Microencapsulation of *Zanthoxylum limonella* Oil (ZLO) in Genipin Crosslinked Chitosan–Gelatin Complex for Mosquito Repellent Application

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ABSTRACT: Essential oil containing chitosan gelatin complex microcapsules crosslinked with genipin were prepared by complex coacervation process. The effects of various parameters such as oil loading, ratio of chitosan to gelatin, degree of crosslinking on oil content, encapsulation efficiency, and the release rate of the essential oil were studied. Scanning electron microscopy study indicated that the surface of the microcapsules were more irregular as the amount of oil loading increased. Thermal stability of microcapsules improved with the increase in the amount of chitosan in chitosan–gelatin matrix as revealed by thermogravimetric analysis. FT-IR spectroscopy and differential scanning calorimetry study indicated that there was no significant interaction between chitosan–gelatin complex and oil. © 2008 Wiley Periodicals, Inc. J Appl Polym Sci 111: 779–785, 2009

Key words: chitosan; gelatin; essential oil; microencapsulation; genipin; mosquito repellent

INTRODUCTION

Recently, considerable efforts are made worldwide to promote the use of environmentally friendly and biodegradable natural insecticides and repellents. A large number of essential oils have been evaluated and found to possess mosquito repellency against various mosquito vectors.[1–7] The essential oil obtained from *Zanthoxylum limonella* (ZLO) has been found to possess mosquito repellent properties against different mosquito vectors.[8] However, the repellency of these plant based products is lower both in efficacy and duration than those of synthetic repellents. Furthermore, essential oils are subjected to environmental deterioration by heat, humidity, light, and oxygen.[9]

Controlled release by microencapsulation seems to be the best way to protect essential oil from environmental damage and thus securing a long shelf-life.[10] The vast majority of publications on microencapsulated repellents are patents.[11] Coacervation,[12–17] molecular inclusion,[18] and spray drying[9,19] techniques are generally used for microencapsulation.

Varieties of crosslinking agents like glutaraldehyde, formaldehyde, epoxy compounds[20–22] are reported to be employed for improving the controlled release behavior. These crosslinking agents can cause physiological toxicity. Therefore, a system is looked for which can produce product having either very less or nil toxicity. Genipin, a natural crosslinker, can react spontaneously with amino acids or proteins. Its toxicity is much less than glutaraldehyde.[23] Chitosan, gelatin, and genipin are naturally occurring materials and have attracted much attention from scientists all over the world. The whole system will be fully biodegradable. Genipin crosslinked alginate-chitosan microcapsule for live cell encapsulation was reported by Chen et al.[24] Chen et al.[25] investigated the fluorogenic characteristics of chitosan–genipin reaction for microencapsulation purposes.

The present work is aimed at to produce chitosan–gelatin complex microcapsules containing ZLO by complex coacervation technique using the natural crosslinker, genipin. Efforts have also been made to study the release characteristics of oil from microcapsules prepared under different conditions

EXPERIMENTAL

Materials

Gelatin type B from Bovine skin with a bloom strength ~ 225 and chitosan with a medium molecular weight with Brookfield viscosity ~ 200 cps were purchased from Sigma-Aldrich (USA). Sodium hydroxide (E. Merck, Mumbai, India), glacial acetic acid (E. Merck, India), Tween 80 (E. Merck), Genipin (Mol. wt. 226.22) (Challenge Bioproducts Co.,

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Taiwan), and silicone oil (Ranbaxy Fine Chemicals, Delhi, India) were used as such received. The core material, essential oil from ZLO, was extracted in our laboratory. DDI (double-distilled deionized) water was used throughout the study. Other reagents used were of analytical grade.

Extraction of essential oil

The seeds of ZLO, a big tree available in Tezpur, were collected and shed dried. Essential oil was obtained by steam distillation of the seeds. The oil obtained was separated from the aqueous phase and dried by treating with anhydrous sodium sulfate. The dried oil was transferred into a dark glass bottle and kept inside the refrigerator at low temperature for subsequent use.

Microencapsulation procedure

To a beaker, certain amount of 2% (w/v) chitosan solution previously made in 1% (v/v) aqueous acetic acid and 2% (w/v) aqueous gelatin solution were taken. Total amount of polymer was kept constant at 1 g. The mixture of polymer solution was stirred by mechanical stirrer under high agitation after adding one drop of silicon antifoaming agent at 40°C. The temperature was maintained at 40°C. To this, essential oil (1–4 mL) was added under high agitation to form an emulsion. Using 0.1*N* NaOH the pH of the emulsion was brought to the range of 5.4–5.9 to attain the maximum coacervation. Once the coacervation took place with the formation of microcapsules, the system was brought to room temperature ($\sim$ 30°C) to harden the microcapsules. The crosslinking of the polymer capsule was achieved by slow addition of certain amount of genipin (0.05–0.5 mmol/g of polymer) solution (0.5% w/v aq. solution). The temperature of the vessel was then raised to 40°C and stirring was continued for about 3–4 h in order to complete the crosslinking reaction. The vessel was then cooled to room temperature. The microcapsules were filtered, washed with 0.3% Tween 80 surfactant solution, 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 1 g of oil could be easily dissolved in 100 mL of water containing 0.3 g Tween 80.

A known concentration of essential oil in DDI water containing 0.3 wt % Tween 80 was scanned in the range of 200–400 nm by using UV visible spectrophotometer. For ZLO having concentration in the range 0.005 to 0.1 g/100 mL, a sharp peak at 256 nm was noticed. The absorbance values at 256 nm obtained with the respective concentrations were recorded and plotted. From the calibration curve, the unknown concentration of ZLO was obtained by knowing the absorbance value

Encapsulation efficiency, oil content, and oil load

A known amount of accurately weighed microcapsules was grounded in a crucible, transferred with precaution to a volumetric flask containing a known amount of 0.3 wt % aqueous Tween 80 solution, and kept for about 3 days with continuous stirring to ensure complete extraction of oil in Tween 80 solution The encapsulation efficiency (%), oil content (%), and oil loading (%) were calculated by using the calibration curve and the following formulae

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

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

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

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; and w_3, total amount of polymer used including crosslinker.

Oil release studies

Oil release studies of encapsulated oil were done by using UV–visible spectrophotometer (UV-2001 Hitachi). A known quantity of microcapsules was placed into a known volume of 0.3 wt % Tween 80 surfactant solution. The microcapsule-Tween 80 mixture was magnetically stirred at a constant rate 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 256 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 0.3 wt % Tween 80 solution was returned to the container.

Scanning electron microscopy study

The samples were deposited on a brass holder and sputtered with gold. Surface characteristics of the microcapsules were studied using scanning electron microscope (model JEOL, JSM-6360) at an accelerated voltage of 10–20 kV and at room temperature.

Thermal properties study

Thermal properties of chitosan, gelatin, ZLO, and ZLO containing microcapsules were evaluated by employing thermogravimetric analyzer (TGA) and differential scanning calorimeter (DSC). TGA study was carried out using TGA (model TA 50, shimadzu) at a heating rate of 10°C/min up to 600°C. DSC study was done in a differential scanning calorimeter (model DSC-60, shimadzu) at a heating rate of 10°C/min upto 400°C. Both the studies were done under nitrogen atmosphere.

Fourier transform infrared (FTIR) study

FTIR spectra were recorded using KBr pellet in a Nicolet (model Impact-410) spectrophotometer. Microcapsules, chitosan, gelatin, and ZLO were each separately finely grounded with KBr and FTIR spectra were recorded in the range of 4000–400 cm^{-1}.

RESULTS AND DISCUSSION

Pure gelatin B solution was scanned between 450 and 600 nm at different pH using UV spectrophotometer. The % transmittances studied in the above wavelength were found to follow more or less similar trend at different pH. For chitosan, the % transmittance at the above scanned wavelength remained unchanged up to a certain pH ($\sim$ 6.00), beyond that the % transmittance decreased due to precipitation.

Chitosan–gelatin mixture of different ratios showed the trend similar to those of chitosan. However, in the case of both chitosan and chitosan/gelatin mixture, the maximum absorption occurred at lower wavelength. Therefore all the successive measurements were done at 450 nm and reported.

To optimize the coacervation behavior, the study of phase separation behavior is essential. This was determined by measuring the coacervate yield as well as turbidity.

Gelatin and chitosan solutions were mixed at different ratio (1 : 1 to 1 : 40) at room temperature under stirring condition. The pH of the solution, prepared at different ratio, was varied from 5.0 to 6.0. In this pH range, no precipitation of chitosan occurred and also it was above the isoelectric point of gelatin. Turbidity would appear due to the formation of coacervate particles. The change in transmittance due to turbidity was monitored using UV spectrophotometer at 450 nm. The pH at which maximum absorption noticed was recorded. The coacervate yield was measured at different pH by decanting the supernatant and drying the coacervate phase. The optimum ratio of gelatin–chitosan and pH at which maximum coacervation observed were 1 : 10 and 5.9, respectively. Similar results were reported by Lopez and Bodmeier.[26]

Oil release studies

Effect of variation of oil loading on release rate

The effect of variation of oil loading on encapsulation efficiency and release rate for 1 : 1 chitosan–gelatin microcapsules are presented in Table I and Figure 1. The more the oil-load, the higher was the release rate. The lower in encapsulation efficiency might be due to the higher % of oil loss during isolation of microcapsules.

At low oil load, small oil droplets were formed as the dispersive force of the stirrer was more effective. The % of chitosan–gelatin mixture was enough to encapsulate properly the oil droplets. With the increase in oil-load, the dispersive force of the stirrer became less efficient which resulted in the formation of large oil droplets. At this stage, chitosan–gelatin mixture could be able to encapsulate the large oil droplets only at the expense of decrease in thickness

TABLE I
Effect of Variation of Oil Loading, Chitosan to Gelatin Ratio, and Genipin Concentration on the Behavior of Microcapsules

Sample particulars					
Chitosan (gm) : Gelatin (gm)	Genipin (mmol)	ZLO (mL)	Oil load (%)	Oil content (%)	Encapsulation efficiency (%)
0.25 : 1	0.05	4	308.75	26.45 ± 1.20	34.14 ± 1.74
0.66 : 1	0.05	4	308.75	25.15 ± 0.57	32.47 ± 1.07
2 : 1	0.05	4	308.75	30.65 ± 0.54	39.56 ± 1.74
4 : 1	0.05	4	308.75	37.65 ± 0.75	48.60 ± 1.14
1 : 1	0.05	1	77.20	22.32 ± 0.15	48.31 ± 0.84
1 : 1	0.05	2	154.37	23.68 ± 0.51	44.3 ± 0.35
1 : 1	0.05	3	231.56	28.0 ± 0.12	32.87 ± 0.96
1 : 1	0.10	4	280.27	42.0 ± 0.45	54.22 ± 1.12
1 : 1	0.20	4	236.60	45.34 ± 0.87	58.53 ± 1.36
1 : 1	0.50	4	161.26	46.50 ± 0.64	60.05 ± 0.89

Total polymer = 1 g; genipin = (0.05–0.5 mmol/gm of polymer; oil = (1–4 mL); water = 100 mL; Temperature = (40 ± 1)°C.

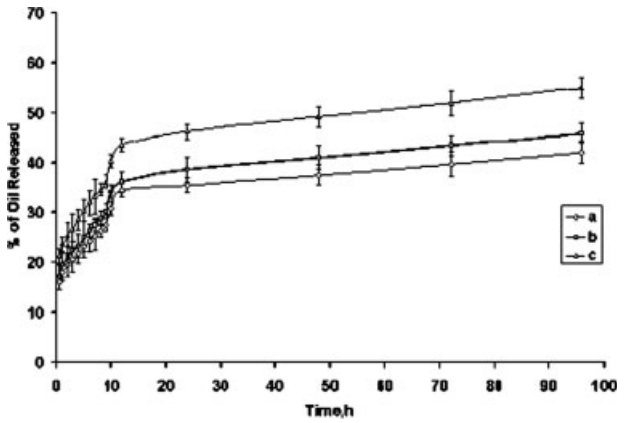

Figure 1 Effect of variation of oil loading on release profile. (a) Polymer 1.0 gm, crosslinker 0.05 mmol, ZLO 1.0 mL; (b) polymer 1.0 gm, crosslinker 0.05 mmol, ZLO 2.0 mL; (c) polymer 1.0 gm, crosslinker 0.05 mmol, ZLO 3.0 mL.

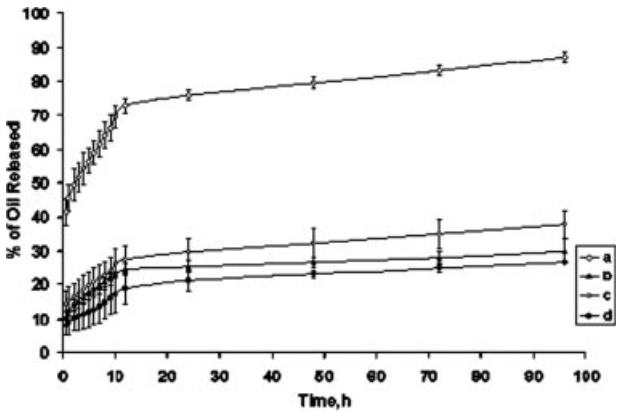

Figure 2 Effect of variation of chitosan to gelatin ratio on release profile. Total polymer 1.0 gm, ratio of chitosan to gelatin in (a) 0.25 : 1; (b) 0.66 : 1; (c) 2 : 1; (d) 4 : 1; ZLO 4.0 mL; crosslinker 0.05 mmol.

of microcapsule wall. Besides this, the amount of chitosan–gelatin mixture might not be sufficient to encapsulate all the oil droplets. Some oil droplets might present without encapsulation. These oil droplets might get exhausted during recovery of microcapsules. As wall thickness decreased, the diffusional path for the oil became short[27,28] which resulted in an increase of release rate.

Again oil content (%) was found to increase with the increase in the % of oil load. At low oil load, many of the microcapsules probably contained few oil droplets indicating that there was an abundance of encapsulating polymer for the oil present. As oil load (%) increased, the number of oil droplets in the microcapsules increased which resulted in an increase in oil content.

Effect of variation of chitosan/gelatin ratio on release rate

The effect of variation of chitosan–gelatin ratio on oil loading, encapsulation efficiency and release rate are shown in Table I and Figure 2. The release rate of oil was governed by the % of chitosan present in the chitosan–gelatin mixture. With the increase in the concentration of chitosan in chitosan–gelatin mixture, the release rate was found to decrease. Again an increase in the viscosity of the chitosan–gelatin mixture was noticed with the increase in the concentration of chitosan.

The higher viscosity might decrease the dispersive force of the stirrer. As a result large oil droplets were formed. The decrease in surface area could be responsible for the decrease in release rate. Moreover, chitosan has more average moieties of primary amine groups than gelatin. Chitosan could react with genipin to form sufficient crosslink bridges compared with gelatin. This might also play a role in reduction of release rate. Similar observations were reported by Kim et al.[29] during the study of the release behavior of triclosan encapsulated within chitosan–gelatin microcapsules.

Both oil content (%) and encapsulation efficiency were also found to increase with the increase in the chitosan concentration. As explained earlier, the increase in viscosity of the medium resulted in the formation of large oil droplets. These large oil droplets had a tendency to coalesce at higher oil load to form further large oil droplets and therefore more oil could be encapsulated with the same amount of encapsulating material.

Effect of variation of concentration of genipin on release rate

Results showing the oil load (%), oil content (%) and encapsulation efficiency are shown in Table I. The release profile of the oil is shown in Figure 3. The trend shown by both oil loading and oil content was as per expectation. Encapsulation efficiency increased

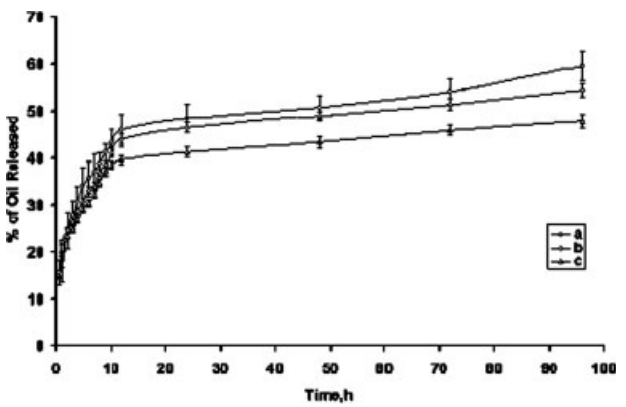

Figure 3 Effect of variation of crosslinker concentration on release profile. (a) Polymer 1.0 gm, crosslinker 0.1 mmol, ZLO 4.0 mL; (b) polymer 1.0 gm, crosslinker 0.2 mmol, ZLO 4.0 mL; (c) polymer 1.0 gm, crosslinker 0.5 mmol, ZLO 4.0 mL.

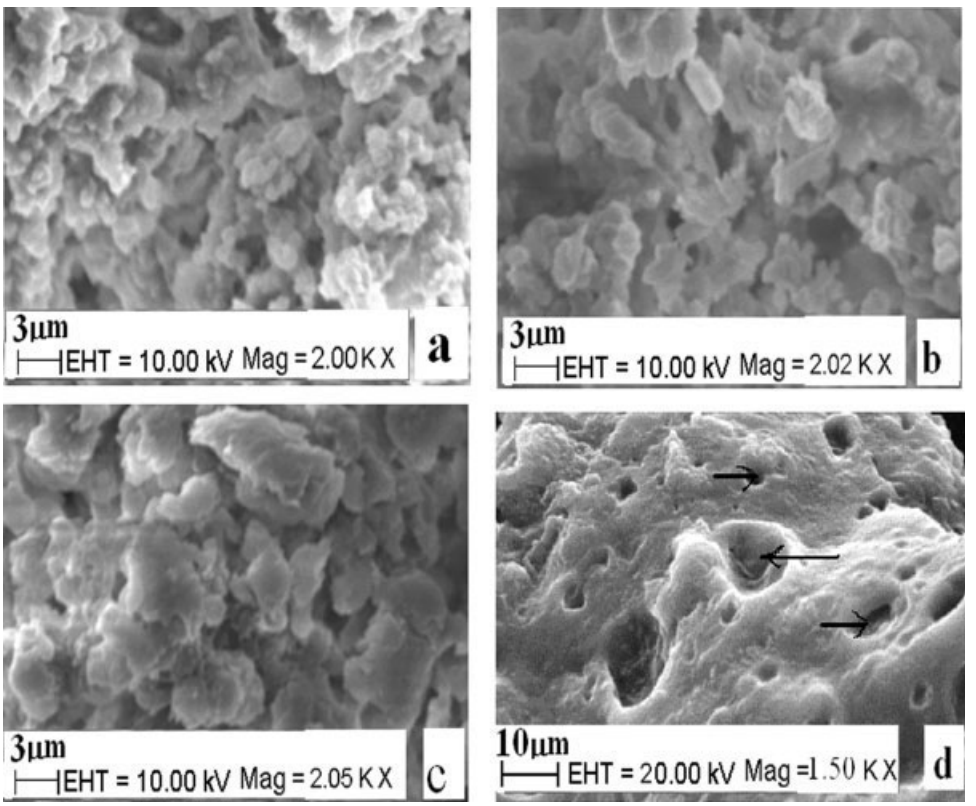

Figure 4 Scanning electron micrographs of microcapsules prepared with oil load (%) (a) 154.37, (b) 308.75, (c) 617.02, (d) 154.37 (after oil release).

with the increase in genipin concentration. The concentration of genipin was varied from 0.1 to 0.50 mmol/g of polymer mixture. The increased efficiency was due to the higher oil retention capacity of the microcapsules caused by the formation of crosslinking. The crosslinking reaction took place between genipin, gelatin, and chitosan. The release rate of oil was found to decrease as the % of genipin increased. The microcapsule wall became compact as degree of crosslinking increased. This resulted in the decrease of diffusion rate through the microcapsule wall. Similar findings were cited in the literature.[10,30]

Scanning electron microscopy study

Scanning electron microscopy (SEM) micrographs of genipin crosslinked chitosan–gelatin microcapsules having different percentage of oil content are shown in Figure 4. At low oil loading [Fig. 4(a)], the surface of the microcapsules appeared smooth compared with those of microcapsules prepared at higher oil loading [Fig. 4(b,c)]. At higher oil loading, a bursting look was observed due to the presence of large percentage of oil. Similar observations were reported in the literature.[10,14] The surface of the microcapsules became more irregular as the percentage of oil loading increased. Figure 4(d) shows the micrograph of the microcapsules after release of substantial amount of oil. The surface of the microcapsules contained a significant number of pin holes (arrow marked). These pin holes might be formed due to the release of oil by diffusion. Moreover, on physical verification, the microcapsules prepared at higher oil loading appeared oily and agglomerated where as those prepared at low oil loading appeared dry and powdery.

Thermogravimetric analysis

Table II shows the initial decomposition temperature (T_i) and residual weight (RW, %) of virgin polymers (chitosan and gelatin), ZLO and ZLO containing

TABLE II
Thermal Analytical Data for Virgin Polymer and Oil Containing Microcapsules

Sample particulars			
Gelatin (gm) : Chitosan (gm)	Oil (mL)	T_i(°C)	RW (%) at 600 °C
1 : 0	–	236	8.75
1 : 0.5	4.0	175	9.33
1 : 1	4.0	190	23.06
0 : 1	–	273	37.74
–	Oil[a]	–	–

T_i, Initial decomposition temperature; RW, residual weight.
[a] Started decomposing from the very beginning.

TABLE III
Temperature of Decomposition (T_D) at Different Weight Loss (%) of Virgin Polymer and Oil Containing Microcapsules

Sample particulars		Temperature of decomposition (T_D) (°C) at different weight loss (%)				
Gelatin (gm) : Chitosan (gm)	Oil (mL)	20	40	60	70	80
1 : 0	–	286	331	394	438	529
1 : 0.5	4	236	300	354	370	446
1 : 1	4	200	302	366	448	–
0 : 1	–	294	320	543	–	–
–	Oil	90	115	155	–	228

microcapsules. Both the Ti (°C) and RW (%) were found to increase with the increase in chitosan concentration in the chitosan–gelatin mixture. The decomposition of ZLO started at an early stage and there was no residue at 600°C.

Temperature of decomposition (T_D) values of ZLO/chitosan/gelatin microcapsules, chitosan, gelatin and oil at different weight loss (%) are shown in Table III. T_D values for the microcapsules increased with the increase in the % of chitosan in the microcapsules. This observed high values might be due to the decreasing chance of elimination of small molecules like NH_3, CO_2, etc. with the formation of crosslinking by genipin. Gelatin contains lower % of lysine and arginine residues as primary amine groups. Chitosan contains glucosamine unit in larger percentage. Genipin could react with the primary amine group of gelatin and glucosamine unit of chitosan. The reaction rate of chitosan and genipin was reported more compared with that of gelatin.[31] So chitosan could form more crosslink bridges compared with that of gelatin and thereby would lead to more thermally stable microcapsules.

The difference in T_i values for various samples could be explained on the basis of their difference in rate of decomposition. The crosslinking reaction of genipin with chitosan was higher compared with that of gelatin as per explanation given above. Moreover oil decomposed at fast rate. Both of these influenced the rate of decomposition and were responsible for different T_i values.

FTIR study

FTIR spectra of chitosan, gelatin, ZLO, and chitosan/gelatin microcapsules containing ZLO were recorded and presented in Figure 5. The spectrum of chitosan displayed a strong amide characteristic peak at 1632 cm^{-1}. Similarly gelatin spectrum also

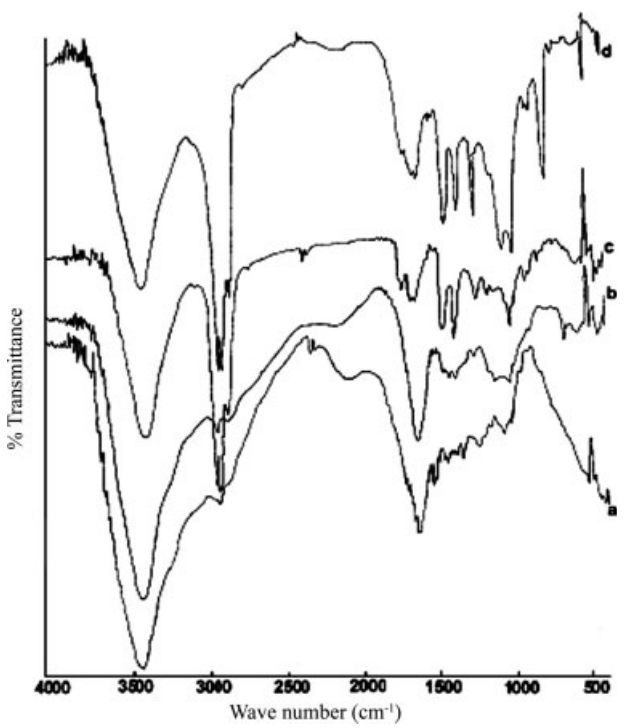

Figure 5 FTIR spectra of (a) gelatin B, (b) chitosan, (c) oil, (d) oil containing microcapsules.

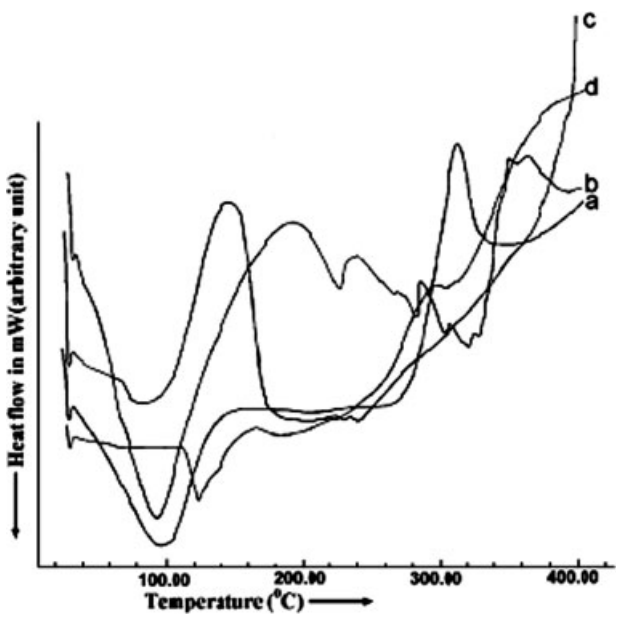

Figure 6 DSC thermograms of (a) chitosan, (b) gelatin B, (c) oil, (d) oil containing microcapsules.

showed an amino band at 1547 cm^{-1} and carbonyl peak at 1624 cm^{-1}. In ZLO, the peaks appeared between 1638 and 1720 cm^{-1} were due to carbonyl stretching band. Besides this, the other notable peaks appeared at 1457 cm^{-1} and 1378 cm^{-1} were due to CH_2 asymmetric deformation and CH_2 symmetric deformation. In the microcapsules, the carbonyl band shifted to 1641 cm^{-1} indicating an interaction between chitosan and gelatin complex. The position of these peaks remained almost unchanged when compared with that of spectrum of ZLO. The position of other peaks which were due to CH_2 asymmetric deformation was also remained unchanged. This suggested that there was no significant interaction between ZLO and chitosan gelatin complex.

Differential scanning calorimetry study

The DSC thermogram of pure chitosan (a), pure gelatin B (b), oil (c), and oil loaded chitosan/gelatin microcapsules (d) are presented in Figure 6. Pure chitosan showed peaks at 98°C, 271°C, and 340°C, respectively. Pure gelatin B showed peaks at 95°C and some multiple peaks in the temperature range 226–323°C. Pure oil showed a peak at 90°C and another broad peak having average peak temperature at 200°C. Oil encapsulated chitosan/gelatin microcapsules showed a sharp peak at 120°C and another two peaks (in shoulder form) having average peak temperature at 240°C and 320°C. The peaks appeared in the temperature range 95–98°C were due to the removal of moisture. The position of one peak appeared in the thermogram (not shown) of physical mixture of chitosan/gelatin/oil at 95°C was found to disappeared and a new peak appeared (shown at 120°C) when genipin was used (crosslinked samples). The position of other two peaks in the thermogram of physical mixture remained almost unchanged irrespective of addition of genipin. The peaks found at 240°C and 320°C in crosslinked oil loaded microcapsules were mainly due to the decomposition of oil and chitosan–gelatin complex respectively. The position of these peaks exhibited in both the thermograms of physical mixture and crosslinked microcapsules suggested that a low compatibility in thermal properties existed in the relation between oil and gelatin-chitosan complex.

CONCLUSIONS

The oil from ZLO can be encapsulated successfully in the chitosan–gelatin matrix using genipin as crosslinker. The release rate of oil depends on oil content, crosslinking density, polymer concentration, etc. The release rate increases with the increase in the oil loading. The higher the percentage of genipin, the lower is the release rate. The release rate has also been found to decrease as the concentration of chitosan in the chitosan–gelatin mixture increases. SEM study shows that the surface of the microcapsules became irregular due to presence of oil. Thermal stability has been found to be improved with the increase in the percentage of chitosan in the chitosan/gelatin matrix. A low compatibility in thermal properties in the relation between oil, gelatin, and chitosan exists as revealed by DSC study. FTIR study shows that there is no remarkable interaction between polymer and oil.

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