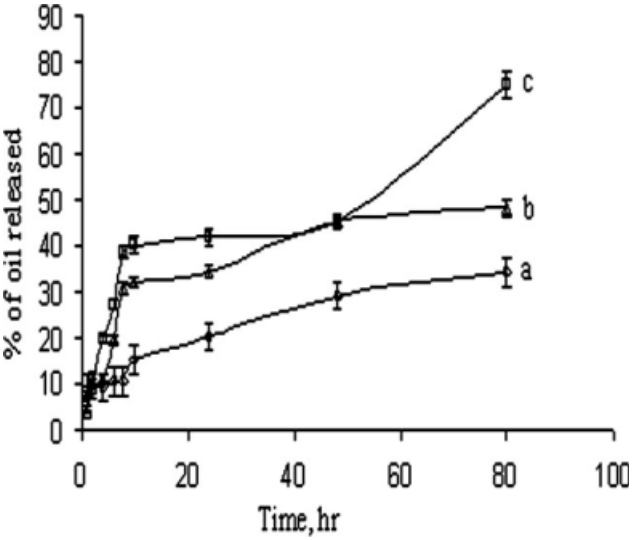

Figure 5 Effect of variation of polymer concentration on release profile [a: polymer 1.156 g; cross-linker 4.625 mmol; NSO 2.04 g, b: polymer 0.68 g; cross-linker 2.5 mmol; NSO 2.04 g, c: polymer 0.408 g; cross-linker 1.875 mmol; NSO 2.04 g].

was observed in the case of 12.5 mmol concentration of glutaraldehyde.

The increase in encapsulation efficiency (%) could be due to the improvement of oil retention capacity of the microcapsules caused by the formation of cross-linking. The cross-linking reaction took place between glutaraldehyde and polyelectrolyte complex of carrageenan and chitosan. The release rate of oil was found to decrease as the % of glutaraldehyde increased. The microcapsule wall became compact as degree of cross-linking increased. This resulted in the decrease of diffusion rate through the microcapsule wall. Similar findings were cited in the literature.[20]

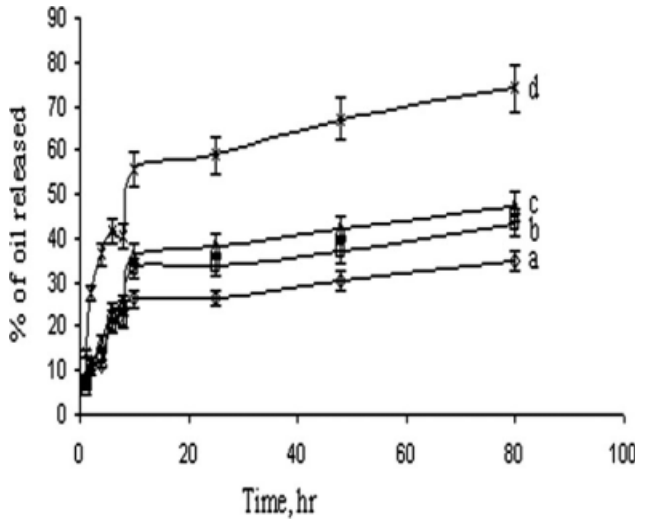

Figure 4 Effect of variation of cross-linker concentration on release profile [a: polymer 0.68 g; cross-linker 12.5 mmol; NSO 1.36 g, b: polymer 0.68 g; cross-linker 7.5 mmol; NSO 1.36 g c: polymer 0.68 g; cross-linker 2.5 mmol; NSO 1.36 g, d: polymer 0.68 g; cross-linker 1.5 mmol; NSO 1.36 g].

Effect of variation of polymer concentration

Table I shows the results of the effect of variation of total polymer concentration on oil loading, oil content, and encapsulation efficiency. In all the studied experiments, the ratio of polymer to cross-linker was kept fixed. As expected, both oil loading (%) and oil content (%) decreased with the increase in total polymer content. Encapsulation efficiency increased initially and then leveled off. With the increase in polymer content, more and more polymer would be available to encapsulate the oil vesicles and thereby efficiency increased. The excess polymer after complete encapsulation would enhance the thickness of the microcapsule. The release profile is shown in Figure 5. The release rate was found to decrease with the increase in polymer concentration. The increase in wall thickness of the microcapsules might be responsible for this type of behavior.[18]

FTIR study

FTIR spectra of Chitosan (curve-a), Carrageenan (curve-b), chitosan-carrageenan polyelectrolyte complex (curve-c), NSO(curve-d), physical mixture of (NSO+ chitosan–carrageenan polyelectrolyte

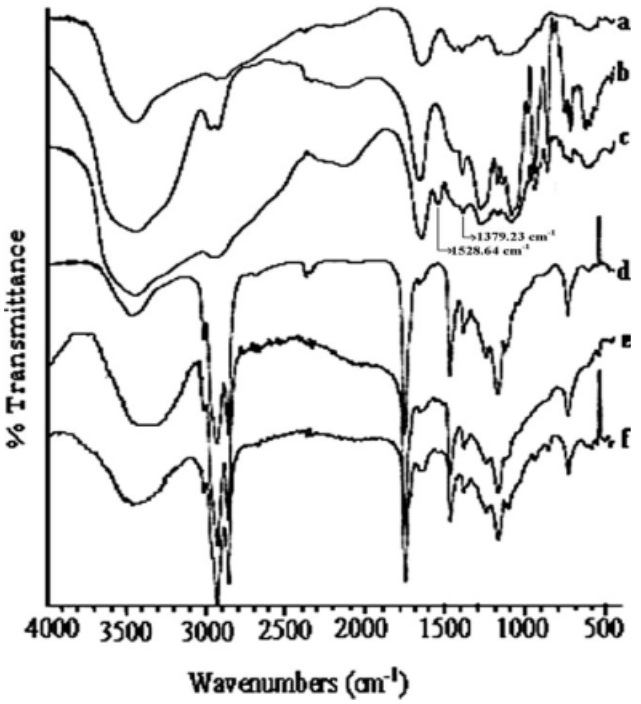

Figure 6 FTIR spectra of (a) chitosan (b) carrageenan (c) polyelectrolyte complex of chitosan and carrageenan (d) NSO (e) physical mixture of (NSO+ polyelectrolyte complex of chitosan and carrageenan) (f) NSO loaded microcapsules.

complex) (curve-e), and NSO loaded chitosan–carrageenan microcapsules (curve-f) are shown in Figure 6. The spectrum of chitosan showed a strong absorption band at 1635.33 cm^{-1} assigned to NH bending. The other notable peaks appeared at 3435, 2920, 1425 and 1384, 1330, 1170, 1075, and 1030 cm^{-1}, were due to O—H + N—H stretching vibration, CH_3 symmetric + CH_2 asymmetric vibration, CH_3 +CH_2 bending vibration, vibration of C—N group, C—O—C asymmetric vibration, C—O(—C—OH—) vibration, C—O(—CH_2—OH—) vibration respectively. All these above peaks appeared in the spectrum of chitosan were observed in the spectrum of carrageenan except the peaks corresponding to nitrogen atom related groups. Besides this, the other notable absorption bands appeared in the spectrum of carrageenan at 1379.23, 1265.70, and 846.33 cm^{-1} were due to sulphonic acid group, C—O stretching band and glycosydic linkages. The appearance of a new band at 1528.64 cm^{-1} due to NH_3^+ groups and reduction of intensity of the absorption band of sulphonic acid groups in the spectrum of chitosan–carrageenan complex indicated the formation of strong polyelectrolyte complex.[12] The absorption bands appeared in the spectrum of NSO at 1745.90, 1463.04, and 1163.85 cm^{-1} were due to carbonyl stretching, CH_2 asymmetric deformation, and C—C stretching vibration. The position of these bands in the physical mixture as well as in the NSO loaded microcapsules remained almost unchanged indicating the absence of any significant interaction between NSO and chitosan–carrageenan polyelectrolyte complex.

Thermal property study

DSC thermograms of neat chitosan–carrageenan polyelectrolyte complex (curve-a), NSO (curve-b), NSO loaded microcapsules (curve-c), and physical mixtures of (NSO+ chitosan–carrageenan polyelectrolyte complex) (curve-d) are shown in Figure 7. In the physical mixture, the ratio of NSO to neat chitosan–carrageenan polyelectrolyte complex was kept similar to that of microcapsules loaded with NSO. The endotherm appeared in all the thermograms except that of oil at around 100°C was due to the removal of moisture. The thermogram of NSO showed an endothermic peak at around 220°C. Both the NSO loaded microcapsules and physical mixture of complex and NSO did not show any remarkable difference in their thermograms. In both the thermograms, the endothermic peak due to NSO appeared almost in the similar position. These results indicated that there was no significant interaction between NSO

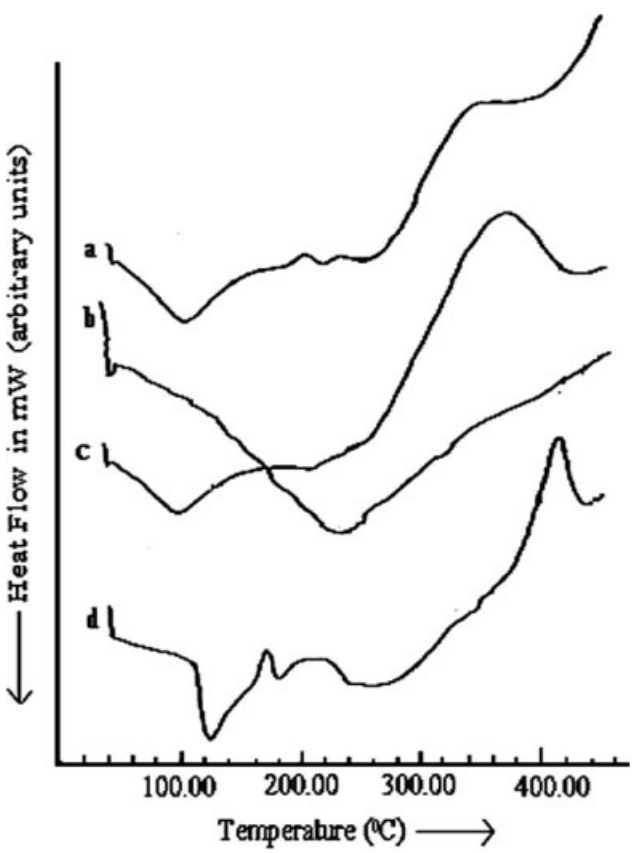

Figure 7 DSC thermograms of (a) neat chitosan–carrageenan polyelectrolyte complex (b) NSO (c) NSO loaded microcapsules (d) physical mixture of (NSO+ chitosan–carrageenan polyelectrolyte complex).

and chitosan–carrageenan complex. The results also suggested a low compatibility in thermal properties in the relation between NSO and chitosan–carrageenan polyelectrolyte complex.

CONCLUSION

The release rate of NSO was found to be dependent on polymer concentration, cross-linker concentration, and oil content. The encapsulation efficiency, oil content, and release rate of NSO increased with the increase in oil loading. The higher the polymer and cross-linker concentration, the lower was the release of NSO from microcapsules. SEM study showed a change in the surface characteristics of the microcapsules due to variation of NSO loading. FTIR results confirmed the formation of chitosan–carrageenan polyelectrolyte complex. It also showed that there was no interaction between chitosan–carrageenan polyelectrolyte complex and NSO. DSC study showed the poor compatibility in thermal properties in the relation between chitosan–carrageenan polyelectrolyte complex and NSO. All these results indicated that chitosan–carrageenan polyelectrolyte complex could be used as an efficient matrix for encapsulation of NSO.

References

1. Isman, M. B. Annu Rev Entomol 2006, 51, 45.
2. Aminabhavi, T. M.; Kulkarni, A. R.; Soppimath, K. S.; Balundgi, R. H.; Mehta, M. H.; Dave, A. M. Polym News 1998, 23, 246.
3. Dave, A. M.; Mehta, M. H.; Aminabhavi, T. M.; Kulkarni, A. R.; Soppimath, K. S. Polym Plast Technol Eng 1999, 38, 673.
4. Hudson, S. M.; Smith, C. In Biopolymer from Renewable Resources; Kalpan, D. L., Ed.; Springer: Berlin, 1988; p 96.
5. Roberts, G. A. F. Chittin Chemistry; Macmillan Press: London, 1992.
6. Bubnis, W. A.; O'Hare, K. T. Proc Int Symp Control Release Bioact Mater 1998, 25, 820.
7. Bhise, K. S.; Dhumal, R. S.; Chauhan, B.; Paradkar, A.; Kadam, S. S. AAPS Pharm Sci Tech 2007, 8; article 44, E1.
8. Bonferoni, M. C.; Rossi, S.; Ferrari, F.; Bettinetti, G. P.; Caramella, C. Int J Pharm 2000, 200, 207.
9. Graham, H.; Baker, Y.; Nojoku-obi, A. J Pharm Sci 1963, 52, 192.
10. Miyazaki, S.; Nakayama, A.; Oda, M.; Takada, M.; Attwood, D. Int J Pharm 1995, 118, 257.
11. Tapia, C.; Costa, E.; Sapag-Hagar, J.; Valenzuela, F.; Basualto, C. Drug Dev Ind Pharm 2002, 28, 217.
12. Tapia, C.; Escobar, Z.; Costa, E.; Sapag-Hagar, J.; Valenzuela, F.; Basualto, C.; Gai, M. N.; Yazdani-Pedram, M. Eur J Pharm Biopharm 2004, 57, 65.
13. De la Torre, P. M.; Enobakhare, Y.; Torrado, S. Biomaterials 2003, 24, 1499.
14. Tomida, H.; Nakamura, C.; Kiryu, S. Chem Pharm Bull Tokyo 1994, 42, 979.
15. Sakiyama, T.; Chu, C. H.; Fujii, T.; Yano, T. J Appl Polym Sci 1993, 50, 2021.
16. Kumber, S. G.; Kulkarni, A. R.; Aminabhabi, T. R. J Microencapsulation 2002, 19, 173.
17. Maji, T. K.; Baruah, I.; Dube, S.; Hussain, M. R. Bioresour Technol 2007, 98, 840.
18. Shu, X. Z.; Zhu, K. Z. J Microencapsulation 2001, 18, 237.
19. Senjokovic, R.; Jalsenjak, I. J Pharm Ind Pharmacol 1981, 33, 279.
20. Dinarvand, R.; Mahmoodi, S.; Farboud, E.; Salehi, M.; Artyabi, F. Acta Pharm 2005, 55, 57.